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ISBN 0-612-11575-5

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UNIVERSITY OF OTTAWA

For Henry, Mom and Dad for all their love and support...

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ACKNOWLEDGEMENTS

I would like to thank the following people who either directly or indirectly contributed to the body of work covered in this thesis:

Henry for his patience, understanding, encouragment and support.

Dr. T. Durst for his patience in teaching me what I needed to learn, for listening to my opinions even though I may have been wrong and for encouraging me when I needed encouragment.

Dr. T.J. Arnason for involving me in an interesting and rewarding project which has allowed me to learn more about insects and secondary metabolities, meet many interesting people and see many beautiful sights.

My parents, my brothers Greg and Keith, and sister Christine for their love and support.

Yvonne Lear for her friendship, understanding and support.

Corine Bensimon for her patience, skill and determination, the X-ray work on the spirocaracolitones could not have been completed without her.

Adriana Laliberte for her friendship and assistance in the work she was involved in while she was our Co-op High school student and a summer student.

Terry, Dana, Claude, Denise, Maya, Gabrielle, Rafik and Rob for the entertaining and interesting conversations and experiences.

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Abbreviations and Symbols

Ac	acetyl
atm	atmosphere
(br)	broad
°C	degrees celcius
CH_2Cl_2	methylene chloride
CI	chemical ionization
cm^{-1}	wavenumber
COSY	Correlation Spectroscopy
d	doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMF	dimethylformamide
ECB	European corn borer
EI	electron impact
equiv.	equivalents
Et	ethyl
EtOAc	ethyl acetate
FAB	fast atom bombardment
h	hours
Hex	hexane
hν	electromagnetic irradiation
Hz	hertz

IC_{50}	concentration to inhibit growth by 50 %
IR	infrared
J	coupling constant
M	molar
[M]+	molecular ion, $[M]^{+\cdot}$
m	multiplet
Me	methyl
m/e	mass/charge ratio
MeOH	methanol
mg	milligram
min	minute
mL	milliliter
MP	melting point
mp	melting point
MW	molecular weight
N	normality
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
Prep TLC	preparative thin layer chromatography
q	quartet

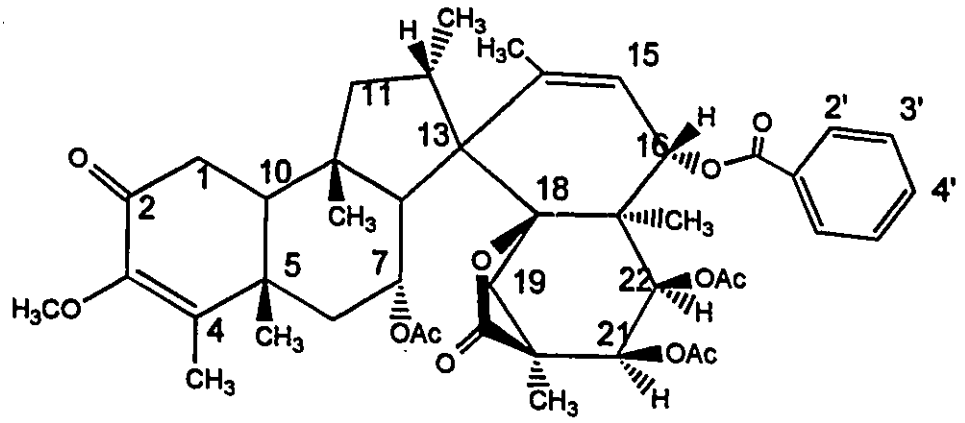
RBF	round bottom flask
rt	room temperature
s	singlet
spp.	species
t	triplet
THF	tetrahydrofuran
tlc	thin layer chromatography

ABSTRACT

This thesis describes the investigation of the bioactivity of triterpenoids, limonoids and spiro-triterpenoids, isolated from the order of the Rutales. The focus of this research has been towards the development of botanical insecticides with non-neurotoxic modes of action in insects. It follows the recent development of Neem (*Azadirachta indica*, Meliaceae) as a botanical insecticide containing a limonoid azadirachtin as its main active compound. Because so few Meliaceae members have been examined for insecticidal activity the project was initiated by the screening of over sixty ethanol extracts of various parts of twenty species (collected from Costa Rica and the Tropical Museum in Miami, Florida) for bioactivity against the European corn borer (*Ostrinia nubilalis*) and the Variegated cutworm (*Peridroma saucia*). The data suggest that a majority of the extracts studied inhibited growth significantly; some are more active than neem leaf extracts. *Swietenia mahogani* bark, *Trichilia glabra* bark, *T. hirta* leaves, *T. americana* bark, *T. trifolia* wood, *T. pleana* and *Azadirachta indica* wood showed potent activity against the cutworm. *Ruptiliocarpon caracolito* bark, *Cedrela odorata* leaves, *Aphanamixis polystachys* wood, *T. glabra* wood and *T. pleana* bark showed good activity against the corn borer larvae.

Bioassay guided fractionation of the most active crude extract, the bark of *Ruptiliocarpon caracolito*, has resulted in the isolation of six very novel spiro-CD-triterpenoids, the spirocaracolitones. The spirocaracolitones, at 100 ppm, were screened for antifungal activity against *Fusarium* using a hyphal growth bioassay. After 48 h a high

degree of inhibition of growth(59-79%) was observed. These novel triterpenoids exhibited no antimalarial activity when screened against *Plasmodium falciparum*. A study of the effects of these compounds, incorporated into artificial diet, on the neonate life cycle of the European corn borer was conducted. All the spirocaracolitones effected the growth and the development of the insects but spirocaracolitone B and spirocaracolitone C were substancially more active than the other spirocaracolitones.

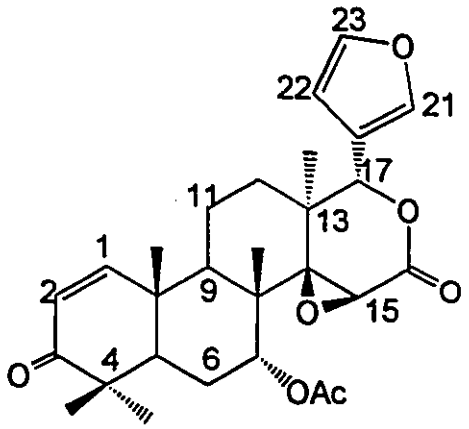


SPIROCARACOLITONE

Two postulates dealing with the biosynthesis of these unique spiro-compounds are also presented. Both commence with a friedelin derivative because canophyllol was isolated in large quantities from the hexane extract. They envisage generation of a carbocation at C12 followed by migration of the C23 methyl group. this results in the formation of a carbocation at C13. The spirosystem results from migration of the C8-C14 bond.

An investigation of the structure/activity relationships of gedunin was conducted to determine the moieties responsible for its antimalarial and/or antifeedant properties. Ten derivatives of gedunin were prepared (1,2-dihydrogedunin, 1,2-

epoxygedunin, 1,2-dihydro-3β-gedunol, 3β-acetoxy-1,2-dihydrogedunin, 7-deacetylgedunin, 7-ketogedunin, hexahydrogedunin, tetrahydrogedunin, 21-acetylgedunin, 23-acetylgedunin) along with five other limonoids (limonin, epilimonol, nomilin, obacunone, hirtin) closely related to gedunin were evaluated for antifeedant activity (*Ostrinia nubilalis*) and antimalarial activity (*Plasmodium falciparum*). Evaluation of these compounds for antimalarial activity presented no increase in activity however it was clearly determined that alterations to the enone in ring A and at C7 of gedunin resulted in large losses of activity. Evaluation of the effects of these limonoids on the neonate life cycle of the European corn borer at 5 and 50 ppm incorporation into the artificial diet did not produced dramatic effects on the growth and development of the larvae. At these concentrations structure/activity relationships could not be proposed.



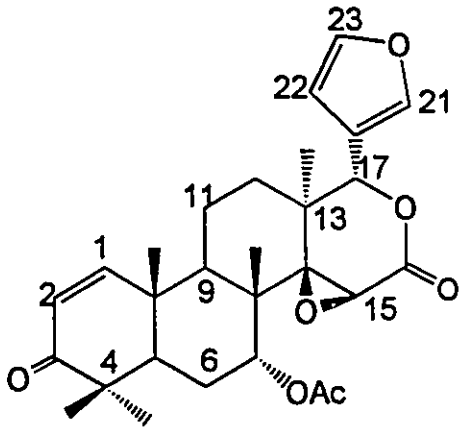
GEDUNIN

CHAPTER 1

ISOLATION AND PREPARATION OF GEDUNIN ANALOGS

1.1.0 INTRODUCTION

The derivatization of gedunin and other D-seco limonoids described in this chapter was designed to investigate the structural requirements necessary for bioactivity against malaria and the European corn borer. Knowledge of structure-activity relationships could lead to a rational strategy for improving the bioactivity of this compound and other D-seco limonoids. Gedunin was chosen as a candidate for this project because it was reported to cause growth reduction and mortality in neonates of the European corn borer at a dietary concentration of 50 ppm and significant feeding deterency on leaves at 500 ppm.[1] It also possesses bioactivity comparable to quinine against malarial plasmodia[2]. It is a constituent of *Cedrela odorata*, a principle timber species of Central America which generates large quantities of sawdust and wood waste available for commercial extraction.



GEDUNIN

Initial modifications focused on ring A to determine if the α,β-unsaturated ketone, a moiety well known to react with biological nucleophiles, was necessary for activity.

1,2-Dihydrogedunin, 1,2-epoxygedunin, 1,2-dihydro-3β-gedunol and its acetate, were prepared to investigate this aspect. Changes to the B ring were focused on modifications at the 7 position: thus both 7-deacetylgedunin and 7-ketogedunin were prepared. The final set of modifications were directed at the furan ring, a common feature of limonoids. Four compounds, hexahydrogedunin, tetrahydrogedunin, 21-acetylgedunin and 23-acetylgedunin were therefore prepared.

The citrus limonoids limonin, epilimonol, nomilin and obacunone were included in this study because they contain a D-seco ring. Hirtin was added to the screening list because its bioactivity has not been investigated in detail and it represents another class of limonoids referred to as the tetracarbocyclics.

The introductory section of this chapter reviews the isolation history of the naturally occuring compounds used in our screen. The synthesis of known and new gedunin analogs will be discussed in a subsequent section.

1.1.1 GEDUNIN

In West Africa a number of large trees commonly used for timber belong to the genus *Entandrophragma* (Meliaceae). One species, *Entandrophragma angolense*, was known as a valuable timber called "gedunohor". Phytochemical investigation of this species resulted in the isolation of a saturated lactone, which was named gedunin, or a methyl ester called methyl angolensate.[3] These compounds were not usually found in the same specimen. Alkaline hydrolysis of gedunin resulted in the formation of furan 3-aldehyde, a product also obtained from alkaline hydrolysis of limonol, therefore the structure of gedunin was postulated

to be that of a D-seco limonoid and therefore similar to limonol. By comparing spectroscopic data of gedunin to limonin and through structural modifications, the structure of gedunin was proposed in 1961.[4] Confirmation of the constitution and relative stereochemistry of gedunin was obtained in 1962 from the X-ray analysis of dihydrogedun-3β-yl iodoacetate, a derivative prepared from gedunin.[5]

Gedunin has been isolated in varying yields from various tissues, mainly the wood, of ten species belonging to the Meliaceae family.(Table 1.1.1) The wood of *Cedrela odorata* and the seeds of *Trichilia trifolia* were found to have the highest gedunin content. It is however important to note that the gedunin content has been documented to be variable within the same species. Gedunin was present in 70-80 % of the Nigerian population of *E. macrophyllum*, the remainder contained methyl angolensate or a mixture of the two.[6] Gedunin could not be found in *E. angolense* specimens which were collected in West Africa even though gedunin had been identified in the same species collected in Nigeria.[7] The variation of gedunin content is seen also in the case of *Cedrela odorata* in Table 1.1.1.

1.1.2 GEDUNIN DERIVATIVES

1.1.2.1 1,2-DIHYDROGEDUNIN

Isolation of 1,2-dihydrogedunin from Meliaceae species is not very common. In the 1950's *Guarea thompsonii* was determined to contain a nonvolatile ketone which, following the isolation and characterization of limonin and then gedunin, was identified as 1,2-dihydrogedunin.[17] Investigation of the heartwood and wood of Nigerian pearwood *(G. thompsonii)*, collected in Nigeria, resulted in the isolation of 1,2-dihydrogedunin in 0.88% and 0.4-1.16% yields, respectively.[17] A year later timber of *G. thompsonii*, also collected in

Nigeria, was determined to contain 0.03% 1,2-dihydrogedunin.[18] These appear to be the only citations of the isolation of this compound from the Meliaceae family.

TABLE 1.1.1: ISOLATION OF GEDUNIN FROM MELIACEAE MEMBERS

PLANTS	TISSUES	% YIELD	COLLECTION SITE	REFERENCE
Azadirachta indica A. Juss.	seeds	0.067	Nigeria	8
Cabralea eichleriana DC.	seeds	NA	Brazil	9
Cedrela odorata L.	heartwood heartwood sapwood	0.17 0.64 0.014	Jamaica Jamaica Brazil	10 11 12
Entandophragma angolense (Welw.) C.DC.	heartwood	0.12	Nigeria	3
Entandophragma congoense A. Chev.	timber	0.20	Africa	6
Entandophragma delevoyi De Wild	timber	0.02	Africa	6,7
Entandophragma macrophyllum A. Chev.	timber	0.15	Africa	6
Melia azadirachta L.	seed oil berries	NA NA	NA India	13 14
Trichilia trifolia L.	seeds	0.65	Venezuela	15
Xylocarpus granatum Koenig	timber seeds	0.10 0.007	Africa Africa	7 16

NA= information not given in reference

1.1.2.2 7-DEACETYLGEDUNIN

Both 7-deacetylgedunin and 7-ketogedunin were of interest because they are gedunin derivatives that have been altered only in the B ring. The first of these, 7-deacetylgedunin, has been isolated from seven Meliaceae species but only in trace amounts.(Table 1.1.2.2) It is interesting to note that this derivative has not been found in *Cedrela odorata* or *Azadirachta indica*, both of which have been reported to contain gedunin. However 7-deacetylgedunin has been isolated from a *Khaya* sp. which is known to contain another D-seco limonoid called khivorin, which differs from gedunin only in the A ring.[19]

TABLE 1.1.2.2: ISOLATION OF 7-DEACETYLGEDUNIN FROM MELIACEAE MEMBERS

PLANTS	TISSUES	% YIELD	COLLECTION SITE	REFERENCE
Cabralea eichleriana DC.	seed	NA	Brazil	9
Chukrasia tabularis A. Juss.	seeds	0.0017	NA	20
Khaya ivorensis A. Chev.	bark	NA	Africa	21
Khaya grandifolia C. DC.	root bark	0.03	Nigeria	19
Melia azadirachta L.	berries seed oil	NA NA	India NA	14 13
Pseudocedrela kotschyii (Schweinf.) Harms.	wood	NA	Nigeria	22
Trichilia trifolia L.	seeds	0.017	Venezuela	15

NA=information not given in reference

1.1.2.3 7-KETOGEDUNIN

From a literature review (Table 1.1.2.3) it can be seen that 7-ketogedunin has been isolated from a number of genera of the Meliaceae family. The percent yields indicate that 7-ketogedunin is not the major limonoid in any of these plants. Several of the plants that contain 7-deacetylgedunin also contained 7-ketogedunin. A number of these species have also yielded the D-seco limonoids gedunin or khivorin.

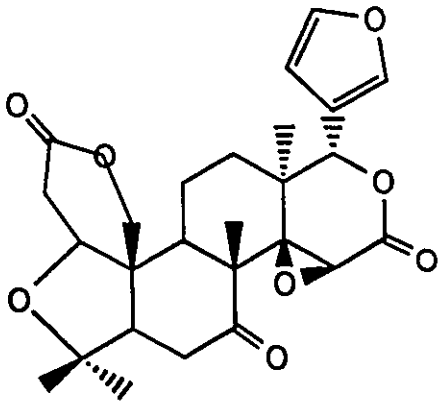
1.1.3 CITRUS LIMONOIDS: LIMONIN, NOMILIN, OBACUNONE AND EPILIMONOL

Limonin, nomilin and obacunone, A,D-seco limonoids, have been isolated from all parts of *Citrus* spp. (Rutaceae). The Rutaceae and Meliaceae families are related in two ways: both are well known to contain limonoids and both belong to the order of the Rutales. Limonin was first isolated in 1841 but eluded structural elucidation until much later.[32] Around the 1940's an intense interest in limonin was prompted by the observation that citrus juices developed a bitter taste with standing.[33,34] This bitterness was not due to the presence of the flavonoid naringin, which had been found earlier to be responsible for the immediate bitterness of fruit juices.[35]

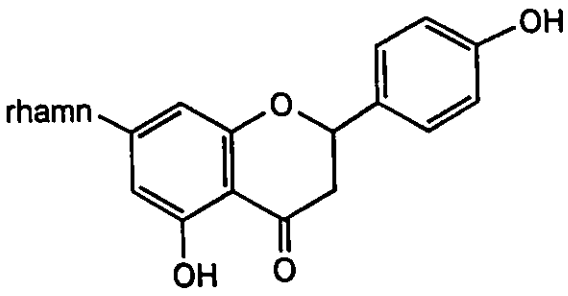
TABLE 1.1.2.3: ISOLATION OF 7-KETOGEDUNIN FROM MELIACEAE MEMBERS

PLANTS	TISSUES	% YIELD	COLLECTION SITE	REFERENCE
Cabralea eichleriana DC.	seeds	NA	Brazil	9
Carapa guyanensis Aubl.	seeds	NA	Brazil	23, 24, 25
Cedrela odorata L.	heartwood heartwood	NA 0.034	Nigeria Jamaica	26 11
Guarea guidonia L. (*G. trichilioides*)	root bark	0.04	French Guiana	27
Khaya ivorensis A. Chev.	timber	NA	Nigeria	21
Khaya senegalensis (Desr.) A. Juss.	heartwood bark timber root	0.0003 0.0002 0.04 0.02	Nigeria Nigeria Nigeria Nigeria	28 28 18 19
Pseudocedrela kotschyii (Schweinf.) Harms.	heartwood wood	NA NA	Nigeria Nigeria	26 22
Swietenia mahagoni Jacq.	seeds	0.0115	Indonesia	29
Trichilia schomburgkii subsp schomburkii C. DC.	stems	0.00075	Guyana	30
Xylocarpus molluccensis (sensu Mabberley)	seeds	NA	Australia	31

NA=information not given in reference



LIMONIN

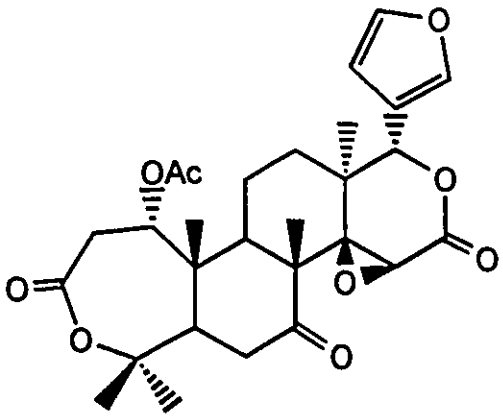


NARINGIN

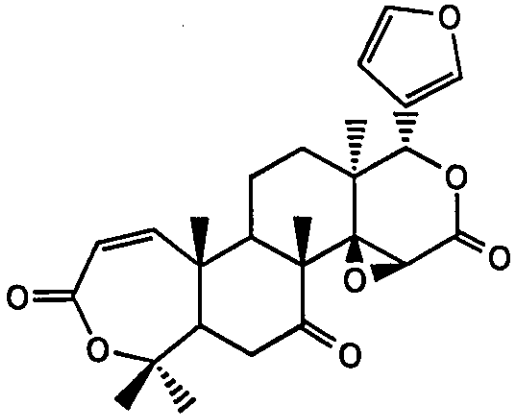
During this time period a number of reports in the literature addressed the constitution of limonin through chemical modifications[32,36,37,38] Finally, in 1960 the X-ray crystal structure of epi-limonol iodoacetate, a derivative prepared from limonin, was determined.[39] The structural determination of limonin was an important accomplishment because it led to the structural elucidation of other related limonoids which had been previously isolated.

Further investigations into the bitterness of fruit juices has suggested that limonic acid, present in the carpellary membrane and albedo tissues of the fruit, is released into the citrus juices during expression and is converted, with time, into limonin by a D-ring lactone hydrolase also present in the juice.[35] The two other limonoids, nomilin and obacunone, have also been isolated as minor bitter principles from citrus species.[32,40] Tests revealed that limonin was exceedingly bitter, nomilin was bitter but obacunone was not bitter.[35] The

removal or decrease in production of these bitter principles in fruit juices is a topic of economic importance and ongoing research.



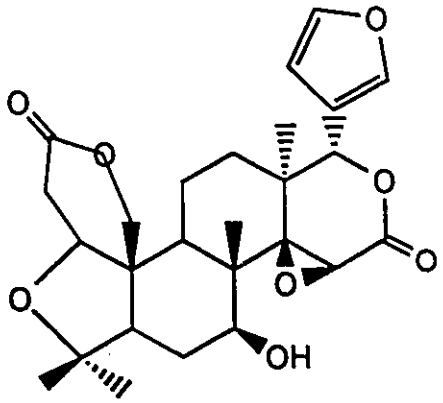
NOMILIN



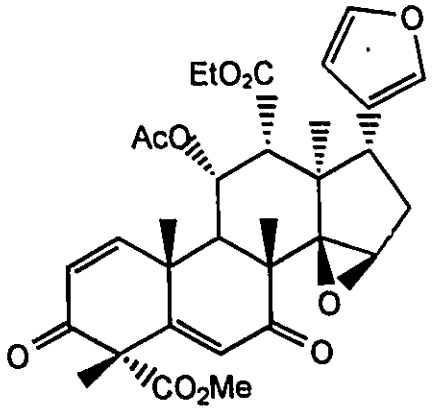
OBACUNONE

More recent interest in these citrus limonoids has centered around the antifeedant activity of these compounds towards various insect pests[35] such as the Spruce budworm[41] and the Colorado potato beetle.[42] This presents an option for insect control that is quite attractive because these limonoids could be extracted from citrus seeds which are an unwanted by-product of the citrus juice industry.

Limonin, nomilin and obacunone were included in these structure activity relationship studies because of there structural similarity to gedunin and because of their relative availability. Epilimonol, a derivative of limonin which has not been extensively screened for antifeedant activity, was prepared for testing.



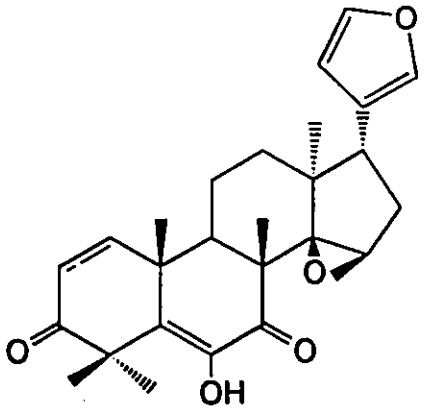
EPILIMONOL



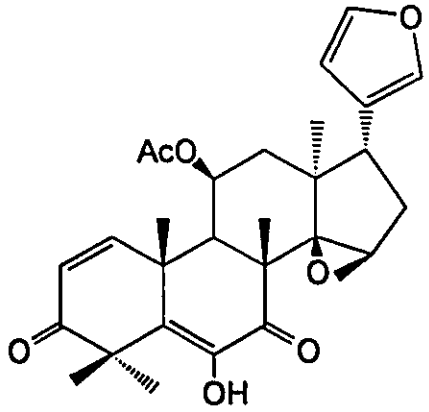
HIRTIN

1.1.4 HIRTIN

Hirtin belongs to the tetracarbocyclic class of limonoids which includes cedrelone and anthothecol.[43] The isolation of hirtin from the seeds and leaves of *Trichilia hirta* L.(Jamaica) was first reported in 1966.[44] A more recent investigation of *T. hirta* from Brazil did not report the isolation of hirtin but a number of other tetracarbocyclic limonoids.[45] Hirtin has recently[46] been shown to have significant growth inhibitory action against *Peridroma saucia* (fall army worm) and *Melanchra picta* (zebra catepillar). Since this compound was available from our collaborators at UBC, it was decided to include it in the study so that its antifeedant activity against the European corn borer and its antimalarial activity against *Plasmodium falciparum* could be evaluated and compared to that of the D-seco limonoids.



CEDRELONE



ANTHOTHECOL

1.2.0 DISCUSSION OF ISOLATION AND SYNTHESIS OF GEDUNIN DERIVATIVES

1.2.1 GEDUNIN

Cedrela odorata wood was selected as the source of gedunin because of our access to this timber species in Central America and because it was reported to contain relatively high quantities of gedunin compared to other species from which it had been isolated (see Table 1.1.1). Also, as mentioned earlier, *C. odorata* is a principle timber species of Central America which generates large quantities of sawdust and wood waste. If a bioactive gedunin derivative was found which could be obtained by simple chemical modifications of gedunin, then an investigation of the gedunin content of this species would be needed to determine if eventual commercial extraction would be feasible.

On the first collection trip to Central America, July 1991, samples of *Cedrela odorata* leaves and bark were collected in Costa Rica and, wood and bark in Belize. Unfortunately when the extracts of the Costa Rican samples were analyzed in the laboratory no gedunin was present. The bark of the sample from Belize contained very little gedunin. Fortunately the *C. odorata* wood collected in Belize contained gedunin. Another collection made in Belize in May 1992 consisted of planks of *C. odorata* from which gedunin was isolated in a 0.11% yield when extracted in April of 1993. When gedunin was isolated from a sample of these planks in March of 1994 the yield was only 0.04%. It is difficult to say if the content of gedunin decreases with storage time because the samples were not analyzed quantitatively. As reported by other researchers,[11] the presence of gedunin in this species is variable.

The melting point of the gedunin isolated was 215-218 °C which was in agreement with the literature value of 218 °C.[3] The key features in the IR spectrum were the carbonyl stretch for the D-ring lactone and the 7-acetate at 1741 cm^{-1}, the C=O stretch at 1669 cm^{-1} for the α,β-unsaturated ketone in ring A and absorptions at 1503 and 875 cm^{-1} which indicated the presence of a β-substituted furan ring.[4]

The complete assignment of all the protons and carbons of gedunin have been published in a workbook entitled Structure Elucidation by Modern NMR.[47] The authors used 2D NMR experiments such as COSY, HC COSY, COLOC and NOE difference spectra to in making the assignments. The proton and carbon spectra of the gedunin which was isolated, were in very good agreement with those reported in the workbook. For the proton and carbon nmr spectra of gedunin see Figure 1.2.1a and 1.2.1b respectively. All of the nmr spectra for gedunin and its analogs, discussed in this chapter, are located in the Appendix located at the end of this chapter.

Either a very weak or no molecular ion peak was obtained when gedunin and its derivatives were submitted for EI/MS analysis. For this reason most of the MS analysis were obtained by CI mass spectrometry. The fragmentation patterns for some of these compounds including gedunin, were published for EI/MS analysis in 1967.[48] Even though molecular ions were often absent, a sufficient number of D-seco limonoids gave molecular ions and fragmentation patterns that could be partly rationalized. Often the loss of methyl radicals and water from parent ions was observed. Acetic acid was lost from compounds containing acetyl moieties and sometimes this was followed by the loss of a methyl group to

give an (M-75) fragment. All the D-seco limonoids analyzed showed the loss of CO_2 apparently from a rearrangement of the epoxylactone in ring D.[48]

Even though the intensities of the peaks of the CI spectra were different from those observed in EI spectra reported in the literature, similarities in fragment losses were observed. In the case of the CI/MS of gedunin, key ions were observed at 483, 423, 379 and 149 m/e. Loss of 60 units from the $(MH)^+$ peak was indicative of the loss of acetic acid because gedunin contained an acetyl group at C-7. Subsequent loss of CO_2 from the 423 m/e fragment yielded fragment 379 m/e. The base peak was observed at 149 m/e.

1.2.2 MODIFICATIONS OF THE A RING OF GEDUNIN

1.2.2.1 1,2-DIHYDROGEDUNIN

The reduction of the 1,2 double bond in gedunin was accomplished via hydrogenation using 5% Pd/Carbon to yield 1,2-dihydrogedunin mp of 233-235 °C (literature mp of 236 °C [18]). In the ^1H nmr (Figure 1.2.2.1a) of the product the vinylic protons H-1 and H-2 at 7.07 ppm and 5.84 ppm respectively, were absent. The ^{13}C nmr spectrum, in Figure 1.2.2.1b, agreed with that published in the literature.[49] The complete assignment of all the proton shifts had not been reported in the literature and therefore was determined using 2D nmr experiments (ie. COSY, DEPT, HMQC). In the IR spectrum, as expected, the α,β-unsaturated carbonyl at 1669 cm^{-1} was shifted to 1703 cm^{-1} because of the loss of conjugation. Fragment losses in the CI/MS spectrum, which are reported in the experimental section, agreed with those observed in the literature.[48]

1.2.2.2 1,2-EPOXYGEDUNIN

The isolation of 1,2-epoxygedunin from a plant has not been reported to date. The preparation of 1,2-epoxygedunin from gedunin was accomplished using an alkaline hydrogen peroxide solution following the literature procedure except that LiOH was used in our experiment.[14,50] The α-configuration of the epoxide was expected because of the steric hindrance of the β face of the molecule by the C-4 and C-10 β-axial methyl groups.[50]

Characterization of the product was initiated by comparison of characteristic proton resonances to those reported in the literature.[51] The ^{13}C nmr shift assignments were determined from inspection of COSY, DEPT and HMQC spectra of the product and by comparison to the ^{1}H and ^{13}C shifts of the parent compound, gedunin (see Figure 1.2.2.2b for the carbon nmr spectrum of 1,2-epoxygedunin). The FAB/MS contained a $(M+1)^+$ peak at 499 m/e which is in agreement with the molecular weight of 498. The IR spectrum revealed that the carbonyl stretch of the 3-ketone was now located at 1702 cm^{-1}.

From the proton nmr it can be seen that the product that we obtained was primarily the α epoxide.Unfortunately the product also contained a small amount of an inpurity, possibly the corresponding β epoxide, which we were unable to separate from the α epoxide. The presence of this impurity was reflected in the melting point, 130-140 °C, which was lower than the literature value of 217-219 °C.[50] The $[\alpha]_D^{23}$ for the product was +65° ($CHCl_3$) as compared to +80° reported in the literature.

1.2.2.3 1,2-DIHYDRO-3β-GEDUNOL

The synthesis of 1,2-dihydro-3β-gedunol from gedunin required two steps. Gedunin was first hydrogenated to obtain 1,2-dihydrogedunin which was treated with $NaBH_4$ to reduce the saturated 3-ketone. A mixture of epimeric alcohols was obtained but the major product was the β isomer which was purified and characterized. In the literature $NaBH_4$ reduction(0.9 equiv.) of gedunin directly was reported to yield 1,2-dihydrogedunol, [4] however the reference did not include a complete characterization of the product. At the time of publication this reduction of an unsaturated ketone was considered uncommon and in later references the desired product is made by way of 1,2-dihydrogedunin. [17] This intermediate was reduced using the Meerwein-Ponndorf-Verlag reduction to yield the α-alcohol. The β-isomer was obtained by way of a prolonged reaction time of the Meerwein-Ponndorf-Verlag reaction or more easily by a $NaBH_4$ reduction. [17]

The CI/MS of 1,2 dihydro-3β-gedunol yielded an $(MH)^+$ peak at 487 m/e which agreed with the molecular weight of 486 for the desired compound. Inspection of the IR spectrum revealed the presence of an OH stretch at 3607 cm^{-1} and the loss of the carbonyl stretch for the 3-ketone at 1703 cm^{-1}. Unfortunately the melting point obtained for the product, 135-140 °C, did not agree with the literature value of 209-211 °C.[17] The $[\alpha]_D^{23}$, -17.5° (c=0.0004, $CHCl_3$), however agreed with the literature value of -15° ($CHCl_3$) [17] thus confirming further the presence of the desired product.

The complete assignment of the proton and/or carbon nmr shifts of 1,2-dihydro-3β-gedunol, Figure 1.2.2.3a and Figure 1.2.2.3b respectively, have not been reported in the literature. The two possible 3-ol isomers were reported to be differentiated by the

variation in the multiplicity and chemical shift of H-3. Identification of the β isomer was based on the shift of H-3(3.21 ppm)[51] and the observation that this proton appeared as a doublet of doublets. The resonance of H-3 for the α isomer occurred at 3.41 ppm as a triplet. The investigation of COSY, HMQC and DEPT 2D nmr spectra afforded the complete carbon and proton assignments of 1,2-dihydro-3β-gedunol given in the Experimental section.

1.2.2.4 3β-ACETOXY-1,2-DIHYDROGEDUNIN

The acetylation of 1,2-dihydro-3β-gedunol resulted in the formation of 3β-acetoxy-1,2-dihydrogedunin which had a melting point of 125-127 °C (literature value was 132-134 °C[17]). The CI/MS displayed a $(MH)^+$ peak at 529 m/e and the IR spectrum revealed the disappearance of the OH stretch of the corresponding alcohol. The $[\alpha]_D^{23}$ value was determined to be -7.5° (c=0.0028, $CHCl_3$) as compared to the literature value of -13° ($CHCl_3$).[17]

The carbon and proton shifts were assigned through comparison of the spectra of the acetate to those of the corresponding alcohol and gedunin. For proton and carbon nmr spectra of 3β-acetoxy-1,2-dihydrogedunin see Figures 1.2.2.4a and 1.2.2.4b respectively. Confirmation of most of the assignments was made possible through the examination of 2D nmr experiments (COSY, HETCORE, DEPT).

1.2.2.5 3-GEDUNOL

The preparation of the allylic alcohols, 3β-gedunol (40% yield) and 3α-gedunol(9% yield) was reported to have been accomplished by the Meerwein-Ponndorf reduction of gedunin.[52] This reaction was initially reported by Ohochuku *et al* to yield 3α-

gedunol as the major product.[53] A more recent publication has pointed out the error in this assignment identifying the major product as the β isomer.[52] In our laboratory the synthesis of 3-gedunol was attempted initially using, a more recent approach to the reduction of unsaturated ketones, the combination of $NaBH_4$ and $CeCl_3.7H_2O$.[54] Unfortunately this resulted in the formation of what is thought to be a diol resulting from the reduction of both the unsaturated ketone and the lactone carbonyl moieties. An attempt at the Meerwein-Ponndorf reduction resulted in the isolation of starting material. Time and lack of additional starting material, gedunin, has not permitted another attempt at this reaction under drier conditions.

1.2.3 MODIFICATIONS OF THE B RING OF GEDUNIN

1.2.3.1 7-DEACETYLGEDUNIN

Mild alkaline hydrolysis of gedunin yielded 7α-deacetylgedunin in a reasonable yield. An accurate melting point was difficult to obtain because the product started to decompose at 250 °C. This agreed with the literature melting point range of 250-272 °C which also mentioned the compound's decomposition at these temperatures.[4] The loss of the singlet at 2.08 ppm in the proton nmr spectrum for the 7-acetate protons indicated that the hydrolysis was successful. The $(MH)^+$ ion in the CI/MS spectrum, also the base peak, appeared at 441 m/e which agreed with the molecular weight of 440 for 7-deacetylgedunin. The presence of an 7-alcohol was confirmed by the absorption at 3604 cm^{-1} in the IR spectrum.

The ^1H nmr of 7-deacetylgedunin (Figure 1.2.3.1a) has been assigned partially in the literature.[51] Because the ^{13}C shifts (in Figure 1.2.3.1b) have been previously assigned[49] the missing ^1H shifts were assigned with the help of DEPT and HMQC spectra. It is interesting to note that the 7α-alcohol is responsible for the downfield shift of H-15 from 3.50 ppm to 3.89 ppm.

1.2.3.2 7-KETOGEDUNIN

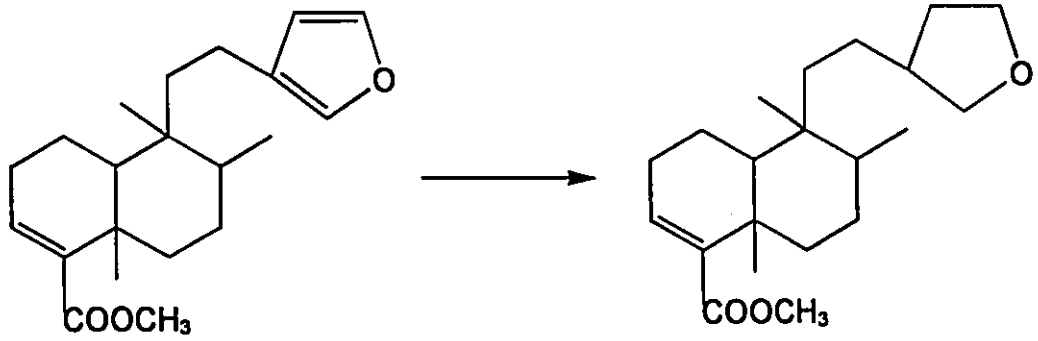
The desired product, 7-ketogedunin[18], was obtained in two steps from gedunin. Mild hydrolysis of gedunin generated 7-deacetylgedunin which was oxidized to 7-ketogedunin using chromium trioxide in pyridine. Inspection of the CI/MS revealed a (MH)+ ion at 485 m/e and a base peak at 425 m/e. In the IR spectrum a new absorption at 1714 cm^{-1} was observed because of the presence of the 7-keto group. The mp of 260-262 °C agreed with the

literature value of 263-264 °C. [18] The proton and carbon shifts in Figures 1.2.3.2a and 1.2.3.2b agreed with those published in the literature for 7-ketogedunin. [29,49,51]

1.2.4 MODIFICATIONS OF THE β-SUBSTITUTED FURAN RING OF GEDUNIN

1.2.4.1 HEXAHYDROGEDUNIN

The selective hydrogenation of the furan moiety of gedunin was the original objective of this research. In the literature, selective hydrogenation of the furan moiety of methylhardwickiic acid, which was accomplished with 5% Rh-C or 70%Rh-30%Pt oxide in glacial acetic acid, left the conjugated olefin intact.[55,56] Because the epoxide in gedunin was suspected to be sensitive to glacial acetic acid the hydrogenation was attempted in different solvents such as ethyl acetate and acetone with 5%Rh-C as the catalyst. Hydrogenation for 18 h at 1 atm of H_2 gave 1,2-dihydrogedunin. Similar results were observed after 18 h using 13.6 atm of H_2 with 5%Pd-C as the catalyst. Hydrogenation of gedunin with PtO_2 in a 10:1 ratio also resulted in the production of 1,2-dihydrogedunin as the only product.



METHYL HARDWICKIATE TETRAHYDROMETHYL HARDWICKIATE

At this stage it was decided to reduce both the α,β-unsaturated ketone double bond and the furan double bonds to obtain hexahydrogedunin and then re-introduce the 1,2-

double bond to yield tetrahydrogedunin. This hydrogenation was accomplished using 5%Rh-C in ethyl acetate, at 1 atm of hydrogen for 96 h.

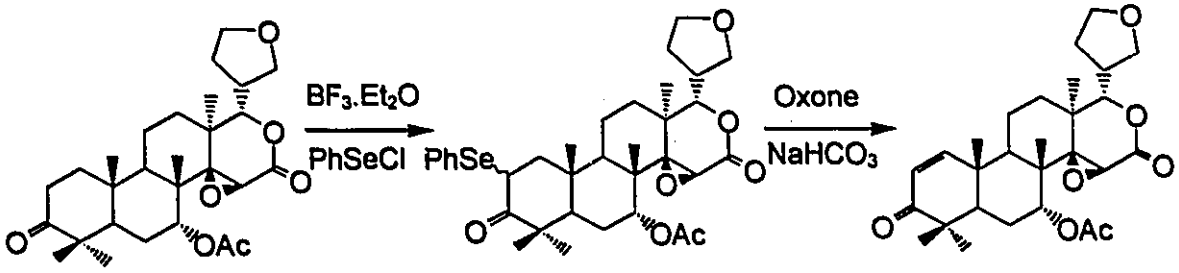
Complete characterization of the purified product, mp 253-255 °C, was conducted because hexahydrogedunin had not been isolated or previously described in the literature. The CI/MS revealed an $(MH)^+$ peak at 489 m/e which sequentially lost 60 and 18 m/e, coinciding with acetic acid and water, to give fragments 429 and 411 m/e respectively. The melting point was determined to be 253-255 °C. The IR spectrum verified the loss of the furan and ring A double bonds by the lack of absorptions at 1669, 1503 and 875 cm^{-1} and the presence of a saturated ketone absorption at 1704 cm^{-1}.

Carbon and proton shift assignments were largely accomplished through the comparison of the spectra to those of 1,2-dihydrogedunin and the later synthesized tetrahydrogedunin (Figure 1.2.4.1b and Figure 1.2.4.1a). Assignment of H-20, 21, 22 and H-23 protons agreed with those determined for tetrahydrogedunin. The chemical shifts of H-1 and H-2 agreed with those assigned in 1,2-dihydrogedunin. A HMQC experiment was used to determine the corresponding carbon shift assignments. The quaternary carbon assignments were determined by comparison to other gedunin derivatives however verification of these assignments would be accomplished using HMBC and INADEQUATE nmr experiments.(see Experimental section)

1.2.4.2 TETRAHYDROGEDUNIN

Starting with hexahydrogedunin, prepared above, the double bond α to the carbonyl in ring A was reintroduced using a two step procedure. This transformation, the

conversion of ketones to α,β-enones using a selenoxide elimination has been reviewed in the literature by Reich *et al.*[57] The first step involved α-phenylselenylation of the 3 ketone using $BF_3.Et_2O$ and phenylselenium chloride in THF.[58] Oxidative deselenylation of the product in methanol with oxone and a small amount of $NaHCO_3$ afforded tetrahydrogedunin in a very low yield. Optimization of this synthesis was not pursued because of the limited amount of starting material which was available.



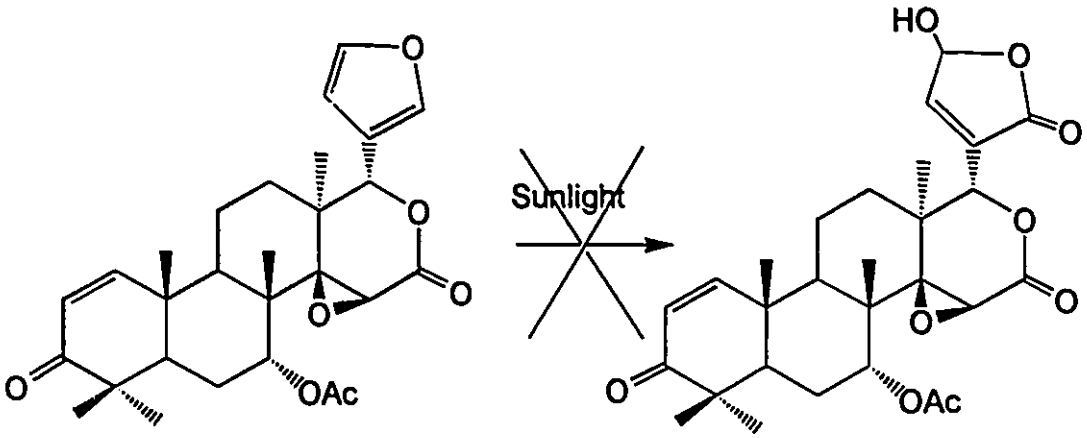
Tetrahydrogedunin has not been reported in the literature and therefore required full characterization. The purified product, mp 260-262 °C, showed the expected $(MH)^+$ peak at 487 m/e in its CI/MS. The reintroduction of the 1,2 double bond was verified by the presence of the carbonyl absorption in the IR spectrum at 1669 cm^{-1} and, the H-1 and H-2 proton resonances at 7.12 and 5.87 ppm, respectively (Figure 1.2.4.2a).

Assignment of the proton and carbon (Figure 1.2.4.2.b) nmr resonances for rings A, B and C mimicked those observed for gedunin. The multiplet at 4.51 ppm was assigned to H-17 because of its coupling with H-20, which appeared as a new resonance at 2.46 ppm. Assignment of H-20, 21, 22, and H-23 protons were based on multiplicity of peaks and on the shifts observed for 2-substituted tetrahydrofuran compounds. Two dimensional

nmr experiments: COSY, HMQC and DEPT, made it possible to verify the new proton resonances and determine the corresponding carbon shifts assignments.

1.2.4.3 21-ACETYLGEDUNIN AND 23-ACETYLGEDUNIN

Several attempts were made at derivatizing the β-substituted furan ring of gedunin. The oxidation of gedunin with NBS in dioxane and water[10] to yield the corresponding butenolide was attempted on a small scale. The result was the isolation of three products which did not correspond to the desired product. Photolysis of gedunin in methanol to photogedunin[10] was also tried but was not successful in our hands.

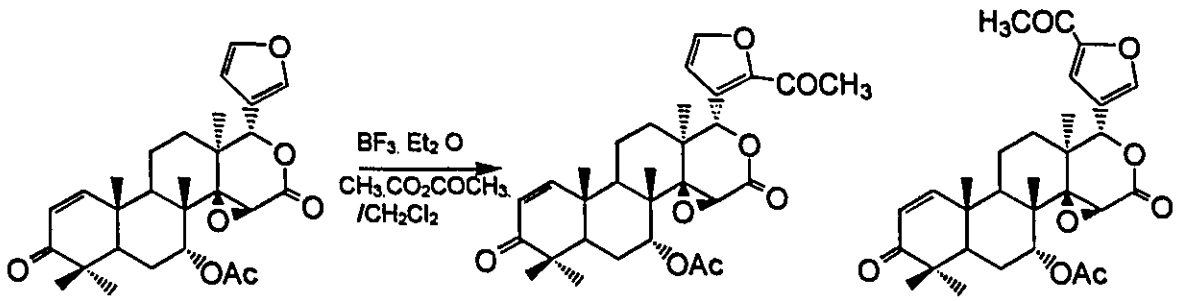


GEDUNIN PHOTOGEDUNIN

Efforts were then focused on the possible bromination of the β-substituted furan of gedunin. A survey of the literature revealed that gedunin or other D-seco limonoids had not been brominated previously. The attempt involved the addition of 1 equiv. of Br_2 in DMF to a solution of gedunin dissolved in DMF.[59] Unfortunately no reaction occurred. The next unsuccessful attempt involved the addition of NBS and Amberlite IRP-64 exchange resin to a solution of gedunin dissolved in toluene.[60] After 4.5 days the reaction was stopped

because no starting material remained. The ^1H nmr spectra of the two products clearly indicated the absence of the 1,2 double bond but all the furan resonances were still intact.

Our last attempt at the derivatization of the β-substituted furan gedunin met with success. In 1949 a procedure for the acylation of furan using $BF_3.Et_2O$ and acetic anhydride was reported by Levine *et al.*[61] The reaction of gedunin with 1.6 equiv. of $BF_3.Et_2O$ and 2.1 equiv. of acetic anhydride for 21 h resulted in the isolation of two products: the more polar product was identified as 21-acetylgedunin (17%) and the less polar as 23-acetylgedunin (49%) on the basis of their MS and ^1H nmr.



GEDUNIN 21-ACETYLGEDUNIN 23-ACETYLGEDUNIN

The CI/MS of both acyl derivatives revealed an $(MH)^+$ peak at 525 m/e which agreed with the anticipated molecular weights. The IR spectra of the products did not differ from that of gedunin when the major absorptions were compared. The loss of one of the three furan hydrogens from gedunin was clearly apparent in the proton nmr spectra of the acetylated products. The keys features in the ^1H nmr of the 21-derivative (Figure 1.2.4.3a) that verified this assignment were the presence of a doublet at 6.68 ppm for H-22 and coupling to it was H-23 appearing as a multiplet at 7.49 ppm. A resonance for H-21 was not present in the ^1H nmr of this derivative.

The ^{1}H nmr of the 23 derivative (Figure 1.2.4.3b) had furan resonances which clearly indicated this assignment. H-21 and H-22 appeared at 7.53 and 7.06 ppm as a triplet and multiplet, respectively, because of long range coupling to each other (W coupling) and possibly H-17. A third resonance for H-23 was not observed. The remainder of the proton and carbon shifts for each derivative were assigned by comparison to those for gedunin and through the use of HMQC and DEPT 2D nmr's.

1.2.5 RELATED D-SECO LIMONOIDS

1.2.5.1 CITRUS LIMONOIDS: LIMONIN, NOMILIN, OBACUNONE AND EPILIMONOL

Limonin was isolated from citrus seeds however, a ^{1}H nmr spectrum of the mother liquor from the crystalization step was devoid of any additional furan resonances. This suggested that nomilin and obacunone were not present. Samples of these limonoids were donated by citrus limonoid researchers. Epilimonol, 7β-limonol, was prepared via $NaBH_4$ reduction of the 7-keto group of limonin.[32] The α-isomer, limonol, has been prepared in the literature using the Meerwein-Ponndorf reduction of limonin.[62]

The melting points of limonin and epilimonol were both in agreement with literature values.[32] Those for obacunone and nomilin were not in very good agreement with the literature values because both had a tendency to decompose before the melting point was reached. The CI/MS of limonin and epilimonol both gave $(MH)^+$ peaks that were the base peaks for the spectra. Quasimolecular ions 471 and 473 m/e agreed with the molecular weights of limonin and epilimonol, respectively. The mass spectra for nomilin and obacunone were not determined.

The ^{1}H nmr resonances for limonin and epilimonol matched those which had been assigned in the literature by Dreyer.[63] The remainder of the proton assignments were determined using COSY spectra. Shift assignments to carbons were determined largely by inspection of HMQC and DEPT spectra, however comparison of shifts to related limonoids was used to assign the quaternary carbons. Epilimonol was not soluble in chloroform so the spectra were obtained in acetone d_6. The ^{1}H and ^{13}C nmr spectra of obacunone agreed with

those published in the literature.[64,65] Comparison of literature spectra of obacunone and deacetylnomilin[67] to those obtained for nomilin allowed for the complete assignment of all the proton and carbon resonances for nomilin.

1.2.6 HIRTIN

A melting point of 148-151 °C(decomp.), which was in agreement with the literature melting point of 159-160 °C[44], was obtained for the sample of hirtin that was obtained from UBC(see experimental section). The ^{1}H nmr spectrum contained the resonances that had been assigned to hirtin when it was first isolated. Unfortunately no other ^{1}H or ^{13}C nmr assignments could be found in the literature. Comparison of the nmr spectra of cedrelone and limonoids related to hirtin, and the use of COSY, HMQC and DEPT spectra aided the assignment of all the carbon and proton resonances.[45]

1.3.0 EXPERIMENTAL

GENERAL: Melting points were determined by use of a Thomas Hoover Capillary melting point apparatus and are uncorrected. Mass spectra were obtained using an VG 7070E or a Kratos concept 2H instrument. IR spectra were recorded in methylene chloride solution employing a Bomem-Michelson MB-100 FT/IR spectrophotometer. Optical rotation values were determined using a Perkin Elmer Polarimeter (model 241) set at the sodium D line(589 nm). The samples were, for the most part, analyzed in spectroscopic grade chloroform. ^{1}H and ^{13}C nmr spectra were obtained on a Bruker AMX-500 spectrometer with the shifts reported in ppm. The multiplicities of the nmr signals were reported employing the following abbreviations, singly or in combination: singlet(s), broad singlet(brs), doublet(d), triplet(t), quartet(q), multiplet(m).

Solvents for extractions and chromatographic purifications were routinely distilled prior to use. THF was dried by distillation from a benzophenone ketyl. Thin layer chromatography(tlc) was carried out using Kieselgel 60 F_{254} precoated 0.25 mm plates. Visualization was facilitated by UV irradiation followed by the charring of a tlc which had been dipped in a 5 % H_2SO_4 in methanol solution, containing a small amount of isocratin. Preparative tlc (Prep TLC) chromatography was carried out using Kieselgel 60 F_{254} precoated 0.50 mm plates. Silica gel 270-400 Mesh was used for flash chromatography.

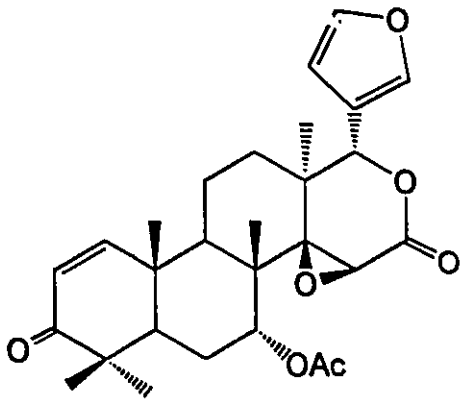
1.3.1 ISOLATION OF GEDUNIN

Planks of *Cedrela odorata* wood obtained from Belize were ground into sawdust using Dado sawblades installed on a benchsaw. Approximately 5 L of 95% ethanol was then added to the 726 g of sawdust. After 18-24 h, the 95% ethanol was decanted off from the sawdust, at this time more ethanol was added. This ethanol extraction was carried out three times in total and the decanted ethanol extracts were condensed using a rotary evaporator. The temperature of the bath for the rotary evaporator did not exceed 45 °C. The condensed extract was then freeze dried.

The freeze dried extract (18.82g) was dissolved in a mixture of 1:1 95% ethanol/water and extracted three times with hexane in a seperatory funnel. The remaining ethanol/water layer was put onto a rotary evaporator to remove most of the ethanol. The resulting aqueous solution was then extracted four times with methylene chloride. Crude silica gel (Mesh 270-400) column chromatography was then conducted on the dried methylene chloride extract. Elution with CH_2Cl_2: EtOAc mixtures 100:0 to 95:5 afforded all of the gedunin present in the crude extract.

Fractions containing gedunin, tlc comparison with standard, were pooled and the solvent was removed to yield 0.9g of a gedunin-rich sample. Flash chromatography (Silica gel Mesh 230-400) of this sample employed the same solvent system used in the chromatography mentioned above. Gedunin rich fractions were identified using tlc (3:1 Hex/EtOAc). Again the gedunin rich fractions were combined and the solvent removed by evaporation.

Quartz shaped crystals of gedunin (0.283g) were obtained by crystallization of the combined gedunin containing fractions from methanol. The sample was verified to be gedunin via comparison of its mp and spectroscopic data (IR, ^{1}H nmr, ^{13}C nmr and MS) with that reported in the literature.[3,4,14,47,48] Gedunin was obtained in 0.04 % yield.



$C_{28}H_{34}O_7$ MW=482 g/mole

MP 215-218 °C(literature 218 °C) [14]

MS (CI/ether): 483 $[MH]^+$(82.0), 423(51.1), 379(19.4), 299(14.3), 149(100).

IR (CH_2Cl_2) ν (cm-1): 2952, 1741, 1669, 1503, 875.

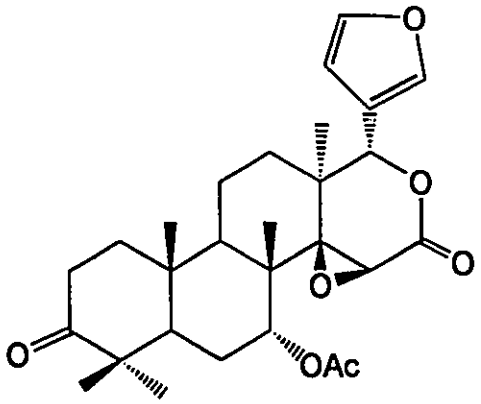
^{1}H NMR ($CDCl_3$) δ(ppm) : 7.07(d, J=10.2 Hz, H-1), 5.84(d, J=10.2 Hz, H-2), 2.14(dd, J=2.5, 13.3 Hz, H-5), 1.92(dt, J=15.1. 3.1 Hz, H-6α), 1.80(m, H-6β), 4.53(dd, J=3.4, 2.1 Hz, H-7), 2.46(dd, J=6.2, 12.8 Hz, H-9), 2.00(m, H-11α), 1.81(m, H-11β), 1.57(ddd, J=2.5, 10.8, 13.3 Hz, H-12α), 1.70(m, H-12β), 3.50(s, H-15), 5.59(s, H-17), 1.22(s, Me-18), 1.20(s, Me-19), 7.39(d, J=1.3 Hz, H-21,23), 6.31(dd, J=1.3 Hz, H-22), 1.04(s, Me-28), 1.05(s, Me-29), 1.13(s, Me-30), 2.08(s, Me-32).

^{13}C NMR ($CDCl_3$) δ(ppm): 156.9(C-1), 126.0(C-2), 203.9(C-3), 44.0(C-4), 46.0(C-5), 23.3(C-6), 73.2(C-7), 42.6(C-8), 39.5(C-9), 40.0(C-10), 15.0(C-11), 26.0(C-12), 38.7(C-13), 69.8(C-14), 56.9(C-15), 167.4(C-16), 78.3(C-17), 17.7(C-18), 19.7(C-19), 120.4(C-20), 143.1(C-21), 109.9(C-22), 141.2(C-23), 27.2(C-28), 21.2(C-29), 18.3(C-30), 169.9(C-31), 21.0(C-32).

1.3.2 PREPARATION OF GEDUNIN DERIVATIVES

1.3.2.1 PREPARATION OF 1,2-DIHYDROGEDUNIN

Hydrogenation of gedunin (200 mg) in the presence of 5% Pd/Carbon (24 mg) in acetone and at 1 atm of hydrogen yielded 1,2-dihydrogedunin after a reaction time of 18 h. It was difficult to follow the progress of this reaction because the Rf of the starting material and the product were the same. The catalyst was filtered off from the product using a sintered glass filter. Purification of the product was accomplished using Prep TLC eluted with 1:1 EtoAc/Hex to give a yield of 90% of the desired product. This procedure was previously described in the literature.[3,67]



$C_{28}H_{36}O_7$ MW=484 g/mole

MP 233-235 °C (literature 237-238 °C)[17]

MS (CI/ether): 485$[MH]^+$(25.9), 425(100), 407(27.2), 381(32.5), 301(56.7).

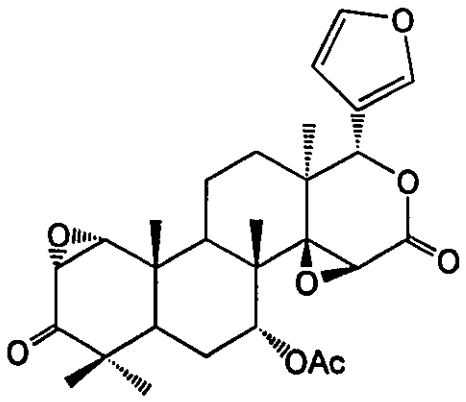
IR (CH_2Cl_2) ν (cm-1): 2949, 1739, 1703, 1504, 876.

^{1}H NMR ($CDCl_3$) δ(ppm): 2.55(m, H-1), 2.42(ddd, J=3.7, 7.4, 16.1 Hz, H-1), 1.47(m, H-2), 1.90(ddd, J=3.9, 7.7, 13.1 Hz, H-2), 1.82(m, H-5), 1.74 and 1.85(m, H-6), 4.50(br s, H-7), 2.28(dd, J=6.9, 12.0 Hz, H-9), 1.70(m, H-11), 1.51 and 1.64 (m, H-12), 3.49(s, H-15), 5.58(s, H-17), 7.38(d, J=1.4 Hz, H-21,23), 6.31(d, J=1.3 Hz, H-22), 0.98, 1.00, 1.05, 1.09, 1.22(s, Me x 5), 2.09(s, Me-32).

^{13}C NMR ($CDCl_3$) δ(ppm): 33.7(C-1), 38.9(C-2), 215.9(C-3), 46.7(C-4), 47.8(C-5), 23.8(C-6), 73.7(C-7), 42.0(C-8), 44.0(C-9), 37.4(C-10), 15.0(C-11), 25.9(C-12), 38.8(C-13), 69.8(C-14), 56.8(C-15), 167.6(C-16), 78.3(C-17), 120.5(C-20), 143.0(C-21), 109.9(C-22), 141.2(C-23), 15.7, 17.4, 18.0, 20.9, 26.1(Me x 5), 21.1(Me-acetate), 170.0(C=O -acetate).

1.3.2.2 PREPARATION OF 1,2-EPOXYGEDUNIN

An RBF containing gedunin (75 mg) dissolved in 10 mL of 3:1 THF/water was cooled to 0°C. The reaction mixture was allowed to warm to room temperature following the addition of 0.1 mL of 30% H_2O_2 and 2 equiv. of LiOH (7.5 mg). After 3 h the reaction mixture was cooled to 0 °C for the addition of Na_2SO_3. The pH of the room temperature reaction mixture was adjusted to 9 using 5% $NaHCO_3$. The THF was removed from the reaction mixture using a vacuum and the aqueous solution was extracted with methylene chloride three times. The organic layer was dried over $MgSO_4$ and the solvent removed via rotary evaporation. The product was purified using Prep TLC.



$C_{28}H_{34}O_8$ MW=498 g/mole

MP 130-140 °C (literature 217-219 °C)[50]

$[\alpha]_D^{23}$ +65° (c=0.0034, $CHCl_3$) (literature +80°)

MS (FAB MS): 499$[M+1]^+$(7.5), 369(18.2), 277(55.1), 185(87.8), 93(100)..

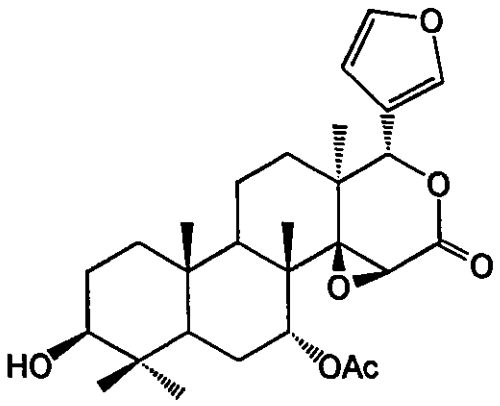
IR (CH_2Cl_2) ν (cm-1): 3005, 1741, 1702, 1504, 876.

^{1}H NMR ($CDCl_3$) δ(ppm): 3.38(d, J=4.6 Hz, H-1), 3.53(d, J=4.7 Hz, H-2), 2.41(dd, J=3.0, 13.8 Hz, H-5), 4.48(m, H-7), 2.76(dd, J=6.6, 12.8 Hz, H-9), 2.05-1.60(m, H-6, H-11, H-12), 3.51(s, H-15), 5.60(s, H-17), 7.39(m, H-21, H-23), 6.32(m, H-22), 2.13(s, acetate Me), 1.28, 1.11, 1.03, 1.01, 0.95(s, Me x 5).

^{13}C NMR ($CDCl_3$) δ(ppm): 56.6(C-1), 62.9(C-2), 211.0(C-3), 44.1(C-4), 38.4(C-5), 22.9(C-6), 73.0(C-7), 42.2(C-8), 38.4(C-9), 39.1(C-10), 15.4(C-11), 25.7(C-12), 38.8(C-13), 69.7(C-14), 57.0(C-15), 167.4(C-16), 78.2(C-17), 120.4(C-20), 143.1(C-21), 109.9(C-22), 141.2(C-23), 15.7, 17.4, 18.4, 20.7, 27.4(Me x 5), 170.1(C-31), 21.1(C-32).

1.3.2.3 PREPARATION OF 1,2-DIHYDRO-3β-GEDUNOL

Reduction of the 3-ketone of 1,2-dihydrogedunin resulted in the preparation of 1,2-dihydro-3β-gedunol. This was accomplished by addition of $NaBH_4$ (1 mg) in ethanol to a solution of 1,2 dihydrogedunin (44mg) dissolved in THF. The reaction mixture was cooled to 10 °C for 2.5 h at which time water was added. Extraction of the reaction mixture with methylene chloride followed by drying over $MgSO_4$ and evaporation of the solvent yielded a product that was predominately the β-isomer. Purification using Prep TLC (1:1 EtOAc/Hex) yielded 1,2-dihydro-3β-gedunol in a 87% yield.



$C_{28}H_{38}O_7$ MW=486 g/mole

MP 135-140 °C (literature 209-211 °C) [17]

$[\alpha]_D^{23}$ -17.5° (c=0.0004, $CHCl_3$) (literature -15°) [17]

MS (CI/ISO): 487$[MH]^+$(72.3), 469(15.2), 427(65.5), 409(100), 303(44.3).

IR (CH_2Cl_2) ν (cm-1): 3607, 2940, 1738, 1605, 875.

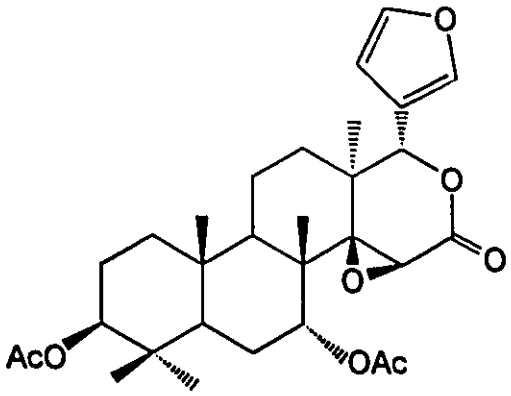
^{1}H NMR ($CDCl_3$) δ(ppm): 1.02 and 1.60 (m, H-1), 1.60 (m, H-2), 3.21(dd, J=4.5, 11.4 Hz, H-3), 1.24 (dd, J=2.3, 13.2 Hz, H-5), 1.60(m, H-6β), 1.89 (ddd, J=2.3, 3.6, 15.0 Hz, H-6), 4.48(dd, J=2.2, 3.4 Hz, H-7), 2.16 (m, H-9), 1.60 (m, H-11), 1.49 and 1.60 (m, H-12), 3.47(s, H-15), 5.57(s, H-17), 7.37(m, H-21, 23), 6.30(m, H-22), 0.74, 0.85, 0.92, 1.04, 1.21(s, Me x 5), 2.09(s, acetate-Me).

^{13}C NMR ($CDCl_3$) δ(ppm): 38.3(C-1), 27.1(C-2)*, 78.4(C-3), 37.7 and 38.2 (C-4 and C-10), 47.9(C-5), 22.8(C-6), 74.2(C-7), 42.1(C-8), 44.8(C-9), 15.0(C-11), 26.0(C-12)*, 38.9(C-13), 69.9(C-14), 56.7(C-15), 167.8(C-16), 78.4(C-17), 120.7(C-20), 142.9(C-21), 110.0(C-22), 141.1(C-23), 15.2, 16.2, 17.3, 18.2, 27.6(Me x 5), 21.2(Me-acetate), 170.0(C=O acetate).

* = assignments are interchangeable

1.3.2.4 PREPARATION OF 3β-ACETOXY-1,2-DIHYDROGEDUNIN

The acetylation procedure involved the addition of pyridine (1 mL) and acetic anhydride (0.25 mL) to 20 mg of 1,2-dihydro-3β-gedunol. After 9 h, water was added to the reaction mixture.The reaction mixture was then extracted with ether three times. After the organic layer had been dried over $MgSO_4$ and condensed it was applied to a Prep TLC for purification. The desired β-acetate was obtained in a 79 % yield.



$C_{30}H_{40}O_8$ MW=528 g/mole

MP 125-127 °C(literature 132-134 °C) [17]

$[\alpha]_D^{23}$ -7.5 ° (c=0.0028, $CHCl_3$) (literature -13°, $CHCl_3$)[17]

MS (CI/ISO): 529$[MH]^+$(88.2), 469(83.8), 451(10.3), 409(100), 345(41.1).

IR (CH_2Cl_2) ν (cm-1): 2950, 1736, 876.

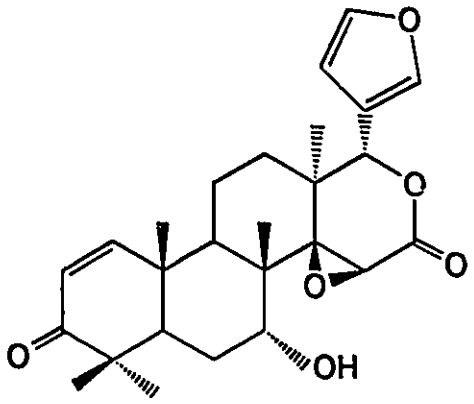
^{1}H NMR ($CDCl_3$) δ(ppm): 1.10 and 1.65(m, H-1), 1.60-1.70(m, H-2), 4.48(m, H-3), 1.38(dd, J=2.1, 13.1 Hz, H-5), 1.60-1.70 and 1.89(m, H-6), 4.48(m, H-7), 2.18(m, H-9), 1.60-1.75(m, H-11), 1.49 and 1.65(m, H-12), 3.47(s, H-15), 5.57(s, H-17), 7.38(m, H-21 and H-23), 6.31(t, J=1.3 Hz, H-22), 0.74, 0.82, 0.94, 1.05, 1.22(s, Me x 5), 2.03(s, Me-3-acetate)*, 2.09(s, Me-7-acetate)*.

^{13}C NMR ($CDCl_3$) δ(ppm): 38.0(C-1), 23.4(C-2), 74.1 and 80.0(C-3 and C-7), 37.14 and 37.7(C-4 and C-10), 48.0(C-5), 22.6(C-6), 42.1(C-8), 44.8(C-9), 14.9(C-11), 25.9(C-12), 38.8(C-13), 69.8(C-14), 56.7(C-15), 167.7(C-16), 78.4(C-17), 120.6(C-20), 142.9(C-21), 109.9(C-22), 141.1(C-23), 16.3, 16.3, 17.3, 18.2, 27.6(Me x 5), 21.1 and 21.2(Me-acetates), 170.0 and 171.0(C=O -acetates).

*= assignments are interchangeable.

1.3.2.5 PREPARATION OF 7-DEACETYLGEDUNIN

The preparation of 7-deacetylgedunin follows, in part, the procedure used by Akisanya et al in 1961.[4] Gedunin (75 mg) was added to 10 mL of methanol in a RBF equipped with a reflux condensor. Once reflux (under N_2) was attained, 1.5 mL of 2N NaOH was added. After 30 min of reflux the reaction mixture was cooled, acidified with 10% H_2SO_4 and extracted three times with EtOAc. The EtOAc layer was dried over $MgSO_4$ and condensed via rotary evaporation. Application of the sample on a Prep TLC plate eluted with the solvent mixture 1:1 EtOAc/Hex yielded purified 7-deacetylgedunin (57.3 mg) in 84 % yield.



$C_{26}H_{32}O_6$ MW=440 g/mole

MP 250-254 °C(decomp.)(literature 250-272 °C)[4]

MS (CI/ISO): 441$[MH]^+$(100), 423(29.2), 405(9.8), 379(10.2), 317(9.8), 299(43.4).

IR (CH_2Cl_2) ν (cm-1): 3604, 2979, 1741, 1667, 1504, 876.

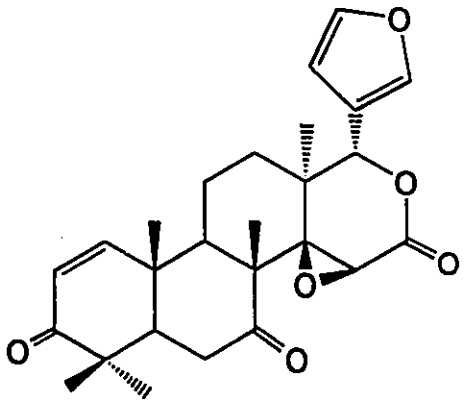
^{1}H NMR ($CDCl_3$) δ(ppm): 7.08(d, J=10.2 Hz, H-1), 5.82(d, J=10.2 Hz, H-2), 3.55(br s, H-7), 2.46(dd, J=2.5, 13.4 Hz, H-5), 2.49(dd, J=6.1, 12.6 Hz, H-9), 3.89(s, H-15), 5.58(s, H-

17), 7.38(m, H-21,23), 6.33(m, H-22), 1.06, 1.07, 1.12, 1.18, 1.21(s, Me x 5), 1.40-2.00(m, H-6, 7-OH, H-11, H-12).

^{13}C NMR ($CDCl_3$) δ(ppm): 157.7(C-1), 125.8(C-2), 204.5(C-3), 44.2(C-4), 44.6(C-5), 27.3(C-6), 69.7(C-7), 43.7(C-8), 38.0(C-9), 40.2(C-10), 15.1(C-11), 26.4(C-12), 38.3(C-13), 70.0(C-14), 57.8(C-15), 168.2(C-16), 78.5(C-17), 120.7(C-20), 143.0(C-21), 110.0(C-22), 141.2(C-23), 17.8, 18.7, 19.9, 21.5, 27.3(Me x 5).

1.3.2.6 PREPARATION OF 7-KETOGEDUNIN

The preparation of the 7-keto derivative of gedunin from 7-deacetylgedunin has been previously reported in the literature.[14] The oxidation of 7-deacetylgedunin (32.4 mg) was accomplished with 65 mg of CrO_3 in 5 mL of pyridine for approximately 21 h. Workup was accomplished by extraction of the reaction mixture with methylene chloride followed by washing of the organic layer with 2% HCl and water. The desired product was obtained in 37% yield following Prep TLC purification (1:3 EtOAc/ methylene chloride).



$C_{26}H_{30}O_6$ MW= 438 g/mole

MP 260-262 °C(literature 262-265 °C) [18]

MS (CI\ISO): 439 $[MH]^+$(100), 423(9.4), 421(4.9), 393(16.4), 315(28.6).

IR (CH_2Cl_2) ν (cm-1): 3055, 2969, 1744, 1714, 1673, 1504, 876.

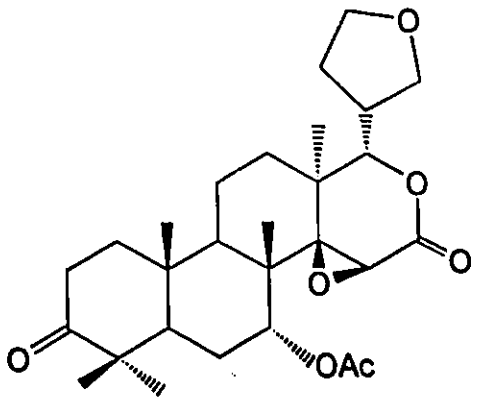
^{1}H NMR ($CDCl_3$) δ(ppm): 7.07(d, J=10.2 Hz, H-1), 5.90(d, J=10.2 Hz, H-2), 2.15(dd, J= 3.3, 14.8 Hz, H-5), 2.39(dd, J=3.2, 13.9 Hz, H-6α), 2.90(t, J=14.4 Hz, H-6β), 2.18(dd, J= 1.5, 11.5 Hz, H-9), 1.76 and 1.96(m, H-11), 1.45 and 1.82(m, H-12), 3.74(s, H-15), 5.42(s, H-17), 7.39(m, H-21), 6.37(dd, J= 0.8, 1.8 Hz, H-22), 7.37(m, H-23), 1.07, 1.10, 1.15, 1.20, 1.33(s, Me x 5).

^{13}C NMR ($CDCl_3$) δ(ppm): 155.9(C-1), 126.5(C-2), 203.3(C-3), 45.2(C-4), 47.6(C-5), 36.7(C-6), 208.2(C-7), 53.4(C-8), 53.6(C-9), 39.6(C-10), 17.2(C-11), 32.2(C-12), 37.7(C-13), 65.6(C-14), 54.6(C-15), 166.9(C-16), 78.0(C-17), 120.2(C-18), 143.1(C-21)*, 109.8(C-22), 141.0(C-23)*, 17.4, 19.8, 20.7, 20.9, 27.0(Me x 5).

*= these assignments could be interchanged

1.3.2.7 PREPARATION OF HEXAHYDROGEDUNIN

Hydrogenation of gedunin (200 mg) in the presence of 5% Rh/Carbon (30 mg), EtOAc and hydrogen (1 atm) yielded hexahydrogedunin after 48 h. Purification of the product was accomplished by flash column chromatography, eluted with 20-50% gradient of EtOAc/Hex, to obtain the desired product in a 36% yield.



$C_{28}H_{40}O_7$ MW=488 g/mole

MP 253-255 °C

MS (CI/ISO): 489$[MH]^+$(7.2), 429(100), 411(40.6), 383(19.5), 301(22.7).

IR (CH_2Cl_2) ν (cm-1): 2939, 1737, 1704.

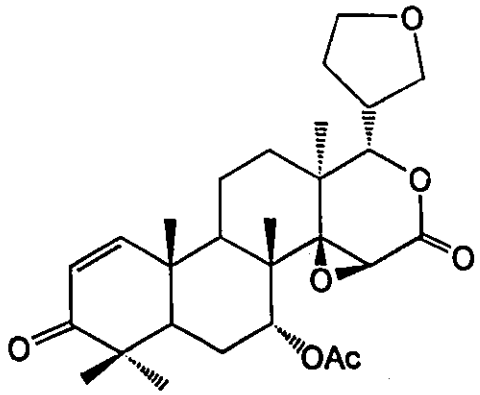
^{1}H NMR ($CDCl_3$) δ(ppm): 2.45 and 2.55(m, H-1), 1.50 and 1.90(m, H-2), 1.82(m, H-5), 1.72 and 1.82(m, H-6), 4.47(m, H-7), 2.26(m, H-9), 1.72 and 1.92(m, H-11), 1.54(m, H-12), 3.40(s, H-15), 4.47(m, H-17), 2.40(m, H-20), 3.34(t, J=8.6 Hz, H-21), 3.85(t, J=8.0 Hz, H-21), 1.92 and 2.07(m, H-22), 3.69(q, J=6.7, 8.5 Hz, H-23), 3.80(m, H-23), 0.99, 0.97, 1.04, 1.04, 1.27(s, Me X 5), 21.0(s, Me-acetate).

^{13}C NMR ($CDCl_3$) δ(ppm): 33.7(C-1), 38.9(C-2), 215.8(C-3), 46.7(C-4), 47.8(C-5), 23.8(C-6), 73.7(C-7), 42.0(C-8), 43.8(C-9), 37.3(C-10), 15.1(C-11), 25.8(C-12), 39.0(C-13), 69.6(C-14), 56.3(C-15), 167.6(C-16), 83.2(C-17), 39.6(C-20), 70.7(C-21), 29.5(C-22), 67.7(C-23), 15.7, 17.9, 18.2, 21.0, 26.0(Me x 5), 20.1(Me-acetate), 169.9(C=O -acetate).

1.3.2.8 PREPARATION OF TETRAHYDROGEDUNIN

The preparation of tetrahydrogedunin was performed in two steps starting with hexahydrogedunin. The first involved the α-phenylselenylation of the ketone in ring A using the chemistry reported by Hernandez *et al.*[58] A 10 mL three necked flask was equipped with septa and flame dried under nitrogen. The starting material, hexahydrogedunin (43.7 mg), was dissolved in two mL of dry THF and added to the reaction flask. Boron trifluoride etherate (1.5 equiv./ 0.164 mL) was then added dropwise to the stirring reaction mixture. This was followed by the addition of 1.75 equiv. of phenylselenium chloride (30 mg) dissolved in 1 mL of dry THF. After 2.5 h, water was added and the reaction mixture extracted with ether 3 or 4 times. The organic layer was dried over $MgSO_4$ and the solvent removed to yield the required α-phenylselenylated product.

The final product, tetrahydrogedunin, was obtained from the α-phenylselenylated product via an oxone oxidative deselenylation. In a RBF the α-phenylselenylated product was combined with 3 mL of methanol, 50 mg of oxone and a small amount of $NaHCO_3$. After the reaction had stirred overnight, water was added and the reaction mixture was extracted with ether. The organic layer was dried over $MgSO_4$ and evaporated to dryness. Application of the product on Prep TLC, eluted with 1:1 EtOAc/Hex, yielded tetrahydrogedunin in an overall yield of 11%.



$C_{28}H_{38}O_7$ MW= 486 g/mole

MP 260-262 °C

MS (CI/ISO): 487$[MH]^+$(80), 427(35), 371(9.2), 279(29.2), 257(10.5).

IR (CH_2Cl_2) ν (cm-1): 3092, 3015, 2956, 1738, 1669.

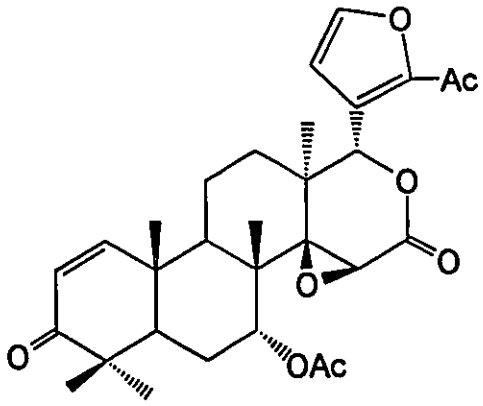
^{1}H NMR ($CDCl_3$) δ(ppm): 7.12(d, J=10.2 Hz, H-1), 5.87(d, J=10.2 Hz, H-2), 2.15(dd, J=2.4, 13.3 Hz, H-5), 1.78 and 1.92(m, H-6), 4.49(brs, H-7), 2.44(m, H-9), 1.83 and 2.07(m, H-11), 1.61(m, H-12), 1.63(d, J=8.8 Hz, H-12), 3.42(s, H-15), 4.51(m, H-17), 2.46(m, H-20), 3.37(t, J=8.6 Hz, H-21), 3.87(t, J=8.0 Hz, H-21), 1.92 and 2.07(m, H-22), 3.71(dt, J= 6.7, 8.5 Hz, H-23), 3.83(dt, J=3.9, 8.4 Hz, H-23), 1.04, 1.05, 1.08, 1.20, 1.29(s, Me X 5), 2.06(s, Me-acetate).

^{13}C NMR ($CDCl_3$) δ(ppm): 156.8(C-1), 126.1(C-2), 203.9(C-3), 44.1(C-4), 46.1(C-5), 23.3(C-6), 73.2(C-7), 42.6(C-8), 39.6(C-9), 40.0(C-10), 15.1(C-11), 26.0(C-12), 38.8(C-13), 69.6(C-14), 56.4(C-15), 167.4(C-16), 83.1(C-17), 39.3(C-20), 70.7(C-21), 29.5(C-22), 67.7(C-23), 18.2, 18.6, 19.7, 21.2, 27.1(Me x 5), 21.0(Me-acetate), 169.8(C=O -acetate).

1.3.2.9 PREPARATION OF 21-ACETYLGEDUNIN AND 23-ACETYLGEDUNIN

The acylation of the furan ring of gedunin at the 21 and 23 positions was accomplished using the approach employed by Levine *et al* for furan in 1949.[61] A flame dried three necked flask was equipped with septa and a nitrogen flow. Gedunin (100 mg), dissolved in 3 mL of methylene chloride, was added to the reaction flask which was then cooled to 0°C. Boron trifluoride etherate (1.6 equiv.\0.040 mL) and acetic anhydride (2.1 equiv.\0.040 mL) were then added to the reaction mixture. The reaction mixture was allowed to warm to room temperature slowly. After 21 h water was added and the reaction mixture was allowed to stir for another 30 min at which time the pH was made basic using Na_2CO_3. The reaction mixture was extracted with methylene chloride, the organic phase washed with water and dried over $MgSO_4$. The final products, 21-acetylgedunin and 23-acetylgedunin, were isolated from the product mixture via Prep TLC purification (eluted with 1:1 EtOAc/Hex) in yields of 17% (Rf= 0.42) and 49% (Rf=0.28) respectively.

21-ACETYLGEDUNIN



$C_{30}H_{36}O_8$ MW= 524 g/mole

MP 245-247 °C

$[\alpha]_D^{23}$ +118.8°(c=0.0032, $CHCl_3$)

MS (CI/ISO): 525 $[MH]^+$(42.5), 509(13.8), 481(20.6), 465(51.7), 139(100).

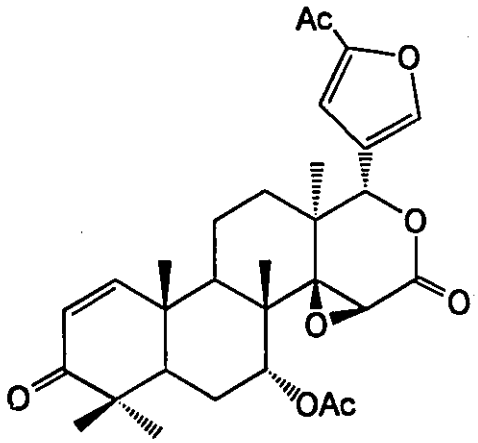
HRMS calcd for $C_{30}H_{36}O_8$ 524.6155 found 524.2406.

IR (CH_2Cl_2) ν (cm-1): 3056, 2976, 1741, 1673, 1586, 822.

^{1}H NMR ($CDCl_3$) δ(ppm): 7.05(d, J= 10.2 Hz, H-1), 5.83(d, J=10.2 Hz, H-2), 2.14(m, H-5), 1.75-1.95(m, H-6), 4.52(br s, H-7), 2.46(dd, J=7.1, 12.3 Hz, H-9), 1.75-1.91(m, H-11), 1.22 and 2.14(m, H-12), 3.51(s, H-15), 6.48(s, H-17), 6.68(d, J=1.7 Hz, H-22), 7.49(m, H-23), 1.04, 1.05, 1.19, 1.20, 1.23(s, Me x 5), 2.09(s, Me-acetate), 2.49(s, Me-acetyl).

^{13}C NMR ($CDCl_3$) δ(ppm): 157.1(C-1), 125.9(C-2), 204.0(C-3), 44.0(C-4), 46.1(C-5), 23.2(C-6), 73.2(C-7), 42.8(C-8), 39.4(C-9), 40.6(C-10), 15.0(C-11), 24.6(C-12), 40.2(C-13), 69.4(C-14), 57.0(C-15), 167.6(C-16), 76.0(C-17), 128.2(C-20), 148.6(C-21), 114.1(C-22), 144.6(C-23), 17.6, 18.5, 19.8, 21.2, 27.1(Me x 5), 21.1(Me-acetate), 27.2(Me-acetyl), 169.9(C=O -acetate), 188.7(C=O -acetyl).

23-ACETYLGEDUNIN



MP 268-271 °C

$[\alpha]_D^{23}$ +75.3° (c=0.0068, $CHCl_3$)

MS (CI/ISO): 525 $[MH]^+$(43.2), 481(39.3), 465(26.4), 423(26.1), 422(30.9), 421(100),

HRMS calcd $C_{30}H_{36}O_8$ for 524.6155 found 524.2421

IR (CH_2Cl_2) ν (cm-1): 2928, 1744, 1676, 1596, 855.

^{1}H NMR ($CDCl_3$) δ(ppm): 7.06(d, J=10.2 Hz, H-1), 5.85(d, J=10.2 Hz, H-2), 2.15(dd, J= 2.4, 13.3 Hz, H-5), 1.79 and 1.95 (m, H-6), 4.54(dd, J= 2.1, 3.4 Hz, H-7), 2.45(m, H-9), 1.84 and 2.00(m, H-11), 1.55 and 1.76(m, H-12), 3.53(s, H-15), 5.61(s, H-17), 7.53(t, J=0.8 Hz, H-21)*, 7.06(m, H-22)*, 1.04, 1.05, 1.14, 1.20, 1.20(s, Me x 5), 2.08(s, Me-acetate), 2.46(s, Me-acetyl).

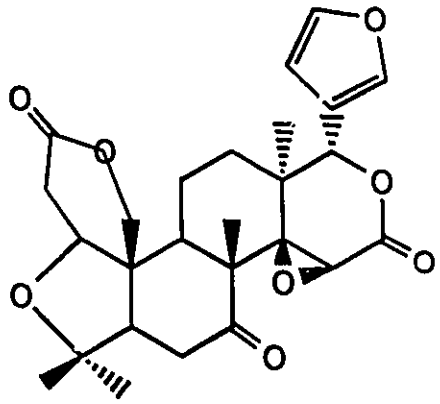
^{13}C NMR ($CDCl_3$) δ(ppm): 156.7(C-1), 126.1(C-2), 203.8(C-3), 44.0(C-4), 46.0(C-5), 23.3(C-6), 73.2(C-7), 42.7(C-8), 40.0(C-9), 39.6(C-10), 14.9(C-11), 26.0(C-12), 38.7(C-13), 69.7(C-14), 56.7(C-15), 166.9(C-16), 77.7(C-17), 123.3(C-20), 144.4(C-21), 116.0(C-22), 153.1(C-23), 17.7, 18.3, 19.7, 21.2*, 27.1(Me x 5), 21.0(Me-acetate)*, 26.1(Me-acetyl),169.8(C=O -acetate), 186.9(C=O -acetyl).

*= assignments are interchangeable.

1.3.2.10 ISOLATION OF LIMONIN

Seeds (174 g) which had been removed from a mixture of old oranges and grapefruit purchased from a local fruit market, were dried in an oven maintained at 40 °C for three days and then ground up using a Wiley Mill. Acetone was added to the ground material

and was filtered off the next day. This extraction was performed three times and the combined acetone extracts were concentrated to a thin syrup. Addition of hexane resulted in the formation of a limonin- containing precipitate. Crystallization of the filtered precipitate using hexane resulted in the isolation of limonin crystals (247.7 mg in a 0.14 % yield). The above procedure is documented in the literature.[32]



$C_{26}H_{30}O_8$ MW=470 g/mole

MP 282-285 °C (decomp) (literature 298 °C)[32]

MS (CI/ISO): 471$[MH]^+$(100), 427(8.4), 425(9.6), 383(4.5), 347(17.5).

^{1}H NMR ($CDCl_3$) δ(ppm): 4.01(s, H-1), 2.66 (dd, J=1.9, 16.8 Hz, H-2), 2.96(dd, J=3.8, 16.8 Hz, H-2), 2.20(dd, J=3.3, 15.9 Hz, H-5), 2.44(dd, J=3.4, 14.5 Hz, H-6), 2.83(dd, J=14.6,15.8 Hz, H-6), 2.53(dd, J=2.9, 12.4 Hz, H-9), 1.75 and 1.85 (m, H-11), 1.50 and 1.75(m, H-12), 4.02(s, H-15), 5.45(s, H-17), 4.44(d, J=13 Hz, H-19), 4.74 (d, J=13 Hz, H-19), 7.38(m, H-21), 6.32(m, H-22), 7.39(m, H-23), 1.05, 1.15, 1.16, 1.27(s, Me x 4).

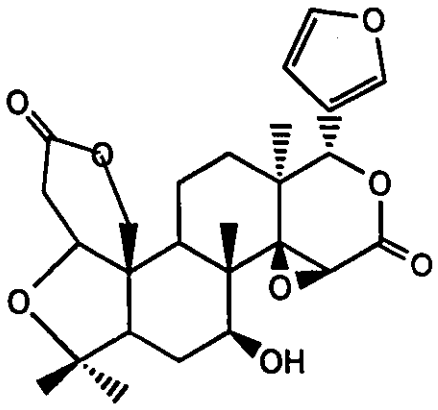
^{13}C NMR ($CDCl_3$) δ(ppm): 79.2(C-1), 35.6(C-2), 169.1(C-3), 80.3(C-4), 60.6(C-5), 36.4(C-6), 206.1(C-7), 46.0(C-8)*, 48.1(C-9), 51.3(C-10)*, 18.9(C-11), 30.9(C-12), 38.0(C-

13), 65.7(C-14), 53.9(C-15), 166.6(C-16), 77.8(C-17), 65.4(C-19), 120.0(C-20), 143.2(C-21), 109.7(C-22), 141.1(C-23). 17.6, 20.7, 21.4, 30.2(Me x 4).

* =assignments are interchangeable

1.3.2.11 PREPARATION OF EPILIMONOL

Epilimonol was prepared, according to the literature[32], via a $NaBH_4$ (15 mg) reduction of limonin (91 mg) in dioxane for 2 h,. The work up consisted of adding water to the reaction mixture followed by extraction with chloroform three times. The dried chloroform extract was purified by Prep plate TLC which was eluted with 40 % acetone/toluene.



$C_{26}H_{32}O_8$ MW=472 g/mole

MP 262-265 °C(decomp)(literature 262-266 °C)[32]

MS (CI/ISO): 473$[MH]^+$(100), 455(15.5), 427(12.8), 349(15.7).

^{1}H NMR (Acetone d_6) δ(ppm): 4.11(m, H-1), 2.64(dd, J=4.0, 16.4 Hz, H-2), 2.71(dd, J=1.5, 16.4 Hz, H-2), 2.14(m, H-5), 1.71 and 1.83(m,H-6), 3.94(dd, J=5.0, 10.4 Hz, H-7),

5.59(br s, 7-OH), 2.49(dd, J=6.7, 12.4 Hz, H-9), 1.88 and 2.14(m, H-11), 1.43(ddd, J=2.4, 9.1, 13.6 Hz, H-12), 1.77(m, H-12), 4.51(s, H-15), 5.61(s, H-17), 4.54(AB quartet, J= 13.5 Hz, H-19), 7.59(m, H-21)*, 6.47(ddd, J=0.3, 0.9, 1.9 Hz, H-22), 7.56(dt, J=0.3, 1.7 Hz, H-23)*, 1.00, 1.05, 1.20, 1.28(s, Me x 4).

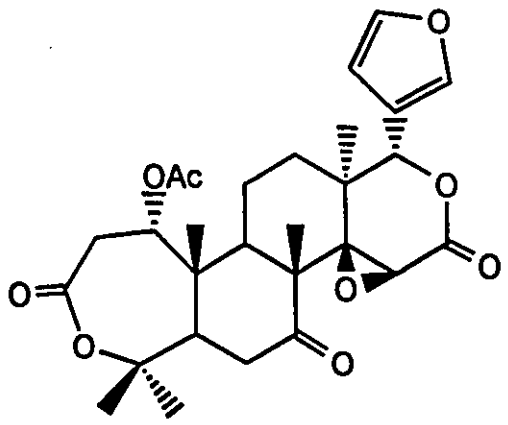
^{13}C NMR (Acetone d_6) δ(ppm):80.4(C-1), 36.4(C-2),170.1(C-3), 80.7(C-4), 58.5(C-5), 29.1(C-6), 78.3(C-7), 44.6^(C-8), 46.4(C-9), 46.5^(C-10), 18.4(C-11), 26.8(C-12), 39.3(C-13), 73.6(C-14), 56.5(C-15), 168.5(C-16), 78.5(C-17), 66.3(C-19), 122.0(C-20), 144.0(C-21)*, 111.0(C-22), 142.5(C-23)*, 14.0, 18.9, 21.9, 30.7(Me x 4).

*,^ = assignments interchangeable

1.3.2.12 NOMILIN, OBACUNONE

Samples of nomilin and obacunone were generously donated by Dr. S. Hasegawa at the US Department of Agriculture, Pasadena, California.

NOMILIN



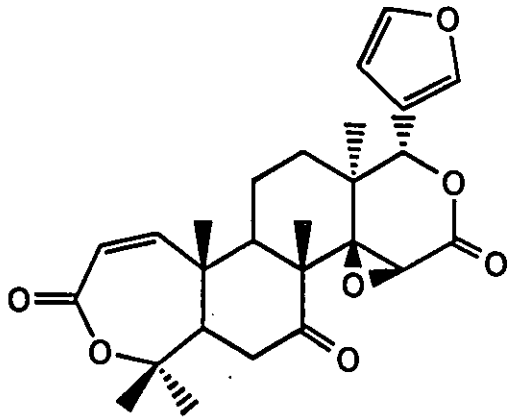
C_{28} $H_{34}O_9$ MW=514 g/mole

MP 232 °C(decomp)(literature 279 °C(decomp))[40]

^{1}H NMR ($CDCl_3$) δ(ppm): 4.99(dd, J=6.5 Hz, H-1), 2.57(m, H-2), 2.57(m, H-5), 3.08(dd, J=7.3, 15.6 Hz, H-6), 3.18(dd, J=1.4, 15.6 Hz, H-6), 2.46(dd, J=2.8, 10.3 Hz, H-9), 1.12 and 1.59(m, H-11 and H-12), 3.78(s, H-15), 5.42(s, H-17), 7.38 and 7.37(s, H-21, 23), 6.30(m, H-22), 1.99(s, acetate), 1.53, 1.45, 1.31, 1.15, 1.07(s, Me x 5)

^{13}C NMR ($CDCl_3$) δ(ppm): 70.7(C-1), 38.8(C-2), 84.3(C-4), 51.0(C-5), 35.3(C-6), 206.7(C-7), 52.9(C-8), 44.4(C-9), 44.2(C-10), 32.3 and 17.2 (C-11 and C-12), 37.5(C-13), 65.4(C-14), 53.4(C-15), 78.0(C-17), 120.1(C-20), 141.0(21), 109.6(C-22), 143.2(C-23), 166.7, 169.1, 169.2(C-3, 16, C=O -acetate), 16.5, 17.1, 20.7, 20.8, 23.4, 33.4(Me-acetate and Me x 5).

OBACUNONE



$C_{26}H_{30}O_7$ MW=454 g/mol

MP 193-195 °C (literature 209-211 °C)[64]

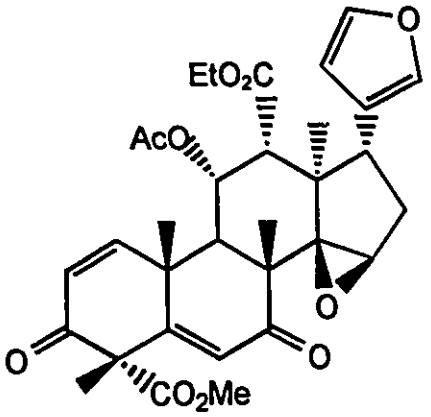
^{1}H NMR ($CDCl_3$) δ(ppm): 6.49(d, J=11.8 Hz, H-1), 5.94(d, J=11.7 Hz, H-2), 2.27(dd, J=5.0, 14.0 Hz, H-5), 2.57(dd, J=5.0, 14.1 Hz, H-6α), 2.96(t, J= 14.1 Hz, H-6β), 3.64(s, H-

15), 5.44(s, H-17), 7.40 and 7.38(m, H-21, 23), 6.34(m, H-22), 1.10, 1.22, 1.43, 1.48, 1.48 (s, Me X 5).

^{13}C NMR ($CDCl_3$) δ(ppm): 156.7(C-1), 123.0(C-2), 166.6(C-3), 83.9(C-4), 57.4(C-5), 39.9(C-6), 207.4(C-7), 53.0(C-8), 49.2(C-9), 43.1(C-10), 19.5(C-11), 32.8(C-12), 37.4(C-13), 65.0(C-14), 53.3(C-15), 166.9(C-16), 78.0(C-17), 120.1(C-20), 143.2(C-21), 109.7(C-22), 141.0(C-23), 32.0(Me-4), 26.8(Me-4), 21.1(Me-8), 16.4(Me-10), 17.0(Me-13).

1.3.2.13 HIRTIN

Hirtin was generously donated by Dr. M. Isman, Department of Plant Science and Botany, University of British Columbia, Vancouver, British Columbia.



$C_{32}H_{36}O_{11}$ MW=596 g/mole

MP 148-151 °C (literature 159-161 °C)[44]

$[\alpha]_D^{23}$ +30.6° (c=0.0080, $CHCl_3$) (literature +26.0°)[44]

^{1}H NMR ($CDCl_3$) δ(ppm): 7.00(d, J= 10.2 Hz, H-1), 6.15(d, J= 10.1 Hz, H-2), 2.96 (brs, H-9), 5.37(br s, H-11), 5.21(br s, H-12), 3.90(s, H-15), 2.28 (m, H-16) and 1.98(ddd, J= 0.6, 11.2, 13.6 Hz, H-16), 2.90(dd, J= 6.7, 11.0 Hz, H-17), 7.10(s, H-21),

6.07(m, H-22), 7.29(m, H-23), 0.77, 1.39, 1.42, 1.81(s, Me x 4), 1.01(t, J= 7.6 Hz, Me-ethyl ester), 2.17(s, Me-acetate), 2.24 and 2.15 (m, CH_2-ethyl ester), 3.75(s, Me-methyl ester).

^{13}C NMR ($CDCl_3$) δ(ppm): 150.7(C-1), 126.8(C-2), 196.2(C-3)*, 59.3(C-4), 129.4(C-5), 141.9(C-6), 195.6(C-7)*, 46.1(C-8), 42.8(C-9), 39.9(C-10), 72.4(C-11), 78.4(C-12), 45.1(C-13), 67.6(C-14), 55.1(C-15), 32.1(C-16), 41.6(C-17), 121.6(C-20), 140.5(C-21), 111.1(C-22), 142.7(C-23), 15.5, 22.5, 22.9, 25.4, (Me x 4), 8.9(Me-ethyl ester), 21.2(Me-acetate), 27.7(CH_2-ethyl ester), 53.0(Me-methyl ester), 169.4(C=O -methyl ester)^, 170.0(C=O -acetate)^, 172.2(C=O -ethyl ester)^.

* , ^ = assignments are interchangeable

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APPENDIX OF CHAPTER 1: NMR SPECTRA OF GEDUNIN ANALOGS

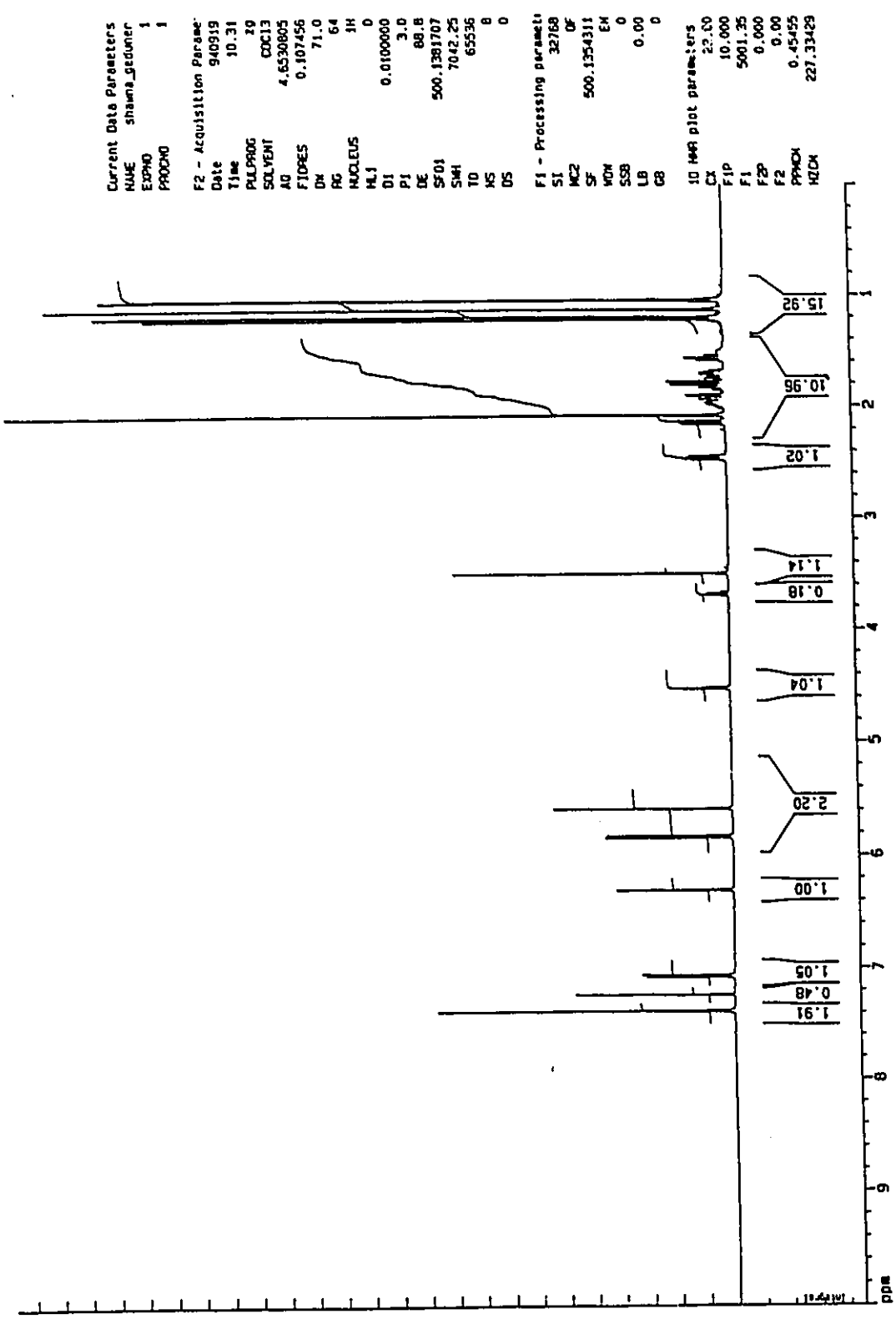


FIGURE 1.2.1a: ^1H NMR OF GEDUNIN

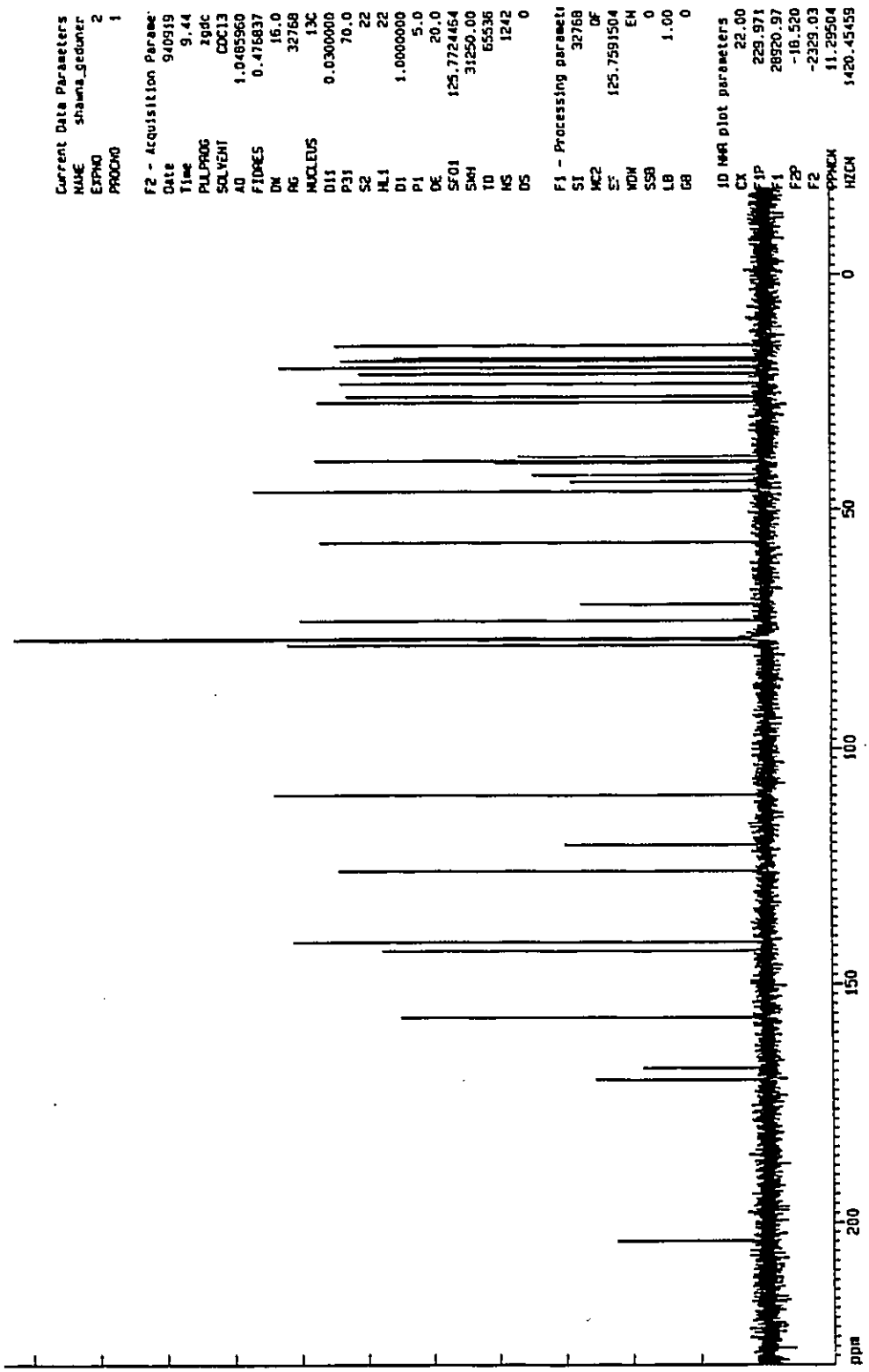


FIGURE 1.2.1b: ^{13}C NMR OF GEDUNIN

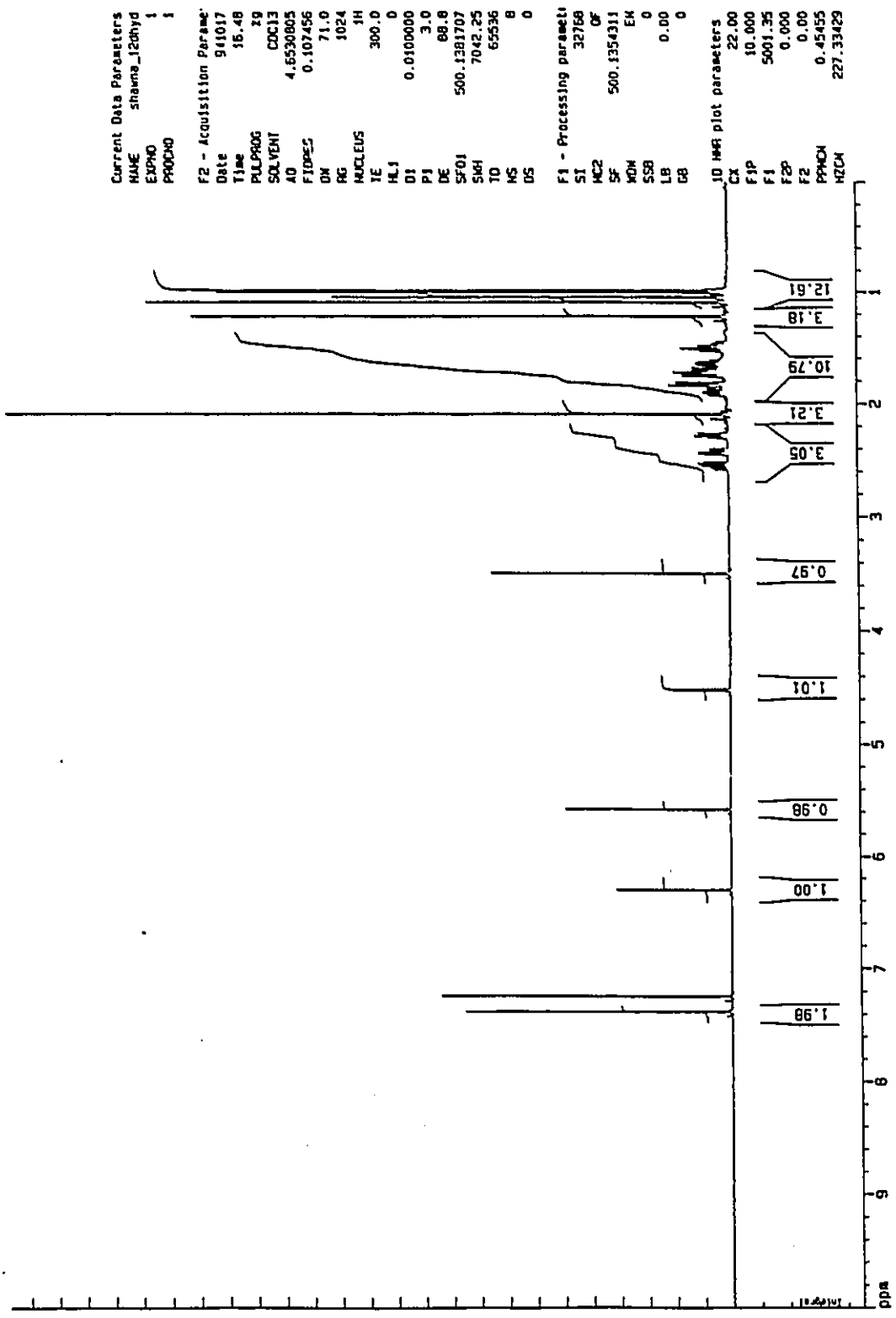


FIGURE 1.2.2.1a: ^{1}H NMR OF 1,2-DIHYDROGEDUNIN

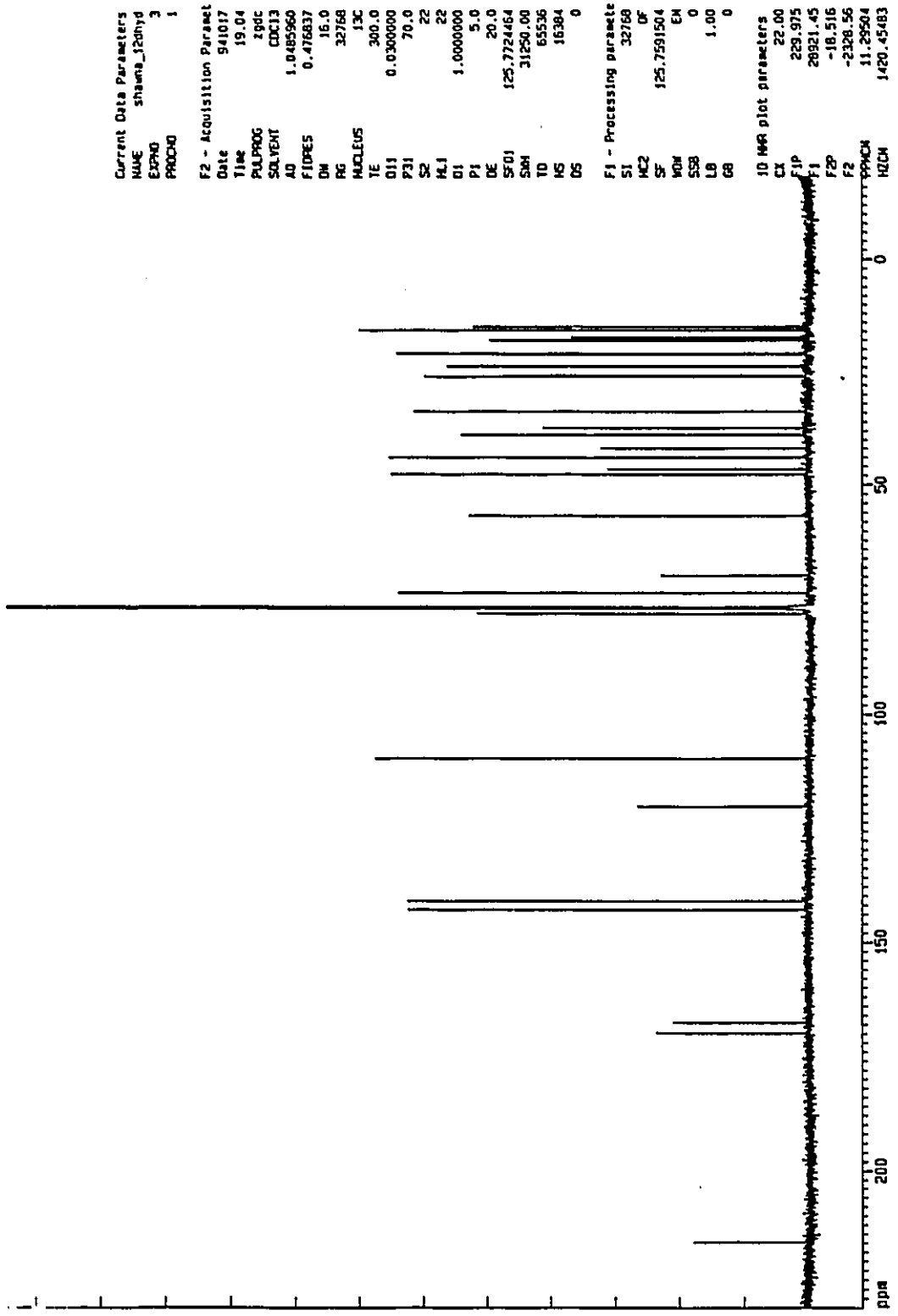


FIGURE 1.2.2.1b: ^{13}C NMR OF 1,2-DIHYDROGEDUNIN

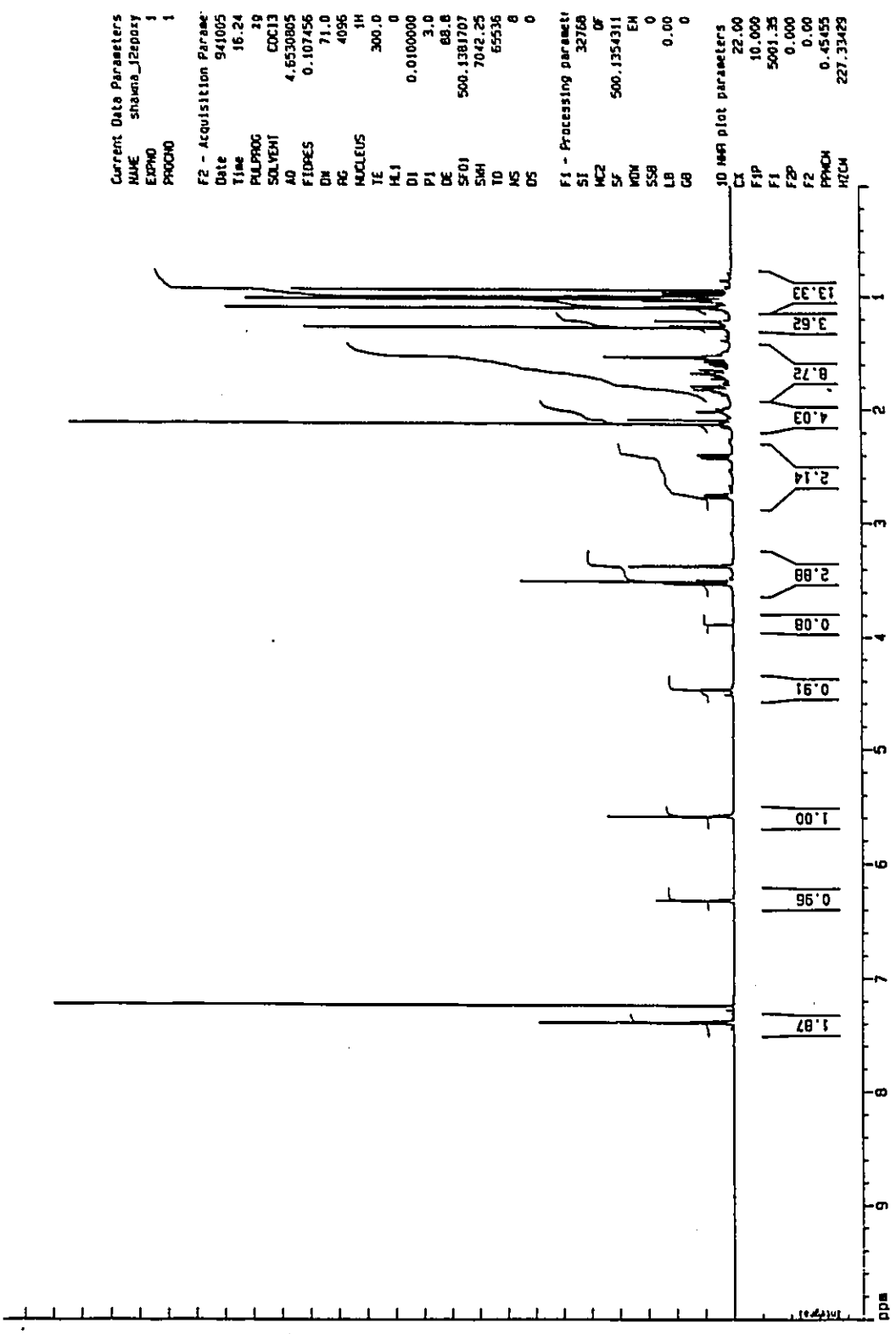


FIGURE 1.2.2.2a: ^{1}H NMR OF 1,2-EPOXYGEDUNIN

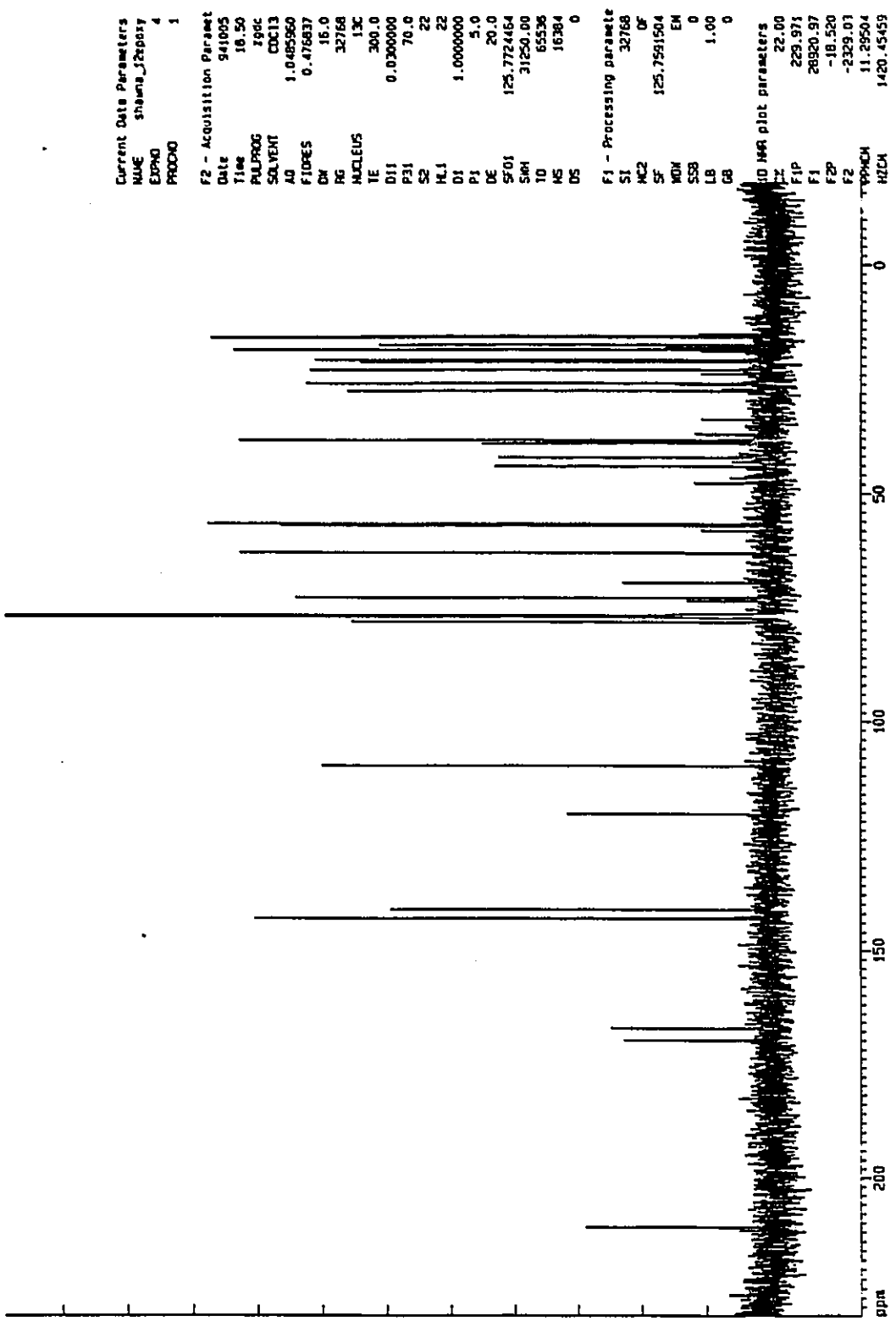


FIGURE 1.2.2.2b: ^{13}C NMR OF 1,2-EPOXYGEDUNIN

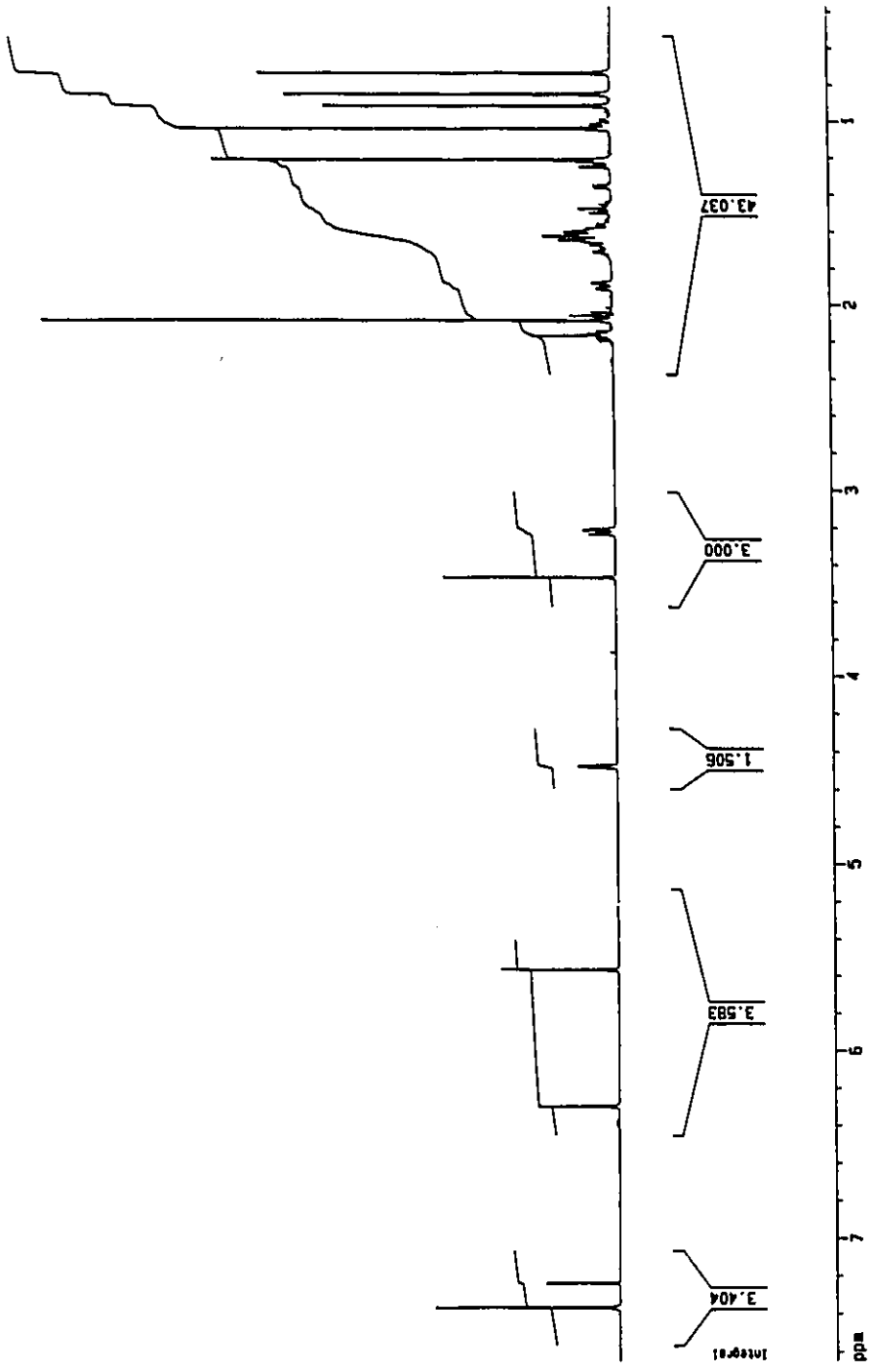


FIGURE 1.2.2.3a: ^{1}H NMR OF 1,2-DIHYDRO-3β-GEDUNOL

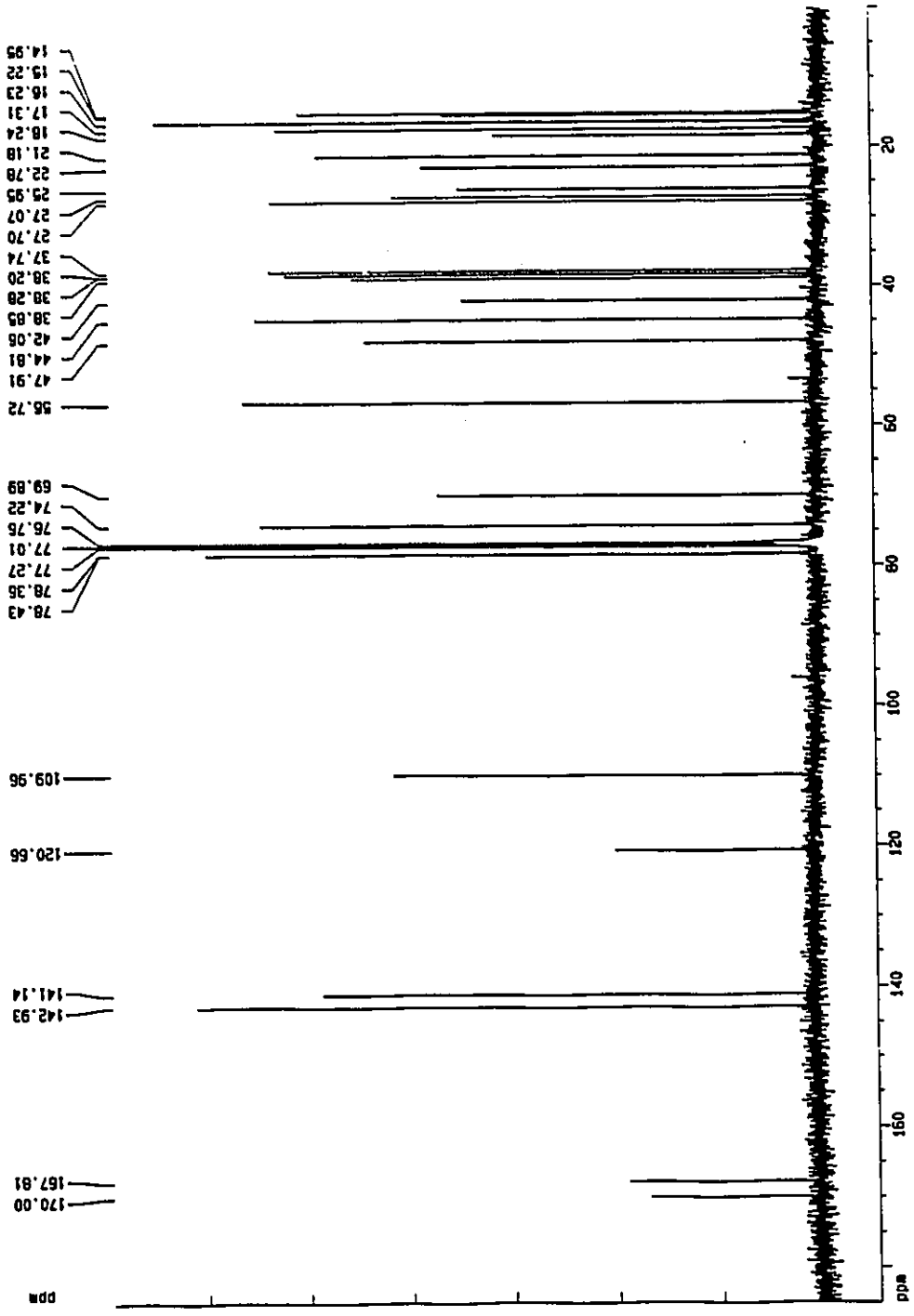


FIGURE 1.2.2.3b: ^{13}C NMR OF 1,2-DIHYDRO-3β-GEDUNOL

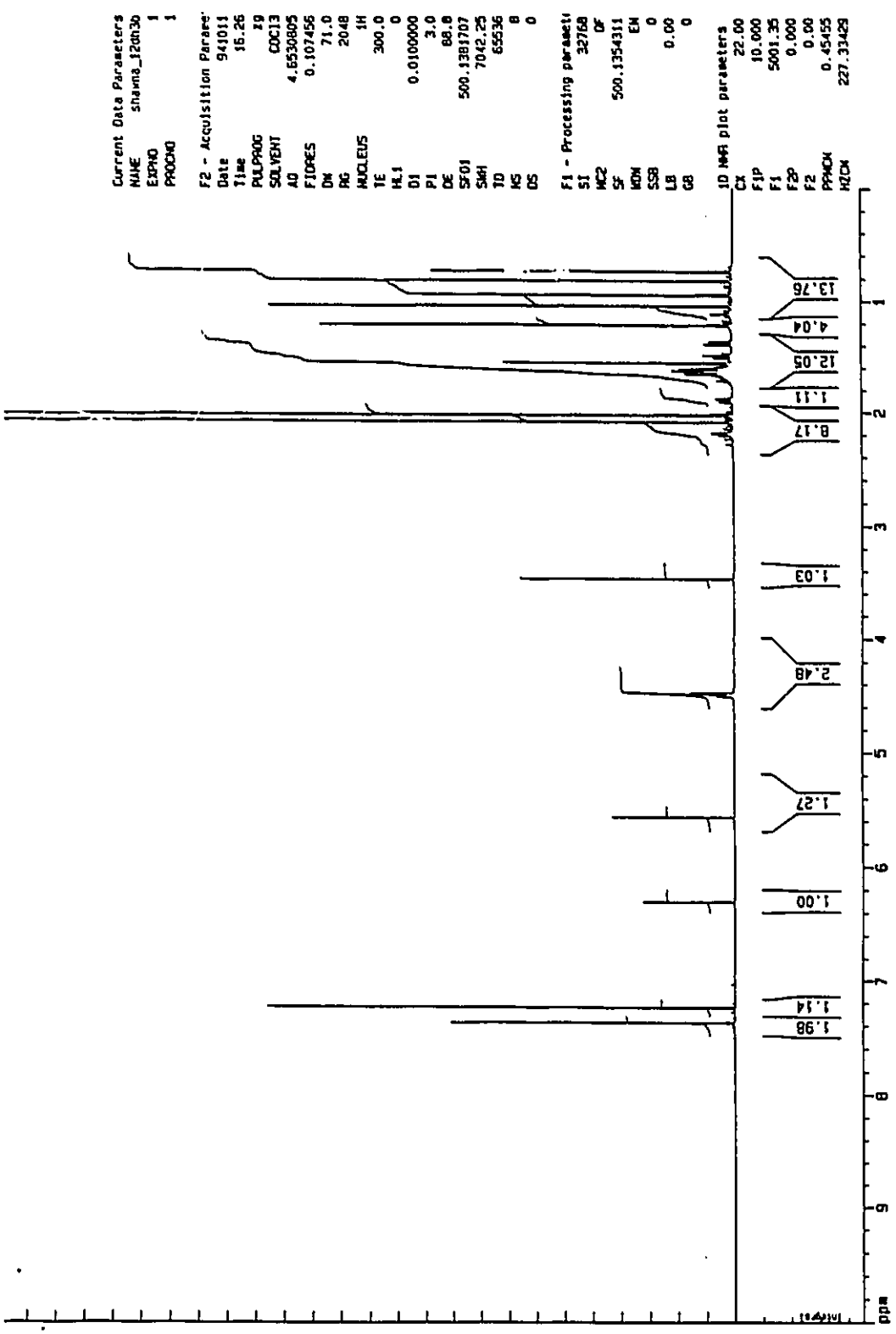


FIGURE 1.2.2.4a: ^{1}H NMR 3β-ACETOXY-1,2-DIHYDROGEDUNIN

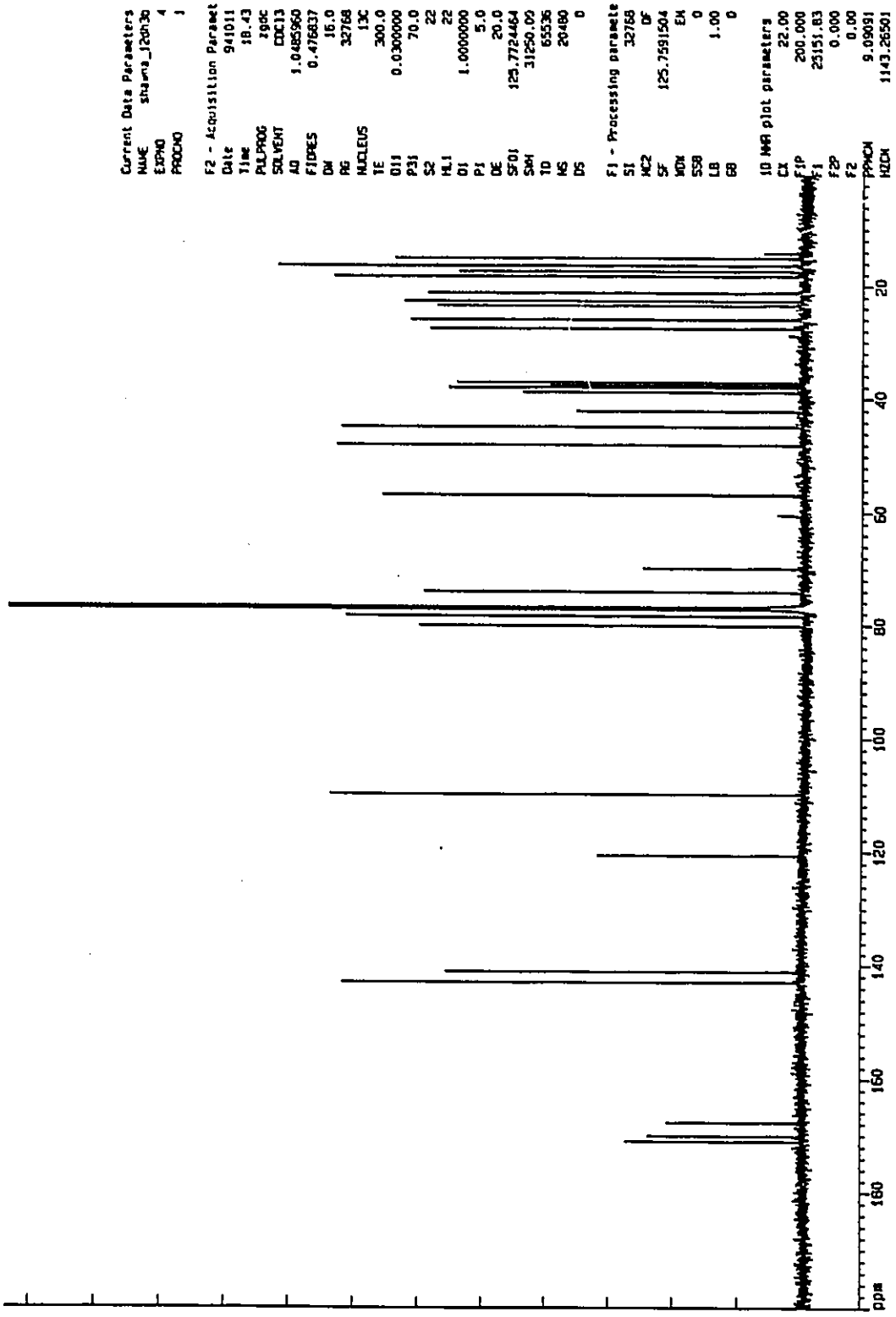


FIGURE 1.2.2.4b: ^{13}C NMR 3β-ACETOXY-1,2-DIHYDROGEDUNIN

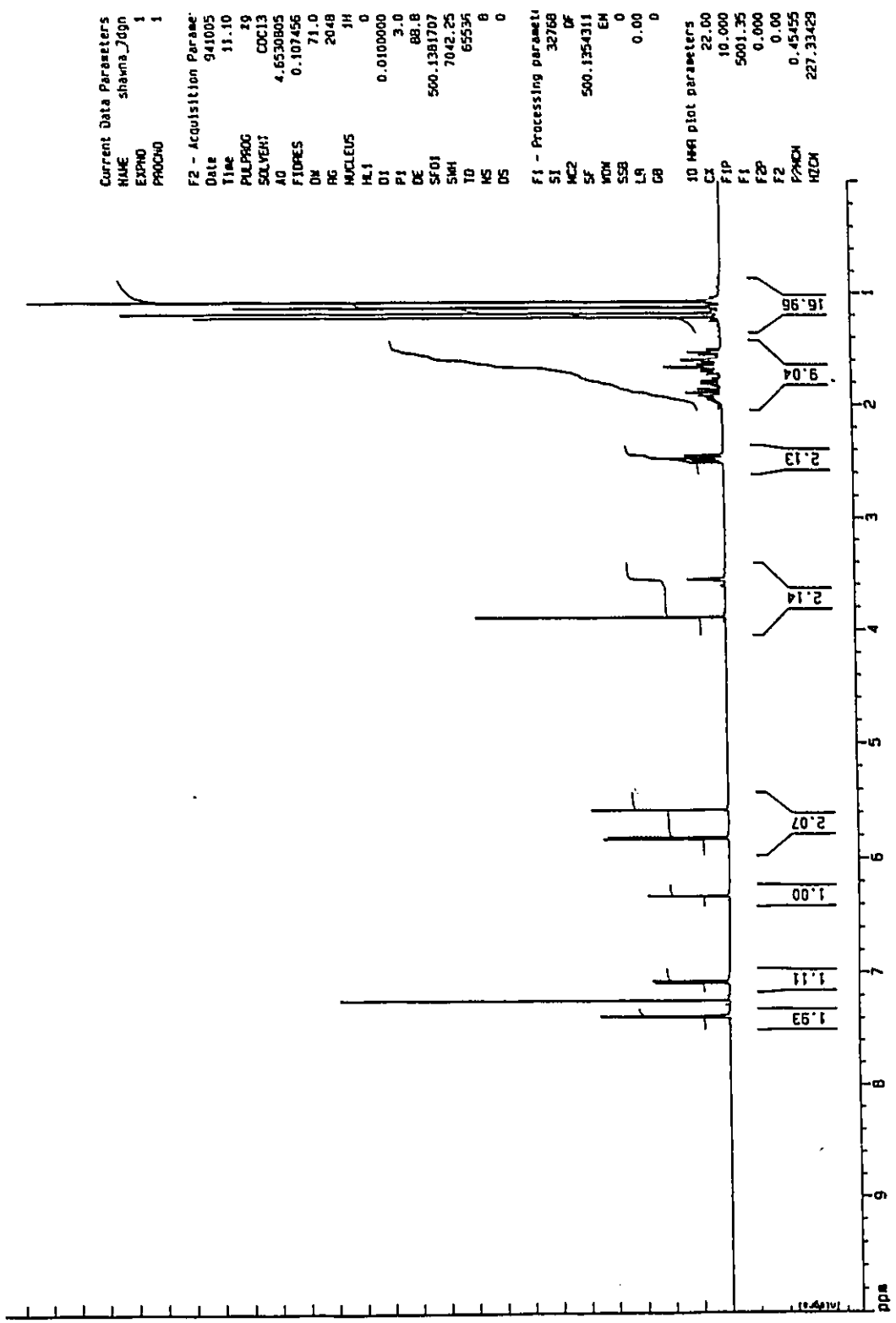


FIGURE 1.2.3.1a: ^{1}H NMR 7-DEACETYLGEDUNIN

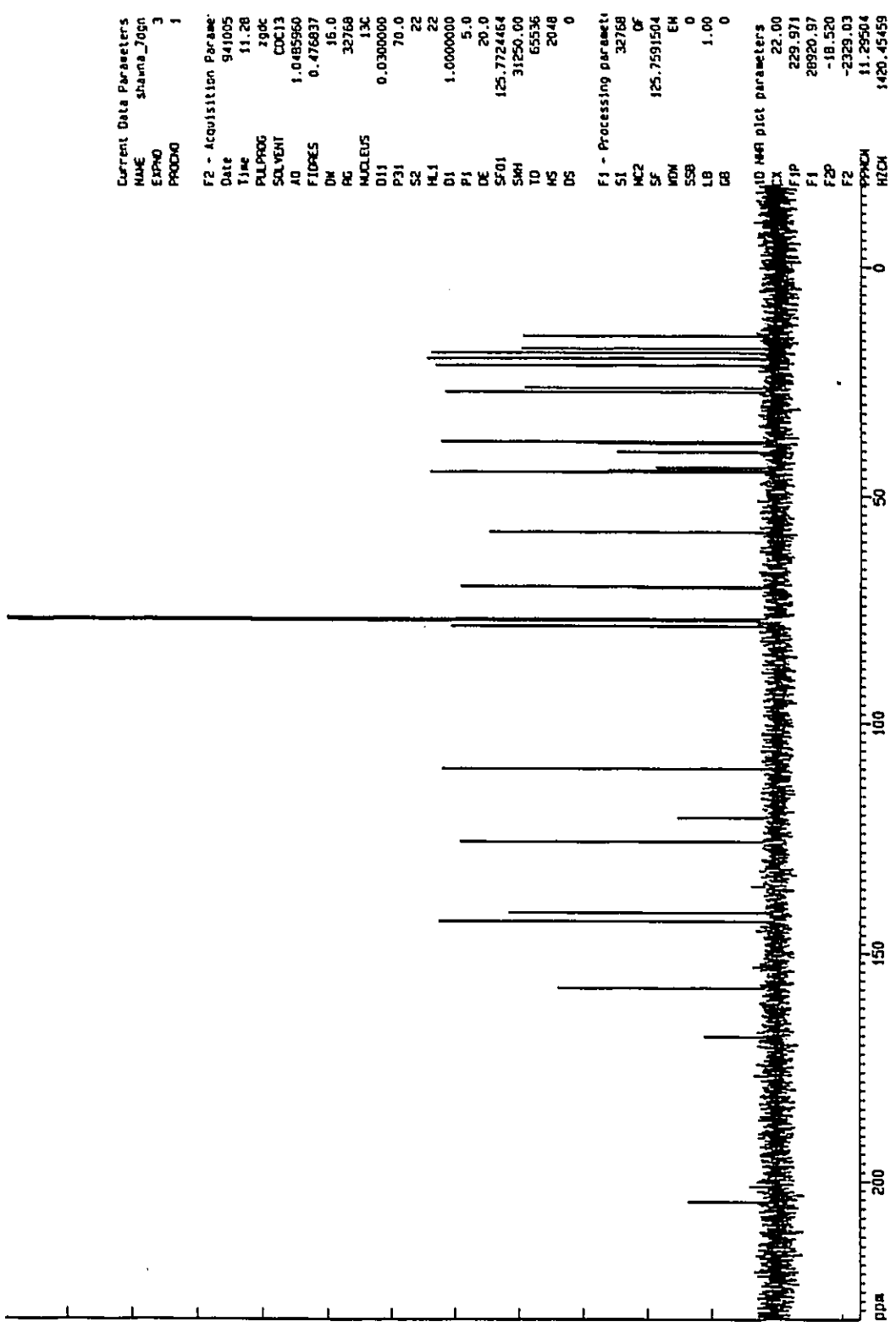


FIGURE 1.2.3.1b: ^{13}C NMR OF 7-DEACETYLGEDUNIN

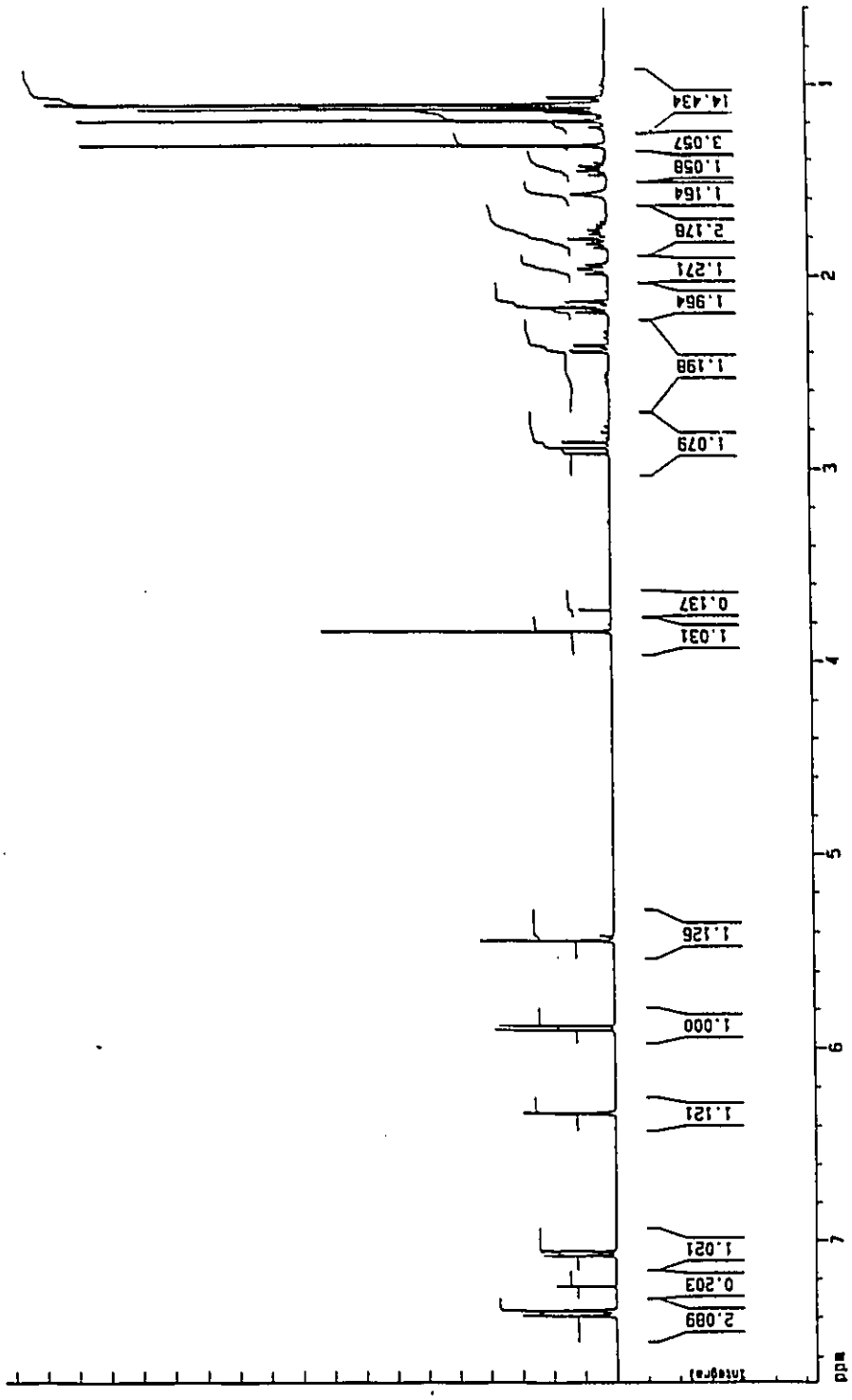


FIGURE 1.2.3.2a: ^{1}H NMR OF 7-KETOGEDUNIN

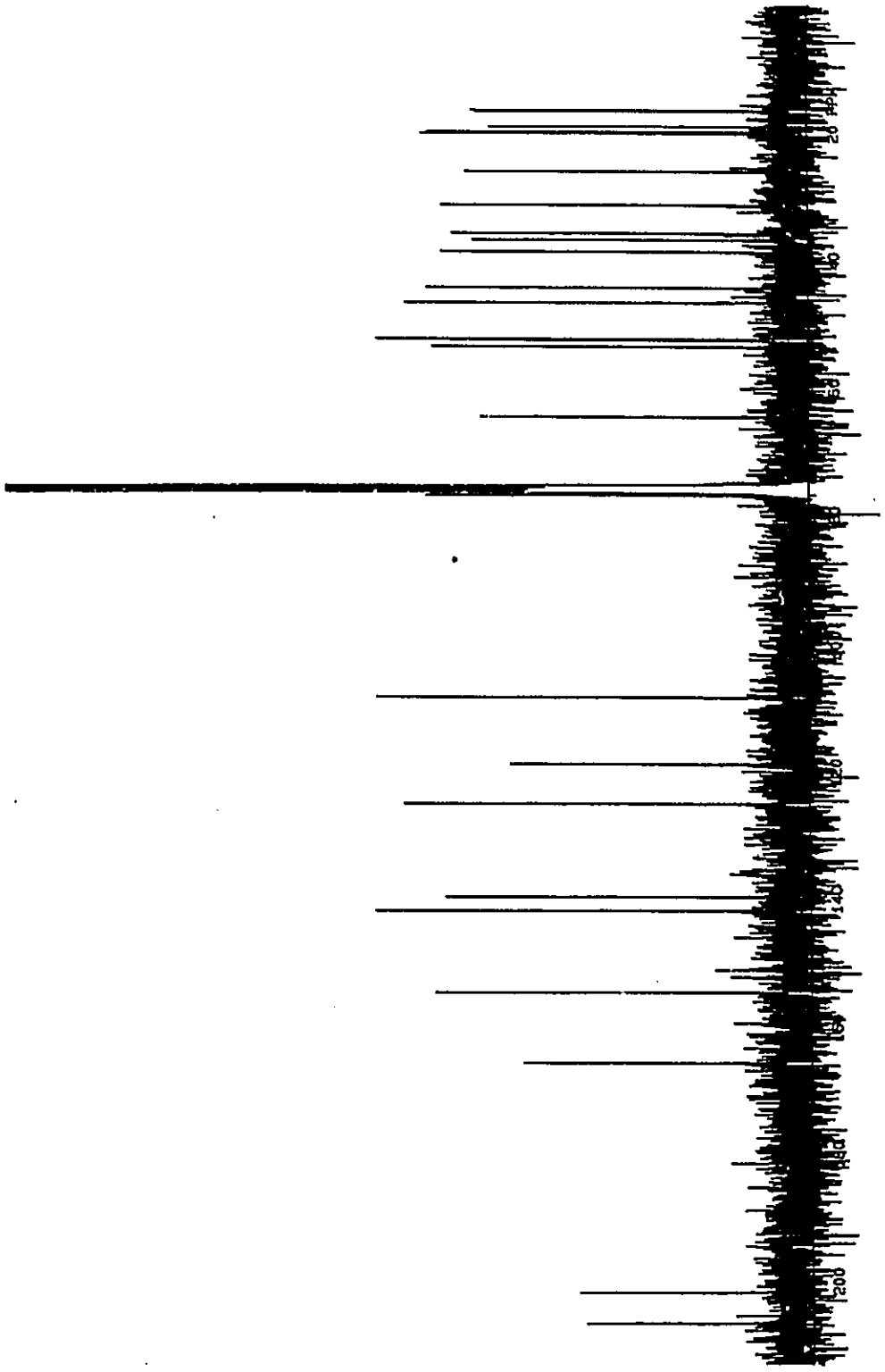


FIGURE 1.2.3.2b: ^{13}C NMR OF 7-KETOGEDUNIN

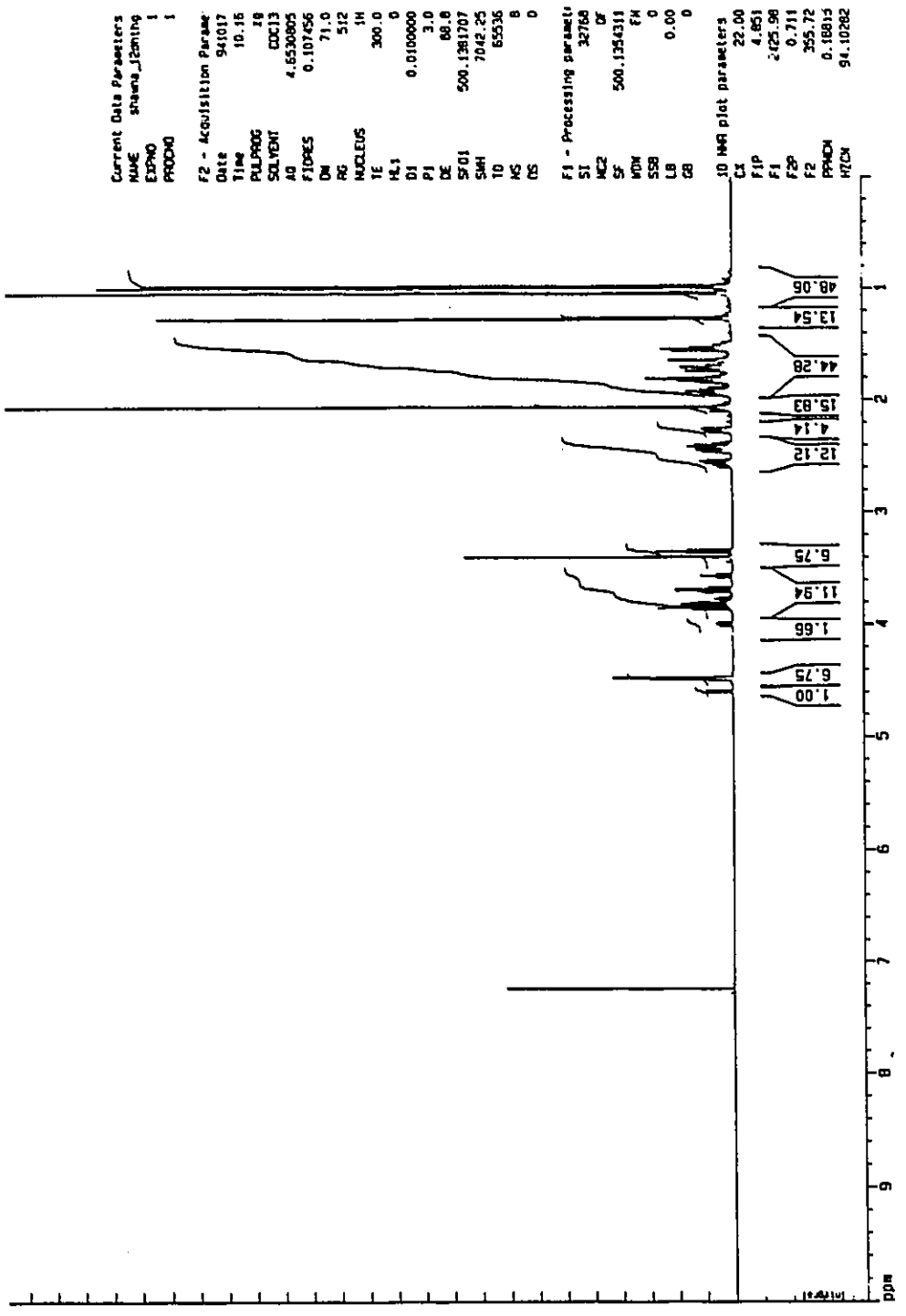


FIGURE 1.2.4.1a: ^{1}H NMR OF HEXAHYDROGEDUNIN

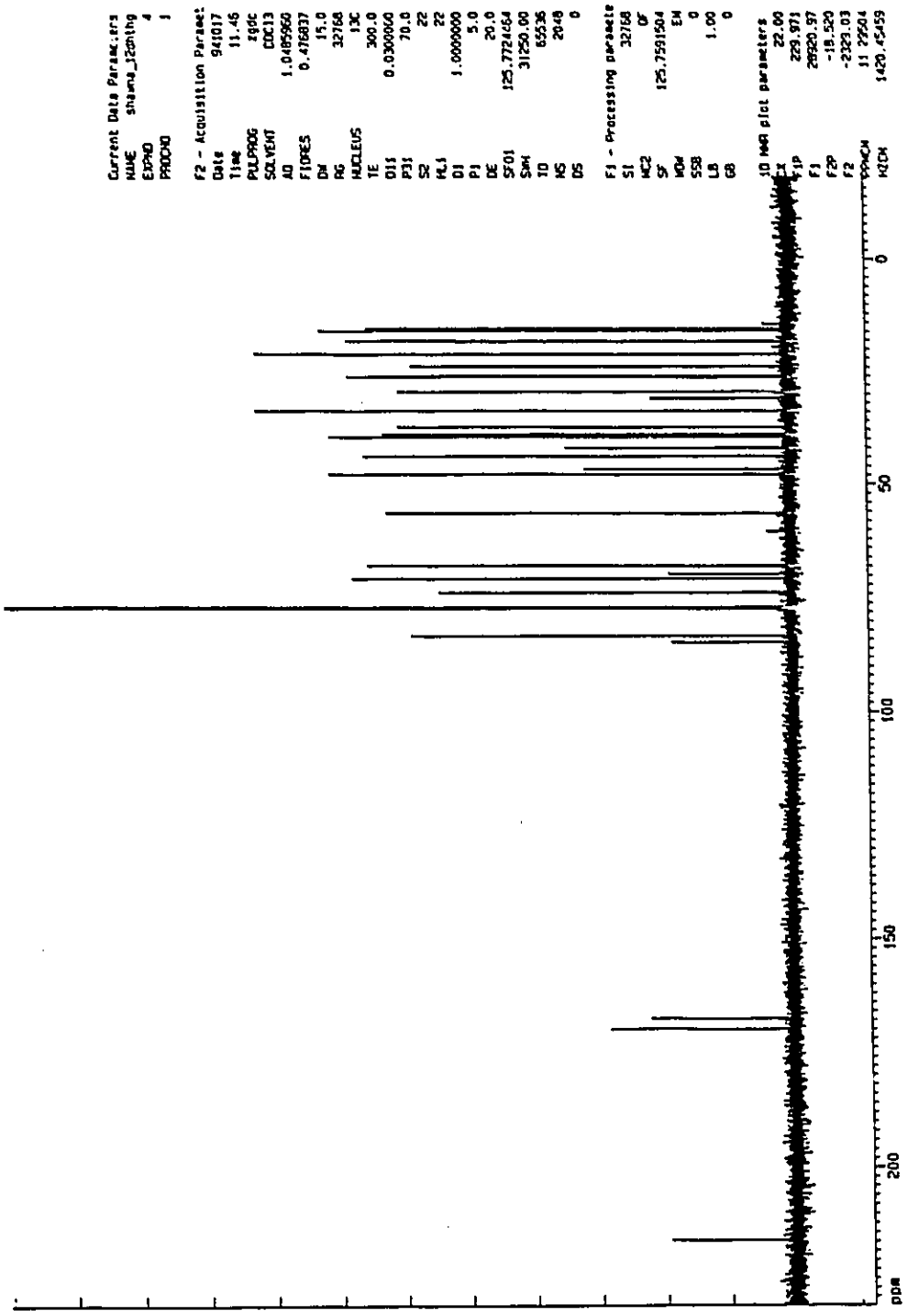


FIGURE 1.2.4.1b: ^{13}C NMR OF HEXAHYDROGEDUNIN

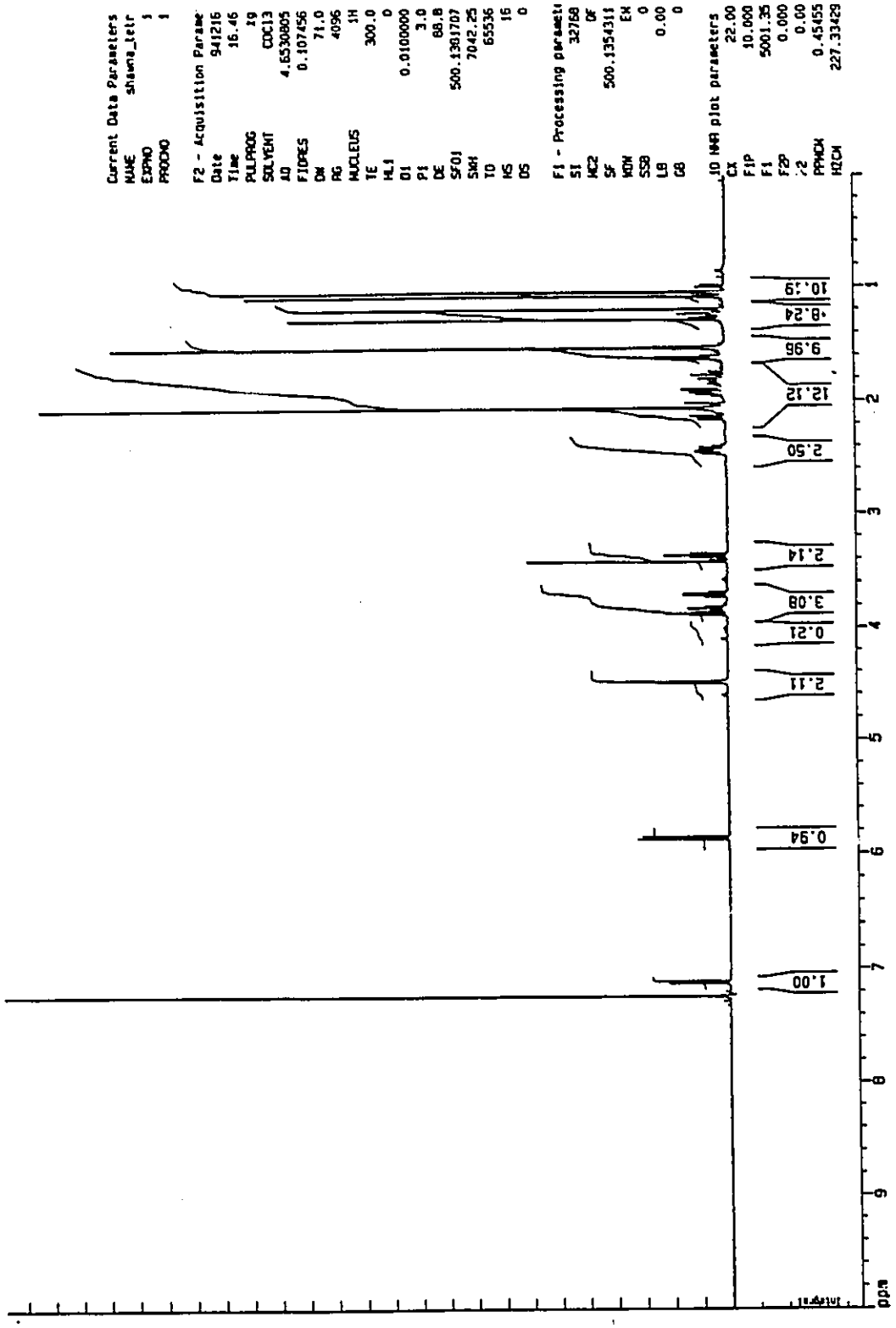


FIGURE 1.2.4.2a: ^{1}H NMR OF TETRAHYDROGEDUNIN

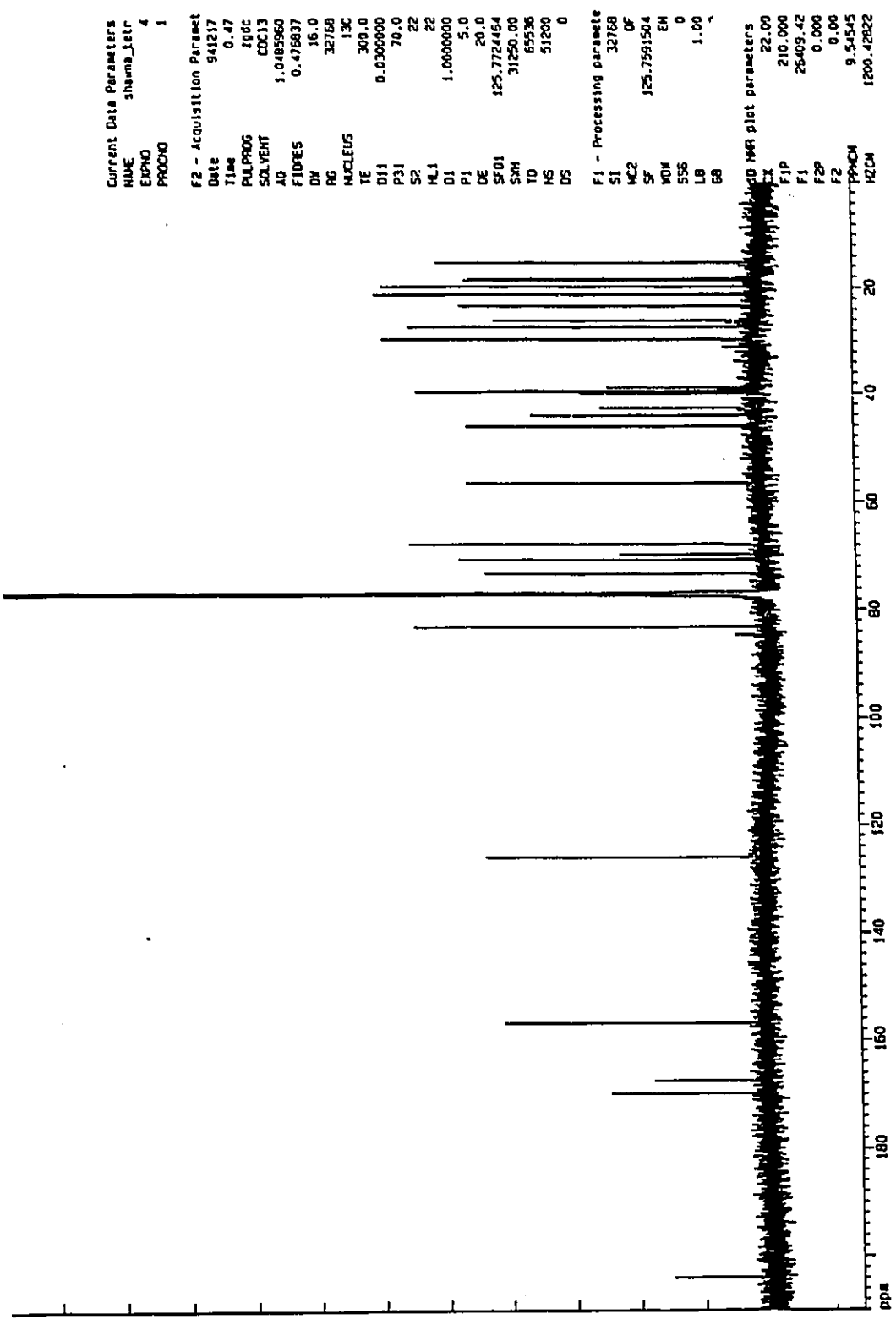


FIGURE 1.2.4.2a: ^{13}C NMROF TETRAHYDROGEDUNIN

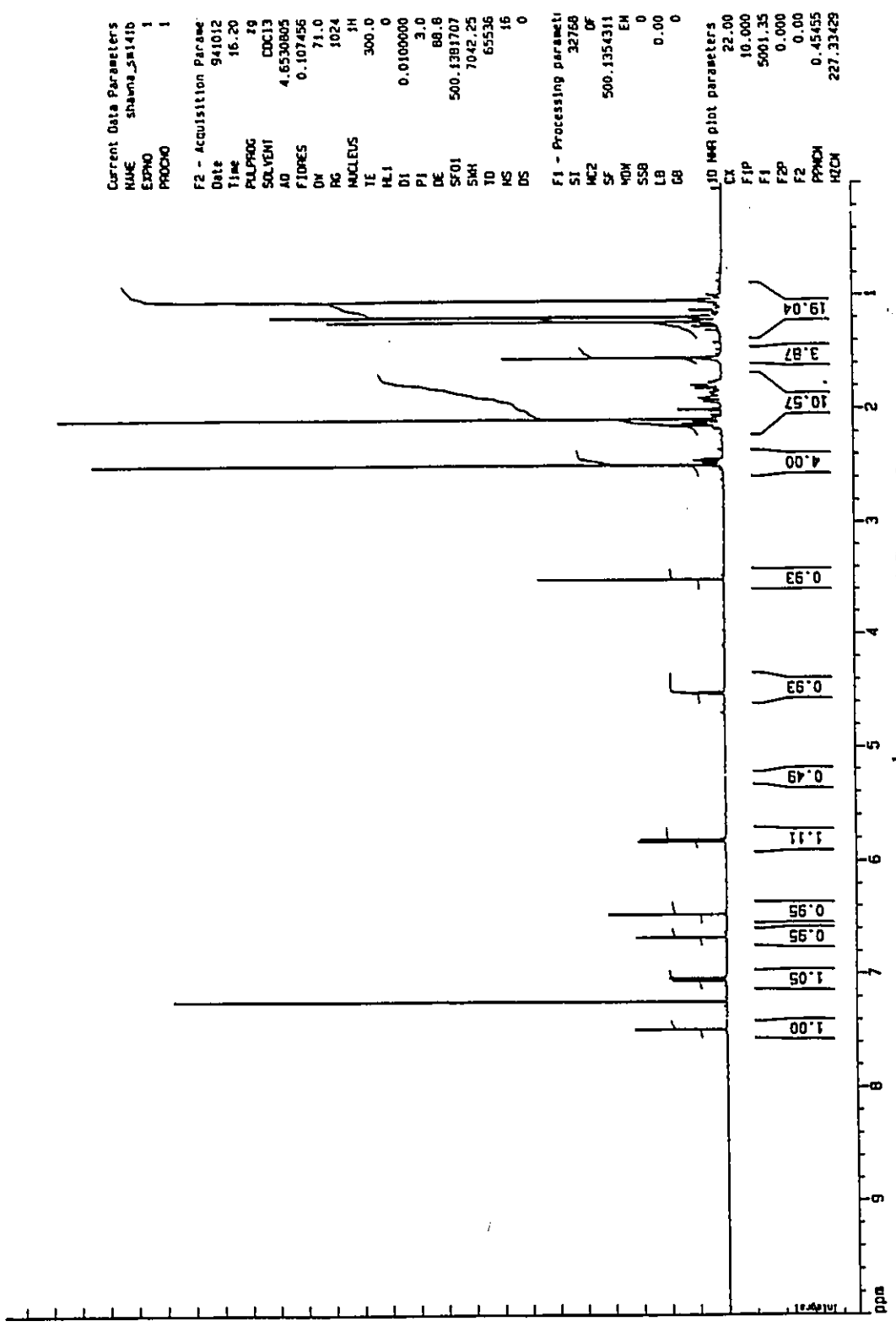


FIGURE 1.2.4.3.a: ^{1}H NMR OF 21-ACETYLGEDUNIN

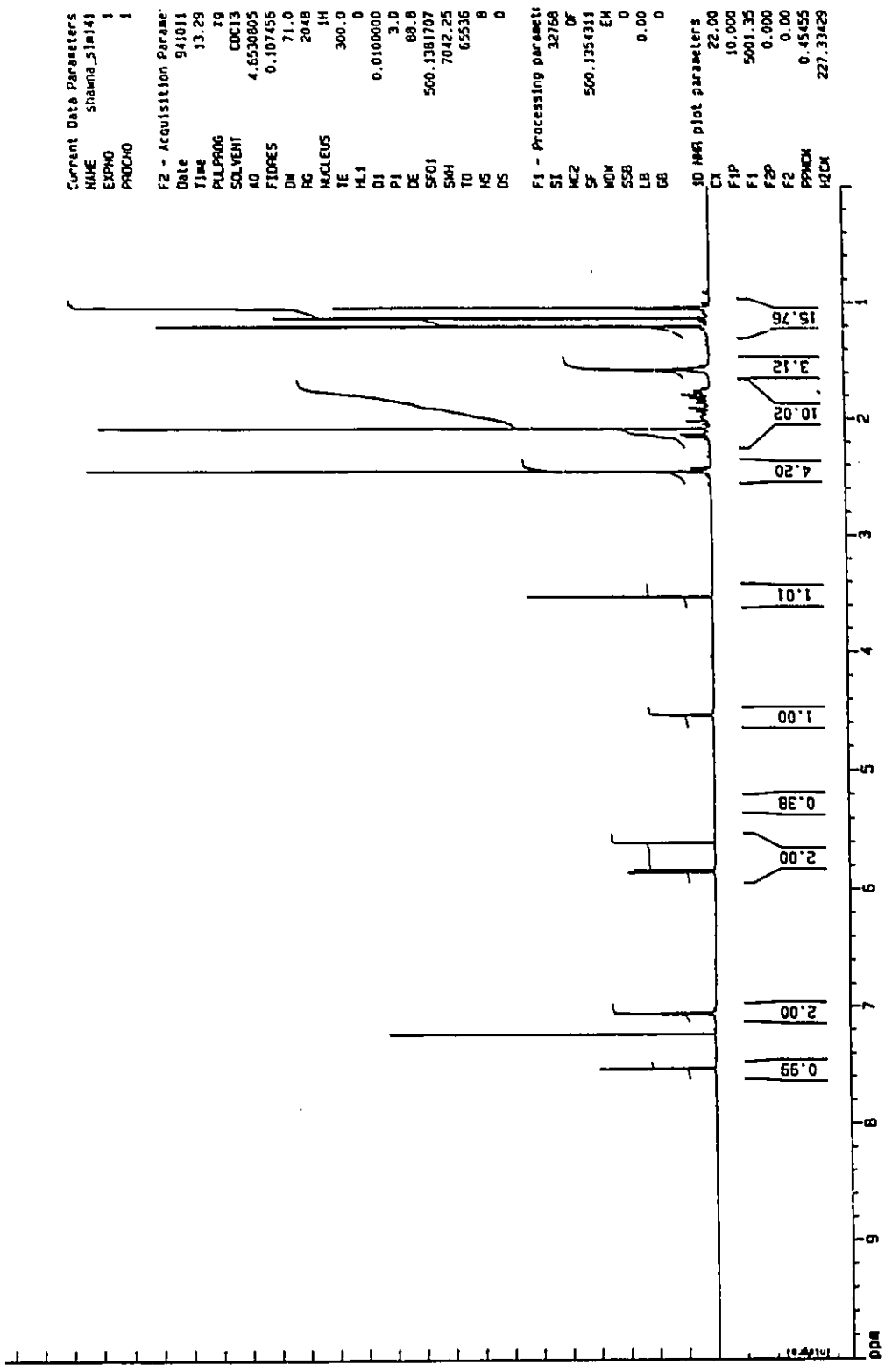


FIGURE 1.2.4.3b: ^{1}H NMR OF 23-ACETYLGEDUNIN

CHAPTER 2

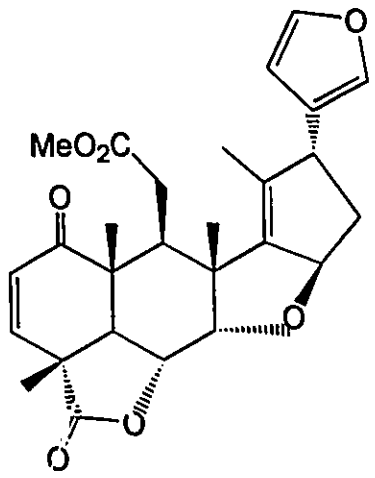
ANTIMALARIAL ACTIVITY OF THE MELIACEAE FAMILY

2.1.0 INTRODUCTION

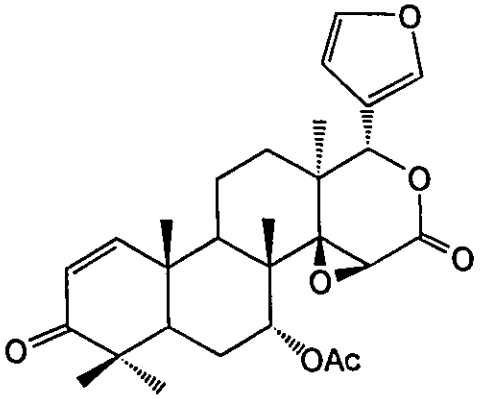
The increase in incidence of malaria in recent years has been attributed to the development of resistance of the malarial protozoa (*Plasmodium falciparum*) to chloroquine and other drugs, and to the development of resistance of the vector mosquitos to presently used insecticides. In tropical America, members of the Meliaceae family, *Cedrela odorata*, *Carapa guianensis* and *Swietenia mahagoni* have been used in traditional medicine for the treatment of fevers,[1] a characteristic symptom of malaria. In West and East Africa a well known member of this family, *Azadirachta indica* , has been known as a reputed folklore antimalarial.[2] The bark and aqueous decoctions of the twigs, stembark and roots have been administered for the treatment of malaria.[3] In Nigeria the leaves of *A. indica* are boiled and the extract is given in uncontrolled doses for the treatment of fevers thought to be due to malaria.[4]

Phytochemical investigations directed at the characterization of the antimalarial principle of the Neem tree, *Azadirachta indica*, were initially conducted by Rochanakij *et al* in 1985.[5] Examination of the leaves of *A. indica* var *siamensis* resulted in the isolation of nimbolide which they associated with the plants antimalarial activity. In 1986 Khalid *et al* screened various limonoids including limonin, nomilin and gedunin for antimalarial activity using *Plasmodium falciparum* in a radioisotope microdilution bioassay to determine the IC_{50} values.[6] For the FCR_{3TC} strain of this plasmodium the IC_{50} values for gedunin, nomilin, and limonin were determined to be respectively, 800 ng/mL, 84100 ng/mL and >100000 ng/mL.

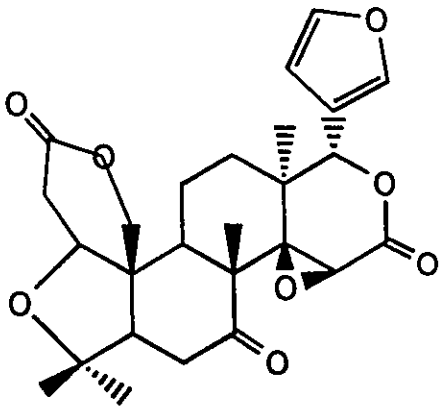
The value for gedunin in this particular screen was roughly equivalent to that of quinine suggesting a promising new antimalarial lead. Three years later, using the same bioassay procedure, they determined that gedunin was the most active antimalarial principle of the bark of *A. indica* and *Melia azadarach*.[7]



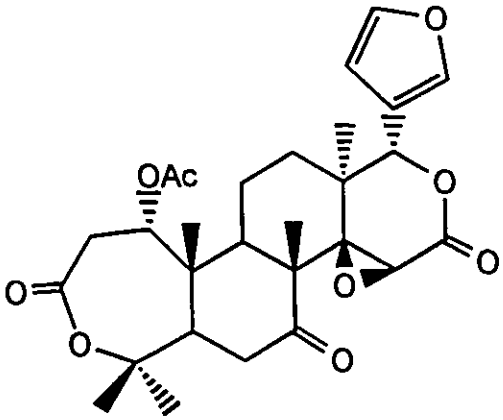
NIMBOLIDE



GEDUNIN



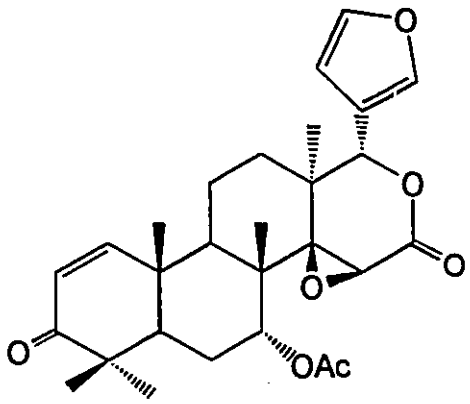
LIMONIN



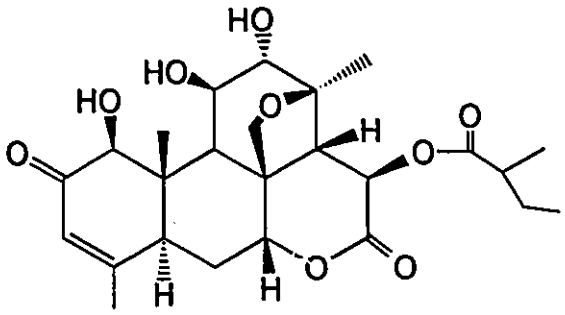
NOMILIN

Both gedunin and nimbolide belong to the class of tetranortriterpenoids referred to as limonoids. They are structurally similar in that both contain α,β-unsaturated ketones in ring A and a furan ring. Research conducted on quassinoids, a class of compounds also displaying high antimalarial activity isolated from the Simaroubaceae family, revealed the importance of an α,β-unsaturated ketone in ring A.[8] The quassinoids are thought to be biosynthetically related to the limonoids.[9]

Comparison of the structure of gedunin to simalikalactone D, a quassinoid with an antimalarial IC_{50} value of 0.9 ng/mL against *P. falciparum* (K1),[10] reveals the presence of α,β-unsaturated ketones in ring A. A more interesting relationship develops when gedunin is treated with alkali resulting in the formation of merogedunin, a quassinoid like compound.[6]

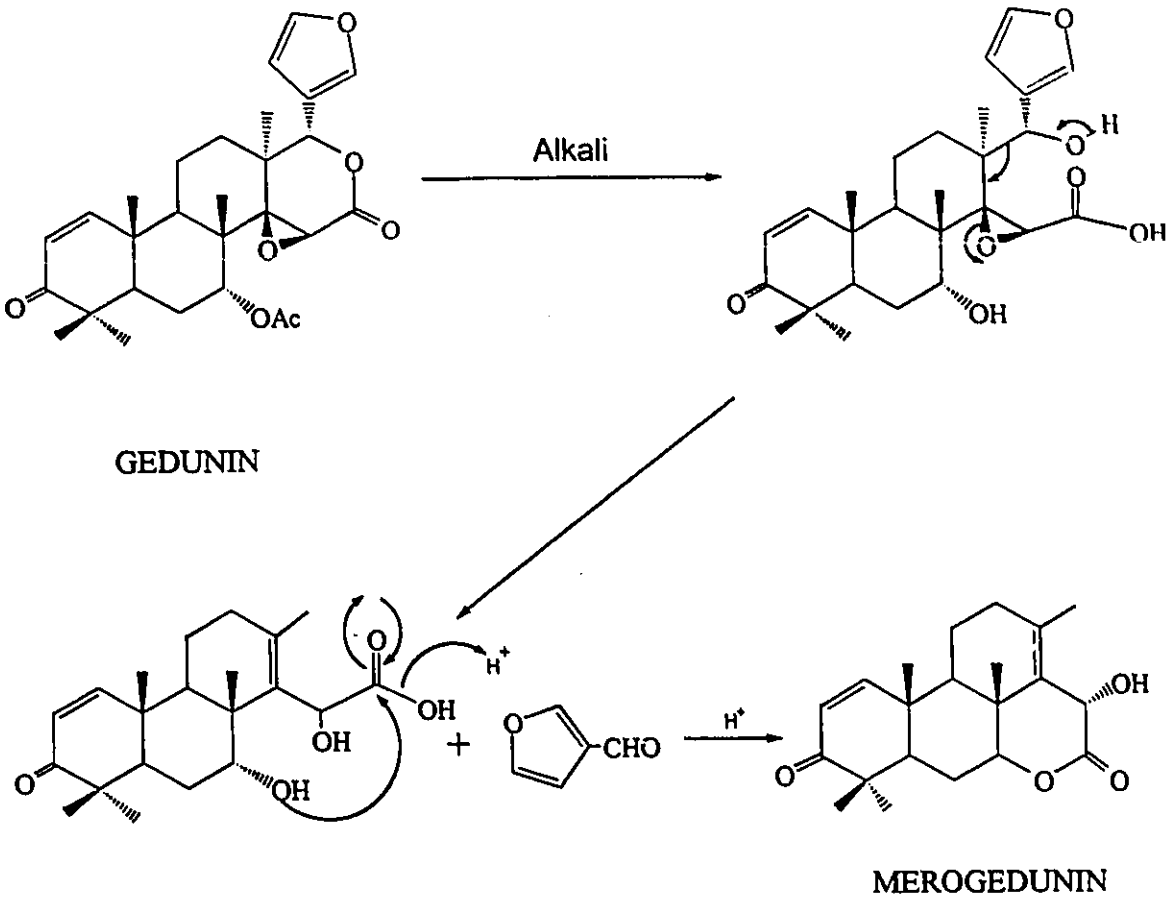


GEDUNIN



SIMALIKALACTONE D

This conversion was initially observed with limonol[11] and in the 1960's became a diagnostic test for assigning an unknown compound to the limonoid class of compounds.[10] Our postulated mechanism for this interconversion involving the 7α-hydroxyl group, the ethylene oxide ring and the D-ring lactone is given in Scheme 2.1.0. Gedunin may be metabolized to this quassinoid-like compound *in situ* and effect its antimalarial activity in this chemical form.[6]



SCHEME 2.1.0: THE INTERCONVERSION OF GEDUNIN TO MEROGEDUNIN

The close resemblance of merogedunin to quassinoids possibly reflects the biosynthetic relationship between limonoids and quassinoids. It has been postulated that the limonoids(C_{26}) are precursors of the quassinoids(C_{20}) through the loss of a C_5 fragment, via the mero-rearrangement described above, and a C-4 methyl group.[10] The families of the Simaroubaceae and Meliaceae are closely related taxonomically as they both belong to the same order of the Rutales.

2.1.1 THE ANTIMALARIAL ACTIVITY OF LIMONOIDS

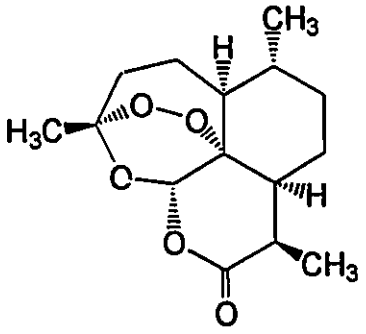
A study which involved the *in vitro* testing of 27 limonoids for antimalarial activity against *P. falciparum* revealed that gedunin was the most active having an IC_{50} value of 720 ng/mL. It was 3 and 20 times less active than chloroquine diphosphate and quinine 2HCl, respectively. Nimbolide had an IC_{50} value of 1740 ng/mL. 1,2-Dihydrogedunin displayed an IC_{50} value of 2630 ng/mL and limonin displayed no antimalarial activity when tested at 50000 ng/mL.[2] Unfortunately there were not enough gedunin related limonoids tested at this time to allow for a more detailed evaluation of the structure/activity relationships of this group of D-seco limonoids. Comparison of gedunin (very active) to 1,2-dihydrogedunin (less active) did however underline the importance of the α,β-unsaturated ketone in ring A.

Cytotoxic evaluation of gedunin demonstrated that it was relatively non-toxic towards GPK cells (keratinocytes from the skin of the ear of black guinea pigs) with an ED_{50} value which was higher than that of quinine. In comparison both nimbolide and 1,2-dihydrogedunin were approximately 20 times more toxic than gedunin. The *in vivo* studies with *P. berghei* revealed that no toxicity was observed for gedunin when it was administered at a level of 90 mg/kg/day.[2]

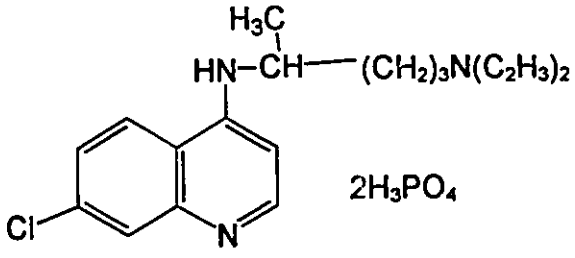
2.2.0 EVALUATION OF ANTIMALARIAL ACTIVITY

2.2.1 ANTIMALARIAL EVALUATION OF MELIACEAE EXTRACTS

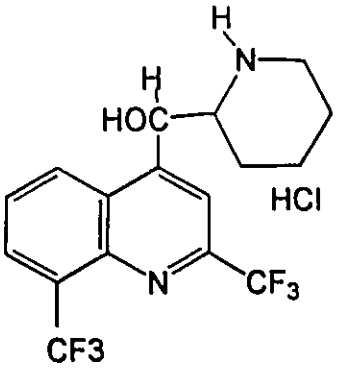
The ethanol extracts that were prepared for the antifeedant bioassay screen (for preparation see Experimental section 3.5.1) against *Ostrinia nubilalis* and *Peridroma saucia* were also submitted for antimalarial activity evaluation against *Plasmodium falciparum* (Clone D6 and W2)(Table 2.2.1). As expected, when testing extracts rather than pure compounds, the activity observed was lower than that of the control drugs artemisinin, chloroquine, mefloquine and quinine. Extracts exhibiting the highest activity against the chloroquine-sensitive strain D6 were the leaves of *Cedrela salvadorensis* and *Chukrasia tabularis*, and the bark and wood respectively of *Trichilia glabra* and *Dysoxylum fraseranum*. The most active extracts against the chloroquine-resistant strain W2 were the leaves of *Chukrasia tabularis* and *Cedrela salvadorensis*, and the wood of *Guarea pyriformis*. The extract of the leaves of *Chukrasia tabularis* was active against both strains of plasmodium and was therefore submitted for cytotoxicity evaluation against a number of human tumor cell lines. The antimalarial activity of this extract was determined to be selective because it was inactive against the tumor cell lines.



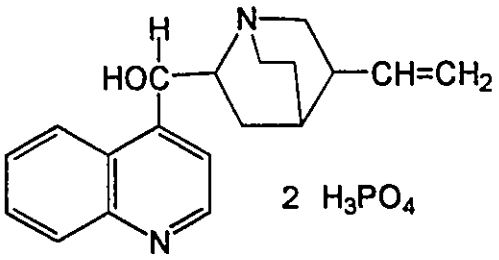
ARTEMISININ



CHLOROQUINE



MEFLOQUINE



QUININE

TABLE 2.2.1: ANTIMALARIAL EVALUATION OF MELIACEAE ETHANOL EXTRACTS

Plant Extract	Plant Part	IC_{50} (ng/mL) Clone D6	IC_{50} (ng/mL) Clone W2
Aphanamixis polystachya Wall & Parker	bark	>20000	>20000
	leaves	>20000	>20000
	wood	16830	>20000
Azadirachta indica A. Juss.	bark	>20000	>20000
	leaves	>20000	>20000
	wood	>20000	>20000
Carapa guianensis Aubl.	bark	>20000	>20000
	leaves	>20000	>20000
	wood	>20000	>20000
Cedrela fissilis Vell.	bark	>20000	>20000
	leaves	>20000	>20000
	wood	>20000	>20000
Cedrela odorata L.	bark	>20000	>20000
	leaves	>20000	>20000
	wood	>20000	>20000

Note: Only results with correlation coefficiants (r) of >0.93 are reported

TABLE 2.2.1:(continued)

Plant Extract	Plant Part	IC_{50} (ng/mL) Clone D6	IC_{50} (ng/mL) Clone W2
Cedrela salvadorensis Standl.	bark fruits leaves leaves	>20000 18650 8330 13800	14950 15250 >20000 13150
Cedrela tonduzii C. DC.	bark/wood	>20000 >20000	>20000 >20000
Cedrela toona (Endlicher)	bark leaves	>20000 >20000 >20000	>20000 >20000 >20000
Chukrasia tabularis A. Juss.	bark leaves wood	>20000 6360 >20000	>20000 3390 >20000
Dysoxylum fraseranum (A. Juss.) Benth.	bark leaves wood	>20000 13080 10180	>20000 >20000 16440
Guarea glabra Vahl.	bark leaves	19710 >20000	>20000 >20000
Guarea pyriformis Pennington	bark leaves wood wood	>20000 >20000 13900 17690	>20000 >20000 13300 15000
Swietenia macrophylla King	bark leaves wood	>20000 >20000 >20000	>20000 >20000 >20000
Swietenia mahagoni (L.) Jacq.	bark leaves wood	>20000 >20000 >20000	>20000 >20000 >20000
Trichilia americana (Sesse & Mocino) Pennington	bark leaves	>20000 >20000	>20000 >20000

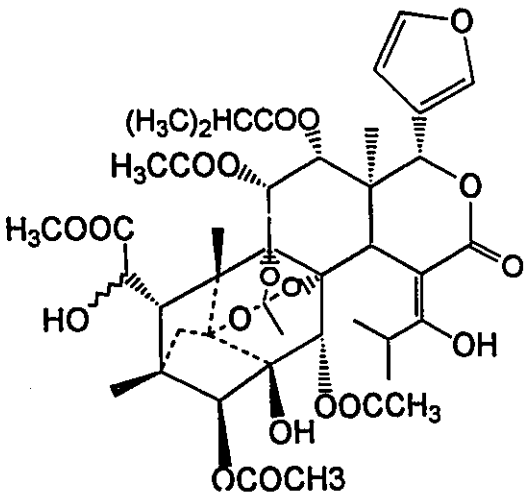
Note: Only results with correlation coefficiants (r) of >0.93 are reported

TABLE 2.2.1:(continued)

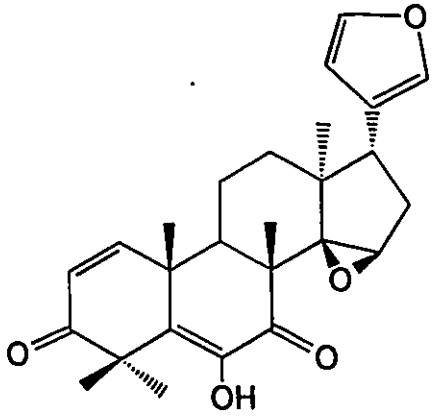
Plant Extract	Plant Part	IC_{50} (ng/mL) Clone D6	IC_{50} (ng/mL) Clone W2
Trichilia glabra L.	bark leaves wood	8750 >20000 >20000	19780 >20000 >20000
Trichilia havanensis Jacq.	bark leaves wood	>20000 >20000 >20000	>20000 >20000 >20000
Trichilia hirta L.	bark leaves wood	>20000 16240 >20000	>20000 >20000 >20000
Trichilia martiana C. DC.	bark leaves	>20000 >20000	>20000 >20000
Trichilia pleeana (A. Juss.) C. DC.	bark leaves wood	>20000 >20000 >20000	>20000 >20000 >20000
Trichilia quadrijuga subsp. cinerascens (C. DC.)Pennington	leaves	>20000	>20000
Trichilia trifolia L.	wood	10290	15200
Artemisinin		2.8	2.9
Chloroquine		1.0 <x <3.1	23.7
Mefloquine		4.4	2.0
Quinine		8.5	56.6

Note: Only results with correlation coefficiants (r) of >0.93 are reported

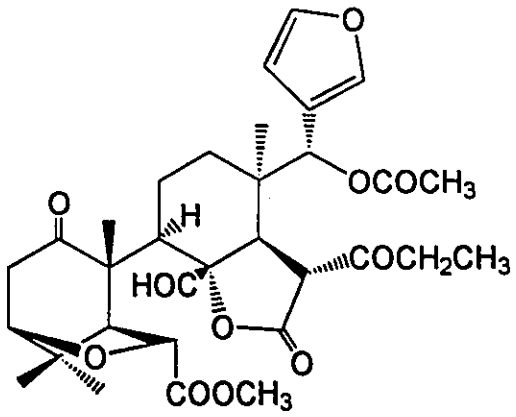
Some of the active species mentioned above have been investigated for limonoid content. The wood and roots of *Chukrasia tabularis* contain limonoids chukrasines A-E and cedrelone.[12,13] Investigations of the genus *Dysoxylum* has resulted in the isolation of limonoids but no work has been reported on the constituents of *D. fraseranum*.[14] Recently phytochemical investigations of the stem bark of *Cedrela salvadorensis* resulted in the isolation of cedranolide.[15] The remaining species: *Trichilia glabra*, and *Guarea pyriformis*, have not yet been investigated but other species in this genera are known to contain limonoids. From this it is quite plausible to suggest that the limonoid constituents could be responsible for the antimalarial activity exhibited. At this point no further fractionation of the extracts has been carried out but the extract of the leaves of *Chukrasia tabularis* certainly warrants further investigation.



CHUKRASINE A



CEDRALONE

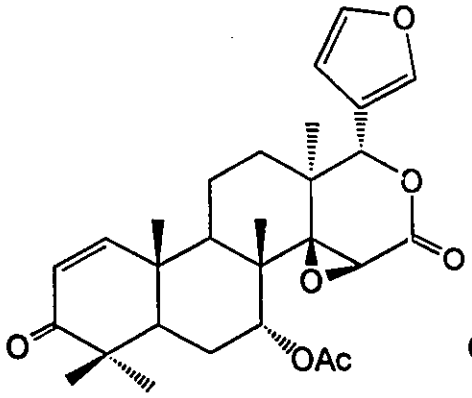


CEDRANOLIDE

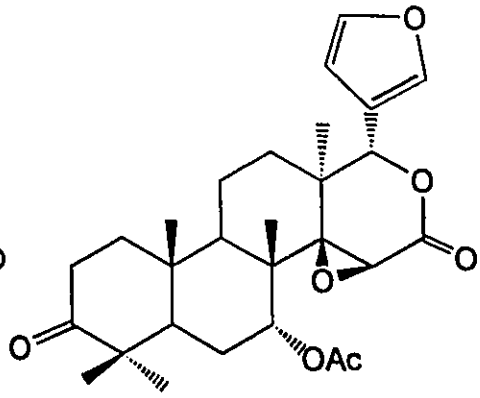
An unexpected result was the lack of activity of the bark, leaf and wood extracts of *Azadirachta indica* and *Cedrela odorata*. These extracts were expected to exhibit some activity because gedunin or other D-seco limonoids have been isolated from both species.[16] The same lack of activity was also observed by Bray *et al*[2] when ethanol extracts of the leaves of both these species were tested *in vitro* against *Plasmodium falciparum* (multi-drug resistant strain (K1)). A possible explanation for this observation is that the limonoid content may vary geographically and/or seasonally. Our collection was performed in Costa Rica whereas Bray et al's collection took place in Ghana.

2.2.2 ANTIMALARIAL STRUCTURE/ACTIVITY RELATIONSHIPS OF GEDUNIN DERIVATIVES

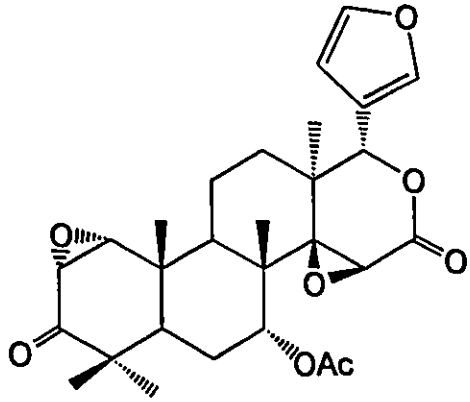
Investigation of the antimalarial activity of the gedunin derivatives (Figure 2.2.2) did not yield a compound with a higher activity than gedunin. However, it did provide for some insight into functionalities required for activity and structure/activity relationships for antimalarial activity of D-seco limonoids. Initially the parasite viability at 10000 ng/mL of



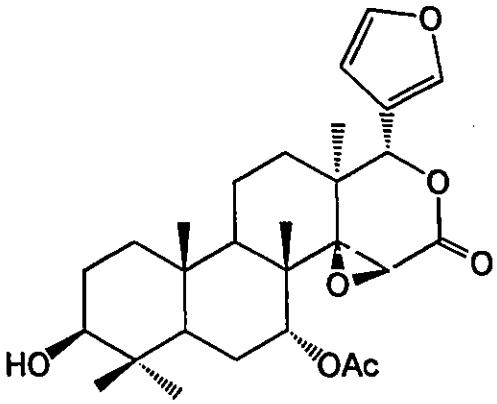
GEDUNIN



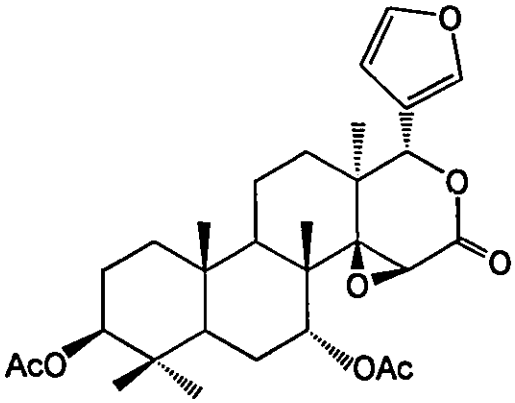
1,2-DIHYDROGEDUNIN



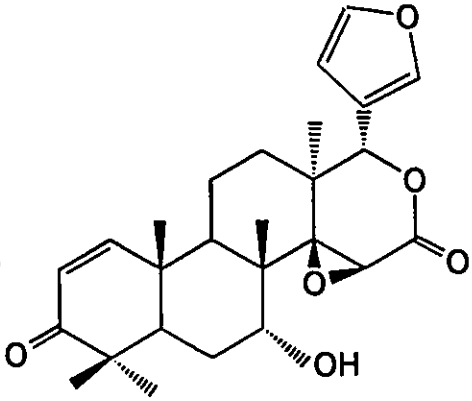
1,2-EPOXYGEDUNIN



1,2-DIHYDRO-3β-GEDUNOL

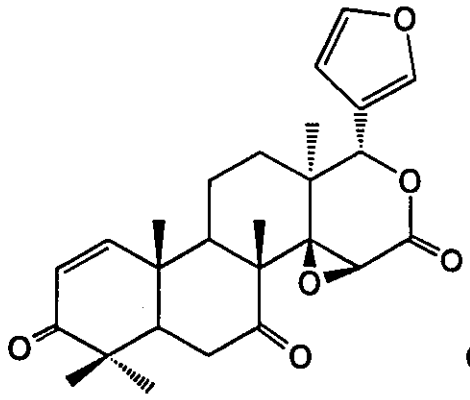


3β-ACETOXY-1,2-DIHYDROGEDUNIN

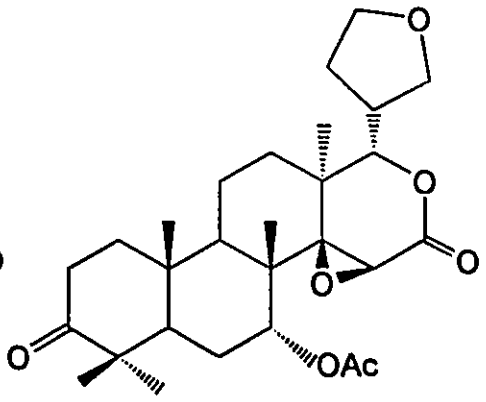


7-DEACETYLGEDUNIN

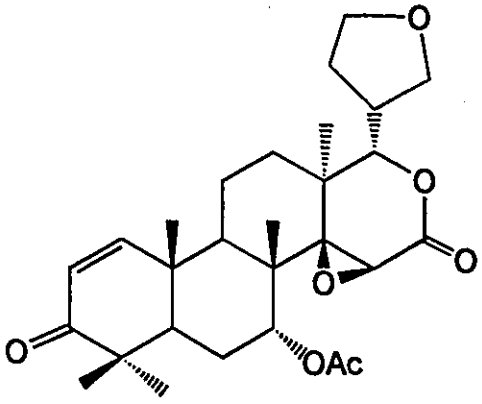
FIGURE 2.2.2: GEDUNIN DERIVATIVES



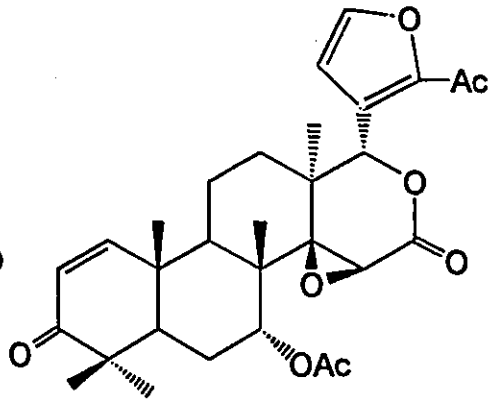
7-KETOGEDUNIN



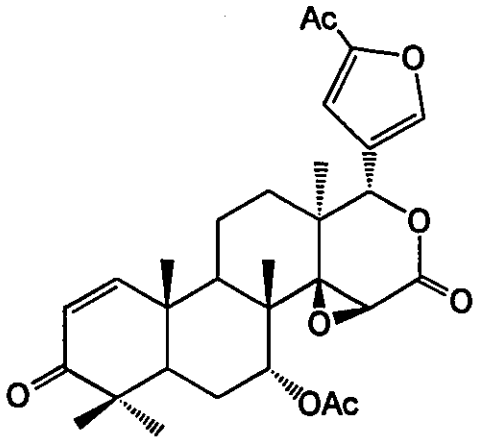
HEXAHYDROGEDUNIN



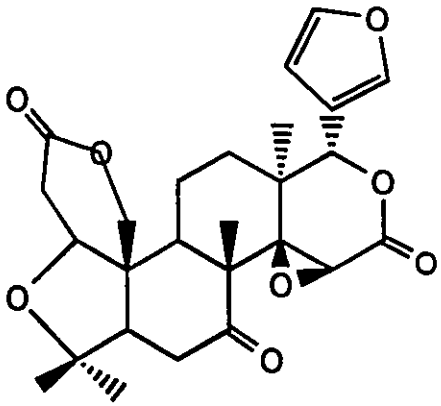
TETRAHYDROGEDUNIN



21-ACETYLGEDUNIN

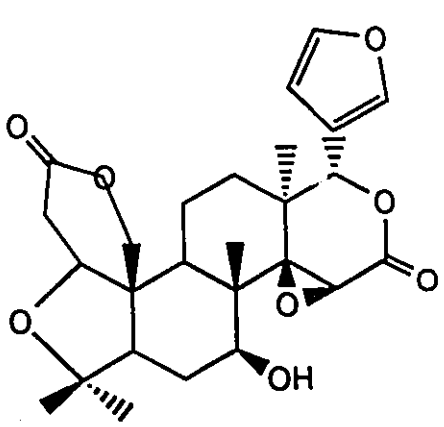


23-ACETYLGEDUNIN

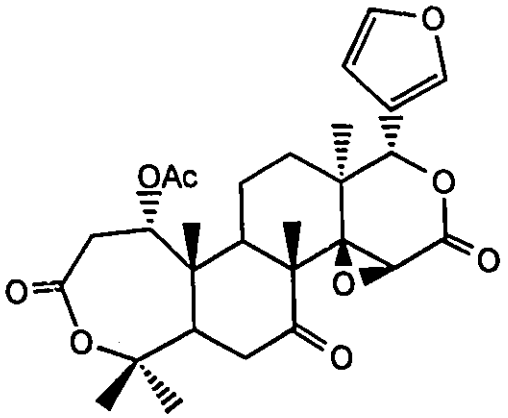


LIMONIN

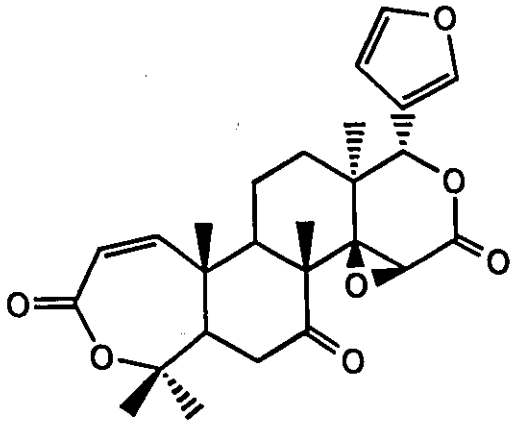
FIGURE 2.2.2 (Continued)



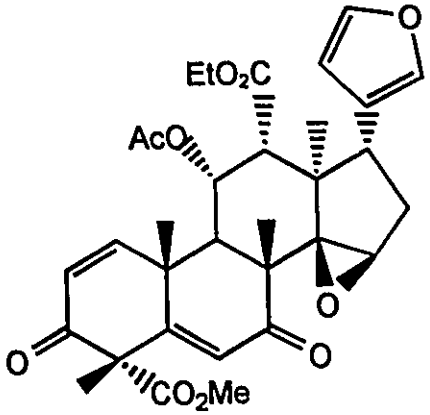
EPILIMONOL



NOMILIN



OBACUNONE



HIRTIN

FIGURE 2.2.2 (Continued)

each compound (Table 2.2.2a), expressed as % of zero drug control, was determined. Only compounds reducing parasite viabilities below 75% were submitted for IC_{50} determinations (Table 2.2.2b). Because the IC_{50} values involve the testing of these compounds at more than one concentration they are more statistically valid for comparison than the parasite viabilities

which are determined at only one concentration. The structure/activity relationships will therefore be discussed in terms of IC_{50} values(Table 2.2.2b).

TABLE 2.2.2a: PARASITE VIABILITIES OF GEDUNIN ANALOGS

COMPOUNDS	PARASITE VIABILITY *Plasmodium falciparum* (expressed as % of zero drug control)	
	Clone D6	Clone W2
Gedunin	6.00	1.40
1,2-Dihydrogedunin	52.00	42.50
1,2-Epoxygedunin	12.40	8.00
1,2-Dihydro-3β-gedunol	52.20	24.70
7-Deacetylgedunin	8.30	4.50
7-Ketogedunin	78.50	72.60
Tetrahydrogedunin	7.70	2.30
21-Acetylgedunin	7.20	1.60
23-Acetylgedunin	6.80	2.50
Hexahydrogedunin	75.00	67.80
Limonin	98.30	87.10
Epilimonol	109.70	95.30
Obacunone	93.40	80.70
Nomilin	104.30	81.20
Hirtin	4.90	0.92

Clone D6= chloroquine-sensitive
Clone W2= chloroquine-resistant
Parasite viabilities were determined at a concentration of 10000 ng/mL

TABLE 2.2.2b: IC_{50} VALUES OF GEDUNIN ANALOGS

COMPOUNDS	*Plasmodium falciparum* IC_{50} (ng/mL)		Relative Toxicity to Gedunin	
	Clone D6	Clone W2	Clone D6	Clone W2
Gedunin	39	20	100	100
1,2-Dihydrogedunin	>10000	840	<0.39	2.38
1,2-Epoxygedunin	2580	980	0.93	2.04
1,2-Dihydro-3β-gedunol	4210	2440	0.93	0.82
7-Deacetylgedunin	2610	1280	1.50	1.56
7-Ketogedunin	>10000	>10000	<0.39	<0.0005
Tetrahydrogedunin	2500	900	1.56	2.22
21-Acetylgedunin	133	39	29.38	51.28
23-Acetylgedunin	832	156	4.69	12.82
Hexahydrogedunin	>10000	2130	<0.39	0.94
Limonin	>10000	>10000	<0.39	<0.0005
Epilimonol	>10000	>10000	<0.39	<0.0005
Obacunone	>10000	>10000	<0.39	<0.0005
Nomilin	>10000	>10000	<0.39	<0.0005
Hirtin	173	96	22.58	20.83
Chloroquine	1.3	29.5	3004.8	67.80
Quinine	14.8	34.9	263.9	57.31
Mefloquine	7.5	1.4	520.8	1428.6
Artemisinin	1.8	0.5	2170.1	4000.0

Relative toxicity: $1/IC_{50}$ normalized to gedunin equaling 100.

Not surprisingly the changes to the α,β-unsaturated ketone moiety in ring A of gedunin revealed its importance for antimalarial activity. When the double bond was reduced, as in 1,2-dihydrogedunin, a large drop in activity was observed in both strains but especially so in the chloroquine sensitive strain (D6). Epoxidation of the 1,2 double bond also resulted in loss of activity. Further reduction of this moiety is tested with the evaluation of the results of 1,2-dihydro-3β-gedunol. Reduction of both the double bond and the ketone, to a saturated

beta alcohol, resulted in substantial losses of activity against both strains, again being more dramatic in strain D6.

The next feature of gedunin to be investigated was the 7-acetate of ring B. This was examined through the testing of 7-deacetylgedunin and 7-ketogedunin. Again losses of activity are observed for both strains however it seems that the presence of a 7-keto functionality eliminates all the antimalarial activity of gedunin. Testing of 7-deacetylgedunin resulted in a loss of activity but not as dramatic as with the 7-keto derivative. The difference in activity between the 7-acetoxy and 7-hydroxy analogs, in both strains, may be due to differences in bioavailability. Limonin, obacunone and nomilin also possess 7-keto functions and similarly exhibited no antimalarial activity. The absence of an OH group or a potential OH group at C-7 could be preventing the interconversion of these limonoids, *in situ*, to their mero-analogs and therefore rendering them inactive. The hypothesis that the mero-interconversion products are related to the antimalarial activity of the limonoids needs to be further investigated. This could be initiated by testing merogedunin for antimalarial activity.

The relatively high level of activity exhibited by hirtin was not anticipated because it did not contain an epoxylactone in ring D and an 7-OH function in ring B. Further investigations of the potential antimalarial activity of related tetracarbocyclic limonoids needs to be addressed in the future.

Alterations to the furan moiety were also investigated and even though losses of activity were observed, the magnitude was much less than what was seen for most of the other derivatives. This is seen especially with 21-acetylgedunin and 23-acetylgedunin. Tetrahydrogedunin exhibited a loss in activity comparable to that observed for 1,2-

epoxygedunin. Comparing 1,2-dihydrogedunin and hexahydrogedunin reveals that the reduction of the double bonds in the furan result in only a marginal increase in loss of activity in strain W2. From the data on the alterations to the furan moiety of gedunin it seems that this section of the molecule is less important for antimalarial activity than the α,β unsaturated ketone in ring A and the 7-acetate function in ring B.

Most of the analogs tested, especially gedunin and hirtin, were found to be more active against the chloroquine-resistant strain W2. For this reason it is believed that these classes of limonoids warrant further investigations as a potential source of new antimalarial agents. The IC_{50} value of gedunin is lower than that of quinine and chloroquine for the chloroquine-resistant strain of *Plasmodium falciparum*. For the chloroquine-sensitive strain it is 2 and 21 times less active than quinine and artemisinin, respectively.

Finally, the IC_{50} values that were determined for gedunin and 1,2-dihydrogedunin by Bray *et al* [2] agreed only qualitatively with those obtained in this study. The reason for this is that the strain of *Plasmodium falciparum* used by Bray *et al* was K1 and therefore different than those used in the present study.

The determination of cytotoxicity of a potential antimalarial drug is an important parameter to determine for the development of an effective and nontoxic drug. It allows for the differentiation between general and selective toxins. The results of the cytotoxicity screening of the gedunin analogues against KB cells, human epidermoid carcinoma cells, are presented in Table 2.2.2c. The KB screen of the compounds tested at 20000 ng/mL and presented as % viability, was conducted to determine which compounds were cytotoxic. Generally, compounds having % viability values lower than 35 % were

submitted for IC_{50} determinations. Hirtin, gedunin, 21-acetylgedunin and 23-acetylgedunin all exhibited some degree of cytotoxicity. The cytotoxicity IC_{50} values for chloroquine (17400 ng/mL), quinine (>20000 ng/mL), mefloquine (3500 ng/mL), and artemisinin (>20000 ng/mL) for the KB cell line were located in the literature.[17] The cytotoxicity for hirtin, a limonoid belonging to the havanensis group, was much higher than the other gedunin analogues and drugs which are currently used to treat malaria. Hirtin therefore appears to be non selective in its mode of antimalarial activity and exhibits the qualities of a general toxin. Further insight into hirtin's cytotoxicity could be gained through the screening of other related limonoids such as cedrelone and the trichilins.

Surprisingly an *in vivo* study of gedunin administered orally or subcutaneously in rats infected with *P. berghei* , another human malaria parasite, in a four day test resulted in no inhibition of parasitaemia.[2] The differences between rat and human systems, and differences between plasmodium species could possibly account for this observation but problems related to reaching the target site or metabolism are more likely causes of differences between the *in vitro* and *in vivo* studies. The investigation of the bioavailability and metabolism of the potential antimalarial agent gedunin, *in vivo* in animals, could also provide avenues for increasing the activity of this and other limonoids.

TABLE 2.2.2c: THE CYTOTOXICITY OF THE GEDUNIN ANALOGS

COMPOUNDS	KB screen	KB
	% Viability	IC_{50} (ng/mL)
Gedunin	32.00	2300
1,2-Dihydrogedunin	71.00	-
1,2-Epoxygedunin	81.00	-
1,2-Dihydro-3β-gedunol	69.00	-
7-Deacetylgedunin	39.00	-
7-Ketogedunin	99.00	-
Tetrahydrogedunin	81.00	-
21-Acetylgedunin	31.00	9400
23-Acetylgedunin	38.00	10900
Hexahydrogedunin	99.00	-
Limonin	NT	-
Epilimonol	NT	-
Obacunone	NT	-
Nomilin	NT	-
Hirtin	4.90	500

NT= not toxic at <20000 ng/mL

- = not toxic at 20000 ng/mL so IC_{50} was not determined.

2.3.0 EXPERIMENTAL

2.3.1 ANTIMALARIAL MICRODILUTION RADIOISOTOPE BIOASSAY

The first step of the bioassay required the addition of cultured *Plasmodium falciparum*, in a red blood cell suspension, to microtiter plates (96 wells per plate) which contained serial dilutions of the gedunin derivatives (drug) to be screened. After 24 h of incubation at 37 °C and an atmosphere of 5% O_2, 5% CO_2, and 90% N_2, [^{3}H]-hypoxanthine was added to each well. Another incubation period of 18 h ensued to allow for the incorporation of the radioisotope into the nucleic acids of the parasites that had survived the drug treatment. Once the incubation was complete, the contents of each well was aspirated onto a glass fiber filter using a semiautomated harvesting device. Each filter was placed into a scintillation vial containing scintillation cocktail and the radioactivity was measured by a scintillation counter.

The above procedure was conducted using two *Plasmodium falciparum* clones: clone D6 was a chloroquine sensitive strain from CDC Sierra Leone whereas clone W2 was a chloroquine-resistant strain obtained originally from CDC Indochina III. The initial screening involved the determination of the parasite viability, expressed as percent of zero-drug control, to each drug tested at 10000 ng/mL. The IC_{50} values were determined only for the drugs which exhibited a percent parasite viability below 75% for one of the clones.

The concentration required to inhibit [^{3}H]-hypoxanthine incorporation by 50 % (IC_{50} value) was determined by linear regression analysis of the dose response curves generated by the serial dilution series of each drug. This brief outline of the antimalarial screening bioassay was originally developed by Desjardins *et al* [18] and has become one of the

most widely used as a test system for the evaluation of potential antimalarial compounds.[17,19,20]

The antimalarial microdilution radioisotope bioassays were conducted in collaboration with Dr. C. Angerhofer in Dr. J.M. Pezzuto's laboratory at the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois.

2.3.2 CYTOTOXICITY ASSAY

The cytotoxicity of the gedunin derivatives was also conducted in Dr. J.M. Pezzuto's laboratory and was determined using the immortal cell line KB. Details of this procedure are given in the paper by K. Likhitwitayawuid *et al.*[20]

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CHAPTER 3

INVESTIGATION OF THE BIOACTIVITY OF TRITERPENOIDS OF THE RUTALES

3.1.0 INTRODUCTION

Besides shaping the natural interactions of plants and insects, phytochemicals from tropical plants include substances that are potentially useful as insect control agents or resistance factors in crop plants. In the past, many insect control agents have been developed from phytochemical leads, obtained largely from tropical plants.[1] Most of the materials developed have been neurotoxins, since these act rapidly and are highly efficacious. However, recently published figures[2] estimate that 504 species of arthropods are now resistant to conventional insecticides/acaricides. Many display multiple resistance, i.e. to more than one insecticide, and at least 48 species are resistant to the synthetic pyrethroids.

The neurotoxic mode of action of almost all conventional insecticides has promoted the rapid development of cross-resistance in insect populations. Research into insecticidal phytochemicals has emphasized non-neurotoxic modes-of-action[3,4] such as antifeedant action, inhibition of molting, growth reduction, loss of fecundity, respiratory inhibition, phototoxicity, etc. These modes-of-action could reduce the risk of cross-resistance in insect populations presently resistant to neurotoxins, as has been demonstrated with plant-derived phototoxins that are effective control agents for malathion-resistant *Culex* larvae.[5] Recent use of standardized plant extracts (botanical insecticides) containing a mixture of

active phytochemicals should reduce the rate of evolution of conventional resistance compared to the selection pressure exerted by a single pure toxin.

The investigation of phytochemicals as useful insect control agents via a botanical route has recently received renewed interest with the development and registration of insecticides based on neem, *Azadirachta indica*.[6-9] Some botanicals may produce "lead" phytochemicals similar to synthetic insecticides, with comparable overall effectiveness, but botanical insecticides have distinctly different properties requiring different research and development paths. Environmental awareness and "green consumerism" has created a favorable environment for the development of botanicals because they are generally non-persistent, and their natural origin and those with softer modes-of-action are perceived favorably by the public.

Some of the disadvantages that must be addressed with botanicals include: location of the best source of material, quality control of standardized extracts, the relatively high instabilities of botanicals which may require more frequent applications compared to synthetics and unfavorable market registration conditions.

3.1.1 THE MELIACEAE AS A NEW SOURCE OF BOTANICAL INSECTICIDES

Because botanicals are produced by living plants, supply can be an important issue. For example, neem is very abundant in India but is far less so in the American tropics. One of the objectives of this research was to determine alternative botanicals from the neotropical (Tropical America) flora. One potential source is the large amount of sawdust and

bark waste containing insecticidal limonoids which can be obtained from tropical timber species of the Rutales (especially Meliaceae) from Central America.

Seed extracts of several Meliaceae species[10,11] have been reported to have potent growth-reducing activity to insects, but we have concentrated our efforts on the more accessible wood, bark and leaves. A screen of methanolic leaf extracts of 21 species of the Meliaceae for feeding deterrency and growth inhibition against *Peridroma saucia* revealed that *Aglaia odorata* and *Turreae holstii* were as active as *Melia azedirachi* and *Azadirachta indica*.[12] *Trichilia hirta* was found to be five times less active than *A. indica* while *Cedrela odorata* and *Guarea glabra* were both found to be approximately twenty times less active.

3.1.2 LIMONOIDS: A POTENTIAL SOURCE OF INSECTICIDAL COMPOUNDS

Reports of the marked insect antifeedant and growth regulating activity of azadirachtin, a C-seco limonoid isolated from *Azadirachta indica*, has led to an increase in interest in the limonoids as a class of potential insecticidal and insect growth regulating compounds[13] A recent review by Champagne *et al* summarized the effects of seventy eight naturally occurring limonoids on insect feeding, growth, and development.[14]

An understanding of the structure-activity relationships of various limonoids was difficult to determine since many of the investigators used different bioassay species and/or larval stages. The data obtained via these different bioassay approaches could not be readily compared. Qualitatively the tetracarbocyclic limonoids were found to be less active than the most active C-seco limonoids followed next by the D-seco limonoids. Such

comparisons are tentative because of the limited availability of some classes of limonoids (i.e. D-seco limonoids) and because some were tested against only a few insects. In conclusion, the potential of the over 300 limonoids which have been isolated to date as insect control agents, is still relatively unexplored.[14]

3.1.3 THE TEST INSECTS

3.1.3.1 THE EUROPEAN CORN BORER (*Ostrinia nubilalis* (Hubner))

Lepidoptera

Presently the European corn borer(ECB) can be found in Africa, Europe, Asia, the United States and most Canadian provinces. This highly polyphagous species attacks important economic crops such as sweet pepper, beet, bean, potato, oat, soyabean and wheat, however it has a preference for maize (corn).[15,16] It is thought to have been introduced to North America from Europe in 1917, shortly before Canadian farmers experienced the 'corn borer years' from 1923-1949. During this period corn yields dropped by fifty percent. An epidemic infestation of corn in Quebec was reported in 1973.[17] In New York state the ECB have been reported to damage up to 30-42% of the spring wheat crop.

The lifecycle of the ECB begins with the deposition of egg masses on the underside of the corn leaves, by the female moth, which hatch four or five days later. The number of generations of ECB's per year varies from one to four depending on latitude and location.[15,16] The lifecycle includes five larval instars or stages, the first two instars feed on the corn leaves with subsequent instars entering the corn stalk. At this most damaging stage to the plant, the ECB's feed and excavate tunnels in the stalk which weakens the plant and

possibly causes it to fall over. With favorable weather and photoperiod conditions the larvae pupate and emerge as adults approximately a week later.[18] When winter is approaching the mature larvae go into diapause inside the corn stalks and emerge sometime in May or June.[15]

3.1.3.2 THE VARIEGATED CUTWORM (*Peridroma saucia* (Hubner))

Lepidoptera

Like the European corn borer, the variegated cutworm is thought to have been introduced from Europe into Canada before 1905. It is a sporadic pest of tomato, tobacco, pepper, cabbage, cauliflower, lettuce, turnips, carrot, onion, bean, pea, corn, celery, spinach, cucumber, rhubarb, alfalfa, oats and wheat crops in Canada. In its larval stage it causes damage to crops by actually cutting off the seedlings. However most of crop damage is ensues when the larvae eat the foliage and/or bore into the marketable parts of the plant which mainly occurs from June to August. Sometimes a second generation of cutworm is observed during the growing season. This pest feeds at night and hides under the soil surface during the day.

3.1.4 THE TEST FUNGI: *FUSARIUM GRAMINEARUM, F. MONILIFORME*, AND *F. SUBGLUTINANS*

Fusarium causes vascular wilt, a plant disease appearing as a wilting, browning and dying of the leaves and shoots of plants followed by the death of the plant. This genus infects annual vegetable crops, flowers, herbaceous perennial ornaments and weeds.[20,21] *Fusarium* is a soil inhabitant which infects its host by penetrating directly or through wounds

in the roots. The fungus then spreads through the plant releasing spores and toxins which are carried to other parts of the plant.

Fusarium produces toxins in corn and other grains which are frequently in stored crops. These toxins cause mycotoxicoses when fed to livestock or humans. Swine are the most sensitive animals to these toxins. *F. moniliforme*, primarily in molded corn, produces zearalenone as its toxin[21] while *F. graminearum* (*Gibberella zeae*) produces deoxynivalenol or vomitoxin.[20] Wheat for human consumption must be essentially free of mycotoxins.

Infection of crops with various species of *Fusarium* can therefore result in large losses to farmers both due to infestations in the fields (decreased harvest) and in storage. These factors if sufficiently severe may make crops unsuitable for consumption.

3.1.5 THE BIOACTIVITY OF GEDUNIN

Some limonoids which are structurally far less complex than azadirachtin have potentially useful bioactivity. A study on the effect of ten limonoids isolated from the Meliaceae on *Ostrinia nubilalis* larvae showed that the tetracarbocyclic limonoids cedrelone and anthothecol, and the D-seco-limonoid nomilin had the highest antifeedant at 50 μg/g fresh weight of leaves.[22] Gedunin exhibited antifeedant activity at 500 μg/g fresh weight of leaves. All of the above mentioned limonoids caused mortality and growth reduction in larvae by 24 days when they were included in neonate life cycle studies at a concentration of 50 ppm. Gedunin caused a mortality of 70-80% while nomilin caused less than 50%.

An earlier study revealed that gedunin possessed growth-inhibitory activity against cotton lepidopteran pests *Pectinophora gossypiella*, *Spodoptera frugiperda*, and

Heliothis zea.[1] Nomilin, obacunone and especially limonin displayed lower bioactivity in these three test species. 7-Ketogedunin, obtained by deacetylation of gedunin followed by oxidation, resulted in a dramatic loss of activity in both *S. frugiperda* and *H. zea* while the activity towards *P. gossypiella* remained roughly the same. 7-Deacetylgedunin was slightly more active towards *P. gossypiella* but less active towards the other two species tested.

Gedunin did not exhibit very high antifeedant activity against the Mexican bean beetle, *Epilachna varivestis*. In contrast khivorin, a D-seco limonoid with a saturated A-ring, exhibited a twenty fold increase in activity relative to gedunin.[23]

It is clear from these studies that the activity is variable but little is known in a systematic way about the structure-activity relationships for antifeedant and growth reducing activity of gedunin and related compounds. The wide range of activity indicates that the gedunin skeleton is a promising source of bioactivity requiring further investigation.

One approach to exploiting the lead structure of gedunin has been to synthesize and investigate the activity of fragments related to gedunin and limonin.[24] Research conducted by Bentley et al on the structure-activity relationships of the citrus limonoid limonin as an antifeedant against the Colorado potato beetle larvae revealed that the furan and epoxide function in ring D were critical for activity.[25] This observation led to the design and synthesis of the model antifeedant compounds in Figure 3.1.5 which were screened for activity against *L. decemlineata* larvae in a no-choice assay.[26] These compounds exhibited bioactivity very similar to that of limonin. In 1991 a number of compounds based on this model were prepared by Mateos et al.[27] Unfortunately the antifeedant activity of these compounds has not been published in the literature to date.

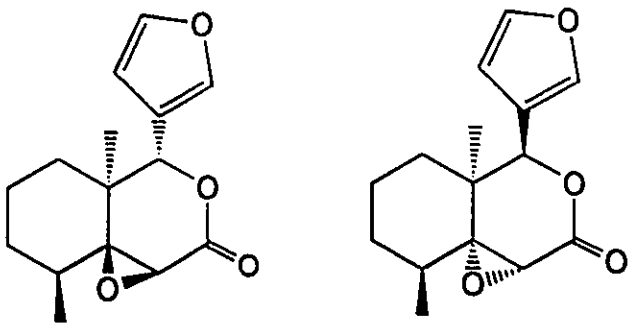


FIGURE 3.1.5: MODEL ANTIFEEDANT COMPOUNDS

3.2.0 RESULTS AND DISCUSSION

3.2.1 ANTIFEEDANT SCREENING RESULTS

The screening of lyophilized ethanolic extracts of 25 species of Meliaceae, including the common indigenous tropical American genera *Cedrela*, *Guarea*, *Carapa*, *Swietenia* and *Trichilia* against two robust economic insects, second instar European corn borer (*Ostrinia nubilalis*) and neonate variegated cutworm (*Peridroma saucia*) are presented in Table 3.2.1. It has been previously demonstrated that corn borer larvae display little feeding aversion to plant chemicals in their diet, when fed soft artificial diets because their chemosensilla become plugged with diet.[28] Thus, the results for the corn borer represent primarily physiological growth reduction only, while the data for the variegated cutworm result from feeding deterrency and/or physiological growth reduction.

The data suggest that a majority of the extracts from the plants studied in the Meliaceae inhibit growth significantly; some are more active than neem leaf extracts. *Swietenia mahogoni* bark, *Trichilia glabra* bark, *T. hirta* leaves, *T. americana* bark, *T. trifolia* wood, *T. pleana* wood and *Azadirachta indica* wood showed potent activity against the cutworm. *Ruptiliocarpon caracolito* bark, *Cedrela odorata* leaves, *Aphanamixis polystachys* wood, *T. glabra* wood and bark, and *T. pleana* bark showed good activity against the corn borer larvae.

Comparison of the activity of the extracts suggests that the wood and bark are generally more chemically defended than the leaves. Loss of foliage is less expensive than replacement of structural materials such as wood. Of the indigenous Central American genera of Meliaceae, foliage extracts of *Cedrela odorata*, *C. tonduzii*, *Trichilia americana*, *T.*

glabra, *Swietenia macrophylla* and *S. mahogoni* showed significant growth reducing activity in the corn borer, but none of the leaf extracts of the *Guarea* spp. had significant activity. Our bioassay results mirror to observations in the field, where *Cedrela* spp. and *Trichilia* spp. generally have leaves with little insect damage while those of the *Guarea's* are often found with considerable feeding damage. *Guarea* leaves are much thicker and tougher than other genera. As suggested by Champagne[14], *Guarea* may use structural defenses because its leaves are long lived, while the other genera rely on chemical defenses. In the literature, A,D-seco limonoids, such as obacunone, found in *Guarea* spp. are considered low in biological activity against many insects. Apo-euphol limonoids such as trichilins found in *Trichilia* spp. are known to be among the most active limonoids towards insects, next to the C-seco limonoids which include azadirachtin.

TABLE 3.2.1. Effect of Meliaceae extracts administered in diet on the growth and development of second instar European corn borer, *Ostrinia nubilalis* and neonate variegated cutworm, *Peridroma saucia.*

Plant	Tissues	*Ostrinia nubilalis* (Concentration in diet = 0.4% fresh weight, n=15) RGR[@] (% of control)	*Peridroma saucia* (Concentration in diet= 0.2% fresh weight, n=20) Larval Weight (% of control)	Collection Site
Aphanamixis polystachya Wall & Parker	bark leaves wood	70.2 a-h* 83.6 a-f 35.5 h-l*	NT 80.5 k-o 89.4 i-l	Fairchild Tropical Garden Miami, Florida.
Azadirachta indica (neem) A. Juss.	bark leaves	73.8 a-h* 85.1 a-f	10.4 x-z* #*	Fairchild Tropical Garden Miami, Florida.
Carapa guianensis Aubl.	bark leaves wood	70.8 a-h* 62.2 a-i* 93.7 a-c	NT NT NT	Puerto Viejo, Limon, Costa Rica.
Cedrela fissilis Vell.	bark leaves wood	68.8 a-h 90.8 a-c 79.0 a-g	35.4 t-w* 32.5 u-w* 85.5 j-n	Fairchild Tropical Garden Miami, Florida.

TABLE 3.2.1 (continued)

Plant	Tissues	*Ostrinia nubilalis* (Concentration in diet = 0.4% fresh weight, n=15) RGR[a] (% of control)	*Peridroma saucia* (Concentration in diet= 0.2% fresh weight, n=20) Larval Weight (% of control)	Collection Site
Cedrela odorata L.	bark leaves wood	63.6 a-i* 74.6 a-h* 43.8 f-l*	96.0 j-k 87.9 i-l 63.6 o-r*	Carara, Costa Rica.
Cedrela salvadorensis Standl.	bark fruits leaves	49.1 d-l* 68.6 a-h* 83.3 a-f	75.5 l-p* 75.8 a* 192.0 l-p*	Ciudad Colon San Jose, Costa Rica.
Cedrela tonduzii(younger) C. DC.	leaves wood	90.9 a-i 83.1 a-f	18.3 w-z* #	outside Heredia, Costa Rica
Cedrela tonduzii(older) C. DC.	leaves	69.5 a-h*	99.4 f-k	outside Heredia, Costa Rica
Cedrela toona (Endlicher)	bark leaves	79.1 a-g* 84.4 a-d	150.8 b* 109.6 e-h*	Fairchild Tropical Garden Miami, Florida.

TABLE 3.2.1(continued)

Plant	Tissues	*Ostrinia nubilalis* (Concentration in diet = 0.4% fresh weight, n=15) RGR[a] (% of control)	*Peridroma saucia* (Concentration in diet= 0.2% fresh weight, n=20) Larval Weight (% of control)	Collection Site
Chukrasia tabularis A. Juss.	bark leaves wood	58.5 b-j* 65.4 a-h* 55.4 c-j*	124.2 c-l* 73.6 l-q* 117.3 d-f	Fairchild Tropical Garden, Miami, Florida.
Dysoxylum fraseranum (A. Juss.) Benth.	bark leaves	83.9 a-f 89.1 a-d	153.4 b* 130.3 c-d*	Fairchild Tropical Garden Miami, Florida.
Guarea glabra Vahl.	bark leaves	70.7 a-h 78.3 a-g	89.0 i-l 67.14 m-r*	La Pacifica Canas, Guanacosta, Costa Rica.
Guarea pterorachis Harms.	leaves twigs wood	82.2 a-f 77.1 a-d 83.0 a-f	NT NT NT	Costa Rica
Guarea pyriformis Pennington	bark leaves wood	82.4 a-f 81.4 a-f 23.4 j-l*	125.3 c-l* 151.5 b* 63.3 o-r*	Reserva Biologica, Carara, Costa Rica.

TABLE 3.2.1(continued)

Plant	Tissues	*Ostrinia nubilalis* (Concentration in diet = 0.4% fresh weight, n=15) RGR[a] (% of control)	*Peridroma saucia* (Concentration in diet= 0.2% fresh weight, n=20) Larval Weight (% of control)	Collection Site
Swietenia macrophylla King	bark leaves wood	79.4 a-g 76.5 a-h* 55.4 b-j*	155.5 b* 52.7 r-t* 113.8 d-g	La Pacifica Canas, Guanacosta, Costa Rica.
Swietenia mahagoni (L.) Jacq.	bark leaves wood	63.3 a-i* 69.5 a-h* 61.7 a-i*	12.1 x-z* 75.1 l-p* 74.7 l-p*	Fairchild Tropical Garden Miami, Florida.
Trichilia americana (Sesse & Mocino)Pennington	bark leaves	85.1 a-f 75.7 a-h*	24.8 v-y* 43.2 s-v*	Costa Rica
Trichilia glabra L.	bark leaves wood	29.0 i-l* 55.8 c-l* 46.1 e-l*	9.4 x-z* 54.1 q-t* #*	La Pacifica, Guanacosta, Costa Rica.
Trichilia havanensis Jacq.	leaves wood	107.5 a 102.1 ab	64.3 o-r* 104.0	outside Heredia, Costa Rica.
Trichilia hirta L.	leaves wood	84.6 a-d 72.5 a-h*	5.3 y-z* #*	Fairchild Tropical Garden Miami, Florida.

TABLE 3.2.1 (continued)

Plant	Tissues	*Ostrinia nubilalis* (Concentration in diet = 0.4% fresh weight, n=15) RGR[@] (% of control)	*Peridroma saucia* (Concentration in diet= 0.2% fresh weight, n=20) Larval Weight (% of control)	Collection Site
Trichilia martiana C. DC.	bark leaves	81.2 a-f 94.2 a-c	NT 86.2 j-m	Ciudad Colon San Jose Costa Rica.
Trichilia pleeana (A. Juss.) C. DC.	bark leaves wood	55.7 b-j* 89.5 a-c 67.9 a-h*	8.3 x-z* 63.6 o-r* 66.7 m-r*	Reserva Biologica Carara, Costa Rica.
Trichilia quadrijuga subsp. cinerascens (C. DC.) Pennington	leaves	93.8 a-c	27.8 v-x*	Reserva Biologica Carara, Costa Rica.
Trichilia trifolia L.	wood	71.9 a-g	6.4 y-z*	Costa Rica
Ruptiliocarpon caracolito Hammel and Zamora	bark	19.5 l*	51.5 r-u*	Peninsula de Osa, Costa Rica.

Note: Means followed by the same letter are not significantly different in Tukey's multiple range test(P=0.05). For *Ostrinia nubilalis* the mean (s.d.) of the RGR of the control= 1.486(0.226). Identifications of all Costa Rican material was undertaken by L. Poveda and P. Sanchez, Fairchild Tropical Garden collections by garden staff. @ -RGR= relative growth of larvae; * = significantly different from the control; # = all insects died before assessment; NT = not tested.

3.2.2 THE ISOLATION AND IDENTIFICATION OF SPIROCARACOLITONES FROM *RUPTILIOCARPON CARACOLITO.* THE FIRST CD SPIRO-TRITERPENOIDS.

3.2.2.1 SPIROCARACOLITONE

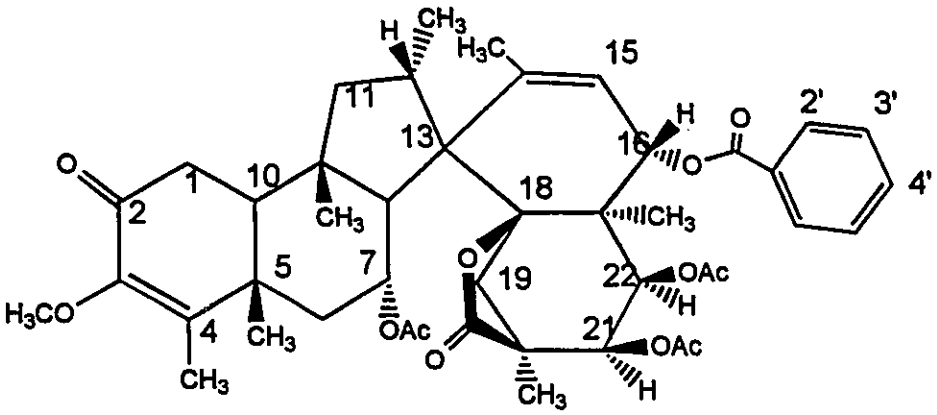
The bark from the tree *Ruptiliocarpon caracolito* was found to give the most active extract, with an RGR of 19.5 %, in the screening of various parts of twenty five species of the Meliaceae against the European corn borer (see Table 3.2.1). As a result of the similarities of the wood of *R. caracolito* to *Trichilia* spp. (Meliaceae) and floral similarities, notably the filament tube, this unusual species was first thought to be a member of the Meliaceae.[29] Recent studies suggest its position as a unique American genus and species of the family, Lepidobotryaceae with close affinity to the monotypic African genus *Lepidobotrys*. *R. caracolito* is an endemic species which was found near Golfito and in the Osa peninsula which are humid lowland tropical rainforests located in Costa Rica. The species is so unusual that it was recently reported in the National Geographic as an "unfamiliar tree" belonging to a family of trees previously known only in Africa but discovered in Costa Rica.[30]

The freeze dried ethanol extract of the bark of *R. caracolito* was separated into hexane, dichloromethane and water soluble portions which were bioassayed for activity against the European corn borer (Table 3.2.2.1a). The dichloromethane soluble fraction, which contained most of the activity, was subjected to silica gel column chromatography which resulted in the collection of ten major fractions. These fractions were then screened for activity against the ECB. The two fractions responsible for most of the activity, fractions four and five, were combined and condensed. The major component of this fraction was isolated by

Preparative HPLC (see Experimental for details) to yield a compound with a melting point of 215-218 °C and $[\alpha]^{25}D$= +39.34 (c=0.0061, CH_2Cl_2). The structure of spirocaracolitone($C_{44}H_{54}O_{12}$) was established using nmr and single X-ray.(see Appendix 3.2.2.1 for Crystallographic data)

TABLE 3.2.2.1a:

	Ostrinia nubilalis (n=15) RGR(% of control)
Hexane solubles	94.6
Dichloromethane solubles	30.0
Water solubles	71.4
Ruptiliocarpon caracolito extract	26.2



SPIROCARACOLITONE

Initial inspection of the proton nmr of spirocaracolitone in Figure 3.2.2.1a revealed the presence of resonances characteristic of a benzoate, methoxy, and three acetate groups at 7.88-7.39, 3.62, and 1.91, 1.94, 2.28 ppm respectively. The occurrence of these groups was consistent with infrared frequencies observed at 1750 cm^{-1}(acetate), 1722 cm^{-1} (benzoate), and the sequential loss of m/e = 60(acetic acid) and 122(benzoic acid) from the $(M+H)^+$ ion in the FAB mass spectrum.[775($(M+H)^+$, (20.4)), 715($(M+H-60)^+$, (2.1)), 593($M+H-60-122)^+$, (4.4)) and 533(($M+H-60-122-60)^+$, (3.0))]. The benzoate and the three acetate groups were each attached to a secondary carbon since the remaining hydrogens on those carbon atoms appeared at 6.02, 5.31, 5.23 and 5.20 ppm, respectively.(Figure 3.2.2.1a)

The infrared showed two additional carbonyl stretching frequencies at 1780 cm^{-1}, eventually assigned to a γ-lactone ring , and at 1674 cm^{-1}, consistent with an α,β-unsaturated ketone. The ^{13}C nmr spectrum (Figure 3.2.2.1b) confirmed the presence of three acetate methyl, two vinylic methyl and a methoxy group. In addition five C-methyl groups, four of which were on quaternary carbons, were observed. Interestingly, only four of the 44 carbons of this molecule were present as methylene groups. Subtraction of the carbons associated with the acetate, benzoate and methoxy groups left 30 carbon atoms thus suggesting that the unknown was a triterpene.

The AB system occurring at 5.23 and 5.20 ppm with a coupling constant of 4.4 Hz was indicative of an axial-equatorial or an equatorial-equatorial interaction between two geminal protons attached to the acetate bearing carbons. The doublet of triplets occurring at 5.31 ppm with J= 3.4 and 10.9 Hz was consistant with an axial hydrogen in a six membered

ring coupled to two other axial hydrogens and one equatorial hydrogen. This was eventually assigned as the axial hydrogen at C-7.

Other isolated spin systems were identified in the proton nmr, however the connectivity between these systems was not obvious without the use of more sophisticated (i.e. INADEQUATE) nmr experiments. These experiments would have been very time consuming since we had relatively small amounts of material. Fortunately crystals suitable for X-ray analysis were obtained after a number of attempts at crystallization from a methanol/water mixture.

Once the structure had been determined by single crystal X-ray structure analysis, the assignment of most of the proton and carbon resonances were determined. Assignment of as many resonances as possible was conducted since a good understanding of the spectra of spirocaracolitone could possibly aid in the structural determinations of the remaining five unknown compounds. A listing of the assignment of proton resonances of spirocaracolitone is given in Table 3.2.2.1b. These spin systems are shown only as partial structures since spirocaracolitone has a rather complicated structure.

Six isolated proton spin systems were identified. The signal at 2.59 ppm was assigned to H-1ax because it showed coupling constants of 14.3 and 17.2 Hz due to an axial-axial vicinal coupling with H-10 and geminal coupling with H-1eq. This is shown in partial structure i. The 14.3 Hz axial-axial coupling constant is larger than what was expected on the basis of the Karplus relationship. H-1eq showed the expected axial-equatorial coupling constant of 3.4 Hz in addition to the 17.2 Hz geminal coupling. The signal for H-10 was

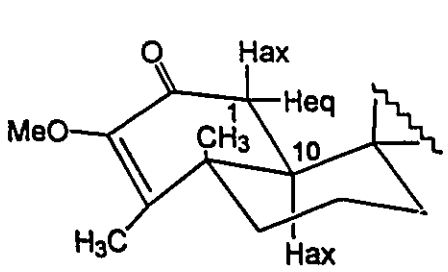
TABLE 3.2.2.1b: ^{1}H NMR SHIFTS of SPIROCARACOLITONE AND SPIROCARACOLITONE A

PROTON	Spirocaracolitone (ppm)	Spirocaracolitone A (ppm)
H-1eq	2.34(dd, J=3.4, 17.2 Hz)	2.34(m)
H-1ax	2.59(dd, J= 14.3, 17.2 Hz)	2.56(dd, J=14.2, 17.1 Hz)
H-6	1.45-1.54(m)	1.18(m)
H-6	2.11(dd, J=3.2, 12.2 Hz)	2.35(m)
H-7	5.31(dt, J=3.2, 10.9 Hz)	5.27(dt, J=3.7, 10.4 Hz)
H-8	2.30(m)	2.05(m)
H-10	1.80-1.85(m)	1.74(dd, J=3.4, 14.2 Hz)
H-11	1.35-1.55(m)	1.28(m)
H-11	~	1.52(dd, J=5.7, 12.4 Hz)
H-12	2.92(m)	2.56(m)
H-15	5.45(brs)	5.95(d, J=3.7 Hz)
H-16	6.02(m)	5.18(d, J=3.7 Hz)
H-19	2.43(d, J=12.9 Hz)	2.32(m)
H-19	2.62(d, J=12.5 Hz)	2.49(d, J=12.7 Hz)
H-21	5.20(d,J=4.4 Hz)*	1.78(d, J=14.4 Hz)
H-21	~	2.06(m)
H-22	5.23(d,J=4.4 Hz)*	4.89(dd, J=1.3, 4.4 Hz)
H'-2	7.89(m)	~
H'-3	7.39(m)	~
H'-4	7.52(m)	~
C-4-Me	1.83(s)	1.82(s)
C-12-Me	1.23(d, J=7.5 Hz)	1.35(d, J=8.3 Hz)
C-14-Me	1.87(brs)	NA
Me	1.18(s)	1.13(s)
	1.22(s)	1.22(s)
	1.23(s)	1.35(s)
	1.47(s)	1.55(s)
	~	1.92(s)
C-3-OMe	3.61(s)	3.57(s)
Me-acetate	1.91(s)	1.96(s)
	1.94(s)	2.08(s)
	2.28(s)	2.14(s)
	~	2.15(s)

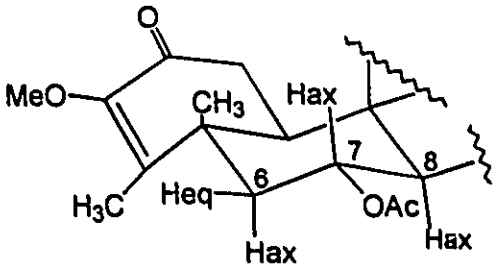
NOTE: * = assignments interchangeable

NA = could not be assigned

centered at 1.83 ppm. Using an HMQC spectrum (Figure 3.2.2.1c) C-1 and C-10 were respectively assigned to resonances at 37.2 and 55.9 ppm.



i

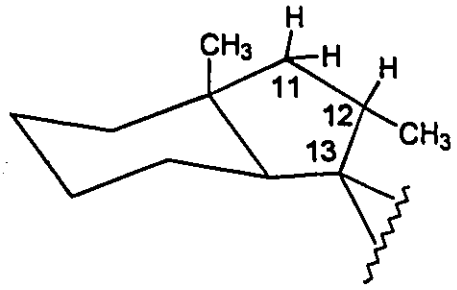


ii

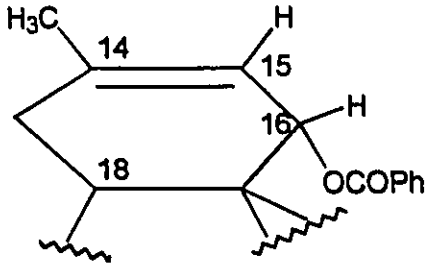
The system due to the four hydrogens on carbons 6, 7 and 8 is given in partial structure ii. Proton 7 was assigned to the dt at 5.31 ppm because its coupling constants dictated one axial-equatorial and two axial-axial interactions. Inspection of the COSY spectrum (Figure 3.2.2.1d) allowed for the assignment of 1.45-1.54 multiplet and the doublet of doublets at 2.11 ppm to the H-6 proton resonances. H-6eq was assigned to the resonance at 2.11 ppm because of its geminal coupling constant of 12.2 Hz and its equatorial-axial coupling constant of 3.2 Hz with H-7. H-6ax was expected to appear as a doublet of doublets or a triplet but overlap with other resonances made it impossible to identify it clearly in the multiplet at 1.45-1.54 ppm. H-8 was assigned to the multiplet at 2.30 ppm using the COSY spectrum in Figure 3.2.2.1d.

The isolated spin system in partial structure iii shows the relationship between H-11, H-12 and the C-12 methyl group. It was not possible to assign the individual hydrogens on C-11. The C-12 methyl group occurred at 1.23 pm as a doublet with a coupling constant of 7.5 Hz which from the COSY spectrum coupled to one hydrogen multiplet at 2.92 ppm (H-12). In turn this multiplet also coupled to a two hydrogen multiplet at 1.35-1.55 ppm

identified as the diastereotopic protons on C-11. Carbon resonances of 50.5, 40.0 and 17.9 ppm were assigned to C-11, C-12 and the C-12 methyl group respectively using the HMQC nmr spectrum.



iii

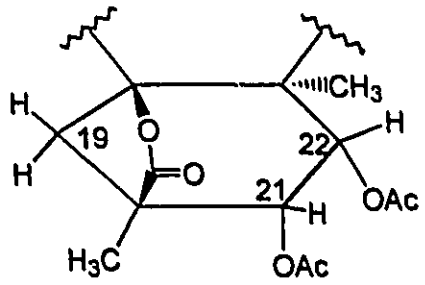


iv

The allylic system in ring D is given in partial structure iv. The one hydrogen narrow multiplet appearing at 6.02 ppm was assigned to H-16 and exhibited a rather low field position because the proton was both allylic and on the carbon bearing the benzoate group. Coupling of this proton to the low field broad singlet at 5.45 ppm (H-15) was detected in the COSY spectrum. H-15 also exhibited long range coupling to the C-14 olefinic methyl group at 1.87 ppm. The carbon resonances for C-14, C-15, C-16 and the C-14 methyl group were assigned as 136.6, 125.5, 69.9 and 23.9 ppm respectively from inspection of the HMQC spectrum.

The final two spin systems, each consisting of a pair of doublets in the proton nmr spectrum, are shown in the partial structure v. Those due to the methylene group at C-19 were located at 2.43 and 2.62 ppm with the expected geminal coupling constant of 12.7 Hz. The hydrogens on carbons 21 and 22 occurred at 5.20 and 5.23 ppm with coupling constants of 4.4 Hz which were consisted with the axial-equatorial arrangement of these protons. It was

not possible to determine the individual assignments for C-21 and C-22 because of the congestion in the HMQC spectrum in this area. C-19 was assigned to the carbon resonance at 42.4 ppm.



V

Assignment of quaternary carbons, especially the spiro carbon C-13, was accomplished by the use of an HMBC nmr experiment (Figure 3.2.2.1e). The identification of the spiro carbon, C-17, C-18 and C-20 at 57.6, 47.2, 90.5 and 43.9 ppm respectively, were determined through the comparison of H-C-C and H-C-C-C correlations of H-11, 12, 15, 16, 18, 21 and H-22. Difficulties were often encountered because some of the expected correlations were not represented in the spectrum due to weak coupling interactions. The assignment of many of the carbon resonances was necessary to aid in the assignment of shifts in the spectra of the other five spirocaracolitones.

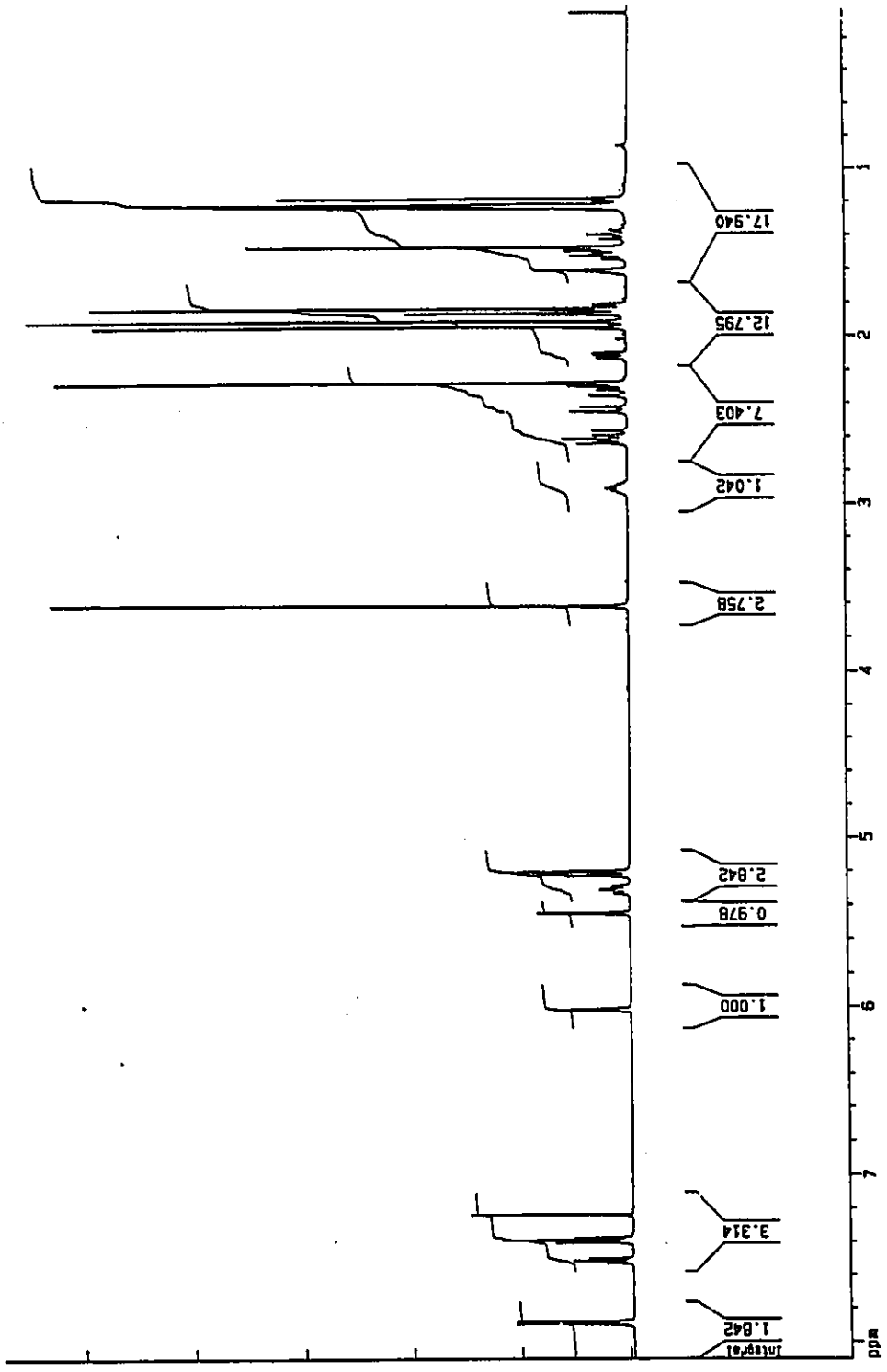


FIGURE 3.2.2.1a: ^{1}H NMR OF SPIROCARACOLITONE

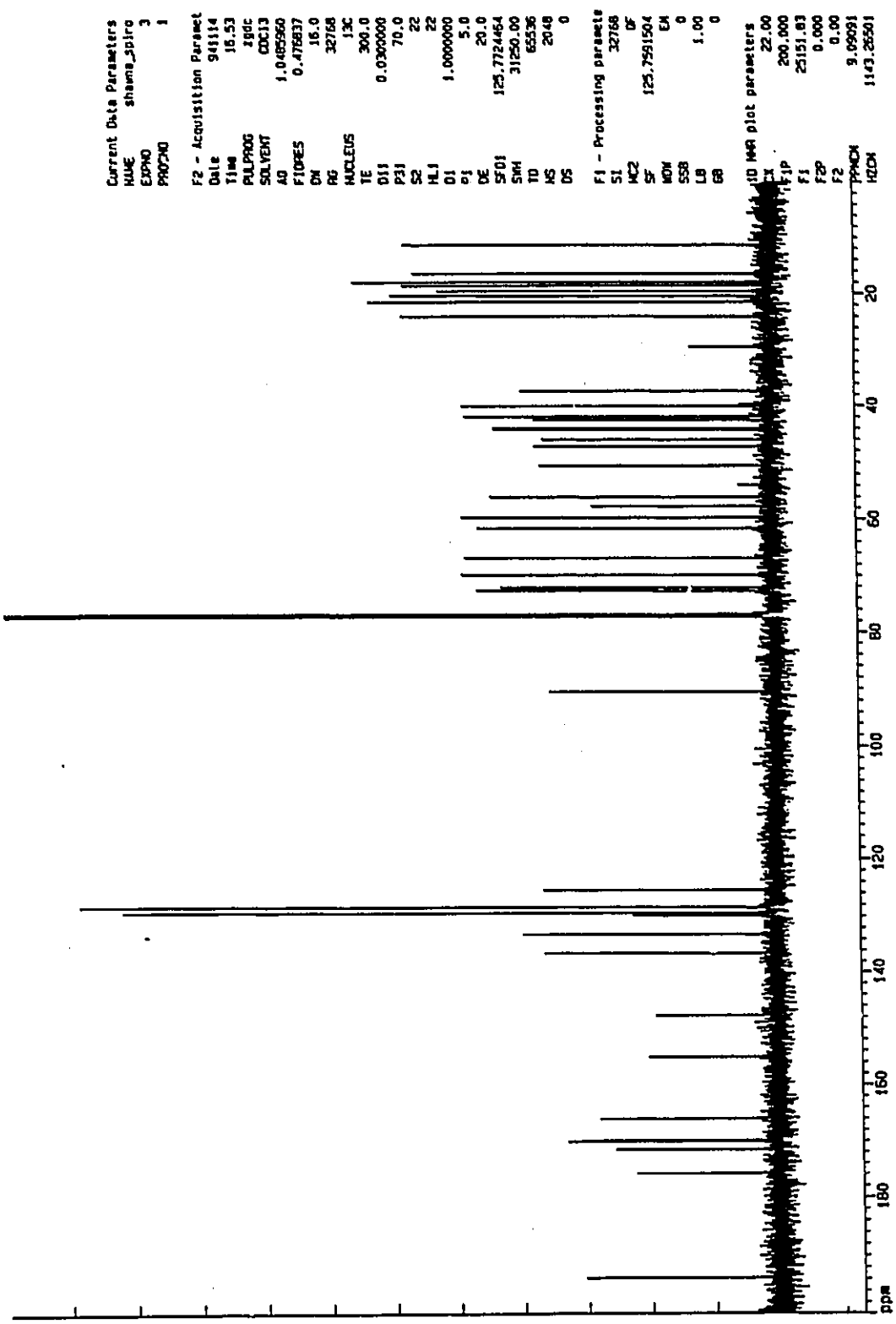


FIGURE 3.2.2.1b: ^{13}C NMR OF SPIROCARACOLITONE

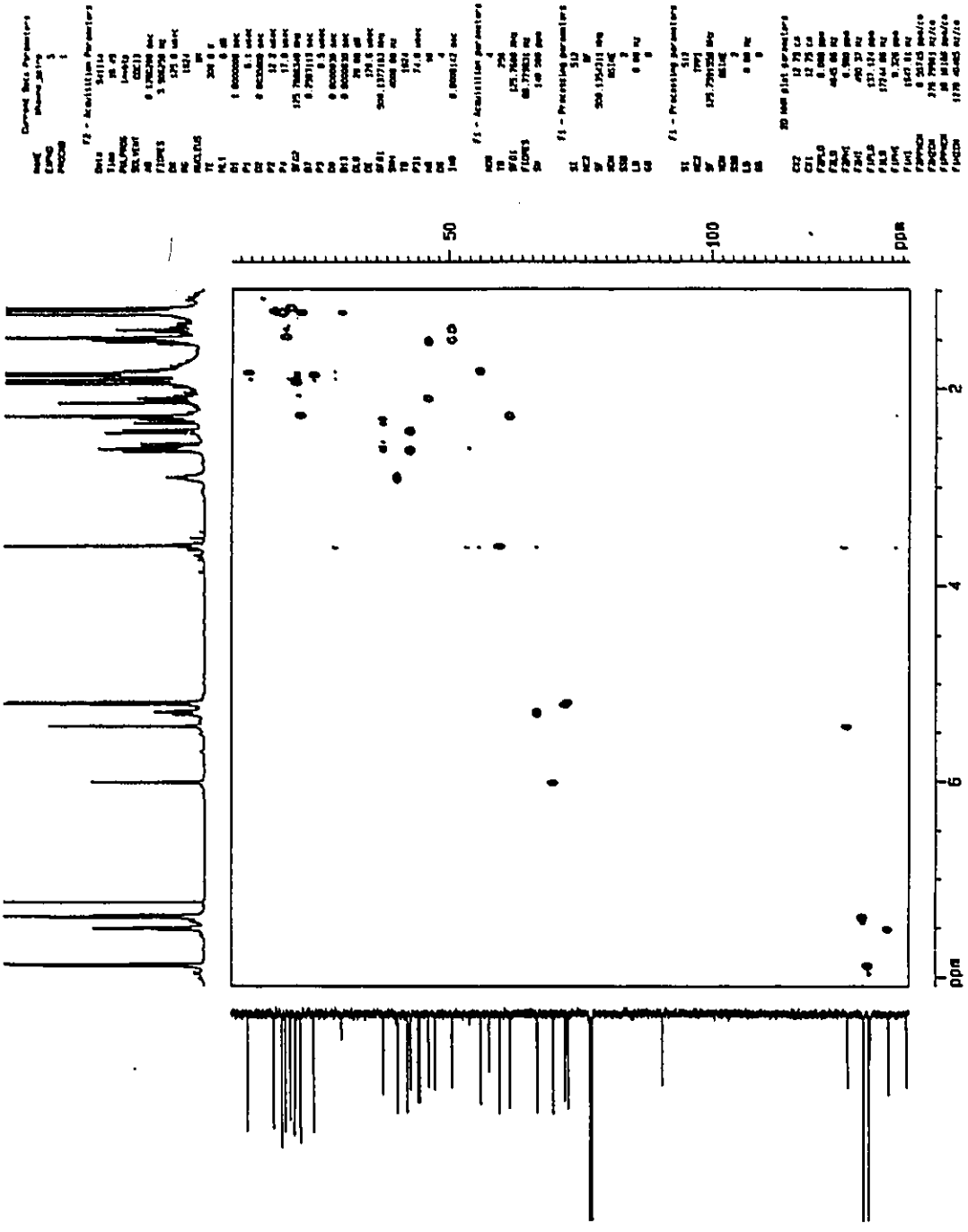


FIGURE 3.2.2.1c: HMQC OF SPIROCARACOLITONE

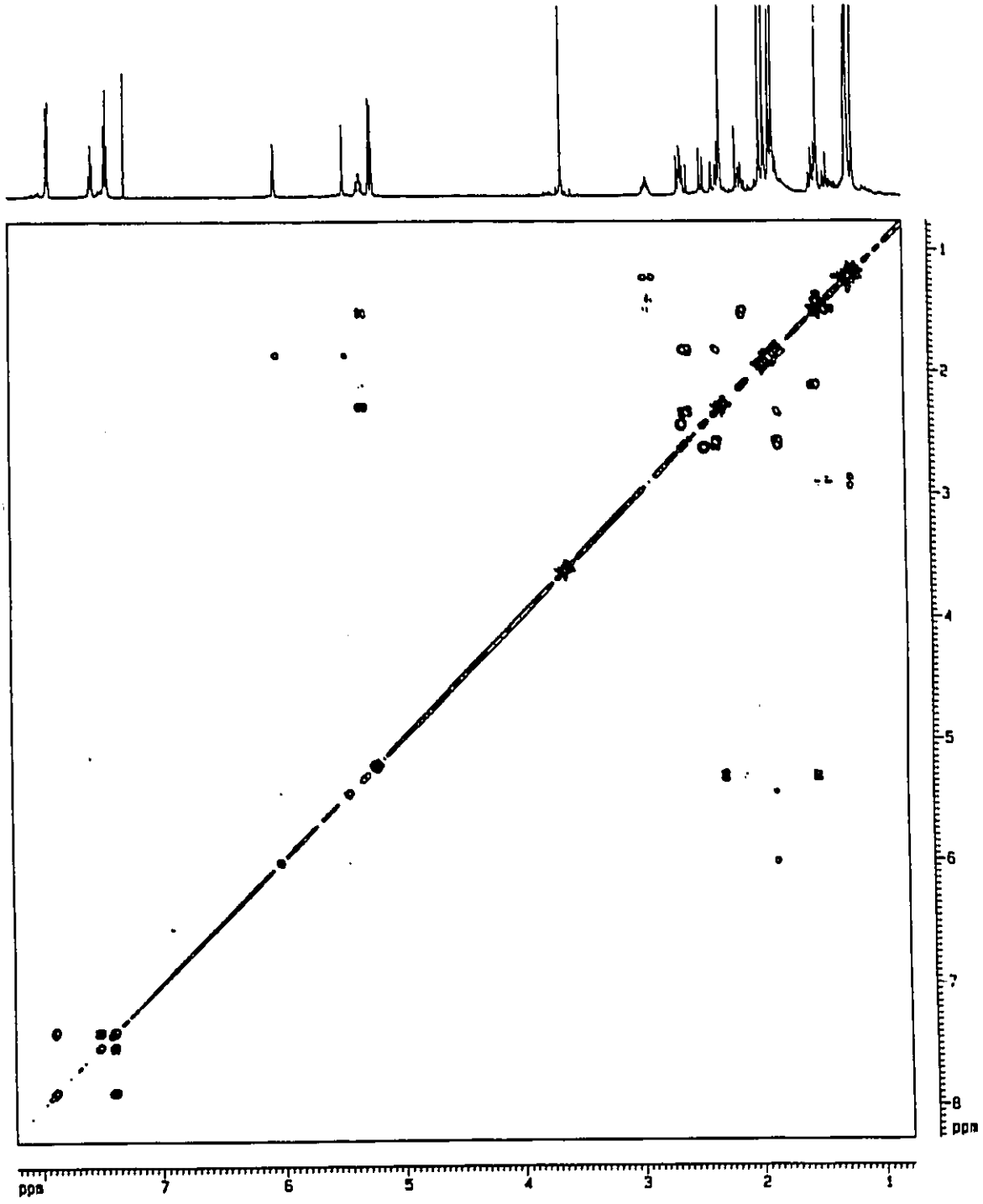


FIGURE 3.2.2.1d: COSY OF SPIROCARACOLITONE

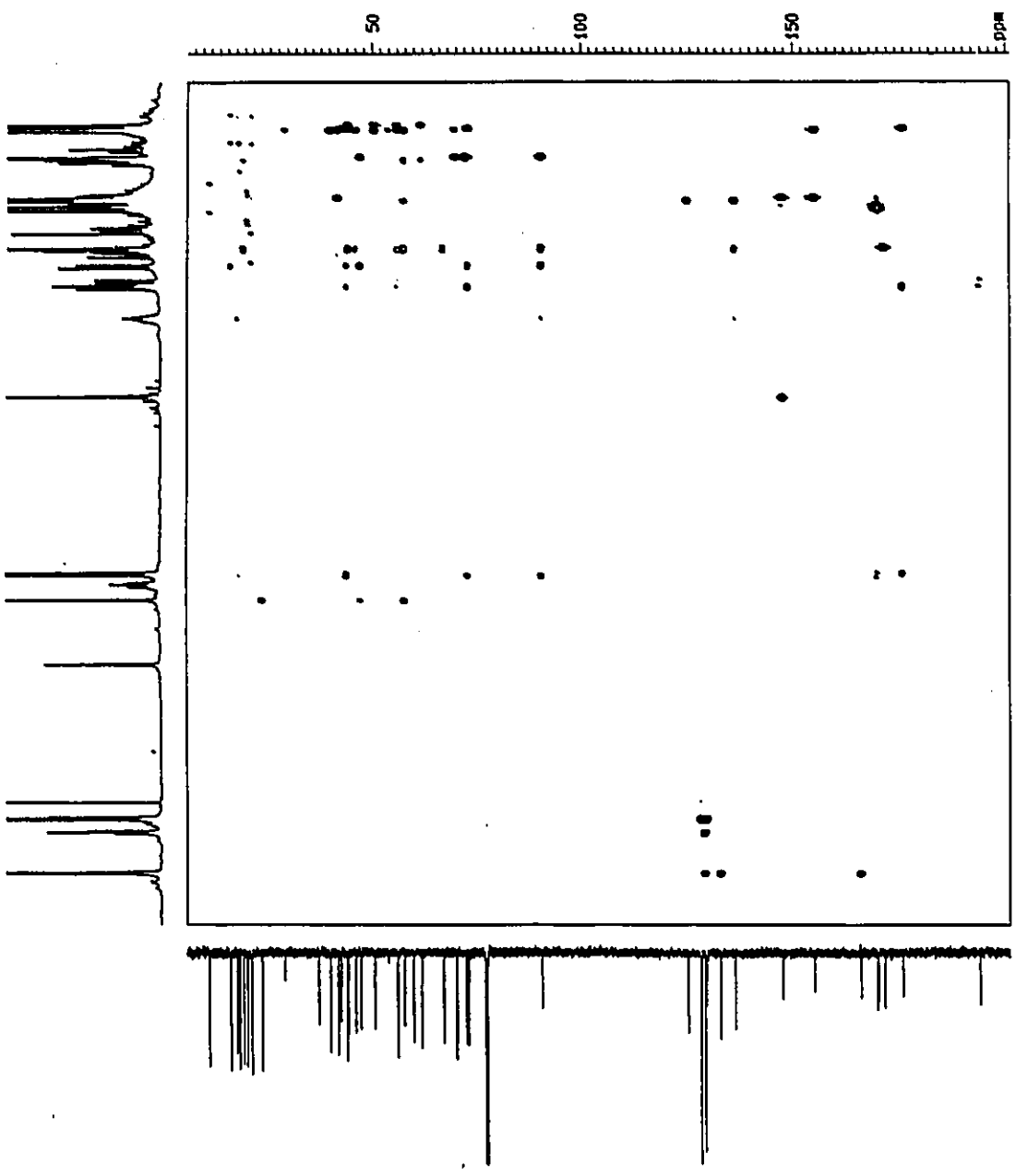
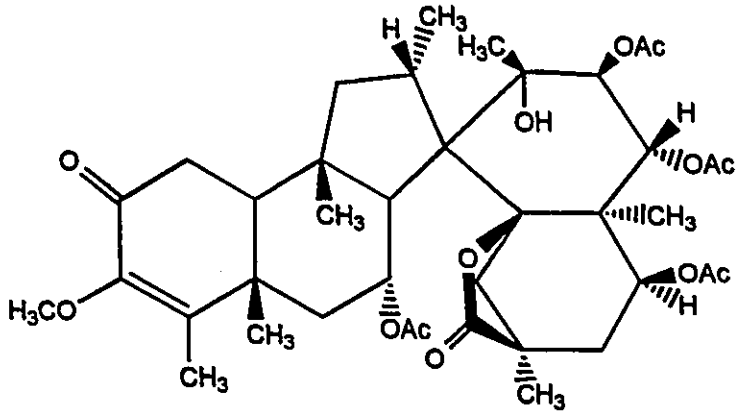


FIGURE 3.2.2.1e: HMBC OF SPIROCARACOLITONE

3.2.2.2 SPIROCARACOLITONE A

When spirocaracolitone was tested alone against the European corn borer it did not fully explain the activity of the active fraction suggesting that the initially observed activity was present in another fraction of the dichloromethane extract or the activity was a result of synergism with other components. Investigation of the components of the active fraction of the bark of *R. caracolito* from a subsequent collection has resulted in the isolation of five pure compounds. The first of these to be described is spirocaracolitone A.

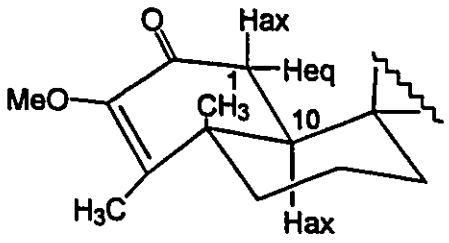


SPIROCARACOLITONE A

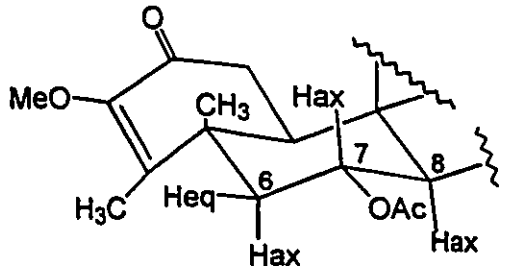
The ^{1}H nmr of the spirocaracolitone A (Figure 3.2.2.2a), when compared to that of spirocaracolitone, was missing both the benzoate and the H-15 resonances(Table 3.2.2.1b). Further inspection of the nmr data revealed the presence of four acetate, one methoxy and six methyl groups in the unknown structure. The presence of an alcohol was suspected because of the broad singlet at 4.13 ppm in the proton nmr and the absorption in the IR spectrum at 3531 cm^{-1}. Elucidation of the structure was accomplished through X-ray

analysis of crystals of spirocaracolitone A obtained from a methanol/water solution. (see Appendix 3.2.2.2 for X-ray data)

The signals due to H-1ax, H-1eq and H-10 in partial structure i occurred at 2.56, 2.34 and 1.74 ppm, respectively. As in spirocaracolitone the specific assignments were based on a large 14.2 Hz axial-axial and 3.4 Hz equatorial-axial coupling constants. Unfortunately H-1ax appeared as a multiplet because of the overlap with the one of the H-6 resonances at 2.35 ppm thus making the determination of the coupling constants and multiplicities impossible for both. From the HMQC (Figure 3.2.2.2b) spectrum the C-1 and C-10 carbon resonances were determined to occur at 37.0 and 55.1 ppm which were very similar to those observed for spirocaracolitone.



i

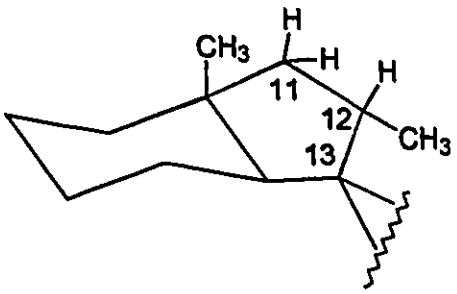


ii

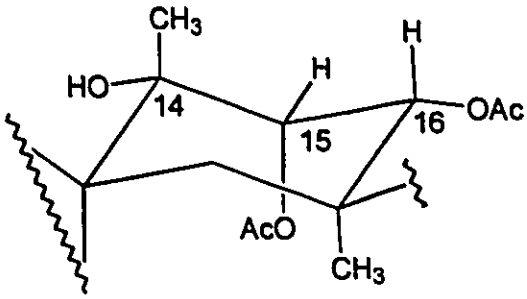
As in spirocaracolitone, H-7 appeared as a doublet of triplets at 5.27 ppm (J=3.7 and 10.4 Hz) which was consistent with two large axial-axial and one axial-equatorial interaction with the neighboring protons at C-6 and C-8 (see partial structure ii above). H-6ax and H-6eq were located at 1.18 and 2.35 ppm as multiplets again with a chemical shift difference of 1.2 ppm between the axial and equatorial protons. The assignment of carbons 6, 7 and 8 respectively to resonances at 47.1, 70.2 and 65.0 ppm were determined by inspection

of the HMQC spectrum (Figure 3.2.2.2b) and were in general agreement to those observed for spirocarcolitone.

The proton resonances for H-11, H-12 and the C-12 methyl group, in partial structure iii, in spirocaracolitone A and spirocaracolitone as expected were quite similar. Resonances at 1.35(d, J= 8.3 Hz), 2.56(m, 1H), 1.28(m, 1H) and 1.52(dd, J=5.7, 12.4 Hz, 1H) were assigned to the C-12 methyl group, H-12, H-11eq and H-11ax respectively. Carbon resonances 52.8, 37.8 and 26.8 ppm (Figure 3.2.2.2c) were assigned to C-11, C-12 and the C-12 methyl group respectively, using the HMQC spectrum.



iii

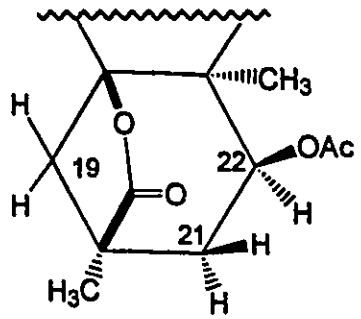


vi

Ring D of spirocaracolitone A differed from that of spirocaracolitone as the double bond has been oxidized with the addition of a C-14 alcohol and a C-15 acetate (see partial structure vi). Also C-16 was substituted with an acetate and not a benzoate as in spirocaracolitone. H-15 and H-16 were assigned to the doublets occurring at 5.95 and 5.18 ppm (J=3.7 Hz) respectively. The magnitude of this coupling constant suggested an axial-equatorial or a equatorial-equatorial interaction between these two protons. Inspection of the X-ray drawing confirmed the presence of a axial-equatorial interaction as shown in vi. H-15 was assigned to the resonance at 5.95 ppm because it was on a carbon bearing an acetate and

on the carbon adjacent to C-14, which had an alcohol substituent. Carbon resonances at 77.5 and 65.4 ppm were assigned to C-15 and C-16 using the HMQC spectrum. A broad one proton singlet at 4.13 ppm was assigned to the the C-14-OH because this proton did not couple to any of the carbon resonances in the HMQC spectrum.

The only difference between the E-ring of spirocaracolitone A and spirocaracolitone was that the former did not have an acetoxy group at C-21. (see partial structure vii). The methylenic protons on C-19 were represented at 2.32 and 2.49 ppm as a multiplet and a doublet, respectively. Because of overlap with other proton resonances the coupling constant of the expected doublet for one of C-19's proton at 2.32 ppm could not be determined, however a J value of 12.7 Hz was determined for the other H-19 resonance. This value agreed with that observed in spirocaracolitone for the same geminal coupling.



vii

The last spin system to be considered was that of methylene group at C-21 and the proton at C-22. H-22 was assigned to a one proton doublet of doublets at 4.89 ppm which had coupling constants of 1.3 and 4.4 Hz. The magnitude of these values suggested the axial-equatorial and equatorial-equatorial interactions to the protons on C-21 which is the case when the C-22 acetate group is in the beta position. From the COSY spectrum the protons on C-21 were located at 1.78 (d, J=14.4 Hz) and 2.06 ppm (m) (Figure 3.2.2.2d). Assignment of

the resonances for C-21 and C-22 at 37.7 and 74.3 ppm was determined from the HMQC spectrum.

The quaternary carbons in rings A-C were assigned via comparison to those of spirocaracolitone. Assignment of C-13, C-14, C-17 and C-20 were determined from the HMBC spectrum (Figure 3.2.2.2e) via a process of elimination to be respectively 66.2, 76.7, 45.3 and 40.9 ppm.

The CI mass spectrum of spirocaracolitone A yielded a [MH]+ peak at 731 m/e which corresponded to the molecular weight predicted by the X-ray structure of 730 g/mole. Losses of 60 units from ions at 731, 671, 611 and 551 m/e were indicative of the loss of acetate moieties in the form of acetic acid. Spirocaracolitone A melted at 240°C with decomposition and exhibited a $[\alpha]^{25}D$ of - 22.17 in methylene chloride(c=0.0046).

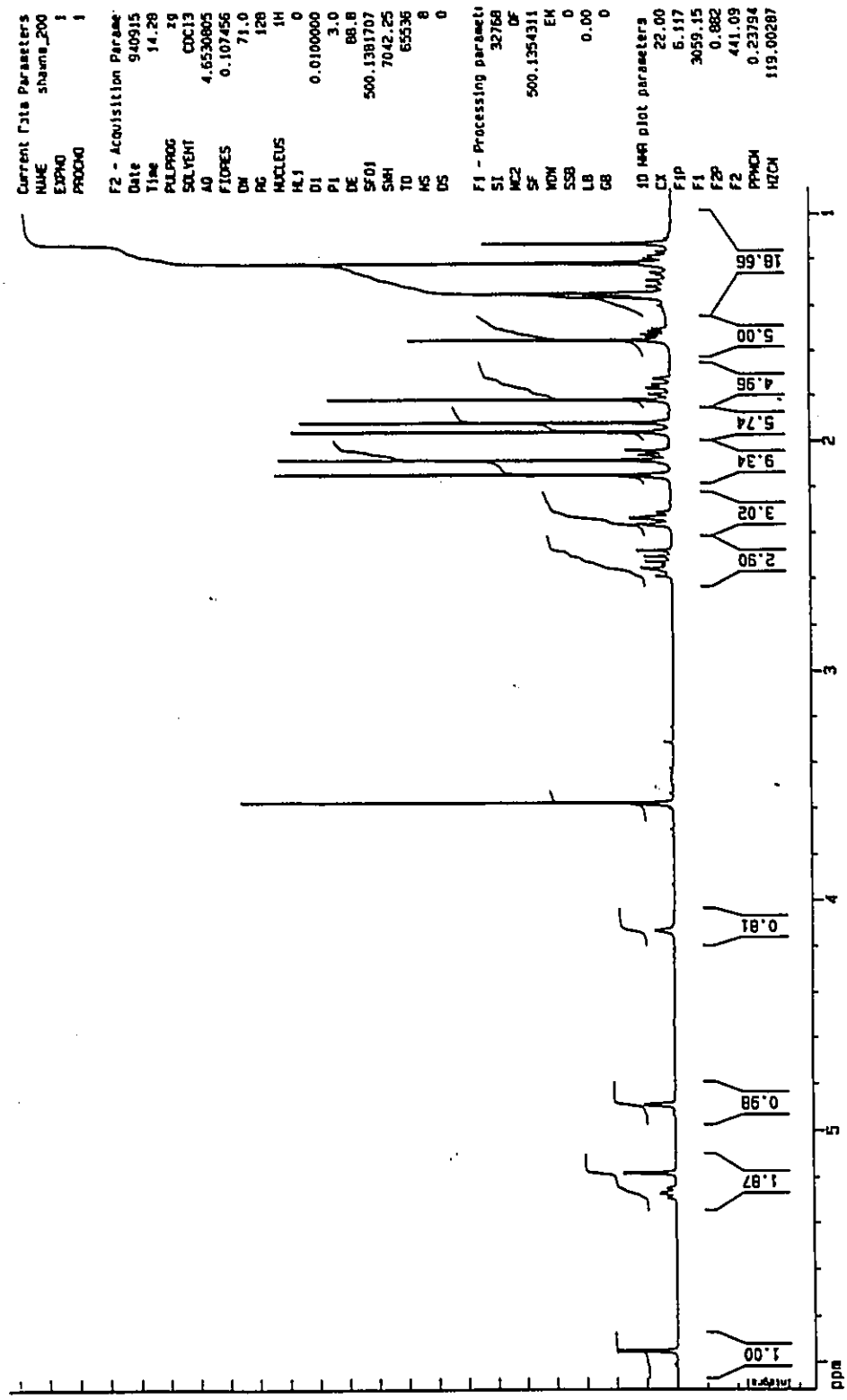


FIGURE 3.2.2.2a: ^{1}H NMR OF SPIROCARACOLITONE A

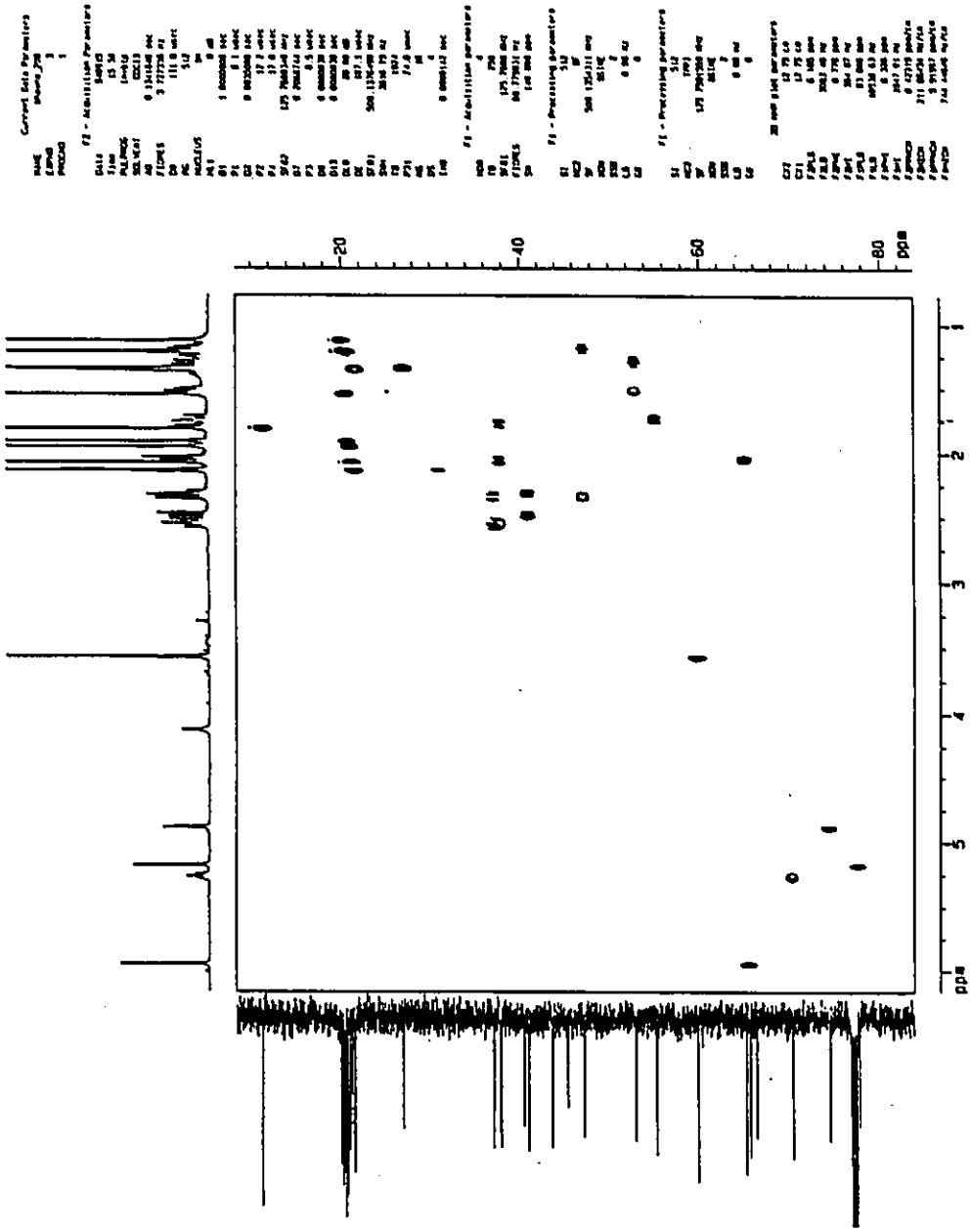


FIGURE 3.2.2.2b: HMQC OF SPIROCARACOLITONE A

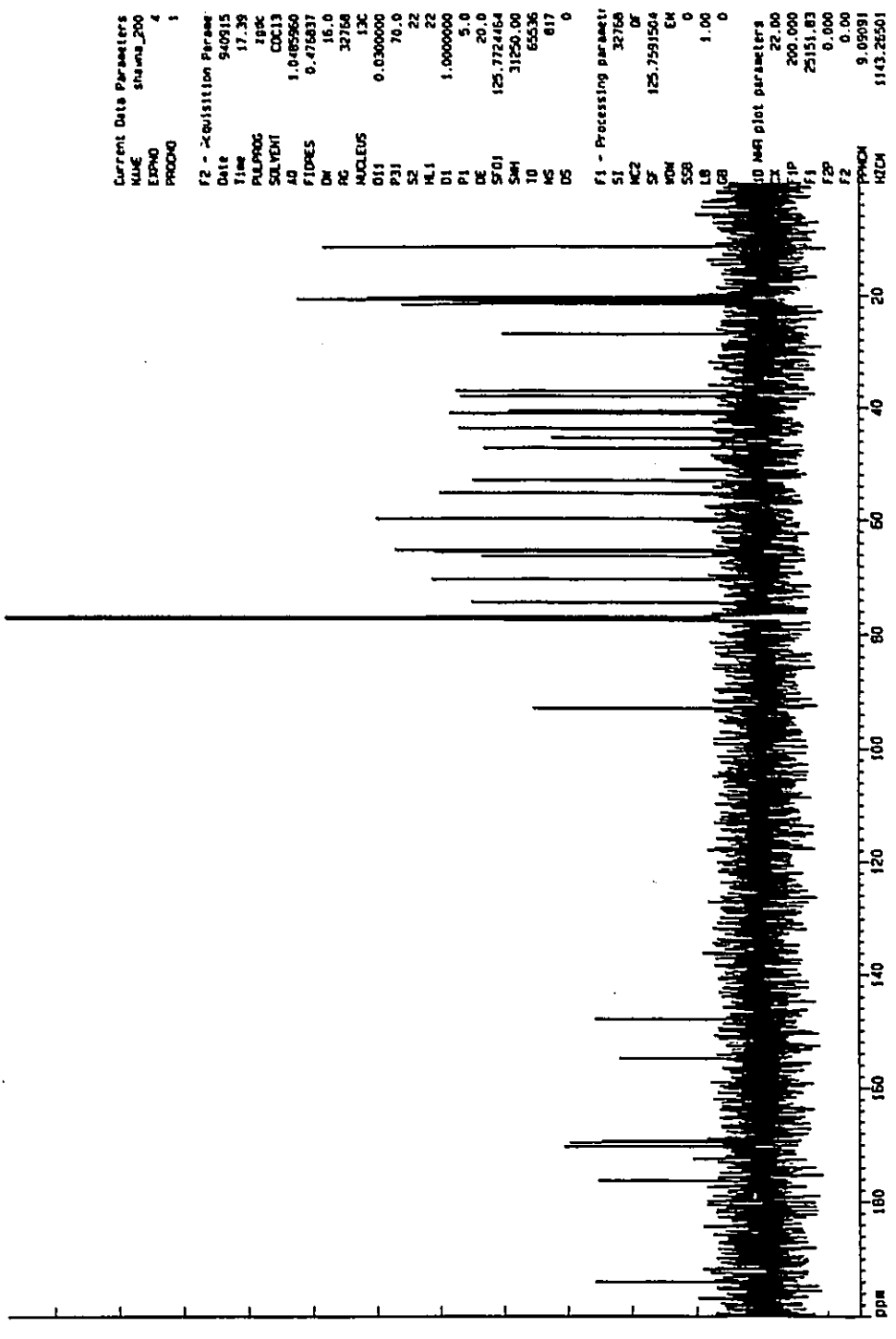


FIGURE 3.2.2.2c: ^{13}C NMR OF SPIROCARACOLITONE A

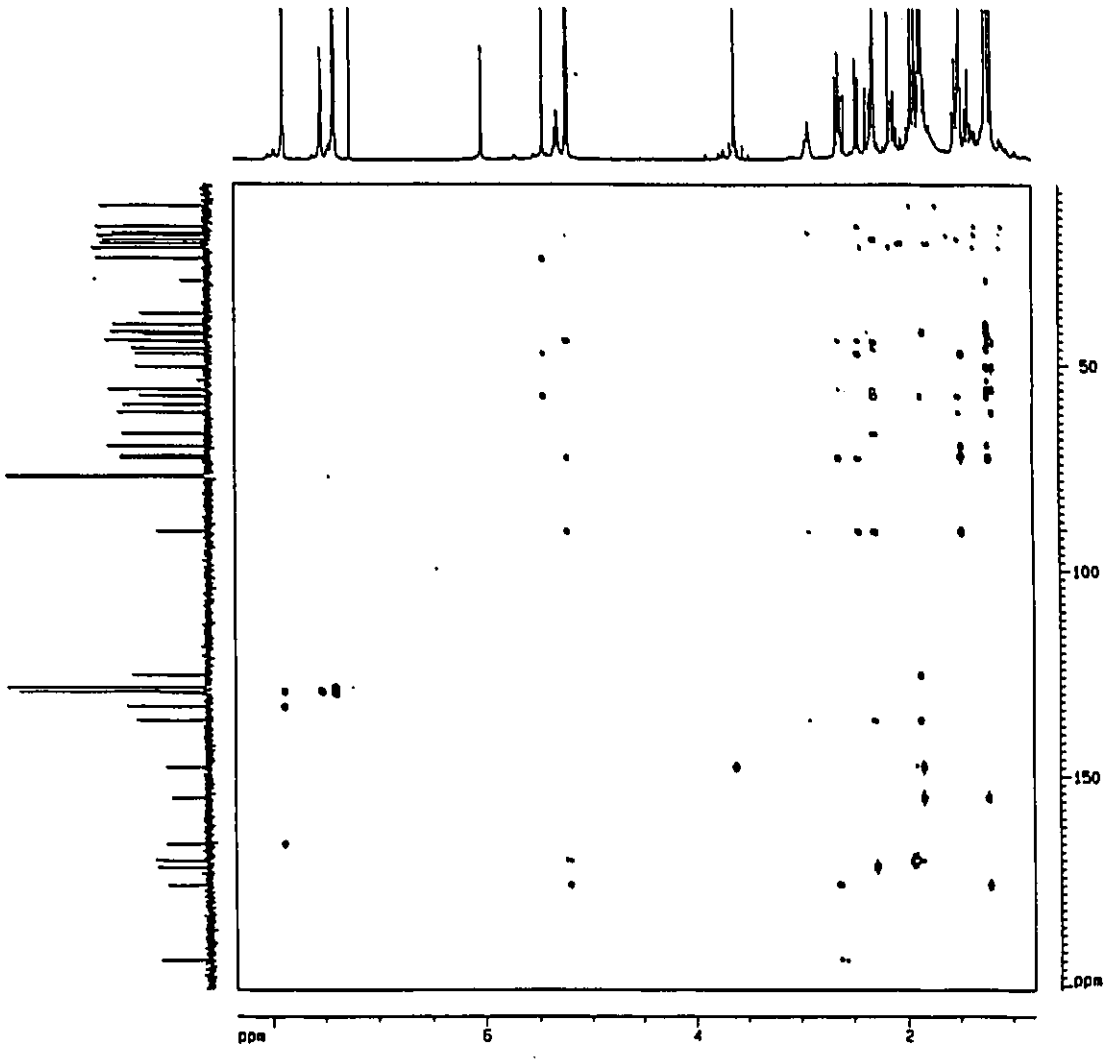


FIGURE 3.2.2.2d: HMBC OF SPIROCARACOLITONE

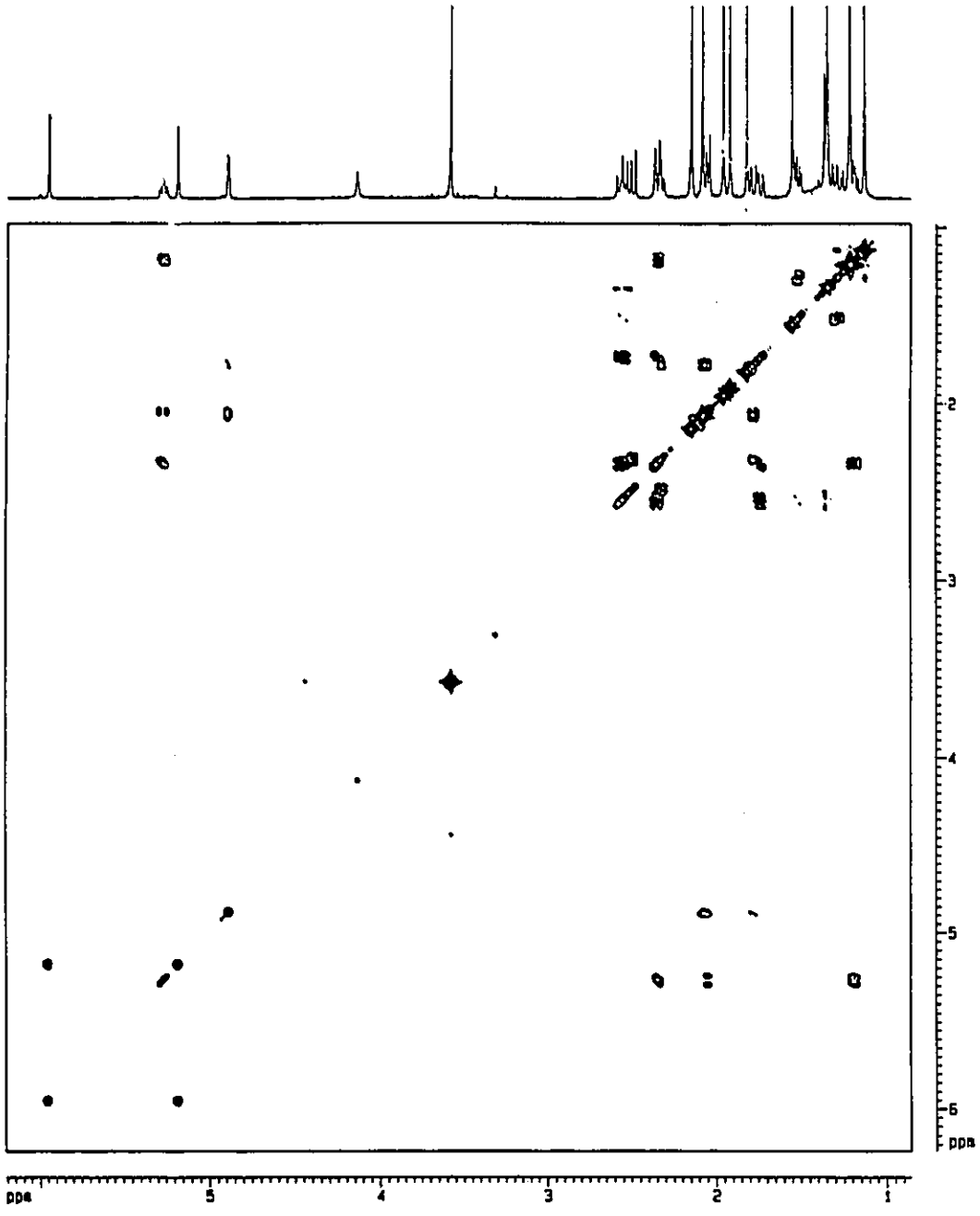


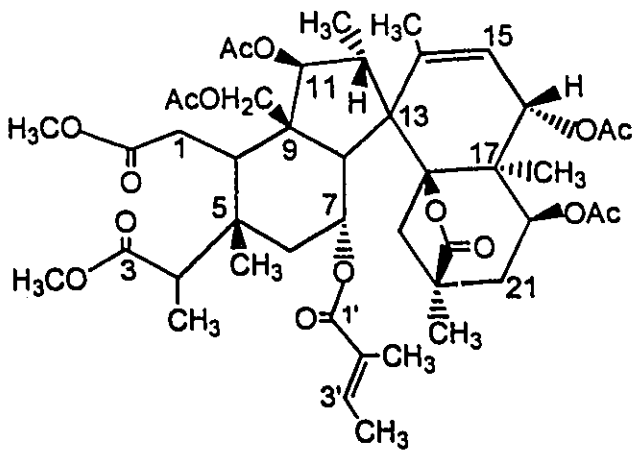
FIGURE 3.2.2.2e: COSY OF SPIROCARACOLITONE A

3.2.2.3 SPIROCARACOLITONE B

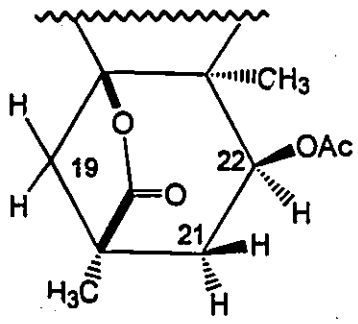
Inspection of the proton nmr of spirocaracolitone B (Figure 3.2.2.3a) revealed that this compound had some similarities to spirocaracolitone and spirocaracolitone A but also some significant differences. Most importantly, spirocaracolitone B lacked the 1674 cm^{-1} absorption in the IR due to the α,β-enone system in ring A. Also missing was the C-4 vinylic methyl and the benzoate group. New features included a tigloyl group revealed by the presence of a doublet of quartets at 7.07 ppm. Comparison of the proton and carbon resonances of those assigned to the tigloyl group in azadiractin further sustantiated this claim.[31] Also present in the proton nmr were eight methyl groups with resonances at 0.93, 1.02, 1.12, 1.22, 1.33, 1.80, 1.81, and 1.91 ppm. Four acetate and two methoxy methyl groups were also detected at 1.91, 1.94, 1.95, 2.07, and 3.58 and 3.59, respectively.

The presence of the E ring lactone, acetates and an α,β-unsaturated ester were suggested by the respective absorptions at 1783, 1738 and 1697 cm^{-1} in the IR spectrum. Sequential losses of m/e=60(acetic acid) from the initially suspected $[M+H]^+$, 759 m/e, in the CI MS were observed. The tigloyl group and three of the acetates were each attached to a secondary carbon because of the presence of resonances at 5.58, 5.22, 5.71, and 4.90 ppm.

Also present were two methyl ester resonances and four acetoxy groups. The presence of the olefinic hydrogen (H-15 at 5.21ppm) and the C-14-methyl group at 1.80 ppm showed that the C-ring in this new compound was similar to spirocaracolitone and not oxygenated as in spirocaracolitone A. X-ray analysis of crystals of spirocaracolitone B obtained from methanol/water, revealed the structure of a spiro-triterpenoid which had undergone cleavage in ring A.(see Appendix 3.2.2.3 for X-ray data).



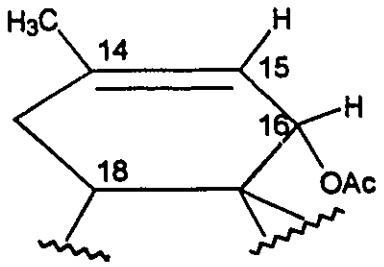
To facilitate the assignment of carbon and proton resonances the structure was broken down intc nine spin systems. This discussion will start with the portion of spirocaracolitone B which is the same as spirocaracolitone A, ring E given in the partial structure vii. Using the COSY spectrum (Figure 3.2.2.3b), the doublet at 4.90 ppm with a coupling constant of 2.4 Hz was assigned to H-22 and determined to couple to the protons on C-21, a one hydrogen doublet at 1.85 ppm (J= 14.8 Hz) and a resonance located at 1.93 ppm which was largely obscured by acetate resonances. The size of these coupling constants and the carbon resonances for C-21 and C-22 (39.7 and 73.3 ppm determined, respectively, from the HMQC spectrum (Figure 3.2.2.3c)) agreed with those determined for spirocaracolitone A.



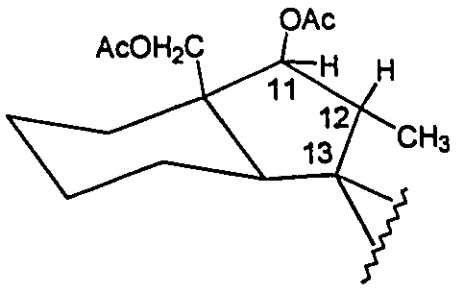
vii

The determination of the resonances of the protons on C-19 was not as obvious as was the case in spirocaracolitone and spirocaracolitone A. The structure of spirocaracolitone B contained five methylene groups which were located at 31.4, 39.7, 41.7, 42.7 and 63.9 ppm in the carbon spectrum(Figure 3.2.2.3d) using the DEPT spectrum (Figure 3.2.2.3e). C-19 was assigned to the resonance at 42.7 ppm because the proton resonances associated with this carbon from the COSY spectrum did not couple to any other proton resonances. The resonance at 63.9 ppm was too far down field to be assigned to this carbon. From the HMQC spectrum the proton resonances for C-19 were assigned to a two hydrogen multiplet at 2.37 ppm. Carbon resonances for C-17, C-18, C-20 and C-30 were assigned as 47.8, 90.5, 40.6 and 177.4 ppm respectively, via comparison to those obtained for spirocaracolitone A.

The D-ring of spirocaracolitone B, shown in partial structure viii, very closely resembled that of spirocaracolitone except it was substituted at C-16 with an acetate and not a benzoate group. H-16 was assigned to the one hydrogen triplet at 5.71 ppm (J=2 Hz) because it coupled in the COSY spectrum with a vinylic proton at 5.21(brs) ppm which was therefore assigned to H-15. Inspection of the HMQC spectrum allowed for the identification of C-15 and C-16 resonances as 126.7 and 69.6 respectively which closely agreed with those assigned in spirocaracolitone. Carbon resonances of C-14 (134.5 ppm) and the C-14-methyl group (23.1 ppm) were assigned by comparison to spirocaracolitone.



viii

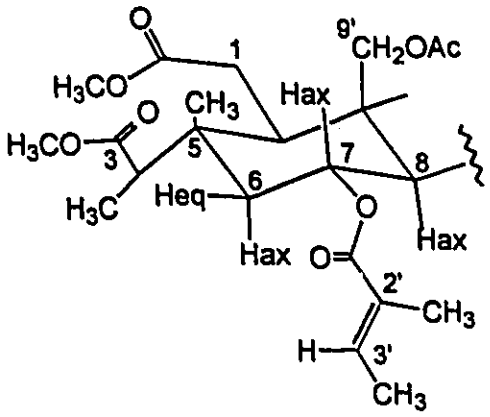


ix

The C-ring of the other two spirocaracolitones did not contain a C-11 acetate group as seen in the partial structure ix of spirocaracolitone B. The C-12-methyl group, 1.12 ppm, was identified in the mass of methyl resonances because it appeared as a doublet with the characteristic coupling constant of 7.2 Hz. From the COSY spectrum it coupled to a characteristic multiplet at 2.79 ppm which was assigned to H-12. Further coupling of this proton was observed to a doublet at 5.22 ppm. The shift of this proton agreed with that expected for a proton on a carbon bearing an acetate group such as H-11. The coupling constant was estimated at 13 Hz as it was difficult to determine which peaks belonged to the doublet because of the closeness of another resonance. Carbon resonances were assigned to C-11, C-12 and the C-12-methyl group using the HMQC spectrum as 82.2, 44.4 and 12.7 ppm respectively.

The methylene group at C'-9, in partial structure x, was assigned the carbon resonance of 63.9 ppm because it was adjacent to an acetate group. From the HMQC spectrum the proton resonances were identified as doublets occurring at 4.60 and 4.91 with a geminal coupling constant of 12.9 Hz. The tigloyl group at C-7 consisted of two vinylic

methyl groups (C'-2 and C'-3) and one vinylic hydrogen (H'-3) assigned to resonances at 1.91(s), 1.81(d) and 7.07 (dq) ppm respectively. H'-3 coupled to the C'-3 methyl group and the C'-4 methyl group with J values of 7.0 and 1.4 Hz respectively. The HMQC spectrum dictated that the carbon resonances for C'-2- methyl, C'-3 methyl and C'-2 were respectively 12.0, 14.8 and 128.4 ppm. The C'-2 was assigned to the 128.4 ppm resonance based on comparison to other tigloyl containing compounds.



x

Proton 7 was assigned to the dt at 5.58 ppm because its coupling constants dictated one axial-equatorial (J=4.4 Hz) and two axial-axial interactions (J=10.9 Hz). From the COSY spectrum the H-6 protons were assigned to a doublet of doublets at 1.70 ppm (H-6eq, J=4.4, 12.9 Hz) and a multiplet at 2.39 ppm (H-6ax). The H-8 resonance was assigned to a multiplet at 2.74 ppm which was overlaping with that of H-10. The corresponding carbon shifts were assigned from inspection of the HMQC spectrum. The downfield shift of H-7 from 5.27 or 5.31 ppm, in spirocaracolitone and spirocaracolitone A, to 5.58 ppm was anticipated because of the presence of the tigloyl group and not the acetate group as seen in the other spiro-structures.

The proton on C-4 was assigned to the one hydrogen quartet at 2.34 ppm because it coupled, from the COSY spectrum, only to a methyl group at 0.93 ppm with a coupling constant of 7.2 Hz. The corresponding carbon shifts were determined to be 47.6 and 15.0 ppm respectively for C-4 and the C-4 methyl group (HMQC spectrum). The final spin system to be discussed is that of C-1 and C-10. The methylene group at C-1 was assigned to 31.4 ppm by a process of elimination. From the HMQC spectrum the diastereotopic H-1 protons were identified as appearing at 2.39 as a multiplet and 3.00 ppm as a doublet of doublets (J=6.0, 18.3 Hz). The COSY spectrum allowed for the identification of H-10 at 2.74 ppm as a multiplet and the HMQC spectrum assigned C-10 to the resonance at 51.2 or 54.4 ppm.

The ^{1}H nmr however predicted the presence of two methoxy resonances, at 3.58 and 3.59 ppm, instead of one which was expected from looking at the crystal structure. The monoacid form of spirocaracolitone B, given in the X-ray structure, is thought to have been present in small quantities in the sample and preferentially crystallized out over the methyl ester form even though the methyl ester form was present in a higher concentration. Spirocaracolitone B is thought to have been isolated in the methyl ester form.

A peak corresponding to the molecular weight of spirocaracolitone B, 858 g/mole, was not found in CI mass spectrum however an ion corresponding to $[M-99]^{+}$, 759 m/e, was present and was postulated to be spirocaracolitone B missing its tigloyl moiety. Spirocaracolitone B had a melting point of 231.0-232.5 °C and an $[\alpha]^{25}D$ of -6.76 in methylene chloride (c= 0.0037).

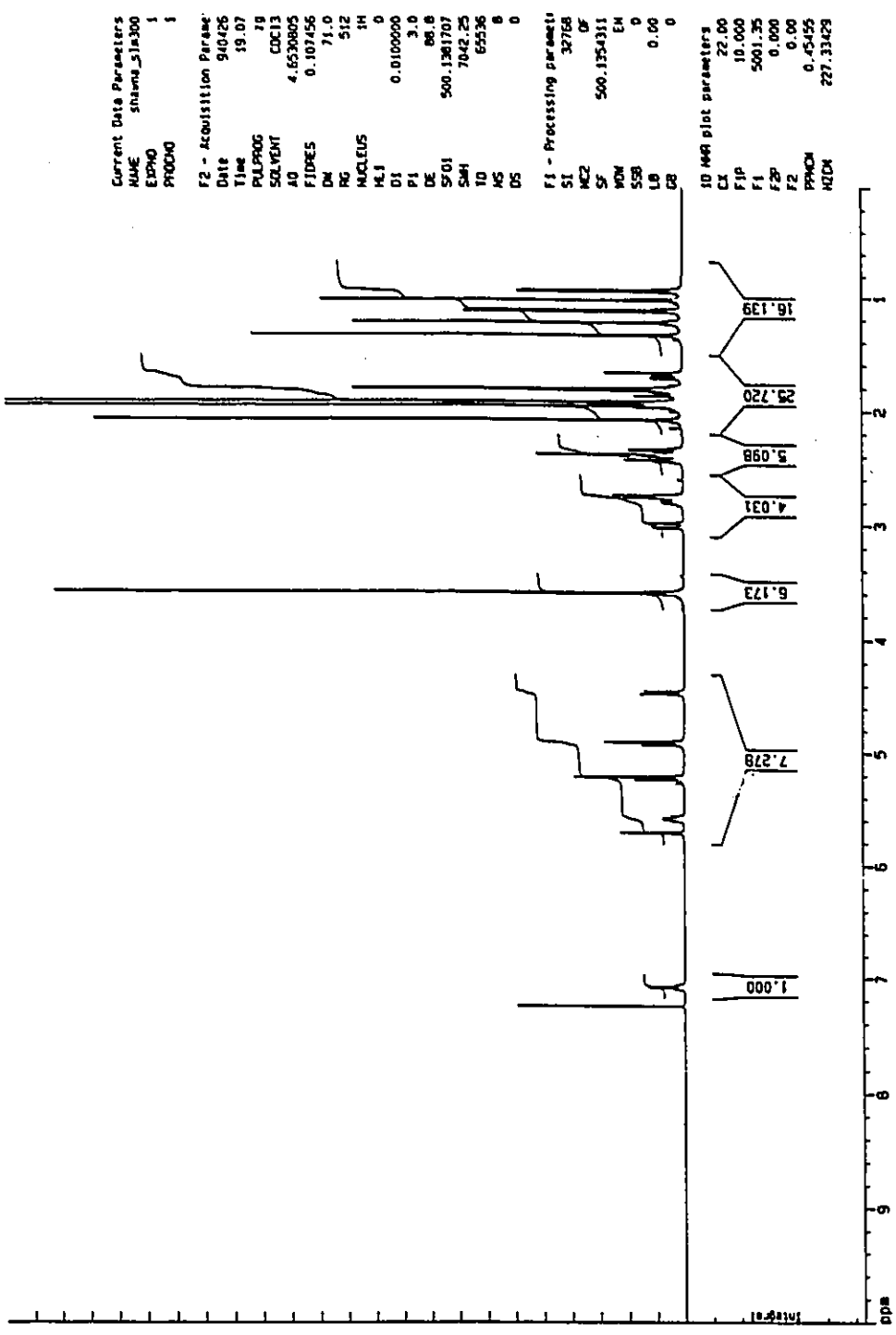


FIGURE 3.2.2.3.a ^{1}H NMR SPIROCARACOLITONE B

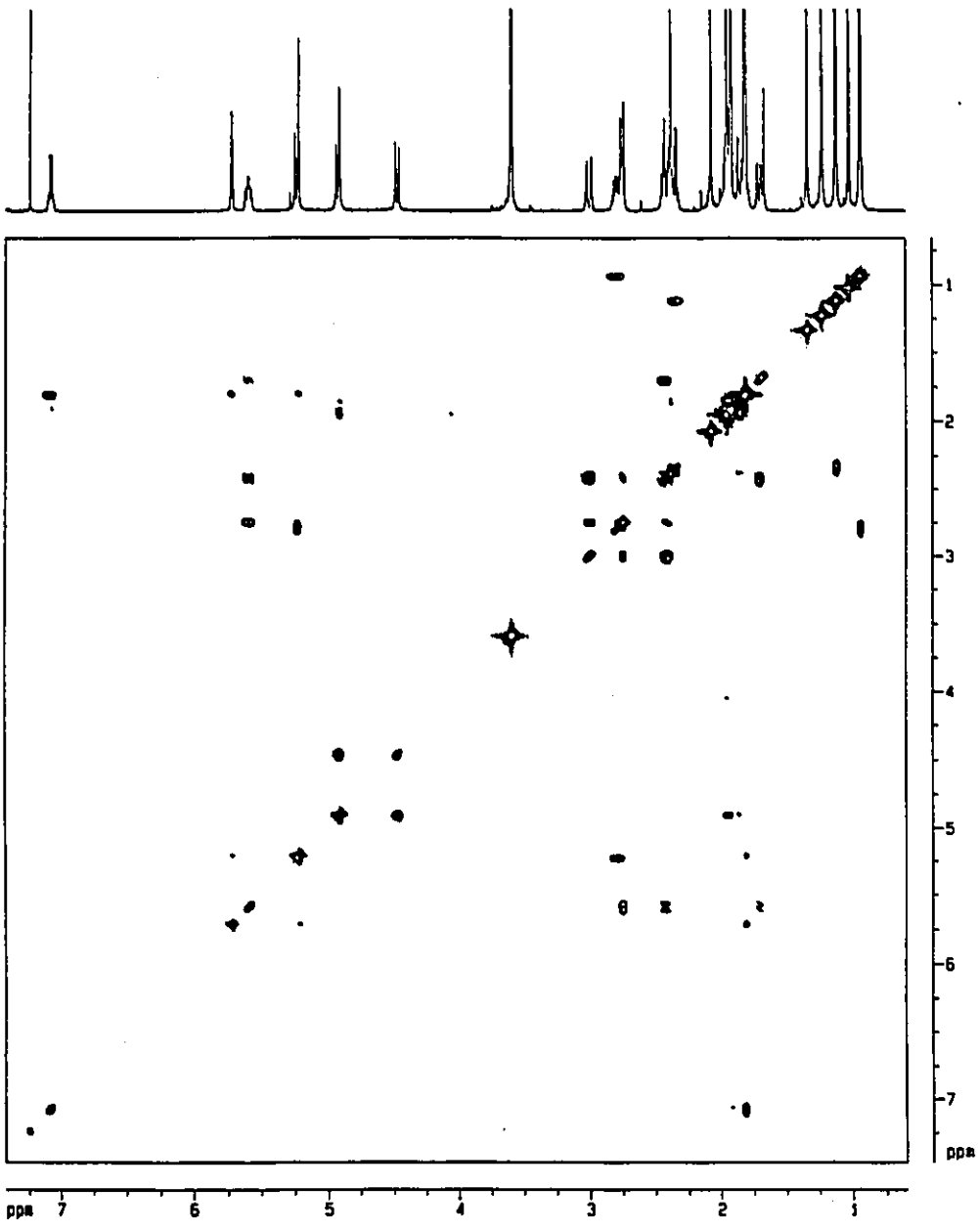


FIGURE 3.2.2.3b: COSY OF SPIROCARACOLITONE B

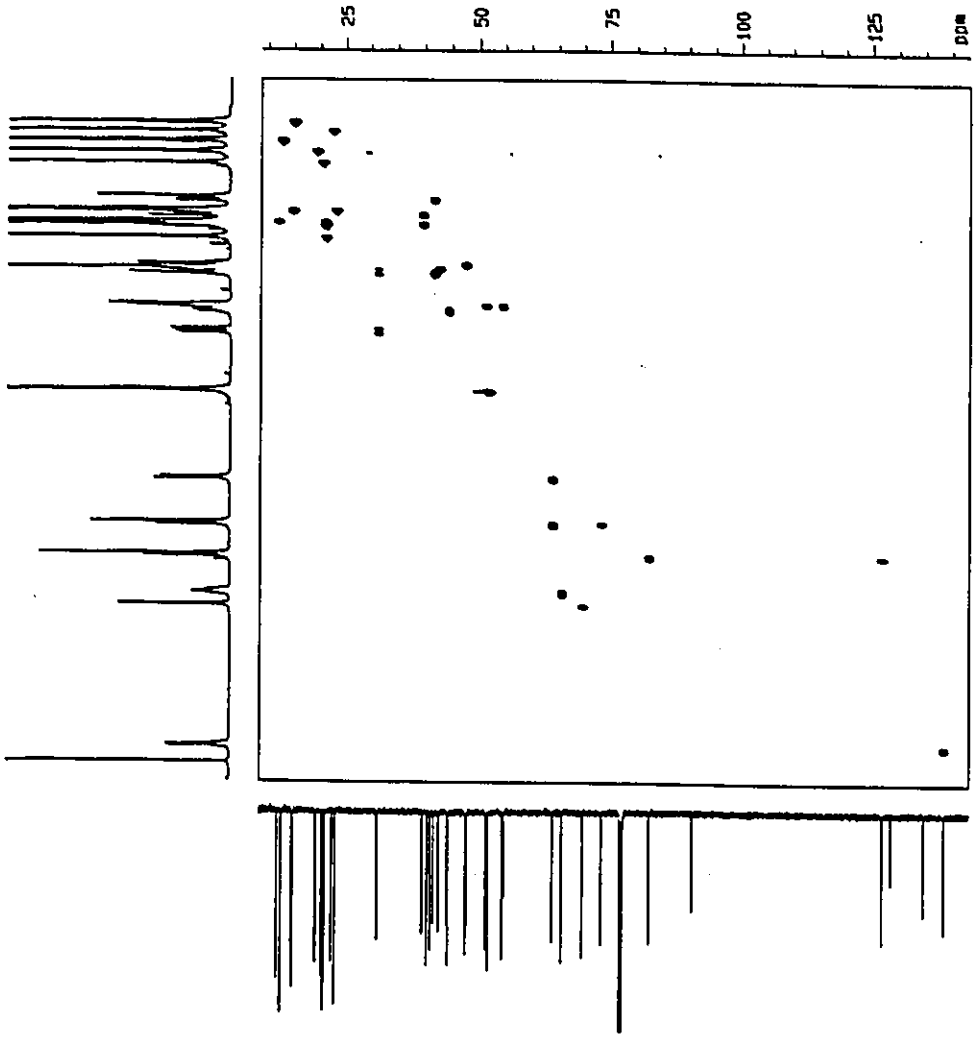


FIGURE 3.2.2.3c: HMQC OF SPIROCARACOLITONE B

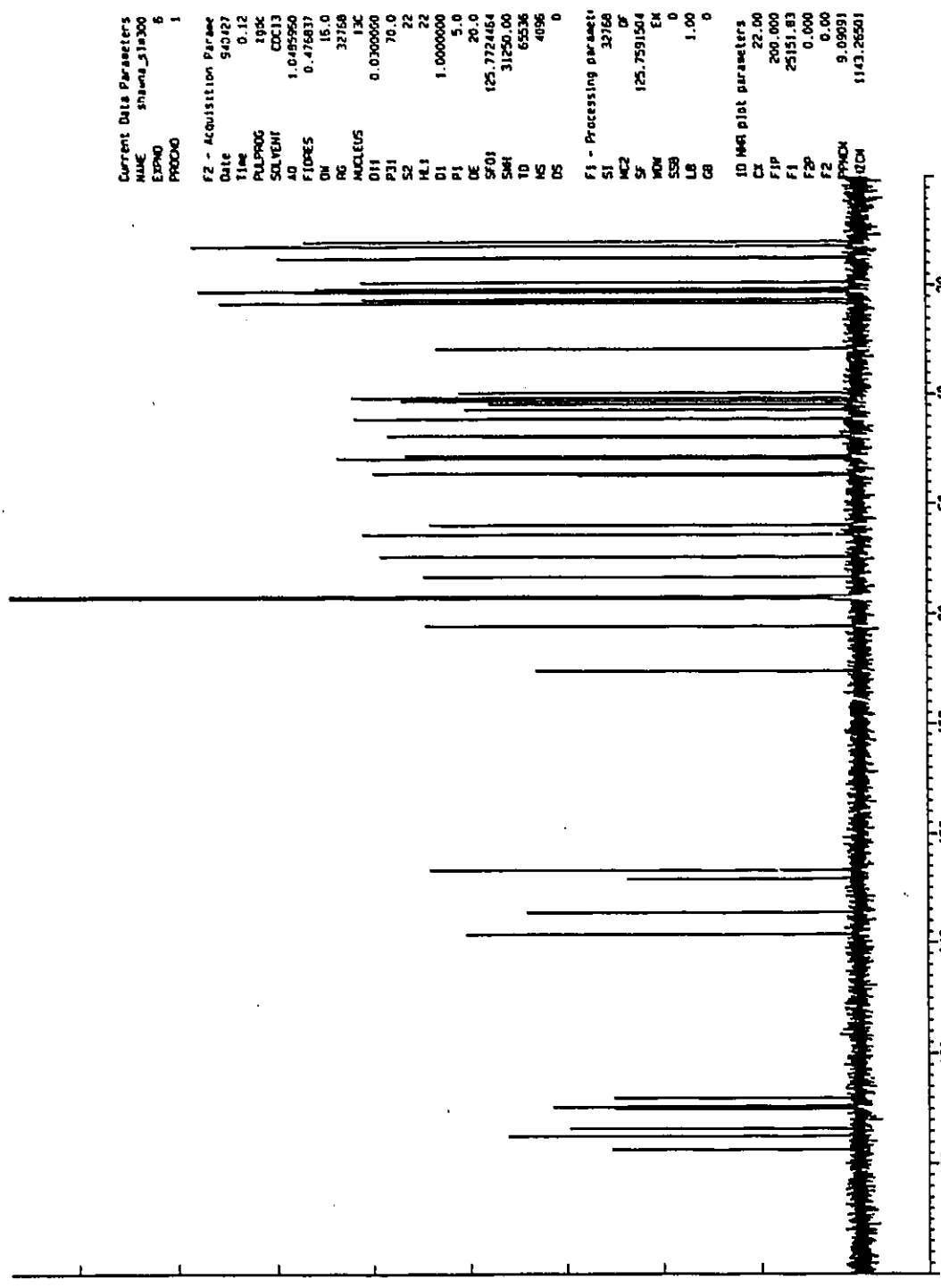


FIGURE 3.2.2.3d: ^{13}C NMR OF SPIROCARACOLITONE B

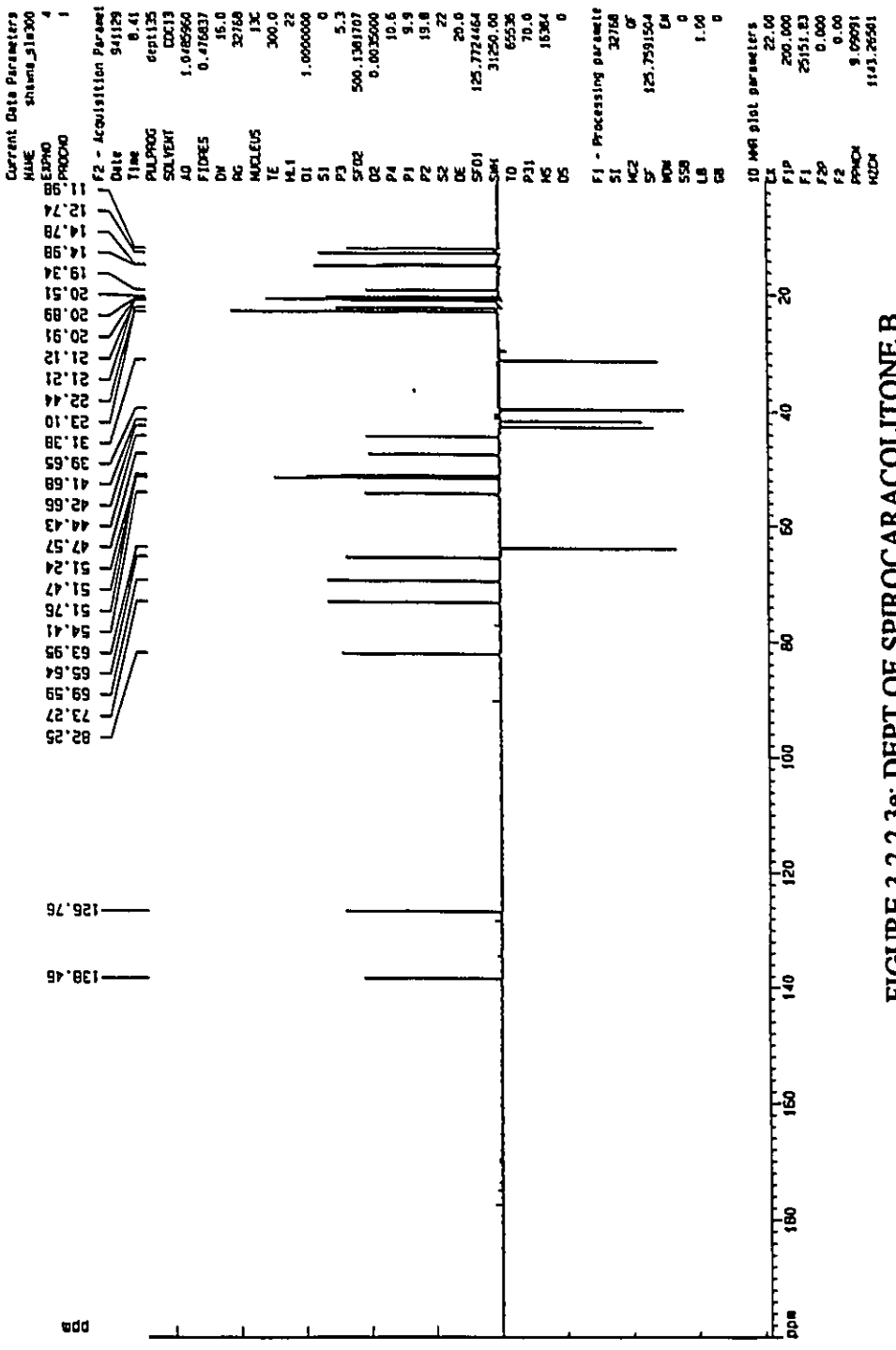
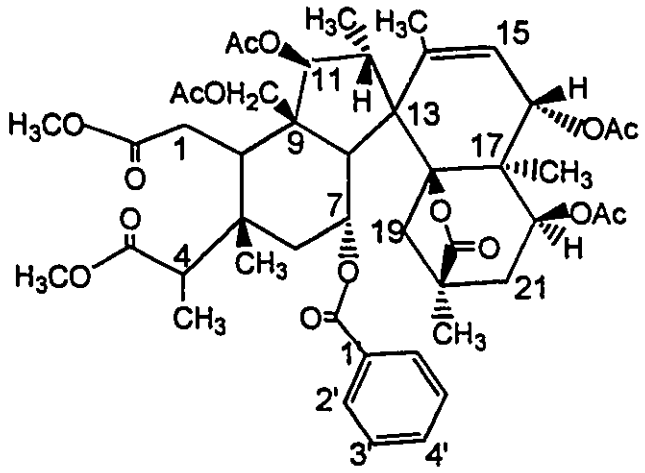


FIGURE 3.2.2.3e: DEPT OF SPIROCARACOLITONE B

3.2.2.4 SPIROCARACOLITONE C

The similarities in the proton and carbon spectra of spirocaracolitone B and spirocaracolitone C (Figure 3.2.2.4a and Figure 3.2.2.4b) made it possible to propose a structure for spirocaracolitone C without X-ray analysis (see Table 3.2.2.4). The same skeleton seen in spirocaracolitone B was proposed for C because of these similarities. The characteristic doublet of triplets for H-7 was located at 5.78 ppm which was in a different location than H-7 observed for spirocaracolitone and spirocaracolitone B (5.31 and 5.58 ppm respectively). This downfield shift suggested that the benzoate group, at 8.26-7.53 ppm, was located at C-7. The remainder of the structure is postulated to be the same as that observed for spirocaracolitone B.



SPIROCARACOLITONE C

The triplet observed at 5.58 ppm was assigned to H-16 because a triplet with the same coupling constant was observed for H-16 in spirocaracolitone B at 5.71 ppm. The carbon shift assignment to this proton, determined to be 69.3 ppm from the HMQC (Figure 3.2.2.4c), matched the carbon shift in spirocaracolitone B. The shift of H-15 in spirocaracolitone B was located at 5.21 ppm. The resonance at 5.12 ppm was

TABLE 3.2.2.4: ^{1}H NMR DATA OF SPIROCARACOLITONE B AND C

PROTON	Spirocaracolitone B (ppm)	Spirocaracolitone C (ppm)
H-1	2.39(m)	2.43(m)
H-1	3.00(dd, J=6.0, 18.3 Hz)	3.01(dd, J=6.0, 18.2 Hz)
H-4	2.34(q, J=7.2 Hz)	2.36(m)
H-6eq	1.70(dd, J=4.4, 12.9 Hz)	1.86(m)
H-6ax	2.39(m)	2.56(dd, J=10.3, 12.8 Hz)
H-7	5.58(dt, J=4.4, 10.9 Hz)	5.78(dt, J=4.6, 10.9 Hz)
H-8	2.74(m)	2.82(m)
H-10	2.74(m)	2.87(d, J=11.7 Hz)
H-11	5.22(d, J~13 Hz)	5.26(d, J=12.1 Hz)
H-12	2.79(m)	2.82(m)
H-15	5.21(brs)	5.12(d, J=1.0 Hz)
H-16	5.71(t, J=2 Hz)	5.58(t, J=2.0 Hz)
H-19	2.37(m)	2.41(m)
H-21	1.85(d, J=14.8 Hz)	1.89(m)
H-21	1.93(m)	1.95(m)
H-22	4.90(d, J=2.4 Hz)	4.88(dd, J=1.3, 4.4 Hz)
H'-3	7.07(dq, J=1.4, 7.0 Hz)	~
C'-2-Me	1.91(s)	~
C'-3-Me	1.81(d, J=7.0 Hz)	~
Benzoate(H'2,3,4)	~	8.26(m), 7.41(m), 7.53(m)
C-4-Me	0.93(d, J=7.4 Hz)	0.95(d, J=7.4 Hz)
C-12-Me	1.12(d, J=7.2 Hz)	1.11(d, J=7.2 Hz)
C-14-Me	1.80(s)	1.85(d, J=1.3 Hz)
Me	1.02(s)	1.05(s)
	1.22(s)	1.21(s)
	1.33	1.38(s)
C'-9-CH2	4.60(d, J=12.9 Hz)	4.51(d, J=12.8 Hz)
	4.91(d, J=12.9 Hz)	4.91(d, J=12.8 Hz)
Me-acetate	1.91(s)	1.78(s)
	1.94(s)	1.97(s)
	1.95(s)	2.00(s)
	2.07(s)	2.09(s)
C-2 and C-3-OMe	3.58(s)	3.60(s)
	3.59(s)	3.61(s)

assigned to H-15 in spirocaracolitone C because the COSY (Figure 3.2.2.4d) showed it coupling only to H-16 and because the carbon shift, obtained from the HMQC (Figure 3.2.2.4c), agreed with the carbon shift assigned to C-15 in spirocaracolitone B. These assignments indicated that the unsaturation present in ring D of spirocaracolitone B was also present in spirocaracolitone C.

The presence of two methoxy resonances suggested the presence of the same A ring cleavage observed in spirocaracolitone B. Comparison of the proton and carbon shifts of the two structures, around the cleaved A ring, resulted in matches for the shifts and coupling constants thus confirming the presence of this feature in spirocarcolitone C. Three sets of doublets corresponding to methyl groups were located at 1.85, 1.11 and 0.95 ppm. These resonances corresponded to those observed for C-14-Me, C-12-Me and C-4-Me in spirocaracolitone B which occurred at 1.81, 1.12 and 0.93 ppm respectively. The remaining proton and carbon resonances agreed with those observed for spirocaracolitone B.

The molecular weight of the proposed structure for spirocaracolitone C, 880 g/mole, agreed with the parent peak observed at 881 m/e $[M+H]^+$ in the FAB MS. Subsequent loss of acetic acid or benzoic acid from the structure explains the ions observed at 821 and 759 m/e respectively. Losses of 60 m/e were also observed for peaks at 821, 759, and 699 m/e. The IR spectrum of spirocaracolitone C contained absorptions corresponding to carbonyl stretches for the E ring lactone and acetates at 1783 and 1737 cm^{-1} respectively. The melting point of spirocaracolitone was determined to be 145-151 °C and a $[\alpha]^{25}D$ of -13.45 was observed in methylene chloride (c= 0.0055).

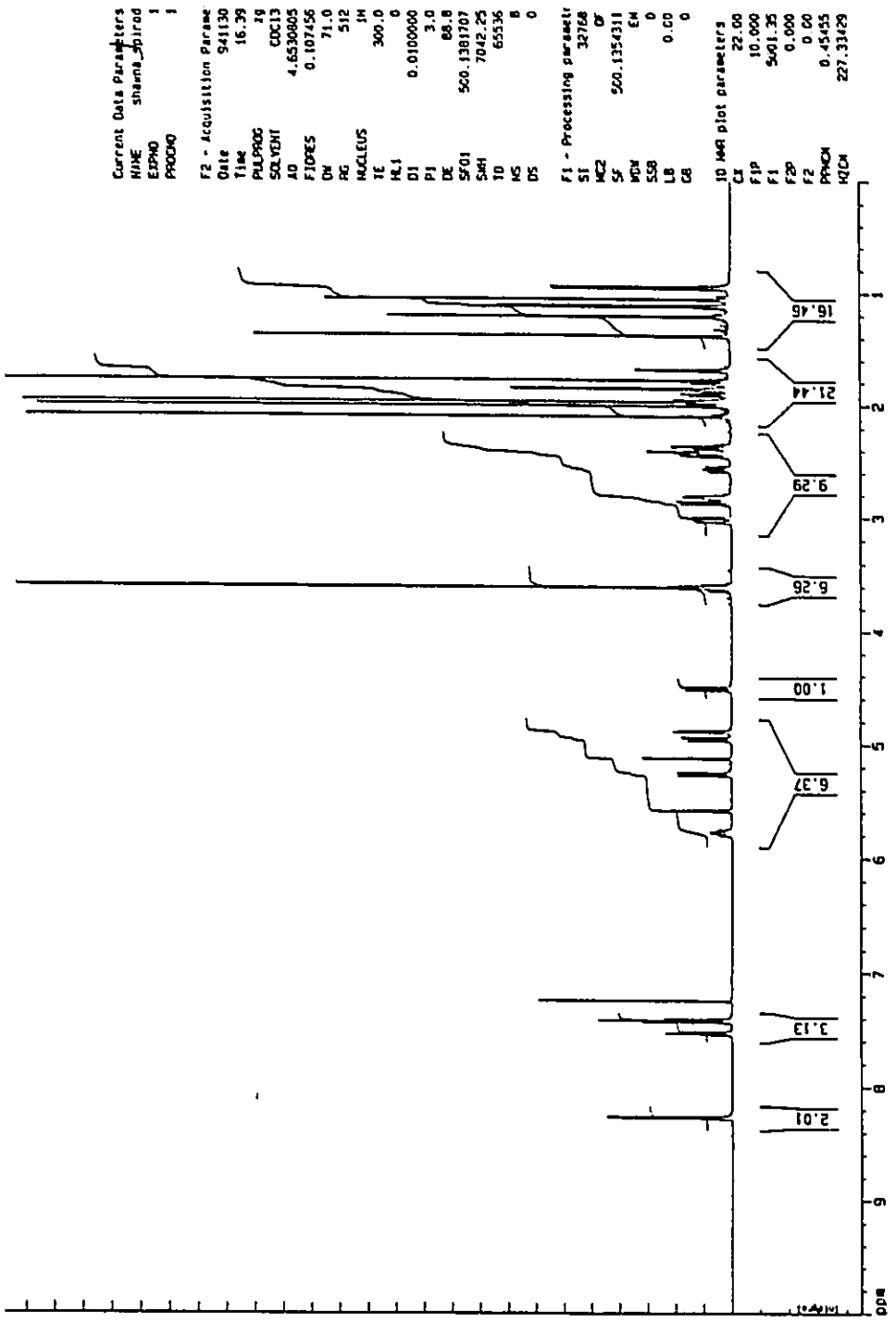


FIGURE 3.2.2.4a: ^{1}H NMR OF SPIROCARACOLITONE C

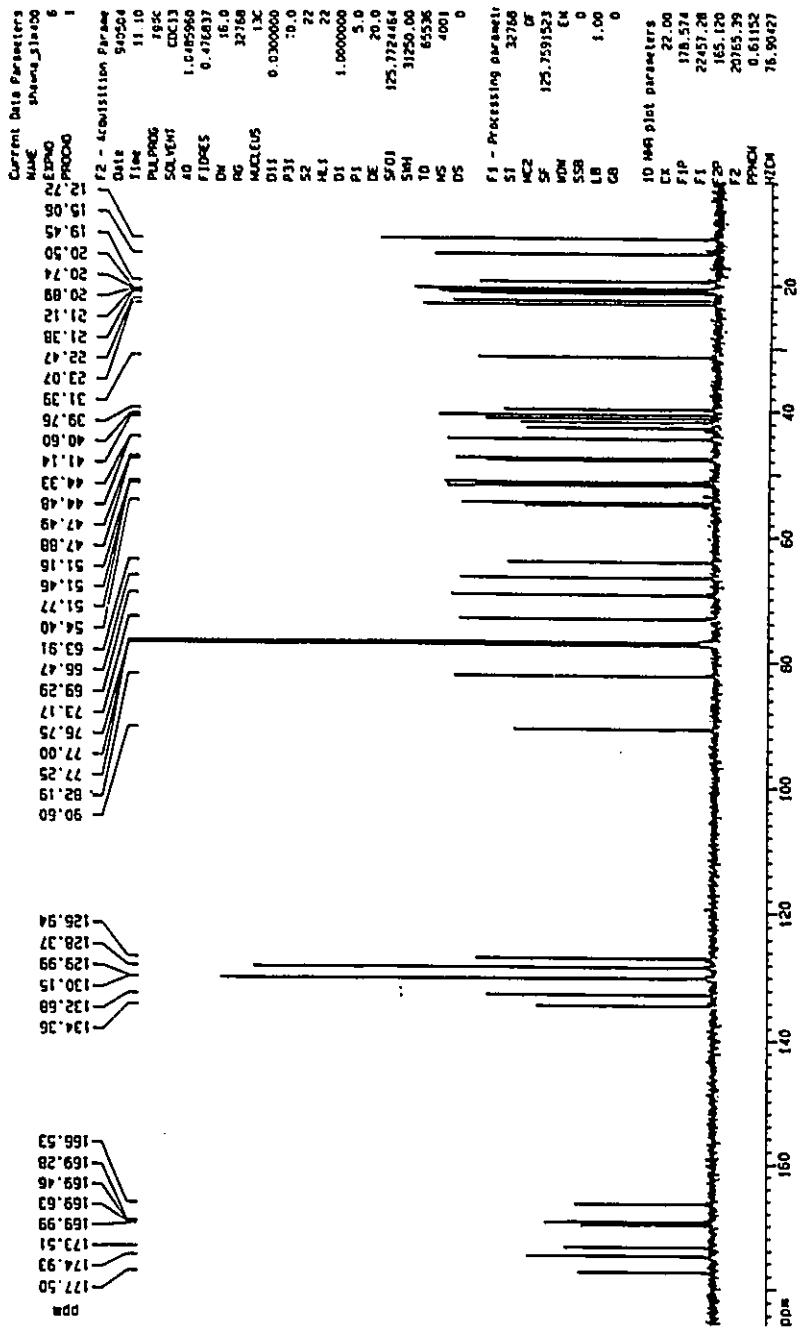


FIGURE 3.2.24 b: ^{13}C NMR OF SPIROCARACOLITONE C

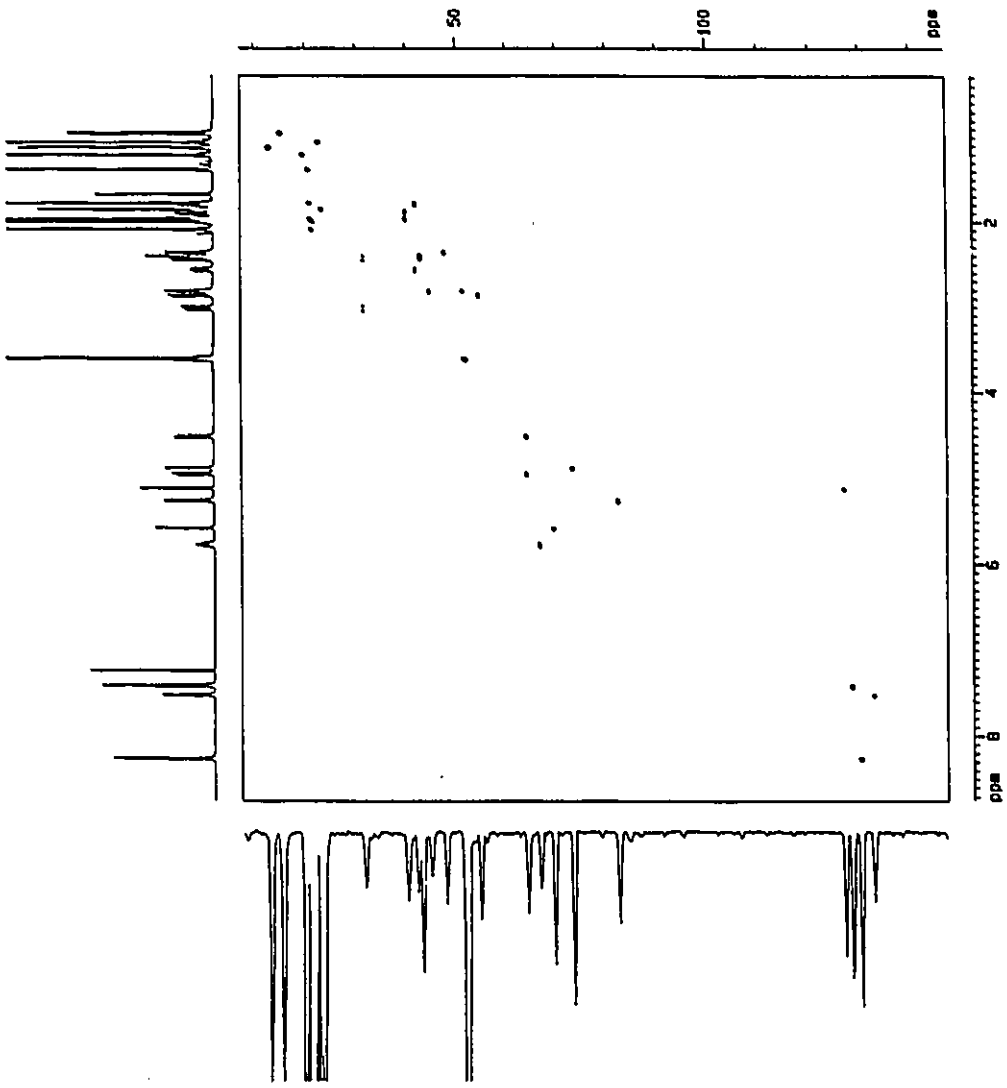


FIGURE 3.2.2.4c: HMQC OF SPIROCARACOLITONE C

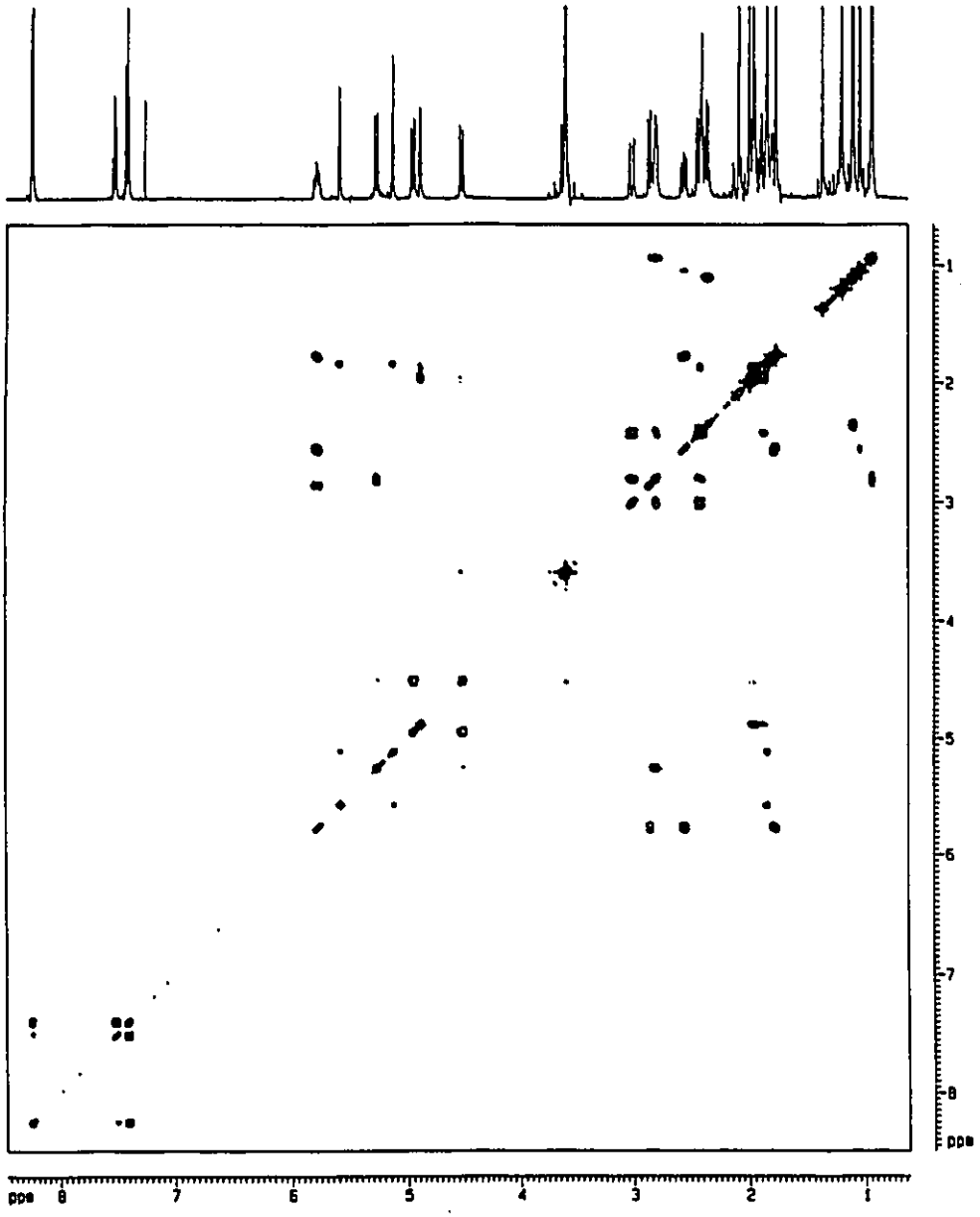
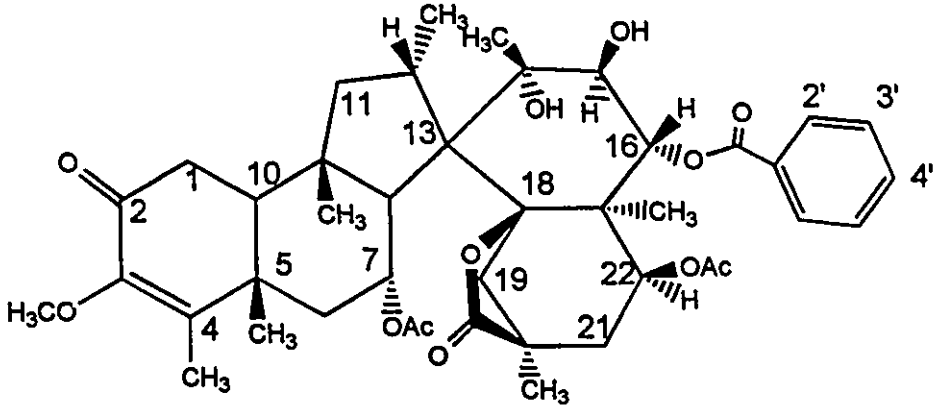


FIGURE 3.2.2.4d: COSY OF SPIROCARACOLITONE C

3.2.2.5 SPIROCARACOLITONE D

The postulated structure for spirocaracolitone D differed only from the structure of spirocaracolitone A in that a benzoate group at C-16 and an alcohol at C-15 replace the acetates that were at these respective positions in spirocaracolitone A. The skeleton was postulated to be similar to spirocaracolitone A because of the similarities in the number of methyne, methylene, methyl and quaternary carbons indicated to be present from the inspection of the ^{1}H nmr (Figure 3.2.2.5a), ^{13}C nmr (Figure 3.2.2.5b) and DEPT (Figure 3.2.2.5c) spectra. Comparison of the proton (Table 3.2.2.5) and carbon shifts of carbons one to 13 revealed matches to all those exhibited for spirocaracolitone and spirocaracolitone A therefore substantiating the presence of this half of the molecule (see Table 3.2.2.5). Coupling constants for the proton-proton interactions also agreed with those of spirocaracolitone and spirocaracolitone A.



SPIROCARACOLITONE D

The presence of alcohol moieties was confirmed by the IR absorption at 3542 cm^{-1} and also by the broad peaks at 1.65 and 3.31 ppm in the ^{1}H nmr which disappeared from the proton spectrum with the addition of D_2O. Proton and carbon shifts for C-18 to C-22 also

compared favorably to those of spirocaracolitone A. The benzoate group, 7.40-7.98 ppm in the proton nmr, and the additional alcohol were placed at C-16 and C-15 respectively because changes in their proton shifts occurred but the coupling constant between these two protons remained the similar to what was observed in spirocaracolitone A (Table 3.2.2.5).

Unsaturation at C-14-15 was ruled out because the parent peak in the FAB MS was 751 m/e and not 749 m/e. The molecular weight of 750 g/mole agreed with that calculated for the proposed structure. Loss of the benzoate and acetate fragments were represented in the mass spectrum. Spirocaracolitone D melted at 251-256 °C with decomposition and had a $[\alpha]^{25}D$ of -21.67 in methylene chloride (c=0.0024).

TABLE 3.2.2.5: ^{1}H NMR DATA OF SPIROCARACOLITONE A, D AND E

PROTON	Spirocaracolitone A (ppm)	Spirocaracolitone D (ppm)	Spirocaracolitone E (ppm)
H-1eq	2.34(m)	2.35(dd, J=3.5, 17.3 Hz)	2.33(dd, J=3.5, 17.4 Hz)
H-1ax	2.56(dd, J=14.2, 17.1 Hz)	2.55(dd, J=14.3, 17.2 Hz)	2.53(dd, J=14.2, 17.2 Hz)
H-6	1.18(m)	1.24(m)	1.14(m)
H-6	2.35(m)	2.24(m)	2.45(dd, J=3.0, 12.9 Hz)
H-7	5.27(dt, J=3.7, 10.4 Hz)	5.43(dt, J=3.1, 10.4 Hz)	4.13(dt, J=3.2, 10.4 Hz)
H-8	2.05(m)	1.98(d, J=10.4 Hz)	1.83(d, J=7.8 Hz)
H-10	1.74(dd, J=3.4, 14.2 Hz)	1.70(dd, J=3.4, 14.1 Hz)	1.67(dd, J=3.3, 14.3 Hz)
H-11	1.28(m)	1.24(m)	1.29(m)
H-11	1.52(dd, J=5.2, 12.4 Hz)	1.54(m)	1.50(dd, J=5.8, 12.0 Hz)
H-12	2.56(m)	2.79(m)	2.61(m)
H-15	5.95(d, J=3.7 Hz)	4.53(brs)	5.45(d, J=3.3 Hz)
H-16	5.18(d, J=3.7 Hz)	6.01(d, J=4.8 Hz)	6.40(d, J=3.3 Hz)
H-19	2.32(m)	2.27(m)	2.33(m)
H-19	2.49(d, J=12.7 Hz)	2.49(d, J=12.8 Hz)	2.58(d, J=12.8 Hz)
H-21	1.78(d, J=14.4 Hz)	1.86(d, J=14.9 Hz)	1.81(m)
H-21	2.06(m)	2.00(m)	2.11(dd, J=4.5, 14.9 Hz)
H-22	4.89(dd, J=1.3, 4.4 Hz)	4.92(dd, J=1.5, 4.4 Hz)	4.92(d, J=4.4 Hz)
Benzoate (H'-2,3,4)	~	7.95(m), 7.43(m), 7.56(m)	7.83(m), 7.35(m), 7.48(m)
C-4-Me	1.82(s)	1.82(s)	1.86(s)
C-12-Me	1.35(d, J=8.3 Hz)	1.31(d, J=7.3 Hz)	1.40(d, J=7.2 Hz)
Me	1.13(s)	1.18(s)	1.09(s)
	1.22(s)	1.19(s)	1.17(s)
	1.35(s)	1.25(s)	1.20(s)
	1.55(s)	1.53(s)	1.45(s)
	1.92(s)	1.53(s)	1.70(s)
Me-acetate	1.96(s)	2.03(s)	1.97(s)
	2.08(s)	2.15(s)	2.06(s)
	2.14(s)	~	~
	2.15(s)	~	~
C-3-OMe	3.57(s)	3.58(s)	3.58(s)
C-14-OH	4.13(brs)	1.65(brs)*	~
C-15-OH	~	3.31(brs)*	~

NOTE: * = shifts interchangeable. ~ = protons absent in structure or in proton nmr.

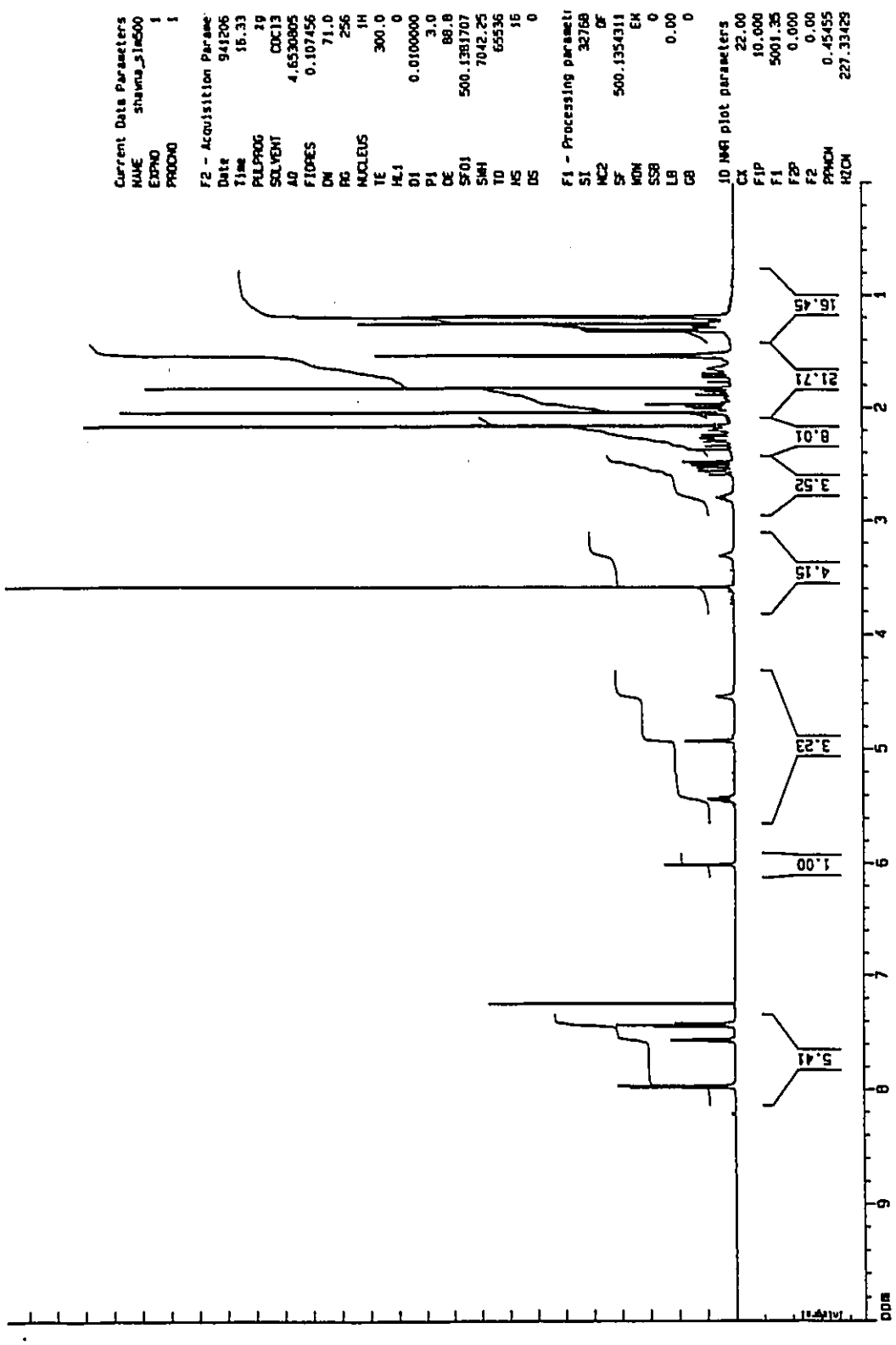


FIGURE 3.2.2.5a: ^{1}H NMR OF SPIROCARACOLITONE D

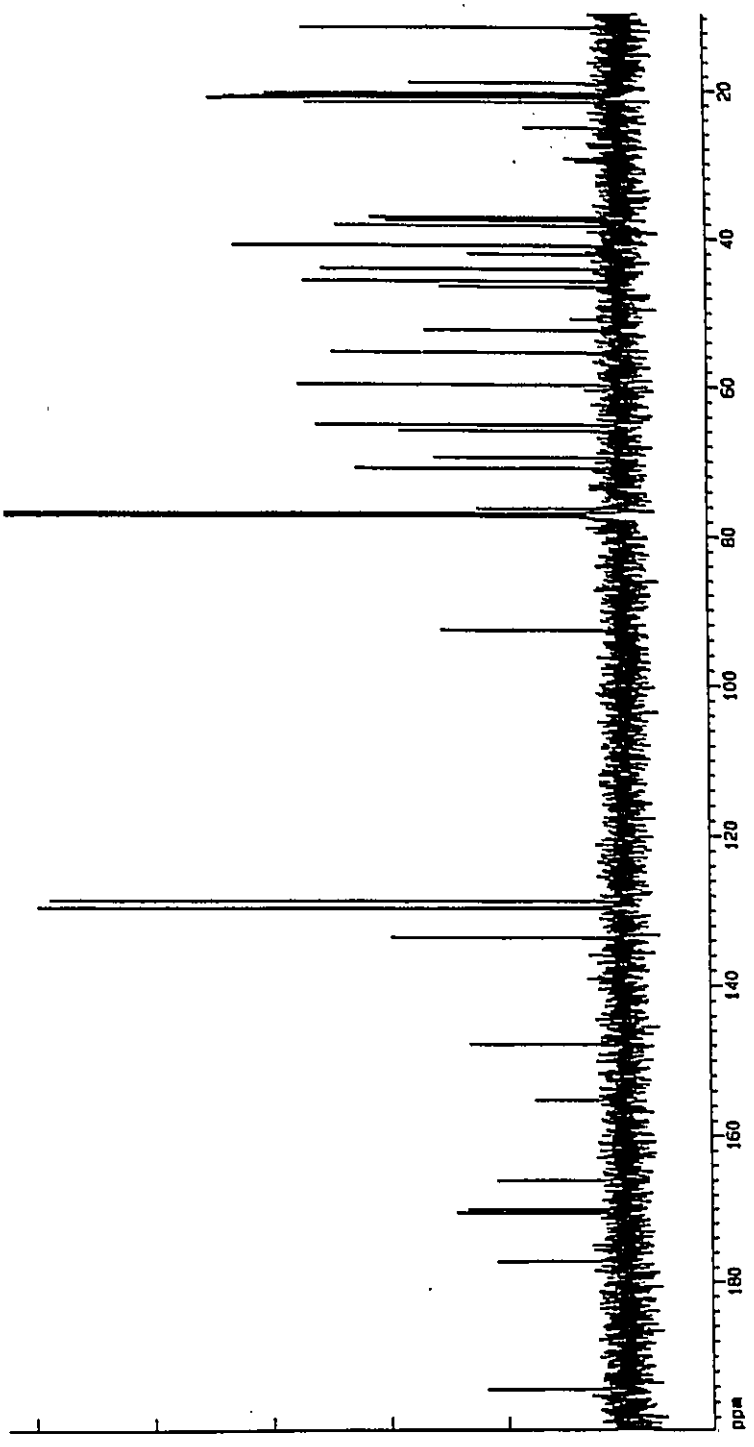


FIGURE 3.2.2.5b: ^{13}C NMR OF SPIROCARACOLITONE D

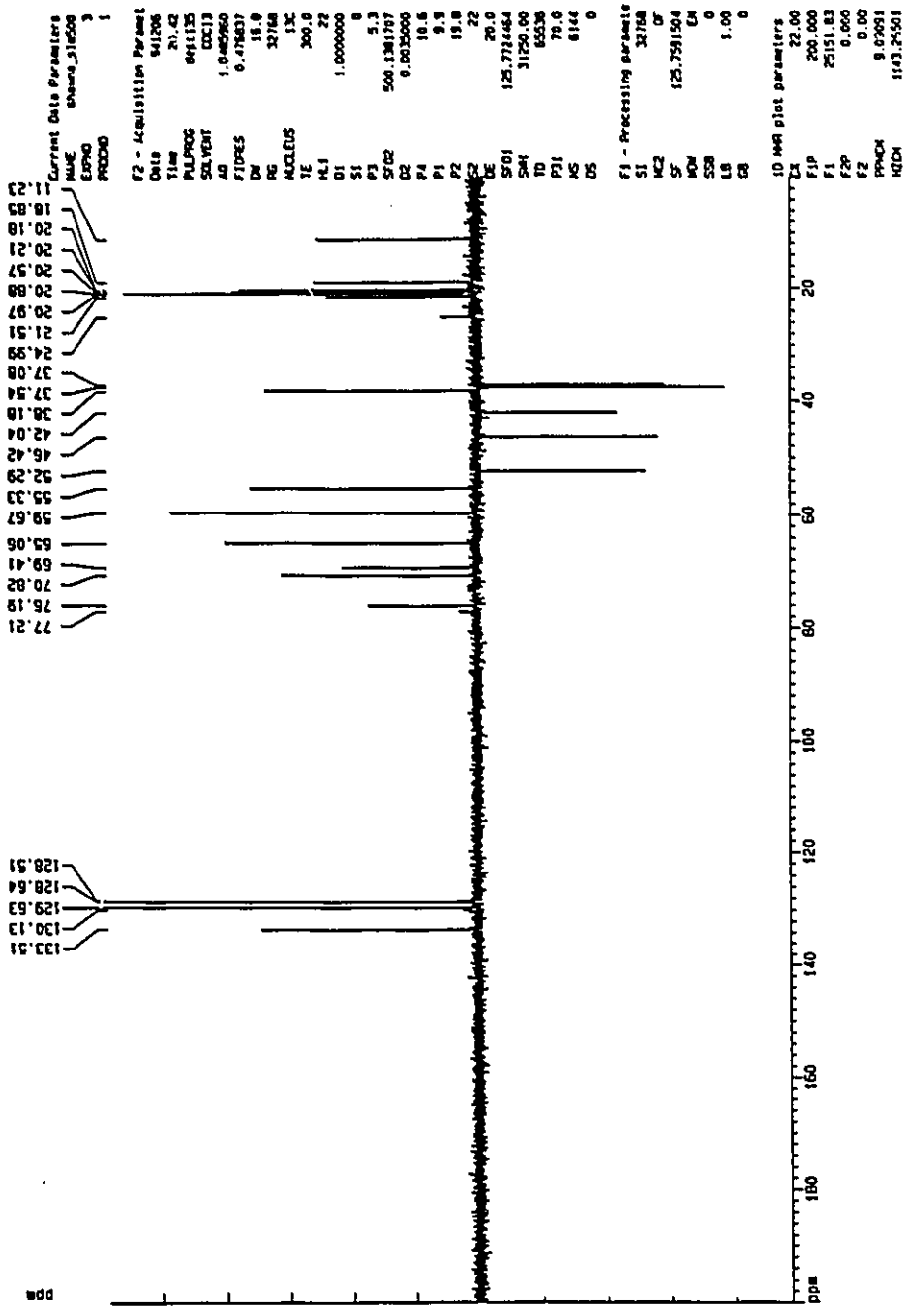
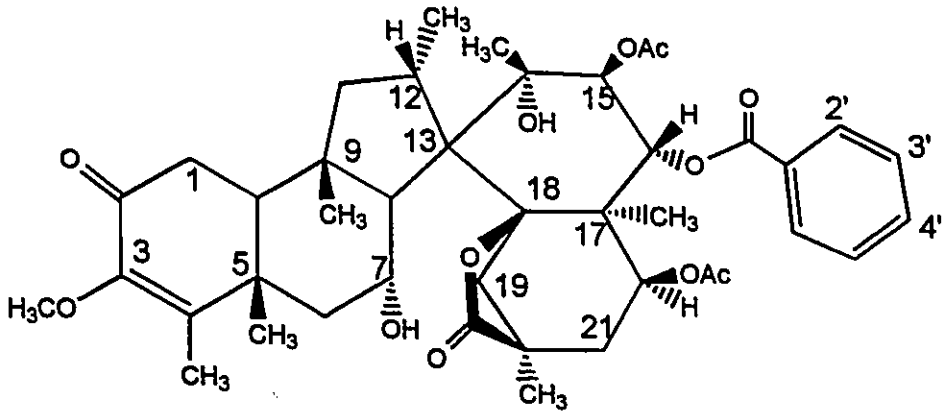


FIGURE 3.2.2.5c: DEPT OF SPIROCARACOLITONE D

3.2.2.6 SPIROCARACOLITONE E

The proposed structure for spirocaracolitone E differs from that of spirocaracolitone A in that an alcohol at C-7 and a C-16 benzoate replace the acetates that were at these respective positions in spirocaracolitone A. Comparison of the proton (Figure 3.2.2.6a) and carbon (Figure 3.2.2.6b) nmr spectra of spirocaracolitone E to that of spirocaracolitone A and D revealed many similarities therefore making the assignment of the structure of spirocaracolitone E relatively easy. The similarities in the proton assignments can be seen in Table 3.2.2.5.



SPIROCARACOLITONE E

The presence of the C-7 and C-14 alcohols was not detected in the proton nmr but were determined to be present from the absorptions in the IR at 3539 and 3301 cm-1. An alcohol was assigned to C-7 because the chemical shift of H-7 appeared at 4.13 ppm and not at 5.27 or 5.43 ppm which was the case in spirocaracolitone A and D. From the FAB MS the molecular weight of spirocarcolitone E was determined to be 750 g/mole, an $[M+H]^+$ of 751 m/e, which agreed with the proposed structure. A cluster was observed at 1502 m/e which represented $2[M+H]^+$. IR absorptions at 1775, 1738 and 1675 cm^{-1} confirmed the presence of

the lactone in ring E, acetates and benzoate and the enone respectively. Spirocaracolitone E decomposed at 228 °C and had an $[\alpha]^{25}D$ of -20.43(c=0.0046) in methylene chloride.

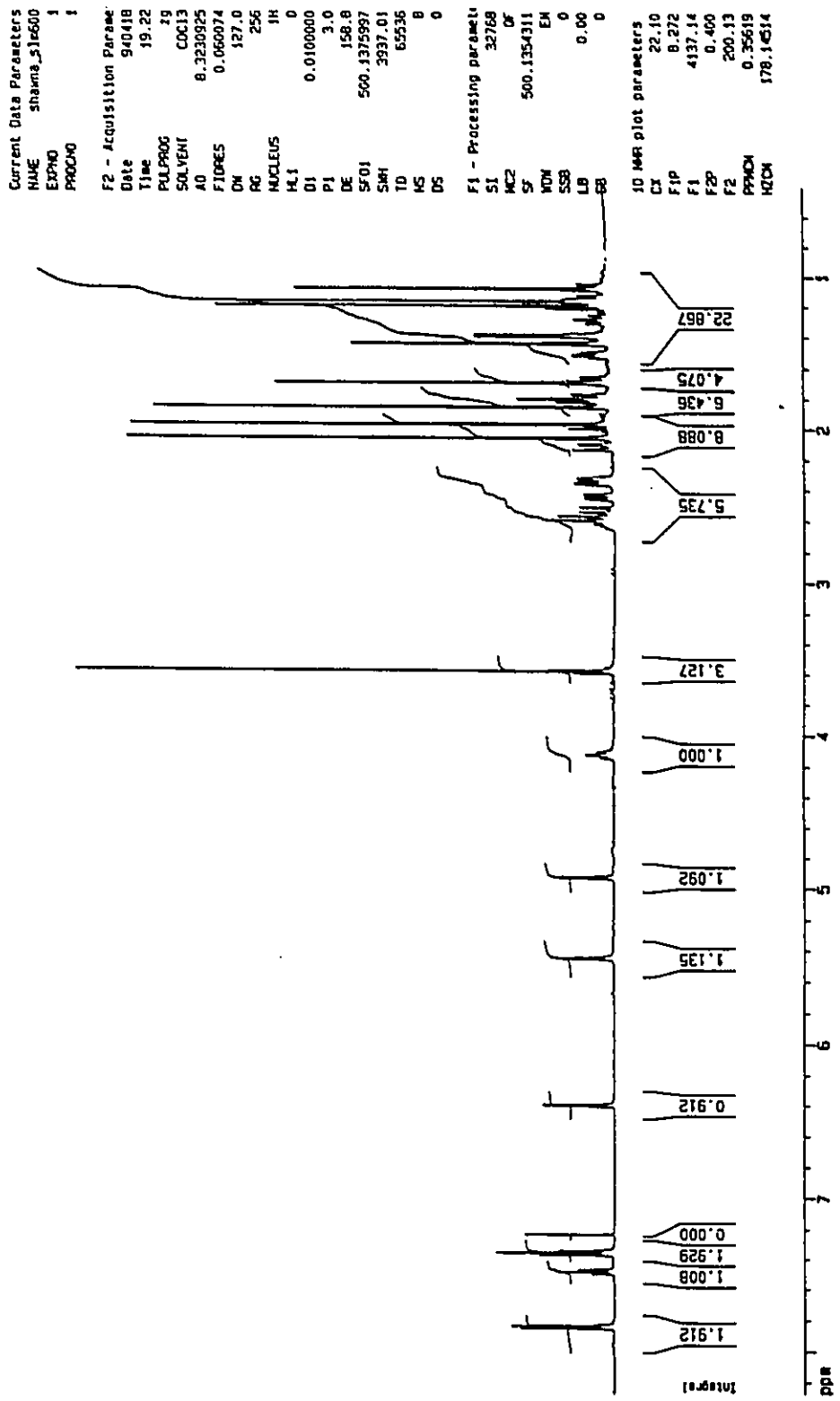


FIGURE 3.2.2.6a: ^{1}H NMR OF SPIROCARACOLITONE E

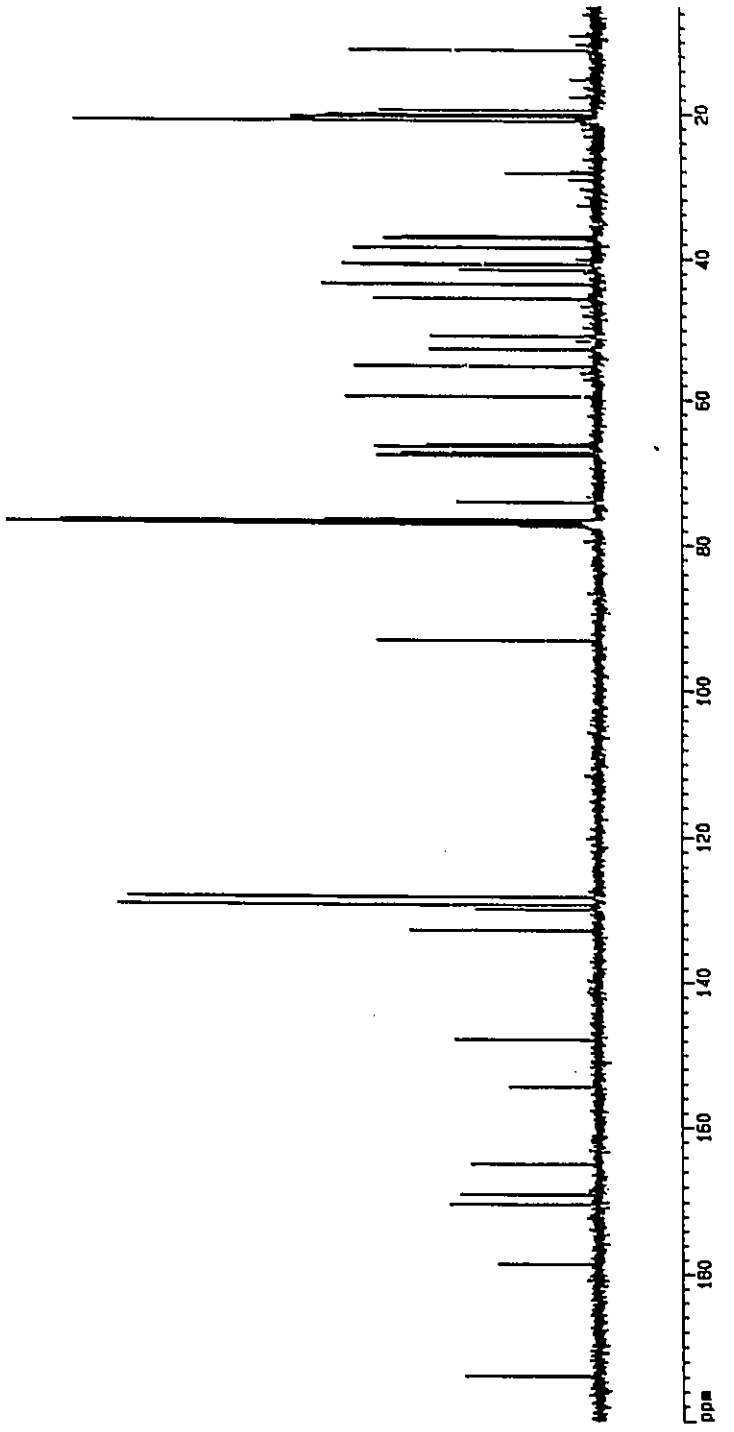
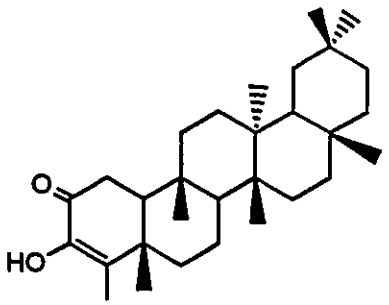


FIGURE 3.2.2.6b: ^{13}C NMR OF SPIROCARACOLITONE E

3.2.3 BIOSYNTHETIC CONSIDERATIONS

The structure of the spirocaracolitones are most interesting from a biosynthetic point of view since several of their structural features are rare or have not been described previously in pentacyclic triterpenoids. There appear to be no examples of such species bearing a C-12 methyl group or a spiro CD ring system and it is highly probable that these two features are biogenetically interdependent. Spiro systems in triterpenoids are rare. A recent example which has a spiro BC ring system is spirosupinanonediol isolated from *Euphorbia supina*.[32] The oxidation pattern of ring A is also unusual but examples do exist, e.g. 3-hydroxyfriedel-3-en-2-one, isolated from the bark of *Quercus suber*.[33] The presence of the C-30-C-18 γ-lactone, the C-21 and C-22 cis diacetates and the C-16 allylic benzoate are features which have been individually reported previously[34] but not in combination.

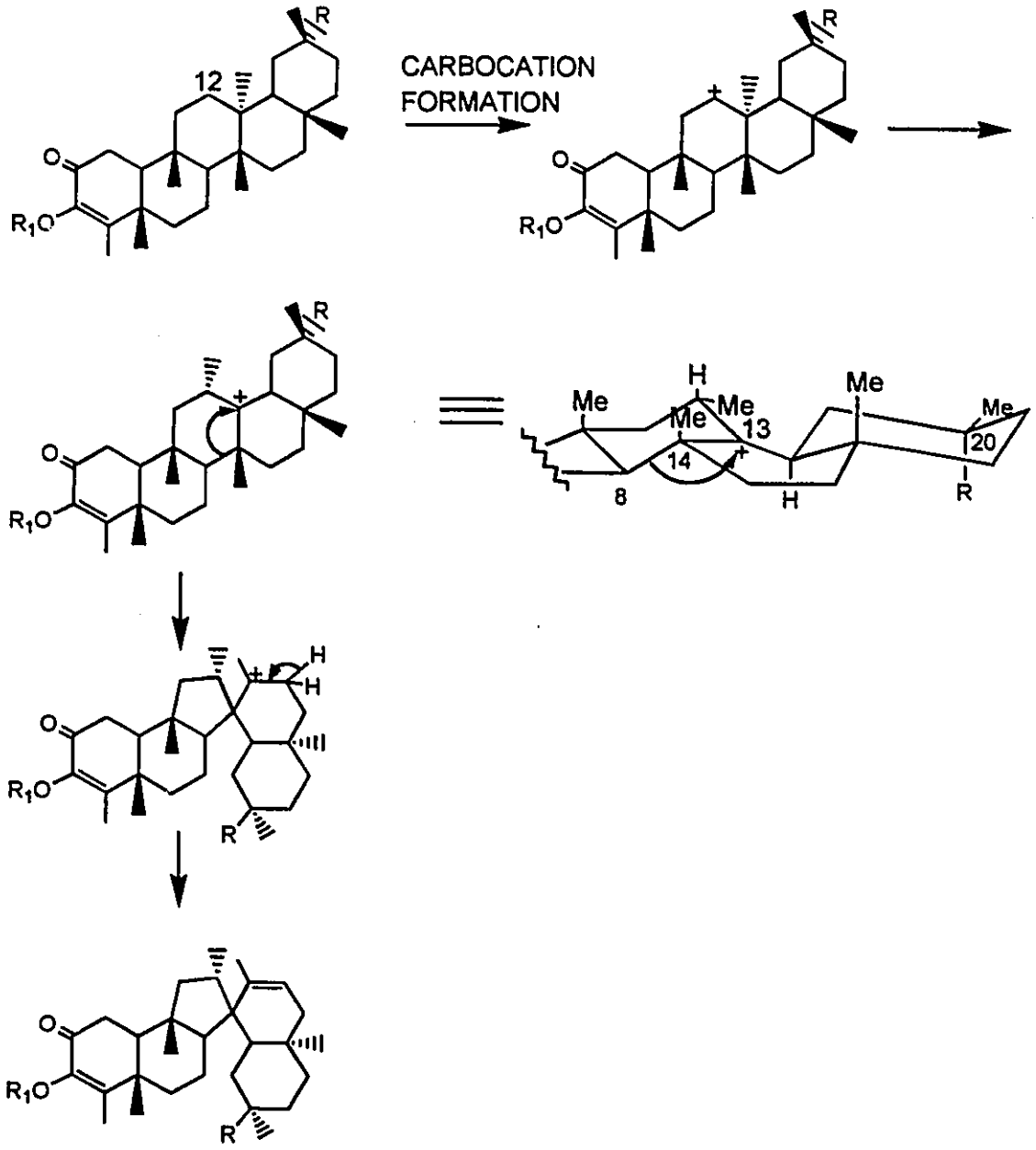


3-HYDROXYFRIEDEL-3-EN-2-ONE

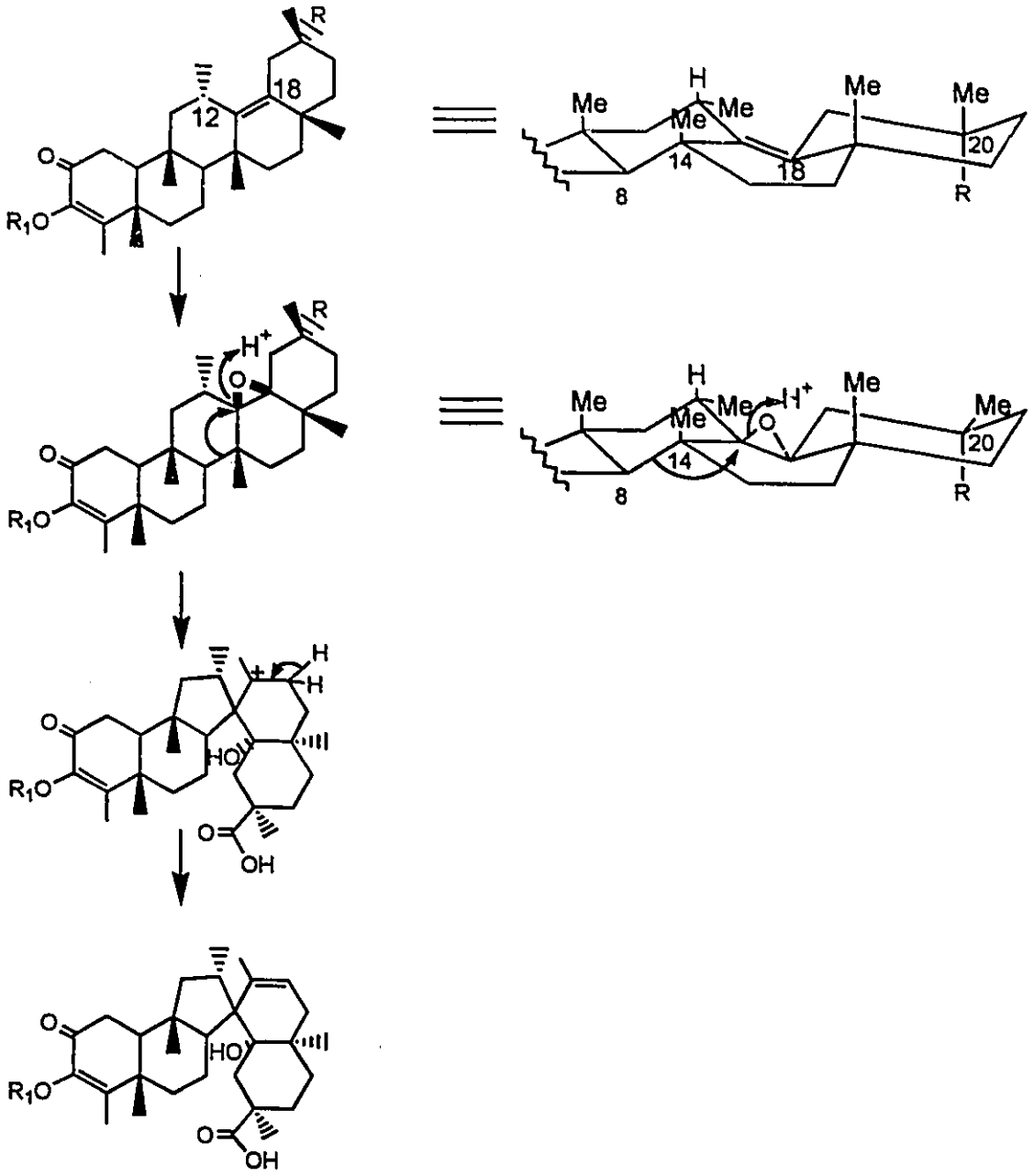
The spirocaracolitones are belived to be rearranged highly oxidized friedelin derivatives. It is interesting to speculate on the genesis of the spiro ring system. This can plausibly be derived from a species such as 3-hydroxyfriedel-3-en-2-one or friedelin itelf in which generation of a carbocation at C-12 is followed by migration of the α-C-13 methyl group. Carbocation formation at C-12 might possibly result from enzymatic hydroxylation at C-12 followed by loss of water or via protonation of a friedelin having a C-11-C-12 double

bond (Scheme 3.2.3a). The newly created C-13 carbocation could undergo a C-8-C-14 bond migration to generate the CD spiro ring system. Enzymatic hydroxylation at C-7, C-16, C-18, C-20 and C-21, and lactonization at C-18-C-30 would result in the final product spirocaracolitone.

Alternatively, a C-13-C-18 double bond could be formed from the C-13 carbocation and through the loss of H-18. Epoxidation of this bond from the β side followed by acid catalyzed ring opening and migration of the antiperiplanar C-8-C-14 bond also generates the spiro system at C-13 (Scheme 3.2.3b). This pathway also results in the formation of a tertiary hydroxyl group at C-18 which could readily be displaced by the C-30 carboxylate to form the γ-lactone. The feature resulting from the epoxide intermediate needs to be balanced by the requirement for epoxidation of the C-13-C-18 double bond from what appears by examination of molecular models the more hindered side, the same side occupied by the C-14 and C-17 axial methyl groups. Enzymatic hydroxylation at C-7, C-16, C-20 and C-21, and C-18-C-30 lactonization via a C-18 carbocation would result in the formation of spirocaracolitone.



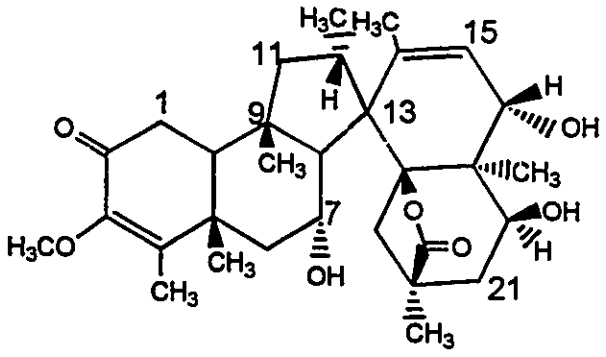
SCHEME 3.2.3a: FORMATION OF THE SPIROCARACOLITONE RING SYSTEM VIA C-8-C-14 BOND MIGRATION TO A C-13 CARBOCATION



SCHEME 3.2.3b: FORMATION OF THE SPIROCARACOLITONE RING SYSTEM VIA A C-13-C-18 EPOXIDE

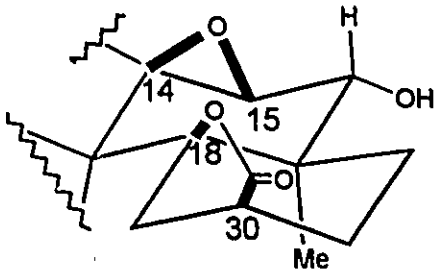
The sequence for the introduction of various oxygen atoms in the spiro-compounds is not known. The oxygenation of ring A could occur either before or after the formation of the spiro ring system. A similar situation holds for the oxidation of one of the methyl groups at C-20 to a carboxylic acid and the other various hydroxylations. It seems reasonable that the hydroxylation at C-16 occured as an allylic hydroxylation after the introduction of the C-14-C-15 double bond.

Amongst the spiro compounds themselves one can postulate the following relationship. If a "parent" spiro species is assumed then one could expect it should contain the features shown in the following structure. Further hydroxylation at C-21, benzoylation at C-16 and acetylation of the remaining hydroxyl groups could lead to spirocaracolitone. From the parent molecule spirocaracolitone B and C, which differ only in the presence of a tigloyl or benzoyl group at C-7, could be obtained after oxidative opening of the A ring possibly by initial hydration, Baeyer-Villiger oxidation and subsequent ring opening. Whether the ring A modification preceeds or follows the C-11 methylene group and the C-9 methyl hydroxylation steps is unknown.

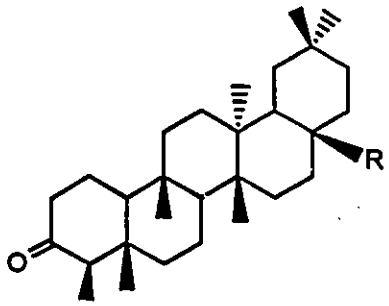


PARENT SPIRO SPECIES

Finally, the formation of spirocaracolitone A, D and E from the parent spiro species is readily envisaged as the result of an epoxidation of the double bond at C-14-C-15 forming the partial structure below. Trans diaxial ring opening of this epoxide with water leads to the oxygenation pattern, including the stereochemistry at C-14 and C-15 in all three compounds which differ only in the degree of acylation or benzylation.



Chromatography of the hexane extract has afforded the friedelin derivative canophyllol. Thus *R. caracolito* appears to be a source of compounds belonging to the friedelin group. Carefull re-examination of the hexane and less polar CH_2Cl_2 bark extracts or extracts of other parts of this tree could result in the identification of possible precursors or intermediates to help secure the biosynthesis of these unusual spiro-compounds.



FRIEDELIN (R=CH_3)
CANOPHYLLOL (R=CH_2OH)

3.3.0 EVALUATION OF THE EFFECTS OF THE SPIROCARACOLITONES ON THE NEONATE LIFE CYCLE OF THE EUROPEAN CORN BORER

Inspection of the graph of the mean larval weights of ECB exposed to spirocaracolitones (Figure 3.3.0) revealed that spirocaracolitone and spirocaracolitones B-D caused significant growth reduction in ECB larvae when incorporated at 50 ppm into diets fed to larvae beginning at the neonate stage. The highest growth reduction was elicited by spirocaracolitone C. All of the spirocaracolitone compounds caused some larval mortality at 5 ppm but at 50 ppm the effect was more pronounced. (Table 3.3.0a) Spirocaracolitone C at 50 ppm caused 21 larval deaths out of the 30 larvae in the study. No strong trends were observed in the number of incomplete larval intermediates or pupal deaths.

Prelongation of the development time of ECB larvae to pupation and decrease in pupal weight were observed, as compared to controls, for spirocaracolitone C and spirocaracolitone B at 50 ppm (Table 3.3.0b). The results for the number of days to pupation were not influenced by gender. A difference in the male and female pupal weights was observed in the control and similarly in the test compounds except in the case of spirocaracolitone C where there were no male pupae alive at this stage of the study. Reduction in adult weight was observed for spirocaracolitone B but was not observed for spirocaracolitone C because its value represented a female population only. An overall reduction in the adult female weights were observed for all the test compounds but a marked reduction in the male weights was observed only for spirocaracolitone and spirocaracolitone B.

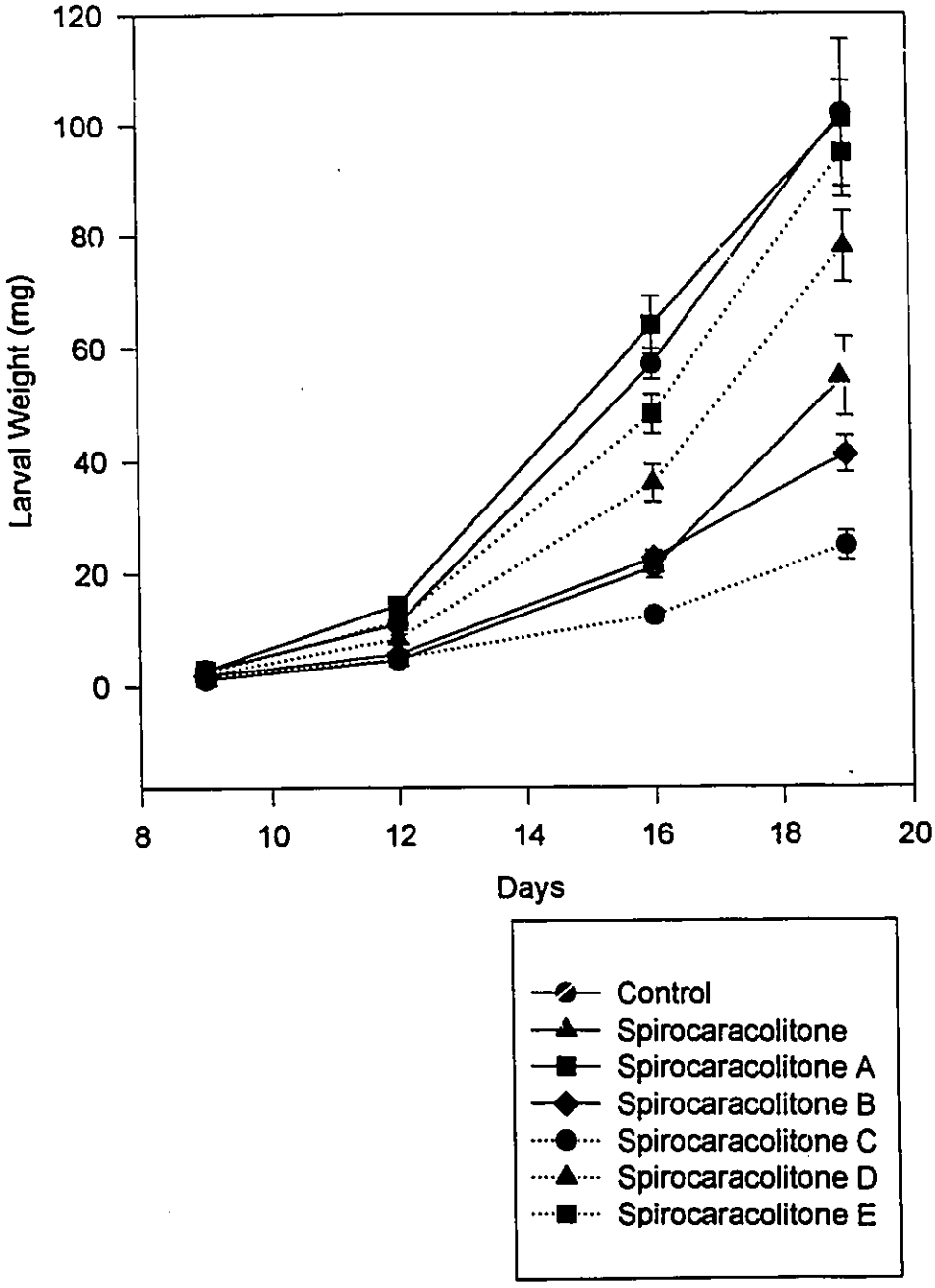


FIGURE 3.3.0: MEAN LARVAL WEIGHTS OF ECB EXPOSED TO SPIROCARACOLITONES(50ppm) IN DIET.

TABLE 3.3.0a : EFFECTS OF SPIROCARACOLITONES ON THE DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)(n=30)

COMPOUND	Concentration (PPM)	Number of Incomplete Larval Intermediates	Number of Larval Deaths	Number of Pupal Deaths
CONTROL	5	1	-	3
SPIROCARACOLITONE	5	1	2	2
SPIROCARACOLITONE A	5	5	4	1
SPIROCARACOLITONE B	5	1	4	2
SPIROCARACOLITONE C	5	3	2	3
SPIROCARACOLITONE D	5	2	3	1
SPIROCARACOLITONE E	5	1	2	1
SPIROCARACOLITONE	50	-	11	5
SPIROCARACOLITONE A	50	5	4	1
SPIROCARACOLITONE B	50	1	10	-
SPIROCARACOLITONE C	50	-	21	1
SPIROCARACOLITONE D	50	2	7	1
SPIROCARACOLITONE E	50	2	3	1

TABLE 3.3.0b : EFFECTS OF SPIROCARACOLITONES AT 50 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUNDS	Days to Pupation (Days)	Days to Pupation		Pupal Weight (mg)	Pupal Weight		Adult Weight (mg)	Adult Weight	
		Female (Days)	Male (Days)		Female (mg)	Male (mg)		Female (mg)	Male (mg)
CONTROL	23.1 (0.24)	23.1 (0.30)	22.8 (0.28)	86.1 (2.9)	103 (3.6)	76.7 (1.7)	44.4 (3.2)	62.5 (2.8)	34.1 (0.94)
SPIROCARACOLITONE	26.6 (0.38)	26.5 (0.33)	26.0 (0.89)	84.7 (4.6)	100 (6.3)	73.1 (3.5)	41.0 (4.4)	52.5 (4.3)	25.7 (0.54)
SPIROCARACOLITONE A	22.9 (0.36)	22.8 (0.29)	22.0 (0.66)	87.2 (3.0)	99.1 (1.8)	71.9 (2.0)	45.1 (3.5)	56.2 (1.2)	29.3 (2.0)
SPIROCARACOLITONE B	29.3 (0.74)	30.8 (2.7)	27.6 (0.93)	72.7 (3.5)	85.3 (4.4)	65.6 (2.8)	34.1 (4.0)	51.6 (3.5)	25.4 (1.7)
SPIROCARACOLITONE C	34.8 (3.3)	32.0 -	~	61.1 (7.7)	67.5 -	~	42.8 -	42.8 -	~
SPIROCARACOLITONE D	25.6 (0.49)	26.1 (0.81)	24.4 (0.42)	81.6 (2.5)	89.4 (2.5)	74.4 (3.1)	44.0 (3.0)	51.2 (2.4)	35.0 (4.2)
SPIROCARACOLITONE E	24.3 (0.35)	25.7 (0.47)	23.6 (0.40)	83.9 (2.8)	98.1 (3.1)	74.1 (2.1)	41.5 (2.2)	51.7 (2.5)	35.0 (1.4)

Note: () = standard error

- = standard error could not be calculated as n=1

~ = no survivors

A considerable (38 %) lowering of the maximum larval weight was observed for spirocaracolitone C (65 mg vs. 104 mg for control)(Table 3.3.0c). Spirocaracolitone B reduced the maximum larval weight by 20 %. The pupal period was extended by one day by spirocaracolitone and spirocaracolitone B but was unaffected by spirocaracolitone A and E. The percent of neonates which achieved pupation decreased compared to the control group for all the test compounds except for spirocaracolitone E. The most dramatic effect was seen in spirocaracolitone C where only 13% of the neonates pupated followed by spirocaracolitone B and spirocaracolitone which had pupation ratios of 57 % and 63 %, respectively. Adult emergence was reduced very dramatically by spirocaracolitone C where only 3% reached emergence. Spirocaracolitone B and spirocaracolitone reduced adult emergence by approximately 40 % compared to the control.

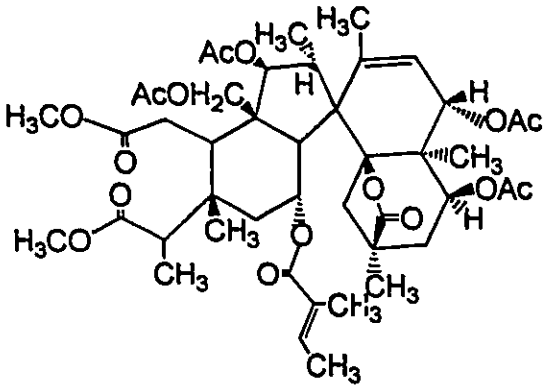
Of all the spiro-triterpenoids tested at 50 ppm, spirocaracolitone C was the most effective in reducing the growth and developmental parameters discussed above. Second in overall effect were spirocaracolitone B and spirocaracolitone. Growth studies were conducted at two dietary concentrations. The data for the developmental parameters observed at 5 ppm incorporation into the diet appear in Appendix 3.3.0 and are not discussed here because the 50 ppm results were more significant. The results for this study at 50 ppm mimic more closely the concentration in which these spiro-triterpenoids actually occur in the bark as the isolated yields ranged from 0.01% to 0.005%. None of the compounds tested were as active as azadirachtin which reduced growth in the European corn borer to the same extent at 1 ppm.[22]

TABLE 3.3.0.c: EFFECT OF SPIROCARACOLITONES AT 50 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

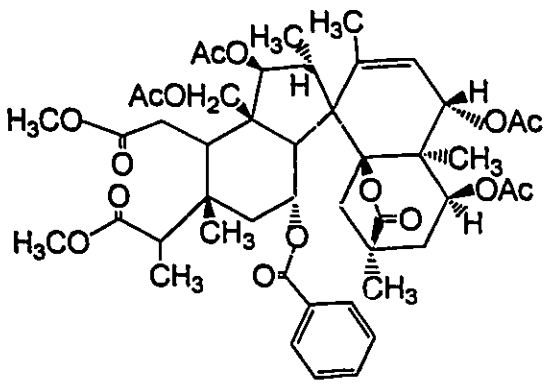
COMPOUNDS	Maximum Larval Weight(mg)	Duration of Pupal Period(days)	% Pupation	Days to Adult Emergence	% Adult Emergence	Sex ratio of Adults female/total
CONTROL	104 (4.7)	8.46 (0.29)	90	31.4 (0.26)	73	0.36
SPIROCARACOLITONE	93.2 (6.5)	9.43 (0.43)	63	35.6 (0.48)	47	0.57
SPIROCARACOLITONE A	99.0 (4.6)	8.88 (0.30)	77	31.4 (0.34)	57	0.59
SPIROCARACOLITONE B	84.2 (4.0)	9.92 (0.62)	57	38.6 (0.94)	40	0.33
SPIROCARACOLITONE C	65.1 (4.4)	8.00 -	13	40.0 -	3	1
SPIROCARACOLITONE D	101 (2.9)	9.22 (0.22)	73	34.6 (0.54)	60	0.56
SPIROCARACOLITONE E	104 4.9	8.39 (0.29)	90	32.8 (0.43)	77	0.39

Note: () = standard error
- = standard error could not be calculated as n=1

The most active spiro-compounds, spirocaracolitone C and B, are the most highly oxidized in the group. In addition to cleavage of the A ring resulting in two methyl esters, the methyl group attached to C-9 and the C-11 carbon have been hydroxylated (and acetylated) in comparison to spirocaracolitone, spirocaracolitone A, D and E. Spirocaracolitone B and C contain seven ester groups compared to four or less for spirocaracolitone A, D and E. The other common feature of the more active spiro-compounds is the C-14-C-15 double bond. In the less active derivatives this group has been converted into a tertiary alcohol and a secondary acetate. The difference between spirocaracolitone B and C is that the C-7 hydroxyl group bears a tigloyl group in spirocaracolitone B verses a benzoate group in spirocaracolitone C. It is somewhat remarkable that the latter is significantly more active.



SPIROCARACOLITONE B



SPIROCARACOLITONE C

The bioassay guided fractionation as well as the significant activity of the pure compounds at dietary concentrations comparable to those found in the diet treated with crude extract suggest that the spirocaracolitones are the principle actives in the extract. The reasons for the co-occurence of these six spiro-analogues is unknown at this point but other workers

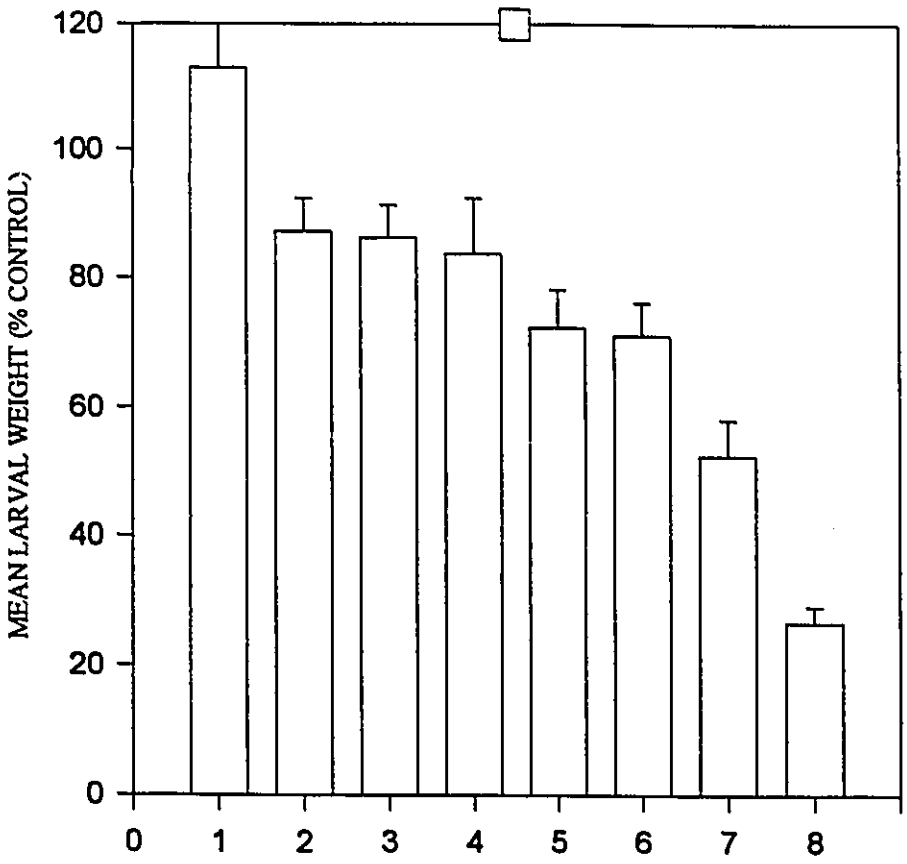
have shown that insecticidal synergisms occur between co-occuring secondary compounds of the same class(i.e. furanocoumarins).[35] Phytochemical redundancy is also a viable strategy for reducing insect resistance, since for example resistance to pure azadirachtin can occur in insects in a few generations while neem extracts containing many limonoids do not induce resistance in insects exposed up to 25 generations.[36]

The effect of the spirocaracolitones on reducing insect growth, increasing development time and mortality are similar to the action of other terpenoids such as the sesquiterpene lactone tenulin[37] and limonoids.[22] The phytochemical mode of action of the spirocaracolitones remains to be investigated, but is likely due to a combination of antifeedant action and postdigestive toxicity as found in other terpenoids.

3.3.1 EVALUATION OF THE EFFECTS OF THE GEDUNIN ANALOGS ON THE NEONATE LIFE CYCLE OF THE EUROPEAN CORN BORER

Eight of the gedunin analogs prepared in chapter one were evaluated for bioactivity using the neonate life cycle study of the European corn borer. The data for the effects of these analogs on the developmental parameters of the European corn borer at 5 ppm and 50 ppm are given in Appendix 3.3.1. This will not be discussed here as few significant differences from the control were observed in these parameters.

The bar graph in Figure 3.3.1 presents the mean larval weights of European corn borer larvae at day 19 of the neonate life cycle study with 50 ppm incorporation of the gedunin analogs into the diets. The data are presented as percent of the control. The most active analog in reducing the larval weight was 1,2-epoxygedunin (27 %) followed by 23-acetylgedunin(52 %) which were both significantly more active than gedunin (71 %). Interestingly 23-acetylgedunin was alot more active than 21-acetylgedunin (84 %). It is difficult to speculate further on structure/activity relationships because all of the analogs were not tested. Evaluation of all the analogs using an alternative test, one designed to evaluate antifeedant activity, and additional insect species is planned for the immediate future.



1 =1,2-DIHYDRO-3B-GEDUNOL
2 = 1,2-DIHYDROGEDUNIN
3 = HEXAHYDROGEDUNIN
4 = 21-ACETYLGEDUNIN
5 = 7-DEACETYLGEDUNIN
6 = GEDUNIN
7 = 23-ACETYLGEDUNIN
8 = 1,2-EPOXYGEDUNIN

FIGURE 3.3.1: MEAN LARVAL WEIGHT OF ECB AT DAY 19 WITH 50 PPM INCORPORATION OF GEDUNIN ANALOGS IN DIET

3.4.0 ANTIMALARIAL AND ANTIFUNGAL ACTIVITY OF THE SPIROCARACOLITONES

The results shown in Table 3.4.0a demonstrate that the spirocaracolitones did not exhibit a high degree of antimalarial activity compared to standard antimalarials. The activity of the spirocaracolitones was approximately twenty to forty times less than that exhibited by gedunin

TABLE 3.4.0a : ANTIMALARIAL ACTIVITY OF THE SPIROCARACOLITONES

Compound	Clone D6 IC_{50} (ng/mL)	Clone W2 IC_{50} (ng/mL)
Spirocaracolitone	870	870
Spirocaracolitone A	>10000	>10000
Spirocaracolitone B	>10000	>10000
Spirocaracolitone C	>10000	>10000
Spirocaracolitone D	>10000	4800
Spirocaracolitone E	>10000	>10000
Gedunin	39	20
Chloroquine Quinine Mefloquine Artemisinin	4.28 21.9 14.1 4.07	42.5 61.0 5.8 2.65

The spirocaracolitones were also screened for antifungal activity against various strains of *Fusarium* using a hyphal growth bioassay in Table 3.4.0b. After 24 h the spirocaracolitones at 100 ppm (100 ug/mL) inhibited hyphal growth of these fungus from 15 to 65 % but after 48 h a much greater inhibition was observed ranging from 49 to 75 %. *Fusarium moniliforme* was the least affected by these compounds, especially spirocaracolitone D, during the first 24 h but a high degree of inhibition of growth was seen for this fungi after 48 h (59-70 %). The antifungal activity of these compounds is significant and suggests that at naturally occurring levels in bark may provide antifungal protection. However the activity is far less than many potent natural products such as alpha terthienyl which inhibits fungal growth of *Fusarium* at 100 ppb.[38]

TABLE 3.4.0b : INHIBITION OF FUNGAL HYPHAL GROWTH BY THE SPIROCARACOLITONES

	Percent growth inhibition of *Fusarium graminearium*		Percent growth inhibition of *Fusarium moniliforme*		Percent growth inhibition of *Fusarium subglutinans*	
Compounds	24 h	48 h	24 h	48 h	24 h	48 h
Spirocaracolitone	62	68	30	62	56	66
Spirocaracolitone A	61	57	38	61	50	63
Spirocaracolitone B	47	49	15	59	37	71
Spirocaracolitone C	65	72	46	68	36	66
Spirocaracolitone D	72	78	51	70	41	75
Spirocaracolitone E	59	64	20	68	48	73

3.5.0 EXPERIMENTAL

GENERAL: Melting points were determined by use of a Thomas Hoover Capillary melting point apparatus and are uncorrected. Mass spectra were obtained using an VG 7070E or a Kratos concept 2H instrument. IR spectra were recorded in methylene chloride solution employing a Bomem- Michelson MB-100 FT/IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AMX-500 spectrometer with the shifts reported in ppm. The multiplicities of the NMR signals were reported employing the following abbreviations, singly or in combination: singlet(s), broad singlet(br s), doublet(d), triplet(t), quartet(q), multiplet(m). Rotation measurements were determined using a Perkin Elmer Polarimeter (Model 241) set on the sodium D line (589 nm). The samples were dissolved in spectroscopic grade methylene chloride.

Solvents for extractions and chromatographic purifications were routinely distilled prior to use. Thin layer chromatography (tlc) was carried out using Kieselgel 60 F_{254} precoated 0.25 mm plates. Visualization was facilitated by UV irradiation followed by the charring of a tlc which had been dipped in a 5 % H_2SO_4 in methanol solution, containing a small amount of isocratin. Preparative tlc (Prep TLC) chromatography was carried out using Kieselgel 60 F_{254} precoated 0.50 mm plates. Silica gel 270-400 Mesh was used for flash chromatography.

Analytical HPLC work was performed on a Varian 9012 HPLC (tertiary pump system) equiped with a Varian Variable wavelength UV-VIS detector and a 5μm Techsphere ODS (reverse phase) column (4.6mm X 25 cm). Semi-preparative HPLC was performed on a Beckman analytical HPLC which was upgraded to a semi-preparative

scale through the installation of, higher capacity pumpheads. The Beckman Variable UV detector also had to be upgraded with a flowcell capable of handling the required flowrates. A semi-preparative column, 20 mm X 25 cm, packed with Techsphere 5µm ODS packing (HPLC Technology) was used with this system.

Preparative work was performed on the LC-908 JAIGEL Recycling HPLC equipped with a fixed wavelength UV detector (254 nm) and a 15µm particle size reverse phase column (ODS-S-343-15). Precolumns were used with all HPLC columns so to extend the life of the columns. HPLC grade acetonitrile (Omnisolv) was purchased from BDH and filtered through Millipore filters (for organic solvents) before use. Water was obtained from a Millipore water filtration system and was used fresh everyday. Degassing of the solvents was necessary only with the use of the semi-preparative system.

3.5.1 PREPARATION OF ETHANOL EXTRACTS

Plant specimens were collected from a variety of locations in Costa Rica and from the Fairchild Tropical Garden Miami, Florida. Leaf, bark fruit and wood samples collected in Costa Rica were put into 1 L Nalgene bottle which were then topped up with 95% ethanol before they were transferred to Canada. In our laboratory the ethanol was filtered off from the specimens and specimens were allowed to dry in a fumehood overnight. Dried bark, fruit and wood samples were ground up using a Wiley Mill and leaf samples were chopped up using a blender. The ethanol which had earlier been decanted off from the samples was then added back to the ground up specimens. The plant specimens were extracted with ethanol three times over a period of three days. The

combined extracts, for each sample, were evaporated in vacuo, placed on a pump for 12 h and freeze dried. Freshly collected samples were air dried, ground up and the extracts prepared as outlined above.

3.5.2 INSECT BIOASSAYS

3.5.2.1 EUROPEAN CORN BORER (*OSTRINIA NUBILALIS*)

The culture of the European corn borer was maintained at 26 °C during photophase, 19 °C during scotophase, 80 % relative humidity and on a photoperiod of 16L:8D. The first step in the preparation of diet for the bioassay involved the mixing together of dry mix (98.5 g), Fumidil B (0.4 g), methyl paraben solution (4.0 mL) and propionic-phosphoric acid solution (4.6 mL) with distilled water (87 mL).[39] The bioassay diet did not contain corngrits, which were present in the maintenance diet.

To obtain a more homogeneous diet a suspension of agar (14.9 g) in distilled water (433 mL) was then heated over an open flame until all of the agar had dissolved. This solution was added to a mixer containing distilled water (178 mL) and stirred until the temperature of the solution had dropped to 58 °C. The dry mix paste, which was prepared earlier, was then added in portions to the stirring agar mixture. When all of the dry mix paste had been added and the temperature of the diet had cooled to 45-50 °C, it was dispensed into beakers in 50 g aliquots and allowed to cool further to 40 °C. At this time 2 mL of a 1:1 95% ethanol/water solution, containing 0.2 g of extract resulting in a diet concentration of 0.4%, was added and stirred into the diet. A 2 mL solution of 95% ethanol/water 1:1 was added to the control diet. As soon as the diet

appeared to be homogeneous it was decanted into 1 cm^3 square molds and allowed to cool.

The diet cubes were removed from the mold and placed in a jar and refrigerated overnight.The next day the diet was allowed to come to room temperature while second instar larvae were being selected from the *Ostrinia nubilalis* culture. Larvae were individually weighed and placed on a diet cube in a three dram glass vial which was then stoppered with absorbant cotton. Each extract/control was replicated fifteen times. After the larvae had incubated in the growth chamber for five days, the final weights were recorded and the relative growth rate as % control (RGR) was determined.

$$\text{RGR} = \frac{(\text{FINAL WT.} - \text{INITIAL WT.})/((\text{FINAL WT.} + \text{INITIAL WT.})/2)}{\text{MEAN RGR OF CONTROL(N=15)}} \times 100$$

3.5.2.2 VARIEGATED CUTWORM (*PERIDROMA SAUCIA*)

Insect cultures of *Peridroma saucia* were reared on artifical diet (Bioserv Inc., Frenchtown, New Jersey) and maintained at 21 °C with a photoperiod of 16L:8D. Plant extracts were suspended in the carrier solvent methanol and incorporated into the diets at a concentration of 0.2% fresh weight of diet. Control diets were prepared using methanol. Two neonate larvae were then placed in a compartment of a tray with approximately 1 g of prepared diet (control or treated). This test was replicated twenty times for each extract and control. The larvae were incubated in a growth chamber at 25 °C with a photoperiod of 16L:8D for 10 days. Each larva was then weighed and the mean

weights for each group were expressed as a percentage of the control.[14] The screening of extracts for biological activity against the variegated cutworm was conducted in the laboratory of Dr. M. Isman at the Departments of Plant Science and Botany, University of British Columbia, Vancouver, B.C..

3.5.3 ISOLATION OF SPIROCARACOLITONES

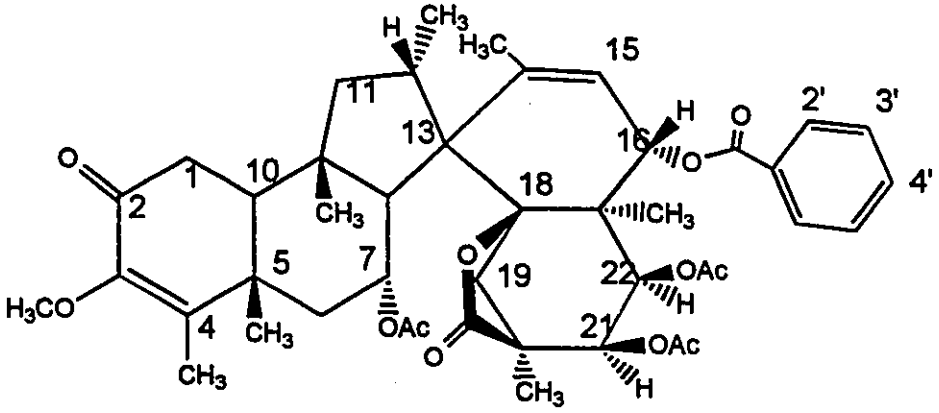
3.5.3.1 SPIROCARACOLITONE

Our collaborators in Costa Rica collected 1.3 kg *Ruptiliocarpon caracolito* bark in June 1992, ground it up with a Wiley mill and added 95% ethanol to the ground material before it was transported to our laboratory. The ground bark was extracted with 95% ethanol three times over the course of three days. The combined extracts were condensed using a rotary evaporator followed by freeze drying to remove any remaining moisture. A portion of the extract was reconstituted in a 1:1 ethanol/water solution and extracted three times with hexane. Most of the ethanol of the ethanol/water layer was then removed in vacuo so that the layer could be further extracted with methylene chloride (four times) without the formation of an emulsion. The methylene chloride layers were combined and condensed using a rotary evaporator.

Flash column chromatography of the methylene chloride extract, eluted with CH_2Cl_2/EtOAc, in a gradient of 0 to 100%, resulted in the collection of a fraction containing spirocaracolitone. Purification of this fraction was attempted using a variety of solvent systems on Prep TLC's and flash columns. Eventually a single spot by TLC was obtained but by nmr the compound contained a number of impurities. This was

substantiated when a number of peaks were observed when the sample was injected into a Varian analytical HPLC equiped with a 5 µm reverse phase column. The analytical HPLC was therefore used to develop a solvent system which would allow for the purification of spirocaracolitone using a Preparative HPLC.

Purification of spirocaracolitone was accomplished using a Beckman Analytical HPLC which had been upgraded to a semi-preparative scale via the installation of higher capacity pump heads. Isocratic elution of a semi-preparative 5 µm reverse phase column with 50% water/ 50 % acetonitrile, at a flow rate of 10 mL/min and a UV detector set at 205 nm allowed for the collection of a peak at a retention time of 1 h which was identified as spirocarocolitone. The isolated yield of spirocaracolitone, of this bark collection, was approximately 0.01%.



$C_{44}H_{54}O_{12}$ MW= 774 g/mole

MP 215-218 °C

$[\alpha]^{25}D$ +39.34(c=0.0061, CH_2Cl_2)

MS FAB MS: 775$[M+H]^+$(20.4), 715(2.1), 593(4.4), 533(3.0), 473(2.1), 105(100).

IR (CH_2Cl_2) $\nu(cm^{-1})$: 1780, 1750, 1722, 1674.

^{1}H NMR ($CDCl_3$) δ(ppm): 2.34(dd, J=3.4, 17.2 Hz, **H-1eq**), 2.59(dd, J=14.3, 17.2 Hz, **H-1ax**), 1.45-1.54(m, **H-6**), 2.11(dd, J=3.2, 12.2 Hz, **H-6**), 5.31(dt, J=3.2, 10.9 Hz, **H-7**), 2.30(m, **H-8**), 1.80-1.85(m, **H-10**), 1.35-1.55(m, **H-11**), 2.92(m, **H-12**), 5.45(brs, **H-15**), 6.02(m, **H-16**), 2.43(d, J=12.9 Hz, **H-19**), 2.62(d, J=12.5 Hz, **H-19**), 5.20 and 5.23(d, J=4.4 Hz, **H-21** and **H-22**), 1.83(s, **C-4-Me**), 1.23(d, J= 7.5 Hz, **C-12-Me**), 1.87(brs, **C-14-Me**), 1.22(s, **C-20-Me**), 1.18, 1.23, 1.47 (s, **C-5, 7, 17 -Me**), 3.61(s, **C-3- OMe**), 1.91, 1.94, 2.28(s, **Me-acetate**), 7.89(m, **H'-2**), 7.39(m, **H'-3**), 7.52(m, **H'-4**).

^{13}C NMR ($CDCl_3$) (ppm): 37.2(**C-1**), 193.9(**C-2**), 147.7(**C-3**), 155.1(**C-4**), 44.2(**C-5**)*, 46.0(**C-6**), 66.8(**C-7**), 61.6(**C-8**), 41.9(**C-9**)*, 55.9(**C-10**), 50.5(**C-11**), 40.0(**C-12**), 57.6(**C-13**), 136.6(**C-14**), 125.5(**C-15**), 69.9(**C-16**), 47.2(**C-17**), 90.5(**C-18**), 42.4(**C-19**), 43.9(**C-20**), 72.7(**C-21**), 72.1(**C-22**), 175.9(**C-30**), 129.8(**C-1'**), 129.4(**C-2'**), 128.4(**C-3'**), 133.2(**C-4'**), 11.3(**C-4-Me**), 17.9(**C-12-Me**), 23.9(**C-14-Me**), 16.3(**C-20-Me**), 18.5, 19.4, 21.4 (**C-5, 9, 17-Me**), 59.7(**C-3-OMe**), 20.2, 20.4, 21.3(**C-7, 21, 22 Me-acetate**),169.8, 170.1, 171.6 (**C=O-acetate**), 166.0(**C=O-benzoate**).

*= assignments are interchangeable

3.5.3.2 SPIROCARACOLITONES A-E

The remaining spirocaracolitones were isolated from *Ruptiliocarpon caracolito* bark which had been collected in Golfito, Costa Rica on July, 20, 1993. The bark was received preserved in 95% ethanol and in 1L nalgene square bottles. The ethanol was decanted and put aside while the bark was allowed to dry in the fume hood overnight. When the bark was dry it was ground into sawdust sized particles using a Wiley mill. The ethanol decanted off in the beginning was then added back to the ground bark and if necessary more ethanol was added to insure that the bark was completely wetted with ethanol. Extraction of the bark with 95% ethanol was performed three times. The extracts were combined and the solvent was removed using a rotary evaporator.

Following freeze drying, a portion of the extract was reconstituted in a 1:1 solution of ethanol/water. This was transferred into a seperatory funnel and extracted four times with hexane. The ethanol/water layer was put on a rotary evaporator until most of the ethanol was removed. The remaining aqueous layer was extracted with methylene chloride four times. Evaporation of the methylene chloride resulted in a fraction which weighed approximately 20% of the initial ethanol extract weight used.

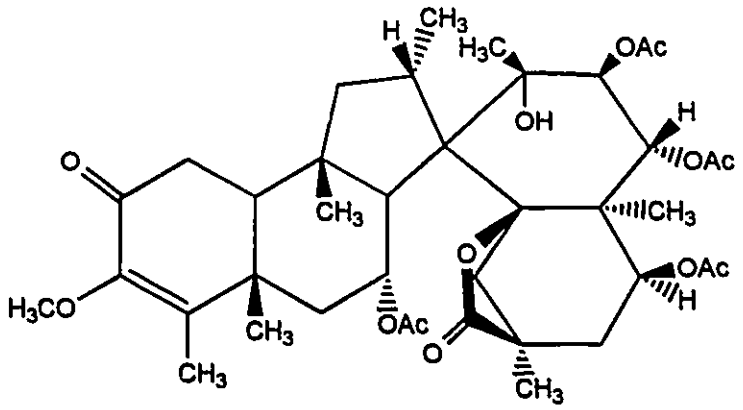
Flash column chromatography of the methylene chloride extract eluted with a gradient with methylene chloride to ethyl acetate, 0 to 100%, resulted in the collection of several fractions. Flash chromatography of the major fraction using acetone/methylene chloride elution of 0 to 25% resulted in six combined portions. Spirocaracolitone E was obtained pure after Prep plate and column chromatography of the third fraction followed by crystallization from methanol (0.01% yield). Further flash and Prep plate

chromatography of the remaining fractions did not result in the isolation of purified components.

The isolation of the remaining spirocaracolitones was eventually achieved using a JAIGEL Recycling HPLC equipped with a reverse phase column, eluted with a solvent system of acetonitrile/water (either 80/20 or 60/40), at a flow rate of 5 mL/min and monitored by a UV detector set a 254 nm. Often 3-6 cycles were required before suitable separation of the components was obtained. The isolated yields of Spirocaracolitones A-D ranged from 0.005 to 0.008% however the actual content of these components in the bark is expected to be higher because losses were incurred during this developmental isolation procedure. A more efficient isolation procedure can probably be developed from this experience.

Throughout the isolation process the purity of fractions was monitored by NMR and using a Varian Analytical HPLC equiped with a 5 μm reverse phase column operated at a flow rate of 0.9 mL/min and a UV detector set at a wavelength of 205 nm. (For details on the HPLC systems see the General Experimental section)

SPIROCARACOLITONE A



$C_{39}H_{54}O_{13}$ MW= 730 g/mole

MP 240 °C(decomp)

$[\alpha]^{25}D$ -22.17(c=0.0046, CH_2Cl_2)

MS (CI/ISO): 731(31.1) $[MH]^+$, 671(25.5), 611(40.8), 551(43.7), 491(27.5), 109(100.0).

IR (CH_2Cl_2) $\nu(cm^{-1})$: 3531, 2940, 1787, 1741, 1674.

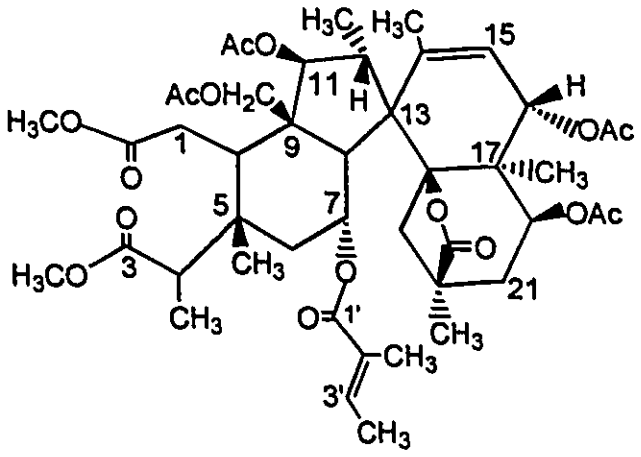
^{1}H NMR ($CDCl_3$) δ(ppm): 2.34(m, **H-1eq**), 2.56(dd, J=14.2, 17.1, **H-1ax**), 1.18 and 2.35(m, **H-6**), 5.27(dt, J=3.7, 10.4 Hz, **H-7**), 2.05(m, **H-8**), 1.74(dd, J=3.4, 14.2 Hz, **H-10**), 1.28(m, **H-11eq**), 1.52(dd, J=5.7, 12.4 Hz, **H-11ax**), 2.56(m, **H-12**), 5.95(d, J=3.7 Hz, **H-15**), 5.18 (d, J=3.7 Hz, **H-16**), 2.32(m, **H-19**), 2.49(d, J=12.7 Hz, **H-19**), 1.78(d, J=14.4 Hz, **H-21**), 2.06(m, **H-21**), 4.89(dd, J=1.3, 4.4 Hz, **H-22**), 4.13(brs, **C-14-OH**), 3.57(s, **C-3-OMe**), 1.82(s, **C-4-Me**), 1.35(d, J=8.3 Hz, **C-12-Me**), 1.13, 1.22, 1.35, 1.55, 1.92, 1.96, 2.08, 2.14, 2.15(s, **Me** X 5 and **Me-acetate** X 4).

^{13}C NMR ($CDCl_3$) δ(ppm): 37.0(**C-1**), 194.0(**C-2**), 147.8(**C-3**), 154.7(**C-4**), 43.6(**C-5**), 47.1(**C-6**), 70.2(**C-7**), 65.0(**C-8**), 43.6(**C-9**), 55.1(**C-10**), 52.8(**C-11**), 37.8(**C-12**),

66.2(**C-13**), 76.7(**C-14**), 65.4(**C-15**)*, 77.5(**C-16**)*, 45.3(**C-17**), 92.9(**C-18**), 40.4(**C-19**), 40.9(**C-20**), 37.7(**C-21**), 74.3(**C-22**), 176.3(**C-30**), 59.7(**C-3-OMe**), 11.2(**C-4-Me**), 26.8(**C-12-Me**), 19.9, 20.1, 20.3, 20.4, 20.6, 20.8, 21.1, 21.4(**Me** X 5 and **Me-acetate** X 4), 169.2, 169.5, 170.1, 170.2(**C=O-acetate** X 4)

* = assignments are interchangeable

SPIROCARACOLITONE B



$C_{45}H_{62}O_{16}$ MW= 858 g/mole

MP 231-232.5 °C

$[\alpha]^{25}D$ -6.76(c=0.0037, CH_2Cl_2)

MS (CI/ISO): 759$[MH-100]^+$(30.7), 699(34.2), 639(25.1), 579(20.7), 519(12.8), 101(100).

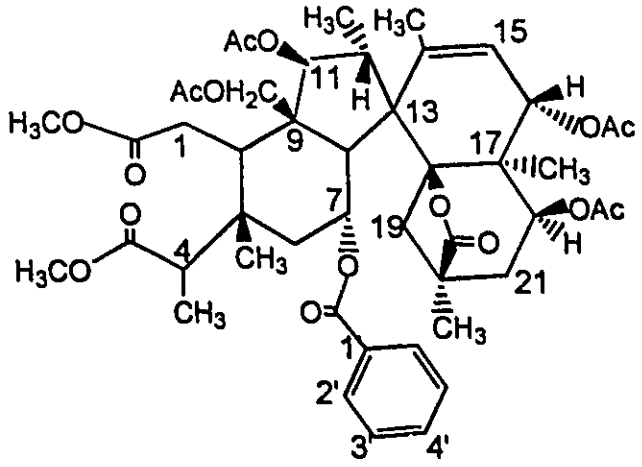
IR (CH_2Cl_2) $\nu(cm^{-1})$: 2957, 1783, 1738, 1697.

^{1}H NMR ($CDCl_3$) δ(ppm): 2.39(m, **H-1**), 3.00(dd, J=6.0, 18.3 Hz, **H-1**), 2.34(q, J=7.2 Hz, **H-4**), 1.70(dd, J=4.4, 12.9 Hz, **H-6eq**), 2.39(m, **H-6ax**), 5.58(dt, J=4.4, 10.9 Hz, **H-7**), 2.74(m, **H-8**), 2.74(m, **H-10**), 5.22(d, J~13 Hz, **H-11**), 2.79(m, **H-12**),

5.21(brs, **H-15**), 5.71(t, J=2 Hz, **H-16**), 2.37(m, **H-19**), 1.85(d, J=14.8 Hz, **H-21**), 1.93(m, **H-21**), 4.90(d, J=2.4 Hz, **H-22**), 0.93(d, J=7.4 Hz, **C-4-Me**), 1.12(d, J=7.2 Hz, **C-12-Me**), 1.80(s, **C-14-Me**), 1.02, 1.22, 1.33(s, **C-5, 17, 20-Me**), 4.60 and 4.91(d, J=12.9 Hz, **C'-9-CH_2**), 7.07(dq, J=1.4, 7.0 Hz, **H'-3**), 1.91(s, **C'-2-Me**), 1.81(d, J=7.0 Hz, **C'-3-Me**), 1.91, 1.94, 1.95, 2.07(s, **Me-acetate** X 4), 3.58 and 3.59(s, **C-2-OMe** and **C-3-OMe**).

^{13}C NMR ($CDCl_3$) δ(ppm): 31.4(**C-1**), 47.6(**C-4**), 41.1 and 44.3(**C-5** and **C-9**), 41.7(**C-6**), 65.7(**C-7**), 51.2 and 54.4 (**C-8** and **C-10**), 82.2(**C-11**), 44.4(**C-12**), 54.8(**C-13**), 134.5(**C-14**), 126.7(**C-15**), 69.6(**C-16**), 47.8(**C-17**), 90.5(**C-18**), 42.7(**C-19**), 40.6(**C-20**), 39.7(**C-21**), 73.3(**C-22**), 63.9(**C'-9-$\underline{C}H_2$**), 15.0(**C-4-Me**), 12.7(**C-12-Me**), 23.1(**C-14-Me**), 128.4(**C'-2**), 138.5(**C'-3**), 12.0(**C'-2-Me**), 14.8(**C'-3-Me**), 19.3, 20.5, 22.4(**C-5, 17, 20-Me**), 20.9, 21.0, 21.1, 21.2(**Me-acetate** X 4), 177.4(**C-30**), 174.9, 173.5, 170.1, 169.7, 169.6, 169.5, 168.1(**C=O** X 7), 51.5(**C-2-OMe** and **C-3-OMe**).

SPIROCARACOLITONE C



$C_{47}H_{60}O_{16}$ MW= 880 g/mole

MP 145-151 °C

$[\alpha]^{25}D$ -13.45(c=0.0055, CH_2Cl_2)

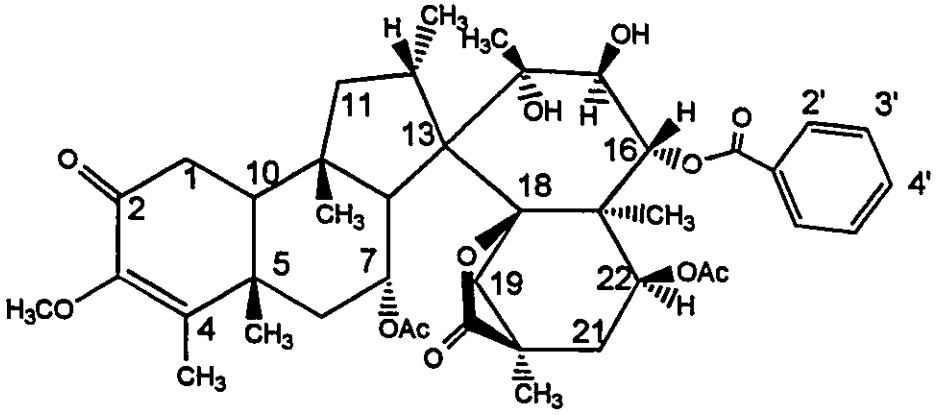
MS FAB MS: 881$[M+H]^+$(1.8), 821(1.3), 761(4.5), 759(34.0), 699(11.5), 639(7.5), 105(100).

IR (CH_2Cl_2) $\nu(cm^{-1})$: 2950, 1783, 1737.

^{1}H NMR ($CDCl_3$) δ(ppm): 2.43(m, **H-1**), 3.01(dd, J=6.0, 18.2 Hz, **H-1**), 2.36(m, **H-4**), 1.86(m, **H-6eq**), 2.56(dd, J=10.3, 12.8 Hz, **H-6ax**), 5.78(dt, J= 4.6, 10.9 Hz, **H-7**), 2.82(m, **H-8**), 4.51 and 4.91(d, J=12.8 Hz, **C'-9-CH_2**), 2.87(d, J=11.7 Hz, **H-10**), 5.26(d, J=12.1 Hz, **H-11**), 2.82(m, **H-12**), 5.12(d, J=1.0 Hz, **H-15**), 5.58(t, J= 2.0 Hz, **H-16**), 2.41(m, **H-19**), 1.89 and 1.95(m, **H-21**), 4.88(dd, J= 1.3, 4.4 Hz, **H-22**), 3.60 and 3.61(s, **C-2-OMe** and **C-3-OMe**), 0.95(d, J=7.4 Hz, **C-4-Me**), 1.11(d, J=7.2 Hz, **C-12-Me**), 1.85(d, J=1.3 Hz, **C-14-Me**), 1.05, 1.21, 1.38(s, **C-5, 17, 20-Me**), 1.78, 1.97, 2.00, 2.09(s, **Me-acetate** X 4), 8.26(m, **H'-2**), 7.41(m, **H'-3**), 7.53(m, **H'-4**).

^{13}C NMR ($CDCl_3$) δ(ppm): 31.4(**C-1**), 47.5(**C-4**), 41.1 and 44.3(**C-5** and **C-9**), 41.7(**C-6**), 66.5(**C-7**), 51.2(**C-8**), 54.4(**C-10**), 82.2(**C-11**), 44.5(**C-12**), 54.8(**C-13**), 134.5(**C-14**), 127.0(**C-15**), 69.3(**C-16**), 47.9(**C-17**), 90.6(**C-18**), 42.7(**C-19**), 40.6(**C-20**), 39.8(**C-21**), 73.2(**C-22**), 177.5(**C-30**), 63.9(**C-9-$\underline{C}H_2$**), 15.1(**C-4-Me**), 12.7(**C-12-Me**), 23.1(**C-14-Me**), 51.5 and 51.8(**C-2-OMe** and **C-3-OMe**), 19.5, 20.5, 22.5(**C-5, 17, 20-Me**), 20.7, 20.9, 21.1, and 21.4(**Me-acetate X 4**), 169.3, 169.5, 169.6, 170.0, 173.5, 174.9(**C=O X 6**), 130.0(**C'-1**), 130.2(**C'-2**), 128.4(**C'-3**), 132.7(**C'-4**), 166.5(**C=O-Benzoate**).

SPIROCARACOLITONE D



$C_{42}H_{54}O_{12}$ MW= 750 g/mole

MP 251-256 °C(decomp)

$[\alpha]^{25}D$ -21.67(c=0.0024, CH_2Cl_2)

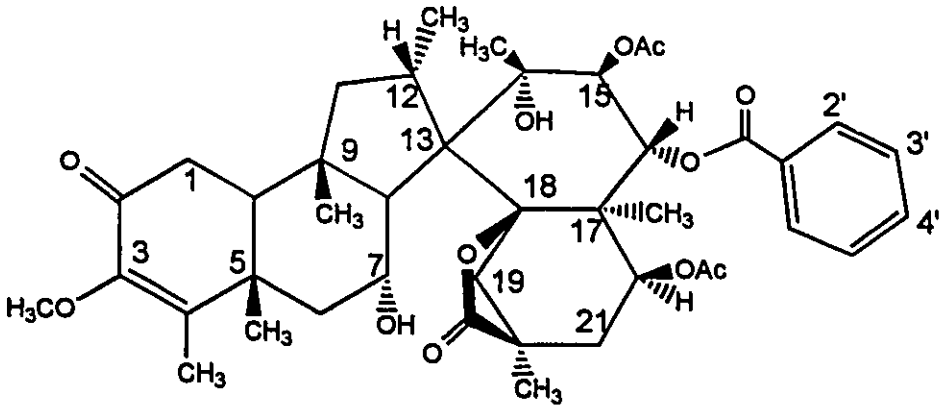
MS FAB MS: 751$[M+H]^+$(12.6), 691(4.5), 631(1.0), 569(2.3), 509(3.4), 93(100).

IR (CH_2Cl_2) ν(cm^{-1}): 2968, 1781, 1731, 1674.

^{1}H NMR ($CDCl_3$) δ(ppm): 2.35(dd, J=3.5, 17.3 Hz, **H-1eq**), 2.55(dd, J=14.3, 17.2 Hz, **H-1ax**), 1.24 and 2.24(m, **H-6**), 5.43(dt, J=3.1, 10.4 Hz, **H-7**), 1.98(d, J=10.4 Hz, **H-8**), 1.70(dd, J=3.4, 14.1 Hz, **H-10**), 1.24 and 1.52(m, **H-11**), 2.79(m, **H-12**), 4.53(brs, **H-15**), 6.01(d, J=4.8 Hz, **H-16**), 2.27(m, **H-19**), 2.49(d, J=12.8 Hz, **H-19**), 1.86(d, J= 14.9 Hz, **H-21**), 2.00(m, **H-21**), 4.92(dd, J=1.5, 4.4 Hz, **H-22**), 1.82(s, **C-4-Me**), 1.31(d, J=7.3 Hz, **C-12-Me**), 1.65 and 3.31(brs, **C-14-OH** and **C-15-OH**), 3.58(s, **C-3-OMe**), 1.18, 1.19, 1.25, 1.53, 1.53(s, **Me** X 5), 2.03 and 2.15(s, **Me-acetate**), 7.95(m, **H'-2**), 7.43(m, **H'-3**), 7.56(m, **H'-4**).

^{13}C NMR ($CDCl_3$) δ(ppm): 37.1(**C-1**), 194.3(**C-2**), 147.8(**C-3**), 155.2(**C-4**), 44.1(**C-5**), 46.4(**C-6**), 70.8(**C-7**), 65.1(**C-8**), 40.9(**C-9**)*, 55.4(**C-10**), 52.3(**C-11**), 38.2(**C-12**), 65.9(**C-13**), 76.8(**C-14**), 73.5(**C-15**), 69.4(**C-16**), 45.7(**C-17**), 92.7(**C-18**), 42.1(**C-19**), 40.8(**C-20**)*, 37.6(**C-21**), 76.2(**C-22**), 177.1(**C-30**), 11.2(**C-4-Me**), 20.6(**C-12-Me**)*, 18.9, 20.2, 20.2, 20.9(**Me** X 5), 59.7(**C-3-OMe**), 21.0 and 21.5(**Me-acetate**), 129.4(**C'-1**), 129.6(**C'-2**), 128.6(**C'-3**), 133.5(**C'-4**), 165.9(**C=O-benzoate**), 170.5, 170.0(**C=O-acetates** X 2).

SPIROCARACOLITONE E



$C_{42}H_{54}O_{12}$ MW= 750 g/mole

MP 228 °C(decomp)

$[\alpha]^{23}D$ -20.43(c=0.0046, CH_2Cl_2)

MS FAB MS: 1502(9.0), 751$[M+H]^+$(2.4), 733(5.1), 691(1.0), 631(1.3), 611(3.8), 551(6.3), 509(7.9), 105(100).

IR (CH_2Cl_2) ν(cm^{-1}): 3539, 3301, 2938, 1775, 1738, 1675.

^{1}H NMR ($CDCl_3$) δ(ppm): 2.33(dd, J=3.5, 17.4 Hz, **H-1eq**), 2.53(dd, J=14.2, 17.2 Hz, **H-1ax**), 1.14(m, **H-6ax**), 2.45(dd, J=3.0, 12.9 Hz, **H-6eq**), 4.13(dt, J=3.2, 10.4 Hz, **H-7**), 1.83(d, J=7.8 Hz, **H-8**), 1.67(dd, J=3.3, 14.3 Hz ,**H-10**), 1.29(m, **H-11**), 1.50(dd, J=5.8, 12.0 Hz, **H-11eq**), 2.61(m, **H-12**), 5.45 and 6.40(d, J=3.3 Hz, **H-15** and **H-16**), 2.33(m, **H-19**), 2.58(d, J=12.8 Hz, **H-19**), 1.81(m, **H-21ax**), 2.11(dd, J=4.5, 19.5 Hz, **H-21eq**), 4.92(d, J=4.4 Hz, **H-22**), 7.83(m, **H'-2**), 7.35(m, **H'-3**), 7.48(m, **H'-4**), 1.86(s, **C-4-Me**), 1.40(d, J=7.2 Hz, **C-12-Me**), 3.58(s, **C-3-OMe**), 1.09, 1.17, 1.20, 1.45, 1.70(s, **Me X 5**), 1.97 and 2.06(s, **Me-acetate**).

^{13}C NMR ($CDCl_3$) δ(ppm): 37.0(**C-1**), 193.9(**C-2**), 147.9(**C-3**), 154.5(**C-4**), 43.6(**C-5**)*, 51.0(**C-6**), 67.4(**C-7**), 67.8(**C-8**), 41.0(**C-9**)^, 55.4(**C-10**), 52.9(**C-11**), 38.6(**C-12**), 66.2(**C-13**), 76.5(**C-14**), 66.6 and 77.5(**C-15** and **C-16**), 45.6(**C-17**)*, 93.3(**C-18**), 41.6(**C-19**), 40.8(**C-20**)^, 37.3(**C-21**), 74.1(**C-22**), 178.6(**C-30**), 11.2(**C-4-Me**), 21.0(**C-12-Me**), 19.6, 20.0, 20.2, 20.5, 28.3(**Me** X 5), 59.6(**C-3-OMe**), 21.0 and 21.0(**Me-acetate**), 130.0(**C'-1**), 129.4(**C'-2**), 128.3(**C'-3**), 132.9(**C'-4**), 165.0(**C=O-benzoate**), 169.0, 170.3(**C=O-acetates** X 2).

*,^ = assignments are interchangeable

3.5.4 NEONATE LIFE CYCLE STUDY

The European corn borer was reared as previously described in section 3.5.2.1. The test compounds, were dissolved in 1 mL of ethanol and incorporated into 100 g of diet, at concentrations of 5 and 50 ppm, when the diet had cooled to 40 °C. The final ethanol concentration in the diets was 1% for all concentrations including the controls. For each compound concentration, 30 neonate larvae were placed individually in 3 dram glass vials plugged with absorbant cotton and containing a treated diet cube. Larvae were incubated in a growth chamber with 26 °C/19 °C day/night temperature regime, 90% R.H. and a L/D:18/6 photoperiod. Larval mortality and weight were recorded at regular intervals during their growth and fresh diet was placed in the vials when necessary. Days to pupation, pupal weight, days to emergence and adult weight and sex were some of the parameters that were recorded during the study. After most of the adults had emerged the study was discontinued.[17]

The neonate life cycle studies were conducted by N. Donskov, J. Arnason and myself in the Chemical Ecology laboratory, Dept. of Biology, University of Ottawa, Ottawa, Ontario.

3.5.5 ANTIFUNGAL BIOASSAY

This antifungal bioassay assessed the effect of the spirocaracolitones on inhibiting the hyphal growth of *Fusarium graminearum* Swabe (DAOM 194276), *F. moniliforme* (DAOM 195167) and *F. subglutinans* (DAOM 194909). Two well microscope slides, four slides per compound per fungus, were inverted on a filter paper in 150 X 17 mm Petri plates and sterilized in an autoclave. The test compounds were dissolved in ethanol at a concentration of 1mg/mL (1000 ppm). Using sterile technique, 100 µL of melted PDA (potato dextrose agar) and 10 µL of the test compound were added to each well, stirred and allowed to solidify. The final concentration of the compound in the media was therefore 100 ppm. The controls had 10 µL of ethanol added to them.

Blocks, approximately 0.9 mm^3, containing hyphae were cut from the actively growing margins of the cultures and placed in the center of each well. Before the plates were incubated the area of the colonies were determined (μm^2) using a micrometer installed into the lense of a stereomicroscope. The filter paper in each Petri plate containing four slides was saturated with water and the covers replaced. The plates were placed in plastic bags and incubated at 22 °C for 24 and 48 h (16L:8D) at which time the

area of the colonies was determined. Activity was evaluated by calculating the % growth of % control and then the % inhibition of growth.

The antifungal screening was conducted at Dr. A. Picman's laboratory, by R. Assabgui and myseelf, using the technique developed in her laboratory[40,41] at the Plant Research Centre, Central Experimental Farm, Agriculture Canada, Ottawa,

3.6.0 REFERENCES

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APPENDIX 3.2.2.1 : X-RAY DATA OF SPIROCARACOLITONE

Space Group and Cell Dimensions Monoclinic, P 21
a 10.853(7) b 16.713(7) c 13.071(5)
beta 96.15(4)
Volume 2357(3)A**3

Empirical formula : O15 C49 H68

Cell dimensions were obtained from 24 reflections with 2Theta angle in the range 40.00 - 47.00 degrees.

Crystal dimensions : 0.20 X 0.10 X 0.20 mm

FW = 897.07 Z = 2 F(000) = 963.90

Dcalc 1.264Mg.m-3, mu 0.08mm-1, lambda 0.70930A, 2Theta(max) 46.9

The intensity data were collected on a Rigaku diffractometer, using the theta/2theta scan mode.
The h,k,l ranges used during structure solution and refinement are :--
Hmin,max -12 12; Kmin,max 0 17; Lmin,max 0 14

No. of reflections measured	3832
No. of unique reflections	3614
No. of reflections with Inet > 2.5sigma(Inet)	2980

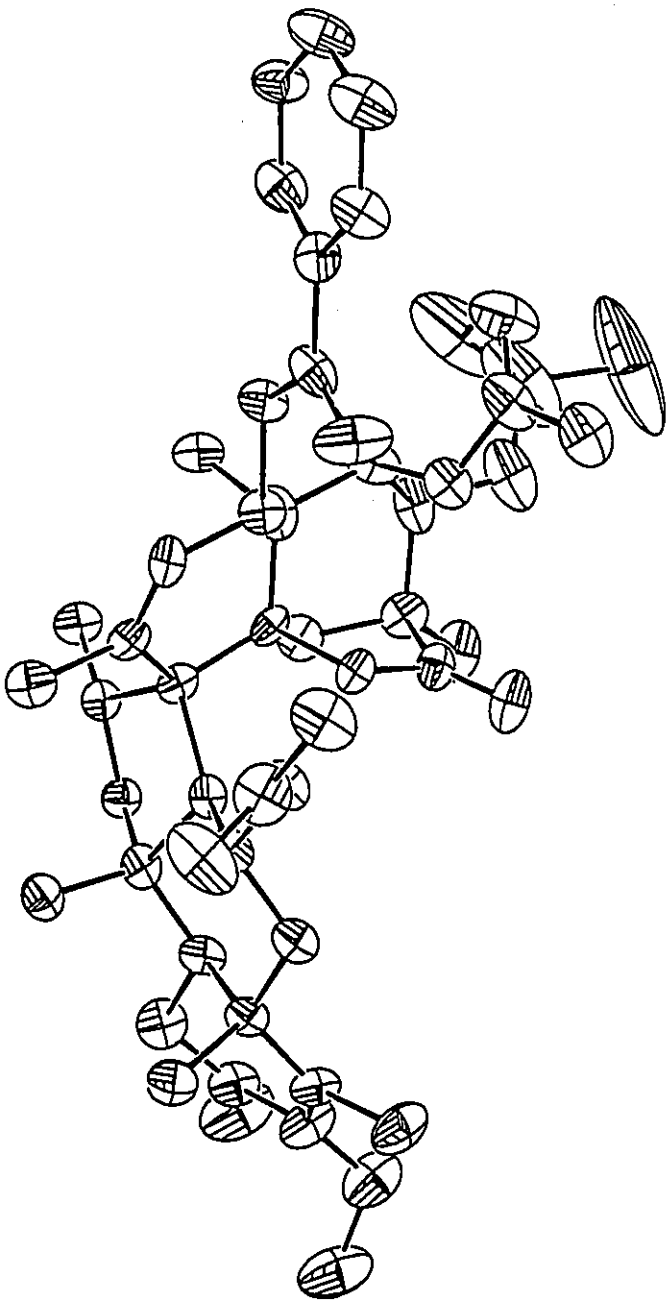
Merging R-value on intensities 0.017
No correction was made for absorption

The last least squares cycle was calculated with
118 atoms, 538 parameters and 2980 out of 3614 reflections.
Weights based on counting-statistics were used.
The weight modifier K in KFo**2 is 0.000100

The residuals are as follows :--
For significant reflections, RF 0.104, Rw 0.092 GoF 10.66
For all reflections, RF 0.128, Rw 0.092.
where RF = Sum(Fo-Fc)/Sum(Fo),
Rw = Sqrt[Sum(w(Fo-Fc)**2)/Sum(wFo**2)] and
GoF = Sqrt[Sum(w(Fo-Fc)**2)/(No. of reflns - No. of params.)]
The maximum shift/sigma ratio was 0.196.

In the last D-map, the deepest hole was -0.430e/A**3,
and the highest peak 0.670e/A**3.

Secondary ext. coeff. 1.206358 sigma 0.057052



Torsion angles

C37	O2	C3	C2	-69.0(14)	C37	O2	C3	C4	122.1(19)
C3	O2	C37	H37C	-178.8(26)	C3	O2	C37	H37A	-59.3(14)
C3	O2	C37	H37B	57.4(14)	C35	O3	C7	C6	-98.4(14)
C35	O3	C7	C8	143.1(15)	C35	O3	C7	H7	21.6(8)
C7	O3	C35	O4	2.2(8)	C7	O3	C35	C36	-179.3(18)
C21	O6	C18	C13	146.5(15)	C21	O6	C18	C17	-89.5(12)
C21	O6	C18	C19	25.2(9)	C18	O6	C21	O5	-176.8(17)
C18	O6	C21	C20	0.0(7)	C33	O7	C22	C20	145.9(18)
C33	O7	C22	C23	-89.3(16)	C33	O7	C22	H22	26.6(11)
C22	O7	C33	O8	-1.4(12)	C22	O7	C33	C34	-180.0(26)
C31	O10	C23	C17	-136.7(16)	C31	O10	C23	C22	101.1(14)
C31	O10	C23	H23	-17.3(8)	C23	O10	C31	O9	-1.0(9)
C23	O10	C31	C32	-178.8(17)	C24	O11	C16	C15	97.9(12)
C24	O11	C16	C17	-140.1(14)	C24	O11	C16	H16	-22.4(7)
C16	O11	C24	O12	-4.5(8)	C16	O11	C24	C25	175.3(15)
C10	C1	C2	O1	-145.9(20)	C10	C1	C2	C3	39.3(10)
H1A	C1	C2	O1	-25.5(9)	H1A	C1	C2	C3	159.8(22)
H1B	C1	C2	O1	93.2(18)	H1B	C1	C2	C3	-81.5(15)
C2	C1	C10	C5	-61.4(12)	C2	C1	C10	C9	165.4(19)
C2	C1	C10	H10	49.8(11)	H1A	C1	C10	C5	178.9(20)
H1A	C1	C10	C9	45.6(10)	H1A	C1	C10	H10	-69.9(13)
H1B	C1	C10	C5	59.4(11)	H1B	C1	C10	C9	-73.8(13)
H1B	C1	C10	H10	170.6(23)	O1	C2	C3	O2	3.2(9)
O1	C2	C3	C4	172.5(23)	C1	C2	C3	O2	178.3(21)
C1	C2	C3	C4	-12.4(9)	O2	C3	C4	C5	176.6(20)
O2	C3	C4	C38	-7.7(9)	C2	C3	C4	C5	7.8(8)
C2	C3	C4	C38	-176.5(20)	C3	C4	C5	C6	-148.2(18)
C3	C4	C5	C10	-29.1(10)	C3	C4	C5	C39	93.6(15)
C38	C4	C5	C6	36.3(10)	C38	C4	C5	C10	155.4(17)
C38	C4	C5	C39	-82.0(13)	C3	C4	C38	H38C	179.4(23)
C3	C4	C38	H38A	-60.4(14)	C3	C4	C38	H38B	59.9(13)
C5	C4	C38	H38C	-4.7(6)	C5	C4	C38	H38A	115.5(19)
C5	C4	C38	H38B	-124.1(20)	C4	C5	C6	C7	167.2(17)
C4	C5	C6	H6A	46.8(10)	C4	C5	C6	H6B	-71.8(13)
C10	C5	C6	C7	48.6(10)	C10	C5	C6	H6A	-71.8(12)
C10	C5	C6	H6B	169.7(19)	C39	C5	C6	C7	-76.5(12)
C39	C5	C6	H6A	163.1(19)	C39	C5	C6	H6B	44.6(10)
C4	C5	C10	C1	55.4(11)	C4	C5	C10	C9	-170.3(17)
C4	C5	C10	H10	-56.6(11)	C6	C5	C10	C1	176.8(17)
C6	C5	C10	C9	-48.8(10)	C6	C5	C10	H10	64.8(11)
C39	C5	C10	C1	-61.5(11)	C39	C5	C10	C9	72.8(12)
C39	C5	C10	H10	-173.5(19)	C4	C5	C39	H39C	-60.2(12)
C4	C5	C39	H39A	60.8(12)	C4	C5	C39	H39B	-179.9(20)
C6	C5	C39	H39C	179.3(20)	C6	C5	C39	H39A	-59.7(11)
C6	C5	C39	H39B	59.5(11)	C10	C5	C39	H39C	57.8(11)
C10	C5	C39	H39A	178.9(20)	C10	C5	C39	H39B	-61.9(11)
C5	C6	C7	O3	178.4(17)	C5	C6	C7	C8	-60.2(10)
C5	C6	C7	H7	57.5(10)	H6A	C6	C7	O3	-60.7(10)
H6A	C6	C7	C8	60.7(11)	H6A	C6	C7	H7	178.4(21)

H6B	C6	C7	O3	56.8(10)	H6B	C6	C7	C8	178.3(19)
H6B	C6	C7	H7	-64.0(11)	O3	C7	C8	C9	-172.3(16)
O3	C7	C8	C13	-37.8(8)	O3	C7	C8	H8	79.2(12)
C6	C7	C8	C9	68.4(11)	C6	C7	C8	C13	-157.0(16)
C6	C7	C8	H8	-40.1(9)	H7	C7	C8	C9	-49.7(9)
H7	C7	C8	C13	84.8(13)	H7	C7	C8	H8	-158.3(20)
C7	C8	C9	C10	-67.4(11)	C7	C8	C9	C11	174.8(16)
C7	C8	C9	C40	59.8(11)	C13	C8	C9	C10	151.6(16)
C13	C8	C9	C11	33.8(8)	C13	C8	C9	C40	-81.2(12)
H8	C8	C9	C10	41.1(9)	H8	C8	C9	C11	-76.7(12)
H8	C8	C9	C40	168.3(18)	C7	C8	C13	C12	-142.5(15)
C7	C8	C13	C14	-25.4(8)	C7	C8	C13	C18	97.9(13)
C9	C8	C13	C12	-9.9(7)	C9	C8	C13	C14	107.1(13)
C9	C8	C13	C18	-129.6(14)	H8	C8	C13	C12	100.6(14)
H8	C8	C13	C14	-142.3(17)	H8	C8	C13	C18	-19.0(6)
C8	C9	C10	C1	-169.9(17)	C8	C9	C10	C5	58.9(10)
C8	C9	C10	H10	-54.2(10)	C11	C9	C10	C1	-62.5(11)
C11	C9	C10	C5	166.2(16)	C11	C9	C10	H10	53.1(10)
C40	C9	C10	C1	59.2(11)	C40	C9	C10	C5	-72.0(12)
C40	C9	C10	H10	174.9(19)	C8	C9	C11	C12	-43.7(9)
C8	C9	C11	H11A	-162.2(18)	C8	C9	C11	H11B	75.5(12)
C10	C9	C11	C12	-155.3(17)	C10	C9	C11	H11A	86.2(14)
C10	C9	C11	H11B	-36.2(8)	C40	C9	C11	C12	80.0(12)
C40	C9	C11	H11A	-38.5(9)	C40	C9	C11	H11B	-160.8(19)
C8	C9	C40	H40C	57.1(11)	C8	C9	C40	H40A	175.8(19)
C8	C9	C40	H40B	-63.8(12)	C10	C9	C40	H40C	-178.8(20)
C10	C9	C40	H40A	-60.1(11)	C10	C9	C40	H40B	60.3(11)
C11	C9	C40	H40C	-53.4(10)	C11	C9	C40	H40A	65.3(12)
C11	C9	C40	H40B	-174.2(20)	C9	C11	C12	C13	41.2(9)
C9	C11	C12	C41	-179.0(16)	C9	C11	C12	H12	-68.0(11)
H11A	C11	C12	C13	159.5(18)	H11A	C11	C12	C41	-60.7(11)
H11A	C11	C12	H12	50.2(9)	H11B	C11	C12	C13	-77.9(12)
H11B	C11	C12	C41	61.9(11)	H11B	C11	C12	H12	172.8(21)
C11	C12	C13	C8	-19.2(8)	C11	C12	C13	C14	-136.6(15)
C11	C12	C13	C18	94.2(13)	C41	C12	C13	C8	-153.1(15)
C41	C12	C13	C14	89.5(13)	C41	C12	C13	C18	-39.7(9)
H12	C12	C13	C8	91.4(13)	H12	C12	C13	C14	-26.0(7)
H12	C12	C13	C18	-155.2(17)	C11	C12	C41	H41C	-179.9(19)
C11	C12	C41	H41A	-60.9(11)	C11	C12	C41	H41B	59.7(11)
C13	C12	C41	H41C	-47.2(9)	C13	C12	C41	H41A	71.9(12)
C13	C12	C41	H41B	-167.5(19)	H12	C12	C41	H41C	68.1(12)
H12	C12	C41	H41A	-172.8(21)	H12	C12	C41	H41B	-52.2(9)
C8	C13	C14	C15	117.8(15)	C8	C13	C14	C42	-61.3(10)
C12	C13	C14	C15	-134.0(16)	C12	C13	C14	C42	46.9(9)
C18	C13	C14	C15	-3.0(8)	C18	C13	C14	C42	177.9(16)
C8	C13	C18	O6	-33.5(7)	C8	C13	C18	C17	-149.5(15)
C8	C13	C18	C19	76.9(11)	C12	C13	C18	O6	-140.9(14)
C12	C13	C18	C17	103.2(13)	C12	C13	C18	C19	-30.5(8)
C14	C13	C18	O6	90.0(12)	C14	C13	C18	C17	-26.0(8)
C14	C13	C18	C19	-159.6(16)	C13	C14	C15	C16	-1.3(7)
C13	C14	C15	H15	178.3(18)	C42	C14	C15	C16	177.9(17)
C42	C14	C15	H15	-2.6(7)	C13	C14	C42	H42C	-1.4(6)
C13	C14	C42	H42A	118.8(17)	C13	C14	C42	H42B	-118.9(17)
C15	C14	C42	H42C	179.5(21)	C15	C14	C42	H42A	-60.4(12)

C15	C14	C42	H42B	61.9(12)
C14	C15	C16	C17	34.5(9)
H15	C15	C16	O11	-26.2(5)
H15	C15	C16	H16	94.6(15)
O11	C16	C17	C23	60.0(10)
C15	C16	C17	C18	-57.3(10)
C15	C16	C17	C43	63.4(11)
H16	C16	C17	C23	-58.4(11)
C16	C17	C18	O6	-63.1(10)
C16	C17	C18	C19	-169.0(17)
C23	C17	C18	C13	174.9(16)
C43	C17	C18	O6	176.9(17)
C43	C17	C18	C19	71.0(12)
C16	C17	C23	C22	151.0(17)
C18	C17	C23	O10	-83.6(12)
C18	C17	C23	H23	156.2(19)
C43	C17	C23	C22	-88.8(14)
C16	C17	C43	H43C	-60.4(11)
C16	C17	C43	H43B	180.0(20)
C18	C17	C43	H43A	176.8(19)
C23	C17	C43	H43C	179.9(20)
C23	C17	C43	H43B	60.4(11)
O6	C18	C19	H19A	-160.8(18)
C13	C18	C19	C20	-156.0(16)
C13	C18	C19	H19B	-36.8(8)
C17	C18	C19	H19A	-52.1(10)
C18	C19	C20	C21	40.7(9)
C18	C19	C20	C44	161.0(17)
H19A	C19	C20	C22	47.0(10)
H19B	C19	C20	C21	-78.5(13)
H19B	C19	C20	C44	41.9(10)
C19	C20	C21	O6	-25.5(8)
C22	C20	C21	O6	86.8(13)
C44	C20	C21	O6	-148.5(18)
C19	C20	C22	C23	62.0(11)
C21	C20	C22	O7	76.1(12)
C21	C20	C22	H22	-164.3(19)
C44	C20	C22	C23	-170.1(18)
C19	C20	C44	H44C	180.0(21)
C19	C20	C44	H44B	60.2(12)
C21	C20	C44	H44A	52.0(11)
C22	C20	C44	H44C	56.8(12)
C22	C20	C44	H44B	-63.1(12)
O7	C22	C23	C17	-164.9(18)
C20	C22	C23	O10	77.6(12)
C20	C22	C23	H23	-163.2(19)
H22	C22	C23	C17	78.4(13)
O11	C24	C25	C26	173.2(19)
O12	C24	C25	C26	-7.0(9)
C24	C25	C26	C27	-177.4(20)
C30	C25	C26	C27	1.8(10)
C24	C25	C30	C29	175.6(21)
C26	C25	C30	C29	-3.5(10)
C25	C26	C27	C28	0.1(9)

C14	C15	C16	O11	153.3(17)
C14	C15	C16	H16	-85.9(14)
H15	C15	C16	C17	-145.0(18)
O11	C16	C17	C18	-178.4(16)
O11	C16	C17	C43	-57.7(10)
C15	C16	C17	C23	-178.9(17)
H16	C16	C17	C18	63.1(11)
H16	C16	C17	C43	-176.1(19)
C16	C17	C18	C13	55.3(10)
C23	C17	C18	O6	56.6(10)
C23	C17	C18	C19	-49.4(10)
C43	C17	C18	C13	-64.7(11)
C16	C17	C23	O10	33.0(8)
C16	C17	C23	H23	-87.3(14)
C18	C17	C23	C22	34.4(9)
C43	C17	C23	O10	153.1(17)
C43	C17	C23	H23	32.9(8)
C16	C17	C43	H43A	60.3(11)
C18	C17	C43	H43C	56.0(11)
C18	C17	C43	H43B	-63.6(11)
C23	C17	C43	H43A	-59.3(11)
O6	C18	C19	C20	-40.7(8)
O6	C18	C19	H19B	78.5(12)
C13	C18	C19	H19A	83.9(13)
C17	C18	C19	C20	68.1(11)
C17	C18	C19	H19B	-172.8(18)
C18	C19	C20	C22	-73.4(12)
H19A	C19	C20	C21	161.1(19)
H19A	C19	C20	C44	-78.6(14)
H19B	C19	C20	C22	167.5(19)
C19	C20	C21	O5	151.2(19)
C22	C20	C21	O5	-96.6(16)
C44	C20	C21	O5	28.1(10)
C19	C20	C22	O7	-176.7(18)
C19	C20	C22	H22	-57.2(11)
C21	C20	C22	C23	-45.2(10)
C44	C20	C22	O7	-48.7(11)
C44	C20	C22	H22	70.8(13)
C19	C20	C44	H44A	-61.3(12)
C21	C20	C44	H44C	-66.7(13)
C21	C20	C44	H44B	173.5(21)
C22	C20	C44	H44A	175.4(21)
O7	C22	C23	O10	-45.8(9)
O7	C22	C23	H23	73.4(13)
C20	C22	C23	C17	-41.4(10)
H22	C22	C23	O10	-162.6(19)
H22	C22	C23	H23	-43.4(8)
O11	C24	C25	C30	-5.9(7)
O12	C24	C25	C30	173.9(20)
C24	C25	C26	H26	4.4(7)
C30	C25	C26	H26	-176.5(21)
C24	C25	C30	H30	-3.7(7)
C26	C25	C30	H30	177.2(22)
C25	C26	C27	H27	178.1(23)

H26	C26	C27	C28	178.4(23)	H26	C26	C27	H27	-3.7(1)
C26	C27	C28	C29	-0.4(9)	C26	C27	C28	H28	179.3(25)
H27	C27	C28	C29	-178.4(24)	H27	C27	C28	H28	1.3(0)
C27	C28	C29	C30	-1.3(10)	C27	C28	C29	H29	177.1(24)
H28	C28	C29	C30	179.0(24)	H28	C28	C29	H29	-2.6(1)
C28	C29	C30	C25	3.2(9)	C28	C29	C30	H30	-177.4(23)
H29	C29	C30	C25	-175.1(22)	H29	C29	C30	H30	4.3(1)
O9	C31	C32	H32C	179.6(25)	O9	C31	C32	H32A	-58.8(14)
O9	C31	C32	H32B	61.7(14)	O10	C31	C32	H32C	-2.6(5)
O10	C31	C32	H32A	118.9(21)	O10	C31	C32	H32B	-120.6(21)
O7	C33	C34	H34C	1.1(7)	O7	C33	C34	H34A	120.7(36)
O7	C33	C34	H34B	-118.5(36)	O8	C33	C34	H34C	-177.5(46)
O8	C33	C34	H34A	-57.8(24)	O8	C33	C34	H34B	62.9(25)
O3	C35	C36	H36C	0.3(5)	O3	C35	C36	H36A	119.7(21)
O3	C35	C36	H36B	-119.2(21)	O4	C35	C36	H36C	178.8(25)
O4	C35	C36	H36A	-61.8(14)	O4	C35	C36	H36B	59.3(14)
C9	C40	H40C	H42C	-64.9(8)	H40A	C40	H40C	H42C	175.0(14)
H40B	C40	H40C	H42C	56.9(7)	C14	C42	H42C	H40C	34.2(6)
H42A	C42	H42C	H40C	-86.9(10)	H42B	C42	H42C	H40C	152.9(14)
C40	H40C	H42C	C42	29.6(12)					

Table of Atomic Bond Angles in Degrees

C3-O2-C37	113.2(17)	O7-C22-C20	111.2(14)
C7-O3-C35	116.5(12)	O7-C22-C23	107.5(12)
C18-O6-C21	112.1(10)	O7-C22-H22	108.7(12)
C22-O7-C33	113.3(17)	C20-C22-C23	113.5(12)
C23-O10-C31	116.3(12)	C20-C22-H22	108.4(13)
C16-O11-C24	119.3(10)	C23-C22-H22	107.3(14)
C2-C1-C10	109.0(14)	O10-C23-C17	108.4(11)
C2-C1-H1A	109.1(13)	O10-C23-C22	106.6(12)
C2-C1-H1B	110.0(16)	O10-C23-H23	110.4(12)
C10-C1-H1A	110.3(15)	C17-C23-C22	113.2(12)
C10-C1-H1B	110.1(13)	C17-C23-H23	109.2(14)
H1A-C1-H1B	108.3(15)	C22-C23-H23	109.1(12)
O1-C2-C1	123.9(15)	O11-C24-O12	121.0(14)
O1-C2-C3	116.9(16)	O11-C24-C25	113.9(13)
C1-C2-C3	118.9(13)	O12-C24-C25	125.0(14)
O2-C3-C2	118.1(14)	C24-C25-C26	119.1(15)
O2-C3-C4	122.4(15)	C24-C25-C30	122.7(14)
C2-C3-C4	118.6(15)	C26-C25-C30	118.2(15)
C3-C4-C5	124.5(14)	C25-C26-C27	121.0(16)
C3-C4-C38	115.6(15)	C25-C26-H26	119.5(16)
C5-C4-C38	119.8(13)	C27-C26-H26	119.4(14)
C4-C5-C6	112.2(13)	C26-C27-C28	121.1(15)
C4-C5-C10	107.5(11)	C26-C27-H27	119.6(17)
C4-C5-C39	105.5(13)	C28-C27-H27	119.3(17)
C6-C5-C10	108.4(12)	C27-C28-C29	118.3(16)
C6-C5-C39	108.8(11)	C27-C28-H28	120.5(15)
C10-C5-C39	114.6(12)	C29-C28-H28	121.2(16)
C5-C6-C7	114.3(12)	C28-C29-C30	121.3(16)
C5-C6-H6A	108.7(12)	C28-C29-H29	118.6(16)
C5-C6-H6B	108.9(13)	C30-C29-H29	120.0(15)
C7-C6-H6A	107.8(13)	C25-C30-C29	120.1(15)
C7-C6-H6B	108.1(12)	C25-C30-H30	120.3(16)
H6A-C6-H6B	108.9(13)	C29-C30-H30	119.6(16)
O3-C7-C6	108.7(12)	O9-C31-O10	123.0(14)
O3-C7-C8	112.0(12)	O9-C31-C32	126.7(16)
O3-C7-H7	110.6(10)	O10-C31-C32	110.3(15)
C6-C7-C8	107.4(11)	C31-C32-H32C	110.5(15)
C6-C7-H7	109.3(13)	C31-C32-H32A	111.6(15)
C8-C7-H7	108.7(12)	C31-C32-H32B	110.0(17)
C7-C8-C9	107.0(12)	H32C-C32-H32A	109.1(18)
C7-C8-C13	125.3(12)	H32C-C32-H32B	107.0(15)
C7-C8-H8	103.2(11)	H32A-C32-H32B	108.6(15)
C9-C8-C13	112.2(11)	O7-C33-O8	125.5(22)
C9-C8-H8	103.3(12)	O7-C33-C34	105(3)
C13-C8-H8	103.2(12)	O8-C33-C34	129(3)
C8-C9-C10	105.8(11)	C33-C34-H34C	111(3)
C8-C9-C11	98.8(12)	C33-C34-H34A	113.0(23)
C8-C9-C40	118.6(12)	C33-C34-H34B	111(4)
C10-C9-C11	113.7(11)	H34C-C34-H34A	106(4)
C10-C9-C40	112.3(13)	H34C-C34-H34B	107.0(25)
C11-C9-C40	107.1(12)	H34A-C34-H34B	106(3)

C1-C10-C5	110.3(13)	O3-C35-O4	122.5(16)
C1-C10-C9	116.8(13)	O3-C35-C36	112.6(15)
C1-C10-H10	105.0(12)	O4-C35-C36	125.0(14)
C5-C10-C9	114.6(11)	C35-C36-H36C	109.9(13)
C5-C10-H10	103.8(12)	C35-C36-H36A	109.8(16)
C9-C10-H10	104.8(13)	C35-C36-H36B	110.5(18)
C9-C11-C12	102.6(11)	H36C-C36-H36A	108.6(18)
C9-C11-H11A	110.6(13)	H36C-C36-H36B	108.4(17)
C9-C11-H11B	111.3(12)	H36A-C36-H36B	109.7(13)
C12-C11-H11A	111.0(12)	O2-C37-H37C	112.7(22)
C12-C11-H11B	111.3(13)	O2-C37-H37A	109.1(16)
H11A-C11-H11B	109.7(13)	O2-C37-H37B	110.8(18)
C11-C12-C13	108.7(11)	H37C-C37-H37A	107.7(19)
C11-C12-C41	111.7(11)	H37C-C37-H37B	110.0(18)
C11-C12-H12	104.7(12)	H37A-C37-H37B	106.3(22)
C13-C12-C41	123.6(13)	C4-C38-H38C	109.8(15)
C13-C12-H12	102.7(10)	C4-C38-H38A	110.2(16)
C41-C12-H12	103.2(12)	C4-C38-H38B	109.9(15)
C8-C13-C12	97.4(11)	H38C-C38-H38A	109.1(16)
C8-C13-C14	112.1(11)	H38C-C38-H38B	108.7(17)
C8-C13-C18	107.5(11)	H38A-C38-H38B	109.2(16)
C12-C13-C14	111.7(11)	C5-C39-H39C	110.8(13)
C12-C13-C18	115.4(10)	C5-C39-H39A	110.5(14)
C14-C13-C18	111.8(12)	C5-C39-H39B	110.2(14)
C13-C14-C15	122.7(13)	H39C-C39-H39A	109.1(15)
C13-C14-C42	121.0(13)	H39C-C39-H39B	108.2(14)
C15-C14-C42	116.3(13)	H39A-C39-H39B	108.0(14)
C14-C15-C16	123.5(13)	C9-C40-H40C	110.1(14)
C14-C15-H15	117.5(13)	C9-C40-H40A	110.1(13)
C16-C15-H15	119.0(14)	C9-C40-H40B	110.8(13)
O11-C16-C15	110.6(12)	H40C-C40-H40A	107.8(13)
O11-C16-C17	106.6(12)	H40C-C40-H40B	109.2(14)
O11-C16-H16	109.8(11)	H40A-C40-H40B	108.8(14)
C15-C16-C17	111.9(12)	C12-C41-H41C	109.5(12)
C15-C16-H16	109.0(13)	C12-C41-H41A	108.7(13)
C17-C16-H16	108.8(12)	C12-C41-H41B	110.0(14)
C16-C17-C18	104.7(12)	H41C-C41-H41A	109.1(15)
C16-C17-C23	110.0(12)	H41C-C41-H41B	109.5(14)
C16-C17-C43	110.7(13)	H41A-C41-H41B	110.1(13)
C18-G17-C23	112.9(13)	C14-C42-H42C	108.4(13)
C18-C17-C43	111.9(12)	C14-C42-H42A	111.0(13)
C23-C17-C43	106.7(13)	C14-C42-H42B	109.6(14)
O6-C18-C13	107.9(11)	H42C-C42-H42A	109.4(15)
O6-C18-C17	103.7(11)	H42C-C42-H42B	107.9(14)
O6-C18-C19	99.4(10)	H42A-C42-H42B	110.5(14)
C13-C18-C17	116.4(11)	C17-C43-H43C	109.7(13)
C13-C18-C19	116.0(12)	C17-C43-H43A	110.0(13)
C17-C18-C19	111.2(12)	C17-C43-H43B	109.4(13)
C18-C19-C20	103.5(11)	H43C-C43-H43A	109.7(13)
C18-C19-H19A	111.8(13)	H43C-C43-H43B	109.1(13)
C18-C19-H19B	111.0(13)	H43A-C43-H43B	108.9(13)
C20-C19-H19A	111.5(13)	C20-C44-H44C	109.0(15)
C20-C19-H19B	111.0(14)	C20-C44-H44A	109.7(13)
H19A-C19-H19B	108.0(12)	C20-C44-H44B	110.5(15)

C19-C20-C21	99.6(11)	H44C-C44-H44A	108.5(16)
C19-C20-C22	107.2(13)	H44C-C44-H44B	109.0(14)
C19-C20-C44	115.8(13)	H44A-C44-H44B	110.0(16)
C21-C20-C22	109.5(12)	O13-C46-C45	123.4(22)
C21-C20-C44	112.0(13)	O13-C46-C47	119.3(22)
C22-C20-C44	111.8(13)	C45-C46-C47	117.3(21)
O5-C21-O6	124.2(14)	O14-C49-C48	102(4)
O5-C21-C20	128.8(14)	C40-H40C-H42C	133.1(8)
O6-C21-C20	106.8(11)	C42-H42C-H40C	170.3(9)

APPENDIX 3.2.2.2 : X-RAY DATA OF SPIROCARACOLITONE A

Space Group and Cell Dimensions Orthorhombic P 212121
a 12.971(5) b 26.027(9) c 11.838(6)
Volume 3997(3)A**3

Empirical formula : O13 C39 H53 + 1/3 H2O

Cell dimensions were obtained from 24 reflections with 2Theta angle
in the range 40.00 - 50.00 degrees.

Crystal dimensions : 0.20 X 0.20 X 0.20 mm

FW = 737.83 Z = 4 F(000) = 1580.74

Dcalc 1.226Mg.m-3, mu 0.06mm-1, lambda 0.70930A, 2Theta(max) 50.0

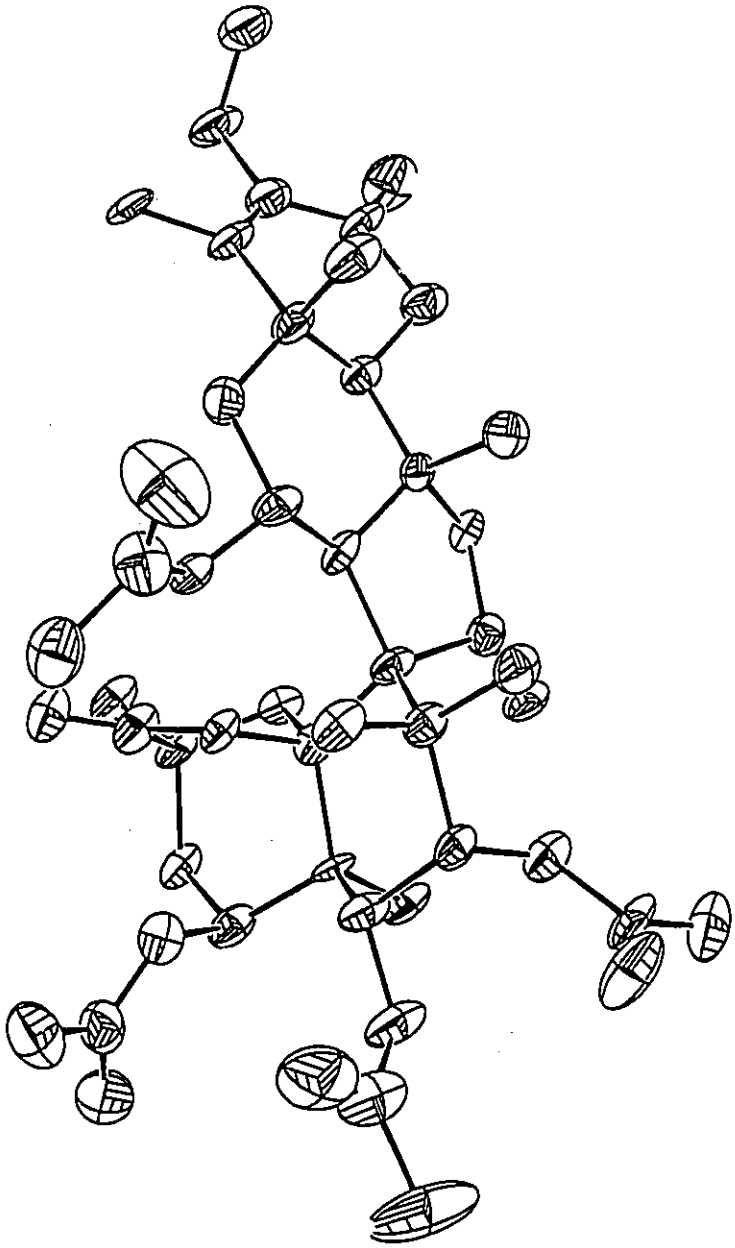
The intensity data were collected on a Rigaku diffractometer,
using the theta/2theta scan mode.
The h,k,l ranges used during structure solution and refinement are :--
Hmin,max 0 15; Kmin,max 0 30; Lmin,max 0 14
No. of reflections measured 3938
No. of unique reflections 3938
No. of reflections with Inet > 2.5sigma(Inet) 2637
Merging R-value on intensities 0.000
No correction was made for absorption

The last least squares cycle was calculated with
106 atoms, 474 parameters and 2637 out of 3938 reflections.
Weights based on counting-statistics were used.

The residuals are as follows :--
For significant reflections, RF 0.103, Rw 0.131 GoF 6.31
For all reflections, RF 0.143, Rw 0.132.
where RF = Sum(Fo-Fc)/Sum(Fo),
Rw = Sqrt[Sum(w(Fo-Fc)**2)/Sum(wFo**2)] and
GoF = Sqrt[Sum(w(Fo-Fc)**2)/(No. of reflns - No. of params.)]
The maximum shift/sigma ratio was 0.068.

In the last D-map, the deepest hole was -0.500e/A**3,
and the highest peak 0.870e/A**3.

Secondary ext. coeff. 0.752218 sigma 0.308352



APPENDIX 3.2.2.3 : X-RAY DATA OF SPIROCARACOLITONE B

Space Group and Cell Dimensions Monoclinic, P 21
a 14.024(3) b 11.816(4) c 15.760(4)
beta 90.72(5)
Volume 2611.3(11)A**3

Empirical formula : O16 C45 H62 +1/2 H20 + 1 EtOH

Cell dimensions were obtained from 24 reflections with 2Theta angle
in the range 40.00 - 50.00 degrees.

Crystal dimensions : 0.20 X 0.20 X 0.20 mm

FW = 906.99 Z = 2 F(000) = 968.47

Dcalc 1.154Mg.m-3, mu 0.08mm-1, lambda 0.70930A, 2Theta(max) 49.9

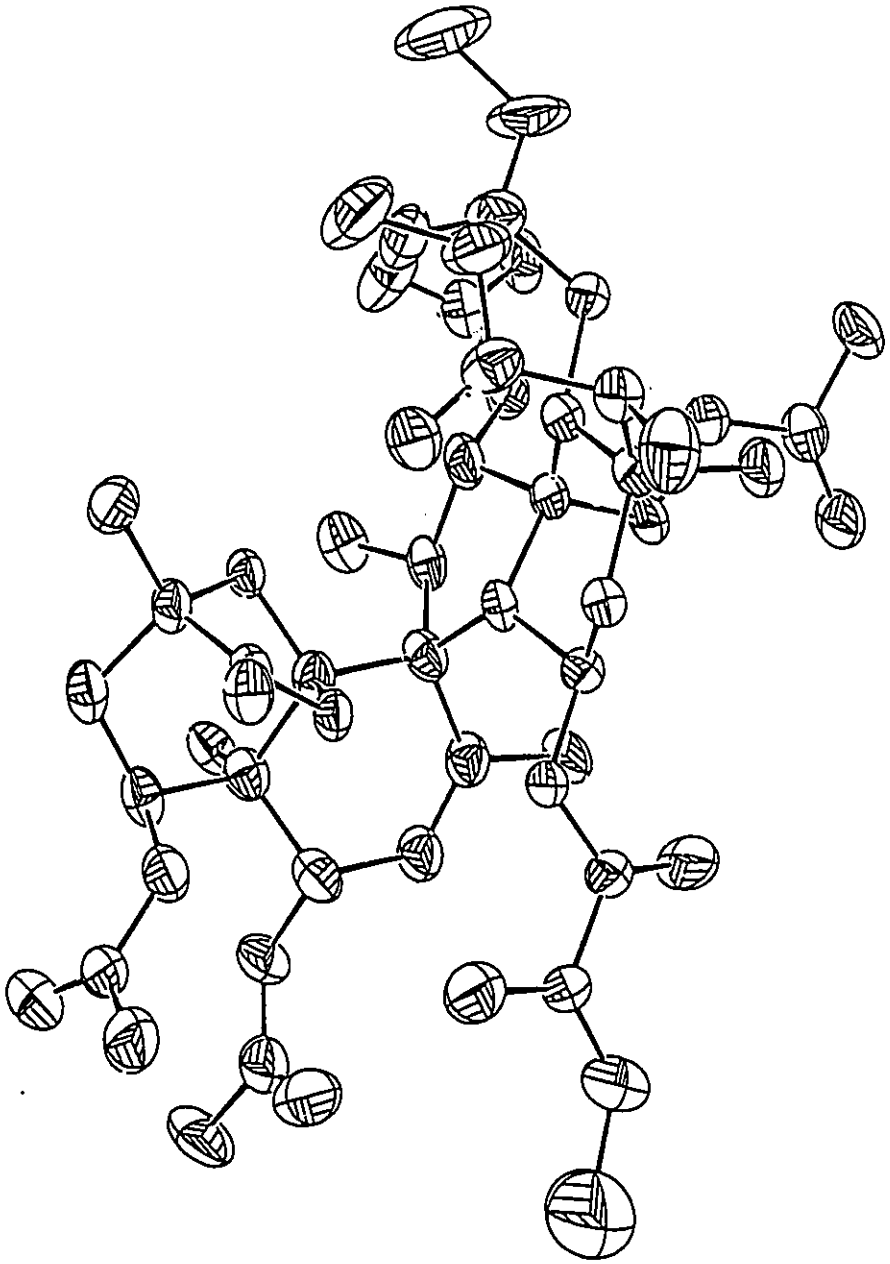
The intensity data were collected on a Rigaku diffractometer,
using the theta/2theta scan mode.
The h,k,l ranges used during structure solution and refinement are :--
Hmin,max -15 15; Kmin,max 0 14; Lmin,max 0 18
No. of reflections measured 4716
No. of unique reflections 4512
No. of reflections with Inet > 2.5sigma(Inet) 3274
Merging R-value on intensities 0.012
No correction was made for absorption

The last least squares cycle was calculated with
130 atoms, 578 parameters and 3274 out of 4512 reflections.
Weights based on counting-statistics were used.
The weight modifier K in KFo**2 is 0.000100

The residuals are as follows :--
For significant reflections, RF 0.074, Rw 0.105 GoF 4.11
For all reflections, RF 0.102, Rw 0.106.
where RF = Sum(Fo-Fc)/Sum(Fo),
Rw = Sqrt[Sum(w(Fo-Fc)**2)/Sum(wFo**2)] and
GoF = Sqrt[Sum(w(Fo-Fc)**2)/(No. of reflns - No. of params.)]
The maximum shift/sigma ratio was 0.563.

In the last D-map, the deepest hole was -0.290e/A**3,
and the highest peak 0.660e/A**3.

Secondary ext. coeff. 0.299567 sigma 0.065733



APPENDIX 3.3.0 : EFFECTS OF SPIROCARACOLITONES(5 PPM IN DIET) ON THE DEVELOPMENTAL PARAMETERS OF THE EUROPEAN CORN BORER.

APPENDIX 3.3.0: EFFECTS OF SPIROCARACOLITONES AT 5 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUNDS	Days to Pupation (Days)	Days to Female (Days)	Pupation Male (Days)	Pupal Weight (mg)	Pupal Weight Female (mg)	Pupal Weight Male (mg)	Adult Weight (mg)	Adult Weight Female (mg)	Adult Weight Male (mg)
CONTROL	23.1 (0.24)	23.1 (0.30)	22.8 (0.28)	86.1 (2.9)	104 (3.6)	76.7 (1.7)	44.4 (3.2)	62.5 (2.8)	34.1 (0.94)
SPIROCARACOLITONE	23.4 (0.18)	23.3 (0.23)	23.3 (0.37)	91.5 (3.3)	103 (3.5)	77.6 (2.1)	46.1 (3.4)	58.2 (2.5)	30.0 (0.84)
SPIROCARACOLITONE A	24.1 (0.47)	24.0 (0.40)	22.8 (0.26)	87.3 (4.6)	106 (4.8)	71.8 (2.5)	46.1 (3.6)	61.8 (2.9)	33.3 (1.8)
SPIROCARACOLITONE B	25.2 (0.74)	24.7 (1.1)	25.4 (1.1)	84.0 (3.9)	88.8 (7.4)	79.1 (2.7)	42.5 (3.3)	53.0 (4.3)	32.0 (2.1)
SPIROCARACOLITONE C	24.4 (0.33)	23.6 (0.42	23.6 (0.31)	86.0 (2.8)	96.8 (3.2)	74.6 (1.2)	40.4 (2.8)	53.2 (2.6)	31.1 (1.1)
SPIROCARACOLITONE D	22.9 (0.44)	22.8 (0.45)	22.4 (0.65)	82.7 (2.8)	95.4 (2.5)	71.3 (2.3)	44.0 (2.6)	54.7 (1.9)	33.3 (1.6)
SPIROCARACOLITONE E	23.8 (0.69)	23.3 (0.21)	23.2 (0.22)	81.3 (3.6)	108 (5.3)	75.0 (1.8)	37.6 (2.8)	60.4 (1.6)	30.5 (1.1)

Note: () = standard error calculated as standard deviation/$(N)^{1/2}$

APPENDIX 3.3.0: EFFECT OF SPIROCARACOLITONES AT 5 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUNDS	Maximum Larval Weight(mg)	Duration of Pupal Period(days)	% Pupation	Days to Adult Emergence	% Adult Emergence	Sex ratio of Adults female/total
CONTROL	104 (4.7)	8.46 (0.29)	90	31.4 (0.26)	73	0.36
SPIROCARACOLITONE	105 (5.1)	9.24 (0.32)	83	32.6 (0.39)	72	0.57
SPIROCARACOLITONE A	99.0 (4.6)	8.60 (0.12)	87	32.0 (0.30)	67	0.45
SPIROCARACOLITONE B	98.7 (5.2)	9.36 (0.33)	83	33.1 (0.54)	76	0.50
SPIROCARACOLITONE C	99.8 (3.3)	9.58 (0.23)	90	33.2 (0.28)	63	0.42
SPIROCARACOLITONE D	97.4 (5.1)	9.04 (0.36)	90	31.6 (0.38)	80	0.50
SPIROCARACOLITONE E	97.4 (5.3)	10.2 (0.)	93	33.4 (0.26)	83	0.24

APPENDIX 3.3.1: EFFECTS OF GEDUNIN ANALOGS ON THE DEVELOPMENTAL PARAMETERS OF THE EUROPEAN CORN BORER

APPENDIX 3.3.1: EFFECTS OF GEDUNIN ANALOGS AT 5 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUNDS	Days to Pupation (Days)	Days to Female (Days)	Pupation Male (Days)	Pupal Weight (mg)	Pupal Weight Female (mg)	Pupal Weight Male (mg)	Adult Weight (mg)	Adult Weight Female (mg)	Adult Weight Male (mg)
CONTROL(1)	24.8 (0.45)	25.3 (0.68)	24.0 (0.53)	67.5 (4.0)	76.6 (4.7)	53.2 (5.53	34.0 (2.3)	39.8 (2.2)	23.7 (2.2)
GEDUNIN	26.1 (0.56)	26.3 (0.7)	26.3 (1.5)	68.1 (2.9)	74.8 (3.5)	60.3 (3.6)	30.9 (2.6)	35.6 (2.6)	19.0 (1.1)
1,2-DIHYDROGEDUNIN	24.9 (0.45)	26.6 (0.69)	23.9 (0.39)	67.0 (2.5)	77.0 (4.1)	61.8 (1.4)	26.8 (2.2)	37.0 (2.7)	19.7 (1.0)
HEXAHYDROGEDUNIN	23.8 (0.26)	24.1 (0.42)	23.4 (0.29)	64.8 (2.0)	65.3 (2.9)	60.4 (3.0)	26.7 (1.8)	32.6 (1.9)	19.5 (0.85)
1,2-EPOXYGEDUNIN	26.8 (0.64)	27.2 (0.88)	26.6 (1.1)	62.9 (2.2)	69.3 (3.1)	56.0 (2.2)	27.0 (2.2)	33.2 (2.6)	19.5 (1 6)
5'-ACETYLGEDUNIN	28.3 (0.90)	30.2 (1.8)	26.4 (0.88)	59.7 (2.9)	68.6 (3.7)	52.5 (2.1)	25.3 (1.7)	31.3 (1.3)	19.2 (1.4)
CONTROL(2)	23.4 (0.34)	24.0 -	23.5 (0.59)	66.1 (2.2)	84.9 -	63.3 (1.6)	32.8 (1.8)	51.6 -	31.6 (1.4)
7-DEACETYLGEDUNIN	24.6 (0.89)	24.1 (0.76)	24.6 (1.1)	73.4 (3.0)	80.1 (4.0)	61.6 (2.9)	38.2 (2.6)	45.4 (3.4)	31.0 (1.6)
2'-ACETYLGEDUNIN	23.7 ()	23.7 (0.73)	23.3 (0.63)	72.1 (3.3)	84.9 (3.4)	60.1 (2.6)	38.5 (2.7)	50.5 (1.9)	28.5 (1.7)
1,2-DIHYDRO-3-β-GEDUNOL	24.7 ()	24.8 (0.43)	23.8 (0.65)	74.3 (2.6)	82.8 (2.9)	62.7 (2.1)	43.2 (2.7)	49.7 (2.0)	32.9 (4.3)

Note: () = standard error calculated as standard deviation/$(N)^{1/2}$

APPENDIX 3.3.1: EFFECTS OF GEDUNIN ANALOGS AT 50 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUNDS	Days to Pupation (Days)	Days to Female (Days)	Pupation Male (Days)	Pupal Weight (mg)	Pupal Weight Female (mg)	Pupal Weight Male (mg)	Adult Weight (mg)	Adult Weight Female (mg)	Adult Weight Male (mg)
CONTROL(1)	24.8 (0.45)	25.3 (0.68)	24.0 (0.53)	67.5 (4.0)	76.6 (4.7)	53.2 (5.5)	34.0 (2.3)	39.8 (2.2)	23.7 (2.2)
GEDUNIN	26.6 (0.74)	27.5 (0.72)	24.9 (1.0)	65.5 (3.0)	73.5 (2.5)	63.7 (4.1)	29.5 (2.2)	36.4 (2.2)	22.0 (1.5)
1,2-DIHYDROGEDUNIN	24.6 (0.43)	25.1 (0.56)	24.2 (0.70)	63.3 (2.2)	69.2 (2.5)	57.3 (1.3)	26.7 (1.9)	32.8 (1.8)	17.8 (1.4)
HEXAHYDROGEDUNIN	25.0 (0.64)	25.1 (1.1)	23.4 (0.43)	67.4 (3.0)	78.1 (2.5)	59.1 (2.4)	30.9 (2.0)	35.8 (1.8)	23.2 (1.7)
1,2-EPOXYGEDUNIN	30.3 (1.1)	30.8 (1.7)	27.6 (0.69)	68.9 (2.9)	79.1 (4.5)	62.4 (2.2)	27.5 (3.2)	42.8 (2.6)	19.9 (1.7)
5'-ACETYLGEDUNIN	26.8 (0.52)	27.6 (0.78)	26.1 (0.74)	60.8 (2.8)	67.5 (4.3)	53.6 (2.4)	24.3 (2.1)	30.1 (3.1)	18.6 (1.1)
CONTROL(2)	23.8 (2.2)	24.0 -	23.5 (0.59)	66.1 (2.2)	84.9 -	63.3 (1.6)	32.8 (1.8)	51.6 -	31.6 (1.4)
7-DEACETYLGEDUNIN	26.0 (2.1)	25.7 (1.2)	26.0 (0.70)	70.8 (3.0)	88.5 (8.1)	64.8 (1.8)	34.4 (2.8)	53.4 (5.1)	29.7 (1.1)
2'-ACETYLGEDUNIN	25.4 (3.0)	26.6 (1.1)	23.4 (0.57)	71.2 (3.0)	79.0 (4.4)	64.8 (2.1)	38.0 (2.5)	45.0 (2.5)	29.9 (1.9)
1,2-DIHYDRO-3-β-GEDUNOL	24.9 (5.3)	23.8 (0.40)	23.3 (0.53)	75.8 (3.4)	85.8 (4.8)	66.9 (1.8)	43.2 (2.7)	51.1 (3.1)	32.3 (1.3)

Note: () = standard error calculated as standard deviation/$(N)^{1/2}$

APPENDIX 3.3.1: EFFECT OF GEDUNIN ANALOGS AT 5 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUNDS	Maximum Larval Weight(mg)	Duration of Pupal Period(days)	% Pupation	Days to Adult Emergence	% Adult Emergence	Sex ratio of Adults female/total
CONTROL(1)	92.0 (4.5)	8.40 (0.37)	90	33.0 (0.79)	83	0.64
GEDUNIN	78.2 (4.0)	8.67 (0.80)	73	34.9 (0.75)	60	0.67
1,2-DIHYDROGEDUNIN	78.3 (3.3)	8.00 (0.31)	77	33.0 (0.52)	73	0.41
HEXAHYDROGEDUNIN	73.4 (2.9)	8.45 (0.51)	77	32.2 (0.66)	67	0.55
1,2-EPOXYGEDUNIN	75.9 (3.0)	8.36 (0.60)	83	35.1 (0.77)	73	0.55
5'-ACETYLGEDUNIN	66.3 (3.9)	6.50 (0.33)	73	34.8 (0.90)	60	0.50
CONTROL(2)	72.8 (3.6)	8.13 (0.24)	67	31.7 (0.68)	53	0.06
7-DEACETYLGEDUNIN	87.7 (4.9)	8.25 (0.28)	73	32.6 (0.82)	57	0.50
2'-ACETYLGEDUNIN	89.5 (3.8)	8.78 (0.65)	77	32.3 (0.58)	73	0.45
1,2-DIHYDRO-3-β-GEDUNOL	91.6 (5.8)	9.14 (0.51)	77	34.7 (1.2)	73	0.67

Note: () = standard error calculated as standard deviation/$(N)^{1/2}$

APPENDIX 3.3.1: EFFECT OF GEDUNIN ANALOGS AT 50 PPM ON DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUNDS	Maximum Larval Weight(mg)	Duration of Pupal Period(days)	% Pupation	Days to Adult Emergence	% Adult Emergence	Sex ratio of Adults female/total
CONTROL(1)	92.0 (4.5)	8.40 (0.40)	90	33.0 (0.79)	83	0.64
GEDUNIN	74.3 (4.5)	9.32 (0.57)	77	35.6 (0.69)	63	0.53
1,2-DIHYDROGEDUNIN	76.6 (2.7)	8.37 (0.48)	93	32.7 (0.54)	90	0.59
HEXAHYDROGEDUNIN	79.4 (3.6)	9.44 (0.67)	77	35.2 (0.97)	60	0.61
1,2-EPOXYGEDUNIN	78.1 (5.0)	9.80 (0.76)	63	38.5 (0.83)	50	0.33
5'-ACETYLGEDUNIN	70.3 (3.8)	6.61 (0.23)	63	33.4 (0.70)	60	0.50
CONTROL(2)	72.8 (3.6)	8.13 (0.24)	67	31.7 (0.68)	53	0.06
7-DEACETYLGEDUNIN	91.3 (4.0)	9.00 (0.31)	69	34.9 (0.56)	59	0.20
2'-ACETYLGEDUNIN	76.2 (4.7)	8.73 (0.43)	57	33.9 (0.96)	50	0.53
1,2-DIHYDRO-3-β-GEDUNOL	88.1 (5.8)	8.93 (0.47)	57	32.5 (0.52)	50	0.43

Note: () = standard error calculated as standard deviation/$(N)^{1/2}$

APPENDIX 3.3.1: EFFECTS OF GEDUNIN ANALOGS ON THE DEVELOPMENTAL PARAMETERS OF *OSTRINIA NUBILALIS* (EUROPEAN CORN BORER)

COMPOUND	Concentration (PPM)	Number of Incomplete Larval Intermediates	Number of Larval Deaths	Number of Pupal Deaths	Number of Adults Emerged Deformed
CONTROL(1)	5	-	3	1	1
GEDUNIN	5	-	8	3	1
1,2-DIHYDROGEDUNIN	5	-	7	1	-
HEXAHYDROGEDUNIN	5	-	7	2	1
1,2-EPOXYGEDUNIN	5	-	5	3	-
5'-ACETYLGEDUNIN	5	-	8	4	-
CONTROL(2)	5	1	9	3	1
7-DEACETYLGEDUNIN	5	1	7	5	1
2'-ACETYLGEDUNIN	5	-	7	1	-
1,2-DIHYDRO-3-β-GEDUNOL	5	2	5	1	2
CONTROL(1)	50	-	3	1	1
GEDUNIN	50	-	7	4	-
1,2-DIHYDROGEDUNIN	50	-	2	1	-
HEXAHYDROGEDUNIN	50	-	6	5	-
1,2-EPOXYGEDUNIN	50	-	11	2	-
5'-ACETYLGEDUNIN	50	-	11	1	-
CONTROL(2)	50	1	9	3	1
7-DEACETYLGEDUNIN	50	3	6	3	1
2'-ACETYLGEDUNIN	50	1	12	1	-
1,2-DIHYDRO-3-β-GEDUNOL	50	-	12	1	1

CLAIMS TO ORIGINAL RESEARCH

1) The synthesis of a number of new gedunin analogs : 1,2-epoxygedunin, tetrahydrogedunin, 2'-acetylgedunin and 5'-acetylgedunin and hexahydrogedunin and the evaluation of the antimalarial (*Palsmodium falciparum*) structure/activity relationships those and other gedunin analogs prepared and isolated in Chapter 1.

2) The evaluation of the antimalarial activity (*Plasmodium falciparum*) of ethanol extracts of over twenty species of the Meliaceae family collected in Costa Rica and Miami, Florida.

3) The screening of twenty species, over sixty ethanol extracts, of the order of the Rutales (primarily the Meliaceae family) for antifeedant activity against the European corn borer (*Ostrinia nubilalis*) and the variegated cutworm (*Peridroma saucia*).

4) The isolation and identification of six spirocaracolitones, spiro-CD-triterpenoids which represent a new class of compounds, from the bark of *Ruptiliocarpon caracolito*.

5) The evaluation of the effects of the newly isolated spirocaracolitones at 5 and 50 ppm in the diet on the developmental parameters of the European corn borer (*Ostrinia nubilalis*).

6) The screening of the spirocaracolitones for antifungal (*Fusarium*) and antimalarial activity (*Plasmodium falciparum*).

7) The evaluation of the effects of the gedunin analogs at 5 and 50 ppm in the diet on the developmental parameters of the European corn borer (*Ostrinia nubilalis*).

8) Postulations of the biosynthetic origin of this unique spiro feature in the spirocaracolitones isolated.

PUBLICATIONS

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