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I dedicate the research presented in this thesis to the memory

of my maternal grandfather, Emil Zöldy,

who was a creative, kind, gentle and loving man, and who has always inspired me

to succeed and achieve in both life and love.

Nagyon szeretlek nagypapa.

Life is essentially a cheat and its conditions are those of defeat ...

the redeeming things are not "happiness and pleasure"

but the deeper satisfactions that come out of struggle.

F. Scott Fitzgerald, Oct. 5, 1940

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ABBREVIATIONS AND SYMBOLS

Δ	heat
9-BBN	9-borabicyclo[3.3.1]nonane
abs.	absolute
Ac	acetate
Bn	benzyl
brs	broad singlet
°C	degrees celcius
calcd.	calculated
cf.	compare
CH_2Cl_2	methylene chloride
chp.	chapter
cm^{-1}	wavenumber
^{13}C NMR	carbon-13 nuclear magnetic resonance
COSY	correlation spectroscopy
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
dd	doublet of doublets
DEPT	distortionless enhancement polarization transfer
DMAP	N,N-dimethylaminopyridine
DMF	N,N-dimethylformamide
E_2	bimolecular elimination

EI	electronic ionization
Elem. Anal.	elemental analysis
eq.	equivalents
Et_3N	triethylamine
Et_2O	diethyl ether
EtOH	ethanol
g	gram
gl. AcOH	glacial acetic acid
h	hour
HCl	hydrochloric acid
HMPA	hexamethylphosphoramide
HMQC	heteronuclear multiple quantum coherence spectroscopy
^{1}H NMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	hertz
IC_{50}	concentration to inhibit growth by 50%
INADEQUATE	incredible natural abundance double quantum transfer experiment
IR	infrared
IUPAC	International Union of Pure and Applied Chemistry
J	coupling constant
KOH	potassium hydroxide

LDA	lithium diisopropylamide
LHMDS	lithium hexamethyldisilazide
m	multiplet
M	molar
m/z	mass to charge ratio
Me	methyl
MeLi	methyllithium
MeOH	methanol
MeOTf	methyl triflate
mg	milligram
min	minutes
mL	milliliter
mp	melting point
Ms	methanesulfonyl
MS	low resolution mass spectroscopy
MW	molecular weight
ng	nanogram
NMPD	N-methylpyrrolidinone
nOe	nuclear Overhausser effect
PDC	pyridinium dichromate
Ph	phenyl
ppm	parts per million

q	quartet
RT	room temperature
s	singlet
S_N2	bimolecular nucleophilic substitution
t	triplet
TBAA	tetrabutylammonium acetate
TBHP	*tert*-butylhydroperoxide
THF	tetrahydrofuran
tlc	thin layer chromatography
Ts	*p*-toluenesulfonyl
$TsNHNH_2$	*p*-toluenesulfonylhydazide
TsOH	*p*-toluenesulfonic acid

ABSTRACT

This thesis describes the first total synthesis of 2-hydroxyandrosta-1,4-diene-3,16-dione, trichiliasterone A, **1**, the transformation of **1** into 2-methoxyandrosta-1,4-diene-3,16-dione **1a**, and the partial synthesis of trichiliasterone B, 3β-hydroxypregna-2,16-dione **2**. Steroids **1** and **2** were isolated from the wood of the Costa Rican tree, *Trichilia hirta*, and both elicited insect growth inhibition in the European corn borer, *Ostrinia nubilalis* and the variegated cutworm, *Peridroma saucia*. Steroid **1a** was isolated from *Trichilia americana*.

In addition to the syntheses of **1** and **2**, derivatives of gedunin **74**, a limonoid currently under development as an antimalarial agent, were prepared. The 1-benzylmercaptogedunin derivative **78** was prepared in connection with the postulated molecular mode of action of gedunin **74**. The derivative, 7-hydroxygedunin **75** was prepared to test its stability under acidic conditions. Finally, 7-methoxygedunin **77** was prepared and tested for antimalarial activity against a chloroquine resistant (W2) and chloroquine sensitive (D6) strain of *Plasmodium falciparum*.

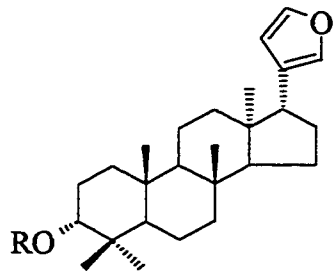
Introduction

Chapter 1

1.1 Isolation, Structure and Background.

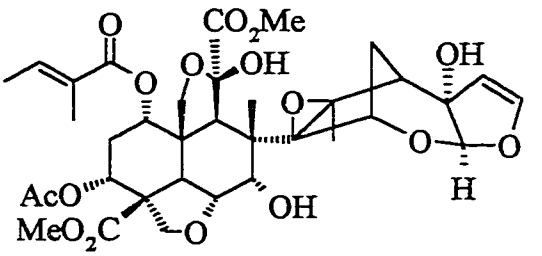
The woody tropical plants of the Meliaceae (Mahogany family) belonging to the plant order Rutales, are known for their production of limonoids as secondary metabolites.[1] Limonoids, also referred to as tetranortriterpenoids, consist of 26 carbons; the basic skeleton is shown in figure 1.1.1. Oxidative insertions and carbon-carbon bond cleavages lead to many interesting structure variations.

Figure 1.1.1 - The skeleton of parent tetranortriterpenoids isolated from species of Meliaceae



These secondary metabolites possess a wide range of biological activity, including bactericidal, anti-viral, anti-fungal and many are medicinally active in animals and humans.[1] More recently, azadirachtin (see figure 1.1.2), a c-seco-tetranortriterpenoid isolated from the Indian neem tree, *Azadirachta indica* A. Juss, has attracted worldwide attention for its insect anti-feedant and growth regulating activity.[2] There is an increasing need for natural pest control agents like azadirachtin to protect crops, and the limonoids appear to be a promising group of compounds.

Figure 1.1.2- The structure of Azadirachtin.

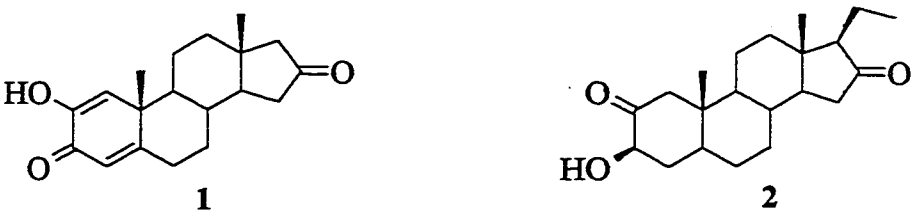


Several years ago, members of our research group screened various Meliaceae species that exist in Central America for natural insecticidal activity.[3] They found the Costa Rican tree, *Trichilia hirta*, to be a promising source of such botanical insecticides. Specifically, the ethanol extracts of the wood and bark exhibited growth inhibitory activity on *Ostrinia nubilalis* (the second instar European corn borer), and *Peridroma saucia* (the neonate variegated cutworm). Further, the activity was attributed to either one or both of the compounds found in the extract. The two biologically active compounds were separated by partitioning the ethanol extract into methylene chloride and other organic solvents. The methylene chloride sample showed the highest insect growth inhibition. It was freeze-dried and purified by column chromatography and preparatory reverse phase HPLC. Unfortunately, only 9 mg and 11 mg respectively of these two compounds labeled **1** and **2** (see figure 1.1.3) were obtained from the 40 g ethanol extract.

A variety of spectral data including ^{1}H NMR, ^{13}C NMR, DEPT, IR, ^{1}H-^{1}H COSY and HMQC suggested that the structure of compound **1**, referred to as trichiliasterone A, was 2-hydroxyandrosta-1,4-diene-3,16-dione. The structure of compound **2** was 3β-

hydroxypregna-2,16-dione, and it is referred to as trichiliasterone B. To the authors' surprise, the ^{1}H NMR spectrum of compound **1** and **2** indicated that both compounds lacked the aromatic resonance characteristic of the furan ring; thus neither compound was a limonoid.

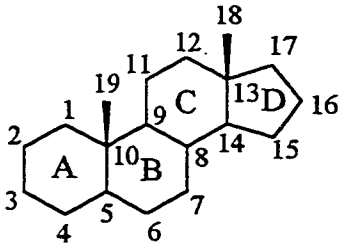
Figure 1.1.3- The structure of 2-hydroxyandrosta-1,4-diene-3,16-dione **1** and 3β-hydroxypregna-2,16-dione **2** isolated from *Trichilia hirta*.



1 **2**

The nomenclature of the steroids in this thesis conforms to IUPAC regulation which has recently been summarized.[4] Figure 1.1.4 illustrates the numbering system and the naming of the rings. Furthermore, all steroids presented herein, have the 5α and 14α configuration unless otherwise stated. Thus, the AB and CD ring fusions are *trans*.

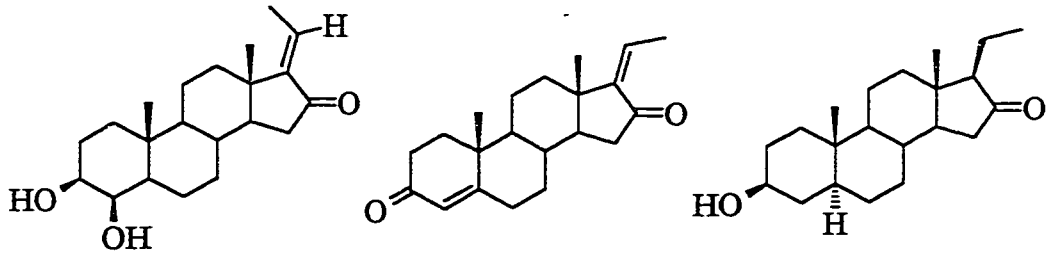
Figure 1.1.4- IUPAC number system for the steroid skeleton.



Confirmation of the structures came from a ^{13}C-^{13}C 2D-NMR INADEQUATE experiment that verified the carbon skeleton of compound **2**. The INADEQUATE experiment determines the carbon skeleton of a molecule by probing the carbon-carbon bonds directly in which only adjacent pairs of ^{13}C atoms are detected.[5] In an unlabelled compound the probability of detection is only 0.012% since the natural abundance of ^{13}C is 1.1%. To overcome this low sensitivity, larger sample sizes are required, typically greater than 100 mg, which in this case was impossible as there was only 11 mg of steroid **2** isolated.[3] This problem was resolved by combining the ^{13}C Nano.nmr™ probe technology with FRED™, a spectral-interpretation NMR software program, since both serve to increase the sensitivity of INADEQUATE.

Neither of these two plant steroids have been reported previously. It is noteworthy that both compounds possess a 16-oxo functionality, which is rare in steroids originating from plant sources. There are few examples of other plant derived steroids that have a 16-oxo substituent (see figure 1.1.5). Lansisterone *E* **3** was isolated from the species *Lansium anamallayanum* Bedd, which like *Trichilia hirta* belongs to the Meliaceae.[6] Lansisterone *E* **3** or 3β,4β-dihydroxy-5α-pregn-(E)-17(20)-en-16-one is isolated in 0.016% yield from the leaves of the native Indian plant.[6] One of the first 16-oxo-steroids to be isolated[7] and synthesized[8] was pregna-4-(Z)-17(20)-diene-3,16-dione **4** or *Z*-guggulsterone which is its trivial name. Like lansisterone, *Z*-guggulsterone **4** was also isolated from a tree native to India, *Commiphora mukul*. Its trivial name originated from the Sanskrit word 'guggulu' which is the name given to the gum-resin that this

Figure 1.1.5- The Structures of other Plant Steroids possessing 16-oxo functionality.



3 **4** **5**

tree exudes.[7] The gum resin has been a part of an ancient Indian form of medicine called 'Ayurveda' and is used to treat various body disorders and infections. The gum contains 0.45% of guggulsterone **4**, and both **4** and the gum have anti-inflammatory, anti-rheumatic and hypocholestremic/hypolipaemic activity.[7] As figure 1.1.5 shows, another 16-oxo-steroid that has been isolated is 3β-hydroxy-5α-pregnan-16-one **5**.[9] It occurs in the roots of *Solanum hainanense* Hance, a Vietnamese plant and from a 100 g sample of powdered roots, researchers have obtained only 25 mg of compound.[9]

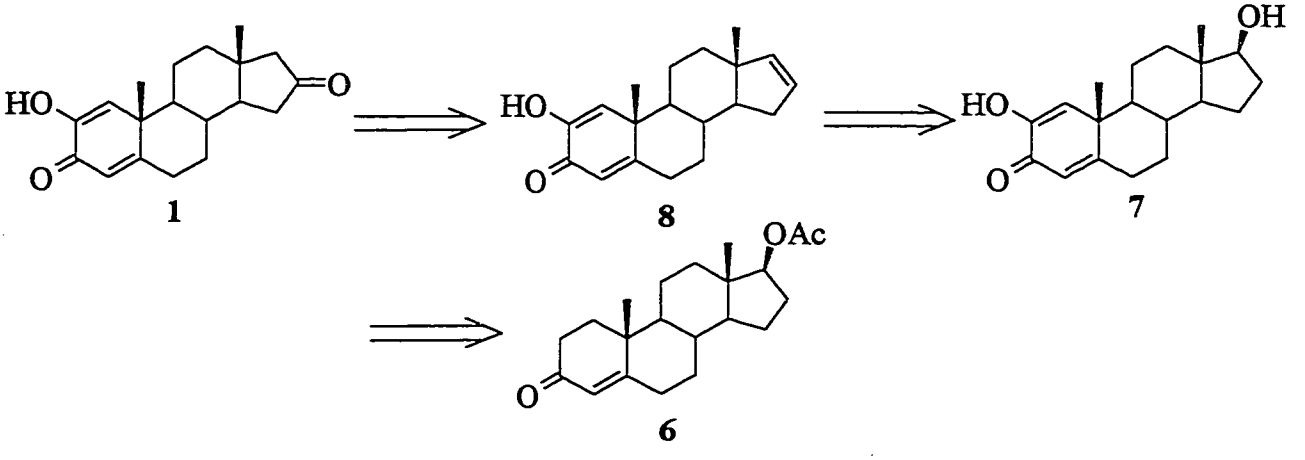
The small amounts of **1** and **2** obtained in the initial isolation rendered isolation to be a non-viable route to the significant amounts needed to carry out a variety of biological screens. Thus, it was decided to initiate a synthesis of trichiliasterone A and B from steroid precursors. The 16-oxo functionality present in these compounds poses a key synthetic problem, in that, the readily available steroid precursors have an oxygen atom at

C-17 not C-16. Hence, a formal oxygen atom transposition must be included in the synthetic scheme.

1.2 The Retrosynthetic Approaches to 2-hydroxyandrosta-1,4-diene-3,16-dione 1.

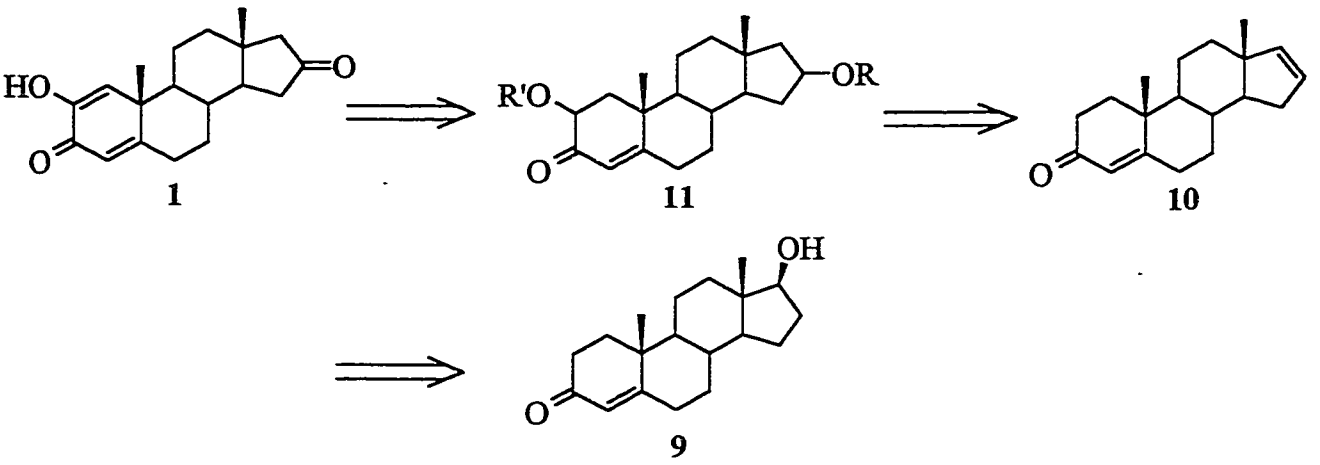
A key decision in the syntheses of both compounds was whether to derivatize first ring A, and then deal with ring D or vice versa. In our initial approach to **1**, we chose to derivatize ring A first (scheme 1.2.1), utilizing the acetate of testosterone **6** as the starting material. The A ring of **6** could easily be functionalized to the 2-hydroxyandrosta-1,4-dien-3-one array of intermediate **7**. The 17β-hydroxyl substituent would then be converted to a potential leaving group, which upon elimination would afford a double bond between C-16 and C-17 of intermediate **8**. Upon regioselective hydration and oxidation of **8**, the required 16-oxo substituent of **1** would be obtained.

Scheme 1.2.1 - A Retrosynthetic Approach to 2-hydroxyandrosta-1,4-diene-3,16-dione **1**.



An alternate sequence was also devised in which a 16-hydroxyl group would first be introduced into the D-ring of testosterone **9**. The 16-hydroxyl substituent would result from the regioselective hydration of the alkene intermediate **10**. Protection of the 16-hydroxyl group, followed by partial elaboration of ring A would lead to intermediate **11**. At this point, oxidation of the hydroxyl group in ring D, and subsequently of that in ring A would lead to **1.**

Scheme 1.2.2- An Alternate Retrosynthetic Approach to 2-hydroxyandrosta-1,4-diene-3,16-dione **1**.

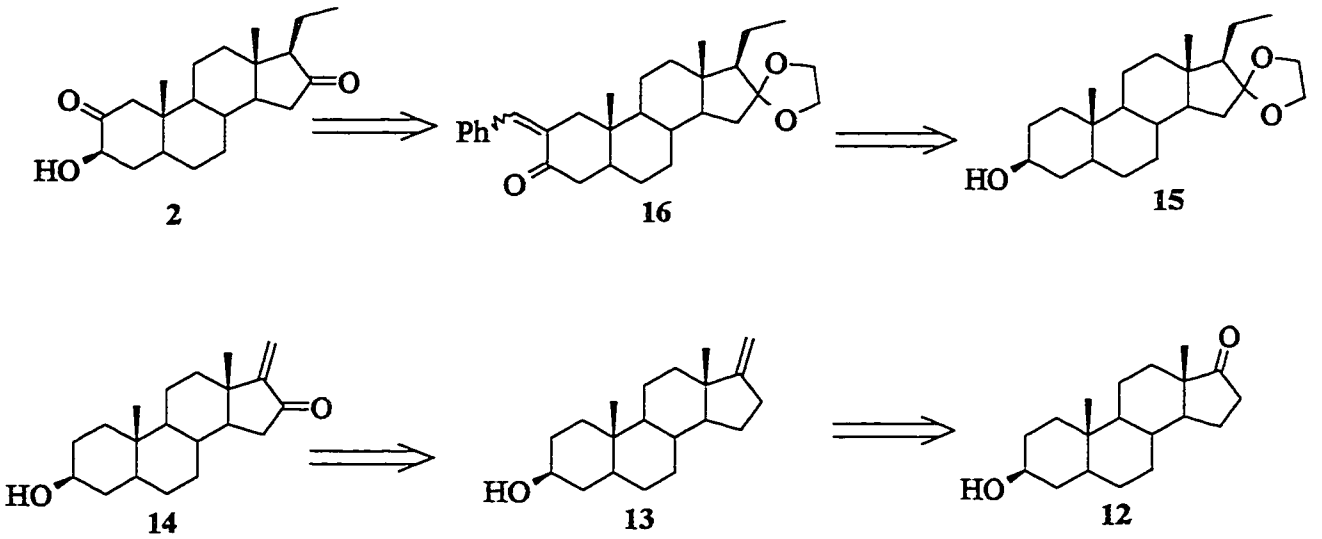


1.3 The Retrosynthetic Approach to 3-hydroxypregna-2,16-dione 2.

In the case of steroid **2**, we chose to work on ring D of isoandrosterone **12** first, because the 17-oxo substituent would easily be converted to the 17(20)-exocyclic double bond of **13**. Allylic oxidation of **13** would give the enone **14** which then would undergo a 1,4-conjugate addition. The resulting pregnone derivative would then be protected as the

ketal **15** and the 3β-hydroxyl group would be oxidized. Aldol condensation of the 3-oxo substituent with benzaldehyde with subsequent dehydration would yield intermediate **16**. Reduction of the 3-oxo group followed by ozonolysis of the exocyclic alkene would lead to the 2-oxo-3-hydroxyl array of **2**.

Scheme 1.3.1- The Retrosynthetic Approach to 3-hydroxypregna-2,16-dione **2**.



Results and Discussion

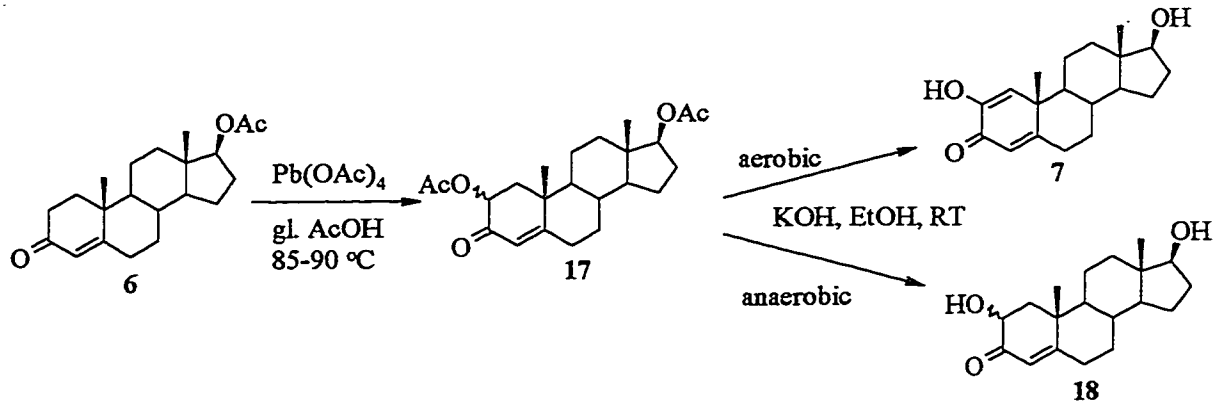
Chapter 2

The Total Synthesis of 2-hydroxyandrosta-1,4-diene-3,16-dione 1.

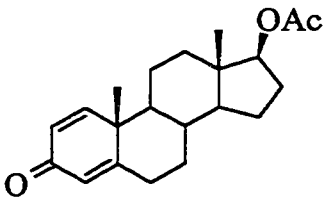
2.1 Initial Attempts (scheme 1.2.1)

In 1953, Sondheimer and co-workers reported that an acetoxy group can be placed adjacent to the carbonyl group of testosterone acetate **6** using lead tetraacetate in hot glacial acetic acid.[10] When other researchers attempted to hydrolyze the 2α–and 2β-hydroxytestosterone diacetate epimers **17**, they obtained 2,17β-dihydroxy-androsta-1,4-dien-3-one **7** instead of the expected 2-hydroxytestosterones **18**.[11,12] This outcome was due to air oxidation of **17** and indeed if one needed to obtain the 2-hydroxytestosterones **18**, strict anaerobic conditions were required.[11]

Scheme 2.1.1- The procedure for obtaining 2,17β-dihydroxyandrosta-1,4-dien-3-one 7.



The simplicity with which testosterone acetate **6** was derivatized to steroid **7**, prompted us to begin the synthesis of trichiliasterone A, **1**, with this effective two step procedure. Essentially, we obtained the A-ring functionality of steroid **1** by heating testosterone acetate **6** with 1.1 molar equivalents of lead tetraacetate for 22 hours in glacial acetic acid at 85°C. The reaction afforded a crude mixture that was purified by column chromatography and the 2α- and 2β,17β-diacetoxytestosterone isomers, **17** (see scheme 2.1.1) were obtained in 44% yield. A byproduct, 17β-hydroxyandrosta-1,4-dien-3-one **19** was obtained in 11% yield and it was identified by its ^{1}H NMR spectrum and by

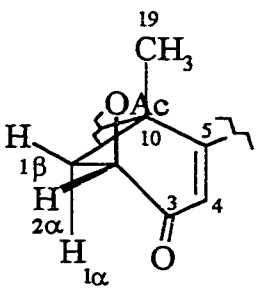


19

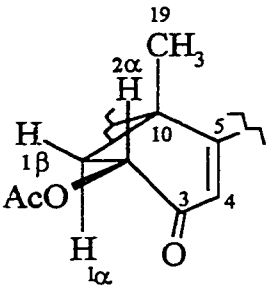
comparison of its melting point of 153-154°C with the literature value of 152.5-155.2°C.[13a]

From the ^{1}H NMR spectrum of **17** it was evident that the 2α- and 2β–isomers were obtained as a 4:1 mixture, but the identification of whether the 2α- or the 2β-isomer was the major product by coupling constants was not possible. The coupling constants for H-2 of the major isomer was 14.0 and 5.5 Hz, and the coupling constants for H-2 of the minor isomer were similar in magnitude at 12.7 and 6.0 Hz. These coupling constants were unexpected in that the β-isomer should have shown two small coupling constants for H-2, whereas the α-isomer should have shown a large and a small coupling constant for

H-2. These expectations stemmed from the fact that the A-ring of most 4-en-3-one steroids exist as a 1α, 2β half-chair conformation (see below).[13b] In this conformation, C-3, C-4, C-5 and C-10 are coplanar, with C-1 below the plane and C-2 above the plane. If the β-isomer exists in this conformation, then there are two small dihedral angles between H-2α and H-1α,β, which would result in two small coupling constants. If the α-isomer exists in the half chair conformation, then a large and a small coupling constant is expected due to the large dihedral angle between H-2β and H-1α, and the small dihedral angle



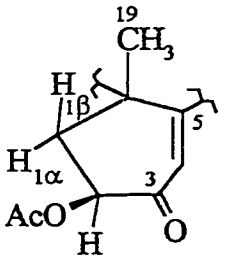
2β-OAc isomer **17**



2α-OAc isomer **17**

(both isomers depicted in 1α,2β **half-chair** conformation)

between H-2β and H-1β. A plausible explanation for the unexpected large and small coupling constants seen for **both** isomers could be that the β-isomer exists in a slightly distorted half-chair conformation called the 1α sofa (see below), whereas the α isomer exists in the half-chair. The 1α sofa conformation has C-2 through C-5 and C-10

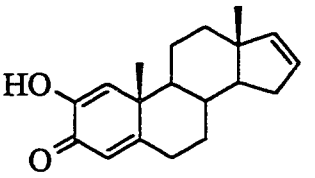


2β-OAc isomer **17** depicted in the 1α-**sofa** conformation

coplanar, with C-1 below the plane.[13b] In the sofa conformation, the dihedral angle between H-2α and H-1β for the β-isomer is large and thus, a large coupling constant as was observed can be expected.

The second step in the synthesis of trichilasterone A, **1**, was to oxidize the mixture of 2α- and 2β,17β-diacetoxytestosterone **17** by Clarke's method.[11] The isomer mixture was stirred with potassium hydroxide in ethanol in an open vessel for four days at room temperature to afford **7** in 100% yield (see scheme 2.1.1). The melting point of **7** was 204-206°C which closely matched the literature value of 207-209.[13a] With the A-ring of trichiliasterone A complete, our attention turned to the derivation of ring D.

As indicated in the retrosynthetic approach 1.2.1, it was proposed that the transposition of the oxygen atom at C-17 to C-16 would occur via an alkene intermediate, namely 2-hydroxyandrosta-1,4,16-trien-3-one **8**. To obtain **8**, the 17-hydroxyl moiety of steroid **7** needed to be converted to a mesylate, so that it could be eliminated under basic

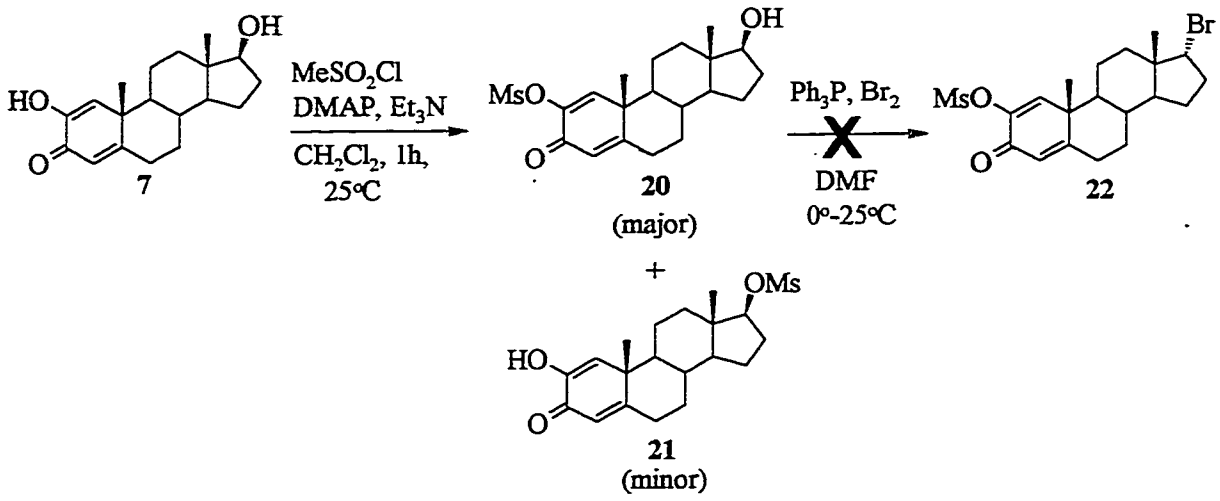


8

conditions without interference from the ring A functionality. However, mesylation of **7**, with one molar equivalent methanesulfonyl chloride as shown in scheme 2.1.2, afforded a mixture of the 2- and 17-mesylated steroids, **20** and **21**. We also tried to derivatize selectively the 17-hydroxyl substituent of **7** as a tosylate but this attempt also failed.

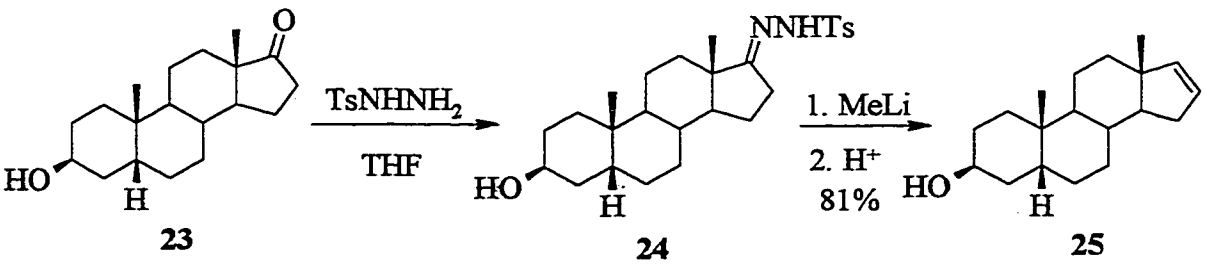
Careful column chromatography of the mesylation reaction of **7** afforded the pure

Scheme 2.1.2-Initial attempts of derivatizing the D-ring with the A-ring in place.



2-mesylated steroid **20.** Unfortunately, attempted transformation of **20** into the 17-bromo derivative **22** using triphenylphosphine and bromine in dimethylformamide[14,15] was unsuccessful. It was anticipated that the brominated intermediate **20** would undergo an E_2-elimination with DBU to afford the desired alkene intermediate **8**. Since the bromination was not successful, this route to the alkene intermediate **8** was abandoned.

The second approach envisaged for the preparation of **8** was via a Shapiro reaction. This required that the 17-hydroxyl substituent of **7** be oxidized to the ketone followed by conversion to its tosyl hydrazone and subsequent treatment with methyllithium. As an example, Fetizon and co-workers[16] have converted 5β-isoandrosterone **23** to **25** by first forming the hydrazone **24,** which upon treatment with

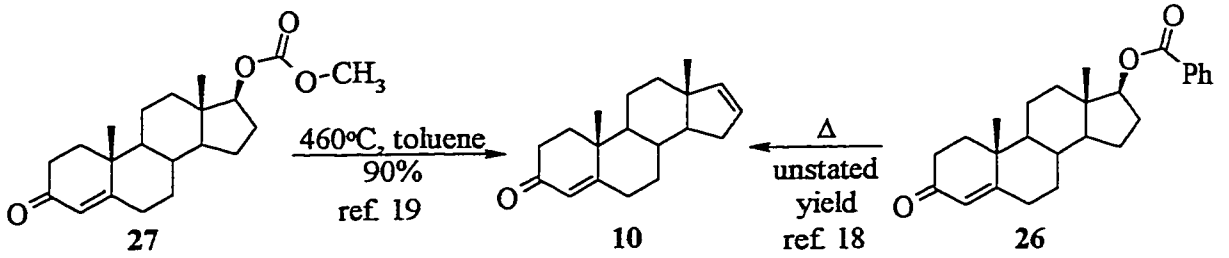


two molar equivalents of methyllithium and subsequent protonation gave 3β-hydroxy(5β)androst-16-ene **25**. However, oxidation of **7** to the corresponding 17-oxo substituent using Swern's procedure[17] led to a complex mixture of products. The use of pyridinium dichromate (PDC) also gave none of the desired product, possibly due to a reaction between PDC and the enol functionality of ring A. Thus, the Shapiro route to the alkene intermediate **8** was also abandoned. At this point, it was decided that the alternate route to trichiliasterone A (scheme 1.2.2) would be pursued, since the 'completed' form of ring A in **7** interfered with attempts at obtaining the alkene intermediate **8**.

2.2 Transformation of the D ring of Testosterone (scheme 1.2.2).

It has been shown that both testosterone benzoate[18] **26** and the methyl carbonate of testosterone[19] **27** readily undergo thermolysis to give androsta-4,16-dien-3-

Scheme 2.2.1-Pyrolysis of testosterone derivatives **26** and **27**.

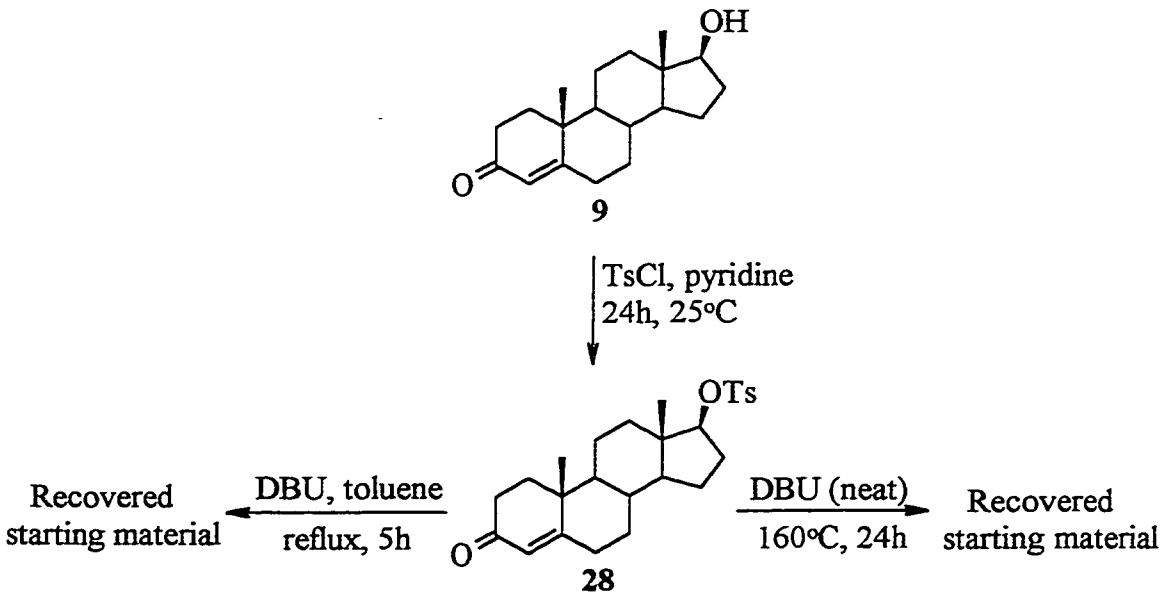


one **10**. Thus, the pyrolysis of testosterone acetate **6** was attempted by heating it for 2 hours at 450 °C. However only starting material was obtained; this may have been due to

a lack of appropriate equipment. To obtain **10** from **27**, Ohloff and his colleagues heated a 10 m long Pyrex© glass column through which a solution of steroid **27** was carried with the flow of N_2 (11 ml/min.) at a rate of approximately 1 g/h.[19] In contrast, we used a much shorter column and did not have control over the flow.

Since the pyrolysis route to steroid **10** was not successful, it was decided to attempt a base-catalyzed elimination[20] of toluenesulfonic acid from the testosterone tosylate **28,** which was prepared according to the method of Sondheimer and co-workers.[21] However, when **28** was heated with DBU neat at 160°C or in refluxing toluene, only starting material was recovered (see scheme 2.2.2). A subsequent and extensive literature review showed that others have also tried base-mediated eliminations of tosyl testosterone **28**. Wilkinson and co-workers[22] reported that the reaction of **28** with potassium *t*-butoxide in *t*-butanol gave in approximately equal amounts, starting

Scheme 2.2.2-Attempts at preparing androsta-4,16-dien-3-one **10**.



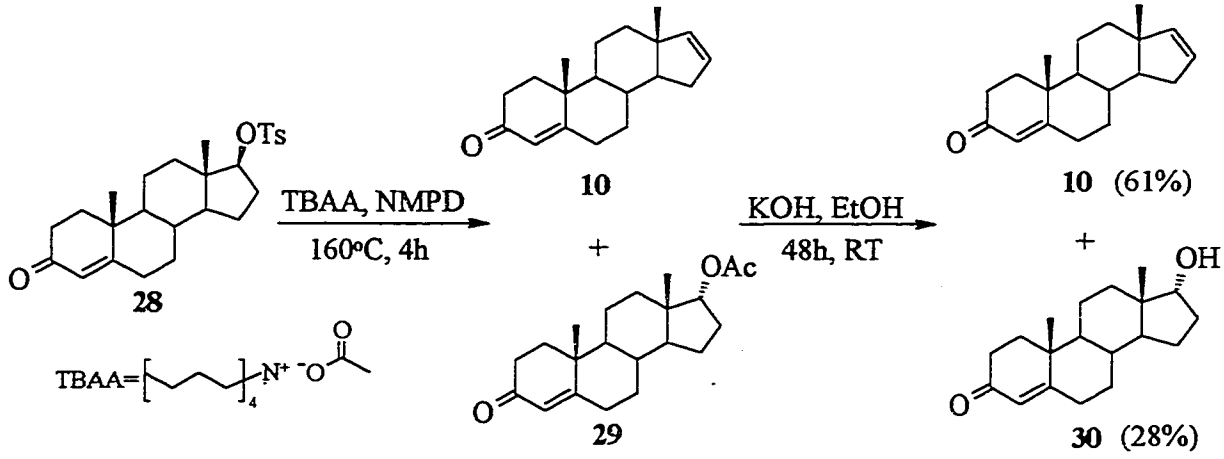
material and testosterone-17α-*t*-butyl ether. When they tried the same reaction using DMSO as the solvent, they were successful in correcting the solvent's nucleophilicity interference, but recovered only starting material.[22]

During the early 1960's, there was great interest in studying the effect of the remote C-17 substituents on the nucleophilic addition to the enone system in androst-4-en-3-one steroids. In particular, a variety of 17α-substituted derivatives of testosterone were needed for these studies. Henbest and Jackson[23] set out to synthesize the 17-α-acetate of testosterone **29** by an S_N2 reaction involving the heating of tosyl testosterone **28** and tetrabutylammonium acetate [24,25] in *N*-methylpyrrolidinone at 160°C for 4 hours. They obtained the 17-α-acetate of testosterone as the minor product (34%). The major product, obtained in 57% yield, was androsta-4,16-dien-3-one **10** the compound of interest to us. Henbest and Jackson stated that the *N*-methylpyrrolidinone was crucial for the formation of **10**. They also proved that the 17α-acetate of testosterone **29** was not the intermediate to **10**, because when they heated **29** and tetrabutylammonium acetate in *N*-methyl-pyrrolidone (NMPD) they found that **29** remained unchanged. Thus, steroid **10** is not derived from the 17α-acetate **29**.

We used Henbest and Jackson's method to obtain **10** with several modifications to simplify the isolation and purification of **10**. First, we distilled the *N*-methylpyrrolidinone from the crude mixture of products **10** and **29** upon the completion of the reaction. Somewhat surprisingly, compounds **10** and **29** had very similar Rf values on alumina in a variety of solvent systems. Silica gel could not be used for chromatography because of the instability of **10** on such a medium.

To facilitate column chromatography, the 17α-acetate of **29** was hydrolyzed to the more polar 17α-hydroxyl-androst-4-en-3-one **30** (see scheme 2.2.3). After chromatography using a 3:1 petroleum ether/diethyl ether solvent system, androsta-4,16-dien-3-one **10** was obtained in 61% yield as colourless oily crystals. The ^{1}H-NMR spectrum (figure 2.2.1-chp. 2 appendix) and IR spectrum of **10** matched that reported by

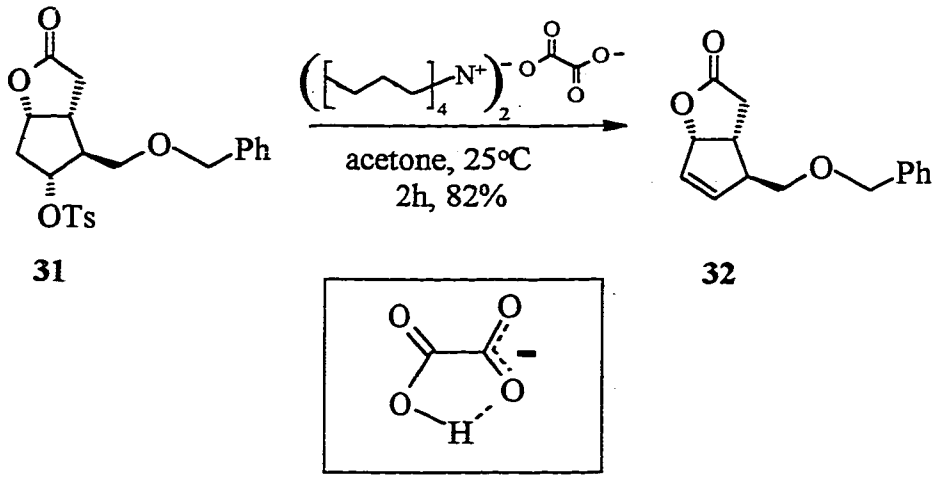
Scheme 2.2.3-Preparation of androsta-4,16-dien-3-one **10**.



Ohloff and co-workers.[19] Recrystallization of **10** in a 1:1 mix of isopropyl ether and petroleum ether gave colourless crystals that melted at 131-135°C [literature mp. 131-133°C].[23] Wilkinson and co-workers reported that androsta-4,16-dien-3-one **10** underwent partial decomposition when stored at room temperature. We also had this problem, and we found that even if **10** was stored in diethyl ether at -35°C, it would begin to decompose in two month's time. Wilkinson et. al[22] stated that decomposition might be due to rearrangements[26,27] caused by the reactivity of the C-16 alkene.

The thermal instability and relatively low yield of **10** prompted us to attempt to optimize the TBAA/NMPD reaction. It has been reported that the use of tetrabutylammonium oxalate in this type of reaction dramatically favours the elimination over the substitution pathway. For example, the unsaturated lactone **32** was obtained from the tosylate **31** upon treatment with five molar equivalents of tetrabutylammonium oxalate in acetone at 25°C for 2 hours.[28] The preference for elimination has been attributed to the internal stability of the oxalate anion caused by hydrogen bonding (scheme 2.2.4). Little or no substitution product was obtained because the stability of the oxalate anion hindered nucleophilic attack.

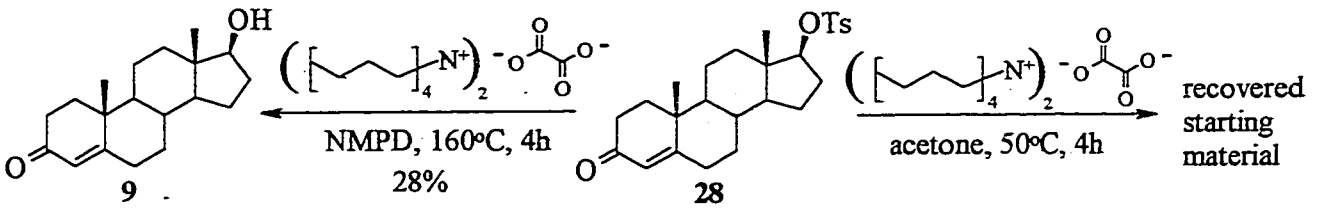
Scheme 2.2.4-Preference for E_2 over S_N2 pathway by the use of tetrabutylammonium oxalate.



However, when 17β-tosyl testosterone **28** and tetrabutylammonium oxalate was refluxed in acetone for 4 hours, only starting material was recovered. A change of solvent to *N*-methylpyrrolidinone did not improve the reaction, as testosterone **9** was isolated in 28%

yield (see scheme 2.2.5). Thus, it was decided to continue the use of Henbest and Jackson's method for obtaining androsta-4,16-dien-3-one **10**.

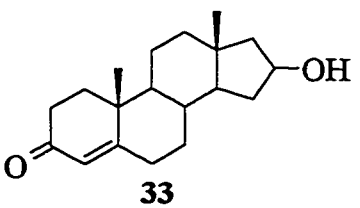
Scheme 2.2.5-Attempts at optimizing the yield of androsta-4,16-dien-3-one **10**.



9 **28**

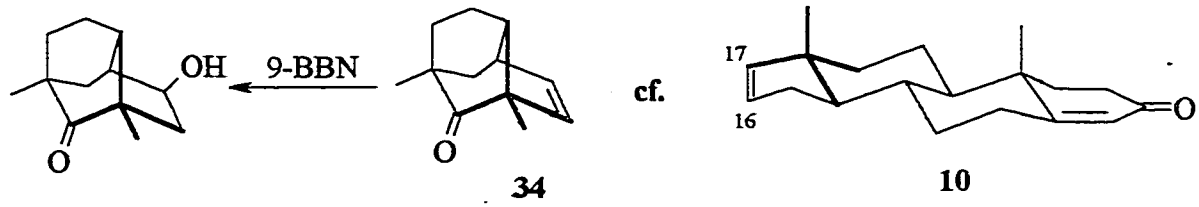
2.3 The Preparation of 16-hydroxyandrost-4-en-3-one 33.

The next step in the synthesis was to oxygenate ring D at the C-16 position. Hydroboration-oxidation was chosen as a suitable method because of the high regioselectivity of 9-BBN, the boron hydride reagent.[29] The bulkiness of 9-BBN would dictate its addition to the less congested end of the double bond, namely C-16. By adding to C-16, the bulky boron hydride would be further from the neopentyl-like center at C-13 and give upon subsequent oxidation at C-16, the desired 16-hydroxyandrost-4-en-3-one **33**, and not testosterone **9**.



33

The method of Chang and Chang[30] was used because they reported successful hydroboration-oxidation of a neopentyl type system **34** which is structurally similar to **10**.



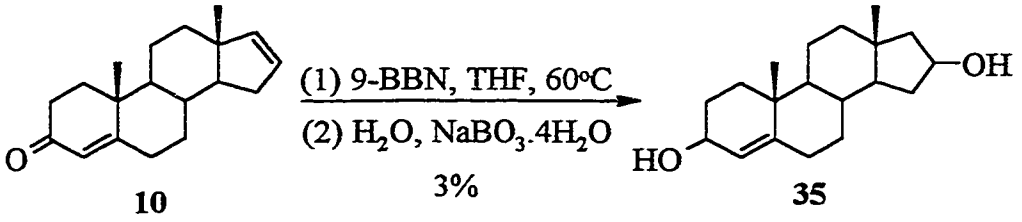
34 **10**

The method was slightly modified in that a sodium perborate/water[31] system was used to oxidize the organoborane instead of the traditional alkaline hydrogen peroxide system. There was a potential for the hydrogen peroxide anion to add in 1,4-conjugate fashion[32] to the α,β-unsaturated system of **10**, whereas the milder sodium perborate ($NaBO_3.4H_2O$) would not.

However, when **10** was heated with excess 9-BBN in THF at 60°C for two hours followed by oxidation at room temperature with $H_2O/NaBO_3.4H_2O$, a complex mixture was obtained. ^1H NMR analysis of the separated compounds indicated that most had multiple C-18 and C-19 methyl peaks and a large *hump* in the methylene region. It was speculated that the hump may be due to non-hydrolyzed borate ($B(OR)_3$) species. An attempt at resolving this problem was to use tetrabutylammonium fluoride as a phase-transfer catalyst to facilitate the biphasic oxidation step. Unfortunately, the tetrabutylammonium fluoride did not facilitate the hydrolysis of the borate esters as was evident by the ^1H NMR spectra obtained.

Only one compound was isolated and characterized from the 9-BBN hydroboration-oxidation reaction. Steroid **35** was obtained in 3% yield and its structure

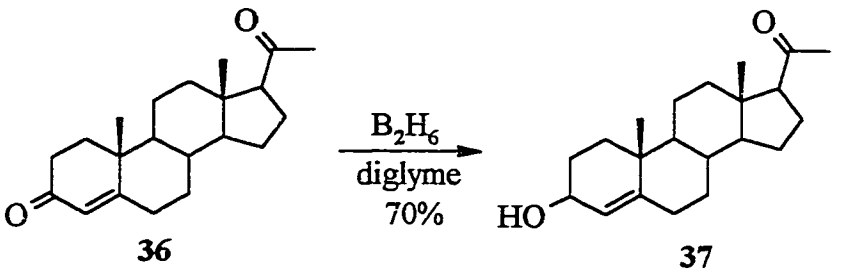
indicated that the α,β-unsaturated double bond of **10** had undergone hydroboration, but not oxidation. In 1977, Pizey reported that although borane reduction of α,β-unsaturated



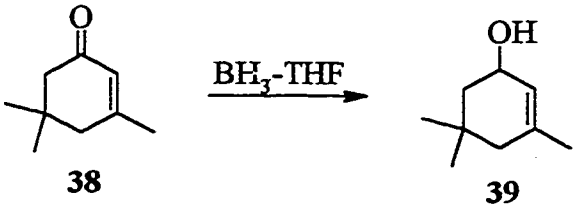
10 **35**

systems was rare, allylic alcohols **37** and **39** did arise when such β-disubstituted compounds as **36** and **38** were treated with a boron hydride (see scheme 2.3.1).[33,34]

Scheme 2.3.1-Hydroboration of β-disubstituted α,β-unsaturated systems.

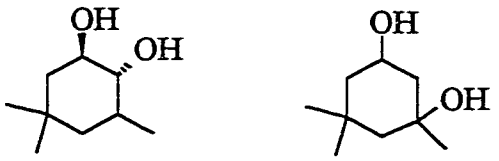


36 **37**



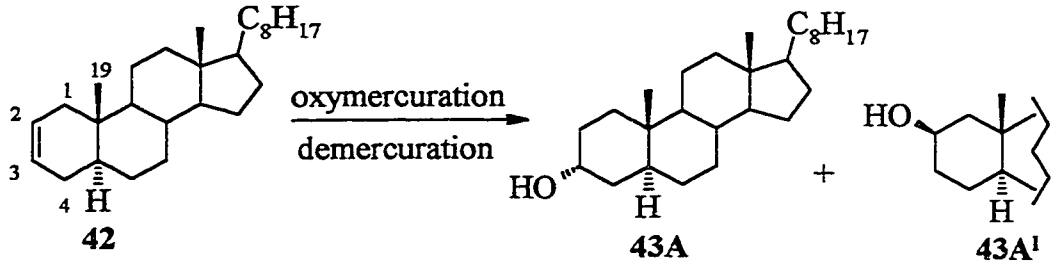
38 **39**

Furthermore, when the organoborane of **38** underwent alkaline-peroxide oxidation, the *trans*-1,2-diol **40** and 1,3-diol **41** were obtained.[32] Thus, it can be speculated that the complex mixture that we obtained when treating **10** with 9-BBN/$NaBO_3.4H_2O$ was in fact derivatives of **10** in which ring A had either the 3,4-diol or 3,5-diol substitution.



40 **41**

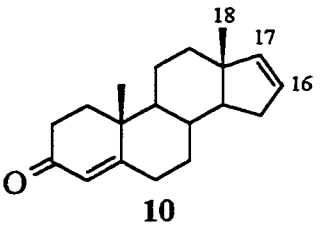
Since hydroboration-oxidation was not successful it was decided to try oxymercuration-demercuration. Literature precedent has shown that oxymercuration is highly susceptible to steric factors.[35,36] Oxymercuration-demercuration of 5α-cholest-2-ene **42** for instance, gave a 70:30 mixture of the 3α-and 2β-cholestanols **43A**, **43A**[1].[37] The ring opening of the three-membered mercurium ion by H_2O occurred preferentially at



42 **43A** **43A**[1]

70:30

c.f

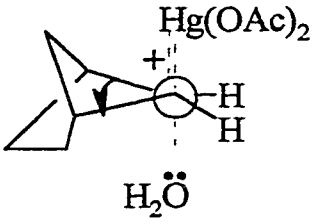


10

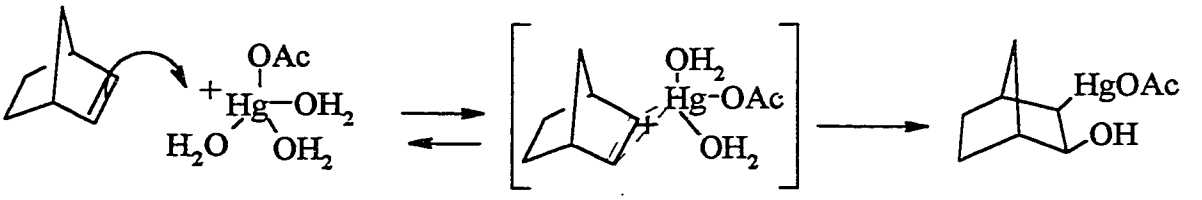
C-3 rather than C-2, because C-3 was further from the 19β-methyl group. In comparison, it was anticipated that the 18β–methyl group of **10** would provide similar steric hindrance to the incoming H_2O nucleophile.

The fact that the 3α-hydroxycholestane **43A** rather than the 3β-epimer was the major product has been attributed to *trans*-diaxial addition.[35] It has been suggested that the mercuric salt adds to the more hindered β-face of the alkene, followed by *trans* attack

by water to give the α-isomer.[35] However, it is also known that strained olefins such as norbornene can undergo *cis*-oxymercuration in which the water adds to the β-mercurium ion from the same side giving the β-isomer.[38,39] The *cis*-addition of the H_2O nucleophile during oxymercuration of strained olefins, is due to the resistance of the molecule to twist about the carbon-carbon bond by approximately 30^0. This twist is necessary for *trans*-opening of the mercurium ion by water as depicted below, and this can readily be



accomplished in simple acyclic or medium-sized ring olefins. One possible mechanism for the *cis*-ring opening of the mercurium ion in rigid systems, is by ligand migration of

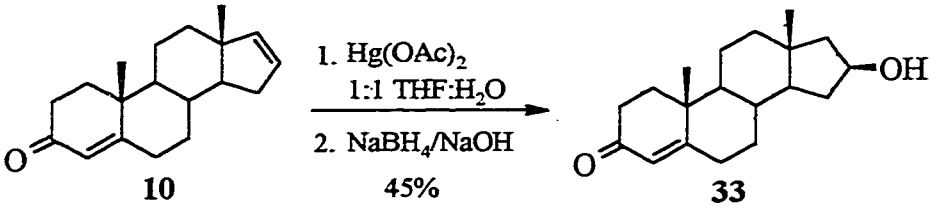


water rather than *trans*-attack by water.[38] Thus, the mercurium ion is opened from the same side resulting in *cis*-oxymercuration.

The oxymercuration-demercuration of steroid **10** was carried out by using Brown and Geoghegan's method[40] in which **10** was stirred with one molar equivalent mercuric acetate in a 1:1 mixture of THF and H_2O for 3 days. The resulting steroidal oxymercurium salt was reduced with an alkaline solution of sodium borohydride to give

steroid **33** in 45% yield (see scheme 2.3.2). In an attempt to optimize the yield of **33,** the THF/H_2O ratio was changed from 1:1 to 9:1 because steroid **10** was only partially soluble

Scheme-2.3.2- The Oxymercuration-Demercuration of androsta-4,16-dien-3-one **26**.



10 **33**

in the 1:1 mixture. It was anticipated that a more homogenous environment would improve the yield. However, similar yields were obtained regardless of the THF/H_2O ratio.

The demercuration step was also a concern, because the sodium borohydride reduction of the steroidal oxymercurium salt could also result in the reduction of the enone in ring A.[41, 42] However, the ^1H NMR spectrum of **33** (see figure 2.3.1-chp. 2 appendix) showed a singlet at δ 5.70 which corresponded to the olefinic H-4, thus proving the α,β–unsaturated ketone was in tact. The resonance for H-16 appeared as a multiplet at δ 4.41-4.36. The ^{13}C NMR spectrum (figure 2.3.2-chp. 2 appendix) showed key quaternary carbons at δ 199.48 (conjugated carbonyl, C-3) and 171.15 (olefinic C-5). The ^{13}C peak for C-4 was at δ 123.78. The IR spectrum of **33** showed the hydroxyl stretch at 3624 cm^{-1}, the conjugated ketone stretch at 1675 cm^{-1} and the C=C stretch at 1618 cm^{-1}.

The stereochemistry of the hydroxyl group at C-16 of **33** was determined by an nOe difference experiment to be β. When the multiplet corresponding to H-16 was

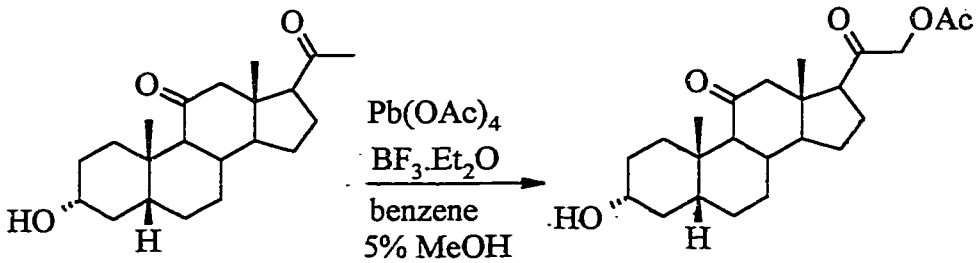
irradiated, no enhancement was observed for the singlet corresponding to the β-oriented C-18 methyl group at δ 0.98. Thus, H-16 and the C-18 methyl group were not on the same side of the ring, indicating that the 16-hydroxyl group was β. This stereochemistry was unexpected as it was anticipated that the mercuric acetate would add to the more hindered β face of **10**, followed by *trans* attack by water to form the 16α–hydroxyl substituent. The β-orientation of the 16-hydroxyl group suggests that either *cis*-oxymercuration occurred, or that the mercurium ion formed on the less hindered α face of **10**, followed by *trans* attack by water.

At this point in the synthesis, it was decided to study a readily available model system to ensure that the proposed five subsequent steps of the synthesis would indeed work

2.4 The Model System for the Synthesis of 2-hydroxyandrosta-1,4-diene-3,16-dione 1.

Testosterone **9** was used as the model compound because it was the closest in structure to 16β-hydroxyandrost-4-en-3-one **33**. The first step in the model synthesis was the lead tetraacetate acetoxylation of **9** at C-2. However, upon reviewing the literature, it was found that a free hydroxyl group in a compound such as **9** would acetylate in the presence of lead tetraacetate in glacial acetic acid.[43] To remedy this situation, others have carried out the acetoxylation under milder conditions. For example, 3α-hydroxy-5β-pregnane-11,20-dione **44** underwent lead tetraacetate oxidation in the presence of

catalytic boron trifluoride etherate ($BF_3.Et_2O$) and 5% methanol to produce 21-acetoxy-3α–hydroxy-5β-pregnane-11,20-dione **45** in 70% yield.[44] It has been proposed that under



44 **45**

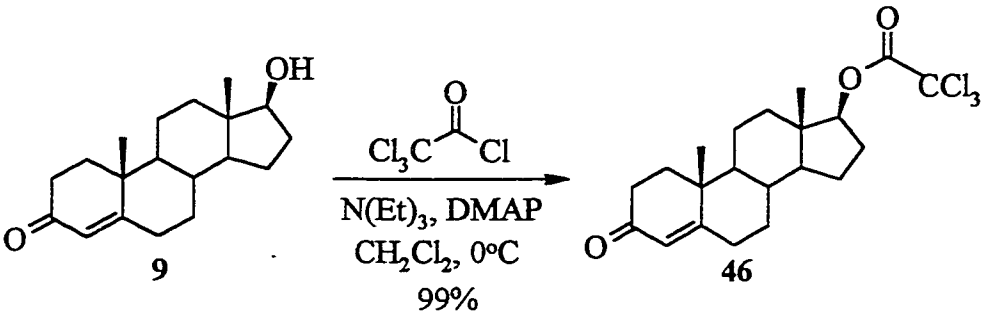
these conditions the Lewis acid forms the protic species, $H^+[BF_3.OMe]^-$ which facilitates the enolization of the ketone.[45]

Consequently, the acetoxylation can be carried out at room temperature rather than the usual 70°C or higher when no Lewis acid is present.[45] At 70°C, radicals and other species are formed in the presence of lead tetraacetate and side-reactions are unavoidable.[45] Needless to say, this method of acetoxylation was quite attractive, not only in the sense of milder conditions, but also it would eliminate the need to protect the 17-hydroxyl group of **9** and later the 16-hydroxyl group of **33**. However, when the acetoxylation of testosterone **9** was attempted under these conditions at room temperature and at 50°C, only starting material was recovered in both cases.

Since the acetoxylation of **9** was not successful, hydroxyl protecting groups were investigated. It was decided that the 17-hydroxyl of testosterone **9** would be protected as a trichloroacetate. Schwarz[46] showed that the trichloroacetate of a steroid was stable to acidic conditions and that it could be selectively cleaved by base over an acetate. Testosterone **9** was smoothly converted to the 17β-trichloroacetate **46** in 99% yield by treating **9** with trichloroacetyl chloride in the presence of base at 0°C for 30 minutes

(scheme 2.4.1). The ^{1}H NMR spectrum of **46** (figure 2.4.2-chp. 2 appendix) showed the triplet signal of H-17 downfield at δ 4.74 whereas in the ^{1}H NMR spectrum for testosterone **9** (see figure 2.4.1-chp. 2 appendix), this triplet was at δ 3.65. Furthermore, the ^{13}C NMR spectrum of **46** showed a quaternary carbon at δ 161.57 that corresponded to the ester carbonyl carbon. The IR spectrum showed an ester (C=O) stretch at 1763

Scheme 2.4.1-The preparation of 17β-trichloroacetoxyandrost-4-en-3-one **46**.

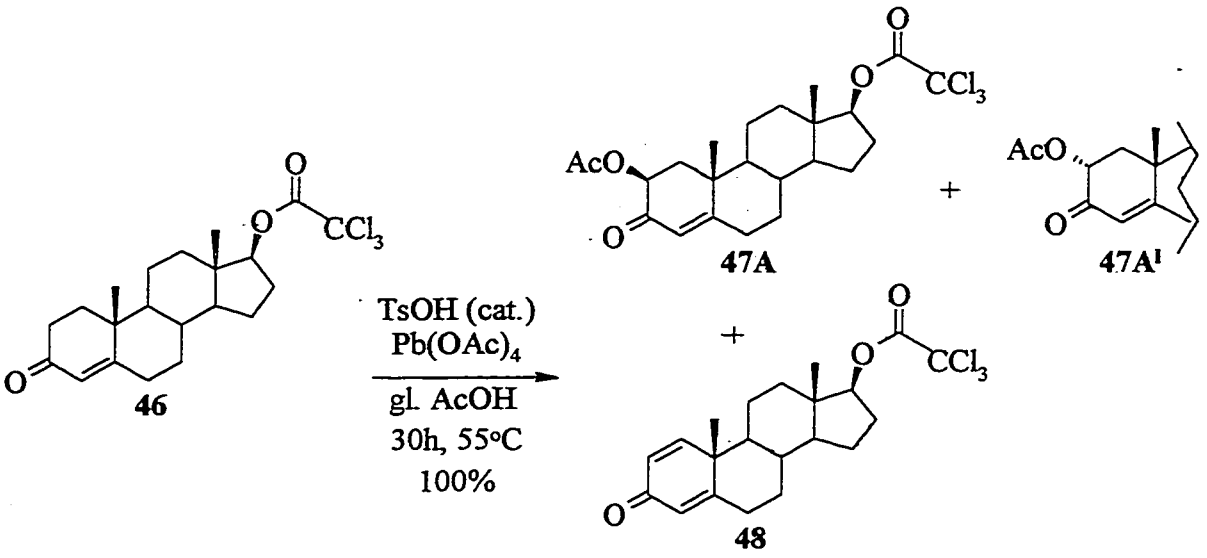


cm^{-1} that accompanied the enone carbonyl stretch at 1671cm^{-1}. The white crystalline solid had a melting point of 151-152°C. The molecular ion was calculated to be 432.1028 and the HRMS of **46** showed a M$^+$ ion at *m/z* 432.1012.

The acetoxylation of **46** with $Pb(OAc)_4$ was the next step and we used the method previously mentioned in section 2.1 (ref. 10). Acetoxylation of **46** in acetic acid at 55°C in the presence of a catalytic amount of *p*-toluenesulfonic acid gave an inseparable mixture of 2β- and 2α-acetoxy-17β-trichloroacetoxyandrost-4-en-3-one **47A**, **47A^1** and 17β-trichloroacetoxyandrosta-1,4-dien-3-one **48** (see scheme 2.4.2). The integration of the ^{1}H NMR spectrum (see figure 2.4.4-chp. 2 appendix) showed that 62% of the mixture was a 1:1 mix of **47A** + **47A^1** and 38% was **48**. The A-ring proton resonances for **48** were at δ

7.03 (d, J=10.4 Hz, H-1), 6.21 (d, J=10.4 Hz, H-2), and 6.05 (s, H-4). The ^{1}H NMR spectrum showed the key A-ring proton resonances for **47A** and **47A^1** at δ 5.78

Scheme 2.4.2-Preparation of 2-acetoxy-17β-trichloroacetoxyandrost-4-en-3-one **47A&A^1**.

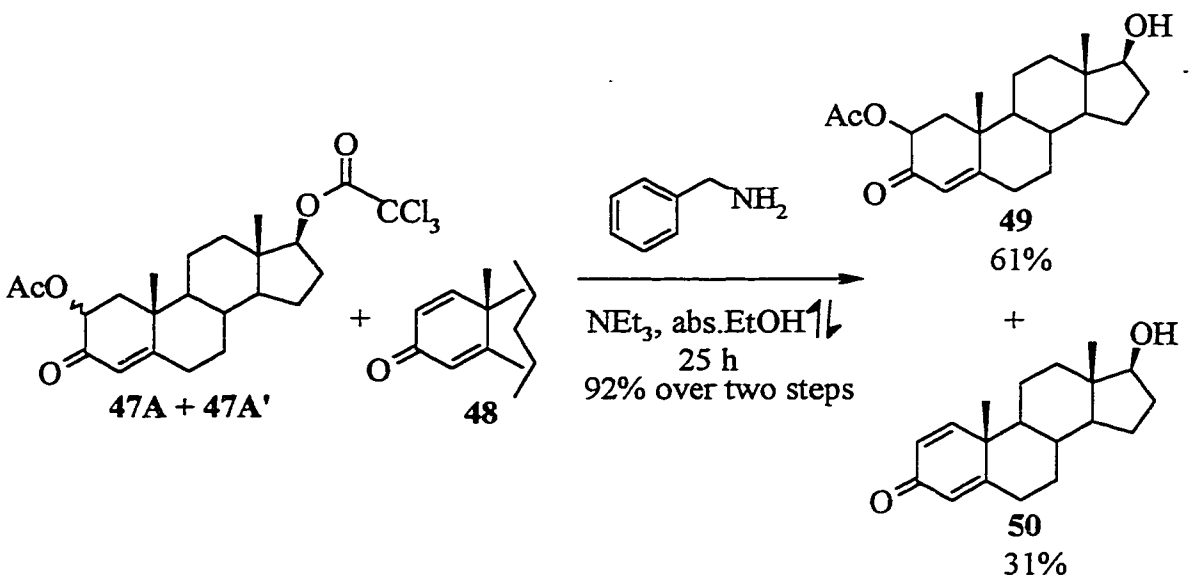


(s, H-4), 5.75 (s, H-4), 5.43 (dd, J= 14.1, 5.5 Hz, H-2) and 5.28 (dd, J= 12.5, 4.2 Hz, H-2). The assignment of the proton resonances as belonging to either **47A** or **47A^1** was not possible, because the stereochemistry of each compound could not be established based on coupling constants. Both the 2α and 2β-isomers showed a large and small coupling constant for H-2, and as was discussed in section 2.1, this is the result of the different conformations in which the 2α and 2β-isomers exist.

After the crude mixture of **47A, 47A^1** and **48** had been purified by column chromatography to remove acetic acid, the trichloroacetate was cleaved selectively by refluxing the mixture with benzylamine and triethylamine in absolute ethanol. Cleavage of the trichloroacetate was complete after 25 hours of refluxing the mixture (see scheme

2.4.3). The desired 2-acetoxy-17β-hydroxyandrost-4-en-3-one **49** was separated from 17β-hydroxyandrosta-1,4-dien-3-one **50** by column chromatography. Steroid **49** was

Scheme 2.4.3-Preparation of 2-acetoxy-17β-hydroxyandrost-4-en-3-one **49**.

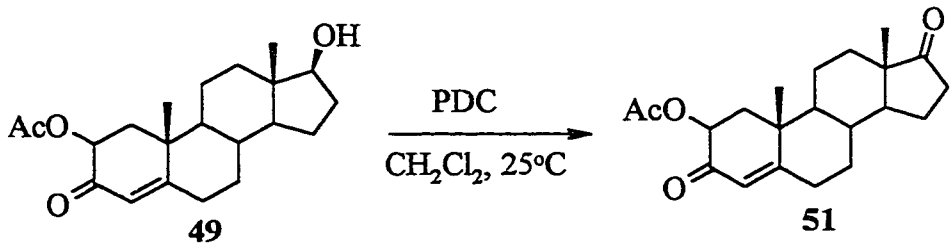


obtained in 61% yield over the two steps as a pale yellow solid with a melting point of 183-185°C. It was apparent from the ^{1}H NMR spectrum of **49** (see figure 2.4.5-chp. 2 appendix) that epimerization had occurred at C-2 as there were resonances for only one major isomer. The ^{1}H NMR spectrum of **49** showed key resonances at δ 5.69 (s, 1H, H-4), 5.38 (dd, J=14.1, 5.5 Hz, 1H, H-2), 3.58 (t, J=8.7 Hz, H-17) and 2.11 (s, 3H, 2-OAc methyl). The molecular ion was calculated to be 346.2145 and the HRMS of **49** showed a M^+ ion at *m/z* 346.2159. The IR spectrum of **49** further proved the successful cleavage of the trichloroacetate group, because it showed a hydroxyl stretch at 3443 cm^{-1}.

The 17β-hydroxyl group of **49** was oxidized to the corresponding ketone by the use of pyridinium dichromate (PDC). The product, 2-acetoxyandrost-4-ene-3,17-dione **51** was obtained in 78% yield as a white solid which melted at 202.5-204°C (see scheme

2.4.4). The ^{1}H NMR spectrum of **51** (see figure 2.4.8-chp. 2 appendix) lacked the triplet at δ 3.58 which corresponded to H-17 of **49**, and the ^{13}C NMR spectrum showed a

Scheme 2.4.4-Preparation of 2-acetoxyandrost-4-ene-3,17-dione **51**.

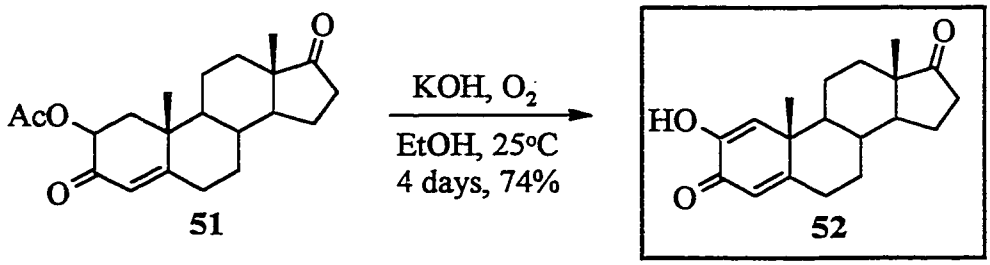


49 **51**

quaternary carbon at δ 219.86 (see figures 2.4.8 and 2.4.9-chp. 2 appendix). Both of these observations verified the conversion of the secondary alcohol to the carbonyl group at C-17. The molecular ion was calculated to be 344.1988 and the HRMS of **51** showed a M^{+} ion at *m/z* 344.2008.

The final step of the model synthesis was tandem deacetoxylation and oxidation as previously described in section 2.1. Steroid **51** was hydrolyzed by stirring the acetate with potassium hydroxide in 95% ethanol at room temperature in an open vessel. The air

Scheme 2.4.5-Synthesis of 2-hydroxyandrosta-1,4-diene-3,17-dione **52**.



51 **52**

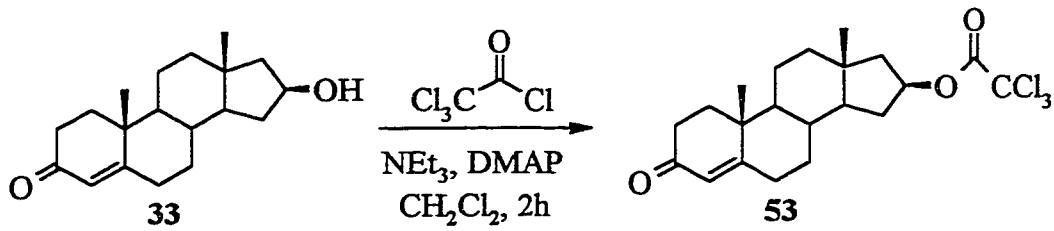
oxidation of the solution took place over four days. The oxidized product, 2-hydroxyandrosta-1,4-diene-3,17-dione **52** was obtained in 74% yield as a white solid with a melting point of 141-143°C.

The ^{1}H NMR spectrum of **52** (see figure 2.4.10-chp. 2 appendix) showed resonances at δ 6.32 (broad s, 2-OH), 6.26 (s, H-1) and 6.15 (s, H-4). The ^{13}C NMR spectrum (see figure 2.4.11-chp. 2 appendix) showed resonances at δ 219.73 (C-17), 181.43 (C-3), 172.07 (C-5), 146.17 (C-2), 123.82 (C-1) and 121.24 (C-4). The IR spectrum of **52** showed a hydroxyl stretch at 3371 cm^{-1} and a ketone stretch at 1736 cm^{-1}, the latter being characteristic of a carbonyl group on a five-membered ring.[47] The IR spectrum also showed an α,β-unsaturated carbonyl stretch at 1643 cm^{-1} and an olefinic stretch at 1613 cm^{-1}. Thus, the model system showed that the last five steps of the proposed eight step synthesis of trichiliasterone A were feasible as 2-hydroxyandrosta-1,4-diene-3,17-dione **52** was obtained in 35% overall yield from testosterone **9**.

2.5 The Five Final Steps of the Total Synthesis of 2-hydroxyandrosta-1,4-diene-3,16-dione 1.

Once the model system (section 2.4) was complete, we returned to the synthesis of **1**, and smoothly protected the 16β-hydroxyl group of **33** to yield the corresponding trichloroacetate **53** (see scheme 2.5.1). The yield of 16β-trichloroacetoxyandrost-4-ene-3-one **53** was 83 % and 11 % of starting material **33** was also recovered. As expected, the signal of H-16 in the ^{1}H NMR spectrum of **53** (see figure 2.5.2-chp. 2 appendix) was

Scheme 2.5.1-The Preparation of 16β-trichloroacetoxyandrost-4-ene-3-one **53**.

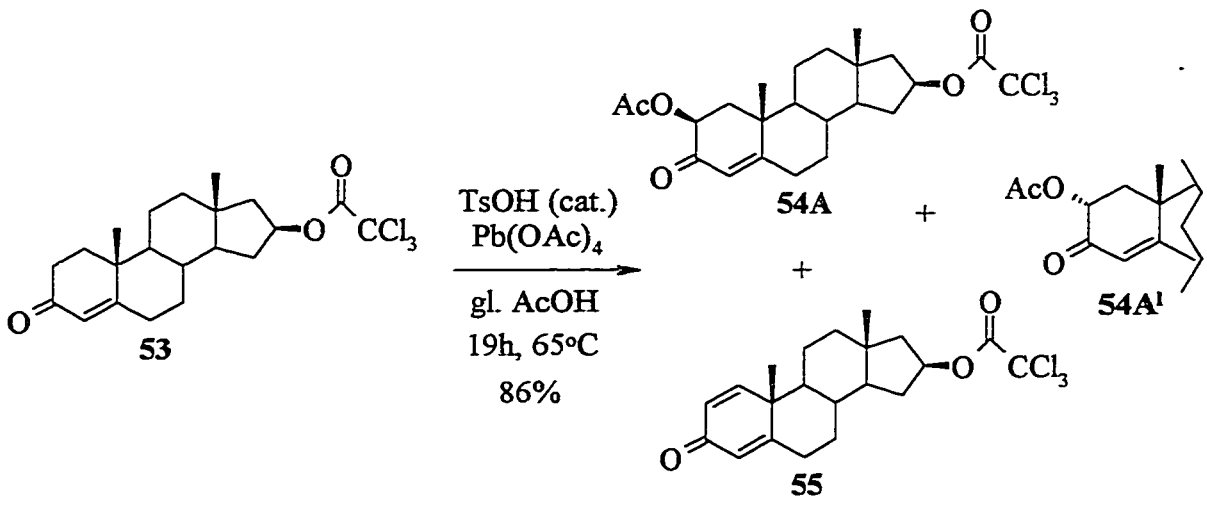


considerably downfield at δ 5.28, compared to the same proton of steroid **33** which resonated at δ 4.38 (see figure 2.3.1-chp. 2 appendix). The IR spectrum of **53** showed the ester carbonyl stretch at 1759 cm^{-1} and the conjugated enone stretch was at 1669 cm^{-1}. The melting point of the pale yellow solid was 147-149°C, and the calculated molecular ion for **53** was 432.1028 which was comparable to the M^+ ion at *m/z* 432.1049 in the HRMS.

The next step was the acetoxylation of **53**. We found that unlike in the model system, the reaction would only proceed to completion when we added two molar equivalents of lead tetraacetate and a catalytic amount of *p*-toluenesulfonic acid. These conditions afforded an inseparable mixture of 2β- and 2α-acetoxy-16β-trichloroacetoxyandrost-4-en-3-one **54A**, **54A'** and 16β-trichloroacetoxyandrosta-1,4-dien-3-one **55** (see scheme 2.5.2). The integration in the ^1H NMR spectrum (see figure 2.5.3-chp. 2 appendix) showed that 78% of the mixture was **54A** + **54A'** and 22% was **55**. Furthermore, the ratio of **54A** to **54A'** was judged to be approximately 1:1 based on the integration of the peaks due to H-4 in both isomers. The spectrum showed key proton resonances for **54A** and **54A'** at δ 5.74 (s, H-4), 5.71 (s, H-4), 5.74-5.22 (m, H-2 for **54A** and **54A'** *and* H-16 for **54A** and **54A'** and **55**), 2.12 (s, 2-OAc) and 2.10 (s, 2-OAc). The

A-ring proton resonances for **55** were at δ 7.01 (d, J=10.1 Hz, H-1), 6.19 (dd, J= 10.1, 2.0 Hz, H-2), and 6.04 (s, H-4).

Scheme 2.5.2-Acetoxylation of 16β-trichloroacetoxyandrost-4-ene-3-one **53**.

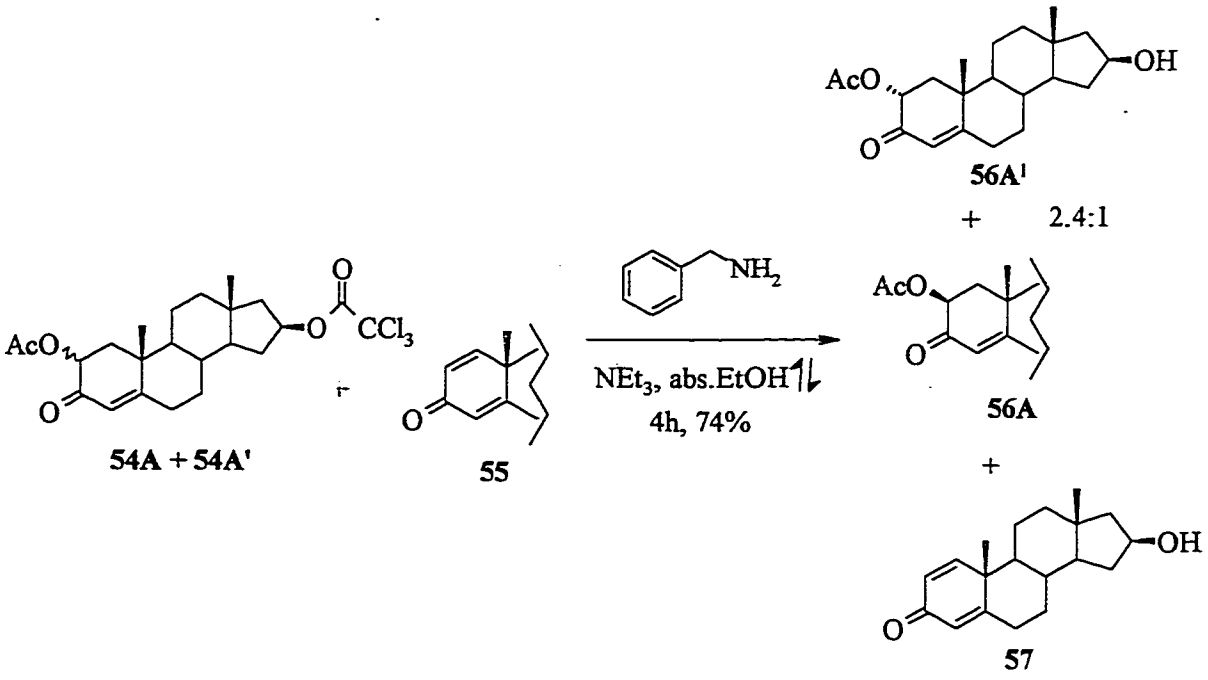


The crude mixture of **54A**, **54A**^{1} and **55** was purified by column chromatography to remove acetic acid and then was taken on to the deprotection step. After refluxing the mixture with benzylamine and triethylamine in absolute ethanol for *only* four hours (cf. to model system compound **47**, which took 25h) cleavage of the trichloroacetate was complete and the C-2 acetate group remained intact (see scheme 2.5.3). The resulting 2β- and 2α-acetoxy-16β-hydroxyandrost-4-en-3-one **56A** and **56A**^{1} were separated from the byproduct 16β-hydroxyandrosta-1,4-dien-3-one **57**.

Steroids **56A** and **56A**^{1} were obtained in a combined yield of 47% as a white solid that melted at 188-190°C. The ^{1}H NMR spectrum of **56A** and **56A**^{1} (see figure 2.5.4-chp. 2 appendix) showed a 2:1 mixture of a major and minor isomer based on the integration observed for H-2 of both isomers. The major and minor isomers could not be assigned as 2α or 2β because the coupling constants observed for H-2 of each isomer were similar in

magnitude. The key proton resonances for the major isomer were at δ 5.71 (d, J=1.5 Hz, H-4), 5.41 (dd, J=14.1, 5.4 Hz, H-2) and 2.13 (s, 2-OAc methyl). The key proton resonances for the minor isomer were at δ 5.74 (d, J=1.2 Hz, H-4), 5.28 (dd, J=13.2, 4.5 Hz, H-2) and 2.11 (s, 2-OAc methyl). The molecular ion for **56A** and **56A'** was calculated to be 346.2145 and the HRMS showed a M^+ ion at *m/z* 346.2141. The byproduct, 16β-hydroxydrosta-1,4-dien-3-one **57** was obtained in 27% yield as a white solid that melted at 173-175°C. The HRMS molecular ion for **57** was observed at *m/z* 286.1899 which was comparable to the calculated value of 286.1934.

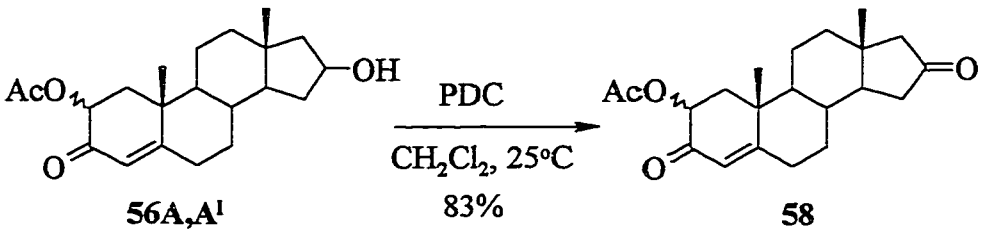
Scheme 2.5.3-Preparation of 2α-,2β-acetoxy-16-hydroxyandrost-4-en-3-one **56A&A'**.



The mixture of steroids **56A,A'** was oxidized at the C-16 position with pyridinium dichromate (PDC) at room temperature for 20 hours to afford 2α– and 2β-

acetoxyandrost-4-ene-3,16-dione **58** in 83% yield (see scheme 2.5.4). The ^{1}H NMR spectrum of **58** lacked the multiplet at δ 4.37-4.35 corresponding to H-16 of **56A,A^{1}**, and the ^{13}C NMR spectrum showed a quaternary carbon at δ 217.26 that indicated the carbonyl group at C-16 (see figures 2.5.7 and 2.5.8-chp. 2 appendix). The infrared frequency at 1743 cm-1 further confirmed the five-membered ring ketone. The melting point of **58** was 201-202°C and the HRMS showed a M^{+} ion at *m/z* 344.1987 which almost matched the calculated value of 344.1988.

Scheme 2.5.4-Oxidation of 2α-,2β-acetoxy-16-hydroxyandrost-4-en-3-one **56A,A^{1}**.

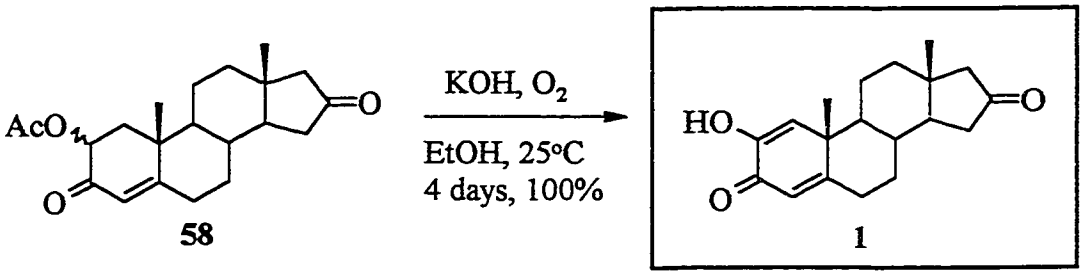


56A,A^{1} **58**

The final step of the synthesis was tandem deacetoxylation and oxidation as previously described for the model system (see section 2.4). When steroid **58** was stirred with potassium hydroxide in 95% ethanol at room temperature in an open vessel for four days, we obtained 2-hydroxyandrosta-1,4-diene-3,16-dione **1** in quantitative yield after column chromatography. The white solid had a melting point of 188-189°C and an $[\alpha]_D^{25}$ value of -220.4 (c=0.84, CH_2Cl_2).

Both the ^{1}H NMR spectrum (see figure 2.5.9-chp. 2 appendix) and the ^{13}C NMR

Scheme 2.5.5-Synthesis of 2-hydroxyandrosta-1,4-diene-3,16-dione 1.



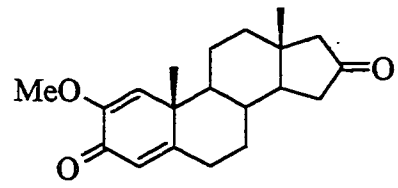
spectrum (see figure 2.5.11-chp. 2 appendix) of the synthetic trichiliasterone A, **1** were essentially identical to the ^{1}H NMR (see figure 2.5.10) and ^{13}C NMR (figure 2.5.12) spectra obtained for the naturally produced trichiliasterone A.[3] Key proton resonances included δ 6.32 (broad s, 2-OH), 6.29 (s, H-1) and 6.17 (d, J=1.4Hz, H-4).[3] The ^{13}C NMR spectrum showed key resonances at 217.14 (C-16), 181.46 (C-3), 172.04 (C-5), 146.23 (C-2), 123.64 (C-1) and 121.29 (C-4). The DEPT (figure 2.5.13), ^{1}H-^{1}H correlation (figure 2.5.14) and the ^{1}H-^{13}C correlation (figure 2.5.15) spectra of the synthetic **1** have been included in the appendix for this chapter.

The IR spectrum of the synthetic **1** had a hydroxyl stretch at 3349 cm^{-1} and a ketone stretch at 1738 cm^{-1}, the latter being characteristic of a five-membered ring ketone.[47] The IR spectrum also showed an α,β-unsaturated carbonyl stretch at 1641 cm^{-1} and an olefinic stretch at 1616 cm^{-1}. Elemental analysis of the synthetic **1** was calculated for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05 and it was found C, 74.84 and 8.36 for hydrogen content. The HRMS showed a M^+ ion at *m/z* 300.1726 which matched exactly the calculated value.

Thus, 2-hydroxyandrosta-1,4-diene-3,16-dione **1** was successfully synthesized in 6% overall yield via eight steps from testosterone **9**.

2.6 A Derivative of Trichiliasterone A, 1a isolated from *Trichilia americana.*

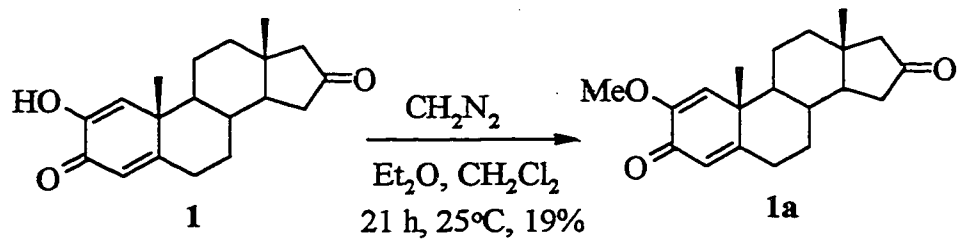
Recently, 2-methoxyandrosta-1,4-diene-3,16-dione **1a** was isolated from *Trichilia americana* by a member of our research group. The structure of **1a** was elucidated



1a

by comparison to the ^{1}H and ^{13}C NMR spectroscopic properties of **1**. The structure was verified by converting **1** to **1a** by reacting **1** with diazomethane for 21 hours at room temperature (scheme 2.6.1). The yield of **1a** was 19% and 65% of starting material was recovered. The ^{1}H NMR spectrum of the synthetic **1a** matched that of the isolated sample. The HRMS of **1a** showed a M^{+} at *m/z* 314.1893 which was similar to the calculated value of 314.1883.

Scheme 2.6.1-Transformation of Trichiliasterone A, 1 to the Methyl Enol Ether Derivative **1a**.



1 **1a**

2.7 EXPERIMENTAL

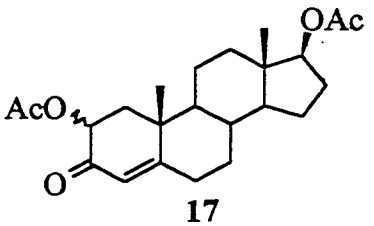
GENERAL: Melting points were determined by use of a Thomas Hoover Capillary melting point apparatus and they were uncorrected. Mass spectra were obtained by using a VG 7070E or a Kratos concept 2H instrument and all compounds were electronically ionized. Infrared spectra were obtained by preparing compounds as thin methylene chloride films on a potassium bromide plate, and the data was recorded by 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). ^{1}H and ^{13}C NMR spectra were obtained from either a Bruker AMX-500 spectrometer or a Varian Gemini-200 spectrometer. The samples for the most part were run in spectroscopic grade deuterated chloroform. The multiplicities of the NMR signals were reported with the following abbreviations: singlet (s), broad singlet (brs), doublet (d), triplet (t), doublet of doublets (dd), quartet (q) and multiplet (m). Assignment of proton resonances for some of the steroids was facilitated by comparison to a literature review on related steroids.[48] In addition, the assignments shown were based on the analysis of ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectra.

Solvents used for reactions and chromatographic purifications were routinely distilled prior to use. Methylene chloride was dried by distillation from calcium hydride. THF was dried by distillation from benzophenone ketyl and similarly *N*-methyl-pyrrolidone was dried over barium oxide. Reactions were monitored by thin-layer chromatography (tlc) and Kieselgel 60 F_{254} precoated 0.25 mm thick glass plates were used. Visualization

was facilitated by UV irradiation followed by charring the tlc plate which was dipped in a molybdate solution. The molybdate solution was prepared by dissolving ammonium molybdate (2.5 g) and cerric sulfate hydrate (1.0 g) in acidified distilled water [H_2O (90 mL); H_2SO_4 (10 mL)]. Silica gel 270-400 mesh was used for flash chromatography and neutral Brockman I activated 150 mesh aluminum oxide was used for column chromatography.

PREPARATION OF 2α– AND 2β,17β-DIACETOXYANDROST-4-EN-3-ONE (17) AND 17β-ACETOXYANDROSTA-1,4-DIEN-3-ONE (19).

Lead tetraacetate (2.95 g, 0.00687 mol), recrystallized from hot glacial acetic acid, was dissolved in glacial acetic acid (40 mL) at 50°C. The mixture was stirred for 15 min, a solution of 17β-acetoxyandrost-4-en-3-one **6** (2.00 g, 0.00624 mol) in glacial acetic acid (20 mL) was added and the mixture was warmed to 85°C. The reaction mixture was stirred for 22 h, cooled to room temperature, diluted with ethyl acetate (100 mL) and washed with a saturated aqueous solution of sodium bicarbonate (3 x 20 mL). The organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a clear brown oil. Flash chromatography (100 g of silica gel, 6:1 hexanes/ethyl acetate) yielded **17** (1.06 g, 44%) as a yellow solid and **19** (0.72 g, 11%) as a white solid.



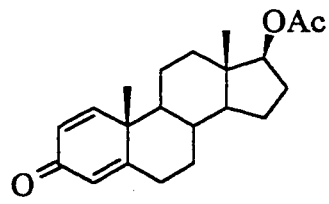
17

$C_{23}H_{32}O_5$ MW=387.49 g/mole

mp 171-197°C (lit. mp:[13] 172-198°C)

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): **major**: 5.74 (s, 1H, H-4), 5.43 (dd, J=14.0, 5.5 Hz, 1H, H-2), 2.16 (s, 3H, H-23, 2-OAc), **minor**: 5.77 (s, 1H, H-4), 5.29 (dd, J=12.7, 6.0 Hz, 1H, H-2), 2.14 (s, 3H, H-23, 2-OAc), **major + minor**: 4.58 (t, J=5.1 Hz, 2H, H-

17), 2.49-0.80 (m, 34H), 2.03 (s, 6H, H-21,17-OAc), 1.31 (s, 6H, H-19), 0.82 (s, 6H, H-18).



19

$C_{21}H_{28}O_3$ MW=328.45 g/mole

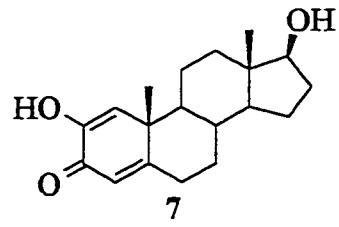
mp 153-154°C (lit. mp:[13] 152.5-155.2°C)

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 7.04 (d, J=10.3 Hz, 1H, H-1), 6.23 (d, J= 10.3 Hz, 1H, H-2), 6.06 (s, 1H, H-4), 4.58 (t, J=5.1 Hz, 1H, H-17), 2.50-0.80 (m, 15H), 2.03 (s, 3H, H-21, 17-OAc), 1.22 (s, 3H, H-19), 0.85 (s, 3H, H-18).

PREPARATION OF 2,17β-DIHYDROXYANDROSTA-1,4-DIEN-3-ONE (7).

A solution of 2α– and 2β,17β–diacetoxyandrost-4-en-3-one **17** (1.77 g, 0.00458 mol) in 95% ethanol (90 mL) was stirred at room temperature for 30 min. A solution of potassium hydroxide (5.14 g, 0.0915 mol) in water (173 mL) was added and the mixture was stirred vigorously for 1 h. Stirring was stopped and the flask was left open to air for four days. The reaction mixture was diluted with ethyl acetate (180 mL), neutralized with 5% aqueous hydrochloric acid and washed with brine (50 mL). The aqueous layer was extracted with ethyl acetate (50 mL) and the combined extracts were dried (anhydrous

magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a pale yellow solid. The solid was washed with 1:1 hexanes/ethyl acetate yielding **7** (1.38 g, 100%) as a white solid.



7

$C_{19}H_{26}O_3$ MW=302.42 g/mole

mp 204-206°C (lit. mp:[11] 207-209°C)

MS [EI, *m/z* (%)]: 302 [MH^+] (49), 284 (16), 147 (100).

HRMS calcd. for $C_{19}H_{26}O_3$: 302.1883; found: 302.1853.

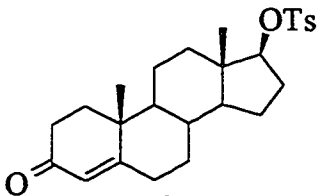
IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3389 (OH), 1638 (C=O).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 6.33 (brs, 1H, H-2), 6.29 (s, 1H, H-1), 6.14 (d, J=1.2 Hz, 1H, H-4), 3.61 (t, J=8.5 Hz, 1H, H-17), 2.48-2.37 (m, 2H), 2.14-1.92 (m, 3H), 1.83 (dt, J=11.9, 3.1 Hz, 1H), 1.76 (dq, J=13.4, 2.9 Hz, 1H), 1.68-1.51 (m, 3H), 1.47-1.40 (m, 1H), 1.30 (dq, J=13.4, 2.9 Hz, 1H), 1.68-1.51 (m, 3H), 1.47-1.40 (m, 1H), 1.30 (dq, J=5.8, 12.2 Hz, 1H), 1.22 (s, 3H, H-19), 1.08-0.87 (m, 4H), 0.79 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 181.59 (C-3), 173.01 (C-5), 146.05 (C-2), 124.49 (C-1), 120.98 (C-4), 81.37 (C-17), 53.71, 50.06, 44.11, 43.16, 36.31, 35.41, 33.44, 32.77, 30.31, 23.49, 22.88, 19.64, 11.10.

PREPARATION OF 17β-TOLUENESULFONYLOXYANDROST-4-EN-3-ONE (28).

p-Toluenesulfonyl chloride (990 mg, 5.19 mmol) was added to a solution of testosterone **9** (992 mg, 3.46 mmol) in pyridine (4.0 mL) at room temperature. The reaction mixture was stirred for 29 h, poured over crushed ice (20 g) and the precipitate was filtered by suction. The resulting solid was washed with 5% aqueous hydrochloric acid (2 x 1 mL) and water (1 x 1 mL). The solid was allowed to dry by suction for 30 min yielding **28** (1.49 g, 98%) as a white solid.



28

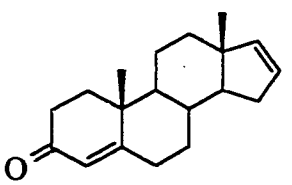
$C_{26}H_{34}O_4S$ MW=442.59 g/mole

mp 165-167°C (lit. mp:[20] 163-168°C)

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 7.76 (d, J=8.3 Hz, 2H), 7.31 (d, J=8.3 Hz, 2 H), 5.69 (s, 1H, H-4), 4.24 (t, J=7.8 Hz, 1H, H-17), 2.43 (s, 3H), 2.41-1.16 (m, 11H), 1.15 (s, 3H, H-19), 0.99-0.85 (m, 8H), 0.83 (s, 3H, H-18).

PREPARATION OF ANDROSTA-4,16-DIEN-3-ONE (10) AND 17α-HYDROXYANDROST-4-EN-3-ONE (30).

Tetrabutylammonium acetate (17.4 g, 0.0578 mol) was added to a clear yellow solution of 17β-toluenesulfonyloxyandrost-4-en-3-one **28** (4.15 g, 0.00938 mol) in dry N-methylpyrrolidinone (90 mL) at room temperature and warmed to 160°C. The reaction mixture was stirred 4 h, dissolved in ether (200 mL) and washed with water (3 x 50 mL). The aqueous layer was extracted with ether (2 x 100 mL) and the combined extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo. The N-methylpyrrolidinone was removed by vacuum distillation (54°C, 5 mm Hg) and the resulting residue was dissolved in 95% ethanol (60 mL). A 0.54 M aqueous solution of potassium hydroxide (57 mL) was added to the ethanol solution and the mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with ethyl acetate (120 mL) and neutralized to pH 7.0 with 5% aqueous hydrochloric acid. The organic layer was separated and washed with water (2 x 50 mL) and brine (1 x 50 mL), dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a yellow solid. Column chromatography (200 g of neutral alumina, 2:1 petroleum ether/ether) yielded **10** (1.54 g, 61%) as a pale yellow solid and **30** (0.75 g, 28%) as a white solid



10

$C_{19}H_{26}O$ MW=270.42 g/mole

mp 131-135°C (lit. mp:[23] 131-133°C)

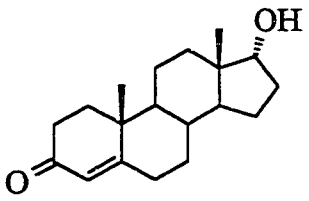
MS [EI, *m/z* (%)]: 270 [MH^+] (68), 147 (100), 91 (78).

HRMS calcd. for $C_{19}H_{26}O$: 270.1985; found: 270.1994.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1660 (C=O), 1615 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 5.83-5.82 (m, 1H, H-17), 5.71 (s, 1H, H-4), 5.69-5.67 (m, 1H, H-16), 2.43-0.78 (m, 17H), 1.19 (s, 3H, H-19), 0.79 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 199.42 (C-3), 171.22 (C-5), 143.49 (C-16 or C-17), 129.22 (C-16 or C-17), 123.83 (C-4), 55.39, 54.43, 45.30, 38.76, 35.56, 35.54, 34.15, 33.90, 32.81, 31.99, 31.88, 20.84, 17.19, 16.88.



30

$C_{19}H_{28}O_2$ MW=288.41 g/mole

mp 166-168°C

MS [EI, *m/z* (%)]: 288 [MH^+] (64), 228 (53), 147 (100).

HRMS calcd. for $C_{19}H_{28}O_2$: 288.2090; found: 288.2093.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3410 (OH), 1654 (C=O), 1609 (C=C).

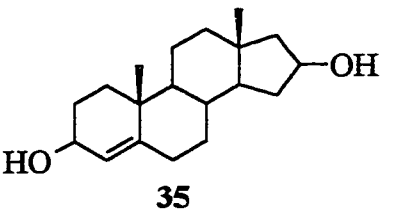
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 5.68 (s, 1H, H-4), 3.71 (d, J=5.9 Hz, 1H, H-17), 2.40-2.21 (m, 4H), 2.14-2.11 (m, 1H), 2.02-1.97 (m, 1H), 1.86-1.81 (m, 1H), 1.76-

1.34 (m, 10H), 1.21-1.14 (m, 1H), 1.15 (s, 3H, H-19), 1.06 (dq, J=12.6, 0.8 Hz, 1H), 0.93 (dt, J=12.0, 4.1 Hz, 1H), 0.66 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 199.52 (C-3), 171.36 (C-5), 123.75 (C-4), 79.54 (C-17), 53.58, 48.14, 45.06, 38.60, 35.80, 35.69, 33.88, 32.86, 32.30, 32.25, 31.14, 24.50, 20.52, 17.38, 16.86.

PREPARATION OF 3,16-DIHYDROXYANDROST-4-ENE (35).

A 0.5 M solution of 9-BBN in tetrahydrofuran (21.4 mL, 0.0107 mol) was added dropwise to a solution of androsta-4,16-dien-3-one **10** (876 mg, 0.00324 mol) in dry tetrahydrofuran (22 mL) at room temperature. The reaction mixture was warmed to 60°C and allowed to stir for 2 h. The reaction mixture was cooled to room temperature and water (22 ml) and sodium perborate tetrahydrate (5,76 g, 0.0374 mol) were added. The mixture was stirred for 3 h, diluted with ethyl acetate (100 mL) and washed with a saturated aqueous solution of sodium carbonate (25 mL) and brine (25 mL). The organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a cloudy oil. Flash chromatography (90 g of silica gel, 2:1 hexanes/ethyl acetate) yielded **35** (29 mg, 3%) as a white solid.



35

$C_{19}H_{30}O_2$ MW=290.45 g/mole

mp 105-106°C

MS [EI, *m/z* (%)]: 290 [MH^+] (1), 105 (80), 91 (100).

HRMS calcd. for $C_{19}H_{30}O_2$: 290.2247; found: 290.2245.

IR (5% in CCl_4, solution cell) ν (cm^{-1}): 3645 (OH), 1659 (C=C).

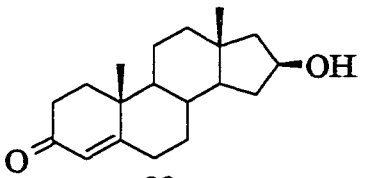
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 5.44 (d, J=3.4 Hz, 1H, H-4), 4.44 (dt, J=7.4, 6.4 Hz, 1H, H-16), 4.04 (brs, 1H, H-3), 2.22-2.11 (m, 1H, H-6), 2.05-1.99 (m, 3H, H-6, H-7), 1.74-1.08 (m, 16H), 1.00-0.78 (m, 1H), 0.95 (s, 3H, H-19), 0.71 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 150.03 (C-5), 120.84 (C-4), 71.76 (C-16), 64.22 (C-3), 54.18, 51.91, 51.74, 41.78, 38.61, 37.64, 37.24, 35.65, 32.98, 32.33, 31.63, 27.82, 21.12, 18.55, 18.09.

PREPARATION OF 16β-HYDROXYANDROST-4-EN-3-ONE (33).

Water (10 mL) and mercuric acetate (1.43 g, 0.00450 mol) were added to a solution of androsta-4,16-dien-3-one **10** (1.22 g, 0.00450 mol) in tetrahydrofuran (10 mL) at room temperature. The reaction mixture was stirred 3 days, 3.0 M aqueous sodium hydroxide (10 mL) was added followed by 0.5 M sodium borohydride in 3.0 M aqueous sodium hydroxide (10 mL) added in 2 mL aliquots. The mixture was stirred for 2 min, stirring was stopped and mercury was allowed to settle. Salt was added to saturate the aqueous layer followed by tetrahydrofuran (6 mL). The mixture was stirred for 30 min, filtered through a cotton plug with suction and the layers were separated. The organic layer was washed with water (3 x 10 mL) and the combined aqueous extracts were extracted with ether (1 x 50 mL). The combined organic extracts were dried (anhydrous

magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a foamy yellow solid. Column chromatography (154 g of neutral alumina, 9:1 petroleum ether/ether) yielded **33** (0.584 g, 45%) as a white solid and starting material **10** (0.220 g, 18%) as a pale yellow solid.



33

$C_{19}H_{28}O_2$ MW=288.41 g/mole

mp 153.5-155.5°C

MS [EI, *m/z* (%)]: 288 [MH^+] (13), 171 (42), 43 (100).

HRMS calcd. for $C_{19}H_{28}O_2$: 288.2090; found: 288.2087.

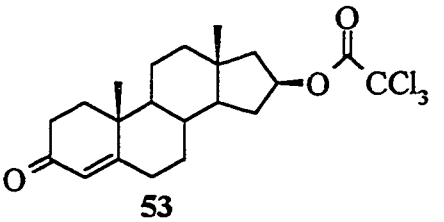
IR (5% in CCl_4, solution cell) ν (cm^{-1}): 3624 (OH), 1675 (C=O), 1618 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 5.70 (s, 1H, H-4), 4.41-4.36 (m, 1H, H-16), 2.43 -2.14 (m, 5H), 2.02-1.97 (m, 1H), 1.85-1.80 (m, 1H), 1.76-1.40 (m, 8H), 1.31 (dt, J=13.1, 5.5 Hz, 1H), 1.17 (s, 3H, H-19), 1.17-0.88 (m, 4H), 0.98 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 199.48 (C-3), 171.15 (C-5), 123.78 (C-4), 71.72 (C-16), 53.83, 53.42, 51.10, 39.96, 38.64, 38.60, 37.04, 35.62, 35.32, 33.90, 32.77, 32.18, 20.62, 18.83, 17.34.

PREPARATION OF 16β-TRICHLOROACETOXYANDROST-4-EN-3-ONE (53).

Triethylamine (69 μL, 0.50 mmol), trichloroacetyl chloride (44 μL, 0.40 mmol) and N,N-dimethylaminopyridine (4.1 mg, 0.040 mmol) were added to a solution of 16β-androst-4-en-3-one **33** (96 mg, 0.33 mmol) in dry methylene chloride (754 μL) at 0°C. The reaction mixture was stirred for 30 min at 0°C and 2 h at room temperature, poured into water (10 mL) and extracted with methylene chloride (3 x 25 mL). The combined extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording an orange solid. Flash chromatography (6 g of silica gel, 9:1 hexanes/ethyl acetate) yielded **53** (120 mg, 83%) as a yellow solid and starting material **33** (20 mg, 11%) as a white solid.



53

$C_{21}H_{27}Cl_3O_3$ MW=432.10 g/mole

mp 147-149°C

MS [EI, *m/z* (%)]: 432 [MH^+] (7), 147 (75), 32 (100).

HRMS calcd. for $C_{21}H_{27}Cl_3O_3$: 432.1028; found: 432.1049.

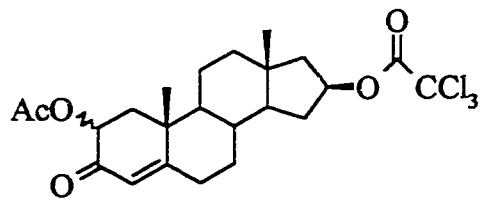
IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1759 (C=O), 1669 (C=O), 1615 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 5.69 (s, 1H, H-4), 5.29-5.27 (m, 1H, H-16), 2.43-2.26 (m, 5H), 2.01-1.98 (m, 1H), 1.99-1.74 (m, 3H), 1.67-1.44 (m, 6H), 1.16 (s, 3H, H-19), 1.15-0.99 (m, 4H), 0.95 (s, 3H, H-18).

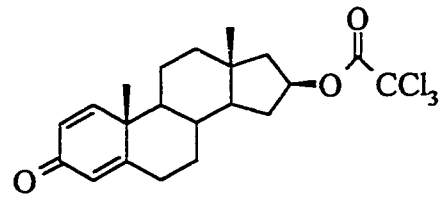
^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 199.22 (C-3), 170.45 (C-5), 161.39 (C-20), 123.96 (C-4), 80.24 (C-16 and C-21), 53.65, 53.01, 47.33, 39.88, 38.58, 38.08, 35.63, 35.25, 33.87, 33.83, 32.59, 31.98, 20.56, 17.83, 17.30.

PREPARATION OF 2β-ACETOXY-16β-TRICHLOROACETOXY-ANDROST-4-EN-3-ONE (54A), 2α-ACETOXY-16β-TRICHLOROACETOXY-ANDROST-4-EN-3-ONE (54A^1) AND 16β-TRICHLOROACETOXYANDROSTA-1,4-DIEN-3-ONE (55).

Lead tetraacetate (719 mg, 1.61 mmol), recrystallized from hot glacial acetic acid, was dissolved in glacial acetic acid (9 mL) at 50°C. The reaction mixture was stirred for 15 min, a solution of 16β-trichloroacetoxyandrost-4-en-3-one **53** (631 mg, 1.50 mmol) and *p*-toluenesulfonic acid (approximately 10 mg) in glacial acetic acid (8 mL) was added and the mixture was warmed to 60 to 70°C. The reaction mixture was stirred for 19 h, cooled to room temperature, diluted with ethyl acetate (125 mL) and washed with water (2 x 10 mL) and a saturated aqueous solution of sodium bicarbonate (4 x 10 mL). The organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a mixture of **54A**, **54A^1** and **55** (620 mg, 86%) as a pale yellow solid.



54A + 54A^{I}



55

$C_{23}H_{29}Cl_3O_5$ MW=491.84 g/mole

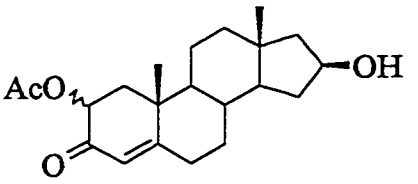
$C_{21}H_{25}Cl_3O_3$ MW=431.79 g/mole

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): **compound 54A+54A^{I}**: 5.74 (s, 1H, H-4), 5.71 (s, 1H, H-4), 2.12 (s, 3H, H-23, 2-OAc), 2.10 (s, 3H, H-23, 2-OAc), **compound 55**: 7.01 (d, J=10.1 Hz, 1H, H-1), 6.19 (dd, J=10.1, 2.0 Hz, 1H, H-2), 6.04 (s, 1H, H-4), **compound 54A+54A^{I}+55**: 5.74-5.72 (m, 5H, H-2 **54A+A^{I}** and H-16 **54A+A^{I}+55**), 2.55-0.85 (m, 49H), 1.29 (s, 3H, H-19), 1.21 (s, 3H, H-19), 1.17 (s, 3H, H-19), 0.96 (s, 3H, H-18), 0.93 (s, 6H, H-18 x2).

PREPARATION OF 2β-ACETOXY-16β-HYDROXYANDROST-1-EN-3-ONE and 2α-ACETOXY-16β-HYDROXYANDROST-1-EN-3-ONE (56A, 56A^{I}) AND 16β-HYDROXYANDROSTA-1,4-DIEN-3-ONE (57).

A solution of 2α- and 2β-acetoxy-16β–trichloroacetoxyandrost-4-en-3-one **54A** and **54A^{I}** and 16β-trichloroacetoxyandrosta-1,4-dien-3-one **55** (250 mg, 0.51 mmol) in absolute ethanol (20 mL) was warmed to 30°C and stirred for 1 h. Triethylamine (0.49 ml, 3.5 mmol) and benzylamine (0.41 mL, 3.8 mmol) were added and the mixture was heated at reflux for 4 h. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with 5% aqueous hydrochloric acid (3 x 10 mL) and water (3 x 10 mL). The

organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording an orange solid. Flash chromatography (32 g of silica gel, 4:1 hexanes/ethyl acetate) yielded a 1:2.4 mixture of **56A** and **56A^1** (176 mg, 47%) as a yellow solid and **57** (39 mg, 27%) as a white solid.



56A,A^1

$C_{21}H_{30}O_4$ MW=346.47 g/mole

mp 188-190°C

MS [EI, *m/z* (%)]: 346 [MH^+] (2), 260 (92), 122 (100).

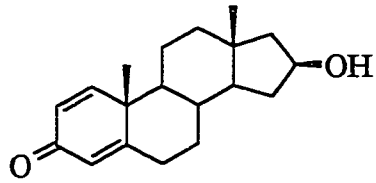
HRMS calcd. for $C_{21}H_{30}O_4$: 346.2145; found: 346.2141.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3484 (OH), 1748 (C=O), 1686 (C=O), 1614 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): **major**: 5.71 (d, J=1.5 Hz, 1H, H-4), 5.41 (dd, J=14.1, 5.4 Hz, 1H, H-2), 2.13 (s, 3H, H-21, 2-OAc), 1.30 (s, 3H, H-19), **minor**: 5.74 (d, J=1.2 Hz, 1H, H-4), 5.28 (dd, J=13.2, 4.5 Hz, 1H, H-2), 2.11(s, 3H, H-21, 2-OAc), 1.17 (s, 3H, H-19), **major + minor**: 4.37-4.25 (m, 2H, H-16), 2.51-2.14 (m, 8H), 2.11 1.95-1.70 (m, 8H), 1.60-1.25 (m, 12H), 1.08-0.85 (m, 8H), 0.96 (s, 6H, H-18 x2).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): **major**: 193.63 (C-3), 170.81 (C-5), 121.73 (C-4), 71.60 (C-2 or C-16), 71.21 (C-2 or C-16), **minor**: 193.50 (C-3), 170.22 (C-5),

120.46 (C-4), 71.65 (C-2 or C-16), 70.37 (C-2 or C-16), **major + minor**: 173.09 (C-20 x2), 54.30, 53.26, 53.23, 50.99, 50.66, 41.37, 41.09, 40.66, 40.39, 39.91, 38.45, 38.42, 37.39, 36.95, 36.91, 35.49, 34.80, 34.68, 32.80, 32.32, 32.04, 22.25, 22.14, 20.90, 20.84, 20.48, 18.96, 18.79, 18.05.



57

$C_{19}H_{26}O_2$ MW=286.42 g/mole

mp 173-175°C

MS [EI, *m/z* (%)]: 286 [MH^+] (47), 147 (24), 122 (100).

HRMS calcd. for $C_{19}H_{26}O_2$: 286.1934; found: 286.1899.

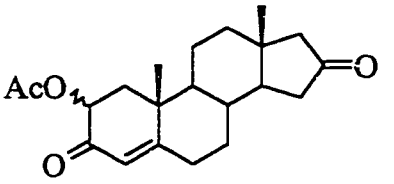
IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3407 (OH), 1695 (C=O), 1617 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 7.03 (d, J=10.2 Hz, 1H, H-1), 6.14 (dd, J=10.2, 1.9 Hz, H-2), 6.04 (s, 1H, H-4), 4.42-4.32 (m, 1H, H-16), 2.49-2.40 (m, 1H), 2.39-2.29 (m, 1H), 2.25-2.11 (m, 2H), 1.98-0.79 (m, 12H), 1.22 (s, 3H, H-19), 1.00 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 186.34 (C-3), 169.10 (C-5), 153.83 (C-1), 127.46 (C-2), 123.83 (C-4), 71.66 (C-16), 53.33, 53.07, 51.05, 43.64, 40.27, 38.52, 37.23, 35.33, 33.83, 32.80, 22.57, 18.95, 18.70.

PREPARATION OF 2α- AND 2β-ACETOXYANDROST-4-EN-3,16-DIONE (58).

Pyridinium dichromate (89mg, 0.24 mmol) was added to a solution of 2α- and 2β-acetoxy-16β-hydroxyandrost-4-en-3-one **56A**, **56A**[1] (55 mg, 0.16 mmol) in dry methylene chloride (1mL) at room temperature. The reaction mixture was stirred for 20 h, diluted with ether (2 mL), filtered through anhydrous magnesium sulfate and celite in a sintered glass funnel. The product was concentrated in vacuo affording **58** (46 mg, 83%) as a white solid.



58

$C_{21}H_{28}O_4$ MW=344.45 g/mole

mp 201-202°C

MS [EI, *m/z* (%)]: 344 [MH^+] (3), 258 (100), 122 (69).

HRMS calcd. for $C_{21}H_{28}O_4$: 344.1988; found: 344.1987.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1743 (C=O), 1690 (C=O), 1617 (C=C).

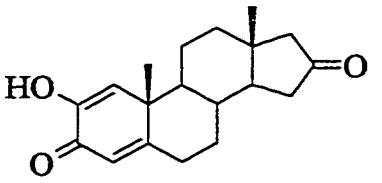
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): **major**: 5.75 (d, J=1.5 Hz, 1H, H-4), 5.44 (dd, J=14.1, 8.7 Hz, 1H, H-2), 2.43-2.11 (m, 3H), 2.15 (s, 3H, H-21), 1.98-1.04 (m, 14H), 1.34 (s, 3H, H-19), 0.92 (s, 3H, H-18), **minor**: 5.78 (d, J=1.3 Hz, 1H, H-4), 5.30 (dd,

J=12.5, 5.0 Hz, 1H, H-2), 2.13 (s, 3H, H-21, 2-OAc), all other peaks buried under the major isomer.

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): only major isomer reported: 217.26 (C-16), 193.41 (C-3), 170.21 (C-5 or C-20), 169.73 (C-5 or C-20), 122.08 (C-4), 71.11 (C-2), 55.48, 53.99, 50.90, 41.20, 40.64, 39.03, 38.82, 37.61, 35.11, 32.08, 31.99, 21.95, 20.84, 18.06, 17.91.

PREPARATION OF 2-HYDROXYANDROSTA-1,4-DIENE-3,16-DIONE (1).

A solution of 2α- and 2β-acetoxyandrost-4-ene-3,17-dione **58** (118 mg, 0.343 mmol) in 95% ethanol (14 mL) was stirred at room temperature for 30 min. A solution of potassium hydroxide (192 mg, 3.43 mmol) in water (7 mL) was added and the mixture was stirred vigorously for 1 h. Stirring was stopped and the flask was left open to air for four days. The reaction mixture was diluted with ethyl acetate (28 mL), neutralized with 5% aqueous hydrochloric acid and washed with water (1 x 10 mL) and brine (1 x 10 mL). The aqueous layer was extracted with ethyl acetate (1 x 20 mL) and the combined extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a pale yellow solid. Flash chromatography (10 g of silica gel, 3:1 hexanes/ethyl acetate) yielded **1** (103 mg, 100%) as a white solid.



1

$C_{19}H_{24}O_3$ MW=300.40 g/mole

mp 188-189°C

$[\alpha]_D^{25}$ -220.4° (c=0.84, CH_2Cl_2)

MS [EI, *m/z* (%)]: 300 [MH^+] (57), 163 (100), 137 (80).

HRMS calcd. for $C_{19}H_{24}O_3$: 300.1726; found: 300.1726.

Elem. Anal. calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05; found: C, 74.84; H, 8.36.

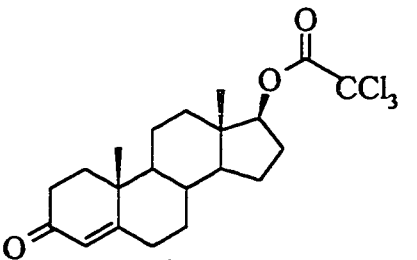
IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3349 (OH), 1738 (C=O), 1641 (C=O), 1616 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 6.32 (brs, 1H, 2-OH), 6.29 (s, 1H, H-1), 6.17 (d, J=1.4 Hz, 1H, H-4), 2.50 (ddt, J=13.4, 5.1, 1.4 Hz, 1H), 2.43 (ddd, J=13.4, 4.6, 2.6 Hz, 1H), 2.19 (dd, J=18.1, 7.6 Hz, 1H), 2.13 (d, J=18.8 Hz, 1H), 2.02-1.71 (m, 7H), 1.48 (ddd, J=13.5, 10.9, 7.6 Hz, 1H), 1.39 (dt, J=12.9, 4.4 Hz, 1H), 1.26 (s, 3H, H-19), 1.23-1.06 (m, 2H), 0.94 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 217.14 (C-16), 181.46 (C-3), 172.04 (C-5), 146.23 (C-2), 123.64 (C-1), 121.29 (C-4), 55.46, 53.39, 50.70, 44.04, 39.29, 39.25, 37.76, 34.85, 34.05, 32.62, 22.85, 19.73, 18.08.

PREPARATION OF 17β-TRICHLOROACETOXYANDROST-4-EN-3-ONE (46).

Triethylamine (0.725 mL, 0.00520 mol), trichloroacetyl chloride (0.464 mL, 0.00416 mol) and N,N-dimethylaminopyridine (0.0424 g, 0.000347 mol) were added to a solution of testosterone **9** (1.00g, 0.00347 mol) in dry methylene chloride (4 mL) at 0°C. The reaction mixture was stirred for 30 min, poured into water (10 mL) and extracted with methylene chloride (3 x 20 mL). The combined extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a white solid. Flash chromatography (108 g of silica gel, 5:1 hexanes/ethyl acetate) yielded **46** (1.48 g, 99%) as a white solid.



46

$C_{21}H_{27}Cl_3O_3$ MW=433.80 g/mole

mp 151-152°C

MS [EI, *m/z* (%)]: 432 [MH^+] (6), 286 (74), 124 (100).

HRMS calcd. for $C_{21}H_{27}Cl_3O_3$: 432.1028; found: 432.1012.

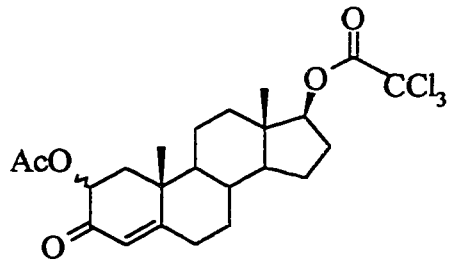
IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1763 (C=O), 1671 (C=O), 1617 (C=C).

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 5.71 (d, J=1.2 Hz, 1H, H-4), 4.74 (d, J=6.9 Hz, 1H, H-17), 2.60-2.10 (m, 5H), 2.09-0.80 (m, 14H), 1.23 (s, 3H, H-19), 0.91 (s, 3H, H-18).

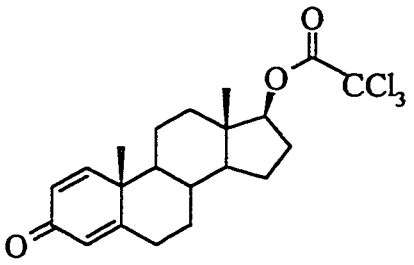
^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 198.92 (C-3), 170.30 (C-5), 161.57 (C-20), 123.78 (C-4), 90.00 (C-21), 87.29 (C-17), 53.30, 49.77, 42.98, 38.33, 36.21, 35.46, 35.07, 33.69, 32.41, 31.16, 26.61, 23.49, 20.39, 17.17, 11.67.

PREPARATION OF 2β-ACETOXY-17β-TRICHLOROACETOXY-ANDROST-4-EN-3-ONE and 2α-ACETOXY-17β-TRICHLOROACETOXY-ANDROST-4-EN-3-ONE (47A, 47A^l) AND 17β-TRICHLOROACETOXY-ANDROSTA-1,4-DIEN-3-ONE (48).

Lead tetraacetate (96 mg, 0.22 mmol), recrystallized from hot glacial acetic acid, was dissolved in glacial acetic acid (1 mL) at 50°C. The reaction mixture was stirred for 15 min, a solution of 17β-trichloroacetoxyandrost-4-en-3-one **46** (85 mg, 0.20 mmol) and *p*-toluenesulfonic acid (approximately 5 mg) in glacial acetic acid (1 mL) was added and the mixture was warmed to 55 to 65°C. The reaction mixture was stirred for 30 h, cooled to room temperature, diluted with ethyl acetate (40 mL) and washed with water (2 x 10 mL) and a saturated aqueous solution of sodium bicarbonate (3 x 10 mL). The organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a 62:38 (**47A**+**47A^l**/**48**) mixture of **47A**, **47A^l** and **48** (98 mg, 100%) as a yellow oil which crystallized as yellow oily prisms in the freezer.



47A + 47A^{I}



48

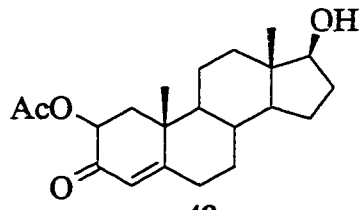
$C_{23}H_{29}Cl_3O_5$ MW=491.84 g/mole $C_{21}H_{25}Cl_3O_3$ MW=431.79 g/mole

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): **compound 47A+47A^{I}**: 5.78 (s, 1H, H-4), 5.75 (s, 1H, H-4), 5.43 (dd, J=14.1, 5.5 Hz, 1H, H-2), 5.28 (dd, J=12.5, 4.2 Hz, 1H, H-2), 2.14 (s, 3H, H-21, 2-OAc), 2.12 (s, 3H, H-21, 2-OAc), **compound 48**: 7.03 (d, J=10.4 Hz, 1H, H-1), 6.21 (d, J=10.4 Hz, 1H, H-2), 6.05 (s, 1H, H-4), **compound 47A+47A^{I}+48**: 4.75 (m, 3H, H-17), 2.60-0.80 (m, 49H), 1.31 (s, 3H, H-19), 1.23 (s, 3H, H-19), 1.19 (s, 3H, H-19), 1.17 (s, 3H, H-18), 0.93 (s, 3H, H-18), 0.90 (s, 3H, H-18).

PREPARATION OF 2-ACETOXY-17β-HYDROXYANDROST-4-EN-3-ONE (49) AND 17β-HYDROXYANDROSTA-1,4-DIEN-3-ONE (50).

A solution of 2α- and 2β-acetoxy-17β-trichloroacetoxyandrost-4-en-3-one **47A** and **47A^{I}** and 17β-trichloroacetoxyandrosta-1,4-dien-3-one **48** (480 mg, 0.978 mmol) in absolute ethanol (20 mL) was warmed to 60 to 70°C and stirred for 1h. Triethylamine (0.136 ml, 1.08 mmol) and benzylamine (0.117 mL, 0.978 mmol) were added and the mixture was heated at reflux for 25 h. The reaction mixture was diluted with ethyl acetate (100 mL) and washed and water (3 x 50 mL). The organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo

affording a yellow-brown foamy solid. Flash chromatography (38 g of silica gel, 3:1 hexanes/ethyl acetate) yielded **49** (190 mg, 61% over two steps) as a yellow solid and **50** (77 mg, 31% over two steps) as a white solid.



49

$C_{21}H_{30}O_4$ MW=346.47 g/mole

mp 183-185°C

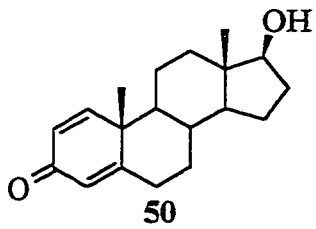
MS [EI, *m/z* (%)]: 346 [MH^+] (1), 260 (24), 122 (100).

HRMS calcd. for $C_{21}H_{30}O_4$: 346.2145; found: 346.2159.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3443 (OH), 1746 (C=O), 1689 (C=O), 1616 (C=C).

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 5.69 (s, 1H, H-4), 5.38 (dd, J=14.1, 5.5 Hz, 1H, H-2), 3.58 (t, J=8.7 Hz, 1H, H-17), 2.40-0.70 (m, 18H), 2.11 (s, 3H, H-21, 2-OAc), 1.28 (s, 3H, H-19), 0.73 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 193.65 (C-3), 170.98 (C-5 or C-21), 170.22 (C-5 or C-21), 121.62 (C-4), 81.23 (C-2), 71.12 (C-17), 54.22, 50.20, 42.64, 41.30, 40.55, 36.12, 34.98, 32.23, 31.29, 30.15, 23.12, 20.83, 20.38, 18.00, 10.96.



50

$C_{19}H_{26}O_2$ MW=286.41 g/mole

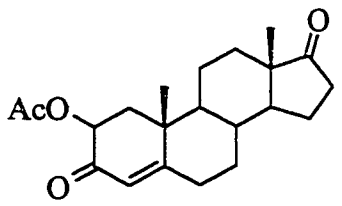
mp 99-101°C

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3418 (OH), 1648 (C=O), 1618 (C=C).

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 7.18 (d, J=10.1 Hz, 1H, H-1), 6.23 (d, J=10.1 Hz, 1H, H-2), 6.07 (s, 1H, H-4), 3.63 (t, J=6.7 Hz, 1H, H-17), 2.58-2.25 (m, 2H), 2.20-0.70 (m, 20H), 1.24 (s, 3H, H-19), 0.82 (s, 3H, H-18).

PREPARATION OF 2-ACETOXYANDROST-4-ENE-3,17-DIONE (51).

Pyridinium dichromate (254 mg, 0.671 mmol) was added to a solution of 2-acetoxy-17β-hydroxyandrost-4-en-3-one **49** (155 mg, 0.447 mmol) in dry methylene chloride (3 mL) at room temperature. The reaction mixture was stirred for 20 h, diluted with ether (2 mL), filtered through anhydrous magnesium sulfate and celite in a sintered glass funnel . The product was concentated in vacuo affording a yellow solid. Flash chromatography (25 g of silica gel, 3:1 hexanes/ethyl acetate) yielded **51** (120 mg, 78%) as a white solid.



51

$C_{21}H_{28}O_4$ MW=344.45 g/mole

mp 202.5-204°C

MS [EI, *m/z* (%)]: 344 [MH^+] (4), 258 (100), 243 (39).

HRMS calcd. for $C_{21}H_{28}O_4$: 344.1988; found: 344.2008.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1741 (C=O), 1690 (C=O), 1614 (C=C).

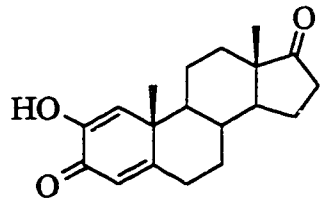
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 5.69 (s, 1H, H-4), 5.37 (dd, J=14.3, 5.2 Hz, 1H, H-2), 2.50-0.70 (m, 17H), 2.09 (s, 3H, H-21, 2-OAc), 1.28 (s, 3H, H-19), 0.85 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 219.86 (C-17), 193.25 (C-3), 170.00 (C-5 or C-20), 169.87 (C-5 or C-20), 121.82 (C-4), 70.89 (C-2), 53.99, 50.44, 47.22, 41.16, 40.42, 35.48, 34.38, 31.90, 30.92, 30.41, 21.45, 20.71, 19.97, 17.90, 13.49.

PREPARATION OF 2-HYDROXYANDROSTA-1,4-DIENE-3,17-DIONE (52).

A solution of 2α-acetoxyandrost-4-ene-3,17-dione **51** (90 mg, 0.26 mmol) in 95% ethanol (7 mL) was stirred at room temperature for 30 min. A solution of potassium hydroxide (150 mg, 2.6 mmol) in water (5 mL) was added and the mixture was stirred vigorously for 1 h. Stirring was stopped and the flask was left open to air for four days.

The reaction mixture was diluted with ethyl acetate (14 mL), neutralized with 5% aqueous hydrochloric acid and washed with water (1 x 10 mL) and brine (1 x 10 mL). The aqueous layer was extracted with ethyl acetate (1 x 25 mL) and the combined extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated in vacuo affording a pale yellow solid. Flash chromatography (5 g of silica gel, 2:1 hexanes/ethyl acetate) yielded **52** (57.6 mg, 74%) as a white solid.



52

$C_{19}H_{24}O_3$ MW=300.40 g/mole

mp 141-143°C

MS [EI, *m/z* (%)]: 300 [MH^+] (58), 137 (73), 32 (100).

HRMS calcd. for $C_{19}H_{24}O_3$: 300.1726; found: 300.1749.

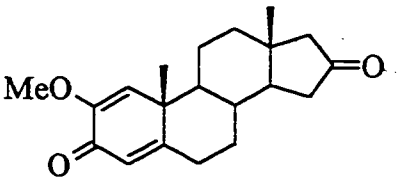
IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3371 (OH), 1736 (C=O), 1643 (C=O), 1613 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 6.32 (brs, 1H, 2-OH), 6.26 (s, 1H, H-1), 6.15 (s, 1H, H-4), 2.49-2.40 (m, 3H), 2.09-2.01 (m, 2H), 1.93-1.81 (m, 3H), 1.75-1.53 (m, 3H), 1.25-1.19 (m, 2H), 1.23 (s, 3H, H-19), 1.10-1.01 (m, 2H), 0.90 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 219.73 (C-17), 181.43 (C-3), 172.07 (C-5), 146.17 (C-2), 123.82 (C-1), 121.24 (C-4), 53.49, 50.35, 47.71, 43.91, 35.59, 34.93, 32.60, 32.52, 31.18, 22.46, 21.87, 19.64, 13.75.

PREPARATION OF 2-METHOXYANDROSRA-1,4-DIENE-3,16-DIONE (1a).

A solution of diazomethane in ether (1 mL, approximately 2.8% w/v prepared from nitrosomethylurea) was added to 2-hydroxyandrosta-1,4-diene-3,16-dione **1** (21 mg, 0.071 mmol) in methylene chloride (5 mL) at 5°C. The reaction mixture was stirred for 21 h at room temperature and was open to air to facilitate the evaporation of excess diazomethane and solvent. The pale yellow solid was purified by flash chromatography (2 g of silica gel, 3:1 hexanes/ethyl acetate) yielding **1a** (4.2 mg, 19%) as a white solid and **1** (14 mg, 65%) as a white solid.



1a

$C_{20}H_{26}O_3$ MW=314.42 g/mole

HRMS calcd. for $C_{20}H_{26}O_3$: 314.1883; found: 314.1893.

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 6.11 (d, J=1.2 Hz, 1H, H-4), 5.93 (s, 1H, H-1), 3.66 (s, 3H, H-20), 2.51-2.38 (m, 2H), 2.23-2.12 (m, 2H), 2.07-1.71 (m, 5H), 1.53-1.37 (m, 2H), 1.27 (s, 3H, H-19), 1.26-1.07 (m, 4H), 0.95 (s, 3H, H-18).

Appendix for Chapter 2

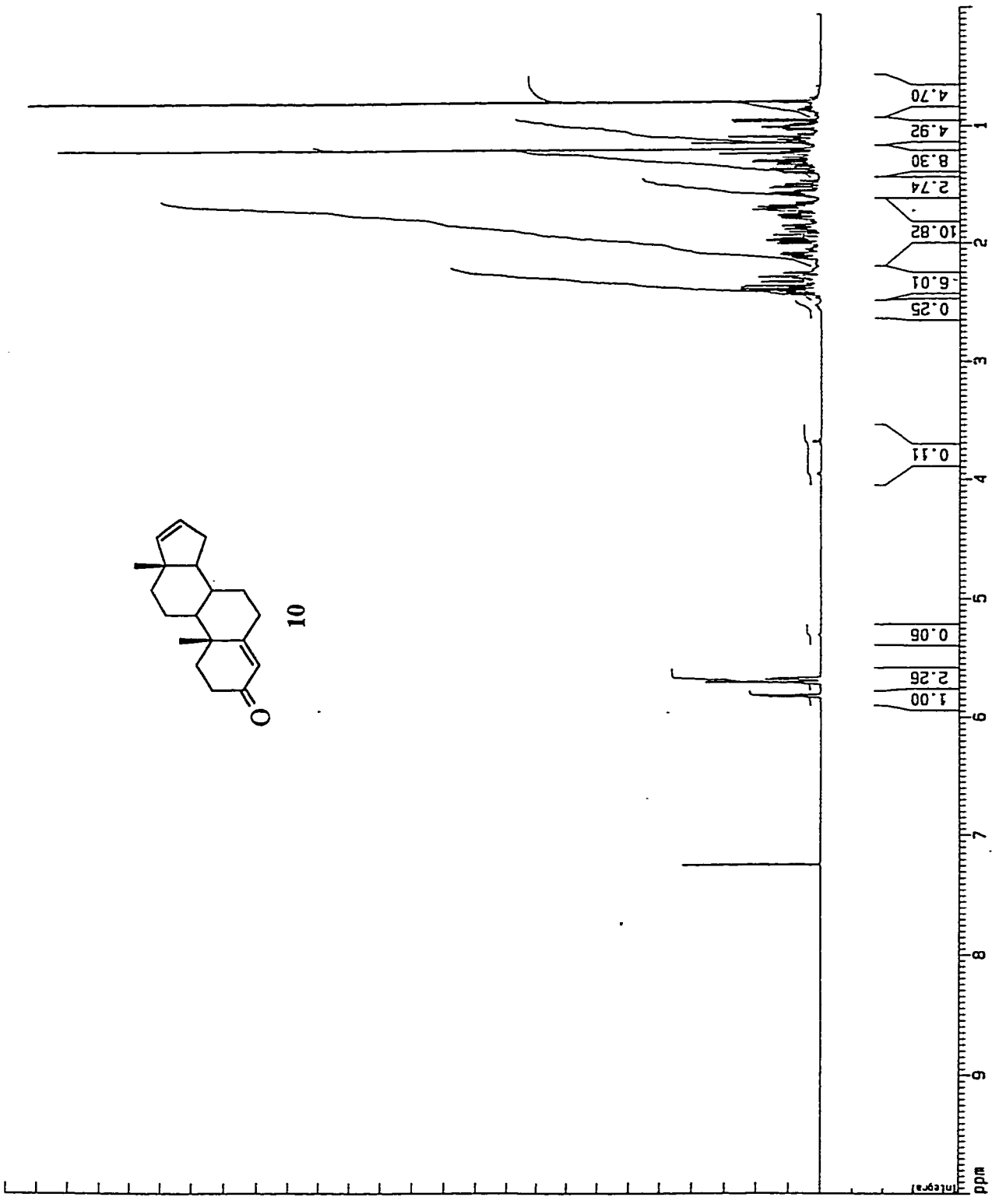


FIGURE 2.2.1: ^{1}H NMR SPECTRUM OF ANDROSTA-4,16-DIEN-3-ONE (10)

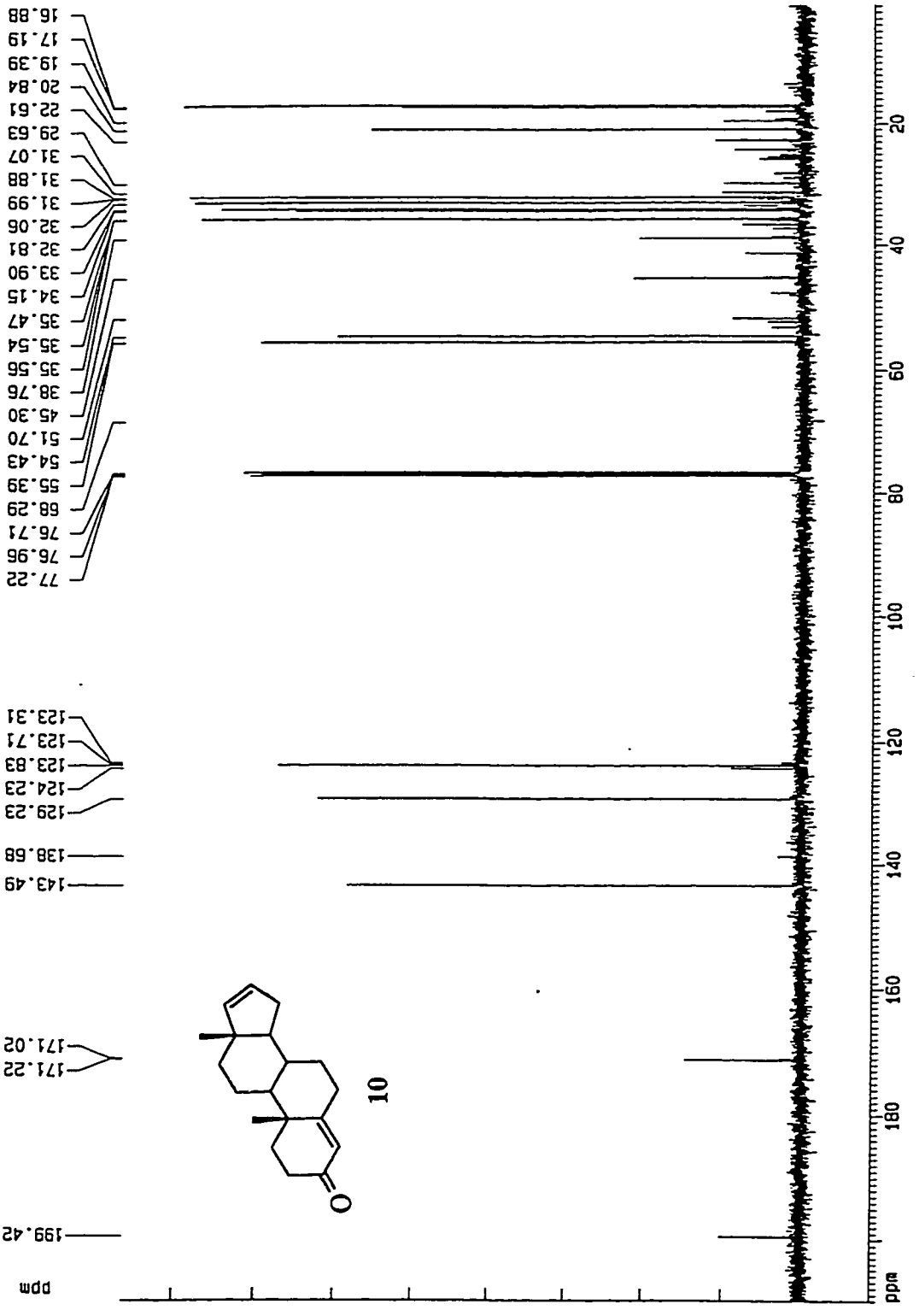


FIGURE 2.2.2: ^{13}C NMR SPECTRUM OF ANDROSTA-4,16-DIEN-3-ONE (10)

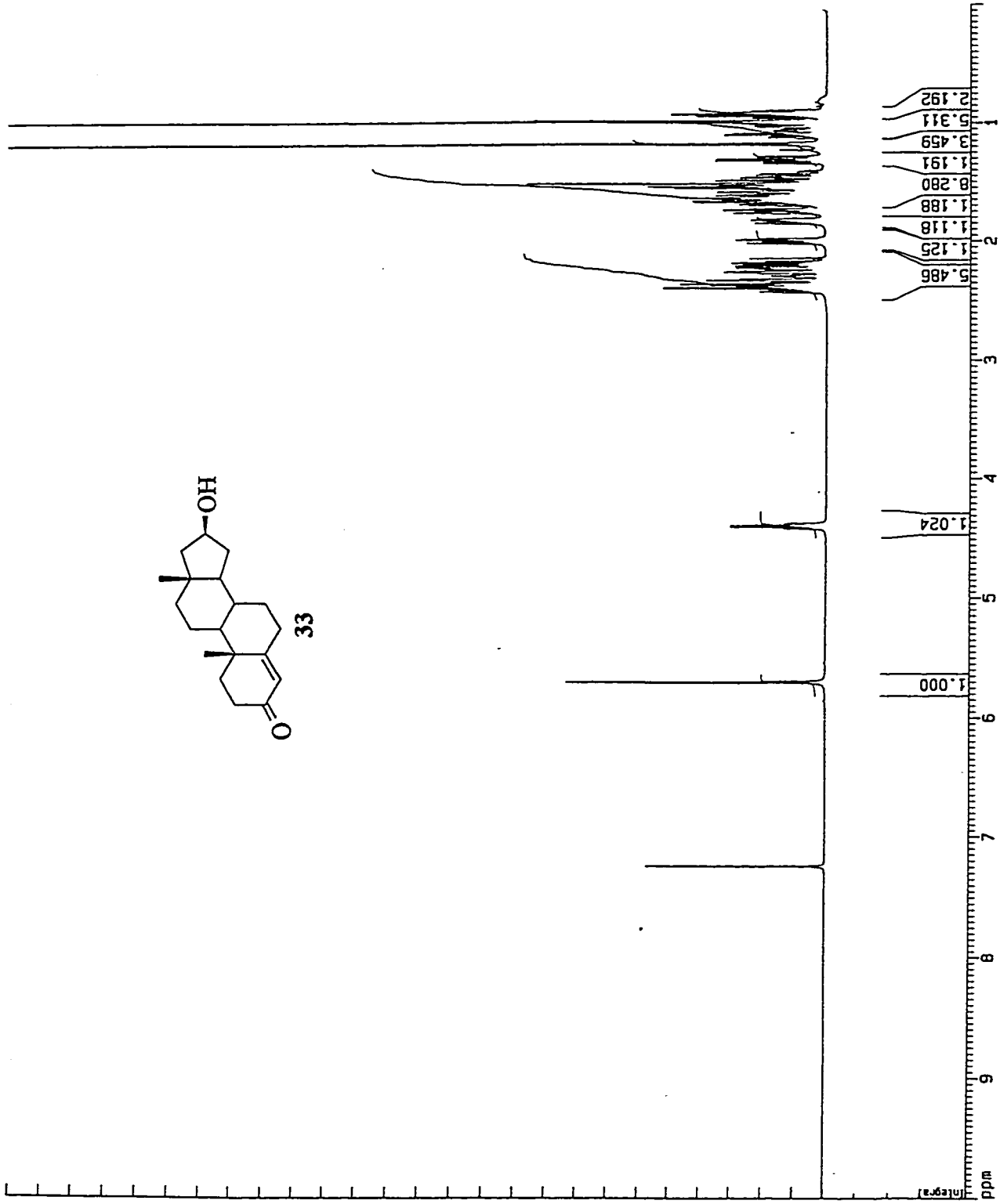


FIGURE 2.3.1: ^{1}H NMR SPECTRUM OF 16β-HYDROXYANDROST-4-EN-3-ONE (33)

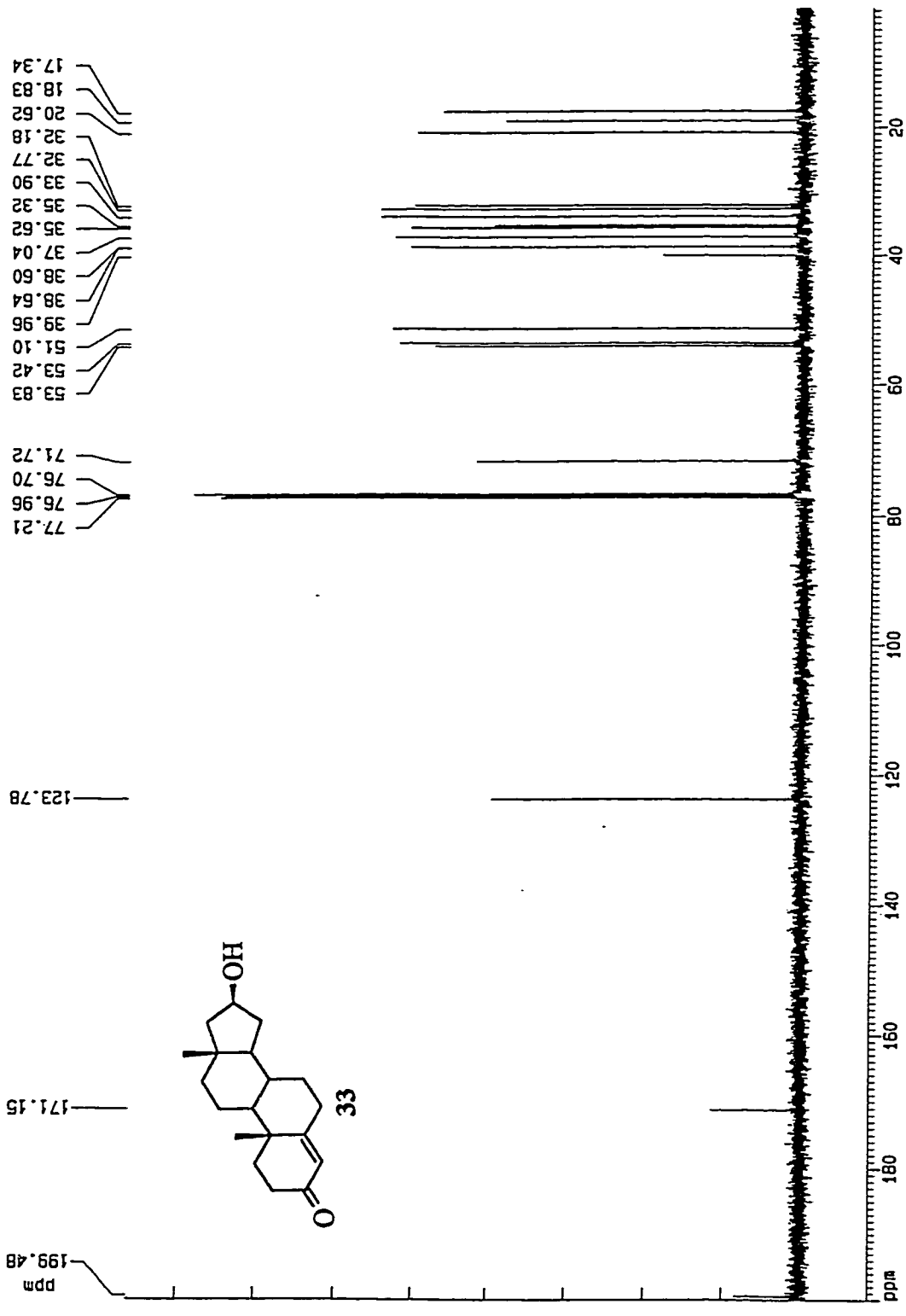


FIGURE 2.3.2: ^{13}C NMR SPECTRUM OF 16β-HYDROXYANDROST-4-EN-3-ONE (33)

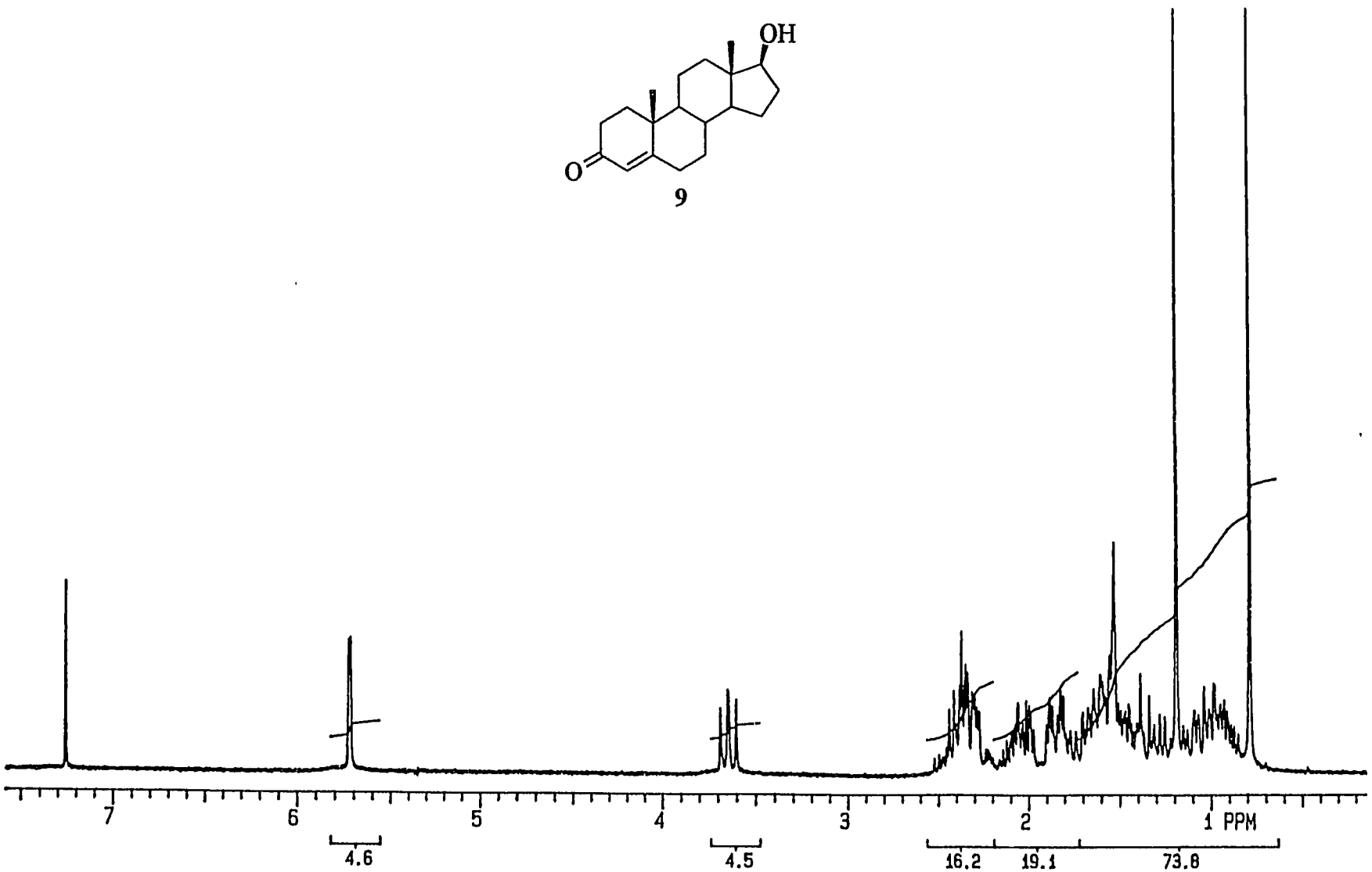


FIGURE 2.4.1: ^{1}H NMR SPECTRUM OF 17β-HYDROXYANDROST-4-EN-3-ONE (9) (TESTOSTERONE)

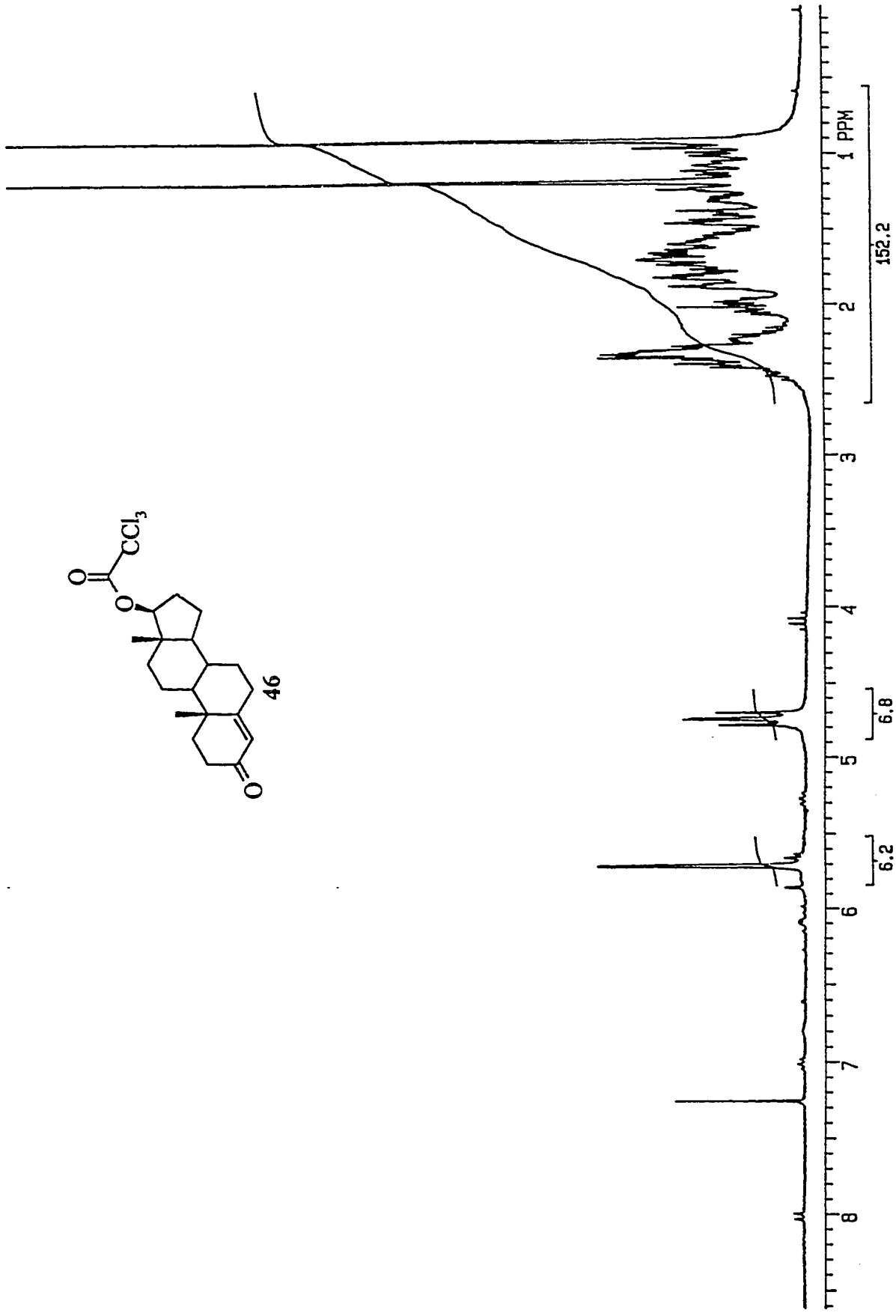


FIGURE 2.4.2: ^{1}H NMR SPECTRUM OF 17β-TRICHLOROACETOXYANDROST-4-EN-3-ONE (46)

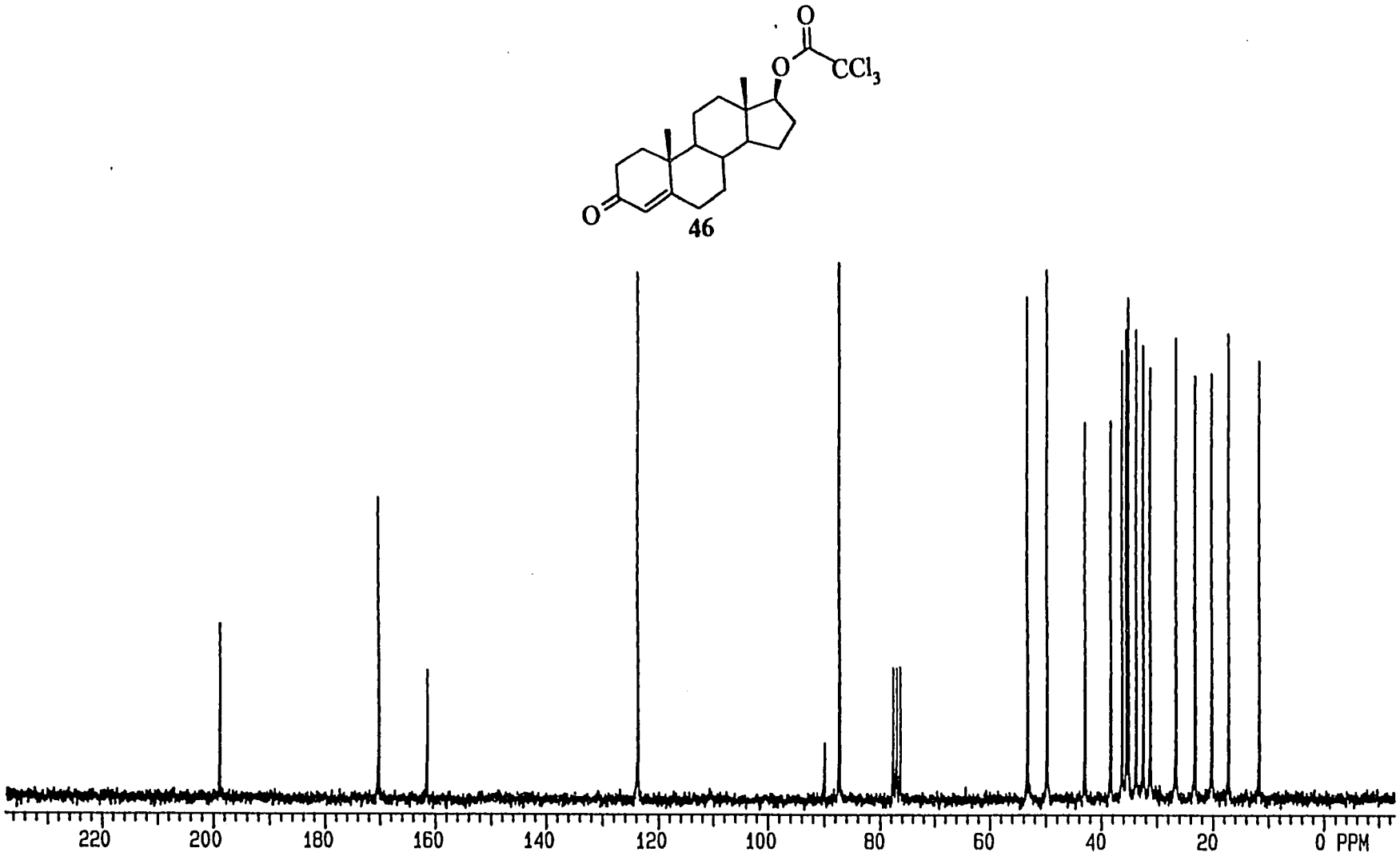


FIGURE 2.4.3 ^{13}C NMR SPECTRUM OF 17β-TRICHLOROACETOXYANDROST-4-EN-3-ONE (46)

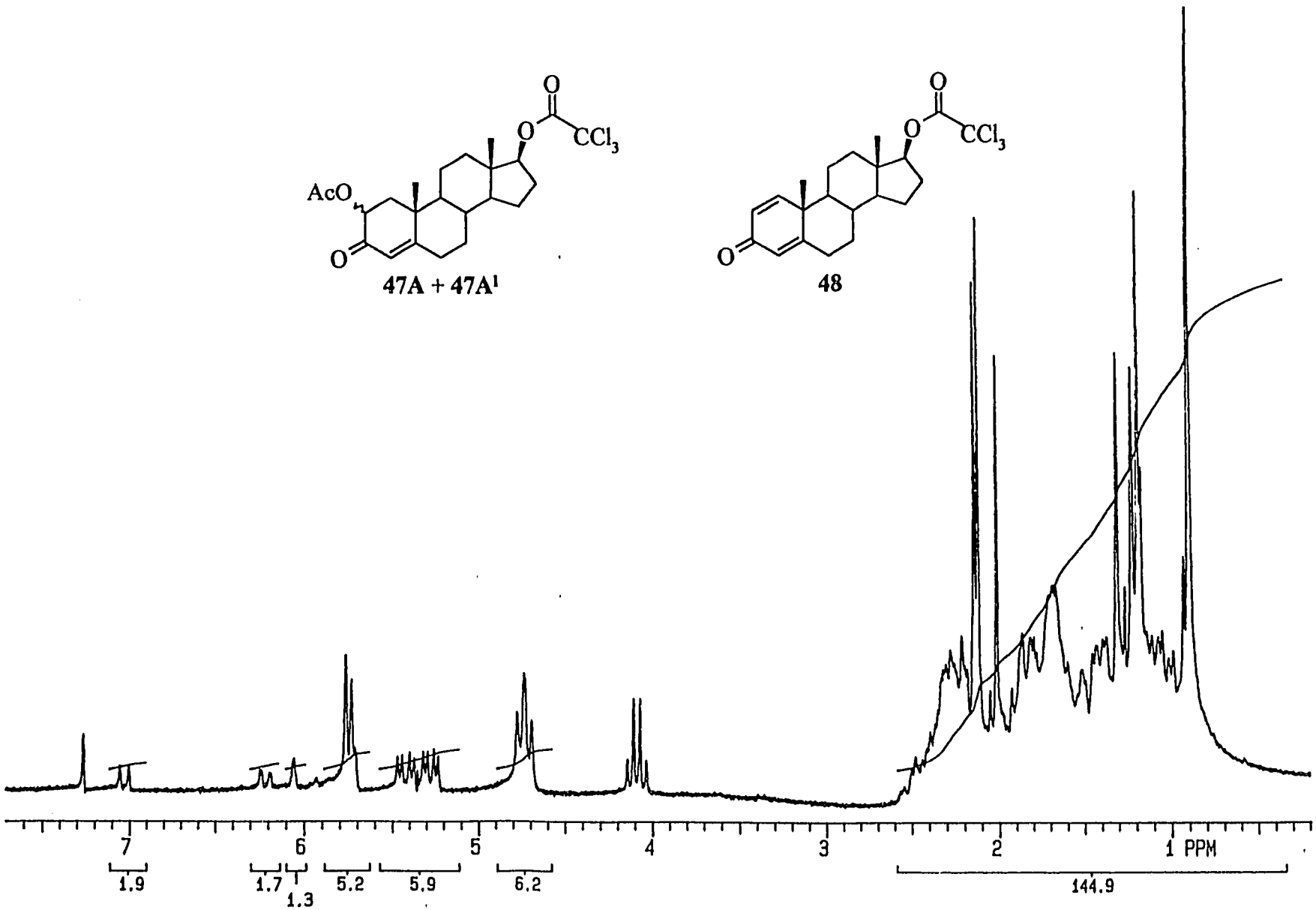


FIGURE 2.4.4: ^{1}H NMR SPECTRUM OF 2β- AND 2α-ACETOXY-17β-TRICHLOROACETOXYANDROST-4-EN-3-ONE (47A AND 47A^1) AND 17β-TRICHLOROACETOXYANDROSTA-1,4-DIENE-3-ONE (48)

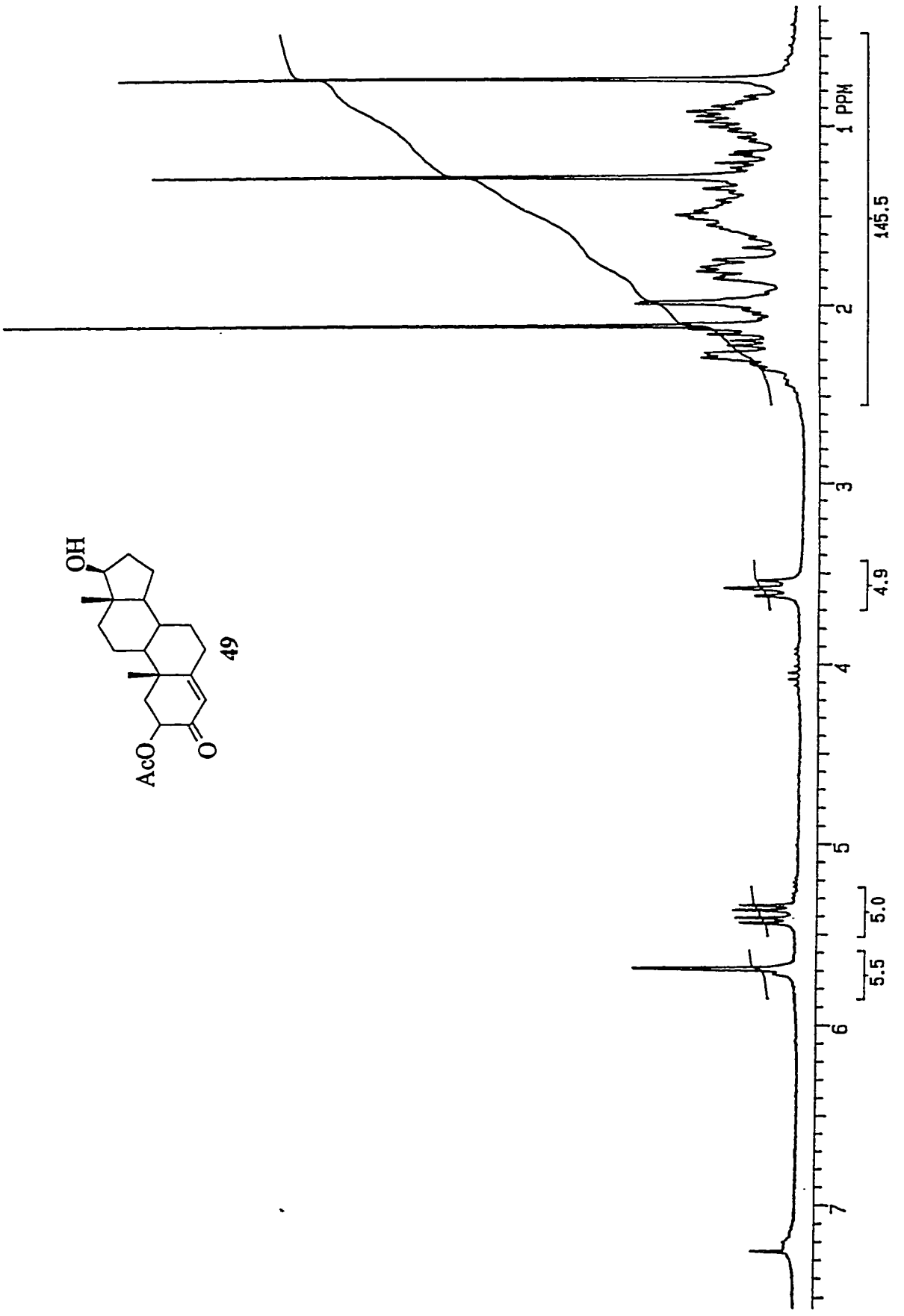
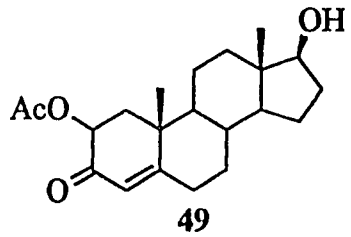


FIGURE 2.4.5: ^{1}H NMR SPECTRUM OF 2-ACETOXY-17β-HYDROXYANDROST-4-EN-3-ONE (49)



49

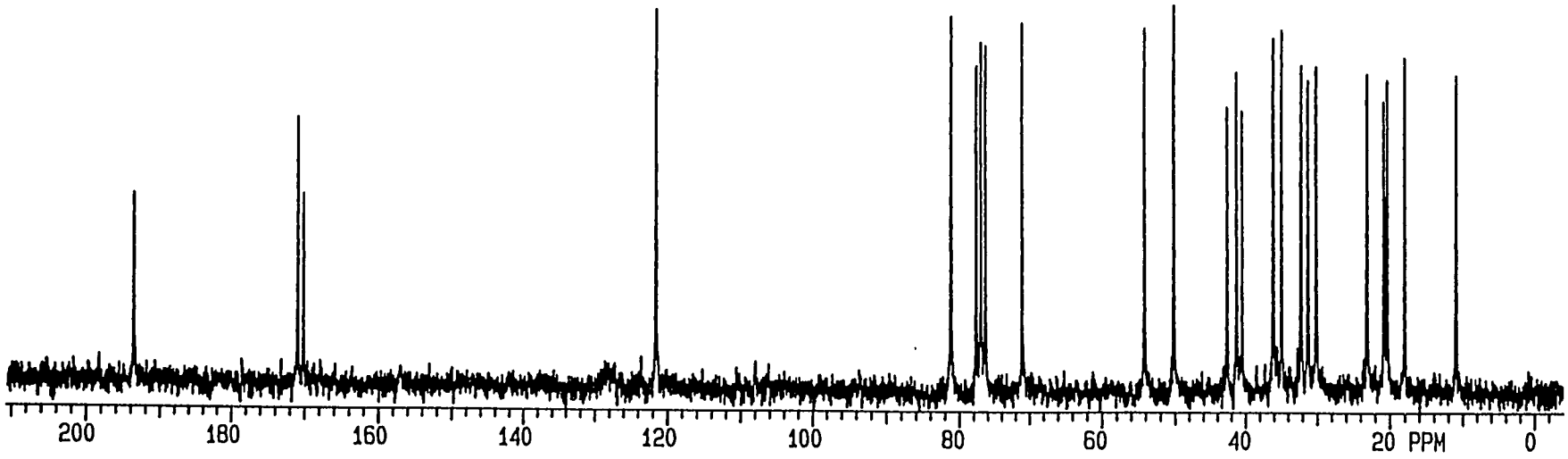


FIGURE 2.4.6: ^{13}C NMR SPECTRUM OF 2-ACETOXY-17β-HYDROXYANDROST-4-EN-3-ONE (49)

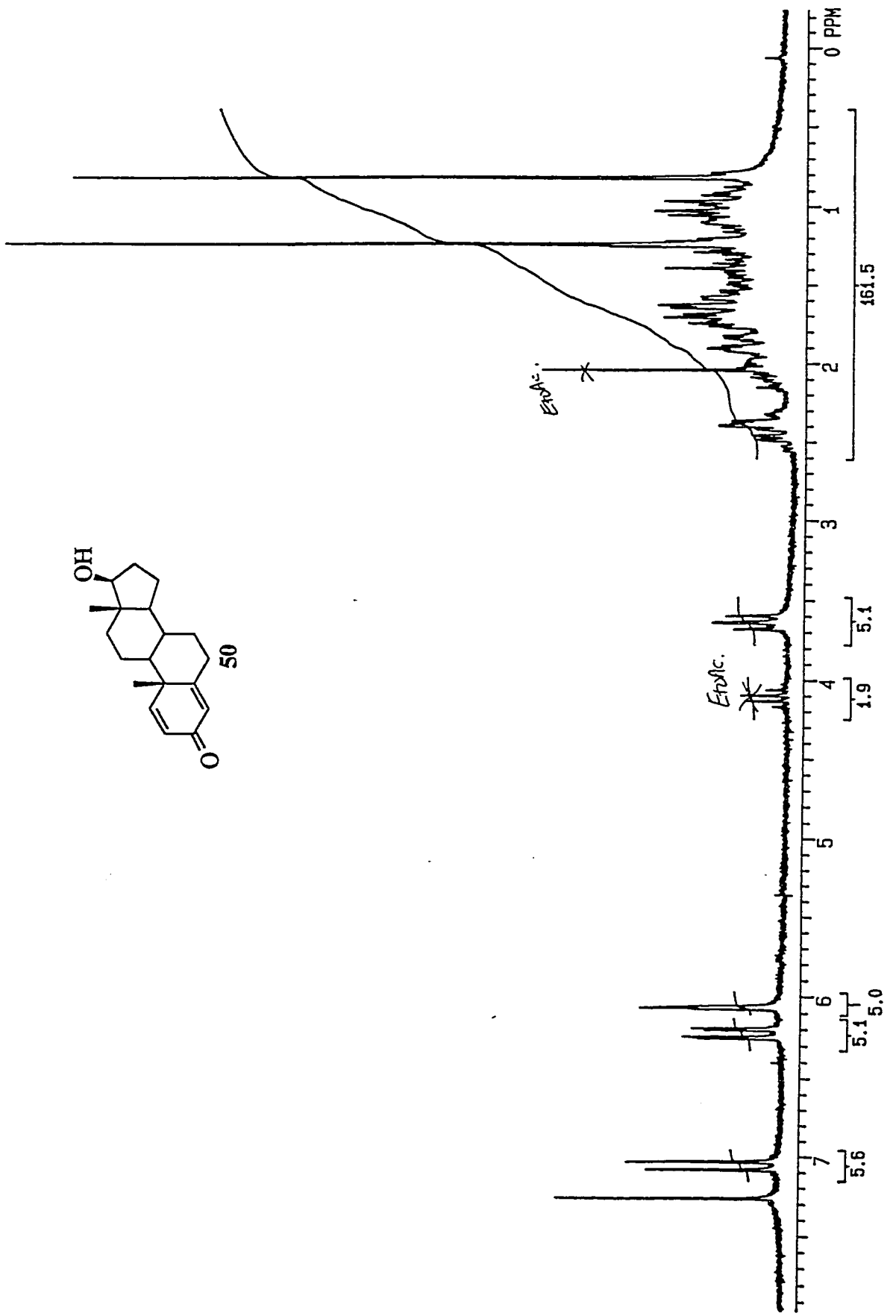


FIGURE 2.4.7: ^{1}H NMR SPECTRUM OF 17β-HYDROXYANDROSTA-1,4-DIEN-3-ONE (50)

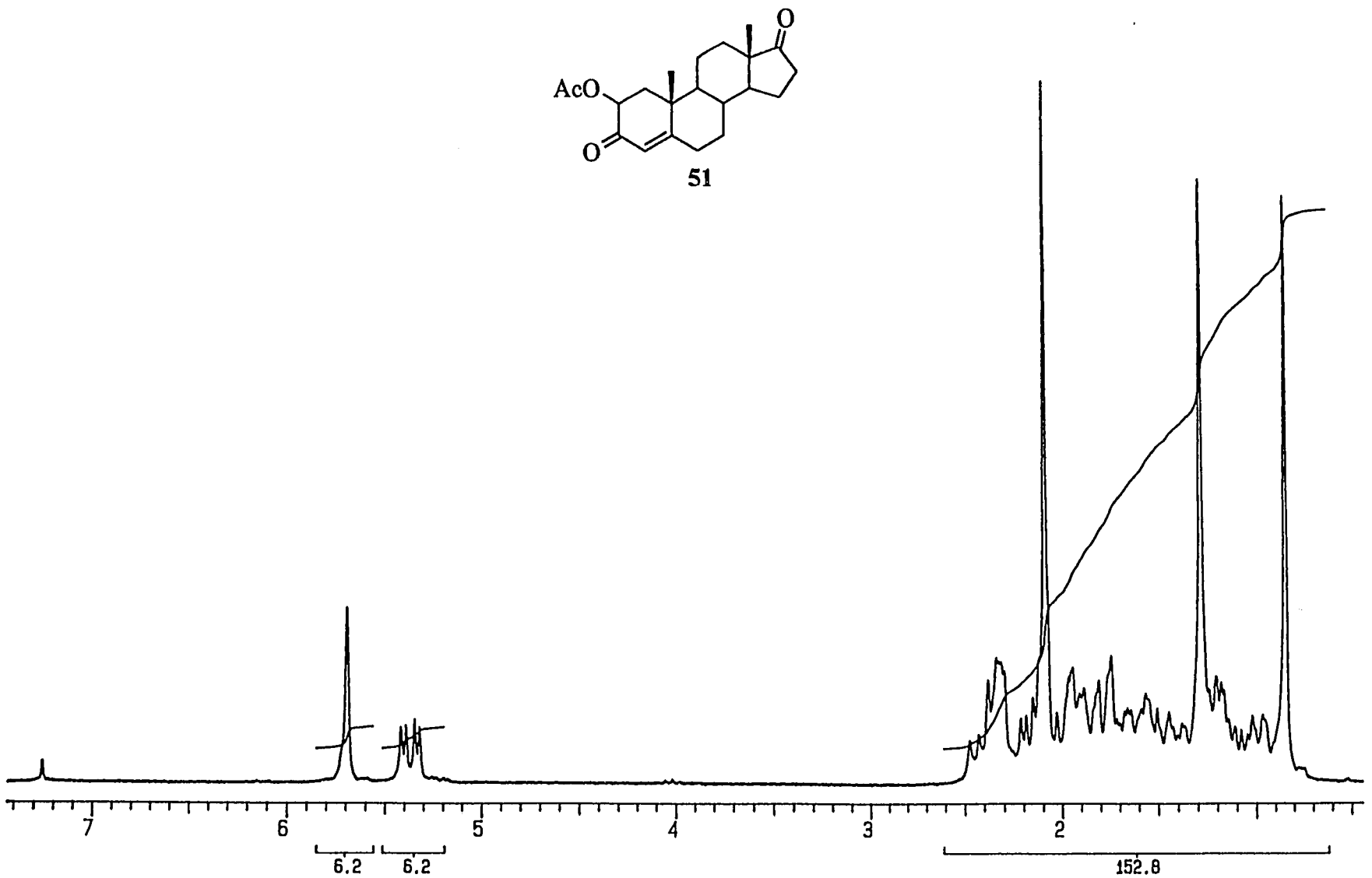
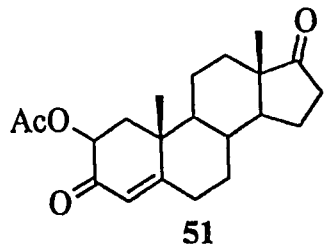


FIGURE 2.4.8: ^{1}H NMR SPECTRUM OF 2-ACETOXYANDROST-4-ENE-3,17-DIONE (51)



51

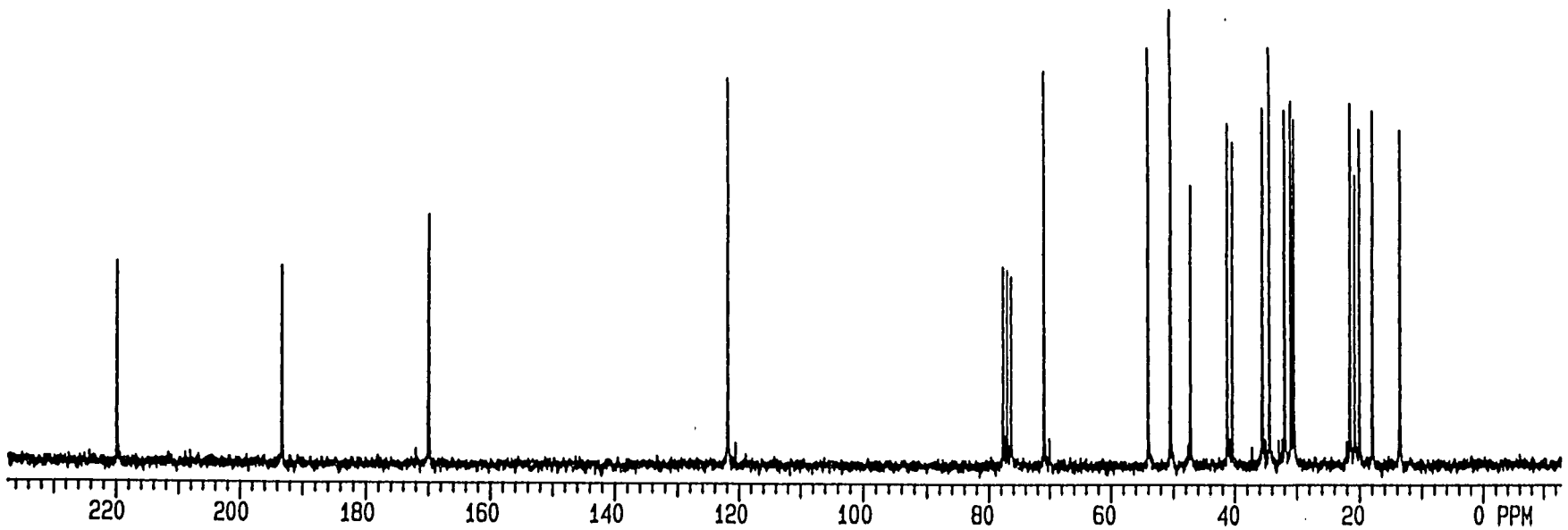


FIGURE 2.4.9: ^{13}C NMR SPECTRUM OF 2-ACETOXYANDROST-4-ENE-3,17-DIONE (51)

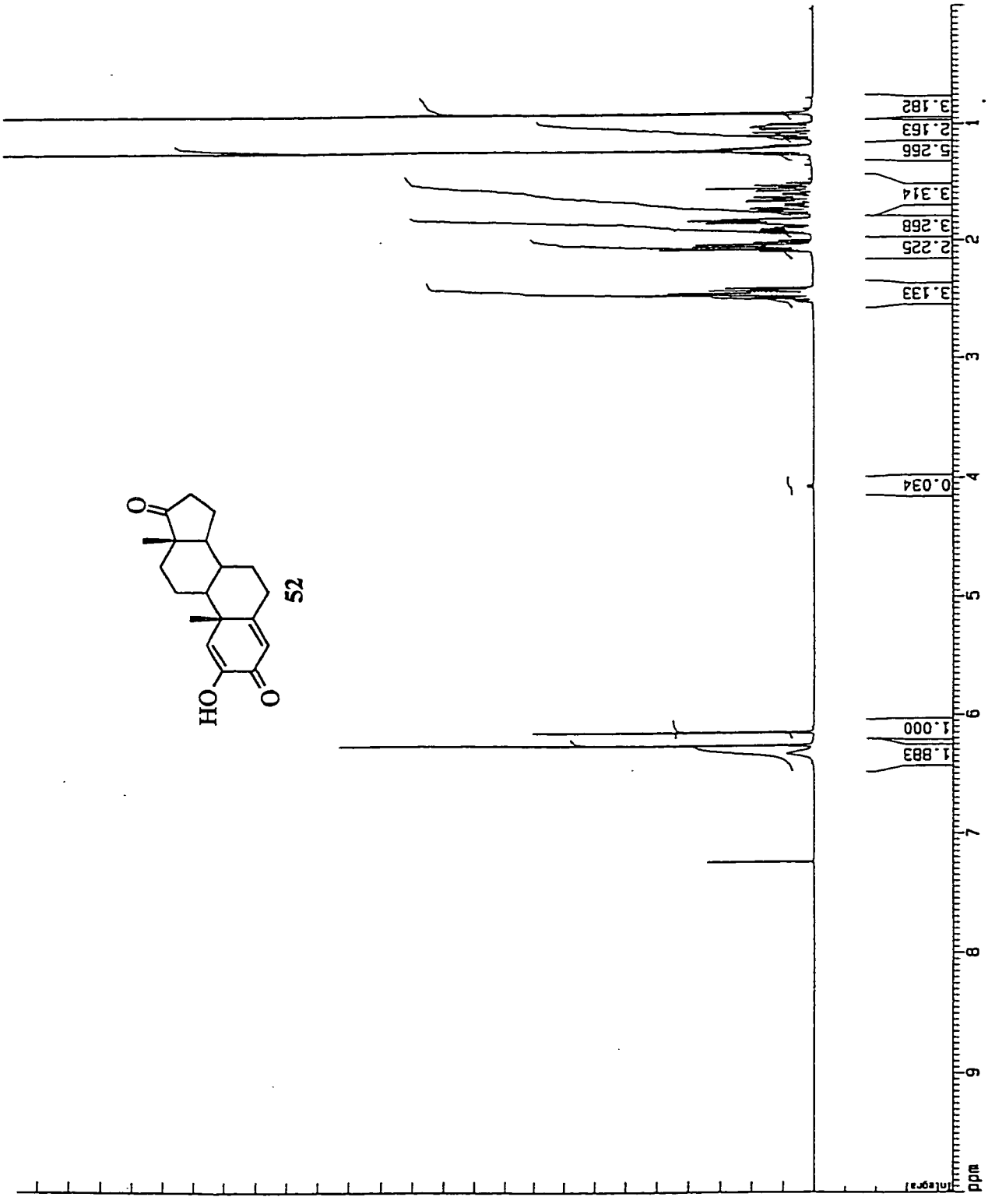


FIGURE 2.4.10: ^{1}H NMR SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIENE-3,17-DIONE (52)

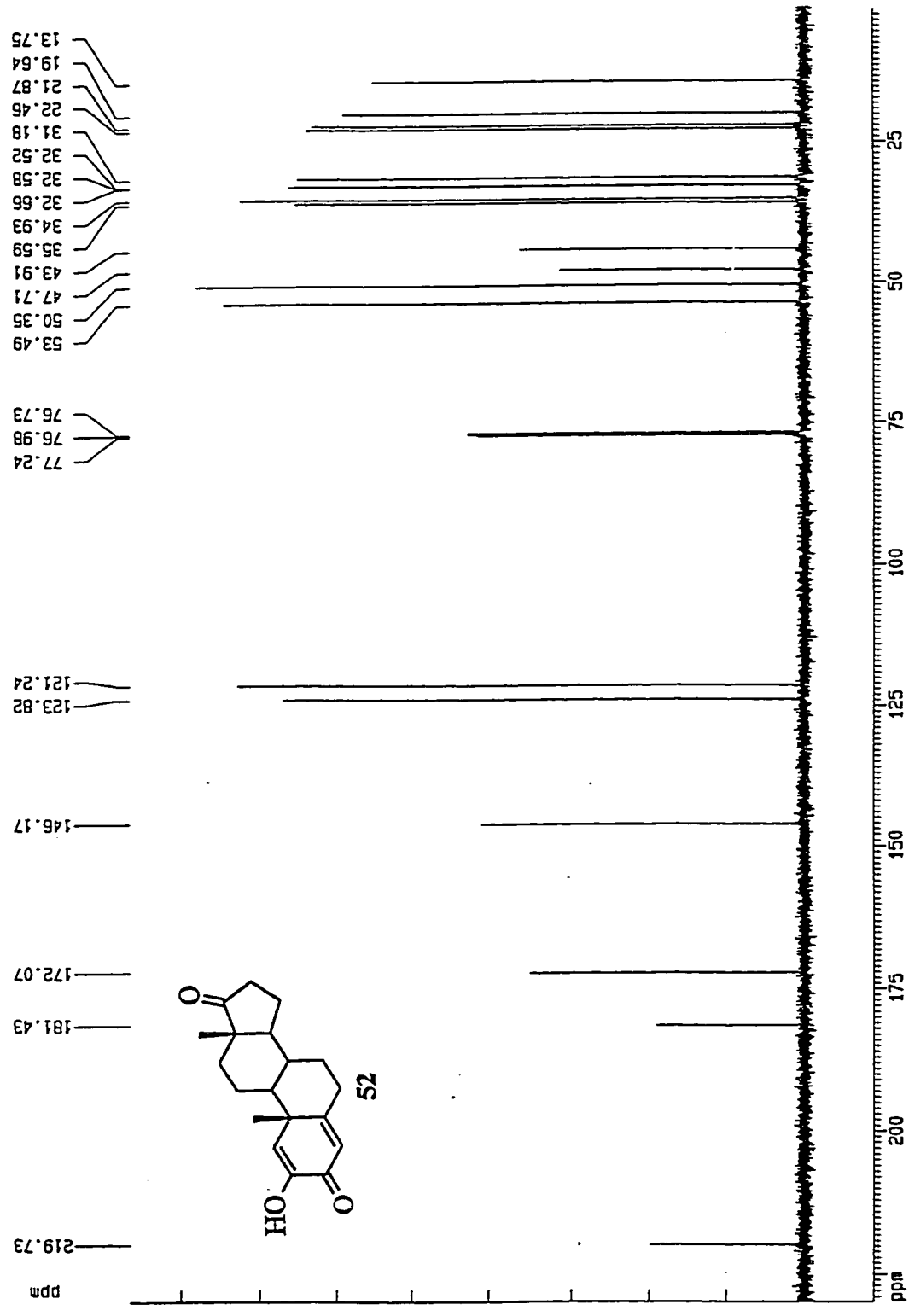


FIGURE 2.4.11: ^{13}C NMR SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIENE-3,17-DIONE (52)

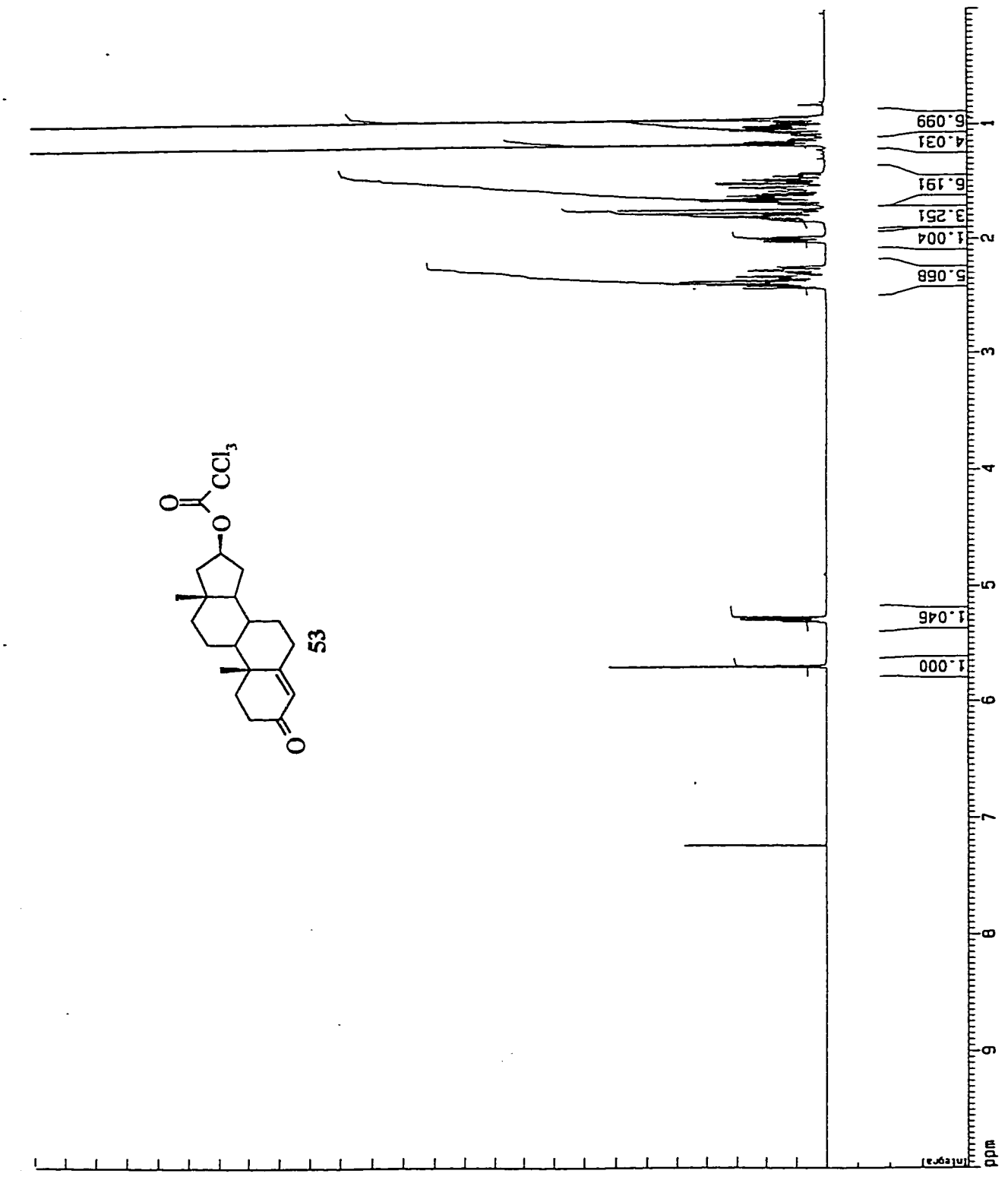


FIGURE 2.5.1: ^{1}H NMR SPECTRUM OF 16β-TRICHLOROACETOXYANDROST-4-EN-3-ONE (53)

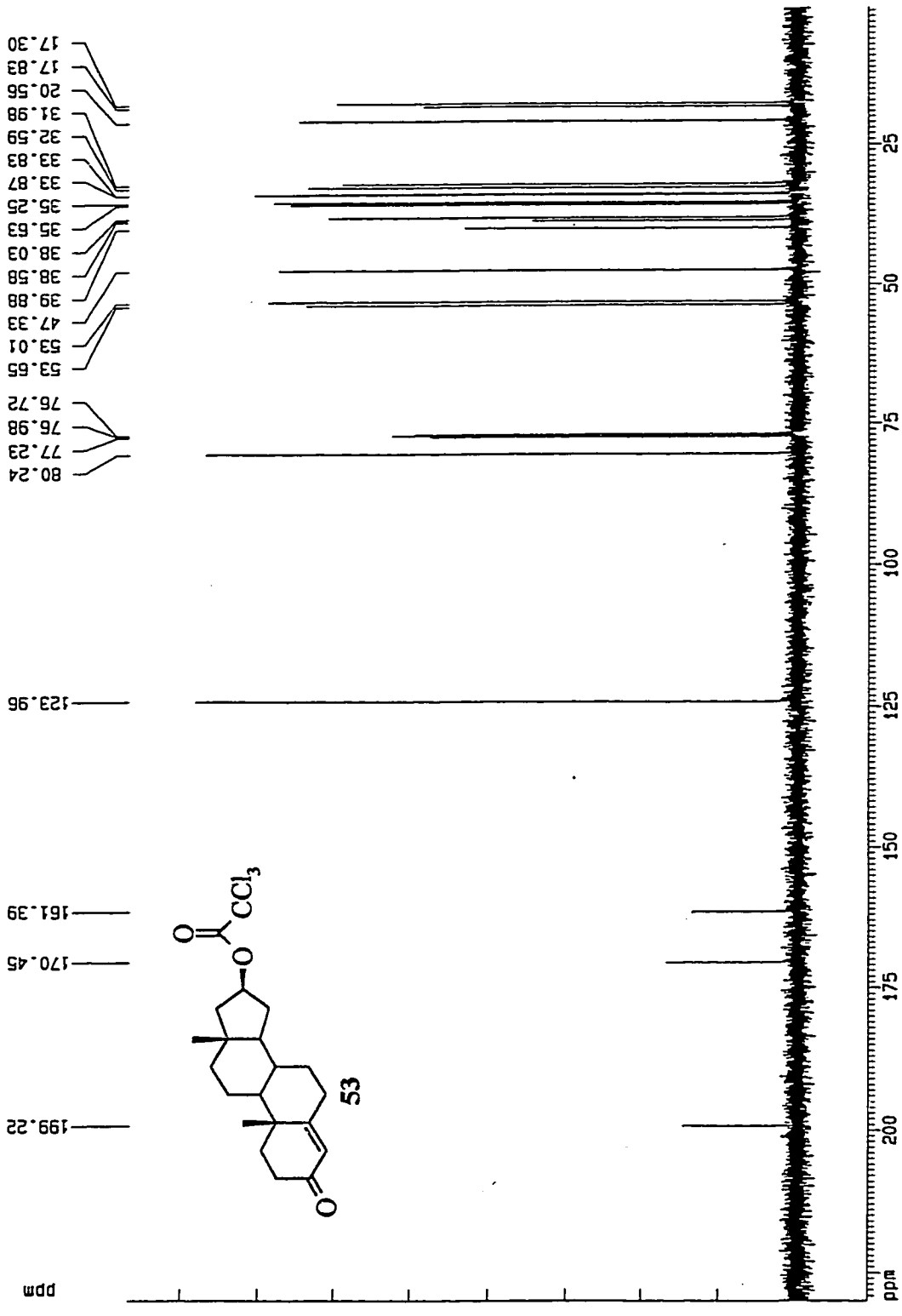


FIGURE 2.5.2: ^{13}C NMR SPECTRUM OF 16β-TRICHLROACETOXYANDROST-4-EN-3-ONE (53)

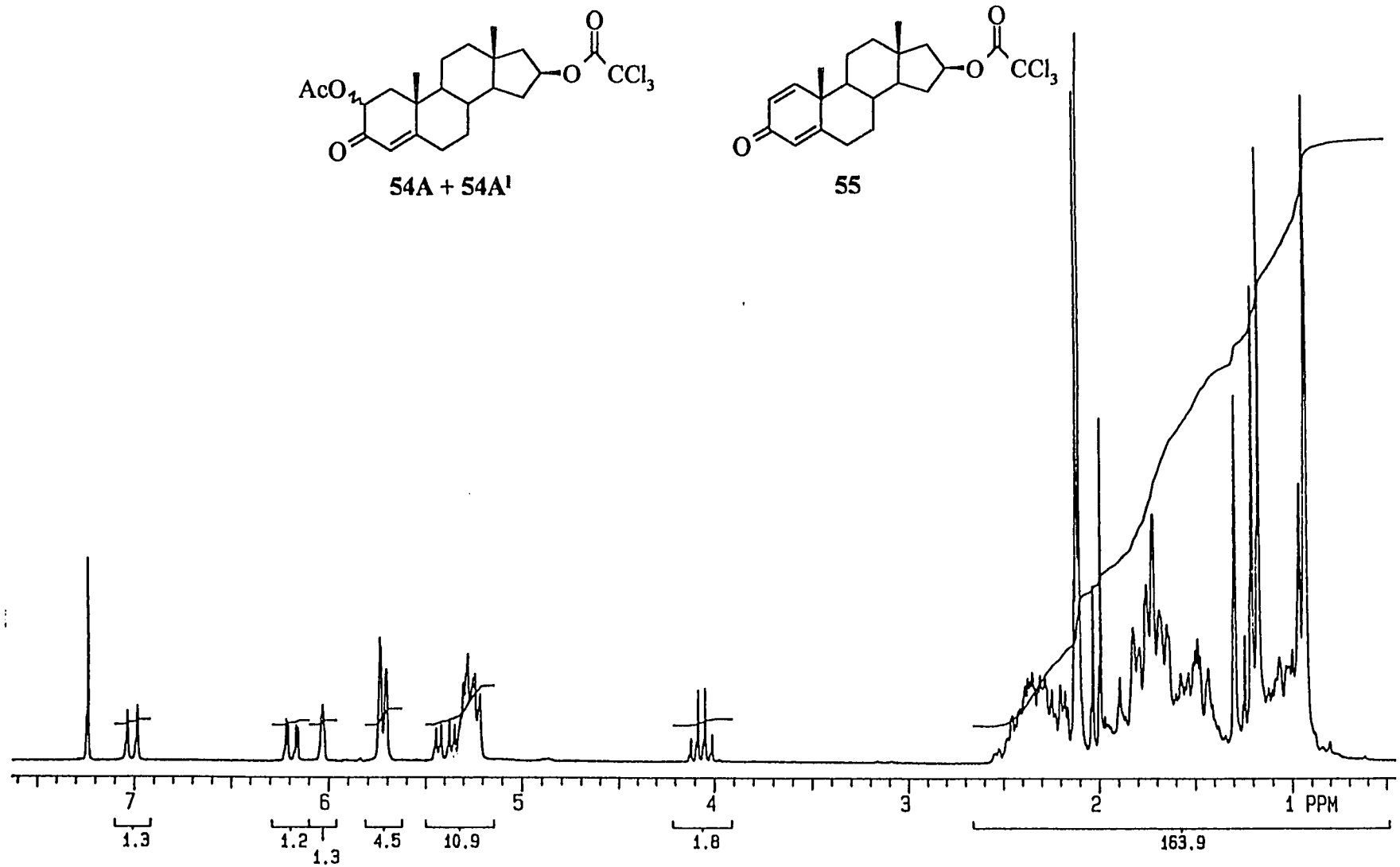


FIGURE 2.5.3: ^{1}H NMR SPECTRUM OF 2β-AND 2α-ACETOXY-16β-TRICHLOROACETOXYANDROST-4-EN-3-ONE (54A, 54A^1) AND 16β-TRICHLOROACETOXYANDROSTA-1,4-DIEN-3-ONE (55)

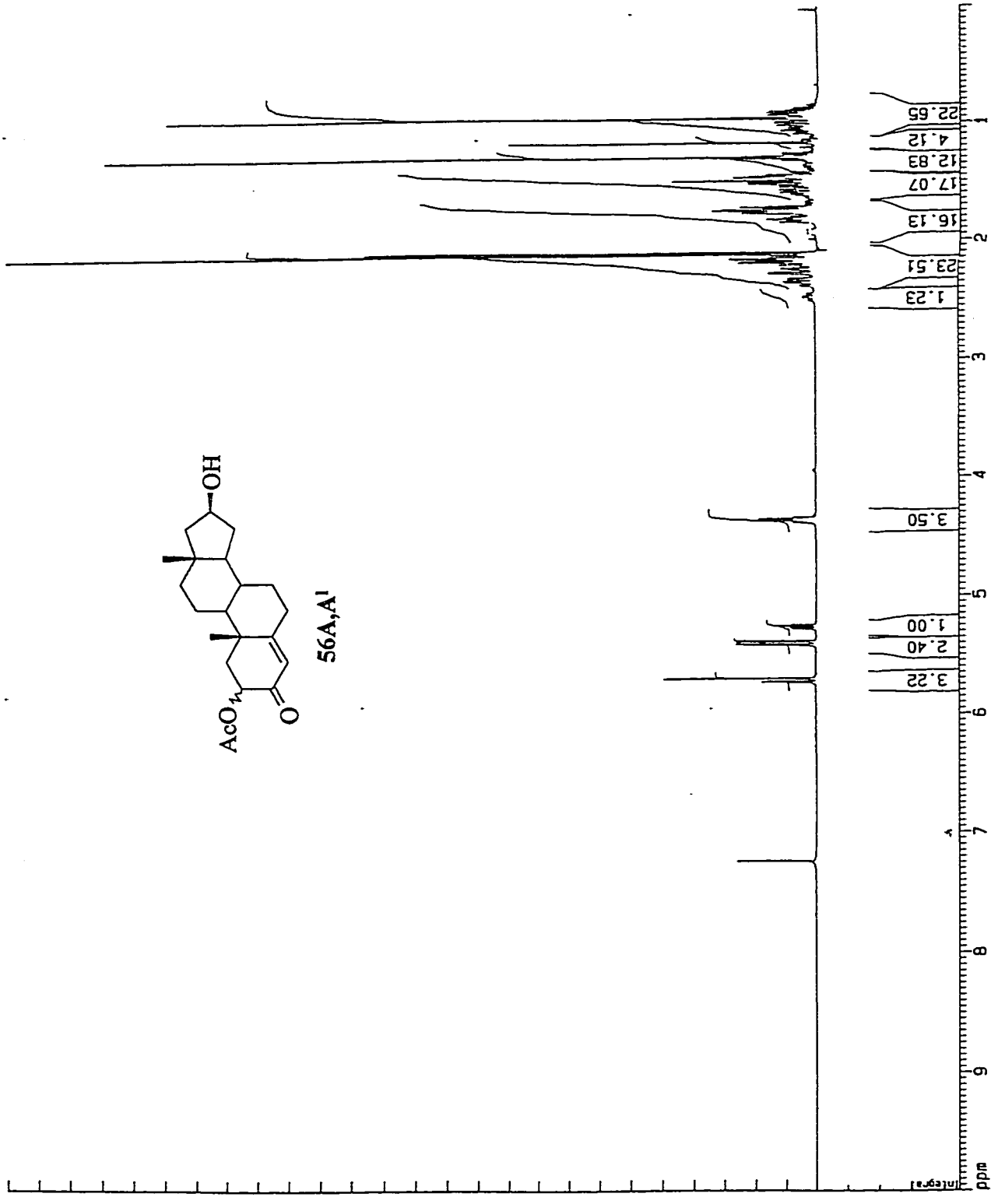


FIGURE 2.5.4: ^{1}H NMR SPECTRUM OF 2β-AND 2α-ACETOXY-16β-HYDROXYANDROST-4-EN-3-ONE (56A, 56A^1)

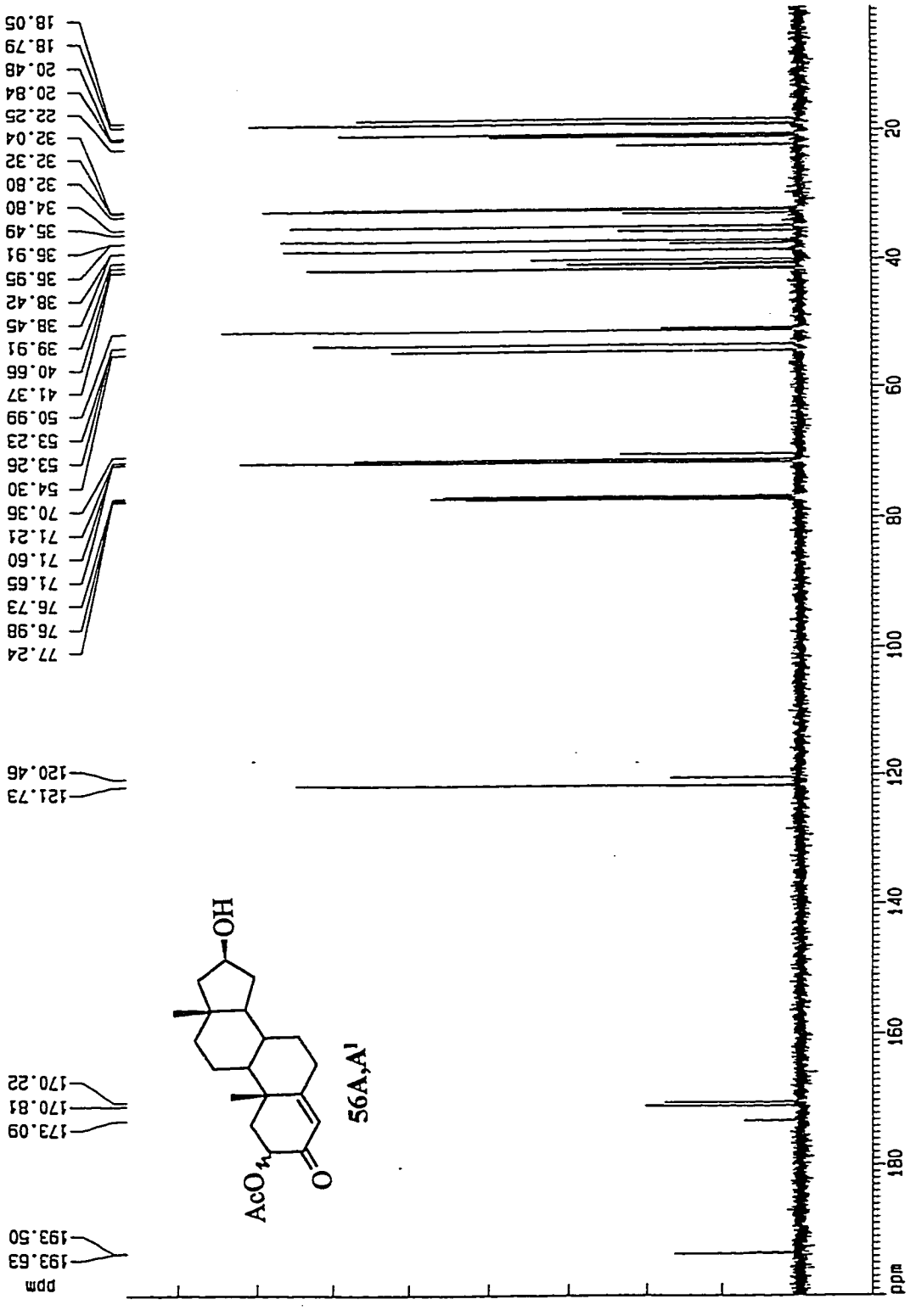


FIGURE 2.5.5: ^{13}C NMR SPECTRUM OF OF 2β-AND 2α-ACETOXY-16β-HYDROXYANDROST-4-EN-3-ONE (56A, 56A^{1})

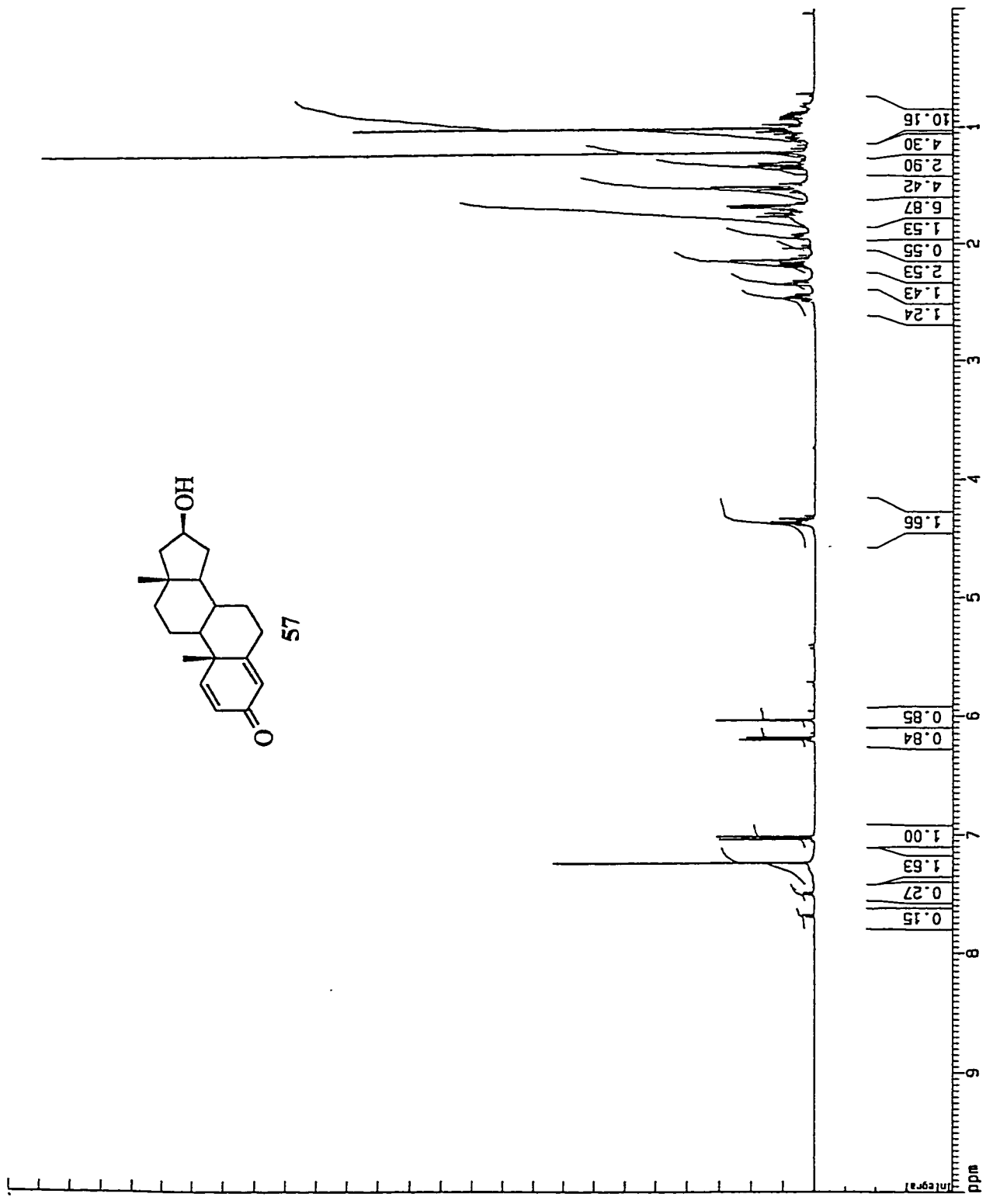


FIGURE 2.5.6: ^{1}H NMR SPECTRUM OF 16β-HYDROXYANDROSTA-1,4-DIEN-3-ONE (57)

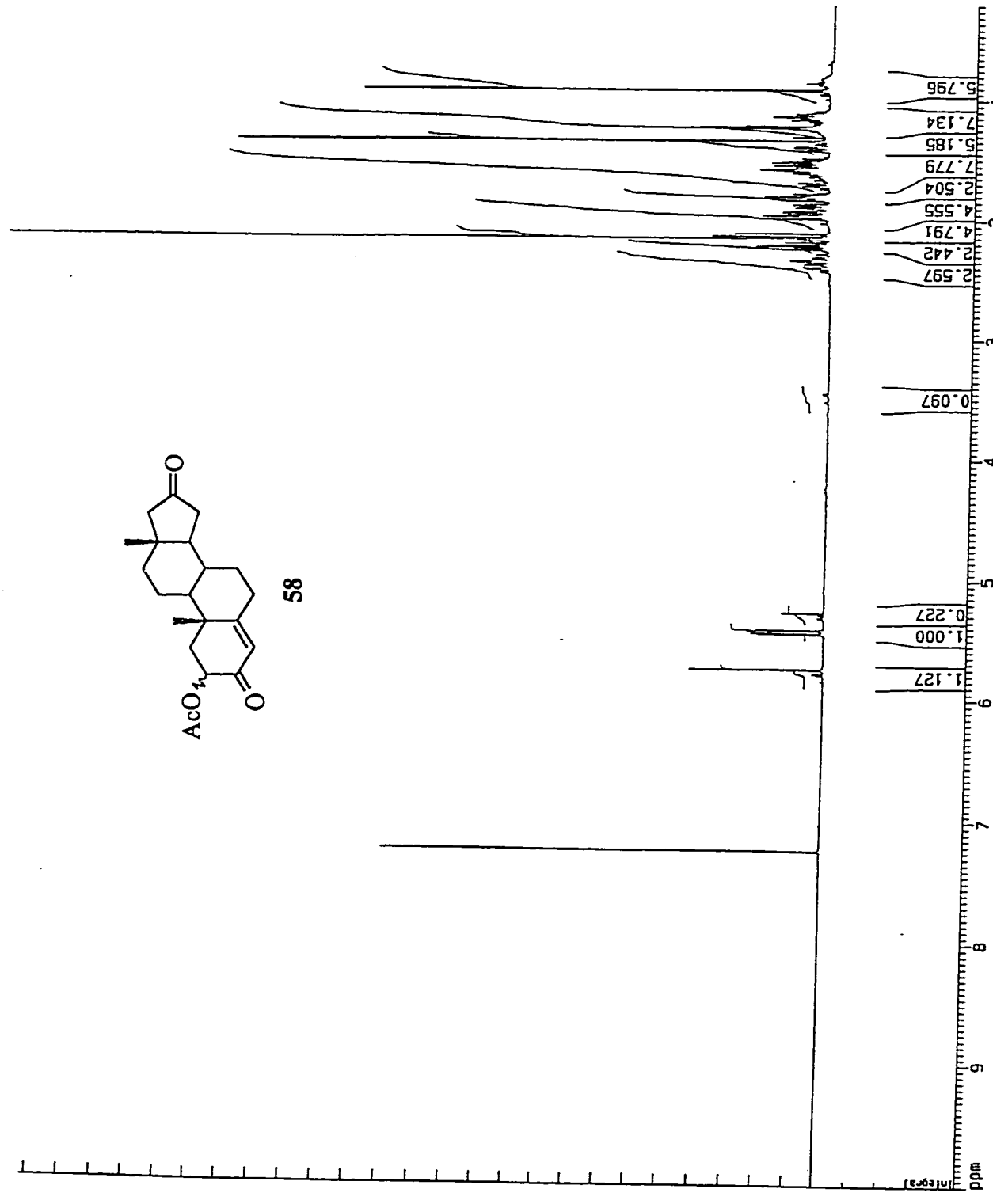


FIGURE 2.5.7: ^{1}H NMR SPECTRUM OF 2β- AND 2α-ACETOXYANDROST-4-EN-3,17-DİONE (58)

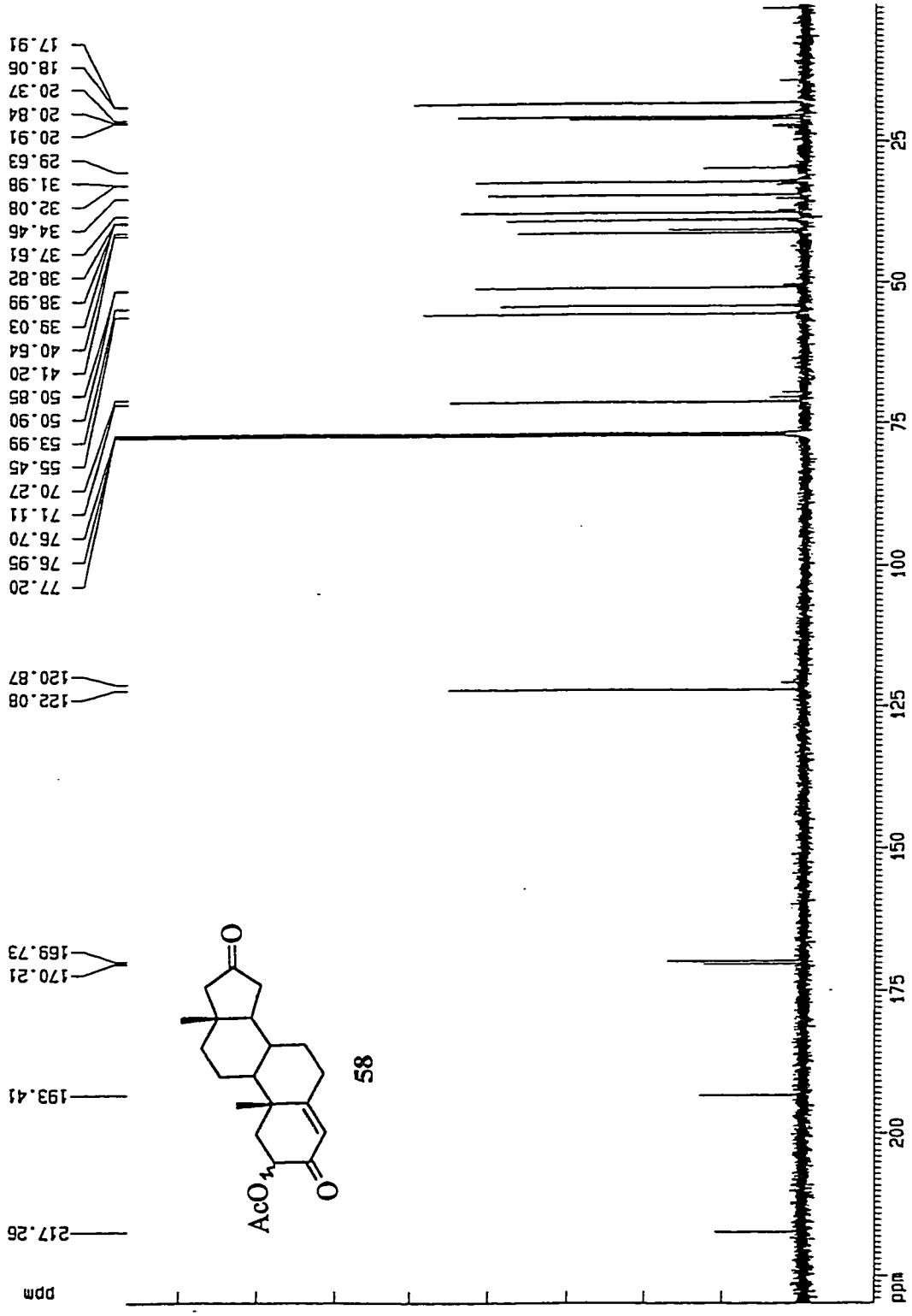


FIGURE 2.5.8: ^{13}C NMR SPECTRUM OF 2β- AND 2α-ACETOXYANDROST-4-EN-3,17-DIONE (58)

FIGURE 2.5.9: ^{1}H NMR SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIEN-3,16-DIONE (1)

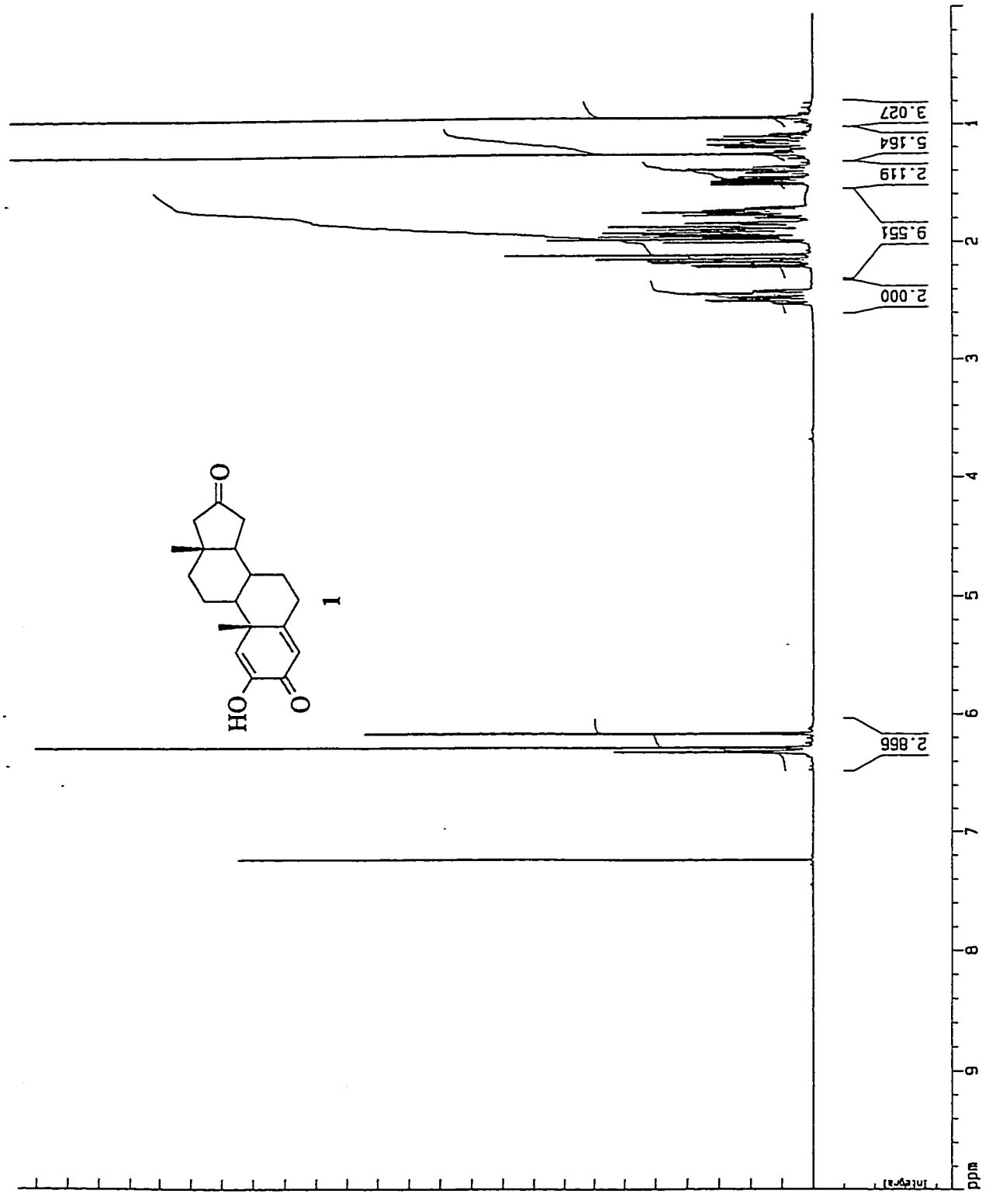


FIGURE 2.5.10: ^{1}H NMR SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIEN-3,16-DIONE (1) ISOLATED FROM *TRICHILIA HIRTA*

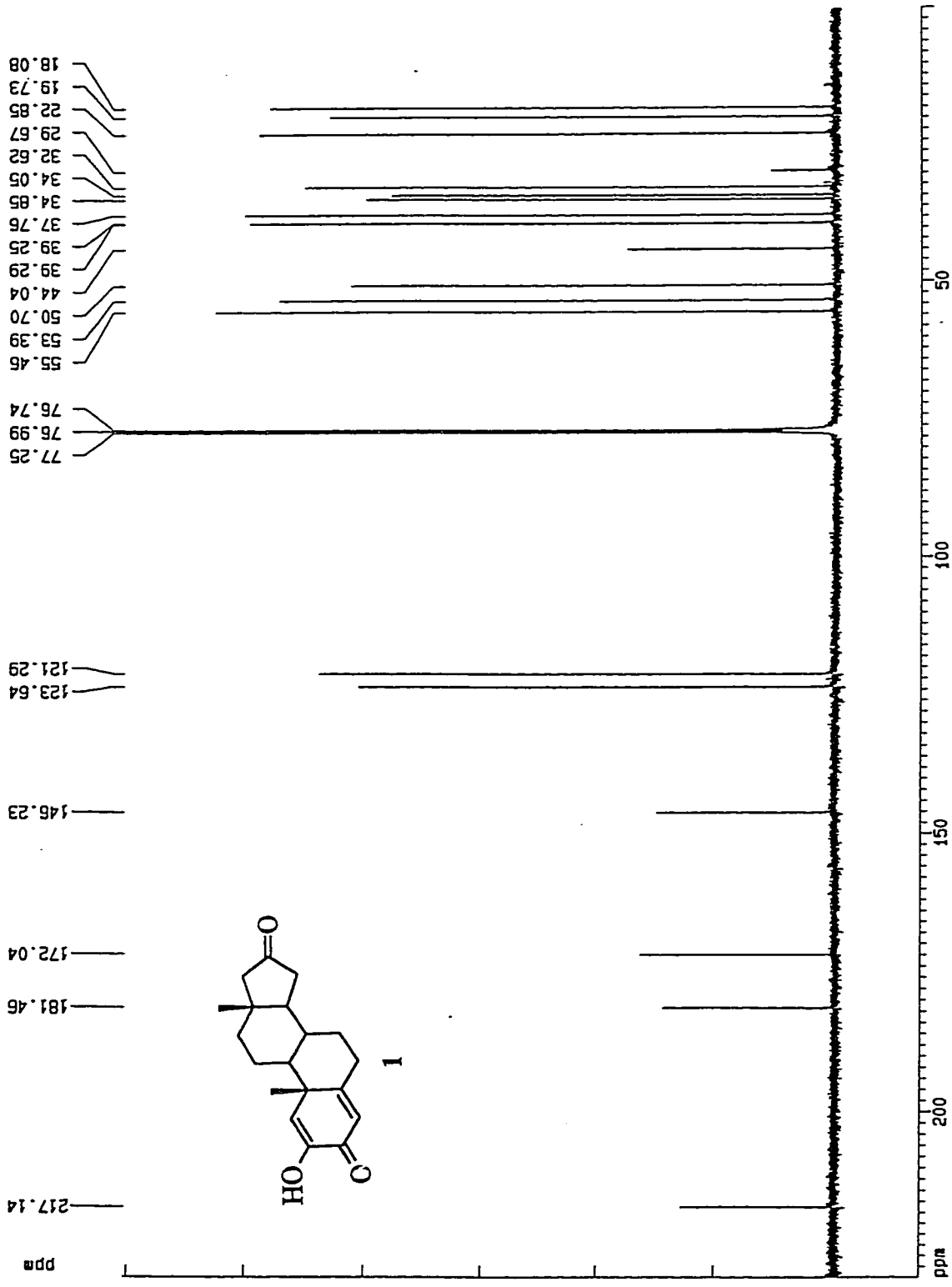


FIGURE 2.5.11: ^{13}C NMR SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIEN-3,16-DIONE (1)

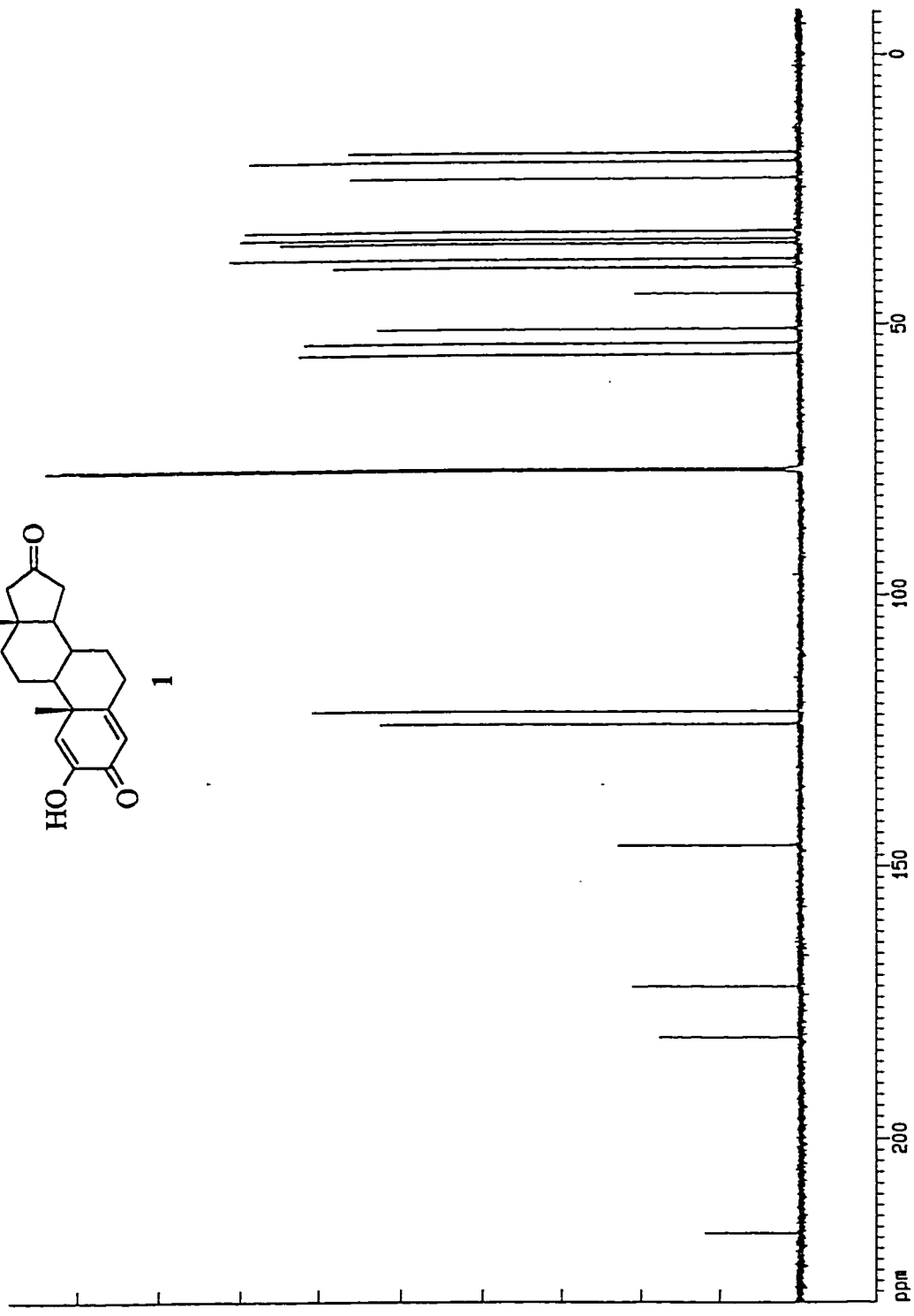


FIGURE 2.5.12: ^{13}C NMR SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIEN-3,16-DIONE (1) ISOLATED FROM *TRICHILIA HIRTA*

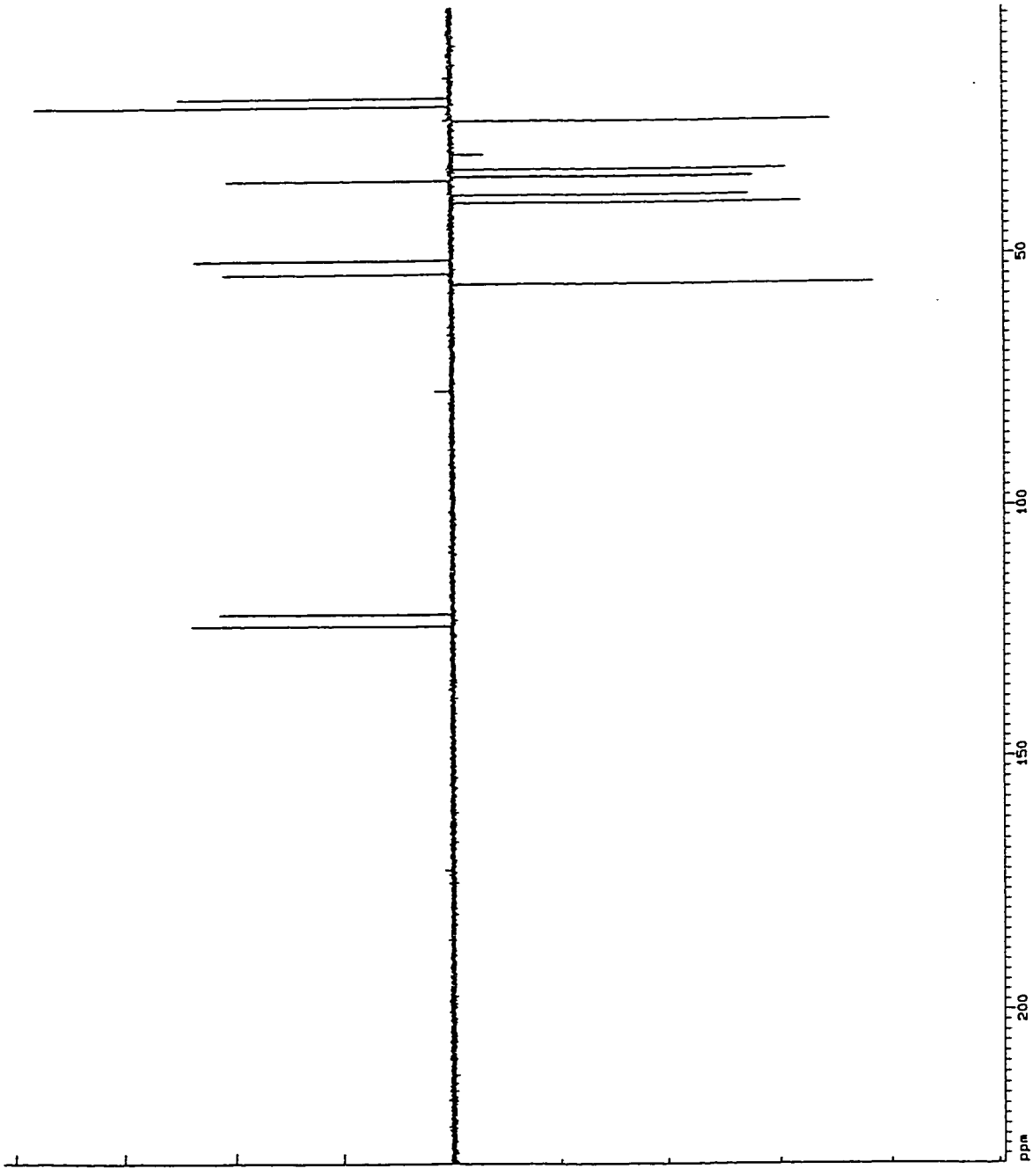


FIGURE 2.5.13: DEPT SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIEN-3,16-DIONE (1)

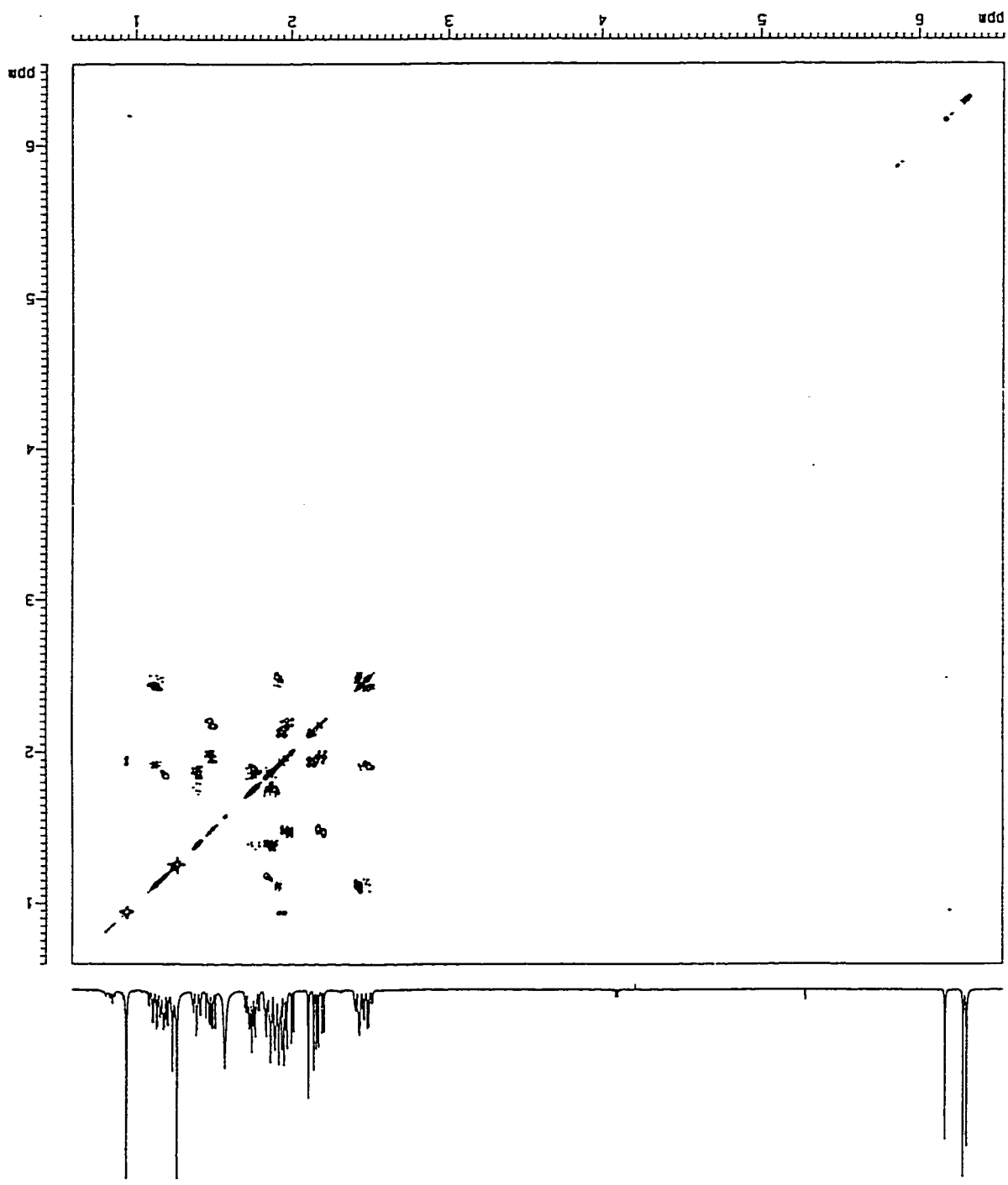


FIGURE 2.5.14: ^{1}H-^{1}H CORRELATION SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIEN-3,16-DIONE (1)

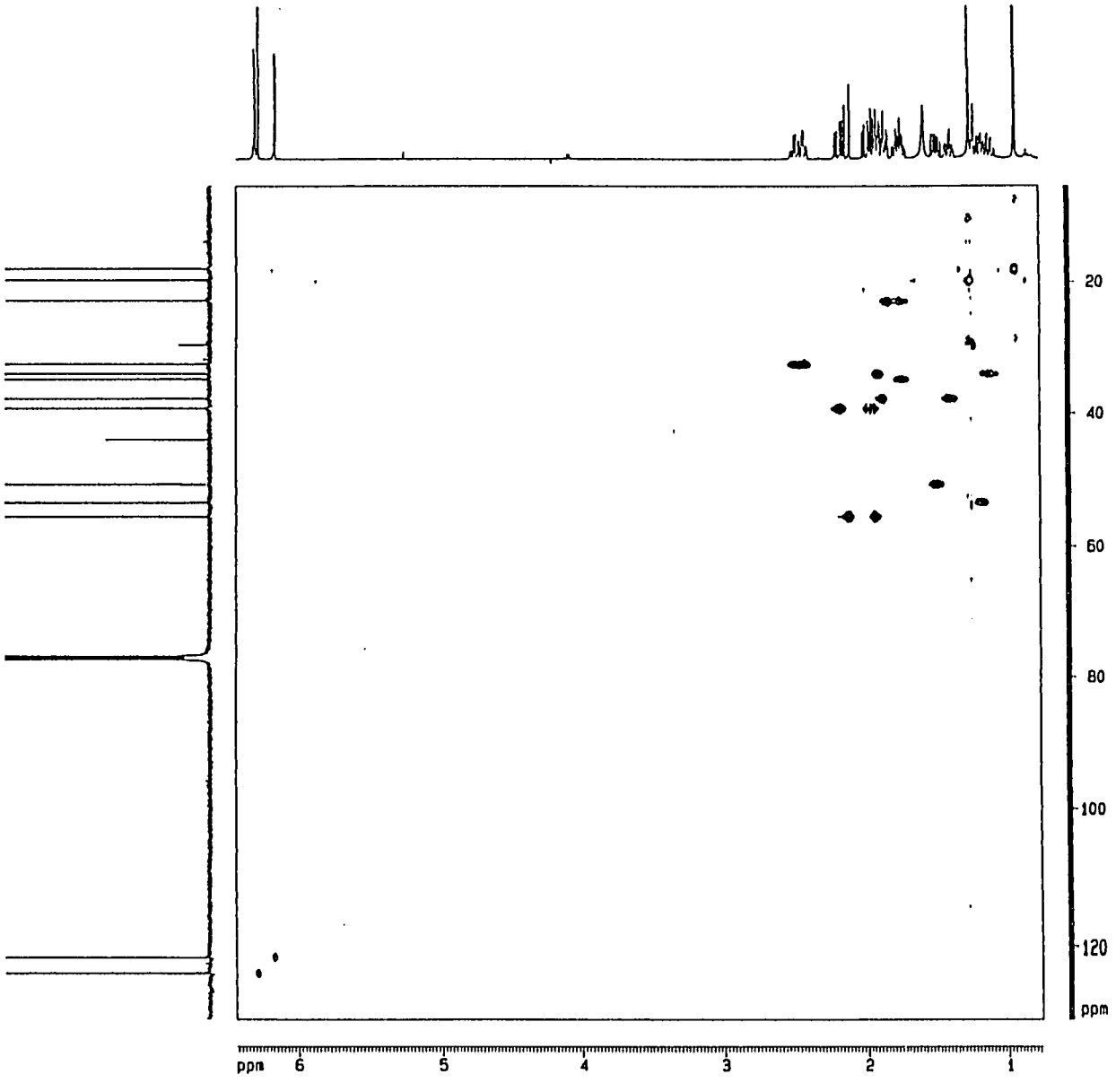


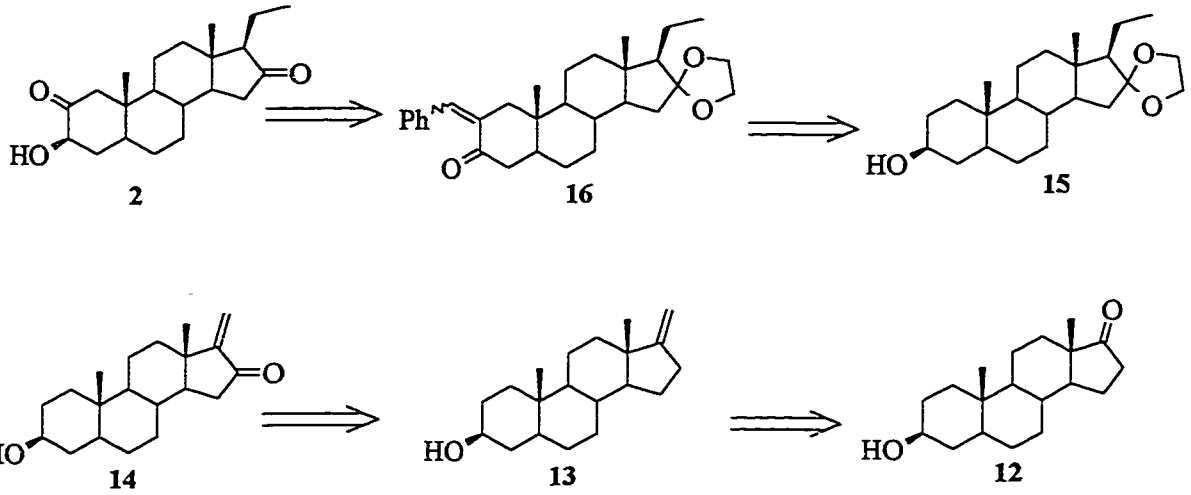
FIGURE 2.5.15: ^{1}H-^{13}C CORRELATION SPECTRUM OF 2-HYDROXYANDROSTA-1,4-DIEN-3,16-DIONE (1)

Chapter 3

The Partial Synthesis of 3-hydroxypregna-2,16-dione 2.

3.1 Transformation of ring D of isoandrosterone 12.

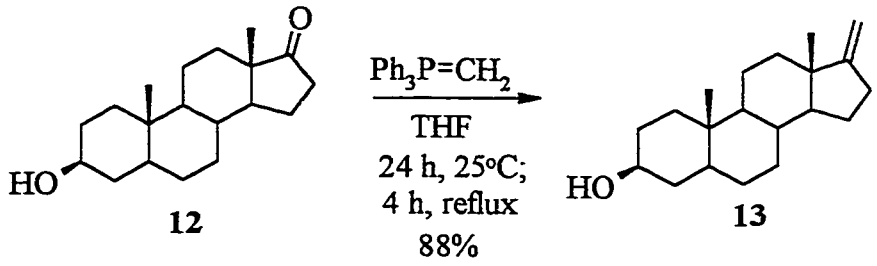
Recalling the retrosynthetic scheme 1.3.1 which has been reproduced below, the strategy proposed for the synthesis of 3-hydroxypregna-2,16-dione **2** or trichiliasterone B was to first transform ring D of isoandrosterone **12**, followed by the transformation of



ring A. The first step towards trichiliasterone B was to generate an exocyclic double bond at C-17 of isoandrosterone **12** by means of the Wittig reaction. Wittig methylenation of isoandrosterone **12** has been previously reported by Sondheimer and Mechoulam.[49] They obtained 3β-hydroxyandrost-17(20)-ene **13** in 58% yield, using a five-fold excess of methylenetriphenylphosphorane. The yield of **13** was only 32% when three moles of the ylide per mole of **12** was employed.

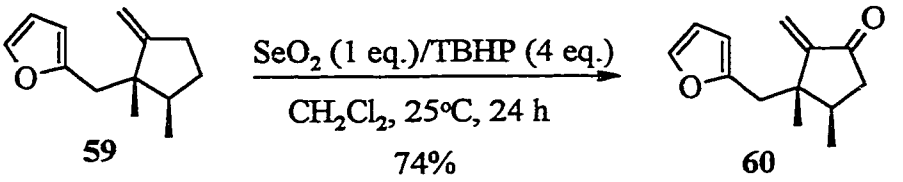
In our hands, the use of a five molar equivalents of methylenetriphenyl-phosphorane per isoandrosterone **12** afforded 3β-hydroxyandrost-17(20)-ene **13** in 88%

Scheme-3.1.1-Methylenation of isoandrosterone **12**.



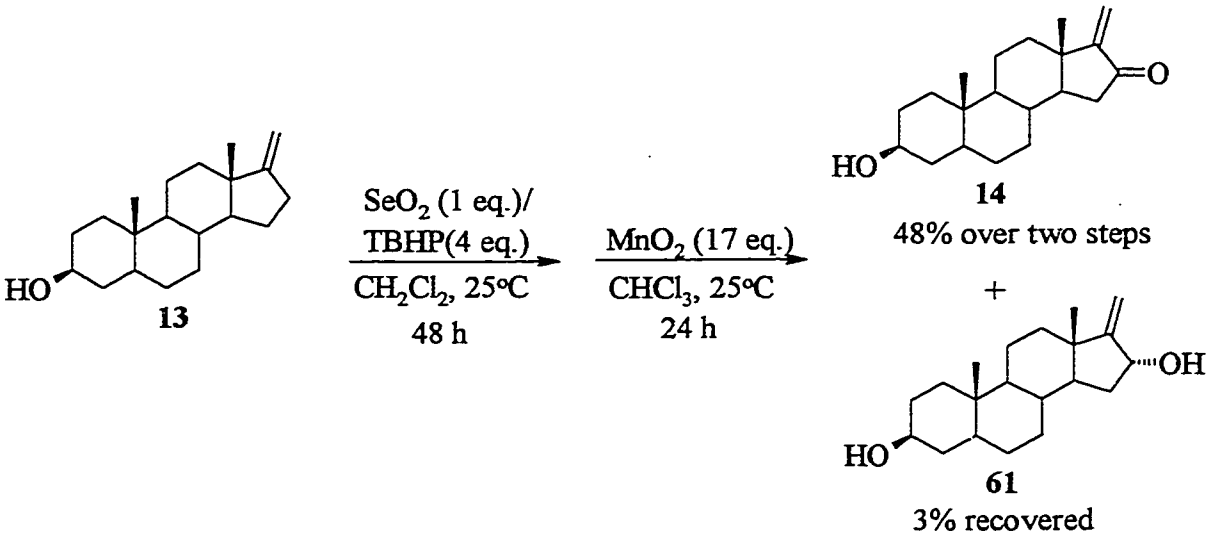
yield. The ^{13}C NMR spectrum (see figure 3.1.2-chp. 3 appendix) of **13** showed two olefinic carbons; one quaternary at δ 161.92 (C-17) and a methylene carbon at δ 100.56 (C-20). The ^{1}H NMR spectrum (figure 3.1.1-chp. 3 appendix) supported the ^{13}C NMR spectrum because it showed a multiplet at δ 4.58 which corresponded to the two olefin hydrogens attached to C-20. The melting point of the white crystalline solid was 144-145°C and matched exactly the previously reported value.[49]

With steroid **13** in hand, the next step was to oxidize the allylic carbon-hydrogen bonds of C-16 to afford the α,β-unsaturated enone **14** (see scheme 3.1.2). Selenium(IV) oxide in combination with *t*-butyl hydroperoxide (TBHP) over longer reaction times, has proven to be an effective system for oxidizing exocyclic double bonds to the corresponding α,β-unsaturated ketone or aldehyde.[50] For example, allylic oxidation of the exocyclic double bond of **59** with SeO_2-TBHP over 24 hours afforded the enone **60** in 74% yield.[51]



Although 3β-hydroxyandrost-17(20)-en-16-one **14** was obtained when **13** was reacted with selenium (IV)-TBHP, over oxidation to **14** was not complete and 3β,16α-dihydroxyandrost-17(20)-ene **61** accompanied **14**. Consequently, the crude mixture was stirred with manganese dioxide (MnO_2) for 24 hours at room temperature to further oxidize[52] the allylic alcohol of steroid **61** to give the enone **14** (see scheme 3.1.2). After column chromatography, enone **14** was obtained as a white solid with a melting point of 148-150° C in 48% yield over the two steps. The allylic alcohol **61** was obtained in 3% yield as white solid with a melting point of 204-205°C.

Scheme 3.1.2-Allylic Oxidation of 3β-hydroxyandrost-17(20)-ene **13**.



The ^{13}C NMR spectrum of **14** (see figure 3.1.4-chp. 3 appendix) showed a quaternary resonance at δ 207.02 (C-16), and two olefinic resonances at δ 156.85 and

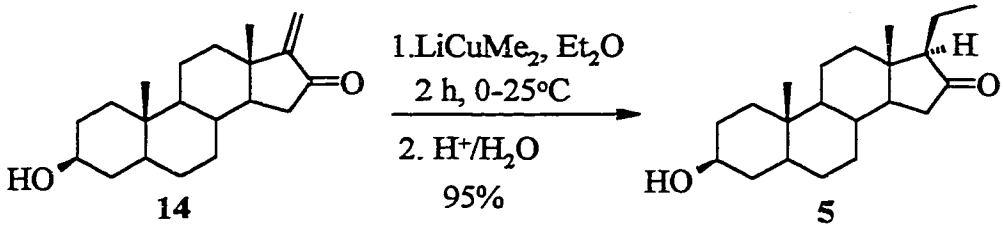
111.88 which corresponded to C-17 and C-20 respectively. The ^{1}H NMR spectrum (figure 3.1.3-chp. 3 appendix) showed olefinic proton singlets at δ 5.77 and 4.98 corresponding to H-20 and the resonanace for H-3 occurred at δ 3.58 as a multiplet. The hydroxyl stretch in the IR spectrum occurred at 3393 cm^{-1} and the stretch corresponding to the five-membered ring α,β-unsaturated ketone at C-16 was at 1725cm^{-1}. The HRMS of **14** showed a M^+ ion at *m/z* 302.2238, which was similar to the calculated value of 302.2247.

The M^+ ion for diol **61** was observed at *m/z* 304.2402, confirming the molecular formula of $C_{20}H_{32}O_2$. The IR spectrum showed two hydroxyl stretches at 3413 cm^{-1} and 3190 cm^{-1} in addition to the C=C stretch at 1656 cm^{-1}. The ^{1}H NMR spectrum of **61** (see figure 3.1.5-chp. 3 appendix) showed two olefinic singlets at δ 5.01 and 4.84 which corresponded to the C-20 methylene hydrogens. The two carbinol hydrogens at C-3 and C-16 were assigned the following resonances: δ 4.63 (broad s, H-16) and 3.58 (m, H-3). These assignments were based on the ^{1}H-^{1}H connectivity seen between the broad singlet at δ 4.63 (H-16) and the olefinic protons at δ 5.01 and 4.84.

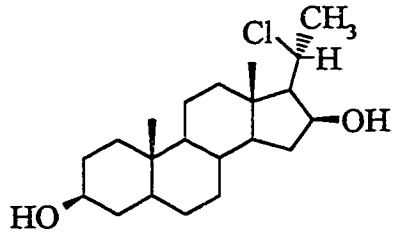
The stereochemistry of the C-16 hydroxyl group of **61** was expected to be α on the basis of a more hindered approach of the SeO_2 to the β-face of ring D due to the C-18 methyl substituent. This assignment was confirmed as α by an nOe difference experiment, in which irradiation of H-16 at δ 4.63 led to a 1.2% signal enhancement for the C-18 methyl group at δ 0.76. The nOe indicated that the C-18 methyl group and H-16 were on the same side of the ring in **61**.

It was expected that 1,4-conjugate addition of lithium dimethylcuprate ($LiCuMe_2$) to the enone functionality of **14** would result in the C-17 ethyl moiety (see scheme 3.1.3). Steroid **14** reacted with 2.3 molar equivalents of $LiCuMe_2$[53] in diethyl ether at 0°C with subsequent quenching of the enolate to give 3β-hydroxypregnan-16-one **5** in 95% yield.

Scheme 3.1.3-The Synthesis of the Naturally Occurring 3β-hydroxypregnan-16-one **5**.



Interestingly, this represents the synthesis of the 16-keto plant steroid **5** presented in figure 1.1.5 which is different from the synthesis previously reported.[9b] Our synthesis of **5** required four discrete steps beginning with isoadrosterone **12** and occurred in 40% overall yield. The synthesis of **5** by Adam and Schreiber[9b] was accomplished in two steps in 42% overall yield from (20*S*)-20-chloro-3β,16β-dihydroxypregnane (shown below).



(20S)-20-chloro-3β,16β-dihydroxypregnane

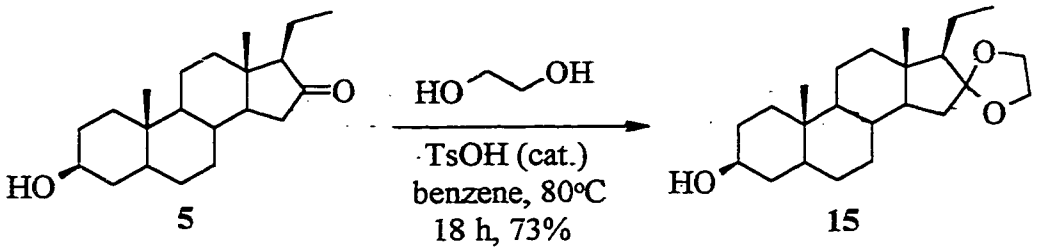
The ^{1}H NMR spectrum of **5** (see figure 3.1.7-chp.3 appendix) closely matched the chemical shifts and multiplicities that were previously seen with the natural product.[9a] Specifically, the multiplet at δ 3.57 corresponded to the carbinol H-3, and the ethyl (CH_3) group resonated as a triplet at δ 1.00 (J=7.5 Hz). The ^{13}C NMR spectrum (figure 3.18-chp. 3 appendix) of **5** which was not previously reported showed a quaternary carbon at δ 219.64 (C-16), a methine carbon at δ 71.11 (C-3) and the three methyl carbons at δ 13.41, 13.38 and 12.27. The mass spectrum had an identical fragmentation pattern to the natural sample and the M^+ ion in the HRMS was at *m/z* 318.2537 which was comparable to the calculated value 318.2560. The IR spectrum of **5** showed the hydroxyl stretch at 3338 cm^{-1} and the five-membered ring ketone stretch at 1739 cm^{-1}. The melting point of 152-153°C obtained for the white solid was identical to that reported previously.[9]

The β-configuration of the C-17 ethyl group was proven by an nOe difference experiment. When the hydrogens of the C-18 methyl (δ 0.66, s) were irradiated, there was no enhancement in the signal for the doublet of doublets corresponding to H-17 at δ 1.86. Thus, H-17 and the C-18 methyl hydrogens must be located on opposite sides of ring D. The selective irradiation of H-17 to see if there was no nOe with the H-18 methyl could not be carried out because the chemical shift of H-17 was close to the multiplet at δ 1.84-1.46.

With the pregnanone skeleton firmly established, the final step was to protect the 16-oxo functionality as a ketal (see scheme 3.1.4). The 16-oxo functionality of **5** was smoothly converted to the ethylene ketal derivative **15** by refluxing **5** and ethylene glycol with a catalytic amount of *p*-toluenesulfonic acid in benzene for 18 hours. A white solid

was obtained in 73% yield with a melting point of 159-161°C. The ^{13}C NMR spectrum of **15** (see figure 3.1.10-chp. 3 appendix) indicated that the C-16 carbonyl signal of **5** at δ 219.64 (see figure 3.1.8-chp. 3 appendix) was replaced with the quaternary C-16 signal at

Scheme 3.1.4-Preparation of 3β-hydroxy-16-ethylenedioxypregnane **15**.



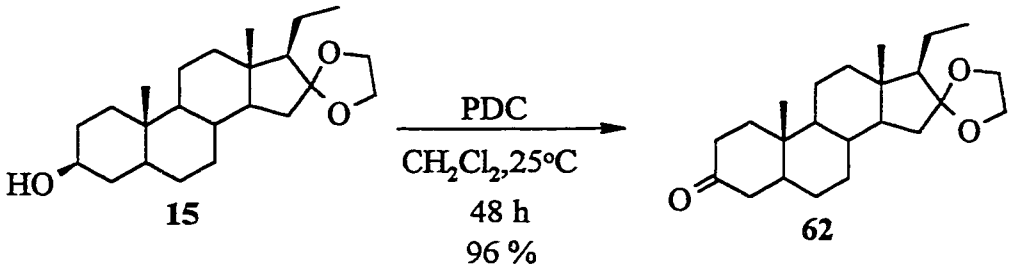
δ 117.51. Also, the ^{1}H NMR spectrum of **15** showed the resonances of the ethylene ketal moiety as a series of multiplets at δ 3.91-3.85, 3.84-3.79, and 3.73-3.66 which collectively integrated to four hydrogens. At this point, work on ring D was complete and our attention turned to the transformation of ring A.

3.2 Transformation of Ring A towards the Synthesis of 3β-hydroxypregna-2,16-dione 2.

The sixth step of the proposed ten step synthesis of trichiliasterone B, **2** was the oxidation of the 3β-hydroxyl group of steroid **15**. The oxidizing agent used was PDC and 16-ethylenedioxypregnan-3-one **62** was obtained in 96% yield as a white crystalline solid with a melting point of 149-151°C (see scheme 3.2.1). The ^{13}C NMR spectrum (see figure 3.2.2-chp.3 appendix) showed the C-3 carbonyl carbon at δ 211.89 and C-16 at δ 117.44.

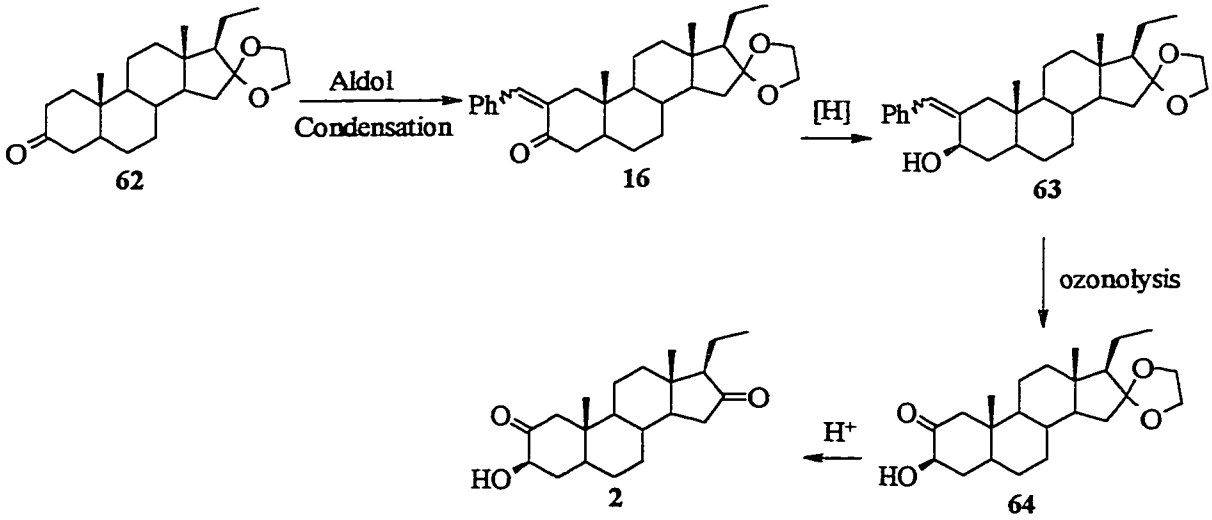
The IR spectrum of **62** showed a C=O stretch at 1701 cm^{-1} and four strong bands at 1171.81, 1155.15, 1092.61, and 1024.56 which was due to the ketal group. The calculated molecular ion for **62** was 360.2666 and the HRMS showed a M^+ ion at *m/z* 360.2679.

Scheme 3.2.1-Oxidation of 3β-hydroxy-16-ethylenedioxypregnane **15**.

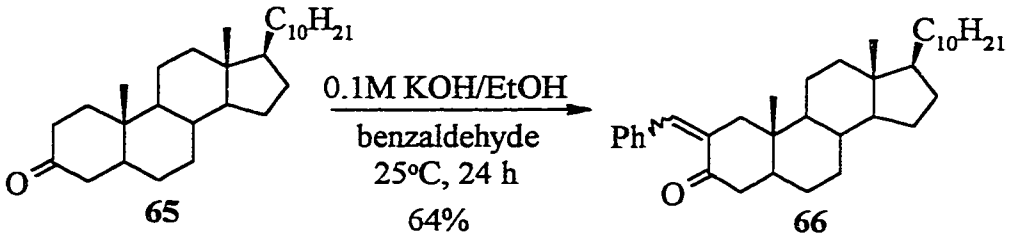


According to scheme 3.2.2, the final four steps of the synthesis of trichiliasterone B required an aldol condensation of **62** with benzaldehyde to afford the benzylidene derivative **16**, reduction of the 3-oxo group, ozonolysis of the exocyclic double bond at C-

Scheme 3.2.2-The Four Final Steps of the Total Synthesis of 3-hydroxypregna-2,16-dione **2**.



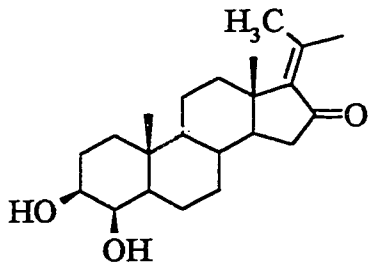
2 and finally, hydrolysis of the C-16 ketal. Initial attempts at carrying out the aldol condensation as planned were not successful.. It was anticipated that condensation would occur at C-2 because C-3-oxo-steroids preferentially enolize at C-2.[54] Barton and co-workers reported that stigmastanone **65** was smoothly converted to its benzylidene



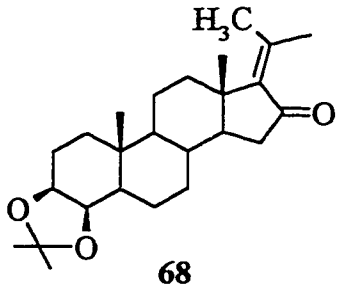
65 **66**

derivative **66** by stirring **65** with excess ethanolic potassium hydroxide and four molar equivalents of benzaldehyde in the dark at room temperature for 24 hours.[55] However, the same reaction conditions with steroid **62,** gave a complex mixture from which none of the desired benzylidene derivative **16** was isolated. Time did not permit variation of the benzaldehyde to steroid **62** ratio, nor careful formation of the Δ-2 enolate with LDA followed by trapping with benzaldehyde. Since 2 g of **62** are available, it is anticipated that another researcher in the group could readily complete the synthesis of trichiliasterone B.

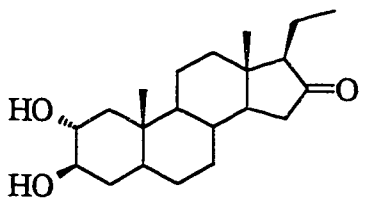
During the writing of this thesis, we came across a report by McLaughlin and co-workers[57] describing the isolation of steroids **67**, **68** and **69** from the root bark of *Melia*



67



68



69

volkensii which occurs in Kenya, Africa. All three compounds showed interesting biological activity, including insecticidal activity against yellow fever mosquito larvae (*Aedes aegypti*). Compounds **67** and **68** were active against six different human tumor cell lines. In particular, **68** showed activity equivalent to andriamycin against the PC-3 prostate cell line. In contrast, the C-17 ethyl derivative **69** was weakly active, showing only marginal selectivity for the MCF-7 (breast) cell line. We speculate that the activity of **67** and **68** may be due to the α,β-unsaturated enone system in ring D. The partial synthesis of trichiliasterone B, **2** could possibly be used towards the syntheses of **67** and **68**, as well as derivatives which could then be tested for anti-cancer activity. Needless to say, testing of the structurally similar intermediates obtained during the partial synthesis of **2** will hopefully be carried out.

3.3 Anti-feedant testing of 2-hydroxyandrosta-1,4-diene-3,16-dione 1.

Since the synthesis of trichiliasterone B **2** has not been completed, only trichiliasterone A (2-hydroxyandrosta-1,4-diene-3,16-dione) **1** has been tested for insect growth inhibition. The testing of steroid **1** followed the method described by Arnason and co-workers[57] and it was tested at two concentrations; 10 ppm and 100 ppm. The larvae of European corn borer, *Ostrinia nubilalis* were placed individually in glass vials containing a cube of artificial diet containing 10 or 100 ppm concentration of **1**. The larvae were weighed every four days and larval weight gains and mortality were recorded. After 40 days, no significant growth reduction in the insects was observed at either concentration

level.[58] Thus, 2-hydroxyandrosta-1,4-diene-3,16-dione **1** did not elicit anti-feedant activity in the European corn borer.

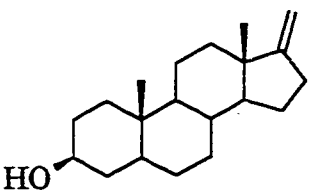
Experimental for Chapter 3

3.4 EXPERIMENTAL

GENERAL: See experimental section 2.7 for general procedures.

PREPARATION OF 3β-HYDROXYANDROST-17(20)-ENE (13).

A 2.2 M solution of *n*-butyllithium in hexane (3.80 mL, 8.00 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (2.95 g, 8.00 mmol) in dry tetrahydrofuran (45 mL) at 0°C. The reaction mixture was stirred for 1.5 h, a solution of isoandrosterone **12** (480 mg, 1.65 mmol) in dry tetrahydrofuran (30 mL) was added dropwise followed by three tetrahydrofuran washes (17 mL) to ensure the steroid was added. The reaction mixture was allowed to stir overnight at room temperature and at reflux for 4 h. The mixture was cooled to room temperature, poured into water (40 mL) and extracted with ether (2 x 60 mL). The combined extracts were washed with water (20 mL), 5% aqueous hydrochloric acid (20 mL), water (20 mL), dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated *in vacuo* affording a yellow-off white crystalline solid. Chromatography (80 g of neutral alumina, 9:1 hexanes/ethyl acetate) yielded **13** (418 mg, 88%) as a white solid.



13

$C_{20}H_{32}O$ MW=288.48 g/mole

mp 144-145°C

MS [EI, *m/z* (%)]: 288 [MH^+] (75), 273 (93), 41 (100).

HRMS calcd. for $C_{20}H_{32}O$: 288.2454; found: 288.2449.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3313 (OH), 1655 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 4.59 -4.57 (m, 2H, H-20), 3.55 (septet, J=4.9 Hz, 1H, H-3), 3.44 (brs, 1H, 3-OH), 2.47-2.41 (m, 1H, H-16), 2.20-2.17 (H-16), 1.78-1.51 (m, 8H), 1.42-1.14 (m, 8H), 1.11-0.87 (m, 2H), 0.79 (s, 3H, H-19), 0.73 (s, 3H, H-18), 0.74-0.60 (m, 2H).

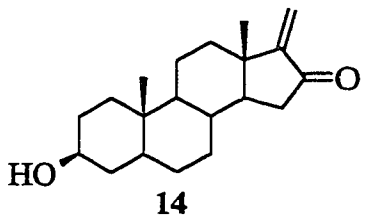
^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 161.92 (C-17), 100.56 (C-20), 71.21 (C-3), 54.54, 54.40, 50.65, 44.87, 44.07, 38.12, 36.98, 35.68, 35.56, 35.42, 31.86, 31.44, 29.34, 28.61, 21.08, 18.47, 12.28.

PREPARATION OF 3β-HYDROXYANDROST-17(20)-EN-16-ONE (14) AND 3β,16α-DIHYDROXYANDROST-17(20)-ENE(61).

t-Butyl hydroperoxide (70%, 0.800 mL, 7.77 mmol) was added to a suspension of selenium dioxide (151 mg, 1.34 mmol) in methylene chloride (10 mL) at room temperature. The reaction mixture was stirred for 30 min, a solution of 3β-hydroxyandrost-17(20)-ene **13** (393 mg, 1.36 mmol) in methylene chloride (5.0 mL and 3 x 1 mL washes) was added and the reaction mixture was stirred for 48 h. Benzene (10 mL) was added to the solution and methylene chloride and benzene-water azeotrope were removed *in vacuo*. Methylene chloride (100 mL) was added and washed with 10%

aqueous potassium hydroxide (4 x 20 mL) and brine (1 x 20 mL). The solvent was removed *in vacuo* affording a white residue. To destroy excess *t*-butyl hydroperoxide the residue was dissolved in cold acetic acid (1.5 mL) and dimethyl sulfide (3.0 mL). The reaction mixture was stirred for 4 h at room temperature, cooled to 0°C and 10% aqueous potassium carbonate (3 to 4 mL) was added to neutralize the cloudy solution. The mixture was diluted with ethyl acetate (50 mL) and the layers were separated. The organic layer was washed with water (2 x 20 mL) and brine (20 mL), dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated *in vacuo* affording a mixture of **14** and **61** as a white solid (338 mg).

The white solid was dissolved in chloroform (80 mL) to which was added activated manganese dioxide (1.60 g, 18.4 mmol) at room temperature. The reaction mixture was stirred for 24 h before being filtered through a plug of celite within a sintered glass funnel. The dark celite cake was washed with hot chloroform (100 mL) and the solvent was removed *in vacuo* affording a yellow solid. Flash chromatography (18 g of silica gel, 5:1 hexanes/ethyl acetate) yielded **14** (199 mg, 48%) as a white solid and **61** (11 mg, 3%) as a white solid.



14

$C_{20}H_{30}O_2$ MW=302.46 g/mole

mp 148-150°C

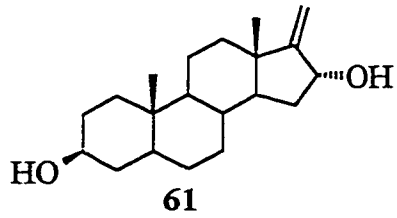
MS [EI, *m/z* (%)]: 302 [MH^+] (32), 284 (100), 269 (37).

HRMS calcd. for $C_{20}H_{30}O_2$: 302.2247; found: 302.2238.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3393 (OH), 1725 (C=O), 1645 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 5.77 (s, 1H, H-20), 4.98 (s, 1H, H-20), 3.58 (m, 1H, H-3), 2.30-1.98 (m, 2H), 1.93-1.90 (m, 1H), 1.83-1.79 (m, 1H), 1.77-1.54 (m, 6H), 1.49-1.21 (m, 8H), 1.17-1.09 (m, 1H), 1.01-0.93 (m, 1H), 0.93 (s, 3H, H-19), 0.89-0.76 (m, 1H), 0.84 (s, 3H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 207.02 (C-16), 156.85 (C-17), 111.88 (C-20), 71.16 (C-3), 54.45, 49.43, 44.85, 42.88, 38.09, 38.01, 36.73, 35.76, 35.36, 34.50, 31.99, 31.42, 28.42, 20.77, 19.16, 12.33.



61

$C_{20}H_{32}O_2$ MW=304.48 g/mole

mp 204-205°C

MS [EI, *m/z* (%)]: 304 [MH^+] (20), 286 (18), 69 (100).

HRMS calcd. for $C_{20}H_{32}O_2$: 304.2404; found: 304.2402.

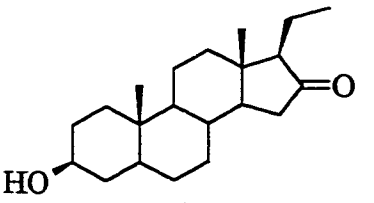
IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3413 (OH), 3190 (OH), 1656 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 5.01 (s, 1H, H-20), 4.84 (s, 1H, H-20), 4.63 (brs, 1H, H-16), 3.58 (m, 1H, H-3), 1.81-1.54 (m, 6H), 1.43-1.21 (m, 12H), 1.13-0.85 (m, 3H), 0.81 (s, 3H, H-19), 0.76 (s, 3H, H-18), 0.72-0.67 (m, 1H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 166.14 (C-17), 103.55 (C-20), 72.68 (C-3 or C-16), 71.19 (C-3 or C-16), 54.61, 51.43, 44.85, 44.48, 38.13, 36.89, 35.80, 35.59, 35.52, 34.96, 31.74, 31.45, 28.51, 20.82, 19.05, 12.27.

PREPARATION OF 3β-HYDROXYPREGNAN-16-ONE (5).

A 1.4 M solution of methyllithium in ether (31.4 mL, 0.0439 mol) was added dropwise to a suspension of copper (I) iodide (4.18 g, 0.0220 mol) in dry ether (235 ml) at 0°C. After 2 min, an orange solution of the cuprate was formed and a solution of 3β-hydroxyandrost-17(20)-en-16-one **14** (2.85 g, 0.00942 mol) in dry ether (300 ml and 4 x 25 mL washes) was added dropwise over 30 min. The reaction mixture was warmed to room temperature and allowed to stir for 2 h before quenching with 0.2 M aqueous sulfuric acid (200 mL) and a saturated aqueous solution of ammonium chloride (100 mL). The reaction mixture was stirred vigorously for 1h, diluted with methylene chloride (750 mL) and water (150 mL) and the layers were separated. The aqueous layer was extracted with methylene chloride (3 x 100 mL) and the combined extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated *in vacuo* affording a green solid. Flash chromatography (111 g of silica gel, 9:1 hexanes/ethyl acetate followed by 1:1 hexanes/ethyl acetate) yielded **5** (2.84 g, 95%) as a white solid.



5

$C_{21}H_{34}O_2$ MW=318.50 g/mole

mp 152-153°C

MS [EI, *m/z* (%)]: 318 [MH^+] (68), 232 (100), 215 (51).

HRMS calcd. for $C_{21}H_{34}O_2$: 318.2560; found: 318.2537.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3338 (OH), 1739 (C=O).

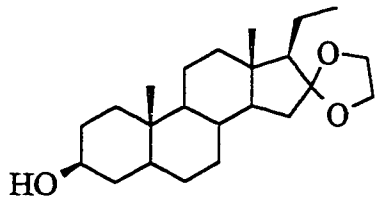
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 3.57 (m, 1H, H-3), 2.18 (dd, J=18.3, 7.5 Hz, 1H), 1.86 (dd, J=8.6, 2.8 Hz, 1H, H-17), 1.84-1.46 (m, 10H), 1.43-1.18 (m, 8H), 1.14-1.08 (m, 1H), 1.00 (t, J=7.5 Hz, 3H, H-21), 1.00-0.89 (m, 2H), 0.81 (s, 3H, H-19), 0.81-0.77 (m, 1H), 0.66 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 219.64 (C-16), 71.11 (C-3), 65.30, 54.34, 50.49, 44.79, 42.10, 38.46, 38.20, 38.04, 36.68, 35.61, 34.48, 32.20, 31.35, 28.40, 20.69, 17.55, 13.41, 13.38, 12.27 .

PREPARATION OF 3β-HYDROXY-16-ETHYLENEDIOXYPREGNANE (15).

p-Toluenesulfonic acid (5 mg) and ethylene glycol (0.46 mL, 8.25 mmol) were added to a solution of 3β-hydroxypregnan-16-one **5** (110 mg, 0.344 mmol) in dry benzene (12 mL) in a 25 mL round bottom flask equiped with a Dean-Stark trap at room

temperature. The reaction mixture was heated at 110°C (bath temperature) for 18 h before being cooled to room temperature. The opaque solution was washed with a saturated aqueous solution of sodium bicarbonate (5 mL) and water (2 x 5 mL). The combined aqueous washes were extracted with methylene chloride (2 x 10 mL) and the combined organic extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated *in vacuo* affording an off-white solid. Chromatography (11 g of neutral alumina, 9:1 hexanes/ethyl acetate followed by 2:1 hexanes/ethyl acetate) yielded **15** (91 mg, 73%) as a white solid.



15

$C_{23}H_{38}O_3$ MW=362.56 g/mole

mp 159-161°C

MS [EI, *m/z* (%)]: 362 [MH^+] (18), 113 (58), 99 (100).

HRMS calcd. for $C_{23}H_{38}O_3$: 362.2822; found: 362.2830.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3412 (OH), 1171, 1095 and 1022 (ketal C-O).

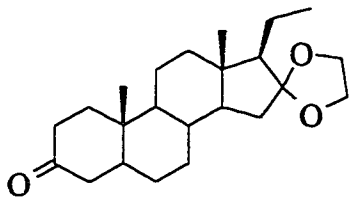
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 3.91-3.85 (m, 2H, ketal), 3.84-3.79 (m, 1H, ketal), 3.73-3.66 (m, 1H, ketal), 3.54 (septet, J=5.0 Hz, H-3), 1.81-1.62 (m, 6H), 1.58-

1.19 (m, 13H), 1.16-1.05 (m, 3H), 0.99-0.85 (m, 2H), 0.86 (t, J=7.5 Hz, 3H, H-21), 0.77 (s, 3H, H-19), 0.68 (s, 3H, H-18).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 117.51 (C-16), 71.19 (C-3), 64.81, 62.86, 60.75, 54.46, 52.11, 44.81, 42.15, 39.94, 38.64, 38.13, 36.82, 35.55, 34.64, 32.03, 31.44, 28.58, 20.75, 16.96, 13.49, 12.92, 12.86.

PREPARATION OF 16-ETHYLENEDIOXYPREGNAN-3-ONE (62).

Pyridinium dichromate (154 mg, 0.410 mmol) was added to a solution of 3β-hydroxy-16-ethylenedioxypregnane **15** (59.5 mg, 0.164 mmol) in dry methylene chloride (1.0 mL) at room temperature. The reaction mixture was stirred for 48 h, diluted with ether (2.0 mL), filtered through anhydrous magnesium sulfate and celite in a sintered glass funnel and rinsed with ether (100 mL). The product was concentrated *in vacuo* affording a white solid. Chromatography (7 g of neutral alumina, 4:1 hexanes/ethyl acetate) yielded **62** (60 mg, 96%) as a white solid.



62

$C_{23}H_{36}O_3$ MW=360.54 g/mole

mp 149-151°C

MS [EI, *m/z* (%)]: 360 [MH^+] (12), 113 (82), 99 (100).

HRMS calcd. for $C_{23}H_{36}O_3$: 360.2666; found: 360.2679.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1707 (C=O), 1172, 1155, 1092 and 1024 (ketal C-O).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 3.92-3.86 (m, 2H, ketal), 3.85-3.81 (m, 1H, ketal), 3.72-3.67 (m, 1H, ketal), 2.38-2.20 (m, 3H), 2.05 (dt, J=15.0, 3.7 Hz, 1H), 1.98 (ddd, J=13.2, 6.4, 2.1 Hz, 1H), 1.81 (dd, J=12.5, 7.0 Hz, 1H), 1.74 (dt, J=12.5, 7.0 Hz, 2H, H-20), 1.63-1.22 (m, 11H), 1.18-1.08 (m, 2H), 0.98 (s, 3H, H-19), 0.96-0.86 (m, 1H), 0.88 (t, J=7.0 Hz, 3H, H-21), 0.78 (dt, J=12.3, 4.0 Hz, 1H), 0.72 (s, 3H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 211.89 (C-3), 117.44 (C-16), 64.88, 62.93, 60.76, 53.92, 51.94, 46.61, 44.64, 42.17, 39.98, 38.54, 38.35, 38.16, 35.75, 34.56, 31.69, 28.83, 20.96, 16.98, 13.50, 12.94, 11.45.

Appendix for Chapter 3

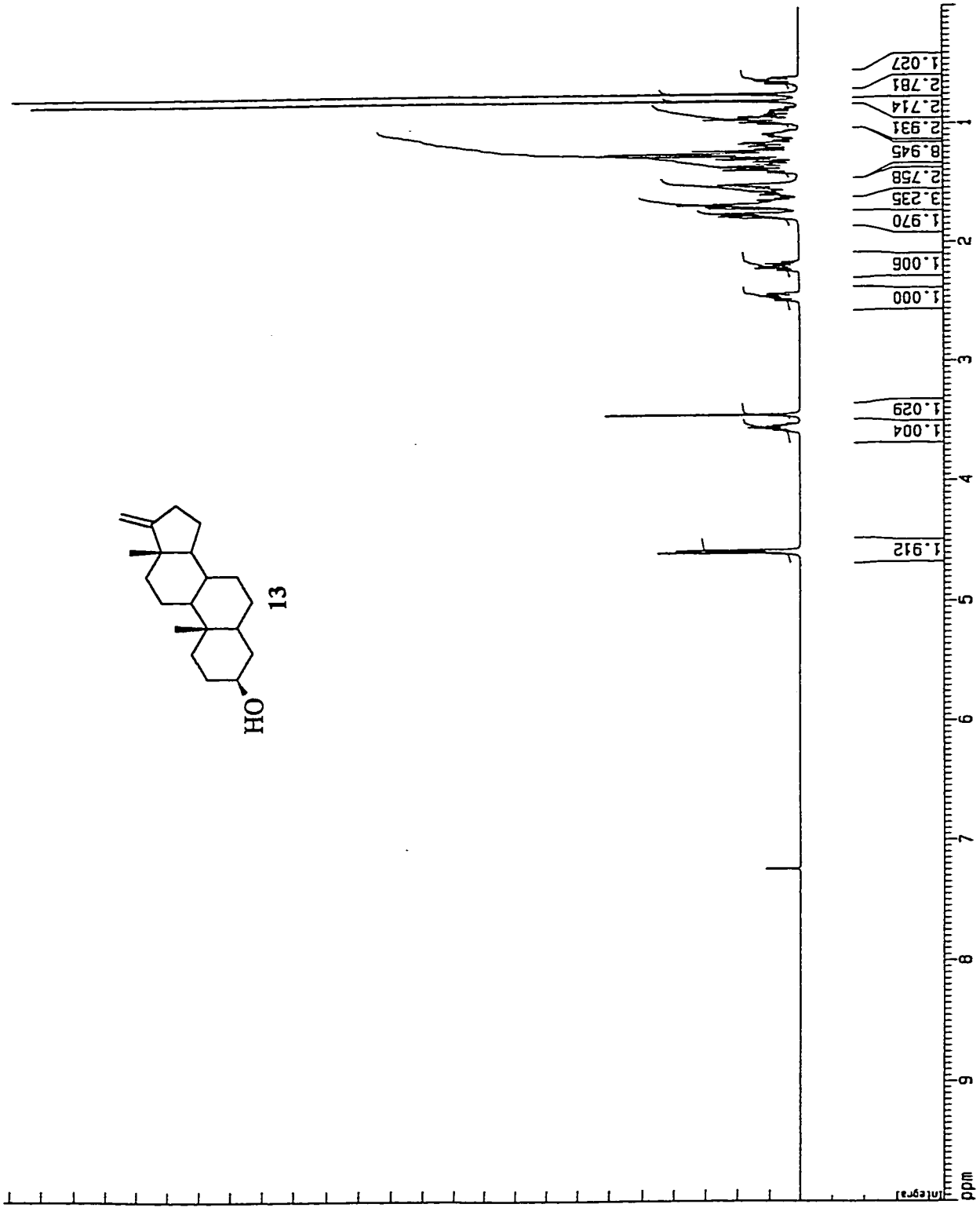


FIGURE 3.1.1: ^{1}H NMR SPECTRUM OF 3β-HYDROXYANDROST-17(20)-ENE (13)

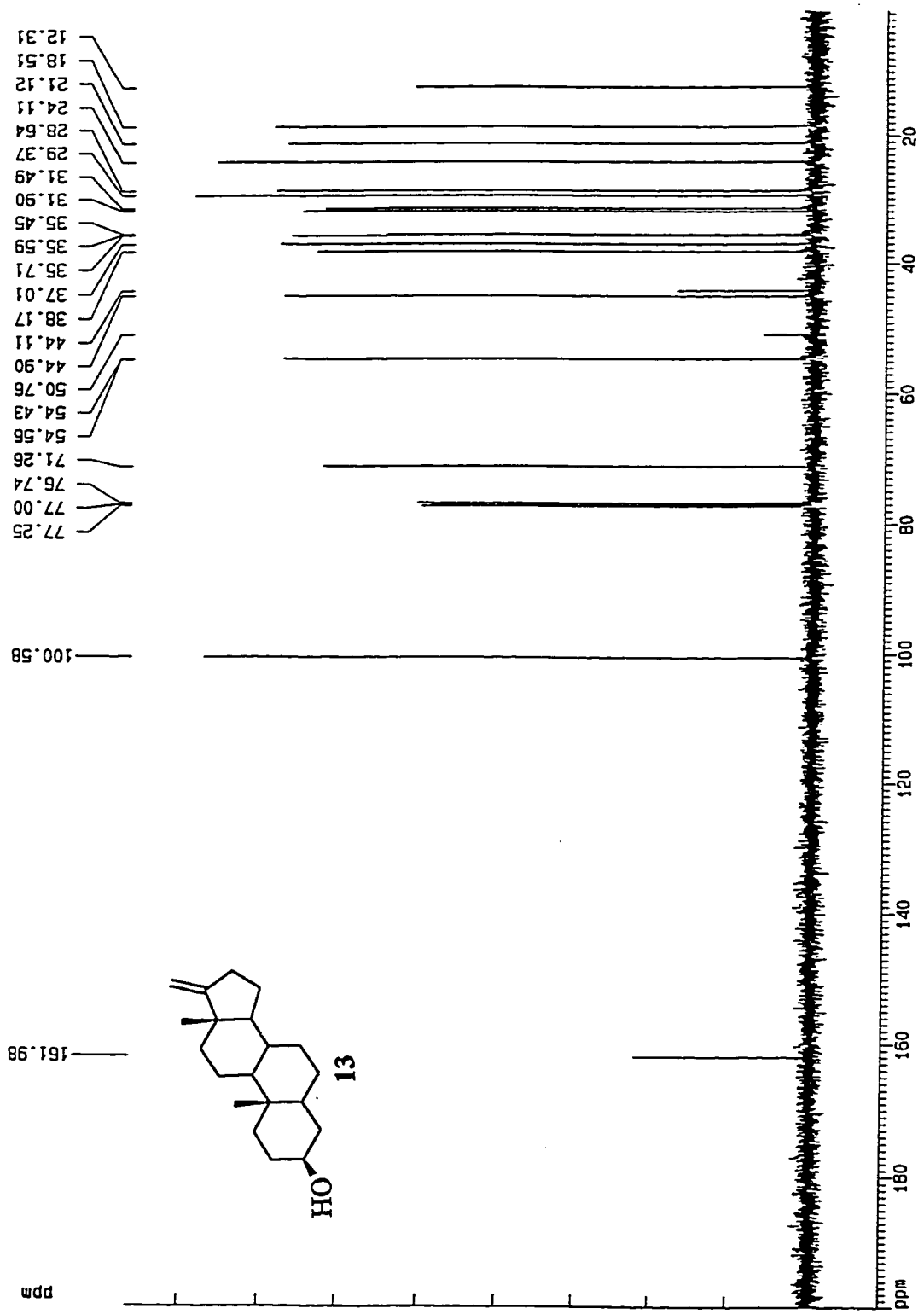


FIGURE 3.1.2: ^{13}C NMR SPECTRUM OF 3β-HYDROXYANDROST-17(20)-ENE (13)

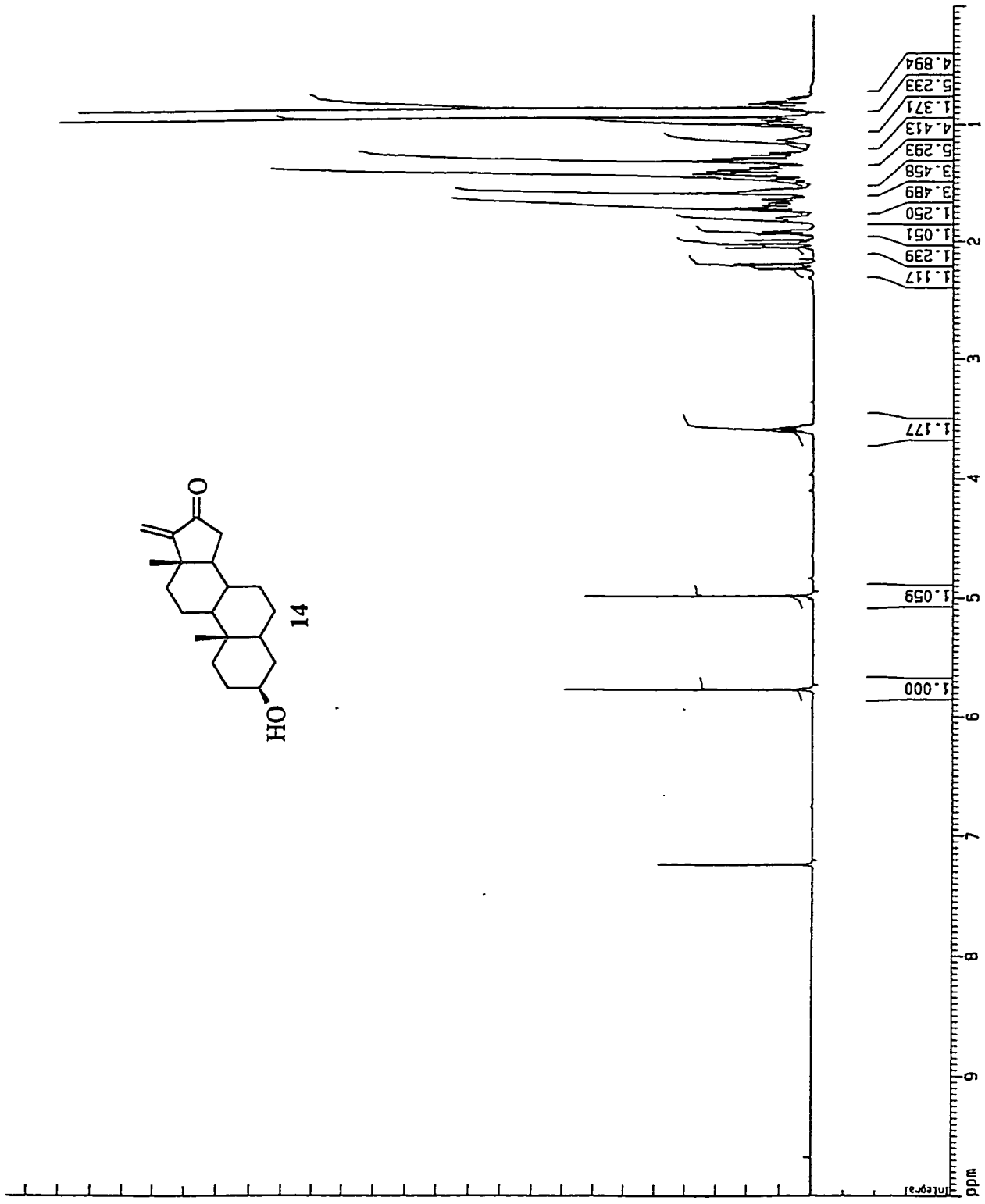


FIGURE 3.1.3: ^{1}H NMR SPECTRUM OF 3β-HYDROXYANDROST-17(20)-EN-16-ONE (14)

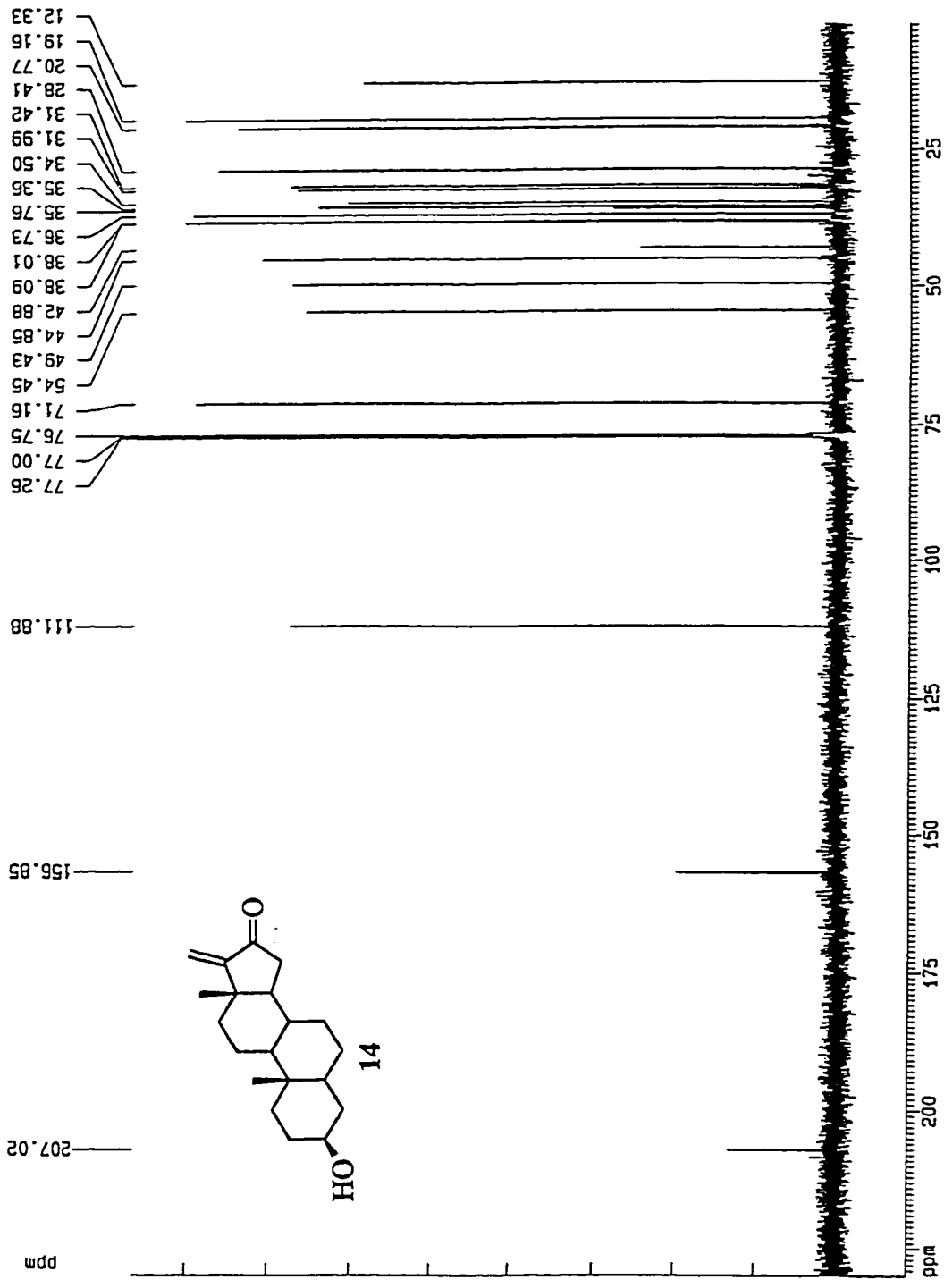


FIGURE 3.1.4: ^{13}C NMR SPECTRUM OF 3β-HYDROXYANDROST-17(20)-EN-16-ONE (14)

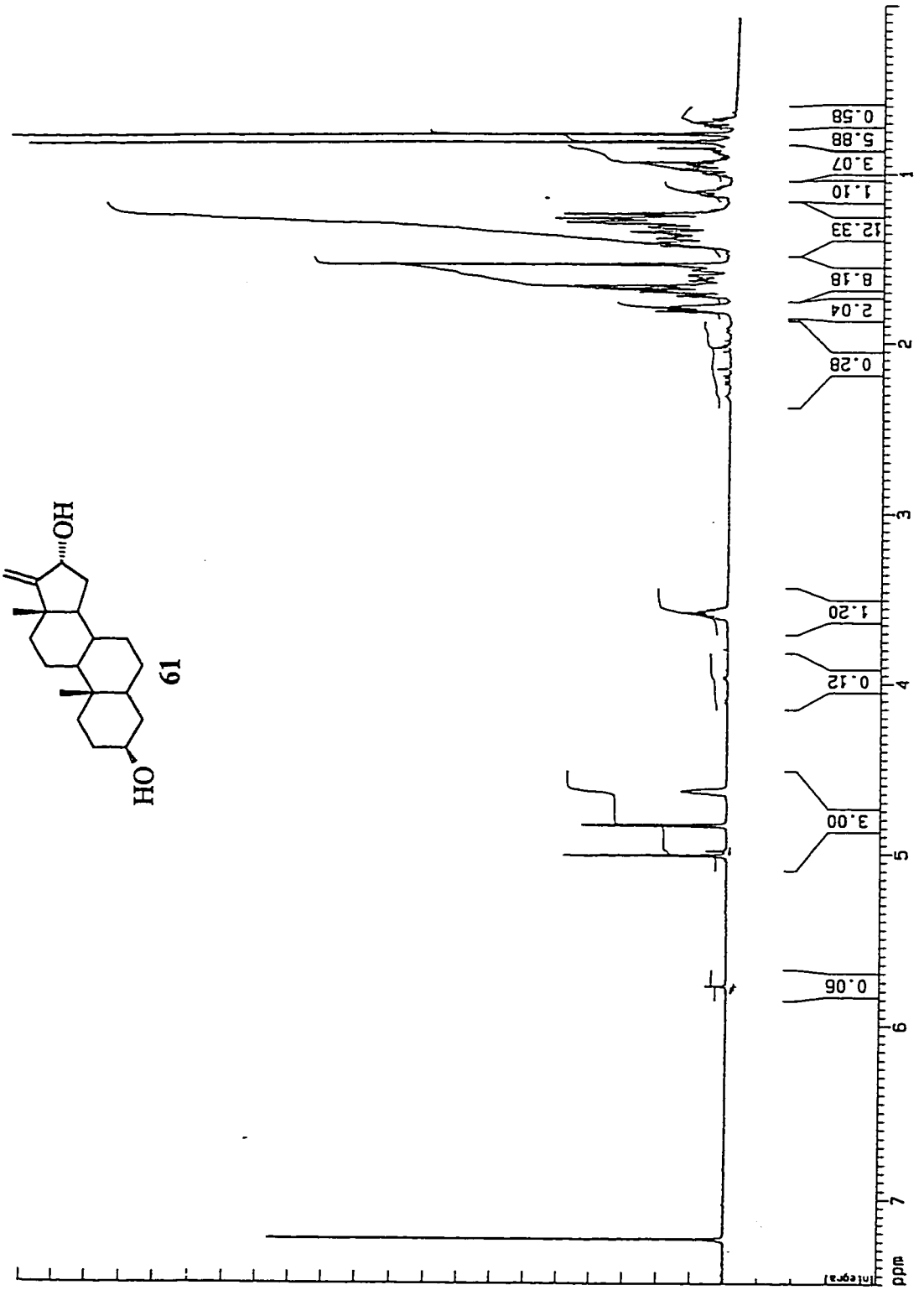


FIGURE 3.1.5: ^{1}H NMR SPECTRUM OF 3β,16α-DIHYDROXYANDROST-17(20)-ENE (61)

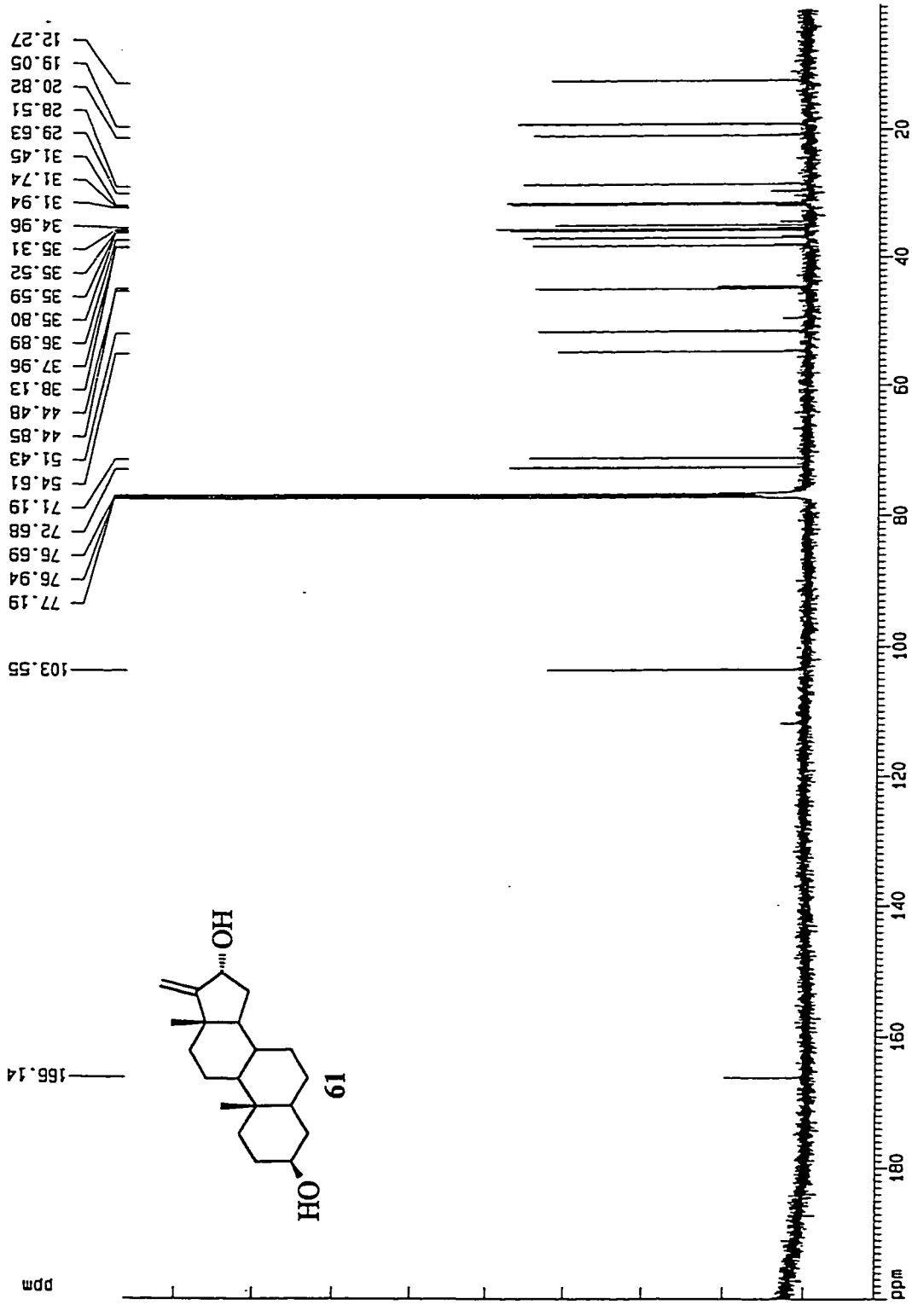


FIGURE 3.1.6: ^{13}C NMR SPECTRUM OF 3β,16α-DIHYDROXYANDROST-17(20)-ENE (61)

FIGURE 3.1.7: ^{1}H NMR SPECTRUM OF 3β-HYDROXYPREGNAN-16-ONE (5)

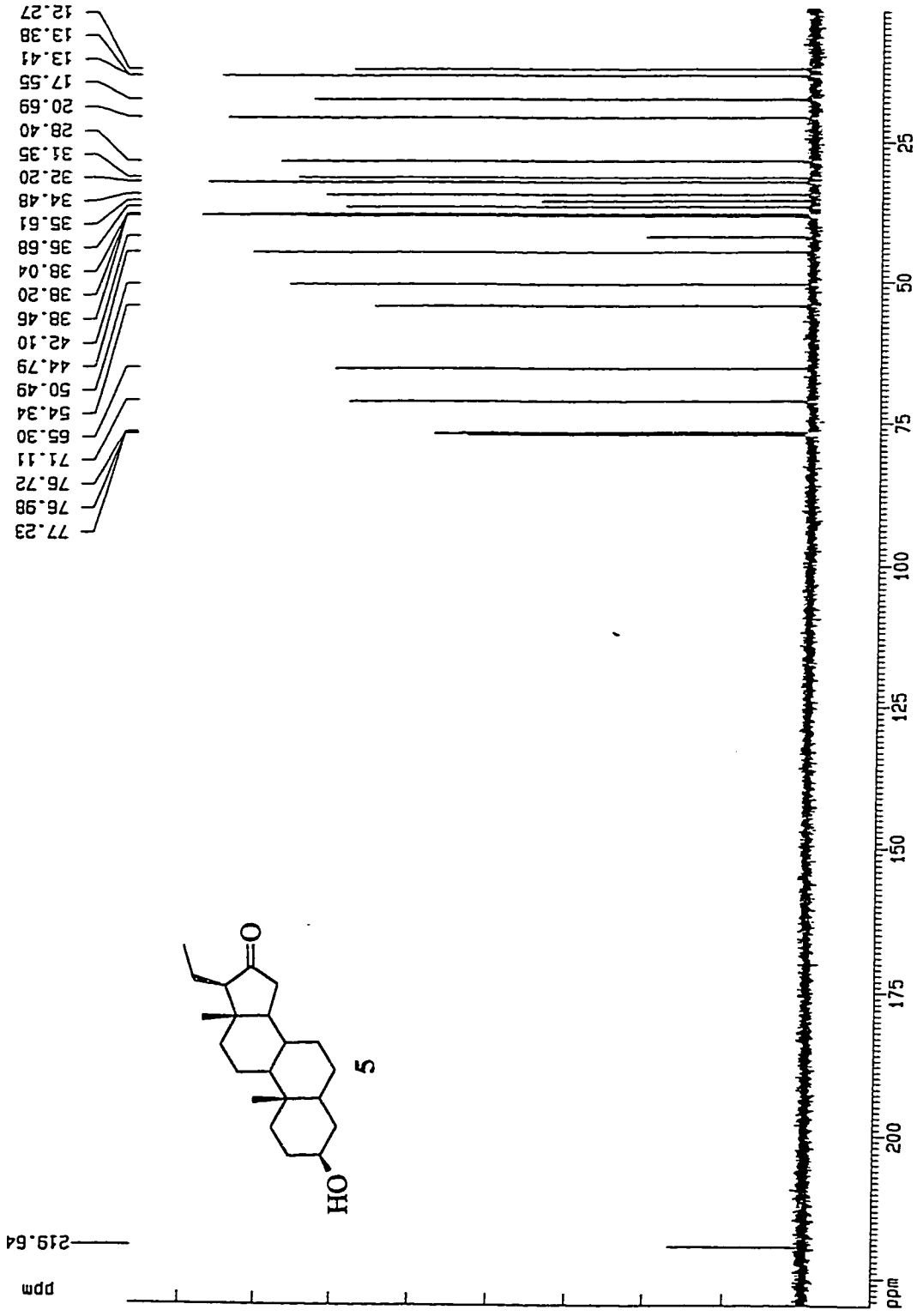


FIGURE 3.1.8: ^{13}C NMR SPECTRUM OF 3β-HYDROXYPREGNAN-16-ONE (5).

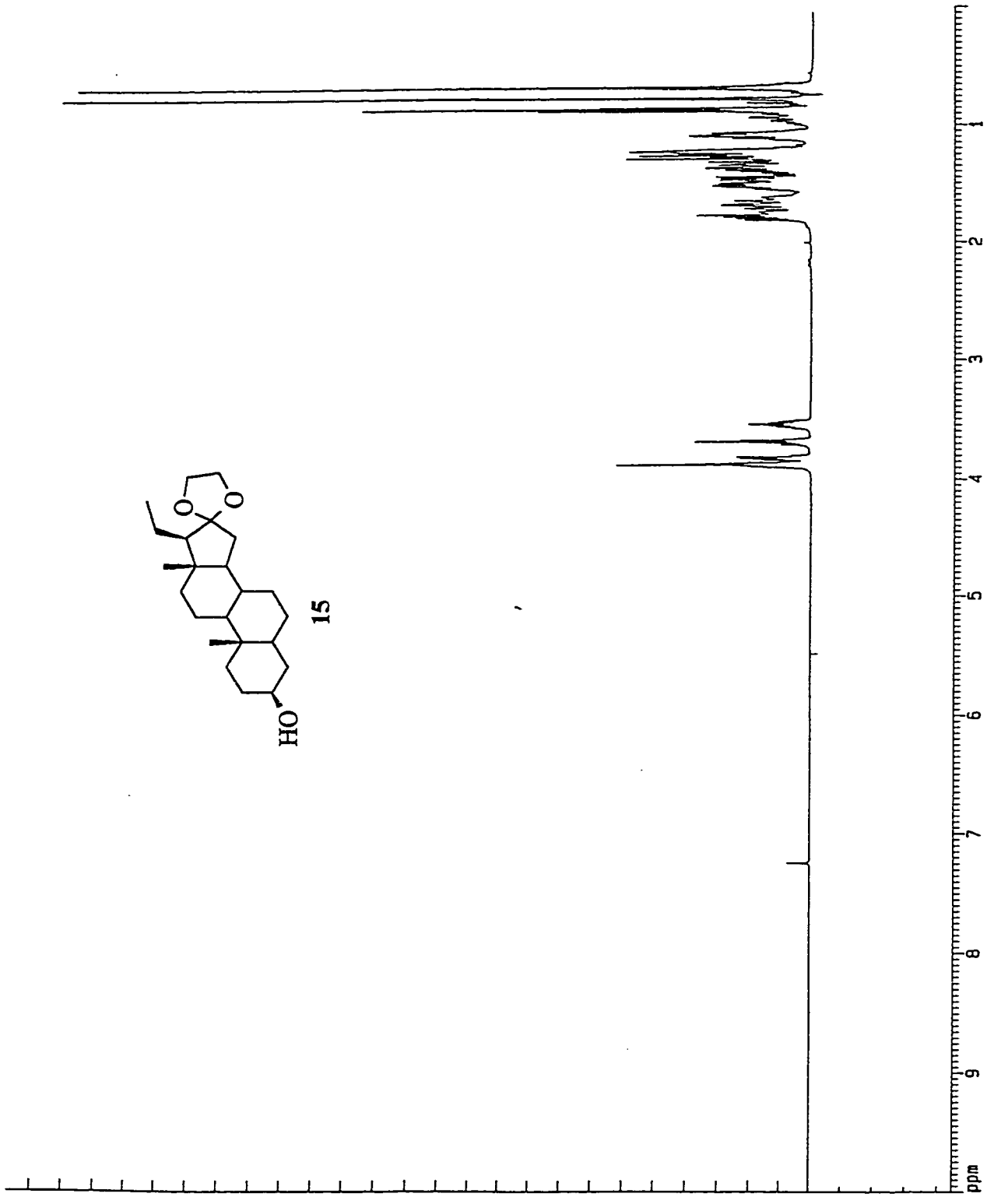


FIGURE 3.1.9: ^{1}H NMR SPECTRUM OF 3β-HYDROXY-16-ETHYLENEDIOXYPREGNANE (15)

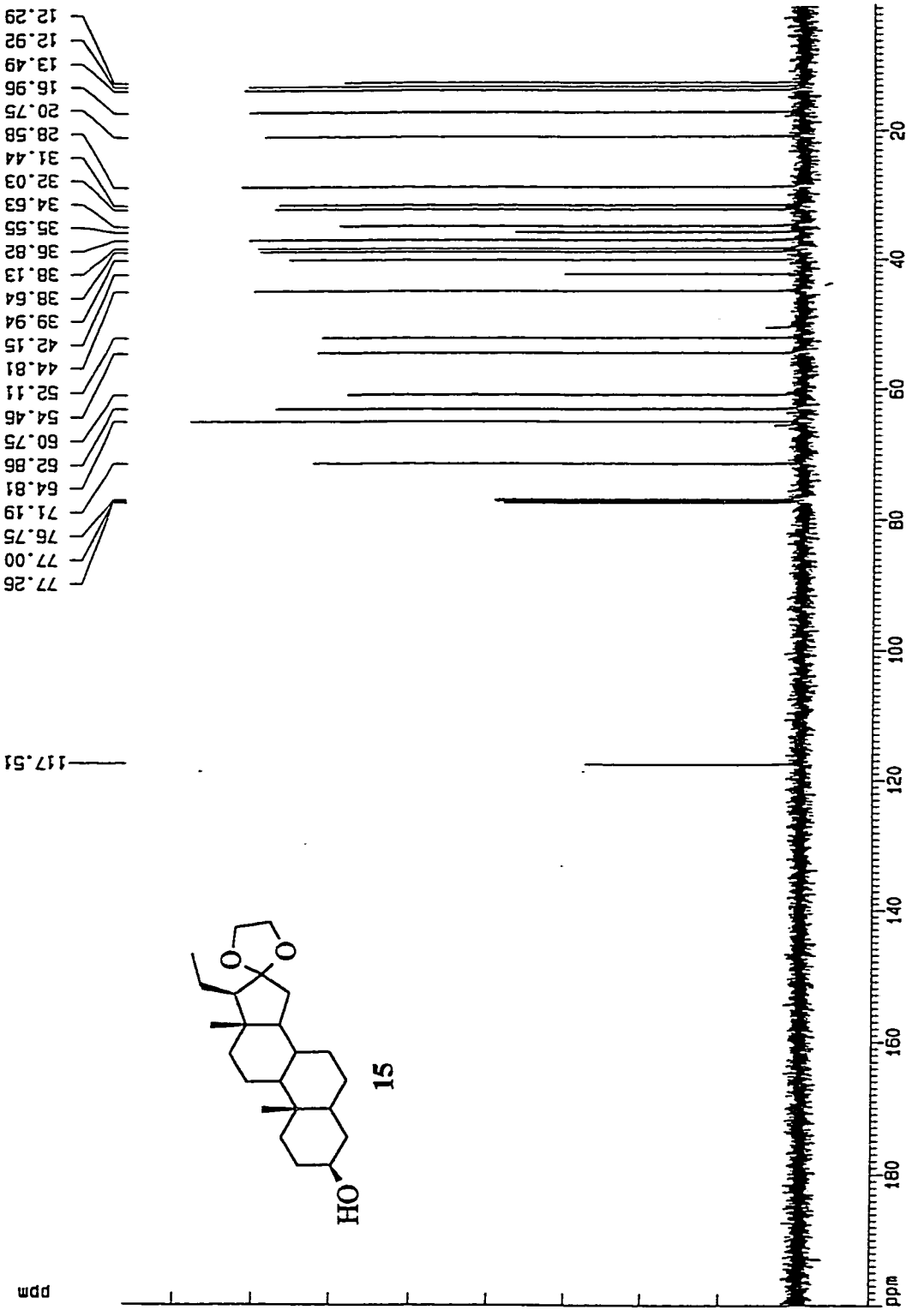


FIGURE 3.1.10: ^{13}C NMR SPECTRUM OF 3β-HYDROXY-16-ETHYLENEDIOXYPREGNANE (15)

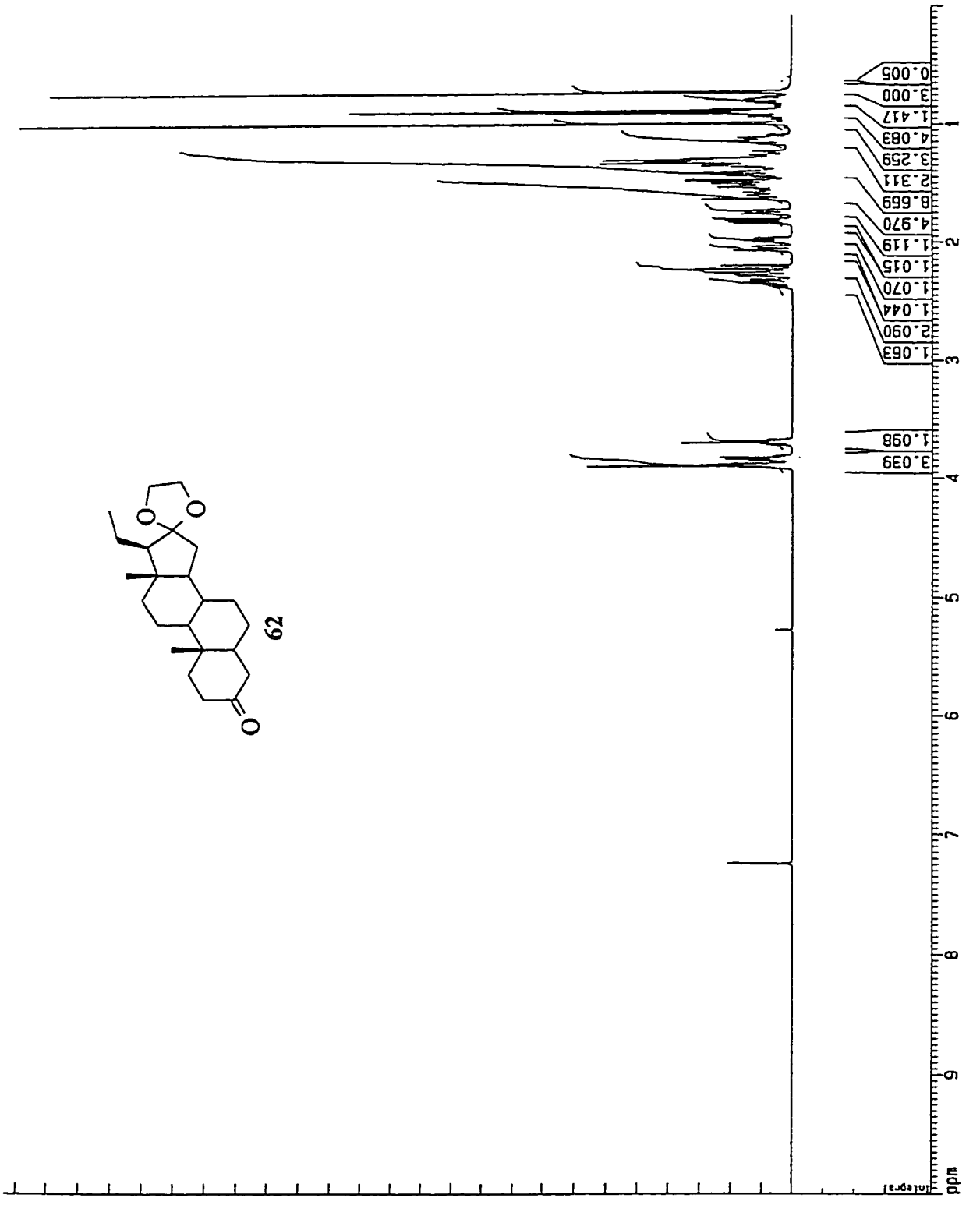


FIGURE 3.2.1: ^{1}H NMR SPECTRUM OF 16-ETHYLENEDIOXYPREGNAN-3-ONE (62)

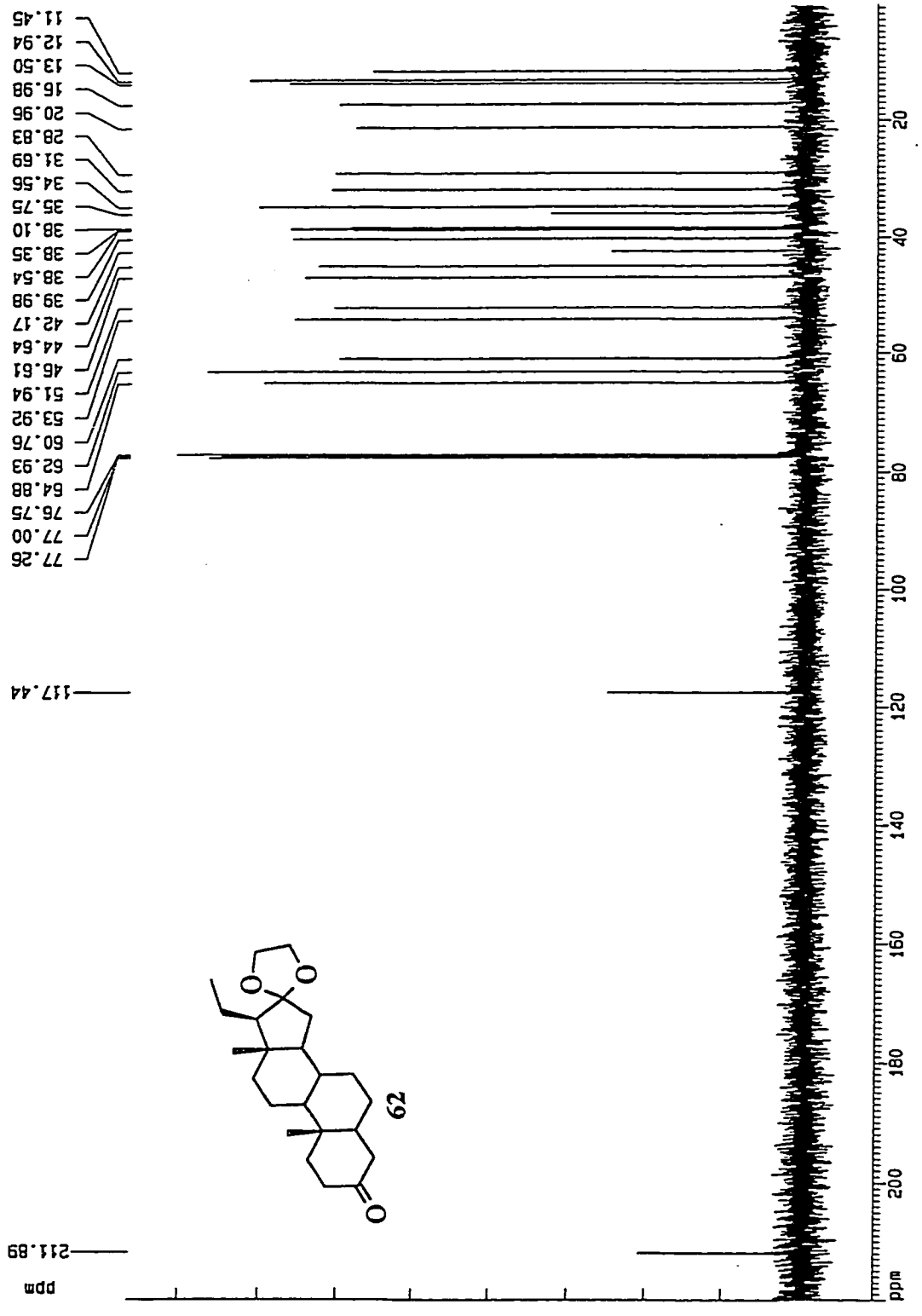


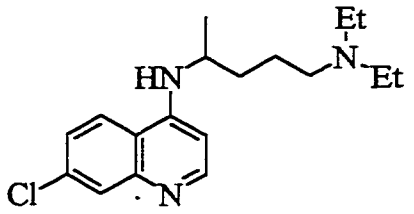
FIGURE 3.2.2: ^{13}C NMR SPECTRUM OF 16-ETHYLENEDIOXYPREGNAN-3-ONE (62)

Chapter 4

Derivatives of the Antimalarial Agent, Gedunin.

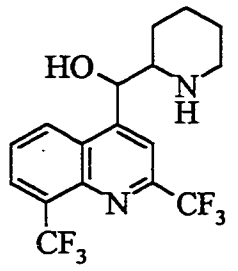
4.1 Introduction

In 1997, the World Health Organization estimated that malaria killed between 1.5 and 2.7 million people worldwide.[59] The resurgence of malaria in many of the endemic regions of the world is due to the multi-drug resistance of the malarial protozoan *Plasmodium falciparum.* Resistance to chloroquine **70**, mefloquine **71** and quinine **72** have been reported in a large number of the endemic regions of the world including Africa,



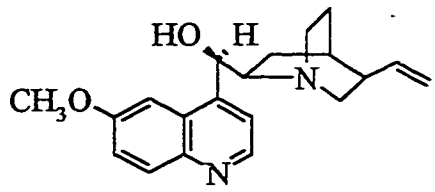
chloroquine

70



mefloquine

71



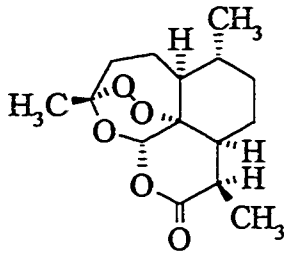
quinine

72

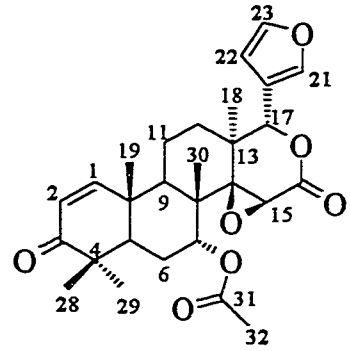
Asia, Central and South America.[59] The search for alternative antimalarials that are structurally unrelated to quinine has yielded such promising compounds as artemisinin **73** which is currently being used as a clinical treatment of malaria. Artemisinin **73** occurs in the leaves of the Chinese shrub *Artemisia annua* L and is a sesquiterpene lactone.[60]

Gedunin **74** a D-seco limonoid, also shows antimalarial activity[61] and it is isolated from the wood of *Cedrela odorata* a Central American tree.[62] It is also found in the

leaves of the neem tree, and natives of Togo, West Africa have traditionally used the tea brewed from these leaves to alleviate fever, a symptom of malaria. Various structure-



artemisinin
73

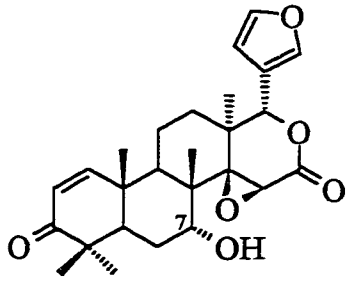


gedunin
74

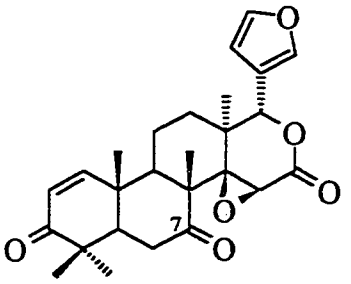
activity relationships of gedunin **74** have been studied, and it has been found that the level of antimalarial activity is dependent on the α,β-unsaturated ketone in ring A.[63] When the double bond of the enone system of **74** was reduced, as in 1,2-dihydrogedunin, a large drop in activity was observed in both the chloroquine resistant (W2), and especially in the chloroquine sensitive (D6) strain of *Plasmodium falciparum*. The IC_{50} value of 1,2-dihydrogedunin for the W2 strain was 840 ng/mL, and for the D6 strain was greater than 10,000 ng/mL.[63] The IC_{50} value of gedunin **74** for the same W2 strain was 20 ng/mL and for the same D6 strain was 39 ng/mL. Epoxidation of the C1-C2 double bond also resulted in loss of activity, as did reduction of both the C1-C2 double bond and the ketone at C-3. It is hypothesized that the importance of the α,β-unsaturated ketone of gedunin **74** is due to the 1,4-conjugate addition of the parasite's glutathione to this enone functionality. The covalent adduct would inhibit the parasite's glutathione from acting as an anti-oxidant which is essential for cellular maintenance within the parasite. The

proposed 1,4-conjugate addition to the enone functionality of gedunin **74** has been tested with benzyl mercaptan and the results are described in section 4.2.

The antimalarial activity of gedunin **74** was also found to be dependent on the substitution at C-7. Loss of activity was observed when 7-hydroxygedunin **75** and 7-oxogedunin **76** were tested in vitro against chloroquine resistant (W2) *Plasmodium falciparum*.[63] The presence of the 7-keto functionality seemed to eliminate all antimalarial activity as the IC_{50} value for **76** was greater than 10 000 ng/mL compared to the IC_{50} value of gedunin at 20 ng/mL. . The difference in activity between gedunin **74** and the 7-hydroxygedunin **75** was not as dramatic since the IC_{50} value observed for **75** was 1280 ng/mL. It was speculated that the difference in activity between gedunin **74** and



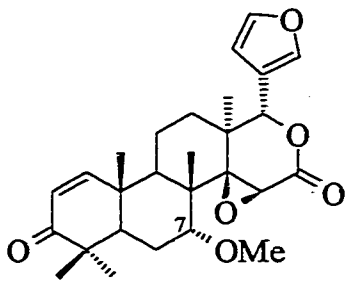
75



76

7-hydroxygedunin **75** was possibly due to differences in bioavailability.[63]

To support this speculation the stability of both gedunin **74** and 7-hydroxygedunin **75** under acidic conditions has been investigated and presented in section 4.3. In addition, it was anticipated that an ether derivative of gedunin **74** at C-7 would lead to a more stable derivative since metabolism of gedunin by an esterase leads to the less active 7-hydroxygedunin **75**. The 7-methoxy derivative **77** was synthesized and tested in vitro for

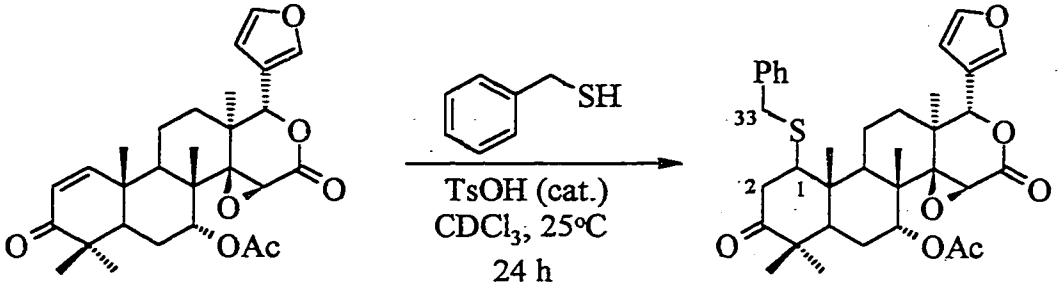


77

activity against both chloroquine resistant (W2) and chloroquine sensitive (D6) strains of *Plasmodium falciparum* (see section 4.4).

4.2 The Mode of Action of Gedunin 74 as an Antimalarial Agent.

The feasibility of the proposed 1,4-conjugate addition of the malaria parasite's glutathione to the α,β-unsaturated ketone of gedunin **74** was tested by mixing benzyl mercaptan with gedunin in the presence of a catalytic amount of *p*-toluenesulfonic acid. The reaction was monitored by ^{1}H NMR spectroscopy and proceeded to completion



74 **78**

within 24 hours at room temperature. The isolated yield of 1-benzylmercaptogedunin **78**, mp 88-90°C, was 46%. The molecular ion was calculated to be 606.2652 and the HRMS showed a M^+ ion at *m/z* 606.2628. Key signals in the ^{1}H NMR spectrum of **78** (see figure

4.2.1-chp. 4 appendix) included a broad singlet at δ 7.16 which corresponded to the phenyl protons and a pair of doublets at δ 3.76 and 3.50 which corresponded to the benzylic protons at C-33. The resonance of H-1, α to the benzyl mercapto moiety was buried in a multiplet at δ 2.69-2.65 which also included the resonance for one of the two protons of the methylene group at C-2. The other proton attached to C-2 appeared as a multiplet at δ 3.11-3.06.

Additional evidence of the benzyl mercapto group came from the ^{13}C NMR spectrum (see figure 4.2.2-chp. 4 appendix) and the DEPT experiment because C-1 was identified as a methine carbon that resonated at δ 49.09. The peak for C-2 was at δ 40.00 and the DEPT experiment indicated it to be a methylene carbon, further proving the absence of a double bond between carbons 1 and 2. In addition there were four peaks at δ 137.82, 129.02, 128.41, and 127.29 which corresponded to the six carbons of the phenyl group. The benzylic methylene carbon resonated at δ 34.92.

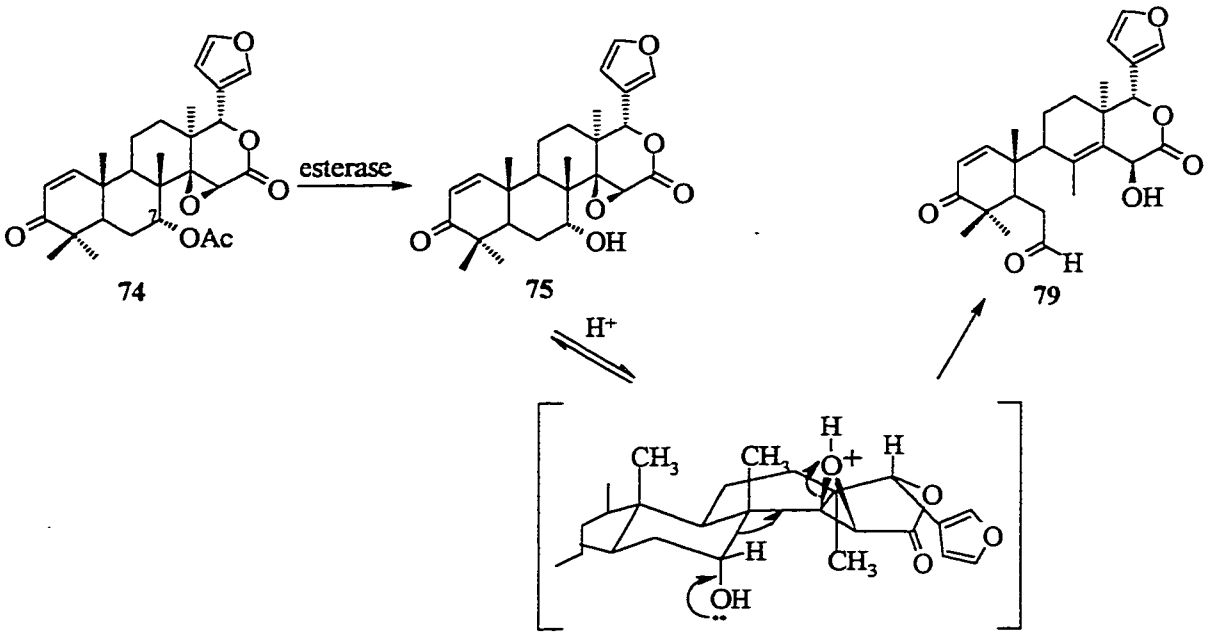
Thus, it has been shown that benzyl mercaptan can add in 1,4-conjugate fashion to gedunin **74**. This chemical observation supports the hypothesis that within the malarial parasite, the thiol moiety of glutathione may react with gedunin to produce a covalent adduct. Such an adduct could lead to the breakdown of cellular maintenance within the parasite, because glutathione would no longer be able to act as a radical scavenger.

4.3 The Bioavailability of Gedunin 74 as an Antimalarial Agent.

It has been speculated that lower bioavailability was the cause for the lack of antimalarial activity observed when 7-hydroxygedunin **75** and 7-oxogedunin **76** were

tested against *Plasmodium falciparum*. An alternate hypothesis to explain the lowered activity of **75** involves an acid catalyzed fragmentation of **75** leading to the inactive metabolite **79** (see scheme 4.3.1). It is postulated that esterase mediated hydrolysis of the acetate group at C-7 of gedunin **74** affords **75** which in turn undergoes an acid catalyzed fragmentation.

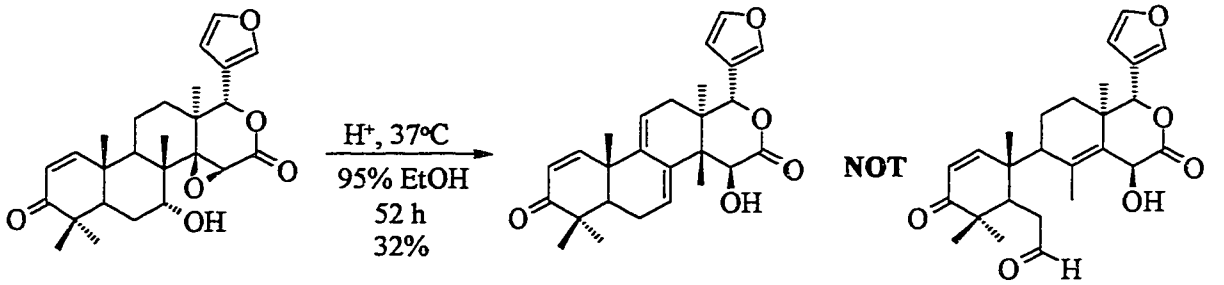
Scheme 4.3.1-Postulated in vivo metabolism of Gedunin.



In order to investigate the possibility of such a fragmentation, gedunin **74** was first converted to 7-hydroxygedunin **75** by dissolving gedunin in methanol to which was added six molar equivalents of 1.0M sodium methoxide. The solution was allowed to stir for 42 hours at room temperature. Following column chromatography, 7-hydroxygedunin **75** was obtained in 91% yield. This was considerably higher than the 47% yield previously reported by Akisanya and coworkers in 1961[64] or the 84% yield reported by MacKinnon.[62] The ^{1}H and ^{13}C and mass spectra of **75** matched that previously reported.[62]

With 7-hydroxygedunin **75** in hand, the possibility of acid catalyzed fragmentation of **75** was investigated by dissolving **75** in a 1.5M solution of HCl in 95% ethanol. The solution was stirred for 52 hours at 37°C at which time starting material was still present, but a major product was evident from the tlc plate. The two main features in the ^{1}H NMR spectrum (see figure 4.3.1-chp. 4 appendix) that indicated that the product was not **79** was the lack of both an aldehyde proton resonance at ~δ 10.0 and a vinyl methyl peak at ~δ 1.7. In addition there were six olefinic carbons in the ^{13}C spectrum which was two more than expected for **79** (see figure 4.3.2-chp. 4 appendix). The mass spectrum of the product showed an intense M^+ ion at *m/z* 422 (100%) instead of the calculated value of 440.2199 for $C_{26}H_{32}O_6$ which is the molecular formula for **79**. The difference in the expected M^+ (440) and the observed M^+ (422), indicated that 7-hydroxygedunin had lost a molecule of water to give rise to **80** whose molecular formula is $C_{26}H_{30}O_5$ (see scheme 4.3.2).

Scheme 4.3.2-Acid catalyzed fragmentation of 7-hydroxygedunin**75**.

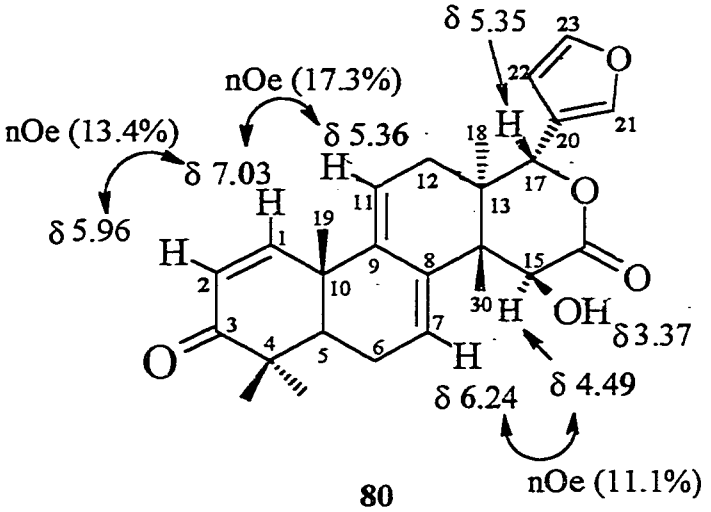


75 **80** **79**

The key resonances in the ^{1}H NMR spectrum which facilitated the structure elucidation of the unknown as **80** are summarized in figure 4.3.3. Many of the assignments were based on careful analysis of the ^{1}H-^{1}H (figure 4.3.4) and ^{1}H-^{13}C (figure

4.3.5) correlation spectra. In addition, the assignment of H-11 and H-7 were based on two different nOe difference experiments. When the resonance at δ 7.03 corresponding to H-1 was irradiated, a 17.3% enhancement was observed for the olefinic proton at δ 5.36 and **no** enhancement was observed at the other olefinic proton resonance at δ 6.24. Thus, the broad singlet at δ 5.36 must belong to H-11 and not to H-7. There was also a 13.4% enhancement observed for the doublet at δ 5.96 when H-1 was irradiated, and this signal was assigned to H-2 because it showed a correlation to H-1 in the ^{1}H-^{1}H correlation experiment (figure 4.3.4-chp. 4 appendix).

Figure 4.3.3 -Key ^{1}H NMR spectral features of compound **80**.



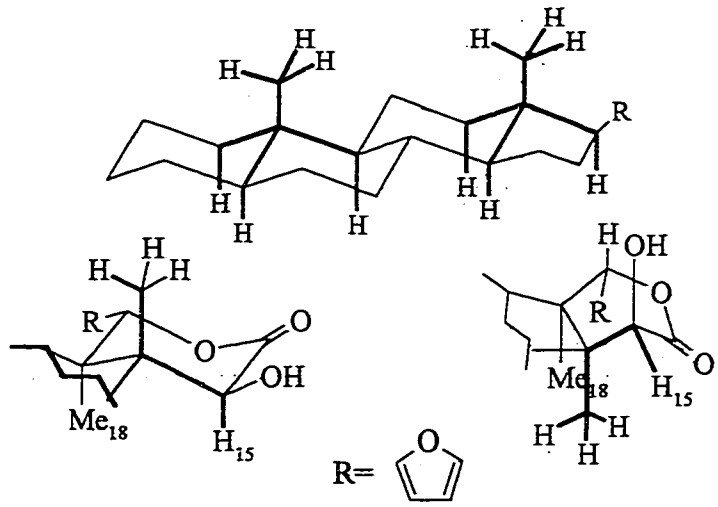
In the second nOe difference experiment, the methine proton at C-15 which resonated at δ 4.49 was irradiated and two enhancements were observed. The broad singlet at δ 6.24 was enhanced by 11.1% and this peak corresponded to the olefinic proton

attached to C-7 (see figure 4.3.3). The other nOe observed was for the methyl singlet at δ 0.91 which has been *tentatively* assigned as the C-18 methyl and this signal was enhanced by 7.6%.

At this point, the stereochemistry of the C-D ring junction indicated in figure 4.3.3 must be addressed. The C-18 methyl group has been assigned α because when the methyl group at δ 0.91 was irradiated, an nOe was observed for the furan ring resonances at δ 7.40 (0.8%) and δ 6.35 (1.6%) which corresponded to H-21/H-23 and H-22 respectively. With the structure for **80** as shown in figure 4.3.3, the only methyl group that could elicit an nOe with the furan protons was C-18. Further evidence that the C-18 methyl group is α comes from the fact that when the C-18 methyl group is irradiated an nOe of 3.3 % is observed for the singlet at δ 4.49 corresponding to H-15 which is known to be α.

The β assignment of the C-30 methyl group shown in figure 4.3.3 is tentative because an nOe difference experiment could not be conducted in which H-17 would be irradiated to see if there was an nOe with the C-30 methyl group. The inability to do such an experiment stemmed from the fact that both H-11 and H-17 resonated at very similar chemical shifts, δ 5.36 and 5.35, which made it impossible for selective irradiation. The only evidence that the C-30 methyl group was β instead of α came from the ^{1}H-^{1}H NMR experiment (see figure 4.3.4-chp. 4 appendix), in which H-15 showed a correlation to the signal at δ 1.19-1.18 which was identified to be two overlapped methyl resonances. It was not possible to identify the two overlapped methyl resonances, but if one was to assume that one of the two methyl groups was indeed C-30, then the correlation observed in figure 4.3.4 can be easily explained. It is known that for decalin systems, long range

coupling exists between the angular methyl groups and protons that lie in a W pattern from the angular methyl (see below).[65] Thus, the C-30 methyl group was β because a W pattern cannot be established between an α-oriented C-30 methyl and the known α H-15.



β C-30 methyl
(W pattern present)

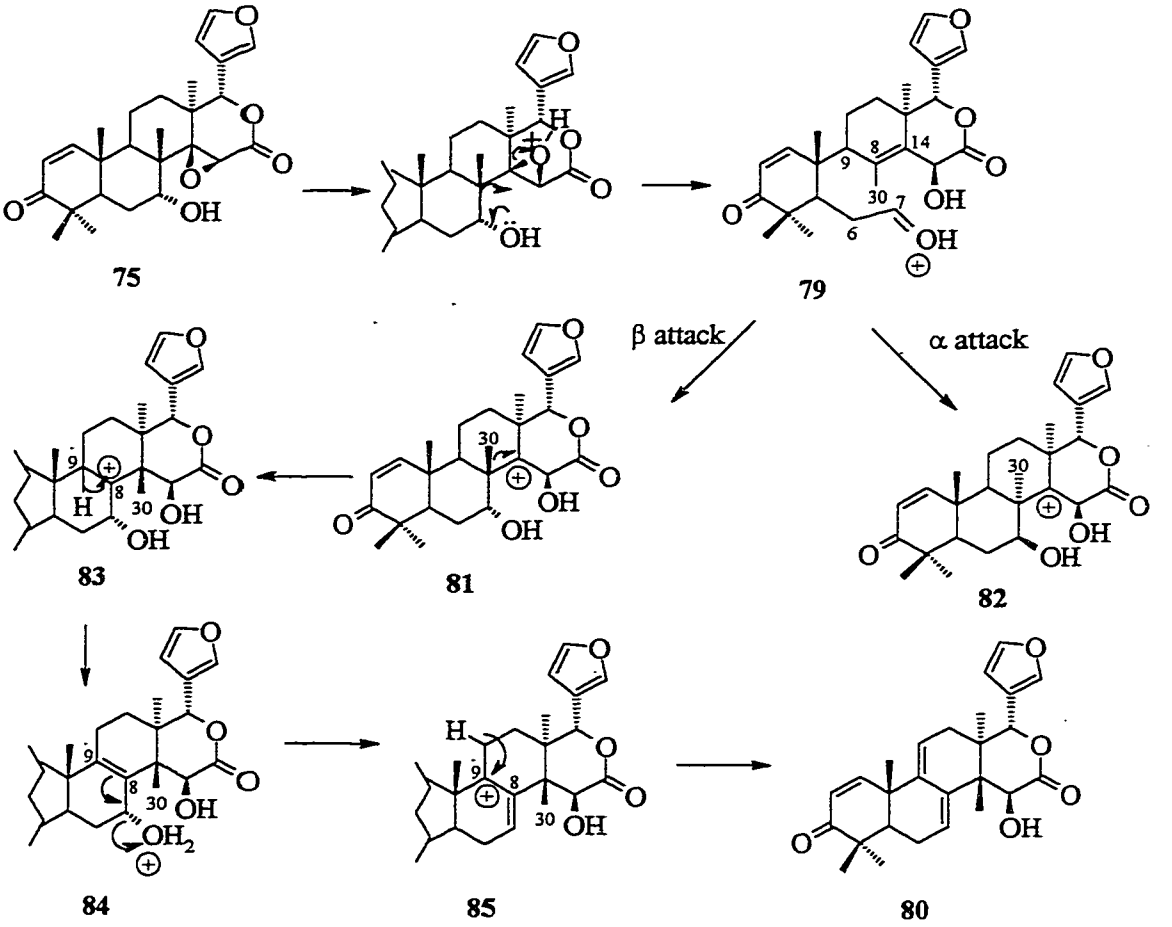
α C-30 methyl
(W pattern absent)

Ideally a crystal structure of **80** would have clearly shown the orientation of the C-18 and C-30 methyl groups, but when crystallization was attempted in several different solvent systems, each attempt either failed to give appropriate crystalline material or the material obtained was a powder. An attempt to make the crystalline semicarbazone of the α,β-unsaturated ketone of **80** also failed as the material obtained was a powder.

With the tentative structure of **80** in hand, a mechanism for the acid catalyzed rearrangement of 7-hydroxygedunin **75** can be proposed. Scheme 4.3.3 shows the lone pair of the 7-hydroxyl group opening the protonated epoxide via a Grob fragmentation to give the protonated intermediate **79**. The intermediate **79** then undergoes ring closure by the attack of the C-8-C-14 double bond on the oxonium ion at C-7. The attack could potentially occur from either the α or β face of the oxonium ion. Intermediate **81**

leads to the structure proposed for **80** since the next step is the *cis*-migration of the C-30 methyl group to C-14. The next step in the mechanism is the formation of a double bond between C-8 and C-9 shown in intermediate **83** with loss of a proton. Loss of water and

Scheme 4.3.3-Proposed Mechanism for the Formation of Compound **80**.

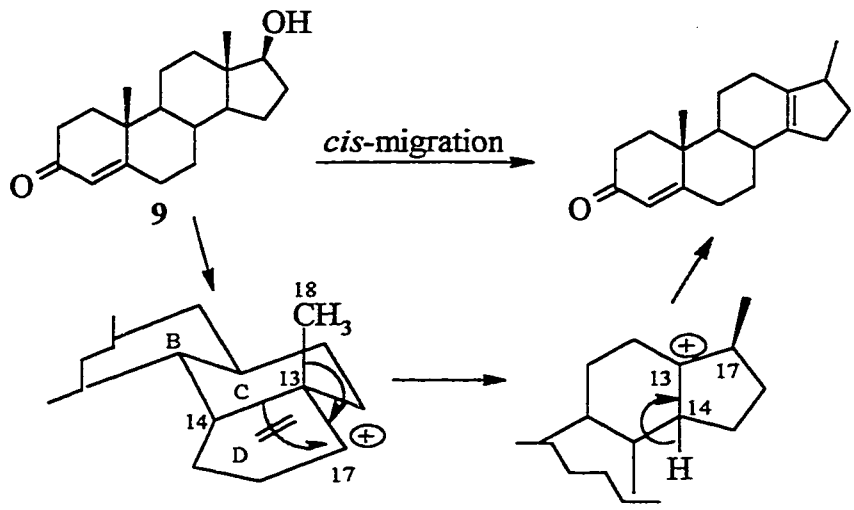


double bond migration leads to intermediate **85** which then loses a proton from C-11 to form the conjugated π system of **80**.

Precedence for *cis*-migration exists in that the C-18 methyl group of testosterone **9** has been shown to undergo *cis*-migration during the dehydration of the protonated 17β-

hydroxyl group (see figure 4.3.7).[27] Despite, the stereoelectronic requirement that a *trans* coplanar arrangement of the migrating moiety relative to the leaving water molecule is necessary, *cis*-migration is favoured. Indeed the stereoelectronic requirement would be satisfied if the C-14-C13 bond migrated instead of the C-18 methyl group, but the higher

Figure 4.3.7-Dehydration of Testosterone **9**: evidence of *cis*-migration.



energy of the resulting *trans*-fused cyclobutane structure must prevent its formation.[27] Thus, the C-18 methyl migrates resulting in a carbocation at C-13 which leads to the formation of a double bond between C-13 and C-14.

Since 7-hydroxygeduinin **75** was not stable under acidic conditions as was evident by the formation of **80**, the stability of gedunin **74** under acidic conditions was also investigated. Gedunin was dissolved in 95% ethanol and excess concentrated hydrochloric acid was added to give a 1.5M solution. The solution was stirred for 52 hours at 37°C and only starting material was recovered. Thus, gedunin was stable under the acidic conditions and it is possible that the 7-acetyl moiety prevents the initial Grob

fragmentation shown in scheme 4.3.3. Thus, it was postulated that an ether moiety at C-7 would also prevent the fragmentation of the derivative and perhaps would yield a derivative with improved antimalarial activity.

4.4 The Antimalarial Activity of 7-methoxygedunin 77.

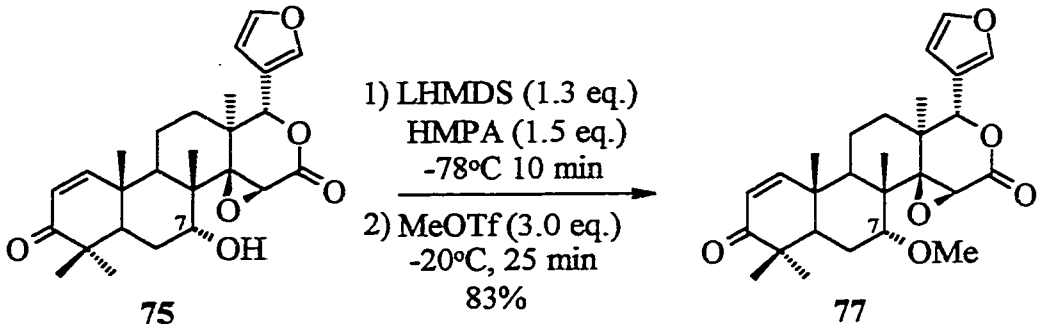
The preparation of 7-methoxygedunin **77** from 7-hydroxygedunin **75** proved to be quite challenging as a variety of methods were attempted before **77** was obtained. The method for carbohydrate methylation[66] was attempted because it has been shown to be milder to base-labile substituents. It involved methylating **75** with 10 molar equivalents of methyl triflate in the presence of excess 2,6-di-*tert*-butyl-4-methylpyridine. The solution was refluxed for 24 hours and only starting material was recovered.

The subsequent attempt involved initial deprotonation of the hydroxyl moiety with sodium hydride (1.0 molar equivalent) at 0°C in toluene and subsequent addition of methyl iodide (1.2 molar equivalents) with gradual warming to room temperature. The solution was allowed to stir for 48 hours at room temperature, but only starting material was recovered. The same reaction was attempted with the use of methyl triflate instead of methyl iodide in the presence of excess 2,6-di-*tert*-butyl-4-methylpyridine, but the same result was obtained.

The last attempt involved following the method described by Koga et al.[67] and it proved to be successful. The hydroxyl group was deprotonated at -78°C in THF with lithium hexamethyldisilazide (LHMDS) in the presence of hexamethylphosphoramide (HMPA) to ensure a naked anion. The anion was quenched at -20°C with excess methyl triflate (MeOTf) and the solution was stirred for 25 minutes (see scheme 4.4.1). The

reaction was complete after 25 minutes and 10% sodium hydroxide followed by distilled water was immediately added. The crude product was purified by column chromatography and 7-methoxygedunin **77** was obtained in 83% yield as a pale oily solid.

Scheme 4.4.1-Preparation of 7-methoxygedunin **77**.



75 **77**

The ^{1}H NMR spectrum (see figure 4.4.1-chp. 4 appendix) of **77** showed the methyl singlet of the 7-methoxy group at δ 3.28 and the H-7 proton appeared as a sharp multiplet at δ 2.89-2.88. The key signals in the ^{13}C NMR spectrum (see figure 4.4.2-chp. 4 appendix) included the α,β-unsaturated ketone, C-3 at δ 204.48, the lactone carbonyl carbon, C-16 at δ 168.04, the ether carbon, C-7 at δ 79.22 and the carbons of the epoxide, C-14 and C-15 at δ 70.07 and δ 57.36 respectively. The IR spectrum showed the lactone carbonyl stretch at 1744 cm^{-1} and the α,β-unsaturated ketone at 1668 cm^{-1}. The HRMS showed a weak M^{+} ion (1.0%) at *m/z* 454.2334 which was in agreement with the calculated value of 454.2356.

The antimalarial activity of 7-methoxygedunin **77** together with gedunin **74** and 7-hydroxygedunin **75** was evaluated against a chloroquine sensitive clone (D6) of

Plasmodium falciparum and a chloroquine resistant clone (W2). The standards in the test included chloroquine **70**, mefloquine **71**, quinine **72** and artemisinin **73**. The IC_{50} values have been summarized in table 4.4.1.[68] Gedunin and derivatives **75** and **77** exhibited larger

Table 4.4.1-Antimalrial activity of Gedunin **74** and Derivatives **75** and **77** against chloroquine-sensitive (D6) and chloroquine-resistant (W2) clone of *Plasmodium falciparum*.

Compound	IC_{50} value (ng/mL) Clone D6	Clone W2
Gedunin **74**	418	424
7-methoxygedunin **77**	1341	1221
7-hydroxygedunin **75**	1896	2068
chloroquine **70**	8	54
mefloquine **71**	7	3
quinine **72**	18	51
artemisinin **73**	7	4

IC_{50} values than any of the four standards. The 7-methoxy derivative **77** showed lower antimalarial activity than gedunin **74** in both the chloroquine resistant and sensitive clones. But the activity of **77** was greater than the activity shown by 7-hydroxygedunin **75** in the case of both clones. Thus, methylating the hydroxyl group at C-7 restored some of the antimalarial activity that was lost in the case of 7-hydroxygedunin **75**.

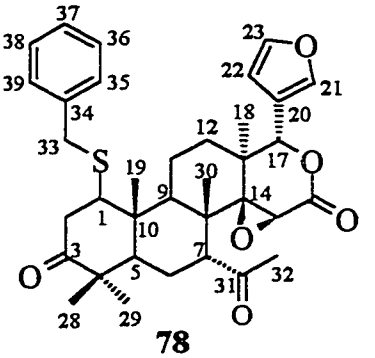
Experimental for Chapter 4

4.5 EXPERIMENTAL

GENERAL: See experimental section 2.7 for general procedures.

PREPARATION OF 1-BENZYLMERCAPTOGEDUNIN (78).

To a solution of gedunin (10 mg, 0.027 mmol) in deuterated chloroform in an NMR tube was added one crystal of *p*-toluenesulfonic acid. The mixture was shaken for 20 min and benzyl mercaptan was added. The reaction mixture was monitored by ^1H NMR and the reaction was complete after 24 h at room temperature. The reaction mixture was diluted with methylene chloride (15 mL) and washed with water (3 x 5 mL). The organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated *in vacuo* affording an orange semi-solid. Flash chromatography (5 g of silica gel, 9:1 hexanes/ethyl acetate) yielded **78** (6 mg, 46%) as a yellow solid.



78

$C_{35}H_{42}O_7S$ MW=606.78 g/mole

mp 88-90°C

MS [EI, *m/z* (%)]: 606 [MH^+] (2), 483 (34), 299 (100).

HRMS calcd. for $C_{35}H_{42}O_7S$: 606.2652; found: 606.2628.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1732 (C=O), 1706 (C=O), 1650 (C=C).

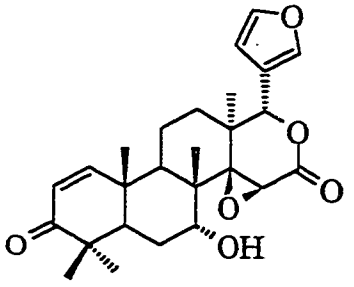
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 7.45 (brt, J=1.6 Hz, 1H, H-21 or H-23), 7.39 (brs, 1H, H-21 or H-23), 7.16 (brs, 5H, H-35,36,37,38,39), 6.32 (s, 1H, H-22), 5.53 (s, 1H, H-17), 4.43 (s, 1H, H-17), 3.76 (d, J=13.8 Hz, 1H, H-33), 3.50 (d, J=13.8 Hz, 1H, H-33), 3.45 (s, 1H, H-15), 3.11-3.06 (m, 1H, H-2), 2.89 (dd, J=12.7, 6.3 Hz, 1H, H-9), 2.69-2.65 (m, 2H, H-1 and H-2), 2.08 (m, 3H, H-32), 2.05 (dd, J=12.7, 3.0 Hz, 1H, H-5), 1.83 (dt, J=15.1, 3.0 Hz, 1H, H-6), 1.73-1.67 (m, 1H, H-6), 1.58-1.45 (m, 1H), 1.44-1.40 (m, 2H), 1.27-1.18 (m, 1H), 1.23 (s, 3H), 1.17-1.10 (m, 1H), 1.05 (s, 3H), 1.02 (s, 3H), 0.98 (s, 3H), 0.95-0.80 (m, 3H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 212.89 (C-3), 169.96 (C-31), 167.60 (C-16), 142.89 (C-21 or C-23), 141.21 (C-21 or C-23), 137.82 (C-34), 129.01 (C-37), 128.41 (2 aromatic carbons), 127.29 (2 aromatic carbons), 120.69 (C-20), 109.96 (C-22), 78.49 (C-17), 73.33 (C-7), 69.32 (C-14), 56.76 (C-15), 49.09 (C-1), 46.78 (quaternary), 42.97 (C-5), 42.01 (quaternary), 41.31 (quaternary), 40.00 (C-2), 38.74 (quaternary), 37.91 (C-9), 34.92 (C-33), 25.67 (CH_2), 24.98 (CH_3), 23.54 (C-6), 22.00 (CH_3), 21.02 (CH_3), 18.28 (CH_3), 17.02 (CH_3), 16.33 (CH_3), 13.32 (CH_2).

PREPARATION OF 7-HYDROXYGEDUNIN (75).

A 1.0 M solution of sodium methoxide in methanol (1.0 mL, 1.0 mmol, see note below) was added to a solution of gedunin (75 mg, 0.16 mmol) in methanol (10 mL). The reaction mixture was stirred for 42 h and amberlite resin (approximately 25 mg, washed with water and methanol) was added. The pH changed from 9.0 to 7.0 after 15 min and the resin was removed by filtration through a sintered glass funnel. The solvent was removed *in vacuo* affording a yellow solid. Flash chromatography (5 g of silica gel, 3:1 hexanes/ethyl acetate) yielded **75** (63 mg, 91%) as white crystals (mp: 251-253°C, lit.[62]: 250-254°C). The spectra obtained matched those previously reported.[62]

Note: A 1.0 M sodium methoxide in methanol stock solution was prepared by the addition of sodium metal (1.15 g, 0.05 mol) to methanol (50.0 mL) at 0°C. The solution was stirred at 0°C until most of the sodium had reacted. The solution was warmed to room temperature and allowed to stir for an additional 1 h.

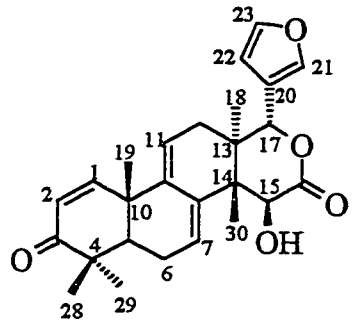


75

$C_{26}H_{30}O_5$ MW=422.52 g/mole

PREPARATION OF COMPOUND 80.

Concentrated hydrochloric acid (1.6 mL) was added to 7-hydroxygedunin **75** (133 mg, 0.302 mmol) in 95 % ethanol (12.6 mL) at room temperature. The reaction mixture was heated to 37°C, allowed to stir for 52 h and neutralized to pH 7.0 with 2.0 M aqueous sodium hydroxide. The mixture was diluted with ether (100 mL) and washed with water (2 x 20 mL) and brine (20 mL). The organic layer was dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated *in vacuo* affording a yellow oily solid. Flash chromatography (5 g of silica gel, 9:1 hexanes/ethyl acetate) yielded **80** (41 mg, 32%) as a white solid.



80

$C_{26}H_{30}O_5$ MW=422.52 g/mole

mp 120-122°C

MS [EI, *m/z* (%)]: 422 [MH^+] (100), 162 (72), 143 (56).

HRMS calcd. for $C_{26}H_{30}O_5$: 422.2094; found: 422.2070.

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 3459 (OH), 1731 (C=O), 1670 (C=O), 1634 (C=C), 1616 (C=C).

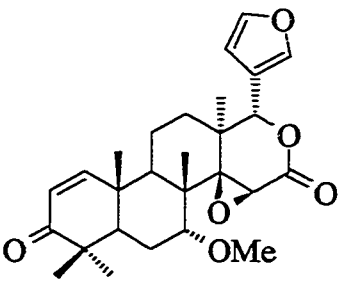
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 7.40 (s, 1H, H-21 or H-23), 7.39 (s, 1H, H-21 or H-23), 7.03 (d, J=10.4 Hz, 1H, H-1), 6.35 (brs, 1H, H-22), 6.24 (brs, 1H, H-7), 5.96 (d, J=10.4 Hz, H-2), 5.36 (brs, 1H, H-11), 5.35 (s, 1H, H-17), 4.49 (s, 1H, H-15), 3.37 (s, 1H, 15-OH), 2.32-2.26 (m, 2H, H-6), 2.22-2.15 (m, 1H, H-12), 1.99 (dd, J=10.4, 6.1 Hz, 1H, H-5), 1.70 (dd, J=18.1, 5.3 Hz, 1H, H-12), 1.19 (s, 3H, Me), 1.18 (s, 3H, Me), 1.11 (s, 3H, Me), 1.08 (s, 3H, Me), 0.91 (s, 3H, Me).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 203.82 (C-3), 174.93 (C-16), 152.36 (C-1), 143.13 (C-21 or C-23), 140.87 (C-8 or C-9), 140.55 (C-21 or C-23), 135.70 (C-8 or C-9), 125.72 (C-2), 123.69 (C-7), 120.42 (C-20), 112.03 (C-11), 109.55 (C-22), 80.03 (C-17), 70.96 (C-15), 45.63 (C-5), 45.11 (quaternary), 44.06 (quaternary), 39.20 (quaternary), 38.95 (quaternary), 34.32 (C-12), 23.84 (C-6), 23.49 (CH_3), 21.23 (CH_3), 20.11 (CH_3), 16.07 (CH_3), 14.82 (CH_3).

PREPARATION OF 7-METHOXYGEDUDIN (77).

A 2.5 M solution of *n*-butyllithium in hexanes (26 μL, 0.066 mmol) was added to a solution of hexamethyldisilazane (15 μL, 0.071 mmol) in dry tetrahydrofuran (0.5 mL) at -78°C. The solution was stirred for 5 min and hexamethylphosphoramide (13 μL, 0.076 mmol) was added. The solution was stirred for 5 min and 7-hydroxygedunin **77** (22 mg, 0.051 mmol) in dry tetrahydrofuran (0.4 mL + 2 x 0.3 mL washes) was added via cannula. The reaction mixture was stirred for 25 min at -78°, warmed to -20° C and methyl triflate (17 μL, 0.076 mmol) was added. The reaction mixture was stirred for 25 min, quenched with 10% aqueous sodium hydroxide (1 mL), poured into water (5 mL) and extracted

with ethyl acetate (3 x 15 mL). The combined organic extracts were dried (anhydrous magnesium sulfate), filtered through a sintered glass funnel and concentrated *in vacuo* affording a yellow oil. Flash chromatography (4 g of silica gel, 9:1 hexanes/ethyl acetate) yielded **77** (19 mg, 83%) as a pale yellow solid.



77

$C_{27}H_{34}O_6$ MW=453.52 g/mole

mp 128-130°C

MS [EI, *m/z* (%)]: 454 [MH^+] (1), 331 (43), 299 (100), 149 (41).

HRMS calcd. for $C_{27}H_{34}O_6$: 454.2356; found: 454.2334

IR (CH_2Cl_2, thin film) ν (cm^{-1}): 1744 (C=O), 1668 (C=O), 1617 (C=C).

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 7.37 (d, J= 4.2 Hz, 2H, H-21 + H 23), 7.08 (d, J=10.2 Hz, 1H, H-1), 6.32 (d, J= 0.7 Hz, 1H, H-22), 5.80 (d, J=10.2 Hz, 1H, H-2), 5.56 (s, 1H, H-17), 3.59 (s, 1H, H-15), 3.28 (s, 3H, 7-OMe), 2.89-2.88 (m, 1H, H-7), 2.42 (dd, J=12.5, 5.8 Hz, 1H, H-9), 2.26 (dd, J=13.0, 1.9 Hz, 1H, H-5), 1.93-1.48 (m, 6H, H-6, H-11 + H-12), 1.17 (s, 3H, Me), 1.16 (s, 3H, Me), 1.13 (s, 3H, Me), 1.08 (s, 3H, Me), 1.05 (s, 3H, Me).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 204.48 (C-3), 168.04 (C-16), 157.72 (C-1), 142.90 (C-21 or C-23), 141.12 (C-21 or C-23), 125.69 (C-2), 120.70 (C-20), 109.94 (C-22), 79.22 (C-7), 78.39 (C-17), 70.07 (C-14), 57.36 (C-15), 55.58 (C-31, OMe), 44.54 (C-5), 44.17 (quaternary), 44.09 (quaternary), 40.01 (quaternary), 38.74 (C-9), 38.31 (quaternary), 27.39 (CH_3), 26.42 (CH_2), 21.49 (CH_3), 20.23 (CH_2), 19.88 (CH_3), 18.50 (CH_3), 17.77 (CH_3), 15.01 (CH_2).

Appendix for Chapter 4

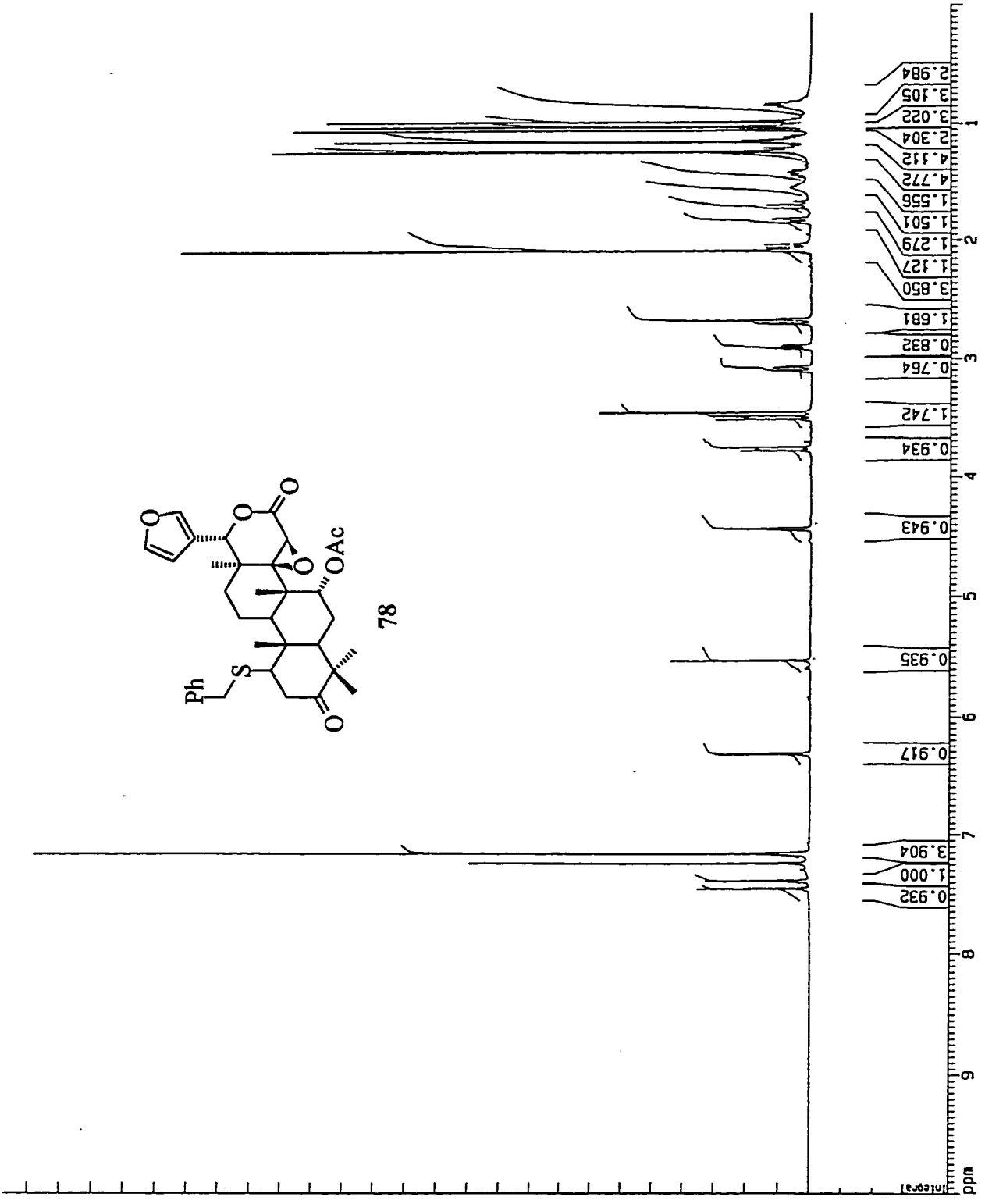


FIGURE 4.2.1: ^{1}H NMR SPECTRUM OF 1-BENZYLMERCAPTOGEDUNIN (78)

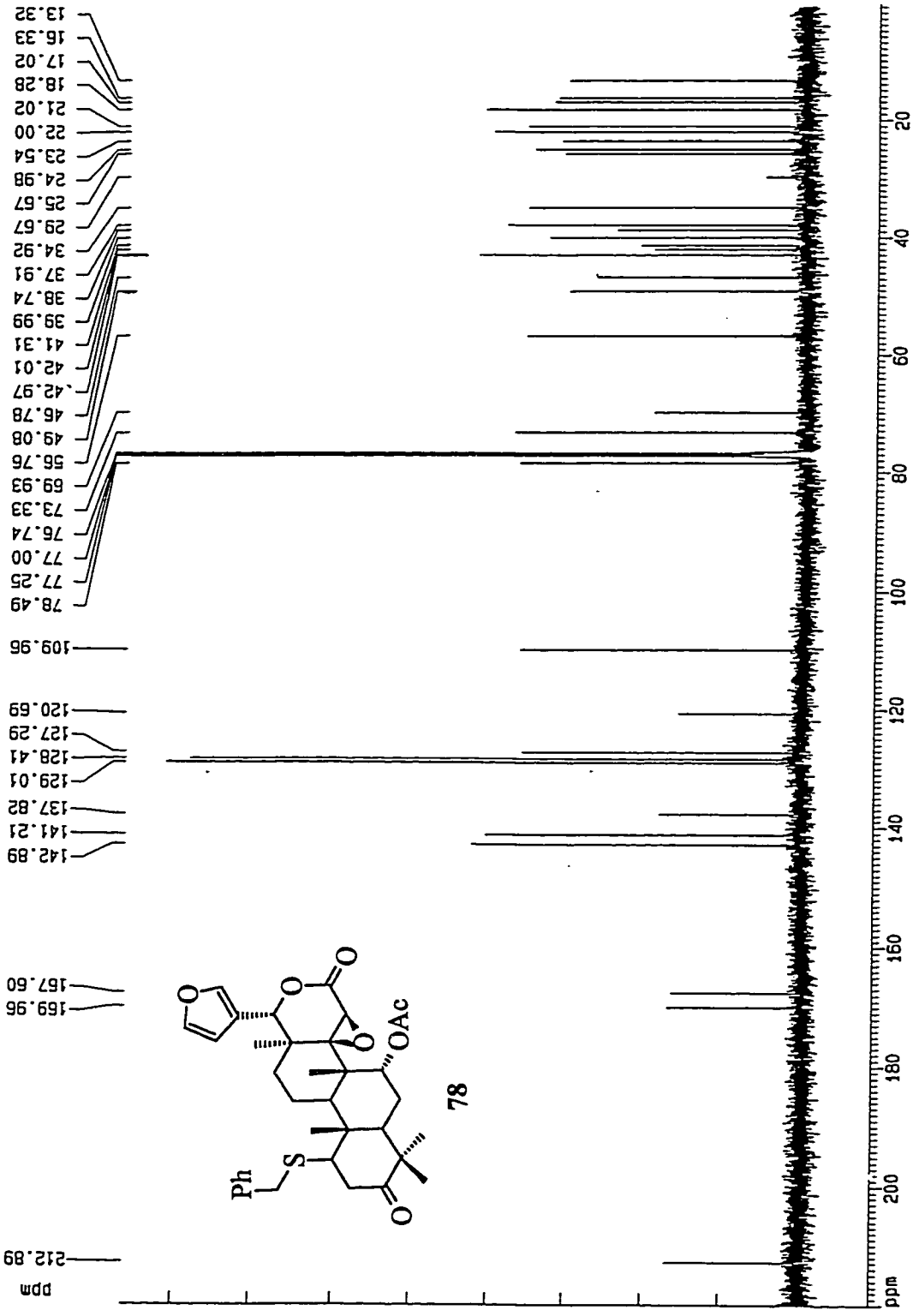


FIGURE 4.2.2: ^{13}C NMR SPECTRUM OF 1-BENZYLMERCAPTOGEDUNIN (78)

FIGURE 4.3.1: ^{1}H NMR SPECTRUM OF COMPOUND 80

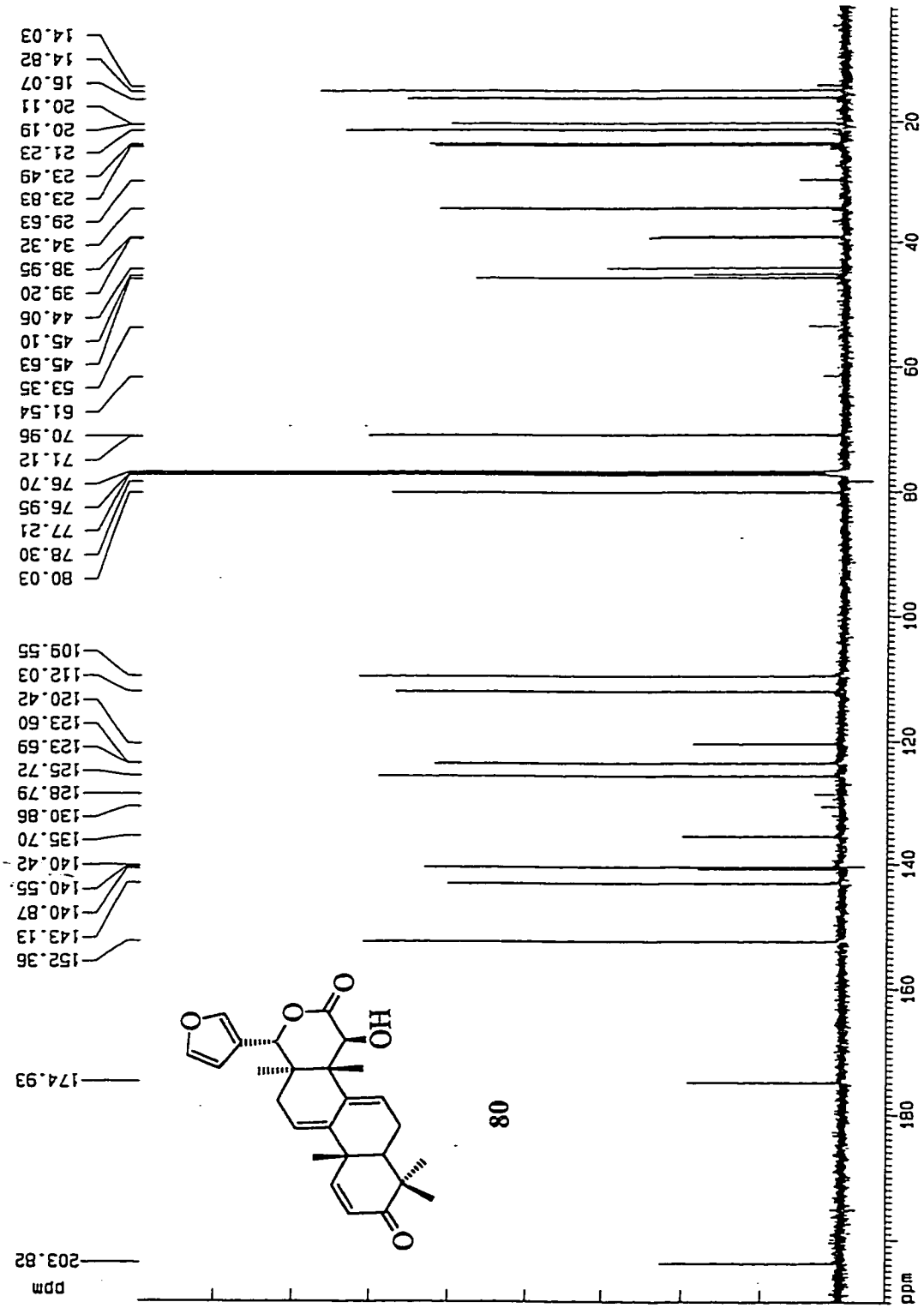


FIGURE 4.3.2: ^{13}C NMR SPECTRUM OF COMPOUND 80

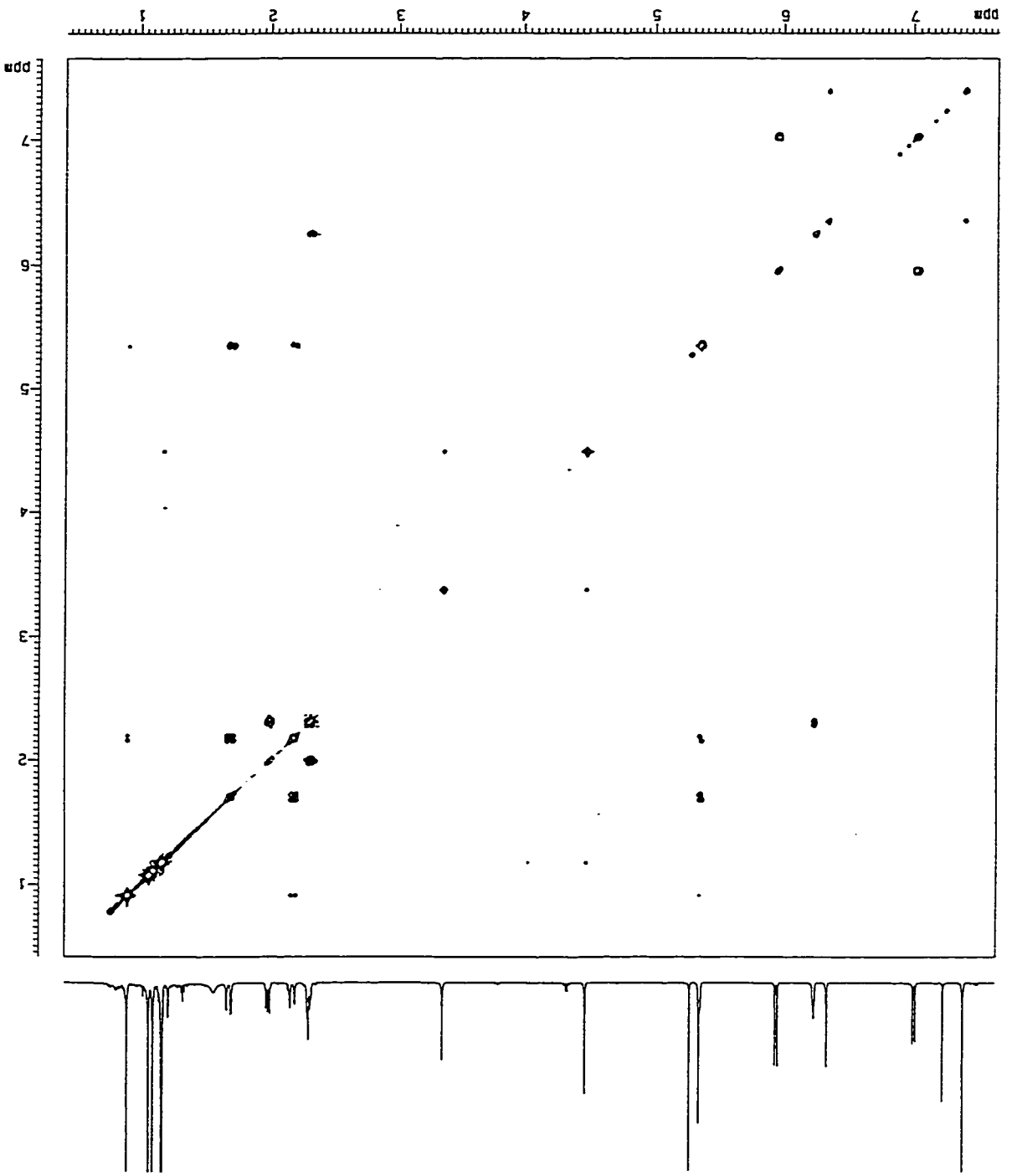


FIGURE 4.3.4: ^{1}H-^{1}H CORRELATION SPECTRUM OF COMPOUND 80

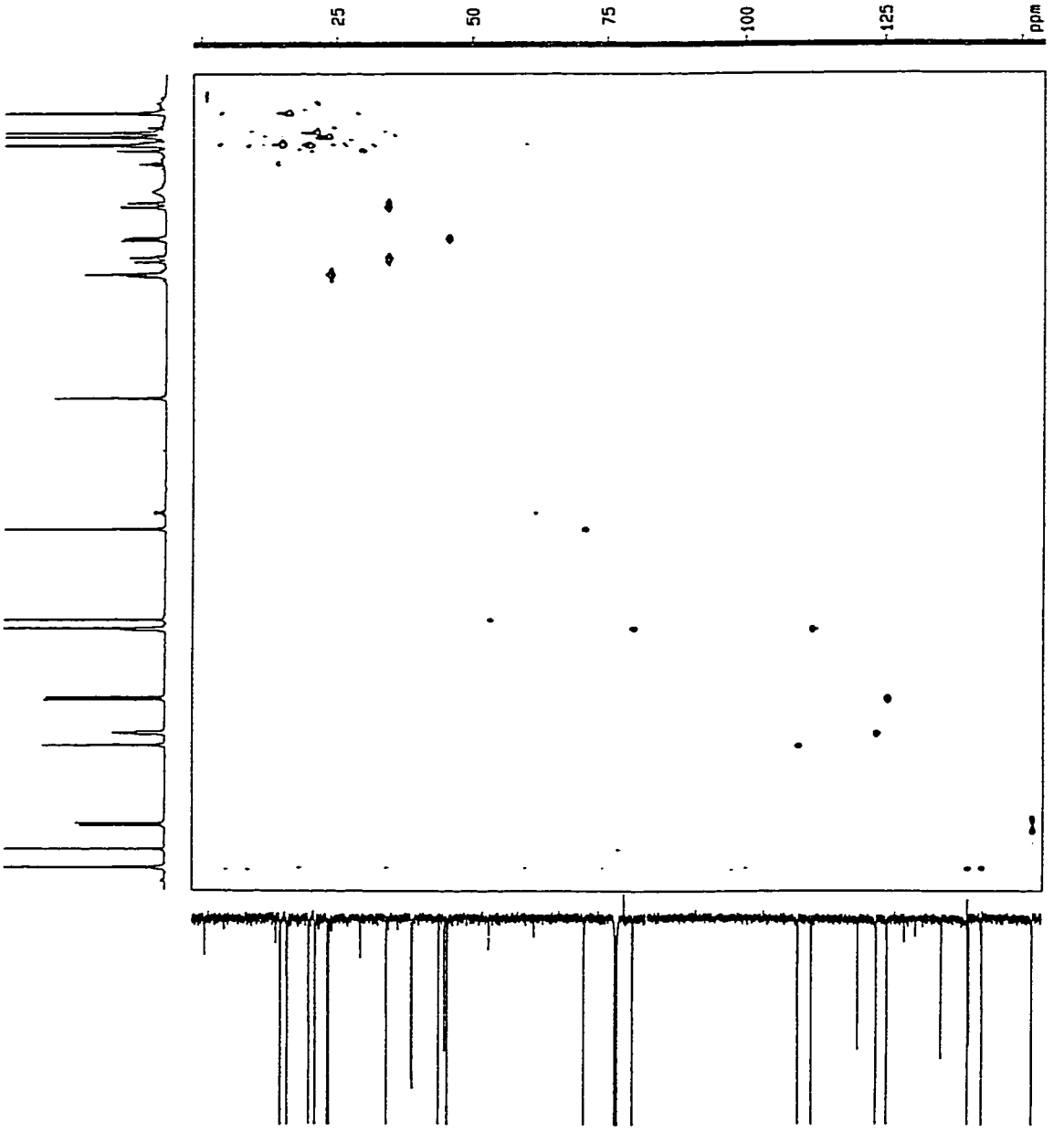


FIGURE 4.3.5: ^{1}H-^{13}C CORRELATION SPECTRUM OF COMPOUND 80

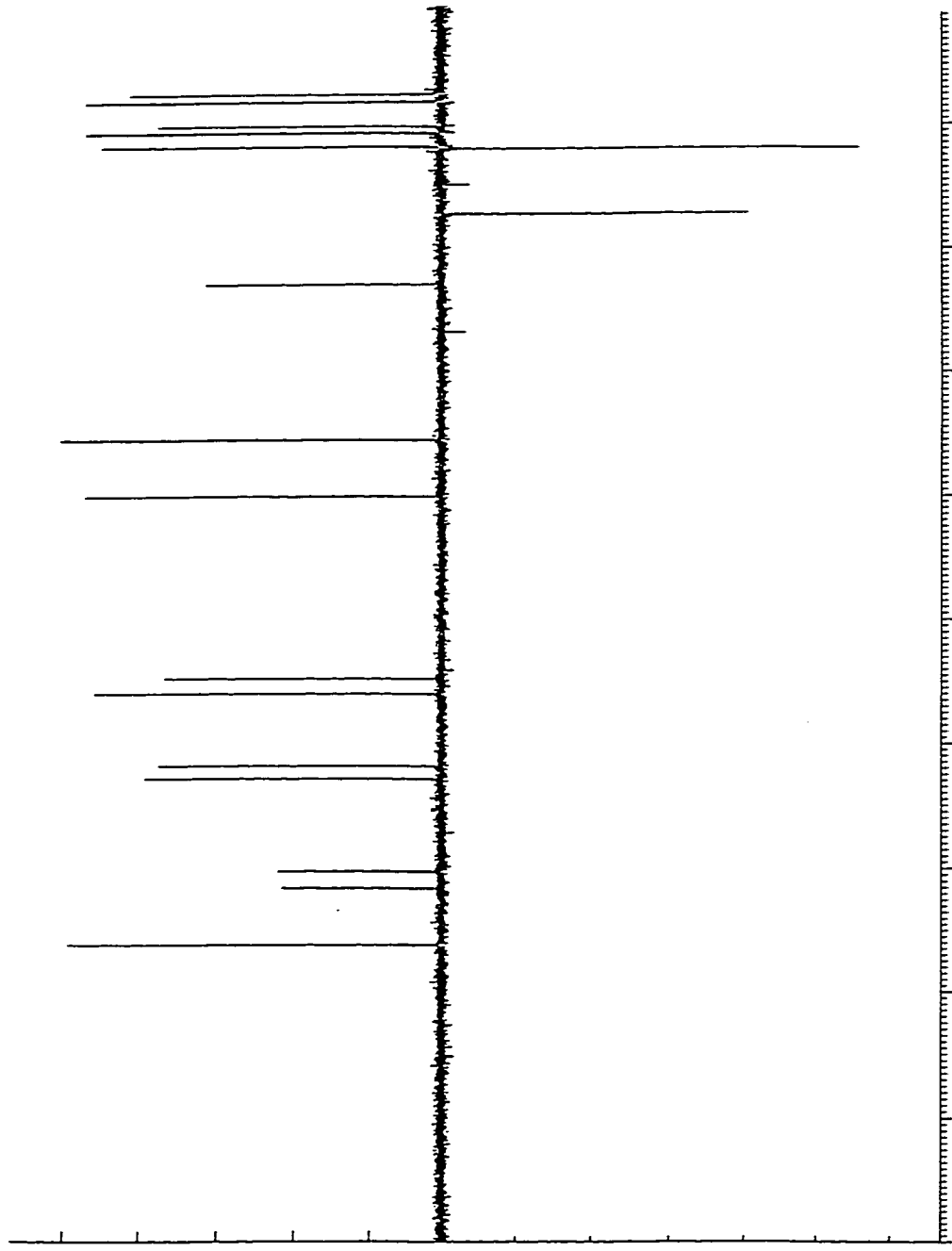


FIGURE 4.3.6: DEPT SPECTRUM OF COMPOUND 80

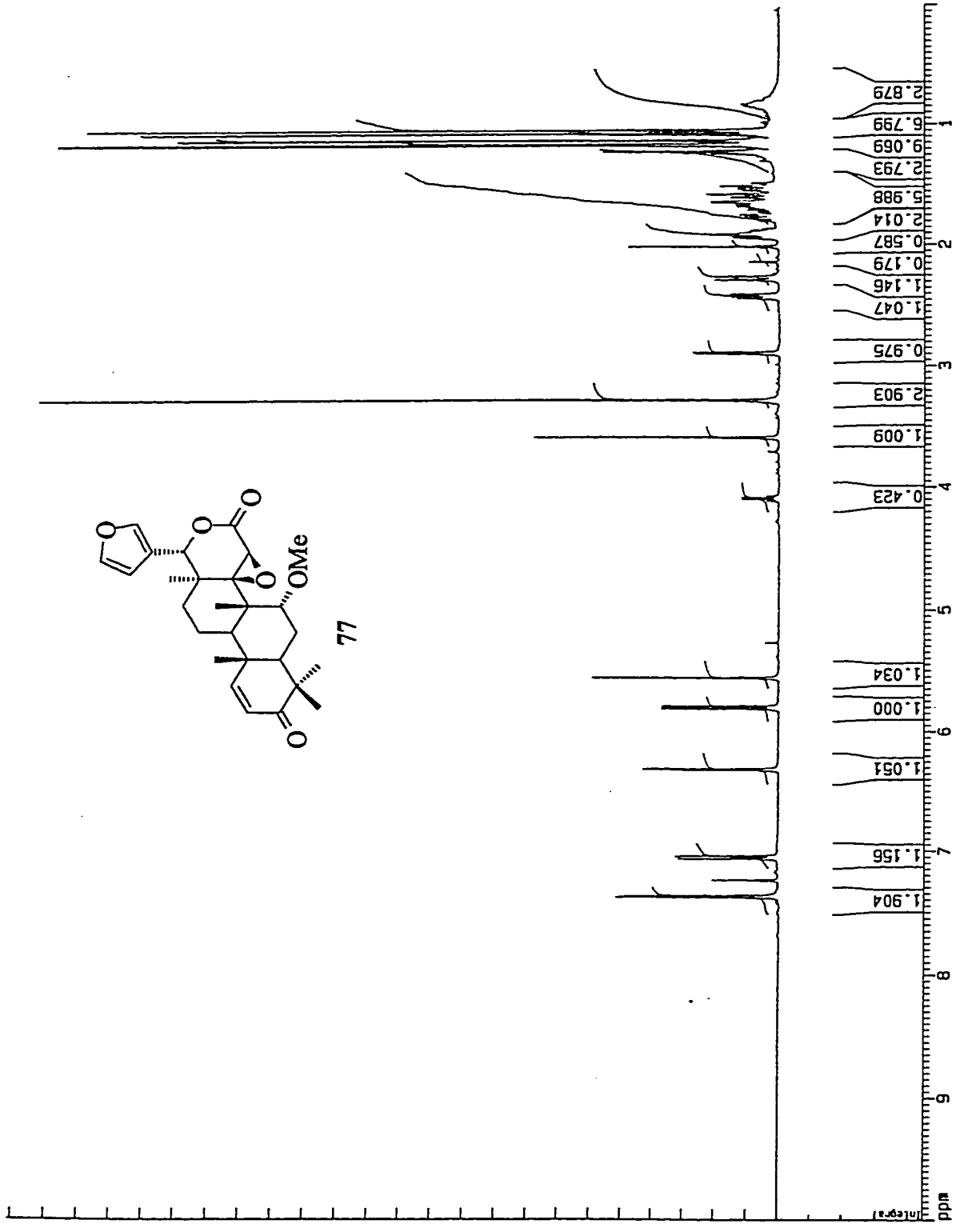


FIGURE 4.4.1: ^{1}H NMR SPECTRUM OF 7-METHOXYGEDUNIN (77)

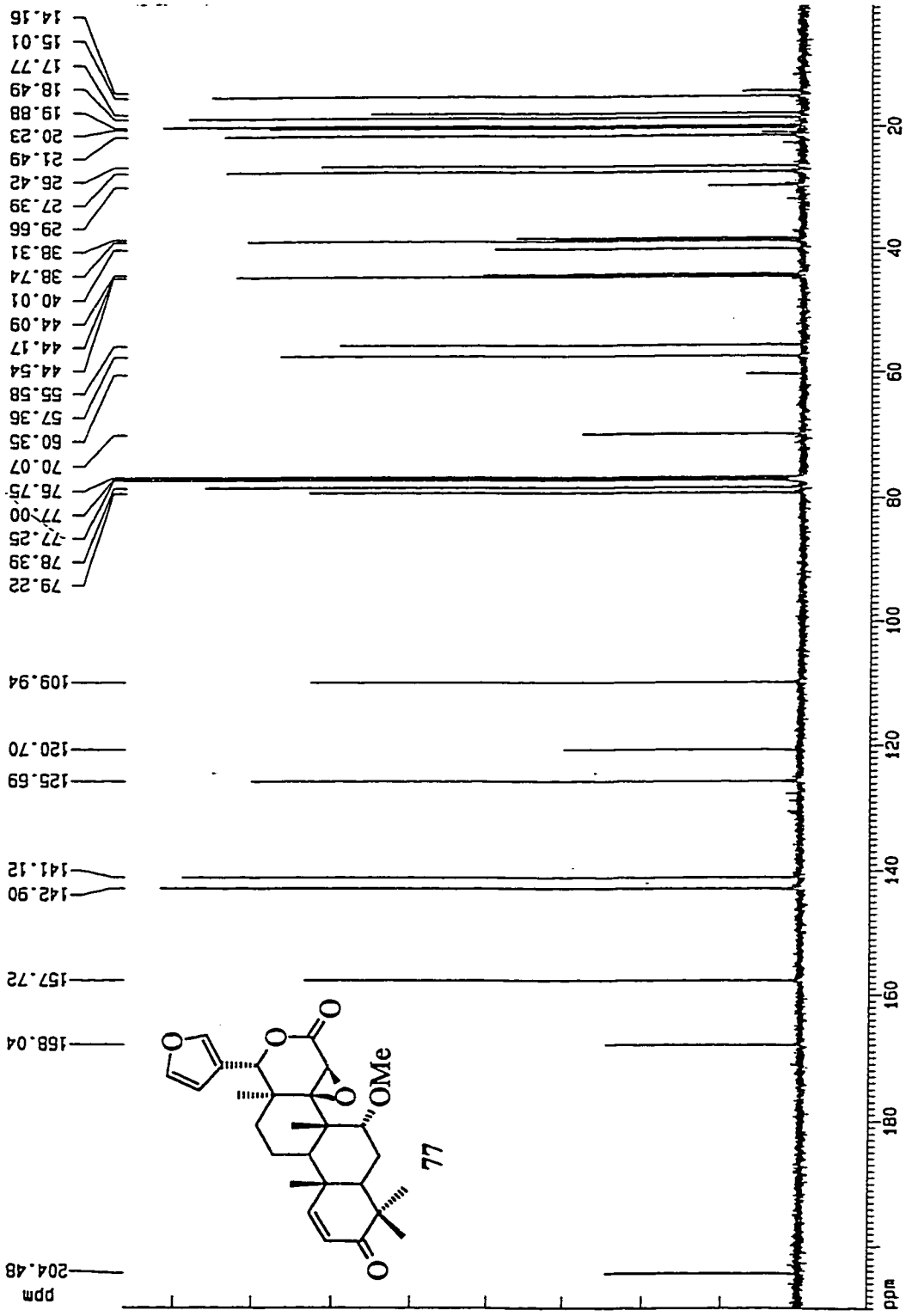


FIGURE 4.4.2: ^{13}C NMR SPECTRUM OF 7-METHOXYGEDUNIN (77)

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CLAIMS TO ORIGINAL RESEARCH

1) The first total synthesis of trichiliasterone A, 2-hydroxyandrosta-1,4-diene-3,16-dione **1** in 6% overall yield via eight steps from testosterone **9**.

2) The transformation of trichiliasterone A, **1**, into 2-methoxyandorsta-1,4-diene-3,16-dione, **1a**.

3) The partial synthesis of trichiliasterone B, 3β-hydroxypregna-2,16-dione **2**.

4) An alternate synthesis of the naturally occurring 3β-hydroxypregnan-16-one **5**.

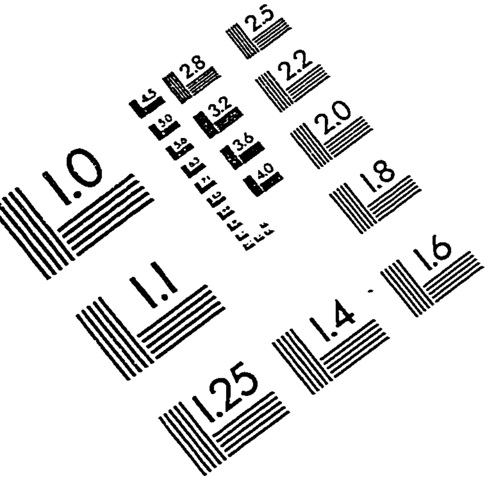
5) The preparation of 1-benzylmercaptogedunin **78**.

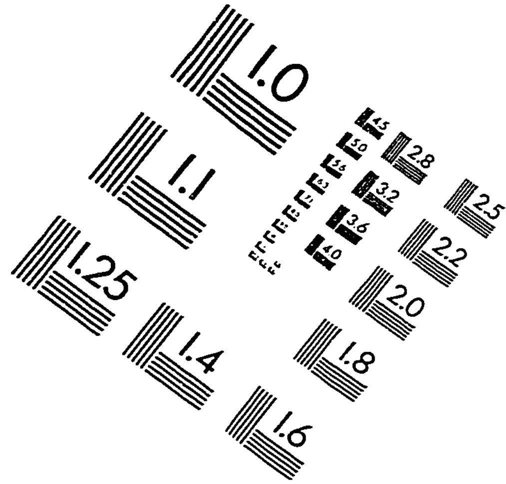
6) The preparation and structure elucidation of compound **80**, which was the product of the acid catalyzed rearrangement of 7-hydroxygedunin **75**.

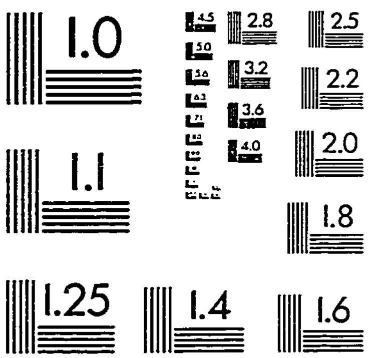
7) The postulated mechanism for the acid catalyzed rearrangement of 7-hydroxygedunin **75**.

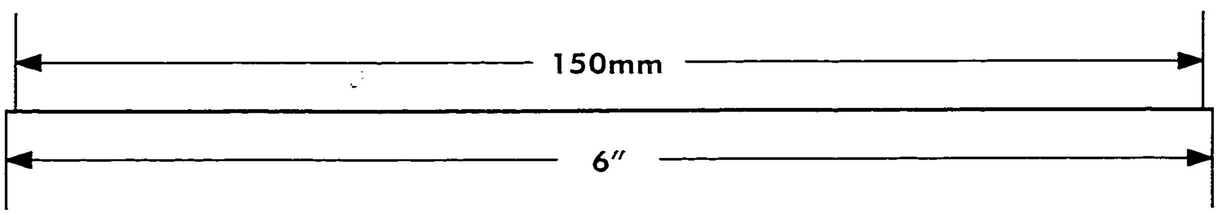
8) The preparation of 7-methoxygedunin **77**.

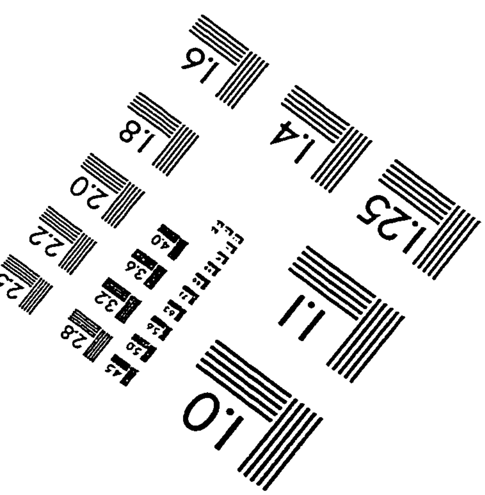
IMAGE EVALUATION TEST TARGET (QA-3)

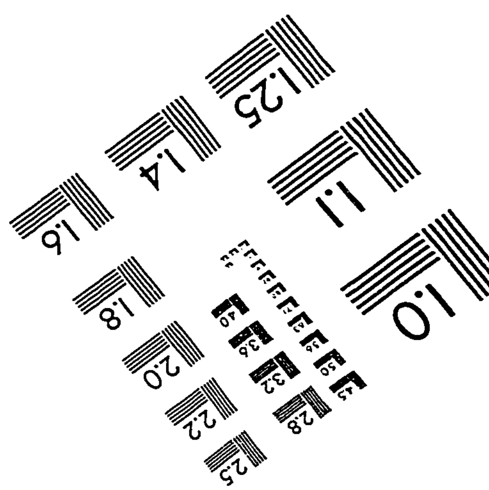












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