(THE NaH PROCEDURE FOR METHYLATION.)

THE METHYLATION OF N-SUBSTITUTED AMINO ACIDS AND OF SOME PHENOLIC AND ALCOHOLIC HYDROXYL GROUPS

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LIST OF ABBREVIATIONS

DMF	N,N-dimethylformamide
hr	hour
i.r.	infrared
MeAA	N-methylamino acid
min	minute
mol	mole
n.m.r.	nuclear magnetic resonance
THF	tetrahydrofuran
w	weight
μmol	micromole

Protecting Groups

Cbz	carbobenzoxy (benzyloxycarbonyl)
Et	ethyl
IsoPro	isopropyl
Me	methyl

TABLE OF CONTENTS

		Page No.
	Summary	iv
I.	INTRODUCTION	
	A. Methylation of Substituted Amino Acids........	1
	B. Methylation of Phenolic and Alcoholic Hydroxyl Groups...........................	10
II.	RESULTS AND DISCUSSION	
	A. Methylation of Substituted Amino Acids........	13
	B. Methylation of Phenolic and Alcoholic Hydroxyl Groups...........................	20
III.	GENERAL DISCUSSION	28
IV.	REAGENTS AND METHODS	
	A. Reagents....................................	35
	1. List of Suppliers........................	35
	(i) Reagents from commercial suppliers.........	36
	(ii) Amino acids.............................	37
	(iii) Amino acid derivatives..................	37

Page No.

Purification and Preparation of Some Reagents.. 37

B. Methods.. 39

1. Chemical.. 39

2. Spectroscopic.. 40

3. Chromatographic.. 41

V. EXPERIMENTAL

A. Preparation of Starting Materials.. 43

B. The NaH Procedure for Methylation under Refluxing Conditions.. 43

C. The NaH Procedure for Methylation at Room Temperature.. 47

1. Methylation of carbobenzoxyleucine - Deprotection with 30% HBr in glacial acetic acid.. 47

2. Methylation of N-carbobenzoxy-O-benzyl tyrosine at room temperature.. 48

Figure 1, n.m.r. of N-methyltyrosine.. 49

D. The NaH Procedure for Methylation as Applied to Methylate Phenolic Hydroxyl Groups.. 51

1. Methylation of o-catechol at room temperature.. 51

Figure 2, n.m.r. of O,O'-dimethyl catechol.. 52

2. Methylation of 2,6-di-tert-butyl-4-methyl phenol at 80°C.. 53

Figure 3, n.m.r. of 2,6-di-tert-butyl-4-methyl-anisole.. 54

Page No.

E. The NaH Procedure for Methylation as Applied to Methylate Alcoholic Hydroxyl Groups.............. 55

1. Methylation of benzyl alcohol at 80°C.......... 55

Figure 4, n.m.r. of methyl benzyl ether............ 56

2. Methylation of triphenyl carbinol at 23°C...... 57

Figure 5, n.m.r. of triphenyl carbinol methyl ether... 58

VI. REFERENCES.. 59

Summary

Previous work in our laboratory had shown that N-protected, N methylamino acids can be prepared by methylating with MeI/NaH in THF/DMF at 80°C. During this reaction the carboxyl group is also esterified. An extension of this procedure to N-carbobenzoxy-tyrosine has shown that the N-methylation proceeded just as well at room temperature in the absence of DMF, and what is even more significant, that the carboxyl group is not esterified under these conditions. It transpired that this had a very important implication, since it has been shown by others in our laboratory that saponification of a protected N-methylamino acid ester causes racemization. Our finding therefore allows the synthesis of stereochemically pure N-carbobenzoxy, N-methylamino acids which were not accessible by the previous methods.

The use of MeI/NaH in THF at room temperature for the methylation of some phenols and alcohols was also investigated. It was found that a few severely hindered compounds, such as triphenyl carbinol and 2,6-di-tert-butyl-p-cresol, which cannot be easily methylated by the usual procedures, could be readily methylated by this procedure.

I. INTRODUCTION

The purpose of this thesis is to explore the applicability of the "NaH procedure for methylation" for the methylation of amino acids with oxygen functional groups. This interest was pursued further to include phenolic and alcoholic hydroxyl groups of diverse organic compounds.

The following introduction to this thesis explains the background information for both of these studies under the titles of "Methylation of Substituted Amino Acids" and "Methylation of Phenolic and Alcoholic Hydroxyl Groups".

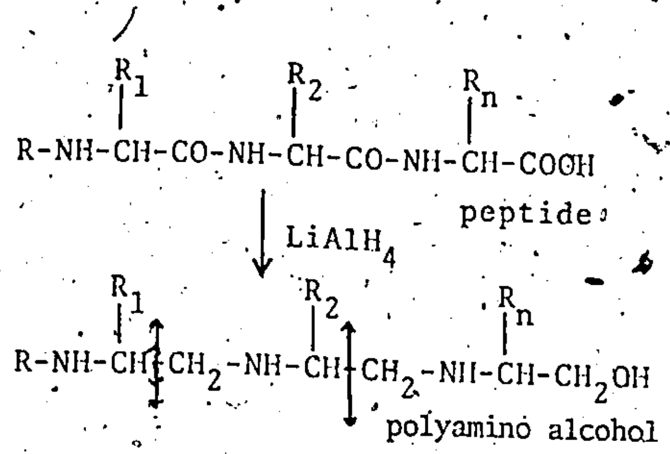
I. INTRODUCTION

A. Methylation of Substituted Amino Acids

Key information leading to increased understanding of a number of important biological processes has been provided by the determination of the amino acid sequence in particular of proteins. Present common practice for such determinations involves enzymatic digestion of the protein followed by column and paper chromatographic separation and purification of the oligopeptide products. The sequence of amino acids in these peptides is then determined by a stepwise degradation procedure which in one way can be monitored by total hydrolysis, with amino acid analysis by

column chromatography, a time consuming procedure. The introduction of mass spectrometry for this purpose was to provide a saving of time and on the whole to simplify sequencing.

Preliminary studies on the use of mass spectrometry for the sequencing of peptides have been done on peptides after reducing the peptide bonds and terminal carboxyl with $LiAlH_4$ to obtain the polyamino alcohols (Biemann et al., 1959). The reason for using polyamino alcohols was that



the fragments resulting from the facile cleavage of the carbon-carbon bond α to the amino group, as shown above, form a repetitive unit which should yield valuable information about the structure of the parent peptide. Also, the conversion of the polyamides into polyamino groups rendered an increase of volatility which is a very important factor to be considered in mass spectrometry. Free peptides,

because of their low volatility and tendency to undergo irregular fragmentation, are not suitable for direct mass spectrometric analysis (Budzikiewicz et al., 1964). Their mass spectra are complex and difficult to interpret when it comes to assigning the ions to their corresponding peaks.

For the purpose of obtaining sufficiently volatile derivatives and simplified mass spectra later studies used N-acetyl derivatives of oligopeptide methyl (ethyl, or tert-butyl) esters which are readily prepared (Kiryushkin et al., 1966; Barber et al., 1965a; Bricas et al., 1965; Barber et al., 1965b; Weygand et al., 1965).

The fragmentation studies of N-acyl peptide esters showed that the unit sequence in the peptide follows quite readily from an analysis of the amino acid type of fragmentation, which involves the rupture of the amide bonds beginning at the C-terminus as shown (Wulfson et al., 1965;

$$\mathrm{R{-}CO} \,|\, \mathrm{NH{-}\overset{R_1}{CH}{-}CO} \,|\, \mathrm{NH{-}\overset{R_2}{CH}{-}CO} \cdots|\cdots \mathrm{NH{-}\overset{R_n}{CH}{-}CO} \,|\, \mathrm{OR}$$

N-acyl peptide derivative esterified

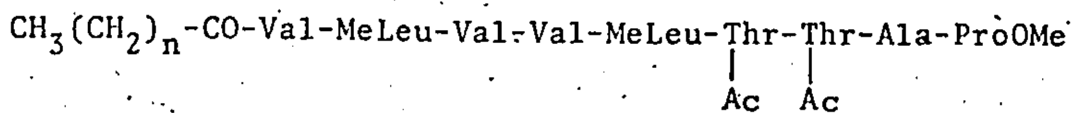
Heyns and Grutzmacher, 1963a, 1963b).

The assumption that the amino acid fragmentation pattern alone was sufficient for the amino acid sequence

determination did not generally hold. According to the data compiled on model substances the mass spectra did not necessarily exhibit every possible sequence peak. Moreover, the fact that some amino acids lose their side chains can complicate matters, and these exceptions must be taken into account when trying to elucidate peptide sequences (Shemyakin et al., 1966).

The usefulness of mass spectrometry for the determination of the sequence of amino acids was recognized. This recognition was strengthened when mass spectrometry was successfully applied for the structural investigations or confirmation of earlier suggested formulae in a series of natural peptidolipids. The first of this series was fortuitine, an acyl nonapeptide methyl ester (Vilkas et al., 1963; Lederer, 1964; Barber et al., 1965c).

Fortuitine is a peptidolipid isolated from Mycobacterium fortuitum and contains equimolar quantities of eicosanoic and docosanoic acids. The final structure was shown to be:



n = 18,20

Fortuitine, the nonapeptide, gave a good spectrum whereas Gramicidin A, the pentadecapeptide, gave no spectrum at all being apparently insufficiently volatile (Barber et al., 1965c). The reason for the low volatility encountered in this study was thought to be the hydrogen bonding due to the presence of the -CO-NH- groups (the peptide bonds). The satisfactory volatility of fortuitine could be explained by the presence of three tertiary amide bonds (due to two MeLeu and one Pro present in the chain). These observations suggested that if a procedure leading to permethylation of the -CO-NH- groupings of oligopeptide derivatives could be found, the resulting modified peptide might be more volatile.

Work on a large number of N-permethylated peptides made it apparent that the mass spectral fragmentation was in all cases simplified. The spectra consisted of "sequence determining" peaks which resulted from -CO-NCH$_3$- cleavage, the intensities of successive peaks decreasing in a regular manner toward higher mass. Therefore the amino acid sequence could be determined within minutes by measuring the mass differences between the major peaks of the spectrum (Thomas et al., 1968). Thus no computer aided interpretation of high resolution spectra as it was introduced to simplify the interpretation of non-methylated peptides would be necessary (Barber et al., 1966; Bieman et al., 1966;

Senn et al., 1966).

The methylation method first used was that of Kuhn which had originally been used for polysaccharides (Kuhn et al., 1955), which employs silver oxide and methyl iodide in DMF (Das et al., 1967). Another method which came into use was the Hakomori method which was first designed for glycolipids (Hakomori, 1964). This method uses the methylsulfinyl carbanion (Corey and Chaykovsky, 1962) as the base for the permethylation of peptides with methyl iodide.

In our laboratory, a new method was recently conceived which employs sodium hydride as the alkylating base with methyl iodide (Coggins, 1970). This method will be known as the NaH procedure for methylation throughout this thesis. Sodium hydride had not been used previously for N-methylation; but it has been used for the alkylation of amides and urethanes, and these were the only two cases recorded for its use (Fones, 1949; Dannley and Lukin, 1957).

This new method was designed for the synthesis of N-methylamino acids. Commercially available carbobenzoxy-amino acids serve as the starting material for N-methylation which is achieved by using methyl iodide as the alkylating agent, sodium hydride as the alkylating base in a tetrahydrofuran, dimethylformamide mixture as the solvent system and

refluxing the reaction flask for 24 hr in an 80°C wax bath. The use of appropriate work-up provides N-methylamino acid esters, carbobenzoxy, N-methylamino acids and other derivatives suitable for peptide synthesis. At about the same time the methylation of N-substituted amino acids with methyl iodide and silver oxide in dimethylformamide giving the corresponding N-substituted-N-methylamino acid methyl ester as final product was reported (Olsen, 1970).

Carbobenzoxy leucine was used as the model compound for the comparison of reagents and conditions already available for permethylation to the new NaH procedure for alkylation (Table I) (Coggins, 1970). The results are summarized as follows: using sodium hydride, the methylation reaction goes to near completion and all of the starting material is accounted for; using silver oxide, the reaction was incomplete but all the starting material was accounted for; using methyl sulfinyl carbanion, the reaction was incomplete and not all the starting material could be accounted for. Therefore, from these and other observations it was suggested that the NaH procedure for methylation which was designed to synthesize N-methylamino acids be used for the permethylation of peptides for mass spectrometric studies. This method was used just for that purpose by

Table I

Comparison of existing permethylating reagents to the new NaH procedure for methylation

Products %	Base	**Sodium Hydride	***Silver Oxide	****Methylsulfinyl Carbanion
	*Ratio of reagents	8:3:1	16:4:1	8:4:1
MeLeu		96	66	23
Leu		1.1	27.1	27.7
Total		97	93	51

* Methyl iodide: Alkylating base: Cbz Leu

** Method of Benoiton and Coggins (1971)

*** Method of Thomas et al. (1968)

**** Method of Vilkas et al. (1968)

Lederer's group (Franek et al., 1969).

This NaH procedure for methylation originally designed for the synthesis of N-methylamino acid is of great importance in this capacity since N-methylamino acids have been shown to be present in nature both in peptides and proteins. Fortuitine, a peptidolipid isolated from Mycobacterium fortuitum already mentioned, contains two N-methylleucines in its peptide chain (Barber et al., 1965a). Also, ε-N-methyllysine was found and isolated from a natural source, the flagellar protein of the bacterium Salmonella typhimurium (Ambler and Rees, 1959). Histones from various higher animals and wheat germ (Murray, 1964), and also rabbit muscle myosin (Kuchl and Adelstein, 1969) were found to contain ε-N-methyllysine. These are just a few of many references showing the presence of an N-methylamino acid in a natural peptide or protein. N-Methylamino acids, sarcosine and N-methylvaline, form an integral part of the structure of Actinomycin D (C_1) (Vining and Waksman, 1954; Brockmann and Grone, 1954). Actinomycins are peptide antibiotics and some of the most potent antitumor agents known (Farber, 1966). The NaH procedure for methylation can provide standards for the identification of these amino acid derivatives. This method can also supply the starting methylated amino acids necessary for the laboratory synthesis

of these mentioned and other peptides and proteins.

B. Methylation of Phenolic and Alcoholic Hydroxyl Groups

The best known method for the methylation of alcoholic hydroxyl groups is known as the Williamson synthesis which utilizes methyl halides or dimethylsulfate under basic conditions (Williamson, 1852). This method has been widely used in chemistry for the methylation of alcohols. It involves the formation of the sodium alcoholate, by dissolving the alcohol in NaOH, and treating it with methyl iodide or dimethylsulfate (Pschorr and Dickhauser, 1911). However, this method gave poor yields and modifications were required. An improved method, with respect to yield, used sodium naphthalene to form the sodium alcoholate and the methylation was completed by the addition of dimethyl sulfate or methyl iodide (Scott et al., 1936).

The methylation of phenol by using NaOH and then Me_2SO_4 resulted in a 72-75% yield of the anisole (Hiers and Hager, 1929; Lewis et al., 1930). The alcohols most readily etherified by the alkaline Me_2SO_4 method were derivatives of methanol containing C_6H_6 residues (Brown et al., 1930; Auwers, 1931).

Dehydration of the alcohols into their respective

ethers by the use of sulfuric acid and heat is a non-specific method, especially if a mixture of alcohols is present. For example, if two alcohols are heated with sulfuric acid and water, a mixture of three respective ethers results (two symmetrical and one a mixed ether) (Senderens, 1924, 1925, 1926).

Catalytic preparations by dry methods consisted of heating the alcohol in the presence of calcined alum ($Al_2(SO_4)_3$) (Mahle and de Godon, 1919, 1920). Boron trifluoride (BF_3) came to be used as a catalyst for the alkylations of phenols and alcohols (Sowa et al., 1935).

The etherification of triphenyl carbinol by butyl alcohol and aromatic alcohols under the catalysis of p-toluenesulfonic acid was reported (Pratt and Draper, 1949). Also, p-toluenesulfonic acid was used for the formation of symmetrical ethers from long chain saturated aliphatic alcohols (Perron and Paquot, 1949).

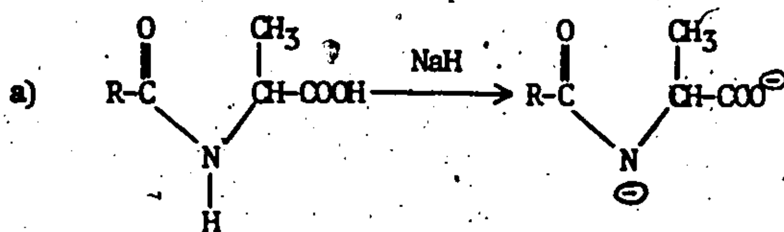
Diazomethane (CH_2N_2) became known as the best methylating agent for acidic hydroxyls. The more weakly acidic phenols and alcohols are methylated at a slower rate. Aluminum alkoxides have been employed as catalysts for the methylation of alcohols with diazomethane but generally with no preparative value (Meerwein and Hinz, 1930). Other

catalysts were introduced giving a wider applicability for methylations with diazomethane; boron trifluoride-diethyl ether (BF_3-Et_2O) (Müller and Rundel, 1958) and fluoroboric acid (FBA) (Neeman, 1959). In principle diazomethane in the presence of a catalyst should methylate alcohols, however exceptions do exist and one example will be shown.

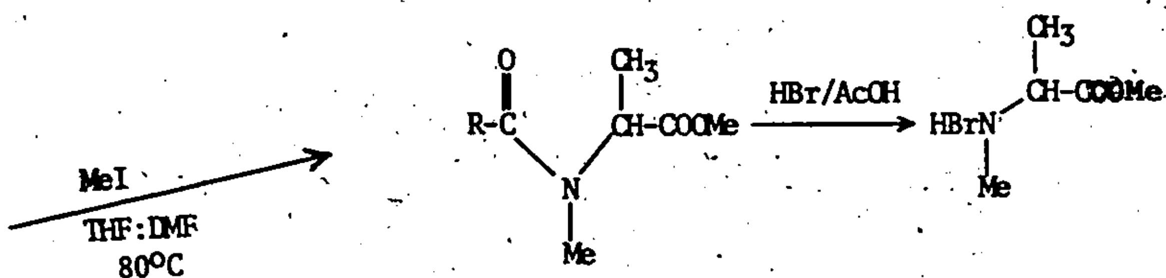
II. RESULTS AND DISCUSSION

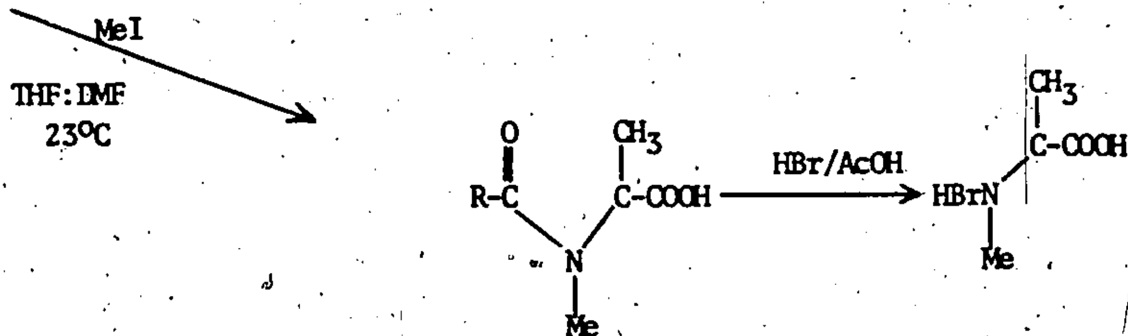
Summations of the NaH Procedure for Methylation

a)

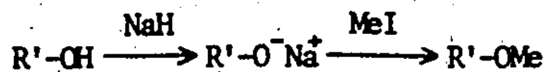


N-Substituted Amino Acid





b) Alcoholic and Phenolic Hydroxyl Groups



THF:DMF
80°C and 23°C

II. RESULTS AND DISCUSSION

A. Methylation of Substituted Amino Acids

The starting point of the work compiled in this thesis was the NaH procedure for methylation established by this laboratory (This thesis, see p. 43 for a detailed description). This procedure entails the dissolving of an N-substituted amino acid in the THF:DMF solvent mixture, adding MeI and NaH in a ratio of 8:3:1 (MeI:NaH:Starting material in moles) and refluxing for 24 hr at 80°C. The product by this procedure is the N-substituted, N-methylamino acid methyl ester.

We came to be interested in the polyfunctional amino acids with emphasis on tyrosine and threonine. Carbobenzoxythreonine was methylated under the established conditions of the NaH procedure for methylation, that is refluxing for 24 hr at 80°C. The n.m.r. of the final product isolated showed that the carbobenzoxythreonine had broken down during the reaction. The proximity of the hydroxyl group which results in β elimination is thought to be the answer. Therefore, the hydroxyl must be protected to obtain N-methylation of threonine.

Carbobenzoxytyrosine, when methylated under these conditions, yielded the N-carbobenzoxy, O,N-dimethyltyrosine

methyl ester (Table II). Also, the methylation of carbobenzoxyleucine and carbobenzoxyphenylalanine was achieved under the set conditions (Table II).

As already mentioned the driving force to further inquiries of this procedure was our interest in tyrosine. The question which arose was whether carbobenzoxytyrosine could be selectively methylated at either its hydroxyl or amino group. The use of dicarbobenzoxytyrosine as starting material was attempted, expecting to obtain only the N-methyltyrosine methyl ester as final product. However, under the established conditions the O-carbobenzoxy came off resulting in a dimethylated tyrosine methyl ester as detected by ir. spectra. Therefore a better protecting group for the hydroxyl group was necessary if N-methylation of tyrosine is the object.

The question concerning the reaction temperature came into focus, that is, it was hoped that the two groups, the hydroxyl and amino groups of tyrosine, would selectively methylate at different temperatures. This led to the trial run of methylating carbobenzoxytyrosine using the set materials (Coggins, 1970) but varying the reaction temperature. This reaction was allowed to stand at room temperature (23°C) being stirred magnetically for 24 hr and not under refluxing conditions. The reaction flask was fitted with a condenser

Table II

Methylation of carbobenzoxyamino acids at 80°C

Starting Material	**Ratio of Reagents (moles)	MeAAMe ester·HBr	
		% Yield (w)	m.p. ^{o}C
CbzLeu	8:3:1	65	105-106
CbzPhe	8:3:1	81	132-133
CbzTyr	8:4:1	*72	138-140

* O,N-dimethyltyrosine methyl ester hydrobromide

** Methyl Iodide: Sodium Hydride: Substituted Amino Acid

and drying tube. The result proved to be very interesting, though it did not answer the question of possible selectivity between the amino and hydroxyl groups. The product of this run was N-carbobenzoxy-N,O-dimethyltyrosine and not its methylester form as identified by ir. spectra.

Therefore, following this discovery, carbobenzoxy-leucine and carbobenzoxyphenylalanine were methylated by the newly found NaH procedure for methylation at room temperature confirming the earlier finding (Table III). The absence of any ester form was quantitatively verified upon amino acid analysis (Table IVb), using the data compiled in Table IVa for the purpose of identification. These two substituted amino acids were methylated both with THF:DMF and THF only as solvent systems at room temperature with no significant differences in the yields of the final products. Therefore the addition of DMF in the solvent system proves to be unnecessary. NaH can be employed in its commercial form, that is as a dispersion in oil. This oil does not interfere in the work-up if HBr/AcOH is used for deprotection. However, for all other instances it may be more desirable to use NaH which has been washed of its oil by means of dry benzene. Washed NaH should be used to dry the solvent for reactions where washed NaH is used in the reaction.

Table III

Methylation of carbobenzoxyamino acids
at room temperature

Starting Material	*Ratio of Reagents (moles)	MeAA HBr	
		% Yield	m.p. ^{o}C
CbzLeu	8:3:1	75	167-170
CbzPhe	8:2:1	78	179-182

* Methyl Iodide: Sodium Hydride: Substituted Amino Acid

*** Table IV

Amino acid analysis
Column 0.9 x 50 cm of AA-15 resin
0.35N sodium citrate buffer pH 4.25, 57°
Buffer flow rate 34 ml/hr
*Methylation at room temperature

Amino Acid	Yield of N-methyl Amino Acid HBr % (w)	Residual Amino Acid** %
Leu	75	1
Phe	78	0

* Conditions of Table III

** Amino acid content of methylated product

*** Amino acid analyzer data - standards
column 0.9 x 50 cm of AA-15 resin
0.35N sodium citrate buffer pH 4.25, 57°

Amino Acid	Constants		Time (m)	
	@@68 ml/hr	@@34 ml/hr	@@68 ml/hr	@@34 ml/hr
@MeLeu	0.74	13.76	34	61
Leu	23.9	40.5	51	100
@MePhe	0.66	24.8	61	104
Phe	22.7	39.0	78	147

@ Compounds obtained from J.R. Coggins

@@ Buffer flow rate

The immediate benefit of this new result was the fact that one obtains the N-carbobenzoxy-N-methylamino acid instead of the methyl ester form which requires saponification to obtain the free carboxyl form. Saponification was often incomplete, therefore this new NaH procedure for methylation at room temperature results to be very useful in avoiding this cumbersome step.

To obtain N-methyltyrosine as initially planned at the commencement of this study N-carbobenzoxy-O-benzyl tyrosine was used as starting material, for when the di-carbobenzoxytyrosine was used as starting material the carbobenzoxy on the hydroxyl came off in the process of the reaction or under the conditions of this reaction. The N-carbobenzoxy-O-benzyl tyrosine was methylated with a ratio of reagents, 8:3:1, in THF only at room temperature for a 24 hr period. After the regular work-up procedure the product was deprotected by hydrogenation and by means of nmr spectra was identified as N-methyltyrosine. Upon amino acid analysis this final product was shown to contain 6% of free tyrosine.

B. Methylation of Phenolic and Alcoholic Hydroxyl Groups

The original interest in the flexibility of the NaH

procedure for methylation established in this laboratory, and its applicability to methylate the hydroxyl function of some protected amino acids, led to further exploration of this procedure with respect to hydroxyl groups of other organic compounds.

p-Nitrophenol was the first compound contained in this section to be methylated in THF:DMF for 24 hr under refluxing conditions and a ratio of reagents of 4:2:1 (Table V). The final product p-nitroanisole was identified by i.r. spectra. It was difficult to isolate the p-nitroanisole due to the presence of the oil in which the NaH had been dispersed. Therefore, it was decided to wash the NaH of its oil just before introducing it into the reaction mixture. Thus, the amounts of NaH, used both as a reagent in the reaction and to dry the solvent prior to its use were washed with dry benzene.

The methylation of p-nitrophenol was repeated, but with the washed NaH and a 90% yield of p-nitroanisole (mp. 54°C) was obtained (Table V).

At this stage of the study, the interesting result of the NaH procedure for methylation at room temperature was discovered with respect to substituted amino acids. As a result, it was decided that the reaction at room temperature

Table V

Alkylation of phenolic hydroxyls

Starting Material	Yield of Alkylated Product % by Weight				Alkylating Agent	Ratio of Reagents moles
ROH	THF:DMF 80°	THF 80°	THF:DMF 23°	THF 23°		Alkyl agent:NaH:ROH
Phenol	75	74	76	74	MeI	8:2:1
"	73	72	70	69	EtI	8:2:1
"	66	80	66	22	IsoProI	8:2:1
p-cresol	60	51				8:2:1
Catechol (o)			88	84	MeI	8:3:1
p-nitrophenol	90	0	85	0	MeI	4:2:1
m-nitrophenol	88	96	87	95	MeI	4:2:1
2,6-di-tert-butyl-4-methylphenol	87	80	87	86	MeI	8:2:1
	*97				MeI	8:2:1

* 1 hr refluxing in wax bath

would also be studied with respect to phenolic and alcoholic hydroxyl groups. Also, it was decided to test THF alone, and THF:DMF side by side as solvent systems to note any existing differences.

p-Nitrophenol methylated at room temperature in the THF:DMF solvent system gave an 85% yield of p-nitroanisole. However, when THF alone was used as a solvent for the methylation of p-nitrophenol, both under refluxing conditions and at room temperature, 84% of the p-nitrophenol was recovered unreacted. Subsequently, m-nitrophenol was methylated both at room temperature and 80°C in both solvent systems. The yields of the respective anisole were in a range of 87 to 95% (Table V). When comparing p-nitro and m-nitrophenol, it can be concluded that not in all preparatory work is DMF dispensable as part of the solvent system for the NaH procedure for methylation. In the case of p-nitrophenol, its presence is essential and this may be attributed to a question of solubility, DMF being more polar than THF. Also the more acidic nature of p-nitrophenol due to its electronic configuration may contribute to this interesting result of requiring DMF in the solvent system to react in this methylation reaction.

Phenol was alkylated using MeI, EtI, and IsoProI as alkylating agents for the purpose of comparing these agents under the various conditions of the NaH procedure for methylation (Table V). The yields of final products, as identified by n.m.r. spectra, were good with the exception of the low 22% yield for isopropylation in THF alone at room temperature (Table V).

p-Cresol was methylated under the refluxing conditions of this procedure in THF:DMF and THF with yields of 50% of methylated product. However, the purity of the starting material p-cresol was in question and this case was not pursued further.

A hindered phenol, 2,6-di-tert-butyl-4-methylphenol, whose methylation was reported in literature (Cohen, 1957), consisted of forming the Na salt in liquid ammonia, adding toluene after evaporating the ammonia and transferring the reaction mixture into a pressure bottle into which MeI was added thereupon replacing the air by nitrogen, the bottle was sealed and the mixture was heated on a steam bath for 18 hr. This reference concerning its lengthy methylation prompted the use of this hindered phenol to test the NaH procedure for methylation. (A detailed preparation is recorded in the Experimental section of this thesis). The yields of the

2,6-di-tert-butyl-4-methylanisole ranged from 80 to 87% for the various conditions of this procedure. Refluxing the 2,6-di-tert-butyl-4-methylphenol in THF:DMF for 1 hr resulted in a 97% yield of the respective anisole (Table V). Thus it was shown how easily the methyl ether of this hindered phenol can be prepared by the NaH procedure for methylation.

As a result of an inquiry into the suitability of the NaH procedure for methylation to methylate the two ortho-hydroxyls of 2-hydroxyestradiol glutathione led into the use of o-catechol as a representative starting material for trial methylation. o-Catechol was methylated at room temperature in both THF and THF:DMF solvent systems yielding 84 and 88% of the O,O'-di-methylcatechol as identified by n.m.r. spectra. However, the problem of solubility still questions the applicability of this NaH procedure for methylation to methylate the two ortho-hydroxyls of 2-hydroxyestradiol glutathione.

Interest was aroused by the reported failure of diazomethane to methylate tri-phenylcarbinol (Neeman, 1959). Therefore, tri-phenylcarbinol was methylated in THF alone at room temperature and refluxing condition using two ratios of reagents (Table VI). The final methylated product, as identified by n.m.r. spectra, was isolated in 74 to 85% yield

Table VI

Methylation of alcoholic hydroxyls

Starting Material	% of Methylated Product		Ratio of Reagents
	THF 80°	THF 23°	MeI:NaH:Starting Material
n-heptanol	35%*	40%*	8:3:2
cyclohexanol	66%*	64%*	8:3:2
benzyl alcohol	90	74	8:3:2
triphenyl carbinol	74 85	85 81	8:3:2 4:2:1

* calculated as amount methylated from nmr. spectra of crude product

with a melting point corresponding to literature (82-83°C, Wang, 1963).

To somewhat complete this part on alcoholic hydroxyl groups it was thought interesting to attempt to methylate an aliphatic primary, a cyclic and an aromatic primary alcohol. Thus n-heptyl, cyclohexanol and benzyl alcohol were methylated in THF alone at 23°C and 80°C. The products were not purified but their identity was established by n.m.r. analysis. Inspection of the n.m.r. curves also allowed the calculation of the relative amounts of starting material and product, giving an indication of the yield (Table iv).

III. GENERAL DISCUSSION

III. GENERAL DISCUSSION

The starting point of the work compiled in this thesis was the NaH procedure for methylation under refluxing conditions, established in this laboratory (Coggins, 1970). When preparing a *N*-methylamino acid according to the conditions of this procedure, the reaction mixture, consisting of a substituted amino acid dissolved in a THF:DMF mixture with MeI and NaH, was allowed to react for a 24 hr period at 80°C. Thus the methylation of a *N*-carbobenzoxyamino acid at 80°C results in the formation of *N*-carbobenzoxy-*N*-methylamino acid methyl ester as final product.

In the course of this study it was found that using the established set of conditions for the NaH procedure for methylation and allowing the reaction mixture to stand at *room temperature*, and not refluxing at 80°C, the final product resulting from a *N*-carbobenzoxyamino acid is the *N*-carbobenzoxy *N*-methylamino acid, and not its methyl ester.

Standard procedures for the synthesis of peptides have been established and widely reviewed (Greenstein and Winitz, 1961; Bodanszky and Ondetti, 1966).

The specific condensation of one amino and one carboxyl

function of two amino acids or peptide derivatives under the mediation of some coupling agent forms a peptide. Specificity is attained when prior to coupling, groups on the amino acids or peptides which would otherwise react and render the condensation non-specific are blocked with an appropriate protective group. Once the required amide bonds in the peptide have been formed, these protective groups are removed by suitable procedures. The problem of racemization during coupling, due to the formation of oxazolone derivatives, can be overcome by synthesizing the peptide stepwise from the C-terminus using suitably N-protected amino acids for coupling to the growing peptide chain.

It now transpires that two widely used and racemization-free reactions, for the deprotection of amino acid derivatives, cause considerable racemization of N-methylamino acid derivatives (Benoiton and McDermott, 1972b). Deprotection of N-carbobenzoxy-N-methylamino acids with anhydrous acid, HBr ~~in glacial~~ acetic acid, results in racemization. However, no racemization was observed when water was added to the acetic acid. Also, deprotection of N-methylamino acid methyl esters, whether uncombined or present at the C-terminus in a peptide, by saponification resulted in racemization under conditions when saponification of amino acid esters

gave little or no racemization.

Under the refluxing conditions of the NaH procedure for methylation and also the reported methyl iodide, silver oxide in DMF procedure for methylation (Olsen, 1970); the final product is the *N*-substituted-*N*-methylamino acid methyl ester form, which for deprotection would require saponification. This deprotection procedure, as presently known, would result in a considerable degree of racemization (Benoiton and McDermott, 1972b). Therefore, under the refluxing conditions of the NaH procedure for methylation and the methyl iodide, silver oxide procedure it would be impossible to obtain an optically pure *N*-methylamino acid.

The NaH procedure for methylation at room temperature avoids the requirement of the saponification step for deprotection, since the final product is the *N*-substituted-*N*-methylamino acid and not its methyl ester. This procedure at room temperature can be successfully employed to obtain optically pure *N*-methylamino acids. And when a *N*-methylamino acid is required as the C-terminus of a peptide some other protective group for the carboxyl function can be used which would not require saponification for deprotection, for example *t*-butyl or benzyl esters can be used.

Because our products had been obtained by deprotection with HBr/acetic acid it is likely that they were partially racemized. For this reason, their optical purity was not investigated. McDermott, in our laboratory, however, has shown by circuitous methods (by preparing dipeptides and analyzing for the diastereomeric products with an analyzer) that our new methylation procedure does give optically pure compounds.

The racemization of N-substituted-N-methylamino acids during saponification might proceed by direct α-proton abstraction. For in N-monosubstituted amino acid derivatives such as carbobenzoxy leucine ester, the N-H group is generally more acidic than the α-C-H, and so will ionize first, thus protecting the α-C-H from ionization, that is protecting against racemization. No such effect exists for the N-substituted-N-methylamino acids. The case of racemization of N-substituted-N-methylamino acids may be due to the absence of this protective effect of the ionization of the -N-H which is present in unmethylated amino acid derivatives (Benoiton and McDermott, 1972b).

The NaH procedure for methylation at room temperature was used to prepare N-methyltyrosine from N-carbobenzoxy-O-benzyl tyrosine as starting material in THF as solvent. However, upon amino acid analysis it was found that the final product, after deprotection by hydrogenation, contained not only N-methyltyrosine but also 6% tyrosine. Thus the methylation was incomplete. Purification of this mixture and isolation of the N-methyltyrosine is a hard task due to the presence of two very similar compounds. Whether this procedure is useful in preparing the N-methyltyrosine is hard

to evaluate for an insufficient amount of starting material was available. A change in the ratio of reagents or the use of THF:DMF as solvent might result in an increase of final methylated product.

The use of either THF or THF:DMF as solvent systems for the methylation of substituted amino acids appeared to make little or no difference in the yields of final product. Also the use of either solvent system for methylations at room temperature appeared to have no effect on the form of the methylated product, which was the substituted N-methylamino acid and not its methyl ester.

In choosing between solvent systems, THF or THF:DMF, for the methylation of hydroxyl containing compounds, one must keep in mind the fact that once the reaction mixture of the NaH procedure for methylation has reacted for the specified period evaporation of the solvents is the next step. This must be kept in mind especially if the final methylated product has an unknown or reported boiling point lower than either solvent THF (b.p. 65°C) and DMF (b.p. 153°C). Due to the rather high boiling point of DMF, in many cases THF was used as the solvent system for methylation reactions.

However, there appears to be no choice as to which solvent system to use to methylate p-nitrophenol at either

room temperature or under refluxing condition; since in THF alone no methylated product was detected at either condition with a recovery of 85% of p-nitrophenol. In this case the presence of DMF is imperative, whereas for the methylation of m-nitrophenol no such difference exists. DMF appears to be necessary to solubilize the more acidic compound, p-nitrophenol. The NaH procedure for methylation has proven to be an efficient procedure for the methylation of phenolic hydroxyls. o-Catechol was methylated at the two o-hydroxyls giving a yield of 84-88% of the O,O'dimethylcatechol. The methylation of the hindered phenol, 2,6-di-tert-butyl-4-methyl-phenol, ranged from 80 to 87% yield of the anisole when reaction mixture was allowed to react for 24 hr. When the methylation reaction was allowed to react for 1 hr in the THF:DMF solvent system under refluxing conditions this hindered phenol gave a 97% yield of the anisole (Table V).

In contrast to the diazomethane procedure, the NaH procedure proved to be very efficient in methylating triphenyl carbinol, a yield of 74-85% being attained. Good yields of methylated product have also been obtained for the cylic and aromatic primary alcohol, cyclohexanol (64-66%) and benzyl alcohol (74-90%) respectively. However,

the NaH procedure is not very efficient for methylation of an aliphatic primary alcohol, e.g. heptyl alcohol, a yield of only 35-40% being obtained (Table VI).

IV. REAGENTS AND METHODS

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A. Reagents

1) *List of Suppliers:*

1.	A	Aldrich Chemical Company, Milwaukee, Wisconsin, U.S.A.
2.	B	J.T. Baker, Phillipsburg, New Jersey, U.S.A.
3.	BDH	BDH Chemicals, Poole, Dorset, England.
4.	E	Eastman Organic Chemicals, Rochester, New York, U.S.A.
5.	F	Fisher Scientific, Fair Lawn, New Jersey, U.S.A.
6.	Fl	Fluka AG, Buchs SG, Switzerland.

7	G	General Biochemicals, Chagrin Falls, Ohio, U.S.A.
8	MCB	Matheson, Coleman, and Bell, Norwood, Ohio, U.S.A.
9	S	Sigma Chemical Corporation, Saint Louis, Missouri, U.S.A.
10	P	Pierce Chemical Company, Rockford, Illinois, U.S.A.

(i) Reagents from commercial suppliers

Benzyl alcohol B

Catechol (o) F

Carbobenzoxy chloride P

Cresol F

Cyclohexanol F

2,6-Di-tert-butyl-4-methyl-phenol A

Ethyl iodide B

Heptyl alcohol E

2-Iodopropane E

Methyl iodide F

m-Nitrophenol S

p-Nitrophenol F

Phenol F

10% Palladium on powdered charcoal MCB

Sodium hydride, dispersion in oil BDH, Fl

Trityl chloride A

All other reagents were reagent grade.

(ii) *Amino acids*

L-Leucine G

L-Phenylalanine G

(iii) *Amino acid derivatives*

N-Carbobenzoxy tyrosine P

N-Carbobenzoxy-O-benzyl tyrosine S

Purification and Preparation of Some Reagents.

N,N-Dimethylformamide:

This solvent was purified by shaking with solid KOH and then CaO, followed by distillation (Leader and Gormley, 1951).

Dry Benzene:

Benzene, reagent grade, was kept over sodium metal.

30% Hydrogen Bromide in Glacial Acetic Acid:

This was prepared by passing dry HBr gas into cooled glacial acetic acid in a flask, protected with a drying tube, until the expected weight increase occurred. Saturated HBr in AcOH is about 36-37%.

Sodium Hydride:

NaH is commercially available in the form of a dispersion in oil. NaH dispersions from different commercial suppliers have been the subject of a study carried out in this laboratory (Benoiton and McDermott, 1972a). The reason for this study was to see how accurate were the stated percentages on the labels of the commercially available NaH dispersions. It must be noted that NaH dispersions do contain a certain amount of NaOH, which can increase upon frequent usage of the dispersion; this in turn may decrease the efficiency of the NaH procedure for methylation. The NaH used throughout this work was a 50% dispersion in oil, which when tested was shown to contain 47% of NaH. In all preparations an excess of NaH was used to ensure that enough was present.

Tetrahydrofuran:

THF when purified by shaking with solid KOH and

then distilled over $LiAlH_4$ (Corey, 1966) tends to peroxidise readily. Therefore, THF supplied by Baker which contains hydroxytoluene was used without purification.

B. Methods

1) *Chemical:*

Hydrogenation:-

Hydrogenations were carried out at 1 atm pressure in an apparatus similar to that described by Wiberg (1960) with palladium on powdered charcoal as catalyst.

Melting point:-

Melting points were determined using a Hoover Capillary Melting Point Apparatus (from A.H. Thomas, Philadelphia). Values given are uncorrected.

Pre-drying THF:DMF and THF:-

The THF:DMF and THF alone solvent systems were pre-dried immediately before use with NaH dispersion or washed NaH. NaH dispersion, 0.2 g, per 10 ml of solvent was used to dry either solvent system used for the alkylation of carbobenzoxyamino acids which were to be deprotected

with HBr/AcOH. Otherwise the same amount of NaH dispersion was suspended in dry benzene, to rid of its oil, and filtered before being used to dry either solvent system. The solvent was used immediately after filtration.

Refluxing conditions:-

In all alkylating procedures the flask was fitted with a condenser and drying tube. The alkylations at 80°C were done by immersing the flask in a wax bath (80°C), under which condition THF refluxes vigorously. For alkylations done at room temperature a magnetic stirrer was used to provide continual vigorous mixing.

2) *Spectroscopic*:

Infrared spectra were obtained using a Unicam Model SP200 spectrophotometer. The spectra were scanned in a range 650-5000 cm^{-1} and the polystyrene bands at 1495 and 1603 cm^{-1} were used as references. Oils were run as liquid films between NaCl discs and solids as KBr discs (0.5-1.0 mg of sample in 250 mg of KBr).

Nuclear magnetic resonance spectra were obtained using a Varian Model T60 spectrometer. The probe temperature

was $34^{o}C$ and the samples were run as 10% w/v solutions in deuterochloroform or trifluoracetic acid. Tetramethylsilane 1% v/v was added to the solvents as an internal standard. Spectra were run in the range 0-10τ in trifluoracetic acid, and in the range -2-10τ in deuterochloroform solutions.

3) *Chromatographic:*

Amino acid analysis:-

Amino acid analyses were carried out using a Beckman Model 120B automatic amino acid analyzer according to the method of Spackman et al. (1958). The instrument was fitted with three columns: a long column (0.9 x 50 cm) containing AA-15 resin (Beckman Instruments, Palo Alto, California), a short column (0.9 x 7 cm) containing AA-27 resin (Beckman) and a third column (0.9 x 15 cm) containing Aminex A-5 resin (Bio Rad Laboratories, Richmond, California). Three different eluting buffers were used: 0.20 N sodium citrate, pH 3.28; 0.20 N sodium citrate, pH 4.25; 0.35 N sodium citrate, pH 5.28.

The individual amino acids were determined by the H x W method of Spackman et al. (1958) involving the following formula.

$$\mu mol = \frac{H \times W}{C}$$

H = net height of the peak

W = counting the number of dots above the half height of the peak

C = a constant for each amino acid

mol = concentration of amino acid

Constants of the common amino acids were determined using a calibration mixture (from Bio Rad) and for others standard solutions were prepared and used. Samples were applied after adjusting their pH to 2.2, and when dilution was necessary, 0.2N sodium citrate buffer (pH 2.2) was used so that the final concentration of the sample was in a range 0.1-1.0 μmol/ml.

Due to the rather low color yield of N-methylamino acids with ninhydrin at the normal rate of flow of buffer (68 ml/hr); this fact makes their detection rather difficult. However, it was found that at half-normal rate of flow of eluting buffer (34 ml/hr) the ninhydrin color constants for N- and α-C-methylamino acids increased from 5 to 30 times (Benoiton and Coggins, 1970). Therefore, all methylated amino acids for the purpose of assessing product purity and yield were analyzed on the amino acid analyzer at half-normal rate of flow of eluting buffer.

V. EXPERIMENTAL

V. EXPERIMENTAL

(All experiments were carried out on the L-isomer of the amino acids, unless otherwise indicated. All the compounds prepared were characterized by n.m.r. and i.r. spectroscopy).

A. Preparation of Starting Materials

1) *N-Carbobenzoxyleucine:*

This amino acid derivative was prepared by the method of Bergmann and Zervas as described in Greenstein and Winitz (1961), procedure 10-20, p. 891. The product was a colourless oil obtained in a 75% yield.

2) *Triphenyl carbinol:*

Triphenyl carbinol was prepared from trityl chloride by the procedure found in Vogel, p. 816 (1964). The m.p. of the product (160-162°C) corresponded to the literature value. The final product, triphenyl carbinol, had the expected n.m.r. spectrum.

3) *O,N-Dicarbobenzoxy tyrosine:*

This amino acid derivative was prepared by a method described in Literature (Neumann et al., 1959). The product was obtained in 60% yield, m.p. 117°C.

B. The NaH Procedure for Methylation under Refluxing Conditions

The procedure for the methylation of substituted amino acids as it was devised in this laboratory is described below (Benoiton and Coggins, 1971). This

procedure was the starting point of this thesis and many reference have been made to it throughout the text.

a) The preparation of N-acyl or N-carbobenzoxy-N-methylamino acid methyl ester.

The N-acyl or N-carbobenzoxyamino acid (0.010 mole) was dissolved in a mixture of THF (50 ml) and DMF (5 ml) which had been previously dried by treating with sodium hydride dispersion (1 g) for 5 minutes and then filtered. Then methyl iodide (5 ml; 0.080 mole) was added followed by sodium hydride dispersion (1.44 g; 0.030 mole). The order of addition must be kept as reported herein. For it was shown that whenever NaH was added before the MeI a substantial amount (35-60%) of the starting material was unaccounted for (Coggins, 1970). The mixture, bubbling rapidly with the evolution of hydrogen was refluxed from a wax bath at 80°C using a condenser fitted with a drying tube. After 24 hr the solvents were evaporated off and the residual solid treated with ether (100 ml) and water (25 ml). The aqueous layer was discarded and the ether layer further washed with water (1x25 ml). After drying ($MgSO_4$) the ether was evaporated to give two immiscible

oils (one being the oil from the NaH dispersion).
The expected i.r. spectra were given by all products at this stage.

The ratio of reagents which gave the best yields of the N-methylamino acid methyl ester and the least amount of unreacted amino acid derivative was 8 moles of MeI, to 3 moles of NaH, to 1 part amino acid derivative, that is a mole ratio of 8:3:1. All other ratios of these reagents used in subsequent alkylations are reported in the same order throughout this thesis.

b) Preparation of N-acyl or N-carbobenzoxy-N-methylamino acids.

To the suspension of the two immiscible oils of the above preparation, were added 2 N NaOH (20 ml) and methanol (40 ml). The mixture was stirred magnetically at 35°C for 3 hr. The methanol was evaporated off, water was added and the mixture was washed with ether (2 x 20 ml), cooled at 0°C and brought to pH 2 with 4 N HCl. The product was extracted into ethyl acetate (3 x 40 ml), the combined extracts were dried ($MgSO_4$), and the solvent was removed by evaporation. The residue was crystallized from water or

from ethyl acetate - light petroleum, bp. 30-60°C.

c) Preparation of N-methylamino acid methyl ester hydrobromides.

A solution of N-carbobenzoxy-N-methylamino acid methyl ester in 30% HBr in glacial acetic acid (20 ml) was left for 2 hr at room temperature. The mixture was evaporated and then re-evaporated with ether. The crystalline residue was dissolved in water (25 ml) and washed with ether (2 x 10 ml). The water was evaporated under vacuum at 40°C and the residual oil re-evaporated twice with methanol. After drying under vacuum over P_2O_5 the residue was crystallized from methanol (20 ml) by the addition of ether (100 to 300 ml) and recrystallized from methanol-ether.

C. <u>The NaH Procedure for Methylation at Room Temperature</u>

1) *Methylation of carbobenzoxyleucine - Deprotection with 30% HBr in glacial acetic acid:*

The carbobenzoxyleucine (0.01 mole, 2.65 g) was dissolved in THF (50 ml) which had been previously dried by treating with NaH dispersion (1 g) for 5 minutes and then filtered. Then MeI (0.08 mole, 5 ml) was added followed by NaH dispersion (0.03 mole, 1.44 g of 50% NaH dispersion in oil). The mixture which at this point was bubbling rapidly with the evolution of hydrogen was allowed to stand at room temperature, 23°C, the reaction flask being fitted with a condenser and drying tube. Continual mixing was supplied by the use of a magnetic stirrer. After 24 hr the solvent was evaporated and the residual solid treated with ether (100 ml) and water (25 ml). The aqueous layer was discarded and the ether layer further washed with water (1 x 25 ml). After drying ($MgSO_4$) the ether was evaporated to give the two immiscible oils (one being the oil from the NaH dispersion).

To the residual immiscible oils was added 30% HBr in glacial acetic acid (20 ml) and the reaction mixture was allowed to stand at room temperature for 2 hr. The mixture was evaporated and then re-evaporated with ether. The crystalline residue was dissolved in water (25 ml) and washed with ether (2 x 10 ml). The aqueous solution was brought to dryness <u>in vacuo</u> at 40°C and the residual oil was freed from water by twice evaporating its solution in methanol to dryness.

After drying under vacuum over P_2O_5 the residue was crystallized from methanol (20 ml) by the addition of ether (100 to 300 ml) and recrystallized from methanol-ether (1.7 g, m.p. 167-170°C) (Table III).

The residue upon amino acid analysis was identified as N-methyl leucine hydrobromide, also verifying the absence of any methyl ester form (Table IVb).

2) *Methylation of N-carbobenzoxy-O-benzyl tyrosine at room temperature:*

To N-carbobenzoxy-O-benzyl tyrosine (0.001 mole, 0.405 g) in THF (15 ml), previously dried with washed NaH was added MeI (0.008 mole, 0.5 ml) followed by NaH (0.003 mole, 0.144 g of 50% NaH dispersion washed with dry benzene). The reaction flask fitted with a condenser and drying tube was left at room temperature (23°C) for 24 hr. The use of a magnetic stirrer provided continual mixing. The solvent was removed by evaporation; the residue was distributed between ether (15 ml) and water (5 ml); the water layer was discarded and the ether layer was washed with water (1 x 5 ml), dried ($MgSO_4$) and evaporated down. The residue was hydrogenated in acetic acid using 10% palladium on powdered charcoal as catalyst for 24 hr. The solution was filtered and evaporated down. The product (0.11 gm) was identified by n.m.r. spectroscopy (see Fig. 1, p. 49) as N-methyltyrosine. Upon amino acid analysis the presence of 6% tyrosine was detected; the methylation was incomplete.

SWEEP OFFSET (Hz): (0)	MANUAL	AUTO	SAMPLE: N-methyltyrosine
SPECTRUM AMPLITUDE: 25	SWEEP TIME (SEC): 50 ~~25~~	(250)	
INTEGRAL AMPLITUDE: 3	SWEEP WIDTH (Hz): 25 50 100 250 ~~500~~	(500)	
SPINNING RATE (RPS): 40	FILTER: 1 ~~2~~ 3 4 5 6 7 8	(2)	
	RF POWER LEVEL: 0.06	(.05)	SOLVENT: $CDCl_3$ + CF_3COOH

REMARKS: N-Carbobenzoxy-O-benzyl-tyrosine methylated in THF at 23°C. Deprotected by hydrogenation.

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analytical instrument division

DATE:

OPERATOR:

60 MHz NMR
SPECTRUM NO.

SUMMATIONS OF THE NAH PROCEDURE FOR METHYLATION

D. The NaH Procedure for Methylation as Applied to Methylate Phenolic Hydroxyl Groups

1) *Methylation of o-catechol at room temperature (detailed):*

The o-catechol (0.01 mole, 1.10 g) was dissolved in THF (50 ml) which had previously been dried by treating with washed NaH (1 g of NaH dispersion suspended in dry benzene and filtered) for 5 minutes and then filtering MeI (0.08 mole, 5 ml) was added followed by NaH (0.03 mole, 1.44 g 50% NaH dispersion washed with dry benzene and filtered). The mixture which at this point was bubbling rapidly with the evolution of hydrogen was allowed to stand at room temperature, 23°C, the reaction flask being fitted with a condenser and drying tube. Continual mixing was supplied by the use of a magnetic stirrer. After 24 hr the solvent was evaporated and the residual solid treated with ether (100 ml) and water (25 ml). The aqueous layer was discarded and the ether layer further washed with water (1 x 25 ml). After drying ($MgSO_4$) the ether was evaporated and the residual liquid (1.1 g) was identified by n.m.r. spectrum (see Fig. 2, p. 52) as O,O'dimethyl catechol. Yield 84%.

The above methylation of o-catechol was also carried out using THF (50 ml) and DMF (5 ml) as the solvent system.

OCH$_3$ } a
OCH$_3$
b { (ring protons)

b

a

Hz: 500 400 300 200 100 0

PPM (δ): 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0

SWEEP OFFSET (Hz): 0
SPECTRUM AMPLITUDE: 3.2
INTEGRAL AMPLITUDE: 6
SPINNING RATE (RPS): 42

MANUAL
SWEEP TIME (SEC): 50 | 100 | AUTO (250)
SWEEP WIDTH (Hz): 25 | 50 | 100 | 250 | 500 (500)
FILTER: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 (2)
RF POWER LEVEL: 0.05 (.05)

SAMPLE: 0,0 dimethyl catechol

SOLVENT: $CDCl_3$

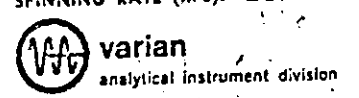
REMARKS: o-Catechol methylated in THF at 23°C for 24 hr

60 MHz NMR
SPECTRUM NO.

varian
analytical instrument division

DATE:

OPERATOR:



Yield 88 % (Table V). The respective n.m.r. spectrum showed a greater amount of impurities present.

2) *Methylation of 2,6-di-tert-butyl-4-methyl phenol at 80°C*

To 2,6-di-tert-butyl-4-methyl-phenol (0.01 mole, 2.2 gm) in THF (50 ml) and DMF (5 ml), previously dried with washed NaH was added MeI (0.08 mole, 5 ml) followed by NaH (0.02 mole, 0.96 gm of 50% NaH dispersion washed with dry benzene). The reaction flask fitted with a condenser and drying tube was placed in a wax bath at 80°C for 24 hr. The solvent was removed by evaporation, the residue was distributed between ether (100 ml) and water (25 ml), the water layer was discarded and the ether layer was washed with water (25 ml), dried ($MgSO_4$) and evaporated down. The residual oil (2.04 gm), on the basis of its n.m.r. spectrum (see Fig. 3, p. 54) was identified as 2,6-di-tert-butyl-4-methyl anisole. Yield 87%.

The above preparation was repeated at 23°C, yield 87%. This hindered phenol was also methylated using THF (50 ml) as solvent system both at 80°C, yield 80%, and 23°C, yield 86%.

The 2,6-di-tert-butyl-4-methylphenol was also methylated in THF:DMF, using the above reported procedure,

d c b a

8.0 7.0 6.0 5.0 PPM (δ) 4.0 3.0 2.0 1.0 0

SWEEP OFFSET (Hz): 0 (200)
SPECTRUM AMPLITUDE: 6.3
INTEGRAL AMPLITUDE: 3
SPINNING RATE (RPS): 46

MANUAL
SWEEP TIME (SEC): 50
SWEEP WIDTH (Hz): 25 50 100 250
FILTER: 2 3 4 5 6 7 8
RF POWER LEVEL: 0.05

AUTO (250) (500) (.2) (.05)

SAMPLE: 2,6-di-*tert*-butyl-4-methyl-anisole

SOLVENT: $CDCl_3$

REMARKS: 2,6-di-tert-butyl-4-methyl-phenol methylated in THF:DMF at 80°C for 24 hr.

60 MHz NMR
SPECTRUM NO.

OPERATOR:

DATE:

varian analytical instrument division

at 80°C for 1 hr. A 97% yield of the respective anisole was obtained.

All the above products were identified on the basis of their n.m.r. spectra.

E. The NaH Procedure for Methylation as Applied to Methylate Alcoholic Hydroxyl Groups

1) *Methylation of benzyl alcohol at 80°C:*

To benzyl alcohol (0.01 mole, 1.03 ml, sp. gr. 1.045) in THF (50 ml) which was previously dried using washed NaH, was added MeI (0.04 mole, 2.5 ml) followed by NaH (0.015 mole, 0.72 gm of 50% NaH dispersion washed with dry benzene). The reaction flask fitted with a condenser and drying tube was placed in a wax bath at 80°C for 24 hr. The solvent was removed by evaporation, the residue was distributed between ether (100 ml) and water (25 ml), the water layer was discarded and the ether layer was washed with water (25 ml), dried ($MgSO_4$) and evaporated down. The residual liquid (1.1 g) was identified as methyl benzyl ether (b.p. 174°C, sp. gr. 0.971) on the basis of its n.m.r. spectrum (see Fig. 4, p. 56). Yield 90%. The above procedure was repeated at 23°C and a 74% yield of the methyl benzyl ether was obtained. (Table VI).

500 400 300 200 100 0 Hz

CH_2OCH_3 (b: CH_2; a: OCH_3; c: ring)

c b a

8.0 7.0 6.0 5.0 PPM (δ) 4.0 3.0 2.0 1.0 0

SWEEP OFFSET (Hz): (200)

SPECTRUM AMPLITUDE: 8.3

INTEGRAL AMPLITUDE: 3

SPINNING RATE (RPS): 42

MANUAL

SWEEP TIME (SEC): 50 | 25 (X)

SWEEP WIDTH (Hz): 25 | 50 | 100 | 250 | 500 (X)

FILTER: 1 (X) | 2 | 3 | 4 | 5 | 6 | 7 | 8

RF POWER LEVEL: 0.05

AUTO ☐

(250)

(500)

(2)

(.05)

SAMPLE: Methyl benzyl ether

SOLVENT: $CDCl_3$

REMARKS: Benzyl alcohol methylated in THF at 80°C for 24 hr.

60 MHz NMR

SPECTRUM NO.

DATE:

OPERATOR:

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2) *Methylation of triphenyl carbinol at 23°C*

To triphenyl carbinol (0.01 mole, 2.6 g) in THF (50 ml) previously dried with washed NaH was added MeI (0.04 mole, 2.5 ml) followed by NaH (0.02 mole, 0.96 gm of 50% NaH dispersion washed with dry benzene). The reaction flask fitted with a condenser and drying tube was left at room temperature (23°C) for 24 hr. The use of a magnetic stirrer provided continual mixing. The solvent was removed by evaporation; the residue was distributed between ether (100 ml) and water (25 ml); the water layer was discarded and the ether layer was washed with water (25 ml) dried ($MgSO_4$) and evaporated down. The residual solid (2.36 gm) was recrystallized from methanol (2.3 gm) and by means of its n.m.r. spectrum (Fig. 5, p. 58) was identified as triphenyl carbinol methyl ether. Yield 85%, m.p. 82-83°C.

The above procedure was carried out at 80°C and a 81% yield of the methyl ether was obtained.

b { (C6H5)3 C-OCH3 } a

b

a

PPM (δ)	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0

Hz	500	400	300	200	100	0

SWEEP OFFSET (Hz): (0)
SPECTRUM AMPLITUDE: 8
INTEGRAL AMPLITUDE: 2
SPINNING RATE (RPS): 40

MANUAL
SWEEP TIME (SEC): 50
SWEEP WIDTH (Hz): 25 | 50 | 100 | 250 | 500
FILTER: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8
RF POWER LEVEL: 0.05

AUTO
(250)
(500)
(2)
(.05)

SAMPLE: Triphenyl carbinol methyl ether

SOLVENT: $CDCl_3$

REMARKS: Triphenyl carbinol methylated in THF at 23°C.

60 MHz NMR
SPECTRUM NO.

DATE:

OPERATOR:

varian
analytical instrument division

VI. REFERENCES

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