STUDIES DIRECTED TOWARDS SYNTHESIS OF BRASSINOSTEROIDS AND CHLORAMPHENICOL

A THESIS

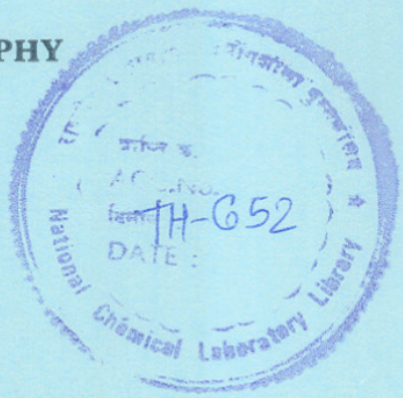
SUBMITTED TO THE

UNIVERSITY OF POONA

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

(IN CHEMISTRY)



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NOVEMBER 1990

Dedicated to

(Late) DR. V. N. GOGTE

CERTIFICATE

Certified that the work incorporated in the thesis entitled "STUDIES DIRECTED TOWARDS THE SYNTHESIS BRASSINOSTEROIDS AND CHLORAMPHENICOL" by Mrs. V.S. Pore was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged.

B.G. Hazra

(Dr.B.G. Hazra)
Supervisor

ACKNOWLEDGEMENT

I take this opportunity to express my sincere gratitude to Dr.B.G. Hazra, Senior Scientist, National Chemical Laboratory, for suggesting the problems, for his inspiring guidance and constant encouragement throughout the course of this investigation.

I acknowledge my grateful thanks to Drs. S. Rajappa and A.S. Rao for encouragaement and discussions during this work. My thanks are also due, to all colleagues for cheerful co-operation.

The services of the co-workers from the spectroscopy, gas-liquid chromatography, microanalysis and library are gratefully acknowledged. My thanks are also due, to Shri H. Nagarajan for typing my thesis. I am highly obliged to Mr. M.N. Joglekar and his colleagues for drawing formulae charts and NMRs.

Finally, I am thankful to the Director, National Chemical Laboratory, for allowing me to submit this work in the form of a thesis for Ph.D.

VSPore

(Mrs. V.S. Pore)

C O N T E N T S

GENERAL REMARKS

1. All melting points and boiling points are uncorrected.

2. All extracts were finally dried over anhydrous sodium sulfate.

3. All reactions were followed by t.l.c. on glass plate coated with silica gel slurry.

4. IR spectra were recorded on a Perkin-Elmer spectrophotometer model 599B using NaCl optics. ν max values are given in cm^{-1}.

5. NMR spectra were recorded on Varian T-60 or Jeol T-60 or Brucker WH-90 or FT-80A Varian spectrophotometer or Brucker MSL-300 using TMS as internal standard. Chemical shifts are given in δ ppm.

6. Mass spectra were taken on Finnigan Mat 1020C mass spectrometer at 70 ev.

7. GLC was recorded on Carlo-Erba:Fracto-vap No.2450, Column: Apiezon L 5% on 80-100 mesh chromosorb WHP at 235°C, Integrator: 3390A Hewlett Packard, Carrier gas: Nitrogen , Flow rate: 35 ml/min, Detector: F.I.D.

8. All optical rotations were measured on JASCO-181- digital Polarimeter using sodium light (9893A) source. Concentrations are expressed in g/100 ml of the solution.

9. In the description of NMR signals the abbreviations s, d, t, q, m, bs, dd, bd mean singlet, doublet, triplet, quartet, multiplet, broad singlet, doublet of a doublet, broad doublet respectively.

10. Pet.ether refers to the fraction boiling between 60-80°C.

11. The numbers assigned to the compounds, charts and figures in each chapter of this thesis refer only to that particular chapter.

CHAPTER I

SYNTHESIS OF BRASSINOSTEROIDS

SUMMARY

This chapter deals with the synthesis of brassinosteroid intermediates. It is divided into three sections (A) Synthesis of (20S)-3α,5-cyclo-6β-methoxy-pregnane-20-carboxyaldehyde (38), involving a key step, namely ene reaction on (Z)-3β-p-toluenesulfonoxy-pregna-5,17(20)-diene (75). Thus, dehydroepiandrosterone (73) was converted into tosylate (75) in two steps. Ene reaction on this tosylate (75) with paraformaldehyde in presence of acetic anhydride using different Lewis acids and cation exchange resins as catalyst afforded stereospecifically the C-22 acetate (76) in excellent yield. Use of cation exchange resin in ene reaction is reported for the first time. This ene reaction product (76) was converted into known C-22 aldehyde (38) by simple reactions.(B) A new short route for a formal total synthesis of dolicholide (4), dolichosterone (9), 28-norbrassinolide (3) and a few other brassinolide analogues is described. The key intermediate, (2R, 3S)-2,3,22-triacetoxy-23,24-dinor-5α-cholan-6-one (87) has been utilised for the synthesis of (4), (9), (3) and few other brassinolide analogues by two independent groups at Japan. This intermediate (87) has been synthesised by these two groups in eleven to fourteen steps starting from stigmasterol. This section deals with stereoselective synthesis of this important compound (87) in nine steps starting from dehydroepiandrosterone (73). (C) Synthesis of 3α,5-cyclo-6β-methoxy-22-acetoxy-24-nor-5α-cholan-23-al (94) starting from the aldehyde (38) has been described. Configurational assignment of the corresponding glycidic ester (92) at C-22, C-23 and also (2R, 3S, 22R, 23S, 24R)-22-hydroxy-23,24-epoxy-2,3-isopropylidenedioxy-6,6-ethylenedioxy-5α-cholestane (54) and its (2R, 3S, 22S, 23R, 24S)-isomer (55) by ^{13}C-NMR spectroscopy has been studied.

INTRODUCTION

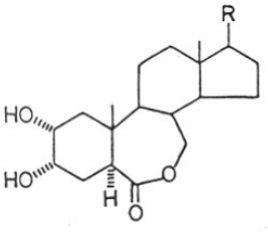
Brassinosteroids are a new class of plant growth regulators. In 1979, United States Department of Agriculture scientists isolated 4 mg of a new steroid named brassinolide (**1**) from 40 kg of bee collected pollen of the rape plant (Brassica napus L.). It produced a powerful growth accelerating effect when applied to young pinto bean plants. The increase in growth was both due to increased cell elongation and cell division. Its structure was elucidated as (2R, 3S,22R, 23R. 24S)-2, 3, 22, 23-tetrahydroxy-24-methyl-B-homo-7-oxa-5∝-cholestan-6-one (**1**) by spectroscopic data including X-ray[1]. "It is the first naturally occurring steroid with an unprecedented seven membered B-ring lactone and two vicinal diol functions at ring A and the side chain. The discovery of brassinolide (**1**) is the most important discovery of the plant physiologists and biochemists, since the discovery of gibberellic acid which cause increase in growth due to cell expansion only.

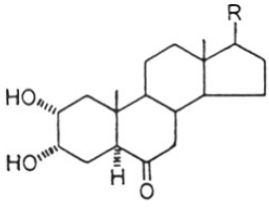
Because of the scarcity, interesting novel structural features and dramatic ability to accelerate the plant growth much effort has been devoted in recent years to the search for further natural brassinosteroids, to the synthesis of brassinolide (**1**) and its analogues and to the study of their biological activity and physiological function.

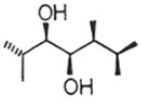
Structure and occurrence

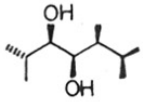
Since the discovery of brassinolide (**1**) as many as 16 further native brassinosteroids have been found in species of different plant families[2,3]. Structurally they exhibit eitehr 7-membered 7-oxa lactone B-ring but differing in the C-24 substitution of side chain moiety (**1-5**, chart I) or they are the corresponding 6-oxo steroids with normal six membered B-ring (**6-13**). 6-Deoxocastasterone (**14**) and 6-deoxodolichosterone (**15**) and 6-deoxohomodolichosterone

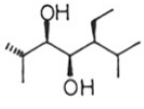
CHART I

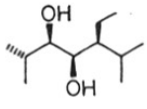


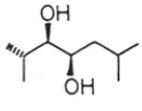


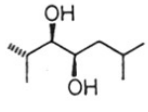
Brassinolide, (1) R = 

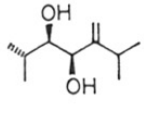
Castasterone, (6) R = 

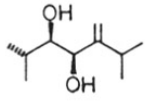
Homobrassinolide, (2) R = 

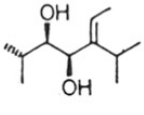
Ethylbrassinone, (7) R = 

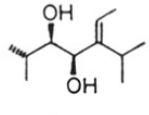
28-Norbrassinolide, (3) R = 

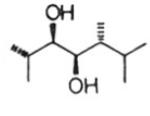
Brassinone, (8) R = 

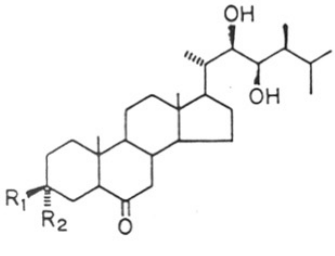
Dolicholide, (4) R = 

Dolichosterone, (9) R = 

Homodolicholide, (5) R = 

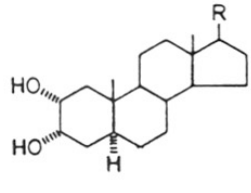
Homodolichosterone, (10) R = 

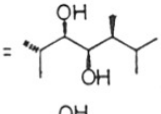
24-Epicastasterone, (11) R = 

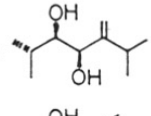


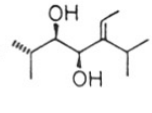
2-Deoxycastasterone, (Typhasterol, 12) R_1 = H ; R_2 = OH

Teasterone (13) R_1 = OH ; R_2 = H



6-Deoxocastasterone, (14) R = 

6-Deoxodolichosterone, (15) R = 

6-Deoxohomodolichosterone, (16) R = 

(16) lack the B-ring oxygen function. All these brassinosteroids are (2R, 3S, 22R, 23R)-tetrols wih exception of 2-deoxycastasterone (typhasterol, 12) and teastserone (13) which show only one (3R)- or (3S)-hydroxy group in ring A, respectively.

The native brassinosteroids are widely distributed in plant kingdom. The detected amounts vary from 0.1 ng/kg (ethyl brassinone, 7, from fruit of Brassica campestris var. pekinensis) upto 100 μg/kg (brassinolide, 1, from pollen of Brassica napus).

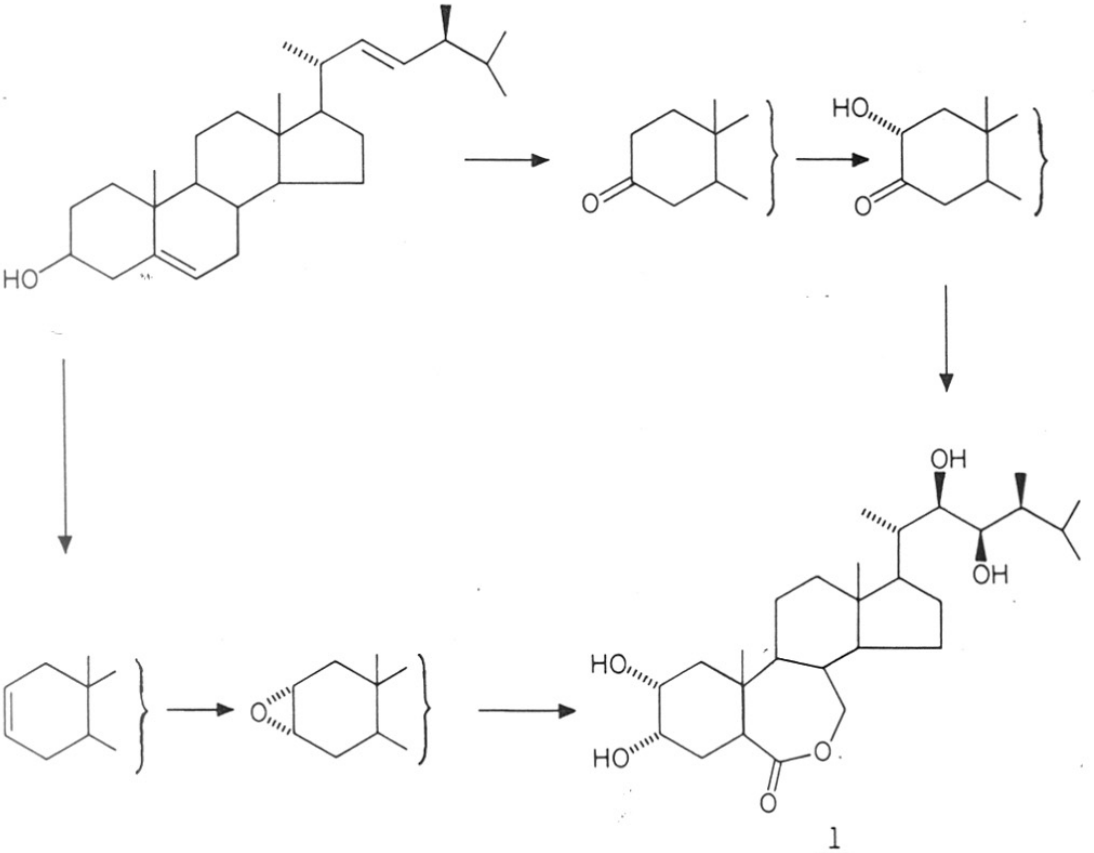
Biosynthesis

No results concerning the biosynthesis of brassinosteroids have been published to date. The biosynthesis presumably commence from the corresponding sterols, e.g. brassicasterol or 24-methylenecholesterol for the C_{28}-brassinosteroids. One of the most important points in brassinolide biosynthesis is the conversion of (3S)-hydroxy group to (2R, 3S)-dihydroxy group. The possible biosynthetic pathways for this via ketol or epoxide intermediates have been discussed[4] (Chart II). The 6-oxo type brassinosteroids are assumed to be biosyntehtic precursors of the corresponding 7-oxalactone compounds especially castasterone (6) for brassionolide (1) and brassinone (8) for 28-nor-brassinolide (3). The 6-deoxobrassinosteroids (14), (15) and (16) have been considered as precursors for their respective 6-oxo analogues (6), (9) and (10) respectively, and (3S) -hydroxy compound teasterone (13) was assumed to be the biosynthetic intermediate to typhasterol (12) and further to castasterone (6) and brassinolide (1) on the basis of their common occurrence.

Synthesis of Brassinosteroids

Soon after the structure elucidation of brassinolide (1), its first synthesis were published[5,6]. Since that time brassinostseroid synthesis has remained a field of tremendous activity. For the chemical synthesis of

CHART - II



1

POSSIBLE BIOSYNTHETIC PATHWAYS OF THE (2R, 3S)-2,3-Dihydroxy MOIETY OF BRASSINOLIDE

brassinosteroids, starting from a suitable steroidal precursor, the typical A/B ring functionalisation and the construction of the dihydroxylated side chain moiety including the asymmetric centres at C-22, C-23 and C-24 are necessary.

A/B functionalisation

In nearly all syntheses of brassinostroids a Δ^5-3β-hydroxy starting steroid (**17**) is transformed into Δ^2-6-oxo compound (**24**) which is a suitable intermediate for the synthesis of both 6-oxo and 6-oxo-7-oxalactone type brassinosteroids (chart III). This Δ^2-6-oxo compound (**24**) obtained by different methods as shown in chart III, when reacted with molar amount of osmium tetr oxide or catalytic amount of OsO_4-NMO, results in stereospecific hydroxylation to the (2R, 3S)-dihydroxy-6-oxo compounds (**29**), thus yielding the typical A/B structural feature of the native brassinosteroids (**6-11**). Subsequent Baeyer-Villiger oxidation of (**29**) gives upto 90% of the desired 7-oxalactones (**30**).

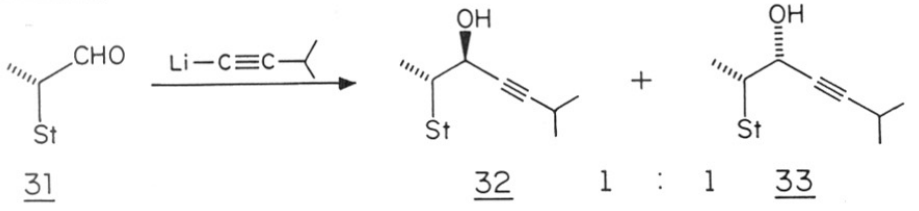
Side chain construction

The starting material for most of the brassinolide syntheses is C-22 aldehyde, which is readily available from dinorcholenic acid or stigmasterol. Out of the several reported syntheses of brassinosteroids, a few representative examples are described here.

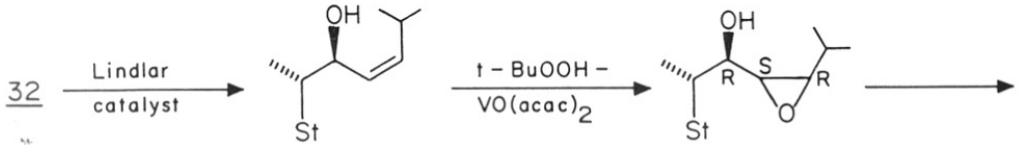
Ishiguro *et al.*[5,7] used C-22 aldehyde (**31**) with a THP-ether protected 3β-hydroxy group which was reacted with 3-methyl but-1-ynyl Li to give a 1:1 ratio of epimeric alcohols at C-22, (**32**) and (**33**) (chart IV, scheme 1). Reduction of (22R)-compound (**32**) with Lindlar catalyst to *cis* allylic alcohol (**34**) followed by Sharpless epoxidation with t-butyl hydroperoxide-oxovanadium acetylacetonate led stereospecifically to (23S, 24R)-epoxide (**35**). Attempts

CHART - IV

SCHEME 1

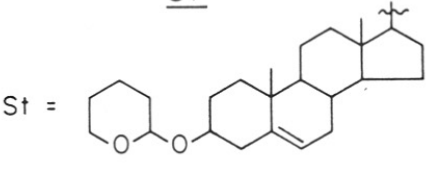


31 32 1 : 1 33

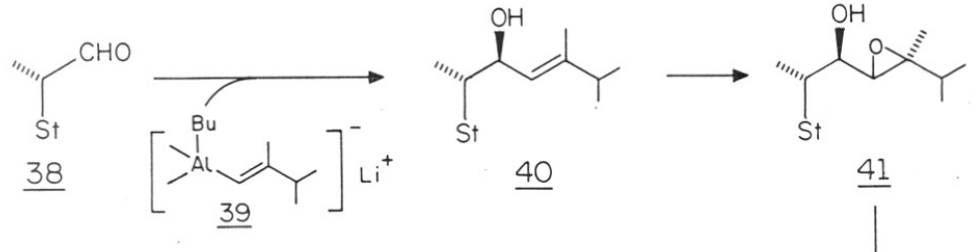


34 35

36 37



SCHEME 2



38 39 40 41

42

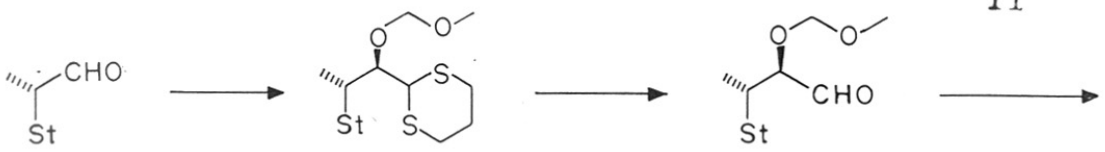
to introduce methyl group at C-24 with Me_3Al, Me_2CuLi or MeMgBr/CuI, were unsuccessful. Therefore, the nitrile alcohol (**36**) was prepared and cyano function was transformed in a further sequence to (24S)-methyl group of brassinolide side chain (**37**).

The brassinolide synthesis by Fung and Siddall[6] involves stereoselective alkylation of a 3,5-cyclo-6-methoxy aldehyde (**38**), with $Li^+[Me_2BuAlCH{=}CMeCHMe_2]^-$ (**39**) to yield the major (22R)-alcohol (**40**) (chart IV, scheme 2). Epoxidation of this compound (**40**) wtih MCPBA to (**41**) followed by anti-Markovnikov reduction with $LiBH_4$-BF_3.THF gave after inversion at C-24, the preferred (22R, 23R, 24S) brassinolide side chain (**42**). Thompson et al.[8] have synthesised brassinolide (**1**) and three of its isomers (22R, 23R, 24R)-, (22S, 23S, 24S)- and (22S, 23S, 24R)-22,23 dihydroxy-24-methyl steroids from a C-24 epimeric 60:40 mixture of 22-dehydrocampesterol (24S-methyl) and brassicasterol (24R-methyl) from oysters.

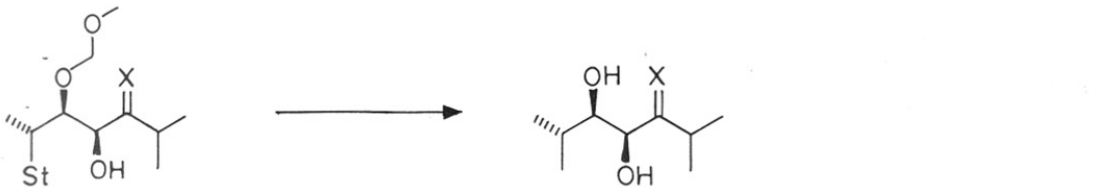
A quite variable method for the brassinosteroid side chain construction has been developed by Takatsuto and Ikekawa[9-11] using the chelation controlled Grignard reaction for a stereoselective introduction of the desired (22R, 23R) diol function (chart IV, scheme 3). Thus from C-22 aldehyde (**43**) with 1,3-dithiane lithium, the (22R)-dithian was obtained and its MOM ether (**44**) was treated with HgO-BF_3 to yield the C-23 aldehyde (**45**). Chelation controlled Grignard reaction with iso-Bu-MgBr and removal of the MOM group gave the 28-norbrassinolide side chain (**47a**). Similarly, Grignard addition of $CH_2{=}C(MgBr)CHMe_2$ permitted syntehsis of dolicholide side chain (**47b**). With iso-Pr-MgBr, the brassinosteroid analogues of the 26,27-bisnor series are available.

One stereoselecstive route to brassinolide side chain by Hayami et al.[12] involves reaction of aldehyde (**38**) with the lithium salt of 2-(Me_2PhSi)-1-

SCHEME 3



43 44 45

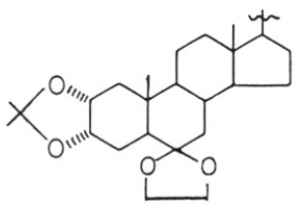


46 47

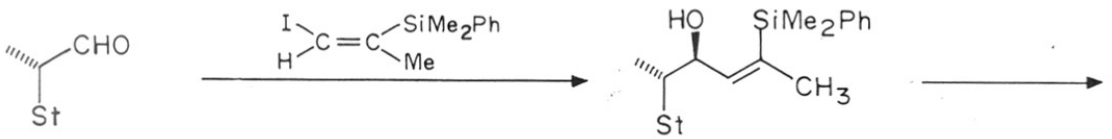
a, X = H_2
b, X = CH_2

a, X = H_2
b, X = CH_2

St =



SCHEME 4



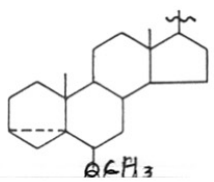
38 48

a = 22 S
b = 22 R

49 50

a, R = H
b, R = CH_2Ph

St =

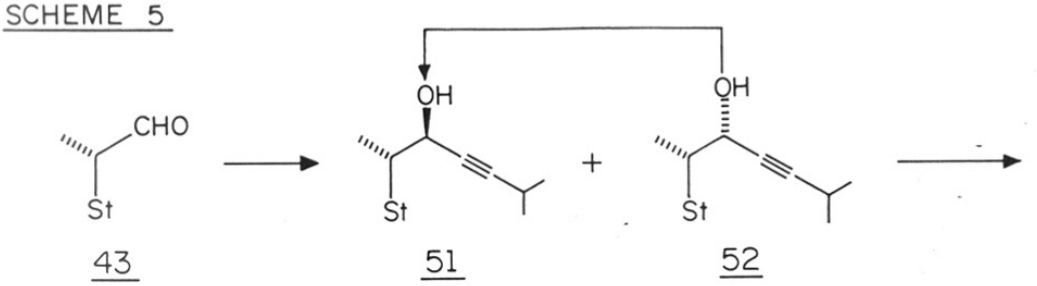


iodo-1-propene to give the major (22S)-allylic alcohol (48a) and the minor (22R)-isomer (48b) (chart IV, scheme 4). Silyl group assisted Sharpless epoxidation and elimination of the Me_2PhSi function with n-Bu_4NF led to an epoxy alcohol (49a) which was transformed to the benzyl ether (49b). The completion of the side chain moiety was achieved upon alkylation of compound(49b)with (iso-Pr)$_2$Cu(CN)Li$_2$ followed by Li-NH_3 dprotection to give(50).

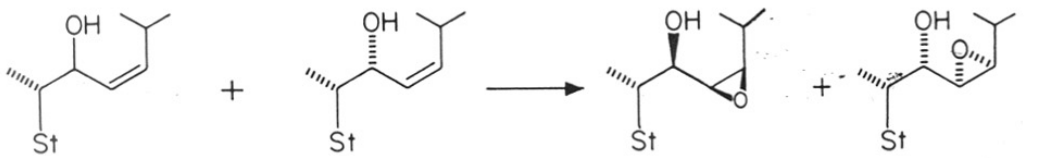
Another syntehsis described by K. Mori et al.[13,14] is suitable for large scale preparation of brassinolide (chart IV, scheme 5). The starting aldehyde (43) was prepared from abundantly available stigmasterol as reported earlier[15] by the author. Addition of $LiC{\equiv}CPr^i$ to (43) yielded a distereomeric mixture of two alkynyl alcohols (51) and (52). Catalytic partial hydrogenation of the mixture of (51) and (52) over P-2 Ni in the presence of ethylenediamine gave a diastereomeric mixture of an allylic alcohol (53) and its (22S)-isomer (53A). The mixture was epoxidized with MCPBA to give a mixture of two epoxides (54) and its (22S)-isomer (55). They were separated by silica gel chromatography. From the mixture of alkynyl alcohols (51) and (52), the desired (51) was separated by crystallization. The mixture after separation of maximum amount (51), was subjected to Mitsunobu reaction to transform more of unwanted (52) to (51). The crucial ring cleavage of the epoxide (54) was effected with 10 eq of Me_3Al in the presence of n-BuLi to give the required brassinolide side chain (56).

A very recent synthesis by Zhou Wei-Shan et al.[16] seems to be elegant method for the construction of the side chain of brassinolide (1). (chart IV, scheme 6). The aldehyde (57) obtained from hyodeoxycholic acid was treated with isobutyl carbonium arsonium ylid to form α,β-unsaturated ketone (58). Epoxidation of (58) with H_2O_2-NaOH afforded α,β-epoxyketone (59). The Wittig-

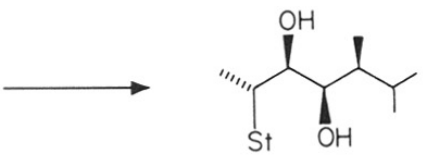
SCHEME 5



43 51 52



53 53 A 54 55



56

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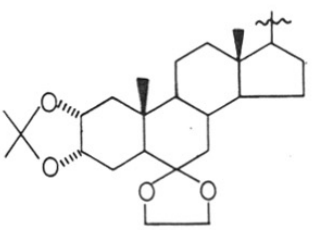
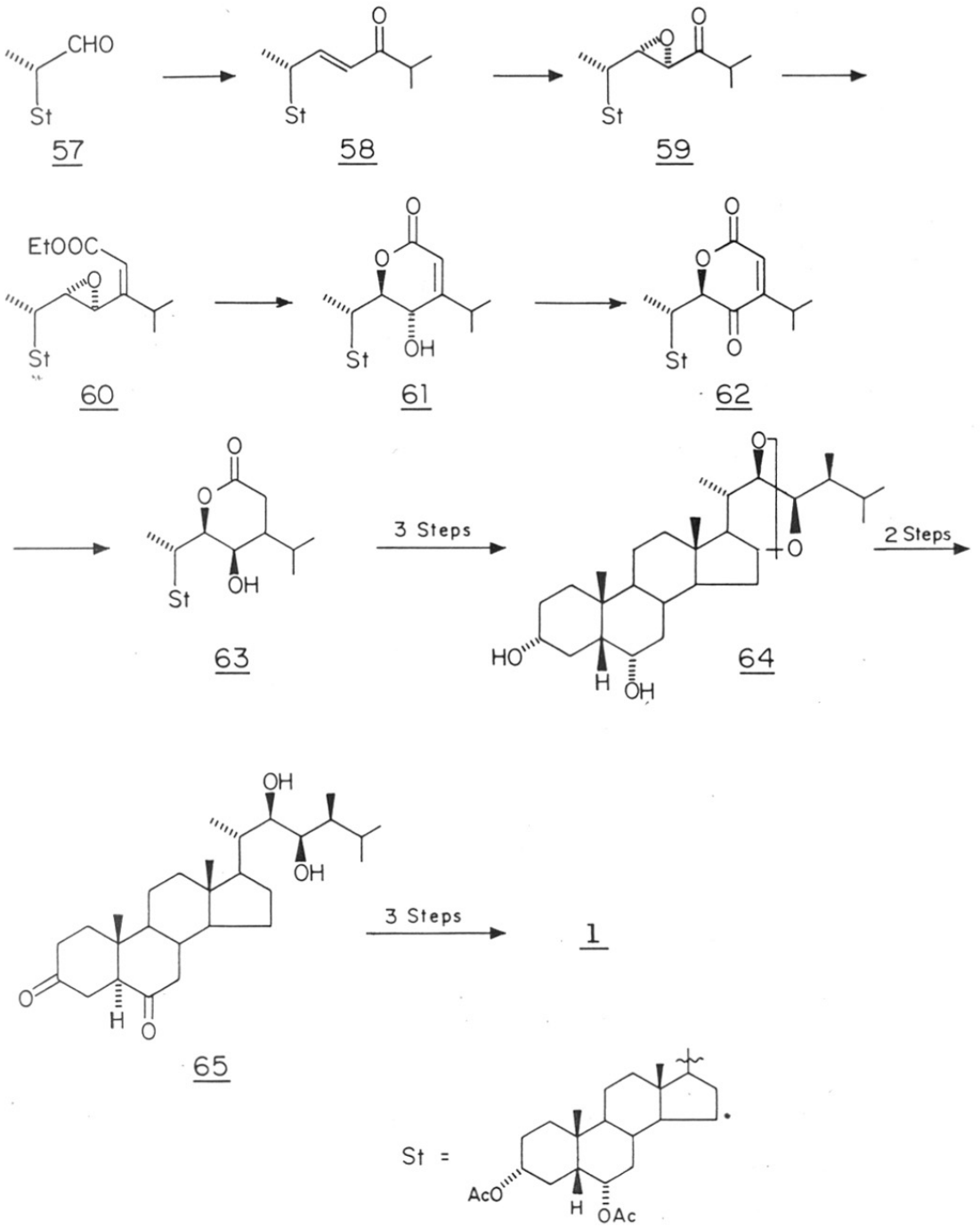


SCHEME 6


CHO
St
57
58
59
EtOOC
60
OH
61
62
63
3 Steps
HO
H
64
2 Steps
65
3 Steps
1
St =
AcO
OAc

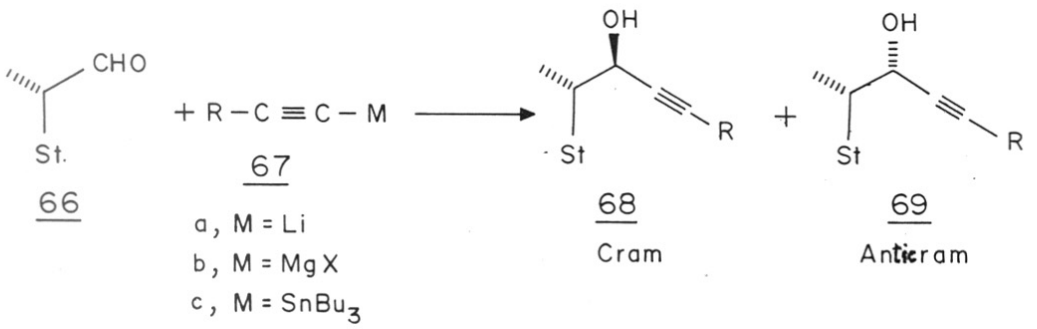
Horner reaction of ethoxycarbonyl methyl phosphonic acid dimethyl ester with (59), furnished a mixture of Z- and E- α,β-unsaturated-γ,δ-α-epoxy acid ester Z-(60) and E-(60) in the ratio 10:1. This key intermediate Z-(60) was lactonised under acidic conditions to give α,β-unsaturated-δ-lactone (61) formed by the carboxylate-aided epoxide ring opening of Z-(60), with the inversion of the configuration at C-22. The (S)-configuration of C-23 could be easily converted into (R)-by successive oxidation and reduction to get the compound (63). Reduction of lactone (63) with di-isobutyl aluminium hydride (DIBAH) afforded a hemiacetal and it was converted into 22,23-acetonide which was decarbonylated with tris-(triphenylphosphine) rhodium chloride to give the known (24S)-methyl derivative (64). Oxidation of this compound (64) with PDC followed by acid treatment afforded compound (65) with the required brassinolide side chain.

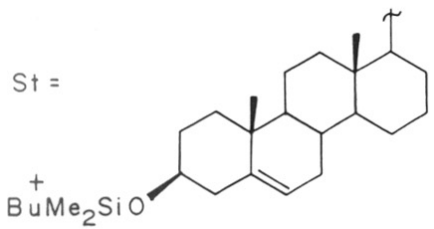
Mc Morris et al.[17] have described the stereoselective synthesis of brassinolide which involves construction of the side chain by reaction of aldehyde (38) with the anion of 2,3-dimethylbutenolide.

One-step stereochemical determination of contiguous four acyclic chiral centres at carbons 21, 22, 23 and 24 on the steroidal side chain has been described by Kametani et al.[18]. The method for the construction of side chain of brassinosteroids, using metal acetylides produces a mixture of cram and anti-cram isomers with low diastereoselectivity. Yoshinori et al.[19] found that the reaction of aldehyde (66) with stannyl acetylenes (67c) in the presence of $TiCl_4$ produces the cram isomer (68) with high distereoselectivity. (at least 85:15) (chart IV, scheme 7).

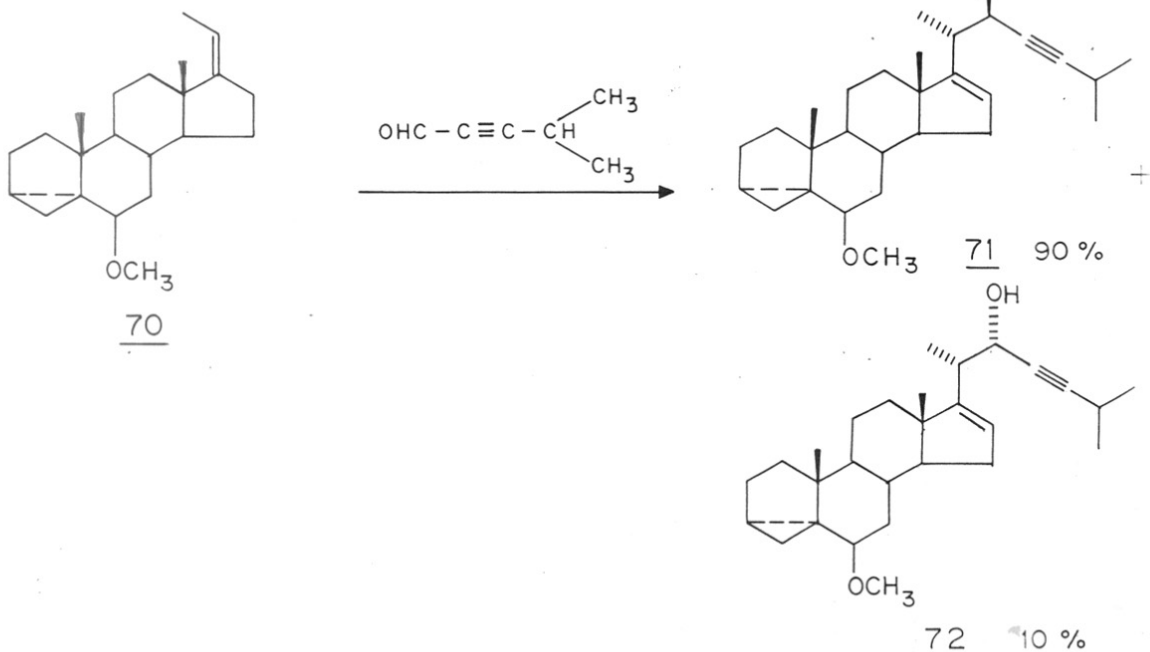
Another highly stereocontrolled synthesis of brassinolide side chain was reported by Takeshi Nakai et. al.[20]. The ene reaction of Z-Δ17(20)steroidal olefin (70) with acetylenic aldehyde in the persence of Me_2AlCl produce

SCHEME-7





SCHEME-8



(20S, 22R)-22-hydroxy-23,24-acetylenic side chain (**71**) in more than 90% yield (chart IV, scheme 8).

Structure -Activity relationship

Brassinosteroids are potent plant growth promoters in a number of different test systems. They show high activity in the bean second internode bio-assay and rice lamina inclination test. For structure activity relationships most of the native brassinosteroids have been tested. It was found that following structural features are necessary for a high activity- a trans A/B ring system (C-5 alpha-hydrogen), a 6-ketone or a 7-oxa-6-ketone system in ring B, cis ∝-oriented hydroxyl groups at C-2 and C-3, cis hydroxy groups at C-22 and C-23 as well as an alkyl substituent at C-24.

PRESENT INVESTIGATION

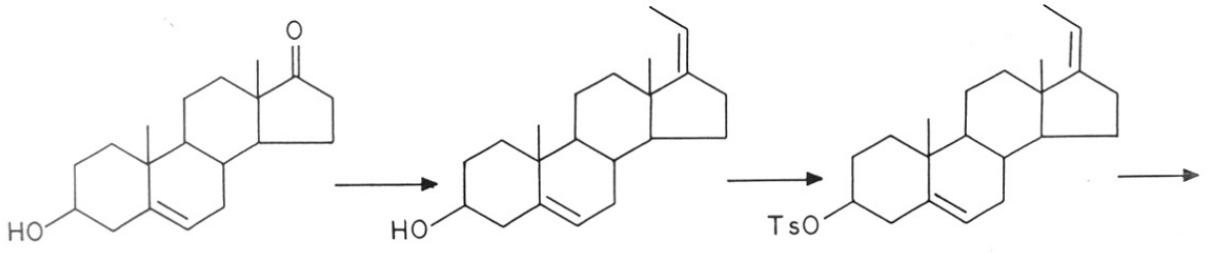
This chapter deals with three aspects-

(A) Study of ene reaction on (Z)-3β-p-toluene sulfonyloxy-pregna-5,17(20)-diene and synthesis of (20S)-6β-methoxy-3α,5-cyclo-5α-pregnane-20-carboxyaldehyde. (B) Stereoselective synthesis of a key intermediate (2R, 3S)-2,3,22-triacetoxy-23,24-dinor-5α-cholan-6-one. (C) Synthesis of 3α,5-cyclo-6β-methoxy-22-acetoxy-24-nor-5α-cholan-23-al. Configurational assignment of the corresponding glycidic ester at C-22, C-23 and also (2R, 3S, 22R, 23S, 24R)-23,24-epoxy-6,6-ethylene-dioxy-22-hydroxy-2,3-isopropylidenedioxy-5α-cholestane and its (2R, 3S, 22S, 23R, 24S)-isomer at C-22, C-23 and C-24 by ^{13}C-NMR spectroscopy.

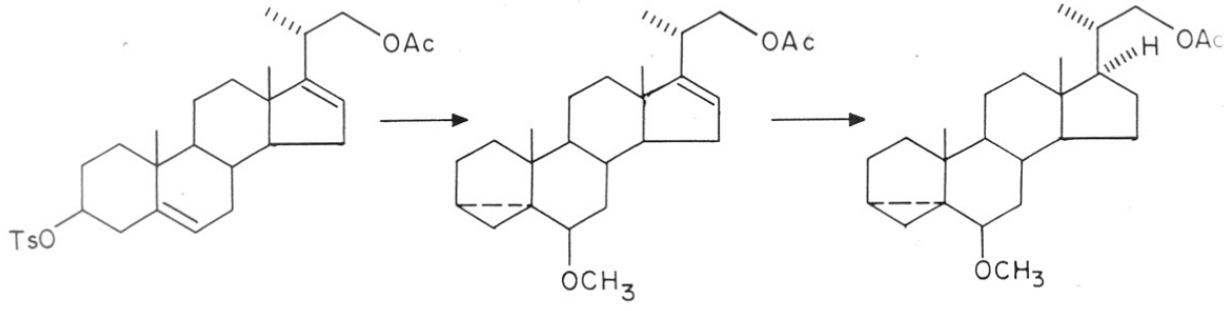
Section A

In connection with the synthesis of brassinosteroids[3], the intermediate (20S)-6β-methoxy-3α,5-cyclo-5α-pregnane-20- -carboxyaldehyde (**38**) was needed. This aldehyde (**38**) is also the starting material for the synthesis of a large number of biologically active products[21-24,6,12,17]. Compound (**38**) has been prepared from stigmasterol in 3 steps[21-24] in varying yields. This aldehyde (**38**) has been synthesised by highly efficient route, starting from 3β-hydroxy-androst-5-en-17-one (**73**) involving ene reaction with paraformaldehyde on (Z)-3β-p-toluenesulfonyloxy-pregna-5,17(20) diene (**75**). The starting material (**73**) is readily available *via* microbial degradation[25] of abundant plant sterols. This 17-ketosteroid (**73**) was converted to (Z)-3β-hydroxy-pregna-5,17(20)diene (**74**) in 89% yield *via* Wittig reaction using 2 equivalents of $(C_6H_5)_3PCH_2CH_3I$, prepared from triphenylphosphine and ethyl iodide and simple work up procedure. Compound (**74**) was prepared earlier[26] using large excess of $(C_6H_5)_3PCH_2CH_3Br$ (6 equivalents), prolonged reaction period and in lesser yield. Compound (**74**) on treatment with p-toluenesulfonyl chloride in pyridine furnished the tosylate (**75**) in 90% yield. This tosylate (**75**) was subjected to ene reaction with paraform-

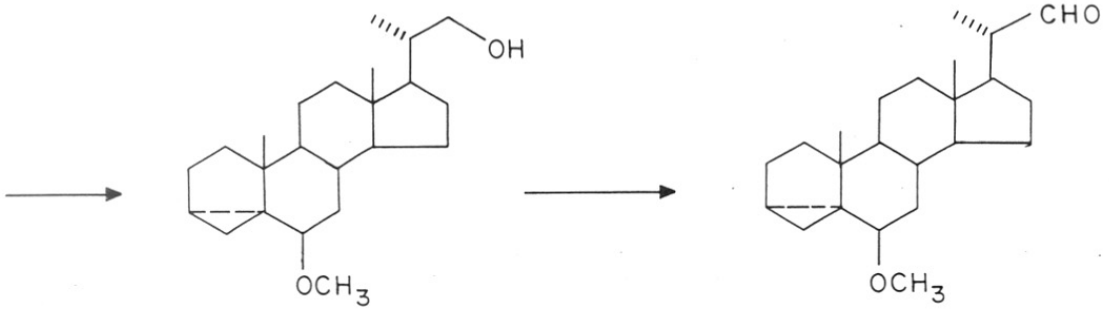
CHART V



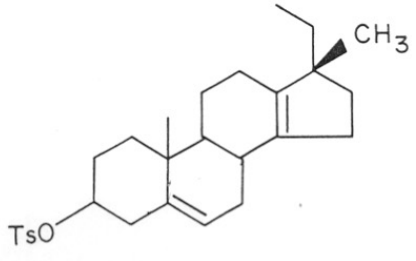
73 74 75



76 77 78



79 38



80

aldehyde as enophile using 10 mole percent of borontrifluoride etherate in the presence of acetic anhydride to afford a mixture of products from which the required known acetate (**76**) was isolated in 38% yield. In order to improve the yield of the acetate (**76**), a comprehensive study of the ene reaction using various cation exchange resins and different Lewis acids as catalysts has been carried out (Table 1). The use of cation exchange resin as a catalyst in the ene reaction has not been reported so far. Diene (**75**) when treated with paraformaldehyde using Amberlist-15 resin as a catalyst in the presence of acetic anhydride a mixture of (**76**) and Wagner-Meerwein rearrangement product (**80**) were isolated. The percentage of compounds (**76**) and (**80**) depends on the concentration of resin and reaction time (Table and experimental). The structure of compound (**80**) has been assigned from its spectral data and elemental analysis. In this reaction Indion-130 resin was used as a catalyst. After the reaction was over, catalyst was filtered and to the filtrate 2 equivalents of pyridine and 1.5 equivalents of acetic anhydride were added. Both the products (**76**) and (**80**) were formed in different proportions depending upon the reaction time and the concentration of the catalyst used. The resin Tulsion CXO-18, did not catalyse this reaction. Using Tulsion T-42 and Zeo-Karb-225 resins and continuing the reaction for 20 and 35 hr respectively, the resins were filtered and the reaction mixture was treated with pyridine followed by addition of acetic anhydride, the required acetate (**76**) was isolated in 96 and 93% yield respectively. The filtered catalyst was reused after activation. The ene reaction carried out using cation exchange resin, stereospecifically generate the natural (S)-configuration at C-20 was confirmed by converting the acetate (**76**) to the known[21-24] aldehyde (**38**), having (S)-configuration at C-20 and also by comparison of the spectral data and mixture melting point of acetate (**76**) with authentic compound (**76**) prepared according to earlier[27] report. When various Lewis acids were used as catalysts e.g. $AlCl_3$, $BF_3.Et_2O$ and $SnCl_4$ the acetate (**76**) was isolated in 78 to 95% yields on quenching the reaction mixture after short interval

Table 1

75 + paraformaldehyde + Ac_2O + Lewis acid/Resin $\xrightarrow{25 \text{ to } 30°}$ 76 + 80

3 equiv.[a] 2 equiv.

Entry	Lewis Acid/Resin	Conc.[b] parts/mole %	Time hrs.	Quenching[c] condition	% Yield[d] Acetate 76	Product 80
1	Amberlyst[e]-15	5.0	0.2	A	36	41
		0.5	2.0	A	40	37
		0.3	30.0	A	75	15
2	Indion[f]-130	5.0	1.0	B	41	46
		0.5	7.0	B	47	40
3	Tulsion[g] T-42	5.0	20.0	B	96	-
4	Zeo-Karb[h]-225	5.0	35.0	B	93	-
5	Tulsion[i] CXO-18	5.0	48.0		No Reaction	
6	$AlCl_3$	60.0	18.0	C	82	-
7	$SnCl_4$	9.0	1.0	C	78	8
8	$BF_3.Et_2O$	10.0	5.0	D	38	-
		10.0	0.75	C	95	-

a Calculation is based on assuming paraformaldehyde as a trimer.

b Concentrations of Lewis acids are presented in mole % and concentrations of resins are presented in parts of resin per part of the substrate.

c Quenching conditions:

A Catalyst was filtered and the reaction mixture was treated with water and extracted with CH_2Cl_2

B Catalyst was filtered and to the reaction mixture were added 2 equivalents of pyridine and 1.5 equivalents of AC_2O.

C The reaction mixture was quenched with 2 equivalents of pyridine and treated with 1.5 equivalents of AC_2O.

D Reaction mixture was treated with water and worked up as usual.

d Isolated yields, the products were identified by ^{1}H-NMR spectra, mass spectra and elemental analysis.

e Amberlyst-15 strong acid (20-50 mesh, Fluka AG).

f Indion-130, strongly acidic cation exchanger [Ion Exchange (India) Ltd., Bombay].

g Tulsion, T-42(H), strongly acidic cation exchanger (Tulsi Fine Chemicals, Pune, India).

h Zeo-karb-225, strongly acidic cation exchanger [Ion exchange (India) Ltd., Bombay].

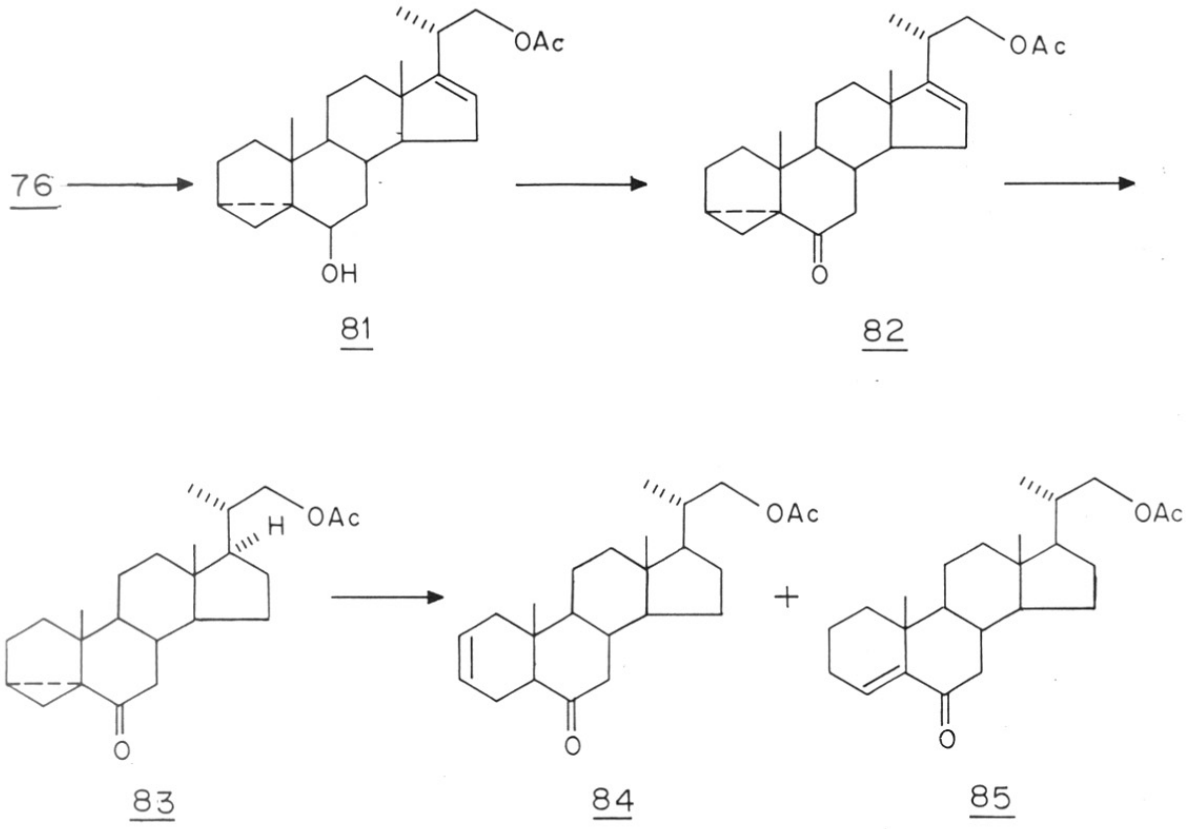
i Tulsion CXO-18, weakly acidic cation exchanger.

with pyridine and acetic anhyride (Table and experimental). This acetate (76) was converted into the aldehyde (38) following simple reaction sequence. Thus the acetate (76) on treatment with anhydrous methanol in the presence of dry pyridine, afforded the 3,5-cyclo compound (77) in 89% yield. This compound (77) on hydrogenation over Pd/C furnished the saturated compound (78)with natural configuration at C-17 in 98% yield. The hydrolysis of compound (78) with methanolic KOH afforded the known[41] alcohol (79) in 96% yield. This C-22 alcohol (79) was oxidized using pyridinium chlorochromate to get the known aldehyde (38) in 95% yield. The conversion of 17 keto steroid (73) to the aldehyde (38) in seven steps is achieved in 59% overall yield. Thus the transformation of dehydroepiandrosterone (73) to aldehyde (38) constitutes a new route to this important aldehyde (chart V).

Section B

A key intermediate (2R, 3S)-2,3,22-triacetoxy-23,24-dinor-5α-cholan-6-one(87)has been converted by Mori et al.[28] into brassinolide analogue. These investigators have syntehsised compound (87) in eleven steps starting from stigmasterol. Ikekawa et al.[9,10] have converted this intermediate (87) into the naturally occurring brassinosteroids namely dolicholide (4), dolichosterone (9) and 28-norbrassinolide (3). They have prepared this triacetate (87) in fourteen steps starting from stigmasterol. This section deals with stereoselective synthesis of this important compound (87) in nine steps starting from dehydroepiandrosterone (73). The acetate (76), prepared from 17-ketosteroid (73) in three steps as described earlier in section A (chart V), was solvolysed using potassium acetate in aqueous acetone[29] to give 3α,5-cyclo-6-hydroxy steroid (81). The crude i-alcohol (81) was oxidized with Jones reagent to give the corresponding 6-keto compound (82), which on hydrogenation over 10% Pd/C afforded the saturated product

CHART VI


76
OAc
OH
81
OAc
O
82
H
OAc
O
83
OAc
O
84
+
OAc
O
85
84
HO
HO
OAc
O
86
AcO
AcO
OAc
O
87

(83). This (83) was heated with lithium bromide and pyridinium p-toluenesulfonate in DMF to give a mixture of two rearranged[30] unsaturated ketones (84). and (85). This mixture was chromatographed on silica gel and was eluted with ethyl acetate-pet.ether (5:95). Compound (84) was isolated in earlier fractions and compound (85) in later fractions. The structure of compound (85) was assigned from ^{1}H-NMR values and its refusal to convert into compound (84) on heating it for a longer period with pyridinium p-toluenesulfonate, LiBr in DMF. Although its IR value (1708 cm^{-1}) is not in agreement with α,β-unsaturated carbonyl absorption. Formation of this type of compound (85) is not uncommon[30] in literature. The double bond in ring A of compound (84) was hydroxylated with catalytic amount of OsO_4 and excess N-methylmorpholine-N-oxide (NMO) in aqueous acetone[31] to give the (2R, 3S)-dihydroxy compound (86) in 94% yield. This dihydroxy compound (86) on heating at 60°C for 8 hr with pyridine and acetic anhydride was converted into required triacetate (87). This forms a new short route (chart VI) for a formal total synthesis of dolicholide (4), dolichosterone (9) and 28-norbrassinolide (3) and a few brassinolide analogue.

Section C

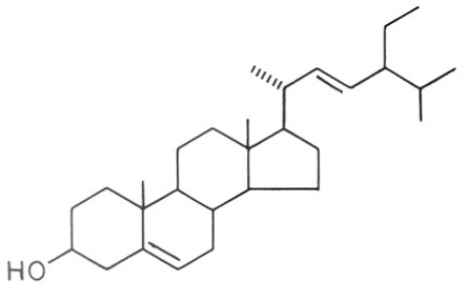
Since the discovery of brassinolide (1) has the remarkable biological activity, much efforts has been devoted to isolation of related natural products and to the synthesis of brassinolide (1) and its analogues. For the synthesis of these steroids, the introduction of -OH groups at C-22 and C-23 with the required stereochemistry, is of great importance. In some synthetic sequences the C-22, C-23 epoxides are crucial intermediate[15]. In other approaches (22 R) hydroxy-(23 S, 24 R)-epoxide is an ideal intermediate[13,14]. In view of the striking sensitivity of carbon shifts to steric effect in cyclic systems allow the determination of the configuration of epoxides[32-34] and since there is a

somewhat restricted rotation of the steroidal side chain, Sierra et al.[35] carried out an analysis of some C-22, C-23 epoxides by ^{13}C-NMR spectroscopy. They found that C-22 and C-23 of (R,R)-epoxide show very similar chemical shifts ($\Delta\delta$ =0.1ppm) whereas in the (S,S)-epoxide those carbons are clearly distinguishable ($\Delta\delta$ = 4.6 ppm). Carbon 21 shows very similar chemical shifts in both compounds while an upfield shift of about 2.5 ppm observed for C-17 of (R,R)-epoxide in comparison with the same carbon of (S,S)-epoxide.

The present investigation in this direction deals with assigning the configuration at C-22 and C-23 of the glycidic ester (**92**) by ^{13}C-NMR spectroscopy. This study also unravel the stereochemical assignments at C-22, C-23 and C-24 of 22-hydroxy-23,24-epoxy steroids (**54**) and (**55**).

Stigmasteryl i-methyl ether (**90**) was prepared according to literature[29] procedure as follows. Stigmasterol (**88**) was converted into its tosylate (**89**) using p-toluenesulfonyl chloride in pyridine. This tosylate (**89**) when refluxed with dry methanol in the presence of fused potassium acetate,afforded stigmasteryl i-methyl ether (**90**). From this i-methyl ether (**90**), the 22,23-diol (**91**) was obtained by reaction with catalytic amount of OsO_4 in aqueous THF using N-methyl morpholine-N-oxide. The 22,23-diol (**91**) on treatment with lead (IV) acetate in dry benzene gave the aldehyde (**38**) in quantitative yield. This aldehyde (**38**) was prepared earlier[21-24] from stigmasteryl i-methyl ether (**90**) by ozonolysis in varying yields. The aldehyde (**38**) on treatment with ethylchloroacetate in the presence of potassium t-butoxide gave the glycidic ester (**92**). Out of the four possible diastereoisomers this ester (**92**) was obtained as a mixture of two diastereoisomers in 70:30 ratio as revealed from 300 MHz proton NMR. From this mixture the major isomer was separated by crystallisation from ethanol (chart VII).

CHART VII



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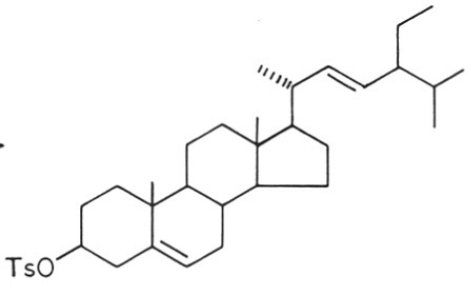

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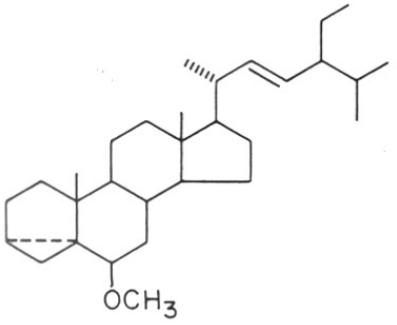

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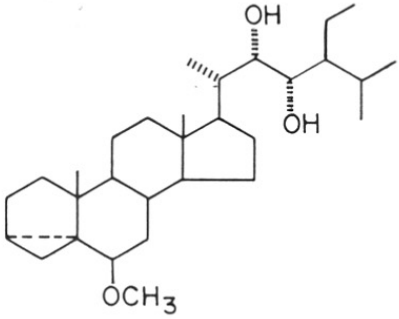

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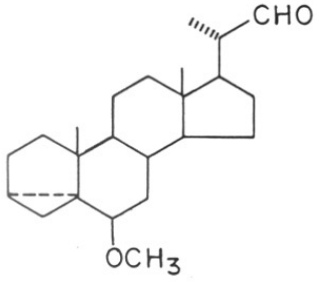

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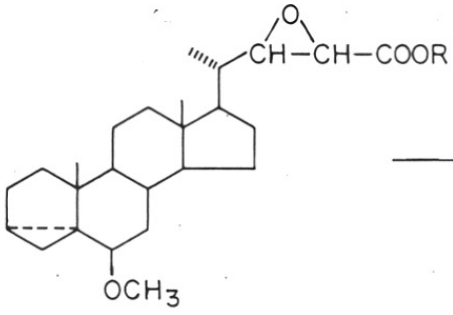
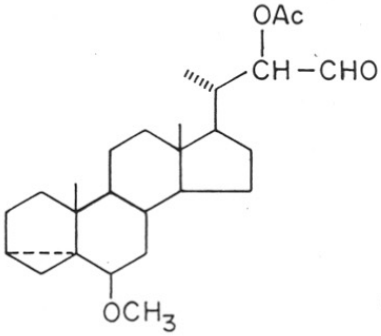


92, R = Et 93, R = Na

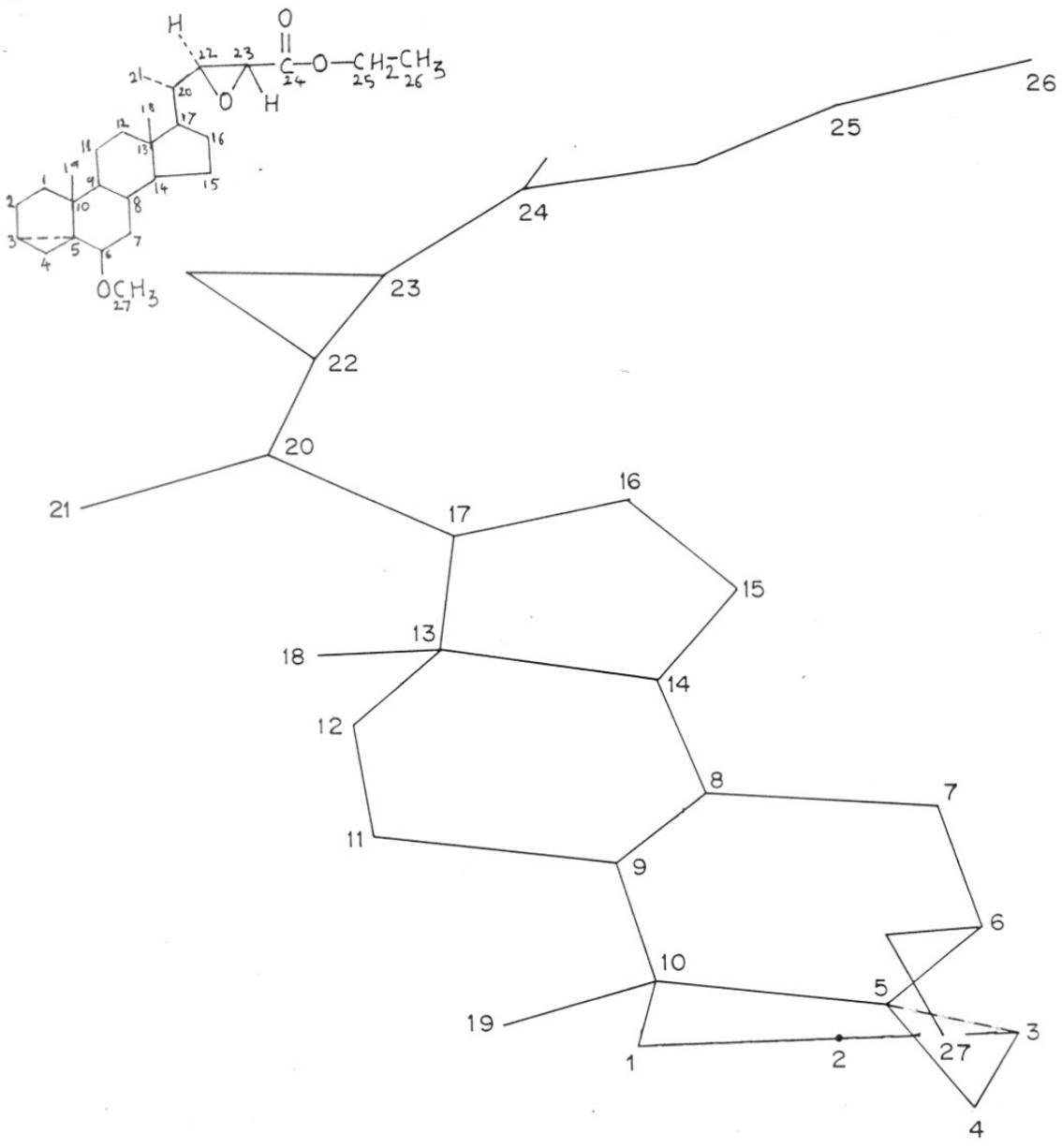


94

Dreiding model study on (R,R)-epoxide of this type of compound shows that the oxirane ring has a paralle 1,3 interaction[35] (trans)- with the hydrogens of C-21 and C-17. Consequently, the signals of both carbons C-21 and C-17 should be shielded by well known oxirane effect[32]. In the case of (S,S)-epoxide the oxirane ring steill has a parallel 1,3-interaction (trans)- with the C-21 hydrogen but not with that of C-17. Therefore, only C-21 should suffer the shielding effect.

There is no difference in ^{13}C-NMR values of carbon atom C-21 of crystallised isomer of glycidic ester (92) and the corresponding isomer in the mixture. There is a deshielding effect at C-17 of the crystallised isomer by 1.72 δ than the corresponding isomer in the mixture. From these observations the crystallised glycidic ester isomer (92) do not possess C-22, C-23 (R,R) or (S,S)- configuration.

In glycidic ester condensation the predominant stereoisomer produced appears to depend upon whether the aldol condensation step or the subsequent ring closure step is rate limiting[36]. The favoured production of the stereoisomer with the ester function trans to large group at β-carbon. According to this, the glycidic ester prepared has a trans oxirane ring with C-22, C-23 (S,R)- or (R,S)- configuration. Looking at the Dreiding model of C-22 (S)- C-23 (R)-oxirane ester, there is a parallel 1,3 interaction (trans)- with C-21 hydrogen but no such interaction with C-17 hydrogen. On the contrary, the oxygen atom of the oxirane ring is very close (cis)- to C-17 hydrogen, suggesting a deshielding effect on C-17. The crystallised isomer (92) has C-17 value at lower field ($\Delta\delta$=1.72 ppm) compared to the other isomer present in the mixture. So the crystallised isomer has C-22 (S)-, C-23 (R)- configuration. This is confirmed by X-ray analysis (see fig.).



VIEW OF THE MOLECULE ALONG [0,1,0] PLANE.

Table 2

Carbon shifts of compounds **54** and **55**

Carbon number	**54**	**55**
1	39.37	39.40
2	72.65[a]	72.71[a]
3	72.60[a]	72.64[a]
4	40.72	40.83
5	45.23	45.30
6	107.27[b]	107.31[b]
7	42.45	42.52
8.	32.71	32.71
9.	51.73	51.93
10	42.05	42.82
11	20.53	20.54
12	27.44	27.66
13	37.75	37.80
14	55.54	55.48
15	21.72	21.77
16	23.82	24.13
17	39.79	41.01
18	11.60	11.74
19	13.14	13.18
20	26.75	22.84
21	12.44	13.03
22	70.43	68.24
23	63.04	63.45
24	60.12	56.39
25	52.68	52.79
26	18.68[c]	18.48[c]
27	20.15[c]	20.07[c]
28	26.31[d]	26.36[d]
29	28.38[d]	28.42[d]
30	109.43[b]	109.42[b]
31	63.90[e]	63.98[e]
32	65.22[e]	65.28[e]

[a-e]The assignments for these signals within a vertical column may be reversed.

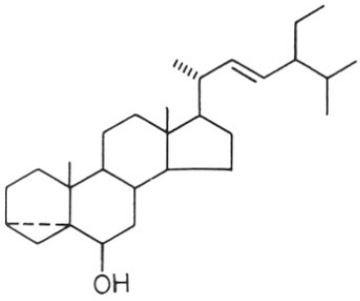
The probable ^{13}C chemical shift data of the mixture of C-22, C-23- (R, S) and (S,R)-glycidic ester and the crystallised isomer C-22 (S)-, C-23 (R)-glycidic ester is presented in Table 2.

The crude glycidic ester (92) was converted into its sodium salt (93) with sodium in ethanol. This sodium salt (93) on treatment with $Pb(OAc)_4$ in benzene containing pyridine gave 22-acetoxy-23-aldehyde (94) which may be a mixture of (22R)- and (22S)- acetoxy -23-aldehyde. Attempts were not made to get these isomer in pure form.

The compound (2R, 3S, 22E)-2,3 isopropylidene-dioxy-6,6-ethylenedioxy-5α-stigmast-22-ene (100) was prepared according to literature[15] procedure. Stigmasterol (88) was converted into its tosylate (89) as described earlier in this section. This tosylate (89) was solvolysed using aqueous acetone in the presence of potassium acetate to get i-stigmasterol (95)[29] (chart VIII). The crude alcohol (95) was oxidised with Jones reagent in acetone to afford the i-ketone (96). The arrangement of i-ketone (96) using LiBr and pyridinium tostylate in DMF gave 2,22-diene (97)[30]. The double bond in ring A was oxidised fairly rapidly (3 hr) with catalytic amount of OsO_4 and excess N-methylmorpholine-N-oxide (NMO) in aqueous acetone[31] to give highly crystalline 2,3-diol (98). On treatment with 2,2-dimethoxy propane and p-toluene sulfonic acid the keto-diol (98), was converted to the corresponding acetone (99) in quantitative yield. The carbonyl group in compound (99) was then protected by converting it into 2,3-isopropylidenedioxy-6,6-ethylenedioxy compound (100) with 2,2-dimethoxy-1,3-dioxalane in the presence of PTSA. From this (100), the 22,23-diol (101) was obtained by reaction with catalytic amount of OsO_4 in aqueous THF using NMO. This 22,23-diol (101) on treatment with $Pb(OAc)_4$ in dry benzene gave the aldehyde (43) in quantitative yield. Aldehyde (43) was prepared

CHART VIII

89 →



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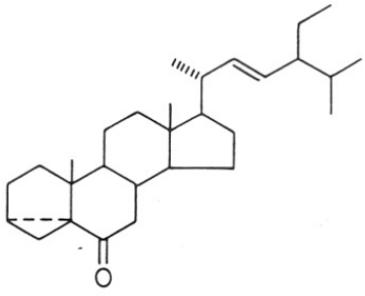

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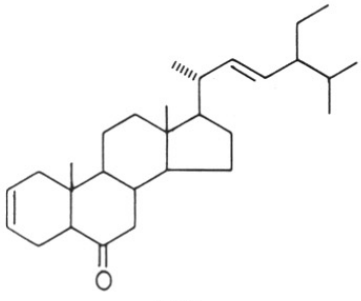

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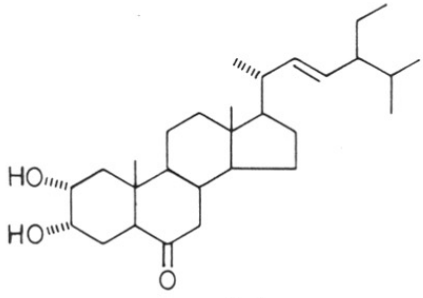

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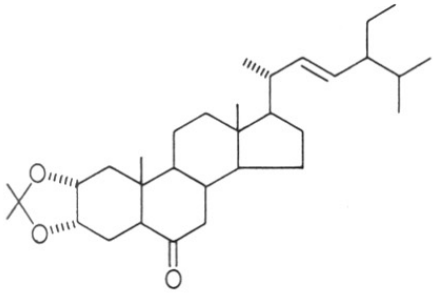
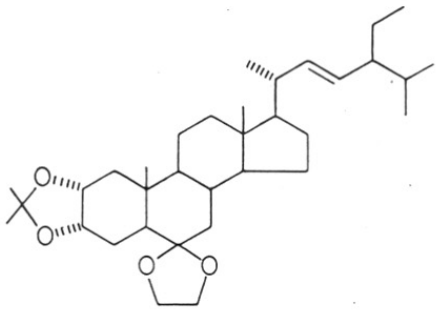
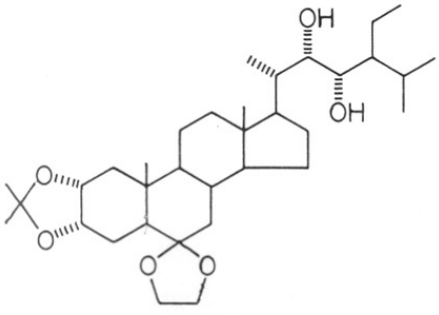


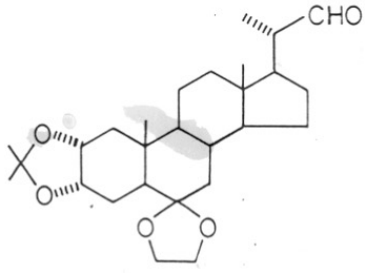
99 →



100 →



101 →



43

earlier[15] from the compound (100) by ozonolysis. From this aldehyde (43), the epoxy alcohols (54) and (55) were prepared by known procedure[13,14]. Addition of LiC≡C-Pr^{i} prepared in situ from 1,1-dibromo-3-methyl-1-butene and n-BuLi, to aldehyde (43), yielded a diastereomeric mixture of two alkynyl alcohols (51) and (52). Catalytic hydrogenation of the mixture of (51) and (52) over P-2 Ni[37] in the presence of ethylenediamine gave a diastereomeric mixture of an allylic alcohol (53) and its (22 S)-isomer (53A). The mixture was epoxidised with MCPBA to give a mixture of two epoxides (54) and its (22S)-isomer (55). These were separated by silica gel chromatography. The undesired compound (55) was eluted in earlier fractions and the desired (54) in later fractions (chart IV, scheme 5).

G. Lukacs et al. have reported the utility of ^{13}C-NMR spectsroscopy to determine the absolute configuration at C-22 of steroids substituted at this centre and having the cholestane side chain[38]. They found that ^{13}C chemical shifts at carbons 17, 20, 21 and 22 of the C-22(S)-hydroxy compound are at higher field than that of C-22 (R)-hydroxy compound.

Komeno et al.[32] found that chemical shift of carbon, γ from oxygen, bearing an axial hydrogen atom, is strongly dependent upon the configuration of the epoxide ring. If the epoxide oxygen and the axial hydrogen in γ position are cis to one another, the carbon atom bearing the hydrogen is strongly shielded (3.5 to 6 ppm). However, in the case of trans relationship, the chemical shift of γ carbon is only slightly affected.

In C-22(R)-epoxy alcohol (54) and C-22(S)-epoxy alcohol (55), the ^{13}C-NMR spectrum shows that values of carbon at 21 are similar for both the compounds ($\Delta\delta$=0.6 ppm) while carbons at 17,20 and 22 are different. These are due to two effects, one due to C-22 hydroxy group and other due to C-23, C-24

epoxy ring.

In the case of carbon 20 of C-22(R) epoxy alcohol (**54**), the hydroxy group at C-22 has pronounced deshielding effect (β-effect) than the C-23, C-24 epoxide oxygen though it is cis (γ effect). So net effect is deshielding of carbon 20 in C-22 (R)-epoxy alcohol (**54**). In case of carbon 20 of C-22(S)-epoxy alcohol (**55**), there is shielding effect due to hydroxy group at C-22 and no effect due to C-23, C-24 trans epoxide. So net effect is shielding of carbon 20 in C-22 (S)-epoxy alcohol (**55**).

The carbon 22 in C-22 (S)-epoxy alcohol (**55**) is at higher field than that of C-22(R)- comnpound which is in agreement with the literature[38].

The carbon 17 of C-22(R)- compound (**54**) is observed to be at higher field, than the same carbon atom of C-22(S)- compound (**55**). This can be explained on the following basis. In compound (**54**), due to C-22(R)- hydroxy group, carbon at 17 is less effectively deshielded than the C-23, C-24 cis epoxide shielding effect. So net effect is shielding of carbon 17. In compound (**55**), due to C-22 (S)-hydroxy group, carbon 17 is shielded and C-23, C-24 trans epoxide has no effect. So net effect is again shielding of C-17 carbon atom. But here, shielding effect of epoxide is more than that of hydroxy group so carbon 17 of compound (**54**) is at higher field than that of compound (**55**).

According to literature[35] the carbons of epoxide ring of (R,R)- compound show very similar chemical shift ($\Delta\delta$=0.1 ppm) whereas in the (S,S)-epoxide those carbons are clearly distinguishable ($\Delta\delta$= 4.6 ppm). In compound (**54**) the difference between the ^{13}C values of the carbons of epoxide ring i.e. C-23 and C-24 is 2.92δ while that of compound (**55**) is 7.06δ i.e. difference in (R,R)-epoxide (**54**) and (S,S)-epoxide (**55**) is 4.04δ. This observed difference ($\Delta\delta$ = 4.04) is in agreement with the literature value.

The probable ^{13}C chemical shift data of compounds (**54**) and (**55**) is presented in Table 3.

Table 3

Carbon shifts of compound 92

Carbon number	(22S, 23R)-	(22S, 23R)- + (22R,23S)
C_1	33.15	33.58
C_2	24.08	24.40
C_3	21.19	21.61
C_4	12.91	12.56 (13.15)
C_5	34.96	35.47
C_6	82.08	82.59
C_7	34.94	35.08
C_8	30.32	30.65
C_9	47.83	48.35
C_{10}	43.17	43.60
C_{11}	22.48	22.78
C_{12}	39.78	40.22
C_{13}	42.85	40.41
C_{14}	54.70	55.00
C_{15}	24.72	25.06
C_{16}	27.12	27.40
C_{17}	53.46	51.74 (54.02)
C_{18}	12.16	12.43
C_{19}	19.04	19.26
C_{20}	38.68	38.85
C_{21}	16.56	16.20 (16.73)
C_{22}	55.84	56.29
C_{23}	63.00	62.93 (63.19)
C_{24}	168.95	169.05
C_{25}	61.18	61.24 (61.37)
C_{26}	13.98	14.27
C_{27}	56.36	56.62

EXPERIMENTAL

Section A

3β-Hydroxy-(Z)-pregna-5,17(20)-diene (74)

Potassium t-butoxide (7.68 g, 68.6 mmol) was prepared by dissolving potassium metal (2.68 g) in t-butanol (43 ml) by heating under reflux for 1.5 hr. It was cooled to room temperature and to it $(C_6H_5)_3$ P-CH_2CH_3I (28.7 g, 68.6 mmol) and THF (40 ml) were added. The mixture was stirred for 0.5 hr and to it, solution od dehydroepiandosterone (**73**, 9.88 g, 34.31 mmol) in THF (40 ml) was added. The reaction mixture was then refluxed for 6.5 hr. It was cooled to room temperature. To the reaction mixture 50% methanol in water (150 ml) was added. It was stirred for 20 minutes and then extracted with hexane (3 x 100 ml), hexane extract was washed with water (2 x 50 ml) and brine (50 ml). It was dried over anhydrous sodium sulfate. After removal of solvent a gummy compound was obtained (16.2 g). To this gummy mass, methanol (50 ml) was added and it was warmed to get clear solution. This solution was then cooled and to it methyl iodide (8 ml) was added and kept overnight at room temeprature. The solvent was removed under reduced pressure to get the 3β-hydroxy-(Z)-pregna-5,17(20)-cliene (**74**, 6.75 g). From the mother liquor more (**74**) was obtained by column chromatography over silica gel (2.53 g), combined yield 9.28 g, 89%; m.p.136-137°C (lit.[39] m.p. 136-138°C); IR(nujol) 3280 and 1060 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.88 (s, 3H, CH_3-18), 1.04 (s, 3H, CH_3-19), 1.6 (d, 3H, CH_3-21, J=6 Hz), 3.52 (m, 1H, CH-3), 5.04 (m, 1H, CH-20), 5.28 (m, 1H, CH-6).

3β-p-Toluenesulfonoxy-(Z)-pregna-5,17 (20) diene (75)

To a soloution of (**74**, 2.53 g, 8.4 mmol) in dry pyridine (15 ml) was added p-toluenesulfonyl chloride (3 g, 15.8 mmol). Reaction mixture was

kept in dark for 48 hr. The reaction mixture was then poured in ice cooled solution of 5% sodium bicarbonate (200 ml). The compound 3β-p-toluenesulfonoxy-(Z)-pregna-5,17 (20) diene (**75**) was isolated by filtration. It was crystallized from ether-petroleum ether, yield 3.1 g, 82%; m.p.119-120°C (lit.[27] m.p.119-119.5°C); IR (nujol) 1605, 1200, 1180, 1110, 980, 960, 905 880 and 825 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.87 (s, 3H, CH_3-18), 0.98 (s, 3H, CH_3-19), 1.65 (d, 3H, CH_3-21, J=7 Hz), 2.44 (s, 3H, CH_3- Ar), 4.29 (m, 1H, CH-3), 5.13 (m, 1H, CH-20), 5.32 (bd, 1H, CH-6), 7.33 and 7.78 (AB pattern, 4H, Ar, J=8 Hz).

(20S)-3 β -p-Toluenesulfonoxy-23,24,dinor-5,16-diene-5α-cholan-22 - acetate (76)

Paraformaldehyde (0.5 g) and acetic anhydride (1 ml) were added to a stirred solution of (**75**, 2.27 g, 5 mmol) in methylene chloride (40 ml). Borontrifluoride etherate (0.06 ml, 10 mole%) in methylene chloride (0.25 ml) was added dropwise to the reaction mixture. It was then stirred at room temperature for 45 min. Pyridine (10 ml) and acetic anhydride (5 ml) were added to the reaction mixture and then it was stirred overnight. Reaction mixture was poured on crushed ice and extracted with methylene chloride (3 x 50 ml). Methylene chloride extract was washed successively with water (2 x 25 ml), saturated sodium bicarbonate solution (2 x 25 ml), water (2 x 25 ml), 2% cold hydrochloric acid (2 x 25 ml), water (2 x 25 ml) and finally with brine. It was dried over anhydrous sodium sulfate. Removal of solvent afforded compound (**76**) as white solid (2.53 g, 95%). It was crystallized from hexane, m.p.107-108°C, (lit.[27] m.p.109-110°C); IR ($CHCl_3$) 1740 (OAc), 1605, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.76 (s, 3H, CH_3-18), 0.93 (s, 3H, CH_3-19), 1.04 (d, 3H, CH_3-21, J=6 Hz), 2.02 (s, 3H, OAc), 2.44 (s, 3H, CH_3-Ar), 3.4-

4.2 (m, 2H, CH_2-22), 4.27 (m, 1H, CH-3), 5.2-5.4 (m, 2H, CH-6 and CH-16), 7.29 and 7.76 (AB pattern, 4H, Ar, J=10 Hz).

(20S)-3β-p-Toluenesulfonoxy-23,24-dinor-5,16-diene-5α-cholan-22-acetate (76) and Wagner-Meerwein rearranged product (80)

To a stirred suspension of Amberlyst-15 resin (0.570 g) and paraformaldehyde (0.025 g), a solution of compound (**75**, 0.114 g) in methylene chloride (3 ml) was added followed by acetic anhydride (0.05 g). After 10-12 minutes resin was filtered and filtrate was diluted with methylene chloride and washed with water. Methylene chloride layer was dried over anhydrous sodium sulfate. Removal of solvent afforded a mixture of two compounds. It was chromatographed over silica gel. Elution with ethyl acetate-petroleum ether (4:96) gave compound (**80**, 0.052 g, 41%); IR ($CHCl_3$) 1600, 1370, 1190, 1180, 945 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.78 (t, 3H, CH_3-21, J=7 Hz), 0.96 (s, 6H, CH_3-17 and CH_3-19), 2.44 (s, 3H, CH_3-Ar), 4.29 (m, 1H, CH-3), 5.32 (m, 1H, CH-6), 7.27 and 7.76 (AB pattern, 4H, Ar, J=10 Hz); MS 454 (M^+), 439, 425, 296, 289, 282, 267, 253 (100%), 197, 172, 157, 143; further elution with ethyl acetate-petroleum ether (6:94), gave the compound (**76**, 0.042 g, 36%), m.p.107-108°C. Its IR, NMR, TLC and mixed m.p. were identical to that of compound (**76**) obtained earlier in BF_3-etherate catalysed reaction.

It was found that when concentration of resin is reduced from 5 parts to 0.5 parts per part of the compound, the ratio of products (**76**) and (**80**) changes to 40:37. If the concentration of the resin is further reduced to 0.3 parts, the ratio of (**76**) and (**80**) changes to 75:15.

(20S)-3α,5-Cyclo-6β-methoxy-23,24-dinor-5α-cholan-16-ene-22-acetate (77)

To a solution of compound (**76**, 1.7 g, 3.2 mmol) in dry methanol (15 ml), dry pyridine (1 ml) was added and the reaction mixture was refluxed

for 2 hr. Methanol was removesd under reduced pressure. The reaction mixture was diluted with water and extracted with ether. The ether extract was washed successively with water, 2% hydrochloric acid, water, saturated sodium bicarbonate solution, water and finally with brine Removal of solvent afforded compound (**77**) as thick oil (1.072 g, 86%); IR (neat) 1735 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.8 (s, 3H, CH_3-18), 1.04 (s, 3H, CH_3-19), 1.07 (d, 3H, CH_3-21, J=7 Hz), 2.02 (s, 3H, OAc), 2.81 (m, 1H, CH-6), 3.37 (s, 3H, OCH_3), 3.48-4.27 (m, 2H, CH_2-22), 5.24-5.48 (m, 1H, CH-16).

(20S)-3α,5-Cyclo-6β-methoxy-23,24-dinor-5α-cholan-22-acetate (78)

The compound (**77**, 1.062 g, 2.75 mmol) in ethanol (35 ml) was hydrogenated in Parr hydrogenator using 10% Pd/C (0.150 g) for 6 hr at 30 psi. The catalyst was filtered and solvent was removed under reduced pressure. The compound (**78**) was obtained as thick oil (1.045 g, 98%). It was crystallized from ethyl acetate-petroleum ether, m.p.123-124°C (lit.[40] m.p. 124-125°C); IR (nujol) 1740 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.67 (s, 3H, CH_3-18), 0.93 (d, 3H, CH_3-21, J=7 Hz), 0.96 (s, 3H, CH_3-19), 1.98 (s, 3H, OAc), 2.69 (m, 1H, CH-6), 3.24 (s, 3H, OCH_3), 3.53-4.13 (m, 2H, CH_2-22).

(22S)-3α,5-Cyclo-6β-methoxy-23,24-dinor-5α-cholan-22-ol (79)

A solution of potassium hydroxide (0.112 g, 2 mmol) in methanol (10 ml) was added to compound (**78**, 0.378 g, 0.97 mmol). The reaction mixture was stirred at room temperature for 16 hr. Methanol was removed under reduced pressure. On usual work up, it afforded the compound (**79**) as thick liquid (0.322 g, 96%); IR (neat) 3495 (-OH), 1460, 1380, 1100 and 1020 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.84 (s, 3H, CH_3-18), 1.00 (d, 3H, CH_3-21, J=6 Hz), 1.04 (s, 3H, CH_3-19), 2.76 (m, 1H, CH-6), 3.28 (s, 3H, OCH_3), 3.5 (d, 2H, CH_2-22, J=6 Hz).

(20S)-3α,5-Cyclo-6β-methoxy-5α-pregnane-20-carboxyaldehyde (38)

To a stirred suspension of potassium acetate (0.025 g) and pyridinium chloro chromate (0.185 g) in methylene chloride (5 ml), a solution of compound (**79**, 0.160 g, 0.46 mmol) in methylene chloride (1 ml) was added dropwise. The reaction mixture was stirred at room temperature for 1 hr. To the reaction mixture ether was added and it was filtered. The residue was washed thoroughly with ether. The filtrate was washed twice with brine, dried over anhydrous sodium sulfate and evaporated to afford the compound (**38**) as thick liquid (0.151 g, 95%); IR (neat) 2700, 1730, 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$), 0.76 (s, 3H, CH_3-18), 1.00 (s, 3H, CH_3-19), 1.11 (d, 3H, CH_3-21, J=7 Hz), 2.74 (m, 1H, CH-6), 3.29 (s, 3H, OCH_3), 9.51 (d, 1H, CHO, J=3 Hz).

Section B

(20S)-3α,5-Cyclo-6β-hydroxy-23,24-dinor-5α-cholan-16-ene-22-acetate (81)

Compound (**76**, 2.0 g, 3.8 mmol prepared earlier as described in Section A) was dissolved in acetone (40 ml). To it anhydrous potassium acetate (1 g, 10.2 mmol) and water (10 ml) were added. The reaction mixture was refluxed on water bath for 3 hr. Solvent was removed and the reaction mixture was extracted with pet-ether. Pet-ether extract was washed with water followed by brine and dried over anhydrous sodium sulfate. Removal of solvent gave compound (**81**) as thick liquid (1.4 g, 99.3%); IR (nujol) 3460 (-OH), 1750 (OAc), 1235 and 1020 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.84 (s, 3H, CH_3-18), 1.09 (s, 3H, CH_3-19), 1.13 (d, 3H, CH_3-21, J=7 Hz), 2.04 (s, 3H, OAc), 3.27 (t, 1H, CH-6, J=2 Hz), 3.64-4.18 (m, 2H, CH_2-22), 5.42 (bs, 1H, CH-16); MS 324, 310 (100%), 295, 269, 173.

(20S)-3α,5-Cyclo-23,24-dinor-5α-cholan-16-ene-22-acetoxy-6-one (82)

To a stirred and cooled (10°C) solution of compound (**81**, 1.4 g) in acetone (45 ml), Jones reagent (1.4 ml, 8N) was added dropwise, keeping the temperature

below 15°C. After the addition, the reaction mixture was stirred for 5 minutes more and to it methanol was added to destroy excess Jones reagent. Solvents were removed completely and reaction mixture was extracted with ether (3 x 75 ml). Ether layer was washed successively with water (2 x 25 ml), saturated sodium bicarbonated solution (2 x 25 ml), water (2 x 25 ml) and brine. Ether extract was dried and evaporated to give the compound (82) as thick liquid (1.31 g). This crude product was chromatographed over silica gel to get pure ketone (82, 0.81 g, 62%); IR (neat) 1740 (OAc) and 1685 (>C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.8 (s, 3H, CH_3-18), 1.04 (s, 3H, CH_3-19), 1.13 (d, 3H, CH_3-21, J=7 Hz), 2.04 (s, 3H, OAc), 2.47 (d, 2H, CH_2-7, J=10 Hz), 3.84-4.29 (m, 2H, CH_2-22), 5.42 (bs, 1H, CH-16); MS 310 (100%) 295, 269, 173, 161, 145, 123 and 105.

(20S)-3α,5-Cyclo-23,24-dinor-5α-cholan-22-acetoxy-6-one (83)

Compound (82, 0.5 g) in ethanol (20 ml) was hydrogenated in Parr apparatus using 10% Pd/C (0.1 g) for 6 hr at 30 psi. The catalyst was filtered and solvent was removed to give compound (83) as oil (0.466 g, 93%); IR (neat) 1735 (OAc), 1690 (>C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.76 (s, 3H, CH_3-18), 0.93 (d, 3H, CH_3-21, J=7 Hz), 1.0 (s, 3H, CH_3-19), 2.04 (s, 3H, OAc), 2.44 (d, 2H, CH_2-7, J=10 Hz), 3.6-4.29 (m, 2H, CH_2-22); MS 372 (M^+, 100%), 354, 344, 314, 297, 269, 136, 121, 91, 79.

(20S)-23,24-Dinor-5α-cholan-22-acetoxy-2-ene-6-one (84) and its isomer (85)

A mixture of compound (83, 0.410 g, 1.1 mmol), lithium bromide (0.041g, 0.47 mmol) and pyridinium p-toluenesulfonate (0.041 g, 0.16 mmol) in DMF (5 ml) was refluxed for 0.5 hr. Reaction mixture was cooled to room temperature, cold water was added to it and then it was extracted with ethyl acetate. Ethyl acetate extract was washed successively with water (2 x 25 ml), saturated

sodium bicarbonate solution (2 x 10 ml), water (2 x 25 ml) and brine. It was dried over anhydrous sodium sulfate. Removal of solvent gave a foamy solid (0.425 g) which was a mixture of two compounds. It was chromatographed over silica gel column. Elution with EtOAc:petroleum ether (5:95) gave compound (**84**) as white solid (0.146 g) m.p. 135°C; IR (nujol) 1736 (OAc), 1708 (>C=O) and 1235 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.71 (s, 6H, CH_3-18 and CH_3-19), 0.96 (d, 3H, CH_3-21, J=7 Hz), 2.04 (s, 3H, OAc), 3.64-4.29 (m, 2H, CH_2-22), 5.58 (m, 2H, CH-2 and CH-3); MS 372 (M^+), 357 (100%), 344, 297, 229, 175, 159, 147, 133, 121, 107, 93, 79; Analysis found C, 77.18; H, 9.62. $C_{24}H_{36}O_3$ require C, 77.37; H, 9.74%.

Further elution with same solvent system gave an oily product (0.148g); IR ($CHCl_3$) 1732 (OAc), 1708 (>C=O) and 1220 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.71 (s, 6H, CH_3-18 and CH_3-19), 1.02 (d, 3H, CH_3-21, J=7 Hz), 2.04 (s, 3H, OAc), 3.6-4.29 (m, 2H, CH_2-22), 5.56 (m, 1H, CH-4); MS 372 (M^+, 100%), 357, 344, 297, 269, 229, 175, 159, 147, 136, 121, 107, 93, 79; Analysis found C, 77.09; H, 9.59. $C_{24}H_{36}O_3$ require C, 77.37; H, 9.74%. This oily compound (0.14 g), LiBr (0.014 g), pyridinium p-toluenesulfonate (0.014 g) in DMF (3 ml) was refluxed for 4 hr. The starting material remained unchanged.

(2R,3S)-2,3-Dihydroxy-22-acetoxy-23,24 dinor-5α-cholan-6-one (86)

A solution of OsO_4 (0.016 g) in t-BuOH (0.2 ml) was added to a solution of compound (**84**, 0.109g, 0.29 mmol) in acetone (5.5 ml). Then NMO (0.109g) and water (0.2 ml) were added to it. The reaction mixture was stirred under nitrogen for 1.5 hr. A solution of sodium bisulfite (0.1 g) in water (1 ml) was added to the reaction mixture. It was stirred for 0.5 hr and then filtered through celite. Filtrate was evaporated to dryness. The residue was extracted with chloroform. On usual work up, it afforded compound (**86**) as a white solid (0.112 g, 94%). It was crystallized from

methanol, m.p. 212°C; IR (nujol) 3520, 3440, 1735 (OAc), 1705 (>C=O) and 1270 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.67 (s, 3H, CH_3-18), 0.76 (s, 3H, CH_3-19), 0.93 (d, 3H, CH_3-21, J=7 Hz), 1.82 (bs, 2H, OH), 2.04 (s, 3H, OAc), 2.64 (dd, 1H, CH-5, J=4, 13 Hz), 3.6-4.29 (m, 2H, CH_2-22), 3.6-3.89 (m, 1H, CH-2), 3.93-4.09 (m, 1H, CH-3); MS 406 (M^+), 391, 346, 328, 304 (100%), 286, 277, 272, 263, 245, 175, 133, 121, 107, 93, 81; Analysis Found C, 70.48; H, 9.27; $C_{24}H_{38}O_5$ require C, 70.9; H, 9.42%.

(2R, 3S)-2,3,22-Triacetoxy-23,24-dinor-5α-cholan-6-one (87)

A mixture of compound (**86**, 0.09 g), pyridine (1 ml) and acetic anhydride (0.8 ml) was heated at 60-65°C, with stirring for 8 hr. On usual work up it gave compound (**87**) as a gummy solid. It was chromatographed on silica gel column. Elution with EtOAc-petroleum ether (15:85) gave solid, which was recrystallized from methanol, m.p. 165°C (lit.[9,10] m.p. 212°C; lit.[28] m.p.182-185°C) (yield 0.071 g, 66%); IR (nujol) 1745 (-OAc), 1720 (>C=O), 1265, 1240, 1165, 1050 and 1030 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.69 (s, 3H, CH_3-18), 0.82 (s, 3H, CH_3-19), 0.96 (d, 3H, CH_3-21, J=7 Hz), 1.96 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.07 (s, 3H, OAc), 3.6-4.31 (m, 2H, CH_2-22), 4.89 (m, 1H, CH-2), 5.31 (m, 1H, CH-3); MS 490 (M^+), 430, 388 (100%), 355, 306, 260, 245, 227, 215, 175, 147, 135, 121, 105, 93, 81.

Section C

Stigmasteryl tosylate (89)

Stigmasterol (**88**, 25.0 g, 60 mmol) was converted into its tosylate using p-toluene sulfonyl chloride (30 g) and pyridine (300 ml); yield 34.0g, 99%; IR (nujol) 1600, 1195, 1175, 1095, 960 and 940 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 2.44 (s, 3H, CH_3-Ar), 4.3 (m, 1H, CH-3), 5.02 (m, 2H, CH-22 and 23), 5.27 (m, 1H, CH-6), 7.24 and 7.74 (AB pattern, 4H, Ar, J=9 Hz).

i-Stigmasterol methyl ether (90)

Stigmasteryl tosylate (20 g) was dissolved in dry methanol (450 ml). To it fused potassium acetate (10 g) was added. The reaction mixture was refluxed for 5 hr. On usual work up it gave i-stigmasterol methyl ether (**90**) (14.69 g; 98%); IR (neat) 1460, 1380, 1100 and 965 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 2.55 (m, 1H, CH-6), 3.2 (s, 3H, OCH_3), 5.0 (m, 2H, CH-22 and 23).

(24S)-24-Ethyl-3α,5-cyclo-6βmethoxy-5α-cholest-22,23-diol (91)

A solution of OsO_4 (0.8 g) in t-BuOH (26.2 ml) was added to a solution of stigmasterol-i-methyl ether (**90**, 15 g) in THF (300 ml). Then NMO (11.25g) and water (75 ml) was added to it. The mixture was stirred at room temperature for 6 days under nitrogen.Additional amount of NMO (1 g x 2) was added during that period. Excess of OsO_4 was reduced with sodium bisulfite solution. The mixture was filtered through celite and the filtrate was concentrated in vacuo. The residue was extracted with ethyl acetate (3 x 100 ml). Ethyl acetate extract was washed successively with water (2 x 50 ml), 5% cold HCl (2 x 50 ml), water (2 x 50 ml), saturated $NaHCO_3$ solution (2 x 50 ml), water (2 x 50 ml) and brine. It was dried over anhydroud sodium sulfate. Removal of solvent afforded 22, 23-diol (**91**) as foamy solid (15.8 g, 97%). It was crystallized from ethyl acetate-petroleum ether, m.p.122-123°C; $[\alpha]_D^{25}$ + 37.8° (c = 2.3, $CHCl_3$); IR (nujol) 3570, 3430 and 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.76-1.11 (18H , CH_3 at 18, 19, 21, 26, 27 and 29), 2.78 (m, 1H, CH-6), 3.31 (s, 3H, OCH_3), 3.62 (m, 2H, CH-22 and CH-23); Analysis found C, 78.53; H, 10.96. $C_{30}H_{52}O_3$ require C, 78.26; H, 11.3%.

(20S), 3α,5-Cyclo-6β-methoxy-5α-pregnane-20-carboxyaldehyde (38)

To a stirred solution of 22,23-diol (**91**, 1.4 g, 3 mmol) in dry benzene (15 ml) $Pb(OAc)_4$ (1.46 g, 3.3 mmol) was added in small portions during

15 minutes at 15°C Stirring was continued for 1.5 hr (starting material completely reacts as shown by TLC). The reaction mixture was then filtered and the residue was washed thoroughly with ether-petroleum ether, 1:1 (20 ml). Filtrate was taken in separatory funnel and it was washed successively with water (2 x 15 ml), sodium bicarbonate solution (2 x 10 ml), water (2 x 25 ml) and brine. It was dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure at low temperature afforded sufficiently pure aldehyde (**38**, 1.03 g, 98%). Its IR and NMR were identical with that of the aldehyde prepared from dehydroepiandrosterone (**73**) in section A (chart V).

3α,5-Cyclo-6β-methoxy-5α-pregnane-20-ethyl glycidate (92)

The aldehyde (**38**, 1.0 g, 2.9 mmol) and ethyl chloroacetate (0.37 g, 3 mmol) in dry benzene (5 ml) were taken in a 50 ml two necked flask fitted with addition funnel and a thermometer. It was cooled below 15°C and a solution of potassium t-butoxide prepared from potassium (0.120 g, 3 mmol) in t-BuOH (2.6 ml) was added dropwise during 20 minutes under nitrogen atmosphere. Stirring was continued for 2 hr. Reaction mixture was then poured on ice and extracted with ether. After usual workup it afforded the glycidic ester (**92**) as a gummy solid (1.23g, 98%). It was crystallized from ethanol m.p.119°C; $[\alpha]_D^{25}$ + 39.7 (c = 1.99, $CHCl_3$); IR (nujol) 1760, 1740 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.71 (s, 3H, CH_3-18), 1.03 (s, 3H, CH_3-19), 1.13 (d, 3H, CH_3-21, J=7 Hz), 1.29 (t, 3H, CH_3-CH_2, J=7 Hz), 2.77 (t, 1H, CH-6, J=2 Hz), 2.87 (dd, 1H, CH-22, J=2, 7 Hz), 3.31 (d, 1H, CH-23, J=2 Hz), 3.32 (s, 3H, OCH_3), 4.24 (q, 2H, CH_2-CH_3, J=7 Hz); MS 430 (M^+), 415, 398, 375 (100%), 293, 255, 213, 199, 185, 173, 159, 145, 135, 121, 105, 91, 79, 71, 67, 55; ^{13}C-NMR ($CDCl_3$) 168.95 (C-24, >C=O), 82.08 (C-6), 62.99 (C-23), 55.84 (C-22), 54.70 (C-14), 56.36 (OCH_3), 61.18 (O-CH_2-CH_3), 53.46 (C-17), 47.83 (C-9), 42.85 (C-13), 43.17 (C-10), 39.78 (C-12), 38.68 (C-20), 34.97

(C-5), 34.95 (C-7), 33.15 (C-1), 30.32 (C-8), 27.12 (C-16), 24.73 (C-15), 24.08 (C-2), 22.48 (C-11), 21.19 (C-3), 19.04 (C-19), 16.56 (C-21), 13.18 ($O-CH_2-\underline{CH_3}$), 12.91 (C-4), 12.16 (C-18); Analysis found C, 75.03; H, 9.84; $C_{27}H_{42}O_4$ require C, 75.34; H, 9.76%.

3α, 5-Cyclo-6β-methoxy-5α-pregnane-20-sodium glycidate (93)

To the ethyl glycidate (**92**, 0.9 g, 2.1 mmol), cold solution of sodium ethoxide prepared from sodium (0.048, 2.1 mmol) in ethanol (1 ml) was added. It was stirred in ice-bath and 3 drops of water were added to it. The thick white pasty suspension was allowed to stand overnight at room temperature. Solvent was evaporated to dryness. Residue was washed with dry ether (yield 0.6 g, 67.6%); m.p.268-272°C(d); $[\alpha]_D^{25}$ + 31.3° [c=2.3, ethanol:water (1:1)]; IR (nujol) 1622 ($\overline{COO}$) cm^{-1}.

3α,5-Cyclo-6β-methoxy-22-acetoxy-24-nor 5α-cholan-23-al (94)

A mixture of sodium salt (**93**, 0.6 g, 1.4 mmol), pyridine (0.237 g, 3 mmol), $Pb(OAc)_4$ (1·33 g,3 mmol) and dry benzene (10 ml) was stirred under nitrogen for 0.5 hr at room temeprature and under reflux for 5 hr. The reaction mixture was cooled to room temeprature, treated with ethylene glycol (1 ml) to decompose excess $Pb(OAc)_4$ and then extracted with ether. Ether extract was washed successively with water, 2% cold HCl, water, sodium bicarbonate solution, water and brine. It was dried over anhydrous sodium sulfate and the solvent was removed to furnish crude acetoxy aldehyde (**94**, 0.505 g). It was chromatographed over silica gel. Elution with ethyl acetate:petroleum ether (10:90) afforded acetoxy aldehyde (**94**, 0.1 g) as an isomeric mixture at C-22; IR (neat) 2860, 1735 (OAc) 1700 (CHO), 1455, 1370, 1225 and 1080 cm^{-1}; ^{1}H-NMR ($CDCl_3$) (0.69) 0.72 (s, 3H, CH_3-18), 0.99 (bs, 3H, CH_3-19), (1.14) 1.17 (d, 3H, CH_3-21, J=7 Hz), (2.08) 2.16 (s, 3H, OAc), 2.74 (m, 1H, CH-6), 3.29 (s, 3H, OCH_3), 4.98 (d, 1H, CH-22, J=4 Hz), (9.43) 9.51 (s, 1H, CHO); MS 416 (M^+), 401, 384, 361, 342, 315, 298,

283, 275, 213, 159, 145, 133, 119, 105, 91, 79, 67, 55, 43 (100%).

i-Stigmasterol (95)

To a solution of stigmasteryl tosylate (**89**, 34.0 g, 0.059 mol) in acetone (660 ml), fused potassium acetate (33 g, 0.34 mole) and water (165 ml) were added. The reaction mixture was refluxed for 21 hr to get crude i-stigmasterol (**95**) as a gum (24.7 g, 100%); IR (neat) 3595, 3410, 1450, 1380, 1210 and 1010 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.68-1.12 (18H, CH_3-18, 19, 21, 26, 27 and 29), 3.2 (m, 1H, CH-6), 5.04 (m, 2H, CH-22 and CH-23).

(24s)-24-Ethyl-3α,5-cyclo-5α-cholest-22E-en-6-one (96)

Jones reagent (16.2 ml, 8N) was added dropwise to a stirred and cooled solution of i-stigmasterol (**95**, 24.7 g, 0.059 mol), in acetone (425 ml). The reaction mixture was stirred for 5 minutes after completion of addition. Excess CrO_3 was destroyed with methanol. Acetone was removed under reduced pressure and the residue on usual work up with ether gave i-ketone (**96**, 23.0 g, 99%); its IR, NMR data are in agreement with the literature[15] values.

(24S)-24-Ethyl-5α-cholesta-2,22E-dien-6-one (97)

A mixture of compound (**96**, 23.0 g, 0.055 mol), pyridinium p-toluenesulfonate (2.3 g, 0.009 mol), lithium bromide (2.3 g, 0.026 mol) in DMF (230 ml) was refluxed for 3 hr. On cooling the reaction mixture, solid was separated. This solid was filtered, washed and dried (16.0 g). Filtrate and the washings were collected together and extracted with ethyl acetate. On usual work up it afforded a gummy solid (6.7 g). This total product was found to be a mixture of three compounds. From this mixture compound (**97**) was separated by column chromatography. On elution with ethyl acetate-petroleum ether (1:99), compound (**97**) was isolated in earlier fractions followed by 3, 22(E)- and 4,22(E)-dienes. IR and NMR data of compound (**97**) was in agreement with the reported[15,41] values.

(2R,3S,24S)-2,3-Dihydroxy-24-ethyl-5α-cholest-22 E -en-6-one (98)

A solution of OsO_4 (0.095 g) in t-BuOH (5.84 ml) was added to a solution of compound (**97**, 3.0 g) in acetone (150 ml). The NMO (3 g) and water (5.4 ml) were added to it. The mixture was stirred at room temeprature for 5 hr. The solid obtained was filtered (1.8 g). Filtrate was treated with $NaHSO_3$ solution and concentrated in vacuo. The residue was extracted with chloroform in usual way to give a gummy solid (1.2 g). The combined product (3.0 g) was crystallized from EtOH to get pure (**98**) as a crystalline solid (2.5 g, 78%); m.p.235°C; its IR and NMR match with the reported[15] values.

(2R,3S,24S)-2,3-isopropylidenedioxy-24-ethyl-5α-cholest-22E-en-6-one (99)

To a solution of compound (**98**, 2.5 g, 5.6 mmol) in methylene chloride (45 ml), 2,2-dimethoxypropane (3.5 ml) and PTSA (0.09 g) were added. The reaction mixture was stirred at room temperature for 2 hr. After addition of powdered K_2CO_3, the mixture was stirred for 5 minutes. It was then washed with $NaHCO_3$ solution followed by water and brine. Methylene chloride extract was dried over anhydrous sodium sulfate and concentrated in vacuo to give compound (**99**) as thick oil (2.7 g). This was crystallized from ethanol, m.p.156°C (lit.[15] m.p.158-159°C); its IR, NMR are in agreement with the literature[15] values.

(2R,3S,24S)-2,3-Isopropylidenedioxy-24-ethyl-6,6-ethylenedioxy-5α-cholest-22E-ene(100)

To a solution of compound (**99**, 2.7 g, 5.8 mmol) in 2,2-dimethyl-1,3-dioxalane (30 ml), PTSA (0.17 g) was added and the solution was stirred and heated under reflux for 3 hr. The mixture was neutralised by addition of K_2CO_3 and it was diluted with ether. The ether solution was washed with saturated $NaHCO_3$ solution followed by water and brine. It was dried over anhydrous sodium sulfate and concentrated in vacuo to give compound (**100**) as a gum (2.9 g); its IR and

and NMR are in agreement with the reported[15] one.

(2R,3S,24S)-2,3-Isopropylidenedioxy-24-ethyl-6,6-ethylenedioxy-5∝-cholest-22, 23-diol (101)

A solution of OsO_4 (0.032 g) in t-BuOH (1.5 ml) was added to a solution of (2R,3S,22E)-2,3-isopropylidenedioxy-6,6-ethylenedioxy-5 ∝ -cholestan-22-ene (**100**, 1.006 g) in THF (17 ml). Then NMO (0.63 g) and water (3.5 ml) was added to it. The mixture was stirred at room temperature for 6 days under nitrogen. Additional amount of NMO (0.2 g x 2) was added during that period. Then excess of OsO_4 was reduced with sodium-bi-sulfite solution. On usual work up it gave the 22,23-diol (**101**, 1.03 g) as foammy solid; IR (nujol) 3460 (-OH), 1245, 1220, 1175 and 1055 cm^{-1}; ^{1}H-NMR ($CDCl_3$): 0.71 (s, 3H, CH_3-18), 0.83 (s, 3H, CH_3-19), 0.94 (d, 3H, CH_2-21 J=7 Hz), 1.23 and 1.38 (s, acetonide), 3.51-4.31 (m, 8H); MS 562 (M^+), 547, 513, 448, 431, 389, 371, 359, 333, 317, 235 (100%), 207, 178, 99.

(2R,3S,20S)-2,3-Isopropylidenedioxy-6,6-ethylenedioxy-5 ∝ -pregnane-20-carboxy-aldehyde (43)

To a stirred and cooled solution (15°C) of 22,23-diol (**101**, 1.7 g, 0.003 mol) in dry benzene (30 ml), $Pb(OAc)_4$ (1.5 g, 0.0033 mol) was added in small portions during 15 minutes. Stirring was continued for 1.5 hr. It was then worked up as described earlier for the $Pb(OAc)_4$ oxidation of (**91**), afforded known[15] aldehyde (**43**, 1.3 g, 100%) as an amorphous solid; IR (nujol) 2700, 1715, 1245, 1218, 1180, 1085 and 1060 cm^{-1}; ^{1}H-NMR ($CDCl_3$): 0.71 (s, 3H, CH_3-18), 0.83 (s, 3H, CH_3-19), 1.16 (d, 3H, CH_3-21, J=7 Hz), 1.36 (s, 3H), 1.51 (s, 3H), 3.92 (m, 4H), 4.16 (m, 1H, CH-2), 4.27 (m, 1H, CH-3), 9.56 (d, 1H, -CHO, J=4Hz).

A mixture of (2R,3S,22R)-and (2R,3S,22S)-6,6-ethylenedioxy-22-hydroxy-2,3-isopropylidenedioxy-5α-cholest-23-yne (51) + (52)

A solution of n-BuLi in n-hexane (1.5 molar, 6.7 ml) was added dropwise to a stirred and cooled solution of 1,1-dibromo-3-methyl-1-butene (1.26 g) in dry THF (24 ml) at -70°C to -60°C under nitrogen. The reaction mixture was warmed to -40°C and stirring was continued for 1 hr at -45°C to -35°C. Then it was again cooled to -70°C. A solution of (43, 1.2 g, 0.0027 mol) in dry THF (12 ml) was added & reaction mixture was stirred at -70 to -60°C for 1 hr. The reaction temeprature was raised to 0°C. The reaction was quenched by addition of saturated NH_4Cl solution. The reaction mixture was extracted with ether. The ether solution was washed with water and dried over anhydrous Na_2SO_4. On removal of solvent, it afforded a mixture of compounds (51) and (52) (1.5 g, 100%); IR (nujol) 3450, 1240, 1210, 1170, 1080 and 1050 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.67 (s, 3H, CH_3-18), 0.82 (s, 3H, CH_3-19), 1.13 (d, 3H, CH_3-21, J=8 Hz), 1.18 (d, 6H, isopropyl), 1.33 (s, 3H), 1.49 (s, 3H), 3.89 (m, 4H), 4.11 (m, 1H), 4.26 (m, 1H), 4.42 (bs, 1H).

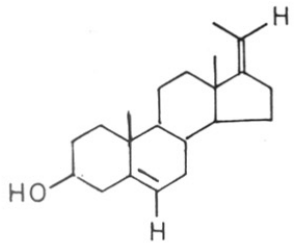
A mixture of (2R,3S,22R,23Z)- and (2R,3S,22S,23Z)-6,6-ethylenedioxy-22-hydroxy-2,3-isopropylidenedioxy-5α-cholest-23-ene (53) + (53A)

P_2/Ni catalyst was prepared from 1 molar $NaBH_4$ in ethanol and $Ni(OAc)_2 4H_2O$ (1.027 g, 0.041 mol) in 95% ethanol (8.3 ml). To this were added under nitrogen, ethylenediamine (0.54 ml)and a solution of (51) and (52) (3.17 g, 0.0061 mol) in 95% ethanol (50 ml). The mixture was stirred for 8 hr . under hydrogen at room temeprature. It was then diluted with ether and filtered. The filtrate was concentrated in vacuo. The residue was dissolved in ether. The ether solution was washed with water, dried and concentrated in vacuo to give compound (53) + (53A) as a foamy solid (3.06 g, 96%); IR (Nujol) 3450 (-OH), 1250, 1225,

1185, 1100 and 1070 cm^{-1}; ^{1}H-NMR ($CDCl_3$): 0.62 (s, 3H, CH_3-18), 0.76 (s, 3H, CH_3-19),0.8-2.33 (m), 1.31 (s, 3H), 1.49 (s, 3H), 3.61-4.56 (m, 7H), 5.11-5.49 (m, 2H).

(2R,3S,22R,23S,24R)-23,24-Epoxy-6,6-ethylenedioxy-22-hydroxy-2,3-isopropylidene-dioxy-5α-cholestan (54) and its (2R,3S,22S,23R,24S)-isomer (55)

MCPBA (50 %, 5 g) was added to a stirred solution of (**53**) + (**53A**) (3.05g) in dry CH_2Cl_2 (140 ml). The stirring was continued for 5 hr. The reaction mixture was washed with 1N NaOH solution followed by water and brine. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give crude product (2.83 g). It was chromatographed on silica gel. Elution with EtOAc:n-hexan (10:90) gave the undesired (22S)-isomer (**55**) in earlier fractions (0.440 g); IR (nujol) 3455 (-OH), 1250, 1220, 1180, 1095 and 1070 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.71 (s, 3H, CH_3-18), 0.83 (s, 3H, CH_3-19), 0.84-2.3 (m), 1.3 (s, 3H), 1.63 (s, 3H), 2.66 (dd, 1H, CH-24, J=4 and 9 Hz), 3.03 (t, 1H, CH-23, J=4 Hz), 3.63 (m, 1H, CH-22), 3.69-4.32 (m, 6H); MS 532 (M^+), 517, 499, 431, 417, 359, 321 (100%), 303, 249, 239, 99 ; and the desired (22R)-isomer (**54**) in later fractions (0.614 g); IR(nujol) 3455 (-OH), 1250, 1225, 1185, 1095 and 1065 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.67 (s, 3H, CH_3-18), 0.84 (s, 3H, CH_3-19), 0.85-2.4 (m), 1.3 (s, 3H), 1.48 (s, 3H), 2.66 (dd, 1H, CH-24, J=4 and 9 Hz), 3.04 (t, 1H, CH-23, J=4 Hz), 3.58 (d, 1H, CH-22, J=7 Hz), 3.69-4.32 (m, 6H); MS 532 (M^+), 517, 448, 433, 359, 321 (100%), 249, 237, 207, 181, 125, 99.



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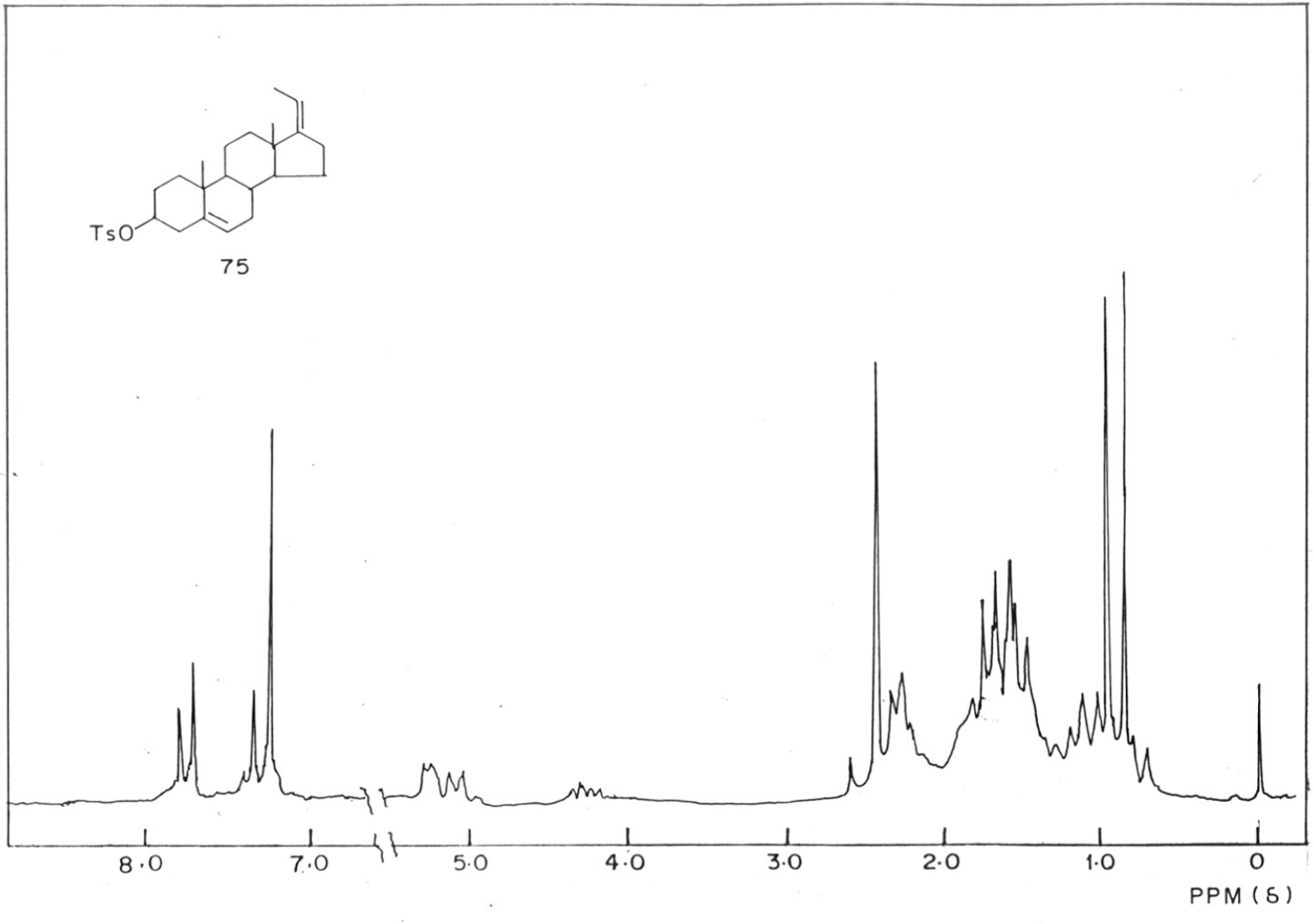
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75
8·0
7·0
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4·0
3·0
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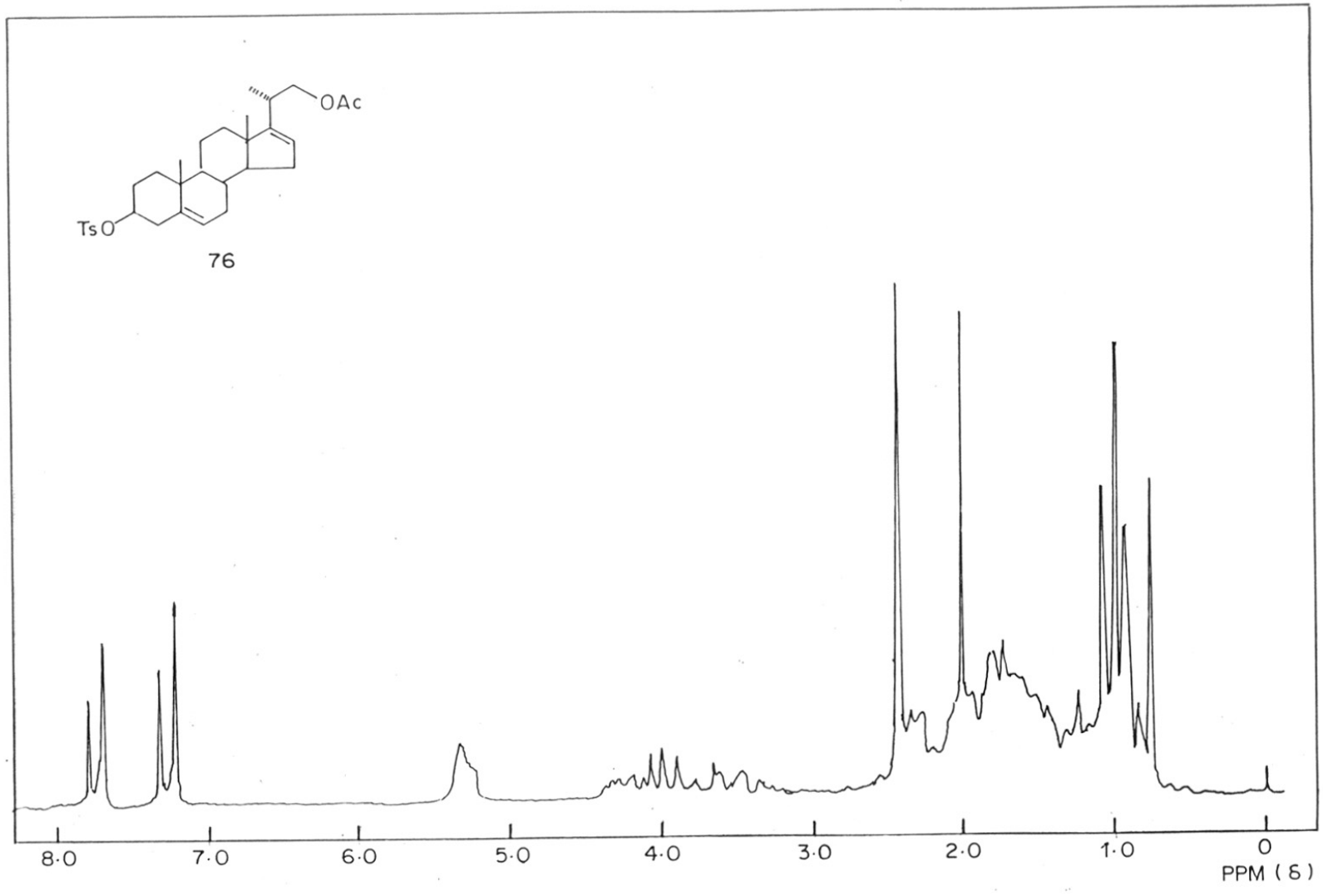
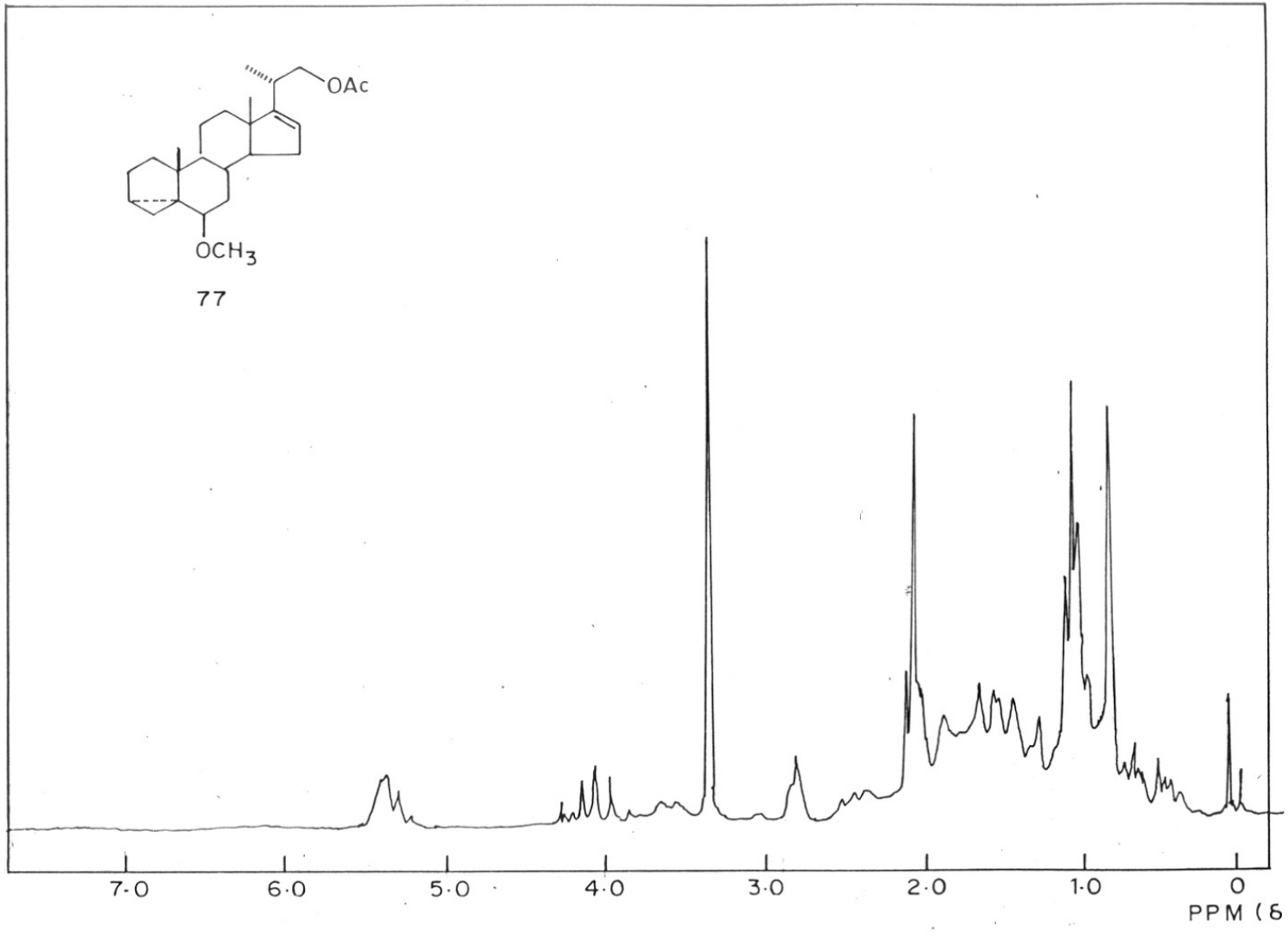
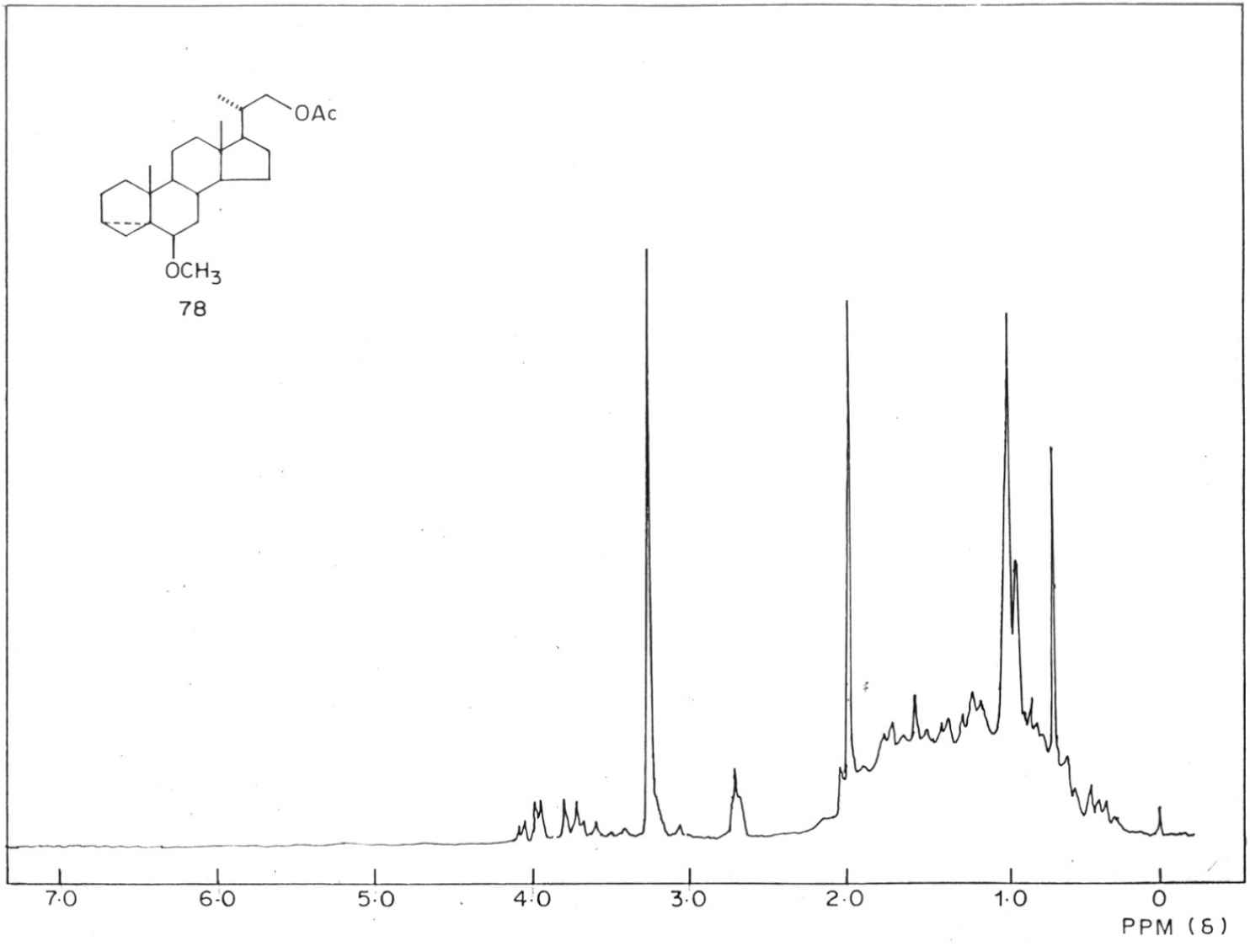
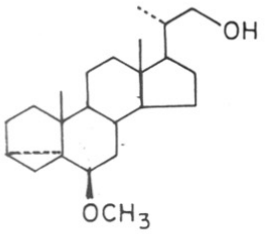
0
PPM (δ)


OAc
TsO
76
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)


OAc
OCH3
77
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)


OAc
OCH3
78
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)



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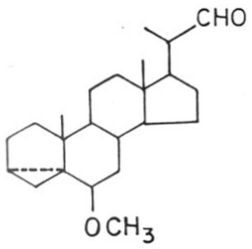

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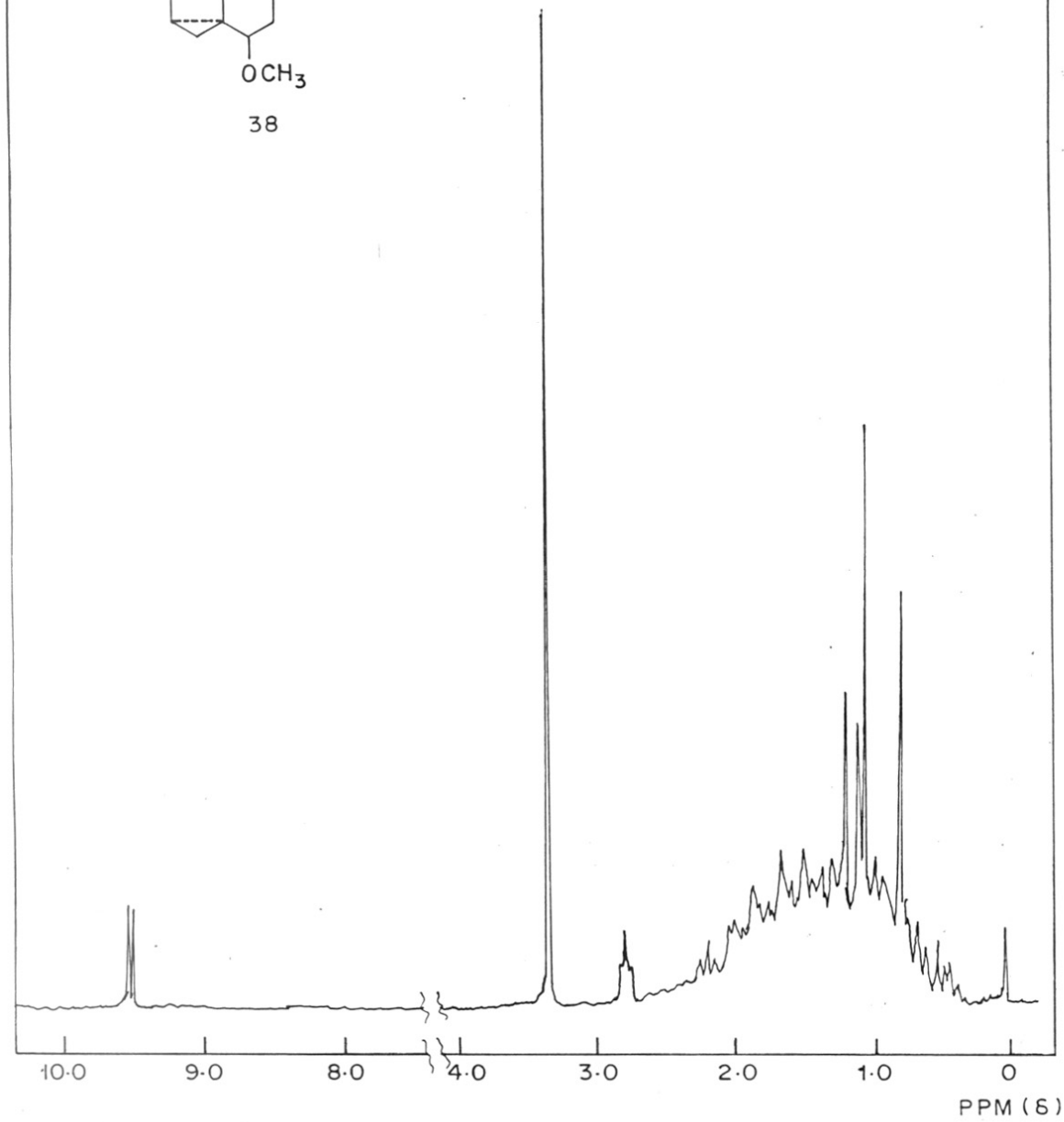

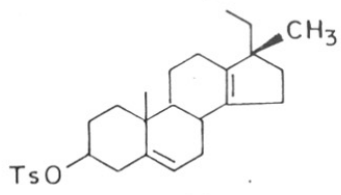

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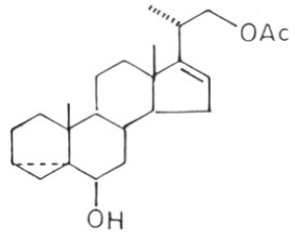

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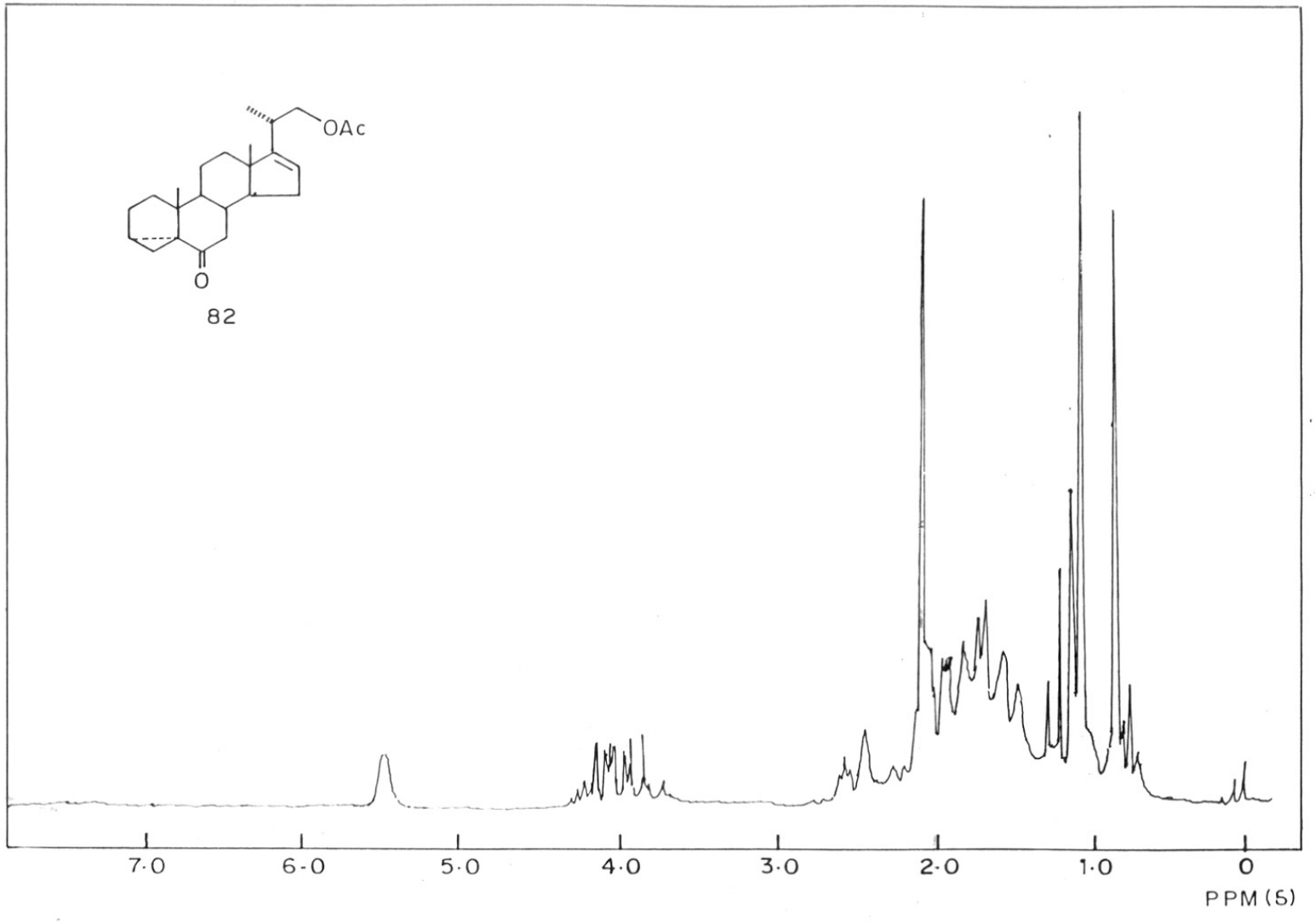
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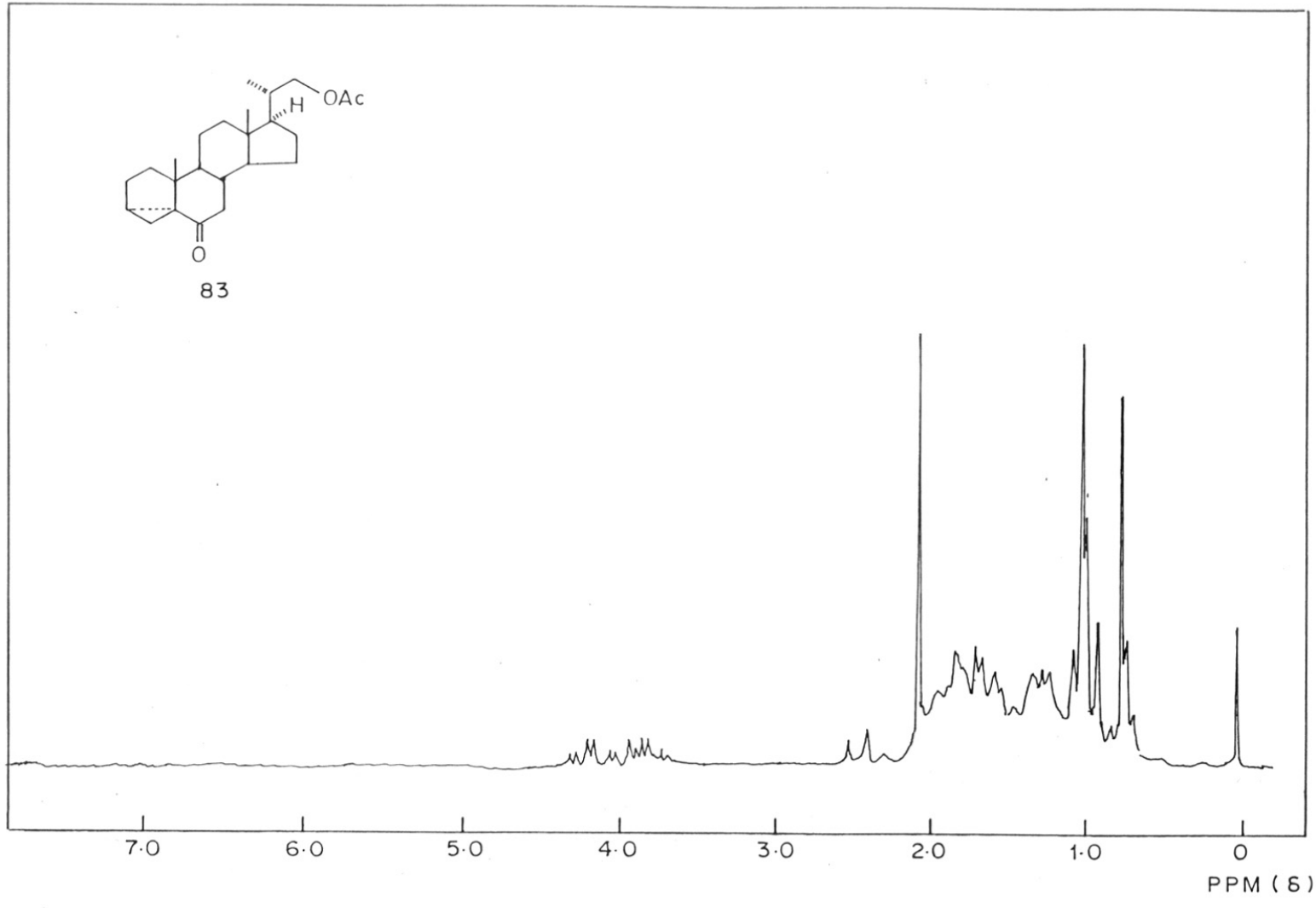
O
82
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)



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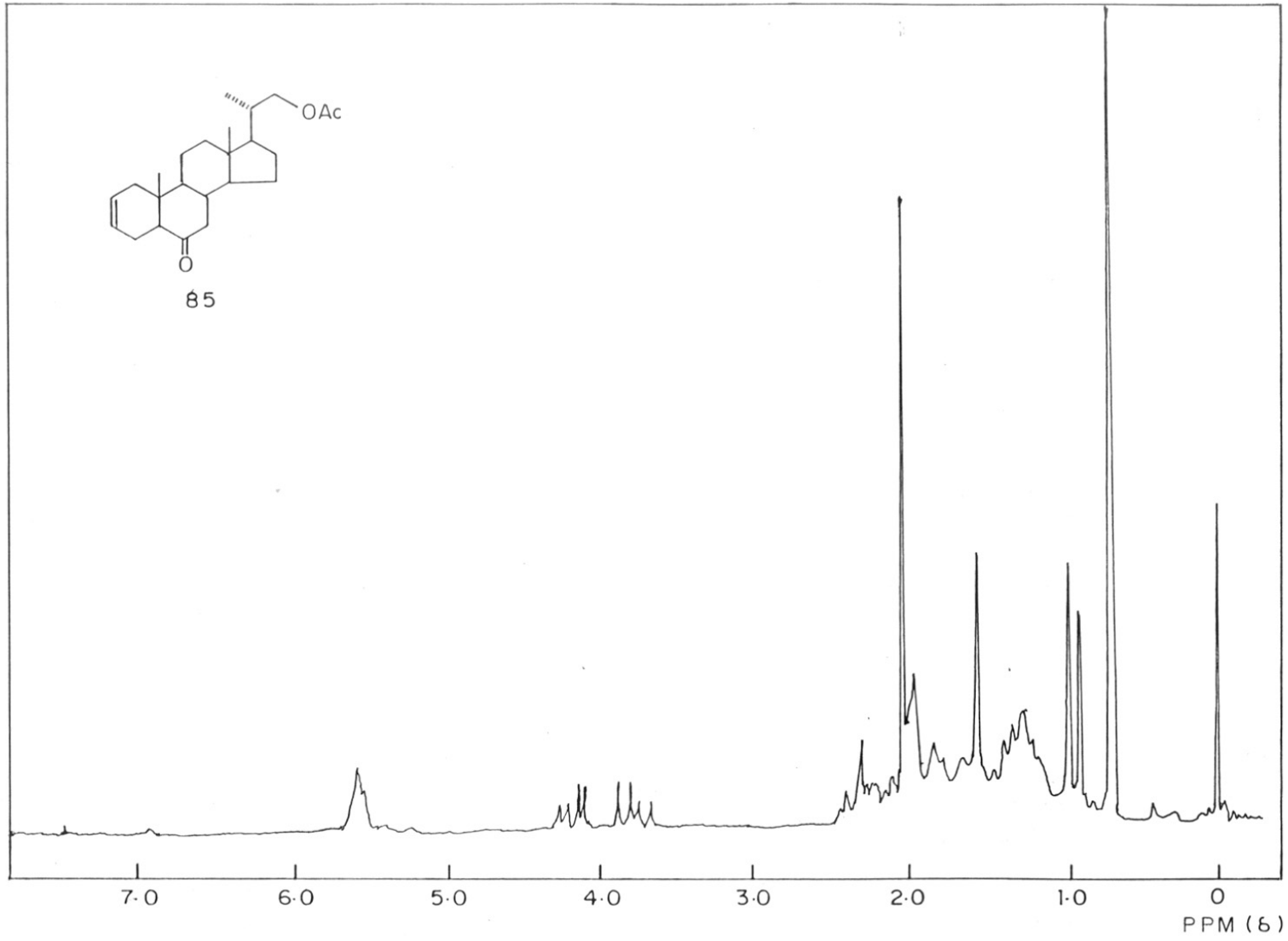
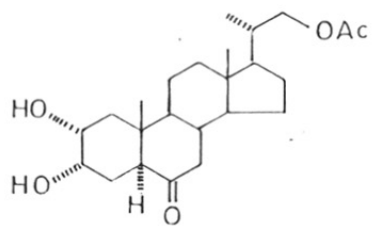

OAc
O
85
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)



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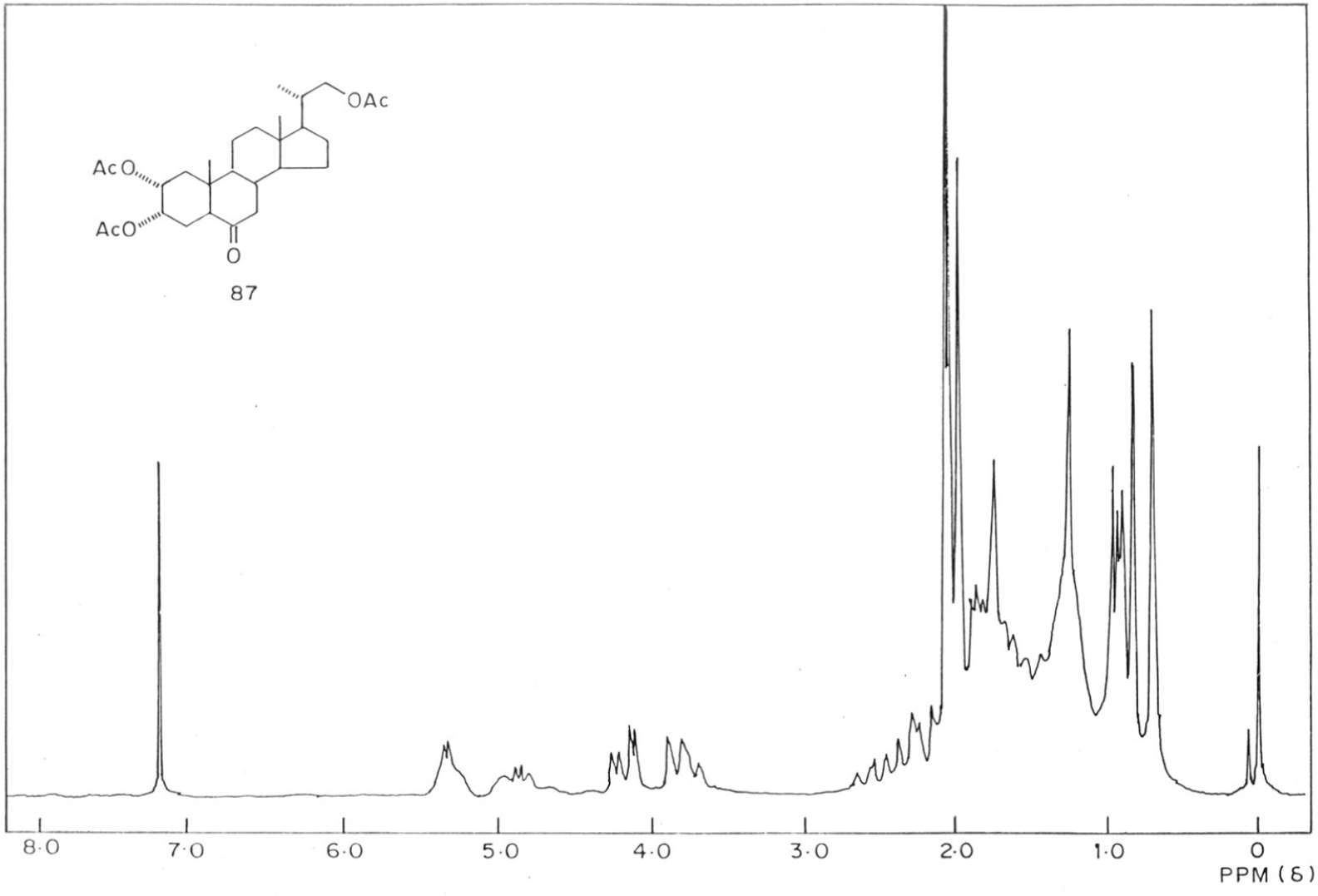
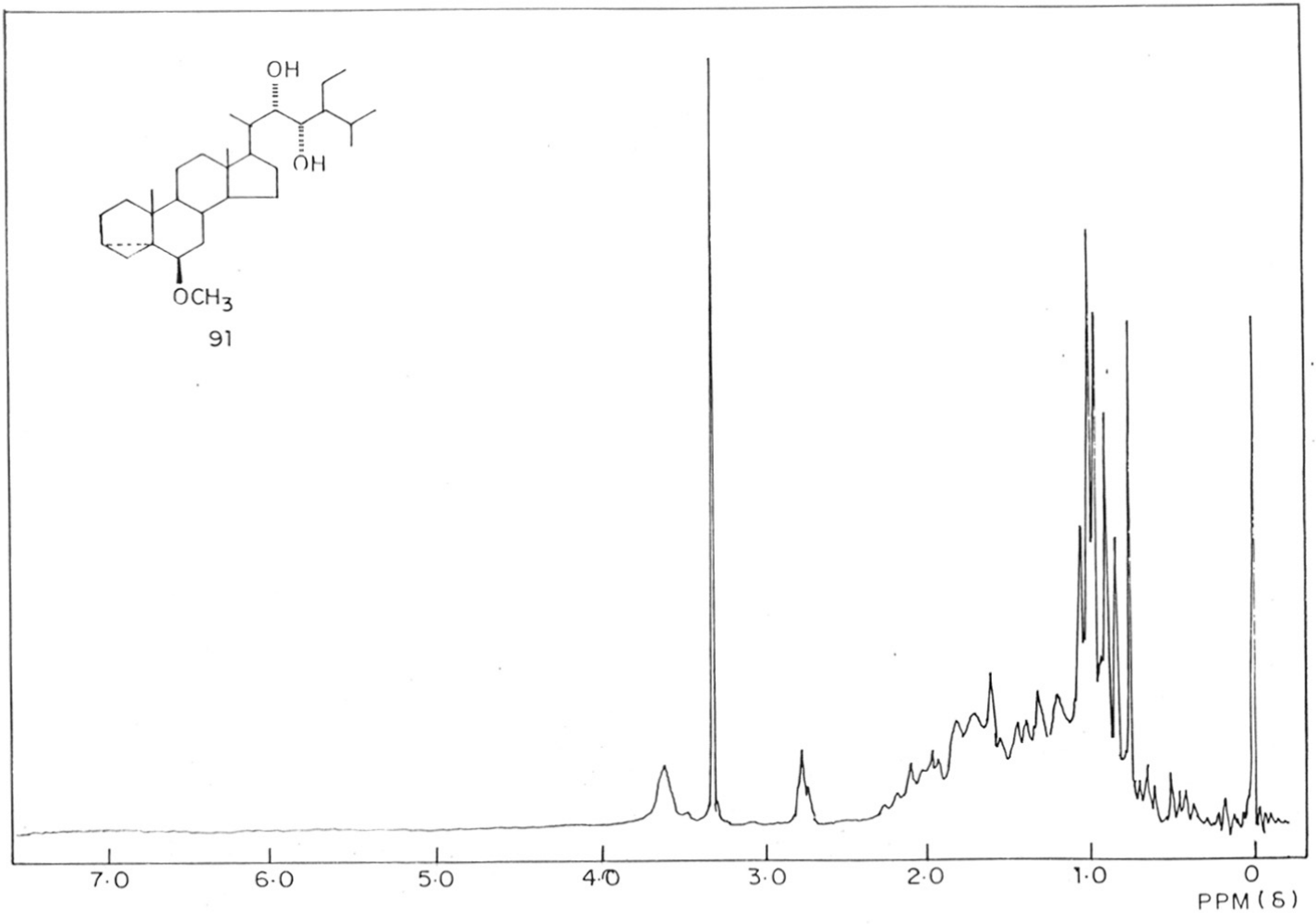
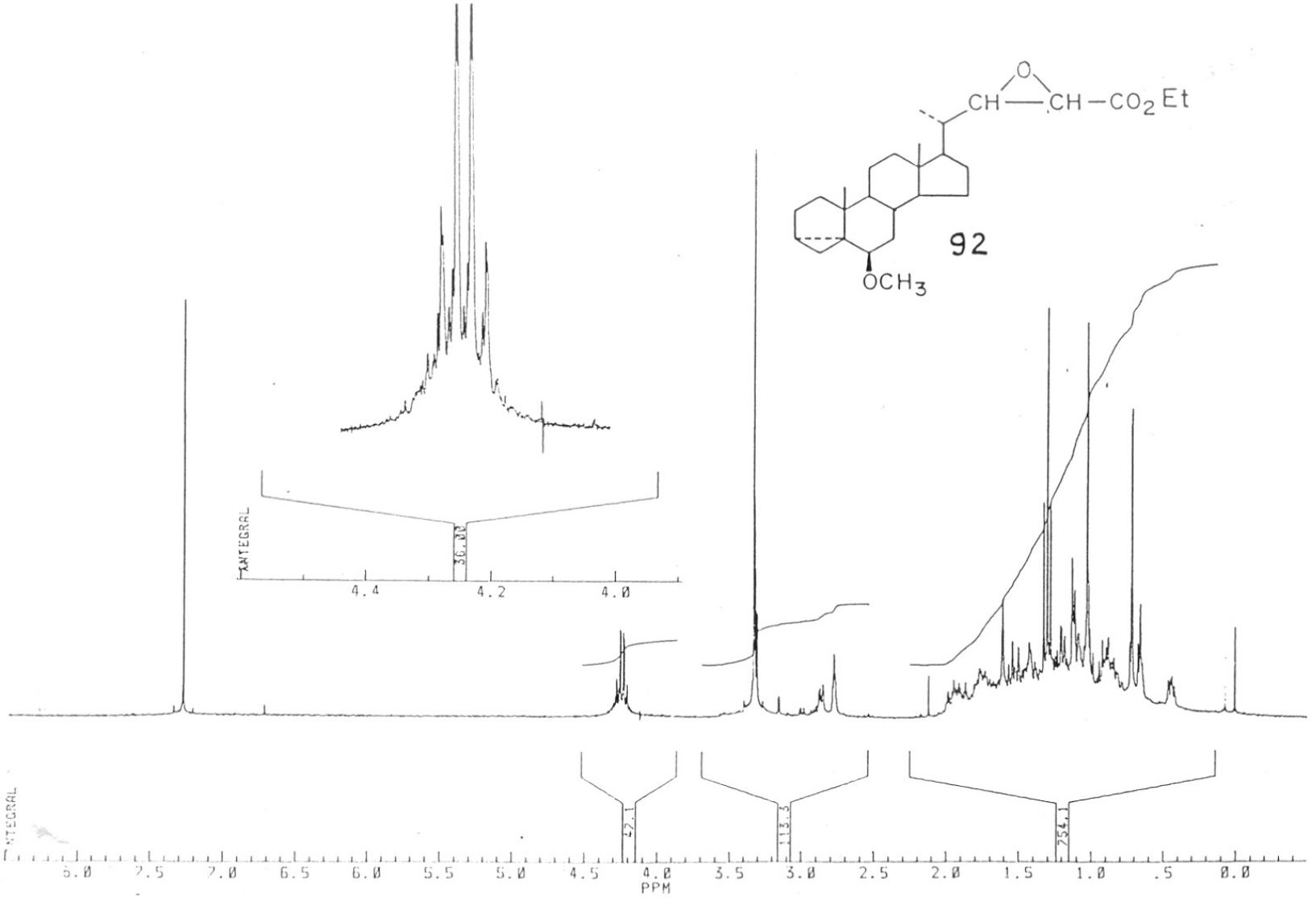
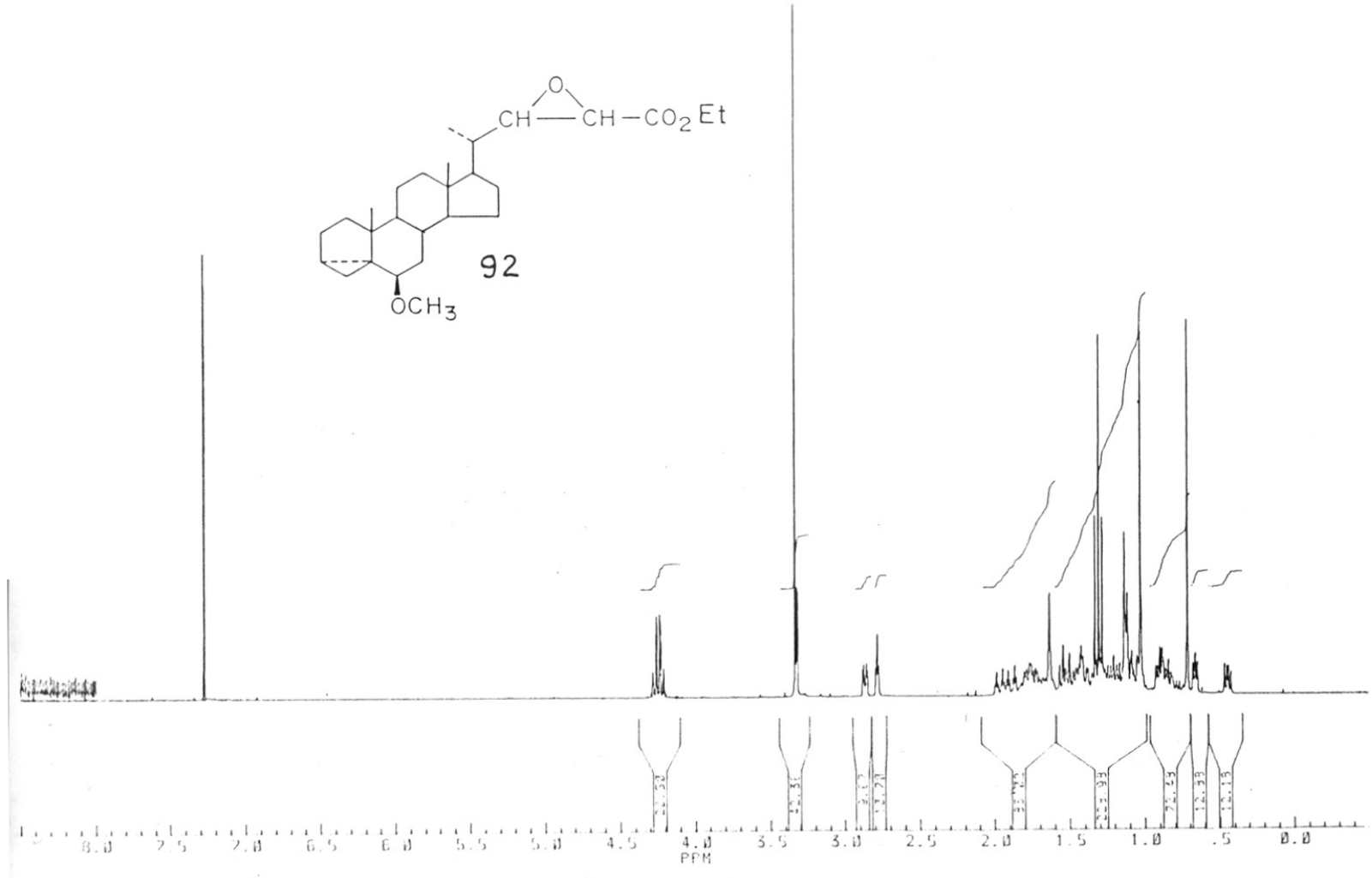
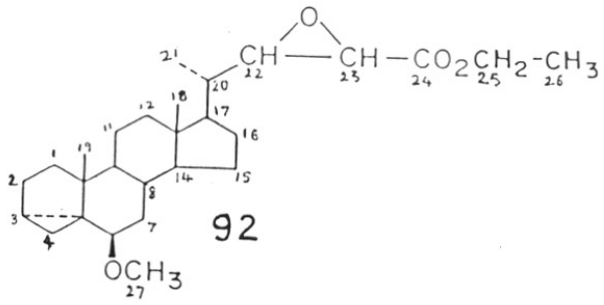

OAc
AcO
AcO
O
87
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)


OH
OH
OCH3
91
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)

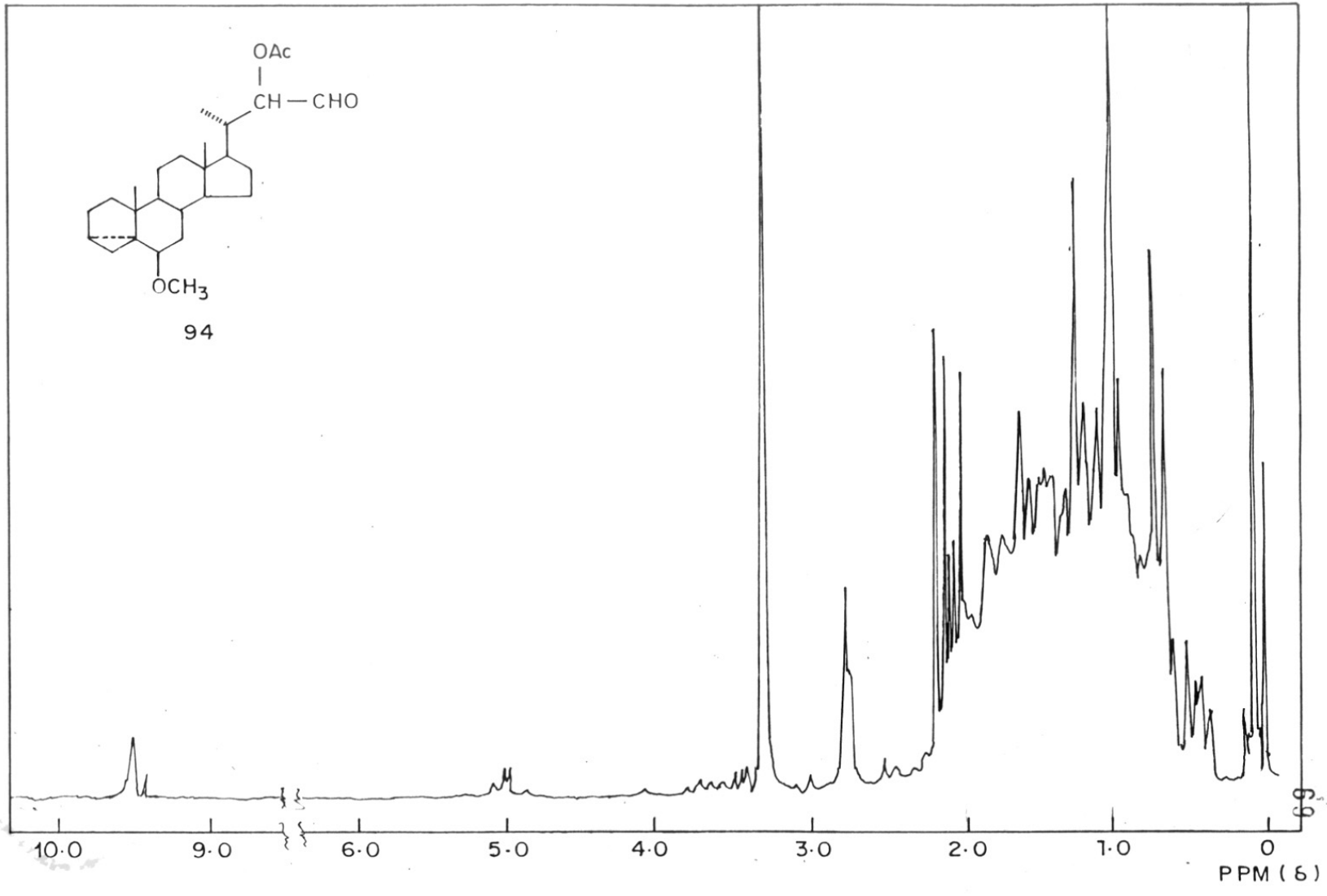

O
CH — CH — CO2 Et
OCH3
92
INTEGRAL
36.00
4.4
4.2
4.0
INTEGRAL
42.1
113.3
254.1
5.0
2.5
2.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM


CH — CH — CO2 Et
O
OCH3
92
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM


O
CH
CH — CO2CH2-CH3
21
22
23
24
25
26
20
17
16
12
18
11
19
1
2
3
4
14
15
8
7
OCH3
27

92

CHCl3
160
140
120
100
80
60
40
20
PPM


OAc
CH — CHO
OCH3
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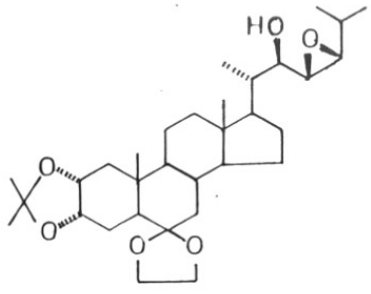
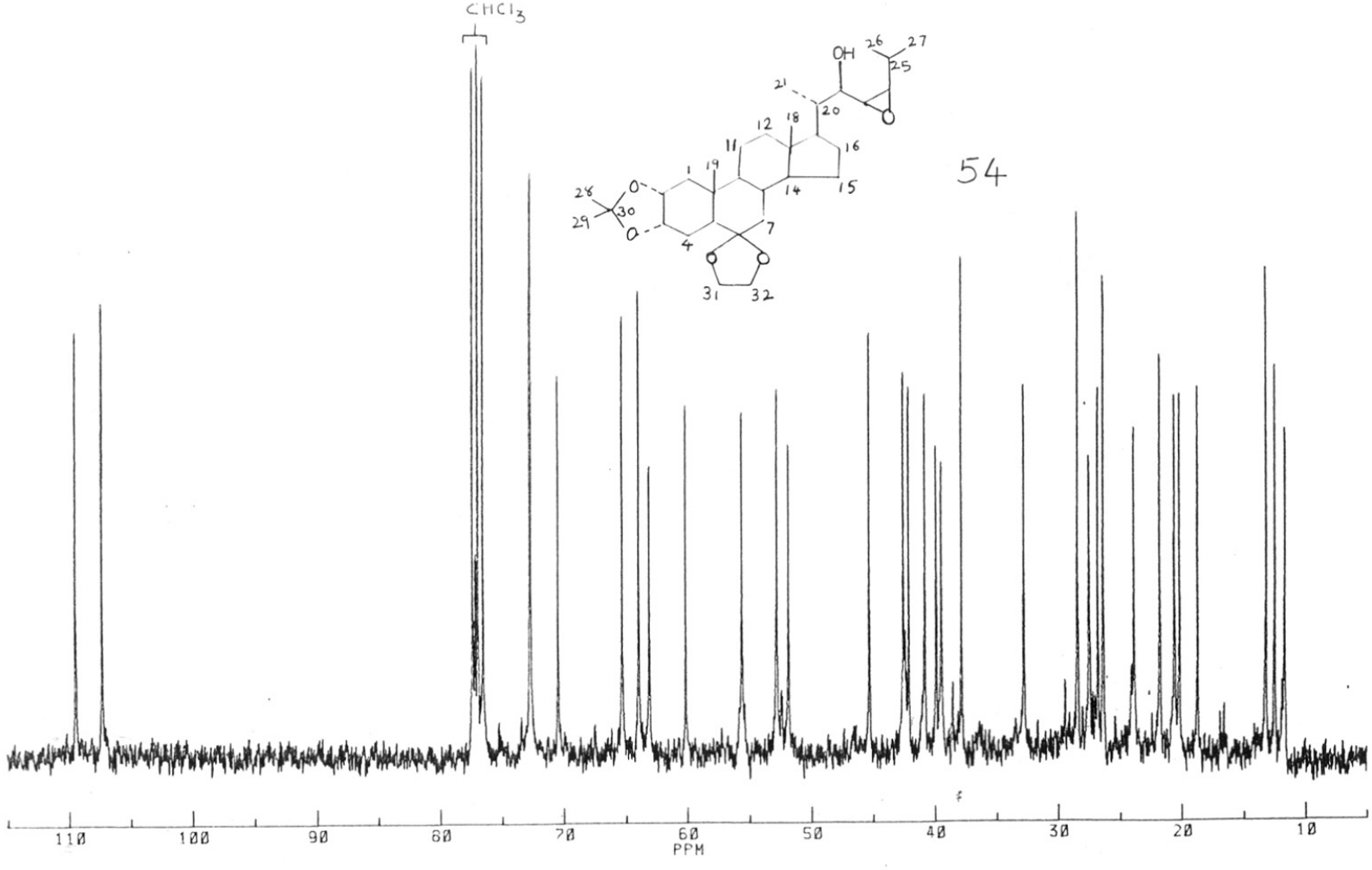
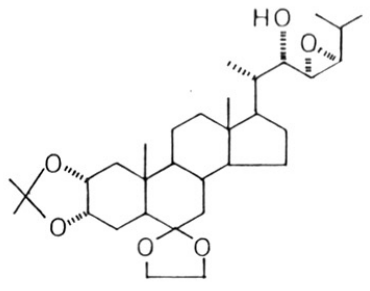
10·0
9·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)



54


CHCl3
OH
26
27
25
21
20
18
12
11
16
1
19
14
15
28
29
30
O
O
4
7
31
32
110
100
90
80
70
60
50
40
30
20
10
PPM

54



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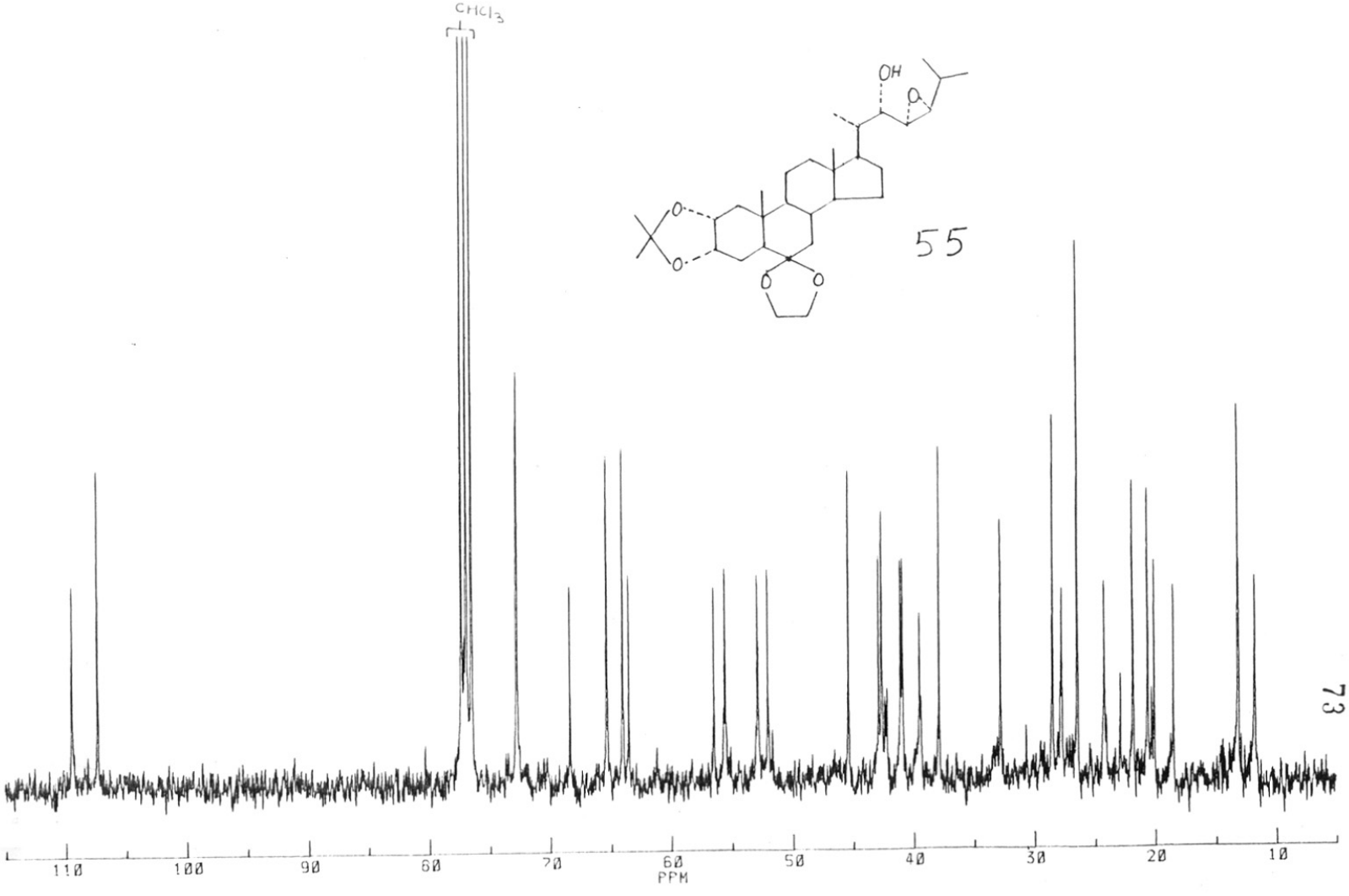
CHCl3
OH
O
O
O
O
O
55
110
100
90
80
70
60
PPM
50
40
30
20
10

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CHAPTER II

STUDIES ON CHLORAMPHENICOL AND RELATED COMPOUNDS

SUMMARY

This chapter deals with a highly efficient and practical route for the synthesis of chloramphenicol (1). An improved procedure for dichloroacetylation of primary and secondary amines via dichloroketene has been found out and it was applied for the synthesis of chloramphenicol intermediate (43). Nitration of 4-phenyl-5-dichloroacetamido-1,3-dioxane (43) has been studied critically. Nitration of 4-phenyl-5-formamido-1,3-dioxane (53) furnishes a higher percentage of *para* isomer while nitration of bromodioxane (41) furnishes higher pecentage of *meta* isomer than nitration of (43). Cyclic formal of chloramphenicol (48) was regioselectively cleaved to (45) using *p*-toluene sulfonic acid and acetic anhydride and this (45) was converted into chloramphenicol (1).

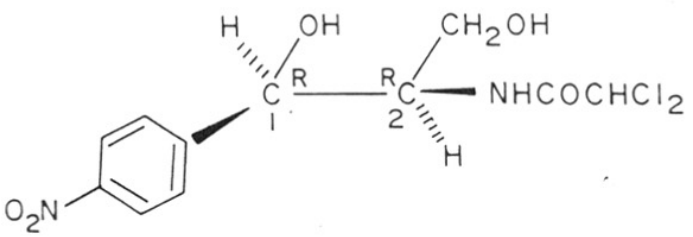
INTRODUCTION

Chloramphenicol (1) is the first broad spectrum antibiotics[1]. It was discovered independently by a group at Parke-Devis and Company (Ehrlich et al., 1947), another group at the University of Illinois (Gottlieb et al., 1948) and one in Japan (Umezawa et al., 1948). Chloramphenicol was originally isolated from aerobic broth cultures of an actinomycete, streptomyces venezuelae. It is antimicrobially active against a wide range of gram-positive and gram-negative bacteria, rickettsiae, the lymphogranuloma-psittacosis group and vibriocholerae . It is particularly active against Salmonella typhi and Hemophilus influenzae.

Chloramphenicol is proved to be a relatively simple compound with the structrue as shown in Chart I (1). The molecule may be considered as comprising of two parts, the 2-acylamido-1,3-propanediol side chain containing two asymmetric carbon atoms, and the p-nitrophenyl group. Out of the four possible stereoisomers, the (D)-threo (1R, 2R)- is by far the most active against microorganisms. The stereochemical configuration of the side chain is thus rather specific in conferring antimicrobial properties. The aromatic part is less specific, as shown by the moderate activity of analogues in which the nitro group is replaced by iodine or by methylsulfonyl group. Thiamphenicol, the analog in which the nitro group is replaced by a methylsulfonyl group, has an antibiotic spectrum similar to that of the natural compound, but significant differences in therapeutic response have led to considerable use in Europe.

Because of the simple structure, chemical syntheses were soon developed, and furnished the product more economically than did the microbiological route. As a result, chloramphenicol became the only widely used antibiotic

CHART-I



CHLORAMPHENICOL

1

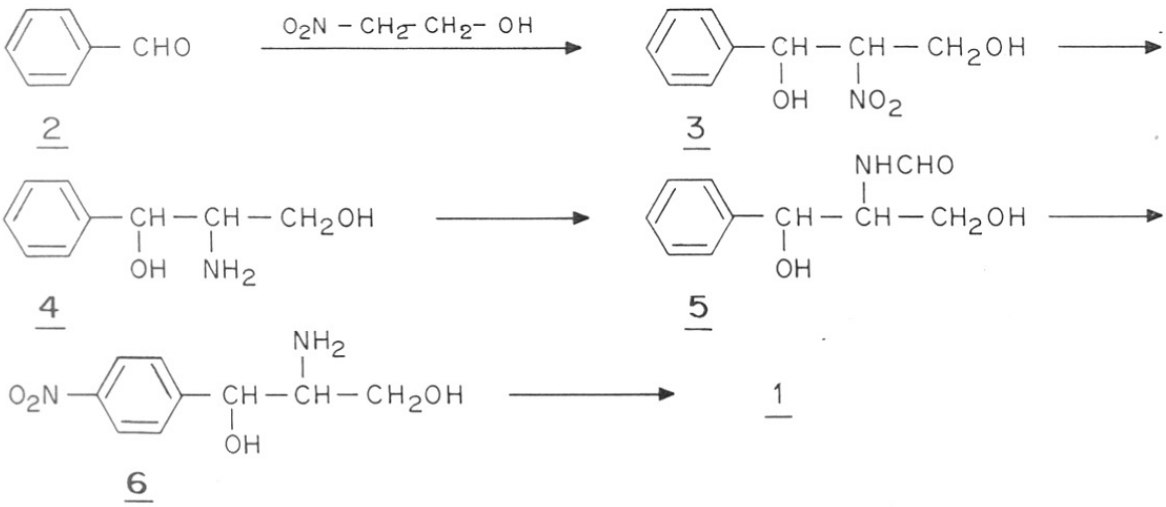
to be made commercially by a wholly chemical process. Chloramphenicol is used as its ester such as palmitate or succinate because they are essentially microbiologically inactive until hydrolysed in vivo to the free antibiotic. One of the characteristics of chloramphenicol leading to rapid and effective therapy is its high rate of absorption after oral administration, wide distribution in body tissues and outstanding ability to penetrate to the brain and cross other lipid barriers. It is also readily cleared from the blood stream by conjugation with glucuronic acid in the liver and excretion in urine.

Although it is very useful clinical agent and is preferred antibiotic for treating typhoid fever and other systemic salmonella infections, its use has been curtailed because of the incidence of bone marrow suppression during prolonged therapy. Because of the hazard of aplastic anemia, clinical use of it is restricted to serious infections by organisms resistant to other agents.

Synthesis

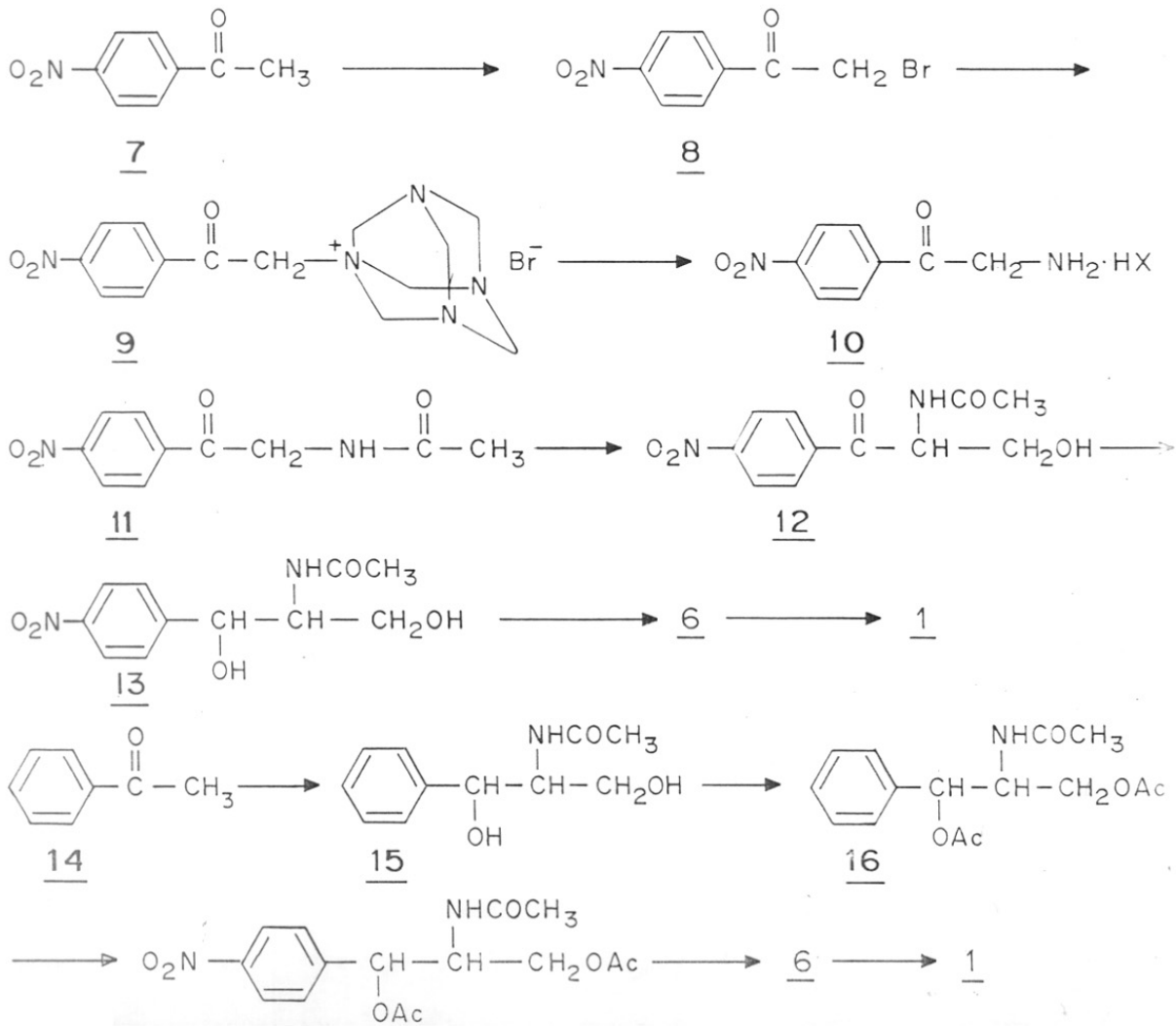
Four synthetic procedures account for most worldwide production. The first synthesis (Chart I, Scheme 1) involved the addition of benzaldehyde (2) to β-nitroethanol to yield[2] 2-nitro-1-phenyl-1,3-propanediol (3) in an uneconomical (D,L)-erythro:(D,L)-threo isomer ratio of about 2:1. Chemists at Boehringer Mannheim G.m.b.H. devised a technique for obtaining exclusively the (D,L)-threo racemate. Reduction of it to the aminodiol(4),swing resolution, subsequent nitration, and dichloroacetylation have made this route the process of choice throughout the 1960s and 1970s. Recently[3] a method is devised to convert unwanted (1S, 2S)-aminodiol (4) into racemic compound which in turn can be converted into required (1R, 2R)- isomer by

SCHEME - 1



2 3 4 5 6 1

SCHEME - 2



7 8 9 10 11 12 13 6 1

14 15 16 6 1

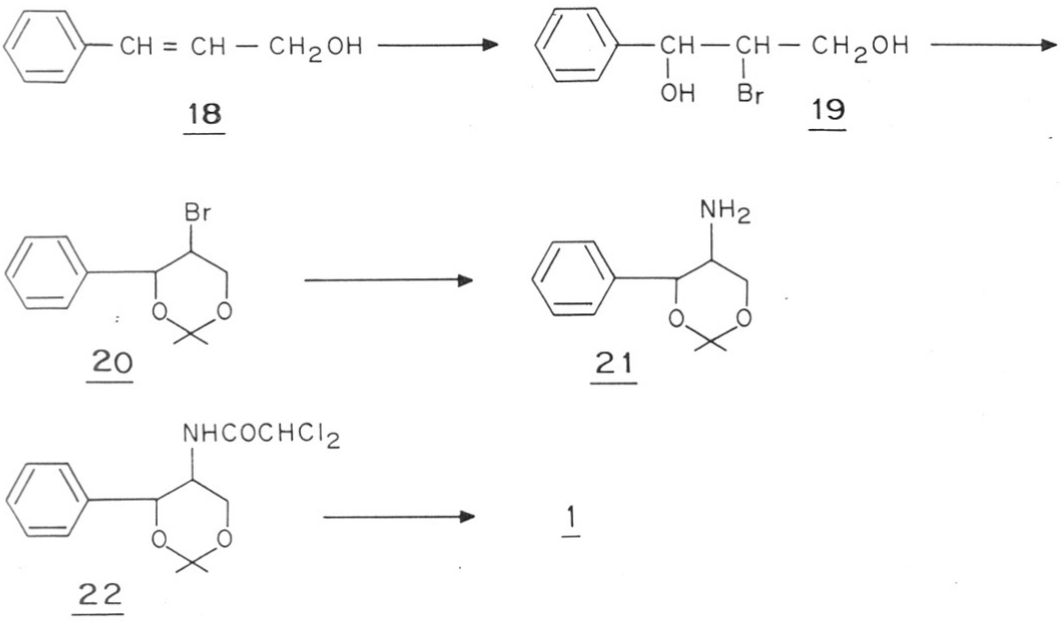
swing resolution.

In the second synthesis (Chart I, Scheme 2), acetophenone (14) or p-nitroacetophenone (7) is used as starting material[4]. The bromination of (7) to (8) may be carried out in variety of inert solvents, yield >90%. The formation of hexamethylene tetramine complex (9) is followed by an alcoholic acid hydrolysis to obtain the corresponding mineral acid salt (10). Acetylation of (10) is carried out in cold water with acetic anhydride and mild alkali to get (11). The addition of formaldehyde to (11) occurs in presence of sodium bicarbonate. The Meerwin-Pondorf reduction of (12) yields almost exclusively the (D,L)-threo racemate (13). If acetophenone (14) is used as starting material, the hydroxyl groups of the compound (15) which is analagous to structure (13) are protected by acetylation and the triacetyl derivative (16) is nitrated with fuming nitric acid to give (17). Acid hydrolysis of either (13) or (17) yields the racemic base (6) which is resolved into its optical antipodes by crystallization of its salts with an optically active acid, e.g., tartaric acid in methanol. From this (6), chloramphenicol (1) is obtained by dichloroacetylation with methyl dichloroacetate in overall yield of about 6%.

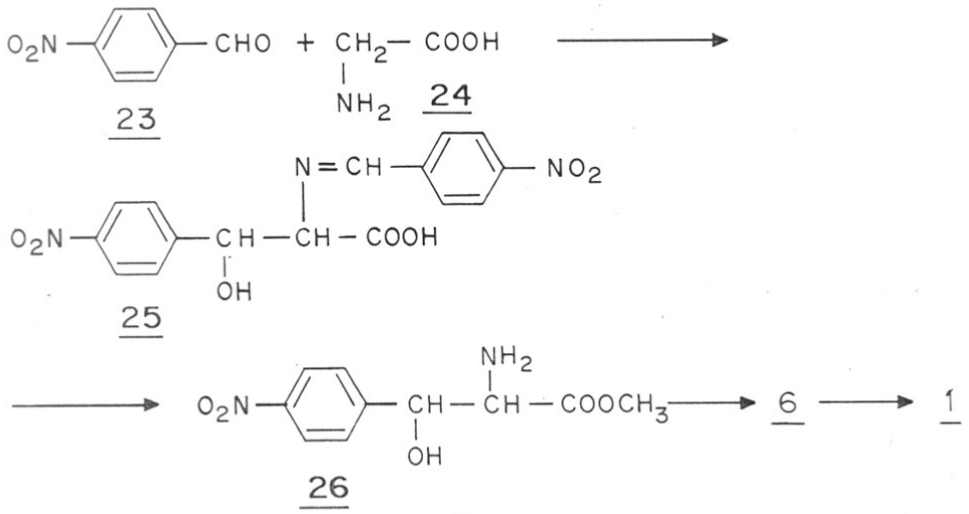
A process (Chart I, Scheme 3) involved cinnamyl alcohol (18) as starting material, was used for a considerable time in Western Europe[5]. Compound (18) is converted to its bromohydrin (19) and in turn to the bromodioxane (20). Reaction with ammonia gives aminodioxane (21) which is resolved, acetylated to (22), nitrated and gently hydrolysed to chloramphenicol (1).

A procedure, which has been utilized in Eastern Europe (Chart I, Scheme 4) involves use of phenyl serine derivative[6]. p-Nitrobenzaldehyde

SCHEME - 3



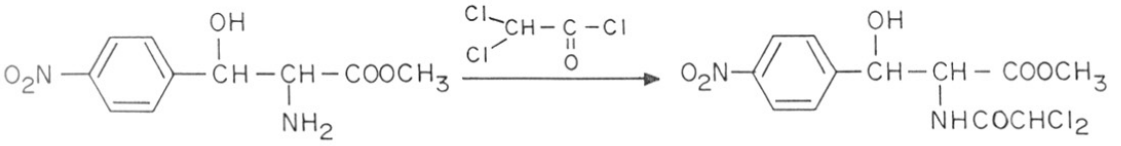
SCHEME - 4



(23) is condensed with glycine (24) to obtain the schiff's base of (D,L)-threo-p-nitrophenyl serine (25). Cleavage of it followed by esterification gives (D,L)-threo-p-nitrophenyl serine methyl ester (26) which may be resolved by (D)-tartaric acid and the ester of correct configuration (corresponding to (L)-p-nitrophenyl serine) reduced by sodium or calcium borohydride to the desired p-nitrophenyl serinol (6) which in turn is converted to chloramphenicol (1). There is a patent report in which β-bromostyrene is converted to chloramphenicol[7].

Very recently (Chart I, Scheme 5) synthesis of chloramphenicol via an enzymatic enantioselective hydrolysis of (D,L)-threo-p-nitrophenyl serine methyl ester (26) has been reported[8]. Methyl (±) threo-N-dichloroacaetyl β-(p-nitrophenyl) serine (27) was enantioselectively hydrolysed by enzyme subtilisin. This enzymatic kinetic resolution gave the corresponding (2S, 3R)-acid (29) and the unhydrolysed (2R, 3S)-ester (28) in high yield and high optical purity. Reduction of (2S, 3R)-acid (29) by borane methyl sulfide complex gave (+)-chloramphenicol (1). Reduction of the (2R, 3S)-ester (28) by LAH gave enantiomer (-)-chloramphenicol (1)

SCHEME-5



26

(±) - threo

27

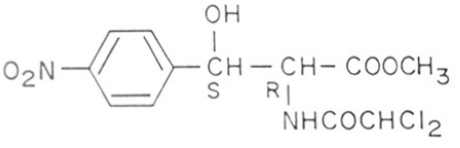
(±) - threo

27

pH 7.7
Subtilisin

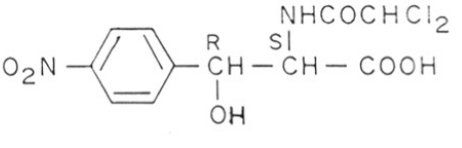
99 %

96 %



28

(+)-thero
ester

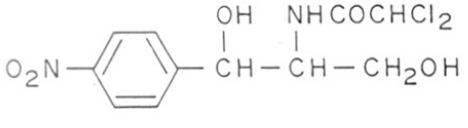


29

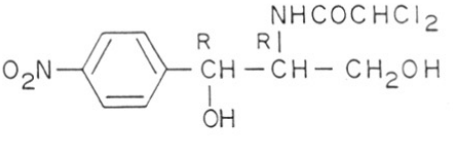
(—) - thero
acid

LAH / dioxane /
ether / 0°C

Me_2S / BH_3
THF / 0°C



(—) - 1



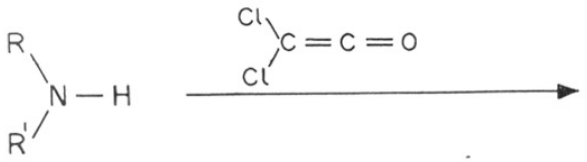
(+) - 1

PRESENT INVESTIGATION

The present investigation for the synthesis of chloramphenicol (1) involves three aspects- (A) an improved procedure for the dichloroacetylation of 4-phenyl-5-amino-1,3-dioxane (42); (B) Nitration studies on 4-phenyl-5-dichloroacetamido-1,3-dioxane (43), 4-phenyl-5-formamido-1,3-dioxane (53) and 4-phenyl-5-bromo-1,3-dioxane (41) and (C) regioselective cleavage of cyclic formal (48) and (56).

One route[7] has been chosen (Chart III) in which known amino dioxane (42) has been utilized for the synthesis of chloramphenicol. Aminodioxane (42) was prepared starting from commercially available styrene (37). Styrene (37) was brominated and the dibromo compound (38) on treatment with water in methyl ethyl ketone furnished the bromohydrin (39). On heating with catalytic amount of p-toluenesulfonic acid compound (39) transformed smoothly to β-bromostyrene (40). Prins reaction on (40) with paraformaldehyde and p-toluenesulfonic acid gave the trans bromodioxane (41). Both cis and trans β-bromostyrenes give only trans bromodioxane[10]. This (41) in methanol and ammonia afforded the (DL)-threo-aminodioxane (42). The amino group in compound (42) was protected by dichloroacetylation to furnish compound (43). The compound (43) is an ideal intermediate for chloramphenicol synthesis because the three carbon side-chain with proper threo configuration is constructed on the benzene ring. This (43) is a safer intermediate for nitration than aminodiol (4) after blocking amino group but keeping hydroxy groups free (Chart I, Scheme 1), since alcohols are known to be readily oxidized by nitric acid. Moreover protection of hydroxyl groups does not involve any extra step.

CHART II



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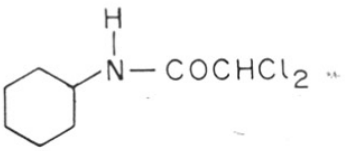

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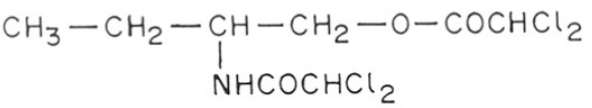

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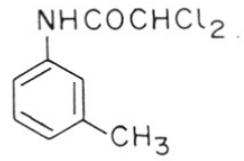

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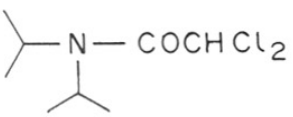

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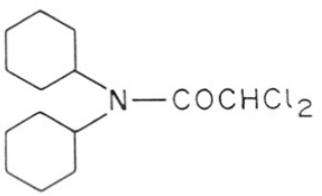
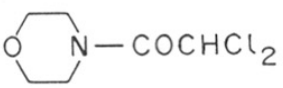
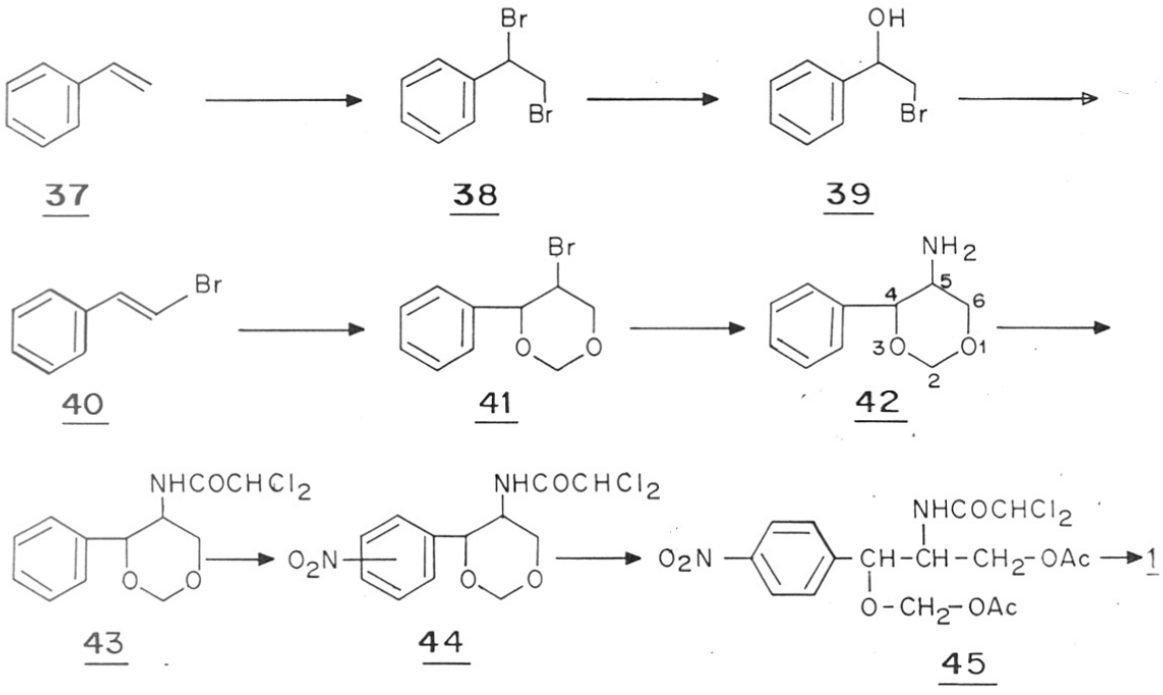


35



36

CHART - III


Br
Br
OH
Br
37
38
39
Br
Br
NH2
4
5
6
3
O
O
1
2
40
41
42
NHCOCHCl2
O2N
NHCOCHCl2
O2N
NHCOCHCl2
CH-CH-CH2-OAc
O-CH2-OAc
1
43
44
45

Section A

4-Phenyl-5-amino-1,3-dioxane (42) was reported to produce 92% yield of 4-phenyl-5-dichloroacetamido-1,3-dioxane (43) on treatment with dichloroacetyl chloride using benzene as solvent and pyridine as a base[7]. When this procedure was repeated only 60-62% yield of dichloroacetamide(43)was obtained and remaining aminodioxane (42) was recovered after basifying the water layer. This clearly shows that a competition reaction between pyridine and substrate amine with the liberated hydrogen chloride takes place which results in low yields of amidation product (43). The dichloroacetylation of a few primary and secondary amines with dichloroketene has been studied in order to establish a suitable condition for dichloroacetylation of (42)

Dichloroacetamido moiety ($NHCOCHCl_2$) is prevalent in number of biologically active molecules. Chloramphenicol and diloxanide, an antiamebic agent with this moiety are of commercial importance. Some compounds containing this group act as agents for protecting useful plants against preemergent herbicide application[12-14]. α,α -Dichloroacetamides have been prepared by reaction of amine or its hydrochloride with dichloroacetyl chloride[13-15]. The disadvantage of this reaction is that the substrate amine reacts with the liberated hydrogen chloride. Although pyridine has been used as base in solvents such as benzene and acetone[15,16],the competition between pyridine and the usually more basic substrate amine,lowers the yield of dichloroacetamide . The use of methyl dichloroacetate results in low to moderate yields and requires prolonged reaction time[2,17]. A patent described the preparation of dichloroacetylation of primary and secondary amines using chloral hydrate, precipitated calcium carbonate and hazardous chemical like sodium cyanide[18].

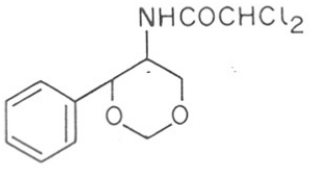
Ketenes have been known since the synthesis of diphenylketene by Staudinger in 1905[19]. During next twenty years attempts were made to prepare

some halogenated ketenes. These ketenes could not be detected and were described as unstable compounds which polymerise readily even at low temperature. Preparation of dichloroketene was independently reported[20] from three different laboratories in 1965-66. A systematic study of the use of dichloroketene for dichloroacetylation of amines has not been reported in literature. So the dichloroacetylation of number primary and secondary amines using dichloroketene which is generated in situ according to literature[21] procedure has been studied. Thus simultaneous addition of chloroform solution of triethyl amine and dichloroacetyl chloride to the amine in chloroform is essential to obtain the high yield of dichloroacetamides reported here. The advantages of this procedure are that the reaction is very fast and clean, it involves simple workup procedure and products obtained require no further purification. All the primary and secondary amines used gave much increased yields of corresponding dichloroacetamides than reported earlier (Chart II). In case of 2-aminobutanol, with one molar equivalent of dichloroketene, no selectivity was observed. O- and N-diacylated product (32) was obtained in 33% yield. Use of two molar equivalents of dichloroketene afforded 98% of (32). Dichloroacetylation of 4-phenyl-5-amino-1,3-dioxane (42) using dichloroketene methodology gave the corresponding dichloroacetamide[11] (43) in 98% yield.

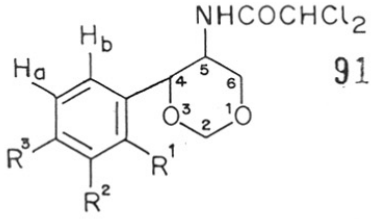
Section B

Compounds containing aromatic ring on nitration afford a mixture of ortho, meta and para nitro derivatives. According to the patent report[7], the nitration of 4-phenyl-5-dichloroacetamido-1,3-dioxane (43) with fuming nitric acid at -20°C furnished the para-isomer (48) in 88% yield. When this experiment was repeated, compound (44) was obtained in 95% yield which

CHART IV



Fuming HNO_3, −20°C

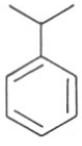


43

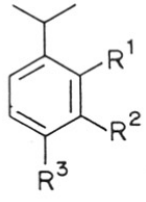
46 $R^1 = NO_2$; $R^2, R^3 = H$

47 $R^2 = NO_2$; $R^1, R^3 = H$

48 $R^3 = NO_2$; $R^1, R^2 = H$



HNO_3

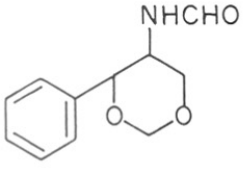


49

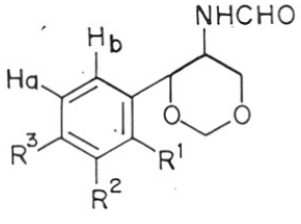
50 $R^1 = NO_2$; $R^2, R^3 = H$

51 $R^2 = NO_2$; $R^1, R^3 = H$

52 $R^3 = NO_2$, $R^1, R^2 = H$



Fuming HNO_3

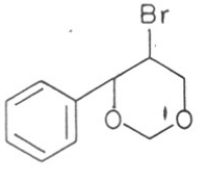


53

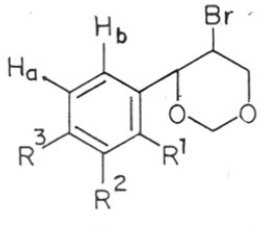
54 $R^1 = NO_2$; $R^2, R^3 = H$

55 $R^2 = NO_2$; $R^1, R^3 = H$

56 $R^3 = NO_2$; $R^1, R^2 = H$



Fuming HNO_3



41

57 $R^1 = NO_2$; $R^2, R^3 = H$

58 $R^2 = NO_2$; $R^1, R^3 = H$

59 $R^3 = NO_2$; $R^1, R^2 = H$

was found to be a mixture of nitro products ortho (46), meta (47) and para (48) (Chart IV). This nitration product (44) according to G.C. analysis contains 35% ortho (46), 19% meta (47) and 46% para (48). TLC of nitration mixture (44) showed two spots. The higher Rf represents ortho isomer which can be isolated by column chromatography followed by crystallization. The lower Rf spot consists of a mixture of meta and para isomers. Although they showed very similar Rf on TLC they could be separated by tedious and careful column chromatography using silica to compound ration. It was observed that there was no effect of concentration of nitric acid, temperature, time or solvent on percentage of ortho, meta and para nitro products. This was revealed by the fact that carrying the reaction at -45°C to 0°C for 30 mins to 3 hrs, using chloroform as a solvent or without it, the percentage of ortho, meta and para remained almost constant (Table).

Study with Drieding model of the substrate (43) shows that the steric requirement of the molecule is comparable to that of isopropyl benzene (49). There is moderate crowding at ortho positions and para position is relatively free. Nitration of isopropyl benzene (49) is known[23] to give all the three nitro products ortho (50) 30%, meta (51) 7.5% amd para (52) 62%. The relative increase of meta product (20%) in nitration of (43) may be mainly due to (a) electron withdrawing dichloroacetamido moiety at C-5 position and probably to a much lesser extent (b) the presence of oxygen at C-3 position which forms oxonium salt in fuming nitric acid. There is ample precedent in literacture[24] for this type of relatively high meta isomer formation in the nitration. Nitration of 4-phenyl-5-dichloroacetamido-1,3-dioxane (43) using variety of reagents were carried out but the percentage of para isomer could not be increased (Table). So other substrates for nitration were searched and the nitration of N-formyl dioxane (53) and bromodioxane(41)was studied.

Table: Nitration of 4-phenyl-5-dichloroacetamido-1,3-dioxane (43)

Entry	Reagent	Solvent	Temp.°C	Time	Compound % of (43)	(46)	(47)	(48)
1	2	3	4	5	6	7	8	9
1.	Fuming HNO_3[a]	$CHCl_3$	-30to -20	15 min.	5.6	33.3	17.3	43.8
				1 hr.	0.3	35.8	17.2	47.0
				2 hr	0.2	34.5	19.2	46.2
				3 hr	0.2	37.2	19.6	44.5
2.	Fuming HNO_3	$CHCl_3$	-10 to 0	1 hr	-	39.7	19.4	40.8
				2 hr	-	38.5	18.5	43.1
3.	Fuming HNO_3 + AC_2O	-	-10 to 0	2 hr	-	50.0	14.8	37.4
4.	Fuming HNO_3	-	-50 to -40	2 hr	0.2	35.4	19.9	44.4
5.	Fuming HNO_3	-	-10 to -5	2 hr	-	38.0	18.7	43.0
6.	Con.HNO_3(70%)	-	-10 to 50	2 hr	100	-	-	-
7.	Con.HNO_3(70%)+ con.H_2SO_4 (98%) 1:1	-	-10 to 0	2 hr	0.4	37.0	22.0	39.4
8.	H_2SO_4(50%)+ $NaNO_2$ in H_2O	sulfolane	5 to 30	8 hr	100	-	-	-
9.	Con.HNO_3(70%) + fuming HNO_3 1:1	-	-10 to 25	2 hr	-	43.0	18.0	38.8
10.	$AgNO_3$-BF_3Et_2O[30]	Acetonitrile	30	24 hr	100	-	-	-
11.	$AgNO_3$-$TiCl_4$ or $AlCl_3$	CH_2Cl_2	30	24 hr	100	-	-	-
12.	Fuming HNO_3-polyphosphoric acid[31]	$CHCl_3$	30	4 hr	-	41.6	18.8	39.6
13.	Tetrabutylammonium nitrate, trifluoro acetic anhydride 18-crown-6[32]	CH_2Cl_2	44	10 hr	100	-	-	-
14.	t-$BuONO_2$-Polyphosphoric acid[30]	Acetonitrile	30	4 hr	14.0	34.0	17.0	32.0
15.	t-$BuONO_2$-$TiCl_4$	CH_2Cl_2	30	24 hr	19.5	22.2	18.0	31.1
16.	Fuming HNO_3-P_2O_5[33]	-	-10 to 0	1 hr	-	39.3	19.4	41.1
17.	Fuming HNO_3-p-toluene sulfonic acid[33]	-	30	24 hr	62.0	16.7	6.5	11.9

1	2	3	4	5	6	7	8	9
18.	Fuming HNO_3-sulfonated Polystyrane resin (acidic)[33]	-	30	48 hr	30.4	27.4	13.7	26.7
19.	Con.HNO_3(70%)- Zeolite catalyst	$CHCl_3$	30	24 hr	100	-	-	-
20.	Fuming HNO_3-Zeolite catalyst	$CHCl_3$	30	3 hr	-	36.3	25.0	37.4

a. Fuming HNO_3 used (sp.gr. 1.52) 9 mole/mole substrate.
The percentage of o, m and p isomers was determined by GC analysis.
The percentage of crude nitro products peak height and retention time were compared with the peak height and retention time of known weight of pure **(43)**, **(46)**, **(47)** and **(48)**

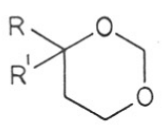
GC analysis of the reaction products obtained by nitration of (53) and (41) with fuming nitric acid showed that bromodioxane (41) gave higher meta isomer (58) 30% and less, the required para isomer (59) 38.5%, while N-formyl dioxane (53) gave minimum meta isomer (55) 16.9% with maximum para isomer (56) 55.3%. This is obvious because compound (41) is more effective in withdrawing the electrons from aromatic ring than compound (53), since in (41) the carbon atom carrying partial positive charge is separated from aromatic ring by only one carbon atom. In (53) the nitrogen atom carrying partial positive charge is separated from aromatic ring by two carbon atoms. In compound (43) there are two electron withdrawing chlorine atoms and as a result there is greater withdrawl of electrons from aromatic ring in (43) as compared to (53).

The above study shows that nitration of 4-phenyl-1,3-dioxane derivatives (43), (41) and (53) furnished mixture of ortho, meta and para nitro isomers and for the synthesis of chloramphenicol N-formyl derivative (53) is a better intermediate for nitration, than N-dichloroacetyl derivative (43) since the former gives the required para isomer in higher yield.

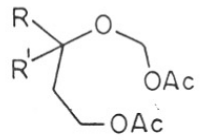
Section C

There are several reports on the ring cleavage of 4-p-nitrophenyl-5-dichloroacetamido-1,3-dioxane (48) and related compounds[25-27]. Bailey has shown[26] recently that formals (60) undergo highly regioselective ring cleavage furnishing the hemiacetals (61) (Chart V). It has been claimed[7] that the reaction of 4-p-nitrophenyl-5-dichloroacetamido-1,3-dioxane (48) with acetic anhydride in presence of anhydrous zinc chloride furnishes the hemiacetal (64). Now, it has been observed that this reaction furnishes a mixture of (45) 15% and oxazoline derivative (63) 85% from ^{1}H-NMR.

CHART V



60

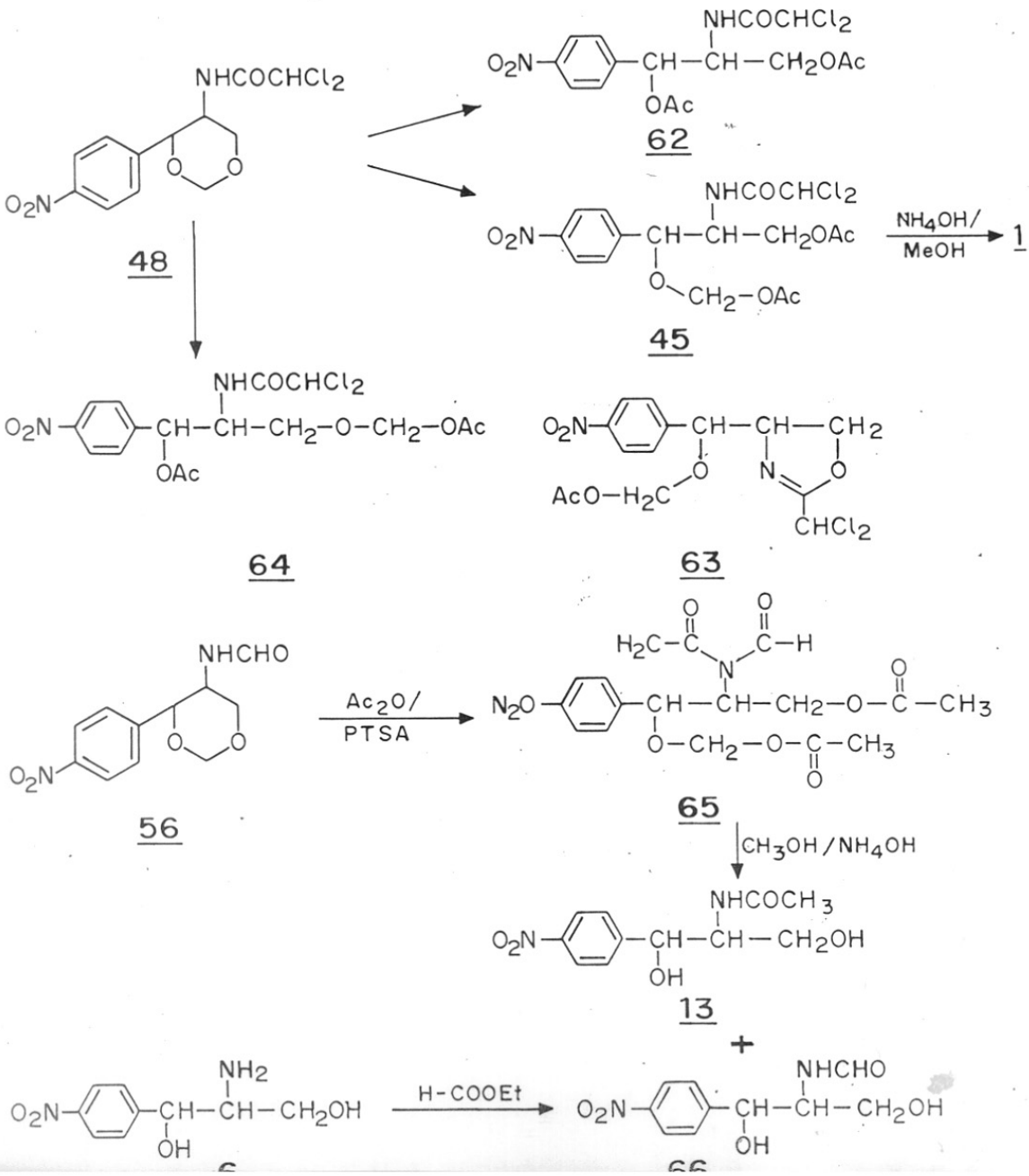


61

a) R = H , R' = CH_3

b) R = R' = CH_3

c) R = H , R' = C_6H_5



This mixture of (45) and (63) furnishes chloramphenicol (1) on treatment with methanol-aqueous ammonia.

Acetals related to (60) but having substituents at C-2 are reproted[27] to undergo ring cleavage on heating with 75% acetic acid. However, under these conditions (48) was remained unchanged.

When a mixture of (48), acetic anhydride and sulfuric acid was kept at room temperature for 24 hrs, there was practically no reaction, but under vigorous conditions (70°, 24 hrs) the product obtained in moderate yield was diacetate (62). Probably the hemiacetal (45) formed initially is transformed to diacetate (62) under the conditions employed. The conversion of acetals to acetates is reported[25] in the literature.

It has been observed that the most efficient method to cleave the compound (48) is acylative cleavage with acetic anhydride in presence of p-toluenesulfonic acid as a catalyst which furnishes 97% yield of hemiacetal (45). This (45) in methanol when treated with aqueous ammonia gave chloramphenicol (1) in 90% yield. The cleavage of 4,p-nitrophenyl-5-formamido-1,3-dioxane (56) under similar conditions has also been studied. The product obtained was hemiacetal (65) which on treatment with methanol-aqueous ammonia gave a mixture of two compounds (13) and (66). This mixture was separated by crystallization. Compound (13) was found to be identical with known[2] (13) and compound (66) was identical with a compound prepared from 1-p-nitrophenyl-2-amino-1,3-propanediol (6) using ethylformate.

EXPERIMENTAL

Section A

Cis (±)-4-phenyl-5-dichloroacetamido-1,3-dioxane (43)

In a three necked flask fitted with two pressure equalized dropping funnels, a solution of (±)-(42), b.p.95/0.4 mm (lit.[7] b.p.100/0.4 mm, 1.01 g, 5.7 mmol) in chloroform (20 ml) was cooled to 0°C. To this, solutions of dichloroacetyl chloride (0.64 ml, 6.1 mmol) in chloroform (10 ml) and triethylamine (1.12 ml, 7.8 mmol) in chloroform (10 ml) were added simultaneously during 20 minutes with stirring, keeping the temperature below 10°C. The reaction mixture was stirred for further period of 30 minutes at 10°C. It was then diluted with more chloroform. The chloroform solution was separated and washed successively, with water (2 x 25 ml), 2% cold HCl (2 x 25 ml), water (2 x 25 ml), saturated sodium bicarbonate solution (2 x 10 ml), water (2 x 25 ml) and brine (2 x 25 ml). It was then dried over anhydrous sodium sulfate. Removal of solvent afforded dichloroacetamide (43) (1.61 g, 98%) m.p.99°C. This was recrystallised from chloroform-petroleum ether. m.p.102°C (lit.[7], m.p.104°C); IR (Nujol) 3320, 3290, 1675 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.64 (bs, 1H), 4.07 (d, 2H, J=2 Hz), 4.22 (m, 1H), 4.98 (d, 1H, J=2 Hz), 4.95 and 5.29 (AB pattern, 2H, J_{AB}=6 Hz), 5.65 (s, 1H), 7.28 (s, 5H).

Cis(-)-(4R)-phenyl-(5R)-dichloroacetamido-1,3-dioxane (43)

In a similar way dichloroacetylation of (-)-aminodioxane (42) b.p. 113°C/1 mm $[\alpha]_D^{30}$ = -64.4° (C=1.8, EtOH) [lit.[28] b.p.114-115°C/1mm $[\alpha]_D$ -66.2° (C=1, EtOH)] furnished (-)-(43) m.p.89-90°C, $[\alpha]_D^{25}$ -20.2° (C=1.1, EtOH); identified by comparison (TLC, NMR) with (±)-(43).

Dichloroacetylation of primary and secondary amines

A General Procedure

In a three necked round bottom flask fitted with a thermometer and two pressure equalized dropping funnels a solution of an amine (50 mmol) in chloroform (40 ml) was cooled to 0°C. To this, solutions of dichloroacetyl chloride (56 m mol) in chloroform (10 ml) and triethylamine (56 mmol) in chlorofom (10 ml) were added simultaneously during 20 minutes, with stirring, keeping the temperature below 10°C. The reaction mixture was stirred at 10°C for a further period of 30 minutes. It was then poured in separatory funnel and was diluted with chloroform (50 ml). The chloroform solution was washed successively with water, 2% cold HCl, water, saturated sodium bicarbonate water and brine. It was then dried over anhydrous sodium sulfate and product was obtained on removal of chloroform. Analytical samples were prepared by recrystallization. The following dichloroacetamides were prepared using the above general procedure.

Acetamide – 2,2-dichloro-N-isopropyl (30)

Yield: 99%, m.p.127°C (lit.[13], yield 45%, m.p.127°C);IR (nujol) 3275 (-NH), 1670 (NHCO), cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.22 (d, 6H, J=6.3 Hz),1.62 (s, 1H), 4.04 (m, 1H), 5.8 (s, 1H); MS 169 (M^+), 154, 86(100%).

Acetamide 2,2-dichloro-N-cyclohexyl (31)

Yield: 98%, m.p.138%(lit.[18], yield: 82%, m.p.140°C); IR (nujol) 3250(NH), 1660 (NHCO) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.03-2.13 (m, 10H), 3.75 (m, 1H), 5.91 (s, 1H), 6.42 (bs, 1H); MS 209 (M^+), 174, 166, 128, 83 (100%).

Acetamide – 2,2-dichloro-N(2-O-dichloroaceatyl hydroxybutyl (32)

Yield: 9 8%, m.p. 50°C; IR (nujol) 3300 (NH), 1770 (O-CO), 1680 (NHCO) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.03 (t, 3H, J=7 Hz),1.64 (m, 2H), 3.93 – 4.48

(m, 3H), 5.93 (s, 1H), 6.03 (s, 1H), 7.17 (m, 1H); MS 309 (M^+), 226, 182, 168 (100%), 152, 116, 83; Analysis Found: C, 31.00; H, 3.75; N, 4.77. $C_8H_{11}Cl_4NO_3$ require C, 30.89; H, 3.57; N, 4.50.

Acetamide-2,2-dichloro-N-(3-methylphenyl) (33)

Yield: 94%, m.p.98°C (lit.[22] m.p.99°C); IR (nujol) 3250 (-NH), 1680 (NHCO) cm^{-1}; ^{1}H-NMR-($CDCl_3$) 1.59 (s, 1H), 2.36 (s, 3H), 6.0 (s, 1H), 6.89 to 7.42 (m, 4H); MS 217 (M^+), 182, 146, 134 (100%), 106, 91, 83, 77.

Acetamide-2,2-dichloro-N,N-diisopropyl (34)

Yield: 85%, m.p.81°C (lit.[13] yield 62%, m.p.81°C); IR(nujol) 1665 (N-CO) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.29 (d, 6H, J=6.5 Hz), 1.42 (d, 6H, J=6.5 Hz), 3.51 (m, 1H), 4.35 (m, 1H), 6.15 (s, 1H); MS 211 (M^+), 196, 176, 154 (100%), 128, 86.

Acetamide -2,2-dichloro-N,N-dicyclohexyl (35)

Yield: 78%, m.p.85°C; IR (nujol) 1660 (N-CO) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.11-2.09 (m, 18H), 2.33 (m, 2H), 2.98 (m, 1H), 3.85 (m, 1H), 6.11 (s, 1H); MS 291 (M^+), 256 (100%), 210, 83; Analysis Found: C, 57.77; H, 7.95; N, 4.89. $C_{14}H_{23}Cl_2NO$ require C, 57.54; H, 7.93; N, 4.79.

Morpholine-4-dichloroacetyl (36)

Yield: 78%, m.p.65°C (lit.[18], yield 38%, m.p.64°C); IR(nujol) 1665 (N-CO-) cm^{-1}; ^{1}H-NMR ($CDCl_3$)3.70 (bs, 8H), 6.15 (s, 1H); MS 197 (M^+), 182, 162, 153, 132, 114 (100%), 86, 83, 70.

Section B

Cis (±)-4-p-nitrophenyl-5-dichloroacetamido-1,3-dioxane (48)

In a 100 ml two necked flask equipped with magnetic stirring bar, addition funnel and thermometer, fuming nitric acid (12.6 ml, 304 mmol,

d = 1.52) was cooled to -30°C. To it a solution of cis (±)-4-phenyl-5-dichloroacetamido-1,3-dioxane (**43**) (9.6 g, 33 mmol) in dry chloroform (20 ml) was added dropwise at -30 to -25°C during 30 minutes with stirring. The stirring was continued for 3 hr at -25°C to -20°C. Small portions of reaction mixture were taken at inervals 15 min, 1 hr, 2 hr and 3 hr for GC analysis. The nitration mixture was poured over crushed ice and extracted with chloroform. The chloroform extract was washed with water, sodium bicarbonate and finally with water. It was dried over anhydrous sodium sulfate. Removal of solvent furnished the nitro product (**44**) (10.22 g, 92%, m.p.134-136°C). This on GC analysis was found to be a mixture of all the three nitro products ortho, meta and para (o=34.5%, m=19% and p=46.4%). On three crystallizations from methanol pure (±)-(**48**) was obtained m.p.159-160°C (lit.[7] m.p.164°C); IR (nujol) 3320 (NH), 1680 (NH-CO-), 1515, 1345 and 1175 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.59 (bs, 1H NH), 4.12 (d, 2H, CH_2-6, J=2 Hz), 4.33 (m, 1H, CH-5), 5.04 (bs, 1H, CH-4), 4.97 and 5.35 (AB pattern, 2H, CH_2-2, J= 6 Hz), 5.66 (s, 1H, $CHCl_2$), 7.48 and 8.18 (AB pattern, 4H, ArHa, R^2 and Hb,R^1, J=8 Hz); MS 304, 306, 251, 221, 191, 183, 177, 164, 154 (100%).

Cis(-)-(4R)-p-nitrophenyl-(5R)-dichloroacetamido-1,3-dioxane (48)

In a similar way nitration of (-)-(**43**) afforded (-)-(**44**) which was also a mixture of o, m and p. It was thrice crystallized from methanol to get pure (-)-(**48**), m.p.161-162°C $[\alpha]_D^{32}$ -65.6° (C=2.47, chloroform), -14.4 (C=2.08, methanol); TLC and NMR were in agreement with that of (±)-(**48**); Found C, 42.94; H, 3.59; N, 8.15, $C_{12}H_{12}Cl_2N_2O_5$ require C, 43.00; H, 3.61; N, 8.36.

The mother liquor after crystallization of cis (-)-(4R) p-nitrophenyl-(5R)-dichloroacetamido-1,3-dioxane (**48**) on column chromatography over silica

gel and elution with hexane-ethylacetate (75:25) gave ortho isomer followed by a mixture of meta and para isomers. Pure ortho isomer (**46**) was obtained by crystallization from methanol. m..p. 98°C; $[\alpha]_D^{26}$ -4.5° (C=2.8, chloroform); IR (nujol) 3250, (NH) 1670 (NHCO), 1540, 1510, 1365, 1330, 1165, 1085, 1025, 990 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.61 (bs, 1H, NH), 4.12 (m, 2H, CH_2-6), 4.52 (bd, 1H, CH-5), 4.98 and 5.28 (AB pattern, 2H, CH_2-2, J=6 Hz), 5.60 (s, 1H, $CHCl_2$), 5.63 (bs, 1H, CH-4), 7.28-7.68 (m, 3H), 8.06 (bd, 1H); MS 339, 337, 335, 308, 306, 304, 182, 153 (100%), 135, 118; Analysis Found: C, 42.82; H, 3.74; N, 8.04. $C_{12}H_{12}Cl_2N_2O_5$ require C, 43.00; H, 3.61; N, 8.36).

The mixture of meta and para isomers were rechromatographed over large excess of silica gel (120 times) and on elution with hexane:ethyl acetate (75:25) afforded meta isomer (**47**) as low melting solid, m.p. 48°C; $[\alpha]_D^{24}$ - 32° (C=1, chloroform); IR (nujol) 3400, (NH), 1690 (NHCO-), 1530, 1345, 1175 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.64 (s, 1H NH), 4.13 (d, 2H, CH_2-6, J=2 Hz), 4.35 (bd , 1H, CH-5), 5.07 (d, 1H CH-4, J=2 Hz), 5.01 and 5.34 (AB pattern, 2H, CH_2-2, J=6 Hz), 5.67 (s, 1H, $CHCl_2$), 7.4-7.73 (m, 2H, Ha and Hb), 8.15 (bd, 1H, R^3H), 8.26 (bs, 1H, R^1H); MS 304 (M^+ -CH_2O), 221, 177, 153 (100%), 118; Analysis Found: C, 43.24; H, 3.88; N, 8.07. $C_{12}H_{12}Cl_2N_2O_5$ require C, 43.00; H, 3.61; N, 8.36.

Further elution with hexane-ethylacetate (75:25) furnished same para isomer (-)- (**48**) which was isolated by recrystallization from nitration mixture earlier.

Cis (±) 4-phenyl-5-formamido-1,3-dioxane (53)

This was prepared by refluxing cis (±)-4-phenyl-5-amino-1,3-dioxane (**42**) (1.084 g) with ethyl formate (4 ml) for 13 hr; m.p.101-102°C; IR (nujol) 3310 (NH), 1675 (NH-CO-) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 4.05 (d, 2H, CH_2-6 J=2 Hz),

4.4 (m, 1H, CH-5), 4.95 (d, 1H, CH-4, J=3 Hz), 4.9 and 5.2 (AB pattern, 2H, CH_2-2 J=6 Hz), 6.45 (bd, 1H-NH), 7.28 (s, 5H Ar), 7.92 (s, 1H, CHO); MS 207 (M^+), 177, 162, 132, 118, 105, 91, 77, 71 (100%).

Cis (±)-4-p-nitrophenyl-5-formamido-1,3-dioxane (56)

A 50 ml two necked round bottom flask equipped with magnetic stirring bar, addition funnel and thermometer was charged with fuming nitric acid (8 ml, 193 mmol, d=1.52). It was cooled to -20°C. Solution of (±)-(**53**), m.p.101-102°C (4 g, 19 mmol) in dry chloroform (15 ml) was added dropwise during 20 minutes maintaining the temperature -20 to -15°C. TLC showed mostly starting material. So temperature of the reaction mixture was raised to 10°C and it was stirred at this temperature for 1.5 hr. Reaction mixture was then poured over ice and extracted with chloroform. Chloroform solution was washed successively with water (2 x 50 ml) saturated sodium bicarbonate (2 x 50 ml), water (2 x 50 ml) and brine. It was dried over anhydrous sodium sulfate and evaporated to furnish crude nitration product (4.26 g, 95%). G.C. analysis of this product shows that it is a mixture of ortho (**54**) 25.7%, meta (**55**) 16.9% and para (**56**) 55.3%. This mixture on crystallization from chloroform-petroleum ether furnished pure para isomer(**56**), m.p.159°C; IR (nujol) 3220 (NH), 1680 (NH-CO-), 1650, 1560, 1340, 1160 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 4.06 (d, 2H, CH_2-6, J=2 Hz), 4.5 (m, 1H, CH-5), 5.04 (bs, 1H, CH-4), 4.96 and 5.26 (AB pattern, 2H, CH_2-2, J=7 Hz), 6.24 (bd, 1H, NH), 7.92 (s, 1H, CHO), 7.44 and 8.15 (AB pattern, 4H, Ar-H, J=8 Hz); MS 222 (M -CH_2O), 101, 71 (100%); Analysis Found: C, 52.45; H, 4.91; N, 10.78. $C_{11}H_{12}N_2O_5$ require C, 52.38; H, 4.8; N, 11.11.

(±)-4-p-Nitrophenyl-5-bromo-1,3-dioxane (59)

Nitration of (±)-(41) (2.42 g, 10 mmol) in dry chloroform (10 ml) was carried out in a similar way for 3 hrs at -10°C. Usual workup afforded (±)-4-p-nitrophenyl-5-bromo-1,3-dioxane (2.8 g, 94%). This on G.C. analysis was found to be a mixture of ortho (57) 31.5%, meta (58) 30% and para (59) 38.5% isomers. This mixture on recrystallization from chloroform-pet.ether gave pure para isomer (±)-(59). m.p. 132-133° (lit.[29] m.p.132-135°C); IR (nujol) 1615, 1520, 1350, 1170, 1090, 1055, 1020, 1005, 940, 850, 775 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 3.89 (m, 1H, H 6a), 4.42 (m, 1H, H 6e), 4.08 (m, 1H, H 5a), 4.67 (m, 1H, H 4a), 4.92 (m, 1H, H 2a), 5.27 (m, 1H, H 2e), 7.64 and 8.28 (AB pattern, 4H Ar-H, J=8 Hz); ^{13}C ($CDCl_3$) 46.7 (C-5), 72.2 (C-6), 83.7 (C-4), 94.6 (C-2), 123.8 (C meta to nitro), 129 (C ortho to nitro), 144.8 (C para to nitro), 148.7 (C attached to nitro).

Section C

threo (±)-1-(Acetoxymethoxy)-3-acetoxy-2-dichloroacetamido-1-p-nitrophenyl-propane (45)

A mixture of (±)-(48) (1.68g, 5 mmol), acetic anhydride (20 ml) and p-toluenesulfonic acid (0.1 g) was heated at 100-110°C for 10 hr with stirring. The reaction mixture was then poured on ice and extracted with ethylacetate. The ethylacetate extract was washed successively with water, saturated sodium bicarbonate solution and finally with water. It was dried over anhydrous sodium sulfate and evaporated to furnish hemiacetal (±)-(45) (2.12 g, 97%). This was crystallized from chloroform-petroleum ether, m.p.132°C; IR (nujol) 3330, 1752, 1682, 1555, 1525, 1360 and 1230 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.9 (s, 3H), 2.05 (s, 3H), 4.25 (m, 3H), 4.95 (bs, 1H), 5.05 and 5.45 (AB pattern, 2H, J=6 Hz), 5.7 (s, 1H), 6.65 (bd, 1H), 7.52 and 8.22 (AB pattern, 4H,

J=8 Hz); MS 347, 349, 287, 275, 225, 212, 195, 170 and 153 (100%). On similar treatment (-)-(48) afforded threo-(1R)-(acetoxymethoxy)-(2R)-dichloroacetomido-3-acetoxy-1-p-nitrophenyl propane (-)-(45) as viscous oil. TLC, IR and NMR are in agreement with (±)-(45).

threo(±)-2,2-Dichloro-N-[2-hydroxy-1(hydroxymethyl)-2-(4-nitrophenyl)ethyl] acetamide-chloramphenicol (±)-(1)

To a solution of hemiacetal diacetate (±)-(45) (0.2 g, 0.46 mmol) in methanol (4 ml), aqueous ammonia (4 ml, 25% solution) was added and it was stirred at room temeprature for 1.5 hr. Methanol was removed under reducesd pressure and the product was isolated with ethylacetate in usual way to furnish (±)-(1) (0.138 g, 93%), m.p.144°C (lit.[7] m.p.144-147°C); IR (nujol) 3390, 3290, 3210, 1680, 1510 and 1350 cm^{-1}; ^{1}H-NMR (d_6 acetone) 3.0 (bm, 2H), 3.73 and 3.80 (AB pattern, 2H, J=4 Hz), 4.18 (m, 1H), 5.11 (m, 1H), 5.33 (bs, 1H), 6.36 (s, 1H), 7.71 and 8.20 (AB pattern, 4H, J=8 Hz); MS 170, 172, 153 (100%), 136 and 118.

threo (+)-2,2-Dichloro-N-[2 – Hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide (1)

Treatment of (-)-(45) with methanol and aqueous ammonia furnished natural chloramphenicol (1) m.p.150°C; $[\alpha]_D^{29}$ + 17.5 (C=5.4, EtOH); its IR, NMR and TLC are in agreement with authentic chloramphenicol.

threo (-)-(1R, 3) Diacetoxy-(2R)-dichloroacetomido-1-p-nitrophenyl propane (62)

A mixture of (-) (48) (0.48 g, 1.3 mmol), conc. H_2SO_4 (0.8 ml) and acetic anhydride (8 ml) was heated at 70°C for 24 hr. It was then cooled, sodium acetate (1.5 g) added and acetic anhydride was removed under reduced pressure. On usual workup it afforded diacetate (62) (0.37 g, 62%), m.p.

138-140°C; $[\alpha]_D^{27}$ -8.39 (C=0.262, EtOH) (lit.[2] m.p.141-142°C); IR (nujol) 3210, 1744, 1690, 1524, 1460, 1450, 1375, 1352, 1230, 1055, 860 and 820 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 2.09 (s, 3H), 2.19 (s, 3H), 4.10 (m, 2H), 4.6 (m, 1H), 5.87 (s, 1H), 6.04 (d, 1H, J=6 Hz), 6.94 (bd, 1H, J=10 Hz), 7.51 and 8.21 (AB pattern, 4H, J=9 Hz) (authentic sample of diacetate was prepared from authentic chloramphenicol, m.p., NMR and $[\alpha]_D$ of compound (62) was found to be in agreement with that prepared from chloramphenicol).

Mixture of hemiacetal diacetate (±)-(45) and oxazoline derivative (±)-(63)

A mixture of (±)-(48) (0.67 g, 2 mmol), fused zinc chloride (0.08g, 0.58 mmol) and acetic anhydride (1.6 ml, 16.9 mmol) was stirred at room temperatuare for 24 hr. The starting material remained unchanged. It was then heated at 100°C for 1 hr and the reaction mixture was poured on crushed ice. On usual workup it afforded viscous oil (0.75 g), IR(nujol) 3460, 3340, 1750, 1685, 1610, 1530 and 1355 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.9 (s, 0.45H), 2.05 (s, 0.45H), 2.1 (s, 2.5H), 4.25 (m, 0.45H), 4.38 (m, 2.5H), 5.09 (d, 1H, J=5 Hz), 5.5 and 5.75 (AB pattern, 2H, J=4 Hz), 6.07 (s, 1H), 7.52 and 8.22 (AB pattern, 4H, J=8 Hz); MS 376 (M^+), 341, 317, 303, 193.

This mixture of hemiacetal diacetate (±)-(45) and oxazoline derivative (±)-(63) on treatment with aqueous ammonia solution in methanol furnished (±)-chloramphenicol. Its TLC, IR and NMR are in agreement with that of (±)-chloramphenicol (1).

threo (±)-1-Acetoxymethoxy)-3-(acetoxy)-2-(N-formyl-N-acetyl) amino-1-p-nitrophenyl propane (±)- (65)

A mixture of (±)-(56) (1.008 g, 4 mmol), acetic anhydride (8 ml) and PTSA (.06 g) on similar treatment to that of (±)- (48) afforded (±)-(65) (1.4 g, 88%); m.p.134°C; IR (nujol) 1750, 1680, 1525, 1350, 1225 and 755 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.82 (s, 3H), 1.96 (s, 3H), 2.5 (s, 3H), 3.82 –

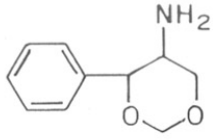
4.49 (m, 3H), 4.77 and 5.18(AB pattern, 2H, J=6 Hz), 4.31 (d, 1H, J=10 Hz), 7.62 and 8.27 (AB pattern, 4H, J=8 Hz), 9.16 (s, 1H); MS 307 (M $-OCH_2OCOCH_3$), 279, 265, 237, 205, 194, 177, 172, 153, 144, 135, 130, 113, 103, 88, 84 (100%) 71; ^{13}C 170.94 (C-10, $NCOCH_3$), 169.4, 169.18 (C-4 and C-7, CO), 162.36 (C-9, NCOH), 147.84 (C-15, ipso-NO_2), 144.32 (C-12, ipso), 128.48 (C-14 and C-16, ortho to nitro), 123.2 (C-13 and C-17, meta to nitro), 84.92 (C-6, O-CH_2), 75.24 (C-1, benzylic), 59.85 (C-3, CH_2), 55.44 (C-2, CH-N), 22.44 (C-11, $NCOCH_3$), 19.8 (C-8, O-CH_2-O-$COCH_3$), 19.38 (C-5, O-$COCH_3$); Analysis Found: C, 51.13; H, 5.22; N, 6.74. $C_{17}H_{20}N_2O_9$ require C, 51. 52; H, 5.05; N, 7.07.

threo (±)-N-(2-hydroxy)-1-(hydroxymethyl)-2-[(p-nitrophenyl) ethyl] acetamide (13)

The compound (±) - (65) on treatment with methanol-aqueous ammonia afforded mixture of two compounds (13) and (66). On crystallization from EtOAc-acetone, compound (±)- (13) separated out and the compound (±)- (66) remained in the mother liquor. The compound (13) has m.p.159°C (lit.[2] m.p.159-164°C); IR (nujol) 3360, 3220, 1670, 1530, 1465, 1380 cm^{-1}; ^{1}H-NMR (d_6 acetone) 1.78 (s, 3H), 2.82 (s, 2H,OH), 3.64 (m, 2H), 4.09 (m, 1H), 5.18 (d, 1H), 7.0 (bd, 1H, -NH), 7.67 and 8.16 (AB pattern, 4H, J=8 Hz); Analysis Found: C, 52.24; H, 5.70; N, 11.58. $C_{11}H_{14}N_2O_5$ require C, 51.92; H, 5.55; N, 11.02.

threo (±)-N-(2-Hydroxy)-1(hydroxymethyl)-2[(p-nitrophenyl) ethyl] formamide (66)

Compound (66) is a gummy solid; IR (nujol) 3520, 3220, 1650, 1510, 1460, 1375 cm^{-1}; ^{1}H-NMR (d_6 acetone) 3.91 (s, 2H,OH), 3.73 (m, 2H), 4.24 (m, 1H), 5.24 (d, 1H, J=2 Hz), 7.27 (bd, 1H, -NH), 7.71 and 8.24 (AB pattern 4H, J=8 Hz), 8.07 (s, 1H,CHO). This (66) was found to be identical with the authentic sample prepared from 1-p-nitrophenyl-2-amino-1,3-propane diol [L-base (6)] using excess of ethyl formate.



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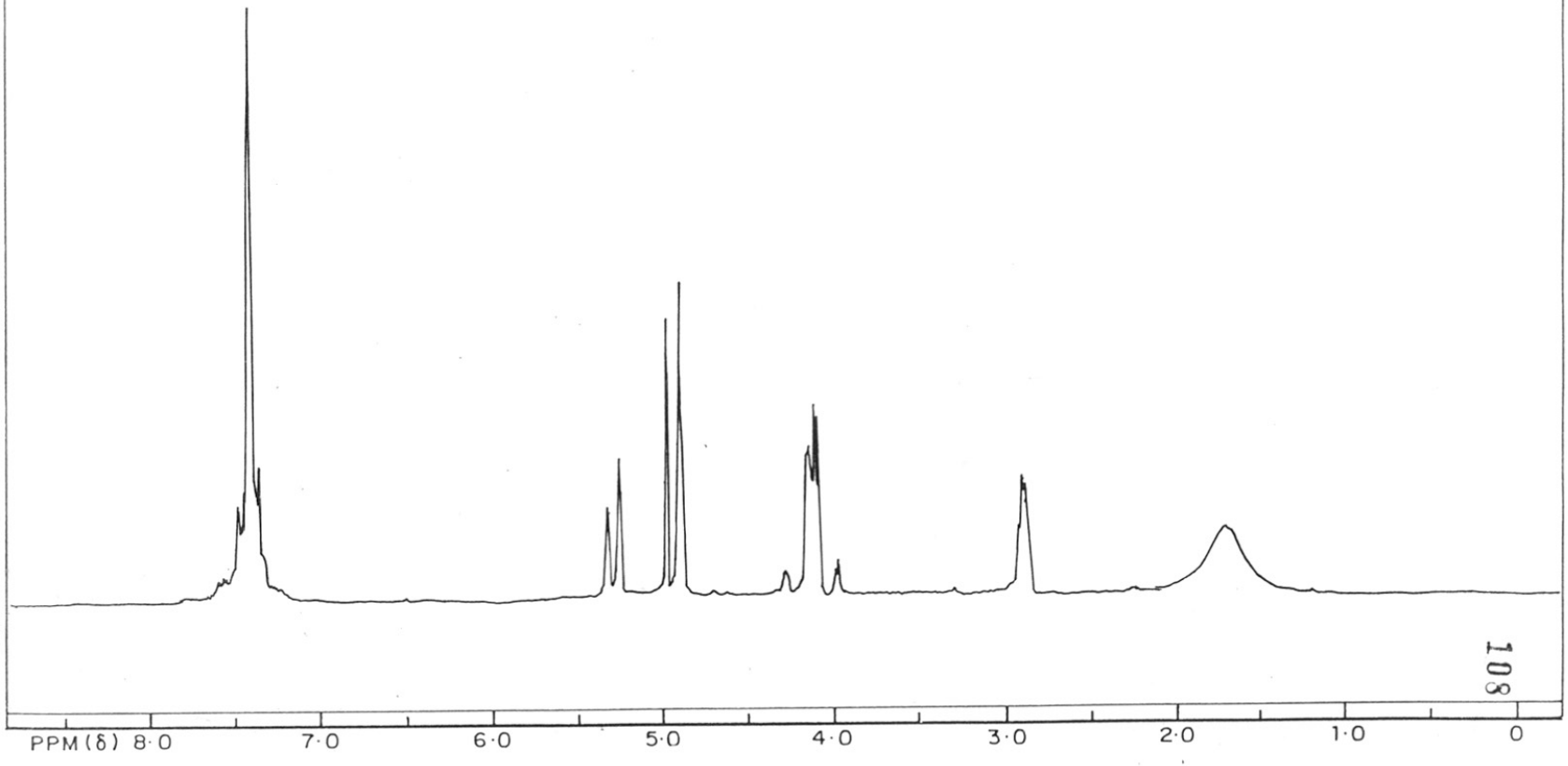

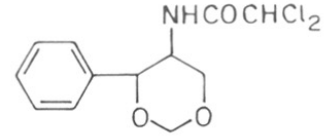

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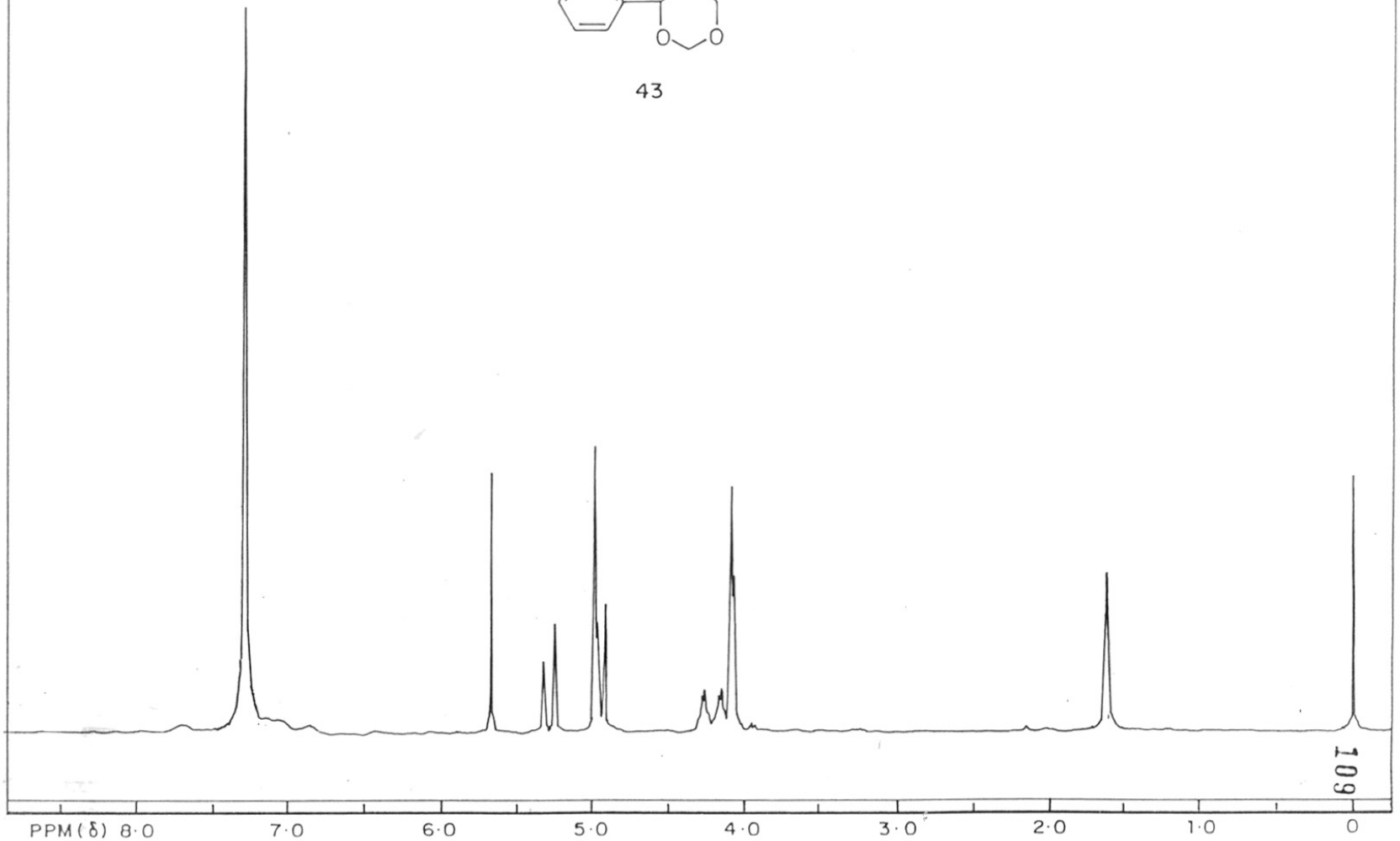

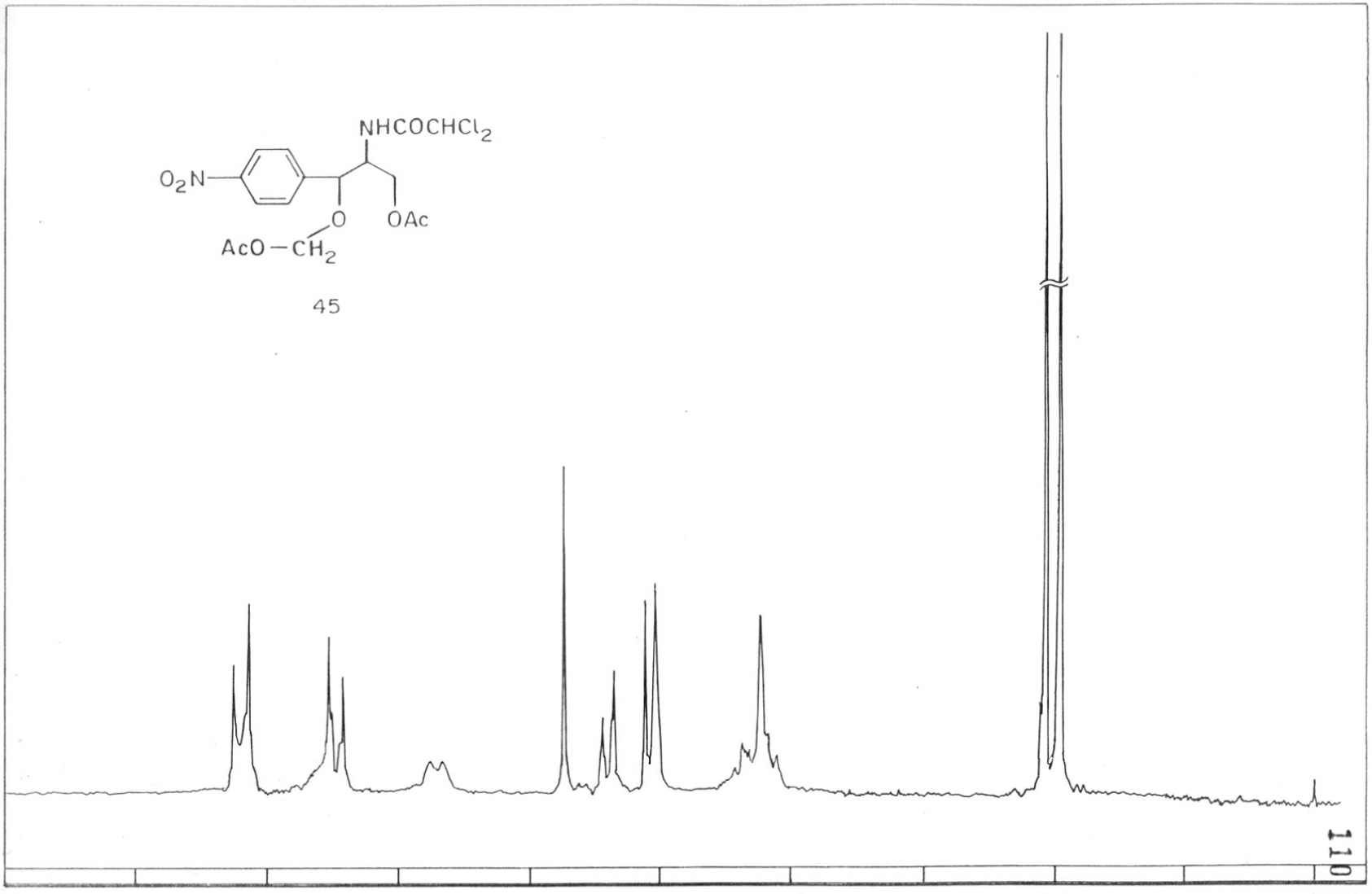
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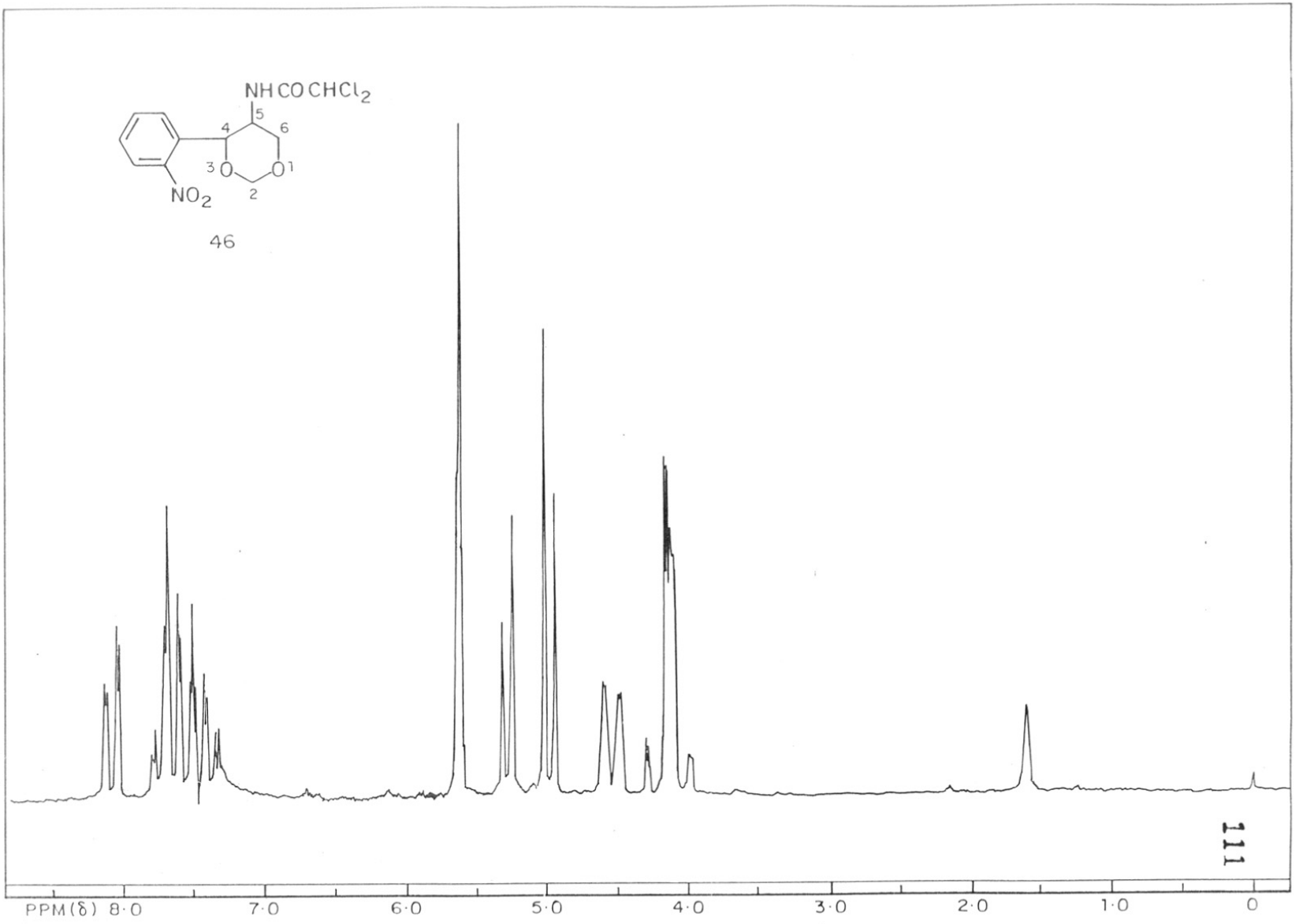

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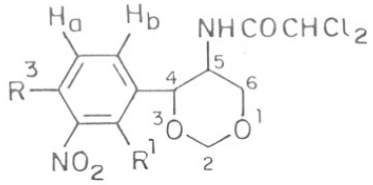

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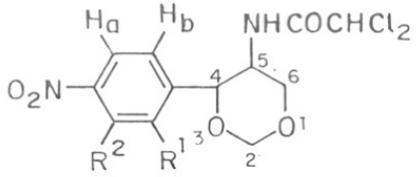
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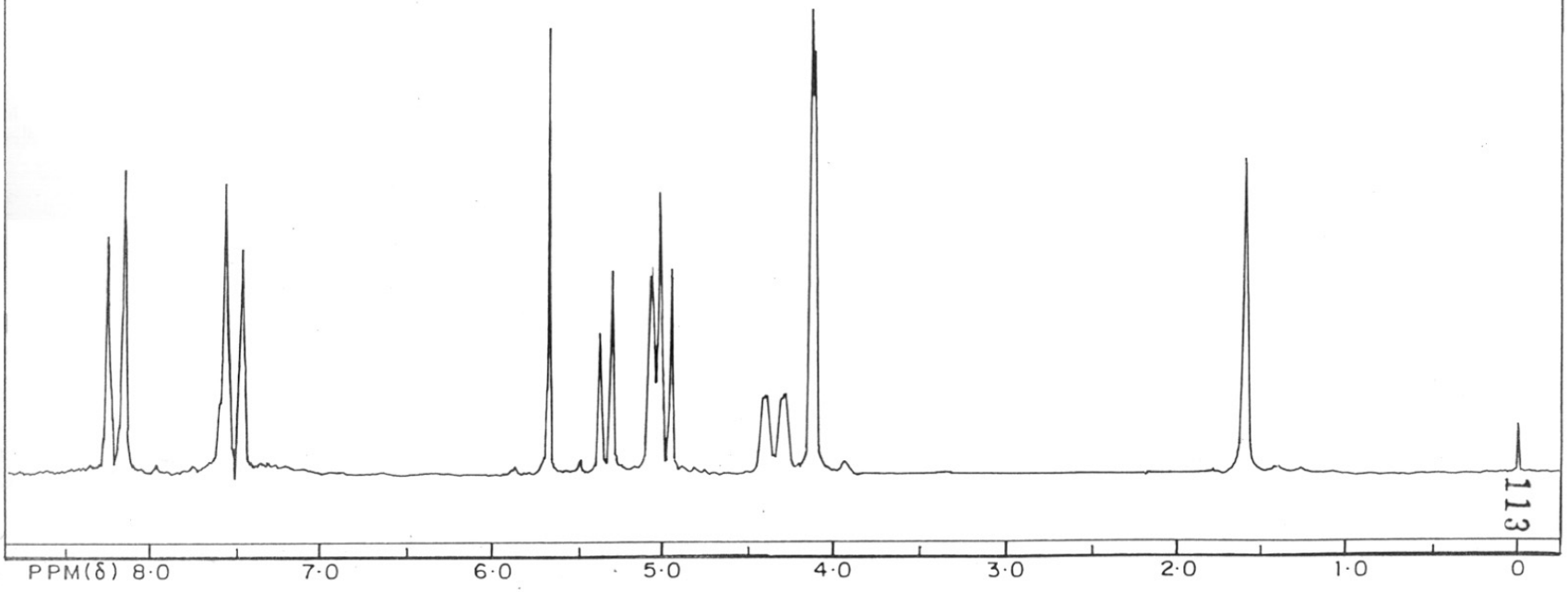
Hb
NHCOCHCl2
O2N
4
5
6
O
O
1
2
3
R2
R1

48


113
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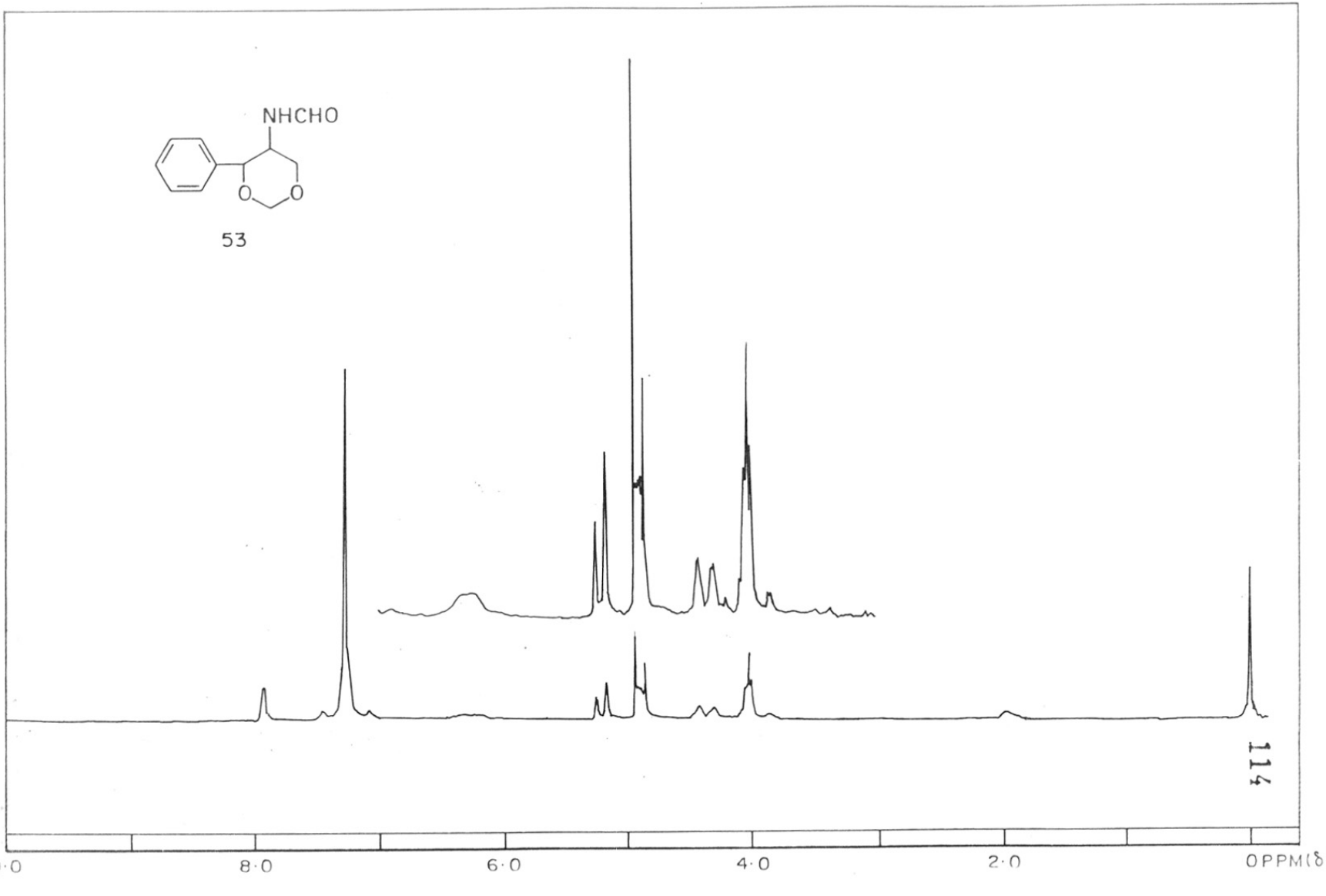
6·0
5·0
4·0
3·0
2·0
1·0
0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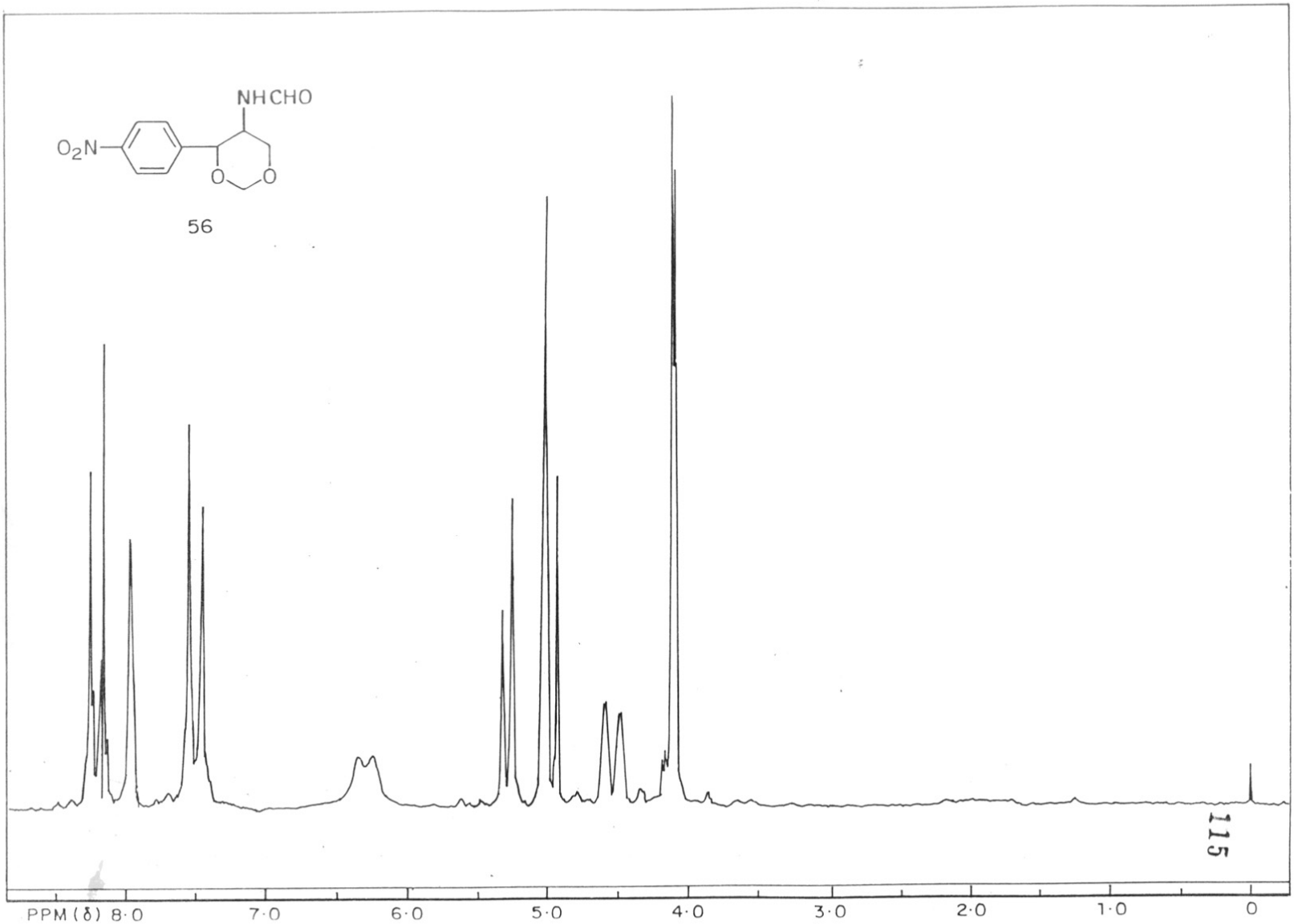
O
O
53
114
10·0
8·0
6·0
4·0
2·0
0 PPM(δ)


NHCHO
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O
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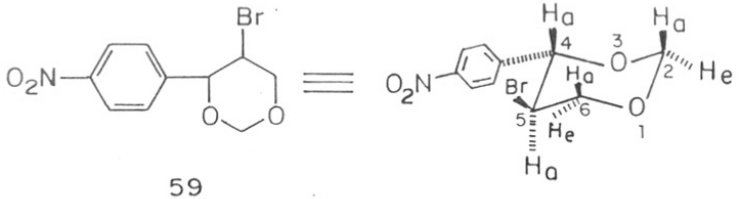
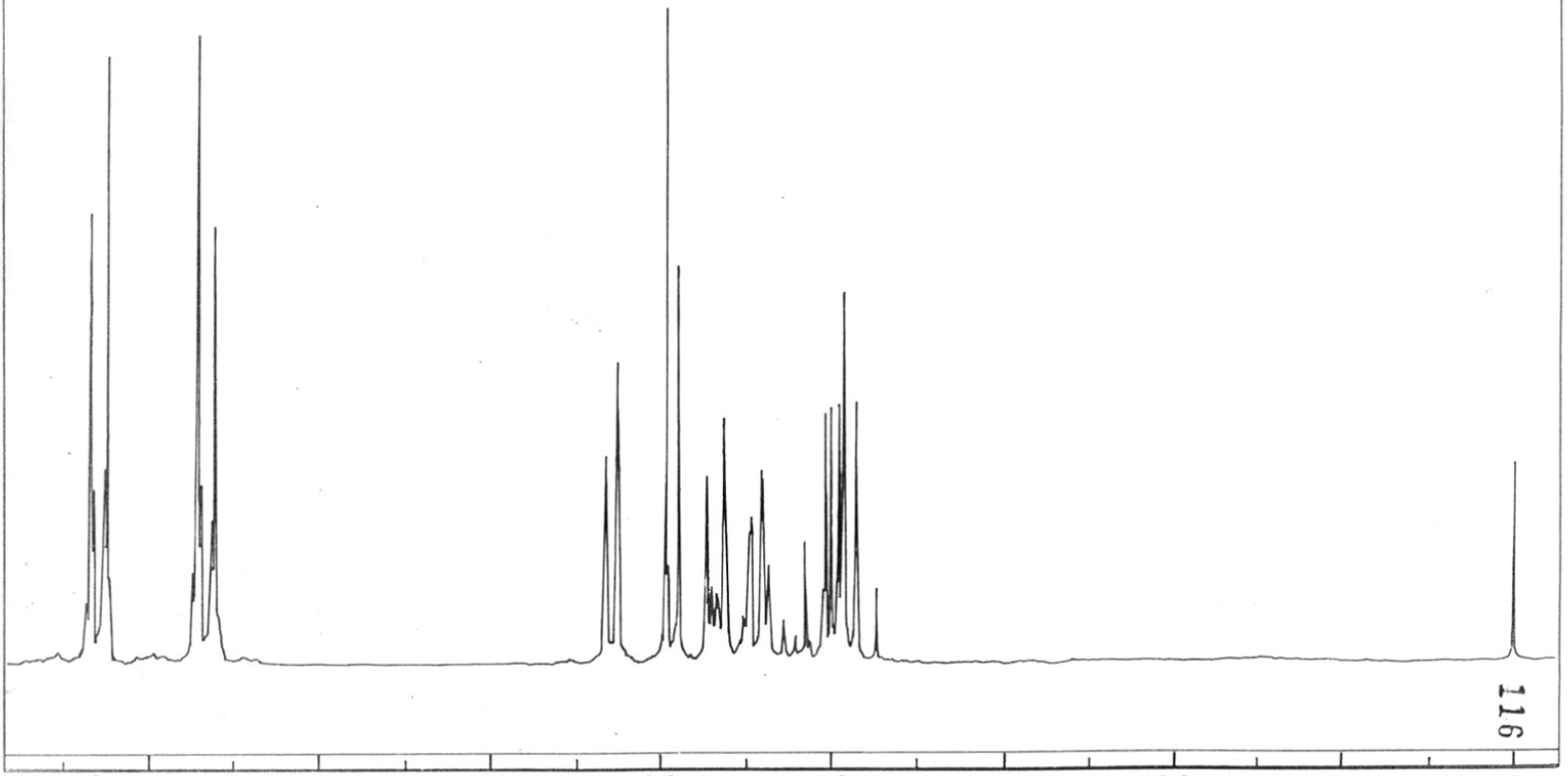
5·0
4·0
3·0
2·0
1·0
0


Br
O_2N
O
O
Ha
4
3
O
Ha
2
He
O_2N
Br
Ha
6
5
He
O
1
Ha

59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4·0
3·0
2·0
1·0
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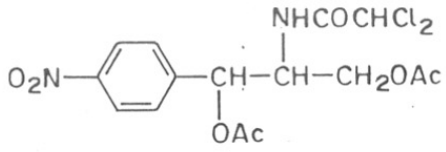

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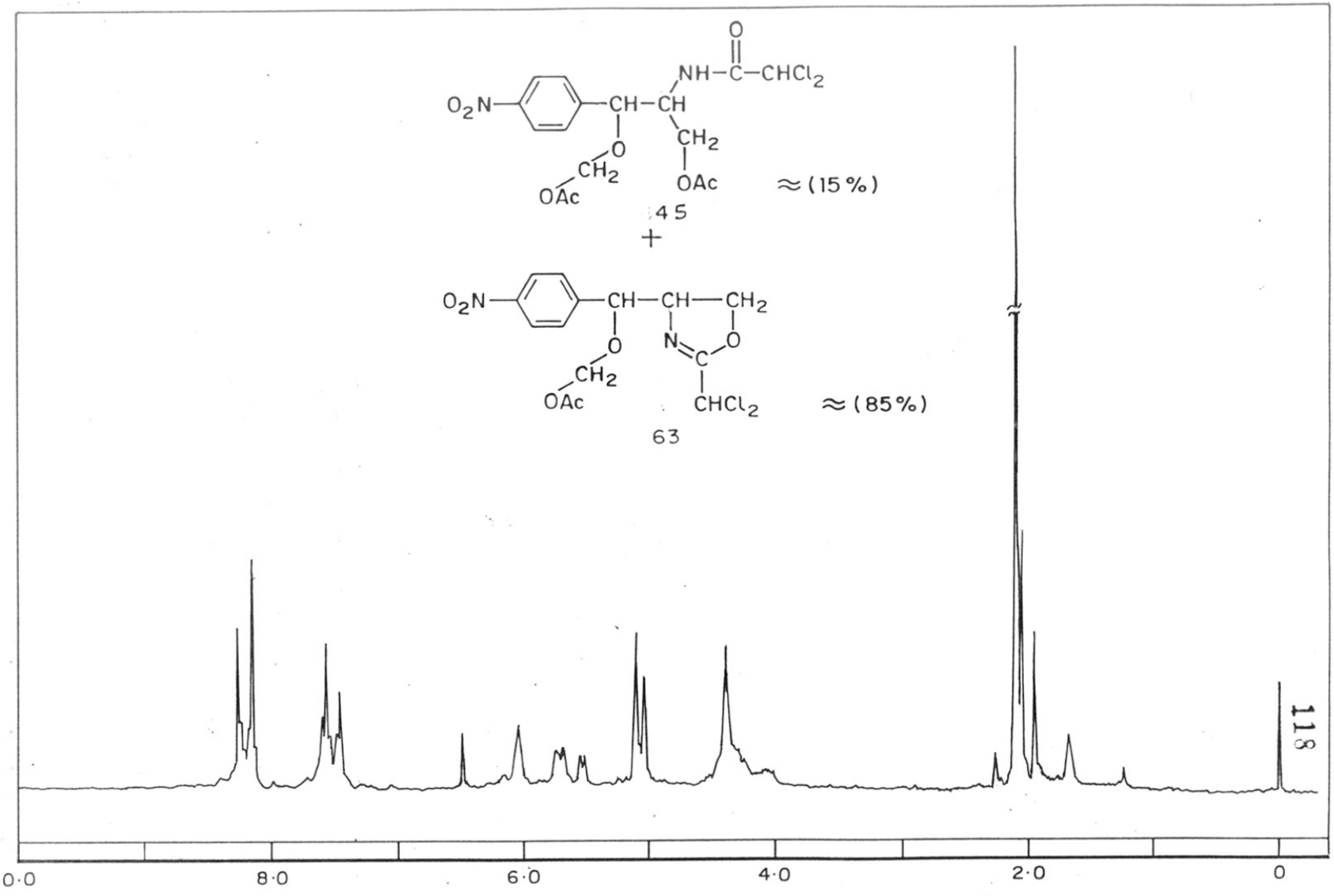
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NH–C–CHCl2
O2N
CH–CH
O
CH2
OAc
CH2
OAc
≈(15%)
45
+
O2N
CH–CH–CH2
O
N=C–O
CH2
OAc
CHCl2
≈(85%)
63
118
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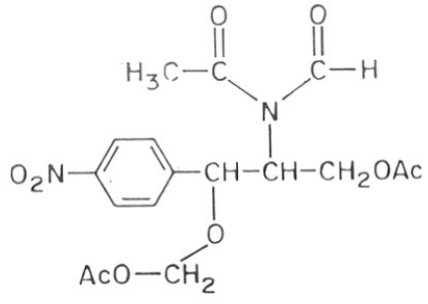
8·0
6·0
4·0
2·0
0


O
O
H3C—C
C—H
N
O2N
CH—CH—CH2OAc
O
AcO—CH2

65

119
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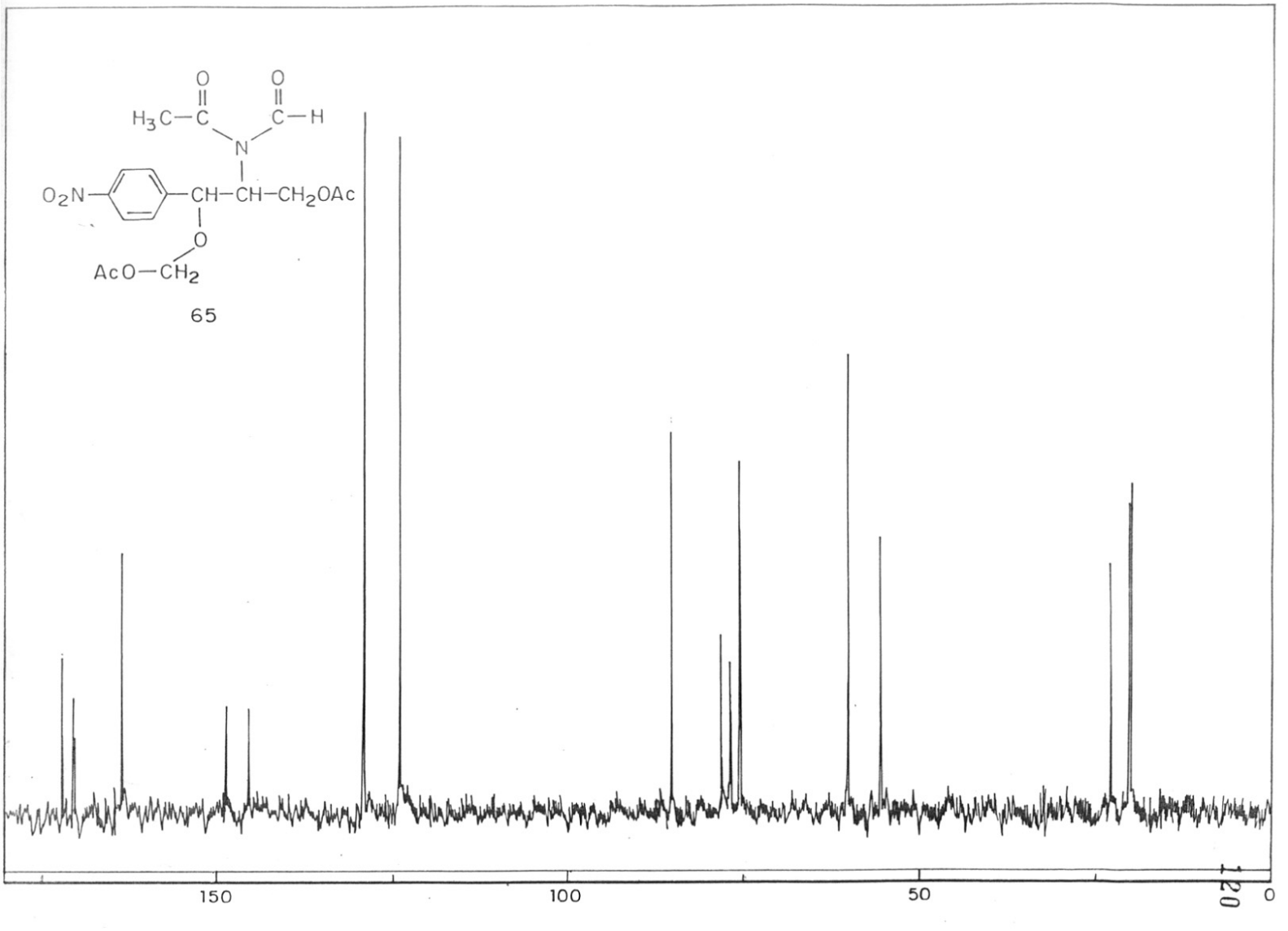
9·0
8·0
7·0
5·0
4·0
3·0
2·0
1·0



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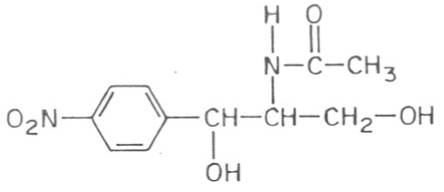
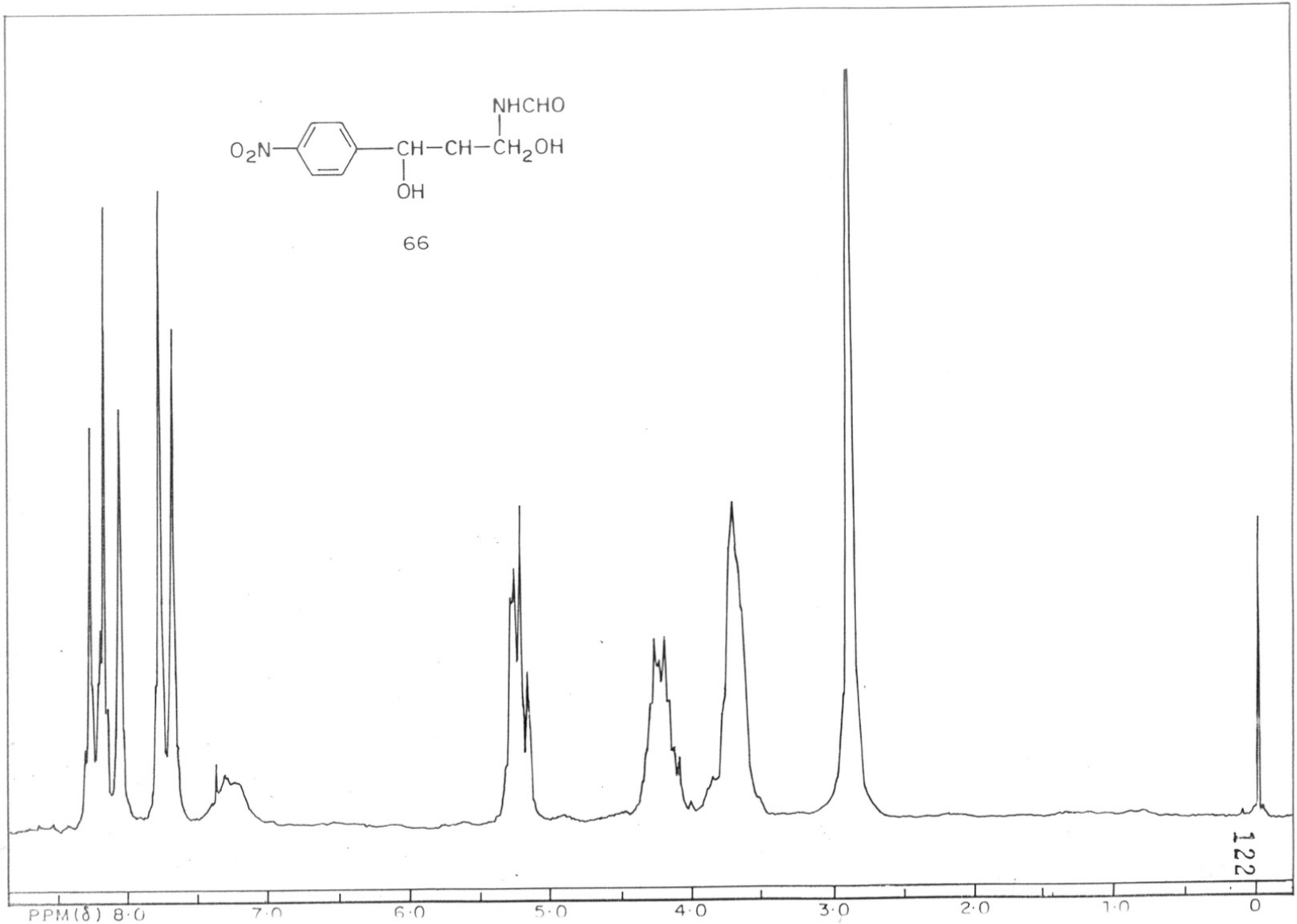


13


NHCHO
O2N
CH–CH–CH2OH
OH
66
122
PPM(δ) 8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0

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CHAPTER III

ALKYLATION OF METHYL NITROACETATE UNDER PTC

SUMMARY

It has been found that C-alkylation of methyl nitroacetate (13) can be best achieved by using triethyl benzyl ammonium chloride (TEBA) as phase transfer catalyst, potassium bicarbonate as base and DMF as a solvent. In the reaction of methyl nitroacetate (13) with benzyl bromide (27), methyl-2-nitro-3-phenyl propanoate (31) was the major product. However, O-alkylated product (38) and C,O-dialkylated product (39) also were formed in minor quantities.

In order to study the effect of substitution in aromatic nucleus of benzyl bromide (27) on product pattern, p-chloro (28), p-methoxy (29) and p-nitro (30) benzyl halides were reacted with methyl nitroacetate. Compound (28) and (29) furnished the C-alkylated products in good yields, however compound (30) afforded dialkylated products (37) and (41). In order to establish the generality of the reaction, simple alkyl halides, such as n-butyl bromide was treated with methyl nitroacetate (13) to give methyl-2-nitrohexanoate (43). Substituted ethyl nitroacetate (44) on reaction with benzyl bromide (27) gave ethyl-2-nitro 2-methyl-3 phenyl propanoate (45) in good yield.

INTRODUCTION

Alkyl nitroacetates are promising intermediates for a number of compounds such as amino acids, nitroparaffins, amines and their derivatives[1]. The presence of active methylene group makes these compounds unique intermediates for the formation of carbon-carbon bonds. Consequently, their reactivity often parallels that of other compounds containing an active methylene group. The nitro group is readily convertible to amino group and ester group is convertible to alcohol or methyl group. The alkyl nitroacetates are thus valuable intermediates for the synthesis of esters of 2-nitroalkanoic acids, nitro alcohols, nitro amines, halonitro compounds, di- and trinitro compounds, oxazolidines, oxazoles, amino acids, amino alcohols etc.[2]. The factors influencing the alkylation of alkyl nitroacetates have not been investigated thoroughly. In recent past, there have been some attempts towards the alkylation of alkyl nitroacetates by classical methods[3-9] which led to C-alkylated product in low yields (9-25%).

Hass and Bender[3] have shown that alkylation of nitroparaffin salts may occur as C-alkylation forming new carbon-carbon bond or O-alkylation, leading to an unstable nitronic ester which breaks down into oxime and a carbonyl compound. Substituted benzyl halides when reacted with nitroparaffins yielded mainly substituted benzaldehydes which were attributed to formation of O-alkylation product.

Boyd and Kelly[4] have reported alkylation of salts of ethyl nitromalonate (1) and ethyl nitroacetate (9) with allyl bromide (3) and crotyl chloride (4) (chart I). Thus potassium salt of ethyl nitromalonate (2) was heated in ethanol with allyl bromide (3) and crotyl chloride (4) to get ethyl allyl-

CHART I

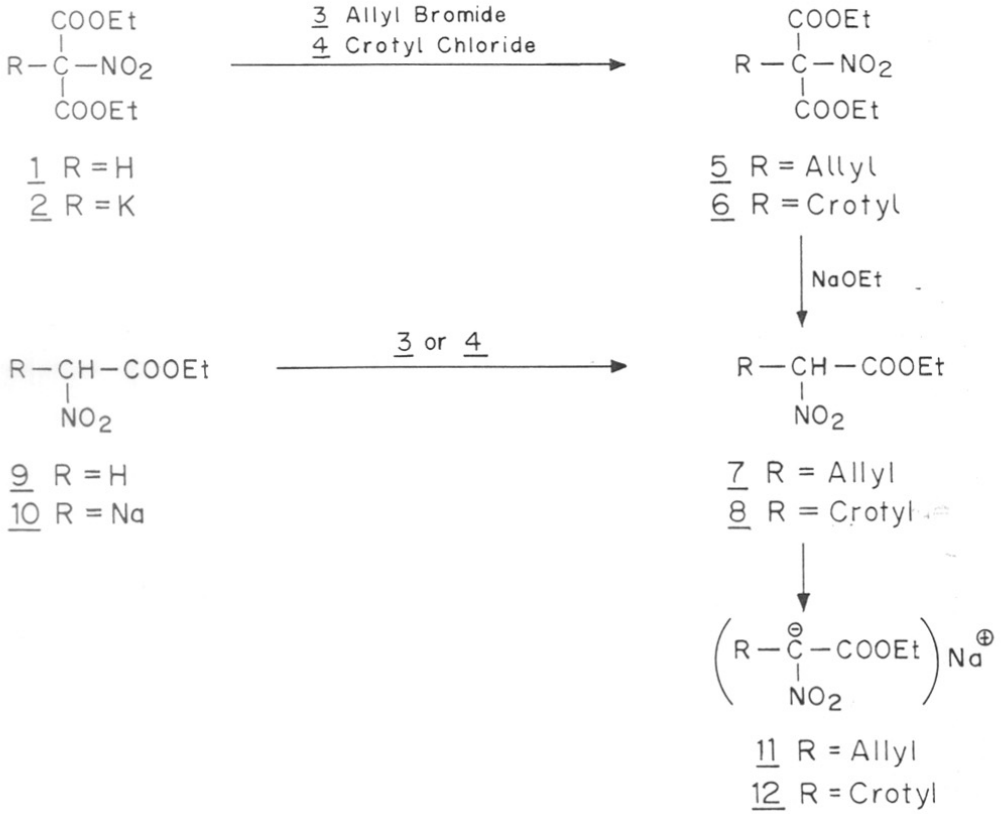
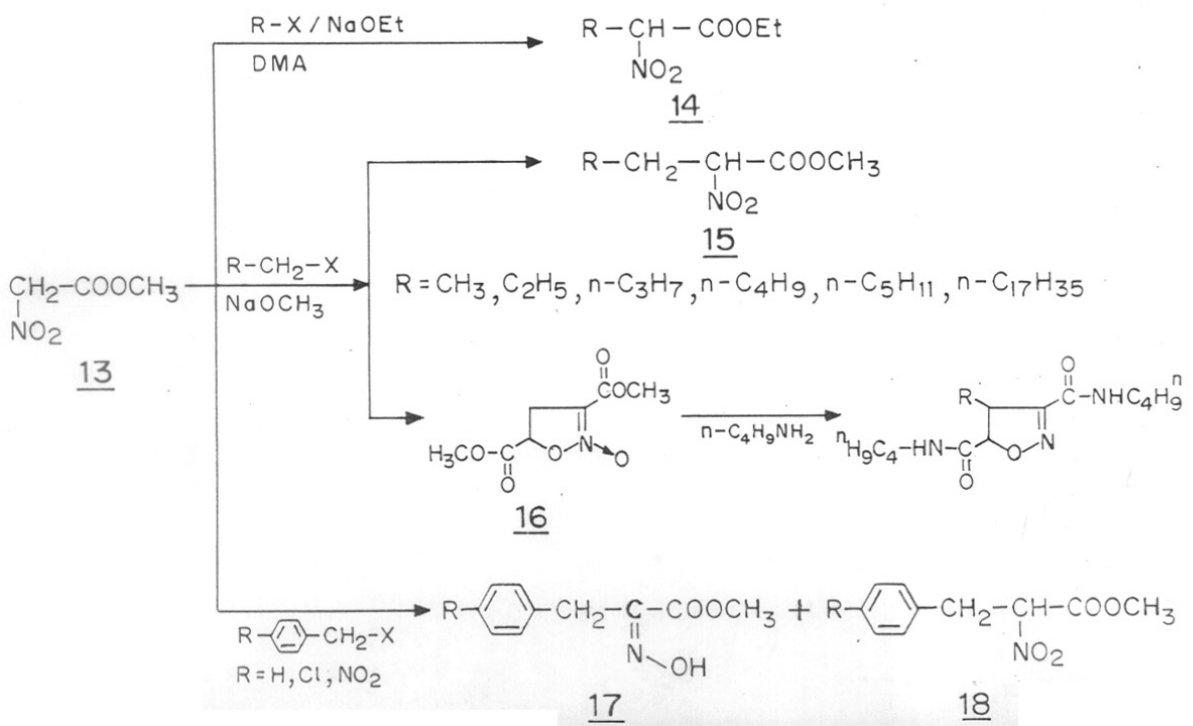


CHART II



nitromalonate (5) and ethyl crotylnitromalonate (6). These (5) and (6) on decarboethoxylation with sodium ethoxide gave ethyl allylnitroacetate (7) and ethyl crotylnitroacetate (8). Similarly sodium salt of ethyl nitroacetate (10) was reacted with allyl bromide (3) and crotyl chloride (4) to get ethyl allylnitroacetate (7) and ethyl crotylnitroacetate (8). These were then converted into their sodium salts (11) and (12) respectively. The yields of alkylation are very low (10-11%). Zen and Kaji[5,6] have synthesized α - nitrodicarboxylates by C-alkylation of methyl nitroacetate with halo ester of carboxylic acid. They have synthesised (DL)-aspartic acid, glutamic acid, alanine, valine, phenyl alanine, lysine etc. It has been found that C-alkylation reaction of nitroacetates with halides take place only in dipolar aprotic solvents such as DMF or dimethylacetamide.

A number of intermediates (14) (chart II) for the synthesis of amino acids were prepared via C-alkylation of methyl nitroacetate (13) with substituted alkyl halides, with yields ranging from 11 to 88%[7]. This alkylation failed in protic solvents. Later it was reported that, use of n-alkyl iodides, in addition to C-alkylated products (15), gave O-alkylated products which were isolated as isoxazoline N-oxides (16) in 32-43%.

The study of alkylation of methyl nitroacetate was extenderd to p-substituted benzyl halides to produce 3-phenyl-2-hydroxyiminopropanoates (17) (10-17%) and 3-phenyl-2-nitropropanoates (18) (20-37%)[8].

Bocharova et al have reported[9,10] alkylation of ethyl nitroacetate with alkyl halides in presence of triethylamine to give 9-17% C-alkylated product.

The poor C-nucleophilicity of nitronate anions (20) can be dramatically improved by formation of α,α doubly deprotonated species (21)[11]. Solutions

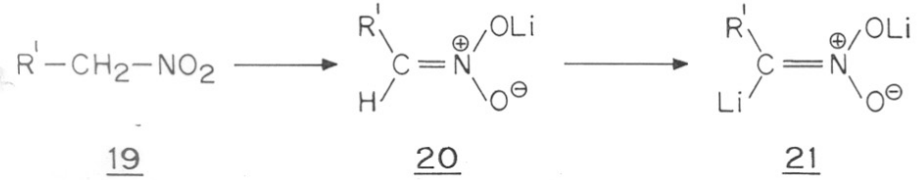
of (21) are obtained by addition of two equivalents of n-butyl lithium to a solution of primary nitroalkane (19) in dry THF at -78°C in the presence of at least two equivalents of hexamethyl phosphoramide (HMPA). The dilithio derivatives (21) are smoothly alkylated to nitro alkanes (22) by primary alkyl and benzyl bromides and iodides in yields of 50-75% (chart III).

Because of great synthetic interest, the alkylation of carbanion is among the most extensively studied phase transfer catalysis (PTC) reactions[12]. It was shown that the PTC method has many advantages over more conventional procedure which require not only expensive bases such as sodium amide, potassium tertiary butoxide, n-butyl lithium, metal hydrides etc. but also anhydrous solvents such as absolute ether, benzene, dimethyl sufloxide, dimethyl formamide etc. In many cases PTC is simpler than other procedures and because it is highly selective, it gives good yields of purer products. Triethyl benzyl ammonium chloride (TEBA) is the most commonly used catalyst for such transformations.

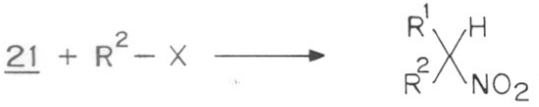
Alkylation reaction of nitroacetate appears to commence with ambident anion and consequently carbon/oxygen atom can be alkylated as shown in chart IV[13]. The extent of C-versus O-alkylation in alkyl nitroacetates depend upon the position of equilibrium between the two anionic species (23) and (24) generated from methyl nitroacetate (13) and their relative nucleophilicity towards the substrate.

To explain the preferential C-alkylation under PTC conditions, it is reasonable to assume that the reactive methylene and the halide ion from PTC form a hydrogen bonded complex (25) in which polarization towards carbanion occurs without the generation of full negative charge. Implication of this type of intermediate has been reported in the literature[14]. This phenomenon also curbs the equilibration with species (26) encouraging thereby preferential C-alkylated product (chart IV).

CHART III



19 20 21

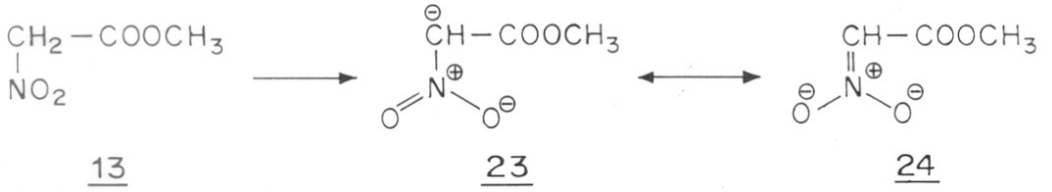


22

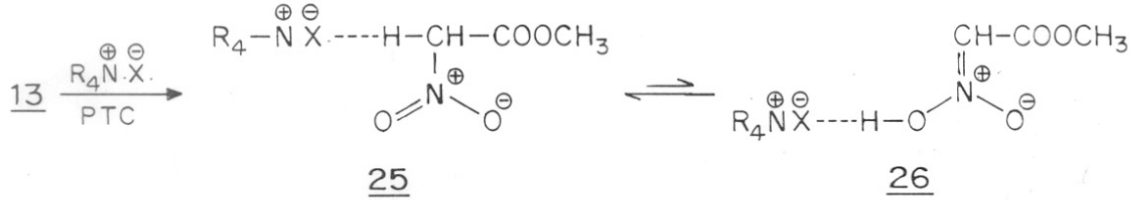
$R^1 = H, C_2H_5, n\text{-}C_5H_{11}, C_6H_5, C_6H_5S$

$R^2 = C_2H_5, n\text{-}C_4H_9, n\text{-}C_5H_{11}, n\text{-}C_6H_{13}, C_6H_5CH_2$

CHART IV



13 23 24



25 26

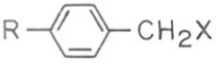
PRESENT INVESTIGATION

Alkylation of alkyl nitroacetates has been carried out using phase transfer catalyst. It was found that alkylation of methyl nitroacetate (13) by benzyl bromide (27) using TEBA as catalyst and sodium hydroxide as base in polar and non-polar aprotic solvents like THF, methylene chloride, benzene, DMF etc. resulted in a complex reaction mixture. When bases like potassium carbonate and sodium carbonate were used in solvents like THF and DMF, it was found that nitro ester reacts with carbonates. It can be recovered after acidification. Here the alkylated product obtained is mainly C,O-dibenzyl (39) with little C,C-dibenzyl (35). When base like calcium hydroxide in THF was used, most of the nitro ester remained unreacted and no desired C-alkylated product (31) was obtained. With the use of optically active bases like sodium-potassium tartarate and dipotasium tartarate, the reaction was very slow and the product (31) was obtained in low yield with no optical induction. When potassium bicarbonate was used as a base in THF without TEBA catalyst only O-alkylated product (38) was isolated. In this reaction when TEBA was used, it showed formation of C-alkylated product (31). But when instead of THF, DMF was used as solvent only C-alkylated product (31) was obtained in 72% yield. Among the different phase transfer catalysts like tetra n-butyl ammonium chloride, tetra n-propyl ammonium chloride and TEBA; TEBA was found to be suitable for C-alkylation than others (Table).

Thus C-alkylation can be best achieved by using TEBA as PT catalyst, dimethyl formamide as solvent and potassium bicarbonate as base.

In order to study the effects of substitution in aromatic nucleus of (27) on product pattern, p-chlorobenzyl chloride (28), p-methoxybenzyl bromide (29) and p-nitrobenzyl bromide (30) were reacted with methyl nitroacetate (13) under

CHART V

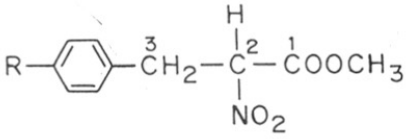


27 R = H , X = Br

28 R = Cl , X = Cl

29 R = OCH_3 , X = Br

30 R = NO_2 , X = Br

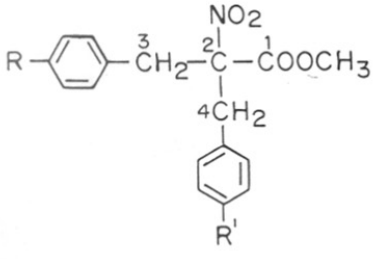


31 R = H

32 R = Cl

33 R = OCH_3

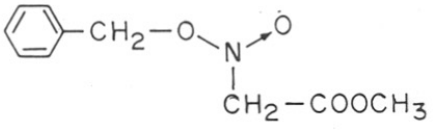
34 R = NO_2



35 R = R' = H

36 R = Cl , R' = H

37 R = R' = NO_2



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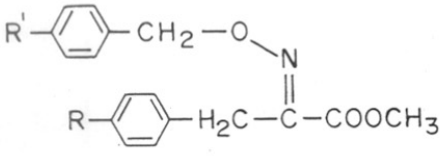
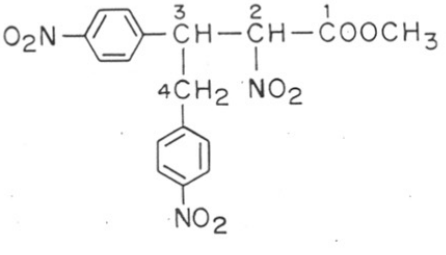


39 R = R' = H

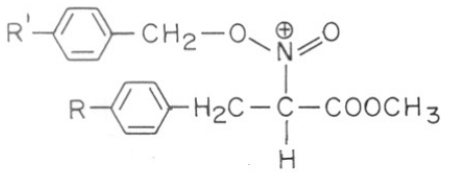
40 R = Cl , R' = H



41

Contd.....

CHART V Contd.



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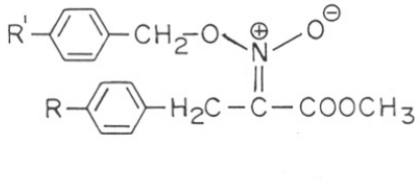

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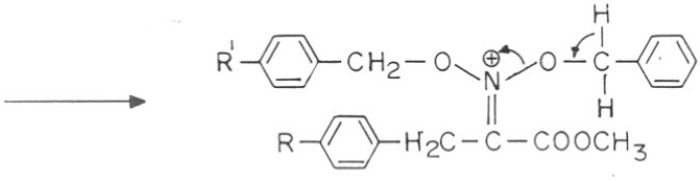


C

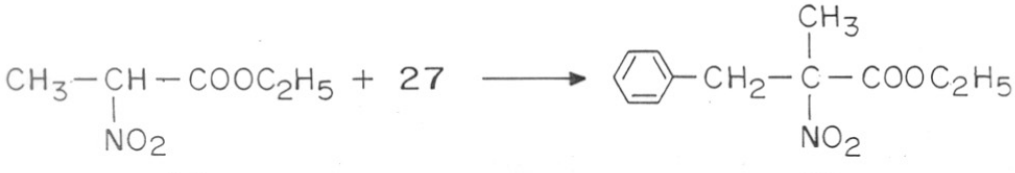
39 or 40

39 R = R' = H

40 R = Cl, R' = H

42

43



44

45

the best conditions mentioned above. p-Chloro (28) and p-methoxy (29) compounds furnished the anticipated methyl-2-nitro-3-phenyl propanoates (32) and (33) in good yields (70% and 75% respectively). However, p-nitrobenzyl bromide (30) gave only dialkylated products methyl-2-nitro-2-(p-nitro)-benzyl 3-(p-nitro) phenyl propanoate (37) and methyl-2-nitro-3,4-bis (p-nitro phenyl)butanoate (41). It appears that compound (34) which itself was not found in the reaction mixture is the precursor for both (37) and (41). This is logical since carbanions at C_2 and C_3 in compound (34) are more stabilized due to nitro group as compared to (31). Intermediacy of compounds (31) and (32) was confirmed by converting (31) to C,C-dialkylated compound (35) under drastic conditions. Similarly (32) when reacted with benzyl bromide (27) furnished (36) and C,O-dialkylated compound (40). Structures of (39) and (40) were evident from the absence of NO_2 peaks and presence of C=N peak in IR. This was ably supported by mass and PMR spectra. These compounds are formed by elimination of Ph-CHO from the C,O,O-trialkylated species (C) (chart V). In order to establish the generality of the reaction, simple alkyl halides such as n-butyl bromide (42) was treated with (13) to give methyl 2-nitrohexanoate (43) in 72% yield. Substituted ethyl nitroacetate (44) yielded ethyl 2-nitro-2-methyl-3-phenyl propanoate (45) also in good yields (78%).

EXPERIMENTAL

Methyl nitroacetate (13) was prepared according to the procedure mentioned in the literature[15]. p-Methoxy benzyl bromide (29) and p-nitro benzyl bromide (30) were prepared by standard procedures from corresponding toluenes, using N-bromo succinimide and benzoyl peroxide in carbon tetra chloride[17]. The compounds used had following physical constants.

$Ph\text{-}CH_2Br$ b.p. 195°C (lit.[18] b.p. 199°C/749 mm).

p-CH_3 O-C_6H_4-CH_2 Br b.p. 120°C/5 mm (lit.[17] b.p. 106°C/2.5 mm)

p-NO_2-C_6H_4-CH_2 Br m.p. 97°C (lit.[18] m.p. 100°C)

p-Cl-C_6H_4-CH_2 Cl m.p. 28°C (lit.[19] m.p. 29°C, b.p. 114-117°C/30 mm)

General procedure for C-alkylation of methyl nitroacetate (13)

Alkyl halide (10 mmol) was added to a stirred solution of methyl nitroacetate (10 mmol) in dimethyl formamide (10 ml) containing TEBA (0.04 mmol) and anhydrous potassium bicarbonate (5 mmol). The reaction mixture was then stirred at 60°C for 16 hr. DMF was removed under reduced pressure and the mixture was diluted with water. It was then extracted with ether. Ether layer was washed with water and dried over anhydrous sodium sulfate. Removal of solvent furnished an oil, which was purified by distillation or column chromatography.

Methyl-2-nitro-3-phenyl propanoate (31)

Benzyl bromide (27, 1.71 g, 10 mmol), methyl nitroacetate (13, 1.21 g, 10 mmol), potassium bicarbonate (0.5 g, 5 mmole) TEBA (0.011 g, 0.04 mmol) afforded 1.49 g of (31), yield 70%; b.p. 80-90°C/0.4 mm; IR (neat) 1750, 1560, 1360 cm^{-1} ; ^{1}H-NMR ($CDCl_3$) 3.5 (d, 2H, CH-3, J=8 Hz), 3.7 (s, 3H $COOCH_3$),

5.28 (t, 1H, CH-2 J=8 Hz), 7.12-7.25 (m, 5H, Ar); MS 209 (M^+), 91 ($Ph-CH_2$, 100%).

The PMR of crude reaction mixture shows the presence of O-alkylated product (38) and C,O-dialkylated product (39).

Methyl-2-nitro-3-p-chlorophenyl propanoate (32)

p-Chlorobenzyl chloride (28, 1.61 g, 10 mmol), methyl nitroacetae (13, 1.21 g, 10 mmol) furnished 1.1g of (32), 70% yield after purification by column chromatography over silica gel using benzene : ethyl acetate (90:10); Rf 0.52 (EtOAc:benzene,5:95); IR (neat)1760, 1570, 1370 cm^{-1}; ^1H-NMR ($CDCl_3$) 3.4 (d, 2H, CH_2-3, J=8 Hz), 3.8 (s, 3H, $COOCH_3$), 5.25 (t, 1H, CH-2, J=8 Hz), 7.11 – 7.52 (m, 4H, Ar); MS 243 (M^+), 196 ($M-HNO_2$), 125 ($Cl-C_6H_4-CH_2$, 100%).

Methyl-2-nitro-3-p-methoxy phenyl propanoate (33)

p-Methoxybenzyl bromide (29, 0.402 g, 2 mmol), methyl nitroacetate (13, 0.238 g, 2 mmol), TEBA (0.0056 g, 0.02 mmol), potassium bicarbonate (0.1 g, 1 mmol) in DMF (10 ml) was stirred at 50°C for 4 hr , gave 0.352 g of (33), yield 74%; b.p.110°C/0.02 mm; IR (neat) 1750, 1560, 1360 cm^{-1}; ^1H-NMR ($CDCl_3$) 3.33 (d, 2H, CH_2-3, J=8 Hz), 3.76 (s, 3H), 3.8 (s, 3H), 5.25 (t, 1H, CH-2, J=8 Hz), 6.7 and 7.06 (AB pattern, 4H, J=10 Hz); MS 239 (M^+), 121 ($MeO-C_6H_4-CH_2$, 100%) 107 ($MeO-C_6H_4$); Analysis Found C, 55.31; H, 5.60; N, 5.93. $C_{11}H_{13}NO_5$ require C, 55.2; H, 5.43; N, 5.85.

Methyl-2-nitro-2-(p-nitrobenzyl)-3-(p-nitro phenyl) propanoate (37) and methyl-2-nitro-3,4-bis (p-nitrophenyl) butanoate (41)

p-Nitrobenzyl bromide (30, 2.16 g, 10 mmol), methyl nitroacetate (13, 1.21 g, 10 mmol), potassium bicarbonate (0.5 g, 5 mmol), TEBA (0.011 g, 0.04 mmol) in DMF (15 ml) was stirred for 2 hr at 50°C. Crude reaction mixture showed three spots on TLC. It was passed over silica gel column. Elution with benzene:EtOAc (90:10) afforded methyl-2-nitro-3,4-bis (p-nitrophenyl)

butanoate (41), 0.5 g, as an oil, b.p. 120°C/0.03 mm; IR (neat) 1760, 1530, 1350 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 3.66 (d, 2H, CH-4, J=10 Hz), 3.87 (s, 3H, $COOCH_3$) 5.37 (d, 1H, CH-2, J=10 Hz), 5.44 (m, 1H CH-3), 7.31 to 8.35 (m, 8H, Ar). The positions of CH-2 and CH-3 were confirmed by decoupling; Analysis Found C, 52.12; H, 4.13; N, 10.62. $C_{17}H_{15}N_3O_8$ require C, 52.44; H, 4.08; N, 10.8.

Further elution with benzene:EtOAc (85:15) gave compound (37), 0.25g, m.p.165°C; IR (nujol) 1735, 1520, 1360 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 3.8 (s, 4H, CH_2-3 and CH_2-4), 4.05 (s, 3H, $COOCH_3$), 7.26 and 8.14 (AB pattern,8H, J=8 Hz); Analysis Found C, 52.81; H, 4.33; N, 11.21. $C_{17}H_{15}N_3O_8$ require C, 52.44; H, 4.08; N, 10.8.

Further elution with benzene:EtOAc (80:20 to 70:30) a compound was isolated. It showed no aromatic protons. It may be polymer of nitro ester. It was not investigated further.

Methyl-2-nitro-2-benzyl-3-phenyl propanoate (35)

Benzyl bromide (27, 0.427 g, 2.5 mmol) was added to a stirred solution of methyl-2-nitro-3-phenyl propanoate (31, 0.523 g, 2.5 mmol) in DMF (10 ml) containing TEBA (0.011 g, 0.04 mmol) and potassium carbonate (0.346 g, 2.5 mmol). The reaction mixture was stirred at 60°C for 16 hr. Usual work up afforded 0.950 g crude product. It was passed over silica gel column. Elution with benzene:EtOAc (90:10) furnished methyl-2-nitro-2-benzyl-3-phenyl propanoate (35), as an oil; Rf 0.72 (benzene:pet.ether-75:25); IR (neat) 1760, 1550, 1370 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 3.44 (s, 4H, CH_2-3 and CH_2-4), 3.62 (s, 3H, $COOCH_3$) 7.0 - 7.37 (m, 10H, Ar); MS 299 (M^+), 91 ($C_6H_5CH_2$, 100%).

Methyl-2-nitro-2-benzyl-3 – (p-chlorophenyl) propanoate (36) and compound (40)

Reaction of benzyl bromide (**27**, 0.171 g, 1 mmol) and methyl-2-nitro-3(p-chlorophenyl)-propanoate (**32**, 0.243 g, 1 mmol)on similar treatment as described above, afforded a mixture of two compounds (0.3 g) which were separated by column chromatography over silica gel. Elution with benzene: EtOAc (90:10) afforded methyl-2-nitro-2-benzyl-3(p-chlorophenyl)propanoate (**36**) as an oil; Rf 0.75 (EtOAc:benzene, 5:95); IR (neat) 1750, 1550, 1360 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 3.42 (s, 2H, CH_2-4), 3.48 (s, 2H, CH_2-3), 3.68 (s, 3H, $COOCH_3$), 7.022 to 7.8 (m, 9H, Ar); MS 333 (M^+), 125 ($Cl\text{-}C_6H_4\text{-}CH_2$), 91 ($C_6H_5\text{-}CH_2$, 100%).

On further elution with benzene:EtOAc (85:15 to 80:20) compound (**40**) was obtained as an oil, Rf 0.53 (EtOAc:benzene, 5:95); IR (neat) 1730, 1600(C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 3.81 (s, 3H, $COOCH_3$), 3.88 (s, 2H, CH_2-3), 5.31 (s, 2H, OCH_2), 7.15 to 7.35 (m, 9H, Ar); MS 317 (M^+), 125 ($Cl\text{-}C_6H_4CH_2$), 91 ($C_6H_5\text{-}CH_2$, 100%).

O-Alkylated product (38)

Benzyl bromide (**27**, 1.71 g, 10 mmol) was added to a stirred solution of methyl nitroacetate (**13**, 1.2 g, 10 mmol) in THF (10 ml) containing potassium bicarbonate (0.5 g, 5 mmol). The reaction mixture was refluxed for 12 hrs. The solvent was removed. Reaction mixture on usual work up gave an oil. It was distilled under reduced pressure, at 80-90°C/0.01 mm, 1.4 g (yield 70%); ^{1}H-NMR ($CDCl_3$) 3.8 (s, 3H,$COOCH_3$), 4.00 (s, 2H, CH_2-2), 4.5 (s, 2H, OCH_2), 7.33 (s, 5H, Ar).

C,O-Dialkylated product (39)

Benzyl bromide (**27**, 1.71 g, 10 mmol) was added to a stirred solution of methyl nitroacetate (**13**, 1.2 g, 10 mmol) in THF (15 ml) containing TEBA

(0.0056 g, 0.02 mmol), potassium carbonate (2.8 g, 20 mmol). The reaction mixture was refluxed for 2 hrs. On usual work up it afforded an oil, which on column chroamtography over silica gel gave C,O-dibenzyl compound (**39**) as an oil, 0.9 g (62%), b.p.80°C/0.36 mm;Rf 0.35 (benzene); IR (neat) 1730, 1600 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$) 3.81 (s, 3H, $COOCH_3$), 3.93 (s, 2H, CH_2-3), 5.25 (s, 2H, OCH_2), 7.12 (s, 5H, Ar), 7.31 (s, 5H, Ar); MS 283 (M^+), 224 ($M-COOCH_3$), 91 ($C_6H_5CH_2$, 100%).

Methyl-2-nitro-hexanoate (43)

Reaction was done according to general procedure for C-alkylation. Reaction was completed within 3 hr . On usual work up it gave an oil, which was chromatographed on silica gel to give the product (**43**) (yield 71.4%); IR (neat) 1770, 1570, 1380 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.8 to 1.6 (m, 7H, alliphatic), 2.2 (m, 2H, CH_2-3), 3.8 (s, 3H, $COOCH_3$), 5.1 (m, 1H, CH-2); MS 175 (M^+).

Ethyl-2-nitro propanoate (44)

It was prepared according to literature procedure, b.p.95°C/3 mm (lit.[16] b.p.55°C/1 mm); IR (neat) 1760, 1560, 1350 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.31 (t, 3H, $-CH_2-\underline{CH_3}$, J=7.5 Hz), 1.81 (d, 3H, J=8 Hz), 4.25 (q, 2H, $-\underline{CH_2}CH_3$, J=7.5 Hz), 5.13 (q, 1H, CH-2, J=8 Hz); MS 147 (M^+), 101 ($M-NO_2$), 74 (M -COOEt).

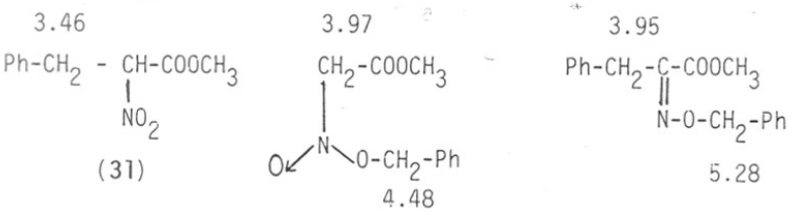
Ethyl-2-nitro-2-methyl-3-phenyl propanoate (45)

Benzyl bromide (**27**, 0.281 g, 1.64 mmol) was added to a stirred solution of ethyl 2-nitro propanoate (**44**, 0.230 g, 1.64 mmol) in DMF (10 ml) containing TEBA (0.011 g, 0.04 mmol) and potassium carbonate (0.230g, 1.64 mmol). The reaction mixture was heated at 50°C for 1.5 hr . Usual work up yielded an oil (0.39 g) which was passed over silica gel column. Elution

with benzene:EtOAc (90:10) gave the product, ethyl-2-nitro-2-methyl-3-phenyl propanoate (**45**) as an oil (0.286 g, 77%), Rf 0.25 (benzene); IR (neat) 1750, 1550, 1360 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 1.28 (t, 3H, CH_2-CH_3, J=6 Hz), 1.68 (s, 3H, CH_3-2), 3.55 (d, 2H, CH_2-3, J=7 Hz), 4.28 (q, 2H, -CH_2-CH_3, J=6 Hz), 7.07 – 7.4 (m, 5H, Ar); MS 237 (M^+), 91 ($C_6H_5CH_2$, 100%).

Table:

The percentage of different compounds (**31**), (**38**) and (**39**) by ^{1}H-NMR study-

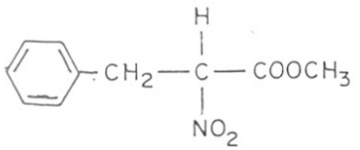


(**31**) (**38**) (**39**)

C-alkylated O-alkylated C,O-dialkylated

Signals corresponding to protons shown above are compared.

Catalyst	C-alkylated			O-alkylated			C,O-dialkylated		
	δ	inte-gration	%	δ	inte-gration	%	δ	inte-gration	%
TEBA	3.46	8	66.66	3.97 (4.48)	2	16.66	3.95 (5.28)	2	16.66
Tetra-n-butyl ammonium chloride	3.46	10	47.6	4.02	4	19.04	3.96	7	33.33
Tetra-n-propyl ammonium chloride	3.36	6	37.5	3.89	4	25.00	3.85	6	37.5



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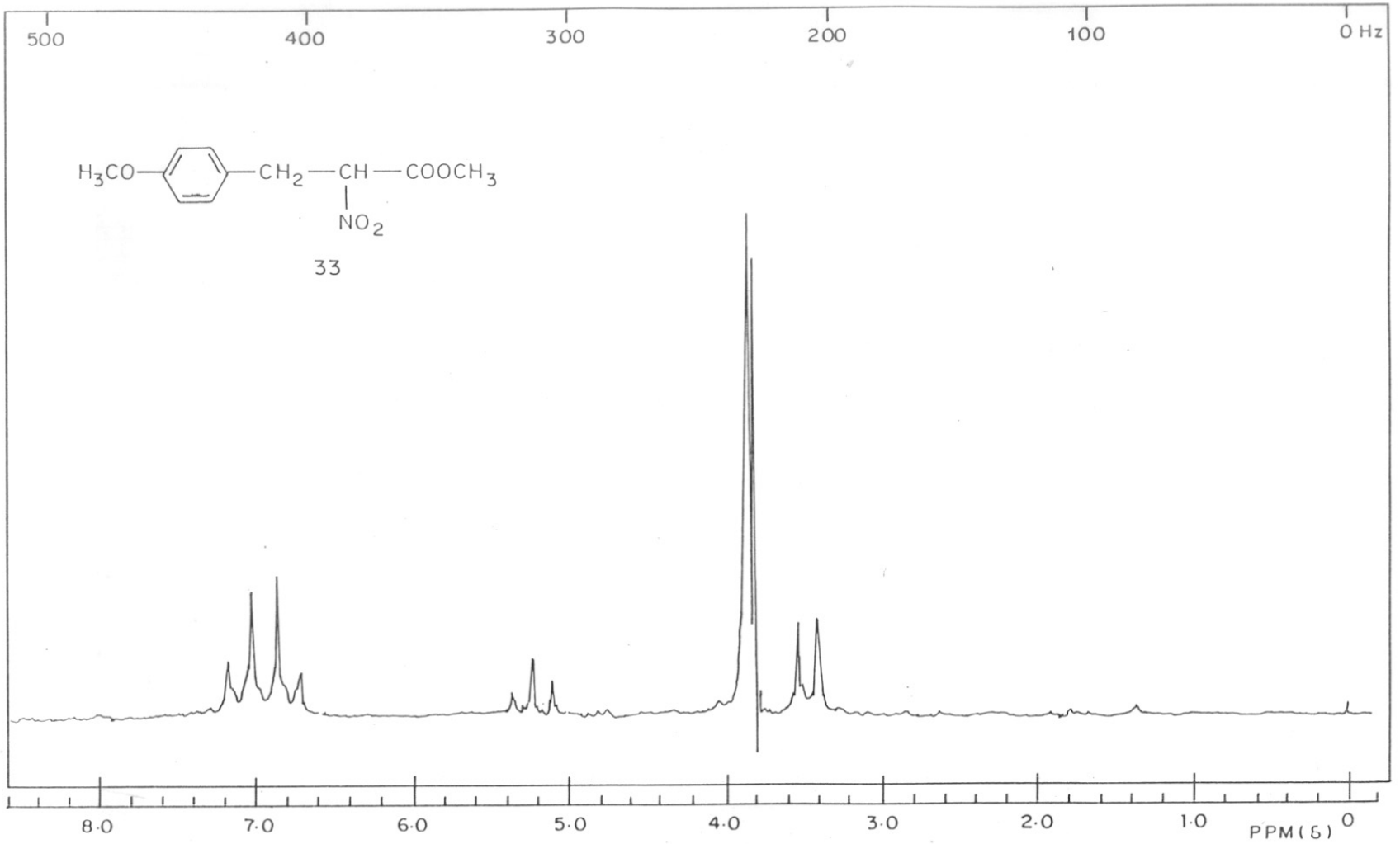
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400
300
200
100
0 Hz
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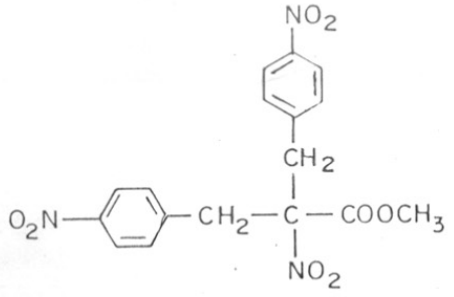
CH2
CH
COOCH3
NO2
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM(δ)

33



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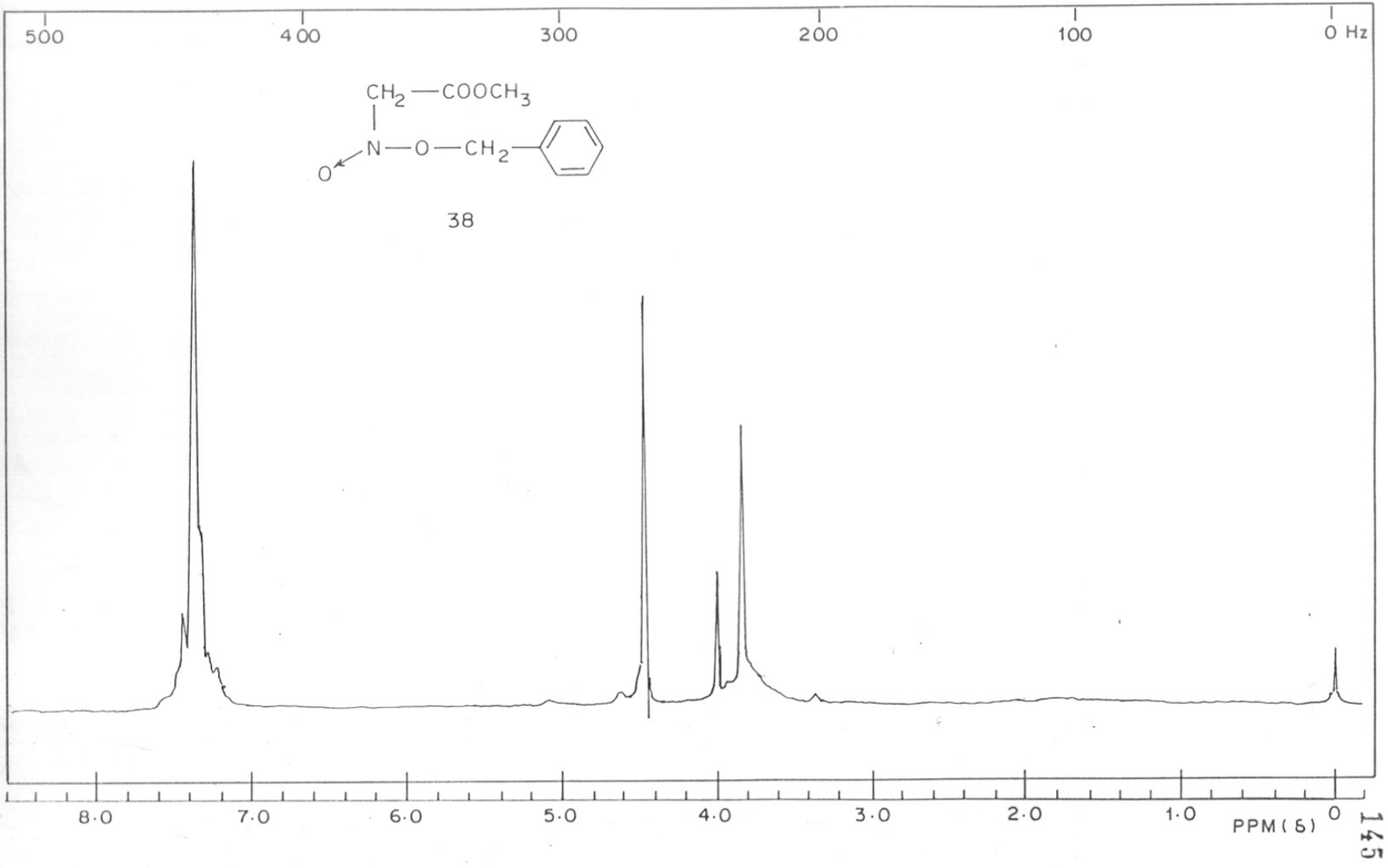
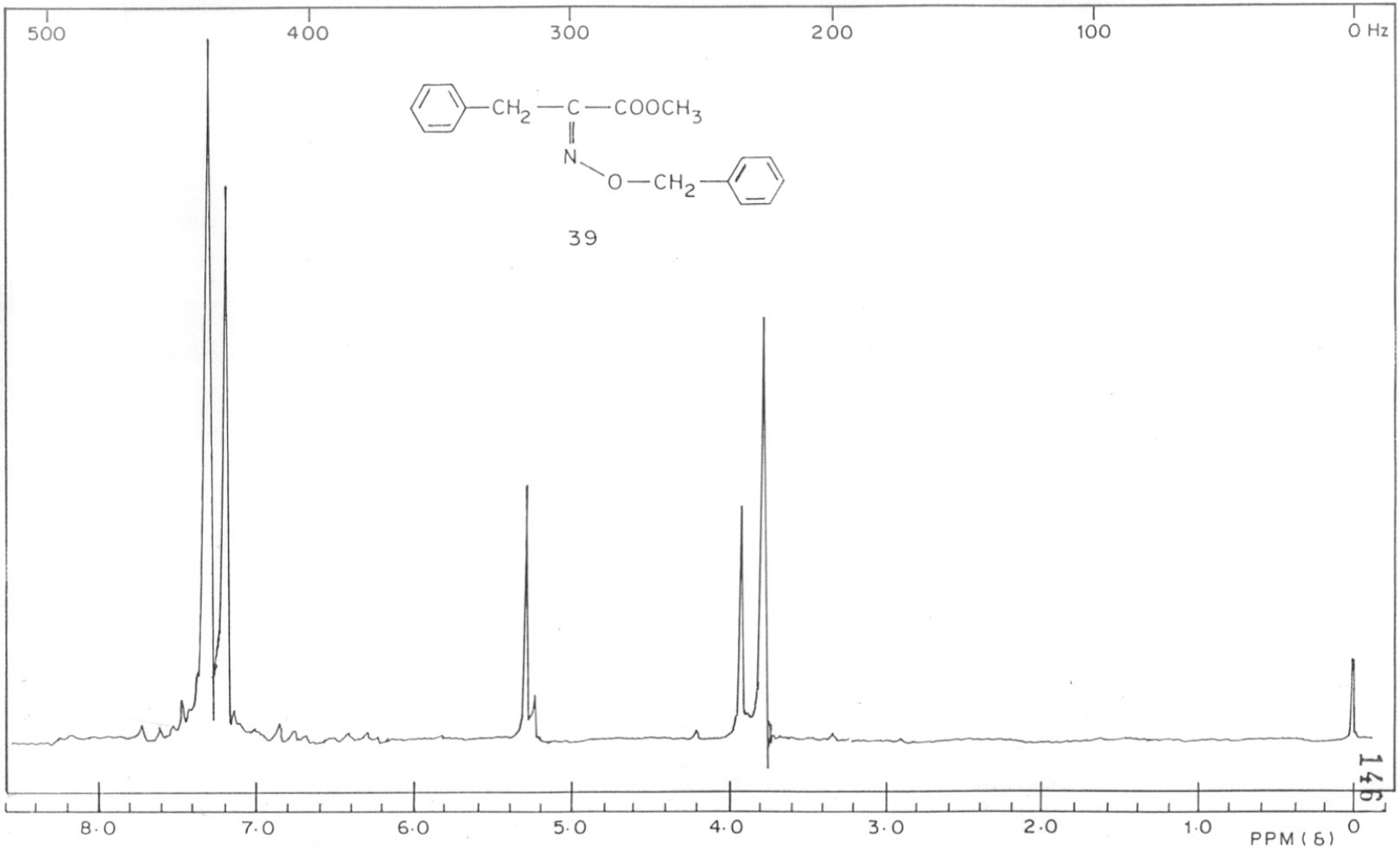
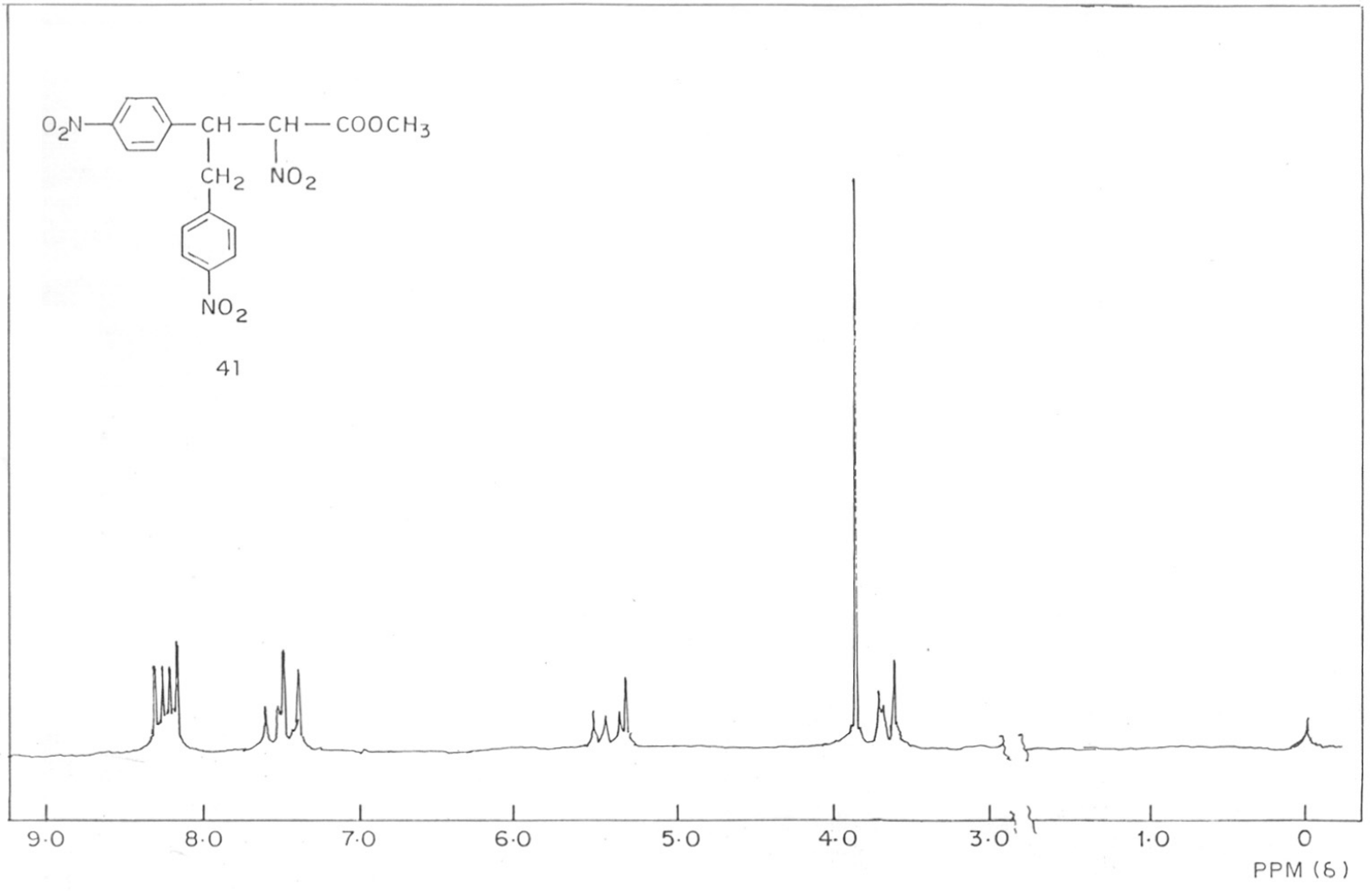

500
400
300
200
100
0 Hz
CH2—COOCH3
N—O—CH2
O
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM(δ)

38


500
400
300
200
100
0 Hz
CH2 — C — COOCH3
N
O — CH2
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)

39



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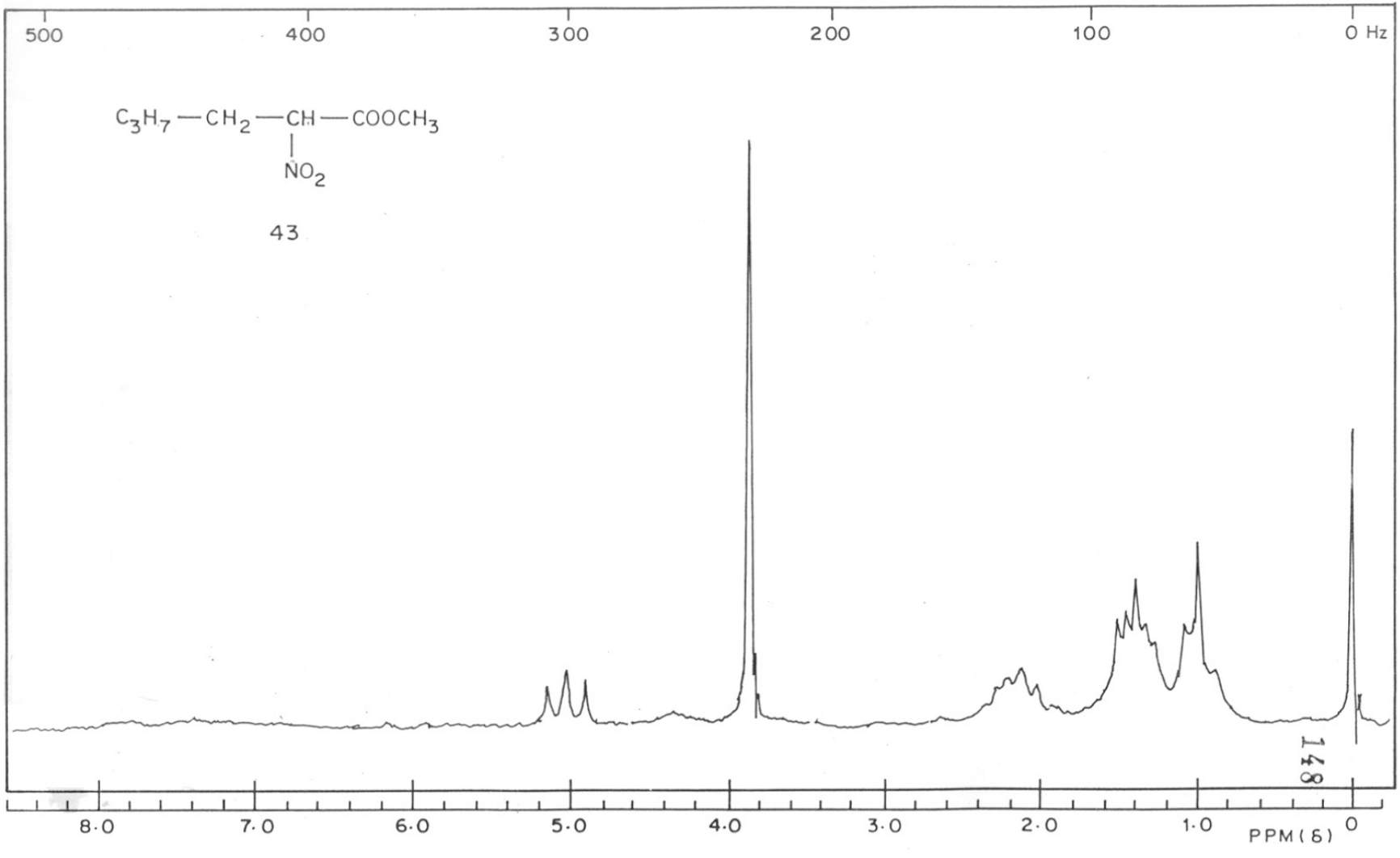
500
400
300
200
100
0 Hz
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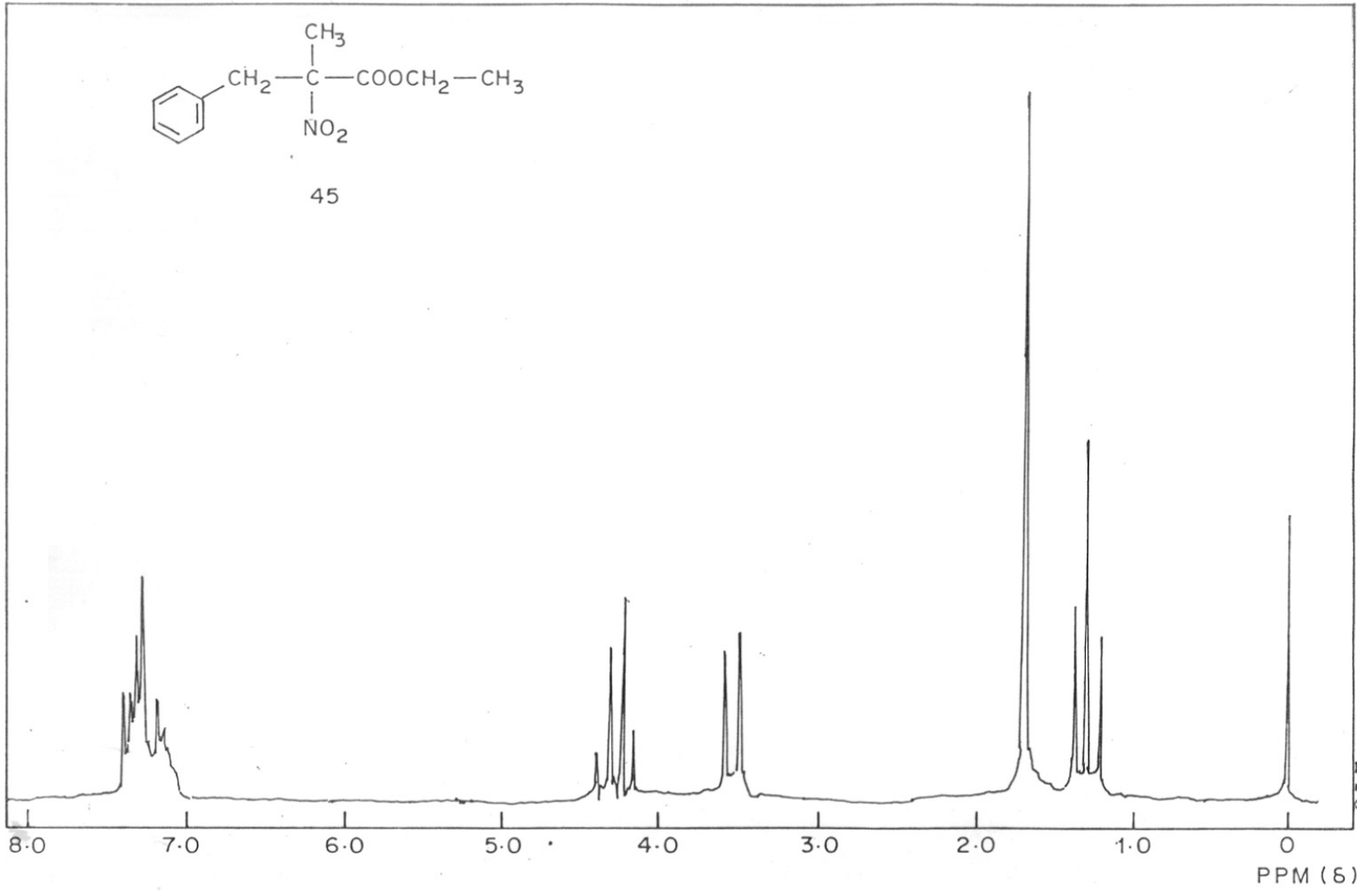
1.48
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ)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NO2
45
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
PPM (δ)

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LIST OF PUBLICATIONS

Research Publications

1. Alkylation of alkyl nitroaceatates under PTC conditions,
(Late) V.N. Gogte, A.A. Natu and V.S. Pore,
Synthetic Communications, **17**(12), 1421-1429 (1987).

2. Synthesis of optically active β-ketosulfides catalysed by quinine/quinidine and homogenous catalysts,
N.N. Bhongale, V.S. Pore and A.A. Natu,
Synthetic Communications, **18**(13), 1597-1606 (1988).

3. An improved procedure for the dichloroacetamidation of primary and secondary amines,
B.G. Hazra, V.S. Pore and S.P. Maybhate,
Org.Prepr.Proc.Int., **21**(3), 355-358 (1989).

4. Regioselective acylative cleavage of cyclic formal of chloramphenicol,
B.G. Hazra, V.S. Pore, S.P. Maybhate, M.V. Natekar and A.S. Rao,
Synthetic Communications, **19**, 1763-1770 (1989).

5. Resin catalysed ene reaction on 3β-toluene p-sulfonoxy-(Z)-pregna-5, 17(20)-diene: Synthesis of (20S)-6β-methoxy-3α,5-cyclo-5α-pregnane-20-carboxyaldehyde,
B.G. Hazra, P.L. Joshi, V.S. Pore,
Tetrahedron Lett., 1990 (in press).

Patents taken

1. An improved process for the preparation of 2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl] acetamide- A chloramphenicol intermediate,
B.G. Hazra, V.S. Pore, S.P. Maybhate and R.B. Mitra,
Patent Appl. No.991/DEL/87.

2. An improved process for preparation of 4-phenyl-5-dichloroacetamido-1,3-dioxane- A chloramphenicol intermediate,
B.G. Hazra, V.S. Pore, S.P. Maybhate and M.V. Natekar,
Patent Appl. No.1053/DEL/87.