Chapter – III

Action of NBS on diosgenin acetate 92 in dimethyl sulphoxide (DMSO)

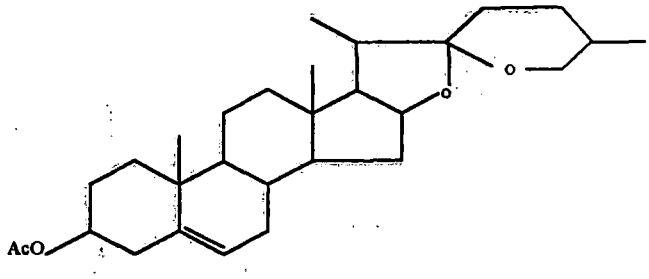
A solution of diosgenin acetate **92** (m.p 193^{0}C) (3 g) in $CHCl_3$ (150 ml) was mixed with DMSO (75 ml) and the solution was cooled to 20^{0}C. NBS (3g) was added to the mixture in small portions and the mixture was kept in dark for seven days .The mixture was then extracted with chloroform and the extract was washed several times with water and dried over Na_2SO_4. The solvent was removed by distillation and the residue was chromatographed over silica gel. The column was eluted with solvents of increasing polarity.

The first fraction eluted with petrol: benzene (2:3) furnished a compound, which on crystallization from $CHCl_3$-MeOH afforded solid **A** with m.p. 230^{0}C and molecular formula $C_{29}H_{45}O_5Br$.

The second fraction eluted with petrol: benzene (1:4) furnished a compound which on crystallization afforded solid **B** m.p. 252^{0}C and molecular formula $C_{29}H_{42}O_6Br_2$.

The third fraction eluted with benzene: ethyl acetate (2:3) furnished a compound which on crystallization afforded solid **C** m.p. 189^0 C and molecular formula $C_{27}H_{41}O_4$ Br.

The most polar fraction **D** eluted by chloroform: methanol (3:2) was difficult to crystallize. It was acetylated with pyridine-acetic anhydride and purified by chromatography and crystallized from chloroform-methanol. The acetate $D_{(a)}$ had m.p. 205-6^{0}C and the molecular formula $C_{33}H_{48}O_8Br_2$.



92

CHARACTARIZATION OF A

Compound **A** was analyzed for $C_{29}H_{45}O_5Br$, m.p 230^{0}C and $[\alpha]_D$ 52.6^0. The compound responded to Beilstein test for halogen. The presence of hydroxy group was indicated by a peak at 3350 cm^{-1} ***(fig.19)*** in the IR spectrum of the compound and peaks at 1710 and 1240 cm^{-1} indicated acetyl group. A negative TNM test proved the absence of double bond. John's oxidation of **A** gave back the starting material suggesting that the hydroxyl group is at the tertiary C-5 position. Attempt to acetylate **A** (Py-Ac_2O) regenerated diosgenin acetate in 100% yield suggesting that the two leaving groups (OH and Br) are in *trans* disposition.

The mass spectrum ***(fig.20)*** of compound **A** showed molecular ion peak at M^+ 554. The other peaks appeared at *m/z* 537, 525, 519, 455, 395, 377, 345, 298, 281, 267, 39 (base peak) 105, 95, 69 and 67. The peak at *m/z* 455 may be attributed to loss of one HBr from peak at *m/z* 537. The fragment at *m/z* 455 lost one AcOH to give a peak peak at *m/z* 395, *m/z* 377 resulted when the fragment at *m/z* 395 lost one water molecule. The base peak at *m/z* 139 may be due to formation of fragment 'e' as in **Scheme X**.

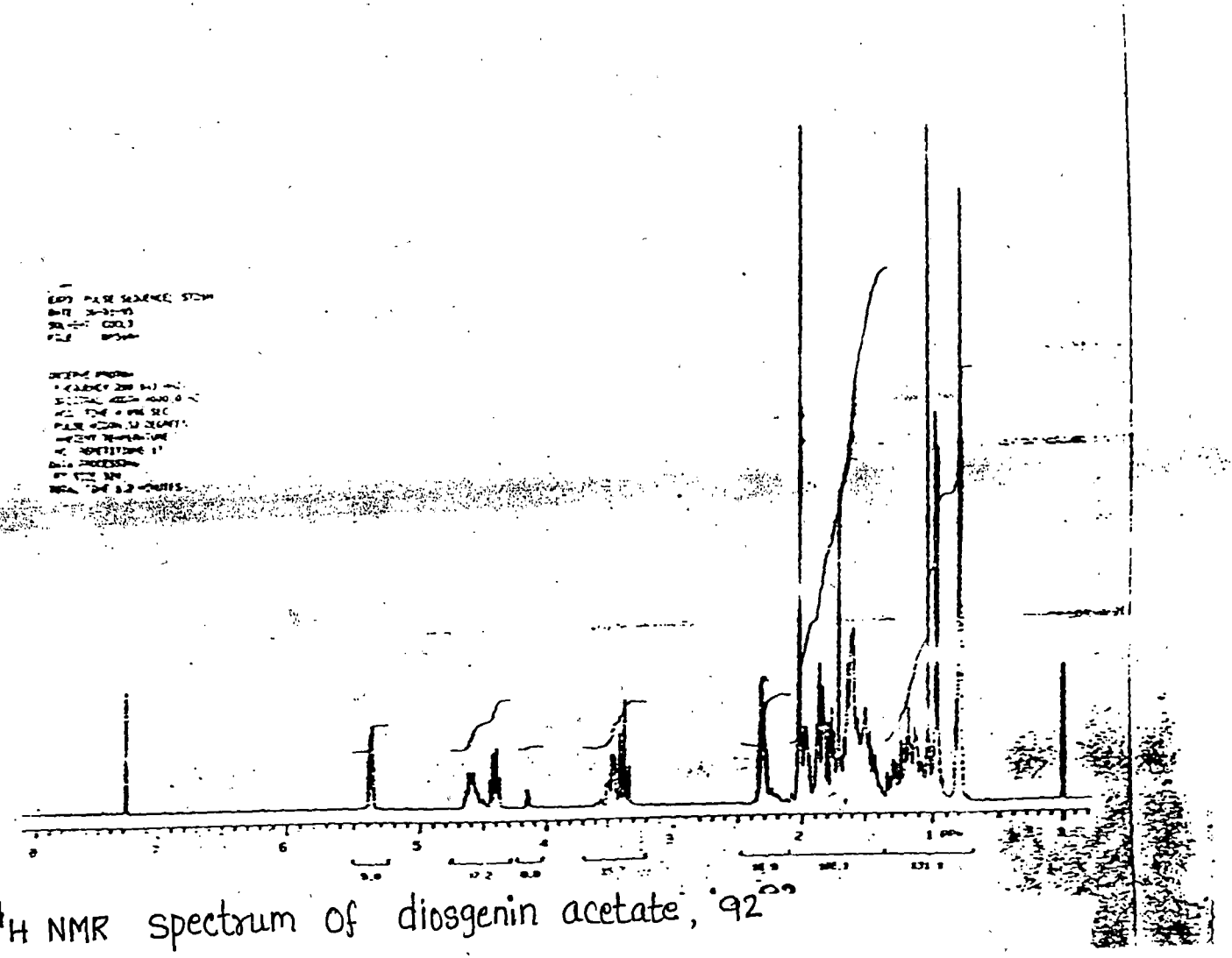


Fig 17A. ^1H NMR Spectrum of diosgenin acetate, '92

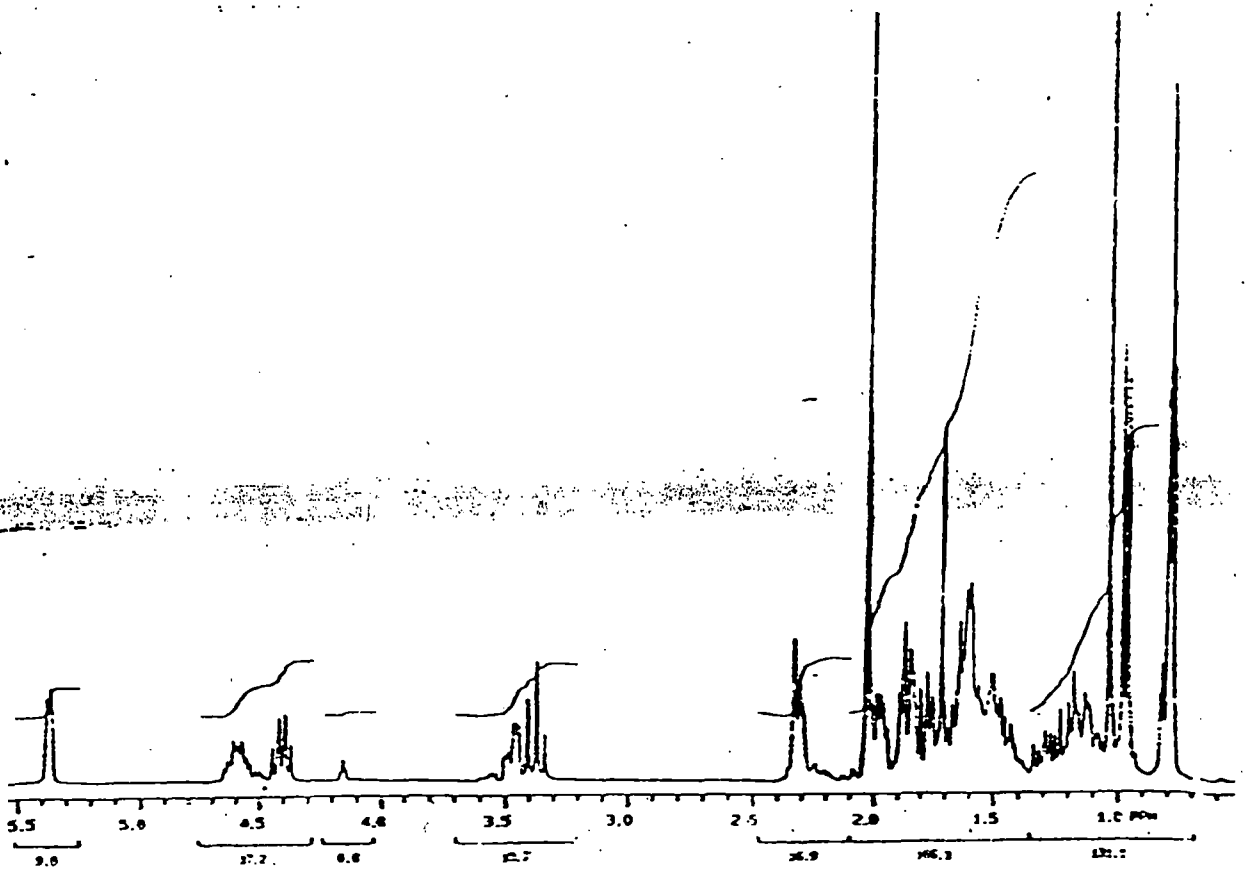


Fig 17B. ^1H NMR spectrum of diosgenin acetate, 92

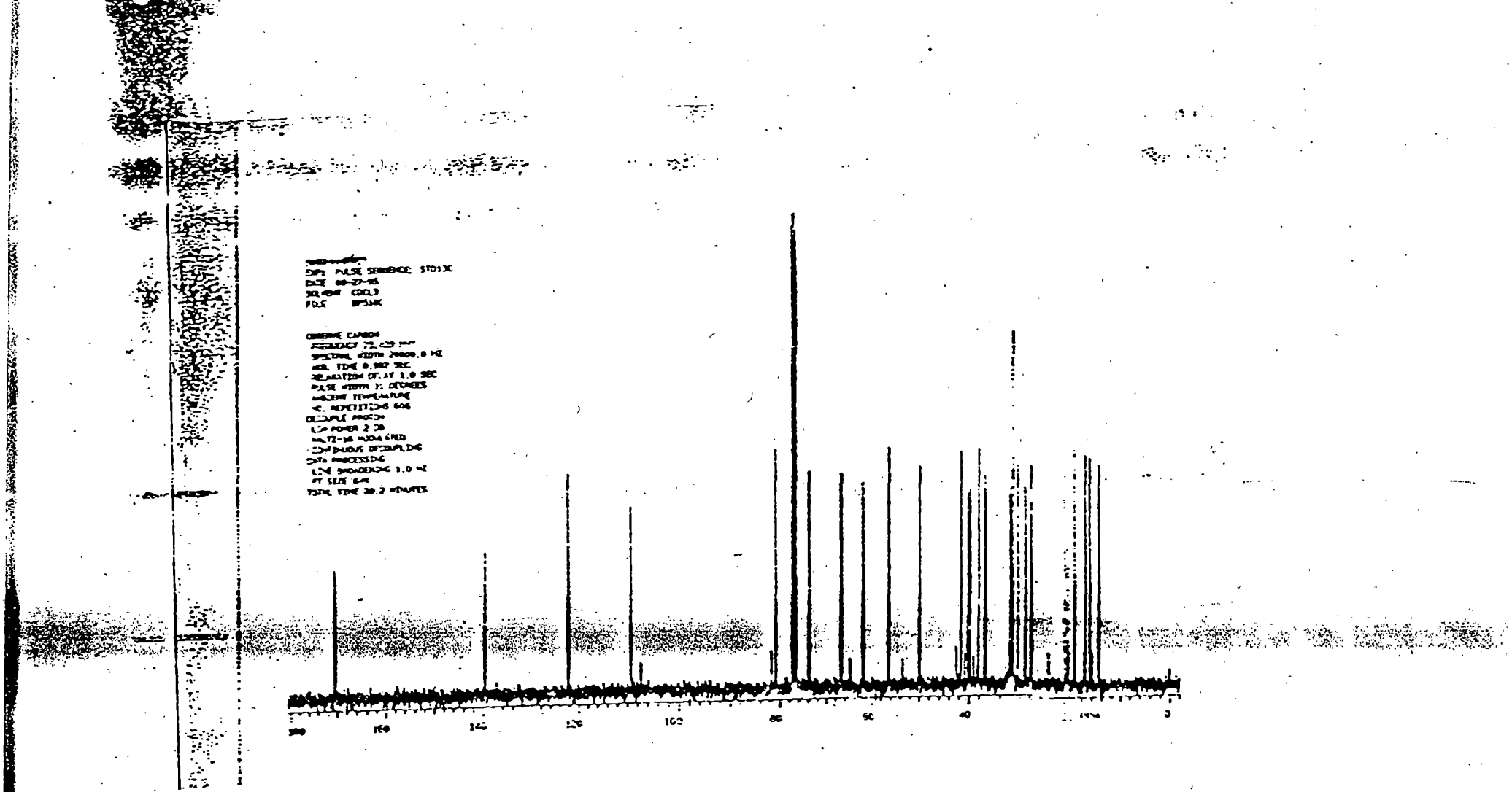


Fig 18. ^{13}C NMR spectrum of diosgenin acetate, 92

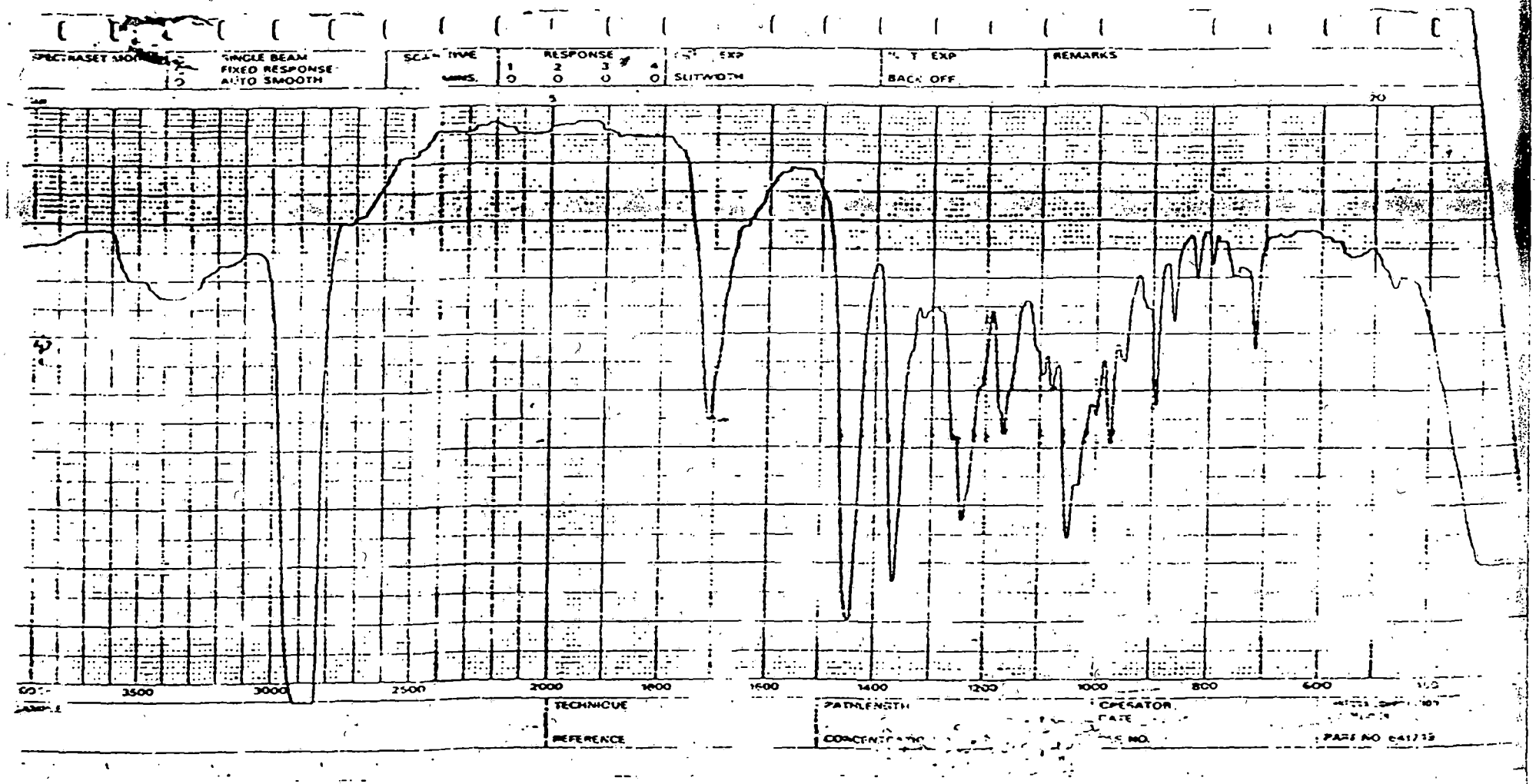


Fig. 19. IR spectrum of 6α-bromo 5β-hydroxy diosgenin 3β-acetate, 93

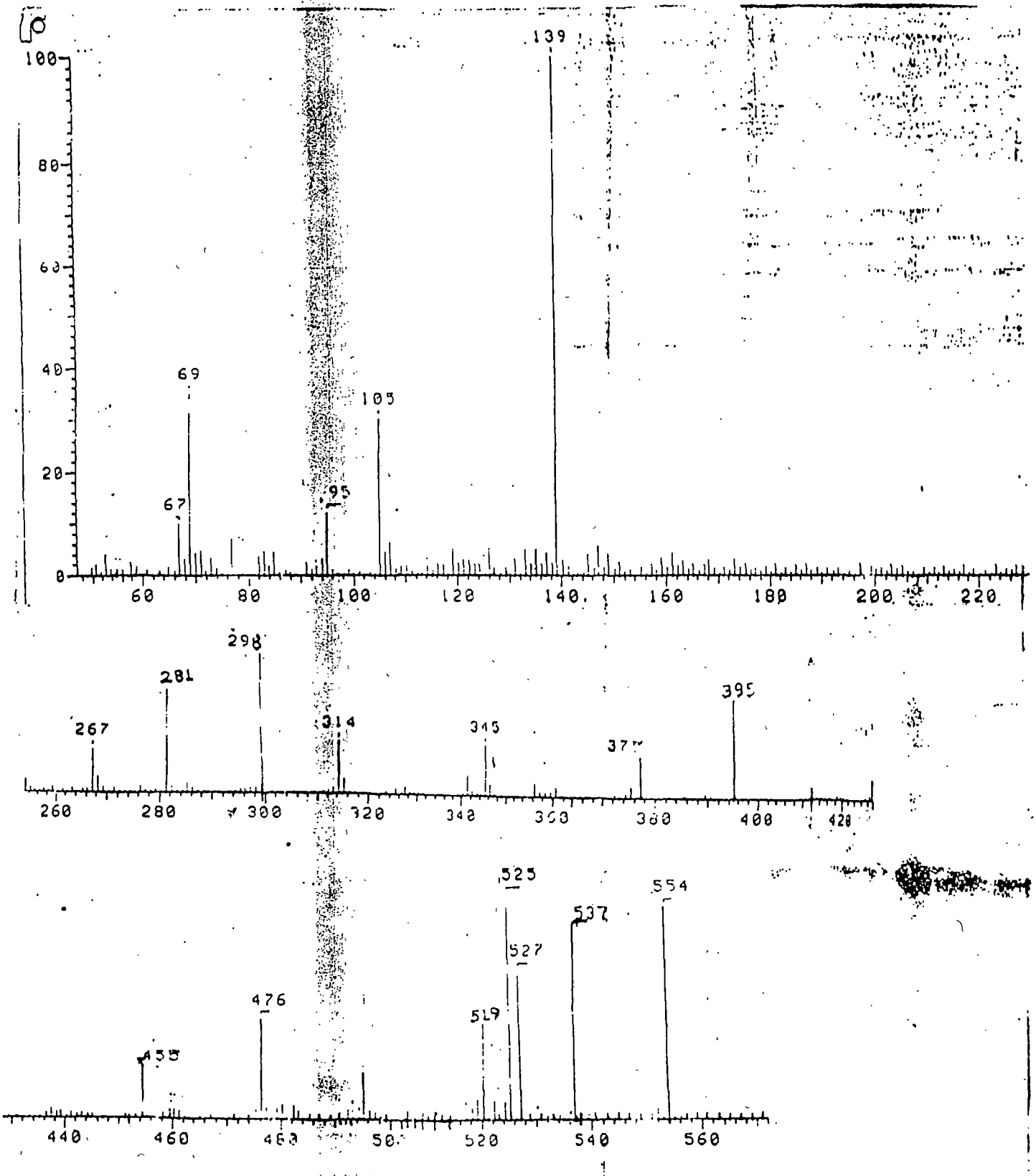


Fig 20. Mass spectrum of 6α-bromo-5β-hydroxy diosgenin 3β-acetate, 93

The structure of **A** was further studied by its ^{1}H NMR spectrum (***Fig.21A & 21B***). ^{1}H NMR of compound **A** contained two doublets centered at δ0.81 and 0.92 ppm, each pair integrated for three protons with same coupling constant (J= 6.5 Hz.) for two secondary methyls , two singlets at δ 0.72 and 1.0 ppm for two tertiary methyls and the singlets at δ 2.09ppm is probably due to the acetoxy methyl present in staring compound **92**, but the heptate due to the methine proton at C-3 has vanished in compound **A** and instead a singlet like one proton peak with width at half height of 5 Hz. appeared at δ 5.3ppm . One sharp singlet at δ 3.0 ppm was due to the hydroxy proton whereas a proton that appeared as doublet centered at δ 4.51 ppm with J value of 14 Hz. and 5Hz. must be due to an axially oriented proton having one axial and one equatorial neighboring proton. Considering that the axial proton is C-6 proton the bromine atom that may cause sufficient downfield shift should be equatorially oriented, but stereo chemistry of hydroxy group attached at C-5 position could be either above or below the plane. The singlet like peak at δ 5.3 ppm if considered to be due to C-3 methine proton suggests that acetoxy group is axially oriented in **A**. Such a change in stereochemistry at C-3 position could be possible only if A/B ring juncture is assumed to be *cis* fused .

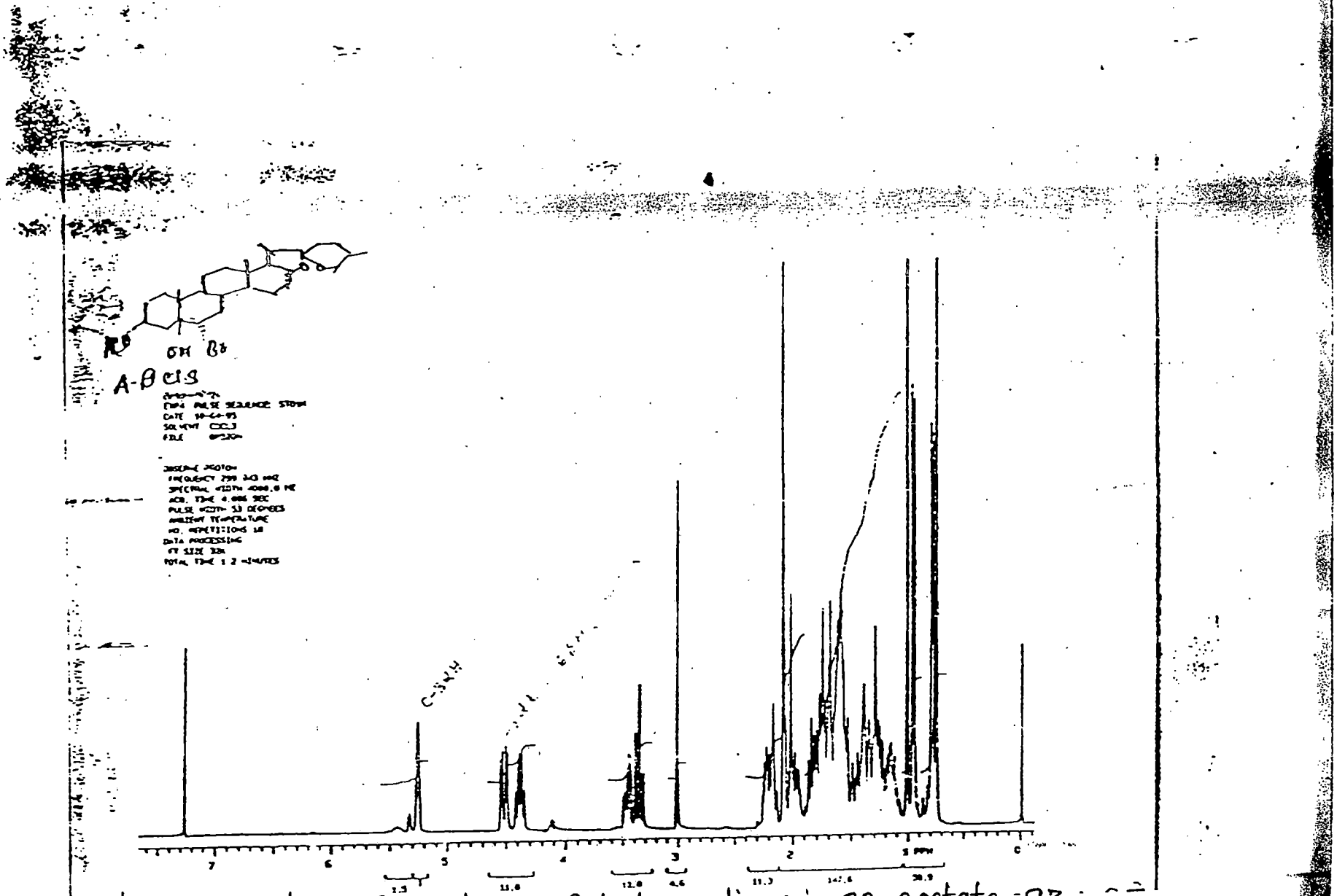


Fig 2 A. ^1H NMR spectrum of 6α-bromo 5β-hydroxy diosgenin 3β-acetate, 93

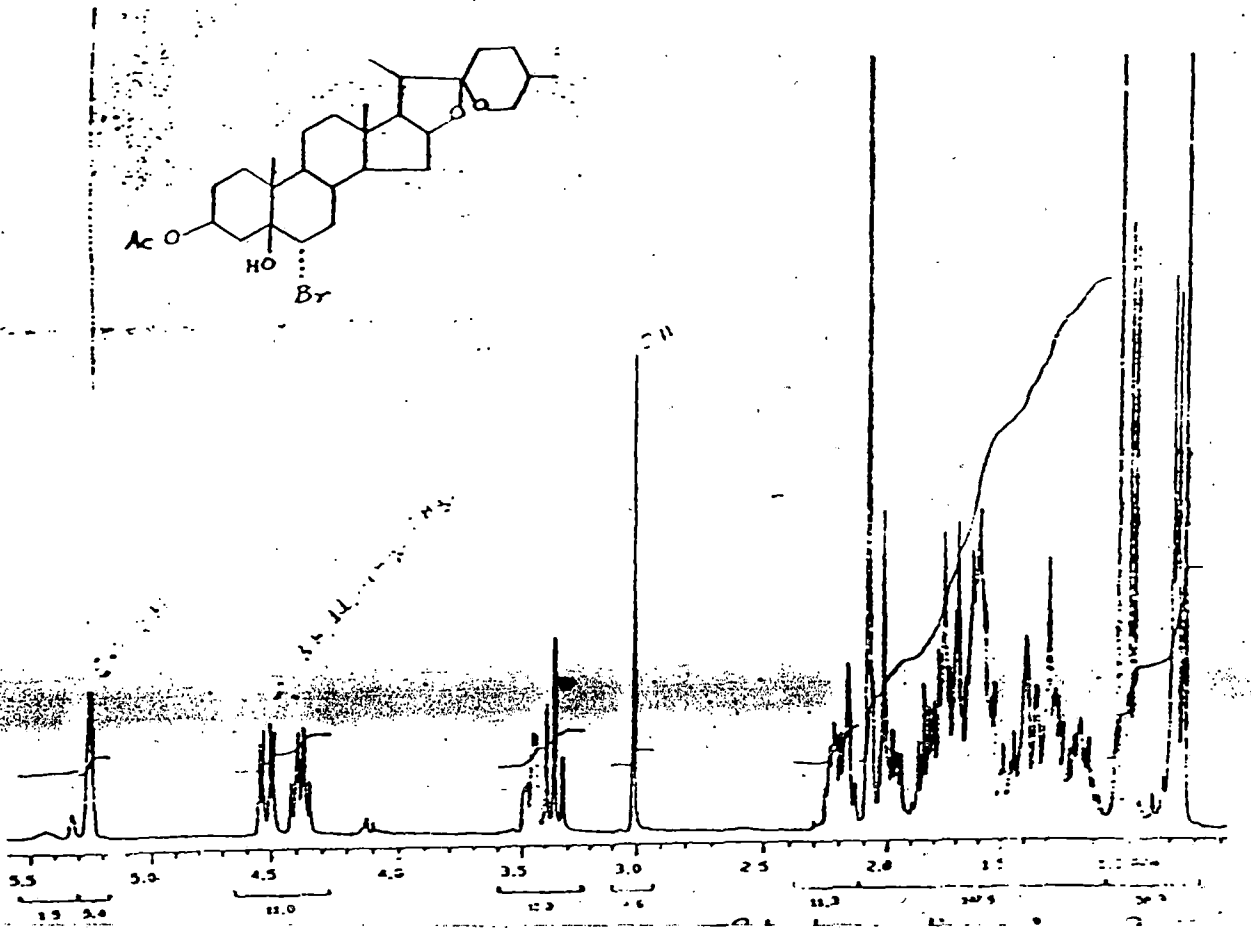
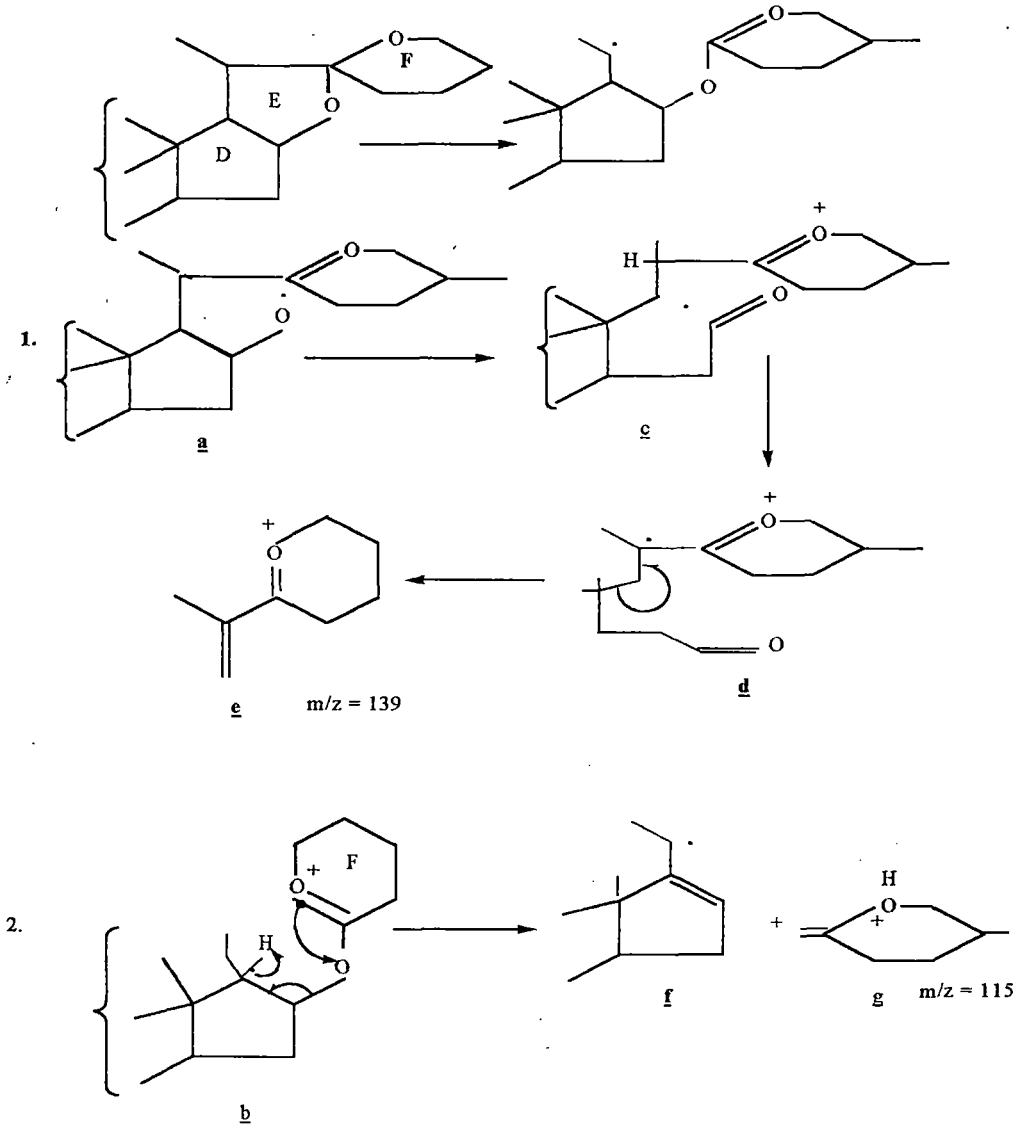


Fig 21B. ^1H NMR spectrum of 6α-bromo 5β-hydroxy diosgenin 3β-acetate. 93

^{13}C NMR spectrum (***Fig. 22A & 22B***) contained 29 resonance peaks and showed presence of 5-CH_3 carbons, 10-CH_2 carbons, 9-CH carbons as quartets, triplets, doublets and the rest as singlets respectively. A comparison of ^{13}C NMR data of **A** with that of diosgenin acetate and 6α-bromo 5β-hydroxy coprostane 3β-yl acetate is given in **Table III**.

Scheme X



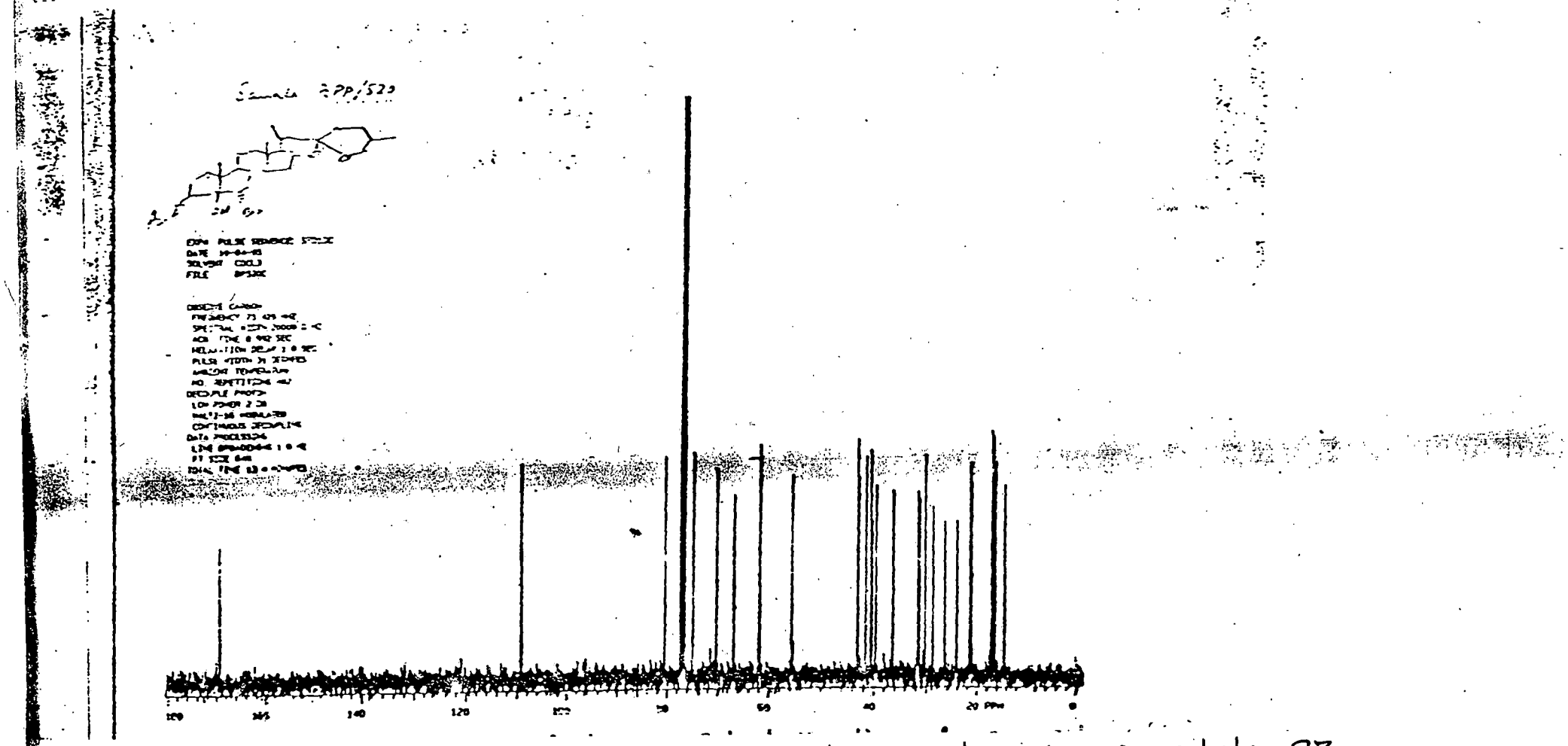


Fig 22A. ^{13}C NMR Spectrum of 6α-bromo 5β-hydroxy diosgenin 3β-acetate, 93

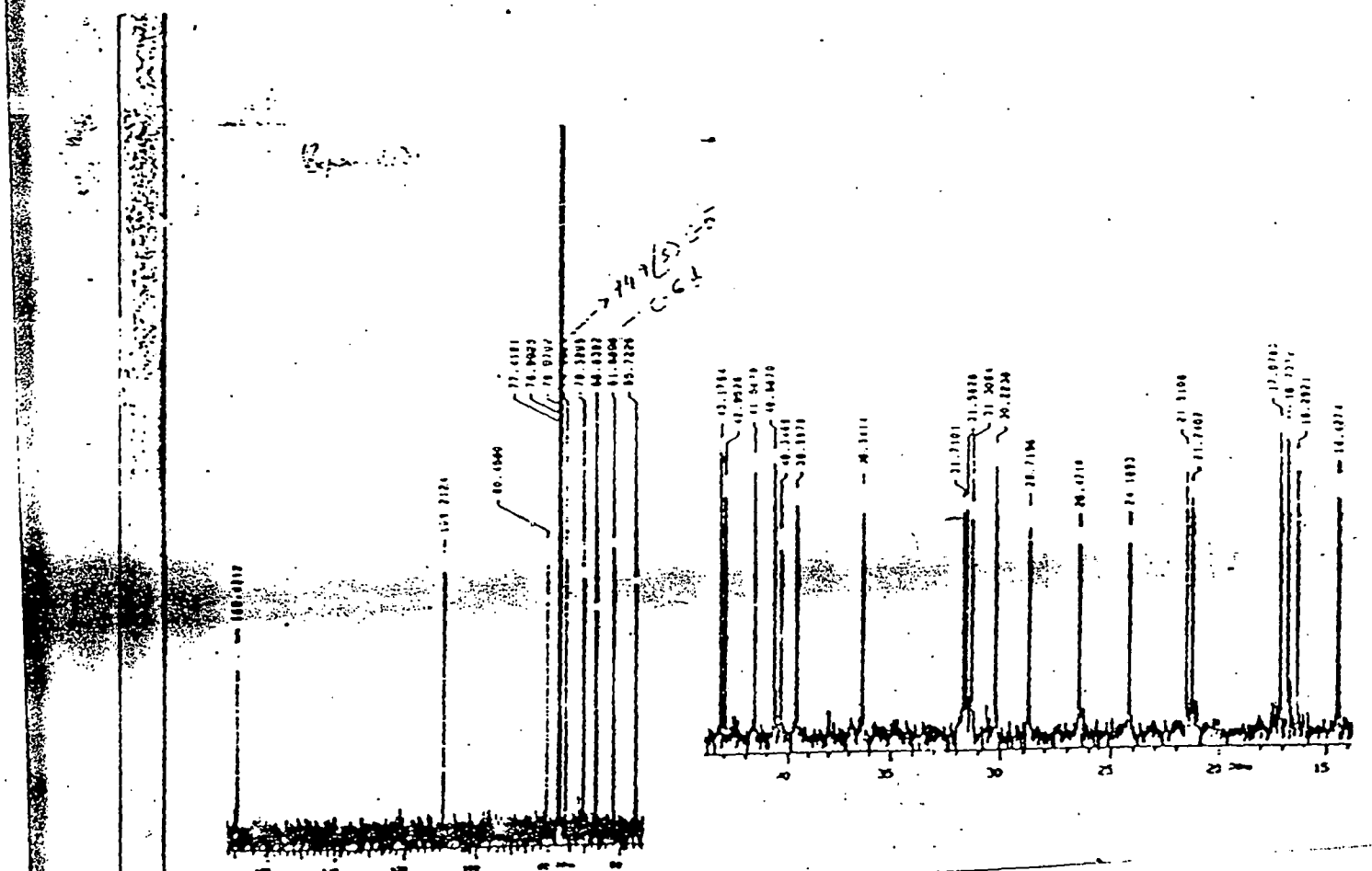
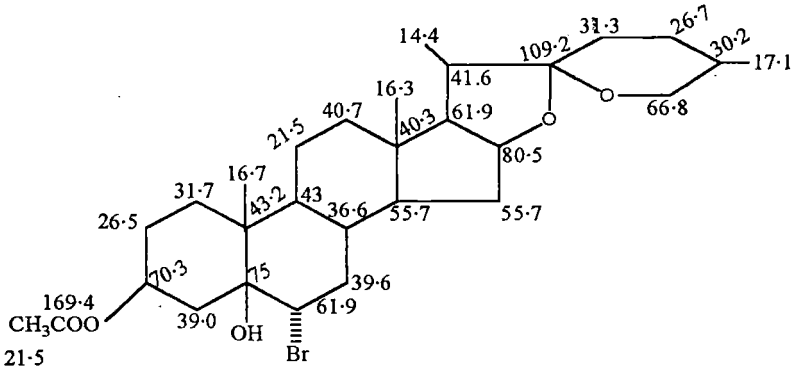


Fig 22B. ^{13}C NMR spectrum of 6α-bromo 5β-hydroxy diosgenin 3β-acetate, 93

Table III

Carbon	Diosgenin acetate	Compound no. 93	6 bromo -3 hydroxy coprostane 3 β-yl acetate
1	36.9	31.7	31.8
2.	31.4	26.5	26.6
3.	73.8	70.3	70.5
4.	41.5	39.0	39.5
5.	139.7	75	75.1
6.	122.3	61.9	62.4
7.	32	39.6	39.5
8.	31.4	36.6	36.8
9.	50	43	42.9
10.	36.7	43.2	43.2
11.	20.8	21.5	21.6
12.	39.7	40.7	40.4
13.	40.2	40.3	42.7
14.	58.4	55.7	56.1
15.	56.4	55.7	24.0
16.	80.7	80.5	28.1
17.	62.1	61.9	56.1
18.	16.3	16.3	12.0
19.	19.3	16.7	16.7
20.	41	41.6	35.7
21.	14.5	14.4	18.6
22.	109.2	109.2	36.1
23.	31.4	31.3	23.8
24.	28.8	26.7	39.7
25.	20.3	30.2	28.0
26.	66.8	66.8	22.6
27.	17.1	17.1	22.8
-OCOCH$_3$	21.5	21.5	21.4
-OCOCH$_3$	170.5	169.4	171.0

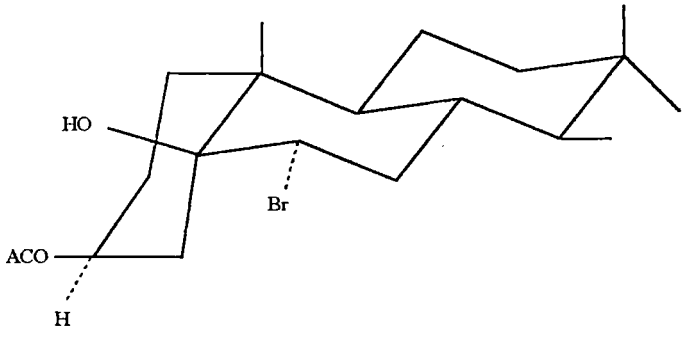
Thus from spectral analysis **A** was designated as 6α-bromo 5β-hydroxy diosgenin 3β- acetate and was assigned structure **93**.



93

6α-bromo 5β-hydroxy diosgenin 3β- acetate.

The conformational structure of **93** may be drawn as shown below.



93

CHARACTERISATION OF B

Compound **B** was analyzed for $C_{29}H_{42}O_6Br_2$ m.p 220^{0}C. Compound **B** responded to positive test for halogen. The IR spectrum of Compound **B** (***fig. 23***) showed peaks at 3350 cm^{-1} for hydroxyl 1700 cm^{-1} for six membered ketone and 1710 cm^{-1} and 1240 cm^{-1} for acetate carbonyl group, thus accounting for all the six oxygen atoms.

The mass spectrum of compound **B** (***fig. 24***) showed maximum peak at *m/z* 630 which showed that there is loss of water molecule from the molecular ion M^+. The other peaks appeared at *m/z* 615,568,566,531,488,473,458,374,341,296, 139(base peak) 115,69,55. The peak at *m/z* 615 is due to the loss of methyl group from m/z 630. The peak at *m/z* 506 is due to loss of AcOH from fragment at *m/z* 566. The peak at *m/z* 488 resulted when fragment at *m/z* 506 lost one water molecule. The peak at *m/z* 473 may be attributed to loss of methyl group from *m/z* 488. The peak at *m/z* 139(base peak) is due to fragmentation of a to e and that at *m/z* 115 is due to fragmentation of b to g (**Scheme X**). The peak at *m/z* 341 is due to the loss of fragment e from m/z 488 and the peak at *m/z* 374 is due to loss of fragment g from *m/z* 488. The fragmentation pattern of compound **B** is similar to those of previously reported diosgenin derivatives (**Scheme XI a**).

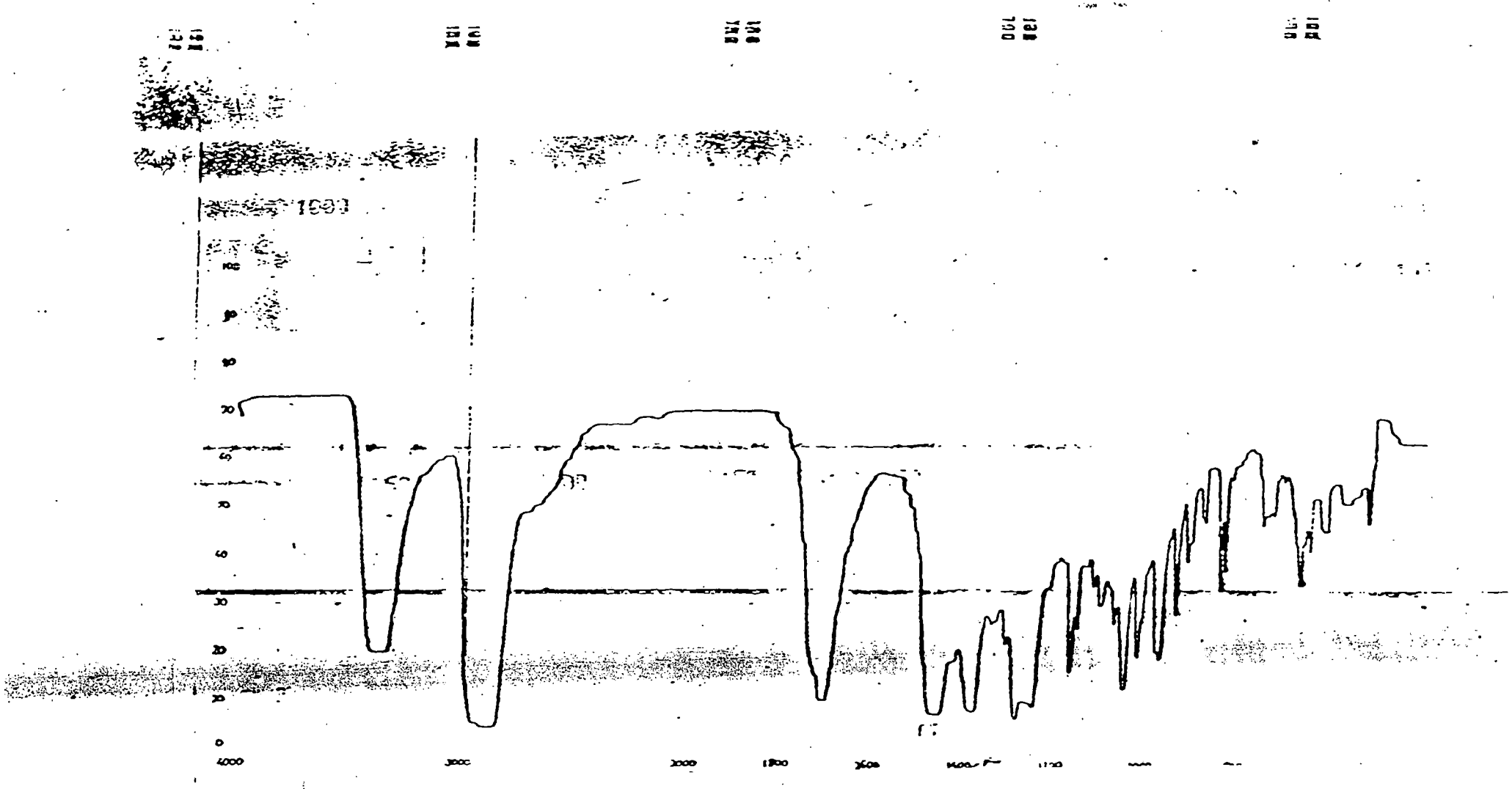


Fig 23. IR spectrum of 7,7-dibromo-5α-hydroxy 6-keto diosgenin 3β-acetate, 94

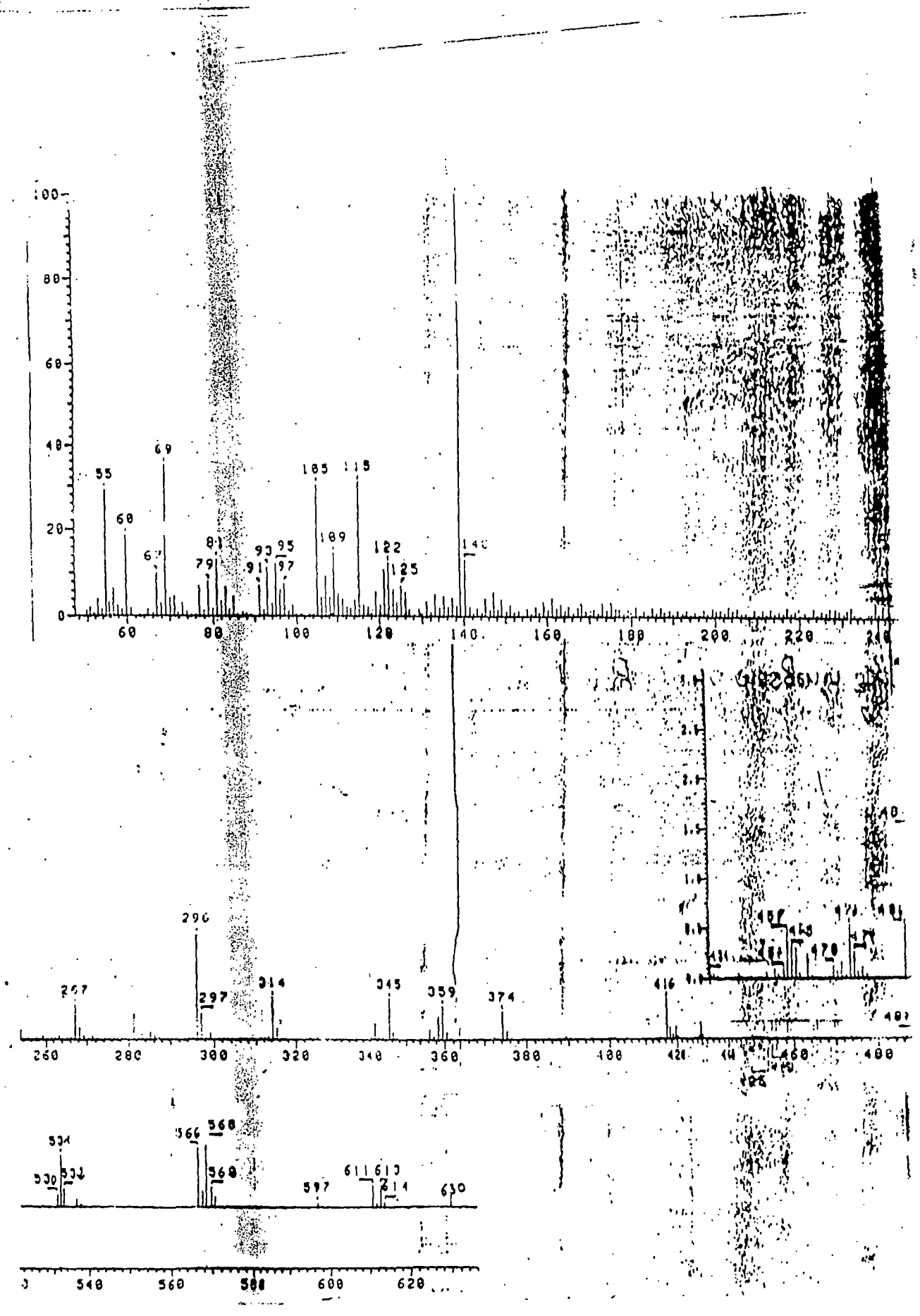
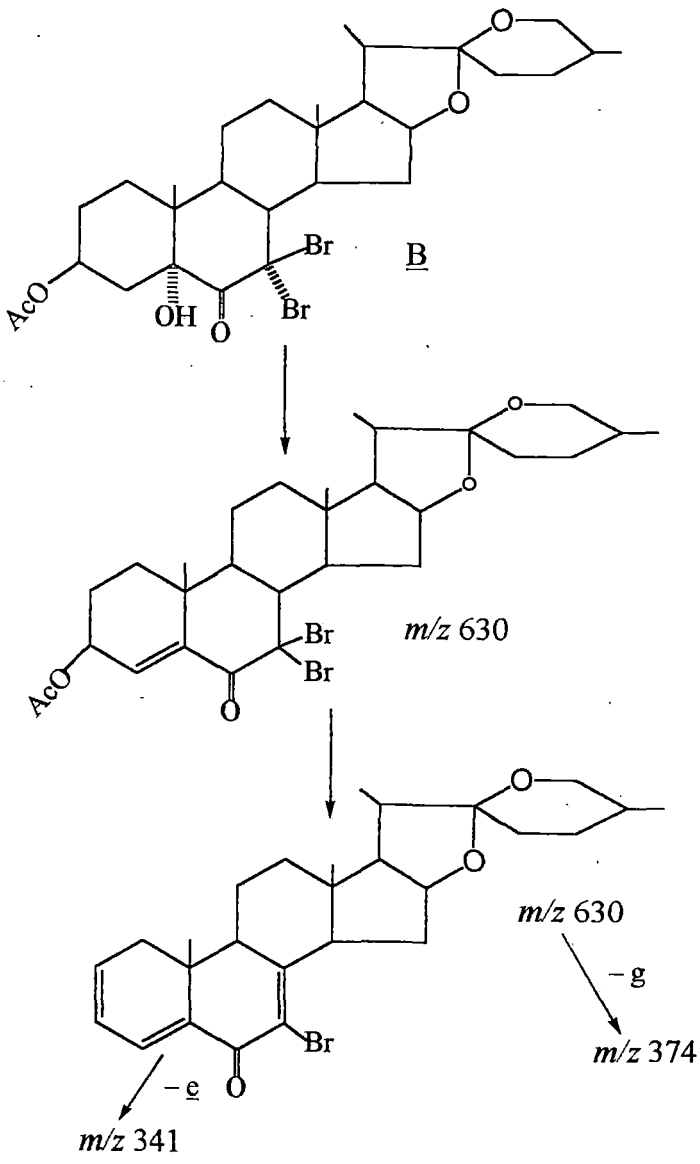


Fig 24. Mass spectrum of 7,7-dibromo .5α-hydroxy 6-keto diosgenin 3β-acetate, 94

Scheme XIa

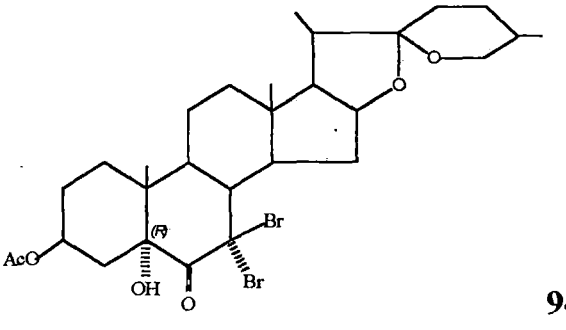
Fragmentation of B



^{1}H NMR spectrum ***(fig. 25 A and fig. 25 B)*** of compound **B** showed the presence of a triplet at δ 3.5ppm. due to equatorial hydrogen at C-26. A quartet at δ 4.41ppm. was attributed to axial hydrogen at C-26. The spectra showed a multiplet at δ 3.47 ppm. which could be due to presence of axial hydrogen at C-16 position. A triplet at δ 2.76ppm. was the result of axial hydrogen at C-8 position, a heptet at δ 5.03 ppm. indicated the presence of axial hydrogen at C-3.A sharp singlet at δ 2.01ppm. showed the presence of acetoxy methyl protons. A one-proton singlet at δ 2.27ppm. was due to a tertiary hydroxy group that was supported by IR spectrum ***(fig. 23).***

The tertiary nature of the hydroxy group was confirmed by its refusal to undergo oxidation with Cr O_3 – Py as well as acetylation with Py – Ac_2O that gave back **B**.

Thus from the spectral analysis compound **B** was designated as 7,7 dibromo - 5α-hydroxy- 6 keto diosgenin-3β acetate and was assigned structure **94**.



94

7,7 dibromo 5α-hydroxy-6-keto diosgenin-3β acetate.

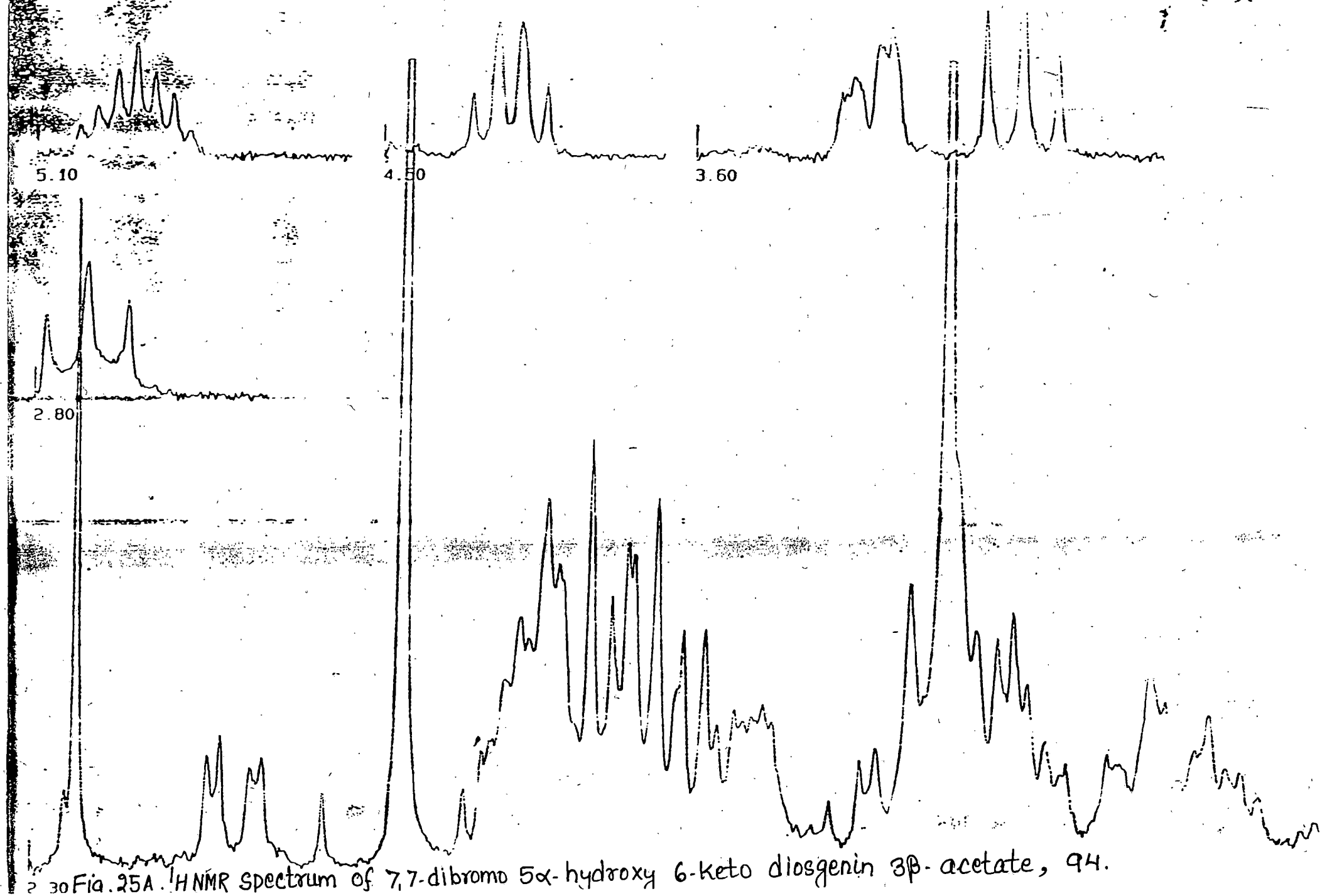


Fig. 25A. ^1H NMR spectrum of 7,7-dibromo 5α-hydroxy 6-keto diosgenin 3β-acetate, 94.

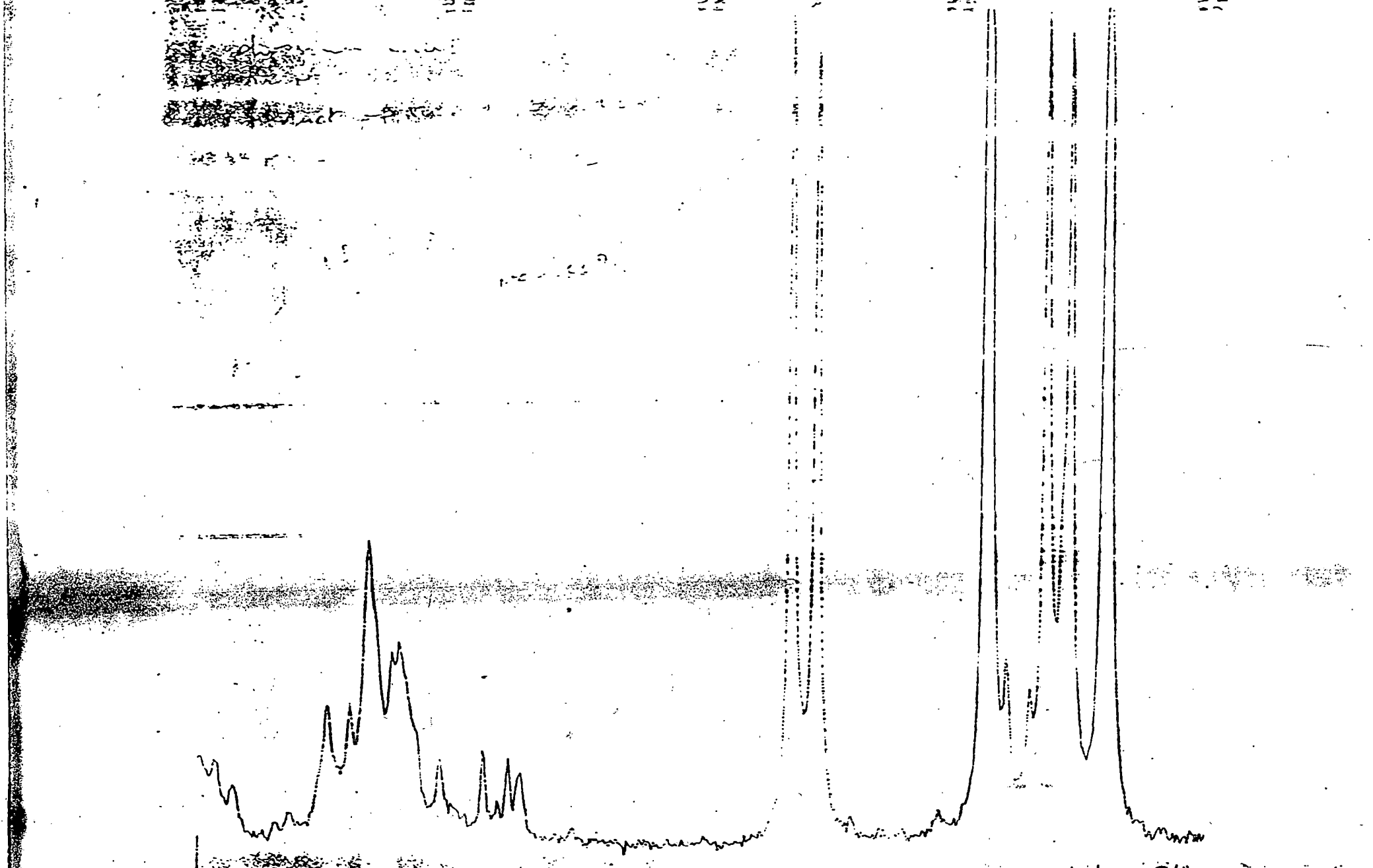


Fig 25B. ^1H NMR spectrum of 7,7-dibromo, 5α-hydroxy 6-keto diosgenin 3β-acetate, 94

Characterization of C

Compound **C** was analyzed for $C_{27}H_{41}O_4Br$, m.p. 188^{0}C . The compound gave positive Beilstein test for halogen. The IR spectrum of the compound showed a broad peak at 3400-3650cm^{-1} indicating the presence of hydroxy group. The absorption at 1705 cm^{-1} indicated the existence of a six membered ring ketone in **C** . The UV spectra of the compound **C** showed no remarkable absorption peak in the region 220 to 300 nm. which suggest the absence of α,β-unsaturated carbonyl group.

The mass spectrum (***fig.26***) of compound **C** showed molecular ion peak at M^+509. The other peaks appeared at *m/z* 415, 413, 402 400, 371, 359, 316, 287,140, 139 (base peak). The peak that appeared at *m/z* 430 is attributed to loss of Br atom from peak at M^+ 509. The peak at *m/z* 400 is due to loss of CH_3 from *m/z* 415.Another peak appeared at *m/z* 371 which may be attributed to the loss of CH_3CH_2 from *m/z* 400. The base peak appeared at *m/z* 139 which is due to formation of fragment e as in **Scheme X**.

^{1}H NMR spectrum of compound **C** (***fig.27***) showed the presence of the two doublets at δ 0.77 and 0.95 ppm. with J value of 6.5 Hz which could be due to secondary methyl protons at C-20 and C-25 that couples with α-hydrogen . The peak at δ 3.76ppm. that appeared as a broad hump with coupling at half height of 10 Hz is due to 3α-H germinal to hydroxyl grouping.The quartets at δ 4.46 ppm and 3.37 ppm coupled with each other and are due to axial hydrogen and equatorial hydrogen at C-26. The double doublets at δ 2.69 ppm. and 2.73 ppm. with coupling constants of 10 and 3Hz indicated that the protons at C-7 axial and equatorial are coupled to each other and with C-8 proton.

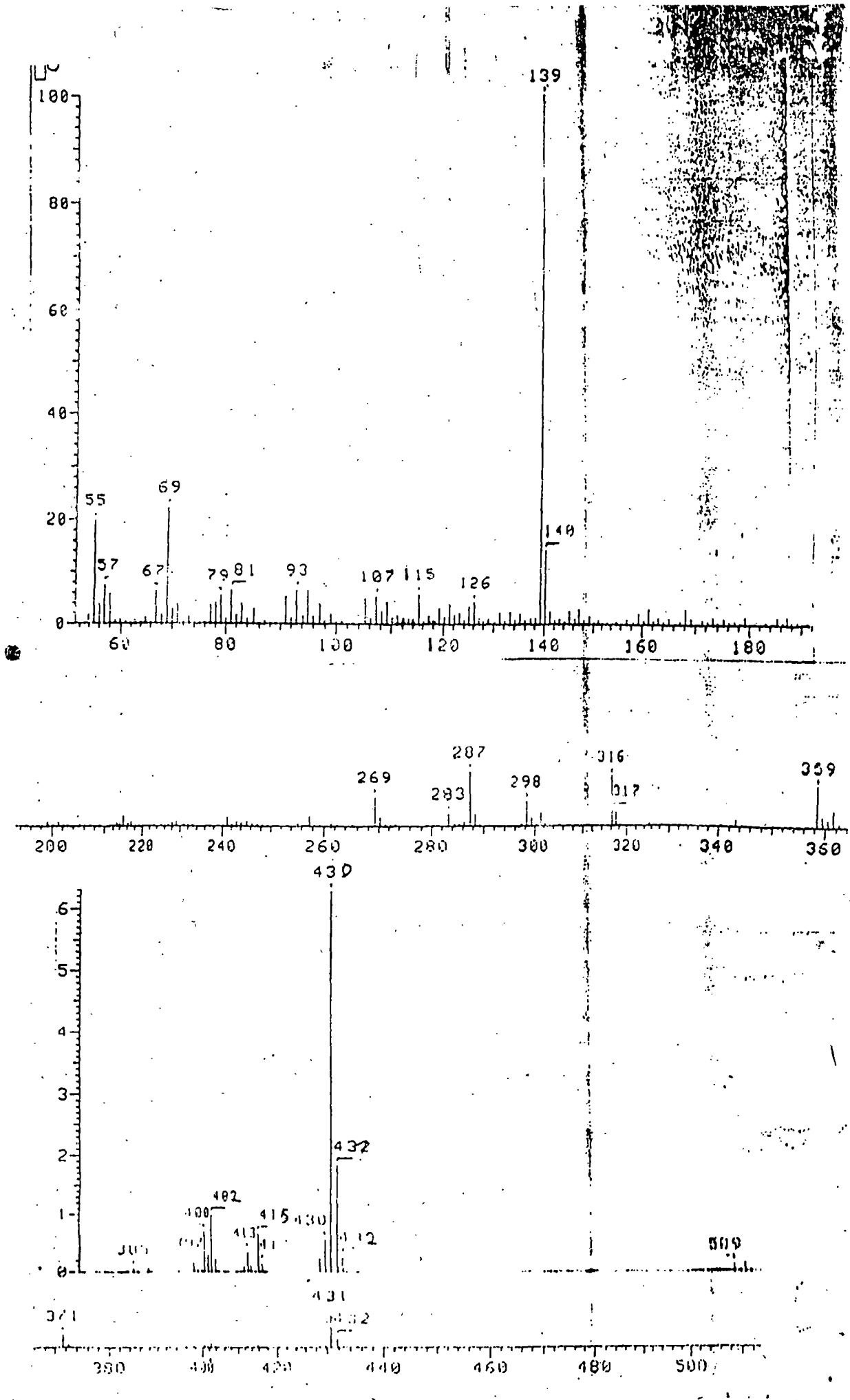


Fig 26. Mass spectrum of diosgenin 5α-bromo 3β-hydroxy-6-one, 95

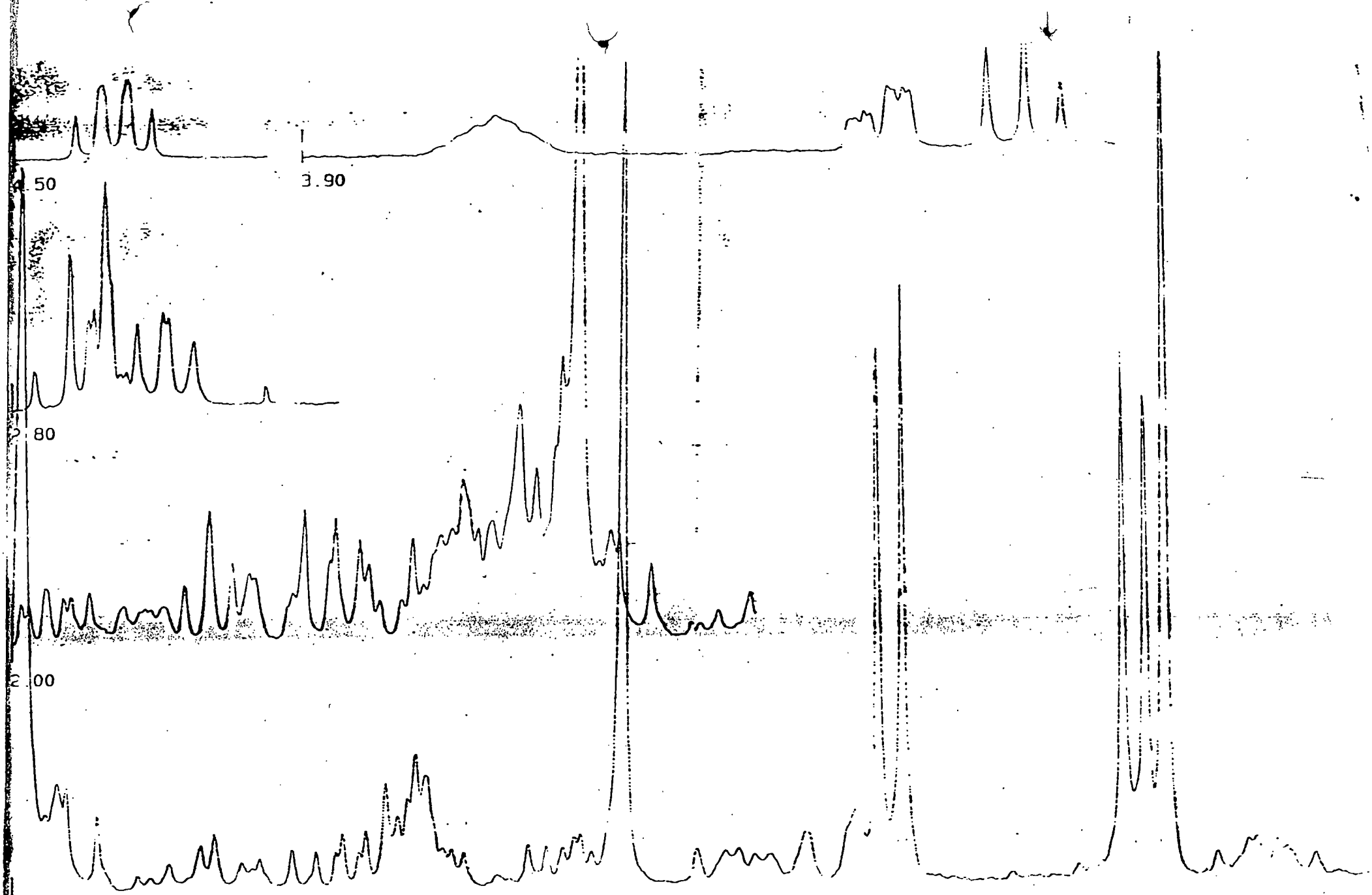
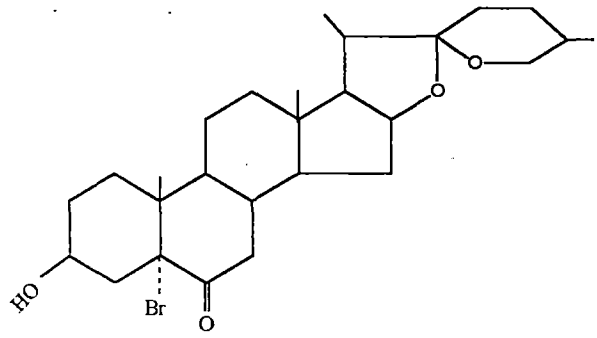


Fig 27: ^{1}HNMR spectrum of diosgenin 5α-bromo 3β-hydroxy-6-one, 95

Acetylation of **C** with acetic anhydride-pyridine furnished a solid of m.p. 232-33°. It was analyzed for $C_{30}H_{43}O_5Br$. It showed the presence of acetoxyl carbonyl group at 1725 and 1240 cm^{-1} and six membered ring ketone at 1705cm^{-1} .

Thus from spectral analysis compound **C** was designated as diosgenin-5α-bromo-3β-hydroxy-6-one was assigned structure **95**.



95

Diosgenin- 5α-bromo-3β- hydroxy-6-one.

Characterization of compound Da

The polar compound **D** that was acetylated to **Da** was analyzed for $C_{33}H_{48}O_8Br_2$ m.p. 205 to 206^{0}C. The compound gave a positive Beilstein test indicating the presence of bromine in the compound. IR spectra of the compound showed peaks at 1710, 1230cm^{-1} indicating the presence of acetoxy group. The ^{1}H NMR spectra of compound $\mathbf{D_a}$ was very instructive in elucidating its structure . ^{1}H NMR spectrum of the compound ***(fig.28)*** showed three sharp singlets at δ 2.08,2.095 and 2.11ppm. each one integrated for three protons indicating the presence of three acetate group in the compound. The heptet at δ 5.45ppm. showed the presence of 3α- proton as axial showing that A/B ring juncture is *trans* fused in **Da**. A singlet at δ 3.07ppm. is due to a proton that is attached to carbon containing electron withdrawing group such as Br or acetate.

The mass spectrum of compound **Da** ***(fig 29)*** showed the presence of the peak at *m/z* 674(M^+734-AcOH).Other peaks appeared at *m/z* 668,,659,671, 576,674,598,578,576, 572, 556, 554, 537, 535, 518, 476, 456, 454, 412,(base peak), 397, 291 ,282, 253, 139, 115, 69. The peak at *m/z* 614 is due to loss of AcOH from *m/z* 674 and that at *m/z* 572 is due to loss of CH_3CO from m/z 614. The peak at m/z 659 may be attributed to loss of CH_3 from *m/z* 674 and that at *m/z* 554 is due to loss of CH_3 COOH from *m/z* 614. The loss of HBr from *m/z* 658 resulted in appearance of peak at *m/z* 576 which lost AcOH to give another peak at *m/z* 516. The fragment at *m/z* 658 lost one bromine to give peak at *m/z* 578 which on losing one CH_3COOH gave peak at *m/z* 518. The fragment at *m/z* 518 lost one CH_2CO to give peak at *m/z* 476. The fragment at *m/z* 454 resulted when fragment at *m/z* 614 lost two bromine atoms. The base peak at *m/z* 412 resulted when *m/z* 454 lost CH_2CO . The base peak at *m/z* 412 lost one CH_3 to give a peak at *m/z* 397. The peak at *m/z* 139 and 115 were possibly due to the formation of fragments e and g as in **Scheme X**. The fragmentation pattern of **Da** is shown in **Scheme XIb**.

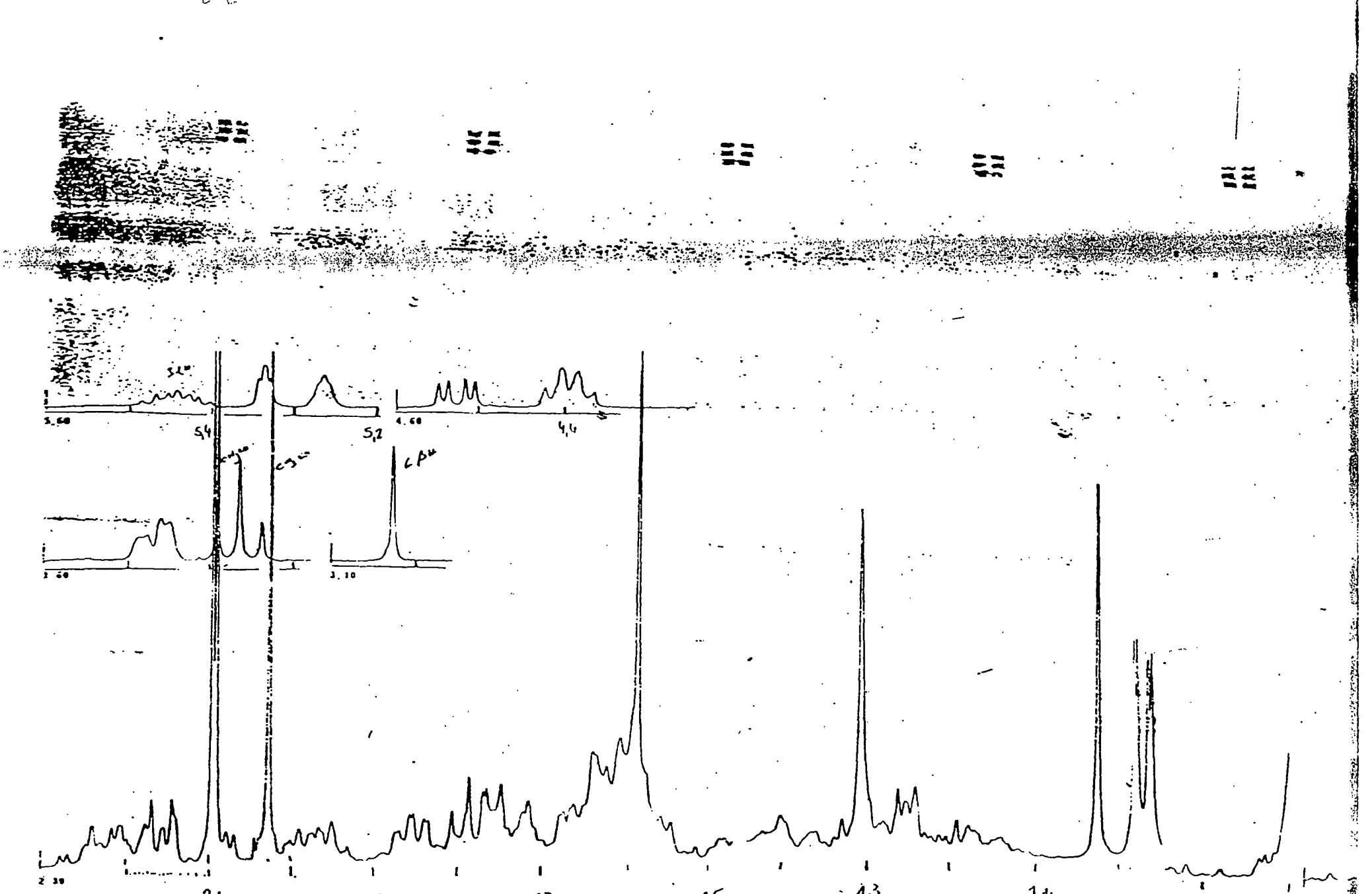


Fig 28. ^1H NMR spectrum of diosgenin 7,7-dibromo 3β, 5α, 6β-triacetate, 96

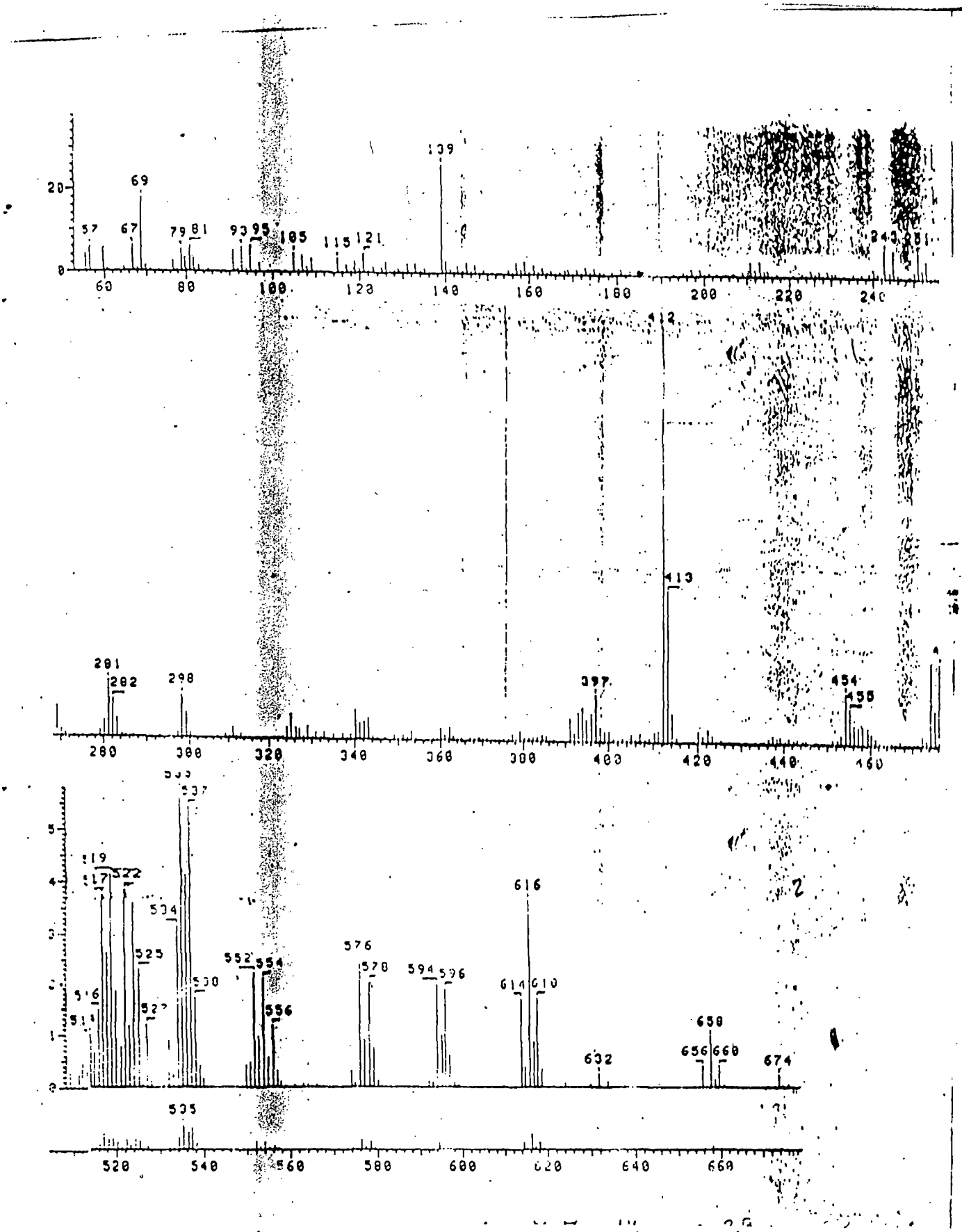
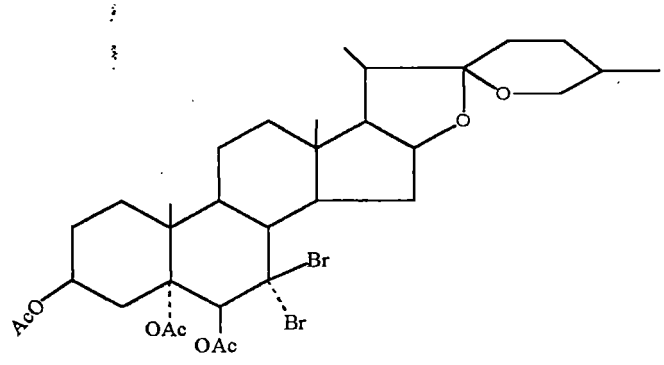


Fig 29. Mass spectrum of diosgenin 7,7-dibromo, 3β, 5α, 6β triacetate, 96

Thus, from the spectral analysis compound $\mathbf{D_a}$ was designated as diosgenin 7,7dibromo 3β,5α,6β-triacetate and was assigned structure **96**.

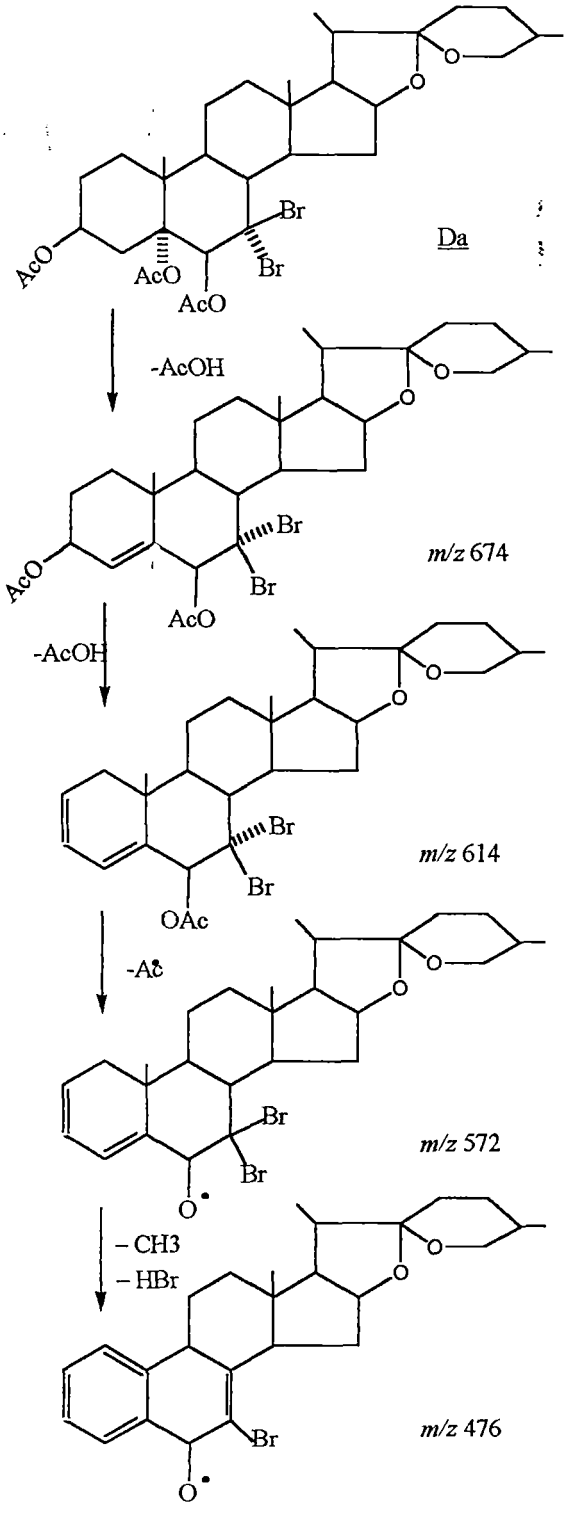


96

Diosgenin- 7, 7 dibromo-3β, 5α, 6β - triacetate

Scheme XI (b)

Fragmentation of Da



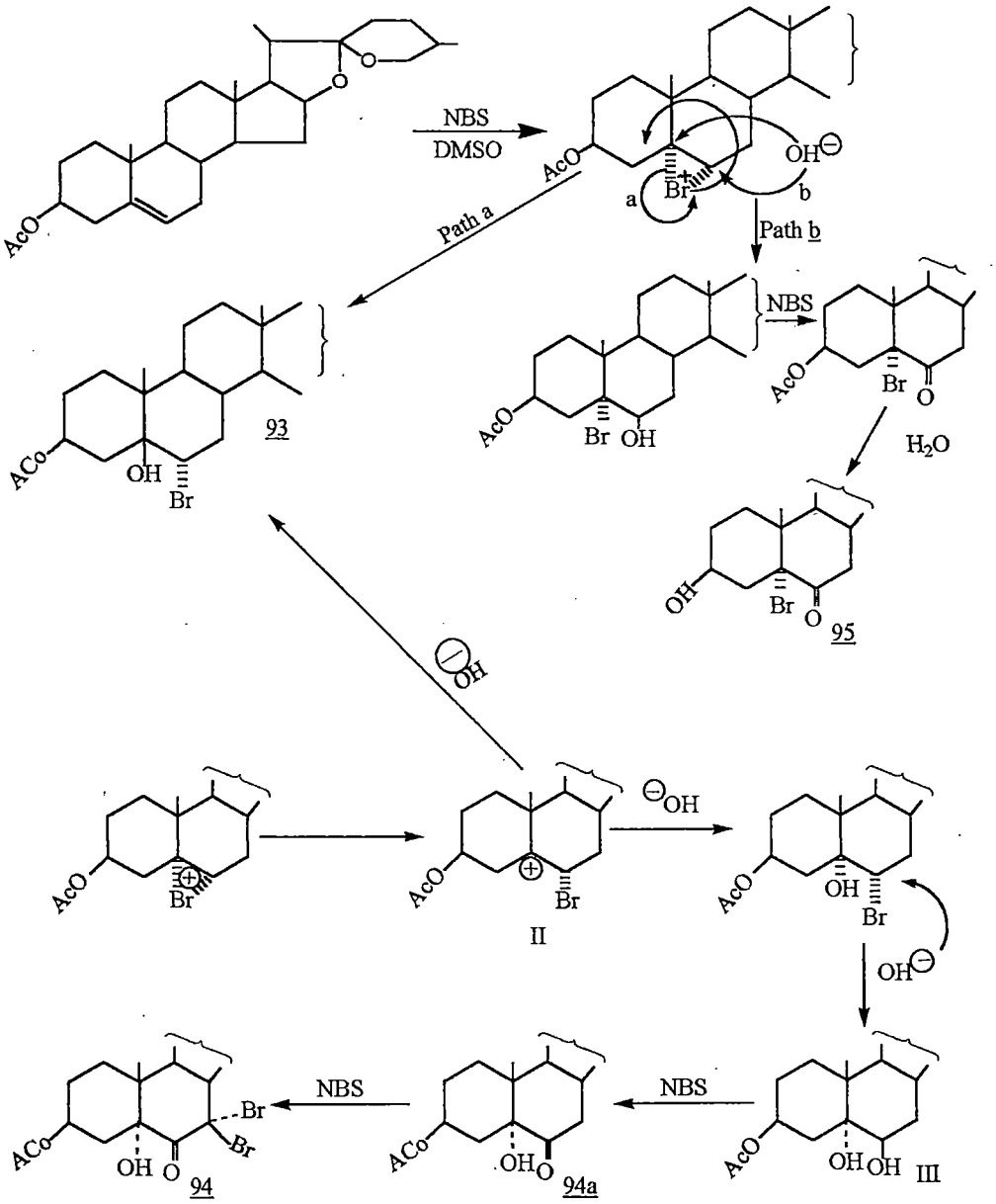
Mechanism for the formation of 93-96

The diosgenin acetate **92** was expected to behave in a typical manner with N-bromosuccinimide in dimethyl sulfoxide and was expected to afford compound similar to that of cholesteroyl acetate **45**. Though **92** yielded compound **93** in the highest percentage (80%) the formation of the other two compounds **94** and **96** needs to follow some other route than observed in case of cholesteroyl acetate **45**. In this later case no bromination product was formed that contain bromine at C-7 carbon, thus showing that allylic bromination is practically absent in cholesteroyl acetate. The following hypothetical route is suggested for the formation of **94** and **96**.

N-bromosccinimide in dimethyl sulfoxide forms brominium ion that attacks the olefinic double bond to form the cation **I** which may undergo nucleophilic attack by the hydroxyl ion either to furnish **93** or $\mathbf{I_a}$. Under the reaction condition $\mathbf{I_a}$ probably affords **95** by oxidation with excess NBS followed by hydrolysis of 3-acetoxyl group. The cation **I** could also rearrange to form the carbocation at C-5 that would subsequently undergo nucleophilic hydrolysis at C-6 to yield the *trans*–diol **III** which with excess NBS furnishes the oxidized product **94a** that finally gives rise to the formation of dibromide as observed in the previous cases. The formation of **93, 94** and **95** are represented in the Scheme **XIIa** whereas that of **96**(and also **94**) is represented in **Scheme XII(b)**. The main step in **XII(b)** is allylic bromination of the C-5(6) olefinic double bond which initially occurs before the bromonium ion attacks to offer the 7,7-dibromide. This is followed by brominium ion attack of the olefinic bond that subsequently undergoes hydrolysis to afford **96a** or get oxidized to **94**.

Scheme XII (a)

Mechanism for the formation of **93**, **94** and **95**.



Scheme XII (b)

Mechanism for the formation of compound **94** and **96a.**