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Spectral Studies of Steroids Using Two-Dimensional NMR Spectroscopy

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Abstract: *During our four decades in the field of steroids research we have faced time and again various stereochemical problems while interpreting spectral data of the synthesized compounds. 2D-NMR spectroscopy now has given a new spectral technique to overcome the conformational uncertainty and justified the assignments of the spectral data. While writing this chapter we are fully aware of the fact that avoiding mathematical and other difficult parameter we are trying to study 2D-NMR spectroscopy taking the examples of steroidal compounds. For this purpose, we have selected steroidal compounds such as 3β-hydroxycholest-5-ene[6] (VI) 2α, 7α-dibromocholest-4-ene-3, 6-dione[7] (VII), 6-oxa-B-homo-5α-cholestan-7-one[8] (VIII), 3β-acetoxy-6-amino (N, N dimethyl)-cholest-5-ene[9] (IX), 3β-chloro-5α-cholestan-6-one[10] (X) and 3β-acetoxy-5, 6β-dibromo-5α-cholestane[11] (XI), for homo and hetero, nuclear cosy NMR spectral studies.*

Keywords: *^{1}H-^{1}HNMR, 2-DNMR,^{13}CNMR, Spectroscopy, Steroids*

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I. Introduction

All the NMR spectra described up to now have two dimensions, the abscissa which corresponds to the frequency axis, from which one reads off the chemical shifts and the ordinate which gives the intensities of the signals. However, when we speak of a two-dimensional, 2D-NMR spectrum we understand this to mean a spectrum in which both the abscissa and the ordinate are frequency axis, with the intensities constitute a third dimension. Ifchemical shifts are plotted along one of the frequency axes and coupling constants along the other, we call this a two-dimensional (shift)-correlated NMR spectrum. From the practical NMR spectroscopist's point of view themost important 2D spectra of the latter type are those which show either ^{1}H-^{1}H or ^{1}H- ^{13}C chemical shift correlations.

During our four decades in the field of steroids research we have faced time and again various stereochemical problems while interpreting spectral data of the synthesized compounds. 2D-NMR spectroscopy now has given a new spectral technique to overcome the conformational uncertainty and justified the assignments of the spectral data. While writing this chapter we are fully aware of the fact that avoiding mathematical and other difficult parameter we are trying to study 2D-NMR spectroscopy taking the examples of steroidal compounds. For this purpose, we have selected steroidal compounds such as 3β-hydroxycholest-5-ene[6] (VI) 2α, 7α-dibromocholest-4-ene-3, 6-dione[7] (VII), 6-oxa-B-homo-5α-cholestan-7-one[8] (VIII), 3β-acetoxy-6-amino (N, N dimethyl)-cholest-5-ene[9] (IX), 3β-chloro-5α-cholestan-6-one[10] (X) and 3β-acetoxy-5, 6β-dibromo-5α-cholestane[11] (XI), for homo and hetero, nuclear cosy NMR spectral studies.

II. Experimental

All melting points were observed on a Kofler hot block apparatus and are uncorrected. I. R. spectra were determined in KBr with JASCO A-100 spectrophotometer. NMR spectra were recorded in $CDCI_3$ on Varian XL-400 MHz. Mass spectra were measured on a JEOL JMS DX-300 mass spectrometer. TLC plates were coated with silica gel. A 20% aqueous solution of perchloric acid was used as spraying agent. Light petroleum ether refers toa fraction of b.p. 60-80°. NMR values were given in ppm (s = singlet, d = doublet, dd = double doublet, t = triplet).

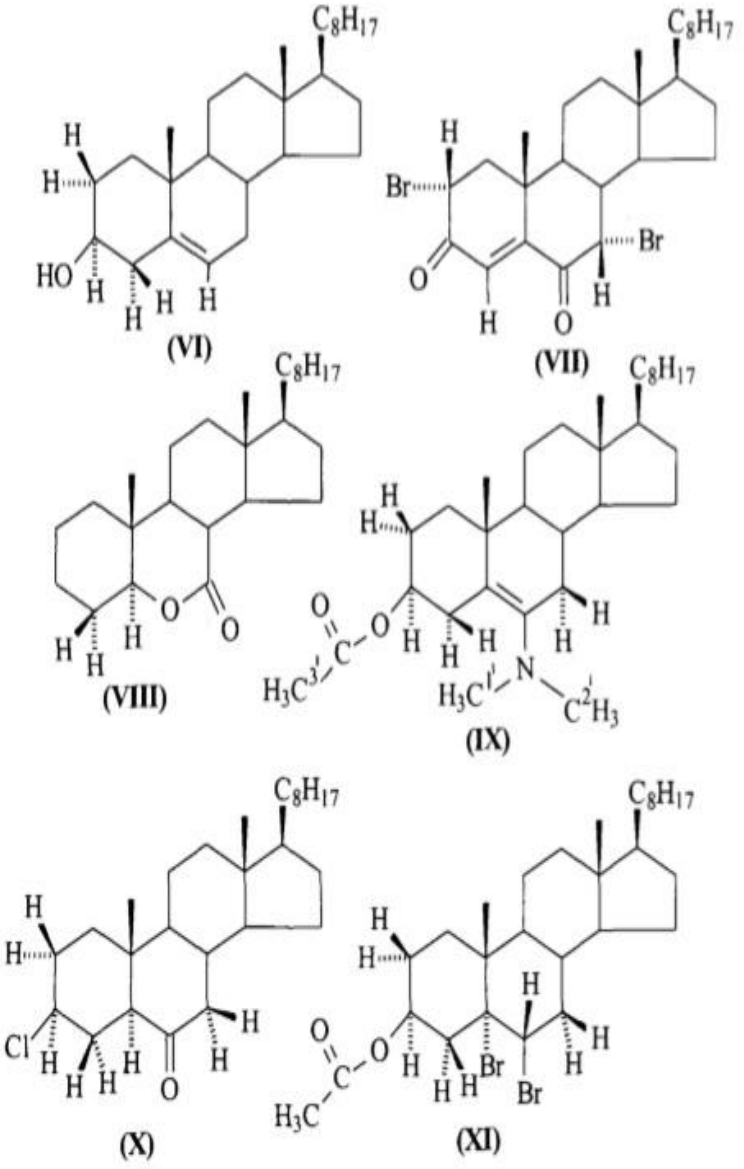
III. Result and Discussion

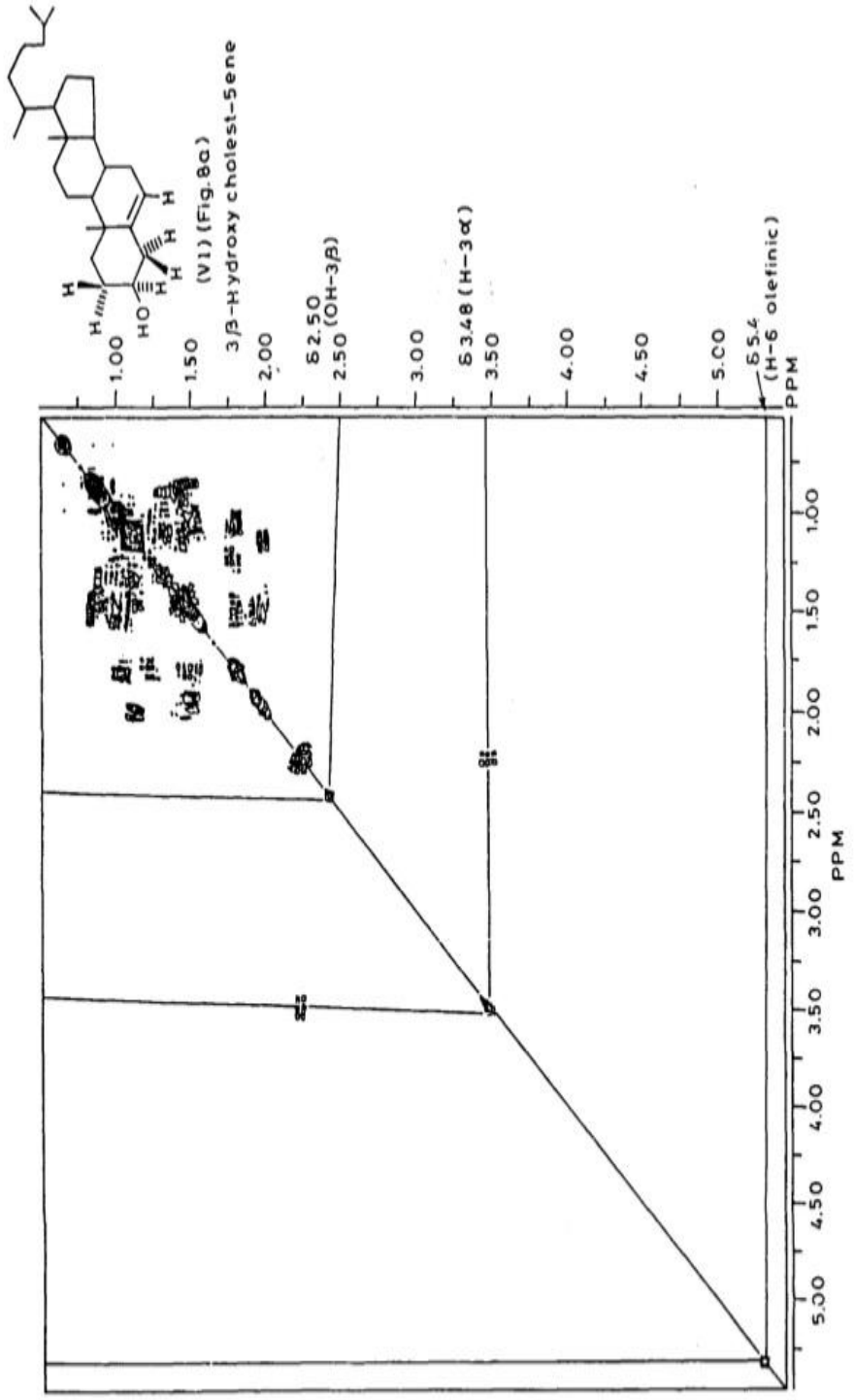
^{1}H- ^{1}H-NMR Homonuclear Cosy Spectral Studies of 3β-Hydroxy-Cholest-5-ene (VI) (Fig.8a):

In ^{1}H- ^{1}H-NMR Homonuclear cosy spectral studies of 3β-hydroxy-cholest-5-ene (VI), normal one-dimensional spectrum is plotted alongside with the two axes (F1 and F2) of cosy spectrum which identify the complete chain of coupling protons. The important and pertinent point in ^{1}H- ^{1}H-NMR homonuclear cosy spectral are diagonal peak multiplets centered around the same F1 and F2 frequency coordinates which indicate the chemical shifts and cross peak multiplets which are centered around different F1 and F2 coordinates and

indicate the coupling. If there is no cross peak multiplets, there is no coupling and the peak in ^{1}H-NMR spectrum must be singlet. ^{1}H- ^{1}H NMR homonuclear cosy spectral studies of 3β-hydroxy-cholest-5-ene (VI) exhibits a contour at δ 5.4 (H-6 olefmic). The contour at δ 3.48 for (H-3α) as diagonal peak multiplets has cross peak multiplets on either side of the diagonal. These peaks indicate coupling of H-3α with H-2 and H-4 protons. A singlet at δ 2.50 is of hydroxyl proton.

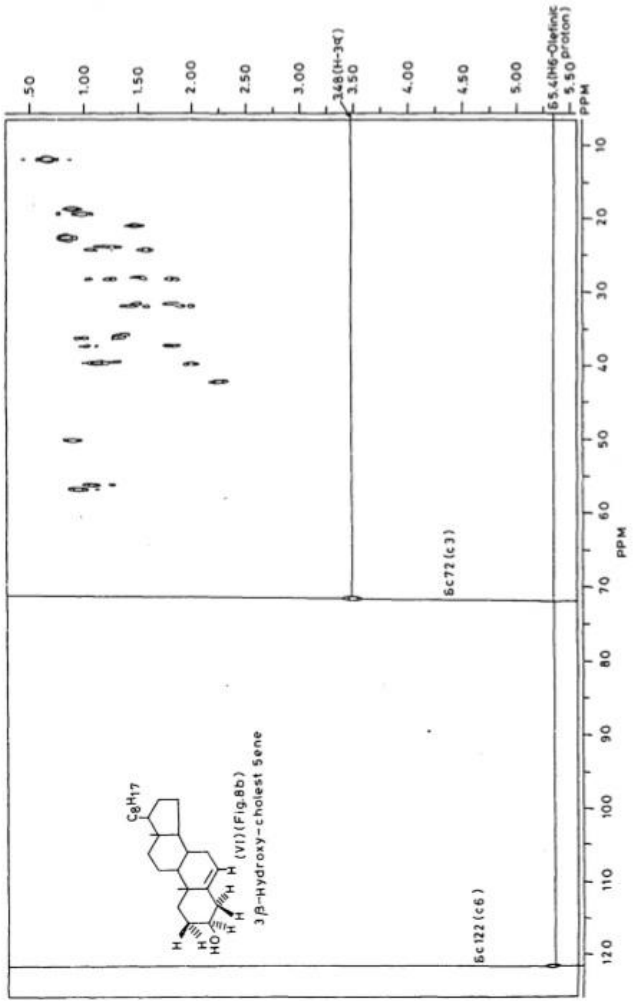
Scheme I:





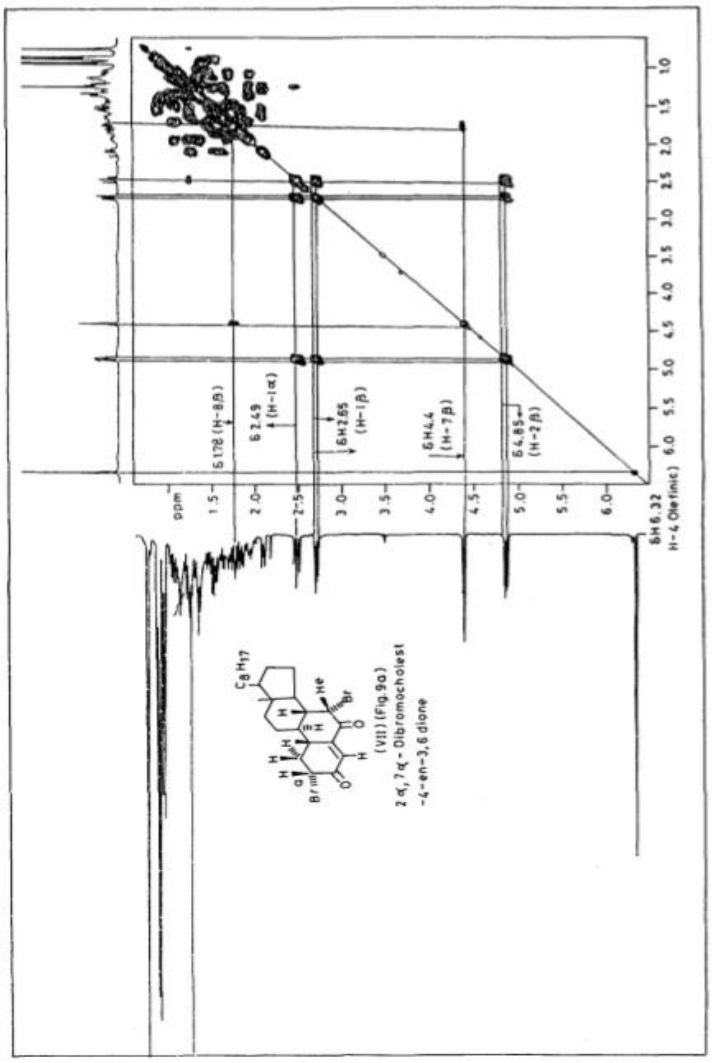
^{1}H-^{13}C-NMR Heteronuclear Cosy Spectral Studies of 3β-hydroxy-cholest-5-ene (VI) (Fig,8b):

^{1}H- ^{13}C-NMR Heteronuclear cosy spectral studies of 3β-hydroxycholest-5-ene (VI) correlates the signals obtained in ^{1}H-NMR spectrum to ^{13}C-NMR spectrum. The position of ^{13}C-NMR signals was plotted along Xaxis (F1) and ^{1}H-NMR chemical shifts to Y axis (F2). The cosy spectrum thus obtained correlates the signals at δ 5.4 (H-6 Olefnic proton) to δc 122 (C6), δ3.48 (mc, H-3α, axial) to δc 72 (C3).

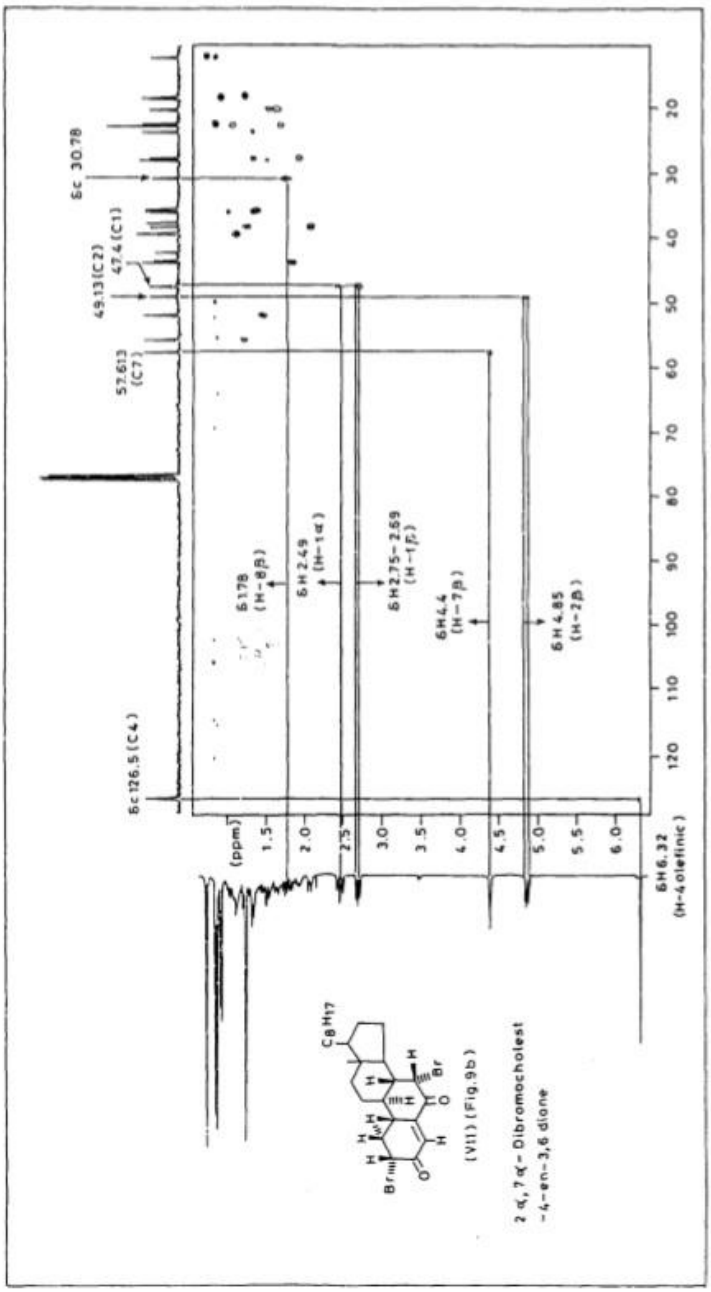


2D-NMR spectral study of 2α.7α-dibromocholest-4-en-3-6-dione (VII) (Fig. 9a, b):
2D-NMR spectral study of 2α, 7α-dibromocholest-4-en-3, 6-dione (VII) was studied with the interest of its application to configurational study at bromine containing carbons.

'H. 'H-NMR Homonuclear Cosy Spectrum of 2α. 7α-dibromocholest-4-en-3. 6-dione (VII) (Fig. 9a):
The 2D-NMR homonuclear cosy spectrum of dibromoketone (VII) (Fig. 9a) explained the stereochemistry of bromine atom at C2 and C7 through the coupling of the protons at C2 and C7 whose chemical shift and multiplicity are given. If we study the ^{1}H- ^{1}H-NMR homonuclear cosy spectrum (Fig.9a) we find a sharp intense singlet appearing on diagonal at δ 6.32. There is no connecting cross peak multiplet and therefore no proton is coupling with this proton which is assigned to C4-H (olefnic proton). A double doublet at δ 4.85 (Jae=5Hz and Jaa = 14 Hz) is assigned to C2-proton. The Cosy spectrum shows so called cross peak multiplets at δ 2.49 and 2.65 which means that the C2 proton is coupled to two protons at CI whose chemical shifts are δ2.49 (H-lα) and 82.65 (H-Iβ). The coupling constants implies that C2-proton is axial (β-oriented) and bromine is equatorial (α-oriented). A doublet at δ4.4 was assigned to H-7β. The correlation spectrum (Fig. 9a) gave cross peak multiplets at δ1.75 (assigned to (H-8β)). This indicates that the doublet at δ 4.4 is (J = 3 Hz) coupled with H-8β which is axial. Since the J value is 3 Hz therefore H-7-proton is equatorial (β-oriented) and C7-bromine is axial (α-oriented).

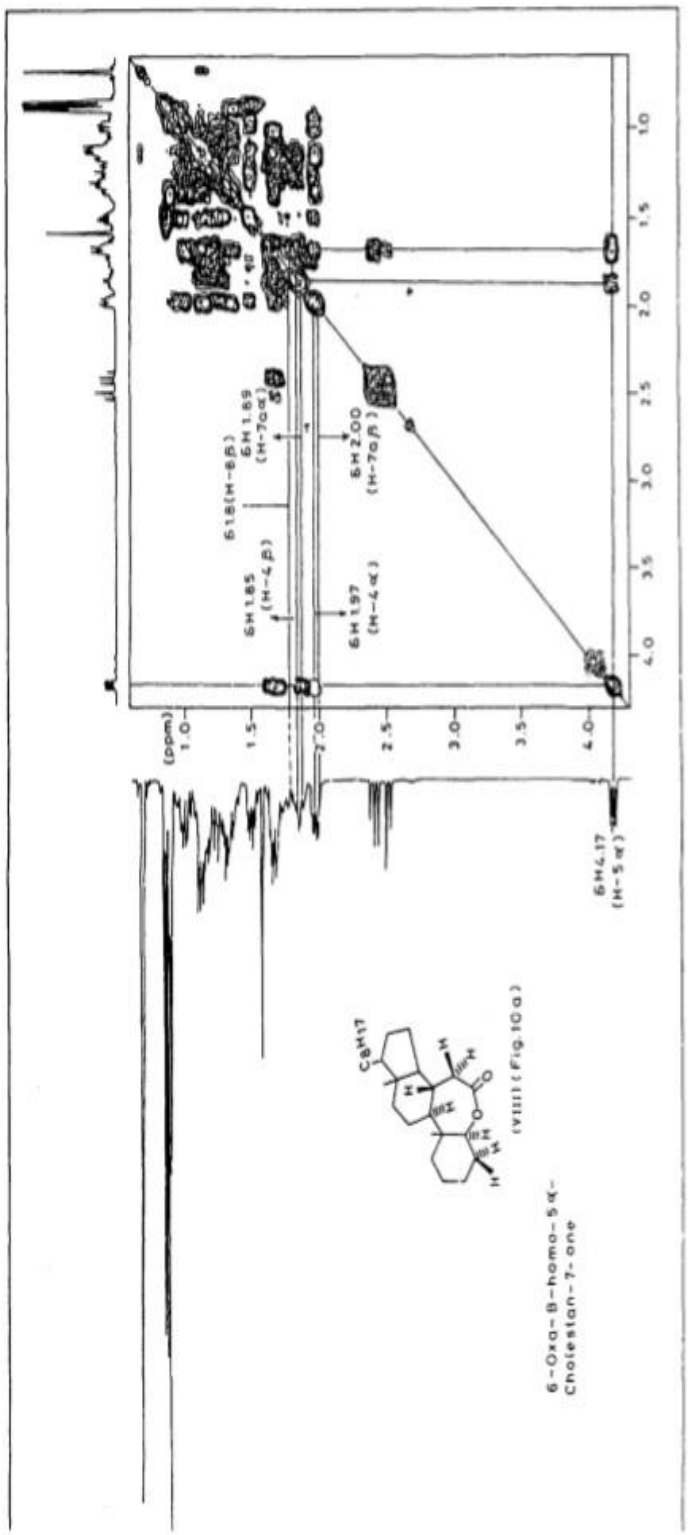


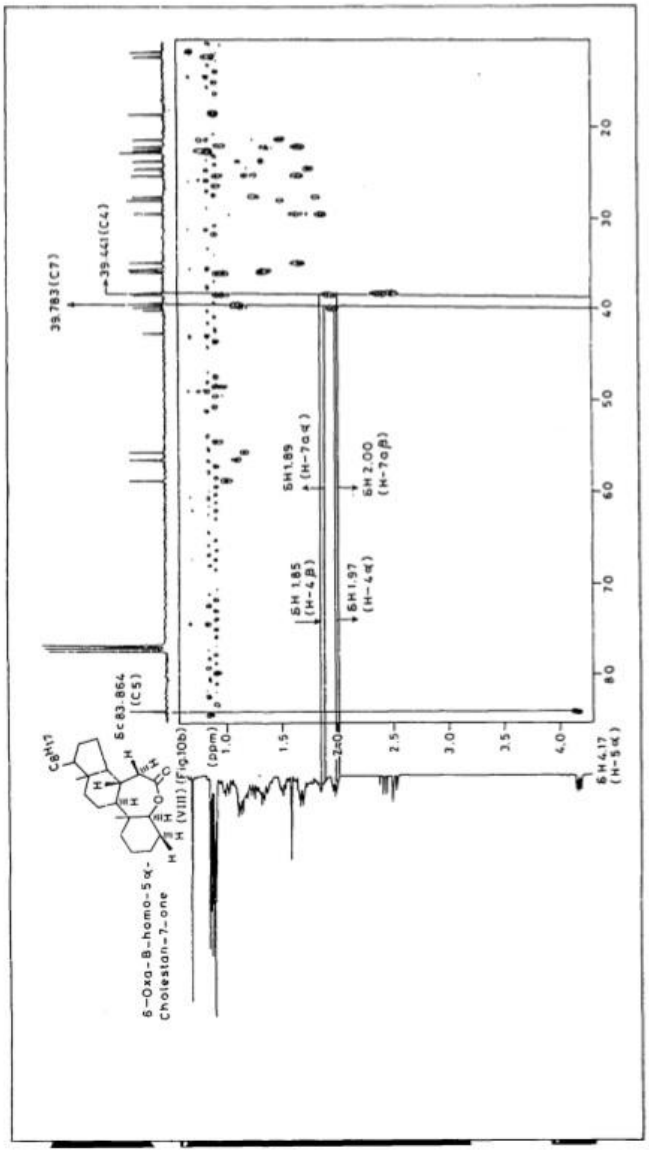
Study of ^{1}H-^{13}C-heteronuclear Cosy Spectrum of 2α,7α-dibromo-cholest-4-en-3, 6-dione (VII) (Fig. 9b): The signal at δ 6.32 (s, H-4olefinic) is correlated to δc 126.5 (C4), δ4.85 (H-2β) correlated to δc 49.13 (C2). H-7β appeared as a doublet at δ4.4 due to the coupling with H-8β and correlated to δc 57.613 (C7), δ1.78 (H-8p) is correlated to δc 30.78 (C8). The Cl-H (α and β) are at 82.65 and 2.49 and correlated for CI at δc 47.4.



2D-NMR spectral study of 6-oxa-β-homo-5α-choIestan-7-one (VIII) (Fig, 10a):
For^{1}H- ^{1}H-NMR homonuclear cosy spectrum of the lactone (VIII), normal one-dimensional spectrum is plotted alongside the two axes (F1 and F2) of cosy spectrum, which reveal and identify the complete chain of coupled protons.

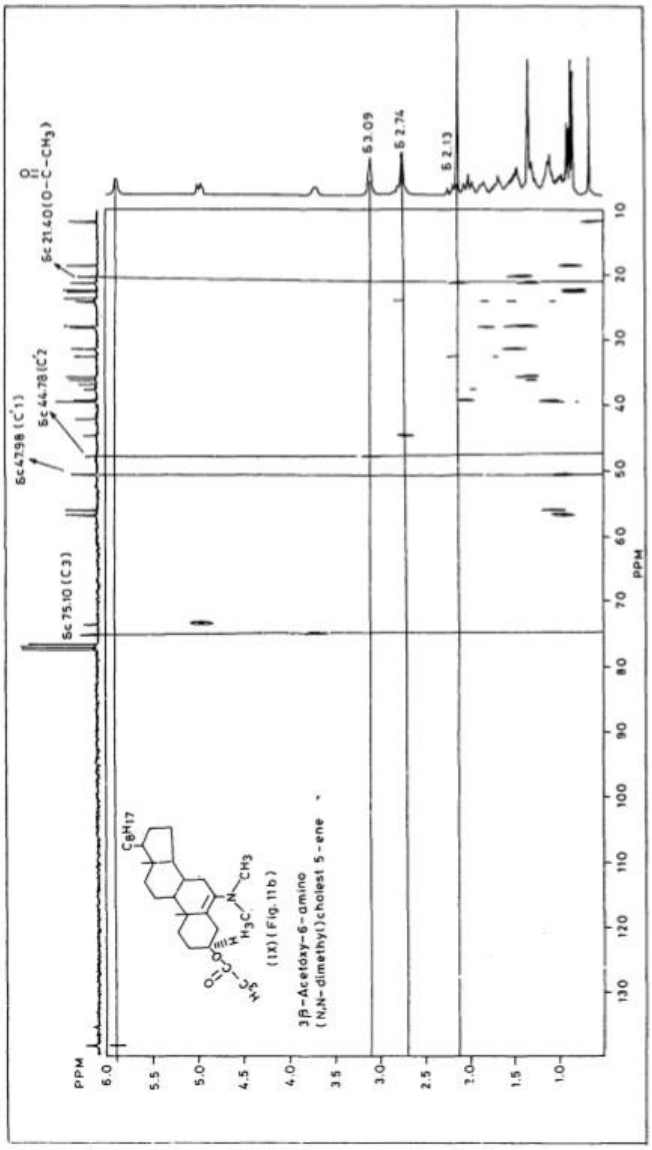
^{1}H-^{1}H-NMR homonuclear of cosy spectrum of 6-oxa-β-homo-5α-cholestan-7-one (VIII) (Fig. l0a):
In the ^{1}H- ^{1}H-NMR homonuclear cosy spectrum of lactone (VIII), the H-5α proton appeared at δ 4.17 in considerable downfield because of the presence of-O-CO- moiety adjacent to C5 as double doublet coupled by H-4α and H-4β protons with Jaa = 13 Hz, Jae = 5.5 Hz. The coupling is confirmed by cosy spectrum where two cross peak multiplets at δ1.85 (H-4β) and δ 1.97 (H-4α) were obtained. The H-7αβat δ 2.00 appeared as double doublet with Jea = 4.5 and Jgem = 13.5 Hz), andH-7aα is found at δ 1.89 also as double doublet with Jaa = 15 and Jgem =13.5 Hz). TheH-7α protons are coupled with H-8β proton (at δ1.80) which is axial in nature.


δH 1.89 (H-7αα)
δH 2.00 (H-7αβ)
δH 1.85 (H-4β)
δH 1.97 (H-4α)
δH 4.17 (H-5α)
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
C8H17
(VIIIII (Fig.10a)
6-Oxa-B-homo-5α-Cholestan-7-one



^{1}H-^{13}C-Heteronuclear NMR cosy spectrum of 6-oxa-β-homo-5α-cholestan-7-one (VIII) (Fig.10b):
The ^{1}H-NMR signal at δ 4.17 (H-5α) is correlated at δc 83.864 (C5), the ^{1}H-NMR peaks at δ 1.85 (H-4β) and δ 1.97 (H-4α) are correlated to δc 39.441 (C4) and two another proton signals at δ 2.0 {H-7aβ) and δ 1.89 (H-7aα) were correlated at δc 39.783 (C7), 81.80 (H-8β) is correlated to δc 30.78 (C8). Thus, the ^{1}H-NMR values for protons in C4, C5 and C7 were correlated with ^{1}H- ^{13}C-NMR hetero-nuclear cosy spectrum (Fig. 10b).

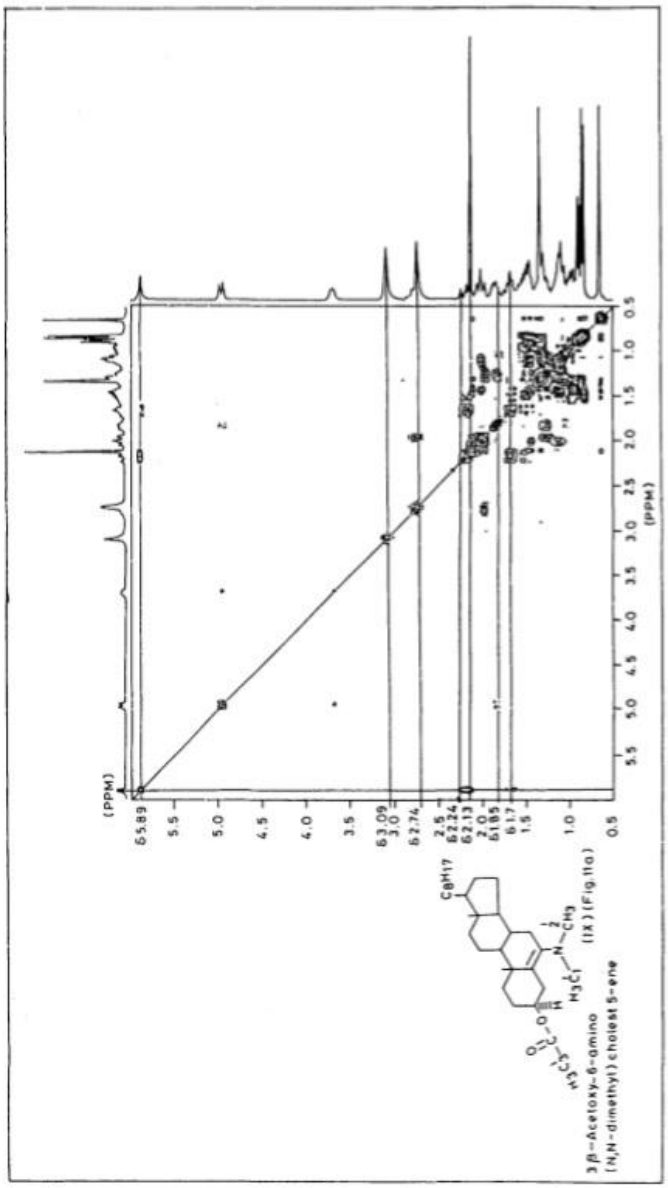
2D-NMR spectral study of 3β-acetoxv-6-amino (N, N-dimethyl) cholest-5-ene (IX)(Fig. 11):
^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-acetoxv-6- amino (N, N-dimethyl) cholest-5-ene (IX) (Fig, 11a):
^{1}H-^{1}H-NMR homonuclear cosy spectrum of 3β-acetoxy-6-amino (N, N-dimethyl) cholest-5-ene exhibits a diagonal peak multiplet at δ5.89 and so called cross-peak multiplets at δ2.24, 1.85 and 1.70. It implies that H-3α proton is coupled by other protons whose shift is δ 2.24, 1.85 and 1.70. It seems that both the equatorial protons at H-4α and H-2α are equivalent appeared at δ2.24 and the H-4β and H-2β appeared at δ1.70 and 1.85 respectively.



This conclusion is drawn from ^{1}H- ^{1}H-NMR-connectivities by cosy spectrum (Fig. 11a). Out of the two-N-methyl protons, one methyl protons are appearing as singlet at δ3.09 because cosy spectrum (Fig. 11a) does not show any cross-peak multiplet correlating this peak. Second N-methyl protons gave a doublet at 62.74 cosy NMR spectrum (Fig. 11a) has a cross-peak multiplet at δ1.85. This shift corresponds to H-7β with J=4 Hz in the equatorial proton present at C7 coupled with the N-methyl protons. The acetate methyl protons had given a peak at δ 2.13 since there is no cross peak multiplet and therefore peak appeared as single

^{1}H-^{13}C-NMR heteronuclear cosy spectrum of 3p-acetoxv-6-ammo (N, N-dimethyl;> cholest-5-ene (IX) (Fig. 11b):

In the ^{1}H- ^{13}C-NMR heteronuclear cosy spectrum of 3(3β-acetoxy-6amino (N, N-dimethyl) cholest-5-ene (IX), the peak at δ 5.89 (H-3α) is correlated at δc75.10 (C3). In the similar manner the two N-methyl peaks at δ3.09 and 2.74 are correlated at δc 47.98 (C'l) and 44.78 (C'2). The peak at δ2.13 (s, -O-CO-CH_3) is correlated to δc 21.40 (H_3C'3-COO).

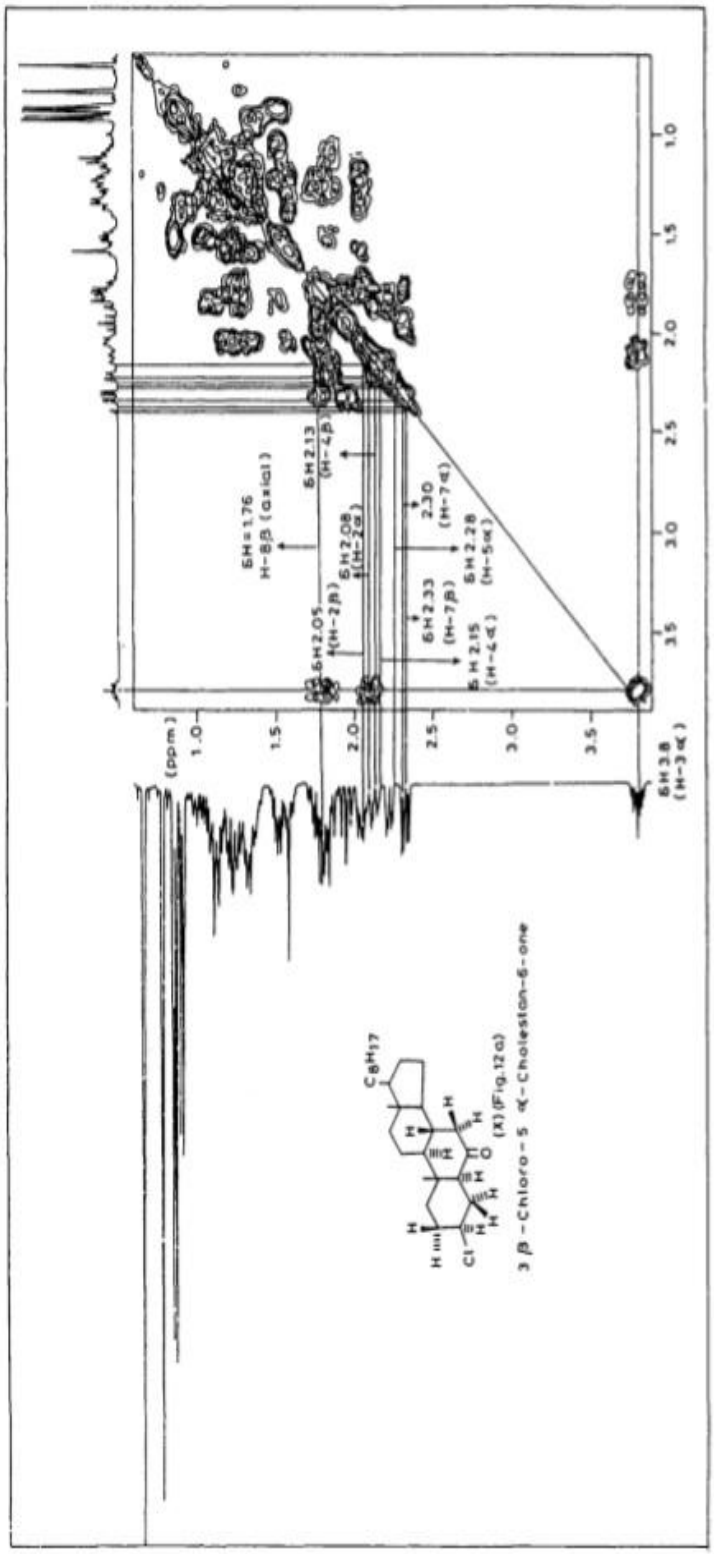


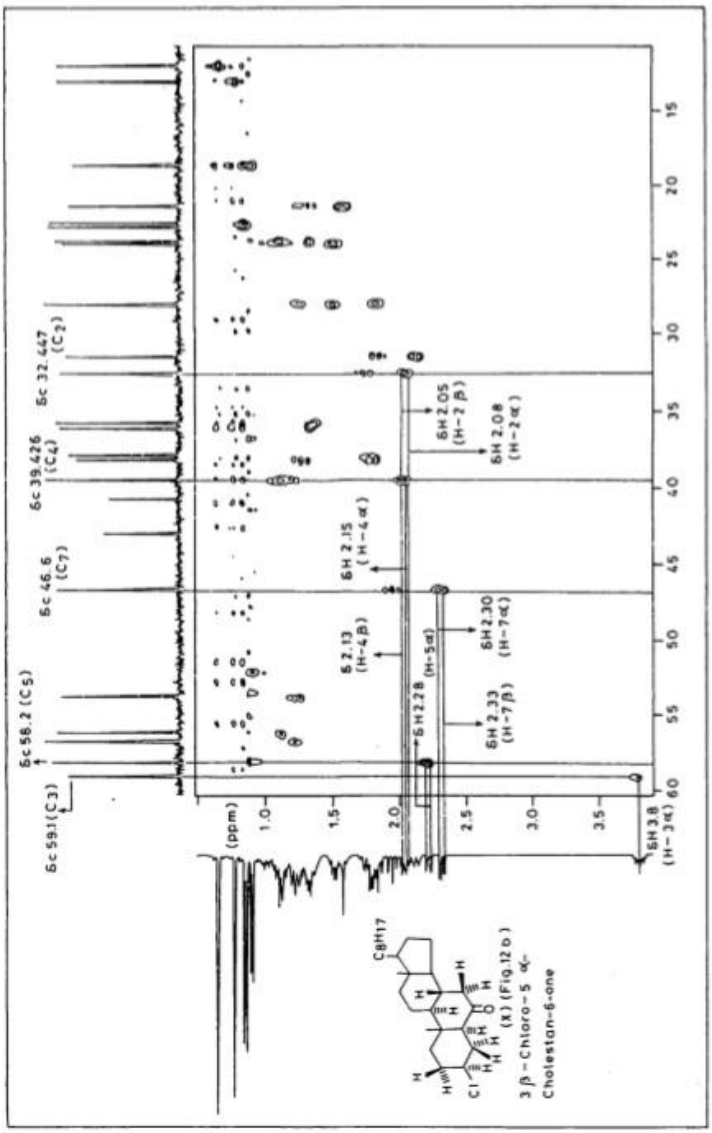
^{1}H-^{1}H-NMR Homonuclear Cosy Spectrum of 3β-chloro-5α-cholestan-6-one (X) (Fig. 12a):

^{1}H- ^{1}H-NMR homonuclear cosy spectrum of 3β chloro-5α-cholestan-6-one (X) (Fig. 12a), explained that H-3α proton at δ3.8 appeared as multiplet is coupled by H-2β (δ 2.05), H-2a (δ 2.08), H-4α (δ 2.15) and H-4β (δ2.13). similarly, H-5α proton at δ2.28 is coupled by H-4α (2.15) and H-4β (δ 2.13) because of presence of cross peak multiplets, on these chemical shifts. There isH-8β (δ1.76) and gem coupling between H-7α (δ 2.30) and H-7β (δ 2.33).

^{1}H-^{13}C-NMR Heteronuclear Cosy Spectrum of 3β-chloro-5α cholestan-6-one (7) (X) (Fig. 12h):

^{1}H-^{13}C-NMRcosy spectrum (Fig. 12b) correlates δ 3.8 (H-3α) to δc 59.1 (C3), δ 2.28 (H-5α) to δc 58.2 (C5), δ2.33 and 2.30 (H-7β and H-7α) to δc 46.6 (C7), δ2.15 and 2.13 (H-4α and H-4β) to δc 39.426 (C4) and finally δ 2.05 (H-2β) and 2.08 (H-2α) to δc 32.447 (C2).


δH 1.76
H-8β (axial)
δH 2.13
(H-4β)
δH 2.08
(H-2α)
δH 2.05
(H-2β)
2.30
(H-7α)
δH 2.28
(H-5α)
δH 2.33
(H-7β)
δH 2.15
(H-4α)
δH 3.8
(H-3α)
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
C_8H_{17}
Cl
H
O
(X) (Fig.12a)
3β-Chloro-5α-Cholestan-6-one

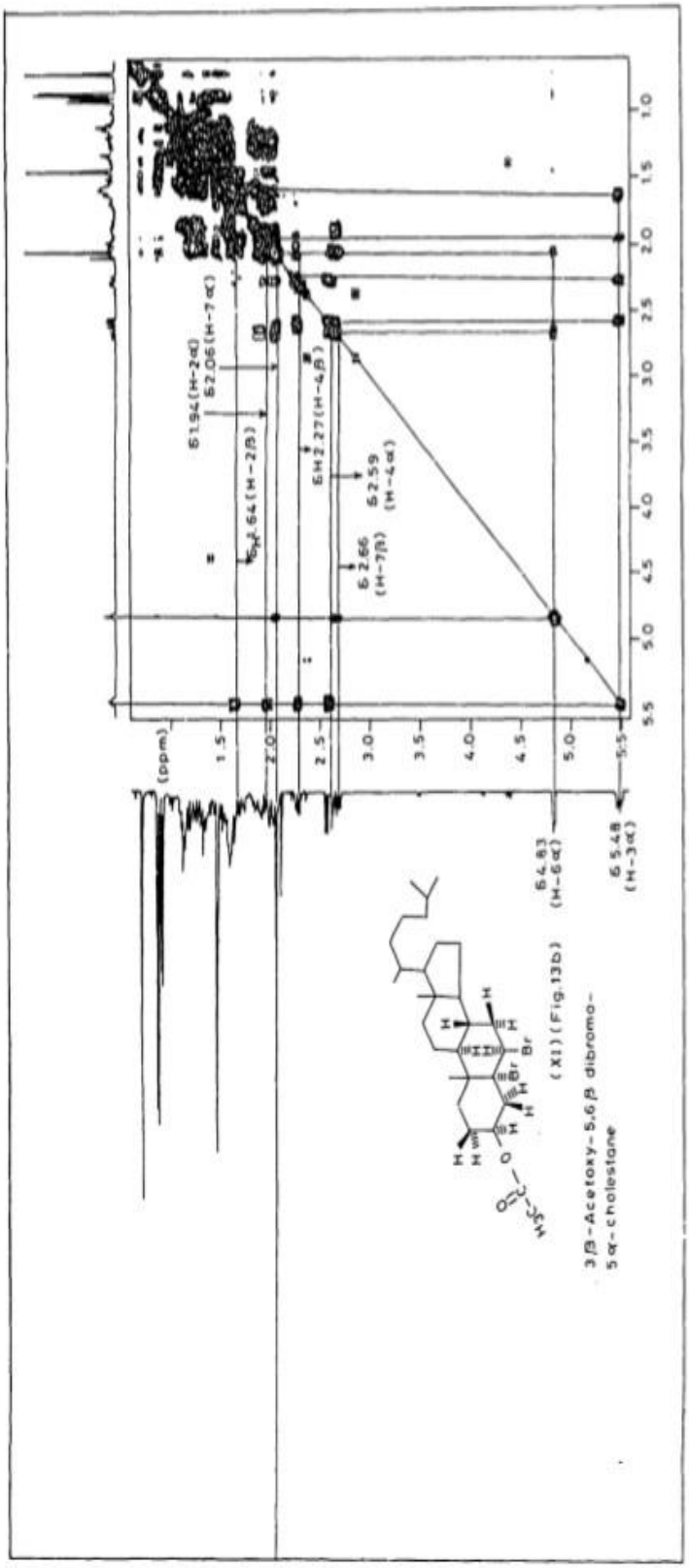


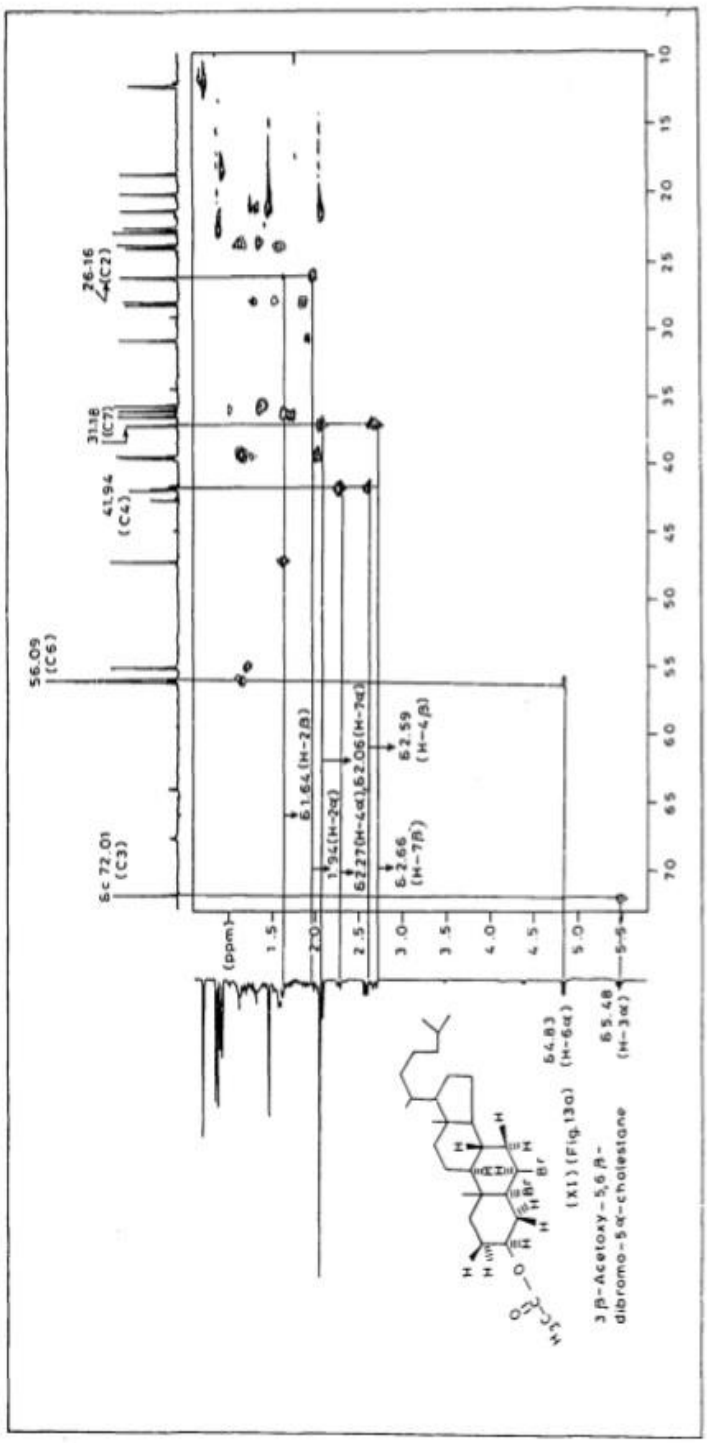
^{1}H-^{1}H-NMR Homonuclear Cosy Spectrum of 3β- acetoxy 5, 6β-dibromo-5α- cholestane(XI) (Fig. 13a):

^{1}H-^{1}H-NMR homonuclear cosy spectrum (Fig. 13a) gave a multiplets at δ5.48 well separated from the rest of the spectrum assigned to (H-3α), the cosy spectrum shows so called cross peak multiplets at δ1.94 (H-2a), 1.64 (H2β), 2.59 (H-4α) and 2.27 (H-4β), this means that H-3α is coupled to C_2 and C_4 proton (both axial and equatorial), a peak found at δ4.83 is assigned to (H-6α). The cosy spectrum (Fig.l3a) exhibits cross peaks multiplets at δ2.66 and δ2.06. The (H-6α) which is equatorial in nature is coupled by C7 axial and equatorial proton appearing at δ2.06 and δ2.66 respectively. A peak at δ2.66 assigned to H-7β is coupled by H-6α (δ 4.83), H-7β (δ 2.66) and H-7α (δ 2.06). the peak at δ2.06 assigned to H-7α is similarly coupled by H-6α (δ4.83) andH-8β (δl.88).

^{1}H-^{13}C-NMR Heteronuclear Cosy Spectrum of 3β-acetoxv,5.6β dibromo-5α- cholestane (XI) (Fig. 13b):

^{1}H-^{13}C NMR heteronuclear cosy spectrum (Fig. 13b) correlates the chemical shift δ and δc and there by explaining a definite position of proton in the spectrum, δ5.48 (H-3α) is correlated to δc 72.01 (C3), δ 4.83 (H-6α) to δc 56.09 (C6), δ 2.66 (H-7β) δ 2.06 (H-7α) to δc 31.18 (C7), δ 2.27 (H-4α), δ 2.59 (H-4β) are correlated to 8c 41.94 (C4). Similarly, 8 1.94 (H-2a) and 81.64 (H-2P) are correlated to 8c 26.16 (C2). So, by this heteronuclear one bond correlation of 3β-acetoxy-5, 6β-dibromo-5α-cholestane (XI) (Fig. 13b) complete assignment of different protons can be done.


δ 1.94 (H-2α)
δ 2.06 (H-7α)
δ 2.64 (H-2β)
δ 2.27 (H-4β)
δ 2.59 (H-4α)
δ 2.66 (H-7β)
δ 4.63 (H-6α)
δ 5.48 (H-3α)
(ppm)
3β-Acetoxy-5,6β dibromo-5α-cholestane
(XII) (Fig.13b)



IV. Conclusion

This study covers the comprehensive analysis of homo and hetro cosy nuclear spectroscopy of selected steroids. It will further help to understand the contour position and proton coupling. ^{1}HNMR and ^{13}C NMR detail studies help us to inculcate the position of protons in the complex steroidal molecule.

Acknowledgements

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Ethical Statement

There is no such use of animals or humans in this research work.

Conflict of interest

There is no conflict todeclare.

Funding Statement

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