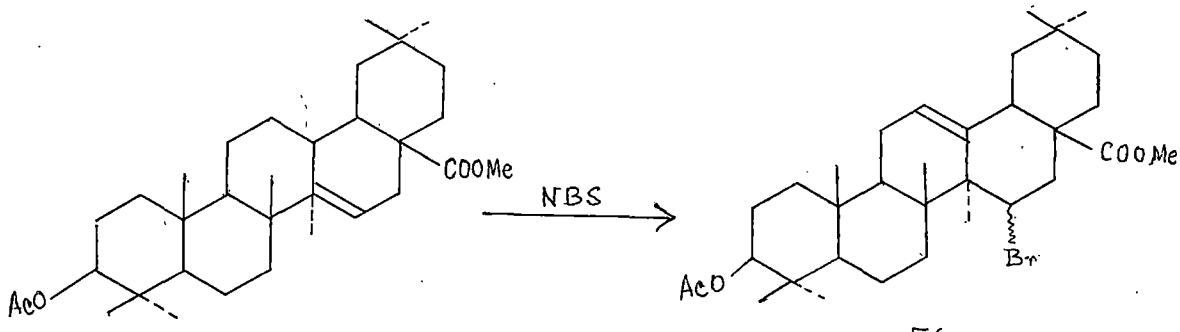
CHAPTER—II

RESULTS AND DISCUSSION

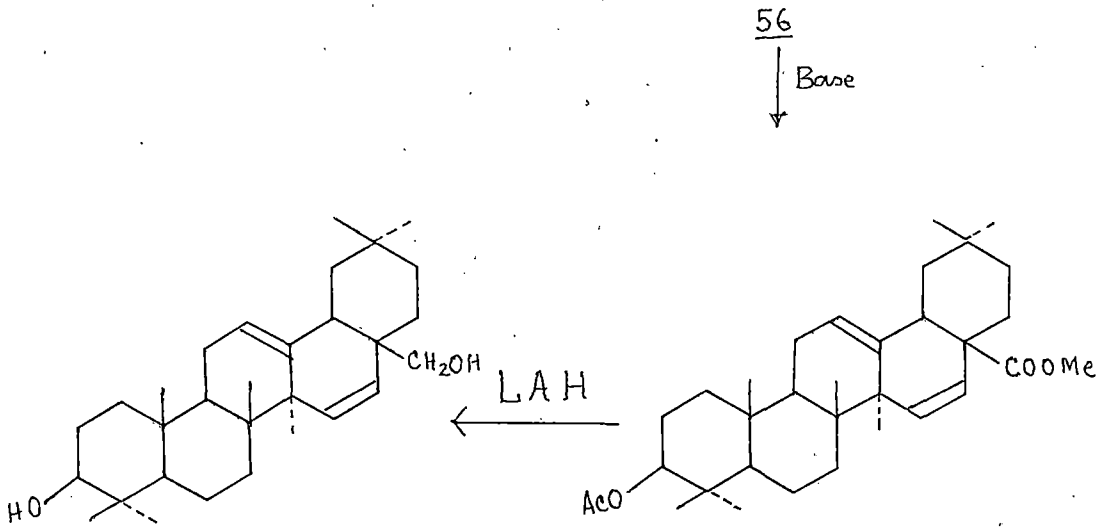
In connection with the partial synthesis of some triterpenoids the preparation of 15-bromo acetyl methyl oleanolate 56 was required. It was found that when taraxeryl acetate was treated with N-bromosuccinimide in presence of dimethyl sulfoxide[36] produced 15-bromo- β -amyrin acetate in good yield. Interested by this result we planned to prepare 15-bromo acetyl methyl oleanolate 56 from acetyl methyl aleuritolate[37] 55 by treatment with N-bromosuccinimide in presence of dimethyl sulfoxide. The bromo compound so formed would be dehydrobrominated by means of dimethyl aniline to produce 3-acetoxy methyl oleanolate-11(12), 15(16)-diene 57. This would produce aegiceradiol[38] 58 by means of lithium aluminium hydride reduction. The scheme which we had planned initially is given below (Scheme—5).

Scheme—5



55 56

Scheme contd.....



58 57

(i) N-bromosuccinimide treatment on acetyl methyl aleuritolate in presence of dimethyl sulfoxide:

For the preparation of 15-bromo acetyl methyl oleanolate, acetyl methyl aleuritolate was taken in chloroform and dimethyl sulfoxide was mixed. To the resulting solution N-bromo-succinimide was added in portions with constant shaking. After keeping the reaction mixture in dark for twelve hours and then usual work up yielded a gummy mass. The mass after column chromatography and crystallisation afforded a colourless crystal 60, $C_{32}H_{49}O_4Br$, m.p. 280°—82°, $[\alpha]_D$ +19.51°. The compound showed positive test for halogen but developed no colouration with TNM showing the absence of any double bond in the compound. IR spectrum (Fig.1) of the compound revealed that the compound possessed a γ -lactone as indicated by the presence of a peak at

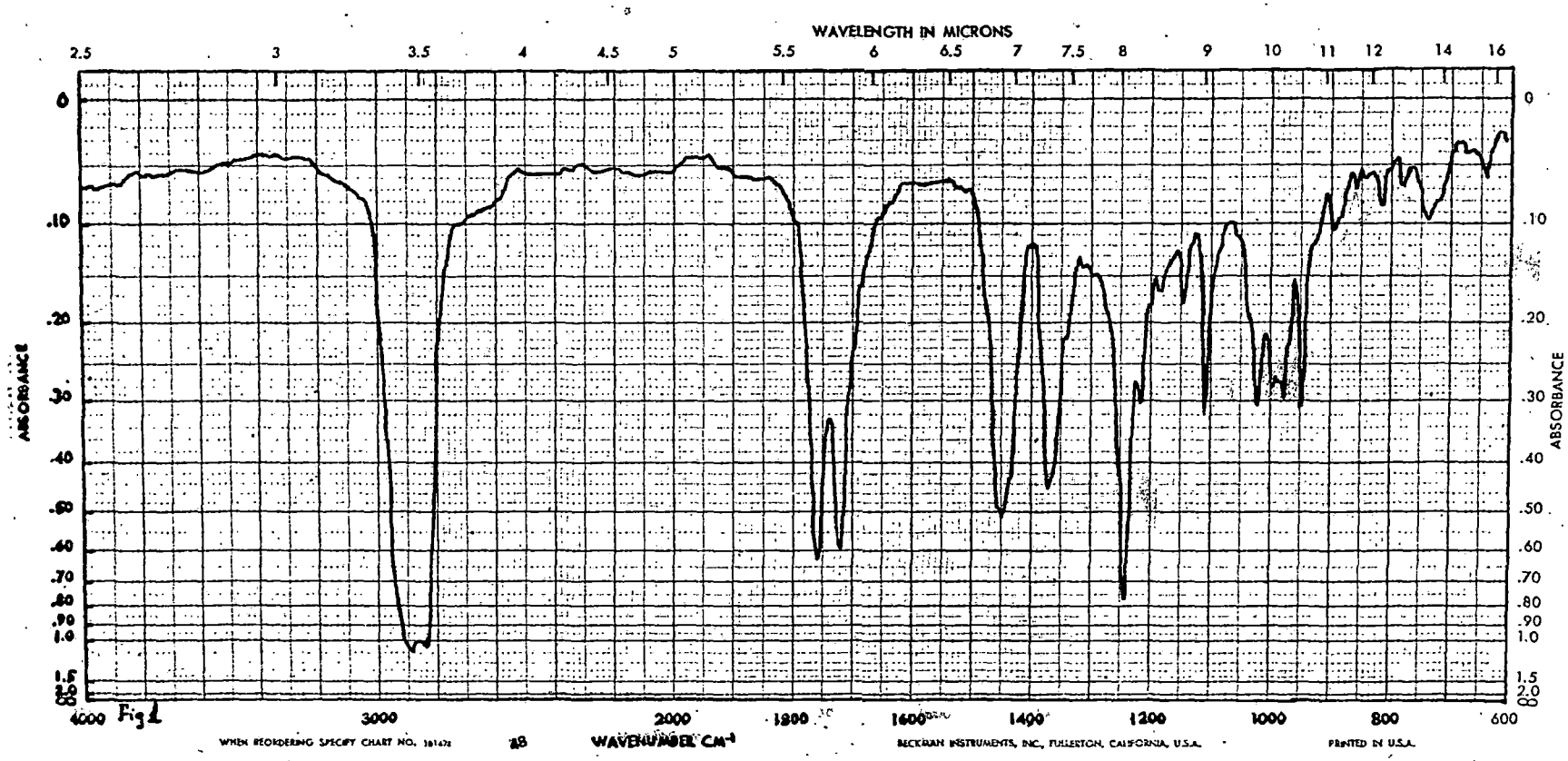


Fig.1: IR spectrum of 15-bromo oleanolic lactone acetate 60, obtained from acetyl methyl aleuritolate.

1760 cm^{-1}, other peaks at 1720 cm^{-1} and 1240 cm^{-1} were due to acetate functional group. The compound showed no UV absorption in the region 200—350 nm. PMR spectrum (Fig.2) of the compound indicated the absence of any vinyl and methoxy carbonyl proton originally present in the original compound 55. So, it was first assumed that during the reaction condition the methoxy carbonyl group was hydrolysed and lactonisation occurred on the double bond. In order to prove the assumption we treated acetyl methyl betulanate with NBS in dimethyl sulfoxide under the same condition when the starting methyl ester was recovered suggesting that no hydrolysis occurred on NBS treatment.

The reaction of N-bromosuccinimide in dimethyl sulfoxide was repeated with acetyl aleuritolic acid 59 and the same γ-lactone was obtained which was confirmed by the m.m.p. and IR comparison.

To establish the structure of the bromo lactone the following reactions were carried on the compound 60.

Treatment of the bromolactone 60 with dimethyl aniline:

The bromolactone 60 was refluxed (4 hrs) with freshly distilled dimethyl aniline for dehydrobromination and after usual work up a compound was obtained which on crystallization from chloroform-methanol mixture, furnished a solid

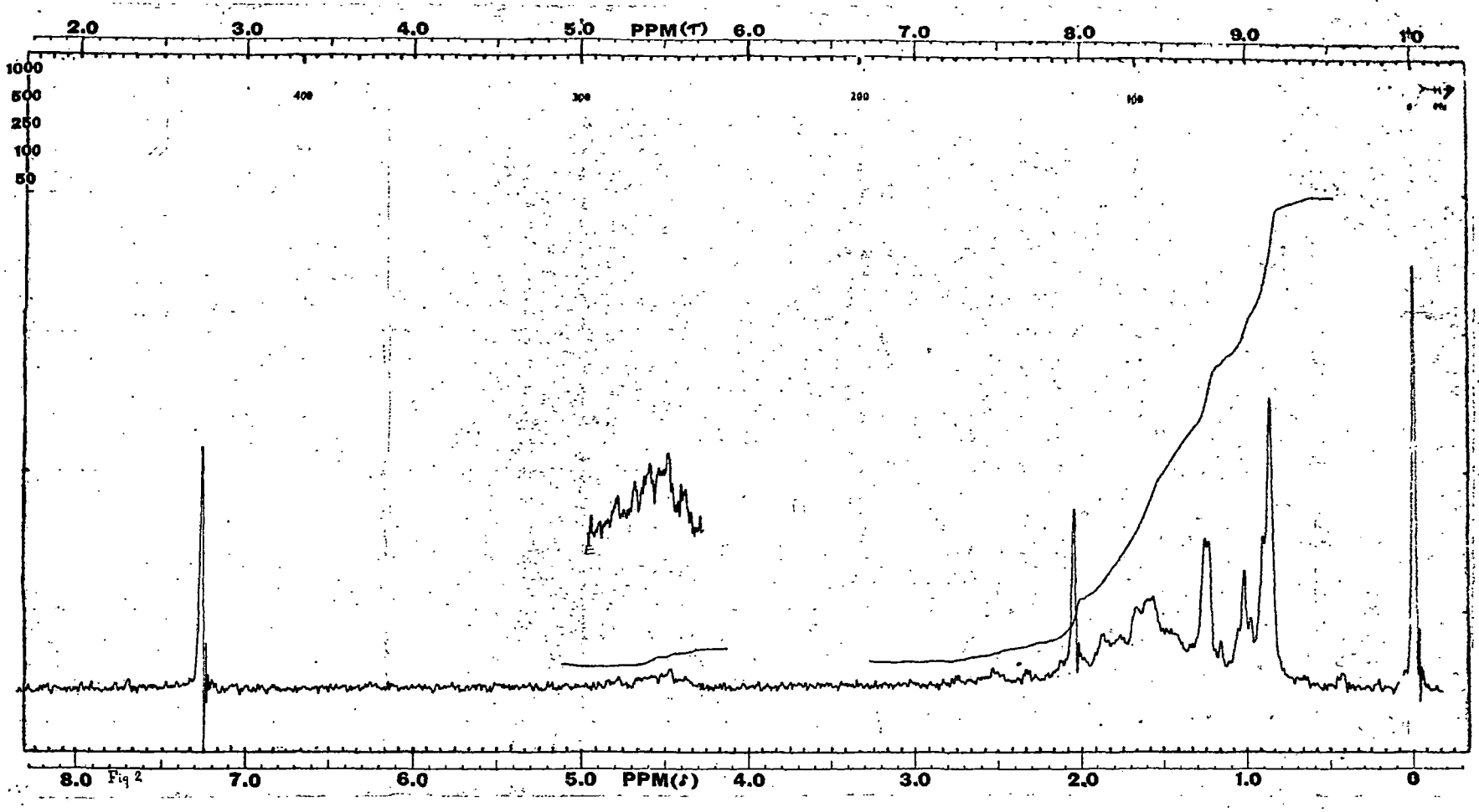


Fig.2: PMR spectrum of 15-bromo-oleanolic lactone acetate 60.

61, $C_{32}H_{48}O_4$, m.p. 308—10°. The compound gave negative Beilstein test for bromine, no UV absorption was observed in the region 220—350 nm; IR (nujol) spectrum (Fig.3) showed the presence of γ-lactone at 1770 cm^{-1}, the acetate group ν_{max} 1730, 1250 cm^{-1} and the disubstituted double bond at 900 cm^{-1}. In the PMR spectrum (Fig.4) of 61, the signals at 0.8 to 1.3 ppm showed the presence of seven tertiary methyl groups, the peak at 2.04 ppm was due to acetoxy methyl protons and the multiplet at 4.5 ppm was due to axial proton geminal to the C—3—acetyl group. Appearance of AB quartet at 5.5 ppm with coupling constant 10 Hz indicated the presence of the grouping —C—C(H)=C(H)—C— .

The unsaturated lactone so obtained was hydrogenated in a mixture of ethyl acetate and acetic acid solution using Adam's catalyst in Parr' Hydrogenation apparatus under pressure of 40 PSI. After usual work up the solid obtained was chromatographed and crystallised from chloroform and methanol mixture. The crystallised product so obtained had m.p. 293—94°, $[\alpha]_D$ +19°, was found to be identical with an authentic sample of oleanolic lactone acetate[39] 62 (by m.m.p. and IR comparison). The authentic sample of oleanolic lactone was prepared by the method of Barton et al[39] from acetyl oleanolic acid. The formation of oleanolic lactone acetate clearly

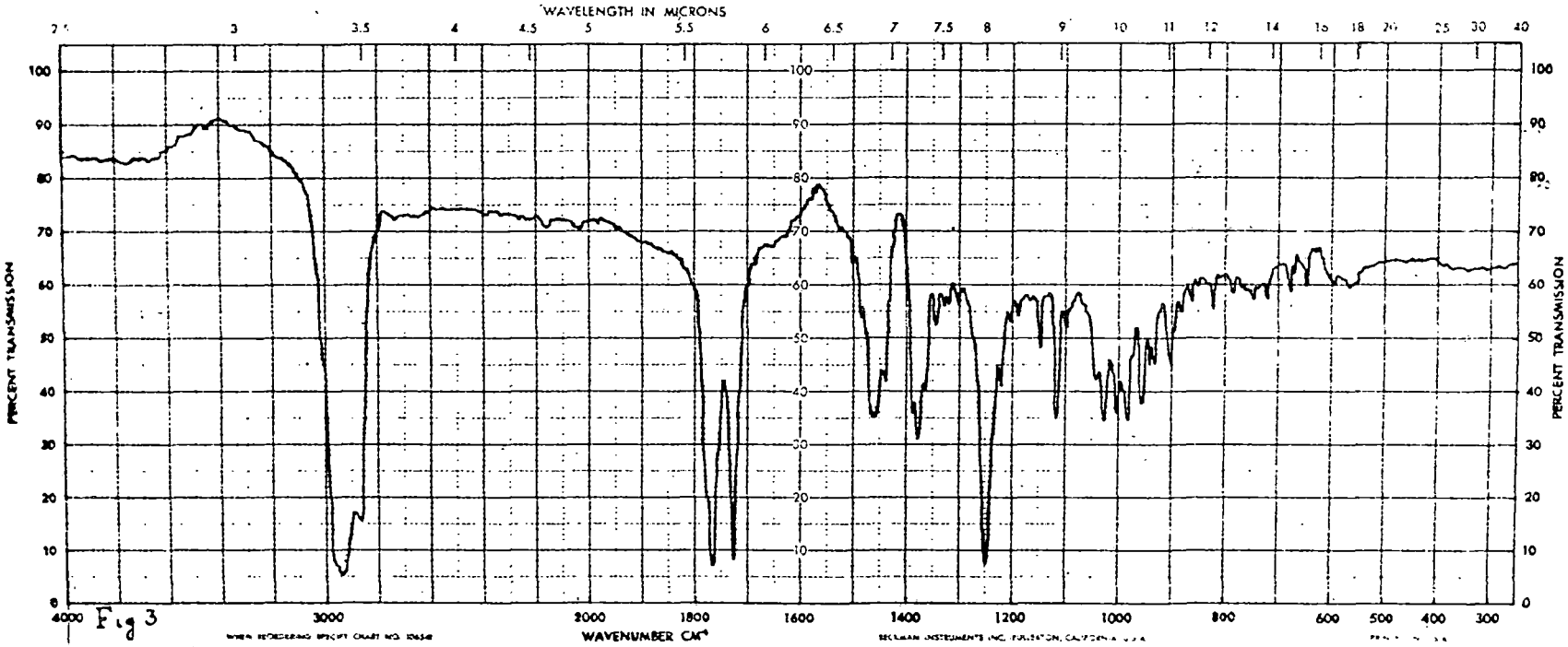


Fig.3: IR spectrum of 15,16-dehydro oleanolic lactone acetate, 61.

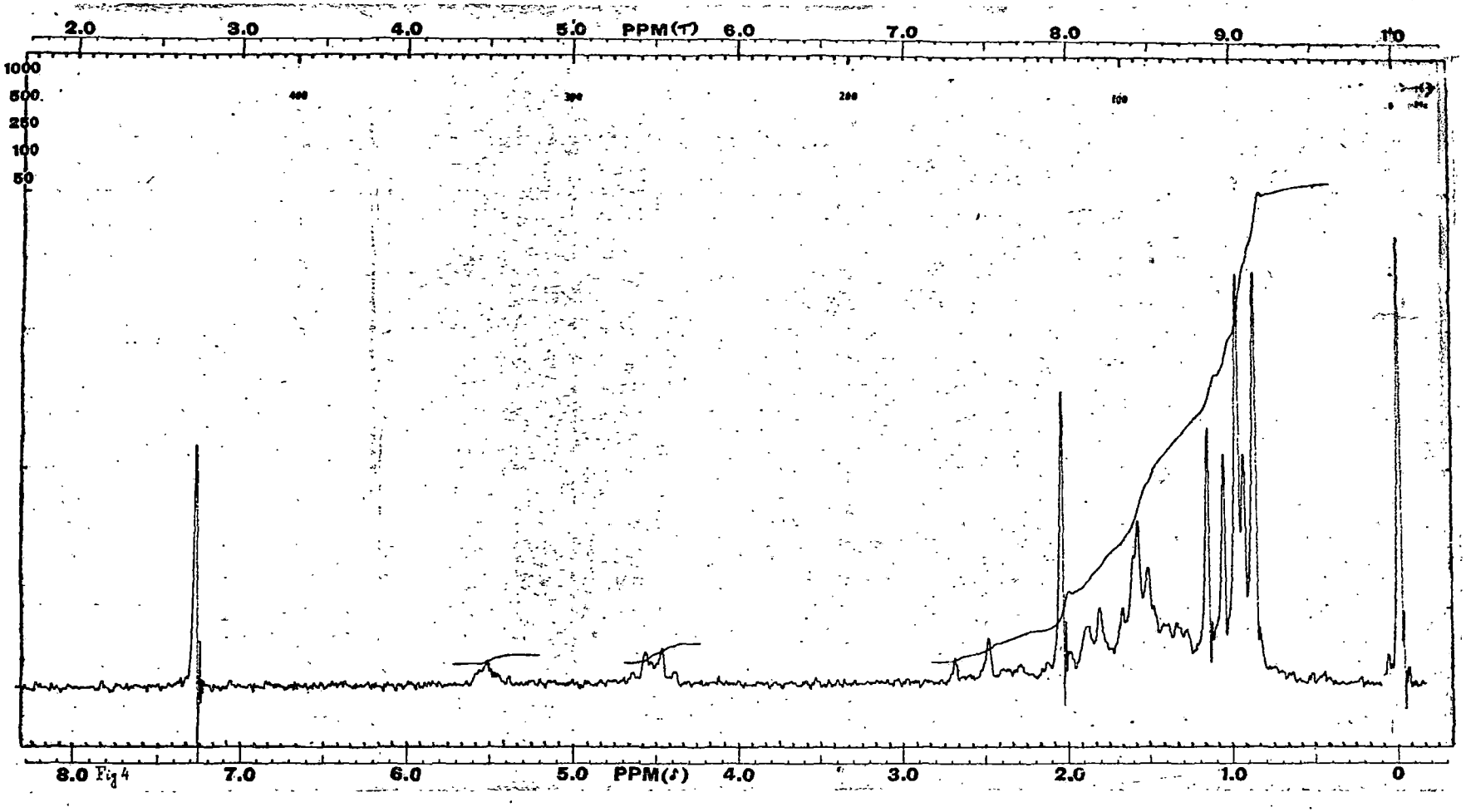
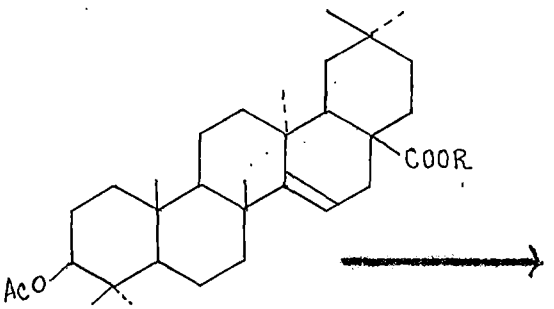


Fig.4: PMR spectrum of 15,16-dehydro oleanolic lactone acetate, 61.

illustrates that the lactyl oxygen is attached to C—13 carbon atom and is β oriented. The whole reaction can be shown in Scheme—6.

Scheme—6



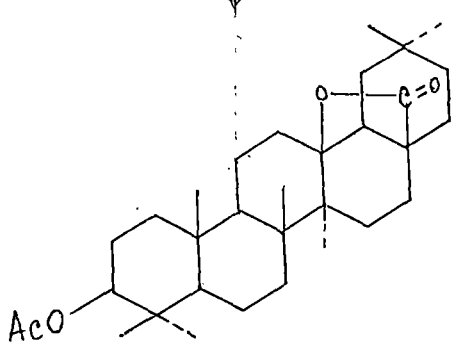
55, R = Me
59, R = H

→ Bromolactone 60

60 —Dimethyl Aniline→ Dehydrobrominated γ lactone with a double bond

61

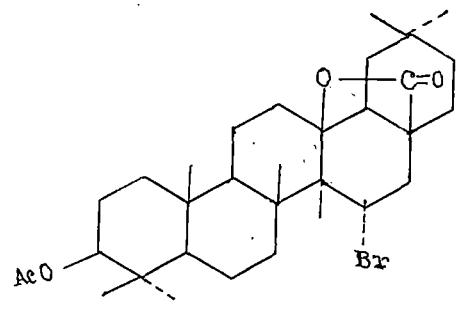
↓ H_2, under pressure



62

So the position of attachment of the lactonic functional group in the bromo lactone 60 was clear. But the position of bromine atom was not clear. To ascertain the position of bromine atom in the bromolactone, lithium aluminium hydride reduction on the lactone 61 was done which furnished a compound, $C_{30}H_{48}O_2$, m.p. 185—86° that was identical with an authentic specimen of aegiceradiol[38] 58 (mmp and IR comparison). The formation of aegiceradiol which contains one of the double bonds at C—15—16 position clearly indicates the position of bromine atom either at C—15 or C— 16 position. The position of the bromine atom should be at C—15 position since the double bond in the original acetyl methyl aleuritolate was at C—14—15 position. So entrance of bromine atom should be at C—15 position as in the case of taraxeryl acetate which gave the 15 bromo β -amyrin acetate 44 with NBS[36]. In the lithium aluminium hydride reduction the lactone ring was affected with the formation of a tertiary hydroxyl group at C—13 which undergoes dehydration producing C—12—13 double bond and $-CH_2OH$ group at C—17 position.

So the structure of the bromolactone can be assigned as 60.



60

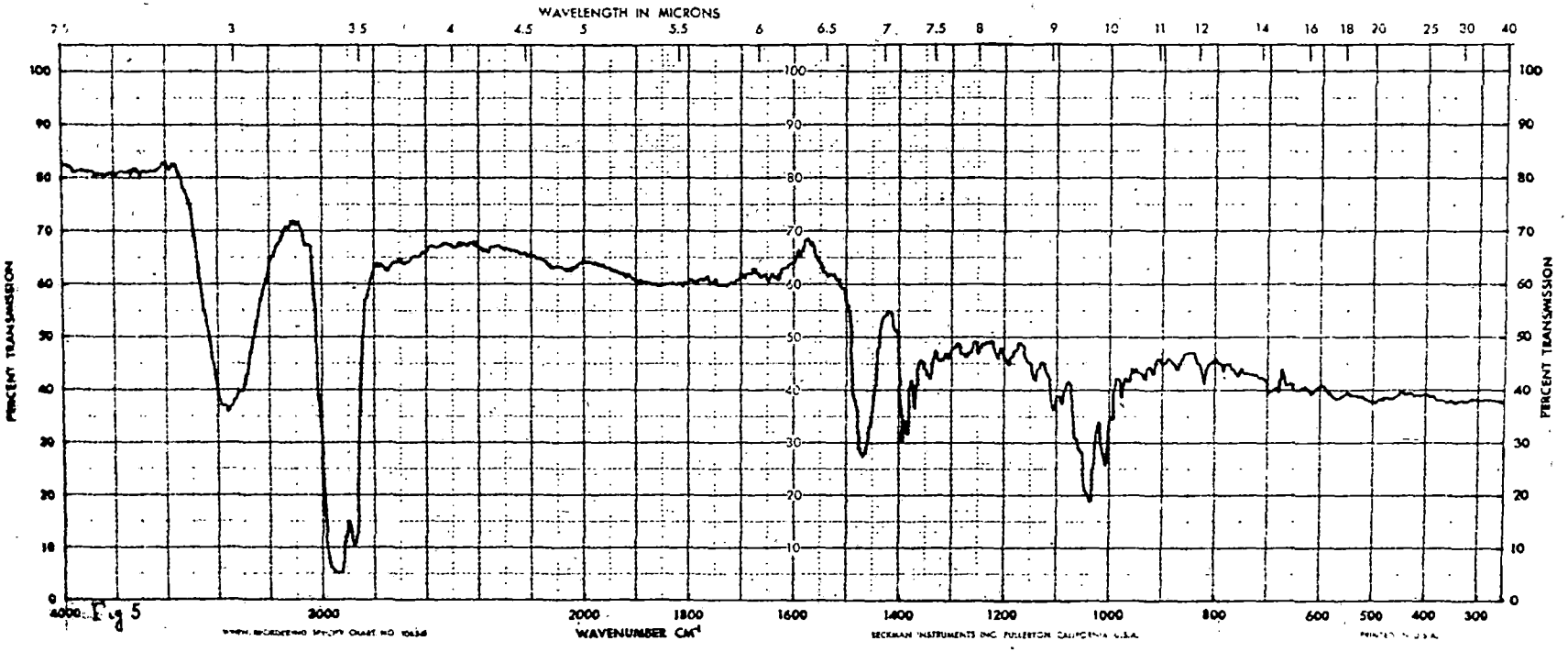
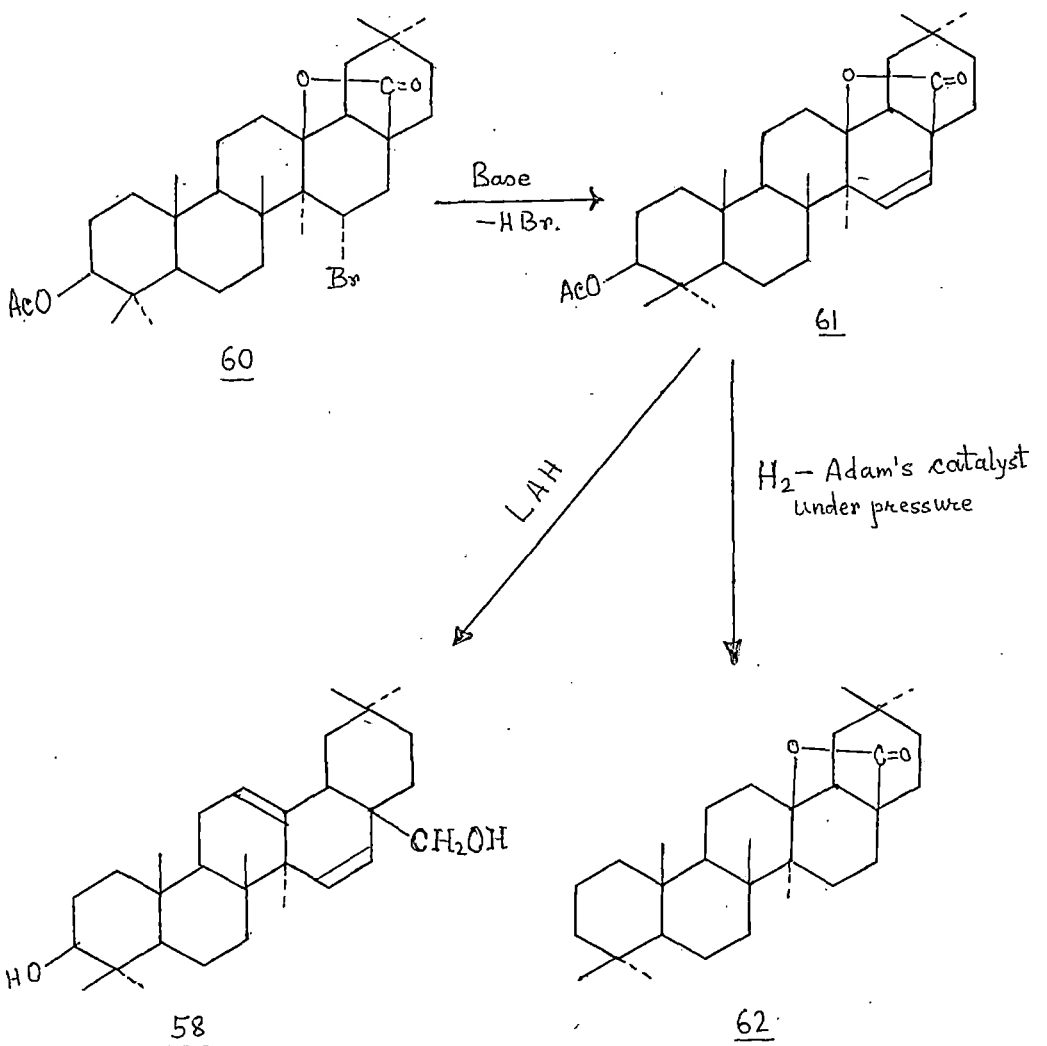


Fig.5: IR spectrum of aegeceradiol, 58.

Thus, the above sequence of reactions on the bromoketone 60 can be represented in the following way (Scheme—7):

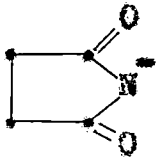
Scheme—7

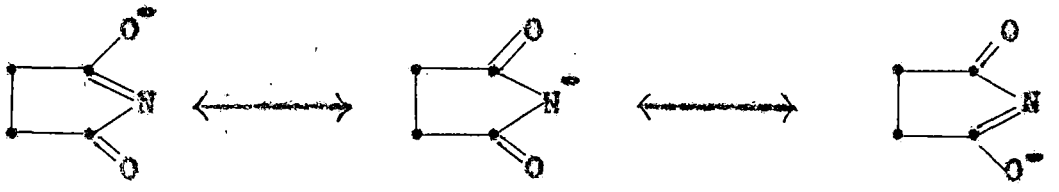


60 61 58 62

A study of the Dreiding model of the lactone 60 showed the bromine atom at C—15 position should be oriented ∝-equatorial. This stearochemistry was confirmed by the PMR spectral data. The proton centred at 4.3 ppm (d,d) with coupling constants 14Hz (Ja, a) and 3Hz (Ja, e) was due to the axial proton geminal to the bromine which must have an axial and equatorial protons on the vicinal carbon. This confirmed the presence of bromine atom at C—15 equatorialy oriented.

Mechanism of formation of the bromolactone 60:

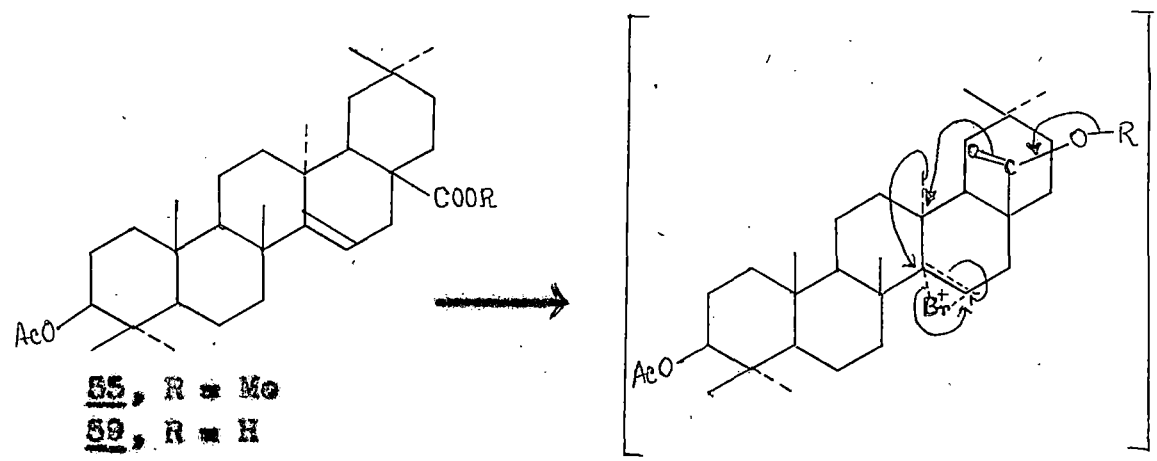
It is well known that dimethyl sulfoxide is a very polar solvent and it is expected that it probably dissociates to  anion and Br^+ cation; the anion being stable due to two other resonating structures.



The bromonium ion thus produced, attacks the olefinic double bond at C—14—15 position forming a three membered charged intermediate which immediately rearranges

to the stable form by the opening of the ring system with concerted migration of C—13 methyl to C—14 position and attack of the C=O⟶ II bond to the vacant C—13 position forming the lactyl bond with loss of CH_3 or H as cation from the COOH group. The mechanism can be shown in Scheme—8.

Scheme—8



55, R = Me
59, R = H

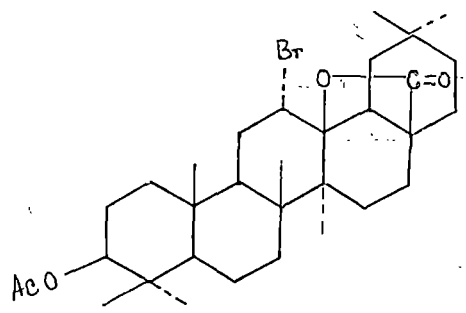
Since bromine atom is bulky it assumes to enter in the equatorial position so as to have minimum steric interactions.

So treatment of NBS in presence of dimethyl sulfoxide on triterpene acid or ester produced bromolactone. As no systematic work was reported on the action of N-bromosuccinimide in presence of dimethyl sulfoxide we were interested whether the reaction could be applied to other triterpene

acids or esters as well. For this purpose the reaction was performed on two other triterpenoids, one belonging to oleanane skeleton viz. acetyl methyl oleanolate and the other to the lupane skeleton viz. acetyl methyl betulenate.

(11) Treatment of N-bromosuccinimide in presence of dimethyl sulfoxide on acetyl methyl oleanolate 63:

Acetyl methyl oleanolate 63 was taken in chloroform and dimethyl sulfoxide was added. To the resulting solution N-bromosuccinimide was added in portions as mentioned in the case of acetyl methyl aleuritolate. After usual work up and chromatography followed by crystallisation a product was isolated 65, $C_{32}H_{49}O_4Br$, m.p. 215—16°, IR(nujol) spectrum (Fig.6) showed a peak at 1770 cm^{-1} indicating the presence of γ -lactone and other peaks at 1725 cm^{-1} and 1240 cm^{-1} were due to acetate functional group. It gave positive Beilstein test for bromine. The compound 65 was found to be identical with 3 β -acetyl-12 α -bromo olean-28→ 13 olide[40], (m.m.p. and IR comparison) prepared by the treatment of 3 β -acetyl oleanolic acid 64 with bromine in acetic acid. So the structure of the bromolactone can be assigned as 65.



65

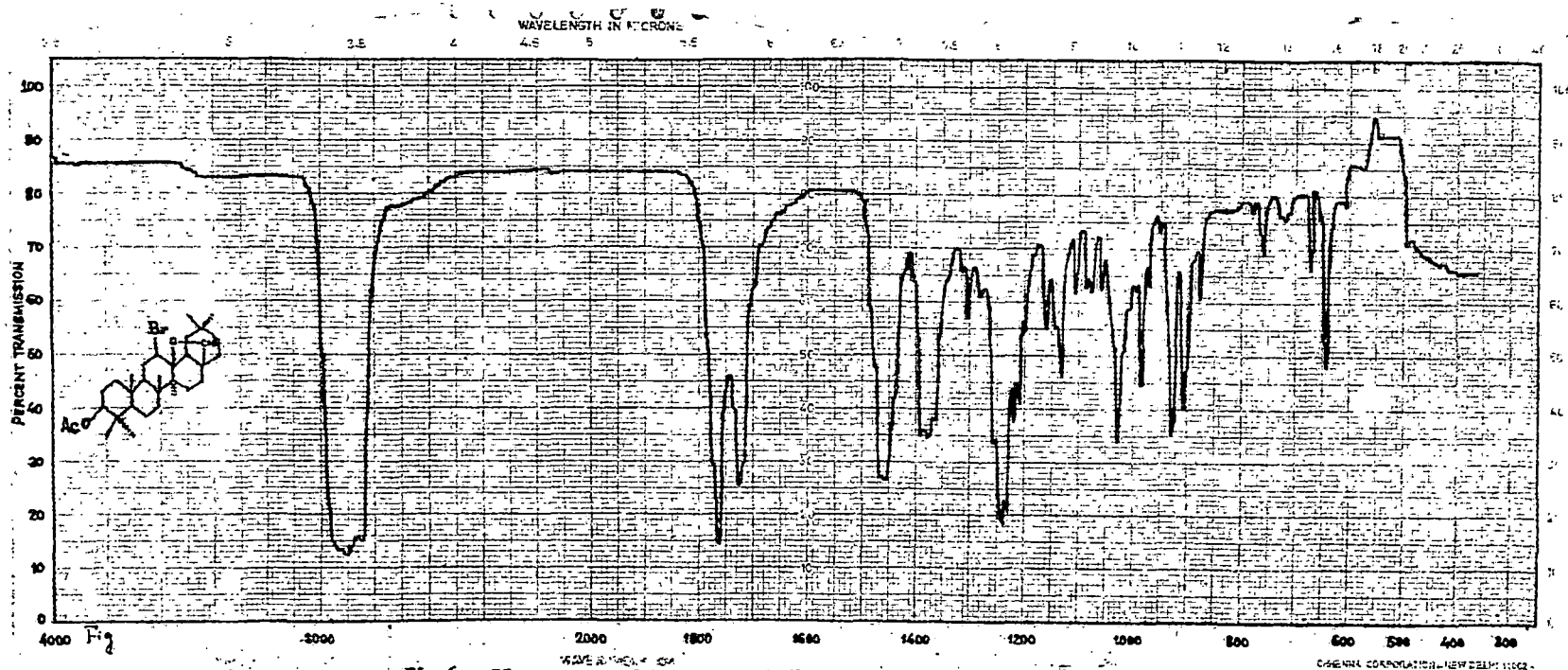


Fig 6 : IR spectrum of bromolactone 65, obtained from acetyl methyl oleanolate.

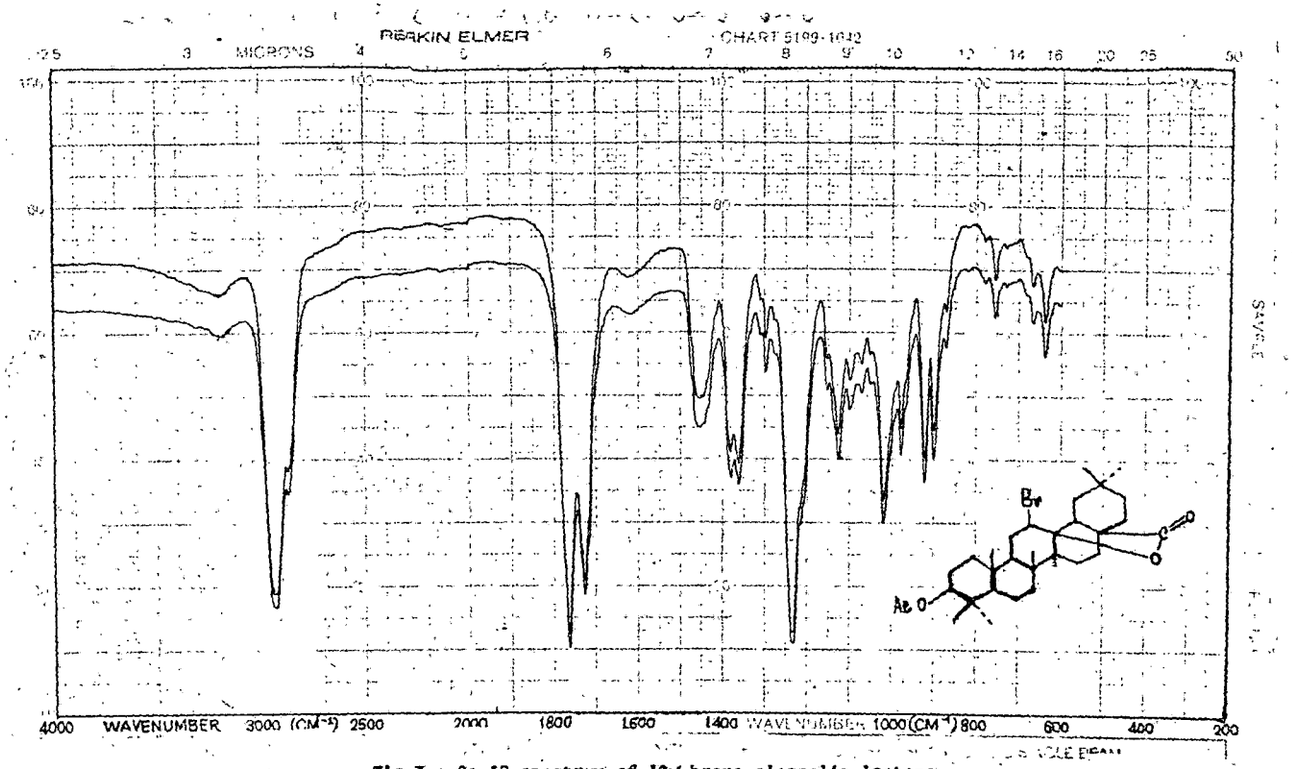
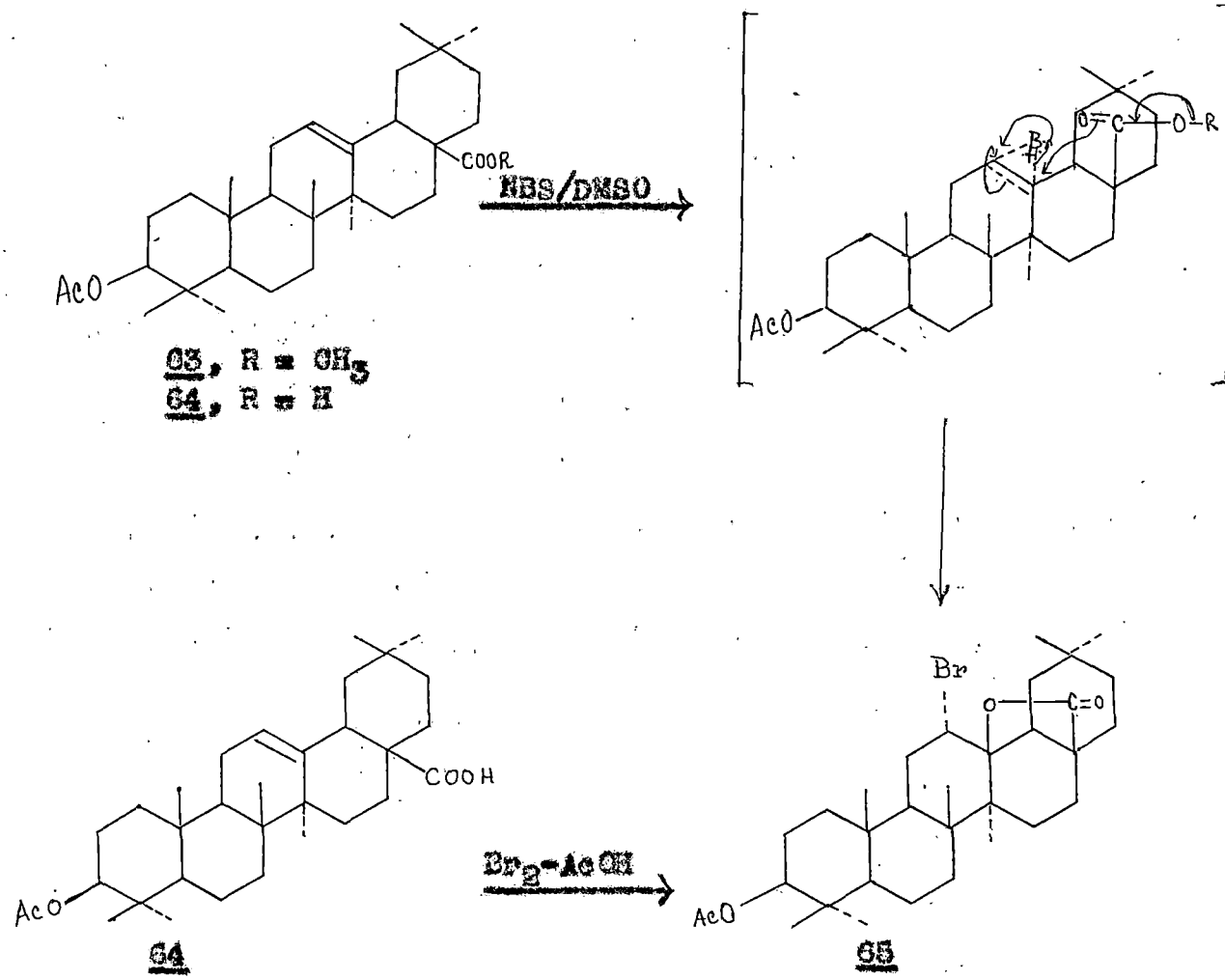


Fig 7 : Co-IR spectrum of 12α-bromo-oleanolic lactone acetate 65, with authentic specimen.

The mechanism (Scheme—9) of formation of 65 can be anticipated as given in case of lactonisation of acetyl methyl aleuritolate, mentioned earlier.

It was found that 3β-acetyl oleanolic acid 64 also formed the same bromolactone 65 on treatment with N-bromo-succinimide in dimethylsulfoxide (by m.m.p. and IR comparison).

Scheme—9



63, R = CH_3
64, R = H

64

65

The reaction of N-bromosuccinimide in presence of dimethylsulfoxide was carried out on a third compound 3β -acetyl methyl betulenate[41] 66. In 3β -acetyl methyl betulenate 66 the double bond is in the form of isopropenyl and the ring D of the triterpene ester is five membered.

(iii) Treatment of NBS in presence of dimethylsulfoxide on methyl 3β -acetyl betulenate[41] 66:

3β -acetyl methyl betulenate 66 was taken in distilled chloroform and dimethyl sulfoxide was added to it. To the resulting solution N-bromosuccinimide was added in portions as was done in earlier cases. Following the same procedure and work up a mass was isolated which on chromatography on a silica gel column afforded three different bromo compounds.

The first compound which was obtained from the column on elution with petroleum (4):benzene (1) was crystallised form $CHCl_3$—MeOH. The crystallised product gave positive test for bromine. Analysis showed the compound had the molecular formula $C_{33}H_{51}O_4Br$ m.p. 235—36°, $[\alpha]_D$ +42.55°. IR spectrum (Fig.8) showed peaks at 1735 cm^{-1} (—COOCH$_3$) 1725 cm^{-1} (—OCOCH$_3$), 1260 cm^{-1} (—CH$_2$Br), 1660, 875 cm^{-1} (=CH$_2$).

PMR spectrum of the compound showed 5-tertiary methyl groups in the region 0.87—1.02 ppm, three hydrogens of —OCOCH$_3$ group was found to be at 2.06 (singlet), two

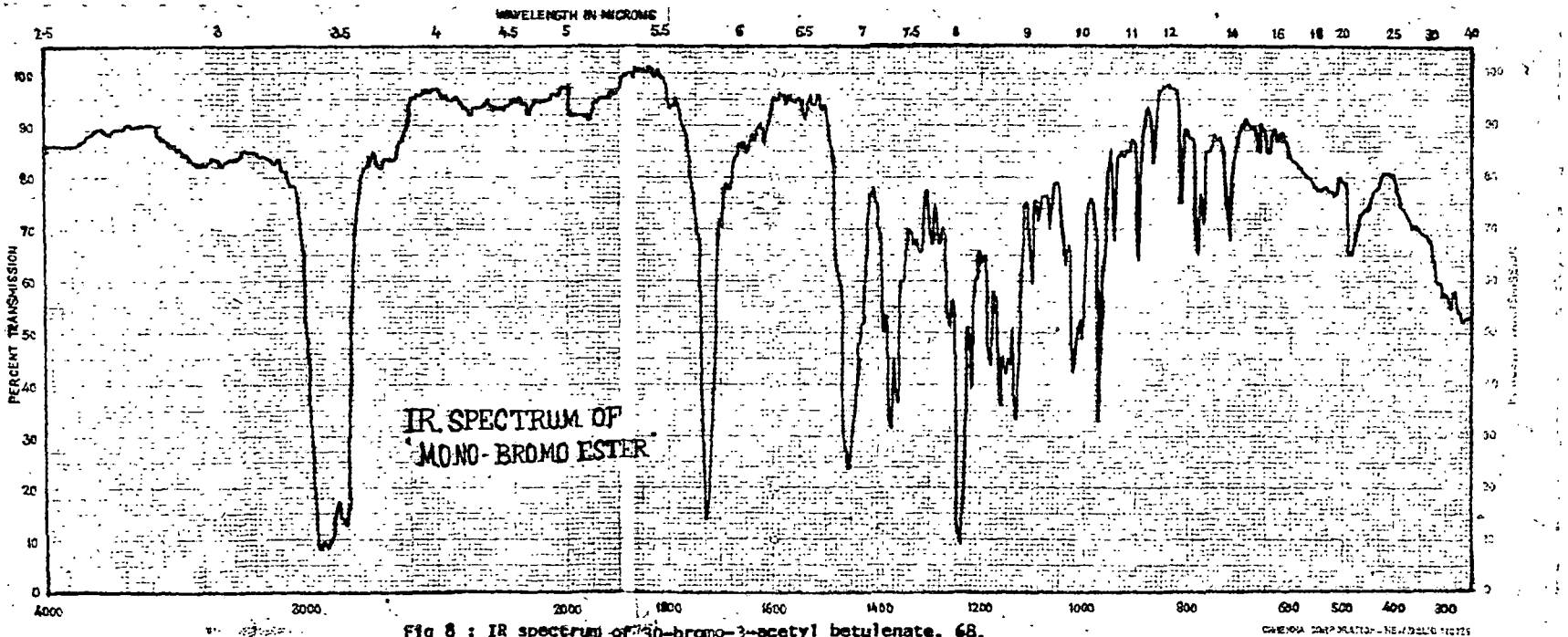
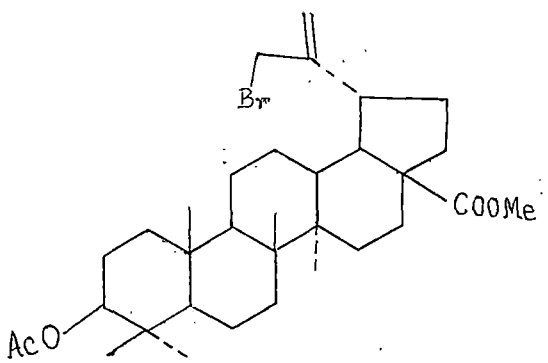


Fig 8 : IR spectrum of 30-bromo-3-acetyl betulenate, 68.

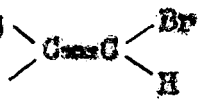
hydrogens as singlet at 3.9 ppm for $-CH_2Br$. At 5.1 ppm two hydrogens were obtained as multiplet due to presence of $=CH_2$. The proton geminal to the 3-acetyl group appeared as a multiplet at 4.5 ppm. From these spectral data the structure of the brome compound has been assigned structure 68 as methyl 30 brome-3 β -acetyl betulenate.

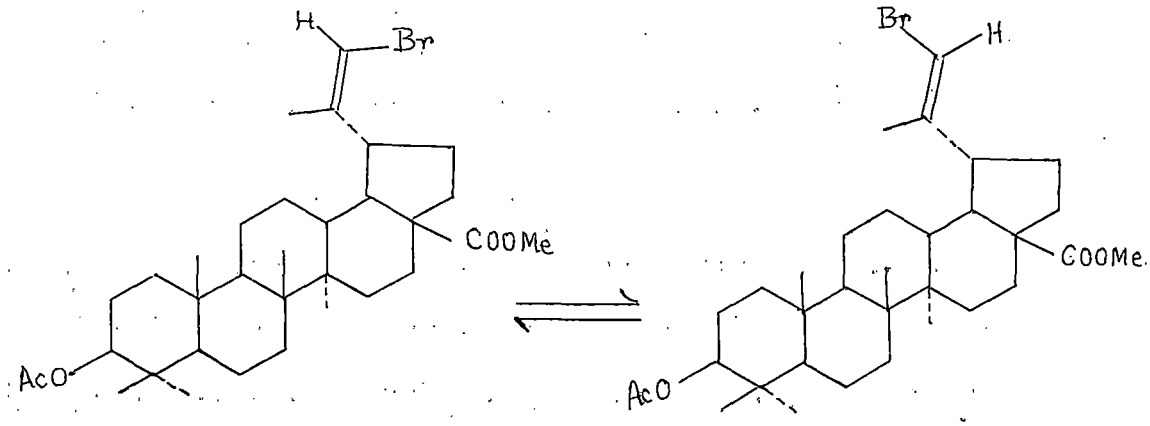


68

The structure has been confirmed by debrominating the compound 68 by treatment with Zn dust and acetic acid and the product that was isolated was found to be identical with methyl 3 β -acetyl betulenate 66 (m.m.p. 200—210).

Further elution of the column with petroleum (4): benzene (1) furnished a second brome compound. This on crystallisation from chloroform-methanol mixture was analysed for $C_{33}H_{51}O_4Br$, m.p. 228—30°, $[\alpha]_D$ +36°. IR spectrum showed

the presence of ester group at 1735 cm^{-1}, acetate carbonyl at 1725, 1240 cm^{-1} and methylene double bond at 1640 cm^{-1} and 900 cm^{-1}. The compound gave positive Beilstein test for bromine. The structure of the compound was derived from the ^1H NMR (Fig.9) spectrum. The peaks in the region 0.78—1.0 ppm indicated the presence of five tertiary methyl groups, the two singlets at 1.7 and 1.78 ppm that was integrated for 3 protons suggested the presence of a methyl group on a double bond, the singlets at 2.05 and 3.7 ppm that were integrated for three protons each showed the existence of acetoxy methyl and carbomethoxyl groups in the compound. The multiplet at 4.48 ppm was due to the proton geminal to the C—3 acetyl group. The presence of singlet at 5.76 and 5.97 ppm integrated for one proton suggested the cis trans isomerism of  group. Hence the structure 68a and 68b is suggested for this bromo compound.



68a 68b

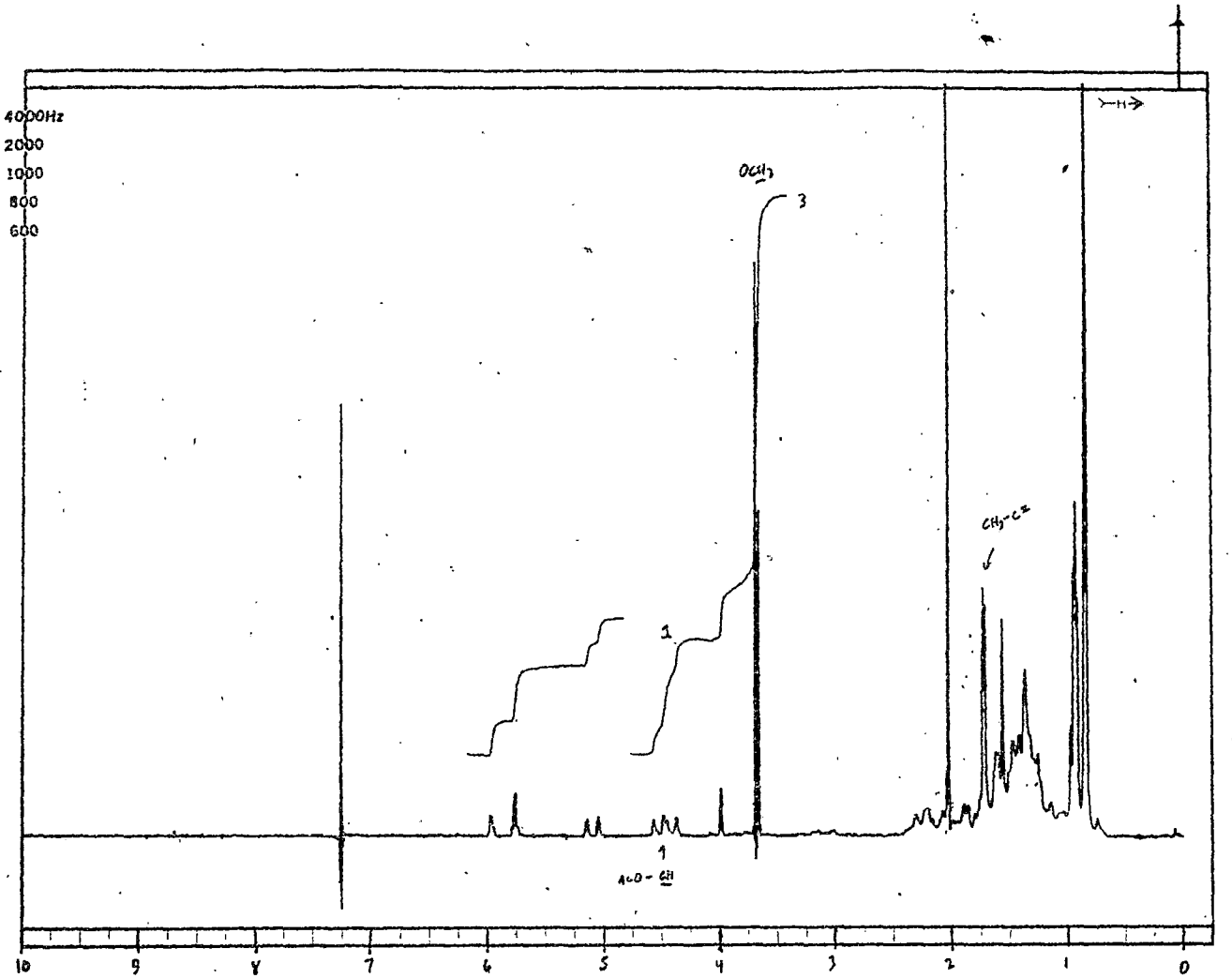


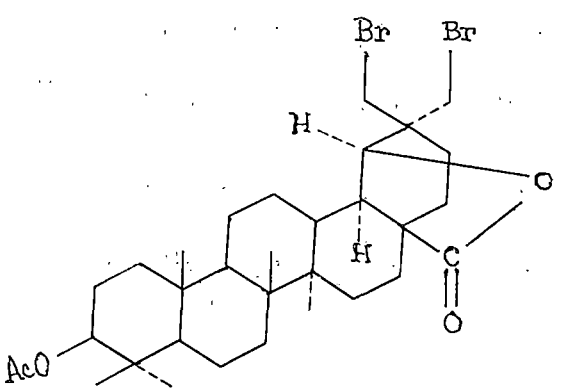
Fig.9: PMR spectrum of 68a ⇌ 68b.

Besides these the presence of peak at 3.9 for CHBr and doublet at 5.1 ppm for $=CH_2$ indicated that there is still some 68 present as impurity.

With the increase of polarity of the solvent petroleum ether (2):benzene (3), the third compound was obtained from the column which was crystallised from a mixture of chloroform-methanol. After crystallisation the compound obtained, gave positive Beilstein test for halogen that indicated the existence of bromine in the compound. The compound was analysed for $C_{32}H_{48}O_4Br_2$, m.p. 303—4°, $[\alpha]_D$ + 47°, showed a negative cotton (ϵ = -0.99) effect in the CD at 218 nm.

IR spectrum (Fig.10) of the compound showed peaks at ν_{max} 1780 cm^{-1} (γ-lactone), 1720, 1240 cm^{-1} (—OCOCH$_3$), 1260 cm^{-1} (—CH_2Br),

The structure of the dibromolactone was proposed to be 69 on the basis of IR, NMR and mass spectral studies.



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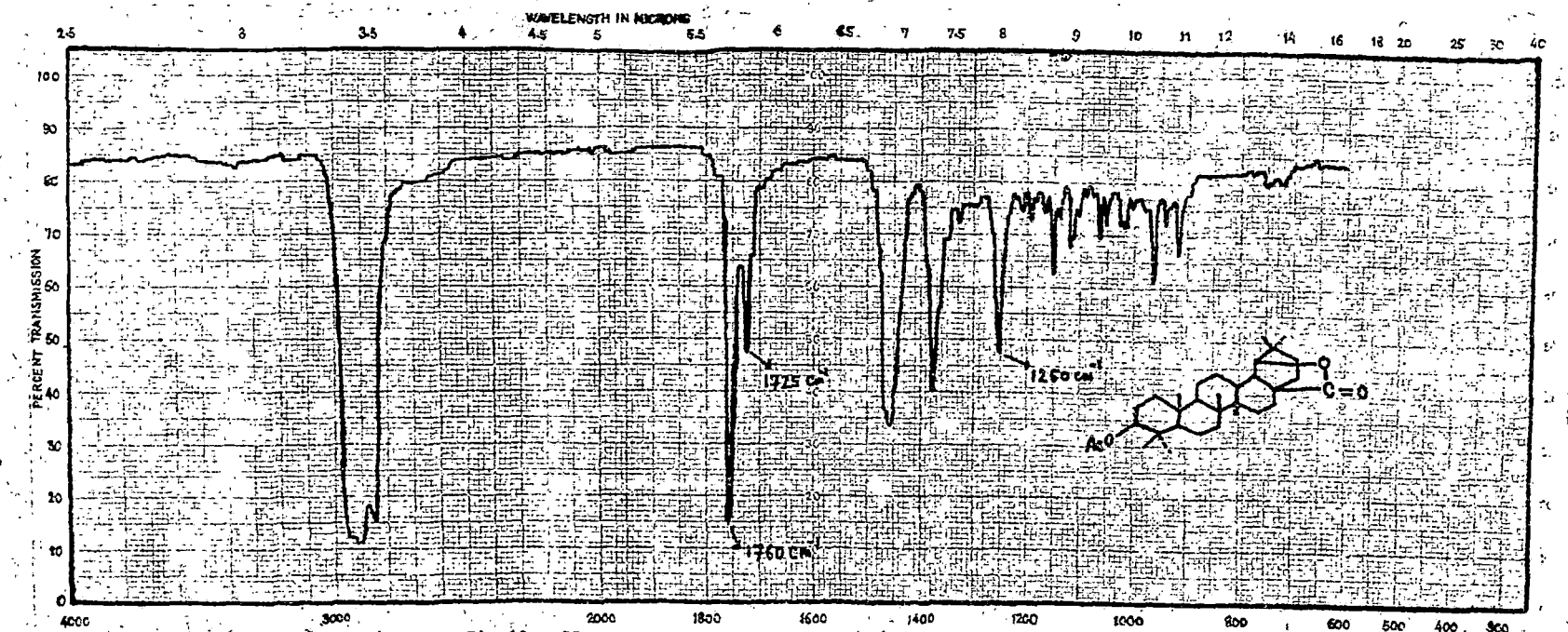


Fig 10 : IR spectrum of dibromolactone 69, obtained from acetyl methyl betulenate.

PMR spectrum (Fig.11) of the compound showed the presence of 5 tertiary methyl groups in the region 0.87 to 0.92 ppm. The singlet at 2.03 ppm integrated for three protons was due to the acetoxy methyl group. Between the region 3.44 to 3.78 ppm there were four peaks. The multiplets centred at 3.44 and 3.65 ppm showed the presence of one proton each. The doublets at 3.73 and 3.78 ppm also showed the presence of one proton each. These peaks were due to 4 hydrogens of C—28—$\underline{H}_2$Br and C_{30}—$\underline{H}_2$Br. The methylene protons present at C_{29} and C_{30} were non equivalent and their position was fixed due to hindered rotation of these groups containing the bulky and highly electronegative bromine atoms which were at the farthest position. The coupling constant was found to be 11 Hz.

At the region 4.34 ppm one peak which was found to be singlet containing only one hydrogen was assumed for the $\underline{H}$—C—OCO i.e. the C—19 proton adjacent to the lactonic oxygen. This value of the PMR spectrum suggested that this proton had either no neighbouring proton or the conformation was such as to have no coupling with the neighbouring proton. A study of the Dreiding model of the compound 69 showed that the dihydral angle between the C—18 and C—19 protons was almost 90° to make it strain free model if the C—18 proton was α-oriented. So the C—19 proton should also be α-oriented.

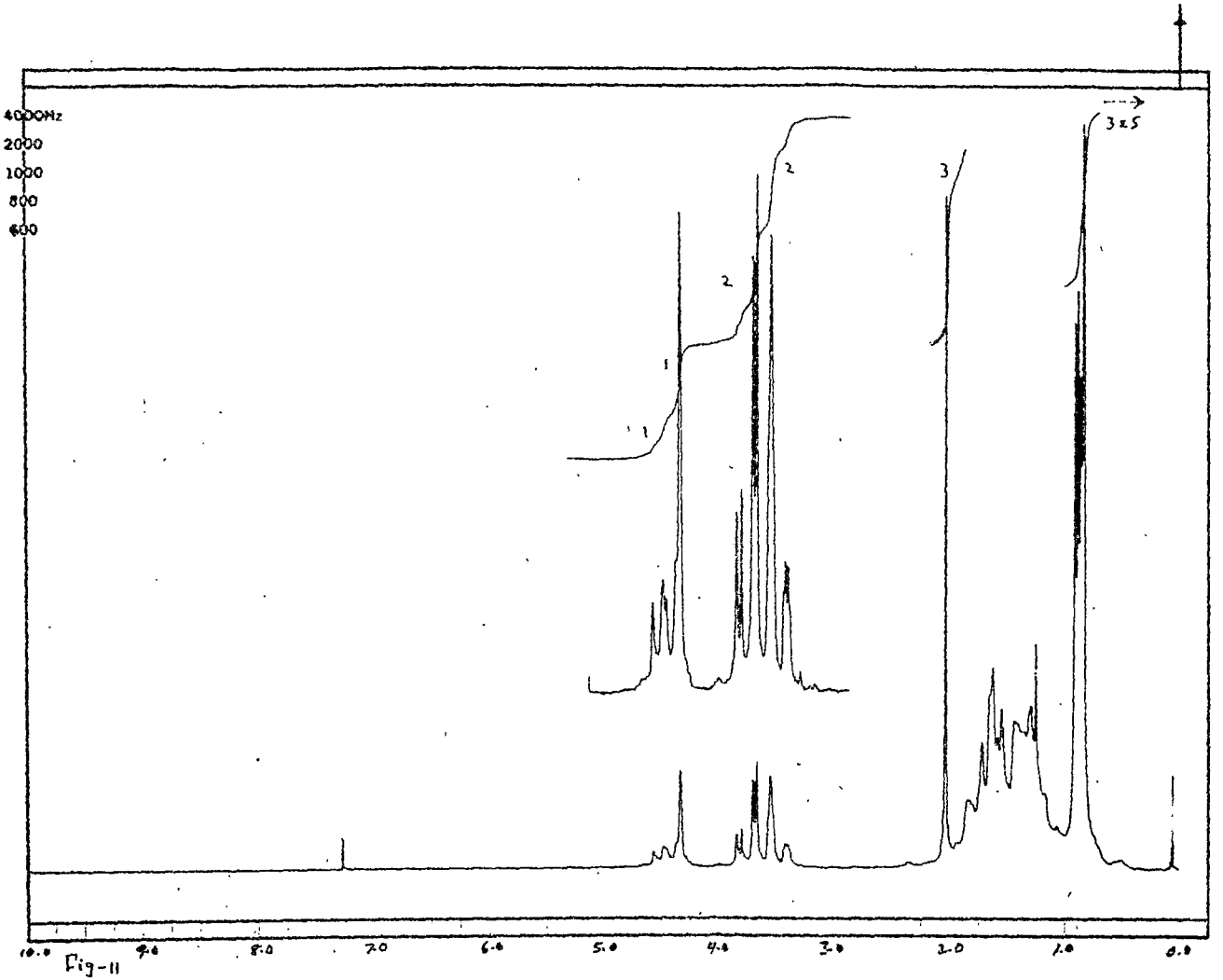


Fig.11: PMR spectrum of dibromo lactone 69.

The peak centred at 4.5 ppm which was found to be a multiplet for only one proton was due to the proton of H—C—O—$COCH_3$.

^{13}C NMR spectrum (Fig.12) of the compound 69 showed a peak at 178.48 ppm which was singlet revealed the presence of a lactonic carbonyl group in a five membered ring. The peak at 171 ppm which was also a singlet indicated the presence of an acetoxy carbonyl group, doublets at 80.77 and 60.95 ppm were due to C—H groups bonded to acetoxy and lactyl oxygen atoms.

Mass spectrum of the compound 69:

Mass peaks (Fig.13) of the compound 69 i.e. 3β-acetyl-29,30-dibromo-18α-olean-28→19β-olide is given in the following way. In the following explanation M_1H represents the protonated molecular mass with the two bromine atoms of isotopic mass 81. M_2H represents the mass with the isotopic bromine 79 and 81 and M_3H for the isotopic mass 79. The molecular ion peaks at 659 (M_1H^+, 10%), 657 (M_2H^+, 19%) and 655 (M_3H^+, 10%) were obtained. The peaks at 599, 597 and 595 might be due to loss of acetic acid from the molecular ion peaks (M_1H^+—CH_3COOH, M_2H^+—CH_3COOH, M_3H^+—CH_3COOH respectively). The peak at 503 might be due to 599—$CH_3{}^{81}Br$ and 597—$CH_3{}^{79}Br$ whose abundance was found to be 5%. The peak

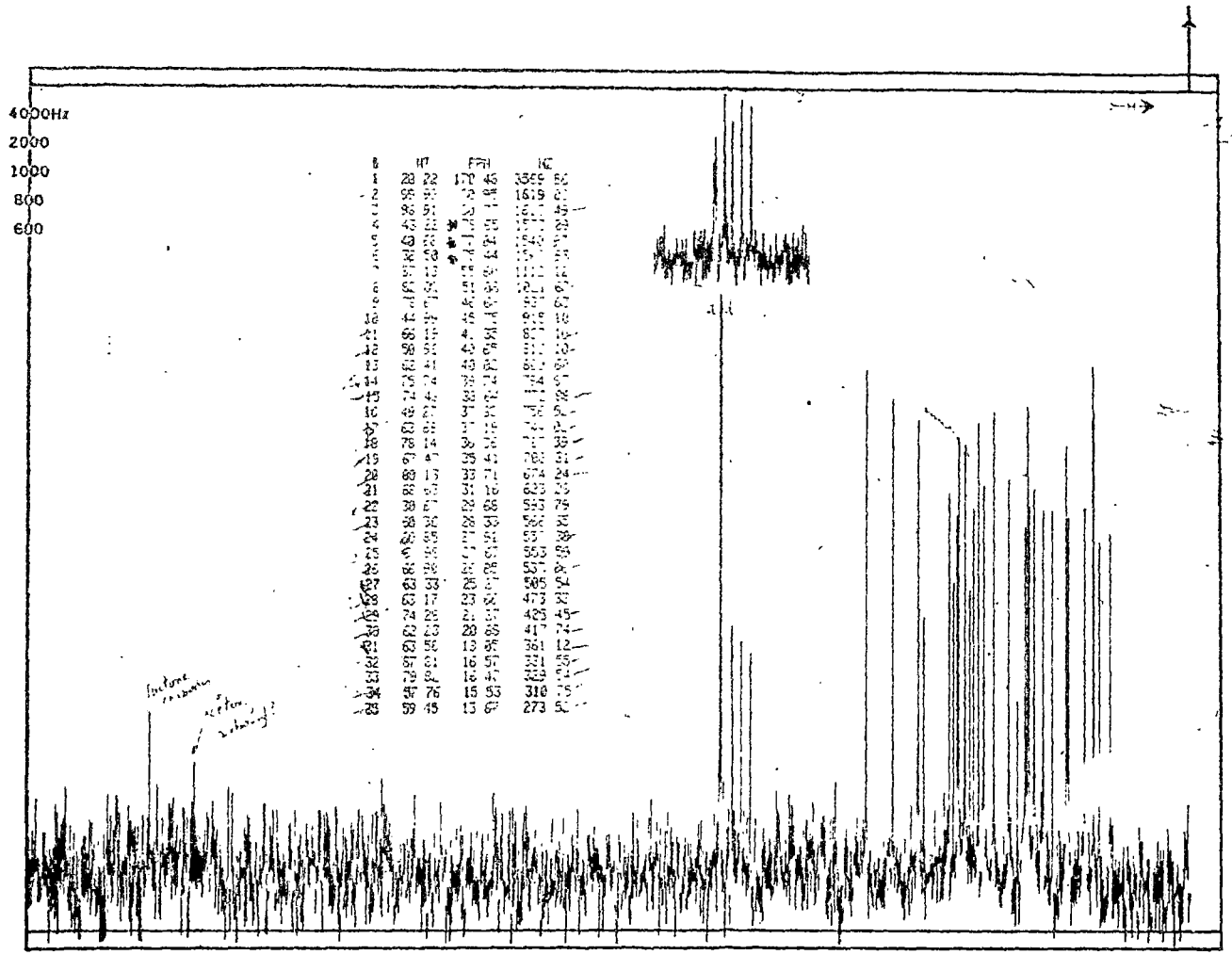


Fig.12: ^{13}C NMR spectrum of dibromolactone 69.

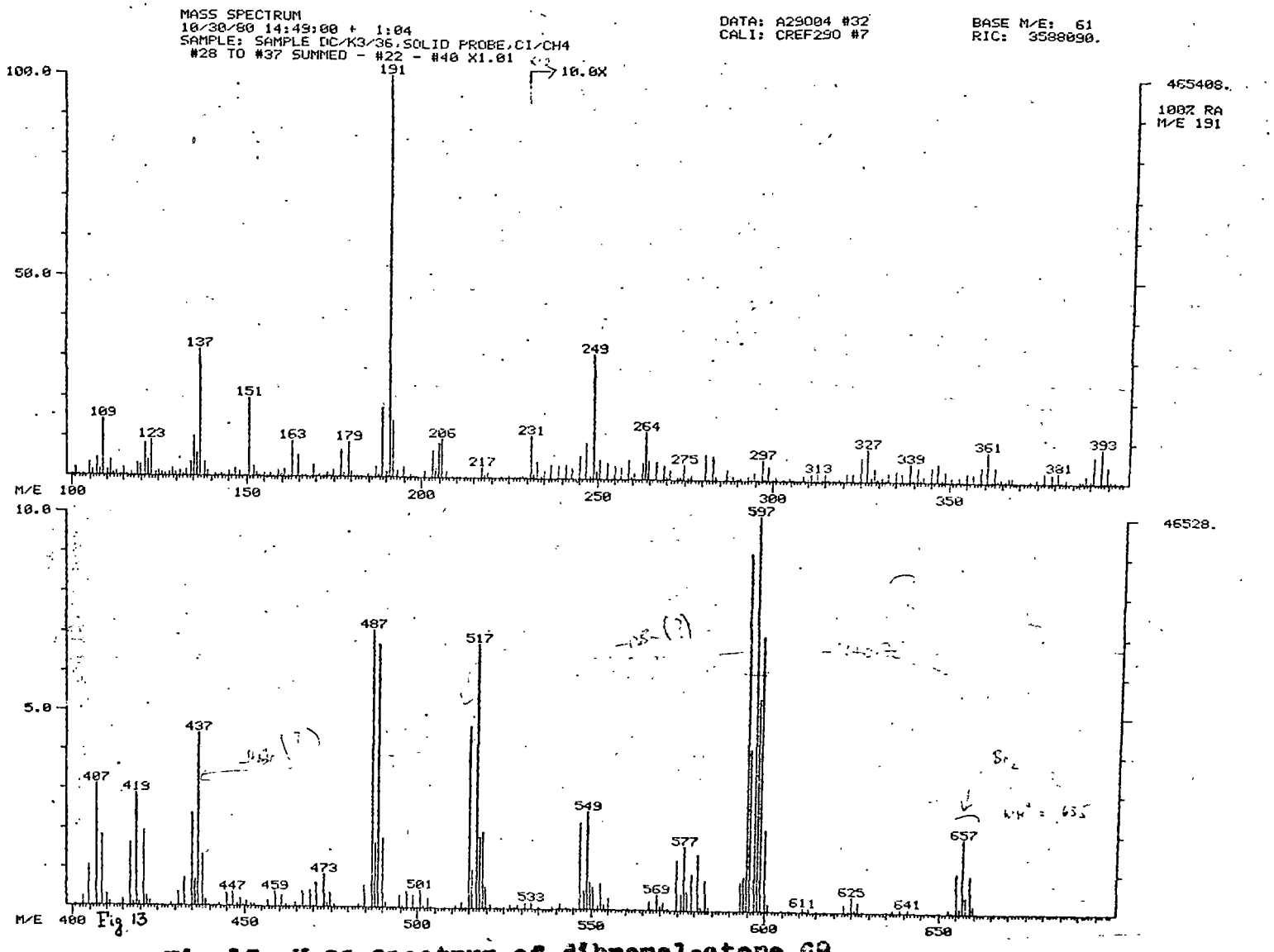


Fig.13: Mass spectrum of dibromolactone 69.

at 501 was assumed for 597—$CH_3{}^{81}Br$ and 595—$CH_3{}^{79}Br$ also of same abundance (5%). The fragment 407 would be for 503—$CH_3{}^{81}Br$ or 501—$CH_3{}^{79}Br$ whose abundancy was 31%. The peak at 517 might be due to 599—$H^{81}Br$ and 597—$H^{79}Br$. The other fragments were 515 (597—$H^{81}Br$ and 595—$H^{79}Br$), 489 (517—CO), 487 (515—CO), 457 (MH—Br_2), 435 (517—$H^{81}Br$ and 515—$H^{79}Br$), 407 (503—$CH_3{}^{81}Br$ and 501—$CH_3{}^{79}Br$ or 457—$H^{79}Br$ and 439—$H^{81}Br$), 249, 191 (base), 189.

The structure of the dibromolactone 69 was finally proved by the following reactions. The dibromolactone was attempted for dehydrobromination by refluxing with dimethyl aniline. It was found that the compound resisted dehydrobromination and the starting material was recovered. This was one supporting evidence of the presence of bromine aton at C—29 and C—30 positions.

Debromination reactions on the dibromolactone 69 was also carried out with the help of Raney Nickel. This reaction furnished a compound 70, $C_{32}H_{50}O_4$, m.p. $\rangle$ 360°, $[\alpha]_D$ +59° was found to be identical with an authentic specimen of 3β-acetyl-oleanan-28→19β olide[42] (m.m.p. and IR comparison). The authentic specimen of 70 was prepared from acetyl betulinic acid. From these observation the structure of dibromolactone can be assigned as 3β-acetyl 29,30 dibromo-18α-H-oleanan-28→19β olide 69.

Treatment of 3β-acetyl betulenic acid[41] 67 with N-bromosuccinimide and dimethyl sulfoxide:

3β-acetyl betulenic acid 67 was taken in chloroform and dimethyl sulfoxide was added. To the resulting solution N-bromosuccinimide was added in portions as in the previous cases. After usual work up the mixture was separated as acid and neutral parts by treatment with alkali. Chromatography followed by crystallisation of the neutral part furnished a compound, $C_{32}H_{48}O_4Br_2$, m.p. 305—4°, was found to be identical (m.m.p. and CO—IR) with 69 i.e. the dibromolactone.

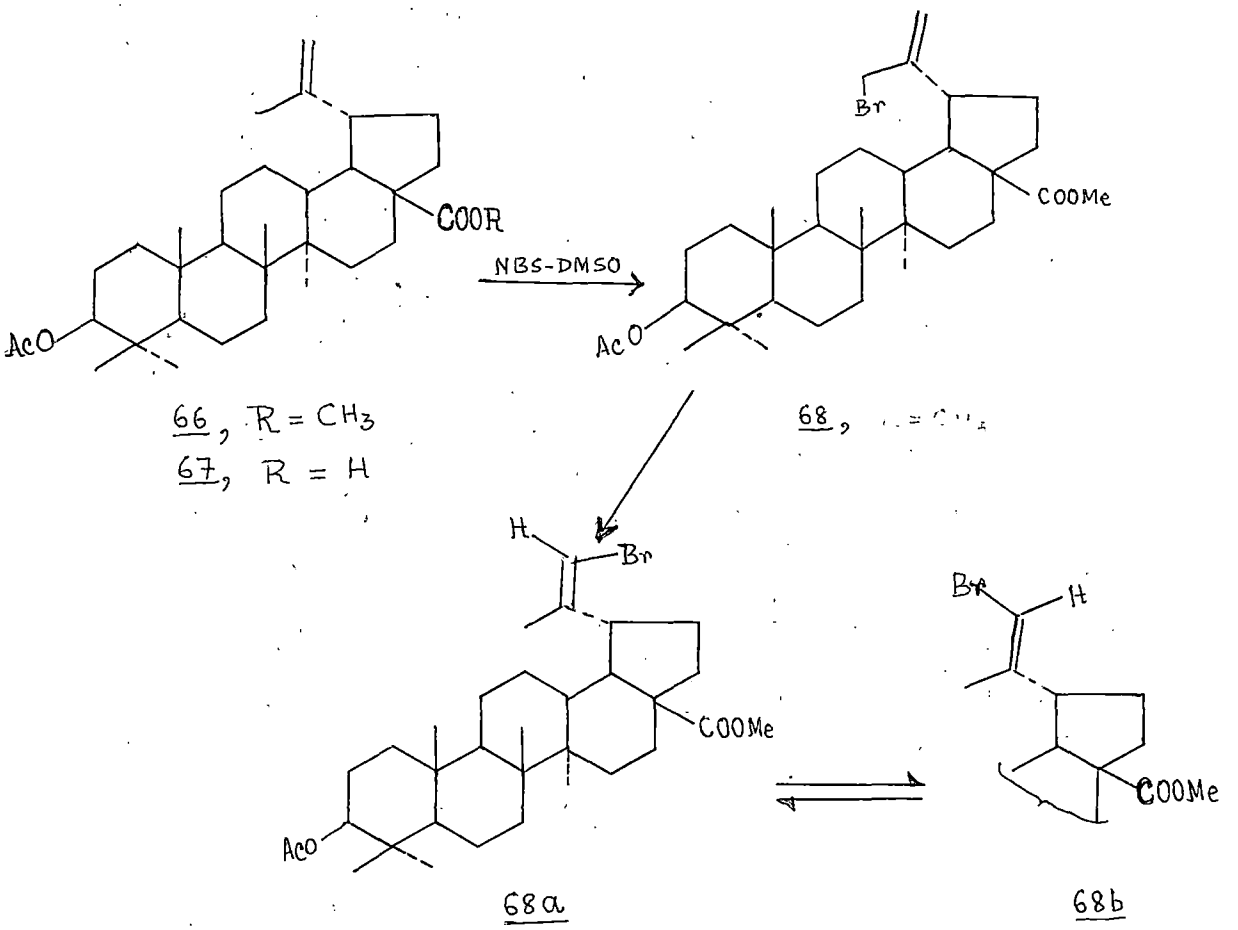
The alkali soluble part was acidified, extracted with ether and esterified with diazomethane. The product so obtained was chromatographed which on elution with solvent petroleum ether(4):benzene(1) furnished two compounds. The first compound was found identical with 68 and the second compound was identified as 68a $\rightleftharpoons$ 68b as discussed in the previous case.

Mechanism of bromination along with lactonisation:

When 66 or 67 was treated with N-bromosuccinimide in presence of dimethyl sulfoxide, first the allylic brominated product 68, was formed. This allylic brominated product might undergo isomerisation to furnish the isomeric cis-trans

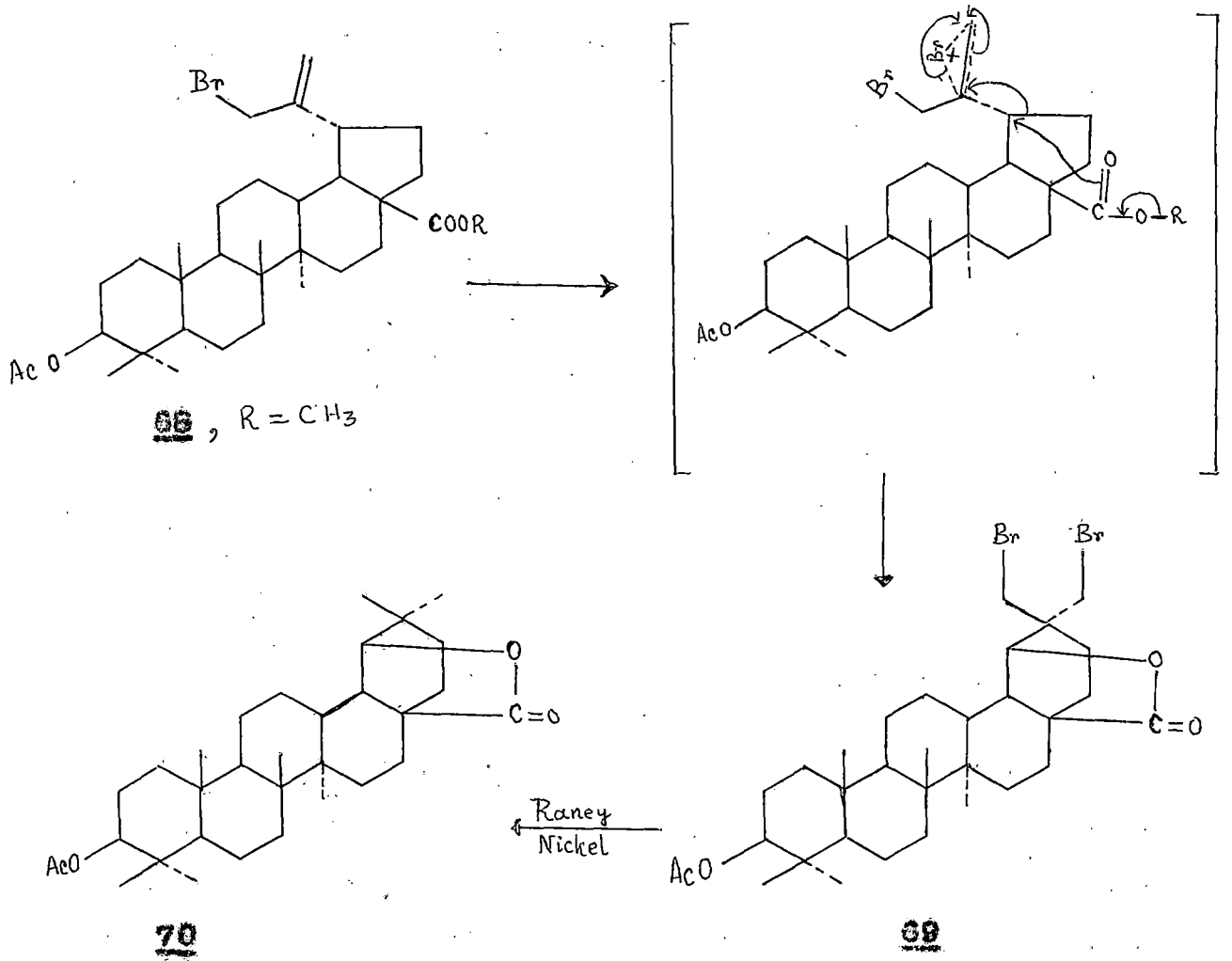
29-bromo compounds 68a ⇌ 68b. Further attack of the bromonium ion on the C—20—30 double bond of 68 caused simultaneously ring expansion and lactonisation. This fact was supported by the experiment with allylic brominated product 68. The isolated compound 68 was treated with N-bromosuccinimide in presence of dimethyl sulfoxide and the same bromolactone 69 was obtained. Again the recovery of starting material in the reaction of 3 β -acetyl methyl betulanate 71 with NBS suggested that the attack of bromonium ion on the double bond initiated the lactonisation (Scheme—10).

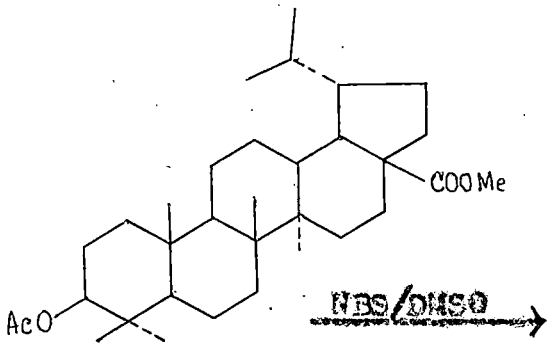
Scheme—10



68a 68b

The allylic brominated product 68 in the presence of NBS would be attacked by the bromonium ion on the olefinic bond at C—20—30 position forming a three membered charged intermediate which immediately rearranged to the stable form by opening of ring system with concerted migration of bond C-19-21 to C-20-21 position and attack of C=O→π bond to the vacant C-19 position forming the lactyl bond with loss of CH_3 or H as cation from the COOR group.





No reaction

71

The above findings show that the esters can be lactonized with NBS in DMSO as their corresponding acids if there is a double bond placed at an appropriate position in the compound. This is perhaps the first finding where the ester group is directly involved in lactone formation without being hydrolysed.