Chapter IV

Experimental

Preparation of acetyl betulinic acid 47a

A mixture of betulinic acid (12 g), pyridine (100 ml) and acetic anhydride (100 ml) was heated on the water bath for 3 hours. The reaction mixture was cooled and poured in ice cold water. The solid thus obtained, was collected by filtration and crystallised from a mixture of chloroform-methanol to give needle shaped crystals of acetyl betulinic acid 47a, m.p. 288-90°, $(\alpha)_D$ + 18.5° (lit. m.p. 290-2° $(\alpha)_D$ + 20.1°).

Found :	C, 76.81; H, 9.72%
Calculated for $C_{32}H_{50}O_4$:	C, 77.06; H, 10.04%

Hydrogenation of 47a : Preparation of acetyl betulanic acid 47b

Acetyl betulinic acid (8 g), dissolved in a mixture of ethyl acetate and acetic acid (100 ml each) was shaken in an atmosphere of hydrogen in presence of PtO_2 catalyst (0.2 g) for 3 hours until absorption of hydrogen ceased. Ethyl acetate was removed by distillation and the solution was diluted with water whereby a white solid (7.5 g) separated out which was collected by filtration. Crystallisation from a mixture of chloroform and methanol furnished crystals, m.p. 304-5° (lit. m.p. 311-12.5°).

Found : C, 76.65; H, 10.55%

Calculated for $C_{32}H_{52}O_4$: C, 76.80; H, 10.40%

U.V. (95% ethanol) : transparent in the region 220 to 300 mμ

I.R. (KBr) : 1242, 1730 and 1700 cm^{-1}.

Lead tetra-acetate oxidation of acetyl betulanic acid 47b : Preparation of hydrocarbon mixture 52a and diacetate 53a:

A mixture of acetyl betulanic acid (6.6 g), dry benzene (60 ml), dry pyridine (3 ml) and lead tetraacetate (9 g.)was heated under reflux under nitrogen atmosphere for 3 hours. The cooled mixture was filtered and the filtrate was concentrated under vacuum to yield a pale yellow gum (6 g.). The ether was dissolved in benzene (20 g) and poured on a column of alumina (500 g) deactivated with 20 ml of a 10% aqueous acetic acid. The chromatogram was developed with petroleum.

Table 1

Chromatography of above gum (6 g)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (1000 ml)	1-20	White solid m.p. 165-73°
Petroleum (300 ml)	21-26	Nil
Petroleum:benzene (4:1) (500 ml)	27-36	White solid m.p. 202-5°

Elution with more polar solvent did not afford any crystalline material

Solid of fractions 1-20 (table 1) (4 g) were collected and was rechromatographed on active alumina (400 g). The chromatogram was developed with petroleum. The solid fraction (4 g) dissolved in benzene (12 ml) was poured on the column and eluted with the following solvents.

Rechromatography of white solid fractions 1-20 (4 g) (Table I):

Table II

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (400 ml)	1-8	Nil
Petroleum:benzene (9:1) (800 ml)	9-24	White solid m.p. 168-74° (same m.p. for each fractions)

Further elution with more polar solvents did not yield any solid

The solid from fractions 9-24 (Table II) (3.8 g) was crystallised from a mixture of chloroform and methanol when crystals of 52a m.p. 170-6°, $(\alpha)_D$ + 28.27° were obtained.

Found : C, 82.14; H, 10.62%

Calc. for $C_{31}H_{50}O_2$: C, 81.94; H, 11.01%

I.R. (KBr) : 1728, 1246 (acetate) 900 and 870 cm^{-1} (trisubstituted double bond).

NMR (60 Mc) : δ.78 to δ.98 (7 methyl), δ1.98 (-O.CO$\underline{CH}_3$), δ 4.4 ($\underline{H}$-C-OCOCH_3), δ5.14 (vinylic proton).

Tetranitromethane : Yellow colour

T.L.C. (benzene:methanol::4:1) : two very close spots R_f 0.82 and 0.81

Estimation of double bond : Perbenzoic acid titration of hydrocarbon mixture 52a

Hydrocarbon mixture 52a (0.055 g) was dissolved in chloroform (5 ml) in a volumetric flask (25 ml). A solution of perbenzoic acid in chloroform (5 ml) was pipetted out and added to the solution and the volume made upto 25 ml by addition of chloroform. A blank solution was similarly prepared by taking 5 ml of perbenzoic acid solution as above in a 25 ml volumetric flask and the volume made up to 25 ml with chloroform. Perbenzoic acid was prepared by the method of Mayer and Manley[28]. 5 ml aliquot portions were taken from each of the above solutions and titrated against standard sodium thiosulphate solution as shown in the table III below.

Strength of sodium thiosulphate solution = 0.0296N

Results

Table III

Time of interval	Blank	Thio required ml.	Reaction mixture	Thio required	Diff. in ml.	No. of double bond
5 minutes	Blank (5 ml) + 2% KI (10 ml) + ACOH (2 ml) + starch soln.	4.8	Aliquot (5 ml) + 2% KI (10 ml) +ACOH (2 ml) starch soln.	4.6	0.2	.26
30 minutes	"	4.8	"	4.3	0.5	.68
1 hour	"	4.8	"	4.1	0.7	.98
2 hours	"	4.8	"	4.1	0.7	.98
4 hours	"	4.8	"	4.1	0.7	.98
8 hours	"	4.8	"	4.1	0.7	.98
16 hours	"	4.8	"	4.1	0.7	.98

Calculation showed that one mole equivalent of perbenzoic acid was consumed within 1 hour, showing thereby that the hydrocarbon mixture 52a contains one double band.

Isolation of the diacetate 53a

Solid fractions 27-36 (1.4 g. Table I) were crystallised from a mixture of chloroform and methanol when fine crystals of 53a m.p. 207-9°, $(\alpha)_D$ + 23.01° were obtained.

Found :	C, 77.21; H, 10.27%
Calculated for $C_{33}H_{54}O_4$:	C, 77.04; H, 10.50%
I.R. (KBr)	: 1726, 1244 cm^{-1} (acetate)
NMR (60 Mc)	: δ 0.78-0.97 (7 CH_3), δ 2.1 (3H, singlet, acetate), δ 4.5 (1H, multiplet, H-C-OCOCH$_3$), δ 1·96 (3H, singlet, acetate)
Mass spectrum	: m/e 514, 454, 411, 249, 189, 175.

Perbenzoic acid titration of the diacetate 53a : Estimation of double bond :

The diacetate 53a (0.072 g) was dissolved in chloroform (5 ml) in a 25 ml volumetric flask. A solution of perbenzoic acid in chloroform (5 ml) pipetted out and added to the solution and the volume made upto 25 ml by addition of chloroform. A blank solution was similarly prepared by taking 5 ml of perbenzoic acid solution as above in a 25 ml volumetric flask and the volume made up to 25 ml with chloroform. 5 ml aliquot portions were taken and titrated

from each of the above solutions against standard sodium thiosulphate solution as shown in the Table IV below.

Strength of sodium thiosulphate solution = 0.0265(N).

Results

Table IV

Time of interval	Blank	Thio required ml.	Reaction mixture	Thio required	Diff. in ml.	No. of double bond
5 minutes	Blank (5 ml) +2% KI (10 ml)+ACOH (2 ml) + starch	4.3	Aliquot (5 ml) +2% KI (10 ml) +ACOH (2 ml) starch soln.	4.3	0.0	0.0
30 minutes	"	4.3	"	4.3	0.0	0.0
1 hour	"	4.3	"	4.3	0.0	0.0
4 hours	"	4.3	"	4.3	0.0	0.0
8 hours	"	4.3	"	4.3	0.0	0.0
16 hours	"	4.3	"	4.3	0.0	0.0
30 hours	"	4.3	"	4.3	0.0	0.0
48 hours	"	4.3	"	4.3	0.0	0.0

It was found that there was no uptake of perbenzoic acid even in 48 hours indicating absence of unsaturation in 53a.

Hydrolysis of 52a and preparation of alcohol 52b

To a solution of 1.5 g. of the acetate 52a in benzene (20 m) 5% methanolic potassium hydroxide solution (100 ml) was added , and the mixture was refluxed on water bath for 3 hours. After removal of benzene and methanol, the residue was diluted with water when white solids separated out. The solid was filtered and crystallised from a mixture of chloroform and methanol to afford needles of crystals m.p. 110-4°, $(\alpha)_D$ + 36.6°.

Found :	C, 84.22; H, 11.34%
Calculated for $C_{29}H_{48}O$	C, 84.46; H, 11.65%
I.R. ($CHCl_3$) :	3400 (hydroxyl), 890, 870, 815 cm^{-1} tri-substituted double bond.

Chromic acid-pyridine oxidation of 52b : Preparation of the ketone 52C

The alcohol 52b (1.1 g.) dissolved in pyridine (10 ml) was added to a CrO_3-Py complex prepared from pyridine (12 ml) and CrO_3 (1.2 g.) at 10°C and the mixture was allowed to stand for 15 hours. Excess of CrO_3 was destroyed by adding methanol (4 ml), diluted with ethyl acetate and filtered. Ethyl acetate was removed and the concentrate was taken up in ether. The organic layer was washed with hydrochloric acid (5%), then with water until neutral and dried

(Na_2SO_4). Removal of ether gave a gummy residue (1 g.). The above residue was chromatographed over a column of active alumina (60 g.) The chromatogram was prepared with petroleum and the product dissolved in benzene (5 ml) was poured on the column and eluted with the following solvents (Table V).

Table V

Eluent	Fractions 50 ml each	Residue
Petroleum (100 ml)	1-2	Nil
Petroleum:benzene (4:1) (150 ml)	3-5	Solid, m.p. 140-6°

Further elution with more polar solvents did not yield any solid.

Fractions 3-5 (0.75 g. Table V) on crystallization from chloroform and methanol furnished crystals of 52C, m.p. 145-50°, $(\alpha)_D$ + 48.19°. Further crystallisation did not raise the melting point.

Found : C, 85.02; H, 11.05%

$C_{29}H_{46}O$ requires : C, 84.87; H, 11.22%

U.V. (95% ethanol) : 285 mμ (ε 75)

I.R. (KBr) : 1705 (6-ring ketone), 887, 875, 810 cm^{-1} (trisubstituted double bond)

Huang Minlon reduction of ketone 52C : Preparation of the hydrocarbon 52d

A mixture of ketone 52C (0.6 g), diethylene glycol (200 ml) and hydrazine hydrate (90%, 10 ml) was refluxed for 30 minutes. Solid KOH (0.8 g) was added and the mixture was further refluxed for one hour. The condenser was removed and the mixture was heated to 195°. After refluxing for another 2½ hours the reaction mixture was cooled and diluted with water when a solid separated out. The solid (0.5 g.) was chromatographed over a column of active alumina (40 g.). The chromatogram was developed with petroleum. The solid (0.5 g.) dissolved in petroleum (5 ml) was placed over the column and was eluted with the following solvents (Table VI).

Table VI

Eluent	Fractions 25 ml each	Residue on evaporation
Petroleum (100 ml)	1-4	Solid m.p. 152-5°
Further elution with more polar solvents did not afford any solid		

Fractions 1-4 (Table VI) were collected (0.35 g.) which on crystallization from chloroform and methanol afforded crystals of 52d (revised 54) m.p. 156-8°, $(\alpha)_D$ - 7.00° (lit.[27] m.p. 153-4°, $(\alpha)_D$ - 12.0°).

Found : C, 87.44; H, 11.98%

$C_{29}H_{48}$ requires : C, 87.87; H, 12.12%

I.R. (KBr) : 1680, 812, 800 cm^{-1}, (trisubstituted double bond)

NMR (60 Mc) : δ 0.75 to δ 1.05 (7 CH_3) and δ 5.2 (vinylic proton)

Acid isomerisation of hydrocarbon mixture 54 to $\Delta^{13(18)}$ hydrocarbon 55:

The hydrocarbon mixture 54 (0.3 g.) was dissolved in acetic acid (21 ml) and 2N sulfuric acid (2.1 ml) was added to it. The reaction mixture was refluxed for 2½ hours. It was then cooled in ice bath and diluted with water. The solid which separated out was collected by filtration. The yellow solid (0.2 g.) was chromatographed on an active alumina (20 g.) column. The column was developed in petroleum and the substance dissolved in petroleum was placed on the column (Table VII).

Table VII

Chromatograph of above yellow solid (0.2 g.)

Eluent	Fractions 25 ml each	Residue on evaporation
Petroleum (100 ml)	1-4	Crystalline solid m.p. 183-5°C

Further elution with more polar solvents did not afford any solid

The fractions 1-4 (Table VII) were combined and crystallised from chloroform and methanol mixture to afford fine prisms of 55, m.p. 192-3°, $(\alpha)_D$ + 67.2 (lit.[27] m.p. 188-9°, $(\alpha)_D$ + 59°).

Found :		C, 87.53; H, 12.01%
Calculated for $C_{29}H_{48}$:		C, 87.87; H, 12.12%
I.R. (KBr)	:	845, 1615-25 cm^{-1} (broad)
NMR (60 Mc)	:	δ 0.62 to δ 1.00 (7 CH_3), no peak corresponding to vinylic proton
Mass spectrum	:	m/e 396, 353, 204, 191, 161.

LAH reduction of 53a : Preparation of diol 53b

The diacetate 53a (0.2 g) was dissolved in ether (50 ml) and to it added LAH (0.5 g.) in ether (30 ml). The reaction mixture was refluxed for 3 hours. Excess reagent was destroyed with saturated sodium sulphate in cold. It was then extracted with ether, washed with water and dried (Na_2SO_4). Evaporation of the solvent left a white residue (0.15 g.) which was recrystallised from methanol to furnish crystals m.p. 230-2°.

Found :		C, 80.65; H, 11.71%
Calculated for $C_{29}H_{50}O_2$:		C, 80.93; H, 11.63%
I.R. (Nujol)	:	3440 cm^{-1} (hydroxy)
Mass spectra	:	m/e 430, 412, 369, 207, 193, 189, 175

Acetylation of 53b : Preparation of hydroxy acetate 53c

The diol 53b (0.1 g) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) at room temperature in the usual manner. The reaction mixture on usual work up afforded a solid (0.08 g) which was crystallised from a mixture of chloroform and methanol to furnish crystals m.p. 270-1°.

Found :		C, 78.68; H, 11.12%
Calculated for $C_{31}H_{52}O_3$	:	C, 78.81; H, 11.01%
I.R. (Nujol)	:	3500 (hydroxy), 1730 and 1245 cm^{-1} (acetate).

Attempted chromic acid oxidation of 53C

The hydroxy acetate 53c (0.2 g) was dissolved in pyridine (5 ml) and was added to CrO_3-Py complex prepared from pyridine (2 ml) and CrO_3 (0.2 g.) and was kept at room temperature for 20 hrs. The crude product (140 mg) obtained by working up in the usual way was chromatographed over an alumina column (.10 g.) deactivated by 0.4 ml of 10% aqueous acetic acid. The chromatogram was prepared with petroleum ether and the product dissolved in benzene (6 ml) was poured on the column. It was eluted with the following solvents (table X).

Table X

Eluent	Fractions 50 ml each	Residue
Petroleum	1-2	Nil
Petroleum:benzene (3:1)	3-4	Nil
Petroleum:benzene (1:1)	5-6	Solid, m.p. 266-8°C

Further elution with more polar solvent did not yield any material

Fractions 5-6 (table X) (120 mg) on recrystallisation from chloroform and methanol furnished crystals m.p. 269-71°. It was found to be identical with the starting material by m.m.p. determination and I.R. comparison.

References

1. G. Buchi, R.E. Erikson and N. Wakabayashi; J. Amer. Chem. Soc. 83, 927, 1961.

2. K. Nakanishi, Y. Y. Lin, H. Kakisawa, H.Y. Hsu and H.C. Hsill; Tetrahedron Letters, No. 22, 1451, 1963.

3. C.R. Bennet and R.C. Cambie; Tetrahedron, 23, 927, 1967.

4. L.H. Zalkow and D.R. Brannon; J. Chem. Soc., 5497, 1964.

5. J.W. Huffman and P.G. Arapakos; J. Org. Chem; 30, 1604, 1965.

6. W.A. Ayer, C.E. McDonald and J.B. Stothers; Canad. J. Chem., 41, 1113, 1963.

7. W.H. Starnes; J. Amer. Chem. Soc. 86, 5603, 1964.

8. C.A. Grob and A. Weiss, Helv. Chim. Acta., 43, 1340, 1960.

   J. Kazan and F.D. Greene, J. Org. Chem., 28, 2965, 1963.

9. W.A. Mosher and C.L. Kehr., J. Amer. Chem. Soc., 75, 3172, 1953; 82, 5342, 1960.

   W.A. Mosher, C.L. Kehr and L.W. Wright, J. Org. Chem., 26, 1044, 1961.

10. M.S. Kharasch, H.N. Friedlander and W.H. Urry, J. Org. Chem., 16, 533, 1951.

11. D. Benson, L.H. Sutcliffe and J. Walkby, J. Amer. Chem. Soc., 81, 4488, 1959.

12. J. Halpern and S.M. Taylor, Discussions Faraday Soc., 29, 3453, 1964.

13. E.J. Corey and J. Casanova Jr, J. Amer. Chem. Soc., 85, 165, 1963.

14. S. Winstein and D. Trefan, J. Amer. Chem. Soc., 74, 1147, 1952.

15. J.A. Berson, D.T. Olsen and J.S. Walia, J. Amer. Chem. Soc., 82, 5000, 1960.

16. L.L. McCoy and A. Zagalo, J. Org. Chem., 25, 824, 1960.

17. J.K. Kochi, J. Amer. Chem. Soc., 87, 165, 3609, 1965.

18. S. Mon and J. Lodge, J. Org. Chem., 29, 3453, 1964.

R.E. Patch; ibid, 28, 276, 1963.

J.P. Cordner and K.K. Pausacher, J. Chem. Soc., 107, 1953.

H.B. Henbest, Ann. Rept. Progr. Chem. (Chem. Soc. London) 53, 146, 1956.

R.M. Monarty and K. Kapadia, Tetrahedron Letters, 1965, 1964; 465, 1965.

19. K. Heusler and J. Kalvoda, ibid, 1001, 1964.

V.M. Micovic et al. ibid, 2091, 1963; Tetrahedron 20, 2279, 1964.

20. D. Hauser, K. Schaffner and O. Jeger, Helv. Chim. Acta., 47, 1883, 1964.

D. Hauser, K. Heusler, J. Kalvoda, K. Schaffner and O. Jeger, ibid, 47, 1961, 1964.

K. Heusler and J. Kalvoda, Angew. Chem. Inter. Ed. Engl., 3, 525, 1964.

21. F.D. Greene et al. J. Org. Chem. 28, 55, 1963.

C. Walling and A. Padwa, J. Amer. Chem. Soc., 85, 1593, 1963.

22. P. Gray and A. Williams. Chem. Rev., 59, 239, 1959.

J.K. Kochi, J. Amer. Chem. Soc., 84, 1193, 1962.

23(a) E.J. Corey and R.W. White, ibid, 80, 6686, 1958.

(b) P.D. Bartlett and T.G. Traylor, ibid, 83, 85, 1961.

80, 4954, 1958.

(c) R.N. Sneen and N.P. Matheny ibid, 86, 5503, 1964.

24. J. Simonsen and W.C.J. Ross, 'The Terpenes' Cambridge University Press, 1967, Vol. V, p. 317.

25. J. Simonsen and W.C.J. Ross, "The Terpenes" Cambridge University Press, 1967, Vol. IV, p. 300.

26. H. Budzikiewicz, J.M. Willson and C. Djerassi, J. Amer. Chem. Soc., 85, 3688, 1963.

27. B. Dietrich and O. Jeger, Helv. Chim. Acta., 33, 711, 1950.

28. J.R. Mayer and N.C. Manley, J. Org. Chem., 29, 2099, 1964.