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CHEMICAL EXAMINATION OF *CENTELLA ASIATICA* LINN—III.

CONSTITUTION OF BRAHMIC ACID*

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Abstract—The structure of brahmic acid has been elucidated to be 2α,3β,6β,23-tetrahydroxy-28-oic-urs-12-ene (Ia) by physical and chemical data, degradative studies and a direct correlation with asiatic acid.

INTRODUCTION

IN EARLIER communications[1,2] we reported the isolation of the triterpene acids, brahmic acid, iso-brahmic acid and betulic acid, along with two saponins, brahmoside and brahminoside, from this plant. These saponins were shown to be tri- and tetra-glycosides of brahmic acid. The present paper describes further studies on brahmic acid leading to the elucidation of its structure. It was shown earlier that brahmic acid, $C_{30}H_{48}O_6$, is an α-amyrin derivative containing three acetylable hydroxyls one of which is a primary hydroxyl. It contained an inert double bond, a glycol group and it yielded a methyl ester which could not be hydrolysed under normal conditions.

RESULTS AND DISCUSSION

In the mass spectrum the molecular ion (M^+) at *m/e* 518 confirmed the molecular formula given earlier. The fragment *m/e* 262 (95 per cent), obtained as the result of retro-Diels-Alder (RDA) fragmentation,[3] undergoes a further loss of 59 mass units ($COOCH_3$) yielding the ion *m/e* 203 (100 per cent) (confirmed by metastable at 157·3).

This species loses 70 mass units to give the highly ionized ion *m/e* 133 (75 per cent) (confirmed by metastable ion at (87·1) and, as usual, is accompanied by an intense ion peak *m/e* 189 (81 per cent) formed from species *m/e* 262 by a one step concerted elimination of $COOCH_3$ and another 14 mass units, presumably those of C-17. Another fragment of *m/e* 249 (35 per cent) arises from the molecular ion (M^+) by one hydrogen transfer and the cleavage of allylically activated bond. All the above fragments pertain to rings C, D and E, and clearly indicate that the double bond is present at C_{12-13}, the carbmethoxy group is attached to C-17 and there is no hydroxy group in these rings of methyl brahmate.

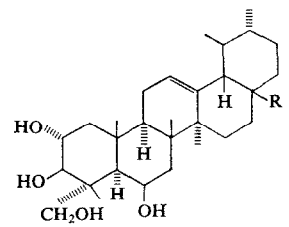
The second component, *m/e* 256, of RDA fragmentation arising out of rings A and B is present to a very little extent (5·6 per cent) but its dehydrated fragment, *m/e* 238 (30 per cent),

* Communication No. 1169 from the Central Drug Research Institute, Lucknow.

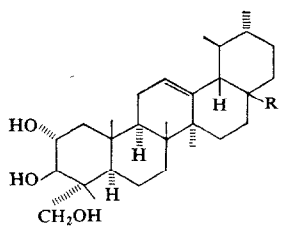
[1] R. P. RASTOGI, B. SARKAR and M. L. DHAR, *J. Sci. Ind. Res.* **19B**, 252 (1960).
[2] R. P. RASTOGI and M. L. DHAR, *Indian J. Chem.* **1**, 267 (1963).
[3] H. BUDZIKEIWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

is fairly abundant. There is another ion, *m/e* 255 (6·5 per cent), which is formed from the molecular ion (M^+) by the transfer of one hydrogen from the methyl at C-8 and the fragmentation of ring C.

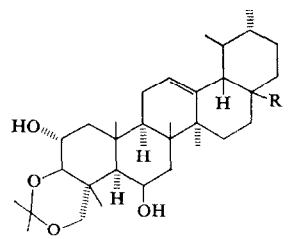
Methyl brahmate (Ib) could be hydrolysed only under the very drastic conditions described for a carbmethoxy group at C-17 in triterpenoids;[4] this confirms the placement of the carboxyl group at C-17 in brahmic acid. Ib formed an isopropylidene derivative (III) indicating the presence of 1,3-glycol system in the molecule. Its reduction with $LiAlH_4$ to a crystalline pentol, named as "brahmol" (Ic), which formed a crystalline tetra-acetate confirmed that brahmic acid contains three acetylable and one unacetylable hydroxyl groups.



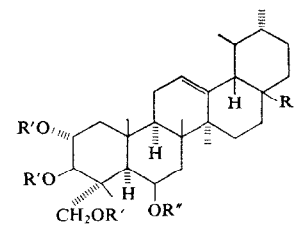
(Ia) R=COOH
(Ib) R=$COOCH_3$
(Ic) R=CH_2OH



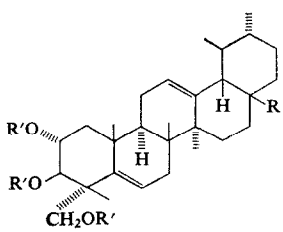
(IIa) R=COOH
(IIb) R=$COOCH_3$



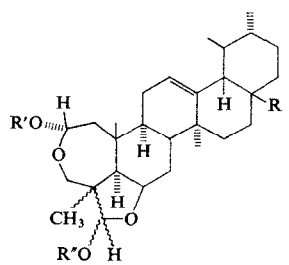
(III) R=$COOCH_3$



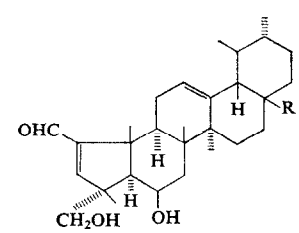
(IVa) R=$COOCH_3$, R′=$COCH_3$; R″=H
(IVb) R=$COOCH_3$, R′=$COCH_3$, R″=$COCH_3$
(IVc) R=CH_2OAc, R′=$COCH_3$, R″=H



(V) R=$COOCH_3$, R′=$COCH_3$



(VIa) R=$COOCH_3$, R′=R″=H
(VIb) R=CH_2OH, R′=R″=H
(VIc) R=$COOCH_3$, R′=R″=$COCH_3$



(VIIa) R=$COOCH_3$
(VIIb) R=CH_2OH

Both methyl brahmate (Ia) and brahmol (Ic) consumed 1 mole of sodium periodate and the oxidation product did not show i.r. absorption in 1700 cm^{-1} region indicating the absence of a keto group in a six-membered ring. These have been shown to be dihemiacetal derivatives (VIa, VIb) on the basis of acetyl derivative and NMR data. The hemiacetal formation

[4] C. DJERASSI, G. H. THOMAS and H. MONSIMER, *J. Am. Chem. Soc.* **77**, 3579 (1955).

was confirmed by the chromic acid oxidation of VIa to a dilactone derivative showing i.r. bands at 1760, 1728 cm^{-1} for γ and seven member lactones respectively and 1742 cm^{-1} for the ester group. A similar observation has been reported recently in the case of arjunolic acid[5] and seems to be of diagnostic potentiality for C-2, C-3 and C-23 trihydroxy triterpenes. The isopropylidene derivative (III), on the other hand, did not react with sodium periodate. This establishes that one of the hydroxyls of the 1:2 glycol system is also a component of the 1:3 glycol which is responsible for the acetonide formation. In view of the above, the structures of centoic acid etc.,[6] which were proposed mainly on the assumption of the periodate oxidation product as a keto-aldehyde, are now open to doubt.

VIa formed a monosemicarbazone presumably as a result of the opening of only one hemiacetal ring under the reaction conditions. This was proved by the unreactivity of the diacetyl derivative (VIc) to semicarbazone formation under similar conditions of reaction because of the stabilization of the hemiacetal rings. Both VIa and VIb also undergo cyclization[7] in the presence of catalytic amounts of piperidine in acetic acid to give VIIa and VIIb containing α:ß-unsaturated aldehyde group. Such a condensation could only occur if as an initial step the hemiacetal rings open up to give the dialdehyde which contains at least one methylene α to either of the aldehyde groups. In brahmic acid the possible positions for the glycol system which would satisfy these requirements are 2,3 and 5,6. The latter alternative is untenable because the isopropylidene derivative of such a compound would still be oxidizable with periodate and the resultant product would be a keto-aldehyde. On the other hand, a 2,3 glycol system would be consistent with the observed facts, and thus in brahmic acid the three hydroxyls are assigned to C-2, C-3 and C-23 as in the case of asiatic acid. A study with the stereomodels show that the five-membered hemiacetal ring would be easily formed if the fourth hydroxyl is situated ß on C-6.

The acetylation of methyl brahmate (Ia) under different conditions gave products A, B and C as shown in the Table 1.

TABLE 1. ACETYLATION OF METHYL BRAHMATE WITH ACETIC ANHYDRIDE

Conditions	Derivatives formed	R_f (TLC)*
Pyridine at room temperature	A, C (1:15)†	0·72 and 0·43
p-Toluene sulphonic acid at room temperature	A, B (1:20)†	0·72 and 0·66
Sodium acetate at reflux temperature	B (A, C in small amounts)	0·66, 0·72 and 0·43

* Solvent system—benzene:methanol (98:2).
† Figures in parentheses indicate the ratio of the derivatives.

The acetyl derivative C analysed for triacetate (IVa). Its NMR spectrum indicated the existence of three AcO groups (3H at 1·98, 2·03 and 2·06 ppm) one of which is primary (2H at 3·74 and 3·95 ppm, AB quartet) and a carbomethoxyl function (3H doublet at 3·62 ppm, $J=1$ c/s. The hydroxyl H appeared as a broad signal at 4·38 ppm whereas the two protons on C-2 and C-3 bearing the AcO groups and one olefinic proton on C-12 showed up as multiplet in the region of 4·83–5·56 ppm.

[5] S. SASAKI, H. C. CHIANG, K. HABAGUCHI, H. Y. HSU and K. NAKANISHI, *Bull. Chem. Soc., Japan* **39**, 1816 (1966).
[6] S. C. BHATTACHARYYA, *J. Indian Chem. Soc.* **33**, 579, 630, 893 (1956).
[7] R. B. WOODWARD, F. SONDHEIMER, D. TAUB, K. HEUSLER and W. M. MCLAMORE, *J. Am. Chem. Soc.* **74**, 4223 (1952).

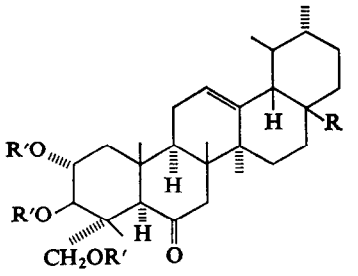
The product B analysed for tetraacetyl methyl brahmate (IVb) while A analysed for anhydrotriacetyl methyl brahmate (V). The structure of V was confirmed by its NMR spectrum showing three AcO groups (6H at 1·98 and 3H at 2·03 ppm) along with a four proton multiplet (4·91–5·75 ppm) unlike that of compound C. This additional proton is attributed to the vinylic proton produced as a result of creation of a trisubstituted double bond. No hydroxyl proton was present in the spectrum.

The conversion of triacetyl methyl brahmate (IVa) into its anhydro derivative (V) was readily and quantitatively achieved by thionyl chloride–pyridine or methane sulphonyl chloride–sulphur dioxide.[8] With phosphorus oxychloride the conversion to V was to the extent of 60 per cent only. These acetylation experiments clearly established that the fourth and unacetylable hydroxyl group of brahmic acid was secondary and hindered in nature. The keto triacetyl methyl brahmate reported earlier,[1] was found to show two i.r. absorption peaks at 1675 and 1707 cm^{-1}, arising from a α:ß-unsaturated ketone and a six-membered carbonyl respectively and was, therefore, a diketo derivative (IX). The oxidation product of tetraacetyl methyl brahmate (IVb) under similar conditions showed a carbonyl absorption at 1675 cm^{-1} in the i.r. spectrum thus confirming that it was the 11-keto derivative. The oxidation of triacetyl methyl brahmate (IVa) with CrO_3–AcOH at room temperature for 15 min and TLC examination of the reaction mixture showed two major spots, the diketo (IX, R_f 0·73) and monoketo (VIIIa, R_f 0·81) derivatives, along with a minor component. In order to obtain the triacetyl methyl brahmonate (VIIIa) exclusively the chromic acid oxidation was carried out either in acetone,[9] pyridine[10] or a mixture of AcOH and chloroform.[11] VIIIa showed i.r. absorption at 1707 cm^{-1} but did not yield any carbonyl derivative, thereby showing that the keto grouping is very hindered in nature. Further oxidation of VIIIa by CrO_3–AcOH gave rise to the diketo derivative (IX).

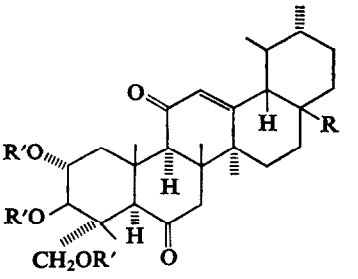
It would thus appear that brahmic acid is in fact a hydroxy asiatic acid and this correlation was confirmed by reducing triacetyl methyl brahonate (VIIIa) by Barton's modification of Wolff Kishner method.[12] The reduction product was methylated and a crystalline product was obtained which was found to be identical with methyl asiatate (IIb).

The experimental data produced so far limits the possibility of the location of the fourth unacylable hydroxyl group in ring B at C-6, since the C-7 hydroxyl would be unhindered and acylable whereas the one present axially at C-6 would exhibit the properties as shown in the case of brahmic acid. This possibility was confirmed by cyclodehydration of methyl brahmonate (VIIIb) under acidic conditions leading to the formation of a cyclic enol ether involving the carbonyl at C-6 and the hydroxymethyl group at C-23, as shown in case of terminolic acid.[13] The resultant product (Xa) showed a single spot on TLC (R_f 0·92), analysed for $C_{31}H_{46}O_5$ and formed a diacetate (Xb). Its i.r. spectrum did not show the carbonyl absorption of the original compound but gave a sharp ester peak at 1728 cm^{-1} with an inflexion at about 1712 cm^{-1}. There was no absorption in the region of 1660 cm^{-1} (enol ether). Similar observation in the case of the analogous product from methyl terminolate has been explained on the assumption that the normal vinyl ether band is displaced towards longer wavelength due to the rigid and highly substituted system and consequently merges in the strong ester absorption.

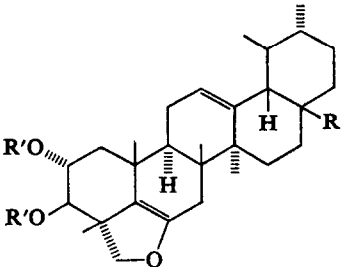
[8] G. G. HAZEN and D W. ROSENBURG, *J. Org. Chem.* **29**, 1930 (1964).
[9] S. W. PELLETIER, *Tetrahedron* **14**, 76 (1961).
[10] G. I. POOS, G. E. ARTH, R. E. BEYLER and L. H. SARETT, *J. Am. Chem. Soc.* **75**, 422 (1953).
[11] A. ZURCHEV, O. JEGER and L RUZICKA, *Helv. Chim. Acta* **37**, 2145 (1964)
[12] D. H. R. BARTON, D. A. J. IVES and B. R. THOMAS, *J. Chem. Soc.* 2056 (1955).
[13] K. E. KING and T. J. KING, *J Chem. Soc* 4469 (1956).



(VIIIa) R = $COOCH_3$; R′ = $COCH_3$
(VIIIb) R = $COOCH_3$; R′ = H
(VIIIc) R = CH_2OAc; R′ = $COCH_3$
(VIIId) R = CH_2OH; R′ = H



(IX) R = $COOCH_3$; R′ = $COCH_3$



(Xa) R = $COOCH_3$; R′ = H
(Xb) R = $COOCH_3$; R′ = $COCH_3$
(Xc) R = CH_2OH; R′ = H

In order to verify this assumption it was necessary to prepare the cyclic enol ether from a derivative of brahmic acid which did not contain the ester grouping. Thus, tetraacetyl brahmol (IVc) was oxidized to the mono-keto derivative (VIIIc) and deacetylated to VIIId. The latter cyclized smoothly to the enol ether (Xc), $C_{30}H_{46}O_4$, showing a sharp peak of medium intensity at 1710 cm^{-1} which could only be due to the vinyl ether grouping. Compound Xc was also obtained by $LiAlH_4$ reduction of methyl anhydrobrahmonate (Xa). Further, since Djerassi *et al.*[14] have shown that the cyclic enol ether formation is only feasible with an equatorial substituent at C-4, the assignment of the hydroxymethyl group would be α (equatorial).

The structure of Xb was finally proved by its NMR spectrum, which exhibited two AcO groups (3H at 1·94 and 2·00 ppm) and a carbomethoxyl group (3H doublet at 3·54 ppm, $J = 1$ c/s). The methylene protons at C-23 appeared as AB quartet (3·92 and 4·23 ppm) and in the region 4·70–5·68 ppm are found one olefinic proton at C-12 and two protons at C-2 and C-3 as a multiplet.

Thus, brahmic acid is 6-hydroxy asiatic acid. The stereo-chemistry at various centres has already been worked out by Polonsky and co-workers[15] in the case of asiatic acid (IIa) and it has been shown above that the hydroxy group at C6 is ß (axial). The complete stereo-formula for brahmic acid would, therefore, be 2α, 3ß, 6ß, 23-tetrahydroxy-28-oic-urs-12-ene (Ia).

[14] C. Djerassi, D. B. Thomas, A. L. Livingston and C. R. Thompson, *J. Am. Chem. Soc.* **79**, 5292 (1957).
[15] J. Polonsky, *Bull. Soc. Chim. Fr.* **19**, 649, 1015 (1952); **20**, 173 (1953); J. Polonsky, E. Sach and E. Lederer, *Bull. Soc. Chim. Fr.* **28**, 1586 (1961).

EXPERIMENTAL

All m.p.'s are uncorrected and were taken on a Kofler block. The u.v. spectra were recorded on a Unicam instrument in absolute ethanol. I.r. spectra were taken on Perkin–Elmer Infracord Model 137. NMR spectra were recorded on Varian A-60 and A-100 instruments in $CDCl_3$ with TMS as internal standard. Unless mentioned otherwise, benzene–MeOH, 98:2 (S1) and 90:10 (S2), have been used as solvent systems in TLC on silica gel G plates, and chlorosulfonic acid–glacial AcOH (1:2) as spray reagent.

Methyl Brahmate Acetonide (III)

Methyl brahmate* (1b) (250 mg), acetone (3 ml) and anhyd. $CuSO_4$ (2 g) were shaken together overnight at room temperature The $CuSO_4$ was filtered and evaporation of the filtrate gave a residue which showed two spots on TLC (S_2), R_f 0·81, 0·31 (unreacted material) The reaction mixture was separated on preparative TLC (S_2) and the component Rf 0 81, 150 mg, was obtained as a colourless powder, m p. 130–135°. (Found: C, 72·86; H, 9·43. $C_{34}H_{54}O_6$ required: C, 73 1; H, 9 65 per cent.)

Hydrolysis of Methyl Brahmate (Ib)

The compound (200 mg) was refluxed in ethylene glycol (20 ml) containing NaOH (4·8 g) for 16 hr. The hydrolysate was acidified and the resultant gelatinous ppt was filtered. It was obtained as colourless powder from MeOH, m p. 290–293°, 120 mg. On TLC (S_2) it showed a single spot R_f 0 16, identical with brahmic acid.

Lithium Aluminum Hydride Reduction of Methyl Brahmate (Ib)

Methyl brahmate (400 mg) in ether (30 ml) soln. was added to $LiAlH_4$ (800 mg) in ether (50 ml) and the reaction mixture was stirred for 4 hr. After standing overnight, the excess of reagent was decomposed with AcOEt the solution was acidified and extracted with the same solvent. The organic layer was washed, dried and freed of the solvent. The colourless residue (320 mg) yielded crystalline brahmol (Ic) from EtOH–$CHCl_3$, m.p. 200–205° TLC (S_2) R_f 0·20 (Found: C, 73·17; H, 10·08. $C_{30}H_{50}O_5$ required: C, 73 46, H, 10 2 per cent)

Acetylation of Brahmol (Ic)

Brahmol (125 mg) was acetylated with Ac_2O (2 ml) and pyridine (2 ml) at room temperature overnight. After the usual working of the reaction mixture, the residue crystallized from MeOH as colourless needles (IVc), 132 mg, m p. 214°, TLC (S_1) R_f 0·25; ν_{max} (KBr) 3400 cm^{-1} (—OH), 1735 cm^{-1} (acetyl). (Found: C, 69 1; H, 8·81; acetyl, 24 4. $C_{38}H_{58}O_9$ required: C, 69·3; H, 8 81 acetyl (for 4), 26 15 per cent)

Sodium Periodate Oxidations

(*a*) *Methyl brahmate (Ib)*. To a soln. of methyl brahmate (32 mg) in AcOEt–EtOH (1:1, 5 ml), $NaIO_4$ soln. (22·3 mg/ml, 3 3 ml) was added. The clear soln. was allowed to stand overnight and the excess of the reagent was titrated against N/10 Na_3AsO_3 soln., along with a suitable blank. $NaIO_4$ consumed = 12 16 mg; calculated (for 1 mol) = 13·18 mg.

The above experiment was repeated with 150 mg of methyl brahmate. After the decomposition of the excess of the reagent with ethylene glycol, the mixture was diluted with H_2O, extracted with ether and worked up in the usual manner. The residue was obtained as colourless powder (VIa) from dilute EtOH, m.p. 120–122°, 135 mg, R_f 0 79 (S_2) on TLC. ν_{max} (KBr) 1730 cm^{-1} (ester) NMR ppm 3 00 (2H, broad, —OH), 3·60 (3H, —$COOCH_3$), 3·70 to 4·70 (3H, —OCH_2— and —OCH< of hemiacetal rings), 4 8–5·70 (3H, two hemiacetal H and one olefinic H). (Found: C, 72·2; H, 9 2. $C_{31}H_{48}O_6$ required: C, 72 09; H, 9·30 per cent.)

VIa (50 mg) was dissolved in MeOH (2·0 ml) containing H_2O (0·56 ml) and semicarbazide HCl-ide (82 mg) and pyridine (0·06 ml) were added to it The soln. was kept at room temperature for 2 hr, then diluted with H_2O and the ppt collected It was purified by preparative TLC using AcOEt and was obtained as colourless powder from EtOH, m.p. 150–152°, 51 mg, R_f 0·41 (S_2) on TLC. ν_{max} (KBr) 1690 cm^{-1} (amide). (Found: N, 7·3 $C_{32}H_{51}O_6N_3$ required: N, 7 32 per cent.)

VIa (100 mg) was acetylated overnight with Ac_2O and pyridine at room temperature. After the usual working, VIc was obtained as colourless powder from dilute MeOH, 90 mg, m.p. 83–85°, R_f 0·75 on TLC (S_1). ν_{max} (KBr) 1745, 1730 cm^{-1} (ester and acetyl). NMR ppm 2 06 (6H, —$OCOCH_3$), 3 60 (3H, —$COOCH_3$), 3·70–4·80 (3H, —OCH_2— and —OCH< of hemiacetal rings), 5 40 (1H, olefinic), 5·80–6·15 (2H, hemiacetal protons) (Found: C, 69 55, H, 8·82. $C_{35}H_{52}O_8$ required C, 70·0; H, 8·66 per cent.)

VIa (25 mg) was oxidized with CrO_3 in acetone for 3 hr at room temperature. After the usual working, the reaction product was purified by preparative TLC in benzene:MeOH (3 per cent). The major product was obtained as colourless powder, m p. 140°, 12 mg, R_f 0 73 on TLC (S_1) ν_{max} (KBr) 1728 cm^{-1} (seven-membered lactone), 1742 cm^{-1} (ester), 1760 cm^{-1} (γ-lactone)

* Methyl brahmate, m.p. 168–171°, was prepared from brahmic acid,[1] m p. 293°. Its m p. was inadvertantly printed as 213° in earlier reference.

(*b*) *Methyl brahmate acetonide (III).* The isopropylidene derivative (26 mg) was dissolved in AcOEt–EtOH (1:1, 2·5 ml) and reacted with $NaIO_4$ soln. (22·3 mg/ml, 1·5 ml) under the conditions given above. The periodate estimation showed that it was not consumed and the original substance was recovered from the reaction mixture.

(*c*) *Brahmol (Ic).* Brahmol (50·6 mg) in AcOEt–EtOH (1:1, 4 ml) was added to $NaIO_4$ soln. (22·3 mg/ml, 3 ml), and after 6 hr consumed 22·45 mg; required (for 1 mol) 22·1 mg.

This experiment was repeated with 200 mg of brahmol. The reaction product was obtained as a colourless powder (VIb) from dilute EtOH, m.p. 172–174°, 178 mg, R_f 0·46 on TLC (S_2). (Found: C, 73·36; H, 9·47. $C_{30}H_{48}O_5$ required: C, 73·77; H, 9·81 per cent.)

Cyclization of the Periodate Oxidation Products

(*a*) *VIa to VIIa.* VIa (25 mg) was dissolved in dry benzene (20 ml) and glacial AcOH and piperidine (5 drops each) were added to it. The soln. was heated at 60° for 1 hr in N_2 atm. Anhyd. $MgSO_4$ (50 mg) was then added and reaction continued for another 30 min. The soln. was diluted with H_2O and extracted with ether. The organic layer was washed, dried, evaporated and the residue was obtained as colourless powder (VIIa) from dilute EtOH, m.p. 103–104°, 20 mg, TLC (S_2), R_f 0·63; ν_{max} (KBr) 1735 cm^{-1} (ester), 1687 cm^{-1} (—C═C—CHO). λ_{max}, 238 nm (logϵ 3·97). (Found: C, 74·37; H, 9·06. $C_{31}H_{46}O_5$ required: C, 74·69; H, 9·23 per cent.)

(*b*) *VIb to VIIb.* VIb (30 mg) was reacted with piperidine and glacial AcOH in a similar manner. On working up, VIIb was obtained as colourless powder, 25 mg, m.p. 145–147°, R_f 0·30 on TLC (S_2); ν_{max} (KBr) 1687 cm^{-1} (—C═C—CHO). λ_{max}, 238 nm (logϵ, 3·99). (Found: C, 76·62; H, 9·46. $C_{30}H_{46}O_4$ required: C, 76·59; H, 9·78 per cent.)

Acetylation of Methyl Brahmate (Ib)

(*a*) *Acetic anhydride-pyridine.* Methyl brahmate (Ib) (400 mg), Ac_2O (5 ml) and pyridine (5 ml) were mixed together and allowed to stand at room temperature for 18 hr. After the usual working, the residue was chromatographed over alumina (12 g, grade III). The benzene–$CHCl_3$ (4:1) eluate gave a residue (216 mg) showing two spots (A and C) on TLC (S_1), R_f 0·72 and 0·43. The benzene-$CHCl_3$ (1:1) and $CHCl_3$ eluates gave a residue which showed only one spot (C), R_f 0·43, on TLC (S_1) and afforded a colourless powder (IVa) from dilute MeOH, 200 mg, m.p. 128–129°, ν_{max} (KBr) 3400 cm^{-1} (—OH), 1745 cm^{-1} (acetyl). NMR ppm 1·98, 2·03 and 2·06 (3H each, —$OCOCH_3$), 3·62 (3H, —$COOCH_3$, doublet, J=1 c/s), 3·74 and 3·95 (2H, AB quartet, CH_2OAc, J=12 c/s), 4·38 (IH, broad, —OH), 4·83–5·56 (3H multiplet, 1 olefinic H and 1H each at C2 and C3). (Found: C, 68·7; H, 8·9; acetyl 18·8. $C_{37}H_{56}O_9$ required: C, 68·9; H, 8·69; acetyl (for 3), 20·0 per cent).

The mixture of A and C was separated on preparative TLC using solvent system S_1 giving A (12 mg) and C (190 mg). Compound A (V), R_f 0·72, on TLC (S_1) was obtained as a colourless powder, m.p. 112°; ν_{max} (KBr) 1745 cm^{-1} (acetyl). NMR: ppm 1·98, 2·03 (6H, 3H, —$OCOCH_3$), 3·62 (3H, —$COOCH_3$, doublet, J=1 c/s), 4·91–5·75 (4H multiplet, 2 olefinic H and 1H each on C2 and C3). (Found: C, 71·05; H, 8·41. $C_{37}H_{54}O_8$ required: C, 70·92; H, 8·62 per cent.)

(*b*) *Acetic anhydride; p-toluene sulphonic acid.* Methyl brahmate (Ib) (200 mg) was treated with Ac_2O (5 ml) and p-toluene sulphonic acid (275 mg) for 24 hr at room temperature. The reaction product, obtained in the usual manner, showed two components (A and B) which were isolated by preparative TLC (S_1). The compound A, R_f 0·72, 12 mg, m.p. 112°, was identical with (V), obtained above. The derivative B (IVb), R_f 0·66, was obtained as colourless powder from dilute EtOH, 240 mg, m.p. 166°, ν_{max} (KBr) 1745 cm^{-1} (acetyl). (Found: C, 67·71; H, 8·55. $C_{39}H_{58}O_{10}$ required: C, 68·21; H, 8·45 per cent).

(*c*) *Acetic anhydride–sodium acetate.* Methyl brahmate (Ib) (150 mg) Ac_2O (3 ml) and anhyd. AcONa (150 mg) were refluxed together for 4 hr. The product obtained on working up of the reaction mixture, showed three spots (A, B and C) on TLC (S_1), R_f 0·72, 0·66 and 0·43. The separation was achieved by chromatography over silica gel and B (IVb) was found to be major component, 135 mg, m.p. 166°.

Dehydration of Triacetyl Methyl Brahmate (IVa)

(*a*) *Thionyl chloride–pyridine.* Triacetyl methyl brahmate (IVa, 150 mg) was taken in pyridine (0·4 ml) thionyl chloride (0·4 ml) was added to it and kept at room temperature overnight. The soln. was diluted with ice-water and extracted with ether. The organic layer was washed, dried and evaporated to give a residue which was obtained as a colourless powder from dilute MeOH, 140 mg, m.p. 112°, R_f 0·72, on TLC (S_1). On admixture with anhydrotriacetyl methyl brahmate (V), it melted at 112° and gave i.r. spectrum which was supimposable.

(*b*) *Mesyl chloride–sulphur dioxide.* A solution of IVa (100 mg) in natural collidine (3·3 ml) and DMF (8 ml) was cooled to 10° and mesyl chloride (10 ml) containing 3·5 per cent anhd. SO_2 was added with stirring during a period of 2 min. The temperature rose quickly but the soln. was maintained at 25–30° and after 5 min excess of the reagent was decomposed with H_2O. The reaction product was worked up to give V as colourless powder, 89 mg, m.p. 112°.

(*c*) *Phosphorus oxychloride–pyridine.* IVa (60 mg), $POCl_3$ (2 ml) and pyridine (7 ml) were allowed to stand overnight at room temperature and then heated on a water bath for 1 hr. The mixture was poured in ice and

extracted with $CHCl_3$. The solvent layer was washed, dried and evaporated to give a residue which was chromatographed over alumina (2 g, grade III). Benzene:$CHCl_3$ (6:1) eluate gave V 37 mg, m.p. 112°. The benzene–$CHCl_3$ (1:1) and $CHCl_3$ eluates gave the starting material (IVa), 20 mg.

Oxidation of Triacetyl Methyl Brahmate (IVa)

(*i*) *Chromum trioxide–acetic acid.* Triacetyl methyl brahmate (IVa) (150 mg) was dissolved in glacial AcOH (5 ml) and reacted overnight with CrO_3 (120 mg) in glacial AcOH (10 ml) containing H_2O (1 ml). The excess of the reagent was decomposed with sulphurous acid and the mixture worked up as usual. The residue gave a colourless powder (IX) from dilute EtOH, 140 mg, m.p. 165°, single spot on TLC (S_1), R_f 0 73; ν_{max} (KBr) 1675 cm^{-1} (—C═C—C═O), 1707 cm^{-1} (inflexion, —C═O). λ_{max}, 249 mμ (log ε 3·98). (Found: C, 67·24; H, 7·81. $C_{37}H_{52}O_{10}$ required: C, 67·68; H, 7·92 per cent.)

(ii) *Chromium trioxide–pyridine.* A soln. of IVa (200 mg) in pyridine (2 ml) was added to CrO_3-pyridine complex (72 mg) (CrO_3 in 2 ml pyridine) at room temperature. After 1 hr, the reaction mixture was diluted with H_2O, extracted with ether and worked up to give a residue, 170 mg. It gave a colourless powder (VIIIa) from dilute EtOH, m p. 126°, R_f 0 81 (TLC S_1); ν_{max} 1745 cm^{-1} (acetyl), 1707 cm^{-1} (acetyl), 1707 cm^{-1} (—C═O). (Found: C, 69 57; H, 8 91. $C_{37}H_{54}O_9$ required: C, 69 15, H, 8·41 per cent.)

Triacetyl methyl brahmonate (VIIIa) on further oxidation with CrO_3–AcOH, according to the procedure described above in (i), gave a product, m.p. 165°, TLC (S_1), R_f 0 73, which was identical with the diketo derivative obtained directly from IVa.

(iii) *Chromium trioxide–acetone.* IVa (50 mg) in acetone (8 ml) was added to CrO_3 (40 mg) soln. in the same solvent and after 3 hr the reaction mixture was worked up. The residue gave VIIIa as colourless powder from dilute EtOH, 48 mg, m.p. 125–126°.

(iv) *Chromium trioxide–acetic acid–chloroform.* IVa (50 mg) in glacial AcOH (20 ml) and $CHCl_3$ (2 ml) was added to a CrO_3 (113 mg) soln. in AcOH–water (4:1, 5 ml). The reaction mixture was worked up after 3 hr and gave VIIIa, 42 mg, m.p. 126°.

Oxidation of Tetraacetyl Methyl Brahmate (IVb)

Tetraacetyl methyl brahmate (IVb) (50 mg) in glacial AcOH–H_2O (4:1,5 ml) was reacted overnight with CrO_3 (65 mg). After the usual working up of the reaction mixture, a colourless powder was obtained from dilute EtOH, 42 mg, m.p. 153–154°, TLC (S_1), R_f 0·33; ν_{max} (KBr) 1745 cm^{-1} (acetyl), 1675 cm^{-1} (—C═C— —C═O). (Found: C, 66·24; H, 8·05. $C_{39}H_{56}O_{11}$ required: C, 66·8; H, 8·0 per cent.)

Wolff–Kishner Reduction of Triacetyl Methyl Brahmonate (VIIIa)

Na (100 mg) in diethylene glycol (5 ml) was heated to 180° and anhyd. N_2H_4 was distilled in until the mixture refluxed at 180°. The soln. was cooled, VIIIa (200 mg) was added and then refluxed overnight. The temperature was then raised to 210° by distilling off some N_2H_4 and refluxing continued for 24 hr. The mixture was diluted with H_2O, extracted with methyl ethyl ketone and the organic layer freed of the solvent. The residue was methylated with ethereal CH_2N_2 and the product was chromatographed over alumina (4 g, grade III). The AcOEt–MeOH (2:1) eluate gave a residue which crystallized as colourless needles (IIb) from dilute EtOH, 33 mg, m.p. 216–218°, TLC (S_2), R_f 0·38. (Found: C, 74·06; H, 9·77. $C_{31}H_{50}O_5$ required: C, 74·10; H, 9 96 per cent.) On admixture with methyl asiatate (m.p. 216–218°), its m.p. is not depressed.

Cyclodehydration of the Keto Derivatives

(*a*) *Methyl brahmonate (VIIIb).* VIIIa (320 mg) was refluxed with alcoholic KOH for 2 hr and the hydrolysate, on working up, gave VIIIb which was obtained as a colourless powder from dilute alcohol, 256 mg, m.p. 135–137°, R_f 0·64 (TLC S_2); ν_{max} (KBr) 1745 cm^{-1} (ester), 1720 cm^{-1} (—C═O). (Found: C, 71·9; H, 9·2. $C_{31}H_{48}O_6$ required: C, 72·09; H, 9 3 per cent.)

VIIIb (150 mg) was allowed to stand overnight in dry MeOH (20 ml) containing conc. HCl (5 drops) at room temperature. On dilution with H_2O, a ppt was obtained which was filtered and washed with H_2O. This ppt yielded anhydromethyl brahmonate (Xa) as a colourless powder from dilute EtOH, 140 mg, m.p. 108–110°, TLC (S_2) R_f 0·92; ν_{max} (KBr) 1728 cm^{-1} (ester), inflexion at 1712 cm^{-1}. (Found: C, 76·90; H, 9·2. $C_{31}H_{46}O_5$ required: C, 76·7; H, 9·23 per cent.)

Xa (50 mg) was reacted with Ac_2O (2 ml) in pyridine (2 ml) solution at room temperature. After the usual working, the acetyl derivative (Xb) was obtained as a colourless powder, 52 mg, m.p. 87–89°, TLC (S_1) R_f 0·80; ν_{max} (KBr) 1745 cm^{-1} (acetyl), inflexion at 1712 cm^{-1}. NMR ppm 1·94, 2·00 (3H each, —$OCOCH_3$), 3·54 (3H, $COOCH_3$ sharp doublet, J=1 c/s), 3·92 and 4·23 (2H, AB quartet, —CH_2O—, J=10 c/s), 4 70–5·68 (3H, multiplet, 1 olefinic H and 1H each on C2 and C3). (Found: C, 71·79; H, 8·17. $C_{35}H_{50}O_7$ required: C, 72·16; H, 8·59 per cent.)

(*b*) *Keto brahmol (VIIIc).* Tetraacetylbrahmol (IVc) (50 mg) was oxidized with CrO_3 (60 mg) in acetone (8 ml) at room temperature for 3 hr. Excess of the reagent was decomposed with H_3SO_3 and the reaction mixture was worked up to give a residue which yielded a colourless powder (VIIIc) from dilute EtOH, 48 mg, TLC (S_1) R_f 0·39; ν_{max} 1745 cm^{-1} (acetyl), 1720 cm^{-1} (—C═O).

The keto derivative VIIIc was refluxed with 5 per cent alcoholic KOH for 2 hr when the deacetylated product (VIIId) was obtained as a colourless powder, 40 mg, TLC (S_2) R_f 0·48; ν_{max} 3400 cm^{-1} (hydroxy), 1720 cm^{-1} (keto).

The keto brahmol (VIIId) (40 mg) was kept with conc. HCl (5 drops) in dry MeOH (15 ml) soln. at room temperature. The reaction mixture on addition of H_2O gave a ppt which was purified by dilute EtOH as a colourless powder of the anhydro product (Xc), 34 mg, m.p. 132–134°, TLC (S_1) R_f 0·78; ν_{max} 3400 cm^{-1} (—OH), 1710 cm^{-1} (—C=C—O—). (Found: C, 76·4; H, 9·5. $C_{30}H_{46}O_4$ required: C, 76·59; H, 9·78 per cent.) The cyclic enol ether (Xc) was also obtained when anhydro methyl brahmonate (Xa, 50 mg) was added to a slurry of LAH (20 mg) in ether (15 ml) and the mixture was stirred at room temperature for 1 hr. The reaction mixture was worked up in the usual manner and the residue was separated by preparative TLC into Xc, 20 mg, and the original material, 12 mg.

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