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A REINVESTIGATION OF THE TRITERPENES OF *CENTELLA ASIATICA**

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Abstract—Apart from the constituents reported earlier, the isolation of asiatic acid, asiaticoside, *meso*-inositol and a new oligosaccharide, "centellose", is described from the Indian variety and their relative proportions determined in the plant material. The periodate oxidation product of asiatic acid has been reinvestigated and its structure has been assigned as a hemiacetal derivative.

INTRODUCTION

IN EARLIER communications[1,2] we reported the isolation of the triterpene acids betulic acid, brahmic acid and isobrahmic acid along with two saponins, brahmoside and brahminoside, from this plant. These saponins were shown to be tri- and tetraglycosides of brahmic acid. The constitution of brahmic acid was elucidated as 2α,3β,6β,23-tetrahydroxy-28-oic-urs-12-ene.[3] Earlier references to the chemical constituents have been cited in the review[4] by Basu and Rastogi on triterpene saponins and sapogenins.

RESULTS AND DISCUSSION

The earlier procedure[1] for the isolation of the chemical constituents has been modified (see below). The benzene-insoluble triterpene fraction (A) was found to be a mixture of brahmic (Ia) and asiatic (IIa) acids.† A facile separation of these acids was achieved as methyl esters although the methylated mixture contained eight products. The major constituents, methyl brahmate (Ib) and methyl asiatate (IIb) along with their respective 3-*O*-methyl derivatives (Ic, IIc) were separated by chromatography. The remaining four components are assumed to be di- and tri-*O*-methyl derivatives considering their higher R_f values. This assumption was confirmed by the fluoboric acid catalysed methylation[5] of Ib and IIb when the same products were obtained. It was observed that methylation products of Ia gave violet spots whereas those derived from IIa gave brown spots on spraying with $ClSO_3H$–AcOH reagent. The acids, Ia and IIa, in the fraction A were quantitatively estimated through acetylation and were found to be present in the ratio of 2:1.

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* Part IV in the series "Chemical Examination of *Centella asiatica*" and communication No. 1303 from Central Drug Research Institute, Lucknow.

† Isobrahmic acid reported by us earlier (Ref. 1) proved to be a mixture of brahmic and asiatic acids.

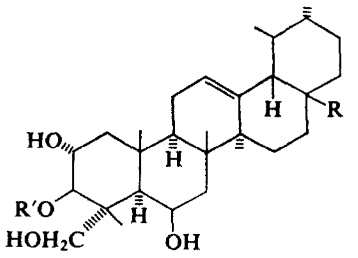
[1] R. P. RASTOGI, B. SARKAR and M. L. DHAR, *J. Sci. Indust. Res.* **19B**, 252 (1960).

[2] R. P. RASTOGI and M. L. DHAR, *Indian J. Chem.* **1**, 267 (1963).

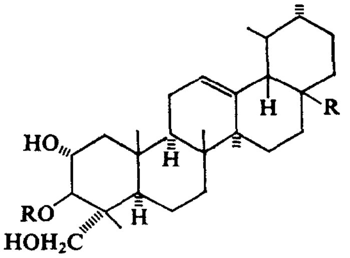
[3] BHAGIRATH SINGH and R. P. RASTOGI, *Phytochem.* **7**, 1385 (1968).

[4] N. BASU and R. P. RASTOGI, *Phytochem.* **6**, 1249 (1967).

[5] J. POLONSKY, E. SACHS and E. LEDERER, *Bull. Soc. Chim. Fr.* **28**, 1586 (1961).



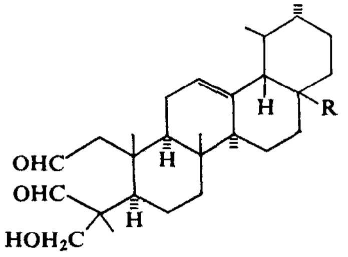
(Ia) R = COOH; R' = H
(Ib) R = $COOCH_3$; R' = H
(Ic) R = $COOCH_3$; R' = CH_3



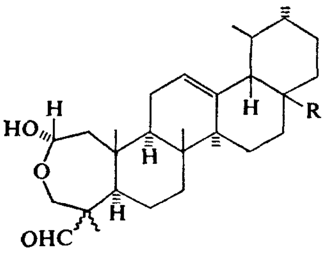
(IIa) R = COOH; R' = H
(IIb) R = $COOCH_3$; R' = H
(IIc) R = $COOCH_3$; R' = CH_3

FIG. 1.

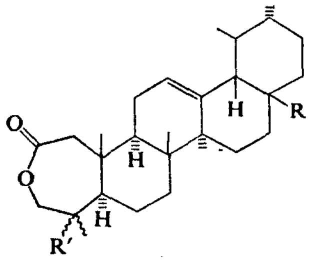
Methyl asiatate (IIb) consumed 1 mole of $NaIO_4$ as expected and gave a product, designated by Polonsky[6, 7] as dialdehyde (III), which was not oxidized with H_2O_2 to a dicarboxylic acid. This oxidation product has now been reinvestigated. It analysed for $C_{31}H_{48}O_5$ and yielded a monosemicarbazone. Its NMR shows one proton singlet at 9·98 ppm for an aldehyde group, two protons multiplet in the region 5·2–5·44 ppm due to an olefinic proton and



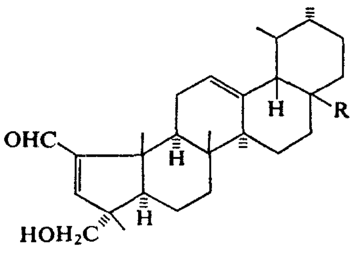
(III) R = $COOCH_3$



(IV) R = $COOCH_3$



(V) R = $COOCH_3$, R' = COOH
(VI) R = R' = $COOCH_3$



(VII) R = $COOCH_3$

FIG. 2.

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[6] J. POLONSKY, *Bull. Soc. Chim., Fr.* **19**, 649 (1952).

[7] J. SIMONSON and W. C. J. ROSS, "*The Terpenes*", Vol. V, p. 61, Cambridge University Press (1957).

a hemiacetal H and a two-proton quartet at 3·74 and 3·93 ppm ($J = 14$ c/s) for the geminal protons at C-23 thus indicating it to be a hemiacetal (IV). This would be in analogy with similar findings reported in the case of brahmic acid.[3]

Further proof for the structure of IV was obtained by chromic acid oxidation which afforded a monocarboxylic acid (V) having a 7-membered lactone ring. Methylation of V gave a crystalline methyl ester (VI) which shows two $COOCH_3$ groups at 3·62 and 3·7 ppm, an AB quartet at 4·62 and 4·04 ppm ($J = 13$ c/s) due to geminal protons at C-23 and a singlet at 2·66 ppm of two protons at C-1. In the presence of catalytic amounts of piperidine and acetic-acid, the hemiacetal ring of IV opens up and recyclization[3] takes place to give an α:β-unsaturated aldehyde (VII).

The slimy deposit (B) was fractionated and was found to consist of brahmoside, brahminoside[1, 2] and asiaticoside[8] in the yields of 3·8, 1·6 and 2·4 per cent respectively.

The hygroscopic residue (C) was a complex mixture of water-soluble components from which brahminoside, glucose, *meso*-inositol and a new oligosaccharide, centellose, have been isolated. Centellose formed a crystalline acetate and yielded glucose on hydrolysis.

EXPERIMENTAL

All m.ps are uncorrected. NMR spectra were recorded on Varian A-60 instrument in $CDCl_3$ with TMS as internal standard. Unless mentioned otherwise, benzene–methanol 98:2 (S_1), 90:10 (S_2), ethyl acetate–methanol–water 9:1:1 (S_3) and 6:1·5:0·75 (S_4) have been used as solvents and the R_fs refer to TLC on silica gel G plates with $ClSO_3H$–glacial AcOH (1:2) or 2% Ce $(SO_4)_2$ in 2NH_2SO_4 as spray reagents.

Isolation of the Mixture of Triterpene Acids

The powdered plant material (10 kg) was repeatedly extracted with alcohol and the total extract was concentrated to about 3 l. On allowing the concentrate to stand in the cold for 3–4 days, a voluminous precipitate separated out which was centrifuged and washed with cold water. The precipitate was extracted with hot benzene (500 ml) to remove the lipid fraction along with betulic acid. Maceration of the benzene-insoluble fraction with 33% alcohol (400 ml) and filtration gave an insoluble powder (A) (77 g) which showed two spots on TLC (S_2), R_f 0·16 and 0·26.

Isolation of Glycosides

The total dilute alcoholic solution was treated with aq. lead acetate in the usual manner and the pH of the final filtrate was adjusted to 6·6 with K_2CO_3 and then concentrated under reduced pressure. The concentrate was kept in the cold for a few days when it deposited a slimy mass (B) which was decanted and washed with small amounts of cold water. The washings were mixed with the decantate. This deposit (95 g) gave three spots on TLC (S_3), R_f 0·21, 0·36 and 0·48.

A portion of this material (5 g) was chromatographed on silica gel (175 g) and eluted with solvent S_3. Eluates 4, 5 (2·75 g) showed two spots, R_f 0·21 and 0·36, and eluate 6 (1·0 g) showed two spots, R_f 0·36 and 0·48. These eluates were again chromatographed separately on silica gel when single fractions were obtained. The residues of fractions containing component R_f 0·48 were mixed and crystallized as colourless needles, m.p. 242–246° (decomp.), 2·0 g (brahmoside, R_f 0·48). The fractions containing component R_f 0·36 were crystallized from alcohol–acetone, m.p. 230–233° (decomp.), 1·25 g, and did not depress the m.p. of asiaticoside (R_f 0·36). The fractions containing component R_f 0·21 crystallized from methanol–acetone, m.p. 216–221° (decomp.), 0·85 g (brahminoside R_f 0·21).

The decantate, obtained after the separation of the deposit B, was saturated with NaCl when a dark brown syrup separated out. It was washed with brine and then freed of the solvent. The residue was dissolved in methanol and partially precipitated with ether and this process was repeated four times to remove most of the inorganic material and resinous impurities. The total methanol–ether solution was evaporated, the residue dissolved in water and passed over Amberlite resins IR-4B and IRC-50. The eluate was evaporated to an orange-coloured, hygroscopic residue (C) which showed six spots on TLC.

A portion of this residue (16 g) was chromatographed over silica gel (225 g) and 250-ml fractions were collected (Table 1).

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[8] P. BOITEAU, A. BUZAS, E. LEDERER and J. POLONSKY, *Nature*, **163**, 258 (1949); *Idem. Bull. Soc. Chim. Biol., Paris* **31**, 46 (1949).

TABLE 1. CHROMATOGRAPHY OF THE HYGROSCOPIC RESIDUE (C)

Fraction no.	Eluate	Wt. (g)	TLC (S_4) spots	Substances isolated
1–3	EtOAc-MeOH-H_2O (8:2:1)	—	—	—
4–10		2·71	0·68, 0·53	Brahminoside (0·68)
11–15		1·27	0·53	Glucose
16–30		3·72	0·53, 0·37	
31–34	EtOAc-MeOH-H_2O (6:2:1)	0·68	0·20, 0·11	
35–40		2·2	0·11	Centellose
41–44		0·21	0·11*, 0·07	*meso*-Inositol
45–48		—	—	

* Faint streak.

The residue from fractions 41–44 crystallized from methanol as colourless needles, m.p. 230°, 150 mg. It was optically inactive, formed an acetate, m.p. 216°, and was identified as *meso*-inositol by i.r. spectrum and mixed m.p. determination with an authentic sample.

Centellose was obtained from the residue of fractions 35–40 as a colourless hygroscopic powder on maceration with acetone, 2·0 g, R_f 0·11 (S_4).

Centellose (300 mg) was acetylated and chromatographed on silica gel. Centellose acetate was eluted with $CHCl_3$: EtOAc (1 :1) and crystallized from alcohol as colourless needles, m.p. 148–150°, 230 mg, $(\alpha)_D = +100°$ ($C = 1·0$, $CHCl_3$), R_f 0·27 (S_1). Centellose (50 mg) was heated with 10 N H_2SO_4 for 6 hr on a water bath. The hydrolysate was neutralized with $BaCO_3$ and chromatographed on paper. It showed a single spot which was identified as glucose by comparative chromatography in three solvents.

Separation of the Triterpene Acids

The acid mixture (A) (200 mg) was separated by preparative TLC using multiple development with solvent S_2, when brahmic acid (120 mg), m.p. 290–293°, and asiatic acid (55 mg), m.p. 238–242°, were obtained.

For separations on larger scale, the acid mixture (10 g) was reacted with ethereal CH_2N_2. On removal of solvent a viscous residue was obtained which showed eight spots on TLC. It was chromatographed on alumina (140 g, grade III) in benzene solution. The ethyl acetate : methanol (2 :1) eluates 7, 8 containing two components were mixed and solvent removed. The residue crystallized in aq. alcohol as colourless needles, 4·06 g, m.p. 168°. It showed one spot (S_2), R_f 0·31 (methyl brahmate, R_f 0·31), ν_{max} (KBr) 1735 cm^{-1} (—$COOCH_3$), 3400 cm^{-1} (—OH). Found: C, 71·77; H, 10·0; M.W. (mass), 518. $C_{31}H_{50}O_6$ required: C, 71·8; H, 9·6%, M.W. 518. On admixture with methyl brahmate (m.p. 168°) the m.p. was not depressed.

The mother liquor was made slightly turbid with water, allowed to stand in the cold and the colourless crystalline deposit was filtered, 2·68 g, m.p. 216–218°, R_f 0·38 (S_2) (methyl asiatate, R_f 0·38), ν_{max} (KBr) 1735 (—$COOCH_3$), 3400 cm^{-1} (—OH). Found: C, 74·02; H, 9·85; M.W. (mass), 502. $C_{31}H_{50}O_5$ required: C, 74·10; H, 9·96 per cent; M.W. 502.

The methyl ester, m.p. 216°, was acetylated to give colourless powder, m.p. 106–108°, R_f 0·89 (S_1), ν_{max} (KBr) 1740 cm^{-1} (acetyl and —$COOCH_3$), NMR: ppm 1·96, 2·01, 2·08 (3H each, $OCOCH_3$), 3·60 (3H, $COOCH_3$), 3·6, 3·86 (2H AB qu, CH_2OAC, $J = 12$ c/s) and 4·83–5·50 (3H m, 1 olefinic H and 1H each on C-2 and C-3). Found: C, 70·52; H, 8·20. $C_{37}H_{56}O_8$ required: C, 70·7; H, 8·91 per cent.

The above triacetyl derivative (50 mg) was oxidized with chromic acid (65 mg) in glacial HOAc (5 ml), yielding a colourless powder, m.p. 255–256°, R_f 0·86 (S_1) (reported m.p. of 11-keto-triacetyl methyl asiatate 256–262°). ν_{max} (KBr) 1740 ($COOCH_3$), 1667 cm^{-1} (—C=C—CO); λ_{max} 250 mμ (log ϵ 4·1). Found: C, 68·7; H, 8·19. $C_{37}H_{54}O_9$ required: C, 69·15; H, 8·41 per cent.

The residue from $CHCl_3$ and ethyl acetate eluates 3, 4, and 5 (2·91 g), showing six spots was rechromatographed over alumina (100 g). The $CHCl_3$ and $CHCl_3$–ethyl acetate (1:1) fractions gave a residue which crystallized from alcohol as colourless needles, m.p. 251–252°, 0·36 g. Found: C, 74·13; H, 9·54. $C_{35}H_{52}O_5$ required: C, 74·4; H, 10·07 per cent. There was no depression in m.p. on admixture with 3-*O*-methyl ether of methyl asiatate (IIc). The subsequent ethyl acetate and ethyl acetate–methanol (10:1) fractions were evaporated and gave colourless needles (Ic) from alcohol, m.p. 243–244°, 0·65 g. Found: C, 71·95; H, 9·47. $C_{32}H_{52}O_6$ required: C, 72·17; H, 9·77 per cent.

Estimation of Brahmic and Asiatic Acids

The mixture of acids (4·0 g) was kept overnight in pyridine (20 ml) and acetic anhydride (20 ml) and the product (4·02 g), showing two spots on TLC (S_1), R_f 0·69 and 0·22, was chromatographed on silica gel (100 g). The $CHCl_3$ eluate gave a residue (1·43 g) with R_f 0·69, whereas the $CHCl_3$–ethyl acetate (5:1) eluate gave a residue (2·56 g) with R_f 0·22. Saponification of the former residue with alcoholic NaOH (5 per cent) gave asiatic acid (IIa) (35·8 per cent) and of the latter brahmic acid (Ia) (64·16 per cent).

Periodate Oxidation of Methyl Asiatate (IIb)

To a solution of IIb (150 mg) in ethyl acetate–ethanol (1:1, 15 ml), $NaIO_4$ solution (8 ml, 22·3 mg/ml) was added. After standing overnight the excess of $NaIO_4$ was decomposed with ethylene glycol and the resulting solution was diluted with water and extracted with ether. The ether layer was washed, dried and evaporated. The residue was obtained as a powder (IV) from dilute methanol, m.p. 115–117°, 137 mg, R_f 0·50 (S_1). ν_{max} 1730, inflexion at 1720 cm^{-1} (—$COOCH_3$ and —CHO), NMR: ppm 2·90 (1H, broad —OH), 3·60 (3H, $COOCH_3$), 3·74 and 3·93 (2H, AB qu $J = 14$ c/s, —O—CH_2), 5·20–5·44 (2H m, 1 olefinic H and 1 hemiacetal H) and 9·98 (1H, CHO). Found: C, 74·23; H, 9·60. $C_{31}H_{48}O_5$ required: C, 74·4; H, 9·6 per cent.

IV gave a semicarbazone, purified by preparative TLC (S_2), as a colourless powder, 36 mg, m.p. 193–195°, R_f 0·60 (S_2). ν_{max} 1730 (ester), 1680 cm^{-1} (amide). Found: N, 7·67. $C_{32}H_{51}O_5N_3$ required: N, 7·54 per cent.

IV (500 mg) was oxidized with CrO_3 (500 mg) in acetone (20 ml) for 4 hr. The product was chromatographed over silica gel (20 g) and eluted with benzene–methanol (98·5:1·5). Fractions 10–20 (20 ml each) were combined, evaporated to a viscous material which gave a colourless powder (V) from alcohol, R_f 0·29 (S_1), 306 mg, m.p. 122–124°, ν_{max} 1710–1735 cm^{-1} broad band (—$COOCH_3$, 7-membered lactone and —COOH). Found: C, 72·1; H, 8·57; neutral equivalent, 514. $C_{31}H_{46}O_6$ required: C, 72·37; H, 8·94 per cent; M.W. 516.

V (55 mg) was reacted with an ethereal solution of CH_2N_2 and kept overnight at 0°. The reaction product was chromatographed over silica gel (5 g) and eluted with benzene–methanol (99·7; 0·3). The major component, eluted out in fractions 8–9 (15 ml each), was crystallized from alcohol as colourless needles (VI), R_f 0·37 in benzene–methanol (199:1) m.p. 232–234°, 38 mg. ν_{max} 1742 ($COOCH_3$), 1721 cm^{-1} (7-membered lactone). NMR: ppm 2·66 (2H s, —CO—CH_2—), 3·60, 3·70 (3H each, —$COOCH_3$), 4·62, 4·04 (2H, AB qu, $J = 13$ c/s, —O—CH_2—), 5·30 (1H, olefinic, $J = 3·5$ c/s). Found: C, 72·57; H, 9·02. $C_{32}H_{48}O_6$ required: C, 72·72; H, 9·09 per cent.

IV (50 mg) in dry benzene (5 ml) and glacial AcOH and piperidine (5 drops each) was heated at 60° for 1 hr in N_2. Anhyd. $MgSO_4$ (50 mg) was then added and reaction continued for another 30 min. The mixture was diluted with water. The organic layer was washed, dried and evaporated. The residue (46·0 mg), by preparative TLC (S_1) gave VII as a colourless powder from alcohol, 33 mg, m.p. 80–81°, R_f 0·60 (S_1), ν_{max} 1735 (ester), 1698 (—C═C—CHO) and 1595 cm^{-1} (—C═C—), λ_{max} 237 nm (log ϵ 3·90). Found: C, 77·42; H, 9·98. $C_{31}H_{46}O_4$ required: C, 77·17; H, 9·54 per cent.

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