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SAPOGENINS OF *CLERODENDRON SERRATUM*

CONSTITUTION OF A NEW PENTACYCLIC TRITERPENE ACID, SERRATAGENIC ACID

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Abstract—The sapogenin mixture obtained from the bark of *Clerondendron serratum* has been found to contain three major triterpenoid constituents, viz. oleanolic acid, queretaroic acid, and a new acid, named serratagenic acid, for which the structure 3β-hydroxy-Δ^{12}-oleane 28,29-dioic acid has been deduced.

THE bark of *Clerodendron serratum* Spreng (Fam. Verbenaceae) is used in India as a drug. In addition to the presence of glucose[1] and a high yield of D-mannitol,[2] we have found the drug to be fairly rich in saponins. Hydrolysis of the saponin with 4N aqueous methanolic sulphuric acid and examination of the product showed the presence of five acidic sapogenins (TLC) and the following sugars, D-glucose, L-rhamnose and D-xylose (paper chromatography).

Chromatography of the sapogenins gave oleanolic acid, characterized as its acetate. The other genins could not be obtained pure by this method. Hence the whole mixture was esterified with diazomethane and the product was chromatographed. Three pure substances were isolated—methyl oleanolate characterized as its acetate, a substance referred to as B and a third referred to as substance C.

Substance C(I), $C_{31}H_{50}O_4$, gave positive Liebermann–Burchard and TNM tests and had IR absorptions characteristic of pentacyclic triterpenes. It contained one ester group and two alcoholic functions. It formed a diacetate (Ib) (M^+ 570) and was reduced by LAH to a triol. The ester group could be hydrolysed with alkali only under drastic conditions. SeO_2 oxidation of the diacetate yielded a compound which showed UV absorption maxima and log ε values typical of a heteroannular diene; this reaction characterized substance C as a member of β-amyrin group. Jones' oxidation of C yielded a compound (Id) which answered the Zimmermann colour reaction and showed an NMR signal at δ 9·6 (aldehydic proton). The Zimmermann colour reaction located one of the OH groups of the parent compound at the usual 3 position while the formation of the aldehyde identified the second alcoholic group as primary in nature. The retro Diels–Alder fragment (D–E rings) obtained in the mass spectrum of the diacetate (Ib) located the primary alcoholic group on ring E so that it should be assigned position 29 or 30. The difficult hydrolysability of C and the position of the highest C-Me signal in the NMR spectrum of Ib and the course of the mass spectral fragmentation located the ester group at position 28.[3] Thus substance C should be oleanolic acid with an additional OH function at position 29 or 30. A comparison of the physical data of C and several derivatives with the values available in the literature

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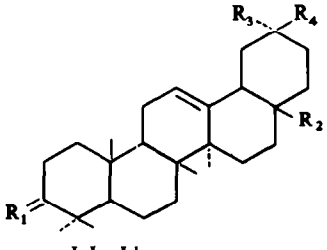
indicated that substance C corresponds to methyl queretaroate. It may be mentioned here that queretaroic acid is known to occur only in certain cacti[4] and has so far not been isolated from any other source.

Substance B (II) $C_{32}H_{50}O_5$ (analysis and mass spectrum) also gave positive Liebermann–Burchard and TNM tests. It had IR absorptions characteristic of pentacyclic triterpenes. It had two ester groups and one alcoholic function. It was reduced by LAH to a triol (IId). SeO_2 oxidation of the acetate gave a compound which had UV absorption maxima and log ε values, indicating that substance B also belongs to the β-amyrin group. One of the two ester groups was hydrolysed by alkali easily while the other required drastic conditions. Jones' oxidation of B gave IIe which gave a positive Zimmermann colour reaction (keto group at position 3). The ester group, which was difficult to hydrolyse, could be assigned the position 28 from the position of the highest C-Me signal in the NMR spectrum and the course of mass spectral fragmentation of II. The easily hydrolysable ester group should be at 23, 29 or 30; C-24 ester is known to be difficult to hydrolyse and 25, 26, 27 are left out from general considerations. The keto diester (IIe) mentioned earlier was hydrolysed easily to a semi-ester (IIf), which was stable even on boiling in aqueous methanolic HCl medium. Had the carboxyl group been at position 23 it should decarboxylate being a β-keto acid. Hence the choice for the location for this hydrolysable ester group lies between 29 and 30. The retro Diels–Alder fragment (D/E rings) in the mass spectrum of B clearly confirms this. Position 30 is ruled out since the properties of IId and IIf do not correspond to the compounds having the same structures described by Djerassi *et al.*[4] Thus by elimination the easily hydrolysable ester group in II should be in position 29 (α and equatorial). A substance with this structure has been described by Tursch *et al.* during their study of mesombryanthemoidigenic acid[5] (III). A direct comparison of the two compounds was kindly carried out by Professor Tursch by using mixed m.p. and TLC and were found to be identical.

Further, treatment of compound IIa with acetic anhydride and a drop of perchloric acid gave a neutral product which had UV absorption maximum at 270 mμ (ethanol). Formation of a compound with this UV absorption maximum has been reported in two previous cases, viz. katonic acid[6] and 3-keto-olean-Δ^{12}-ene 28,29-dioic acid-28-methyl ester;[5] both these substances have a 12:13 double bond, and C-29 in the form of —COOH. Hence compound B was identified as the dimethyl ester of 3β-hydroxy-olean-Δ^{12}-ene 28,29-dioic acid. For convenience of reference it is suggested that it may be called serratagenic acid.

The structure proposed here for serratagenic acid was originally proposed for



I, Ia–Id
II, IIa–IIf
III

spergulagenic acid (from *Mollugo spergula*) by Chakrabarthi *et al.*[7] based on a wrong assumption that the —COOH located at C-20 is equatorial and should therefore be derived from C-29. But as a result of direct comparison of one of its derivatives with a substance of well proved structure prepared by Prof. Tursch, the earlier conclusion has been found to be wrong and the —COOH is now considered as derived from C-30.[8]

TABLE

Compound	R_1	R_2	R_3	R_4
I	αH, βOH	CO_2CH_3	CH_3	CH_2OH
Ia	αH, βOH	CO_2H	CH_3	CH_2OH
Ib	αH, βOAc	CO_2CH_3	CH_3	CH_2OAc
Ic	αH, βOH	CH_2OH	CH_3	CH_2OH
Id	=O	CO_2CH_3	CH_3	CHO
II	αH, βOH	CO_2CH_3	CO_2CH_3	CH_3
IIa	αH, βOH	CO_2CH_3	CO_2H	CH_3
IIb	αH, βOH	CO_2H	CO_2H	CH_3
IIc	αH, βOAc	CO_2CH_3	CO_2CH_3	CH_3
IId	αH, βOH	CH_2OH	CH_2OH	CH_3
IIe	=O	CO_2CH_3	CO_2CH_3	CH_3
IIf	=O	CO_2CH_3	CO_2H	CH_3
III	αH, βOH	CO_2H	CH_2OH	CH_3

EXPERIMENTAL

The material used in the investigation was obtained locally. The powdered material was extracted with pet. ether and hot alcohol. From the unsaponifiable part of the pet. ether extract, β-sitosterol, m.p. 136–137°, $[\alpha]_D$ −34·2° (*c*, 1·16) was isolated and characterized as its acetate m.p. 132°, $[\alpha]_D$ −39·6° (*c*, 1·03). The alcoholic extract on concentration gave large amounts of D-mannitol. After the removal of D-mannitol, the extract was further concentrated, a large amount of water added and extracted with n-butanol. This extract was concentrated, the residue dissolved in MeOH and the saponin precipitated with acetone. This procedure was repeated thrice when an almost colourless amorphous powder (0·5% yield) was obtained.

The saponin was refluxed with 4N 50% methanolic H_2SO_4 aq for 4 hr and after removal of MeOH the aqueous soln was extracted with ether. The aqueous portion was examined in the usual manner and D-glucose, L-rhamnose and D-xylose were identified. The ether soln was extracted with 5% NaOH aq and the alkaline soln and the precipitated Na salt were subjected to appropriate treatment to set free the acids. The sapogenin mixture (15% yield) was repeatedly treated with diazomethane and the ester mixture was chromatographed on a column of silica gel. The following three substances were isolated: (1) methyl oleanolate eluted by benzene, (0·01% yield), (2) dimethyl ester of serratagenic acid eluted by benzene–$CHCl_3$ (3:1), (0·003% yield) and (3) methyl queretaroate, eluted by $CHCl_3$ (0·003% yield). Methyl oleanolate, $C_{31}H_{50}O_3$, m.p. 198–200°, $[\alpha]_D$ +70·2° (*c*, 1·16), acetate, $C_{33}H_{52}O_4$, m.p. 224–225°, $[\alpha]_D$ +66·2° (*c*, 1·05) (comparison with authentic samples by m.m.p. and TLC).

Methyl queretaroate (I), colourless needles from MeOH, m.p. 226–228°. $[\alpha]_D$ +77·8° (*c*, 0·77), IR bands at 3448, 1735, 1667, 1385, 1366, 890 and 825 cm^{-1}. (Found: C, 77·2; H, 10·7; OMe, 6·1. $C_{31}H_{50}O_4$ requires: C, 76·5; H, 10·2 and OMe (one), 6·0%).

Diacetate (Ib) (Ac_2O–pyridine method), from MeOH–$CHCl_3$ shining flakes, m.p. 204–206°, $[\alpha]_D$ +71·9° (*c*, 1·03). (Found: C, 73·5; H, 10·0. $C_{35}H_{54}O_6$ requires: C, 73·7; H, 9·5%). The NMR spectrum in $CDCl_3$ showed the following signals (ϕ values in ppm): 5·32 (m) (1, 12-H), 4·08 (s), (2, —CH_2OAc), 3·65 (s), (3, —COOCH_3), 2·08 (s) (3), 2·06 (s) (3) (2, —OCOCH_3), 1·28 (s) (3),

* All rotations were taken in chloroform solution except when otherwise stated.

1·02 (s) (3), 0·95 (s) (3) 0·88 (s) (6) and 0·75 (s) (3) (six tertiary methyls). Mass spectrum: (percentage abundance) *m/e* 570 (5·0%), 510 (17·0), 320 (44·5), 261 (14·0), 260 (43·0), 247 (50·0), 246 (17·5), 201 (100·0), 200 (26·0), 190 (57·0), 189 (39·0), 187 (40·0), 133 (17·0).

Queretaroic acid (Ia). Compound I (100 mg) was refluxed with 25% KOH in ethylene glycol (10 ml) on a sand bath for 15 hr. The product was poured into excess water, acidified and worked in the usual manner. Crystallization from MeOH gave needles, m.p. >310°, $[\alpha]_D$ +77·9° (*c*, 1·26, pyridine). (Found: C, 75·8; H, 10·3. $C_{30}H_{48}O_4$ requires: C, 76·3; H, 9·9%).

LAH *reduction of* I *to triol* Ic. Compound I (50 mg) was refluxed in THF (20 ml) with LAH (50 mg) for 3 hr. The reaction mixture was cooled, excess reagent decomposed with moist EtOAc, acidified with dil H_2SO_4 and extracted with ether. After passing through a small column of neutral Al_2O_3 the triol crystallized as needles from acetone, m.p. 275–276°. $[\alpha]_D$ +85·5° (*c*, 0·61, pyridine). (Found: C, 78·8; H, 11·3. $C_{30}H_{50}O_3$ requires: C, 78·6; H, 10·9%).

SeO_2 *oxidation of* Ib. Compound Ib (30 mg) was refluxed in glacial AcOH (5 ml) with freshly sublimed SeO_2 (50 mg) for 6 hr. The solvent was removed under reduced press and the reddish-brown solid was chromatographed over neutral Al_2O_3. Elution with $CHCl_3$ followed by crystallization from MeOH gave pale yellow needles, m.p. 240–245°. (Found: C, 73·6; H, 9·7. $C_{35}H_{52}O_6$ requires: C, 73·9; H, 9·2%); λ_{max}^{EtOH} 242, 250 and 260 mμ (log ε: 4·38, 4·43 and 4·26 respy).

Jones' oxidation of I *to* Id. The product was crystallized from acetone as colourless needles, m.p. 210–211°, $[\alpha]_D$ +102° (*c*, 1·06). IR absorption bands at 1739, 1721, 1387, 860 and 830 cm^{-1}. The NMR spectrum in $CDCl_3$ showed an aldehydic signal at 9·6 δ. (Found: C, 76·9; H, 10·0. $C_{31}H_{46}O_4$ requires: C, 77·2; H, 10·0%).

Dimethyl ester of serratagenic acid (II). Colourless needles from MeOH, m.p. 202–204°, $[\alpha]_D$ +35·7° (*c*, 0·842). (Found: C, 75·01; H, 10·0; OMe, 12·8. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·7; OMe (two) 12·0%); IR absorption bands at 3448, 1739, 1667, 1385, 1366, 875 and 825 cm^{-1}. The NMR spectrum in $CDCl_3$ showed the following signals (δ values in ppm); 5·32 (m) (1, 12-H), 3·67 (s) (3), 3·60 (s) (two — $COOCH_3$) 1·28 (s) (3), 1·17 (s) (3), 1·03 (s) (3), 0·97 (s) (3), 0·83 (s) (3) and 0·76 (s) (3) (six tertiary methyls). Mass spectrum: (percentage abundance) *m/e* 514 (7·0%), 307 (13·0), 306 (59·0), 247 (37·0), 246 (12·0), 233 (10·5), 207 (30·0), 189 (9·0), 187 (100·0), 173 (28·5), 133 (15·0).

Acetate IIc (Ac_2O–pyridine method) crystallized from MeOH–$CHCl_3$ as shining needles, m.p. 274–276°, $[\alpha]_D$ +35·2° (*c*, 1·1). (Found: C, 72·9; H, 9·5. $C_{34}H_{52}O_6$ requires: C, 73·4; H, 9·4%).

Partial hydrolysis of II *to* IIa. Compound II (50 mg) was hydrolysed by boiling in 10% methanolic KOH (25 ml) for 6 hr. The product was worked out as usual. Crystallization from MeOH gave needles, m.p. 252–253°, $[\alpha]_D$ +49·7° (*c*, 1·01). (Found: C, 73·9; H, 9·9; OMe, 5·9. $C_{31}H_{48}O_5$ requires C, 74·4; H, 9·6; OMe (one), 6·0%). Compd II was reformed on treatment with ethereal diazomethane.

Serratagenic acid (IIb). Compound II (30 mg) was hydrolysed by refluxing with 25% KOH in ethylene glycol (6 ml) for 15 hr. The product crystallized from MeOH as needles of IIb, m.p. >310°, $[\alpha]_D$ +37·1° (*c*, 1·24, pyridine); IR absorption bands at 3450, 1710, 1667, 1370 cm^{-1}. (Found: C, 73·8; H, 10·0. $C_{30}H_{46}O_5$ requires: C, 74·1; H, 9·5%).

LAH *reduction of* II *to triol* IId. The product was crystallized from acetone as colourless needles, m.p. 287–289°, $[\alpha]_D$ +47·6° (*c*, 0·72). (Found: C, 78·8; H, 11·3. $C_{30}H_{50}O_3$ requires: C, 78·6; H, 10·9%).

SeO_2 *oxidation of* IIc. The brownish-red product was chromatographed on a column of neutral Al_2O_3 and then crystallized from MeOH as pale yellow needles, m.p. 198–200°, λ_{max}^{EtOH} 243, 251 and 260 mμ (log ε 4·35, 4·50 and 4·23 respectively). (Found: C, 73·3; H, 9·8. $C_{34}H_{50}O_6$ requires: C, 73·7; H, 9·5%).

Jones' oxidation of II *to* IIe. The product was purified by passing over a column of silica gel and crystallized twice from acetone as colourless needles, m.p. 130–132°, $[\alpha]_D$ +68·5° (*c*, 1·10). IR absorption bands at: 1735, 1718, 1385, 1366 cm^{-1}. (Found: C, 75·4; H, 9·8. $C_{32}H_{48}O_5$ requires: C, 75·0; H, 9·4%).

Hydrolysis of IIe *to* IIf. IIe was hydrolysed with 10% methanolic KOH. The product failed to crystallize even after passing through a column of silica gel. Its m.p. was over a range 120–125° but TLC in two different solvents showed it to be a single entity. The very low R_f value of the product when compared with that of IIe showed that it contained the —COOH group. Treatment with ethereal diazomethane gave back IIe as shown by TLC. The hydrolysis product (IIf) was recovered unchanged on refluxing with 2N methanolic HCl aq for 2 hr.

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