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A NEW SAPONIN OF OLEANOLIC ACID FROM *PERESKIA GRANDIFOLIA**

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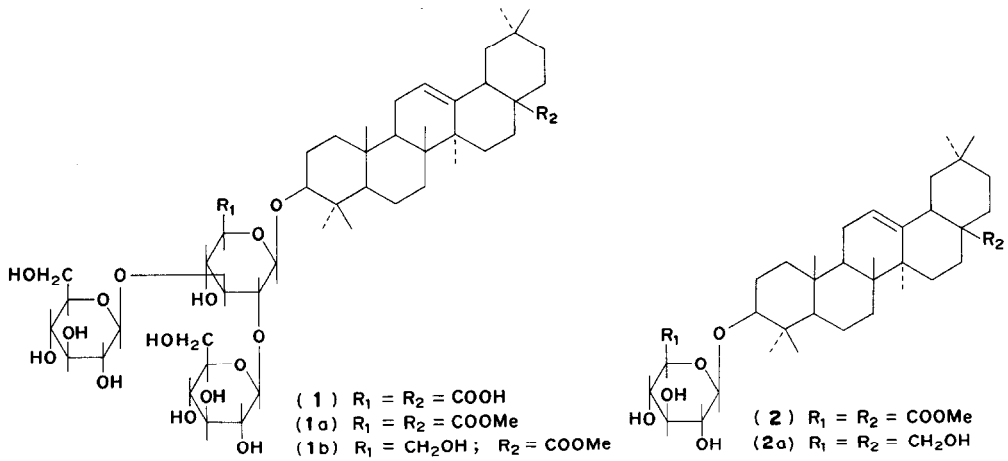
Key Word Index—*Pereskia grandifolia*; Cactaceae; oleanolic acid saponin.

Plant. Pereskia grandifolia Haw. (Syn. *Pereskia grandiflora*) (N.O. Cactaceae). *Uses.* to reduce swellings.[1] *Previous work.* None.

The dried powdered fruits after removal of the seeds, were defatted with petrol, $CHCl_3$ and then extracted with 90% EtOH. From the EtOH extract a saponin mixture was isolated which on hydrolysis gave only oleanolic acid. The saponin mixture on treatment with CH_2N_2 followed by chromatographic separation yielded a single dimethyl ester (**1a**). On complete hydrolysis, **1a** afforded methyl oleanolate as the sapogenin and D-glucose and D-glucuronic acid as sugars. Isolation of only methyl oleanolate without any significant amount of the acid established the fact that in the dimethyl ester the sugars are linked to the C-3 hydroxyl group of oleanolic acid. **1a** on partial hydrolysis with 1% H_2SO_4 furnished a product which on further treatment with CH_2N_2, followed by chromatography, yielded methyl oleanolate and dimethyl ester of oleanolic acid-β-D-glucuronopyranoside (**2**).[2,3] The identity of (**2**) was confirmed by its reduction with $LiAlH_4$ to erythrodiol-3-β-D-glucoside (**2a**), which on hydrolysis yielded erythrodiol and D-glucose. Furthermore (**1a**) on reduction with $NaBH_4$ in H_2O at room temperature[4] yielded methyl oleanolate glucoside (**1b**), which on hydrolysis furnished methyl oleanolate and D-glucose. Quantitative analysis showed that 1 mol of (**1b**) contains 1 mol of methyl oleanolate and 3 mol of D-glucose. Again (**1b**) on complete premethylation by Hakomori's method[5] followed by hydrolysis yielded 4,6-di-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose in the



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ratio 1:2. The presence of the former indicated that it is linked to two other D-glucose units through 1 → 2 and 1 → 3 linkages. This conclusion was also supported by periodate oxidation of (**1b**).

With regard to the configuration of saponin glycosidic linkages it is a general observation that D-sugars occur with β-glycosidic and L-sugars with α-glycosidic linkages.[6] Consequently one could expect β-glycosidic linkages in the dimethyl ester of the saponin. The calculation of the molecular rotation of (**1a**) on the basis of Klyne's rule[7] also supports this assumption. The calculated $[M]_D$ of (**1a**) is −9° and is in reasonable agreement with the observed value of −31·5°.

From these results, the dimethyl ester and the parent saponin can be represented as (**1a**) and (**1**) respectively.

EXPERIMENTAL

The following solvents were used for PC: (a) *n*-BuOH–HOAc–H_2O (4:1:4), upper layer; (b) *n*-BuOH–pyridine–H_2O (6:4:3); (c) EtOAc–pyridine–H_2O (8:2:1); (d) *n*-BuOH–EtOH–H_2O (4:1:5). Spray reagents were: (1) satd soln of aniline oxalate in H_2O[8] and (2) aniline hydrogen pthalate.

Isolation of a saponin mixture and one of its dimethyl ester. The EtOH extract from the fruits was dissolved in H_2O (500 ml), filtered and the filtrate extracted with Et_2O and then with *n*-BuOH (4 × 100 ml). The *n*-BuOH extract was taken in MeOH and poured into a large vol. of Et_2O. The ppt was collected and the process was repeated until a colourless powder was obtained (7·5 g). Zeisel's estimation showed the absence of methoxyl. The saponin mixture in MeOH (500 ml) was treated with excess of an Et_2O soln of CH_2N_2. The product (3·2 g) was chromatographed over a silica gel. Elution with $CHCl_3$–MeOH (17:3) gave a homogeneous compound, crystallized from 85% MeOH, when saponin dimethyl ester (**1a**) was obtained in micro needles, m.p. 275–280° (dec.), $[\alpha]_D^{27}$ −3·2° (EtOH) (Found: C, 60·65; H, 8·24; OMe, 6·9. $C_{50}H_{80}O_{19}$ requires: C, 60·97; H, 8·13; OMe, 6·3%).

Hydrolysis of **1a**. 100 mg **1a** was refluxed with 6% H_2SO_4 in 80% MeOH for 8 hr and gave methyl oleanolate (30 mg) (m.p., m.m.p., IR, TLC) D-glucose and D-glucuronic acid (identified by PC in solvents a–e).

Partial hydrolysis of **1a**. **1a** (500 mg) was refluxed with 50 ml 1% H_2SO_4 in MeOH for 1 hr and the product was treated with excess of ethereal CH_2N_2 and chromatographed over silica gel. C_6H_6 eluted methyl oleanolate (80 mg) (m.p., m.m.p., IR) and $CHCl_3$–MeOH (99:1) eluted dimethyl ester of oleanolic acid β-D-glucuronopyranoside **2**(165 mg) (crystallized from MeOH–ether mixture), m.p. 202–205°, $[\alpha]_D^{27}$ + 18° (EtOH) (Found: C, 69·25; H, 9·40. Calc. for $C_{38}H_{60}O_9$: C, 69·10; H, 9·2%). **2** on further hydrolysis furnished methyl oleanolate and D-glucuronic acid.

Reduction of **2** *to* **2a**. **2** (50 mg) was reduced with $LiAlH_4$ in boiling THF (100 ml) for 20 hr. The product was crystallized from MeOH–ether mixture when erythrodiol-3-β-D-glucoside (**2a**) was obtained m.p. 107–108°, $[\alpha]_D^{27}$ +27·3° (EtOH). **2a** on usual hydrolysis yielded erythrodiol and D-glucose.

Reduction of (**1a**) *to* (**1b**). A soln of $NaBH_4$ (300 mg) in H_2O (200 ml) was added to a soln of **1a** (300 mg) in H_2O (75 ml) and kept overnight. The product (**1b**) was an amorphous powder (200 mg), m.p. 229–234° $[\alpha]_D^{27}$ −6·5° (EtOH).

Quantitative estimation of sapogenin and sugars in **1b**. 24·45 mg **1b** was hydrolysed with 7% H_2SO_4 in MeOH (5 ml) in a sealed tube, and both the sapogenin and the sugars were estimated. Methyl oleanolate was found to be 45·4% (required 46·5%). The syrup containing sugars was estimated by Dubois method[9] and the percentage of D-glucose was found to be 52·1%, which corresponded to a trioside (required 53·4%).

Permethylation of **1b** *and hydrolysis.* 50% NaH dispersion in oil (*ca.* 250 mg) was suspended in DMSO (25 ml) and kept in an oil bath at 80° for 1 hr. A soln of **1b** (150 mg) in DMSO (5 ml) was then added gradually and the mixture was kept at 80° for 1 hr with constant stirring. It was cooled in ice and MeI (3 ml) was added in drops. The syrup (120 mg) produced was hydrolysed and the methylated sugars were identified by PC (solvent d) as 2,3,4,6-tetra-*O*-methyl-D-glucose and 4,6-di-*O*-methyl-D-glucose having R_G 1·00 and 0·45 respectively. Further the methyl sugars were estimated quantitatively by alkaline hypoiodide method[10] and the molar ratio of 4,6-di-*O*-methyl-D-glucose: 2,3,4,6-tetra-*O*-methyl-D-glucose was found to be 1·00:2·10.

Periodate oxidation of **1b**. A soln of **1b** (20 mg) in EtOH (10 ml) was treated with an equal vol. of 0·1 $NaIO_4$. After 50 hr the liberation of formic acid and periodate uptake became constant corresponding to 2·2 and 3·98 mol respectively.

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