unidentified compound had the same retention time as the TMSi ether of lanosterol (RR_t 0.98), but the acetylated derivative (RR_t 0.93) was separated from the acetylated lanosterol (RR_t 0.99). Cycloartenol was completely separated from 31-norcyclolaudenol (RR_t of acetylated derivative 1.03) and 24-methylene cycloartanol from cyclolaudenol (RR_t of acetylated derivative 1.20).

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OBTUSILOBICININ, A NEW SAPONIN FROM *ANEMONE OBTUSILOBA*

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Key Word Index—*Anemone obtusiloba;* Ranunculaceae; obtusilobicinin; olean-12-ene-28-oic-3-*O*-(α-L-arabinofuranosyl 1→2) (α-L-arabinofuranosyl 1→2-*O*-α-L-rhamnopyranosyl 1→4)-β-D-glucopyranoside.

INTRODUCTION

Several saponins [1–6] have been isolated from different species of the genus *Anemone*, but, surprisingly, complete structures of very few of them have been elucidated [5, 6]. The presence of two saponins, obtusilobin and obtusilobinin [6], has already been reported in *Anemone obtusiloba* [7].

RESULTS AND DISCUSSION

Obtusilobicinin (**1**), $C_{52}H_{84}O_{20}$, gave all the tests of saponins [8]. Hydrolysis with 7% H_2SO_4 yielded oleanolic acid (IR, ^{1}H NMR, MS) [9–12], D-glucose, L-rhamnose and L-arabinose (co–PPC). The sugars were found to be present in the ratio of 1:1:2 as revealed by colorimetric estimation [13] and the genin content was found to be 44.0% (quantitative hydrolysis) against 44.35% calculated for one unit of oleanolic acid and four units of sugars per molecule of obtusilobicinin. Thus, a molecule of obtusilobicin contains one unit each of oleanolic acid, D-glucose, L-rhamnose and two units of L-arabinose.

It is evident from the structure of oleanolic acid that the —OH at C-3 and the —COOH at C-17 are available for glycosidic linkage with sugar residues. The saponin could not be hydrolysed with 5 N NH_4OH, which is a specific reagent [14] for the hydrolysis of sugar esters, indicating that sugars were not present in ester combination with the —COOH group of oleanolic acid. This led to the conclusion that all the sugar units were linked as a tetroside unit to the —OH

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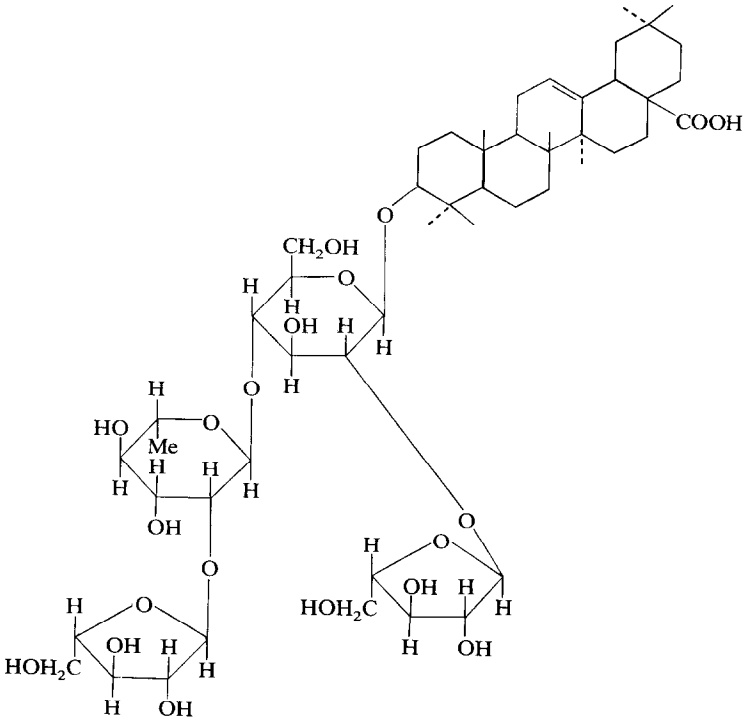

1

at C-3 of oleanolic acid.

The sequence of sugar moieties in obtusilobicinin was determined by its partial hydrolysis to produce two prosapogenins designated as PS_1 and PS_2. Hydrolysis of prosapogenin PS_1 yielded oleanolic acid and D-glucose, whereas hydrolysis of PS_2 yielded oleanolic acid, D-glucose and L-rhamnose, indicating that L-arabinose moieties were the end sugars and D-glucose was the first sugar in the saponin.

To determine the exact sugar linkages, the saponin and the prosapogenins PS_1 and PS_2 were permethylated [15] and hydrolysed. The prosapogenin PS_1, on the previous treatment, released 2,3,4,6-tetra-*O*-methyl-D-glucose indicating that C-1 of the D-glucose moiety was involved in the formation of a glycosidic linkage with C-3 of the genin, whereas PS_2 released 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose, indicating that C-1 of L-rhamnose was linked with C-4 of D-glucose. The saponin on permethylation and hydrolysis yielded oleanolic acid, 3,6-di-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-L-rhamnose and 2,3,5-tri-*O*-methyl-L-arabinose. As it had already been established that two arabinose moieties were present in the saponin, the release of arabinose as its trimethyl ether from the saponin further supported the fact that both arabinose moieties were the end sugars. The release of D-glucose and L-rhamnose as their respective trimethyl ethers (2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose) from PS_2 and as their respective dimethyl ethers (3,6-di-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-L-rhamnose) from the saponin led to the conclusion that both the L-arabinose moieties were linked by C-1′ independently to C-2 of L-rhamnose and D-glucose, respectively, in the saponin.

The saponin obtusilobicinin, on hydrolysis with diastase, yielded L-rhamnose, L-arabinose and a prosapogenin PS_1 which on almond emulsin hydrolysis released D-glucose indicating that the rhamnose and arabinose moieties were involved in the formation of an α-glycosidic linkage and glucose was involved in the β-glycosidic linkage.

The exact configurations of the sugar linkages in the saponin were established by a consideration of the molecular rotation values in the light of Klyne's rule. The different possible combinations of the sugar linkages are shown [16–18] in Table 1. The observed $[M]_D$ value for the saponin was $-236.4°$. The $[M]_D$ value of the genin is known to be $+355°$. The difference, $-591.4°$, is close to the first combination shown in Table 1. Therefore, the exact configuration of the sugar linkages was D-glucose-β, L-rhamnose-α and L-arabinose-α.

After elucidating the nature of the glycosidic linkage and the position of attachment of all the sugars in the saponin, a complete structure to obtusilobicinin may be assigned by taking into consideration the easy elimination of L-arabinose on partial hydrolysis of the saponin, which indicated that L-arabinose was present as a furanoside [19]. Furthermore, the release of L-arabinose as 2,3,5-tri-*O*-methyl-L-arabinose from permethylated saponin, L-rhamnose as 2,3,4-tri-*O*-methyl-L-rhamnose from permethylated PS_2 and D-glucose as 2,3,4,6-tetra-*O*-methyl-D-glucose from the permethylated PS_1 clearly suggests that D-glucose and L-rhamnose moieties were present as pyranosides and L-arabinose as a furanoside. This fact was also confirmed by the periodate oxidation of obtusilobicinin,

Table 1. Possible combinations of the sugar linkages in obtusilobicinin

Combinations of methyl glycosides	Total $[M]_D$ values
β-D-glu + α-L-rh + α-L-ar + α-L-ar	−66−110−205−205 = −586°
β-D-glu + α-L-rh + α-L-ar + β-L-ar	−66−110−205− 77 = −458°
β-D-glu + α-L-rh + β-L-ar + β-L-ar	−66−110− 77− 77 = −330°
β-D-glu + β-L-rh + α-L-ar + α-L-ar	−66+168−205−205 = −308°
β-D-glu + β-L-rh + α-L-ar + β-L-ar	−66+168−205− 77 = −180°
β-D-glu + β-L-rh + β-L-ar + β-L-ar	−66+168− 77− 77 = − 52°

PS_2 and PS_1. Hence, obtusilobicinin **1** is olean-12-ene-28-oic-3-*O*-(α-L-arabinofuranosyl 1 → 2) (α-L-arabinofuranosyl 1 → 2-*O*-α-L-rhmnopyranosyl 1 → 4)-β-D-glucopyranoside.

EXPERIMENTAL

Extraction and isolation. The defatted powdered plant (5 kg) was exhaustively extracted with EtOH. The EtOH extract (4.6 l.) was concd *in vacuo*. The residue was washed successively with Et_2O, $CHCl_3$ and Me_2CO and was finally dissolved in MeOH, filtered and the filtrate poured into excess Et_2O, whereby a brown mass precipitated. The ppt. was again dissolved in a little MeOH and was adsorbed onto a column of Si gel and eluted successively with a mixture of MeOH and Me_2CO (1:2), and MeOH. The MeOH and Me_2CO eluate gave 2 fractions which yielded obtusilobin and obtusilobinin [6], whereas the MeOH eluate after the removal of the solvent yielded a brown mass. The brown mass was dissolved in a minimum amount of MeOH and poured into excess Et_2O with constant stirring, whereupon a brown mass precipitated. On repeating this process several times, a light brown ppt. of obtusilobicinin (**1**) was obtained, (1.55 g) mp 226°, R_f 0.52 (PPC; BuOH–HOAc–H_2O, 4:1:5). (Found: C, 60.82; H, 8.26. Calc. for $C_{52}H_{84}O_{20}$: C, 60.70; H, 8.17%.)

Identification of sugars in the hydrolysate, isolation and study of the sapogenin from obtusilobicinin (**1**). Obtusilobicinin (300 mg) was hydrolysed by refluxing with 7% H_2SO in EtOH (100 ml) for 5 hr. The product was poured into H_2O (300 ml) and the EtOH was removed by distillation *in vacuo*. After the removal of EtOH, the aq. hydrolysate was kept overnight in a refrigerator, whereupon the sapogenin settled at the bottom and was separated by filtration, purified by the K-salt method [20] and crystallized from $CHCl_3$ to yield white crystals (132 mg), mp 308–310°, $[\alpha]_D^{26}$ + 78° ($CHCl_3$). (Found: C, 78.98; H, 10.82. Calc. for $C_{30}H_{48}O_3$: C, 78.94; H, 10.52%.) IR ν_{max}^{KBr} cm^{-1}: 3420, 2900, 2840, 1701, 1464, 1390, 1366, 1347, 1325, 1305, 1264, 828, 818, 804; MS *m/e*: 456 (M^+), 441, 411, 410, 395, 300, 248, 207, 203 (base peak), 189, 175, 133; methyl ester: $C_{31}H_{50}O_3$, mp 198–199°; MS *m/e* 470 (M^+), 455, 411, 410, 262, 249, 207, 203 (base peak), 189, 133, ^1H NMR ($CDCl_3$): δ 0.75 (3H), 0.80 (3H), 0.97 (6H), 1.00 (6H), 1.16 (3H), 3.60 (3H), 5.28 (1H). The aq. hydrolysate, after being neutralized with $BaCO_3$, on PPC (BuOH–HOAc–H_2O, 4:1:5; spray—aniline hydrogen phthalate) revealed the presence of D-glucose (R_f 0.18), L-rhamnose (R_f 0.36) and L-arabinose (R_f 0.20).

Quantitative estimation of sugars in the saponin hydrolysate. The ratio of sugars in the saponin was determined colorimetrically [13] in a Klett–Summerson photoelectric colorimeter using a blue filter (420 nm) with the help of standard curves of authentic sugars. Then solns (10, 20, 30, 40, 50, . . . , 100 μg in 0.02 ml H_2O) of each of 3 sugars, D-glucose, L-rhamnose and L-arabinose were applied on Whatman No. 1 filter papers (50 × 55 cm, spot distances 4 cm). The chromatograms were developed by descending technique with BuOH–HOAc–H_2O (4:1:5) for 20 hr, dried in air, sprayed with aniline hydrogen phthalate and dried at 110° for 15 min. The coloured spots were cut out in equal rectangles, eluted by immersing in 50% HOAc (5 ml each) and the colour intensity of each eluate measured. The sugars in the hydrolysate of **1** were assayed as described above.

Partial hydrolysis of **1**; *isolation of oleanolic acid-β-D-glucopyranoside* (PS_1) *and oleanolic acid-β-D-glucorhamnopyranoside* (PS_2). **1** (1 g) and 1% H_2SO_4 in MeOH (100 ml) were kept for 4 days at room temp. MeOH was removed and H_2O (25 ml) was added. The aq. soln was extracted with BuOH which was concd and chromatographed over a column of Si gel using a mixture of $CHCl_3$–EtOH (1:1) as solvent, whereupon 2 prosapogenins, PS_1 and PS_2, were obtained.

Permethylation of **1**; *oleanolic acid-β-D-glucopyranoside* (PS_1) *and oleanolic acid-β-D-glucorhamnopyranoside* (PS_2) *and hydrolysis of the permethylated derivatives.* The glycosides (100 mg each) were treated with MeI (2 ml) and Ag_2O (1 g) in DMF (5 ml) separately for 50 hr at room temp. The mixtures were filtered and the residue washed with a little DMF. The filtrate was evapd to dryness and the residue taken in EtOH (30 ml). The syrups obtained after the removal of EtOH were hydrolysed with Killiani's mixture (HOAc–HCl–H_2O, 7:3:10) [21] and the product worked up in the usual way. The products were analysed by PPC (BuOH–EtOH–H_2O, 5:1:4) [22–24]. The hydrolysate from **1** contained 3,6-di-*O*-methyl-D-glucose (R_g = 0.91); 3,4-di-*O*-methyl-L-rhamnose (R_g = 0.84) and 2,3,5-tri-*O*-methyl-L-arabinose (R_g = 0.95); PS_1 hydrolysate contained 2,3,4,6-tetra-*O*-methyl-D-glucose (R_g = 1.0); PS_2 hydrolysate contained 2,3,6-tri-*O*-methyl-D-glucose (R_g = 0.83) and 2,3,4-tri-*O*-methyl-L-rhamnose (R_g = 1.01).

Periodate oxidation of **1**, *oleanolic acid-β-D-glucoside* (PS_1) *and oleanolic acid glucorhamnoside* (PS_2). Periodate oxidation was carried out by the method in ref. [25]. 50 mg of PS_1 or PS_2 were dissolved in 25 ml EtOH, and 25 ml 0.25 M sodium metaperiodate soln added. The oxidation was allowed to proceed at room temp. for 60 hr. Aliquots (5 ml) were withdrawn in duplicate from the reaction mixture at different intervals of time and analysed for periodate and formic acid.

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3-*EPI*KATONIC ACID FROM GUAR MEAL, *CYAMOPSIS TETRAGONOLOBA*

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Key Word Index—*Cyamopsis tetragonoloba;* Leguminosae; guar; seed meal; saponin; pentacyclic triterpene; 3-*epi*katonic acid; 11-deoxo-18β-liquiritic acid; 3β-hydroxyolean-12-ene-29-oic acid.

INTRODUCTION

Guar, *Cyamopsis tetragonoloba* L. (syn. *C. psoraloides*) is a drought-tolerant annual herb indigenous to the Indian subcontinent. The plant is also grown commercially in the drier areas of U.S., South Africa and Australia because the seeds are a source of the valuable guar gum, a galactomannan which is used in confectionery and cosmetics. After milling to remove the endosperm which contains most of the gum, the residual meal (45% protein) is used as a stock feed. However, the incorporation of guar meal in poultry diets is limited by its adverse effect on chick growth and on egg production. Verma [1] working at the Poultry Research Centre, showed that guar meal contained about 10% saponin and that the addition of cholesterol to a laying-hen diet containing guar meal improved egg production.

This paper reports on the isolation and identification of the major sapogenin obtained by acid hydrolysis of the saponin extracted from guar meal.

RESULTS AND DISCUSSION

Although the saponin has not been obtained in a pure crystalline state, acid hydrolysis of the crude product gave 5% by weight of ether-soluble material from which a crystalline acidic sapogenin, mp 283–284°, was isolated by recrystallization from acetone. The MS and ^{13}C NMR were consistent with those of a 3β-hydroxypentacylic triterpene acid, distinct from oleanolic acid. Comparison ot the mps and $[\alpha]_D$, of several derivatives of the guar sapogenin with those of 3-*epi*katonic acid [2] indicated a similarity. The identity of guar sapogenin was established as 3-*epi*katonic acid or 11-deoxo-18β-liquiritic acid **1** [mmp of acetate