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OBTUSILOBININ AND OBTUSILOBIN, TWO NEW TRITERPENE SAPONINS FROM *ANEMONE OBTUSILOBA*

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Key Word Index—*Anemone obtusiloba*; Ranunculaceae; obtusilobinin; obtusilobin; saponins; oleanolic acid derivatives.

Abstract—Obtusilobinin and obtusilobin, two new saponins, have been isolated from the ethanolic extract of *Anemone obtusiloba* (Ranunculaceae). The structural elucidation of obtusilobinin and obtusilobin have showed them to be olean-12-ene-28-oic-3-*O*-(α-L-arabinofuranosyl 1→2) (α-L-rhamnopyranosyl 1→4)-β-D-glucopyranoside and olean-12-ene-28-oic-3-*O*-α-L-rhamnopyranosyl 2→1-*O*-α-L-arabinofuranoside, respectively.

INTRODUCTION

Several saponins [1–5] have been isolated from different species of the genus *Anemone*, but surprisingly, very few complete structures have been elucidated [5]. We have therefore examined *Anemone obtusiloba* [6] with a view to a detailed study of the saponin and other constituents of the plant. The ethanol extract of the plant afforded two saponins, provisionally named as obtusilobinin and obtusilobin.

RESULTS AND DISCUSSION

Obtusilobinin, $C_{47}H_{76}O_{16}$, gave all the tests of saponin [7] and on hydrolysis with 7% H_2SO_4, yielded oleanolic acid (IR, ^1H NMR, MS) [8–11], D-glucose, L-rhamnose and L-arabinose (co-PPC). The sugars were found to be present in equimolar proportion (1:1:1) as revealed by colorimetric estimation [12] and the genin content was found to be 50.7% (quantitative hydrolysis) against 50.89% calculated for one unit of oleanolic acid and three units of sugars per molecule of obtusilobinin. Thus a molecule of obtusilobinin contained one unit each of oleanolic acid, D-glucose, L-rhamnose and L-arabinose.

From the structure of oleanolic acid, it is evident that only the —OH at C-3 and —COOH at C-17 were available for linkage with sugar residues. The saponin was not hydrolysed with 5N NH_4OH, which is a specific reagent [13] for the hydrolysis of sugar esters, indicating that sugars were not present in ester combination with the —COOH group. This led to the conclusion that all three sugar units were linked as a trioside unit to the —OH at C-3.

The sequence of the sugar moieties in obtusilobinin was determined by partial hydrolysis which resulted in two prosapogenins designated Ps_1 and Ps_2. Hydrolysis of prosapogenin Ps_1 yielded oleanolic acid, D-glucose and L-rhamnose, but Ps_2 gave oleanolic acid and D-glucose. This showed L-arabinose to be the end sugar and D-glucose to be the first sugar in the saponin.

The saponin was permethylated [14] and hydrolysed to yield three methylated sugars, 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-L-rhamnose and 3,6-di-*O*-methyl-D-glucose. The release of L-arabinose and L-rhamnose as their respective trimethyl ethers led to the conclusion that L-arabinose and L-rhamnose were linked independently to different hydroxyls of the same glucose moiety in the saponin. The prosapogenin Ps_2 on permethylation and hydrolysis yielded oleanolic acid and 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 the genin. Permethylation of prosapogenin Ps_1 followed by hydrolysis yielded oleanolic acid, 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose, indicating that C-1 of the L-rhamnose unit was linked to C-4 of the D-glucose moiety. Hence prosapogenins Ps_1 and Ps_2 may be represented by structures **1** and **2**, respectively. The production of 3,6-di-*O*-methyl-D-glucose, 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-L-rhamnose on hydrolysis of permethylated saponin clearly suggested that the C-2 and C-4 of the glucose moiety were involved in the formation of glycosidic linkage with C-1 each of L-arabinose and L-rhamnose, respectively. The saponin, obtusilobinin, on hydrolysis with diastase, yielded L-rhamnose, L-arabinose and a prosapogenin Ps_2 which on hydrolysis with almond–emulsin released D-glucose, indicating that rhamnose and arabinose moieties were involved in the formation of an α-glycosidic linkage and glucose in a β-glycosidic linkage.

The exact configuration of sugar linkages in the saponin was established by the consideration of the molecular rotation values in the light of Klyne's rule [15–17] and the four possible combinations of the sugar linkages are shown in Table 1.

The observed $[M]_D$ value for the saponin was −29.5°. The $[M]_D$ value of the genin is known to be +355°. The difference (−384.5°) is close to the first combination of 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 position of attachment of all the sugars in the saponin, a complete structure to obtusilobinin was assigned by considering the elimination of L-arabinose during partial hydrolysis of the saponin, which indicated that L-arabi-

Table 1. Possible combinations of the sugar linkages in obtusilobinin

Combinations of methyl glycoside	Total $[M]_D$ values (degrees)
β-D-glu + α-L-rh + α-L-ar	−66 − 110 − 205 = −381
β-D-glu + α-L-rh + β-L-ar	−66 − 110 − 77 = −253
β-D-glu + β-L-rh + α-L-ar	−66 + 168 − 205 = −103
β-D-glu + β-L-rh + β-L-ar	−66 + 168 − 77 = −25

nose was present as a furanoside [18]. Furthermore, release of L-arabinose as 2,3,5-tri-*O*-methyl-L-arabinose and L-rhamnose as 2,3,4-tri-*O*-methyl-rhamnose on hydrolysis of permethylated obtusilobinin, and 2,3,4,6-tetra-*O*-methyl-D-glucose from the hydrolysis of permethylated Ps_2 clearly suggest that D-glucose and L-rhamnose moieties were present as pyranosides, and L-arabinose as furanoside. The above fact was also confirmed by the periodate oxidation of obtusilobinin and prosapogenin Ps_1 (**1**). Hence obtusilobinin (**3**) is olean-12-ene-28-oic-3-*O*-(α-L-arabinofuranosyl 1→2) (α-L-rhamnopyranosyl 1→4)-β-D-glucopyranoside.

Obtusilobin, $C_{41}H_{66}O_{11}$, gave on hydrolysis oleanolic acid, L-rhamnose and L-arabinose. The sugars were found to be equimolar in proportion (1:1) as revealed by colorimetric estimation [12] and the yield of the sapogenin was found to be 61.54% (quantitative hydrolysis) against 62.12% calculated for one unit of oleanolic acid and two units of sugars per molecule of obtusilobin.

The sequence of the sugar moieties in the saponin was determined by partial hydrolysis which resulted in a prosapogenin, Ps_3, characterized as olean-12-ene-28-oic-3-*O*-α-L-rhamnopyranoside (by mmp with an authentic sample, isolated from *A. narcissiflora*) and L-arabinose.

The sugar linkages were established by permethylation of prosapogenin and the saponin, followed by their hydrolysis, whereupon 2,3,4-tri-*O*-methyl-L-rhamnose was obtained from prosapogenin and 3,4-di-*O*-methyl-L-rhamnose and 2,3,5-tri-*O*-methyl-L-arabinose from the saponin. These results led to the conclusions that C-1 of the L-arabinose moiety was linked to C-2 of L-rhamnose and C-1 of L-rhamnose was linked to C-3 OH of oleanoic acid.

The saponin obtusilobin yielded, on hydrolysis with diastase oleanolic acid, L-rhamnose and L-arabinose indicating that both the sugars were involved in the formation of α-glycosidic linkages. The exact configuration of the sugar linkages was established by consideration of the molecular rotation values in the light of Klyne's rule [15–17] and the four possible combinations of both the sugar linkages are shown in Table 2.

Table 2. Possible combinations of the sugar linkages in obtusilobin

Combinations of methyl glycosides	Total $[M]_D$ values (degrees)
α-L-rh + α-L-ar	−110 − 205 = −315
α-L-rh + β-L-ar	−110 − 77 = −187
β-L-rh + α-L-ar	+168 − 205 = −37
β-L-rh + β-L-ar	+168 − 77 = +91

The observed $[M]_D$ value for the saponin was −36.7° and the $[M]_D$ value of the sapogenin was known to be +355°. The difference −318.3° was close to the first combination of sugar linkage, hence the nature of the linkage was established as rhamnose-α and arabinose-α.

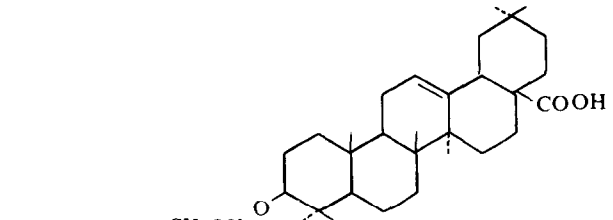
After establishing the nature of the glycosidic linkage and position of attachment of both the sugars in the saponin, a complete structure to obtusilobin was assigned by considering the easy elimination of L-arabinose during the partial hydrolysis of obtusilobin and the release of 2,3,5-tri-*O*-methyl-L-arabinose on hydrolysis of the permethylated obtusilobin, which clearly suggest that arabinose was present as the furanoside [18] leaving only its anomeric —OH for glycosidic linkage with the L-rhamnose unit. The results of periodate oxidation of obtusilobin and prosapogenin Ps_3 further confirmed the presence of L-rhamnose as pyranoside and L-arabinose as furanoside. Hence, obtusilobin (**4**) is olean-12-ene-28-oic-3-*O*-α-L-rhamnopyranosyl 2 → 1-*O*-α-L-arabinofuranoside.

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 was poured into excess Et_2O whereby a brown mass was pptd. The ppt. was dissolved in MeOH and, adsorbed onto a column of Si gel and eluted with a mixture of MeOH and Me_2CO (1:2) to give 2 fractions. Both fractions were redissolved in MeOH and poured in excess Et_2O separately whereupon a light brown mass was pptd. Both compounds were crystallized from MeOH to yield obtusilobinin (2.2 g), mp 220° and obtusilobin (2.05 g), mp 190° and the purity checked by PPC (BuOH–HOAc–H_2O, 4:1:5: R_f 0.55 and 0.66, respectively). [Found (obtusilobinin): C, 60.46; H, 9.38. $C_{47}H_{76}O_{16}$ requires: C, 61.94; H, 8.48%. Found (obtusilobin): C, 66.86; H, 9.32. $C_{41}H_{66}O_{11}$ requires: C, 67.02; H, 8.99%].

Identification of sugars in the hydrolysate, isolation and study of sapogenin from obtusilobinin and obtusilobin. Obtusilobinin and obtusilobin (1 g each) were hydrolysed separately by refluxing with 7% H_2SO_4 in EtOH (100 ml) for 5 hr on a steam bath. The products were poured in H_2O (500 ml) and EtOH was removed by distillation *in vacuo*. The sapogenins were separated from the aq. hydrolysates and purified as the K salt [19]. They were crystallized from $CHCl_3$ into colourless cystals (0.507 and 0.615 g, respectively), mp of both 308–10°, $[\alpha]_D^{26}$ +78 ($CHCl_3$). Both sapogenins were found to be the same compound by TLC ($CHCl_3$–C_6H_6–EtOAc, 1:2:3; spray—30% $SbCl_3$ in $CHCl_3$; R_f 0.35). (Found: C, 77.98; H, 10.86. $C_{30}H_{48}O_3$ requires: 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). Both hydrolysates were neutralized with $BaCO_3$. The neutral aq. hydrolysate from obtusilobinin revealed the presence of D-glucose (R_f 0.18), L-rhamnose (R_f 0.36) and L-arabinose (R_f 0.20), and the hydrolysate from obtusilobin revealed the presence of L-rhamnose and L-arabinose by PPC (BuOH–HOAc–H_2O, 4:1:5; spray—aniline hydrogen phthalate).

Quantitative estimation of sugars in the saponin hydrolysate. The ratio of sugars in the saponin was determined colorimetrically [12] in a Klett–Summerson photoelectric colorimeter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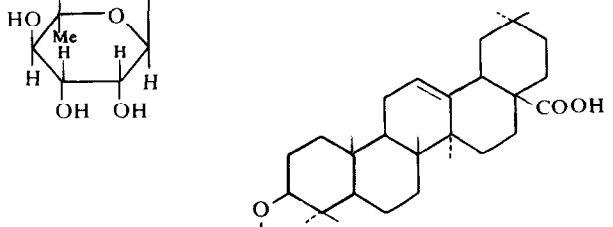
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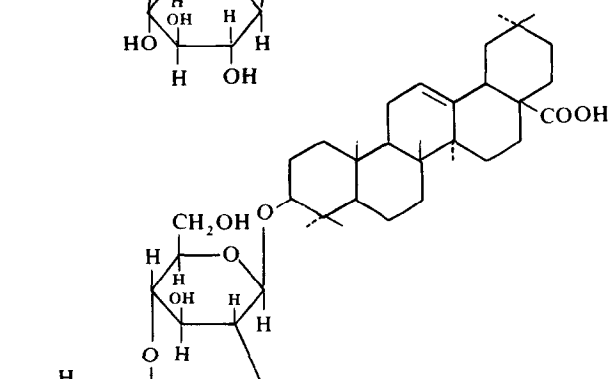
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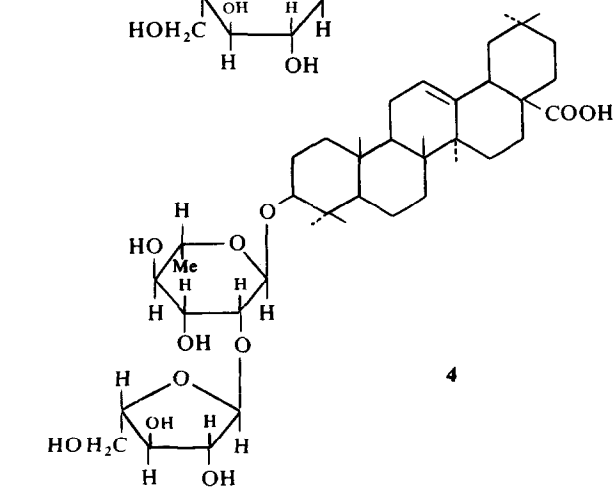
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using a blue filter (420 nm), with the help of standard curves of authentic sugars. Ten solns (10, 20, 30, 40, 50–100 μg in 0.02 ml H_2O) of each of 3 sugars, D-glucose, L-rhamnose and L-arabinose, were applied on Whatman No. 1 filter papers (50 × 55 cm, spot distances 4 cm). The chromatograms were developed by the descending technique with BuOH–HOAc–H_2O (4:1:5) for 24 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 hydrolysates of obtusilobinin and obtusilobin (see method above) were assayed as described above.

Partial hydrolysis of obtusilobinin (**3**) *and obtusilobin* (**4**): *isolation of oleanolic acid-β-D-glucorhamnopyranoside* (**1**), *oleanolic acid-β-D-glucopyranoside* (**2**) *and oleanolic acid-α-L-rhamnopyranoside.* Obtusilobinin and obtusilobin (1 g each) and 1% H_2SO_4 in MeOH (80 ml) were kept for 4 days separately at 34°. MeOH was removed and H_2O (20 ml) was added. The aq. solns were extracted with BuOH. The BuOH extract of the acid hydrolysate of obtusilobinin, on concn to a syrup and CC over Si gel, solvent $CHCl_3$–EtOH (1:1), yielded 2 prosapogenins, Ps_1 (**1**) and Ps_2 (**2**). The BuOH extract of the acid hydrolysate of obtusilobin on concn and CC over Si gel, solvent $CHCl_3$–EtOH (1:1), yielded a prosapogenin identified as oleanolic acid α-L-rhamnopyranoside mp 206 208°, $[\alpha]_D^{26}$ +39.2° (MeOH). (Found: C, 70.78; H, 9.98. $C_{36}H_{58}O_7$ requires: C, 71.76; H, 9.63%).

Permethylation of obtusilobinin (**3**), *oleanolic acid-β-D-glucorhamnopyranoside* (**1**), *oleanolic acid-β-D-glucopyranoside* (**2**), ***obtusilobin* (4) *and oleanolic-α-L-rhamnopyranoside and the hydrolysis of the permethylated derivatives.*** The glycosides (80 mg each) were treated with MeI (2 ml) and Ag_2O (1 g) in DMF (4 ml) separately for 48 hr at room temp. The contents were filtered and the residue washed with a little DMF. The filtrate was evapd to dryness and the residue taken up in EtOH (30 ml). The syrups obtained after removal of EtOH were hydrolysed with Killiani's mixture (HOAc–HCl–H_2O, 35:15:50) [20] and the product worked up in the usual way. The products were analysed by PPC (BuOH–EtOH–H_2O, 5:1:4) [21–23]. The hydrolysate from **3** contained 3,6-di-*O*-methyl-D-glucose (R_g) 0.91; 2,3,4-tri-*O*-methyl-L-rhamnose (R_g) 1.01 and 2,3,5-tri-*O*-methyl-L-arabinose (R_g) 0.95; **1** hydrolysate contained 2,3,6-tri-*O*-methyl-D-glucose (R_g) 0.83 and 2,3,4-tri-*O*-methyl-L-rhamnose; **2** hydrolysate contained 2,3,4,6-tetra-*O*-methyl-D-glucose (R_g) 1.0; **4** hydrolysate contained 3,4-di-*O*-methyl-L-rhamnose (R_g) 0.84 and 2,3,5-tri-*O*-methyl-L-arabinose and the oleanolic acid-α-L-rhamnopyranoside hydrolysate contained 2,3,4-tri-*O*-methyl-L-rhamnose.

Periodate oxidation of obtusilobinin (**3**), *oleanolic acid-glucorhamnoside* (**1**), *obtusilobin* (**4**) *and oleanolic acid-rhamnoside.* **Periodate oxidations were carried out separately by the method of ref. [24]. 50 mg of each were dissolved in 25 ml EtOH and 25 ml** 0.25 M sodium metaperiodate soln added. The oxidation was allowed to take place 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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REFERENCES

1. Luft, G. (1926) *Monatsh.* **47**, 259.
2. Balansard, J. and Delphant, J. (1945) *Med. Trop.* **5**, 322.
3. Beinfait, A. (1960) *Bull. Ordre Pharm.* **15**, 167.
4. **Huang, W. Y., Chen, W.-K., Chou, Y.-L. and Chu, J.-H. (1962) *Hua Hsuch Hsuch Pao* 28, 126; (1963) *Chem. Abstr.* 59, 1692.**
5. Tiwari, K. P. and Singh, R. B. (1978) *Phytochemistry* **17**, 1991.
6. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants* p. 18. CSIR, New Delhi.
7. **Sannie, C. H. (1948) *Analyt. Biochem. Med.* 9, 175.**
8. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
9. Wilson, R. G. and Williams, D. H. (1969) *Tetrahedron* **25**, 155.
10. Djerassi, C. (1953) *J. Am. Chem. Soc.* **75**, 2254.
11. Tandon, S. P. and Tiwari, K. P. (1967) *Proc. Natl. Acad. Sci. India Sect. A* **37**, 173.
12. Misra, S. B. and Rao, V. K. M. (1960) *J. Sci. Ind. Res. Sect. C (India)* **19**, 173.
13. Hariharan, V. and Rangaswamy, S. (1970) *Phytochemistry* **9**, 409.
14. **Kuhn, R., Trischmann, H. and Low, I. (1955) *Angew. Chem.* 67, 32.**
15. Klyne, W. (1950) *Biochem. J.* **47**, 4.
16. Seshadri, T. R. and Vydeeswaran, S. (1972) *Indian J. Chem.* **10**, 589.
17. Dutta, T. and Basu, U. P. (1968) *Indian J. Chem.* **6**, 471.
18. Jones, J. K. N. and Smith, F. (1946) *Advances in Carbohydrate Chemistry* Vol. 2, p. 243. Academic Press, New York.
19. **Dodge, F. D. (1918) *J. Am. Chem. Soc.* 40, 1917.**
20. Kiliani, H. (1930) *Ber. Dtsch. Chem. Ges.* **63B**, 2866.
21. Adams, G. A. (1960) *Can. J. Chem.* **38**, 280.
22. Lederer, E. and Lederer, M. (1957) *Chromatography* p. 251. Elsevier, Amsterdam.
23. Williams, S. C. and Jones, J. K. N. (1967) *Can. J. Chem.* **45**, 275.
24. Hirst, E. L. and Jones, J. K. N. (1949) *J. Chem. Soc.* 1659.