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A TRITERPENOID SAPONIN FROM *FICARIA RANUNCULOIDES* TUBERS

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Key Word Index—*Ficaria ranunculoides*; Ranunculaceae; triterpenoid saponin; 3-*O*-(α-arabinopyranosyl-1′) 28-*O*-[β-glucopyranosyl 1‴ → 6″ (α-rhamnopyranosyl-1‴ → 4″) β-glucopyranosyl 1″]-hederagenin.

Abstract—One of the minor saponins extracted from the tubers of *Ficaria ranunculoides* and purified by fermentation may be 3-*O*-(α-arabinopyranosyl-1′)28-*O*-[β-glucopyranosyl-1‴ → 6″(α-rhamnopyranosyl-1‴ → 4″)β-glucopyranosyl-1″]-hederagenin. On the basis of chemical degradation and spectral analysis, the structure of this new saponin is proposed.

INTRODUCTION

Ficaria ranunculoides Moench tubers have been reported to contain saponins which show local anti-haemorrhoidal activity [1, 2]. On acid hydrolysis, these saponins release two triterpenoid sapogenins (hederagenin and oleanolic acid) and three sugar moieties (rhamnose, arabinose and glucose) [3, 4]. The most abundant saponin is a hederagenin glucoside, the polyglucosidic chain of which is linked to the C-3 hydroxyl group of the aglycone [5].

In a previous communication, we described the isolation of another saponin and its purification using fungal strains. We showed this saponin, on acid hydrolysis, to give hederagenin, rhamnose and glucose [6]. We now report the evidence leading to the elucidation of the structure of this new saponin.

RESULTS AND DISCUSSION

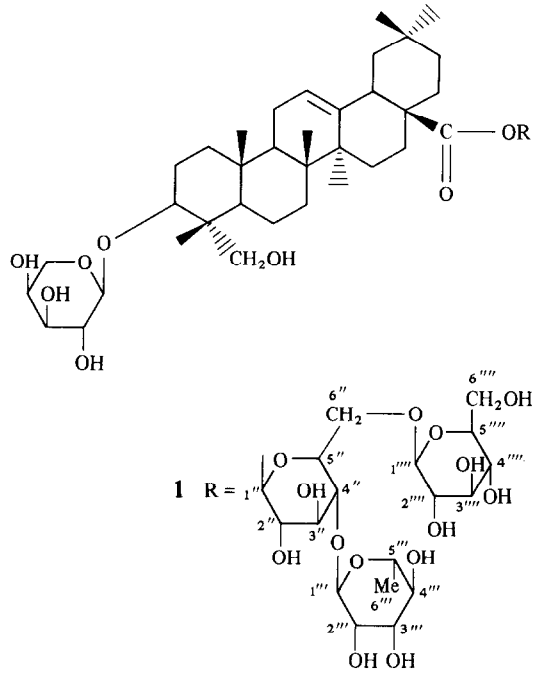
Saponin **1** is a white, amorphous, hygroscopic solid. It was not possible to obtain the anhydrous form even after drying at 100° and 0.1 mmHg. Consequently, its MW could not be calculated on the basis of elemental analysis. This phenomenon has been described by numerous investigators for various saponins, for example asiaticoside [7]. However, mass spectrometry by fast atomic bombardment (FAB) yielded a molecular peak at m/z 1097 $[M + Na]^+$ indicating a MW of 1074.

The ^{13}C NMR spectrum of the saponin showed 47 peaks, six of which were double-intensity signals indicating a total of 53 carbon atoms. Among these peaks, the chemical shift at 176.4 ppm could be assigned to a carbon ester (IR ν_{max} 1740 cm^{-1}); those at 144 and 122.7 ppm to two double-bonded trisubstituted carbons (C=CH) (^1H NMR: δ5.36, 1H, $W_{1/2}$ = 9 Hz), and those at 106.2, 104.4, 102.4, and 95.6 ppm to four anomeric carbons of four sugar molecules. These initial assignments suggested that the saponin contained a triterpene molecule and four sugar molecules, and that the five molecules were linked by either ether or ester bonding. Acetylation of the saponin confirmed this hypothesis and afforded compound **2** which had a trisubstituted double bond (^1H NMR: δ5.32, 1H, $W_{1/2}$ = 8.5 Hz) and 13 acetylated hydroxyl groups (^1H NMR: δ1.98–2.32, 2 × 6 H and 9 × 3H).

Alkaline hydrolysis of saponin **1** formed a precipitate which was insoluble in ether but soluble in *n*-butanol. On TLC, this compound showed a spot (violet, R_f = 0.75) different from that of **1** (violet, R_f = 0.45) and it was probably a prosapogenin (**3**).

Mass spectrometry of **3** by fast atomic bombardment yielded a molecular peak at m/z 627 $[M + Na]^+$ indicating a MW of 604. This MW was in agreement with the formula $C_{35}H_{56}O_8$ found by elemental analysis.

The ^{13}C NMR spectrum of **3** confirmed the existence of



3 R = H

35 carbons (33 peaks, two of which had double-intensity signals). Among these peaks, there was, at 180.1 ppm, the carbon of a carboxyl group; at 144.8 and 122.5 ppm there were two double-bonded trisubstituted carbons (C=CH) (^{1}H NMR: δ5.50, 1H, $W_{1/2}$ = 8.5 Hz); at 106.2 ppm there was an anomeric carbon (^{1}H NMR: δ5.03, *d*, *J* = 7 Hz); and at 74.6, 73.0, 69.4, and 64.6 ppm there were four carbons (three CH and one CH_2) of a pentose (C′-3, C′-2, C′-4, C′-5). These five chemical shifts correspond to the five carbons of a C′$_1$-ether arabinopyranosyl group. Acid hydrolysis of this prosapogenin (**3**) produced hederagenin (**5**) and arabinose (**6**). The chemical shifts in the ^{13}C NMR spectrum of **3** corresponded to those of the 3-*O*-α-arabinopyranosyl hederagenin reported by Aoki *et al.* [8].

Acidic hydrolysis of saponin **1** also gave hederagenin (**5**) and sugars identified as arabinose (**6**), rhamnose (**7**) and glucose (**8**) in the ratio 1:1:2. It can be concluded, therefore that saponin **1** has a saccharide chain bonded to the C-28 carboxyl of the prosapogenin by an ester group and that this chain is formed by a rhamnose molecule and two glucose molecules. The chain could be either linear or branched, and in each case the monosaccharide directly esterified at the C-28 carboxyl group could be either glucose or rhamnose.

The ^{13}C NMR spectral analysis removed the ambiguity. If one subtracts the chemical shifts due to the hederagenin substituted at C-3 by an arabinose (δ106.2, 72.8, 74.3, 69.3, 64.4 corresponding to C′-1, C′-3, C′-2, C′-4, C′-5, respectively), there remained 18 peaks which were attributed to three sugars: two substituted on C-1 and one substituted on C-1, C-4 and C-6 (Table 1).

The C″-1 carbon was bonded to the C-28 carboxyl group, and the C″-4 and C″-6 carbons were etherified by C‴-1 and C⁗-1 of the other two sugars. This result precluded the possibility of a linear saccharide chain, but did not permit a choice between the following two structures (A and B).

(A) Rhamnose (1 → 6)—Glucose (1 → 4)—Glucose–OC-28

(B) Glucose (1 → 6)—Glucose (1 → 4)—Glucose–OC-28

These two possible structures for saponin **1** corresponding to chains (A) or (B), respectively, have not previously been described in the literature. After comparisons with the values of the chemical shifts of the carbons corresponding to saikosaponine C described by Tori *et al.* [9], we propose chain (**B**).

In addition, the studies performed by Barthomeuf *et al.* (unpublished results) have demonstrated the existence of gentiobiose accompanying the saponin in the alcohol extract of *Ficaria* tubers. The presence of this diholoside, which may be a precursor or a degradation product of the saponin, would also point to chain (**B**), where the glucose molecules are linked by a 1 → 6 glycosidic bond.

Thus, the saponin isolated from tubers of *Ficaria ranunculoides* described here may be 3-*O*-(α-arabinopyranosyl-1′)28-*O*-[β-glucopyranosyl-1⁗ → 6″ (α-rhamnopyranosyl-1‴ → 4″) β-glucopyranosyl-1″]-hederagenin.

EXPERIMENTAL

Mps are uncorr. Analytical TLC was carried out on silica gel (Schleicher and Schüll F 1500, 0.25 mm) TLC (1) employed the following solvents: $CHCl_3$–MeOH (90:10) (solvent A); $CHCl_3$–MeOH–H_2O (61:32:7) (solvent B); $CHCl_3$–MeOH (98:2) (solvent C). The compounds were visualized by spraying with 10% H_2SO_4 followed by heating (110°, 10 min). TLC (2) was performed on cellulose (Merck, 0.1 mm) with EtOAc–pyridine–H_2O (2:2:1) as developing solvent. The spots were visualized by spraying with aniline hydrogen phthalate and then heating (110°, 10 min).

GLC was run with operating conditions GC (1) for sapogenin: stainless steel column (0.60 m × 3 mm) packed with 3% SE 52 on Chromosorb GAW (80–100 mesh), column temp. 260°, carrier gas: N_2, 1 bar; injection temp. 270°; FID detector: 270°; or with operating conditions GC (2) for sugars: stainless steel column (2.5 m × 3 mm) packed with 10% UCW on Gas Chrom Q (100–200 mesh), 150° isothermal for 35 min, then temp. programmed to 220° at 2°/min, carrier gas: N_2, 2 bars at 150°; injection temp. 250°; FID detector 250°. Sapogenin and sugars were analysed as their TMSi derivatives prepared in pyridine soln. For sugars, the first peaks of the chromatogram were sometimes obscured by the large tailing of pyridine. Before injection of the samples into the GLC the pyridine was removed by the method of Yamakawa and Ueca [10].

IR spectra were taken in KBr pellets (saponin, prosapogenin and sapogenin) and in $CHCl_3$ soln (acetylated derivatives).

^{1}H NMR spectra were recorded at 400 MHz [11, 12] in pyridine-d_5 (saponin, prosapogenin and sapogenin) and in $CDCl_3$ soln (acetylated saponin and acetylated prosapogenin). ^{13}C NMR spectra were recorded at 62.87 MHz in pyridine-d_5. The chemical shifts are given in ppm; TMS was used as internal standard.

Saponin (**1**). Mp > 190° (decomp.); TLC (1) solvent B R_f = 0.45 (violet spot); IR ν_{max}^{KBr} cm^{-1}: 3500–3300 (OH sugars), 1740 (CO ester); MS (FAB) *m/z*: 1.097 [M + Na]$^+$; ^{1}H NMR: δ0.83, 0.84, 0.90, 0.95, 1.09, 1.14 (3H × 6, *s*, Me-24, 25, 26, 27, 29, 30). 5.36 (1H, *m*, $W_{1/2}$ = 9 Hz, H-12). ^{13}C NMR: δ38.7 (*s*, C-1), 26.0 (*d*, C-2), 82.0 (*s*, C-3), 43.3 (*s*, C-4), 47.4 (*s*, C-5), 18.3 (*s*, C-6), 33.2 (*d*, C-7), 39.8 (*s*, C-8), 47.4 (*s*, C-9), 36.8 (*s*, C-10), 23.6 (*s*, C-11), 122.7 (*s*, C-12), 144.0 (*s*, C-13), 42.0 (*s*, C-14), 28.1 (*s*, C-15), 23.2 (*s*, C-16), 46.9 (*d*, C-17), 41.6 (*s*, C-18), 46.9 (*d*, C-19), 30.6 (*s*, C-20), 33.2 (*d*, C-21), 32.7 (*s*, C-22), 66.7 (*s*, C-23), 13.4 (*s*, C-24), 16.1 (*s*, C-25), 17.4 (*s*, C-26), 26.0 (*s*, C-27), 176.4 (*s*, C-28), 33.1 (*s*, C-29), 23.6 (*s*, C-30), 106.2 (*d*, C-1′), 74.3 (*d*, C-2′), 72.8 (*d*, C-3′), 69.3 (*d*, C-4′), 64.4 (*d*, C-5′); δC″, C‴ and C⁗: see Table 1.

Acetylation of **1**. Saponin **1** was treated with Ac_2O–pyridine at 25° for 12 hr. Removal of reagents gave a residue which was chromatographed on silica gel CC with C_6H_6, then C_6H_6–Et_2O (85:15) and (75:25). The last solvent yielded the acetylated saponin **2** as an amorphous powder: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH; ^{1}H NMR: δ0.73, 0.89, 0.90, 0.95, 1.00, 1.10 (3H × 6, *s*, Me-24, 25, 26, 27, 29, 30), 1.97, 1.98, 2.00, 2.01, 2.02, 2.04, 2.05, 2.06, 2.08, 2.10, 2.13 (6H × 2 and 3H × 9, *s*, 13 OAc), 5.32 (1H, *m*, $W_{1/2}$ = 8.5 Hz, H-12).

Alkaline hydrolysis of saponin **1**. Saponin **1** was refluxed with 5% NaOH (15 ml) for 1.5 hr. The reaction gave an Et_2O-

Table 1. ^{13}C NMR chemical shifts (ppm) of the saponin **1** sugar moiety linked to the C-28 carboxyl group

	C-1	C-2	C-3	C-4	C-5	C-6
Rhamnose C‴	102.4	72.4	72.2	73.6	70.1	18.3
Glucose C⁗	104.8	73.6	78.4	70.5	76.6	61.1
Glucose C″	95.4	75.0	76.3	78.4	76.0	68.9
$\Delta\delta$ = C⁗ − C″	+9.4	−1.4	+2.1	−7.9	+0.6	−7.8

insoluble ppt. The reaction mixture was extracted with *n*-BuOH. Removal of the solvent, *in vacuo*, to dryness gave a residue (57 mg), which was chromatographed on silica gel CC with $CHCl_3$, then $CHCl_3$–MeOH (98:2) and (97:3). One fraction of the last solvent afforded prosapogenin **3**: mp > 210° (decomp.); TLC (1), solvent B, R_f = 0.75 (violet); MS (FAB): 627 $[M+Na]^+$. (Found: C, 67.33; H, 9.54; O, 23.13. Calc. for $C_{35}H_{56}O_8 \cdot H_2O$: C, 67.52; H, 9.32; O, 23.15%.) IR ν_{max}^{KBr} cm^{-1}: 3500–3300 (OH sugars), 2650 (COOH), 1700 (COOH); ^1H NMR: δ0.87, 0.89, 0.95, 0.98, 1.19 (6H × 1 and 3H × 3, *s*, Me-24, 25, 26, 27, 29, 30), 5.03 (1H, *d*, *J* = 7 Hz, H-1'), 5.50 (1H, *m*, $W_{1/2}$ = 8.5 Hz, H-12). ^{13}C NMR spectrum of **3** [13] was the same as that of 3-*O*-arabinopyranosyl hederagenin [8]. The aq. mother liquor was neutralized with Amberlite IR C 50 and evapd *in vacuo* to dryness. The residue (oligosaccharide moiety) (55 mg) was hydrolysed with 5% $HClO_4$ (2 ml) under a N_2 atmosphere in a sealed tube, at 120° for 8 hr to provide rhamnose (**7**) and glucose (**8**), identified by co-TLC (1), solvent B, R_f (**7**) = 0.49 (yellow) and R_f (**8**) = 0.27 (yellow); co-TLC (2), R_f (**7**) = 0.76 (orange) and R_f (**8**) = 0.49 (orange) and by co-GC (2): R_t (**7**) = 24 and 33 min and R_t (**8**) = 52.2 and 56.7 min identical to authentic samples.

Acetylation of prosapogenin **3**. Acetylation of **3** in the same manner as saponin **1** gave a residue, which was chromatographed on silica gel CC with C_6H_6, then C_6H_6–Et_2O (94:6) and (92:8). The last solvent afforded acetylated prosapogenin **4** as an amorphous powder: TLC (1), solvent C, R_f = 0.7 (violet). (Found: C, 67.20; H, 8.64; O, 24.31. Calc. for $C_{43}H_{64}O_{12}$: C, 66.83; H, 8.29; O, 24.87%.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 1740 (OCO-Me), 1700 (COOH); ^1H NMR: δ0.71, 0.72, 0.89, 0.91, 0.92, 1.09 (3H × 6, *s*, Me-24, 25, 26, 27, 29, 30), 2, 2.05, 2.09, 2.11 (3H × 4, *s*, 4OAc), 2.83 (1H, *dd*, H-3), 5.28 (1H, *m*, *J* = 8.5 Hz, H-12).

Acid hydrolysis of saponin **1**. Saponin **1** (100 mg) was hydrolysed with 5% $HClO_4$ (2.5 ml) at 120° for 8 hr, under a N_2 atmosphere, in a sealed tube. The ppt. was extracted with Et_2O. Removal of solvent gave sapogenin **5** (41 mg), identified as hederagenin by co-TLC (1), solvent A, R_f = 0.55 (pink) and co-GC (1), R_t = 19.2 min; IR and ^1H NMR data identical to an authentic sample. The aq. mother liquor was neutralized with Amberlite IR 45 (OH) and evapd to dryness *in vacuo*. The presence of arabinose (**6**), rhamnose (**7**), and glucose (**8**) was established in the residue by co-TLC (**1**), solvent B, R_f (**6**) = 0.36, R_f (**7**) = 0.49, R_f (**8**) = 0.27 (yellow); co-TLC (2), R_f (**6**) = 0.54 (pink), R_f (**7**) = 0.76 (orange), R_f (**8**) = 0.49 (orange); and by co-GC (2), R_t (**6**) = 20.5, 24.75 and 28.6 min; R_t (**7**) = 24.0 and 33.0 min; R_t (**8**) = 52.2 and 56.7 min, identical to known samples.

Acid hydrolysis of prosapogenin **3**. Prosapogenin **3** was H_2O-insoluble. To a soln of **3** (100 mg) in EtOH–H_2O (75:25) (25 ml) was added 2 ml H_2SO_4 and the mixture was refluxed for 3 hr. EtOH evapn and addition of H_2O gave a ppt., which was extracted with Et_2O. After removal of the solvent, the Et_2O extracts gave the sapogenin **5** (79 mg) identical to hederagenin in the same way as described above (co-TLC, co-GC, IR, ^1H NMR with an authentic sample). After neutralization with Amberlite IR 45 (OH) and evapn to dryness, the mother liquor furnished a residue in which arabinose (**6**) was characterized by co-TLC and co-GC in the same manner as that described above.

Quantitative sugar estimation in the saponin **1** *hydrolysate*. This was carried out by the method of Misra and Rao [14] on the residue provided by neutralization and evapn to dryness of the saponin **1** acid hydrolysate. The aq. soln (2 ml) of the residue (25 mg) was chromatographed on paper (Whatman No. 1) for 20 hr with *n*-BuOH–pyridine–C_6H_6–H_2O (5:3:3:1) as developing solvent. The sugar spots, visualized by spraying with aniline hydrogen phthalate and then heating (110°, 15 min), were eluted with 50% HOAc and estimated colorimetrically at 420 nm. The average value of the experiments gave the arabinose, rhamnose, glucose ratio as 1:1:2.

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