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AQUILEGIFOLIN: A TRITERPENOID GLYCOSIDE FROM *THALICTRUM AQUILEGIFOLIUM**

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Key Word Index—*Thalictrum aquilegifolium*; Ranunculaceae; triterpenoid glycoside; aquilegifolin.

Abstract—A new triterpenoid glycoside, aquilegifolin, has been isolated from *Thalictrum aquilegifolium* and the structure established by chemical transformations, spectral analysis and comparison with closely related compounds.

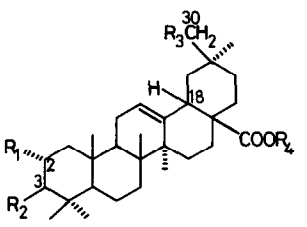
INTRODUCTION

In continuation of our studies on the constituents of Ranunculaceae plants [1, 2], we now report the isolation and structure determination of a new triterpenoid glycoside, aquilegifolin, from *Thalictrum aquilegifolium*. The structure of aquilegifolin has been shown to be 2α,3β-diacetoxy-30-hydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside.

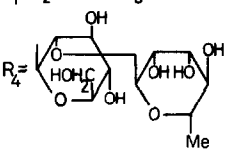
RESULTS AND DISCUSSION

Aquilegifolin (**1**) was analysed for $C_{46}H_{72}O_{16}\cdot H_2O$ and its IR spectrum showed hydroxyl (3400 cm^{-1}) and carbonyl (1740, 1725 cm^{-1}) absorptions. The ^1H NMR spectrum of **1** revealed the presence of two acetyl (δ2.00, 2.10) groups. Treatment of **1** with 5% KOH in methanol at room temperature gave **2**; the ^1H NMR spectrum showed no signal due to an acetyl group. Acetylation of both **1** and **2** afforded the same compound (**3**); the mass spectrum suggested the presence of a rhamno-glucoside linkage (*m/z* 561, 273).

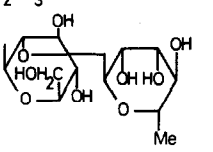
Acid hydrolysis of **1** with 10% H_2SO_4–aq. dioxane yielded glucose, rhamnose and a triterpene (**4**, $C_{30}H_{48}O_5$). The IR spectrum of **4** showed a carboxyl group (1685 cm^{-1}) absorption. The same compound (**4**) was also afforded by alkaline hydrolysis of **1** at refluxing temperature. Compound **2**, therefore, must be a rhamno-glucosyl ester of a triterpene acid **4** and **1** should be a diacetate of **2**. Methylation of **4** with diazomethane gave the methyl ester (**5**), which was converted to the triacetate (**6**) by acetic anhydride–pyridine treatment. The molecular formula of **6** was revealed to be $C_{37}H_{56}O_8$ by high resolution mass spectroscopy. The IR spectrum of **6** showed no hydroxyl absorption, therefore the eight oxygens can be attributed to one carbomethoxyl and three acetoxyls, that is, $C_{29}H_{44}(OAc)_3$ (COOMe). Considering that the ^1H NMR spectrum of **6** exhibited one proton triplet on a trisubstituted double bond around δ5.28, it should be a pentacyclic triterpene. Prominent ions in the mass spectrum of **6** at *m/z* 320, 260 and 201 corresponded with the fragmentation observed with urs-12-enes or olean-12-enes having a carbomethoxyl group at C-17 and an acetoxyl group on rings D/E [3]. The assignment of the carboxyl group to the C-17 position was supported by CD curves of **5** ($[\theta]_{218}$ −6954) which was very similar to that of a series of Δ^{12}-triterpene-28-carboxylic acids [4]. The presence of a hydroxyl group on ring B was ruled out by the fact that **5** formed the triacetate under mild conditions. In the ^1H NMR spectrum of **6** the signal due to the C-18 proton appeared as a quartet (J = 14 and 6 Hz) around δ2.85. Therefore **6** must have the oleanane skeleton [5]. The ^1H NMR spectrum of **6** also indicated the presence of one primary and two secondary acetoxyl groups: δ4.00, 4.07 (each 1H, *d*, J = 12 Hz, CH_2OAc), 4.74 (1H, *d*, J = 10 Hz,



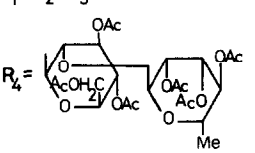
1 R_1=R_2=OAc, R_3=OH



2 R_1=R_2=R_3=OH



3 R_1=R_2=R_3=OAc



4 R_1=R_2=R_3=OH, R_4=H

5 R_1=R_2=R_3=OH, R_4=Me

6 R_1=R_2=R_3=OAc, R_4=Me

7 R_1=H, R_2=R_3=OAc, R_4=Me

8 R_1=R_2=OH, R_3=H, R_4=Me

9 R_1=R_2=OAc, R_3=H, R_4=Me

*Part 2 in the series "Studies on the Constituents of Ranunculaceae Plants". For Part 1 see ref. [2].

>CHOAc), 5.10 (1H, *dt*, $\bar{J}$ = 4 and 12 Hz, >CHOAc). On irradiation at the frequency of the methine proton doublet at δ4.74, another methine proton multiplet at δ5.10 simplified to a quartet like signal. Therefore, two secondary acetoxyl groups must be vicinal to each other and exist in a di-equatorial conformation according to the coupling constant (10 Hz) of the methine proton at δ4.74. The methine group bearing the proton doublet at δ4.74 also must be adjacent to a quarternary carbon. The ^{13}C NMR spectrum of **6** showed a signal due to C-13 at δ143.3, which supported the conclusion that it had an oleanane skeleton [6]. From the above ^{1}H NMR spectral data and the biogenetic considerations, the positions of the acetoxyl groups were revealed to be C-2, C-3 and C-29, or C-2, C-3 and C-30. Comparing the ^{13}C NMR spectral data of **6** with those of methyl diacetylqueretaroate (**7**) [7] having the acetoxyl group at C-30, methyl maslinate (**8**) and methyl diacetylmaslinate (**9**), the signals due to C-19, C-20, C-21, C-29 and C-30 of **6** are in good agreement with those of **7** (Table 2). Compound **4** thus can be formulated as 2α, 3β, 30-trihydroxyolean-12-en-28-oic acid and **2** as the 28-rhamno-glucosyl ester of 4.

From the ^{13}C NMR spectral data of **1** and **2** (Table 1), it is clear that there is a sugar moiety composed of a glucose molecule and a rhamnose molecule linked to the C-28 carboxyl group of **4** by a β-glycosidic linkage through C-1 of the glucose (δ94.7), to which the rhamnose (δ101.0) is linked by an α-glycosidic linkage [7–9]. The anomeric carbon signal of β-monoglycosyl ester has been generally found to appear near δ95.5 [10–12], but the signal due to the ester glucosyl anomeric carbon of **2** was observed at somewhat higher field (δ94.7). This strongly suggested that the sugar moiety of **2** would be a β-neohesperidosyl (= 2-*O*-(α-rhamnosyl)-β-glucopyranosyl) group, because on going from β-glucose to β-neohesperidose, the anomeric carbon signal of the reducing unit is found to be shielded by the substitution at its vicinal hydroxyl group [13]. The ^{1}H NMR spectrum of **2** showed signals of two anomeric protons assignable to an anomer proton of rhamnose (δ4.81, 1H, *s*) and an ester glycosidic glucose (δ5.06, 1H, *d*, *J* = 7 Hz) which supported the presence of α-rhamnosyl and β-glucosyl linkages. Since it is a general rule that the glycosidic linkages of sugars in the D- and L-series are β and α, respectively [14–16], the above data indicated that **2** is the 28-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside of **4**.

The positions of the acetyl groups of **1** could be

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (δ, pyridine-d_5)*

C	1	2	C	1	2
1	42.3	47.8	27	26.1	26.0
2	73.3	68.6	28	176.5	176.5
3	79.7	83.7	29	28.9	28.8
4	39.4	39.8	30	65.2	65.3
5	55.6	55.9	OCOMe	21.3	
6	18.7	18.9		21.3	
7	33.1	33.2	OCOMe	170.7	
8	40.0	40.0		171.2	
9	47.9	48.2	1^{G}	94.7	94.7
10	38.6	38.5	2^{G}	79.7	79.8
11	23.6	23.8	3^{G}	75.1	75.1
12	122.5	122.5	4^{G}	71.3	71.3
13	144.0	144.0	5^{G}	78.8	78.9
14	42.3	42.3	6^{G}	62.0	62.0
15	28.5	28.5	1^{R}	101.0	101.0
16	22.9	23.4	2^{R}	72.2	72.4
17	47.1	47.1	3^{R}	72.2	72.2
18	41.5	41.6	4^{R}	73.8	73.8
19	41.8	41.8	5^{R}	69.7	69.7
20	35.6	35.6	6^{R}	18.7	18.7
21	30.0	29.9			
22	32.1	32.1			
23	29.1	29.3			
24	17.4	17.4			
25	16.8	17.0			
26	18.0	17.6			

*^{G} refers to glucose, ^{R} to rhamnose.

Table 2. ^{13}C NMR spectral data of compounds **6**, **7**, **8** and **9** (δ, $CDCl_3$)

C	6	7*	8	9
1	43.9	38.1	46.9	44.0
2	70.0	23.4	68.5	70.1
3	80.6	80.7	83.8	80.7
4	39.4	37.7	39.9	39.5
5	54.9	55.2	55.8	55.0
6	18.2	18.2	18.8	18.4
7	32.4	32.6	33.1	32.7
8	39.4	39.3	39.8	39.8
9	47.5	47.5	48.0	48.2
10	38.1	36.9	38.5	38.4
11	23.5	23.4	23.4	23.5
12	122.5	122.7	122.8	122.4
13	143.3	142.9	144.1	144.3
14	41.6	41.4	41.9	41.9
15	27.5	27.5	28.0	28.0
16	23.2	23.4	23.8	23.7
17	46.4	46.4	46.9	46.9
18	40.7	40.7	41.8	41.7
19	41.4	41.4	46.0	46.0
20	33.8	33.7	30.8	30.8
21	29.2†	29.3‡	33.9	33.9
22	31.8†	31.7‡	32.7	32.9
23	28.4	28.0	28.3	28.3
24	16.8	16.7	16.9	17.0
25	16.4	15.4	16.8	16.6
26	17.6	16.7	17.7	17.8
27	26.0	25.9	26.1	26.1
28	177.7	177.4	177.9	177.8
29	27.8	27.8	33.1	33.1
30	67.7	67.7	13.6	23.6
CO_2Me	50.2	50.2	51.6	51.6
OCOMe	20.9	20.9		20.8
	20.9	21.2		21.0
	21.1			
OCOMe	170.5	170.6		170.3
	170.9	170.9		170.5
	171.3			

*Data taken from ref. [7].

†‡Assignments bearing the same superscript in each spectrum may be reversed.

determined by a comparison of the ^{13}C NMR spectral data of **1** and **2**. Comparing the ^{13}C NMR spectrum of **1** with that of **2**, all signals due to sugar carbons of **1** are in good agreement with those of **2**. The acetyl groups, therefore, should be on the aglycone moiety, and C-2, C-3 and C-30 might each contain the acetoxyl groups. The signals due to C-30 and C-20 of **1** coincide well with those of **2**, but C-1, C-2, and C-3 of **1** are quite different from those of compound **2**. From the above data, the acetoxyl groups must be attached to C-2 and C-3. It is known that acetylation of a hydroxyl group shifts the signal of the carbon bearing the hydroxyl group to a downfield position and that of the contiguous carbon moves upfield. However, in the case of **1** the shifts are not in agreement with this generally known acetylation shift because of the vicinal acetoxyl groups. The ^{13}C NMR spectral data of the aglycone moieties of **1** and **2** were compared with those of **8** and **9** to confirm the positions of the acetyl groups of **1** (Tables 1 and 2). The signals due to C-1, C-3 and C-4 of **9** were shifted upfield by −2.9, −3.1 and −0.4 ppm, respectively, and the signal of C-2 was shifted downfield by +1.6 ppm. The shifts of C-1, C-2, C-3 and C-4 (by −5.5, +2.7, −4.0 and −0.4 ppm, respectively) of **1** are in good accordance with those of **9**. Thus we conclude that aquilegifolin is 2α,3β-diacetoxy-30-hydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside.

EXPERIMENTAL

All mps are uncorr. IR spectra were recorded on KBr discs. ^1H NMR spectra were run at 200 and 100 MHz, and the ^{13}C NMR spectra at 50 and 25 MHz with TMS as internal standard. Mass spectra (70 eV) were taken with a direct inlet. Plants were collected at Yamanashi prefecture, Japan, in 1981.

Extraction and isolation of **1**. Dried leaves (285 g) of *Thalictrum aquilegifolium* were extracted with hot MeOH and the MeOH soln was concd *in vacuo* to 150 ml. The methanolic extract was extracted with 150 ml of 5% HOAc. The aq. HOAc soln was extracted with EtOAc. The EtOAc extract was chromatographed on a silica gel column and the$CHCl_3$–MeOH (10:1) eluate gave crude **1**, which was purified by preparative TLC with $CHCl_3$–MeOH–H_2O (40:18:3), 122 mg.

Aquilegifolin (**1**). Recrystallized from MeOH–H_2O, mp 195–197°, $[\alpha]_D^{23}$ −5.88° (*c* 0.13, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1725. ^1H NMR (pyridine-d_5): δ 2.00 and 2.10 (each 3H, *s*, Ac × 2). (Found: C, 61.32; H, 8.25. $C_{46}H_{72}O_{16}\cdot H_2O$ requires: C, 61.44; H, 8.30%.)

Alkaline hydrolysis of **1** *at room temp.* Treatment of **1** (21 mg) with 5% KOH in MeOH for 15 min at room temp. gave 16 mg of **2**. Recrystallization from MeOH–H_2O afforded fine crystals of **2**, mp 232–234°. IR ν_{max}^{KBr} cm^{-1}: 3350, 1740. ^1H NMR (pyridine-d_5): δ 4.81 (1H, *s*, rhamnose H-1), 5.06 (1H, *d*, *J* = 7 Hz, glucose H-1). (Found: C, 61.91; H, 8.40. $C_{42}H_{68}O_{14}\cdot H_2O$ requires: C, 61.92; H, 8.66%.)

Alkaline hydrolysis of **1** *by reflux.* Compound **1** (18 mg) was refluxed with 5% KOH for 5 hr to give **4**.

Peracetate of **1** *and* **2**. Treatment of **1** and **2** with Ac_2O–pyridine overnight at room temp. afforded **3**. Recrystallization from MeOH gave fine crystals of **3**, mp 141–143°. IR ν_{max}^{KBr} cm^{-1}: 1735. MS *m/z*: 561, 273. ^1H NMR ($CDCl_3$): δ 1.96, 1.98, 2.04, 2.06, 2.08, 2.10, 2.12, 2.14 (27H, each *s*, Ac × 9).

Acid hydrolysis of **1**. Compound **1** (82 mg) was refluxed with 2 N H_2SO_4 (8 ml)–dioxane (4 ml)–H_2O (4 ml) for 7 hr and the reaction mixture was extracted with EtOAc. The EtOAc extract was purified by preparative TLC with EtOAc to give 25 mg of **4**, amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3400, 1685. MS *m/z*: 488.3487 $[M]^+$, calc. for $C_{30}H_{48}O_5$, 488.3499, 264, 234 (base peak), 201. The aq. layer of the hydrolysate was neutralized with ion-exchange resin (IRA-410) and evaporated. Trimethylsilylation followed by GLC [1.5% OV-1 on Shimalite W (80–100 mesh)] showed the presence of D-glucose and L-rhamnose (molar ratio 1:1).

Methyl ester of **4**. A soln of **4** (20 mg) in MeOH was treated with CH_2N_2 to yield 18 mg of **5**, amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3340, 1722. MS *m/z*: 502.3650 $[M]^+$, calc. for $C_{31}H_{50}O_5$, 502.3655, 278, 201 (RDA).

Triacetate of **5**. Treatment of **5** (15 mg) with Ac_2O–pyridine overnight at room temp. afforded **6** (15 mg). Recrystallization from EtOAc–hexane gave colourless needles, mp 211–213°. IR ν_{max}^{KBr} cm^{-1}: 1740, 1730, 1240. MS *m/z*: 628 $[M]^+$, 568.3724 $[M-AcOH]^+$, calc. for $C_{35}H_{52}O_6$, 568.3761, 508 $[568-AcOH]^+$, 320 (RDA), 260 $[320-AcOH]^+$, 201 $[260-MeOCO]^+$. ^1H NMR ($CDCl_3$): δ 0.72, 0.89, 0.91, 0.92, 1.05, 1.14 (each 3H, *s*, Me), 1.98, 2.06, 2.07 (each 3H, *s*, Ac), 2.85 (1H, *q*, *J* = 14 Hz, 6 Hz, H-18), 3.61 (3H, *s*, OMe), 4.00, 4.07 (each 1H, *d*, *J* = 12 Hz, H_2-30), 4.74 (1H, *d*, *J* = 10 Hz, H-3), 5.10 (1H, *dt*, *J* = 4 and 12 Hz, H-2), 5.28 (1H, *br s*, H-12).

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