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CYCLOARTANE-TYPE GLYCOSIDES FROM THALICTRI HERBA

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Key Word Index—*Thalictrum* sp.; Thalictri Herba; Ranunculaceae; cycloartane glycosides; thalictoside.

Abstract—Two new cycloartane-type glycosides, designated as thalictosides V and IX, were isolated from the methanolic extract of Thalictri Herba (Takatogusa), the dried aerial parts of *Thalictrum* sp. plants. Their chemical structures have been characterized as 3-*O*-monodesmoside and the 3,21-di-*O*-bisdesmoside of 3β,22ξ,30-trihydroxycycloart-24-en-21-oic acid, by chemical and spectroscopic evidence.

INTRODUCTION

In the preceding paper [1] on the chemical constituents of Thalictri Herba (Takatogusa), the dried aerial parts of *Thalictrum* sp. plants, we described the isolation and structural determination of two cycloartane-type and three oleanane-type triterpene glycosides. In a continuing study on the glycosidic constituents of this plant material, we obtained, in addition, two new cycloartane-type glycosides, named thalictosides V (**1**) and IX (**2**). This paper deals with their structural characterization.

RESULTS AND DISCUSSION

The methanol extract of Thalictri Herba was partitioned into a benzene–water solvent system. Diaion HP-20 column chromatography of the water-soluble portion provided the glycosidic constituents, which were further purified using a combination of Sephadex LH-20, silica gel and ODS column chromatography to furnish the two thalictosides, V and IX (**1** and **2**).

Thalictoside V (**1**), obtained as a powder, showed a $[M-H]^-$ peak at *m/z* 941 in the negative FAB-mass spectrum, and proton signals owing to a cyclopropane methylene at δ0.21 and 0.80, three tertiary methyl groups at δ0.94, 1.27 and 1.52, two olefinic methyl groups at δ1.63 and 1.69 on a double bond, two secondary methyl groups at δ1.63 (J = 6.2 Hz) and 1.70 (J = 6.2 Hz), three anomeric protons at δ4.96 (1H, *d*, J = 7.7 Hz), 5.47 (1H, *br s*) and 6.68 (1H, *br s*), and one olefinic proton at δ5.61 (1H, *br t*) in the ^{1}H NMR spectrum. These spectral data indicated that **1** was a cycloartane triglycoside derivative. Besides, the chemical shifts of the aglycone moiety, except for the signals owing to the side-chain moiety and the C-17 on the D-ring, and of the sugar residue in the ^{13}C NMR spectrum of **1** showed coincidence with those of thalictoside III (**3**). Moreover, the ^{1}H–^{1}H COSY of **1** exhibited cross-peaks between the olefinic proton at δ5.61 and the methylene proton at δ2.65, and between the signal at δ2.65 and the hydroxy methine proton at δ4.03. Therefore, it became clear that the hydroxy methine próton at δ4.03 and the two olefinic methyl groups were located at C-22 and C-25, respectively. No signal owing to a C-21 methyl group was observed in the ^{1}H NMR spectrum. Thus, the C-21 signal was deduced to be a carboxyl. Thereupon, **1** was treated with diazomethane to afford the methyl ester (**4**), which showed a clustered $[M]^+$ at *m/z* 979.5242 $[C_{49}H_{80}O_{18}Na]^+$ in the HR-FAB-mass spectrum. The ^{1}H and ^{13}C NMR spectra of **4** showed signals owing to one new methoxy group (δ_H3.79 and δ_c52.4) and one ester carbonyl carbon (δ_c174.9). From the above evidence, the structure of **1** was concluded to be 3β,22ξ,30-trihydroxycycloart-24-en-21-oic acid 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside.

Thalictoside IX (**2**), obtained as a powder, showed a clustered $[M]^+$ at *m/z* 1259.6036 $[C_{59}H_{96}O_{27}Na]^+$ in the HR-FAB-mass spectrum. The ^{1}H NMR spectrum displayed signals owing to one cyclopropane methylene group [δ0.20, 0.75 (each *br s*, H_2-19)], three tertiary methyl groups [δ0.89 (*s*, H_3-28), 1.17 (*s*, H_3-18) and 1.52 (*s*, H_3-29)], two olefinic methyl groups [δ1.66 (*s*, H_3-26) and 1.71 (*s*, H_3-27)], two secondary methyl groups [δ1.65 (*d*, J = 5.9 Hz, Rha H_3-6) and 1.70 (*d*, J = 6.2 Hz, Rha H_3-6)], and one olefinic proton [δ5.58 (*br t*, H-24)]. Based on the above evidence, the structure of **2** was considered to be analogous to that of **1**. Furthermore, the ^{1}H NMR spectrum of **2** suggested the presence of five anomeric proton signals at δ4.92 (1H, *d*, J = 7.3 Hz), 4.97 (1H, *d*, J = 7.0 Hz), 5.49 (1H, *br s*), 6.25 (1H, *d*, J = 7.7 Hz) and 6.71 (1H, *br s*). The ^{13}C NMR spectrum revealed the presence of one ester carbonyl carbon signal at δ173.5

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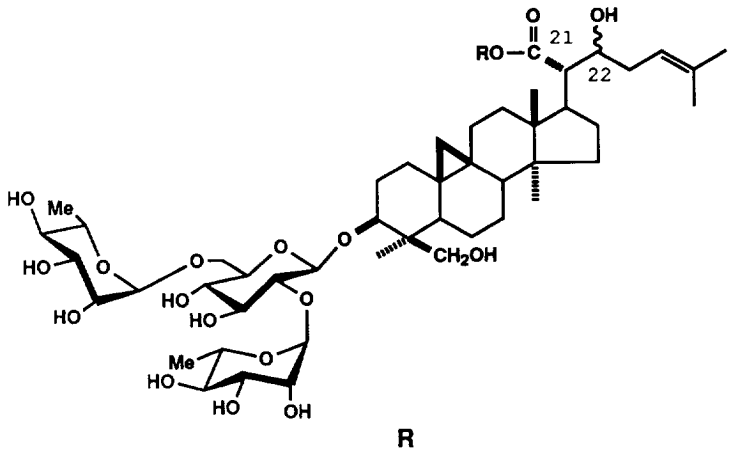

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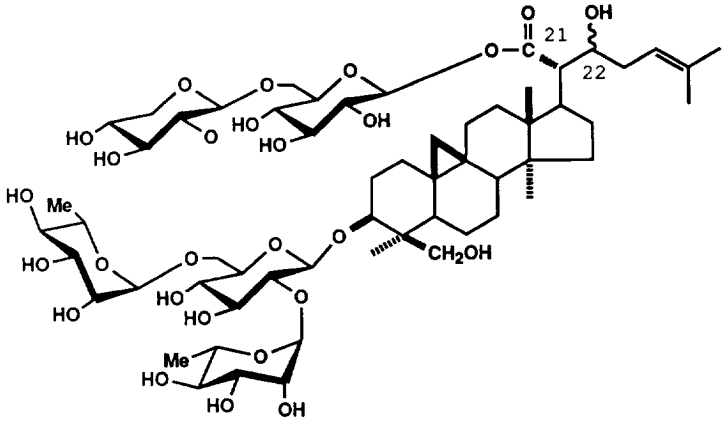

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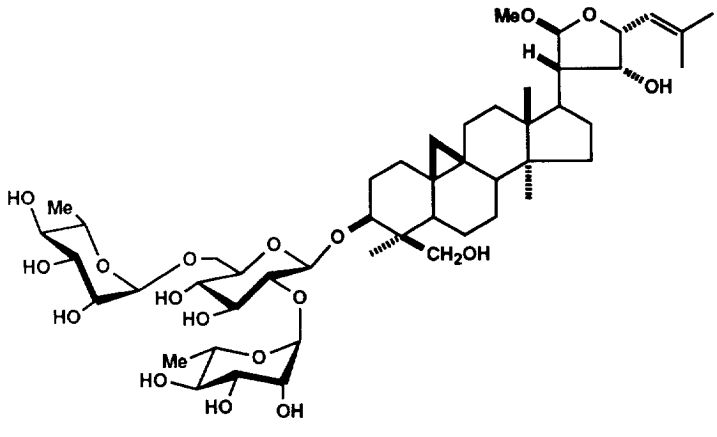

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and five anomeric carbon signals at δ96.1, 100.9, 102.6, 105.4 and 105.7. The anomeric carbon signal at δ96.1 could be assigned to the sugar attached to the carboxyl group as an ester linkage. On selective cleavage of the ester-glycoside linkage with anhydrous LiI and 2,6-lutidine in anhydrous methanol [2], **2** provided an anomeric mixture of methyl oligoglycoside and a prosapogenin. In the ^{13}C NMR spectral data for the methyl ester (**5**) of the prosapogenin, all carbon signals were in good agreement with those of **4**. The anomeric mixture of methyl glycosides obtained by selective cleavage of the ester-glycoside linkage of **2** on acid hydrolysis gave glucose and xylose. Meanwhile, the negative FAB-mass spectrum of **2** gave a peak at *m/z* 1235 owing to $[M - H]^-$, which was higher

by 249 mu (hexose + pentose) than that of **1**. Furthermore, in the ^{13}C NMR spectrum of **2**, signals owing to the sugar moiety linked to the C-21 carboxyl group of the aglycone could be assigned to β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside. From the above evidence, the structure of **2** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranosyl 3β,22ξ,30-trihydroxycycloart-24-en-21-oic acid 21-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.

EXPERIMENTAL

General. ^1H and ^{13}C NMR chemical shifts are given on a δ (ppm) scale with TMS as int. standard. FAB-MS were measured in a 3-nitrobenzyl alcohol matrix. TLC was performed on pre-coated Kieselgel 60 F_{245} (Merck) and detection was achieved by spraying with 10% H_2SO_4 followed by heating. CC was carried out on Kieselgel (230–400 mesh, Merck), Sephadex LH-20 (Pharmacia), ODS (PrePAK-500/C_{18}, Waters) and Diaion HP-20 (Mitsubishi).

Isolation of triterpenes. Thalictri Herba (4.9 kg, purchased from Uchida Wakanyaku) was extracted with MeOH and the extract partitioned between benzene and H_2O (1:1). The H_2O-soluble portion (605.1 g) was subjected to Diaion HP-20 CC with MeOH–H_2O (0 → 30 → 50 → 70 → 90 → 100%) to afford seven frs (1–7). Fr. 7 (22.1 g) was then chromatographed on Sephadex LH-20 with MeOH to provide four frs (8–11). Fr. 9 (14 g) was further sepd by silica gel CC with $CHCl_3$–MeOH–H_2O (45:10:1 → 40:10:1 → 14:6:1) to give six frs (12–18). Fr. 17 was subsequently purified by ODS CC with MeOH–H_2O (50 → 60%), followed by silica gel CC with $CHCl_3$–MeOH–H_2O (40:10:1), to furnish thalictoside V (**1**) (13 mg). Fr. 18 was also purified by ODS CC with MeOH–H_2O (50 → 60%), followed by silica gel CC with $CHCl_3$–MeOH–H_2O (14:6:1) to give thalictoside IX (**2**) (133 mg).

Thalictoside V (**1**). Powder. $[\alpha]_D^{25}$ −16.5° (MeOH; *c* 0.23). Neg. FAB-MS (*m/z*): 941 $[M - H]^-$. ^1H NMR (pyridine-d_5): δ0.21, 0.80 (each 1H, *br s*, H_2-19), 0.94, 1.27, 1.52, 1.63 and 1.69 (each 3H, *s*, H_3-28, H_3-18, H_3-29, H_3-26 and H_3-27), 1.63 (3H, *d*, *J* = 6.2 Hz, Rha H_3-6), 1.70 (3H, *d*, *J* = 6.2 Hz, Rha H_3-6), 2.65 (2H, *m*, H_2-23), 3.59 (1H, *br d*, *J* = 13.6 Hz, H-3), 4.03 (1H, *m*, H-22), 4.96 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.47 (1H, *br s*, Rha H-1), 5.61 (1H, *br t*, H-24), 6.68 (1H, *br s*, Rha H-1). ^{13}C NMR (pyridine-d_5): see Table 1.

Table 1. ^{13}C NMR data for **1**–**5** (δ ppm, in pyridine-d_5)

C	1	2	3	4	5		1	2	3	4	5
1	30.5	30.7	30.8	30.5	30.7	3-*O*-					
2	30.0	30.5	30.3	30.0	30.2	Glc C-1	105.2	105.4	105.4	105.2	105.3
3	89.4	89.7	89.7	89.4	89.6	2	80.0	80.1	80.1	80.0	80.1
4	45.2	45.4	45.4	45.2	45.3	3	76.1	76.2	76.3	76.1	76.2
5	47.9	48.4	48.2	47.8	48.0	4	72.6	72.8	72.8	72.7	72.7
6	22.6	22.8	22.9	22.5	22.7	5	76.4	76.5	76.6	76.4	76.5
7	26.7	26.3	27.7	26.5	26.7	6	68.1	68.4	68.2	68.1	68.2
8	48.2	48.4	48.5	48.1	48.3	Rha C-1	100.7	100.9	100.9	100.8	100.9
9	19.8	19.9	20.1	19.7	19.9	2	71.9	72.0	72.2	71.6	71.7
10	26.2	26.4	26.4	26.3	26.4	3	72.0	72.3	72.3	72.0	72.1
11	26.2	26.7	26.5	26.2	26.3	4	74.3	74.5	74.4	74.3	74.4
12	36.2	35.9	35.8	35.8	35.9	5	69.1	69.1	69.5	69.0	69.1
13	45.2	45.6	45.5	45.2	45.3	6	18.5	18.6	18.5	18.6	18.7
14	48.8	48.7	48.8	48.5	48.6	Rha C-1	102.3	102.6	102.5	102.3	102.5
15	32.0	32.1	32.2	32.1	32.2	2	71.9	72.3	72.2	71.9	72.0
16	26.9	27.0	27.0	26.7	26.8	3	72.2	72.4	72.3	72.2	72.3
17	45.9	45.4	48.8	45.7	45.8	4	73.8	74.0	74.0	73.9	74.0
18	19.6	19.7	18.7	19.5	19.6	5	69.6	69.8	69.8	69.8	69.7
19	29.8	30.0	30.1	29.9	30.0	6	18.3	18.5	18.5	18.4	18.4
20	52.3	53.1	54.8	50.6	51.1	21-*O*-					
21	*	173.5	108.7	174.9	175.0	Glc C-1		96.1			
22	72.2	72.3	76.7	72.2	72.3	2		73.8			
23	35.2	35.5	79.0	35.3	35.4	3		78.5			
24	122.2	122.4	122.6	121.9	121.9	4		71.2			
25	132.8	133.2	136.1	133.0	133.1	5		77.7			
26	25.8	26.0	26.0	25.8	25.9	6		69.6			
27	18.0	18.3	19.8	17.9	18.0	Xyl C-1		105.7			
28	18.5	18.7	18.8	18.2	18.3	2		74.8			
29	19.7	19.9	19.9	19.7	19.9	3		78.1			
30	60.5	60.6	60.7	60.7	60.7	4		71.0			
OMe			54.6	52.4	52.5	5		66.8			

*Unobserved.

Thalictoside IX (**2**). Powder. $[\alpha]_D^{25}$ − 14 (MeOH; *c* 1.00). Neg. FAB-MS (*m/z*): 1235 $[M - H]^-$. HR-FAB-MS (*m/z*): 1259.6028 $[M + Na]^+$ (Calcd for $C_{59}H_{96}O_{27}Na$ 1259.6028). ^1H NMR (pyridine-d_5) δ:0.20, 0.75 (each 1H, *br s*, H_2-19), 0.89, 1.17, 1.52, 1.66 and 1.71 (each 3H, *s*, H_3-28, H_3-18, H_3-29, H_3-26 and H_3-27), 1.65 (3H, *d*, *J* = 5.9 Hz, Rha H_3-6), 1.70 (3H, *d*, *J* = 6.2 Hz, Rha H_3-6), 2.75 (2H, *m*, H_2-23), 3.59 (1H, *br d*, *J* = 8.4 Hz, H-3), 4.92 (1H, *d*, *J* = 7.3 Hz, Xyl H-1), 4.97 (1H, *d*, *J* = 7.0 Hz, Glc H-1), 5.49 (1H, *br s*, Rha H-1), 5.58 (1H, *br t*, H-24), 6.25 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 6.71 (1H, *br s*, Rha H-1). ^{13}C NMR (pyridine-d_5): see Table 1.

Methylation of compound **1** *with* CH_2N_2. Thalictoside V (**1**, 13 mg) was dissolved in MeOH (1 ml). An Et_2O soln of CH_2N_2 (10 ml) was added and evapd after 1.5 hr. The residue (20 mg) was subjected to silica gel CC with CH_3Cl–MeOH–H_2O (90:10:1) to furnish the carboxy Me ester of thalictoside V (**4**, 15 mg) as a powder. $[\alpha]_D^{25}$ − 14.5 (MeOH; *c* 0.50). HR-FAB-MS (*m/z*): 979.5242 $[M + Na]^+$ (Calcd for $C_{49}H_{80}O_{18}Na$ 979.5241). ^1H NMR (pyridine-d_5): δ0.27, 0.82 (each 1H, AB *q*, *J* = 4.0 Hz, H_2-19), 0.88, 1.11, 1.55, 1.64 and 1.69 (each 3H, *s*, H_3-28, H_3-18, H_3-29, H_3-26 and H_3-27), 1.66 (3H, *d*, *J* = 6.2 Hz, Rha H_3-6), 1.73 (3H, *d*, *J* = 6.2 Hz, Rha H_3-6), 3.79 (3H, *s*, H-OMe), 4.99 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.50 (1H, *br s*, Rha H-1), 5.56 (1H, *br t*, H-24), 6.72 (1H, *br s*, Rha H-1). ^{13}C NMR (pyridine-d_5): see Table 1.

Selective cleavage of ester-glycoside linkage of compound **2**. After a mixt. of thalictoside IX (**2**, 35 mg) in 2,6-lutidine (2 ml) containing dry MeOH (1 ml) and LiI (60 mg) was heated to 110° for 8 hr, the reaction mixt. was diluted with 50% MeOH (10 ml) and passed through an Amberlite MB-3 (10 ml) column. The eluate was concd *in vacuo* and the resulting product chromatographed on Diaion HP-20 using 20% MeOH and MeOH as eluents. The 20% MeOH eluate was concd *in vacuo* and the resulting product in 2 N HCl–MeOH (2 ml) was heated at 100° for 1.5 hr and then neutralized with 3% KOH–MeOH to detect glucose and xylose on TLC. The MeOH eluate was concd and dissolved in MeOH (1 ml). An Et_2O soln of CH_2N_2 (5 ml) was added and evapd after 1.5 hr. The residue (25 mg) was subjected to silica gel CC with CH_3Cl–MeOH–H_2O (90:10:1) to furnish the carboxy Me ester of the prosapogenin (**5**, 13 mg) as a powder. $[\alpha]_D^{25}$ − 14.5° (MeOH; *c* 1.30). ^{13}C NMR (pyridine-d_5): see Table 1.

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