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AN 18-NORSPIROSTANOL GLYCOSIDE FROM *TRILLIUM TSCHONOSKII*

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Key Word Index—*Trillium tschonoskii*; Liliaceae; 18-norspirostanol glycoside; epitrillenoside CA; epitrillenogenin.

Abstract—An 18-norspirostanol derivative isolated from the aerial parts of *Trillium tschonoskii* was shown to be 1-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-acetyl-α-L-arabinopyranosyl]-21-*O*-acetyl-epitrillenogenin.

INTRODUCTION

A group of unique steroidal glycosides, 18-norspirostanol oligosides, are found in *Trillium kamtschaticum Pall.* [1–3], *Trillium tschonoskii* MAX. [4, 5] and *Paris quadriforia* L. The 18-norspirostanol tetraglycoside, trillenoside A [1, 2] is the major steroidal glycoside (*ca* 0.38%) in *T. kamtschaticum*, a plant from which we have now obtained a new penta-*O*-acetyl 18-norspirostanol derivative.

RESULTS AND DISCUSSION

The new 18-spirostanol glycoside, designated as epitrillenoside CA (**1**), was isolated as a white powder, $[\alpha]_D$ −94.3° (methanol). Its IR spectrum showed absorptions due to hydroxyl (3400 cm^{-1}) and ester (1720 cm^{-1}) groups and an α,β-unsaturated ketone system (1690, 1620 cm^{-1}). On acid hydrolysis **1** furnished epitrillenogenin, $C_{26}H_{36}O_8$, mp 286–288°, $[\alpha]_D$ −210.1° (methanol), together with arabinose and rhamnose. The ^1H NMR spectrum of **1** showed the presence of five acetyl groups (δ2.01, 2.06, 2.07, 2.17 and 2.18), a total of four protons [δ5.07 (1H, *t*, *J* = 10 Hz), 5.21 (1H, *dd*, *J* = 1.8, 3.4 Hz), 5.33 (1H, *m*), 5.47 (1H, *dd*, *J* = 3.4, 10 Hz)] adjacent to acetoxyl groups and a rhamnosyl anomeric proton [δ5.45 (1H, *d*, *J* = 1.8 Hz)]. Saponification of **1** gave a product **3**, $C_{37}H_{54}O_{16}$, $[\alpha]_D$ −108.2° (methanol), identical with epitrillenoside C [3], 1-*O*-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-epitrillenogenin. Therefore, **1** was presumed to be a pentaacetyl derivative of **3**. An ion at *m/z* 273 in the mass spectrum of **1** suggested the presence of a per-*O*-acetylated rhamnosyl group as the terminal sugar residue. Thus, the ^1H NMR signals at δ5.21, 5.47 and 5.07 could be assigned to the protons at H-2, H-3 and H-4, respectively, by comparison with those of methyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside and by spin decoupling experiments. One (1H, *m*, δ5.33) of the remaining two acetoxy methine protons was assigned to H-4 of the arabinopyranosyl residue by reference to those of methyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside. A comparative study of the ^{13}C NMR spectra (Table 1) of **1** and **2** allowed the assignment of the last acetyl group. Thus the signals due to C-21 and C-20 were shifted by +1.9 and −4.0 ppm, respectively, indicating that the acetyl group was linked to C-21. Therefore, epitrillenoside CA (**1**) is 1-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-acetyl-α-L-arabinopyranosyl]-21-*O*-acetyl-epitrillenogenin.

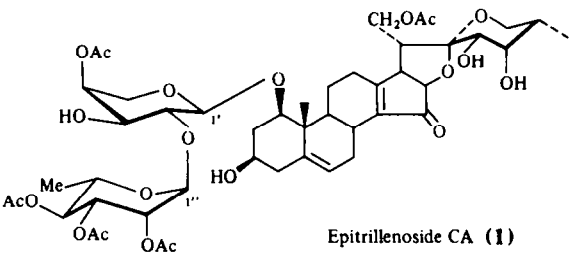
EXPERIMENTAL

Mps (Kofler apparatus): uncorr. ^1H NMR 200 MHz, with TMS as internal standard; ^{13}C NMR: 50.1 MHz; CC: silica gel Merck 60; Plant material was collected in June 1984, in prefecture Kumamoto (Japan).

Extraction and isolation of epitrillenoside CA (**1**). The fresh aerial parts (376 g) of *T. tschonoskii* were extracted with MeOH and evapd *in vacuo* to give a residue (42 g), which was chromatographed repeatedly over silica gel using $CHCl_3$–MeOH–H_2O (7:3:0.5, 10:2:0.1) and $CHCl_3$–MeOH–EtOAc–H_2O (2:2:5:1) to afford epitrillenoside CA (320 mg).

Epitrillenoside CA (**1**). White powder, $[\alpha]_D^{18}$ −94.5° (*c* 0.84; MeOH). ^1H NMR ($CDCl_3$): δ0.81 (3H, *d*, *J* = 6.8 Hz, 25-Me), 1.04 (3H, *s*, 10-Me), 1.24 (3H, *d*, *J* = 7.3 Hz, rha 5-Me), 2.01, 2.06, 2.07, 2.16, 2.18 (5 × OAc), 5.07 (1H, *t*, *J* = 10 Hz, rha 4″-H), 5.21 (1H, *dd*, *J* = 1.8, 3.4 Hz, rha 2″-H), 5.33 (1H, *m*, ara 4′-H), 5.45 (1H, *d*, *J* = 1.8 Hz, rha 1″-H), 5.47 (1H, *dd*, *J* = 3.5, 10 Hz, rha 3″-H) and 5.63 (1H, *dd*, *J* = 1.2, 5.4 Hz, 6-H). (Found: C, 58.81; H, 6.62. $C_{47}H_{64}O_{21}$ requires: C, 58.50; H, 6.69%.)

Acid hydrolysis of **1**. Epitrillenoside CA (**1**), 250 mg, was hydrolysed with hot 2 N HCl–MeOH (10 ml) for 1.5 hr. The reaction mixture was neutralized with 3% KOH–MeOH and the resulting salt was filtered off. The filtrate was subjected to silica gel CC ($CHCl_3$–MeOH–H_2O, 9:2:0.1) to furnish an aglycone [colourless needles (25 mg, from dil. MeOH), mp 286–288° (decomp.); $[\alpha]_D$ −210.1° (*c* 0.45; MeOH); IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 1703, 1634 (enone), 1000, 985, 980, 943, 905 and 865; (Found: C, 65.34; H, 7.63. $C_{26}H_{36}O_8$ requires: C, 65.53, H,



Epitrillenoside CA (**1**)

Table 1. ^{13}C NMR data for compounds 1 and 3 (50.1 MHz, $CDCl_3$)

C	3	1	C	3	1
Sapogenol 1	78.5	85.3	24	69.6	69.4
2	43.4	37.4	25	35.8	33.8
3	68.2	67.8	26	61.6	61.4
4	43.1	41.9	27	12.9	12.1
5	140.2	139.4	Ara 1′		100.4
6	124.6	125.7	2′		74.9
7	29.9	29.7	3′		73.3
8	31.7	31.4	4′		67.6
9	48.6	47.4	5′		66.4
10	43.4	41.8	Rha 1′		96.4
11	24.8	24.8	2″		69.0
12	28.3	28.9	3″		70.1
13	175.4	174.6	4″		71.5
14	138.8	137.3	5″		66.2
15	204.1	204.1	6″		17.4
16	82.2	81.5	CO		160.9
17	48.9	47.8			170.4
19	13.0	13.3			170.8 × 2
20	50.0	46.0			171.7
21	61.3	63.2	Me		20.8
22	113.7	110.8			20.9
23	72.3	71.9			21.0 × 3

7.62] identical with epitrilleriogenin and the methyl glycosides of α-L-rhamnopyranoside (R_f 0.41, $CHCl_3$–MeOH–H_2O, 8:2:0.2) and α-L-arabinopyranoside (R_f 0.34).

Alkaline hydrolysis of **1**. A soln of **1** (130 mg) in 3% KOH–MeOH (5 ml) was refluxed for 15 min. The soln was neutralized with 1N HCl–MeOH, and evapd to dryness *in vacuo*. The residue was treated with MeOH and the soluble part was passed through a Sephadex LH-20 column (solvent MeOH) to give the glycoside, as a white powder (42 mg), $[\alpha]_D$ −108.2° (*c* 1.01; MeOH); IR ν_{max}^{KBr} cm^{-1}: 3600–3200 (OH), 1690, 1622 (enone), 945, 934, 915, 870 and 850; ^{13}C NMR (pyridine-d_5): δ84.3, 37.2, 68.1, 43.0, 139.2, 124.6, 29.3, 31.8, 47.6, 42.4, 25.2, 28.2, 176.5, 138.6, 204.6, 82.2, 47.9 (C-1-17), 13.8, 49.8, 61.3, 113.6, 72.2, 69.9., 35.6, 61.3, 12.8 (C-19-27), 100.6, 75.6, 74.0, 69.0, 67.2 (C-1′–C-5′), 101.3, 72.2, 72.2, 74.8, 69.4 and 18.8 (C-1″–C-6″). (Found: C, 56.31; H, 7.38. $C_{37}H_{54}O_{16} \cdot 2H_2O$ requires: C, 56.19; H, 7.39.)

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REFERENCES

1. Nohara, T., Nakano, A., Miyahara, K., Komori, T. and Kawasaki, T. (1975) *Tetrahedron Letters* **18**, 4381.
2. Nohara, T., Komori, T. and Kawasaki, T. (1980) *Chem. Pharm. Bull.* **28**, 1437.
3. Fukuda, N., Imamura, N., Saito, E., Nohara, T. and Kawasaki, T. (1981) *Chem. Pharm. Bull.* **29**, 325.
4. Nakano, K., Nohara, T., Tomimatsu, T. and Kawasaki, T. (1982) *J. Chem. Soc. Chem. Commun.* 789.
5. Nakano, K., Nohara, T., Tomimatsu, T. and Kawasaki, T. (1983) *Phytochemistry* **22**, 1047.