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A FLAVONOL GLYCOSIDE AND A SESQUITERPENE CELLOBIOSIDE FROM *TRILLIUM TSCHONOSKII*

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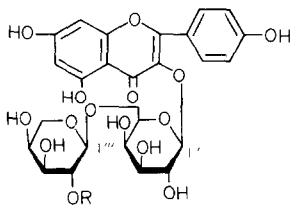
Key Word Index—*Trillium tschonoskii*; Liliaceae, flavonoid, kaempferol glycoside monoacetate, sesquiterpene cellobioside

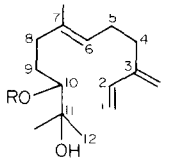
Abstract—The underground and aerial organs of *Trillium tschonoskii* afforded two new compounds in addition to dioscin, methyl protodioscin and four 18-norspirostanol oligosides previously reported. These were identified as 3-*O*-[2‴-*O*-acetyl-α-L- arabinopyranosyl-(1 → 6)-β-D-galactopyranosyl]kaempferol and 7,11-dimethyl-3-methylen-1,6-dodecadien-10,11-diol 10-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside

INTRODUCTION

Previously, we reported the isolation and characterization of Ts-a (dioscin) and Ts-b (methylprotodioscin) [1] as major components and the 18-norspirostanol oligosides. Ts-c [2], Ts-d, Ts-e and Ts-g [3] as minor ones from the underground parts of *Trillium tschonoskii* Max. We have now isolated two additional compounds, Ts-f (**1**) and Ts-x (**3**) from this plant and performed their structural elucidation



1 R = Ac
2 R = H



3 R = Cellobiose
4 R = Cellobiose (acetate)
5 R = H
6 R = Ac
8 R = Cellobiose (methyl ether)

RESULTS AND DISCUSSION

Ts-f (**1**) showed IR absorptions due to an acetoxyl group (1725 cm^{-1}), a hydroxyl group (3400 cm^{-1}), an α,β-unsaturated ketone (1650, 1601 cm^{-1}) and, an aromatic ring (900, 850, 770 cm^{-1}) On acid hydrolysis **1** yielded kaempferol, galactose and arabinose A comparative study of the ^{13}C NMR spectra of the desacetyl compound (**2**) of **1**, kaempferol, methyl β-D-galactopyranoside and methyl α-L-arabinopyranoside suggested that the L-arabinopyranosyl residue was linked to the hydroxyl at C-6 of the D-galactopyranosyl residue and its diglycosyl moiety combined with the hydroxyl at C-3 of kaempferol [4]. As regards the location of the acetyl linkage, the ^1H NMR spectrum of **1** revealed that it was attached to the hydroxyl at C-2 of the terminal arabinosyl residue. That is, a double doublet signal ($J = 7, 8$ Hz) at δ 5.70 in the chemical shift region of the methine adjacent to the acetoxyl was coupled with an arabinosyl anomeric proton signal at δ 4.72 (*d*, $J = 7$ Hz) (by spin decoupling experiment) Consequently, Ts-f (**1**) is 3-*O*-[2‴-*O*-acetyl-α-L-arabinopyranosyl-(1 → 6)-β-D-galactopyranosyl] kaempferol.

Ts-x (**3**) showed 27 carbons due to three methyls (δ 16.1, 25.2, 26.7), four methylenes (δ 27 0, 31 0, 31.7, 36.3), three double bonds [trisubstituted δ 135.9 (*s*), 124.8 (*d*), disubstituted 113.5 (*t*), 146.4 (*s*), monosubstituted 116.2 (*t*), 139.4 (*d*)] and two oxygen-bearing carbons, H–C–O– and >C–O– [δ 89 9 (*d*), 71 9 (*s*)] together with two hexosyl sugar residues (δ 62 0, 62 4, 71 5, 74 7, 74.9, 76 5, 76.7, 78 1, 78.4, 81 1, 104.9, 105 6) in the ^{13}C NMR spectrum The hepta-acetate (**4**) of **3**, colorless needles, mp 132, $[\alpha]_D$ −17.6° ($CHCl_3$), exhibited a terminal peracetylated hexosyl–hexosyl cation at *m/z* 619 and a terminal peracetylated hexosyl cation at *m/z* 331 Therefore, Ts-x (**3**) must be a sesquiterpene diglycoside. Enzymic hydrolysis of **3** afforded an aglycone (**5**), colorless oil (n_D 1.491), $[\alpha]_D$ +15.4° and D-glucose. The aglycone (**5**) showed absorptions due to the diene in the IR and UV spectra (ν_{max}^{KBr} cm^{-1}. 1630, 1590, 987, 895; λ_{max}^{EtOH} nm· 225) The ^1H NMR spectrum of **5** showed two methyls, δ 1.15 and 1.20 (each *s*), a vinyl methyl, 1.62 (*br s*), a carbinol methine, 3 36 (*dd*, $J = 3, 9$ Hz), H_a, H_b>C=C<H_c, R [H_a 5.06 (*d*, $J = 11$ Hz), H_b 5.24 (*d*, $J = 18$ Hz), H_c 6 40 (*dd*, $J = 11, 18$ Hz)], a vinylidene 5.02 (*s*) and a vinyl proton 5.22 (*m*) Since the methyl signal at δ 1 62 changed into a sharp singlet when the vinyl proton at δ 5.22 was irradiated, the methyl and vinyl protons are vicinal to a double bond.

Moreover, the chemical shift (δ 1.60 in carbon tetrachloride) of the vinyl methyl suggested the double bond to be *trans* [5] The diene structure was supposed to be $-\overset{\overset{CH_2}{\|}}{C}-CH=CH_2$ on the basis of the IR (1590 cm^{-1}) and UV (225 nm, ε = 28 000) absorptions [6, 7] of Ts-x (**3**). If it were a diene $=\overset{\overset{Me}{|}}{C}-CH=CH_2$ structure, the absorptions, 1640 cm^{-1} in the IR, 232–238 nm in the UV, would be anticipated [8]. The acetate (**6**) of **4**, colorless oil, $[\alpha]_D$ +3.5° ($CHCl_3$), showed the molecular ion at *m/z* 280 $C_{17}H_{28}O_3$ in the mass spectrum. The ^1H NMR spectrum of **6** exhibited one acetyl signal at δ 2.08 and its acetyl carbinol proton at δ 4.75 (*dd*, *J* = 5, 10 Hz) Its IR spectrum still showed a hydroxyl absorption (3580 cm^{-1}) Therefore, one of two hydroxyls in **5** is tertiary and the other secondary. Sodium periodate oxidation of **5** followed by coupling with 2,4-dinitrophenylhydrazine gave a hydrazone (**7**), mp 64–68°, MS *m/z* 358 (M^+, $C_{18}H_{22}O_4N_4$). From the above data the structure of the aglycone (**5**) of Ts-x(**3**) is as shown. The permethylate (**8**), colorless oil, MS *m/z* 660 $[M]^+$, 423 (terminal permethylated hexosyl–hexosyl cation), 219 (terminal permethylated hexosyl cation), derived from Ts-x (**3**) by Kuhn's method [9], yielded on methanolysis a mixture of methyl glycosides of 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranose and 2,3,6-tri-*O*-methyl-α-D-glucopyranose. Since the carbinol methine δ 3.22 (*dd*, *J* = 4, 8 Hz) at C-10 in the ^1H NMR spectrum of **4** appeared at 4.75 (*dd*, *J* = 5, 10 Hz) in that of **6**, the glycosyl residue should be bound with the hydroxyl at C-10 of **5**. Moreover, the ^1H NMR spectrum of **8** exhibited two anomeric proton signals at δ 4.27, 4.28 (both *d*, *J* = 8 Hz) both suggesting β-configurations Consequently, Ts-x (**3**) is 7,11-dimethyl-3-methylen-1, 6-dodecadien-10, 11-diol 10-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside

EXPERIMENTAL

Isolation of Ts-f (**1**) Fresh aerial parts of *Trillium tschonoskii* Max (2 9 kg) were extracted with refluxing MeOH (6 l) and the extract was concd under red pres to give a residue (141 g), which was partitioned between *n*-BuOH and H_2O The organic layer was evaporated *in vacuo* and the residue (26 g) was repeatedly chromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 8 2 0 2 → 7 3 0 5, $CHCl_3$–*n*-BuOH–MeOH–H_2O, 7 3·3 0 5) to give Ts-f (**1**) (287 mg)

Ts-f (**1**) A pale yellow powder (dilute MeOH), $[\alpha]_D^{26}$ − 53 9° (pyridine, *c* 1 52) ^1H NMR (C_5D_5N) δ 1 93 (3H, *s*, OAc), 4 72 (1H, *d*, *J* = 7 Hz, arabinosyl anomeric proton), 4 76 (1H, *t*, *J* = 8 Hz, H-2″ of galactose), 5 70 (1H, *dd*, *J* = 7, 8 Hz, H-2‴ of arabinose), 6.07 (1H, *d*, *J* = 8 Hz, galactosyl anomeric proton), 6 70 (1H, *d*, *J* = 2 Hz, H-6), 6.80 (1H, *d*, *J* = 2 Hz, H-8), 7 18 (2H, *d*, *J* = 8 Hz, H-3′, H-5′), 8 55 (2H, *d*, *J* = 8 Hz, H-2′, H-6′) ^{13}C NMR (DMSO-d_6) aglycone (kaempferol C-2–C-10, C-1′–C-6′), δ 156 4, 133 3, 177 4, 161 2, 98 9, 164 3, 93 7, 156.2, 104 0, 120.7, 130 8, 115 1, 160 1, 115 1, 130 8, galactose (C-1″–C-6″), 101 9, 71 0, 72 7, 68 5, 75 4, 67.0, arabinose (C-1‴–C-5‴); 100 4, 72 0, 70 3, 67 8, 65 5, Ac, 20 3, 169 2

Alkaline treatment of **1** Compound **1** (90 mg) was saponified with 5% KOH–MeOH (2 ml) for 30 min at room temp The soln was neutralized with 1 N HCl–MeOH and evaporated to dryness *in vacuo* The residue was chromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 7 3 0 2) to give an acetyl free compound (**2**) (80 mg), a yellow powder, mp 254–259°, $[\alpha]_D^{27}$ − 42 7° (pyridine, *c* 0 38), ^{13}C NMR (DMSO-d_6) aglycone (kaempferol C-2–C-10, C-1′–C-6′), δ 156 3, 133 1, 177 3, 161 0, 98 7, 164 3, 93 6, 156 3, 103 8, 120 7, 130 8, 115 0, 159 9, 115 0, 130 8, galactose (C-1″–C-6″), 101 6, 70 9, 72 9, 68 1, 74 1, 66 4, arabinose (C-1‴–C-5‴), 102.6, 72 4, 70 3, 66 4, 64 8

Permethylate of **2** Treatment of **2** (39 mg) with CH_2N_2 followed by Kuhn's methylation gave a permethylate as a yellow powder, MS *m/z* 707 $[M+H]^+$, 515, 470, 440, 412, 378, 342, 329, 328, 310, 282, 175 ^1H NMR (C_5D_5N) δ 4 18 (1H, *d*, *J* = 7 Hz, arabinosyl anomeric proton), 5 68 (1H, *d*, *J* = 8 Hz, galactosyl anomeric proton), 6 31 (1H, *d*, *J* = 2 Hz, H-6), 6 45 (1H, *d*, *J* = 2 Hz, H-8), 6 95 (2H, *d*, *J* = 8 Hz, H-3′, H-5′), 8 14 (2H, *d*, *J* = 8 Hz, H-2′, H-6′)

Methanolysis of permethylate Permethylate (8 mg) was methanolysed with 1 N HCl–MeOH (1 ml) in the usual manner to yield a mixture of methylated sugars, identical with methyl-2,3,4-tri-*O*-methylarabinopyranoside (R_f 0 51) and 2,3,4-tri-*O*-methylgalactopyranoside (R_f 0 32, 0 30) on TLC (solvent, *n*-hexane–Me_2CO, 1 1).

Isolation of Ts-x hepta-acetate (**4**) The MeOH extractive (316 g) of underground parts of *T. tschonoskii* Max (3 5 kg) was partitioned between *n*-BuOH and H_2O The organic layer was evaporated *in vacuo* and the residue (126 g) was repeatedly subjected to CC on Si gel ($CHCl_3$–MeOH, 20 1, $CHCl_3$–MeOH–H_2O, 9 2·0 2 → 7 3 0 2) to afford a fraction (1 0 g) which was then acetylated with Ac_2O–pyridine (each 10 ml) at room temp overnight and purified by Si gel CC (*n*-hexane–EtOAc, 3 1 → 3:2) to yield a hepta-acetate (**4**) (112 6 mg) of **3** Colorless needles, mp 132°, $[\alpha]_D^{20}$ − 17 6° ($CHCl_3$, *c* 1.02) MS *m/z* 619 $[C_{26}H_{35}O_{17}]^+$, 559, 457, 331 $[C_{14}H_{19}O_9]^+$, 271, 202, 169, 109, 98 ^1H NMR ($CDCl_3$) δ 1 09 (6H, *s*, Me_2-11), 1 57 (3H, *s*, vinylmethyl), 1 8–2 3 (OAc), 3 22 (1H, *dd*, *J* = 4, 8 Hz, H-10), 4 98 (2H, *s*, vinylidene-3), 5 04 (1H, *d*, *J* = 10 Hz, H-1), 5 22 (1H, *d*, *J* = 17 Hz, H-1′), 5 25 (1H, *s*, H-6), 6 35 (1H, *dd*, *J* = 11, 17 Hz, H-2) (Found. C, 57 51, H, 7 10 $C_{41}H_{60}O_{19}$ requires C, 57 47, H, 7 01%)

Ts-x (**3**) Compound **4** (90 mg) was saponified with 3% KOH–MeOH (5 ml) at room temp. for 20 min The soln was neutralized with 1 N HCl–MeOH and evaporated to dryness *in vacuo*. The residue was passed through a Sephadex LH-20 column (MeOH) to give Ts-x (**3**) (35 mg) as a colorless oil, $[\alpha]_D^{25}$ − 21 9° (MeOH; *c* 1 00) ^{13}C NMR (C_5D_5N) δ 16 1 (*q*), 25 2 (*q*), 26 7 (*q*), 27 0 (*t*), 31 0 (*t*), 31 7 (*t*), 36 3 (*t*), 62 0 (*t*), 62 4 (*t*), 71 5 (*d*), 71 9 (*d*), 74 7 (*d*), 74 9 (*d*), 76 5 (*d*), 76 7 (*d*), 78 1 (*d*), 78 4 (*d*), 81 1 (*d*), 89.9 (*d*), 104 9 (*d*), 105 6 (*d*), 113 5 (*t*), 116 2 (*t*), 124 8 (*d*), 135 9 (*s*), 139 4 (*d*), 146 4 (*s*)

Enzymic hydrolysis of **3** A mixture of **3** (300 mg) and glycosidase *Turbo cornutus* (200 mg) in H_2O (10 ml) was incubated at 37° for 1 hr then extracted with Et_2O The ethereal layer was concd and applied to a Si gel column to afford the aglycone (**5**) (65 mg), colorless oil, n_D^{20} 1 491, $[\alpha]_D^{15}$ + 15 4° ($CHCl_3$, *c* 3 31), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3550 (OH), 1630, 1590, 987, 895 ($CH_2=CH-\overset{|}{C}=CH_2$) UV λ_{max}^{EtOH} nm 225 (ε = 28 000). ^1H NMR ($CDCl_3$) δ 1.15, 1 20 (each 3H, *s*, Me_2-11), 1 62 (3H, *s*, vinylmethyl), 1 95–2 22 (4H, *s*, H_2-4 and H_2-5), 3.36 (1H, *dd*, *J* = 3, 9 Hz, H-10), 5.02 (2H, *s*, vinylidene-3), 5 06 (1H, *d*, *J* = 11 Hz, H-1), 5 22 (1H, *br s*, H-6) 5 24 (1H, *d*, *J* = 18 Hz, H′-1), 6 40 (1H, *dd*, *J* = 11, 18 Hz, H-2). The aq layer was evaporated *in vacuo* to give a residue which was passed through a Sephadex LH-20 column using MeOH as solvent to afford a colorless syrup identical with D-glucose, R_f 0 23 on PPC, solvent the upper layer of *n*-BuOH–pyridine–H_2O (6 2 3) + pyridine (1), $[\alpha]_D^{23}$ + 42 3° (H_2O, *c* 1 25)

The monoacetate (**6**) *of* **5** Compound **5** (80 mg) was acetylated with Ac_2O–pyridine (1 1, 6 ml) in the usual manner to give the monoacetate (**6**) (44 mg), a colorless oil, $[\alpha]_D^{20}$ + 3 5° ($CHCl_3$, *c*

2 12), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 1725, 1245 (OAc), 1630, 1590, 990, 895 ($CH_2=CH-\overset{|}{C}=CH_2$) MS *m/z* 280 $[C_{17}H_{28}O_3]^+$, $[M]^+$, 262 $[C_{17}H_{26}O_2]^+$, 220 $[C_{15}H_{24}O]^+$, 202 $[C_{15}H_{22}]^+$, 187 $[C_{14}H_{19}]^+$, 159 $[C_{12}H_{15}]^+$, 149 $[C_{11}H_{17}]^+$, 134 $[C_{10}H_{14}]^+$, 120 $[C_8H_{12}]^+$ ^{1}H NMR ($CDCl_3$): δ 1.18 (6H, *s*, Me_2-11), 1.59 (3H, *s*, vinylmethyl), 2.08 (3H, *s*, OAc), 2 18–2 20 (4H, *s*, H_2-4 and H_2-5), 4.75 (1H, *dd*, *J* = 5, 10 Hz, H-10), 4 98 (2H, *s*, vinylidene-3), 5.02 (1H, *d*, *J* = 11 Hz, H-1), 5 20 (1H, *d*, *J* = 18 Hz, H'-1), 6.34 (1H, *dd*, *J* = 11, 18 Hz, H-2).

$NaIO_4$ *oxidation of* **5** To a soln of **5** (10 mg) in 90% MeOH (3 ml), $NaIO_4$ (15 mg) was added and stirred for 3 hr at room temp. The reaction mixture was concd under red. pres., diluted with H_2O and extracted with Et_2O The residue reacted with 2,4-dinitrophenylhydrazine (12 ml) in the presence of EtOH (2 ml) and one drop conc. HCl for 10 min on the hot bath After neutralization with 3% KOH–MeOH, the residue was subjected to Si gel CC (*n*-hexane–EtOAc, 5:1) to afford the hydrazone, orange crystals (6 mg), mp 64–65°, MS *m/z* 358 $[C_{18}H_{22}N_4O_4]^+$, $[M]^+$, ^{1}H NMR ($CDCl_3$)· δ 1.67 (3H, *s*, vinylmethyl), 2 20–2.25 (4H, *s*, H_2-4 and H_2-5), 5 00 (2H, *s*, vinylidene-3), 5.06 (1H, *d*, *J* = 11 Hz, H-1), 5 20 (1H, *d*, *J* = 18 Hz, H'-1), 6.26 (1H, *dd*, *J* = 11, 18 Hz, H-2), 6 1–6 5 (1H, *m*, H-10), 7 51 (1H, *dd*, NH), 7.92 (1H, *d*, *J* = 10 Hz, arom H), 8.30 (1H, *dd*, *J* = 3, 10 Hz, arom. H), 9.12 (1H, *d*, *J* = 3 Hz, arom H).

Methylation of **3**. After **3** (50 mg), DMF (2 ml), Ag_2O (2 g) and MeI (15 ml) were stirred for 19 hr at room temp., the reaction mixture was filtered and washed with $CHCl_3$ The filtrate was evaporated to give a residue, which was purified by Si gel CC (*n*-hexane–EtOAc, 1.1) to afford a permethylate (**8**) of **3**, a colorless oil. R_f 0.39 (*n*-hexane–EtOAc, 1 1). MS *m/z* 660 $[M]^+$, 423 (terminal permethylated hexosylhexose), 219 (terminal permethylated hexose). ^{1}H NMR ($CDCl_3$) δ 1 12 (6H, *s*, Me_2-11), 1.60 (3H, *s*, vinylmethyl), 2.18–2.21 (4H, H_2-4 and H_2-5), 3.34–3.59 (OMe), 4.27, 4 28 (each 1H, *d*, *J* = 8 Hz, 2 × glucosyl anomeric proton), 4 98 (2H, *s*, vinylidene-3), 5 04 (1H, *d*, *J* = 11 Hz, H-1), 5.21 (1H, *br s*, H-6), 5 20 (1H, *d*, *J* = 18 Hz, H'-1), 6.36 (1H, *dd*, *J* = 11, 18 Hz, H-2)

Methanolysis of **8**. Compound **8** (20 mg) was methanolized with 1 N HCl–MeOH (4 ml) for 2 hr. The reaction mixture was neutralized with 3% KOH–MeOH and passed through a Sephadex LH-20 column eluting with MeOH to give methyl-2,3,4,6-tetra-*O*-methyl-α- and β-glucopyranoside and methyl-2,3,6-tri-*O*-methyl-β-D-glucopyranoside.

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