The air-dried aerial parts of the plants were powdered and extracted with cold $Me_2CO$ for 1 week. The solvent was removed from the extracts and the residues chromatographed on Si gel columns. The acids were methylated with $CH_2N_2$ in $Et_2O$ soln. GC of the methyl esters was performed on a 1.5 m × 3 mm column packed with 3% OV-1 on Chromosorb at 260°, detector and injector temp. 300°, carrier gas $N_2$ at 20 ml/min.

Methyl oleanolate and methyl ursolate were identified by their GC, MS and $^1$H NMR data. Methyl maslinate and methyl 3-epi-maslinate were isolated and identified by comparison with the physical, MS and $^1$H NMR data in the lit. [2, 4, 5].

The separation of micromeric acid from ursolic and oleanolic acids was as described previously [7], by chromatography of the mixture of the acetylated methyl esters on a Si gel–$AgNO_3$ column. Methyl acetylmicromerate was identified by mmp, MS, and GC, in comparison with an authentic specimen.

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# STEROL AND TRITERPENOID GLYCOSIDES FROM THE ROOTS OF *PATRINIA SCABIOSAEFOLIA*

WON SICK WOO, JAE SUE CHOI, OTTO SELIGMANN* and HILDEBERT WAGNER*

Natural Products Research Institute, Seoul National University, Korea; *Institut für Pharmazeutische Biologie der Universität München, West Germany

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**Key Word Index**—*Patrinia scabiosaefolia*; Valerianaceae; triterpenoid glycosides; sterol glucosides; 2′-*O*-acetyl-3-*O*-α-L-arabinopyranosyl-hederagenin.

**Abstract**—From the roots of *Patrinia scabiosaefolia*, oleanolic acid, hederagenin, 3-*O*-α-L-arabinopyranosyl-oleanolic acid, 3-*O*-α-L-arabinopyranosyl-hederagenin, 2′-*O*-acetyl-3-*O*-α-L-arabinopyranosyl-hederagenin and a mixture of sitosterol and campesterol-D-glucosides were isolated.

*Patrinia scabiosaefolia* is a perennial weed, roots of which have been used as an indigenous medicine for edema, appendicitis, endometritis and inflammation and several pertaining pharmacological activities were reported [1]. On the other hand, a methanol extract caused a significant prolongation of hexobarbital-induced sleeping time, elevation of serum transaminase activities and severe histopathological changes in the liver in mice [2].

In the course of a search for the hepatotoxic substances, six compounds numbered **1**–**6** in order of increasing polarity were isolated from the chloroform-soluble fraction of a methanol extract of the roots.

Compound **1**, mp 310°, $[\alpha]_D^{23}$ +58.6° and **2**, mp 330–334°, $[\alpha]_D^{20}$ +90° were identified as oleanolic acid and hederagenin, respectively, by direct comparison with authentic samples. Compound **3**, mp 226–229°, $[\alpha]_D^{20}$ +68° gave a positive reaction in the Libermann–Burchard and Molish tests and showed ester (1725 and

1230 $cm^{-1}$), free acid (1690 $cm^{-1}$) and glycoside bond (1000–1100 $cm^{-1}$) absorptions in its IR spectrum.

Acid hydrolysis of **3** gave hederagenin (mmp, co-TLC) and L-arabinose (co-TLC). Alkaline hydrolysis of **3** yielded **6**, mp 228–231°, $[\alpha]_D^{25}$ + 67.2°, which afforded hederagenin (mmp, co-TLC) and L-arabinose (co-TLC) on acid hydrolysis. Methanolysis of the permethylated product of **6**, mp 163–165°, $[M]^+$ at *m/z* 674, gave 23-*O*-methylhederagenin methylester, mp 203–206° (mmp, co-TLC) and methyl 2,3,4-tri-*O*-methyl-L-arabinopyranoside (GC).

Therefore, the structure of **6** was elucidated as 3-*O*-L-arabinopyranosyl-hederagenin.

The α-orientation of the glycosidic linkage was suggested not only from the *J* values of the anomeric proton signals of its permethylated product ($J$ = 7 Hz) and its acetate ($J$ = 6 Hz), but also from the molecular rotation difference (− 17.6°) between **6** and hederagenin ($[\phi]_D$ of methyl-α-L-arabinoside = + 28.37°, that of the β form = + 402.62°) [3]. The $^1$H NMR spectrum of **3** showed one acetyl group at δ 2.1, and the acetate of **3**, mp 223–227°, was identical with the acetate of **6**. The presence of an intense peak at *m/z* 175 in the mass spectrum of **3**, which corresponds to the fragment of arabinosyl monoacetate ($C_7H_{11}O_5$), suggested that an acetyl group was situated at its sugar moiety. Treatment of **3** with acetone in the presence of cupric sulphate afforded an acetonide, mp 174–176°, indicating that the attachment point of the acetyl group was the 2′-hydroxyl of **6**. As expected, a base peak due to isopropylidene arabinosyl acetate ($C_{10}H_{15}O_5$) appeared at *m/z* 215 in its mass spectrum.

The $^{13}$C NMR chemical shifts of **3** were also in agreement with the formulation of **3** as 2′-*O*-acetyl-3-*O*-α-L-arabinosyl-hederagenin. A comparison of the spectrum of **3** with that of **6**, showed that all of the signals due to the sapogenin moiety of both compounds appeared at almost the same positions. With regard to the sugar carbon region, on going from **6** to **3** the signal for C-2′ is displaced downfield by + 1.7 ppm while signals due to C-1′ and C-3′ are shielded by − 2.1 and − 2.0 ppm, respectively. Such changes in the chemical shifts of sugar carbons can only be explained if the hydroxyl group at the 2′-position is acetylated. This compound has not previously been reported in nature.

Compound **4**, mp 256–258°, $[\alpha]_D^{23}$ + 50°, afforded oleanolic acid (mmp, co-TLC) and L-arabinose (co-TLC) on acid hydrolysis. This compound was identified, by means of permethylation followed by methanolysis and NMR data analysis of its acetate, as 3-*O*-α-L-arabinopyranosyl-oleanolic acid. The molecular rotation difference between **4** and oleanolic acid was + 26.55°. This compound has only been reported in the flower buds of *Fatsia japonica* [4].

Compound **5**, mp 276–279°, on acid hydrolysis gave D-glucose (co-TLC) and a genin, mp 128–131°, which was identified as a mixture of sitosterol (64.5%) and campesterol (35.5%) (MS, GC).

## EXPERIMENTAL

*Isolation.* The roots of *Patrinia scabiosaefolia* were extracted with MeOH. The MeOH extract was concd and defatted with *n*-hexane and then extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and separated by chromatography on a Si gel column with $CHCl_3$–MeOH–7% HOAc (5:1:1) to give six compounds (**1**–**6**).

Compound **1** (oleanolic acid), mp 310°, needles from MeOH; $[\alpha]_D^{23}$ + 58.6° (MeOH; *c* 1.04) (mmp, co-TLC, MS); methylacetate, mp 220–222° (mmp, co-TLC, NMR).

Compound **2** (hederagenin), mp 330–334°, needles from MeOH–$CHCl_3$, $[\alpha]_D^{20}$ + 90° (pyridine; *c* 0.04) (mmp, co-TLC, MS); methyl acetate, mp 192–193° (mmp, co-TLC, NMR).

Compound **3**, mp 226–229°, prisms from MeOH, $[\alpha]_D^{20}$ + 68° (MeOH; *c* 0.05); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400(OH), 1725 and 1230 (acetate), 1690 (acid), 1000–1100 (glycoside); $^1$H NMR ($CD_3OD$ + TFA): δ 0.6–1.17 (Me × 6), 2.1(3H, *s*, MeCO), 4.45(1H, *d*, $J$ = 7 Hz, anomeric H); $^{13}$C NMR (DMSO-$d_6$) sugar: δ 102.7(C-1′), 72.9(C-2′), 70.8(C-3′), 68.4(C-4′), 66.2(C-5′); MS *m/z* (rel. int.): 646 $[M]^+$ (0.06), 248 $[RDA]^+$ (100), 203 $[RDA - COOH]^+$ (84.8), 175[arabinosyl acetate]$^+$ (64.6).

Compound **4**, mp 256–258°, needles from MeOH, $[\alpha]_D^{23}$ + 50° (MeOH; *c* 0.07); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400(OH), 1700(acid), 1000–1100(glycoside).

Compound **5**, mp 276–279°, needles from MeOH.

Compound **6**, mp 228–231°, needles from MeOH, $[\alpha]_D^{25}$ + 67.2° (MeOH; *c* 0.64); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400(OH), 1700(acid), 1000–1100(glycoside); $^{13}$C NMR (DMSO-$d_6$) sugar: δ 104.8(C-1′), 72.8(C-3′), 71.2(C-2′), 67.6(C-4′), 65.1(C-5′).

*Hydrolysis of compound* **3**. Compound **3** (30 mg) was heated in 5% $H_2SO_4$ (5 ml) for 5 hr. After cooling, the ppt was filtered and crystallized from $CHCl_3$–MeOH to yield needles, mp 330–334°, identified as **2** by mmp and co-TLC with an authentic sample. The filtrate was neutralized with $BaCO_3$, filtered and evaporated under red. pres. The residue was found to be L-arabinose by TLC (cellulose; pyridine–EtOAc–HOAc–$H_2O$, 36:36:7:21; $R_f$ 0.38).

Another sample (30 mg) was heated in 5% KOH (5 ml) for 4 hr. After cooling, the reaction mixture was acidified with dilute HCl and extracted with BuOH. BuOH soln was washed with $H_2O$, evaporated under red. pres. and crystallized from MeOH to yield needles, mp 228–231°, which were identical with **6** (mmp, co-TLC).

*Acetylation of compound* **3**. Compound **3** (200 mg) was acetylated with $Ac_2O$ and pyridine in the usual way. The reaction product was crystallized from MeOH to give needles, mp 223–227°; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1750 and 1230; $^1$H NMR ($CDCl_3$): δ 0.70–1.10 (Me × 6), 2.0, 2.07, 2.1, 2.12 (3H each, *s*, MeCO × 4), 4.38 (1H, *d*, $J$ = 6 Hz, anomeric H), which was identical with the acetate obtained from **6** (see below).

*Acetonide formation of compound* **3**. Compound **3** (10 mg) in dry $Me_2CO$ (3 ml) was stirred overnight in the presence of dry $CuSO_4$ (10 mg) and filtered. After removal of the solvent under red. pres. the residue was subjected to prep. TLC ($C_6H_6$–dioxane HOAc, 90:25:4; $R_f$ 0.6), to yield an acetonide, mp 174–176°; MS *m/z* (rel. int.): 686 $[M]^+$ (0.07), 248 $[RDA]^+$ (81.9), 215 [isopropylidene arabinosyl acetate]$^+$ (100), 203 $[RDA - COOH]^+$ (62.8).

*Acid hydrolysis of compound* **4**. A soln of **4** (30 mg) in 5% $H_2SO_4$–EtOH (5 ml) was refluxed for 5 hr and concd under red. pres. to remove EtOH. After addition of $H_2O$ the resulting ppt was filtered and crystallized from MeOH to afford needles, mp 310°, identified as **1** (mmp, co-TLC). L-Arabinose was detected in the filtrate by TLC in the same way as above.

*Acetylation of compound* **4**. Compound **4** (30 mg) was acetylated as above and crystallized from MeOH to give prisms, mp 148–150°; $^1$H NMR ($CDCl_3$): δ0.84–1.15 (Me × 7), 2.05 (6H, *s*, MeCO × 2), 2.08 (3H, *s*, MeCO), 4.21 (1H, *d*, $J$ = 6 Hz, anomeric H).

*Permethylation of compound* **4** *followed by methanolysis.* Permethylation of **4** (30 mg), according to the method described in ref. [5], followed by purification by CC with $C_6H_6$–$Et_2O$–MeOH (8:2:0.5) and crystallization from MeOH yielded prisms, mp 170–172°, MS *m/z* (rel. int.): 644 $[M]^+$ (0.2),

453 $[M-PMAra]^+$ (61.6), 262 $[RDA]^+$ (47.4), 203 $[RDA-COOMe]^+$ (100), 175 $[PMAra]^+$ (33.4).

The permethylether (10 mg) was refluxed in 5% HCl–MeOH (3 ml) for 5 hr. The reaction mixture was poured onto ice, filtered and the ppt was crystallized from MeOH to give methyl oleanolate, mp 201–202° (mmp, co-TLC). The filtrate was extracted with $CHCl_3$. The $CHCl_3$ soln was evaporated. The residue was found to be methyl-2,3,4-tri-*O*-methyl-L-arabinopyranoside by GC (column, 5% NPGS, 4 mm × 1.5 m; column temp., 180°; injector temp., 200°; $N_2$, 45 ml/min; $R_t$, 3.6 min).

*Hydrolysis of compound* **5**. A soln of **5** (20 mg) in 5% HCl–MeOH (5 ml) was refluxed for 5 hr and concd under red. pres. to remove MeOH. After addition of $H_2O$ the resulting ppt was filtered and crystallized from MeOH to yield a sterol mixture, mp 128–131°; MS *m/z* (rel. int.): 414 $[M_1]^+$ (100), 400 $[M_2]^+$ (37.8), 329 $[M_1-C_5H_9O]^+$ (100), 315 $[M_2-C_5H_9O]^+$ (27.1), 303 $[M_1-C_7H_{11}O]^+$ (47.6), 289 $[M_2-C_7H_{11}O]^+$ (20.4), 275 $[M_1-C_9H_{15}O]^+$ (22.7), 273 $[M-\text{side chain}]^+$ (79.6), 261 $[M_2-C_9H_{15}O]^+$ (11.1), 255 $[M-\text{side chain}-H_2O]^+$ (76.9), which was found to consist of sitosterol (64.5%, $R_t$ 3.4 min) and campesterol (35.5%, $R_t$ 2.8 min) by GC (column, 3% OV-I, 4 mm × 1.5 m; column temp., 270°; injector temp., 300°; $N_2$, 45 ml/min). The filterate was hydrolysed with conc. HCl, neutralized with $Ag_2CO_3$ and subjected to TLC (cellulose; pyridine–EtOAc–HOAc–$H_2O$, 36:36:7:21); only D-glucose ($R_f$ 0.27) was detected.

*Acid hydrolysis of compound* **6**. Acid hydrolysis of **6** (50 mg) under the same conditions as for **3** yielded **2** and L-arabinose.

*Acetylation of compound* **6**. Acetylation of **6** (50 mg) under the same conditions as for **3** gave the same acetate as that prepared from **3**.

*Permethylation of compound* **6** *followed by methanolysis*. Permethylation of **6** (30 mg) according to the method of ref. [5] gave a permethylated product, mp 163–165°; MS *m/z* (rel. int.): 674 $[M]^+$ (0.24), 483 $[M-PMAra]^+$ (26.2), 262 $[RDA]^+$ (64.3), 203 $[RDA-COOMe]^+$ (100), 175 $[PMAra]^+$ (43.2); $^1H$ NMR ($CDCl_3$): $\delta$ 0.68–1.09 (Me × 6), 3.3, 3.41, 3.47, 3.55, 3.6 (3H each, *s*, OMe × 5), 4.17 (1H, *d*, $J$ = 7 Hz, anomeric H).

The product (10 mg) was hydrolysed with 5% HCl–MeOH as above to yield 23-*O*-methylhederagenin methylester, mp 203–206°, identified by direct comparison with an authentic sample. By GC under the same conditions as above, methyl-2,3,4-tri-*O*-methyl-L-arabinopyranoside was detected in the filtrate freed from the genin.

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# 18-NORSPIROSTANOL DERIVATIVES FROM *TRILLIUM TSCHONOSKII*

KIMIKO NAKANO, TOSHIHIRO NOHARA, TOSHIAKI TOMIMATSU and TOSHIO KAWASAKI*

Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi 1-78, 770 Tokushima, Japan; *Faculty of Pharmaceutical Sciences, Kyushu University, Maedashi 3-1-1, Higashi-ku, 812 Fukuoka, Japan

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**Key Word Index**—*Trillium tschonoskii*; Liliaceae; 18-norspirostanol oligoside; acylated glycoside.

**Abstract**—Three 18-norspironstanol oligoglycosides partly acylated in their sugar moieties were isolated from the underground parts of *Trillium tschonoskii*. Their structures were characterized, as 1-*O*-[2″,3″,4″-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-epitrillenogenin-24-*O*-acetate, 1-*O*-[2″,3″,4″-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-epitrillenogenin and 1-*O*-[2″,4″-di-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-epitrillenogenin-24-*O*-acetate.

## INTRODUCTION

Previously, we reported the structural characterizations of Ts-a (dioscin), Ts-b (methyl proto-dioscin) [1] and Ts-c [2] isolated from the underground parts of *Trillium tschonoskii*. Our continuing study of this plant has led to the isolation of three additional 18-norspirostanol derivatives [3–6]. They are novel steroidal oligosides, designated Ts-d (**1**), Ts-e (**2**) and Ts-g (**3**), and this paper