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Coumarins and Triterpenoid Glycosides from the Roots of *Patrinia scabiosaefolia*

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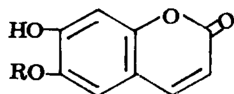
Abstract □ From the roots of *Patrinia scabiosaefolia* (Valerianaceae), scopoletin (1), esculetin (2), oleanonic acid (3), 3-O-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl oleanolic acid (4) and 3-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl hederagenin (5) were isolated and characterized by spectral data.
Keyword □ *Patrinia scabiosaefolia*, Valerianaceae, Scopoletin, Esculetin, Oleanonic acid, Triterpenoid glycosides, ^{13}C NMR.

In the previous paper[1], the isolation of 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 from the roots of *Patrinia scabiosaefolia* (Valerianaceae) was reported. In a course of continuous work on the plant, additional five compounds were isolated from the same plant part.

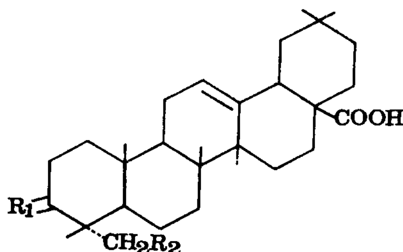
Compound **1**, mp 201~202°, showed hydroxyl (3340cm^{-1}), α,β-unsaturated ketone (1703 cm^{-1}) and aromatic ring (1608, 1565 and 1510 cm^{-1}) absorption bands in its IR spectrum and showed absorption peaks characteristic of a coumarin at 230, 250, 255, 261, 298 and 345 nm in its UV spectrum (in MeOH) which were shifted by addition of NaOMe (242, 255, 261, 278 and 393 nm). The MS spectrum showed a molecular ion at m/z 192 (100%) and other fragment ions at m/z 177 (M^+-CH_3, 12.3), 164 (M^+-CO, 50) and 149 (177−CO, 100). The ^{1}H NMR spectrum showed a pair of ortho coupled doublets of one proton each at δ7.58 (J=9Hz, H-4) and 6.27 (J=9Hz, H-3), two singlets of one proton each at 6.95 (H-5) and 6.82 (H-8) in addition to a methoxy signal at 3.97. These spectral data were in agreement with those for the structure of 6-methoxy-7-hydroxycoumarin (=scopoletin). It was further identified by direct comparison with an authentic sample (IR, NMR, mmp and co-TLC).

Compound **2**, mp 267~268°, showed hydroxyl (3200cm^{-1}), α,β-unsaturated ketone (1662 cm^{-1}) and aromatic ring (1610 and 1560cm^{-1}) absorption bands in its IR spectrum and absorption peaks characteristic of a coumarin at 231, 260, 302 and 354nm in its UV spectrum (in MeOH) which were shifted by addition of NaOMe (242, 280 and 400nm). The MS spectrum showed a molecular ion at m/z 178 (100%) and other fragment peaks at m/z 150 (M^+-CO, 61) and m/z 28 (CO, 41.3). The ^{1}H NMR spectrum showed a pair of ortho coupled doublets of one proton each at δ7.83 (J=9Hz, H-4) and 6.14 (J=9Hz, H-3), two singlets of one proton each at 6.97 (H-5) and 6.70 (H-8). These spectral data were in agreement with those for the structure of 6,7-dihydroxy coumarin (=esculetin). It was also identified by direct comparison with an authentic sample (IR, NMR, mmp and co-TLC).

Compound **3**, mp 156~158°, gave a positive



1 R=Me
2 R=H



	R_1	R_2
3	=O	H
4	···H, Ara-Rha	H
4p	···H, Ara	H
5	···H, Ara-Rha	OH
5p	···H, Ara	OH

reaction in the Liebermann-Burchard and Zimmermann tests and showed carbonyl absorption ($1695cm^{-1}$) in its IR spectrum. The MS spectrum showed a molecular ion at m/z 454 (4.07%) and other ions at m/z 248 (RDA, 100%) and 203 (RDA-COOH, 94.8%) indicating that compound **3** has an α-amyrin or a β-amyrin skeleton with one carboxyl group at rings D and E. The ^1H NMR spectrum of compound **3**-methylester exhibited seven angular methyl signals at δ 0.80 (3H), 0.97 (6H), 1.03(3H), 1.05(3H), 1.08(6H), a carbomethoxyl signal at 3.66 (3H), an olefinic proton at 5.33 (IH, brs) and a multiplet centered at 2.42 (2H) assignable to methylene protons neighboring to ketone. The ketone group must be located at C-3 by biosynthetic consideration. From the above results, structure of compound **3** was elucidated as 3-oxo oleanolic acid (=oleanonic acid). It was further identified by direct comparison with an authentic sample (IR, MS, mmp and co-TLC).

Compound **4**, $C_{41}H_{66}O_{11}$. $1\frac{1}{2}$ H_2O, mp 242~244°, gave a positive reaction in the Liebermann-Burchard and Molisch tests and showed hydroxyl ($3400cm^{-1}$), free acid ($1700cm^{-1}$) and glycoside ($1000\sim1100cm^{-1}$) absorption bands in its IR spectrum. Acid hydrolysis of compound **4** gave oleanolic acid as the genin identified by direct comparison with an authentic sample (mmp and co-TLC). L-Arabinose and L-rhamnose were detected as the sugar components in the hydrolysate free of the aglycone. Acetylation of compound **4** followed by methylation gave a methylacetate, mp 110-112°, showing five acetate methyl signals in its NMR spectrum. Partial hydrolysis of compound **4** yielded a prosapogenin (**4p**) consisting of oleanolic acid and arabinose, together with oleanolic acid and unchanged compound **4.** Compound **4p** was identified as oleanolic acid-3-O-α-L-arabinopyranoside by direct comparison with an authentic sample[1]. Interglycosidic linkage was determined to be (1 →2) by the glycosylation shift of ^{13}C NMR spectrum (Table I) and by the formation of methyl 3, 4-di-O- methyl arabinopyranoside and methyl 2, 3, 4-tri-O-methyl rhamnopyranoside from permethylated compound **4**, mp 207~208°. The α-orientation of the glycosidic linkage was suggested not only from the J value of the anomeric proton signal of permethylated compound **4** (Experimental) but also from the molecular rotation difference between compounds **4** and **4p** (Table II). Therefore, the structure of compound **4** was elucidated as 3-O-α-L-rhamno-

Table I: ^{13}C NMR chemical shift of sugars in triterpenoid glycosides (δ from TMS in DMSO-d_6).

	4p	5p	4	5
Arabinose				
C-1	105.5	104.8	103.7	102.8
2	71.0	71.2	74.8	74.5
3	72.7	72.8	72.2	72.6
4	67.4	67.6	67.3	67.7
5	64.6	65.1	63.6	64.2
Rhamnose				
C-1	99.9*		100.2	100.0
2	70.6		70.6	70.5
3	70.6		70.6	70.5
4	72.3		72.2	72.2
5	68.0		68.4	68.3
6	17.7		17.9	17.9

* Values for rhamnoside in Re-prosapogenin III from ref. 3.

Table II: $[M]_D$ of triterpenoid glycosides and related substances.

Substance	$[M]_D$	Difference	Reference
Compound **4**	+55.05°		
Prosapogenin **4p**	+294.0°	−238.94°	1
Compound **5**	+168.75°		
Prosapogenin **5p**	+407.2°	−238.45°	1
Me-α-L-rhamno-pyranoside	−110°		4
Me-β-L-rhamno-pyranoside	+168°		4

pyranosyl (1→2)-α-L-arabinopyranosyl-oleanolic acid.

Compound 5, $C_{41}H_{66}O_{12} \cdot \frac{1}{2} H_2O$, mp 256~258°, gave a positive reaction in the Liebermann-Burchard and Molisch tests and showed hydroxyl ($3400cm^{-1}$), free acid ($1685cm^{-1}$) and glycoside ($1000\sim1100cm^{-1}$) absorption bands in its IR spectrum. Acid hydrolysis of compound **5** gave hederagenin as the genin identified by direct comparison with an authentic sample (mmp and co-TLC) and L-arabinose and L-rhamnose detected as the sugar components by TLC. Acetylation of compound **5** followed by methylation afforded a methylacetate, mp 134~136°, showing six acetate methyl signals in its NMR spectrum. Partial hydrolysis of compound **5** yielded a prosapogenin (**5p**) consisting of hederagenin and arabinose, together with hederagenin and unchanged compound **5**. Compound **5p** was identified as hederagenin-3-O-α-L-arabinopyranoside by direct comparison with an authentic sample[1]. Interglycosidic linkage was determined to be (1→2) by the glycosylation shift of ^{13}C NMR spectrum (Table I) and by the formation of methyl 3,4-di-O-methyl arabinopyranoside and methyl 2,3,4-tri-O-methyl rhamnopyranoside (GC) from permethylated compound **5**, mp 126~127°. The α-orientation of the glycosidic linkage was suggested not only from the J value of the anomeric proton signal of permethylated compound **5** (Experimental) but also from the molecular rotation difference between compounds **5** and **5p** (Table II). Therefore, the structure of compound **5** was elucidated as 3-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl hederagenin.

EXPERIMENTAL METHODS

All melting points were determined on a Mitamura-Riken apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer model 243 polarimeter. IR spectra were obtained with a Beckman model IR-20A spectrophotometer and NMR spectra were taken on a Varian model FT-80A with TMS as internal standard. Mass spectra were obtained with Hewlett Packard model 5985B GC/MS spectrometer. Elemental Analyses were taken on a Shimadzu Model UM-3 Universal microanalyzer.

The UV spectra were run on a Shimadzu model MPS-50L recording spectrophotometer.

Isolation

The roots of ***P. scabiosaefolia*** were extracted with MeOH. The MeOH extract was partitioned with hexane, $CHCl_3$ and BuOH successively. The $CHCl_3$ phases were combined and concentrated in vacuo and separated by chromatography on a Si gel column with $CHCl_3$ -MeOH-7%HAc (5 : 1 : 1) followed by Sephadex LH-20 with MeOH to give compounds **1** and **3**. The BuOH phases were combined and concentrated in vacuo and separated by chromatography on a Si gel column with $CHCl_3$-MeOH-H_2O (52:28:8) to afford 10 fractions. Fr. 1 was concentrated and rechromatograhed on a Si gel column with $CHCl_3$-MeOH-7%HAc (25:8:5) and Sephadex LH-20 with MeOH to give compounds **2**, **4** and **5**. Other fractions are under rechromatography for isolation of hepatotoxic substances.

Compound **1** (scopoletin), mp 201∼202°, colorless needles from MeOH. $FeCl_3$ positive.

Compound **2** (esculetin), mp 267∼268°, yellowish needles from MeOH. $FeCl_3$ positive.

Compound **3** (oleanonic acid), mp 156-158°, $[\alpha]_D^{23}+84°$ (MeOH, c 0.1), colorless needles from MeOH,

Compound **4**, mp 242-244°, $[\alpha]_D^{25}+7.5°$ (MeOH, c 0.4), colorless needles from MeOH. Anal. Cal. for $C_{41}H_{66}O_{11}\cdot 1\frac{1}{2}\ H_2O$: C, 64.63; H, 9.13, Found : C, 64.57; H, 8.76.

Compound **5**, mp 256-258°, $[\alpha]_D^{25}+22.5°$ (MeOH, c 0.4), colorless needles from MeOH. Anal. Cal. for $C_{41}H_{66}O_{12}\cdot \frac{1}{2}\ H_2O$: C, 64.65; H, 8.66, Found : C, 64.8; H, 8.89.

Methylation of Compound 3

Compound **3** (50 mg) was treated with ethereal diazomethane in the usual manner and crystallized from MeOH to give colorless needles, mp 172-174°; IR $\nu_{max}^{KBr}cm^{-1}$: 1725 (ester), 1700 (six-membered ketone).

Acid Hydrolysis of Compound 4

A soln of **4** (20mg) in 5% H_2SO_4-EtOH (15ml) was refluxed for 5 hr. The reaction mixture was worked up as usual. The ppt was recrystallized from MeOH, mp 310°, which was identified as oleanolic acid by IR, mmp and co-TLC with an authentic sample. L-Arabinose and L-rhamnose were detected in the aqueous layer after neutralization ($BaCO_3$) by TLC (cellulose; pyridine : EtOAc : HOAc : H_2O=36:36:7:21, R*f* 0.38 and 0.46).

Acetylation of Compound 4

Compound **4** (30mg) was treated with Ac_2O (1ml) and pyridine (0.5ml) for overnight and worked up as usual. The reaction product was crystallized from hexane to give colorless prisms, mp 142-144°; IR $\nu_{max}^{KBr}cm^{-1}$: 1760, 1220(acetate).

Methylation of Compound 4-peracetate

Methylation of **4**-peracetate (30mg) with ethereal diazomethane in the usual manner and crystallization from MeOH gave colorless prisms, mp 110-112°; IR $\nu_{max}^{KBr}cm^{-1}$: 1755, 1240, 1220 (ester); ^1H NMR ($CDCl_3$) : 0.75-1.05 (Me×7), 1.21 (3H, d, J=7Hz, 6-Me of rhamnose), 2.00, 2.07, 2.12, 2.13, (MeCO×5), 3.61(3H, s, OMe), 4.56(1H, d, J=6Hz, anomeric H), 5.10 (1H, br.s, $W\frac{1}{2}$=4Hz, anomeric H), 5.22(1H, br.s, olefin).

Permethylation of Compound 4 Followed by Methanolysis

Permethylation of **4**, according to the method described by Brimacombe[2], followed by purification by CC with C_6H_6-Et_2O (4 : 1) and crystallization from hexane yielded colorless needles, mp 207-208°; IR $\nu_{max}^{KBr}cm^{-1}$:1725 (ester). ^1H NMR ($CDCl_3$): 0.70-1.10 (Me×7), 1.16 (d,

J=6Hz, 6-Me of rhamnose), 3.45, 3.47, 3.49, 3.52, 3.61 (OMe×6), 4.41(1H, d, J=6Hz, anomeric H), 5.14(1H, br.s, $W\frac{1}{2}$=4.5Hz, anomeric H), 5.28(1H, br.s, olefin). The permethylether (10mg) in 5% HCl-MeOH (5ml) was refluxed for 3hr. The reaction mixture was concentrated, diluted with water and filtered. The ppt was crystallized from MeOH to yield a colorless needles of methyl oleanolate, mp 201-202° (mmp and co-TLC). The filtrate was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated in vacuo and methyl-2, 3, 4-tri-O-methylrhamnopyranoside (T_R 2.6 and 1.8) and methyl 3, 4-di-O-methylarabinopyranoside (T_R 7.2 and 6.2) were detected by GC (column, 5% NPGS on Gas chrom Q, 4mm×1.5m, column temp. 160°, detector temp. 180°, carrier gas 40ml/min, chart speed 1cm/min).

Partial Hydrolysis of Compound 4

Compound **4** (50mg) in 0.5 N-H_2SO_4 in 75% EtOH was refluxed for 30min. After neutralization with 0.5% KOH, the reaction products were extracted with n-BuOH and column chromatographed ($CHCl_3$-MeOH-7% HAc=25:8:5, lower phase) to yield oleanolic acid (15mg), oleanolic acid-3-arabinoside (**4p**) (10mg), mp 246-248° and unchanged **4** (10mg).

Acid Hydrolysis of Compound 5

A soln of **5** (20mg) in 5% H_2SO_4-EtOH (10ml) was refluxed for 5hr. The reaction mixture was worked up as usual. The ppt was recrystallized from MeOH, mp 330-334°, which was identified as hederagenin by IR, mmp and co-TLC with an authentic sample. L-Arabinose and L-rhamnose were detected in the aqueous layer after neutralization ($BaCO_3$) by TLC (the same conditions, as above, Rf 0.38 and 0.46).

Acetylation of Compound 5

Compound **5** (30mg) was treated with Ac_2O (1ml) and pyridine (0.5ml) for overnight and worked up as usual. The reaction product was crystallized from hexane to give colorless prisms, mp 168-171°; IR $\nu_{max}^{KBr}cm^{-1}$: 1740, 1230 (acetate).

Methylation of Compound 5-peracetate

Methylation of **5**-peracetate (30mg) with ethereal diazomethane in the usual manner and crystallization from hexane gave colorless prisms, mp 134-136°; IR $\nu_{max}^{KBr}cm^{-1}$: 1750, 1240, 1220 (ester); ^1H NMR ($CDCl_3$); 0.73-1.12 (Me×6), 1.23 (3H, d, J=7Hz, 6-Me of rhamnose), 1.98, 2.07, 2.12, 2.13 (MeCO×6), 3.63(3H, s, OMe), 4.46(1H, d, J=7Hz, anomeric H), 5.10 (1H, br.s, $W\frac{1}{2}$=4Hz, anomeric H), 5.23 (1H, br.s, olefin).

Permethylation of Compound 5 Followed by Methanolysis

Permethylation of **5** according to the method described in ref. (2), followed by purification by CC with C_6H_6-Et_2O (4 : 1) and crystallization from hexane yielded colorless needles, mp 126~127°; IR $\nu_{max}^{KBr}cm^{-1}$: 1725 (ester); ^1H NMR ($CDCl_3$); 0.69-1.10(Me×6), 1.24(3H, d, J=6.5Hz, 6-Me of rhamnose), 3.29, 3.42, 3.45, 3.48, 3.51, 3.60 (OMe×7), 4.27 (1H, d, J=6Hz, anomeric H), 5.14(1H, br.s, $W\frac{1}{2}$=4Hz, anomeric H), 5.25(1H, br.s, olefin). The permethylether (10mg) in 5% HCl-MeOH (5ml) was refluxed for 3 hr. The reaction mixture was concentrated, diluted with water and filtered. The ppt was crystallized from MeOH to yield a colorless needles of 23-methyl hederagenin methylester, mp 203~206° (mmp and co-TLC). The filtrate was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated in vacuo and methyl 2, 3, 4-tri-O-methylrhamnopyranoside (T_R 2.6 and 1.8) and methyl 3, 4-di-O-methylarabinopyranoside (T_R 7.2 and 6.2) were detected (the same conditions as above).

Partial Hydrolysis of Compound 5

Compound **5** (50mg) in 0.5 N-H_2SO_4 in 75%

EtOH was refluxed for 30min. After neutralization with 0.5% KOH, the reaction products were extracted with n-BuOH and column chromatographed ($CHCl_3$-MeOH- 7%HAc=25: 8: 5, lower phase) to yield hederagenin (15mg), hederagenin-3-arabinoside (**5p**) (10mg), mp 228 ~231° and unchanged **5** (10mg).

ACKNOWLEDGEMENT

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