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COUMARIN GLYCOSIDES OF *SESELI MONTANUM**

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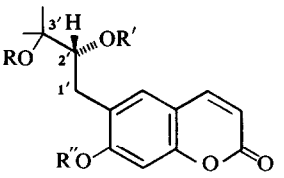
Key Word Index—*Seseli montanum*; Umbelliferae; new coumarin glycosides; peucedanol; ulopterol; apterin; stereochemistry.

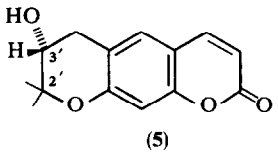
Abstract—Three new coumarin glycosides isolated from roots of *Seseli montanum* were shown to be the 3′-*O*-, 2′-*O*-, and the 7-*O*-β-D-glucopyranosides respectively of 2′(*R*)-6-(2′,3′-dihydroxy-3′-methylbutyl)-7-hydroxycoumarin. The structures were elucidated by spectroscopic and chemical methods. (*R*)-Configuration was assigned to the aglycone, also known as (+)-peucedanol, and to its 7-methyl ether, (+)-ulopterol, by chemical correlation. Additionally, apterin was obtained and characterized.

The umbellifer *Seseli montanum* L. is widely distributed in the southern part of Europe. In an earlier report by Bohlmann *et al.* on its content of acetylenic compounds [1], this species was stated also to contain coumarins. We now report a re-investigation of the roots, which has led to the isolation of four coumarin glycosides, three of which are new.

The glycosides were obtained upon methanol extraction of the dried plant material and subsequent fractionations by column chromatography on polyamide and cellulose columns. The main glycoside (**1**) ($C_{20}H_{26}O_{10}$, elemental analysis, FD–MS) was isolated in substantial quantity. Upon acid hydrolysis of **1**, D-glucose was the only sugar component detected. The presence of a coumarin skeleton in **1** was suggested by the blue fluorescence, by bands in the IR-spectrum at 1732 and 1700 (C=O), 1624 (C=C), 1573 and 1494 cm^{-1} (aromatic) and by the UV spectrum, which is very similar to that of 7-hydroxycoumarin. A strong bathochromic shift upon addition of NaOAc revealed the presence of a free phenolic OH group. The PMR-spectrum (D_2O) showed characteristic coumarin doublets at δ7.57 and δ6.00 (J = 9.6 Hz) and two one-proton singlets at δ7.08 and δ6.38, attributable to the aromatic protons in a 7-hydroxy-6-substituted coumarin. Also observable, outside the region of the sugar protons, was a broad two-proton multiplet centred at δ2.6, obviously the AB-part (J_{AB} = 14 Hz) of a partially hidden ABX-pattern and two three-proton singlets at δ1.29 and δ1.27. Considering also the elemental composition of **1**, these signals could be assigned to the benzylic methylene group and the *gem*-dimethyls, respectively, of a 3,4-dioxygenated isopentyl side chain at C-6. In confirmation of this assignment, the aglycone (**4**) obtained by acid hydrolysis of **1** showed physical data (UV, IR, PMR, [α], mp) in accordance with those reported for (+)-peucedanol [2] and, additionally, a side product formed by acid catalyzed cyclization of **4** during the hydrolysis, was shown to be (−)-3′-hydroxy-3′,4′-dihydroxanthyletin (**5**) by comparison with an authentic sample. This cyclization product, (**5**) to which *R*-configuration has earlier been assigned [3], also provided a clue to the hitherto unknown stereochemistry of (+)-peucedanol (**4**), which must be *R* also.

From the facile formation of the peracetyl derivative of





(**5**)

1, R = Glc, R′ = R″ = H
2, R′ = Glc, R = R″ = H
3, R″ = Glc, R = R′ = H
4, R = R′ = R″ = H
6, R = R′ = H, R″ = Me

1 under condition where **4** only afforded a diacetate, the presence of a free tertiary OH group at C-3′ in **1** is unlikely. Also a comparison of δ-values for methine and for *gem*-dimethyl protons in these acetates and in the triacetate of **4**, prepared under forcing conditions, shows shift differences, conceivable only with C-3′-*O* as the position of sugar attachment in **1**. Lastly, the PMR-spectrum of **1** showed the configuration of the glucosidic linkage to β by the J value (7.3 Hz) of the doublet, arising from the anomeric proton, at δ4.64. Hence, **1** is the 3′-*O*-β-D-glucopyranoside of 2′(*R*)-6-(2′,3′-dihydroxy-3′-methylbutyl)-7-hydroxycoumarin.

Two glycosides (**2**) and (**3**), isomeric with **1**, were obtained in minor quantity. The spectral data of these compounds and the results of the acid hydrolysis, together established **2** as the 2′-*O*-β-D-glucopyranoside of **4** with a free phenolic OH group, and **3** as the 7-*O*-β-D-glucopyranoside of **4**. Whereas the PMR-data indicate β-configuration in **2**, as clearly as in the case of **1**, the anomeric proton of **3** only show an ill-resolved doublet δ 4.99. A β-configuration also for this compound was inferred, however, from the width of the signal ($W_{\frac{1}{2}}$ = 9 Hz) and from the difference in molecular rotations,

* Part 21 in a series 'Constituents of Umbelliferous Plants'. For Part 20 see Nielsen, B. E. and Jensen, E. (1976) *Phytochemistry* **15**, 1049.

$\Delta[M]_D$ (**3** and **4**) − 193°, compared with $[M]_D$ values of the phenyl D-glucopyranosides, α. 491, β. − 152 (MeOH) [4].

(+)-(*R*)-Peucedanol (**4**) and its enantiomer have been obtained earlier from one umbellifer [2] and from one rutaceous plant [5] respectively, but this is the first report on its occurrence in glycosidic form. Ulopterol, the 7-methyl ether of peucedanol, has been reported to occur in members of the same plant families, described either as the dextrorotatory form [6, 7], or without specification of optical rotation [8, 9]. Now, *R*-configuration (**6**) has been assigned to (+)-ulopterol, as this was the enantiomer obtained upon methylation of **1** with diazomethane and hydrolysis of the resultant glycoside.

Finally a coumarin glycoside (**7**) was obtained, which from the results of acid hydrolysis and from an inspection of the PMR-spectrum appeared to be a mono-β-D-glucopyranoside of *cis*-9-hydroxy-8,9-dihydrooroselol. The identity of **7** with the known 1′-*O*-β-D-glucopyranoside of this aglycone, apterin [10], was established by comparison with an authentic sample. Some corrections to the data originally reported for this compound is given in the Experimental. The detection of apterin in several umbellifers was recently reported [11].

EXPERIMENTAL

Mps were corr. and determined in capillary tubes. PMR spectra were obtained at 90 MHz using acetonitrile (δ2.00) for D_2O solutions, and TMS otherwise, as int. refs. Optical rotations were measured on a Perkin–Elmer 141 polarimeter. MS were obtained with an EI/FI/FD ion source. A high temp. carbon activated tungsten wire emitter (emitter → catode 10.5 kV) was used in FD–MS, an el. energy of 70 eV in EI–MS, sample temp. 200–250° throughout. D-Glucose was identified by TLC, and by the D-glucose oxidase test. The plant material was grown at the Botanic Garden of Copenhagen, and its identity verified by Dr. K. Rahn.

Isolation of coumarin glycosides. Dried and ground roots (218 g) were extracted with MeOH. An aq. soln of the MeOH concentrate (35 g) was extracted with EtOAc and evapd. The residue (26 g) was chromatographed on a polyamide column (220 g) with a H_2O → MeOH gradient, giving a series of fractions (total *ca* 1.9 g) containing blue-fluorescent compounds. Rechromatography on polyamide columns and on columns of microcryst. cellulose with C_6H_6–EtOAc–AcOH–H_2O (2.5 2 1) as eluent afforded crystalline **7**, **3**, **1** and **2**, mentioned in order of elution from polyamide.

(R)-*Peucedanol 3′-O-β-D-glucopyranoside* (**1**) 790 mg (from EtOH), ill-def. mp (Found: C, 56.10; H, 6.38. $C_{20}H_{26}O_{10}$ requires C, 56.33; H, 6.07%). $[\alpha]_D^{23}$ + 16.9°, $[\alpha]_{436}^{23}$ + 63.0° (MeOH; *c* 1.2). λ_{max}^{MeOH} nm (log ε). 332 (4.11), 256 (*sh*) (3.46), 247 (*sh*) (3.55), 222 (4.14), NaOAc shift. λ_{max} 332 → 378. IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1732 (C=O), 1700 (*sh*) (C=O), 1624 (C=C), 1573 and 1494 (aromatic). PMR (δ in D_2O) 7.57 (1H, *d*, *J* = 9.6 Hz, H-4), 7.08 (1H, *s*, H-5), 6.38 (1H, *s*, H-8), 6.00 (1H, *d*, *J* = 9.6 Hz, H-3), 2.76 (1H, *bd*, *J* = 14 Hz, H_a-1′) 2.37 (1H, *dd*, *J* = 14, *ca* 10 Hz, H_b-1′) 1.29 and 1.27 (6H, *ss*, *gem*-dimethyls), 4.64 (1H, *d*, *J* = 7.3 Hz, anomeric H, overlapping DOH shifted by add. of CF_3COOD) 3.9–3.1 (7H, *m*, other sugar protons and H-2′). FD–MS, *m/e*. 427 (M^+ + 1]. EI–MS, *m/e*, (rel. int.) 264 [M^+ aglycon] (18), 246 [M_a^+-H_2O] (27), 228 [M_a^+-$2H_2O$] (2), 206 (11), 188 (7), 187 (8), 176 (86), 175 (100), 163 (9), 147 (12).

Acid hydrolysis of **1**. 166 mg **1** was hydrolysed in 0.25 N HCl for 1 hr at 100°. Upon extraction several times with CH_2Cl_2 D-glucose was detected in the aq. soln. The CH_2Cl_2 extractives were chromatographed on a Si gel column. Elution with a solvent gradient, CH_2Cl_2 → CH_2Cl_2–EtOAc (1 1) with further add. of MeOH (0 → 2%) afforded: (a) (*R*)-peucedanol (**4**), 70 mg, mp 177.5–178° (EtOAc) (lit. [2] 174–175°) $[\alpha]_D^{23}$ + 50.2° (EtOH 96%, *c* 0.5), $[\alpha]_D^{23}$ + 53.2° and $[\alpha]_{436}^{23}$ + 140.7° (MeOH; *c* 0.5) (lit. [2] $[\alpha]_D^{20}$ + 31.2° (EtOH); *c* 0.6) and for the enantiomer [5] $[\alpha]_D$ − 47° (EtOH; *c* 0.7)). PMR, IR and UV spectra as reported [2]. (*b*) (*R*)-3′-Hydroxy-3′,4′-dihydroxanthyletin (**5**), 5 mg, mp 181.5° (petrol) $[\alpha]_D^{23}$ − 107° (C_5H_5N; *c* 0.1) (lit. [3] mp 180.5–181.5°, $[\alpha]_D^{23}$ − 102° (C_5H_5N, *c* 0.5)). Identical (TLC, IR) with an authentic sample.

Acetylation of **1** *and* **4**. Treatment of **1** with Ac_2O–C_6H_5N (2 1) for 2.5 hr at 24°, evapn of reagents at red. press and purification on a Si gel column with CH_2Cl_2–EtOAc mixtures as eluents afforded non-crystalline **1** hexaacetate. PMR (δ in $CDCl_3$) 7.64 (1H, *d*, *J* = 9.5 Hz, H-4), 7.34 (1H, *s*, H-5), 7.07 (1H, *s*, C-8), 6.36 (1H, *d*, *J* = 9.5 Hz, H-3), 5.18 (*dd*, H-2′, by decouplings), 3.03 (1H, *dd*, *J* = 14.5, *ca* 3 Hz, H_a-1′), 2.73 (1H, *dd*, *J* = 14.5 *ca* 10 Hz, H_b-1′), 1.27 and 1.25 (6H, *ss*, *gem*-dimethyls), 4.76 (1H, *d*, *J* = 7.5 Hz, anomeric H), other sugar protons at 5.4–4.9 and 4.2–3.6, acetyl singlets at 2.40 (3H, phenolic), 2.05 (12H) and 1.86 (3H). IR (CCl_4) no OH absorption. Acetylation of **4** as for **1** afforded a diacetate (96% yield). PMR (partial, δ in $CDCl_3$) 5.07 (1H, *dd*, H-2′), 2.94 (2H, *m*, H-1′), 1.95 (1H, *s*, exchangeable by add. of D_2O, —OH), 1.30 (6H, *s*, *gem*-dimethyls), acetyl singlets at 2.39 (3H, phenolic) and 1.92 (3H). Further acetylation of **4** diacetate by refluxing with Ac_2O–CaH_2 C_6H_6 at 110° for 22 hr [12], working up as usually and chromatography on a Si gel column with CH_2Cl_2–EtOAc mixtures as eluents, afforded **4** triacetate (65% yield). PMR (partial, δ in $CDCl_3$) 5.45 (1H, *dd*, H-2′) 2.87 (2H, *m*, H-1′) 1.57 nd 1.53 (6H, *ss*, *gem*-dimethyls), acetyl singlets at 2.41 (3H, phenolic) 1.97 and 1.89 (6H).

7-O-Methylation of **1** *and acid hydrolysis.* A MeOH soln of **1** (101 mg) was treated with an excess of ethereal CH_2N for 24 hr. Upon evapn. and chromatography on cellulose and Si gel columns with C_6H_6–EtOAc–AcOH–H_2O (2 5 2 1) as the eluent, amorphous **1** 7-Me ether (69 mg) was obtained. $[\alpha]_D^{23}$ + 39° and $[\alpha]_{436}^{23}$ + 122° (MeOH, *c* 2.0). λ_{max}^{MeO} nm (log ε). 330 (4.10), 298 (*sh*) (3.83), 253 (*sh*) (3.63), 224 (4.24), no NaOAc shift. IR ν_{max}^{KBr} cm^{-1} 3400 (—OH), 1726 and 1711 (C=O), 1624 (C=C), 1575 and 1502 (aromatic). PMR (δ in D_2O) 7.53 (1H, *d*, *J* = 9.5 Hz, H-4), 6.98 (1H, *s*, H-5) 6.32 (1H, *s*, H-8), 5.96 (1H, *d*, *J* = 9.5 Hz, H-3), 2.63 (1H, *bd*, *J* = 14 Hz, H_a-1′), 2.20 (1H, *dd*, *J* = 14 Hz, *ca* 10 Hz, H_b-1′), 1.27 and 1.22 (6H, *ss*, *gem*-dimethyls) 4.67 (1H, *d*, *J* = 7.5 Hz, anomeric H, overlapping DOH shifted by add. of CF_3COOD), 4.0–3.1 (10H, *m*, other sugar protons, H-2′ and —OMe (δ 3.62)). FD–MS *m/e* 441 [M^+ + 1]. Acid hydrolysis as for **1** afforded (+)-(*R*)-ulopterol (**6**) mp 141.5–142° (EtOAc) (lit. [6] 136–138°). $[\alpha]_D^{24}$ + 98° and $[\alpha]_{436}^{24}$ + 267° (MeOH; *c* 0.3) lit. [6] $[\alpha]_D^{22}$ + 81.8° (MeOH, *c* 0.07) $[\alpha]_D^{24}$ + 73° ($CHCl_3$; *c* 0.2) (lit. [7] $[\alpha]_D$ + 57° ($CHCl_3$)). UV, IR, PMR-spectra as reported [6–9].

(R)-*Peucedanol 2′-O-β-D-glucopyranoside* (**2**). 20 mg (from EtOH) ill-def. mp $[\alpha]_D^{23.5}$ + 13.4° and $[\alpha]_{436}^{23.5}$ + 51.3° (MeOH; *c* 1.2) λ_{max}^{MeOH} nm (log ε). 331 (4.08), 257 (3.50), 247 (3.57), 223 (4.09), NaOAc shift. λ_{max} 331 → 377 IR ν_{max}^{KBr} cm^{-1}. 3400 (OH), 1734, 1711 (*sh*) (C=O), 1625 (C=C) 1577 and 1496 (aromatic) PMR (δ in D_2O). 7.67 (1H, *d*, *J* = 9.5 Hz, H-4), 7.18 (1H, *s*, H-5), 6.52 (1H, *s*, H-8), 6.06 (1H, *d*, *J* = 9.5 Hz, H-3), 2.81 (1H, *bd* *J* = 14 Hz, H_a-1′), 2.44 (1H, *dd*, *J* = 14, *ca* 10 Hz, H_b-1′) 1.28 (6H, *s*, *gem*-dimethyls), 4.65 (1H, *d*, *J* = 7.3 Hz, anomeric H, overlapping DOH shifted by add. of CF_3COOD), 4.0–3.1 (7H, *m*, other sugar protons and H-2′). FD–MS, *m/e* 427 [M^+ + 1]. EI–MS, *m/e* (rel. int.). 264 [M^+ aglycon] (27), 246 [M_a^+-H_2O] (32), 228 [M_a^+-$2H_2O$] (9), 213 (10), 206 (9), 188 (31), 187 (33), 176 (92), 175 (100), 163 (26), 147 (18). Upon acid hydrolysis (0.25 N HCl, 2 hr, 100°) D-glucose and **5** were detected and **4** isolated (UV, IR, [α], mp)

(R)-*Peucedanol 7-O-β-D-glucopyranoside* (**3**). 55 mg mp 205–205.5° (EtOH). (Found: C, 56.30; H, 6.15. $C_{20}H_{26}O_{10}$ requires C, 56.33; H 6.07%). $[\alpha]_D^{24}$ − 12.3° and $[\alpha]_{436}^{24}$ + 4.0° (MeOH, *c* 1.0). λ_{max}^{MeOH} nm (log ε). 326 (4.10), 294 (3.91), 251, (3.48), 222 (4.27), no shift with NaOAc. IR ν_{max}^{KBr} cm^{-1} *ca* 3400 (OH), 1711 (C=O), 1627 (C=C), 1569 and 1500 (aromatic). PMR (δ in D_2O) δ 7.58 (1H, *d*, *J* = 9.5 Hz, H-4) 7.12 (1H, *s*, H-5) 6.82 (1H, *s*, H-8), 6.03 (1H, *d*, *J* = 9.5 Hz, H-3), 2.93 (1H, *bd*, *J* = 13.5 Hz, H_a-1′), 2.27 (1H, *dd*, *J* = 13.5, *ca* 10 Hz, H_b-1′), 1.23 (6H, *s*, *gem*-dimethyls),

4.99 (1H, *bd*, $W_{\frac{1}{2}}$ 9 Hz, anomeric H), 4.1–3.4 (7H, *m*, other sugar protons and H-2'). FD–MS, *m/e*. 427 [$M^+ + 1$]. EI–MS, *m/e* (rel. int.): 264 [$M^+_{aglycon}$] (36), 246 [M_a^+-H_2O] (8), 213 (3), 206 (21), 189 (11), 188 (8), 187 (10), 176 (100), 175 (90), 163 (30), 147 (13). Upon acid hydrolysis (0.25 N HCl, 2 hr, 100°) D-glucose and **5** were detected and **4** isolated (UV, IR, [α], mp).

Apterin (**7**). 47 mg (EtOH), ill-def. mp $[\alpha]_D^{24}$ + 213° (MeOH; *c* 0.4), $[\alpha]_D^{24}$ + 228° (H_2O; *c* 0.1) (lit. [10] $[\alpha]_D^{25}$ − 229° (H_2O; *c* 0.9)). λ_{max}^{MeOH} nm (log ε). 324 (4.14), 259.5 (3.38), 249 (3.40), 218 (4.15). D-Glucose was the only sugar detected upon acid hydrolysis. Except for an upfield shift (0.50 ppm) of all signals, the PMR-spectrum (D_2O) was as reported [10] (the high δ-values reported presumably are with external (capillary) TMS as reference, instead of internal standard). Identity was established by comparison with authentic apterin by IR-spectroscopy and by TLC. The authentic sample in our hands showed $[\alpha]_D^{24}$ + 227° (H_2O; *c* 0.3) opposite to the reported value.

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