Journal of Natural Products
Vol. 51, No. 1, pp. 136-141, Jan-Feb 1988

NATIVE AMERICAN FOOD AND MEDICINAL PLANTS, 8. WATER-SOLUBLE CONSTITUENTS OF *LOMATIUM DISSECTUM*[1]

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ABSTRACT.—A continuing investigation of the umbellifer *Lomatium dissectum* has resulted in the isolation of a known flavonoid [**1**] and three coumarin glycosides [**2–4**], two of which are previously unreported. One of these new compounds [**4**] contains apiose, a sugar uncommon in the coumarins. The ichthyotoxicity of the plant extracts has been traced to the tetronic acids isolated earlier in this study.

We recently reported (1) the isolation and identification of a mixture of tetronic acids responsible for the antimicrobial activity in the CH_2Cl_2-soluble extracts of the umbellifer *Lomatium dissectum* Nutt. When our antimicrobial screens also indicated inhibition of bacterial and fungal growth by the H_2O-soluble extracts, we initiated a search for the constituent(s) responsible. Our efforts have resulted in the isolation of a flavonoid glycoside and three coumarin glycosides, the subjects of this report.

Initial fractionation of the H_2O-soluble extracts was accomplished by low pressure, gradient elution reversed-phase (C_{18}) chromatography. The antimicrobial activity was found in a large fraction eluted with MeOH-H_2O (3:2). Gel permeation chromatography of that fraction through Sephadex LH-20 cleanly separated the active minor constituent from a substantial uv-active fraction.

Inspection of the ^{1}H-nmr spectrum of the antimicrobial compound revealed six aromatic/olefinic protons, along with signals typical of a sugar residue. Decoupling experiments indicated 1,2,4-trisubstituted and 1,2,3,5-tetrasubstituted benzene rings and an isolated hydrogen on an sp^2 carbon. These partial structures and uv absorptions at 349 and 253 nm suggested a flavonoid. The molecular formula, $C_{21}H_{20}O_{12}$, was obtained from fabms and ^{13}C-nmr data. The aromatic substitution patterns and characteristic shifts in the uv spectra upon addition of NaOMe, NaOAc, NaOAc/H_3BO_3, $AlCl_3$, and $AlCl_3$/HCl (2) confirmed that the molecule was a flavonoid and indicated that this antimicrobial constituent was 7-*O*-β-D-glucosyl luteolin [**1**]. The ^{13}C-nmr resonances were in accord with literature values (3).

When our attention was directed to a report (4) linking the reputed ichthyotoxicity of *L. dissectum* (5) to coumarin glycosides, we continued separation studies on the residual uv-active material from the flavonoid isolation. Although we were able to isolate three coumarin glycosides by reversed-phase (C_{18}) hplc, the resolution was not sufficient to permit scale up, even to reasonable semipreparative levels. However, application of centrifugal countercurrent chromatography (cccc) (6) resulted in excellent resolution of three coumarin glycosides, **2–4**; preparative separations of up to 400 mg of a mixture of the three compounds were possible (Figure 1).

The ^{13}C-nmr spectra (Table 1) indicated that the three compounds shared the same aglycone. In each compound, two pairs of *ortho*-coupled aromatic protons in the ^{1}H-nmr, uv absorptions near 342, 258, and 248 nm and an ir absorption at 1745 cm^{-1} were reminiscent of the khellactone coumarins we had encountered earlier in our work on *Musineon divaricatum* (7), but the signals for the modified prenyl substituents were different. The three spin system at δ 4.67 (1H, t) and 3.28 (2H, d) did not fit the dihydropyran system found in the khellactones but could be more readily accommodated

[1]For Part 7, see VanWagenen and Cardellina (1).

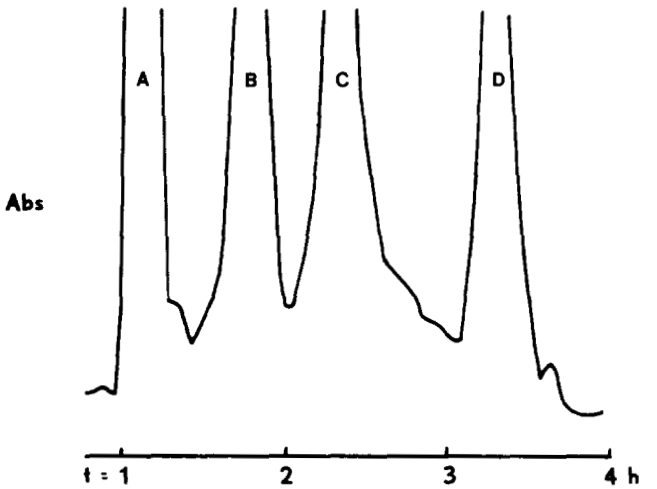
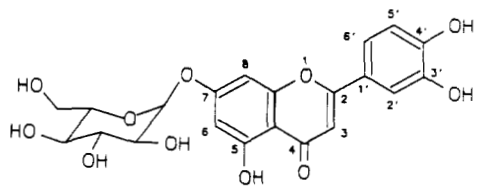


FIGURE 1. High speed countercurrent chromatogram with a 440-mg mixture applied to column; solvent system $CHCl_3$-MeOH-H_2O (13:23:16); flow rate 4 ml/min; mobile phase lower; detection 254 nm. Peak A, **2** (163 mg); B, unidentified (15 mg); C, **4** (55 mg); D, **3** (108 mg).

by a dihydrofuran system. The methyl singlets near δ 1.30 had to be attached to an sp^3 carbon bearing oxygen. These data suggested structure **5** for the aglycone; this was confirmed by hydrolysis of **2–4** to give columbianetin, **5**, identical in all respects to the coumarin obtained previously from the hydrolysis of columbianin (8).

With the aglycone of **2–4** assigned, inspection of the ^{13}C-nmr data revealed that the compounds differed in both number and type of sugar residues (Table 2). Compound **2**, with four pairs of carbon resonances (δ 75.9 and 74.6; 75.7, 2C; 73.2, 2C;



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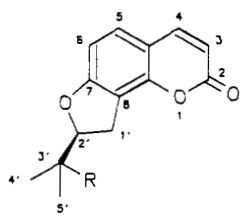
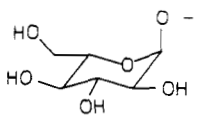
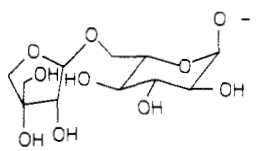
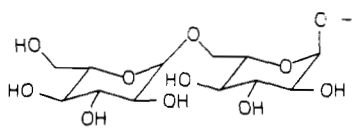


2 R= 

4 R= 

3 R= 

5 R=OH

TABLE 1. ^{13}C-nmr Data for Aglycone Portions of **2–4**[a] and of the Aglycone **5**.[b]

Carbon	Compound			
	2	**3**	**4**	**5**
2	163.9	163.7	164.3	163.7
3	110.7	110.7	110.8	112.2
4	146.2	146.0	146.6	143.9
4a	113.3	113.2	113.6	113.1
5	129.6	129.5	129.6	128.7
6	107.2	107.2	107.2	106.6
7	163.3	163.2	163.5	161.0
8	113.7	113.5	114.5	114.0
8a	150.3	150.1	150.6	151.3
1′	27.0	27.0	26.9	27.5
2′	90.8	90.8	91.3	91.3
3′	79.1	79.1	79.3	71.8
4′/5′	22.1/21.6	22.0/21.3	23.3/22.4	26.0/24.0

[a]In D_2O.
[b]In $CDCl_3$.

69.6 and 69.4) with similar chemical shifts to **3** (δ 75.8, 75.6, 73.3, and 69.7), suggested that it was simply a disaccharide composed of the same sugar residue as **3**. Prominent peaks in the fabms for **2** at *m/z* 409, 247, and 229, indicating losses of $C_6H_{12}O_5$, $C_{12}H_{20}O_{10}$, and $C_{12}H_{22}O_{11}$ from the molecular ion at *m/z* 571 ($C_{26}H_{34}O_{14}+H$) also pointed to the disaccharide nature of **2**. The carbon resonances of both **2** and **3** were, for the most part, in agreement with literature values (9) for β-D-glucosyl residues, but the chemical shift of the acetal carbon linked to the aglycone in both cases (δ 97.1) seemed anomalous. The upfield shifts from the expected values would seem to be due to shielding by the γ-carbons on the tertiary alkoxy group (10). Indeed, hydrolysis of **3**, the most abundant compound, with methanolic HCl gave α-methyl-D-glucose, identical in all respects with an authentic sample. Coumarin **2**, then, was columbianin (11) and **3**, the previously unknown monoglucoside.

TABLE 2. ^{13}C-nmr Data for Carbohydrate Residues of **2–4**, and **6**.

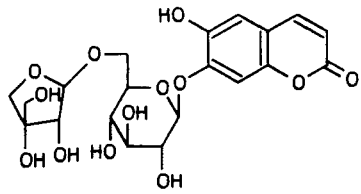
Carbon	Compound					
	2[a]	**3**[a]	**4**[a]	**3**[b]	**4**[b]	**6**[b,c]
1″	97.1	97.1	97.6	97.4	97.4	100.8
2″	73.2	73.3	73.4	73.5	73.3	73.4
3″	75.7	75.6	75.6	76.6	75.3	75.4
4″	69.6	69.7	69.0	70.1	70.0	69.9
5″	75.9	75.8	74.1	76.9	75.1	76.1
6″	68.4	60.6	66.2	60.9	67.3	63.5[d]
1‴	103.0		108.7		109.1	109.3
2‴	73.2		76.6		76.8	75.7
3‴	74.6		78.5		76.8	78.7
4‴	69.4		73.5		73.5	73.0
5‴	75.7		63.8		63.3	67.5[d]
6‴	60.8					

[a]In D_2O.
[b]In DMSO-d_6.
[c]From Forgacs *et al.* (12).
[d]Should be reversed.

The ^{13}C-nmr spectrum of **4** was distinguished from **2** and **3** in that resonances for only eleven sugar carbons were observed. In the ^{1}H nmr, a pair of vicinal methines (δ 4.47 and 3.59, each 1H, d, J=3.2) and two isolated methylene groups suggested a pentose branched at C-3; apiose is the known sugar of this type. A ^{1}H-^{13}C correlation experiment permitted assignment of the carbons of the pentose residue (Table 2), and the data matched those previously reported for apiose (12). A 10.4% nOe between the branching methylene (C-5″) and the (C-2″) methine and the magnitude of the H-1″ to H-2″ coupling confirmed that this sugar was, indeed, a β-linked D-apiose. The remaining glycosyl carbon signals were appropriate for a 6-linked β-D-glucosyl unit and matched data reported for diospyroside [**6**] from *Diospyros sapota* (12). The structure of **4** was, therefore, 6′-(β-D-apiosyl)-β-D-glucosyl-columbianetin, a novel coumarin glycoside. Mild hydrolysis of **4** gave D-apiose, $[\alpha]_D$ +7.0°, with a positive Sandermann test (13).

An interesting anomaly in the ^{1}H nmr (D_2O) of **4** was a broad doublet at δ 2.20; ^{1}H-^{13}C and ^{1}H-^{1}H correlation experiments revealed that this signal represented one of the protons on C-6 of the glucose unit. Apparently, the preferred conformation in D_2O provides for substantial anisotropic shielding of this proton; in pyridine-d_5, the signal appears in the more typical δ 3.5–3.8 range.

Apiose is fairly widespread in the plant kingdom (14). Primarily found in polysaccharides, it has been reported in just over a dozen secondary metabolites, mostly flavonoid and phenolic glycosides. Diospyroside [**6**] (12) and adicardin (15) appear to be the only other apiose-containing coumarin glycosides.



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The flavonoid **1** is largely responsible for the modest antimicrobial activity observed in the water-soluble extracts; 0.5 mg/disk elicited a 3-mm zone of inhibition toward *Xanthomonas campestris*, a Gram-negative plant pathogen. Some additional activity was observed in a still smaller fraction comprised of a complex mixture of other flavonoid glycosides.

Despite intimations that coumarins would be responsible for the ichthyotoxicity of extracts of *L. dissectum* (4), we found no toxicity to McBride cutthroat trout or goldfish in either the crude water solubles at 1 g/liter or in any of the coumarin glycosides at 10 mg/liter. We did find, however, that the tetronic acids we isolated earlier from the organic solubles (1) were quite toxic to the trout; an LD_{50} of 0.6 mg/liter was determined.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained with a Bruker WM-250 spectrometer with $CDCl_3$ or D_2O as solvent and internal standard for ^{1}H-nmr spectra and D_2O or DMSO-d_6 with TMS as external standard for ^{13}C-nmr spectra. Mass spectral analyses were performed on VG Instruments MM16F and 7070 EHF mass spectrometers. Ir spectra were recorded with a Nicolet 5DX spectrophotometer; uv spectra were determined with a Varian G34 spectrophotometer. All centrifugal countercurrent chromatography was done with a P.C. Inc. Multi-Layer Coil Separator-Extractor, using a semi-preparative 255-ml-capacity column (1.6 mm i.d. × 130 m).

SEPARATION OF THE CONSTITUENTS OF *L. DISSECTUM*.—The collection, extraction, and initial partitioning of *L. dissectum* have been described (1). A 3.65-g portion of the crude H_2O solubles was applied to a bed (2.5 × 30 cm) of reversed-phase silica (C_{18}) and eluted under pressure (12 psi N_2) with an H_2O/MeOH gradient. A 225-mg portion of fraction C (715 mg), eluted with H_2O-MeOH (3:2), was permeated through Sephadex LH-20 (2.5 × 38 cm) with MeOH-H_2O (4:1) to afford two fractions. Fraction B, 13 mg, was 7-*O*-β-D-glucosyl luteolin [**1**]. A 30-mg portion of fraction A (211 mg) was subjected to cccc using H_2O-MeOH-$CHCl_3$ (16:23:13), with the lower phase as the mobile phase. The flow rate of the mobile phase and the spinning rate of the instrument were maintained at 4 ml/min and 900 rpm, respectively, throughout the run. Three compounds eluted from the stationary phase: **2** (8 mg), **3** (15 mg), and **4** (3 mg).

CHARACTERIZATION OF 7-*O*-β-D-GLUCOSYL LUTEOLIN [**1**].—Fine needles from dilute EtOH, mp 240–241°, $[\alpha]_D$ −58° (*c* = 1.5, DMSO); λ max (MeOH) 347 nm (ε 14,800), 263sh (12,600) 253 (13,800); (NaOMe) 393, 296sh, 262; (NaOAc): 402, 365, 266sh, 258; (NaOAc/H_3BO_3) 372, 256; ($AlCl_3$) 425, 328, 296sh, 272; ($AlCl_3$/HCl) 384, 363, 294sh, 270; fabms *m/z* (rel. int.) $[MH]^+$ 449 (7), 269 (64); ^1H nmr (CD_3OD) δ 7.29 (1H, dd, *J* = 8.5, ca. 0.5), 7.28 (1H, d, ca. 0.5), 6.78 (1H, d, 8.5), 6.60 (1H, d, 1.5), 6.47 (1H, s), 6.37 (1H, d, 1.5), 4.95 (1H, d, 1.5), 3.83 (1H, m), 3.65–3.18 (6H, overlapping m); ^{13}C nmr (DMSO-d_6) δ 181.9 (C-4), 164.5 (C-2), 163.0 (C-7 or C-9), 161.2 (C-7 or C-9), 156.8 (C-5), 150.1 (C-4′), 145.8 (C-3′), 121.3 (C-1′), 119.2 (C-6′), 116.0 (C-5′), 113.5 (C-2′), 105.4 (C-10), 103.9 (C-3), 99.9 (C-8 or C-1″), 99.6 (C-8 or C-1″), 94.8 (C-6), 77.2 (C-3″ or C-5″), 76.4 (C-3″ or C-5″), 73.7 (C-2″), 69.6 (C-4″), 60.6 (C-6″).

COLUMBIANIN [**2**].—Prisms from hot MeOH, mp 273–275, $[\alpha]_D$ +118° (*c* = 1.0, H_2O); λ max (H_2O) 327 nm (ε 14,000), 259 (4000), 250 (3400); fabms *m/z* (rel. int.) $[MH]^+$ 571 (86), 409 (82), 247 (100), 229 (55); ^1H nmr (D_2O): δ 7.60 (1H, d, *J* = 9.5), 7.14 (1H, d, 8.4), 6.56 (1H, d, 8.4), 5.94 (1H, d, 9.5), 474 (1H, t, 9.4), 4.03 (1H, d, 7.7), 3.63 (1H, d, 11.9), 3.44 (2H, br d, 11.5), 3.25–2.90 (11H, overlapping m), 1.19 (3H, s), 1.06 (3H, s); ^{13}C nmr (D_2O) see Tables 1 and 2.

β-D-GLUCOSYL-COLUMBIANETIN [**3**].—An amorphous white solid, mp 114–118°, $[\alpha]_D$ +210° (*c* = 1.0, H_2O); λ max (H_2O) 327 nm (ε 21,900), 259 (6000), 250 (5000); fabms *m/z* (rel. int.) $[MH]^+$ 409 (30), 247 (100), 229 (100); ν max ($CHCl_3$) 3420, 2920, 1733, 1619 cm^{-1}; ^1H nmr (D_2O) δ 7.52 (1H, d, *J* = 9.5), 7.07 (1H, d, 8.4), 6.51 (1H, d, 8.4), 5.87 (1H, d, 9.5), 4.67 (1H, t, 9.2), 4.51 (1H, d, 7.7), 3.33–2.91 (8H, overlapping m), 1.16 (3H, s), 1.05 (3H, s); ^{13}C nmr see Tables 1 and 2.

β-D-GLUCOSYL-6′-(β-D-APIOSYL) COLUMBIANETIN [**4**].—A white solid, mp 112–114°; $[\alpha]_D$ +75° (*c* = 1.0, H_2O); λ max (H_2O) 327 nm (ε 15,100), 259 (4500), 250 (3800); fabms *m/z* (rel. int.): $[MH]^+$ 541 (55), 409 (72), 247 (100), 229 (100); ^1H nmr (D_2O): δ 7.70 (1H, d, *J* = 9.5), 7.21 (1H, d, 8.4), 6.60 (1H, d, 8.4), 6.03 (1H, d, 9.5), 4.71 (1H, t, 9.8), 4.56 (1H, d, 8.0), 4.47 (1H, d, 3.2), 3.59 (1H, d, 3.2), 3.54 (1H, d, 10.2), 3.46 (1H, d, 10.2), 3.33 (2H, s), 3.22–3.18 (2H, overlapping m), 3.08 (2H, d, 9.8), 3.00 (1H, d, *J* = 10.2), 2.93 (2H, overlapping m), 2.20 (1H, d, 10.2), 1.29 (3H, s), 1.09 (3H, s); ^{13}C nmr see Tables 1 and 2.

HYDROLYSIS OF **3**.—A solution of 60 mg of **3** in 10 ml of 10% methanolic HCl was refluxed for 4 h, after which time the solvent was removed in vacuo to give an amorphous white solid. This material was partitioned between 35 ml H_2O and 4 × 35 ml $CHCl_3$. The $CHCl_3$-soluble component (37 mg) was purified by permeation through Bio-Beads S-X8 (137 × 2.5 cm) with CH_2Cl_2-cyclohexane (3:2), which afforded 34 mg of a colorless solid. Recrystallization of this material from EtOAc gave **5**, large prisms, mp 162–163 K; $[\alpha]_D$ +151° (*c* = 1.0, $CHCl_3$); λ max (EtOH) 329 nm (ε 14,100), 263 (6000), 254 (5000), 219 (15,000); eims *m/z* (rel. int.) 246 (48), 213 (14), 189 (15), 188 (88), 187 (100), 175 (15), 160 (27); hrms *m/z* 246.0889 (calcd for $C_{14}H_{14}O_4$, 246.0892); ^1H nmr ($CDCl_3$) δ 7.59 (1H, d, *J* = 9.5), 7.22 (1H, d, 8.4), 6.71 (1H, d, 8.4), 6.16 (1H, d, 9.5), 4.76 (1H, t, 9.0), 3.28 (2H, d, 9.0), 1.73 (1H, br s) 1.33 (3H, s), 1.21 (3H, s); ^{13}C nmr ($CDCl_3$) see Table 1. The aqueous phase was lyophilized to give α-methyl-D-glucose as a glassy solid. Recrystallization gave fine crystals, mp 143–144°, $[\alpha]_D$ +95° (*c* = 1.0, H_2O); ^{13}C nmr (D_2O) δ 99.4, 73.2, 71.7, 71.3, 69.7, 60.7, 55.1. These data were identical with those obtained for authentic α-methyl-D-glucose (see below).

PREPARATION OF α-METHYL-D-GLUCOSE.—D-Glucose (100 mg) was refluxed in the same manner as **3** above, in 10 ml of 10% methanolic HCl for 4 h. Removal of solvent in vacuo and recrystallization from hot H_2O afforded needles, mp 144–145, $[\alpha]_D$ +99° (*c* = 1.0, H_2O); ^{13}C nmr (D_2O) δ 99.4, 73.3, 71.7, 71.4, 69.8, 60.8, 55.2.

ACETYLATION OF **3**.—To 15 mg of **3** dissolved in 2 ml pyridine was added 1 ml Ac_2O and a few crystals of DMAP. This solution was stirred at 85° for 4 h. The resulting mixture was evaporated in vacuo and the resulting tar was washed through a small bed of silica with 250 ml CH_2Cl_2-hexane (1:1). The eluate

was evaporated to give a light brown solid (23 mg). Several recrystallizations from hexane/EtOAc gave **6**, fine needles, mp 163–164°, $[\alpha]_D$ +145° (c=1.0, $CHCl_3$); eims *m/z* (rel. int.) 576 (12), 331 (27), 229 (66), 213 (22), 187 (54); hrms *m/z* 576.1838 (calcd for $C_{28}H_{32}O_{13}$, 576.1842); ^1H nmr ($CDCl_3$) δ 7.60 (1H, d, J=9.5), 7.23 (1H, d, 8.2), 6.69 (1H, d, 8.2), 6.18 (1H, d, 9.5), 5.17 (1H, t, 9.4), 4.99 (1H, t, 9.4), 4.89 (1H, t, 8.0), 4.81 (1H, t, 8.1), 4.74 (1H, t, 9.2), 3.98 (1H, dd, 10.1, 5.6), 3.58 (1H, d, 10.1), 3.49 (1H, m), 3.28 (2H br d, 9.2), 2.01 (3H, s), 1.99 (3H, s), 1.97 (3H, s), 1.96 (3H, s), 1.34 (3H, s), 1.26 (3H, s).

HYDROLYSIS OF **4**.—A solution of 70 mg of **4** in 10 ml of 1% HCl was stirred at 80° for 1 h until tlc (Si gel, $CHCl_3$-MeOH, 3:1) indicated formation of the aglycone **5** (**5**: R_f 0.85; **4**: R_f 0.45). This solution was immediately chromatographed through Bio-Gel SM-2 (22×2.5 cm) with an H_2O/MeOH gradient. Fraction 1 was D-apiose; $[\alpha]_D$ +7° (c=1.0, H_2O); λ max ($H_2O+H_2SO_4$) 276.5 nm, λ max (H_2SO_4+fructose) 449 nm, λ max (H_2SO_4+fructose+cysteine) 463 nm.

ACKNOWLEDGMENTS

We thank Dr. Stephen Grode of Upjohn for conducting the 2D-nmr experiments, Mr. Patrick Plantenburg for identifying the plant specimens, Ms. Robyn Klein for bringing reference 4 to our attention, and Charlie Smith and William Dwyer of the Bozeman Fish Hatchery for providing the trout for the assays. This work was supported by a Minorities Biomedical Research Support Grant from the National Institutes of Health.

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Received 18 September 1987