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PHENOLIC CONSTITUENTS FROM *ONOSMA HETEROPHYLLA*

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ABSTRACT.—Two new phenolic compounds, together with apigenin, luteolin, chrysoeriol, and quercetin, have been isolated from the aerial parts of *Onosma heterophylla* (Boraginaceae). These compounds were characterized by chemical and spectral data as 1-methyl-2-phenylethyl isoferulate [**1**] and 1-methyl-3-(3′-methoxy-4′-hydroxyphenyl)-propyl caffeate [**2**].

Onosma heterophylla Griseb. (Boraginaceae) is a shrub that grows wild in northern Greece. The Boraginaceae family is known to yield alkannin and its esters, which display the ability to regenerate necrotic tissue and are regarded as a new class of drugs (1,2).

In our previous papers the isolation, from the roots of *O. heterophylla*, of isohexenylnaphthazarins (3), lipids (4), and pyrrolizidine alkaloids was reported (5). From the aerial parts, two new phenolic compounds **1** and **2**, along with apigenin, luteolin, chrysoeriol, and quercetin, were isolated. This paper describes the isolation and structural elucidation of these new compounds.

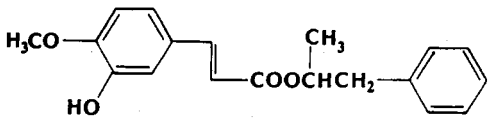
RESULTS AND DISCUSSION

Aerial parts of the plant (which previously had been extracted first with petroleum ether and then with CH_2Cl_2) were extracted with MeOH. The MeOH residue was dissolved in H_2O, and the solution was partitioned with Et_2O, EtOAc, and *n*-BuOH. The Et_2O residue was chromatographed on polyamide and Sephadex LH-20 columns to afford four known flavonoids, apigenin, luteolin, chrysoeriol, and quercetin, as well as compounds **1** and **2**. The known flavonoids were identified by comparing their mp's, uv-vis [standard shift reagents (6)], and ms data (7).

Compound **1**, whose blue fluorescence became more intense with NH_3 fumes, showed an uv spectrum characteristic of a cinnamic ester with a phenylalkyl and alcohol, exhibiting an absorption maximum at 329 nm and a bathochromic shift to 371 nm with addition of MeONa (8,9). Absorption bands in the ir spectrum indicated the presence of -OH (3500 cm^{-1}) and C=O belonging to α,β-unsaturated ester (1720 and 1645 cm^{-1}).

Examination of the ^{1}H-nmr spectrum suggested that the most likely structure of this compound is **1** as illustrated, with two aromatic moieties, two *trans*-olefinic protons, and an aliphatic carbon chain. The ^{1}H-nmr spectrum showed an ABX system [δ 7.03 ppm (d, J=1.9 Hz, 1H), 6.98 (dd, J=1.9 Hz, 8 Hz, 1H)] and a one-proton doublet at δ 6.73 (J=8 Hz). The last proton was overlapped with a broad multiplet (δ 6.71–6.80) attributable to a monosubstituted benzene ring. Also, an AB system [δ 6.25 (d, J=16 Hz, 1H) and 7.47 (d, J=16 Hz, 1H)] was assigned to *trans*-olefinic protons. Additionally, a broad multiplet (δ 5.15, 1H) was due to the methine proton. A singlet (δ 3.67, 3H) was attributed to the MeO of the isoferuloyl moiety. Two doublet of doublets at δ 2.30 (J=6.5 Hz, J_{gem}=14.3 Hz, 1H, H_b) and at δ 2.66 (J=6 Hz, J_{gem}=14.3 Hz, 1H, H_a) were assigned to the benzyl protons. Finally, a doublet at δ 1.28 (J=6.7 Hz, 3H) was attributed to the Me group.

In the eims of **1**, two characteristic



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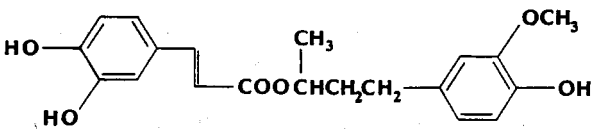
fragments were derived from the molecular ion (*m/z* 312) by a McLafferty rearrangment; these were observed at *m/z* 194 $[(MeO)(HO)C_6O_3CH{=}CHCOOH]^{+\cdot}$ and at *m/z* 118 $[MeCH{=}CHC_6H_5]^{+\cdot}$. Confirming the isoferuloyl structure were the fragments at *m/z* 194, 178 $[(MeO)(HO)C_6H_3CH_2CH\text{-}C{\equiv}O]^+$, 163 $[178-Me]^+$, 135 $[163-CO]^+$, and 55 (base peak). The base peak $[CH_2{=}CH\text{-}C{\equiv}O]^+$ likely arose from the *m/z* 178 fragment by elimination of the 3-hydroxy-4-methoxy-phenyl radical. Confirming the alcohol moiety of the molecule were fragments at *m/z* 136 $[HOCH(Me)CH_2C_6H_5]^+$, 118, 91, 77, and 45. The *m/z* 45 ion $[MeCH{=}OH]^+$ likely arose from the *m/z* 136 fragment by elimination of the benzyl radical. The *m/z* 91 (benzyl) and *m/z* 77 (phenyl) ions were derived from the *m/z* 118 fragment.

Compound **2** showed a blue fluorescence that became more intense with NH_3 fumes, similar to compound **1**. Additionally, the uv spectrum of **2** was similar to that of **1**. Absorption bands in the ir spectrum indicated the presence of an OH (3550 cm^{-1}) and a C=0 belonging to α,β-unsaturated ester (1720 and 1640 cm^{-1}).

The ^{1}H-nmr spectrum of **2** showed two ABX systems: [δ 7.05 (d, J=2 Hz, 1H), δ 6.98 (dd, J=2 Hz, 8 Hz, 1H), δ 6.74 (d, J=8 Hz, 1H) and δ 6.64 (d, J=2 Hz, 1H) overlapping with a doublet (1H) of J=8 Hz at δ 6.63; δ 6.47 (dd, J=2 Hz, 8 Hz, 1H)]. The first ABX system was attributed to a caffeoyl moiety and the second to a 1,3,4-trisubstituted benzene. Also, an AB system [δ 6.23 (d, J=16.1 Hz, 1H) and δ 7.47 (d, J=16.1 Hz, 1H)] was assigned to trans-olefinic protons. Additionally, a broad multiplet (δ 5.06, 1H) due to the methine proton and a singlet (δ 3.62, 3H) were attributed to the MeO attached to the second benzene ring. Two triplets [δ 2.28 (1H, H_b, J_{ab}=14.1 Hz) and δ 2.65 (1H, H_a, J_{ax_2}=6.4 Hz)] were assigned to the benzyl protons, and a broad multiplet (δ 1.98–2.05, 2H) was attributed to the methylene group adjacent to the benzyl group. Finally, a doublet at δ1.26 (3H, J=6.8 Hz) was attributed to the Me group.

In the eims of **2**, two characteristic fragments arose from the molecular ion (*m/z* 358) by a McLafferty rearrangement; these were at *m/z* 180 $[(HO)(OH)C_6H_3CH{=}CHCOOH]^{+\cdot}$ and *m/z* 178 $[MeCH{=}CHCH_2C_6H_3(OMe)(OH)]^{+\cdot}$. Confirming the caffeoyl structure were fragments at *m/z* 180, 164 $[(HO)(HO)C_6H_3CH_2\text{-}CH\text{-}C{\equiv}O]^+$, 135 $[163-CO]^+$, 109 $[135-C_2H_2]^+$, and 55 $[CH_2{=}CH\text{-}C{\equiv}O]^+$ (base peak). Confirming the alcohol moiety of the molecule were fragments at *m/z* 196 $[HOCH(Me)CH_2CH_2C_6H_3(OMe)(OH)]^+$, 181 $[196-Me]^+$, 178, 137 $[CH_2C_6H_3(OMe)(OH)]^+$, and 123 $[C_6H_3(OMe)(OH)]^+$. The fragments *m/z* 149 $[178-COH]^+$ and 107 $(137-CH_2O)^+$ were indicative of the *p*-OH group in the benzene ring of the phenylalkylalcohol.

Acidic hydrolysis (3 N HCl) of **1** and **2** did not yield any products, as was evidenced by chromatographic controls of aqueous solutions (for sugars). On the



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other hand, mild alkaline hydrolysis (2 N NaOH) of these compounds yielded isoferulic and caffeic acid, which were chromatographically identified (10,11). These findings, in combination with the above mentioned spectral data, are in accordance with the proposed structures of **1** and **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined using a Kofler hot stage instrument and are uncorrected. Uv-vis spectra were measured on a Hitachi U-2000 spectrophotometer. Ir spectra were recorded on a Perkin-Elmer 567 spectrophotometer. ^{1}H-nmr spectra were obtained with a Brüker AMX-400 instrument using TMS as internal standard. Eims were obtained with a VG-305 instrument at ionizing voltage of 70 eV. Tlc was performed on Cellulose plates (Merck) using various developing solvents.

PLANT MATERIAL.—The plant material is the same as described in a previous paper (3).

EXTRACTION AND ISOLATION.—Air-dried aerial parts of the plant (400 g) were extracted (Soxhlet) first with petroleum ether and then with CH_2Cl_2 to remove lipids and chlorophylls. The remaining plant material was extracted thoroughly with MeOH and, after evaporation of the solvent, a residue (20 g) remained. This residue was redissolved in 500 ml of boiling H_2O and filtered, and the aqueous filtrate was partitioned with Et_2O (6×200 ml), EtOAc (6×200 ml), and *n*-BuOH (6×200 ml).

The yellowish Et_2O residue (1.2 g) was chromatographed on a 4×30 cm polyamide (MN-SC6) column using PhMe-MeCOEt-MeOH (progressive increase of MeCOEt and MeOH). Elution with PhMe-MeCOEt-MeOH (80:15:5) afforded **1**. Elution with PhMe-MeCOEt-MeOH (65:20:15) afforded a mixture of **2** and apigenin, which were separated on a 1×50 cm Sephadex LH-20 (MeOH) column.

Elution with PhMe-MeCOEt-MeOH (55:20:25) afforded a mixture of chrysoeriol and luteolin, which were then separated on a 1×50 cm Sephadex LH-20 (MeOH) column. Chrysoeriol was eluted first followed by luteolin; subsequent elution with PhMe-MeCOEt-MeOH (40:20:40) afforded quercetin.

All the above-mentioned compounds were purified on Sephadex LH-20 (MeOH) columns and identified by comparisons of spectral and chromatographic data.

1-Methyl-2-phenylethyl isoferulate [**1**].—Compound **1** was isolated as a yellowish viscous solid (8 mg) that could not be induced to crystallize. Tlc was performed on cellulose with: (A) $CHCl_3$-HOAc-H_2O (100:90:10) R_f 0.73; (B) EtOAc-HOAc-H_2O (80:20:40, organic phase) R_f 0.95; (C) HOAc-H_2O (15:85) R_f 0.12. Fluorescence: blue-blue (+NH_3). Uv λ max nm (MeOH) 329, 289, (MeOH+MeONa) 371, 309 sh; ir ν max (Nujol) cm^{-1} see text; ^{1}H nmr (400 MHz, DMSO-d_6) see text; eims *m/z* (%) $[M]^+$ 312 (0.5), 195 (1.8), 194 (13.3), 193 (2.9), 179 (1.2), 178 (8.6), 177 (1.5), 164 (3.5), 163 (20.8), 162 (3.6), 149 (11.6), 136 (6.9), 135 (9.2), 134 (8.7), 118 (5.6), 117 (26.6), 105 (13.8), 92 (4.4), 91 (20.1), 77 (32.0), 71 (30.8), 69 (45.7), 57 (81.5), 55 (100), 45 (47.7).

1-Methyl-3-(3'-methoxy-4'-hydroxyphenyl)-propyl caffeate [**2**].—Compound **2** was isolated as a yellowish viscous solid (9 mg) that also could not be induced to crystallize. Tlc was performed on cellulose with (A) $CHCl_3$-HOAc-H_2O (100:90:10) R_f 0.66; (B) EtOAc-HOAc-H_2O (80:20:40, organic phase) R_f 0.89; (C) HOAc-H_2O (15:85) R_f 0.08. Fluorescence: blue-blue (+NH_3). Uv λ max nm (MeOH) 330, 286, (MeOH+MeONa) 372, 310 sh, 264 sh; ir ν max (Nujol) cm^{-1} see text; ^{1}H nmr (400 MHz, DMSO-d_6) see text; eims *m/z* (%) $[M]^+$ 358 (0.6), 196 (1.6), 195 (1.0), 181 (1.7), 180 (3.8), 179 (1.7), 178 (5.0), 167 (7.7), 164 (4.5), 163 (4.9), 151 (5.5), 149 (28.4), 137 (12.6), 136 (5.4), 135 (5.8), 123 (18.3), 110 (7.5), 109 (13.4), 107 (70.0), 71 (41.3), 69 (44.7), 57 (83.0), 55 (100).

MILD ALKALINE HYDROLYSIS OF COMPOUNDS **1** AND **2**.—Hydrolysis was accomplished with NaOH (2 N) for 2 h at room temperature in the dark. After acidification of the hydrolysate, the hydrolysis products were extracted with Et_2O. The Et_2O residue was dissolved in a minimum quantity of MeOH, and the MeOH solution was subjected to tlc with standard procedures (10,11) and compared with authentic samples of isoferulic (Roth) and caffeic acid (Fluka). The two corresponding alcohols migrated as dark-blue spots near the front of the developing solvent.

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