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CHEMICAL CONSTITUENTS OF *BUXUS SEMPERVIRENS*

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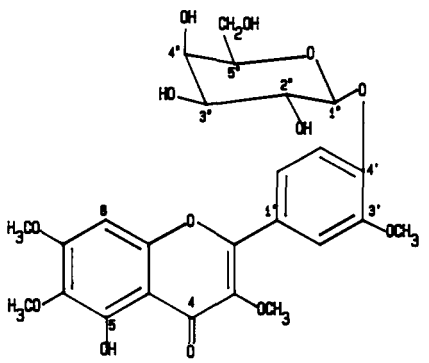
ABSTRACT.—A new flavonoid glycoside **1** is isolated from the leaves of *Buxus sempervirens*, and its structure is determined on the basis of spectral studies. It is the first flavonoid glycoside to be isolated from the genus *Buxus*. We also report the isolation of methyl syringate from this plant. The isolation and ^{13}C-nmr spectra of buxpsiine and cyclomicrophylline A are also described.

Buxus sempervirens L. (Buxaceae) is widely distributed in Eurasia. The H_2O extracts of the plant find extensive use in the indigenous system of medicine for the treatment of many diseases (1). Previously, we have reported a number of new steroidal alkaloids and two known flavonoids isolated for the first time from the genus *Buxus* (2–5). In the present paper, we describe the isolation and structure elucidation of a new flavonoid glycoside, galactobuxin [**1**], along with methyl syringate [**2**]. The isolation and ^{13}C-nmr spectra of two known bases, buxpsiine [**3**] and cyclomicrophylline A [**4**], are also described.

RESULTS AND DISCUSSION

Galactobuxin [**1**] was isolated from the EtOH extract of the leaves of *B. sempervirens*. Uv spectra of the glycoside and aglycone showed a hypsochromic shift between band I of the glycoside (339 nm) and that of the aglycone (350 nm), indicating glycosylation at the 4′-hydroxyl group (6). Confirmation was secured by bathochromic shift of 61 nm for the aglycone in the presence of NaOMe; no such corresponding shift occurred for the glycoside. The presence of a 5-hydroxy-4-keto function was indicated by bathochromic shifts encountered both in the glycoside (30 nm) and in the aglycone (34 nm) when the uv spectrum was recorded with addition of $AlCl_3$ (6).

The ir spectrum afforded an absorption at 1650 cm^{-1}, indicating the presence of a conjugated carbonyl group. Other intense absorptions were observed at 3350 (OH), 1600 (C=C ar.), 1100 (C-O-C) cm^{-1}. Positive fabms showed the molecular ion peak at *m/z* 536.1527, corresponding to the molecular formula $C_{25}H_{28}O_{13}$ and indicating



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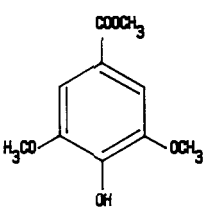

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twelve degrees of unsaturation in the molecule. A large peak in the eims at *m/z* 373 (75%) was due to the aglycone part of the molecule.

The ^{1}H-nmr spectrum of **1** had four 3H singlets at δ 3.73–3.92 for methoxyl groups. The anomeric proton of the sugar moiety appeared at δ 5.00 as a doublet indicating its α disposition (7) while other sugar protons resonated in the region δ 3.2–3.8. The signals at δ 4.52, 5.03, 5.06, and 5.29 were due to the hydroxyl protons of the sugar moiety. A one-proton singlet at δ 6.95 was assigned to H-8. The C-5′ proton appeared at δ 7.27 as a doublet while H-2′ appeared at δ 7.69 as another doublet. The C-6′ proton afforded a double doublet at δ 7.92. The 5-OH proton appeared at δ 12.35. These assignments were confirmed by ^{1}H^{1}H-COSY, long-range COSY, NOESY, and nOe difference measurements (8,9).

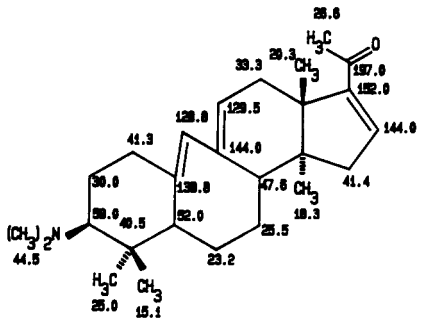
Irradiation of H-8 resulted in 14.6% nOe of the 7-OMe protons while a 4.2% nOe was observed in the reverse direction. Irradiation at the 3-OMe protons resulted in a 8.4% nOe of H-2′, while irradiation of H-2′ resulted in 7.3% nOe of the 3-OMe protons and 12.5% nOe of the 3′-OMe protons. Similarly irradiation of the 3′-OMe protons resulted in a 9.4% nOe of the H-2′ and 5.2% nOe of the H-5″ in compound **1**.

The ^{13}C-nmr spectrum (DMSO, 100 MHz) of **1** indicated the presence of 25 carbons in the molecule. The multiplicity assignments were made by carrying out the DEPT pulse sequence with the last polarization pulse angle θ = 45°, 90°, and 135° (9–11). The anomeric carbon resonated at δ 99.64, which indicated an α configuration for the anomeric proton and hence a β-glycoside linkage (12). The ^{13}C-nmr spectral assignments shown in Table 1 were made on the basis of a hetero COSY experiment and comparison with similar reported compounds (12,13).

The 2D direct ^{1}H/^{13}C chemical shift correlation (hetero COSY) experiment provided further insights into the structure. The hetero COSY spectrum showed a strong cross peak at δ 60.0/3.21 indicating that the protons resonating at δ 3.21 (H-6″) are attached to the carbon resonating at δ 60.0 (C-6″). Another strong peak linking the signal of the C-8 carbon with the signal at δ 6.95 (H-8) was observed, which confirmed the assignment of these signals. The anomeric carbon C-1″ was coupled with the proton at δ 5.00, which confirmed that the signal at δ 5.00 was due to H-1″. The C-2′, C-5′, and C-6′ carbons were coupled to the protons resonating at δ 7.69 (H-2′), 7.27 (H-5′), and 7.67 (H-6′). These interactions were further confirmed from the ^{1}H-detected (inverse) ^{1}H/^{13}C correlation experiment (HMBC) (14,15).

Acid hydrolysis of **1** gave a sugar which was identified as galactose by comparison with an authentic sample and by its ^{13}C-nmr spectrum (9). The aglycone obtained was identified by ^{1}H-nmr spectroscopy at 4′,5-dihydroxy-3,3′,6,7-tetramethoxyflavone, a compound reported by us previously (2).

The second compound was identified as methyl syringate [**2**] by comparison of its



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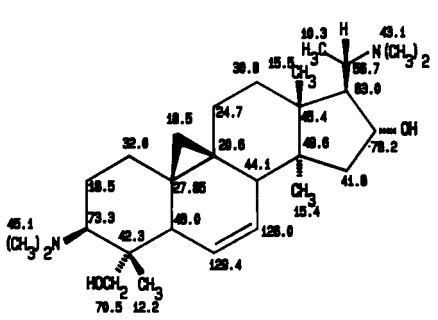

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TABLE 1. ^{13}C-nmr Chemical Shift Assignments of Galactobuxin [**1**].

Carbon	Chemical shift	Carbon	Chemical shift
C-2	151.73	C-1″	99.64
C-3	138.16	C-2″	73.10
C-4	178.22	C-3″	76.78
C-5	151.53	C-4″	69.60
C-6	132.25	C-5″	77.10
C-7	158.68	C-6″	60.00
C-8	91.47	3′-OMe	55.93
C-9	155.15	3-OMe	60.51
C-10	105.55	6-OMe	60.59
C-1′	123.23	7-OMe	56.43
C-2′	112.36		
C-3′	148.64		
C-4′	148.99		
C-5′	115.00		
C-6′	121.71		

data with those reported previously (16). This compound has been synthesized, but it has not been isolated previously from any source.

The third and fourth compounds were identified as buxpsiine [**3**] and cyclomicrophylline A [**4**] by comparison of their spectral data with those reported earlier (17,18). The compound **4** has been isolated from *B. sempervirens* for the first time.

EXPERIMENTAL

PLANT MATERIAL.—The leaves of *B. sempervirens* (dry wt 10 kg) were collected from the Beynam forest, Ankara, Turkey in September 1986. The plant was identified by Prof. Mehmet Koyuncu, Department of Pharmacognosy, Gazi University, and a voucher specimen (GUE 1243) was deposited in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara.

EXTRACTION AND ISOLATION.—The EtOH extract of the leaves of *B. sempervirens* was concentrated and extracted into 10% HOAc. Partial separation of the compounds was achieved by extraction into $CHCl_3$ at different pH values. The fraction obtained at pH 9 was chromatographed on a Si gel column with $CHCl_3$, then $CHCl_3$/MeOH mixtures.

Galactobuxin [**1**].—The fraction eluted with $CHCl_3$-MeOH (80:20) afforded galactobuxin [**1**] as an amorphous solid: $[\alpha]^{25}D$ −42 (c = 0.1, MeOH); uv λ max (MeOH) 339, 273, 253 sh; (MeOH + $AlCl_3$) 369, 283, 264; (MeOH + $AlCl_3$ + HCl) 366, 380, 264 nm; fabms 536.1527 (calcd 536.1530 for $C_{25}H_{28}O_{13}$); eims *m/z* (%) $[M]^+$ 373 (75), 358 (80), 340 (60), 149 (70), 73 (85), 69 (100); ^{1}H nmr (DMSO-d_6, 400 MHz) δ 3.2–3.8 (6H, m, sugar protons), 3.73 (3H, s, 3-OMe), 3.82 (3H, s, 3′-OMe), 3.87 (3H, s, 6-OMe), 3.92 (3H, s, 7-OMe), 4.52 (1H, m, 6″-OH), 5.00 (1H, d, $J_{1,2}$ = 7.4 Hz, H-1″), 5.03 (1H, bs, 4″-OH), 5.06 (1H, bs, 3″-OH), 5.29 (1H, bs, 2″-OH), 6.95 (1H, s, H-8), 7.27 (1H, d, $J_{5',6'}$ = 9.7 Hz, H-5′), 7.67 (1H, dd, $J_{6',5'}$ = 9.7 Hz, $J_{6',2'}$ = 2.3 Hz, H-6′), 7.69 (1H, d, $J_{2',6'}$ = 2.3 Hz, H-2′); ^{13}C nmr (DMSO-d_6, 100 MHz) see Table 1.

Acid hydrolysis of **1** (15 mg) was carried out with 10% HCl (25 ml) at 100° for 1 h. The sugar was identified as galactose by comparison with an authentic sample on tlc using EtOAc-HOAc-MeOH-H_2O (5:1.5:1.5:2). The aglycone was identified as 4′,5-dihydroxy-3,3′,6,7-tetramethoxyflavone by comparing its spectral data with those of the original compound. Uv λ max (MeOH) 350, 256, 252 sh nm; ir ν max ($CHCl_3$) 3400, 2915, 1650, 1600 cm^{-1}; ^{1}H-nmr (400 MHz, $CDCl_3$) δ 3.86 (3H, s, OMe), 3.95 (3H, s, OMe), 3.96 (3H, s, OMe), 3.98 (3H, s, OMe), 6.50 (1H, s, H-8), 7.05 (1H, d, J = 8.5 Hz, H-5′), 7.65 (1H, dd, $J_{5',6'}$ = 8.5 Hz, $J_{6',2'}$ = 2.1 Hz, H-6′), 7.70 (1H, d, $J_{2',6'}$ = 2.1 Hz, H-2′), 10.52 (1H, s, OH).

Methyl syringate [**2**].—The fraction obtained on elution with $CHCl_3$-MeOH (90:10) afforded compound **2** (10 mg, amorphous) upon preparative tlc (Si gl) in Me_2CO-hexane (4:1): uv λ max (MeOH) 215, 276 nm; ir ν max ($CHCl_3$) 3500 (OH), 1700 (C=O), 1610 (C-H ar.), 1335 (C-O-C); ^{1}H nmr (400 MHz, $CDCl_3$) δ 3.86 (3H, s, OMe), 3.89 (6H, s, 2 OMe), 5.89 (1H, s, OH), 7.32 (2H, s, aromatic protons); eims *m/z* (%) $[M]^+$ 212 (80), 197 (50).

Buxpsiine [**3**].—The fraction obtained on elution with $CHCl_3$-MeOH (85:15) afforded compound **3**, which was purified on preparative tlc (Si gel), in Me_2CO-hexane-diethylamine (5:20:1), to afford an amorphous solid (12 mg): $[\alpha]^{25}D$ +98 (c = 0.2, $CHCl_3$); uv λ max (MeOH) 239, 244 nm; ir ν max ($CHCl_3$) 1655, 2925 cm^{-1}; eims *m/z* (%) $[M]^+$ 381 (15), 84 (25), 71 (100), 58 (33); ^1H nmr (400 MHz, $CDCl_3$) δ 0.73 (3H, s, Me), 0.74 (3H, s, Me), 0.93 (3H, s, Me), 1.02 (3H, s, Me), 2.26 (3H, s, Me), 2.28 (6H, s, NMe), 5.58 (1H, m, H-11), 5.92 (1H, s, H-19), 6.66 (1H, m, H-16); ^{13}C nmr (100 MHz, $CDCl_3$) see structure **3**.

Cyclomicrophylline A [**4**].—The fraction obtained on elution with $CHCl_3$-MeOH (85:15) also afforded **4**, which was purified on preparative tlc (Si gel) in Me_2CO-hexane-diethylamine (5:25:1) to afford an amorphous solid (15 mg): $[\alpha]^{25}D$ −92° (c = 0.2, $CHCl_3$); ir ν max ($CHCl_3$) 3400 (OH), 1600 (C=C) cm^{-1}; ^1H-nmr (400 MHz, $CDCl_3$) δ 0.93 (3H, s, Me), 0.94 (3H, s, Me), 1.08 (3H, s, Me), 1.13 (3H, d, J = 6.4 Hz, Me-21), 0.14 (1H, d, J = 4.2 Hz, H-19α), 0.78 (1H, d, J = 4.2 Hz, H-19β), 2.38 (6H, s, NMe_2), 2.64 (6H, s, NMe_2), 3.56 (1H, d, J = 10.5 Hz, H-31α), 3.86 (1H, d, J = 10.6 Hz, H-31β), 4.52 (1H, m, H-16β), 5.42–5.50 (2H, m, H-6, H-7); eims *m/z* (%) $[M]^+$ 444.3716 (58), 373 (60), 149 (40), 72 (100).

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