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ALKALOIDS FROM THE LEAVES OF *BUXUS SEMPERVIRENS*

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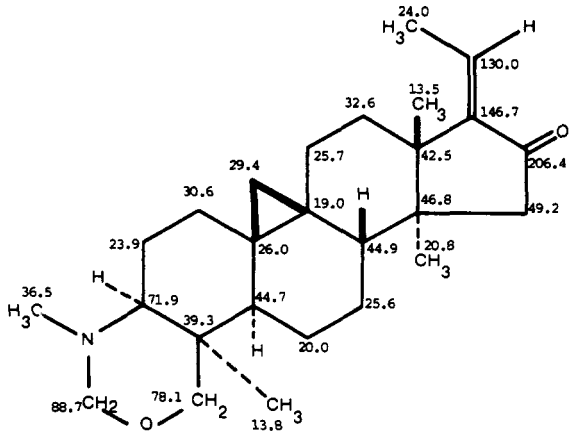
Buxus sempervirens L. (Buxaceae) is a shrub that occurs extensively in Eurasia. *Buxus* species extracts have been used in indigenous medicine for a variety of purposes, including as a febrifuge and for the relief of rheumatism and malaria (1). Previous work on *Buxus* species has resulted in the isolation of over 135 steroidal alkaloids. In continuation of our studies on various *Buxus* species (2–11), we now report the isolation and structure of a new alkaloid, (−)-sempervirone [**1**]. Two other bases isolated afforded spectral data consistent with the structures corresponding to (−)-cyclomicrobuxinine [**2**] (12) and (−)-cyclosuffrobuxinine M [**3**] (14). All these compounds have been isolated from the so-called weakly basic alkaloidal fraction of *B. sempervirens* leaves, extractable between pH 3.0 and 5.0.

(−)-Sempervirone [**1**], $C_{26}H_{39}NO_2$, $[\alpha]^{20}D$ −65 ($CHCl_3$) showed a uv maximum at 243 nm, characteristic of an α,β-unsaturated ketone. The ir spectrum exhibited strong absorptions at 1715 and 1632 cm^{-1} (α,β-unsaturated cyclopentanone) (14).

The ^{1}H-nmr spectrum (400 MHz, $CDCl_3$) displayed three 3-H singlets at δ 0.94, 1.16, and 1.32, corresponding to Me-32, Me-30, and Me-18, respectively. An AB quartet centered at δ 0.43 and 0.72 ($J_{19\alpha,19\beta}$ = 4.5 Hz) was assigned to α- and β-cyclopropyl methylene protons. A 3-H doublet at δ 1.83 (J = 7.5 Hz) was due to Me-21 coupled with the vinylic H-20, which in turn appeared as a quartet at δ 6.55 (J = 7.5 Hz) (15). Additionally, a 3-H singlet at δ 2.16 was attributed to the *N*-Me group. An AB quartet resonating at δ 3.16 and 3.77 ($J_{31\alpha,31\beta}$ = 10.6 Hz) was assigned to the C-31 methylenic protons, while another AB quartet at δ 3.63 and 4.46 ($J_{33\alpha,33\beta}$ = 7.5 Hz) was due to the C-33 methylenic protons of the tetrahyooxazine ring (3).

Two-dimensional ^{1}H-nmr measurements (COSY 45°, NOESY) (16) fully agreed with the proposed structure **1** for (−)-sempervirone. The COSY-45° spectrum established the coupling interactions between the geminal and vicinal protons. Thus, the COSY spectrum showed geminal interactions between C-19α and β protons as well as between C-31α and β methylenic protons. The NOESY spectrum served to show the relative stereochemistry at several key points in the molecule. Strong NOESY cross peaks were observed between the 18-methyl and 21-allylic methyl protons. This observation supported an *E* geometry for the enone system. The ^{13}C-nmr spectrum ($CDCl_3$, 100 MHz) of (−)-sempervirone [**1**] showed four signals at δ 13.45, 13.79, 20.82, and 23.96 which have been assigned to the methyl carbons C-18, C-30, C-32, and C-21, respectively. The C-31 methylene carbon appeared at δ 78.07, and the C-33 methylene carbon resonated at δ 88.66 due to the deshielding influence of the nitrogen and oxygen atoms, respectively. The assignments to the various carbons, confirmed by DEPT and GASPE experiments, are presented around structure **1**. The mass spectrum of compound **1** included the molecular ion at *m/z* 397.2982, in agreement with the molecular formula $C_{26}H_{39}NO_2$ (calcd 397.2981). The peak at *m/z* 382.2748 resulted from the loss of a



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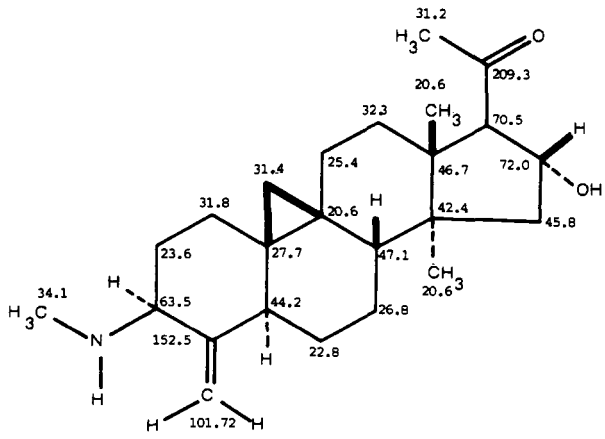
methyl group from the molecular ion. A considerably larger peak at *m/z* 127.1002 corresponded to the cleavage of ring A along with the attached tetrahydrooxazine ring. This fragment is common in compounds bearing a nonsubstituted tetrahydrooxazine ring (3). The base peak at *m/z* 71.0496, having the formula C_4H_9N (calcd 71.0496), was due to the cleavage of ring A along with a part of the nitrogen-containing side ring, accompanied by an intramolecular proton transfer (3). These data led to structure **1** for (−)-sempervirone.

Our second compound was identified to have the structure corresponding to (+)-cyclomicrobuxinine [**2**] by comparison of its spectral data (ms, nmr, uv, and ir) with those reported in the literature (12,13) and by the consistency of the spectral data with structure **2**. Presently the ^{13}C-nmr assignments, confirmed by DEPT experiments (17), are presented around structure **2**.

Our third base was identified as (−)-cyclosuffrobuxinine M [**3**], previously isolated from *Buxus microphylla* (14), by spectral assignments, and by comparison of the spectral data with those reported in the literature. We have ob-



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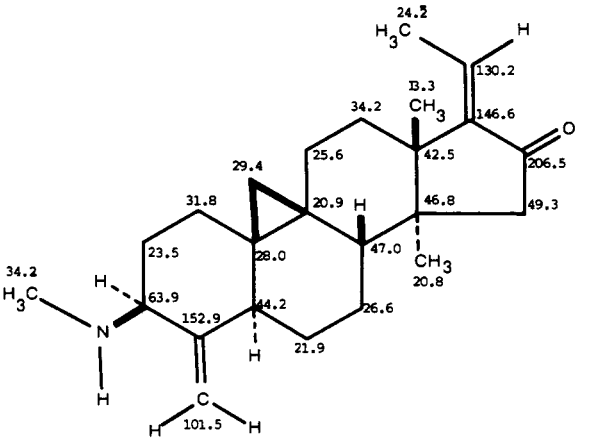

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tained it for the first time from the leaves of *B. sempervirens*. Its ^{13}C-nmr assignments are presented around structure **3**.

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 Dr. Bilge Sener, Department of Pharmacognosy, Gazi University, and a voucher specimen was deposited in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara.

EXTRACTION AND PURIFICATION.—The EtOH extract of air-dried leaves was evaporated to a gum. The total alkaloids (500 g) were obtained by extraction into 10% HOAc. Partial separation of the alkaloids was achieved by extraction into $CHCl_3$ at different pH values. The fraction obtained at pH 3.5 (20 g) was loaded on a Si gel column (300 g). Elution was carried out with $CHCl_3$ and then with $CHCl_3$/MeOH. Three main fractions were obtained: Fraction A, $CHCl_3$-MeOH (98:2); fraction B, $CHCl_3$-MeOH (90:10); fraction C, $CHCl_3$-MeOH (85:15).

(−)-SEMPERVIRONE [**1**].—Fraction A was further purified by preparative tlc (Si gel) in C_6H_{14}-Me_2CO (2:1) to afford (−)-sempervirone [**1**] as a white amorphous solid (6.0 mg), $[\alpha]^{20}D$ −65° (c = 0.5, $CHCl_3$); λ max (MeOH) 243 nm (log ε 3.8); ν max ($CHCl_3$) 1715, 1638 (α,β-unsaturated ketone/cyclopentenone), 1150 (C-O) cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 0.43 (1H, d, $J_{19\alpha,19\beta}$ = 4.5 Hz, H-19α), 0.72 (1H, d, $J_{19\beta,19\alpha}$ = 4.5 Hz, H-19β), 0.94 (3H, s, Me-32), 1.16 (3H, s, Me-30), 1.32 (3H, s, Me-18), 1.83 (3H, d, $J_{21,20}$ = 7.5 Hz, Me-21), 2.16 (3H, s, *N*-Me), 3.16 (1H, d, $J_{33\alpha,33\beta}$ = 10.6 Hz, H-33), 3.63 (1H, d, $J_{31\alpha,31\beta}$ = 7.5 Hz, H-31), 3.77 (1H, d, $J_{33\beta,33\alpha}$ = 10.6 Hz, H-33), 4.46 (1H, d, $J_{31\beta,31\alpha}$ = 7.5 Hz, H-31), 6.55 (1H, q, J = 7.5 Hz, H-20); *m/z* (rel. int.) $[M]^+$ $C_{26}H_{39}NO_2$ 397.2980 (31), $C_{25}H_{36}NO_2$ 382.7441 (27), $C_7H_{13}NO$ 127.1002 (45), $C_5H_{11}N$ 85.0699 (65), C_4H_{10} 72.1311 (29), C_4H_9N 71.0859 (100), C_4H_8N 70.0658 (53), C_3H_8N 58.0579 (90).

(+)-CYCLOMICROBUXININE [**2**].—Fraction B was further chromatographed by preparative tlc (Si gel) in C_6H_{14}-Me_2CO-Et_2NH (7:3:2) to afford a colorless, amorphous solid **2** (20 mg), $[\alpha]^{20}D$ +162 (c = 1.5, $CHCl_3$); λ max (MeOH) terminal; ν max ($CHCl_3$) 3600 (N-H), 3390 (O-H), 1695 (ketone carbonyl) cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 0.06 (1H, d, $J_{19\alpha,19\beta}$ = 4.6 Hz, H-19α), 0.30 (1H, d, $J_{19\beta,19\alpha}$ = 4.6 Hz, H-19β), 0.90 (3H, s, Me), 1.20 (3H, s, Me), 2.14 (3H, s, Me), 2.49 (3H, s, *N*-Me), 2.89 (1H, dd, $J_{3,2}$ = 11.8 Hz, $J_{3,2}$ = 4.4 Hz, H-3), 3.01 (1H, d, $J_{17,16}$ = 6.6 Hz, H-17), 4.58 (1H, s, =C-H), 4.85 (1H, s, =C-H), 4.89 (1H, ddd, $J_{16,17}$ = 6.6 Hz, $J_{16,15}$ = 7.6 Hz, $J_{16,15}$ = 2.0 Hz, H-16); ^{13}C nmr (100 MHz, $CDCl_3$) see structure **2**; *m/z* (rel. int.) $[M]^+$ $C_{24}H_{37}NO_2$ 371.2831 (calcd 371.2824) (19), $[M-Me]^+$ 356 (95), $[M-Ac]^+$ 328 (13), C_3H_7N 57 (100).

(−)-CYCLOSUFFROBUXININE M [**3**].—Fraction C was purified further by tlc (Si gel) in solvent system C_6H_{14}-Me_2CO-Et_2NH (6:4:2) to afford **3** (10 mg), $[\alpha]^{20}D$ −56 (c = 1.8, $CHCl_3$); λ max (MeOH) 243 nm (log ε 3.2); ν max ($CHCl_3$) 3610 (NH), 1705, 1640 (α,β-unsaturated ketone/cyclopentenone); ^{1}H nmr (400 MHz, $CDCl_3$) δ 0.13 (1H, d, $J_{19\alpha,19\beta}$ = 4.5 Hz, H-19α), 0.38 (1H, d, $J_{19\beta,19\alpha}$ = 4.5 Hz, H-19β), 0.97 (3H, s, Me), 1.32 (3H, s, Me), 1.82 (3H, d, $J_{21,20}$ = 7.5 Hz, Me-21), 2.54 (3H, s, *N*-Me), 2.97 (1H, br d, $J_{3,2}$ = 10.4 Hz, H-3), 4.84 (1H, s, =C-H), 4.86 (1H, s, =C-H), 6.57 (1H, q, H-20); ^{13}C nmr (100 MHz, $CDCl_3$) see structure **3**; *m/z*

(rel. int.) $[M]^+$ $C_{24}H_{35}NO$ 353.2725 (calcd 353.2718), $[M-Me]^+$ 338 (8), C_4H_9N 71 (60), C_3H_8N 58 (100).

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