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NEW ALKALOIDS FROM *BUXUS SEMPERVIRENS*

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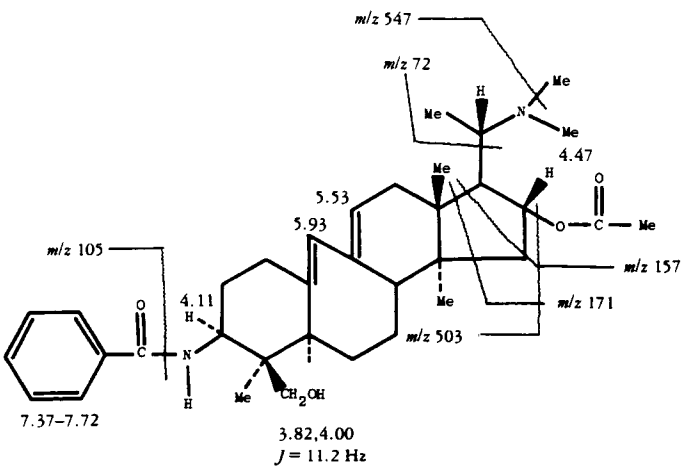
ABSTRACT.—*Buxus sempervirens* of Turkish origin has yielded two new steroidal alkaloids, (−)-*O*-acetyl-*N*-benzoylbuxidienine [**1**] and (+)-nor-16α-acetoxybuxabenzamidienine [**2**]. The structures have been determined through spectroscopic studies.

Buxus sempervirens L. (Buxaceae) is a shrub that is widely distributed in Eurasia and abundantly found in Turkey. H_2O extracts of this plant have found extensive use in indigenous medicine (1). We have previously investigated alkaloids from the leaves of *B. sempervirens* (2–4), and the present study has led to the isolation and structure elucidation of two new steroidal alkaloids, (−)-*O*-acetyl-*N*-benzoylbuxidienine [**1**] and (+)-nor-16α-acetoxybuxabenzamidienine [**2**]. Their structures have been elucidated through extensive spectroscopic studies. The compounds have been isolated from the weakly basic alkaloidal fraction of *B. sempervirens* leaves, obtained at pH 3.5.

(−)-*O*-Acetyl-*N*-benzoylbuxidienine [**1**], $C_{35}H_{50}N_2O_4$, showed uv absorption maxima at 238 (log ε 4.31), 245 (4.29), and 255 sh (4.16) nm indicating the presence of a 9(10↦19) *abeo* diene system and an aromatic ring (5,6). The ir spectrum showed intense absorptions at 3400 (OH), 3350 (NH), 1720 (ester C=O), 1660 (unsaturated amide C=O), and 1610 cm^{-1} (C=C).

The ^{1}H-nmr spectrum (400 MHz, $CDCl_3$) displayed three 3-H singlets at δ 0.75, 0.91, and 1.24, corresponding to three tertiary methyl groups. A 3-H doublet resonated at δ 0.91 ($J_{21,20}$ = 6.5 Hz) due to Me-21. A 3-H singlet at δ 2.09 was due to the acetyl methyl group while a 6-H broad singlet at δ 2.26 was due to the NMe_2 group. Two AB doublets centered at δ 3.82 and 4.00 ($J_{31\alpha,31\beta}$ = 11.24 Hz) were due to the two methylenic protons of the 4β-hydroxymethyl group. A multiplet centered at δ 4.47 was assigned to the 16β proton, geminal to the acetoxy group. The 3α proton appeared as a multiplet at



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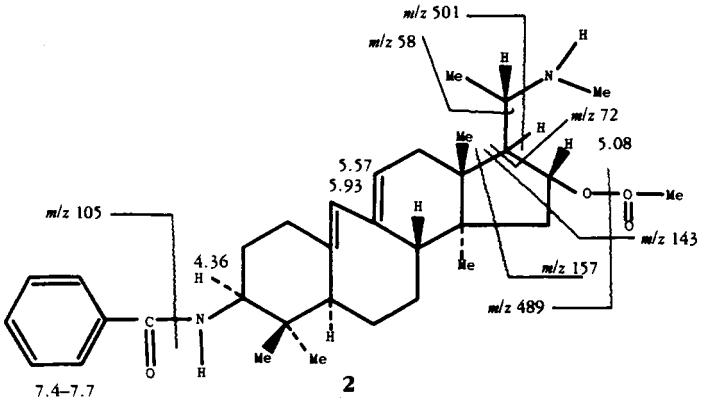

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δ 4.11, while the amidic-NH proton appeared as a doublet at δ 5.74 ($J_{3,NH}$ = 9.6 Hz). A singlet at δ 6.01 was ascribed to the olefinic proton at C-19, while a multiplet at δ 5.53 was assigned to the C-11 olefinic proton (7). The aromatic protons appeared as two groups of 3H and 2H multiplets centered at δ 7.37 and 7.72, respectively.

The ^{1}H-nmr spectrum of **1** was also rerun in pyridine-d_5 (8). It is known that under these conditions the protons adjacent to the hydroxy group will suffer pronounced paramagnetic shifts. This was found to occur for the 31-methylenic protons (shifts from δ 3.82 and 4.00 to δ 4.00 and 4.50, respectively) and allowed the determination of the geminal coupling constant ($J_{31\alpha,31\beta}$ = 11.7 Hz). Similarly the Me-30 shifted from δ 0.91 to 1.07, which served to establish its chemical shift. These paramagnetic shifts argue convincingly in favor of the proposed 31 position for the hydroxyl group (9,10). ^{13}C-nmr values are given in Table 1.

The high resolution mass spectrum of **1** showed the molecular ion at *m/z* 562.37363, corresponding to the molecular formula $C_{35}H_{50}N_2O_4$ (calcd 562.3738). The peak at *m/z* 547.35312 resulted from the loss of a methyl group from the molecular ion. A considerably larger peak at *m/z* 503.36099 was due to the loss of the acetate group from the molecular ion. A large peak at *m/z* 105.03389 corresponded to the benzoyl cation. Compound **1** showed a base peak

TABLE 1. ^{13}C-nmr Values of Compounds **1** and **2**.[a]

Carbon	Compound	
	1	**2**
C-1	38.01	37.58
C-2	29.77	29.73
C-3	49.34	50.97
C-4	43.50	40.50
C-5	50.68	51.44
C-6	25.34	25.61
C-7	29.53	29.20
C-8	55.80	55.86
C-9	135.50	135.20
C-10	138.25	138.33
C-11	126.78	126.70
C-12	30.22	30.10
C-13	39.76	37.58
C-14	47.29	47.72
C-15	43.07	39.99
C-16	75.68	79.53
C-17	70.56	68.32
Me-18	11.63	11.64
C-19	126.78	126.83
C-20	60.50	62.58
Me-21	18.05	24.08
Me-30	21.02	21.01
Me-31	—	17.70
CH_2OH-31	65.03	—
Me-32	16.64	16.57
NMe_2	45.34	—
NHMe	—	45.40
MeCOO	40.15	46.80
MeCOO	170.25	170.30
CONH	167.11	167.14
C-1′	138.24	143.91
C-2′	128.59	127.95
C-3′	128.79	128.63
C-4′	131.34	131.33
C-5′	130.43	130.74
C-6′	128.59	128.55

[a]The ^{13}C-nmr spectra were obtained in $CDCl_3$ at 100 MHz.

at *m/z* 72.0812 which arose by the cleavage of the ring-D nitrogen-containing side chain (10). In the light of these data, structure **1** was assigned to this compound.

Our second compound, (+)-nor-16α-acetoxybuxabenzamidienine [**2**], $C_{34}H_{48}N_2O_3$, showed uv and ir spectra closely approximating those of **1** (see Experimental), indicating the presence of the same chromophores and functionalities (5,6).

The ^{1}H-nmr spectrum (400 MHz, $CDCl_3$) of **2** showed four 3-H singlets at δ 0.67, 0.75, 0.86, and 0.99, corresponding to four tertiary methyl groups. The Me-21 appeared as a doublet at δ 1.26 ($J_{21,20}$ = 6.48) while a 3-H singlet at δ 1.76 was due to acetyl methyl group. The N-Me resonated at δ 2.43 as a 3-H singlet. It appears downfield as compared to NMe_2 (12). The 16β and 3α protons appeared as multiplets at δ 5.08 and 4.36, respectively (3,12). The C-19 olefinic proton resonated at δ 5.57 as a multiplet (3,12). The amidic-NH proton appeared as a doublet at δ 6.34 ($J_{3\alpha,NH}$ = 8.52 Hz). The aromatic protons appeared as two groups of 3H and 2H multiplets centered at δ 7.40 and 7.70, respectively. The ^{13}C-nmr assignments, confirmed by DEPT experiments (11,13) are presented in Table 1.

The mass spectrum of **2** included the molecular ion at *m/z* 532.3665 in agreement with the molecular formula $C_{34}H_{48}N_2O_3$ (calcd 532.3664). The peak at *m/z* 501.3243 resulted from the loss of an $MeNH_2$ from the molecular ion. The peak at *m/z* 105.0339 was due to the benzoyl cation. The base peak at *m/z* 72.1311 was due to the cleavage of the ring-D nitrogen-containing side chain (10). A large peak at *m/z* 58 was due to the cleavage of the nitrogen-containing side chain from ring D, which confirmed the presence of the monomethylamino group (10). These studies led to structure **2** for this compound.

EXPERIMENTAL

PLANT MATERIAL.—The leaves of *B. sempervirens* (dry wt 10 kg) were collected from the Beynam Forest, Ankara, Turkey, in February 1988. The plant was identified by Dr. Bilge Sener, Department of Pharmacognosy, Gazi University, and a voucher specimen (2,3) 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 carried out 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), and elution was carried out first with $CHCl_3$ and then with $CHCl_3$/MeOH. Several fractions were obtained. Two fractions chosen for study were fraction A [$CHCl_3$-MeOH (10:1), 2 g] and fraction B [$CHCl_3$-MeOH (8:1), 2 g].

(−)-O-*Acetyl*-N-*benzoylbuxidienine* [**1**].—Fraction A was subjected to preparative tlc (Si gel) in C_6H_{14}-Me_2CO-Et_2NH (25:5:1) to afford **1** as a white amorphous solid (20 mg): $[\alpha]^{20}D$ −5 (*c* = 0.2, $CHCl_3$), λ max (MeOH) 232, 245, 255 sh nm; ν max ($CHCl_3$) 3400 (OH), 3350 (NH), 1720 (ester C=O), 1660 (amide C=O), 1610 (C=C) cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 0.75 (3H, s, Me-32), 0.91 (3H, s, Me-18), 1.24 (3H, s, Me-30), 0.93 (3H, d, $J_{21,20}$ = 6.5 Hz, Me-21), 2.09 (3H, s, OAc), 2.26 (6H, s, NMe_2), 3.82 (1H, d, $J_{31\alpha,31\beta}$ = 11.2 Hz, H-31α), 4.00 (1H, d, $J_{31\beta,31\alpha}$ = 11.2 Hz, H-31β), 4.11 (1H, m, H-3α), 4.47 (1H, m, H-16β), 5.53 (1H, m, H-11), 5.74 (1H, d, $J_{3\alpha,NH}$ = 9.6 Hz, N-H), 6.01 (1H, s, H-19), 7.37–7.72 (5H, m, ArH); *m/z* (rel. int.) $[M]^+$ ($C_{35}H_{50}N_2O_4$) 562.3736 (calcd 562.3738) (50), $[M-Me]^+$ 547.3531 (25), $[M-OAc]^+$ 503.3610 (20), $[C_7H_5O]^+$ 105.034 (30), $[C_4H_{10}N]^+$ 72.0812 (100).

(+)-*Nor-16-acetoxybuxabenzamidienine* [**2**].—Fraction B was further chromatographed by preparative tlc (Si gel) in C_6H_{14}-Me_2CO-Et_2NH (20:5:1) to afford a white amorphous solid **2** (25 mg): $[\alpha]^{20}D$ +17.5 (*c* = 0.4, $CHCl_3$); λ max (MeOH) 230, 245, 254 sh nm; ν max ($CHCl_3$) 3350 (NH), 1725 (ester C=O), 1650 (amide C=O), 1605 (C=C) cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 0.67 (3H, s, Me-32), 0.75 (3H, s, Me-31), 0.86 (3H, s, Me-18), 0.99 (3H, s, Me-30), 1.26 (3H, d, $J_{21,20}$ = 6.5 Hz, Me-21), 1.76 (3H, s, OAc), 2.43 (3H, s, NMe), 4.36 (1H, m, H-3α), 5.08 (1H, m, H-16β), 5.57 (1H, m, H-11), 5.93 (1H, s, H-19), 6.34 (1H, d,

$J_{3\alpha,NH}$ = 8.52 Hz, NH), 7.40–7.70 (5H, m, ArH); *m/z* (rel. int.) $[M]^+$ ($C_{36}H_{52}N_2O_3$) 532.3665 (calcd 532.3664) (10), $[M-MeNH_2]^+$ 501.3243 (10), $[C_7H_5O]^+$ 105.034 (45) $[C_4H_{10}N]^+$ 72.1311 (100), $[C_3H_8N]^+$ 58.0579 (50).

Overall spectral data of **2** closely resemble those of 16α-acetoxybuxabenzamidienine reported earlier by us from the leaves of *Buxus papillosa* (9).

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