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SEMPERVIRAMIDINE—A NEW STEROIDAL ALKALOID FROM *BUXUS SEMPERVIRENS*

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Key Word Index—*Buxus sempervirens*; Buxaceae; steroidal alkaloids; (+)-semperviramidine; (+)-16α-acetoxybuxabenzamidienine.

Abstract—From leaves of *B. sempervirens* a new steroidal alkaloid (+)-semperviramidine has been isolated and structurally elucidated as (20*S*)-16α-acetoxy-3β-benzoylamino-20-dimethylamino-4,4,14-trimethyl-9β,19-cyclo-5α-pregnane (**1**) by spectroscopic methods. In addition, the known alkaloid (+)-16α-acetoxybuxabenzamidienine (**2**) was isolated from this plant for the first time.

INTRODUCTION

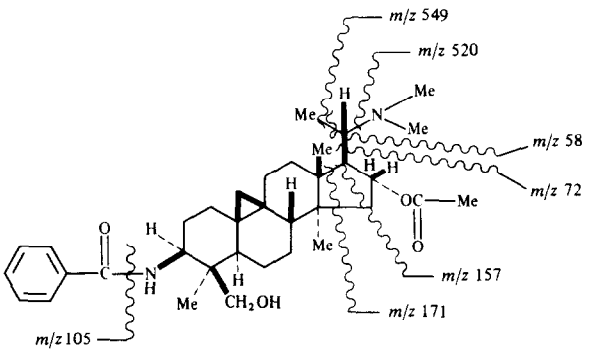
Buxus sempervirens L. (Buxaceae) is a shrub widely distributed in Eurasia and North America, which is abundantly found in Turkey. Water extracts of this plant have found extensive use in the indigenous system of medicine [1]. Continuing our investigations on the leaves of *B. sempervirens*, we report here the isolation and structure determination of a new steroidal alkaloid, (+)-semperviramidine (**1**). Its structure has been elucidated through extensive spectroscopic studies. In addition to this, the already known steroidal alkaloid (+)-16α-acetoxybuxabenzamidienine (**2**) [2] has also been isolated.

RESULTS AND DISCUSSION

The crude alkaloids were isolated from the concentrated ethanolic extracts of the leaves of *B. sempervirens* by extraction at different pH values. The fraction obtained at pH 3.5 was subjected to column chromatography on silica gel. Further purification by preparative TLC resulted in the isolation of compounds **1** and **2**.

(+)-Semperviramidine (**1**), $C_{35}H_{52}N_2O_4$, showed UV absorption maxima at 225 nm, characteristic of a secondary benzamidic chromophore [3, 4]. The IR spectrum displayed absorptions at 3350 (O–H), 1721 (ester carbonyl), 1657 (amide carbonyl) and 1598 (C=C) cm^{-1} [4]. The ^1H NMR spectrum of **1** ($CDCl_3$, 400 MHz) showed two AB doublets which resonated at δ0.38 and 0.55 ($J_{19\alpha,19\beta}$ = 3.7 Hz) and were assigned to the 19α- and β-cyclopropyl protons. Three 3-H singlets at δ0.80, 0.98, 1.15 were assigned to the three tertiary methyl groups. A doublet resonated at δ0.93 ($J_{21,20}$ = 6.7 Hz) due to the 20-methyl group (or 21-H_3). A three-proton singlet at δ2.09 was due to the acetate methyl group while the $N(Me)_2$ group appeared as a 6-H broad singlet at δ2.23. Two AB doublets centred at δ3.82 and 4.00 ($J_{31\alpha,31\beta}$ = ~9.0 Hz) were due to the two methylenic protons of the 4β-hydroxymethyl groups. A multiplet centred at δ4.38 was assigned to the 16β proton, geminal to the acetoxy group. The 3α proton appeared as a multiplet at δ4.18, while the amidic -NH proton appeared as a clean doublet at δ5.85 ($J_{3\alpha,NH}$ = 9.8 Hz). Two multiplets integrating for 3H and 2H appeared at δ7.43 and 7.68 were due to 3′/4′/5′ and 2′/6′ aromatic protons, respectively.

In accord with all the other related *Buxus* alkaloids, the 3-aminated substituent has been placed in a *beta* configuration. Furthermore, whenever biogenetic oxidation of one of the two 4-methyl substituents of ring A occurs, it is always the 4β-methyl (C-31) group that is effected [3]. The ^1H NMR spectrum of **1** was also rerun



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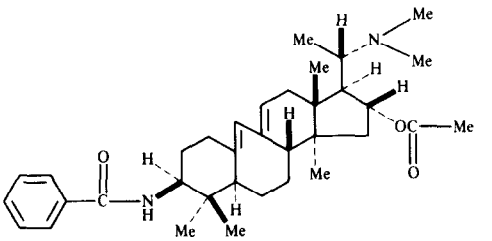

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in pyridine-d_5 [6]. It is known that under these conditions the protons adjacent to the hydroxy group will suffer pronounced paramagnetic shifts. This was found to occur for the 31-methylenic protons (from δ3.82 and 4.00 and δ4.00 and 4.35 respectively) and allowed the determination of the geminal coupling constant ($J_{31\alpha,31\beta}$ = 12.0 Hz). Similarly, the 30-methyl protons were shifted from δ0.98 to 1.22, which served to establish their chemical shift (since the 18 and 32 methyls are not expected to undergo the marked downfield shift in d_5-pyridine). These paramagnetic shifts argue convincingly in favour of the proposed 31 position for the hydroxyl function [2, 5].

The high resolution mass spectrum of **1** showed the molecular ion at *m/z* 564.3915 corresponding to the molecular formula $C_{35}H_{52}N_2O_4$, indicating the presence of eleven double bond equivalents in the molecule. A considerably large peak at *m/z* 549.36753 was due to the loss of the methyl group from molecular ion while *m/z* 520.3455 represented the loss of $N(Me)_2$ group. A large peak at *m/z* 105.0339 corresponded to the benzoyl cation. Compound **1** showed a base peak at *m/z* 72.0812 which arose by the cleavage of the ring D nitrogen-containing side chain [5]. Peaks at *m/z* 171.1167 and 157.1031 arose by the cleavage of ring D and established the 16-position of the acetate group. The overall mass fragmentation pattern was very close to (+)-*N*-benzoyl-16α-acetoxycloxobuxidine-F [7]. In the light of this data structure **1** was assigned to the new alkaloid.

Our second compound was identified as the known 16α-acetoxybuxabenzamidienine (**2**) by TLC and spectroscopic comparison with an authentic sample. This compound has not been found previously in *B. sempervirens*, but had been isolated from the leaves of *B. papilosa* [2].

EXPERIMENTAL

The ^{1}H NMR spectra were recorded at 400 MHz. TLC experiments were performed on silica gel (GF-254) precoated plates (E. Merck).

The plant material was collected from the Beynam forest, Ankara/Turkey, in Sept. 1986, and was identified by Prof. Bilge Sener (Pharmacognosy Department, Gazi University, Ankara).

The EtOH extract of air-dried leaves was evapd to a gum. The alkaloids (50 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 silica gel column (300 g). Elution was carried out first with $CHCl_3$ and then with $CHCl_3$–MeOH. Several fractions were obtained. Fraction A, $CHCl_3$–MeOH (9:1), 2.5 g; Fraction B, $CHCl_3$–MeOH (4:1), 2 g.

(+)-*Semperviramidine* (**1**). Fraction A was subjected to repeated prep. TLC using the system acetone–petrol (40–60°)–$NHEt_2$ (5:15:1) to supply amorphous **1** (6 mg); $[\alpha]_D^{20} = +33°$ ($CHCl_3$); UV λ_{max} (MeOH) nm: 225 nm; IR ν_{max} ($CHCl_3$) cm^{-1}: 3350 (O–H), 1721 (amide carbonyl), 1657 (ester carbonyl), 1598 (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.38 (1H, *d*, $J_{19\alpha,19\beta}$ = 3.7 Hz, H-19α), 0.55 (1H, *d*, $J_{19\beta,19\alpha}$ = 3.7 Hz, H-19β), 0.80 (3H, *s*, Me), 0.93 (3H, *d*, $J_{21,20}$ = 6.7 Hz, Me-21), 0.98 (3H, *s*, H_3-30), 1.15 (3H, *s*, Me), 2.09 (3H, *s*, C(=O)–Me), 2.23 [6H, *s*, $N(Me)_2$], 3.82 (1H, *d*, $J_{31\alpha,31\beta}$ = 12.1 Hz, H-31α), 4.00 (1H, *d*, $J_{31\beta,31\alpha}$ = 12.1 Hz, H-31β), 4.18 (1H, *m*, H-3), 4.38 (1H, *m*, H-16), 5.85 (1H, *d*, $J_{NH,3}$ = 9.8 Hz, –NH), 7.43–7.68 (5H, *m*, ArH); MS *m/z* (int.,%): 564.3915 ($C_{35}H_{52}N_2O_4$, calcd. 564.3926, 3), 549.3675 ($C_{34}H_{49}N_2O_4$, calcd, 549.3692, (1) 520.3455 ($C_{33}H_{46}NO_4$, calcd, 520.3426, (2) 171.1167 ($C_9H_{17}NO_2$, calcd, 171.1259, (4) 157.1013 ($C_8H_{15}NO_2$, calcd, 157.1102, (5), 105.0703 (C_7H_5O, calcd, 105.0340, (10) 73 (2), 72.0812 ($C_4H_{10}N$, calcd, 72.0813, 100), 58.0650 (C_3H_8N, calcd, 58.0656, 10).

16α-*Acetoxybuxabenzamidienine* (**2**). Fraction B was subjected to prep. TLC (silica gel) in solvent system, acetone: petrol (40–60°): $NHEt_2$ (5:10:1) to afford amorphous **2** (10 mg); $[\alpha]_D^{25} = +6°$ ($CHCl_3$); UV λ_{max} (MeOH) nm: 225, 230sh, 237, 245, 254sh; IR ν_{max}($CHCl_3$) cm^{-1}: 3400 (NH), 1718 (ester carbonyl), 1652 (amide carbonyl), 1600 (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.70 (3H, *s*, Me), 0.85 (3H, *s*, Me), 0.86 (3H, *s*, Me), 0.86 (3H, *d*, $J_{21,20}$ = 6.8 Hz, H_3-21), 1.80 (3H, *s*, C(=O)–Me), 2.30 [6H, *s*, $N(Me)_2$], 2.60 (1H, *m*, H-20), 4.37 (1H, *m*, H-3), 5.06 (1H, *m*, H-16), 5.53 (1H, *br s*, H-11), 5.90 (1H, *m*, H-19), 6.36 (1H, *d*, $J_{NH,3}$ = 8.5 Hz, N*H*), 7.37–7.72 (5H, *m*, ArH); MS *m/z* (rel. int.): 546.3729 ($C_{35}H_{50}N_2O_3$, calcd. 546.3720, 3), 531 (4), 503 (20), 171 (3), 157 (8), 148 (40), 72 (100), 71 (10), 58 (3).

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