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New Steroidal Alkaloids from the Roots of *Buxus sempervirens*

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Phytochemical studies on an EtOH-soluble extract of the roots of *Buxus sempervirens* of Turkish origin have resulted in the isolation of three new steroidal alkaloids, (+)-semperviraminol (**1**), (+)-buxamine F (**2**), and (+)-17-oxocycloprotobuxine (**3**), along with two known steroidal alkaloids, (+)-buxoxybenzamine (**4**) and (+)-buxapapillinine (**5**). The structures of **1**−**3** were elucidated with the aid of spectroscopic studies. Compounds **4** and **5** exhibited phytotoxic activity against *Lemna minor*.

Buxus alkaloids are a unique steroid−triterpenoid class of alkaloids having a pregnane-type basic skeleton with a 4-dimethyl-9β,10β-cycloartenol system and a C-20 degraded side chain.[1] This type of alkaloid has shown interesting pharmacological activities such as antimalarial,[2] antituberculosis,[2] and anti-HIV effects.[3] *Buxus sempervirens* L. (Buxaceae), commonly known as "boxwood", is found abundantly in Turkey. Our previous phytochemical studies on this plant have resulted in the isolation of more than 20 new steroidal alkaloids.[4−8] In a continuation of our search for new bioactive steroidal alkaloids from various species of the genus *Buxus*, we have isolated three new steroidal alkaloids, (+)-semperviraminol (**1**), (+)-buxamine F (**2**), and (+)-17-oxocycloprotobuxine (**3**), along with two known steroidal alkaloids, (+)-buxoxybenzamine (**4**) and (+)-buxapapillinine (**5**), isolated from an ethanolic extract of the roots of *B. sempervirens* of Turkish origin. The structures of these compounds were established on the basis of extensive spectroscopic studies. Compounds **4** and **5** were shown to exhibit significant phytotoxic activity against *Lemna minor* L.

(+)-Semperviraminol (**1**), $C_{35}H_{52}N_2O_4$, was isolated as a colorless amorphous solid. The UV spectrum showed an absorption maximum at 226 nm, indicating the presence of a secondary benzamide chromophore.[9] The IR spectrum displayed intense absorption bands at 3416 (OH), 3315 (NH), 1709 (ester carbonyl), 1645 (α,β-unsaturated amide carbonyl), and 1600 (C=C) cm^{-1}. The HREIMS of **1** showed a molecular ion peak at *m*/*z* 564.3892, which provided the elemental formula $C_{35}H_{52}N_2O_4$ (calcd 564.3895) and indicated the presence of 11 degrees of unsaturation. Compound **1** showed the base peak at *m*/*z* 72.0814 ($C_4H_{10}N$, calcd 72.0813), indicating the presence of *N*,*N*-dimethylamino group at C-20, as the side chain of ring D.[10]

The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of **1** featured four three-proton singlets at δ 0.85, 0.91, 0.94, and 1.22 due to the protons of four methyl groups attached to quaternary carbon atoms. A three-proton doublet at δ 1.29 ($J_{21,20}$ = 6.6 Hz) was due to the C-21 secondary methyl protons, while acetyl methyl protons resonated at δ 2.06 and were assigned to the C-6 acetoxy group. A six-proton broad singlet at δ 2.27 was assigned to the *N*,*N*-dimethyl protons. The C-3 methine proton resonated as a double doublet at δ 3.95 ($J_{3\alpha\ 2\beta}$ = 9.7 Hz, $J_{3\alpha,NH}$ = 9.5 Hz). The C-2 methine proton, geminal to a hydroxyl group, resonated at δ 4.00 ($J_{2\beta,3\alpha}$ = 9.7 Hz, $J_{2\beta,1}$ = 5.4 Hz). A one-proton doublet of double doublets at δ 4.77 ($J_{6\beta,5\alpha}$ = 9.9 Hz, $J_{6\beta,7\alpha}$ = 9.7 Hz, $J_{6\beta,7\beta}$ = 4.9 Hz) was due to the C-6 methine proton, geminal to an acetoxy group. An olefinic doublet resonating at δ 5.69 ($J_{1,2\beta}$ = 5.4 Hz), ascribed to the C-1 methine proton, was also observed in the ^{1}H NMR spectrum of **1**. The exchangeable amide NH resonated at δ 6.14

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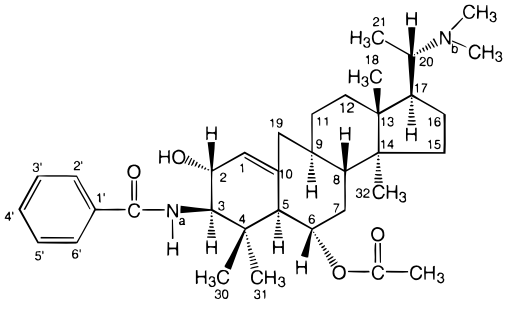

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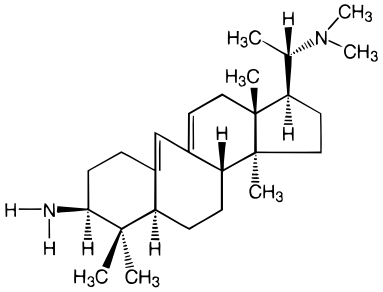

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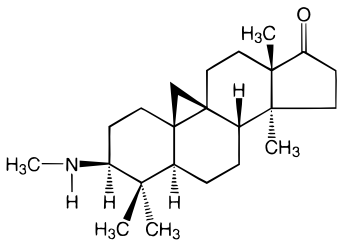

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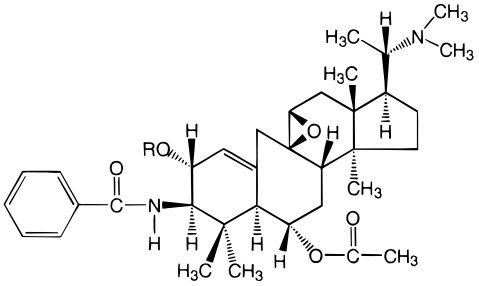

4 R = H
5 R = OAc

($J_{NH,3\alpha}$ = 9.5 Hz), and this signal disappeared when the ^{1}H NMR spectrum was recorded in CD_3OD.[11] Two sets of two- and three-proton multiplets at δ 7.37−7.65 were attributed to the aromatic protons. The COSY-45° and HOHAHA spectra of **1** revealed the presence of three isolated spin systems in the molecule, that is, spin system "a" (a phenyl moiety), "b" (H-1−H_2-12), and "c" (H_2-15−H_3-21), which are shown in Figure 1.[12,13] The spin system "b" started with the C-1 olefinic proton (δ 5.69), which exhibited vicinal coupling with H-2 (δ 4.00). H-2 showed cross peaks with H-3 (δ 3.95) which, in turn, showed cross peaks with the amide NH (δ 6.14). Allylic couplings of olefinic H-1 (δ 5.69) with H_2-19 (δ 2.64 and 1.70) and H-5 (δ 2.50) were observed in the HOHAHA spectra (40 and 60 ms). H-5 exhibited a COSY-45° interaction with H-6 (δ 4.77), which, in turn, showed cross peaks with H_2-7 (δ 1.69 and 0.89). H_2-7 exhibited vicinal couplings with H-8 (δ 1.56). The latter showed cross peaks with H-9 (δ 1.77). The

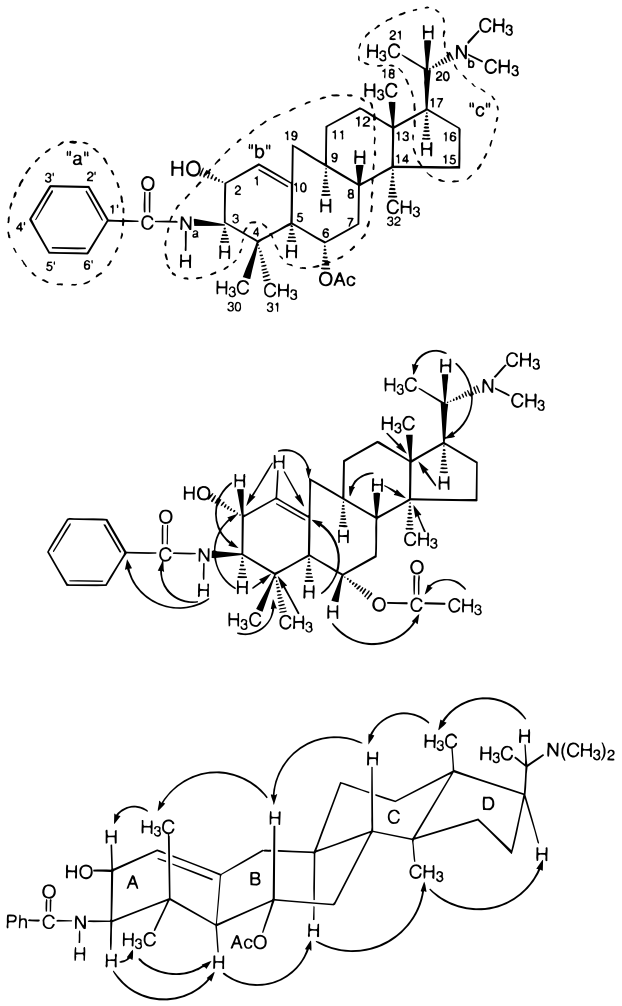

Figure 1. Spin systems "a"−"c" obtained from COSY-45° and HOHAHA spectra, important HMBC interactions, and probable conformations of rings A−D of compound **1** as obtained from the NOESY spectrum.

H-9 signal showed vicinal couplings with H_2-19 (δ 2.64 and 1.70) and H_2-11 (δ 1.75 and 1.27), while H_2-11 showed ^{1}H−^{1}H spin correlations with H_2-12 (δ 1.65 and 0.99). On the basis of similar observations from the COSY-45° and HOHAHA spectra for other coupled protons, spin systems "a" and "c" were also deduced.

The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of **1** showed the resonances of all 35 carbon atoms in the molecule. DEPT spectra revealed the presence of 14 methine, six methylene, eight methyl, and seven quaternary carbon atoms in **1**. The olefinic C-1 resonated at δ 134.0, while the C-2 signal appeared at δ 67.9 and C-3 resonated at δ 61.5. Their downfield chemical shift values were indicative of the presence of geminal hydroxyl and benzamide groups, respectively. Another downfield aliphatic signal at δ 77.9 was due to the acetoxy-bearing C-6. Complete ^{13}C NMR chemical shift assignments and ^{1}H−^{13}C one-bond shift correlation of each protonated carbon atom of **1**, as determined from the HMQC spectrum, are presented in Table 1.

The HMBC spectrum of **1** was also very useful for determining the ^{13}C NMR chemical shift assignments of quaternary carbon atoms in the molecule and the establishment of structure **1** from substructures "a"−"c" obtained from the COSY-45° and HOHAHA spectra. H-1 (δ 5.69) exhibited HMBC interactions with C-2 (δ 67.9), C-5 (δ 49.8), and C-10 (δ 134.5). H-3 (δ 3.95) showed long-range het-

Table 1. $^{1}H-^{13}C$ One-Bond Shift Correlations of Compounds **1** and **2** as Determined from HMQC Spectra and Complete ^{13}C NMR Chemical Shift Assignments of Compound **3**

	1		**2**		**3**
carbon	δ_C	δ_H	δ_C	δ_H	δ_C
C-1	134.0 d	5.69 (d, J = 5.4 Hz)	34.4 t	2.13 (m), 1.72 (m)	28.4 t
C-2	67.9 d	4.00 (dd, J = 9.7, 5.4 Hz)	30.1 t	1.75 (m), 1.49 (m)	30.2 t
C-3	61.5 d	3.95 (dd, J = 9.7, 9.5 Hz)	69.2 d	3.57 (dd, dd, J 9.8, 4.2 Hz)	61.3 d
C-4	45.0 s		40.9 s		39.7 s
C-5	49.8 d	2.50 (d, J = 9.9 Hz)	49.5 d	2.25 (m)	47.8 d
C-6	77.9 d	4.77 (ddd, J = 9.9, 9.7, 4.9 Hz)	25.4 t	1.89 (m), 1.32 (m)	21.3 t
C-7	35.6 t	1.69 (m), 0.89 (m)	27.7 t	1.98 (m), 1.43 (m)	26.9 t
C-8	41.2 d	1.56 (m)	49.8 d	2.15 (m)	49.6 d
C-9	41.3 d	1.77 (m)	138.2 s		22.7 s
C-10	134.5 s		134.1 s		26.0 s
C-11	25.9 t	1.75 (m), 1.27 (m)	129.7 d	5.89 (br s)	27.3 t
C-12	24.6 t	1.65 (m), 0.99 (m)	38.5 t	2.20 (m), 1.84 (m)	35.1 t
C-13	39.4 s		44.5 s		44.2 s
C-14	49.3 s		45.7 s		45.6 s
C-15	26.7t	1.95 (m), 1.45 (m)	33.0 t	2.00 (m), 1.68 (m)	32.5 t
C-16	27.1 t	2.00 (m), 1.60 (m)	27.0 t	1.93 (m), 1.41 (m)	29.4 t
C-17	53.5 d	2.55 (m)	51.4 d	2.64 (m)	209.6 s
C-18	12.3 q	0.85 (s)	14.3 q	0.74 (s)	18.2 q
C-19	44.6 t	2.64 (dd, J = 13.8, 9.8 Hz) 1.70 (dd, J = 13.8,4.4 Hz)	129.8 d	5.96 (s)	19.6 t
C-20	63.3 d	1.93 (m)	62.0 d	2.68 (m)	
C-21	10.2 q	1.29 (d, J = 6.6 Hz)	9.8 q	0.85 (d, J = 6.5 Hz)	
C-30	13.5 q	0.91 (s)	15.4 q	0.84 (s)	14.0 q
C-31	14.0 q	0.94 (s)	16.9 q	0.91 (s)	16.4 q
C-32	16.7 q	1.22 (s)	17.3 q	1.07 (s)	16.9 q
N_a-CH_3					44.7 q
$N_b(CH_3)_2$	37.4 q	2.27 (br s)	38.9 q	2.20 (s)	
$OCOCH_3$	21.7 q	2.06 (s)			
$OCOCH_3$	174.3 s				
OCNH	165.7 s				
C-1′	131.8 s				
C-2′	126.8 d	7.65 (dd, J = 6.8, 1.5 Hz)			
C-3′	128.6 d	7.37 (dd, J = 7.3, 6.8 Hz)			
C-4′	129.6 d	7.40 (dd, J = 7.3, 1.5 Hz)			
C-5′	128.6 d	7.37 (dd, J = 7.3, 6.8 Hz)			
C-6′	126.8 d	7.65 (dd, J = 6.8, 1.5 Hz)			

eronuclear multiple-bond couplings with the amide carbonyl carbon (δ 165.7), C-2 (δ 67.9), and C-4 (δ 45.0). Important HMBC interactions are shown in Figure 1.

The relative stereochemistry at various chiral centers was established with the aid of the NOESY spectrum and from the $^{1}H-^{1}H$ coupling constants. The NOESY spectrum of **1** showed cross peaks between H-2 (δ 4.00) and H_3-30 (δ 0.91), which also exhibited NOE interactions with H-6 (δ 4.77). The latter showed a cross peak with H-8 (δ 1.56), which also exhibited a NOE with H_3-18 (δ 0.85) and, in turn, showed a cross peak with H-20 (δ 1.93). It has already been reported in the literature that H-8 is invariably β-oriented in this class of alkaloids.[14] This suggested β-orientation of the H-2, H-6, H_3-18, and H_3-30 substituents. The *trans*-diaxial $^{1}H-^{1}H$ coupling constants of H-2β/H-3α ($J_{2\beta,3\alpha}$ = 9.7 Hz), H-6β/H-5α, and H-6β/H-7α ($J_{6\beta,5\alpha}$ = 9.9 Hz, $J_{6\beta,7\alpha}$ = 9.7 Hz) also favored β-orientation of H-2 and H-6. H-3 (δ 3.95) showed a NOESY interaction with H-5 (δ 2.50), which, in turn, exhibited a NOE with H-9 (δ 1.77). H-5 has invariably α-orientation in *Buxus* alkaloids.[14] These observations obtained from the NOESY spectrum suggested the α-orientation of H-3 and H-9. The probable conformations of rings A−D as obtained from the NOESY spectrum are shown in Figure 1. These spectroscopic studies characterized compound **1** (semperviraminol) as (20*S*)-2α-hydroxy-6α-acetoxy-3β-(benzoylamino)-20-(dimethylamino)-9,10-*seco*-buxa-1(10)-ene.

(+)-Buxamine F (**2**), $C_{26}H_{44}N_2$, was also isolated as a colorless amorphous solid. The UV spectrum showed maximum absorptions at 238 and 245 with shoulders at 231 and 252 nm, which are characteristic of a 9(10→19) *abeo*-diene system in *Buxus* alkaloids.[10] The IR spectrum showed absorption bands at 3316 (NH), 2913 (CH), and 1607 (C=C) cm^{-1}. The HREIMS of compound **2** showed the molecular ion peak at m/z 384.3499, which is in agreement with the elemental formula $C_{26}H_{44}N_2$ (calcd 384.3504), and indicated the presence of six degrees of unsaturation. The base peak at m/z 72.0812 ($C_4H_{10}N$, calcd 72.0813) was due to the cleavage of the ring D side chain containing a *N*,*N*-dimethyl amino substituent.[10]

The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **2** showed four three-proton singlets at δ 0.74, 0.84, 0.91, and 1.07, due to the protons of four methyl groups attached at quaternary carbon atoms. A doublet, integrating for three protons resonated at δ 0.85 ($J_{21,20}$ = 6.5 Hz), was due to the protons of the C-21 secondary methyl group, which also exhibited cross peaks with the C-20 methine proton (δ 2.68) in the COSY-45° spectrum. The *N*,*N*-dimethyl protons resonated as a broad six-proton singlet at δ 2.20. The ^{1}H NMR spectrum also exhibited two olefinic signals at δ 5.89 (broad singlet) and 5.96 (sharp singlet), which were assigned to the C-11 and C-19 olefinic protons, respectively. H-11 exhibited a COSY-45° interaction with H_2-12 (δ 2.20 and 1.84). The C-11 and C-19 signals appeared at δ 129.7 and 129.8, respectively, in the ^{13}C NMR spectrum. H-11 (δ 5.89) exhibited cross peaks with C-11 (δ 129.7) and H-19 (δ 5.96) with C-19 (δ 129.8) in the HMQC spectrum. Complete ^{13}C NMR chemical shift assignments as well as direct $^{1}H-^{13}C$ one-bond shift correlations of each protonated carbon atom of **2** are shown in Table 1. H-11 (δ 5.89) showed HMBC interactions with C-9 (δ 138.2) and C-12 (δ 38.5), while H-19 (δ 5.96) exhibited long-range

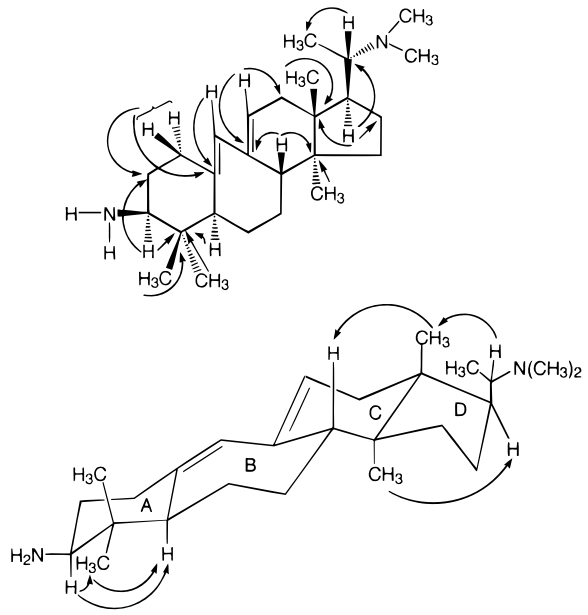

Figure 2. Important HMBC interactions, probable conformations of rings A−D, and observed NOE interactions of compound **2**.

heteronuclear interactions with C-9 (δ 138.2) and C-10 (δ 134.1). Important HMBC interactions of **2** are shown in Figure 2.

The stereochemistry at various chiral centers of **2** was established with the help of the NOESY spectrum, as discussed previously for compound **1**, and probable conformations of rings A−D of **2** as obtained from the NOESY spectrum are presented in Figure 2. Based on these spectroscopic studies, compound **2** (buxamine F) was characterized as (20*S*)-3β-(amino)-20-(dimethylamino)-9,10-*seco*-buxa-9(11),10(19)-diene.

(+)-17-Oxocyclocyprotobuxine (**3**), $C_{23}H_{37}NO$, was isolated as a colorless gum. The UV spectrum showed only terminal absorption, indicating the lack of any conjugated chromophore in the molecule. The IR spectrum displayed intense absorption bands at 2903 (CH) and 1723 (cyclopentanone) cm^{-1}. The HREIMS of **3** showed the molecular ion peak at *m*/*z* 343.2880, which provided an elemental formula $C_{23}H_{37}NO$ (calcd 343.2875) and indicated the presence of six degrees of unsaturation. The ion at *m*/*z* 328.2643 ($C_{22}H_{34}NO$, 328.2640) was due to the loss of a methyl group from the molecular ion. *Buxus* alkaloids containing either a dimethylamino group or a methylamino group at C-20 of the ring D side chain usually exhibit a base peak at *m*/*z* 72 or 58, while those *Buxus* alkaloids having an amino functionality at C-3 show a very prominent ion at *m*/*z* 71 (for a dimethylamino group) or 57 (for a methylamino group).[10] Compound **3** showed the base peak at *m*/*z* 57.0576 (C_3H_7N, 57.0578), which presumably arose by the cleavage of ring A along with the nitrogen-containing side chain, indicating the presence of methylamino group at C-3.[10] The absence of an ion at *m*/*z* 72 or 58 in the mass spectrum of **3** indicated the absence of an amino functionality at C-17 as a side chain of ring D.

The ^1H NMR spectrum ($CDCl_3$, 400 MHz) of **3** (Experimental Section) resembled that of **2**, except that it lacked signals for an olefinic H-11, and the H-19, C-20/*N*,*N*-dimethyl, and C-21 methyl protons. However, the presence of a set of two AB doublets at δ 0.15 and 0.43 ($J_{19\alpha,19\beta}$ = 4.3 Hz) in the ^1H NMR spectrum, which were assigned to the C-19 methylene protons, indicated the presence of a 9β,10β-cyclopropane system in the molecule.[10] The signal at δ 19.6 in the ^{13}C NMR spectrum was due to C-19. Complete ^{13}C NMR chemical shift assignments of **3** are shown in Table 1. The ^1H and ^{13}C NMR spectra did not show the resonance of any signal for a C-21 methyl group or a *N*,*N*-dimethyl group, indicating the absence of a C-17 side chain. The mass spectrum also did not exhibit any ion at *m*/*z* 58 or 72, which further confirmed some modification at C-17. However, the signal at δ 209.6 in the ^{13}C NMR spectrum was due to a carbonyl carbon (C-17). The presence of a carbonyl functionality in the molecule was also evident from the IR spectrum, which showed an intense absorption band at 1723 cm^{-1}. This spectral data suggested the presence of a carbonyl functionality at C-17. Any other location of carbonyl group would not have satisfied the observed spectral data. On the basis of this spectral data, structure **3** was proposed for 17-oxocyclocyprotobuxine [3β-(methylamino)-9β,10β-cyclobuxa-17-one], a new steroidal alkaloid.

Two known steroidal alkamines, (+)-buxoxybenzamine (**4**) and (+)-buxapapillinine (**5**), were isolated for the first time from the roots of *B. sempervirens*. The UV, IR, ^1H NMR, ^{13}C NMR, and MS of compounds **4** and **5** were nearly identical with those of (+)-buxoxybenzamine and (+)-buxapapillinine reported in the literature.[15,16] Compounds **4** and **5** were previously isolated from *B. papillosa*.[15,16] Compound **4** showed 86%, 57%, and 57% phytotoxic activity against *Lemna minor* L. at 500, 50, and 5 ppm, respectively, while compound **5** exhibited values of 86%, 71%, and 57% at these same dose levels, as determined by the method described by McLaughlin et al.[17] Compounds **1**−**3** were inactive in this assay when tested at the same concentrations as **4** and **5**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Polatronic D polarimeter. The UV spectra were recorded on a Shimadzu UV 240 instrument. The IR spectra were recorded on a JASCO-IRA1 IR spectrophotometer. The ^1H NMR spectra were recorded in $CDCl_3$ on AM300, AM400, and AM500 Bruker NMR spectrometers at 300, 400, and 500 MHz, while ^{13}C NMR spectra were recorded on AM300 and AM400 Bruker NMR spectrometers at 75 and 100 MHz with TMS as internal standard. Mass spectral measurements were conducted on a Varian-MAT 312 double-focusing mass spectrometer connected to a DEC PDP 11/34 computer system. The purity of the samples was checked by TLC (Si gel, GF_{254} precoated plates).

Plant Material. The roots of *B. sempervirens* were collected from Beynam Forest, Ankara, Turkey, in September 1990. This plant was identified by Prof. Mehmet Koyuncu, Department of Pharmacognosy, Gazi University, Ankara, Turkey. A voucher specimen (GUE #1243) has been deposited in the herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Extraction and Isolation. The roots of *B. sempervirens* (10 kg) were dried, crushed, and extracted with EtOH (100 L) at room temperature. An EtOH extract of the *B. sempervirens* was concentrated under reduced pressure to a gum (160 g), which was dissolved in distilled H_2O. The aqueous extract was extracted with $CHCl_3$ at different pH values, to achieve partial separation of alkaloids, and three $CHCl_3$-soluble fractions at pH 3.5, 7.0, and 9.5 were obtained. The pH was adjusted by addition of 10% HOAc and NH_4OH solution.

The $CHCl_3$ extract (18.3 g) obtained at pH 9.5 was loaded onto a Si gel column and eluted with pure petroleum ether (40−60 °C), mixtures of petroleum ether−$CHCl_3$, and then with mixtures of $CHCl_3$−MeOH. Three fractions, F-1, F-2, and F-3, were obtained on elution of the Si gel column with petroleum ether−$CHCl_3$ (3:7), petroleum ether−$CHCl_3$ (2:8), and $CHCl_3$−MeOH (6:4), respectively. Fraction F-3 (25.6 mg) was subjected to preparative TLC using petroleum ether−Et_2O−diethylamine (5:4:0.1) to afford compound **1** (R_f 0.43),

while compound **2** (R_f 0.56) was purified from fraction F-2 (18.9 mg) through preparative TLC using petroleum ether−Et_2O−diethylamine (3:6:0.1). Fraction F-1 (10.3 mg) was also subjected to preparative TLC using petroleum ether−Me_2CO−diethylamine (4:5:0.1) to isolate compound **3** (R_f 0.71).

The acidic fraction (2.7 g) obtained at pH 3.5 was also loaded onto a Si gel column, and elution was effected with petroleum ether−$CHCl_3$ (0−100%) and then $CHCl_3$−MeOH (0−100%) to obtain various fractions. Fraction F-4 (100 mg), obtained on elution with 30% petroleum ether and 70% $CHCl_3$, was subjected to preparative TLC using petroleum ether−Me_2CO−diethylamine (3:7:0.1) to purify compound **4** (R_f 0.73). Fraction F-5 (110 mg) was obtained on elution of the same column with 20% petroleum ether−80% $CHCl_3$. This fraction was also subjected to preparative TLC using petroleum ether−Et_2O−diethylamine (2:7:0.2) to yield compound **5** (R_f 0.87).

(+)-Semperviraminol (1): colorless, amorphous solid (12.9 mg); $[\alpha]^{20}_D$ +97° (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} 226 nm (log ϵ 2.3); IR ($CHCl_3$) ν_{max} 3416, 3315, 1709, 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; EIMS *m*/*z* 564 [M^+] (3), 549 (5), 504 (4), 105 (45), 72 (100); HREIMS *m*/*z* 564.3892 ($C_{35}H_{52}N_2O_4$, calcd 564.3895), 549.3658 ($C_{34}H_{49}N_2O_4$, calcd 549.3660), 504.3681 ($C_{33}H_{48}N_2O_2$, calcd 504.3884), 105.0338 (C_7H_5O, calcd 105.0340), 72.0814 ($C_4H_{10}N$, calcd 72.0813).

(+)-Buxamine-F (2): colorless, amorphous solid (13.6 mg); $[\alpha]^{20}_D$ +49° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 231 (sh) (log ϵ 3.2), 238 (log ϵ 3.4), 245 (log ϵ 2.9), 252 (sh) (log ϵ 3.1) nm; IR ($CHCl_3$) ν_{max} 3316, 2913, 1607 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 384 [M^+] (1), 369 (3), 43 (20), 72 (100); HREIMS *m*/*z* 384.3499 ($C_{26}H_{44}N_2$, calcd 384.3504), 369.3272 ($C_{25}H_{41}N_2$, calcd 369.3269), 72.0812 ($C_4H_{10}N$, calcd 72.0813), 43.0236 (C_2H_5N, calcd 43.0234).

(+)-17-Oxocycloprotobuxine (3): colorless gum (5.5 mg); $[\alpha]^{20}_D$ +48° (*c* 0.73, $CHCl_3$); UV (MeOH) λ_{max} 203 nm (log ϵ 1.85); IR ($CHCl_3$) ν_{max} 2903, 1723 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.15 (1H, d, $J_{19\alpha,19\beta}$ = 4.3 Hz, H-19α), 0.43 (1H, d, $J_{19\beta,19\alpha}$ = 4.3 Hz, H-19β), 0.86 (3H, s, H_3-18), 0.95 (3H, s, H_3-31), 1.02 (3H, s, H_3-30), 1.22 (3H, s, H_3-32), 2.16 (3H, s, N−CH_3), 2.94 (1H, dd, $J_{3\alpha,2\beta}$ = 8.9 Hz, $J_{3\alpha,2\alpha}$ = 3.8 Hz, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 343 [M^+] (10), 328 (7), 57 (100); HREIMS *m*/*z* 343.2880 ($C_{23}H_{37}NO$, calcd 343.2875), 328.2643 ($C_{22}H_{34}NO$, calcd 328.2640), 57.0576 (C_3H_7N, calcd 57.0578).

Phytotoxic Assay. This assay was performed against *Lemna minor* L. (duckweed) at the Plant Screening Section, H. E. J. Research Institute of Chemistry, by the procedure described by McLaughlin et al.[17]

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