New Steroidal Alkaloids from the Roots of *Buxus sempervirens*

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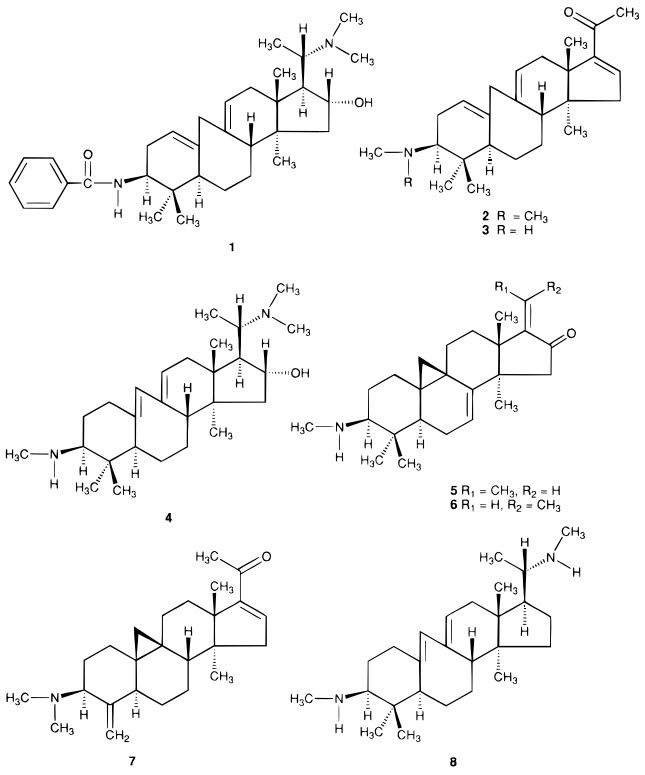
Phytochemical studies on the ethanolic extract of the roots of *Buxus sempervirens* L. of Turkish origin have resulted in the isolation of four new steroidal alkaloids, (+)-16α-hydroxy-N_a-benzoylbuxadine [(20*S*)-16α-hydroxy-3β-(benzoylamino)-20-(dimethylamino)-9,10-*seco*-buxa-9(11),1(10)-diene] (**1**), (+)-semperviraminone [3β-(dimethylamino)-20-oxo-9,10-*seco*-buxa-9(11),1-(10),16(17)-triene] (**2**), (+)-N_a-demethylsemperviraminone [3β-(methylamino)-20-oxo-9,10-*seco*-buxa-9(11),1(10),16(17)-triene] (**3**), and (+)-buxaminol-C [(20*S*)-16α-hydroxy-3β-(methylamino)-20-(dimethylamino)-9,10-*seco*-buxa-9(11),10(19)-diene] (**4**). Four known steroidal alkamines, (−)-(*E*)-cyclobuxaphylamine [(20*E*)-16-oxo-3β-(methylamino)-9β,10β-cyclobuxa-7(8),17(20)-diene] (**5**), (−)-(*Z*)-cyclobuxaphylamine [(20*Z*)-16-oxo-3β-(methylamino)-9β,10β-cyclobuxa-7(8),17(20)-diene] (**6**), (+)-cyclomicrobuxeine [3β-(dimethylamino)-20-oxo-9β,10β-cyclobuxa-4(30),16(17)-diene] (**7**), and (+)-papilamine [(20*S*)-3β,20-bis(methylamino)-9,10-*seco*-buxa-9(11),10(19)-diene] (**8**), were also isolated for the first time from this species. Their structures were elucidated on the basis of extensive spectroscopic studies.

Buxus sempervirens L. (Buxaceae) is an herb that is widely distributed in Eurasia and occurs abundantly in Turkey. Aqueous extracts of the plant have found extensive use in the treatment of various ailments in folk medicine.[1] The ethanolic extract of *B. sempervirens* (boxwood) containing cyclobuxine D and buxamine has been reported to be active against the human immunodeficiency virus (HIV) and other diseases in which the tumor necrosis factor is involved.[2] Our previous phytochemical studies on *B. sempervirens* have resulted in the isolation of over 20 new steroidal alkaloids.[3−6] The present paper describes the isolation and structure elucidation of four new steroidal alkaloids, (+)-16α-hydroxy-N_a-benzoylbuxadine (**1**), (+)-semperviraminone (**2**), N_a-demethylsemperviraminone (**3**), and (+)-buxaminol-C (**4**) along with four known steroidal alkamines, (−)-(*E*)-cyclobuxaphylamine (**5**), (−)-(*Z*)-cyclobuxaphylamine (**6**), (+)-cyclomicrobuxeine (**7**), and (+)-papilamine (**8**), isolated for the first time from the ethanolic extracts of the roots of *B. sempervirens*. Their structures were established on the basis of extensive spectroscopic studies.



Results and Discussion

(+)-16α-Hydroxy-N_a-benzoylbuxadine (**1**), $C_{33}H_{48}N_2O_2$, was isolated as a colorless amorphous solid from ethanolic extracts of the roots of *B. sempervirens*. The UV spectrum showed a maximum absorption at 228 nm, indicating the presence of a secondary benzamide chromophore.[7] The IR spectrum displayed intense absorptions at 3416 (NH), 3310 (OH), 1656 (aromatic amide carbonyl), and 1594 (C=C) cm^{-1}.

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) of **1** featured four 3H singlets at δ 0.74, 0.96, 0.97, and 1.23 due to the four tertiary methyl groups. A doublet centered at δ 1.29 ($J_{21,20}$ = 6.6 Hz) was assigned to the C-21 secondary methyl protons. The $N(CH_3)_2$ protons appeared as a 6H broad "singlet" at δ 2.27. Another broad "singlet" at δ 2.77 was assigned to the C-19 methylene protons flanked by the quaternary C-9 and C-10 carbons, whereas two-proton overlapping multiplets centered at δ 4.15 were ascribed to the C-3 and C-16 methine protons, geminal to the benzamidic nitrogen and hydroxyl group, respectively. The C-1 olefinic proton resonated as a broad singlet at δ 5.27, while another 1H broad singlet at δ 5.39 was ascribed to the C-11 olefinic proton. A 1H doublet centered at δ 6.13

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($J_{NH,3\alpha}$ = 9.8 Hz) was due to the amidic NH. The chemically equivalent *ortho* C-2′/C-6′ protons appeared as a 2H double doublet at δ 7.80 (J_1 = 7.1 Hz, J_2 = 1.4 Hz), while the *meta* C-3′/C-5′ protons resonated as a 2H double doublet at δ 7.39 (J_1 = 7.1 Hz, J_2 = 6.9 Hz). Another 1H double doublet at δ 7.43 (J_1 = 6.9 Hz and J_2 = 1.4 Hz) was due to the *para* C-4′ proton.

The COSY-45° and HOHAHA spectra revealed the presence of four isolated spin systems in the molecule.[8,9] The spin system "a" comprises the phenyl moiety, while the spin system "b" starts with the C-1 olefinic proton (δ 5.27), which showed COSY-45° interactions with the C-2 methylene protons (δ 2.10 and 2.00). The C-2 protons showed vicinal coupling with the C-3 methine proton (δ 4.15), which in turn showed cross-peaks with the amidic NH (δ 6.13). The allylic couplings of the C-19 protons (δ 2.77) with the C-1 and C-11 olefinic protons were also observed in the COSY-45° spectrum. The coupling between the olefinic C-11 proton (δ 5.39) and the C-12 protons (δ 2.15 and 1.65) was also apparent in the spectrum. The C-16 methine proton (δ 4.15) (spin system "c") exhibited vicinal couplings with the C-17 methine (δ 1.97) and C-15 methylene protons (δ 2.00 and 1.25). The former further showed coupling with the C-20 methine proton (δ 1.99), which in turn exhibited coupling with the C-21 methyl protons (δ 1.29). The spin system "*d*" was mostly traced from the HOHAHA spectra in which the C-1 olefinic proton (δ 5.27) exhibited allylic coupling with the C-5 methine proton (δ 2.20). The latter showed COSY-45° interactions with the C-6 methylene protons (δ 1.90 and 1.50), which in turn showed cross-peaks with the C-7 methylene protons (δ 1.49 and 1.32). The COSY-45° interactions of the C-7 methylene protons with the C-8 methine proton (δ 1.95) were also observed in the spectrum.

The ^{13}C-NMR spectrum ($CDCl_3$) of **1** showed resonances for all 33 carbons in the molecule. The multiplicities of the carbon signals were determined by DEPT experiments, which showed the presence of seven CH_3, six CH_2, 13 CH, and by difference from the broadband spectrum, seven quaternary carbons. Signals at δ 14.0, 15.3, 15.5, 16.6, and 17.0 could be assigned to the C-21, C-18, C-30, C-31, and C-32 methyl carbons. The olefinic C-1 appeared at δ 125.0, while C-11 resonated at δ 124.9. The downfield signal at δ 76.8 was attributed to C-16. The downfield chemical shift of C-16 was assigned to a geminal hydroxyl functionality. Complete ^{13}C-NMR chemical shift assignments are presented in Table 1.

The 2D inverse HMQC and HMBC NMR spectra of **1** were particulalry informative. The HMQC spectrum showed direct connectivity of H-1 (δ 5.27) with C-1 (δ 125.0). Two ^{1}H/^{13}C couplings of the protons at δ 4.15 were with the C-3 (δ 49.9) and C-16 (δ 76.8) carbons. The ^{1}H/^{13}C one-bond chemical shifts determined by the HMQC spectrum are presented in Table 1.

The HMBC spectrum of **1** was very useful for the chemical shift assignments of the quaternary carbon atoms and for building up of structure **1** from various substructures a−d. The H-2′/H-6′ protons (δ 7.80) (fragment a) showed HMBC interactions with the amidic carbonyl (δ 166.4). H-3 (δ 4.15) (fragment "b") also showed cross-peaks with the amidic carbonyl carbon (δ 166.4). These observations helped to connect C-1′ (fragment a) with the C-3 (fragment b) through the amidic bond. The presence of an aromatic amide bond was also confirmed from the IR spectrum, which afforded a strong absorption band at 1656 cm^{-1}. The allylic long-range heteronuclear interactions of H-1 (δ 5.27) (fragment b) and H-5 (δ 2.20) (fragment d) with the quaternary C-10 (δ 134.0) were also observed in the HMBC spectrum and helped to connect fragment b with d through the vinylic quaternary C-10. H-5 (δ 2.20) and H-3 (δ 4.15) exhibited HMBC interactions with the C-4 (δ 44.7), which suggested that C-3 (fragment b) is connected with C-5 (fragment d) through the quaternary C-4 (δ 44.7). The HOHAHA spectrum (100 ms) showed allylic coupling of H-11 (δ 5.39) with H-8 (δ 1.95) and indicated that C-8 (fragment d) is linked to C-11 (fragment b) through quaternary C-9. H-12 (δ 2.15 and 1.65) (fragment b) and H-17 (δ 1.97) showed HMBC cross-peaks with the quaternary C-13 (δ 49.5), while H-8 (δ 1.95) and H-15 (δ 2.00 and 1.25) exhibited cross-peaks with the C-14 (δ 46.4). These observations helped to connect C-12 (fragment b) with C-17 (fragment c) through the quaternary C-13, while C-8 (fragment d) was linked with C-15 (fragment c) through the quaternary C-14. Important HMBC interactions are shown in Figure 1.

The relative stereochemistries at various chiral centers were established with the help of the NOESY spectrum. H-3α exhibited a NOE cross-peak with H-5α. The C-5 methine proton is invariably α-oriented in *Buxus* alkaloids.[10] The probable conformations of rings A, B, C, and D, as obtained from the NOESY spectrum and important NOE interactions of **1**, are shown in Figure 2.

Table 1. ^{1}H/^{13}C One-Bond Correlations in (+)-16α-Hydroxybenzoylbuxadine (**1**) As Determined from the HMQC Spectrum

carbon no.	^{13}C-NMR chemical shift (δ)	multiplicity (DEPT)	^{1}H-NMR chemical shift (δ)
C-1	125.0	CH	5.27
C-2	29.6	CH_2	2.10 and 2.00
C-3	49.9	CH	4.15
C-4	44.7	−C−	
C-5	47.2	CH	2.20
C-6	26.7	CH_2	1.90 and 1.50
C-7	27.4	CH_2	1.49 and 1.32
C-8	48.4	CH	1.95
C-9	126.2	−C−	
C-10	134.0	−C−	
C-11	124.9	CH	5.39
C-12	29.5	CH_2	2.15 and 1.65
C-13	49.5	−C−	
C-14	46.4	−C−	
C-15	36.6	CH_2	2.00 and 1.25
C-16	76.8	CH	4.15
C-17	47.0	CH	1.97
C-18	15.3	CH_3	0.74
C-19	45.2	CH_2	2.77
C-20	62.0	CH	1.99
C-21	14.0	CH_3	1.29
C-30	15.5	CH_3	0.96
C-31	16.6	CH_3	0.97
C-32	17.0	CH_3	1.23
$N(CH_3)_2$	38.9	CH_3	2.27
$N_aC{=}O$	166.4	−C−	
C-1′	141.1	−C−	
C-2′	126.8	CH	7.80
C-3′	128.5	CH	7.39
C-4′	132.4	CH	7.43
C-5′	128.5	CH	7.39
C-6′	126.8	CH	7.80

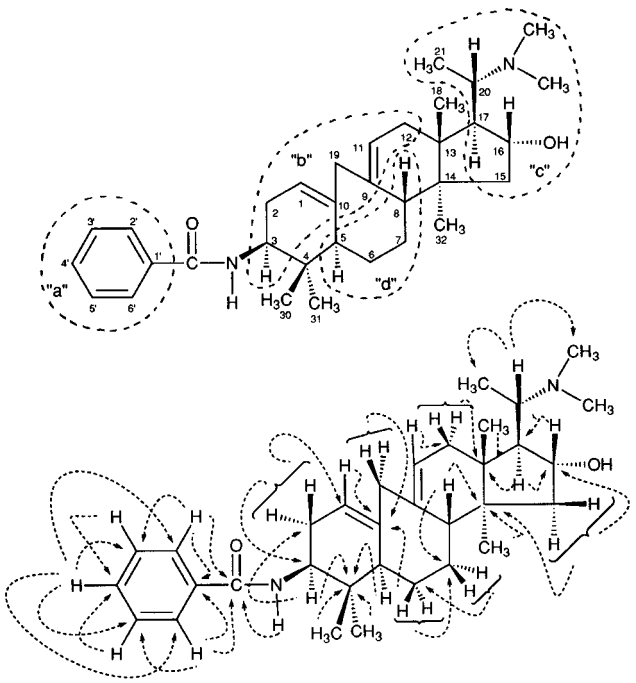

Figure 1. Isolated spin systems (a−d) and important HMBC interactions for compound **1**.

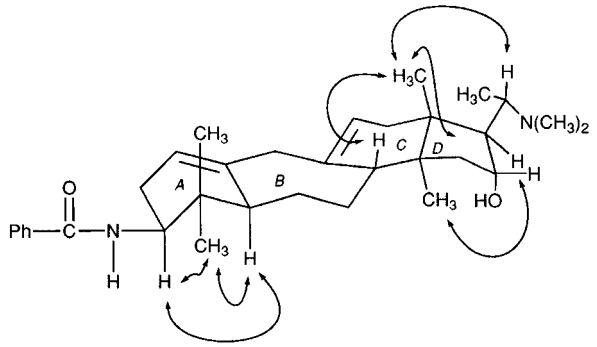

Figure 2. Probable conformation and observed NOE interactions for **1**.

The HREIMS of **1** showed a molecular ion at *m/z* 504.3708, which was in agreement with the molecular formula $C_{33}H_{48}N_2O_2$ (calcd 504.3716), establishing 11 degrees of unsaturation in the molecule. The peak at *m/z* 489.3487 indicated the loss of a methyl group from the molecular ion. A peak at *m/z* 183.1620, *retro* Diels−Alder cleavage of ring C, indicated a double bond in ring C. Another peak at *m/z* 105.0384 indicated the loss of a benzoyl cation. Compound **1** gave a base peak at *m/z* 72.0807 consistent with the *N*,*N*-dimethylamino substituent at C-20 of the ring D side chain.

Compound **1** showed antibacterial activity against *Proteus mirabllis*, *Salmonella typhi*, *Shigella flexnerii*, *Proteus vulgaris*, and *Escherichia coli* at concentrations of 200 μg/100 μL and antifungal activity against *Stachybotrys atra*, *Microsporum canis*, and *Epidermophyton floccosum* at concentrations of 200 μg/μL. Compound **1** also exhibited 46%, 28.6%, and 20.3% phytotoxicity against *Lemna minor* L. at concentrations of 500, 50, and 5 ppm, respectively.

(+)-Semperviraminone (**2**), $C_{26}H_{39}NO$, was isolated as a colorless gum. The UV spectrum showed an absorption at 240 nm suggestive of an α,β-unsaturated carbonyl chromophore.[11] The IR spectrum afforded strong absorptions at 1654 (α, β-unsaturated carbonyl) and 1595 (C=C) cm^{-1}.

The ^{1}H-NMR spectrum of **2** featured four tertiary methyl signals at δ 0.71, 0.73, 0.91, and 0.92. A 3H sharp singlet centered at δ 2.26 was assigned to the C-21 methyl protons α to the C-20 carbonyl function. Another 6H singlet at δ 2.27 was due to the *N*-methyl protons at C-3 of ring A. The C-11 olefinic proton resonated as a broad singlet at δ 5.65, while another 1H broad singlet at δ 5.89 was ascribed to the C-1 olefinic proton. A 1H double doublet centered at δ 6.66 (J_1 = 3.9 Hz, J_2 = 2.1 Hz) was assigned to the C-16 vinylic proton. The allylic C-19 methylene protons resonated at δ 2.98.

The COSY-45° spectrum of **2** showed cross-peaks between the C-1 olefinic proton (δ 5.89) and the C-2 methylene protons (δ 1.90 and 1.75), which in turn showed COSY-45° interactions with the C-3 methine proton (δ 2.93). The vicinal coupling of H-11 (δ 5.65) with the C-12 methylene protons (δ 1.85 and 1.45) was also observed. H-16 (δ 6.66) showed cross-peaks with the C-15 methylene protons (δ 1.60 and 1.35). The NOESY spectrum exhibited cross-peaks between H-16 (δ 6.66) and C-21 methyl protons (δ 2.26), confirming the *cisoid* conformation of the C-21 methyl group along the C-17/C-20 bond.

The HREIMS of **2** showed the molecular ion at *m/z* 381.3037 corresponding to the molecular formula $C_{26}H_{39}NO$ (calcd 381.3031), indicating eight double-bond equivalents in the molecule. The peak at *m/z* 366.2799 was due to loss of a methyl group from the molecular ion. Another peak at *m/z* 338.2846 was indicative of the loss of an acetyl group, while the fragment at *m/z* 136.0879 arose by the *retro* Diels−Alder cleavage of the ring C, which further indicated the presence of a double bond in the ring C. The base peak at *m/z* 71.0733 was consistent with a dimethylamino substituent at C-3 of ring A. These spectroscopic studies led to structure **2** for this new steroidal base.

(+)-N_a-Demethylsemperviraminone (**3**), $C_{25}H_{37}NO$, was isolated as a colorless gummy material. The ^{1}H-NMR, UV, and IR spectra of compound **3** were distinctly similar to those of **2**. The ^{1}H-NMR spectrum of compound **3**, however, featured a 3H singlet at δ 2.73 for the *N*-methyl protons.

The HREIMS of **3** showed the molecular ion at *m/z* 367.2868, which corresponded to molecular formula $C_{25}H_{37}NO$ and suggested the presence of eight degrees of unsaturation. The base peak at *m/z* 57.0577 was due to the loss of $[CH_2CH{=}NHCH_3]^{\cdot+}$ from M^+. The spectrum also exhibited a similar fragmentation pattern as that of **2** but many ions were at 14 amu lower *m/z* values. These spectroscopic studies led to the conclusion that compound **3** was the N_a-demethyl derivative of (+)-semperviraminone (**2**).

(+)-Buxaminol C (**4**), $C_{27}H_{46}N_2O$, was also isolated as a colorless gummy material. The UV spectrum showed absorptions at 235 and 247 nm with shoulders at 230 and 251 nm, indicating the presence of a 9(10→19) *abeo* diene system.[11] The IR spectrum displayed intense absorptions at 3621 (OH), 3309 (NH), 2931 (CH), and 1623 (C=C) cm^{-1}.

The ^{1}H-NMR spectrum of **4** showed four 3H singlets at δ 0.67, 0.68, 0.87, and 1.01 for the four tertiary methyl protons. A 3H doublet centered at δ 0.95 ($J_{21,20}$

= 6.6 Hz) was due to the C-21 secondary methyl protons. The $N_b(CH_3)_2$ protons resonated as a 6H singlet at δ 2.26, while the N_a-methyl protons also appeared as a 3H singlet at δ 2.44. A 1H multiplet at δ 4.42 was ascribed to the C-16 methine proton, geminal to the hydroxyl group. The C-11 olefinic proton resonated as a broad singlet at δ 5.50, while another 1H sharp singlet at δ 5.91 was ascribed to the C-19 vinylic proton.

The HREIMS of **4** showed the molecular ion at *m/z* 414.3612 in agreement with the molecular formula $C_{27}H_{46}N_2O$ (calcd 414.3609). The peak at *m/z* 399.3372 was due to the loss of a methyl group from the M^+. Compound **4** showed the base peak at *m/z* 72.0811, which arose by the characteristic cleavage of the *N*,*N*-dimethylamino substituent-containing side chain on ring D. The peak *m/z* 57.0576 was due to the cleavage of ring A. These spectroscopic studies led to assignment of structure **4** to this new steroidal alkaloid.

Four known steroidal alkamines, (−)-(*E*)-cyclobuxaphylamine (**5**), (−)-(*Z*)-cyclobuxaphylamine (**6**), (+)-cyclomicrobuxeine (**7**), and (+)-papilamine (**8**), were also isolated for the first time from the roots of *B. sempervirens*. The UV, IR, ^{1}H-NMR, and mass spectra of compounds **5**, **6**, **7**, and **8** were nearly identical with those of (*E*)-cyclobuxaphylamine, (−)-(*Z*)-cyclobuxaphylamine, (+)-cyclomicrobuxeine, and (+)-papilamine reported in the literature.[12,14] Compounds **5**, **6**, and **8** were previously isolated from *B. papillosa*,[12,13] while compound **7** was obtained from *B. microphylla*.[14]

Experimental Section

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

Plant Material. The roots of *B. sempervirens* (10 kg) 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 no. 1243) was deposited in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Extraction and Isolation. The roots of *B. sempervirens* (8 kg) were dried, crushed, and extracted with ethanol (100 L) at room temperature. An ethanolic extract of the air dried roots of the *B. sempervirens* was concentrated to a gum (160 gm) under reduced pressure, which was then dissolved in distilled H_2O. The aqueous extract was extracted with $CHCl_3$ at different pH values, in order 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% acetic acid or ammonium hydroxide. The $CHCl_3$ fraction obtained at pH ~3.5 (2.7 gm) was loaded onto a Si gel column (500 gm) and eluted with pure petroleum ether (40−60 °C), mixtures of petroleum ether−$CHCl_3$, and then with mixtures of $CHCl_3$−MeOH of increasing polarities. The fraction obtained on elution with 20% petroleum ether−80% $CHCl_3$ was subjected to preparative TLC using petroleum ether−diethyl ether−diethylamine (9:1:0.5) to yield **1** (R_f = 0.89). The fraction obtained on elution of the Si gel column with 90% $CHCl_3$−10% MeOH by preparative TLC using petroleum ether−diethyl ether−diethylamine (9:1:0.5) as solvent system afforded compounds **2** (R_f = 0.58) and **3** (R_f = 0.56). Compounds **5** and **6** were isolated from a fraction that was obtained on elution of the Si gel column with 60% $CHCl_3$−40% MeOH. This fraction on preparative TLC using petroleum ether−acetone−diethylamine (7.5:2.5:0.5) as the developing solvent yielded the known compounds **5** (R_f = 0.73) and **6** (R_f = 0.68). The fraction obtained on elution of the Si gel with 70% $CHCl_3$−30% MeOH was subjected to preparative TLC using petroleum ether−acetone−diethylamine (7.5:2.5:0.1) to afford compound **7** (R_f = 0.54).

The basic fraction (18.3 g) obtained at pH ~9.5 was also loaded on a Si gel column (500 g), and elution was made with petroleum ether, petroleum ether−$CHCl_3$, $CHCl_3$, and then with increasing polarities of $CHCl_3$−MeOH. The fraction obtained on elution with 20% petroleum ether−80% $CHCl_3$ was subjected to TLC using petroleum ether−diethyl ether−diethylamine (3:6:01) as solvent system to yield **4** (R_f = 0.43) and **8** (R_f = 0.53).

Antibacterial Activity. Antibacterial activities were performed by the agar well diffusion method using Mueller Hinton Agar medium. The radius of the each well was 5 mm for both 100 μg/100 μL and 200 μg/100 μL using ampicillin as standard antibiotic.[15]

Antifungal Activity. Antifungal activity of the compounds was measured by the tube dilution method.[16] A solution of each compound in DMSO was added to molten Sabouraud dextrose Agar (SDA) to prepare slants. The slants were inoculated with the fungi and incubated at 29 °C for 7 days. Inhibition of the growth was observed, and MIC values were determined against a standard antifungal compound (griseofulvin) on the 8 day.

(+)-16α-Hydroxy-N_a-benzoylbuxadine (**1**) was obtained as a colorless amorphous solid (13 mg): $[\alpha]^{20}_D$ +123° (*c* 0.8, $CHCl_3$); UV(MeOH) λ_{max} 228 nm; IR ($CHCl_3$) ν_{max} 3416, 3310, 1656, 1594 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) see Table 1; ^{13}C-NMR ($CDCl_3$, 75 MHz) δ see Table 1; EIMS *m/z* 504 $[M]^+$ (3.4), 489 (7.5), 183 (14), 105 (45), 72 (100); HREIMS *m/z* 504.3708 (calcd for $C_{33}H_{48}N_2O_2$, 504.3716), 489.3487 (calcd for $C_{33}H_{45}N_2O_2$, 489.3485), 183.1620 (calcd for $C_{11}H_{21}NO$, 183.1623), 105.0384 (calcd for C_7H_5O, 105.0340), 72.0807 (calcd for $C_4H_{10}N_2$, 72.0813).

(+)-Semperviaminone (**2**) was isolated as a colorless gummy material (6.3 mg): $[\alpha]^{20}_D$ +0.49° (*c* = 1.20, $CHCl_3$); UV (MeOH) λ_{max} 240 nm; IR ($CHCl_3$) ν_{max} 1654, 1595 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, CH_3), 0.72 (3H, s, CH_3), 0.91 (3H, s, CH_3), 0.92 (3H, s, CH_3), 2.26 (3H, s, O=CCH_3), 2.27 (6H, s, $N_a(CH_3)_2$), 2.98 (2H, br s, H_2-19), 5.65 (1H, br s, H-11), 5.89 (1H, br. s, H-1), 6.66 (1H, dd, J_1 = 3.9 Hz, J_2 = 2.1 Hz, H-16); EIMS *m/z* 381 $[M]^+$ (46), 366 (25), 338 (25), 136 (35), 71 (100); HREIMS *m/z* 381.3037 (calcd for $C_{26}H_{39}NO$, 381.3031), 366.2799 (calcd for $C_{25}H_{36}NO$, 366.2797),

338.2846 (calcd for $C_{24}H_{36}N$, 338.2848), 136.0879 (calcd for $C_9H_{12}O$, 136.0888), 71.0733 (calcd for C_4H_9N, 71.0735).

(+)-N_a-Demethylsemperviraminone (**3**) was obtained as a colorless gummy material (5.9 mg): $[\alpha]^{20}_D$ +0.45° (c = 0.76, $CHCl_3$); UV (MeOH) λ_{max} 240 nm; IR ($CHCl_3$) ν_{max} 1656, 1645 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.73 (3H, s, CH_3), 0.92 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.31 (3H, s, CH_3), 2.26 (3H, s, O=CCH_3), 2.73 (3H, s, N_a-CH_3), 2.99 (2H, br s, H_2-19), 5.62 (1H, br s, H-11), 5.80 (1H, br s, H-1), 6.66 (1H, dd, J_1 = 3.6 Hz, J_2 = 2.3 Hz, H-16); EIMS m/z 367 $[M]^+$ (15), 352 (9), 324 (25), 136 (35), 47 (100); HREIMS m/z 367.2868 (calcd for $C_{25}H_{37}$-NO, 367.2875), 352.2644 (calcd for $C_{24}H_{34}NO$, 352.2640), 324.2688 (calcd for $C_{23}H_{34}N$, 324.2691), 136.0884 (calcd for $C_9H_{12}O$, 136.0888), 57.0577 (calcd for C_3H_7N, 57.0578).

(+)-Buxaminol C (**4**) was isolated as a colorless gummy material (5.4 mg): $[\alpha]^{20}_D$ +88° (c = 0.65, $CHCl_3$); UV (MeOH) λ_{max} 230 (sh), 235, 247, 251 (sh) nm; IR ($CHCl_3$) ν_{max} 3621, 3309, 2931, 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.67 (3H, s, CH_3), 0.68 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.95 (3H, d, $J_{21,20}$ = 6.6 Hz, H-21), 1.01 (3H, s, CH_3), 2.26 (6H, s, $N_b(CH_3)_2$), 2.44 (3H, s, N_aCH_3), 2.97 (1H, m, H-3), 4.42 (1H, m, H-16), 5.50 (1H, br s, H-11), 5.91 (1H, s, H-19); EIMS m/z 414 $[M]^+$ (4), 399 (3.6), 72 (100), 57 (40); HREIMS m/z 414.3612 (calcd for $C_{27}H_{46}N_2O$, 414.3609), 399.3372 (calcd for $C_{26}H_{43}N_2O$, 399.3375), 72.0811 (calcd for $C_4H_{10}N$, 72.0812), 57.0576 (calcd for C_3H_7N, 57.0578).

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