New Steroidal Alkaloids from *Buxus longifolia*

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Four new steroidal alkaloids, (+)-cyclovirobuxeine F (**1**), *N*-benzoyl-*O*-acetylbuxalongifoline (**2**), buxasamarine (**3**), and (+)-cyclobuxamidine (**4**), along with two known steroidal bases, 16α-acetoxybuxabenzamidienine (**5**) and *trans*-cyclosuffrobuxinine (**6**), were isolated from the leaves of *Buxus longifolia*. The alkaloids **1**−**4** showed significant antibacterial activity.

Extracts of *Buxus* species have been used in the indigenous system of medicine for the treatment of a number of diseases including skin diseases, rheumatism, and malaria.[1] Phytochemical investigations on various *Buxus* species have resulted in the isolation of a large number of steroidal bases.[2−6] Our previous work on the leaves of *Buxus longifolia* Boiss (Buxaceae) of Turkish origin has resulted in the isolation of three new steroidal alkaloids.[7,8] Herein we describe the isolation and structure determination of four new steroidal bases from the same plant source, (+)-cyclovirobuxeine F [(20*S*)-16α-acetoxy-3β-(benzoylamino)-20-(dimethylamino)-31,6α-epoxy-9,10-*seco*-buxa-9(11),10(19)-diene] (**1**); *N*-benzoyl-*O*-acetylbuxalongifoline [(20*S*)-16α-acetoxy-3β-(benzoylamino)-20-(dimethylamino)-31-oxo-9,10-*seco*-buxa-9(11),10(19)-diene] (**2**); buxasamarine [(20*S*)-16α-hydroxy-3β-(dimethylamino)-20-(methylamino)-buxane] (**3**); and (+)-cyclobuxamidine [(20*S*)-3β-(*N*-methylacetylamino)-20-(methylamino)-31-norbuxa-4(30)-ene] (**4**), along with two known steroidal bases, 16α-acetoxybuxabenzamidienine [(20*S*)-16α-acetoxy-3β-(benozylamino)-20-(dimethylamino)-9,10-*seco*-buxa-9(11),-10(9)-diene] (**5**) and *trans*-cyclosuffrobuxinine [3β-(methylamino)-16-oxo-31-norbuxa-4(30),17(20)(*cisoid*)-diene] (**6**), from leaves of *B. longifolia*. Alkaloids **5** and **6** are reported for the first time from this plant.

Results and Discussion

The EtOH extract of the leaves of *B. longifolia*, when processed by column chromatography and preparative TLC, led to the isolation of four new alkaloids, (+)-cyclovirobuxeine F (**1**), *N*-benzoyl-*O*-acetylbuxalongifoline (**2**), buxasamarine (**3**), and (+)-cyclobuxamidine (**4**).

(+)-Cyclovirobuxeine F (**1**) had the molecular composition $C_{35}H_{48}N_2O_4$ as inferred from the HREIMS, which exhibited the M^+ at *m*/*z* at 560.3531 (calcd 560.3539), indicating 13 double-bond equivalents in the molecule. The MS gave an ion at *m*/*z* 545 resulting from loss of a CH_3 group from the M^+. Ions at *m*/*z* 171 and 157 were due to cleavage of ring D along with the nitrogen-containing side chain at C-17. These ions suggested that C-16 is the probable position of the acetoxy substituent on ring D.[4] The base peak at *m*/*z* 72 represents the trimethyliminium cation, $H_3C-CH=N^+(CH_3)_2$. An ion at *m*/*z* 105 is consistent with a $Ph-C\equiv O^+$ fragment.

The ^{1}H-NMR spectrum of **1** showed three tertiary CH_3 singlets at δ 0.69, 0.73, and 1.90, while the secondary CH_3 resonated as a doublet at δ 1.41 ($J_{21,20}$ = 6.0 Hz). These observations favor a cycloartenol-type triterpenoid skeleton, as described in other *Buxus* alkaloids.[3−6] The amidic NH doublet at (δ 6.60) and 5H multiplets between δ 7.45 and 7.85 indicated a benzamidic substituent. The amidic NH showed coupling with a methine proton that resonated as a multiplet at δ 4.85 attributed to H-3, which showed vicinal couplings with H-2 (δ 1.70, 1.81), which were in turn coupled with the H-1 (δ 2.67). Two AB doublets resonating at δ 3.64 and 3.70 ($J_{31\alpha,31\beta}$ = 8.6 Hz) were assigned to an oxygen-bearing CH_2 (H-31). The downfield CH multiplet at δ 4.90 and a CH_3 singlet at δ 2.13 indicated an acetate function probably at C-16 on ring D. Another downfield multiplet at δ 4.86 was assigned to the CH proton of the oxygen-bearing C-6. The ethereal nature of the C-31 oxygen was initially inferred from the molecular composition, which showed four oxygen atoms. Three of these were accounted for by acetate and amide functionalities. The remaining oxygen was accounted for by an ether link between two carbon atoms that appeared downfield at δ 78.6 and 75.2. This was further confirmed by ^{1}H-NMR in pyridine-d_5. The C-31 CH_2 protons showed only a slight paramagnetic shift from δ 3.64 to 3.74 and from δ 3.70 to 3.76. The C-6 CH also showed only a slight paramagnetic shift from δ 4.86 to 5.00. These weak paramagnetic shifts also indicated the presence of an ether linkage between C-31 and C-6, inasmuch as more pronounced solvent-induced downfield chemical shifts (ca. 1.0 ppm) would have been expected in case of an −OH substitutent as reported earlier.[4,9] The H-3 (δ 4.85), showed a weak *W* (1,3-*cis*-diaxial) coupling with the H-5 (δ 2.07) in the COSY-45° spectrum. Couplings among H-5/H-6, H-6/H-7, and H-7/H-8 were also observed in the COSY spectrum. A broad singlet that appeared at δ 5.46 was assigned to the vinylic proton (H-11), and the broad singlet at δ 5.87 was assigned to the olefinic proton (H-19). H-19 showed a weak allylic coupling with H-11 (olefinic), which in turn showed vicinal coupling with H_2-12 (δ 2.10). These observations suggested the presence of an *abeo*-diene system, which was confirmed by the characteristic UV absorptions.[10]

A broad-band decoupled ^{13}C-NMR spectrum of **1**

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Table 1. ^{1}H- and ^{13}C-NMR Chemical Shift Data ($CDCl_3$) of Compounds **1**, **2**, and **3**

	1		**2**		**3**	
carbon	^{13}C δ (mult, DEPT)	^{1}H δ (mult, J = Hz)	^{13}C δ (mult, DEPT)	^{1}H δ (mult, J = Hz)	^{13}C δ (mult, DEPT)	^{1}H δ (mult, J = Hz)
1	37.5 (CH_2)	2.67 (m)	39.1 (CH_2)	1.80 (m)	127.0 (CH)	5.62 (d) ($J_{1,2}$ = 10.5)
2	34.2 (CH_2)	H_α = 1.70 (m) H_β = 1.70 (m)	25.1 (CH_2)	2.05 (m) 2.20 (m)	128.0 (CH)	5.40 (m)
3	56.6 (CH)	4.85 (m)	56.8 (CH)	4.30 (m)	71.3 (CH)	2.50 (m)
4	44.8 (C)		48.7 (C)		41.3 (3)	
5	42.2 (CH)	2.07 (m)	50.5 (CH)	1.40 (m)	48.7 (CH)	1.87 (m)
6	75.2 (CH)	4.86 (m)	29.7 (CH_2)	1.24 (m) 1.32 (m)	20.0 (CH_2)	1.50 (m)
7	26.8 (CH_2)	2.10 (m)	36.7 (CH_2)	1.82 (m) 1.90 (m)	31.1 (CH_2)	1.25 (m)
8	47.6 (CH)	2.00 (m)	42.1 (CH)	2.00 (m)	44.1 (CH)	2.00 (m)
9	134.7 (C)		134.0 (C)		19.0 (C)	
10	133.5 (C)		137.0 (C)		20.0 (C)	
11	127.3 (CH)	5.46 (m)	126.0 (CH)	5.54 (m)	24.9 (CH_2)	1.85 (m) 1.92 (m)
12	43.7 (CH_2)	2.10 (m)	37.5 (CH_2)	2.15 (m) 2.25 (m)	31.7 (CH_2)	1.70 (m)
13	46.2 (C)		44.0 (C)		45.3 (C)	
14	48.8 (C)		47.0 (C)		49.7 (C)	
15	32.9 (CH_2)	1.50 (m) 1.61 (m)	42.6 (CH_2)	2.12 (m)	42.1 (CH_2)	2.00 (m)
16	75.2 (CH)	4.90 (m)	79.3 (CH)	5.10 (m)	77.7 (CH)	4.24 (m)
17	53.6 (CH)	2.05 (m)	56.4 (CH)	2.03 (m)	59.8 (CH)	1.75 (m)
18	16.0 (CH_3)	0.69 (s)	16.7 (CH_3)	0.85 (s)	17.8 (CH_3)	0.90 (s)
19	128.4 (CH)	5.87 (s)	128.0 (CH)	5.90 (s)	18.25 (CH_2)	−0.11 (d) ($J_{19\alpha,19\beta}$ = 4.1) 0.70 (d) ($J_{19\beta,19\alpha}$ = 4.1)
20	65.5 (CH)	3.20 (m)	65.4 (CH)	3.00 (m)	58.8 (CH)	2.85 (m)
21	12.4 (CH_3)	1.41 (d) ($J_{21,20}$ = 6.0)	11.2 (CH_3)	1.28 (d) ($J_{21,20}$ = 6.5)	15.1 (CH_3)	1.12 (d) ($J_{21,20}$ = 6.1)
30	19.9 (CH_3)	1.90 (s)	21.0 (CH_3)	1.80 (s)	26.0 (CH_3)	1.15 (s)
31	78.6 (CH_2)	Hα = 3.64 (d) ($J_{31\alpha,31\beta}$ = 8.6) H_β = 3.70 (d) ($J_{31\beta,31\alpha}$ = 8.6)	201.0 (CH)	9.53 (s)	15.5 (CH_3)	1.05 (s)
32	17.5 (CH_3)	0.73 (s)	17.6 (CH_3)	0.84 (s)	16.5 (CH_3)	0.77 (CH_3)
N_a-CH_3					31.7 (CH_3)	2.27 (s)
N_b-CH_3	36.0 (CH_3)	2.65 (s)	46.7 (CH_3)	2.70 (s)	32.6 (CH_3)	2.40 (s)
N_b-CH_3	43.4 (CH_3)	2.65 (s)				
C-1′	132.0 (C)		137.0 (C)			
C-2′	128.7 (CH)	7.85 (m)	128.0 (CH)	7.71 (m)		
C-3′	130.1 (CH)	7.45 (m)	129.3 (CH)	7.40 (m)		
C-4′	131.0 (CH)	7.75 (m)	131.3 (CH)	7.46 (m)		
C-5′	130.1 (CH)	7.45 (m)	129.3 (CH)	7.40 (m)		
C-6′	128.7 (CH)	7.85 (m)	128.0 (CH)	7.71 (m)		
Ph-CO-N	171.3 (C)		166.0 (C)			
H_3C-CO-	177.3 (C)		170.3 (C)			
H_3C-CO	21.2 (CH_3)	2.13 (s)	21.5 (CH_3)	2.07 (s)		

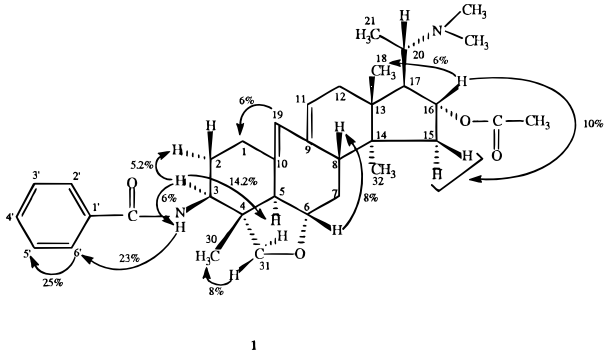
showed 35 carbon resonances. The DEPT spectra indicated that there were seven CH_3, six CH_2, fourteen CH, and (by difference from the broad-band decoupled spectrum) eight quaternary carbon atoms. Signals for the vinylic C-11 and C-19 appeared at δ 127.3 and 128.4. Two downfield quaternary signals at δ 171.3 and 177.3 were assigned to the benzamidic and ester carbonyl carbons. One-bond ^{1}H−^{13}C correlations were established by heteronuclear multiple quantum coherence (HMQC) NMR experiments.[11−14] The chemical shifts and various proton−carbon connectivities are given in Table 1.

The long-range ^{1}H−^{13}C connectivities were determined from an HMBC spectrum.[12,14] A CH carbon resonating at δ 56.6 (C-3) was coupled to protons resonating at δ 3.64 and 3.70 (H-31α and β) and 1.70 (H-2α). The C-4 quaternary carbon resonating the δ 44.8 exhibited couplings with the protons resonating at δ 4.85 (H-3) and 2.07 (H-5), confirming that C-3 is linked to C-4. Similarly, the olefinic C-10 (δ 133.5) exhibited couplings with the protons resonating at δ 2.67 (H-1) and 2.07 (H-5). The quaternary carbon at δ 46.2 (C-13) exhibited heteronuclear interaction with the protons at δ 2.10 (H_2-12). The acetoxy-bearing C-16 CH proton (δ 4.90) showed $^2J_{CH}$ couplings with C-17 (δ 53.6) and C-15 (δ 32.9), and ^3J coupling with C-20 (δ 65.5) indicated that the acetoxy group was present at the C-16 position. ^{1}H−^{13}C long-range connectivities in **1** are summarized in Table 2.

NOE difference NMR measurements were carried out to determine the relative stereochemistry at various asymmetric centers in **1**. For example when H-8β (δ 2.00) was irradiated, an 8% NOE was observed at δ 4.86 (H-6), indicating the β-orientation of H-6, and therefore, C-6 ether linkage is α-oriented. When H-31 (δ 3.70) was irradiated, an 8% NOE was observed on H-30 (δ 1.90), which is β-oriented; therefore, H-31 was inferred to be α-oriented. Similarly, when H-3 (δ 4.85) was irradiated, a 14.2% NOE was observed at δ 2.07 (C-5α) and a 5.2% NOE at δ 1.70 (equatorial H-2α), indicating α- (pseudo axial) stereochemistry of the C-3 proton. Irradiation of H-16 (δ 4.90) resulted in a 6% NOE at δ 0.69 (βCH_3-18) and no NOE effect on the α-oriented C-17 proton, indicating that the proton at C-16 was β-oriented and that the acetoxy substituent was α-oriented. Other

Table 2. HMBC Correlations for **1** and **3**

1			**3**		
^{1}H (δ)	$^2J_{HC}$ (δ)	$^3J_{HC}$ (δ)	^{1}H (δ)	$^2J_{HC}$ (δ)	$^3J_{HC}$ (δ)
H-2′, H6′ (7.85)		171.3 (amide, C=O)			
		131.0 (C-4′)	H-1 (5.62)	20.0 (C-10)	48.7 (C-5)
H3′ (7.75)	131.0 (C-4′)		H-2 (5.40)	127.0 (C-1)	20.0 (C-10)
				71.33 (C-3)	
H-3′,5′ (7.75)	128.7 (C-2′,C-6′)	132.0 (C-1′)	H-16 (4.24)	59.8 (C-17)	45.35 (C-13)
				42.1 (C-15)	49.7 (C-14)
H-11 (5.46)	43.7 (C-12)	47.6 (C-8)	H-3 (2.50)	128.0 (C-2)	127.0 (C-1)
				41.3 (C-4)	
H-16 (4.90)	32.9 (C-15)	65.5 (C-20)			
	53.6 (C-17)				
H-3 (4.85)	44.8 (C-4)	78.6 (C-31)	H-8 (2.00)	19.0 (C-9)	45.3 (C-13)
		171.3 (amide, C=O)		49.7 (C-14)	
				31.1 (C-7)	
H-31 βH (3.70)	44.8 (C-4)	19.9 (C-30)	H-5 (1.87)	20.0 (C-6)	31.1 (C-7)
		56.6 (C-3)		41.3 (C-4)	127.0 (C-1)
		75.2 (C-6)			
H-31 αH (3.64)	44.8 (C-4)	56.6 (C-3)	H-11 (1.85)	31.7 (C-12)	18.2 (C-19)
		42.2 (C-5)		19.0 (C-9)	
		75.2 (C-6)			
H-1 (2.67)	133.5 (C-10)				
	34.2 (C-2)				
H-12 (2.10)	46.2 (C-13)		H-12 (1.70)	45.3 (C-13)	59.8 (C-17)
H-5 (2.07)	44.8 (C-4)		H-7 (1.25)	44.1 (C-8)	48.7 (C-5)
	133.5 (C-10)			20.0 (C-6)	
H-8 (2.00)	48.8 (C-14)		H-30 (1.15)	41.3 (C-4)	15.5 (C-31)
					71.3 (C-3)
H-2α (1.70)	56.6 (C-3)		H-31 (1.05)	41.3 (C-4)	48.7 (C-5)

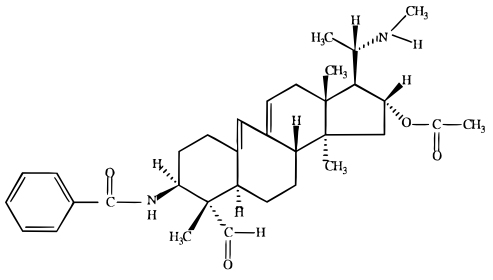


1

Figure 1. NOE connectivities observed in **1**.

important NOE interactions are delineated around Figure 1.

The second compound, *N*-benzoyl-*O*-acetylbuxalongifoline (**2**), was found to be similar to (+)-buxanaldinine present in the literature.[4] The M^+ at *m/z* 546.3461 (calcd 546.3455) in HREIMS, corresponding to molecular formula $C_{34}H_{46}N_2O_4$, and indicating 13 double-bond equivalents in the molecule. The mass fragmentation pattern of **2** was distinctly similar to the known alkaloid 16α-acetoxybuxabenzamidienine (**5**). Both compounds gave a fragment ion at *m/z* 105 due to cleavage of a benozyl group. Compound **2** has an N_b-CH_3 group at C-20 position, while **5** has a N_b-$(CH_3)_2$ group. Thus, the fragment ions obtained from cleavage of the C-17/C-20 bond have different *m/z* values. Compound **2** gave the base peak at *m/z* 58, due to the dimethyliminium ion $H_3C-CH=^+NHCH_3$, while **5** gave the base peak at *m/z* 72 due to the trimethyliminium ion $H_3C-CH=N^+-(CH_3)_2$. Similarly, compound **2** yielded two fragment ions at *m/z* 143 and 157, due to the cleavage of ring D, which indicated an acetate group at C-16. Compound **5**, on the other hand gave two fragment ions at *m/z* 157 and 171 due to the similar cleavages of ring D. The only difference in the composition of the fragment ions was the lack of one CH_3 group in **2**. The ^{1}H-NMR spectrum of **2** was also found to be distinctly similar to **5**, with



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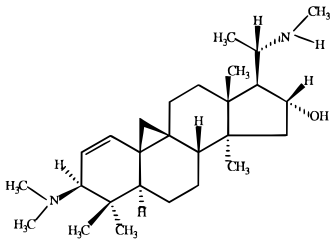

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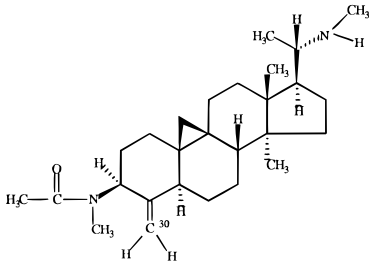

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Table 3. Antibacterial Activity of Compounds **1**, **2**, **3** and **4**

test bacteria	compound	zone of inhibition in nm 100 μg/100 μL	zone of inhibition in nm 200 μg/100 μL	MIC
Salmonella typhi	**1**	7	8	100 μg/100 μL
	2	6	8	100 μg/100 μL
	3	6	8	100 μg/100 μL
	4	6	6	150 μg/100 μL
	ampicillin[a]	10	11	30 μg/100 μL
Shigella flexneri	**1**	7	7	100 μg/100 μL
	2	6	6	200 μg/100 μL
	3		7	150 μg/100 μL
	ampicillin	11	11	30 μg/100 μL
Pseudomonas aeruginosa	**1**	6	8	10 μg/100 μL
	2	8	9	50 μg/100 μL
	3	7	9	50 μg/100 μL
	ampicillin	11	11	40 μg/100 μL
Escherichia coli	**4**		6	200 μg/100 μL
	ampicillin	11	12	50 μg/100 μL

[a] Ampicillin was used as standard drug.

the difference that the ^{1}H-NMR spectrum of **2** showed the presence of an aldehydic group (δ 9.53), most likely at C-4,[4] and lack of one N-CH_3.

^{13}C-NMR spectra of **2** showed resonances for all 34 carbons, including six CH_3, six CH_2, 14 CH, and eight quaternary carbon atoms. There were three signals of carbonyl carbons, that is, the carbon of acetyl carbonyl appeared at δ 170.3, amide carbonyl at δ 166.0, and aldehyde carbonyl carbon at δ 201.0. The chemical shifts of various carbons and their one-bond heteronuclear connectivities are presented in Table 1. The above spectroscopic studies led to structure for this new steroidal base, *N*-benzoyl-*O*-acetylbuxalongifoline.

A HREIMS of **3** showed the M^+ at *m/z* 414.3600 (calcd 414.3609) corresponding to the molecular formula $C_{27}H_{46}N_2O$, indicating six double-bond equivalents in the molecule. The base peak at *m/z* 58 was attributed to the fragment ion, $H_3C-CH=^+NC\ CH_3$. Two fragment ions at *m/z* 101 and 115 indicated that the OH group was at C-16 in ring D.[5]

The ^{1}H-NMR spectrum of **3** revealed four tertiary CH_3 groups at δ 0.77, 0.90, 1.05, and 1.15, while the secondary CH_3 resonated as a doublet at δ 1.12 ($J_{21,20}$ = 6.1 Hz). A doublet at δ 5.62 was assigned to the vinylic proton (H-1), and it showed coupling with the H-2 vinylic proton (δ 5.40); H-3 (δ 2.50) showed vicinal coupling with H-2 (δ 5.40) and *W* (1,3-*cis*-diaxial) coupling with H-5 (δ 1.87). The H-19Hα resonated as a very upfield doublet at δ −0.11 and showed geminal coupling with H-19β. Geminal coupling between H_2-11 (δ 1.85, 1.92) and their vicinal couplings with H_2-12 (δ 1.70) were also observed. A multiplet at δ 4.24 was assigned to the H-16, which showed couplings with the H-17 (δ 1.75) and H-15 (δ 2.00).

DEPT and broad-band decoupled ^{13}C-NMR spectra of **3** showed resonances of all eight CH_3, six CH_2, eight CH, and five quaternary carbon atoms. A characteristic feature of the broad-band ^{13}C-NMR spectrum was the appearance of downfield olefinic signals at δ 127.0 (C-1) and 128.0 (C-2). The ^{13}C-NMR chemical shifts and one-bond heteronuclear correlations (HMQC) of **3** are presented in Table 1. Table 2 summarizes HMBC interactions.

The spectral data of (+)-cyclobuxamidine (**4**) was similar to that reported for (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine.[15] The HREIMS of **4** exhibited M^+ at *m/z* 412.3129, corresponding to the molecular formula $C_{27}H_{44}N_2O$ (calcd 412.3119) and indicating seven double-bond equivalents in the molecule. The base peak at *m/z* 58 represented the dimethyliminium ion $H_3C-CH=N^+$-HCH_3). Ions at *m/z* 85 and 99 were due to cleavage of the C-13,17/C-15,16 and C-13,17/C-14,15 bonds of ring D along with the nitrogen-containing side chain.

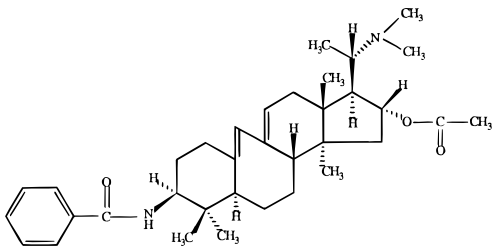
The ^{1}H-NMR spectrum of **4** revealed two tertiary CH_3 groups as two sharp 3H singlets at δ 1.20 and 0.87. The H-21 proton resonated as a doublet at δ 0.98 ($J_{21,20}$ = 6.0 Hz). The appearace of two AB doublets at δ 1.02 and 0.30 ($J_{19\alpha,19\beta}$ = 4.3 Hz) was characteristic of H-19 cyclopropyl protons. H-3 appeared as a multiplet at δ 4.45 and showed *W* (1,3-*cis*-diaxial coupling with H-5 proton in the COSY-45° spectrum. The ^{1}H-NMR spectrum of **4** displayed doubling of many peaks, each integrating for nearly half of the actual integration. For instance, N_a-CH_3 appeared as singlets at δ 2.99 and 3.10. Similarly, the methyl of N_aCOCH_3 showed two singlets of δ 2.01 and 2.15. The exomethylene protons (H-30) also showed doubling of signals and resonated at δ 4.56/4.60 and 4.65/4.70. It was suspected that this doubling of signals in the spectrum of **4** was due to its existence of two conformers (amidic rotamers)[15] at room temperature. Stereochemical assignments at the various asymmetric centers on the skeleton were made on biosynthetic considerations and are in agreement with the known *Buxus* alkaloids.[16] The above spectroscopic studies led to structure **4** for this new steroidal alkaloid, (+)-cyclobuxamidine.

Two known compounds 16α-acetoxybuxabenzamidienine (**5**)[4] and *trans*-cyclosuffrobuxinine (**6**)[16] were isolated for the first time from the leaves of *B. longifolia*. These compounds were earlier isolated from *Buxus sempervirens*.

Compounds **1**−**3** showed antibacterial activity against *Salmonella typhi*, *Shigella flexneri*, and *Pseudomonas aeruginosa*, while compound **4** was active against *Salmonella typhi* and *Escherichia coli* (Table 3) when assayed by the tube broth dilution method.

Experimental Section

General Experimental Procedures. The MS spectra were recorded on a Varian MAT 312 double-focusing spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker AM 400 NMR spectrometer. UV spectra were recorded on a Shimadzu UV-240 instrument. The IR spectra were recorded on a JASCO IRA-1



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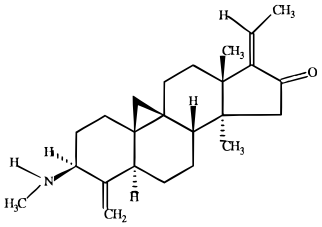

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IR spectrophotometer. Specific rotations were measured on a Polartronic D polarimeter. Purities of the samples were checked on precoated TLC (Si gel, G_{254}) plates.

Plant Material. Leaves of *Buxus longifoia* Boiss (10 kg) were collected in Hatay-Antakya in southeastern Turkey, near St. Peter's Church, in June 1990. The plant was identified by one of us (B.S.), and a voucher specimen was deposited in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Extraction and Isolation. An EtOH extract (818 g) of *B. longifolia* leaves was evaporated to a gum and redissolved in H_2O. Partial separation of the alkaloids was carried out by extraction with $CHCl_3$ at different pH values. The $CHCl_3$ extract obtained at pH 5.0 (12 g) was loaded onto a Si gel column (70−230 mesh, 360 g), which was eluted with mixture of petroleum ether−$CHCl_3$ in order of increasing polarity. The fraction obtained by eluting the column with petroleum ether−$CHCl_3$ (1.5:8.5) was found to contain three major compounds, by TLC, which were further purified by preparative TLC (Si gel) using petroleum ether−$CHCl_3$−Et_2NH (8:1.8:0.2) to afford compounds **1**, **3**, and **4**.

The $CHCl_3$ extract of pH 7.0 (35.2 g) was also loaded on a Si gel column (70−230 mesh, 1 kg), which was eluted with increasing polarities of mixtures of petroleum ether−$CHCl_3$. The fraction obtained by eluting the column with petroleum ether−$CHCl_3$ (6:4) was further purified by preparative TLC (Si gel) in petroleum ether−$CHCl_3$−Et_2NH (8:1.8:0.2) to give **2** along with two known steroidal bases, (+)-16α-acetoxybuxabenzamidienine (**5**) and *trans*-cyclosuffrobuxinine (**6**).

(+)-Cyclovirobuxeine F (1): white amorphous solid; $[\alpha]^{22}_D$ +7° (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 254 (4.35), 246 (4.40), 238 (4.32), 230 (4.31) nm; IR ($CHCl_3$) ν_{max} 3406, 2813, 1712, 1610, 1358 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS *m/z* 560 [M^+], 545 (10), 171 (11), 157 (11.4), 72 (100), 105 (23.5); HREIMS *m/z* 560.3531 (calcd for $C_{35}H_{48}N_2O_4$, 560.3539).

***N*-Benzoyl-*O*-acetylbuxalongifoline (2):** white amorphous solid; $[\alpha]^{22}_D$ +16° (*c* 0.065, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 254 (sh) (4.32), 245 (4.30), 235 (4.29), 229 (4.28) nm; IR ($CHCl_3$) ν_{max} 3348, 2812, 1718, 1664, 1626 cm^{-1}; ^{1}H- ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS *m/z* 546 [M^+] (10), 531 (8), 58 (100), 144 (10), 157 (25), 105 (32); HREIMS *m/z* 546.3461 (calcd for $C_{34}H_{46}N_2O_4$, 560.3455).

Buxasamarine (3): white amorphous solid; $[\alpha]^{22}_D$ +23° (*c* 0.13, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3456, 2902, 1362 cm^{-1}; ^{1}H- ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data, see Table 1; EIMS *m/z* 414 [M^+] (15), 399 (10), 58 (100), 101 (12), 115 (15); HREIMS *m/z* 414.600 (calcd for $C_{27}H_{46}N_2O$, 414.3609).

(+)-Cyclobuxamidine (4): white amorphous solid; $[\alpha]^{22}_D$ +24° (*c* 0.041, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (4.36) nm; IR ($CHCl_3$) ν_{max} 3673, 2918, 1685, 1607 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, s, *tert*-Me), 0.98 (3H, d, $J_{21,20}$ = 6.0 Hz, Me-21), 1.20 (3H, s, *tert*-Me), 2.01, 2.15 (3H, s, $COCH_3$), 2.73 (3H, s, CH_3-N_b), 2.99, 3.10 (3H, s, CH_3-N_a), 4.45 (1H, m, H-3), 4.56, 4.60 (1H, s, H-30), 4.65, 4.70 (1H, s, H-30); EIMS *m/z* 412 [M^+] (12), 99 (3), 113 (2), 58 (100); HREIMS *m/z* 412.3129 (calcd for $C_{27}H_{44}N_2O$, 412.3119).

(+)-16α-Acetoxybuxabenzamidienine (5): white amorphous solid; $[\alpha]^{22}_D$ +8° (*c* 0.015, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 254 (4.40), 245 (4.35), 237 (4.32), 230 (4.30), 225 (4.28) nm; IR ($CHCl_3$) ν_{max} 3520, 1720, 1650, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.75 (3H, s, *tert*-Me), 0.84 (3H, s, *tert*-Me), 0.85 (3H, s, *tert*-Me), 0.90 (3H, d, $J_{21,20}$ = 6.5 Hz, Me-21) 1.85 (3H, s, $COCH_3$), 2.30 [6H, s, N, $(CH_3)_2$], 2.80 (1H, m, H-20), 4.35 (1H, m, H-3), 5.06 (1H, m, H-16), 5.45 (1H, s, H-11), 5.90 (1H, m, H-19), 6.50 (1H, d, $J_{NH,3}$ = 8.2 Hz), 7.72−7.30 (5H, m, Ar-H); EIMS 546 [M^+] (25), 531 (18), 171 (45), 157 (50), 105 (25), 72 (100); HREIMS *m/z* 546.3467 (calcd for $C_{35}H_{50}N_2O_3$, 545.63451).

***trans*-Cyclosuffrobuxinine (6):** white amorphous solid; $[\alpha]^{22}_D$ −42° (*c* 0.012, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 243 (3.90) nm; IR ($CHCl_3$) ν_{max} 3450, 3040, 1734, 1730, 1680, 1660, 1465 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.60, 0.91 (each 1H, d, $J_{19\alpha,19\beta}$ = 4.2 Hz, H-19), 1.12 (3H, s, *tert*-Me), 1.21 (3H, s, *tert*-Me), 1.82 (3H, d, $J_{21,20}$ = 6.2 Hz, Me-21) 4.84 and 4.86 (each 1H, s, C=CH_2), 5.50 (1H, q, H-20); EIMS 353 [M^+] (20), 383 (15), 325 (30), 70 (40), 57 (100); HREIMS *m/z* 353.2758 (calcd for $C_{24}H_{35}NO$, 353.2718).

Bioassay Procedure. Pure compounds were assayed for antibacterial activity against *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) values were determined by the tube broth dilution method[17] using nutrient broth. Sensitive bacterial cultures (0.1 mL of 10^4−10^6 cells/mL) were added to the tubes containing 1 mL of different concentrations of the test sample, and 1 mL of nutrient broth. A similar procedure was followed for the standard antibiotic ampicillin. Inhibition of growth was observed after 24 h of incubation at 37 °C.

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