New Triterpenoidal Alkaloids from *Buxus sempervirens*

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Buxus sempervirens, (+)-16α, 31-Diacetylbuxadine and (–)-N_b-Demethylcyclomikuranine

Phytochemical studies on the ethanolic extract of the roots of *Buxus sempervirens* of Turkish origin have resulted in the isolation of two new triterpenoidal alkaloids, (+)-16α, 31-diacetylbuxadine (**1**), (–)-N_b-demethylcyclomikuranine (**2**) along with three known natural products, (–)-cyclomikuranine (**3**), (–)-cyclobuxophylline-K (**4**) and (+)-buxaquamarine (**5**) isolated for the first time from this species of genus *Buxus*. The structures of these new natural products were established on the basis of extensive spectroscopic studies. Compound **1** exhibited antibacterial activity against human pathogenic bacteria and weak phytotoxic activity against *Lemna minor* Linn.

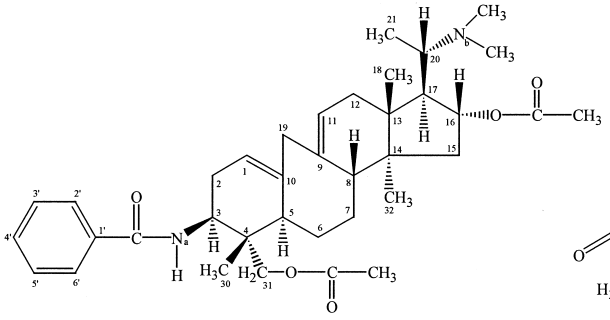
Introduction

Buxus alkaloids are unique steroid-triterpenoid class of alkaloids having a pregnane type basic skeleton with the C-4ß methyl, 9ß,10ß cycloartenol system and a C-20 degraded side chain (Ata, 1995). These types of alkaloids have shown interesting pharmacological activities. For instance, cycloprotobuxine-A has shown protective effect against cardiac arrythmia induced by oubain (LD_{50} 5 mg/kg) and positive inotropic effect on isolated guinea pig myocardium (Cordell 1981; Wang *et al*., 1989 and 1992). *Buxus sempervirens* L (Buxaceae) commonly known as "boxwood" is abundantly found in Turkey. The crude ethanolic extract of the plant is reported to be active against the human immunodeficiency Virus (HIV) and other diseases in which the tumor necrosis factor is involved and it has also been reported in the literature that trail dose administration of *B. sempervirens* preparation (SPV30) in HIV-infected asymptomatic patients have shown a delay in the progression of HIV disease and no severe side-effects were observed (Durant *et al*., 1998). Previously over fifty new steroidal alkaloids have been reported in the literature by our and other research groups (Atta-ur-Rahman *et al*., 1999 and Loru *et al*., 2000). In this paper, we report the isolation and structure elucidation of two new triterpenoidal alkaloids, (+)-16α,31-diacetylbuxadine (**1**) and (–)-N_b-demethylcyclomikuranine (**2**) along with three known triterpenoidal alkaloids, (–)-cyclomikuranine (**3**), (–)-cyclobuxophylline-K (**4**) and (+)-buxaquamarine (**5**), isolated for the first time from *Buxus sempervirens*. The structures of these new and reported compounds were elucidated with the aid of extensive spectroscopic studies. Compound **1** exhibited antibacterial activity against human pathogenic bacteria and weak phytotoxic activity against *Lemna minor* Linn.

Results and Discussion

(+)-16α,31-Diacetylbuxadine (**1**), $C_{37}H_{52}N_2O_5$, was isolated as a colourless gummy material. The UV spectrum showed absorption maxima at 226 nm indicating the presence of secondary benzamide chromophore (Kupchan *et al*., 1969). The IR spectrum displayed absorption bands at 3301 (NH), 1710 (ester carbonyl), 1642 (α,β-unsaturated amide carbonyl) and 1590 (C=C) cm^{-1}.

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of **1** showed the resonance of three three-proton singlets at δ 0.78, 0.90 and 1.00 due to the three quaternary methyl groups. A three-proton doublet at δ 0.89 ($J_{21,20}$ = 6.5 Hz) was ascribed to the C-21 secondary methyl protons. Two singlets integrating



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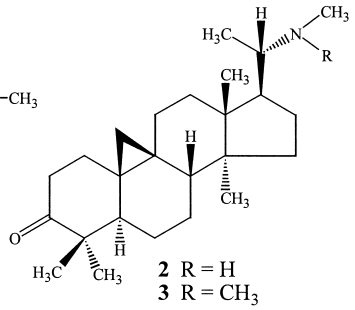
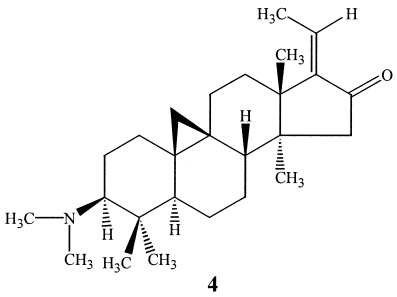


2 R = H
3 R = CH_3



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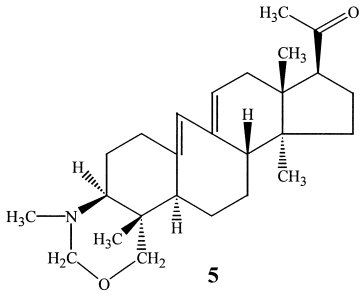

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for three-protons each appeared at δ 2.00 and 2.08 were due to the two sets of methyl protons of the acetyl groups. The *N*,*N*-dimethyl protons resonated as six-proton broad singlet at δ 2.28. A set of two AB doublets at δ 3.84 and 3.94 ($J_{31\alpha,31\beta}$ = 11.0 Hz) were due to the C-31 methylene protons. Its downfield value was due to the presence of a geminal acetoxy group. Another one-proton multiplet at δ 5.20 was assigned to the C-16 methine proton, geminal to the acetoxy functionality. The olefinic signals at δ 5.31 and 5.47 were also observed in the ^{1}H-NMR spectrum which were ascribed to the C-11 and C-1 olefinic protons, respectively. The ^{1}H-NMR spectrum also featured a one-proton multiplet at δ 4.45 and a one-proton doublet at δ 6.15 ($J_{NH,3\alpha}$ = 9.7 Hz) which were due to the C-3 methine and amidic NH protons, respectively. The aromatic protons resonated as two sets of two- and three-proton multiplets at δ 7.40 and 7.75.

The COSY-45° and HOHAHA spectra (20, 60 and 100 ms) greatly facilitated the ^{1}H-NMR chemical shift assignments (Atta-ur-Rahman 1989 and Atta-ur-Rahman and Choudhary 1996). The C-1 olefinic proton (δ 5.47) showed vicinal coupling with the C-2 methylene protons (δ 2.10 and 2.50) which in turn exhibited COSY-45° interactions with the C-3 methine proton (δ 4.45). The latter also showed cross-peaks with the amidic NH (δ 6.15). The C-11 olefinic proton (δ 5.31) showed cross-peaks with the C-12 methylene protons (δ 1.90 and 2.20). The allylic coupling of the C-19 methylene protons (δ 2.96 and 2.99) with the C-1 and C-11 olefinic protons were observed in the COSY-45° spectrum. The allylic coupling of the C-5 (δ 2.35) and C-8 (δ 1.50) methine protons with the C-1 and C-11 olefinic protons were observed in the HOHAHA spectra, respectively. The C-16 methine proton (δ 5.20) showed vicinal coupling with the C-15 methylene (δ 1.45 and 1.82) and C-17 methine (δ 2.15) protons in the COSY-45° spectrum while the latter also exhibited ^{1}H-^{1}H spin correlations with the C-20 methine proton (δ 1.55). The C-20 methine proton exhibited cross-peaks with the C-21 methyl protons (δ 0.89).

The ^{13}C-NMR spectrum ($CDCl_3$, 100MHz) of **1** showed resonances for all thirty seven carbon atoms in the molecule. The DEPT spectra were also recorded to establish the multiplicity of each carbon signals present in the broad band spectrum. DEPT spectra revealed the presence of thirteen methine, seven methylene and eight methyl carbons in the molecule. Subtraction of DEPT spectra from the broadband spectrum indicated the presence of nine quaternary carbon atoms in the molecule. The olefinic C-1 and C-11 carbons appeared at δ 120.5 and 118.1 respectively. Two aliphatic downfield signals at δ 78.6 and 70.6 were due to C-16 and C-31, respectively, their downfield chemical shift values being consistent with the presence of geminal acetoxy functionalities. The complete ^{13}C-NMR chemical shift assignments of **1** are shown in Table I.

Table I. ^{1}H/^{13}C One-bond shift correlation of (+)-16α, 31-diacetylbuxadine (**1**) as determined from HMQC spectrum.

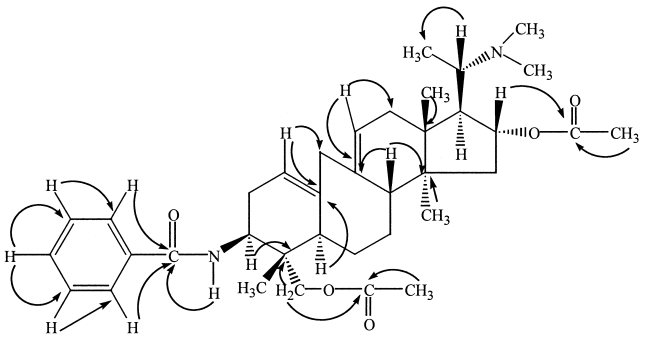
	1		**2**	
Carbon	δ_C	δ_H	δ_C	δ_H
C-1	120.5(d)	5.47(br s)	33.9(t)	2.10(m)
				1.71(m)
C-2	43.0(t)	2.50(m)	38.4(t)	1.73(m)
		2.10(m)		1.69(m)
C-3	63.8(d)	4.45(m)	201.1(s)	– – –
C-4	44.4(s)	– – –	41.2(s)	– – –
C-5	48.0(d)	2.35(m)	50.1(d)	2.25(m)
C-6	26.9(t)	1.99(m)	25.4(t)	1.69(m)
		1.22(m)		1.30(m)
C-7	29.8(t)	1.85(m)	26.6(t)	1.87(m)
		1.22(m)		1.31(m)
C-8	49.8(d)	1.50(m)	50.4(d)	2.10(m)
C-9	131.6(s)	– – –	22.0(s)	– – –
C-10	134.7(s)	– – –	25.5(s)	– – –
C-11	118.1(d)	5.31(br s)	25.9(t)	1.99(m)
				1.33(m)
C-12	37.5(t)	2.20(m)	27.2(t)	1.65(m)
		1.90(m)		1.21(m)
C-13	38.9(s)	– – –	44.9(s)	– – –
C-14	41.0(s)	– – –	46.6(s)	– – –
C-15	33.1(t)	1.82(m)	32.6(t)	1.90(m)
		1.45(m)		1.25(m)
C-16	78.6(d)	5.20(m)	30.4(t)	1.44(m)
C-17	48.1(d)	2.15(m)		1.19(m)
C-18	16.4(q)	0.78(s)	14.0(q)	0.99(s)
C-19	49.9(t)	2.99(br s)	19.6(t)	0.56(d,J = 4.3Hz)
		2.96(br d)		0.73(d,J = 4.3Hz)
C-20	52.9(d)	1.55(m)	60.0(d)	2.30(m)
C-21	15.2(q)	0.89(d,J = 6.5Hz)	10.2(q)	1.14(d,J = 6.5Hz)
C-30	17.3(q)	0.90(s)	16.4(q)	1.00(s)
C-31	70.6(q)	3.94 (d,J = 11.0Hz)	17.0(q)	1.04(s)
		3.84(d,J= 11.0Hz)		
C-32	18.3(q)	1.00(s)	17.6(q)	1.12(s)
N-CH_3	– – –	– – –	39.8(q)	2.35(s)
N$(CH_3)_2$	39.6(q)	2.28(br s)		
O$COCH_3$	21.0(q)	2.00(s)		
O$COCH_3$	22.0(q)	2.08(s)		
O$COCH_3$	170.4(s)	– – –		
O$COCH_3$	171.3(s)	– – –		
NHCO	166.6(s)	– – –		
C-1′	131.4(s)	– – –		
C-2′	126.5(d)	7.75(m)		
C-3′	129.1(d)	7.40(m)		
C-4′	132.0(d)	7.49(m)		
C-5′	129.1(d)	7.40(m)		
C-6′	126.5(d)	7.75(m)		

Multiplicity was determined from DEPT spectra.

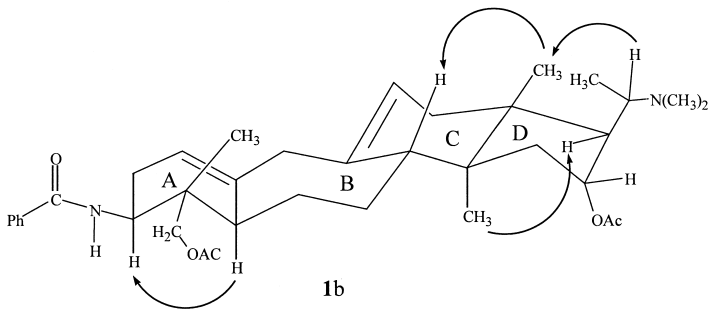
The HMQC spectrum of **1** helped to determine $^{1}H/^{13}C$ one-bond shift correlations. The C-1 olefinic proton (δ 5.47) showed $^{1}H/^{13}C$ direct one-bond connectivity with C-1 (δ 120.5). Similarly the C-11 olefinic proton (δ 5.31) exhibited a cross-peak with C-11 (δ 118.1). The allylic C-19 methylene protons (δ 2.96 and 2.99) showed $^{1}H/^{13}C$ one-bond shift correlations with C-19 (δ 49.9). The C-16 methine (δ 5.20) and C-31 methylene (δ 3.84 and 3.94) protons showed cross-peaks with C-16 (δ 78.6) and C-31 (δ 70.6), respectively, in the HMQC spectrum. The complete $^{1}H/^{13}C$ one-bond shift correlations of **1** as determined from the HMQC spectrum are presented in Table I.

The HMBC spectrum helped to determine the ^{13}C-NMR chemical shift assignments of quaternary carbon atoms and to establish the overall structure. The C-1 olefinic proton (δ 5.47) showed cross-peaks with C-2 (δ 43.0), C-5 (δ 48.0), C-10 (δ 134.7) and C-19 (δ 49.9). The C-11 olefinic proton (δ 5.31) exhibited HMBC interactions with C-8 (δ 49.8), C-9 (δ 131.6), C-12 (δ 37.5) and C-19 (δ 49.9). The C-3 methine proton (δ 4.45) showed long-range heteronuclear shift correlations with C-2 (δ 43.0), C-4 (δ 44.4) and with the amidic carbonyl carbon (δ 166.6). The C-8 methine (δ 1.50) and C-12 methylene (δ 1.90 and 2.20) protons exhibited cross-peaks with the quaternary C-14 (δ 41.0) and C-13 (δ 38.9), respectively. The C-15 methylene (δ 1.45 and 1.82) and C-17 methine (δ 2.15) protons showed cross-peaks with the quaternary C-14 (δ 41.0) and C-13 (δ 38.9), respectively. Similarly the C-16 methine proton (δ 5.20) showed HMBC interactions with C-15 (δ 33.1), C-17 (δ 48.1) and with the C-16 acetyl carbonyl carbon (δ 171.3). The C-31 acetyl methyl protons (δ 2.00) showed cross-peaks with the C-31 acetyl carbonyl carbon (δ 170.4) while the C-31 methylene protons (δ 3.84 and 3.94) also exhibited heteronuclear multiple bond connectivity with C-4 (δ 44.4) and with the C-31 acetyl carbonyl carbon (δ 170.4). Important HMBC interactions are shown around structure **1**a.

The stereochemistry at various chiral centers was established with the help of the NOESY spectrum. The C-16 methine proton (δ 5.20) showed NOE interaction with the C-20 methine proton (δ 1.55) which exhibited cross-peaks with the C-18 methyl protons (δ 0.78). H_3-18 showed cross-peaks with H-8 (δ 1.50). It has already been



1a



1b

reported in the literature that H-8 is invariably β-oriented in this class of alkaloids (Brown and Kupchan, 1962). This suggested β-orientation of H-16, H-20 and the C-18 methyl group. H-3 (δ 4.45) showed cross-peaks with H-5 (δ 2.35). H-5 is invariably α-oriented in *Buxus* alkaloids (Brown and Kupchan, 1962) indicating an α-orientation of C-3 H and β-orientation of the amino functionality. Probable conformations of rings A, B, C and D of **1** as obtained from NOESY spectrum are presented in structure **1**b.

The high-resolution electron-impact mass spectrum (HREIMS) of **1** showed the molecular ion peak at *m/z* 604.4216 which was in agreement with the molecular formula $C_{37}H_{52}N_2O_5$ (calcd. 604.4217) indicating the presence of thirteen degrees of unsaturation in the molecule. The ion at *m/z* 589.3946 ($C_{36}H_{49}N_2O_5$, calcd. 589.3949) was due to the loss of a methyl group from the M^+. The peak at *m/z* 171.1256 ($C_9H_{17}NO_2$, calcd. 171.1259) characteristically arose by the cleavage of ring D along with the attached substituents and consistent with the acetoxy group at C-16 (Atta-ur-Rahman and Choudhary, 1988). The ion at *m/z* 225.1729 ($C_{13}H_{23}NO_2$, calcd. 225.1728) could result by the *retero* Diels-Alder cleavage of ring C which further suggested the presence of a C=C bond in the ring C. The ion at *m/z* 105.0343 (C_7H_5O, calcd. 105.0340) was due to the benzoyl cation. The base peak at *m/z* 72.0810 ($C_4H_{10}N$, calcd. 72.0813) represented the trimethyliminium ion (Budzikiewicz, 1972). These spectroscopic studies led to structure **1** for this new triterpenoidal alkaloid.

Compound **1** exhibited antibacterial activity against *Shigella flexnerii*, *Proteus mirabilis*, *P. vulgaris*, *Corynebacterium hoffmanni*, *Klebsiella pneumoniae*, *Streptococcus fecalis*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* with observed zones of inhibition of 5, 7, 11, 4, 14, 5, 12, 19 and 10 mm in diameter, respectively, at concentration of 200 μg/100 μl applied to each disk. Compound **1** has also showed 62%, 44% and 16% phytotoxic activity against *Lemna minor* L. 500, 50 and 5 ppm, respectively, as determined by the methods described by McLaughlin *et al.* (1991).

The second compound (−)-N_b-demethylcyclomikuranine (**2**) $C_{25}H_{41}NO_2$, was also isolated as a colourless amorphous material. The UV and IR spectra of compound **2** resembled those reported for (−)-cyclomikuranine (**3**) (Nakano *et al.*, 1966). The ^{1}H-NMR spectrum of **2** was also distinctly similar to that (−)-cyclomikuranine (**3**) except for the presence of N_b-methyl protons which resonated as three-proton singlet at δ 2.35. Complete ^{1}H and ^{13}C-NMR chemical shift assignments were established from the data obtained from HMQC spectrum and are shown in Table I.

The HREIMS of **2** afforded M^+ at *m/z* 387.3129 which provided the molecular formula $C_{25}H_{41}NO_2$, indicating the presence of six double bond equivalent in the molecule. The ion at *m/z* 372 ($C_{24}H_{38}NO_2$) was due to the loss of a methyl group from the molecular ion. The base peak at *m/z* 58 was due to the dimethyliminium cation. The mass spectrum of **2** showed fragments and losses as expected for demethylcyclomikuranine. Based on these spectroscopic studies, compound **2** was characterized as (−)-N_b-demethylcyclomikuranine, a new natural product.

In addition to these two new natural products, three known triterpenoidal alkaloids, (−)-cyclomikuranine (**4**), (−)-cyclobuxophylline K (**5**) and (+)-buxaquamarine (**6**) were also isolated for the first time from the roots of *B. sempervirens.* The IR, UV, ^{1}H-NMR and mass spectra of compounds **3**–**5** were identical to those of (−)-cyclomikuranine, (−)-cyclobuxophylline-K (Nakano *et al.*, 1966) and (+)-buxaquamarine (Atta-ur-Rahman *et al.*, 1985), respectively, reported in the literature. Previously compounds **3** and **4** were isolated from *B. microphylla* (Nakano *et al.*, 1966) while **5** was purified from *B. papillosa* (Atta-ur-Rahman *et al.*, 1985).

Experimental

General

The mass spectrometric measurements were conducted on a Varian MAT 312 double focussing mass spectrometer connected to a DEC PDP 11/34 computer system. The ^{1}H-NMR spectra were recorded in $CDCl_3$ on a AM 400 and AM 500 Bruker NMR spectrometers at 400 and 500 MHz while ^{13}C-NMR was recorded on a AM 400 Bruker NMR spectrometer at 100 MHz with TMS as internal standard. The IR spectra were recorded on a Jasco-IRA1 IR spectrophotometer. The UV spectra were recorded on a Shimadzu UV 240 in-

strument. The optical rotations were measured on a Polatronic D polarimeter (Hitachi) and the purities of the samples were checked on TLC (silica gel, GF 254 precoated plates purchased from Merck).

Antibacterial activity

Testing for antibacterial activity was performed at the Plant Screening Section of H. E. J. Research Institute of Chemistry, University of Karachi by using standard disk diffusion assays. Ampicilline and tobramycin were used as standard antibiotics.

Phytotoxic Assay

This assay was performed against *Lemna minor* L. by the procedure described by McLaughlin *et al.* (1991).

Plant material

The roots of *B. sempervirens* (10 kg) were collected from Beynam forest, Ankara, Turkey in September 1991 and stored in cold room. The plant was identified by Prof. Mehmet Koyuncu, Department of Pharmacognosy, Gazi University, Ankara, Turkey. A voucher specimen (GUE # 1243) was deposited in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Extraction and isolation

The roots of *B. sempervirens* (10 kg) were dried, crushed and extracted with ethanol (100 l) at room temperature. An ethanolic extract of *B. sempervirens* was concentrated to a gum (160 g) under reduced pressure and distilled water was added. The aqueous extract was extracted with chloroform at different pH values, in order to achieve partial separation of the alkaloids and three chloroform-soluble fractions at pH 3.5, 7.0 and 9.5 were obtained. The pH was adjusted by the addition of 10% acetic acid or ammonium hydroxide solutions.

The chloroform extract obtained at pH 3.5 (2.7 g) was loaded onto a silica gel column (1 kg). The column was eluted with petrol ether (40–60°)-chloroform (0–100%) and chloroform-methanol (0–100%). On elution with petrol ether (40–60°)-chloroform (4:6, v/v) and chloroform-methanol (8:2), two fractions F-1 and F-2 were obtained. Fraction F-1 was subjected to TLC using petrol ether (40–60°)-acetone-diethylamine (6:5:0.1, v/v/v) as solvent system to afford (+)-16α,31-diacetylbuxadine (**1**) as a colourless gummy material (87 mg, R_f = 0.68). Similarly preparative TLC of fraction F-2 using petrol ether (40–60°)-acetone-diethylamine (9.5:0.5:0.1, v/v/v) as developing solvent afforded (–)-N_b-demethylcyclomikuranine (**2**) (6.3 mg, R_f = 0.58) and (–)-cyclomikuranine (**3**) (6.0 mg, R_f = 0.61) as colourless gums.

The chloroform extract obtained at pH 9.5 (18.3 g) was also loaded onto a silica gel column (500 gm). The column was again eluted with petrol ether (40–60°)-chloroform (0–100%) and then with chloroform-methanol (0–100%) to afford various fractions. The fraction which was obtained on elution with pet. ether (40–60°)-chloroform (4:6, v/v) was subjected to preparative TLC using petrol ether (40–60°)-diethylether-diethylamine (3:6:0.1, v/v/v) as eluent to yield (–)-cyclobuxophylline-K (**4**) (4.3 mg, R_f = 0.74) and (+)-buxaquamarine (**5**) (11.7 mg, R_f = 0.49) as colourless amorphous solids.

(+)-16α,31-Diacetylbuxadine (**1**)

$[\alpha]^{20}_D$ = +107° (*c* = 0.26, $CHCl_3$); UV λ_{max} (MeOH): 226 nm; IR ν_{max} ($CHCl_3$) cm^{-1}: 3301 (NH), 1710 (ester carbonyl), 1642 (α,β-unsaturated amide carbonyl), 1590 (C=C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: See Table I; ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: see Table I; HREIMS *m/z* (rel. int.%): 604.4216 ($C_{37}H_{52}N_2O_5$, calcd. 604.4217, 2.8), 589.3946 ($C_{36}H_{49}N_2O_5$, calcd. 589.3949, 4.7), 225.1729 ($C_{13}H_{23}NO_2$, calcd. 225.1728, 10.8), 171.1256 ($C_9H_{17}NO_2$, calcd. 171.1259, 11.9), 105.0343 (C_7H_5O, calcd. 105.0340, 45), 72.0810 ($C_4H_{10}N$, calcd. 72.0813, 100).

(–)-N_b-Demethylcyclomikuranine (**2**)

$[\alpha]^{20}_D$ = –23° (*c* = 0.26, $CHCl_3$); UV λ_{max} (MeOH): 203 nm; IR ν_{max} ($CHCl_3$) cm^{-1}: 3430 (OH), 1720 (six-membered ketone); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: See Table I; ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: See Table I; HREI MS *m/z* (rel. int.%): 387.3129 ($C_{25}H_{41}NO_2$, calcd. 387.3127, 12.9), 372.2911 ($C_{24}H_{39}NO_2$, calcd. 372.2902, 15), 71.0731 (C_4H_9N, calcd. 71.0735, 85), 58.0659 (C_3H_8N, calcd. 58.0657, 100).

(–)-Cyclomikuranine (**3**)

$[\alpha]^{20}_D$ = –85° (*c* = 0.31, $CHCl_3$); UV λ_{max} (MeOH): 205 nm; IR ν_{max} ($CHCl_3$) cm^{-1}: 3436 (OH), 1725 (six-membered ketone); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.57 (1H, d, $J_{19\alpha,19\beta}$ = 4.3 Hz, H-19α), 0.73 (1H, d, $J_{19\beta,19\alpha}$ = 4.3 Hz, H-19ß), 0.99 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.13 (3H, s, CH_3), 1.15 (1H, d, $J_{21,20}$ = 6.6 Hz, 21-CH_3), 2.25 (6H, s, $N(CH_3)_2$), 4.45 (1H, m, H-16ß); EI MS *m/z* (rel. int.%): 401 ($C_{26}H_{43}NO$, 12.9), 386 ($C_{35}H_{40}NO_2$, 15), 72 ($C_4H_{10}N$, 100).

(–)-Cyclobuxophylline-K (**4**)

$[\alpha]^{20}_D$ = –42° (*c* = 0.65, $CHCl_3$); UV λ_{max} (MeOH): 244 nm; IR ν_{max} ($CHCl_3$) cm^{-1}: 1724, 1645 (α,β-unsaturated C=O, C=C), 1593 (C=C); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.29 (1H, d, $J_{19\alpha,19\beta}$ = 4.4 Hz, H-19α), 0.60 (1H, d, $J_{19\beta,19\alpha}$ = 4.4 Hz, H-19ß), 0.80 (3H, s, CH_3), 0.96 (3H, s, CH_3), 0.98 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.85 (3H, d, $J_{21,20}$ = 7.5 Hz, 21-CH_3), 2.31 (6H, s, $N(CH_3)_2$), 6.59 (1H, q, $J_{20,21}$ = 7.5 Hz, H-20); HREI MS *m/z* (rel. int.%): 383.5417 ($C_{26}H_{41}NO$, calcd. 397.5415, 42), 368.3425 ($C_{25}H_{38}NO$, calcd. 368.3423, 35), 71.0737 (C_4H_9N, calcd. 71.0735, 100).

(+)-Buxaquamarine (**5**)

$[\alpha]^{20}_D$ = +48° (*c* = 0.25, $CHCl_3$); UV λ_{max} (MeOH): 230 (sh), 238, 245 and 253 (sh) nm; IR ν_{max} ($CHCl_3$) cm^{-1}: 2839 (CH), 1685 (C=O), 1599 (C=C); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.68 (3H, s, CH_3), 0. 75 (3H, s, CH_3), 1.06 (3H, s, CH_3), 2.00 (3H, s, O=CO-CH_3), 2.13 (3H, s, *N*-CH_3), 3.27 (1H, d, $J_{31\alpha,31\beta}$ = 10.4 Hz, H-31α), 3.31 (1H, d, $J_{33\alpha,33\beta}$ = 9.6 Hz, H-33α), 3.85 (1H, d, $J_{31\beta,31\alpha}$ = 10.4 Hz, H-31ß), 4.45 (1H, $J_{33\beta,33\alpha}$ = 9.6 Hz, H-33ß), 5.60 (1H, br. s, H-11), 6.00 (1H, s, H-19); HREI MS *m/z* (rel. int.%): 397.2345 ($C_{26}H_{39}NO_2$, calcd. 397.2343, 10), 382.2641 ($C_{25}H_{36}NO_2$, calcd. 382.2641, 9), 127.0645 ($C_7H_{13}NO$, calcd. 127.0645, 100).

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