PERGAMON

Phytochemistry 54 (2000) 951–957

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Four steroidal alkaloids from the leaves of *Buxus sempervirens*

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Received 10 August 1999; received in revised form 5 January 2000

Abstract

Four new steroidal alkaloids, N_{20}-formylbuxaminol E [(20S)-16α-hydroxy-20-(formylamino)-3β-(dimethylamino)-9,10-seco-buxa-9(11),10(19)-diene] (**1**), O_{16}-syringylbuxaminol E [(20S)-16α-syringoyl-3β-(dimethylamino)-20-(amino)-9,10-seco-buxa-9(11),10(19)-diene] (**2**), N_{20}-acetylbuxamine G [(20S)-20-(acetylamino)-3β-(methylamino)-9,10-seco-buxa-9(11),10(19)-diene] (**3**) and N_{20}-acetylbuxamine E [(20S)-20-(acetylamino)-3β-(dimethylamino)-9,10-seco-buxa-9(11),10(19)-diene] (**4**) were isolated from the leaves of *Buxus sempervirens*. Their structures were determined mainly on the basis of 2D NMR studies.

Keywords: *Buxus sempervirens*; Buxaceae; Boxwood; Steroidal alkaloids; N_{20}-Formylbuxaminol E; O_{16}-Syringylbuxaminol E; N_{20}-Acetylbuxamine E; N_{20}-Acetylbuxamine G

1. Introduction

Buxus sempervirens L. (Buxaceae) is widely distributed in Eurasia, and North America. A lot of interest is shown in Buxaceae because of the abundance of their steroidal alkaloids. Investigators took interest in this plant for several decades with regard to its medicinal properties and chemical constituents. The extracts of *Buxus* species are used in folk medicine for curing various diseases and more recently in the treatment of Human Immunodeficiency Virus infections as described by Valmet (1983), Durant et al. (1996, 1998).

Many compounds extracted from leaves and roots of *B. sempervirens* have been characterised before by several groups (Atta-Ur-Rahman and Muzaffar, 1988; Tomko and Voticky, 1973; Cerny and Sorm, 1967). The structures of most of them contain either the buxane (9,19-cyclobuxus) or the 9,10-seco-buxa-9(11), 10(19) diene skeleton affording them a well defined ring junctions. Therefore, *buxus* alkaloids known to date mainly differ by the substituents groups usually located on C3, C16 and C20 positions. Recently, a new type of steroidal derivative has been described by Fourneau et al. (1997), the spirobuxus alkaloids. The present report describes the isolation and the structure elucidation by spectroscopic studies of four new steroidal alkaloids, N_{20}-formylbuxaminol E (**1**), O_{16}-syringylbuxaminol E (**2**), N_{20}-acetylbuxamine G (**3**) and N_{20}-acetylbuxamine E (**4**). These compounds are members of the 9,19-cyclo-9,10 secobuxus structural type.

2. Results and discussion

The mixture of alkaloids was obtained from a hydroalcoholic extract of *B. sempervirens* leaves. After separation of alkaloids by extraction at different pH

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00036-4

values, the fraction obtained at pH 5 was chromatographed to yield compounds **1** and **2**, whereas the fraction obtained at pH 4 yielded compounds **3** and **4** which only differ by one methyl group (Fig. 1).

Each compound was submitted to UV, IR, mass, and NMR spectroscopic analyses. The assignment of ^{1}H and ^{13}C resonances of the spectra was carried out from the analysis and combination of DQF-COSY, TOCSY, DEPT, HSQC-PEP, HSQC-TOCSY and HMBC data.

The UV spectrum of compound **1** showed absorptions at 238, 245 and 254 nm, characteristic of 9(10 → 19)*abeo*-diene system this is in agreement with results obtained later (Stauffacher, 1964; Voticky et al., 1976b). The IR spectrum displayed bands at 3334 (OH/N–H), 1667 (amide carbonyl) and 1647 (C═C) cm^{-1}.

The high-resolution mass spectrum (FAB (+) / HR) of **1** showed a $[M+H]^{+}$ at *m/z* 429.3500 (calc. 429.3481) which corresponds to the molecular formula $C_{27}H_{44}N_2O_2$ and suggests the presence of seven degrees of unsaturation. The EI mass spectrum showed the base peak at *m/z* 71 with peaks at *m/z* 58 and 84, indicative of a C3-dimethylamino grouping in agreement with data obtained by Voticky et al. (1976a). The peak at *m/z* 399 resulted from the loss of the formyl group from the $[M]^{+}$.

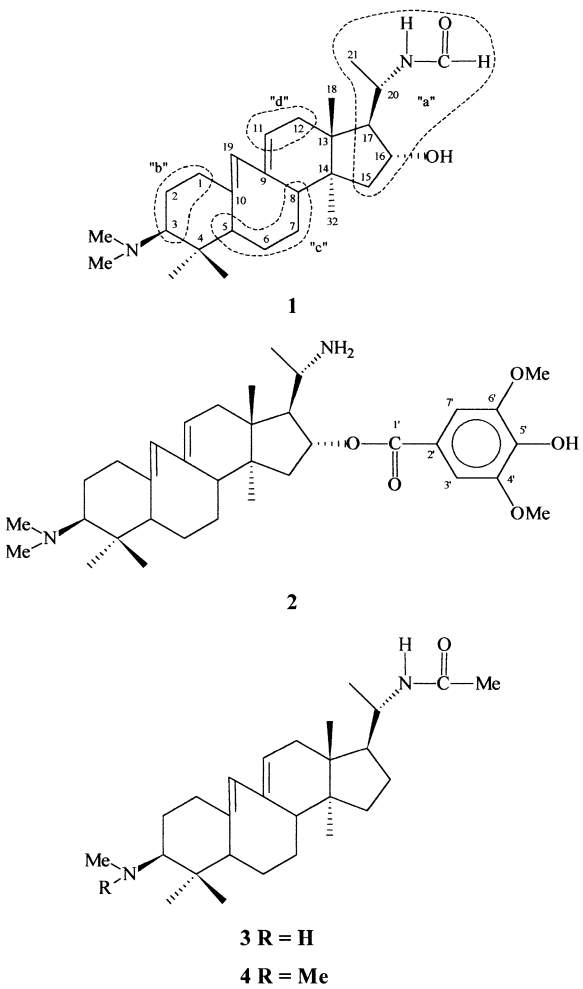

Fig. 1. Structure of the four new alkaloids isolated from *Buxus sempervirens*.

The ^{1}H-NMR spectrum of **1** revealed the presence of four 3H singlets corresponding to methyl groups at δ 0.69, δ 0.77, δ 0.90 and δ 1.00, along with one 3H doublets at δ 1.20 ($J_{21,20} = 6.3$ Hz) unambiguously assigned to H-21. The *N*,*N*-dimethyl protons resonated as a 6H singlet at δ 2.28. Two-proton multiplets centered at δ 4.09 and 4.18 were ascribed to the C-20 and C-16 methine protons, geminal to the amidic nitrogen and hydroxyl group, respectively. The H-11 and H-19 olefinic protons were clearly identified as the broad singlet at δ 5.50 and the sharp singlet at δ 5.88, respectively.

The ^{13}C- NMR spectrum of **1** showed 27 resonances, while the DEPT spectrum showed the presence of seven CH_3, six CH_2, nine CH and by difference from the ^{13}C spectrum, five quaternary carbons.

To facilitate the assignment several proton spin systems, isolated by quaternary carbons, were delineated (Fig. 1). COSY and TOCSY allowed their assignment. Then, these partial structures (spin system "a", "b", "c" and "d") were linked together by using HMBC connectivities between protons of these various spin systems and quaternary carbons on the basis of the 9,10-seco-buxa-9(11), 10(19) diene skeleton.

The COSY and TOCSY spectra allowed us to identify the four spin systems of the molecule. The spin system "a" starts with the formyl proton (δ 8.12), which showed COSY interactions with the N–H amidic proton (δ 6.33). Moreover, this spin system includes the H-16 signal low field shifted (δ 4.18) due the hydroxyl group. Therefore, based on the COSY and TOCSY connectivities the H-20, H-21, H-17, H-16 and H-15 signals were easily identified. This assignment was confirmed by HSQC and HSQC-TOCSY experiments. The C-1 methylene protons (δ 2.09 and 2.26) (spin system "b", mostly traced from the TOCSY spectra) exhibited vicinal coupling with the C-2 methylene protons (δ 1.50 and 1.73), which in turn exhibited coupling with the C-3 methine proton. The C-5 methine proton (δ 1.94) (spin system "c") showed coupling with the C-6 methylene protons (δ 1.36 and 2.11). The latter showed TOCSY interactions with the C-8 methine proton (δ 2.00). The isolated spin system "d" was traced from the COSY spectra in which interactions of the C-11 methine proton (δ 5.50) with the C-12 methylene protons (δ 1.97 and 2.24) were observed.

Finally, the methyl signals at δ 15.16, 17.13, 18.00, 21.01 and 24.99 were assigned to the C-31, C-18, C-32, C-21 and C-31 carbons, respectively. The two down-

field methine signals at δ 76.96 and 71.56 were assigned to C-16 and C-3 carbons, respectively.

Complete ^{1}H- and ^{13}C-NMR chemical shifts and important HMBC interactions are presented in Table 1.

The UV spectrum of compound **2** showed absorptions at 221, 236, 245, 254 and 278 nm. The absorptions at 236, 245 and 254 nm were characteristic of 9(10 → 19)*abeo*-diene system. The IR spectrum displays bands at 3364 and 1330 (aromatic OH), 1694 (ester carbonyl), 1462 (aromatic C=C), 1219, 1118 and 1007 (aromatic ether) cm^{-1}.

The high-resolution mass spectrum (FAB (+) / HR) of **2** showed a $[M+H]^{+}$ at *m/z* 581.3956 (calc. 581.3954) which corresponds to molecular formula $C_{35}H_{52}N_2O_5$ and suggests the presence of eleven degrees of unsaturation. The EI mass spectrum showed a fragmentation pattern, with peaks at *m/z* 58, 71, 84, indicative of a C3-dimethylamino grouping. The peak at *m/z* 181 indicates the loss of a syringoyl cation.

The ^{1}H NMR spectrum of **2** showed four 3H singulets at δ 0.69, δ 0.80, δ 0.93, δ 0.98 and a 3H doublet centered at δ 1.16 ($J_{21,20} = 6,3$ Hz) characteristic of the H-21. The N_3-dimethyl protons appeared as a 6H singlet at δ 2.28. Two 1H multiplet centered at δ 5.40 and δ 3.13 were assigned to the C-16 methine proton geminal to the ester group and to the C-20 methine proton geminal to the NH_2 group. A broad singlet at δ 5.50 and a singlet at δ 5.90 were typically representative of the H-11 and the H-19 vinylic protons, respectively. Due to the symmetry of the aromatic ring of the syringoyl group the *ortho* C-3′/C-7′ protons appeared as a 2H singlet at δ 7.10, while the C-4′/C-6′ methoxy groups resonated as a 6H singlet at δ 3.82.

The ^{13}C-NMR spectrum of **2** displays 35 resonances and the DEPT spectrum showed the presence of nine methyl, six methylene, 10 methine and by difference from the ^{13}C spectrum, 10 quaternary carbons. Signals easily identified were that of the carbonyl of the syringoyl ester at δ 166.28, as well as those of the four vinylic carbons at δ 138.45 for C-9, δ 137.16 for C-10 and δ 128.33 for both C-11 and C-19. The chemical equivalent *ortho* C-3′/C-7′ and *meta* C-4′/C-6′ of the syringoyl group appeared at δ 106.76 and δ 147.16, whereas the C-2′ and *para* C-5′ appeared at δ 120.29 and δ 140.69. Two downfield shifted signals at δ 71.63 and δ 79.47 were assigned to C-3 and C-16.

The COSY spectrum of **2** showed a crosspeak between the C-16 methine proton (δ 5.40) and the C-17 methine proton (δ 2.14). The C-17 methine proton (δ 2.14) showed COSY interaction with the C-20 meth-

Table 1
^{1}H- and ^{13}C-NMR chemical shifts and HMBC connectivities measured for **1** in $CDCl_3$ (20°C)

Position	Group	δ ^{1}H (ppm)	δ ^{13}C (ppm)	HMBC connectivities[a]
1	CH_2	2.09–2.26	41.39	19
2	CH_2	1.50–1.73	23.15	
3	CH	2.10	71.56	30, 31, *N*–$(Me)_2$
4	C		43.47	2, 5, 30, 31
5	CH	1.94	52.03	1, 6, 7, 19, 30, 31
6	CH_2	1.36–2.11	30.17	7, 8
7	CH_2	1.28–1.44	25.73	6, 8
8	CH	2.00	49.26	6, 7, 19, 32
9	C		138.90	7, 8
10	C		137.05	5
11	CH	5.50 s.	127.96	12, 19
12	CH_2	1.97–2.24	37.92	18
13	C		44.50	11, 15, 18
14	C		47.59	8, 15, 18, 32
15	CH_2	1.40–2.02	45.43	32
16	CH	4.18	76.96	15, 17
17	CH	1.83	59.70	18, 21
18	CH_3	0.77 *s*	17.13	12
19	CH	5.88 *s*	128.29	5
20	CH	4.09	47.07	17, 21, CO–H
21	CH_3	1.20 *d*	21.01	
30	CH3	1.00 *s*	24.99	31
31	CH_3	0.69 *s*	15.16	5, 30
32	CH_3	0.90 *s*	18.00	8, 15
N_3–$(Me)_2$	CH_3	2.28 *s*	44.63	3
C_{20}–HN	HN	6.32 *d*		
N–CO–H	H	8.12 *s*		
N–CO	CO		160.77	

[a] Protons correlating with the carbon resonance.

ine proton (δ 3.13) which in turn showed interaction with the C-21 methyl proton (δ 1.16). The TOCSY spectrum showed a crosspeak between the C-16 methine proton (δ 5.40) and the C-15 methylene proton (δ 1.52 and 2.15). The C-11 olefinic proton (δ 5.50) showed interaction with the C-12 methylene proton (δ 2.06 and 2.33) and the C-19 vinylic proton (δ 5.90).

Complete ^{1}H and ^{13}C-NMR chemical shifts of **2** are presented in Table 2.

The UV spectrum of compound **3** showed absorptions at 238 nm, 245 nm and 254 (*sh*) nm. The IR spectrum displayed bands at 3289 (N–H), 1665 (amide carbonyl), 1646 (C=C) cm^{-1}. The EI mass spectrum showed the $[M]^+$ at *m/z* 412 while the high resolution spectrum (FAB (+) / HR) gave the exact mass $[M+H]^+$ at *m/z* 413.3537 (calc. 413.3532) corresponding to the molecular formula $C_{27}H_{44}N_2O$. The base peak at *m/z* 57 was due to the loss of $[CH_2CH{=}NHCH_3]^+$ from $[M]^+$ (Atta-Ur-Rahman et al., 1997). The peak at *m/z* 369 and 397 resulted, respectively, from the loss of CH_3CO and methyl group from the $[M]^+$.

The ^{1}H-NMR spectrum of **3** showed four tertiary methyl groups as singlets at δ 0.66, δ 0.74, δ 0.77 and δ 1.06, and one doublet centered at δ 1.11 ($J_{21,\,20} = 6.3$ Hz) characteristic of the H-21 methyl group. Another singlet integrating for three protons at δ 1.95 was ascribed to the N_3-methyl protons. A broad singlet at δ 5.50 and a singlet at δ 5.93 were typical of H-11 and H-19 vinylic protons, respectively. All these chemical shifts are in agreement with the typical 9,19-cyclo-9,10 secobuxus skeleton. The position of the NHCO-Me was proved by the following: the amidic NH proton afforded a doublet at δ 5.23 ($J_{20,\,NH} = 9.3$ Hz) which exhibited a COSY interaction with H-20 at δ 4.00 which in turn exhibited an interaction with H-17. Therefore, the H-20 signal appeared as a broad multiplet. The methyl group adjacent to the carbonyl afforded a three-proton singlet at δ 1.92 and in HMBC spectrum showed long range connectivities with the carbonyl.

The ^{13}C NMR spectrum of **3** displays 27 resonances and the DEPT spectrum showed the presence of seven methyl, seven methylene and seven methine groups.

Table 2
^{1}H- and ^{13}C-NMR chemical shifts and HMBC connectivities measured for **2** in $CDCl_3$ (20°C)

Position	Group	δ ^{1}H (ppm)	δ ^{13}C (ppm)	HMBC connectivities[a]
1	CH_2	2.09–2.27	41.45	19
2	CH_2	1.50–1.74	23.22	3
3	CH	2.1	71.63	30, 31, *N*–Me
4	C		43.44	3, 5, 30, 31
5	CH	1.94	52.09	1, 7, 19, 30, 31
6	CH_2	1.35–2.08	30.17	7, 8
7	CH_2	1.26–1.42	25.73	6, 8
8	CH	2.05	49.17	6, 7, 19, 32
9	C		138.45	7, 8
10	C		137.16	1, 5, 19
11	CH	5.50	128.33	1, 5, 11, 12, 19
12	CH_2	2.06–2.33	38.04	18
13	C		44.50	15, 18, 32
14	C		47.33	8, 16, 18, 32
15	CH_2	1.52–2.15	43.29	32
16	CH	5.40	79.47	15, 17
17	CH	2.14	59.24	18, 21
18	CH_3	0.80	17.02	12
19	CH	5.90	128.33	1, 5, 11, 12, 19
20	CH	3.13	48.62	16, 21
21	CH_3	1.16 *d*	22.96	
30	CH_3	0.98	25.03	31
31	CH_3	0.69	15.21	3, 5, 30
32	CH_3	0.93	17.40	8, 15
N_3–$(Me)_2$	CH_3	2.28	44.59	3
O-C_1·O	CO		166.28	3′, 7′
2′	C		120.29	3′, 7′
3′-7′	CH	7.10	106.76	
4′-6′	C		147.16	
5′	C		140.69	3′, 7′
4′,6′-OMe	CH_3	3.82	56.26	

[a] Protons correlating with the carbon resonance.

Typical signals of **3** were those due to NHCO (δ 168.85), C-9 (δ 138.63), C-10 (δ 135.99), C-11 (δ 128.99), C-19 (δ 129.23), and C-3 (δ 68.61). Complete ^{1}H and ^{13}C chemical shifts are presented in Table 3, along with the HMBC connectivities.

The overall spectral behaviour of **4** was very similar to that of **3**. Briefly, the UV spectrum of compound **4** showed absorptions at 238 nm, 245 nm and 254 (*sh*) nm. The IR spectrum displays bands at 3280 (N–H), 1663 (amide carbonyl), 1641 (C=C) cm^{-1}. The high resolution spectrum (FAB (+) / HR) gave the exact mass $[M+H]^{+}$ at *m/z* 427.3654 (calc. 427.3688) corresponding to the molecular formula $C_{28}H_{46}N_2O$. NMR data of compounds **3** and **4** showed that the carbon 3 are substituted by *N*-methyl and *N*-dimethyl group, respectively. The *N*-methyl signal of the compound **3** is characterised by signal at 2.5 ppm (^{1}H) and 35.4 ppm (^{13}C) whereas for the compound **4**, chemical shift values of the *N*-methyl signal [1H (2.3 ppm) an ^{13}C (44.45 ppm)] clearly indicate the presence of a *N*-dimethyl group. This is in agreement with the mass spectroscopy data for these two compounds, 412 and 426 respectively. Therefore, as most of their chemical shifts of **4** are very similar to those of the compound **3**, only ^{13}C chemical shifts which differ more than 0.2 ppm are given: C1 (δ 41.08), C2 (δ 30.02), C3 (δ 72.00), C5 (δ 51.80), C6 (δ 23.11), $N(CH_3)_2$ (δ 44.45). They are all located near the carbon 3, in agreement with the difference of structure of the substituent group.

3. Experimental

3.1. Extraction and isolation

Air dried leaves of *Buxus sempervirens* (2.360 kg), were extracted by EtOH–H_2O–CH_3COOH (10:9:1). The alkaloids (20 g) were obtained by a liquid/liquid extraction with CH_2Cl_2 after evaporation of EtOH and alkalinisation of the crude extract.

Partial separation of the alkaloids was carried out by extraction with McIlvaine's solutions at different pH values. The fraction obtained at pH 5 (1.9 g) was loaded onto a neutral alumina column (75 g) and eluted with mixtures of CH_2Cl_2–MeOH of increasing

Table 3
^{1}H- and ^{13}C-NMR chemical shifts and HMBC connectivities measured for **3** in $CDCl_3$ (20°C)

Position	group	δ ^{1}H (ppm)	δ ^{13}C (ppm)	HMBC connectivities[a]
1	CH_2	2.11–2.23	40.34	19
2	CH_2	1.41–1.94	28.72	1
3	CH	2.22	68.61	5, 30, 31, *N*–CH_3
4	C		41.27	5, 6, 30, 31
5	CH	1.97	51.37	1, 6, 7, 19, 30, 31
6	CH_2	1.37–2.14	30.25	5, 7, 8
7	CH_2	1.24–1.49	25.64	
8	CH	2.04	49.53	6, 7, 11, 19, 32
9	C		138.63	7, 8
10	C		135.99	1, 5, 19
11	CH	5.50 *s*	128.99	12, 19
12	CH_2	2.00–2.10	38.19	17, 18
13	C		43.39	11, 15, 18, 32
14	C		48.85	8, 18, 32
15	CH_2	1.38–1.44	33.03	16, 32
16	CH_2	1.48–1.83	26.65	17
17	CH	1.71	51.96	18, 21
18	CH_3	0.77 *s*	16.02	17
19	CH	5.93 *s*	129.23	1, 11
20	CH	4.00	48.76	17, 21
21	CH_3	1.11 *d*	21.45	
30	CH_3	1.06 *s*	24.89	31
31	CH_3	0.74 *s*	14.84	5, 30
32	CH_3	0.66 *s*	17.13	15
N_3–Me	CH_3	2.50 *s*	35.42	
C_{20}–HN	HN	5.23 *d*		
N–CO	C		168.85	CO–Me, HN
CO–Me	CH_3	1.92 *s*	23.78	HN

[a] Protons correlating with the carbon resonance.

polarities. The fraction eluted with CH_2Cl_2–MeOH (95-5) was chromatographed on a column of silica gel with mixtures of CH_2Cl_2–MeOH–diethylamine (95:5:0.5) to yield **1**. The fraction eluted with MeOH was subjected to prep TLC using the system toluene–acetone–EtOH (40:40:6) saturated with NH_3 to yield **2**. The fraction obtained at pH 4 (0.7 g) was chromatographed on a column of neutral alumina (60 g) with CH_2Cl_2–MeOH in order of increasing polarity. The fraction eluted with CH_2Cl_2–MeOH (99-1) was subjected to prep TLC (silica gel) using the system toluene–acetone–EtOH (40:40:6) saturated with NH_3 to supply amorphous **3**. The fraction eluted with CH_2Cl_2–MeOH (99.5-0.5) was subjected to prep TLC (silica gel) using the system petroleum ether (40–60°C)-Et_2O–Et_2NH (8:2:0.5) to supply amorphous **4**.

3.2. Mass spectroscopy

MS measurements were conducted on a Finnigan Mat Incoo 500E mass spectrometer.

3.3. Infra Red spectroscopy

IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR spectrometer. Optical rotations were measured on a Polarimeter Type AA-5 Optical Activity.

3.4. NMR spectroscopy

The samples were solubilized in $CDCl_3$ and all NMR experiments were recorded at 20°C on a Bruker AMX spectrometer, operating at 600 MHz for ^{1}H nucleus and 125 MHz for ^{13}Cnucleus. Chemical shifts are quoted relative to the $CDCl_3$ resonance fixed at 7.24 ppm for the proton and 77.0 ppm for the carbon. The multiplicity of carbon signals was established by using the DEPT experiment.

DQF-COSY spectra (Rance et al., 1983) were collected into a 800×1024 data matrix with 32 scans per t_1 value and TOCSY (Rance, 1987) spectra were collected with a mixing time of 80 ms into a 512×1024 data matrix with 16 scans per t_1 value.

HSQC (Bodenhausen and Ruben, 1980) experiments were recorded with a delay of 3.5 ms ($^1J_{CH} = 143$ Hz) and HSQC-TOCSY (Lerner and Bax, 1986) experiment with a mixing time for proton-proton transfer of 80 ms (400×1024) with 32 scans per t_1 value to identify respectively, the one-bond carbon-proton and the network of proton-proton connectivities. The HMBC (Bax and Summers, 1986) experiment (400×1024) was recorded with a delay of 50 ms with 64 scans per t_1 value to identify long-range proton-carbon connectivities.

All data were processed with the UXNMR software. For DQF-COSY and TOCSY data one zero filling and a $\pi/4$ phase-shifted sine bell window function were applied in both dimensions before Fourier transform. For HSQC, HSQC-TOCSY and HMBC data, a zero filling and a $\pi/2$ phase-shifted sine bell window function were applied in F2 and F1 dimensions prior to processing.

N_{20}-formylbuxaminol E (**1**) was obtained as a colorless gummy material (7 mg), $[\alpha]_D$: + 13° (*c* 0.15 in MeOH). UV λ_{max}: 238, 245, 254 (*sh*) nm. IR ν_{max} 3334, 2931, 1667, 1647 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1. EIMS *m*/*z* (rel. int): 428 $[M]^+$ (7), 399 $[M\text{-}COH]^+$ (2), 385 $[M\text{-}43]^+$ (13), 84 (35), 71 (100), 58 (33), 44 (63).

O_{16}-syringylbuxaminol E (**2**) was isolated as a colorless gummy material (17 mg), $[\alpha]_D$: + 83° (*c* 0.53 in EtOH). UV λ_{max}: 221, 236, 245, 254 (*sh*), 278 nm. IR ν_{max} 3364, 2942, 1694, 1462, 1330, 1219, 1118, 1007 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 2. EIMS *m*/*z* (rel. int): 580 $[M]^+$ (4), 181 (46), 84 (33), 71 (72), 58 (35), 44 (100).

N_{20}-acetylbuxamine G (**3**) was isolated as a colorless gummy material (8 mg), $[\alpha]_D$: + 20° (*c* 0.41 in MeOH). UV λ_{max}: 238, 245, 254 (*sh*) nm. IR ν_{max} 3289, 2932, 1665, 1646 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 3. EIMS *m*/*z* (rel. int):412 $[M]^+$ (10), 397 (4), 369 (14), 57 (100).

N_{20}-acetylbuxamine E (**4**) was obtained as a colorless gummy material (7 mg), $[\alpha]_D$: + 8° (*c* 0.53 in MeOH). UV λ_{max}: 238, 245, 254 (*sh*) nm. IR ν_{max} 3280, 2974, 1663, 1641 cm^{-1}. EIMS *m*/*z* (rel. int):426 $[M]^+$ (4), 383 (8), 84 (48), 71 (79), 58 (33), 44 (100).

Acknowledgements

The work was partially supported financially by Conseil Régional Provence Alpes Cotes d'Azur.

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