New Triterpenoidal Alkaloids from *Buxus hyrcana*

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Phytochemical studies on the methanolic extract of the leaves of *Buxus hyrcana* pojark, collected in Iran, have resulted in the isolation of two new triterpenoidal alkaloids, (+)-2α,16β,31-triacetylbuxiran (**1**), (+)-2α,16β,31-triacetyl-9-11-dihydrobuxiran (**2**). Spectroscopic methods were used to elucidate the structures of these new natural products.

Key words: Buxus hyrcana, Tripenoidal Alkaloid, Buxiniran

Introduction

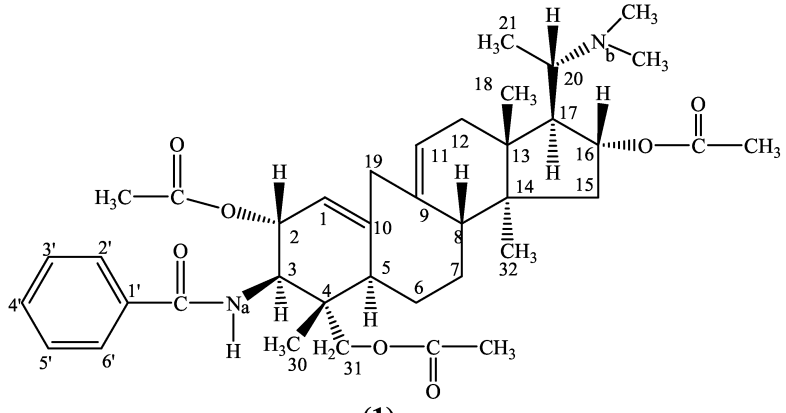
The genus *Buxus* is a rich source of steroidal alkaloids which have shown interesting biological activities. For instance, cycloprotobuxine-A has shown a protective effect against cardiac arrythmia induced by oubain (LD_{50} 5 mg/kg) and positive inotropic effect on isolated guinea pig myocardium [1 – 3]. The crude ethanolic extract of *Buxus semeprvirens* has been reported to exhibit anti Human Immunodeficiency Virus (HIV) activity [4]. Previous chemical studies on different plants of genus *Buxus* have yielded over 80 triterpenoidal alkaloids [5].

Buxus hyrcana is abundant in Iran and previously, over ten compounds have been isolated from this species [6 – 7]. (+)-*N*-Tigloylbuxahyrcanine, purified from *B. hyrcana*, has shown acetylcholinesterase and butyrocholinesterase inhibitory activities [6 – 7]. Our recent chemical studies on the methanolic extract of *B. hyrcana* have resulted in the isolation of two new triterpenoidal alkaloids, (+)-2α,16β,31-triacetylbuxiran (**1**), (+)-2α,16β,31-triacetyl-9-11-dihydrobuxiran (**2**). Spectroscopic methods were used to establish the structures of these new compounds.

Results and Discussion

(+)-2α,16β,31-Triacetylbuxiran (**1**) was purified as a colorless amorphous solid. Its IR spectrum displayed intense bands at 3456 (amidic NH), 1715 (ester carbonyl), 1660 (amidic carbonyl), and 1597 (C=C) cm^{-1}. The UV spectrum showed maximum absorption at 227 nm, indicating the presence of a secondary benzamide chromophore [8]. The High Resolution Electron Impact Mass Spectrum (HREIMS) of compound **1** exhibited the molecular ion peak at *m/z* 662.3897, which provided the molecular formula $C_{39}H_{54}N_2O_7$ (calcd. 662.3931). The ^1H and ^{13}C NMR data also supported this molecular formula indicating the presence of fourteen degrees of unsaturation in compound **1**. The base peak at *m/z* 72 ($C_4H_{10}N$) suggested the presence of a *N*, *N*-dimethylamino group at C-20 [9 – 11]. Another intense ion at *m/z* 105 (C_7H_5O) was due to the loss of a benzoyl cation [9 – 11].

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of **1** displayed three 3H singlets at $\delta = 0.82$, 0.91 and 0.92 due to the C-18, C-30 and C-32 tertiary methyl protons, respectively. A secondary C-21 methyl resonated as a doublet at $\delta = 0.89$ ($J_{20,21} = 6.5$ Hz). A six-proton broad singlet at $\delta = 2.37$ was ascribed to the N_b-dimethyl protons. The C-3 resonated as doublets of double doublet at $\delta = 4.21$ ($J_{3\alpha,2\beta} = 12.5$ Hz, $J_{3\alpha,NH} = 9.5$ Hz, $J_{3\alpha,5\alpha} = 0.5$ Hz), while the amidic NH resonated as a doublet at $\delta = 6.51$ ($J_{NH,3\alpha} = 9.5$ Hz). The C-31 methylene protons resonated as two sets of AB doublets at $\delta = 3.92$ and 4.10 ($J = 11.0$ Hz). A broad doublet at $\delta = 2.76$ ($J_{19\alpha,19\beta} = 15.7$ Hz) was due to H-19α, while H-19β appeared as a multiplet at $\delta = 2.58$. Two aliphatic downfield signals at $\delta = 4.75$ and 5.26 were attributed to the C-16 and C-2 methine protons, respectively. Their downfield chemical shift values were indicative of the presence of geminal acetoxy functionalities on C-2 and C-16. The C-1 and C-11 olefinic protons resonated at $\delta = 5.33$ and



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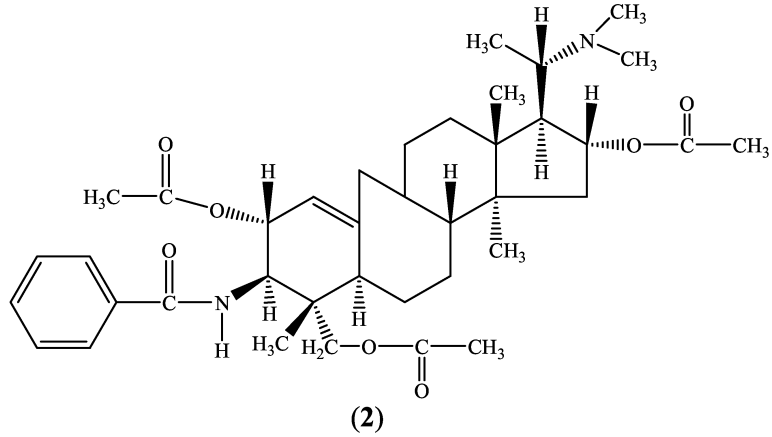


(**2**)

5.37, respectively. The aromatic protons of the benzoyl moiety appeared as two sets of multiplets centered at $\delta = 7.26 - 7.69$.

The COSY 45° spectrum was very informative with regard to the assignment of the ^{1}H NMR chemical shift assignments correctly. The C-3 methine proton ($\delta = 4.21$) showed vicinal couplings with the amidic NH ($\delta = 6.51$) and C-2 methine proton ($\delta = 5.26$). The latter in turn displayed COSY 45° interactions with the C-1 methine proton ($\delta = 5.33$). The C-11 methine proton ($\delta = 5.37$) showed cross-peaks with the C-12 methylene protons ($\delta = 1.96$ and 2.03). Allylic couplings of C-19 methylene protons ($\delta = 2.58$ and 2.76) with the C-1 and C-11 protons were also observed in the COSY 45° spectrum. The C-16 methine proton ($\delta = 4.75$) exhibited ^{1}H-^{1}H spin correlations with the C-15 methylene ($\delta = 1.38$ and 1.60) and C-17 methine ($\delta = 1.85$) protons. The latter showed cross-peaks with the C-20 methine proton ($\delta = 2.32$), which in turn exhibited vicinal couplings with the C-21 methyl protons ($\delta = 0.89$).

The ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of **1** showed signals of all thirty nine carbons atoms and a very careful interpretation of this spectrum showed that most of the carbons have similar chemical shift as those of *Buxus* alkaloids. Complete ^{13}C NMR chemical shift assignments and ^{1}H/^{13}C one-bond shift correlations of all protonated carbon atoms of **1**, as determined from the HMQC spectrum, are presented in Table 1. The HMBC spectrum showed long-range heteronuclear couplings and it was very helpful to assign ^{13}C NMR chemical shift assignments of quaternary carbon atoms of compound **1**. Important HMBC interactions of **1** are shown in Fig. 1.

The stereochemistry at various chiral centers was established with the help of a NOESY spectrum and ^{1}H-^{1}H coupling constants. The C-16 methine proton ($\delta = 4.75$) showed NOE interaction with the C-20 methine proton ($\delta = 2.32$), which exhibited cross-peaks with the C-18 methyl protons ($\delta = 0.82$). H_3-18 also showed cross-peaks with H-8 ($\delta = 2.13$). It has already been reported in the literature that H-8 is in-

Table 1. ^{1}H and ^{13}C NMR chemical shift assignments and ^{1}H/^{13}C one-bond shift correlations determined from HMQC spectra of compounds **1** – **2**.

† Multiplicities were determined by DEPT spectrum.

	1		**2**	
Carbon	^{1}H	^{13}C†	^{1}H	^{13}C†
1.	5.33 (br s)	123.7(d)	5.39 (br s)	124.5(d)
2.	5.26 (m)	68.2(d)	5.30 (m)	67.2(d)
3.	4.21(ddd, J=12.5,9.5 and 0.5 Hz)	62.1(d)	4.25(ddd, J=12.5,9.5 and 2.5 Hz)	65.3(d)
4.	–	39.7 (s)	–	40.8(s)
5.	1.25 (m)	42.7(d)	1.30 (m)	41.5(d)
6.	1.81, 1.47 (m)	27.2(t)	1.80 and 1.40 (m)	26.7(t)
7.	1.85 and 1.29 (m)	27.09(t)	1.89 and 1.25 (m)	27.2(t)
8.	2.13(m)	31.4(d)	2.25 (m)	31.3(d)
9.	–	124.2(s)	1.67 (m)	29.2(d)
10.	–	124.0 (s)	–	123.9(s)
11.	5.37 (br s)	119.10	2.11 and 1.68 (m)	29.9(t)
12.	2.03 and 1.96(m)	29.1(t)	2.12 and 1.91 (m)	29.2(t)
13.	–	30.0(s)	–	30.3(s)
14.	–	31.9(s)	–	31.8 (s)
15.	1.60 and 1.38 (m)	28.9(t)	1.68 and 1.37(m)	29.9(t)
16.	4.75(m)	66.7(d)	4.76(m)	66.8(d)
17.	1.85(m)	30.4(d)	1.84(m)	30.5(d)
18.	0.82(s)	11.0(q)	0.84(s)	10.9(q)
19.	2.76(br d, J = 15.7 Hz) 2.58 (br s)	39.4(t)	2.77(br d, J = 15.7 Hz) 2.58 (br. s)	39.5(t)
20.	2.32 (m)	60.3(d)	2.31(m)	62.1(d)
21.	0.89(d, J = 6.5Hz)	8.7 (q)	0.87(d, J =Hz)	8.3(q)
30.	0.91(s)	14.1(q)	0.89(s)	14.0(q)
31.	3.92 (d, J = 11.0 Hz) 4.10 (d, J = 11.0 Hz)	68.9(t)	3.90 (d, J = 10.9 Hz) 4.10(d, J = 10.9 Hz)	68.9(t)
32.	0.92 (s)	14.1(q)	0.90 (s)	14.3(q)
1'.	–	132.5(s)	–	132.3(s)
2'.	7.26(m)	128.80(d)	7.24(m)	128.7(d)
3'.	7.51(m)	129.8(d)	7.49(m)	129.7(d)
4'.	7.69(m)	131.0	7.75(m)	130.8(d)
5'.	7.51(m)	129.8(d)	7.49(m)	129.7(d)
6'.	7.26(m)	128.80(d)	7.24(m)	128.7(d)
$N(CH_3)_2$	2.37(br s)	38.6(q)	2.38(br s)	39.0(q)
NHCO	–	167.8(s)	–	168.0(s)
$OCOCH_3$	2.01	23.1(q)	1.99(s)	23.0(q)
$OCOCH_3$	2.02	22.9(q)	2.00(s)	22.8(q)
$OCOCH_3$	2.05	23.6(q)	2.04(s)	23.5(q)
$OCOCH_3$	–	174.2(s)	–	174.0(s)
$OCOCH_3$	–	174.2(s)	–	174.0(s)
$OCOCH_3$	–	174.4(s)	–	174.02(s)

variably β-oriented in this class of alkaloids [12]. This suggested β-orientation of H-16, H-20 and the C-18 methyl group. H-3 ($\delta = 4.21$) showed cross-peaks with H-5 ($\delta = 1.25$). H-5 is invariably α-oriented in *Buxus* alkaloids [12] indicating an α-orientation of C-3 H and β-orientation of the amino functionality. The C-2 methine proton resonated as a double doublet at $\delta = 5.26$ ($J_{2\beta,3\alpha} = 12.5$ Hz, $J_{2\beta,1} = 5.2$ Hz). The coupling constant between H-2 and H-3 ($J_{2\beta,3\alpha} = 12.5$ Hz) was indicative of *trans-diaxial* orientations of these two protons. We already assigned an α-orientation for H-3 and this coupling constant data led us to assign the β-orientation for H-2 and an α-stereochemistry for the C-2 acetoxy functionality. The probable conformations of rings A, B, C and D, as obtained from the NOESY spectrum, are shown in Fig. 2. These spectroscopic studies helped to establish structure **1** for this new alkaloid.

Our second compound, (+)-2α,16α,31-triacetyl-9,11-dihydrobuxiran (**2**) was isolated as a colorless amorphous solid. Its IR and UV spectra were nearly identical to those of compound **1** indicating the presence of same functional groups and chromophore. The ^{1}H and ^{13}C NMR spectra were also distinctly similar to those of compound **1** except for the chemical shift values of C-9 and C-11. The C-9 methine proton appeared at $\delta = 1.67$ and C-11 methylene protons resonated at $\delta = 1.68$ and 2.11. The H-9 dis-

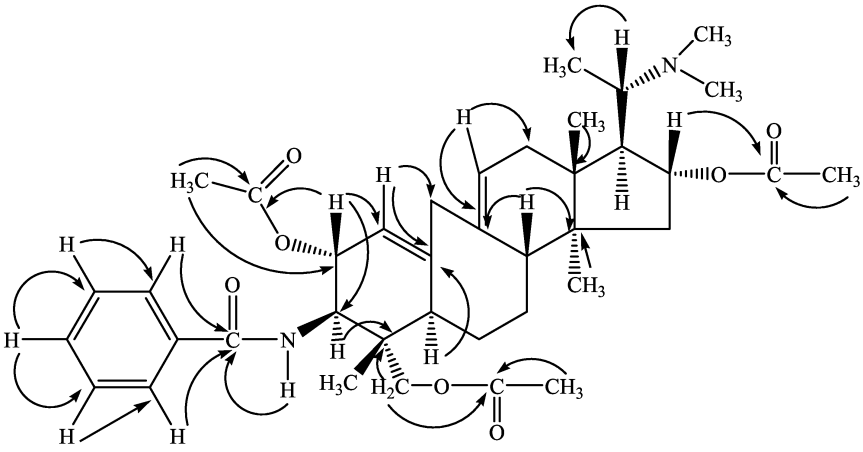
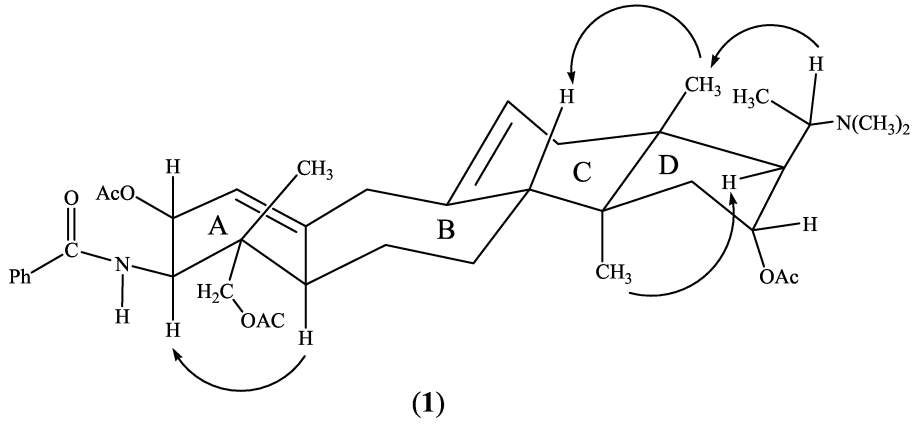


Fig. 1. Important $^{1}H/^{13}C$ long-range couplings of Compound **1**, as observed in HMBC spectrum.



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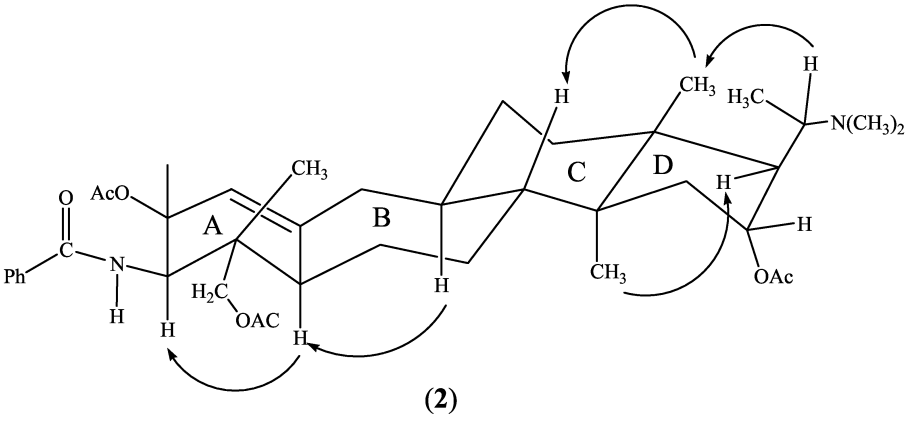

(**2**)

Fig. 2. Probable conformations of Rings A, B, C and D in compounds **1** and **2**, as obtained from NOESY spectrum and important NOE interactions.

played vicinal couplings with C-11 methylene protons ($\delta = 1.68$ and 2.11) and the later showed cross-peaks with C-12 methylene protons ($\delta = 2.12$ and 1.91) in the COSY 45° spectrum. In the ^{13}C NMR spectrum, the C-9 and C-11 resonated at $\delta = 29.2$ and 29.9, respectively. The upfield chemical shift values of these two carbon atoms indicated that C-9 and C-11 were sp^3 hybridized methylene carbon atoms. This was further confirmed by the HMQC spectrum in which H_2-11 ($\delta = 1.68$ and 2.11) showed $^{1}H/^{13}C$ one-bond shift cor-

relations with C-11 ($\delta = 29.9$) and H-9 ($\delta = 1.67$) displayed cross peak with the C-9 ($\delta = 29.2$). This spectral data suggested to us that compound **2** is a 9,11-dihydro analog of compound **1**. This was also confirmed by the mass spectrum, which exhibited a molecular ion peak at *m/z* 664.4091. This provided molecular formula $C_{39}H_{56}N_2O_7$ (calcd 664.4088) and indicated the presence of thirteen degrees of unsaturation in compound **2**. Additionally, the mass spectrum of **2** showed that each ion was appearing at 2 amu higher than those of compound **1**, as it can be expected for the dihydro derivative of **1**.

After confirming that compound **2** was a 9,11-dihydro derivative of compound **1**, we used NOESY spectrum to establish the stereochemistry at all chiral centers. The NOE data was similar to that of compound **1**. This helped us to assume that most of the chiral centers have same stereochemistry as those of compound **1**. H-9 ($\delta = 1.67$) showed cross-peaks with C-5 methine ($\delta = 1.30$) and C-14 methyl ($\delta = 0.90$) protons. This helped us to assign α-stereochemistry for C-9 methine proton. Based on these spectroscopic studies, structure **2** was established for this new steroidal alkaloid.

Experimental Section

General

The mass spectrometric measurements were conducted on Shimadzu (GC-14A) and INCOSSO, FINNIGA-MAT Mass spectrometers. The ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-500 AVANCE NMR spectrometers at 500 MHz while ^{13}C NMR was recorded on the same Instrument at 125 MHz with TMS as Internal standard. The IR and UV spectra were recorded on a Shimadzu IR-240 and Shimadzu UV 240 spectrophotometers, respectively. The optical rotations were measured on a Polatronic D polarimeter (Hitachi). The column chromatography was carried out using silica gel (type 60 of mesh size 7 – 230 purchased from Merck) and the purities of the samples were checked on TLC (silica gel, GF 254 pre-coated plates purchased from Merck).

Plant material

The leaves of *Buxus hyrcana* pojark (5 kg) were collected by one of us, in August 2004. Prof. Jahad Sazandegi, Mazandaran state, Iran, identified this plant and a voucher specimen (No.B-530) was deposited in the herbarium of the Shaheed Beheshti University, Tehran, Iran.

Extraction of plant material

The leaves of *B. hyrcana* were dried and extracted with methanol (5 l) at 25 °C. The solvent was evaporated under reduced pressure to prepare gum (381.1 g). This extract was dissolved in distilled water and this extract was defatted with hexane. This defatted extract was extracted with chloroform at different pH values (3.5, 7.0 and 9.5).

Isolation of compounds **1** *and* **2**

The chloroform extract (28.1 g) obtained at pH 9.5 was subjected to column chromatography. The column was eluted with pet. ether (40 – 60 °C)-chloroform (0 – 100%) and then with chloroform-methanol (0 – 100%). A fraction obtained on elution of silica gel column with pet ether (40 – 60 °C)-chloroform (40:60) was subjected to preparative silica gel TLC using pet ether (40 – 60 °C)-acetone-diethylamine (6:4:0.1) as a mobile phase to afford compounds **1** and **2**.

*(+)-2α,16β,31-Triacetylbuxiran (**1**)*

$[\alpha]_D^{25} = +107$ (c = 0.26, $CHCl_3$). – UV/vis (MeOH): $\lambda_{max} = 227$ nm. – IR ($CHCl_3$): $\tilde{\nu} = 3456$ (amidic NH), 2912 (CH), 1715 (ester carbonyl), 1660 (amidic carbonyl) and 1597 (C=C) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ see Table 1. – ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ see Table 1. – MS (EI, 70 eV): *m/z* (%) = 662 (M+, 10%), 647 (M^+-CH_3, 12%), 105 (C_7H_5O, 45%), 72 ($C_4H_{10}N$, 100%). – MS (HREI): $m/z = 662.3897$ (M^+, $C_{39}H_{54}N_2O_7$, calcd. 662.3931).

*(+)-2α,16β,31-Triacetyl-9,11-dihydrobuxiran (**2**)*

$[\alpha]_D^{25} = +89$ (c = 0.39, $CHCl_3$). – UV(MeOH): $\lambda_{max} =$ 226 nm. – IR ($CHCl_3$): $\tilde{\nu} = 3453$ (amidic NH), 2910 (CH), 1712 (ester carbonyl), 1665 (amidic carbonyl) and 1600 (C=C) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ see Table 1; – ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ see Table 1. – MS (EI, 70 eV): *m/z* (%) = 664 (M+, 8%), 649 (M^+-CH_3, 9%), 105 (C_7H_5O, 55%), 72 ($C_4H_{10}N$, 100%). – MS (HREI): $m/z = 664.4091$ (M^+, $C_{39}H_{56}N_2O_7$, calcd. 664.4088).

Acknowledgements

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