A New Triterpenoid Alkaloid from *Buxus sempervirens*

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A new triterpenoid alkaloid, 16-deacetoxy-hyrcamine (**1**), together with six analogs, was isolated from the leaves and stems of *Buxus sempervirens*. Their structures were elucidated based on spectral methods and by comparison of the spectral data with those reported previously. All isolated compounds were evaluated for their growth inhibitory activity against human cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480. Compounds **1** – **4** and **6** showed modest cytotoxic activity against A-549, MCF-7, and SW480.

Key words: Buxus sempervirens, Triterpenoid Alkaloid, 16-Deacetoxy-hyrcamine, Cytotoxicity

Introduction

Buxus sempervirens, of the family Buxaceae, is an indigenous medicinal plant used in China. The *Buxus* is a rich source for triterpenoid alkaloids, and more than 220 *Buxus* alkaloids have been isolated from different species [1]. Among these *Buxus* alkaloids from *B. sempervirens*, cyclobuxine was found as the first compound in this genus [2]. Buxenine-G is the first cytotoxic *Buxus* alkaloid [3], and spirofornabuxine constitutes a novel type by its original spiro junction at C-10 and by the presence of a cycloheptatriene ring [4]. In order to discover new secondary metabolites with diverse structures and promising activities, chemical and activity investigations on this plant were carried out. As a result, a new triterpenoid alkaloid, 16-deacetoxy-hyrcamine (**1**), and six known ones (**2** – **7**) (Fig. 1) were isolated. The cytotoxic activities of all isolated compounds were also evaluated.

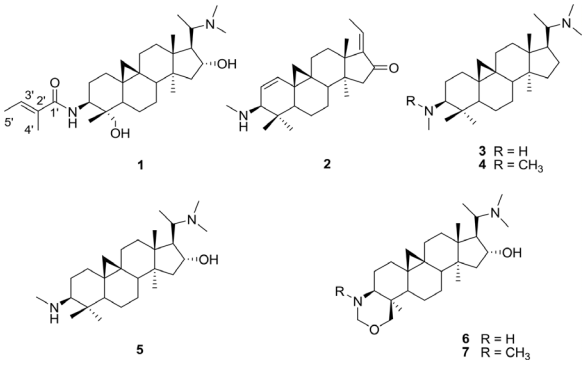

Fig. 1. Chemical structures of **1** – **7**.

Result and Discussion

16-Deacetoxy-hyrcamine (**1**) was obtained as a colorless powder, and the molecular formula was established to be $C_{31}H_{53}N_2O_3$ on the basis of HR-ESI-MS at m/z = 501.4056 $[M+H]^+$ (calcd. 501.4061). The IR spectrum suggested the presence of amide ketone (1661 cm^{-1}) functions.

The assignment of the ^1H and ^{13}C NMR spectroscopic data of **1** (Table 1) was based on HSQC, HMBC data, and on ROESY, ^1H, ^1H-COSY spectra.

The ^1H, ^{13}C NMR and DEPT spectra displayed 31 carbon resonances comprising a typical tigloyl group located at C-3 [δ_H = 6.45 (q, J = 12.0, H-3′), 1.86 (s, H-4′), 1.77 (d, J = 12.0, H-5′); δ_C = 170.4 (s, C-1′), 131.2 (s, C-2′), 131.7 (d, C-3′), 12.5 (q, C-4′), 14.0 (q, C-5′)], and the deducatioin was supported by heteronuclear multiple bond connectivity (HMBC) correlations: H-3′ to C-1′, C-2′, C-4′, C-5′; H-4′ to C-1′, C-2′, C-3′; H-5′ to C-2′, C-3′, and H-3 (δ_H = 4.07) to C-1′ (Fig. 2). Six methyls (including one secondary and three tertiary methyls, and two $N(CH_3)_2$), nine methylenes [including characteristic signals for a cyclopropane CH_2 at $\delta_{H/C}$ = 0.36, 0.54; 30.3 (C-19) and one hydroxymethylene at $\delta_{H/C}$ = 2.95, 3.35; 64.1 (C-30)], six methines (of which one was oxygenated), and five quaternary carbons. The NMR data of **1**, assigned tentatively as a triterpenoid alkaloid, were

Table 1. ^{1}H and ^{13}C NMR data of **1** (at 500/125 MHz, resp., in $CDCl_3$).

No.	δ_C	δ_H	No.	δ_C	δ_H
1	32.6 (t)	1.24 (m), 1.69 (m)	16	77.2 (d)	4.53 (m)
2	26.0 (t)	1.31 (m)	17	65.0 (d)	3.20 (m)
3	51.1 (d)	4.07 (m)	18	20.2 (q)	1.16 (s)
4	46.0 (s)		19	30.3 (t)	0.36, 0.54 (d, J = 5.0 Hz, H-19)
5	40.3 (d)	1.98 (m)	20	56.8 (d)	2.07 (m)
6	20.7 (t)	1.12 (m), 1.75 (m)	21	11.0 (q)	1.18 (d, J= 8.0 Hz, H-21)
7	27.8 (t)	1.68 (m)	30	64.1 (t)	3.35, 2.95 (AB, J = 12.5 Hz)
8	47.9 (d)	1.41 (m)	31	11.4 (q)	0.62 (s)
9	19.1 (s)		32	19.3 (q)	1.00 (s)
10	25.6 (s)		1′	170.4 (s)	
11	25.7 (t)	1.14 (m)	2′	131.2 (s)	
12	32.2 (t)	1.38 (m), 1.52 (m)	3′	131.7 (d)	6.45 (q, J = 12.0 Hz)
13	44.5 (s)		4′	12.5 (q)	1.86 (s)
14	47.5 (s)		5′	14.0 (q)	1.77 (d, J = 12.0 Hz)
15	44.4 (t)	1.84 (m)			

Table 2. Cytotoxicity of compounds with IC_{50} values (μM).

No.	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	17.60	> 40	19.70	13.65	11.07
2	5.26	15.73	17.32	12.07	3.35
3	19.08	32.45	14.99	15.46	12.35
4	15.98	> 40	35.74	16.06	13.98
5	> 40	> 40	> 40	> 40	> 40
6	16.77	28.07	16.24	15.98	12.79
7	22.41	> 40	30.43	> 40	13.57
Cisplatin	1.00	17.05	26.75	14.97	16.88
Taxol	< 0.008	< 0.008	< 0.008	< 0.008	< 0.008

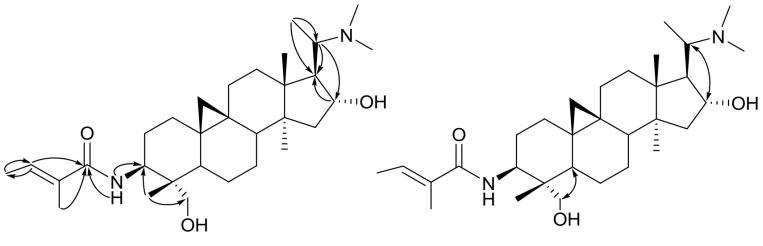

Fig. 2. Key HMBC and ROESY correlations of **1**.

similar to those of hyrcamine [5]. The distinct difference between them was that an acetoxy group in hyrcamine was replaced by a hydroxyl in **1**. Therefore, compound **1** was elucidated as 16-deacetoxyhyrcamine.

The identity of the six known compounds, buxithienine (**2**) [6], cycloprotobuxine C (**3**) [7, 8], cycloprotobuxine A (**4**) [9], cyclovirobuxine C (**5**) [7], cyclobuxoxazine (**6**) [10], and cyclobuxoxazine A (**7**) [11] was established on the basis of their spectroscopic data as well as by comparison with literature data.

All obtained compounds were tested for cytotoxic activity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 human cell lines. Results are expressed as IC_{50} values in μM. As summarized in Table 2, compounds **1** – **4** and **6** showed cytotoxicity against A-549, MCF-7, and SW480, while **7** showed cytotoxicity only to the SW480. Compound **5** was non-cytotoxic, with IC_{50} values > 40 μM for all cell lines.

Experimental Section

General

Optical rotations were obtained with a Horiba SEAP-300 polarimeter. NMR spectra were measured on Bruker AM-400 and DRX-500 instruments (Bruker, Zürich, Switzerland) with TMS as internal standard. HR-ESI-MS data were recorded on a VG Auto Spec-3000 spectrometer. Infrared spectra were recorded on an Shimadzu IR-450 instrument by using KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (200 – 250 μm thickness. F_{254} Si-gel 60 and F_{254} RP-18 Si-gel-60, Qingdao Marine Chemical, Inc.). Silica gel (200 – 300 mesh, Qingdao Marine Chemical, Inc.), amino silica gel (75 – 100 μm, Fuji Silysia Chemical LTD, Japan), C-18 (25 – 40 μm, Fuji Silysia Chemical LTD, Japan),

and Sephadex LH-20 (Pharmacia) were used for column chromatography.

Plant material

The leaves and stems of *B. sempervirens* were collected at Kunming (Yunnan), China, in August 2009. The sample was identified by Prof. Xun-Gong of the Kunming Institute of Botany, and a voucher specimen (KIB 20090821) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dried and powdered stems and leaves of *B. sempervirens* (10.0 kg) were extracted with Me_2CO (3×20 L, each one day) at r.t. and filtered. The filtrate was evaporated to yield an extract, which was partitioned between EtOAc and 0.01 N HCl. The aqueous layer was alkalinized to pH = 10.0 with 2 N NaOH followed by exhaustive extraction with $CHCl_3$. The $CHCl_3$-soluble fraction (26 g) was chromatographed on a silica gel column, eluted with $CHCl_3$-MeOH (50 : 1, 20 : 1, 10 : 1, 2 : 1), to give four major fractions (Fr. 1 – 4). Fr. 1 (3 g) was separated by a Sephadex LH-20 gel column eluted with MeOH and then further purified by silica gel column chromatography (CC), using PE-EtOAc-Et_2NH (1000 : 10 : 3) as the mobile phase to yield **3** (18 mg), **4** (23 mg), **5** (8 mg), and **7** (10 mg). Fr. 2 (1 g) was subjected to silica gel using PE-Me_2CO (20 : 1, 10 : 1, 5 : 1) as solvent to afford fractions Fr. 2.1 – 3. Fr. 2.2 (300 mg) was separated on C-18 gel CC by aqueous MeOH (60 – 90 %) and subjected to a silica gel column using $CHCl_3$-Me_2CO (20 : 1) as eluent to yield **6** (12 mg). Fr. 3 (2.3 g) was chromatographed on silica gel using $CHCl_3$-MeOH (20 : 1, 5 : 1) as solvent and repeatedly separated on amino silica gel CC, eluted with $CHCl_3$-MeOH (50 : 1, 10 : 1), to give **1** (8 mg). Fr. 4 (4.1 g) was chromatographed on amino silica gel using $CHCl_3$-MeOH (20 : 1, 5 : 1) as solvent to afford Fr. 4.1 and Fr. 4.2. Fr. 4.1 (1 g) was separated on Sephadex LH-20 (MeOH), to give **2** (23 mg).

*16-deacetoxy-hyrcamine (**1**)*: colorless powder. – UV (MeOH) λ_{max} ($\lg \varepsilon_{max}$) = 242 (2.72), 228 (2.44) nm. $[\alpha]_D^{24} = -11.6$ (c = 1.52, MeOH). – IR (KBr): ν = 2925, 1661 cm^{-1}. – ^1H, ^{13}C NMR data: see Table 1. – MS ((+)-ESI): m/z = 501. – HRMS ((+)-ESI): m/z = 501.4056 (calcd. 501.4061 for $C_{31}H_{54}N_2O_3$, $[M+H]^+$).

Cytotoxicity test

Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells with the MTT (MTT, sigma, USA) method described as before [12], and using cisplatin (DDP, sigma, USA) as control. Cell growth inhibition curves were graphed, and the IC_{50} value of each compound was calculated by the Reed and Muench method [13]. The cell lines used in this experiment were: promyelocytic leukemia HL-60, hepatocellular carcinoma SMMC-7721, alveolar basal epithelial carcinoma A549, breast adenocarcinoma MCF-7, and colon cancer SW480. The cells lines were obtained from the Shanghai cell bank of China.

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