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THREE STEROIDAL ALKALOIDS FROM *BUXUS MICROPHYLLA*

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Three new steroidal alkaloids have been isolated from the leaves and stems of *Buxus microphylla* Sieb et Zucc. They are buxmicrophylline B, C and D. Their structures were elucidated by extensive analysis of the spectral data.

Keywords: *Buxus microphylla*; Buxmicrophylline B, C and D

INTRODUCTION

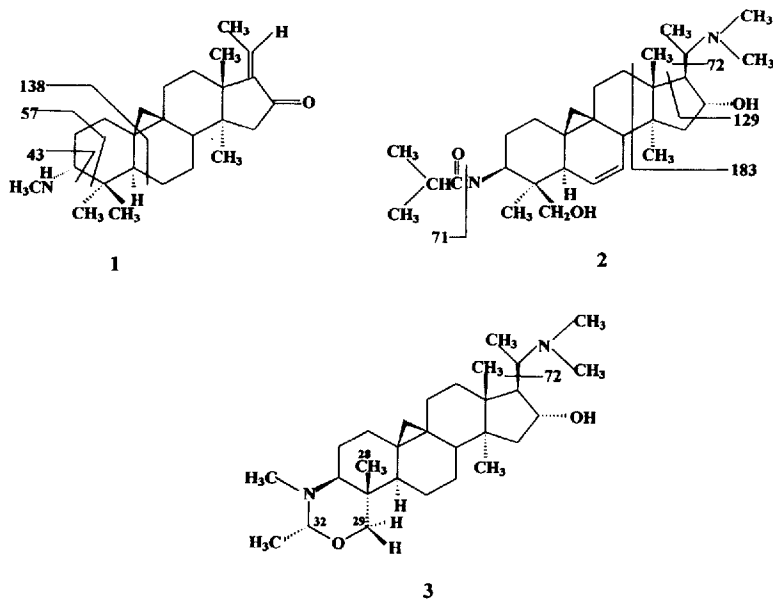
Buxus microphylla Sieb et Zucc is an evergreen shrub which is found in the southwest town Kunming, Yunnan province of China. Working on the leaves and stems of *B. micrylla* Sieb. et Zucc has resulted in isolation of three steroidal alkaloids. They all have the pentacyclic 9- β -19-cyclo-5- α -pregnane systems. In this paper, we report the isolation and structural elucidation of three steroidal alkaloids from the leaves and stems of this plant.

RESULTS AND DISCUSSION

Buxmicrophylline B (**1**) was assigned the molecular formula $C_{25}H_{39}NO$ (HRMS). The ion of highest mass $[M]^+$ was observed at m/z 369 in EIMS

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and shifted to $[M+1]^+$ m/z 370 in FABMS. The IR and UV absorptions appeared at $1710, 1640\text{ cm}^{-1}$ and 249 nm , which indicated an α,β -unsaturated carbonyl function. The $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) spectrum of **1** showed five methyl signals as singlets at δ 1.81, 1.38, 1.23, 0.92 and 0.91 which were assigned to 21- CH_3 , H-18, H-28, H-30 and H-29. The cyclopropyl methylene protons appeared as two doublets at δ 0.57 and 0.32 ($J=4.2\text{ Hz}$). The doublet at δ 1.81 ($J=7.5\text{ Hz}$) was attributed to the 21- CH_3 group, splitted by H-20, which in turn appeared as a quartet at δ 6.54 ($J=7.5\text{ Hz}$). A singlet at δ 2.21 was assigned to the N- CH_3 group. The base peak at m/z 57 also resulted from the cleavage of ring A as shown in structure **1**. Compound **1** was very similar to (–)-E-buxenone [1]. The only difference was the chemical shift data of C-1 and C-5 at δ 25.92 and 44.17. It suggested that the 3-H should be of β orientation. Otherwise, the chemical shifts should be at δ 30 and 47 respectively [2]. The structure of buxmicrophylline B was thus assigned to be **1**.



Buxmicrophylline C (**2**) has a molecular formula $\text{C}_{30}\text{H}_{50}\text{N}_2\text{O}_3$ based on HRMS. Its IR spectrum indicated the presence of hydroxyl group ($3400, 3250, 1090, 1040\text{ cm}^{-1}$), amide group (1540 cm^{-1}) and carbon-carbon double bond ($3100, 1630\text{ cm}^{-1}$). The $400\text{ MHz } ^1\text{H-NMR}$ spectrum of **2** showed that two olefinic protons at δ 5.65 (1H, d, $J=10.5\text{ Hz}$, H-7) and

5.35 (1H, dd, $J = 10.5, 3.2$ Hz, H-6). 3-H and 16-H were at δ 4.08 (1H, m) and 4.03 (1H, septet, $J = 2.8, 7.2, 10.0$ Hz) respectively, thus 16-H should be of β orientation [3]. Two coupled signals at δ 2.95 (1H, d, $J = 13.4$ Hz) and 3.47 (1H, d, $J = 13.4$ Hz) suggested two protons attached to C-28. The difference of their chemical shifts indicated that one proton was shielded and another was deshielded by the carbonyl group. In the ^{13}C -NMR spectrum of **2**, C-28 shifted upfield from δ 73.7 to 63.8 and also C-19 shifted upfield from δ 30.4 to 18.8. It can be explained that the two carbons were shielded by the carbonyl group and carbon-carbon double bond respectively. The mass spectrum of **2** showed the base peak at m/z 71 due to the fragment $(\text{CH}_3)_2\text{CH}-\text{C}=\text{O}$. Loss of the trimethyliminium side chain accounted for m/z 72. Other important peaks are m/z 129, 183. Therefore, the structure of buxmicrophylline C is **2**.

Buxmicrophylline D (**3**) was obtained as colorless needles. The HREIMS of **3** exhibited the M^+ peak at m/z 458.3839 corresponding to the molecular formula $\text{C}_{29}\text{H}_{50}\text{N}_2\text{O}_2$. Based on its IR, ^1H -, ^{13}C -NMR spectra, compound **3** has no unsaturated group, so it should have six rings according to the calculation of its unsaturation degrees ($\text{UN} = 6$). Its IR spectrum indicated the presence of hydroxyl group ($3500, 3420, 3280\text{ cm}^{-1}$). The 400 MHz ^1H -NMR spectrum of **3** showed the presence of two coupled signals at δ 4.27 (1H, q, $J = 5.6$ Hz) and 1.28 (3H, d, $J = 5.6$ Hz) characteristic of $\text{CH}-\text{CH}_3$, also a signal at δ 4.02 (septet, $J = 2.8, 7.2, 10.0$ Hz) should be assigned to H-16 β . The signals at δ 3.30 (1H, d, $J = 10.8$ Hz) and 3.74 (1H, d, $J = 10.8$ Hz) were assigned to H-29 α and H-29 β respectively. Study of the $^1\text{H}-^1\text{H}$ COSY spectrum showed that H-3 did not couple with H-32. So H-32 should be the β orientation. Based on the chemical shift of C-28 at δ 11.47, it can be concluded that the heterocycle is linked with ring-A in *trans* form. Then the structure of compound **3** can be determined as shown.

We cannot find the signals of methyls connected with 21-N of compounds **2** and **3** in their ^1H - and ^{13}C -NMR spectra. After we had finished this paper, Dr. Yu Shishan and his coworkers reported that they met the same problem as ours. They discovered it is due to the presence of a little acid [4].

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a WC-1 micro-melting point apparatus and are uncorrected. The IR spectra were recorded on a MIDAC FT-IR

interferometer. NMR spectra were taken in CDCl_3 using TMS as internal standard on a Bruker AM-400 spectrometer; 2D-NMR experiments were carried out with standard pulse sequences. MS spectra were measured with VG Auto Spec-3000 mass spectrometer.

Plant Material

Buxus microphylla Sieb. et Zucc was collected at Kunming (Yunnan), China and identified by Prof. Feng Guomei of the Kunming Institute of Botany, where a voucher specimen is deposited.

Extraction and Isolation

The dried and powdered stems and leaves (13 kg) were extracted with hot EtOH. Filtration and evaporation of the solvent yielded 2440 g of residue, which was dissolved by 5% AcOH solution, then extracted with CHCl_3 at different pH values to give fractions A at pH 7 (78 g) and B at pH 10 (80 g). Fr. A (78 g) was chromatographed on a column of silica gel (800 g) eluting with cyclohexane containing gradually increasing amounts of $\text{NH}(\text{Et})_2$ to afford buxmicrophylline B (30 mg) and C (24 mg). Fr. B was dealt with by the same steps as Fr. A and buxmicrophylline D was isolated.

Buxmicrophylline B (1) $\text{C}_{25}\text{H}_{39}\text{NO}$ (anal: 369.3006, calcd. 369.3030), colorless needles, m.p. 149–151°C. UV (CCl_3) λ_{max} (log ϵ) 249(2.77) nm; IR (KBr) ν_{max} : 3400, 2980, 1710, 1640 cm^{-1} ; $^1\text{H-NMR}$ δ (ppm): 6.54 (1H, q, $J=7.5$ Hz, H-20), 2.21 (3H, s, N- CH_3), 1.81 (3H, s, H-21), 1.38 (3H, s, H-18), 1.23 (3H, s, H-28), 0.92 (3H, s, H-30), 0.91 (3H, s, H-29), 0.57 (1H, d, $J=4.2$ Hz, H-19 β), 0.32 (1H, d, $J=4.2$ Hz, H-19 α); EIMS m/z : $[\text{M}]^+$ 369(8), 355(93), 341(67), 327(72), 286(84), 272(28), 232(62), 218(83), 138(91), 57(100), 43(98); $^{13}\text{C-NMR}$: Table I.

Buxmicrophylline C (2) $\text{C}_{30}\text{H}_{50}\text{N}_2\text{O}_3$ (anal: 486.3955, calcd. 486.3900), colorless needles, m.p. 229–230°C. IR (KBr) ν_{max} : 3400, 3250, 3100, 2940, 2920, 1630, 1540, 1450, 1370, 1350, 1090, 1040 cm^{-1} ; $^1\text{H-NMR}$ δ (ppm): 5.65 (1H, d, $J=10.5$ Hz, H-7), 5.35 (1H, dd, $J=10.5, 3.2$ Hz, H-6), 4.08 (3H, m, H-3), 4.03 (1H, septet, $J=2.8, 7.2, 10.0$ Hz, H-16), 3.47, 2.95 (2H, dd, $J=13.4$ Hz, H-28), 2.61 (1H, m, H-17), 1.16 (6H, d, $J=7.0$ Hz, $(\text{CH}_3)_2$), 0.99 (3H, s, H-18), 0.89 (3H, s, H-30), 0.85 (3H, d, $J=6.5$ Hz, H-21), 0.57 (3H, s, H-29), 0.70 (1H, d, $J=4.0$ Hz, H-19 β), 0.21 (1H, d, $J=4.07$ Hz, H-19 α); EIMS m/z : $[\text{MH}]^+$ 487(37), 422(35), 396(17), 171(33), 157(37),

TABLE I ^{13}C -NMR spectral data of compounds 1-3 (δ ppm in CDCl_3)

C	1	2	3
1	25.9	30.4	31.7
2	25.6	27.6	26.0
3	66.8	50.2	63.5
4	42.6	44.1	37.1
5	44.2	39.3	47.2
6	20.7	129.0	20.2
7	27.9	125.8	25.4
8	45.1	43.5	45.1
9	22.6	21.2	19.3
10	28.6	28.0	26.0
11	25.0	24.8	27.5
12	31.5	31.9	33.1
13	46.7	45.2	45.0
14	46.7	49.6	47.4
15	49.1	41.6	44.5
16	206.2	79.1	78.9
17	146.6	62.6	62.6
18	20.7	18.6	18.7
19	29.5	18.8	30.6
20	129.8	56.7	57.2
21	14.7	10.1	9.6
28	13.1	63.8	11.5
29	23.9	11.7	77.3
30	20.7	15.4	20.9
N-CH ₃	40.1	*	*
COCH(CH ₃) ₂		178.1	
		35.9	
		19.5	
		20.1	
CH-CH ₃			85.4
			21.8

*Signals are not discernible.

183(41), 129(26), 105(56), 88(61), 71(100), 72(80), 43(67); ^{13}C -NMR: Table I.

Buxmicrophylline D (3) $\text{C}_{29}\text{H}_{50}\text{N}_2\text{O}_2$ (anal: 458.3839, calcd. 458.3872), colorless needles, m.p. 228–230°C. IR (KBr) ν_{max} : 3500, 3420, 3280, 2950, 2930, 2855, 1440, 1370, 1350, 1145, 1070, 1040, 1000, 940 cm^{-1} ; ^1H -NMR δ (ppm): 4.27 (1H, q, $J=5.4$ Hz, H-32), 4.02 (1H, septet, $J=2.8, 7.2, 10.0$ Hz, H-16), 3.74, 3.30 (2H, dd, $J=10.8$ Hz, H-29), 2.62 (1H, m, H-3), 1.28 (3H, d, $J=5.6$ Hz, 32-CH₃), 1.25 (3H, d, $J=6.6$ Hz, H-21), 0.58 (1H, d, $J=4.0$ Hz, H-19 β), 0.31 (1H, d, $J=4.0$ Hz, H-19 α); EIMS m/z : $[\text{MH}]^+$ 458(13), 444(25), 429(19), 356(23), 326(17), 173(21), 119(31), 91(42), 72(100); ^{13}C -NMR: Table I.

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