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TRITERPENOID ALKALOIDS FROM *Buxus rugulosa*

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*A new triterpenoid alkaloid, buxrugulosamine (**1**), together with four known, ones was isolated from the ethanolic extract of the leaves and twigs of* Buxus rugulosa. *Their structures were determined by analysis of spectral data or comparing them with authentic samples.*

Key word: *Buxus rugulosa*, triterpenoid alkaloids.

A lot of interest is shown in Buxaceae because of the abundance of their triterpenoid alkaloids. Investigators took interest in these plants for several decades with regard to its medicinal properties and chemical constituents. Some alkaloids have been used in the treatment of malaria, rheumatism, skin infections [1], and cardiovascular diseases [2-3]. *Buxus rugulosa*, a member of this family collected from Panzihua in Sichuan province of China [4], has been chemically studied for the first time.

In this study, a new triterpenoid alkaloid, buxrugulosamine (**1**), together with cyclobuxosuffrine K (**2**), cyclobuxophylline O (**3**), N_{20}-acetylbuxamine G (**4**), N_{20}-acetylbuxamine E (**5**), was extracted from the leaves and twigs of *B. rugulosa*. Their structures were determined predominantly by spectral data or comparing them with authentic samples.

The leaves and twigs of *B. rugulosa* were extracted with 95% methanol and the extract was treated as described in the Experimental section. The crude materials were separated by chromatography using silica gel.

Compound **1** was obtained as a whiter powder. The molecular formula $C_{27}H_{46}N_2O$ was determined from the quasimolecular ion peak at *m/z* 437.3506. $[M+Na]^+$ in the HRESIMS. The IR spectrum displayed intense bands at 3536 (NH, amide), and 1648 (C=O, amide) cm^{-1}. The ^1H NMR ($CDCl_3$) spectrum of **1** showed the presence of four methyl singlets at δ 1.03, 0.93, 0.90, and 0.77 for the C-30, C-32, C-31, and C-18 tertiary methyl groups, an N-methyl singlet at δ 2.47, and an acetyl methyl singlet δ 1.94, respectively, while a methyl resonated as a doublet at δ 1.12 (d, 6.0 Hz, 21-CH_3). Cyclopropylmethylene protons H-19 exhibited an AB quartet at δ 0.55 (d, 3.2 Hz), 0.33 (d, 4.1 Hz) [5].

The ^{13}C NMR spectra of **1** showed resonances for all 27 carbons including seven CH_3, nine CH_2, five CH, and six quaternary carbons by DEPT analysis. The ^{13}C NMR values for all the carbons in **1** were assigned on the basis of DEPT, HMQC, and HMBC spectra. The NHCO-Me at C-3 was proved by the NH proton with a large coupling constant at δ 5.52 (d, J = 8.1 Hz), which exhibited HMBC correlations with the carbonyl carbon (δ 168.7) and C-4 (δ 39.9). The N-CH_3 was located at C-20 by the HMBC correlations of N-CH_3/C-20 (δ 53.3). Therefore, the H-20 signal appeared as a broad multiplet. On the basis of biogenetic precedents, the configuration of the *Buxus* alkaloids is predicted as 5α,8β,9β,10β,13β,14α, and 20α [6]. So, compound **1** was elucidated as shown in Fig. 1.

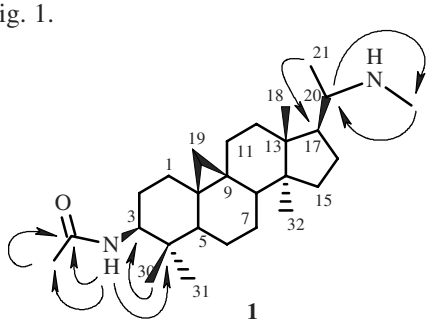

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Fig. 1. Structure and major HMBC (→) correlations of **1**.

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Compounds **2** and **3** were identified by comparing their spectral data and behavior on TLC with those of authentic samples.

Compounds **4** and **5** were identified from their spectral data, which were in agreement with the published values [7].

EXPERIMENTAL

Melting points were determined using an XRC-1 melting point apparatus (Sichuan University Science Instruments Factory) and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 automatic polarimeter. UV and IR spectra were obtained on a Lambda 35 spectrometer and a Perkin Elmer FT-IR spectrometer, respectively. NMR spectra were recorded on a Bruker Advance 600 spectrometer with TMS as internal standard. ESIMS was carried out on a Finnigan-LCQ^{DECA} mass spectrometer.

Extraction and Isolation. The air-dried and powdered leaves and twigs of *B. rugulosa* (5 kg) were soaked with 95% ethanol (5 L × 2.7 days each) at room temperature. The solvent was evaporated under reduced pressure to give 123 g residue, which was suspended in water (400 mL), defatted with petroleum ether (200 mL × 2), and extracted with $CHCl_3$ (250 mL × 6) at pH values 3.0 and 9.0 to give the corresponding fractions A (83.1 g) and B (25.1 g), respectively. Fraction B was divided into five subfractions B1-B4 by silica gel column chromatography and eluted with petroleum $CHCl_3$–MeOH–CH_3COOH (50:1:0.5, 20:1:0.5 each 300 mL). B2 (1.1 g) was separated by silica gel column chromatography and eluted with $CHCl_3$–EtOAc–CH_3COOH (30:1:0.5, 500 mL) to afford **1** (15 mg), **2** (18 mg), and **3** (45 mg). Compounds **4** (26 mg), and **5** (13 mg) were obtained from B3 (2.8 g) by column chromatography with petroleum ether-EtOAc (10:1, 1.5 L) as solvents.

Buxrugulosamine (**1**): white needle ($CHCl_3$); mp 174-176°C; $[\alpha]_D^{20}$ +42.1° (*c* 0.31, $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3536, 2952, 2931, 2874, 1648, 1542, 1456, 1374, 1270, 1131, 1095, 604; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.52 (1H, d, J = 8.1, 3-NH), 4.00 (1H, m, 20-H), 2.47 (3H, s, NCH_3), 2.00 (1H, m, H-3), 1.94 (3H, s, $COCH_3$), 1.12 (3H, d, J = 6.0, H-21), 1.03 (3H, s, H-30), 0.93 (3H, s, H-32), 0.90 (3H, s, H-31), 0.77 (3H, s, H-18), 0.55 (1H, d, J = 3.2 Hz, H-19a), 0.33 (1H, d, J = 4.1, H-19b); ^{13}C NMR (600 MHz, $CDCl_3$, δ): 168.7 ($COCH_3$), 68.8 (C-3), 53.3 (C-20), 49.1 (C-17), 49.1(C-14), 48.7 (C-5), 48.5 (C-8), 47.9 (C-13), 44.8 (NCH_3), 39.9 (C-4), 35.5 (C-1), 35.2 (C-12), 32.7 (C-15), 32.3 (C-2), 29.7 (C-19), 26.8 (C-16), 26.7 (C-7), 26.5 (C-10), 26.2 (C-11), 26.0 (C-30), 25.8 (C-9), 23.6 ($COCH_3$), 21.3 (C-6), 19.9 (C-21), 19.3 (C-18), 18.4 (C-32), 15.1 (C-31). ESIMS (positive): *m/z* 415.3 $[M+H]^+$, HRESIMS (positive mode) *m/z* 437.3506 $[M+Na]^+$ (calcd. for $C_{27}H_{46}N_2ONa$, 437.3508).

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