Short Communication

Short and Efficient Approach Towards Buxozine-C, an Alkaloid from *Buxus sempervirens*

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Buxus alkaloids display a wide range of interesting pharmacological activities. Here, we present a short and efficient approach towards one of the alkaloids, buxozine-C. The first synthesis has been achieved with 91% yield starting from cyclovirobuxine-D by forming a tetrahydro-oxazine ring with formaldehyde at room temperature.

Keywords: Buxozine-C / *Buxus* alkaloids / Cyclovirobuxine D / Semisynthesis / Tetrahydro-oxazine

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Introduction

The *Buxus* plants are rich source of triterpenoid alkaloids. Previous phytochemical studies on *Buxus* species have resulted in the isolation of more than 200 of such compounds [1]. In the indigenous system of medicine, extracts of genus *Buxus* have been used for the treatment of several disorders [2]. Various substituted dibasic alkaloids with a cyclopropane ring in positions 9, 19 and substituents in positions 4 or 14 have been described [3]. Cyclovirobuxine-D **1**, a primary alkaloid extracted from the plant *Buxus microphylla*, is widely used clinically in China for the treatment of cardiovascular and cerebrovascular diseases [4]. Despite cyclovirobuxine-D prospects as a cardiovascular agent and the considerable excitement surrounding its potential therapeutic value, several problems associated with the drug hamper its widespread usage. Central among them is the issue of bioavailability deficiency [5]. Research on the congeners is one of the feasible approaches to define the structure-activity relationship for *Buxus* alkaloids.

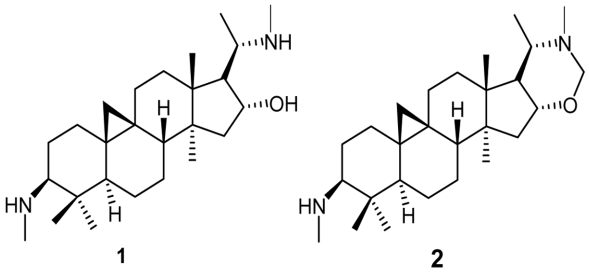

Figure 1. Chemical structure of cyclovirobuxine-D and buxozine-C.

Of all these congeners, buxozine-C **2** has been taken as the most difficult during the conventional purification process because of the scanty contents. Compound **2**, the first *Buxus* alkaloid possessing a tetrahydro-oxazine ring joined to position 16α, 17β of the androstane skeleton, was first isolated from *Buxus sempervirens* L. and its structure elucidation reported by Votickÿ in 1977 [6].

Within the alkaloid components of the plant extracts, the biological activity of buxozine-C **2** is unknown because of resource deficiency. So, the partial synthesis of alkaloid **2** is essential for the research on structure-activity relationship for natural *Buxus* alkaloids. The semisynthesis of compound **2** has not been recorded before. Thus, a simple synthesis of buxozine-C **2** starting from cyclovirobuxine-D **1** was developed.

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Results and Discussion

Wood (excluding the bark) extract containing 66% cyclovirobuxine-D **1**, 24% cyclobuxine-D, and 10% other *Buxus* alkaloids, was purchased from Jiangsu Tenglong Biological Technological Co. Ltd, China. The crude alkaloid was then purified as described in the literature [7] followed by recrystallization from acetone. The melting point of compound **1** purified is 220–221°C (acetone, decomp.), similar to that reported in the literature (Brown *et al*., 221–224°C) [8].

Closure of compound **1** with an excess of formaldehyde in the presence of ethanol as solvent at room temperature afforded alkaloid **2** in 91% yields.

In this *Buxus* alkaloid **2**, the closure of the side chains of cyclovirobuxine-D **1** forms a tetrahydro-oxazine ring for the biogenetic cause. The postulated formulation of compound **2** is in accordance with the number of acid hydrogen found in the molecule [6]. Characteristically, the TOF-MS displayed a peak of $[M+H]^+$ at 415.3 ($C_{27}H_{46}N_2OH^+$, requires 415.36). The ^{1}H-NMR showed the presence of the methylene group (C-27) between two hetero atoms at 4.23 (d, J = 9.6 Hz) and 4.53 (d, J = 9.6 Hz), while the ^{13}C-NMR displayed the same group at 88.5. The IR spectrum exhibited vibration of a C–O–C bond (1100 cm^{-1}) and a *tert*-amino group (1175 cm^{-1}).

Experimental

General

Melting points are uncorrected. IR spectra were recorded in KBr on Nicolet Impact 410 spectrophotometer (Nicolet, Madison, WI, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 300 MHz on Bruker AV-300 and at 500MHz on Bruker AV-500 NMR spectrometers (Bruker, Rheinstetten, Germany), respectively. TOF-MS was recorded on Agilent 1100 LC-MS mass spectrometer (Agilent, Palo Alto, CA, USA). Assignment of C atoms in ^{13}C-NMR spectra was based on previous studies with *Buxus* alkaloids [9, 10]. Analytical TLC was carried out on 0.25 mm silica gel plates using $CHCl_3$-EtOH-Et_3N (8 : 1 : 1). Visualization was done in I_2.

Chemistry

*Buxozine-C **2***

To a solution of alkaloid **1** (402 mg, 1 mmol) in ethanol (5 mL), formaldehyde (37% aqueous solution) (0.15 mL, 2 mmol) was added, and the mixture was stirred at room temperature. After stirring for 15 min, TLC analysis indicated the absence of unreacted compound **1** (R_f 0.53). The solvent was evaporated under reduced pressure, and the residue was extracted with CH_2Cl_2, washed with water and brine, and dried over $MgSO_4$. The solvent was removed and the residue was purified by recrystallization from ether to afford alkaloid **2** as white powder (376 mg, 91%), m. p. 138–139°C (ether, decomp.) (Votickÿ *et al.,* m.p. 137°C). R_f 0.76. IR (KBr) ν_{max} cm^{-1}: 3414, 2958, 2926, 2862, 2823, 2782, 2764, 1656, 1639, 1455, 1383, 1377, 1355, 1336, 1256, 1175, 1152, 1100, 1039, 1029, 1003, 987, 897, 839, 591. ^{1}H-NMR ($CDCl_3$) (only diagnostic peaks listed): δ (ppm) = 4.53 (1H, d, J = 9.6 Hz, H-27), 4.23 (1H, d, J = 9.6 Hz, H-27), 3.70 (1H, m, H-16), 2.80 (1H, m, H-20), 2.36 (3H, s, H-23), 2.28 (3H, s, H-24), 1.12 (3H, s, H-22), 1.10 (3 H, s, H-18), 1.05 (3H, s, H-26), 0.96 (3H, s, H-25), 0.79 (3H, m, H-21), 0.58 (1H, d, J = 3.8 Hz, H-19), 0.28 (1H, d, J = 4.0 Hz, H-19). ^{13}C-NMR ($CDCl_3$): δ (ppm) = 88.5 (C-27), 83.6 (C-16), 71.8 (C-3), 59.1 (C-20), 49.6 (C-17), 49.1 (C-14), 48.1 (C-8), 46.7 (C-24), 44.4 (C-13), 43.5 (C-4), 41.9 (C-5), 41.3 (C-10), 34.5 (C-12), 33.4 (C-15), 31.2 (C-1), 31.0 (C-19), 27.4 (C-26), 26.7 (C-23), 26.6 (C-6), 26.1 (C-7), 23.4 (C-2), 21.3 (C-9), 20.4 (C-11), 19.7 (C-22), 19.3 (C-18),17.4 (C-25), 16.0 (C-21). TOF-MS m/z: 415.3 ($C_{27}H_{46}N_2OH^+$).

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