Jusbetonin, the First Indolo[3,2-*b*]quinoline Alkaloid Glycoside, from *Justicia betonica*

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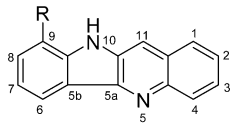
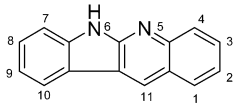
Received August 28, 2003

A new indolo[3,2-*b*]quinoline alkaloid glycoside, jusbetonin (**1**), and three known alkaloids, namely, 10*H*-quindoline (**2**), 6*H*-quinindoline (**3**), and 5*H*,6*H*-quinindolin-11-one (**4**), have been isolated from the leaves of *Justicia betonica*. The structure of **1** was established on the basis of 1D and 2D NMR (^{1}H−^{1}H COSY, HMQC, and HMBC) and HRFABMS data. Compound **1** is the first example of a glycosylated indolo[3,2-*b*]quinoline alkaloid, while compound **4** was isolated for the first time from a natural source.

The genus *Justicia* (Acanthaceae) comprises about 300 species, which are widely distributed in the tropical regions of the world.[1] Lignans are the major group of compounds reported from this genus. A few species, namely, *J. adhatoda*,[2−5] *J. gendarussa*,[6] and *J. ghiesbreghtiana*,[7,8] have yielded several bioactive alkaloids. Vasicine and vasicinone have been reported as the active constituents from *J. adhatoda*.[2−5] During our continuing phytochemical studies on *Justicia* plants,[9] we found an unexamined species, *J. betonica*, possessing morphological similarities to *J. adhatoda*. This similar plant morphology, coupled with the importance of *J. adhatoda* alkaloids, prompted us to examine the chemical constituents of *J. betonica*, resulting in the isolation of a novel indolo[3,2-*b*]quinoline alkaloid glycoside, jusbetonin (**1**), along with three known related alkaloids, namely, 10*H*-quindoline (**2**),[10,11] 6*H*-quinindoline (**3**),[12−14] and 5*H*,6*H*-quinindolin-11-one (**4**).[15] Although compound **4** was obtained during the reactions of sodioisatin with *ortho*- and *para*-nitrobenzyl chlorides,[15] there are no detailed spectroscopic data recorded in the literature on this alkaloid. The present isolation of **4** marks its first reported occurrence from a natural source. Herein, we report on the isolation and structure elucidation of **1** from a leaf extract of *J. betonica*.



1 R = OGlc
2 R = H



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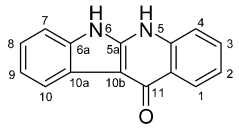

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Compound **1** was isolated as a yellow amorphous powder. Its molecular formula, $C_{21}H_{20}N_2O_6$, was deduced from HRFABMS (*m*/*z* 397.1397 [M + H]$^+$, calcd 397.1400) and ^{13}C NMR data. The IR absorption at 3422 cm^{-1} and the ^{1}H NMR peak at δ 11.19 (1H, s) implied the presence of an NH group in **1**. The ^{1}H NMR spectral data of **1** showed eight aromatic protons resonating between δ 7.21 and 8.30. In addition, it showed a series of signals between δ 3.20 and 3.79, characteristic of a sugar moiety. The presence of one anomeric proton at δ 4.99 (d, J = 7.7 Hz) indicated that **1** is a monoglycoside. In the ^{1}H NMR spectrum of **1**, the proton signals at δ 8.18, 8.10, 7.65, and 7.56 were assigned to an AA′BB′ spin system (1,2-disubstituted benzene ring), the signals at δ 8.02, 7.43, and 7.21 were assigned to an ABC spin system (1,2,3-trisubstituted benzene ring), and the deshielded singlet at δ 8.30 was assigned to a methine proton from a pyridine ring. These assignments were supported by correlations observed in the ^{1}H−^{1}H COSY NMR spectrum. The ^{13}C NMR spectrum showed seven quaternary carbons, eight methine carbons, and six aliphatic sugar carbons. As expected, the NH proton signal at δ 11.19 did not show any connectivity with any of the carbons in the HMQC spectrum. The HMQC spectrum also revealed that all eight protons were connected to different carbons, so it was concluded that the second nitrogen atom present in compound **1** is tertiary. In the HMBC spectrum of **1**, the proton signal at δ 8.30 (H-11) showed correlations with the carbon signals at δ 145.7 (C-5a), 143.4 (C-4a), and 127.5 (C-1) and the N-H signal at δ 11.19 showed correlations with the carbon signals at δ 145.7 (C-5a) and 122.6 (C-5b). These three-bond correlations, along with the ^{1}H−^{1}H COSY data, led to the proposal of an indoloquinoline nucleus with the two nitrogen atoms on opposite sides of a linear tetracyclic system, namely, an indolo[3,2-*b*]quinoline skeleton. A careful comparison of the ^{1}H and ^{13}C NMR data with those of indolo[3,2-*b*]quinolines revealed that compound **1** exhibited a close resemblance to 10*H*-quindoline,[10,11] except for the presence of an additional sugar moiety. The lack of any proton signal attributable to H-9 in the ^{1}H NMR spectrum of **1**, in comparison with 10*H*-quindoline, suggested that the sugar moiety is attached at C-9. This was further supported by an HMBC correlation between the anomeric proton (δ 4.99) and the highly deshielded carbon signal at δ 143.7 (C-9). A careful comparison of the ^{13}C NMR data of the sugar unit with those of other glycosides recorded in the literature[16] suggested that the sugar unit in **1** is glucose. Further, H-1′ showed a correlation with C-5′ in the HMBC spectrum, confirming the pyranoside form for glucose. The configuration of the glucose unit was established as β from the coupling constant of the anomeric

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10.1021/np030392y CCC: $27.50
Published on Web 01/13/2004

Table 1. NMR Data of Jusbetonin (**1**)[a]

position	δ_C	δ_H (*J* Hz)	HMBC (H to C)
1	127.5	8.10 d (7.4)	C-3, C-4a, C-11
2	124.9	7.56 dd (7.4, 7.2)	C-4, C-11a
3	126.2	7.65 dd (8.4, 7.2)	C-1, C-4a
4	128.6	8.18 d (8.4)	C-2, C-3, C-11a
4a	143.4		
5a	145.7		
5b	122.6		
6	115.3	8.02 d (7.6)	C-8, C-9a
7	119.8	7.21 dd (8.0, 7.6)	C-5b, C-9
8	115.8	7.43 d (8.0)	C-6, C-9, C-9a
9	143.7		
9a	134.8		
10a	132.3		
11	113.7	8.30 s	C-1, C-4a, C-5a
11a	126.7		
N-H		11.19 s	C-5a, C-5b, C-10a
1′	102.5	4.99 d (7.7)	C-5′, C-9
2′	73.6	3.34−3.47 m	
3′	76.2	3.34−3.47 m	
4′	70.0	3.20−3.27 m	
5′	77.3	3.34−3.47 m	
6′	60.9	3.54 m	
		3.79 d (11.0)	

[a]Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).

proton (H-1′) located at δ 4.99 (d, J = 7.7 Hz). On the basis of the above, the structure of **1** could be derived as 9-[β-D-glucopyranosyloxy]-10*H*-indolo[3,2-*b*]quinoline and was named jusbetonin. This is the first glycoside among the naturally occurring linear indoloquinoline alkaloids.

It is interesting that linearly fused indoloquinoline alkaloids (indolo[3,2-*b*]quinoline and indolo[2,3-*b*]quinoline) are rarely isolated from natural sources, but due to their pronounced biological importance,[17−20] several synthetic derivatives have been prepared and evaluated for their cytotoxic activities[21] and DNA-damaging properties.[22]

Experimental Section

General Experimental Procedures. Melting points were measured on a Buchi-540 melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco P-1020 polarimeter. IR spectra were obtained on a Perkin-Elmer BX FT-IR spectrometer. NMR spectra were run on a GE Ω 500 or a Varian Unity Inova 400 spectrometer. FABMS was performed on a JEOL SX 102/DA-6000 and HRFABMS on a JEOL JMS-700 mass spectrometer. Silica gel (ACME, 100−200 or finer than 200 mesh) was used for column chromatography.

Plant Material. The whole plant material was collected from Balinaidu Kandriga, Chittoor District, India, during March 1997 and authenticated as *Justicia betonica* L. (Acanthaceae) by Research and Specimen Cell, National Institute of Science Communication, CSIR, New Delhi. A voucher specimen is on deposit at NISCOM (NISCOM Field No. 1795) and at the Department of Chemistry, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation. The shade-dried and milled leaf material (1.62 kg) was extracted with MeOH (5 × 10 L) at room temperature. The MeOH extract was concentrated under reduced pressure and fractionated between EtOAc and MeOH using a Soxhlet apparatus. The EtOAc fraction (50 g) was subjected to silica gel chromatography using mixtures of petroleum ether−EtOAc (19:1, 9:1, 4:1, and 2:1) to give 10*H*-quindoline (**2**, 130 mg), 6*H*-quinindoline (**3**, 10 mg), and 5*H*,6*H*-quinindolin-11-one (**4**, 20 mg). Column chromatography of the MeOH-soluble portion (105 g) over silica gel column chromatography using a gradient solvent system of $CHCl_3$−MeOH (4:1 to 1:1) afforded jusbetonin (**1**, 10 mg).

Jusbetonin (1): yellow amorphous powder; mp > 300 °C; $[\alpha]^{25}_D$ −48.2° (*c* 0.54, MeOH); IR (KBr) ν_{max} 3422, 2927, 1629 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 397 $[M + H]^+$ (51), 307 (37), 254 (25), 232 (29), 176 (81), 154 (100), 136 (100); HRFABMS *m*/*z* 397.1397 (calcd for $C_{21}H_{21}N_2O_6$ $[M + H]^+$, 397.1400).

5*H*,6*H*-Quinindolin-11-one (4): pink amorphous powder; mp > 300 °C; IR (KBr) ν_{max} 3334, 1616, 1416, 1208 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 12.15 (1H, s, NH-5), 11.35 (1H, s, NH-6), 9.32 (1H, d, J = 7.8 Hz, H-1), 7.80 (1H, d, J = 7.5 Hz, H-10), 7.44 (1H, dd, J = 7.7, 7.4 Hz, H-8), 7.30 (1H, dd, J = 7.8, 7.6 Hz, H-3), 7.14 (1H, dd, J = 7.8, 7.8 Hz, H-2), 7.11 (1H, d, J = 7.7 Hz, H-7), 7.07 (1H, d, J = 7.6 Hz, H-4), 6.94 (1H, dd, J = 7.5, 7.4 Hz, H-9); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 189.1 (C-11), 172.8 (C-5a), 152.8 (C-6a), 142.2 (C-11a), 139.5 (C-10b), 137.2 (C-8), 129.8 (C-3), 126.0 (C-1), 125.1 (C-10), 122.1 (C-2), 121.6 (C-9), 120.5 (C-10a), 112.9 (C-7), 110.2 (C-4), 108.1 (C-4a); HREIMS *m*/*z* 234.0795 (calcd for $C_{15}H_{10}N_2O$ $[M]^+$, 234.0793).

Acknowledgment. The authors thank Sri G. Ganga Raju, Laila Impex, Vijayawada, India, for encouragement, and Prof. Ching-Fong Shu, National Chiao Tung University, Hsinchu, Taiwan, for the HRFABMS.

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NP030392Y