A NEW BISBENZYLISOQUINOLINE ALKALOID FROM PHAEANTHUS VIETNAMENSIS AND ITS ANTIBACTERIAL ACTIVITY≠

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Abstract —— A new bisbenzylisoquinoline alkaloid (-)-(1S,1´R)-O,O´-dimethylgrisabine (1) was isolated from the leaves of Phaeanthus vietnamensis Ban. Its structure was determined on the basis of the extensive 2-D and 1-D nmr long-range heteronuclear correlations. Its antibacterial activity is also described.

Phaeanthus vietnamensis Ban. (Annonaceae) is an endemic plant of central Vietnam used in traditional medicine as a healing agent[1]. Recently, we have reported on the isolation of alkaloids from the leaves of this plant[2]. The structure elucidation of the main alkaloid of P. vietnamensis is described in the present communication.

The alkaloid 1, $[\alpha]_D^{26}$ -26^0 (c 0.19, $CHCl_3$) was isolated as an amorphous solid[3]. The ms exhibited a M^+ at m/z 638.3325 corresponding to the molecular formula $C_{39}H_{46}N_2O_6$ (calcd 638.3356, confirmed by CI ms). The ms of 1 is typical of that of a bisbenzylisoquinoline alkaloid containing only a single tail-to-tail ether bridge[4,5]. Base peak at m/z 206 ($C_{12}H_{16}NO_2$) confirms the presence of two methoxyls in each of the head units of 1. The ^1H nmr spectrum of 1 (Table 1)

≠ Dedicated to the memory of the late Professor Tetsuji Kametani.

shows two N-methyl singlets, five methoxyls, and four singlets of isolated aromatic protons. The ms and substitution pattern of alkaloid 1 appear to be identical to those of O,O-dimethylgrisabine, which was prepared by methylation of the alkaloid grisabine (2)[6]. To determine unambiguously the structure of alkaloid 1, complete proton and carbon assignments in its nmr spectra were performed. These data have not previously been reported in studies on the nmr of bisbenzylisoquinolines[7]. COSY experiments revealed three AA´BB´ systems (two aliphatic, one aromatic), two aliphatic ABX systems, and aromatic ABC system in the molecule of 1. The chemical shifts of overlapped multiplets were obtained from the projection of proton 2D J-resolved spectra[8]. Delayed COSY experiment[9] identified two pairs of para-situated aromatic protons. The low-field members of the aromatic AA´BB´ system which is long-range coupled to the AB part of one ABX system was assigned to H-10´ and H-14´. The corresponding X-part of latter system is therefore due to H-1´. The aromatic singlet exhibiting a long-range coupling to H-1´ thus represents H-8´. Using the already established link between the para-protons, the signal of H-5´ was found. The latter is coupled to both H-4´ protons whose crosspeaks then define protons H-3´. An N-methyl group that is coupled to one H-3´ is assigned to N(2´)-Me. The protons of the other monomer of the molecule were assigned similarly. Methoxyl resonances were identified on the basis of their long-range couplings to the vicinal aromatic protons: C(6)-OMe to H-5, C(7)-OMe to H-8, C(6´)-OMe to H-5´, C(7´)-OMe to H-8´, and C(12)-OMe to H-13.

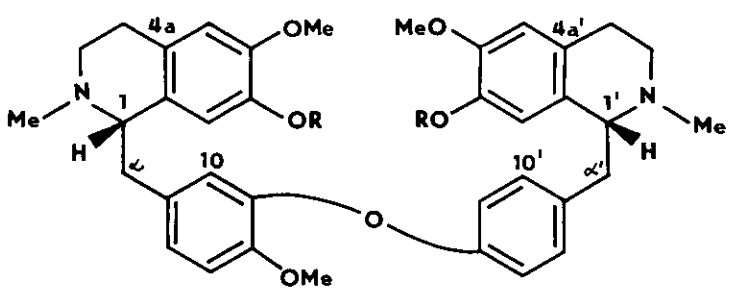
Nearly all carbons signals were resolved at 100 MHz. Besides the two groups magnetically equivalent nuclei (C-10´ and C-14´, C-11´ and C-13´), there are only three other overlaps: methoxyls at 55.49 and 55.75 ppm and aromatic carbons at 112.24 ppm. Many signals are grouped in pairs, reflecting the nearly symmetrical nature of the molecule. Having completed the proton assignment, the assignment of protonated carbons by heteronuclear 2D correlation[10,11] was straightforward. Quaternary carbons were assigned by heteronuclear ^{1}H, ^{13}C 2D correlation (optimized for J=10 and J=5 Hz) based on the ^{2}J and ^{3}J (C,H) couplings. The signal exhibiting a crosspeak with H-8´ was assigned to C-4´a, that giving a crosspeak with H-5´ to C-8´a. Similarly, C-4a and C-8a were found. The signal at 131.93 ppm coupled to H-14 and so represents C-9; the assignment of 132.52 ppm signal to C-9´ is obtained by elimination. The carbon resonating at 144.49 ppm, coupled

to both H-10 and H-13, was assigned to C-12. Methoxyl bearing carbons C-6 and C-7 (also C-6′ and C-7′) exhibited couplings to their corresponding methoxyls and to H-5 and H-8 (H-5′ and H-8′, respectively). The carbon assigned to C-12 (149.97 ppm) displayed couplings to H-10, H-14, and C(12)-OMe. The most downfield signal exhibited crosspeaks with H-10′, H-14′, H-11′, and H-13′ and must be due to C-12′. The alkaloid thus has the structure 1.

It is very rewarding to see that a complete ^{1}H and ^{13}C signal assignment in a nearly symmetric dimeric benzylisoquinoline alkaloid can be achieved by judicious use of modern 2D nmr techniques.

The CD spectrum of 1 in MeOH displays three negative Cotton effects at 282 nm ($\Delta\varepsilon$ -2.6), 246 (-6.2), and at 216 (-10.7). Thus the 1S,1′R absolute configuration for alkaloid 1 is directly assignable[12].

The alkaloid part of the extract from the leaves of P. vietnamensis exhibits antimicrobial activity. The main alkaloid 1 showed a potent antibacterial action with minimal inhibitory concentration (MIC) of 62.5 ppm against Bacillus subtilis[13].



1 : R = Me

2 : R = H

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) nmr data of (-)-(1S,1´R)-O,O´-dimethylgrisabine (1) in $CDCl_3$ (δ ppm, J = Hz).

2.560	s (3H)	N-Me	24.08 t	C-4´
2.615	s (3H)	N´-Me	24.30 t	C-4
2.635	mt (1H)	H-4^{u}	40.13 t	C-α
2.676	mt (1H)	H-4´^{u}	40.25 t	C-α´
2.731	dd (1H, J=13.4, 8.6)	H-α^{u}	41.32 q	N´-Me
2.759	dd (1H, J=13.2, 9.0)	H-α'^{u}	41.56 q	N-Me
2.879	mt (1H)	H-3^{u}	45.66 t	C-3´
2.901	mt (1H)	H-4^{d}	45.87 t	C-3
2.928	mt (1H)	H-3´^{u}	55.49 q (2C)	7,7´-OMe
2.968	mt (1H)	H-4´^{d}	55.75 q (2C)	6,6´-OMe
3.255	mt (1H)	H-3^{d}	56.06 q	12-OMe
3.265	dd (1H, J=13.4, 4.1)	H-α^{d}	64.46 d	C-1
3.328	mt (1H)	H-3´^{d}	64.57 d	C-1´
3.363	dd (1H, J=13.2, 4.2)	H-α'^{d}	111.07 d	C-8
3.513	s (3H)	7´-OMe	111.19 d	C-5´
3.562	s (3H)	7-OMe	111.24 d (2C)	C-5,8´
3.791	dd (1H, J=8.6, 4.1)	H-1	112.70 d	C-13
3.792	s (3H)	12-OMe	116.67 d (2C)	C-11´,13´
3.821	s (3H)	6-OMe	122.70 d	C-10
3.834	s (3H)	6´-OMe	124.22 s	C-4a´
3.881	dd (1H, J=9.0, 4.2)	H-1´	124.67 s	C-4a
5.875	s (1H)	H-8´	126.19 d	C-14
5.951	s (1H)	H-8	126.80 s	C-8a´
6.545	s (1H)	H-5	127.08 s	C-8a
6.574	s (1H)	H-5´	130.90 d (2C)	C-10´,14´
6.700	d (1H, J=2.0)	H-10	131.93 s	C-9
6.770	AA´BB´ (2H, J=8.7)	H-11´,13´	132.52 s	C-9´
6.857	dd (1H, J=8.4, 2.0)	H-14	144.49 s	C-12
6.889	d (1H, J=8.4)	H-13	146.52 s	C-7´
6.995	AA´BB´ (2H, J=8.7)	H-10´,14´	146.56 s	C-7
			147.76 s	C-6
			147.81 s	C-6´
			149.97 s	C-12
			156.58 s	C-12´

^{u} upfield proton, ^{d} downfield proton

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Received, 22nd May, 1989