DEHATRINE, AN ANTIMALARIAL BISBENZYLISOQUINOLINE ALKALOID FROM THE INDONESIAN MEDICINAL PLANT *BEILSCHMIEDIA MADANG*, ISOLATED AS A MIXTURE OF TWO ROTATIONAL ISOMERS

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Through bioassay-guided separations of the chemical constituents of the Indonesian medicinal plant *Beilschmiedia madang* BL. a bisbenzylisoquinoline alkaloid was obtained as the major antimalarial principle. The physicochemical properties of the alkaloid were consistent with the proposed structure of dehatrine (**1**). However, the alkaloid isolated by us was shown to be a mixture of two rotational isomers. The X-ray crystallographic analysis of **1** has shown that two rotamers are incorporated in a single crystal in 1:1 ratio. The complex NMR spectrum of **1** has also been defined as a mixture of two rotamers by extensive use of 2D (COSY and COLOC) techniques. Dehatrine (**1**) has been shown to significantly inhibit the growth of cultured *Plasmodium falciparum* K1 strain (cholorquine resistant) with similar activity to quinine.

KEYWORDS *Beilschmiedia madang*; dehatrine; bisbenzylisoquinoline; rotational isomer; antimalarial substance; Indonesian medicinal plant

Although several effective antimalarial agents (chloroquine, artemisine, *etc.*) have been developed, millions of people are still suffering from malaria, especially in some tropical areas of the world, mainly due to the spread of malarial pathogens having multi-drug resistance. As a continuation of our chemical characterization studies of Indonesian medicinal plants,[1] we have been investigating the antimalarial principles of the wood of *Beilschmiedia madang* BL. (Lauraceae), collected in July 1990 in the Kepahiang area, Bengkulu, Sumatera Island, Indonesia.[2] The plant is called "Medang Kohat" in that area, and the decoction of the wood has been used as an anti-malarial preparation. This paper deals with the chemical characterization of the major antimalarial bisbenzylisoquinoline alkaloid from the wood, identical with dehatrine (**1**), which has been noted to exist in a 1:1 mixture of the rotational isomers either in crystal or in solution.

The methanol extract of the wood was partitioned into a $CHCl_3$-MeOH-water (4:4:3) mixture. The crude alkaloid fraction (0.58 % from the wood), obtained from the $CHCl_3$ layer in the usual manner, was subjected to silica gel column chromatography to give an antimalarial major alkaloid, mp 146-148 °C (from MeOH), $[\alpha]_D$ +26° (*c*=1.6, $CHCl_3$, 22 °C), (0.06 % from the wood), the physicochemical properties of which are consistent with the proposed structure of a bisbenzylisoquinoline alkaloid dehartine (**1**), which was isolated from *Dehaasia triandra* (Lauraceae).[3] Thus, the IR spectrum (KBr) of the alkaloid showed absorption bands due to imino (1640 cm^{-1}) and phenyl ether (1240, 1060 cm^{-1}) groups and aromatic ring (1600, 1500 cm^{-1}), whereas the high-resolution FAB and SIMS spectra substantiated the molecular formula as $C_{37}H_{38}N_2O_6$. From this evidence, the alkaloid has been presumed to be a bisbenzylisoquinoline alkaloid; however, the ^1H and ^{13}C NMR spectra of the alkaloid taken in various solvents were very complex ones, while the HPLC of the alkaloid secured purity of the compound. Then the alkaloid was subjected to Birch reduction (Na/liq. NH_3), which provided two benzyltetrahydroisoquinoline compounds: *l-O,O,N*-trimethylcoclaurine (**2**)[4] and *dl*-coclaurine (**3**).[5]

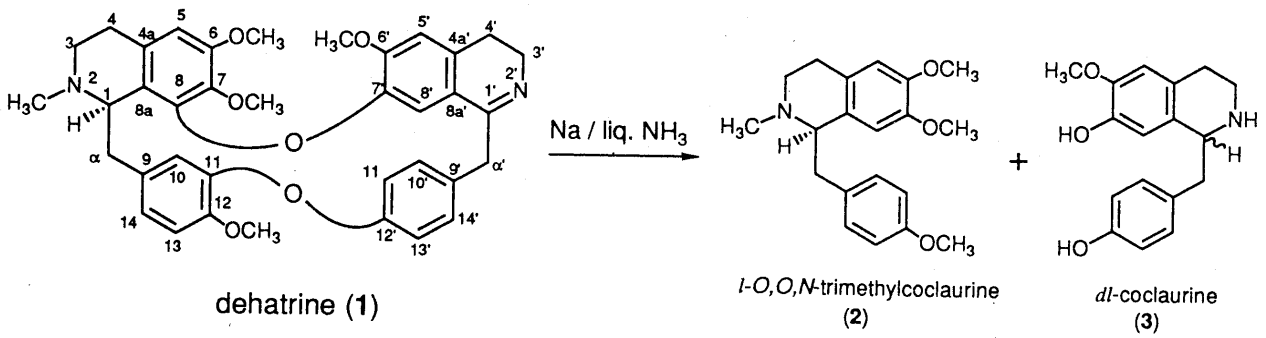

Fig. 1

Finally, the alkaloid was recrystallized from ethanol at room temperature (24 °C) to furnish colorless prisms suitable for X-ray crystallographic analysis. The crystal data were as follows: monoclinic, space group $P2_1$, a=15.354(2)Å, b=13.485(1)Å, c=15.944(2)Å, β=98.637(9)°, V= 3263.7(6)Å^3, Z=2, D_x=1.276 g/cm^3, μ(Cu /Kα)=6.69 cm^{-1}, F(000)=1328. The reflectional intensities within 2θ=110° were collected on a Rigaku automatic four-circle diffractometer with graphite-monochromated Cu/Kα radiation and collected for the Lorentz and polarization factors. The 5489 independent reflections having [$|Fo| > 3\sigma(|Fo|)$] were used for the structure determination and refinement. The present discrepancy indexes R and Rw are 0.0876 and 0.0917,. respectively.

The X-ray analysis has confirmed that the structure of the alkaloid isolated by us is identical with dehatrine (**1**)[3] which is a head-to-head type bisbenzylisoquinoline alkaloid (two monomeric units linked through two ether bonds between C8 and C7' and C11 and C12'). The analysis has also elucidated that the molecule of **1** takes two rotational isomeric forms in a 1:1 ratio in the crystal. The PLUTO drawings of two rotamers are shown in Fig. 2 as conformers A and B. In each conformer, the rotational angle of two benzene rings, linked through an ether bond between C11 and C12', is about 90°. On the other hand, the rotational angle of each isoquinoline ring, linked between C8 and C7' through an ether bond, differs significantly, *i.e.*, about 30° in conformer A, while it is almost perpendicular in conformer B.

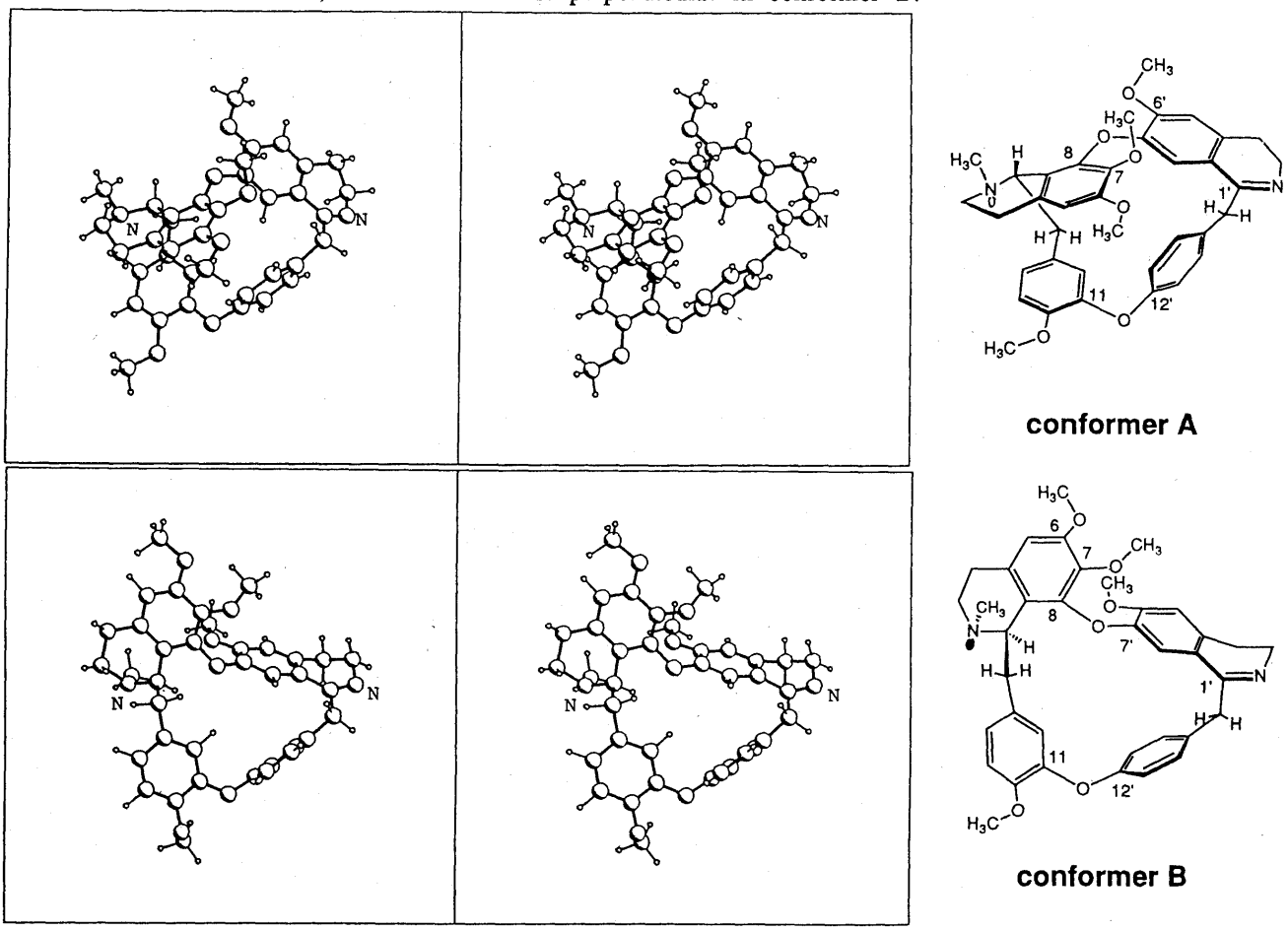

Fig. 2. PLUTO Drawings of Dehatrine (**1**)

As mentioned above, dehatrine (**1**) itself is a previously reported compound. However, neither the presence of two rotational isomers nor its property of showing a complex NMR spectrum has been reported before,[3] except in the papers by Inubushi *et al.*,[6] in which they described the synthesis of **1** and mentioned the appearance of doubled methoxyl signals in the ^{1}H NMR spectrum, presumably due to the presence of conformational isomers without an examination in detail.

We have taken the ^{1}H NMR spectra of **1** at various temperatures, and the resulting changes in the methoxyl regions are shown in Fig. 3. At 27 °C, the spectrum was complicated, but in accordance with rising temperature, the spectrum gradually changed to a simpler one. When the solution temperature was once raised to 60 °C and re-cooled to 27 °C, the re-measured spectrum was completely identical with the spectrum initially taken at 27 °C. These findings suggest that dehatrine (**1**) may take thermally exchangeable conformers in solution.

In order to assign the complex ^1H NMR and ^{13}C NMR spectra of dehatrine (**1**), we have utilized extensively 2D NMR techniques (^1H-^1H COSY, ^1H-^{13}C COSY, and COLOC), and the resulting assignments are shown in Table I. At the present stage of investigation, however, we are unable to exactly correlate two conformers (conformer A and B) in the crystal with two conformers (provisionally named rotamers I and II) in the NMR-measured solution.

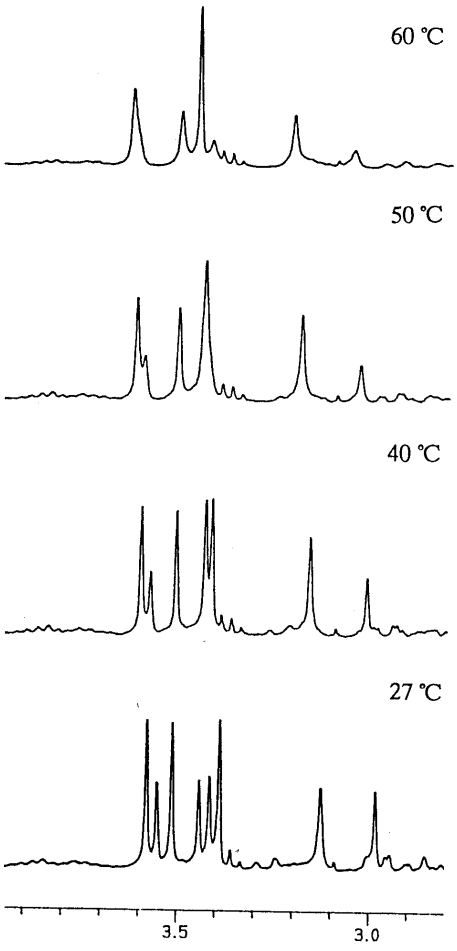

Fig. 3 (taken at 270 MHz in C_6D_6)

Table I. ^1H (270 MHz) and ^{13}C (67.8 MHz) NMR Data for **1** (at 27 °C in $CDCl_3$, δ in ppm)

Rotamer I				Rotamer II		
COLOC observed between	^1H NMR	^{13}C NMR	location	^{13}C NMR	^1H NMR	COLOC observed between
8a	3.94-3.96	59.7(d)	C-1	62.1(d)	3.65	
		*a	C-3	43.8(t)	2.87	
		*a	C-4	22.4(t)		
-----	-----	129.9(s)	C-4a	129.9(s)	-----	-----
6,7,8a,	6.39	106.9(d)	C-5	105.8(d)	6.32	4,6,7,8a,
-----	-----	151.3(s)	C-6	151.1(s)	-----	-----
-----	-----	138.7(s)	C-7	137.1(s)	-----	-----
-----	-----	147.4(s)	C-8	147.4(s)	-----	-----
-----	-----	123.0(s)	C-8a	120.1(s)	-----	-----
-----	-----	135.0(s)	C-9	132.4(s)	-----	-----
11,12,14	6.03	114.1(d)	C-10	116.3(d)	*b	
-----	-----	150.0(s)	C-11	149.2(s)	-----	-----
-----	-----	146.5(s)	C-12	146.4(s)	-----	-----
	*b	110.7(d)	C-13	111.1(d)	*b	
	6.74	122.7(d)	C-14	123.0(d)	*b	
9,10,14	2.41-2.50	39.1(t)	C-α	40.4(t)	(2.24-2.30)	9,10,14
	2.75				(2.66-2.70)	
1	2.44	42.8(q)	N-CH_3	42.4(q)	2.22	1
-----	-----	166.0(s)	C-1'	166.8(s)	-----	-----
	(3.68-3.86)	47.1(t)	C-3'	47.1(t)	(3.68-3.86)	
	(4.10-4.13)				(4.10-4.13)	
(4a')	(2.58-2.72)	25.7(t)	C-4'	25.8(t)	(2.58-2.72)	(5',8a')
-----	-----	133.5(s)	C-4a'	134.4(s)	-----	-----
4',6',7',8a'	6.62	110.6(d)	C-5'	110.0(d)	6.53	4',6',7',8a'
-----	-----	150.5(s)	C-6'	151.6(s)	-----	-----
-----	-----	143.7(s)	C-7'	143.2(s)	-----	-----
		*a	C-8'	117.9(d)	*b	
-----	-----	121.1(s)	C-8a'	120.5(s)	-----	-----
-----	-----	135.6(s)	C-9'	135.8(s)	-----	-----
12',14'	6.98	130.0(d)	C-10'	130.0(d)	*b	
	*b	*c (d)	C-11'	*c (d)	*b	
-----	-----	153.3(s)	C-12'	153.1(s)	-----	-----
	*b	*c (d)	C-13'	123.0(d)	7.18	9'
10',12'	7.36	130.7(d)	C-14'	131.3(d)	7.49	10'.12'
	4.06-4.13	44.2(t)	C-α'	43.8(t)	4.20	
6	3.89	55.8(q)	6-OCH_3	55.8(q)	3.78	6
7	3.50	60.2(q)	7-OCH_3	60.4(q)	3.30	7
12	3.92	55.9(q)	12-OCH_3	55.9(q)	3.91	12
6'	3.69	55.7(q)	6'-OCH_3	55.3(q)	3.52	6'

*a: unidentified, *b: observed at 6.78-6.81 with 9H intensity,
*c: observed at 123.1, 123.6, or 123.7.

Recently, some bisbenzylisoquinolines have been shown to be antimalarials.[7] Dehatrine (**1**) here isolated has been found to exhibit a potent inhibitory activity (IC_{50}: 0.17 μM, IC_{90}: 3.6 μM) against the proliferation of malarial pathogen *Plasmodium falciparum* (K1 strain; a chloroquine resistant strain in human erythrocytes)[8] with almost the same activity as quinine (IC_{50}: 0.27 μM and IC_{90}: 1.5 μM for the same pathogen). Therefore, it may be concluded that dehatrine (**1**) is a responsible constituent of the antimalarial folk medicine "Medang Kohat".

We are currently continuing the chemical investigation of the other constituents of this antimalarial wood. We have so far isolated several minor bisbenzylisoquinoline alkaloids, some of which also gave complex NMR spectra similar to dehatrine (**1**). The details will be reported in due course.

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