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Stilbenoids in the stem bark of *Hopea parviflora*

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Abstract

From the bark of *Hopea parviflora*, two stilbenoids, named (+)-parviflorol and (−)-ampelopsin A, were isolated in addition to three known compounds: (+)-balanocarpol, (−)-ε-viniferin and (−)-hopeaphenol. Their structures were determined by analysis of spectral data, including 2D NMR and NOE experiments, respectively. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Hopea parviflora; Dipterocarpaceae; Stilbenoids; (+)-Parviflorol; (−)-Ampelopsin A; (+)-Balanocarpol; (−)-ε-Viniferin; (−)-Hopeaphenol

1. Introduction

Many stilbene derivatives have been isolated from plants belonging to families of the Vitaceae, Leguminosae and Dipterocapaceae (Sotheeswaran & Pasupathy, 1993), and various biological activities including chemopreventive (Jang et al., 1997), hepatoprotective (Oshima et al., 1995) and topoisomerase II inhibitory (Nozaki et al., 1997) properties have been described. Stilbenoids are, therefore, potentiality useful lead compounds. In previous papers, we reported the structural determination of stilbenoids in the genera *Sophora* (Leguminosae) (Ohyama, Tanaka & Iinuma, 1994a, 1994b; Ohyama, Ichise, Tanaka & Iinuma, 1996; Ohyama, Tanaka, Iinuma & Burandt, 1998) and *Parthenocissus* (Vitaceae) (Tanaka, Iinuma & Murata, 1998; Tanaka, Ohyama, Morimoto, Asai & Iinuma, 1998). In this paper, we report the isolation and structure determination of stilbenoids isolated from the bark of *Hopea parviflora* Bedd. (Dipterocarpaceae).

2. Results and discussion

The bark of *H. parviflora* was extracted with acetone and methanol, the extracts, which afforded five stilbene oligomers.

Compound **1**, $[\alpha]_D$ +62°, obtained as a yellow solid, gave an M^+ ion at m/z 380 in the EIMS corresponding to the molecular formula $C_{21}H_{16}O_7$. Compound **1** reacted positively with $FeCl_3$ and Gibbs reagents. Its ^{1}H-NMR spectrum showed the presence of one set of *ortho*-coupled aromatic protons assignable to a 4-hydroxyphenyl group [δ 6.86 (2H, *d*, $J = 8.6$ Hz, H-2a and 6a) and 6.49 (2H, *d*, $J = 8.6$ Hz, H-3a and 5a)], two sets of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring [δ 6.10 (1H, *d*, $J = 2.5$ Hz, H-12a), 6.74 (1H, *br d*, $J = 2.5$ Hz, H-14a); δ 6.73 (1H, *d*, $J = 1.9$ Hz, H-4b), 7.41 (1H, *d*, $J = 1.9$ Hz, H-6b), an aliphatic hydroxyl group [δ 5.20 (1H, *d*, $J = 4.0$ Hz, C8a-OH which was correlated with

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Table 1
^{1}H- and ^{13}C-NMR spectral data for **1**–**3**[a]

No.	**1**		**2**		**3**	
	δH (J, Hz)	*δC*	*δH*	*δC*	*δH*	*δC*
1a		131.1		130.9		133.5
2(6)a	6.86 *br d* (8.6)	130.5	6.88 *d* (8.3)	128.7	6.75 *d* (8.3)	131.5
3(5)a	6.49 *d* (8.6)	115.0	6.62 *d* (8.3)	115.4	6.42 *d* (8.3)	114.2
4a		155.8		156.0[b]		155.8[c]
7a	5.15 *br s*	48.4	5.45 *d* (4.9)	43.8	4.90 *br s*	50.3
8a	5.32 *br s*	74.8	5.42 *br d* (4.9)	71.2	5.40 *br s*	73.2
9a		148.8		140.2		140.8
10a		111.7		118.9		113.8
11a		168.8		160.1		159.7
12a	6.10 *d* (2.5)	102.1	6.14 *br d* (1.9)	97.1	6.20 *br s*	95.1
13a		164.5		158.8[d]		159.2
14a	6.74 *br d* (2.5)	107.1	6.64 *d* (1.9)	110.5	6.26 *d* (2.0)	104.4
1b		141.5		132.5		133.7
2b		122.1	7.09 *d* (8.8)	129.9	7.50 *d* (8.3)	130.5
3b		156.2	6.75 *d* (8.8)	116.0	6.95 *d* (8.3)	116.4
4b	6.73 *d* (1.9)	108.1		158.4[b]		158.6[c]
5b		157.2	6.75 *d* (8.8)	116.0	6.95 *d* (8.3)	116.4
6b	7.41 *d* (1.9)	110.5	7.09 *d* (8.8)	129.9	7.50 *d* (8.3)	130.5
7b		195.7	5.42 *d* (11.3)	88.3	5.69 *d* (9.3)	93.5
8b			4.15 *br d* (11.3)	49.5	5.16 *br d* (9.3)	52.3
9b				143.1		142.8
10b	118.2			118.2		120.4
11b	157.2			157.2		157.4
12b			6.42 *d* (2.5)	101.6	6.09 *br s*	102.0
13b				158.8[d]		156.9
14b			6.21 *br s*	105.5	5.96 *d* (2.3)	106.8
OH	5.20 *d* (4.0, C-8a) 7.87, 8.48, 8.65 9.15 each *br s* 14.10 *s* (C-3b)		3.57 *br s* (C-8a) 8.08, 8.17, 8.26 8.32, 8.40 *each br s*		4.36 *d* (4.4, C-8a) 7.74 *br s* (C-4b) 7.85 *br s* (C-13a) 7.97 *br s* (C-11b) 8.04 *br s* (C-13b) 8.56 *br s* (C-4a)	

[a] Measured in acetone-*d*6 (400 and 100 MHz). All protons were assigned by HH-, HH long-range COSY and difference NOE spectrum. All carbons were assigned by CH COSY and COLOC spectrum. The assignment of hydroxyl groups of **3** was determined by difference NOE spectrum.

[b,c] Interchangeable.

[d] Overlapping.

H-8a in the HH long range COSY spectrum) and five phenolic hydroxyl groups [*δ* 7.87, 8.48, 8.65, 9.15 and 14.10 (chelated)]. Two mutually coupled benzylic methine protons were also observed at *δ* 5.15 (1H, *br s*, H-7a) and 5.32 (1H, *br s*, H-8a). In the HH long range COSY spectrum, a methine proton (H-7a) had a correlation with H-2(6)a on ring A1, and H-8a was correlated with H-14a on the ring A2. In its ^{13}C-NMR spectrum, 19 carbon signals were observed with the resonance at *δ* 195.7 assigned to a carbonyl group (C-7b). All protonated carbons were assigned by the CH COSY spectrum as shown in Table 1. In the COLOC spectrum, significant correlations were observed between C-2(6)a/H-7a, C-1a/H-8a, C-9a/H-8a and C-9a/H-7a, respectively, as shown in Fig. 1. These results

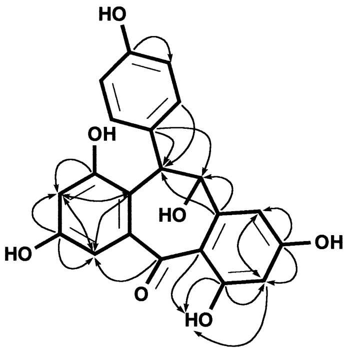


Fig. 1. CH long-range correlations in the COLOC spectrum of **1** ($J = 8$ Hz).

indicated that a stilbene skeleton (ring A1-C7a-C8a-ring A2) existed in the structure of **1**. In addition, a carbon signal (C-12a) had a long = range correlation with the chelated OH (C11a-OH) through ^{3}J in the COLOC spectrum. Thus, the chelated hydroxyl group was attached to ring A2. A *meta*-coupled proton (H-6b) was observed at lower field (δ 7.41), indicating that it was located at the *peri*-position relative to the carbonyl group. A long range correlation was also observed between C-7b and H-6b. The results indicated, therefore, that **1** had a unit [A2-CO(C-7b)-B1]. Although, no correlation was observed between B1 ring carbons and H-7a (and/or H-8a), C-7a and C-2b could be linked as shown in the molecular formula of **1**. Finally, the planar structure of **1** could be illustrated as shown in Fig. 1. In the DIFNOE experiments, NOEs were observed between H-2(6)a/C-8a-OH and H-7a/H-8a. Thus, the aliphatic hydroxyl group and the ring A1 are in a *cis* relationship. Hence, the relative stereo structure of **1** was confirmed as shown in Fig. 2.

Compound **2**, $[\alpha]_D - 170°$, obtained as a colorless solid, gave an $[M-H]^-$ ion at *m*/*z* 469 in the negative ion FABMS corresponding to molecular formula $C_{28}H_{22}O_7$. The ^{1}H-NMR spectrum showed the presence of two sets of *ortho*-coupled protons assignable to two 4-hydroxyphenyl groups [δ 6.88 (2H, *d*, $J = 8.3$ Hz, H-2a and 6a), 6.62 (2H, *d*, $J = 8.3$ Hz, H-3a, 5a); δ 7.08 (2H, *d*, $J = 8.8$ Hz, H-2b and 6b), 6.75 (2H, *d*, $J = 8.8$ Hz, H-3b and 5b)], two sets of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring [δ 6.64 (1H, *d*, $J = 1.9$ Hz, H-14a), 6.14 (1H, *br d*, $J = 1.9$ Hz, H-12a); δ 6.21 (1H, *br s*, H-14b), 6.42 (1H, *d*, $J = 2.5$ Hz, H-12b)], an aliphatic hydroxyl group [δ 3.57 (1H, *br s*)] and five phenolic hydroxyl groups (δ 8.08, 8.17, 8.26, 8.32 and 8.40). Two sets of mutually coupled benzylic methine protons were also observed [δ 4.15 (1H, *br d*, $J = 11.3$ Hz, H-8b), 5.42 (1H, *d*, $J = 11.3$ Hz, H-7b); δ 5.42 (1H, *br d*, $J = 4.9$ Hz, H-8a), 5.45 (1H, *d*, $J = 4.9$ Hz, H-7a)]. In the ^{13}C-NMR spectrum, all protonated carbons were assigned from the CH COSY spectrum as shown in Table 1. One set of benzylic methine protons (δ 4.15 and 5.42) was assigned to protons of the dihydrofuran ring. In the HH long-range COSY spectrum, long-range correlations were observed between the methine protons and aromatic protons as follows; H-7a/H-2(6)a, H-7b/H-2(6)b and H-8a/H-14a. respectively. In the decoupling experiment, H-8a was observed as a sharp doublet when, the aliphatic hydroxyl signal (δ 3.57) was irradiated. This result indicated that the aliphatic hydroxyl group was substituted at C-8a. In the COLOC spectrum (Fig. 2), significant correlations were observed between C-2(6)a/H-7a, C-1a/H-8c, C-9a/H-7a, C-9a/H-8a and C-10a/H-8a, demonstrating the presence of a stilbene skeleton (ring A1-C7a-C8a-ring A2). On the other hand, the CH long-range correlations observed between C-2(6)b/H-7b, C-7b/H-8b, C-9b/H-8b, C-10b/H-8b and C-14b/H-8b indicated the presence of another stilbene skeleton (ring B1-C7b-

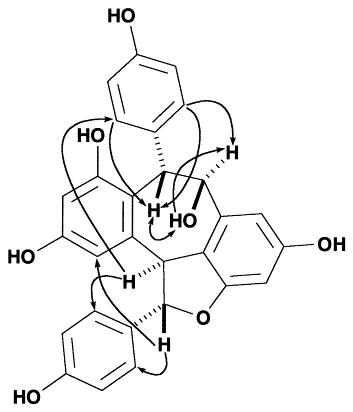


Fig. 3. NOEs in the difference NOE spectrum of **2**.

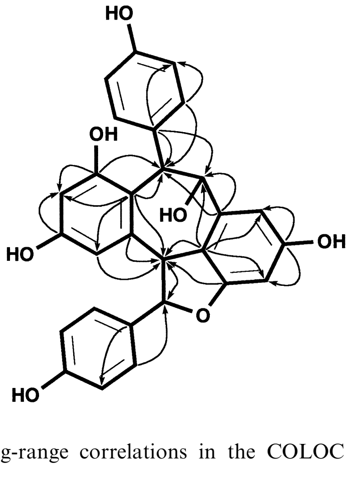


Fig. 2. CH long-range correlations in the COLOC spectrum of **2** ($J = 8$ Hz).

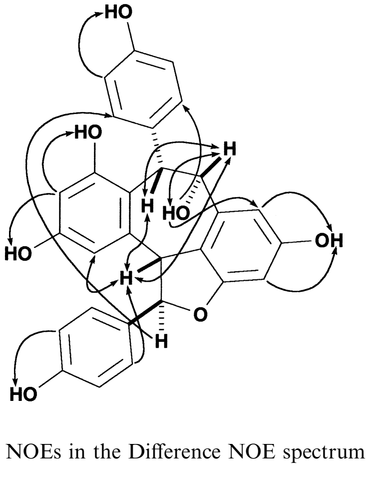
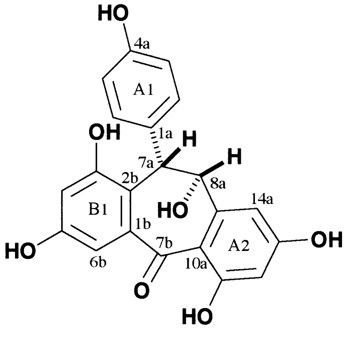
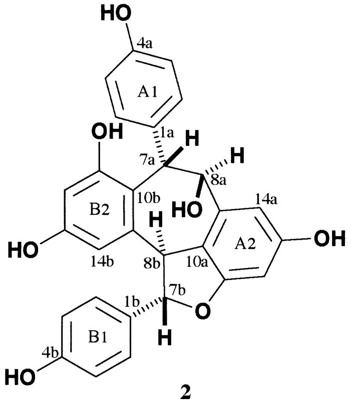


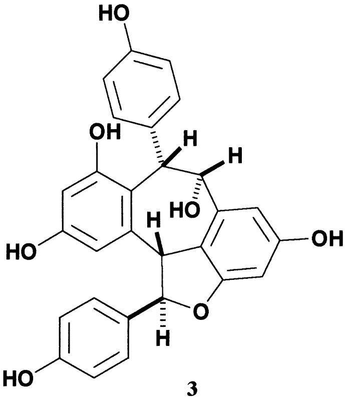
Fig. 4. NOEs in the Difference NOE spectrum of **3**.



1



2



3

C8b-ring B2). The two stilbene skeletons could be connected at C-7a/C-10b and C-10a/C-8b as indicated by CH long-range correlations (C-10a/H-8b, C-11a/H-8b, C-10b/H-7a and C-11b/H-7a) in the COLOC spectrum. The planar structure of **2** was, therefore, illustrated as shown in Fig. 2. In the NOE experiments (Fig. 3), significant NOEs were also observed between H-2a(6a)/H-8a, H-7a/C-8a-OH, H-7b/H-14b and H-8b/H-2b(6b). These results indicated that the ring A1/H-8a and ring B1/H-8b were in a *cis* relationship to each other. A further NOE was observed between H-8b/H-2(6)a, which indicated that ring A1 and H-8b were in its same orientation (Fig. 4). Consequently, the relative stereo structure was confirmed as shown in Fig. 3. Thus, compound **2** has the same relative structure as ampelopsin A (Oshima, Ueno & Hikino, 1990) isolated from *Ampelopsis brevipedunculata* var. *hancei*; however, its optical rotation was opposite to that of ampelopsin A.

Compound **3**, $[\alpha]_D$ +3° was obtained as colorless solid and gave an $[M\text{-}H]^-$ ion at *m/z* 469 in the negative ion FABMS corresponding to the molecular formula $C_{28}H_{22}O_7$. The analysis of HH long range COSY, CH COSY and COLOC spectrum indicated that **3** had the same planar structure as for **2**. In the NOE experiments, significant NOEs were observed between H-7a/H-8a, C-8a-OH/H-2(6)a, H-2(6)b/H-8b, H-8a/H-8b, H-7b/H-2(6)a and H-8b/H-2(6)b, indicating that the orientations of ring A1/C8a-OH, ring B1/H-8b, H-8a/H-8b and H-7a/H-8b were in a *cis* relationship. Therefore, **3** was a stereo isomer of **2** at the C-7a position. This compound, named balanocarpol, had been previously isolated from *Balanocarpus zeyanicus* and *Hopea. jocunda* (Diyasena, Sotheeswaran, Surendrakumar, Balasubramania, Bokel & Krau, 1985), although its NMR assignment was incomplete. In this paper, a detail NMR balanocarpol analysis of users 2D NMR spectroscopy is given, as shown in Table 1. The absolute stereochemistry of **1**, **2** and **3** has not yet been determined.

Two other known compounds were isolated, these being determined to (−)-ε-viniferin (Langcake & Pryce, 1977) and (−)-hopeaphenol (Coggon et al., 1965; Kawabata, Fukushi, Hara & Mizutani, 1992) following analysis of spectral data and comparison with authentic samples.

3. Experimental

3.1. Extraction and isolation

The dried bark (1 kg) of *Hopea parviflora*, collected in India in 1997, was sequentially extracted at room temperature with acetone with acetone and MeOH (each, 5l × 3 times), respectively. The extracts were then concentrated in vacuo. An aliquot (20 g) of the acetone extract (ca. 200 g) was subjected to silica gel chromatography eluted with $CHCl_3$–MeOH. The $CHCl_3$–MeOH (5:1) eluted fractions were combined and further applied to silica gel which were eluted with benzene-acetone-EtOH (6:1:1) to afford 8 fractions. The fraction 4 was further purified by preparative TLC using the solvent systems as follows: benzene–acetone–EtOH (6:1:1) and $CHCl_3$–acetone–AcOEt (2:1:1). Repeated preparative TLC gave **1** (54 mg), **2** (32 mg), **3** (45 mg) and ε-viniferin (230 mg), respectively. The $CHCl_3$–MeOH (3:1) eluted fraction was subjected to further silica gel and Sephadex LH 20 (solvent:acetone) chromatography to afford (−)-hopeaphenol (1.2 g).

Compound **1** (*parviflorol*) Yellow solid. $[\alpha]_D$ + 62° ($c = 0.18$, MeOH), UV (nm, MeOH): 338. EMS *m/z* (rel. int.): 380 (100).

Compound **2** [(−)-*ampelopsin A*] Colorless solid. $[\alpha]_D$ − 170° ($c = 0.09$, MeOH). Negative ion FABMS: *m/z* 469 $[M\text{-}H]^-$. UV (nm, MeOH): 285.

Compound **3** [(+)-*balanocarpol*] Colorless solid. $[\alpha]_D$ + 30° ($c = 0.03$, MeOH). UV (nm, MeOH): 283. Negative ion FABMS: *m/z* 469 $[M\text{–}H]^-$.

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