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Antimalarial preracemosols A and B, possible biogenetic precursors of racemosol from *Bauhinia malabarica* Roxb.

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Abstract

Racemosol and demethylracemosol, together with their possible biogenetic precursors, preracemosol A and preracemosol B, were isolated from the roots of *Bauhinia malabarica* Roxb. While only racemosol and demethylracemosol exhibited cytotoxicity against KB and BC cell lines, all four compounds exhibited moderate antimalarial activity.

Keywords: *Bauhinia malabarica*; Leguminosaes; Bibenzyls; Cytotoxic compounds; Antimalarial compounds; Biosynthesis; Racemosol biosynthesis

1. Introduction

As part of an on-going research program on biologically active substances from Thai bioresources conducted at the National Center for Genetic Engineering and Biotechnology (BIOTEC), we have screened biological activities of extracts from plants and microorganisms. We found that the root extracts of *Bauhinia malabarica* Roxb. exhibited in vitro antimalarial activity, and, the active constituents were isolated and identified: these are racemosol (**1**) and its derivative (**2**), the bibenzyls preracemosol A (**3**) and preracemosol B (**4**), respectively. Racemosol (**1**) and its derivative (**2**), were previously isolated from *B. racemosa* and *B. rufescens*, respectively (Anjaneyulu et al., 1986; Maillard et al., 1991). The biosynthetic pathway to these tetracyclic substances, involving stilbene related (bibenzyl) compounds as precursors, was proposed by Hostettmann and coworkers, as outlined in Fig. 1 (Maillard et al., 1991). The co-existence of compounds **1** and **2**, and their possible biogenetic precursors, **3** and **4**, within the same plant, *B. malabarica*, provides supporting evidence for the biosynthetic pathway proposed earlier (Maillard et al., 1991).

2. Results and discussion

Compounds **1**–**4** were obtained from the root extract of *B. malabarica* after purification by Sephadex LH-20 column chromatography, followed by preparative HPLC (reversed phase C_{18} column). The yields of compounds **1**–**4** were 0.0004%, 0.0005%, 0.0003%, and 0.0002% (dry weight), respectively. The spectral data of racemosol (**1**) and demethylracemosol (**2**) were identical in all respects to those published (Anjaneyulu et al., 1986; Maillard et al., 1991).

The exact mass (at m/z 343.1906, calcd. for $C_{21}H_{27}O_4$ $(M+H)^+$, 343.1909) obtained from the ESITOF mass spectrum of preracemosol A (**3**) gave the molecular formula as $C_{21}H_{26}O_4$. The ^1H and ^{13}C NMR spectra (Table 1) of preracemosol A (**3**) were generally similar to that of preracemosol B (**4**). The partial structure from C-1′ to C-8′ of preracemosol A (**3**) was established employing the HMBC technique (correlations as seen on the HMBC spectrum of **3**: H-5′ to C-1′ and C-3′; H-6′ to C-2′ and C-4′; H-7′ to C-2′ and C-1; and H-8′ to C-2 and C-1′). The ^1H NMR spectral data for preracemosol

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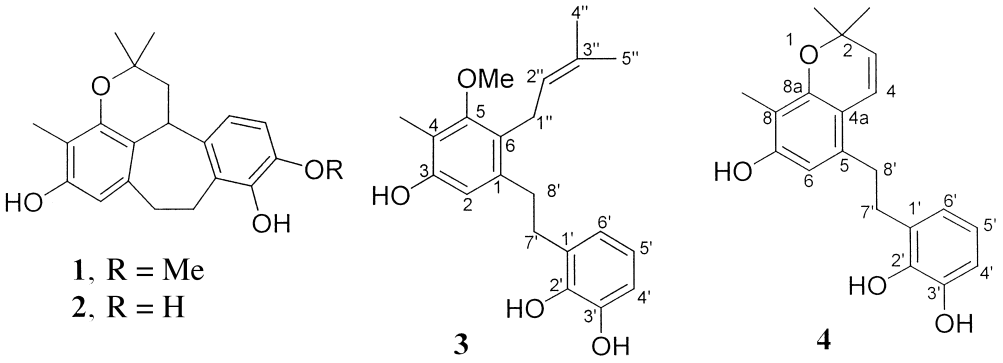

A (**3**) showed a typical set of proton signals for a prenyl moiety, which was readily confirmed by analysis of the ^{1}H–^{1}H COSY and HMBC spectral data. The ^{1}H–^{1}H COSY spectrum of **3** revealed that H-2″ was coupled to H-1″, and H-2″ also showed an allylic coupling to H-4″ and H-5″, whilst the HMBC data demonstrated correlations of H-1″ to C-1, C-5 and C-3″; H-2″ to C-4″; H-4″ and H-5″ to C-2″ and C-3″; H-2 to C-8′; 4-Me protons to C-3, C-4 and C-5; and 5-OMe protons to C-5, respectively. The NOESY spectrum of preracemosol A (**3**) allowed the assignment of H-4″ (δ_H 1.62) and H-5″ (δ_H 1.70); a cross peak between H-4″ and H-2″ was observed, but none between H-5″ and H-2″. The NOESY spectral data of **3** also confirmed the position of the 5-OMe group, showing the correlation of 5-OMe protons to H-2″.

An empirical formula $C_{20}H_{22}O_4$ of **4** was established from the ESITOF mass spectrum of **4** (accurate mass ion at *m*/*z* 327.1588, calcd. for $C_{20}H_{23}O_4$ $(M+H)^+$, 327.1596). The ^{1}H and ^{13}C NMR spectral data of preracemosol B (**4**) (Table 1) revealed two methylene groups (H-7′ and H-8′, both at δ_C 32.3 and δ_H 2.65) bridged between two aromatic rings. In addition, HMBC data for preracemosol B (**4**) conclusively demonstrated correlations of methylene protons (H-7′ or H-8′) to C-1′ and C-5; H-6 to C-8′; and H-6′ to C-7′, respectively. The HMBC spectrum of preracemosol B (**4**) also showed correlations of H-4′ and H-6′ to C-2′; H-5′ to C-3′; and H-6′ to C-1′. The presence of a chromene unit (C-1 to C-8) in preracemosol B (**4**) was readily confirmed by analysis of the HMBC spectrum, where correlations of *gem* Me protons and H-4 to C-2; H-3 and H-6 to C-4a; H-4 and 8-Me to C-8a; 8-Me to C-7; and 8-Me and H-6 to C-8 were observed.

While compounds **3** and **4** showed no cytotoxicity, compounds **1** and **2** exhibited cytotoxicity against KB (EC_{50} at 15.0 μg/ml for **1** and 5.6 μg/ml for **2**) and BC (EC_{50} at 6.1 μg/ml for **1** and 3.6 μg/ml for **2**) cell lines. Compounds **1**–**4** also exhibited moderate antimalarial activities with EC_{50} values of 0.9, 2.0, 18.0 and 3.0 μg/ml, respectively.

The presence of preracemosol A (**3**) and preracemosol B (**4**) in *B. malabarica* supports Hostettmann and coworkers' postulation that they are biogenetic precursors of the tetracyclic racemosol (**1**) and its derivative (**2**) (Fig. 1). The presumed enzymatic cyclization of **3** (and **4**) leads to **1** and **2** (Fig. 1). Though a number of substances, including flavonoids, steroids, chalcones, lignanes, were isolated from the plant Genus *Bauhinia* (Achenbach et al., 1988; Iribarren and Pomilio, 1983; Gupta et al., 1980), this is the first report on bibenzyl constituents in this genus.

3. Experimental

3.1. General

The ^{1}H, ^{13}C, DEPTs, ^{1}H–^{1}H COSY, NOESY, HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 8.0 Hz as well as 4.0 Hz) spectra were recorded on a Bruker DRX 400, operating at 400.1 MHz for proton and 100.6 MHz for carbon. The ESITOF mass spectra were obtained from a Micromass LCT mass spectrometer, while the IR spectra were measured on a Perkin–Elmer 2000 spectrometer. An HPLC pump (Waters 600) was equipped with an UV-photodiode array detector (Waters 996).

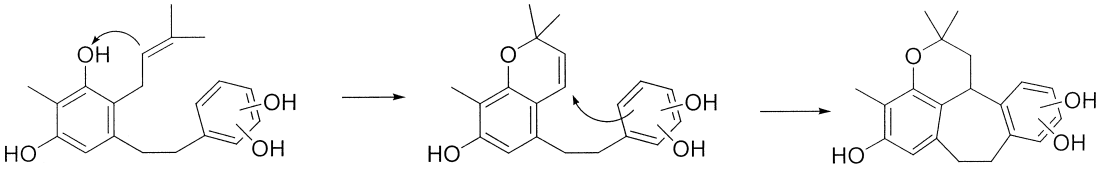

Fig. 1. Possible biosynthetic pathway to the tetracyclic compounds (**1** and **2**).

Table 1
^1H (400 Mz) and ^{13}C (100 Hz) NMR spectral data for **3** and **4**

	3			**4**		
Position	δ_C	δ_H, *J* (Hz)[a]	δ_H, *J* (Hz)[b]	δ_C	δ_H, *J* (Hz)[a]	δ_H, *J* (Hz)[b]
1	138.8	–	–	–	–	–
2	111.3	6.53, *s*	6.55, *s*	74.7	–	–
3	154.2	–	–	126.6	5.52, *d*, 10.0	5.50, *d*, 10
4	114.3	–	–	118.6[c]	6.58, *d*, 10.0	6.57, *d*, 10
4a	–	–	–	110.9	–	–
5	157.2	–	–	135.5	–	–
6	122.5	–	–	107.9	6.24, *s*	6.26, *s*
7	–	–	–	155.6	–	–
8	–	–	–	109.0	–	–
8a	–	–	–	151.5	–	–
1′	128.7	–	–	128.5	–	–
2′	143.1	–	–	143.1	–	–
3′	144.8	–	–	143.8	–	–
4′	120.0	6.51, *dd*, 7.4, 2.0	6.74, *dd*, 8, 1.8	119.3[c]	6.52, *brd*, 7.8	6.73, *dd*, 8, 1.8
5′	118.6	6.54, *brt*, 7.6	6.70, *brt*, 7.7	113.2	6.63, *brt*, 7.7	6.69, *brt*, 7.7
6′	113.2	6.64, *dd*, 7.4, 2.0	6.65, *dd*, 8, 1.8	120.2[c]	6.52, *brd*, 7.8	6.65, *dd*, 8, 1.8
7′	32.8	2.65, *m*	2.80, *s*	32.3	2.65, *m*	2.82, *s*
8′	32.0	2.64, *m*	2.80, *s*	32.3	2.65, *m*	2.82, *s*
1″	24.6	3.23, *d*, 6.3	3.30, *d*, 6.3	–	–	–
2″	124.9	5.00, *t*, 6.3	5.10, *t*, 6.3	–	–	–
3″	129.6	–	–	–	–	–
4″	25.5	1.62, *s*	1.66, *s*	–	–	–
5″	17.8	1.70, *s*	1.74, *s*	–	–	–
2-Me	–	–	–	27.4	1.34, *s*	1.37, *s*
–	–	–		27.4	1.34, *s*	1.37, *s*
4-Me	9.2	2.01, *s*	2.07, *s*	–	–	–
8-Me	–	–	–	8.0	1.91, *s*	2.07, *s*
5-OMe	60.0	3.59, *s*	3.70, *s*	–	–	–
OH		8.10, *brs*	–	–	8.15, *brs*	–
		9.05, *brs*	–		9.09, *brs*	–
		9.15, *brs*	–		9.13, *brs*	–

[a] Acquired in DMSO-d_6.
[b] Acquired in $CDCl_3$.
[c] May be interchanged in the same column.

3.2. Plant specimen, extraction and isolation of *1–4*

Roots of *B. malabarica* Roxb. (Leguminosae) were collected from Nakhon Sawan Province in January 1998, and the plant specimen was identified by comparison with the authentic sample (voucher specimen no. BK32699) deposited at the Herbarium of the Royal Forest Department, the Ministry of Agriculture and Cooperatives, Bangkok, Thailand. Ground roots of *B. malabarica* (1.2 kg) were macerated in CH_2Cl_2 (6 liters), and the crude extract was subjected to Sephadex LH-20 column chromatography (eluted with MeOH). Fractions (ca 100 ml each) were collected, from which fractions 8 and 9 were pooled, evaporated to dryness, and further purified by a preparative HPLC (C_{18} reversed phase column, Prep Nova Pak, Waters) using H_2O–MeCN (60:40, v/v) as eluent, yielding **1** (50 mg) and **2** (77 mg). Fractions 10–12 obtained from the Sephadex column were combined and subsequently purified by a preparative HPLC using C_{18} reversed phase column, eluted with H_2O:MeCN (50:50, v/v), to give **3** (25 mg) and **4** (32 mg).

3.3. Bioassay

The parasite *Plasmodium falciparum* (K1, multidrug resistant strain) was cultured continuously according to the method of Trager and Jensen (1976). Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al. (1979). Effective concentration (EC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]hypoxanthine by *P. falciparum*. An EC_{50} value of 0.16 μg/ml (3.1 μM) was observed for the standard sample, chloroquine diphosphate, in the same test system. The cytoxicity employed the colorimetric method

(Skehan et al., 1990). Ellipticine was used as a reference substance, exhibiting the activity toward BC and KB cell lines, both with the EC_{50} of 0.3 μg/ml.

3.4. Preracemosol A (**3**)

Brown viscous oil; IR ν_{max} cm^{-1}: 3340, 2924, 1608, 1480, 1419, 1291, 1101, 1004; UV ($CHCl_3$) λ_{max} nm: 221, 279; ESITOF MS: *m/z* 343.1906, calcd. for $C_{21}H_{27}O_4$ $(M+H)^+$, 343.1909; ^1H and ^{13}C NMR: see Table 1.

3.5. Preracemosol B (**4**)

Brown viscous oil; IR ν_{max} cm^{-1} : 3340, 2975, 1598, 1480, 1424, 1127, 999; UV ($CHCl_3$) λ_{max} nm: 220, 278; ESITOF MS: *m/z* 327.1588, calcd. for $C_{20}H_{23}O_4$ $(M+H)^+$, 327.1596; ^1H and ^{13}C NMR: see Table 1.

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