

# Lakoochins A and B, New Antimycobacterial Stilbene Derivatives from *Artocarpus lakoocha*

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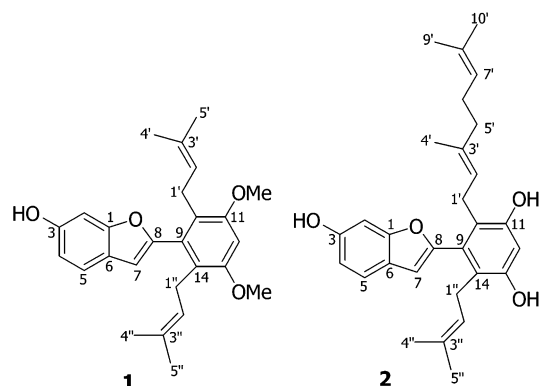
Received September 23, 2003

Two new stilbene derivatives, lakoochins A (**1**) and B (**2**), were isolated from the roots of *Artocarpus lakoocha*. The structures of **1** and **2** were elucidated by analysis of their spectral data. Lakoochins A (**1**) and B (**2**) exhibited antimycobacterial activity with the respective MIC values of 12.5 and 50  $\mu\text{g/mL}$ . While **1** was cytotoxic against the BC (breast cancer) cell line (IC<sub>50</sub> 6.1  $\mu\text{g/mL}$ ) but inactive (at 20  $\mu\text{g/mL}$ ) toward KB (nasopharyngeal carcinoma) cells, compound **2** possessed cytotoxicity against the BC and KB cell lines with IC<sub>50</sub> values of 3.1 and 6.1  $\mu\text{g/mL}$ , respectively.

*Artocarpus lakoocha* (Moraceae), "Ma-Haad" in Thai, is widely distributed throughout Thailand. The extremely hard and durable heartwood of *A. lakoocha* has extensive use in local construction and has been especially favored as railroad ties since the early days of the railroad system in this country. A totally different and quite valuable property of the heartwood is its use as a very effective Thai folklore medicine for the eradication of tapeworms. The medication is prepared as a cream-colored froth obtained by boiling the chopped heartwood with water for 2–3 h. Under the microscope, the dried froth, "Puak-Haad" as it is called in folkloric medicine, can actually be seen to consist of tiny crystals of 2,4,3',5'-tetrahydroxystilbene.<sup>1</sup> Apart from this stilbene, lectins and a flavonol glycoside have been found as constituents of this plant.<sup>2–5</sup> Although *A. lakoocha* was recently proved scientifically to be effective for the treatment of taeniasis,<sup>6,7</sup> few reports on the biological activities of metabolites from the plant have been recorded to date.<sup>1–7</sup> Upon reinvestigation, we noted that a crude extract of *A. lakoocha* roots exhibited antimycobacterial activity with a minimum inhibitory concentration (MIC) of 50  $\mu\text{g/mL}$ . Chemical investigation of the active crude extract led to the identification of two new stilbene derivatives, named lakoochins A (**1**) and B (**2**). We report herein the isolation, characterization, and biological activities of lakoochins A (**1**) and B (**2**).

Lakoochins A (**1**) and B (**2**) were obtained after purification of a CH<sub>2</sub>Cl<sub>2</sub> extract of *A. lakoocha* roots with chromatographic techniques (Sephadex LH-20 and silica gel).

Lakoochin A (**1**) was obtained as an off-white semisolid, and its molecular formula C<sub>26</sub>H<sub>30</sub>O<sub>4</sub> was deduced from the ESITOFMS. The <sup>1</sup>H NMR spectrum of **1** showed an ABX aromatic spin system ( $\delta_{\text{H}}$  6.97, d,  $J = 2.1$  Hz; 6.78, dd,  $J = 8.1$  and 2.1 Hz; and 7.4, d,  $J = 8.1$  Hz), two downfield singlets ( $\delta_{\text{H}}$  6.54 and 6.61), two methyl ether singlets (both resonances at  $\delta_{\text{H}}$  3.87), and two sets of signals for a prenyl group (both at  $\delta_{\text{H}}$  3.15, d,  $J = 6.8$  Hz, 2  $\times$  2H; 5.05, br t,  $J = 7.1$  Hz, 2  $\times$  1H; 1.39, s, 2  $\times$  3H; and 1.58, s, 2  $\times$  3H). Analysis of the <sup>13</sup>C, DEPT, and HMQC spectral data of lakoochin A (**1**) also revealed symmetrical methoxy and prenyl groups. Interpretation of the HMBC spectrum of **1**



placed two prenyl and methoxy groups in an aromatic ring, resulting in the formation of a symmetrical ring C (HMBC correlations were seen from H-12 to C-10, C-11, C-13, and C-14; the OMe-11 protons to C-11; the OMe-13 protons to C-13; H-1' to C-9, C-10, and C-11; and H-1'' to C-9, C-13, and C-14). An upfield shift of C-12 ( $\delta_{\text{C}}$  97.3) and the NOESY correlation between the OMe protons (OMe-11 and OMe-13) and H-12 confirmed the positions of the two OMe groups and H-12 in **1**. A gross structure for lakoochin A (**1**) was established by analysis of the HMBC spectral data, indicating that the symmetrical unit was bridged with an ABX aromatic ring through the double bond at C-7/C-8 (key HMBC correlations were from H-7 to C-1, C-6, C-8, and C-9). The NOESY spectrum of **1** revealed the proximity of H-5 and H-7 and also assisted in the assignment of H-4' (or H-4'') and H-5' (or H-5''), where the correlation between H-2' (or H-2'') and H-5' (or H-5'') was observed. Complete assignment of protons and carbons in **1** was by analysis of HMBC, <sup>1</sup>H–<sup>1</sup>H COSY, and NOESY spectra (Table 1). Accordingly, the structure of lakoochin A (**1**) was established as an isoprenylated derivative of 2-arylbenzofuran.

Lakoochin B (**2**) (off-white semisolid) exhibited a molecular formula of C<sub>29</sub>H<sub>34</sub>O<sub>4</sub> by ESITOFMS. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of lakoochin B (**2**) were similar to those of lakoochin A (**1**). However, unlike in **1**, there were no methyl ether groups in **2**, but instead broad signals of exchangeable hydroxyl protons were evident in the <sup>1</sup>H NMR spectrum of **2**. Additional prenyl signals were also observed in the <sup>1</sup>H and <sup>13</sup>C NMR spectra of **2**. On the basis of these spectral data, lakoochin B (**2**) was established as a desmethyl prenyl derivative of lakoochin A (**1**). Analysis of the <sup>1</sup>H–<sup>1</sup>H COSY

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**Table 1.**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (100 MHz) NMR Spectral Data ( $\text{CDCl}_3$ ) of Lakoochins A (**1**) and B (**2**)

position	lakoochin A ( <b>1</b> )			lakoochin B ( <b>2</b> )		
	$\delta_{\text{C}}$ , mult.	$\delta_{\text{H}}$ , mult., $J$ in Hz		$\delta_{\text{C}}$ , mult.	$\delta_{\text{H}}$ , mult., $J$ in Hz	
1	155.6, s			155.6, s		
2	98.2, d	6.97, d, 2.1		98.3, d	7.01, d, 2.1	
3	153.3, s <sup>a</sup>			153.3, s		
4	111.5, d	6.78, dd, 8.1, 2.1		111.8, d	6.83, dd, 8.4, 2.1	
5	120.8, d	7.4, d, 8.1		120.9, d	7.44, d, 8.4	
6	123.2, s			122.1, s		
7	106.2, d	6.54, s		106.4, d	6.58, s	
8	153.0, s <sup>a</sup>			153.1, s <sup>b</sup>		
9	131.8, s			131.4, s		
10	122.5, s			120.1, s <sup>c</sup>		
11	156.3, s			153.8, s <sup>b</sup>		
12	97.3, d	6.61, s		105.6, d	6.55, s	
13	156.3, s			154.0, s		
14	122.5, s			120.2, s <sup>c</sup>		
1'	26.5, t	3.15, d, 6.8		27.4, t	3.19, d, 6.8	
2'	123.6, d	5.05, br t, 7.1		122.3, d <sup>d</sup>	5.21, br t, 6.5	
3'	130.3, s			138.0, s		
4'	17.6, q	1.39, s		16.0, q	1.64, s	
5'	25.7, q	1.58, s		39.6, t	2.03, m	
6'				26.3, t	2.08, m	
7'				123.7, d	5.05, br t, 6.6	
8'				132.0, s		
9'				17.6, q <sup>e</sup>	1.60, s	
10'				25.6, q	1.69, s	
1''	26.5, t	3.15, d, 6.8		27.4, t	3.19, d, 6.8	
2''	123.6, d	5.05, br t, 7.1		122.4, d <sup>d</sup>	5.21, br t, 6.5	
3''	130.3, s			134.2, s		
4''	17.6, q	1.39, s		17.7, q <sup>e</sup>	1.64, s	
5''	25.7, q	1.58, s		1.71, s		
OH-3				5.37, br s		
OH-11				5.43, br s		
OH-13				5.43, br s		
OMe-11	55.9, q	3.87, s				
OMe-13	55.9, q	3.87, s				

<sup>a-e</sup> Are exchangeable in the same column.

and HMBC spectral data readily indicated a head-to-tail linkage of the additional prenyl moiety in **2**. The  $^1\text{H}$ – $^1\text{H}$  COSY spectrum revealed that the H-5' methyl group in **1** was replaced by a methylene group in **2**; allylic coupling between H-5' and H-2' was also observed in **2**. The HMBC spectrum of lakoochin B (**2**) demonstrated correlations from H-4' to C-3' and from H-2' to C-5', confirming the head-to-tail addition of the second prenyl unit. Unlike that of **1**, ring C of **2** was unsymmetrical, whereupon slight differences in the chemical shifts of C-10 ( $\delta_{\text{C}}$  120.1) and C-14 ( $\delta_{\text{C}}$  120.2) and those of C-11 ( $\delta_{\text{C}}$  153.8) and C-13 ( $\delta_{\text{C}}$  154.0) were observed. The NOESY spectrum indicated *trans* geometry of the C-2'/C-3' double bond in **2**, demonstrating an intense cross-peak between H-2' and H-5'. Again the NOESY spectrum also assisted in the assignment of H-9', H-10', H-4'', and H-5''. Analysis of the HMBC,  $^1\text{H}$ – $^1\text{H}$  COSY, and NOESY spectra led to complete assignments of the protons and carbons in lakoochin B (**2**) (Table 1). The structure of lakoochin B (**2**) was established as a derivative of 2-arylbenzofuran.

Lakoochins A (**1**) and B (**2**) exhibited antimycobacterial activity with respective MIC values of 12.5 and 50  $\mu\text{g}/\text{mL}$ . While **1** was cytotoxic against the BC (breast cancer) cell line ( $\text{IC}_{50}$  6.1  $\mu\text{g}/\text{mL}$ ) but inactive (at 20  $\mu\text{g}/\text{mL}$ ) toward KB (nasopharyngeal carcinoma) cells, compound **2** possessed cytotoxicity against the BC and KB cell lines with  $\text{IC}_{50}$  values of 3.1 and 6.1  $\mu\text{g}/\text{mL}$ , respectively.

## Experimental Section

**General Experimental Procedures.** UV spectra were recorded on a Cary 1E UV–vis spectrophotometer. IR spectra were measured on a Perkin-Elmer 2000 spectrometer. The  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT,  $^1\text{H}$ – $^1\text{H}$  COSY, NOESY, HMQC, and HMBC NMR experiments were carried out on a Bruker DRX 400 NMR spectrometer, operating at 400 MHz for proton and 100 MHz

for carbon. The ESITOFMS were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for determination of the accurate masses.<sup>8</sup>

**Plant Material.** *Artocarpus lakoocha* was collected in September 2002, from Nakhon Sawan Province, Thailand, and identified by Panarat Charoensai. A voucher specimen (no. BRU522) was deposited at the BIOTEC, Pathumthani, Thailand.

**Extraction and Isolation.** Air-dried ground roots of *A. lakoocha* (3 kg) were macerated in  $\text{CH}_2\text{Cl}_2$  (10 L) for 48 h. The extract was filtered and evaporated to yield 30 g of a crude extract. The  $\text{CH}_2\text{Cl}_2$  extract was partially purified by Sephadex LH-20 column chromatography (MeOH as eluent), from which 15 fractions (80 mL each) were collected. Fractions 7 and 8 were combined and further purified by silica gel column chromatography (eluted with hexane–EtOAc, 80:20), yielding lakoochin A (**1**) (15 mg) and lakoochin B (**2**) (32 mg).

**Lakoochin A (1):** off-white semisolid; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 209 (4.76), 239 (4.35), and 295 (4.26) nm; IR (neat)  $\nu_{\text{max}}$  3329, 3007, 2965, 2929, 2856, 1625, 1586, 1489, 1461, 1438, 1321, 1115, 1033, 823  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; ESITOFMS  $m/z$  405.2032  $[\text{M} - \text{H}]^-$ , calcd for  $[\text{C}_{24}\text{H}_{32}\text{O}_6 - \text{H}]^-$ , 405.2066.

**Lakoochin B (2):** off-white semisolid; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 205 (4.49), 250 (3.77), and 296 (3.88) nm; IR (neat)  $\nu_{\text{max}}$  3427, 3009, 2972, 2917, 2857, 1625, 1602, 1489, 1440, 1304, 1145, 1112, 966, 824  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; ESITOFMS  $m/z$  445.2375  $[\text{M} - \text{H}]^-$ , calcd for  $[\text{C}_{29}\text{H}_{34}\text{O}_4 - \text{H}]^-$ , 445.2379.

**Bioassays.** Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).<sup>9</sup> The mycobacterium *M. tuberculosis* H37Ra was cultured in Middle-brook 7H9 broth. The standard drugs, isoniazid and kanamycin sulfate, used as reference compounds for the antimycobacterial assay, showed MIC values of 0.040–0.090 and 2.0–5.0  $\mu\text{g}/\text{mL}$ , respectively. The MIC values of the reference compounds were determined in the same experiment as experimental samples. Cytotoxicity was determined by employing the colorimetric method described by Skehan and co-workers.<sup>10</sup> The reference compound, ellipticine, exhibited activity toward the Vero, KB, and BC cell lines with  $\text{IC}_{50}$  ranges of 0.2–0.3  $\mu\text{g}/\text{mL}$ .

**Acknowledgment.** Financial support from the Biodiversity Research and Training Program (BRT) to S.R. and from The Thailand Research Fund (TRF) to P.K. is gratefully acknowledged. A.P. acknowledges Postgraduate Education and Research in Chemistry (PERCH) for facilities at Mahidol University. Y.T. thanks the National Center for Genetic Engineering and Biotechnology for the Senior Research Fellowship Award.

**Supporting Information Available:**  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT135,  $^1\text{H}$ – $^1\text{H}$  COSY, NOESY, HMQC, and HMBC NMR spectral data of lakoochins A (**1**) and B (**2**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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