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

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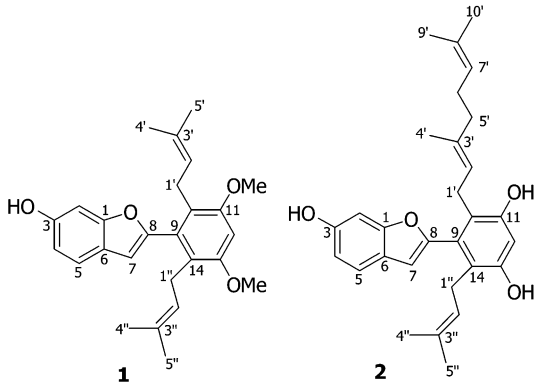
Two new stilbene derivatives, lakoochins A (**1**) and B (**2**), were isolated from the roots of *Artocarpus lakoocha*. The structures of **1** and **2** were elucidated by analysis of their spectral data. Lakoochins A (**1**) and B (**2**) exhibited antimycobacterial activity with the respective MIC values of 12.5 and 50 μg/mL. While **1** was cytotoxic against the BC (breast cancer) cell line (IC_{50} 6.1 μg/mL) but inactive (at 20 μg/mL) toward KB (nasopharyngeal carcinoma) cells, compound **2** possessed cytotoxicity against the BC and KB cell lines with IC_{50} values of 3.1 and 6.1 μg/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.[1] Apart from this stilbene, lectins and a flavonol glycoside have been found as constituents of this plant.[2−5] Although *A. lakoocha* was recently proved scientifically to be effective for the treatment of taeniasis,[6,7] few reports on the biological activities of metabolites from the plant have been recorded to date.[1−7] Upon reinvestigation, we noted that a crude extract of *A. lakoocha* roots exhibited antimycobacterial activity with a minimum inhibitory concentration (MIC) of 50 μg/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_2Cl_2 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 $C_{26}H_{30}O_4$ was deduced from the ESITOFMS. The ^{1}H NMR spectrum of **1** showed an ABX aromatic spin system (δ_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 (δ_H 6.54 and 6.61), two methyl ether singlets (both resonances at δ_H 3.87), and two sets of signals for a prenyl group (both at δ_H 3.15, d, J = 6.8 Hz, 2 × 2H; 5.05, br t, J = 7.1 Hz, 2 × 1H; 1.39, s, 2 × 3H; and 1.58, s, 2 × 3H). Analysis of the ^{13}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 (δ_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, ^{1}H−^{1}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 $C_{29}H_{34}O_4$ by ESITOFMS. The ^{1}H and ^{13}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 ^{1}H NMR spectrum of **2**. Additional prenyl signals were also observed in the ^{1}H and ^{13}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 ^{1}H−^{1}H COSY

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectral Data ($CDCl_3$) of Lakoochins A (**1**) and B (**2**)

	lakoochin A (**1**)		lakoochin B (**2**)	
position	δ_C, mult.	δ_H, mult., *J* in Hz	δ_C, mult.	δ_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[a]		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[a]		153.1, s[b]	
9	131.8, s		131.4, s	
10	122.5, s		120.1, s[c]	
11	156.3, s		153.8, s[b]	
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[c]	
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[d]	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[e]	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[d]	5.21, br t, 6.5
3″	130.3, s		134.2, s	
4″	17.6, q	1.39, s	17.7, q[e]	1.64, s
5″	25.7, q	1.58, s	25.7, q	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		

[a–e] 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}H−^{1}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 (δ_C 120.1) and C-14 (δ_C 120.2) and those of C-11 (δ_C 153.8) and C-13 (δ_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}H−^{1}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 μg/mL. While **1** was cytotoxic against the BC (breast cancer) cell line (IC_{50} 6.1 μg/mL) but inactive (at 20 μg/mL) toward KB (nasopharyngeal carcinoma) cells, compound **2** possessed cytotoxicity against the BC and KB cell lines with IC_{50} values of 3.1 and 6.1 μg/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}H, ^{13}C, DEPT, ^{1}H−^{1}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.[8]

Plant Material. *Artocarpus lakoocha* was collected in September 2002, from Nakhon Sawan Province, Thailand, and identified by Panarat Charoenchai. 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 CH_2Cl_2 (10 L) for 48 h. The extract was filtered and evaporated to yield 30 g of a crude extract. The CH_2Cl_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) λ_{max} (log ϵ) 209 (4.76), 239 (4.35), and 295 (4.26) nm; IR (neat) ν_{max} 3329, 3007, 2965, 2929, 2856, 1625, 1586, 1489, 1461, 1438, 1321, 1115, 1033, 823 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESITOFMS *m/z* 405.2032 $[M - H]^-$, calcd for $[C_{24}H_{32}O_6 - H]^-$, 405.2066.

Lakoochin B (2): off-white semisolid; UV (MeOH) λ_{max} (log ϵ) 205 (4.49), 250 (3.77), and 296 (3.88) nm; IR (neat) ν_{max} 3427, 3009, 2972, 2917, 2857, 1625, 1602, 1489, 1440, 1304, 1145, 1112, 966, 824 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESITOFMS *m/z* 445.2375 $[M - H]^-$, calcd for $[C_{29}H_{34}O_4 - H]^-$, 445.2379.

Bioassays. Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[9] 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 μg/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.[10] The reference compound, ellipticine, exhibited activity toward the Vero, KB, and BC cell lines with IC_{50} ranges of 0.2−0.3 μg/mL.

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Supporting Information Available: ^{1}H, ^{13}C, DEPT135, ^{1}H−^{1}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.

References and Notes

(1) Mongolsuk, S.; Robertson, A.; Towers, R. *J. Chem. Soc.* **1957**, 2231−2233.
(2) Ratanabanangkoon, K.; Poopyruchpong, N.; Charoensiri, K. *J. Sci. Soc. Thailand* **1976**, *2*, 202−205.
(3) Chauhan, J. S.; Kumari, G. *Planta Med.* **1979**, *37*, 86−88.
(4) Wongkham, S.; Wongkham, C.; Boonsiri, P.; Simasathiansophon, S.; Trisonthi, C.; Atisook, K. *Phytochemistry* **1995**, *40*, 1331−1334.
(5) Chatterjee, B. P.; Ahmed, H.; Chowdhury, S. *Carbohydr. Res.* **1988**, *180*, 97−110.
(6) Charoenlarp, P.; Radomyos, P.; Harinasuta, T. *Southeast Asian J. Trop. Med. Public Health* **1981**, *12*, 568−570.
(7) Charoenlarp, P.; Radomyos, P.; Bunnag, D. *J. Med. Assoc. Thai* **1989**, *72*, 71−73.
(8) Eckers, C.; Wolff, J. C.; Haskins, N. J.; Sage, A. B.; Giles, K.; Bateman, R. *Anal. Chem.* **2000**, *72*, 3683−3688.
(9) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.
(10) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107−1112.

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