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FOUR STILBENES FROM *SALACIA LEHMBACHII*

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Key Word Index—*Salacia lehmbachii*; Celastraceae; bark; stilbenes; stilbene dimer; lehmbachol.

Abstract—New stilbene derivatives designated lehmbachols A–D were isolated from the bark of *Salacia lehmbachii*, collected in Papua New Guinea. Their structures were established from spectroscopic evidence as 1-[1-methoxy-1-(3-methoxy-4-hydroxyphenyl)methyl]-2-(3,5-dimethoxyphenyl)-3-(3-methoxy-4-hydroxyphenyl)-4,6-dihydroxy-2,3-dihydro-1H-indene and its diastereoisomer, 1-[1-ethoxy-1-(3-methoxy-4-hydroxyphenyl)methyl]-2-(3,5-dimethoxyphenyl)-3-(3-methoxy-4-hydroxyphenyl)-4,6-dihydroxy-2,3-dihydro-1H-indene and 2-(3-methoxy-4-hydroxyphenyl)-5,7-dihydroxy-8-(3,5-dimethoxy-4-hydroxyphenyl)-2,3,9,10-tetrahydro-8H-indeno [1,2-c] furan, respectively.

INTRODUCTION

In previous papers [1–4], we reported on the structural elucidation of new sesquiterpenes with intersecting bioactivity, isolated from *Tripterygium wilfordii* var. *regelii* (Celastraceae). Continuing our chemical study on the Celastraceae, we have now carried out investigations into the constituents of *Salacia lehmbachii* collected in Papua New Guinea. It has been reported that some *Salacia* species have been used in native medicine, e.g. *S. prinoides* is used to treat diabetes in Western Ghats of India [5]. However, there are few publications about *Salacia* [6, 7] and no reports on *S. lehmbachii* have previously been published. In this paper, we report the structural elucidation of four new stilbene derivatives, lehmbachols A (**1**), B (**2**), C (**3**) and D (**4**) obtained from the plant, along with five known compounds.

RESULTS AND DISCUSSION

An ethanol extract (66.6 g) of the bark *S. lehmbachii* was partitioned between ethyl acetate and water, and the ethylacetate soluble part (13.0 g) was separated and purified by column chromatography to obtain each component. Nine compounds (**1**–**9**) were isolated from the bark.

Lehmbachol A (**1**) has the molecular formula $C_{31}H_{30}O_9$ by positive FAB-mass spectrometry. Its IR spectrum showed the presence of hydroxyl (3403 cm^{-1}) and aromatic groups (1606 cm^{-1} and 1515 cm^{-1}). The ^1H NMR spectrum of compound **1** (Table 1) showed 11 protons belonging to aromatic rings and three methoxyl groups, including two (δ 3.72 and 3.61) bonded to aromatic rings, and four protons at high field (δ 2.71, 3.32, 3.93 and 4.16). Three methoxyl carbons, four methine carbons (δ 47.4, 60.6, 60.8 and 90.3) and 24 aromatic carbons, which were assignable to four benzene rings, were also seen in the ^{13}C NMR spectrum (Table 2). From the coupling pattern and integration in the ^1H NMR spectrum, four aromatic rings (A, B, C and D) were recognized. Ring C has symmetrical 1,3,5-substitution because it showed an A_2X pattern (δ 5.37 (2H, *d*, J = 2.1 Hz) and 6.00 (1H, *d*, J = 2.1 Hz)). All of the other rings are unsymmetrical, i.e., ring A has 1,2,3,5-substitution and both rings B and D have 1,3,4-substitution. The partial structure —CH—CH—CH—CH— was suggested from the ^1H–^1H COSY spectrum of compound **1**.

In the HMBC spectrum of compound **1**, the proton signal at H-7 (δ 2.71) was correlated with the carbon signal C-1″ (δ_C 152.1; ring C), the proton signal at H-10 (δ 3.93) with the carbon signals C-2′ (δ_C 113.3) and C-6′ (δ_C 122.8; ring B), the proton signal H-8 (δ 4.16) with the carbon signals C-1‴ (δ_C 139.7; ring D) and C-9 (δ_C 123.7; ring A). From these data the connections between C-7 and ring C, C-10 and ring B, and C-8

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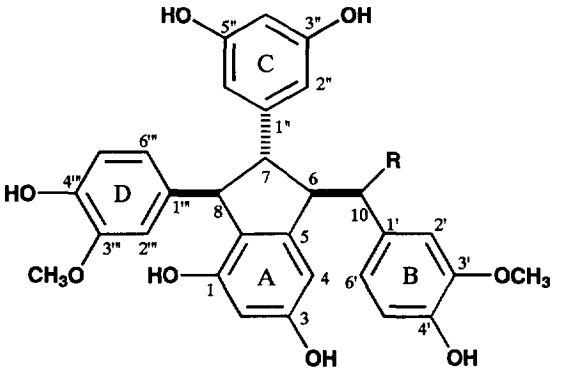

1, 2: R= OCH_3
3 : R= OCH_2CH_3

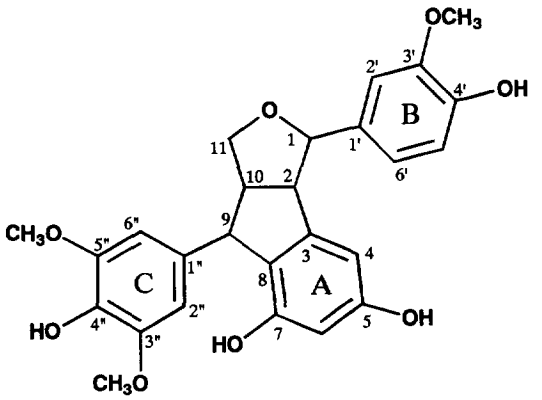

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Table 1. ^1H NMR data for compounds **1**–**3** (δ in methanol-d_4)

	1	**2**	**3**
2	6.25, 1H, *d*, *J* = 2.1 Hz	6.14, 1H, *d*, *J* = 2.0 Hz	6.25, 1H, *d*, *J* = 2.1 Hz
4	6.67, 1H, *d*, *J* = 2.1 Hz	6.11, 1H, *d*, *J* = 2.0 Hz	6.69, 1H, *d*, *J* = 2.1 Hz
6	3.32, 1H, *dd*, *J* = 4.0, 8.0 Hz	3.52, 1H, *dd*, *J* = 5.2, 7.0 Hz	3.30, 1H, *dd*, *J* = 2.9, 9.3 Hz
7	2.71, 1H, *dd*, *J* = 4.0, 4.0 Hz	3.16, 1H, *dd*, *J* = 4.9, 5.2 Hz	2.71, 1H, *dd*, *J* = 2.9, 3.1 Hz
8	4.16, 1H, *d*, *J* = 4.0 Hz	4.13, 1H, *d*, *J* = 4.9 Hz	4.18, 1H, *d*, *J* = 2.9 Hz
10	3.93, 1H, *d*, *J* = 8.0 Hz	4.00, 1H, *d*, *J* = 7.0 Hz	4.00, 1H, *d*, *J* = 9.3 Hz
2′	6.32, 1H, *d*, *J* = 1.6 Hz	6.81, 1H, *d*, *J* = 1.6 Hz	6.31, 1H, *d*, *J* = 1.7 Hz
5′	6.65, 1H, *d*, *J* = 8.0 Hz	6.69, 1H, *d*, *J* = 8.0 Hz	6.63, 1H, *d*, *J* = 7.8 Hz
6′	6.38, 1H, *dd*, *J* = 1.6, 8.0 Hz	6.58, 1H, *dd*, *J* = 1.6, 8.0 Hz	6.38, 1H, *dd*, *J* = 1.7, 7.8 Hz
2″, 6″	5.73, 2H, *d*, *J* = 2.1 Hz	*ca* 4.8, 2H, *m*	5.73, 2H, *d*, *J* = 2.1 Hz
4″	6.00, 1H, *d*, *J* = 2.1 Hz	*ca* 4.8, 1H, *m*	5.99, 1H, *d*, *J* = 2.1 Hz
2‴	6.51, 1H, *d*, *J* = 1.9 Hz	6.38, 1H, *d*, *J* = 1.7 Hz	6.53, 1H, *d*, *J* = 1.8 Hz
5‴	6.68, 1H, *d*, *J* = 8.2 Hz	6.61, 1H, *d*, *J* = 8.0 Hz	6.69, 1H, *d*, *J* = 7.9 Hz
6‴	6.39, 1H, *dd*, *J* = 1.9, 8.2 Hz	6.33, 1H, *dd*, *J* = 1.7, 8.0 Hz	6.41, 1H, *dd*, *J* = 1.8, 7.9 Hz
10-OMe	3.09, 3H, *s*	3.06, 3H, *s*	–
10-OEt	–	–	*ca* 3.25, 1H, *m*
	–	–	3.18, 1H, *m*
	–	–	1.11, 3H, *t*, *J* = 6.9 Hz
3′-OMe	3.61, 3H, *s*	3.61, 3H, *s*	3.61, 3H, *s*
3‴-OMe	3.72, 3H, *s*	3.70, 3H, *s*	3.72, 3H, *s*

and rings D and A, were determined. Because clear long-range correlations between H-8 and C-5 (δ_C 150.7), H-6 (δ 3.32) and C-9 were observed, a cyclopentane ring constructed by C-5, 6, 7, 8 and 9 could be elucidated. The bonding positions of methoxyl and hydroxyl groups on the aromatic rings were elucidated by HMBC and NOESY spectra. The methoxyl group at high-field (δ 3.09) gave a long-range correlation with C-10. Consequently, all carbons and protons were assigned as shown in Tables 1 and 2; thus, the planar structure of lehmbachol A was determined as shown in formula **1**.

The relative structure of compound **1** was revealed by NOESY experiment in the following manner.

(1) H-6, H-7 and H-8 are not diaxial, since correlations between H-6 and H-7, H-7 and H-8 were observed. Thus, the possible orientations of H-6, 7 and 8 are four, namely, axial (ax)–equatorial (eq)–ax, ax–eq–eq, eq–eq–ax, eq–ax–eq and eq–eq–eq.

(2) Correlations are observed between H-2″ (H-6″) and H-6, H-7, H-8; two configurations, **1a** and **1b** (Fig. 1), are possible.

(3) H-2‴ (H-6‴) showed correlations with H-7 and H-8. As a result, the partial structure was determined to be **1b** and the relative configuration, except for C-10, was thus proposed for lehmbachol A.

Lehmbachol B (**2**) exhibited a $[M-H]^-$ at *m/z* 545 ($[M-H]^-$) in the negative ion FAB-mass-spectrum and the molecular formula $C_{31}H_{30}O_9$ was proposed. The ^{13}C NMR data of compound **2** (Table 2) were

Table 2. ^{13}C NMR data for compounds **1–3** (δ in methanol-d_4)

	1	2	3
1	156.3	156.3	156.3
2	103.6	103.5	103.5
3	160.1	159.6	160.0
4	107.1	107.0	107.2
5	150.7	148.2	150.9
6	60.6	61.5	61.8
7	60.8	60.9	60.8
8	57.4	58.0	57.2
9	123.7	124.8	123.7
10	90.3	88.9	88.3
1′	133.8	133.1	134.5
2′	113.3	113.5	113.2
3′	149.6	149.7	149.5
4′	148.0	148.0	147.8
5′	116.5	116.3	116.5
6′	122.8	124.0	122.6
1″	152.1	151.2	152.3
2″	107.2	107.8	107.1
3″	160.1	160.3	160.0
4″	102.1	102.3	102.0
5″	160.1	160.3	160.0
6″	107.2	107.8	107.1
1‴	139.7	139.8	139.7
2‴	113.4	112.7	113.3
3‴	149.6	149.6	149.6
4‴	146.5	146.3	146.5
5‴	116.7	116.5	116.7
6‴	121.8	122.1	121.8
10-OMe	57.7	57.7	–
10-OEt	–	–	65.9
	–	–	16.4
3′-OMe	57.3	57.2	57.0
3‴-OMe	57.1	57.2	57.2

very similar to those of **1**, suggesting that it has the same planar structure as compound **1**. In the NOESY spectrum of compound **2**, the nOe correlations for the cyclopentane ring were the same as lehmbachol A (**1**). Therefore, we suggested that compound **2** is a diastereomer with a different orientation at C-10.

The negative-ion FAB-mass spectrum of lehmbachol C (**3**) gave the molecular formula $C_{32}H_{32}O_9$. The IR and NMR spectra indicated the existence of hydroxyl groups and four aromatic rings. The ^{13}C NMR spectrum of compound **3** (Table 2) is very similar to that at lehmbachol A (**1**) except for the number of methoxyl and ethoxyl groups (**1**, $CH_3O \times 3$; **3**, $CH_3O \times 2$, $CH_3CH_2O \times 1$). Furthermore, we investigated the NMR spectra, including two-dimensional NMR spectra (COSY, HMQC, HMBC), of compound **3**, which disclosed that the skeleton is the same as compound **1** and that an ethoxyl group instead of a methoxyl group is at the C-10 position. It is possible that lehmbachol C (**3**) is an artefact.

Lehmbachol D (**4**) showed $[M]^+$ at m/z 466 and the molecular formula $C_{26}H_{26}O_8$ in the high-resolution EI-mass spectrum. Its NMR spectrum indicated the presence of three aromatic rings (A, B and C) and three methoxyl groups. Ring C had a symmetrical four-substitution pattern (δ 6.32 (2H, *s*)) ring A an asymmetrical 1,2,3,5-substitution (δ 6.25 (*d*, $J = 1.6$ Hz) and 6.19 (*d*, $J = 1.6$ Hz)) and ring B a 1,3,4-substitution (δ 6.97 (*d*, $J = 1.8$ Hz), 6.87 (*dd*, $J = 1.8$, 8.1 Hz) and 6.80 (*d*, $J = 8.1$ Hz)), respectively.

In the ^1H NMR spectrum (Table 1), one methoxyl signal (δ 3.87) bonded to ring A or B, and two methoxyl signals (δ 3.73) bonded to ring C, were observed. All proton signals in the high field were assigned from a COSY spectrum. In the HMBC spectrum, it was confirmed that ring B was connected to C-1 and that ring C was connected to C-9. Because long-range correlations from the proton signals H-9, H-2 to the carbon signals C-8 (δ_C 123.9), C-3 (149.4) were detected, a cyclopentane ring moiety constructed by C-2 (δ_C 60.8), C-3, C-8, C-9 (53.0) and C-10 (57.5) was proposed. The protons and carbons at position 11 (δ 4.49, 3.56; δ_C 75.9) and position 1 (4.69; 90.3) had only long-range correlations with each other, and so a tetrahydrofuran ring comprising C-1, C-2, C-10 (δ_C 57.5) and C-11 was suggested. Consequently, a fusion ring by an aromatic ring (ring A) and a tetrahydrofuran ring was elucidated. The connecting positions of the methoxyl and hydroxyl groups were determined by long-range correlations. Consequently, all protons and carbons were finally assigned and the planar structure for lehmbachol D was determined as shown in formula **4**.

Compounds **5–8** were shown to be aromatic compounds from their NMR spectral data. They were the known stilbene derivatives, resveratol, isorhapontigenin [8], gnetol [9] and gnetin D [10]; they were identified by comparison with authentic samples and/or literature data. Compound **9** was identified as lariciresinol [11], previously isolated from *Araucaria angustifolia*.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Other spectroscopic procedures were the same as described in ref. [1].

Plant material. The bark of *S. lehmbachii* was obtained from the National Park, Central Province of Papua New Guinea.

Extraction and fractionation. The EtOH extract from 1.2 kg of air-dried material (66.6 g) was partitioned between EtOAc and H_2O to obtain the EtOAc-sol. part (13 g). This was loaded onto a silica gel column and eluted with *n*-hexane-EtOAc to obtain three frs, 1 (1.2 g), 2 (61 mg) and 3 (1.3 g). Fr. 1 was fractionated by silica gel CC to obtain resveratol (113 mg) and isorhapontin (467 mg). Fr. 2 was purified in

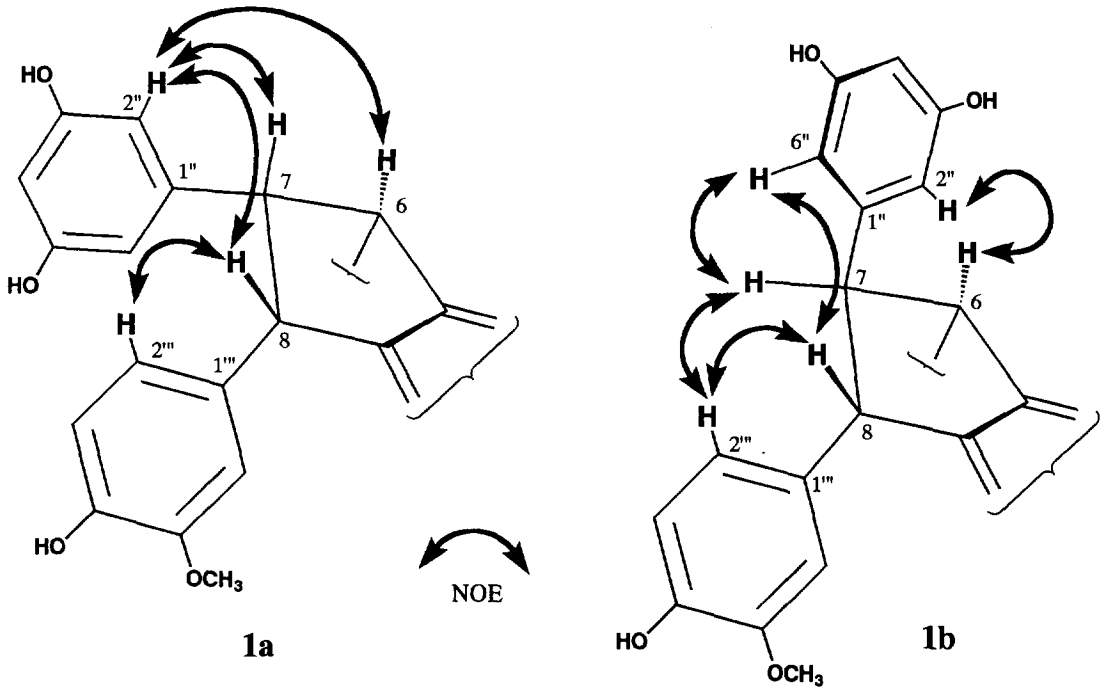

Fig. 1. Possible configurations of compound **1**.

the same manner to yield gnetol. Fr. 3 was fractionated by CC on silica gel and/or Sephadex LH-20, repeatedly, to obtain lehmbachol A (**1**, 15 mg), lehmbachol B (**2**, 4 mg), lehmbachol C (**3**, 5 mg), lehmbachol D (**4**, 14 mg), gnetin D (40 mg) and lariciresinol (9 mg).

Lehmbachol A (**1**). Amorphous yellowish powder. $[\alpha]_D^{25}$ −8.6° (MeOH, *c* 0.99). HR pos. FAB-MS: *m/z* 547.1933 $[M+H]^+$, calcd for $C_{31}H_{30}O_9$, 547.1968. IR ν_{max}^{KBr} cm^{-1}: 3403, 2926, 1606, 1515, 1273. UV λ_{max} nm (log ε): 281 (4.0). ^1H and ^{13}C NMR: Tables 1 and 2, respectively.

Lehmbachol B (**2**). Amorphous yellowish powder. $[\alpha]_D^{25}$ −4.2° (MeOH, *c* 0.96). HR neg. FAB-MS: *m/z* 545.1774 $[M-H]^-$, calcd for $C_{31}H_{30}O_9$, 545.1811. IR ν_{max}^{KBr} cm^{-1}: 3392, 2935, 1605, 1515, 1274. UV λ_{max} nm (log ε): 281 (3.9). ^1H and ^{13}C NMR: Tables 1 and 2, respectively.

Lehmbachol C (**3**). Amorphous yellowish powder. $[\alpha]_D^{25}$ −7.4° (MeOH, *c* 0.27). HR neg. FAB-MS: *m/z* 559.2003 $[M-H]^-$, calcd for $C_{32}H_{32}O_9$, 559.1941. IR ν_{max}^{KBr} cm^{-1}: 3400, 2935, 1605, 1515, 1273. UV λ_{max} nm (log ε): 281 (3.9). ^1H and ^{13}C NMR: Tables 1 and 2, respectively.

Lehmbachol D (**4**). Amorphous yellow powder. $[\alpha]_D^{25}$ +25.1° (MeOH, *c* 0.86). HR EI-MS: *m/z* 466.1633 $[M]^+$, calcd for $C_{26}H_{26}O_8$, 466.1627. IR ν_{max}^{KBr} cm^{-1}: 3402, 2938, 1614, 1517, 1462, 1274, 1216. UV λ_{max} nm (log ε): 315 (3.2), 281 (3.8). ^1H NMR (MeOH-d_4): δ 6.97 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.87 (1H, *dd*, *J* = 1.8, 8.1 Hz, H-6′), 6.80 (1H, *d*, *J* = 8.1 Hz, H-5′), 6.32 (2H, *s*, H-2, 6″), 6.25 (1H, *d*, *J* = 1.6 Hz, H-4), 6.19 (1H, *d*, *J* = 1.6 Hz, H-6), 4.69 (1H, *d*, *J* = 4.7 Hz, H-1), 4.49 (1H, *dd*, *J* = 8.4, 8.4 Hz, H-11 α), 4.14 (1H, *br s*, H-9), 3.87 (3H, *s*, 3′-OCH_3), 3.77 (1H, *dd*, *J* = 4.7, 9.0 Hz, H-2), 3.73 (6H, *s*, 3″, 5″-OCH_3), 3.56 (1H, *dd*, *J* = 8.4, 8.4 Hz, H-11β), 3.05 (1H, *ddd*, *J* = 1.6, 8.4, 8.4 Hz, H-10). ^{13}C NMR (MeOH-d_4): δ: 161.0 (*s*, C-5), 157.2 (*s*, C-7), 150.0 (3C, each *s*, C-3′, 3″, 5″), 149.4 (*s*, C-3), 148.1 (*s*, C-4′), 138.9 (*s*, C-1″), 136.0 (*s*, C-1′), 135.6 (*s*, C-4″), 123.9 (*s*, C-8), 121.1 (*d*, C-6′), 117.0 (*d*, C-5′), 111.9 (*d*, C-2′), 106.5 (2C, *d*, C-2″, 6″), 104.2 (*d*, C-4), 103.8 (*d*, C-6), 90.3 (*d*, C-1), 75.9 (*t*, C-11), 60.8 (*d*, C-2), 57.6 (2C, *q*, 3″, 5″-CH_3), 57.5 (*q*, C-10), 57.4 (*q*, 3′-CH_3), 53.0 (*d*, C-9).

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