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DIARYLDIMETHYLBUTANE LIGNANS FROM *MYRISTICA ARGENTEA* AND THEIR ANTIMICROBIAL ACTION AGAINST *STREPTOCOCCUS MUTANS**

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Key Word Index—*Myristica argentea*; Myristaceae; mace; lignans; 1,4-diaryl-2,3-dimethylbutanes; antibacterial activity; dental caries prevention.

Abstract—Four 1,4-diaryl-2,3-dimethylbutane type lignans were isolated from the aril of *Myristica argentea*. In addition to two known lignans, namely *erythro*-austrobailignan-6 and *meso*-dihydroguaiaretic acid, two new compounds were determined to be *rel*-1-(4-hydroxy-3-methoxyphenyl)-4-(3,4-methylenedioxyphenyl)-2,3-dimethylbutan-1-ol (myristargenol A) and *rel*-1,4-di(4-hydroxy-3-methoxyphenyl)-2,3-dimethylbutan-1-ol (myristargenol B) by chemical and spectroscopic methods.

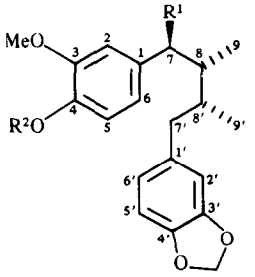
INTRODUCTION

The aril of *Myristica argentea* Warb., (Papuan mace) is used as a spice and a traditional folk medicine in South-East Asia. Although the constituents of the common mace (*M. fragrans* Houtt.) have been investigated [2–7], few chemical and biological studies on *M. argentea* have been carried out so far. This paper describes the isolation and structural elucidation of four lignans from the aril of *M. argentea* and their antibacterial effect against a cariogenic bacteria, *Streptococcus mutans*.

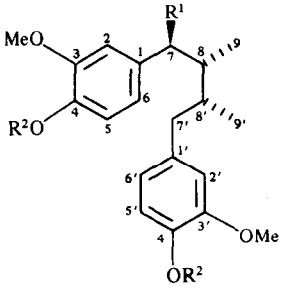
RESULTS AND DISCUSSION

The dichloromethane-soluble portion of the 50% ethanol extract of the aril of *M. argentea* was separated by silica gel CC to afford four 1,4-diaryl-2,3-dimethylbutane lignans (**1**–**4**). Compound **1**, $C_{20}H_{24}O_4$ (M^+, *m/z* 328.1675), was obtained as colourless needles. The assignment of all protons and carbons was achieved by double resonance and C-H 2D COSY techniques. The structure of **1** was determined to be *erythro*-1-(4-hydroxy-3-methoxyphenyl)-4-(3,4-methylenedioxyphenyl)-2,3-dimethylbutane on the basis of spectral data, optical rotation [8,9] and the physical data of its methyl (**1a**, M^+, 342), acetyl (**1b**, M^+, 370) and 3,5-dinitrobenzoyl (**1c**, M^+, 494) derivatives.

The second major compound (**2**, $C_{20}H_{26}O_4$) was identified as *erythro*-1,4-di(4-hydroxy-3-methoxyphenyl)-2,3-dimethylbutane (*meso*-dihydroguaiaretic acid) on the basis of its spectral data. [10–13].



1 R^1 = H, R^2 = H
1a R^1 = H, R^2 = Me
1b R^1 = H, R^2 = Ac
1c R^1 = H, R^2 = 3,5-diNO_2PhCO
3 R^1 = OH, R^2 = H
3a R^1 = OAc, R^2 = Ac



2 R^1 = H, R^2 = H
4 R^1 = OH, R^2 = H

*Part 2 in the series 'Lignans of Papuan Mace (*Myristica argentea* Warb.)'. For Part 1 see ref. [1].

Myristargenol A (**3**) was isolated as colourless needles. The presence of hydroxyl groups was indicated by the presence of IR absorption bands at 3450 and 3150 cm^{-1}. The EIMS showed a molecular peak at *m/z* 344, accompanied by characteristic peaks at *m/z* 326 $[M-H_2O]^+$, 137 $[CH_2C_6H_3(OH)(OMe)]^+$ and 135 $[CH_2C_6H_3(OCH_2O)]^+$. The base peak at *m/z* 153 suggested the presence of vanillyl group bearing an α-hydroxyl group. The ^{13}C NMR spectrum of **3** was similar to that of lignan **1**, except for the changes in the signals of C-7 (δ77.13) and C-9 (δ11.43) due to the presence of a hydroxyl group on C-7 and its γ-effect to the C-9 methyl group, respectively (Table 1). In the ^1H NMR spectrum of **3**, the differences of the chemical shifts compared to those of **1** were mainly observed for the protons of the vanillyl moiety. A large lower field shift was observed for H-7 (δ4.42) and slightly smaller shifts for H-2 and H-6. A signal at δ2.33 which was shifted from δ1.73 and was assigned to H-8′ established the intramolecular hydrogen bonding of a hydroxyl group (C-7, OH) with H-8′ (Table 2). To confirm the complete structure and relative stereochemistry, myristargenol A (**3**) was subjected to X-ray analysis after conversion to its diacetate (**3a**). The stereoscopic view of **3a** is shown as Fig. 1. Thus, the structure of **3** was established to be *rel*-1-(4-hydroxy-3-methoxyphenyl)-4-(3,4-methylenedioxyphenyl)-2,3-dimethylbutan-1-ol.

Compound **4**, named myristargenol B, was obtained as colourless crystals with the molecular formula $C_{20}H_{26}O_5$. The spectral data were very similar to those of lignans **2** and **3**, except for the differences caused by the presence of a hydroxyl group and one more methoxyl group instead of methylenedioxy group, respectively. The ^1H NMR spectrum showed signals for a benzylic methine substituted by oxygen at δ4.42 (*d*, *J* = 9.8Hz). Two methoxyl groups at δ3.90 and a pair of 1,3,4-trisubstituted aromatic protons revealed the presence of two 4-hydroxy-3-methoxyphenyl groups (Table 2). The ^{13}C NMR spectrum (Table 1) and the mass fragmentation pattern (*m/z* 346 $[M]^+$, 328 $[M-H_2O]^+$, 153 [CH(OH) C_6H_3(OH)(OMe)]$^+$, 137 [vanillyl moiety]$^+$) supported the structure of **4**. On the basis of these data, the

Table 1. ^{13}C NMR data of compounds **1**–**4** (100 MHz, $CDCl_3$, δ-values)

C	1	2	3	4
1	133.8	133.8	136.6	136.3
2	111.5	111.5	109.0	108.8
3	146.3	146.4	146.7	146.0
4	143.6	143.6	145.2	144.9
5	114.0	114.0	114.0	113.8
6	121.7	121.8	120.0	119.9
7	38.9	38.9	77.1	77.2
8	39.3	39.2	45.1	45.3
9	16.2	16.2	11.4	11.7
1′	135.7	133.8	136.1	133.9
2′	109.4	111.5	109.6	111.5
3′	147.5	146.4	147.5	146.4
4′	145.5	143.6	145.5	143.3
5′	107.9	114.0	108.0	113.8
6′	121.8	121.8	121.9	121.6
7′	39.1	38.9	37.2	37.3
8′	39.4	39.2	35.2	35.3
9′	16.1	16.2	17.8	18.2
O–Me	55.9	55.9	56.0	56.0
O–Me		55.9		56.1
O–CH_2–O	100.7		100.7	

Table 2. ^1H NMR spectral data of compounds **1**–**4** (400 MHz, $CDCl_3$ δ-values)

H	1	2	3	4
2	6.62 *d*	6.61 *d*	6.89 *d*	6.89 *d*
5	6.82 *d*	6.82 *d*	6.87 *d*	6.87 *d*
6	6.64 *dd*	6.65 *dd*	6.79 *dd*	6.80 *dd*
7a	2.25 *dd*	2.28 *dd*	4.42 *d*	4.43 *d*
7b	2.72 *dd*	2.73 *dd*		
8	1.73 *m*	1.75 *m*	1.85 *ddq*	1.86 *ddq*
9	0.84 *d*	0.84 *d*	0.61 *d*	0.62 *d*
2′	6.65 *d*	6.61 *d*	6.74 *d*	6.73 *d*
5′	6.72 *d*	6.82 *d*	6.73 *d*	6.83 *d*
6′	6.61 *dd*	6.65 *dd*	6.66 *dd*	6.71 *dd*
7′a	2.29 *dd*	2.28 *dd*	2.15 *dd*	2.16 *dd*
7′b	2.72 *dd*	2.73 *dd*	2.86 *dd*	2.88 *dd*
8′	1.73 *m*	1.75 *m*	2.33 *dddq*	2.37 *dddq*
9′	0.83 *d*	0.84 *d*	0.88 *d*	0.89 *d*
OMe	3.87 *s*	3.85 *s*	3.90 *s*	3.90 *s*
OMe		3.85 *s*		3.88 *s*
O-CH_2-O	5.92 *s*		5.92 *s*	
Ar-OH	5.45 *s*	5.46 *s*	5.62 *s*	5.61 *s*
Ar-OH		5.46 *s*		5.47 *s*

J (Hz) **1**: 2,6 = 1.7; 5,6 = 8.1; 7a, 7b = 13.6; 7a,8 = 9.3; 7b,8 = 5.1; 8,9 = 6.6; 2′,6′ = 1.7; 5′,6′ = 7.8; 7′a,7′b = 13.6; 7′a,8′ = 9.3; 7′b,8′ = 5.1; 8′,9′ = 6.8. **2**: 2,6 = 1.5; 5,6 = 8.1; 7a,7b = 13.5; 7a,8 = 9.3; 7b,8 = 5.1; 8,9 = 6.6. **3**: 2,6 = 1.7; 5,6 = 8.1; 7,8 = 9.8; 8,9 = 7.1; 8,8′ = 2.7; 2′,6′ = 1.5; 5′,6′ = 7.6; 7′a,7′b = 13.1; 7′a,8′ = 10.6; 7′b,8′ = 3.7; 8′,9′ = 6.8. **4**: 2,6 = 1.7; 5,6 = 8.1; 7,8 = 9.5; 8,9 = 6.7; 8,8′ = 2.9; 2′,6 = 1.7; 5′,6′ = 7.8; 7′a,7′b = 13.1; 7′a,8′ = 10.5; 7′b,8′ = 4.0; 8′,9′ = 7.1.

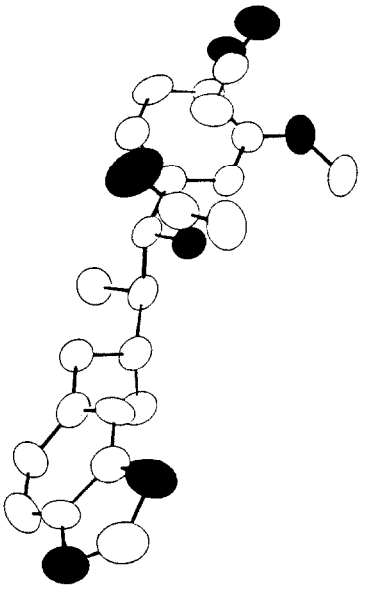


Fig. 1. X-ray stereoscopic view of diacetyl miristalgenol A (**3a**).

Table 3. Antimicrobial activity of lignans against *Streptococcus mutans*

Lignan	100	50	25	12.5 (ppm)
1	+	+	+	+
2	−	−	−	+
3	+	+	+	+
4	+	+	+	+

+, growth was observed.
−, growth was not observed (inhibited).

structure of **4** was concluded to be *rel*-1,4-di(4-hydroxy-3-methoxyphenyl)-2,3-dimethylbutan-1-ol. Only a few compounds related to the structure of **3** and **4** are known [14].

The antibacterial activity of the lignans were measured in Bacto brain heart infusion broth against *Streptococcus mutans*, which causes tooth caries. As shown in Table 3, lignan **2** showed potent antibacterial action with a minimal inhibitory concentration (MIC) of 25 ppm.

EXPERIMENTAL

Mps: uncorr; MS: direct insertion probe at 70 eV; NMR: $CDCl_3$, ^{13}C at 100.7 MHz, ^1H at 400.5 and 60 MHz.

Extraction and isolation. Powdered aril of *Myristica argentea* (550 g) was extracted (×3) with 50% EtOH (1.2 l) at room temp. After filtration and evapn of the solvent, the residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble layer (19 g) was fractionated to afford a weakly acidic fraction (13 g), which was subjected to CC on silica gel to give 19 fractions on elution with C_6H_6–Me_2CO.

1-(4-*hydroxy*-3-*methoxyphenyl*)-4-(3,4-*methylenedioxyphenyl*)-2,3-*Dimethylbutane* (**1**). Colourless needles (1 g), mp 68.5–69.0°; $[\alpha]_D^{24}$ + 4.8° ($CHCl_3$; *c* 1.0); UV λ_{max}^{EtOH} nm (log ε): 285 (3.85), 231 (4.03), 205 (4.63); IR ν_{max}^{film} cm^{-1}: 3500, 1609, 1240, 1036, 933; MS *m/z* (rel. int.): 328 $[M]^+$ (31), 179 (48), 137 (100), 135 (68).

Methyl ether of **1**. UV λ_{max}^{EtOH} nm (log ε): 284 (3.91), 231 (4.19), 205 (4.77); IR ν_{max}^{film} cm^{-1}: 1610, 1593, 1240, 1035, 933; ^1H NMR (60 MHz, $CDCl_3$): δ0.83 (6H, *d*, *J* = 6.6 Hz, H-9, H-9′), 1.45–1.95 (2H, *m*, H-8, H-8′), 2.02–2.93 (4H, *m*, H-7, H-7′), 3.86 (6H, *s*, -OMe), 5.91 (2H, *s*, $-OCH_2O-$), 6.45–6.90 (6H, *m*, H-Ar); MS *m/z* (rel. int.): 342 $[M]^+$, 206, 151 (100), 135.

meso-*Dihydroguaiaretic acid* (**2**). Mp 84.5–85.0°; $[\alpha]_D^{24}$ 0° ($CHCl_3$; *c* 1.0), UV λ_{max}^{EtOH} nm (log ε): 282 (3.95), 228 (4.29), 207 (4.65); IR ν_{max}^{Nujol} cm^{-1}: 3500, 1605, 1272, 1235, 1150, 1028; MS *m/z* (rel. int.): 330 $[M]^+$ (100), 206 (19), 194 (16), 137 (77).

Myristargenol A (**3**). Mp 133°; $[\alpha]_D^{24}$ + 9.9° (EtOH; *c* 0.81); UV λ_{max}^{MeOH} nm (log ε): 283 (3.49), 230 (3.73), 203 (4.44); IR ν_{max}^{film} cm^{-1}: 3450, 3150, 1600, 1250, 1045, 1030, 930; MS *m/z* (rel. int.): 344 $[M]^+$ (3), 326 (35), 239 (11), 191 (34), 164 (18), 162 (11), 159 (38), 153 (100), 137 (5), 135 (11).

Diacetyl myristargenol A (**3a**). Mp 137°; $[\alpha]_D^{24}$ + 23.3° $CHCl_3$; *c* 0.81). IR ν_{max}^{Nujol} cm^{-1}: 1760, 1740, 1600, 1240, 1195, 1035; ^1H NMR (60 MHz, $CDCl_3$): δ0.66 (3H, *d*, *J* = 7.0 Hz, H-9), 0.86 (3H, *d*, *J* = 7.0 Hz, H-9′), 1.7–2.3 (3H, *m*, H-7′a, H-8, H-8′), 2.07 (3H, *s*, -OAc), 2.29 (3H, *s*, -OAc), 2.80 (1H, *dd*, *J* = 4.0, 13.0 Hz, H-7′b), 5.66 (1H, *d*, *J* = 9.6 Hz, H-7), 5.91 (2H, *s*, $-OCH_2O-$), 6.4–7.0 (6H, *m*, H-Ar); MS *m/z* (rel. int.): 428 $[M]^+$ (30), 368 (11), 255 (34), 195 (42), 164 (55), 163 (44), 162 (100), 153 (54), 135 (64).

Myristargenol B (**4**). Mp 93°; $[\alpha]_D^{24}$ + 14.2° ($CHCl_3$; *c* 0.79); UV λ_{max}^{MeOH} nm (log ε): 281 (3.27), 228 (3.64), 203 (4.28); IR ν_{max}^{Nujol} cm^{-1}: 3400, 1610, 1265, 1230, 1150, 1120, 1035, 1020; MS *m/z* (rel. int.): 346 $[M]^+$ (4), 241 (4), 194 (4), 192 (3), 191 (6), 164 (23), 159 (12), 153 (100), 137 (33).

X-ray analysis of **3a**. $C_{24}H_{28}O_7$, orthorhombic, space group $P2_12_12_1$; *a* = 10.972 (6), *b* = 12.035 (6), *c* = 17.226 (5) Å, D_x = 1.25 g/cm³, *z* = 4 and μ (MoK_α) = 1.0 cm $^{-1}$. The cell dimensions and intensities were measured on a Syntex R3 four-circle diffractometer with graphite-monochromated MoK_α radiation with ω-scan mode within 2θ less than 45°. A total of 1707 independent reflections were collected, among which 1425 reflections ($I \geq 1.96\sigma(I)$) were stored as observed. The structure was solved by the direct method using MULTAN in the Syntex XTL program [15]. The refinement of atomic parameters was carried out by a block-diagonal least-squares method. Thermal parameters were refined anisotropically for all the non-hydrogen atoms and isotropically for the hydrogen atoms. The final R-value was 0.064.

Antimicrobial assay procedure. Each sample was dissolved in EtOH to a concentration of 0.5%. The soln (200, 100, 50, 25 μl) was dissolved in Bacto brain heart infusion agar soln (10 ml) in a petri dish. The final concentration of the lignans was 100, 50, 25 and 12.5 μg/ml, respectively. After the agar medium solidified, *Streptococcus mutans* RIMD3125001 was inoculated on to the plate. Antimicrobial activity was evaluated by measurement of the inhibition of growth after incubation for 18 hr at 37°.

REFERENCES

1. Nakatani, N. and Ikeda, K. (1987) *Chem. Express* **2**, 627.
2. Shulgin, A. T. (1963) *Nature* **197**, 379.
3. Shulgin, A. T. and Kerlinger, H. O. (1964) *Naturwissenschaften* **51**, 360.
4. Sammy, G. M. and Nawar, W. W. (1968) *Chem. Ind.* 1279.
5. Isogai, A., Murakoshi, S., Suzuki, A. and Tamura, S. (1973) *Agric. Biol. Chem.* **37**, 889.
6. Hattori, M., Hada, S., Watahiki, A., Ihara, H., Shu, Y-Z., Kakiuchi, N., Mizuno, T. and Namba, T. (1986) *Chem. Pharm. Bull.* **34**, 3885.
7. Hattori, M., Hada, S., Kawata, Y., Tezuka, Y., Kikuchi, T. and Namba, T. (1987) *Chem. Pharm. Bull.* **35**, 3315.
8. Woo, W. S., Shin, K. H., Wagner, H. and Lotter, H. (1987) *Phytochemistry* **26**, 1542.
9. Murphy, S. T., Ritchie, E. and Taylor, W. C. (1975) *Aust. J. Chem.* **28**, 81.
10. Schrecker, A. W. and Hartwell, J. L. (1955) *J. Am. Chem. Soc.* **77**, 432.
11. Schrecker, A. W. (1957) *J. Am. Chem. Soc.* **79**, 3823.
12. King, F. E. and Wilson, J. G. (1964) *J. Chem. Soc.* 4011.
13. Ikeya, Y., Taguchi, H., Yoshioka, I. and Kobayashi, H. (1979) *Chem. Pharm. Bull.* **27**, 1583.
14. Lopes, L. M. X., Yashida, M. and Gottlieb, O. R. (1984) *Phytochemistry* **23**, 2647.
15. Germain, G., Main, P. and Woolfson, M. M. (1971) *Acta. Crystallogr. Sect. A* **27**, 368.