Monoamine Oxidase Inhibitory Naphthoquinone and/or Naphthalene Dimers from Lemuni Hitam (*Diospyros* sp.), a Malaysian Herbal Medicine

Emi Okuyama,*[,a] Masayuki Homma,[a] Yoko Satoh,[a] Haruhiro Fujimoto,[a] Masami Ishibashi,[a] Mikio Yamazaki,[a] Motoyoshi Satake[b], and Azizi Bin Aiyub Ghazali[c]

Faculty of Pharmaceutical Sciences, Chiba University,[a] 1–33 Yayoi-cho, Inage-ku, Chiba 263–8522, Japan, National Institute of Health Sciences,[b] 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan, and Universiti Sains Malaysia,[c] 11800 Pulau Pinang, Malaysia. Received June 7, 1999; accepted July 15, 1999

From the extract of a Malaysian herbal medicine, Lemuni Hitam (*Diospyros* sp.), which exhibited monoamine oxidase (MAO) inhibition, three new naphthoquinone and/or naphthalene dimers (lemuninols A—C, 1—3) were isolated together with 4,6-dihydroxy-5-methoxy-2-methyl-naphthalene (8) and six known monomers (4—7, 9 and 10). The structures were determined by spectroscopic methods including 2D-NMR techniques. Among them, lemuninol A showed 45% inhibition of MAO (mouse liver) at 5.0×10^{-6} g/ml, and lemuninols B and C and a naphthoquinone (9) indicated weak activity. Some related quinones were also tested for their MAO inhibitory activities.

Key words *Diospyros* sp.; quinone; monoamine oxidase inhibition; Ebenaceae; Malaysian herbal medicine; Lemuni Hitam

A Malaysian herbal medicine, Lemuni Hitam, which is the black-colored heartwood of *Diospyros* species (Ebenaceae), is used as a tonic in a decoction, although it is said that taking it in large dosage causes toxicity. In the genus Diospyros, naphthalenes and naphthoquinones have been isolated from the fruit,[1,2] stem,[3] root,[4] heartwood,[5,6] *etc*., and recently some biological activities such as ichthyotoxicity, germination inhibition, antifungal activity,[2] platelet aggregation inhibition,[7] and antiprotozoal activity[8] were reported. This paper deals with the isolation and structures of three new naphthoquinone and/or naphthalene dimers (lemuninols A—C, **1**—**3**) together with 4,6-dihydroxy-5-methoxy-2-methyl-naphthalene (**8**) and six known monomers from Lemuni Hitam, and their monoamine oxidase (MAO) inhibitory activity. The MAO inhibition of some other quinones is also discussed.

Isolation and the Structures MAO inhibition is related to treatment of emotional disturbance such as anti-depression, and to neuroprotection including anti-Parkinson's disease.[9—12] The ethyl acetate and the methanol extracts of Lemuni Hitam exhibited MAO inhibitory activity (about 60%) at 2.5×10^{-4} g/ml. Both extracts were independently applied to silica gel chromatography, and the fractions showing a similar TLC pattern were combined together to give fr. 1-A to fr. 1-G. By further separation using Sephadex LH-20 and HPLC-ODS, compound **1** was obtained from fr. 1-E, and compounds **2** and **3** from fr. 1-D. These new compounds were named lemuninols A—C, respectively. Fractions 1-A and 1-B gave compounds **4**—**7** and **8**—**10**, respectively, by application of preparative TLC (silica gel) and HPLC-ODS.

Lemuninol A (**1**) is a dark-red powder which shows a $FeCl_3$ positive (reddish-purple) spot on TLC. The molecular formula of $C_{24}H_{20}O_6$ (mw 404) was determined by high-resolution (HR)-FAB-MS. The ^{1}H- and ^{13}C-NMR (in acetone-d_6) indicated 18 aromatic carbons with two methyl and two methoxy groups at δ 1.85/δ 13.8 and δ 2.22/δ 21.8, and at δ 3.91/δ 56.66 and δ 4.13/δ 56.70, respectively. Observation of two carbonyl carbons at δ 182.7 and δ 186.1 suggested the quinone moiety. In the ^{1}H-NMR, there are six aromatic protons; three multiplets at δ 7.49—7.52 and δ 7.76—7.80, two meta-coupled at δ 6.46 (dd, J=1.5, 0.5 Hz) and δ 6.70 (br s), and one isolated proton at δ 6.66 (s). Phenolic OHs were assigned at δ 8.33 and δ 9.20. Interpretation of these data and two dimentional (2D)-NMRs such as pulsed field gradient heteronuclear multiple quantum coherrence (PFG-HMQC) and PFG-heteronuclear multiple-bond correlation (HMBC) spectra together with the nuclear Overhauser effects (NOEs) of δ 4.13/δ 6.66 and 9.20, δ 3.91/δ 7.49—7.52 and δ 2.22/δ 6.70 revealed the presence of two components, 2,5-dihydroxy-4-methoxy-7-methyl-naphthalene and 5-methoxy-2-methyl-1,4-naphthoquinone (Fig. 2). These components were connected by the NOE observed between the methyl group at δ 1.85 in the quinone and the proton at δ 6.70 in the naphthalene.

Lemuninol B (**2**), a white powder showing a bluish-purple spot by the $FeCl_3$ reagent on TLC, has the molecular formula

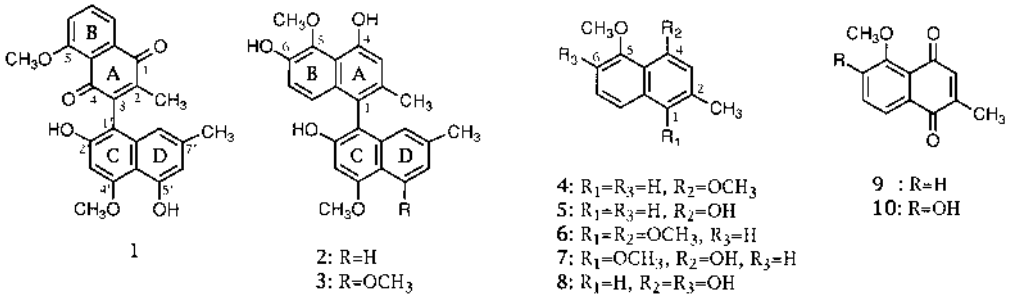

Fig. 1. Structures of the Isolated Compounds **1**—**10**

* To whom correspondence should be addressed.

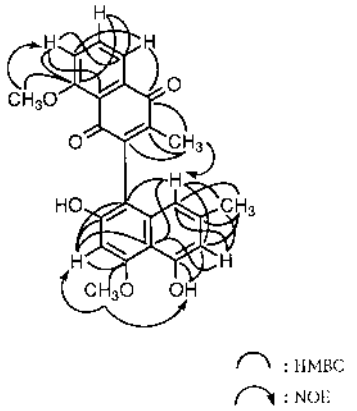

Fig. 2. HMBC and NOE Correlations of Lemuninol A (**1**)

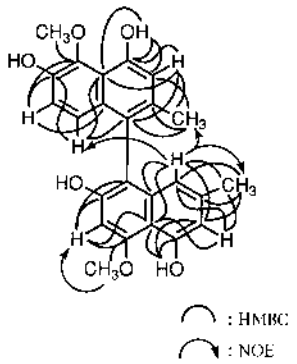

Fig. 3. HMBC and NOE Correlations of Lemuninol B (**2**)

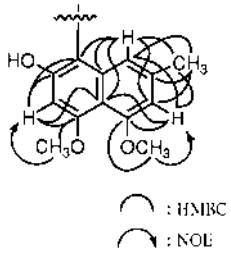

Fig. 4. HMBC and NOE Correlations of Lemuninol C (**3**)

of $C_{24}H_{22}O_6$ determined by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra suggested that **2** had the same C,D-ring as that of **1**, although a proton of 8′-H was high-field shifted to δ 6.17 (dd, J=1.5, 0.7 Hz). The quinone carbonyl carbons were not observed in the ^{13}C-NMR of **2**, but there were two additional aromatic carbons (Table 1). The ^{1}H-NMR signals according to the A, B-ring in **2** were assigned to three aromatic protons at δ 6.80 (s) and δ 6.81 and 6.96 (each d, J=9.0 Hz), and two hydroxy groups at δ 8.48 and 9.55 together with a methyl group and a methoxy group at δ 1.99 and δ 4.12, respectively, to characterize 4,6-dihydroxy-5-methoxy-2-methyl-naphthalene by 2D-NMR techniques (Fig. 3). The NOEs between δ 6.17 (8′-H) and δ 6.81 (8-H), 1.99 (2-CH_3) and 2.08 (7′-CH_3) confirmed the dimerized structure of **2**.

The spectral data of lemuninol C (**3**) are very similar to those of **2**, except that the molecular ion was 14 mass units higher (*m/z* 420) in the EI-MS spectrum. The ^{1}H- and ^{13}C-NMR indicated an additional methoxy group instead of a hydroxy group at δ 9.24 in **2**. The 2D-NMRs such as PFG-HMBC shown in Fig. 4 and NOE between H-8′ and H-8 revealed the structure of **3**.

Table 1. ^{13}C-NMR Data of Lemuninols A—C (**1**—**3**)

C-	**1**[a)]	**1**[b)]	**2**[a)]	**3**[a)]
1	186.1	185.6	122.8	123.4
2	145.6[c)]	146.1[d)]	136.0	136.0
3	145.4[c)]	144.8[d)]	113.8	113.8
4	182.7	185.1	153.73[e)]	153.6
5	160.6	159.7	141.4	141.4
6	119.1	117.6	145.2	145.1
7	135.1	134.9	120.0	119.9
8	119.2	119.3	123.9	124.0
9	135.7	134.6	131.9	132.0
10	121.7	120.3	118.2	118.2
1′	108.8	107.5	111.7	110.7
2′	152.9	151.6	153.74[e)]	154.1
3′	97.3	96.7	97.4	98.4
4′	158.6	157.5	158.0	159.3
5′	155.9	155.0	155.9	158.8
6′	110.4	110.2	110.3	106.5
7′	139.1	138.9	138.6	137.3
8′	115.3	114.2	115.7	117.1
9′	136.7	135.0	138.1	138.6
10′	109.3	108.6	109.6	112.2
2-CH_3	13.8	14.1	20.1	20.1
5-OCH_3	56.66	56.4	62.0	62.0
4′-OCH_3	56.70	55.6	56.7	56.2
5′-OCH_3				56.3
7′-CH_3	21.8	22.0	21.9	22.0

a) in acetone-d_6, *b*) in $CDCl_3$, *c*—*e*) interchangeable.

Compounds **4**—**8** and **9**, **10** were estimated to be naphthalenes and naphthoquinones, respectively, by ^{13}C-NMR, as shown in Table 2. Among them, compounds **5**, **7**, **8** and **10** showed reddish-purple color by $FeCl_3$ on TLC. The substituents and the positions in each compound were determined by ^{1}H- and ^{13}C-NMR including 2D-NMR techniques, and the compounds were finally identified to be 4,5-dimethoxy-2-methyl-, 4-hydroxy-5-methoxy-2-methyl-, 1,4,5-trimethoxy-2-methyl-, 4-hydroxy-1,5-dimethoxy-2-methyl- and 4,6-dihydroxy-5-methoxy-2-methyl-naphthalene for **4**—**8**, and 5-methoxy-2-methyl-, and 6-hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone for **9**, **10**, respectively (Fig. 1). To the best of our knowledge, **8** has not been reported as a natural product, and **6** is the first isolation from *Diospyros* sp.

MAO Inhibition of the Isolated Compounds and the Other Quinones A summary of the MAO inhibitory activity of the isolated compounds is shown in Table 3. Among them, lemuninol A (**1**) is the most potent, and lemuninols B and C (**2**, **3**) and a naphthoquinone **9** showed weak activity. Lemuninol A (**1**) has the same C, D-ring as that of **2**, and the A,B-chromophore in **1** is the same structure as that of **9**. Comparing between **2** and **3**, methylation of 5′-OH in D-ring did not affect the activity. The A, B-chromophore in both **2** and **3** is the same as that in the structure of **8** which has no activity. From these data, each monomer part in **1**—**3** does not seem to be responsible for its full activity.

To obtain some more information on the active structure of **1**, simple quinones were tested for their MAO inhibition (Table 4). Considering the structures of the active quinones, **11**, **14**, **15**, **16** and **18**, they have a 2-methyl-1,4-quinone (not hydroquinone like **22**) moiety except for benzoquinone (**11**)

Talbe 2. ^{13}C-NMR of Compounds **4**—**10**

C-	**4**[a]	**5**[b]	**6**[a]	**7**[b]	**8**[a]	**9**[b]	**10**[a]
1	120.4	118.3	147.2	146.0	118.9	185.8	184.8
2	137.0	137.9	126.4	128.4	134.9	145.4	147.3
3	109.1	112.3	109.6	112.7	112.7	137.9	137.4
4	158.2	154.2	153.0	150.2	153.8	184.4	185.1
5	158.3	156.2	157.3	156.4	141.3	159.4	147.9
6	106.2	103.1	105.8	103.5	145.2	117.7	159.1
7	127.3	125.7	126.5	125.8	120.2	134.6	121.1
8	120.7	121.3	114.5	115.7	125.2	119.4	125.4
9	138.6	136.9	131.4	130.5	132.2	134.4	125.8
10	116.9	113.3	117.1	114.2	117.3	120.0	126.1
1-OCH_3			60.9	61.0			
2-CH_3	21.7	21.6	16.1	15.9	21.4	15.7	15.9
4-OCH_3	56.32[c]		56.9				
5-OCH_3	56.37[c]	56.0	56.4	56.1	61.9	56.5	64.5

a) in acetone-d_6, *b*) in $CDCl_3$, *c*) interchangeable.

Table 3. MAO Inhibitory Ratios of Compounds **1**—**10** from Lemuni Hitam

Compound	Inhibitory ratio (%)		
	2.5×10^{-5} g/ml	1.0×10^{-5} g/ml	5.0×10^{-6} g/ml
1	75	62	45
2	20	9	4
3	21	15	6
4	−1	−5	−5
5	−1	−3	−1
6	0	1	1
7	5	1	−1
8	5	0	−3
9	26	12	9
10	2	3	2
Clorgyline	—	82	—

Table 4. MAO Inhibitory Ratios of Some Related Quinones

Compound	Inhibitory ratio (%)		
	2.5×10^{-5} g/ml	1.0×10^{-5} g/ml	5.0×10^{-6} g/ml
11	74	44	7
12	−1	−7	−3
13	30	9	5
14	87	78	66
15	82	44	1
16	54	22	5
17	8	−6	−1
18	72	60	38
19	30	8	−2
20	69	4	1
21	8	3	2
22	19	8	3
23	30	5	1

11: *p*-benzoquinone, **12**: *p*-hydroquinone, **13**: 1,4-naphthoquinone, **14**: menadione (vitamin K3), **15**: 2,5-dimethyl-*p*-benzoquinone, **16**: 2,6-dimethyl-*p*-benzoquinone, **17**: 2-isopropyl-5-methyl-*p*-benzoquinone, **18**: tetramethyl-*p*-benzoquinone, **19**: 2-chloro-*p*-benzoquinone, **20**: tetrachloro-*p*-benzoquinone, **21**: 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, **22**: trimethyl-*p*-hydroquinone, **23**: 2-phenyl-*p*-benzoquinone.

itself. The 2-methyl group cannot substitute with aromatized carbons such as **23**, and with halogens in **19**—**21** for MAO inhibition. Some substituents such as an isopropyl group in **17** and a 6-hydroxy group in **10** counteract the inhibition. A methoxy group at the *peri*-position also seems to reduce the activity in the case of **9**. Although some naphthoquinone monomers showed MAO inhibitory activity, the quinone or naphthalene component in **1** did not explain sufficiently the activity of the dimerized compounds, which possibly need some active structure.

Experimental

UV spectra were measured on a Hitachi U-3400 spectrometer. EI-MS and HR-FAB-MS spectra were recorded with JEOL JMS AM-20 and JEOL HX-110 A spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL JNM-A500 and -A400 spectrometers with tetramethylsilane or a solvent as internal standard. Column chromatography was performed on Sephadex LH-20, BW-127ZH, Nacalai Silica Gel 60 and Chromatorex ODS (100—200 mesh) and TLC by Merck RP-18F254S and Silica Gel 60 F_{254}. Sensyu Pak, ODS-4251-S, ODS-5251-N and ODS-Pegasil were used for HPLC.

Materials Lemuni Hitam was collected by Azizi in 1994 and was identified as *Diospyros* sp. by Satake. The voucher specimen (LNP19409-01) is kept in the Laboratory of Natural Products, Faculty of Pharmaceutical Sciences, Chiba University.

Isolation Lemuni Hitam, 5.41 g, was cut in small pieces and was extracted successively with ethyl acetate and methanol at room temperature. Both extracts (171 mg and 229 mg, respectively) showed MAO inhibitory activity (about 60% inhibition at 2.5×10^{-4} g/ml) and were independently separated by silica gel column chromatography (ch. 1) using *n*-hexane/acetone as an eluent. Similar fractions from each extract by TLC were combined together to give fr. 1-A to 1-C eluted with *n*-hexane/acetone 5 : 1, fr. 1-D to 1-F with 2 : 1, and fr. G with methanol. Further separation of fr. 1-E (52 mg) and fr. 1-D (61 mg) was made independently by Sephadex LH-20 column chromatography with methanol (ch. 2, 4) and then by ODS-HPLC with acetonitrile/water 1 : 1 as an eluent (ch. 3, 5). Compound **1**, 19 mg, was obtained from the former fraction, and compounds **2** and **3**, 19 and 4 mg, respectively, from the latter. By silica gel chromatography (ch. 6) of fr. 1-A, 85 mg, using an eluent of *n*-hexane/ethyl acetate 5 : 1, fr. 6-B was obtained, which was then separated by preparative TLC (ch. 7) on silica gel with *n*-hexane/acetone 5 : 2 to give fr. 7-A (38 mg), 7-B (22 mg) and 7-C (2 mg). Fraction 7-A was separated into compounds **4** and **5**, 15 and 4 mg, respectively, by ODS-HPLC (ch. 8, 9) with acetonitrile/water 1 : 1, and fr. 7-B to compounds **6** and **7** (each 8 mg). Fraction 1-B was applied to preparative TLC (ch. 10) on silica gel with *n*-hexane/ethyl acetate 5 : 3, and each fraction was then purified by ODS-HPLC (ch. 11—13) using an eluent of acetonitrile/water 7 : 13 to yield compounds **8** (6 mg), **9** (2 mg) and **10** (11 mg).

Lemuninol A (**1**): Dark-red amorphous powder. HR-FAB-MS (NBA/PEG 200+400) *m/z*: 405.1350 $(M+H)^+$ (err. +1.2 mmu for $C_{24}H_{21}O_6$), 404.1290 (M^+) (err. +3.0 mmu for $C_{24}H_{20}O_6$). EI-MS *m/z* (%): 404 (M^+, 62), 389 (100), 387 (36), 374 (7), 359 (4), 331 (3) 329 (2), 202 (10). ^{1}H-NMR ($CDCl_3$) δ: 1.88 (3H, s, 2-CH_3), 2.28 (3H, s, 7′-CH_3), 3.49 (3H, d, J=2.9 Hz, 4′-OCH_3), 3.91 (3H, s, 5-OCH_3), 6.27 (1H, s, 3′-H), 6.54 (1H, br s, 8′-H), 6.58 (1H, br s, 6′-H), 6.63 (1H, br s, 2′-OH), 7.26 (1H, d, J=8.6 Hz, 6-H), 7.67 (1H, dd, J=8.6, 7.6 Hz, 7-H), 7.82 (1H, d, J=7.6 Hz, 8-H), 9.19 (1H, s, 5′-OH). ^{1}H-NMR (acetone-d_6) δ: 1.85 (3H, s, 2-CH_3), 2.22 (3H, br s, 7′-CH_3), 3.91 (3H, s, 5-OCH_3), 4.13 (3H, s, 4′-OCH_3), 6.46 (1H, dd, J=1.5, 0.5 Hz, 6′-H), 6.66 (1H, s, 3′-H), 6.70 (1H, br s, 8′-H), 7.49—7.52 (1H, m,

6-H), 7.76—7.80 (2H, m, 7, 8-H), 8.33 (1H, br s, 2′-OH), 9.20 (1H, s, 5′-OH). UV λ_{max} (ethanol) nm (log ε): 210 (4.61), 237 (4.72), 267 (sh, 4.09), 298 (3.83), 309 (3.86), 334 (sh, 3.74), 342 (3.78), 389 (3.60). UV λ_{max} (ethanol+NaOH) nm: 251, 302, 315, 361.

Lemuninol B (**2**): White amorphous powder. HR-FAB-MS (glycerol/PEG 200+400) *m/z*: 407.1467 $(M+H)^+$ (err. −2.7 mmu for $C_{24}H_{23}O_6$), 406.1417 (M^+) (err. +0.1 mmu for $C_{24}H_{22}O_6$). EI-MS *m/z* (%): 406 (M^+, 100), 391 (18), 203 (2). ^{1}H-NMR (acetone-d_6) δ: 1.99 (3H, s, 2-CH_3), 2.08 (3H, s, 7′-CH_3), 4.12 (3H, s, 5-OCH_3), 4.17 (3H, s, 4′-OCH_3), 6.17 (1H, dd, J=1.5, 0.7 Hz, 8′-H), 6.43 (1H, br d, J=1.5 Hz, 6′-H), 6.70 (1H, s, 3′-H), 6.80 (1H, s, 3-H), 6.81 (1H, d, J=9.0 Hz, 8-H), 6.96 (1H, d, J=9.0 Hz, 7-H), 7.40(1H, br s, 2′ or 6-OH), 8.48 (1H, br s, 6 or 2′-OH), 9.24 (1H, s, 5′-OH), 9.55 (1H, br s, 4-OH). UV λ_{max} (ethanol) nm (log ε): 235 (4.89), 298 (sh, 4.03), 308 (4.04), 346 (4.00). UV λ_{max} (ethanol+NaOH) nm: 250, 304, 364.

Lemuninol C (**3**): White amorphous powder. EI-MS *m/z* (%): 420 (M^+, 100), 405 (14), 203 (1), 189 (7). ^{1}H-NMR (acetone-d_6) δ: 1.98 (3H, s, 2-CH_3), 2.12 (3H, s, 7′-CH_3), 3.89 (3H, s, 5′-OCH_3), 3.94 (3H, s, 4′-OCH_3), 4.12 (3H, s, 5-OCH_3), 6.28 (1H, dd, J=1.2, 1.0 Hz, 8′-H), 6.55 (1H, d, J=1.2 Hz, 6′-H), 6.64 (1H, s, 3′-H), 6.80 (1H, s, 5-H), 6.80 (1H, d, J=9.3 Hz, 8-H), 6.95 (1H, d, J=9.3 Hz, 7-H), 7.29 (1H, br s, 2′ or 6-OH), 8.51 (1H, br s, 6 or 2′-OH), 9.55 (1H, s, 4-OH). UV λ_{max} (ethanol) nm: 235, 298 (sh), 308, 341, 348 (sh). UV λ_{max} (ethanol+NaOH) nm: 244 (sh), 250, 302, 304 (sh), 340, 361 (sh).

4: White amorphous powder. EI-MS *m/z* (%): 202 (M^+, 100), 129 (45), 115 (30), 101 (9). ^{1}H-NMR (acetone-d_6) δ: 2.41 (3H, d, J=0.7 Hz, 2-CH_3), 3.87 (3H, s, 4 or 5-OCH_3), 3.88 (3H, s, 5 or 4-OCH_3), 6.75 (1H, d, J=1.5 Hz, 3-H), 6.81 (1H, dd, J=7.1, 1.7 Hz, 6-H), 7.16 (1H, br s, 1-H), 7.27 (1H, dd, J=8.3, 1.7 Hz, 8-H), 7.31 (1H, dd, J=8.3, 7.1 Hz, 7-H). The UV spectrum is identical with the published data of the compound isolated from *Diospyros* sp.[13]

5: White amorphous powder. EI-MS *m/z* (%): 188 (M^+, 100), 173 (42), 145 (59), 127 (15), 115 (45), 102 (6). ^{1}H-NMR ($CDCl_3$) δ: 2.42 (3H, s, 2-CH_3), 4.04 (3H, s, 5-OCH_3), 6.70 (1H, br d, J=7.6 Hz, 6-H), 6.73 (1H, d, J=1.5 Hz, 3-H), 7.08 (1H, br s, 1-H), 7.26 (1H, dd, J=8.3, 7.6 Hz, 7-H), 7.32 (1H, dd, J=8.3, 0.7 Hz, 8-H), 9.22 (1H, s, 4-OH). The UV data is identical with that of the compound isolated from *D. melanoxylon*.[5]

6: White amorphous powder. EI-MS *m/z* (%): 232 (M^+, 53), 217 (100), 201 (4), 189 (20), 174 (11), 159 (3), 145 (6), 128 (13), 115 (18), 103 (9). ^{1}H-NMR ($CDCl_3$) δ: 2.42 (3H, s, 2-CH_3), 3.83 (3H, s,1-OCH_3), 3.93 (3H, s, 4-OCH_3), 3.96 (3H, s, 5-OCH_3), 6.65 (1H, s, 3-H), 6.82 (1H, br d, J=7.5 Hz, 6-H), 7.39 (1H, t-like, J=8.1 Hz, 7-H), 7.66 (1H, dd, J=8.6, 1.0 Hz, 8-H). UV λ_{max} (ethanol) nm (log ε): 230 (4.71), 297 (sh, 3.82), 304 (3.86), 319 (sh, 3.77), 322 (3.81), 333 (sh, 3.70), 337 (3.81). The UV and ^{1}H-NMR are almost identical with the reported data of trimethoxy-hydroplumbagine.[14]

7: White amorphous powder. EI-MS *m/z* (%): 218 (M^+, 84), 203 (100), 188 (15), 175 (19), 160 (18), 131 (15), 115 (33), 103 (11). ^{1}H-NMR ($CDCl_3$) δ: 2.38 (3H, s, 2-CH_3), 3.81 (3H, s,1-OCH_3), 4.04 (3H, s, 5-OCH_3), 6.69 (1H, s, 3-H), 6.75 (1H, br d, J=7.6 Hz, 6-H), 7.33 (1H, t-like, J=8.1 Hz, 7-H), 7.66 (1H, br d, J=8.6 Hz, 8-H), 9.05 (1H, s, 4-OH). UV λ_{max} (ethanol) nm (log ε): 230 (4.65), 300 (sh, 3.79), 309 (3.85), 324 (3.86), 339 (3.89). The ^{1}H-NMR data is identical with the compound isolated from *D. melanoxylon*, although the UV was not the same.[5]

8: White amorphous powder. EI-MS *m/z* (%): 204 (M^+, 54), 189 (77), 161 (50), 143 (14), 131 (14), 115 (100), 102 (23). ^{1}H-NMR (acetone-d_6) δ: 2.34 (3H, br s, 2-CH_3), 4.05 (3H, s, 5-OCH_3), 6.61 (1H, d, J=1.7 Hz, 3-H), 7.04 (1H, br s, 1-H), 7.15 (1H, d, J=8.9 Hz, 7-H), 7.41 (1H, d, J=8.9 Hz, 8-H), 8.51 (1H, br s, 6-OH), 9.30 (1H, br s, 4-OH). UV λ_{max} (ethanol) nm: 230, 239 (sh), 278 (sh), 290, 301, 336, 346.

9: Yellow amorphous powder. EI-MS *m/z* (%): 202 (M^+, 100), 187 (3), 173 (15), 156 (6), 145 (8), 131 (14), 128 (17), 115 (50), 104 (53). ^{1}H-NMR ($CDCl_3$) δ: 2.14 (3H, d, J=1.4 Hz, 2-H), 4.00 (3H, s, 5-OCH_3), 6.74 (1H, q, J=1.4 Hz, 3-H), 7.29 (1H, dd, J=8.3, 1.2 Hz, 6-H), 7.66 (1H, dd, J=8.3, 7.7 Hz, 7-H), 7.76 (1H, dd, J=7.7, 1.2 Hz, 8-H). This compound has already been isolated from *D. melanoxylon*, and the UV data is identical.[5] It was also identified by comparison with the published ^{13}C-NMR data except for the opposite assignment of C-6 and 8.[15]

10: Yellow amorphous powder. EI-MS *m/z* (%): 218 (M^+, 100), 200 (6), 171 (53), 147 (18), 131 (11), 120 (24), 115 (20), 103 (13). ^{1}H-NMR (acetone-d_6) δ: 2.08 (3H, d, J=1.5 Hz, 2-CH_3), 3.85 (3H, s, 5-OCH_3), 6.71 (1H, q, J=1.5 Hz, 3-H), 7.25 (1H, d, J=8.5 Hz, 7-H), 7.79 (1H, d, J=8.5 Hz, 8-H). UV λ_{max} (ethanol) nm: 213, 262, 399. The UV and ^{1}H-NMR data are almost identical with those of the compound from *D. celebica*.[6]

Assay Male ddY strain mice (4 weeks old) obtained from Japan SLC, Hamamatsu, Japan, were used after conditioning about a week. Each mouse liver was homogenized with 4 volumes of 1.15% KCl under ice-cooling. The homogenates were centrifuged at 3800 rpm for 10 min, and the supernatant was used for assay. The assay of MAO inhibitory activity was carried out as previously described.[16,17] Samples dissolved in dimethyl sulfoxide (DMSO) were added to the incubation medium (final concentration of DMSO: <3%). The fluorescence intensity of 4-hydroxyquinoline formed from the substrate, kynuramine (kynuramine hydrobromide, Sigma), was measured at 380 nm with excitation at 315 nm. As a blank test, the reaction was carried out without the substrate, which was later applied. A control solution was made in which a sample was added after the incubation was stopped. Clorgyline (Aldrich) was used as a positive control.

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