Manuifolins D, E, and F: New Isoflavonoids from *Maackia tenuifolia*

Jia-Feng Zeng,[†] Guo-Lin Li,[†] Jing-Kang Shen,[†] Da-Yuan Zhu,*[,†] Ke Chen,[‡] and Kuo-Hsiung Lee*[,‡]

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China, Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill, North Carolina 27599

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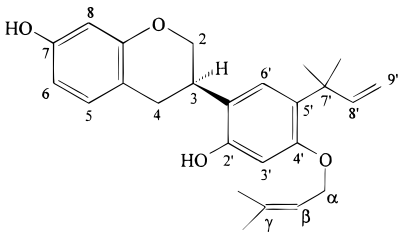
Chemical examination of roots of *Maackia tenuifolia* yielded three new isoflavans, manuifolins D, E, and F, along with the known (6a*R*,12a*R*)-pterocarpin and (6a*R*,l2a*R*)-maackiain. The new compounds were established as (3*R*)-5′-(1,1-dimethyl-2-propenyl)-4′-*O*-(3-methyl-2-butenyl)-7,2′-dihydroxyisoflavan (**1**), (3*R*)-6,5′-bis(1,1-dimethyl-2-propenyl)-7,2′,4′,-trihydroxyisoflavan (**2**), and (3*R*)-5′-(1-isopropylethenyl)-8-(3-methyl-2-butenyl)-7,2′,4′-trihydroxyisoflavan (**3**), respectively, by spectroscopic methods.

Although the plant *Maackia amurensis* (Leguminosae) has been well studied, little research has been done on the related species *Maackia tenuifolia* (Hemsl.) Hand.-Mazz.,[1,2] which is distributed in Jiangsu, Anhui, and Zhejiang Provinces of China and has been used as an antitumor drug and fungicide in Chinese folk medicine. In a previous paper,[3] we reported the isolation and identification of two isoflavans, manuifolins A and B, from this plant. Further examination of the CH_2Cl_2-soluble portion of the ethanolic extract gave three new isoflavans, manuifolins D (**1**), E (**2**), and F (**3**), along with the known (6a*R*,12a*R*)-pterocarpin and (6a*R*,12a*R*)-maackiain. The known compounds were identified by spectral comparison with literature data.[4,5] In this paper, we report the isolation and structural elucidation of the three new compounds.



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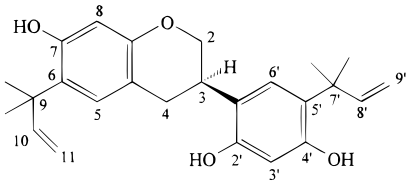

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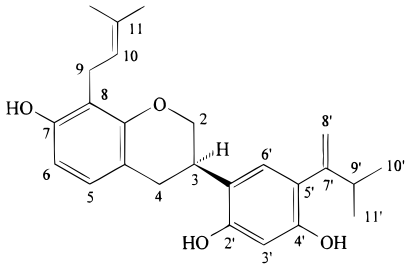

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Manuifolin D (**1**) was obtained as a white powder. HRMS of **1** indicated its molecular formula to be $C_{25}H_{30}O_4$. In the UV spectrum, bands at 210 (4.86) and 290 (4.09) nm were characteristic of isoflavans.[6] The ^{1}H NMR spectrum of **1** showed isoflavan heterocyclic protons at δ 2.85 (1H, br dd), 3.00 (1H, dd), 3.42 (1H, m), 4.03 (1H, t), 4.29 (1H, br d). Signals at δ 1.38 (6H, s), 4.90 (1H, d), 4.91 (1H, d), and 6.13 (1H, dd) and signals at δ 1.70 (3H, s), 1.77 (3H, s), 4.44 (2H, d), and 5.46 (1 H, br t) revealed the presence of a 1,1-dimethyl-2-propenyl group and a 3-methyl-2-butenyl group in this molecule. Signals for methylene protons (δ 4.44) and an olefinic proton (δ 5.46) in the 3-methyl-2-butenyl unit were shifted downfield compared with those of manuifolin A,[3] suggesting this group was attached to an oxygen atom. The chemical shift data were in agreement with those reported in the literature.[7] In the aromatic region, signals for three protons constituting an ABX system and two singlets were seen. The ^{1}H NMR data and RDA fragment ions at *m*/*z* 123 and 272 suggested the C_5 unit should be at C-5′ and a hydroxy group and the O−C_5 group should be located at C-2′ and C-4′, respectively. Careful comparison of the ^{1}H NMR spectrum of **1** with that of manuifolin A revealed that signals of the geminal olefinic protons in the 1,1-dimethyl-2-propenyl group of **1** shifted upfield to δ 4.90 (1H, d) and 4.91 (1H, d), suggesting that the O−C_5 unit was located at C-4′. Steric interaction then could result in upfield shifts of neighboring protons. This consideration was confirmed by HMBC experiments, in which correlation was observed between methylene protons of the O−C_5 unit and C-4′. Thus, **1** is determined to be 5′-(1,1-dimethyl-2-propenyl)-4′-*O*-(3-methyl-2-butenyl)-7,2′-dihydroxyisoflavan. The unambiguous assignments

* Authors to whom correspondence should be addressed. D. Y. Zhu: State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China. Tel: 86-21-64311833. FAX: 86-21-64370269. K. H. Lee: Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill, NC 27599. Tel: (919) 962-0066. E-mail: KHLEE@UNC.EDU. FAX: (919) 966-3893.

† Shanghai Institute of Materia Medica.

‡ School of Pharmacy, UNC.

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Table 1. ^{13}C NMR,[a] HMBC (H → C), and Long-Range HETCOR (C → H) of Compounds **1**, **2**, and **3** (in $CDCl_3$)

carbon no.	**1**	HMBC	carbon no.	**2**	long-range HETCOR	**3**	long-range HETCOR
2	69.95 t	3H	2	69.81 t		70.03 t	
3	32.19 d	2H, 4H, 6′H	3	32.21 d	4H, 6′H	31.82 d	6′H
4	30.42 t	3H, 5H	4	30.56 t	2H, 5H	30.92 t	
4a	114.83 s	4H, 8H	4a	114.19 s	4H, 8H	114.44 s	4H, 6H
5	130.41 d		5	127.08 d	4H	127.51 d	
6	107.92 d		6	124.85 s	8H, 7-OH, 9 2×CH_3	108.06 d	
7	154.77 s	5H	7	153.69 s	5H, 8H, 7-OH	153.50 s	5H, 9H,
8	103.19 d		8	105.14 d	7-OH	114.35 s	9H, 10H
8a	155.09 s	5H	8a	154.01 s	5H, 8H	152.33 s	2H, 4H, 5H
1′	117.65 s	3H, 3′H, 6′H	9	39.77 s	5H, 11H	22.33 t	
2′	152.28 s	3H, 3′H, 6′H	CH_3	27.20 q	10H		
3′	101.33 d	α-H,	CH_3	27.20 q	10H		
4′	156.84 s	3′H, 6′H, α-H	10	148.22 d		122. 13 d	9H, 11 2×CH_3
5′	129.27 s	7′ 2×CH_3	11	113.23 t[b]		134.22 s	
6′	126.25 d	3H, 3′H	CH_3			17.83 q	11-CH_3
7′	40.20 s	6′H, 9′H	CH_3			25.77 q	11-CH_3
CH_3	27.13 q	6′H, 8′H	1′	119.46 s	4H, 3′H, 2′-OH	119.78 s	3′H, 2′-OH
CH_3	27.18 q	6′H, 8′H	2′	153.32 s	3′H, 6′H, 2′-OH	152.88 s	6′H
8′	148.09 d	7′ 2×CH_3, 9′H	3′	104.89 d	4′-OH	103.93 d	4′-OH
9′	109.74 t		4′	153.94 s	3′H, 6′H, 4′-OH	153.81 s	6′H, 4′-OH
α	65.22 t	3′H, γ 2×CH_3	5′	124.42 s	3′H, 4′-OH, 7′ 2×CH_3	121.70 s	10′-OH
β	119.98 d	α-H, γ 2×CH_3	6′	125.33 d		127.44 d	
γ	136.93 s	α-H	7′	39.83 s	6′H, 9′H	150.11 s	10′H, 11′H
CH_3	18.24 q	γ-CH_3	CH_3	26.97 q			
CH_3	25.69 q	γ-CH_3	CH_3	27.01 q			
			8′	148.14 d	7′ 2×CH_3	111.25 t	11′H
			9′	113.37 t[b]		42.05 d	8′H, 6′H
			10′			18.47 q	
			11′			20.86 q	8′H

[a] ^{13}C data assignments of compounds **1**, **2**, and **3** were made combining DEPT and HETCOR experiments. [b] Interchangeable assignments.

of carbon signals in ^{13}C NMR were based on HETCOR and HMBC experiments.

Manuifolin E (**2**), $C_{25}H_{30}O_4$ (HRMS), was obtained as a light yellow gum. The isoflavan nature of **2** was shown by UV and ^1H NMR data. Two sets of signals characteristic of a 1,1-dimethyl-2-propenyl group and four aromatic proton singlets were observed in the ^1H NMR spectrum. The chemical shifts of the aromatic protons and biogenetic considerations suggested that two C_5 units should be attached at C-5′ and C-6, and the molecule must be oxygenated at C-2′, C-4′, and C-7, which was supported by RDA fragment ions at *m*/*z* 191 and 204 in the EI mass spectrum. The substitution mode was further confirmed by long-range HETCOR experiment, in which correlations between C-9 and H-5, C-7′ and H-6′ were observed. Thus, **2** is determined as 6,5′-bis(1,1-dimethyl-2-propenyl)-7,2′,4′-trihydroxyisoflavan.

Manuifolin F (**3**), $C_{25}H_{30}O_4$ (HRMS), was obtained as a colorless gum. The ^1H NMR spectrum revealed the presence of a 3-methyl-2-butenyl unit. Signals at δ 1.35 (3H, d, *J* = 7.0 Hz), l.62 (3H, d, *J* = 4.7 Hz), 3.41 (1H, m), and 5.00, 5.06 (each 1H, br s) were assignable to a 1-isopropylethenyl group, which was confirmed by ^{13}C NMR data and long-range HETCOR experiment (*vide infra*). This is the first time this C_5 unit has been found in an isoflavan. The signals of two ortho-coupled doublets and two singlets in aromatic region were also observed. Chemical shifts and RDA fragment ions *m*/*z* 191 and 204 indicated that two C_5 units were located at C-8 and C-5′ or C-6 and C-3′. The assignment of the 3-methyl-2-butenyl group at C-8 was supported by comparison of ^1H NMR and ^{13}C NMR data of **1** with those of manuifolin A.[3] The chemical shifts of the hydrogen and carbon atoms in the A ring of both molecules were very similar. Then another C_5 unit must be attached to C-5′. Unambiguous evidence was provided by long-range HETCOR experiment, in which correlations between H-10 and C-8 and H-9 and C-8 were observed. Correlations between C-7′ and methyl proton (δ 1.35, 1.62) and C-9′ and two geminal olefinic protons were also seen (see Table 1). Thus, the structure of **3** was established as 5′-(1-isopropylethenyl)-8-(3-methyl-2-butenyl)-7,2′,4′ -trihydroxyisoflavan.

Compounds **1**, **2**, and **3** showed positive Cotton effects in the 270−300 nm region in their CD spectra as it is observed in (3*R*)-isoflavans.[8,9] Combining these results with the occurrence of the biogenetically relevant compounds (6a*R*,12a*R*)-pterocarpin and (6a*R*,12a*R*)-maackiain in the same plant allowed us to assign the C-3 stereochemistry of these compounds as the *R* configuration.

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler micro melting point apparatus and are uncorrected. ^1H NMR (at 400 MHz) and HMBC spectra were recorded on a Bruker AM-400 spectrometer. ^{13}C NMR (at 75 MHz), HETCOR and long-range HETCOR spectra were taken on a Bruker AC-300 spectrometer. Chemical shifts were given in δ (ppm), and the solvent signal was used as reference (δ 7.24 ppm for ^1H and δ 77.00 for ^{13}C, respectively). EIMS and HRMS were performed at 70 eV on a MAT-95 mass spectrometer. CD were obtained with a JASCO DIP-181 DIGITAL polarimeter. UV spectra were measured on a Shimadzu UV-250 instrument using MeOH as solvent. CC and TLC were carried out using silica gel obtained from Qingdao Ocean Chemical Co.

Plant Material. *Maackia tenuifolia* was collected in Linan County, Zhejiang Province, China. A Voucher specimen [LMT 8503] has been deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The extraction and fractionation of the plant material were described in ref 3. Chromatography of the CH_2Cl_2 soluble portion of the ethanolic extract with $CHCl_3$−MeOH (40:1) gave fractions 31−40, in which the precipitates were filtered and recrystalized to give 40 mg of (6a*R*,12a*R*)-pterocarpin. The fractions 76−84 were further purified by CC with CH_2Cl_2−acetone (20:1) and then on Sephadex LH-20 to give 30 mg of **1** and 80 mg of (6a*R*,12a*R*)-maackiain. The 116−128 fractions eluted with $CHCl_3$−MeOH (30:1) were further purified by CC with cyclohexane−acetone(3:1) and then on Sephadex LH-20 and preparative TLC to give 40 mg of **2** and 65 mg of **3**.

(3*R*)-(−)-Manuifolin D (1). $C_{25}H_{30}O_4$; white powder; mp 186 °C; $[\alpha]^{10}_D$ −35.05° (MeOH, *c* 0.0713); UV λ^{MeOH}_{max} nm (log ϵ) 210 (4.86), 285 (4.13), 290 (4.09); EIMS *m*/*z* 394 $[M]^+$ (4), 326 (20), 272 (3), 204 (28), 191 (25), 189 (35), 123 (22); HRMS *m*/*z* 394.2144 $[M]^+$ (calc for $C_{25}H_{30}O_4$ *m*/*z* 394.2144); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (6H, s, Me_2-7′), 1.70, 1.77 (each 3H, s, Me_2-γ), 2.85 (1H, br dd, *J* = 15.6, 5.0 Hz, H-4_{eq}), 3.00 (1H, dd, *J* = 15.6, 11.0 Hz, H-4_{ax}), 3.42 (1H, m, H-3), 4.03 (1H, t, *J* = 10.3 Hz, H-2_{ax}), 4.29 (1H, br d, *J* = 10.3 Hz, H-2_{eq}), 4.44 (2H, d, *J* = 6.4 Hz, CH_2-α), 4.90 (1H, d, *J* = 10.8 Hz, H_a-9′), 4.91 (1H, d, *J* = 17.6 Hz, H_b-9′), 5.46 (1H, br t, *J* = 6.4 Hz, H-β), 6.13 (1H, dd, *J* = 17.6, 10.8 Hz, H-8′), 6.32 (1H, s, H-3′), 6.33 (1H, d, *J* = 2.5 Hz, H-8), 6.36 (1H, dd, *J* = 8.1, 2.5 Hz, H-6), 6.92 (1H, d, *J* = 8.1 Hz, H-5), 6.95 (1H, s, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1; CD (MeOH, *c* 0.0135) $[\theta]_{205}$ +10890, $[\theta]_{220}$ 0, $[\theta]_{233}$ −7260, $[\theta]_{243}$ 0, $[\theta]_{258}$ +2145, $[\theta]_{270}$ +1155, $[\theta]_{283}$ +3300, $[\theta]_{299}$ 0.

(3*R*)-(−)-Manuifolin E (2): $C_{25}H_{30}O_4$; light yellow gum; $[\alpha]^{245}_D$ −34.42° (MeOH, *c* 0.1947); UV λ^{MeOH}_{max} nm (log ϵ) 207 (4.56), 286 (3 .74); EIMS *m*/*z* 394 $[M]^+$ (56), 227 (30), 204 (20), 191 (46), 189 (26), 137 (71), 118 (67), 91 (44); HRMS *m*/*z* 394.2148 $[M]^+$ (calc for $C_{25}H_{30}O_4$ *m*/*z* 394.2144); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33, 1.34, 1.39, 1.40 (each 3H, s, Me_2-7′, Me_2-9), 2.90 (1H, br dd, *J* = 15.6, 4.2 Hz, H-4_{eq}), 3.03 (1H, dd, *J* = 15.6, 10.4 Hz, H-4_{ax}), 3.49 (1H, m, H-3), 4.04 (1H, t, *J* = 10.1 Hz, H-2_{ax}), 4.31 (1H, br d, *J* = 10.1 Hz, H-2_{eq}), 5.27, 5.28 (each 1H, d, *J* = 10.6, Ha-11, Ha-9′), 5.32, 5.33 (each 1H, d, *J* = 17.7 Hz, Hb-11, Hb-9′), 6.14, 6.16 (each 1H, dd, *J* = 17.7, 10.6 Hz, H-10, H-8′), 6.28 (1H, s, H-3′), 6.36 (1H, s, H-8), 6.94 (2H, s, H-5, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1. CD (MeOH, *c* 0.0050) $[\theta]_{197}$ 0, $[\theta]_{201}$ −12210, $[\theta]_{205}$ 0, $[\theta]_{209}$ +8580, $[\theta]_{225}$ +3960, $[\theta]_{252}$ +7755, $[\theta]_{277}$ +3630, $[\theta]_{310}$ 0.

(3*R*)-(−)-Manuifolin F (3): $C_{25}H_{30}O_4$; colorless gum; $[\alpha]^{20}_D$ −11.65° (MeOH, *c* 0.3313); UV λ^{MeOH}_{max} nm (log ϵ) 206 (4.73), 286 (3.73); EIMS *m*/*z* 394 $[M]^+$ (62), 204 (53), 191 (80), 107, 91, 69; HRMS *m*/*z* 394.2145 $[M]^+$ (calc for $C_{25}H_{30}O_4$ *m*/*z* 394.2144); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, d, *J* = 7.0 Hz, Me-10′), 1.62 (3H, d, *J* = 4.7 Hz, Me-11′), 1.72, 1.79 (each 3H, s, Me_2-11), 2.88 (1H, br d, *J* = 15.6 Hz, H-4_{eq}), 3.01 (1H, dd, *J* = 15.6, 11.0 Hz, H-4_{ax}), 3.38 (2H, d, *J* = 7.0 Hz, H-9), 3.41 (2H, m, H-3, H-9′), 4.01 (1H, br t, *J* = 10.2 Hz, H-2_{ax}), 4.36 (1H, br d, *J* = 10.2 Hz, H-2_{eq}), 5.00 (1H, br s, Ha-8′), 5.06 (1H, br s, Hb-8′), 5.23 (1H, br t, *J* = 7.0 Hz, H-10), 6.26 (1H, s, H-3′), 6.39 (1H, d, *J* = 8.4 Hz, H-6), 6.80 (1H, s, H-6′), 6.81 (1H, d, *J* = 8.4 Hz, H-5); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1; CD (MeOH, *c* 0.002 75) $[\theta]_{202}$ −9900, $[\theta]_{210}$ +10230, $[\theta]_{230}$ 0, $[\theta]_{235}$ −1320, $[\theta]_{239}$ 0, $[\theta]_{255}$ +3300, $[\theta]_{270}$ +2640, $[\theta]_{282}$ +2640, $[\theta]_{305}$ 0.

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