Pergamon

PII: S0031-9422(96)00294-4

Phytochemistry, Vol. 43, No. 4, pp. 893–896, 1996

0031-9422/96 $15.00 + 0.00

TWO ISOPRENOID-SUBSTITUTED ISOFLAVANS FROM ROOTS OF *MAACKIA TENUIFOLIA*

JIA-FENG ZENG,* GUO-LIN LI, XIN XU and DA-YUAN ZHU

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Science,
Shanghai 200031, China

(*Received in revised form* 15 *April* 1996)

Key Word Index—*Maackia tenuifolia*; Leguminosae; roots; isoflavan; tenuifolins A and B; 4-(1,1-dimethyl-2-propenyl)-1,3-dihydroxybenzene.

Abstract—Two new isoprenoid-substituted isoflavans, named tenuifolins A and B, along with a known compound, 4-(1,1-dimethyl-2-propenyl)-1,3-dihydroxybenzene, were isolated from the roots of *Maackia tenuifolia*. Their structures were elucidated by spectroscopic methods. Copyright © 1996 Published by Elsevier Science Ltd

---

INTRODUCTION

Maackia tenuifolia, distributed in Jiangsu, Anhui and Zhejiang provinces of China, has been used as antitumour drug and fungicide in Chinese folk medicine. Previous studies of this species have resulted in the isolation and synthesis of several new bioactive flavonoids [1–3]. Our investigation of the methylene dichloride extract of the roots of the title plant provided two new prenylated isoflavans, tenuifolins A and B, and 4-(1,1-dimethyl-2-propenyl)-1,3-dihydroxybenzene, which has not previously been reported as a natural product. Their structures were elucidated using spectroscopic methods.

RESULTS AND DISCUSSION

The methylene dichloride extract was subjected to silica gel column chromatography eluting with petrol–ethyl acetate gradients. Three compounds (**1–3**) were obtained from the petrol–ethyl acetate (4:1) fraction by repeated silica gel and Sephadex LH20 column chromatography.

Tenuifolin A (**1**), $C_{26}H_{32}O_4$ (by HR-mass spectrum), was obtained as a gum. The ^{1}H NMR spectrum exhibited characteristic signals for an isoflavan skeleton (H-2, H-3 and H-4). This spectrum also showed the presence of a 3-methyl-2-butenyl, 1,1-dimethyl-2-propenyl and methoxyl groups. The signals of two singlet aromatic protons (B ring) and two *ortho*-coupled aromatic protons (AX-type, A ring) were also observed. The EI-mass spectrum of compound **1** showed the characteristic fragment ions at *m/z* 191 (**1a**, A ring) and 218 (**1b**, B ring), which were caused by the usual retro-Diels–Alder fragmentation, indicating that the 3-methyl-2-butenyl group or 1,1-dimethyl-2-propenyl group and a hydroxyl group were located on the A ring, while a hydroxyl group, the methoxyl group and the 1,1-dimethyl-2-propenyl group or 3-methyl-2-butenyl group were on the B ring. The presence of the two singlet protons on the B ring was determined by NOE measurement of compound **1**. Enhancement of the single aromatic signal (δ 6.41) by 9.5% was observed when the methoxyl protons (δ 3.76) were irradiated. In the HMBC spectrum, a correlation observed between C-7′ (δ 39.80) and H-6′ (δ 6.95) suggested that the 1,1-dimethyl-2-propenyl group is attached to C-5′. The position of the methoxyl group was determined at C-2′ by a correlation between the protons of methoxyl group and C-2′, which showed correlation with H-3 (δ 3.52) and H-3′ (δ 6.41). The location of 3-methyl-2-butenyl group at C-8 and a hydroxyl group at C-7 was deduced by a correlation observed between C-4 (δ 30.99) and the *ortho*-coupled aromatic proton (δ 6.81, H-5), which resonated at a slightly lower field strength. Consequently, compound **1** was determined as 5′-(1,1-dimethyl-2-propenyl)-8-(3-methyl-2-butenyl)-2′-methoxy-7,4′-dihydroxyisoflavan. The absolute configuration of compound **1** was assigned to be 3*R* from its CD spectrum with a positive Cotton effect at 281 nm [4, 5]. Thus, the structure of tenuifolin A was characterized as formula **1**.

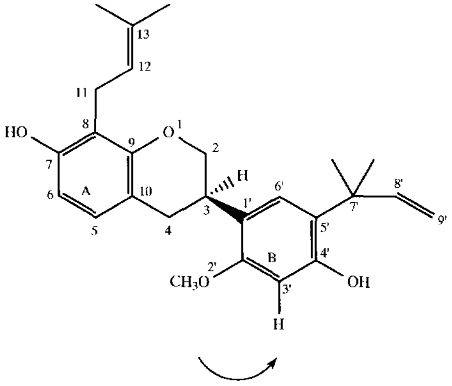
Tenuifolin B (**2**), $C_{25}H_{30}O_4$ (by HR-mass spectrum), was obtained as a gum. Its ^{1}H NMR spectrum was very similar with that of compound **1**, except for the presence of two prenyl groups, four *ortho*-coupled aromatic protons and the lack of a methoxyl group. The mass spectrum showed the characteristic fragment ions at *m/z* 191 (**2a**, A ring) and 204 (**2b**, B ring). In NOE experiments (in DMSO), the enhancements of proton signals, δ 8.19, δ 9.01 and δ 9.06, of three hydroxyl

---

*Author to whom correspondence should be addressed.

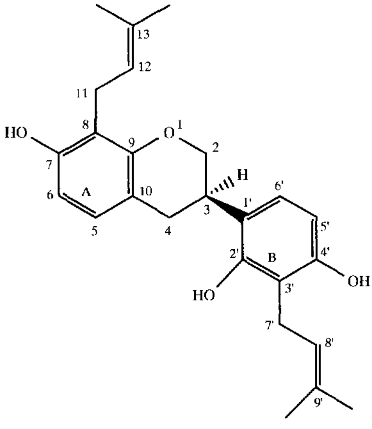
groups by 4.3%, 4.6% and 4.6%, respectively, were observed when the methylene protons (δ 3.37 and 3.43) of two prenyl groups were irradiated simultaneously, indicating that B ring was substituted as shown in **2b**; the other 3-methyl-2-butenyl group and a hydroxyl group were adjacent on the A ring. In the ^{13}C NMR spectrum of compound **2**, chemical shifts of the carbon atoms of A-ring were very similar to those of relevant atoms of compound **1**. All the above evidence suggested that compound **2** was 8,3′-diprenyl-7,2′,4′-trihydroxyisoflavan. The absolute configuration of compound **2** was assigned to be 3*R* from its CD spectrum, with a positive Cotton effect at 283 nm [4, 5].

Compound **3** was obtained as a light yellow oil. Its IR spectrum showed a broad absorption band at 3400 cm^{-1} due to hydroxyl groups and absorption bands at 1620 cm^{-1}, 1600 cm^{-1}, 1505 cm^{-1} and 1450 cm^{-1} due to a benzene skeleton. The EI-mass spectrum of **3** showed a [M]$^+$ at *m/z* 178. The ^{1}H NMR spectrum in CDCl$_3$ exhibited signals for protons of a 1,1-dimethyl-2-propenyl group and three aromatic protons. The ^{1}H NMR spectrum of compound **3** in

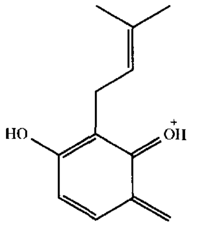


NOE 9.5%

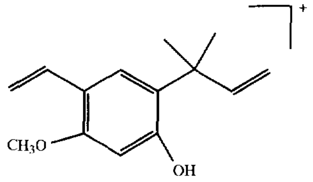
1



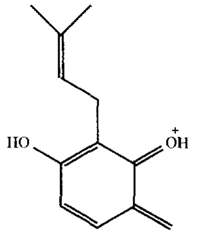
2



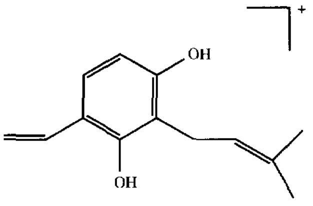
1a



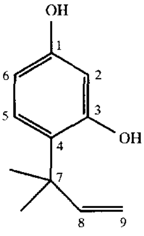
1b



2a



2b



3

Table 1. NMR data of compounds **1** and **2** in $CDCl_3$

C		**1**		
		(C)	(HMBC)	**2** (C)
2		70.2	3H, 4H	70.1
3		31.7	6′H, 4H, 2H	31.8
4		31.0	5H, 2H	30.9
5		127.5	4H	127.5
6		108.0		108.0
7		153.5	5H, 6H, 11H	153.6
8		114.3	6H, 11H	114.3
9		152.4	4H, 5H, 6H, 2H, 11H	152.3
10		114.5	3H, 4H, 6H	114.5
11		22.4		22.3[b]
12		122.2	11H, $13CH_3$, $13CH_3'$	122.1
13		134.0	11H, $13CH_3$, $13CH_3'$	134.2
	$-CH_3$	17.9[a]	12H, $13CH_3'$	17.9[d]
	$-CH_3'$	25.8[a]	12H, $13CH_3$	25.8[d]
1′		121.3	6′H, 3′H, 2H, 3H, 4H	120.6
2′		157.2	6′H, 3′H, OCH_3, 3H	153.7[c]
	OCH_3	55.4		
3′		100.8		113.2
4′		154.2	6′H, 3′H	152.7[c]
5′		123.5	3′H, 8′H, $7'CH_3$, $7'CH_3'$	107.6
6′		124.9	3H	125.1
7′		39.8	6′H, 8′H, 9′H	22.8[b]
	$-CH_3$	27.0	8′H, $7'CH_3'$	
	$-CH_3'$	27.0	8′H, $7'CH_3$	
8′		148.2	9′H, $7'CH_3$, $7'CH_3'$	121.2
9′		113.3		136.3
	$-CH_3$			17.8[d]
	$-CH_3'$			25.8[d]

[a–d] Interchangeable assignments.

DMSO showed the presence of protons (δ 9.08 and 8.99) of two hydroxyl groups. The position of the above groups on the benzene ring was deduced from the chemical shifts of three aromatic protons. Because the signal of H-2 was in an upper field and H-5 in a relatively lower field, only structure **3** was possible. Hence, compound **3** was determined as 4-(1,1-dimethyl-2-propenyl)-1,3-dihydroxybenzene. Compound **3** has been reported in the literature [6] to be synthesized but without spectral data. This is the first report of the natural occurrence of compound **3**.

EXPERIMENTAL

General. NMR: 400 MHz for ^1H and 100 MHz for ^{13}C; chemical shifts were given in δ with solvent signal used as ref. EI-MS and HR-MS: 70 eV. UV: in MeOH. IR: film. CC and TLC was carried out using silica gel (Qingdao Ocean Chemical Co.).

Plant material. Roots of *M. tenuifolia* (Hemsl.) Hand-Mazz$_{33}$. were collected in March 1985, from Linan County, Zhejiang Province, China, and were identified at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Science by Associate Prof. S. H. Jiang where a voucher specimen has been deposited [LMt 8503].

Extraction and isolation. Powdered roots (37 kg) were extracted with 95% EtOH and the concd EtOH extract dissolved in 80% aq. MeOH. After successive extraction with petrol, benzene and CH_2Cl_2, it was condensed and dissolved in CH_2Cl_2. The mixt. was filtered and the CH_2Cl_2 soln evapd *in vacuo* to give a brown gum (1 kg). The CH_2Cl_2-soluble portion (400 g) was subjected to silica gel CC, eluting with petrol–EtOAc (8:1), (4:1), (2:1), (1:1), (0:1) and 95% EtOH to yield 200 frs. Frs 49–68 were repeatedly chromatographed on silica gel and sephadex LH-20 columns with the solvent systems, petrol–Me_2CO (10:3), CH_2Cl_2–EtOAc (20:1), $CHCl_3$–MeOH (40:1), $CHCl_3$–MeOH (1:1) to give compounds **1**, **2** and **3**.

Tenuifolin A (**1**). $C_{26}H_{32}O_4$, gum. $[\alpha]_D^{23.5}$ $-1.321°$ (MeOH; *c* 0.1060). UV λ_{max}^{MeOH} nm: 214, 225 (shoulder), 284. EI-MS (rel. int): *m/z*: 409 $[M+1]^+$ (10), 408 $[M]^+$ (36), 218 (33), 205 (43), 203 (50), 191 (26), 151 (100), 135 (17); HR-MS, *m/z* 408.2312 $[M]^+$ (calc. for $C_{26}H_{32}O_4$ *m/z*: 408.2301). ^1H NMR (400 MHz, $CDCl_3$): δ 1.35 (6H, *s*, Me_2-7′), 1.72, 1.79 (each 3H, *s*, Me_2-13), 2.86 (1H, *br dd*, $J = 16$, 5 Hz, H-4), 3.00 (1H, *dd*, $J = 16$, 11 Hz, H-4), 3.38 (2H, *d*, $J = 6$ Hz, H-11), 3.52 (1H, *m*, H-3), 3.76 (3H, *s*, O-Me), 4.01 (1H, *t*, $J = 10$ Hz, H-2), 4.32 (1H, *br d*, $J = 10$ Hz, H-2), 5.23 (1H, *br t*, $J = 6$ Hz, H-12), 5.28 (1H, *d*, $J = 11$ Hz, H-9′), 5.34 (1H, *d*, $J = 18$ Hz, H-9′), 6.15 (1H, *dd*, $J = 18$ Hz, 11 Hz, H-8′), 6.38 (1H, *d*, $J =$ 8 Hz, H-6), 6.41 (1H, *s*, H-3′), 6.81 (1H, *d*, $J = 8$ Hz, H-5), 6.95 (1H, *s*, H-6′). ^{13}C NMR (100 MHz, CDCl$_3$): see Table 1. CD (MeOH; *c* 0.02875), $[\theta]_{220}$ +10890, $[\theta]_{229}$ 0, $[\theta]_{236}$ −7590, $[\theta]_{255}$ 0, $[\theta]_{281}$ +2301, $[\theta]_{295}$ 0.

Tenuifolin B (**2**). $C_{25}H_{30}O_4$, gum. $[\alpha]_D^{23.5}$ 1.314° (MeOH; *c* 0.1047). UV λ_{max}^{MeOH} nm: 216, 280. EI-MS *m/z* (rel. int.): 394 $[M]^+$ (23), 284 (29), 204 (16), 191 (36), 175 (12), 149 (51), 135 (28); HR-MS, *m/z* 394.2116 $[M]^+$ (calc. for $C_{25}H_{30}O_4$ *m/z*: 394.2144). ^{1}H NMR (400 MHz, CDCl$_3$). δ 1.72, 1.77, 1.79, 1.83 (each 3H, *s*, Me$_2$-13, Me$_2$-9′), 2.85 (1H, *br dd*, J = 16, 5 Hz, H-4), 2.96 (1H, *dd*, J = 16, 11 Hz, H-4(, 3.37, 3.43 (each 2H, *d*, J = 7 Hz, H-11, H-7′), 3.46 (1H, *m*, H-3), 3.96 (1H, *t*, J = 10 Hz, H-2), 4.35 (1H, *br*, *d*, J = 10 Hz, H-2), 5.24 (2H, *m*, H-12, H-8′), 6.35, 6.37 (each 1H, *d*, J = 8.28, 8.17 Hz, H-5′, H-6), 6.80, 6.81 (each 1H, *d*, J = 8.17, 8.14 Hz, H-5, H-6′). ^{13}C NMR (100 Hz, CDCl$_3$): see Table 1. CD (MeOH; *c* 0.01675), $[\theta]_{220}$ −30360, $[\theta]_{255}$ 0, $[\theta]_{283}$ +2640, $[\theta]_{295}$ 0.

4-*(*1,1-*Dimethyl*-2-*propenyl)*-1,3-*dihydroxybenzene* (**3**). $C_{11}H_{14}O_2$, light yellow oil, IR ν_{max} cm^{-1}: 3400 (OH, *br*), 1620, 1600, 1505, 1450 (benzene skeleton). EI-MS *m/z* (rel. int.): 178 $[M]^+$ (26), 163 $[M - CH_3]^+$ (100), 135 (12), 123 (17), 107 (8), 95 (8), 77 (4), 69 (5). ^{1}H NMR (400 MHz, CDCl$_3$: δ 1.39 (6H, *s*, Me$_2$-7), 5.28 (1H, *d*, J = 10 Hz, H-9), 5.33 (1H, *d*, J = 18 Hz, H-9), 6.15 (1H, *dd*, J = 18, 10 Hz, H-8), 6.34 (1H, *d*, J = 2.4 Hz, H-2), 6.38 (1H, *dd*, J = 8.5, 2.7 Hz, H-6), 7.08 (1H, *d*, J = 8.5 Hz, H-5). ^{1}H NMR (400 MHz, DMSO): δ 1.35 (6H, *s*, Me$_2$-7), 4.86 (1H, *d*, J = 11 Hz, H-9), 4.87 (1H, *d*, J = 17 Hz, H-9), 6.10 (1H, *br d*, J = 8 Hz, H-6), 6.19 (1H, *dd*, J = 17, 11 Hz, H-8), 6.24 (1H, *br s*, H-2), 6.85 (1H, *d*, J = 8 Hz, H-5), 8.99, 9.08 (each 1H, *br s*, OH × 2).

REFERENCES

1. Zhou, B. N., Shi, C. S., Dommise, R. A. and Pieters, L. (1989) *Farmaceutisch Tijdschr. Belg.* **66e**, 55.
2. Shen, J. H., Wang, L. and Zhou, B. N. (1991) *Chin. Chem. Letters* **2**, 629.
3. Shen, J. H., Shi, C. S., Ma, X. J. and Zhou, B. N. (1992) *Nat. Prod. Letters* **1**, 99.
4. Verbit, L. and Clark-Lewis, J. W. (1968) *Tetrahedron* **24**, 5519.
5. Kurosawa, K., Ollis, W. D., Redman, B. T., Sutherland, I. O., Alves, H. M. and Gottlieb, O. R. (1978) *Phytochemistry* **17**, 1423.
6. Harwood, L. M., Oxford, A. J. and Thomson, C. (1987) *J. Chem. Soc., Chem. Commun.* **21**, 1615.