Two New Sesqueneolignans from the Bark of *Illicium dunnianum*

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Two new sesqueneolignans, named dunnianol (**1**) and isodunnianol (**2**), respectively, were isolated from the bark of *Illicium dunnianum* together with the known biphenylneolignan, magnolol (**3**). Compounds **1** and **2** have a similar triphenyl-type neolignan structure to that of macranthol (**4**), which we previously isolated from the pericarps of *I. macranthum*. Their structures were elucidated by means of the detailed analysis of their ¹H- and ¹³C-nuclear magnetic resonance spectra of **1**, **2**, and their acetylation and methylation products, including two-dimensional NMR spectra, comparing with those of magnolol, isomagnolol and macranthol.

Keywords *Illicium dunnianum*; Illiciaceae; sesqueneolignan; dunnianol; isodunnianol; magnolol; honokiol; isomagnolol; macranthol; triphenyl-neolignan

The constituents of the families of the Magnoliales, especially the genus of Magnoliaceae¹⁾ and Lauraceae,²⁾ are characterized by the presence of a series of unusual biphenylneolignans. While Illiciaceae once belonged to Magnoliaceae, no biphenyls have been isolated so far. As a part of a research program dealing with screening toxic sesquiterpenes from *Illicium* plants,³⁾ we previously isolated a triphenylneolignan, macranthol (**4**),⁴⁾ which has a non-symmetrical structure, from the bark of *Illicium macranthum*. The presence of a symmetrical structure of compound "magnolol (**3**)", and non-symmetrical structure of compound "honokiol (**5**)" in biphenyls prompted us to reinvestigate the other *Illicium* plants to isolate the symmetrical triphenylneolignan.

We previously reported the isolation of three pseudoani-

satin-like sesquiterpenes, 6-deoxypseudoanisatin, dunnianin and 6-deoxydunnianin, from the bark of *Illicium dunnianum* TUTCHER (Illiciaceae),⁵⁾ which is one of the toxic *Illicium* plants in China. Reinvestigation of the MeOH extract of the bark of *I. dunnianum* resulted in the isolation of two new triphenyl-neolignans (**1**) and (**2**) together with a well-known biphenyl compound, magnolol (**3**).

The EtOAc soluble part of the MeOH extract (180 g) of the bark of *I. dunnianum* (1.19 kg) was subjected to counter-current distribution using the solvent system of EtOAc-H₂O to give fractions of 1—68 as we reported before.⁵⁾ Of these fractions, 6-deoxypseudoanisatin, dunnianin and 6-deoxydunnianin were isolated from fractions of 41—55. Separation and purification of fractions 56—63 by repeated silica gel chromatography afforded crystalline compound (**1**) (998.5 mg) and oily compound (**2**) (118.8 mg), which we named dunnianol and isodunnianol, respectively, along with magnolol (**3**) (28.0 mg).

Dunnianol (**1**) was obtained as colorless needles, mp 132—133 °C, and its molecular formula, C₂₇H₂₆O₃, the same as that of **4**, was determined by mass spectrum (MS) (*m/z*: 398 [M⁺]) and elemental analysis, and responded positively to the iron(III) chloride test. The feature of the proton nuclear magnetic resonance (¹H-NMR) spectrum of **1** was very similar to that of macranthol (**4**) indicating a type of triphenylneolignan for **1**. But, the carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum of **1** showed only fifteen signal peaks in spite of the molecular formula indicated 27 carbons, suggesting it to be a symmetrical structure for **1**.

Dunnianol (**1**) afforded a methyl-derivative (**6**) by treatment with CH₃I and K₂CO₃ in acetone, and an acetyl-derivative (**7**) with a mixture of 4,4'-dimethylamino-pyridine (DMAP), Ac₂O and pyridine, respectively. The molecular ion peak of these compounds in MS suggested that these are trimethoxy (*m/z*: 440) and triacetoxy (*m/z*: 524) derivatives, respectively. These results indicated **1** has three phenolic hydroxyl groups. The symmetrical structure of **1** was also supported by the methoxy- and acetoxy-methyl signals in the ¹H-NMR spectra of **6** and **7**, respectively; *i.e.* the signals of three methoxy groups appeared as a 3H-singlet at δ_H 3.19 and a 6H-singlet at δ_H 3.78 in the ¹H-NMR spectrum of **6**, and the same property was shown in the case of acetoxy signals (δ_H 1.66 (3H, s) and 2.08 (6H, s)) in the ¹H-NMR spectrum of **7**.

Fig. 1

Fig. 2. Structure of Dunnianol (**1**) and the ¹H-¹³C Long-Range Correlations

TABLE I. ^1H -NMR Data for Dunnianol (**1**) and Isodunnianol (**2**) (400 MHz, in CDCl_3 ; J (Hz) in Parentheses)

Protons	Dunnianol (1)	Protons	Isodunnianol (2)
3,5	7.17 s	3	6.72 d (1.8)
7	3.46 d (6.6)	5	6.90 d (1.8)
8	5.99	7	3.29 d (6.7)
	ddt (18.7, 11.7, 6.6)	8	5.90 ddt (16.1, 9.5, 6.7)
9a	5.13 dd (18.7, 1.8)	9a	5.03 dd (9.5, 1.6)
9b	5.14 dd (11.7, 1.8)	9b	5.04 dd (16.1, 1.5)
3',3''	7.14 d (2.2)	2',6'	6.99 d (8.4)
5',5''	7.15 dd (8.4, 2.2)	3',5'	7.19 d (8.4)
6',6''	6.97 d (8.4)	7''	3.39 d (5.9)
7',7''	3.39 d (6.6)	8'	5.98 ddt (16.1, 9.6, 5.9)
8',8''	5.98	9'a,9''a	5.09 dd (9.6, 1.5)
	ddt (17.2, 10.0, 6.6)	9'b,9''b	5.10 dd (16.1, 1.5)
9'a,9''a	5.07 dd (10.0, 1.9)	3''	7.12 d (2.2)
9'b,9''b	5.10 dd (17.2, 1.9)	5''	7.13 dd (8.8, 2.2)
		6''	7.01 d (8.8)
		7''	3.39 d (5.9)
		8''	5.95 ddt (16.1, 9.6, 5.9)

Assignments were aided by ^1H - ^1H 2D COSY and HMQC spectra.

TABLE II. ^{13}C -NMR Data for Dunnianol (**1**) and Isodunnianol (**2**) (100 MHz, in CDCl_3),^{a)} and Related Compounds, Magnolol (**3**)^{b)} and Isomagnolol (**10**)^{c)}

Carbons	1	3	2	10
1	147.7		141.7	143.9
2	125.4		144.2	145.9
3	131.3		117.8	119.2
4	134.0		132.7	132.6
5			126.5	124.8
6			125.5	116.2
7	39.4		39.5	
8	137.2		137.3	
9	116.2		115.6	
1'	151.4	151.0	154.6	155.4
2'	124.4	124.5	118.5	117.9
3'	131.6	131.4	130.1	129.9
4'	133.2	133.3	135.9	135.3
5'	130.0	129.8	130.1	129.9
6'	117.2	116.8	118.5	117.9
7'	39.4		39.5	
8'	137.5		137.8	
9'	115.8		116.0	
1''			151.8	
2''			125.0	
3''			131.0	
4''			133.3	
5''			129.7	
6''			117.5	
7''			39.5	
8''			137.1	
9''			116.0	

a) Assignments were made with the aid of HMQC and HMBC spectra. b, c) Data were taken from references 6 and 2c, respectively.

The detailed analysis of ^1H - and ^{13}C -NMR spectra of **1** was undertaken with the aid of ^1H - ^1H 2-dimensional correlation spectroscopy (2D COSY), heteronuclear multiple bond correlation (HMBC), and heteronuclear multiple quantum coherence (HMQC) spectroscopies. As a result of these assignments, it was elucidated that the carbon signals for B- (and C-) ring were almost identical with those of magnolol (**3**),⁶⁾ and positions 3 and 5 in the A-ring have protons. Thus, considering the symmetrical structure, two

Fig. 3. Structure of Isodunnianol (**2**) and the ^1H - ^{13}C Long-Range Correlations

phenolic arylpropanoids (B- and C-rings) are linked to positions 2 and 6 of the A-ring. Therefore, the structure of dunnianol (**1**) was determined as shown in Fig. 2.

Isodunnianol (**2**), a colorless oil, was also positive to the iron(III) chloride test. The molecular formula of **2**, $\text{C}_{27}\text{H}_{26}\text{O}_3$, was the same as those of **1** and **4**. The ^1H -NMR spectrum of **2** also resembled those of macranthol (**4**) and dunnianol (**1**), suggesting it to be a triphenyl-sesquienolignan. In spite of the same molecular formula as those of **1** and **4**, compound **2** afforded dimethoxy- (**8**) and diacetoxy-derivatives (**9**) suggesting the presence of only two phenolic hydroxyl groups. Thus, another oxygen should be included in an ether-linkage.

According to the unambiguous assignments of the ^1H - and ^{13}C -NMR spectra of **2** with the aid of ^1H - ^1H 2D COSY, HMBC, and HMQC spectra, it was clarified that the carbon signals for aromatic C-ring were almost identical with those of magnolol (**3**),⁶⁾ and the carbon signals for A and B-rings resembled those of isomagnolol (**10**), which is the isomer of magnolol (**3**), and was isolated from the roots of *Sassafras randaiense* (Lauraceae).^{2c)} The presence of a *p*-substituted benzene ring (B-ring) was also supported by AA'BB' signals at δ_{H} 6.99 (2H, d, $J=8.4$ Hz) and 7.19 (2H, d, $J=8.4$ Hz), and the proton signals for a catechol moiety (A-ring) appeared as AB type signals at δ 6.72 and 6.90 (each 1H, d, $J=1.8$ Hz), respectively. On the other hand, the carbon signal of isomagnolol at δ_{C} 116.2 corresponding to the position C-6 in A-ring showed a distinct downfield shift to δ_{C} 125.5 in the ^{13}C -NMR spectrum of **2**, indicating the C-ring links to position 6 in the A-ring.

To confirm a linkage pattern of three arylpropanoids in the structure of **2**, nuclear Overhauser effect (NOE) experiments were performed on the methylation product (**8**). Two methoxyl signals were shown in the ^1H -NMR spectrum of **8** at δ_{H} 3.59 and 3.77. When the signal at δ_{H} 3.77 was irradiated, a doublet signal at δ_{H} 6.89 (1H, $J=8.0$ Hz, H-6'') in the C-ring was enhanced. An irradiation of the signal at δ_{H} 3.59 produced NOEs at δ_{H} 6.93 (2H, d, $J=8.4$ Hz, H-2', 6') in the B-ring and at δ_{H} 7.08 (1H, d, $J=2.6$ Hz, H-3'') in the C-ring. Therefore, these results confirmed the structure proposed for **2**.

Biphenyls are of interest because of the known pharmacological activities.⁷⁾ Although macranthol (**4**) was found to be biologically non-active by our research, the above compounds obtained here are now under investigation.

Experimental

The melting point was determined on a Yanagimoto micro melting point

apparatus and is uncorrected. ^1H - and ^{13}C -NMR spectra were taken with JEOL GX-400 spectrometers. NOE and 2D COSY experiments were performed on this apparatus. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard. Electron impact mass spectrum (EI-MS) was recorded on a JEOL JMS-DX-303 spectrometer. Infrared (IR) spectra were recorded on a JASCO IR-180 or Shimadzu IR-408. Ultraviolet (UV) absorption maxima were measured on a Hitachi 323 spectrometer.

Extraction and Isolation The bark (1.19 kg) of *I. dunnianum* was extracted with methanol (6 l), and successive separation was undertaken as previously reported.⁵⁾ The EtOAc-soluble part was fractionated by counter-current distribution using the solvent system of EtOAc–H₂O to give fractions of 1–68. 6-Deoxypseudoanisatin, dunnianin and 6-deoxydunnianin were obtained from the residue from fractions of 21–55. Repeated silica gel column chromatography [*n*-hexane–EtOAc (7:3)] of fractions of 56–63 afforded magnolol (**3**) (28.0 mg), a crude compound of **1**, and an oily compound **2**, isodunnianol (118.8 mg). The crude compound of **1** was recrystallized from *n*-hexane–EtOAc to give colorless needles, dunnianol (**1**) (998.5 mg).

Although **3** was not obtained as crystals, this compound was suggested as magnolol because its MS showed a molecular ion at m/z 266 and the appearance of ^1H -NMR spectrum of **3** was identical with that of the figure reported in the reference.^{1c)}

Dunnianol (1) Colorless needles from *n*-hexane–EtOAc, mp 132–133 °C, EI-MS m/z : 398 (M^+). IR ν_{max} (KBr) cm^{-1} : 3260, 3080, 2970, 2900, 1638, 1495, 1415, 970, 910, 610. UV λ_{max} (EtOH) nm (ϵ): 223 (49800), 246 (29000), 296 (6300), 340 (5700). Anal. Calcd for $\text{C}_{27}\text{H}_{26}\text{O}_3$: C, 81.38; H, 6.58. Found: C, 81.29; H, 6.70.

Methylation of Dunnianol (1) **1** (20 mg) was dissolved in a mixture of K_2CO_3 (0.5 g), CH_3I (0.6 ml) and dry acetone (20 ml), and the mixture was refluxed for 3 h. After the solution was cooled down, a grain of K_2SO_3 was filtered off. The filtrate was evaporated to dryness under reduced pressure, and the residual oil was chromatographed on silica gel [*n*-hexane–EtOAc (1:9)] to give a methylated compound (**6**) as an oil (16 mg). EI-MS m/z : 440 (M^+), 426 ($\text{M}^+ - 14$). ^1H -NMR δ (CDCl_3): 3.19 (3H, s, –OMe (C-1)), 3.35 (4H, d, $J=7.0$ Hz, H-7', 7''), 3.40 (2H, d, $J=7.0$ Hz, H-7), 3.78 (6H, s, –OMe (C-1', 1'')), 5.02–5.17 (6H, m), 5.92–6.08 (3H, m), 6.90 (2H, d, $J=8.1$ Hz, H-6', 6''), 7.07 (2H, s, H-3, 5), 7.13 (2H, dd, $J=8.1, 2.6$ Hz, H-5', 5''), 7.14 (2H, d, $J=2.6$ Hz, H-3', 3'').

Acetylation of Dunnianol (1) **1** (10 mg) was dissolved in a mixture of dry pyridine (0.5 ml) and Ac_2O (0.5 ml) with a grain of DMAP (2 mg), and the solution was left overnight at room temperature, then evaporated to dryness under reduced pressure. This residue was chromatographed on silica gel [*n*-hexane–EtOAc (1:9)] to give an oily acetylated compound (**7**) (9 mg). EI-MS m/z : 524 (M^+), 482, 440, 398. ^1H -NMR δ (CDCl_3): 1.66 (3H, s, –OAc), 2.08 (6H, s, –OAc), 3.39 (4H, d, $J=7.0$ Hz, H-7', 7''), 3.41 (2H, d, $J=8.8$ Hz, H-7), 5.06–5.14 (6H, m), 5.91–6.03 (3H, m), 7.08 (2H, d, $J=8.1$ Hz, H-6', 6''), 7.12 (2H, s, H-3, 5), 7.14 (2H, d, $J=2.2$ Hz, H-3', 3''), 7.20 (2H, dd, $J=8.1, 2.2$ Hz, H-5', 5'').

Isodunnianol (2) Colorless oil, $\text{C}_{27}\text{H}_{26}\text{O}_3$, EI-MS m/z : 398 (M^+), 264 ($\text{M}^+ - p\text{-allylphenoxy group}$). IR ν_{max} (Nujol) cm^{-1} : 3350, 3080, 2900, 1637, 1605, 1590, 1425, 997, 910.

Methylation of Isodunnianol (2) **2** (25 mg) was methylated with the same manner as **1** to give a methylated compound (**8**) as an oil (22 mg).

EI-MS m/z : 426 (M^+). ^1H -NMR δ (CDCl_3): 3.32 (2H, d, $J=6.6$ Hz, H-7), 3.35 (4H, d, $J=6.2$ Hz, H-7', 7''), 3.59 (3H, s, –OMe (C-1'')), 3.77 (3H, s, –OMe (C-1)), 5.02–5.08 (6H, m), 5.89–6.03 (3H, m), 6.80 (1H, d, $J=1.8$ Hz, H-3), 6.87 (1H, d, $J=1.8$ Hz, H-4), 6.89 (1H, d, $J=8.0$ Hz, H-6''), 6.93 (2H, d, $J=8.4$ Hz, H-2', 6'), 7.08 (1H, d, $J=2.6$ Hz, H-3''), 7.12 (2H, d, $J=8.4$ Hz, H-3', 5'), 7.13 (1H, dd, $J=8.0, 2.6$ Hz, H-5''), ^{13}C -NMR δ (CDCl_3): 148.5 (C-1), 148.6 (C-2), 120.5 (C-3), 133.6 (C-4), 126.8 (C-5), 131.8 (C-6), 155.3 (C-1'), 117.3 (C-2', 6'), 129.6 (C-3', 5'), 135.4 (C-4'), 156.3 (C-1''), 127.5 (C-2''), 131.5 (C-3''), 134.0 (C-4''), 128.7 (C-5''), 111.0 (C-6''), 55.8 (–OMe (C-1')), 60.8 (–OMe (C-1)), 39.3, 39.5, 39.6, 115.5, 115.6, 116.0, 137.1, 137.7, 137.9 (signals of allyl groups).

Acetylation of Isodunnianol (2) **2** (15 mg) was acetylated with the same manner as **1** to give an acetylated compound (**9**) as an oil (11 mg). EI-MS m/z : 482 (M^+), 440, 398. ^1H -NMR δ (CDCl_3): 1.97 (3H, s, –OAc), 2.08 (3H, s, –OAc), 3.33 (2H, d, $J=7.0$ Hz), 3.36 (2H, d, $J=6.6$ Hz), 3.40 (2H, d, $J=6.6$ Hz), 5.04–5.12 (6H, m), 5.86–6.02 (3H, m) (allyl protons), 6.82 (1H, d, $J=2.2$ Hz, H-3), 6.87 (1H, d, $J=2.2$ Hz, H-5), 6.94 (2H, d, $J=8.8$ Hz, H-2', 6'), 7.08 (1H, d, $J=8.1$ Hz, H-6''), 7.13 (2H, d, $J=8.8$ Hz, H-3', 5'), 7.15 (1H, d, $J=2.2$ Hz, H-3''), 7.21 (1H, dd, $J=8.1, 2.2$ Hz, H-5'').

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