Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 4243–4249

Structure and neurotrophic activity of novel sesqui-neolignans from the pericarps of *Illicium fargesii*

Miyako Moriyama,[a] Jian-Mei Huang,[b] Chun-Shu Yang,[b] Hideaki Hioki,[a] Miwa Kubo,[a] Kenichi Harada[a] and Yoshiyasu Fukuyama[a,*]

[a]*Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan*
[b]*Faculty of Chinese Pharmaceutical Sciences, Beijing University of Chinese Medicine, Beijing 100029, China*

Received 22 February 2007; revised 10 March 2007; accepted 12 March 2007
Available online 14 March 2007

Abstract—The structures of three new sesqui-neolignans, fargenin (**1**) and fargenones A and B (**2** and **3**), together with two previously known sesqui-neolignans, macranthol (**4**) and isodunnianol (**5**), isolated from the methanol extract of the pericarps of *Illicium fargesii* were elucidated on the basis of spectroscopic data. Compounds **1**–**3** are the second three examples of the sesqui-neolignans that contain a variety of non-aromatic rings, and their plausible biosynthetic routes from chavicol are proposed. Among the isolated sesqui-neolignans, isodunnianol has been found to exhibit neurite outgrowth-promoting activity at the concentration ranging from 0.1 to 10 μM in the primary cultured rat cortical neurons.

1. Introduction

Illicium species that belong to the sole genus of the family Illiciaceae are distributed mainly in the southern parts of China,[1] and most of them are considered to be toxic plants. *seco*-Prezizaane-type sesquiterpenes, prenylated C6–C3 compounds and sesqui-neolignans are the secondary metabolites characteristic of the *Illicium* plants.[2] Among them, anisatin is well-known as one of the neurotoxic components in the *Illicium* plants,[3] whereas merrilactone A,[4] an anislactone-type sesquiterpene isolated from *Illlicium merrillianum*, and jiadifenin,[5] a majucin-type sesquiterpene isolated from *Illlicium jiadifengpi*, exhibit neurite outgrowth-promoting activity in the primary cultured rat cortical neurons. Additionally, bicycloillicinone asarone acetal[6] and tricycloillicinone,[7] prenylated C_6–C_3 compounds isolated from *Illlicium tashiroi*, are able to enhance ChAT (choline acetyltransferase) activity in the primary cultured P10 rat septal neurons. Thus, the *Illicium* species have been regarded as one of the attractive plant sources to find chemically and biologically intriguing secondary metabolites. In our ongoing search for neurotrophic substances from the *Illicium* species, three new sesqui-neolignans named fargenin (**1**), fargenone A (**2**), and fargenone B (**3**), together with two known sesqui-neolignans, macranthol (**4**)[8] and isodunnianol (**5**),[9] were isolated from the pericarps of *Illicium fargesii* collected in Yunnan, China. In this paper, we now report the structure elucidation of new compounds **1**–**3**, their plausible biosynthetic routes, and the neurotrophic property of the isolated sesqui-neolignans (Fig. 1).

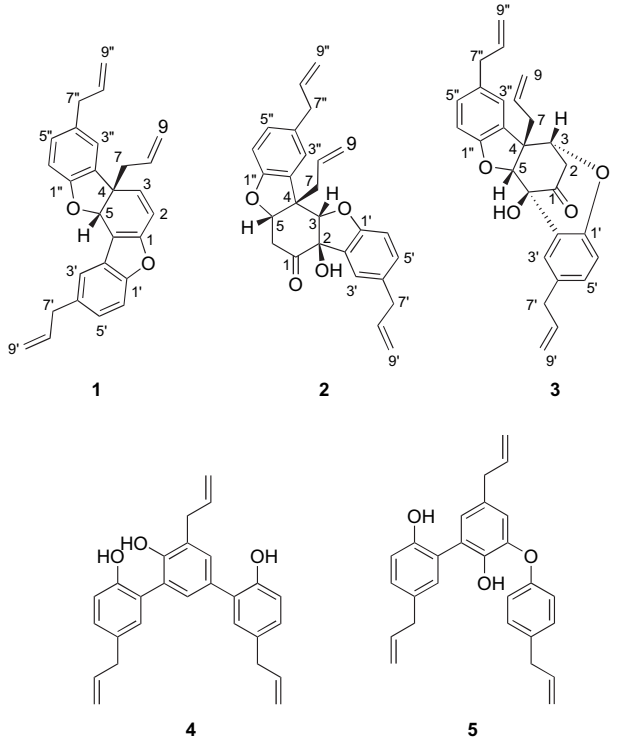


Figure 1. Sesqui-neolignans **1**–**5** isolated from *Illicium fargesii*.

Keywords: *Illicium fargesii*; Sesqui-neolignan; Fargenin; Fargenones A, B; Neurotrophic activity.
* Corresponding author. Tel.: +81 88 622 9611; fax: +81 88 655 3051; e-mail: fukuyama@ph.bunri-u.ac.jp

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.03.063

2. Results and discussion

The methanol extract of the pericarps of *I. fargesii* was purified by a variety of chromatographic methods to afford three new sesqui-neolignans **1**–**3** along with the previously known macranthol and isodunnianol.

Fargenin (**1**) was obtained as an amorphous white solid. The molecular formula of fargenin **1** was established as $C_{27}H_{24}O_2$ by high-resolution EIMS at *m/z* 380.1777 (M^+, Δ −0.1 mmu), indicating 16 degrees of unsaturation. Its IR spectrum displayed absorption due to an aromatic moiety at 1636 cm^{-1}. The ^1H NMR spectrum (Table 1) of **1** showed signals corresponding to three allyl groups [δ_H 2.66 (2H, ddd, *J*=7.1, 1.1, 1.1 Hz, H-7), 5.04 (ddt, *J*=17.3, 3.0, 1.1 Hz, H-9), 5.05 (ddt, *J*=10.2, 3.0, 1.1 Hz, H-9), 5.61 (ddt, *J*=17.3, 10.2, 7.1 Hz, H-8); δ_H 3.51 (2H, dt, *J*=6.9, 1.6 Hz, H-7′), 5.08 (ddt, *J*=16.8, 3.6, 1.6 Hz, H-9′), 5.09 (dt, *J*=10.2, 3.6, 1.6 Hz, H-9′), 6.04 (ddt, *J*=16.8, 10.2, 6.9 Hz, H-8′); δ_H 3.35 (2H, dt, *J*=6.8, 1.6 Hz, H-7″), 5.13 (ddt, *J*=16.8, 3.6, 1.6 Hz, H-9″), 5.14 (ddt, *J*=9.9, 3.6, 1.6 Hz, H-9″), 5.96 (ddt, *J*=16.8, 9.9, 6.8 Hz, H-8″)], two 1,2,4-trisubstituted benzene rings and a disubstituted double bond with a *Z*-geometry at δ_H 5.98 (d, *J*=9.9 Hz, H-3) and 6.51 (d, *J*=9.9 Hz, H-2). The ^{13}C NMR spectroscopic data (Table 1) of **1** showed the presence of twenty seven carbons including three quaternary sp^2 oxygen-bearing carbons at δ_C 152.3, 153.9, and 157.2, a quaternary olefinic carbon at δ_C 108.7, an oxy-methine carbon at δ_C 81.9, and a quaternary sp^3 carbon at δ_C 51.2. These structure fragments (bold line in Fig. 2), as being revealed by analysis of HMQC and ^1H–^1H COSY spectra, were assembled into a whole structure on the basis of the following HMBC analyses. HMBC correlations of H-2 at δ_H 6.51 to C-6 at δ_C 108.7, H-3 to C-4 at δ_C 51.2 and C-1 at δ_C 152.3, and H-5 to C-6, C-1, and C-3 at δ_C135.5 led to the formation of a cyclohexadiene ring. In the HMBC experiment, both the H-7 and H-3″ protons correlated to C-4 and further H-5 showed a cross-peak to the deshielded sp^2 C-1″ resonated at δ_C 157.2, thereby suggesting that both one allyl group and one 4-allylbenzene unit were connected to the C-4 position of this cyclohexadiene ring, and a dihydrofuran ring should be closed between C-5 and C-1″. Further HMBC correlations of the H-3′ proton resonated at δ_H 7.51 to C-6 suggested that the second 4-allylbenzene connects via C-2′ to C-6. These spectroscopic data indicate that fargenin (**1**) belongs to a rarely occurred sesquineolignan derived from three 4-allylphenols (chavicol), one of which is modified to a non-aromatic cyclohexadiene ring.[10] The much deshielded oxygenated carbons of C-1 and C-1′ at δ_C 153.9 and the fact that no hydroxyl group was present together with one remaining unsaturation degree requirement suggested that a dihydrofuran ring was formed between C-1 and C-1′ as depicted in Figure 2, thereby culminating in the proposal of a plane structure **1**. However an alternative structure **1′** was not excluded by the observed HMBC correlations, because the H-3 and H-5 signals appeared at the same chemical shift at δ_H 5.98. If HMBC cross-peaks from H-3 were swapped for those from H-5, two possible structures **1** and **1′** would be consistent with the analysis of the HMBC spectra.

Table 1. ^1H (600 MHz) and ^{13}C NMR (150 MHz) data of **1** in $CDCl_3$

Position	δ_H	δ_C
1		152.3
2	6.51 (d, 9.9)	115.0
3	5.98 (d, 9.9)	135.5
4		51.2
5	5.98 (s)	81.9
6		108.7
7	2.66 (2H, ddd, 7.1, 1.1, 1.1)	44.7
8	5.61 (ddt, 17.3, 10.2, 7.1)	132.1
9	5.04 (ddt, 17.3, 3.0, 1.1); 5.05 (ddt, 10.2, 3.0, 1.1)	119.2
1′		153.9
2′		127.1
3′	7.51 (d, 1.9)	119.2
4′		135.4
5′	7.10 (dd, 8.5, 1.9)	125.1
6′	7.37 (d, 8.5)	111.2
7′	3.51 (2H, dt, 6.9, 1.6)	40.2
8′	6.04 (ddt, 16.8, 10.2, 6.9)	137.9
9′	5.08 (ddt, 16.8, 3.6, 1.6); 5.09 (ddt, 10.2, 3.6, 1.6)	115.7
1″		157.2
2″		131.7
3″	7.03 (d, 1.9)	123.6
4″		132.5
5″	6.93 (dd, 8.1, 1.9)	128.5
6″	6.77 (d, 8.1)	109.8
7″	3.35 (2H, dt, 6.8, 1.6)	39.7
8″	5.96 (ddt, 16.8, 9.9, 6.8)	137.9
9″	5.13 (ddt, 16.8, 3.6, 1.6); 5.14 (ddt, 9.9, 3.6, 1.6)	115.6

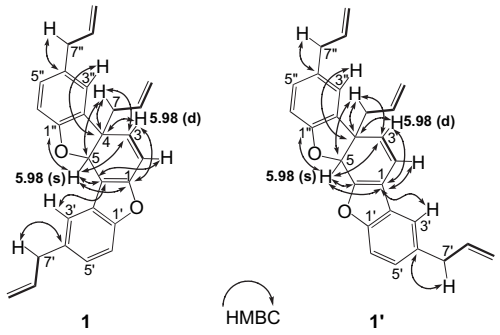


Figure 2. HMBC correlations and two possible plane structures **1** and **1′** for fargenin.

However, the NOESY data (Fig. 3) permitted us to distinguish **1** from **1′**. The allylic H-7 showed the NOE correlations to H-3, H-3″, and H-5, the latest one of which further showed an NOE correlation to H-3′. In contrast, these NOE data applied to the structure **1′** not only ruled out the possibility of **1′** because the spatial distance between H-5 and H-3′ is too far to observe NOE to each other, but also

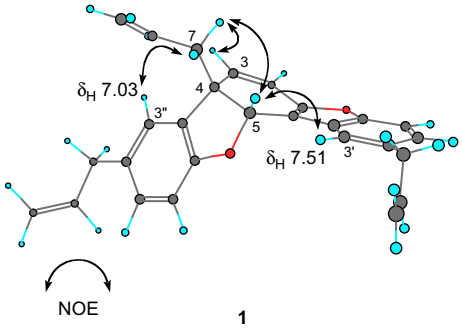


Figure 3. Representative NOESY correlations of fargenin (**1**).

Table 2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of **2** and **3** in $CDCl_3$

Position	**2**		**3**	
	δ_C	δ_H	δ_C	δ_H
1	205.6		204.3	
2	83.6		39.5	3.13 (dd, 20.0, 2.3) 3.30 (dd, 20.0, 4.2)
3	93.6	5.27 (s)	75.6	4.75 (dd, 4.2, 2.3)
4	51.4		52.8	
5	88.5	5.07 (dd, 6.3, 1.4)	93.4	4.71 (s)
6	40.6	2.54 (dd, 14.0, 1.4); 2.82 (dd, 14.0, 6.3)	81.0	
7	42.5	2.69 (dd, 13.5, 8.0); 2.76 (dd, 13.5, 3.9)	43.9	2.37 (dd, 13.7, 7.7); 2.50 (dd, 13.7, 6.5)
8	132.1	5.62 (dddd, 15.7, 11.5, 8.0, 3.9)	131.5	5.55 (dddd, 16.8, 10.2, 7.7, 6.5)
9	119.9	5.14 (dd, 11.5, 2.1); 5.15 (dd, 15.7, 2.1)	120.5	5.13 (dd, 16.8, 1.4); 5.17 (dd, 10.2, 1.4)
1′	157.6		150.1	
2′	128.1		119.9	
3′	124.1	6.88 (d, 1.9)	127.1	7.37 (d, 2.2)
4′	133.7		132.8	
5′	131.5	7.10 (dd, 8.4, 1.9)	130.2	6.82 (dd, 8.2, 2.2)
6′	110.7	6.86 (d, 8.4)	118.0	6.38 (d, 8.2)
7′	39.5	3.28 (2H, dt, 6.9, 1.4)	39.3	3.24 (2H, dt, 6.9, 1.0)
8′	137.2	5.88 (ddt, 17.6, 11.0, 6.9)	137.5	5.87 (ddt, 16.8, 10.2, 6.9)
9′	116.0	5.03 (ddt, 17.6, 3.4, 1.4); 5.04 (ddt, 11.0, 3.4, 1.4)	115.6	4.95 (ddt, 16.8, 2.7, 1.0); 5.00 (ddt, 10.2, 2.7, 1.0)
1″	157.4		158.7	
2″	125.7		128.7	
3″	125.5	6.93 (d, 1.8)	124.0	6.96 (d, 1.4)
4″	132.4		132.0	
5″	130.5	7.00 (dd, 8.2, 1.8)	129.4	6.82 (dd, 8.0, 1.4)
6″	109.6	6.72 (d, 8.2)	109.2	6.49 (d, 8.0)
7″	39.5	3.31 (2H, dt, 6.5, 1.6)	39.6	3.31 (2H, dt, 6.3, 1.4)
8″	137.4	5.92 (ddt, 16.8, 10.2, 6.5)	138.1	5.94 (ddt, 16.5, 10.2, 6.3)
9″	115.5	5.00 (ddt, 16.8, 3.6, 1.6); 5.02 (ddt, 10.2, 3.6, 1.6)	115.4	5.04 (ddt, 16.5, 3.3, 1.4); 5.05 (ddt, 10.2, 3.3, 1.4)
2-OH		3.64 (s)		
6-OH				4.40 (s)

proved the relative stereochemistry regarding C-4 and C-5 to be cis. Thus, on the basis of the above spectroscopic data coupled with the oxidative assembly of three chavicols, the structure of fargenin was rigorously characterized as **1**.

Fargenone A (**2**) gave the molecular formula $C_{27}H_{26}O_4$, as determined by high-resolution EIMS at *m/z* 414.1839 (M^+, Δ 0.8 mmu). Its IR spectrum displayed absorptions due to a hydroxyl group at 3488 cm^{-1}, a carbonyl group at 1730 cm^{-1}, and an aromatic moiety at 1638 and 1612 cm^{-1}. The NMR data (Table 2) of **2** showed the presence of three allyl groups and two 1,2,4-trisubstituted benzene units, indicating being similar to those of **1** except for the presence of a hydroxyl group, the proton signal of which resonated at δ_H 3.64 (s) exchangeable with D_2O, as well as of a carbonyl group at δ_C 205.6 (s). The ^{13}C NMR data showed signals for three oxygen-bearing sp^3 carbons at δ_C 83.6 (s), 88.5 (d), and 93.6 (d), two oxygen-bearing sp^2 carbons at δ_C 157.4 (s) and 157.6 (s), and a carbonyl carbon at δ_C 205.6 (s). These data implied that **2** is another sesqui-neolignan comprising two C6–C3 units and one non-aromatic C6–C3 unit. Analysis of H–H COSY and HMQC provided a structure fragment HC(5)–C(6)H_2 as denoted by the bold lines in Figure 4a, in addition to three allyl groups and two kinds of 1,2,4-trisubstituted benzene rings. Three allyl groups were connected to C-4, C-4′, and C-4″ from the HMBC correlations (H-7/C-4 at δ_C 51.4, H-7′/C-4′ at δ_C 133.7 and H-7″/C-4″ at δ_C 132.4). These spectroscopic data together with the biogenesis of the sesqui-neolignans form oxidative coupling of three 4-allylphenol (chavicol) units postulated that the two original 4-allylphenol units would be connected to the third modified non-aromatic ring with the allyl group at the C-4 position. The hydroxyl proton signal resonated at δ_H 3.64 showed the HMBC correlation to a quaternary C-2 at δ_C 83.6, indicating the hydroxyl group being placed on the C-2 position. Additionally, this hydroxyl proton showed the HMBC correlations to C-1 at δ_C 205.6 and C-3 at δ_C 93.6, and in turn the H-3 and H-5 signals correlated to C-1, C-2, and C-4 and to C-1 and C-4, respectively. As a result, these HMBC data suggested that the third center C6–C3 unit was a cyclohexanone ring not only having the allyl and hydroxyl groups at C-4 and C-2, respectively, but also containing three consecutive quaternary carbons C-2, C-3, and C-4. Since

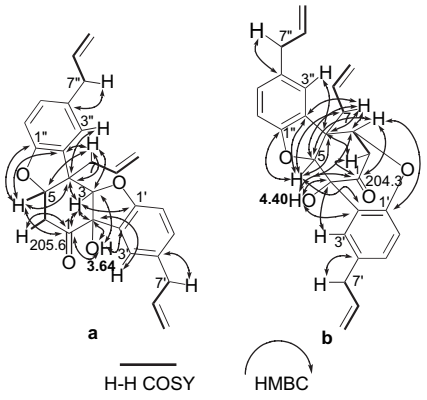


Figure 4. HMBC correlations **a** and **b** for fargenones A (**2**) and B (**3**).

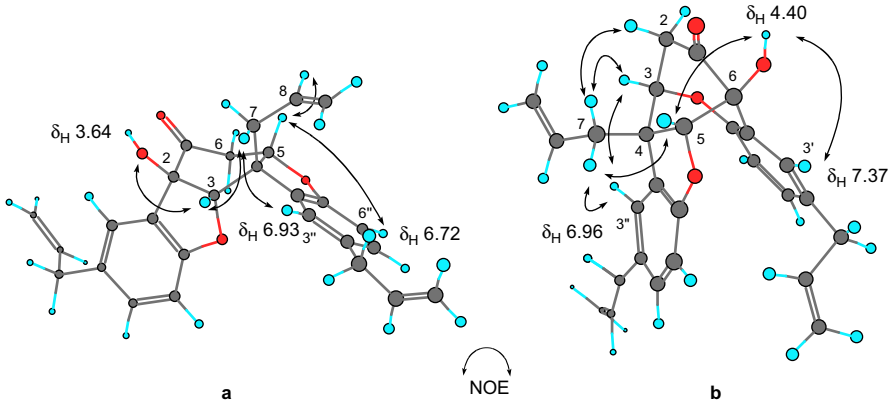
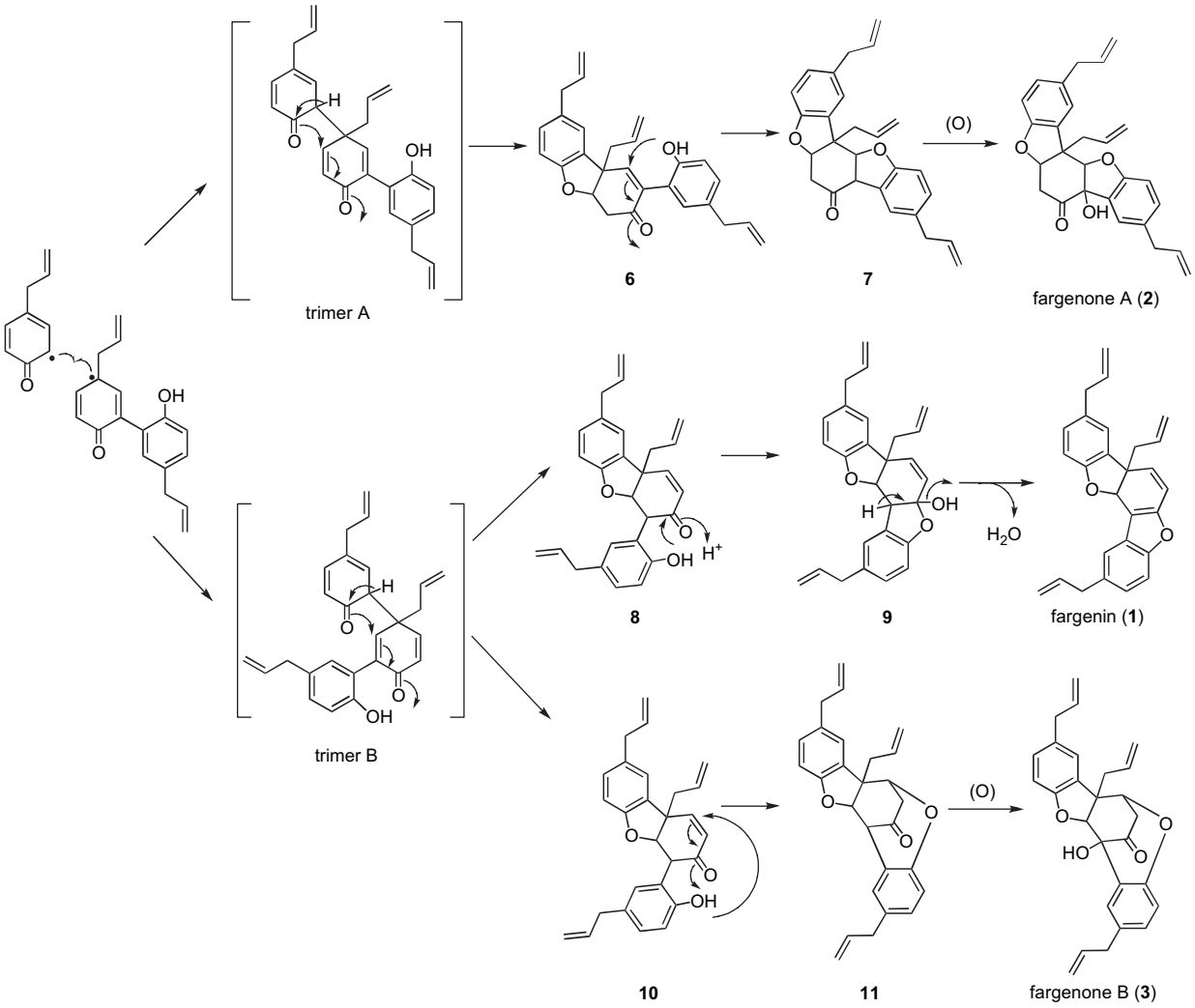


Figure 5. Representative NOESY correlations **a** and **b** for fargenones A (**2**) and B (**3**).

two unsaturation equivalents were left, the presence of two rings was suggested. The aromatic H-3′ and the hydroxyl proton attached at C-2 showed HMBC correlations to C-2 and C-2′, respectively, indicating the linkage between C-2 and C-2′. Additionally, the HMBC correlation between the oxy-methine H-3 resonated at δ_H 5.27 (s) and the deshielded C-1′ at δ_C 157.6 suggested that C-3 and C-1″ were connected through an oxygen to form one dihydrofuran ring. The presence of another dihydrofuran ring was revealed from the HMBC correlations (H-7/C-5 and C-2″ and H-5/C-1″). Thus, these spectroscopic data culminated in proposing the plane structure **a** in Figure 4 for fargenone A. The cis stereochemistry at the C-2 and C-3 ring junctions was assigned by the NOESY correlations observed between OH-2 and H-3, the latter of which was cis disposed to the allyl group at C-4 due to the observation of an NOE between H-3 and one (δ_H 2.76) of the allylic methylene H-7. In addition, the cis stereochemistry at the C-4 and C-5 ring junctions was defined on the basis of an NOE correlation between the oxy-methine H-5 and the olefinic H-8 as depicted in Figure 5a. These NOE data confirmed that all the ring junctions took the same spatial disposition. Thus, the structure of fargenone A was established as **2**.

Compound **3** having the same molecular formula $C_{27}H_{24}O_6$ as that of **2**, obtained from high-resolution EIMS at *m/z* 414.1837 (M^+, Δ 0.6 mmu), exhibited physical data very similar to those of **2**. Analysis of its NMR data (Table 2) with H–H COSY and HMQC showed the presence of the same structure fragments as those of **2**, such as two 1,2,4-trisubstituted benzene rings, three allyl groups, and CH_2–CH–O as depicted by the bold line in Figure 4b in addition to hydroxyl and carbonyl groups although their chemical shift values, in particular, assignable to the center cyclohexanone ring were considerably different from those of **2**. These spectroscopic data implied that **3** would be a variant of the middle C6–C3 ring in the previously discussed structures **1** and **2**. In the HMBC spectra, the hydroxyl proton resonated at δ_H 4.40 showed cross-peaks to the C-5 oxy-methine at δ_C 93.4, the C-6 quaternary carbon at δ_C 81.0, and the C-1 carbonyl at δ_C 204.3, the latter of which in turn correlated to the methylene H-2 and the oxy-methine H-3 at δ_H 4.75 (dd, J=4.2, 2.3 Hz). These HMBC data resulted in C-1 being connected to C-2 and C-6 as well as in the bond formation between C-3 and C-4. The allylic methylene H-7 showed HMBC correlations to the quaternary C-4 at δ_C 52.8 and two kinds of the oxy-methines at δ_C 75.6 and 93.4, accounting for one allylic group attached to C-4 and the connection of C-4 with C-3 and C-5. Thus, the HMBC data corroborated the center C6–C3 unit to be a 3,4,5,6-tetrasubstituted cyclohexanone ring as depicted in Figure 4b. Taking two remaining unsaturation requirements into consideration, the molecule of **3** must have two additional rings like in the cases of **1** and **3**. The connection of C-2″ with C-4 was revealed from HMBC correlation of H-5 and H-7 to C-2″ at δ_C 128.7, and also an ether linkage between C-1″ and C-5 was unambiguously confirmed by the HMBC correlation of H-5 to C-1″ together with consideration of the deshielded δ_C 93.4 and 158.7 assignable to C-5 and C-1″. Moreover, it was rationally explained on the basis of the HMBC correlations of H-3′ to C-6, and H-5 and OH-6 to C-2′ as well as of H-3 to C-1′ together by considering the deshielded C-1′ and C-3 resonated at δ_C 150.1 and 75.6 that C-6 and C-2′ was bonded and also an ether linkage was formed between C-1′ and C-3. These spectroscopic data allowed us to give the pentacyclic plane structure **b** as depicted in Figure 4. The relative configuration of **3**, which was shown in Figure 5b, was clarified from NOESY correlations with H-5/H-7 and OH-6, OH-6/H-3′, H-7/H-2 and H-3, and H-3″/H-3 and H-7. Thus, the structure of fargenone B was determined as **3**.

Since macranthol (**4**) consisting of three C6–C3 units was first reported in 1989 by Kouno et al., only three examples of this natural products, dunnianol and isodunnianol (**5**) from *Illicium dunnianum*.[8–10] and simonsinol from *Illicium simonsii*[11] have been documented in the literatures. A group of these unique natural products have been commonly named sesqui-neolignan according to a naming system of a group of terpenoids. It should be noted that the sesqui-neolignans have occurred solely in *Illicium* species, and have been never found in other plants. The sesqui-neolignans are presumably assembled by oxidative cross-coupling of three C6–C3 units in an *ortho–ortho* or an *ortho–para* fashion.[10] When resultant trimers can undergo aromatization in a dehydration step, macranthol (**4**) and isodunnianol (**5**) are produced. On the other hand, fargenin (**1**), fargenones A (**2**), and B (**3**) would be derived from the trimer A or B, which is the cross-coupled product at the *para* and *ortho* positions between 4-allylphenol and magnolol. Since the coupled trimer A or B contains a non-aromatic cyclohexadienone ring



Scheme 1. Plausible biosynthetic route of **1**–**4**.

as the center unit, fargenin (**1**), fargenones A (**2**), and B (**3**) would be formed by a tandem intramolecular 1,4-addition and subsequent oxidation. As summarized in Scheme 1, an intramolecular oxy-Michael addition of one phenol to the center dienone (trimer A) would lead to **6**, which in turn would repeat an intramolecular oxy-Michael addition of the other phenol to the center enone followed by oxidation to give rise to fargenone A (**2**). Fargenin (**1**) would be biosynthesized from the trimer B via **8** and **9** by an intramolecular oxy-Michael addition of the phenol attached at the C-4 position of the center ring to the dienone, followed by acetalization with 4-allylphenol attached at the C-2 position of the center ring and dehydration, whereas fargenin B (**3**) would be produced from the trimer B in similar courses (**10** and **11**) as shown in Scheme 1.

In the previous papers,[12] we reported that honokiol and magnolol, which belong to biphenyl-type neolignans, exhibited neurotrophic activity in the primary cultured rat cortical neurons. Since the sesqui-neolignans **1**–**5** share the same C6–C3 unit as honokiol and magnolol in the molecule, we expected that they may have likewise neurotrophic property in the primary cultured rat cortical neurons. As a result, isodunnianol (**5**) was found to significantly promote neurite outgrowth of the cultured rat cortical neurons in the range of concentration from 0.1 to 10 μM, but the other compounds **1**–**4** exhibited no efficacy at the same concentration.[13] As shown in Figure 6, the measurement of longest neurite extending from each cell body indicates that **5** can promote neurite outgrowth as effectively as bFGF, basic fibroblast growth factor. It should be noted that two C6–C3 units of isodunnianol (**5**) precisely correspond to the molecule of magnolol. This consistency may be responsible for neurite outgrowth-promoting activity of **5**.

In conclusion, we have isolated three new sesqui-neolignans (**1**–**3**) from *I. fargesii*, which are the second three examples of sesqui-neolignans bearing non-aromatic C6–C3 units, and have proposed a plausible biosynthetic route leading to these sesqui-neolignans. The present chemical studies indicate that *I. fargesii* is rich in C6–C3 compounds but elaborates few *seco*-prezizaane-type sesquiterpenes compared with other *Illicium* species.[14,15] More interestingly, isodunnianol (**5**) shows potential as candidates of non-peptidal neurotrophic agents useful for treatment of neurodegenerative diseases.[16]

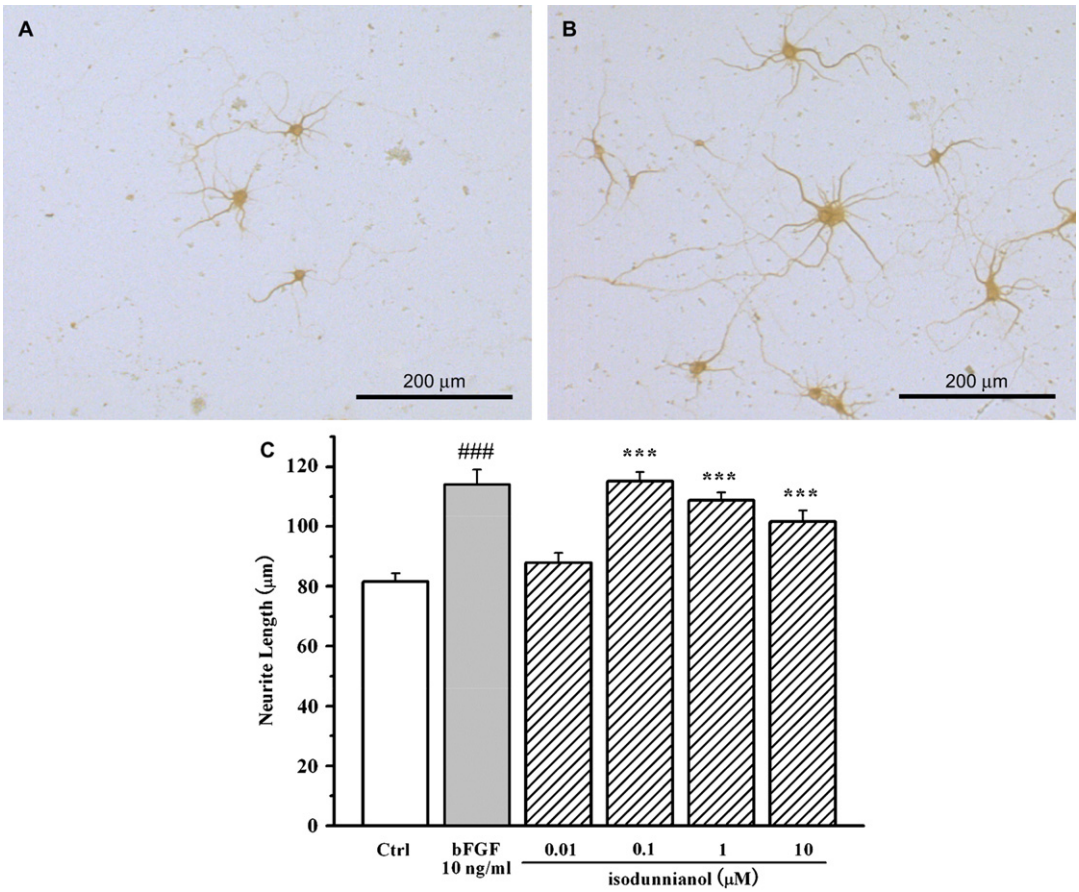


Figure 6. Neurite outgrowth-promoting activity of isodunnianol (**5**) in the primary cultured rat cortical neurons. (**A**): morphology of neurons in control groups, (**B**): morphology of neurons in 0.1 μM **5**, (**C**): quantitative analysis of dose-dependent manner. In each group, the average length of the primary dendrite-like process of 80 neurons was measured. Data were expressed as means±SE. The difference between groups was tested with one-way ANOVA followed by Bonferroni post hoc means comparison or Student's *t*-test. ###, ***, $p<0.001$ vs control. Data presented here were derived from one of the two repeated experiments with similar results. Bar=200 μm.

3. Experimental

3.1. General experimental procedure

Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. IR spectra were measured on a JASCO FT-IR 5300 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on a Varian Unity 600 instrument. Chemical shifts are given as δ (ppm) with TMS as an internal standard. MS were recorded on a JEOL AX-500 instrument. Column chromatography was carried out on Kieselgel 60 (70–230 mesh) and Wako gel C-300.

3.2. Plant material

The pericarps of *I. fargesii* were collected in Yunnan, China, in August 1998. A voucher specimen has been deposited at Beijing University of Chinese Medicine.

3.3. Extraction and isolation

The dried pericarps of *I. fargesii* were extracted with MeOH to yield 600 g of MeOH extract. The extract (240 g) was chromatographed over a silica gel (Kieselgel 60) eluting with a step gradient of CH_2Cl_2 (100%), CH_2Cl_2/EtOAc (9:1), CH_2Cl_2/EtOAc (1:1), CH_2Cl_2/EtOAc (1:9), EtOAc (100%), EtOAc/MeOH (9:1), and EtOAc/MeOH (1:1) to give eight fractions (1–8). Fraction 1 (4.4 g) was first subjected to a silica gel (Wako gel C-300) column chromatography eluting with *n*-hexane/EtOAc (4:1) to give fractions (9–16). Fraction 11 (1.6 g) was separated by a silica gel chromatography with *n*-hexane/EtOAc (4:1) to give fractions (17–22), and fraction 19 (760 mg) subjected to a silica gel (Wako gel C-300) chromatography eluting with *n*-hexane/CH_2Cl_2 (4:1) to give fractions (23–27). Fraction 25 (80 mg) was purified by preparative silica gel TLC [*n*-hexane/CH_2Cl_2 (1:1)] to give fargenin (**1**) (5.7 mg). Fraction 12 was separated by a silica gel chromatography with *n*-hexane/CH_2Cl_2 (4:1) to give fraction (32–41), and fraction 41 (376 mg) was separated by a silica gel chromatography with *n*-hexane/CH_2Cl_2 (2:1) to give fraction (42–48). Additionally, fraction 46 was subjected to a silica gel chromatography with *n*-hexane/EtOAc (15:1) to give fractions (49–58), followed by preparative reversed-phase TLC [MeOH (100%)] of fraction 51 to give fargenone A (**2**) (3.5 mg). Fraction 2 (1.1 g) was first subjected to a silica gel (Wako gel C-300) chromatography eluting with *n*-hexane/EtOAc (10:1) to give fractions (59–66). Fraction 64 and 65 were separated by preparative reversed-phase ODS TLC [MeOH/H_2O (98:2)] to give fargenone B (**3**) (8.4 mg).

3.3.1. Fargenin (1). Amorphous solid; $[\alpha]_D^{12}$ −12.7 (*c* 0.80, $CHCl_3$); IR (film) ν_{max} 1636 (aroma.) cm^{-1}; UV (EtOH) λ_{max} 293 nm (ε 3300), 306 nm (ε 3200); EIMS *m/z* (rel int.) 380 $[M]^+$ (57), 339 (21), 298 (100); HREIMS *m/z* 380.1777 (M^+, calcd for $C_{27}H_{24}O_2$, 380.1776); ^1H and ^{13}C NMR data see Table 1.

3.3.2. Fargenone A (2). Amorphous powder; $[\alpha]_D^{21}$ +14.2 (*c* 1.53, $CHCl_3$); IR (film) ν_{max} 3488 (OH), 1736 (C═O), 1638, 1612 (aroma.) cm^{-1}; UV (EtOH) λ_{max} 292 nm (ε 3100); EIMS *m/z* (rel int.) 414 $[M]^+$ (37), 373 (9), 199 (52); HREIMS *m/z* 414.1839 (M^+, calcd for $C_{27}H_{26}O_4$, 414.1831); ^1H and ^{13}C NMR data see Table 2.

3.3.3. Fargenone B (3). Amorphous powder; $[\alpha]_D^{22}$ −19.2 (*c* 1.54, $CHCl_3$); IR (film) ν_{max} 3463 (OH), 1730 (C═O), 1638, 1612 (aroma.) cm^{-1}; UV (EtOH) λ_{max} 286 nm (ε 2200); EIMS *m/z* (rel int.) 414 $[M]^+$ (70), 373 (2), 197 (6); HREIMS *m/z* 414.1837 (M^+, calcd for $C_{27}H_{26}O_4$, 414.1831); ^1H and ^{13}C NMR data see Table 2.

3.4. Neurotrophic activity in the primary cultured rat cortical neurons

After freshly isolated cortical neurons were cultured in poly-L-lysine-coated 24-well plates at a density of 5000 cells cm^{-2} for 24 h, culture medium was changed from DMEM/10% FBS to Neurobasal medium/2% B27 with various concentrations of test samples and bFGF as positive control. After further culture for 6 days, the neurons were fixed with 4% paraformaldehyde in PBS for 20 min, and permeated with 0.1% Triton X-100 in PBS for 20 min after endogenous peroxidase activity was blocked by freshly prepared 0.3% H_2O_2 for 20 min. For anti-MAP2 staining, the neurons were incubated in primary antibody [anti-MAP2 (1:1000)] overnight at 4 °C followed by incubation with horse peroxidase-conjugated second antibody [SimpleStain PO (1:2)] for 1 h, and then peroxidase was developed with 200 μl substrate SimpleStain DAB solution. The length of primary (longest) process stained in each procedure was determined with image analysis software system LuminaVision 1.0/MacScope 2.6. At least 80-well immunohistochemically stained neurons, which did not grow on or near glial cells and made no connection to more than two cells, were selected for measurements of their primary neurite length.

Acknowledgements

We thank Dr. M. Tanaka and Ms. Y. Okamoto for measurements of NMR and MS spectra. This work was supported by a Grant-in-Aid for Priority Area (No. 18032085) from the Ministry of Education, Science, Sports, Culture, and Technology of Japan.

References and notes

1. Richard, M. K.; Saunders, F. L. S. *Bot. J. Linn. Soc.* **1995**, *117*, 333–352.
2. For reviews of chemical constituents of the *Illicium* species, see: (a) Fukuyama, Y.; Huang, J.-M. *Illicium, Pimpinella and Foeniculum*; Jodral, M. M., Ed.; CRC: Florida, FL, 2004; pp 31–68; (b) Fukuyama, Y.; Huang, J.-M. *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 2005; Vol. 32, pp 395–429.
3. Lane, J. F.; Koch, W. T.; Leeds, N. S.; Gorin, G. *J. Am. Chem. Soc.* **1952**, *74*, 3211–3215.
4. Huang, J.-M.; Yokoyama, R.; Yang, C.-S.; Fukuyama, Y. *Tetrahedron Lett.* **2000**, *41*, 6111–6114.
5. Yokoyama, R.; Huang, J.-M.; Yang, C.-S.; Fukuyama, Y. *J. Nat. Prod.* **2002**, *65*, 527–531.
6. Fukuyama, Y.; Hata, Y.; Kodama, M. *Planta Med.* **1997**, *63*, 275–277.
7. Fukuyama, Y.; Shida, N.; Kodama, M. *Chem. Pharm. Bull.* **1995**, *43*, 2270–2272.
8. Kouno, I.; Hashimoto, A.; Kawano, N.; Yang, C.-S. *Chem. Pharm. Bull.* **1989**, *37*, 1291–1292.
9. Kouno, I.; Morisaki, T.; Hara, Y.; Yang, C.-S. *Chem. Pharm. Bull.* **1991**, *39*, 2606–2608.
10. Sy, L.-K.; Brown, G. D. *Phytochemistry* **1996**, *43*, 1417–1419.
11. Kouno, I.; Iwamoto, C.; Kameda, Y.; Tanaka, T.; Yang, C.-S. *Chem. Pharm. Bull.* **1994**, *42*, 112–114.
12. Fukuyama, Y.; Nakade, K.; Minoshima, Y.; Yokoyama, R.; Zhai, H.; Mistumoto, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1163–1166.
13. Macranthol (**4**) showed no neurite outgrowth-promoting activity in spite of having the honokiol unit in the molecule, but exhibited moderate neuroprotetcive activity at 10 μM in the rat cortical neurons cultured in the N2 medium.[12]
14. Sy, L.-K.; Sauders, R. M. K.; Brown, G. D. *Phytochemistry* **1997**, *44*, 1099–1108.
15. Ward, R. S. *Nat. Prod. Rep.* **1995**, *12*, 183–205.
16. Xie, Y.; Long, F. M. *J. Mol. Neurosci.* **2002**, *19*, 107–111.