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PRENYLATED C_6-C_3 COMPOUNDS FROM ROOT BARK OF *ILLICIUM ANISATUM*

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Key Word Index—*Illicium anisatum*; Illiciaceae; prenylated C_6-C_3 compounds; 11-*epi*-illicinone E; 2,3-dehydro-5,6-di-*O*-methylillifunone E; 2,3-dihydroillicinone E; 4,12-di-*O*-methylillifunone C.

Abstract—On examination of the constituents from the root bark of *Illicium anisatum*, six prenylated C_6-C_3 compounds have been isolated, including two known ones. The structures were elucidated by means of spectroscopic analysis. © 1997 Elsevier Science Ltd

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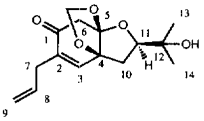
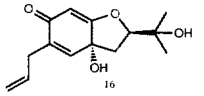
INTRODUCTION

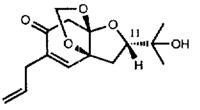
We have described the isolation and structural determination of the structurally novel constituents classified into three groups, sesquiterpene, triphenyl-neolignan and glyceryl C_6-C_3, from Chinese *Illicium* plants [1-9]. On the other hand, Japanese star-anise, *I. anisatum*, is well known as a toxic plant and sesquiterpenes, anisatin, neoanisatin [10, 11] and 2-oxyneoanisatin [12], of the constituents from the fruits, has shown to be responsible for its toxicity. As the root bark of this species was also suggested to have a convulsive component [13], we have examined the constituents of this part. Although none of the toxic component was isolated, four new prenylated C_6-C_3 compounds (**3**–**6**) named 11-*epi*-illicinone E, 2,3-dehydro-5,6-di-*O*-methylillifunone E, 2,3-dihydroillicinone E and 4,12-di-*O*-methylillifunone C, respectively, were isolated together with two known compounds, illicinone E (**1**) and 2,3-dehydroillifunone C (**2**). These types of compounds have been frequently isolated only from *Illicium* plants [14-21]. We now describe the isolation and structural determination of the four new prenylated C_6-C_3 compounds.



1 Illicinone E



2 2,3 Dehydroillifunone C



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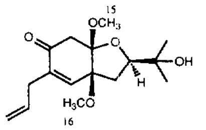

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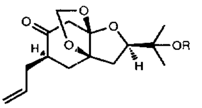
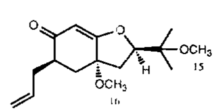


5 R=H
7 R=CH_3: 13-*O*-Methyl-2,3-dihydroillicinone E



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RESULTS AND DISCUSSION

The MeOH extract of the root bark of *I. anisatum* was separated by Sephadex LH-20 column chromatography with MeOH to give three fractions. The middle part of these fractions was fractionated by CHP-20P column chromatography with methanol–water to give four fractions. Of these, the fourth fraction was purified by MPLC affording six compounds (**1**–**6**). Compound **1** was identified by illicinone E, by comparison with ^1H and ^{13}C NMR data described in the literature [18]. Compound **2** was identified in a similar way as 2,3-dehydroillifunone C [20].

Compound **3** had the molecular formula $C_{15}H_{20}O_5$ based on the ion peak at m/z 280 in the mass spectrum and the presence of 15 carbon signals in its ^{13}C NMR spectrum of **3**. The IR spectrum revealed the presence of a hydroxyl (3500 cm^{-1}) and an α,β-conjugated carbonyl (1685 cm^{-1}) groups. The NMR data indicated the presence of an allyl, an isolated methylene [δ_H 2.80 ($d, J = 17$ Hz) and 3.20 ($d, J = 17$ Hz); δ_C 42.0], a methylenedioxy group [δ_H 4.94, 5.21 (each s); δ_C 94.8] and a dimethyl carbinol group [δ_H 1.11, 1.20; δ_C 24.6,

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signal for a methoxyl group. Hence, the structure of compound **5** was determined to be 2,3-dihydroillicinone E. The configuration of the C-2 position was analysed from the coupling constant between H-2 and H3a, H-3b protons, being the same as that of **7**.

The EI-mass and the ^{13}C NMR spectra of compound **6** gave the molecular formula $C_{16}H_{24}O_4$. The IR spectrum showed the absorption band for an α,β-conjugated carbonyl group at 1645 cm^{-1}. The NMR spectra of **6** indicated the presence of two methoxyl groups [δ_H 3.26 (3H, *s*) and 3.32 (3H, *s*); δ_C 50.2 and 51.8], a dimethyl carbinol group [δ_H 1.25 (3H, *s*) and 1.18 (3H, *s*); δ_C 20.4 and 21.7], an allyl group and a carbonyl group (δ_C 199.5). In the H-H COSY spectrum, the proton connectivity of an allyl group was extended to methine and methylene groups. These features were typical of those of a 5,6-dehydroillifunone-type of structure. Therefore, the two methoxyl groups were located at the C-4 and C-12 positions, respectively. The lower shift value of the H-11 proton signal at δ_H 4.58 indicated the presence of a 1,3-*syn*-methoxyl group, i.e. a 4*S*, 11*R* system. If the configurations of C-2 and C-4 were on the same side (2*S*, 4*S* or 2*R*, 4*R*), the carbon chemical shift of C-2 would increase by 45 ppm, whereas if they were on opposed side (2*R*, 4*S* or 2*S*, 4*R*), the shift would be *ca* 40 ppm [20]. The carbon chemical shift of C-2 in the ^{13}C NMR spectrum of **6** was δ_C 40.2, confirming that the configuration of C-2 was on the opposite side to the 4-methoxyl group. Consequently, the structure of **6** was determined to be 4,12-di-*O*-methylillifunone C.

EXPERIMENTAL

General. ^1H (500 MHz) and ^{13}C (125 MHz) NMR: $CDCl_3$, TMS as int. standard. CC: silica gel (Merck, 70–230 mesh), Diaion CHP-20P (Mitsubishi Kasei Co. Ltd.) and Sephadex LH-20. TCL: precoated silica gel F_{254} plates.

Plant material. Root bark of *I. anisatum* L., identified by Dr N. K. (ex-professor of pharmacognosy in Nagasaki University), was collected on a small mountain of Yamaguchi prefecture, Japan. A voucher specimen is deposited at our botanical garden in Nagasaki University.

Extraction and isolation. Chopped root bark (207.3 g) was immersed in MeOH at room temp. for one week. The MeOH extract was evapd *in vacuo* to give a crude brown extract (27.7 g), which was fractionated by CC on Sephadex LH-20 using MeOH to afford 3 frs. Of these frs, fr.2 was sepd by CC on Diaion CHP-20P developing with MeOH–H_2O (7:3) to give 5 frs (I–V). Fr.IV was further fractionated and repeatedly purified by CC on silica gel using $CHCl_3$–MeOH (49:1) to yield compounds **1** (3 mg), **2** (24.9 mg), **3** (29.1 mg), **4** (8.8 mg), **5** (6 mg) and **6** (4.9 mg), respectively.

11-Epi-*illicinone E.* (**3**). Oil. $[\alpha]_D^{15}$ $-7.8°$ (MeOH; *c* 2.08). $C_{15}H_{20}O_5$. EI-MS *m/z* 280 $[M]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1685 (conj. C = O), 1642 (C = C). ^{13}C and ^1H NMR: Tables 1 and 2.

2,3-*Dehydro*-5,6-*di*-O-*methylillifunone E* (**4**). Oil. $[\alpha]_D^{15}$ $+68.3°$ (MeOH; *c* 1.38). $C_{16}H_{24}O_5$. EI-MS *m/z* 296 $[M]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1682 (conj. C = O), 1640 (C = C). ^{13}C and ^1H NMR: Tables 1 and 2.

2,3-*Dihydroillicinone E* (**5**). Oil. $[\alpha]_D^{15}$ $+16.1°$ (MeOH; *c* 0.55). $C_{15}H_{22}O_5$. EI-MS *m/z* 282 $[M]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1718 (C = O), 1640 (C = C). ^{13}C and ^1H NMR: Tables 1 and 2.

4,12-*Di*-O-*methylillicinone C* (**6**). Colourless amorphous powder. $[\alpha]_D^{15}$ $+13.0°$ (MeOH; *c* 0.36). $C_{16}H_{24}O_4$. EI-MS *m/z* 280 $[M]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1645 (conj. C = O). ^{13}C and ^1H NMR: Tables 1 and 2.

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Table 1. ^{13}C NMR (125 MHz; $CDCl_3$) spectral data of compounds **1**, **3**–**6**

C	**1***	**3**	**4**	**5**	**6**
1	194.6	194.1	194.4	208.1	199.5
2	138.3	138.8	141.2	43.7	40.2
3	138.8	138.0	142.0	34.3	34.5
4	85.4	85.6	81.3	88.4	74.7
5	110.4	110.9	102.4	111.3	177.3
6	44.7	46.3	42.0	47.4	101.2
7	33.2	33.1	33.0	34.7	34.1
8	134.1	134.1	134.5	135.1	136.3
9	117.9	117.8	117.6	117.9	116.9
10	35.9	37.3	36.2	36.2	33.6
11	84.1	86.1	83.7	84.9	90.1
12	71.0	70.2	70.9	71.1	80.8
13	24.3	24.3	24.5	24.1	21.7
14	27.0	27.5	25.6	27.5	20.4
O-CH_2-O	94.3	94.8		92.8	
OMe			49.0		50.2
OMe			52.8		51.8

* Good agreement with data of ref. [18].

25.6]. On the other hand, the ^1H and ^{13}C NMR spectra were similar to those of illicinone E (Tables 1 and 2), indicating that **3** was also a prenylated C_6-C_3 compound; this was also supported by HMBC correlations. All of the carbon signals of **3** appeared at similar values to those of illicinone E, except for two signals of C-10 and C-11, suggesting that **3** is a stereoisomer of illicinone E. The C-11 signal of **3** shifted to lower field by 2 ppm compared with that of illicinone E, and the H-11 signal of **3** shifted 0.46 ppm to lower field compared with illicinone E. For the reason of the anisotropic effect by oxygens of a methylene dioxy group [20], the H-11 proton signal may shift to lower field. Thus, the structure of compound **3** was concluded to be the 11-epimer of illicinone E.

Compound **4** had the molecular formula $C_{16}H_{24}O_5$ judging from the ion peak at *m/z* 296 in the mass spectrum and 16 carbon signals in the ^{13}C NMR spectrum. The IR spectrum showed absorption bands at 3500 and 1682 cm^{-1}, indicating the presence of a hydroxyl group and an α,β-conjugated carbonyl group. The ^1H and ^{13}C NMR spectra also strongly resembled to those of illicinone E, except for the appearance of two methoxyl signals [δ_H 3.44 (δ_C 49.0) and δ_H 3.39 (δ_C 52.8)], in place of the methylenedioxy group of illicinone E. The positions of the two methoxyl groups were determined as follows. One of the methoxyl signals at δ_H 3.44 was correlated with the C-5 signal of δ_C 104.4, and the signal of δ_H 3.39 had connectivity with the C-4 signal of δ_C 81.3 in the HMBC spectrum, indicating that the two methoxyl groups are located at the C-4 and C-5 positions, respectively. As the signal of H-11, δ_H 3.62, appeared at a similar position to that of illicinone E, the configurations of two methoxyl groups were the same as those of the methylenedioxy group of illicinone E and the configuration at C-11 of **4** was also the same as that of illicinone E. Accordingly, the structure of **4** was concluded to be 2,3-dehydro-5,6-di-*O*-methylillifunone E, based on the structure of illifunone E [20].

Compound **5** had the molecular formula $C_{15}H_{22}O_5$. The IR spectrum showed absorption for a carbonyl group (1718 cm^{-1}). The NMR data indicated the presence of an allyl, methylenedioxy group [δ_H 5.10 (2H, *s*); δ_C 92.8] and a dimethyl carbinol group [δ_H 1.27 (3H, *s*) and 1.11 (3H, *s*); δ_C 24.1 and 27.5]. All these data strongly resembled those of 13-*O*-methyl-2,3-dihydroillicinone E (**7**) [18], except for the absence of the

Table 2. ^1H NMR (500 MHz; $CDCl_3$) spectral data of compounds **1**, **3**–**6**

C	**1***	**3**	**4**	**5**	**6**
2	—	—	—	2.22 (*dd*, 8, 4)	2.57 (*m*)
3a	6.59 (*t*, 1)	6.52 (*s*)	6.41 (*d*, 1)	2.18 (*dd*, 12, 8)	1.56 (*dd*, 15, 13.5)
3b				2.26 (*dd*, 12, 4)	2.55 (*dd*, 15, 4.5)
6a	2.92 (*d*, 17)	2.80 (*d*, 17)	2.66 (*d*, 17)	2.80 (*d*, 16)	5.46 (*s*)
6b	3.04 (*d*, 17)	3.28 (*d*, 17)	3.20 (*d*, 17)	2.92 (*d*,16)	—
7	3.02 (*dd*, 7, 3)	3.00 (*dd*, 12, 3)	3.06 (*d*, 7)	2.20 (*m*)	2.16 (*m*)
				2.63 (*m*)	2.74 (*m*)
8	5.80 (*ddt*, 17, 10, 3)	5.78 (*ddd*, 17, 10, 3)	5.83 (*ddt*, 17, 10, 7)	5.73 (*ddt*, 17, 10, 2)	5.75 (*ddt*, 17, 10, 3)
9a	5.10 (*dd*, 17, 1.5)	5.09 (*dd*, 17, 1)	5.09 (*dd*, 17, 2)	5.12 (*m*)	5.05 (*dd*, 10, 2)
9b	5.13 (*dd*, 10, 1.5)	5.12 (*dd*, 10, 1)	5.13 (*dd*, 10, 2)	5.14 (*m*)	5.07 (*dd*. 17, 2)
10a	2.13 (*dd*. 14, 7)	2.14 (*dd*, 14, 11)	2.04 (*dd*, 12, 6)	2.09 (*dd*, 14, 7)	1.82 (*dd*, 14, 10)
10b	2.58 (*dd*, 14, 9)	2.38 (*dd*, 14, 5)	2.57 (*dd*, 12. 11)	2.47 (*dd*. 10, 10)	2.39 (*dd*, 14, 5)
11	3.80 (*dd*, 9, 7)	4.26 (*dd*, 11, 5)	3.62 (*dd*, 11, 6)	3.74 (*dd*, 10, 7)	4.58 (*dd*, 10, 5)
13	1.14 (*s*)	1.12 (*s*)	1.11 (*s*)	1.11 (*s*)	1.18 (*s*)
14	1.27 (*s*)	1.31 (*s*)	1.20 (*s*)	1.27 (*s*)	1.25 (*s*)
O-CH_2-O	5.13 (*s*)	4.94 (*s*)	—	5.10 (*s*)	—
	5.28 (*s*)	5.21 (*s*)			
OMe (15)	—	—	3.44 (*s*)	—	3.32 (*s*)
OMe (16)	—	—	3.39 (*s*)	—	3.26 (*s*)

* Good agreement with data of ref. [18].