Isolation of Neoanisatin Derivatives from the Pericarps of *Illicium majus* with Other Constituents

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Further studies on the constituents of *Illicium majus* have resulted in the isolation of five new neoanisatin derivatives and two known phytoquinoides together with two known neolignans. Two of the neoanisatin derivatives are 1-hydroxyl, which are the first examples isolated anisatin derivatives so far. Another one, 2-oxoneoanisatin, exhibited picrotoxin-like convulsions in mice, the same as anisatin. The other two 3,4-dehydroxy-2-oxoneoanisatin isomers could not be separated from each other, even with high performance liquid chromatography.

Keywords *Illicium majus*, Illiciaceae; pericarps; 1-hydroxylneoanisatin; 2-oxoneoanisatin; neolignan; phytoquinoid

In the course of our investigation of the toxic constituents of the pericarps of *Illicium majus* HOOK. f. *et* THOMS., which is one of the toxic *Illicium* plants in China, we reported the isolation and structure determination of sesquiterpene lactones.[1-3] In these reports, we described the isolation of anisatin and other anisatin-like sesquiterpenes, which we call "majucin-type" compounds. This paper deals with the isolation and structure determination of the neoanisatin derivatives and other constituents from the pericarps of *I. majus*.

As we described before,[2] the MeOH extract of *I. majus* was divided into *n*-hexane-, AcOEt-, *n*-BuOH- and H_2O-soluble parts. Then, the AcOEt-soluble part was partitioned between AcOEt and H_2O with a counter-current distribution apparatus to give five fractions (I—V). The isolation of fractions II, III and IV afforded pseudomajucin,[1] anisatin, neomajucin, 2,3-dehydroneomajucin,[2] and six 2-oxyneomajucins.[3] The investigation of fraction V afforded **1** (16.5 mg), **2** (132 mg), a mixture of **4** and **5** (10 mg in the total amount) and two known phytoquinoides (**6**) (61 mg) and (**7**) (11 mg), respectively. From the *n*-BuOH-soluble part (286 g), a large amount of shikimic acid was removed by repeated column chromatography on Amberlite IRA-400; and subsequent chromatography on silica gel gave pseudomajucin glucoside[1] and two known neolignans, **8** (83 mg) and **9** (180 mg), respectively, together with a neoanisatin derivative (**3**) (10 mg).

Compound **1** was obtained as a colorless oil, $[\alpha]_D$ −16.9° (c=0.41, dioxane), and has the molecular formula, $C_{15}H_{20}O_8$, the same as anisatin, as analyzed by electron impact mass spectrum (EI-MS), and carbon counts in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. Absorptions due to a hydroxyl group at 3320 cm^{-1}, β-lactone at 1820 cm^{-1}, and δ-lactone at 1720 cm^{-1} were shown in the infrared (IR) spectrum of **1**. The presence of the β-lactone moiety was also suggested by the signal at δ_C 168.1 in the ^{13}C-NMR spectrum, and the characteristic small coupling constant (J=6.2 Hz) of AB type doublet signals at δ_H 4.49 and 4.90 in the proton ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**. The ^{13}C-NMR spectral data of **1** (Table II), analyzed by the heteronuclear multiple bond correlation (HMBC) spectrum, were similar to those of anisatin, except for the signals due to C-1 and C-3. The C-1 signal, revealed by HMBC spectrum, was assigned as a singlet at δ_C 81.3, and the C-3 signal was triplet at δ_C 33.2. In connection with these results, one of two methyl signals, H-15 (δ_H 1.63), appeared as a singlet and shifted to a lower field in the ^{1}H-NMR spectrum of **1** compared to the doublet H-15 signal of anisatin. These findings indicated that **1** is substantiated at C-1 by a hydroxy group in the structure of neoanisatin.

The relative stereochemistries at C-1 and C-6 can be deduced from a consideration of the results of the differential nuclear Overhauser effect (NOE) experiments as 1β-methyl and 6β-methyl, respectively; *i.e.* irradiation of H-15 signal produced NOE enhancements at the signals of H-10, H-8β and H-2β. Whereas, irradiation of H-10 gave expected NOEs at H-15 and H-8β. On the other hand, irradiation of H-12 caused NOEs at H-14a signal along with an H-7 signal as shown in Fig. 2, indicating that the configuration of C-12 methyl is β. Consequently, the structure of **1** was established as 1-hydroxyneoanisatin. It is the first example of a 1-hydroxyl group in the anisatin-type structure.

Compound **3** was obtained as a colorless oil, $[\alpha]_D$ −7.6° (c=0.49, dioxane), and has the molecular formula, $C_{15}H_{20}O_7$, as analyzed by EI-MS, and thus has one oxygen less than the molecular formula of **1**. In the IR spectrum of **3**, absorptions due to a hydroxyl group at 3350 cm^{-1}, β-lactone at 1820 cm^{-1}, and δ-lactone at 1725 cm^{-1} were demonstrated. The presence of the β-lactone moiety was also indicated by the characteristic small coupling constant (J=6.2 Hz) of AB type signals at δ_H 4.54 and 4.92 in the ^{1}H-NMR spectrum, which also showed a singlet methyl signal at δ_H 1.63 and a doublet methyl signal at δ_H 1.30 (J=7.3 Hz). Although these spectral features were very similar to those of neoanisatin, two-dimensional proton–proton correlation spectroscopy (2D ^{1}H–^{1}H COSY) of **3**

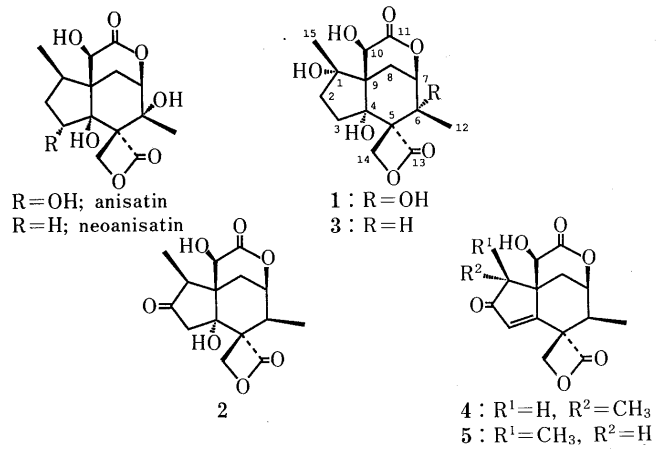

Fig. 1

TABLE I. ^{1}H-NMR Data for Anisatin and Compounds **1**—**5** (400 MHz, δ from TMS in Pyridine-d_5; J (Hz) in Parentheses)

Protons	Anisatin	1	2	3	4	5
1	2.73	—	3.20	—	3.01	2.38
	ddq (12.4, 11.0, 7.3)		q (7.3)		q (7.7)	q (7.3)
2α	2.18	2.39	—	2.46	—	—
	ddd (14.8, 11.0, 4.4)	ddd (13.9, 9.1, 6.9)		ddd (13.9, 9.2, 7.3)		
2β	2.37	2.52	—	2.56	—	—
	ddd (14.8, 12.4, 9.5)	ddd (13.9, 11.7, 2.5)		ddd (13.9, 12.1, 2.6)		
3α	—	2.24	2.85	2.27	6.50	6.59
		ddd (12.0, 9.1, 2.5)	d (17.6)	ddd (12.1, 9.2, 2.6)	s	s
3β	5.57	2.88	3.63	2.91	—	—
	dd (9.5, 4.4)	ddd (12.0, 11.7, 6.9)	d (17.6)	td (12.1, 7.3)		
6	—	—	3.33	3.27	2.47	2.56
			qd (7.3, 2.0)	qd (7.3, 2.2)	qd (7.0, 1.8)	qd (7.0, 1.5)
7	4.44	4.52	4.56	4.56	4.71	4.67
	dd (3.7, 2.0)	dd (3.6, 2.2)	ddd (4.0, 2.2, 2.0)	dt (3.3, 2.2)	dt (4.0, 1.8)	dt (4.4, 1.5)
8α	2.73	3.17	2.74	2.82	1.94	2.02
	dd (14.7, 2.0)	dd (15.0, 2.2)	dd (13.9, 2.2)	dd (14.3, 2.2)	dd (14.3, 1.8)	dd (13.9, 1.5)
8β	2.04	2.11	2.14	2.04	2.42	2.60
	dd (14.7, 3.7)	dd (15.0, 3.6)	dd (13.9, 4.0)	dd (14.3, 3.3)	dd (14.3, 4.0)	dd (13.9, 4.4)
10	4.49	4.61	4.53	4.53	4.60	4.55
	br d (4.4)	br s	s	br d (4.8)	s	s
10-OH	8.70	8.40	—	8.61	—	—
	br d (4.4)	br s		br d (4.8)		
H-12	1.79	1.80	1.32	1.30	1.30	1.30
	s	s	d (7.3)	d (7.3)	d (7.0)	d (7.0)
14a	4.50	4.49	4.59	4.54	4.77	4.75
	d (6.2)	d (6.2)	d (6.6)	d (6.2)	d (5.5)	d (5.5)
14b	5.06	4.90	4.90	4.92	4.80	4.56
	d (6.2)	d (6.2)	d (6.6)	d (6.2)	d (5.5)	d (5.5)
15	1.10	1.63	1.32	1.63	1.14	1.43
	d (7.3)	s	d (7.3)	s	d (7.7)	d (7.3)

Assignments were aided by ^{1}H–^{1}H 2D COSY spectra.

TABLE II. ^{13}C-NMR Data for Anisatin and Compounds **1**—**5** (100 MHz, δ from TMS in Pyridine-d_5)

Carbons	Anisatin	1	2	3	4	5
1	37.5 d	81.3 s	48.8 d	81.4 s	47.1 d	51.7 d
2	42.1 t	41.4 t	214.5 s	41.7 t	207.6 s	206.4 s
3	71.2 d	33.2 t	46.2 t	32.8 t	129.6 d	130.6 d
4	85.5 s	86.9 s	77.6 s	83.9 s	170.3 s	167.7 s
5	65.3 s	66.9 s	66.4 s	67.2 s	63.5 s	63.1 s
6	74.9 s	74.7 s	35.2 d	34.9 d	39.7 d	38.7 d
7	81.7 d	81.8 d	78.9 d	79.4 d	78.2 d	78.2 d
8	27.6 t	24.4 t	31.2 t	28.4 t	33.8 t	36.4 t
9	50.6 s	53.7 s	50.5 s	54.1 s	50.5 s	49.6 s
10	70.1 d	70.5 d	69.8 d	70.9 d	71.2 d	69.6 d
11	174.8 s	174.2 s	174.6 s	174.8 s	173.2 s	173.9 s
12	22.1 q	22.5 q	12.8 q	12.5 q	12.5 q	12.8 q
13	168.7 s	168.1 s	171.5 s	171.3 s	170.3 s	170.3 s
14	65.3 t	65.5 t	64.1 t	63.9 t	66.9 t	68.2 t
15	13.7 q	23.2 q	8.0 q	23.3 q	10.2 q	8.8 q

Assignments were made with the aid of HMQC and HMBC spectra.

showed the connectivity of H-12-H-6-H-7-H-8α,β and H-2α,β-H-3α,β. Thus, **3** should possess a 1-hydroxyl group the same as the structure of **1**, but has no 6-hydroxyl group; *i.e.* **3** was presumed to be a 6-dehydroxy-derivative of **1**, and the carbon connectivities of this structure was supported by HMBC spectrum of **3**.

The configurations of C-15 and C-12 methyl groups were deduced from NOE experiments. When irradiated at the frequency of H-14a, the signals of H-14b and H-12 were enhanced. On the other hand, the irradiation of the H-15 methyl signal caused the enhancement of the signals of

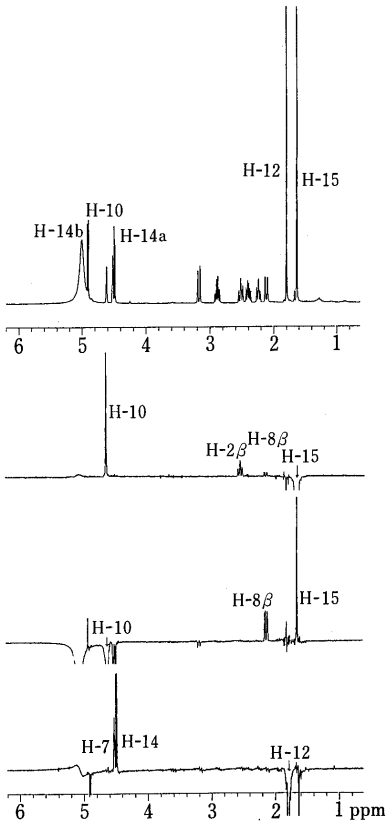

Fig. 2. ^{1}H-NMR Spectrum of **1** and NOE Spectra

TABLE III. ^1H and ^{13}C Correlations Shown in the HMBC Spectra of Compounds **1**—**5**[a]

Protons	1	2	3	4	5
H-1	—	—	C-8, 9, 10, 15	C-2, 15	C-2, 8, 9, 15
H-2β	b)	b)	—	—	—
H-2α	C-1	b)	—	—	—
H-3β	b)	b)	C-2	C-1, 2, 9	C-1, 2, 9
H-3α	b)	b)	C-4, 9	—	—
H-6	—	b)	C-5, 12, 13, 14	C-4, 5, 13, 14	C-5, 12, 13, 14
H-7	b)	b)	b)	b)	b)
H-8β	b)	C-6, 7	b)	b)	C-4
H-8α	C-9, 10	C-10	C-9, 10	C-9, 10	C-9, 10
H-10	C-4, 9, 11	C-4, 9, 11	C-1, 4, 9, 11	C-1, 4, 9, 11	C-4, 9, 11
H-14a	C-4, 6	C-4	C-4, 13	C-4, 13	C-4, 13
H-14b	C-4, 6, 13	b)	C-4, 13	C-4, 13	C-4, 13
H-15	C-1, 2, 9	C-1, 2, 9	C-1, 2, 9	C-1, 2, 9	C-1, 2

a) Measured in pyridine-d_5. b) No correlations were seen.

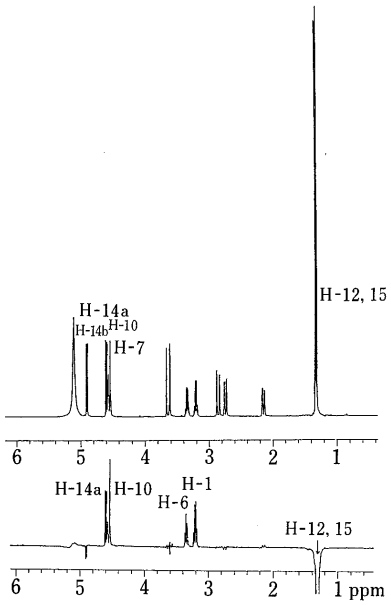

Fig. 3. ^1H-NMR Spectrum of **2** and NOE Spectrum

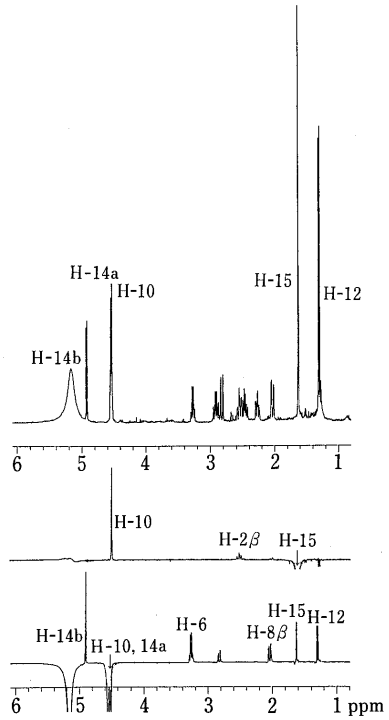

Fig. 4. ^1H-NMR Spectrum of **3** and NOE Spectra

H-10 and H-2β, as summarized in Fig. 4. Consequently, the structure of compound **3** was determined to be a 6-dehydroxy compound of **1**.

Compound **2**, colorless prisms, mp 211—213 °C; $[\alpha]_D$ +67.4° (c=0.23) has the molecular formula, $C_{15}H_{18}O_7$, by elemental analysis and EI-MS. The IR spectrum of **2** showed absorptions due to hydroxyl groups at 3480 and 3460 cm^{-1}, and a δ-lactone carbonyl group at 1730 cm^{-1}, along with characteristic absorptions due to a β-lactone carbonyl group at 1825 cm^{-1} and a cyclopentanone carbonyl group at 1745 cm^{-1}. The presence of a β-lactone moiety was supported by the signals of an AB quartet at δ_H 4.59 and 4.90 (each doublet) with a small coupling constant (J=6.6 Hz) in the ^1H-NMR spectrum, which also suggested the presence of two secondary methyl groups in CD_3OD solution as two doublet signals at δ_H 1.05 (3H, d, J=7.0 Hz) and δ_H 1.22 (3H, d, J=7.3 Hz), and in C_5D_5N solution as one doublet signal at δ_H 1.32 (6H, d, J=7.3 Hz). In the ^1H–^1H 2D COSY of **2**, the connectivity was clarified between H-15-H-1, H-3α-H-3β, and H-6-H-7-H-8α,β, respectively. The assignment of signals in the ^{13}C-NMR spectrum and the connectivity of carbons and hydrogens clarified with the aid of HMBC spectrum suggested that **2** is also an anisatin-type compound. These results propose that **2** is a 2-oxo-6-deoxyneoanisatin-derivative.[5]

TABLE IV. Dose-Dependent Mortality Induced by Compound **2**

Dose	Mortality
1.000	1/10
1.250	3/10
1.500	7/10
2.000	8/10
4.000	10/10

The configurations at C-1 and C-6 were confirmed as 1β and 6β-methyl by an NOE experiment, which showed enhancement between the signals due to H-15 and H-10, whereas an NOE observed between the H-12 signal and one of the H-14 signals (δ_H 4.59) as shown in Fig. 3. These observations demonstrated that **2** has the same configura-

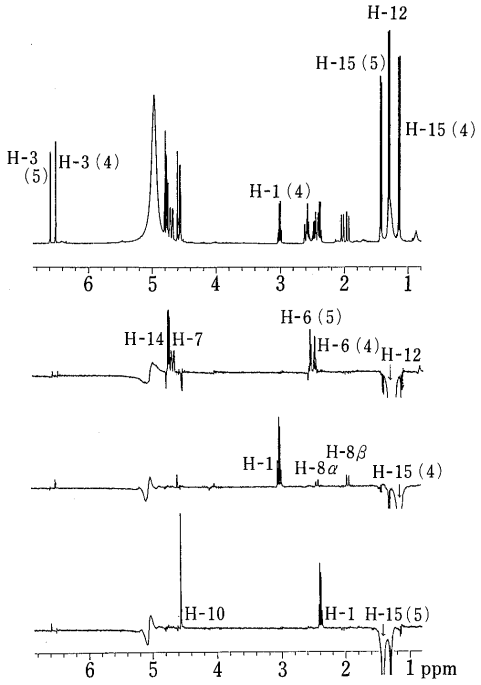

Fig. 5. ^{1}H-NMR Spectrum of a Mixture of **4** and **5**, and NOE Spectra

tion as that of neoanisatin; thus, the structure of **2** was determined to be 2-oxo-6-deoxyneoanisatin.

The toxicity of this compound was estimated as 1.46 mg/kg, which was examined using ddY-strain mice weighing 25—28 g, with 10 animals in each dose group. When the mice were treated with this compound, the animals exhibited picrotoxin-like convulsions, in a dose-dependent manner, which is shown in Table IV. As we reported before,[2,3] majucin-type compounds have no toxicity except for neomajucin, which is almost ten times less toxic than the toxicity of anisatin. We also confirmed that pseudoanisatin has no toxicity, as was reported by Lane *et al.*[4] Accordingly, the above result suggested that strong toxicity may be dependent upon the possession of β- and γ-lactone moieties in the anisatin-type structure, although other anisatin-type compounds reported herein were not examined because of their small quantities.

Compounds **4** and **5** were obtained as an epimeric mixture in spite of an attempt to purify each with high performance liquid chromatography (HPLC). However, it was possible to analyse their ^{1}H- and ^{13}C-NMR spectra by comparing the NMR spectra of the mixtures of **4**:**5**=2:1 and **4**:**5**=1:1. These mixtures were crystallized from AcOEt and showed a molecular ion peak at *m/z* 292 indicating a molecular formula of $C_{15}H_{16}O_6$. In the ^{1}H-NMR spectrum of this mixture, two sets of AB quartet signals with a small coupling constant (J=5.5 Hz) were seen, suggesting **4** and **5** have a β-lactone group. Two sets of two doublet methyl signals (H-12 and H-15 signals, and H-12 signals overlapping each other) were also seen in this spectrum, and the carbon signals in the ^{13}C-NMR spectrum of the mixture were very similar to those of compound **2** except for two sets of two olefinic carbon signals at δ_C 129.6 (d), 170.3 (s) and δ_C 130.6 (d), 167.7 (s). The olefinic singlet proton signals were seen at δ_H 6.50 and 6.59, respectively. These features indicated that **4** and **5** are a 3,4-dehydroxy compound of **3**, and therefore this mixture was the epimeric

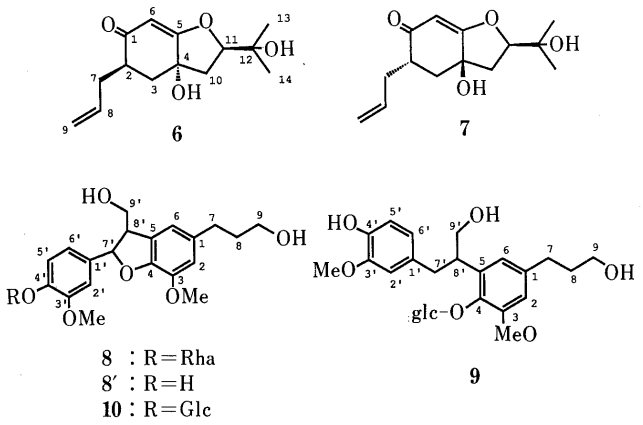

Fig. 6

mixture of C-1 α-methyl and C-1 β-methyl compounds.

To resolve the configuration at C-1, NOE experiments were performed in the ^{1}H-NMR spectrum of the 1:1 mixture. When the signal of H-15 (δ_H 1.14) in those proton signals ascribable to compound **4** was irradiated, the signals of H-1, H-8α and H-8β were enhanced. On the other hand, irradiation of H-15 (δ_H 1.43) belonging to compound **5** caused enhancements of the signals of H-1 and H-10, as shown in Fig. 5. These results indicated that **4** is the 3,4-dehydroxy 15α-methyl compound of **2**, and **5** is the 3,4-dehydroxy compound of **2**. In the majucin-type compounds, there were the same isomers as compounds **4** and **5**. But, it was possible to separate them from each other in the case of the majucin-type compounds.[3]

Compound **6** was isolated as a colorless oil. Its molecular formula was determined as $C_{14}H_{20}O_4$ by an analysis of MS (*m/z*: 252 [M^+]) and carbon counts in the ^{13}C-NMR spectrum. The IR spectrum of **6** showed the absorptions due to hydroxy groups (3580 and 3350 cm^{-1}) and an α,β-unsaturated carbonyl group (1640 cm^{-1}). The ^{1}H–^{1}H 2D COSY of **6** revealed the connectivity of CH_2=CH–CH_2–CH–CH_2– and –O–CH–CH_2–, and the ^{1}H-NMR spectrum showed the presence of a *tert*-dimethyl group and an α proton of α,β-unsaturated olefine. Compound **7**, which was obtained as colorless needles, mp 110—112 °C, has the same molecular formula as **6**, and the IR and ^{1}H- and ^{13}C-NMR spectra strongly resembled those of **6**. These results suggested that **6** and **7** should be analogous compounds each other. The structure, which satisfied these data and the partial structures derived from the ^{13}C–^{1}H long-range correlations in the HMBC spectra (in C_5D_5N solution) of **6** and **7**, was assigned for the structure of illifunone, which belongs to phytoquinoid and was obtained from *I. tashiroi* and *I. arborescens*,[6] both of which are the same species as *I. majus*. So far, four stereoisomers of illifunone, illifunone A, B, C and D, have been isolated from these plants in connection with the stereochemistry at the positions of C-2 and C-4. These compounds were easily distinguished from each other based on the consideration of the chemical shifts of axial H-3 and H-11 proton signals in the ^{1}H-NMR spectra of these compounds. Comparisons of the spectral and physical data of **6** and **7** with those of illifunones A, B, C and D, suggested that **6** is identical to illifunone C, and **7** to illifunone A. Phytoquinoid is constituted of a C_6–C_3 unit and isoprene, and is a rare compound found only in higher plants.

Compound **8** was isolated as a viscous oil, $[\alpha]_D$ −51.8° (c=0.26, MeOH). Its molecular formula was deduced $C_{26}H_{34}O_{10}$ by fast atom bombardment mass spectrum (FAB-MS) (m/z: 529 [M^++Na]) and by carbon counts in the ^{13}C-NMR spectrum. Acid hydrolysis of **8** with hesperidinase afforded an aglycone (**8'**) and a sugar. The sugar is rhamnose as corroborated by Avicel thin layer chromatography (TLC) developed with an authentic sample of rhamnose. In the ^{1}H-NMR spectrum of **8**, two methoxy signals were seen. In the ^{13}C-NMR spectrum of **8**, twelve aromatic and six aliphatic carbon signals were shown except for the carbon signals of rhamnose and methoxy groups. The ^{1}H–^{1}H 2D COSY spectrum of **8** suggested that the aromatic rings are 1,3,4-tri-substituted and 1,3,4,5-tetra-substituted benzene rings. Thus, **8** was proven a rhamnoside of dihydrodehydroconiferyl alcohol. The linkage position of rhamnose to dihydrodehydroconiferyl alcohol was clarified as 4' by HMBC spectrum of **8**. This compound was previously obtained from *Pinus massoniana* by O. Theander *et al.*[7] and from *Epimedium diphyllum* by A. Ueno *et al.*,[8] and the spectral data of **8**, including the optical rotation value, were approximately identical to those of the former compound.

The configurations at C-7' and C-8' were not specified in the compound obtained by O. Theander *et al.*, but A. Ueno *et al.* suggested C-7 and C-8 to be *R* and *S*, respectively, by circular dichroic (CD) spectroscopy. Although their proposal was derived from the results of 2-aryl-3-methyl-2,3-dihydrobenzofuran derivatives, it seems that these results cannot be applied to the derivatives of dihydrodehydroconiferyl alcohol. The plane structure of the aglycone of their compounds was identical to the aglycone of compound **10**, which was obtained by T. Yamauchi *et al.*[9] from *Trachelospermum asiaticum*, but they did not determine the configuration at C-7 and C-8, and suggested that this compound may have the same stereoisomeric structure as that obtained by Takemoto *et al.*[10] or by Satake *et al.*[11] comparing their optical rotation value. The negative optical rotation value of **8'** ($[\alpha]_D$ −3.9°) suggests that **8** has the same stereoisomeric structure as that of the aglycone ($[\alpha]_D$ −3.2°) of **10**, rather than that obtained by Theander *et al.* ($[\alpha]_D$ +4.7°). Since the sugar moiety was characterized as an α-linked rhamnose, **8** was deduced to be the 4'-*O*-α-D-rhamnoside of dihydrodehydroconiferyl alcohol.

Compound **9** was obtained as a colorless syrup, and its molecular formula was deduced as $C_{26}H_{36}O_{11}$ from FAB-MS (m/z: 525 [M^++1]) and carbon counts in the ^{13}C-NMR spectrum. The presence of two benzene rings, two methoxy groups and a glucose moiety was clarified by its ^{1}H- and ^{13}C-NMR spectra, and six extra aliphatic carbons were also suggested by the ^{13}C-NMR spectrum. These findings indicated that **9** is also lignan. The ^{1}H-NMR spectrum of **9** indicated that one of two benzene rings is 1,3,4-tri-substituted and the other is 1,3,4,5-tetra-substituted ones, and the ^{1}H–^{1}H COSY spectrum suggested the connectivity of –CH_2–CH–CH_2O– and –CH_2–CH_2–CH_2O– moieties. The linkage position of glucose was elucidated by HMBC spectrum as 4. All this data and the HMBC correlations demonstrated the structure as shown in Fig. 6. This compound was isolated from *Epomedium grandiflorum* by A. Ueno *et al.*,[12] and named icariside E_3, whose ^{1}H- and ^{13}C-NMR spectral data and optical rotation value were identical with those of **9**.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL GX-400 and JEOL FX-90Q spectrometers. NOE and 2D COSY experiments were performed on the former apparatus. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard. EI-MS and FAB-MS were recorded on a JEOL JMS-DX-303 spectrometer. IR spectra were recorded on a JASCO IR-180 or Shimadzu IR-408. CD spectrum was taken on a JASCO J-500A apparatus. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. Medium-pressure liquid chromatography (MPLC) was carried out on a JASCO 880-PU pump using a Kusano Si-5 column and a Kusano ODS-20 column.

Isolation The MeOH extract of the pericarps (1.5 kg) of *I. majus* was extracted with AcOEt and *n*-BuOH successively, after extraction with *n*-hexane. The AcOEt-soluble part was subjected to counter-current distribution using the solvent system of H_2O and AcOEt to give five fractions (I—V) as described before. Of these fractions, fraction V was newly investigated to give compound **1** (16.5 mg), **2** (132 mg), and **4** and **5** as a 1:1 mixture (10 mg total) together with compounds **6** (61 mg) and **7** (11 mg), respectively. The separation of the *n*-BuOH soluble part (286 g) was performed, after the exclusion of a large amount of shikimic acid, to give three fractions (I—III). From fraction I, compound **3** (10 mg) was obtained and purified by a Kusano Si-5 column. Fraction III was subjected to a Sephadex LH-20 column and ODS-MPLC using a solvent system of H_2O–MeOH (4:6), successively, to give compounds **8** (83 mg) and **9** (180 mg).

Compound 1 Colorless oil. $[\alpha]_D^{23}$ −16.5° (c=0.41, dioxane). EI-MS m/z: 328 (M^+), $C_{15}H_{20}O_8$. IR ν_{max}^{Nujol} cm^{-1}: 3320 (OH), 1820 (β-lactone), 1720 (δ-lactone).

Compound 2 Colorless prisms from $CHCl_3$–AcOEt, mp 211—213 °C. $[\alpha]_D^{21}$ +67.4° (c=0.23, dioxane). EI-MS m/z: 310 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3480, 3460 (OH), 1825 (β-lactone), 1745 (cyclopentenone), 1730 (δ-lactone). *Anal.* Calcd for $C_{15}H_{18}O_7 \cdot 1/2H_2O$: C, 56.42; H, 6.00. Found: C, 56.26; H, 5.95.

Compound 3 Colorless oil. $[\alpha]_D^{20}$ −7.6° (c=0.49, dioxane). EI-MS m/z: 312 (M^+), $C_{15}H_{20}O_7$. IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH), 1820 (β-lactone), 1725 (δ-lactone).

Compounds 4 and 5 Colorless needles (as a 1:1 mixture). EI-MS m/z: 292 (M^+), $C_{15}H_{16}O_6$.

Compound 6 (Illifunone C) Colorless oil. $[\alpha]_D^{17}$ +61.9° (c=0.29, $CHCl_3$). EI-MS m/z: 252 (M^+), $C_{14}H_{20}O_4$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}:3580, 3350 (OH), 1640 (α,β-unsaturated ketone), 1185. ^{1}H-NMR (400 MHz, C_5D_5N) δ: 3.19 (1H, m, H-2), 1.87 (1H, t, J=13.2 Hz, H-3β), 2.59 (1H, dd, J=13.2, 4.4 Hz, H-3α), 5.64 (1H, s, H-6), 2.36 (1H, m, H-7a), 2.92 (1H, m, H-7b), 5.86 (1H, m, H-8), 5.02 (1H, m, H-9a), 5.10 (1H, m, H-9b), 2.38 (1H, dd, J=12.8, 10.3 Hz, H-10α), 2.52 (1H, dd, J=12.8, 4.8 Hz, H-10β), 5.09 (1H, dd, J=10.3, 4.8 Hz, H-11), 1.33 (3H, s, H-13 or H-14), 1.52 (3H, s, H-14 or H-13). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.71 (1H, t, J=12 Hz, H-3), 5.27 (1H, s, H-6), 5.86 (1H, m, H-8), 5.06 (1H, m, H-9a), 4.92 (1H, m, H-9b), 1.95 (1H, dd, J=13, 10 Hz, H-10a), 2.36 (1H, dd, J=13, 5 Hz, H-10b), 4.68 (1H, dd, J=10, 5 Hz, H-11), 1.17 (3H, s, H-13 or H-14), 1.37 (3H, s, H-14 or H-13). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δ: 200.6 (s, C-1), 39.9 (d, C-2), 38.5 (t, C-3), 75.7 (s, C-4), 179.4 (s, C-5), 100.1 (d, C-6), 34.0 (t, C-7), 135.5 (d, C-8), 116.6 (t, C-9), 38.8 (t, C-10), 91.0 (d, C-11), 70.2 (s, C-12), 24.4 (q, C-13 or C-14), 26.8 (q, C-14 or C-13).

Compound 7 (Illifunone A) Colorless needles from *n*-hexane–$CHCl_3$, mp 110—112 °C. $[\alpha]_D^{16}$ −83.4° (c=0.58, $CHCl_3$). EI-MS m/z: 252 (M^+), $C_{14}H_{20}O_4$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3340 (OH), 1640 (α,β-unsaturated ketone), 1180. ^{1}H-NMR (400 MHz, C_5D_5N) δ: 3.14 (1H, m, H-2), 1.72 (1H, t, J=12.8 Hz, H-3β), 2.56 (1H, dd, J=12.8, 4.4 Hz, H-3α), 5.66 (1H, s, H-6), 2.43 (1H, m, H-7a), 2.88 (1H, m, H-7b), 5.89 (1H, m, H-8), 5.04 (1H, m, H-9a), 5.12 (1H, m, H-9b), 2.25 (1H, dd, J=13.9, 9.5 Hz, H-10α), 2.56 (1H, dd, J=13.9, 1.1 Hz, H-10β), 4.61 (1H, dd, J=9.5, 1.1 Hz, H-11), 1.31 (3H, s, H-13 or H-14), 1.55 (3H, s, H-14 or H-13). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.63 (1H, t, J=12 Hz, H-3), 5.33 (1H, s, H-6), 5.71 (1H, m, H-8), 5.08 (1H, m, H-9a), 4.94 (1H, m, H-9b), 2.17 (1H, dd, J=14, 9 Hz, H-10a), 2.40 (1H, dd, J=14, 2 Hz, H-10b), 4.42 (1H, dd, J=9, 2 Hz, H-11), 1.28 (3H, s, H-13 or H-14), 1.50 (3H, s, H-14 or H-13). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δ: 200.5 (s, C-1), 40.6 (d, C-2), 39.1 (t, C-3), 73.3 (s, C-4), 180.4 (s, C-5), 99.2 (d, C-6), 34.2 (t, C-7), 136.0 (d, C-8), 116.4 (t, C-9), 38.0 (t, C-10), 90.4 (d, C-11), 71.5 (s, C-12), 26.8(q,

C-13 or C-14), 26.9 (q, C-14 or C-13).

Compound 8 Amorphous powder. $[\alpha]_D^{20}$ −51.8° (c=0.26, MeOH). FAB-MS m/z: 529 $[M^+ + Na]$, $C_{26}H_{34}O_{10}$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2930, 1605, 1510, 1460, 1420. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.72 (1H, s, H-2), 6.73 (1H, s, H-6), 2.62 (2H, t, J=7.5 Hz, H-7), 1.81 (2H, m, H-8), 3.57 (2H, t, J=6.4 Hz, H-9), 7.03 (1H, d, J=1.8 Hz, H-2′), 7.08 (1H, d, J=8.4 Hz, H-5′), 6.92 (1H, dd, J=8.4, 1.8 Hz, H-6′), 5.55 (1H, d, J=6.0 Hz, H-7′), 3.46 (1H, m, H-8′), 3.75 (1H, dd, J=11.0, 7.5 Hz, H-9′a), 3.85 (1H, dd, J=11.0, 5.0 Hz, H-9′b), 3.86 (3H, s, –OMe), 3.80 (3H, s, –OMe), 5.34 (1H, d, J=1.8 Hz, rha-1″), 4.06 (1H, dd, J=3.5, 1.8 Hz, rha-2″), 3.87 (1H, J=9.5, 3.5 Hz, rha-3″), 3.44 (1H, J=9.5 Hz, rha-4″), 3.78 (1H, dq, J=9.5, 6.2 Hz, rha-5″), 1.21 (3H, d, J=6.2 Hz, rha-6″). ^{13}C-NMR (100 MHz, CD_3OD) δ: 131.7 (s, C-1), 114.3 (d, C-2), 145.3 (s, C-3), 147.5 (s, C-4), 127.6 (s, C-5), 118.0 (d, C-6), 32.9 (t, C-7), 35.8 (t, C-8), 62.3 (t, C-9), 138.8 (s, C-1′), 111. 4 (d, C-2′), 152.1 (s, C-3′), 146.6 (s, C-4′), 119.6 (d, C-5′), 119.2 (d, C-6′), 88.5 (d, C-7′), 55.6 (d, C-8′), 65.2 (t, C-9′), 56.9 (q, –OMe), 56.6 (q, –OMe), 101.4 (d, rha-1″), 72.2 (d, rha-2″), 72.1 (d, rha-3″), 73.9 (d, rha-4″), 70.8 (d, rha-5″), 18.0 (q, rha-6″).

Hydrolysis of 8 Hesperidinase (Sigma Chemical Co.) (70 mg) was added to a solution of **8** (30 mg) in H_2O (5 ml), which was shaken at 37 °C for 2 d, then extracted with AcOEt. The AcOEt soluble portion was dried over Na_2SO_4, then evaporated under reduced pressure to give the residue. This was purified by SiO_2 column chromatography using the solvent of $CHCl_3$–MeOH (1 : 1) to give **8′** (16 mg). The water layer was concentrated under reduced pressure, and was examined by Avicel TLC (Funakoshi Yakuhin Co., Ltd.) developed with n-BuOH–pyridine–H_2O (6 : 4 : 3) together with an authentic sample of rhamnose.

Compound 8′ Colorless oil. $[\alpha]_D^{16}$ −3.9° (c=0.37, acetone). CD (c=0.0016 mol/l, MeOH) $[\theta]$ (nm): −3838 (294), −10800 (242). ^{1}H-NMR (400 MHz, C_5D_5N) δ: 7.06 (1H, br s, H-2), 6.92 (1H, br s, H-6), 2.87 (2H, t, J=7.7 Hz, H-7), 2.08 (2H, m, H-8), 3.92 (2H, t, J=6.2 Hz, H-9), 7.33 (1H, d, J=1.8 Hz, H-2′), 7.20 (1H, d, J=8.1 Hz, H-5′), 7.25 (1H, dd, J=8.1, 1.8 Hz, H-6′), 6.06 (1H, d, J=6.6 Hz, H-7′), 3.96 (1H, m, H-8′), 4.20 (1H, dd, J=11.0, 6.6 Hz, H-9′a), 4.27 (1H, dd, J=11.0, 5.5 Hz, H-9′b), 3.63, 3.83 (each 3H, s, –OMe). ^{13}C-NMR (100 MHz, C_5D_5N) δ: 130.2 (s, C-1), 113.8 (d, C-2), 144.7 (s, C-3), 147.4 (s, C-4), 136. 1(s, C-5), 117.6 (d, C-6), 32.7 (t, C-7), 36.0 (t, C-8), 61.5 (t, C-9), 133.9 (s, C-1′), 110.9 (d, C-2′), 148 (s, C-3′), 148.8 (s, C-4′), 116.5 (d, C-5′), 119.8 (d, C-6′), 88.4 (d, C-7′), 55.1 (d, C-8′), 64.4 (t, C-9′), 55.9, 56.3 (each q, –OMe × 2).

Compound 9 Amorphous powder. $[\alpha]_D^{18}$ −61.1° (c=0.26, MeOH). FAB-MS m/z: 525 $[M^+ + H]$, $C_{26}H_{36}O_{11}$. IR ν_{max}^{KBr} cm^{-1}: 3385, 2930, 1590, 1520, 1460, 1430. ^{1}H-NMR (400 MHz, C_5D_5N) δ: 7.05 (1H, d, J=1.8 Hz, H-2), 7.16 (1H, d, J=1.8 Hz, H-6), 2.85 (2H, br t, J=7.3 Hz, H-7), 2.05 (2H, m, H-8), 3.90 (2H, t, J=6.2 Hz, H-9), 6.78 (1H, d, J=1.8 Hz, H-2′), 6.99 (1H, d, J=7.7 Hz, H-5′), 6.88 (1H, dd, J=7.7, 1.8 Hz, H-6′), 3.11 (1H, dd, J=13.9, 9.1 Hz, H-7′a), 3.46 (1H, dd, J=13.9, 5.9 Hz, H-7′b), 4.79 (1H, m, H-8′), 3.64, 3.66 (each 3H, s, –OMe), 5.40 (1H, d, J=7.3 Hz, glc-1″). ^{13}C-NMR (100 MHz, C_5D_5N) δ: 139.6 (s, C-1), 113.3 (d, C-2), 152.5 (s, C-3), 143.2 (s, C-4), 139.1 (s, C-5), 122.3 (d, C-6), 32.9 (t, C-7), 35.7 (t, C-8), 61.5 (t, C-9), 132.4 (s, C-1′), 111.3 (d, C-2′), 148.2 (s, C-3′), 146.0 (s, C-4′), 116.0 (d, C-5′), 119.8 (d, C-6′), 39.2 (t, C-7′), 42.3 (d, C-8′), 66.9 (t, C-9′), 55.7, 55.9 (each q, –OMe), 105.7 (d, glc-1″), 76.2 (d, C-2″), 78.4 (d, C-3″), 71.1 (d, C-4″), 78.2 (d, C-5″), 62.4 (t, C-6″).

Acknowledgment We are grateful to Mr. Y. Ohama for valuable assistance in measuring the NMR spectra.

References

1) I. Kouno, N. Baba, M. Hashimoto, N. Kawano, C.-S. Yang, and S. Sato, *Chem. Pharm. Bull.*, **37**, 2427 (1989).
2) I. Kouno, N. Baba, M. Hashimoto, N. Kawano, M. Takahashi, H. Kaneto, C.-S. Yang, and S. Sato, *Chem. Pharm. Bull.*, **37**, 2448 (1989).
3) I. Kouno, N. Baba, M. Hashimoto, N. Kawano, M. Takahashi, H. Kaneto, and C.-S. Yang, *Chem. Pharm. Bull.*, **38**, 422 (1990).
4) J. F. Lane, W. T. Koch, N. S. Leeds, and G. Gorin, *J. Am. Chem. Soc.*, **74**, 3211 (1952).
5) C.-S. Yang, M. Hashimoto, N. Baba, M. Takahashi, H. Kaneto, N. Kawano, and I. Kouno, *Chem. Pharm. Bull.*, **38**, 291 (1990).
6) K. Yakushijin, T. Tohshima, E. Kitagawa, R. Suzuki, J. Sekikawa, T. Morishita, H. Murata, S.-T. Lu, and H. Furukawa, *Chem. Pharm. Bull.*, **32**, 11 (1984).
7) L. N. Lundgren, Z. Shen, and O. Theander, *Acta. Chem. Scan., Ser. B*, **39**, 241 (1985).
8) T. Miyase, A. Ueno, N. Takizawa, H. Kobayashi, and H. Oguchi, *Phytochemistry*, **28**, 3483 (1989).
9) F. Abe and T. Yamauchi, *Chem. Pharm. Bull.*, **34**, 4340 (1986).
10) T. Takemoto, T. Miyase, and G. Kusano, *Phytochemistry*, **14**, 1890 (1978).
11) T. Satake, T. Murakami, Y. Saiki, and C.-M. Chen, *Chem. Pharm. Bull.*, **26**, 1616 (1978).
12) T. Miyase, A. Ueno, N. Takagi, H. Kobayashi, and H. Oguchi, *Chem. Pharm. Bull.*, **36**, 2475 (1988).