Phenylpropanoids from the Barks of *Illicium difengpi*

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4-*O*-(2-Hydroxy-1-hydroxymethylethyl)-dihydroconiferyl alcohol and its 6-*p*-coumaroyl-glucoside, 4-*O*-(1-carboxy-2-hydroxyethyl) dihydroconiferyl alcohol and rhamnosyl glucoside of 2-hydroxy safrole have been isolated from the barks of *Illicium difengpi*. Their structures were determined on the basis of the nuclear magnetic resonance spectral data and chemical evidences.

Keywords *Illicium difengpi*; Illiciaceae; bark; phenylpropanoid; dihydroconiferyl alcohol; 2-hydroxy safrole; glycerol; glyceric acid

We have reported on the isolation and structure determination of the constituents of Japanese and Chinese toxic *Illicium* plants.[1] *Illicium difengpi* K.I.B. *et* K.I.M., which is a non-toxic plant distributed in the southern part of China, has been used as an antiarthritic agent in China. This crude drug was included in "China's Pharmacopoeia" edited in 1977,[2] but excluded from its edited edition in 1985, because no constituent of this plant has been isolated. To clarify the constituent of *I. difengpi*, and as a part of our investigation on the constituent of *Illicium* species, we have examined the bark of this plant.

The bark (2.2 kg) of *I. difengpi*, collected at Guangxi, China, was extracted with MeOH three times. The MeOH extract was defatted with *n*-hexane, then partitioned between AcOEt and H_2O, and between *n*-BuOH and H_2O, successively. Although the other *Illicium* plants, which we examined, contained sesquiterpenes in the AcOEt soluble part, the AcOEt soluble parts of the MeOH extract of *I. difengpi* did not afford anything. The *n*-BuOH soluble part (81.5 g) was subjected to a subsequent separation as stated in the experimental section, then afforded compounds **1** (10.3 mg) as an amorphous powder and **2** (15.0 mg) as a colorless oil. Compounds **3** (159.7 mg) and **4** (628.6 mg), which were purified by a Kusano prepacked octadecyl silica (ODS) column, were also obtained as a colorless syrup and a white powder, respectively.

The molecular formula of compound **1** ($C_{13}H_{20}O_5$), an amorphous powder, was revealed by fast atom bombardment mass spectrum (FAB-MS) (*m/z* 257 [$M^+ + H$]) and by carbon counts in the carbon-13 (^{13}C-) nuclear magnetic resonance (NMR) spectrum. The proton (^1H-) NMR spectrum of **1** demonstrated signals due to three aromatic protons at δ_H 6.85 (d, 1H, $J=2.2$ Hz), 6.73 (dd, 1H, $J=2.2$, 8.1 Hz) and 6.99 (d, 1H, $J=8.1$ Hz), one methoxy group at δ_H 3.84 (s, 3H), two methylene groups at δ_H 1.79—1.83 (m, 2H), and 2.63 (t, 2H, $J=7.7$ Hz) and one hydroxymethyl group at δ_H 3.56 (t, 2H, $J=6.6$ Hz). The detailed analysis of these data and the ^{13}C-NMR spectrum of **1** with the aid of two-dimensional proton–proton correlation spectroscopy (2D ^1H–^1H COSY) and the heteronuclear multiple bond correlation (HMBC) spectrum indicated the presence of dihydroconiferyl alcohol moiety in the structure of **1**. The other proton and carbon signals at δ_H 3.74, 3.76 (each d, 2H, $J=5.1$ Hz); (H_2-1′ and H_2-3′) or (H_2-3′ and H_2-1′), 4.15 (quintet, 1H, $J=5.1$ Hz); (H-2′) and δ_C 62.1 (C-1′ and C-3′), 83.3 (C-2′) demonstrated a glycerol moiety.

Acetylation of **1** with Ac_2O and pyridine gave triacetate (**1a**), whose ^1H-NMR spectrum showed a remarkably down field shift of methylene protons of three primary hydroxyl groups. Thus, the C-2′ position of a glycerol moiety should link to the phenolic oxygen of the dihydroconiferyl alcohol moiety. This compound was isolated as a 9-*O*-rhamnopyranosyl derivative by Inada *et al.* at the same time.[3] The ^1H- and ^{13}C-NMR spectral data for **1** were almost identical with those of the aglycone moiety of "ampelopsisrhamnoside" except for the H_2-9 and C-9 signals.

Compound **2**, a colorless syrup, $[\alpha]_D$ −17.5° (MeOH) had the molecular formula $C_{17}H_{26}O_6$ from FAB-MS (*m/z* 327 [$M^+ + 1$]) and carbon counts in the ^{13}C-NMR spectrum of **2**. Its infrared (IR) spectrum suggested the absorption (1740 cm^{-1}) due to an ester carbonyl group. In the ^1H-NMR spectral data of **2**, three aromatic protons at δ_H 6.86 (d, 1H, $J=2.2$ Hz), 6.70 (dd, 1H, $J=2.2$, 8.1 Hz), and 6.83 (d, 1H, $J=8.1$ Hz), one methoxy group at δ_H 3.95 (s, 3H), two methylene groups at δ_H 1.77—1.84 (m, 2H), and 2.63 (t, 2H, $J=7.7$ Hz) and one hydroxymethyl group at δ_H 3.55 (t, 2H, $J=6.6$ Hz) were observed. Such data including ^{13}C-NMR data closely resembled those of **1**, suggesting the presence of dihydroconiferyl alcohol moiety in the structure of **2**. However, the ^{13}C-signals at δ_C 171.8 (s), 81.2 (d), and 63.8 (t) indicated the glyceric acid moiety instead of the glycerol moiety in **1**, and the other signals at δ_C 66.1 (t), 31.7 (t), 20.0 (t), and 14.0 (q), together with the result of the proton connectivities in the 2D ^1H–^1H COSY spectrum, suggested the *n*-butyl alcohol moiety, which should link as an ester group with glyceric acid. The linked position of glyceric acid and dihydroconiferyl alcohol is in the same manner as that of **1** judging from the ^{13}C-signals at δ_C 81.2 (d) and 63.8 (t). Thus, the structure of **2** is shown in Fig. 1. The *n*-butyl ester moiety might be an artefact in the course of the separating work.

The molecular formula, $C_{26}H_{34}O_{12}$, of compound **3**, a colorless syrup, $[\alpha]_D$ −20.2° (MeOH), was determined by FAB-MS (*m/z* 561 [$M^+ + Na$]) and carbon counts in the ^{13}C-NMR spectrum of **3**. Its ^1H- and ^{13}C-NMR spectra also demonstrated the dihydroconiferyl alcohol moiety along with the presence of *p*-hydroxybenzoic acid; *i.e.* the A_2B_2 type proton signals at δ_H 7.89 (d, 2H, $J=8.8$ Hz), 6.81 (d, 2H, $J=8.8$ Hz) and ^{13}C-signals at δ_C 122.2 (s), 132.9 (2C, d), 116.2 (2C, d), 163.5 (s), and 168.1 (s). Acetylation of **3** in Ac_2O–pyridine afforded an acetate (**3a**) that has one phenolic and five alcoholic acetoxy groups, suggesting from the ^1H-NMR spectrum. By acid hydrolysis of **3** in 2 N HCl/EtOH–H_2O, three compounds **3b**, **3c**, and **3d** were

TABLE I. ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR Data for Compounds **1**, **1a** and **2** (δ from TMS in CD_3OD; J (Hz) in Parentheses)

Positions	**1**		**1a**	**2**	
	^{13}C	^{1}H	^{1}H	^{13}C	^{1}H
1	138.3 s	—	—	138.6 s	—
2	114.2 d	6.85 (d, 1H, J=2.2)	6.85 (d, 1H, J=1.8)	114.3 d	6.86 (d, 1H, J=2.2)
3	152.0 s	—	—	151.3 s	—
4	146.9 s	—	—	146.6 s	—
5	119.5 d	6.99 (d, 1H, J=8.1)	6.95 (d, 1H, J=8.1)	118.1 d	6.83 (d, 1H, J=8.1)
6	121.9 d	6.73 (dd, 1H, J=8.1, 2.2)	6.72 (dd, 1H, J=8.1, 1.8)	121.7 d	6.70 (dd, 1H, J=8.1, 2.2)
7	32.7 t	2.63 (t, 2H, J=7.7)	2.65 (b t, 2H, J=7.7)	32.7 t	2.63 (t, 2H, J=7.7)
8	35.5 t	1.79—1.83 (m, 2H)	1.91—1.99 (m, 2H)	35.5 t	1.77—1.84 (m, 2H)
9	62.2 t	3.56 (t, 2H, J=6.6)	4.06 (t, 2H, J=6.6)	62.2 t	3.55 (t, 2H, J=6.6)
–OMe	56.5 q	3.84 (s, 3H)	3.82 (s, 3H)	56.6 q	3.95 (s, 3H)
1′	62.1 t	3.74 (d, 2H, J=5.1)[a]	4.30 (d, 2H, J=5.1)	171.8 s	—
2′	83.3 d	4.15 (qui, 1H, J=5.1)	4.55 (qui, 1H, J=5.1)	81.2 d	4.70 (t, 1H, J=5.7)
3′	62.1 t	3.76 (d, 2H, J=5.1)[a]	4.30 (d, 2H, J=5.1)	63.8 t	3.95 (br d, 2H, J=5.7)
1″				66.1 t	4.16 (m, 2H)
2″				31.7 t	1.56—1.63 (m, 2H)
3″				20.0 t	1.33 (sex, 2H, J=7.3)
4″				14.0 q	0.90 (t, 3H, J=7.3)
–OAc			2.02 (s, 3H)		
–OAc			2.03 (s, 3H)		
–OAc			2.03 (s, 3H)		

a) Assignments are interchangeable. Assignments were made with the aid of the ^{13}C–^{1}H 2D COSY, and HMBC spectra. Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; qui, quintet; sex, sextet; m, multiplet.

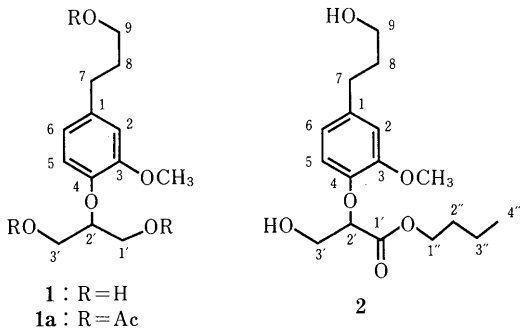

Fig. 1

detected. **3c** was identified as *p*-hydroxybenzoic acid by comparison with an authentic sample. **3d** was distinguished as glucose by Avicel thin-layer chromatography (TLC) with the authentic sample of glucose. The ^{1}H- and ^{13}C-NMR spectra of **3b** were completely identical with those of **1**, thus indicating that **3** is a glucoside of **1**. The signals of methylene protons of four primary hydroxy groups in the structure of **3** were easily ascribed, respectively, by the 2D ^{1}H–^{1}H COSY and the heteronuclear multiple quantum coherence (HMQC) spectroscopy. As a result, the H_2-9 signal and the H_2-3′ signal did not shift to lower field compared to those of **1**, indicating that a glucose linked to position C-1′ of the glycerol segment. The *p*-hydroxy benzoyl moiety should link to C-6″ of glucose, because the H_2-6″ and C-6″ signals of glucose shifted considerably lower field. Therefore, the structure of **3** was assigned as in the figure.

The stereochemistry of the asymmetric centers of C-2′ in the glycerol moiety of compounds **2** and **3** were not clarified.

Compound **4**, $C_{22}H_{30}O_{12}$, $[\alpha]_D$ −58.9° (MeOH), FAB-MS (*m/z* 487 $[M^+ + 1]$), was obtained as a white powder. The ^{1}H-NMR spectrum of **4** showed the signals at δ_H 5.03 (dd, 1H, J=17.2, 1.8 Hz), 4.98 (dd, 1H, J=9.3, 1.8 Hz), 4.02 (ddd, 1H, J=17.2, 9.3, 6.4 Hz) and 3.40 (d, 2H, J=6.4 Hz), indicating the presence of a 2-propenyl

TABLE II. ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR Data for Compound **3** (δ from TMS in CD_3OD; J (Hz) in Parentheses)

Positions	^{13}C	^{1}H
1	138.4 s	—
2	114.1 d	6.79 (d, 1H, J=1.8)
3	152.0 s	—
4	146.5 s	—
5	119.9 d	6.98 (d, 1H, J=8.1)
6	121.8 d	6.61 (dd, 1H, J=8.1, 1.8)
7	32.7 t	2.59 (t, 2H, J=7.7)
8	35.5 t	1.74—1.81 (m, 2H)
9	62.1 t	3.55 (t, 2H, J=6.4)
Gly-1′	69.6 t	4.02 (dd, 1H, J=11.0, 4.8)
		3.85 (dd, 1H, J=11.0, 5.5)
Gly-2′	81.6 d	4.31—4.41 (m, 1H)
Gly-3′	62.2 t	3.71 (dd, 1H, J=11.7, 5.1)
		3.76 (dd, 1H, J=11.7, 5.1)
Glu-1″	104.8 d	4.38 (d, 1H, J=8.1)
Glu-2″	75.0 d	3.26 (d, 1H, J=8.1)
Glu-3″	77.8 d	3.38—3.62 (m, 2H)
Glu-4″	71.8 d	
Glu-5″	75.5 d	3.58—3.62 (m, 1H)
Glu-6″	64.8 t	4.61 (dd, 1H, J=11.7, 2.2)
		4.31—4.41 (m, 1H)
1‴	122.2 s	—
2‴,6‴	132.9 d	7.89 (d, 2H, J=8.8)
3‴,5‴	116.2 d	6.81 (d, 2H, J=8.8)
4‴	163.5 s	—
7‴	168.1 s	—
–OMe	56.5 q	3.79 (s, 3H)

Assignments were made with the aid of the ^{13}C–^{1}H 2D COSY, and HMBC spectra.

side-chain group. The signals of aromatic protons were seen at δ_H 6.59 and 6.79 (each s, 1H) together with a methylene dioxy group at δ_H 5.87 (d, 1H, J=1.1 Hz) and 5.88 (d, 1H, J=1.1 Hz), thus **4** should have a phenylpropanoid moiety. The ^{13}C-NMR spectrum of **4** also exhibited the carbon signals due to glucose and rhamnose moieties, as was confirmed by the acid hydrolysis of **4** with 2 N HCl. Thus, **4** is a diglycosyl phenylpropanoid. When **4** was hydrolyzed

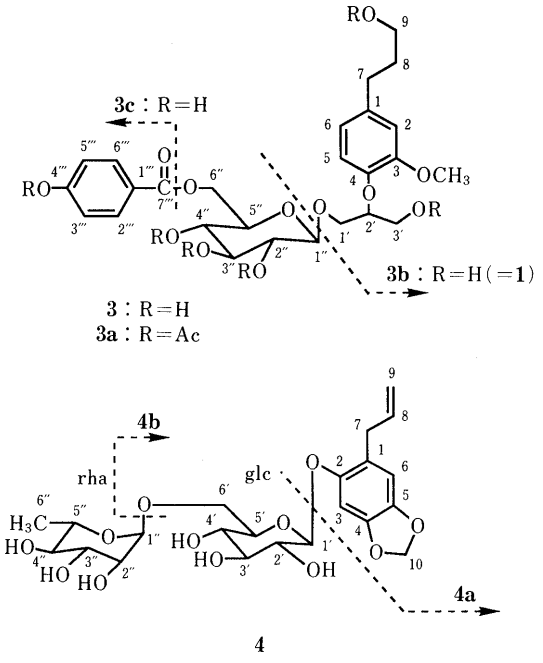

Fig. 2

TABLE III. ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR Data for Compounds **4** and **4a** (δ from TMS in CD_3OD; J (Hz) in Parentheses)

Positions	**4** ^{13}C	**4** ^{1}H	**4a** ^{13}C	**4a** ^{1}H
1	124.2 s	—	116.8 s	—
2	150.9 s	—	148.6 s	—
3	101.2 d	6.79 (s, 1H)	98.7 d	6.58 (s, 1H)
4	147.6 s	—	146.8 s	—
5	144.4 s	—	141.6 s	—
6	109.8 d	6.59 (s, 1H)	109.5 d	6.42 (s, 1H)
7	34.9 t	3.40 (d, 2H, J=6.4)	35.0 t	3.31 (d, 2H, J=6.2)
8	138.9 d	4.02 (ddd, 1H, J= 17.2, 9.3, 6.4)	136.4 d	5.96 (ddd, 1H, J= 17.6, 9.5, 6.2)
9a	115.6 t	5.03 (dd, 1H, J= 17.2, 1.8)	116.4 t	5.17 (dd, 1H, J= 17.2, 1.8)
9b		4.98 (dd, 1H, J= 9.3, 1.8)		5.15 (dd, 1H, J= 9.9, 1.8)
10a	102.4 t	5.88 (d, 1H, J=1.1)	101.0 t	5.88 (s, 2H)
10b		5.87 (d, 1H, J=1.1)		
Glc-1′	104.4 d	4.65 (d, 1H, J=7.7)		
Glc-2′	75.0 d			
Glc-3′	78.2 d			
Glc-4′	71.6 d			
Glc-5′	76.8 d			
Glc-6′a	68.1 t	4.01 (dd, 1H, J= 11.0, 1.5)		
Glc-6′b		3.59 (d, 1H, J=11.0)		
Rha-1″	102.2 d	4.72 (d, 1H, J=1.5)		
Rha-2″	72.1 d	3.87 (dd, 1H, J= 3.3, 1.5)		
Rha-3″	72.4 d	3.68 (dd, 1H, J= 9.5, 3.3)		
Rha-4″	74.0 d			
Rha-5″	69.8 d	3.57—3.65 (m, 1H)		
Rha-6″	18.0 q	1.23 (d, 3H, J=6.2)		
Glc-2′-5′, Rha-4″		(3.31—3.56 (m, 5H))		

with crude hesperidinase for 24 h at 37 °C, it gave colorless needles (**4a**). **4a**, mp 77—78 °C, showed the molecular ion peak at m/z 178 in electron impact (EI)-MS. According to the ^{1}H- and ^{13}C-NMR spectra of **4a**, as shown in Table III, **4a** has 2-propenyl and methylenedioxy groups along with a phenolic hydroxyl group. Two aromatic protons appeared as a singlet at δ_H 6.42 and 6.58 (each 1H), indicating **4a** has a 1,2,4,5-substituted benzene ring. Intensive analysis of the HMBC spectrum of **4a** suggesged that **4a** is 2-allyl-4,5-methylenedioxyphenol. This compound was synthesized and reported by Alexander *et al.*, in 1959,[4] and the melting point of **4a** was identical with the reported value.

On the other hand, the hydrolysis of **4** with crude hesperidinase for 6 h at 37 °C afforded compound **4b**, whose FAB-MS showed the molecular ion at m/z 341 [M^++1]. Acetylation of **4b** with Ac_2O and pyridine gave compound **4c**. In the ^{1}H-NMR spectrum of **4c**, four acetyl methyl groups were seen at δ_H 2.10, 2.07, 2.05 and 2.03 (each 3H, s). Therefore, the linking order of **4a**, glucose and rhamnose was established as in the order of **4a**→glucose→rhamnose. Rhamnose should link to glucose at position C-6′, because the signal of C-6 in the ^{13}C-NMR spectrum of **4** appeared at δ_C 68.1. The anomeric proton signal of glucose appears as a doublet signal (J=7.7 Hz) and the carbon signal at δ_C 104.4, indicating the β-linkage of glucose to allylphenol. There is good agreement between the carbon signals due to the sugar moiety (rutinose) of **4** (Table III) and those of the glucose–(glucose–rhamnose) moiety of the hydrolyzed product of capsianoside A isolated by Nohara *et al.*, in CD_3OD solution.[5] As a result of this evidence, **4** was revealed to be 2-allyl-4,5-methylenedioxyphenol-1-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside.

Experimental

The melting point was determined on a Yanagimoto micro melting point apparatus and is uncorrected. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL JNM-GX-400 and JEOL JNM-FX-90Q spectrometers. 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 and Shimadzu IR-408. 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 barks (2.2 kg) of *I. difengpi* was extracted with AcOEt and *n*-BuOH successively, after extraction with *n*-hexane. The *n*-BuOH soluble part (81.5 g) was dissolved in the solvent mixture of *n*-hexane–AcOEt (1 : 9), and the soluble part was again dissolved in acetone to afford the acetone soluble portion, which was further partitioned between AcOEt and H_2O. The AcOEt soluble part was chromatographed over Toyopearl HW-40 using MeOH, repeatedly, to provide fractions A, B, C, D, E, F and G. Fraction B was subjected to a column of Toyopearl HW-40 (solvent, H_2O : MeOH = 1 : 3) followed by repeated silica gel chromatography (solvent; $CHCl_3$: MeOH : H_2O = 8 : 2 : 0.1), which afforded compounds **1** (10.3 mg) and **2** (15.0 mg). Subsequent column chromatography of fractions C and D gave compounds **3** (159.7 mg) and **4** (628.6 mg), which were purified by a Kusano prepacked ODS column (solvent, H_2O : MeOH = 4 : 6), respectively.

Compound 1 An amorphous powder, $C_{13}H_{20}O_5$, FAB-MS m/z: 257 [M^++1]. IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH), 1590, 1510, 1375.

Acetylation of 1 **1** (5 mg) was dissolved in a mixture of dry pyridine (0.5 ml) and Ac_2O (0.5 ml), 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–AcOEt (1 : 9)] to give an oily acetylated compound (**1a**) (4 mg). EI-MS m/z: 382 (M^+). ^{1}H-NMR: see Table I.

Compound 2 A cololress syrup, $C_{17}H_{26}O_6$, FAB-MS m/z: 327 [M^++1], $[\alpha]_D^{15}$ −17.5° (c=0.75, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH), 1740 (CO), 1590.

Compound 3 A colorless syrup, $C_{26}H_{34}O_{12}$, FAB-MS *m/z*: 561 $[M^+ + Na]$, $[\alpha]_D^{20}$ −20.2° (*c* = 1.32, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH), 1735 (CO), 1600.

Acetylation of 3 **3** (16 mg) was dissolved in a mixture of 1 ml of dry pyridine and 1 ml of Ac_2O. After standing over night, the solvent was evaporated under reduced pressure to give a residue, which was chromatographed on silica gel (solvent; $CHCl_3$: MeOH = 99 : 1). An acetate **3a** (16.6 mg), syrup, was finally purified by Kusano Si-5 column with the same solvent. **3a**: EI-MS *m/z*: 790 $[M^+]$; δ (CD_3OD) (90 MHz): 1.84, 1.96, 1.97, 2.01, 2.02, 2.30 (each 3H, s), 2.60 (2H, br t, *J* = 8.2 Hz), 3.79 (3H, s), 3.8—5.4 (13H, m), 4.44 (1H, d, *J* = 7.1 Hz), 6.62 (1H, dd, *J* = 8.2, 1.7 Hz), 6.79 (1H, d, *J* = 1.7 Hz), 6.91 (1H, d, *J* = 8.2 Hz), 7.21, 8.06 (each 2H, d, *J* = 8.8 Hz).

Acid Hydrolysis of 3 Compound **3** (10 mg) was dissolved in a mixture of 2 N HCl (0.5 ml) and EtOH (1 ml), which was refluxed for 2 h. After 10 ml of H_2O was poured into the reaction mixture, it was neutralized with $AgCO_3$, then the precipitates were filtered off. The filtrate was partitioned between H_2O and AcOEt three times to give an organic layer and a water layer. The latter including **3d** was concentrated under the reduced pressure, then examined by Avicel TLC (solvent, *n*-BuOH : pyridine : H_2O = 6 : 4 : 3, upper layer) with an authentic sample of glucose. The former was evaporated to dryness after drying over Na_2SO_4, and the residue was chromatographed on silica gel using the solvent system of $CHCl_3$–MeOH–H_2O (75 : 25 : 0.1) to give a trace amount of compounds **3b** and **3c**. **3c** was identified as *p*-hydroxybenzoic acid by comparison of its ^{1}H-NMR spectrum and TLC with those of the authentic sample. **3b** was corroborated to **1** by its TLC, ^{1}H-NMR spectrum and FAB-MS.

Compound 4 A colorless powder, $C_{22}H_{30}O_{12}$, FAB-MS *m/z*: 487 $[M^+ + 1]$, $[\alpha]_D^{15}$ −58.9° (*c* = 1.54, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3325 (OH), 1635, 915.

Acid Hydrolysis of 4 **4** (8 mg) was dissolved in aqueous 2 N HCl, and heated over a water bath (*ca.* 70 °C) for 1 h. After cooling down, the reaction mixture was neutralized with $AgCO_3$. The precipitates were filtered off and the aqueous filtrate was extracted with Et_2O three times. The water layer was concentrated under reduced pressure and examined by Avicel TLC (the same solvent as in the case of **3**) with the authentic samples of glucose and rhamnose.

Enzymic Hydrolysis of 4 Crude hesperidinase (Sigma Chemical Co.) (40 mg) was added to a solution of **4** (35 mg) in H_2O (10 ml), which was shaken at 37 °C for 24 h, then extracted with Et_2O three times. The Et_2O 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 *n*-hexane–AcOEt (2 : 1) to give **4a** (7 mg). **4a**; colorless needles, mp 77—78 °C (lit. 76—77 °C).[4] ^{1}H- and ^{13}C-NMR spectra were shown in Table III.

Partial Enzymic Hydrolysis of 4 **4** (32 mg) and crude hesperidinase (40 mg) in H_2O (10 ml) was shaken at 37 °C for 6 h, then extracted with Et_2O three times. The Et_2O 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 (4 : 1) to give a syrup, **4b** (10 mg). FAB-MS *m/z*: 341 $[M^+ + 1]$, δ (CD_3OD) (90 MHz): *ca.* 3.3 (2H, m, overlapped with the signal of solvent), 3.6—4.0 (6H, m), 4.67 (1H, d, *J* = 7.1 Hz, anomeric proton), 4.97 (1H, dd, *J* = 8.8, 1.7 Hz), 5.01 (1H, dd, *J* = 16.0, 1.7 Hz), 5.86 (2H, s, methylene protons of methylene dioxy moiety), 6.02 (1H, m), 6.58, 6.81 (each 1H, s, aromatic protons).

Acetylation of 4b **4b** (5 mg) was dissolved in a mixture of dry pyridine (0.5 ml) and Ac_2O (0.5 ml), and left to stand for 4 h. After a usual treatment, the residue was chromatographed on silica gel with the solvent of $CHCl_3$–MeOH (19 : 1) to give an oily syrup, **4c** (4 mg). EI-MS *m/z*: 508 $[M^+]$, δ ($CDCl_3$) (90 MHz): 2.03, 2.05, 2.07, 2.10 (each 3H, s, –OAc), 3.23 (2H, br d, *J* = 6.3 Hz), 3.89 (1H, m), 4.19 (2H, m, $-CH_2OH$), 4.23 (1H, d, *J* = 6.5 Hz, anomeric proton), 4.8—5.3 (5H, m), 5.78 (1H, m), 5.90 (2H, s, methylene protons of methylene dioxy moiety), 6.61, 6.70 (each 1H, s, aromatic protons).

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