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The Structure of Toxic Components from Leucothoe Keiskei[1]

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Constituents of *Leucothoe Keiskei*, poriolide (**1**) and isoporiolide (**2**) have a novel structure of the macrocyclic biphenyl-type flavonoid glycosides. Results of the spectroscopic analysis and the chemical degradation of these compounds are discussed. The structure of isoporiolide, an isomer of poriolide, was derived from a comparison of the degradation products of poriolide, isoporiolide and their derivatives. On hydrolysis with barium hydroxide, both hexamethylethers (**5** and **17**) of poriolide and isoporiolide gave the identical propiophenone glycoside (**12a**), but the isoporiolide hexamethylether yielded an isomer (**18a**) of the carboxylic acid (**13a**) obtained from the poriolide hexamethylether.

Leucothoe Keiskei Miq. (Ericaceae) is a woody herb which is distributed on the Pacific side of Central Honshu and it is called Iwananten in Japan. A methanolic extract of the plant showed a toxicity but only two triterpenoids, ursolic acid and uvaol had been isolated by Shimada in 1939.[3] This finding prompted us to investigate precisely the constituent of this plant, resulting in the isolation of the toxic principles designated as poriolide and isoporiolide. We describe herein the structural determination of these macrocyclic flavonoid glycosides having a biphenyl-type structure.

Poriolide (**1**), mp 265°, $[\alpha]_D^{20}$ −334° (c=2, acetone) has the molecular formula of $C_{29}H_{26}O_{12}$ from elementary analysis and the high resolution mass spectrum (M^+ 566.144), and its toxicity is represented by the intravenous LD_{50} 1.0 mg/kg in mice. Acetylation of **1** with acetic anhydride in pyridine at 100° gave a hexaacetate (**3**), mp 293—294°, and methylation of **1** with dimethyl sulfate in the presence of barium hydroxide-barium oxide in dimethyl sulfoxide-N,N-dimethylformamide afforded a hexamethylether (**4**), mp 280—282°. On methylation with methyl iodide in the presence of silver oxide in dimethylformamide, poriolide (**1**) yielded a hexamethylether (**5**), mp 270°. The molecular formula and the nuclear magnetic resonance (NMR) spectrum of **5** suggest that a secondary methyl group in addition to six methoxyl methyl groups was introduced into **1** on methylation. From the following results it is clear that the secondary methyl group of **5** is located at the C-3 position adjacent to the carbonyl group. Reduction of **5** with sodium borohydride followed by treatment with *p*-toluenesulfonic acid yielded a 1,2-chromene type compound (**7**), mp 253—255°, whose infrared (IR) spectrum shows an ester absorption band at 1725 cm^{-1}. The doublet at δ 1.48 (J=7.0 Hz) due to the secondary methyl group in the NMR spectrum of **5** changed to a singlet of an olefinic methyl group at δ 2.06 in **7**. In addition, alkaline degradation of **5** gave a propiophenone glycoside (**12a**) (*vide infra*). Methylation of **1** with diazomethane afforded a dimethylether (**8**), mp 222—224°, whose IR and NMR data and the positive ferric chloride test indicate that the dimethylether (**8**) has another phenolic group. Therefore, poriolide (**1**) has three phenolic and three alcoholic hydroxyl groups. Hydrolysis of **1** with sodium hydroxide followed by treatment with hydrochloric acid gave D-glucose in the water-soluble fraction.

The NMR spectrum of **1** reveals the presence of three benzene rings in the molecule, one of which is pentasubstituted and has a methyl group. Observation of a long-range coupling between the methyl protons and the aromatic proton 8-H at δ 6.46 leads to the partial struc-

1) Preliminary report: A. Ogiso, A. Sato, S. Sato, and C. Tamura, *Tetrahedron Letters*, 1972, 3071.
2) Location: *1-2-58, Hiromachi, Shinagawa-ku, Tokyo.*
3) G. Shimada, *J. Pharm. Soc. Japan*, **59**, 619 (1939).

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ture (**A**). The signals at δ 7.83 (2″-H, d, J=2.3 Hz), 6.96 (5″-H, d, J=8.5 Hz), and 8.05 (6″-H, dd, J=2.3 and 8.5 Hz) belong to the benzene ring depicted as the partial structure (**B**). Another is assigned to the benzene ring in the partial structure (**C**). Because irradiation of the signal assignable to a benzylic proton 2-H at δ 5.86 (dd, J=2.5 and 5.7 Hz), which constitutes an ABX pattern with the methylene protons adjacent to the carbonyl group, sharpens the signals of the aromatic protons 2′-H at δ 7.59 (d, J=2.5 Hz) and 6′-H at δ 7.09 (dd, J=2.5 and 8.5 Hz). The IR absorption band at 1640 cm^{-1} in **1** markedly shows the presence of the hydrogen bonding between the carbonyl group and the phenolic group, and the corresponding absorption bands in **4** and **5** shift at 1675 and 1695 cm^{-1} respectively. A nuclear Overhauser effect is observed in the aromatic proton 8-H when the signal of an anomeric proton at δ 5.41 (d, J=7.0 Hz) is irradiated. In addition to these evidences, since (*R*)-poriol (**9a**) and its glycoside, (*R*)-poriolin (**9b**), were isolated from the same plant source, the partial structures (**A** and **C**) could be connected to form a flavanone structure as in **D**.

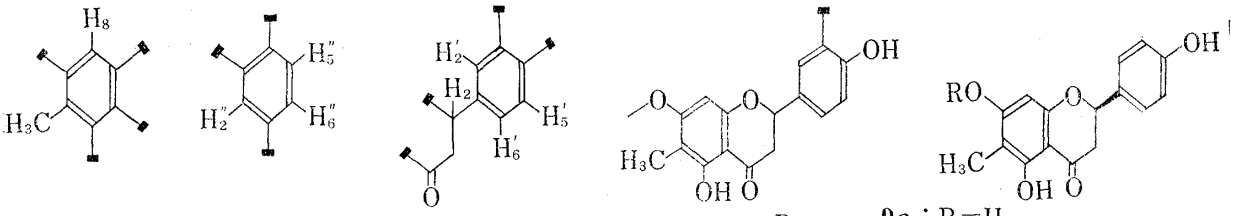

Chart 1

With Sarett reagent, oxidation of the benzylalcohol (**10**), mp 173—174°, obtained by lithium aluminium hydride reduction of **5**, gave a benzaldehyde (**11a**), mp 148—149°, showing absorption bands at 1695 and 1685 cm^{-1} in the IR spectrum. The NMR spectrum of **11a** exhibits a long-range coupling between the aldehydic proton at δ 10.48 and the aromatic proton 2″-H at δ 7.98 (d, J=2.3 Hz). The IR absorption band at 1670 cm^{-1} in **1** indicates the presence of hydrogen bonding between the ester carbonyl and phenolic groups, while the corresponding absorption bands of **4** and **5** appear at 1730 and 1740 cm^{-1} respectively. The above results extend the partial structure (B) to E.

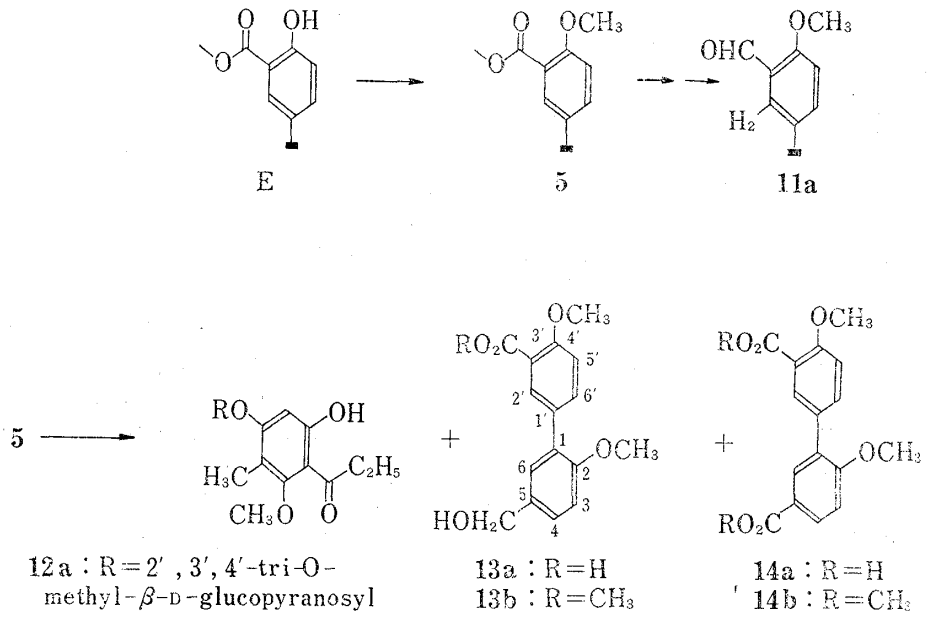

Chart 2

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Table I. NMR Spectrum Data of the Biphenyls

	13b	18b
2-OCH_3	3.79 (s)	3.74 (s)
2'-OCH_3		3.46 (s)
3'-CO_2OH_3	3.83 (s)	3.86 (s)
4'-OCH_3	3.90 (s)	
5-CH_2OH	4.59 (s)	4.58 (d, J=6.0 Hz)
3-H	7.03 (d, J=9.0 Hz)	7.02 (d, J=8.5 Hz)
4-H	7.29 (dd, J=2.5, 9.0 Hz)	7.34 (dd, J=2.3, 8.5 Hz)
6-H	7.30 (d, J=2.5 Hz)	7.19 (d, J=2.3 Hz)
2'-H	7.84 (d, J=2.2 Hz)	
4'-H		7.66 (dd, J=2.1, 8.0 Hz)
5'-H	7.14 (d, J=9.0 Hz)	7.16 (t, J=8.0 Hz)
6'-H	7.66 (dd, J=2.2, 9.0 Hz)	7.46 (dd, J=2.1, 8.0 Hz)

Spectra are measured in deuteroacetone.

Degradation of **5** with barium hydroxide gave the propiophenone glycoside (**12a**), 179—180° and biphenylcarboxylic acids (**13a** and **14a**). The glycoside (**12a**) was easily hydrolyzed with acid to afford 2,4-dihydroxy-6-methoxy-5-methylpropiophenone (**12b**), mp 132—134°. Separation of the acids (**13a** and **14a**) was effected by a preparative thin-layer chromatography of the corresponding methyl esters (**13b** and **14b**). The structure of the methylester (**13b**) having the molecular formula of $C_{17}H_{18}O_5$ was assigned by the NMR spectrum shown in Table I. Irradiation of the signal assignable to the carbinyl methylene group at δ 4.59 sharpens the aromatic protons 4-H at δ 7.29 (dd, J=2.5 and 9.0 Hz) and 6-H at δ 7.30 (d, J=2.5 Hz), furthermore the aromatic protons 3-H at δ 7.03 (d, J=9.0 Hz) and 5'-H at δ 7.14 (d, J=9.0 Hz) exhibit nuclear Overhauser effects (20% and 25%), when the methoxyl methyl signals at δ 3.79 and at δ 3.90 are irradiated respectively. Thus, the alkaline degradation products (**12a** and **13b**) provide evidences that poriolide (**1**) has the structure of a 3'-phenylflavanone-7-O-glucoside. The structure of the aglycone (**15**), mp 212—213°, derived from the hexamethylether (**4**) was proved by the synthesis described in the following paper.[4]

A remaining problem for the structure of **1** is which hydroxyl group of the glucose moiety constitutes the intramolecular ester linkage with the carboxyl group. Although the Dreiding model shows that all of the hydroxyl groups in the glucose moiety are capable of forming an ester linkage, the observation of the nuclear Overhauser effect in the aromatic proton 8-H by irradiation of the signal due to the anomeric proton indicates that the ester linkage is formed at C-6 hydroxyl group. Namely, only in the case of esterification of the C-6 hydroxyl group with the carboxyl group, the anomeric proton and the aromatic proton H-8 are close enough to account for the nuclear Overhauser effect. In the other cases, formation of an ester linkage with either C-2, C-3, or C-4 alcohols, both protons would not be accessible to each other to show the nuclear Overhauser effect in their preferred conformations. This inference is supported by the comparison of the NMR spectrum of the aldehyde (**11a**) with that of its acetate (**11b**), mp 197—198°. In the NMR spectrum of **11b**, only the signals at δ 4.14 (dd, J=6.5 and 11.5 Hz) and at δ 4.32 (dd, J=3.0 and 11.5 Hz) assignable to the methylene protons at C-6 of glucose appear in lower field than those of **11a** (δ 3.2—3.9). Thus, in the poriolide hexamethylether (**5**), C-6 alcohol of glucose has been esterified intramolecularly to form the macrocyclic ester, and the structure of poriolide must be depicted as **1**.

Isoporiolide (**2**), mp 293—295°, $[\alpha]_D^{20}$ −340° (c=2, acetone), LD_{50} 1.6 mg/kg in mice, is an isomer of **1** from the molecular formula of $C_{29}H_{26}O_{12}$ (M^+ 566.144). The aromatic protons appearing at δ 7.74 (4″-H, dd, J=2.0 and 8.0 Hz), 6.96 (5″-H, t, J=8.0 Hz), and 7.79 (6″-H,

4) A. Ogiso, A. Sato, I. Kashida, and Y. Sugimura, *Chem. Pharm. Bull.* (Tokyo), **22**, 144 (1974).

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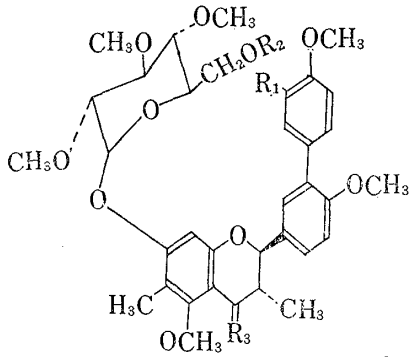
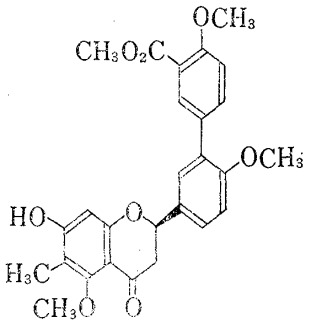


10 : $R_1=CH_2OH$, $R_2=H$, $R_3=\langle^{H}_{OH}$
11a : $R_1=CHO$, $R_2=H$, $R_3=O$
11b : $R_1=CHO$, $R_2=COCH_3$, $R_3=O$



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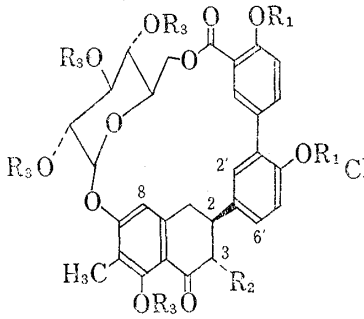

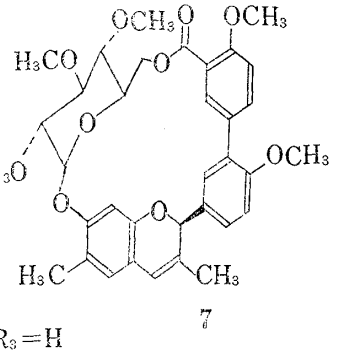

1 : $R_1=R_2=R_3=H$
3 : $R_1=R_3=COCH_3$, $R_2=H$
4 : $R_1=R_3=CH_3$, $R_2=H$
5 : $R_1=R_2=R_3=CH_3$
8 : $R_1=CH_3$, $R_2=R_3=H$

7

Chart 3

dd, $J=2.0$ and 8.0 Hz) in the NMR spectrum of **2** are assigned to a 1,2,3-trisubstituted benzene ring (F) in addition to the presence of the same partial structures (A and C) as those of poriolide. On hydrolysis with sodium hydroxide followed by hydrochloric acid, **2** gave D-glucose. Methylation of **2** with dimethyl sulfate in the presence of barium hydroxide-barium oxide yielded the hexamethylether (**16**), mp 243.5—244.5° and treatment of **2** with methyl iodide in the presence of silver oxide afforded the hexamethylether (**17**) having a secondary methyl group at the C-3 position. The spectral data of these derivatives are very similar to those of the corresponding derivatives of poriolide (**1**).

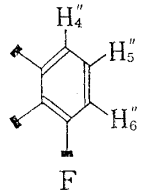

Chart 4

Alkaline degradation of **17** under the same conditions as **5** afforded the propiophenone glycoside (**12a**) and two biphenylcarboxylic acids (**18a** and **19a**). Since the resulting glycoside is identical with **12a** derived from **5**, isoporiolide has the same partial structure with respect to the A ring of flavanone and glucose moiety including the position of the sugar and the ester linkage. In the NMR spectrum of the methylester (**18b**), obtained by esterification of **18a** with diazomethane, there is observed the signal pattern due to the 2,2′,3′,5-tetrasubstituted biphenyl protons as shown in Table I. Furthermore, irradiation of the signal at δ 4.58 assignable to the carbinyl protons sharpens the signals of two aromatic protons at δ 7.19 (6-H) and 7.34 (4-H), in addition, only the aromatic proton at 7.02 (3-H) exhibits a nuclear Overhauser effect (37%) when the methoxyl methyl signal at δ 3.74 is irradiated. Thus the structure (**18b**) was assigned to the methyl ester derived from isoporiolide (**2**) and proved by the

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unambiguous synthesis described in the following paper.[4] Recombination of **12a** and **18a**, together with reference to the circular dichroism (CD) data described below, led to the conclusion that the structure of isoporiolide is designated as **2**. The structure of the aglycone (**20**), mp 201—203° derived from hexamethylether (**16**) was proved by the synthesis described in the following paper.[4]

Notwithstanding the failure in the hydrolysis of **2** with sodium hydroxide, careful hydrolysis with barium hydroxide followed by esterification with diazomethane gave the glycoside methylester (**21a**), which was successfully hydrolyzed to the aglycone (**21b**), mp 215—216°, by incubation with β-glucosidase. Application of the modified octant rule for α,β-unsaturated ketone to flavanones predicts that the flavanones having a 2(*R*)-configuration will show negative Cotton effects near at 330 nm due to a *n*-π* transition and those having a 2(*S*)-configuration exhibit positive ones.[5] In the CD of **21a** and **21b** shown in Fig. 1, the sign of n-π* transition near at 330 nm is negative and that of the π-π* transition near at 285 nm is positive. These signed features are characteristic of the 2(*R*)-configuration as exemplified by (+)-sakuranetin[6] and (+)-naringenin.[7]

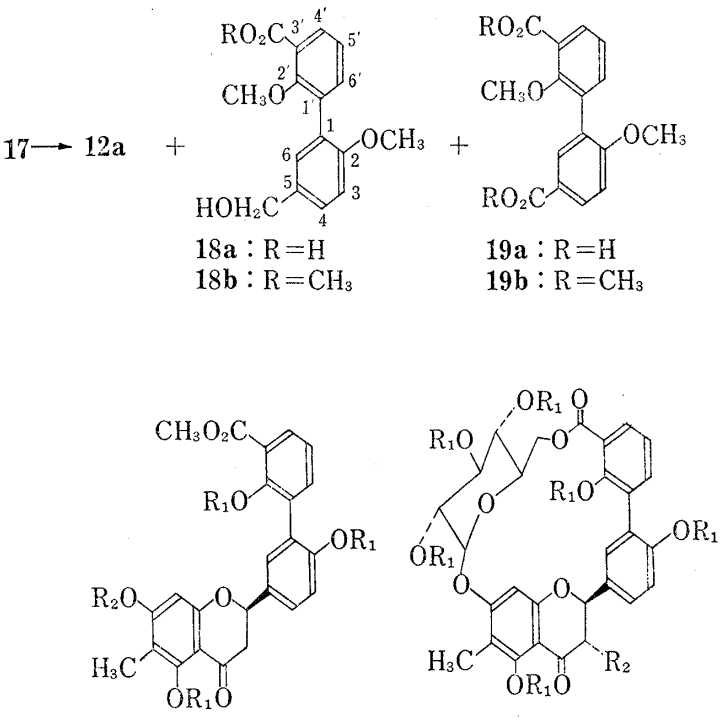

Chart 5

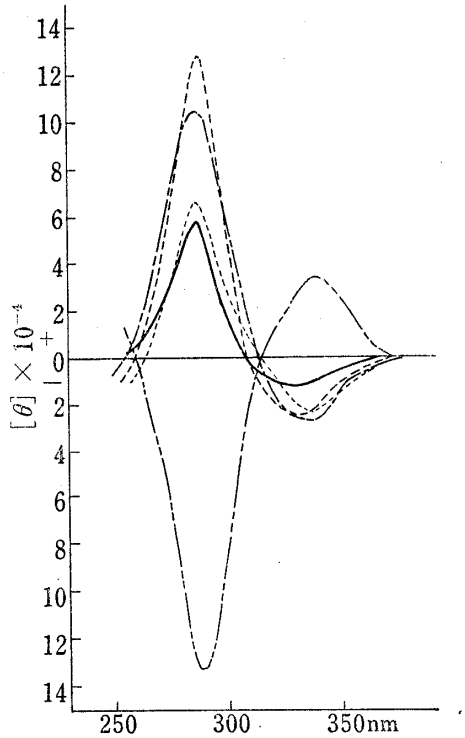

Fig. 1. Circular Dichroism Curves of the Flavanones

Additionally, since (*R*)-poriol (**9a**) and the glucoside, (*R*)-poriolin (**9b**)[8] have the same signed features as **21a** and **21b**, these flavanones have the 2(*R*)-configuration. Poriol isolated by Barton[9] has been determined to be the 2(*S*)-configuration.[5]

5) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).
6) W. Gaffield and A.C. Wais, *Chem. Comm.*, **1968**, 29.
7) H. Arakawa and Y. Masui, *Bull. Chem. Soc. Japan*, **42**, 1452 (1969).
8) A. Ogiso and I. Kashida, *Phytochemistry*, **11**, 3545 (1972).
9) G.M. Barton, *Can. J. Chem.*, **45**, 1020 (1967); *idem*, *Phytochemistry*, **11**, 426 (1972).

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Experimental

The melting points were determined in capillary tubes and uncorrected. The ultraviolet (UV) spectra were measured in 95% ethanol using Beckman DK-2A spectrophotometer. The IR spectra were determined on a JASCO IR-2A spectrophotometer. The NMR spectra were determined with Varian A-60 and HA-100 spectrometer using tetramethylsilane as an internal reference.

Isolation——Dry ground whole plants (10 kg) were extracted thrice with 10 liter of hot methanol. The combined methanolic extracts were concentrated. After addition of water, the concentrate was washed with *n*-hexane. From the *n*-hexane layer, ursolic acid, uvaol and phytosterols were isolated. The aqueous methanolic layer was extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried and evaporated to leave 400 g of an oil. A solution of the oil in a small volume of ethyl acetate was poured into 2 liter of benzene under vigorous stirring. The resulting precipitates were collected and dried to yield 320 g of a brown powder. The benzene layer was evaporated to give 75 g of a dark green oil, which was chromatographed on silica gel (1 kg) eluting with benzene–ethyl acetate (10: 1) to afford (*R*)-poriol, which was recrystallized from methanol giving 4.0 g of colorless needles. The brown powder was dissolved in ethanol and allowed to stand at room temperature to yield 19 g of poriolide, which was recrystallized from ethyl acetate–ethanol giving 17 g of light yellow crystals. The mother liquor was chromatographed on silica gel (ten times amount of the dry weight), elution with ethyl acetate gave 35 g of a mixture of poriolide and isoporiolide, which was recrystallized from ethanol affording 15 g of poriolide. Further elution of the column with ethyl acetate gave a fraction containing flavonoid glycosides. The fraction was treated with an active carbon and chromatographed on polyamide to give 200 g of an amorphous material. Preparative thin-layer chromatography using polyamide developing with methanol–acetic acid–water (90: 5: 5) gave 20 mg of (*R*)-poriolin from 200 mg of the amorphous material. The mother liquor free from crystals of poriolide was chromatographed on magnesium silicate column and elution with acetone gave isoporiolide, which was recrystallized from *n*-hexane–acetone affording 15 g of colorless needles.

Poriolide: mp 265°, $[\alpha]_D^{20°}$ −334° (*c*=2, acetone), LD_{50} 1.0 mg/kg. Mass Spectrum *m/e*: 566.144 (Calcd. 566.142) [M+]. IR ν_{max}^{Nujol} cm^{-1}: 3300, 1675, 1640, 1610, 1600, 1500, 1490. UV λ_{max} nm (ε): 287 (22000). NMR δ [$(CD_3)_2CO$]: 1.91 (3H, s), 3.31 (1H, dd, *J*=2.5, 17.0 Hz), 3.48 (1H, dd, *J*=5.7, 17.0 Hz), 5.86 (1H, dd, *J*=2.5, 5.7 Hz), 5.41 (1H, d, *J*=7.0 Hz), 6.46 (1H, s), 7.83 (1H, d, *J*=2.3 Hz), 6.96 (1H, d, *J*=8.5 Hz), 8.05 (1H, dd, *J*=2.3, 8.5 Hz), 7.59 (1H, d, *J*=2.5 Hz), 6.90 (1H, d, *J*=8.5 Hz), 7.09 (1H, dd, *J*=2.5, 8.5 Hz), 10.66 (1H, s), 12.06 (1H, s). *Anal.* Calcd. for $C_{29}H_{26}O_{12}\cdot H_2O$: C, 59.93; H, 4.72. Found: C, 59.89; H, 4.80.

Isoporiolide: mp 293—295°, $[\alpha]_D^{20°}$ −340° (*c*=2, acetone), LD_{50} 1.6 mg/kg. Mass Spectrum *m/e*: 566.144 (Calcd. 566.142) [M+]. IR ν_{max}^{Nujol} cm^{-1}: 3400, 3205, 1710, 1630, 1580, 1500. UV λ_{max} nm (ε): 287 (18600). NMR δ [$(CD_3)_2CO$]: 1.91 (3H, s), 3.33 (1H, dd, *J*=2.5, 17.5 Hz), 3.46 (1H, dd, *J*=5.5, 17.5 Hz), 5.82 (1H, dd, *J*=2.5, 5.5), 5.49 (1H, d, *J*=7.0 Hz), 6.41 (1H, s), 6.87 (1H, d, *J*=8.5 Hz), 7.02 (1H, dd, *J*=2.5, 8.5 Hz), 7.84 (1H, d, *J*=2.5), 6.96 (1H, t, *J*=8.0 Hz), 7.74 (1H, dd, *J*=2.0, 8.0 Hz), 7.79 (1H, dd, *J*=2.0, 8.0 Hz), 8.98 (1H, s), 12.09 (1H, s). *Anal.* Calcd. for $C_{29}H_{26}O_{12}\cdot H_2O$: C, 59.93; H, 4.72. Found: C, 60.17; H, 4.60.

(*R*)-Poriol: mp 270—275°, $[\alpha]_D^{25°}$ +10° (*c*=3.5, acetone). IR ν_{max}^{Nujol} cm^{-1}: 3350, 3200, 1635, 1625, 1610, 1550. UV λ_{max}nm (ε): 212 (26900), 225 (24300), 294 (18000), 335 (3700). CD (dioxane) $[\theta]^{20°}$ (nm): +58300 (287), −12600 (328). NMR δ ($CDCl_3$): 1.91 (3H, s), 2.72 (1H, dd, *J*=4.0, 17.0 Hz), 3.21 (1H, dd, *J*=12.0, 17.0 Hz), 5.41 (1H, dd, *J*=4.0, 12.0 Hz), 6.02 (1H, s), 6.83 (2H, d, *J*=8.6 Hz), 7.33 (2H, d, *J*=8.6 Hz), 12.45 (1H, s). *Anal.* Calcd. for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93. Found: C, 67.00; H, 4.75.

(*R*)-Poriolin: mp 173—175°, $[\alpha]_D^{20°}$ 0° (*c*=3.5, acetone). IR ν_{max}^{Nujol} cm^{-1}: 3320, 1640, 1620, 1590, 1520. UV λ_{max}nm (ε): 288 (16200), 330 (3300). *Anal.* Calcd. for $C_{22}H_{24}O_{10}$: C, 58.93; H, 5.36. Found: C, 58.81; H, 5.43.

Acetylation of Poriolide (1)——To a solution of 100 mg of poriolide (1) in 1 ml of dry pyridine was added 1 ml of acetic anhydride, and the mixture was heated at 100° for 1 hr. The reaction mixture was poured into ice-water and the resulting precipitates were recrystallized from chloroform to yield 100 mg of the hexaacetate (3) as colorless needles, mp 293—294°. IR ν_{max}^{Nujol} cm^{-1}: 1770, 1750, 1730, 1675. UV λ_{max} nm (ε): 260 (18000), 280 (14300). NMR δ ($CDCl_3$): 2.01 (3H, s), 2.05 (3H, s), 2.13 (6H, s), 2.36 (6H, s). *Anal.* Calcd. for $C_{41}H_{40}O_{18}$: C, 60.00; H, 4.85. Found: C, 60.20; H, 4.77.

Methylation of Poriolide (1) with Dimethyl Sulfate——To a solution of 600 mg of poriolide (1) in 3 ml of dimethylsulfoxide and 3 ml of N,N-dimethylformamide was added 1.02 g of barium oxide and 1.02 g of barium hydroxide octahydrate. To the mixture was added 2.1 ml of dimethyl sulfate at the temperature below 7°. After the addition was finished, the reaction mixture was allowed to stir at room temperature overnight, treated with 2 ml of ammonia, and extracted with chloroform. The chloroform layer was washed with water, dried and evaporated to yield 500 mg of the hexamethylether (4), which was recrystallized from *n*-hexane–chloroform affording colorless needles, mp 280—282°. IR ν_{max}^{Nujol} cm^{-1}: 1730, 1675. UV λ_{max} nm (ε): 264 (21600), 304 (10000). NMR δ ($CDCl_3$): 3.61 (3H, s), 3.65 (3H, s), 3.70 (6H, s), 3.77 (3H, s), 3.98 (3H, s). *Anal.* Calcd. for $C_{35}H_{38}O_{12}$: C, 64.61; H, 5.89. Found: C, 64.67; H, 6.05.

Methylation of Poriolide (1) with Methyl Iodide——To a solution of 1.2 g of poriolide (1) in 32 ml of N,N-dimethylformamide was added 4.8 g of finely ground silver oxide and 8 ml of methyl iodide and the

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mixture was shaken at room temperature for 24 hr. After addition of chloroform, the mixture was filtered and evaporated to afford a light yellow oil, which was chromatographed on silica gel (20 g) on elution with chloroform to separate 1.2 g of the hexamethylether (**5**), which was recrystallized from chloroform–methanol giving 1.1 g of colorless needles, mp 270°. IR ν_{max}^{Nujol} cm^{-1}: 1740, 1695. UV λ_{max} nm (ε): 264 (21100), 302 (9700). NMR δ ($CDCl_3$): 1.48 (3H, d, J=7.0 Hz), 3.63 (3H, s), 3.65 (3H, s), 3.70 (6H, s), 3.73 (3H, s), 3.96 (3H, s). *Anal.* Calcd. for $C_{36}H_{40}O_{12}$: C, 65.05; H, 6.07. Found: C, 64.82; H, 5.84.

Methylation of Poriolide (1) with Diazomethane——To a solution of 1.0 g of poriolide (**1**) in 20 ml of dioxane was added a large excess of an ether solution of diazomethane and the mixture was kept at room temperature overnight. Evaporation of the solvent left 800 mg of a crystalline product, which was recrystallized from acetone giving 650 mg of the dimethylether (**8**) as light yellow needles, mp 222—224°. IR ν_{max}^{Nujol} cm^{-1}: 3450, 1720, 1640. UV λ_{max} nm (ε): 230 (sh) (35800), 260 (17800), 284 (19000). NMR δ (C_5D_5N): 3.65 (3H, s), 3.80 (3H, s), 12.05 (1H, s). *Anal.* Calcd. for $C_{31}H_{30}O_{12}\cdot H_2O$: C, 60.78; H, 5.23. Found: C, 60.78; H, 5.22.

Reduction of Hexamethylether (5) with Sodium Borohydride——To a solution of 50 mg of sodium borohydride in 5 ml of tetrahydrofuran was added a solution of 300 mg of the hexamethylether (**5**) in 10 ml of tetrahydrofuran and the mixture was allowed to stir at room temperature overnight. After addition of glacial acetic acid, the resulting precipitates were filtered and the filtrate was evaporated to give an oil. The chloroform solution of the oil was washed with water, dried and evaporated to yield 230 mg of the reduction product (**6**), which was recrystallized from chloroform–methanol giving 197 mg of colorless needles, mp 241—243°. IR ν_{max}^{Nujol} cm^{-1}: 3620, 1715. UV λ_{max} nm (ε): 230 (41000), 262 (16900), 328 (9000). *Anal.* Calcd. for $C_{36}H_{42}O_{12}$: C, 64.86; H, 6.01. Found: C, 64.59; H, 6.08.

Dehydration of Alcohol (6) with *p*-Toluenesulfonic Acid——A solution of 200 mg of the alcohol (**6**) and 5 mg of *p*-toluenesulfonic acid in 15 ml of benzene was heated under reflux for 10 min. The reaction mixture was extracted with ethyl acetate and the organic layer was washed with satd. sodium bicarbonate solution and with water successively. Drying and evaporation of the solvent gave the styrene compound (**7**), which was recrystallized from *n*-hexane–chloroform giving 145 mg of colorless needles, mp 253—255°. IR ν_{max}^{Nujol} cm^{-1}: 1725, 1610, 1590, 1570, 1495. UV λ_{max} nm (ε): 233 (sh) (44200), 285 (12700). NMR δ ($CDCl_3$): 2.06 (6H, s), 5.53 (1H, s), 7.74 (1H, s). *Anal.* Calcd. for $C_{36}H_{40}O_{11}$: C, 66.66; H, 6.17. Found: C, 66.41; H, 6.14.

D-Glucose from Poriolide (1)——A solution of 50 mg of poriolide in 2 ml of 5% sodium hydroxide was heated on a steam bath for 30 min. The reaction mixture was acidified with 5% hydrochloric acid and extracted with ethyl acetate. The organic layer was evaporated and dissolved in 3 ml of 5% hydrochloric acid–methanol. After heating under reflux for 1 hr, the reaction mixture was diluted with water and washed with ethyl acetate. D-Glucose obtained from the aqueous layer was identified by thin-layer chromatography, vapor phase chromatography of the corresponding trimethylsilylether and measurement of the optical rotation.

Reduction of Hexamethylether (5) with Lithium Aluminum Hydride——To a solution of 100 mg of lithium aluminum hydride in 5 ml of dry tetrahydrofuran was added a solution of 151 mg of the hexamethylether (**5**) in 5 ml of dry tetrahydrofuran. The mixture was stirred at room temperature for 2 hr, and treated with ethyl acetate and water. Filtration of the solids and evaporation of the solvent left 150 mg of an oil. Preparative thin-layer chromatography on silica gel developing with benzene–ethyl acetate (1: 1) gave the reduction product (**10**), which was recrystallized from *n*-hexane–acetone yielding 75 mg of needles, mp 173—174°. IR ν_{max}^{Nujol} cm^{-1}: 3450, 1615, 1600, 1510, 1505. UV λ_{max} nm (ε): 230 (sh) (32900), 258 (14600), 285 (10100). *Anal.* Calcd. for $C_{36}H_{46}O_{12}$: C, 64.46; H, 6.91. Found: C, 64.22; H, 6.95.

Oxidation of Benzylalcohol (10) with Chromium Trioxide in Pyridine——To the Sarett reagent prepared from 100 mg of chromium trioxide and 1 ml of pyridine was added a solution of 100 mg of the benzylalcohol (**10**) in 0.5 ml of pyridine and the mixture was stirred at room temperature overnight. After addition of ethyl acetate, the resulting precipitates were filtered off and the ethyl acetate layer was washed with dil. hydrochloric acid and water. Drying and evaporation of the solvent gave the aldehyde (**11a**), which was recrystallized from *n*-hexane–acetone yielding 95 mg of colorless needles, mp 148—149°. IR ν_{max}^{Nujol} cm^{-1}: 3350, 1695, 1685. UV λ_{max} nm (ε): 226 (sh) (48600), 255 (27000), 324 (6400). NMR δ ($CDCl_3$): 10.48 (1H, s). *Anal.* Calcd. for $C_{36}H_{42}O_{12}$: C, 64.85; H, 6.35. Found: C, 64.79; H, 6.30.

Acetylation of Aldehyde (11a)——To a solution of 40 mg of the aldehyde (**11a**) in 0.5 ml of dry pyridine was added 0.5 ml of acetic anhydride and the mixture was kept at room temperature overnight. The reaction mixture was poured into ice-water to give yellow crystals of the acetate (**11b**), which was recrystallized from acetone–methanol affording 35 mg of colorless needles, mp 197—198°. IR ν_{max}^{Nujol} cm^{-1}: 1750, 1690, 1675, 1610, 1580, 1495. UV λ_{max} nm (ε): 227 (52400), 255 (29600), 326 (6800). NMR δ ($CDCl_3$): 1.87 (3H, s), 1.90 (3H, s), 2.12 (3H, s), 4.14 (1H, dd, J=6.5, 11.5 Hz), 4.32 (1H, dd, J=3.0, 11.5), 10.47 (1H, s). *Anal.* Calcd. for $C_{38}H_{44}O_{13}$: C, 64.40; H, 6.26. Found: C, 64.30; H, 6.20.

Alkaline Degradation of Hexamethylether (5) with Barium Hydroxide——A solution of 1.0 g of the hexamethylether (**5**) in 20 ml of dioxane was added to 20 ml of 10% aqueous barium hydroxide solution and the mixture was refluxed for 24 hr under nitrogen atmosphere. After acidification with 10% hydrochloric acid, the mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with satd. aqueous sodium bicarbonate solution, dried and evaporated to give the propiophenone glycoside (**12a**), which was recrystallized from *n*-hexane–acetone affording 350 mg of needles, mp 179—180°. Acidification of the

NII-Electronic Library Service

sodium bicarbonate extract and extraction with ethyl acetate gave 490 mg of the mixture of carboxylic acids. After esterification with diazomethane, the mixture was chromatographed on silica gel (15 g) to afford 100 mg of the dimethyl ester (**14b**) and 170 mg of the methyl ester (**13b**).

12a: IR ν_{max}^{Nujol} cm^{-1}: 3450, 3300, 1620. UV λ_{max} nm (ε): 276 (14000), 328 (4600). NMR δ ($CDCl_3$): 1.18 (3H, t, J=7.0 Hz), 3.11 (2H, q, J=7.0 Hz), 2.13 (3H, s), 3.57 (3H, s), 3.65 (6H, s), 6.35 (1H, s), 11.71 (1H, s). *Anal.* Calcd. for $C_{20}H_{30}O_9$: C, 57.96; H, 7.30. Found: C, 57.98; H, 7.28.

13b: IR $\nu_{max}^{liq.}$ cm^{-1}: 3510, 1710. UV λ_{max} nm (ε): 255 (13800), 292 (7600). NMR δ [$(CD_3)_2CO$]: 3.79 (3H, s), 3.83 (3H, s), 3.90 (3H, s), 4.59 (2H, s). *Anal.* Calcd. for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.30; H, 5.91.

Hydrolysis of Propiophenone Glycoside (12a)——A solution of 440 mg of the propiophenone glycoside (**12a**) in 80 ml of 5% hydrochloric acid–methanol was heated under reflux for 2 hr. After evaporation of methanol, the reaction mixture was extracted with ether. The ether layer was washed with water, dried and evaporated to dryness. Chromatography of the residue on silica gel (8 g) eluting with benzene–ethyl acetate (3: 1) afforded 2,4-dihydroxy-6-methoxy-5-methylpropiophenone (**12b**), which was recrystallized from *n*-hexane–ether giving 150 mg of pale yellow needles, mp 132—134°. IR ν_{max}^{Nujol} cm^{-1}: 3350, 3300, 1625. UV λ_{max} nm (ε): 284 (13700), 327 (5500). NMR δ ($CDCl_3$): 1.18 (3H, t, J=7.0 Hz), 3.10 (2H, q, J=7.0 Hz), 2.10 (3H, s), 3.75 (3H, s), 6.21 (1H, s), 6.70 (1H, s), 13.40 (1H, s). *Anal.* Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.82; H, 6.53.

Hydrolysis of Hexamethylether (4) with Sodium Hydroxide——A solution of 107 mg of the hexamethylether (**4**) in 3 ml of dioxane was added to 3 ml of 1N sodium hydroxide solution and the mixture was kept at room temperature overnight. The reaction mixture was poured into 10 ml of water and allowed to stand at room temperature for 30 min. After acidification with conc. hydrochloric acid, the mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried and concentrated to dryness and the residue was heated in 10 ml of 3% hydrogen chloride–methanol. The corresponding methyl esters were subjected to a preparative thin-layer chromatography using silica gel. Development with benzene–ethyl acetate (2: 1) gave the desired aglycone (**15**), which was recrystallized from *n*-hexane–acetone affording 30 mg of pale yellow needles, mp 212—213°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3240, 1720, 1670, 1615, 1495. UV λ_{max} nm (ε): 284 (23900). NMR δ (C_5D_5N): 2.39 (3H, s), 2.95 (1H, dd, J=3.5, 16.4 Hz), 3.25 (1H, dd, J=12.4, 16.4 Hz), 3.66 (3H, s), 3.77 (3H, s), 3.83 (3H, s), 4.04 (3H, s), 5.55 (1H, dd, J=3.5, 12.4 Hz). *Anal.* Calcd. for $C_{27}H_{26}O_8$: C, 67.77; H, 5.48. Found: C, 67.70; H, 5.45.

Methylation of Isoporiolide (2) with Dimethyl Sulfate——To a solution of 200 mg of isoporiolide (**2**) in the mixture of 2 ml of dimethyl sulfoxide and 2 ml of N,N-dimethylformamide was added 400 mg of barium oxide and 400 mg of barium hydroxide octahydrate successively. The mixture was treated with 0.8 ml of dimethyl sulfate at room temperature and allowed to stir overnight. The reaction mixture was poured into 10% hydrochloric acid solution and the resulting precipitates were collected and recrystallized from acetone-methanol to give 100 mg of the hexamethylether (**16**) as colorless needles, mp 243.5—244.5°. IR ν_{max}^{Nujol} cm^{-1}: 1720, 1670, 1620, 1600, 1500. UV λ_{max} nm (ε): 274 (17200), 299 (7700), 320 (4500). NMR δ ($CDCl_3$): 2.60 (3H, s), 3.63 (9H, s), 3.71 (3H, s), 3.77 (3H, s). *Anal.* Calcd. for $C_{35}H_{38}O_{12}$: C, 64.61; H, 5.89. Found: C, 64.52; H, 5.85.

Methylation of Isoporiolide (2) with Methyl Iodide——A mixture of 100 mg of isoporiolide (**2**), 500 mg of finely gound silver oxide, and 1 ml of methyl iodide in 3 ml of N,N-dimethylformamide was shaken at room temperature for 4 hr. After addition of chloroform, the resulting precipitates were filtered off and the solvent was evaporated. The residue was subjected to a silica gel chromatography. The eluent with chloroform gave 100 mg of the hexamethylether (**17**) as a colorless amorphous. IR ν_{max}^{Nujol} cm^{-1}: 1710, 1680, 1600, 1570, 1500. UV λ_{max} nm (ε): 276 (17200), 300 (8000), 320 (4400). NMR δ ($CDCl_3$): 1.48 (3H, d, J= 7.2 Hz), 2.63 (3H, s), 3.63 (9H, s), 3.70 (3H, s), 3.75 (3H, s). *Anal.* Calcd. for $C_{36}H_{40}O_{12}$: C, 65.05; H, 6.70. Found: C, 64.95; H, 6.00.

D-Glucose from Isoporiolide (2)——The same procedure as the case of poriolide gave D-glucose identified by several methods.

Alkaline Degradation of Hexamethylether (17) with Barium Hydroxide——A solution of 1.0 g of the hexamethylether (**17**) in 20 ml of dioxane was added to 20 ml of 10% aqueous barium hydroxide solution and the mixture was refluxed for 24 hr under nitrogen atmosphere. After acidification with 10% hydrochloric acid, the mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with satd. aqueous sodium bicarbonate solution, dried and evaporated to give 400 mg of the propiophenone glycoside (**12a**). After acidification of the sodium bicarbonate extract, the mixture of the carboxylic acids was extracted with ethyl acetate and esterified with diazomethane. The mixture of the corresponding methylesters was subjected to a silica gel chromatography. The eluent with benzene–ethyl acetate (3: 1) gave 100 mg of the dimethyl ester (**19b**) and that with benzene–ethyl acetate (1: 1) gave 200 mg of the methyl ester (**18b**). IR $\nu_{max}^{liq.}$ cm^{-1}: 3450, 1720, 1610, 1590, 1500. UV λ_{max} nm (ε): 287 (5200). NMR δ [$(CD_3)_2CO$]: 3.46 (3H, s), 3.74 (3H, s), 3.86 (3H, s), 4.04 (1H, t, J=6.0 Hz), 4.58 (2H, d, J=6.0 Hz). *Anal.* Calcd. for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.35; H, 6.11.

Hydrolysis of Hexamethylether (16) with Sodium Hydroxide——A solution of 50 mg of the hexamethylether (**16**) in 2 ml of dioxane was added to 2 ml of 1N sodium hydroxide solution and the mixture was kept

NII-Electronic Library Service

at room temperature overnight. The reaction mixture was poured into 5 ml of water and allowed to stand at room temperature for 30 min. After acidification with conc. hydrochloric acid, the mixture was extracted with ethyl acetate The organic layer was washed with water, dried and evaporated to dryness. The residue was heated in 5 ml of 3% hydrogen chloride–methanol. The corresponding methyl esters were subjected to a preparative thin-layer chromatography using silica gel. Developing with benzene–ethyl acetate (2: 1) gave the aglycone (**20**), which was recrystallized from *n*-hexane–acetone affording 5 mg of colorless needles, mp 201—203°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3280, 1725, 1680, 1610, 1510. UV λ_{max} nm (ε): 283 (24000). NMR δ [$(CD_3)_2CO$]: 2.03 (3H, s), 2.70 (1H, dd, J=3.0, 17.0 Hz), 3.01 (1H, dd, J=12.0, 17.0 Hz), 3.75 (3H, s), 3.80 (3H, s), 3.87 (3H, s), 5.46 (1H, dd, J=3.0, 12.0 Hz), 7.17 (1H, d, J=7.5 Hz), 7.20 (1H, t, J=8.5 Hz), 7.39 (1H, d, J=2.0 Hz), 7.45 (1H, dd, J=2.0, 8.5 Hz), 7.54 (1H, dd, J=2.0, 8.5 Hz), 7.70 (1H, dd, J=2.0, 7.5 Hz). *Anal.* Calcd. for $C_{23}H_{26}O_8$: C, 67.70; H, 5.48. Found: C, 67.80; H, 5.42.

Hydrolysis of Isoporiolide (2) with Barium Hydroxide——To a solution of 300 mg of isoporiolide (2) in 5 ml of dioxane was added a solution of 500 mg of barium hydroxide octahydrate in 5 ml of water under cooling in ice-water and the resulting precipitates were washed with acetone. The precipitates in ethyl acetate were treated with 10% cold hydrochloric acid and extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried and evaporated to give 289 mg of a carboxylic acid, which was esterified with 1.7 ml of an ether solution of diazomethane. Evaporation of the solvent left 300 mg of a red oil. The oil was subjected to a silica gel chromatography. The eluent with ethyl acetate afforded 130 mg of **21a** as a light yellow amorphous. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1670, 1640, 1600, 1500. UV λ_{max} nm (ε): 286 (18700), 320 (7200). NMR δ ($CDCl_3$): 2.79 (1H, dd, J=3.0, 17.0 Hz), 3.25 (1H, dd, J=13.0, 17.0 Hz), 5.45 (1H, dd, J= 3.0, 13.0 Hz), 3.95 (3H, s). CD (dioxane) $[\theta]^{20°}$ (nm): +107100 (285), −22800 (335). *Anal.* Calcd. for $C_{30}H_{28}O_{13}$: C, 59.93; H, 4.72. Found: C, 60.21; H, 4.92.

Hydrolysis of Glycoside (21a) with β-Glucosidase——A solution of 1.0 g of the glycoside (**21a**) in 4 ml of dioxane was added to 2 liter of sodium acetate buffer adjusted by acetic acid to pH 5.0. The mixture was incubated with 200 mg of β-glucosidase at 37° for 6 days covered with toluene. After removal of toluene, the aqueous layer was extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried and evaporated to yield 1.0 g of a colorless oil, which was chromatographed on silica gel (20 g). The eluent with benzene–ethyl acetate (4: 1) gave 340 mg of **21b**, which was recrystallized from acetone–isopropyl ether to give 300 mg of light yellow prisms, mp 215—216°. The eluent with ethyl acetate yielded 550 mg of the recovered glycoside. IR ν_{max}^{Nujol} cm^{-1}: 3400, 3360, 1675, 1645, 1615, 1505. UV λ_{max} nm (ε): 295 (17500). CD (dioxane) $[\theta]^{20}$ (nm): +128000 (288), −24200 (334). NMR δ [$(CD_3)_2CO$]: 2.75 (1H, dd, J=3.0, 17.0 Hz), 3.19 (1H, dd, J=12.0, 17.0 Hz), 5.45 (1H, dd, J=3.0, 12.0 Hz), 3.98 (3H, s). *Anal.* Calcd. for $C_{24}H_{20}O_8$: C, 66.05; H, 4.62. Found: C, 66.06; H, 4.84.

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