Blood Pressure Measurement——The measurement was performed described as previously.[10] The blood pressures of the rats employed were 80—110 mmHg.

Acknowledgement Thanks are due to Dr. N. Ishikura, Kumamoto University, and Dr. Y. Nishikawa, Tokyo Municipal Laboratories for Medical Sciences, for providing astragalin and isoquercitrin, respectively, and to Mr. M. Nishiuchi, of this University, for collecting the leaves of *Diospyros kaki*.

[Chem. Pharm. Bull. 27(11)2868—2873(1979)] UDC 547.918.02 : 581.192

Elucidation of the Structure of a New Lignan Glucoside from *Olea europaea* by Carbon-13 Nuclear Magnetic Resonance Spectroscopy

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(Received May 30, 1979)

The structure of a new lignan glucoside isolated from the stem of *Olea europaea* LINN. (Oleaceae) was elucidated as (+)-1-acetoxypinoresinol-4′-β-D-glucoside [(1*S*,2*R*,5*R*,6*S*)-1-acetoxy-2-(3′-methoxy-4′-hydroxyphenyl)-6-(3″-methoxy-4″-hydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane 4′-β-D-glucopyranoside] by analysis of the carbon-13 nuclear magnetic resonance and circular dichroism spectra.

Keywords——*Olea europaea* L.; Oleaceae; new lignan glucoside; (+)-1-acetoxypinoresinol-4′-β-D-glucoside; ^{13}C-NMR spectra; MS spectra; CD curves

During an examination of Oleaceae we have investigated the stem constituents of *Olea europaea* LINN., the fruits of which are a rich source of olive oil.

We report here the isolation of a new lignan glucoside (**1**) [(+)-1-acetoxypinoresinol-4′-β-D-glucoside], related to (+)-pinoresinol-β-D-glucoside[2] (**8**), and assign the structure based on carbon-13 nuclear magnetic resonance (^{13}C–NMR) and circular dichroism (CD) data.

The extraction was carried out as described in "Experimental".

Compound **1** was isolated from the chloroform extract as colorless needles, $C_{28}H_{34}O_{13}\cdot 1/2H_2O$, mp 183.5—185°, $[\alpha]_D^{22}$ +7.9° (ethanol).

Infrared (IR) absorption of **1** at 1735 cm^{-1} suggested the presence of one alcoholic acetoxyl, which was confirmed by the appearance of a signal at δ 1.67 in the proton nuclear magnetic resonance (PMR) spectrum.

Deacetylation of **1** with ammonia in methanol gave compound **3**, $C_{26}H_{32}O_{12}\cdot 1.5H_2O$, $[\alpha]_D^{23}$ −1.1° (ethanol) as an amorphous powder.

Acetylation of **1** with acetic anhydride–pyridine gave compound **7** as a colorless syrup, $[\alpha]_D^{20}$ −6.3° (ethanol), mass spectrum (MS) *m/e*, 788 [M$^+$].

The PMR spectrum of **7** showed the presence of five alcoholic acetoxyls (δ 1.67, 2.03, 2.10), one phenolic acetoxyl (δ 2.33), two aromatic methoxyls (δ 3.83, 3.87) and six aromatic protons (δ 6.73—7.27).

The ultraviolet (UV) spectrum of **1** showed absorption maxima at 231 and 279.5 nm. The bathochromic shift of the absorption maximum with sodium ethoxide was very similar to that of **8**, suggesting a pinoresinol-type lignan glucoside structure.

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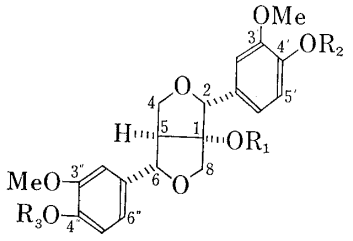
1) Location: *a) Ishikari-Tobetsu, Hokkaido, 061-02, Japan*; *b) Yamashiro-cho, Tokushima, 770, Japan.*
2) M. Chiba, S. Hisada, and S. Nishibe, *Shoyakugaku Zasshi*, **32**, 194 (1978).

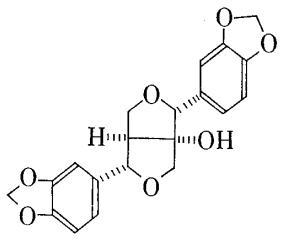
The ^{13}C–NMR spectra of derivatives of **1** were correlated with those of known lignans, **8** and its derivatives, and it was shown that the ^{13}C–NMR shifts could be used to assign the structure of **1**.

Tables I and II present the ^{13}C–NMR data for compounds **1**—**10** and their assignments.

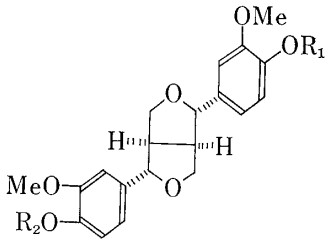
In particular the chemical shifts of the 1′ and 1″ carbon atoms are sensitive both to changes in the substituents on the aromatic rings and to their stereochemistry. The chemical



1: R_1=Ac, R_2=Glc, R_3=H
2: R_1=Ac, R_2=Glc, R_3=Me
3: R_1=H, R_2=Glc, R_3=H
4: R_1=H, R_2=Glc, R_3=Me
5: R_1=H, R_2=Glc $(Ac)_4$, R_3=Me
6: R_1=Ac, R_2=Glc $(Ac)_4$, R_3=Me
7: R_1=Ac, R_2=Glc $(Ac)_4$, R_3=Ac



paulownin



8: R_1=Glc, R_2=H
9: R_1=Glc, R_2=Me
10: R_1=Glc $(Ac)_4$, R_2=Ac

Chart 1

Table I. ^{13}C–NMR Chemical Shifts[a]

	(1)	(2)	(3)	(4)	(8)	(9)
C–1	97.0	96.9	91.2	91.2		
C–5	58.2	58.2	60.8	60.8	53.5	53.5
C–4	69.7	69.7	70.3	70.3		
C–8	73.8	73.7	74.7	74.7	70.9	71.0
C–2	86.1	86.0	86.9	86.8	84.8	
C–6	84.5	84.3	85.4	85.1	85.1	84.8
C–1′	130.3	130.2	131.1	131.1	135.2	135.2
C–1″	131.2	131.7	132.3	133.9	132.1	133.8
C–3′	146.3	146.2	145.9	145.9	145.9	145.8
C–3″	147.5	148.1	147.4	148.3	148.9	148.1
C–4′	148.2	148.3	148.3	148.7		148.7
C–4″		148.7				148.9
C–2′	110.7	110.1	110.7	110.2	110.4	109.9
C–2″	113.0	111.6	112.5	111.6	115.1	110.5
C–5′	114.6	112.9	114.6	112.5	118.1	111.6
C–5″	115.3	114.6	115.1	114.6	118.6	118.6
C–6′	119.0	118.5	118.8	118.4		
C–6″	121.1	121.0	119.7	119.7		
$\underline{C}H_3CO$	20.6	20.5				
$CH_3\underline{C}O$	168.8	168.7				
CH_3O	55.6	55.4	55.6	55.7	55.6	55.4
	55.8	55.7		55.9		55.6
Glc–1	99.9	99.7	100.3	100.3	100.1	100.1
Glc–2	73.2	73.2	73.2	73.2	73.1	73.1
Glc–3	76.9	79.8	76.9	76.9	76.9	76.8
Glc–4	69.7	69.7	69.7	69.7	69.6	69.6
Glc–5	76.9	76.8	76.9	76.9	76.9	76.8
Glc–6	60.7	60.6	60.8	60.8	60.8	60.6

a) The spectra were taken with a JNM-FX 60 spectrometer (15.00 MHz) in DMSO-d_6 with TMS as an internal reference, using micro cells. FT-NMR conditions: spectral width, 4KHz; number of data points, 8192; pulse repeat time, 1.2 sec; number of pulses, 5000—100000: pulse flipping angle, 45°.

Table II. ^{13}C–NMR Chemical Shifts[a]

	Paulownin[6]	(5)	(6)	(7)	(10)
C–1	91.7	91.9	97.2	97.1	54.3
C–5	60.6	60.3	58.7	58.9	54.5
C–4	71.6	71.7	72.5	72.5	72.0
C–8	75.0	74.9	74.9	74.9	
C–2	87.5	87.5	86.8	86.7	85.6
C–6	85.9	85.8	85.6	85.4	
C–1′	129.4	133.1	133.1	133.1	137.8
C–1″	134.8	132.2	132.4	139.1	140.2
C–3′	148.2	146.2	146.0	146.1	145.7
C–3″	147.9	149.3	148.9	151.3	150.9
C–4′		151.1	150.3	150.3	151.3
C–4″		148.9		139.4	139.2
C–2′	106.9	109.9	109.6	110.1	110.0
C–2″	107.5	111.2	111.0	113.7	110.7
C–5′	108.2	111.4	113.8	118.2	117.9
C–5″	108.6	119.0	118.6	119.7	118.8
C–6′	119.8	120.3	119.7	120.6	120.3
C–6″	120.1		120.5	122.9	122.9
$\underline{C}H_3CO$		20.6	20.5 20.7	20.5	20.7
$CH_3\underline{C}O$		169.4 170.3 170.6	169.3 170.1 170.4	169.0 169.3 170.2 170.5	169.1 169.4 170.3 170.6
CH_3O		55.9 56.2	55.9 56.1	55.9 56.1	56.0 56.2
OCH_2O	101.1 101.2				

a) The spectra were taken with a JNM-FX 60 spectrometer (15.00 MHz) in $CDCl_3$ with TMS as an internal reference, using micro cells.
FT-NMR conditions: see Table I.

shifts of the 1,5-, 2,6- and 4,8-carbon atoms are also sensitive to the substituents on the 2,6-diaryl-3,7-dioxabicyclo[3,3,0]octane ring and to the stereochemistry but not to changes in the aryl groups.[3–5]

By comparing the ^{13}C–NMR spectrum of compound **5** (colorless syrup; MS *m/e*, 718 [M$^+$]) with that of a known lignan, paulownin,[6] it was clearly confirmed that **5** contains a 1-hydroxy-2,6-diaryl-3,7-dioxabicyclo[3,3,0]octane ring.

The appreciable differences of chemical shifts for the 1 and 5 carbon atoms, at 91.9 ppm and 60.3 ppm in **5**, 97.0 ppm and 58.2 ppm in **1**, and 97.2 ppm and 58.7 ppm in **6**, respectively, also indicated that one alcoholic acetoxyl group of **1** is attached at the 1 carbon atom.

The aromatic carbon shifts of compounds **1**—**10** suggested that the aryl groups of the new lignan glucoside are 3-methoxy-4-hydroxyphenyl units, and that one of them is linked to a β-D-glucosyl moiety.

To elucidate the position of the glucose linkage in **1**, we applied the method used for the assignments of (+)-epipinoresinol-β-D-glucoside and symplocosin, based on ^{13}C–NMR analysis.[5]

3) A. Pelter, R.S. Ward, E.V. Rao, and K.V. Sastry, *Tetrahedron*, **32**, 2783 (1976).
4) M. Chiba, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.* (Tokyo), **25**, 3435 (1977).
5) M. Chiba, S. Hisada, S. Nishibe, and H. Thieme, *Phytochemistry*, "in press").
6) A.S.R. Anjaneyulu, K.J. Rao, V.K. Rao, L.R. Row, C. Subrahmanyam, A. Pelter, and R.S. Ward, *Tetrahedron*, **31**, 1277 (1975).

Thus, the downfield shifts of the 1″ carbon atom of the 3″,4″-dimethoxyphenyl unit relative to the corresponding one of the 3″-methoxy-4″-hydroxyphenyl unit are 0.5 ppm between **1** and **2**, and 1.6 ppm between **3** and **4** when the ^{13}C–NMR spectra of the glucosides are measured in dimethyl sulfoxide-d_6, indicating the presence of a free phenolic hydroxyl group at the 4″ carbon atom of **1**.

The chemical shifts of the 1′ carbon atom in **1**, **2**, **3** and **4** appeared at 130.3, 130.2, 131.1 and 131.1 ppm, respectively, and are around 5 ppm upfield of the corresponding signal in **8** and (+)-pinoresinol monomethyl ether-β-D-glucoside (**9**).[4,5]

These upfield shifts in **1**—**4** are due to the γ substituent effect of the alcoholic hydroxyl group at the 1 carbon atom.

No differences of chemical shift values for the **1′** carbon atom are observed between **1** and **2**, or between **3** and **4**.

These ^{13}C–NMR data indicate that β-D-glucose is attached to the 4′ carbon atom of **1**.

The fragmentation patterns in the MS of **3** and **4** (Chart 2) are also in good agreement with the results of ^{13}C–NMR analysis.

With regard to the problem of the absolute configuration of **1**, the CD curves of **3** and **4** were compared with those of stereochemically established lignans, **8**, **9** and its analogs,

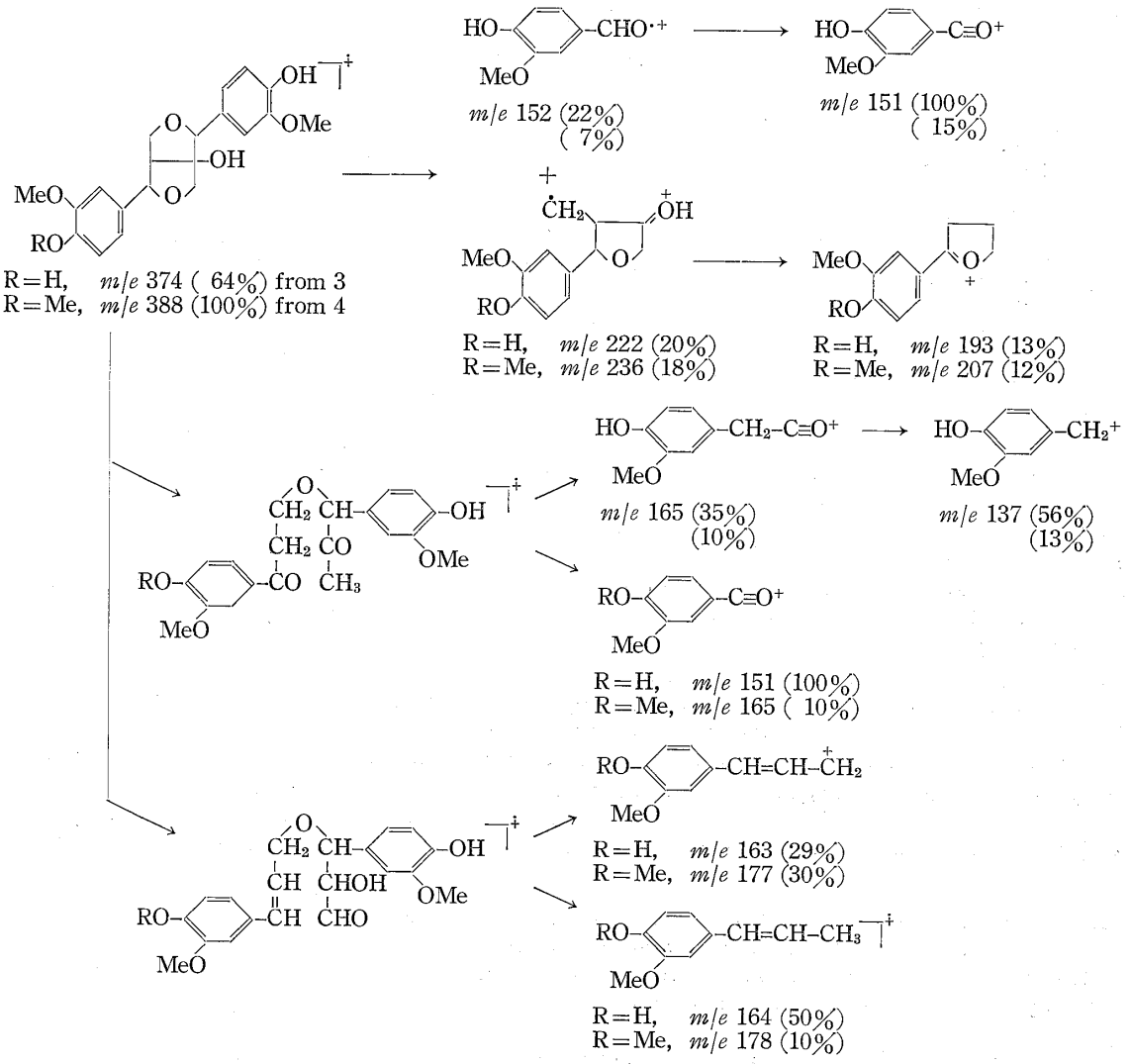


Chart 2. The Mass Fragmentation Patterns of 3 and 4

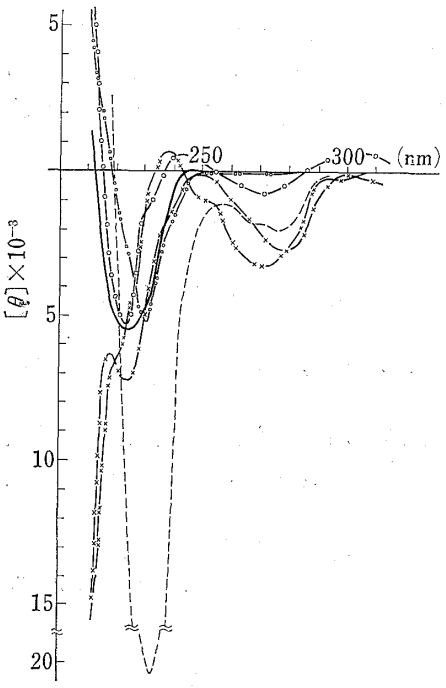


Fig. 1. Circular Dichroism Curves in Ethanol

———— : (+)-1-hydroxypinoresinol-β-D-glucoside (**3**).
---------- : (+)-1-hydroxypinoresinol monomethyl ether-β-D-glucoside (**4**).
—o—o— : (+)-pinoresinol-β-D-glucoside (**8**).
—oo— : (+)-pinoresinol monomethyl ether-β-D-glucoside (**9**).
—×—×— : (−)-pinoresinol-β-D-glucoside.
—××— : (−)-pinoresinol monomethyl ether-β-D-glucoside.

(−)-pinoresinol-β-D-glucoside and (−)-pinoresinol monomethyl ether-β-D-glucoside.[7]

The similarity of the Cotton effects of **3** ($[\theta]^{20}\times10^{-3}$ (nm): −5.56 (225)) and **4** ($[\theta]^{20}\times10^{-3}$ (nm): −20.4 (233)) with those of **8** ($[\theta]^{20}\times10^{-3}$ (nm): −5.31 (223)) and **9** ($[\theta]^{20}\times10^{-3}$ (nm): −5.27 (230)) indicated that they have the same absolute configuration as **8** and **9**.

Therefore, it seems reasonable to assume that the Cotton effect around 230 nm arises from the absolute configuration of the 2,6-diaryl-3,7-dioxabicyclo[3,3,0]-octane ring as in the cases of 2,3-dibenzylbutyrolactone lignans.[8,9]

Consequently, the structure of **1** has been established as (+)-1-acetoxypinoresinol-4′-β-D-glucoside [(1*S*,2*R*,5*R*,6*S*)-1-acetoxy-2-(3′-methoxy-4′-hydroxyphenyl)-6-(3″-methoxy-4″-hydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane 4′-β-D-glucopyranoside].

Recently it was found that **1** shows high inhibitory activity against cAMP-phosphodiesterase *in vitro*.[10]

Pharmacological studies are now in progress.

Experimental

The following instruments were used: melting point, Yanagimoto micro melting point apparatus; optical rotation values, Yanagimoto OR-10 machine; IR spectra, Shimadzu IR-400; UV spectra, Shimadzu UV-210; MS spectra, Shimadzu LKB-9000 at 20 eV using a direct sample inlet into the ion source in all cases; CD curves, Jasco J-40; PMR spectra, JEOL JNM-PMX 60 with tetramethylsilane ($\delta=0$) as an internal reference; ^{13}C-NMR spectra, JEOL FX-60, equipped with a JEC-980 computer.

Precoated thin-layer chromatography (TLC) plates, silica gel 60_{F-254} (Merck), were used for TLC and spots were detected by spraying with 10% H_2SO_4 soln. and heating. Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

The abbreviations used are as follows: s, singlet; m, multiplet.

Isolation——The air-dried and cut stems of *Olea europaea* Linn. (1.2 kg) were extracted four times with hot MeOH. The MeOH solution was evaporated to a small volume under reduced pressure, diluted with water and filtered. The filtrate was extracted successively with ether and $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness.

The $CHCl_3$ extract (4.7 g) was subjected to column chromatography, eluting with $CHCl_3$–EtOH (10: 1 v/v). The fractions (50 ml each) were monitored by TLC using $CHCl_3$–EtOH (4: 1 v/v) as a developer. The fractions showing a TLC spot at *Rf* 0.35 were concentrated.

The residue was purified by preparative TLC and recrystallized from EtOH to give **1** (6.3 mg).

Properties of (+)-1-Acetoxypinoresinol-4′-β-D-glucoside (**1**)——Colorless needles, mp 183.5—185°, TLC *Rf* 0.35 ($CHCl_3$–EtOH=4: 1), $[\alpha]_D^{22}$ +7.9° (c=1.0 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.31), 279.5 (3.83). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 254, 280, 292. IR ν_{max}^{KBr} cm^{-1}: 3325 (OH), 1735 (C=O), 1600, 1590, 1520 (C=C). *Anal.*

7) H. Inouye, Y. Takeda, and H. Nishimura, *Yakugaku Zasshi*, **93**, 44 (1973).
8) S. Nishibe, M. Chiba, and S. Hisada, *Yakugaku Zasshi*, **97**, 1366 (1977).
9) S. Nishibe, M. Chiba, A. Sakushima, S. Hisada, S. Yamanouchi, M. Takido, U. Sankawa, and A. Sakakibara, Symposium Papers, 21st Symposium on the Chemistry of Natural Products, Sapporo, 1978, p. 159.
10) Private communication from Drs. U. Sankawa and T. Nikaido, Faculty of Pharmaceutical Sciences, University of Tokyo.

Calcd. for $C_{28}H_{34}O_{13}\cdot 1/2H_2O$: C, 57.23; H, 6.00. Found: C, 57.68; H, 5.93. PMR (in CD_3OD) δ: 1.67 (3H, s, $OCOCH_3$), 3.87 (6H, s, $2\times CH_3O$), 6.67—7.30 (6H, m, arom. H).

(+)-1-Acetoxypinoresinol Monomethyl Ether-4′-β-D-glucoside (2)——**1** was methylated with diazomethane in the usual way. The crude methylation product was purified by preparative TLC to give **2** as an amorphous powder. TLC *Rf* 0.42 ($CHCl_3$–EtOH=4:1). $[\alpha]_D^{20}$ +9.1° (c=1.6 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.25), 278 (3.68). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (C=O), 1590, 1510 (C=C). *Anal.* Calcd. for $C_{29}H_{36}O_{13}\cdot H_2O$: C, 57.04; H, 6.27. Found: C, 57.53; H, 5.97. PMR (in CD_3OD) δ: 1.67 (3H, s, $OCOCH_3$), 3.83 (9H, s, $3\times CH_3O$), 6.83—7.23 (6H, m, arom. H).

(+)-1-Hydroxypinoresinol-4′-β-D-glucoside (3)——**1** was deacetylated with ammonia in methanol in the usual way. The crude deacetylation product was purified by preparative TLC to give **3** as an amorphous powder. TLC *Rf* 0.19 ($CHCl_3$–EtOH=4:1). $[\alpha]_D^{23}$ −1.1° (c=1.4 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230 (4.23), 279.5 (3.74). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 254, 280, 292. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1590, 1510 (C=C). *Anal.* Calcd. for $C_{26}H_{32}O_{12}\cdot 1.5H_2O$: C, 55.41; H, 6.26. Found: C, 55.39; H, 6.29. PMR (in CD_3OD) δ: 3.90 (6H, s, $2\times CH_3O$), 6.67—7.33 (6H, m, arom. H). CD ($c=2.937\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): −5.56 (225) (negative maximum).

(+)-Hydroxypinoresinol Monomethyl Ether-4′-β-D-glucoside (4)——**2** was deacetylated with ammonia in methanol in the usual way. The crude deacetylation product was purified by preparative TLC to give **4** as an amorphous powder. TLC *Rf* 0.21 ($CHCl_3$–EtOH=4:1). $[\alpha]_D^{23}$ −1.3° (c=1.0 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230 (4.26), 277 (3.76). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1590, 1510 (C=C). *Anal.* Calcd. for $C_{27}H_{34}O_{12}\cdot 1.5H_2O$: C, 56.14; H, 6.46. Found: C, 56.23; H, 6.18. PMR (in CD_3OD) δ: 3.83, 3.87 (9H, each s, $3\times CH_3O$), 6.83—7.27 (6H, m, arom. H). CD ($c=3.331\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): −20.4 (233), −2.1 (275) (negative maximum).

(+)-Hydroxypinoresinol Monomethyl Ether-4′-β-D-glucoside Tetraacetate (5)——**4** was acetylated with acetic anhydride-pyridine in the usual way. The crude acetate was purified by preparative TLC to give **5** as a colorless syrup. MS *m/e*: 718 [M^+], 331, 169, 109. UV λ_{max}^{EtOH} nm (log ε): 226 (4.13), 279 (3.65). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1750 (C=O), 1600, 1590, 1510 (C=C). PMR (in $CDCl_3$) δ: 2.02, 2.06 (12H, each s, $4\times$alcoholic $OCOCH_3$), 3.75, 3.83 (9H, each s, $3\times CH_3O$), 6.80—7.20 (6H, m, arom. H).

(+)-1-Acetoxypinoresinol Monomethyl Ether-4′-β-D-glucoside Tetraacetate (6)——**2** was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC to give **6** as a colorless syrup. MS *m/e*: 760 [M^+], 331, 169, 109. UV λ_{max}^{EtOH} nm (log ε): 229 (4.20), 278 (3.70). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (C=O), 1600, 1590, 1510 (C=C). PMR (in $CDCl_3$) δ: 1.67 (3H, s, alcoholic $OCOCH_3$), 2.02, 2.06 (12H, each s, $4\times$alcoholic $OCOCH_3$), 3.80, 3.88 (9H, each s, $3\times CH_3O$), 6.80—7.20 (6H, m, arom. H).

(+)-1-Acetoxypinoresinol-4′-β-D-glucoside Pentaacetate (7)——**1** was acetylated with acetic anhydride-pyridine in the usual way. The crude acetate was purified by preparative TLC to give **7** as a colorless syrup. $[\alpha]_D^{20}$ −6.3° (c=1.8 in EtOH). MS *m/e*: 788 [M^+], 331, 169, 109. UV λ_{max}^{EtOH} nm (log ε): 220 (4.26), 275 (3.75), 279 (3.74). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (C=O), 1595, 1500 (C=C). PMR (in $CDCl_3$) δ: 1.67 (3H, s, alcoholic $OCOCH_3$), 2.03, 2.10 (12H, each s, $4\times$alcoholic $OCOCH_3$), 2.33 (3H, s, phenolic $OCOCH_3$), 3.83, 3.87 (6H, each s, $2\times CH_3O$), 6.73—7.27 (6H, m, arom. H).

Acknowledgement We are indebted to Prof. Dr. H. Inouye, Faculty of Pharmaceutical Sciences, Kyoto University, for supplying (−)-pinoresinol-β-D-glucoside and (−)-pinoresinol monomethyl ether-β-D-glucoside.

Thanks are also due to Dr. T. Yashiro, Analytical Center of the Faculty of Pharmaceutical Sciences, Nagoya City University, for elemental analyses and to the staff of the Faculty of Pharmaceutical Sciences, Hokkaido University for CD measurement.