Matairesinol-4,4′-di-*O*-β-D-glucopyranoside from *Trachelospermum asiaticum* var. *intermedium*[1]

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A new lignan diglucoside was isolated from the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai (Apocynaceae) and the structure has been determined as matairesinol-4,4′-di-*O*-β-D-glucopyranoside (**1**).

Un nouveau diglucoside de la lignine a été isolé des tiges des *trachelospermum asiaticum* Nakai var. *intermedium* Nakai (Apocynaceae) et la structure a été identifié au matairesinol di-*O*-β-D-glycopyranoside-4,4′ (**1**). [Traduit par le journal]

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In our previous papers (1–4), the structures of four lignan glucosides from the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai were elucidated.

In this paper a new lignan diglucoside, matairesinol-4,4′-di-*O*-β-D-glucopyranoside (**1**) was isolated. The only other lignan diglucoside previously reported is liriodendrin (5). The co-occurrence of matairesinol-4,4′-di-*O*-β-D-glucopyranoside (**1**) and matairesinoside (matairesinol-4′-*O*-β-D-glucopyranoside) are subjects of interest from the biogenetic point of view and suggests the possibility of occurrence of another matairesinol monoglucoside possessing a glucose moiety linked with phenolic hydroxy group at C-4. Actually the isolation of matairesinol monoglucoside (matairesinol-4-*O*-β-D-glucopyranoside) from safflower meal has been reported (6–7).

The chloroform–methanol (2:1, v/v) extract described in the Experimental was subjected to a column chromatography on activated charcoal followed by chromatography on a silica gel column, chloroform–ethanol (3:2, v/v) being used as eluant.

Compound **1**, m.p. 104–106°, was obtained from the fraction showing R_f 0.16 spot on t.l.c. using chloroform–ethanol (3:1) as developer. The i.r. spectrum of **1** showed a γ-lactone band at 1765 cm^{-1}. The peaks at 225 and 279 nm in the u.v. spectrum were unchanged when adding sodium ethoxide, thus indicating the absence of free phenolic hydroxy group.

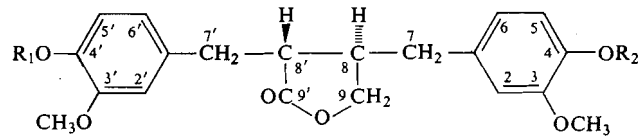
The acid hydrolysis of **1** yielded aglycone (**3**) and D-glucose. The i.r. spectrum of **3** showed a γ-lactone band at 1775 cm^{-1} and the u.v. spectrum showed the peaks at 231.5 and 283 nm, giving bathochromic shift to 250.5 and 298.5 nm with sodium ethoxide. The properties of **3** were identical with those of matairesinol. Compound **3** was identified with an authentic sample by a mixed melting point and i.r. spectra. The presence of D-glucose was proven by paper chromatography.

Compound **1** was treated with acetic anhydride and pyridine at room temperature to give the acetylated derivative (**2**). The n.m.r. spectral data of **2** are as described in the Experimental. The similar assignments of the protons of sugar and aglycone parts in the n.m.r. spectrum are reported in those of matairesinoside pentaacetate (8) and arctiin tetraacetate (9).

Compound **1** was suggested, therefore, to be a matairesinol derivative having two glucose moieties linked with phenolic hydroxy group at C-4 and -4′. The analytical data of **1** was satisfactory for $C_{32}H_{42}O_{16}\cdot H_2O$. The molecular weight determination of **2** by vapor pressure osmometry also agreed with the formula $C_{48}H_{58}O_{24}$. The proof of two glucosidic linkages rather than a glucosyl–glucosidic linkage was achieved as follows. The permethyl ether prepared by the methylation of **1** with sodium hydride, dimethyl sulfoxide, and methyl iodide (Hakomori's method (10)) afforded only methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside on methanolysis with 3% methanolic hydrogen chloride. The glucosyl–glucoside was omitted since it was not the only type of dissacharide moiety possible.

The stereochemistry of the glucosidic linkages could not be determined from the n.m.r. spectrum of **2** (11). The comparison of the experi-

[1]Part IV in the series "Lignans of *Trachelospermum asiaticum* var. *intermedium*".



1 R_1 = glucosyl; R_2 = glucosyl
2 R_1 = $(Ac)_4$ glucosyl; R_2 = $(Ac)_4$glucosyl
3 R_1 = H; R_2 = H

TABLE 1. Values of the specific molecular rotation

Calcd. $[M]_D^\circ$ in ethanol*		Found $[M]_D^\circ$ in ethanol
Lignans		Lignans
4′-β,4-β linkages	−250.7	**1** −168.2
4′-β,4-α or 4′-α,4-β linkages	+119.3	**3** −124.7
4′-α,4-α linkages	+489.3	

*The following $[M]_D$-values were used for the calculation: methyl-α-D-glucopyranoside, +307°, and methyl-β-D-glucopyranoside, −63°.

mental value of the specific molecular rotation ($[M]_D$) of **1** with those calculated with α- and β-glucose moieties are as shown in Table 1.

The $[M]_D$-value and the enzymatic hydrolysis of **1** with emulsin proved the β-linkage of glucose moieties with the aglycone. Thus the structure of **1** has been established as matairesinol-4,4′-di-*O*-β-D-glucopyranoside (4,4′-dihydroxy-3,3′-dimethoxy-lignan-olid(9,9′)-4,4′-di-β-D-glucopyranoside by the nomenclature of Freudenberg and Weinges (12)).

Experimental

All melting points were not corrected. The following equipment was used: i.r. spectra, infrared spectrophotometers IR-S, IR-E, and IRA-2 (Jasco); u.v. spectra, Hitachi recording spectrophotometer Model EPS-3T (ethanol); n.m.r. spectra, JNM-MH-60 (Jeol) with tetramethylsilane (δ = 0) as internal standard in deuteriochloroform; optical rotation, Yanagimoto OR-10; molecular weight, Hitachi Perkin–Elmer 115 molecular weight apparatus with benzil as reference compound; g.l.c., JGC-1100 (Jeol) with flame ionization detector.

The t.l.c. values were obtained with silica gel G (Merck) as adsorbent; the spots were detected by spraying with 10% sulfuric acid and heating. For paper chromatography Toyo filter paper No. 51 (2 cm × 40 cm) was used.

Extraction

The dried stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai (25 kg) were extracted with hot methanol (4 × 36 l) and the methanol solution was evaporated to a small volume, diluted with water, and filtered. The filtrate was extracted successively with light petroleum (4 × 1.5 l), ether (4 × 1.5 l), and chloroform (5 × 1.5 l). The aqueous layer was concentrated to a syrup and extracted with hot ethyl acetate (4 × 1.8 l). The residue was extracted with chloroform–methanol (2:1, v/v) (5 × 1.5 l). The chloroform–methanol extract (82 g) was chromatographed on a column of activated charcoal (Wako, 400 g, 9 cm × 32 cm). Fractions (1 l each) were eluted by methanol–water (1:99, v/v) (nos. 1–2), methanol–water (1:1, v/v) (nos. 3–7), and methanol alone (nos. 8–13), respectively.

Matairesinol-4,4′-di-O-β-D-glucopyranoside (1)

The eluate of fraction no. 10 (1.7 g) was chromatographed on a silica gel column (Mallinckrodt, 100 g, 4.5 cm × 26 cm) with chloroform–ethanol (3:2, v/v) as eluting solvent. Fractions (50 ml each) were monitored by t.l.c. using chloroform–ethanol (3:1, v/v) as developer. The R_f 0.16 fraction was evaporated and final purification was achieved by re-chromatography on a silica gel column to give colorless powder **1**, 51.4 mg, m.p. 104–106°, $[\alpha]_D^{14}$ −24.0 (*c*, 0.5 in ethanol).

Anal. Calcd. for $C_{32}H_{42}O_{16}\cdot H_2O$: C, 54.85; H, 6.33. Found: C, 54.52; H, 6.24.

Spectra: λ_{max} in nm (log ε) 225 (4.09), 279 (3.65); ν_{max} (KBr) 3560–3240 (hydroxyl), 1765 (γ-lactone), 1595, 1510 cm^{-1} (aromatic).

Acid Hydrolysis of Matairesinol-4,4′-di-O-β-D-glucopyranoside (1)

The solution of matairesinol-4,4′-di-*O*-β-D-glucopyranoside (45 mg) in 10% sulfuric acid (25 ml) was heated on a boiling water bath for 2 h. The oily product separated was extracted with ether and the ether solution was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was chromatographed on a silica gel column (Mallinckrodt, 30 g, 2.5 cm × 12 cm) with chloroform–ethyl acetate (4:1, v/v) as eluting solvent. Fractions were monitored by t.l.c. using chloroform–ethyl acetate (1:1, v/v) as developer. The R_f 0.62 fraction was evaporated to dryness. The residue was recrystallized from 30% aqueous acetic acid to give colorless needles, 6 mg, m.p. 118.5–119.5°, $[\alpha]_D^{23}$ −34.8 (*c*, 0.3 in ethanol) an authentic sample $[\alpha]_D^{8}$ −40.0 (in ethanol) (9); lit. $[\alpha]_D^{25}$ −37.9, −35.7, −40.5 (in acetone) (6).

Spectra: λ_{max} in nm (log ε) 231.5 (4.10), 283 (3.79), shifting to 250.5 (4.33), 298.5 (4.03) in sodium ethoxide; ν_{max} ($CHCl_3$) 3560 (hydroxyl), 1775 (γ-lactone), 1610, 1510 cm^{-1} (aromatic).

The product was identified with an authentic sample of matairesinol by a mixed melting point and i.r. spectral comparison.

The water layer was neutralized with barium carbonate and evaporated to dryness. Paper chromatography of this

residue (solvent: butanol – acetic acid – water (4:1:1, v/v), color reagent: aniline hydrogen phthalate) showed only one spot of D-glucose.

Matairesinol-4,4′-di-O-β-D-glucopyranoside Octaacetate (2)

Matairesinol-4,4′-di-*O*-β-D-glucopyranoside (40 mg) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml). The mixture was left standing overnight at room temperature. The reaction product was added to the ice water with stirring and then extracted with ether. The ether solution was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was recrystallized from methanol to give colorless powder, 31.1 mg, m.p. 88–89°.

Anal. Calcd. for $C_{48}H_{58}O_{24}$ (mol. wt. 1018.98): C, 56.58; H, 5.74. Found ((vapor pressure osmometry in chloroform) 976.4): C, 56.77; H, 5.94.

Spectra: λ_{max} in nm (log ε) 227 sh (4.11), 279 (3.66); ν_{max} (KBr) 1760 (γ-lactone and acetyl), 1595, 1515 cm^{-1} (aromatic); n.m.r. 7.10–6.35 (6H, aromatic H, multiplet), 5.50–4.75 (8H, multiplet), 4.30–4.00 (6H, broad singlet), 4.00–3.80 (2H, C-9, multiplet), 3.75 (6H, methoxyl, singlet), 3.00–2.75 (2H, C-8, -8′, broad), 2.75–2.40 (4H, C-7, -7′, broad), 2.02 and 2.05 (24H, acetyl, two singlets).

Gas–Liquid Chromatography on Methanolysate of Permethyl Ether of Matairesinol-4,4′-di-O-β-D-glucopyranoside (1)

The carbanion prepared from sodium hydride (40 mg) and dimethyl sulfoxide (1 ml) was added to solution of matairesinol-4,4′-di-*O*-β-D-glucopyranoside (8 mg) in dimethyl sulfoxide (1 ml) under nitrogen gas and the mixture was stirred at room temperature. After 1 h, methyl iodide (0.2 ml) was added and the mixture was left standing overnight. The water was added to the reaction mixture, which was extracted with chloroform. The chloroform solution was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue (6.6 mg) was heated with 3% methanolic hydrogen chloride in a sealed tube placed in boiling water bath for 10 h. The reaction mixture was diluted with water and extracted with chloroform. The chloroform solution was washed with water, dried, and concentrated. The presence of methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside only in the concentrated solution was demonstrated by g.l.c. (condition: column, 15% poly-butanediol glycol succinate on Celite 545 (2 m × 3 mm); column temperature, 175°; carrier gas, N_2 (30 ml/min); t_R (min), 5.5 and 7.5).

Enzymatic Hydrolysis of Matairesinol-4,4′-di-O-β-D-glucopyranoside (1)

The emulsion (1 mg) (Tokyo Chemical Industry Co.), was added to matairesinol-4,4′-di-*O*-β-D-glucopyranoside (5 mg) in purified water (5 ml) and the mixture was left standing at room temperature for 2 weeks. The mixture was extracted with ether. The ether layer was dried and evaporated. The presence of matairesinol in the residue was proved by co-t.l.c. with an authentic sample. The water layer was evaporated to dryness. In the residue, only the presence of D-glucose was shown by the paper chromatography.

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