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Lignans of *Trachelospermum asiaticum* var. *intermedium*. V.[1] Isolation of Nortrachelogenin-4,4′-di-O-β-D-glucopyranoside

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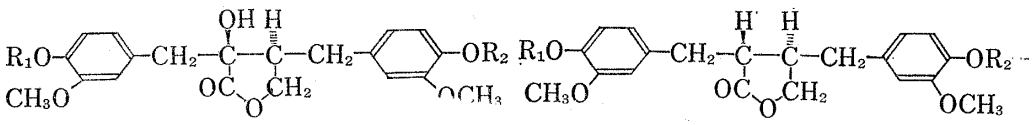
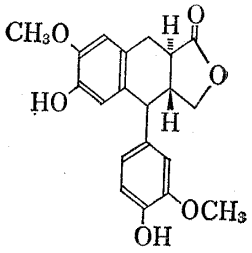
A new lignan diglucoside was isolated from the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai (Apocynaceae) and its structure was established as nortrachelogenin-4,4′-di-O-β-D-glucopyranoside (I). Also the presence of α-conidendrin glucoside was suggested.

In a previous work, six lignan glucosides, arctiin[3] (X), matairesinoside[4] (IX), tracheloside[4] (IV), nortracheloside[5] (III), arctigenin-4′-β-gentiobioside[6] (XI), and matairesinol-4,4′-di-O-β-D-glucopyranoside[1] (VII), were isolated from the stems of *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai. In addition, a new lignan diglucoside was isolated this time from the residue left after the extractions of methanol-soluble fraction of the stems successively with chloroform, ethyl acetate and chloroform–methanol (2: 1) and it was identified as nortrachelogenin-4,4′-di-O-β-D-glucopyranoside (I). The residue was subjected to a column chromatography over activated charcoal followed by chromatography over a silica gel column, successively eluted with chloroform–ethanol (4: 1) and chloroform–ethanol (3: 2).



I: R_1=glucosyl, R_2=glucosyl
II: R_1=$(Ac)_4$glucosyl, R_2=$(Ac)_4$glucosyl
III: R_1=glucosyl, R_2=H
IV: R_1=glucosyl, R_2=CH_3
V: R_1=H, R_2=H
VI: R_1=C_2H_5, R_2=C_2H_5

VII: R_1=glucosyl, R_2=glucosyl
VIII: R_1=$(Ac)_4$glucosyl, R_2=$(Ac)_4$glucosyl
IX: R_1=glucosyl, R_2=H
X: R_1=glucosyl, R_2=CH_3
XI: R_1=gentiobiosyl, R_2=CH_3



XII

Chart 1

The first component of lignan glucosides was isolated from the fractions showing a spot at *Rf* 0.45 on thin-layer chromatography (TLC) using chloroform–ethanol (4: 1) as a developer. Recrystallization from ethanol afforded colorless grains, identified with IV in all respects. When the lignan glucoside without purification was used for acid hydrolysis, a small amount of α-conidendrin (XII), $C_{20}H_{20}O_6$, mp 263.5—264.5°, was obtained from the hydrolyzate, thus suggesting the possibility of the lignan glucoside to be a mixture of IV and a small amount of α-conidendrin glucoside. Separation of α-conidendrin glucoside is now in progress.

The second component of lignan glucosides from the *Rf* 0.28 fraction was suggested to be III by co-TLC with an authentic sample but further purification was not tried.

1) Part IV: S. Nishibe, S. Hisada, and I. Inagaki, *Can. J. Chem.*, **51**, 1050 (1973).
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
3) I. Inagaki, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.* (Tokyo), **16**, 2307 (1968).
4) I. Inagaki, S. Hisada, and S. Nishibe, *Phytochemistry*, **10**, 211 (1971); I. Inagaki, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.* (Tokyo), **20**, 2710 (1972).
5) S. Nishibe, S. Hisada, and I. Inagaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2075 (1972).
6) S. Nishibe, S. Hisada, and I. Inagaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 639 (1973).

The fractions showing a spot at *Rf* 0.09 on TLC using chloroform–ethanol (3: 1) as a developer were evaporated to give a third component of lignan glucosides. The final purification was achieved by re-chromatography over a silica gel column to give colorless powder (I), $C_{32}H_{42}O_{17}\cdot 1.5\ H_2O$, mp 137—140°, $[\alpha]_D^{14}$ −15.9° (ethanol). The infrared (IR) spectrum of I showed a γ-lactone band at 1770 cm^{-1}, and ultraviolet (UV) spectrum of I resembled that of VII (Table I). The absorption maxima at 224 and 279 nm in its UV spectrum remained unchanged when sodium ethoxide was added, indicating the absence of a free phenolic hydroxyl group. Acid hydrolysis of I yielded an amorphous aglycone (V) and D-glucose. The IR and UV spectra of V were identical with those of nortrachelogenin which had already been obtained by the acid hydrolysis of III.[5]

Table I. UV Spectra of Lignan Glucosides λ_{max}^{EtOH} nm (log ε)

I	224 (4.19), 279 (3.75)	VII	225 (4.09), 279 (3.65)
II	226sh(4.15), 279 (3.76)	VIII	227sh(4.11), 279 (3.66)
III	231 (4.09), 283 (3.75)	IX	229 (4.33), 281 (3.79)
IV	230 (4.21), 280 (3.76)	X	230 (4.26), 280 (3.63)
		XI	230 (4.20), 280 (3.79)

Treatment of V with excess diazoethane gave a glassy diethylnortrachelogenin (VI), which gave a crystalline hydroxy-acid, $C_{24}H_{32}O_8$, mp 114—115°, on treatment with 1N sodium hydroxide and which was identified with an authentic sample prepared previously[5] by a mixed melting point and IR spectral comparison. The presence of D-glucose was proved by paper chromatography.

I was treated with acetic anhydride and pyridine in a usual manner to afford an octaacetate (II), $C_{48}H_{58}O_{25}$, mp 124—125°. Its nuclear magnetic resonance (NMR) spectrum showed signals at δ 3.85 (6H, singlet) attributable to two aromatic methoxyls and at δ 2.00 and 2.05 (24H, each singlets) for eight aliphatic acetyls. I was assumed to be nortrachelogenin derivative having glucosyl-aglycone linkages. The presence of two glucosidic linkages was proved in the following way. The permethyl ether prepared by the methylation of I with sodium hydride, dimethyl sulfoxide, and methyl iodide (Hakomori's method[7]) afforded only methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside on methanolysis with 3% methanolic hydrogen chloride, which was demonstrated by gas–liquid chromatography (GLC).

Table II. Values of the Specific Molecular Rotation

Calcd. $[M]_D$ in ethanol		Found $[M]_D$ in ethanol	
Lignans		Lignans	
4′-β,4-β linkages	−188.9°	I	−115.4°
4′-β,4-α or 4′-α,4-β linkages	+181.1°		
4′-α,4-α linkages	+551.1°		

The following $[M]_D$ values were used for the calculation: methyl-α-D-glucopyranoside, +307°, methyl-β-D-glucopyranoside, −63°, and nortrachelogenin (V), −62.9°.[5]

The comparison of the experimental value of the specific molecular rotation ($[M]_D$) of I with those calculated with α- and β-glucose moieties is shown in Table II. $[M]_D$ value and enzymic hydrolysis of I with emulsin proved the β-linkage of glucose moieties with the aglycone. Thus, the structure of I has been established as nortrachelogenin-4,4′-di-O-β-D-glucopyranoside(4,4′,8′-trihydroxy-3,3′-dimethoxy-lignan-olid(9,9′)-4,4′-di-β-D-glucopyranoside by the nomenclature of Freudenberg and Weinges[8]).

7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
8) K. Freudenberg and K. Weinges, *Tetrahedron*, **15**, 115 (1961).

The presence of a series of related lignan glucosides in the same plant is of interest from the biogenetic point of view.

Experimental

All the melting points are not corrected. The following equipment was used: IR spectra, Infrared Spectrophotometer IR-S, IR-E, and IRA-2 (JASCO); UV spectra, Hitachi Recording Spectrophotometer Model EPS-3T; NMR spectra, JNM-MH-60 (JEOL) with tetramethylsilane ($\delta=0$) as internal standard; optical rotation values, Direct Reading Polarimeter Model OR-10 (Yanagimoto); molecular weight, Hitachi Perkin-Elmer 115 molecular weight apparatus with benzil as reference compound; mass spectra, Hitachi Mass Spectrometer Model RMU-6C; gas–liquid chromatography (GLC), JGC-1100 (JEOL) with flame ionization detector. The abbreviation used are as follows: s, singlet; sh, shoulder.

The TLC values were obtained with Kieselgur G nach Stahl (Merck) as adsorbent and the spots were detected by spraying with 10% H_2SO_4 and heating. For paper chromatography (PC) Toyo Roshi No. 51 (2×40 cm) was used. For column chromatography silica gel (100 mesh, Mallinckrodt) was used.

Isolation——The extraction procedure of the air-dried and cut stems (25 kg) was as reported in the previous papers.[1,6] The residue (300 g) left after extractions of MeOH-soluble fraction of the stems successively with $CHCl_3$, AcOEt, and $CHCl_3$–MeOH (2: 1) was chromatographed over a column of activated charcoal (400 g). Fractions (2 l each) were eluted successively with MeOH–H_2O (1: 99) (No. 1—2), MeOH–H_2O (1: 1) (No. 3—5), and MeOH alone (No. 6—19). The residue (10.2 g) from fractions No. 12—19 was chromatographed over a column of silica gel (250 g), successively eluted with $CHCl_3$–EtOH (4: 1) (fractions No. 1—19) and $CHCl_3$–EtOH (3: 2) (fractions No. 20—47). Fractions (100 ml each) were monitored by TLC. The fractions showing a spot at *Rf* 0.45 on TLC using $CHCl_3$–EtOH (4: 1) as a developer were evaporated to give the first component of lignan glucoside. Recrystallization from EtOH afforded colorless grains (IV). The fractions showing a spot at *Rf* 0.28 were evaporated to give the second component of lignan glucosides, which was suggested to be III by co-TLC with an authentic sample. Further purification was not tried. The fractions showing a spot at *Rf* 0.09 on TLC using $CHCl_3$–EtOH (3: 1) as a developer were evaporated to give the third component of lignan glucosides and the final purification was achieved by rechromatography over silica gel column (70 g) using $CHCl_3$–EtOH (1: 1) as a developer to give 41.7 mg of a colorless powder (I).

Tracheloside (IV)——Colorless grains, mp 170—172°. UV λ_{max}^{EtOH} nm(log ε): 230 (4.21), 280 (3.76). IR ν_{max}^{KBr} cm^{-1}: 3560—3240 (OH), 1755 (γ-lactone CO), 1605, 1590, 1510 (aromatic). *Anal.* Calcd. for $C_{27}H_{34}O_{12}\cdot1/2H_2O$: C, 57.95; H, 6.31. Found: C, 57.68; H, 6.21.

This was identified with authentic tracheloside by IR spectrum and a mixed melting point.

α-Conidendrin (XII)——The crude lignan glucoside (862 mg), from which the above IV was obtained, in 10% H_2SO_4 (50 ml) was heated on a water bath for 2 hr. The crude aglycone extracted with ether was submitted to column chromatography using $CHCl_3$–AcOEt (4: 1) as the solvent. Fractions (50 ml each) were monitored by TLC, developing with $CHCl_3$–AcOEt (1: 1). A colorless powder was obtained from fraction No. 9 and was recrystallized from methanol to XII (3.7 mg) as colorless needles, mp 263.5—264.5°. UV λ_{max}^{EtOH} nm(log ε): 231sh (4.20), 284 (3.86). UV $\lambda_{max}^{EtOH+NaOH}$ nm(log ε): 249 (4.30), 300 (4.02). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1765 (γ-lactone CO), 1615, 1605, 1583, 1510 (aromatic). *Anal.* Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.14; H, 5.65. This was identified with authentic α-conidendrin by a mixed melting point and IR spectra. Trachelogenin was obtained from fractions No. 10—11.

Properties of Nortrachelogenin-4,4′-di-O-β-D-glucopyranoside (I)——Colorless powder, mp 137—140°. TLC *Rf*: 0.09 ($CHCl_3$: EtOH=3: 1). $[\alpha]_D^{14}$ −15.9° (c=0.83 in EtOH). UV λ_{max}^{EtOH} nm(log ε): 224 (4.19), 279 (3.75). IR ν_{max}^{KBr} cm^{-1}: 3560—3240 (OH), 1770 (γ-lactone CO), 1595, 1515 (aromatic). *Anal.* Calcd. for $C_{32}H_{42}O_{17}\cdot1.5H_2O$: C, 52.96; H, 6.25. Found: C, 53.05; H, 6.08.

Acid Hydrolysis of Nortrachelogenin-4,4′-di-O-β-D-glucopyranoside (I)——A solution of I (35 mg) in 10% H_2SO_4 (20 ml) was heated on a boiling water-bath for 2 hr. The oily product that separated was extracted with ether, the extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed over a silica gel column with $CHCl_3$–AcOEt (4: 1) as eluting solvent. Fractions were monitored by TLC using $CHCl_3$–AcOEt (1: 1) as a developer. The *Rf* 0.50 fraction was evaporated to give amorphous V. UV λ_{max}^{EtOH} nm(log ε): 230.5 (3.93), 283 (3.61). UV $\lambda_{max}^{EtOH+NaOH}$ nm(log ε): 249.5 (4.09), 297.5 (3.80). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520 (OH), 1780 (γ-lactone CO), 1610, 1510 (aromatic). V was identified with authentic nortrachelogenin by co-TLC and IR spectral comparison. On treatment with excess $C_2H_4N_2$ as usual, V gave a glassy VI. Calcd. for $C_{24}H_{30}O_7$: mol. wt. 430.48. Mass Spectrum *m/e*: 430 (M^+).

The treatment of VI with 1N NaOH followed by careful acidification with 10% H_2SO_4 gave a hydroxy-acid as colorless grains, mp 114—115°. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1705 (CO), 1605, 1590, 1515 (aromatic). *Anal.* Calcd. for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19. Found: C, 63.77; H, 7.21. This hydroxy-acid was identified with an authentic sample prepared previously[5] by a mixed melting point and IR spectral comparison.

The aqueous layer left after extraction of V with ether was neutralized with $BaCO_3$ and evaporated to dryness. Paper chromatography of this residue (solvent: BuOH–AcOH–H_2O (4: 1: 1), BuOH–pyridine–

H_2O (3:2:1.5) and AcOEt–pyridine–H_2O (2:1:2), color reagent: aniline hydrogen phthalate) showed only one spot of D-glucose.

Nortrachelogenin-4,4′-di-O-β-D-glucopyranoside Octaacetate (II)——A mixture of I (25 mg) dissolved in pyridine (0.5 ml) and added with Ac_2O (0.5 ml) was left to stand overnight at room temperature. The reaction mixture was added to ice water with stirring and then the mixture was extracted with ether. The solution was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from MeOH to 17.6 mg of colorless powder (II), mp 124—125°. UV λ_{max}^{EtOH} nm(log ε): 226sh (4.15), 279 (3.76). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1760 (γ-lactone and acetyl CO), 1600, 1510 (aromatic). *Anal.* Calcd. for $C_{48}H_{58}O_{25}$: C, 55.70; H, 5.65; mol. wt. 1034.9. Found: C, 55.59; H, 5.71; mol. wt. (vapor pressure osmometry in $CHCl_3$) 987.4. NMR (in $CDCl_3$) δ: 3.85 (6H, s, methoxyl), 2.00, 2.05 (24H, each s, acetyl).

GLC on Methanolysate of Permethyl Ether of Nortrachelogenin-4,4′-di-O-β-D-glucopyranoside (I)——The carbanion prepared from NaH (40 mg) and Me_2SO (1 ml) was added to a solution of I (10 mg) in Me_2SO (1 ml) in N_2 atmosphere and the mixture was stirred at room temperature. After 1 hr, MeI (0.2 ml) was added and the mixture was left to stand overnight. H_2O was added to the reaction mixture and the solution was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was heated with 3% HCl–MeOH in a sealed tube placed in a boiling water bath for 10 hr. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried, and concentrated. The presence of only methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside in the concentrated solution was demonstrated by GLC (conditions: column, 15% polybutanediol glycol succinate on Celite 545 (3 mm × 2 m); column temperature, 175°; carrier gas, N_2 (30 ml/min); t_R (min), 5.7 and 7.8).

Enzymic Hydrolysis of Nortrachelogenin-4,4′-di-O-β-D-glucopyranoside (I)——Emulsin (1 ml) (Tokyo Chemical Industry Co.,) was added to I (5 mg) in H_2O (5 ml) and the mixture was left standing at room temperature for 2 weeks. The mixture was extracted with ether, the ether solution was dried, and evaporated. The residue after purification was identified as V by co-TLC and UV spectral comparison with the sample obtained by the acid hydrolysis of I. The aqueous layer left after extraction with ether was evaporated to dryness and the residue was found to contain only D-glucose by paper chromatography.

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