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Lignans of *Trachelospermum asiaticum* var. *intermedium*. III.[1] Isolation of a New Lignan Glycoside, Arctigenin-4'-β-gentiobioside

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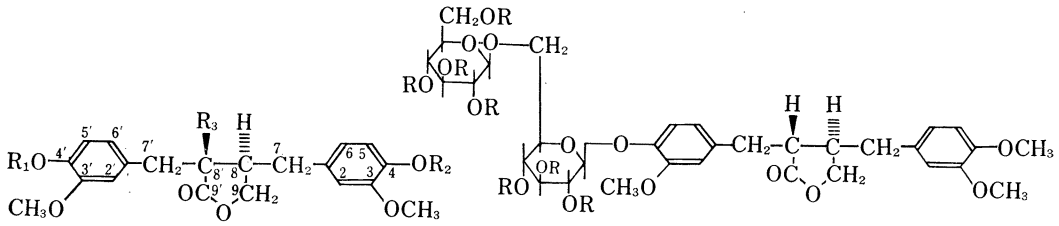
A new lignan glycoside was isolated from the stems of *Trachelospermum asiaticum* NAKAI var. *intermedium* NAKAI (Apocynaceae) and its structure has been determined as 4'-hydroxy-3,3',4-trimethoxy-lignan-olid(9,9')-4'-(6-O-β-D-glucopyranosyl)-β-D-glucopyranoside (=arctigenin-4'-β-gentiobioside) (V), which is a sole example of naturally occuring of lignan having glucosyl glucose moiety.

Four lignan glucoside, arctiin(I),[3] matairesinoside(II),[3] tracheloside(III)[3] and nortracheloside(IV)[1] were isolated from the stems of *Trachelospermum asiaticum* NAKAI var. *intermedium* NAKAI. Among them II, III and IV were new lignan glucosides and were elucidated to be 4,4'-dihydroxy-3,3'-dimethoxy-lignan-olid(9,9')-4'-β-D-glucopyranoside, 4',8'-dihydroxy-3,3',4-trimethoxy-lignan-olid(9,9')-4'-β-D-glucopyranoside and 4,4',8'-trihydroxy-3,3'-dimethoxy-lignan-olid(9,9')-4'-β-D-glucopyranoside, respectively.



I: R_1=glucosyl, R_2=CH_3, R_3=H.
III: R_1=glucosyl, R_2=CH_3, R_3=OH.
VI: R_1=H, R_2=CH_3, R_3=H.

II: R_1=glucosyl, R_2=H, R_3=H.
IV: R_1=glucosyl, R_2=H, R_3=OH.
V: R=H.
VII: R=CH_3CO.

Chart 1

In addition we isolated a new lignan glycoside, arctigenin-4'-β-gentiobioside(V), as a sole example of naturally occuring of lignan glycoside having glucosyl glucose moiety. The present paper is concerned with the isolation and structural determination of V.

The chloroform–methanol (2:1) extract of residue as described in experimental section was column chromatographed on activated charcoal followed by silica gel column chromatography to obtain the crude V. The crystallization from methanol gave colorless crystalline powder(V), $C_{33}H_{44}O_{16}\cdot H_2O$, mp 174—176°, $[\alpha]_D^{26}$ −57.2° (water), in 0.0004% yield from the dried stems.

The ultraviolet (UV) spectrum of V showed absorption maxima at 230 and 280 nm. The infrared (IR) spectrum resembled that of I, suggesting to be one of lignan glycosides.

The acid hydrolysis of V gave aglycone(VI) and D-glucose. The fragmentation pathways of VI in mass (MS) spectrum indicated to be that of arctigenin as shown in Fig. 1. VI was identified with an authentic sample of arctigenin by a mixed fusion, MS and IR spectral comparison.

1) Part II: S. Nishibe, S. Hisada, and I. Inagaki, *Chem. Pharm. Bull.* (Tokyo), "accepted".
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
3) I. Inagaki, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.* (Tokyo), **20**, 2710 (1972).

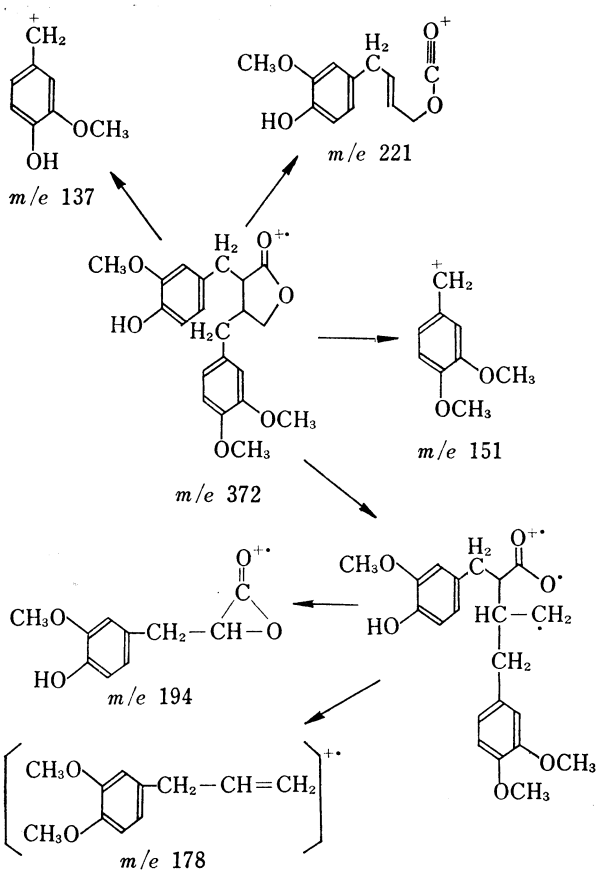


Fig. 1. The Fragmentation Pathways of Arctigenin Mass Spectrum

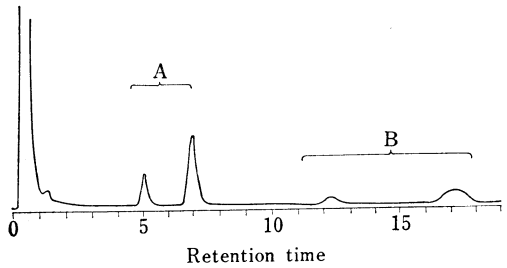


Fig. 2. Gas Chromatogram of the Methanolysate of Permethyl Ether of V

A: methyl 2,3,4,6-tetra-O-methyl-D-glucopyranosides
B: methyl 2,3,4-tri-O-methyl-D-glucopyranosides

D-Glucose was proved by paper chromatography (PC) and gas-liquid chromatography (GLC) as trimethylsilyl ether.

V was treated with acetic anhydride and pyridine at room temperature to give arctigenin-4′-β-gentiobioside heptaacetate (VII), $C_{47}H_{58}O_{23}$, mp 183—184°, $[\alpha]_D^{21}$ −46.7° (chloroform). The nuclear magnetic resonance (NMR) spectrum of VII showed signals attributable to three aromatic methoxyls at δ 3.80 and 3.85 (singlets), seven aliphatic acetyls at δ 1.90 and 2.05 (singlets) and an anomer proton between two glucose moieties at δ 4.55 (doublet, J=6 cps, β-linkage).

Hence V was assumed to be an arctigenin derivative having a glucosyl glucose moiety. The analytical data of V was satisfactory for the formula.

The molecular weight determination of VII by vapor pressure osmometry also agreed with that of the formula.

The permethyl ether prepared by the methylation of V with sodium hydride, dimethyl sulfoxide and methyl iodide (Hakomori's method[4]) afforded on methanolysis with 3% methanolic hydrogen chloride methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,3,4-tri-O-methyl-D-glucopyranoside in almost equal molar ratio as shown in Fig. 2. The fact was confirmed in the comparison with methanolysate of permethyl gentiobiose by GLC.

The enzymatic hydrolysis with emulsion gave VI and D-glucose proving the β-linkage of two glucose moieties and also of the sugar moiety with the aglycone.

Therefore, the structure of V has been established as 4′-hydroxy-3,3′,4-trimethoxylignan-olid(9,9′)-4′-(6-O-β-D-glucopyranosyl)-β-D-glucopyranoside (= arctigenin-4′-β-gentiobioside).

4) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

Experimental

All melting points were 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 (δ=O) 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 thin-layer chromatography (TLC) values were obtained with Kieselgur G nach Stahl (Merck) as adsorbent; the spots were detected by spraying with 10% H_2SO_4 and heating. For PC Toyo Roshi No. 51 (2 cm×40 cm) was used. For column chromatography silica gel (100 mesh, Mallinckrodt) was used.

The abbreviation used are as follows: s, singlet; d, doublet; m, multiplet; br. s, broad singlet.

Isolation——The dried stems of *Trachelospermum asiaticum* NAKAI var. *intermedium* NAKAI (25 kg) were extracted with hot MeOH. The MeOH solution was evaporated to small volume, diluted with water and filtered. The filtrate was extracted successively with petr. ether, ether and chloroform. The aqueous layer was concentrated to a syrup and extracted with hot AcOEt. The residue was extracted with hot $CHCl_3$–MeOH (2: 1). The $CHCl_3$–MeOH extract (82 g) was column chromatographed on activated charcoal (Wako, 400 g). Fractions (1 l each) were eluted by methanol–water (1: 99) (No. 1—2), methanol–water (1: 1) (No. 3—7) and methanol alone (No. 8—13), successively. The eluate of fraction No. 12 (2.2 g) was chromatographed on silica gel column (100 g) with $CHCl_3$–EtOH (3: 2) as eluting solvent. Fractions (50 ml each) were monitored by TLC using $CHCl_3$–EtOH (3: 1) as a developer. The *Rf* 0.22 fraction was evaporated. The residue obtained (129.4 mg) was crystallized from MeOH yielding V (91 mg, 0.0004% yield of dried stems).

Properties of Arctigenin-4′-β-gentiobioside (V)——Colorless crystalline powder, mp 174—176°, $[\alpha]_D^{26}$ −57.2° (c=1.0 in water). UV λ_{max}^{EtOH} nm (log ε): 230 (4.20), 280 (3.79). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1770 (γ-lactone C=O), 1595, 1515 (aromatic). *Anal.* Calcd. for $C_{33}H_{44}O_{16}\cdot H_2O$: C, 55.46; H, 6.49. Found: C, 55.63; H, 6.55.

Acid Hydrolysis of Arctigenin-4′-β-gentiobioside (V)——The solution of V (40 mg) in 10% H_2SO_4 (25 ml) was heated on a boiling water bath for 2 hr. The oily product separated was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried over sodium sulfate and evaporated to dryness. The residue was crystallized from MeOH to give colorless prisms (VI), 18 mg, mp 94—95°. Mass Spectrum *m/e*: parent ion 372 (92%), base peak 137. 151 (74%). VI was identified with an authentic sample of arctigenin by mixed fusion, MS and IR spectral comparison. The water layer was neutralized with barium carbonate and evaporated to dryness. PC of this residue (solvent; butanol–acetic acid–water (4: 1: 1). Color reagent; aniline hydrogen phthalate) showed only one spot of D-glucose. The residue was treated with TMS-HT (hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine) to give trimethylsilyl ether. GLC of the trimethylsilyl ether (condition; column, 3% SE-52 on Chromosorb W (2 m×3 mm). Column temperature, 160°. Carrier gas; N_2 (0.5 kg/cm^2)) showed the presence of α-TMS-glucose and β-TMS-glucose at t_R 53 min and 90 min, respectively.

Arctigenin-4′-β-gentiobioside Heptaacetate (VII)——V (40 mg) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) and left standing overnight at room temperature. The reaction product was added with stirring, to the ice water and then extracted with ether. The ether solution was washed with water, dried over sodium sulfate and evaporated to dryness. The residue (52 mg) was crystallized from MeOH to yield VII (33 mg) as colorless needles, mp 183—184°, $[\alpha]_D^{21}$ −46.7° (c=1.17 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 229 (4.22), 279 (3.81). IR ν_{max}^{KBr} cm^{-1}: no OH, 1760 (γ-lactone and acetyl C=O), 1595, 1515 (aromatic). *Anal.* Calcd. for $C_{47}H_{58}O_{23}$: C, 56.97; H, 5.90; mol. wt. 990.9. Found: C, 57.07; H, 6.06; mol. wt. (vapor pressure osmometry in $CHCl_3$) 976.2. NMR (in $CDCl_3$) δ: 6.35—7.10 (6H, m, aromatic), 4.80—5.40 (6H, m), 4.55 (1H, d, J=6 cps, anomer), 4.25 (2H, br.s), 3.40—4.10 (7H, m), 3.80 and 3.85 (9H, each s, methoxyl), 2.95 (2H, br.s, C-8,8′), 2.60 (4H, br.s, C-7,7′), 1.90 and 2.05 (21H, each s, acetyl).

GLC on Methanolysate of Permethyl Ether of Arctigenin-4′-β-gentiobioside (V)——The carbanion prepared from NaH (200 mg) and DMSO (3 ml) was added to the solution of V (50 mg) in DMSO (5 ml) in the presence of nitrogen gas and the mixture was stirred at room temperature. After 1 hr CH_3I (1 ml) was added and the mixture was left standing overnight. Then water was added to the reaction mixture, which was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried over sodium sulfate and evaporated to dryness. The residue (30 mg) was chromatographed on silica gel (30 g) with $CHCl_3$–AcOEt (4: 1) as eluting solvent. Fractions (25 ml each) were monitored by TLC using $CHCl_3$–AcOEt (1: 1) as a developer. The *Rf* 0.82 fraction was evaporated to give an almost colorless syrup of permethyl ether, whose IR spectrum showed no hydroxy band. The permethyl ether was heated with 3% methanolic hydrogen chloride in sealed tube in a boiling water bath for 10 hr. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried and concentrated. On the comparison with methanolysate of permethyl gentiobiose treated in a similar manner the presence in equal mole ratio of methyl

2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,3,4-tri-O-methyl-D-glucopyranoside in the concentrated solution was demonstrated by GLC (condition: column, 15% poly-butanediol glycol succinate on Celite 545 (2 m×3 mm). Column temperature, 175°. Carrier gas, N_2 (30 ml/min)).

Enzymatic Hydrolysis of Arctigenin-4′-β-gentiobioside (V)——The emulsin (1 mg) (Tokyo Chemical Industry Co.,) was added to V (10 mg) in purified water (10 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 residue was crystallized from MeOH to give colorless prisms. The crystals were identified as VI by mixed melting point and IR spectral comparisons with an authentic sample prepared by the acid hydrolysis of V. The water layer was evaporated to dryness. In the residue, only the presence of D-glucose was shown by PC.

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