[Chem. Pharm. Bull. 25(9)2300—2305(1977)]

UDC 547.918.02 : 581.192

Studies on Rhubarb (Rhei Rhizoma). III.[1] Stilbene Glycosides[2]

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(Received January 17, 1977)

A new stilbene glycoside (I), mp 258°, $[\alpha]_D$ −86.0°, $C_{27}H_{26}O_{12}\cdot 1/2H_2O$, together with 3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside (II) and *d*-catechin (III), was isolated from the Japanese rhubarb, "Shinshū Daiō", and the structure of I was established to be 3,5,4′-trihydroxystilbene 4′-O-β-D-(6″-O-galloyl)-glucopyranoside on the bases of chemical and spectral evidence.

The occurrences of the stilbene glycosides (I, II) and III in commercial Chinese rhubarbs were examined by thin-layer chromatography, which revealed that almost all kinds of rhubarbs contained II and III while I appeared in the limited ones.

Keywords——rhubarb; Polygonaceae; stilbene glycoside; stilbene glycoside gallate; *d*-catechin

In a previous paper,[1] eight anthraquinone glycosides, among which three glycosides were new, have been isolated from Chinese rhubarb (Tōgai Daiō). As a continuative study on rhubarb, we have investigated the phenolic constituents of Japanese rhubarb, "Shinshū Daiō",[4] a hybrid between *Rheum coreanum* Nakai and a variant of *Rheum palmatum* L., resulting in the isolation of a new stilbene glycoside (I), together with 3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside (II)[5] and *d*-catechin (III).[6] This paper concerns the structure elucidation of I and also describes the occurrences of I, II and III in various rhubarbs purchased in the Chinese market.

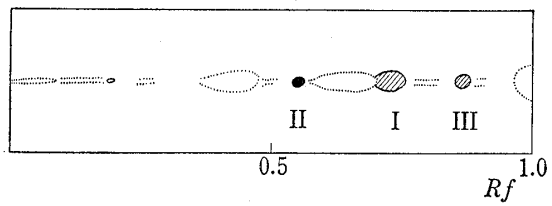


Fig. 1. TLC of the AcOEt Soluble Portion

AcOEt–MeOH–H_2O (20: 3: 2), Kieselgel G.
▨ : $FeCl_3$ reagent.
● : 10% H_2SO_4.
○ : anthraquinone derivatives.

The aqueous suspension of the 60% MeOH extractive of rhubarb was extracted successively with ether, AcOEt and *n*-BuOH. The AcOEt soluble portion, which revealed at least two blue-green spots with $FeCl_3$ reagent and a reddish brown spot with 10% H_2SO_4 reagent besides the spots of anthraquinone derivatives on thin-layer chromatogram (TLC) (Fig. 1), was subjected to silica gel column chromatography to afford glycoside I (I), II (II) and *d*-catechin (III).

I, mp 258°, $[\alpha]_D$ −86.0° (MeOH), $C_{27}H_{26}O_{12}\cdot 1/2H_2O$, shows blue coloration with $FeCl_3$ reagent and emits blue fluorescence under a ultraviolet (UV) lamp (365 nm). The infrared (IR) spectrum of I exhibits the absorption bands due to hydroxyl (3480 cm^{-1}, broad) and ester carbonyl (1690 cm^{-1}) groups. The nuclear magnetic resonance (NMR) spectrum of I shows the signals attributable to sugar protons between δ 3.64—5.04.

1) Part II: H. Okabe, K. Matsuo, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **21**, 1254 (1973).
2) This work was presented at the Annual Meeting of Japanese Society of Pharmacognosy, Chiba, October, 1975.
3) Location: *3-1-1, Maidashi, Higashi-ku, Fukuoka, 812, Japan.*
4) T. Matsuoka and R. Hatta, *J. Takeda Res. Lab.*, **29**, 776 (1970).
5) T. Murakami and K. Tanaka, *Yakugaku Zasshi*, **93**, 733 (1973).
6) *a*) H. Friedrich and J. Höhle, *Arch. Pharm.*, **299**, 857 (1966); *b*) T.K. Chumbalov and L.T. Pashinina, *Uch. Zap. Univ.*, **44**, 87 (1958) [*Chem. Abstr.*, **55**, 14596 (1961)]; *c*) R.P. Biggs, W.L. Cooper, E.O. Hazleton, M. Nierenstein, and P.H. Price, *J. Am. Chem. Soc.*, **53**, 1500 (1931).

Acid hydrolysis of I yielded IV, mp 262—263°, $C_{14}H_{14}O_3$, as the aglycone, along with gallic acid and glucose. IV shows the hydroxyl absorption band (3280 cm^{-1}) in the IR spectrum, and suggests the presence of highly conjugated system (306, 320 nm) in the UV spectrum. The NMR spectrum of IV exhibits nine-proton signals in the olefinic proton region, among which a pair of doublet signals at δ 6.85 and 7.08 are assigned to *trans* olefinic protons as shown by the coupling constants (J=16 Hz), and an A_2B_2-type quartet signal at δ 6.86 and 7.42 (J=9 Hz) represents the presence of *p*-substituted benzene ring. The AX_2-type signals appeared at δ 6.28 (1H, t, J=2 Hz) and 6.55 (2H, d, J=2Hz) are assignable to the protons on the 1,3,5-trisubstituted benzene ring. From these spectral data, the structure of IV is determined to be 3,5,4′-trihydroxystilbene.[7]

Alkaline treatment of I afforded a hydrolysate, mp 262—263°, which revealed the same *Rf* value as that of II on TLC, and was identified with II by the comparison of TLC, IR and NMR spectra.

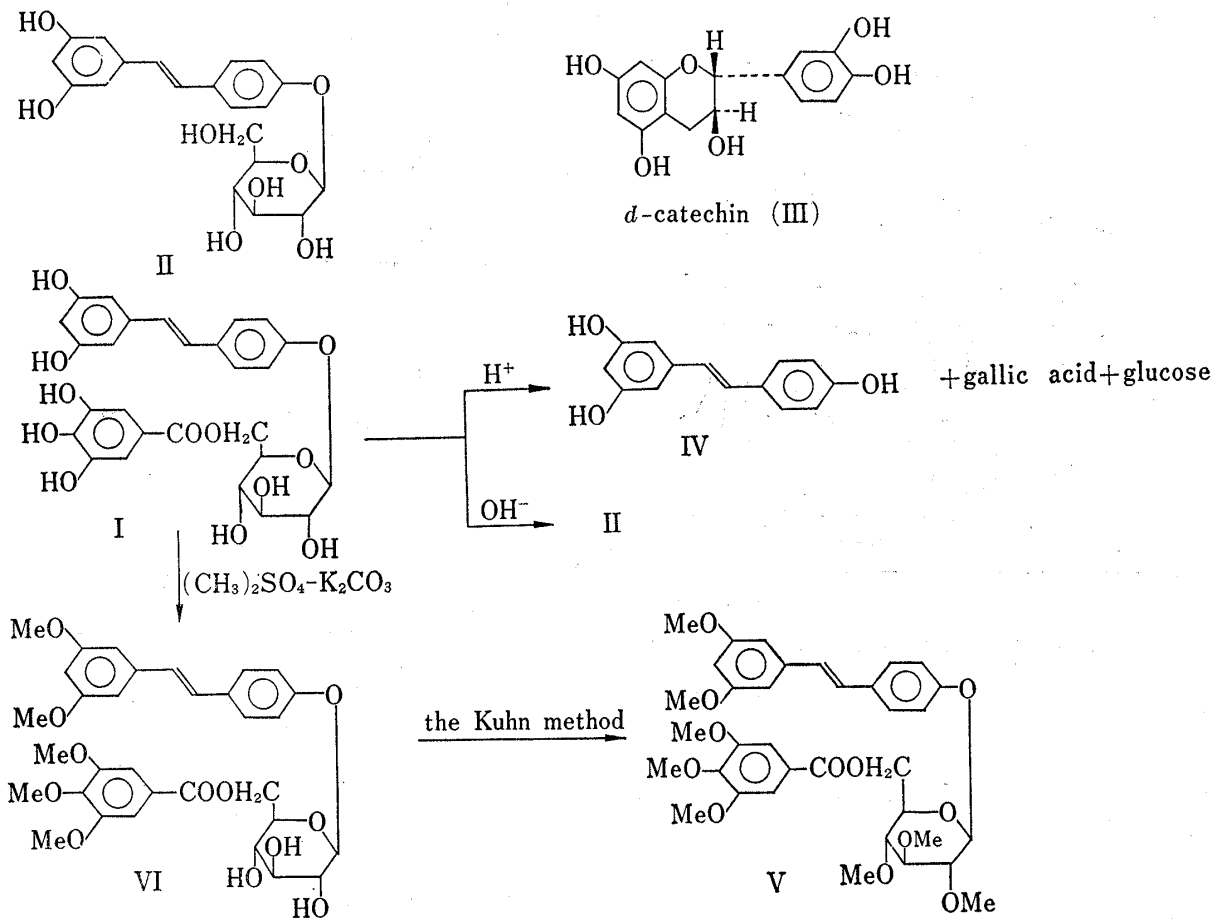


Chart 1

The location of gallic acid was deduced on the following bases. Direct complete methylation of I by the Kuhn method[8] was unsuccessful on account of the instability of I for Ag_2O and alkali, probably due to the decomposition of gallic acid moiety in I with those reagents. However, methylation of I with dimethyl sulfate and potassium carbonate in dry acetone followed by the Kuhn method gave the permethylate (V), mp 119—120°, $C_{35}H_{42}O_{12}$, which shows in the NMR spectrum three aliphatic (δ 3.60—3.68) and five aromatic methoxyl signals (δ 3.84—3.98), thus suggesting that gallic acid is combined to one of hydroxyl groups

7) T. Kariyone, M. Takahashi, T. Ito, and K. Masutani, *Yakugaku Zasshi*, **79**, 219 (1959).
8) R. Kuhn I. Löw, and H. Trischmann, *Chem. Ber.*, **88**, 1492, 1960 (1955).

of glucose moiety. V, after treatment with alkali, was subjected to methanolysis to furnish methyl 2,3,4-tri-O-methyl glucopyranoside as identified by gas–liquid chromatography (GLC). This fact suggests that the location of gallic acid is the C-6 hydroxyl group of glucose moiety. Furthermore, the NMR spectrum of I shows ABX-type signals among which AB portions (δ 4.40, 4.72) are shifted to the downfield as compared to those of II and assigned to protons bearing an ester group, along with a doublet signal at δ 5.04 (J=7 Hz) due to the anomeric proton of glucose unit (Fig. 2,3). Irradiation of the signal at δ 4.40 altered the sextet signal at δ 3.94 to the double doublet signal, while the signals at δ 4.40 and 4.72 were changed to an AB-type quartet on irradiation of the signal at δ 3.94. Since two signals at δ 4.40 and 4.72 were coupled with each other (J=16 Hz, *geminal* coupling), they are reasonably assigned to the C-6 protons of the glucose moiety. These results also support that gallic acid is attached to the C-6 hydroxyl group of glucose unit.

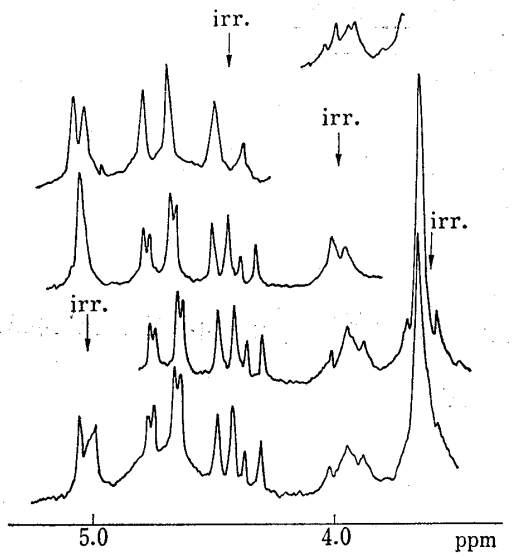


Fig. 2. NMR Spectrum of I (d_6-Acetone)

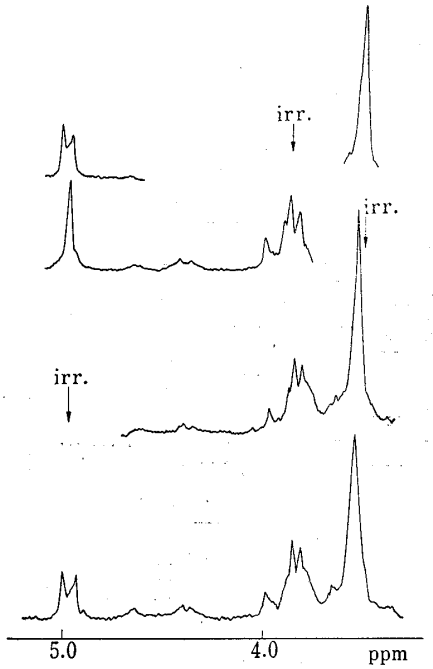
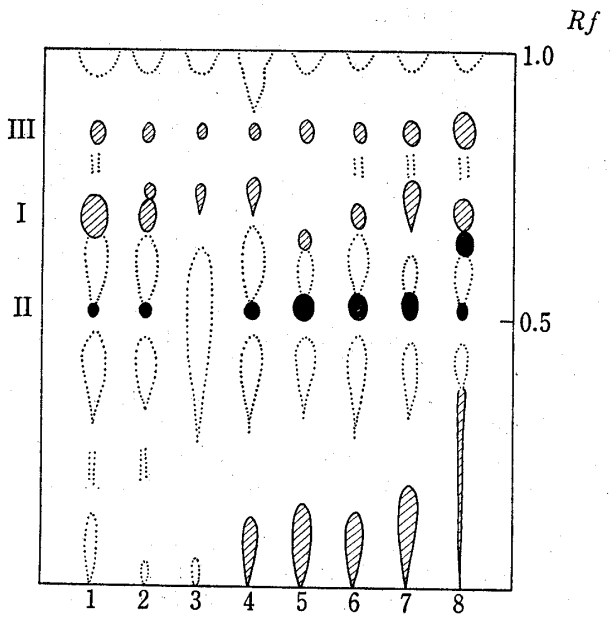


Fig. 3. NMR Spectrum of II (d_6-Acetone+D_2O)

Consequently, the structure of I is characterized to be 3,5,4′-trihydroxystilbene 4′-O-β-D-(6″-O-galloyl)-glucopyranoside. I appears to be the first example of esterified stilbene glycoside so far known.

The occurrences of I, II, and III in commercial Chinese rhubarbs were examined by TLC of AcOEt extracts which were obtained by partition between AcOEt and water of the MeOH extractives (Fig. 4). II and III are detected in almost all the varieties of rhubarbs investigated, while I appears in the limited number of samples, In "The Japanese Pharmacopoeia IX"[9] the chemical evaluation of rhubarb depends upon the occurrence of the stilbene glycoside, rhaponticin, which exhibits on irradiation of UV rays bluish-purple fluorescent spot on silica gel TLC [*Rf* 0.3—0.6, solvent: isopropyl ether–*n*-BuOH–MeOH (27: 7: 3)] and is usually detected in rhubarbs of inferior quality. However, as shown in Fig. 4, almost all kinds of rhubarbs, both inferior and superior ones, contain stilbene derivatives whose structures are closely related to that of rhaponticin, so that it might be difficult to apply the method described in "The Japanese Pharmacopoeia IX" to the chemical evaluation of rhubarb.

9) Hirokawa Publishing Co., Tokyo, 1976, p. D-532.



1 : Shinshū Daiō (信州大黄).
2 : Kinmon Daiō A (錦紋大黄 A 級).
3 : Kinmon Daiō B (錦紋大黄 B 級).
4 : Gaō 1 (雅黄 1 等).
5 : Gaō 2 (雅黄 2 等).
6 : Gaō 3 (雅黄 3 等).
7 : Batei Daiō (馬蹄大黄).
8 : Imo Daiō (芋 大 黄).

I : 3,5,4′-trihydroxystilbene 4′-O-β-D-(6″-O-galloyl)-glucopyranoside.
II : 3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside.
III : *d*-catechin.

Fig. 4. TLC of AcOEt Ext.

AcOEt–MeOH–H_2O(20: 3: 2).
▨ : $FeCl_3$ reagent.
● : 10% H_2SO_4.
○ : anthraquinone derivatives.

Experimental

Melting points were determined by a Yanagimoto Micro Melting Point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter. IR and UV spectra were recorded on a Nihon Bunkō Model DS-301 and Shimadzu SV-50A spectrometer, respectively, and NMR spectra were measured at 100 MHz with a JNR-4H-100 spectrometer and chemical shifts are given on δ (ppm) scale with tetramethylsilane as the internal standard (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet). Mass spectra (MS) were taken on a JMS-01SG mass spectrometer with a direct inlet system. GLC was run on a Shimadzu GC-4BM-PF gas chromatograph with flame ionization detector using glass column (1.5 m×4 mmϕ) packed with 5% 1,4-butanediol succinate on Shimalite W (60—80 mesh). Paper partition chromatography (PPC) for sugars was carried out on Tōyō Roshi No. 50 using aniline hydrogen phthalate as the detector. TLC was conducted on Kieselgel G nach Stahl (Merck) and the spots were visualized by spraying $FeCl_3$ reagent or spraying 10% H_2SO_4 solution followed by heating. Column chromatography was carried out with Kieselgel (70—200 mesh) (Merck). The ratios of solvents and reagents in mixture are given in v/v.

Isolation of Glycoside I (I), II (II) and *d*-Catechin (III)——The air-dried powdered rhizoma (2.05 kg) of Shinshū Daiō cultivated in Hokkaido were mixed with Celite 545 (1.0 kg) and the mixture was percolated with 60% MeOH (19 l). The solvent was evaporated off under reduced pressure to afford a black oily residue (1.23 kg). The suspension of this 60% MeOH extractive (322 g) in water (5 l) was shaken with ether (4 l), AcOEt (7 l) *n*-BuOH, successively. The AcOEt layer was washed with water, dried and evaporated to yield a brown residue (67.1 g), which was dissolved in hot MeOH and resulting crystalline mass mainly composed of anthraquinone glycosides was filtered off. The filtrate (40.0 g), after evaporation of the solvent, was subjected to repeated silica gel column chromatography using AcOEt and $CHCl_3$–MeOH as the solvents to give *d*-catechin (III, 0.3 g), glycoside I (8.0 g) and II (0.9 g).

***d*-Catechin (III)**——Colorless granules, mp 172—174.5°, $[\alpha]_D^{18.5}$ +9.5° (c=1.0, acetone). UV λ_{max}^{MeOH} nm: 281.5. IR ν_{max}^{KBr} cm^{-1}: 3240—3400 (broad), 1630. NMR (d_6-acetone) ppm: 2.52 (1H, q, J=8, 16 Hz, C_4-H), 2.93 (1H, q, J=6, 16 Hz, C_4-H), 3.80—4.18 (1H, m, C_3-H), 4.56 (1H, d, J=8 Hz, C_2-H), 5.85, 6.01 (each 1H, d, J=2 Hz, C_6-, C_8-H), 6.77—6.91 (3H, m, aromatic H).

II——Pale brown needles (H_2O), mp 253—254°, $[\alpha]_D^{21}$ −64.5° (c=0.68, MeOH). UV λ_{max}^{MeOH} nm (log ε): 305 (4.31), 314 (4.30). IR ν_{max}^{KBr} cm^{-1}: 3480, 3380, 1600, 1510, 960. NMR (d_6-acetone) ppm: 3.52 (5H, m, sugar C_2-, C_3-, C_4-, C_6-H), 3.82 (1H, sextet, J=8, 3 Hz, sugar C_5-H), 4.98 (1H, d, J=7 Hz, anomeric H), 6.24 (1H, t, J=2 Hz, aromatic H), 6.56 (2H, d, J=2 Hz, aromatic H), 6.92, 7.10 (each 1H, d, J=16 Hz, *trans* olefinic H), 7.06, 7.50 (4H, A_2B_2-type q, J=9 Hz, aromatic H), 8.33 (s, OH). *Anal.* Calcd. for $C_{20}H_{22}O_8 \cdot 1/2H_2O$: C, 60.43; H, 5.65. Found: C, 60.14; H, 5.52.

Methylation of II with $(CH_3)_2SO_4$ and K_2CO_3——A mixture of II (100 mg), anhydrous K_2CO_3 (2.0 g) and $(CH_3)_2SO_4$ (5 ml) in dry acetone was refluxed for 4 hr. After filtration, the filtrate was concentrated to remove acetone, diluted with water and extracted with AcOEt. The AcOEt layer was washed with water, dried and evaporated to yield an oily residue which was chromatographed over silica gel. Elution with AcOEt afforded colorless needles, mp 96—97°. UV λ_{max}^{MeOH} nm (log ε): 270 sh. (4.34), 305 (4.48). IR ν_{max}^{KBr} cm^{-1}: 3420, 1690, 1590. NMR (d_6-acetone) ppm; 3.40—3.60 (6H, sugar H), 3.80 (6H, s, $2\times OCH_3$), 4.88 (1H, d, $J=7$ Hz, anomeric H), 6.39 (1H, t, $J=2$ Hz, aromatic H), 6.74 (2H, d, $J=2$ Hz, aromatic H), 7.07 (1H, d, $J=16$ Hz, *trans* olefinic H), 7.08 (2H, d, $J=9$ Hz, aromatic H), 7.20 (1H, d, $J=16$ Hz, *trans* olefinic H), 7.51 (2H, d, $J=9$ Hz, aromatic H). The NMR spectrum of this compound was identical with that of II-methylate described in the literature.[5]

Acetylation of II——II (100 mg) was treated with Ac_2O (0.5 ml) and pyridine (0.5 ml) overnight, and the usual working up afforded pale yellow granules (80 mg), mp 175°. UV λ_{max}^{MeOH} nm (log ε): 230 sh. (4.38), 305 (4.47), 316 (4.50). IR ν_{max}^{KBr} cm^{-1}: 1745, 1605, 1515, 975. MS *m/e*: 642 (M^+), 331 (peracetylated glucose residue). NMR ($CDCl_3$) ppm: 2.02, 2.03, 2.05, 2.06 (each 3H, s, $4\times$sugar OAc), 2.23 (6H, s, $2\times$phenolic OAc), 3.72—5.32 (7H, sugar H), 6.80 (1H, t, $J=2$ Hz, aromatic H), 6.92 (1H, d, $J=16$ Hz, *trans* olefinic H), 6.96 (2H, d, $J=9$ Hz, aromatic H), 7.00 (1H, d, $J=16$ Hz, *trans* olefinic H), 7.07 (2H, d, $J=2$ Hz, aromatic H), 7.40 (2H, d, $J=9$ Hz, aromatic H). This hexaacetate was identified with II-acetate by the NMR spectrum appeared in the reference.[5]

I——Pale brown needles ($CHCl_3$–MeOH), mp 258°, $[\alpha]_D^{21}$ −86.0° ($c=1.0$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 301 (4.70), 318 (4.61). IR ν_{max}^{KBr} cm^{-1}: 3480, 1690, 1600, 960. NMR (d_6-acetone) ppm: 3.42—3.80 (3H, m, sugar C_2-, C_3-, C_4-H), 3.94 (1H, sextet, $J=7,3$ Hz, sugar C_5-H), 4.40 (1H, q, $J=7$, 12 Hz, sugar C_6-H), 4.72 (1H, q, $J=3$, 12 Hz, sugar C_6-H), 5.04 (1H, d, $J=7$ Hz, anomeric H), 6.32 (1H, t, $J=2$ Hz, aromatic H), 6.62 (2H, d, $J=2$ Hz, aromatic H), 6.92, 7.09 (each 1H, d, $J=16$ Hz, *trans* olefinic H), 7.12 (2H, d, $J=9$ Hz, aromatic H), 7.28 (2H, s, aromatic H), 7.50 (2H, d, $J=9$ Hz, aromatic H), 8.36 (br. s. OH). *Anal.* Calcd. for $C_{27}H_{26}O_{12}\cdot 1/2H_2O$: C, 56.78; H, 4.78. Found: C, 56.94; H, 5.13.

Acid Hydrolysis of I——A solution of I (300 mg) in 10% H_2SO_4 (5 ml) (H_2O: EtOH=7: 3) was heated on a water bath for 3 hr, and the mixture was extracted with ether. After the aqueous layer was neutralized with $BaCO_3$, the precipitates were filtered off and the filtrate was evaporated under reduced pressure to yield a colorless syrup, which was subjected to PPC [solvent I: *n*-BuOH–AcOH–H_2O (4: 1: 5), upper layer. solvent II: *n*-BuOH–pyridine–H_2O (7: 1: 2), upper layer] and was identified with glucose [*Rf*: 0.13 (solvent I), 0.26 (solvent II)]. The ether solution was washed with aqueous 5% $NaHCO_3$, dried and evaporated to afford pale brown needles (IV, 19 mg), mp 262—263°. UV λ_{max}^{MeOH} nm (log ε): 306 (4.31), 320 (4.30). IR ν_{max}^{KBr} cm^{-1}: 3280 (broad), 1610, 1590, 968. NMR (d_6-acetone) ppm: 6.28 (1H, t, $J=2$ Hz, aromatic H), 6.55 (2H, d, $J=2$ Hz, aromatic H), 6.85, 7.08 (each 1H, d, $J=16$ Hz, *trans* olefinic H), 6.86, 7.42 (each 2H, A_2B_2-type q, $J=9$ Hz, aromatic H), 8.33 (br. s, OH). *Anal.* Calcd. for $C_{14}H_{14}O_3$: C, 73.38; H, 5.30. Found: C, 73.67; H, 5.30. The aqueous 5% $NaHCO_3$ solution was acidified with HCl and extracted with ether. The ether layer was washed with water, dried and evaporated to give colorless plates (6 mg), which was identified with gallic acid (IR, mixed mp).

Alkaline Hydrolysis of I——A solution of I (178 mg) in aqueous 1% KOH (15 ml) was heated on a water bath (85°) under N_2 atmosphere for 20 min. After cooling, the mixture was acidified with 5% HCl and extracted with AcOEt. The AcOEt layer was washed with water, dried and evaporated to give a brown oily residue (98 mg). Crystallization of the residue from $CHCl_3$–MeOH gave pale brown needles, mp 262—263°, which was identified with II (IR, NMR, mixed mp).

Methylation of I with $(CH_3)_2SO_4$ and K_2CO_3——A mixture of I (600 mg), anhydrous K_2CO_3 (8.0 g) and $(CH_3)_2SO_4$ (5 ml) in dry acetone (60 ml) was refluxed for 2 hr, and working up as above gave colorless needles (VI, 414 mg), mp 175—178°. UV λ_{max}^{MeOH} nm (log ε): 273 (4.62), 304 (4.74). IR ν_{max}^{KBr} cm^{-1}: 3480 (broad), 1690, 1590. NMR ($CDCl_3$) ppm: 3.86—3.88 (15H, $5\times OCH_3$), 4.30—5.06 (sugar H), 6.36 (1H, t, $J=2$ Hz, aromatic H), 6.50 (2H, d, $J=2$ Hz, aromatic H), 6.96 (1H, d, $J=16$ Hz, *trans* olefinic H), 7.03 (2H, d, $J=9$ Hz, aromatic H), 7.16 (1H, d, $J=16$ Hz, *trans* olefinic H), 7.30 (2H, d, $J=9$ Hz, aromatic H), 7.36 (2H, s, aromatic H). *Anal.* Calcd. for $C_{32}H_{36}O_{12}$: C, 62.68; H, 5.95. Found: C, 62.74; H, 5.92.

Methylation of VI by the Kuhn Method——To a stirred solution of VI (150 mg) in dimethyl formamide (DMF) (2 ml) was added freshly prepared Ag_2O (1.0 g) and CH_3I (1 ml). The mixture was kept at room temperature for 4 hr, and diluted with $CHCl_3$. Precipitates were filtered off and the filtrate was evaporated under reduced pressure. A colorless oily residue was crystallized from MeOH to form colorless needles (V, 71 mg). The mother liquor, from which the crystals were filtered, was purified by silica gel column chromatography. Benzene–AcOEt (4: 1) eluate afforded further crops of V (67 mg). V, mp 119—120°. UV λ_{max}^{MeOH} nm (log ε): 270 sh. (4.37), 304 (4.49). IR ν_{max}^{KBr} cm^{-1}: 1710, 1590. NMR ($CDCl_3$) ppm: 3.60—3.68 (9H, $3\times OCH_3$), 3.84—3.98 (15H, $5\times OCH_3$), 4.32—4.84 (sugar H), 6.40 (1H, t, $J=2$ Hz, aromatic H), 6.66 (2H, d, $J=2$ Hz, aromatic H), 6.82, 7.02 (each 1H, d, $J=16$ Hz, *trans* olefinic H), 6.98, 7.30 (each 2H, d, $J=9$ Hz, aromatic H), 7.32 (2H, s, aromatic H). *Anal.* Calcd. for $C_{35}H_{42}O_{12}$: C, 63.54; H, 6.46. Found: C, 63.50; H, 6.31.

Alkaline Treatment of V followed by Methanolysis——To a solution of V (10 mg) in dioxane (2 ml) was added aqueous 1% NaOH and heated on a water bath (90°) for 3 hr. The mixture was diluted with water

and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried and evaporated under reduced pressure. The colorless residue was methanolized with 3% HCl–MeOH (3 ml) under reflux for 2 hr. The mixture was neutralized with Ag_2O and the precipitates were filtered off. The filtrate was treated with H_2S gas and the solution, after filtration of black precipitates, was evaporated. The residue was examined by GLC (column temp.: 145°, N_2: 0.8 kg/cm^2) and was identified with methyl 2,3,4-tri-O-methyl-glucopyranoside [t_R (min): 2.40, 3.00].

Acknowledgement The authors are indebted to Drs. M. Goto and T. Matsuoka, Takeda Chemical Industries, Ltd., for the supply of Shinshū Daiō and for their encouragement. They are also grateful to the members of the Analytical Center of this Faculty for IR, UV, NMR and mass spectral measurements, and to the members of the Central Analysis Room of this University for microanalyses. This work was in part supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture which is gratefully acknowledged.