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Studies on Rhubarb (Rhei Rhizoma). IV.[1] Naphthalene Glycosides[2]

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From the Japanese rhubarb, "Shinshū Daiō," two new naphthalene glycosides, I, mp 150—152°, $[\alpha]_D$ −119.0°, $C_{20}H_{24}O_9 \cdot H_2O$ and II, $[\alpha]_D$ −102.0°, $C_{22}H_{24}O_{12} \cdot 3\ 1/2H_2O$ were isolated together with 6-hydroxymusizin 8-O-β-D-glucopyranoside which was found to be identical with the one isolated from aphid, *Aphis nerii*. By the chemical and spectral evidences, the structures of I and II were characterized to be torachrysone 8-O-β-D-glucopyranoside and torachrysone 8-O-β-D-(6′-O-oxalyl)-glucopyranoside, respectively.

Keywords——rhubarb; polygonaceae; naphthalene glycoside; torachrysone glucoside; torachrysone oxalyl glucoside; 6-hydroxymusizin glucoside

In the preceding paper,[1] the authors reported the isolation and the structure elucidation of 3,5,4′-trihydroxystilbene 4′-O-β-D-(6″-O-galloyl)glucopyranoside, 3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside and *d*-catechin from the Japanese rhubarb, "Shinshū Daiō", and the occurrences of the stilbene glycosides in Chinese rhubarbs. Further investigation on phenolic constituents of the above rhubarb has resulted in the isolation of three naphthalene glycosides, one of which is identified with 6-hydroxymusizin 8-O-β-D-glucopyranoside previously isolated from the bright orange aphid, *Aphis nerii*, by Brown, *et al.*,[4] and the other two appear not to be reported to date. This paper deals with the structure elucidation of these naphthalene glycosides.

The aqueous suspension of the MeOH extractives of the fresh rhubarbs was extracted with $CHCl_3$–AcOEt (1:1), AcOEt and *n*-BuOH, successively. The AcOEt soluble portion, which revealed at least three blue-purple spots with Gibbs' reagent on silica gel thin-layer chromatogram, was repeatedly chromatographed over silica gel to furnish glycoside A (I) and C (III). Glycoside B (II) was fairly unstable with silica gel column chromatography, and was separated by cellulose and Sephadex column chromatography followed by preparative thin-layer chromatography (TLC).

Glycoside A (I) forms colorless needles (MeOH), mp 150—152°, $[\alpha]_D$ −109.0° (MeOH), $C_{20}H_{24}O_9 \cdot H_2O$. The ultraviolet (UV) ($\lambda_{max}^{MeOH}$ nm: 235, 240 sh., 260, 270 sh., 314, 326, 340. $\lambda_{max}^{MeOH+AlCl_3}$ nm: 236, 280, 320, 333, 415) and infrared (IR) (ν_{max}^{KBr} cm^{-1}: 3400, 1630) spectra of I suggest the presence of a hydroxynaphthalene skeleton having a chelated carbonyl group. The proton magnetic resonance (PMR) spectrum of I exhibits the presence of an aromatic methyl (δ 2.26), an acetyl (δ 2.52), a methoxyl (δ 3.88) groups and three aromatic protons ascribable to an isolated (δ 7.03) and a pair of *meta*-coupled ones (δ 6.85, 7.01, J=2 Hz). It also shows the proton signals due to the sugar moiety between δ 3.30—5.18 including an anomeric proton signal (δ 5.18, J=7 Hz). Acetylation of I with acetic anhydride and pyridine afforded a pentaacetate (IV), mp 183—184.5°, $C_{30}H_{34}O_{14}$, which reveals one phenolic (δ 2.32) and four aliphatic acetoxyl (δ 2.00, 2.04, 2.07, 2.16) signals on the PMR spectrum and the

1) Part III: G. Nonaka, M. Minami, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2300 (1977).
2) A part of this work was presented at the Annual Meeting of Japanese Society of Pharmacognosy, Hiroshima, November, 1976.
3) Location: *3-1-1, Maidashi, Higashi-ku, Fukuoka, 812, Japan.*
4) K.S. Brown Jr., D.W. Cameron, and U. Weiss, *Tetrahedron Lett.*, **1969**, 471.

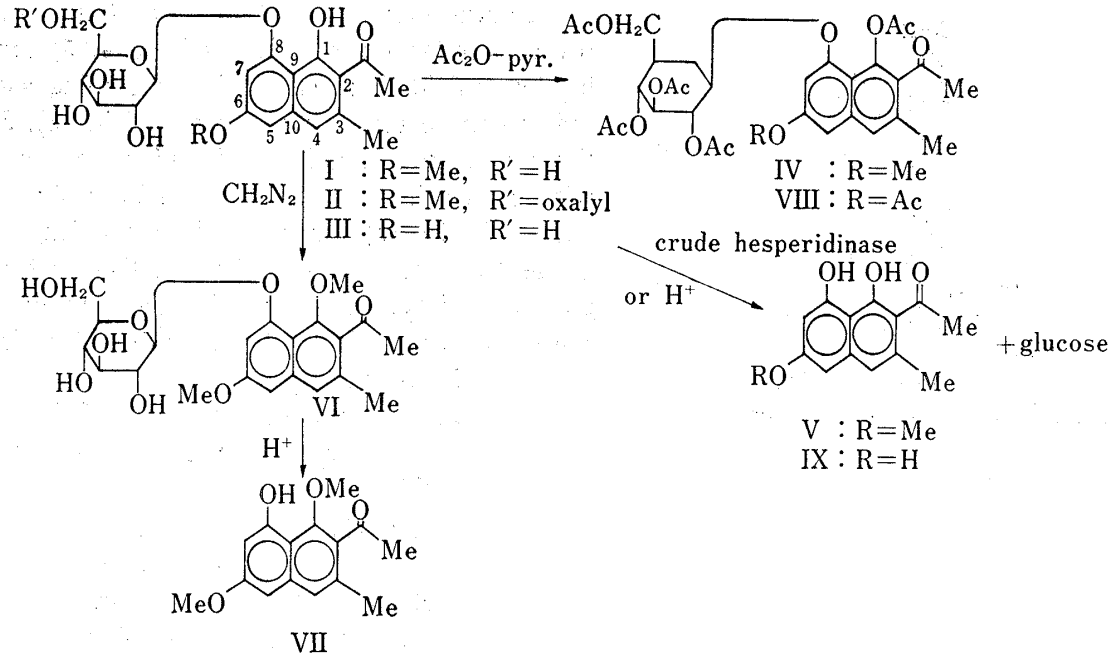

Chart 1

absorption band (1704 cm^{-1}) due to a non-chelated carbonyl group on the IR spectrum. From these spectral and chemical results, the structure of I is presumed to be the glycoside of 2-acetyl-3-methyl-6-methoxy-1,8-dihydroxylnaphthalene, that is, torachrysone glycoside.

Enzymatic hydrolysis of I with crude hesperidinase yielded the aglycone (V), mp 216—217°, $C_{14}H_{14}O_4$, and glucose. V forms invariably yellow-colored needles, and has proved to be identical with torachrysone reported by Shibata, *et al.*[5]

Methylation of I with diazomethane gave the monomethyl ether (VI), which was then subjected to acid hydrolysis to furnish the colorless hydrolysate (VII), mp 63.5—64.5°. The melting point and IR spectrum of VII were completely identical with those of 1-O-methyl torachrysone[5] which is one of the partial methylation products of torachrysone.

Consequently, the position of the glucose moiety is determined to be C-8 hydroxyl group of torachrysone, and I is defined as torachrysone 8-O-β-D-glucopyranoside.

Glycoside B (II) is obtained as a pale yellow powder, $[\alpha]_D$ −102.0° (H_2O), $C_{22}H_{24}O_{12}\cdot 3\ 1/2H_2O$, and shows the very similar UV (λ_{max}^{MeOH} nm: 235, 262, 311, 325, 340) and IR (ν_{max}^{KBr} cm^{-1}: 3400, 1630) spectra to those of I, but II shows absorption band due to an ester carbonyl group (1727 cm^{-1}) on the IR spectrum. The PMR spectrum of II indicates that II contains the similar substituents to I, revealing no additional protons to be detected as compared with those of I. Treatment of II with alkali afforded the colorless hydrolysate, mp 151—153° and oxalic acid. The latter was identified by the co-chromatography over a cellulose plate with the authentic sample. The former hydrolysate showed the same *Rf* value as that of I, and its identity with I was established by the comparison of the IR spectrum and the mixed melting point determination. From these chemical and spectral evidences, II is characterized to be an oxalyl ester of I.

The location of the oxalyl group is deduced to be C-6 hydroxyl group in the glucose moiety by the carbon-13 nuclear magnetic resonance (CMR) spectral comparison between II and I, as described in the preceding communication.[6]

5) S. Shibata, E. Morishita, M. Kaneda, Y. Kimura, M. Takido, and S. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 454 (1969).
6) K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, O. Tanaka, H. Oshio, S. Takagi, M. Yamaki, K. Masuda, G. Nonaka, M. Tsuboi, and I. Nishioka, *Tetrahedron Lett.*, **1977**, 1231.

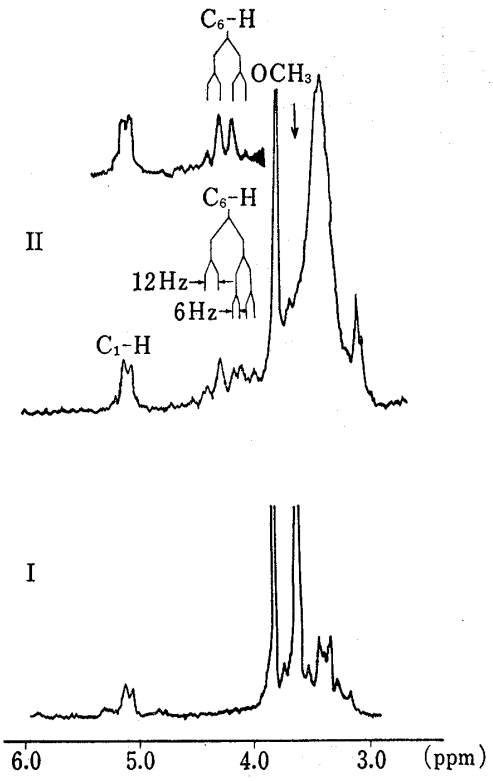

Fig. 1. PMR Spectra of II and I (d_6-DMSO+D_2O)

Furthermore, the PMR spectrum of II reveals the two-proton signals (4.09, 4.35) appearing lower field than those of I and being assignable to the ester bearing protons (Fig. 1). Since the two-proton signals are found to be coupled with each other having a geminal coupling constant (J=12 Hz) by the spin-decoupling technique, they can be assigned to be C-6 protons of glucose moiety. These results also support that the oxalyl group is located in the C-6 hydroxyl group of glucose moiety.

Accordingly, the structure of II is represented by torachrysone 8-O-β-D-(6′-O-oxalyl)-glucopyranoside.

Glycoside C (III) is crystallized as colorless needles (MeOH), mp 209—211°, $[\alpha]_D$ −118.6° (MeOH), $C_{19}H_{22}O_9$, and possesses an *ortho*-chelated hydroxynaphthalene skeleton as shown by UV (λ_{max}^{MeOH} nm: 235.5, 265, 313 sh., 327, 342) and IR (ν_{max}^{KBr} cm^{-1}: 3360, 1650, 1635) spectra. The PMR spectrum of III reveals the similar proton signals to those of I except a methoxyl signal. Acetylation of III with acetic anhydride and pyridine gave the oily hexaacetate (VIII), $C_{31}H_{34}O_{15}$, which exhibits two aromatic (δ 2.36, 6H) and four aliphatic acetoxyl (δ 2.00, 2.04, 2.07, 2.10) signals on the PMR spectrum. Acid hydrolysis of III furnished the pale yellowish-green aglycone (IX), mp 205—206°, $C_{13}H_{12}O_4$, and glucose. The mass spectrum of IX shows the molecular ion peak at *m/e* 232, which is less than that of V by 14 mass units, suggesting that IX might be 6-hydroxymusizin.[4] Methylation of III with diazomethane afforded a colorless oil, which shows two methoxyl signals (δ 3.75, 3.81) on the PMR spectrum, and was identified with the synthetic sample of I-monomethyl ether (VI) by the IR and PMR spectral comparison.

Thus, the structure of III is determined to be 6-hydroxymusizin 8-O-β-D-glucopyranoside.[4]

Glycoside A (I), B (II) and C (III) appear to be the first examples of naphthalene derivatives isolated from rhubarbs, and they seem likely to be biogenetically derived *via* C_{14} polyketide intermediates. It is of interest that glycoside C (III) is proved to be identical with the compound isolated from aphid, *Aphis nerii*, which suck the juice of cardenolide-rich plants such as *Nerium oleander* L. and *Asclepias curassavica* L.

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 Bunko Model DS-301 and Shimadzu SV-50A spectrometer, respectively. PMR and CMR spectra were measured at 100 MHz with a JEOL-C-100H spectrometer and 25 MHz with a JNM-PFT-100 NMR spectrometer, respectively, and chemical shifts are given on δ (ppm) scale with tetramethylsilane as the internal standard (s, singlet; d, doublet; t, triplet; m, multiplet). MS were taken on a JMS-01SG mass spectrometer Column chromatography was carried out using silica gel (70—200 mesh, Merck), polyamide C-200 (Wako Pure Chemical Ind. Ltd.), cellulose (Avicel, Asahi Kasei) and Sephadex LH-20 (Pharmacia Fine Chemicals). TLC was performed on silica gel G nach Stahl (Merck) and Avicel-SF-cellulose (Funakoshi Chemical Ind. Ltd.) plates, and the spots were detected by spraying Gibbs' reagent or spraying 10% H_2SO_4 solution followed by heating. For preparative TLC was used silica gel 60 F_{254} (Merck)

and the spots were examined under UV light. Paper partition chromatography (PPC) for sugars was carried out on Toyo Filter Paper No. 50 using aniline hydrogen phthalate as the detector. The ratios of solvents and reagents in mixture are given in v/v.

Isolation of Glycoside A (I), B (II) and C (III)——The fresh rhizomes (21.6 kg) of Shinshū Daiō were extracted with MeOH four times. The MeOH extractives thus obtained were suspended in water and extracted with $CHCl_3$–AcOEt (1: 1), AcOEt and *n*-BuOH, successively. The AcOEt layer was washed with water, dried and evaporated to yield a dark brown residue (487 g). A part (100 g) of this AcOEt extract was repeatedly chromatographed over silica gel (solvent: $CHCl_3$–MeOH, AcOEt–MeOH) to afford glycoside A (I, 920 mg) and glycoside C (III, 246 mg). Another part (135 g) of the above AcOEt extract was subjected to column chromatography using cellulose (H_2O–MeOH) and Sephadex LH-20 (MeOH), successively, to give crude glycoside B, which was further purified by preparative TLC over silica gel to yield glycoside B (II, 80 mg).

Glycoside A (I)——Colorless needles (MeOH), mp 150—152°, $[\alpha]_D^{21}$ −109.0° (c=0.5, MeOH). UV λ_{max}^{MeOH} nm (log ε): 235 (4.74), 240 sh. (4.70), 260 (4.49), 270 sh. (4.38), 314 (3.39), 326 (3.91), 340 (3.71). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 236, 280, 320, 333, 415. IR ν_{max}^{KBr} cm^{-1}: 3400 (broad), 1630, 1590. PMR (d_6-acetone) ppm: 2.26 (3H, s, Ar–CH_3), 2.52 (3H, s, $COCH_3$), 3.30—5.12 (sugar H), 3.88 (3H, s, OCH_3), 5.18 (1H, d, J=7 Hz, anomeric H), 6.85, 7.01 (each 1H, d, J=2 Hz, aromatic H), 7.03 (1H, s, aromatic H), 9.64 (OH). CMR (d_6-DMSO) ppm: 19.4 (Ar–CH_3), 32.0 ($COCH_3$), 55.2 (OCH_3), 60.5 (glucosyl C-6′), 69.8 (glucosyl C-4′), 73.3 (glucosyl C-2′), 76.1 (glucosyl C-3′), 77.7 (glucosyl C-5′), 101.1, 102.7, 103.0 (glucosyl C-1′, C-5, C-7), 108.5 (C-9), 118.8 (C-4), 123.0 (C-2), 133.6 (C-10), 136.8 (C-3), 150.9 (C-1), 155.3 (C-8), 158.2 (C-6), 204.3 ($COCH_3$). *Anal.* Calcd. for $C_{20}H_{24}O_9 \cdot H_2O$: C, 56.87; H, 5.94. Found: C, 56.33; H, 6.14.

Acetylation of I——I (20 mg) was treated with Ac_2O (1 ml) and pyridine (1 ml) overnight, and working up as usual gave colorless needles (IV, 23 mg), mp 183—184.5°. UV λ_{max}^{MeOH} nm (log ε): 235 (4.90), 300 (4.23), 336 (3.93). IR ν_{max}^{KBr} cm^{-1}: 1755, 1704, 1637, 1615, 1580. PMR ($CDCl_3$) ppm: 2.00, 2.04, 2.07, 2.16 (each 3H, s, sugar $OCOCH_3$), 2.32 (3H, s, phenolic $OCOCH_3$), 2.37 (3H, s, Ar–CH_3), 2.48 (3H, s, $COCH_3$), 3.84 (3H, s, OCH_3), 4.40—5.40 (sugar H), 6.73, 6.79 (each 1H, d, J=2 Hz, aromatic H), 7.39 (1H, s, aromatic H). MS m/e: 618.194 (M^+, Calcd. for $C_{30}H_{34}O_{14}$: 618.190).

Hydrolysis of I with Crude Hesperidinase——A mixture of I (80 mg) and crude hesperidinase (40 mg) in water was incubated at 37° for 30 min. The resulting precipitates were extracted with ether, and the ether layer was washed with water, dried and evaporated. The residue was crystallized from MeOH to yield pale yellow needles (V, 32 mg), mp 216—217°. UV λ_{max}^{MeOH} nm (log ε): 232 (4.45), 272 (4.43), 318 (3.67), 392 (3.65). IR ν_{max}^{KBr} cm^{-1}: 3320, 1630, 1588. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3320, 1635, 1588. PMR ($CDCl_3$) ppm: 2.62 (3H, s, Ar–CH_3), 2.72 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.44 (2H, aromatic H), 6.80 (1H, aromatic H), 10.32 (OH). MS m/e: 246.089 (M^+ Calcd. for $C_{14}H_{14}O_4$: 246.091). The aqueous layer from which the aglycone was separated, was evaporated off *in vacuo*, and the residue was subjected to PPC [solvent: *n*-BuOH–AcOH–H_2O (4: 1: 5), upper layer]. Glucose was identified (Rf: 0.17).

Methylation of I with CH_2N_2——A solution of I (150 mg) in MeOH was added the etheral solution of CH_2N_2, and kept at room temperature for 2 hr. The solvent was evaporated and the residue was purified by silica gel column chromatography to afford a colorless oil (VI, 95 mg). UV λ_{max}^{MeOH} nm (log ε): 232 (4.87), 298 (4.21), 334 (3.92). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (broad), 1700, 1630, 1580. PMR ($CDCl_3$) ppm: 2.28 (3H, s, Ar–CH_3), 2.54 (3H, s, $COCH_3$), 3.32—4.55 (sugar H), 3.75, 3.81 (each 3H, s, OCH_3), 4.76 (1H, d, J=7 Hz, anomeric H), 6.69, 6.86 (each 1H, d, J=2 Hz, aromatic H), 7.22 (1H, s, aromatic H).

Acid Hydrolysis of VI——A solution of VI (97 mg) in 5% H_2SO_4 (5 ml) (H_2O: EtOH=7: 3) was heated on a water bath for 2.5 hr. The mixture was condensed to remove EtOH *in vacuo*, diluted with water and extracted with ether. The ether layer was washed with water, dried and evaporated to furnish colorless needles (VII), mp 63.5—64.5°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1700, 1640, 1580. VII was identified with the authentic sample of 1-O-methyl torachrysone by the comparison of the IR spectrum and mmp.[5]

Glycoside B (II)——A pale yellow powder, $[\alpha]_D^{18}$ −102.0° (c=0.25, H_2O). UV λ_{max}^{MeOH} nm: 235, 262, 311, 325, 340. IR ν_{max}^{KBr} cm^{-1}: 3400 (broad), 1727, 1630, 1590. PMR (d_6-DMSO) ppm: 2.25 (3H, s, Ar–CH_3), 2.53 (3H, s, $COCH_3$), 3.85 (3H, s, OCH_3), 3.10—3.96 (sugar H), 4.09 (1H, q, J=6, 12 Hz, sugar C_6–H), 4.35 (1H, d, J=12 Hz, sugar C_6–H), 5.13 (1H, d, J=6 Hz, anomeric H), 6.93, 6.96, 7.09 (each 1H, aromatic H). CMR (d_6-DMSO) ppm: 19.4 (Ar–CH_3), 32.2 ($COCH_3$), 55.3 (OCH_3), 63.3 (glucosyl C-6′), 69.9 (glucosyl C-4′), 73.2 (glucosyl C-2′), 74.4 (glucosyl C-5′), 75.8 (glucosyl C-3′), 101.2, 102.4, 103.3 (glucosyl C-1′, C-5, C-7), 108.7 (C-9), 118.6 (C-4), 123.2 (C-2), 133.6 (C-10), 136.9 (C-3), 151.1 (C-1), 155.1 (C-8), 158.2 (C-6), 168.9, 170.5 (oxalyl), 204.3 ($COCH_3$). *Anal.* Calcd. for $C_{22}H_{24}O_{12} \cdot 3\ 1/2H_2O$: C, 48.62; H, 5.75. Found: C, 48.85; H, 5.20.

Alkaline Hydrolysis of II——A solution of II (21 mg) in aqueous 1% KOH (1 ml) was kept at room temperature overnight. The mixture was passed through a column of Amberlite CG-120, and eluted with water. The first eluting strong acidic fraction was examined by cellulose TLC [solvent: phenol–H_2O–HCOOH (75: 25: 1)] and was identified with oxalic acid (Rf: 0.35). The second fraction, after evaporation of the solvent, was crystallized from MeOH to yield colorless needles (10 mg), mp 151—153°, which was identified with I by the comparison of the IR spectrum and mmp.

Glycoside C (III)——Colorless needles (MeOH), mp 209—211°, $[\alpha]_D^{21}$ −118.6° (c=0.59, MeOH). *Anal.* Calcd. for $C_{19}H_{22}O_9$: C, 57.85; H, 5.62. Found: C, 57.63; H, 5.63.

Acetylation of III——III (50 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) overnight. The usual working up, and purification by silica gel chromatography (solvent: benzene–AcOEt) afforded a colorless oil (VIII, 70 mg). UV λ_{max}^{MeOH} nm (log ε): 294 (4.01). IR $\nu_{max}^{CHCl_3}$: 1760, 1708, 1638. MS *m/e*: 646.191 (M^+, Calcd. for $C_{31}H_{34}O_{15}$: 646.190), 331 (peracetylated hexose residue), 271, 232 (aglycone), 217.

Acid Hydrolysis of III——A solution of III (97 mg) in 2 N H_2SO_4 (5 ml) (H_2O: EtOH=7: 3) was heated on a water bath for 1.5 hr. The mixture was diluted with water and the resulting precipitates, after filtration, were recrystallized from MeOH to furnish pale yellowish-green needles (IX, 31 mg), mp 205—206°. The aqueous solution from which the aglycone was filtered, was neutralized with $Ba(OH)_2$, and the inorganic precipitates were filtered off. The filtrate was evaporated *in vacuo* to give a colorless syrup, which was subjected to PPC [solvent: *n*-BuOH–AcOH–H_2O (4: 1: 5), upper layer; *n*-BuOH–pyridine–H_2O (6: 2: 3), upper layer], and identified with glucose (*Rf*: 0.21, 0.26, respectively).

Methylation of III with CH_2N_2——To a solution of III (51 mg) in MeOH was added a solution of CH_2N_2-etherate and kept at room temperature for 1.5 hr. After excess CH_2N_2 was decomposed by adding 3 drops of AcOH, the solvent was evaporated off *in vacuo*. The residue was purified by silica gel column chromatography (solvent: $CHCl_3$–MeOH) to yield a colorless oil (38 mg), $[\alpha]_D^{22}$ −85.3° (*c*=0.75, MeOH), which was identified with I-monomethyl ether (VI) by the comparison of the IR and PMR spectra and mmp.

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