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Isolation and Structure of Two New Glucosides, 1-O-β-D-Glucopyranosyl-scyllo-inositol and 1-O-β-D-Glucopyranosyl-proto-quercitol[1,2]

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Two new glucosides, which have been elucidated to be 1-O-β-D-glucopyranosyl-scyllo-inositol (Ia) and 1-O-β-D-glucopyranosyl-proto-quercitol (IIa), were isolated from an aqueous extract of the leaves and branches of *Quercus stenophylla* Makino (Japanese name "Urajirogashi").

Besides these we detected other disaccharides by gas–liquid chromatography (Fig. 1).

We have reported the isolation of three flavones, three tannins, three other phenolic compounds, and a fatty acid from the lead acetate precipitate of an aqueous extract of the leaves and branches of *Quercus stenophylla* Makino (Japanese name "Urajirogashi").[4] In addition, the filtrate from the lead acetate treatment has been shown to contain scyllo-inositol, proto-quercitol, D-glucose and D-fructose.[1] Also we have described the separation of a disaccharide from the same filtrate.[1] We now wish to describe the isolation and structure determination of two new glucosides, 1-O-β-D-glucopyranosyl-scyllo-inositol (Ia) and 1-O-β-D-glucopyranosyl-proto-quercitol (IIa).

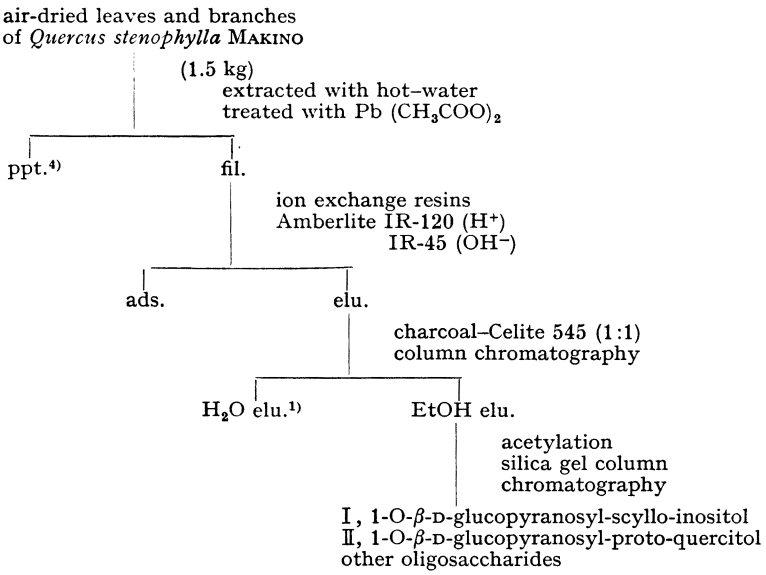


Chart 1

1) This paper constitutes Part IV of the series entitled "Studies on the Constituents of *Quercus spp.*" Part III: Y. Kamano, Y. Tachi, T. Otake, and M. Komatsu, *Yakugaku Zasshi*, **89**, 1302 (1969).
2) This work was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April, 1969.
3) Location: *No. 34-1 Takada-3-chome, Toshima-ku, Tokyo.*
4) Y. Kamano, Y. Tachi, T. Otake, and M. Komatsu, *Yakugaku Zasshi*, **88**, 1235 (1968).

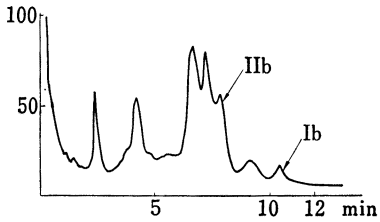
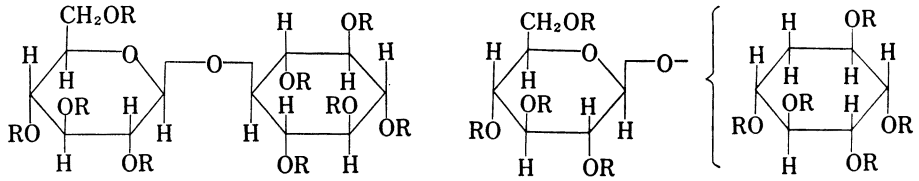


Fig. 1 Gas–Liquid Chromatography of Disaccharide Acetates

condition: column 10% SE-30 on chromosorb W, 265°, N_2 50 ml/min

According to the prior procedure,[1] the filtrate from lead acetate treatment was passed through columns of Amberlite IR-120 (H^+) and Amberlite IR-45 (OH^-) and then chromatographed on charcoal–Celite, as shown in Chart 1. Elution with 5%- and 10%-aqueous alcohol gave a disaccharide fraction as a colorless amorphous solid, which was acetylated with acetic anhydride and pyridiye to yield a colorless amorphous acetate mixture. The acetate mixture was shown by gas–liquid chromatographic analysis to consist of 7 substances (Fig. 1). Therefore, the acetate mixture was submitted to column chromatography on silica gel, giving two crystalline acetates (Ib and IIb).

Compound Ib, mp 237—239°, was obtained as colorless needles from methanol and had the formula $C_{30}H_{40}O_{20}$, and the following spectral properties: ν_{max}^{KBr}: 1755 (s, ester CO), 1260, 1250, 1240—1220 (s, C—O), 908 (s, CH), 898 (s, CH)[5] and 888 cm^{-1} (w, CH): δ (60 Mc, in $CDCl_3$) 1.88—2.11 (27H, 9×—$OCOCH_3$), 3.8—5.4 (13H, multiplet, $>C_5<^{CH_2OAc}_{H}$ and $10\times>C<^{OAc}_{H}$, the C_1-H signal overlapping in this range). These data indicated that Ib is a disaccharide having nine acetoxy groupings. Hydrolysis of Ib with 1N sodium hydroxide solution gave a colorless amorphous solid (Ia), which did not reduce Fehling's solution.



Ia: R=H
Ib: R=Ac

IIa: R=H
IIb: R=Ac

Chart 2

By treatment with β-glucosidase (emulsin)[6] Ia yielded D-glucose and scyllo-inositol in equal amounts. Hydrolysis of Ia with 1N hydrochloric acid also gave both D-glucose and scyllo–inositol. The acetate derivatives were characterized by gas–liquid chromatography (GLC). Therefore, the structure of Ia was shown to be the D-glucoside of scyllo-inositol. Further, β-configuration of the ether linkage was supported by the successful hydrolysis with β-glucosidase. This conclusion was also supported by the infrared (IR) spectrum, which showed a strong band for the C–H stretching vibration at 898 cm^{-1} typical of a β-anomer,[5] and by the nuclear magntic resonance (NMR) spectrum, which did not show any signals associated with the C_1-proton signal of α-anomer of D-glucose at about δ 6.3.[7] The structure of Ia is therefore 1-O-β-D-glucopyranosyl-scyllo-inositol as indicated in Chart 2.

Next, compound IIb, mp 242—244°, was obtained as colorless needles from methanol and found to have the formula $C_{28}H_{38}O_{18}$, and the following spectral properties: ν_{max}^{KBr}: 1770—

5) S.A. Barket, E.J. Bourne, M. Stancey, and D.H. Whiffen, *Chem. & Ind.*, **1953**, 196; S.A. Barket, E.J. Bourne, M. Stancey, and D.H. Whiffen, *J. Chem. Soc.*, **1954**, 171; S.A. Barket, E.J. Bourne, R. Stephens, and D.H. Whiffen, *ibid.*, **1954**, 3468.

6) S. Hattori and H. Imaseki, *J. Am. Chem. Soc.*, **81**, 4424 (1959).

7) Generally, the C_1-proton signal of α-anomer of D-glucose appeared at about δ 6.3 in a lower field than β-anomer (R.U. Lemieux, R.K. Kullning, H.J. Bernstein, and W.G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958)).

1740 (s, ester CO), 1260—1220 (s, C-O), 909 (s, CH), 883 (s, CH, a band suggesting β-configuration of the ether linkage)[5] and 863 cm^{-1} (w, CH); δ (60 Mc, in $CDCl_3$), 2.0—2.13 (26 H, multiplet, $>CH_2$ and $8\times$-$OCOCH_3$), 3.5—5.3 (11H, multiplet, $>C_5<^{CH_2OR}_{H}$ and $9\times>C<^{OAc}_{H}$), 5.39 (1H, doublet, J=4.0 cps, C_1—H). Alkaline hydrolysis IIb gave IIa as a colorless amorphous solid, which did not reduce Fehling's solution. Acidic hydrolysis of IIa gave one mole equivalent each D-glucose and proto-quercitol, which were also obtained by hydrolysis of IIa with β-glucosidase (emulsin).[6] Therefore, the structure of IIa was determined to be 1-O-β-D-glucopyranosyl-proto-quercitol, the location of D-glucose on proto-quercitol, however, being unsolved.

The two glucosides (Ia and IIa) of scyllo-inositol and proto-quercitol were new compounds. Recently a glucoside of inositol was isolated from enzyme extracts of growing cells of *Sporobolomyces singularis* by Gorin, *et al.*,[8] and a galactoside of inositol was also isolated from the same source by Gorin, *et al.*,[8] from juice of the sugar beet (*Beta vulgaris*) by Brown, *et al.*[9] and from vetch seeds by Petek, *et al.*[10] However these inositol in contrast to our results were exclusively the myo-form. Also of biosynthetic interest is the fact that scyllo-inositol, proto-quercitol and D-glucose, have been isolated from *Quercus stenophylla* MAKINO.[1]

Besides the above glucosides we detected several other disaccharides by GLC (Fig. 1), and studies of these materials will be reported in the forthcoming paper.

Experimental

All melting points are uncorrected. IR spectra were determined in KBr pellets using a Nihon Bunko Model DS 301 spectrophotometer. NMR spectra were taken on a Hitachi-Perkin Elmer R-20 spectrometer with tetramethylsilane as an internal standard and are reported in δ values. The solvent used is $CDCl_3$. Molecular weights were determined using a Hitachi-Perkin Elmer-Molecular Weight Apparatus Model 115 (Vapor pressure equilibrium method). The optical rotations were determined using a Nihon Bunko Model DIP-SL.

GLC was run with a Hitachi Model K-53 Apparatus equipped with a hydrogen flame ionization detector using a column (4 mm$\times$1 m) of 10% SE-30 on chromosorb W. Nitrogen was used as carrier gas at flow rate of 50 ml/min. The other gas chromatographic conditions indicated.

Paper chromatography (PPC) was performed on Toyo Roshi No. 50 using solvent system (ascending technique), BuOH–AcOH–H_2O (4:1:5) and 85% phenol, by the same method as described in the previous paper.[1]

Isolation and Acetylation of Disaccharide Fraction——According to the procedure described before,[1] the filtrate of the lead acetate treatment was passed through ion exchange columns Amberlite IR-120 (H^+) (2 liters) and Amberlite IR-45 (OH^-) (2 liters) and then chromatographed on charcoal–Celite (1:1), as shown in Chart 1. Elution with 5%- and 10% aqueous alcohol gave a disaccharide fraction, 2.67 g (0.18%, from plant 1.5 kg), as a colorless amorphous solid, 1.2 g of which was acetylated with acetic anhydride (25 ml) and pyridine (25 ml) at the room temperature for 48 hr. The mixture was poured into 1 liter of the ice–H_2O, extracted with $CHCl_3$, washed with H_2O, 1 N HCl aq., 5% $NaHCO_3$ aq. and H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an acetate mixture, 1.95 g, as a colorless amorphous solid.

GLC of Disaccharide Fraction——A disaccharide acetate mixture obtained above was showed the presence of seven compounds by GLC (column temperature was 265°, t_R (min): 5.0, 6.5, 8.2, 8.8, 9.45 (IIb), 11.1, 12.9 (Ib)), as shown in Fig. 1.

Separation of Disaccharide Fraction by Column Chromatography——A disaccharide acetate mixture (1.9 g) was mixed with 7.5 g of silica gel (Wakogel C-200) in $CHCl_3$ and the mixture, after removal of the solvent, was charged on the top of a column (2.2$\phi\times$55 cm) of silica gel (100 g). The column was successively eluted with C_6H_6–AcOEt mixture and MeOH, as shown in Table I. Elution with C_6H_6–AcOEt (4:1) gave two crystalline compounds, Ib (100 mg) and IIb (82 mg).

1-O-β-D-Glucopyranosyl-scyllo-inositol Acetate (Ib)——A colorless needles from MeOH, mp 237—239°. $[\alpha]_D^{23}$ −33° (c=1.50, $CHCl_3$). Mol. wt. Found: 728. *Anal.* Calcd. for $C_{30}H_{40}O_{20}$: C, 50.00; H, 5.59. Found: C, 50.16; H, 5.50. The assignment of the structure was made by the spectral and physical data described above.

8) P.A.J. Gorin, K. Horitsu, and J.F.T. Spencer, *Can. J. Chem.*, **43**, 2259 (1965).
9) R.J. Brown and R.F. Serro, *J. Am. Chem. Soc.*, **75**, 1040 (1953).
10) F. Petek, E. Villarroya, and J.E. Courtois, *Compt. Rend.*, **263**, 195 (1966).

Table I. Separation of Disaccharide Acetates (1.9 g) by Silica Gel Column Chromatography

Fraction No.	Solvent	Yield (mg)	Compounds
1 —10	C_6H_6:AcOEt (9:1)	137	
11—15	C_6H_6:AcOEt (8:2)	300	
16—20	C_6H_6:AcOEt (8:2)	123 (crystals 82 mg)	IIb
21—31	C_6H_6:AcOEt (8:2)	510	
32—33	C_6H_6:AcOEt (8:2)	143 (crystals 100 mg)	Ib
34—38	C_6H_6:AcOEt (7:3)	20	
39—43	MeOH	560	
Total		1793mg	
Recovery		94.4%	

1 Fraction=200 ml

1-O-β-D-Glucopyranosyl-proto-quercitol Acetate (IIb)——A colorless needles from MeOH, mp 242—244° $[\alpha]_D^{23}$ 0 (c=1.60, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{38}O_{18}$: C, 50.74; H, 5.77. Found: C, 50.67; H, 5.75. The structural assignment of IIb was based on the spectral and physical data described above.

Preparation of 1-O-β-D-Glucopyranosyl-scyllo-inositol (Ia)——To 30 mg of Ib in 5 ml of MeOH, 1 ml of 1 N NaOH aq. was added, and allowed to stand at room temperature overnight. The mixture was passed through a column of Amberlite IR-120 (H^+) (15 ml) and successively a column of Amberlite IR-45 (OH^-) (12 ml). The solution was concentrated *in vacuo* to dryness to give 18 mg of 1-O-β-D-glucopyranosyl-scyllo-inositol (Ia), as a colorless amorphous solid, which did not reduce Fehling's solution.

Hydrolysis of Ia——a) By 1 N HCl aq.: 16 mg of Ia in 2 ml of 1 N HCl aq. was heated on water-bath at 80—95° for 3 hr. The mixture, after cooling to room temperature, was passed through a column of Amberlite IR-45 (OH^-) (20 ml) and the resulting solution was evaporated *in vacuo* to give 13.4 mg of a crystalline material.

This material was shown to consist of two substances by PPC (solvent: *n*-BuOH–AcOH–H_2O (4:1:5)). One of them had a dark brown spot (*Rf* 0.01, reagent: $AgNO_3$–NH_4OH), which was agreed with a spot of scyllo-inositol obtained in the previous paper. The other had a brown spot (*Rf* 0.17, reagent: aniline phthalate), which was identical with D-glucose.

The crystalline material, after acetylation, was separated by GLC (column temp.: 150°) to give D-glucose (t_R: 17 min 51 sec) and scyllo-inositol (t_R: 27 min 12 sec) in equal amounts.

Recrystallization of the crystalline material from MeOH gave scyllo-inositol, mp 347—348° as colorless prisms, which was identical with the authentic sample obtained in the previous paper[1] by mixed mp and comparison of the spectra.

b) By Emulsin (β-Glucosidase): Emulsin was dissolved in acetate (Michaelis) buffer (0.1 M, pH 4.7) to make a 5% solution and heated to 60° for 5 min. Hattori and Imaseki[6] described that the emulsin solution thus treated did not have α-glucosidase activity. To 1 ml of the emulsin solution, 1.4 mg of Ia was added, mixed and allowed to stand at 38° for 2 hr. Semi-quantitative estimation on a paper chromatogram showed that the glucoside, Ia, was hydrolyzed by the emulsin to give both D-glucose (*Rf* 0.17) and scyllo-inositol (*Rf* 0.01) in about equal amounts.

Preparation of 1-O-β-D-Glucopyranosyl-proto-quercitol (IIa)——A mixture of IIb (30 mg) in MeOH (5 ml) and 1 ml of 1 N NaOH aq. was allowed to stand at room temperature overnight. After the mixture was passed through a column of Amberlite IR-120 (H^+) (15 ml) and then a column of Amberlite IR-45 (OH^-) (12 ml), the solution was concentrated *in vacuo* to give 17 mg of 1-O-β-D-glucopyranosyl-proto-quercitol (IIa), as a colorless amorphous solid, which did not reduce Fehling's solution.

Hydrolysis of IIa——a) By 1 N HCl aq.: A solution of IIa (15 mg) in 1 N HCl aq. (2 ml) was heated on water-bath at 80—95° for 3 hr. After cooling, the mixture was passed through a column of Amberlite IR-45 (OH^-) (18 ml) and the resulting solution was evaporated *in vacuo* to give 12 mg of an amorphous solid.

The product was indicated to consist of both D-glucose (*Rf* 0.37, color brown (aniline phthalate)) and proto-quercitol (*Rf* 0.40, color dark brown ($AgNO_3$–NH_4OH)) in about equal amounts by semi-quantitative estimation on PPC (solvent system: 85% phenol).

The product was also separated by GLC (column temp.: 150°) to yield D-glucose (t_R: 17 min 51 sec) and proto-quercitol (t_R: 13 min 30 sec) in equal amounts.

b) By Emulsin (β-Glucosidase): A mixture of the emulsin solution (1 ml) and Ia (1.3 mg) was allowed to stand at 37° for 3 hr. On paper chromatographic analysis in the same way as described above, the solution

was showed that the glucoside IIa was hydrolyzed by the emulsin to give both D-glucose and proto-quercitol n about equal amounts.

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