NOTE**New Egonol Glycoside Having D-Xylose from Immature Seeds of *Styrax obassia* Sieb. et Zucc.****Masahide TAKANASHI^{*1} and Yasuomi TAKIZAWA^{*2}**^{*1} Keiogijuku Yochisha

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Abstract: A new compound, egonol β -primeveroside (**EGX**) was isolated from immature seeds of *Styrax obassia* Sieb. et Zucc., along with three known egonol glycosides and its structure was determined as 3''-O-[6-O-(β -D-xylopyranosyl)- β -D-glucopyranosyl]egonol based on spectroscopic and chemical findings. The major glycoside **EGX** changed to egonol β -gentiobioside (**EG2**) in the maturing season of seeds.

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Key words: *Styrax obassia*, immature seed, egonol glycoside, β -primeveroside, structural determination

1 Introduction

Egonol (**E**) (1) was first isolated from the seed oil of *Styrax japonica* in 1915 and its structure was shown to be 5-(3-hydroxypropyl)-7-methoxy-2-(3',4'-methylene-dioxyphenyl)benzofuran (2). Egonol (**E**) is an effective pyrethrum synergist (3). Its mono-, di- and tri-glucosides, **EG**, **EG2** and **EG3** (4, 5) were isolated from the seeds of *Styrax officinalis* L. (6, 7). The isolation and structural elucidation of three new egonol derivatives, egonol 2-methylbutanoate, 7-demethoxyegonol 2-methylbutanoate and 7-demethoxyegonol from *Styrax obassia* Sieb. et Zucc. were previously reported in this study. The isolation and structural elucidation of a new egonol β -primeveroside (**EGX**) from immature seeds of *Styrax obassia* Sieb. et Zucc. were carried out.

2 Experimental**2.1 General Procedures**

All melting points were determined on a micro mp

apparatus (Yazawa BY-2) and indicated as uncorrected values. Optical rotation was taken on a Perkin-Elmer 241 polarimeter. The UV spectrum was recorded on a Hitachi U-3210 spectrophotometer. IR spectra were taken in KBr on a JASCO A-3 infrared spectrometer. NMR spectra (¹H-NMR: 400 MHz, ¹³C-NMR: 100 MHz) were measured with a JEOL JNM-EX400 spectrometer in pyridine-*d*₅, with TMS as the internal reference. MS spectra (SIMS) were measured with a Hitachi RMU-6M mass spectrometer at an ionization voltage of 70 eV. The analysis of methyl glycoside was conducted by Shimadzu GC-4 CM gas chromatography with a flame ionization detector. GLC was fitted with a 2 m glass column (3 mm inside diameter) packed with 1.5% SE-30 on Gas Chrom-Z 80 mesh. Temperature gradually (5°C/min) rose to 140°C from 200°C, with nitrogen gas (30mL/min) as carrier. Detector temperature: 250 °C. HPLC was carried out with Hitachi L6000 and Hitachi L4200 UV detectors, column (4.6×250 mm): Intersil Ph-3, 5 μ m (GL Science Inc.), monitor: 318 nm, developer: CH₃CN/H₂O (4:5, v/v), flow rate: 1 mL/min.

2·2 Isolation of EGX

Immature fruits (208.4 g) of *Styrax obassia* Sieb. et Zucc. were harvested in June 1982 at Koganei-shi, Tokyo. The seeds (80.4 g) were taken from the fruit, and extracted with $\text{CHCl}_3/\text{MeOH}$ (1:1, v/v, 300mL $\times$ 3) for two weeks at room temperature. The extracts were concentrated to a soft solid (17.14 g) which was refluxed with acetone (100 mL) for 1 h. After cooling, the insoluble part was dried to yield a brown solid (8.76 g). From the acetone soluble part, five known compounds, egonol (**E**), demethoxyegonol, egonol acetate, egonol 2-methylbutanoate and demethoxyegonol 2-methylbutanoate were isolated, and identified with authentic samples by TLC and IR (6, 7).

For the acetone insoluble part, four conspicuous peaks showing RRT=0.32 (**EG3**), RRT=0.40 (**EG2**), RRT= 0.45 (**EGX**) and RRT=0.61 (**EG**) were noted by HPLC using butyl p-hydroxybenzoate as inner standard. The acetone insoluble solid (8.0 g) was washed with EtOH (100 mL) to give a pale brown solid (5.0 g). The solid (1.0 g) was chromatographed on a column (4.5 cm diameter) of silica gel (Merck, Kieselguhr 60, 85.3 g) and eluted with EtOAc/2-propanol/ H_2O (6:2:1, v/v) to give **EG** (5.7 mg), **EG3** (5.0 mg) and **EG2** (14.0 mg) and crude **EGX** (250.0 mg). The crude **EGX** was purified by gel-filtration (TOYOPEAL HW-40, 80% aq EtOH) to give **EGX** (200.0 mg) as needles recrystallized from 80% aq EtOH; mp 220.5~222.0°C; $[\alpha]_D^{20}$ -0.38° (c 1.0, DMSO); IR (cm^{-1}): 3400 (OH), 1600 and 1500 (aromatic); SIMS: m/z 620 (M^+), 489 ($\text{M}-132+\text{H}^+$) and 327 ($\text{M}-132-162+\text{H}^+$); UV λ_{max} (log ϵ): 214 (1.72) and 303 nm (1.13) in 80% aq EtOH; ^1H - and ^{13}C -NMR data, see Table 1.

2·3 Acid Hydrolysis of EGX

A solution of **EGX** (100.0 mg) in 2M H_2SO_4 (2.0 mL) was heated at 95°C for 5 h. The reaction mixture was treated with BaCl_2 , neutralized to pH 3-4 with Ag_2CO_3 and filtered. The solution was chromatographed on a TLC plate (Merck, Art. 8715, treated with 0.5M KH_2PO_4) and developed with 2-propanol/ H_2O (4:1, v/v). The plate was sprayed with anisaldehyde- H_2SO_4 reagent was compared with that of glucose (R_f 0.34) and xylose (R_f 0.43). The hydrolyzate gave a positive test for D-glucose with D-glucose oxidase tape (Shionogi & Co.).

Table 1 NMR Data for **EG2** and **EGX**.

Position	EG2		EGX	
	$^{13}\text{C}, \delta$	$^1\text{H}, \delta$ (J, Hz)	$^{13}\text{C}, \delta$	$^1\text{H}, \delta$ (J, Hz)
2	156.3		156.3	
3	101.4	7.13 s	101.4	7.13 s
3a	131.6		131.6	
4	113.0	7.10 d (1.0)	113.1	7.12 d (1.0)
5	138.6		138.5	
6	108.4	6.85 d (1.0)	108.4	6.84 d (1.0)
7	145.4		145.4	
7a	143.0		143.0	
1'	125.2		125.3	
2'	105.8	7.57 d (1.9)	105.8	7.57 d (1.9)
3'	148.8		148.8	
4'	148.6		148.6	
5'	109.1	6.95 d (8.3)	109.1	6.95 d (7.8)
6'	119.5	7.53 dd (8.3, 1.9)	119.5	7.54 dd (7.8, 1.9)
1''	32.8	2.88 m	32.8	2.88 m
2''	32.6	2.07 m	32.5	2.05 m
3''	69.0	3.73 dt (9.8, 6.3)	68.9	3.73 dt (9.5, 7.2)
		4.27 m		4.26 m
OCH ₂ O	102.0	6.00 s	102.0	6.00 s
7-OCH ₃	56.1	3.97 s	56.1	3.97 s
G1	104.8	4.83 d (7.8)	104.8	4.83 d (7.8)
G2	75.2	4.06 m	75.2	4.06 m
G3	78.6	4.22 m	78.6	4.24 m
G4	71.7	4.21 m	71.7	4.23 m
G5	77.3	4.11 m	77.2	4.10 m
G6	70.2	4.38 dd (11.2, 6.4)	70.0	4.37 dd (11.3, 6.8)
		4.88 dd (11.2, <1)		4.88 dd (11.3, 2.0)
G1'	105.6	5.15 d (7.8)	106.1	5.03 d (7.3)
G2'	75.3	4.09 m	75.0	4.06 m
G3'	78.5	4.26 m	78.3	4.16 dd (10.0, 9.1)
G4'	71.1	4.28 m	71.2	4.23 m
G5'	78.5	3.96 m	67.2	3.70 dd (11.0, 11.0)
				4.37 dd (11.0, 5.6)
G6'	62.8	4.40 dd (11.7, 5.4)		
		4.54 dd (11.7, 2.5)		

Chemical shifts (δ : ppm from TMS) were measured in pyridine- d_5 by JEOL JNM-EX400 spectrometer. All carbons and protons assigned by ^1H - ^1H COSY, ^{13}C - ^1H COSY and HMBC experiments.

2·4 GLC Analysis of Methanolizate

EGX (8.0mg) was dissolved in 6% HCl-MeOH (1.0 mL, Kokusan Kagaku Co.) and heated for 3 h in a shield glass tube. The mixture was neutralized with Ag_2CO_3 , filtered and evaporated to dryness. After trimethylsilylation with BSTFA-pyridine and concentrating, the residue was examined by GLC. Methyl glucoside (RRT: 1.00 and 1.08) and methyl xyloside (RRT:

0.44 and 0.47) were detected by comparison with reference glycosides.

2·5 Methanolysis

EGX (543.0 mg) in 5% HCl-MeOH (10.0 mL) was heated for 4 h at 90°C in a shield glass tube and after cooling to 5°C, the reaction mixture was filtered and the precipitate washed with chilled MeOH. The precipitate was dried under reduced pressure to yield **E** (107.6 mg) as white powder. Aglycone **E** was identical with an authentic egonol in all respects. The filtrate was concentrated to dryness to give a crude oil, which was further purified by column chromatography on silica gel (5 g, CHCl₃-MeOH, 10/1) to afford **E** (8.7 mg) and a mixture (54.5 mg) of methyl glycosides.

2·6 Acetylation of Methyl Glycosides

The above mixture (54.5 mg) of methyl glycosides was treated with acetic anhydride (0.15 mL) in pyridine (2 mL) for 15 h at room temperature. After the addition of MeOH, the mixture was stirred for 30 min and evaporated to dryness. The residual oil was purified by preparative TLC (Merck, Art. 5745) and developed with hexane/acetone (3:1,v/v) to give methyl 2, 3, 4-tri-*O*-acetyl- α -D-xylopyranoside (10.2 mg), methyl 2, 3, 4, 6-tetra-*O*-acetyl- α -D-glucoside (16.3 mg) and a mixture of β -anomers (6.2 mg). Methyl 2, 3, 4-tri-*O*-acetyl- α -D-xylopyranoside: $[\alpha]_D^{25} +113.6^\circ$ (*c* 0.9, CHCl₃) [lit. (8) +118°], and a methyl 2, 3, 4, 6-tetra-*O*-acetyl- α -D-glucoside: $[\alpha]_D^{25} +107.7^\circ$ (*c* 1.0, CHCl₃) [lit. (9) +130.5°].

3 Results and Discussion

Immature seeds of *Styrax obassia* Sieb. et Zucc. were extracted with chloroform-methanol (1:1,v/v) and con-

centrated and the concentrate was refluxed with acetone. A new compound, **EGX** was isolated as a major compound, along with **EG**, **EG2** and **EG3** from the acetone insoluble part. **EGX** showed a strong hydroxyl absorption band at 3400 cm⁻¹ and aromatic absorptions at 1600 and 1500 cm⁻¹ in the IR spectrum. The positive ion SIMS spectrum showed a molecular ion peak at *m/z* 620 (M⁺), and fragment ion peaks indicating loss of one pentose unit at *m/z* 489 (M-132+H)⁺ and loss of pentose and hexose units at *m/z* 327 (M-132-162+H)⁺. By methanolysis of **EGX**, aglycone **E** was isolated and identified with an authentic egonol.

Based on NMR spectra shown in **Table 1**, the structure of **EGX** was proposed as that of egonol glycoside with 31 carbons. ¹H- and ¹³C-NMR chemical shifts were assigned as that of 2D experiments (¹H-¹H COSY, ¹H-¹³C COSY and HMBC). Anomeric proton signals at δ 4.83 (d, *J*=7.8 Hz, G1) and 5.03 (d, *J*=7.3 Hz, G1') led to assignment of the pyranosides as β -form, which was confirmed by comparison with those of **EG2**. The ¹H-NMR spectrum showed a signal at δ 3.97 (3H, s) due to aromatic methoxy protons and signals at δ 7.13 (1H, s, H-3), 7.12 (1H, d, *J*=1.0 Hz, H-4), 6.84 (1H, d, *J*=1.0 Hz, H-6), 7.57 (1H, d, *J*=1.9 Hz, H-2'), 6.95 (1H, d, *J*=7.8 Hz, H-5') and 7.54 (1H, dd, *J*=7.8 Hz, *J*=1.9 Hz, H-6') due to aromatic protons. Signals at δ 4.06-4.88 region were interpreted from the protons of glucopyranoside units, based on ¹H-¹H COSY and ¹³C-¹H COSY. In the ¹³C-NMR spectrum, the anomeric carbon signal of the inner glucose was at δ 104.8 and terminal xylose at δ 106.1. The glucose carbon signals were in a good agreement with those reported for inner glucopyranoside of **EG2**. The G6 carbon signal shifted to lower field compared with 6-OH of glucose. The terminal xylopyranose may thus be concluded to be attached to

Table 2 HPLC Analyses of Egonol Glycosides in Acetone Insoluble Solids from Immature and Mature Seeds.

Egonol Glycosides	RRT ^a	Immaturity ^b (June) (%)	Maturity ^c (October) (%)
EG3	0.32	0.68	1.82
EG2	0.40	2.18	27.65
EGX	0.45	11.28	0.56
EG	0.61	3.39	0.52

^a RRT: Relative retention time to butyl p-hydroxybenzoate. See Experimental.

^b See Experimental.

^c Acetone insoluble solid (1.00 g) was obtained by extraction with CHCl₃/MeOH (12.19 g) from mature seeds (47.71 g) and then acetone treatment.

6-OH of the glucopyranose moiety.

Methyl D-glucopyranoside and methyl D-xylopyranoside were characterized by optical rotation of the peracetates. The structure of **EGX** from the present results is thus shown to be 3''-O-[6-O-(β -D-xylopyranosyl)- β -D-glucopyranosyl]egonol (egonol β -primeveroside).

Egonol glycosides in acetone insoluble parts, extracted from immature (collected on June) and mature (October) seeds of *Styrax obassia* Sieb. et Zucc., were analyzed by HPLC. As shown in **Table 2**, **EG2** and **EGX** contents differed considerably. **EGX** in the immature seeds changed to **EG2** in the maturing season.

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