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MONO- AND TRIGLYCOSYLSTEROLS FROM THE LEAFY STEM OF RICE

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Key Word Index—*Oryza sativa*; Gramineae; rice; glycoside; sterol; sterylglycoside; glycosylsterol; glucosylsitosterol; cellotriosylsitosterol.

Abstract—Two sterylglycosides have been isolated from rice plants and identified as β-D-glucopyranosyl (1 → 3)-sitosterol and β-D-glucopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 3)-sitosterol (cellotriosylsitosterol), respectively.

INTRODUCTION

Monoglycosylsterols are known to occur widely in the plant kingdom [1], but the occurrence of sterylglycosides containing an oligosaccharide has only been reported in a few communications [2–6]. The present paper reports the isolation of monoglycosylsterol and also triglycosylsterol from the leafy stem of the rice plant.

RESULTS AND DISCUSSION

Two sterylglycosides obtained from rice plants gave positive tests with H_2SO_4–HOAc (for sterols) and the anthrone reagent (for sugars). Their acetyl and methyl derivatives showed single spots with the same R_f values on TLC as those for authentic mono- and triglycosylsterols isolated from rice seeds [5].

Acid hydrolysis of the sterylglycosides afforded only 4-demethylsterols in the lipophilic fractions. Analysis by GLC revealed seven sterol peaks, among which sitosterol was preponderant in both lipids (Table 1). A significant amount of cholesterol in the triglycosylsterol was presumed to be not real, because an ion at m/z 369 indicating the presence of cholesterol was very weak in the mass spectrum of the permethylated triglycosylsterol.

The sugar constituent identified in both sterylglycosides was predominantly glucose. The amount of glucose recovered after oxidation with CrO_3 was found to be under 1% for the monoglycosylsterol and *ca.* 10% for the triglycosylsterol. These results imply that all the glycosidic linkages in both lipids were of the β-configuration [7]. GLC of the permethylated sugars prepared from triglycosylsterol gave four peaks, having RR_t values of 0.69, 1, 2.18 and 2.99, respectively, taking the R_t of α-methyl-2,3,4,6-tetra-*O*-methylglucoside as unity. The faster two peaks were identified as β- and α-methyl-2,3,4,6-tetra-*O*-methylglucosides and the slower two as β- and α-methyl-2,3,6-tri-*O*-methylglucosides by direct comparison of the R_t and mass spectra with the respective authentic samples prepared from permethylated maltose. The ratio of the terminal to internal sugars was found to be *ca.* 1:2. From these data, the nature of the linkage in the glycoside moiety was established to be glucose β1 → in the monoglycosylsterol and cellotriosyl β1→ in the triglycosylsterol.

Table 1. Composition of the sterols present in the sterylglycosides obtained from leafy stem of rice (%)

Sterol	Monoglycosyl-sterol	Triglycosyl-sterol
Cholesterol (?)	1.4	22.2
Campesterol	27.5	18.5
Stigmasterol	19.7	16.7
Sitosterol	51.4	42.6
Others*	<0.1	<0.1

* % of the sum for Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol.

Based on these findings and the results reported previously [5, 6], the structures of the two sterylglycosides found in the rice plant were characterized as β-D-glucopyranosyl(1 → 3)-sitosterol and β-D-glucopyranosyl(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl(1 → 3)sitosterol, respectively. The triglycosylsterol is a new glycoside, which was identical with the triglycosylsterol isolated previously from rice seeds in our laboratory [5]. Diglycosylsterol was not recognized in the present work. However, the possibility that a trace amount of the diglycoside was also present could not be excluded, since a few reports have suggested the occurrence of diglycosylsterol in plants [2, 4, 5].

EXPERIMENTAL

Extraction and isolation. Rice straw (2 kg) was exhaustively extracted with $CHCl_3$–MeOH (2:1). The extract was washed with H_2O and concd to dryness [8]. The residue (44 g) was subjected to Si gel CC by sequential elution with $CHCl_3$ and MeOH. The latter fraction was treated with methanolic 0.4 N KOH at 38° for 2 hr to yield alkali-stable lipids [9]. From this fraction two sterylglycosides were isolated by a combination of Si gel CC and prep. TLC on Si gel with $CHCl_3$–MeOH–H_2O

(65:25:4; R_f 0.75 and 0.24, respectively), as described previously [5]. Each glycoside was acetylated and purified by TLC on Si gel with $CHCl_3$–C_6H_6–Me_2CO (80:20:10).

Degradation of sterylglycoside. Each sterylglycoside was heated with methanolic 1 N HCl at 100° for 4 hr. After adding H_2O, the reaction mixture was extracted with Et_2O, and the concd extract was analysed by TLC and GLC (1.5% OV-17) [8]. The residual H_2O–MeOH phase was deionized by passing through a column of ion-exchange resin and dried to yield methylglycosides, which were subjected to GLC with 1.5% SE-30.

Oxidation with CrO_3. The oxidation was carried out to determine the anomeric configuration of the glycosidic linkages [7]. Each glycoside (0.5 mg) was acetylated together with inositol (0.2 mg) as standard. A half of the acetyl derivatives was dissolved into HOAc (0.5 ml) and oxidized with CrO_3 (50 mg) for 20 min at 45° in the ultrasonic bath [5]. The oxidation products and the other half of the unoxidized acetyl derivatives were degraded as described above, and analysed by GLC.

Permethylation. Each sterylglycoside was permethylated in $HOCN(CH_3)_2$ with CH_3I in the presence of BaO and $Ba(OH)_2$ [10]. The permethylates were purified by TLC on Si gel with $CHCl_3$–C_6H_6–Me_2CO (80:20:10). MS (direct-inlet method, 20 eV) of the two sterylglycosides exhibited ions indicating of the presence of 4-demethylsterols, at *m/z* 397 due to sitosterol, at 395 due to stigmasterol and at 383 due to campesterol [5], and ions derived from the methylated sugars at *m/z* 88, 101, 147 etc. [11]. In the spectrum of triglycosylsterol, a diagnostic ion at *m/z* 305 for the 1 → 4 linkage of the internal hexose [12] was observed. Purified products were methanolysed as described above and the methylglycosides were analysed by GLC on 3% neopentyl glycol succinate [6].

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