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Glyceroglycolipids in Rice Grain

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ABSTRACT

Monoglycosyldiglyceride (MGDG) and diglycosyldiglyceride (DGDG) were isolated from rice grain, identified, and examined for composition of fatty acid as well as sugar constituents. MGDG was found to contain seven component acids, palmitic (51.4%), linoleic (29.7%), and oleic (22.0%) being predominant, and one component sugar, galactose. DGDG was observed to have eight component acids, including linoleic (38.8%), oleic (19.2%), palmitic (18.8%), and linolenic (18.2%), and two component sugars, galactose (85.8%) and glucose (14.2%).

Fujino and Sakata (1) recently noted that there are at least three groups of glycolipids in rice grain lipids, namely, glyceroglycolipid, sphingoglycolipid, and sterol glycoside. The present paper describes isolation of two glyceroglycolipids, monoglycosyldiglyceride (MGDG) and diglycosyldiglyceride (DGDG), from rice grain, and their identification and chemical composition.

MGDG and DGDG, found first in wheat flour by Carter et al. (2), are now known to be widely distributed in nature, especially in the kingdom of plants and microorganisms (3).

MATERIALS AND METHODS

Preparation of Crude Glycolipid

Three kilograms of rice grain, harvested at Hokkaido Prefecture in 1969, was milled and extracted with chloroform-methanol (2:1). The extract was washed with water, dehydrated with sodium sulfate, and condensed under reduced pressure to obtain almost 79 g. of total lipid. The lipid was subjected to silicic acid column chromatography by sequential elution with chloroform, acetone, and methanol according to the procedure of Rouser et al. (4). Crude neutral lipid was eluted with chloroform, crude glycolipid with acetone, and crude phospholipid with methanol. Yields of the crude lipid fractionated were 61.1, 7.7, and 9.1 g., respectively.

Isolation of Glyceroglycolipid

The crude glycolipid was subjected to silicic acid column chromatography with a mixture of chloroform and acetone, following the technique of Vorbeck and Marinetti (5). The eluates with chloroform-acetone 8:2, 2:8, and 0:10

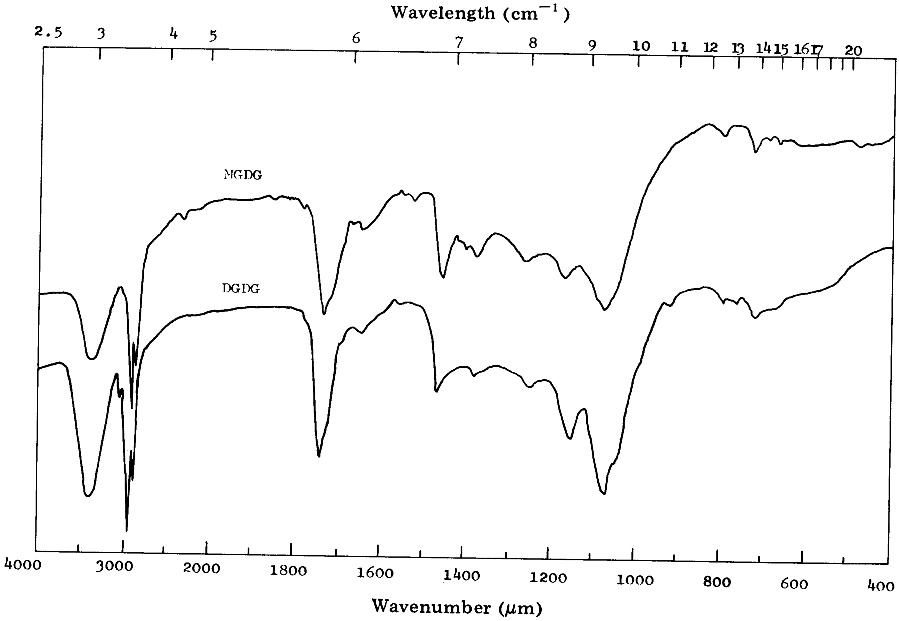

Fig. 1. Infrared spectra (in KBr) of glyceroglycolipids isolated from rice grain.

corresponded approximately to MGDG, DGDG, and sulfur-containing glyceroglycolipid, respectively. Each of the first two was rechromatographed repeatedly to obtain pure MGDG and DGDG, although the latter one could not be purified owing to the small amount. The two glyceroglycolipids thus isolated were identified by thin-layer silica gel G chromatography and by infrared spectrometry.

Methanolysis of Glyceroglycolipid

Five milligrams of glyceroglycolipid was refluxed for 4 hr. with 5% HCl in methanol. After methanolysis, the mixture was cooled and extracted with hexane. The extract was washed with water, dehydrated, and concentrated under reduced pressure to produce methyl esters of the component fatty acids. The residual mixture, after extraction with hexane, was taken to ion-exchange column chromatography with Amberlite IR-4B (OH^-). The effluent was concentrated under reduced pressure to get the constituent sugars as methylglycosides.

Analysis of Components

The fatty acid methyl esters were analyzed as obtained, and the methyl glycosides after trimethylsilylation (6) by gas-liquid chromatography. The analyses were carried out with a Hitachi gas chromatograph (Model 063, Hitachi Seisakusho Co. Ltd., Tokyo), equipped with a hydrogen flame ionization detector. Carrier gas was N_2, and temperature was 175°C. A glass column (0.3 × 200 cm.) was packed with 10% diethyleneglycol succinatepolyester in Chromosorb W for fatty acid methyl esters, and with 5% SE-30 for trimethylsilylether derivatives of methyl glycosides.

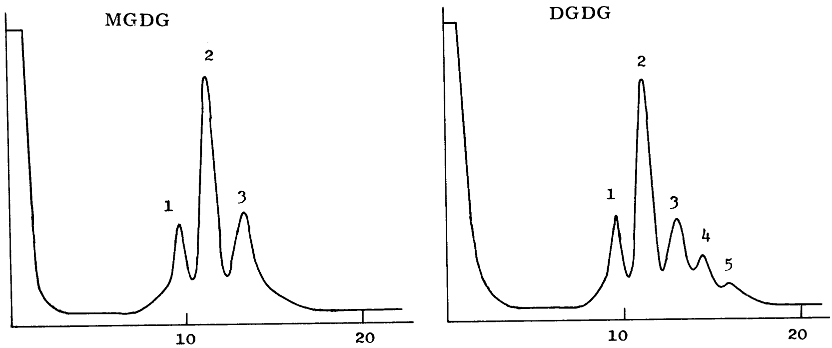

Fig. 2. Gas-liquid chromatogram of trimethylsilyl ether derivatives of methyl glycosides obtained from rice grain glyceroglycolipids. 1, γ-galactose; 2, α-galactose; 3, β-galactose; 4. α-glucose; 5, β-glucose.

RESULTS AND DISCUSSION

Identification of Rice Grain Glyceroglycolipids

The two glyceroglycolipids isolated from rice grain were separately put on a thin-layer silica gel G plate, developed with chloroform-methanol (95:12), and located by spraying with anthrone reagent. Two single spots were revealed on the plate and the R_f values agreed well with those of the standard preparations of MGDG and DGDG from alfalfa (7). Infrared spectra of the rice grain MGDG and DGDG (Fig. 1) gave peaks at 1,735 cm^{-1} for the ester bond, and at 3,440 cm^{-1} and 1,125~1,000 cm^{-1} for the hydroxy group of sugar moieties. The two infrared patterns coincided with those of MGDG and DGDG isolated from spinach by Allen et al.(8).

Component Fatty Acids of Rice Grain Glyceroglycolipids

Composition of the fatty acids analyzed by gas-liquid chromatography is shown in Table I. Seven peaks were found in MGDG, and eight peaks in DGDG. They were identified by comparing the relative retention times with those for standard fatty acid methyl esters. Predominant in MGDG were palmitic (51.4%), linoleic (29.7%), and oleic (22.0%) acids, and in DGDG were linoleic (38.8%), oleic

TABLE I. FATTY ACID COMPOSITION OF RICE GRAIN GLYCEROGLYCOLIPIDS

Fatty Acid	MGDG %	DGDG %
14:0	2.8	0.6
16:0	51.4	18.8
16:1	1.0	0.4
18:0	6.8	2.8
18:1	22.0	19.2
18:2	29.7	38.8
18:3	8.5	18.2
20:0	...	1.0

(19.2%), palmitic (18.8%), and linolenic (18.2%) acids. Patterns of the component acids seem to be not always similar to each other between MGDG and DGDG (9).

Component Sugars of Rice Grain Glyceroglycolipids

Typical gas-liquid chromatographic profiles for the component sugars of rice grain glycolipids are shown in Fig. 2. The peaks were identified by co-chromatography with standard preparations of glucose and galactose. Only galactose was seen in MGDG, while not merely galactose (85.8%) but also glucose (14.2%) was found in DGDG. Galactose is known to be the most common sugar constituent in MGDG and DGDG of higher plants, but glucose is rare. However, glucose, mannose, and glucuronic acid are detected, in addition to galactose, as sugar components in the glycosyldiglycerides of microorganisms (3,10). The nature of combination of galactose and glucose in rice grain DGDG is a problem to be elucidated.

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