10. *Species of Sphingolipids in Rice Grain*

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Previous studies in our laboratory indicated the existence of CM*) and G_1CM in brown rice grain.[1] Recently, we have obtained evidence for the presence of not only these but other oligoglycosyl ceramides in rice bran. The present paper describes comprehensively isolation, chemical analyses and species studies on CM, G_1CM, G_2CM, G_3CM and G_4CM in rice grain.

Experimentals. *Preparation and fractionation of sphingolipids.*[1] Grains of brown rice were polished to separate bran, which was steamed to deactivate the enzymes and extracted with chloroform-methanol and butanol-water to obtain total lipid. The lipid was subjected to silicic acid column chromatography to divide it into neutral lipid, glycolipid and phospholipid fractions. The glycolipid fraction was then applied to silicic acid thin-layer chromatography, mild-alkaline treatment and silicic acid column chromatography to prepare and purify sphingolipids separately. The sphingolipids gave five spots on thin-layer chromatogram; Rf values and IR spectra corresponded to those of CM and $G_{1-4}CM$ isolated from wheat grain.[2] The observation suggested that sphingolipids prepared should be ceramide, mono-, di-, tri- and tetraglycosyl ceramide.

Composition of constituent fatty acids.[1),3),**)] Each sphingolipid was methanolyzed and analyzed for the methyl fatty acids with thin-layer chromatography, gas-liquid chromatography and mass spectrometry. Through the five lipids, the constituent fatty acids were practically not the normal but the 2-hydroxy acids, which amounted at least to nine. Among them, 2-hydroxy lignoceric acid was dominating in CM[1] and $G_{2-4}CM$, whereas 2-hydroxyarachidic acid in G_1CM.[1]

Characterization of component sphingosines.[1),3),**)] Each sphingolipid was methanolyzed to obtain sphingosines which were derived to the 2 or 3 carbons-less aldehydes, acids and alcohols, and to the

*) Abbreviations: CM for ceramide (N-acyl sphingosine), G_1CM for monoglycosyl ceramide (cerebroside), G_2CM for diglycosyl ceramide, G_3CM for triglycosyl ceramide and G_4CM for tetraglycosyl ceramide.

**) The term, sphingosine, is used in the present paper as a comprehensive nomination for sphinganine and its analogues.

hydroxylated compounds. The products were examined by thin-layer chromatography, gas-liquid chromatography and mass spectrometry to characterize sphingosines. Trihydroxy-type sphingosines were preponderant in CM[1] and G_{2-4}CM, whereas dihydroxy-type sphingosines in G_1CM.[1] The exemplary long-chain bases were 4-hydroxy-sphinganine and 4-hydroxy-8-sphingenine in CM, 4,8-sphingadienine and 4-hydroxy-8-sphingenine in G_1CM, and 4-hydroxy-8-sphingenine, 4-hydroxy-sphinganine and 4,8-sphingadienine in G_{2-4}CM, the respective sphingosine firstly mentioned being predominant.

Composition of constituents saccharides.[1),4)] Each sphingoglycolipid was methanolyzed, and the resultant methylglycosides were analyzed with gas-liquid chromatography. In all the four glycolipids, the component saccharides were glucose and mannose, of which glucose tended to decrease whereas mannose to increase, as number of the hexose radicals became higher in the molecules of glycolipid.

Binding position of sugars. Each glycosylceramide was permethylated and methanolyzed to obtain permethylated sugars, which were analyzed with gas-liquid chromatography and mass spectrometry to elucidate binding position of the component sugars.[5)]. In G_1CM, a large amount of the terminal glucose with C-1 linkage and a small quantity of the terminal mannose with C-1 linkage were seen. In G_2CM, the terminal glucose with C-1 linkage, the terminal mannose with C-1 linkage and the internal glucose with C-4,1 linkages were detected in the approximate ratio of 1:1:2, indicating the sugar sequence should be Glc-Glc- or Man-Glc-. In G_3CM, the terminal glucose with C-1 linkage, the terminal mannose with C-1 linkage, the internal glucose with C-4,1 linkages and the internal mannose with C-4,1 linkages were recognized in the approximate ratio of 2:1:3:3, meaning that the sugar sequence should be Glc-Man-Glc- or Man-Man-Glc-. In G_4CM, the proportion of the terminal glucose with C-1 linkage, the terminal mannose with C-1 linkage, the internal glucose with C-4,1 linkages and the internal mannose with C-4,1 linkages was approximately 1:1:2:4, signifying that the sugar sequence should be Glc-Man-Man-Glc- or Man-Man-Man-Glc-.

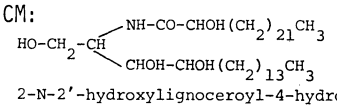
Anomeric configuration of sugars. Each sphingoglycolipid was permethylated, oxidized with chromic acid, methanolyzed and examined as to whether the resultant sugar was decomposed or not.[6)] In the four glycolipids, all the terminal and the internal hexoses were found to practically disappear after chromic oxidation by gas chromatography as well as thin-layer chromatography, demonstrating that all the constituent hexoses were of the β configuration.

Discussions. Judging from the experimental results above mentioned together with the known informations[1),6)] of sphingolipids, the

representative species of CM, G_1CM, G_2CM, G_3CM and G_4CM will be proposed as pictured in Fig. 1.

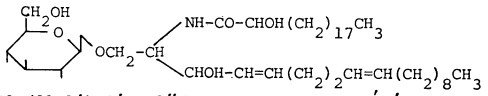
Lipid I was identical with CM in wheat grain.[2] II is universal G_1CM in nature, although the ceramide moiety is various. Monoman-

CM:

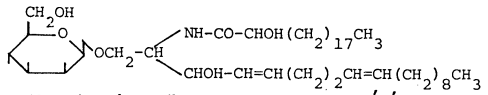


2-N-2'-hydroxylignoceroyl-4-hydroxysphinganine(I)

G_1CM:

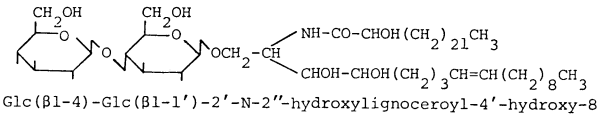


Glc(β1-1')-2'-N-2"-hydroxyarachidoyl-4',8'-sphingadienine(II)

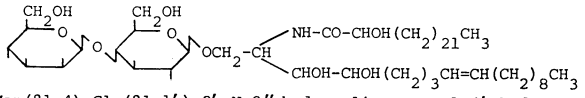


Man(β1-1')-2'-N-2"-hydroxyarachidoyl-4',8'-sphingadienine(III)

G_2CM:

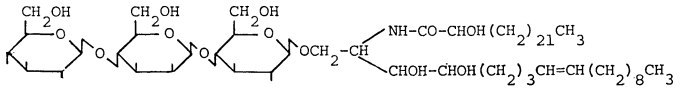


Glc(β1-4)-Glc(β1-1')-2'-N-2"-hydroxylignoceroyl-4'-hydroxy-8'-sphingenine(IV)

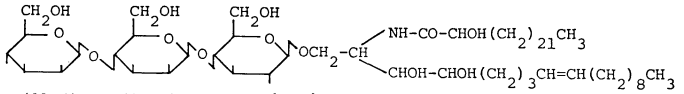


Man(β1-4)-Glc(β1-1')-2'-N-2"-hydroxylignoceroyl-4'-hydroxy-8'-sphingenine(V)

G_3CM:

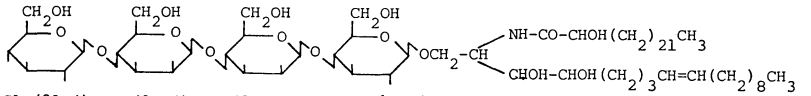


Glc(β1-4)-Man(β1-4)-Glc(β1-1')-2'-N-2"-hydroxylignoceroyl-4'-hydroxy-8-sphingenine(Vl)

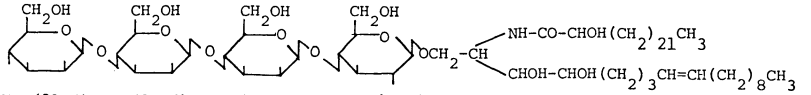


Man(β1-4)-Man(β1-4)-Glc(β1-1')-2'-N-2"-hydroxylignoceroyl-4'-hydroxy-8'-sphingenine(VII)

G_4CM:



Glc(β1-4)-Man(β1-4)-Man(β1-4)-Glc(β1-1')-2'-N-2"-hydroxylignoceroyl-4'-hydroxy-8'-sphingenine(VIII)



Man(β1-4)-Man(β1-4)-Man(β1-4)-Glc(β1-1')-2'-N-2"-hydroxylignoceroyl-4'-hydroxy-8'-sphingenine(IX)

Fig. 1. Representative species of sphingolipids in rice grain.

nosyllipid of III type was recently isolated from fresh-water bivalves.[7] G_2CM V has been detected in wheat[2),6)] and bivalves.[8] VII[2),6)] and IX[2)] were equal respectively to G_3CM and G_4CM of wheat concerning the sugar chains but not always the ceramide radicals. It could be noted that the compounds IV, VI and VIII found in the rice grain are the novel sphingolipids in nature, although the respective lipid has not completely isolated yet.

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