9. *Structural Characterization of Sphingolipids in Wheat Grain*

By Yasuhiko FUJINO and Masao OHNISHI
Department of Agricultural Chemistry, Obihiro University, Obihiro, Hokkaido 080

(Communicated by Sankichi TAKEI, M. J. A., Feb. 12, 1982)

Sphingolipids, a group of the complex lipids, are known to be distributed widely in nature, mainly constituting the biological membranes. The chemical studies have been well carried out on sphingolipids in animal kingdom, but not so much on the lipids in plants and microorganisms. As sphingolipids in wheat flour, $G_{1-3}CM$*) were detected and examined chiefly for the sugar radicals but not in detail for the non-polar moieties.[1)-4)] The authors isolated not only the $G_{1-3}CM$ but also newly CM and G_4CM from wheat grain and characterized the chemical structure of all these sphingolipids.

Experimentals. *Isolation of sphingolipids.*[5)] The whole grains of wheat were milled, extracted with chloroform-methanol and butanol-water, and chromatographed through silicic column to obtain polar lipids. The fraction was purified by mild-alkaline hydrolysis, silicic column chromatography, acetylation and silicic thin-layer chromatography to prepare sphingolipids. The lipids gave five single spots on the thin-layer chromatogram; Rf values and IR spectra corresponded respectively to the known CM and $G_{1-4}CM$ in animals; the approximate molar ratios[6)] of component sphingosine to saccharide on gas-chromatogram were 1 : 0, 1 : 1, 1 : 2, 1 : 3 and 1 : 4, respectively. The findings strongly suggested that the sphingolipids isolated were ceramide, mono-, di-, tri- and tetraglycosylceramide.

Component fatty acids. Each sphingolipid was decomposed by hydrochloric acid in methanol to produce fatty acid methylesters, which were analyzed by thin-layer chromatography, gas-liquid chromatography and mass spectrometry.[5),7)] Normal, 2-hydroxy and 2,3-dihydroxy fatty acids were revealed, 2-hydroxy acids being preponderant. As the 2-hydroxy acids, at least nine ones were observed altogether. Among them, hydroxylignoceric acid was principal in CM, whereas hydroxypalmitic and hydroxyarachidic acids in $G_{1-4}CM$.

*) In the present paper, the following abbreviations are used: CM for ceramide N-acyl sphingosine); G_1CM, G_2CM, G_3CM and G_4CM for mono-, di-, tri- and tetraglycosyl ceramide, respectively. The term, sphingosine, is employed as the general name for the sphinganine and its analogues.

Component sphingosines.[5),7)] Each sphingolipid was degraded by alkaline hydrolysis to give sphingosines, which were identified by thin-layer chromatography, gas chromatography and mass spectrometry of themselves as well as their derivatives. The composition was determined by gas-chromatographic analysis of long-chain aldehydes, derived from sphingosines by oxidation with sodium periodate (Table I). Among nine sphingosines recognized, 4-hydroxysphinganine was rich in CM, whereas 8-sphingenine and 4,8-sphingadienine were major in $G_{1-4}CM$.

Table I. Composition of sphingosines in wheat sphingolipids

Sphingosine	CM	G_1CM	G_2CM	G_3CM	G_4CM
Sphinganine	14%	9%	5%	1%	3%
4-*trans*-sphingenine	<1	1	<1	<1	1
8-*trans*-sphingenine	6	13	21	22	21
8-*cis*-sphingenine		60	50	48	42
4-*trans*-8-*trans*-sphingadienine	1	2	1	2	3
4-*trans*-8-*cis*-sphingadienine	<1	14	11	16	18
4-hydroxy-sphinganine	65	<1	1	1	1
4-hydroxy-8-*trans*-sphingenine	14	<1	3	2	4
4-hydroxy-8-*cis*-sphingenine		1	8	8	14
Dihydroxy type	21	96	88	89	82
Trihydroxy type	79	4	12	11	18

Component saccharides. Each sphingoglycolipid was examined after methanolysis for the sugar constituents with gas-liquid chromatography.[5),7)] Saccharides detected were glucose and mannose, the approximate ratios being 1:0 in G_1CM, 1:1 in G_2CM, 1:2 in G_3CM and 1:2–3 in G_4CM. Then, each sphingoglycolipid was acetylated, treated with chromic acid and methanolyzed.[8)] The most of the component sugar was destroyed, the survival one being in quite a small amount in every case. This implies that all the component hexoses were of the β configuration in the four glycolipids. Further, each sphingoglycolipid was permethylated and decomposed to obtain the methylated sugars, which were analyzed by gas chromatography-mass spectrometry.[9)] In case of G_1CM, only methyl-2,3,4,6-tetramethyl-glucoside meaning terminal glucose with a linkage at C-1 was detected. In cases of $G_{2-4}CM$, not only methyl-2,3,4,6-tetramethylmannoside signifying terminal mannose with a linkage at C-1 but also methyl-2,3,6-trimethyl-mannosides and glucosides, respectively comprising internal mannose and glucose with linkages at C-1 and C-4, were observed, approximate ratios of the former to the latter being 1:1

in G_2CM, 1:2 in G_3CM and 1:3 in G_4CM. The data demonstrated that $G_{1-4}CM$ possessed the sugar chains of Glc(β1-1')-, Man(β1-4)-Glc (β1-1')-, Man(β1-4)-Man(β1-4)-Glc(β1-1')- and Man(β1-4)-Man(β1-4)-Man(β1-4)-Glc(β1-1')-, respectively.

Analyses of ceramide type. Each of CM and G_1CM was directly oxidized with sodium periodate to produce di- and trihydroxy base-containing lipids, which were divided by thin-layer chromatography and analyzed respectively for the component fatty acids by gas-liquid chromatography. Dihydroxy base-containing CM, the minor group of CM, and dihydroxy base-containing G_1CM, the major group of G_1CM, commonly possessed hydroxypalmitic and hydroxyarachidic acids as the main components. On the other hand, trihydroxy base-containing CM, the major type of CM, and trihydroxy base-containing G_1CM, the minor type of G_1CM, commonly included hydroxylignoceric acid as the chief constituents. The fact suggested that the minor and major groups of CM should be biosynthetic precursors of the major and minor types of G_1CM, respectively.

Discussions. Judging from the results mentioned above together with the known findings of sphingolipids,[1)-4)] the principal molecular species of CM and $G_{1-4}CM$ in wheat are depicted as shown in Fig. 1.

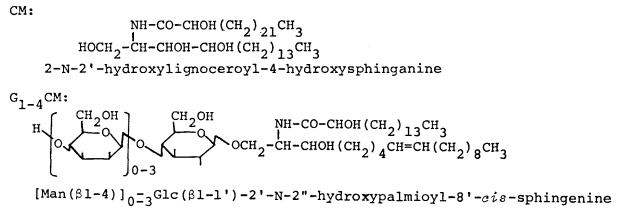

Fig. 1. Major species of sphingolipids in wheat grain.

In sphingolipids of wheat grain, pattern of the component fatty acids was different between CM and $G_{1-4}CM$ but similar among $G_{1-4}CM$, and so was pattern of the constituent sphingosines (Table I). Besides, component sugar was only glucose in G_1CM whereas those were step-wise-increasing mannoses in addition to glucose in $G_{2-4}CM$ (*Component saccharides*). Further, minor and major species of free ceramide were rather identical, respectively, with major and minor species of bound ceramide, G_1CM (*Analyses of ceramide types*). It is conceived from these data that dihydroxy base-containing CM would be preferentially glucosylated to form G_1CM, which is successively mannosylated to generate G_2CM, G_3CM and G_4CM, in metabolism of wheat sphingolipids.

Recently mannose-containing sphingolipids have been isolated

from spermatozoa of the fresh-water bivalves and characterized.[10] The detailed structure of sugar chain in mannosphingolipids seems to be somewhat different between mollusca (bivalves) and higher plants (wheat).

Acknowledgement. This work was supported in part by Grants of Scientific Research in 1980 and 1981 from the Ministry of Education, Science and Culture of Japan.

References

1) Carter, H. E., Hendry, R. R., Nojima, S., Stanacev, N. Z., and Ohno, K. (1961): J. Biol. Chem., **236**, 1912.
2) Carter, H. E., Ohno, K., Nojima, S., Tipton, C. L., and Stanacev, N. Z. (1961): J. Lipid Res., **2**, 215.
3) MacMurray, T. A., and Morrison, W. R. (1970): J. Sci. Food Agric., **21**, 520.
4) Laine, R. A., and Renkonen, O. (1974): Biochemistry, **13**, 2837.
5) Fujino, Y., and Ohnishi, M. (1976): Chem. Phys. Lipids, **17**, 275.
6) Itasaka, O., Sugita, M., Yoshizaki, H., and Hori, T. (1976): J. Biochem., **80**, 935.
7) Ohnishi, M., Ito, S., and Fujino, Y. (1980): Shishitsu Seikagaku Kenkyu, **22**, 379 (in Japanese).
8) Laine, R. A., and Renkonen, O. (1975): J. Lipid Res., **16**, 102.
9) Matsubara, T., and Hayashi, A. (1974): Biomed. Mass Spectrom., **1**, 62.
10) Sugita, M., Yamamoto, T., Masuda, S., Itasaka, O., and Hori, T. (1981): J. Biochem., **90**, 1529.