Note

Investigation of Sphingolipids
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Received October 1, 1984

We had inferred that the main constituent of monoglycosylceramide (G_1 CM) in pea seeds would be glucosyl-hydroxytricosanoyl-sphinganine,¹⁾ but recent investigations in detail showed that the main components of G_1 CM in pea seeds were glucose, hydroxypalmitic acid, and sphingene. This paper describes the constituent compositions and the principal molecular species of G_1 CM together with the other sphingolipids, ceramide (CM) and diglycosylceramide (G_2 CM), in pea seeds.

Alkali-stable lipids were extracted from pea (*Pisum sativum*) seeds, harvested in Hokkaido Prefecture, Japan in 1980, as described before.^{2,3)} Three sphingolipids were separated and monitored by silicic acid TLC with chloroform-methanol-water (65:16:2), using plant CM, G_1 CM, and G_2 CM as references.^{3,4)} The R_f values, color reactions, and infrared spectra of these lipids were identical with those of CM, G_1 CM, and G_2 CM from soybeans³⁾

and wheat grains.⁴⁾ The ratio of the amounts of CM, G_1 CM, and G_2 CM in pea seeds was 1.3:1.0:0.1. G_1 CM was approximately 0.8% of the polar lipid fraction. Thus, the constituents and the molecular species of each lipid were assayed as reported previously.³⁾

The fatty acid composition of pea sphingolipids is shown in Table I. Normal (non-hydroxy), 2-hydroxy, and 2,3-dihydroxy fatty acids were detected in CM, the 2-hydroxy fatty acid being principal as in the case of plant seed CM.²⁻⁵⁾ The major fatty acids in G_1 CM and G_2 CM were also 2-hydroxy ones. The representative 2-hydroxy fatty acids were confirmed by GC-MS as $C_{24:0}$ in CM, $C_{16:0}$, $C_{14:0}$ in G_1 CM, and $C_{22:0}$, $C_{24:0}$ in G_2 CM. The compositions of these acids were different; this has been observed as well in rice grain sphingolipids.⁶⁾ 2,3-Dihydroxy fatty acids, the characteristic components in CM, were chiefly $C_{24:0}$ and $C_{25:0}$.

The main component sphingoid prepared from G_1 CM by acidic hydrolysis was probably sphinganine, judging from the mobility on silica gel TLC with the solvent mixture of chloroform-methanol-2N ammonia (40:10:1). GLC of the *O*-trimethylsilyl ether derivative gave a retention time similar to that of sphinganine.¹⁾ However, GC-MS of the *O*-trimethylsilyl ether derivative^{3,4)} demonstrated that this component was identical with sphingene,⁴⁾ but not with sphinganine. GLC of the degradative products by periodate-permanganate oxidation³⁾ revealed a peak of C_{10} monocarboxylic acid, indicating that the double bond in original sphingoid was located at C-8. Sphingoid composition analyzed by GLC of fatty aldehydes derived from periodate oxidation of CM, G_1 CM, and G_2 CM is shown in Table II. Major sphingoids in CM were 4-hydroxy-8-sphingene and 4-

TABLE I. COMPOSITION OF FATTY ACIDS IN SPHINGOLIPIDS FROM PEA SEEDS (%)

Fatty acid	Ceramide			Monoglycosyl-ceramide		Diglycosyl-ceramide	
	Normal	2-Hydroxy	2,3-Dihydroxy	Normal	2-Hydroxy	Normal	2-Hydroxy
14:0	2.0	0.1	—	2.2	20.7	3.9	6.1
16:0	41.7	5.8	—	39.6	38.8	33.3	21.0
18:0	18.9	14.8	—	24.3	1.3	13.7	7.7
18:1	13.3	—	—	5.5	—	1.7	—
20:0	2.1	2.9	—	5.6	1.5	3.3	3.6
21:0	2.8	0.8	<0.1	1.3	0.7	4.6	1.0
22:0	7.7	19.1	5.5	6.6	19.1	8.6	28.1
23:0	2.1	9.1	9.4	2.2	2.0	8.1	6.6
24:0	7.5	41.8	55.4	7.8	9.5	9.1	25.0
25:0	0.4	4.0	20.9	0.7	<0.1	1.8	0.4
26:0	0.3	1.2	8.8	0.6	<0.1	1.3	0.2
Others	1.2	0.4	<0.1	3.6	1.2	10.6	0.3
*	7	90	3	2	98	—	—

* % of each type of fatty acid for total fatty acid in the respective lipid.

TABLE II. COMPOSITION OF SPHINGOIDS IN SPHINGOLIPIDS FROM PEA SEEDS (%)

Sphingoid	Ceramide	Monoglycosyl-ceramide	Diglycosyl-ceramide
Sphinganine	3.1	1.8	2.0
<i>trans</i> -4-Sphingenine	—	0.4	0.6
<i>trans</i> & <i>cis</i> -8-Sphingenine	1.6	51.1	30.8
<i>trans</i> -4, <i>trans</i> -8-Sphingadienine	—	9.6	12.6
<i>trans</i> -4, <i>cis</i> -8-Sphingadienine	—	0.6	1.3
4-Hydroxysphinganine	35.1	0.8	12.2
4-Hydroxy- <i>trans</i> & <i>cis</i> -8-sphingenine	60.2	35.7	40.5

TABLE III. COMPOSITION OF 2-HYDROXY FATTY ACIDS IN MONOGLYCOSYLCERAMIDE SPECIES FROM PEA SEEDS (%)

2-Hydroxy fatty acid	Dihydroxy base-containing species	Trihydroxy base-containing species
14:0	21.9	9.8
16:0	77.8	31.0
18:0	0.1	2.1
20:0	0.1	2.8
22:0	0.1	34.3
23:0	—	2.1
24:0	<0.1	15.6
Others	0.2	2.3

hydroxysphinganine, whereas those in G₁CM and G₂CM were 8-sphingenine and 4-hydroxy-8-sphingenine. The pattern was somewhat different from those in soybeans,³⁾ wheat grains,⁴⁾ Azuki beans⁵⁾ and mung beans.⁷⁾ Resolution of *cis* and *trans* isomers in 8-sphingenine and 4-hydroxy-8-sphingenine was not done because of the small amount.

G₁CM was fractionated into di- and trihydroxy sphingoid-containing species using TLC^{8,9)} and examined for the respective composition of the fatty acid constituents (Table III). Dihydroxy base-containing species were mainly C₁₆ and C₁₄ acids, and the trihydroxy base-containing species were C₂₂ and C₂₄ acids besides the above two. Maybe dihydroxy sphingoid has a tendency to combine with the shorter chain hydroxy fatty acids and trihydroxy sphingoid with the longer chain ones.³⁾

The component sugar of G₁CM was predominantly glucose (99%), and that of G₂CM was mainly glucose (83%), mannose (12%), and galactose (5%). Although glycosidic linkages could not be analyzed because we had

only a small amount of the compound, the main structure of the sugar moiety was assumed to be Glc(β 1 $\rightarrow$ 1')- in G₁CM and Glc(β 1 $\rightarrow$ 4)-Glc(β 1 $\rightarrow$ 1')- in G₂CM judging from information in the literature.^{4,6,10)}

From the results in the present experiments, the representative molecular species of sphingolipids in pea seeds are *N*-2'-hydroxylignoceroyl-4-hydroxy-8-sphingenine for CM, 1-*O*-glucosyl-*N*-2'-hydroxypalmitoyl-8-sphingenine for G₁CM, and 1-*O*-diglucosyl-*N*-2'-hydroxybehenyl-4-hydroxy-8-sphingenine for G₂CM.

Acknowledgment. This research was supported in part by a Grant-in-Aid for Scientific Research in 1982-83 from the Ministry of Education, Science and Culture of Japan.

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