Occurrence of Free Base of Phytosphingosine in *Candida intermedia* (IFO 0761)[†]

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In the survey for ninhydrin positive lipids in microorganisms, one of the authors has found a new lysine-containing lipid,^{1,2)} the

structure of which was later proved to be an ester of N-acyllysine.³⁾ It had some biological activities.⁴⁾ In order to detect other amino acid-containing lipids, screening test was tried for various microorganisms. A ninhydrin positive lipid was found in one of hydrocarbon-utilizing yeasts, *Candida intermedia* and tentatively named "substance X" in our earlier report⁵⁾ in which lipid composition of *C. intermedia* was analyzed. The substance X was found only when *C. intermedia* was cultured in glucose medium, but not in *n*-paraffin medium, though we had supposed that hydrocarbon might be the more preferable source for lipid synthesis.

The substance X was purely isolated by repeated chromatography on a silicic acid

FIG. 1. IR-Spectra of Phytosphingosine (PhS) from Various Sources (KBr).

[†] Lipid Composition of *Candida intermedia* (IFO 0761). Part II. See reference 5).

column. About 60 mg of the substance X was obtained from 120 g of dried cells of *C.*

intermedia (2~3% of total lipid). It was hardly soluble in chloroform and stable against various treatments with acids, alkalis or other reagents. It melted at 202°C with previous sintering at 60~80°C. IR-Spectrum showed the presence of -OH and/or -NH₂ groups. NMR-Spectrum showed the signal of long methylene chain. N-Benzoyl derivative of the substance X was subjected to oxidation with periodate followed by hydrolysis and produced serine, which showed the presence of the same polar structure (-CHOH-CHOH-CHNH₂-CH₂OH) as phytosphingosine.⁶⁾ From these results the substance X was assumed to be a free base of phytosphingosine. Therefore, comparison of the substance X was made with the authentic sample of free phytosphingosine, which was kindly sent by Dr. K.-A. Karlsson. The melting points and the IR-Spectra (as shown in Fig. 1) of both substances were completely identical. The complete agreement of the IR-absorptions of both samples, especially, in finger print region confirmed us that the substance X must be a free base of phytosphingosine. Phytosphingosine has been known as a skelton structure of many glycolipids of not only plants including yeasts, but also of animal tissues,^{7,8)} however, it has never been found in free form. Among microorganisms, presence of cerebrin⁹⁾ and tetraacetylphytosphingosine¹⁰⁾ has been reported in brewer's yeast and *Hansenula ciferri*, respectively. We prepared another free sample of phytosphingosine by hydrolysis of tetraacetylphytosphingosine of *H. ciferri*. The IR-spectrum of the prepared sample was also identical with those of phytosphingosine sent by Dr. K.-A. Karlsson and of the substance X as shown in Fig. 1. In personal communication Dr. Karlsson wrote that only 3% of the total tetraacetylphytosphingosine was free in *H. ciferri*. In order to check the presence of tetraacetylphytosphingosine or other complex

phytosphingosine in *C. intermedia*, we tried to detect them by thin-layer chromatography in the hydrolysates of lipid extracts of both cells and cultured broth of *C. intermedia*, but none of them was found. Therefore, it was concluded that *C. intermedia* contained only free base of phytosphingosine. Our strain might be a deficient mutant of biosynthetic enzyme of cerebrin or tetraacetylphytosphingosine. Carbon number of the phytosphingosine found in *C. intermedia* is now under investigation.* Complete data on the identification of the substance X will be published elsewhere.

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* Carbon number was recently determined by Gas-liquid chromatography-mass spectrometry. The sample contained C₁₈- and C₂₀-phytosphingosine in the ratio of 9:1.