Presence of Free Bases of C₁₈- and C₂₀-Phytosphingosine in a Yeast, *Candida intermedia* (IFO 0761)

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Phytosphingosine (PhS*) had been thought to be characteristic among plants, but later it was found also among animal tissues.^{1,2)} Many yeasts contain various kinds of sphingolipids,³⁻⁵⁾ but free bases of sphingosine and PhS had never been found in nature. In our survey for ninhydrin positive lipids, PhS was isolated for the first time in the lipid fraction of *Candida intermedia* (IFO 0761).^{6,7)} In this report carbon number was determined by gas-liquid chromatography-mass spectrometry, which also revealed that the PhS was a mixture of C₁₈- and C₂₀- PhS bases in the ratio of 9: 1.

About 60 mg of the PhS was obtained from about 120 g of dried cells of *C. intermedia*.^{6,7)} It was trimethylsilylated⁸⁾ with N,O-bis-(trimethylsilyl)-acetamide, as indicated below; (a) added 1 mg of the sample, 0.2 ml of CH₃CN and 0.2 ml of N,O-bis-(trimethylsilyl)-acetamide in the test tube. (b) Securely capped the tube and placed it in the oil bath. (c) Heated it at 75°C for 30 min, then removed the tube and allowed to cool, then the sample was subjected to gas-mass analysis, which was carried out with the Shimadzu-LKB

9000 instrument equipped with a 0.3 cm × 200 cm glass column packed with 3% OV-1 on chromosorb W, 60~80 mesh.

Operational conditions were as follows; a column temperature of 230°C, an ion source temperature of 290°C, a separator temperature of 270°C, an ionizing electron energy of 70 eV, and accelerator voltage of 3.5 kV. The trimethylsilylated sample of PhS gave two peaks on gas-liquid chromatographic analysis. The areal ratio of them was 9: 1. The main peak was analysed by mass spectrometry as shown in Fig. 1. Mass spectrometric studies of ceramide containing amino di- or tri-ols have been carried out by Hammarström⁹⁾ and other workers.^{10,11)} Based on their results together with ours obtained with the authentic sample, the mass spectra of trimethylsilylated samples were analysed. The molecular peak was absent in Fig. 1, but the molecular weight was indicated by the presence of peaks at *m/e* 590 (M-15) and *m/e* (M-103) formed through loss of a methyl group and -CH₂O-Si(CH₃)₃, respectively. The cleavage between C-2 and C-3 of the long chain base took place producing ions of *m/e* 204, which was the base peak. However, the residual ions of *m/e* 401 was not found, because the presence of the additional trimethylsilyloxy group at C-4 in PhS led to the characteristic cleavage between C-3 and C-4, not observed in spectra of other types of ceramides, for example, sphingosine. The fragment of *m/e* 299 was presumably formed through simple cleavage between C-3 and C-4 with retention of the charge on the oxygen atom at C-4.

From the characteristic peaks described above, we concluded that the main peak must be a free base of C₁₈-PhS.

The minor peak was analysed in the same way and supposed to be a free base of C₂₀-PhS, because all

FIG. 1. Mass Spectrum of the TMS-PhS Obtained from the Main Peak Separated by Gas-liquid Chromatography. The experimental conditions were described in the text. TMS-PhS; trimethylsilylated phytosphingosine.

* Abbreviations used: PhS=phytosphingosine.

FIG. 2. Mass Spectrum of the TMS-PhS Obtained from the Minor Peak Separated by Gas-liquid Chromatography. TMS-PhS; trimethylsilylated phytosphingosine.

the corresponding characteristic ion peaks in Fig. 2 had 28 $(-\text{CH}_2-\text{CH}_2-)$ larger ion peaks than those of C_{18} -PhS. The final conclusion was that the PhS from *C. intermedia* consisted of C_{18} - and C_{20} -PhSs in the ratio of 9:1. PhS has been investigated in detail by Karlsson and his colleagues.^{1,2)} Recently they found that about half of the PhS obtained from spiny dogfish had a branched-chain base.¹²⁾ However, no branched chain was observed in our PhS of *C. intermedia* from its NMR-spectrum (not shown here). Many yeasts contain PhS base as a part of yeast cerebrin.^{3,4)} *Hansenula ciferrii* contains tetraacetyl-PhS,^{5,13)} which was studied extensively,^{14,15)} but only 3% of the tetraacetyl-PhS was free in *H. ciferrii* (K.-A. Karlsson; personal communication). In spite of efforts of many lipid chemists, a free base has never been found in nature. One of the reasons has been believed that the free base is so cytotoxic that it will be promptly converted or detoxicated to non-toxic forms, for example, tetraacetyl-PhS, N-acetylceraamide or other glycolipids etc. Kidder (personal communication) found that the free base had cytotoxic activity against tetrahymena. According to Mizushima (personal communication) 0.03 mg/ml of sphingosine base killed 50% of Ehrlich ascites tumor (1.96×10^6 cells/ml) and it had also haemolytic activity against rat erythrocyte.

C. intermedia might be a deficient mutant of some biosynthetic enzymes of cerebrin or tetraacetyl-PhS etc. Any biosynthetic pathway of PhS base is not yet clear. Stoffel *et al.*¹⁴⁾ and Di Mari *et al.*¹⁶⁾ employed an enzyme system obtained from *H. ciferrii*. For the further study on the biosynthesis of PhS, *C. intermedia* might become useful.

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