Studies on the Conjugated Lipids

Part XV*. Presence of dihydrosphingosine in Sphingomyelin from Horse Spinal Cord

Sir:

It is generally known that sphingomyelin, one of the sphingolipids distributed in animal tissues, contains sphingosine as a fatty base constituent. However, the presence of dihydrosphingosine in the sphingomyelin molecule has not been confirmed definitely, although its presence was suggested by Thannhauser and Boncoddio¹⁾ from iodine number of the component sphingosine base of sphingomyelin from brain and spleen. The present paper describes the isolation and characterization of dihydrosphingosine in sphingomyelin from horse spinal cord.

A pure sample of sphingomyelin (36 g) prepared in this laboratory²⁾ from horse spinal cord was hydrolyzed with 10% methanolic sulfuric acid for eight hours. After removal of fatty acid esters by filtration from the reaction mixture, crude sphingosine was extracted from the filtrate with ether under alkaline condition. Ether having been evaporated, the crude sphingosine was dissolved into methanol and then precipitated as the sulfate with the addition of 1 N ethanolic sulfuric acid. Treatment of the sphingosine sulfate with 100 times volume of methanol gave rise not only to a soluble fraction which consisted of the sulfates of O-methylsphingosine and sphingosine as reported in the litera-

ture³⁾, but also a small amount of methanol insoluble fraction (0.6 g) whose iodine number was found to be 12.7 whereas the iodine number calculated for sphingosine sulfate and dihydrosphingosine sulfate is 72.9 and zero, respectively.

The methanol insoluble fraction (0.5 g) was treated with 0.5 N ethanolic sodium hydroxide to give the free base. Benzoylation of the base with benzoyl chloride in pyridine brought about a brownish gummy precipitate, which was washed with ice-cold ethanol to form a white crystalline material (0.7 g). Repeated recrystallization of the material from acetone and subsequently from methanol yielded a beautiful, needle-like crystal, whose melting point, 143~144°C, and specific rotatory power, $[\alpha]_D^{20} = -28^\circ$ (in chloroform), were practically consonant with those of tribenzoyldihydrosphingosine³⁾.

The tribenzoyl derivative (0.5 g) was once brought back to free base by hydrolysis with 5% methanolic potassium hydroxide. Acetylation of the base with acetic anhydride in pyridine resulted in a white solid (0.35 g), which was repeatedly purified by recrystallization from ethanol to produce a snow-white crystalline powder. The melting point, 100~101°C, and the specific rotation, $[\alpha]_D^{20} = +18^\circ$ (in chloroform) indicated that the product was a pure preparation of triacetyldihydrosphingosine³⁾.

* Part XIV: This Journal, **24**, 692 (1960).

1) S.J. Thannhauser and N.F. Boncoddio, *J. Biol. Chem.*, **172**, 141 (1948).

2) T. Negishi, *Research Bulletin of Obihiro Zootechnical University*, **3**, 10 (1959).

3) H.E. Carter, W.P. Norris, F.J. Glick, G.E. Phillips and R. Harris, *J. Biol. Chem.*, **170**, 269 (1947).

These data safely lead to a conclusion that dihydrosphingosine is one of the component bases in sphingomyelin from horse spinal cord, although a possibility still exists that unsaturated sphingosine is the predominant base in the same substance.

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