Phosphonosphingoglycolipid, a Novel Sphingolipid from the Viscera of *Turbo cornutus*

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This paper reports the occurrence of a new lipid, phosphonosphingoglycolipid, in the viscera tissues of a marine shellfish belonging to Gastropoda, *Turbo cornutus*.

Fresh tissues of the shellfish were divided into viscera and muscle parts. The extraction of lipids from the viscera tissues was performed as described previously. The crude lipids were washed by the Folch's partition method. The lipids recovered in chloroform layer were fractionated by column chromatography on silicic acid using the solvent systems of chloroform-methanol and acetone-methanol. The lipids eluted with chloroform-methanol (3:2, vol/vol) were subjected to mild alkaline hydrolysis, and then rechromatographed on silicic acid by eluting with chloroform-methanol (4:1, vol/vol), acetone-methanol (9:1,vol/vol), chloroform-methanol (3:2, vol/vol) and methanol, successively.

![Fig. 1 TLC of purified phosphonosphingoglycolipid (a), carbohydrate-containing breakdown products obtained from phosphonosphingoglycolipid by degradation with saturated Ba(OH)$_2$ (b), and galactosyl ceramide of ox brain (c) on a silica gel G plate. Development with chloroform-methanol-water (65:25:4, by vol.). Detection with α-naphthol-sulfuric acid.](image)

![Fig. 2 TLC of water-soluble components obtained from phosphonosphingoglycolipid by degradation with 2N-HCl at 100°C for 100 min (a), for 13hr (b), with references: 2-N-methylaminoethylphosphonic acid (c) and galactose (d). Development with n-propanol-conc. ammonia-water (6:3:1, by vol.) on a cellulose plate. Detection with Hanes-Isherwood reagent (A) and AgNO$_3$/NaOH reagent (B).](image)
The lipid obtained in the fraction of chloroform-methanol (3 : 2, vol/vol) showed a guitar-shaped spot positive to the reagent of Dittmer and Lester$^2$ and \( \alpha \)-naphthol-sulfuric acid reagent on TLC (silica gel G) (Fig.-1). IR showed absorption bands expected for sphingolipid containing sugar and phosphonic acid moiety. The analysis of this lipid gave the molar ratio of phosphorus : galactose : fatty acid : long chain base as 1.00 : 1.24 : 0.94 : 1.02$^3$.

Acid hydrolysis of this lipid in 2 N HCl at 100°C for 13 hr yielded galactose and 2-N-methylaminoethylphosphonic acid (MAEP) as water-soluble components. These compounds were identified by TLC on cellulose (Fig.-2) and gas chromatography–mass spectrometry of their trimethylsilyl derivatives$^9$.

Palmitic and 2-hydroxy palmitic acids were major fatty acid components, which may cause the guitar-shaped spot of the lipid on TLC.

Partial alkaline hydrolysis of this lipid with saturated barium hydroxide for 6 hr under refluxing$^4$ liberated cerebroside as the main degradation product (Fig.-1). After purification by silica gel G column chromatography$^5$, the cerebroside was identified as galactosyl ceramide by TLC, IR and GLC of the products obtained on methanolysis. From these results, the skeleton of this lipid have to be galactosyl ceramide.

Partial acidic hydrolysis (2N-HCl, 100°C, 100 min) liberated sugar phosphonate together with MAEP and galactose on cellulose TLC (Fig.-2). This fact shows that MAEP is linked to galactose moiety of cerebroside.

Then, the new lipid is a phosphonic acid derivative of cerebroside, and might be O-[O'-(N-methylaminoethylphosphonyl)-galactosyl] ceramide.

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References
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