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Abstract

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Characterization of glycospingolipids in *Neurospora crassa*.

A family of glycolipids has been identified and partially characterized in mycelia from a wild type strain of Neurospora crassa.

The St. Lawrence 74 strain (IIB stock) was grown for 43 h in Vogel's minimal medium containing sucrose and biotin. The material was washed with water, lyophilized and extracted with 20 ml of chloroform:methanol (2:1;v:v) per gram of dry mycelium (Folch et al., 1957, J. Biol Chem. 226: 497). The mixture was then partitioned with 0.2 vol of 4 mM $MgCl_2$ per ml of extract, and the organic phase thus obtained was evaporated to dryness. Afterwards the material (from about 0.4 g of lyophilized mycelium) was saponified with 0.1 N NaOH in methanol for 30 min at 37°C, dissolved in chloroform and loaded on a column of silicic acid (Unisil, Clarkson, 1.5 x 14 cm) equilibrated with chloroform.

The column was sequentially washed and eluted with 10 column volumes of the following solvents: chloroform, chlorofom:acetone (l:l;v:v), acetone, acetone:methanol (1:1;v:v) and methanol.

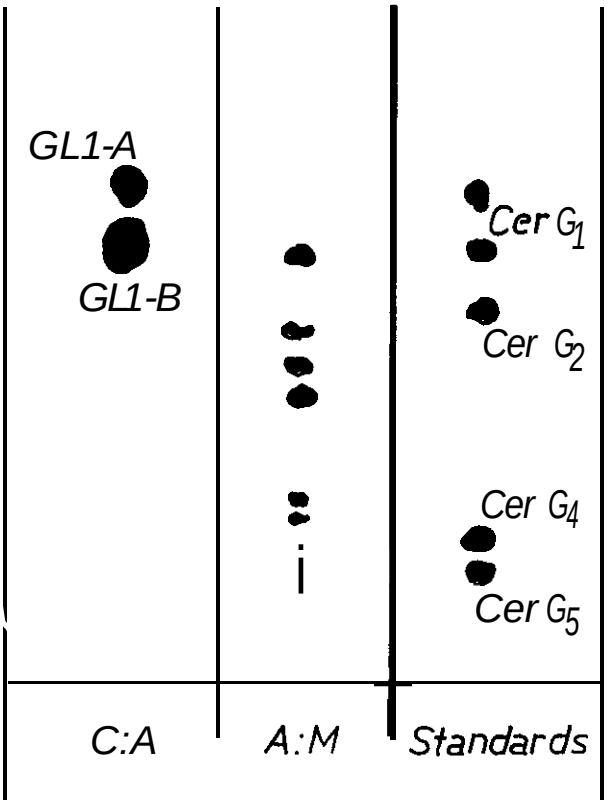


Figure 1. Thin layer chromatography (Silica gel G, Merck) of lipids in fractions C:A and A:M from a silicic acid column. The solvent was chloroform:methanol:water (65:25:4,v:v:v). The plate was stained with the orcinol reagent. $CerG_1$, $CerG_2$, $CerGr_4$, and $CerG_5$, standards of mono, di, tetra and pentahexosyl ceramide (Supelco).

Glycolipids were found in fractions eluting with chloroform:acetone (C:A) and acetone: methanol (A:M). Figure 1 illustrates a thin layer chromatography (TLC) of such fractionsl after staining with the orcinol reagent specific for sugars (Svennnerholm 1965, J. Neurochem. 1: 42). As shown, a glycolipid family having chromatographic mabilities between mono- and pentahexosyl ceramides has been identified.

Further characterization was performed on compounds termed GLI-A and GL1-B in Figure 1. These glycolipids were purified by preparative TLC. Methanalvsis of these comoounds was done with 0.75 N HCl in methanol for 18 h at 80°C. Fatty acid methyl esters were extracted, five times, with hexane, and sphingosines were precipitated from the aqueous phase by adjusting the pH to 10 with ammonium hydroxide. Methyl glycosides remaining in the supernate, were subjected to silylation according to Clamp et al. (1971 Meth. Biochem. Analysis, 19: 233). Gas-liquid chromatography (GLC) of the silyl derivatives was performed on 3% methyl-silicone (SE-30; Supelco) an Anakron ABS with N2 as carrier (30 ml/min; FID selector). A gradient temperature was programmed (4°C/min.) from 140°C to 250°C. Under these conditions the presence of α and β methylglucosides was established.

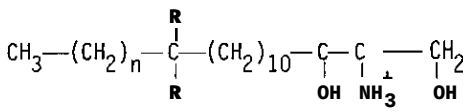
GLC coupled to mass spectrometry (MS) of fatty acid methylesters was performed on 15% diethylglycolsuccinate on Chromosorb W-AW-DMCS, using He as carrier (20 ml/min; FID detector) with a temperature gradient of lO°C/min from 70 to 200°C. The ion source of the spectrometer (Varian CH-7A) was 200°C (70 eV, 1 mA emission). whereas the detector was also at 200°C (19 eV: 0.2 mA emission). Palmitic (35%) stearic (41%) and oleic (24%) acids were identified in GL1-A, whereas 2-hydroxystearic (43%), 2-hydroxyoleic (15%), palmitic (23%) stearic (ll%), and oleic (8%) acids were identified in GL1-B.

Tetra (trimethylsilyl) derivatives of sphingosines were obtained by incubation at room temperature for 20 h with N,O-bis-(trimethylsilyl-trifluoroacetamide) and trimethylchlorosilane. Mixtures were resolved by GLC coupled to mass spectrometry on 2% methylsilicone (OV-101; Supelco) on Chromosorb W-AW-DMCS using He as carrier (12 ml/min; FID detector) with a temperature gradient of lO°C/min from 90 to 280°C. ion source was programmed at 200°C (70 eV, 1 mA emission) and the dectror was at 200°C (22 eV, 0.6 mA emission). Spectra obtained indicated the presence of dihydrosphingosines with structures tentatively assigned in Table 1.

From this evidence it may be concluded that Neurospora mycelium contains a heterogeneous family of monoglucosylceramides having dihydrosphingosines of variable chain lengths and hydroxylated and non-hydroxylated fatty acids. The existence in Neurospora of a

TABLE 1

Tentative structures of Neurospora long chain bases of GL1-A and GL1-B



Glycolipid	Carbon	n	R
GL1-A	I7	2	H
	18	3	H
	20	5	H
GL1-B	21	6	H
	22	7	H
	24	7	CH_3

tetrahexosylceramide have been previously reported by Lester et al. (1947, J. Biol. Chem. 249: 3388). Structure of this compound was established only on basis of GLC or TLC chromatographic mobilities. Other evidences (Kushwaha, et al,. 1976, Lipids, 11: 778) denied the existence of glycolipids in Neurospora mycelium. - - - - Institute de Investigaciones Bioquimicas "Fundacion Campomar" and Departamento de Quimica Organica, Facultad de Ciencias Exactas y Naturales. Obligado 2490, 1428 Buenos Aires, Argentina.