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Abstract

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CHEMISTRY

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THIN-LAYER CHROMATOGRAPHY OF SPHINGOSINE DERIVATIVES

The great difficulties associated with the isolation, individualization, and identification of various natural sphingolipids are well known. One of the main reasons for these difficulties is the absence of suitable chromatographic methods that would make it possible rapidly and clearly to determine individual representatives of the sphingolipids, as well as the products of their partial degradation. This applies fully to the corresponding higher amino alcohols—sphingosine, its analogs, and derivatives. The development of a suitable analytical chromatographic method for sphingosine derivatives is necessary not only for the study of questions concerning the biochemistry of natural sphingosine derivatives, but also for the isolation and individualization of certain new compounds of this type that have recently been found, for example, in plasma sphingolipids ([1]), in the hydrolysate of cytolipin H ([2]), and in other natural objects.

All attempts to apply paper chromatography to these purposes ([3]-[6]) have failed to yield reliable results. This was fully confirmed by our own data as well. In the chromatography of sphingosine and related compounds on paper in various systems, both acidic (with formic or acetic acids) and basic (with ammonia or pyridine), elongated, diffuse spots are formed; moreover, separation of mixtures did not occur, and the values of R_f in all the most varied systems used were always above 0.80. Negative results were also obtained by us in chromatography in a thin layer of cellulose. Somewhat better results are given by the chromatography of N-dinitrophenyl ([7]) and N-succinyl ([8]) derivatives of sphingosine; however, it is very laborious and also not entirely reliable. The best results are given by Sweeley' s method ([1]), based on gas-liquid chromatography of aldehydes obtained by periodate oxidation of sphingosines; however, this method does not possess the necessary specificity, since what is determined is not the sphingosines themselves but the products of their degradation, which may also be formed from impurities of a non-sphingosine type.

In the course of work connected with the study of the cerebroside fraction of

brain and the elucidation of the structure of new cerebrosides ([9]), we encountered the need for a chromatographic method that would make it possible rapidly to determine sphingosine derivatives in very small quantities. For this purpose we applied thin-layer chromatography, which is finding ever broader application in various fields of organic chemistry ([10]) and which we had previously used for the analysis of cerebrosides ([11]). For the chromatography of sphingosine bases and some of their derivatives we used chromatography in a fixed thin layer of silica gel. It turned out that, when suitable solvent systems are used, the method makes it possible clearly to distinguish and separate sphingosine, dihydrosphingosine, their O-methyl ethers, psychosine, and dihydropsychosine (β-D-galactosides of sphingosine and dihydrosphingosine). The chromatographic determination of O-methyl ethers and psychosines is of special significance, since these compounds arise as products of complete or partial hydrolysis of sphingolipids and are common components of the complex mixture of bases that is thereby obtained.

Sphingosine was obtained by Carter' s method during hydrolysis of triacetylsphingosine (m.p. 99–100°, $[\alpha]_D^{25}$ 11.9°; Carter et al. [12]: m.p. 101–102°, $[\alpha]_D^{25}$ 11.7°), obtained by acetylation of crude sphingosine isolated from cerebrosides of ox brain.

Dihydrosphingosine was obtained by hydrogenation of sphingosine over platinum oxide and was purified via the *N*-acetyl derivative (m.p. 117–119°, Carter et al. [12]: m.p. 120–123°). The *O*-methyl ether of sphingosine (isomer I) was isolated from a mixture of sphingosine bases obtained by methanolysis of cerebrosides of ox brain according to Carter et al. [13]; the *p*-oxobenzenesulfonate had m.p. 190–191°, and the hydrochloride m.p. 112–114° (Carter et al. [13]: 193–196° and 116–119°, respectively). The *O*-methyl ether of dihydrosphingosine (isomer I) was obtained by hydrogenation over platinum oxide of the preceding compound, m.p. 61–63°, literature data: 67–68° [13]. Psychosine was obtained by partial hydrolysis of cerebron with $Ba(OH)_2$ in aqueous dioxane [14]. Dihydropsychosine was obtained by hydrogenation of psychosine [14].

Chromatography was carried out on glass plates (13 × 18 cm) coated with a layer of KSK silica gel (200–300 mesh), mixed with gypsum and water (6 g silica gel, 0.35 g gypsum, and 17 ml water); after application of the paste, the plates were dried in air for 4–6 h and for 1 h at 105°. Alcoholic solutions (concentration 2–4 mg in 1 ml) of sphingosine bases were applied to the starting line of the plate at a distance of 1 cm from the lower edge and 1.5 cm from one another. Development was carried out by the ascending method, placing the plate almost vertically in a well-sealed chamber with the solvent system. At 18° chromatography lasted about 4 h; during this time the solvent front traveled a distance of about 15 cm. After drying in air for 5 h, the chromatogram was sprayed with a 0.2% solution of ninhydrin in butanol. Sphingosine, dihydrosphingosine, *O*-methyl ether of dihydrosphingosine, psychosine, and dihydropsychosine appeared as purple spots on a white background, whereas the *O*-methyl ether of sphingosine appeared as a bright-yellow spot.

Fig. 1. Separation of a mixture of sphingosine bases in a thin layer of silica gel. 1—sphingosine (10 μg); 2—dihydrosphingosine (20 μg); 3—O-methyl ether of sphingosine (20 μg); 4—O-methyl ether of dihydrosphingosine (20 μg); M—mixture of sphingosine bases. Solvent system A. Development time 4 h; distance traveled by the solvent system, 14 cm

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The spots of sphingosine, its methyl ether, psychosine, and dihydropsychosine appear 10–15 min after spraying, whereas the spots of dihydrosphingosine and its *O*-methyl ether appear only after 40–50 min, which is very important, since it provides an additional possibility for identifying these compounds. The sensitivity of the method is 15 μg for dihydrosphingosine and its *O*-methyl ether and 10 μg for the remaining sphingosine bases. Heating the chromatogram is not recommended, since although spot development is accelerated, their identification becomes more difficult.

For development of chromatograms we used the following solvent systems: A) butanol—ethyl acetate—11% aqueous ammonia (60 : 32 : 8), B) butanol—ethyl acetate—5% aqueous formic acid (75 : 20 : 5), C) chloroform—methanol (60 : 40), and D—chloroform—methanol (40 : 60). All solvent systems were prepared immediately before chromatography; the components were mixed by volume. The data obtained by chromatography in the indicated systems are given in Table 1 and in Fig. 1. They show that the best separation of sphingosine, dihydrosphingosine, and *O*-methyl sphingosine occurs in system A; *O*-methyl ether

Table 1

Values of R_f^* for sphingosine bases in thin-layer chromatography on silica gel

Compounds	Sensitivity, µg	R_f values in solvent systems A	R_f values in solvent systems B	R_f values in solvent systems C	R_f values in solvent systems D
Sphingosine	10	0.52	0.40	0.24–0.41**	0.42–0.32
Dihydrosphingosine	20	0.44	0.39	0.13–0.10**	0.32–0.26
O-methyl ether of sphingosine	10	0.58	0.34	0.32–0.24**	0.55–0.45
O-methyl ether of dihydrosphingosine	20	0.44	0.24	–	–
Psychosine	5	0.06	0.15	0.06	0.20
Dihydropsychosine	5	0.06	0.15	0.06	0.14

* The R_f values given are averages of 5 determinations; discrepancies between the data of individual determinations, as a rule, did not exceed ±10%.
** Elongated spots. The R_f values of the upper and lower boundaries of the spot are given.

dihydrosphingosine in this system moves together with dihydrosphingosine. In system B, sphingosine and dihydrosphingosine are not separated, but good separation of the mixture of dihydrosphingosine and its O-methyl ether is observed. In systems A and B the sphingosine bases form small round spots; in systems C and D all the bases listed give elongated spots and separation of the mixture practically does not occur. The best system for chromatographic identification of psychosine and dihydropsychosine proved to be system D, but even in this system the mixture of psychosine and dihydropsychosine separates poorly. For complete separation of a mixture of four sphingosine bases, two-dimensional thin-layer chromatography on silica gel proved very convenient. The result of such a separation is shown in Fig. 2. Onto the starting point M of a 20 × 20 cm plate a mixture is applied consisting of 10 µg of sphingosine, 20 µg of dihydrosphingosine, and 20 µg each of their methyl ethers. Onto starting points 1, 2, 3, and 4 are applied, respectively, 10 µg of sphingosine, 20 µg of dihydrosphingosine, 20 µg of the O-methyl ether of sphingosine, and 20 µg of the O-methyl ether of dihydrosphingosine. The chromatogram is developed in the first direction in system A, so that the solvent front does not reach the starting point 4 by 1.5 cm, dried overnight in air, and developed in the second direction in system B, after which it is dried for 5 h in air and sprayed with a ninhydrin

solution. In this case the mixture of sphingosine bases is well separated; each compound gives a well-formed spot.

Fig. 2. Two-dimensional chromatography of sphingosine bases in a thin layer of silica gel: 1—sphingosine; 2—dihydrosphingosine; 3—O-methyl ether of sphingosine; 4—O-methyl ether of dihydrosphingosine; *M*—mixture of sphingosine bases (20 µg each); 1st direction—system A, 2nd direction—system B.

The advantages of the method we propose for thin-layer chromatography of sphingosine bases are its speed and the possibility of working with

small quantities of sphingosine derivatives (up to 10 μg), the possibility of identifying natural sphingosine bases without subjecting them to chemical transformations (acylation, dinitrophenylation) and degradation (periodate oxidation), and the possibility of directly investigating hydrolysates of natural lipids. It should be noted that, for the identification of sphingosine bases by the method of Sweeley ([1]), it is necessary first to separate the sphingosine bases from compounds of nonsphingosine nature, which greatly complicates the work.

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Note: Figure translations are in progress. See original paper for figures.