Journal of Chromatography, 85 (1973) 174–176

CHROM. 6876

Note

The detection of cerebrosides on thin-layer chromatograms with an anthrone spray reagent

C. M. VAN GENT, O. J. ROSELEUR and P. VAN DER BIJL
Gaubius Institute Health Research Organisation TNO, 5d Herenstraat, Leyden (The Netherlands)
(Received April 18th, 1973)

Free sugars can be detected on paper or thin-layer chromatograms in many ways[1,2]. In particular, ammoniacal silver nitrate[3,4], which reacts with reducing sugars, silver metal being deposited as a brownish spot at the location of the sugar on the chromatograms, is used extensively. Alternatively, spray reagents are used which depend for their action on the degradation of the reducing sugars by acid to give furfuraldehyde or one of its derivatives, which can be condensed with an aromatic amine or phenol to give characteristically coloured compounds[2,5]. Spray reagents belonging to this class and used for the present study of detecting cerebrosides on thin-layer chromatograms by virtue of their covalently linked sugar moieties were diphenylamine–*p*-anisidine[6], orcinol[7], aniline hydrogen phthalate[8] and anthrone[9].

EXPERIMENTAL

General experimental conditions

The following solvent systems were used for column chromatography or thin-layer chromatography (TLC): (*a*) methanol–chloroform (1:4), (*b*) methanol–chloroform (1:1), (*c*) methanol–chloroform–water (400:100:2), and (*d*) methanol–chloroform–water (25:75:4) (all v/v).

Source and isolation of cerebrosides

A lipid mixture (*ca.* 50 mg), obtained from small portions of human aortae with methanol–chloroform (1:2, v/v), was subjected to the Folch partitioning procedure to remove non-lipid material and gangliosides[10,11] and subsequently applied to a glass column (20 × 1 cm), packed with silica gel (0.7 g, 100–200 mesh, Mallinckrodt, St. Louis, Mo., U.S.A.). The column was eluted with chloroform (15 ml), solvent systems *a* 7 ml, *b* 7 ml and *c* 20 ml, respectively, and the four fractions thus obtained were dried under a steady stream of nitrogen on a water-bath (50–60°) and weighed.

Fraction *i* (40 mg) was shown by TLC in chloroform and subsequent spraying with 10% (v/v) sulphuric acid[12] to consist of neutral lipid material only.

Fraction *ii* (1.5 mg) consisted mainly of cerebrosides and cephalins, trace amounts of phosphatidic acid and phosphodiglycerides also being present. This was shown by a colorimetric sugar assay[13], phosphorus determination[14] and TLC

against standards on two separate plates in solvent system *d*. One of the plates was sprayed with ninhydrin[15], and the other with an ammonium molybdate–hydrazine–sulphate spray[16], in order to identify lipids containing amino-groups and phosphorus, respectively.

Fraction *iii* (0.3 mg), a buffer fraction between fractions *ii* and *iv*, was shown, by the same methods used for fraction *ii*, to contain lecithins and trace amounts of cerebrosides.

Fraction *iv* (8.0 mg) was shown by the methods used for fraction *ii* to consist mainly of sphingomyelins, lecithins and lysolecithins.

TLC of cerebrosides of fraction (ii)

TLC plates were prepared by immersing microscope slides (76×26 mm) in a silica gel (Camag Type D-O)–chloroform suspension (1:2, w/v). After air-drying, no prior heat-activation being required, the plates were ready for use. A small portion of fraction *ii* in chloroform was applied to a plate in a narrow band *ca.* 1 cm from the bottom and the plate developed in solvent system *d*. After development, the plate was air-dried and sprayed with one of the following reagents: diphenylamine–*p*-anisidine[6], orcinol[7], aniline hydrogen phthalate[8] and anthrone. On heating the plate at 100° for *ca.* 10 min, several bands, which were later shown by extraction followed by a colorimetric sugar assay[13] to consist of cerebrosides, became visible. These bands, which differed in colour and intensity, depending on the spray reagent used, disappeared on cooling. The bands reappeared on heating, but this cycle could be performed only a limited number of times owing to charring of the cerebrosides. All of the above spray reagents were tested in this manner.

RESULTS AND DISCUSSION

Most of the spray reagents tested showed a vague colour development, but spraying with a 10% (v/v) sulphuric acid solution, followed by 1% (w/v) anthrone in benzene, was found to give the best results.

Four distinct, green doublet bands were obtained for the cerebrosides from human aortae (Fig. 1). From the amounts of material applied to several plates, the lower detection limit of the method was estimated to be *ca.* 5 μg. Other lipids, such as phosphatidic acid, phosphodiglycerides and cephalins, also present in fraction *ii*, do not interfere.

This method can, however, only be used qualitatively, as the fairly rapid fading of the bands after heating makes quantitative colour intensity measurements, by densitometry for example, unreliable.

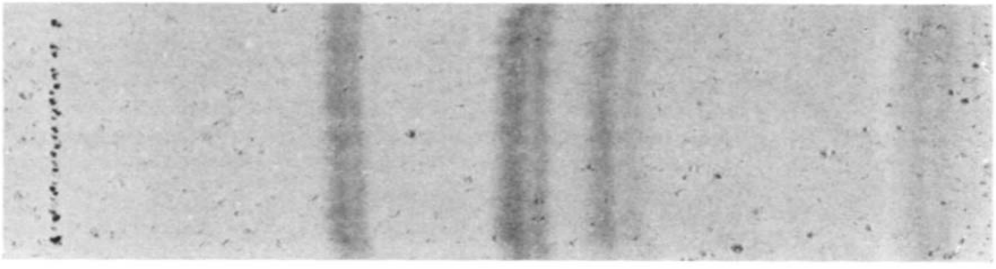

Fig. 1. Thin-layer chromatogram obtained for cerebrosides from human aortae.

REFERENCES

1 W. W. Binkley, *Advan. Carbohyd. Chem.*, 10 (1955) 55.
2 E. Lederer and M. Lederer, *Chromatography*, Elsevier, Amsterdam, London, New York, Lausanne, 1957, p. 253.
3 S. M. Patridge, *Biochem. J.*, 42 (1968) 238.
4 E. F. McFarren, K. Brand and H. R. Ruthkowsky, *Anal. Chem.*, 23 (1951) 1146.
5 W. F. Durham, *Diss. Abstr.*, 19 (1959) 2736.
6 R. W. Baily, *J. Chromatogr.*, 8 (1962) 57.
7 R. Klevstrand and A. Nordal, *Acta Chem. Scand.*, 4 (1950) 1320.
8 S. M. Partridge, *Nature (London)*, 164 (1949) 443.
9 R. Johanson, *Nature (London)*, 172 (1953) 956.
10 J. Folch, M. Lees and G. H. J. Sloane-Stanley, *J. Biol. Chem.*, 226 (1957) 497.
11 C. Entenman, *J. Amer. Oil. Chem. Soc.*, 38 (1961) 534.
12 C. M. van Gent, *Z. Anal. Chem.*, 236 (1968) 344.
13 J. R. Helbert and K. D. Brown, *Anal. Chem.*, 29 (1957) 1464.
14 C. J. F. Böttcher, C. M. van Gent and C. Pries, *Anal. Chim. Acta*, 24 (1961) 203.
15 R. A. Famy, A. Niederwieser, G. Pataki and M. Brenner, *Helv. Chim. Acta*, 44 (1961) 2022.
16 C. M. van Gent, unpublished results.