absolute methanol. The methanol solvent was stripped off in tared round-bottom flasks, on a rotary film evaporator under vacuum with gentle heating by a flow of hot tap water. The weight of each fraction was determined and then it was redissolved in methanol at the rate of 10 mg per ml. Ten microliters of each fraction were spotted onto a 20 cm Brinkmann MN Polygram Sil N-HR/UV_{254} thin-layer chromatography plate 1.5 cm from the bottom and 1 cm apart. A stream of air was blown over the plate to aid evaporation of the solvent and the spots were kept as small as possible. The plate was then developed to within 1 cm of the top of the plate, dried and redeveloped until the isomers were well resolved. Two repetitions were usually sufficient.

When this TLC plate was viewed under ultraviolet light the top cm of the column contained no abscisic acid. The next 5 cm contained 0.25 to 0.30 g of essentially pure crystalline *cis,trans*-abscisic acid, the optically inactive natural isomer. The next 4 cm contained about 0.25 g of *cis,trans*-abscisic acid, which was contaminated with a trace at an estimated 10% of the *trans* isomer. The next 2 cm contained 0.15 to 0.20 g of abscisic acid which appeared to be a 1 to 1 mixture of the isomer. The remaining fractions contained 0.25 to 0.30 g of *trans,trans*-abscisic acid, containing 10% or less of the *cis* isomer. The *cis* isomer after recrystallization from benzene had a melting point of 188°. The *trans* isomer, likewise crystallized from benzene melted at 143°.

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A thin-layer chromatographic separation of cerebrosides and related compounds

As a part of our current studies on cerebroside biosynthesis, a thin-layer chromatography system was desired that would separate a mixture containing cerebrosides and some likely metabolic precursers (*e.g.*, ceramide, psycosine, sphingosine, and fatty acid) from each other. This system could provide a rapid assay for the incorporation of fatty acid or glucose into cerebrosides or into any of the possible intermediates, while separating these products from the radioactive substrates. While numerous thin-layer chromatography systems have been developed that separate one or more of the lipids mentioned above[1–5], a search of the literature revealed no system that

would separate all of these substances on one chromatogram. This note describes a thin-layer chromatography system developed for the desired separations.

Materials and methods

Glass plates (20 × 20 cm) were coated with Silica Gel G (Brinkmann Instruments Inc., Westbury, N.Y.), to a thickness of 0.6 mm using a Desaga-Brinkmann spreader. The plates were air dried and then activated in an oven at 100° for 1 h. The activated plates were stored in a desiccator until used.

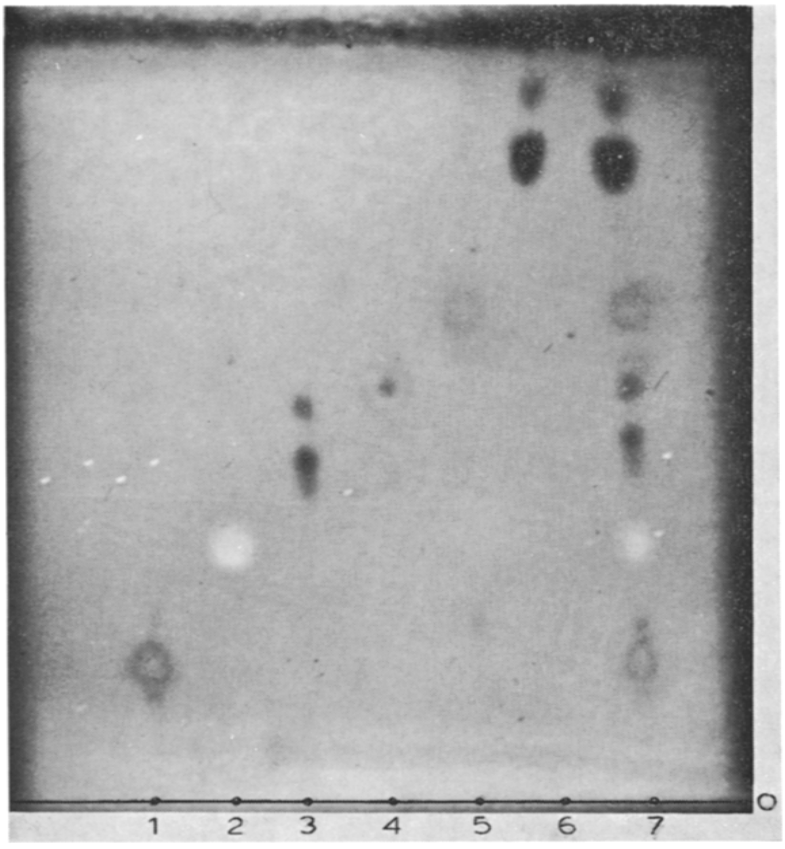

Fig. 1. Picture of a typical chromatogram. From the left the substances are in the same order as listed in Table I. The spots on the right are a mixture of all six substances.

A 10–20 μg sample of each standard was applied to the plate in a small spot. The standard cerebrosides, ceramide, psycosine, and sphingosine were obtained from Applied Science Laboratories (State College, Pa.). Dihydrosphingosine and palmitic acid were purchased from Miles Laboratories (Elkhart, Ind.) and Sigma Chemical Co. (St. Louis, Mo.), respectively. The standards were each applied using a solution of 5 mg/ml in chloroform–methanol (2:1).

Plates were developed in chloroform–methanol–water–conc. ammonia (48:14:1:1). The solvent was equilibrated in the tank for 5 min before use. After development a plate was air dried until no trace of solvent was visible (about 1 min). The plate was then sprayed immediately with a solution of bromthymol blue (0.04 % in 0.01 *N* NaOH). The plate in Fig. 1 was sprayed lightly with the bromthymol blue

solution and dried. This dried plate was then sprayed with sulfuric acid and charred in an oven at 150° for 1 h. When a developed chromatogram was to be counted for radioactivity, it was sprayed only with bromthymol blue.

Results and discussion

The R_F values for the substances studied are presented in Table I. All spots were clearly resolved except for a slight overlap between the faster moving cerebroside spot and dihydrosphingosine. This was not a hindrance since dihydrosphingosine was not used as a radioactive substrate in our studies and it is found only in minute quantities in animal cerebrosides.

TABLE I

R_F VALUES AND COLORS FOR CEREBROSIDES AND RELATED COMPOUNDS

Developed in chloroform–methanol–water–conc. ammonia (48:14:1:1) and sprayed with bromthymol blue (0.04 % in 0.01 *N* NaOH).

Compound	*Color*	R_F *value*[a]
Psycosine	Blue	0.13–0.20
Stearic acid	Red-brown	0.29–0.36
Cerebrosides	Green	0.41–0.54
Dihydrosphingosine	Blue	0.53–0.57
Sphingosine	Blue	0.59–0.65
Ceramide	Green	0.77–0.92

[a] R_F values indicate the range of the visible spots.

Identification of the lipids was aided by the ammonia remaining on the plate when the plate is sprayed with bromthymol blue. The ammonia gave the lipids their characteristic colors as indicated in Table I, while the background assumed a dark blue color when sprayed. As the plate dried, the characteristic yellow background appeared and the color intensity of the lipid spots faded.

The solvent should be used within one-half hour after being mixed in the developing tank. When a longer period of time elapsed, all tested substances gave higher R_F values and less resolution was obtained between the several spots of cerebrosides and ceramides. A similar effect was observed on very humid days.

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