ACTA CHEMICA SCANDINAVICA 16 (1962) 1282–1290

Short Communications

Isolation of Blood Serum Glycolipids

ELISABET SVENNERHOLM and LARS SVENNERHOLM

Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden

In 1958 we described a method for the quantitative determination of plasma cerebrosides and gave figures for their normal plasma concentration [1]. In the same report partial purification of the glycolipids in blood serum by chromatography on magnesium trisilicate and alumina was described. In this process it had soon been evident that the glycolipids with more complex carbohydrate moities were retained on the column. In the method now to be presented the chromatography is performed on silicic acid and ion exchange cellulose.

Materials and methods. Purification of solvents, specifications of silicic acid and DEAE-cellulose and analytical methods for galactose and sulfate were the same as those described by Svennerholm and Thorin [2]. Alumina (standardized according to Brockman) was manufactured by E. Merck AG., Darmstadt. The finest particles were removed by twice suspending the alumina in methanol and decanting the supernatant after 5 min of settling.

Procedure: Preparation of a total serum lipid extract. Normal human blood, without hemolysis, was centrifuged within half an hour after it had been withdrawn. The serum was poured into four volumes of boiling ethanol and stored under ethanol at +4° until it was used. The sampling of serum proceeded for a year. When 17 l of serum had been collected the ethanol extract was evaporated to a volume of about 2 l. The solvent consisted then of about equal volumes of ethanol and water. 60 g of potassium hydroxide pellets were added under gentle stirring, and the extract was stored at +35° for 16 h. After this time the alkaline hydrolysate was extracted three times with 1 l of light petroleum, b.p. 40–45°. The petroleum extracts were pooled and set aside. Then the hydrolysate was acidified with 4 N hydrochloric acid to pH 4–5 and extracted four times with 1 l of chloroform-methanol(4:1, v/v). These extracts were evaporated to dryness (by adding toluene-ethanol 1:1,v/v, several times), redissolved in distilled chloroform and freed from non-lipid contaminants by filtration through a sintered glass filter.

The petroleum extract (P-extract) and the chloroform extract (C-extract) were tested by paper partition chromatography [3]. The P-extract contained mainly cholesterol but also small amounts of glycolipids and sphingomyelins. In the C-extract free fatty acids predominated, but there were also rather large amounts of sphingomyelins and smaller amounts of glycolipids.

Chromatography on silicic acid. The P-extract was evaporated to dryness and redissolved in ethyl ether and filtered. It was added to the top of a 80 g silicic acid column packed in ethyl ether. The column was eluted with 2 l of ethyl ether. The eluate contained large amounts of cholesterol but no glycolipids and sphingomyelins. Afterwards the column was eluted with methanol and samples of about 25 ml were collected on an automatic fraction collector. In the first 250 ml glycolipids were eluted (glycolipid P) and in the following 250 ml sphingomyelins (sphingomyelin P).

The C-extract was divided into four equal volumes and subjected to chromatography on four identical columns with 80 g of silicic acid prepared in chloroform. After the extract had entered the column, it was eluted according to the following scheme:

Chloroform	800 ml
Chloroform-methanol (9:1, v/v)	400 ml
Chloroform-methanol (2:1, v/v)	400 ml
Methanol	800 ml

The chloroform extract was collected in a single batch. It contained only simple lipids (free fatty acids, chloresterol and glycerides). After the eluting solvent had been shifted from chloroform to chloroform-methanol (9:1, v/v) the eluate was collected on the fraction collector and every second fraction was tested by paper chromatography. With chloroform-methanol (9:1, v/v) only glycolipids and autoxidized lipid material were eluted. The more slow moving glycolipids were not eluted until with chloroform-methanol (2:1, v/v) but they were mixed with some sphingomyelins. The methanol eluate contained mainly sphingomyelins. All tubes of the four runnings containing glycolipids but free from sphingomyelins were pooled (glycolipids K 1—4) as the tubes containing sphingomyelins but no detectable amounts of glycolipids (sphingomyelins K 1—4). The tubes containing both glycolipids and sphingomyelins were combined and rechromatographed on a fifth silicic acid column. Glycolipids K 5 and sphingomyelins K 5 were obtained.

Chromatography on alumina. All fractions containing sphingomyelins (sphingomyelin P and K 1—5) were combined, evaporated to dryness, redissolved in 1 l of chloroform-methanol (1:1, v/v), and filtered. The extract was poured onto a column of 50 g of alumina, and the column was eluted with 1 l of chloroform-methanol (1:1, v/v). The total chloroform-methanol extract contained practically all the serum sphingomyelins in pure form. It was later used for gas chromatographic studies of the fatty acid composition of serum sphingomyelins [4]. Afterwards, the alumina was eluted with 1 l of chloroform-ethanol-water (2:5:2,v/v). As this extract contained glycolipids, it was evaporated to 1/4 of its original volume, dialysed against tap water and evaporated to dryness in a rotating evaporator (glycolipid A).

Rechromatography of the glycolipids on silicic acid. As all the glycolipid fractions still contained a large proportion of simple lipids, they were all combined (glycolipid P, K 1—5 and A). They weighed 2.1 g and the hexose content was less than 10 %. They were rechromatographed on 80 g of silicic acid prepared in chloroform. The glycolipids were dissolved in 200 ml of chloroform and poured onto the column. The column was then eluted with: chloroform 800 ml, chloroform-methanol (4:1, v/v) 800 ml, chloroform-methanol (1:1, v/v) 800 ml, and methanol 400 ml. All the eluates were collected in fractions of 25 ml and every second fraction was tested by paper partition chromatography. The pure chloroform eluate did not contain any glycolipids and was discarded. The glycolipids all appeared in the chloroform-methanol eluates, but as there was a distinct difference in mobility between those of tubes 1—40 and those of tubes 41—60 they were divided in two separate fractions (glycolipid I and II), which were evaporated to a small volume and dialysed against running water for 48 h and lyophilized.

Yields:

Glycolipid I	: 477 mg; galactose	16.5 %
Glycolipid II	: 119 mg; galactose	15.0 %

The glycolipid-hexose content of all the serum was 160 mg, and thus the recovery is somewhat more than 60 %. As about 10 % of glycolipids have been used for the chromatographic analyses, the losses due to the isolation are less than 30 %.

Chromatography on DEAE-cellulose. Glycolipids I and II were then subjected to chromatography on DEAE-cellulose in order to divide the glycolipids into a neutral and an acid fraction. Glycolipid I was dissolved in chloroform-methanol (2:1, v/v), poured on a 10 g DEAE cellulose column and eluted as described by Svennerholm and Thorin [2] with 250 ml of (*a*) chloroform-methanol (2:1, v/v), (*b*) same solvent + acetic acid to 5 %, (*c*) same solvent + 1/10 volume of 5 N lithium chloride in water. The neutral eluate was evaporated to dryness without further treatment (glycolipid I A). The other two were evaporated to 1/4 of their original volume, dialysed against running water for 48 h and evaporated to dryness in a rotating evaporator (glycolipid I B and I C, respectively). Glycolipid II was treated in exactly the same way, but only half the amount of cellulose and solvents were used.

Yields:

Glycolipid I	A :	340 mg; galactose	18.0 %
	II A :	16 mg; galactose	21.4 %
	I B :	22 mg; galactose	5.6 %
	II B :	19 mg; galactose	6.3 %
	I C :	74 mg; galactose	8.7 %
	II C :	57 mg; galactose	12.4 %

With paper partition chromatography and thin-layer chromatography on silicic acid acid it could be shown that the fractions A only contained the neutral glycolipids, and that the fractions B contained kephalin B, deranged, (oxidized?) neutral glycolipids and autoxidized lipid material, probably from polyunsaturated fatty acids. The fractions C contained sulfatides and a still unidentified acid lipid. In fraction I C the molar ratio galactose to sulfate was 1.1 : 1.

Preliminary isolation studies indicate that in the neutral fractions there are mono-, di- and trihexosides and neutral aminoglycolipids.

Glucose is the dominating sugar in the monohexosides (cerebrosides), while galactose and glucose occur in about equal amounts in the other neutral glycolipids.

The authors are indebted for financial support given by *Statens Medicinska Forskningsråd* and *Medicinska Fakulteten vid Göteborgs Universitet.*

1. Svennerholm, E. and Svennerholm, L. *Scand. J. Clin. Lab. Invest.* **10** (1958) 97.
2. Svennerholm, L. and Thorin, H. *J. Lipid Research* (Submitted for publication).
3. Beiss, U. and Armbruster, O. *Z. Naturforsch.* **13B** (1958) 79.
4. Stenhagen, E., Svennerholm, E. and Svennerholm, L. *Unpublished experiments.*

Received May 28, 1962.

Hereditary Variation in Content of Isothiocyanates and Thiooxazolidones in Seeds of Rape and Turnip Rape

L.-Å. APPELQVIST

Chemical Division, Swedish Seed Association, Svalöv, Sweden

The use of rapeseed meal in animal nutrition is limited mainly due to its content of glucosides, which in the presence of water are split up and form isothiocyanates and thiooxazolidones. Rather much work has been performed to find the maximum permitted dosage of such meals to various animal species [1]. As rapeseed meal is available in large quantities in several countries considerable effort has been made, *e.g.* in Canada and Sweden, to apply some industrial detoxification process to the meal and thus increase its nutritional value. Even if it is relatively easy to remove the toxic substances industrially [2], it is at present not economically possible as the price for processed rapeseed meal would be to high. If toasting — dry heating — should be efficient in decreasing the toxicity of the meal such high temperatures must be used (130°C for 24 h or 150°C for 5 h [2]) that protein denaturation most probably occurs. Therefore we started this investigation with the object to find out if the hereditary variation in content of these substances are great enough to form a basis for decreasing their content through plant breeding.

The seed samples investigated were taken from the field trials of the Oil Crop Division at the Swedish Seed Association, except for Duro which was received from the General Seed Company, Svalöv. Thus all samples represent carefully handled seed, grown in Sweden under normal conditions.

The determination of isothiocyanates (I) and thiooxazolidones (II) were made essentially according to Wetter [3,4]. All tabulated figures are based upon defatted, dry meal and represent the mean of two analyses. The detailed procedure used in this investigation will be described in a forthcoming, more comprehensive report.

The results thus obtained with rape and turnip rape are shown in Table 1.

Varietal differences in content of these substances in the summerforms of rape and turnip rape have earlier been investigated by Wetter and Craig [5] using seeds grown in different locations in Canada.

Kreula and Kiesvaara [6] investigated the variation in thiooxazolidone content as well in winter as in summer types of rape and turnip rape most probably grown in Finland. Delaveau [7], using a method not quite comparable to the one used in this investigation, has studied varietal differences in content of isothiocyanates in rape and turnip rape, most likely grown in France.

Compared with the figures of Wetter and Craig our results show much greater varietal deviations and lower mean values for both I and II in summer rape. We have found varieties of summer turnip rape with more I and less II than they found. Their findings that the quotient I/II is about 1 in rape and 3.5 in turnip rape can not be of general validity and are most probably due to the rather close relation of the varieties used by them. We have found variations between 0.7 and 1.4 in I/II for summer rape, 2.9 and about 40 in summer turnip rape. The variation reported for the winter types is considerable. It should also be noted that the amount of II is approximately twice as high in winter rape as in summer rape.

The mean value and the variation in content of II in all four groups investigated are not very different from those reported by Kreula and Kiesvaara.

The great variation in content of thiooxazolidone, from 7.9 mg/g for Matador