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PARTITION CHROMATOGRAPHY OF CYCLITOLS ON ION-EXCHANGE RESINS

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SUMMARY

Partition chromatography in aqueous ethanol of cyclitols on an anion exchanger (SO_4^{2-}) and a cation exchanger (Li^+) showed that cyclohexanepentols are held less firmly than cyclohexanehexols and that the distribution coefficients of O-methylated species are lower than those of unsubstituted species. Of 13 species studied, 12 were easily separated on the lithium resin. The unresolved peak was easily resolved on the sulphate resin. The observation that *myo*-inositol is held much more strongly than monosaccharides is used advantageously in a method for its determination in vegetables.

The solubilities of *myo*-inositol and mannitol in aqueous ethanol were compared with their distribution coefficients at various ethanol concentrations. The results showed that the resins exert large salting-in effects.

INTRODUCTION

In a study of the separation of sugars, alditols and anhydroalditols by partition chromatography in aqueous ethanol on ion-exchange resins, a few experiments with *myo*-inositol were included. It was found that this compound was retained much more strongly than any other compound of similar molecular size studied previously[1]. The purpose of the present work was to study the chromatographic behaviour of this and other cyclitols in more detail.

EXPERIMENTAL

The equipment used for the separations and for recording the chromatographic peaks after treatment of the eluate with periodate and determination of the decrease in absorbance at 230 nm was the same as that used in the previous work[1]. The conditions applied during the chromatographic runs are given in the tables and figure legends. Both resins were of the styrene–divinylbenzene type with 8% cross-linkage. The cation exchanger (Durrum DC-2) was of the sulphonic acid type. The anion exchanger was of the trimethylbenzylammonium type (Aminex A-27).

Determinations of *myo*-inositol in dried peas were carried out after acid hydrolysis. The finely divided sample (0.7 g) was mixed with 20 cm^3 of 42% hydrochloric acid at +2° and heated in an open flask at 80° for 4 h. After evaporation

at 35°, the residue was dissolved in water, filtered, and the solution treated with an anion exchanger (HCO_3^-) and a cation exchanger (H^+). After evaporation, the residue was dissolved in 5 cm^3 of 85% ethanol. Aliquots of this solution were analyzed.

The solubilities of mannitol and *myo*-inositol at 30° were determined at various ethanol concentrations by a batch equilibrium technique. Samples of the solution were withdrawn with a pipette furnished with a filter, and after dilution the concentration was determined in the monitor used for analysis of the eluates. The results obtained after stirring for 1, 2 and 3 days with the ethanol solutions gave consistent results.

The distribution of water and ethanol between the resin and the external solution was determined at 30° by the centrifuge method under conditions similar to those applied previously[2]. In order to obviate errors due to the adhering liquid film, the resin particles (Dowex 1-X8) were larger (about 0.5 mm) than those used in the chromatographic runs (about 30 μm). The resin was purified by treating it with sodium hydroxide and hydrochloric acid before being converted into the sulphate form. Trace amounts of ethanol-soluble material were removed by pre-treatment with ethanol. Finally, the resin was washed with water and air-dried at room temperature. The determinations of ethanol were made automatically by chromic acid oxidation[3].

CHROMATOGRAPHIC SEPARATIONS

From the peak elution volumes determined with single species and with mixtures of several cyclitols at different ethanol concentrations, the volume distribution coefficients, D_v, were calculated[4]. The results given in Table I show that the distribution coefficients increase with increased ethanol concentration and that the order of elution of the cyclitols is independent of the ethanol concentration within the range studied. Similar results have been obtained previously with sugars and alditols. The investigated cyclitols belong to four different groups, which are eluted in the following order on the sulphate column:

(1) di-O-methylcyclohexanehexols (1,3-di-O-methyl-*myo*-inositol: dambonitol);
(2) O-methylcyclohexanehexols (O-methylinositols: quebrachitol, pinitol, sequoyitol);
(3) cyclohexanepentols (quercitols);
(4) cyclohexanehexols (inositols).

This order of elution was expected, because previous studies on alditols and sugars showed that with very few exceptions the distribution coefficients increase with an increased number of hydroxyl groups and are lower for O-methylated species than for unsubstituted compounds with the same number of free hydroxyl groups. In agreement with previous observations with sugars and alditols, most diastereoisomers exhibited large differences in their elution behaviour.

The order of elution of the groups was the same for the lithium resin as for the sulphate resin, with the exception that one of the monomethylated cyclohexanehexols was eluted between the cyclohexanepentols. Again, the individual members of each group exhibited distribution coefficients that differed considerably. With most compounds, the separation factors were more favourable with the lithium resin

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS, D_v, DETERMINED AT 75° ON A CATION-EXCHANGE RESIN (Li^+) AND AN ANION-EXCHANGE RESIN (SO_4^{2-}) AT FIVE DIFFERENT ETHANOL CONCENTRATIONS (60–80%, w/w)

Experiments with alditols and aldoses at 75% ethanol for comparison.

*Compound**	*Cation-exchange resin (Li^+)*					*Anion-exchange resin (SO_4^{2-})*				
	60%	*65%*	*70%*	*75%*	*80%*	*60%*	*65%*	*70%*	*75%*	*80%*
1,3-Di-O-methyl-*myo*-inositol (1)	0.14	0.14	0.26	0.40	0.84	0.05	0.08	0.09	0.15	0.17
1D-3-O-Methyl-*chiro*-inositol (58)	0.38	0.39	0.67	0.90	1.79	0.44	0.60	0.91	1.24	1.79
1L-2-O-Methyl-*chiro*-inositol (40)	0.67	0.67	1.01	1.22	2.58	0.40	0.57	0.78	1.16	1.41
1L-1,3,4/2,5-Cyclohexanepentol (65)	0.67	0.67	1.01	1.38	2.69	0.70	0.95	1.37	1.80	2.60
5-O-Methyl-*myo*-inositol (47)	0.67	0.67	1.18	1.58	3.37	0.53	0.66	0.99	1.35	2.05
1,3,5/2,4-Cyclohexanepentol (66)	0.84	0.89	1.51	2.09	4.33	0.82	1.16	1.71	2.44	3.79
1L-*chiro*-Inositol (37)	1.12	1.29	2.13	3.04	6.13	1.71	2.47	3.57	5.66	9.22
muco-Inositol (100)	1.23	1.40	2.30	3.33	7.31	1.16	1.67	2.51	3.80	5.93
allo-Inositol (79)	1.34	1.51	2.53	3.75	8.60	1.08	1.58	2.38	3.48	5.63
neo-Inositol (34)	1.51	1.79	2.98	4.45	10.2	1.29	1.79	2.68	4.38	7.07
scyllo-Inositol (47)	1.51	1.85	3.03	4.65	10.4	2.17	3.19	4.37	8.25	15.1
myo-Inositol (100)	1.51	1.91	3.09	4.98	11.3	1.84	2.72	3.86	6.84	12.1
epi-Inositol (110)	2.24	2.75	4.76	7.48	15.1	1.33	1.88	2.89	4.48	7.36
Xylose				0.89					2.35	
Glucose				1.27					3.69	
Galactose				1.61					3.28	
Xylitol				1.82					1.45	
Mannitol				2.10					2.85	
Glucitol				2.23					2.38	

* The values in parentheses refer to the detector response (area per μmole) relative to that recorded with *myo*-inositol.

than with the sulphate resin. In agreement with observations made previously with sugars and alditols, it was found that species that exhibited unfavourable separation factors on the sulphate resin were very well separated on the lithium resin. Typical examples are *epi*-inositol and *neo*-inositol, which exhibited a separation factor of 1.02 on the sulphate resin and 1.70 on the lithium resin (75% ethanol).

A chromatogram obtained with a mixture of the 13 cyclitols studied is reproduced in Fig. 1. Quebrachitol, 1,3,4/2,5-cyclohexanepentol and sequoyitol showed some overlapping, but with a longer column these compounds were well separated from each other. Compounds 10 and 11 (*neo*-inositol and *scyllo*-inositol) cannot be completely separated on the applied resin (separation factor 1.05) unless very long columns are used. On the other hand, these compounds are very easily separated on the sulphate resin, on which *scyllo*-inositol is held much more strongly than any of the other cyclitols investigated. On this resin, the separation factor in 75% ethanol is 1.9.

The chromatogram in Fig. 2 refers to an experiment with 10 cyclitols on an anion exchanger (SO_4^{2-}). Quebrachitol and pinitol appeared in the same elution band, whereas the other peaks were well separated.

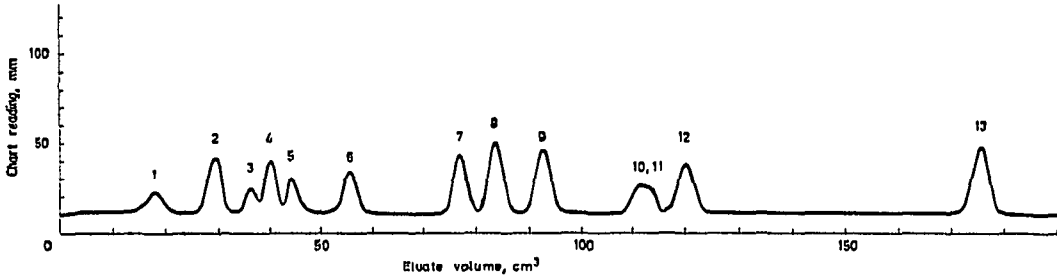

Fig. 1. Separation of various cyclitols (40–60 μg of each) on a lithium resin at 75° in 75% (w/w) ethanol. Resin bed: 720×6.3 mm, Durrum DC-2 (Li^+), 7–14 μm. Nominal linear flow-rate: 1.16 cm/min. (1) 1,3-di-O-methyl-*myo*-inositol (dambonitol); (2) 1D-3-O-methyl-*chiro*-inositol (pinitol); (3) 1L-2-O-methyl-*chiro*-inositol (L-quebrachitol); (4) 1L-1,3,4/2,5-cyclohexanepentol; (5) 5-O-methyl-*myo*-inositol (sequoyitol); (6) 1,3,5/2,4-cyclohexanepentol; (7) 1L-*chiro*-inositol; (8) *muco*-inositol; (9) *allo*-inositol; (10) *neo*-inositol; (11) *scyllo*-inositol; (12) *myo*-inositol; (13) *epi*-inositol.

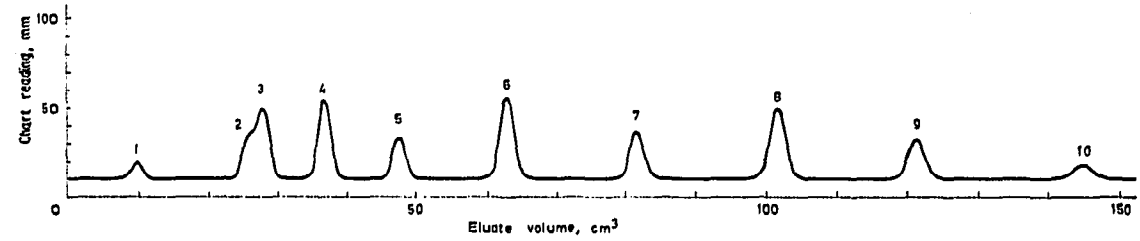

Fig. 2. Separation of various cyclitols on a sulphate resin with 75% (w/w) ethanol at 75°. Resin bed: 1150×4.3 mm, Aminex A-27 (SO_4^{2-}), 12–15 μm. Nominal linear flow-rate: 2.37 cm/min. (1) 1,3-di-O-methyl-*myo*-inositol (dambonitol); (2) 1L-2-O-methyl-*chiro*-inositol (L-quebrachitol); (3) 1D-3-O-methyl-*chiro*-inositol (pinitol); (4) 1,3,4/2,5-cyclohexanepentol; (5) 1,3,5/2,4-cyclohexanepentol; (6) *allo*-inositol; (7) *epi*-inositol; (8) *chiro*-inositol; (9) *myo*-inositol; (10) *scyllo*-inositol.

The above results show that not only *myo*-inositol but also other inositols are sorbed very effectively by the resins in aqueous ethanol. On the sulphate resin, all inositols are held more strongly than the hexitols, but one of the inositols is held less firmly than glucose, which exhibits the highest distribution coefficient of the hexoses. On the lithium column, all hexoses and hexitols are eluted before the inositols.

A chromatogram demonstrating the separation of hexoses and hexitols from *myo*-inositol on a short column is given in Fig. 3. As expected, the hexoses and hexitols could be easily separated as a group on this short column.

The areas of the peaks corresponding to a single cyclitol were directly proportional to the amounts added to the column. In a series of four experiments with *myo*-inositol, in which the amounts applied to the small column varied between 30 and 90 μg, the areas per unit weight of cyclitol exhibited a maximum deviation from the mean of $\pm 1.6\%$. These experiments were made without an internal standard. On the long columns, the reproducibility was somewhat lower, which can be ascribed to variations in flow from the peristaltic pump. The variations can be compensated for by using an internal standard.

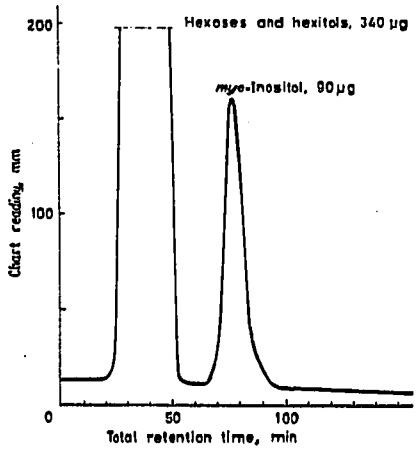

Fig. 3. Separation of *myo*-inositol from a mixture of various hexoses (glucose, galactose and mannose) and hexitols (glucitol and mannitol). Column: 24×6.0 mm, Durrum DC-2 (Li^+), 7–14 µm. Eluent: 85% (w/w) ethanol, 30°. Nominal linear flow-rate: 1.10 cm/min. The total retention time includes the hold-up time in the analyzing system (about 20 min).

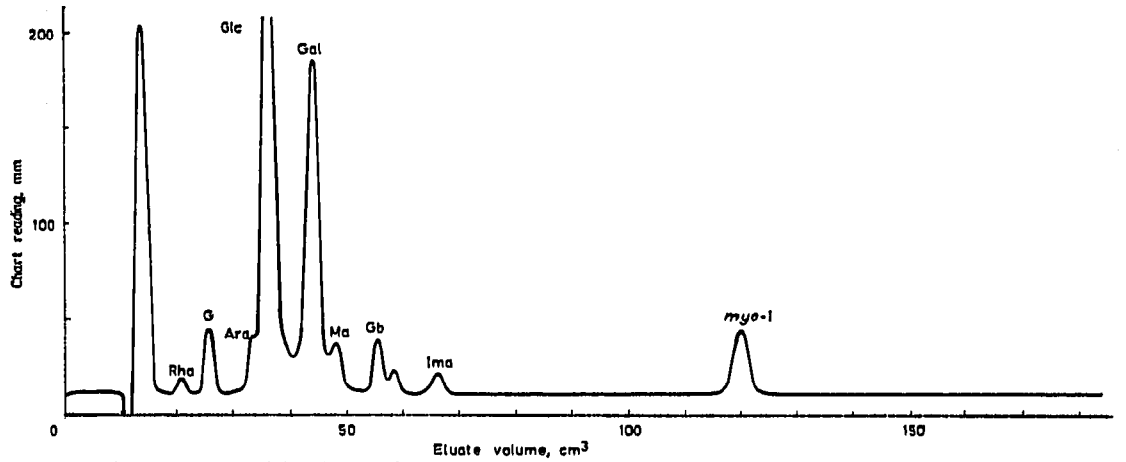

Fig. 4. Chromatographic determination of *myo*-inositol (*myo*-I) in a hydrolyzate of peas on a cation-exchange resin (Li^+). Column: 720×6.3 mm, Durrum DC-2 (Li^+), 7–14 µm. Eluent: 75% (w/w) ethanol, 75°. Nominal linear flow-rate: 1.15 cm/min. Identified sugars: rhamnose (Rha), arabinose (Ara), glucose (Glc), galactose (Gal), maltose (Ma), gentiobiose (Gb) and isomaltose (Ima). (G= glycerol).

It is well known that *myo*-inositol is an important constituent in many materials of biological origin, in which the compound can be present in both the free form and esterified with phosphoric acid. In order to study the possibilities of the suggested separation technique for the determination of *myo*-inositol in biological materials, experiments were made with a solution obtained after acid hydrolysis of dried peas (known to contain the phosphoric ester[5]) and subsequent removal of the mineral acid.

The chromatogram given in Fig. 4 shows that glucose and galactose were the main monosaccharides present. Typical reversion products[6], such as gentiobiose

and isomaltose, were also recorded. These and the other sugars reported in Fig. 4 were identified in separate experiments in which the eluate was analyzed by the orcinol and periodate–formaldehyde methods[6-8]. Both methods showed that the last sugar, isomaltose, was completely eluted at an eluate volume of 70 ml and that on this column a peak that gave a response only in the periodate consumption channel was recorded at 120 ml. The position was the same as that of an authentic sample of *myo*-inositol. Experiments made with a sulphate resin confirmed the presence of *myo*-inositol. The amount recorded in the run on the lithium column was 46.2 μg and that obtained from the sulphate column 43.5 μg, which, calculated on dry weight of the sample, gives the content of *myo*-inositol as 1.7‰. The results show that partition chromatography on ion-exchange resins is a promising method for the determination of cyclitols in biological materials.

COMPARISON BETWEEN OBSERVED AND CALCULATED DISTRIBUTION COEFFICIENTS

As shown previously, the uptake of sugar on to an ion-exchange resin from aqueous ethanol can be related to the fact that the water:ethanol ratio is greater inside the resin than in the external solution. Strongly polar solutes prefer the water-rich phase and therefore accumulate in the resin. As mentioned previously[2], the distribution coefficient can be calculated from solubility data and determinations of the distribution of ethanol and water between the two phases. In the simplest approach, the very crude assumption is made that the resin behaves as an inert solid support. In addition, it is assumed that the activity coefficient of the polar solute is independent of its concentration. Based upon determinations of the ethanol distri-

TABLE II

SOLUBILITIES OF MANNITOL AND *myo*-INOSITOL AND DETERMINATIONS OF THE RESIN SWELLING AND ETHANOL CONCENTRATION IN THE RESIN PHASE AS A FUNCTION OF THE EXTERNAL CONCENTRATION

All determinations at 30°. The resin is not included in the calculation of the mole fraction in the resin phase.

Mole fraction of ethanol in external solution	*Solubility (g per 1000 g of solvent)*		*Ethanol + water after centrifugation (g per g of dry resin)*	*Mole fraction of ethanol in the resin*	*Dry resin (g per cm³ of bed volume)*
	Mannitol	*myo-Inositol*			
0.100	120.3	45.8	0.748	0.0649	0.403
0.200	68.4	17.2	0.712	0.0959	0.407
0.300	41.7	7.25	0.689	0.114	0.410
0.400	24.0	3.20	0.654	0.133	0.420
0 500	12.5	1.36	0.620	0.151	0.425
0.600	6.52	0.540	0.587	0.170	0.444
0.700	3.39	0.195	0.557	0.197	0.459
0.800	–	–	0.490	0.305	0.488
0.900	–	–	0.446	0.506	0.495

bution between the resin phase and aqueous ethanol and solubility measurements of mannitol and *myo*-inositol given in Table II, the weight distribution coefficient was obtained as the ratio between the amount of solute in the ethanol–water mixture held by 1 g of dry resin and the amount of solute in 1 cm^3 of the external solution. The volume distribution coefficients, D_v, were obtained by multiplication by the bed density, *i.e.*, the weight of dry resin per cubic centimetre of bed volume.

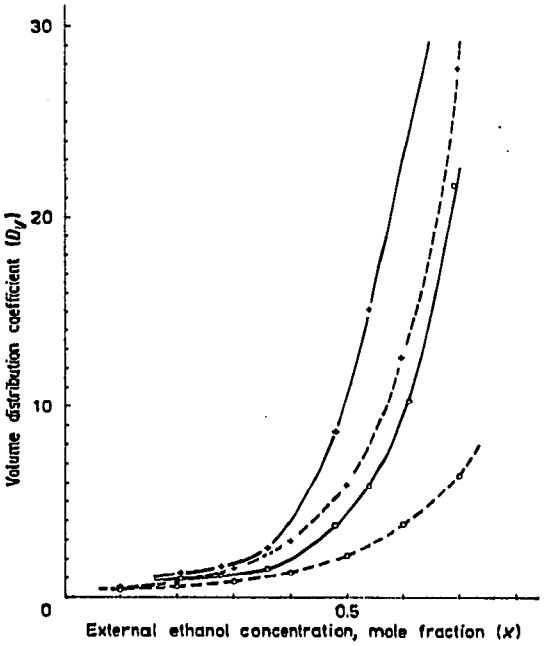

Fig. 5. Observed and calculated volume distribution coefficients, D_v, at 30° as a function of the mole fraction of ethanol in the external solution. Full lines refer to observed values and broken lines to calculated values. ○, Mannitol; +, *myo*-Inositol.

In Fig. 5, the calculated D_v values are given as a function of the ethanol concentration in the external solution, together with the values determined in separate experiments with the same type of resin at the same temperature. As with the observed D_v values, the calculated values increase markedly with increasing ethanol concentration. The values for *myo*-inositol are much higher than those of mannitol, but with both solutes the observed distribution coefficients are much higher than the calculated values, and this holds true for the whole concentration range. The ratio between the observed and calculated values increases with increasing ethanol concentration. Evidently, the salting-in effect of the resin is much more important than the effect of the swelling pressure, which will tend to exclude the solutes from the resin phase[9]. Similar results were obtained in some experiments with glucose reported previously[2].

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