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APPLICATION OF POLYURONIDES AS OPTICALLY ACTIVE ION EXCHANGERS

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SUMMARY

Studies on the chromatographic resolution of the racemic methyl esters of *threo*- and *erythro*-3-amino-2,3-diphenylpropanoic acids, *threo*- and *erythro*-3-amino-1-propanols and the medicinal compound of *threo* configuration ritalin (methyl phenidate) on the polyuronides polygalacturonic (pectic) acid and alginic acid, were carried out.

The resolution of the racemic bases was shown to be possible in the cases being studied. The effectiveness of chromatographic resolution was mainly dependent on the degree of swelling of the polyuronide and the moisture content of the eluent.

A difference was observed in the behaviour of both polyuronides towards the racemic amino bases of *threo* and *erythro* configuration; this difference could help in providing evidence for the *erythro* or *threo* configuration of compounds analogous to those studied in the present work.

INTRODUCTION

The chromatographic resolution of racemic compounds on synthetic optically active ion exchangers was reviewed by Rogozhin and Davankov[1]. These ion exchangers are commonly obtained from synthetic polymers modified by optically active compounds[2,3].

The application of natural optically active ion exchangers to the chromatographic resolution of racemates is of particular interest. The polyuronides appear to be promising compounds in this category. The preliminary results with a detailed description of the method of preparation of the polyuronide and the chromatographic study have been presented in earlier papers[4–7].

This work was carried out with sunflower pectic (polygalacturonic) acid with a 92% polyuronic content and alginic acid with an 83% polyuronic content (Fig. 1). We examined the resolution of racemic amino esters and aminopropanols using column chromatography with organic solvents as eluents. The resolution of the following racemic bases (Fig. 2) was examined: methyl esters of *erythro*- and *threo*-3-amino-2,3-diphenylpropanoic acids (bases 1 and 2), *erythro*- and *threo*-3-amino-1-propanols (bases 3 and 4) and the medicinal compound ritalin [methyl α-phenyl-α-(2-

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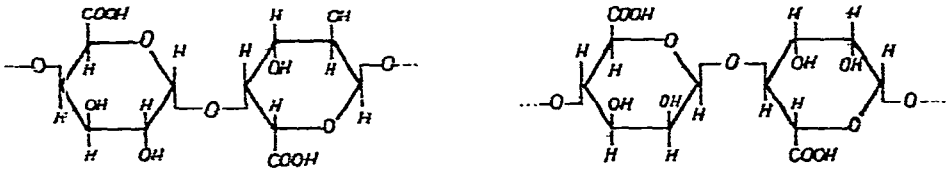


Fig. 1. Structures of ion exchangers.

piperidyl)acetate; methylphenidate][8,9], which is an amino ester of *threo* configuration.

THEORETICAL

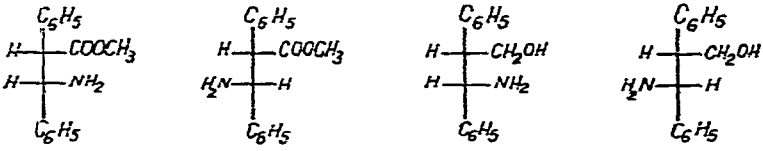
The resolution of a racemic base containing a primary amino group (designated generally as $R_SNH_2 + R_RNH_2$), on a definite polyuronide, the elementary unit of which may be conditionally indicated by RgCOOH, could be effected by the following interaction:

$$B_S + HOOCRg \overset{K_S}{\rightleftharpoons} B_SHOOCRg$$

$$B_R + HOOCRg \overset{K_R}{\rightleftharpoons} B_RHOOCRg$$

The optical resolution of the racemic mixture appears to be feasible under conditions where the equilibrium constants K_S and K_R are sufficiently different from each other.

The influence of various factors such as type and state of the ion exchangers, manner of carrying out the chromatography, rate of elution, concentration of the



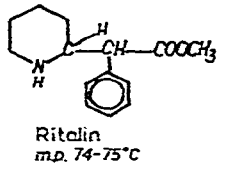


Fig. 2. Structures of racemic bases and ritalin.

racemate in the eluent and type of solvent on the effectiveness of chromatographic resolution was studied.

EFFECTIVENESS OF CHROMATOGRAPHIC RESOLUTION

In order to provide a better comparison of the results obtained in the separate experiments, a quantitative expression of the effectiveness of chromatographic resolution was derived:

$$E_r = \frac{\sum_{i=1}^{n} P_i [\alpha_i]_D^{20}}{[\alpha_0]_D^{20} \sum_{i=1}^{n} P_i} \cdot 100$$

where $[\alpha_i]_D^{20}$ is the absolute value of the specific rotation of a separate fraction, $[\alpha_0]_D^{20}$ is the absolute value of the specific rotation of the pure antipode and P_i is the weight in grams of the same fraction.

For convenience the effectiveness of resolution is expressed as a percentage. The suggested expression, in its physical sense, represents the optical yield for the entire chromatographic process in an average form.

Effect of the degree of swelling of the polyuronide

The polyuronides used are insoluble in water but swell strongly in it. This results in some motion away from each of the polymer chains and permits better exposure of the asymmetric ion-exchange sites. Swollen carbohydrate gels have also been used for the resolution of racemates by other workers: starch columns by Haynes *et al.*[10] and triacetylated cellulose columns by Hesse and Hagel[11].

The effect of this factor using the sunflower pectic acid as the cation exchanger and base 1 (*erythro* ester) was examined at six different degrees of swelling of the polyuronide. With a view to obtaining a desirable degree of swelling, the polyuronide was subjected to a preliminary treatment with an organic solvent (methanol–diethyl ether, 1:1) containing a suitable amount of water. The results are given in Table I and plotted in Fig. 3. It can be seen that the most effective resolution was obtained at a degree of swelling of 23 ml/g. In this instance the chromatogram shows two well shaped peaks for both antipodes.

Effect of moisture content of the solvent

The effect of the hydration of the polyuronide and the moisture content of the eluent was studied using the sunflower polygalacturonic acid as cation exchanger and base 1 (Table II).

Model experiments were carried out on a polyuronide with an optimum degree of swelling (experiments 1–4) and without preliminary swelling (experiment 5). The mixed solvents methanol–diethyl ether (1:1) (experiment 1), absolute methanol–diethyl ether (1:1) (experiments 2 and 4), absolute methanol–diethyl etner (1:1) + 5% water (experiment 3) and absolute methanol–diethyl ether (1:1) + 10% water (experiment 5) were used as eluents. In experiments 2 and 3, after obtaining the desired

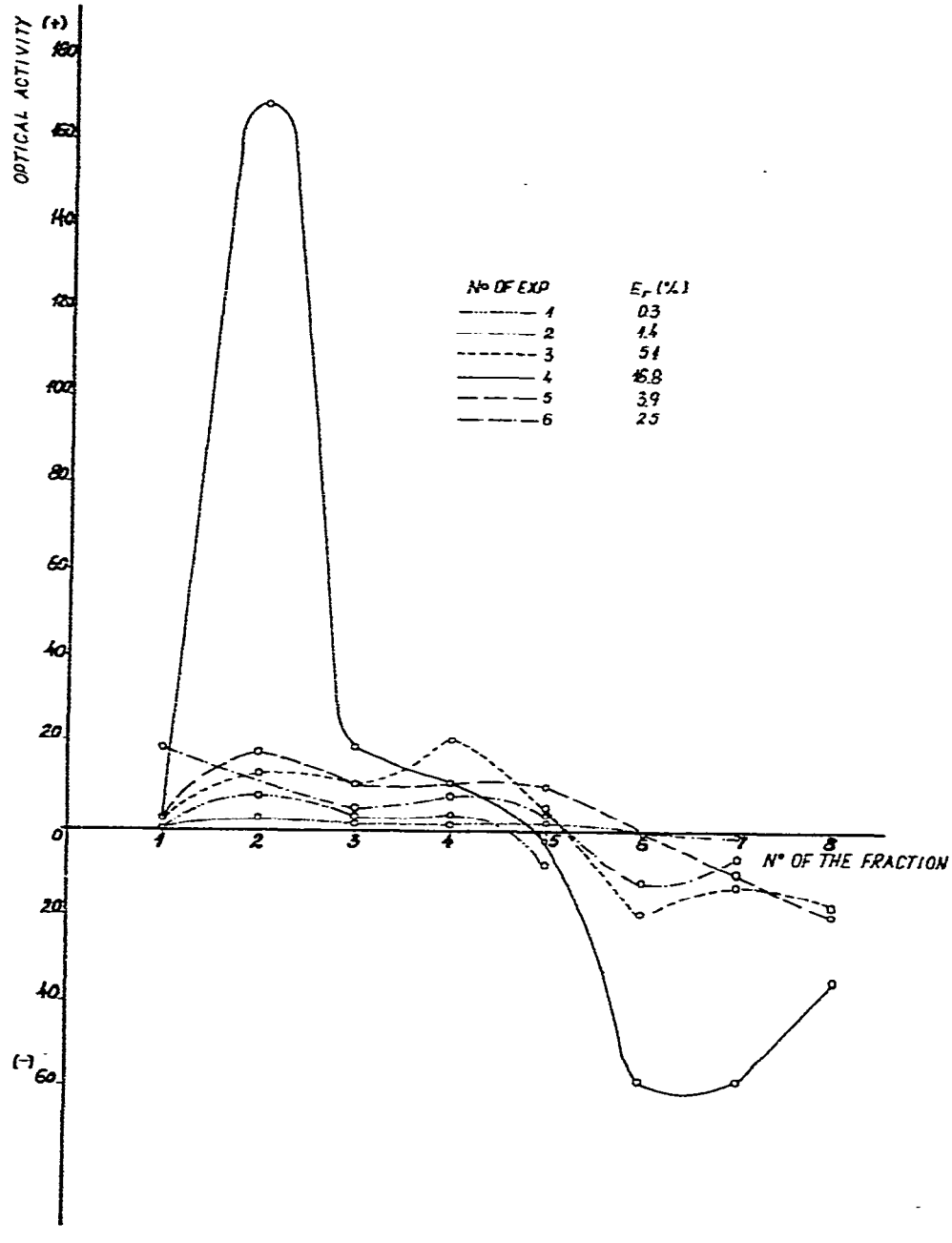


Fig. 3. Effect of degree of swelling of pectic acid on effectiveness of resolution of base I (see Table I).

TABLE I

EFFECT OF DEGREE OF SWELLING OF PECTIC ACID ON EFFECTIVENESS OF RESOLUTION OF BASE I

Fraction No.	*Expt. No.*											
	1		*2*		*3*		*4*		*5*		*6*	
	Degree of swelling (cm^3/g)											
	3.5		*5*		*12*		*23*		*27*		*41*	
	Base (mg)	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$
1	17*	0	4*	0	5*	+3.2	5*	+ 3.1	2*	+1.0	133*	+1.3
2	146*	+0.2	11*	+6.3	20*	+6.4	83*	+20.1	27*	+5.8	98*	+0.6
3	45*	+0.2	19**	+7.3	23*	+5.1	47*	+ 3.7	24*	+6.0	69*	+0.8
4	2*	+0.1	204**	+0.1	35*	+5.8	13*	+ 0.9	24*	+4.5	38*	+1.7
5	4*	+0.1	147**	−0.5	36*	+2.6	21*	+ 0.8	31*	+2.7	13*	+1.4
6	102**	−0.1			123**	−1.4	106**	− 5.6	120**	0	65**	−1.9
7	118**	−0.2			87**	−1.3	89**	− 6.6	81**	−1.2	31**	−2.0
8					92**	−1.9	44**	− 8.3	89**	−2.4		
E_r(%)	0.3		1.4		5.1		16.8		3.9		2.5	

* The volume of each fraction (expts. 1,3–6) was 100 cm^3. In experiment 2 the first fraction was 200 cm^3 and the second fraction 300 cm^3.

** Substance (base) extracted from the column after chromatography was completed.

TABLE II

EFFECT OF HYDRATION OF THE PECTIC ACID AND MOISTURE CONTENT OF THE ELUENT ON EFFECTIVENESS OF CHROMATOGRAPHIC RESOLUTION ON BASE I

Fraction No.	*Expt. No.*									
	1		*2*		*3*		*4*		*5*	
	Base (mg)	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$
1	5*	+ 3.1	60*	+0.9	20*	+ 1.5	9*	+ 1.7	46*	+ 0.4
2	83*	+20.1	41*	+2.5	18*	+ 1.6	37*	+ 6.0	13*	+ 2.9
3	47*	+ 3.7	125**	+3.7	20*	+ 2.5	57*	+ 7.4	15*	+ 3.7
4	13*	+ 0.9	175**	+1.5	27*	+12.8	42*	+14.3	15*	+ 9.7
5	21*	+ 0.8	93**	−1.2	49*	+12.7	36*	+ 2.1	15*	+ 6.7
6	106**	− 5.6			90**	+17.2	39**	0	78**	+15.3
7	89**	− 6.6			174**	− 4.4	118**	− 2.2	120**	− 1.2
8	44**	− 8.3			103**	−12.7	108**	− 4.4	136**	−15.8
E_r(%)	16.8		4.0		19.2		9.3		17.1	

* The volume of each fraction (expts. 1,3–5) was 100 cm^3. In experiment 2 the first fraction was 200 cm^3 and the second fraction 300 cm^3.

** Substance (base) extracted from the column after chromatography was completed.

TABLE III

RESOLUTION OF RACEMIC BASES OF *THREO* AND *ERYTHRO* CONFIGURATION ON POLYGALACTURONIC ACID AND ALGINIC ACID

Acid	*Fraction No.*	*threo-β-Amino-ester*		*threo-β-Amino propanol*		*Ritalin*		*erythro-β-Amino ester*		*erythro-β-Amino-propanol*	
		Base (mg)	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$
Polygalacturonic acid	1	12*	+ 9.3	19*	+2	25*	+3.4	5*	+ 3.1	23*	+1.3
	2	7*	+17.0	5**	+4.3	92*	+4.2	33*	+20.1	5**	+3.0
	3	17**	+26	36**	+4.0	60*	+4.4	47*	+ 3.7	68**	+0.7
	4	76**	+33.2	184**	−0.6	3**	0	13*	+ 0.9	133**	−0.2
	5	160**	− 8.4			2**	0	21*	+ 0.8		
	6	165**	−14.8			235**	−1.8	106**	− 5.6		
	7							89**	− 6.6		
	8							44**	− 3.3		
Alginic acid	1	5*	+ 7.4	11*	0	5*	0	10*	− 3.4	21*	0
	2	33*	+ 9.3	17*	+1	148*	+2.1	5*	−20.0	22*	−4.7
	3	22*	+ 8.3	5**	−5.7	221*	+0.8	25*	−27.7	8**	−4.6
	4	16*	+ 6.7	48**	−0.3	26**	−3.1	34*	−22.9	104**	+0.4
	5	12*	+ 5.5			28**	−4.5	23*	−17.0		
	6	77**	+ 4.2			20**	−3.9	68**	− 2.1		
	7	52**	+ 2.5			12**	−2.0	128**	− 6.1		
	8	103**	− 0.7					114**	+ 9.4		
	9	85**	− 8.1								

* The volume of each fraction was 100 cm^3. In instances when the weight of the base in the fractions was below 5 mg, several fractions were combined.
** Substance (base) extracted from the column after chromatography was completed.

degree of swelling of the polyuronide, a five-fold treatment each with 150 ml of absolute methanol–diethyl ether (1:1) was carried out. This treatment was aimed at extracting and removing the water which hydrated the polymer chains of the polyuronide and its substitution with molecules of the organic solvent.

It can be seen from the data in Table II that the highest resolution was obtained in experiment 3 but the E_r value was similar to those in experiments 1 and 5. This suggests that while both the moisture content in the eluent and the degree of swelling of the polyuronide influence considerably the effectiveness of chromatographic resolution of the base studied, the effect of the first factor is greater.

EFFECT OF THE CONFIGURATION OF THE RACEMIC BASES ON THE COURSE OF CHROMATOGRAPHIC RESOLUTION

Comparative studies were carried out using the two polyuronides pectic (polygalacturonic) acid and alginic acid with racemic bases of *erythro* and *threo* configuration (Fig. 2). The relative configurations of bases 1 and 2 were determined by Kurtev and co-workers[12,13] and the absolute configurations of bases 3 and 4 by Berova and Kurtev[14]. The results, given in Table III, show an interesting difference in the behaviour of both polyuronides towards the *erythro*-β-amino ester (base 1). With the pectic acid the (+)-antipode was eluted first, whereas when alginic acid is used the (−)-antipode was eluted first. This behaviour of both polyuronides was also observed in the chromatography of the racemic *erythro*-β-aminopropanol (base 3). On the other hand, the *threo*-bases 2 and 4 and ritalin (with *threo* configuration) behaved in the same way on both polyuronides with the (+)-antipode eluting first in chromatography.

This interesting behaviour of the two polyuronides could be of assistance in providing evidence for the *erythro* or *threo* configuration of compounds analogous to those studied here.

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