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Chromatographic resolution of racemates on natural optically active ion exchangers

IV. Influence of the solvent on the optical resolution of racemic bases on sunflower pectic acid

In previous papers[1-3], we have shown that pectic acid appears to be a suitable, optically active cation exchanger for the separation of racemic bases by column chromatography. A more detailed study has been made of elution chromatography based on the neutralizing interaction among the molecules of the racemate (R_rNH_2 and R_sNH_2) and the separate unit (R_gCOOH) of the polymeric chain of pectic (polygalacturonic) acid, as a result of which two diastereomeric salts are obtained:

$$R_rNH_2 + R_sNH_2 + 2R_gCOOH \rightleftharpoons R_rNH_3 \cdot OOCR_g + R_sNH_3 \cdot OOCR_g$$

A measurable separation was shown[2] to be feasible provided that the two diastereomeric salts obtained are insoluble in the eluent and that there is a sufficient difference between their equilibrium or dissociation constants. These conditions applied when a mixture of ether and methanol (1:1) was used as the eluent. It was found that the effectiveness of the chromatographic resolution increases with an increase in the degree of swelling of the ion exchanger[3] and depends on the rate of elution[2]. It was also found[3] that the use of this mixed eluent results in better resolution of the antipodes of one of the racemic bases studied. Similar results were obtained in experiments with alginic acid[4]. It was interesting to investigate the extent to which this effect depends on the type of base and on the eluent itself.

The present study was carried out to elucidate the effect of the type of eluent on the effectiveness of chromatographic resolution.

Quantitative expression of the effectiveness of chromatographic resolution

In order to provide a better comparison of the results obtained in the study, we had to derive an objective index to express quantitatively the effectiveness of the chromatographic resolution of racemates. In the literature on ion-exchange chromatography, different indices are used for the objective quantitative determination of the selectivity of a given ion exchanger with respect to a definite pair of ions[4-9]. These indices have not so far been applied to the study of the chromatographic resolution of racemates on optically active ion exchangers, although to a certain extent these investigations are also attempting to find conditions under which the ion exchanger will exhibit selectivity towards one of the antipodes of the racemic mixture. For asymmetric syntheses and the preparative resolution of racemates, the index "stereospecificity" is used as a characteristic parameter of the effectiveness of the action of the asymmetrical reagent[10]. This index cannot be used successfully in the present work as it represents a ratio of one antipode towards the other in a given product of a definite optical purity, $S\phi = (100 + P)/(100 - P)$. Evaluations concerned with the chromatographic resolution of racemates are based on a qualitative discussion of the results of the optical activity of the fractions obtained by chromatography. We have also used the same method to evaluate our results in previous work[1-4]. This approach is not suitable for the purposes of the present investigation.

When chromatographic resolution of a racemate is carried out, a definite number of fractions with different optical activities and different weights is obtained. Consequently, in a mathematical expression of the effectiveness of the chromatographic resolution of a racemate, these components should participate with particular relative weights, in addition to the specific rotation of the pure antipodes. As a result, a total index, E_r, is obtained that gives a quantitative idea of the average separating ability of the ion exchanger under the conditions of chromatography:

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

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

It is evident that the value of E_r will vary between 0 and 100. The maximum value corresponds to a completely resolved racemate, and the zero value to a lack of an optical resolution. Consequently, the E_r value gives, in an average form, the percentage degree of optical resolution in a particular experiment.

Experimental

The experiments were carried out with the methyl esters[11] of (±)-*threo*-3-amino-2,3-diphenylpropanoic acid (base 1) and (±)-*erythro*-3-amino-2,3-diphenylpropanoic acid (base 2). Polygalacturonic acid (2.5 g), obtained[12] from sunflower heads, was introduced into the column (I.D. 18 mm; height 200 mm) after a preliminary swelling in the respective solvent, mixed with water, to the optimum degree of swelling (20 ml/g). The base (0.5 g) was introduced as a 0.5 % solution; the rate of elution was 100 ml/h. The eluates (5 × 100 ml) and adsorbent (3–4 parts) after the resolution were treated in the manner described previously[3]. The successive fractions, each weighing less than 3 mg, were combined into one fraction in order to achieve a more precise determination of the optical activity.

The following solvents were used: methanol, propanol, acetone, ether, chloroform, mixtures of methanol–ether (1:1, 1:2 and 2:1) and a mixture of methanol–chloroform (1:1). The solvents were selected so that liquids of different dielectric constants and different behaviour could be used. We were restricted in our choice by the adopted method as well as by the solubility of the racemic bases used and their salts with the polygalacturonic acid: the solvent had to dissolve well the base but not its polygalacturonate.

The dielectric constants of the methanol–ether and methanol–chloroform mixtures were measured with a laboratory apparatus constructed at the Institute of Biophysics (Higher Medical Institute, Plovdiv) by Dr. P. ATMADJOV, to whom we express our gratitude.

Results and discussion

In Table I are presented the results of the chromatography performed on base 1, and in Table II the results of the experiments with base 2. The results ob-

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TABLE I

CHROMATOGRAPHY ON BASE I

Methanol		*Propanol*		*Acetone*		*Ether*		*Chloroform*		*Methanol–ether (2:1)*		*Methanol–ether (1:1)*		*Methanol–ether (1:2)*		*Methanol–chloroform (1:1)*	
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)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$
7	− 8.9	6	0	248	+1.0	11	+ 2.2	25	+ 4.1	15	+ 3.2	7	+5.7	22	+ 1.1	12	+ 5.5
9	− 4.4	8[a]	0	95	+0.2	51	+15.3	80	+ 7.2	8	+ 8.7	90	+6.7	11	+ 2.9	30[a]	+15.2
14[a]	− 1.5	104[a]	−16	11	0	31	+ 8.6	36	− 1.4	132[a]	+18.3	60	+1.8	70	+17.0	178[a]	−17.2
221[a]	−22	290[a]	+ 5.9	5	0	19	+ 9.1	28	− 4.1	292[a]	− 9.6	41	+1.7	192[a]	+ 2.7	110[a]	−16.2
172[a]	+28			60[a]	−0.9	20	+ 7.1	20[a]	−12.5			30	+2.2	133[a]	− 6.4		
				43[a]	−1.6	97[a]	− 6.7	24[a]	−13.2			22	+1.4				
				34[a]	−2.7	99[a]	− 2.9	16[a]	− 9.0			12	+1.9				
						115[a]	+ 1.9	13[a]	−12.0			112[a]	−2.1				
												77[a]	−2.8				
E_r(%)	12.7		4.6		0.5		3.1		3.7		6.5		1.7		3.5		9.6

[a] Substance (base) extracted from the column after chromatography is completed.

TABLE II

CHROMATOGRAPHY ON BASE 2

Propanol		*Acetone*		*Ether*		*Chloroform*		*Methanol–ether (2:1)*		*Methanol–ether (1:1)*		*Methanol–ether (1:2)*		*Methanol–chloroform (1:1)*	
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)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$
23	+ 8.3	21	+2.4	16	+2.4	14	+ 4.8	15	+1.3	5	+ 3.1	14	+2.6	12	+13.6
55[a]	+10.0	141	+7.5	106	+1.1	147	+ 6.3	8	+6.5	83	+20.1	8	+1.9	20	+12.3
115[a]	0	109	+3.1	33	+2.1	123	+ 0.5	132[a]	+2.5	47	+ 3.7	20[a]	+8.0	76	+ 4.5
91[a]	− 2.9	35	+1.4	17	+3.1	56	− 3.1	270[a]	−1.7	13	+ 0.9	104[a]	+2.4	139	+ 1.1
80[a]	− 6.5	11	0	18	+3.0	31	− 6.3			21	− 0.8	261[a]	−2.2	117[a]	− 4.3
		61[a]	−5.1	8[a]	+0.9	14[a]	− 8.8			106[a]	− 5.6			98[a]	− 5.0
		46[a]	−6.1	26[a]	−0.6	16[a]	− 7.6			89[a]	− 6.6				
		36[a]	−6.1	124[a]	−1.8	12[a]	−10.6			44[a]	− 8.3				
		30[a]	−9.1			10[a]	− 6.2								
E_r(%)	9.5		10.5		3.3		8.7		4.0		16.4		4.9		8.1

[a] Substance (base) extracted from the column after chromatography is completed.

tained indicate that the deduced expression E_r characterizes the effectiveness of the chromatographic optical resolution of a racemate well. Regardless of the fact that the two investigated bases are of identical structure and differ solely in their configurations, the results in Tables I and II show some essential differences in their behaviour towards the solvents investigated. Evidently the effectiveness of the resolution in a given composition of the eluent depends specifically on the configuration of the racemate rather than on its functionality, as suggested by DALGLIESH[13] and KREBS *et al.*[14].

Another important fact is that with the *threo*-base (Table I) in amphiprotic solvents, the (−)-antipode is eluted more rapidly, while in aprotic solvents (ether, chloroform, acetone), the (+)-antipode is eluted first. Another feature of the *threo*-isomer is observed in the experiment with ether: the final fraction has a (+)-rotation like that of the first four fractions. This anomaly cannot be explained by an experimental error, as the experiment was repeated three times and gave the same results each time. Evidently, differences in the rates of interaction and of elution of the two antipodes are involved that ought to lead to the observed conversion. This fact is probably responsible for the relatively low effectiveness of the chromatographic resolution in the same experiment in comparison with those with methanol, the methanol–chloroform mixture or propanol.

TABLE III

DIELECTRIC CONSTANTS AND E_r VALUES

Solvent	*Dielectric constant*	*Base 1*		*Base 2*	
		Effectiveness, E_r (%)	*Yield (%)*	*Effectiveness, E_r (%)*	*Yield (%)*
Methanol	32.6	12.7	85	—	—
Methanol–ether (2:1)	22.7	6.5	91	4.0	85
Acetone	20.7	0.5	99	10.5	98
Propanol	20.1	4.6	82	9.5	73
Methanol–chloroform (1:1)	19.6	9.6	63	8.1	93
Methanol–ether (1:1)	18.5	1.7	91	16.4	82
Methanol–ether (1:2)	15.9	3.5	83	4.9	89
Chloroform	4.8	3.7	49	8.7	84
Ether	4.3	3.1	89	3.3	70

In Table III are given the dielectric constants of the organic eluents used and the values of E_r for the separate experiments. The results show that no strict dependence exists between the dielectric constant of the eluent and the effectiveness of the chromatographic resolution. A certain tendency towards better resolution is observed when amphiprotic solvents are used. It is evident that in the choice of the most suitable eluent, not only the functionality of the racemate is of importance but also its configuration: the separation of the *erythro*-base is accomplished with the highest effectiveness when a methanol–ether (1:1) mixture or acetone is used, whereas the same eluents give a very low degree of separation with the *threo*-isomer.

It must be noted, in conclusion, that in the chromatographic resolution of racemic bases on optically active cation exchangers, the influence of the solvent on

the effectiveness of the chromatography is very complex and depends on the composition of the eluent as well as on the type of racemic base (configuration and functionality).

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