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Note

Chromatographic resolution of racemates on optically active ion exchangers

VI. Partial resolution of racemates on DEAE-Sephadex, QAE-Sephadex and CM-Sephadex

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It has been shown in previous papers[1–5] that the application of polyuronides of various origins to chromatographic resolution of racemic bases by using organic solvents is possible.

It is interesting to examine the behaviour in this type of resolution of artificial ion exchangers on a cellulose or dextran base. Investigations on the application of such ion exchangers for the separation of racemates have not so far been published, although cellulose and neutral Sephadex gels have already been used for partial resolution of racemates[6–8].

EXPERIMENTAL

For our present investigation we used the dextran-type cation exchangers, CM-Sephadex C-25 (capacity, 4.5 ± 0.5 mequiv./g; particle size 40–120 μ) and the anion exchangers, DEAE-Sephadex A-25 (capacity 3.5 ± 0.5 mequiv./g; particle size, 40–120 μ) and QAE-Sephadex A-25 (capacity, 3.0 ± 0.4 mequiv./g; particle size, 40–120 μ), (Pharmacia, Uppsala, Sweden). The methyl ester of (±)-*threo*-3-amino-2,3-diphenylpropanoic acid (base 1) and the methyl ester of (±)-*erythro*-3-amino-2,3-diphenylpropanoic acid (base 2) were used in the experiments with CM-Sephadex C-25 to provide comparative data with that obtained with the polyuronides. (±)-*Threo*-2,3-diphenyl-3-hydroxypropanoic acid was applied during our experiments with the anion exchangers, DEAE-Sephadex A-25 and QAE-Sephadex A-25. The conditions for the experiments are presented in Table I. The operational technique is described in previous publications[1–3].

RESULTS AND DISCUSSION

Experiments with CM-Sephadex C-25

The results given in Table II show that the effectivenes[9] of the chromatographic

TABLE I

EXPERIMENTAL CONDITIONS

Experiments 1, 2 and 3, CM-Sephadex C-25; experiments 4 and 5, DEAE-Sephadex A-25; experiment 6, QAE-Sephadex A-25.

Parameters	*Experiment No.*					
	1	*2*	*3*	*4*	*5*	*6*
Height of column (mm)	55	102	80	130	70	170
Column diameter (mm)	14	12	12	16	12	18
Weight of ion exchanger (g)	5	5.3	3.9	4	2	8
Degree of swelling (ml/g)	1.7	2.3	2.3	7	8	5.3
Form of ion exchanger	Na^+	H^+	H^+	acetate	acetate	acetate
Racemate (g/100 ml)	0.5	0.25	0.25	0.5	0.5	1.0
Solvent	methanol	methanol	methanol	methanol	methanol–diethyl ether (1:1)	methanol
Elution rate (ml/h)	100	100	100	100	120	100
Volume of the fractions (ml)	1–2 × 100 3 × 400	1–2 × 200	1–2 × 200	1 × 900 2 × 450 3 × 150 4–11 × 100	1–6 × 100 7–8 × 150	1–5 × 300

TABLE II

RESOLUTION OF RACEMIC AMINO ESTERS ON CM-SEPHADEX C-25

Experiment No.					
1		*2*		*3*	
Weight of base (mg)	$[\alpha]_D^{20}$	*Weight of base (mg)*	$[\alpha]_D^{20}$	*Weight of base (mg)*	$[\alpha]_D^{20}$
401	− 0.1	9	− 2.4	160	+ 3.0
12	− 2.2	5	−11.2	36	− 8.6
14	− 1.6	120*	− 5.3	11*	− 7.3
70*	+ 1.9	74*	+ 9.0		
E_r (%)	0.8		3.5		8.4
Yield (%)	99		84		82.8

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

resolution (E_r) is too low when cation exchangers in the Na^+ form are used, whereas it increases with elution in the H^+ form.

An interesting fact is observed on comparing the data for resolution of the two diastereomeric bases on the cation exchanger in the H^+ form. With the *threo*-base the (−) stereoisomer is eluted first, while in the experiments with polygalacturonic[2] and alginic[3] acid the (+) antipode is eluted first for the same base. With the *erythro*-base the (+) antipode is eluted initially, as was the case in the experiments with polygalacturic acid. It is notable that with the *threo*-base only a small part (approx. 6%) is eluted and the greater part remains associated on the ion exchanger, while with the *erythro*-isomer the greater part (78%) is eluted. This shows that in methanol the dissociation of the salt formed between the *erythro*-base and the ion exchanger occurs at a considerably higher rate than does the dissociation of the salt resulting from the *threo*-base and the ion exchanger. That difference provides the possibility for effective resolution of both diastereomeric amino esters by chromatography on CM-Sephadex C-25.

Experiments with DEAE-Sephadex A-25 and QAE-Sephadex A-25

The previous experiments on chromatographic resolution of *threo*-2,3-diphenyl-3-hydroxypropanoic acid on the ion exchangers in the Cl^- and OH^- forms gave unsatisfactory results. Some improvement was noted when an ion exchanger in the HCO_3^- form was used. Best resolution was obtained with an ion exchanger in the acetate form. In Table III are given results obtained from this series of investigations.

TABLE III

RESOLUTION OF *threo*-HYDROXY ACID ON DEAE-SEPHADEX A-25 AND QAE-SEPHADEX A-25 IN ACETATE FORM

Experiment No.					
4		*5*		*6*	
Weight of acid (mg)	$[\alpha]_D^{20}$	*Weight of acid (mg)*	$[\alpha]_D^{20}$	*Weight of acid (mg)*	$[\alpha]_D^{20}$
5	0	4	0	16	+5.3
7	+2	66	+2.6	121	+2.7
88	+3.1	82	+1.5	229	−0.8
86	+0.6	61	+0.8	237	−1.3
80	−0.6	41	+1.0	113	−1.3
52	−0.8	21	+1.2	14*	−1.8
37	−1.3	42	−0.6		
43	−1.2	28	0		
40	−1.5	104*	−0.5		
31	−1.9				
20	−2.6				
10*	−0.9				
E_r (%)	2.8		2.1		2.9

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

It is noticeable that, in the experiments with DEAE-Sephadex A-25 and methanol as solvent, over 95% of the chromatographed acid is eluted. Under the same operational conditions, using methanol–diethyl ether (1:1) as solvent, only 70% of the acid is eluted and the rest of it remains in the column. Best resolution is achieved on QAE-Sephadex A-25, but with considerable losses. This increased effectiveness with the more basic anion exchanger shows that with increasing influence of the ion-exchange interaction the chromatographic resolution improves.

CONCLUSIONS

It is evident that the use of dextran-based ion exchangers, like the Sephadex gels, makes possible the resolution of racemic bases (on cation exchangers) and racemic acids (on anion exchangers). Comparing the data from investigations on the chromatographic resolution of racemic bases on polyuronides, it is seen that with Sephadex gel ion exchangers the effectiveness of resolution is markedly lower. This shows that the distance between the asymmetric centre and the reaction centre of the ion exchanger probably influences the effectiveness of chromatographic resolution: with polyuronides they are in close contact, while with dextran based ion exchangers they are separated by 3 or 4 atoms.

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