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Note

Chromatographic resolution of racemates on natural optically active ion exchangers

VIII. Influence of the hydration of the polyuronide and the water content of the eluent on the effectiveness of chromatographic resolution

MARIANA II. POPOVA and CHRISTO G. KRATCHANOV

Department of Organic Chemistry and Biochemistry, Higher Institute of Food Technology, Plovdiv (Bulgaria)

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It has been shown in previous papers[1–3] that in a medium of organic solvents the effectiveness of the chromatographic resolution of racemic bases on natural optically active ion exchangers of the polyuronic type depends considerably upon the degree of swelling. With a view to obtaining a desirable degree of swelling, the polyuronide is subjected to a prelinimary treatment with a selected organic solvent containing a suitable amount of water. After washing with the pure solvent (no water added), chromatography is performed, *i.e.*, the water takes part as a component of solution used only in the preliminary stage (swelling of the ion exchanger). It had not been elucidated whether in the succeeding treatment with organic solvents the hydrating water molecules are replaced by the molecules of the solvent used or whether the water remains in order to hydrate the polymeric chains of the polyuronide. It is known that hydration and dehydration of natural polymers is brought about gradually[4]. On the other hand, having in view the ionic character of the processes proceeding during the chromatographic resolution of racemic bases on optically active cation exchangers, the water, being a strongly polar compound, is expected to exert a significant influence upon the running of the ion exchanger in the organic medium used. In this connection, it was interesting to investigate the influence of hydration of the ion exchanger and the water content of the eluent moisture on the effectiveness of the chromatographic resolution.

EXPERIMENTAL

Model experiments were carried out with sunflower pectic acid, obtained from sunflower heads[5]. The methyl ester of (±)-*erythro*-3-amino-2,3-diphenylpropanoic acid was employed. The experimental conditions are given in Table I. Expriments 1–4 were carried out at the optimal degree of swelling of the ion exchanger[1] achieved by leaving the pectic acid in water–methanol–diethyl ether (1:5:5) for 1 day. Experiment 5 alone was carried out without preliminary swelling of the ion exchanger. In experiments 2 and 3, after the treatment to obtain the optimal degree of swelling, the pectic

TABLE I

EXPERIMENTAL CONDITIONS

Parameter	*Experiment No.*				
	1	*2*	*3*	*4*	*5*
Column diameter (mm)	14.2	18	18	14.2	14.2
Height of column (mm)	120	205	210	140	120
Weight of ion exchanger (g)	0.85	2.17	2.19	1.15	2.0
Degree of swelling (ml/g)	22.4	23.0	23.0	19.0	9.5
Previous treatment of the pectic acid	—	5 times 150 ml of abs. ME (1:1)	5 times 150 ml of abs. ME (1:1)	—	No previous swelling
Racemate (g per 100 ml)	0.5	0.5	0.5	0.5	0.5
Eluent	Usual ME (1:1)	Abs. ME	Abs. ME (1:1) + 5% H_2O	Abs. ME (1:1)	Usual ME + 10% H_2O
Volume of fractions (ml)	100	100	100	100	100

acid was dehydrated by prolongaed treatment (5 days) with a mixture of absolute methanol and absolute diethyl ether (1:1) [abs. ME (1:1) in Table I]. The chromatographic procedure for all samples was performed in the manner described previously[1-3] with a mixture of methanol and diethyl ether (1:1) with different water contents used as the eluent (see Table I).

RESULTS AND DISCUSSION

From the results obtained (Table II) it is evident that the lowest effectiveness of the chromatographic resolution was observed in experiment 2, irrespective of the

TABLE II

INFLUENCE OF HYDRATION OF THE POLYURONIDE AND WATER CONTENT OF THE ELUENT ON THE EFFECTIVENESS OF THE CHROMATOGRAPHIC RESOLUTION ON THE METHYL ESTER OF (±)-*ERYTHRO*-3-AMINO-2,3-DIPHENYLPROPANOIC ACID

Expt. 1		*Expt. 2*		*Expt. 3*		*Expt. 4*		*Expt. 5*	
Base (mg)	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$	*Base (mg)*	$[\alpha]_D^{20}$
5	+ 3.1	60	+0.9	20	+ 1.5	9	+ 1.7	46	+ 0.4
83	+20.1	41	+2.5	18	+ 1.6	37	+ 6.0	13	+ 2.9
47	+ 3.7	125*	+3.7	20	+ 2.5	57	+ 7.4	15	+ 3.7
13	+ 0.9	175*	+1.5	27	+12.8	42	+14.3	15	+ 9.7
21	+ 0.8	93*	−1.2	49	+12.7	36	+ 2.1	15	+ 6.7
106*	− 5.6	494**		90*	+17.2	39*	0.0	78*	+15.3
89*	− 6.6			174*	− 4.4	118*	− 2.2	120*	− 1.2
44*	− 8.3			103*	−12.7	108*	− 4.4	136*	−15.8
408**				491**		446**		438**	
E_r***(%)	17.3		4.0		19.2		9.3		17.1

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

** Total amount of the substance obtained.

*** Total index for the quantitative expression of the effectiveness of chromatographic resolution (see ref. 3).

use of a larger column and a larger amount of the ion exchanger. In this experiment the ion exchanger was subjected to preliminary dehydration and a mixture of absolute methanol and absolute diethyl ether was used as the eluent. A comparison of the results from experiments 1–4 indicates that at the optimal degree of swelling the most important influence upon the chromatographic resolution is the water content of the eluent. In addition, probably in this instance the water is essential for the ion-exchange process taking place. The hydration of the ion exchanger also has an influence (compare the effectiveness of the chromatographic resolutions in experiments 2 and 5), but it is considerably lower than the effect of the water content of the eluent (compare experiments 2 and 3). A comparison of the results from experiments 1 and 5 shows that any preliminary swelling of the ion exchanger is of less importance when the eluent contains sufficient water.

CONCLUSION

The water content of the eluent has been found to exert the greatest influence on the effectiveness of chromatographic resolution. Preliminary hydration and swelling of the ion exchanger has a much smaller influence.

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