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LIQUID CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS OF RACEMIC α AMINOPHOSPHONATES AS THEIR N-3, 5-DINITROBENZOYL DERIVATIVES ON CHIRAL FLUOROCARBINOL STATIONARY PHASES

By

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المتضادات الضوئية لخليط من الفا - أمينو فوسفونات كمشتقات ن - ٣ و٥ - ثنائي نيتروبنزويل بواسطة كروماتوجرافيا السائل على الأعمدة بإستخدام فلوروكربينول كيرالي كوسط ساكن

عادل إبراهيم سليم

في هذا البحث تم فصل المتضادات الضوئية لإسترات الفا - أمينو آرايل (أو الكيل) الفوسفونات بواسطة التحليل الكروماتوجرافي السائل على الأعمدة وذلك بفصلها كمشتقات ن - ٣ و٥ - ثنائي نيترو البنزويل بإستخدام ثلاثة مركبات من فلوروكربينول كيرالي كأوساط ساكنة في عمود الفصل .

Key Words: Chiral stationary phase, α-Aminophosphonate, Fluorocarbinol, Chromatographic separation of enantiomers.

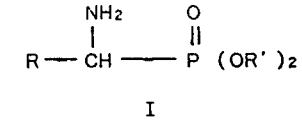
ABSTRACT

The enantiomers of a wide variety of α-aminoaryl (or alkyl) phosphonate esters are separated by liquid chromatography as their N-3,5-dinitrobenzoyl derivatives on three chiral fluorocarbinol stationary phases. In addition the mechanistic interaction involved in the chiral recognition process is described.

INTRODUCTION

Because of the rapidly growing interest in both asymmetric synthesis and the enantiodependent action of medicinal chemicals, the determination of enantiomeric purity of biologically active materials is increasingly important to organic chemists and to medicinal and pharmacological researchers[1]. When applicable, the chromatographic separation of enantiomers on a chiral stationary phase (CSP) is the method of choice for determining the enantiomeric purity[2]. Design and application of chiral stationary phases depend heavily upon an understanding of the interactions involved in the chiral recognition process. An accurate chiral recognition model can be used to predict which enantiomers can be separated on a given chiral column and to assign absolute configurations based upon elution order[3,4].

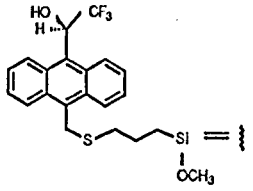
The phosphorous analogues of α-amino phosphonates are being increasingly used in the synthesis of new antibiotics and other biologically active compounds. Although a wealth of information is available on the synthesis of racemic aminophosphonic acids[1], only one compound, the phenylglycine analogue (I, R = Ph, R' = H), has been obtained optically active by synthesis with asymmetric induction[5] or by resolution of the racemate[6]. Glowiak *et al* have determined by X-ray crystallographic analysis the absolute configuration of two aminophosphonic acids analogues of phenylglycine (I, R = Ph, R' = H) and valine (I, R = i-propyl, R' = H)[7].



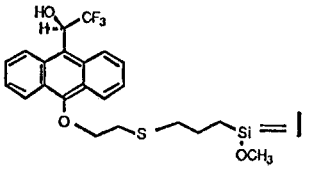
I

In Pirkle laboratory a series of racemic α-aminoaryl (or alkyl) phosphonate derivatives (I) have been synthesised[8] by the reaction of the corresponding aldehyde with lithium bis (trimethylsilyl) amide according to the procedure of Hart et al[9], followed by treatment with trimethyl phosphite.

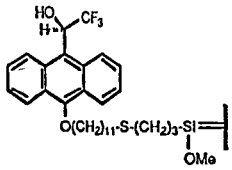
The enantiomeric separation of several alkyl α-aminoaryl (or alkyl) phosphonate as their N-3, 5-dinitrobenzoyl derivatives was described in this text using three chiral fluorocarbinol stationary phases CSP 2, CSP 3[10] and CSP 4[11].



CSP 2



CSP 3



CSP4

In addition a three point interaction model was postulated to describe the chiral recognition process.

EXPERIMENTAL

Apparatus

Chromatography was performed with an Anspec-Bischoff model 2200 HPLC pump, Rheodyne Inc. model 7125 injector with a 20 μL sample loop, Milton Roy (UV Monitor^{TM} D) 254 nm fixed wavelength detector and a Kipp & Zonen model BD 41 chart recorder.

Preparation of 3-Mercaptopropyl Silanized Silica[12]

A 500 ml flask equipped with a Dean-Stark trap and reflux condenser was charged with 15.7 g of 5 μm/300 Å silica gel and 100 ml of benzene. After refluxing for 20 hours, the solvent was removed under reduced pressure and the silica gel was dried *in vacuo* at 80°C for 20 h. 80 ml of 3-mercaptopropyl trimethoxysilane were dissolved in 40 ml of a mixture of benzene/pyridine (1:1) and added dropwise during one hour to a stirred suspension of the above silica gel in 40 ml of a mixture of benzene/pyridine (1:1) while the bath temperature was adjusted at 85°C. After the addition has taken place, the heating under reflux continued for 120 hour. Cooling to room temperature, filtering, washing 5 times with benzene, then 5 times with acetone and two times with diethyl ether and drying under vacuum at 80°C for 20 h to give 16.92 g of 3-mercaptopropyl silanized silica. Elemental analysis; Found: C = 3.78%, H = 0.83%, S = 2.48%. Calculated: 0.79 mmol/g (based on C); 0.82 mmol/g (based on H); 0.77 mmol/g (based on S).

Chiral Stationary Phase 2 [13, 14]:

5.4 g of the above 3-mercaptopropyl silanizd silica were suspended in 30 ml ethanol containing 0.4 g of sodium hydroxide pellets. 3.69 g of enantiomerically pure (IR)-2,2,2-trifluoro-1-[10-(α-bromoethyl)-9-anthryl]ethanol[14] dissolved in 10 ml ethanol were added to the above suspension. Heating under nitrogen was continued for 72 hour with occasional stirring. Cooling to room temperature, filtering, washing three times with methanol, three times with acetone and three times with diethyl ether then drying under vacuum at 80° C for 24 hour gave 5.83 g of the product. Elemental analysis; Found: C = 8.56%, H = 0.94%, F = 2.57 %, S = 1.46 %. Calculated: 0.34 mmol/g (based on C); 0.44 mmol/g (based on H); 0.45 mmol/g (based on F); 0.45 mmol/g (based on S). The modified silica was packed as a methanol slurry into a 250 x 4.6 mm i. d. column using conventional methods.

Chiral Stationary Phase 3 [13, 15]

The above mercaptopropyl silanized silica (5.15 g) and 0.4 g of sodium hydroxide pellets were slurried in 30 ml of ethanol. To the above slurry a solution of 4.25 g of enantiomerically pure (IR)-2,2,2-trifluoro-1-[10-(α-bromoethoxy)-9-anthryl]ethanol[15] in 10 ml ethanol was added and refluxed for 36 hour and worked up as described before to give 5.49 g of modified silica. Elemental analysis; Found: C = 8.35%, H = 0.98 %, F = 1.88 %, S = 1.12 %. Calculated: 0.32 mmol/g (based on C); 0.42 mmol/g (based on H); 0.33 mmol/g (based on F); 0.35 mmol/g (based on S). The modified silica was packed as a methanol slurry into a 250 x 4.6 mm-i.d. column using conventional methods.

Chiral Stationary Phase 4

The synthesis of CSP 4 was described before[11].

Analytes

The various analytes used in this study to compare CSP 2, CSP 3 and CSP 4 were available from prior studies by Pirkle and his coworkers[8].

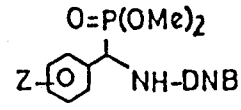
RESULTS AND DISCUSSION

The data in Tables 1 and 2 illustrates the greater enantioselectivity noted for these amino phosphonate derivatives on CSP 4 compared to that noted on CSP 2 and CSP 3. The separation of enantiomeric dimethyl N-3,5-dinitrobenzoyl α-amino phosphonate on chiral fluorocarbinol stationary phases depend most likely on a three point interaction during the chiral recognition process. A postulated mechanistic explanation is described in scheme 1. In this conformation, the amide-ester analytes have two basic centres; the DNB-carbonyl oxygen and the phosphonate oxygen (P = O), with which carbinols of CSP can chelate. These derivatives preferentially populate conformation that places the DNB-carbonyl oxygen near the methine hydrogen (presumably, because of carbinyl hydrogen bonding)[13]. As shown in scheme 1; the diastereomeric solvate, formed between the more strongly retained (R)-aryl-α-amino-phosphonate DNB enantiomer and the (R)-fluorocarbinol CSP, illustrate a three point interaction where the phosphonate oxygen is more basic

than the DNB carbonyl oxygen. The primary hydrogen bonding interaction occurs at the phosphonate oxygen while the second interaction occurs at the DNB carbonyl oxygen. The third interaction appears as π–π interaction between the π-acidic group of DNB and π-basic group (anthryl ring) of CSP.

Table 1

Separation of the enantiomers of dimethyl-N-3,5-dinitrobenzoyl-α-amino substituted benzyl phosphonate on CSP 2, CSP 3 and 4.



CSP/Z	CSP 2		CSP 3		CSP 4	
	α	k'**	α	k'**	α	k'**
H	1.51	8.21	1.71	12.92	1.82	8.77
o-CH_3	1.53	5.97	1.75	9.69	1.85	6.77
m-CH_3	1.62	6.64	1.97	10.0	2.01	7.46
p-CH_3	1.53	6.42	1.78	11.31	1.85	7.62
2,4,6-trimethyl	1.36	5.36	1.64	12.08	1.67	8.31
p-i-prop.	1.62	4.86	1.93	9.23	2.0	6.23
p-Cl	1.33	5.57	1.51	9.77	1.64	6.38
p-NO_2	1.15	13.64	1.23	23.38	1.28	14.15
p-allyl	1.57	7.57	1.72	14.69	1.88	10.0

* Separation factor ($\alpha = \frac{t_2 - t_0}{t_1 - t_0}$)

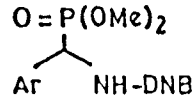
Capacity ratio ($k_1 = \frac{t_1 - t_0}{t_0} = k'$ & $k_2 = \frac{t_2 - t_0}{t_0}$)

where t_1 is the retention time of the first peak.

** 20% 2-propanol/*n*-hexane, 2 ml/min., S-enantiomers is the first eluted one.

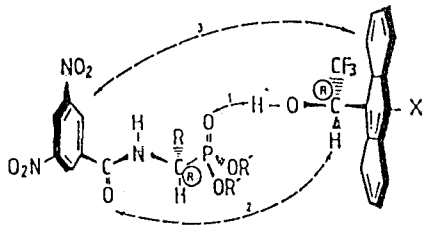
Table 2

Separation of the enantiomers of dimethyl-N-3,5-dinitrobenzoyl-α-amino dry phosphonate on CSP 2, CSP 3, and CSP 4



CSP/Ar	CSP 2		CSP 3		CSP 4	
	α	k'*	α	k'*	α	k'*
Ph	1.51	8.21	1.71	12.92	1.82	8.77
1-naph	1.56	7.21	1.82	10.5	2.09	8.15
2-naph	1.46	8.93	1.65	12.86	1.86	10.25

20% of 2-propanol/*n*-hexane, 2ml/min., S-enantiomer is the first eluted one.



Scheme 1

The three point interaction between the (R)-enantiomer of DNB-substituted aminometylene phosphonates and the (R)-fluorocarbinol stationary phases.

Detailed study of Table 1 and Fig. 1 lead to the following conclusions:

(i) For each CSP; *m*-tolyl- and *p*-isopropylphenyl substituted analytes (scheme 1, R - *m*-tolyl, R' = Me and R = *p*-isopropylphenyl, R' = Me) have the highest degree of recognition (as measured by the α-value) due to the highest (+M) and (+I) effects of these groups which increase the basicity of P = O and thus increase the primary interaction.

(ii) *p*-Nitrophenyl substituted analytes have the lowest α-value followed by chlorophenyl one due to (-I) and (-M) of NO_2 and (-I) of Cl which decrease the basicity of P = O.

(iii) For each analyte the order or degree of recognition was found to be CSP 4> CSP 3> CSP 2. This agrees with our postulation for increasing the π-basic character of anthryl ring through substituents at C_{10}; -O-$(CH_2)_{11}$ of CSP 4 > -O-$(CH_2)_2$ of CSP 3 > -CH_2 of CSP 2 which increase the π–π interaction in CSP **4** with respect to the other CSPs.

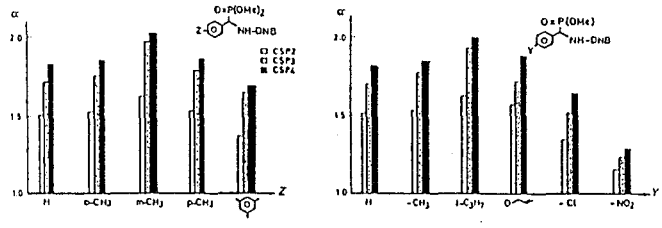


Fig. 1. The effect of substituent on the enantiomeric separation factor of dimethyl-N-3, 5-dinitrobenzoyl substituted α-aminobenzylphosphonate.

Study of Table 2 and Fig. 2 show that the 1-naphthyl substituted analyte has the highest α-value due to the steric effect of 1-naphthyl group (which has more steric bulk than 2-naphthyl and phenyl groups). The three aryl substituted analytes in general have higher α-value due to the steric effect of 1-naphthyl group (which has more steric bulk than 2-naphthyl and phenyl groups). The three aryl substituted analytes in general have higher α-value due to an increase in the basicity of P = O (resonance effect) which enhances the primary interaction.

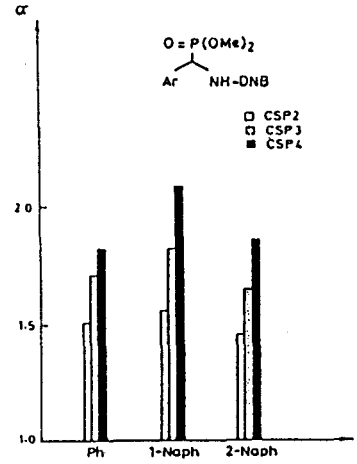
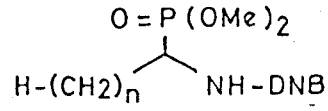


Fig. 2 The effect of aryl type on the separation of the enantiomers of dimethyl N-3,5-dinitrobenzoyl-α-aminoaryl phosphonate.

Table 3

Separation of the enantiomers of dimethyl-N-3,5-dinitrobenzoyl-α-amino substituted benzyl phosphonate on CSP 2, CSP 3 and CSP 4

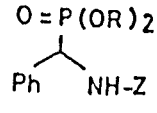


CSP/Ar	CSP 2		CSP 3		CSP 4	
n	α	k'*	α	k'*	α	k'*
2	1.08	6.07	1.13	9.21	1.22	6.5
3	1.13	4.93	1.15	7.87	1.23	5.42
4	1.15	4.29	1.21	6.0	1.27	4.67
5	1.15	3.86	1.23	5.21	1.27	4.17
6	1.16	3.5	1.25	4.93	1.27	3.75
7	1.16	3.14	1.25	4.73	1.27	3.5
8	1.17	2.93	1.26	4.27	1.27	3.08
10	1.2	2.5	1.26	3.53	1.28	2.67

* 20% IPA/hex, 2ml/min., S-enantiomer is the first eluted one.

Table 4

Effect of the type of N-derivative and phosphonate ester on the separation of the enantiomers of dialkyl-α-aminobenzyl phosphonate on CSP 1, CSP 2 and CSP 3



Z		CSP 2		CSP 3		CSP 4	
	R	α	k'*	α	k'*	α	k'*
Benzyl	Me	1.08	4.36	1.23	11.15	1.26	8.92
p-NO_2-benzoyl	Me	1.21	5.71	1.29	8.0	1.37	5.58
DNB	Me	1.51	8.21	1.71	12.92	1.82	8.77
DNB	Et	1.53	5.5	1.84	6.47	1.9	6.15
DNB	i-prop	1.79	2.93	2.0	3.87	2.04	3.69

* 20% IPA/hex, 2ml/min. S-enantiomer is the first eluted one.

Table 3 and Figure 3 illustrate the separation of the enantiomers of dimethyl N-3,5-dinitrobenzoyl-α-aminoalkyl phosphonate on the three chiral fluorocarbinol stationary phases. Study of these data show that these analytes possess a lower degree of recognition (as measured by the α-value) in comparison to that of aryl substituted analytes since alkyl group have lower (+I) while aryl group has higher (+I) and (+M). As the length of alkyl group increases, the α-value is slightly increased; since the increase of alkyl side chain length raises its +I effect by a small amount only; consequently the basicity of P = O will slightly increase. This effect is strong only for methyl and ethyl groups. In addition the capacity factor of the first eluted enantiomer (K') decrease as the length of alkyl group increases due to the increase of its hydrophobic character (and consequently its solubility in the eluent must be increase).

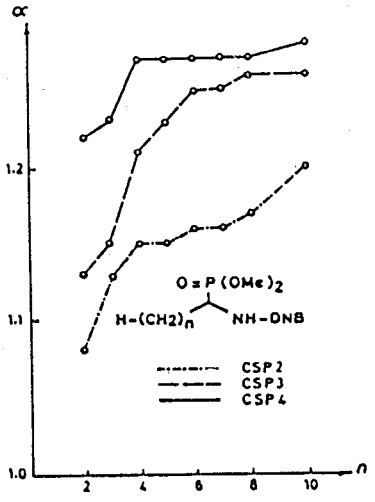


Fig. 3 Separation of the enantiomers of dimethyl N-3,5-dinitrobenzoyl-α-aminoalkyl phosphonate

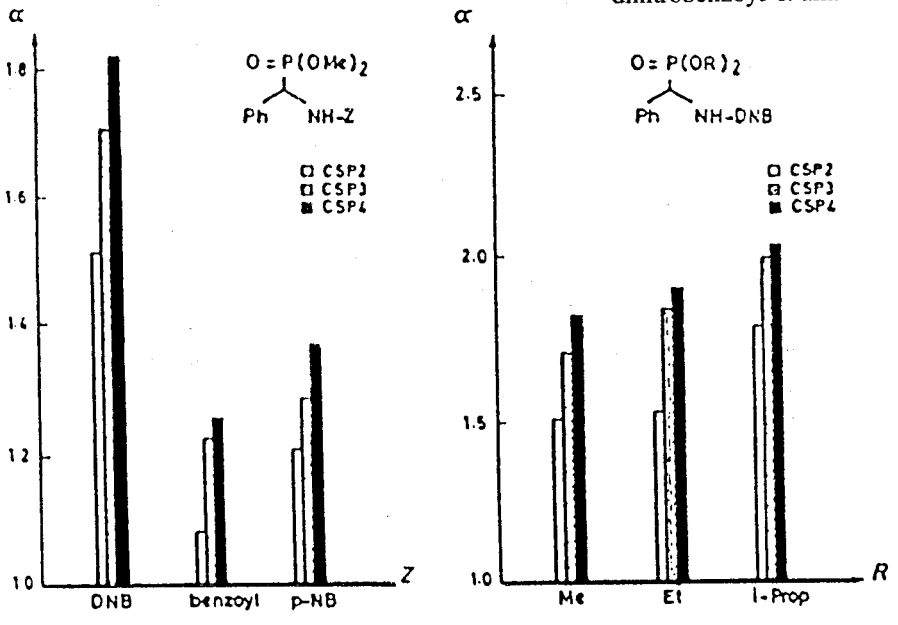


Fig. 4 Effect of the type of N-derivative and phosphonate ester on the separation of the enantiomeris of dialkyl α-amino benzyl phosphonate

Table 4 and Fig. 4 describe the effect of the N-acylated group and the type of phosphonate ester on the enantiomeric separation of racemic dialkyl α-amino benzyl phosphonate derivatives on the three chiral trifluorocarbinol stationary phases. With respect to the type of acylated group the DNB-group is the best one due to an increase in the π-acidic character of its benzene ring which increase the tertiary π–π-interaction, and hence gives higher α-values. In the ester series; iso-propyl-phosphonate esters show higher chiral recognition than ethyl or methyl esters. This phenomenon is attributed to the higher (+I) effect of iso-propyl as compared to ethyl and methyl which increase the basicity of P = O group and enhances the primary interaction.

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