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HPLC With Carbohydrate Carbamate Chiral Phases: Influence of Chiral Phase Structure on Enantioselectivity

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ABSTRACT The enantioselective resolution of *trans*-stilbene oxide and of 23 chiral sulfoxides was investigated on cellulose and amylose tris(arylcarbamate) stationary phases coated on aminopropylated 7 μm spherical silica with 500 Å diameter pores. Cellulose tris-(3,5 dimethylphenylcarbamate) showed good resolving power for many of the sulfoxides and amylose tris-(3,5 dimethoxyphenylcarbamate) showed advantages for the resolution of certain sulfoxides which were not separated on other phases.

KEY WORDS: amylose, cellulose, phenylcarbamate, 3,5-dimethylphenylcarbamate, 3,5-dimethoxyphenylcarbamate, *trans*-stilbene oxide, chiral sulfoxides, resolution

Methods for the direct chromatographic separation of enantiomers are of growing importance, especially for pharmaceuticals and other biologically active compounds.[1,2] In recent years, arylcarbamate derivatives of polysaccharides such as cellulose and amylose, coated onto organosilane-modified silica, have been used successfully as chiral stationary phases for the HPLC resolution of many racemic compounds.[3–9] Chiral recognition is assumed to be due to the formation of interstrand and intrastrand inclusion complexes and binding to the polar carbamate groups, which interact with the solute by hydrogen bonding with NH and C O and by dipole–dipole bonding to the C O moiety.[4] Consequently, the chiral discrimination ability of these phases is influenced by the nature of substituents attached to the phenyl groups in the arylcarbamate functions. Among a wide range of substituted arylcarbamates of cellulose and amylose investigated, the phenyl and 3,5-dimethylphenyl carbamates have generally proved the most successful for the resolution of many classes of racemates, but it has been noted that no simple rules can yet be deduced for predicting the behaviour of such systems and an empirical approach based on experimentation remains necessary.[5]

Compounds containing an unsymmetrically substituted and hence chiral sulfoxide group are of considerable importance in asymmetric synthesis[10] and as drugs.[11] Consequently, methods are required for the rapid, accurate, and sensitive analysis of enantiomeric ratios in compounds where the chirality is due solely to the presence of an asymmetric sulfoxide function. The resolution of sulfoxides by the classical method of formation of diastereoisomers is not easy to perform, because of the difficulty of derivatising the sulfoxide function.

A number of publications have described the successful resolution of sulfoxide enantiomers on chiral HPLC phases. Applications reported have included the analysis of simple (mainly aryl alkyl) sulfoxides[12–16] and of drugs such as albendazole oxide[17,18] and omeprazole[19,20] and also preparative-scale resolution of gram quantities.[15] Chiral phases which have been reported to give good separations include Pirkle phases,[12–15] protein phases[19] and carbohydrate carbamate phases.[5,20]

This paper reports an investigation of the resolution of a series of asymmetric sulfoxides, including diaryl sulfoxides, on arylcarbamate derivatives of cellulose and amylose and explores the influence on resolution of different substituents on the aryl groups.

MATERIALS AND METHODS

Chemicals

Silica (Nucleosil; Camlab, UK) had the following properties: particle size, 7 μm; pore size, 500 Å; pore volume, 0.8 ml/g; surface area, 35 m^2/g.

Racemic mixtures and enantiomers were purchased from Aldrich, UK or were prepared by standards methods. Isocyanates were purchased from Fluorochem UK. Solvents (HPLC grade) were obtained from Rathburn UK.

Equipment

The HPLC system consisted of a Cecil CE-1100 pump, a CE-2012 UV detector operated at 254 nm, Rheodyne 7125 injector fitted with a 20 μl loop, and a Kipp & Zonen BD40 recorder.

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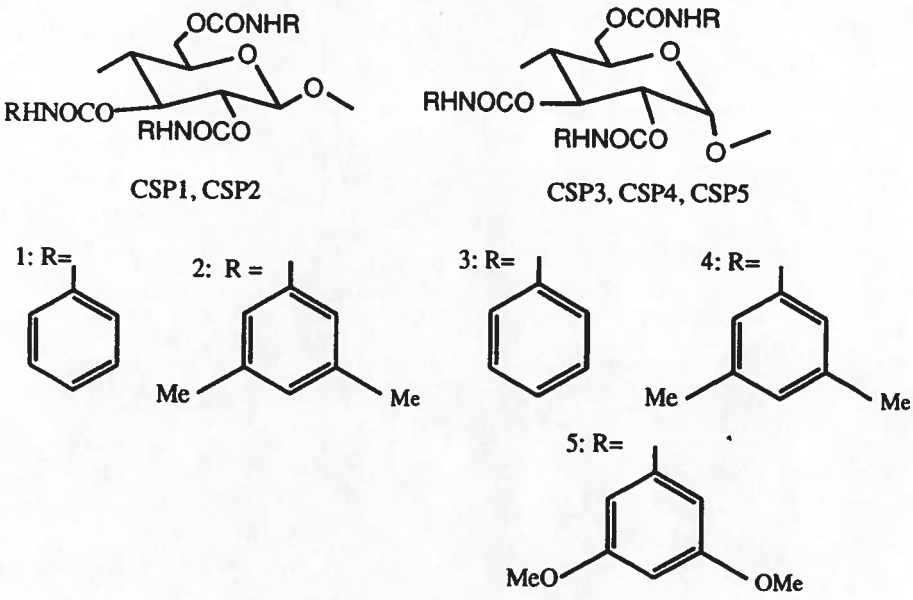


Fig. 1. Structure of chiral stationary phases CSP1–CSP5.

A high pressure slurry packer fitted with a Haskel 780-3 pump was used for column packing.

Preparation of Carbohydrate Carbamates

Amylose and cellulose tris(phenylcarbamate) derivatives were prepared as previously reported.[4] The dried carbohydrate was refluxed in pyridine for 24 h and then 3.5 equivalents of aryl isocynate was added and refluxing continued for a further 72 h. After cooling, the product was isolated as a methanol-insoluble fraction. Yields were 70–100% in all cases.

Preparation of Stationary Phases

Aminopropylated silica (APS) was prepared from silica according to the reported procedure.[21] Silica gel was oven dried (110°C), stored for 24 h in a closed container over saturated LiCl solution, and then reacted with 3-aminopropyltriethoxysilane in toluene at 110°C under reflux for 4 h (elemental analysis: C 1.10%, H 0.38%, N 0.30%).

Each carbohydrate tris(arylcarbamate) derivative (0.675g) was dissolved in 10 ml of THF, the solution added to the silica gel (2.70 g) (previously wetted with THF), and the mixture dried under vacuum on a rotary evaporator to give a 20% w/w loading. The packing material was sieved (38 μm) and packed into a stainless-steel column (150 × 4.6 mm i.d.) at 6000 psi as a slurry (hexane/2-propanol, 50:50, v:v) and equilibrated with hexane/2-propanol (80:20, v:v).

Column Evaluation

The performance of each column packed with a chiral stationary phase (CSP) (Fig. 1) was evaluated initially using *trans*-stilbene oxide as a racemic standard. Dead times (t_0) were estimated using 1,3,5-tri-*tert*-butylbenzene. Hexane/2-propanol (90:10, v:v) was used as mobile phase at a flow rate of 1.0 ml/min.

The series of sulfoxides (Fig. 2) was analysed on the different chiral stationary phases using hexane/2-propanol (90:10, v:v), as mobile phase at a flow rate of 1.0 ml/min.

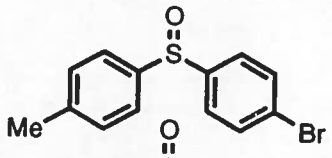
RESULTS AND DISCUSSION

Reactions of cellulose and amylose with aryl isocyanates in refluxing pyridine afforded a series of carbamates (1–5, Fig. 1). In each case, elemental analysis indicated that essentially quantitative derivatisation of all the hydroxyl groups in the carbohydrate had occurred (Table 1).

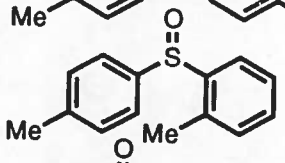
Whereas literature reports[3–9] have referred only to the use of very wide pore silica gels (1000–4000 Å) as supports for carbohydrate carbamate phases, we have found[22] that equally good performance in the resolution of a range of racemates is generally obtained on supports with smaller pore diameters, such as 500 Å. In the present work, Nucleosil 7 μm silica with 500 Å pores was used, after conversion to the aminopropyl derivative, and was coated with the carbamates (**1–5**) by evaporation of THF solutions.

The chiral stationary phases CSP1–CSP5 all showed symmetrical peaks and gave good separations of the enantiomers of *trans*-stilbene oxide (Table 2). The CSP2 phase was the most efficient, whilst the new phase CSP5 based on amylose tris(3,5-dimethoxyphenylcarbamate) gave the second most effective separation.

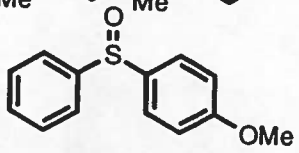
A comparison of the behaviour of the 23 chiral sulfoxides (Fig. 2) on the phenylcarbamate and 3,5-dimethylphenylcarbamate phases (Table 3) indicated that the broadest resolving power was displayed by cellulose tris(3,5-dimethylphenylcarbamate) CSP2. Of the 23 racemic sulfoxides, 17 showed some degree of separation on this column, compared with only 7 on the corresponding amylose tris(3,5-dimethylphenylcarbamate) CSP4, which was the next most effective of these 4 phases. Some of the separations on CSP2 are extremely



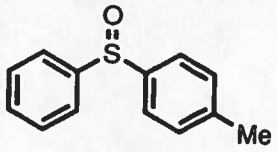
1: p- TOLYL p-BROMO-PHENYL SULFOXIDE



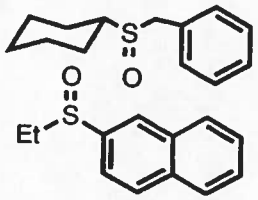
2: 0-TOLYL p-TOLYL SULFOXIDE



3: PHENYL 4-METHOXY-PHENYL SULFOXIDE

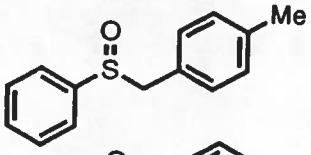


4: PHENYL p-TOLYL SULFOXIDE

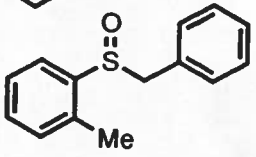


5: BENZYL CYCLOHEHYL SULFOXIDE

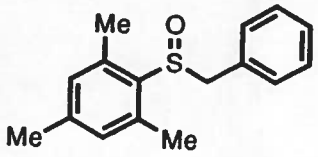
6: ETHYL 2 -NAPHTHYL SULFOXIDE



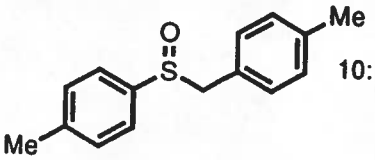
7: PHENYL 4-METHYL-BENZYL SULFOXIDE



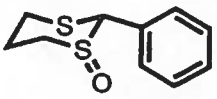
8: BENZYL o-TOLYL SULFOXIDE



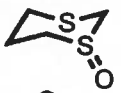
9: BENZYL MESITYL SULFOXIDE



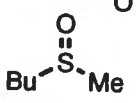
10: 4-METHYLBENZYL p-TOLYL SULFOXIDE



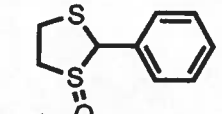
11: trans-2-PHENYL -1,3-DITHIANE SULFOXIDE



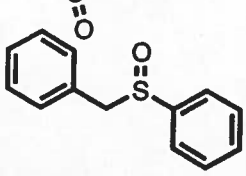
12: 1,3-DITHIANE 1- OXIDE



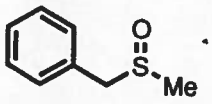
13: METHYL BUTYL SULFOXIDE



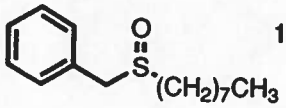
14: cis-, trans-2-PHENYL -1,3- DITHIOLANE SULFOXIDE



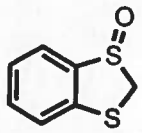
15: PHENYL BENZYL SULFOXIDE



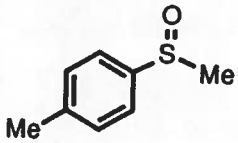
16: METHY BENZYL SULFOXIDE



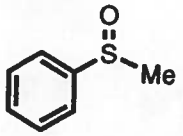
17: n-OCTYL BENZYL SULFOXIDE



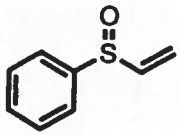
18: 1,3 BENZODITHIOLE -1-OXIDE



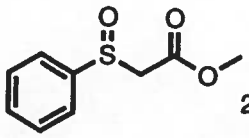
19: METHYL p-TOLYL SULFOXIDE



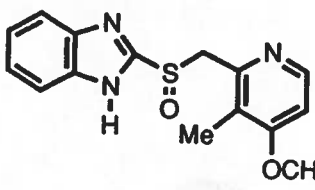
20: METHYL PHENYL SULFOXIDE



21: PHENYL VINYL SULFOXIDE



22: METHYL PHENYL-SULFINYL)ACETATE



23: LANSOPRAZOLE

Fig. 2. Structures of racemic sulfoxides.

TABLE 1. Microanalysis results for carbohydrate carbamates

Phase no.	Carbohydrate	Isocyanate	Calculated (%)			Found (%)		
			C	H	N	C	H	N
1	Cellulose	Phenyl	62.42	4.85	8.09	61.52	4.94	7.93
2	Cellulose	3,5-Dimethylphenyl	65.66	6.18	6.96	64.28	6.12	6.63
3	Amylose	Phenyl	62.42	4.85	8.09	61.82	4.85	7.82
4	Amylose	3,5-Dimethylphenyl	65.66	6.18	6.96	63.90	6.27	6.97
5	Amylose	3,5-Dimethoxyphenyl	56.60	5.29	6.00	56.30	5.29	5.97

TABLE 2. Resolution of *trans*-stilbene oxide enantiomers on chiral stationary phases CSP1–CSP5[a]

	CSP1	CSP2	CSP3	CSP4	CSP5
k'_1	0.67	0.75	0.89	0.36	0.57
α	1.46	1.80	1.60	1.58	1.80
Rs	1.45	2.19	1.50	1.50	1.60

[a] Mobile phase: hexane:2-propanol (90:10), flow rate: 1.0 ml/min.

large, e.g., those for sulfoxides 9 and 11. On the other hand, resolution was generally not observed for the nearly symmetrical diaryl sulfoxides which differ only in substitution at the para position (e.g., compounds 1, 3, 4), nor was any separation observed on any of these 4 phases for ethyl 2-naphthyl sulfoxide (6) or methyl butyl sulfoxide (13).

The new 3,5-dimethoxyphenylcarbamate derivative of amylose, CSP5, showed some interesting differences in selectivity from the above 4 phases. Some degree of separation was observed for 12 of the 23 sulfoxides investigated, placing this phase intermediate between the dimethylphenylcarbamates of amylose and cellulose in its performance. Four of the

TABLE 3. Resolution of sulfoxides (1–23) on carbohydrate carbamate phases eluted with hexane/2-propanol: 90:10 or *95:5 v:v

	CSP1			CSP3			CSP4		
	k'_1	α	Rs	k'_1	α	Rs	k'_1	α	Rs
1	5.93	1.00		7.11	1.00		5.50	1.00	
2	7.00	1.00		7.11	1.00		3.89	1.08	0.25
3	17.0	1.00		11.6	1.00		8.53	1.00	
4				6.40	1.00		5.12	1.00	
5	6.28	1.21	0.43	6.10	1.77	1.85	6.37	1.00	
6	4.59	1.00							
7	7.53	1.25	0.68	6.00	1.00		4.59	1.00	
8	6.73	1.00		4.90	1.00		4.25	1.00	
9	5.57	1.00		4.10	1.16	0.25	2.76	1.00	
10	6.88	1.00		6.7	1.00		4.65	1.00	
11	18.4	1.00		17.6	1.93	2.97	11.5	1.80	2.90
12	17.1	1.00		22.6	1.08				
13	5.2	1.00							
14	3.33	1.00		7.11	1.00		5.70	1.20	1.14
	24.1	1.00		14.7	1.00		10.6	1.20	0.90
15	6.27	1.15	0.98	8.16	1.00		4.57	1.00	
16	11.8	1.00		9.89	1.29	0.94	4.67	1.10	0.55
17	3.18	1.00		5.89	1.30	0.86	2.42	1.07	0.17
18				14.5	1.06	0.43	11.5	1.22	1.38
19	19.9	1.00		7.78	1.00		4.35	1.00	
20	9.90	1.00		6.89	1.00		4.61	1.00	
21	9.77	1.13	0.90	4.10	1.00		2.27	1.00	
22	19.5	1.00		10.7	1.00		3.44	1.00	
23	0.85	1.00					9.92	1.00	

(continued)

TABLE 3. Resolution of sulfoxides (1–23) on carbohydrate carbamate phases eluted with hexane/2-propanol: 90:10 or *95:5 v:v (continued)

	CSP2			CSP5		
	k'_1	α	Rs	k'_1	α	Rs
1	2.92	1.00		7.26*	1.06	0.4
2	0.90	1.10	0.15	8.75	1.00	
3	4.86	1.00		16.55	1.00	
4	2.69	1.00		9.00	1.00	
5	3.00	1.00		5.31	1.30	1.14
6	4.7	1.00		9.50	1.00	
7	2.5	1.18	1.00	6.06	1.00	
8	2.57	1.28	1.25	6.14*	1.28	1.45
9	2.0	2.55	5.37	5.31*	1.36	1.47
10	2.71	1.16	0.86	10.10*	1.20	0.95
11	7.14	1.98	5.44	12.55	1.15	0.60
12	9.71	1.22	1.25			
13	1.12	1.00		2.89	1.00	
14	2.5	1.14	1.11	6.54	1.11	0.59
	6.0	1.15	1.04	13.04	1.22	1.24
15	4.25	1.19	1.25	6.27	1.15	0.98
16	2.57	1.00		11.82	1.00	
17	2.31	1.00		3.18	1.00	
18	9.67	1.16	1.15	20.6	1.00	
19	1.61	1.12	1.00	6.45	1.45	1.86
20	2.05	1.14	0.83	9.90	1.17	0.96
21	2.07	1.17	0.91	9.77	1.13	0.90
22	6.14	1.14	0.86	8.35	1.00	
23	17.7	1.32	1.00	12.83	1.00	

sulfoxides (8, 10, 14, 19) which were resolved on CSP2 showed better resolution on CSP 5 and a further 2 sulfoxides (1, 5), which were completely unresolved on CSP2, showed some separation on CSP5. The partial resolution on CSP5 of the diaryl sulfoxide 1, in which the aryl groups differ only in the exchange of a bromine for a methyl group in the para position, is particularly noteworthy. This separation suggests a recognition model in which there must be a combination of H-bonding between the sulfoxide and carbamate residues and, further, stereoselective interactions of each sulfoxide aryl group with the surrounding environment, probably within the "chiral ravines"[23] formed between the carbohydrate strands.

The amylose tris(3,5-dimethoxyphenylcarbamate) phase CSP5, which is readily prepared from commercially available materials, appears to offer some advantages over the widely used phenylcarbamate and 3,5-dimethylphenylcarbamate phases for the resolution of certain enantiomers and merits further exploration of its potential utility.

ACKNOWLEDGMENTS

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