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# USE OF CHIRAL STATIONARY PHASES FOR THE CHROMATOGRAPHIC DETERMINATION OF ENANTIOMERIC PURITY AND ABSOLUTE CONFIGURATION OF SOME β-LACTAMS

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## SUMMARY

The enantiomers of a variety of β-lactams bearing aryl substituents in the 3- or 4-position have been chromatographically separated on amino acid-derived chiral stationary phases. Separation factors are typically modest. Alternatively, 3- and 4-substituted β-lactams may be easily ring-opened and converted into N-3,5-dinitrobenzoyl ester derivatives of the resulting β-amino acids. The enantiomers of these derivatives are readily separated on amide-derived chiral stationary phases. Aromatic substituents are not essential in the latter instances. Such separations enable accurate and convenient determination of the enantiomeric purity of the original β-lactam and, in many instances, assignment of the absolute configuration of the major enantiomer of the β-amino acid derivatives and hence the β-lactam precursors.

## INTRODUCTION

The recent discovery of carbapenems and related antibiotics has stimulated enormous activity in the area of β-lactam synthesis[1,5]. Owing to the demonstrated enantiodependence of the physiological activity of β-lactams[5], there is a clear need for methods by which β-lactams can be obtained enantiomerically pure and by which their enantiomeric purities and absolute configurations can be determined. Efforts to obtain single enantiomers of β-lactams have been based on asymmetric syntheses[3] or syntheses starting from optically active precursors[5–7]. Simple methods for resolving β-lactams do not exist[7]. Evaluation of optical purity by classical methods, such as polarimetry, has shortcomings and leaves much to be desired in convenience and accuracy[8].

Recently, several chiral stationary phases have been introduced which are capable of resolving a host of racemates both analytically and preparatively[9,10]. We now wish to report that CSP 1, derived from (*S*)-N-3,5-dinitrobenzoylleucine, allows one to separate the enantiomers of β-lactams bearing aromatic substituents in the 3-

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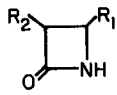
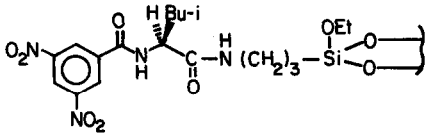
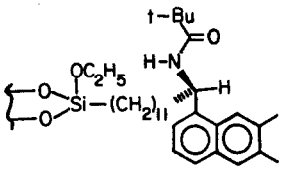
or 4-position. We also wish to report that CSP 2, derived from (*R*)-N-pivaloyl-α-(6,7-dimethyl-1-naphthyl)-11-dodecenylamine, effectively separates the enantiomers of a wide variety of N-3,5-dinitrobenzoyl derivatives of the β-amino acid esters derived from alcoholysis of mono- and disubstituted lactams. Aromatic substituents may be present but are not necessary in the latter case. Thus, enantiomeric purities of β-lactams are now easily determined by high-performance liquid chromatography (HPLC). Moreover, absolute configurations of the β-lactam enantiomers can sometimes be assigned from the elution order of the β-amino acid derivatives without recourse to configurationally known samples.



3a–t



CSP 1



CSP 2

## EXPERIMENTAL

Compounds described herein were available from other studies and have been described elsewhere[2]. β-Lactams 3s, 3t, 3u, 3v were kindly donated by Professor D. H. Hua[4]. The preparation of N-3,5-dinitrobenzoyl amino acid CSPs has been described[9]. Such columns are now commercially available. The preparation of amide CSPs, such as CSP 2, has also been described[10].

Chromatography was performed using an Altex 100 A pump, Altex 210 injector and an Altex Model 165 detector operated at 254 and 280 nm. A Kipp & Zonen BD-41 recorder was used. Isocratic elution was used; mobile phase composition is given in the tables.

## RESULTS AND DISCUSSION

The enantiomers of β-lactams having aromatic substituents in the 3- or 4-positions can be separated directly by chromatography on any of a number of N-

TABLE I

RESOLUTION OF TYPE 3 β-LACTAMS ON CSP

$R_2CH–CHR_1$
| |
CO–NH

| *Compound* | $R_2$ | $R_1$ | | α | $k_1'$* |
|---|---|---|---|---|---|
| 3a | $CH_3CH[Si(CH_3)_2C_6H_5]$ | $C_6H_5$ | *cis* | 1.25 | 1.0 |
| 3b | $(CH_3)_2CH$ | $CH_2COC_6HOCH_3$ | *cis* | 1.19 | 17.8 |
| 3c | $(CH_3)_2N–$ | $C_6H_5$ | *cis* | 1.12 | 2.5 |
| 3d | $(CH_3)_2CH$ | $C_6H_5$ | *cis* | 1.10 | 1.8 |
| 3e | H | $C_6H_5$ | – | Barely resolves | |
| 3f | H | 1-Naphthyl | – | Barely resolves | |
| 3g | $C_6H_5$ | H | – | 1.06 | 1.0 |

* Mobile phase was 20% 2-propanol in hexane.

3,5-dinitrobenzoylamino acid-derived chiral stationary phases. Table I contains data pertinent to the resolution of such β-lactams on (*S*)-CSP 1. Separability factors are modest (α = 1.06–1.3). Even so, preparative resolutions can be effected, thus allowing one to obtain enantiomerically pure or enriched samples. The resolution of γ- and δ-lactams on amino acid-derived chiral stationary phases has been described[9].

The resolution of achirally derivatized aliphatic β-amino acids (*i.e.* 3,5-dinitrobenzamide esters) on amide chiral stationary phases, such as CSP 2, has been reported recently[11]. Since β-lactams undergo ready alcoholysis to afford the corresponding β-amino esters and conversion of these into the 3,5-dinitrobenzamides is trivial, it is possible to determine the enantiomeric purity of a wide variety of β-lactams by chromatography of their ring-opened derivatives on appropriate chiral stationary phases. Table II contains data pertinent to the resolution of such β-lactam derivatives on CSP 2.

Fig. 1 illustrates the use of this approach for the evaluation of the efficacy of an asymmetric synthesis of a β-lactam. A mixture of *cis*- and *trans*-3-isopropyl-4-phenyl-2-azetidinone was prepared by reaction of a homochiral ester with N-trimethylsilylbenzaldimine[6]. Trace a is the chromatogram of the ring-opened β-lactam derivatives from the crude reaction product. The enantiomeric purity of both the *cis* and the *trans* β-lactam isomers is readily apparent. Trace b is the chromatogram similarly obtained from recrystallized material. Trace c similarly stems from the mother liquor of the recrystallization after chromatographic removal of the *trans* isomer. As can be seen, recrystallization gives enrichment in the major β-lactam enantiomer, whereas the mother liquor is depleted of this enantiomer.

*Elution orders*

To relate elution order to absolute configuration, racemic lactam 3e was partially resolved on CSP 1 and the (*R*)-(+) enriched fraction was converted into the N-3,5-dinitrobenzoyl-*n*-octyl ester derivative. The major enantiomer of this β-amino acid derivative is preferentially retained on CSP 2. Similarly, (+)-enriched samples of lactams 3m, 3n, 3o, 3p and 3d afford N-3,5-dinitrobenzoyl derivatives, the major

TABLE II

RESOLUTION OF N-3,5-DINITROBENZOYL OCTYL ESTERS DERIVED FROM TYPE 3 β-LACTAM ON CSP 2

DNB–HN–$CHR_1$–$CHR_2$–$COOC_8H_{17}$
(4)

| *Compound* | $R_2$ | $R_1$ | | α | $k_1'$* |
|---|---|---|---|---|---|
| 4h | $(CH_3)_2CH$ | 2-Furyl | *cis* | 3.08 | 3.9 |
| 4i | $(CH_3)_2CH$ | $C{\equiv}CC_6H_4OCH_3$ | *cis* | 7.00 | 10.0 |
| 4g** | dimethyl | $C_6H_5$ | – | 1.49 | 14.6 |
| 4k | $(CH_3)_3C$ | $C_6H_5$ | *cis* | 8.55 | 10.0 |
| 4l | $CH_3$ | $C_6H_5$ | *trans* | 5.19 | 6.9 |
| 4m | $CH_3$ | $C_6H_5$ | *cis* | 2.43 | 23.2 |
| 4n | $CH_3CH_2$ | $C_6H_5$ | *trans* | 5.41 | 5.0 |
| 4o | $CH_3CH_2$ | $C_6H_5$ | *cis* | 2.78 | 23.7 |
| 4p | $(CH_3)_2CH$ | $C_6H_5$ | *trans* | 7.70 | 3.1 |
| 4d | $(CH_3)_2CH$ | $C_6H_5$ | *cis* | 3.34 | 25.0 |
| 4q | $CH_3CH_2$ | *trans*-$C_6H_5CH{=}CH$ | *cis* | 2.80 | 13.1 |
| 4r | $CH_3CH_2$ | *trans*-$C_6H_5CH{=}CH$ | *trans* | 2.89 | 5.9 |
| 4e | H | $C_6H_5$ | | 2.42 | 17.6 |
| 4f | H | 1-Naphthyl | | 1.16 | 30.0 |
| 4s*** | H | $CH(CH_3)CH_2CH_3$ | $k_1'=9.67$, $k_2'=10.67$, $k_3'=13.10$, $k_4'=13.83$ | | |
| 4t*** | H | 3'-Cyclohexenyl | | 1.11 | 11.0 |
| 4u*** | H | Vinyl | | 1.42 | 15.2 |
| 4v*** | H | Allyl | | 1.27 | 13.2 |
| 4w*** | H | $CH_2CH_2CH_3$ | | 1.22 | 16.0 |

* Mobile phase was 20% 2-propanol in hexane.
** Compound bears geminal methyls in 3-position.
*** Methyl (not octyl) ester.

enantiomers of which are preferentially retained on CSP 2. For both the *cis* and the *trans* series, the magnitude of separability factors observed for the enantiomers increase steadily and smoothly as the size of the 3-substituent increases. Consequently we employ "tracking of absolute configuration"[10] to extend the (4-*R*) assignment to the most retained enantiomers of 4m, 4n, 4o, 4p and 4d. These assignments also apply to the (+)-enantiomers of the corresponding β-lactam precursors.

We have previously found that, for amide CSPs similar to 2, the enantiomers of N-3,5-dinitrobenzoyl analytes have available two competing chiral recognition mechanisms of opposite enantioselectivity. The magnitudes of the separability factors and even elution orders of the enantiomers depend upon the relative contributions to enantiomer separation made by each of the competing processes[10]. The dipole stacking mechanism is dominant for the 3,5-dinitrobenzamides of α-aryl alkylamines. Were this mechanism also dominant for the N-3,5-dinitrobenzoyl-*n*-octyl ester derivatives of the 4-aryl substituted β-lactams shown in Table II, one would expect the enantiomers arising from the β-lactams having the (4-*R*) configuration to be selec-

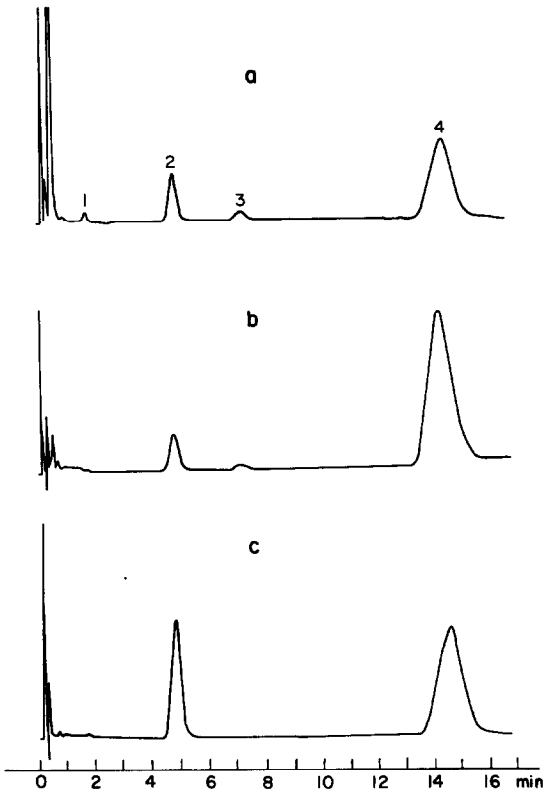
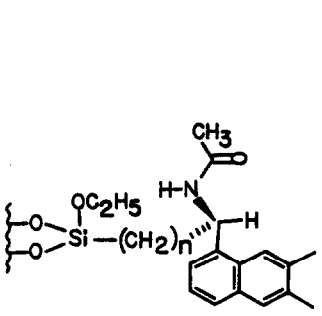



Fig. 1. Evaluation of asymmetric synthesis of β-lactams by chromatography on chiral stationary phases. Peaks 2 and 4 arise from the enantiomers of the 3,5-dinitrobenzamide octyl esters derived from *cis*-3-isopropyl-4-phenyl-2-azetidinone. Peaks 1 and 3 arise similarly from the *trans* isomer. The chromatograms were generated on CSP 2; the mobile phase was 10% water in methanol. (a) Crude reaction mixture, after derivatization of the lactams as the 3,5-dinitrobenzamides of the octyl esters; (b) recrystallized product, similarly derivatized; (c) mother liquor of the recrystallization, similarly treated. The *trans* β-lactam isomer was removed by chromatography on silica gel.

tively retained. Conversely, the "hydrogen bonding" mechanism dominant for N-3,5-dinitrobenzoyl α-amino acid esters, would, if dominant for the aforementioned β-lactam derivatives, selectively retain the enantiomers stemming from the lactams having the (4-*S*) configuration. The elution order observed for the N-3,5-dinitrobenzoyl *n*-octyl ester derivatives of the 4-aryl substituted β-lactams is consistent with the dominance of a "dipole stacking" process.

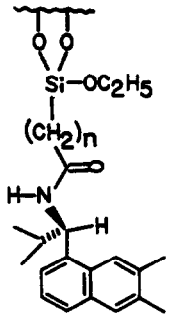
Prior study has shown that changes in the structures of amide CSPs similar to 2 can alter the balance between the two competing processes. For example, shortening the length of the "connecting arm" (the methylene chain which anchors the chiral portion of the CSP to the silica support) is known to reduce the contribution of an intercalative retention process when, as in the present case, nonpolar mobile phases are used. For N-3,5-dinitrobenzoyl derivatives of α-aryl alkyl amines, the dipole stacking process is not an intercalative process on CSPs 2, 5a and 5b. However, the minor competing hydrogen bonding process is intercalative and is suppressed by a

reduction of the length of the connecting arm of the CSP. Hence, larger separability factors and reduced retention times are observed. Thus, comparison of the chromatographic behavior of a series of type 4 β-amino acid derivatives on CSPs 5a and 5b should lead to qualitatively predictable results[10].




CSP **5a** n=11

CSP **5b** n=5




CSP **6a** n=10

CSP **6b** n=4

The type 4 derivatives stemming from the (+)-enriched samples of the β-lactams 3m, 3o, 3p and 3d were chromatographed on CSPs 5a and b. Modest increases in separability factors are typically observed on the short-armed 5b compared to 5a (Table III). Reduced retention is also observed. Since these differences are modest, we infer that the intercalative hydrogen bonding process makes relatively little contribution to the overall retention and chiral recognition of the type 4 analytes. Perusal of the data in Tables II and III reveals an interesting pattern. The type 4 derivatives of *trans* lactams 3d, 3n and 3p show rather larger α values and smaller $k_1'$ values than do their *cis* counterparts. What is the mechanistic significance of this? First, it appears that it is the configuration at the 4-carbon that determines elution order. In other words, the aforementioned type 4 analytes are much like N-3,5-dinitrobenzoyl derivatives of α-aryl alkylamines. However, the β-carboalkoxy group is a possible site for additional interactions and its disposition with respect to the CSP during adsorption will certainly be influenced by the nature of the substituent on the 3-carbon and by the absolute configuration at that position. Initially, one might suspect that the effect of the second chiral center is to give rise to a chiral recognition component that either adds or detracts from the larger contribution originating at the 4-carbon. However, this is not the case. The derivatives of lactams 3e and 3g do not possess chirality at carbon 3; hence, there can be no second contribution to add or detract. Note in Table III that, relative to 4g and 4e, the enantiomers of 4l, 4m, 4o, 4p and 4d all show greater chiral recognition (*i.e.* have larger α values). Relative to 4e, the retention of the enantiomers derived from the *cis* β-lactams is enhanced by the introduction of the 3-alkyl substituent whereas that of the enantiomers from the *trans* β-lactams is reduced. In addition to the bonding interactions which typically occur during dipole–dipole stacking, an additional bonding interaction is thought to occur between the carbonyl oxygen of the analytes carboalkoxy group and the CSPs amide hydrogen. The extent to which this hydrogen bonding occurs depends upon the conformational preference of the analyte. Large 3-alkyl substituents will more

TABLE III

RESOLUTION OF N-3,5-DINITROBENZOYL *n*-OCTYL ESTERS DERIVED FROM SOME TYPE 3 β-LACTAMS ON CSPs 5a, 5b, 6a, 6b USING 20% 2-PROPANOL IN HEXANE

| *Compound* | *CSP 5a* | | *CSP 5b* | | *CSP 6a* | | *CSP 6b* | |
|---|---|---|---|---|---|---|---|---|
| | α | $k_1$ | α | $k_1'$ | α | $k_1'$ | α | $k_1'$ |
| 4e | 2.51 | 7.4 | 3.08 | 5.6 | 1.89 | 9.1 | 1.56 | 6.6 |
| 4g | 3.30 | 4.1 | 4.16 | 4.1 | 2.48 | 5.3 | 2.25 | 3.9 |
| 4l | 4.93 | 3.9 | 5.13 | 3.1 | 4.26 | 4.4 | 2.28 | 3.1 |
| 4m | 2.76 | 10.4 | 3.36 | 6.4 | 3.58 | 8.4 | 3.60 | 5.9 |
| 4n | 4.66 | 3.0 | 5.12 | 2.7 | 5.23 | 2.8 | 2.20 | 2.2 |
| 4o | 3.40 | 7.9 | 3.47 | 8.1 | 3.70 | 10.5 | 3.75 | 6.4 |
| 4p | 5.58 | 1.7 | 4.75 | 1.6 | 6.62 | 1.6 | 2.69 | 1.1 |
| 4d | 4.08 | 8.7 | 4.39 | 6.9 | 5.12 | 10.2 | 4.92 | 6.3 |
| 4k | 8.40 | 5.7 | 9.50 | 5.0 | 11.60 | 5.6 | 9.26 | 3.0 |

effectively control analyte conformation and the resulting rigidity can aid chiral recognition, provided it is a conformation favorable to chiral recognition that is being preferentially populated. Additionally, one can speculate that, because the carboalkoxy and alkyl substituents of the derivatives of the *cis* and *trans* lactams are oriented differently, the extent to which these substituents intercalate between adjacent strands of bonded phase might differ. From the lesser retention of the *trans* lactam derivatives, one would infer that either the carboalkoxy groups are less available for hydrogen bonding or that the adsorption process involves a greater degree of intercalation. However, the comparative behavior on CSPs 5a and b argues against strong intercalative effects on these CSPs.

Additional relevant information was obtained by chromatographing these analytes on acyl-linked CSPs 6a and b. These CSPs produce characteristic responses from analytes which utilize dominant dipole stacking processes, as these are intercalative processes on CSPs 6a and b[10]. CSP 6b, having the shorter connecting arm, should reduce retention relative to 6a, provided intercalation is occurring. The data in table III shows this trend to a considerable degree, thus supporting the dominance of a dipole stacking-like process. Again, the derivatives of the *cis* lactams are more strongly retained than those from the corresponding *trans* lactams and again show the smaller separability factors. This clearly indicates an intrinsic conformational difference between the *cis*- and *trans*-derived analytes and relegates intercalative effects to a modest role in the determination of selectivity and retention on CSP 6a. Note however that while a reduction of the length of the connecting arm of the type 6 CSPs produces but small changes in the separability of the enantiomers derived from the *cis* lactams, considerable reduction in separability for the *trans*-derived analogues results. However, no such reduction is noted for the *trans*-derived analytes as one reduces the length of the connecting arm of the type 5 CSPs. These effects seem to indicate that, on type 6 CSPs, the *trans*-derivated analytes are oriented so as to cause repulsive interaction between the carboakoxy "tail" and the underlying silica support when the length of the connecting arm is reduced. The *cis* lactam-derived analytes are comparatively unaffected by such interactions.

In summary, the dominant chiral recognition mechanism is viewed as a dipole

The urine test can thus be used as a method for personal monitoring of the exposure, which is often a desirable objective, *e.g.* in occupational health care. Another aspect is that the mass spectral identification of the urinary amines also reveals the original degraded polyurethane. The putative polymer, upon heating, should release the same monomeric isocyanate structure and its derivative amine found in the urine of the exposed subjects, an important element in the toxicological evaluation of fire victims.

## CONCLUSION

The mass fragmentographic method described in this paper offers a sensitive and selective means of detecting exogenous primary diamines in urine. It is also well suited for other samples of minimal sizes because of the low detection limit.

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## REFERENCES

1 R. A. Anderson, I. Thomson and W. A. Harland, *Fire Mater.*, 3 (1979) 91.
2 W. D. Woolley and P. J. Fardell, *Fire Res.*, 1 (1977) 11.
3 F. D. Hileman, K. J. Voorhees, L. H. Wojcik, M. M. Birky, P. W. Ryan and I. N. Einhorn, *J. Polym. Sci.*, 13 (1975) 571.
4 C. J. Hilado and P. A. Huttlinger, *J. Thermal Insul.*, 4 (1981) 276.
5 D. A. Purser and W. D. Woolley, *J. Fire Sci.*, 2 (1983) 110.
6 D. M. Russo, P. Sgro and H. J. Schneider, *Neurobehav. Toxicol. Teratol.*, 3 (1981) 265.
7 J. Chambers, J. Jiricny and C. B. Reese, *Fire Mater.*, 5 (1981) 133.
8 A. Zitting, C. Rosenberg, S. Vainiotalo and H. Savolainen, *Fire Mater.*, 6 (1982) 96.
9 C. Rosenberg and H. Savolainen, *J. Chromatogr.*, 323 (1985) 429.
10 C. Rosenberg, *Analyst (London)*, 109 (1984) 859.
11 U.S. Department of Health, Education, and Welfare, *NIOSH Manual of Analytical Methods*, Vol. 1, Washington, DC, 1977, Method 116.
12 B. Masiulanis, *J. Appl. Polym. Sci.*, 29 (1984) 681.
13 J. Drozd, *J. Chromatogr.*, 113 (1975) 303.
14 G. Skarping, L. Renman and B. E. F. Smith, *J. Chromatogr.*, 267 (1983) 315.
15 I. B. Glowinski and W. W. Weber, *J. Biol. Chem.*, 257 (1982) 1424.