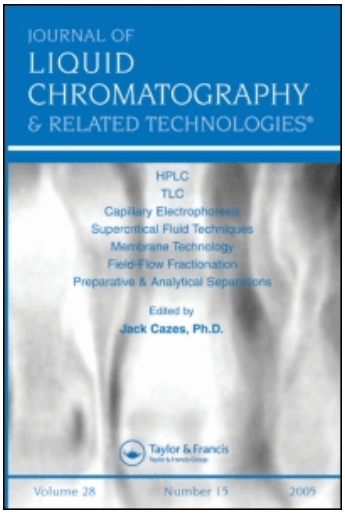

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ANALYTICAL AND PREPARATIVE ENANTIOMERIC SEPARATION OF A SERIES OF C5-CYCLOALKYLAMINE-1,4-BENZODIAZEPIN-2-ONE CCK_B RECEPTOR ANTAGONISTS BY CHIRAL HPLC

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ABSTRACT

A series of C5 cycloalkyl benzodiazepin-2-one CCK_B receptor antagonists are reported in which substitution by tolyl-urea at C3 has generated a chiral centre. As only one of these enantiomers is selective for the CCK_B receptor an analytical separation of the enantiomers was developed to monitor the resolution of compounds by chemical means. It was shown that such compounds may be resolved using a Pirkle-type 3,5-(dinitrobenzoyl)-leucine (DNBL) chiral stationary phase (CSP) to give high α and R_S values. Such high enantioselectivities allowed the separation to be scaled-up sufficiently such that gram quantities of each enantiomer could be isolated by preparative chiral HPLC. An investigation is described in which the effect of substituent, mobile phase composition, temperature and the alternate CSP 3,5-(dinitrobenzoyl)-phenylglycine (DNPBG) is discussed for such compounds.

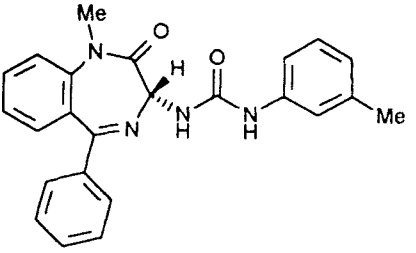

Figure 1. Structure of L-365,260.

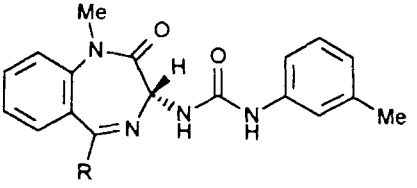

Figure 2. Structure of amidine analogue of L-365,260, where R=cycloalkyl bonded through nitrogen.

INTRODUCTION

Cholecystokinin (CCK) is a 33 amino acid polypeptide hormone which occurs in numerous molecular forms throughout both the central and peripheral nervous systems. CCK exerts a variety of actions on peripheral organs, such as regulating pancreatic secretion and gut motility, and may also function as a neurotransmitter or neuromodulator in the CNS.[1,2] The actions of CCK are mediated by two receptor subtypes designated CCK_A and CCK_B,[3] with the majority of the central receptors being of the CCK_B subtype.

A number of non-peptidic CCK_B receptor antagonists have been reported. Structures based upon the natural product Asperlicin have given rise to a series of 1,4-benzodiazepin-2-ones, notably L-365,260 (Figure 1).[5-7] It was shown that the 3*R*-enantiomer was selective for CCK_B binding over CCK_A.[8] Further modifications of this structure to increase affinity, selectivity and solubility have led to a series of modifications at the C5 position of the benzodiazepine in which the phenyl ring of L-365,260 has been replaced by a cycloalkylamine to form an amidine (Figure 2).[9]

The enantiomeric separation of substituted 1,4-benzodiazepine-2-ones using Pirkle type DNBPG or DNBL chiral stationary phases has already been demonstrated where the C5 substituent was phenyl.[10] However, loss of enantiomeric discrimination was observed in changing to cyclohexyl.

As stereochemistry at C3 is an important factor in determining CCK_B / CCK_A binding selectivity, it is essential to separate the enantiomers of these molecules either to provide enantiomeric purities of resolved material or to preparatively separate racemates. In the present study, the resolution of 1,4-benzodiazepine-2-ones bearing C5-amidine substituents is investigated using both Pirkle DNBPG and DNBL columns. The effect of C5 substituent, temperature and mobile phase composition is considered along with efforts to scale the separation up to provide a preparative method for obtaining individual enantiomers from racemic mixtures.

EXPERIMENTAL

Materials

All compounds described were synthesised in-house with identity and purity confirmed by NMR, MS, HPLC and elemental analysis. HPLC grade methanol, hexane and 1-chlorobutane were obtained from Fisons (Loughborough, UK). Ethanol was obtained from Hayman Limited (Witham, UK).

Instrumentation

An HP1090L series high performance liquid chromatograph was used for the analytical separations (Hewlett Packard, Avondale, USA). The system comprises an autoinjector, consisting of a Rheodyne 7010 injection valve fitted with a 250 µl loop, an autosampler and a binary DR-5 solvent delivery system. Detection was by UV using a built-in filter photometric detector (FPD) and data was processed using an HP DOS ChemStation. Column temperature was regulated using a Violet T-55S column cooler (Flowgen, UK).

Preparative separations were performed using a Shimadzu preparative HPLC supplied by Dyson Instruments (Houghton-le-Spring, UK) consisting of a C-R4A Chromatopac, a SCL-8A system controller, a SIL-8A autoinjector, two LC-8A pumps, a SPD-6A UV detector and a FCV-100B fraction collector.

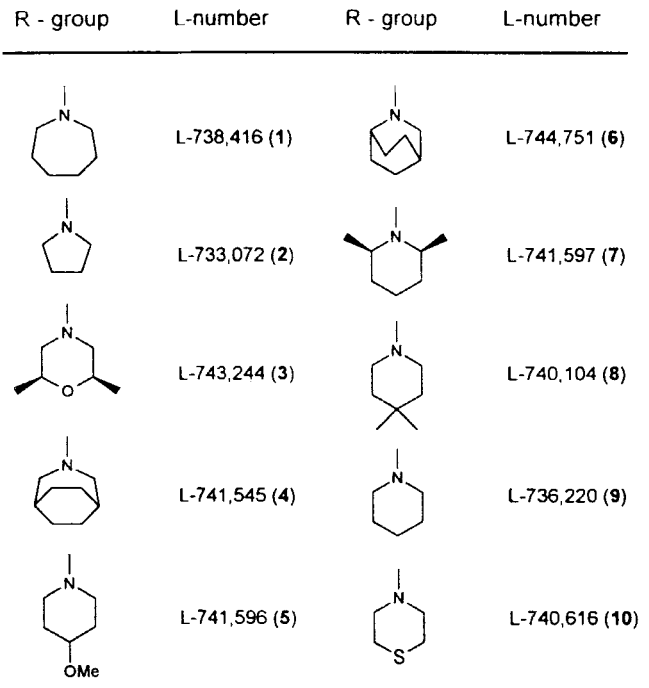

Figure 3. Structure and identification of C5 cycloalkyl substituents.

Chromatographic Conditions

HPLC analysis was performed using columns containing DNBPG or DNBL covalently bonded to silica, supplied by Hichrom (Reading, UK). The dimensions were 250 x 4.6mm i.d. for the analytical columns and 250 x 20mm i.d. for the preparative column with a silica particle size of 5μm. Typical mobile phases were 5% MeOH in 1-chlorobutane or 50% EtOH in hexane. The flow rate analytically was 1.0 mL/min and preparatively 20.0 mL/min. The FPD was set to 230 nm, this being the approximate λ_{max} for these compounds.

Detection for preparative separations was performed at 300nm in an attempt to avoid saturation of the detector. 5μl of a 1mg/mL solution of all compounds were injected for the analytical analyses which were performed at thermostatically regulated temperature.

Table 1

Summary of Capacity Factor, Separation Selectivity and Resolution using a DNBL CSP

Compound L-number	K'_1	K'_2	α	R_S
L-738,416 (1)	1.16	3.95	3.41	16.74
L-733,072 (2)	2.00	3.79	1.90	8.29
L-743,244 (3)	1.13	2.63	2.34	9.57
L-741,545 (4)	1.32	3.03	2.30	10.92
L-741,596 (5)	1.49	3.53	2.37	10.44
L-744,751 (6)	1.68	4.03	2.40	11.42
L-741,597 (7)	1.04	4.32	4.15	17.61
L-740,104 (8)	1.16	2.48	2.14	9.64
L-736,220 (9)	1.31	4.00	3.05	15.22
L-741,616 (10)	1.45	4.00	2.76	13.02

RESULTS AND DISCUSSION

A series of 10 compounds bearing different cycloalkyl C5 substituents was investigated (Figure 3). The effect of varying the C5 substituent was analysed using both the DNBPG and DNBL chiral stationary phases and the α and R_S values for these experiments are presented (Tables 1,2) using a 5% MeOH/ 1-chlorobutane mobile phase. From our previous work with R = cyclohexyl[11] using a DNBL CSP, it was clear that the C5 substituent did not have to be phenyl and hence was not involved in π-π bonding. It was therefore not anticipated that the nature of the C5 substituent would strongly influence the chiral selectivity, as it was felt that this was not significantly contributing towards CSP interactions. Excellent separation selectivities and resolutions were demonstrated for the series with the DNBL CSP proving superior in most cases to the DNBPG, although this was not a general rule. Previous separations of 3-substituted benzodiazepines[10] have shown a similar difference between the phases but not such high stereospecificity. It is suggested that the C3 3-(methylphenyl)-urea substituent contributes significantly to the interactions with the CSP through classical CSP interactions of H-bonding, dipole-dipole and π-π interactions and it is likely that this substituent will dominate the conformation of the molecule to maintain this group psuedo-equatorial.

Table 2

Summary of Capacity Factor, Separation Selectivity, and Resolution using a DNBPG CSP

Compound L-number	K'_1	K'_2	α	R_S
L-738,416 (1)	1.36	1.65	1.21	2.65
L-733,072 (2)	3.31	4.34	1.27	4.54
L-743,244 (3)	1.25	1.40	1.12	1.55
L-741,545 (4)	1.49	1.76	1.18	2.27
L-741,596 (5)	1.65	1.93	1.17	2.88
L-744,751 (6)	2.73	3.80	1.39	5.49
L-741,597 (7)	0.99	1.40	1.42	5.29
L-740,104 (8)	1.32	1.38	1.05	0.66
L-736,220 (9)	1.75	1.86	1.06	0.91
L-741,616 (10)	1.34	1.75	1.31	4.20

In the absence of 'classical' interactions between the CSP and the substituent at C5, one explanation for the differences observed in separation selectivity and resolution upon substituent variation is that the directionality of the classical H-bonds or dipole interactions are altered. If one considers analogue 7, which possesses a cycloalkylamine with 2,6-dimethyl substitution, an α value of 4.15 and an R_S value of 17.61 indicates a significant energy difference between the CSP-interactions of the two enantiomers. Although the amidine group will preferentially try to adopt a planar arrangement, the effect of 2,6-dimethylation will be to partially disrupt the conjugation, hence the substituent will sit slightly out of plane. Assuming that the π-acid/π-base interaction between the benzodiazepine nucleus and the 3,5-(dinitrobenzoyl) group predominates for such CSP's, then this interaction is likely to be restricted by the C5 substituent. Consequently, if one overlays the benzodiazepine portion of each enantiomer, it is found that the directionality of, for example, the C2 carbonyl group of the benzodiazepine is markedly different between these enantiomers, with one projecting above and the other below the plane of the molecule.

This suggests that the carbonyl from only one enantiomer can interact optimally with the CSP, leading to a large difference in interaction energy between enantiomers. This may also be true of other dipoles, H-bond acceptor or H–bond donor group in the molecule. The effect of C5 substitution on

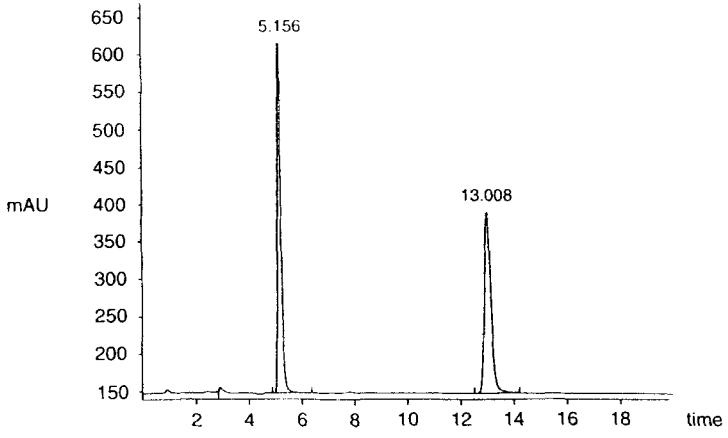

Figure 4. Chromatogram of enantiomeric separation of L-741,597. (Conditions: Hichrom DNBL column (250 x 4.6mm i.d.); mobile phase 5% MeOH in 1-Chlorobutane; Flow 1ml/min; Detect 230nm).

enantiomeric selectivity may therefore be to sterically restrict access to an optimal π-π interaction. The effect of this could therefore be to change the directionality of other substituents on the molecule towards the CSP thereby optimising the energy of interaction for one enantiomer.

It was found that prediction of separation selectivities between the DNBL and DNBPG CSP's was not straightforward. For example, the highest separation selectivity was achieved for 7 on the DNBL phase and this compound also proved to have one of the highest separation selectivities using the DNBPG phase, suggesting that there is some commonality of interactions between the two phases for this compound. However, a major difference between the phases is demonstrated with the C5 pyrrolidine derivative 2. This showed the lowest separation selectivity on the DNBL but one of the highest using DNBPG.

The effect of mobile phase was investigated, as it had been shown that ethanol/hexane mixtures were capable of achieving high separation selectivities, but it was felt that for preparative purposes this would be inadequate due to solubility criteria since these molecules were poorly soluble in such mixtures. Also, since high percentages of ethanol were required for adequate elution times the viscosity of the mobile phase was higher than desired leading to poor mass transport between the phases and consequent

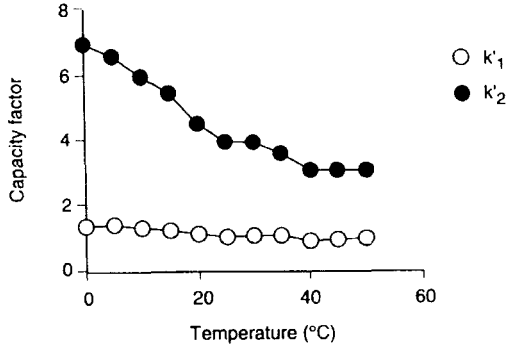

Figure 5. Effect of temperature on capacity factors for L-741,597.

band-broadening particularly of the later eluting enantiomer. Chlorobutane was chosen as the bulk solvent as it displayed increased solubilising properties of the compounds and an increase in polarity over hexane allowing the proportion of organic modifier to be minimised. A typical chromatogram, demonstrating the very high enantioselectivity obtained is shown (Figure 4).

The effect of temperature on capacity factor is demonstrated (Figure 5). The effect of increasing temperature on the capacity factor for the first eluting enantiomer is minimal whereas a steady increase of K'_2 can be demonstrated for decreasing temperature. This is probably due to reducing the degrees of freedom of both the enantiomer and the chiral stationary phase hence the energy of interaction increases leading to an increase in capacity factor. As K'_1 is constant this naturally leads to an increase in α, but the effect of this on R_S is less clear (Figure 6). Resolution appears to reach a maximum at 20-25°C with a rapid decline after this. Resolution would be expected to decrease at higher temperature as K'_2 decreases but not necessarily at lower temperatures. This could be explained by the higher solvent viscocities at lower temperatures, leading again to poor mass-transfer between stationary and mobile phases, resulting in a large increase in the band broadening of both enantiomers even though K'_1 does not change.

Preparative chromatography could be performed for these enantiomers and was indeed the preferred method for resolution of final compounds of this class. This was despite the success of chemical resolution using Edman degradation chemistry of a suitable amino intermediate, as this required further synthetic elaboration to yield the desired compound. Solubility in organic

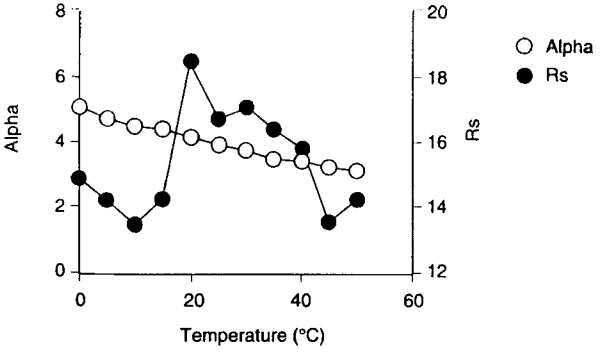

Figure 6. Effect of temperature on separation selectivity and resolution for L-741,597.

solvents was a major issue for these compounds, affecting the choice of mobile phase as described above. It was found that most compounds could be dissolved in a 9/1 chloroform / ethanol mixture as free bases at 10mg/mL. Loadings of 2.5mL, equivalent to 25mg, onto the 20mm i.d. column could then be achieved routinely and the enantiomers separated to baseline. The limiting factor to loading in this case appeared to be volume injected, as larger volumes would cause a large disturbance in peak shape leading to loss of resolution. Where solubility permitted, higher loadings of material in an equivalent volume was achievable with >50mg/run injections being performed. Repeat injections could be made or the process automated if >1g of compound was required. Throughput of this methodology was high with 25mg injections possible every 7 minutes, and peaks were collected in high yield with purities >99% e.e.

As would be expected with the specific stereochemistry of Pirkle phases, the elution order of the enantiomers was entirely predictable with the 3*R* enantiomer always eluting before the 3*S*, making identification of the enantiomer required for CCK_B receptor binding facile.

CONCLUSIONS

In general, the Pirkle DNBL CSP provides the highest selectivity for the 1,4-benzodiazepin-2-ones bearing C5 amidine substituents studied. Use of this CSP allowed facile monitoring of reactions from enantioselective syntheses to determine optical purities and the preparation of material from milligram to gram quantities for biological evaluation.

Although insufficient compounds were available to perform quantitative structural analysis, the compounds that separated well appear to have large bulky groups at C5 that cause this substituent to lie out of the plane of the benzodiazepine portion of the molecule. Additionally it is suggested that the steric effect of the C5 substituents affect the attainment of the dominant π- π interaction and in so doing alter the directionality of interactions of other substituents thereby accentuating the energy differences between the 3*S* and 3*R* enantiomers.

Preparatively, high separation loadings were possible due to the high separation selectivities and good resolutions observed although solubility in suitable organic solvents was a limiting factor. Gram quantities of enantiomers could be prepared using this methodology and this helped to establish chiral HPLC as the method of choice over more classical resolution methods for these molecules.

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REFERENCES

1. R. Y. Wang, Ann. N.Y. Acad. Sci., **537**, 362-379, (1988).

2. M. Albus, Prog. Neuro-Psychopharmacol. Biol. Psychiatry., **12**, S5-S21 (1988).

3. T. Moran, R. Robinson, M. S. Goldrich, P. McHigh, Brain Res., **365**, 175-179 (1986).

4. R. S. Chang, V. J. Lotti, R. L. Monaghan, J. Birnbaum, E. O. Stapely, M. A. Goetz , G. Albers-Schonberg, A. A. Patchett, J. M. Liesch, O. D. Hensens, J. P. Springer, Science, **230**, 177-179 (1985).

5. M. G. Bock, R. M. DiPardo, B. E. Evans, K. E.Rittle, W. L. Whitter, D. F. Veber, P. S. Anderson, R. M. Friedinger, J. Med. Chem., **32**, 16-23, (1989).

6. R. S. Chang , T. B. Chen, M. G. Bock, R. M. Freidinger, R. Chen, A. Rosegay, V. J. Lotti, Mol. Pharmacol, **35**, 803-808 (1989).

7. V. J. Lotti, R. S. Chang, Eur. J. Pharmacol., **162**, 273-280 (1989).

8. M. G. Bock, Drugs Fut., **16**, 631, (1991).

9. G. A. Showell, S. Bourrain, J. G. Neduvelil, S. R.Fletcher, R. Baker, A. P. Watt, A. E. Fletcher, S. B. Freedman, J. A. Kemp, G. R. Marshall, S. Patel, A. J. Smith, V. G. Matassa J. Med Chem., **37**, 719-721 (1994).

10. W. H. Pirkle, A. Tsipouras, J. Chromatogr., **291**, 291-298, (1984).

11. M. S. Chambers. S. C. Hobbs, S. R. Fletcher, V. G. Matassa, P. J. Mitchell, A. P. Watt, R. Baker, S. B. Freedman, S. Patel, A. J. Smith, Bioorg. Med. Chem. Lett., **3 (10)**, 1919-1924 (1993).

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