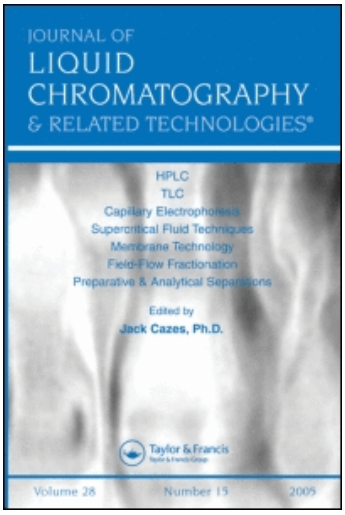

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CHIRAL SEPARATION OF 4,4-DISUBSTITUTED PIPERIDINYL SUBSTANCE P ANTAGONISTS

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CHIRAL SEPARATION OF 4,4-DISUBSTITUTED PIPERIDINYL SUBSTANCE P ANTAGONISTS

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ABSTRACT

The chiral separation of fourteen Substance P antagonists, substituted at a benzylic carbon to generate a chiral centre, was investigated using Chiracel OD-H and Chiralpak AD stationary phases. The nature of an N-substituent, distant from the chiral centre, was found to modulate separation selectivity. Aromatic substitution on the benzyl group also affected separation selectivity, but to a lesser degree. Some complementary character between Chiracel OD-H and Chiralpak AD was seen, but selection of the optimal phase did not appear predictable. The effect of temperature was also unanticipated, with some compounds showing the expected decrease of separation selectivity with temperature, whereas, an example of an improvement in separation selectivity with temperature to give an entropically controlled separation was also observed. This serves to highlight the complex nature of enantioselective interactions using chiral polymers and suggests that, given an unknown compound in this series, adequate separation conditions are difficult to predict.

INTRODUCTION

Substance P (SP), an undecapeptide, is a member of the tachykinin family of peptides and has been shown to act predominantly through the neurokinin-1 (NK_1) receptor. The action of SP at the NK_1 receptor has been implicated in responses, such as the transmission of pain,[1] vasodilation,[2] smooth muscle contraction of the airway neurogenic inflammation,[3] and immune response regulation[4]. This suggests that NK_1 antagonists may find benefit in the treatment of conditions such as, migraine,[5,6] rheumatoid arthritis, and in the maintenance of chronic pain.[7] It has further been suggested that the action of an NK_1 antagonist may be useful in the control of cytotoxic-agent induced emesis, such as that induced in chemotherapeutic treatments,[8-10] Recently, the localisation of substance P in specific brain regions suggested that substance P antagonists may possess useful psychotherapeutic properties, and this has been supported through the finding that the substance P antagonist, MK-0869, demonstrates robust antidepressant effects in the clinic.[11]

There have been a number of recent reports of selective, non-peptidic SP antagonists from many structurally different classes.[12-18] Amongst the findings in terms of structure-activity relationships, are that stereochemical constraints exist around the NK_1 receptor[19,20] and as such, if compounds with chiral centres are to be evaluated then resolution of the individual enantiomers is a prerequisite.

Here, we report a series of 4,4-disubstituted piperidines (see Table 1 for general structure) in which substitution at the benzylic carbon by methyl or hydroxymethyl has generated a chiral centre. Although resolution of these compounds can be achieved by chemical means, the efficiency of this process must be monitored, hence, a direct measure of the enantiomeric excess of the final compounds is required. This has led us to the investigation of chiral HPLC methodologies that would be applicable to a wide range of substituent changes as the structure-activity relationships for these molecules were explored.

In considering general approaches, we felt that, although, inclusion complexes with cyclodextrin[21,22] or interaction with immobilised protein phases[23] may resolve some compounds this was unlikely to be general enough. Indeed, analysis of the molecules show them to contain a paucity of functionality, even for the classical "Pirkle" brush-type interactions of H-bonding, dipole/dipole, and π-acid / π-base, particularly as some molecules contain an electron deficient aromatic ring, whilst others contain an electron rich one.

It is well known that phenyl carbamate derivatised cellulosic and amylosic phases, in which the derivative is adsorbed onto macroporous silica gel, offer a mode of chiral selectivity that may be reasonably broad in scope and have been reported to separate many analytes.[24] Although, one can rationalise the interac-

tions observed in terms of hydrogen bonding through the NH and CO functions of the carbamate moiety, the nature of the interactions is likely to be multimodal, comprising chiral cavities which may change conformation, particularly as a function of temperature.[25] Furthermore, thermodynamic data obtained using cellulose and amylose stationary phases, indicate that whilst separations are commonly enthalpy controlled, entropy controlled separations have been reported, whereby, separation selectivity is seen to improve with increasing temperature.[26,27]

To this end, we have examined fourteen analogues in an attempt to establish HPLC conditions for such molecules that give an improved chance of effecting a separation without undertaking a full-scale optimisation for each compound. In particular, we have looked at whether cellulose should be preferred to amylose when using the same tris-3,5 dimethylphenyl carbamate derivatives (i.e. Chiracel OD-H compared to Chiralpak AD) and the effect of substitution pattern. Additionally, by determining the thermodynamics of the separation, it is considered whether elevated or reduced temperature should be preferentially employed in the first instance.

EXPERIMENTAL

Materials

All compounds described were synthesised in-house with identity and purity confirmed by NMR, MS, HPLC, and elemental analysis. HPLC grade hexane was obtained from Fisher (Loughborough, UK) and AnaLar grade ethanol from BDH (Poole, UK).

Instrumentation

An HP1090M series high performance liquid chromatograph was used for the analytical separations (Hewlett Packard, Avondale, USA). The system is comprised of 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 diode-array detector (DAD) and data was processed using a HP ChemStation (rev. 3.03). Column temperature was regulated using an external column heater/ cooler device, the Violet T-55S (Flowgen, UK).

Chromatographic Conditions

HPLC analysis was performed using columns of derivatised cellulose and amylose adsorbed onto silica gel (Chiracel and Chiralpak columns, Diacel

Chemical Industries, Ltd, Japan). The main phases used were Chiralpak AD (250 x 4.6 mm i.d., 10 μm) and Chiracel OD-H (250 x 4.6 mm i.d., 5 μm). Typical mobile phases were 5-10% ethanol in hexane with a flow rate of 1.0 mL/min. The DAD was set to 210 nm with a bandwidth of 10 nm as this was the λ_{max} for the majority of the compounds. Between 2 and 10 μL of a 1 mg/mL solution in ethanol was analyzed.

RESULTS AND DISCUSSION

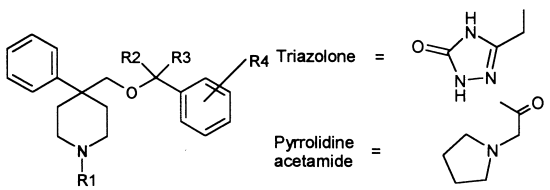
The purpose of the study was to attempt to understand the nature of the interactions which are participating in affording enantioselectivity for this series of molecules and, therefore, be able to make more rational decisions in phase selection and separation conditions in the future. The range of substituents studied is shown in Table 1. Two different N-substituents were utilised, namely triazolone (TZ) and pyrrolidine acetamide (PA). The benzylic carbon, which generates the chiral centre, may be substituted with methyl, hydroxymethyl, or in the case of **11**, both. Aromatic substitution was also varied with both mono- and bis- substitution, mainly with electron withdrawing groups.

The effect of the N-substituent on the chiral recognition process is illustrated by comparing **1** with **12** and **2** with **14,** using a Chiralpak AD column. In both cases, only the N-substituent changes, and in both cases, there is a marked improvement in both separation selectivity and resolution of the PA derivative over the TZ. As these groups are remote from the chiral centre, one could postulate that the effect of the PA is to anchor the molecule in a favourable orientation for chiral recognition, although the capacity factors for PA are smaller than for TZ. Indeed, the PA derivative now contains a urea function leading to restricted rotation about the N-CO-N axis due to the double bond character of the N-C bond. This in turn suggests that there is a more fixed directionality of this carbonyl group which then will lead to a lower energy complex with the CSP should the molecule be able to orient correctly.

The effect of moving from α-methyl to α-hydroxymethyl is less easy to distinguish between the TZ and PA series, although, some tentative conclusions may be drawn. Although, the interaction of the solute and CSP is a net summation of both chiral and achiral interactions, it is assumed that the achiral contributions are essentially equivalent as there is no more than a 2 fold difference in k'_2, hence, the majority of differences observed may be said to be due to enantioselective interactions. If we compare **1** with **10** in the TZ series, although the separation is very poor for both compounds at 10% EtOH, at 5% EtOH it would appear that there is certainly an improvement in selectivity and resolution in the case of the hydroxymethyl analogue. This demonstrates that there must be an extra polar or H-bonding interaction to be gained over the methyl analogue, which may be negated at higher ethanol concentrations.

Table 1

Summary of Separation Selectivity and Resolution Data



					Chiralpak AD				Chiralcel OD-H			
					10% EtOH		5% EtOH		10% EtOH		5% EtOHG	
#	R1	R2	R3	R4	α	R_s	α	R_s	α	R_s	α	R_s
1	Triazolone	CH_3	H	3,5 CF_3	1.17	1.49	1.17	1.80	1.17	0.94	1.11	0.93
2	Triazolone	CH_3	H	3,5 Cl	1.00	---	1.00	---	1.11	0.73	1.09	0.72
3	Triazolone	CH_3	H	2,4 CF_3	1.27	1.09	1.33	1.86	---	---	---	---
4	Triazolone	CH_3	H	2,4 Cl	1.14	1.31	1.18	1.83	1.08	0.37	1.07	0.40
5	Triazolone	CH_3	H	3-1	1.00	---	1.00	---	1.00	---	1.00	---
6	Triazolone	CH_3	H	3-Br	1.00	---	1.00	---	1.05	0.12	1.05	0.17
7	Triazolone	CH_3	H	3-Cl	1.00	---	1.00	---	1.07	0.25	1.06	---
8	Triazolone	CH_3	H	3-OiPr	1.06	0.56	1.09	0.99	1.00	---	1.00	---
9	Triazolone	CH_3	H	3-NH_2	1.09	0.90	1.11	1.39	1.00	---	1.00	---
10	Triazolone	CH_2OH	H	3,5 CF_3	1.00	---	1.36	2.45				
11	Triazolone	CH_3	CH_2OH	3,5 CF_3	1.60	3.05	1.68	4.68				
12	Pyrrolidine acetamide	CH_3	H	3,5 CF_3	1.68	1.39	1.61	1.87				
13	Pyrrolidine acetamide	CH_2OH	H	3,5 CF_3	1.00	---	1.00	---	1.19	1.25	1.11	0.71
14	Pyrrolidine acetamide	CH_3	H	3,5 Cl	1.31	2.28	1.33	1.77	1.13	0.87	1.13	0.82

Indeed, moving to the geminally substituted compound **11**, it can be seen that the effects are almost additive, as this again, shows an improvement in chiral recognition over **10**. However, in this case the bond angles at the chiral centre will also be sterically altered which may be beneficial for chiral recognition.

The effect of aromatic substitution is considered for the TZ series. The 3,5 bis-CF_3 is more poorly resolved than the 2,4 bis-CF_3 and similarly, the 3,5 di-Cl is completely unresolved, whereas the 2,4 di-Cl is well separated. This suggests that should a π-π interaction occur between the stationary phase and analyte, there must be a steric component that is tolerant of 3,5-substitution, but not 2,4-substitution, as the electron densities of these rings are likely to be similar. This may be explicable in terms of the steric hindrance that would be expected with a face-to-face π-π interaction between the 3,5-substituted ring of the analyte and 3,5 di-methyl phenyl ring of the CSP, leading to a reduced interaction but which would not be so pronounced with a 2,4-substituted analyte.

The same argument may be invoked with the 3-halogen substituents (**5**, **6**, and **7**). Moving to more electron donating substituents as in **8** and **9**, separation is observed, but it is clear that electron density, whilst important for the TZ series, is certainly not dominant. For example, compound **2** and compound **14** differ only by the nature of the N-substituent, hence, one would assume that the electron densities are ostensibly the same. However, **2** is not resolved on Chiralpak AD, whereas, **14** is, so it can be concluded that although π-electron density will aid separation, this can be over-ridden by the inclusion of an additional interaction, as in the case of the PA substituent, which would appear to confer more in terms of creating an energy difference between the enantiomers.

In the PA series, we can only compare **12** with **13**; however, this shows the opposite change in stereoselectivity to that seen in the TZ series, with no separation seen for the hydroxymethyl derivative, but good separation seen for the α-methyl analogue. One explanation here would be, that the interaction with the PA function is predominant, which then restricts the ability of the molecule to pick up an additional interaction; in fact, it becomes detrimental. Alternatively, the binding modes between the series may be completely different and one way to explore this is to look at the effect of temperature to see if the trends observed are broadly the same for different substituents.

Since the kinetic (or hydrodynamic) contribution to chromatographic efficiency is reliant on the nature of the packed bed, axial molecular diffusion and mass transfer from mobile to stationary phase and back again is critical. This mass transfer process is, in turn, dependant on the diffusion coefficient which is shown to be proportional to temperature and inversely proportional to viscosity, such that increasing temperature should improve column efficiency by both improving diffusion and decreasing mobile phase viscosity.

However, the thermodynamics of chromatographic processes show that the separation selectivity (α) relies on there being a difference in the free-energy of interaction ($\Delta\Delta G°$) with the stationary phase such that

$$\Delta\Delta G° = RT\ln \alpha \quad (1)$$

where R is the gas constant and T the absolute temperature. This may be expressed in terms of enthalpic and entropic contributions and rewritten in terms of α, such that:

$$\ln \alpha = -\Delta\Delta H°/RT + \Delta\Delta S°/R \quad (2)$$

where $\Delta\Delta H°$ is the difference in enthalpy and $\Delta\Delta S°$ the difference in entropy between the two enantiomers and the stationary phase. One might expect that for chiral chromatography, molecular interactions would be enthalpy controlled and this is indeed the general finding in practice. It follows, therefore, that an increase in temperature would impart additional energy to both the analyte and the stationary phase, thereby, diminishing $\Delta\Delta H°$ and possibly increasing $\Delta\Delta S°$ leading to a reduction in α. Consequently, kinetic and thermodynamic considerations, at first sight appear to be in direct opposition as higher temperatures should favour the former and lower temperatures the latter.

However, examination of equation (**2**) shows that a plot of ln α vs. 1/T should give a straight line of slope $-\Delta\Delta H°/R$ and an intercept of $\Delta\Delta S°/R$. When ln α =0 (i.e. α=1) the enantiomers coelute and at this temperature (T_{iso}) the enthalpic and entropic contributions are balanced such that:

$$T_{iso} = \Delta\Delta H°/\Delta\Delta S° \quad (3)$$

Theory would, therefore, predict that above this temperature the elution order of the enantiomers would reverse and an increase in separation selectivity with temperature will be observed; in such an instance separations are said to be entropically controlled and such separations have only been infrequently reported.[26,27]

We examined the effect of temperature initially on two analogues, **4** and **14,** as examples from the TZ and PA series using a Chiralpak AD column. Temperature was varied between 0°C and 40°C in 10°C increments, and the separation selectivity calculated for each temperature. Plotting 1/T vs. ln α gave the expected linear relationship (Figure 1) for both compounds with α decreasing as a function of temperature, which is consistent with an enthalpy-

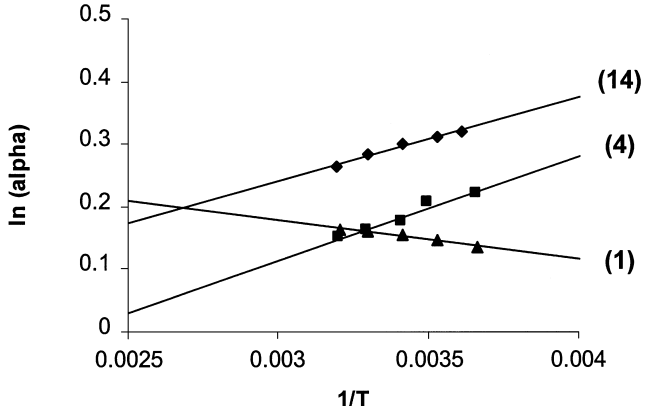


Figure 1. Temperature dependence of the separation selectivity α for compounds **1**, **4**, and **14** on Chiralpak AD, obtained by plotting the natural log of the separation selectivity versus the reciprocal of the temperature (in Kelvin).

controlled process. This suggested that we should investigate subambient temperatures as part of a chiral separation screening protocol. However, in conducting this experiment for compound **1**, the line was found to slope in the opposite direction, such that, separation selectivity increased with increasing temperature. This was unexpected, as the only difference between **1** and **4** is the aromatic substitution pattern; yet clearly, this is altering the mode of interaction with the stationary phase profoundly.

The experiment was then repeated using the same three compounds, **1**, **4**, and **14**, only switching from a Chiralpak AD to a Chiracel OD-H column. The data is summarised in Figure 2. Although, the carbamate derivative is the same for both stationary phases, differences would still be anticipated as the configuration of the glucose units between cellulose and amylose is different as is the higher order structure.[28] For **4,** there is only a very poor separation and, hence, it is difficult to surmise any trend. Intriguingly however, the temperature dependencies for **1** and **14** are opposite to that observed for Chiralpak AD, with **1** now being enthalpy controlled and **14** being entropy controlled. It is, therefore, apparent even from this limited study, that, although, it may be possible to investigate and optimise the dependencies on chiral recognition of a single analyte for this series in some detail, the behaviour of even close analogues on cellulose and amylose based phases are likely to be more difficult to predict.

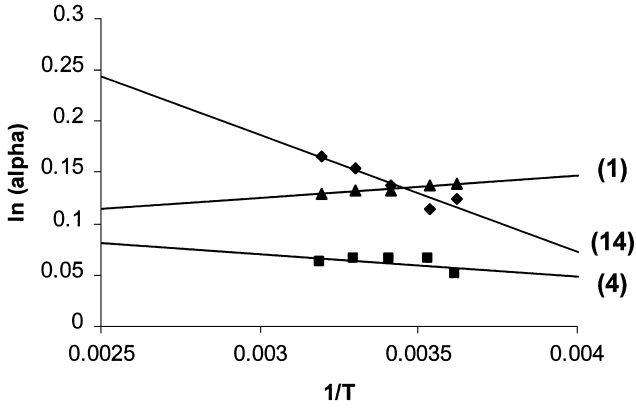


Figure 2. Temperature dependence of the separation selectivity α for compounds **1**, **4**, and **14** on Chiracel OD-H, obtained by plotting the natural log of the separation selectivity versus the reciprocal of the temperature (in Kelvin).

CONCLUSIONS

Although, it was possible, with this series of compounds, to explore the effects of analyte structure on chiral recognition, it is evident that it is unlikely that we can generate general conditions for chiral screening with these phases. The unexpected change, due to only small structural modification, from being an enthalpy to an entropy-controlled separation, suggests that for full optimisation compounds will still need to be treated on a case-by-case basis. Furthermore, the optimisation conditions learned from dealing with cellulose phases do not translate into amylose phases, as for some compounds, equivalent separation selectivities are observed, whereas, for others it is complementary. This is also true of the separation thermodynamics which, for two compounds studied, were found to be opposite for the two phases.

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