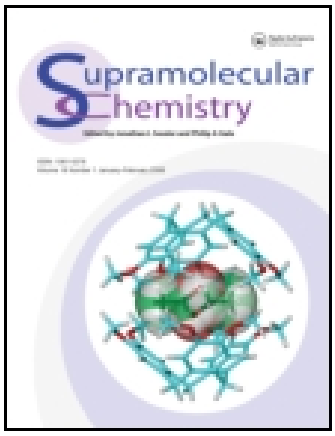

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The enantiomeric separation of a series of fifteen racemic 4,5-disubstituted imidazole compounds was examined by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). These alkaloid analytes are important precursors for the total synthesis of the natural product calcaridine A and other *Leucetta*-derived alkaloids. Therefore, the enantiomeric analysis of these analytes is not only important in the production of enantiomerically pure calcaridine A, but also for a better understanding of the stereochemistry involved in related biosynthetic pathways. Several bonded cyclodextrin (both native and derivatised) stationary phases were evaluated for their ability to separate these racemates via HPLC. Likewise, several cyclodextrin derivatives were evaluated for their ability to separate this set of compounds via CE. Using HPLC, 14 of the 15 racemic compounds were separated. Eight of the analytes were separated using CE with resolutions up to 7.0. Using both HPLC and CE approaches, the entire set of analytes was separated. The optimisation of these separations was discussed and a comparison between the chiral selectors used was made. Lastly, the similarity of the 15 analytes allowed for insight into the mechanism of chiral recognition.

Keywords: enantiomeric separations; chiral separations; high performance liquid chromatography; capillary electrophoresis; cyclodextrin; calcaridine A

Introduction

The synthesis of natural products is of great interest to organic and medicinal chemists. Bioactive molecules and their metabolites, as well as any related chemical derivatives or analogues, are an abundant resource for the development of new pharmaceutical compounds (*1–6*). The intricate structural constitution of many naturally occurring molecules can make synthesis a challenging undertaking. Furthermore, the stereochemical requirements often increase the burden of the task and may require the use of asymmetric synthetic pathways or stereoselective separation techniques. Considering the potential pharmacological importance of natural products, researchers are continually searching for new, undiscovered molecules. Recognising that about 70% of the earth's surface is water, many of these researchers have intuitively turned to the seas and marine organisms in search of these compounds (*7–18*). Currently, one group of organisms which has come under scrutiny is marine sponges (*10–18*).

The Fijian sponge, *Leucetta* sp., has been shown to be a good source of some very interesting alkaloid imidazole compounds, such as calcaridine A, spirocalcaridine A and B, and spiroleucettadine (*11–17*). The latter, spiroleucettadine, has been shown to possess antibacterial properties that are comparable to those of vancomycin and penicillin (*15*). Calcaridine A is thought to be a precursor in the biosynthesis of spiroleucettadine. Recently, calcaridine A has been successfully synthesised through a multi-step process that concluded with an oxidative rearrangement of a 4,5-disubstituted imidazole (*16–17*). Throughout this synthetic pathway, several newly synthesised chiral alkaloids were produced. These chiral precursors were synthesised and used in their racemic form, such that, when the oxidative rearrangement step was performed, the synthetic material was formed as a racemic mixture of calcaridine A. In addition to calcaridine A, the C14-epimer was formed in the oxidative rearrangement. The enantiomeric separation and purification of any of these racemic precursors would allow for the production of enantiomerically pure calcaridine A. Furthermore, enantioselective separation techniques that are applicable to separate these types of compounds may be helpful in understanding when and how proper stereochemistry is introduced throughout the biosynthetic pathways involving these alkaloids. For these reasons, the stereoselective separation of these precursors is of relevance.

Currently, the most commonly used technique for enantiomeric separations is high performance liquid chromatography (HPLC) (*19*). One popular set of chiral HPLC stationary phases is cyclodextrins and their derivatives. Cyclodextrins have been used to separate a vast number of chiral analytes and have shown

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to be extremely applicable towards the separation of racemic analytes containing aromatic functionality (*20–25*). Under reverse phase (RP) solvent conditions, aromatic moieties often form inclusion complexes with the cyclodextrins. Separation by HPLC offers the advantage of preparative capabilities, which could aid in the production of the pure enantiomers of calcaridine A in the long-term.

Additionally, capillary electrophoresis (CE) may offer another means by which these racemic precursors could be resolved. Cyclodextrins and their derivatives dominate the field of chiral selectors used in CE (*26–28*). Though these separations offer limited potential in the preparative production of enantiomerically pure calcaridine A, they could be useful for determining enantiomeric excesses of asymmetric synthetic steps.

In this study, the enantiomeric separation of 15 newly synthesised 4,5-disubstituted imidazoles was explored. Both HPLC and CE were tested and compared for their abilities to separate the enantiomers of these chiral analytes. To our knowledge, none of these compounds have previously been subjected to any chiral chromatographic or electrophoretic technique. Also, the structural similarity of this series of analytes may allow some insight into their chiral recognition by cyclodextrins and provide a basis for exploration of related systems.

Experimental

Materials

HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from VWR (West Chester, PA, USA). Deionised water was produced by a Milli-Q system (Billerica, MA, USA). Ammonium acetate (H_4NOAc), glacial acetic acid (HOAc), triethylamine (TEA), sodium phosphate monobasic and dibasic, phosphoric acid and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). All the chiral analytes listed in Figure 1 were synthesised according to the literature by Koswatta et al. (*16–18*). Note, compound **5** possesses an azide group and was stored away from light. All HPLC columns used were of analytical dimensions (25 cm × 4.6 mm, 5 μm) and were obtained from Advanced Separation Technologies (Whippany, NJ, USA). The columns used were as follows: β-CD (β-cyclodextrin), HP-RSP (high performance hydroxypropyl ether β-cyclodextrin), RSP (hydroxypropyl ether β-cyclodextrin), AC (acetylated β-cyclodextrin), RN and SN (R- and S-naphthylethyl carbamate β-cyclodextrin), DMP (dimethylphenyl carbamate β-cyclodextrin), DNP (2,6-dinitro-4-trifluoromethylphenyl ether β-cyclodextrin) and DM (dimethylated β-cyclodextrin). CE chiral selectors were obtained from Sigma-Aldrich (Milwaukee, WI, USA) and are as follows: SBCD (sulphated β-cyclodextrin), DMBCD (dimethylated β-cyclodextrin) and HPBCD (hydroxypropyl β-cyclodextrin). The untreated fused silica capillaries (50 μm i.d.) for CE were purchased from Polymicro Technologies (Pheonix, AX, USA).

Methods

All HPLC analyses were performed on one of two chromatographic systems, both of which were produced by Shimadzu (Kyoto, Japan). The pumps used were models LC-6A and LC-10A, the detectors were SPD-6A and SPD-10A, and the data were recorded using a SPD-6A chromatographic integrator. In both cases, the samples were injected using a 6-port injector equipped with 10 μL injection loops.

All CE experiments were performed on Beckman Coulter P/ACE MDQ (Fullerton, CA, USA) CE system equipped with an on-column UV–visible detector.

HPLC analyses

All samples were dissolved in MeOH for analyses done in RP and in ACN when the tests were performed in the polar organic mode (PO). The wavelength of detection for all HPLC experiments was 254 nm. H_4NOAc buffers were adjusted to a desired pH using HOAc. The mobile phase compositions listed throughout this manuscript are in volume to volume ratios. All mobile phases were degassed by ultrasonication under vacuum for 5 min before use. The mobile phase flow rate throughout the entire study was 1 ml/min.

CE analyses

Unmodified fused-silica capillary was thermostated at 25°C, with the length being 30 cm to the end and 20 cm to the detection window. UV detection was accomplished at 214 nm. Sodium phosphate, monobasic and dibasic (1:1), was dissolved in deionised water and adjusted with concentrated phosphoric acid to the desired pH. Buffer additives (β-cyclodextrin derivatives) were added directly into the buffer. All samples were dissolved in a solution consisting of 20% methanol and 80% water. Normal polarity (NP) and reversed polarity analyses used voltages of + 7 and − 7 kV, respectively.

At the beginning of each series of experiments, the capillary was rinsed with 1 M sodium hydroxide for 5 min and water for 5 min for conditioning. Between each run, the capillary was flushed with 1 M sodium hydroxide for 2 min, water for 2 min and buffer for 2 min. Then, the sample solution was injected hydrodynamically at 0.5 psi for 5 s.

Calculations

The HPLC retention factors (k_1'), selectivities (α) and resolution (R_s) values were calculated as follows: $k_1' = (t_{r1} - t_0)/t_{r1}$, $\alpha = (t_{r2} - t_0)/(t_{r1} - t_0)$ and

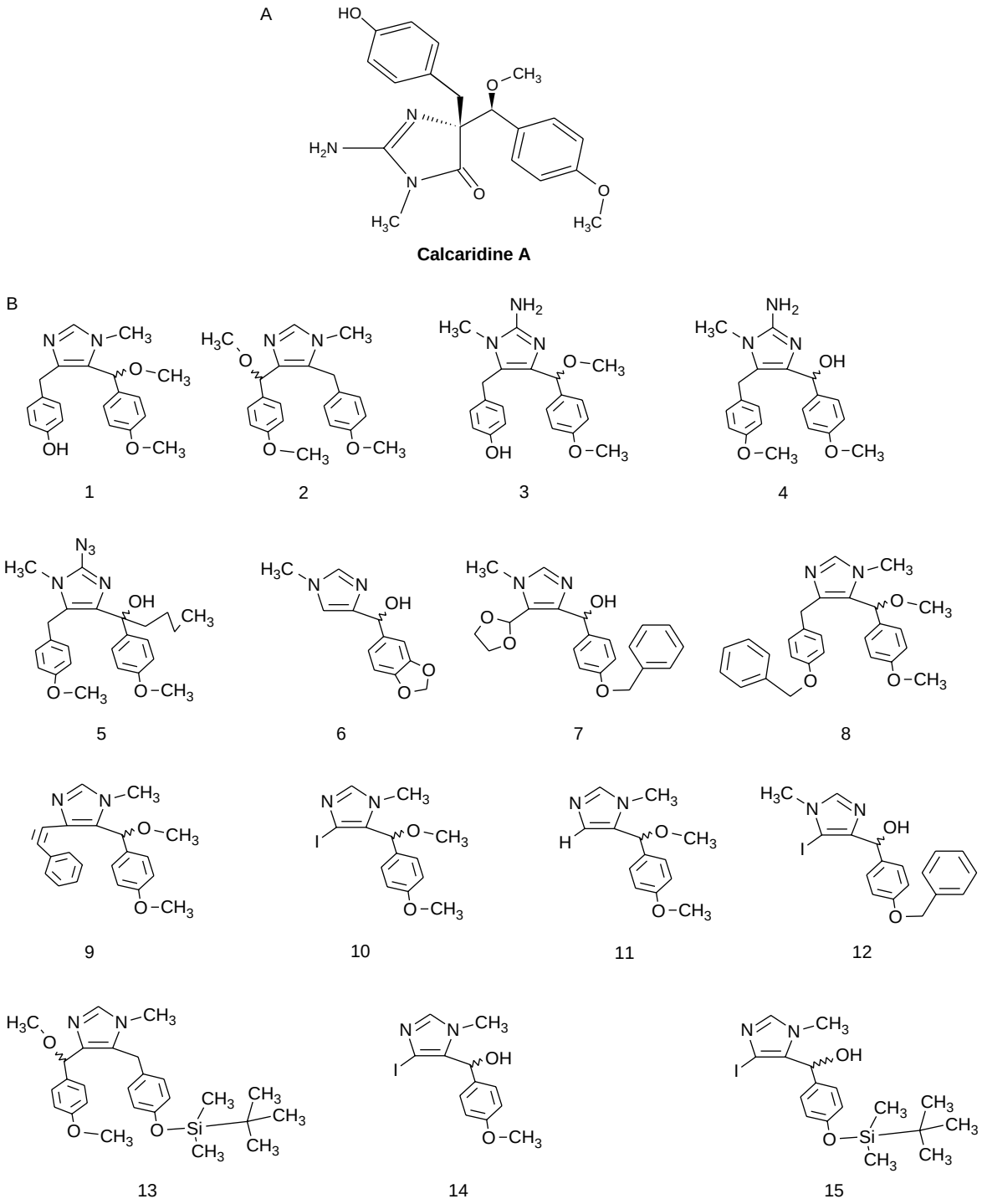

Figure 1. Structure of calcaridine A (A) and the structures of 15 racemates that were subjected to enantioselective HPLC and CE analyses in this study (B).

$R_s = 2^*(t_{r2} - t_{r1})/W_1 + W_2)$, where t_{r1} and t_{r2} are the retention times of the first and second eluting peaks, t_0 is the dead time (found by calculating the void volume of the column) and W_1 and W_2 are the baseline widths of the first and second eluting peaks.

In CE, the migration time of the first peak is represented by t_{m1}. The efficiencies (N), selectivities (α) and resolution (R_s) values were calculated as follows: $N = 16(t_m/W)^2$, $\alpha = \mu_{app1}/\mu_{app2}$, $R_s = 2^*(t_{r2} - t_{r1})/W_1 + W_2)$, where μ_{app1} and μ_{app2} represent the apparent electrophoretic

mobilities of the first and second peaks. Dimethyl sulphoxide was used as the electroosmotic flow (EOF) marker. All other constants are the same as above.

Results and discussion

Analytes

Figure 1(A) shows the structure of calcaridine A. Also, in Figure 1(B), the chemical structures of the 15, racemic synthetic intermediates are shown. As can be seen, this set of intermediates offers systematic changes in functionalities, as well as placement of the stereogenic centre (being alpha to either the 4-position or the 5-position of the imidazole core).

HPLC analyses

A summary of the optimised HPLC separations data is given in Table 1. The optimised conditions were considered to be when the resolution (R_s) values were greatest and the retention factors (k') were satisfactorily small. Enantioselectivity (α) was observed for 14 of the 15 compounds. These α values ranged from 1.08 (compounds **12** and **13**) to 1.29 (compound **6**). Compound **15** was not separable under the conditions used. The R_s values for these compounds ranged from 0.6 (compound **13**) to 2.0 (compound **10**). Seven of the compounds had R_s values greater than or equal to 1.5 and were considered to be baseline separated.

As can be seen in Table 1, the 3,5-dimethylphenyl-derivatised β-cyclodextrin (DMP) chiral stationary phase (CSP) produced the most favourable HPLC separations for 5 of the 15 compounds. The hydroxypropyl-β-cyclodextrin derivatives (HP-RSP and RSP), as well as the native β-cyclodextrin (β-CD) chiral stationary phase, each accounted for three of the baseline separations. In fact, it was the native β-CD phase which produced the greatest R_s value observed in this study ($R_s = 2.0$ for compound **10**). It is important to note that just because a CSP does not appear in Table 1, does not necessarily mean it did not produce any separations, because Table 1 lists only the best results.

A more complete comparison between the nine CSPs tested in this study can be seen in Figure 2, which depicts the total number of partial ($0.4 < R_s < 1.5$) and baseline ($R_s > 1.5$) separations obtained using each chiral selector. The chiral stationary phase that produced the greatest number of separated compounds was the HP-RSP/RSP media. This chiral selector offered enantioselectivity for 66% of the compounds tested. Additionally, this CSP produced four baseline separations; a number that was matched only by the S-naphthylethyl carbamate (SN) derivative of β-cyclodextrin. For this reason, it was determined that this stationary phase should be the recommended starting point when attempting to separate similar analytes. The chiral selectors AC, β-CD, SN, RN and DMP (Figure 2) separated only six to seven of the analytes. Keeping in mind that the DMP phase was previously acknowledged as the one that gave the greatest number of 'best' separations, it is interesting to see that it only ranks modestly compared to other CSPs in terms of total separations. This is a result of the DMP phase being complementary to the other phases tested, which allowed for the separation of certain compounds that were not separable when using the other chiral selectors. The CSPs that performed poorly were the DNP and the DM, both of which only showed partial separations for four analytes and no baseline separations.

In this study, both RP and PO modes were tested. The normal phase was also attempted, but the initial results were very poor. In the RP mode, both ACN and methanol were tested as organic modifiers. In general, using ACN as the organic modifier resulted in sharper peaks with smaller

Table 1. Summary of the optimised HPLC enantiomeric separations.

Analyte no.	CSP[a]	k'_1	α	R_s	Mobile phase
1	HP-RSP	4.46	1.15	1.6	85/15 20 mM H_4OAc/ACN, pH = 4.1
2	DMP	3.53	1.13	1.6	80/20 20 mM H_4OAc/ACN, pH = 4.1
3	RN	11.81	1.06	1.3	75/25 20 mM H_4OAc/ACN, pH = 4.1
4	SN	4.55	1.10	1.6	85/15 20 mM H_4OAc/ACN, pH = 4.1
5	HP-RSP	3.45	1.09	1.1	85/15 20 mM H_4OAc/ACN, pH = 4.1
6	DMP	2.04	1.29	1.8	95/5 20 mM H_4OAc/ACN, pH = 4.1
7	DMP	0.78	1.17	1.3	99/1/.3/.2 ACN/MeOH/HOAc/TEA
8	β-CD	1.55	1.16	1.1	80/20 20 mM H_4OAc/ACN, pH = 4.1
9	β-CD	7.97	1.19	1.6	85/15 20 mM H_4OAc/ACN, pH = 4.1
10	β-CD	3.73	1.27	2.0	75/25 20 mM H_4OAc/ACN, pH = 4.1
11	DMP	4.71	1.14	1.3	95/5 20 mM H_4OAc/ACN, pH = 4.1
12	DMP	4.95	1.08	1.3	80/20 20 mM H_4OAc/ACN, pH = 4.1
13	AC	1.10	1.08	0.6	80/20 20 mM H_4OAc/MeOH, pH = 4.1
14	RSP	5.00	1.09	1.7	85/15 20 mM H_4OAc/ACN, pH = 4.1
15	N/A	–	–	–	–

[a] For a list of the defined CSP abbreviations, see the Experimental section.
Notes: N/A means no enantioseparation was observed for this compound under any condition. For the structures of the analytes, refer to Figure 1.

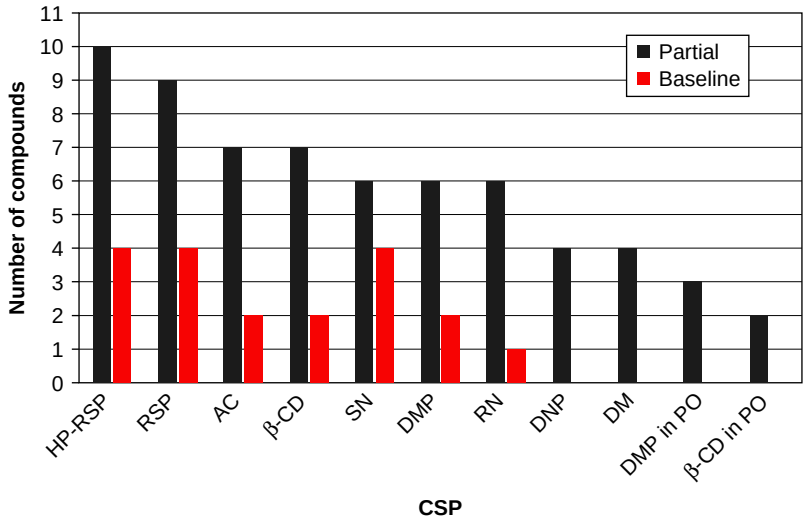

Figure 2. A representation of the total number of partial and baseline separations obtained using HPLC with different chiral stationary phases. Note, 'in PO' means the separations were obtained in the polar organic mode, whereas all other separations were obtained in the RP mode. For a list of the defined CSP abbreviations, see the Experimental section.

retention factors, whereas using methanol yielded the opposite, i.e. broader, longer retained peaks. For this reason, ACN typically resulted in better (higher R_s) separations. As indicated in Table 1, only once did methanol produce a better separation than ACN (compound **13**). Considering that all these compounds have an imidazole core, it was necessary to use a buffer (ammonium acetate in this study). Both the pH and the buffer concentration were examined in the optimisation process. The optimum pH and buffer concentrations were determined to be 4.1 and 20 mM, respectively. Changing the ratio of the aqueous to organic co-solvents was the most important tool in manipulating the separations. When using cyclodextrin-based CSPs in the RP, it is important to allow hydrophobic inclusion complexation to occur (*21*, *23*). The organic modifier competes with the analytes for the non-polar cavity, thus increases in the ACN (or methanol) percentage resulted in smaller retention factors. The occurrence of inclusion complexation with cyclodextrins has been extensively studied (*29*). Throughout this work, optimum aqueous/organic ratios ranged from 75/25 to 95/5.

Conversely, the PO mode does not permit inclusion complexes to form as the high organic content in the mobile phase completely occupies the cyclodextrin cavity (*30*). Rather, the dominant interaction in this operation is through hydrogen bonding. Thus, evaluation in the PO mode was also performed on all the CSPs which possessed free hydroxyl groups. Operating in the PO mode resulted in very few separations for this set of analytes. As listed in Table 1, only one of the optimised separations (compound **7**) was obtained using PO mobile phases. Figure 2 shows all the separations obtained in the PO mode. The only CSPs that showed any enantioselectivity were the DMP and native β-CD stationary phases, which separated just three and two analytes, respectively. However, when compared to the separations obtained in the RP mode, the separations found using the PO mode were of greater efficiency and of lower retention. This is a typical feature of the PO mode and arises from the faster adsorption–desorption kinetics involved in the hydrogen-bonding interactions that occur at the mouth of the cyclodextrin cavity versus the slower kinetics involved with inclusion complexation (*31*). A prime example of this can be seen in Figure 3, where the separation of compound **7** on the DMP CSP is shown. Figure 3(A) shows the separation in PO mode and Figure 3(B) shows the separation in the RP.

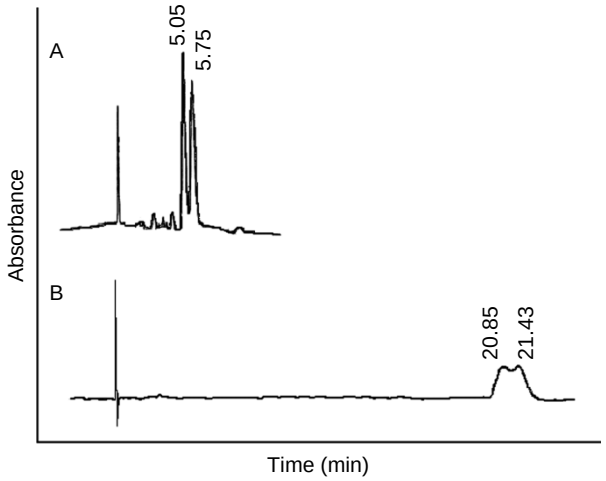

Figure 3. HPLC enantiomeric separations of compound **7** in the polar organic mode (A) and in RP (B). The CSP used was DMP. The mobile phase conditions were: (A) 99/1/.3/.2; ACN/MeOH/HOAc/TEA and (B) 75/25; 20 mM H4NOAc (pH 4.1)/ACN. For other chromatographic conditions see the Experimental section. See Figure 1 for the analyte structure.

Notice the highly efficient separation and short analysis time in the upper chromatogram, while the lower chromatogram is of poorer efficiency and longer retention. Keep in mind that for this set of analytes, this is only one of a few examples for which the PO mode gave the superior separation.

Generally, the main cause for such poor results in the PO mode was the lack of sufficient retention. Usually, the compounds that respond well to being chromatographed in the PO mode were those that had smaller retention factors in reversed phase conditions. Conversely, compounds that retained well in the RP mode were not well retained in the PO mode. An example of this phenomenon is shown in Figure 4, where the retention curves for two different analytes (compounds **1** and **14**) are shown. The left-hand region of the plot shows the retention in RP and the right-hand region shows the retention in the PO mode. Note that the curves cross each other when the separation mode is changed. This indicates that compound **14** relies heavily on the formation of an inclusion complex for retention, whereas compound **1** does not. In the PO mode, when inclusion complexation is not available, compound **1** gives rise to longer retention. Apparently, compound **1** can interact with the CSP through hydrogen bonding more than compound **14** can. Comparing the structure of these two analytes reveals that compound **1** has more possible hydrogen-bonding sites than compound **14**.

Perhaps the most interesting trends, which can lead to insights to the chiral recognition mechanism, were noted in the RP mode. First, it was observed that the analytes which possess aromatic substituents in either the 4-position or 5-position, but not both, were most easily separated. Examples of such analytes include compounds **6**, **10**, **11** and **14**. When these analytes interact with the cyclodextrins via inclusion complexation, there is no competition as to which hydrophobic portion of the analyte will occupy the cyclodextrin's cavity. Thus, the stereogenic centre is always near to the included moiety (i.e. the aromatic ring). Compounds that have hydrophobic aromatic moieties in both the 4-position and 5-position will dynamically compete for inclusion in the cyclodextrin cavity. This being the case, the stereogenic centre may not always be near to the chiral selector. Examples of analytes that posed such difficulties were compounds **1**, **2**, **3**, **4** and **9**. Yet, there were compounds (e.g. compounds **7** and **12**) which possessed only one hydrophobic substituent, but were still difficult to separate. Since these two analytes share an additional terminal benzyl moiety, it is reasonable that this is the cause of these molecules being more challenging to separate. It is evident that the benzyl groups will most easily occupy the stationary phase cavity, which will in turn place the stereogenic centre further from the chiral selector.

Compound **15** was the only analyte that was not separated by any CSP in either mode of operation. In fact, it was difficult in most cases to even get this molecule to elute from the stationary phase. This tenacious retention is surely an effect of the presence of the *tert*-butyldimethylsilane (TBDMS) functionality. However, compound **13** also possesses the TBDMS group and enantioselectivity was observed for it. This finding also supports the previous conclusion that analytes with two hydrophobic portions may compete with each other for the cavity of the cyclodextrin. Hence with compound **13**, the addition of the second substituent allowed for shorter retention and allowed for enantioselectivity to be observed.

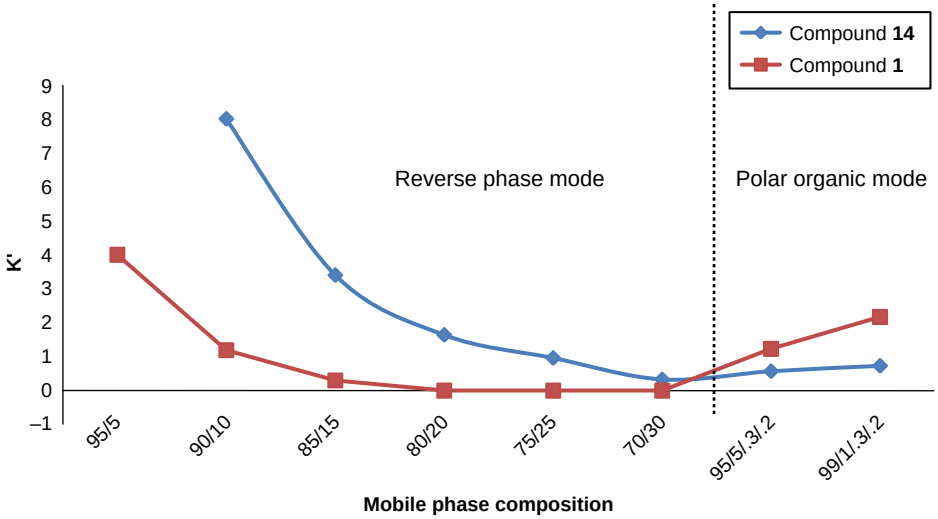

Figure 4. Retention curves for two of the analytes chromatographed using HPLC. The mobile phase composition in the RP region is 20 mM H_4OAc (pH 4.1)/ACN. The mobile phase composition in the polar organic region is ACN/MeOH/HOAc/TEA. The stationary phase was native β-CD. See Figure 1 for analyte structures.

CE analyses

In addition to the evaluation by HPLC, all the analytes were also subjected to enantioselective CE using derivatised cyclodextrin additives as chiral selectors. A summary of the optimised CE separations is given in Table 2. As can be seen, 8 out of the 15 compounds were separated by CE. Enantiomeric selectivity values (α) ranged from 1.02 (compound **7**) to 1.34 (compound **11**). Separation efficiency (N) values were observed from 2000 (compound **11**) to 27,000 (compound **7**). Lastly, resolution values (R_s) ranged from 0.8 (compound **7**) to 7.0 (compound **4**). Of the eight compounds that were separated using CE, five of them were baseline separated, with the worst baseline separation still exhibiting a resolution greater than 2.0.

For the optimised separations shown in Table 2, the background electrolyte composition and pH, as well as the chiral selector used, are also listed. The concentration of the background electrolyte was evaluated at 10 mM intervals from 10 to 100 mM in order to determine the proper concentration. As can be seen, typical optimum concentrations fell between 30 and 70 mM. The pH of the electrolyte was also adjusted for optimum separations. As listed in Table 2, optimised pH values were found to be approximately 3 and 8, depending on which (reversed or NP) mode was being employed. This is discussed in detail below.

Almost certainly, the most effective means by which an enantiomeric separation can be modified in CE is by changing the chiral selector itself. Seven of the eight best separations (Table 2) were obtained using SBCD as the chiral selector. Throughout this work, several cyclodextrin-based chiral selectors were evaluated. Figure 5 compares the efficacy of all the selectors tested. SBCD again shows its superiority by occupying two-thirds of the entire chart, as well as being a 'co-selector' for three other separations. In fact, of the 12 total separations, only 4 of them were obtained using a chiral selector other than pure SBCD and only 1 was found without use of any SBCD. As can be seen, HPBCD was unable to separate any analytes on its own, rather it needed to be used in combination with SBCD to obtain three separations. The only other cyclodextrin derivative determined to be useful for these enantiomeric separations was DMBCD, which separated just one analyte.

Another facet to Figure 5 is the distinction of the separations found in the reverse polarity mode versus those found in the NP mode. Totally 9 of the 12 separations were found in the NP mode, which, therefore, appears to be the mode of choice. Also, when comparing those separations obtained using just SBCD, the NP conditions still give rise to a greater number of separations, but by a narrower margin. In the NP mode, the EOF causes the bulk solution to migrate in the direction of the cathode, whereas the anionic chiral selectors (SBCD) migrate towards the anode (away from the detection window), giving rise to countercurrent interactions between the chiral selector and the analytes. As noted earlier, the optimum pH found in the NP mode was 8.0. This is because higher pH conditions are required for a strong EOF. At this pH, the analytes will migrate and elute as neutral molecules. Conversely, the optimum pH found when using the reverse polarity mode was 2.6. In this mode, the EOF must be strongly suppressed, and the electrophoretic movement of anionic chiral selectors will be towards the anode (towards the detection window). For these reasons, separations done in the NP mode were generally faster, but the resolution was usually lower. This effect is shown in Figure 6, where the separation of compound **1** was performed with both reverse polarity (Figure 6(A)) and NP (Figure 6(B)). Compound **1** was baseline separated under both conditions, but the analysis

Table 2. Summary of the optimised CE enantiomeric separations.

Analyte no.	t_{m1}[a]	N	α	R_s	Condition[b]
1	9.20	19000	1.15	4.8	30 mM SBCD 50 mM phosphate, pH = 2.6
2	12.97	13000	1.21	5.5	50 mM SBCD 50 mM phosphate, pH = 8.0
3	N/A	–	–	–	–
4	19.33	21000	1.22	7.0	70 mM SBCD 50 mM phosphate, pH = 8.0
5	N/A	–	–	–	–
6	12.85	5000	1.08	1.3	60 mM SBCD 50 mM phosphate, pH = 8.0
7	10.14	27000	1.02	0.8	30 mM SBCD 50 mM phosphate, pH = 3.0[c]
8	N/A	–	–	–	–
9	10.78	4000	1.19	2.1	50 mM SBCD 50 mM phosphate, pH = 2.6
10	N/A	–	–	–	–
11	10.39	2000	1.34	2.7	45 mM SBCD 50 mM phosphate, pH = 8.0
12	N/A	–	–	–	–
13	N/A	–	–	–	–
14	N/A	–	–	–	–
15	30.30	14000	1.05	1.3	30 mM DMBCD 50 mM phosphate, pH = 3.6

[a] t_{m1} is listed in minutes.
[b] SBCD and DMBCD stand for sulphated β-cyclodextrin and dimethylated β-cyclodextrin, respectively.
[c] Sample was made and injected quickly to prevent hydrolysis of acetal and ketone formation.
Notes: N/A means no enantioseparation was observed for this compound under any condition. For the structures of the analytes, refer to Figure 1.

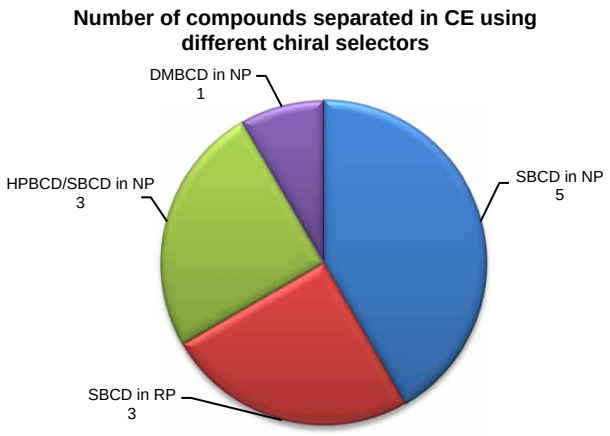

Figure 5. Pie chart representing the distribution of separation among different chiral selectors. Note, the 12 separations represented here are not necessarily unique. DMBCD, SBCD and HPBCD/SBCD stand for dimethyl-β-CD, sulphated-β-CD, and a mixture of hydroxypropyl-β-CD and sulphated-β-CD, respectively. NP and RP denote normal polarity and reversed polarity modes.

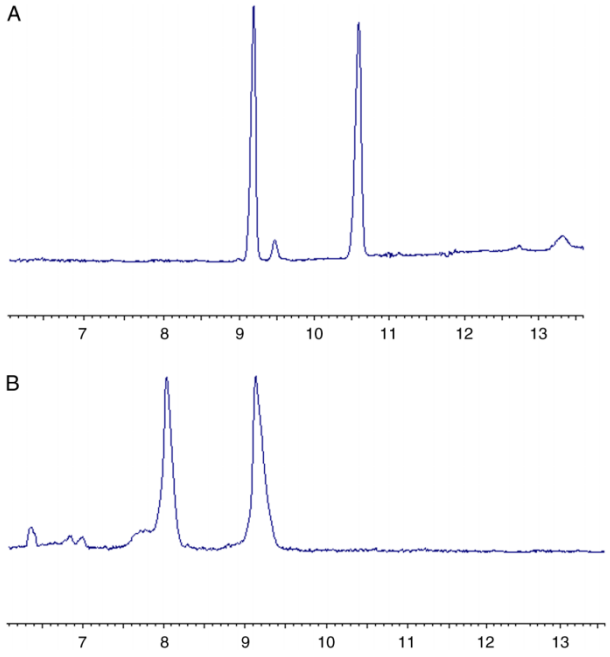

Figure 6. A comparison of the separation of compound **1** by CE in the reverse polarity mode (A) and in the normal polarity mode (B). Conditions were 50 mM phosphate buffer (pH=2.6(A) and 8.0(B)) and 30 mM SBCD. For other conditions, see Experimental section. See Figure 1 for analyte structure.

time is shorter for the separation done in the NP mode. However, it should be noted that for this analyte, the efficiency and resolution were far greater for the separation performed in the reverse polarity mode.

There was one exception to the pH rules outlined above. Compound **15** was separated in the NP mode, yet the optimum pH of the buffer was 3.6. This is the only analyte listed in Table 1 that was separated with a neutral chiral selector (DMBCD). When compound **15** was run using SBCD and a basic buffer, the analyte was not eluted. It can be concluded that the attraction between SBCD and compound **15** was too strong, thus it was not a suitable chiral selector. It is probably not coincidental that compound **15** would not elute during HPLC analyses in RP conditions. Fortunately, the use of DMBCD at acidic pH values allowed for the enantiomeric separation of this analyte. When the pH was low enough to ionise the analyte, the electrophoretic mobility of the positively charged compound **15** was able to overcome the strength of the binding to the slowly moving chiral selector which reaches the detector in a reasonable time.

Comparison between HPLC and CE enantiomeric separations

It is interesting to make a brief comparison of the results obtained using HPLC to those obtained using CE for the enantiomeric separation of these compounds. First, it should be understood that the main importance of this work is the ability to possibly purify one of these precursors for use in the production of enantiomerically pure calcaridine A. From this viewpoint, the HPLC results are more valuable. However, the separations made by CE are not without merit. For example, rapid separations of these compounds may be useful in following the stereochemistry as it is introduced and/or controlled throughout biosynthetic pathways. For such a study, the potentiality for preparative separations is not necessary. Also, rapid enantiomeric excess measurements may be desirable if these analytes are the result of asymmetric syntheses.

Using HPLC, 14 of the 15 compounds were separated, whereas using CE, only 8 were separated. Though HPLC appears to be more broadly applicable to separating these compounds, it should be noted that when a separation was obtained by both separation techniques, the CE method usually produced greater resolution. This is a common observation in the general comparison between HPLC and CE analyses, and this study was no exception. For example, the greatest R_s found using HPLC was 2.0, whereas the best CE separation had an R_s of 7.0. Figure 7 compares some separations obtained using HPLC (Figure 7(A) and (B)) and CE (Figure 7(C) and (D)). The comparison made in the separation of compound **2** by HPLC (Figure 7(A)) and by CE (Figure 7(C)) shows the typical advantage of using CE. However, there were some cases where the HPLC separation was superior. One such example is shown in the separation of compound **6** by HPLC (Figure 7(B)) and by CE (Figure 7(D)). These observations indicate that both techniques have distinct advantages for this set of analytes. Also, it is important to note that the one compound that was not separated by HPLC was separated

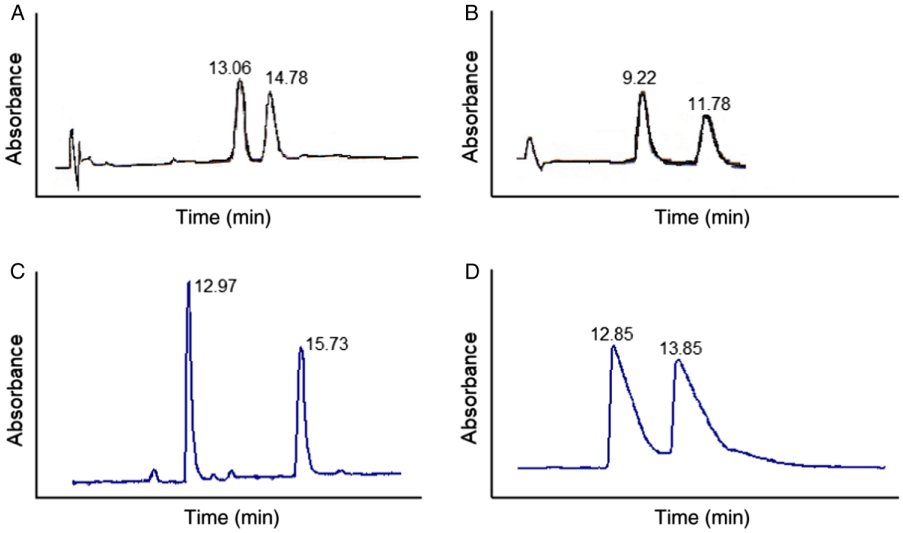

Figure 7. Enantioselective HPLC (A and B) and CE (C and D) analyses of compounds **2** (A and C) and **6** (B and D). The chiral selectors were DMP and SBCD for HPLC and CE, respectively. The HPLC mobile phases were 80/20 (A) and 95/5 (B); 20 mMH4NOAc (pH=4.1)/ACN. The CE experiments were done in normal polarity using 50 mM phosphate buffer (pH=8.0) with 50 mM and 60 mM SBCD (C and D, respectively). See Experimental section for other details. See Figure 1 for analyte structures.

by CE (compound **15**). Furthermore, by using both techniques, the entire set of analytes was separated.

Conclusions

In this study, 15 chiral heterocycles were subjected to enantioselective HPLC and CE analyses using β-cyclodextrin and its derivatives. These compounds are important precursors in the synthesis of calcaridine A. The combination of HPLC and CE allowed for the separation of all 15 analytes. HPLC seems to be more broadly applicable for their separation, whereas the CE separations obtained were generally of high resolution. It was determined that in HPLC, RP separations using the Cyclobond RSP and DMP phases resulted in the greatest number of separations. Throughout the CE analyses, SBCD proved to be the dominant chiral selector and the NP mode produced more separations. In summary, using cyclodextrin-based chiral selectors via HPLC and CE is a viable means for separating this important set of analytes. Furthermore, this work will likely prove useful in future studies in the production of calcaridine A, as well as in the study of other related natural products.

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