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Effects of compound structure and temperature on the resolution of enantiomers of cyclopentenones by liquid chromatography on derivatized cellulose chiral stationary phases

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ABSTRACT

Analytical HPLC methods using derivatized cellulose chiral stationary phases were developed for the separation of the enantiomers of eight cyclopentenone derivatives. The mobile phase and the chiral stationary phase were varied to achieve the best resolution. It was shown that the chemical environment distant from the chiral center had large effects on the separation obtained. The effect of subambient and elevated temperature on the retention, separation and the resolution of the enantiomers was also investigated. Maximum resolution was obtained at elevated temperatures. Three of the compounds were scaled up to preparative loadings to determine the correlation between analytical resolution and preparative resolution. In addition, an example of the sample self-displacement effect observed during the preparative resolution of one of these compounds will be discussed.

INTRODUCTION

Cyclopentenones are important intermediates for numerous natural products including prostaglandins [1,2]. They contain a chiral carbon and exist as enantiomers. There are two approaches to obtaining enantiomerically pure chemicals. These are (1) asymmetric synthesis of the desired isomer and (2) resolution of a racemic mixture into individual isomers. Various synthetic methods to produce optically pure cyclopentenones have been developed [3–5]. In addition, enzymatic methods have been developed to resolve the enantiomers of cyclopentenones [6]. Methods for the resolution of a racemic mixture include recrystallization of diastereomeric salts, formation of diastereomeric derivatives followed by chromatographic resolution on an achiral stationary phase, or direct chromatographic resolution of enantiomers using a chiral stationary phase or a chiral mobile phase additive. Only a limited amount of work using liquid chromatography for the analytical or preparative resolution of cyclopentenone precursors of prostaglandins has been published [2,7–9].

The use of temperature has become an increasingly useful parameter to achieve chiral separations. The effect of temperature on chiral separations using cellulose [10–12], cyclodextrins [13–17], Pirkle type [19,20], and cellulose tris-3,5-dimethylphenyl carbamate (Chiralcel OD) [20–23] chiral stationary phases (CSPs) has been reported. For cellulose triacetate CSP, separation factor α and resolution increase with increasing temperature. For cyclodextrin CSPs, decreasing temperature increases α and can either decrease or increase resolution based on

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the compound being separated. For separations with adequate α values at room temperature, elevated temperatures have been used to increase resolution. For Pirkle type CSPs, α increases with decreasing temperature while resolution again decreases due to poorer mass transfer rates. For Chiralcel OD CSPs, α is relatively unaffected by column temperature, while maximum resolution was obtained at either ambient or subambient temperatures.

Sample self-displacement is a phenomenon that is observed during the preparative purification of a binary mixture under sample overload conditions. Sample self-displacement causes a sharpening of the elution band of the first eluting component of the mixture. This results in a larger yield of pure chemical than would be expected based on the separation seen at analytical loadings. For sample self-displacement to occur, the second eluting component of the binary mixture must be present in equal or greater amounts relative to the first eluting component. The phenomenon of sample self-displacement has been shown to occur for numerous types of chemicals and can be used to increase the throughput of any separation involving a binary mixture [24–26].

EXPERIMENTAL

Materials

The chiral stationary phases used for these studies were obtained from Daicel (Tokyo, Japan) through Baker (Phillipsburgh, NJ, USA) or Regis (Morton Grove, IL, USA) as prepacked analytical (250 mm × 4.6 mm I.D.) and preparative columns (500 mm × 10 mm I.D. and 500 mm × 20 mm I.D.). The prostaglandin precursors were synthesized in the Chemical Development laboratories of Searle (Skokie, IL, USA). The solvents were reagent grade or better and obtained from a variety of sources.

Equipment

The analytical chromatograph consisted of a Waters Assoc. Model 590 solvent delivery system and a U6K injector of Waters Intelligent Sample Processor Model 712 (Milford, MA, USA), a Kratos Model 783 variable wavelength detector (Ramsey, NJ, USA), a Linear Model 585 recorder (Hackensack, NJ, USA), and Digital Equipment Corporation VAX 11/785 computer with Searle chromatography data system. Elevated column temperatures were achieved using a Fiatron CH-30 column heater equipped with a TC-50 temperature controller (Oconomowoc, WI, USA). Subambient column temperatures were achieved either with tap water flowing through a column jacket or by immersing the column in an ice bath. For column temperatures of 0°C, the mobile phase was stored in a refrigerator overnight prior to use and kept in an ice bath during use. For all other temperatures studied the mobile phase was kept at room temperature.

The preparative chromatograph consisted of two Beckman Model 101 pumps with preparative heads, a Model 165 variable wavelength detector with a 5-mm semi-preparative flowcell, a Model 450 data system/controller (Berkeley, CA, USA) and a Kipp and Zonen Model BD41 two channel recorder (Delft, Netherlands). A Rheodyne Model 7125 syringe loading sample injector (Cotati, CA, USA) equipped with a 10-ml loop (Valco, Houston, TX, USA). The column effluent was fractionated using a Gilson Model FC220 fraction collector (Middleton, WI, USA).

RESULTS AND DISCUSSION

Analytical HPLC

Recently in our laboratories, the use of enzymes to resolve racemic mixtures of various cyclopentenones has been explored [6]. In order to monitor the extent of these resolutions, analytical HPLC methods were needed that could be used to determine low levels of the undesired enantiomer. Analytical HPLC methods for the enantiomeric separation of the eight cyclopentenones shown in Figs. 1 and 2 were developed using Chiralcel columns. Chiralcel chiral stationary phases are derivatives of cellulose which are adsorbed on silica gel. For this work Chiralcel OA (acetate derivative), OC (phenylcarbamate derivative), OD (dimethyl phenylcarbamate derivative), OJ (p-methylbenzoate derivative) and OK (tricinnamate derivative) were used (Fig. 3).

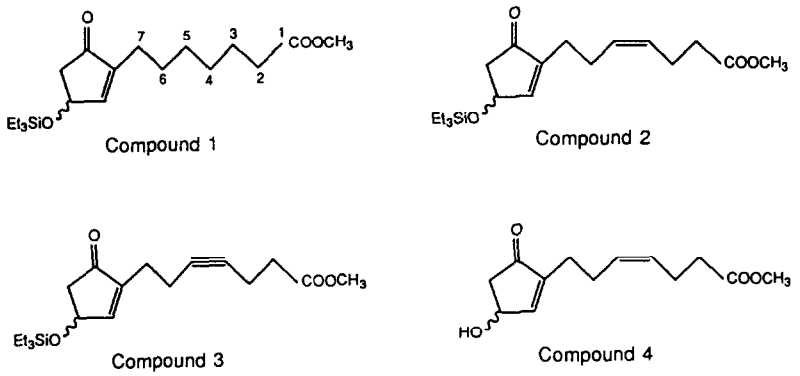

Fig. 1. Structures of cyclopentenone precursors; compounds 1 to 4.

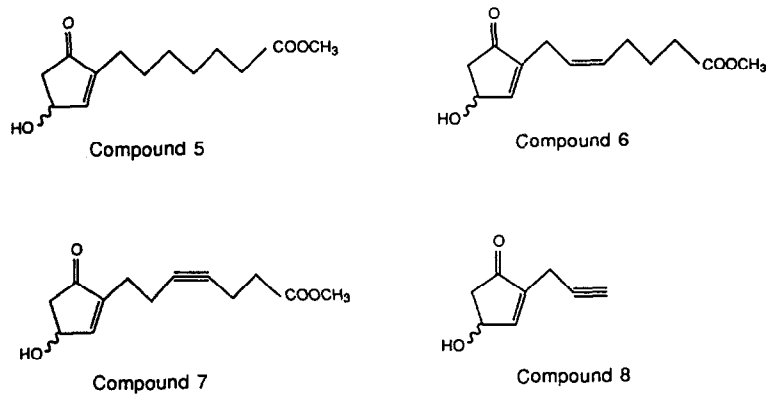

Fig. 2. Structures of cyclopentenone precursors; compounds 5 to 8.

The analytical HPLC separations for compounds 1 to 8 are shown in Figs. 4 and 5. Table I summarizes the capacity factors (k'), separation factor (α), and resolution (R_s) for the enantiomer separation of these eight compounds.

Since the eight cyclopentenone compounds

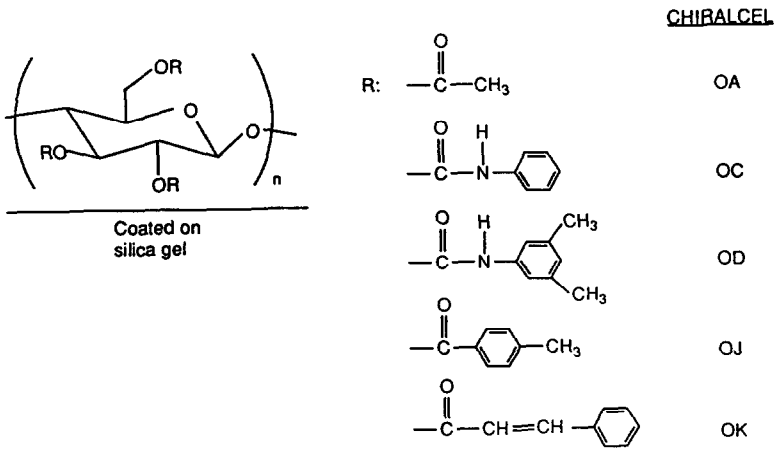

Fig. 3. Structures of various Chiralcel packings.

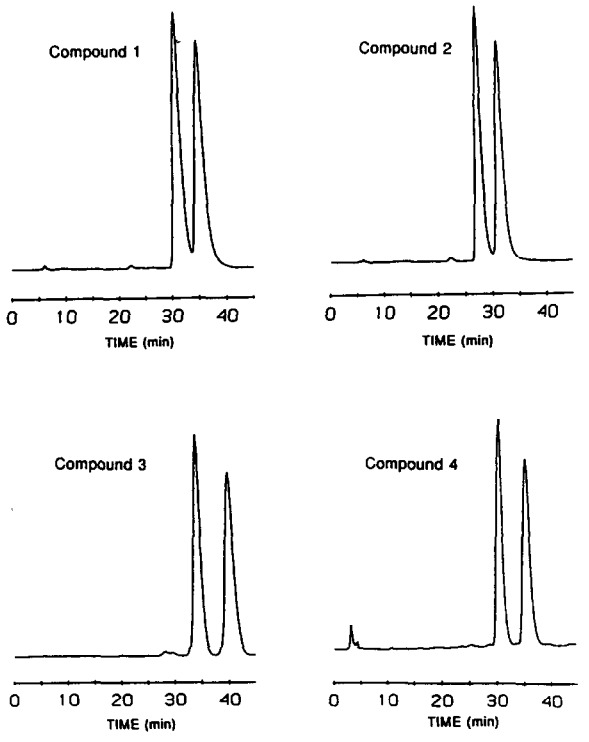

Fig. 4. Analytical HPLC separation of compounds 1 to 4. Analysis conducted on Chiralcel OC (compounds 1 to 3) or Chiralcel OD (compound 4) (250 mm × 4.6 mm I.D.), detection at 215 nm, 0.2 AUFS. Mobile phase, flow-rate: compound 1, 2 and 3 hexane–isopropanol (98:2), 0.5 ml/min; compound 4 hexane–isopropanol (93:7), 0.7 ml/min. All analyses performed at room temperature.

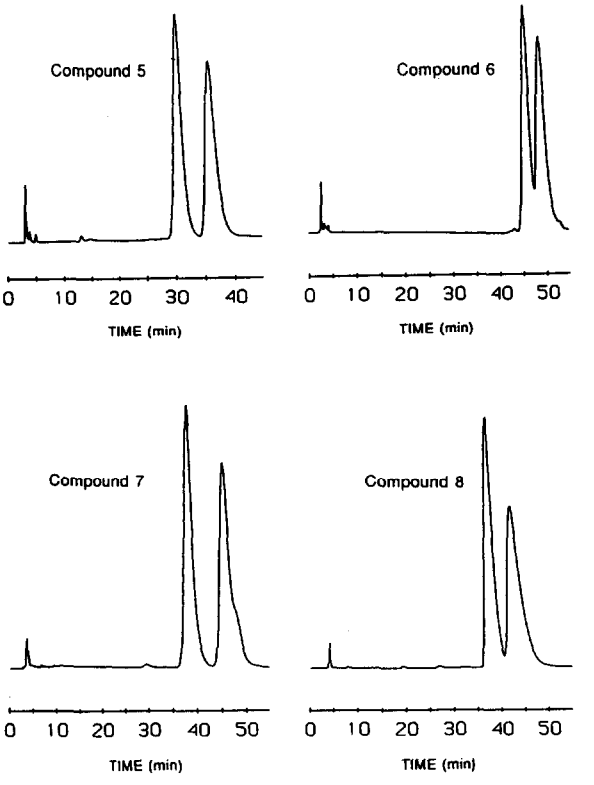

Fig. 5. Analytical HPLC separation of compounds 5 to 8. Detection at 215 nm, 0.2 AUFS. Column, mobile phase, flow-rate: compound 5 Chiralcel OC, hexane–isopropanol (90:10), 1 ml/min; compound 6 Chiralcel OD, hexane–isopropanol (97:3), 1.2 ml/min; compound 7 Chiralcel OC hexane–isopropanol (90:10), 1.0 ml/min; compound 8 Chiralcel OA hexane–isopropanol (96:4), 1.0 ml/min. All analyses performed at room temperature except for compound 5 and 7 which were performed at 50°C.

that were studied are structurally similar, having their chiral center on the same position on the cyclopentenone ring, it is possible to study the effect structural changes distant from the chiral center have on the enantiomeric separation. For example, compounds 1 to 3 all have a triethylsilyl protected group on the chiral center. The only difference between these three compounds is the degree of saturation in the 4,5 position of the upper side chain. Reviewing the data in Table I shows that the degree of saturation in the upper side chain has little effect on retention, but has an effect on α and R_s. From compound 1 (single bond) to compound 3 (triple bond) we see an increase in α of 0.05 (1.16 to 1.21) and an increase in R_s of 1.0 (1.51 to 2.51). The plate number for the separation of compounds 1, 2 and 3 is 2076, 2583 and 3161, respectively. It is apparent that both α and plate number have an effect on resolution.

Another example is the results obtained with compounds 5 and 7, the unprotected analogues of compounds 1 and 3. When a triple bond is present at the 4,5 position (compound 7) α is unchanged, retention is increased (30%) and resolution is increased (2.02 to 2.25).

Other examples of the effect structural changes distant from the chiral center can have on the enantiomeric separation were realized during the method development to produce the analytical HPLC methods shown in Figs. 4 and 5. Compounds 4, 5 and 7 are the unprotected analogues of compounds 2, 1 and 3, respectively.

TABLE I

VALUES FOR ANALYTICAL SEPARATION OF ENANTIOMERS FOR COMPOUNDS 1 TO 8

See Figs. 4 and 5 for HPLC conditions.

Compound	k_1' [a]	k_2' [b]	α	R_s
1[c]	4.14	4.83	1.16	1.51
2[c]	3.59	4.23	1.18	1.85
3[c]	4.68	5.68	1.21	2.51
4[d]	6.06	7.20	1.19	2.42
5[e]	9.08	10.89	1.20	2.02
6[f]	18.50	21.46	1.16	1.02
7[e]	11.72	14.15	1.21	2.25
8[g]	11.33	13.00	1.15	1.16

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.
[c] Chiralcel OC, hexane–isopropanol (98:2).
[d] Chiralcel OD, hexane–isopropanol (93:7).
[e] Chiralcel OC, hexane–isopropanol (90:10).
[f] Chiralcel OD, hexane–isopropanol (97:3).
[g] Chiralcel OA, hexane–isopropanol (96:4).

TABLE II

VALUES FOR ANALYTICAL SEPARATION

Compound	k_1' [a]	k_2' [b]	α	R_s
4[c]	6.07	7.19	1.19	2.00
5[c]	6.16	6.77	1.10	1.32
7[c]	8.40	8.40	1.00	0.00
4[d]	7.07	8.53	1.21	1.31
5[d]	6.47	7.53	1.16	1.23
7[d]	9.27	10.80	1.17	1.28
4[e]	8.32	10.15	1.22	3.49
6[e]	9.14	9.79	1.07	0.89

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.
[c] Conditions: Chiralcel OD, hexane–isopropanol (93:7).
[d] Conditions: Chiralcel OC, hexane–isopropanol (85:15).
[e] Conditions: Chiralcel OD, hexane–isopropanol (94:6).

When the unprotected compounds (compounds 4, 5 and 7) were analyzed using a Chiralcel OC column different results are seen compared to the triethylsilyl (TES) protected compounds (compounds 1, 2 and 3). The protected compounds showed a drastic difference in resolution (Table I), while only slight differences were seen with the unprotected compounds (Table II). In addition, different trends for α *vs.* degree of saturation at the 4,5 position are observed. For the TES protected compounds, α increases as saturation decreases (single bond to triple bond). With the unprotected compounds, the single bond also results in the lowest separation, but the maximum separation is seen for the compound with a double bond in the 4,5 position. This is different from the protected compounds which exhibit the largest separation for the compound with a triple bond in the 4,5 position. An interesting observation is seen when compounds 4, 5 and 7 are analyzed on two different CSPs (Chiralcel OC and Chiralcel OD). These results are summarized in Table II. On the Chiralcel OC column, all three compounds are separated. Only minor differences in α and R_s are observed. An increase in α and resolution is seen from the single bond compound to the triple bond compound, with maximum separation seen for the double bond compound. When the compounds are analyzed on a Chiralcel OD column, the double bond compound again has increased α and R_s relative to the single bond compound, but all separation is lost for the triple bond compound.

The final effect is seen when compounds 4 and 6 are analyzed using a Chiralcel OD column with a mobile phase of isopropanol–hexane (6:94). The only difference between these two compounds is the location of the *trans* double bond in the upper side chain. With compound 4 the double bond is located in the 4,5 position. With compound 6 it is located in the 5,6 position. Evaluation of the data in Table II shows that moving the double bond from the 4,5 position to the 5,6 position results in a pronounced decrease in both α and R_s.

Temperature effects

During HPLC method development it was found that elevated temperature improved the separation of compounds 5 and 7. A review of literature showed that both subambient and elevated temperatures had been used in other laboratories to improve chiral separations [10–23]. To better understand the effects of tempera-

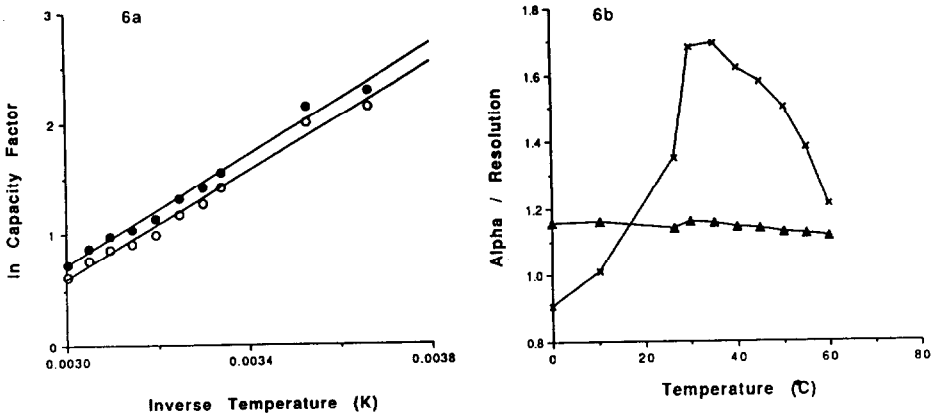

Fig. 6. Effect of temperature on analytical HPLC separation of compound 1. See Fig. 4 for chromatographic conditions. ● = k' for first eluting enantiomer; ○ = k' for second eluting enantiomer; ▲ = α; × = R_s.

ture on the separations of cyclopentenones using derivatized cellulose CSPs, the separation of compounds 1, 2 and 5 were investigated at column temperatures between 0°C and 60°C.

The results of the temperature experiments for compounds 1, 2 and 5 are shown in Figs. 6, 7 and 8, respectively. The effect of temperature on plate number for the three compounds is shown in Fig. 9. For all three compounds, retention decreases with increasing temperature. Good linear correlation is obtained between ln k' and $1/T$. Enantioselectivity (α) is relatively unchanged for all compounds at all temperatures explored. The effect of temperature on enantiomeric resolution is more drastic. For all three compounds, subambient temperatures decrease enantiomeric resolution. Different effects are seen with elevated temperatures for the three compounds studied. For the TES-protected compounds (compounds 1 and 2, Figs. 6 and 7) maximum enantiomeric resolution is obtained at slightly elevated temperatures (30–35°C). Above 35°C resolution decreases for both compounds. For compound 5, no maximum in resolution was seen with increasing temperature. Resolution increased up to 60°C, the maximum temperature explored. Review of the plate numbers obtained at the various temperatures (Fig. 9) shows plate number increasing with temperature for all three compounds studied. The drastic results of this elevation in temperature is shown in Fig. 10. Much sharper peaks are obtained at 60°C, relative to 25°C and 45°C. The effect is not only a faster analysis, but also a lower detection limit

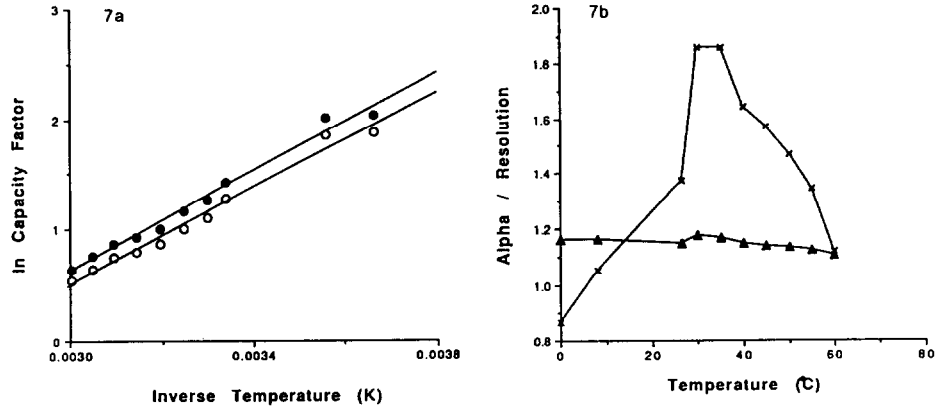

Fig. 7. Effect of temperature on analytical HPLC separation of compound 2. See Fig. 4 for chromatographic conditions. ● = k' for first eluting enantiomer; ○ = k' for second eluting enantiomer; ▲ = α; × = R_s.

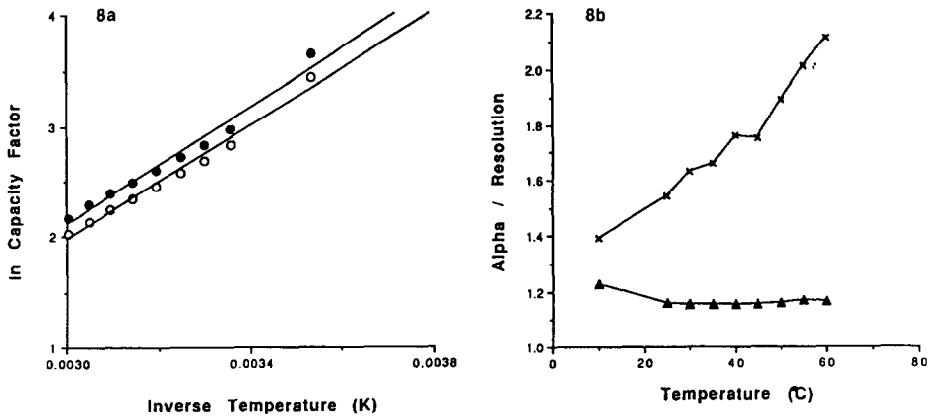

Fig. 8. Effect of temperature on analytical HPLC separation of compound 5. See Fig. 5 for chromatographic conditions. ● = k' for first eluting enantiomer; ○ = k' for second eluting enantiomer; ▲ = α; × = R_s.

for either enantiomer. It must be noted that the manufacturer of Chiralcel columns recommends a maximum temperature of 40°C.

Why is it that temperature has this effect with cellulose based CSPs? The answer lies in the microscopic structure of the stationary phase. The cellulose derivatives are adsorbed onto silica gel, resulting in a coating which is approximately 100 Å thick. This results in poor mass transfer between the solute and the stationary phase. Elevated temperatures improve mass transfer and result in increased resolution. Subambient temperatures have the opposite effect, reducing mass transfer and decreasing resolution. While this explains the trends seen with temperature, it does not explain the difference increasing temperature has on the three compounds studied. Fig. 9 shows that theoretical plates increase with temperature for all three compounds, so it does not appear this is responsible for the differences observed between the compounds. There are two possible reasons why maximum resolution with increasing temperature was seen for compounds 1 and 2, but not for compound 5. The first possibility is the different viscosities of the mobile phases used for the separations. The HPLC conditions for compounds 1 and 2 has a mobile phase of 2% isopropanol in hexane, while compound 5 has a mobile phase of 10% isopropanol in hexane. The mobile phase for

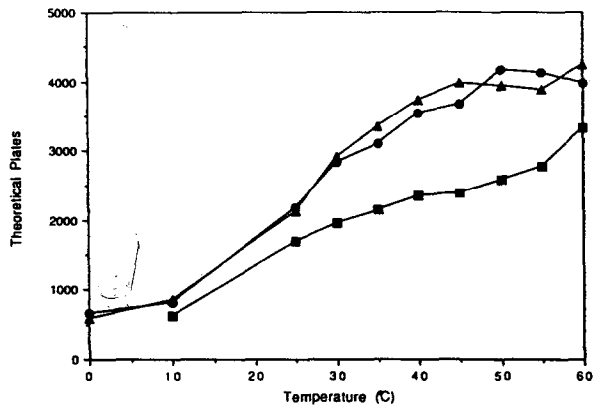

Fig. 9. Effect of temperature on theoretical plates for the analytical separation of compounds 1, 2 and 5. See Figs. 4 and 5 for chromatographic conditions. ● = compound 1; ▲ = compound 2; ■ = compound 5.

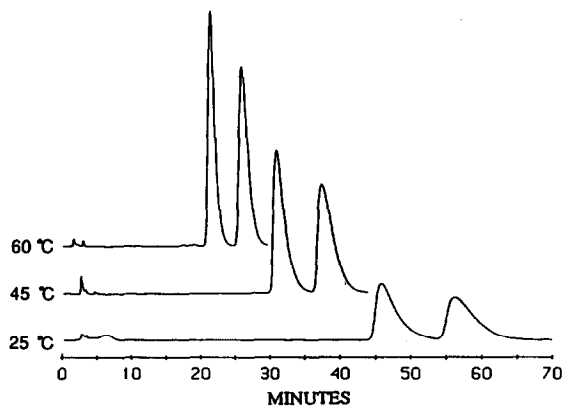

Fig. 10. Analytical HPLC separation of compound 5 at 25, 45 and 60°C. Analysis conducted on Chiralcel OC, isopropanol–hexane (90:10); flow-rate, 1 ml/min; detection at 215 nm, 0.2 AUFS.

compound 5 is more viscous than that for compounds 1 and 2. Since elevated temperature improves chromatographic efficiency by decreasing the viscosity of the mobile phase [27], it follows that a larger temperature effect would be expected for a mobile phase with increased viscosity (compound 5) relative to a lower viscosity mobile phase (compounds 1 and 2). Another possibility is the increased retention seen for compound 5 relative to compounds 1 and 2. The retention of compound 5 at room temperature is approximately 60% longer than those of compounds 1 and 2. At elevated temperatures (above 40°C), the retention for compounds 1 and 2 is small enough ($k' < 3$) that the $[k'/(k'+1)]$ component of the resolution equation causes a drop in resolution. Since the retention of compound 5 is larger, k' never becomes the limiting factor in the resolution and therefore a maximum is never reached. Additional experiments are presently being conducted in our laboratories to determine if either of these two theories are correct.

Preparative chromatography

The preparative resolution of compound 2 has been previously investigated in our laboratories [7]. This work showed that while an isopropanol–hexane mobile phase gave better analytical separation, a mobile phase of ethanol–hexane gave superior results at preparative loadings. The preparative resolution of compounds 1 and 3 were investigated using the method developed for compound 2. [Chiralcel OC (500 mm × 22 mm I.D.), flow-rate, 20 ml/min; mobile phase, ethanol–hexane (1:99); loading 4 mg sample per gram of packing]. Due to the limited separation seen on the preparative UV chromatogram, HPLC analysis of the individual fractions was necessary to determine enantiomeric content. The results from these analyses for compound 2 are shown in Fig. 11. The isolated yields for the preparative resolution of compounds 1, 2 and 3 are summarized in Table III. A good correlation between analytical separation (see Table I) and preparative resolution was obtained. With compound 1 ($\alpha = 1.16$), 37% of the available first eluting enantiomer was isolated. With compound 2 ($\alpha = 1.18$), 54% was isolated, and with compound 3 ($\alpha = 1.21$), 71% of the first eluting enantiomer was isolated. This demonstrates the benefit of developing the best separation prior to scale-up to preparative loadings. An increase in α of only 0.05 resulted in a near doubling of isolated yield for the first eluting enantiomer. In addition, the increased separation allowed the isolation of the second eluting enantiomer of compounds 2 and 3, which was not possible for compound 1.

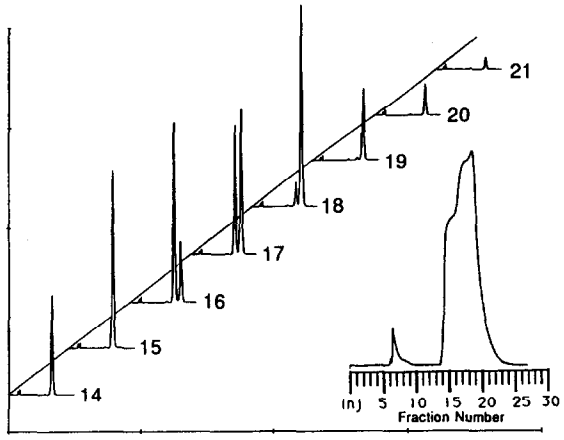

Fig. 11. Analytical HPLC analysis of wet fractions generated during preparative purification of compound 2. See text for preparative conditions. See Fig. 4 for analytical HPLC conditions.

TABLE III

RESULTS OF PREPARATIVE RESOLUTION OF COMPOUNDS 1 TO 3

Chiralcel OC (500 mm × 22 mm I.D.) containing approximately 125 g of packing; flow-rate 20 ml/min; mobile phase, ethanol–hexane (1:99); loading, 4 mg sample per gram of packing.

Compound	First eluting enantiomer	
	%[a]	Mass (mg)
1	37	92
2	54	137
3	71	177

[a] Enantiomeric purity >99.5%.

Sample self displacement effect

The observation of the sample self-displacement effect during the preparative resolution of compound 2 has been previously reported by our laboratories [28]. Since that time, additional investigations into the sample self-displacement effect in chiral separations have been conducted. The results shown in Fig. 12 verified that a sample self-displacement effect was occurring. Individual preparative injections of the *R* and *S* enantiomers of compound 2 show overlapping elution profiles. Based on these profiles, one would except little *R* enantiomer to be isolated from a mixture of the enantiomers. When this mixture is injected onto the column, drastically different elution profiles are obtained. The elution band of the first eluting enantiomer is moved forward and compressed by the large amount of the second eluting enantiomer. The end result is a better recovery of *R* enantiomer than predicted based on the individual elution profiles. The isolated yield of the *R* enantiomer was 54%. This improved yield is consistent with that expected if sample self-displacement is occurring.

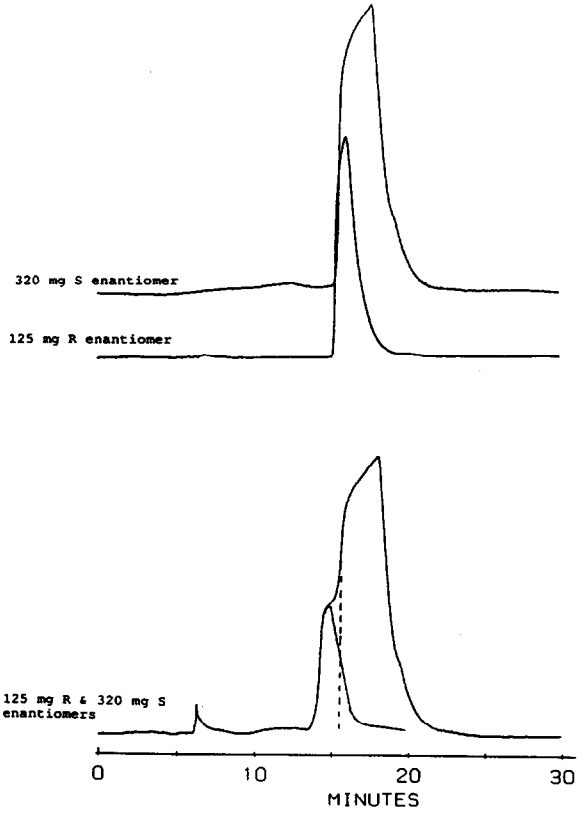

Fig. 12. Elution profiles for individual injections of *R* and *S* enantiomer of compound 2 and elution profile for mixture of *R* and *S* enantiomers.

The effect of differing enantiomeric ratio on the resolution of compound 2 was also investigated. A racemic mixture along with mixtures enriched in both the first eluting and the second eluting enantiomer were explored. The loading (500 mg on a 500 mm × 20 mm I.D. column) was kept constant for all three mixtures. The isolated recoveries of the *R* and *S* enantiomers are summarized in Table IV. The preparative UV traces along with the elution patterns of the enantiomers are shown in Fig. 13. For a racemic mixture, we see a sharpening of the elution band for the first eluting enantiomer. This is caused by the elution of the second enantiomer. The isolated yield of the first eluting *R* enantiomer is 54%. The isolated yield of the second eluting *S* enantiomer is only 17% due to tailing of the first enantiomer into a large portion of the elution band of the second enantiomer. When a mixture that is enriched in the first eluting *R* enantiomer (85/15) is purified, we see a larger, broader elution profile for the *R* enantiomer. This is due to the lack of a large amount of second eluting enantiomer to compress the elution band of the first eluting enantiomer. This results in increased overlap with the *S* enantiomer and an isolated

TABLE IV

RECOVERY AS A FUNCTION OF ENANTIOMER RATIO FOR COMPOUND 2

See text for chromatographic conditions.

Enantiomer ratio (*R*/*S*)[a]	Percent *R* isolated	Percent *S* isolated
50/50	54	17
85/15	50	none
22/78	31	14

[a] Elution order for compound 2 is *R*, *S*.

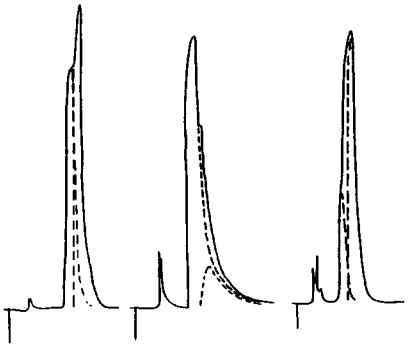

Fig. 13. Elution profiles for mixtures of varying enantiomer ratios for compound 2. See text for discussion.

yield of the *R* enantiomer of only 50%. No pure *S* enantiomer was produced with this sample. Drastic differences are seen when an excess of *S* enantiomer is present in the mixture (22/78). The large amount of the second eluting *S* enantiomer compresses the elution band of the first eluting *R* enantiomer. The isolated yield of the *R* enantiomer is 31%. The isolated yield of the second eluting *S* enantiomer is still poor due to tailing of the *R* enantiomer.

CONCLUSIONS

Analytical and preparative HPLC can be used for the direct resolution of cyclopentenone precursors to prostaglandins. The chemical environment far removed from the chiral center can have some effect on the enantiomeric separation obtained. The use of elevated temperature can be used to increase the enantiomeric separation obtained with cellulose based chiral stationary phases due to increased mass transfer between the solute and the stationary phase. The resolution obtained at preparative loadings is directly related to the analytical separation and small increases in α can cause large increases in isolated yields. Sample self-displacement occurs during the preparative resolution of cyclopentenones. An excess of the second elution enantiomer is required for sample self-displacement to occur.

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