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# Analytical and preparative resolution of enantiomers of prostaglandin precursors and prostaglandins by liquid chromatography on derivatized cellulose chiral stationary phases

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## ABSTRACT

Analytical methods were developed for the separation of the enantiomers of four cyclopentenone precursors of prostaglandins. The resolution obtained is correlated with the chemical environment around the chiral center of the cyclopentenones. The analytical methods were scaled up to preparative loadings and the chromatographic parameters were varied to determine their effect on the preparative separations. The correlation between analytical resolution and preparative resolution was also investigated. In addition to the precursors, the preparative resolution of the enantiomers of a synthetic prostaglandin analogue was investigated.

## INTRODUCTION

Cyclopentenones are important intermediates for numerous natural products including prostaglandins[1,2]. They contain one or more chiral carbon(s) and can 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]. 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 resolution of prostaglandins and their precursors has been published[6,7]. Direct resolution of the enantiomers of a prostaglandin and it's precursors using liquid chromatography on a chiral stationary phase was attempted at both analytical and preparative loadings.

Enisoprost (Fig. 1) is an $E_1$ type prostaglandin analogue. It was first synthesized in 1979 in the medicinal chemistry laboratories of G. D. Searle & Co. The present

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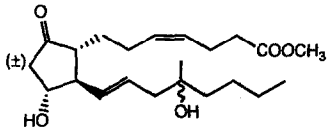


Fig. 1. Structure of enisoprost.

synthesis of enisoprost results in the formation of four isomers (Fig. 2). Since the 11*R*,16*S*-isomer was needed for testing purposes, an attempt was made to synthesize this isomer. Enisoprost is produced by coupling the $\beta$ side chain to the cyclopentenone containing the $\alpha$ side chain (Fig. 3)[8]. Enisoprost can be derived from any of four different cyclopentenone derivatives (Fig. 4)[9]. An asymmetric synthesis of the beta side chain was easily developed[10]. The asymmetric synthesis of the cyclopentenone was significantly more difficult and time consuming, therefore preparative liquid chromatography was investigated to isolate the desired enantiomer from a racemic mixture.

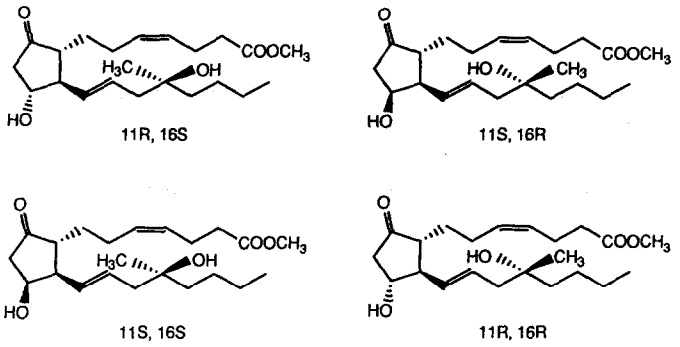


Fig. 2. Structures of four isomers of enisoprost.

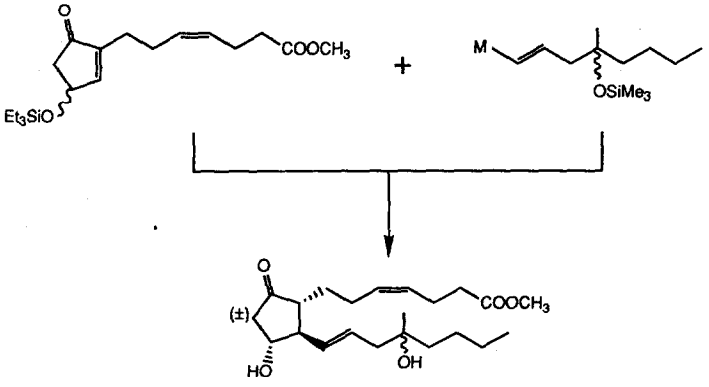


Fig. 3. Synthesis of enisoprost. Et = Ethyl; Me = methyl.

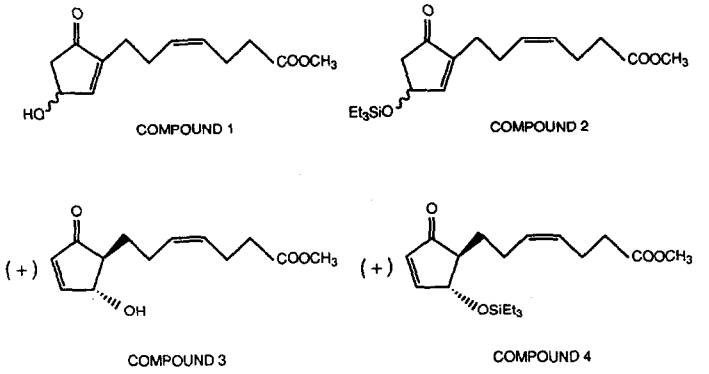


Fig. 4. Structures of four cyclopentenone precursors, compounds 1–4.

In this paper we will report on the use of liquid chromatography for the enantiomeric resolution of cyclopentenone derivatives and a prostaglandin analogue. Analytical high-performance liquid chromatography (HPLC) methods were developed using derivatized cellulose stationary phases. In addition, the preparative resolution of these compounds will be discussed.

## EXPERIMENTAL

### *Materials*

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

### *Equipment*

The analytical chromatograph consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model 590 solvent-delivery system and a U6K injector or Waters intelligent sample processor, a Kratos (Ramsey, NJ, U.S.A.) Model 783 variable-wavelength detector, a Linear (Hackensack, NJ, U.S.A.) Model 585 recorder and a Digital (Equipment Corp.) VAX 11/785 computer with Searle chromatography data system.

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

## RESULTS AND DISCUSSION

### *Analytical HPLC*

Analytical HPLC methods for the enantiomer separation of the four cyclopentenones shown in Fig. 4 were developed using a Chiralcel OC column. Chiralcel OC (Fig. 5) is a phenylcarbamate derivative of cellulose which is adsorbed on silica gel. Cellulose-based phases have mobile phase restrictions since certain solvents can dissolve the cellulose. The manufacturer of these columns recommends the use of alkanes with low percentages (<40%) of alcohols as polar modifiers. The analytical HPLC separations for compounds **1–4** are shown in Fig. 6. Table I summarizes the

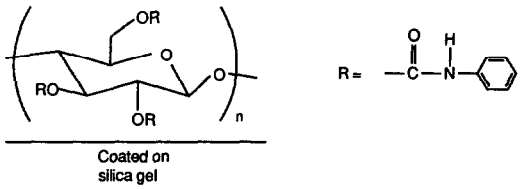


Fig. 5. Structure of Chiralcel OC packing.

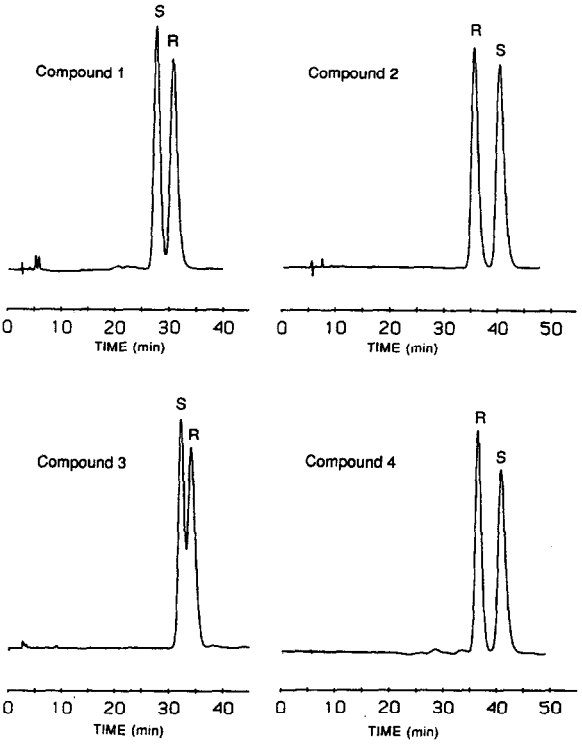


Fig. 6. Analytical HPLC separation of compounds **1–4**. Analysis conducted on Chiralcel OC column (250 × 4.6 mm I.D.), detection at 215 nm, 0.1 a.u.f.s. Mobile phase, flow-rate: compound **1**, hexane–isopropanol (85:15, v/v), 1.0 ml/min; compound **2** and **4**, hexane–isopropanol (99:1, v/v), 0.5 ml/min; compound **3**, hexane–isopropanol (90:10, v/v), 1.0 ml/min.

TABLE I

VALUES FOR ANALYTICAL SEPARATION OF ENANTIOMERS FOR COMPOUNDS 1–4

See Fig. 6 for HPLC conditions.

| *Compound* | $k'_1$[a] | $k'_2$[b] | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| 1 | 9.65 | 10.82 | 1.12 | 1.3 |
| 2 | 5.47 | 6.33 | 1.16 | 1.9 |
| 3 | 11.35 | 12.06 | 1.06 | 0.7 |
| 4 | 5.64 | 6.43 | 1.14 | 1.7 |

[a] Capacity factor for first-eluting enantiomer.
[b] Capacity factor for second-eluting enantiomer.

capacity factors ($k'$), separation factor ($\alpha$), and resolution ($R_s$) for the enantiomer separation of these four compounds.

Further analytical method development was done using compound **2**. Using a Chiralcel OC column, mobile phases of hexane with various alcohols were investigated. The percentage of alcohol present in the mobile phase was varied such that the capacity factors remained approximately constant. The results of these experiments are summarized in Table II. These data show that various straight chain alcohols have no significant effect on resolution. Branched alcohols gave reduced resolution compared to their straight chain analogues.

Different types of derivatized cellulose packings were investigated for the separation of the enantiomers of compound **2**. The derivatized cellulose packings used are shown in Fig. 7. Results are summarized in Table III. These results show that Chiralcel OC packing gave the largest resolution for compound **2**.

Chiral separations occur in liquid chromatography through the formation of diastereomeric complexes between the solute and the chiral stationary phase. With cellulose-based chiral phases these complexes are formed through attractive interactions such as hydrogen bonding, $\pi$–$\pi$ and dipole interactions. One major difference with cellulose-based phases is that the chiral discriminator is located in the cavities or

TABLE II

EFFECT OF POLAR MODIFIER ON ANALYTICAL SEPARATION OF ENANTIOMERS OF COMPOUND **2**

HPLC conditions: Chiralcel OC (250 × 4.6 mm I.D.); flow-rate, 0.5 ml/min; non-polar solvent, hexane; detection, 215 nm, 0.2 a.u.f.s.

| *Polar modifier (%)* | $k'_1$[a] | $k'_2$[b] | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| Ethanol (0.4) | 6.01 | 7.13 | 1.19 | 2.3 |
| *n*-Propanol (0.65) | 5.79 | 6.85 | 1.18 | 2.1 |
| *n*-Butanol (1.0) | 5.08 | 6.07 | 1.19 | 2.2 |
| Isopropanol (1.0) | 5.47 | 6.33 | 1.16 | 1.9 |
| *tert.*-Butanol | 5.67 | 6.52 | 1.15 | 1.5 |

[a] Capacity factor for first-eluting enantiomer.
[b] Capacity factor for second-eluting enantiomer.

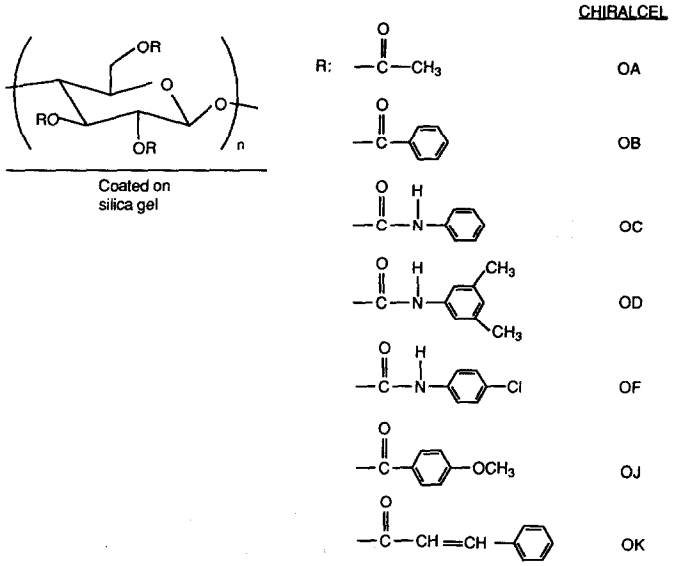


Fig. 7. Structures of various Chiralcel packings.

ravines of the cellulose[11]. In an attempt to understand the chiral mechanism, the effect of differing silyl protecting groups on the separation of the enantiomers of compound **2** was investigated. The protecting groups and the results obtained are summarized in Table IV. The distances for the protecting group are measured from the ether oxygen to the outermost hydrogen. For non-spherical protecting groups, two distances are given. Volume measurements use the outermost distance as the diameter of the protecting group. These data indicate that the bulky diphenyl methyl protecting group causes loss of chiral discrimination. This appears related to the inability of this compound to enter into the cavity of the cellulose and interact with the chiral

TABLE III

EFFECT OF CHIRAL STATIONARY PHASE ON ANALYTICAL SEPARATION OF ENANTIOMERS OF COMPOUND **2**

HPLC conditions: mobile phase, hexane–isopropanol (99:1, v/v); flow-rate, 0.5 ml/min; detection, 215 nm, 0.2 a.u.f.s.

| *Chiralcel packing* | $k'_1$[a] | $k'_2$[b] | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| OA | 1.57 | 1.57 | 1 | 0 |
| OB | 3.34 | 4.10 | 1.20 | 0.6 |
| OC | 5.47 | 6.33 | 1.16 | 1.9 |
| OD | 3.20 | 3.70 | 1.16 | 1.6 |
| OF | >10 | >10 | – | – |
| OJ | 2.78 | 2.99 | 1.08 | 0.8 |
| OK | 4.82 | 5.35 | 1.11 | 0.8 |

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.

TABLE IV

EFFECT OF PROTECTING GROUP ON ANALYTICAL SEPARATION OF ENANTIOMERS OF COMPOUND 2

HPLC conditions: Chiralcel OC (250 × 4.6 mm I.D.); mobile phase, hexane–isopropanol (99:1, v/v), flow-rate, 0.5 ml/min; detection 215 nm, 0.2 a.u.f.s.

| *Protecting group* | *Distance (Å)*[b] | *Volume (Å³)*[c] | $k_1'$[d] | $k_2'$[e] | α | $R_s$ |
|---|---|---|---|---|---|---|
| $(C_6H_5)_2CH_3Si$–[a] | 2.5, 5.8 | 202.3 | 6.8 | 6.8 | 1.0 | 0 |
| $((CH_3)_2CH)_3Si$– | 3.1 | 193.1 | 4.2 | 4.8 | 1.14 | 1.4 |
| $(CH_3)_2[(CH_3)_3C]Si$– | 2.5, 3.9 | 142.9 | 3.4 | 4.0 | 1.18 | 1.7 |
| $(CH_3CH_2)_3Si$– | 3.9 | 144.2 | 5.5 | 6.3 | 1.16 | 1.9 |

[a] Flow-rate 1.0 ml/min.
[b] Distance to outer hydrogen(s).
[c] Volume for protecting group measured using distance to outermost hydrogen as diameter.
[d] Capacity factor for first eluting enantiomer.
[e] Capacity factor for second eluting enantiomer.

discriminator. Similar results using 4-hydroxy-2-cyclopentenone derivatives have been previously reported[12].

*Preparative HPLC*

When developing an analytical method for scale-up to preparative, it is desirable to have an analytical resolution greater than 2 and a $k'$ for the desired compound of less than 5. Table I shows that for compounds **1–4**, the resolution was between 0.7 and 1.9 and the $k'$ ranged from 5.6 to 12.1. A $k'$ of less than 5 is desired because of shorter run times and a corresponding increase in the throughput (g/h of pure chemical) for the purification.

Column eluent was monitored with a UV detector. The increased sample load either reduced or destroyed altogether the separation seen on the preparative chromatogram. Because of this, individual fractions were analyzed using analytical HPLC to determine enantiomeric content. A plot of enantiomer content *versus* fraction number is shown in Fig. 8.

To determine the feasibility of isolating gram quantities of chiral cyclopentenone the analytical separations reported in Fig. 6 were scaled up to preparative loadings. A 500 × 10 mm I.D. column containing approximately 25 g of stationary phase was used for preparative method development. This is approximately a nine-fold increase in column volume compared to the analytical column. A direct scale-up of the analytical methods would allow an injection of only 0.2 mg of a racemic mixture. This sample size was much too small to produce the required amount of chemical. Experiments were undertaken to determine the effect of increasing sample size on the separation. Column loadings of 1, 2 and 4 mg sample per gram of packing were investigated. Preparative loadings greater than 4 mg sample per gram packing were not investigated due to the small degree of separation seen analytically and the need to keep the isolated yields as high as possible. Loadings less than 1 mg sample per gram of packing were not investigated since they would be inefficient to purify gram quantities of material.

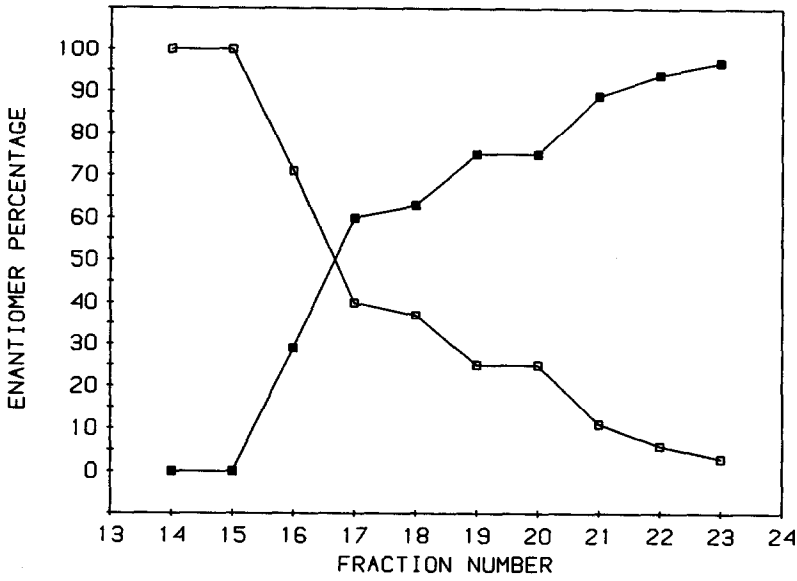


Fig. 8. Enantiomeric purity of individual fractions collected from preparative purification of compound **4**. Purification conducted on Chiralcel OC (500 × 10 mm I.D.) containing approximately 25 g of packing. Loading of 2 mg sample per gram of packing, a mobile phase of hexane–isopropanol (99:1, v/v) and a flow-rate of 4 ml/min was used. Each fraction contains 8 ml. Purification yielded 30% of available first enantiomer (>99.5%) and no second-eluting enantiomer. □ = *R*-enantiomer; ■ = *S*-enantiomer.

From these preparative loading experiments it was determined that the first eluting enantiomer could be isolated pure for three of the four compounds. The compound which showed no separation preparatively, compound **3**, also exhibited the poorest resolution in the analytical HPLC method. The results of these experiments are summarized in Table V. These data show that as loading increases, the amount of first enantiomer produced per hour increases even though the percentage of enantiomer isolated decreases. This results in a method with greater throughput. This is just one of the many trade-offs that must be negotiated when developing an efficient preparative liquid chromatographic method. Another benefit of a larger sample size is that the sample is more concentrated in the column eluent and therefore less solvent needs to be removed to recover the product.

Preparative method development showed that no significant amount of second eluting enantiomer could be isolated at any of the column loadings attempted. In order to isolate the second eluting enantiomer a series of purifications was needed. Each purification would remove a portion of the first eluting enantiomer. The overlap material from each purification, which was enriched in the second eluting enantiomer, would then be repurified to remove additional first eluting enantiomer. This process would be repeated until second eluting enantiomer of sufficient purity was obtained. This approach was used to isolate the second eluting enantiomer of compound **2**. One gram of a racemic mixture was originally chromatographed through multiple injections on a Chiralcel OC column (500 × 20 mm I.D.). From the first purification 270 mg of first-eluting enantiomer were obtained. The fractions containing the

TABLE V

RESULTS OF PREPARATIVE EXPERIMENTS FOR COMPOUNDS 1–4

HPLC conditions: Chiralcel OC (500 × 10 mm I.D.) containing approximately 25 g of packing; flow-rate, 4 ml/min for compounds **2** and **4**, 8 ml/min for compounds **1** and **3**.

| *Compound* | *Mobile phase (hexane–isopropanol)* | *Loading (mg/g)* | *First eluting enantiomer*[a] | | |
|---|---|---|---|---|---|
| | | | *Percent*[b] *isolated* | *mg per injection* | *mg per hour* |
| **1** | 85:15 | 1 | 63 | 7.9 | 15.8 |
| | 85:15 | 2 | 35 | 8.9 | 17.8 |
| | 85:15 | 4 | 18 | 9.6 | 19.2 |
| **1** | 90:10 | 1 | 56 | 7.0 | 14.0 |
| | 90:10 | 2 | 39 | 9.8 | 19.6 |
| | 90:10 | 4 | 34 | 17.0 | 34.0 |
| **2** | 99:1 | 1 | 35 | 4.4 | 8.8 |
| | 99:1 | 2 | 35 | 8.9 | 17.8 |
| | 99:1 | 4 | 37 | 19.0 | 38.0 |
| **3** | 85:15 | 1 | 0 | – | – |
| | 85:15 | 2 | 0 | – | – |
| | 85:15 | 4 | 0 | – | – |
| **4** | 99:1 | 1 | 38 | 5.0 | 10.0 |
| | 99:1 | 2 | 30 | 7.5 | 15.0 |
| | 99:1 | 4 | 29 | 14.6 | 29.2 |

[a] No second-eluting enantiomer isolated.
[b] Purity of first enantiomer > 99.5%.

second-eluting enantiomer were combined, dried and repurified. This procedure of purification, solvent removal and repurification was repeated two times to generate a 391-mg sample which contained 95% of the second eluting enantiomer. This sample was then purified to produce 200 mg of second enantiomer. Using this series of four purifications, 270 mg of first-eluting enantiomer (>99.5%) and 200 mg of second-eluting enantiomer (>98%) were isolated. This represents recoveries of 54 and 40%, respectively. Since the remaining enantiomerically impure chemical from these series of chromatographic purifications was very close to a racemic mixture, it could be rerun through another series of purifications, if desired, to isolate more of the individual enantiomers.

*Preparative resolution of prostaglandin enantiomers*

The preparative resolution of the 11*R*,16*S*- and the 11*S*,16*R*-enantiomers of enisoprost (Fig. 2) was investigated. The analytical separation for these two enantiomers is shown in Fig. 9. This method was scaled up to preparative loadings and the percentage of isopropanol present in the mobile phase varied. The results of these experiments are summarized in Table VI. These results show that both enantiomers could be isolated at all loadings and isopropanol percentages attempted. The $\alpha$ value for this separation is 1.29. This is much better than the $\alpha$ value for the separation of the

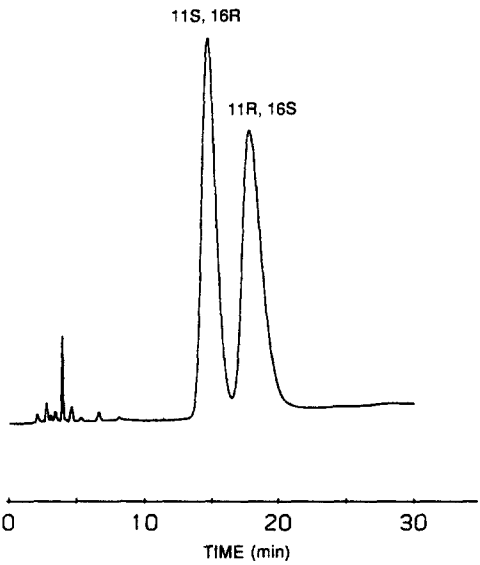


Fig. 9. Analytical HPLC separation of 11*S*,16*R*- and 11*R*,16*S*-enantiomers of enisoprost. Analysis conducted on Chiralcel OC column (250 × 4.6 mm I.D.) with a mobile phase of hexane–isopropanol (85:15, v/v). A flow-rate of 0.5 ml/min and detection at 215 nm, 0.1 a.u.f.s. was used.

prostaglandin precursors (see Table I) and because of this a larger amount of chemical could be isolated at the preparative loadings attempted.

## CONCLUSION

Analytical and preparative HPLC can be used for the direct resolution of the enantiomers of prostaglandins and their precursors. Using cellulose-based phases,

TABLE VI

PREPARATIVE RESOLUTION OF 11*R*,16*S*- AND 11*S*,16*R*-ENANTIOMERS OF ENISOPROST

HPLC conditions: Chiralcel OC (500 × 10 mm I.D.) containing approximately 25 g of packing; flow-rate 8 ml/min.

| *Mobile phase (hexane–isopropanol)* | *Loading (mg/g)* | *First enantiomer*[a] | | *Second enantiomer*[a] | |
|---|---|---|---|---|---|
| | | *(%)*[b] | *weight (mg)* | *(%)*[b] | *weight (mg)* |
| 85:15 | 1 | 72 | 9.0 | 49 | 6.1 |
| 90:10 | 1 | 83 | 10.6 | 61 | 7.7 |
| | 2 | 76 | 19.1 | 31 | 7.8 |
| | 4 | 38 | 19.3 | 16 | 8.2 |
| 95:5 | 1 | 82 | 10.4 | 58 | 7.4 |
| | 2 | 73 | 18.8 | 35 | 9.1 |

[a] Purity > 99.5%.
[b] Percent of enantiomer available.

changing the alcohol in the mobile phase has very little effect on the separation, although branched alcohols reduce the separation obtained with their straight chain analogues. The degrees of separation obtained is related to the ability of the molecule to interact with the stationary phase and "fit" into the cellulose cavity. The analytical separation is directly related to the resolution obtained at preparative loadings.

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