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PREPARATIVE RESOLUTION OF ENANTIOMERS OF PROSTAGLANDIN PRECURSORS BY LIQUID CHROMATOGRAPHY ON A CHIRAL STATIONARY PHASE

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SUMMARY

Preparative liquid chromatographic methods were developed for the chiral resolution of two different cyclopentenone precursors of a synthetic prostaglandin. Various solvent combinations of alcohols and alkanes were investigated to determine the method which has the greatest throughput. The effect of particle size on the chiral resolution was also investigated. In addition, the preparative system was automated to llow for unattended operation for up to 10 h.

INTRODUCTION

Cyclopentenones are important intermediates for numerous natural products including prostaglandins[1,2]. Since they contain a chiral carbon, various synthetic methods to produce optically pure cyclopentenones have been developed[3–5]. Only a limited amount of work on the resolution of the enantiomers of prostaglandin precursors using liquid chromatography (LC) has been published[2]. In 1987 a new synthetic prostaglandin E_1 analogue was synthesized at G. D. Searle and Co. To support the development activities of this compound, multigram quantities of a chiral cyclopentenone precursor were needed. 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. The methods of resolution can include recrystallization, 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. Direct resolution of the enantiomers using LC on a chiral stationary phase was used to isolate the desired enantiomer from a racemic mixture.

In this paper we will discuss the investigations and the method which was used to generate approximately 25 g of the desired enantiomer. We will also discuss the automation of the preparative system to allow for unattended operation and to reduce the manual labor required for this purification.

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EXPERIMENTAL

Materials

The chiral stationary phase, Chiralcel OC, used for these studies was obtained from Daicel (Tokyo, Japan) through J. T. Baker (Phillipsburgh, NJ, U.S.A.) as prepacked analytical (250 mm × 4.6 mm I.D.) and preparative (500 mm × 10 mm I.D. and 500 mm × 20 mm I.D.) columns.

The prostaglandin precursors were synthesized in the Chemical Development Labs. of G. D. Searle and Co. (Skokie, IL, U.S.A.). Prior to the separation of the enantiomers, all prostaglandin precursors were purified on 40-μm silica gel using a mobile phase of ethyl acetate–hexane (10:90 for compound 1, 50:50 for compound 2) to remove minor impurities. The solvents were reagent grade or better and obtained from a variety of sources.

Equipment

The analytical chromatograph consisted of a Spectra-Physics (San Jose, CA, U.S.A.) Model SP8100 pump, a Waters (Milford, MA, U.S.A.) intelligent sample processor Model 712, a Kratos (Ramsey, NJ, U.S.A.) Model 757 variable-wavelength UV detector, a Kipp and Zonen (Delft, The Netherlands) Model BD41 two-channel recorder and a Digital Equipment Corp. (Maynard, MA, U.S.A.) VAX 11/785 computer with a Searle chromatography data station.

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 and Zonen 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 Instruments, Houston, TX, U.S.A.) or a Gilson Model (Middleton, WI, U.S.A.) 401 dilutor in combination with a Rheodyne electrically actuated Model 7010 injector equipped with a Valco 10-ml loop were used for sample injection. Effluent fractions were collected with either a Gilson Model FC220 or Model 201 fraction collector. A Valco electrically actuated six-port valve was used to allow effluent flow to different locations.

RESULTS AND DISCUSSION

Mobile phase selection

The analytical separation of the protected racemic cyclopentenone (compound 1, Fig. 1) is shown in Fig. 2. The elution order of the enantiomers is *R, S*. The capacity factor, k', is suitable for scale-up to a preparative separation and therefore was used as a starting point for preparative method development using a Chiralcel OC column

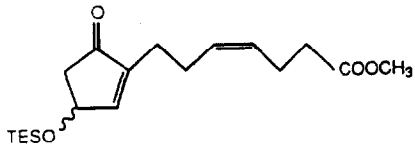

Fig. 1. Structure of triethylsilyl protected cyclopentenone, compound 1.

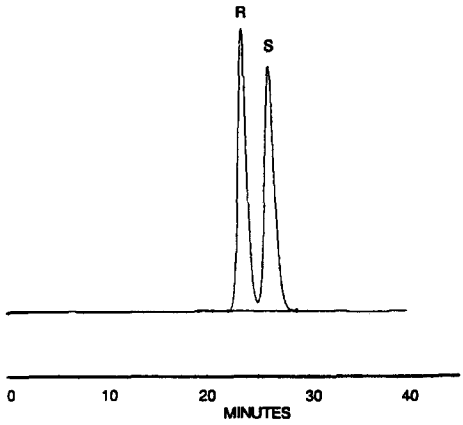

Fig. 2. Analytical HPLC separation of compound 1 on a Chiralcel OC column (250 mm × 4.6 mm I.D.), with a mobile phase of isopropanol–hexane (2:98). A flow-rate of 0.5 ml/min and detection at 215 nm, 0.1 a.u.f.s. were used.

packed with 10-μm particles. Chiralcel OC (Fig. 3) is the phenylcarbamate derivative of cellulose which is adsorbed onto 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. For the solvent selection portion of our method development we investigated different alkanes such as hexane and 1,1,2-trimethylpentane (isooctane) with alcohols such as absolute ethanol, isopropanol and *tert.*-butanol. These experiments were all performed at a loading of 4 mg of sample per gram of packing and at a flow-rate of 20 ml/min. Analytical high-performance LC was used to determine the enantiomer content of the fractions from the preparative separation.

The results of these experiments are summarized in Table I. The object of these experiments was to determine the method that produced the largest amount of pure *R* enantiomer in the shortest time. Based on these criteria the ethanol–hexane system was chosen. The ethanol–isooctane system produced an equal amount of pure *R* enantiomer but had a slightly longer run time. An additional benefit was that the ethanol–hexane system produced more pure *S* enantiomer. A chromatogram of a preparative separation using ethanol–hexane as mobile phase is shown in Fig. 4.

Throughput studies

The throughput is the amount of purified chemical produced per hour.

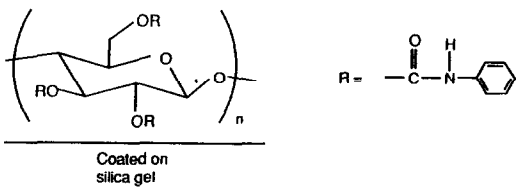

Fig. 3. Structure of the Chiralcel OC packing.

TABLE I
EFFECT OF MOBILE PHASE COMPOSITION ON THE SEPARATION OF COMPOUND 1

Mobile phase[a]	*R enantiomer isolated*[b]		*S enantiomer isolated*[c]		*Run time (min)*
	%	*Weight (mg)*	*%*	*Weight (mg)*	
Ethanol–hexane (1:99)	54	135	17	44	25
Ethanol–isooctane (1:99)	55	137	12	31	30
Isopropanol–hexane (2:98)	37	91	none		30
tert.-Butanol–hexane (2:98)	32	80	none		60

[a] Flow-rate, 20 ml/min; loading, 4 mg sample per g packing.
[b] Purity >99.5% (by high-performance LC).
[c] Purity > 99% (by high-performance LC).

Flow-rate and loading studies help to establish additional parameters required to maximize the throughput. The throughput can be increased by increasing the quantities produced per run and/or by decreasing the run time. For increased quantities, the loading must be increased and for decreased run time, the flow-rate must be increased. Flow-rates of 10, 20 and 40 ml/min and loadings of 2, 4, and 8 mg of sample per g of packing were investigated. The results of these experiments are summarized in Table II.

This table shows that as the loading increases, the amount of chemical produced per hour increases even though the percent of isolated enantiomer decreases. The same trend is seen with increasing flow-rate. Increasing the loading and the flow-rate increases the amount of pure chemical produced per hour and therefore results in a method with a higher throughput.

There are many factors which could influence the decision on which loading and

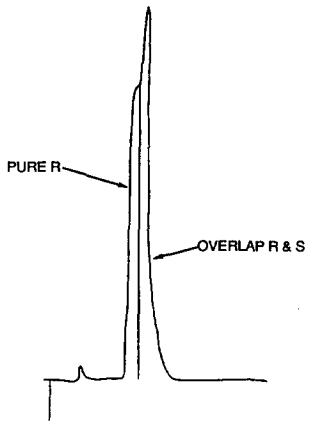

Fig. 4. Chromatogram of the preparative resolution of the enantiomers of compound 1. The purification was conducted on a Chiralcel OC column, 10-μm (500 mm × 20 mm I.D.), with a mobile phase of ethanol–hexane (1:99). A flow-rate of 20 ml/min, detection at 254 nm, 0.5 a.u.f.s. and a loading of 4 mg of sample per gram of packing were used.

TABLE II
FLOW-RATE AND LOADING STUDIES
Mobile phase, ethanol–hexane (1:99).

Flow-rate (ml/min)	*Loading (mg/g)*	*% R isolated*	*mg R/injection*	*mg R/h*
10	4	62	155	233
20	4	54	135	405
40	4	49	123	738
20	2	79	99	297
20	4	54	135	405
20	8	32	162	486

flow-rate to use for purifications. Some of these factors are: cost of the chemical prior to separation, the amount of chemical that must be produced, the added cost and effort of isolating additional chemical from the overlap portions, safety, and equipment capabilities. If the chemical being purified is expensive and relatively small quantities are required, the purification can be run at lower loadings, thus isolating the largest percentage of chemical possible while sacrificing the throughput. If the chemical is relatively inexpensive, higher loadings can be used, allowing larger quantities to be isolated.

Since our chemical was relatively expensive and only 40 g were required for initial development studies, we decided on a loading of 4 mg of sample per g of packing and a flow-rate of 20 ml/min. A flow-rate of 40 ml/min should have been used for greatest throughput. Due to equipment limitations and safety considerations the lower flow-rate of 20 ml/min was used.

Effect of particle size on separation

Packing of 20 μm is available as an alternative to the 10-μm packing for preparative separations. This 20-μm material is sold as prepacked columns or as bulk packing. If this material gave a comparable separation we would be able to pack larger columns, resulting in lower cost and greatly increasing the throughput. The results of these experiments are shown in Table III. This data shows that for compound 1, the separation was greatly reduced when using 20-μm packing. For our separation, use of this packing was not practical.

TABLE III
COMPARISON OF 10- AND 20-μm CHIRALCEL OC
Flow-rate, 20 ml/min; loading, 4 mg of sample per g packing.

Particle size (μm)	*R enantiomer isolated*		*S enantiomer isolated*	
	%	*Weight (mg)*	*%*	*Weight (mg)*
10	54	137	17	44
20	12	29	–	–

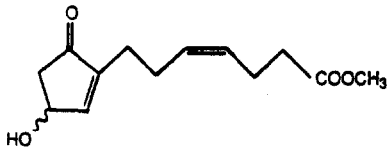

Fig. 5. Structure of unprotected hydroxycyclopentenone, compound 2.

Purification of unprotected hydroxycyclopentenone

In addition to the *R* enantiomer, the *S* enantiomer of compound 1 was required for product development activities. Table I shows that only small amounts of the second eluting *S* enantiomer were isolated during the isolation of the *R* enantiomer. To obtain the *S* enantiomer we took advantage of the fact that the elution order of the enantiomers for the unprotected hydroxycyclopentenone (compound 2, Fig. 5) is reversed from that of the protected hydroxycyclopentenone (compound 1). The elution order for compound 1 is *R*, *S*, and for compound 2 the order is *S*, *R*. During the separation of compound 2, the *S* enantiomer elutes first, allowing more of that enantiomer to be isolated than if it eluted second. The chemistry necessary to interconvert compound 1 and 2 is simple and does not result in racemization. Therefore we could isolate the *S* enantiomer of compound 1 through the isolation of the *S* enantiomer of compound 2 and then conversion to compound 1.

The analytical separation of compound 2 is shown in Fig. 6. Due to the small amounts of the *S* enantiomer needed, only three loadings and two different solvent compositions were investigated. The methods and the results are summarized in Table IV. The method used for purification was hexane–isopropanol (85:15) as mobile phase and a loading of 2 mg of sample per g of packing.

Automation of preparative LC equipment

Method development for the isolation of the *R* enantiomer of compound

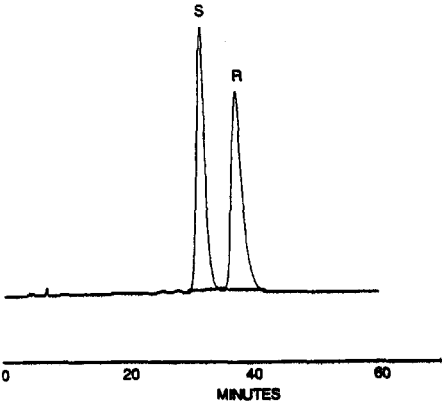

Fig. 6. Analytical HPLC separation of compound 2 on a Chiralcel OC column (250 mm × 4.6 mm), with a mobile phase of isopropanol–hexane (15:85). A flow-rate of 1.0 ml/min and detection at 215 nm, 0.1 a.u.f.s. were used.

TABLE IV
LOADING AND SOLVENT COMPOSITION STUDIES FOR COMPOUND 2
Chiralcel OC, 500 mm × 10 mm I.D., flow-rate 8 ml/min.

Mobile phase (hexane–isopropanol)	*Loading (mg sample/g packing)*	*% S enantiomer isolated*[a]
85:15	1	63
85:15	2	36
85:15	4	19
90:10	1	56
90:10	2	39
90:10	4	34

[a] No *R* enantiomer isolated.

1 showed that a 10-μm Chiralcel OC column was needed for the purification. Since the largest column commercially available was 500 mm × 20 mm I.D., a large number of repetitive injections would be necessary to produce the required amount of chemical. To help reduce the manhours necessary for this operation, automation of pump control, sample injection, and fraction collection is needed. This automation was accomplished with a pump controller, a programmable fraction collector and a 10-ml autosyringe to fill the loop of an electrically actuated injector. The complete preparative system consisted of pumps, an injector, detector, recorder and fraction collector. All were controlled though the use of flags and contact closures from the system controller.

The system controller is able to run programs up to 10 h in length. Multiple

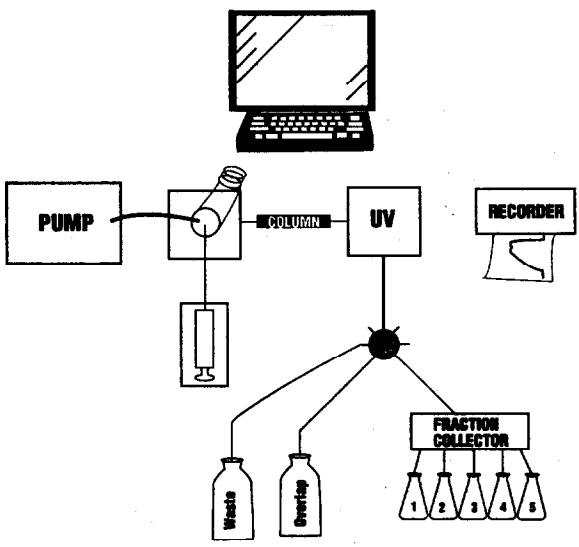

Fig. 7. Schematic of automated preparative system.

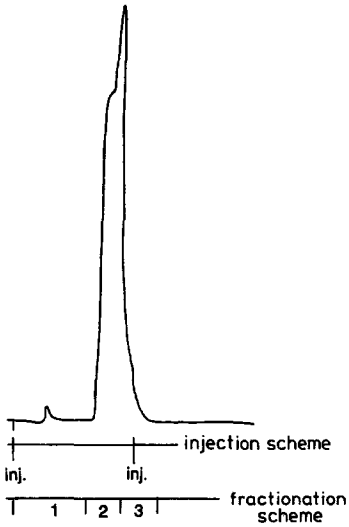

Fig. 8. Chromatogram of automated preparative run of compound 1. The purification was conducted on a Chiralcel OC column, 10-μm (500 mm × 20 mm I.D.), with a mobile phase of ethanol–hexane (1:99). A flow-rate of 20 ml/min, detection at 254 nm, 0.5 a.u.f.s. and a loading of 4 mg of sample per g of packing were used. 1 = waste fraction; 2 = fraction collector; 3 = overlap fraction.

fraction collectors were used because the fraction collectors could only collect 220 fractions for approximately 3.5 h. An electrically actuated six-port valve was installed after the detector allowing multiple fraction collectors to be used. While this allowed for unattended operation, it generated large numbers of fractions that needed analysis and workup.

To reduce the number of fractions, a different fraction collector was used that contained a funnel assembly, allowing for the collection of up to 27 fractions. In addition there was no limit on the fraction size. This allowed the effluent from repetitive injections to be collected into the same fraction vessels. Using this setup we were able to run unattended for up to 10 h and generate only 27 fractions which required analysis. The fractionation scheme was also adapted so that effluent containing impure or no chemical was diverted to a large container. A schematic of the automated system is shown in Fig. 7. A chromatogram from an automated run is shown in Fig. 8.

CONCLUSION

These studies demonstrate that preparative LC on a chiral stationary phase can be used to isolate multigram quantities of pure enantiomers. The separations are usually difficult but can be optimized through the investigation of solvent selectivities, loadings and flow-rate. It was also demonstrated that automation could be utilized to allow for unattended operation of the preparative LC system and greatly reduce the manual labor required for these purifications. We were able to isolate approximately 400 mg of *R* enantiomer per h and to produce a total of 25 g of *R* enantiomer.

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