# Short Communication

# Suitable chiral packing material for the high-performance liquid chromatographic separation of derivatives of 1'-hydroxyeugenol

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ABSTRACT

Plants of the genus *Coreopsis* contain rare phenylpropanoids of the 1'-hydroxyeugenol (Eu) type. To investigate the biological activities of these compounds, 1'-hydroxyeugenol isobutyrate (Eu1) and its derivatives were synthesized. The aim was to separate racemic Eu1 by high-performance liquid chromatography into its enantiomers on a preparative scale, in order subsequently to synthesize the equivalent esters. To control the stereoselectivity of esterification of Eu1, analytical systems investigated for Eu1–Eu5 can be utilized. The best results were obtained on microcrystalline cellulose triacetate (CTA), Pirkle Convent phenylglycine and Chiracel OK columns. Racemic Eu1 could be separated on a preparative scale on CTA.

INTRODUCTION

The genus *Coreopsis* accumulates phenylpropanoids with unusual structures and rare occurrence, and derivatives of 1'-hydroxyeugenol (Eu) (Fig. 1) are of great interest regarding their biological activities [1–5]. Eu2–Eu4 are naturally occurring compounds in the roots of *Coreopsis* species, whereas Eu1 and Eu5 are totally absent in the genus *Coreopsis*. A screening programme for the biological activities of Eu1–Eu5 required large amounts of these compounds. On this account, the racemates of Eu1–Eu5 were synthesized. In addition to Eu2–Eu4, the racemates of Eu1 and Eu5 were also integrated into the screening programme to obtain information about a supposed stucture–activity relationship.

It is well known that many naturally occurring or synthesized optically active compounds with one asymmetric carbon atom produce full biological activities only when they have a high enantiomeric purity. For this reason it was considered useful to test also the optically pure enantiomers of Eu1–Eu5,
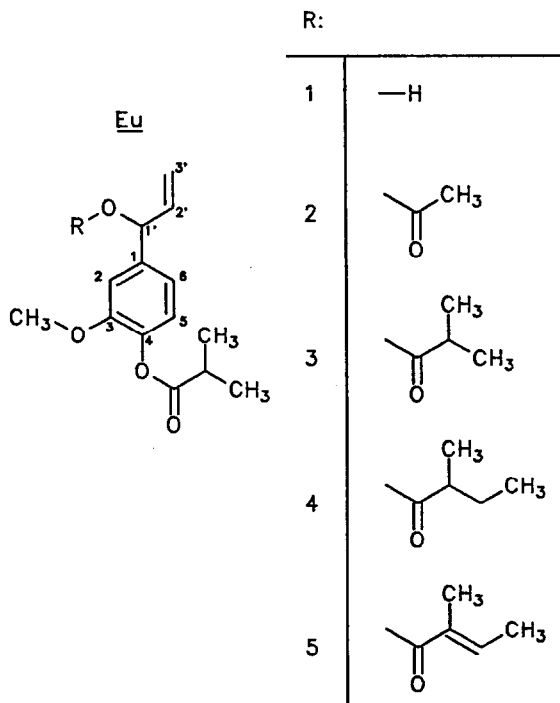
R:

Eu

1    —H

2    (acetyl group, CH₃)

3    (isobutyryl group)

4    (isovaleryl group)

5    (tigloyl group)

Fig. 1. Derivatives of 1'-hydroxyeugenol (Eu). Eu1 = 1'-hydroxy-eugenol isobutyrate; Eu2 = 1'-acetoxyeugenol isobutyrate; Eu3 = 1'-isobutyryloxyeugenol isobutyrate; Eu4 = 1'-isovaleroyloxyeugenol isobutyrate; Eu5 = 1'-tigloxyeugenol isobutyrate.

depending on the results with racemic compounds.

Separation of enantiomers by liquid chromatography on chiral stationary phases has become a practical and useful method for obtaining pure optical isomers. In this paper, we report high-performance liquid chromatographic (HPLC) separations of racemic Eu1 into pure optical enantiomers on a preparative scale and analytical separations of Eu1–Eu5 on different chiral stationary phases.

EXPERIMENTAL

Chiral stationary phases and eluents

Microcrystalline cellulose triacetate (CTA). The column contained CTA prepared by heterogeneous acetylation (250 × 6 mm I.D.) (Macherey–Nagel, Düren, Germany). The injection volume was 20 μl (25 mg of substance/ml of eluent).

For Eu1, Eu4 and Eu5 the eluent was n-hexane–isopropanol (90:10, v/v) at flow-rates of 1.5, 0.5 and 1 ml/min, respectively. For Eu2 the eluent was ethanol at a flow-rate of 1 ml/min. Eu3 was not tested.

Pirkle convent phenylglycine. The column (250 × 10 mm I.D.) contained as stationary phase (R)-(−)-N-3,5-dinitrobenzoylphenylglycine bonded on aminopropyl-functionalized silica gel (Regis, Austin, USA); N = 57 800 theoretical plates. The injection volume was 20 μl (25 mg of substance/ml of eluent) and the flow-rate was 1 ml/min.

For Eu1 and Eu2 the eluent was n-hexane–isopropanol (90:10, v/v) and for Eu3, Eu4 and Eu5 n-hexane–isopropanol (98:2, v/v).

Chiracel OK. The column (250 × 4 mm I.D.) contained as stationary phase cellulose cinnamate on silica gel [6,7] (Daicel Chemical Industries, Deventer, Netherlands); N = 17 500 theoretical plates. The injection volume was 20 μl (1 mg of substance/ml of eluent).

The eluent was n-hexane–isopropanol (98:2, v/v), at a flow-rate of 1.0 ml/min for Eu2 and 0.5 ml/min for Eu1 and Eu3–Eu5.

Preparative separation of Eu1

A CTA column (250 × 20 mm I.D.) (Macherey–Nagel) was used. The injection volume was 250 μl (80 mg of Eu1/ml of eluent). The eluent was n-hexane–isopropanol (90:10, v/v) at a flow-rate of 7 ml/min. The resolution factors were $k'_1$ = 6, α = 1.33 and $R_s$ = 0.4 (for definitions, see Table I).

A 456-mg amount of racemic 1'-hydroxyeugenol isobutyrate was dissolved in the eluent, of which 20 mg were always loaded on to the column. The weighed fractions showed 164 mg of enantiomerically pure (−)-Eu1 and 266 mg of (+)-Eu1.

Equipment

Analytical chromatography was performed using a Kontron (Munich, Germany) Model 420 pump, a Perkin-Elmer (Überlingen, Germany) Model 241 polarimeter and an Erma Optical Works UV detector (wavelength 279 nm) (Besta, Heidelberg, Germany). The effluent from the column was guided through the polarimeter and then through the UV detector.

For preparative chromatography a Latek (Heidelberg, Germany) P700 pump and a Milton-Roy (Hasselroth, Germany) Spectro Monitor 3100 UV detector (flow-rate 7 ml/min, wavelength 279 nm) were used.

## Synthesis of Eu1–Eu5

Grignard reaction of vanillin isobutyrate (obtainable through esterification of vanillin with isobutyryl chloride) with vinylmagnesium bromide gave racemic Eu1 [3]. Esterification of Eu1 with acetyl chloride or isobutyryl chloride yielded Eu2 and Eu3, respectively.

Eu4 and Eu5 were prepared using a method for the direct esterification of carboxylic acids described by Neises and Stegliz [8].

## RESULTS AND DISCUSSION

### Analytical separation of enantiomers

*Microcrystalline cellulose triacetate.* CTA, developed by Hesse and Hagel [9,10], is a useful stationary phase and has been widely employed for the separation of aromatic compounds [11,12]. First we examined the extent of separation using ethanol as the eluent and changing the flow. Using a flow-rate of only 0.1 ml/min we observed a partial separation of Eu1. This was insufficient for transfer to a preparative system, so we used more lipophilic eluents in place of ethanol. Finally, using $n$-hexane–isopropanol (90:10), Eu1 was completely separated into its enantiomers ($k'_1 = 3.43$; $\alpha = 1.42$; $R_s = 0.83$) (Table I). The polarimetric results show that the first peak represents the laevorotatory enantiomer of Eu1 and the second peak the dextrorotatory enantiomer.

All efforts to separate racemic Eu2–Eu5 into the enantiomers using CTA failed.

*Pirkle phenylglycine column.* This chiral stationary phase, described by Pirkle and co-workers [13,14], showed an ability to separate the enantiomers of Eu1 using $n$-hexane–isopropanol (90:10) as eluent. The enantiomer eluted first was (+)-Eu1. It was also possible to separate Eu3–Eu5 when the eluent was changed to $n$-hexane–isopropanol (98:2) (Fig. 2). Resolution of Eu2 on this chiral stationary phase was impossible (Table I). In contrast to Eu1, the laevorotatory enantiomers of Eu3–Eu5 left the column faster than the dextrorotatory enantiomers.

*Chiracel OK column.* With a Chiracel OK column and $n$-hexane–isopropanol (98:2) as eluent (flow-rate 1 ml/min), racemic Eu2 was successfully separated into its antipodes (Table I). The separations of te enantiomers of Eu3 and Eu4 were even more effective when the flow-rate was halved. In all

## TABLE I

### RESULTS OF CHROMATOGRAPHIC RESOLUTION OF Eu1–Eu5 ON DIFFERENT COLUMNS

$k'_1$ (capacity factor for less retained enantiomer) = (retention time − dead time)/dead time; $\alpha$ (separation factor) = (capacity factor for more retained enantiomer)/$k'_1$; $R_s$ (resolution factor) = 2(distance of the two peak positions)/(sum of band widths of the two peaks); for separation conditions, see Experimental. $k'_1$: +/− indicates sign of optical rotation of first eluting enantiomer.

| Column | $k'_1$ | $\alpha'$ | $R_s$ | Substance |
|---|---|---|---|---|
| CTA | 3.43(−) | 1.42 | 0.83 | Eu1 |
| Pirkle | 2.00(+) | 1.25 | 1.00 | |
| Chiracel OK | 0.88 | 1.00 | − | |
| CTA | 2.33(−) | 1.00 | −[a] | Eu2 |
| Pirkle | 1.29(+) | 1.00 | −[a] | |
| Chiracel OK | 5.63(+) | 1.19 | 1.06 | |
| Pirkle | 2.29(−) | 1.12 | 1.14 | Eu3 |
| Chiracel OK | 1.69(+) | 1.78 | 1.75 | |
| CTA | 0.85(−) | 1.00 | −[a] | Eu4 |
| Pirkle | 1.96(−) | 1.09 | 1.00 | |
| Chiracel OK | 2.25(+) | 1.17 | 0.67 | |
| CTA | 1.09 | 1.00 | − | Eu5 |
| Pirkle | 3.14(−) | 1.08 | 1.00 | |
| Chiracel OK | 4.87 | 1.00 | − | |

[a] No separation was observed with the UV detector, although partial separation was obtained with the polarimeter detector.
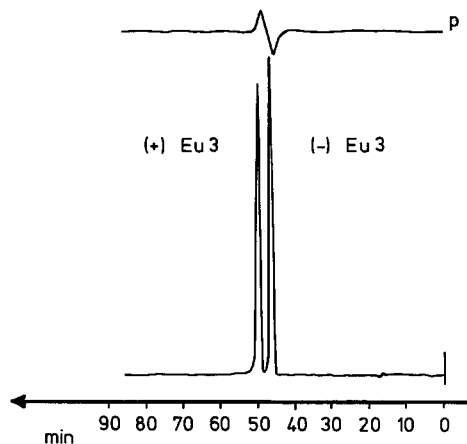


Fig. 2. Analytical separation of Eu3 using the Pirkle convent phenylglycine column (250 × 10 mm I.D.) with $n$-hexane–isopropanol (98:2) as eluent at a flow-rate of 1 ml/min. UV detection at 279 nm. Concentration, 25 mg/ml; injection volume, 20 $\mu$l. Polarimeter: Hg lamp emitting at 302 nm (p = polarimeter curve).

instances the dextrorotatory enantiomer was eluted first. Separation of Eu1 and Eu5 was impossible under these conditions.

The results of the analytical resolution of Eu1–Eu5 on the different chiral stationary phases are summarized in Table I. All racemic compounds were separable chromatographically on one or other of the three columns. The selection of the chiral stationary phases was made empirically, because it is very difficult to determine the structure requirements for a chiral stationary phase based on the structural parameters of the enantiomers. The best results were obtained on the Pirkle phenylglycine column. This column showd a remarkable chiral discrimination against Eu1 and Eu3–Eu5, whereas the CTA column resolved only Eu1 with high efficiency. The latter is unexpected as CTA has been widely employed for the separation of aromatic compounds [12,15,16].

It is also noteworthy that on the Pirkle column the enantiomers of Eu1 were eluted in reversed order compared with the enantiomers of Eu3–Eu5. This indicates that esterification of the alcoholic OH group on the side-chain of Eu1 has the consequence that the chiral discrimination of Eu3–Eu5 on the Pirkle phenylglycine column changed with respect to Eu1.

*Separation of Eu1 on CTA on a preparative scale*

The next objective was to separate the synthetic racemate of Eu1 into its antipodes on a preparative scale.The optimization strategy for preparative chromatography in the form of overload experiments was applied to the direct HPLC separation of the enantiomers. Considering the time–performance factor, we chose a system that allowed only a partial separation. The analysis of the fractions to ascertain the points of intersection and to determine the enantiomeric purity was achieved with the Pirkle phenylglycine column. The chromatography resulted in a high optical purity of (−)-Eu1 and an

enantiomeric excess of (+)-Eu1 of 99%. The mean fractions containing the enantiomers in the ratio 1:1 were separated again.

CONCLUSIONS

With microcrystalline CTA as a chiral stationary phase and *n*-hexane–isopropanol (90:10, v/v) as eluent, racemic Eu1 was successfully separated into its antipodes by HPLC on both an analytical and a preparative scale. It seems to be possible to separate pure (+)-Eu1 and (−)-Eu1 in large amounts using this technique.

Based on the results of the biological tests, the equivalent esters of (+)- and (−)-Eu1 will now be synthesized, and the optical purity of the esterification products will be controlled with these analytical systems investigated for Eu1–Eu5.

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