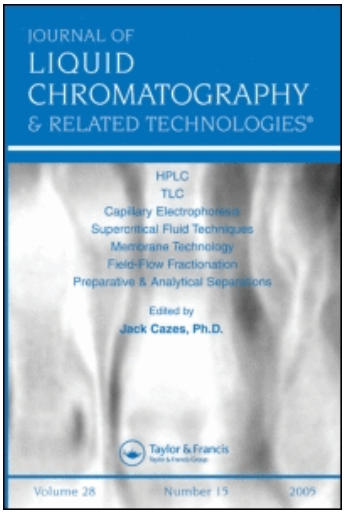

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Semi-Preparative HPLC Separations of *E* and *Z* Isomers of New Aromatic Retinoids

Subramaniam Mohanraj[ab]
[a] Baker Laboratory, Department of Chemistry, Cornell University, Ithaca, NY [b] Department of Chemistry, Oklahoma State University, Stillwater, OK

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SEMI-PREPARATIVE HPLC SEPARATIONS OF E AND Z ISOMERS OF NEW AROMATIC RETINOIDS

Subramaniam Mohanraj (1)
Baker Laboratory, Department of Chemistry
Cornell University
Ithaca, NY 14853

ABSTRACT

Semi-preparative scale HPLC separations of E and Z isomers of new aromatic retinoids with $\alpha \simeq 1$ are reported. Clean separations with quantitative yields were achieved in less than 1 hour by normal phase HPLC using an analytical instrument.

INTRODUCTION

Synthetic retinoids are extremely effective drugs in various types of keratinization disorders (2). In addition, they exert antiinflammatory effects and seem to possess immunomodulatory properties such as lymphocytes and macrophages (2). The application of HPLC to the separation of retinoids continues to expand at a rapid rate (3,4). Much work has been done in developing methods for the analysis and separation of retinoids in μg and ng quantities (4). There are instances (5) that E isomers were crystallized from retinoid mixtures leaving up to 42% of E and Z mixtures in mother liquors. The corresponding Z isomers were not isolated although Z isomers of some retinoids show significant biological activity (6) and 13-cis retinoic

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acid is less toxic than all-trans retinoic acid (2). Crystals obtained after repeated crystallizations of an E and Z retinoid mixture from different solvents were still reported to be a mixture (7). It has also been reported that synthetic retinoids, an E and Z mixture (5a and 6a in reference 7) could not be separated by HPLC using different solvent systems.

During our work on the synthesis of new aromatic retinoids for testing as potential anti-cancer agents, we were faced with the problem of separating the following retinoids in significant quantities: a) methyl (Z)- and methyl (E)-p-[2-(5,6,7,8-tetrahydro-8,8-dimethyl-2-naphthyl)propenyl]benzoates (1 and 2), b) (1E,3Z)- and (1E,3E)-m-[4-(5,6,7,8-tetrahydro-8,8-dimethyl-2-naphthyl)-1,3-pentadienyl]phenols (3 and 4), c) (Z)- and (E)-3-methyl-5-[2-(5,6,7,8-tetrahydro-8,8-dimethyl-2-naphthyl)-propenyl]phenols (5 and 6), and d) methyl (2E,4E,6Z)- and methyl (2E,4E,6E)-3-methyl-7-(5,6,7,8-tetrahydro-8,8-dimethyl-2-naphthyl)-2,4,6-octatrienoates (7 and 8). We have achieved excellent HPLC separations of the retinoid isomers having $\alpha \simeq 1$ (TLC analysis) in a semi-preparative scale. Structures and α (selectivity factor) values of the retinoid isomers are shown in Figure 1.

MATERIALS AND METHODS

HPLC separations were carried out on a Waters Associates ALC 201 liquid chromatograph equipped with a differential refractometer R401 using a Whatman Partisil M9 10/50 column with a chart speed of 1/2" per min. Fractions were characterized by ^{1}H NMR at 300 MHz using a Bruker WM 300 spectrometer and ^{13}C NMR at 22.5 MHz using a Jeol FX 90Q spectrometer. TLC was performed on pre-coated Kieselgel 60 plates (Merck). The synthesis and anti-tumor activity of the new aromatic retinoids will be published elsewhere (8).

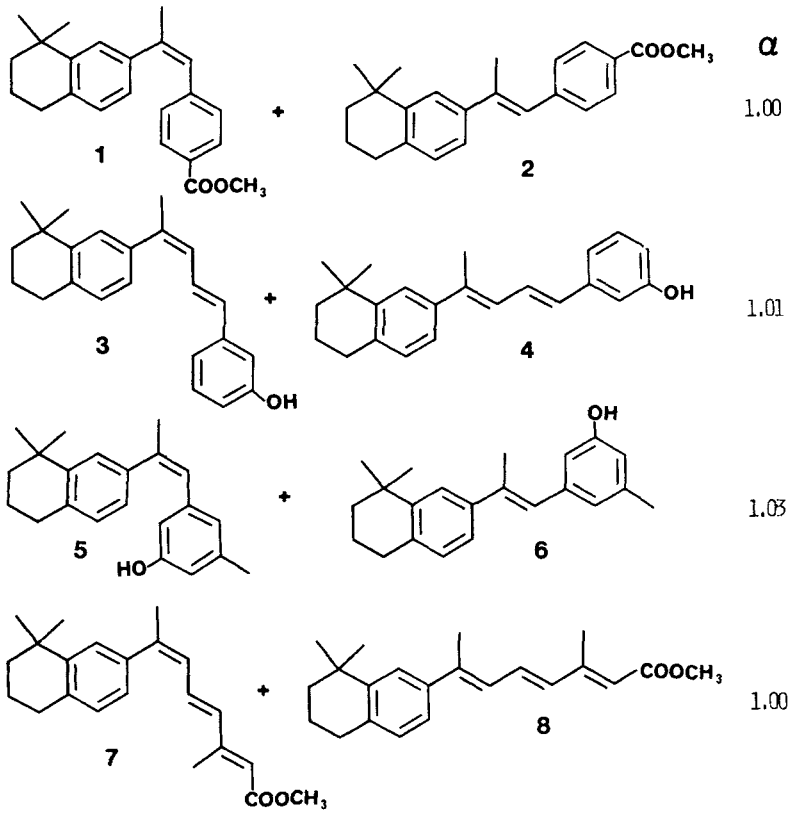

FIGURE 1. Structures and α values of Z and E isomers of new aromatic retinoids.

RESULTS AND DISCUSSION

The mixture of 1 and 2 (ca. 3:2 by NMR analysis) showed a single spot on silica gel plates; Rf 0.22 (solvent system: 4% EtOAc/hexane); selectivity factor α ≃1.00 (TLC analysis). Figure 2a shows the HPLC separation of retinoids 1 and 2 with the solvent system 4% EtOAc/hexane at a flow rate of 1.54 ml/min. Injection of 38 mg of the mixture of 1 and 2 afforded 21 mg of 1 and 14 mg of 2 (92.1% yield) in 43 min.

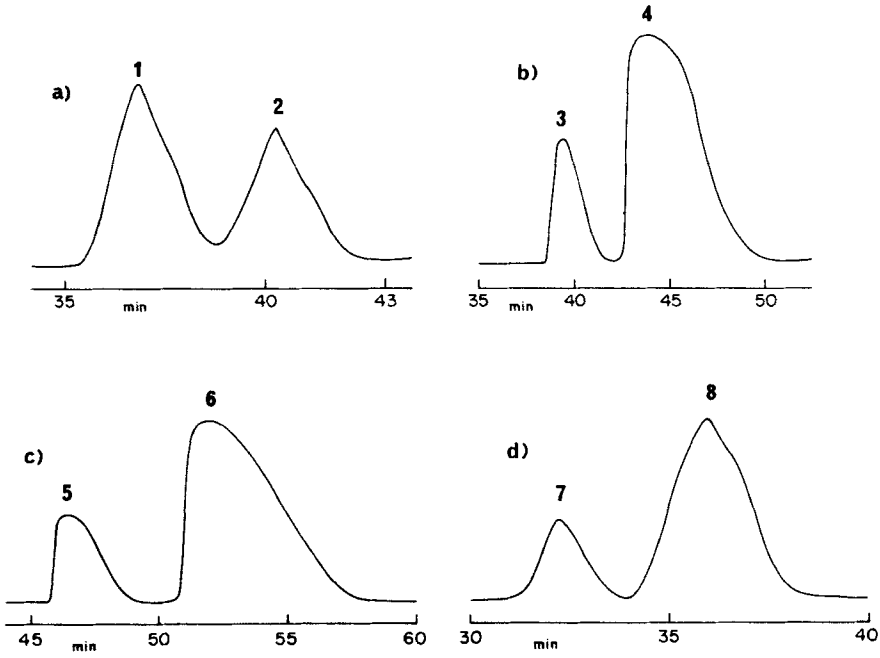

FIGURE 2. Separations of Z and E isomers of retinoids a) 1 and 2; b) 3 and 4; c) 5 and 6; d) 7 and 8.

The mixture of 3 and 4 (ca. 1:4 by NMR analysis) showed almost a single spot on silica gel plates; Rf 0.21 (solvent system: 15% EtOAc/hexane); $\alpha \simeq 1.01$. Figure 2b shows the HPLC separation of retinoids 3 and 4 with the solvent system 15% EtOAc/hexane at a flow rate of 1.2 ml/min. Injection of 64 mg of the mixture of 3 and 4 furnished 13 mg of 3 and 49 mg of 4 (96.9% yield) in 50 min.

The mixture of 5 and 6 (ca. 15:85 by NMR analysis) showed very closely overlapping spots on silica gel plates; Rf 0.205 and 0.21 (solvent system: 12% EtOAc/hexane); $\alpha \simeq 1.03$. Figure 2c shows the HPLC separation of retinoids 5 and 6 with the solvent system 12% EtOAc/hexane at a flow rate of 1.2 ml/min. Injection of 53 mg of the mixture of 5 and 6 gave 8mg of 5 and 43 mg of 6 (96.2% yield) in 1 hour.

The mixture of 7 and 8 (ca. 35:65 by NMR analysis) showed a single spot on silica gel plates; Rf 0.25 (solvent system: 5% EtOAc/hexane); $\alpha \simeq 1.00$. Figure 2d shows the HPLC separation of retinoids 7 and 8 with the solvent system 5% EtOAc/hexane at a flow rate of 1.44 ml/min. Injection of 40 mg of the mixture of 7 and 8 furnished 13 mg of 7 and 24 mg of 8 (92.5% yield) in 40 min.

Excellent separations with yields of 92% were achieved in a) and d) where $\alpha \simeq 1.00$. In b) and c) where $\alpha \simeq 1.01$ and 1.03, 96-97% yields were obtained with clean separations. In all the four cases, Z isomers were eluted first followed by the corresponding E isomers.

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