Journal of Chromatography, 295 (1984) 295–298
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,749

Note

Separation of carotenoids by high-performance liquid chromatography

III*. 1,2-Epoxycarotenoids**

MATTHIAS KAMBER and HANSPETER PFANDER*
Department of Organic Chemistry, Chemical Institutes, University of Berne, Freiestrasse 3, CH-3012 Berne (Switzerland)
(First received September 2nd, 1983; revised manuscript received March 16th, 1984)

Several years ago, Britton and co-workers[1–3] isolated 1,2-epoxycarotenoids from tomatoes. Our investigations of the stereochemistry of these compounds[4,5] and the synthesis of optically active 1,2-epoxycarotenoids[6–8] required an efficient method for the separation of these pigments and especially of *cis/trans* isomers. High-performance liquid chromatography (HPLC) has been ussed extensively for the separation of complex carotenoids mixtures in recent years. For example, the separation of the carotenoids from the hips of *Rosa pomifera* was described by Märki-Fischer *et al.*[9], the separation of the stereoisomers of zeaxanthin and lutein via diastereoisomers was reported by Rüttimann *et al.*[10] and we have used this method for the separation of crocetin derivatives[11,12]. Reviews have been published by Fiksdahl *et al.*[13] and Taylor[14].

In this paper, we report on the separation of 1,2-epoxy-1,2-dihydrolycopene (1), 1′,2′-epoxy-1′,2′-dihydro-γ-carotene (2) and 1,2-epoxy-1′,2′-dihydro-δ-carotene (3) on LiChrosorb RP-18 and the separation of the *cis/trans* isomers of 1 and 2 on buffered silica gel.

EXPERIMENTAL

Separations were carried out with shielding from light.

Apparatus

Two Altex Model 110A pumps, an Altex 420 microprocessor, a Rheodyne Type 7125 sample inlet system, a Uvikon LCD 725 detector and a W + W Tarkan 600 recorder were used. The columns were made of stainless steel 316, with dimensions of 250 × 4.6 mm I.D. for analytical and 250 × 10 mm I.D. for semi-preparative separations.

Solvents

n-Hexane was obtained from Fisons (Loughborough, U. K.) and acetonitrile,

* For Part II, see ref. 12.
** Part of the *Ph.D. Thesis* of M.K., University of Berne, Berne, 1982.

0021-9673/84/$03.00

tert.-butyl methylether, N,N-diisopropylethylamine from Fluka (Buchs, Switzerland).

Conditions for HPLC

Separation of 1, 2 and 3. Pre-packed columns (Merck, Darmstadt, F.R.G., LiChrosorb RP-18, 5 μm) and acetonitrile at a flow-rate of 1.8 ml/min were used on the analytical scale. Volumes of 20 μl of a solution of the three pigments in the mobile phase were injected and detection was carried out at 480 nm. Under these conditions the capacity factors (k') were 4.40 for 1, 5.60 for 3 and 6.40 for 2.

Separation of cis/trans isomers of 1. The analytical column was slurry-packed[15] with LiChrosorb SI 60, 5 μm. The mobile phase was *n*-hexane–*tert.*-butyl methyl ether–N,N-diisopropylethylamine (100:4:0.1) at a flow-rate of 1.4 ml/min. Volumes of 20 μl of a solution of 1 in dichloromethane were injected and the compounds were detected at 500 nm. Under these conditions the k' values were: 3.86 for 7-*cis*-1, 4.57 for 7′-*cis*-1 and 5.12 for all-*trans*-1. The semi-preparative column was also slurry-packed with LiChrosorb SI 60, 5 μm. The mobile phase was the same as on the analytical scale, but at a flow-rate of 2.8 ml/min, and detection was at 520 nm. Amounts of 0.1–0.3 mg of a mixture of 1 in 100 μl of dichloromethane were injected. The k' values were 2.69 for 7-*cis*-1, 2.98 for 7′-*cis*-1 and 3.35 for all-*trans*-1.

Separation of cis/trans isomers of 2. The columns, the mobile phase, the detection and the injected volumes were for the *cis/trans* isomers of 1. For analytical separations the flow-rate was 1.3 ml/min and the k' values were 1.91 for 7′-*cis*-2 and 3.37 for all-*trans*-2. For semi-preparative separations the flow-rate 2.5 ml/min and the k' values were: 1.52 for 7′-*cis*-2 and 1.96 for all-*trans*-2.

RESULTS AND DISCUSSION

Separation of 1, 2 and 3.

As we reported earlier[11], Spherisorb ODS as the stationary phase and acetonitrile as the mobile phase can be used to separate α-, β- and γ-carotene and lycopene. Similar conditions were used to separate 1,2-epoxy-1,2-dihydrolycopene (1,2-epoxy-1,2-dihydro-ψ,ψ-carotene) (1), 1′,2′-epoxy-1′,2′-dihydro-γ-carotene (1′,2′-epoxy-1′,2′-dihydro-β,ψ-carotene) (2) and 1′,2′-epoxy-1′,2′-dihydro-δ-carotene (1′,2′-epoxy-1′,2′-dihydro-ε,ψ-carotene) (3). These three pigments were completely separated within 10 min on LiChrosorb EP-18 with acetonitrile (Fig. 1).

Separation of cis/trans isomers of 1,2-epoxy-1,2-dihydrolycopene (1)

The 1,2-epoxycarotenoids are very sensitive compounds. During the separation on silica gel these pigments are partially degraded, as was shown by repeated injection of the isomers. On the other hand, experiments with reversed-phase material gave no separation. However, using buffered silica-gel (with N,N-diisopropylethylamine) three pure isomers could be obtained without serious loss, namely, 7-*cis*-, 7′-*cis*- and all-*trans*-1 (Fig. 2).

This method was extended to the semi-preparative scale using 250 × 10 mm I.D. columns. With larger columns the loss of 1 increased drastically, owing to the long time required for each run. With the semi-preparative columns 0.1–0.3 mg of the *cis/trans* mixture could be separated within 30 min, which seemed acceptable for obtaining enough of the pure isomers for spectroscopic examinations[8].

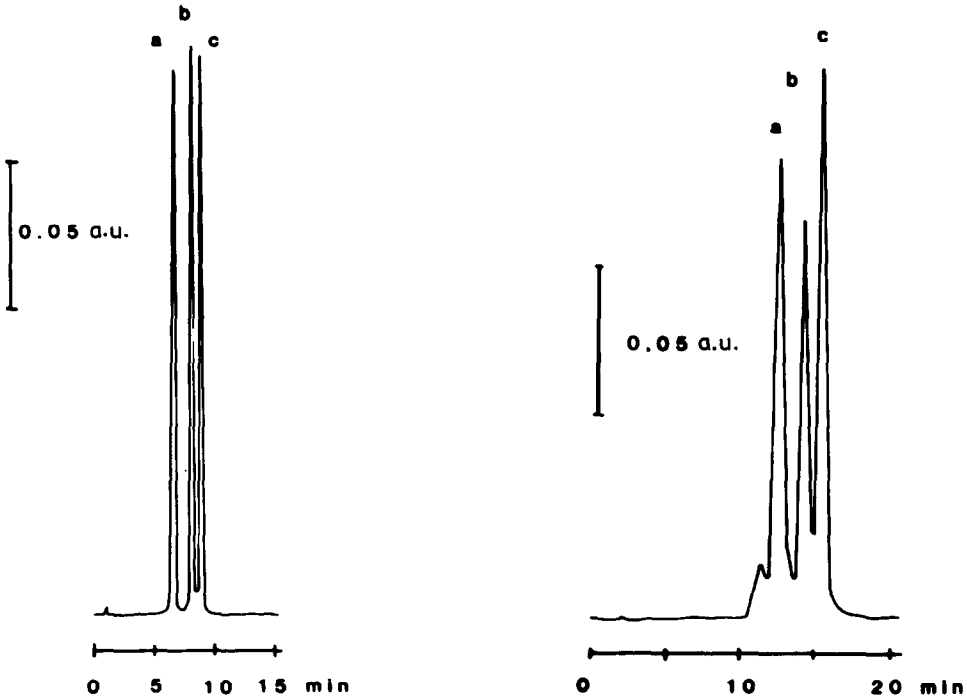

Fig. 1. HPLC of (a) 1,2-epoxy-1,2-dihydrolycopene, (b) 1′,2′-epoxy-1′,2′-dihydro-δ-carotene and (c) 1′,2′-epoxy-1′,2′-dihydro-γ-carotene.

Fig. 2. HPLC of (a) 7-*cis*-1, (b) 7′-*cis*-1 and (c) all-*trans*-1.

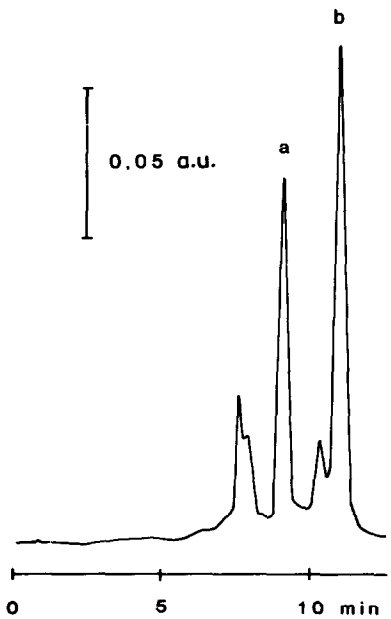

Fig. 3. HPLC of (a) 7′-*cis*-2 and (b) all-*trans*-2.

Separation of cis/trans isomers of 1',2'-epoxy-1',2'-dihydro-γ-carotene (2)

This sensitive pigment was also separated on buffered silica gel. From the synthetic sample the pure 7'-*cis* and the all-*trans* isomers could be separated (fig. 3).

For spectroscopic examinations larger amounts of the isomers were separated with a semi-preparative column.

ACKNOWLEDGEMENTS

This work was supported by the Swiss National Science Foundation. We are also grateful to F. Hoffmann-La Roche & Co., Ltd (Basle, Switzerland) for their support, to Mrs. V. Meyer for experimental assistance in packing the columns and to Dr. M. Läderach for assistance with the manuscript.

REFERENCES

1 G. Britton and T. W. Goodwin, *Phytochemistry*, 8 (1969) 2257.
2 A. Ben-Aziz, G. Britton and T. W. Goodwin, *Phytochemistry*, 12 (1973) 2759.
3 G. Britton and T. W. Goodwin, *Phytochemistry*, 14 (1975) 2530.
4 J. D. Berset, *Lizentiat*, Berne, 1980.
5 J. D. Berset and H. Pfander, *Helv. Chim. Acta*, in press.
6 H. Pfander, M. Kamber and Y. Battegay-Nussbaumer, *Helv. Chim. Acta*, 63 (1980) 1367.
7 H. Pfander and M. Kamber, *Helv. Chim. Acta*, 63 (1980) 1972.
8 M. Kamber, H. Pfander and K. Noack, *Helv. Chim. Acta*, in press.
9 E. Märki-Fischer, U. Marti, R. Buchecker and C. H. Eugster, *Helv. Chim. Acta*, 66 (1983) 494.
10 A. Rüttimann, K. Schiedt and M. Vecchi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 612.
11 H. Pfander, H. Schurtenberger and V. Meyer, *Chimia*, 34 (1980) 179.
12 H. Pfander and M. Rychener, *J. Chromatogr.*, 234 (1982) 443.
13 A. Fiksdahl, J. T. Mortensen and S. Liaaen-Jensen, *J. Chromatogr.*, 157 (1978) 111.
14 R. T. Taylor, *Advan. Chromatogr.*, 22 (1983) 157.
15 V. Meyer, *Praxis der Hochleistungsflüssigchromatographie*, Diesterweg-Salle, Frankfurt a.M., 1979.