CHROM. 14,398

Note

Separation of dolichols and polyprenols by straight-phase high-performance liquid chromatography

R. KENNEDY KELLER*, GRANT D. ROTTLER and W. LEE ADAIR, Jr.

Department of Biochemistry, University of South Florida College of Medicine, Tampa, FL 33612 (U.S.A.)

(Received September 17th, 1981)

Polyprenols and their 2,3-dihydro derivatives, the dolichols, are widely distributed in nature[1,2]. Recently, as part of a project to prepare substrates for the CTP-dependent dolichol kinase, we developed a procedure for converting polyprenols to dolichols by specific chemical reduction of the α-isoprene unit[3]. To monitor the course of reduction and quantify the final product, we investigated the applicability of high-performance liquid chromatography (HPLC). We found that reversed-phase HPLC, a method used exclusively by previous workers to analyze dolichols[4–6], was unsatisfactory for resolving polyprenols from dolichols.

In this paper we demonstrate the separation and quantitation of polyprenols and dolichols by adsorption (straight-phase) HPLC. We show that the broadened peaks observed for each compound are due to partial fractionation of the individual isoprenolog species. In addition, by employing this technique, we show that commercial laboratory rat chow contains considerable amounts of both polyprenols and dolichols.

EXPERIMENTAL

Materials and methods

Solanesol and pig liver dolichol were purchased from Sigma. Individual isoprenologs of dolichol were prepared by semipreparative HPLC as described below. Polyprenol-16 (hexadecaprenol) and polyprenol-19 (nonadecaprenol) were from Calbiochem. Ficaprenol was prepared from leaves of *Ficus elastica* according to Burgos *et al.*[7]. Pinaprenol (a mixture of polyprenols ranging in length from fourteen to eighteen isoprenes) was prepared from *Pinus elliottii* by a similar procedure. α-*trans*-Polyprenol-16, rat chow prenol, and dolichol-11 were prepared according to Keller *et al.*[3]. Geraniol and nerol were from Tridom/Fluka. Citronellol was from Pfaltz and Bauer, Inc.

High-performance liquid chromatography

HPLC was carried out at room temperature on a Laboratory Data Control Constametric II chromatograph equipped with a variable wavelength detector. UV monitoring was at 210 nm. UV signals were integrated on-line with a Perkin-Elmer Minigrator. Straight-phase (adsorption) chromatography was performed at a flow-rate of 2 ml/min on a 25-cm Whatman Partisil-5 column using mixtures of diethyl

ether in hexane as described in the figure legends. Reversed-phase chromatography was carried out on a 25-cm Whatman Partisil-5 ODS column using a mobile phase of isopropanol–methanol (1:1 v/v) and a flow-rate of 1.5 ml/min.

RESULTS AND DISCUSSION

Initial experiments on the chromatographic behavior of prenols used the model compounds nerol, which has an α-*cis*-isoprene unit, geraniol, which is α-*trans*, and citronellol, which has a saturated α-isoprene unit. All three compounds have the same chain length. Fig. 1 shows the separation of these three compounds on straight-phase HPLC. The elution order nerol, citronellol, geraniol suggests that the bulky hydro-'carbon group *cis* to the alcohol moiety sterically hinders association of nerol with the silica support, leading to a relatively early elution time. Apparently the steric effect overcomes the increased polarity due to the double bond. Steric hindrance is apparently not a factor in the association of the hydroxyl groups of citronellol and geraniol with the support and thus these two compounds fractionate according to their respective polarities.

When polyprenols and dolichols were tested on straight-phase HPLC, the same order of elution was found. As shown in Fig. 2, the naturally occurring α-*cis*-polyprenol-16 emerged earlier than dolichol-16, which in turn emerged earlier than synthetically prepared α-*trans*-polyprenol-16. The baseline separation of the *cis*-polyprenols and dolichols allowed us to monitor purity of these compounds during the chemical conversion of polyprenols to dolichols. Detector response was linear from 0.5 to 50 μg.
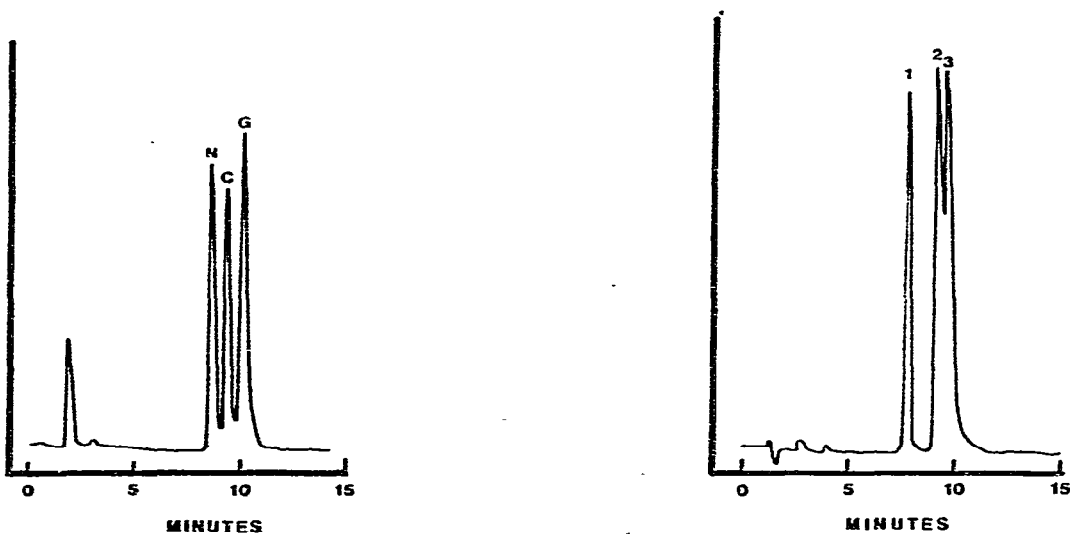

Fig. 1. Straight-phase HPLC of nerol (N), citronellol (C), and geraniol (G). The mobile phase employed was hexane–diethyl ether (90:10).

Fig. 2. Straight-phase HPLC of α-*cis*-polyprenol-16 (1), dolichol-16 (2) and α-*trans*-polyprenol-16 (3). The mobile phase was hexane–diethyl ether (93:7).
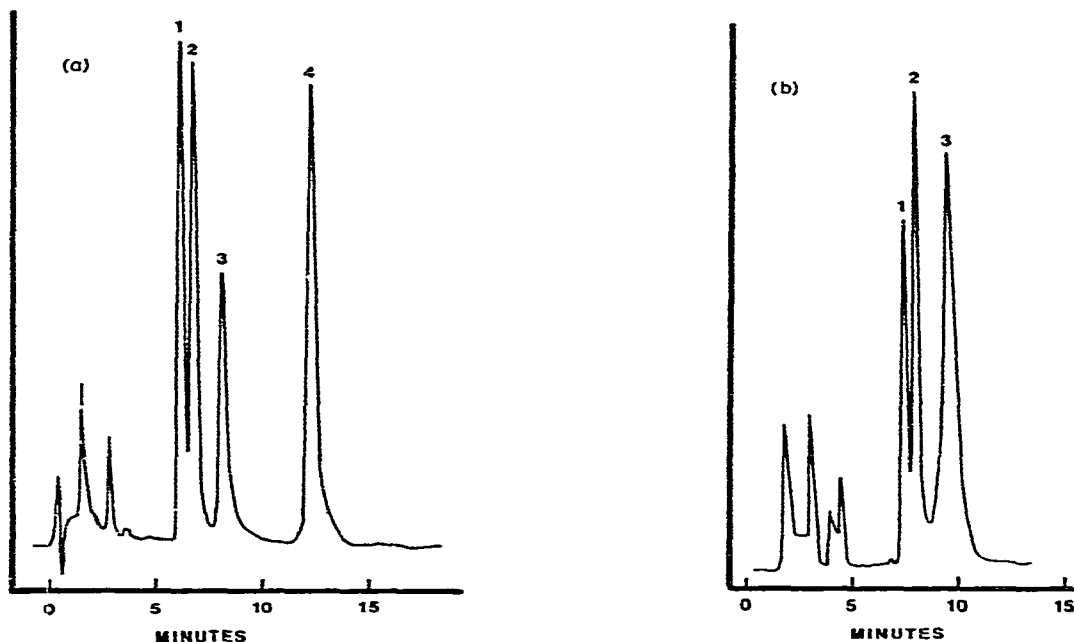
Fig. 3. Straight-phase HPLC of: (a) polyprenol-19 (1), polyprenol-16 (2), ficaprenol (polyprenol-11) (3) and solanesol (4); (b) dolichol-20 (1), dolichol-17 (2), and dolichol-11 (3). The mobile phase was hexane–diethyl ether (93:7).

Straight-phase HPLC of pig liver dolichol, containing a mixture of isoprenologs, gave a broadened peak compared with that of pure dolichol-16 or -19. Similar results were obtained with a polyprenol fraction from pine needles. These results suggested that partial fractionation of prenols into their individual isoprenolog compounds was taking place. To examine this possibility, dolichols and polyprenols of defined chain length were chromatographed. The results show that significant separation of isoprenologs is achieved for each class of prenol (Fig. 3). When the relative elution times were plotted as a function of isoprene number, a linear relationship was obtained (Fig. 4). Interestingly, all-*trans*-solanesol (nine isoprenes) eluted considerably later than would have been predicted based on chain length and the effect of the α-*trans* isoprene. Apparently, internal *trans* residues can also contribute to a lengthened retention time. Although the 25-cm straight-phase column that we have employed was not able to resolve prenols differing by a single isoprene, it may be possible to achieve such separation using tandem columns. However, for the purpose of quantitation, it is convenient to obtain a single broad peak rather than a a resolved isoprenolog pattern since, in the latter case, each peak must be integrated and summed to give the total. Indeed, it is for this reason that straight-phase HPLC is preferable to the reversed-phase mode in simplicity of quantitation.

The ability to resolve polyprenols and dolichols on straight-phase HPLC proved useful in examining the prenol content of commercial laboratory rat chow. Fig. 5 shows that chow contains polyprenols and dolichols in a ratio of 1:4. Quantitation of these peaks indicates that 11.5 μg prenols are present per g of chow. By
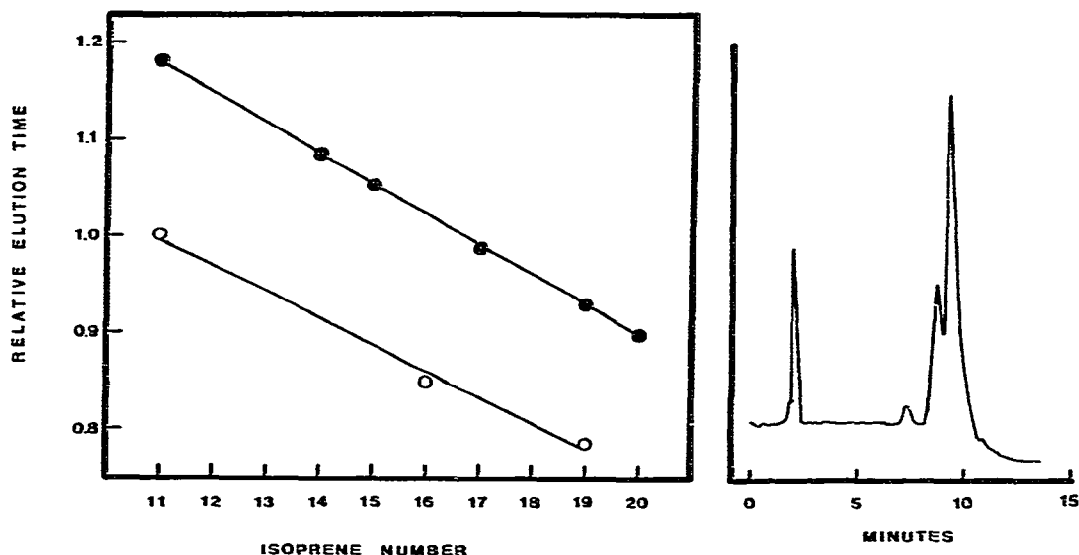
Fig. 4. Plot of relative elution time *vs.* isoprene number for dolichols (●) and polyprenols (○). Data are plotted relative to the elution time of ficaprenol, which was added as an internal standard in all experiments.

Fig. 5. Straight-phase HPLC of high-molecular-weight prenol fraction from rat chow. A high-molecular-weight prenol fraction was prepared using Fractogel 6000[8] and subjected to straight-phase HPLC in hexane–diethyl ether (93:7).

reversed-phase HPLC, the dolichols and polyprenols were found to contain isoprenologs ranging from thirteen to eighteen isoprenes in length[3].

REFERENCES

1 F. W. Hemming, *MTP Int. Rev. Sci. Biochem. Ser One*, 4 (1974) 39.
2 R. A. Morton, in J. Ganguly and R. M. S. Smellie (Editors), *Current Trends in the Biochemistry of Lipids*, Academic Press, New York, 1972, p. 203.
3 R. K. Keller, G. D. Rottler and W. L. Adair, *Biochem. Biophys. Res. Commun.*, submitted for publication.
4 I. A. Tavares, N. J. Johnson and F. W. Hemming, *Biochem. Soc. Trans.*, 5 (1977) 1771.
5 D. J. Freeman, C. A. Rupar and K. K. Carroll, *Lipids*, 15 (1980) 191.
6 R. W. Keenan, N. Rice and R. Quock, *Biochem. J.*, 165 (1977) 405.
7 J. Burgos, F. W. Hemming, J. F. Pennock and R. A. Morton, *Biochem. J.*, 88 (1963) 470.
8 R. K. Keller and W. L. Adair, *J. Biol. Chem.*, submitted for publication.