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Liquid chromatographic separation of isomeric phenanthrols on monomeric and polymeric C_{18} columns

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ABSTRACT

Separations of all five possible isomeric phenanthrols by reversed-phase high-performance liquid chromatography using a monomeric Zorbax C_{18} column and a polymeric Vydac C_{18} column were compared. With identical elution solvents and flow-rates, the phenanthrols were separated with shorter retention times on the latter column. The elution orders of the phenanthrols were different on the two columns. Ultraviolet absorption spectral properties of the phenanthrols in methanol and in alkaline methanol are reported.

INTRODUCTION

Metabolism of phenanthrene yields phenolic products which are formed by spontaneous isomerization of the metabolically formed epoxide intermediates [1–3]. A satisfactory chromatographic method has previously not been available to separate all five possible phenanthrols efficiently. We describe in this paper the reversed-phase high-performance liquid chromatographic (HPLC) separation of all five isomeric phenanthrols by the sequential use of a polymeric Vydac C_{18} column and a monomeric Zorbax ODS (C_{18}) column. Ultraviolet absorption properties of chromatographically pure phenanthrols are reported.

EXPERIMENTAL

Materials

9-Phenanthrol was purchased from Aldrich (St. Louis, MO, U.S.A.). Phenanthrene *trans*-1,2- and *trans*-3,4-dihydrodiols were isolated by normal-phase HPLC (see below) from a mixture of products formed by incubation of phenanthrene with rat liver microsomes [2,3], 1- and 2-phenanthrols were obtained by dehydration of phenanthrene *trans*-1,2-dihydrodiol with dilute HCl in acetone at 37°C for 1 h and 3- and 4-phenanthrols were similarly obtained by dehydration of phenanthrene *trans*-3,4-dihydrodiol. All phenanthrols used for UV absorption spectral measurement were eluted as a single peak by reversed-phase HPLC.

Phenanthrene *trans*-1,2- dihydrodiol, obtained by incubation of phenanthrene

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with rat liver microsomes and isolated by normal-phase HPLC (see below), was dehydrated by acid in acetone to form 1-phenanthrol and 2-phenanthrol, which were separated on the Vydac C_{18} column. The area ratio of the chromatographic peaks, detected at 254 nm, of 1- and 2-phenanthrol was 64:36, which is in close agreement with the ratio of 66:34 reported by Jerina *et al.* [4]. Phenanthrene *trans*-3,4.dihydrodiol, obtained as a metabolite of phenanthrene, was dehydrated by acid in acetone to form 3- and 4-phenanthrol, which were separated on the Vydac C_{18} column. The area ratio of the chromatographic peaks, detected at 254 nm, of 3- and 4-phenanthrol was 65:35, which is consistent with the ratio of 59:41 reported by Jerina *et al.* [4].

High-performance liquid chromatography

HPLC was performed using a Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph consisting of a Model 6000A solvent-delivery system, a Model M45 solvent-delivery system, a Model 660 solvent programmer and a Model 440 absorbance detector. Samples were injected via a Valco (Houston, TX, U.S.A.) Model N60 loop injector. Retention times and ratios of areas under the chromatographic peaks were recorded with a Hewlett-Packard Model 3390A integrator.

Normal-phase HPLC

The dihydrodiol metabolites of phenanthrene were separated on a DuPont (Wilmington, DE, U.S.A.) Zorbax SIL column (25 cm × 6.2 mm I.D.) with tetrahydrofuran–hexane (1:3, v/v) at a flow-rate of 2 ml/min. Under these conditions the retention times were phenanthrene 3.8, phenanthrols 5.0, phenanthrene *trans*-9,10-dihydrodiol 6.8, phenanthrene *trans*-1,2-dihydrodiol 9.0 and phenanthrene *trans*-3,4-dihydrodiol 13.1 min. Efficient separation of the three phenanthrene *trans*-dihydrodiols was also achieved by using a DuPont Golden SIL column (8 cm × 6.2 mm I.D.), eluted with 2.5% of ethanol–acetonitrile (2:1, v/v) in hexane at a flow-rate of 2 ml/min. For the purpose of preparing relatively large amount of phenanthrene dihydrodiol metabolites, normal-phase HPLC is preferred to reversed-phase HPLC [2] because of relative ease of removal or organic solvents.

Reversed-phase HPLC

A monomeric Zorbax ODS column (25 cm × 4.6 mm I.D.; DuPont) or a polymeric Vydac C_{18} columns (25 cm × 4.6 mm I.D.; Separations Group, Hesperia, CA, U.S.A.) was used. Phenanthrols were eluted with methanol–water (3:2 or 1:1, v/v) at a flow-rate of 1.2 ml/min.

Absorption spectra

Ultraviolet absorption spectra of phenanthrols in methanol and in alkaline methanol (*ca.* 0.05 ml of 0.5 *M* NaOH in methanol) were determined using a 1-cm path length quartz cuvette with a Beckman Model 25 spectrophotometer.

RESULTS AND DISCUSSION

Retention times of phenanthrols by reversed-phase HPLC on the monomeric Zorbax ODS column and the polymeric Vydac C_{18} column using methanol-water (3.2, v/v) are shown in Fig. 1. With identical elution solvents and flow-rates, the

phenanthrols are retained longer on the monomeric Zorbax ODS column. Further, the elution orders of the phenanthrols on the monomeric and polymeric columns are different. The elution order on the Zorbax ODS column is 2- <3- <9- <1- <4-phenanthrol, whereas on the Vydac C_{18} column the elution order is 3- <2- <9- <4- <1-phenanthrol. The peaks of the phenanthrols on the monomeric Zorbac ODS column are generally broader (peak width at half height $\geq$1.3 min) and the elution order is similar to that on a DuPont Permaphase ODS (1-m) column [4]. On the other hand, the peaks of the phenanthrols on the polymeric Vydac C_{18} column are relatively sharper and more symmetrical (Fig. 2). By using a lower concentration of methanol (methanol–water, 1:1), 2-, 3.- and 9-phenanthrol are better separated, whereas 4- and 1-phenanthrol are partially separated (Fig. 2). In comparison, 9-phenanthrol is well separated from 2- and 3-phenanthrol on the monomeric Zorbax ODS column (Fig. 1). Similarly, 1- and 4-phenanthrol are also better separated on the monomeric Zorbax ODS column. Hence, it is possible to separate all five isomeric phenanthrols by sequential use of the monomeric and polymeric C_{18} columns.

All phenanthrols have UV absorption maxima at *ca.* 250 nm (Fig. 3). Each phenanthrol has distinct and chracteristic UV absorption properties. Differences in the UV absorption properties of 3-, 4- and 9-phenanthrol in methanol are small and subtle. However, when their UV absorption properties in alkaline methanol are viewed together, the differences become apparent. The UV absorption properties of 1- and 2-phenanthrol in methanol and alkaline methanol are different from each other and from other isomeric phenanthrols. Subtle differences in the UV absorption properties among isomeric phenanthrols are difficult to differentiate if the absorption properties are tabulated in terms of molar absorptivities and wavelengths. The UV absorption spectra described in this paper should be useful in the identification of isomeric phenanthrols.

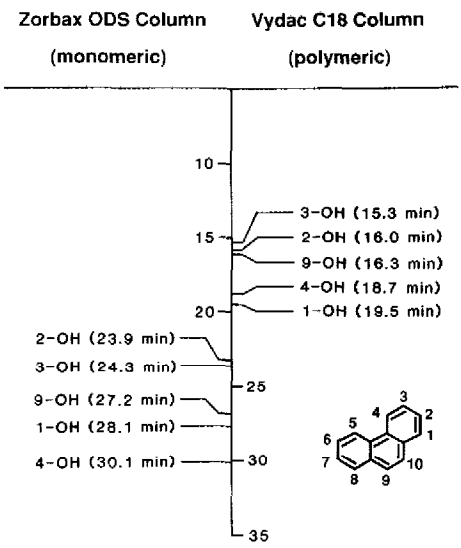

Fig. 1. Retention times of phenanthrols on the monomeric Zorbax ODS and the polymeric Vydac C_{18} columns. Eluent: methanol–water (3:2, v/v) at a flow-rate of 1.2 ml/min.

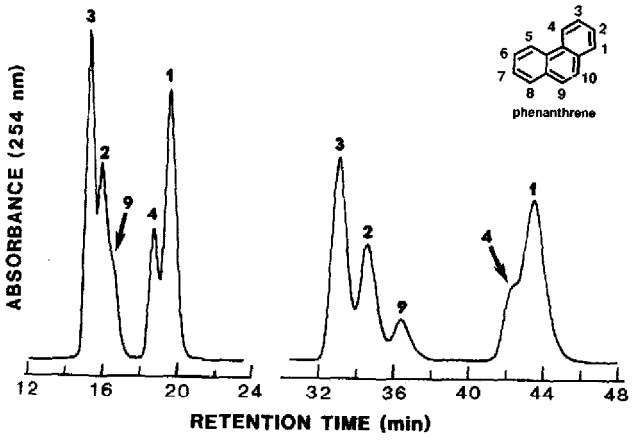

Fig. 2. Reversed-phase HPLC separation of phenanthrols on a polymeric Vydac C_{18} column. Eluents: methanol–water (3:2, v/v) (left) and methanol–water (1:1, v/v) (right) at a flow-rate of 1.2 ml/min. Numbers on peaks indicate the positions of the hydroxyl group on the phenanthrene molecule.

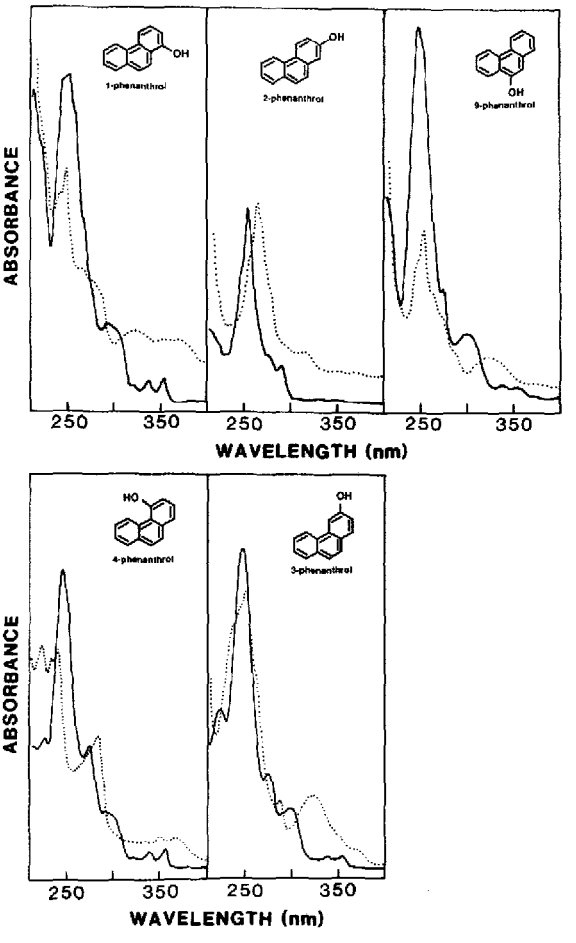

Fig. 3. Ultraviolet absorption spectra of phenanthrols in methanol (solid curves) and in alkaline methano (dotted curves).

We have previously reported the separation of isomeric phenols of chrysene, benz[*a*]anthracene, and benzo[*a*]pyrene using monomeric and polymeric C_{18} columns [5]. It appears that the polymeric Vydac C_{18} column is generally more useful in the separation of isomeric phenols of polycylic aromatic hydrocarbons.

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