

Note

High-pressure liquid chromatography of *n*-alkyl-4-nitrophenylcarbonate esters

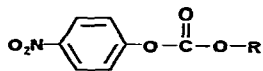
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The highly efficient separation powers of high-pressure liquid chromatography (HPLC) have been demonstrated in many areas of analytical chemistry. The most common method of detection in HPLC is spectrophotometric; therefore, the detection limits of a compound are directly related to its absorptivity. Molecules having high natural absorptivity can be detected in low concentrations by HPLC. However, compounds with low absorptivity values must be derivatized¹ with a strong chromophoric group in order to achieve good detectability. The use of derivatizing reagents to produce molecules of high absorptivity has been applied to many classes of compounds²⁻⁴. The non-destructive spectrophotometric method of detection in HPLC is an additional advantage in the analysis of samples of limited quantity. This allows for sample collection following analysis and the generation of additional analytical data from the sample; however, these data must be generated on the derivatized sample. This technique has been used with good results². However, it requires the development of secondary analytical methods for the derivatized sample since the nature of the sample has been altered by the derivatization process.

The formation of a chromophoric derivative which could be easily hydrolyzed to yield the original sample molecule following HPLC analysis would represent a substantial improvement in this area. Our initial efforts at the production of such a derivative have been directed toward the formation of 4-nitrophenylcarbonate esters of hydroxyl containing compounds. This paper reports the results of our studies on the liquid-chromatographic properties and the ease of hydrolysis of some *n*-alkyl-4-nitrophenylcarbonates ($R = n\text{-C}_1\text{-C}_8$).



EXPERIMENTAL

Apparatus and materials

The liquid chromatograph consisted of a Waters Model 6000 solvent pump, Model U6K injector, Model 440 UV detector and a Varian Model A-25 recorder. UV measurements were carried out on a Hitachi 60 or a Perkin-Elmer 200 double-

beam spectrophotometer. IR spectra were determined on a Beckman 4230 spectrophotometer and NMR spectra were recorded using a Varian T-60A.

All reagents were used as purchased without further purification. The 4-nitrophenylchloroformate was purchased from Aldrich (Milwaukee, Wisc., U.S.A.). Spectrophotometric grade acetonitrile was obtained from Burdick and Jackson Labs., (Muskegon, Mich., U.S.A.).

Synthesis of 4-nitrophenylcarbonate esters

The n -C₁-C₈ (methanol-octanol) aliphatic alcohol esters were prepared according to the following general procedure. A solution of 0.01 mole of the appropriate alcohol, 0.015 mole of 4-nitrophenylchloroformate and 0.03 mole of triethylamine in 100 ml of benzene was refluxed for 2 h, then allowed to cool to room temperature. The solution was added to a separatory funnel and extracted with water, dried (MgSO₄) and evaporated to yield the desired esters.

Chromatographic procedure

Separation by HPLC was accomplished using a μ -Bondapak C₁₈ column (30 cm \times 1/4 in. O.D., Waters Assoc., Milford, Mass., U.S.A.). The mobile phase consisted of a mixture of acetonitrile-water (double distilled) (1:1). The mobile phase flow-rate was 1.5 ml/min producing a back pressure of 1500 p.s.i. and the UV detector was operated at 254 nm. In all runs 5 μ l of an acetonitrile solution of the esters was injected using a 25- μ l syringe. The separations were carried out at ambient temperature.

Hydrolysis study

A $8.71 \cdot 10^{-4}$ M solution of ethyl-4-nitrophenylcarbonate in aqueous acetonitrile was prepared and 10 ml of this solution added to five reaction vials. To each vial was added 0.2 ml of 2 M sodium hydroxide and the resulting solutions were allowed to stand at ambient temperature. The contents of the vials were analyzed by HPLC (5 μ l injected) at various time intervals.

RESULTS AND DISCUSSION

The 4-nitrophenyloxycarbonyl group has been described in recent years as a useful protecting group for alcohols⁵. The esters have been widely used to mask hydroxyl groups in carbohydrates. The desirable characteristics of protecting moieties for certain functional groups in organic synthesis are similar to the desired behaviour for chromophoric derivatives for HPLC. The derivatives should be prepared in a relatively easy, straight-forward manner and exhibit reasonable chemical stability yet be sufficiently labile to allow for the regeneration of the original functional group under mild conditions. In addition the HPLC derivatives should be strong chromophores in the 254 nm region (wavelength used in most fixed-wavelength UV detectors).

This initial study included the synthesis, UV absorption spectra, liquid chromatographic properties, and hydrolysis of a series of n -alkyl-4-nitrophenylcarbonates. The synthesis of the reference n -C₁-C₈ alkyl-4-nitrophenylcarbonates was accomplished according to standard synthetic procedures. The products were purified by

recrystallization or column chromatography. The methyl and ethyl esters are solids while the remaining compounds are liquids at ambient temperature. The esters showed characteristic NMR and IR (carbonyl, 1765 cm^{-1}) spectra.

The UV absorption spectra (Fig. 1) are similar for all esters studied, each showing maximum absorption at 267 nm. This similarity is expected since the chromophore is identical for all compounds. Therefore, the molar absorptivity should be similar for the series of esters and was found to be $1.0 \cdot 10^4$ at 267 nm. The esters are strong absorbers at 254 nm as indicated by a molar absorptivity of $8.1 \cdot 10^3$ (molar absorptivities determined in ethanol). The high molar absorptivity of the 4-nitrophenylcarbonate esters makes for good detectability, thus, a peak of 5:1 signal to noise ratio was obtained from an injection of 10 ng of ethyl-4-nitrophenylcarbonate.

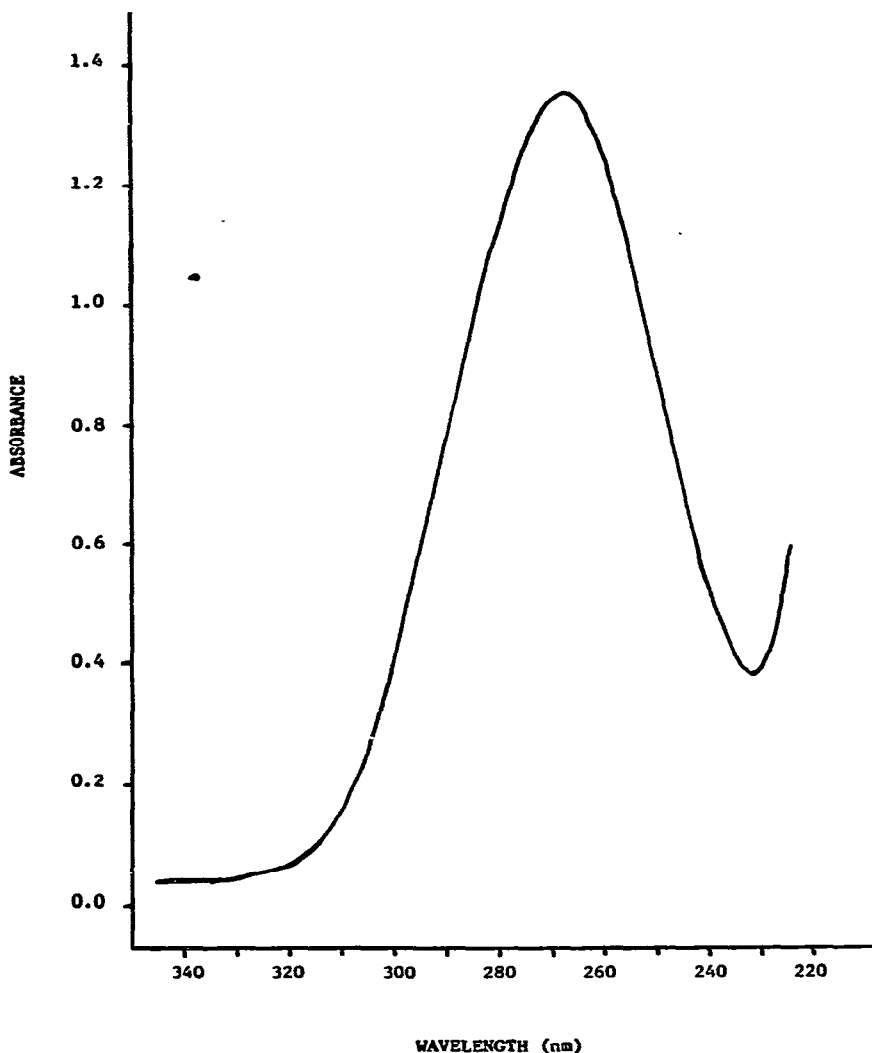


Fig. 1. UV absorption spectrum of ethyl-4-nitrophenylcarbonate.

The HPLC separation of the esters was achieved using a C_{18} reversed-phase column with an isocratic solvent system consisting of equal amounts of acetonitrile and water (Fig. 2). The retention of the esters by the non-polar stationary phase increases with increasing chain length and molecular weight. These observations are consistent with the expected behaviour of a series of homologues on a hydrophobic stationary phase.

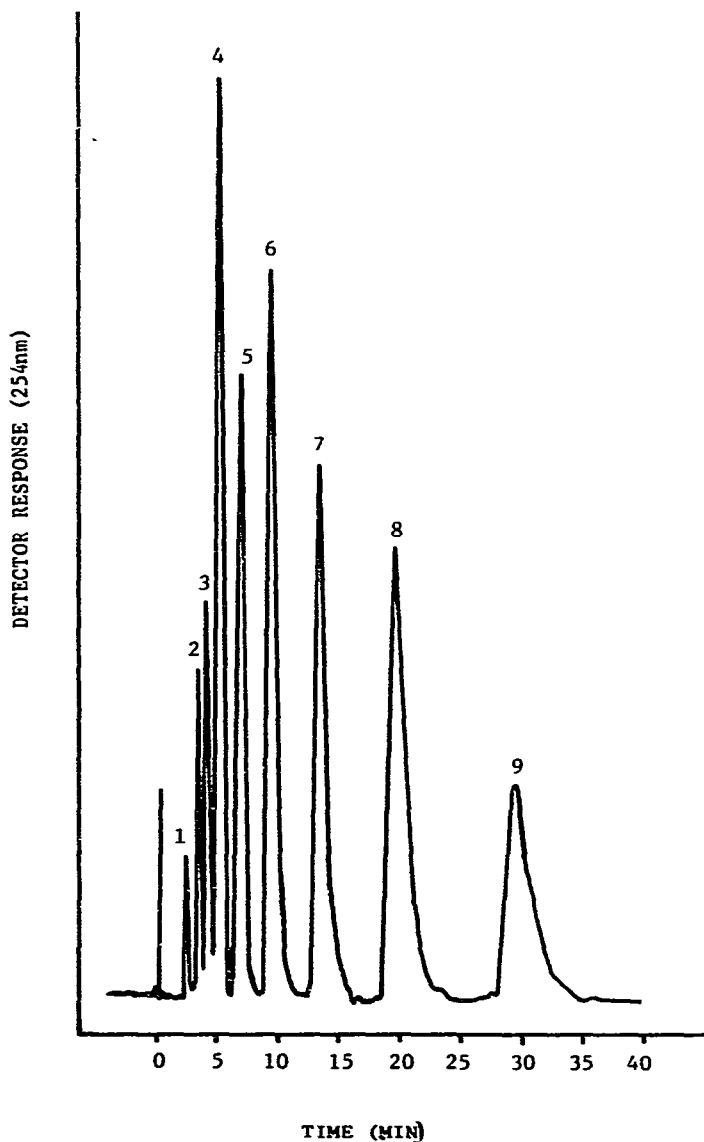


Fig. 2. Water-acetonitrile isocratic elution HPLC of *n*-alkyl-4-nitrophenylcarbonates. Peaks: 1 = 4-nitrophenol, 2 = methyl-4-nitrophenylcarbonate, 3 = ethyl-4-nitrophenylcarbonate, 4 = *n*-propyl-4-nitrophenylcarbonate, 5 = *n*-butyl-4-nitrophenylcarbonate, 6 = *n*-pentyl-4-nitrophenylcarbonate, 7 = *n*-hexyl-4-nitrophenylcarbonate, 8 = *n*-heptyl-4-nitrophenylcarbonate, 9 = *n*-octyl-4-nitrophenylcarbonate.

Carbonate esters are reported⁵ to be readily hydrolyzed under basic conditions. The results of our studies on the hydrolysis of these *n*-alkyl-4-nitrophenylcarbonates indicate that complete hydrolysis can be achieved in 10 min using sodium hydroxide. The study was conducted using an aqueous acetonitrile solvent system in order to

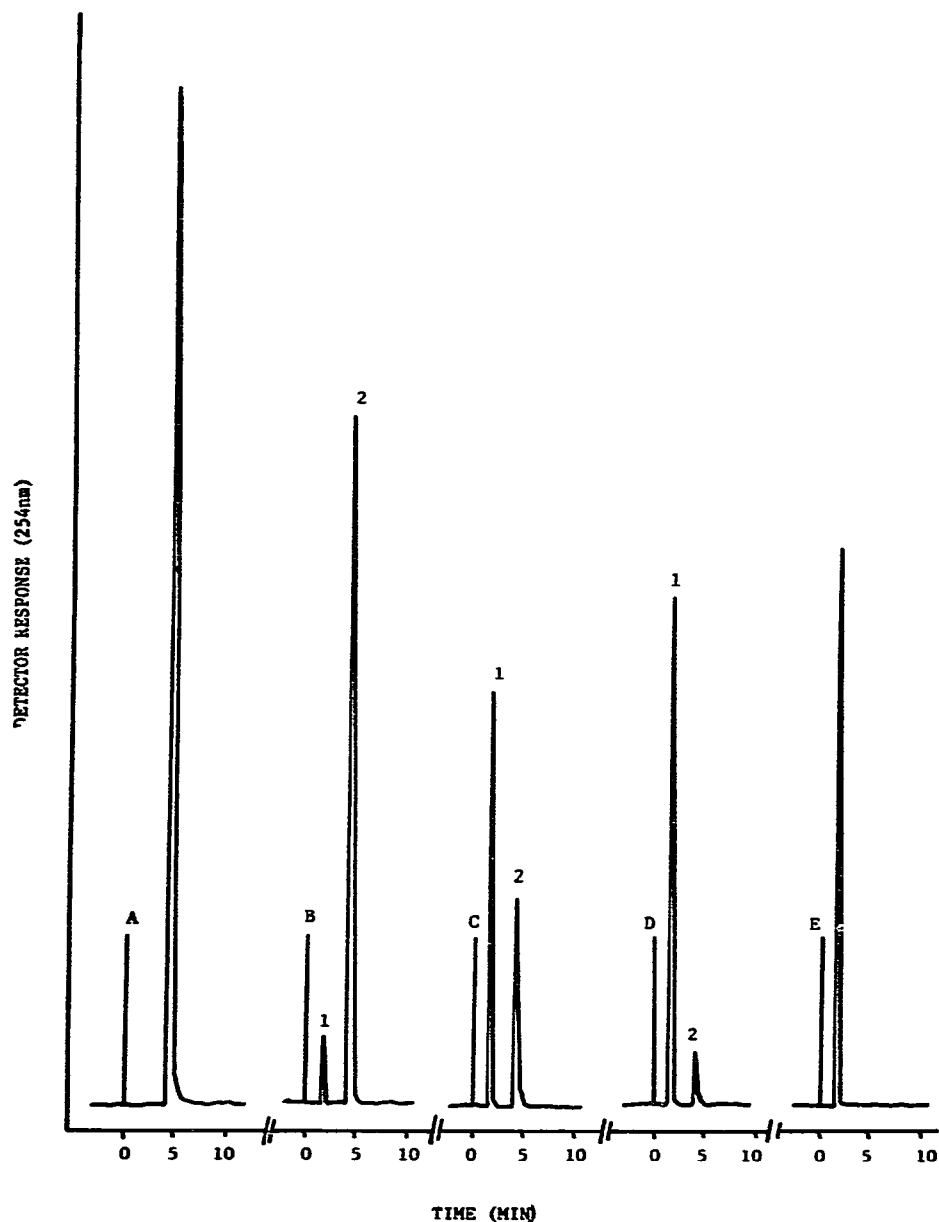


Fig. 3. Hydrolysis of ethyl-4-nitrophenylcarbonate. A, B, C, D and E respectively: 0, 2.5, 5.0, 7.5 and 10.0 min following addition of sodium hydroxide. Peaks: 1 = 4-nitrophenol, 2 = ethyl-4-nitrophenylcarbonate.

simulate the conditions which exist when the sample is collected directly from the HPLC effluent. The hydrolysis process yields the original alcohol and 4-nitrophenol; the disappearance of the ester and the appearance of 4-nitrophenol was followed by HPLC and is shown in Fig. 3. The hydrolysis of the ester was further examined by gas-chromatographic analysis of samples at various time intervals. The disappearance of the ester peak in HPLC was paralleled by the appearance of the alcohol peak as shown by gas chromatography. Although the esters are readily hydrolyzed under basic conditions, the hydrolysis does not occur in the absence of these conditions and hydrolysis products were observed only in those samples previously treated with sodium hydroxide. Thus, these initial studies indicate 4-nitrophenylcarbonate esters of compounds containing a hydroxyl group to be excellent chromophoric derivatives with good chromatographic properties. Furthermore, the original alcohol can be quickly and easily regenerated. The quantitative aspects and further applications of this technique are currently under investigation.

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