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TRACE DETERMINATION OF PHENOLS BY GAS CHROMATOGRAPHY AS THEIR 2,4-DINITROPHENYL ETHERS*

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SUMMARY

Dinitrophenylation of phenols by reaction with 1-fluoro-2,4-dinitrobenzene is shown to yield derivatives amenable to gas chromatography and displaying strongly electron-capturing properties. Various methods of preparation are compared.

INTRODUCTION

Water palatability studies and pesticide residue analysis are two areas in which the gas chromatographic determination of trace quantities of phenols is currently attracting much interest. Free phenols can be determined directly by gas chromatography^{1,2}, but in general their properties preclude detection at the nanogram level. The usual approach to the development of sensitive phenol detection methods, which we have also followed, has been to prepare derivatives that are amenable to gas chromatography and detectable at the nanogram level with either electron-capture, flame-thermionic or flame-photometric detectors.

Phosphorylation of phenols by reaction with dimethyl phosphorochloridithionate has been used to prepare derivatives amenable to detection with the flame-thermionic or flame-photometric detectors³. In our experience, however, removal of excess reagent is difficult and the yield of the phosphorylated product is low when microgram quantities of phenols are reacted in solution, particularly in the case of the less acidic phenols.

In preparing electron-capturing derivatives of phenols, esters such as trifluoroacetates⁴ and chloroacetates⁵, and ethers formed by reaction with the reagent α -bromo-2,3,4,5,6-pentafluorotoluene⁶ have proved useful. In a recent paper⁷ the use of 1-fluoro-2,4-dinitrobenzene for the preparation of strongly electron-capturing derivatives of aromatic amines was described. In this present study, the same reagent was used to prepare the dinitrophenyl ether derivatives of the phenols. These derivatives display useful gas chromatographic and electron-capturing properties. A comparative study of techniques for preparing these phenol derivatives is also described.

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EXPERIMENTAL

Preparation and gas chromatography of 2,4-dinitrophenyl ethers

2,4-Dinitrophenyl ethers of some 40 phenols were prepared in milligram amounts using the procedure of REINHEIMER *et al.*⁸. 5 μ l quantities of solutions containing about 5 ng of derivative in acetone were injected on to a GE XE-60 chromatographic column described previously⁷, with electron-capture detection. Sub-dilutions were also injected to determine the detection limits for the various compounds.

Derivative formation with microgram quantities of phenols

The following three procedures were used to prepare derivatives from 10 μ g quantities of phenols.

(i) 4 ml of acetone containing the phenol(s), 0.1 ml of a saturated sodium methoxide solution in methanol and 1 ml of 1-fluoro-2,4-dinitrobenzene solution (1% w/v in acetone) were pipetted into a 10 ml pear-shaped flask and refluxed for 30 min. The solution was added to 25 ml of an aqueous sodium hydroxide solution (2.5% w/v), washed in with a little water, and the mixture extracted with 25 ml of chloroform. The chloroform extract was dried with anhydrous sodium sulphate and evaporated to low volume⁸. The residue was then dissolved in acetone and a 5 μ l aliquot injected on to the gas chromatographic column for comparison with a series of standards.

(ii) 25 ml of water containing the phenol(s), 0.1 ml of a saturated sodium methoxide solution in methanol and 1 ml of a 1-fluoro-2,4-dinitrobenzene solution (1% w/v in acetone) were shaken together in a separator for 2 min. 25 ml of sodium hydroxide solution (2.5% w/v) and 10 ml of *n*-hexane were added and the solution was shaken for 1 min. The *n*-hexane layer was separated and dried by passage through a short column of anhydrous granular sodium sulphate and a 5 μ l aliquot examined by gas chromatography.

(iii) An acetone solution containing the phenol(s) was spotted on to a thin-layer chromatoplate coated with a 250 μ m thick layer of one of the following adsorbents (Merck): Kieselguhr G, Silica Gel G or Aluminium Oxide G, or alternatively on one of the following papers (Whatman): filter paper No. 541, silica gel loaded paper S.G. 81, aluminium hydroxide loaded paper A.H. 81 or glass fibre paper G.F. 81. The layer or paper was then sprayed with a saturated solution of sodium methoxide in methanol (7 ml was required to spray 400 sq. cm to the required level) followed by a similar quantity of a solution of 1-fluoro-2,4-dinitrobenzene (4% w/v in acetone). The layer or paper was sandwiched between two glass plates clipped tightly together and was heated in an oven at 190° for 40 min. When cool the layer in the area of interest was removed, either by scraping the layer from the plate in the case of the thin-layer adsorbents or by cutting out the spot area where a paper was used. The layer adsorbents were transferred to short columns plugged with cotton wool and the derivatives eluted with two 5 ml portions of acetone. The papers were directly added to tubes containing 10 ml of acetone together with two drops of water with resultant extraction of the derivatives. The yield of derivative from each sample was determined by injecting aliquots of the final solutions on to the gas chromatographic column for comparison with standards.

RESULTS

Table I shows the retention time data and sensitivity to electron-capture detection of the 2,4-dinitrophenyl ethers on a Silicone GE XE-60 column. Linear calibration curves were obtained over the range of 1–10 ng. Table II shows the yields of derivatives obtained by the various preparative procedures; these yields are expressed as percentages of the theoretical yield assuming 10 μg of phenol to react completely to form the corresponding ether. $\text{p}K_{\text{a}}$ values (in aqueous solution) for the various phenols examined, were obtained from literature sources and are included in Table II.

DISCUSSION

The elution sequence of 2,4-dinitrophenyl ethers (see Table I) is influenced by the nature of the phenolic portion of the molecule and, under the conditions specified, spans a sufficiently wide time range to be of value in phenol characterisation. The *ortho*, *meta*, *para* sequence of elution—the “*ortho* effect”—normally associated with the gas chromatography of free isomeric phenols is also displayed by their 2,4-dinitrophenyl ethers although the separation of the isomeric ethers is poorer than that displayed by the corresponding phenols. The electron-capturing properties of the derivatives do not appear to arise from the phenolic portion of these molecules but are associated with the presence of the aromatic nitro groups. The apparent differences in sensitivity (see Table I) arise as a result of peak broadening associated with increasing retention time.

The electron-capturing properties of 2,4-dinitrophenyl ethers compare favourably with phenol derivatives prepared by reaction with chloroacetic anhydride⁵ or α -bromo-2,3,4,5,6-pentafluorotoluene⁶. The elution sequence, in terms of the parent phenols, is different with each of the three derivatives and the 2,4-dinitrophenyl ethers apparently give better separations than the other two groups of compounds. Because of the relatively low volatility of the 2,4-dinitrophenyl ethers, it was necessary to operate the columns at temperatures close to the maximum permissible with electron-capture detectors using tritium as the radioactive source. The absence of any signs of decomposition and the known stability of ethers would indicate that separations at higher temperatures would be possible provided a suitable electron-capture detector were used (*e.g.* one based on ⁶³Ni). This probably opens up the prospect of gas chromatography of the dinitrophenyl derivatives of polyhydric phenols which are known to be formed readily⁸, but were not examined in this work as it was assumed that their retention times would be excessive at 215°.

The results in Table II indicated that the nature of the phenol, together with the procedure used for derivative formation greatly influenced the yield of 2,4-dinitrophenyl ether. Under reflux conditions, with acetone as the solvent, increasing yield was obtained as the acid strength of the phenol increased, whereas a reaction in cold aqueous conditions resulted in a reversal of this behaviour. Under the forcing conditions encountered with the “sandwiched layer” technique, higher yields were generally obtained, with the exception of reactions carried out on an alumina thin layer. The more acidic phenols in general gave the highest yields of derivative but the support exerted a marked influence on the reaction. The most useful support material for

TABLE I

RETENTION TIME DATA AND ELECTRON-CAPTURE SENSITIVITY OF 2,4-DINITROPHENYL DERIVATIVES OF PHENOLS

Conditions: 1% GE XE-60 and 0.1% Epikote 1001 on Chromosorb G, acid-washed, dimethylchlorosilane coated, 60-80 mesh. Temperature: 215°. Column material: glass 140 cm in length, 1.5 mm I.D.

| Parent phenol | Retention time relative to 1-naphthol derivative | Sensitivity ^a to electron-capture detection (g × 10 ⁻⁹) | Parent phenol | Retention time relative to 1-naphthol derivative | Sensitivity (g × 10 ⁻⁹) |
|-----------------------------------|--|--|---------------------------------|--|-------------------------------------|
| Phenol | 16 | 0.10 | 4- <i>tert.</i> -Butylphenol | 38 | 0.10 |
| <i>o</i> -Cresol | 17 | 0.05 | 4-Allylphenol | 38 | 0.1 |
| 4-Fluorophenol | 19 | 0.10 | 4- <i>sec.</i> -Butylphenol | 40 | 0.10 |
| <i>m</i> -Cresol | 20 | 0.05 | 4- <i>tert.</i> -Pentylphenol | 50 | 0.10 |
| 2,5-Xylenol | 20 | 0.05 | 4-Bromophenol | 56 | 0.10 |
| <i>p</i> -Cresol | 21 | 0.05 | 2,4-Dichlorophenol | 58 | 0.10 |
| Thymol | 23 | 0.05 | Eugenol | 62 | 0.10 |
| 2-Isopropoxyphenol | 23 | 0.05 | 4-Iodophenol | 92 | 0.10 |
| 3,5-Xylenol | 23 | 0.05 | 3,5-Dimethyl-4-methylthiophenol | 94 | 0.10 |
| 4-Ethylphenol | 28 | 0.05 | 4- <i>tert.</i> -Octylphenol | 95 | 0.20 |
| 2-Methoxyphenol | 29 | 0.05 | 1-Naphthol | 100 (8.2 min) | 0.20 |
| 3-Ethyl-5-methylphenol | 29 | 0.05 | 2,4,5-Trichlorophenol | 102 | 0.20 |
| 2-Chlorophenol | 29 | 0.05 | 2-Naphthol | 141 | 0.20 |
| 4-Isopropylphenol | 31 | 0.10 | 4-Cyclohexylphenol | 153 | 0.30 |
| 3,4-Xylenol | 31 | 0.10 | 2-Nitrophenol | 168 | 0.50 |
| 3-Chlorophenol | 33 | 0.10 | 3-Nitrophenol | 178 | 0.50 |
| 3,5-Di- <i>tert.</i> -butylphenol | 36 | 0.10 | 4-Nitrophenol | 238 | 0.50 |
| 4-Chlorophenol | 38 | 0.10 | 4-Benzylphenol | 285 | 0.40 |

^a Sensitivity expressed as the weight of derivative producing a peak with height equivalent to 10% full-scale deflection at an amplification producing a noise level of 5% f.s.d.

TABLE II

COMPARATIVE STUDY OF PROCEDURES FOR 2,4-DINITROPHENYL ETHER FORMATION

Procedures: (1) Refluxing in acetone solvent; (2) cold aqueous reaction; (3) sandwiched layer reaction, chromatoplate coated with Kieselguhr G; (4) sandwiched layer reaction, chromatoplate coated with Silica Gel G; (5) sandwiched layer reaction, chromatoplate coated with Aluminium Oxide G; (6) sandwiched layer reaction, filter paper No. 541; (7) sandwiched layer reaction, silica gel loaded paper SG 81; (8) sandwiched layer reaction, aluminium hydroxide loaded paper AH 81; (9) sandwiched layer reaction, glass fibre paper GF 81.

| Compound | <i>pK_a</i> | % Yield (average of six determinations) | | | | | | | | |
|--------------------|-----------------------|---|----|-----|----|---|----|-----|----|----|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Phenol | 10.0 | 6 | 15 | 4 | 27 | 0 | 40 | 51 | 35 | 1 |
| 4-Fluorophenol | 9.88 | 5 | 25 | 10 | 34 | 0 | 43 | 46 | 41 | 6 |
| 4-Chlorophenol | 9.42 | 23 | 20 | 41 | 57 | 0 | 65 | 69 | 64 | 7 |
| 4-Bromophenol | 9.34 | 38 | 19 | 62 | 52 | 0 | 64 | 63 | 59 | 19 |
| 4-Iodophenol | 9.10 | 61 | 20 | 91 | 62 | 0 | 72 | 76 | 76 | 44 |
| 2,4-Dichlorophenol | 7.82 | 34 | 0 | 32 | 46 | 0 | 44 | 57 | 54 | 0 |
| 4-Nitrophenol | 7.20 | 54 | 0 | 100 | 51 | 0 | 27 | 100 | 66 | 80 |

general screening was the paper loaded with silica gel which gave high yields with the minimum dependence on the acidity of the phenol.

The "sandwiched layer" reaction can be used to prepare many derivatives simultaneously—a 20 × 20 cm plate can be spotted with fifty or more samples and after reaction each derivative may be eluted separately. Another advantage of this type of reaction is that only a relatively small amount of the reagent is present on a single spot and the problem of removal of excess reagent encountered frequently with other types of preparation does not usually arise.

The methods described are being applied in this laboratory to the detection and determination of residues of certain pesticides, such as carbamates, which yield phenols on hydrolysis.

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