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ETHER DERIVATIVES FOR THE DETERMINATION OF PHENOLS AND PHENOL-GENERATING PESTICIDES BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

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SUMMARY

The formation of ether derivatives for the gas chromatographic determination of phenols and phenol-generating pesticides provides a simple and sensitive analytical method. The properties of a series of 2,6-dinitro-4-trifluoromethylphenyl ethers were compared with those of previously reported pentafluorobenzyl and 2,4-dinitrophenyl derivatives of phenols; the three types were found to be similar in their ease of preparation, stability, and electron capture response, and formed a complimentary series for trace analysis and identification. Micro-derivatization may be applied to phenols, pesticides which generate phenols on hydrolysis, or both; for illustration, recovery of p.p.b.* levels of carbaryl insecticide and the corresponding 1-naphthol from water via the DNT derivative exceeded eighty percent.

INTRODUCTION

Phenols and their derivatives are important environmental contaminants introduced directly in industrial waste effluent and pesticide formulations and indirectly through environmental transformations of both natural and synthetic chemicals. The direct gas–liquid chromatographic (GLC) analysis of water for trace quantities of phenols is limited by the relative insensitivity of flame ionization detectors[1]. Pre-concentration, by adsorption on charcoal for example[2], extends the limits of detection but, at the same time, results in higher levels of interference which tend to obscure interpretation of the chromatogram[3]. Those phenols containing electron-attracting substituents, such as p-nitrophenol[4] and pentachlorophenol[5], may be determined with considerable sensitivity by electron capture GLC, usually after conversion to more volatile methyl or trimethylsilyl ethers.

A more general approach to the analysis of trace levels of phenols involves conversion to derivatives in which the derivatizing reagent confers both electron capturing and beneficial chromatographic properties. Both ester and ether derivatives have been examined for this purpose; examples of the former include trifluoroacetyl[6], monochloroacetyl[7], and trichloroacetyl[8] derivatives, and 2,4-dinitrophenyl[9, 10] (DNP) and pentafluorobenzyl[11] (PFB) ethers illustrate the latter. Applications of the

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* Throughout this article the American (10^9) billion is meant.

monochloroacetyl[12], trichloroacetyl[8, 13], and dinitrophenyl[10] derivatization procedures have been extended to phenols obtained by hydrolysis of pesticides.

Due to the usual hydrolytic instability of such esters, the ethers appear to be the derivatives of choice for both qualitative and quantitative determination of microgram quantities of phenols[14]. The PFB ethers, for example, elicit excellent electron capture responses and possess relatively short retention times on columns of low polarity which, with their stability and relative ease of formation, has led to their use for rapid qualitative analysis of the phenolic fraction from charcoal adsorption samples of river water[3]. The DNP derivatives, which exhibit much longer retention times, have been successfully adapted to quantitative analysis of carbamate insecticides in vegetables and water by derivatization of the phenolic hydrolysis products[10]; the conversion efficiency of the derivatization procedure was not defined, nor was the extension to quantitative analysis of phenols themselves reported.

We report here the properties and analytical applications of a series of phenol derivatives based on 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene, a reagent previously shown to be useful for formation of electron capturing derivatives of amines produced by hydrolysis of several common pesticides[15]. The micro-derivatization procedure described for these 2,6-dinitro-4-trifluoromethylphenyl (DNT) ethers may be applied, with minor modification, to determination of phenol-generating insecticides, particularly the methylcarbamate insecticides.

EXPERIMENTAL

Materials

4-Chloro-α,α,α-trifluoro-3,5-dinitrotoluene (J. T. Baker), 1-fluoro-2,4-dinitrobenzene (Matheson, Coleman and Bell), pentafluorobenzyl bromide (Aldrich or K & K Laboratories) and picryl chloride (2,4,6-trinitrochlorobenzene, Aldrich) were used as received. Methyl parathion (O,O-dimethyl O-p-nitrophenyl phosphorothioate), carbaryl (1-napthyl methylcarbamate), Landrin (3,4,5-trimethylphenyl methylcarbamate), and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) were pesticide analytical standards used as received from the respective manufacturers. Acetone and methylene chloride were distilled twice from technical grade solvents. Phenols were reagent grade materials from commercial sources or pesticide analytical standards and were used without further purification.

Gas chromatography

Varian Aerograph Models 204 and 1700 gas chromatographs equipped with tritium electron capture detectors and 1/8 in. O.D. glass columns were employed. Carrier gas (nitrogen) flow, 40–60 ml/min; injection port temperature, 260°; detector temperature, 210°. Column packing and oven temperatures are noted in the following sections.

Preparation of derivatives for characterization

Phenols were converted to their ether derivatives by the procedure of REINHEIMER et al.[16]. For example, a solution of 0.72 g (0.0056 mole) of p-chlorophenol, 0.75 g (0.0028 mole) of DNT chloride, and 0.8 ml (0.0058 mole) of triethylamine in 10 ml of acetone was refluxed for 30 min. The resulting mixture was evaporated to

dryness and triturated with 10 ml of 5% hydrochloric acid. After separation by filtration, the solids were mixed with 20 ml of 5% aqueous sodium hydroxide solution, filtered off, and purified by recrystallization from ethanol to give a yellow solid, m.p. 125–126°.

The properties of recrystallized DNT derivatives of phenols are summarized in Table I. No effort was made to maximize yields, since the preparative work was simply to furnish enough sample for characterization. Derivatives were characterized by their IR, NMR, and mass spectra. The NMR spectra (Hitachi Perkin-Elmer

TABLE I

PROPERTIES OF PHENOL DNT DERIVATIVES

Phenol	Yield (%)	m.p. (°C)	Mass spectrum		R_{Aldrin}	
			Parent (abund.)	Diagnostic fragment (abund.)	SE-30[a]	OV-225[b]
Phenol	32	86–87[c]	328 (39)	93 (100)	0.51	2.00
o-Cresol	55	113–114	—	—	0.55	2.00
m-Cresol	51	92–93	—	—	0.59	2.40
p-Cresol	73	113–114[c]	—	—	0.70	3.00
1-Naphthol	80	142–143	378 (95)	143 (100)	2.81	15.93
p-Penylphenol	56	186–187	—	—	7.11	41.75
m-Isopropylphenol	50	93–94	370 (100)	135 (83)	0.91	2.27
3,4,5-Trimethylphenol	80	159–160	370 (52)	135 (100)	1.38	4.50
2,3,5-Trimethylphenol	50	165–166	370 (9)	135 (32)	1.04	2.88
p-Hydroxybenzyl alcohol	36	131–134	—	—	2.28	22.50
2,6-Dimethyl-4-hydroxybenzyl alcohol	44	101–102	—	—	3.11	29.75
p-Methoxyphenol	88	111–112	358 (10)	123 (100)	1.17	3.22
p-Nitrophenol	51	132–133	373 (54)	235 (100)	2.36	33.06
o-Isopropoxyphenol	50	73–74	386 (12)	109 (100)	1.04	3.11
3-Methyl-4-methylthiophenol	85	144–145	388 (19)	153 (100)	2.25	13.25
3-Methyl-4-methylsulfinylphenol	74	187–188	404 (59)	153 (89)	6.78	50.00
3-Methyl-4-methylsulfonylphenol	87	193–194	—	—	7.40	50.00
2,3-Dihydro-7-hydroxy-2,2-dimethylbenzofuran (carbofuran phenol)	91	54–55	398 (53)	163 (100)	1.32	4.16
2,3-Dihydro-7-hydroxy,2,2-dimethyl-3-oxobenzofuran	35	105	412 (68)	149 (100)	1.44	8.38
2,3-Dihydro-3,7-dihydroxy-2,2-dimethylbenzofuran	37	50–54	—	—	2.06	15.80
p-Chlorophenol	95	125–126[c]	362 (30)	127 (100)	0.97	4.66
2,3-Dichlorophenol	53	144–145	396 (22)	161 (100)	1.63	9.66
2,4-Dichlorophenol	50	143–144	—	—	1.41	8.02
2,5-Dichlorophenol	58	168–169	396 (100)	161 (100)	1.31	6.73
2,6-Dichlorophenol	44	125–126	—	—	1.26	4.33
3,4-Dichlorophenol	70	123–124	396 (19)	161 (100)	1.63	9.25
3,5-Dichlorophenol	71	147–148	396 (25)	161 (100)	1.26	6.20
2,4,5-Trichlorophenol	68	117–118	431 (30)	195 (100)	2.00	12.33
Pentachlorophenol	21	190–193	500 (18)	265 (100)	5.92	22.46

[a] Column length, 2 ft; 5% SE-30 on 60/80 Gas Chrom W, AW, DMCS treated; column temperature, 195°.
[b] Column length, 2 ft.; 2% OV-225 on 60/80 Gas Chrom Q, AW, DMCS treated; column temperature, 185°.
[c] MALCHENKO et al.[17] report essentially the same melting point.

Model R-20, deuteriochloroform, TMS internal standard) showed a relatively invariant singlet for the aromatic protons of the DNT ring at 8.3 to 8.6 p.p.m., and were otherwise consistent with the structure expected from the phenol moiety. Mass spectra (CEC Model 21-490, solid probe inlet) were characterized by a relatively abundant parent peak and, generally, a base peak corresponding to the phenol cation formed in the primary cleavage

$$[\text{ArO--DNT}]^{\ddagger} \rightarrow [\text{ArO}]^{+} + [\text{DNT}]^{\cdot}$$

Microscale dervatization technique

To a solution of 10–25 μg of the phenol in 0.2 ml of acetone, contained in a 10-ml volumetric flask, were added 20 μl of a 5 % aqueous solution of potassium hydroxide or potassium carbonate. After brief agitation, 9 ml of acetone and 0.25 ml of a stock solution of 5 mg/ml of DNT chloride, PFB bromide, or DNP fluoride in acetone were added. The volume was adjusted to 10 ml with acetone; the stoppered flask was then shaken vigorously for 30 sec, allowed to stand in the dark for 2 h, and 1–3 μl portions were injected directly into the gas chromatograph. Percent conversions were measured by comparison of peak areas with those from the appropriate amount of standard derivative.

Analysis of insecticides was carried out in this way, with the following modification: after the addition of base, the stoppered flask was allowed to stand in an oil-bath maintained at 80° to a depth of 2 cm for 1 h to effect hydrolysis. After cooling in tap water, 9 ml of acetone were added and the derivatization procedure completed as in the preceding paragraph.

Recovery of phenols and methylcarbamates from deionized water

1 l of deionized water was fortified with 25 μl of a 1 mg/ml standard solution of the appropriate compound in acetone. The mixture was then acidified to pH 2 or less with phosphoric acid and extracted with four 50-ml portions of methylene chloride. The combined extracts were washed with 50 ml of water and filtered through anhydrous sodium sulfate into a 250-ml round bottom flask. The volume was reduced to *ca.* 0.5 ml by distillation through a Snyder column, remaining methylene chloride was removed by twice adding 5 ml of acetone and evaporating to *ca.* 0.5 ml, and the resulting acetone concentrate solution was transferred quantitatively to a 10-ml volumetric flask with 2 ml of acetone and the volume reduced to *ca.* 0.2 ml under a nitrogen stream. DNT derivatization was carried out as outlined in the preceding paragraphs, using potassium carbonate as the base. Recoveries were based on comparison of GLC peak areas with those from standard derivatives prepared at the same time. Unfortified samples were also extracted and derivatized at the same time, to serve as controls; no significant GLC interferences were observed.

Recovery of phenols and methylcarbamates from river water

1 l of river water (from a 22-l sample collected from the American River, Sacramento County, Calif.) was fortified with 25 μl of a 1 mg/ml standard of 1-naphthol in acetone and 25 μl of a 1 mg/ml standard of carbaryl in acetone. Extraction and concentration steps were carried out as in the preceding example. The 0.5 ml acetone concentrate solution was divided into two equal portions; one portion (A) was

subjected to DNT derivatization without hydrolysis, and the other (B) to DNT derivatization after hydrolysis for 1 h at 80°. Recovery of 1-naphthol from A was measured by comparison of the GLC peak area with that from 12.5 μg of 1-naphthol derivatized at the same time. Recovery of carbamate, estimated from the hydrolyzed portion B, was based on comparison of net GLC peak area, after subtraction of area due to the 1-naphthol contained in A, and correction by a factor of 1.40 representing the differential contribution of 1-naphthol and carbaryl to DNT formation, with that from 12.5 μg of 1-naphthol derivatized at the same time. The procedure was repeated using carbofuran phenol and carbofuran, with a correction factor of 1.35. Unfortified controls resulted in no significant GLC interferences.

A small amount of hydrolysis of methylcarbamates was encountered in the derivatization of A when fortified reagent blanks were carried through this procedure. This could be effectively eliminated by diluting the 0.25 ml of acetone concentrate solution A to 9.0 ml prior to addition of base without affecting overall conversion efficiency of the phenol.

RESULTS AND DISCUSSION

All of the phenols examined, including hindered 2,6-substituted compounds, reacted with the DNT chloride reagent to yield crystalline ethers possessing melting points comparable to those of the PFB and DNP derivatives, and agreeing with the few reported by MALCHENKO et al.[17]. A comparison of GLC retention and electron capture response relative to aldrin (Table II) showed that the relative retention times for derivatives of each phenol varied regularly in the order PFB < DNT < DNP. The relative response did not vary greatly among the three series, although PFB responses were consistently higher than the others. The presence of the trifluoromethyl group apparently does not increase electron capture response in the DNT derivatives, but does confer greater volatility compared with the DNP derivatives[15]. The difference in retention times suggests that simultaneous use of the three types of derivatives may be helpful in confirmatory qualitative analysis of phenols whose identities are in question, and offer alternatives where interferences complicate

TABLE II

GAS CHROMATOGRAPHIC RETENTION AND ELECTRON CAPTURE RESPONSE FOR PHENOL PFB, DNT, AND DNP DERIVATIVES[a]

Phenol	PFB		DNT		DNP	
	Retention	Response	Retention	Response	Retention	Response
Phenol	0.14	0.18	0.48	0.14	1.5	0.23
p-Chlorophenol	0.30	0.29	0.89	0.19	2.5	0.31
3,4,5-Trimethylphenol	0.49	0.36	1.19	0.16	3.8	0.20
Carbofuran phenol	0.51	0.30	1.17	0.13	3.7	0.25
p-Nitrophenol	0.92	0.29	2.14	0.10	5.5	0.06
1-Naphthol	0.94	0.39	2.25	0.20	6.3	0.19

[a] Column length, 6 ft.; 5% SE-30 on 60/80 Chrom W, AW, DMCS treated; column temperatures were 195°, 230°, and 250°, respectively, for the PFB, DNT, and DNP derivatives. All values are relative to aldrin (1.00).

analysis of any one of the three types of derivatives. A liquid phase of intermediate polarity (OV-225, Table I), offers a means of increasing the retention time by a factor of 3–5 for DNT derivatives of the less polar phenols, and 10–15 for those of the more polar ones such as p-nitrophenol and p-hydroxybenzyl alcohol.

The reaction of phenols with picryl chloride resulted in formation of picryl ethers providing approximately the same electron capture response as DNT derivatives but with greatly increased retention times. These derivatives may be useful where interferences preclude the use of the more volatile PFB, DNT, or DNP derivatives.

The micro-scale derivatization procedure allows the reaction to proceed at room temperature in a homogeneous medium from which direct GLC injection may be made. The amount of base and reagent were not important variables when employed in excess. Relatively large molar excesses (greater than fifty-fold) were routinely employed to provide for situations in which the amount and number of phenols may be unknown. Sodium bicarbonate and triethylamine were ineffective in catalyzing the microscale reaction; either potassium hydroxide or potassium carbonate could be used, through the percent conversion was found to vary considerably with time (Fig. 1). The high yield obtained almost instantaneously with potassium hydroxide at room temperature decreased rapidly with time, possibly as a result of subsequent hydrolysis of the DNT derivative[18], or air oxidation of the phenoxide. On the other hand, the almost quantitative conversion within 2 h, enhanced stability, and lower reagent background (Fig. 2) recommend the use of potassium carbonate. Whichever base is used, derivatives of standards for quantitative comparison should be prepared at the same time as those of solutions to be measured, to minimize variability due to reaction time.
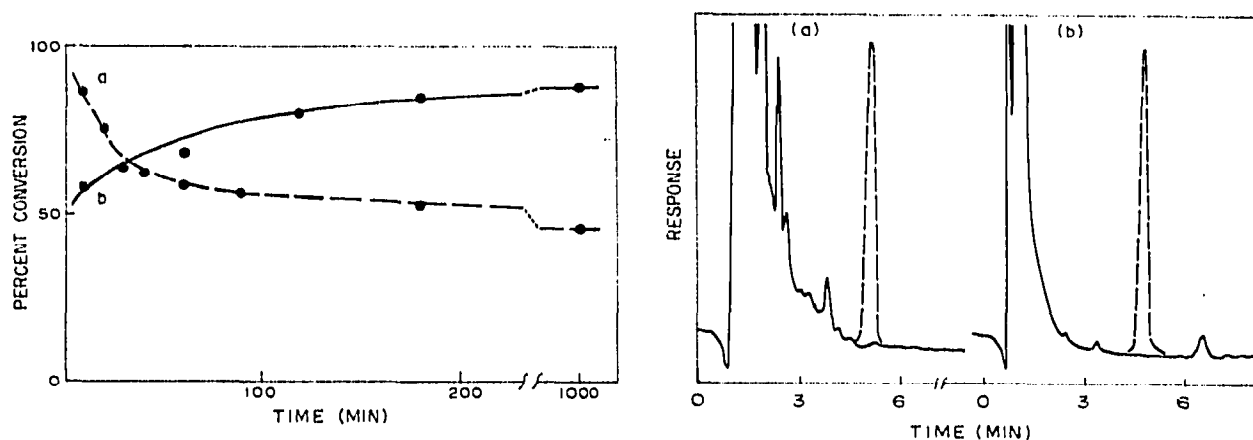

Fig. 1. Variation of percent conversion of 1-naphthol to DNT derivative with time, using (a) potassium hydroxide and (b) potassium carbonate as base.

Fig. 2. Reagent blanks for micro-scale DNT derivatization using (a) potassium hydroxide and (b) potassium carbonate as base, with peak from 2.6 ng of 1-naphthol DNT derivative superimposed. (6-ft. GLC column of 5% SE-30 on 60/80 Chrom W, AW, DMCS treated; column temperature, 250°.)

Conversions regularly exceeded 50 % using either base (Table III); higher yields generally were obtained with the more acidic phenols and with potassium carbonate, consistent with the observations reported with DNP fluoride[9] and the

expectation that ionization of the phenol must precede the coupling step. When substituted in the present procedure, PFB bromide and DNP fluoride gave yields

TABLE III

CONVERSION OF PHENOLS AND PHENOL-GENERATING INSECTICIDES TO DNT-DERIVATIVES

A micro-scale derivatization procedure was used with a single determination in each case.

Phenol	Percent conversion		Insecticide	Percent conversion	
	KOH	K_2CO_3		KOH	K_2CO_3
Phenol	67.2	77.1	—	—	—
p-Chlorophenol	80.0	92.6	—	—	—
3,4,5-Trimethylphenol	62.2	51.4	Landrin	68.0	51.5
Carbofuran phenol	57.4	64.0	carbofuran	43.5	73.2
p-Nitrophenol	53.5	100.0	methyl parathion	49.5	9.4
1-Naphthol	64.7	80.4	carbaryl	67.2	53.0

of derivatives comparable to those obtained with DNT chloride (Table IV), while potassium hydroxide caused a comparable decrease with time for all three derivatives. Thus, the reactivity of the three reagents and the corresponding derivatives appears to be quite similar; and conversions in the present procedure or those previously cited for PFB[3] and DNP[9,10] derivatives will closely reflect reaction conditions. For example, the reported derivatization with DNP fluoride and sodium methoxide in refluxing acetone[9] gave yields considerably lower than those obtained by our procedure.

TABLE IV

COMPARISON OF PFB, DNT, AND DNP DERIVATIZATION OF 1-NAPHTHOL

A micro-scale derivatization procedure was used with a single determination in each case.

Reagent	Percent conversion			
	KOH		K_2CO_3	
	1 h	7 h	1 h	7 h
PFB bromide	84	58	95	100
DNT chloride	86	62	77	93
DNP fluoride	80	60	99	84

Our method can provide for hydrolysis of phenol-generating carbamates and organophosphates by heating the basic solution at 80° prior to addition of derivatizing reagent. The derivatization efficiencies with methylcarbamates (Table III) were similar to those for the phenols themselves regardless which base was employed, but the low conversion (9.4 %) of the organophosphate, methyl parathion, in the presence of potassium carbonate reflected inefficient hydrolysis. When applied to organophosphates a strong base such as potassium hydroxide will be required for high derivatization yields.

Phenols and carbamates in water were recovered and determined efficiently at the 25-p.p.b. level with the DNT procedure (Table V). Substitution of methylene chloride for petroleum ether in the extraction step and its removal through a Snyder column increased the recovery over that of an existing procedure[10,20]. While the lower limit of determination was not investigated, analysis at levels below 2.5 p.p.b. should be feasible in the absence of major background interferences. To further define applicability, a sample of river water was fortified with a methylcarbamate and its corresponding phenol, each at 25 p.p.b. The concentrated extract was divided into two equal portions, one of which was subjected directly to DNT derivatization to measure the phenol, and the other to derivatization following hydrolysis to estimate the ester, allowing for the contribution of the phenol. The results (Table V) indicate that recoveries of phenols were in good agreement with those from deionized water fortified with each compound separately; no interferences were observed. Recoveries of methylcarbamates from river water were somewhat lower than from deionized water, due partly to a small (*ca.* 10 %) contribution of the methylcarbamate to the DNT derivative of the free phenol. Subsequently we found that a slight modification in the derivatization step, described in the EXPERIMENTAL section, eliminated this problem.

TABLE V

RECOVERY OF CARBOFURAN, CARBARYL, AND CORRESPONDING PHENOLS FROM DEIONIZED AND RIVER WATER AS DETERMINED BY DNT DERIVATIZATION

Chemical	Percent recovery	
	Deionized water[a]	River water[b]
1-Naphthol	84.6	90.7
Carbaryl	99.4	62.3
Carbofuran phenol	84.2	85.0
Carbofuran	84.8	53.5

[a] Average of three determinations; 25 μg (25 p.p.b.) fortifications of each compound were analyzed in separate determinations.
[b] Average of three determinations; 25 μg (25 p.p.b.) fortifications of 1-naphthol and carbaryl were determined simultaneously; 25 μg (25 p.p.b.) fortifications of carbofuran phenol and carbofuran were determined simultaneously.

This procedure offers the possibility for determination of phenols and their parent pesticides, either together or separately, and bypasses their resolution prior to the determination step. It allows satisfactory sensitivity for many applications, and provides for resolution and identification of the phenols. The quantitation of of methylcarbamates by difference when both methylcarbamates and phenols are determined simultaneously does, however, introduce additional variability which could lower the precision from that attainable when the compounds are determined separately, following a solvent partition or chromatographic resolution.

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