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CHROMATOGRAPHY OF DINITROPHENOLS

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SUMMARY

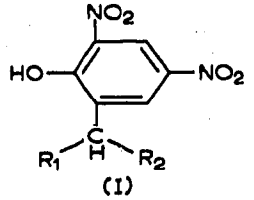
The thin-layer and gas–liquid chromatography of fourteen homologous series of substituted dinitrophenols is reported, and the relationship between chromatographic behaviour and chemical structure discussed.

INTRODUCTION

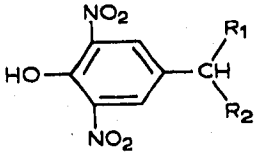
Current literature on the chromatography of dinitrophenols includes the use of reversed-phase chromatography for estimation of pesticide residues[1,2] and a comprehensive evaluation of reversed-phase and adsorption chromatography of nuclear substituted phenols and phenolic ethers[3].

Our earlier paper on the GLC separation of some alkyldinitrophenols[4] coincided with that of HRIVŇÁK AND ŠŤOTA[5], who reported the separation of free dinitrophenolic pesticides on polar (cyclohexanedimethanol succinate/, butanediol succinate/, neopentyl glycol succinate/H_3PO_4) columns.

The present work, which reports the thin-layer (adsorption and reversed-phase), gas–liquid and column chromatographic separations of substituted dinitrophenols (I and II; R_1 = alkyl, cycloalkyl or aryl, R_2 = *n*-alkyl)



(I)



(II)

is part of a comprehensive structure/activity programme involving the control of powdery mildews[6,7].

EXPERIMENTAL

All solvents used were previously dried over molecular sieves.

Reversed-phase TLC

Twenty grams of cellulose powder (Macherey Nagel MN 300) were blended with a solution of 2 ml ethyl oleate in 98 ml dry ether for 2 min and the resulting slurry spread on two 40 × 20 cm plates giving a layer 0.3 mm thick. The plates were air dried for 15 min before use. Compounds were applied as 0.25 μl spots of a 0.025% w/v solution in acetone, one homologous series being spotted on each plate together with three spots of the standard; positions of individual compounds were randomised. Two spots of each sample were applied to the plate, making about eighteen spots in all and enabling 2 in. of the layer to be kept free at each end in order to avoid edge-effects. The mobile phase (40% aqueous ethanol saturated with ethyl oleate) was placed in a paper-lined glass tank insulated with expanded polystyrene and the whole allowed to equilibrate overnight. The plates were held in a rack inside the tank and developed for 2.25 h, by which time the solvent front had travelled 12–13 cm from the origin. Positions of individual spots were detected either by viewing under UV light (254 nm) or by spraying with 2% w/v ethanolic KOH. The dried layers were sprayed with "Neatan" (Merck), covered with "Transpaseal" and the distances travelled by each compound measured. The spots were usually oval in shape and all measurements were made to the leading edge since the estimation of centres of spots was difficult[8]. Use of more concentrated solutions of test compounds invariably led to excessive streaking.

Adsorption TLC

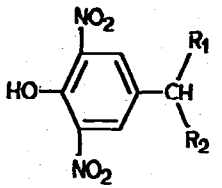
Plates (20 × 20 cm), spread with 0.25 mm thick layers of Silica Gel G (Merck), were dried at 110° for 1 h, cooled, stored in a desiccator and used within 24 h. Compounds were applied as 1 μl of a 0.025% w/v solution in acetone. Each plate carried eight to eleven spots plus two of a standard (4-methyl-2,6-dinitrophenol) at approximately 7 cm from each edge. The plates were developed for 15 cm using light petroleum (b.p. 40–60°)–ether–formic acid (90:10:2) in an insulated glass tank fully lined with chromatography paper and kept at 25–26°. The solvent was changed every 24 h and the tank allowed to equilibrate overnight before use. The dried plates were sprayed with 2% w/v ethanolic potassium hydroxide or ammonia (0.880) to enhance the yellow to orange colour of the spots. Centres of spots were marked, the plate sprayed with "Neatan" and distances from the start to the centres measured. The R_F values from a plate were discarded if the R_F value of the standard was outside the limits 0.14 ± 0.01.

Gas–liquid chromatography

A Varian Aerograph A90-P3 instrument fitted with a flame ionisation detector and the linear temperature programmer 326 was used. The 10 ft. × ¼ in. stainless steel column contained 1% diethylene glycol adipate (stabilized: Analabs C5) and 0.08% orthophosphoric acid on Chromosorb G (AW-DMCS) (80–100 mesh). Using a nitrogen flow rate of 100 ml/min, the working temperatures were: column, 245°, injection port, 275°, and detector, 290°. Two to five microlitres of a 0.075% w/v solution of test material in acetone, together with an internal standard of 4-(1-cyclopentylbutyl)-2,6-dinitrophenol were injected. In cases where the test substance had a very similar retention time to that of the standard (8 min) the value of the latter was determined separately.

TABLE I

R_M VALUES (REVERSED-PHASE CHROMATOGRAPHY) FOR HOMOLOGOUS SERIES OF 4-(1-SUBSTITUTED ALKYL)-2,6-DINITROPHENOLS



(II)

	R_2										
R_1	$-H$	$-CH_3$	$-CH_2CH_3$	$-(CH_2)_2CH_3$	$-(CH_2)_3CH_3$	$-(CH_2)_4CH_3$	$-(CH_2)_5CH_3$	$-(CH_2)_6CH_3$	$-(CH_2)_7CH_3$	$-(CH_2)_8CH_3$	$-(CH_2)_9CH_3$
Hydrogen	−0.51	−0.35	−0.21	−0.03	+0.11	+0.24	+0.38	+0.51	+0.63	+0.78	+0.98
Methyl	−0.35	−0.30	−0.18	−0.03	+0.09	+0.22	+0.35				
Ethyl	−0.21	−0.18	−0.17	−0.05	+0.07	+0.21					
n-Propyl	−0.03	−0.03	+0.03	+0.15	+0.26	+0.39					
n-Butyl	+0.11	+0.09	+0.10	+0.20	+0.33	+0.43					
Cyclobutyl	+0.01	+0.05	+0.15	+0.28	+0.39	+0.49	+0.64				
Cyclopentyl	+0.02	+0.09	+0.16	+0.24	+0.38	+0.52	+0.64				
Cyclohexyl	+0.10	+0.14	+0.20	+0.30	+0.41	+0.54	+0.66				
Cyclohexyl-methyl	+0.36	+0.34	+0.42	+0.56	+0.66	+0.75					
Cyclohexyl-ethyl	+0.44	+0.47	+0.54	+0.66	+0.76						
Phenyl	−0.30	−0.23	−0.11	+0.01	+0.12	+0.27					
Benzyl	−0.09	−0.05	+0.04	+0.15	+0.28	+0.39	+0.54				

Column chromatography

This gave a convenient method for isolation of pure samples. Crude dinitrophenols could be purified by elution from alumina[9], silica gel or magnesium carbonate.

Columns were packed with silica gel (Merck, 0.05–0.2 mm) in light petroleum (b.p. 40–60°) and the dinitrophenol added. Light petroleum (b.p. 40–60°) containing 0.5–1.0% ether eluted mono-nitrated impurities and successive increments in ether content up to 4% gave the pure dinitrophenol.

Recent work has shown that pure specimens of dinitrophenols may be more rapidly eluted from magnesium carbonate (BDH chromatographic grade: dried 1 h at 200°) packed in light petroleum (b.p. 40–60°).

TABLE II

CHROMATOGRAPHIC DATA FOR 2-(1-SUBSTITUTED)-4,6-DINITROPHENOLS

Substituent	R_M *value (adsorption chromatography)*	R_M *value (reversed-phase chromatography)*	*Relative retention (GLC)*
Methyl	+0.48	−0.39	0.19
Ethyl	+0.33	−0.23	0.18
n-Propyl	+0.28	−0.05	0.20
n-Butyl	+0.21	+0.11	0.25
n-Pentyl	+0.15	+0.28	0.31
n-Hexyl	+0.10	+0.42	0.38
n-Heptyl	+0.07	+0.55	0.48
n-Octyl	+0.05	+0.71	0.63
1-Methylheptyl	−0.04	+0.52	0.42
1-Ethylhexyl	−0.08	+0.53	0.31
1-Propylpentyl	−0.12	+0.56	0.32

RESULTS AND DISCUSSION

Reversed-phase TLC

R_M values for the dinitrophenols tested are recorded in Tables I and II; 4-methyl-2,6-dinitrophenol (or the 4,6-dinitroanalogue) was used as the standard since all these structures may be regarded as being derived from this basic one for the calculation of Hansch π functions[7]. A survey of 100 plates showed a mean R_F value of 0.76 for the 4-methyl-2,6-dinitrophenol standard. All values were within $\pm$ 0.03 and 90% within $\pm$ 0.02 of this mean. Plots of R_M *versus* C_n (the number of carbon atoms in the side chain R_2) for the various homologous series all gave straight lines for those compounds which contained a methylene group in this chain, that is for the propyl and succeeding homologues. The regression lines for all series except *n*-alkyl were nearly parallel, with slopes ranging from 0.109 to 0.129. An analysis of variance carried out on the experimental data suggested that the compounds fell into three groups (Table III).

MARTIN[10] stated that for a given solvent system the change in R_M value caused by the introduction of a group R_1 into a molecule is constant provided that

TABLE III

CLASSIFICATION OF HOMOLOGOUS SERIES OF SUBSTITUTED ALKYLDINITROPHENOLS ACCORDING TO THE SLOPES OF THEIR R_M/C_n REGRESSION LINES

From an analysis of variance.

Group	*Homologous series*	*Slope*
1	2-*n*-Alkyl	0.144
	4-*n*-Alkyl	0.148
2	4-*n*-Propylalkyl	0.119
	4-*n*-Butylalkyl	0.111
	4-Cyclohexylalkyl	0.115
	4-Cyclohexylmethylalkyl	0.109
	4-Cyclohexylethylalkyl	0.110
3	4-Methylalkyl	0.129
	4-Ethylalkyl	0.125
	4-Benzylalkyl	0.125
	4-Phenylalkyl	0.125
	4-Cyclobutylalkyl	0.120
	4-Cyclopentylalkyl	0.119

no intramolecular interactions occur that might affect the partition coefficient. In the compounds studied, three such types of interaction are possible:

(1) Change in dipole moment of the molecule caused by the change from the methyl to ethyl and, to some extent, propyl group.

(2) Any change which substantially alters the fatty nature of the molecule.

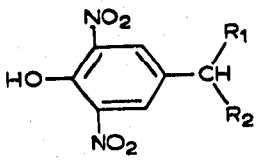
(3) The shielding of polar groups by long chains.

Type 1 behaviour obviously occurs with these dinitrophenols since linearity of the R_M/C_n curve is only achieved for those compounds with propyl and higher alkyl chains. Similarly, addition of a methylene group to methyl and to ethyl causes much greater relative change in the fatty nature of the molecule than with, for example, the change from pentyl to hexyl. A study of molecular models shows that the shielding of polar groups by long chains is feasible only in those compounds with alkyl chains (R_2) of seven or more carbon atoms. Our results suggest that this effect is negligible with all the compounds so far examined with the possible exception of 4-undecyl-2,6-dinitrophenol which had an unusually high R_M value. The differing slope of the R_M/C_n line for the 4-*n*-alkyl series does indicate that the introduction of an α-alkyl substituent has some influence on the overall polarity of the molecule. BARK AND GRAHAM[3] concluded that the partition of the molecule between the non-polar stationary phase and the polar mobile phase is governed by the intermolecular hydrogen bonding of groups in the molecule and polar groups in the latter. The non-polar stationary phase dissolves the non-polar part of the molecule. Our experimental conditions differed from those used by these workers in that we obtained more discrete spots by the use of a higher loading of ethyl oleate in the stationary phase.

The contributions of the R_1 substituents to the R_M values of butyl and succeeding homologues are also reasonably constant (Table IV). These values were obtained by subtraction of the R_M value of the appropriate *n*-alkyl compound from that of the 1-substituted alkyl compound.

TABLE IV

CONTRIBUTIONS (ΔR_M) OF THE R_1 SUBSTITUENTS TO THE R_M VALUES FOR 4-(1-SUBSTITUTED ALKYL)-2,6-DINITROPHENOLS



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R_1 =	ΔR_M when R_2 =				Mean ΔR_M
	$-(CH_2)_2CH_3$	$-(CH_2)_3CH_3$	$-(CH_2)_4CH_3$	$-(CH_2)_5CH_3$	
Methyl	0	−0.02	−0.02	−0.03	−0.02
Ethyl	−0.02	−0.04	−0.03		−0.03
n-Propyl	+0.18	+0.18	+0.15	+0.15	+0.17
n-Butyl	+0.23	+0.22	+0.19		+0.21
Cyclobutyl	+0.31	+0.28	+0.25	+0.26	+0.28
Cyclopentyl	+0.27	+0.27	+0.28	+0.26	+0.27
Cyclohexyl	+0.33	+0.30	+0.30	+0.28	+0.30
Cyclohexylmethyl	+0.59	+0.55	+0.51		+0.55
Phenyl	+0.02	+0.01	+0.03		+0.02
Benzyl	+0.18	+0.17	+0.15	+0.16	+0.17

It is clear that our results agree with those of BARK AND GRAHAM and that the chromatographic behaviour of 4-substituted 2,6-dinitrophenols is in accordance with MARTIN's postulates.

A 2-alkyl substituent is more capable of shielding the hydroxyl group and so affecting the inter- and intra-molecular hydrogen bonding characteristics of the molecule than a 4-alkyl substituent. This is reflected in the higher R_M values of the 4,6-dinitrophenols compared with their 2,6-dinitro analogues (Tables I and II).

Adsorption TLC

The R_M values for members of twelve homologous series are recorded in Table V. Excessive streaking of materials on plates was prevented by the addition of 2% formic or acetic acid to the mobile phase. This procedure gave almost circular spots.

Plots of R_M *versus* C_n were made for the various homologous series but only in the 2- and 4-*n*-alkyl series did these approximate to a straight line and then only for the *n*-hexyl and succeeding homologues. Similar curves were given by plots of the R_F value relative to the standard and by $R'_M \left(= \log \left[\frac{1}{1 \cdot 1 R_F} - 1\right]\right)^{11}$ against C_n. The R'_M term was introduced by SNYDER in an attempt to compensate for reduction of R_F values by the action of solvent gradients. This author concluded, however, that any correlation of R_M values within homologous series is due to a fortuitous cancellation of complex terms. This appears to be the case with substituted dinitrophenols in this system although it is possible that linearity of the R_M/C_n curves would be achieved with higher homologues.

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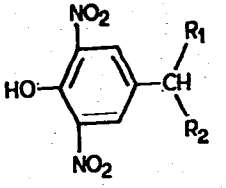
TABLE V

R_M VALUES (ADSORPTION CHROMATOGRAPHY) FOR HOMOLOGOUS SERIES OF 4-(1-SUBSTITUTED ALKYL)-2,6-DINITROPHENOLS

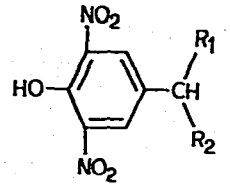


(II)

	R_2										
R_1	$-H$	$-CH_3$	$-CH_2CH_3$	$-(CH_2)_2CH_3$	$-(CH_2)_3CH_3$	$-(CH_2)_4CH_3$	$-(CH_2)_5CH_3$	$-(CH_2)_6CH_3$	$-(CH_2)_7CH_3$	$-(CH_2)_8CH_3$	$-(CH_2)_9CH_3$
Hydrogen	+0.79	+0.63	+0.51	+0.43	+0.38	+0.34	+0.32	+0.32	+0.30	+0.28	+0.26
Methyl	+0.63	+0.49	+0.40	+0.35	+0.31	+0.28	+0.25				
Ethyl	+0.51	+0.40	+0.34	+0.27	+0.22	+0.20					
n-Propyl	+0.43	+0.35	+0.27	+0.21	+0.19	+0.11					
n-Butyl	+0.38	+0.31	+0.22	+0.19	+0.11	+0.08					
Cyclobutyl	+0.43	+0.33	+0.26	+0.21	+0.15	+0.12	+0.10				
Cyclopentyl	+0.37	+0.31	+0.24	+0.17	+0.13	+0.09	+0.05				
Cyclohexyl	+0.32	+0.27	+0.19	+0.16	+0.12	+0.08	+0.04	+0.03			
Cyclohexyl-methyl	+0.36	+0.27	+0.19	+0.11	+0.05	+0.04					
Cyclohexyl-ethyl	+0.34	+0.24	+0.16	+0.09	+0.05						
Phenyl	+0.79	+0.62	+0.53	+0.46	+0.41	+0.39					
Benzyl	+0.71	+0.57	+0.48	+0.41	+0.37	+0.33	+0.29				

TABLE VI

RELATIVE RETENTIONS[a] FOR HOMOLOGOUS SERIES OF 4-(1-SUBSTITUTED ALKYL)-2,6-DINITROPHENOLS



(II)

	R_2										
R_1	$-H$	$-CH_3$	$-CH_2CH_3$	$-(CH_2)_2CH_3$	$-(CH_2)_3CH_3$	$-(CH_2)_4CH_3$	$-(CH_2)_5CH_3$	$-(CH_2)_6CH_3$	$-(CH_2)_7CH_3$	$-(CH_2)_8CH_3$	$-(CH_2)_9CH_3$
Hydrogen	0.25	0.27	0.32	0.42	0.53	0.65	0.85	1.05	1.33	1.72	2.16
Methyl	0.27	0.31	0.36	0.41	0.48	0.60	0.78	0.94	1.18		
Ethyl	0.32	0.36	0.39	0.41	0.49	0.60					
n-Propyl	0.42	0.41	0.41	0.48	0.56	0.64					
n-Butyl	0.53	0.48	0.49	0.56	0.63	0.77					
n-Pentyl	0.64	0.60	0.60	0.62	0.77						
Cyclobutyl	0.69	0.64	0.66	0.74	0.87	1.06	1.25				
Cyclopentyl	0.96	0.91	0.92	1.00	1.16	1.40	1.67				
Cyclohexyl	1.27	1.26	1.17	1.35	1.48	1.87	2.25	2.73			
Cyclohexyl-methyl	1.66	1.30	1.22	1.38	1.62	1.90					
Cyclohexyl-ethyl	2.15	1.73	1.75	1.98	2.28						
Phenyl	2.70	2.11	2.19	2.45	2.75	3.02					
Benzyl	3.09	2.32	2.24	2.39	2.73	3.01					

[a] Relative to 4-(1-cyclopentylbutyl)-2,6-dinitrophenol.

Gas–liquid chromatography

Retention times for members of fourteen homologous dinitrophenol series are given in Tabls VI. The retention times quoted are those relative to that of 4-(1-cyclopentylbutyl)-2,6-dinitrophenol, this standard being more generally applicable than 2-methyl-4,6-dinitro- or 4-methyl-2,6-dinitrophenol. The stationary phase employed in this work (stabilised diethylene glycol adipate) was superior to the LAC-2R-446 used previously[4] in that column temperatures up to 260° could be tolerated, thus enabling the analysis of compounds of higher molecular weight. However this material, which is not cross-linked with pentaerythritol, would not effect the complete separation of dinocap phenol isomers[4].

Plots of log r (relative retention) against C_n were linear for the butyl and succeeding homologues and slopes for regressed lines are recorded in Table VII. Most

TABLE VII

CLASSIFICATION OF HOMOLOGOUS SERIES OF (1-SUBSTITUTED ALKYL)-DINITROPHENOLS ACCORDING TO SLOPES OF THEIR LOG r/C_n REGRESSION LINES

From an analysis of variance.

Group	*Homologous series*	*Slope*
1	4-*n*-Alkyl	0.101
	2-*n*-Alkyl	0.098
2	4-(1-Methylalkyl)	0.091
	4-(1-Ethylalkyl)	0.084
3	4-(1-Cyclohexylalkyl)	0.080
	4-(1-Cyclopentylalkyl)	0.075
	4-(1-Cyclobutylalkyl)	0.075
4	4-(1-*n*-Butylalkyl)	0.065
	4-(1-*n*-Propylalkyl)	0.061
5	4-(1-Benzylalkyl)	0.050
	4-(1-Phenylalkyl)	0.045

series showed a minimum relative retention with the ethyl isomer. An analysis of variance carried out on the regressed data indicated that the homologous series, as far as their GLC properties were concerned, could be classified into five groups. The log r /C_n lines for group 2 were superimposible as were those in group 5, indicating that a difference of one methylene group in R_1 had no effect on the partition of the molecule. The log r/C_n lines for the three 1-cycloalkyl series (Group 3) were equidistant, suggesting that the log r increment for each alicyclic methylene group is constant. However, although the addition of one methylene group to the cyclohexyl (R_1) chain caused no significant increase in log r (cyclohexylmethylalkyl series), that of a further methylene group caused an appreciable increase (cyclohexylethylalkyl series). A similar behaviour was observed between compounds in group 2 and those in group 4, the change from the ethyl (R_1) group to *n*-propyl causing an increase in log r. Addition of a further methylene group (the (1-butylalkyl) series) gave a log r/C_n line which was parallel to that of the 1-propylalkyl series and indicated the possibility of a constant log r increment per methylene group added to R_1 for higher

series. Insufficient data were available to derive a log r/C_n plot for the 1-pentylalkyl series but the existing values for log r were higher than those for the corresponding members in the 1-butylalkyl series.

Relative retention data for n-alkanes with 28, 32, 36, 38 and 40 carbon atoms gave a linear log r/C_n relationship with a slope of 0.118, indicating that a true partition process was taking place.

The failure to attain linear log r/C_n curves in our previous work was almost certainly due to the instability of the column packing above 210°, which precluded the analysis of higher homologues.

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REFERENCES

1 G. YIP AND S. F. HOWARD, *J. Assoc. Offic. Anal. Chemists*, 49 (1966) 1166.
2 H. M. BOGGS, *J. Assoc. Offic. Anal. Chemists*, 47 (1964) 346.
3 *e.g.* L. S. BARK AND R. J. T. GRAHAM, *Proc. S.A.C. Conf., Nottingham 1965*, W. Heffer and Sons, Cambridge, 1966, p. 112.
4 D. R. CLIFFORD AND D. A. M. WATKINS, *J. Gas Chromatog.*, 6 (1968) 191.
5 J. HRIVŇÁK AND Z. ŠŤOTA, *J. Gas Chromatog.*, 6 (1968) 9.
6 *e.g.* D. M. FIELDGATE AND D. WOODCOCK, *J. Sci. Food Agr.*, 19 (1968) 338.
7 D. R. CLIFFORD, A. C. DEACON AND M. E. HOLGATE, *Ann. Appl. Biol.*, (1969) in press.
8 D. L. GUMPRECHT, *J. Chromatog.*, 18 (1965) 336.
9 R. J. W. BYRDE, D. R. CLIFFORD AND D. WOODCOCK, *Ann. Appl. Biol.*, 57 (1966) 223.
10 A. T. P. MARTIN, *Biochem. Soc. Symp. (Cambridge, Engl.)*, 3 (1950) 4.
11 L. R. SNYDER, in J. C. GIDDINGS AND R. A. KELLER (Editors), *Advances in Chromatography*, Vol. 4, Dekker, New York, 1967, p. 3.