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CHLOROBIPHENYLOLS

THIN-LAYER CHROMATOGRAPHY AND COLOUR REACTIONS OF SOME HYDROXYLATED CHLOROBIPHENYLS*

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SUMMARY

The thin-layer chromatographic (TLC) behaviour and the colour reactions of 20 chlorobiphenylols (*i.e.*, chlorohydroxybiphenyls) and the corresponding monohydroxy- and dihydroxybiphenyls have been studied and a number of correlations between structure and chromatographic properties made. Particular attention has been paid to the use of TLC in combination with mass spectrometry for the detection and identification of these compounds using non-destructive complexing spray reagents and dansyl derivative formation.

INTRODUCTION

Chlorobiphenylols (chlorohydroxybiphenyls) have been shown to be major products of chlorobiphenyl metabolism in higher plants, animals and microorganisms[1]. Recently, the synthesis of a number of chlorobiphenylols was reported and some of their chromatographic and spectroscopic properties described[2].

Thin-layer chromatography (TLC) has been shown to be a very valuable method for the separation and purification of these metabolic products of the polychlorobiphenyls. Colour reactions, particularly with complexing reagents, coupled with mass spectrometry have proved to be a very valuable aid to the identification of such compounds[3,4]. The relationships that exist between the TLC properties,

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colour reactions and structures of a number of chlorobiphenylols have been studied and this paper reports the results of these investigations.

TLC methods and colour reactions have also been shown to be useful for the detection and determination of the fungicide 2-hydroxybiphenyl[5] and certain hydroxy compounds found among the metabolites of biphenyl[6–9] and 2-hydroxybiphenyl[10]. The paper chromatography of the hydroxybiphenyls has been reported[11,12] and the reversed-phase chromatography of some hydroxybiphenyls, including a number of chloro-derivatives has also recently been described[13].

EXPERIMENTAL

Chromogenic reagents

The sources of chemicals used for the preparation of the majority of the chromogenic reagents used in this investigation can be found in refs. 14 and 15. The electron acceptor complexing reagents were employed as solutions (0.2–1%) in chloroform or ethyl acetate (*cf.*, ref. 15). Additional chromogenic reagents used were: (a) Millon's reagent, prepared by dissolving mercury (5 g) in fuming nitric acid (sp.gr. = 1.52; 10 ml) and diluting the solution with water (10 ml); (b) metaperiodate reagent[16], a 0.5% (w/v) aqueous solution of analytical-grade potassium metaperiodate; (c) 4-nitrophenylacetonitrile[17], the developed chromatoplates first being sprayed with a 0.2% solution of 4-nitrophenylacetonitrile and, after drying, oversprayed with 2% aqueous alcoholic sodium hydroxide solution; (d) Fremy's salt, a 1% aqueous solution of potassium nitrosodisulphonate; and (e) cerium(IV) ammonium nitrate, a solution of cerium(IV) ammonium nitrate (1 g) in 2 *N* nitric acid (250 ml).

Dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride) was obtained from Aldrich, Milwaukee, Wisc., U.S.A.

Biphenyl derivatives

The hydroxybiphenyl derivatives were obtained from the following sources (suppliers in parentheses): 2- and 4-hydroxybiphenyl, 2,2'- and 4,4'-dihydroxybiphenyl, 3-chloro-4-biphenylol, 3,3',5,5'-tetrachloro-4,4'-biphenyldiol (Aldrich), 2,5- and 3,4-dihydroxybiphenyl (Eastman-Kodak, Rochester, N.Y., U.S.A.); 3,3'-dihydroxybiphenyl was prepared from its dimethyl ether (Aldrich) by demethylation with boron tribromide[18]. All other chlorobiphenylols were prepared in these laboratories[2].

Dansyl derivatives

The dansyl derivatives of 4'-chloro-4-biphenylol and 3-chloro-4-biphenylol were prepared by methods analogous to those used for the synthesis of the dansyl derivatives of hydroxybiphenyls[19] and chlorophenols[20].

Mass spectrometry

Mass spectra of complexes (*cf.*, ref. 15) and dansyl derivatives (*cf.*, ref. 19) were obtained on a DuPont/CEC 21-110B double-focusing mass spectrometer. Direct sample introduction and an ionizing voltage of 70 eV were used.

Thin-layer chromatography

TLC was carried out using the ascending technique at room temperature on Merck (F_{254}) commercially prepared silica plates (0.25 mm) with a fluorescent indicator throughout this investigation. The solvent systems used were: A, benzene–methanol–98% formic acid (90:9:1); B, benzene–methanol (90:9); C, dichloromethane–methanol (95:5); D, dichloromethane–methanol–conc. aq. ammonia (95:5:1); E, carbon tetrachloride–acetone (20:1); F, carbon tetrachloride–acetone–acetic acid (40:2:1); G, benzene–ethyl acetate (25:1); H, benzene–chloroform (1:1); I, chloroform; J, benzene–2-propanol (70:30); K, benzene–2-propanol–conc. aq. ammonia (70:30:4); L, chloroform–*n*-hexane–methanol (45:30:5). Samples of *ca.* 10–30 μg of the biphenyl derivatives were used in each case for the determination of R_F values and for the colour reactions.

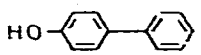
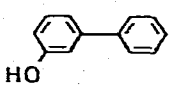
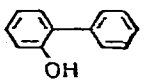
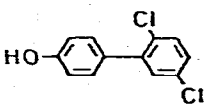
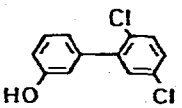
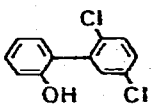
The plates were air dried after development, examined in UV light and sprayed with the chromogenic reagents used in this investigation.

RESULTS AND DISCUSSION

TLC is one of the methods of choice for the separation and purification of polar compounds such as the phenolic metabolites of chlorobiphenyls. No deriva-

TABLE I

R_F VALUES OF THE HYDROXYBIPHENYLS AND THEIR 2',5'-DICHLORO-DERIVATIVES

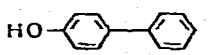
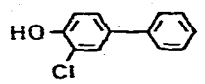
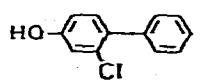
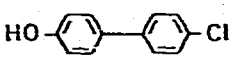
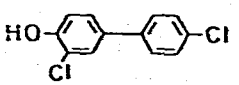
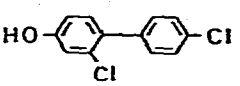
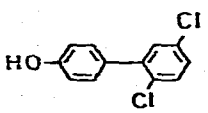
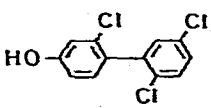
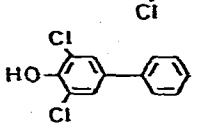
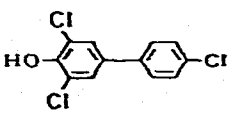
Compound	R_F × 100 *in solvent*										
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*L*
	59	66	92	80	29	50	38	32	38	86	67
	63	70	95	92	38	55	58	35	58	91	75
	78	77	97	95	63	66	74	64	86	91	86
	76	75	88	90	44	64	54	45	54	92	87
	84	75	93	93	47	62	61	52	53	92	79
	80	78	89	95	67	86	69	70	83	88	90

tization is necessary, considerable loading of plates is possible and some structural information can be obtained by the use of selected detection reagents.

The chromatographic behaviour will be discussed by comparing and contrasting small groups of related compounds insofar as the relationships between R_F values and structures are concerned. The colour reactions will, however, be presented in one table. All R_F values reported in the tables are expressed as $R_F \times 100$ and the values reported are the average of those obtained on at least three chromatograms. It should be pointed out, however, that differences in R_F values are sometimes observed when the experiments are carried out under different conditions of atmo-

TABLE II

R_F VALUES OF SOME MONO-, DI- AND TRICHLORO-4-BIPHENYLOLS

Compound	$R_F \times 100$ *in solvent*										
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*L*
	59	66	92	80	29	50	38	32	38	86	67
	87	79	95	85	55	76	75	69	75	89	85
	79	75	95	84	48	70	70	63	81	93	82
	32	65	93	87	31	54	50	35	47	43	74
	80	75	95	80	44	62	65	65	76	93	87
	75	75	93	84	41	62	60	39	47	93	86
	76	75	88	90	44	64	54	45	54	92	87
	83	81	91	84	51	84	67	72	81	91	90
	88	80	98	28	59	85	79	73	83	93	88
	89	82	90	15	51	88	69	74	85	90	90

spheric humidity. These differences can be significant and standard substances should always be employed for comparison purposes.

The R_F values for the three isomeric monohydroxybiphenyls and the corresponding 2',5'-dichloro-derivatives are shown in Table I. Generally, the R_F values obtained increase in the order 4-OH $\lesssim$ 3-OH $<$ 2-OH for both sets of compounds in most solvents. The 2',5'-dichloro-substituted compounds generally show an increase in R_F value over the parent hydroxybiphenyl. This increase is more pronounced for the 4-hydroxy-derivative and is in the order 4-OH $>$ 3-OH $>$ 2-OH for the other isomers.

In Table II, the R_F values for a number of mono-, di- and trichlorobiphenylols are presented. Increasing chlorine substitution usually increases the R_F value of the 4-hydroxybiphenyl derivative. For the monochloro- compounds available, R_F values decrease in the order 3-Cl $>$ 2-Cl $>$ 4'-Cl. A similar effect can be seen with the di- and trichloro- derivatives. The dramatic effect of solvents that contain ammonia on the R_F values of compounds with chlorine groups *ortho* to the hydroxyl group will be discussed later.

The effect of the position of the hydroxyl groups on the R_F values of some isomeric dihydroxybiphenyls is shown in Table III. As would have been expected, all the dihydroxybiphenyls studied so far have lower R_F values than the corresponding monohydroxybiphenyls. Dihydroxybiphenyls with their hydroxyl groups on different rings appear to have lower R_F values than those having both hydroxyl groups on the same ring. The overall order of R_F values is 2,5- $>$ 3,4- $>$ 2,2'- $>$ 4,4'- $\approx$ 3,3'-.

The influence of *o*-chloro- substitution on the R_F values is shown in Table IV for a number of chloro- derivatives of 4-hydroxy- and 4,4'-dihydroxybiphenyls. One chlorine atom *ortho* to the hydroxyl group usually results in a considerable increase

TABLE III

R_F VALUES OF SOME DIHYDROXYBIPHENYLS

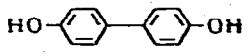
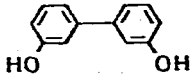
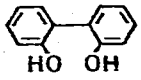
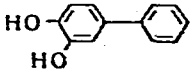
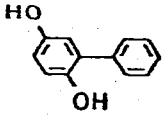
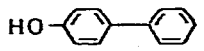
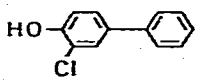
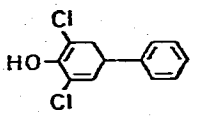
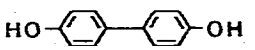
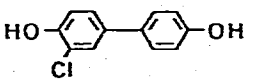
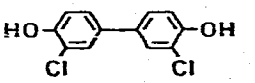
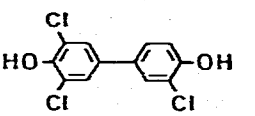
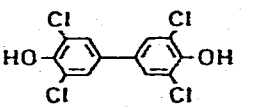
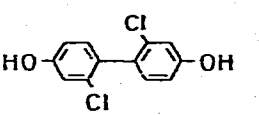
Compound	R_F · 100 *in solvent*										
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*L*
	29	28	46	50	0	5	5	2	5	87	13
	28	33	53	47	7	4	4	2	2	88	20
	35	35	55	55	11	10	11	6	5	87	18
	42	43	69	58	9	20	16	1	16	88	35
	42	47	78	72	16	25	29	20	33	86	42

TABLE IV

R_F VALUES FOR *o*-CHLORO-SUBSTITUTED 4-HYDROXY- AND 4,4'-DIHYDROXY-BIPHENYLS

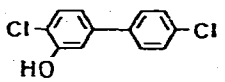
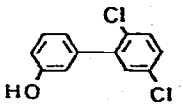
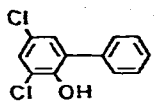
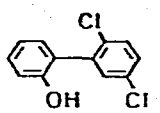
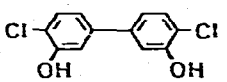
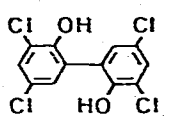
Compound	$R_F \times 100$ *in solvent*											
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
	59	66	92	80	29	50	38	32	38	86	90	67
	87	79	95	85	55	76	75	69	75	89	83	85
	88	80	98	28	59	85	79	73	83	93	48	88
	29	28	46	50	0	5	5	2	5	87	80	13
	57	37	57	47	11	25	12	11	20	89	66	39
	42	42	68	46	11	32	20	22	35	89	45	46
	58	48	73	7	20	50	33	28	47	87	13	59
	60	47	75	0	18	54	64	25	45	90	0	45
	40	41	64	56	14	27	23	10	14	89	78	40

in R_F values. The second *o*-chloro- substituent normally further increases the R_F value although not so significantly.

For any particular 4-hydroxy-(or 4,4'-dihydroxy-)biphenyl possessing an *o*-chloro- substituent the R_F value can be lowered by the addition of ammonia to the solvent system (*e.g.*, compare solvents D and C and solvents K and L). This effect is noticeable with one *o*-chloro- substituent, and becomes significant with two *o*-chloro- groups, one each *ortho* to a different hydroxyl group [*e.g.*, 3,3'-dichloro-4,4'-dihydroxybiphenyl, R_F (solvent) = 0.68 (C), 0.46 (D) and 0.89 (J), 0.45 (K)] and it is dramatic when two chlorine atoms *ortho* to the same hydroxyl group are involved. The R_F value for 3,3',5,5'-tetrachloro-4,4'-biphenyldiol decreased from 0.90 to 0.0

TABLE V

R_F VALUES OF CHLORINATED 2- AND 3-HYDROXYBIPHENYLS

Compound	$R_F \times 100$ *in solvent*											
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
	85	79	95	87	50	70	71	69	79	93	87	86
	84	75	93	93	47	62	67	52	53	92	95	70
	92	76	45	94	79	89	88	42	47	93	85	92
	80	78	89	95	67	86	69	70	83	88	91	90
	43	46	63	27	6	25	9	11	20	89	21	47
	50	6	6	4	0	49	0	0	0	9	43	3

when ammonia was added to solvent system J to give solvent system K. The R_F values for 2,2′-dichloro-4,4′-biphenyldiol are included in Table IV for comparison.

Table V reports the R_F values of some hydroxychlorobiphenyls derived from 2- and 3-hydroxybiphenyl. Because of the limited number of compounds available in these series, correlation of structure with R_F values becomes impossible. It is perhaps worth noting that the retarding effect of ammonia on the R_F values in the developing solvent is greater with 4,4′-dichloro-3,3′-biphenyldiol than in the structurally related 3,3′-dichloro-4,4′-biphenyldiol (*cf.*, Table IV). The R_F values for 3,3′,5,5′-tetrachloro-2,2′-biphenyldiol are generally lower than those of the corresponding 4,4′-dihydroxy-derivative.

Colour reactions

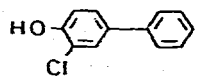
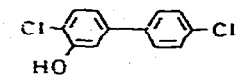
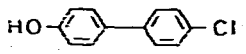
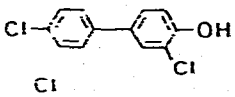
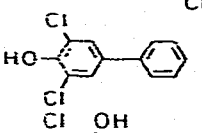
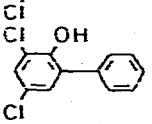
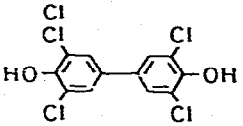
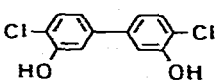
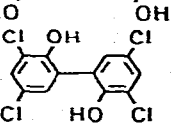
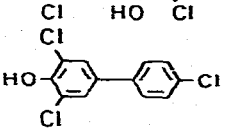
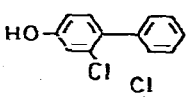
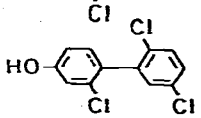
Chlorobiphenylols (chlorohydroxybiphenyls)[1], similarly to chlorophenols[21], do not always give colour reactions typical of compounds containing phenolic hydroxyl groups. The colours obtained by the action of 12 chromogenic reagents on 20 chlorobiphenylols are shown in Table VI, while Table VII presents the colours obtained from 8 hydroxylated biphenyl derivatives by the action of 15 chromogenic reagents.

In the case of the chlorohydroxy derivatives, good stable colours were obtained

TABLE VI

COLOUR REACTIONS OF SOME CHLOROHYDROXYBIPHENYLS

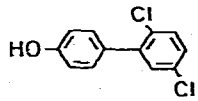
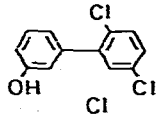
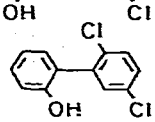
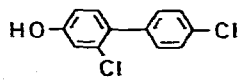
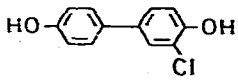
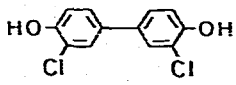
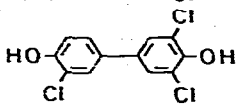
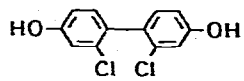
Abbreviations for chromogenic reagents: CNTNF = 9-dicyanomethylene-2,4,7-trinitrofluorene; TetNF = 2,4,5,7-tetranitro-9-fluorenone; TNFCOOH = 4,5,7-trinitro-9-fluorenone-2-carboxylic acid; NNCD = 2-chloro-4-nitrobenzenediazonium naphthalene-2-sulphonate, (1) saturated solution in chloroform and (2) solution in ethanol; TNF = 2,4,7-trinitro-9-fluorenone; TCNE = tetracyanoethylene.

Chlorohydroxybiphenyl derivative		*Chromogenic reagents and colours obtained*			
Substituents	*Structure*	*Nitric acid (conc.)*	*Millon's reagent*	*2,6-Dibromo-quinone-4-chlorimide*	*CNTNF*
3-Chloro-4-hydroxy-		Orange	Orange	Orange	Red-brown
4,4'-Dichloro-3-hydroxy-		Pale orange	Very pale yellow	Green	Colourless
4-Chloro-4'-hydroxy-		Bright yellow	Bright yellow	Pink	Lt. grey
3,4'-Dichloro-4-hydroxy-		Pale yellow	Orange-yellow	Orange-brown	Lt. grey
3,5-Dichloro-4-hydroxy-		Dark orange	Orange-pink	Orange-yellow	Lt. grey
3,5-Dichloro-2-hydroxy-		Dark orange	Cherry red	Pale green	Yellow
3,5,3',5'-Tetrachloro-4,4'-dihydroxy-		Lt. orange	Bright orange	Yellow	Colourless
4,4'-Dichloro-3,3'-dihydroxy-		Pale yellow	Pale yellow	Lt. blue-green	Orange
3,5,3',5'-Tetrachloro-2,2'-dihydroxy-		Pale yellow	White	Orange-yellow	Lt. grey
3,4',5-Trichloro-4-hydroxy-		Orange-rust	Orange-pink	Yellow	Lt. brown
2-Chloro-4-hydroxy-		Yellow	Yellow	Pale orange	Mauve
2,2',3'-Trichloro-4-hydroxy-		Pale yellow	Colourless	Colourless	Lt. grey

TetNF	*TNFCOOH*	*NNCD (1)*	*NNCD (2)*	*Cerium(IV) ammonium nitrate*	*Chloranil*	*TNF*	*TCNE*
Orange	Lt. orange	Colourless	Lt. pink	Colourless	Pink	Orange	Orange
Yellow	Colourless	Orange-yellow	Dark rose	Lt. yellow	Colourless	Yellow	Colourless
Orange	Pink	Pink-orange	Lt. pink	Colourless	Pink	Orange	V. lt. orange
Pink	V. lt. orange	Pink-orange	Lt. pink	Colourless	Pink	Bright orange	Orange
Lt. brown	V. lt. orange	colourless (bleached after 5 min)	White	Pink	Colourless	Yellow-orange	Orange
Yellow	Yellow	Colourless	Colourless	Yellow	Colourless	Yellow-green	Orange
Lt. green	Lt. green	Yellow	Mint green	Orange (intense)	Lt. green	Colourless	Colourless
Orange-yellow	Yellow	Yellow-orange	Dark rose	V. lt. yellow	Lt. yellow	Yellow	Colourless
Lt. brown	Lt. brown	Colourless	Colourless	Yellow	Colourless	Yellow	Colourless
Lt. grey	Lt. yellow	Colourless	White	Pink	Colourless	Beige	Yellow
V. lt. pink	Beige	Lt. pink	V. lt. mauve	Lt. mauve	V. lt. pink	Orange	Colourless
Lt. grey	Lt. grey	Colourless	V. lt. mauve	V. lt. blue-green	Lt. yellow	Colourless	Colourless

(Continued on p. 242)

TABLE VI *(continued)*

Chlorohydroxybiphenyl derivative		*Chromogenic reagents and colours obtained*			
Substituents	*Structure*	*Nitric acid (conc.)*	*Millon's reagent*	*2,6-Dibromo-quinone-4-chlorimide*	*CNTNF*
2,5-Dichloro-4'-hydroxy-		Pale yellow	Lt. yellow-green	Colourless	Grey
2,5-Dichloro-3'-hydroxy-		Colourless	V. lt. beige	V. lt. grey	Lt. pink
2,5-Dichloro-2'-hydroxy-		Yellow	Lt. yellow	Mid-blue	Orange
2,4'-Dichloro-4-hydroxy-		Yellow	V. lt. green-yellow	Lt. orange	Colourless
3-Chloro-4,4'-dihydroxy-		Dark brown	Grey-beige	Lt. beige	Lt. grey
3,3'-Dichloro-4,4'-dihydroxy-		Green-gold	Lt. gold	Beige	Grey
3,3',5-Trichloro-4,4'-dihydroxy-		Gold yellow	Orange-yellow	Lt. yellow	V. lt. grey
2,2'-Dichloro-4,4'-dihydroxy-		Colourless	Beige	Colourless	Pink

with the three inorganic spray reagents used, namely concentrated nitric acid, Millon's reagent and cerium(IV) ammonium nitrate. The nitric acid reagent gave a series of yellow, orange and brown colours, presumably due to the formation of nitro compounds. Millon's reagent gave well defined colours and enabled distinctions to be made between certain pairs of isomers. The cerium(IV) salt solution appeared to give deeper colours with more highly substituted hydroxychlorobiphenyls. The 2,6-dibromoquinone-4-chloroimide reagent (which is closely related to the well known phenol reagent 2,6-N-trichloro-*p*-benzoquinoneimine, *i.e.*, Gibb's reagent), as would have been expected, gave strong colours with all compounds tested.

The stable diazonium salt, *i.e.*, the NNCD reagent, 2-chloro-4-nitrobenzene-diazonium naphthalene-2-sulphonate, gave good colours with many of the chlorobiphenylols examined, coupling strongly, as would have been expected, with compounds with an unsubstituted position *para* to a hydroxyl group.

The electron-acceptor reagents CNTNF, TetNF, TNFCOOH and TNF gave

TetNF	*TNFCOOH*	*NNCD (1)*	*NNCD (2)*	*Cerium (IV) ammonium nitrate*	*Chloranil*	*TNF*	*TCNE*
Lt. grey	Lt. grey	V. lt. yellow	Colourless	Lt. blue	Yellow	Colourless	Lt. yellow
Colourless	Lt. pink	Yellow	Orange	White	Lt. orange	Lt. yellow	Colourless
V. lt. pink	Lt. yellow	Yellow-orange	Orange	Dark gold	Lt. orange		Lt. pink
Colourless	Beige	Pink-orange	Pink	White	Pink	Bright yellow	Colourless
Lt. beige	Beige	Dark beige	Yellow	Green-gold	Lt. mauve	Beige	V. lt. yellow
Lt. beige	Lt. beige	Beige	Yellow	Lt. gold	Lt. mauve	Pink-beige	Colourless
V. lt. grey	Lt. grey	Yellow	Yellow	Orange-yellow	Colourless	Lt. yellow	Colourless
Lt. yellow	Lt. orange	Orange	V. lt. orange	Lt. mauve	Salmon pink	Colourless	Yellow-orange

good colours with the chlorobiphenylols. The hydroxyl group in the hydroxy-compounds makes them better electron donors than the parent non-hydroxylated derivatives, and consequently more highly coloured complexes are usually obtained with the hydroxy-compounds.

The non-chlorinated hydroxy- and dihydroxybiphenyls also react well with most of the above reagents. The use of two reagents, known to detect *o*- and *p*-dihydroxy groups in aromatic compounds, namely potassium periodate[16] and 4-nitrophenylacetonitrile[17], enable 3,4-dihydroxybiphenyl and 2,5-dihydroxybiphenyl to be easily distinguished from each other and from the monohydroxybiphenyls and the other dihydroxybiphenyls included in the series.

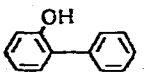
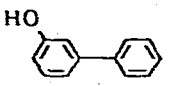
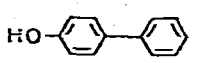
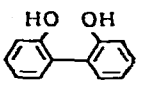
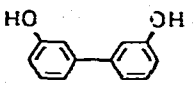
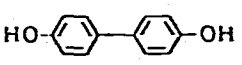
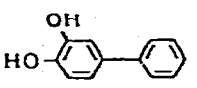
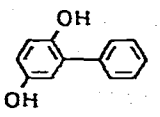
Dansyl derivatives

A method for the detection and identification of hydroxybiphenyls in urine or other biological fluids by TLC via dansyl derivative formation has recently been

TABLE VII

COLOUR REACTIONS OF SOME HYDROXY- AND DIHYDROXYBIPHENYLS

Abbreviations for chromogenic reagents as in Table VI, together with: KIO_4 = potassium metaperiodate; 4NPa = 4-nitrophenylacetonitrile; F. salt = potassium nitrosodisulphonate; NNCD(3) = 0.1% solution in 50% aqueous acetic acid, oversprayed with 0.5 *N* aqueous alcoholic sodium hydroxide solution.

Hydroxy and dihydroxybiphenyl derivatives		*Chromogenic reagents and colours obtained*					
Substituents	*Structure*	*Nitric acid (conc.)*	*Millon's reagent*	*2,6-Dibromo-quinone-4-chloroimide*	*CNTNF*	*TetNF*	*TNFCOOH*
2-Hydroxy-		Yellow-green	Lt. brown	Grey-blue	Lt. mauve	Lt. orange	Lt. pink
3-Hydroxy-		Pea green	Grey	Grey-blue	Pink	Lt. orange	Pink
4-Hydroxy-		Gold-yellow	Lt. green-yellow	Pink	Lt. pink	Lt. orange-brown	Lt. yellow
2,2'-Dihydroxy-		Dark brown	Dark brown	Green	Lt. rose	Lt. yellow-orange	Pink
3,3'-Dihydroxy-		Yellow	Grey-green	Lt. orange	Lt. orange	Lt. yellow	Lt. orange-pink
4,4'-Dihydroxy-		Green-gold	Grey	Colourless	Lt. grey	Grey-brown	Pink
3,4-Dihydroxy-		Bright orange	Orange	Purple	Grey	Beige	Lt. brown
2,5-Dihydroxy-		Yellow	Yellow	Yellow - olive	Mid-grey-green	Beige	Lt. grey brown

described[19] and trace amounts of phenolic metabolites of chlorobiphenyls have been detected and analysed by TLC–mass spectrometry[1], as outlined in Fig. 1. Dansyl derivatives of 4'-chloro-4-hydroxybiphenyl and 3-chloro-4'-hydroxybiphenyl have been prepared in this investigation in order to obtain some preliminary data on the TLC and mass spectrometric properties of these derivatives. The dansyl derivatives of the two chlorobiphenylols could be separated by TLC (silica layers using carbon tetrachloride as the developing solvent: dansyl derivative of 3-chloro-4-biphenylol, R_F = 0.64; dansyl derivative of 4'-chloro-4-biphenylol, R_F = 0.46). The mass spectra of these derivatives are discussed in the following section.

Mass spectra of complexes and dansyl derivatives

It has previously been shown[15] that the mass spectra of some compounds can be obtained directly from the coloured complex formed in the chromogenic detection on thin-layer plates. Thermal dissociation in the ion source and the greatly different

NNCD (1)	*NNCD (3)*	*Cerium(IV) ammonium nitrate*	*Chloranil*	*TNF*	*TCNE*	*KIO_4*	*4NPA*	*F. salt*
Pink	Purple – – yellow	Dark rust	Colourless	Orange yellow	Colourless	Colourless	Colourless	Colourless
Yellow	Mauve – – orange-yellow	White	Colourless	Yellow	Lt. pink	Colourless	Colourless	Colourless
Yellow	Purple – – brown	Lt. blue	Lt. pink	Orange-pink	Lt. blue	Colourless	Colourless	Colourless
Gold	Purple – – orange	Brown	Lt. pink	Yellow	Lt. pink	Brown	Colourless	Lt. yellow
Colourless	Mauve – – orange brown	Brown	Colourless	Yellow-orange	Lt. pink	Lt. yellow	Colourless	Lt. yellow
Beige	Colourless – – yellow brown	Pea green	Grey	Lt. brown	Lt. grey-green	Olive – – brown	Colourless	Rust brown
Dk. pink brown	Colourless – – brown	Mid-brown	Lt. green-grey	Brown	Grey	Orange yellow	Yellow – – violet – – grey	Yellow
Colourless	Colourless – – brown	Lt. blue	Lt. brown	Lt. orange	Navy blue	Yellow – – blue grey	Green – – olive – – brown	Blue

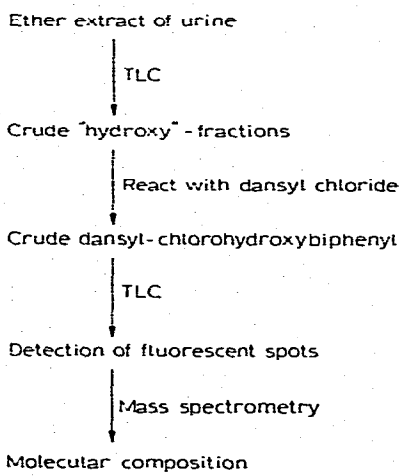


Fig. 1. Scheme for the identification of chlorobiphenylols (chlorohydroxybiphenyls) in biological samples via dansylation and mass spectrometry.

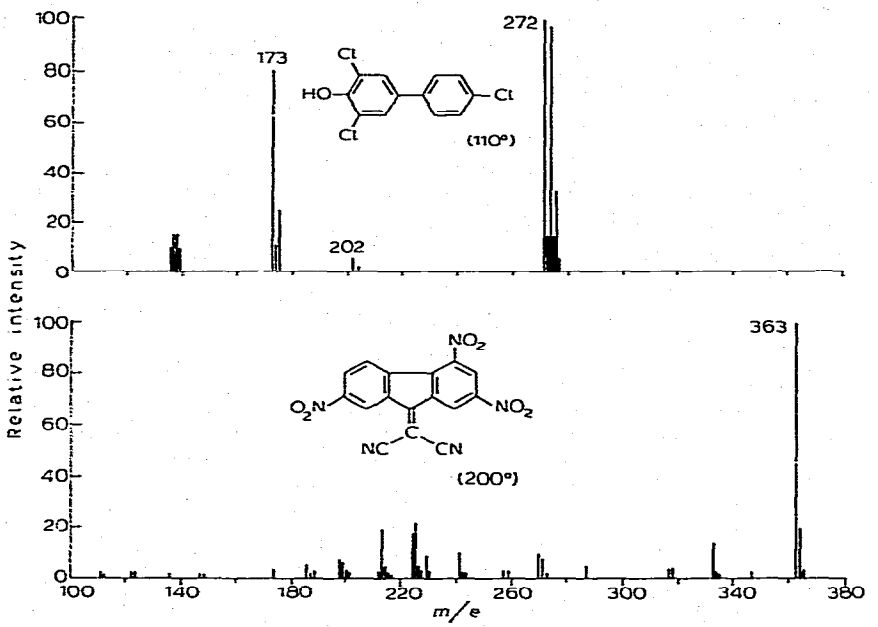


Fig. 2. 70-eV mass spectra of 3,4',5-trichloro-4-biphenylol and 9-dicyanomethylene-2,4,7-trinitrofluorene sublimed from the π-complex in the ion source of the mass spectrometer. Sample temperatures are indicated.

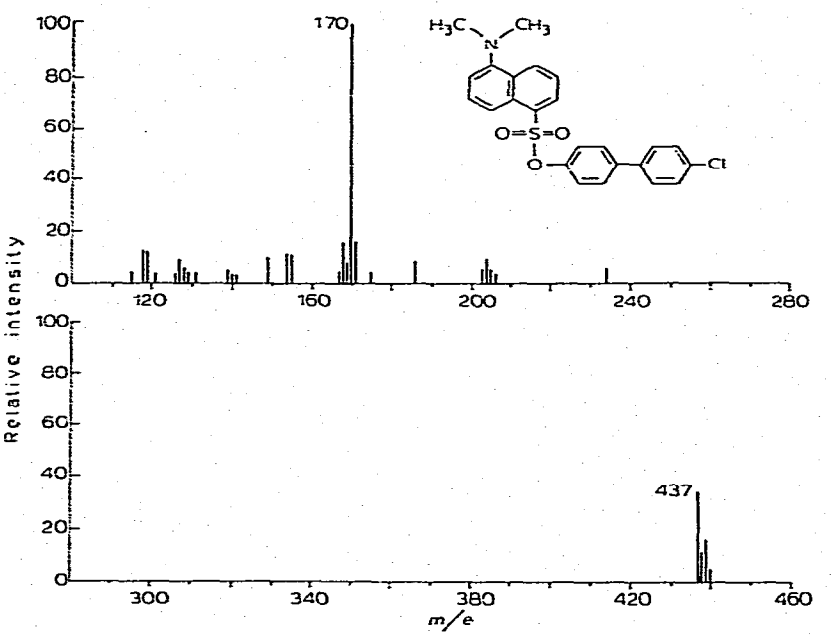


Fig. 3. 70-eV mass spectrum of the dansyl derivative of 4'-chloro-4-biphenylol.

volatilities of detected compounds allows individual spectra of the hydroxy-metabolite to be obtained. An example is given in Fig. 2. During the heating of the probe, the spectrum of the trichlorobiphenylol is obtained at 110°, a temperature much lower than that required for sublimation of the 9-dicyanomethylene-2,4,7-trinitrofluorene (CNTNF).

Mass spectrometry can also be usefully employed for the confirmation of the molecular composition of a hydroxymetabolite via its dansyl derivative. The spectrum of the dansyl derivative of 4'-chloro-4-biphenylol (synthetic sample) is shown in Fig. 3. The usual features of dansyl derivatives (a relatively abundant molecular ion and an abundant ion at *m/e* 170 corresponding to the dimethylaminonaphthalene moiety) are apparent in this spectrum.

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