

## THE COLOUR REACTIONS OF THE HYDROXYSKATOLES

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A number of reports have appeared in the literature, in recent years, of relatively specific colour reactions for the various hydroxyindoles, containing the hydroxyl group in the benzene moiety of the indole ring system. For instance, 5-hydroxyindoles have been reported to give violet colours specifically with 1-nitroso-2-naphthol in the presence of nitrous acid<sup>1</sup>. 6-Hydroxyindoles (and 6-alkoxyindoles), with a free 2-position, were said to give immediate bright red colours with diazonium salts under acid conditions<sup>2</sup>. 7-Hydroxyindoles were, by contrast, reported to give slowly forming brownish-purple colours with the acid diazo reagent, and indoles hydroxylated in the 4- and 5-positions were said to merely give weak brown or yellow colours under these conditions<sup>2</sup>. However, as far as the authors are aware, these colour reactions have only been studied with a relatively small number of hydroxyindole derivatives, particularly in the cases of the 4- and 7-hydroxyindole derivatives. A summary of the various colour reactions that have been employed, to date, in the paper chromatography of the hydroxyindoles (mostly 5-hydroxyindole derivatives) is contained in a recent review on the determination of hydroxyindoles in biological materials by SANDLER<sup>3</sup>.

There are a number of reports in the literature concerning possible relationships between the urinary excretion of relatively large amounts of "6-hydroxyskatole sulphate" and some forms of mental illness (*cf.* reviews by SPRINCE<sup>4</sup> and RODNIGHT<sup>5</sup>). It has recently been shown, however, by enzymatic hydrolysis and thin-layer chromatography, that the substance previously considered to be "6-sulphatoxyskatole" is, in all probability, a mixture of several different hydroxyskatole conjugates<sup>6</sup>. An essential prerequisite for this investigation was a complete understanding of the chromatographic behaviour of all four isomeric hydroxyskatoles. Several paper and thin-layer chromatographic systems have been developed, in these laboratories, for the separation and identification of the various isomeric hydroxyskatoles<sup>7,8</sup>. A preliminary report of the colours given by the 4-, 5-, 6- and 7-hydroxyskatoles with several different chromogenic reagents has already appeared<sup>7</sup>; this communication will describe a much more extensive survey of the colour reactions of this group of compounds.

## EXPERIMENTAL

*Hydroxyskatoles*

The hydroxyskatoles were prepared by the methods described in the literature<sup>9</sup>.

*Chromogenic reagents*

The preparation of the various chromogenic reagents, used in this investigation,

is described in detail, since it has sometimes been observed that relatively minor changes in the method of preparation of a reagent can result in marked changes in the final colour developed with a particular compound.

(i) *Ehrlich's reagent*. A solution of *p*-dimethylaminobenzaldehyde (1 g) in a mixture of concentrated hydrochloric acid (25 ml) and methanol (75 ml).

(ii) *Van Urk's reagent*. A solution of *p*-dimethylaminobenzaldehyde (0.125 g) in 100 ml of 65 % sulphuric acid, containing 0.1 ml of 5 % aqueous ferric chloride.

(iii) *F*<sub>1</sub>. A solution of 4-N,N-bis-(2-chloroethyl)-aminobenzaldehyde\* (1 g) in a mixture of concentrated hydrochloric acid (25 ml) and methanol (75 ml) (cf. REIO<sup>10</sup>).

(iv) *F*<sub>2</sub>. A solution of 4-N,N-bis-(2-chloroethyl)-amino-2-tolualdehyde\* (1 g) in a mixture of concentrated hydrochloric acid (25 ml) and methanol (75 ml) (cf. REIO<sup>10</sup>).

(v) *DMCA*. A solution of *p*-dimethylaminocinnamaldehyde (2 g) in a mixture of 6 *N* hydrochloric acid (100 ml) and ethanol (100 ml). This solution was diluted with 5 volumes of ethanol immediately prior to use (cf. HARLEY-MASON AND ARCHER<sup>11</sup>).

(vi) *Terephthalaldehyde reagent*. A solution of terephthalaldehyde (0.2 g) in a mixture of acetone (90 ml) and glacial acetic acid (10 ml). After spraying with this reagent the plates were heated at 105° for 3 min (cf. CURZON AND GILTROW<sup>12</sup>).

(vii) *Procházká's reagent*. A mixture of 35 % aqueous formaldehyde (10 ml) and 25 % hydrochloric acid (10 ml) diluted with 95 % ethanol (20 ml). After spraying with this reagent the plates were heated at 105° for 5 min (PROCHÁZKA *et al.*<sup>13</sup> and cf. RANDERATH<sup>14</sup>).

(viii) *Xanthyrol reagent*. A solution of xanthyrol (0.2 g) in a mixture of ethanol (90 ml) and concentrated hydrochloric acid (10 ml) prepared immediately before use (cf. DICKMAN AND CROCKETT<sup>15</sup>).

(ix) *1-Nitroso-2-naphthol-nitrous acid reagent*. The plate was first sprayed with a 1 % solution of 1-nitroso-2-naphthol in 95 % ethanol; after drying it was sprayed with freshly prepared 2 *N* hydrochloric acid containing 2 vol. % of a 5 % sodium nitrite solution (UDENFRIEND *et al.*<sup>1</sup> and cf. JEPSON<sup>16</sup>).

(x) *Sodium periodate-picric acid reagent*. A mixture of equal volumes of 10 % aqueous sodium metaperiodate and 3.5 % aqueous picric acid; prepared at 100° to ensure that the reagents remain in solution (cf. CADENAS AND DEFERRARI<sup>17</sup>).

(xi.a) *Gibb's reagent (neutral)*. A solution of N,2,6-trichloro-*p*-quinoneimine (2 g) in ethanol (100 ml).

(xi.b) *Gibb's reagent (basic)*. The plate was first sprayed with the neutral reagent, prepared as described in Section (xi.a), and then exposed to ammonia fumes.

(xii.a) *Folin and Ciocalteu's reagent (neutral)*. This reagent was available commercially (B.D.H.) and was diluted with two volumes of water directly prior to use.

(xii.b) *Folin and Ciocalteu's reagent (basic)*. The plate was first sprayed with the dilute commercial reagent, cf. Section (xii.a), and then exposed to ammonia fumes.

(xiii) *Gold chloride*. The commercially available reagent (Fisher Scientific Co.); a 0.5 % aqueous solution was used directly (cf. ERSPAMER AND BORETTI<sup>18</sup>).

(xiv) *2,2-Diphenyl-1-picrylhydrazyl*. A solution of 2,2-diphenyl-1-picrylhydrazyl\*\* (1 g) in ethanol (100 ml) (cf. HERZMANN AND VENKER<sup>19</sup>).

\* Obtained from the Frinton Laboratories, Vineland, N.J., U.S.A.

\*\* Obtained from the Eastman-Kodak Chemical Company, Rochester, N.Y., U.S.A.

(*xv.a*) *NNCD (acid)*. A solution of 2-chloro-4-nitrobenzenediazonium naphthalene-2-sulphonate\* (0.1 g) in dilute aqueous acetic acid (pH = 2) (100 ml) (*cf.* CARTWRIGHT AND ROBERTS<sup>20</sup>).

(*xv.b*) and (*xv.c*) *NNCD (alkaline)*. After spraying with the NNCD reagent, prepared by the method described above, the plates were sprayed with either: (b) 20 % aqueous sodium carbonate solution or (c) 10 % tetraethylammonium hydroxide solution.

(*xvi.a*) *DSA (acid)*. Ten volumes of a solution of sulphanilic acid (9 g) in concentrated hydrochloric acid (90 ml) and water (900 ml) were mixed, at 0°, with one volume of 5 % aqueous sodium nitrite. After 5 min the excess nitrous acid was destroyed by the addition of excess ammonium sulphamate (*cf.* JEPSON<sup>16</sup>).

(*xvi.b*) *DSA (acid; in the presence of excess nitrous acid)*. A 5 % aqueous solution of sodium nitrite (1.5 ml) was added to a solution of sulphanilic acid (1.5 ml, containing 9 g of sulphanilic acid in 90 ml of concentrated hydrochloric acid per litre of water); a further quantity (6 ml) of the aqueous sodium nitrite solution was then added (*cf.* BLOCK *et al.*<sup>21</sup>). The solution was diluted to 50 ml with water after standing at room temperature for 5 min.

(*xvii*) *NBDF*. A solution of *p*-nitrobenzenediazonium fluoroborate\*\* (0.1 g) in dilute aqueous acetic acid (pH = 2) (100 ml).

#### Colour development

The hydroxyskatoles (10 µg of each isomer)\*\*\* were applied, in methanolic solution, to the origin of Silica gel G plates and the thin-layer chromatograms were developed with one of the solvent systems previously described by the authors for the thin-layer chromatography of the hydroxyskatoles, *i.e.* diisopropyl ether or 1,2-dichloroethane-diisopropylamine (6:1) (*cf.* HEACOCK AND MAHON<sup>8</sup>). After drying in a current of air each developed chromatoplate was sprayed with one of the various colour reagents that are mentioned above. The initial colour development and the subsequent changes in the colour of the spots and the backgrounds were carefully noted.

#### RESULTS AND DISCUSSION

The colours obtained from the hydroxyskatoles by the action of several different chromogenic reagents, after chromatography on Silica gel G using diisopropyl ether as the developing solvent are shown in Table I. The use of the basic solvent system described above (*i.e.* 1,2-dichloroethane-diisopropylamine (6:1)), which, although giving better separations of the four isomeric hydroxyskatoles, can give unsatisfactory results in some cases, since residual traces of this solvent occasionally interfere, quite strikingly, with the colour produced by a particular hydroxyskatole with certain of the chromogenic reagents. This is not always a problem when a strongly

\* Obtained from Hopkins and Williams Ltd., Chadwell Heath, Essex, England.

\*\* Obtained from the Eastman-Kodak Chemical Company, Rochester, N.Y., U.S.A.

\*\*\* It was found, in practice, that although 10 µg of the hydroxyskatoles usually gave well defined colours with most of the chromogenic reagents described above, more satisfactory results were obtained with the reagents (vi), (vii), (ix) and (x) if 20 µg of the hydroxy compound were used.

acidic spray reagent (*e.g.* Ehrlich's reagent) is used, presumably due to rapid and complete neutralisation of any residual amine on the plate. The colours reported are those observed in transmitted light, the plates being viewed on an X-ray viewing screen illuminated by diffused daylight-type fluorescent light. As would have been expected somewhat different colours were sometimes observed when a different light source (*e.g.* tungsten light or daylight) was used to illuminate the chromatograms or when the chromatograms were viewed in reflected light.

Since the hydroxyskatoles are both indolic and phenolic in character they give strong colours with typical indole reagents, such as the Ehrlich reagent (*i.e.* *p*-dimethylaminobenzaldehyde), and also with phenol reagents, including the diazonium salts, Folin and Ciocalteu's reagent and Gibb's reagent (*i.e.* *N*,2,6-trichlorobenzoquinoneimine).

One of the most striking colours obtained from a hydroxyskatole with the Ehrlich reagent was the bright greenish blue colour given by the 6-isomer; 5-hydroxyskatole gave a definite violet colour and the other two isomers gave slightly different shades of greyish blue-violet colours with this reagent.

The colours obtained from the hydroxyskatoles with the Van Urk modification of this reagent (*i.e.* *p*-dimethylaminobenzaldehyde in 65 % sulphuric acid, containing a small quantity of ferric chloride) were fairly well differentiated (see Table I). Initially all the hydroxyskatoles gave yellow colours with the Van Urk reagent, however, the edges of the spots rapidly darkened and the peripheral colours slowly spread inwards and in *ca.* 24 h covered the entire area of the spot. 5-Hydroxyskatole eventually gave a distinct stable blue colour with the Van Urk reagent. This reagent has one practical advantage over the simple Ehrlich reagent; the background of the sprayed chromatograms does not discolour on keeping.

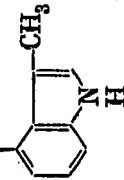
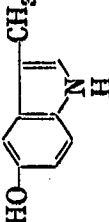
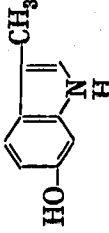
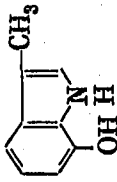
Two relatively new reagents, which are chemically similar to the Ehrlich reagent, *i.e.* 4-*N,N*-bis-(2-chloroethyl)-aminobenzaldehyde ( $F_1$ ) and 4-*N,N*-bis-(2-chloroethyl)-amino-2-tolualdehyde ( $F_2$ ), which have recently been utilised by REIO as location reagents in indole chromatography<sup>10</sup>, gave intense and initially relatively well differentiated colours with the hydroxyskatoles. It is interesting to note, however, that both the reagents ( $F_1$ ) and ( $F_2$ ) gave a greenish-blue coloured product with the 7-isomer, similar to the bluish green coloured derivatives given by the 6-isomer with the simple Ehrlich reagent. These reagents appear to be very sensitive for detection of the hydroxyskatoles and may prove useful in the future for the detection and assay of other hydroxyindole derivatives.

*p*-Dimethylaminocinnamaldehyde, introduced by HARLEY-MASON AND ARCHER as a spray reagent for indoles<sup>11</sup>, gives vivid blue to grey-violet colours with the compounds under investigation. This reagent is undoubtedly very sensitive, but suffers from a number of disadvantages (*cf.* DURKEE AND SIROS<sup>22</sup>); firstly, the colours are not particularly well differentiated and secondly the background is quite unstable, rapidly acquiring a pink colour and finally turning red-brown. This chromogenic reagent is quite unsuitable to use in cases where basic solvents have been used during the running of the chromatograms. The residual traces of developing solvents appear to interfere with the colour development and in such cases the initial background colour is often too intense for the spray to be of any practical utility.

The terephthalaldehyde reagent rapidly gives a very characteristic stable bright blue colour with 6-hydroxyskatole. The other isomers only react relatively

TABLE I

COLOUR REACTIONS OF THE HYDROXYSKATOLES ON SILICA GEL G

Chromogenic reagents		Colours <sup>a, b</sup> given with the hydroxyskatoles			
		4-Hydroxyskatole	5-Hydroxyskatole	6-Hydroxyskatole	7-Hydroxyskatole
					
Ehrlich's reagent		Grey-blue → grey-violet	Violet → blue-violet	Green → blue-green	Grey → blue-grey
Van Urk's reagent <sup>c</sup>		Yellow (grey-brown edge) → grey-brown	Yellow (blue-grey edge) → blue	Yellow (green-brown edge) → olive-green	Yellow (grey-brown edge) → grey
F <sub>1</sub> <sup>d</sup>		Blue	Violet	Blue-violet → blue	Blue-green
F <sub>2</sub> <sup>e</sup>		Grey-blue	Violet	Blue	Blue-green
DMCA <sup>f</sup>		Grey-violet	Blue → blue-violet → violet	Blue-green → grey → grey-brown	Violet → grey-violet
Terephthalaldehyde <sup>g</sup>		Yellow (blue-green edge) → grey	Weak pink → orange-pink → yellow-brown	Blue	Mauve-brown → red-brown
Procházka's reagent <sup>h</sup>		Dull olive green → grey-brown	Violet-brown → red-brown	Blue	Grey-violet → grey-brown
Xanthidrol		Yellow (grey-violet edge) → grey-green (pink-violet edge)	Pink (blue-violet edge) → orange (violet edge) → blue-violet	Yellow-orange (violet edge) → grey-green (violet edge)	Yellow (violet edge) → grey-violet
1-Nitroso-2-naphthol-nitrous acid		Dull grey → grey-green	Grey → grey-violet → violet	Dull orange-brown	Dull grey-violet
Sodium metaperiodate-picric acid		Dark grey	Brown	Grey-brown	Violet-brown

Gibb's reagent (neutral)	Red-brown $\longrightarrow$ brown-violet	Violet	Red-brown $\longrightarrow$ brown	Orange-brown $\longrightarrow$ orange
Gibb's reagent (basic)	Blue-violet	Blue-violet	Blue-violet	Blue-violet
Folin and Ciocalteu's reagent (neutral)	Grey-blue $\longrightarrow$ grey	Grey-blue $\longrightarrow$ grey-violet	Grey-brown $\longrightarrow$ dull green-brown	Grey-violet $\longrightarrow$ grey-blue
Folin and Ciocalteu's reagent (basic)	Grey-blue	Grey-blue	Grey-blue	Grey-blue
Gold chloride	Grey-green $\longrightarrow$ grey	Grey	Greyish olive green	Violet $\longrightarrow$ grey-violet
2,2-Diphenyl-1-picrylhydrazyl	Pale yellow $\longrightarrow$ grey-green	Pale yellow $\longrightarrow$ pink-brown	Pale yellow $\longrightarrow$ grey-green	Pale yellow $\longrightarrow$ violet-brown
NNCD <sup>1</sup> (acid)	Red-brown	Orange	Brick red	Orange
NNCD <sup>1</sup> (sodium carbonate)	Grey-brown	Orange	Violet-brown	Yellow-brown
NNCD <sup>1</sup> (tetraethylammonium hydroxide)	Grey-blue	Orange-brown	Blue	Blue-green
DSA <sup>1</sup> (acid; excess nitrous acid removed)	Grey-brown	Yellow-brown $\longrightarrow$ orange-brown	Pink-brown $\longrightarrow$ bright red	Violet-brown
DSA <sup>1</sup> (acid; in the presence of nitrous acid)	Orange $\longrightarrow$ brown	Orange-brown $\longrightarrow$ brown	Yellow-orange $\longrightarrow$ yellow-brown	Orange $\longrightarrow$ brown

<sup>a</sup> The colours reported are those observed by viewing the developed chromatograms (after spraying) in transmitted light on an X-ray viewing screen with diffused fluorescent light (daylight-type) illumination.

<sup>b</sup> The initial colour produced by the particular reagent is reported together with subsequent major colour changes.

<sup>c</sup> In all cases, the hydroxyskatoles initially gave yellow colours with this reagent. However, after a few minutes the final colour began to develop on the periphery of the spot and within 24 h had covered the entire area of the spot.

<sup>d</sup> F<sub>1</sub> = 4-N,N-bis-(2-chloroethyl)-aminobenzaldehyde.

<sup>e</sup> F<sub>2</sub> = 4-N,N-bis-(2-chloroethyl)-amino-2-tolualdehyde.

<sup>f</sup> DMCA = *p*-dimethylaminocinnamaldehyde.

<sup>g</sup> With the exception of the bright blue colour which develops rapidly with the 6-isomer, the colours produced by all the other isomers form very slowly.

<sup>h</sup> There is an apparent marked difference in some of the colours given by the hydroxyskatoles with this reagent on viewing in reflected or transmitted light. The coloured products obtained from the 4- and 6-hydroxyskatoles appear much greener in reflected light.

<sup>i</sup> NNCD = 2-chloro-4-nitrobenzenediazonium naphthalene-2-sulphonate.

<sup>j</sup> DSA = diazotised sulphanilic acid.

slowly with this reagent, particularly the 5-isomer, which initially gives only a very weak pink colour. After 24 h distinct grey and red-brown colours were shown by the 4- and 7-hydroxyskatoles respectively; after about 48 h the spot due to the 5-isomer developed a yellow-brown colour. The colours eventually obtained from the hydroxyskatoles with terephthalaldehyde were quite distinctive in all four cases and the background appears to be quite stable, however, it suffers the disadvantage of being somewhat less sensitive than many of the other reagents tested.

The Procházka reagent (*i.e.* formaldehyde and concentrated hydrochloric acid) gave a definite blue colour with the 6-isomer; the other isomers giving a series of rather dull brownish colours.

The colours obtained from the hydroxyskatoles with the xanthydrol reagent are markedly affected by the presence of residual traces of basic solvents. In general the colours develop slowly with this reagent; under neutral conditions the 4- and 6-isomers initially gave yellow spots (with grey-violet edges); after 24 h the central area of each spot became grey-green in colour, but the violet edge was still apparent. Deep blue-violet and grey-violet spots were eventually obtained from 5- and 7-hydroxyskatole respectively.

The 1-nitroso-2-naphthol-nitrous acid reagent, reported to give violet colours specifically with 5-hydroxyindoles<sup>1</sup>, did, in fact, give a violet colour with 5-hydroxyskatole. However, the 7-isomer also gave a dull greyish violet colour with this system, which could be confused, by inexperienced observers, with the colour obtained from the 5-isomer. The other two isomers gave muddy grey to brown colours with this reagent. As reported<sup>1,16</sup> previously, this reagent is not very sensitive and in general, the colours produced were relatively dull. The background colour, which was initially yellow, darkened markedly on standing. (The nitrosonaphthol-nitrite reagent appeared to give brighter colours with the hydroxyskatoles on paper than on silica gel and the background was clearer.)

The sodium metaperiodate-picric acid reagent, initially used for the detection of amines<sup>17</sup>, can also be used for the detection of the hydroxyskatoles, although it is relatively insensitive. However, this reagent gave a characteristic strong dark grey colour with the 4-isomer, whilst the other isomers gave dull brown colours with slight grey or violet hues. All the colours were formed on a bright yellow background.

The nature of the colours obtained by the action of Gibb's reagent (*i.e.* N,2,6-trichloroquinoneimine) on the hydroxyskatoles is strongly affected by the presence or absence of traces of bases. Under neutral conditions the 5-isomer gave a distinct violet colour, the other three isomers giving bright red to orange-brown colours. However, traces of base altered these colours in a striking fashion; all the isomers gave blue to grey-violet spots with Gibb's reagent under alkaline conditions. Exposure of the plate to ammonia fumes after, spraying, immediately converted all the spots to a vivid blue-violet colour; spraying with alkaline buffers produced various greyish violet shades. The background rapidly turned brown on exposure to alkali, or if residual traces of basic solvents were present. The alkali sensitivity of these coloured derivatives undoubtedly accounts for the apparent differences in the colours obtained by the action of Gibb's reagent on the hydroxyskatole on paper and on silica as reported in an earlier paper<sup>7</sup>. A neutral running solvent system was used in the case of the separations on formamide paper, whereas a basic solvent system was employed with the silica plates.

Folin and Ciocalteu's reagent gave varying shades of dark grey colours with all four hydroxyskatoles (on a yellow background). Once again, the colours of the products were affected by small quantities of bases; fuming the plates with ammonia, after spraying, converted all the spots to virtually the same grey-blue colour. A quantitative assay procedure for the hydroxyskatoles based on the colours they give with the Folin and Ciocalteu reagent, under alkaline conditions, has recently been described by the authors<sup>23</sup>.

Gold chloride gave a distinct violet colour with 7-hydroxyskatole, whilst the other three isomers gave dull grey-green colours with this reagent.

An interesting reagent which has recently been used for the detection of phenols on paper chromatograms is the stable free radical 2,2-diphenyl-1-picrylhydrazyl<sup>19</sup>. Initially the hydroxyskatoles appear as very pale yellow spots on a mauve background when developed chromatograms are sprayed with this reagent. However, after standing for about 24 h at room temperature the spots due to the 4- and 6-isomers develop grey-green colours, whilst the 5- and 7-hydroxyskatoles respectively gave pink-brown and violet-brown derivatives. During this time the initial bright pink-mauve background colour changes to a dull violet-brown.

It has been reported that 6-hydroxy- and 6-alkoxy-indoles react with diazonium salts, under acid conditions, to give brilliant red coloured products<sup>2, 16, 24</sup>. 6-Hydroxyskatole does not appear to be an exception to this rule, giving a bright red colour with acidified diazonium salts, provided that any excess nitrous acid present in the diazonium salt solution is destroyed before spraying. The DSA reagent (*i.e.* diazotised sulphanilic acid) prepared by the method described by JEPSON<sup>16</sup> gives a bright red colour with 6-hydroxyskatole and grey, orange and violet shades of brown with the 4-, 5- and 7-isomers respectively. However, if the excess nitrous acid is not removed before colour development, merely yellow to orange shades of brown colours are obtained with all four isomers. This accounts for the fact that 6-hydroxyskatole was previously reported by the authors as giving a yellow-brown colour with diazotised sulphanilic acid<sup>7</sup>. The previously reported colours were obtained with diazotised sulphanilic acid in the presence of excess nitrous acid. The reagent was prepared by one of the methods described in BLOCK *et al.*<sup>21</sup>.

A number of stabilised diazonium salts are now available commercially, and their use as location reagents in chromatography avoids the necessity of preparing the diazonium salt before use, and eliminates the possibility of excess nitrous acid being present in the reagent and interfering with the colours produced. One of the most useful compounds of this type is the so-called NNCD reagent (*i.e.* 2-chloro-4-nitrobenzenediazonium naphthalene-2-sulphonate). This reagent in dilute acetic acid gave a vivid brick red colour with 6-hydroxyskatole, and although orange colours were obtained from the 5- and 7-isomers, a definite red-brown colour was also obtained from the 4-isomer, which could possibly be confused with the colour obtained from the 6-isomer. The products obtained from the hydroxyskatoles with the NNCD reagent underwent quite striking colour changes on further spraying with aqueous bases (sodium carbonate or tetraethylammonium hydroxide were used). The coloured derivative of the 5-isomer was the least affected, remaining orange on spraying with the sodium carbonate solution and becoming orange-brown on treatment with the stronger alkali. The most striking colour changes were observed with the product derived from the 7-isomer, which turned yellow-brown with the carbonate solution,



TABLE II  
COLOUR REACTIONS OF THE HYDROXYSKATOLES ON PAPER

<i>Chromogenic reagents</i>	<i>Colours given with the hydroxyskatoles with two diazonium salts</i>			
	<i>4-Hydroxyskatole</i>	<i>5-Hydroxyskatole</i>	<i>6-Hydroxyskatole</i>	<i>7-Hydroxyskatole</i>
NNCD <sup>a</sup> (acid)	Rust	Orange	Brick red	Intense tangerine orange
NNCD/sodium carbonate	Grey-brown	Orange	Dark brown	Olive green
NNCD/tetraethylammonium hydroxide	Grey-blue	Pink → brown	Bright blue → grey-blue	Deep turquoise
NBDF (acid)	Brick red	Orange	Red-violet	Orange-red
NBDF/sodium carbonate	Grey-brown	Brown	Violet-brown	Dark brown
NBDF/tetraethylammonium hydroxide	Grey-blue	Grey-brown	Blue → grey-blue	Blue

<sup>a</sup> NNCD = 2-chloro-4-nitrobenzenediazonium naphthalene-2-sulphonate; NBDF = *p*-nitrobenzenediazonium fluoroborate.

and gave a vivid blue-green colour on spraying with the solution of the strong base. The colour of the product derived from the 6-isomer changed from red to violet-brown on treatment with sodium carbonate, finally turning blue with the strong alkali. This blue product was initially very brightly coloured, however the colour faded quite rapidly, much more rapidly than the corresponding product derived from the 7-isomer. JEPSON *et al.* have reported the formation of green-blue products from 7-hydroxytryptamine and 7-hydroxyindole-3-acetic acid with diazotised *p*-nitroaniline in the presence of sodium carbonate<sup>2</sup>. ACHESON AND HANDS have reported that the 5- and 6-hydroxyskatole give orange and orange-red colours respectively with the Pauly modification of the DSA reagent<sup>25</sup>.

The colour reactions of the hydroxyskatoles with diazonium salts and the subsequent colour changes occurring on treatment with alkali were more readily observed on paper, the colours being much brighter than on thin layers of silica gel. Table II lists the colours obtained from the hydroxyskatoles with the NNCD reagent on paper and the subsequent colour changes observed on treatment with alkali. The colours were basically similar to those observed on silica. Analogous coloured products were obtained from the hydroxyskatoles by the use of another commercially available stable diazonium salt (*p*-nitrobenzenediazonium fluoroborate, *i.e.* the NBDF reagent). The results obtained, with this reagent, are recorded in Table II.

#### CONCLUSIONS

This investigation has shown that there is no shortage of chromogenic reagents for the location of the hydroxyskatoles on developed chromatograms. As would have been expected from previous publications on the colour reactions of hydroxyindole derivatives, 6-hydroxyskatole gave a bright red colour with acid diazo reagents, a deep blue-green colour with the Ehrlich reagent and a violet colour with 1-nitroso-2-naphthol in the presence of nitrous acid.

One of the best colour reagents for 4-hydroxyskatole would appear to be the metaperiodate-picric acid reagent, which gave a distinct dark grey colour with this isomer. The stable blue colour given with the Van Urk reagent and the bright violet colour given with Gibb's reagent (neutral) are two of the most characteristic reactions of the 5-isomer. As well as the distinctive colours given with the acid diazo reagent and Ehrlich's reagent, the stable blue colours given with terephthalaldehyde and the Procházka reagent are very characteristic of the 6-isomer. The most striking colour reaction of the 7-hydroxyskatole was the bright orange obtained with the acid diazo reagents, which changed to a vivid green-blue colour on treatment with strong alkali. The violet colour given with gold chloride also appeared characteristic of the 7-isomer. However, in general, caution should be exercised in deciding the position of the hydroxyl group in a hydroxyindole on the basis of colour reactions alone, since similar colours are often produced with two or more isomers even with some of the most selective reagents.

#### ACKNOWLEDGEMENTS

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#### SUMMARY

The colour reactions given by 4-, 5-, 6- and 7-hydroxyskatole with a number of different chromogenic reagents are reported. The relative specificity of the reagents for the various positional isomers is discussed.

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