9 *The Oxidation of Aromatic Amines. Part VI.* Persulphate Oxidation of Some Carcinogenic Aromatic Amines.*

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The action of alkaline persulphate on a number of aromatic amines, most of which are carcinogenic, gives, as principal products, the sulphuric esters of the corresponding *o*-aminophenols, which yield the free *o*-aminophenols on acid-hydrolysis.

The results of Bonser, Clayson, Jull, and Pyrah [1] suggest that the carcinogenic action of 2-naphthylamine may be due, not to the amine itself, but to 2-amino-1-naphthol formed by the metabolism of the amine in the body. Other carcinogenic aromatic amines may act *via* the corresponding *o*-aminophenols, and in order to test this by the method of bladder implantation [2,3] small amounts of the *o*-aminophenols were required. Many of these aromatic amines can be oxidised with alkaline persulphate in the manner previously described [4,5] to give reasonable yields of the corresponding aminophenyl sulphuric esters, which yield the aminophenols on acid-hydrolysis. The sulphuric esters and, in most cases, the aminophenols are new compounds, and in some cases it has not been possible to establish their identity unequivocally. In the aromatic series, only *o*-carboxy-amines are substituted in the *para*-position to the amino-group by the entering sulphuric ester group; [6] *meta*-substituted products have never been detected. Most of the amines which we oxidised are already *para*-substituted and we have assumed therefore that the products are *ortho*-substituted amines. The amines successfully oxidised in this reaction were 2'-chloro-4-dimethylamino- and 4-dimethylamino-2'-methyl-*trans*-stilbene, 4-dimethylamino-azobenzene and -diphenyl, *NN*-dimethyl-1- and -2-naphthylamine, 3 : 4 : 1-xylidine, and sulphanilamide.

With the stilbenes some oxidation of the double bonds occurred: *o*-chloro- and *p*-dimethylamino-benzoic acid, and *o*-toluic acid, were isolated. 4-Dimethylaminostilbene itself was scarcely attacked by persulphate, the only product isolated being a little benzoic acid. The ultraviolet spectra of the sulphuric esters and aminophenols derived from the substituted dimethylaminostilbenes confirmed the proposed structures (Roe, unpublished work) and were similar to that of 4-amino-3-methoxystilbene.[7] The presence of small amounts of substituted 4-dimethylamino-*cis*-stilbenes was also indicated.

Persulphate oxidations of 2-aminofluorene and 4-amino-4'-fluorodiphenyl (which yielded brown amorphous substances), 2-amino-anthracene, -anthraquinone, and -chrysene, and 4-aminoazobenzene (which failed to react to any appreciable extent) were unsuccessful. 4 : 4'-Diaminodiphenyl sulphone, 2-amino-2' : 3-dimethylazobenzene, and di-2'-chloroethyl-2-naphthylamine yielded sulphuric esters which did not crystallise.

Of the *o*-aminophenols isolated, 2-amino-4 : 5-dimethylphenol [3] and 4-dimethylamino-3-hydroxyazobenzene (unpublished observations) induced cancer of the bladder when implanted into the bladders of mice. Tested under the same conditions 4-dimethylamino-3-hydroxydiphenyl and 1-dimethylamino-2-naphthol were not carcinogenic. The biological tests on the other aminophenols are not yet complete.

It is of interest that, whereas 4-dimethylaminostilbene was not attacked by persulphate,

* Part V, Boyland and Manson, *J.*, 1957, 4689.

[1] Bonser, Clayson, Jull, and Pyrah, *Brit. J. Cancer*, 1952, **6**, 412.
[2] *Idem, ibid.*, 1956, **10**, 533; Bonser, Bradshaw, Clayson, and Jull, *ibid.*, p. 539.
[3] Boyland and Watson, *Nature*, 1956, 177, 837; Allen, Boyland, Dukes, Horning, and Watson, *Brit. J. Cancer*, 1957, **11**, 212.
[4] Boyland, Manson, and Sims, *J.*, 1953, 3622.
[5] Boyland and Sims, *J.*, 1954, 980.
[6] Boyland, Sims, and Williams, *Biochem. J.*, 1956, **62**, 546.
[7] Bergel, *Chem. and Ind.*, 1954, 904.

the 2′-chloro- and the 2′-methyl derivative were oxidised. These substituted 4-dimethylaminostilbenes are more active as growth-inhibitors and carcinogens than 4-dimethyl aminostilbene itself. The compounds which are the more susceptible to persulphate oxidation are the more active biologically.

Experimental

Materials.—The aminostilbenes had been prepared by the methods of Haddow, Harris, Kon, and Roe.[8] *NN*-Dimethyl-2-naphthylamine, m. p. 46°, and 4-dimethylaminodiphenyl, plates (from ethanol), m. p. 120° (lit.,[9] m. p. 123°) (Found: C, 85·3; H, 7·6; N, 7·4. Calc. for $C_{14}H_{15}N$: C, 85·2; H, 7·7; N, 7·1%), were prepared in good yield from the unmethylated amines with trimethyl phosphate.[10] The other amines were commercial products.

Persulphate Oxidations.—The amines, in aqueous-acetone solution, were treated with a 10% excess of sodium or potassium persulphate in the presence of a 20% excess of aqueous sodium or potassium hydroxide as previously described,[4,5] except that with the tertiary amines the persulphate was added as a solid at the beginning of the oxidations. The mixtures were stirred at room temperature for 8 hr., kept overnight, filtered, and treated as described below.

2′-*Chloro*-4-*dimethylamino*-trans-*stilbene*. The solution obtained from the oxidation of the amine (10 g.) was evaporated to 200 ml. under reduced pressure, and the unchanged amine (5·2 g.) filtered off. The filtrate was washed with ether for 4 hr. and the aqueous phase evaporated to 25 ml. under reduced pressure. The solid was filtered off and recrystallised from water, to yield the *sodium salt* of the sulphuric ester in yellow plates (Found: after drying at 25°/0·5 mm: N, 3·7; Cl, 9·0; S, 7·95. $C_{16}H_{15}O_4NClSNa,H_2O$ requires N, 3·6; Cl, 9·0; S, 8·1%). The filtrate was acidified with 2N-sulphuric acid and extracted with ether (3 × 100 ml.), the ethereal solution was extracted with aqueous sodium hydrogen carbonate (2 × 5 ml.), and the aqueous solution was washed with ether and acidified with 2N-sulphuric acid. The solution was kept overnight at 0°, and the solid (23 mg.) filtered off and crystallised from water in needles, to yield *p*-dimethylaminobenzoic acid, m. p. 232—233° (lit.,[11] m. p. 236°). The filtrate was extracted with ether (3 × 50 ml.), and the ether was evaporated to leave a gummy solid which was extracted with boiling light petroleum (b. p. 80—100°). Removal of the solvent afforded a solid (230 mg.), m. p. 130—133°; after three recrystallisations from water it formed needles, m. p. 139—140°, undepressed in admixture with *o*-chlorobenzoic acid.

When a suspension of the sodium salt of the sulphuric ester in water was acidified with concentrated hydrochloric acid, a gum was formed which rapidly crystallised to yield the *sulphuric ester* of 2′-chloro-4-dimethylamino-3-hydroxy-*trans*-stilbene in needles (from aqueous ethanol), m. p. 220—224° (decomp.) (Found: C, 54·4; H, 4·9; N, 3·8; S, 8·7. $C_{16}H_{16}O_4NClS$ requires C, 54·5; H, 4·6; N, 4·0; S, 9·1%). Solutions of the ester had a bright blue fluorescence in ultraviolet light.

The ester (2 g.), suspended in water (50 ml.) and concentrated hydrochloric acid (10 ml.), was heated to 100° for 30 min. A slight excess of aqueous sodium hydrogen carbonate was added to the cooled solution, 2′-*chloro*-4-*dimethylamino*-3-*hydroxy*-trans-*stilbene* (1·25 g.) separating. From aqueous ethanol or from ethyl acetate it formed flat greenish needles, m. p. 137—138° (Found: C, 70·0; H, 6·1; N, 5·2. $C_{16}H_{16}ONCl$ requires C, 70·2; H, 5·9; N, 5·1%). On sublimation at 120—170°/0·2 mm. it formed colourless plates, m. p. 139—139·5°. The aminophenol had a bright blue fluorescence in ultraviolet light.

4-*Dimethylamino*-2′-*methyl*-trans-*stilbene*. When this amine (10 g.) was treated as described above, unchanged starting material (4·9 g.) was recovered. The crude sodium salt (3·6 g.) obtained was suspended in water (25 ml.) and acidified with 10N-sulphuric acid; the *sulphuric ester* of 4-dimethylamino-3-hydroxy-2′-methyl-*trans*-stilbene (3·2 g.) separated as a gum which crystallised; from aqueous ethanol it formed needles, m. p. 183—185° (Found: C, 61·3; H, 5·85; N, 4·4; S, 9·8. $C_{17}H_{19}O_4NS$ requires C, 61·2; H, 5·7; N, 4·2; S, 9·6%). The *sodium salt* (Found: N, 3·7; S, 8·6. $C_{17}H_{18}O_4NSNa$ requires N, 3·95; S, 9·0%) and *potassium salt* (Found: N, 3·85. $C_{17}H_{18}O_4NSK$ requires N, 3·8%) (prepared from the acid) separated from water in pale yellow feathery needles and yellow plates respectively. Solutions of the ester had a bright

[8] Haddow, Harris, Kon, and Roe, *Phil. Trans.*, 1948, *A*, **241**, 147.
[9] Bell and Kenyon, *J.*, 1926, 2709.
[10] Billman, Radike, and Mundy, *J. Amer. Chem. Soc.*, 1942, **64**, 2977.
[11] Harben and Freund, *Ber.*, 1909, **42**, 4815.

blue fluorescence in ultraviolet light. *o*-Toluic acid (165 mg.) was isolated from the mother-liquors from the oxidation in the way already described, separating from water in needles, m. p. and mixed m. p. 101—102°.

The ester (2 g.) was hydrolysed with concentrated hydrochloric acid, to yield 4-*dimethylamino*-3-*hydroxy*-2'-*methyl*-trans-*stilbene* (1·3 g.); from aqueous ethanol it formed light brown plates, m. p. 153·5—154·5° (Found: C, 80·3; H, 7·7; N, 5·7. $C_{17}H_{19}ON$ requires C, 80·6; H, 7·6; N, 5·5%). The aminophenol sublimed at 130°/0·2 mm., as colourless plates, m. p. 153·5—154·5°. Solutions of the aminophenol had a strong blue fluorescence in ultraviolet light.

4-*Dimethylaminoazobenzene*. The amine (10 g.) was treated with potassium persulphate in the usual manner. When the reaction mixture was evaporated to 200 ml. under reduced pressure, unchanged amine (9·2 g.) separated. The filtrate was washed with ether and evaporated to 10 ml. under reduced pressure, the *potassium salt* (510 mg.) of the sulphate ester separating. It crystallised from methanol–ether in orange plates and from water in golden-yellow plates (Found: C, 47·1; H, 4·2; N, 11·6; S, 8·9. $C_{14}H_{14}O_4N_3SK$ requires C, 46·8; H, 3·9; N, 11·7; S, 8·9%).

When the potassium salt was suspended in water and acidified with hydrochloric acid, the *sulphuric ester* of 4-dimethylamino-3-hydroxyazobenzene separated; from aqueous ethanol it formed red plates, decomp. 195—198° (Found: C, 52·7; H, 5·0; N, 13·1; S, 9·7. $C_{14}H_{15}O_4N_3S$ requires C, 52·3; H, 4·7; N, 13·1; S, 10·0%). Hydrolysis of the ester with hydrochloric acid as before afforded 4-*dimethylamino*-3-*hydroxyazobenzene*, separating from light petroleum (b. p. 80—100°) in feathery golden-yellow needles, m. p. 131—131·5° (Found: C, 69·8; H, 6·45; N, 17·6. $C_{14}H_{15}ON_3$ requires C, 69·7; H, 6·3; N, 17·4%).

4-*Dimethylaminodiphenyl*. 4-Dimethylaminodiphenyl (10 g.) was oxidised with sodium persulphate and the reaction mixture was treated in the usual manner. *Sodium* 4-*dimethylamino*-3-*diphenylyl sulphate* (3·3 g.) separated: from water it formed plates (Found: N, 4·6. $C_{14}H_{14}O_4NSNa$ requires N, 4·4%). When a suspension of the salt in water was acidified with concentrated hydrochloric acid 4-*dimethylamino*-3-*diphenylyl hydrogen sulphate* separated which crystallised from ethanol in elongated plates, m. p. 228—231° (decomp.) (Found: C, 57·2; H, 5·2; N, 4·7; S, 10·8. $C_{14}H_{15}O_4NS$ requires C, 57·3; H, 5·1; N, 4·8; S, 10·9%).

The ester (1·2 g.) was hydrolysed as before, to yield 4-*dimethylamino*-3-*hydroxydiphenyl* (0·85 g.), separating from aqueous ethanol in flat needles, m. p. 128—129° (Found: C, 78·6; H, 7·1; N, 6·7). $C_{14}H_{15}ON$ requires C, 78·8; H, 7·1; N, 6·6%).

NN-*Dimethyl*-1-*naphthylamine*. The amine (10 g.) was oxidised as before and the reaction mixture evaporated to 200 ml. under reduced pressure and washed with ether. The pH of the aqueous solution was adjusted to 4 with 2N-sulphuric acid, and the mixture was washed with ether; the brown gum, which first separated, crystallised. 1-*Dimethylamino*-2-*naphthyl hydrogen sulphate* (2·9 g.) was purified by dissolving it in aqueous sodium hydrogen carbonate and acidifying the solution with concentrated hydrochloric acid; it formed plates, m. p. 155—158° (decomp.) (Found: N, 5·25; S, 12·3. $C_{12}H_{13}O_4NS$ requires N, 5·2; S, 12·0%). The *sodium salt* (prepared from the acid) separated from ethanol–ether in pinkish plates (Found: N, 4·7; S, 11·1. $C_{12}H_{12}O_4NSNa$ requires N, 4·8; S, 11·1%). The ester was hydrolysed with hydrochloric acid in the usual manner, the solution was treated with a slight excess of sodium hydrogen carbonate, and the product was isolated with ether. 1-Dimethylamino-2-naphthol crystallised from aqueous ethanol in needles, m. p. 52—53° (lit.,[12] m. p. 54°) (Found: C, 76·6; H, 7·1; N, 7·5. Calc. for $C_{12}H_{13}ON$: C, 77·0; H, 7·0; N, 7·5%). The *benzoyl derivative* separated from aqueous ethanol in needles, m. p. 70—71° (Found: C, 77·9; H, 5·9; N, 4·8. $C_{19}H_{17}O_2N$ requires C, 78·3; H, 5·9; N, 4·8%).

NN-*Dimethyl*-2-*naphthylamine*. The amine (10 g.) was similarly oxidised, yielding 2-*dimethylamino*-1-*naphthyl hydrogen sulphate* (3·7 g.), plates (from 90% aqueous ethanol), m. p. 172—174° (softening at 152°) (Found: C, 53·9; H, 4·9; N, 5·3; S, 12·15. $C_{12}H_{13}O_4NS$ requires C, 53·9; H, 4·9; N, 5·2; S, 12·0%). The ester was hydrolysed with hydrochloric acid in the usual manner to yield a solid which was distilled in a microsublimation apparatus at 65°/0·1 mm. to yield plates, m. p. 33—34°, which rapidly decomposed in air and are presumed to be 2-dimethylamino-1-naphthol. The solid yielded a *benzoyl derivative* which separated from light petroleum (b. p. 80—100°) in pale orange needles, m. p. 111—113° (Found: C, 78·0; H, 5·8; N, 4·9. $C_{19}H_{17}O_2N$ requires C, 78·3; H, 5·9; N, 4·8%).

3 : 4 : 1-*Xylidine*. The amine (10 g.) was oxidised with sodium persulphate, and the reaction

[12] Haworth, Lamberton, and Woodcock, *J.*, 1947, 182.

mixture was treated in the usual manner and finally evaporated to dryness under reduced pressure. The residue was extracted with boiling methanol (3 × 100 ml.), and the combined extracts evaporated to small volume and kept at room temperature overnight. *Sodium 2-amino-4 : 5-dimethylphenyl sulphate* (4·2 g.) was obtained; from aqueous ethanol it formed flat, light brown needles (Found: N, 5·6. $C_8H_{10}O_4NSNa$ requires N, 5·9%). The *hydrogen sulphate* (obtained by acidification of an aqueous solution of the sodium salt) crystallised from ethanol in needles, m. p. 254—257° (decomp.) (Found: C, 44·1; H, 5·2; N, 6·4. $C_8H_{11}O_4NS$ requires C, 44·2; H, 5·1; N, 6·45%). The *potassium salt* (prepared from the acid) separated from aqueous ethanol in plates (Found: N, 5·6. $C_8H_{10}O_4NSK$ requires N, 5·5%).

Acid-hydrolysis of the ester (2 g.) yielded 2-amino-4 : 5-dimethylphenol (1·35 g.), separating from ether in light brown plates, m. p. 169—171° (Found: N, 10·6. Calc. for $C_8H_{11}ON$: N, 10·2%). The aminophenol was purified by sublimation at 170°/0·2 mm., forming colourless plates, m. p. 174—175°, which reddened in air (lit.,[13] m. p. 173—175°, becoming brown at 165°).

Sulphanilamide. Sulphanilamide (10 g.) was oxidised with potassium persulphate as before. The mixture was evaporated to 200 ml. under reduced pressure, filtered, washed with ether, and acidified to pH 3 with 2N-sulphuric acid. The coloured precipitate was separated, and the pH of the filtrate was adjusted to 7 with aqueous 2N-potassium hydroxide. The solution was evaporated to dryness under reduced pressure and the residue was extracted with boiling methanol (5 × 100 ml.). The combined extracts were evaporated to dryness, the residue was dissolved in a little water, and the solution was passed through a column of De-Acidite E ion-exchange resin (5 × 25 cm.), which absorbed most of the sulphuric ester together with some coloured material. The column was washed with methanol, and the ester eluted with aqueous 2N-ammonia (1 l.). The eluate was evaporated to dryness under reduced pressure, the residue was dissolved in boiling methanol (50 ml.), and coloured material was precipitated from the solution by means of ether (50 ml.). Excess of ether was added to precipitate the ammonium salt of the sulphate ester (3·75 g.), which separated as a gum which crystallised. Repeated recrystallisation of the product from methanol–ether afforded *ammonium 2-amino-p-sulphamoylphenyl sulphate* as reddish-brown needles (Found: C, 25·75; H, 3·9; N, 14·2; S, 22·6. $C_6H_{11}O_6N_3S_2$ requires C, 25·3; H, 3·9; N, 14·7; S, 22·5%).

When an aqueous solution of the ammonium salt was acidified with hydrochloric acid, *2-amino-p-sulphamoylphenyl hydrogen sulphate* separated. It was purified by dissolving it in aqueous sodium hydrogen carbonate and acidifying the solution; it formed pinkish plates, decomp. 245—248° (softening at 220°) (Found: C, 26·8; H, 3·2; N, 10·5. $C_6H_8O_6N_2S_2$ requires C, 26·9; H, 3·0; N, 10·4%). The ester separated from methanol–ether in pink solvated plates, m. p. 200—202° (decomp.) (Found, after drying at 100°/0·2 mm. for 4 hr.: C, 28·7, 28·8; H, 4·35, 4·0; N, 9·4, 9·1; S, 21·6. Calc. for $C_6H_8O_4N_2S_2,CH_3{\cdot}OH$: C, 29·0; H, 4·0; N, 9·3; S, 21·35%).

The ester was hydrolysed with hydrochloric acid as before and the solution was neutralised with sodium hydrogen carbonate and extracted with ether for 6 hr. The ether was removed and the residue crystallised from ethanol–chloroform, as described by Thorpe and Williams, in pinkish needles, m. p. 162—163°, undepressed on admixture with 4-amino-3-hydroxybenzenesulphonamide [14] (kindly supplied by Dr. W. V. Thorpe) (Found: N, 15·1. Calc. for $C_6H_8O_3N_2S$: N, 14·9%). When the aminophenol, in aqueous sodium hydroxide or in pyridine, was treated with an excess of benzoyl chloride a *tribenzoyl derivative* was formed, crystallising from aqueous ethanol in feathery needles, m. p. 222° (Found: C, 64·5; H, 4·0; N, 5·6. $C_{27}H_{20}O_6N_2S$ requires C, 64·8; H, 4·0; N, 5·6%); this (m. p. and mixed m. p. 222°) was also obtained when authentic 4-amino-3-hydroxybenzenesulphonamide was similarly treated (Found: C, 64·5; H, 4·1; N, 5·9%).

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[13] Crossley and Bartlett, *J.*, 1913, 1299.
[14] Thorpe and Williams, *Biochem. J.*, 1941, **35**, 61.