173. *The Complex-formation and Rearrangement of* p-*Hydroxylaminobenzenesulphonamide.*

By H. Burton and N. Walker.

Confirmatory evidence has been obtained to show that *p*-hydroxylaminobenzenesulphonamide and sulphanilamide form a complex, m. p. 161°. The rearrangement of *p*-hydroxylaminobenzenesulphonamide in dilute acid solution to *p*-aminophenol and azoxybenzene-4 : 4′-disulphonamide has been carried out.

The recent work of Sevag (*J. Amer. Chem. Soc.*, **1943**, **65**, 110) has considerably clarified the confusion which had arisen regarding the identity of *p*-hydroxylaminobenzenesulphonamide, of which two crystalline forms with different m. p.'s had been reported by various workers (Mayer, *Biol. méd. suppl.*, **1937**, **27**, 45; Bratton, White, and Marshall, *Proc. Soc. Exp. Biol. Med.*, **1939**, **42**, 847; Burton, *Chem. and Ind.*, **1941**, **60**, 449; Sevag and Shelburne, *J. Bact.*, **1942**, **43**, 421). On the grounds of differences in solubilities and salt-forming properties, elementary analysis, oxygen consumption, and the colorimetric estimation of sulphanilamide by Ehrlich's reagent after removal of hydroxylamine by oxidation, Sevag concludes that the form with m. p. 161·5° is a complex of two molecules of *p*-hydroxylaminobenzenesulphonamide with one molecule of sulphanilamide, and that the form, m. p. 140°, is pure *p*-hydroxylaminobenzenesulphonamide. We have obtained additional evidence which supports this constitution for the higher-melting form, namely, the isolation of sulphanilamide after removal of *p*-hydroxylaminobenzenesulphonamide by air oxidation, the isolation of *N*-acetyl-*p*-hydroxylaminobenzenesulphonamide and of N^4-acetylsulphanilamide from the product obtained by acetylating the

higher-melting form, and by the synthesis of the higher-melting complex by crystallisation of a mixture of one molecule of sulphanilamide with two molecules of *p*-hydroxylaminobenzenesulphonamide from warm water.

Since Mayer (*loc cit*) put forward the view that the bacteriostatic action of sulphanilamide is due to its oxidation to *p*-hydroxylaminobenzenesulphonamide various workers have investigated possible oxidation products of sulphanilamide. Thorpe, Williams, and Shelswell (*Biochem. J.*, 1941, **35**, 52) reject James' claim (*ibid.*, 1940, **34**, 640) to have isolated *p*-hydroxylaminobenzenesulphonic acid and acetyl-*p*-hydroxylaminobenzenesulphonamide from urine and suggest that the most likely oxidation products of sulphanilamide to be produced *in vivo* are 4-amino-3-hydroxybenzenesulphonamide and 4-amino-2-hydroxybenzenesulphonamide; further, they state : " If the formation of an *o*- or *m*-aminophenolsulphonamide could be proved it would argue against the correctness of the hydroxylamino hypothesis, in that there is no example in the literature of the transformation of a hydroxylamine into an *o*- or *m*-aminophenol." The latter statement is only partially correct (see Bamberger and Laguth, *Ber.*, 1898, **31**, 1500; Bamberger, *Ber.*, 1900, **33**, 3600). Thorpe *et al.* (*loc. cit.*) also found that if urines, from rabbits which had been relatively highly dosed with sulphanilamide, were boiled with dilute hydrochloric acid for a few minutes, a diazo-reaction after acetylation was no longer given (a solution of *p*-hydroxylaminobenzenesulphonamide still gave the acetylated diazo-reaction after boiling with hydrochloric acid under similar conditions) and attribute this result to an *o*-aminophenol derivative, *e.g.*, 4-amino-3-hydroxybenzenesulphonamide. We have found, however, that *p*-hydroxylaminobenzenesulphonamide, on heating with dilute sulphuric or hydrochloric acid in an atmosphere of carbon dioxide on the water-bath, is converted into azoxybenzene-4 : 4′-disulphonamide and *p*-aminophenol. Thus, the above argument used by Thorpe *et al.* (*loc. cit.*) against the correctness of the hydroxylamine hypothesis of sulphanilamide oxidation is shown not to be a valid one and this possibility must be considered as still open. This result also amplifies the statement of Bratton *et al.* (*loc. cit.*) that in acid solution *p*-hydroxylaminobenzenesulphonamide is fairly stable and apparently goes only into the azoxy-compound; in neutral or alkaline solution decomposition is accelerated and both the azoxy-compound and sulphanilamide are formed. No *p*-aminophenol was detected by the indophenol test by the present authors when the higher-melting complex in faintly alkaline solution was oxidised by air at room temperature.

Experimental.

Microanalyses were carried out by Dr. G. Weiler and Dr. F. B. Strauss of Oxford.

The " complex," m. p. 161·5°, of *p*-hydroxylaminobenzenesulphonamide and sulphanilamide was prepared by Burton's method (*loc. cit.*). *p*-Hydroxylaminobenzenesulphonamide prepared as described by Bratton, White, and Marshall had m. p. 140°, unchanged on recrystallisation from air-free water (Found : C, 38·4; H, 4·5; N, 14·4, 14·8. Calc. for $C_6H_8O_3N_2S$: C, 38·3; H, 4·25; N, 14·9%). We found, however, that the first-named method sometimes gave the hydroxylamine, m. p. 140°, and that the second method occasionally afforded the " complex."

Acetylation of the " Complex."—The " complex " (1·77 g.) and acetic anhydride (1·1 g.), stirred at room temperature, set to a hard white mass. The product was dissolved in hot methyl alcohol (25 ml.); the filtered solution, on cooling, deposited crystalline *N*-acetyl-*p*-hydroxylaminobenzenesulphonamide (0·51 g.), m. p. 220° (decomp.). After recrystallisation from aqueous alcohol it formed small colourless plates, m. p. 224° (decomp.) (Found : C, 41·8; H, 4·4; N, 12·25; S, 14·3. Calc. for $C_8H_{10}O_4N_2S$: C, 41·7; H, 4·35; N, 12·2; S, 13·9%). It gave an intense blood-red colour with dilute ferric chloride solution, as stated by Bratton *et al.* (*loc. cit.*). The methyl-alcoholic filtrate, evaporated to dryness, left a solid (1·45 g.), m. p. 140—160°, which was fractionally crystallised twice from methyl alcohol and finally fractionated from water; after filtration of a little *N*-acetyl-*p*-hydroxylaminobenzenesulphonamide, long colourless needles of N^4-acetylsulphanilamide (4—5 mg.), m. p. 216—217°, not depressed by an authentic specimen, were obtained.

Oxidation of the " Complex " by Air.—The " complex " (0·5 g.) was dissolved in water (25 ml.), 3 drops of concentrated aqueous ammonia added, and air aspirated through the solution for 3 hours at room temperature. Azoxybenzene-4 : 4′-disulphonamide (0·238 g.) was removed and the filtrate, which gave no reaction with cold, ammoniacal silver nitrate and did not show an indophenol reaction, was evaporated to dryness, yielding a residue (0·215 g.), m. p. 155—158°. After twice recrystallising from water, sulphanilamide, m. p. 163—164°, not depressed by an authentic specimen, was obtained.

Synthesis of the " Complex."—*p*-Hydroxylaminobenzenesulphonamide (0·056 g.), m. p. 141—142°, and sulphanilamide (0·025 g.) were placed in the hollow stopper of a Thunberg tube, water (3 ml.) was put in the tube, and it was then evacuated and closed. On warming and mixing the contents of the tube, a clear solution was obtained. When this was cooled in ice, colourless crystals of the " complex " separated, m. p. 161—162°, depressed by sulphanilamide but not by the complex prepared by Burton's method.

Rearrangement of the " Complex."—A solution of the " complex " (3 g.) in air-free water (80 ml.) and concentrated sulphuric acid (4 g.) through which carbon dioxide was slowly passing was heated on the steam-bath for 6 hours. The mixture was cooled, and azoxybenzene-4 : 4′-disulphonamide (0·7 g.), m. p. 295° (decomp.), removed. The cold filtrate was made neutral to Congo-red paper with solid sodium hydrogen carbonate; after a few minutes, nearly pure sulphanilamide (0·47 g.) separated, m. p. 158—160°, and, after recrystallisation from water, m. p. 164°, not depressed by an authentic specimen. The filtrate from the sulphanilamide, on treatment with potassium hydroxide (3 g.) and benzoyl chloride (3 ml.), gave a solid, which was collected, washed with water, and crystallised from alcohol, giving nearly colourless, small plates of *ON*-dibenzoyl-*p*-aminophenol (0·11 g.), m. p. 230—231°, raised to 232° by recrystallisation from methyl alcohol and not depressed by an authentic specimen. The rearrangement was also carried out with 5% hydrochloric acid in place of sulphuric acid and the same products were obtained.

Rearrangement of p-*Hydroxylaminobenzenesulphonamide.*—This (0·5 g.), m. p. 140°, in air-free water (20 ml.) and concentrated sulphuric acid (1 g.) was treated as was the complex in the preceding experiment. The filtrate from the azoxybenzene-4 : 4′-disulphonamide (0·185 g.), m. p. 300° (decomp.), was neutralised with solid sodium hydrogen carbonate and cooled in ice. Nothing separated, but on shaking with sodium hydroxide (2 g.) and benzoyl chloride (2 g.) *ON*-dibenzoyl-*p*-aminophenol (0·072 g.) was obtained; after crystallisation from absolute alcohol it had m. p. 230—231°, not depressed by an authentic specimen.

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