

173. *The Complex-formation and Rearrangement of p-Hydroxylaminobenzene-sulphonamide.*

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Confirmatory evidence has been obtained to show that *p*-hydroxylaminobenzenesulphonamide and sulph-anilamide 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 C₆H₈O₃N₂S : 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 C₈H₁₀O₄N₂S : 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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