The Constitution of Hydroxysulphapyridine and Synthesis of 2-Aminohydroxypyridines

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(Received 9 November 1949)

Hydroxysulphapyridine was isolated as a metabolite of sulphapyridine in the dog by Scudi (1940, 1944) and by Weber, Lalich & Major (1943). In an investigation of the metabolism of sulphapyridine in the rabbit, we obtained a compound which appeared to be identical with the hydroxysulphapyridine produced by the dog (Bray, Neale & Thorpe, 1946). The constitution was not determined, but evidence was obtained that one hydroxyl group had been introduced. Scudi (1944) had suggested that this occupied either position 3 in the pyridine ring or position 2 in the benzene ring. Since, however, hydrolysis of hydroxysulphapyridine gave sulph-anilic acid and a compound believed to be an aminohydroxypyridine it was more reasonable to infer that the hydroxyl was attached to the pyridine ring (Weber *et al.* 1943; Bray *et al.* 1946). In the expectation that hydroxylation might occur in position 5, we synthesized a compound which the literature led us to believe was 2-amino-5-hydroxypyridine. Since this compound was different from that obtained from the rabbit we attempted to synthesize the other three isomers. This investigation revealed that the so-called '2:5'-compound was really the 2:3-isomer. Of the four possible isomers two, 2-amino-3-hydroxypyridine and 2-amino-6-hydroxypyridine, have been obtained by routes which leave little doubt of their configuration. We have not succeeded in preparing 2-amino-5-hydroxypyridine, although we have obtained strong evidence that it does indeed form the aminohydroxypyridine moiety of hydroxysulphapyridine. Since we met with considerable difficulty in the preparation of aminohydroxypyridines and found some discrepancies in the literature, we include in this report a brief summary of some of the syntheses carried out.

EXPERIMENTAL

Synthesis of aminohydroxypyridines

2-Amino-3-hydroxypyridine was prepared by three separate methods.

Method 1. 8-Hydroxyquinoline was oxidized with fuming HNO_3 to yield quinolinic acid, m.p. 190° (decomp.), from which quinolinimide was obtained by treatment with acetic anhydride and acetamide (Sucharda, 1925). The imide, with NaClO , gave 3-aminopyridine-2-carboxylic acid (3-aminopicolinic acid), m.p. 214° , and 2-aminopyridine-3-carboxylic acid (2-aminonicotinic acid) which were separated, the

latter being less soluble in acid solution. 3-Aminopicolinic acid was converted to 3-hydroxypicolinic acid, m.p. $218-220^\circ$, by decomposition of the diazonium salt (Kirpal, 1908) and thence via the methyl ester (Camps, 1902) to 3-hydroxypicolinamide which formed colourless needles, m.p. 194° , and gave a red colour with FeCl_3 . (Found: N, 20.2; $\text{C}_6\text{H}_6\text{O}_2\text{N}_2$ requires N, 20.3%.) This amide on treatment with NaBrO gave 2-amino-3-hydroxypyridine, m.p. $169-170^\circ$, Plazek & Rodewald (1936) give m.p. $164-168^\circ$. (Found: N, 25.2. Calc. for $\text{C}_6\text{H}_6\text{ON}_2$: N, 25.5%.) Yield, 2 g. from 50 g. of 8-hydroxyquinoline. 2-Amino-3-hydroxypyridine gave a purple coloration with FeCl_3 , a negative diazo reaction, and no colour with diazotized 2:5-diaminopyridine in alkaline (NaHCO_3) solution. It formed a picrate, m.p. 257° (decomp.).

Method 2. Pyridine was sulphonated with fuming H_2SO_4 ($2\text{HgO} \cdot \text{HgSO}_4$ catalyst) (cf. Wulff, 1932*a*), and the resulting 3-sulphonate was fused with KOH to give 3-hydroxypyridine, m.p. $127-129^\circ$ (cf. Wulff, 1932*b*). This was nitrated (I. G. Farbenindustrie, 1933). According to Maier-Bode & Altpeter (1934) the product, m.p. $68-69^\circ$, is 6-nitro-3-hydroxypyridine. Plazek & Rodewald (1936), however, state that the product is 2-nitro-3-hydroxypyridine. We concur, since our product, m.p. $67-68^\circ$, on reduction with SnCl_2 and HCl gave 2-amino-3-hydroxypyridine, identical with that prepared by Method 1. Yield, 5 g. from 50 g. of 3-hydroxypyridine (from 133 g. of pyridine).

Method 3. Pyridine was brominated (Englert & McElvain, 1929) and the resulting 3-bromopyridine (b.p. $168-172^\circ$) treated with Na ethoxide (Weidel & Blau, 1885). The product, 3-ethoxypyridine (b.p. $190-192^\circ$), on nitration gave 2-nitro-3-ethoxypyridine (m.p. $31-32^\circ$). This was reduced to 2-amino-3-ethoxypyridine (m.p. $80-81^\circ$), which was de-ethylated by means of HBr and acetic acid at 130° to give 2-amino-3-hydroxypyridine, identical with that given by Methods 1 and 2. Yield, 5 g. from 50 g. of 3-bromopyridine (from 250 g. of pyridine). The procedure in the later stages was essentially that of Koenigs, Gerdes & Sirot (1928). It will be evident from our later experiments that 2-amino-5-hydroxypyridine may be formed in traces as a by-product in this synthesis.

2-Amino-4-hydroxypyridine. Attempts at the synthesis of this compound by the route picolinic acid $\rightarrow$ 4-chloropicolinic acid $\rightarrow$ 4-aminopicolinic acid $\rightarrow$ 4-hydroxypicolinic acid and thence by Hofmann degradation of the amide to 2-amino-4-hydroxypyridine were unsuccessful. In view of the evidence for the identity of the biosynthetic aminohydroxypyridine as the 2:5-isomer further syntheses of the 2:4-isomer were not attempted.

2-Amino-5-hydroxypyridine. Numerous unsuccessful attempts were made to prepare this compound. Two of these are referred to under Methods 2 and 3 for 2-amino-3-hydroxypyridine. Further methods tried included (a) via the benzene-azo compound from 3-hydroxypyridine and benzene

diazonium chloride, (b) from 2-amino-5-chloro-(or-bromo)-pyridine, (c) from 5-hydroxy- α -picoline, (d) from 2-amino-pyridine-5-sulphonic acid, (e) from 2:5-diaminopyridine, and (f) from 2-chloro-5-nitropyridine. Theoretically it would be expected that nitration of 3-ethoxypyridine which as already shown (Method 3 above) gives 2-nitro-3-ethoxypyridine, would result in at least some nitration in the para position. The preparation of 2-amino-3-hydroxypyridine by Method 3 was accordingly repeated but without any purification or recrystallization at any stage. The crude aminohydroxypyridine hydrobromide thus obtained was dissolved in absolute ethanol and the solution poured into an excess of ether, from which the solid hydrobromide crystallized. This was separated and dissolved in a small volume of water. The solution, after neutralization with NaHCO_3 , was continuously extracted with ether to give fractions A (ether-soluble material) and B (residual liquid). The original ethereal mother liquors were evaporated to give a small amount of syrup (fraction C). The composition of these fractions was investigated by means of paper

requires N, 9.2%). It was converted to the *amide*, white needles, m.p. 250°, which gave a bright orange colour with FeCl_3 . (Found: N, 20.1; $\text{C}_6\text{H}_6\text{O}_2\text{N}$ requires N, 20.3%). Yield, 1.1 g. from 50 g. of saccharic acid. Treatment of the amide with NaBrO gave a poor yield of 2-amino-6-hydroxypyridine (m.p. 198–200°). This was identical with the base prepared from a specimen of 2-amino-6-hydroxypyridine sulphate kindly given to us by Dr A. J. Ewins. The compound formed a picrate, m.p. 191° (decomp.), gave a purple coloration with FeCl_3 and a bright orange-yellow colour with diazotized 2:5-diaminopyridine in alkaline solution.

RESULTS AND DISCUSSION

Comparison of synthetic aminohydroxypyridines with that from hydroxysulphapyridine

Some properties of the aminohydroxypyridines obtained and those of the compound from hydroxysulphapyridine are recorded in Table 1. The R_F

Table 1. *Comparison of aminohydroxypyridines*

	M.p. (°)	M.p. of picrate (°)	Colour with FeCl_3	Dichloro- quinonechloro- imide test	Colour on coupling with diazotized 2:5-diamino- pyridine	R_F value (petroleum- butanol)
2-Amino-3-hydroxypyridine	169–170	257	Purple	Deep blue	None	0.68
2-Amino-6-hydroxypyridine	198–200	191	Purple	Deep blue	Orange	0.63
2-Amino-?-hydroxypyridine from hydroxysulphapyridine	34	218	Purple	Colourless	Red	0.22
?-Amino-5-hydroxypyridine from Method 3	—	—	Purple	Colourless	Red	0.22
2-Aminopyridine-1-oxide* (2-imino-1-hydroxy-1:2- dihydropyridine)	161	Unstable	Intense pure blue	—	—	—

* Properties given by Newbold & Spring (1949).

chromatography using a mixture of petroleum, b.p. 90–100° (1 vol.), *n*-butanol (2 vol.) and water (3 vol.) as developing solvent and FeCl_3 for detection. (For apparatus and procedure see Bray, Thorpe & White, 1950.) Compounds with the following R_F values and FeCl_3 colours (in parentheses) were obtained: fraction A, 0.9 (brown) and 0.68 (purple); fraction B, 0.68 (purple) and 0.22 (purple); fraction C, 0.68 (purple) in very small amount.

The compound with R_F value 0.68 was shown to be 2-amino-3-hydroxypyridine, the major product of the reaction. The faster moving compound has not been identified, but the compound with R_F value 0.22 appeared to have properties identical with those of the aminohydroxypyridine from hydroxysulphapyridine. As will be seen later, there is good reason to believe this compound is 2-amino-5-hydroxypyridine. The amounts formed, however, were so small as to leave little hope of isolation of the compound by this method.

2-Amino-6-hydroxypyridine. This was prepared from saccharic acid which, on treatment with HBr , gave dehydromucic acid (Phelps & Hale, 1901). This on reduction with Na amalgam gave dihydrofuran-2:5-dicarboxylic acid (Hill & Wheeler, 1901), which was converted to 2-hydroxypyridine-6-carboxylic acid (6-hydroxypicolinic acid), m.p. 275–278° (decomp.), by autoclaving with NH_4Br at 160–170° (Fischer, Hess & Stahlschmidt, 1912). The *methyl ester* formed white needles, m.p. 80°. (Found: N, 9.1; $\text{C}_7\text{H}_7\text{O}_3\text{N}$

values were determined using the mixture of petroleum, b.p. 90–100°, *n*-butanol and water already mentioned. In addition to the aminohydroxypyridines there is another theoretically possible oxidation product which would result from addition of one atom of oxygen to 2-aminopyridine. Some properties of this compound, 2-aminopyridine-1-oxide (2-amino-1-hydroxy-1:2-dihydropyridine), which has recently been prepared by Newbold & Spring (1949) are also included in Table 1. It is clear that the compound from hydroxysulphapyridine is not 2-amino-3-hydroxypyridine, 2-amino-6-hydroxypyridine or 2-aminopyridine-1-oxide. The negative dichloroquinonechloroimide test would be expected if the hydroxyl were in position 5 since this reagent does not usually give a blue colour with phenols in which the position para to the hydroxyl is substituted (Gibbs, 1927). Scudi's (1944) choice of position 3 as the point of hydroxylation was based upon the observation that hydroxysulphapyridine gave a positive test. We confirm that hydroxysulphapyridine gives a colour with the reagent, but the aminohydroxypyridine obtained from it by hydrolysis does not. In our opinion the colour given by hydroxy-

sulphapyridine is not a typical indophenol colour. It is comparatively feeble in intensity, and the blue colour soon turns purple.

The similarity in the properties of the aminohydroxypyridine from hydroxysulphapyridine and that formed in traces by Method 3 provides evidence that these compounds are in fact 2-amino-5-hydroxypyridine. The only possible isomers which can be formed both by hydroxylation of 2-aminopyridine and by amination (via nitration) of 3-ethoxypyridine are 2-amino-3-hydroxypyridine and 2-amino-5-hydroxypyridine. The compound formed in the rabbit and as a trace product from 3-ethoxypyridine is certainly not 2-amino-3-hydroxypyridine. It is highly probable, therefore, that the pyridine moiety of hydroxysulphapyridine is 2-amino-5-hydroxypyridine and that the constitution of hydroxysulphapyridine is 2-(*p*-aminobenzenesulphonamido)-5-hydroxypyridine.

The configuration of the aminohydroxypyridine of Koenigs, Gerdes & Sirot (1928)

The three syntheses of 2-amino-3-hydroxypyridine render untenable the contention of Koenigs *et al.* (1928) and of Maier-Bode & Altpeter (1934) that 2-amino-5-hydroxypyridine was prepared from the mononitro derivatives of 3-ethoxy- or 3-hydroxypyridine. The evidence of Koenigs *et al.* for the position of the nitro group introduced into 3-ethoxypyridine was the conversion of the resulting aminohydroxypyridine into a dihydroxypyridine which was identical with that prepared by Kudernatsch (1897) by fusion of 3-hydroxypyridine with sodium hydroxide. Kudernatsch's evidence for the 2:5 configuration of this compound was that it did not give the reactions expected of 2:3-dihydroxypyridine, which was then unknown. Plazek & Rodewald (1936) maintained that nitration of 3-hydroxypyridine resulted in the entry of the nitro group into position 2 and not 6, as stated by Maier-

Bode & Altpeter (1934), since the aminohydroxypyridine obtained by reduction formed a phenoxazine derivative with picryl chloride. The compounds which we obtained by the three methods described in the Experimental section were identical. The synthesis from quinolinic acid, and the fact that Methods 2 and 3 involved nitration of pyridines substituted in position 3, leave no doubt that this compound is 2-amino-3-hydroxypyridine. The claims that Methods 2 and 3 lead mainly to 2-amino-5-hydroxypyridine cannot, therefore, be accepted. The compounds derived from the mononitro derivatives of 3-ethoxy- and 3-hydroxypyridine to which Koenigs *et al.* (1928) and Maier-Bode & Altpeter (1934) attributed the 2:5 configuration were actually 2:3 substituted pyridines.

SUMMARY

1. An attempt has been made to identify the aminohydroxypyridine moiety of hydroxysulphapyridine, the metabolite of sulphapyridine in the rabbit.

2. 2-Amino-6-hydroxy- and 2-amino-3-hydroxypyridines have been synthesized and shown to differ from the biosynthetic compound.

3. Unsuccessful attempts were made to prepare 2-amino-4-hydroxy- and 2-amino-5-hydroxypyridine, although evidence was obtained that the latter is a minor product in the preparation of the 2:3-isomer. Claims by earlier workers to have prepared the 2:5-isomer were shown to refer to the 2:3-isomer.

4. Evidence has been obtained that the biosynthetic product is 2-amino-5-hydroxypyridine and hence hydroxysulphapyridine is 2-(*p*-aminobenzenesulphonamido)-5-hydroxypyridine.

We are indebted to the Royal Society for a Government Grant which defrayed part of the cost of this work. Some of the micro-analyses were carried out by Drs Weiler and Strauss, Oxford.

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