[CONTRIBUTION NO. 150 FROM THE CANCER RESEARCH INSTITUTE, NEW ENGLAND DEACONESS HOSPITAL]

The Elbs Peroxydisulfate Oxidation in the Pyridine Series: a New Synthesis of 2,5-Dihydroxypyridine[1]

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RECEIVED FEBRUARY 18, 1958

The Elbs peroxydisulfate oxidation has been applied to 2- and 3-hydroxypyridine. In each case 2,5-dihydroxypyridine is the major product isolated following hydrolysis of the sulfate esters. Small amounts of the *o*-dihydroxypyridines were isolated as well, 2-hydroxypyridine yielding 2,3-dihydroxypyridine, and 3-hydroxypyridine yielding both 2,3- and 3,4-dihydroxypyridines. The degradation of the synthetic 2,5-dihydroxypyridine by cell-free extracts of *Pseudomonas fluorescens* is described.

The recent finding that 2,5-dihydroxypyridine (III) is an intermediate in the oxidation of nicotinic acid by a strain of *Pseudomonas fluorescens*[3] has made a facile synthesis of this compound desirable. Two synthetic routes already have been reported, both involving lengthy procedures. A seven-step synthesis from 2-aminopyridine[4] and an eight-step route from 3-bromopyridine[5] have been described, while the old report[6] of the conversion of 3-hydroxypyridine (II) to 2,5-dihydroxypyridine by alkali fusion has since been shown to yield instead the 2,3-isomer IV.[7]

The application of the Elbs peroxydisulfate hydroxylation reaction[8] offered a direct approach to the desired compound from readily available starting materials. In this reaction, the monosulfate ester of the diphenol is the initial product, acid hydrolysis yielding the diphenol itself. We have found that 2,5-dihydroxypyridine is easily preparable by the Elbs reaction from either 2-hydroxypyridine (I) or 3-hydroxypyridine. Further study of these oxidations has shown that small amounts of the *o*-isomers also are produced according to the scheme of Fig. 1.

The major product in the Elbs oxidation of either 2- or 3-hydroxypyridine was the 2,5-isomer which was isolated easily from both reaction mixtures by virtue of its lower solubility. The 2,3-isomer was isolated from the 2-hydroxypyridine oxidation mixture following removal of III. Both 2,3- and 3,4-dihydroxypyridines were isolated from the mother liquors after removal of III in the oxidation of 3-hydroxypyridine.

The 2,5-dihydroxypyridine produced by the present reaction was subjected to oxidation and degradation by enzyme preparations from *P. fluorescens*, strain N-9; the end-products of the reaction were qualitatively and quantitatively identical to those demonstrated using the biologically synthesized dihydroxypyridine.[3]

(1) This investigation was supported in part by the United States Atomic Energy Commission Contract No. AT (30-1)-901 with the New England Deaconess Hospital.

(2) Postdoctoral Research Fellow of the National Cancer Institute, U. S. Public Health Service.

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(4) R. Adams and T. R. Govindachari, THIS JOURNAL, **69**, 1806 (1947).

(5) H. J. den Hertog, J. P. Wibaut, F. R. Schepman and A. A. Van der Wal, *Rec. trav. chim.*, **69**, 700 (1950). This paper reports syntheses and spectra of all the dihydroxypyridines.

(6) R. Kudernatsch, *Monatsh.*, **18**, 613 (1897).

(7) O. v. Shickh, A. Binz and A. Schulz, *Ber.*, **69**, 2593 (1936).

(8) K. Elbs, *J. prakt. Chem.*, [2] **48**, 179 (1893).

Experimental

Oxidation of 2-Hydroxypyridine: 2,5-Dihydroxypyridine (III).—Thirty-eight grams (0.4 mole) of I (Aldrich Chemical Co.) and 80 g. (2.0 moles) of sodium hydroxide were dissolved in 1500 ml. of water. The solution was cooled to 5° in an ice-bath, 2 g. of ferrous sulfate[9] dissolved in 20 ml. of water was added, and then 135 g. (0.5 mole) of potassium peroxydisulfate was added all at once. The mixture was stirred for 20 hours while the temperature was allowed to rise slowly to 20°. At this time, the phenol concentration (following hydrolysis) had reached a maximum value, as judged by the assay procedure previously described.[3]

The reaction mixture was filtered by gravity, cooled and brought to *p*H 0.75 by the addition of concentrated sulfuric acid. The acidified mixture was then hydrolyzed at 100° under a nitrogen atmosphere for 30 minutes. The red-brown hydrolysate was cooled in an ice-bath and brought to *p*H 6.5 with 10 *N* sodium hydroxide (under nitrogen), then evaporated to dryness *in vacuo*, and finally dried over phosphorus pentoxide. The salt cake was extracted with

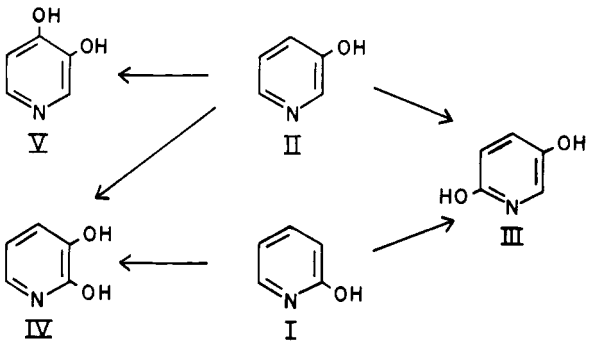

Fig. 1.

isopropyl alcohol in a Soxhlet apparatus until the effluent no longer gave the phenol test. The isopropyl alcohol extract was decolorized with charcoal and then concentrated under nitrogen until crystals began to form. After standing overnight at −10°, the solution deposited 19 g. (42%) of crude III which, after two recrystallizations from ethanol, gave 8 g. of nearly colorless crystals darkening at 230° and decomposing between 250–260° without melting. Additional material may be obtained by working up the mother liquors.

Anal. Calcd. for $C_5H_5NO_2$: C, 54.05; H, 4.54; N, 12.6. Found: C, 54.1; H, 4.7; N, 12.3.

Quantitative measurement of the Folin–Ciocalteu reaction with 0.1 μmole of synthetic III gave $O.D._{760\ m\mu}$ 0.160 as compared with $O.D._{760\ m\mu}$ 0.170 for the biologically prepared compound.[3] The color with the ferric chloride reagent was pinkish-red as reported,[4,5] with an absorption maximum at 510 mμ (ε 488). The spectrum of the compound in water and in 0.1 *N* HCl showed two maxima: $\epsilon^{water}_{320\ m\mu}$ 5620, $\epsilon^{water}_{230.5\ m\mu}$ 7390, $\epsilon^{HCl}_{307\ m\mu}$ 6000, $\epsilon^{HCl}_{224\ m\mu}$ 6100. The diacetyl derivative was prepared according to den Hertog, *et al.*,[5] and gave m.p. 69.5–70° after recrystallization from hexane. The reported[5] melting point is 68°.

2,3-Dihydroxypyridine (IV).—After filtering off III, the isopropyl alcohol mother liquor was concentrated to dryness

(9) F. Mauthner, *ibid.*, **156**, 150 (1940).

and the residue triturated with ethyl acetate. The ethyl acetate extract was then concentrated, diluted with isopropyl alcohol, decolorized with charcoal, and allowed to crystallize. Recrystallization gave a mixture of crystals of III and IV. The mother liquor on standing deposited crystals of IV giving the same ultraviolet spectrum and ferric chloride reaction as IV isolated from the oxidation of II.

Chromatography of the isopropyl alcohol extract of the salt cake in the 1-butanol–hydrochloric acid–water system previously described[3] showed only two spots reacting with ferric chloride which corresponded to III and IV. Hence, it was possible to calculate the ratio of the two isomers in this extract from the spectra of the ferric chloride complexes of the pure compounds. An appropriate aliquot of the isopropyl alcohol extract was treated with the ferric chloride reagent and the optical density at 510 and 700 mμ. determined. The concentration of IV is given by O.D. 700 mμ/1140, while that of III is given by O.D. 510 mμ − [IV]1090/488. The ratio of the *p*-isomer to the *o*-isomer was 11.5:1.

Oxidation of 3-Hydroxypyridine: 2,5-Dihydroxypyridine (III).—Potassium peroxydisulfate (0.19 mole, 51 g.) was added all at once to a cooled solution of 14.2 g. (0.15 mole) of II (Aldrich Chem. Co.) in 500 ml. of 2 *N* sodium hydroxide. The mixture was stirred for two hours at 5°, and the stirring continued for an additional two hours with the cooling bath removed. The mixture was allowed to stand at room temperature for 16 hours and was then adjusted to *p*H 1 with sulfuric acid and refluxed for an hour to hydrolyze the sulfate esters. The cooled solution was adjusted to *p*H 6.5 with 10 *N* sodium hydroxide and then worked up as described above for the reaction with I. The isopropyl alcohol extract yielded 1.9 g. (11%) of III.

2,3- and 3,4-Dihydroxypyridines (IV and V).—After removal of III, an aliquot of the mother liquor was chromatographed in the butanol–hydrochloric acid–water solvent system. The paper chromatograms showed the presence of three compounds reacting with ferric chloride (Table I).

TABLE I

Dihydroxypyridine	Color with $FeCl_3$	R_f	Ultraviolet fluorescence
2,5-	Pinkish-red	0.89	Blue-white
2,3-	Blue	.81	None
3,4-	Purple	.69	None

The mother liquor was concentrated to dryness at reduced pressure and yielded 6.4 g. of residue. This was extracted with 200 ml. of boiling ethyl acetate in two portions. The ethyl acetate-insoluble residue gave the pinkish-red ferric chloride test characteristic of III. On concentration of the ethyl acetate extract, 2.0 g. of unreacted II was recovered. The ethyl acetate solution then was allowed to crystallize at 5° for several days during which time it deposited II together with both IV and V. The major portion of II was separated from the dihydroxypyridines by warming the mixture and decanting the solution of II from the less soluble residue of dihydroxypyridines. The residue was then dissolved in isopropyl alcohol and treated with charcoal. On standing, it deposited 100 mg. of crystals of crude IV which after recrystallization melted at 252–255° dec. The compound showed two maxima in both water and 0.1 *N* HCl: $\epsilon^{water}_{297\,m\mu}$ 8150, $\epsilon^{water}_{233\,m\mu}$ 4900, $\epsilon^{HCl}_{295\,m\mu}$ 8150, $\epsilon^{HCl}_{233\,m\mu}$ 4600.

Anal. Calcd. for $C_5H_5NO_2$: C, 54.05; H, 4.54; N, 12.6. Found: C, 53.7; H, 4.7; N, 12.1.

This product gave a blue color with the ferric chloride reagent showing a maximum at 595–600 mμ (ε 1755). It gave a blue color with the reagent of Folin and Ciocalteu, 0.1 μmole producing an optical density at 760 mμ of 0.160.

After concentration and treatment with benzene, the isopropyl alcohol mother liquor deposited mixed crystals of IV and V. Addition of hexane to this final mother liquor yielded 5 mg. of crystals of V, m.p. 228–235° dec. The spectrum of this material agreed with that obtained from the elution of the R_f 0.69 material from the chromatogram and with the spectrum reported by den Hertog, *et al.*,[5] showing one maximum in water and two in 0.1 *N* HCl; $\epsilon^{water}_{273\,m\mu}$ 10,100, $\epsilon^{HCl}_{268\,m\mu}$ 5130, $\epsilon^{HCl}_{241\,m\mu}$ 3460. It gave a red-purple color with the ferric chloride reagent with a maximum at 535 mμ (ε 1220).

Enzymatic Degradation of 2,5-Dihydroxypyridine.—Synthetic III was submitted to enzymatic oxidation and degradation by a soluble fraction prepared from nicotinic acid-grown cells of *P. fluorescens*.[3] The extracts were diluted to the point at which oxygen uptake ceased at two atoms per μmole of substrate. Oxygen uptake, carbon dioxide evolution and ammonia formation were estimated as previously described.[3] The sum of malate and fumarate was determined in the presence of excess fumarase using lyophilized cells of *Lactobacillus plantarum* as a source of L-malate decarboxylase.[10] Formate was estimated by measuring carbon dioxide evolution catalyzed by the particulate formic oxidase prepared from nicotinic acid-grown *P. fluorescens*.[11] The data agree with those obtained[3] using biologically prepared III (Table II) (eq. 1).

$$C_5H_5NO_2 + O_2 + 2H_2O \longrightarrow HCOOH + NH_3 + HOOCCH{=}CHCOOH \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} HOOCCH_2CHOHCOOH \quad (1)$$

TABLE II

BALANCE SHEET FOR THE OXIDATION OF 2,5-DIHYDROXYPYRIDINE

Both control and experimental vessels contained 0.3 ml. of soluble fraction (26 mg. of protein per ml.), 1.5 ml. of 0.02 *M* phosphate buffer (*p*H 6.8) and 0.05 ml. of 0.04 *M* ferrous sulfate. The experimental vessel was furnished with 0.2 ml. of 0.025 *M* 2,5-dihydroxypyridine, the control with 0.2 ml. of water.

	Micromoles			Moles per mole of substrate oxidized	
Reactants	Control	Exptl.	Difference	Obsd.	Theory[a]
2,5-Dihydroxypyridine	0.0	5.0	5.0		
Oxygen consumed	0.0	4.87	4.87	0.98	1.00
Products					
Carbon dioxide[b]	2.76	2.50	−0.26	−0.05	0.00
Ammonia	6.25	11.10	4.85	0.97	1.00
Sum of malate and fumarate	0.00	5.0	5.0	1.00	1.00
Formate	0.00	5.10	5.10	1.02	1.00

[a] To fit eq. 1. [b] Including initial bound CO_2.

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(10) S. Korkes, in "Methods in Enzymology" ed. by S. P. Colowick and N. O. Kaplan, Vol. 3, Academic Press Inc., New York, N. Y., 1957, pp. 436–437.

(11) E. J. Behrman, Ph.D. Dissertation, University of California, Berkeley, 1957.