potassium hydroxide. The slightly discolored crystals obtained were dissolved in 15 cc. of water; the solution was heated to 80° and neutralized with lithium carbonate, 101% of the theoretical quantity being necessary to reach *p*H 7. On cooling, the colorless needles which separated were filtered off, washed twice with 3-cc. portions of ice-water, and dried at 60°. Yield was 2.72 g. (96%); m. p., 239.5–240°, decomposition above 230°.

Anal. Calcd. for $C_5H_5O_2N$: C, 54.03; H, 4.54; N, 12.61. Found: C, 54.34; H, 4.58; N, 12.68.

The aqueous solution of a test sample was neutral to litmus and gave a violet color with ferric chloride.

The **diacetate** was prepared by the method of Adams, *et al.*[2] The dihydroxypyridine (130 mg.) was heated with 1.0 cc. of acetic anhydride for five minutes. Excess acetic anhydride and acetic acid were removed in a vacuum desiccator over potassium hydroxide. The almost-colorless residue was recrystallized (Nuchar W) from dry ethyl acetate, yielding shiny leaflets, m. p., 138.5–140°.

Anal. Calcd. for $C_9H_9O_4N$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.65; H, 4.88; N, 7.16.

The aqueous solution of a test sample was neutral and gave no color with ferric chloride. On standing, however, the reaction became acid and a violet color was developed with ferric chloride.

A **monoacetate** was isolated when the crude acetylation product was allowed to stand in a vacuum desiccator over potassium hydroxide for fourteen days. Upon extraction with boiling ethyl acetate, a small quantity of the monoacetate remained undissolved; colorless needles, m. p., 145.5–146.5°.

Anal. Calcd. for $C_7H_7O_3N$: N, 9.15. Found: N, 9.30.

The aqueous solution of a test sample gave no color with ferric chloride. On standing, however, a violet color developed. This behavior indicates that the 3-monoacetate has been isolated. Apparently, this compound is not identical with the monoacetate described by Peratoner,[6] m. p. 207–208°.

B. From Leucaenine.—Two grams of leucaenine were pyrolyzed in a vacuum sublimation apparatus at 200–240° (1 mm.).[3] The sublimate (900 mg.) was recrystallized twice from water (Nuchar W), giving colorless crystals which were dried at 60°. Yield was 390 mg.; m. p., 239.5–240°, decomposition above 230°.

Anal. Calcd. for $C_5H_5O_2N$: C, 54.03; H, 4.54; N, 12.61. Found: C, 53.80; H, 4.35; N, 12.90.

Mixed melting point with 3,4-dihydroxypyridine from A was 239.5–240°, decomposition above 230°.

The **diacetate** was prepared as described above: colorless leaflets, m. p., 139–140°.

Anal. Calcd. for $C_9H_9O_4N$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.51; H, 4.36; N, 7.35.

Mixed melting point with the diacetate from A, was 138.5–140°.

Summary

The dihydroxypyridine obtained when leucaenine is pyrolyzed (in the manner described by Adams, *et al.*) has been proved to have the 3,4-structure.

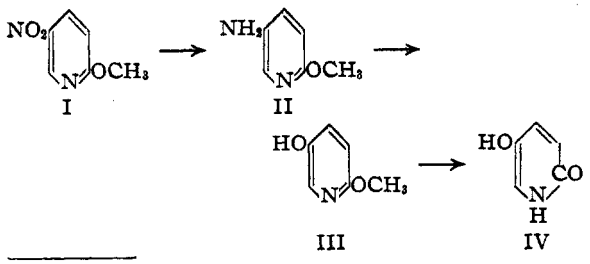
PITTSBURGH 13, PA. RECEIVED FEBRUARY 27, 1947

[CONTRIBUTION FROM NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Structure of Leucenol. III. Synthesis of 2,5-Dihydroxypyridine (5-Hydroxy-2-pyridone)[1]

BY ROGER ADAMS AND T. R. GOVINDACHARI

By the pyrolysis of leucenol, a dihydroxypyridine (hydroxypyridone) was isolated which was assumed to be the unknown 5-hydroxy-2-pyridone because its properties did not conform to those described in the literature for any of the five known isomers. In this Laboratory and in that of Wibaut[2] previous attempts to prepare this compound have failed. A successful synthesis of 5-hydroxy-2-pyridone has now been realized. It is a stable compound resembling the other hydroxypyridones. 2-Methoxy-5-nitropyridine (I) was reduced to the corresponding amino compound (II). This was diazotized and the amino group replaced by hydroxyl to yield 2-methoxy-5-hydroxypyridine (III) which hydrolyzed readily in the presence of hydrobromic acid to give 2,5-dihydroxypyridine (5-hydroxy-2-pyridone) (IV). The yields in all steps except the replacement of the amino group by hydroxyl were very satisfactory. In general diazotization of a 3-aminopyridine gives low yields[3] but in this case only 3–4% yield resulted. No attempts to improve this preparation were made since the amount of 2-methoxy-5-hydroxypyridine obtained was adequate for hydrolysis and characterization of the 5-hydroxy-2-pyridone. The 2-methoxy-5-aminopyridine is reported in the literature both as a crystalline solid,[4] m. p. 135–136°, and as an oil extremely susceptible to oxidation.[5] In this investigation it proved to be a colorless oil easily obtainable in quantitative yields.



I II III IV

The pyridone (IV) begins turning dark at 215° and decomposes without melting at 240–250° (cor.) and a mixture with the leucenol pyrolysate (m. p. 242–244° with decomposition), melts at 210° with decomposition. The synthetic product gives a pinkish red color with ferric chloride, un-

(1) For previous paper see Adams and Jones, THIS JOURNAL, **69**, 1803 (1947); Paper V, *ibid.*, **69**, 1810 (1947).

(2) Bickel and Wibaut, *Rec. trav. chim.*, **65**, 65 (1946).

(3) Schickh, Binz and Schulz, *Ber.*, **69**, 2600 (1936); Parker and Shive, THIS JOURNAL, **69**, 63 (1947).

(4) Rath, *Ann.*, **484**, 52 (1930).

(5) Magidson and Menschikoff, *Ber.*, **58**, 113 (1925).

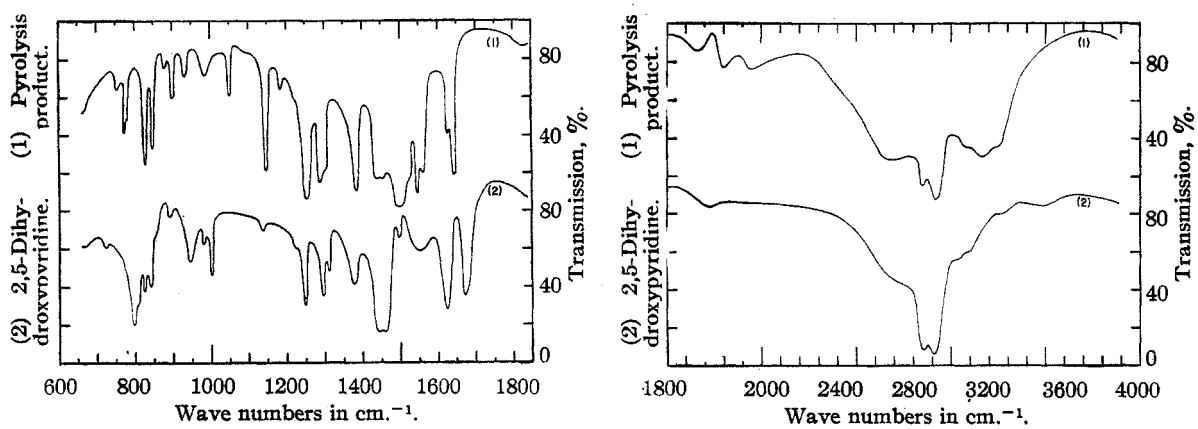
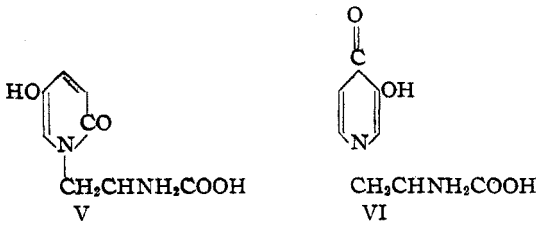


Fig. 1.—Both samples were dried in Abderhalden. To obtain the spectra each sample was ground up in Nujol and placed between two rocksalt plates to reduce the scattering caused by solid particles. The only Nujol absorption bands are the C–H stretching frequencies at 2920 and 2850 cm.$^{-1}$ and the CH_2 and CH_3 deformational frequencies at 1460 and 1375 cm.$^{-1}$.

changed on addition of more reagent whereas the pyrolysate gives first a red which changes to a purple color on addition of more reagent (incorrectly reported in a previous paper[6] as giving directly a purple color). It gives a blue color with Folin's reagent. The infrared absorption spectrum of IV differs from that of the pyrolysate as shown in Fig. I. It must be concluded therefore that the postulated structure of the pyrolysate as 5-hydroxy-2-pyridone is incorrect.

This discovery indicates that the previously proposed structure (V) for leucenol is faulty. Of the two suggested by Wibaut,[7] that with the side chain attached to nitrogen (VI) is probably the right one.

The pyrolysate, on the basis of formula VI, must be 3-hydroxy-4-pyridone, but if this is so the properties now assigned to 3-hydroxy-4-pyridone in the literature must be in error.



The authors are indebted to Mrs. Agatha R. Johnson for the infrared absorption spectra determinations.

Experimental

2-Chloro-5-nitropyridine.—This compound was prepared by the method described by Caldwell and Kornfeld.[8]

2-Methoxy-5-nitropyridine.—A solution of 10 g. of 2-chloro-5-nitropyridine in 100 ml. of absolute methanol was added to a solution of 1.75 g. of sodium in 50 ml. of absolute methanol with good shaking. The solution was refluxed for ten minutes and the methanol was removed by distillation. The residue was steam-distilled, when 2-methoxy-5-nitropyridine passed over as colorless crystals solidifying in the condenser. The steam-distillate was cooled in ice and the material filtered, yield 7.2 g. (74.5%). It was purified by crystallization from dilute ethanol; colorless prisms, m. p. 109° (cor.).

There are two previously described syntheses which give relatively poor yields; from the silver salt of 5-nitro-2-pyridone[4] (44% yield), m. p. 108–109°, and from 5-nitro-2-iodopyridine[5] (33.8% yield), m. p. 110°.

2-Methoxy-5-aminopyridine.—A solution of 5 g. of 2-methoxy-5-nitropyridine in 50 ml. of absolute ethanol was reduced with hydrogen in the presence of 0.1 g. of platinum oxide catalyst at 2–3 atm. pressure. The reduction stopped upon absorption of three moles of hydrogen. The colorless solution was filtered from the catalyst, keeping an atmosphere of nitrogen over the funnel and the filtrate was distilled *in vacuo* to remove the ethanol completely. A light brown oil was left behind. On distillation *in vacuo*, this passed over as a colorless oil, b. p. 125–126° at 10 mm.; yield, 3.9 g. (95%). It had a strong basic odor and had a tendency to get dark by exposure to the atmosphere.

By addition of one mole of picric acid to the ethanolic solution of the amine, both the mono and dipicrates were formed, the dipicrate separating out first and the monopicrate on concentration of the filtrate. Both the picrates were recrystallized from ethanol. The dipicrate formed yellow crystals, m. p. 128°, and the monopicrate, yellow crystals, m. p. 160°.

Anal. Monopicrate: Calcd. for $C_{12}H_{11}N_5O_8$: C, 40.9; H, 3.15; N, 19.9. Found: C, 41.1; H, 3.16; N, 19.56.

Anal. Dipicrate: Calcd. for $C_{18}H_{14}N_8O_{15}$: C, 37.1; H, 2.43; N, 19.2. Found: C, 37.34; H, 2.37; N, 19.35.

2-Methoxy-5-hydroxypyridine.—The crude 2-methoxy-5-aminopyridine obtained by reduction of 10 g. of nitro compound was dissolved in a mixture of 11 ml. of concentrated sulfuric acid and 80 ml. of water. The solution was cooled to below −5° and treated dropwise with good stirring with a solution of 5 g. of sodium nitrite in 20 ml. of water, keeping the temperature below −5°. After the addition, the stirring was continued for thirty minutes at the temperature of diazotization. The solution was then poured into 200 g. of ice and the excess nitrous acid removed by addition of the necessary amount of urea and the mineral acid neutralized (congo red paper) by addition of sodium acetate. The ice-cold solution was then dropped into a solution of 20 g. of copper sulfate in 50 g. of water with vigorous stirring, the temperature being kept at 50°. The temperature was then raised to

(6) Adams, Cristol, Anderson and Albert, THIS JOURNAL, 67, 89 (1945).

(7) Wibaut, *Helv. Chim. Acta*, 29, 1669 (1946).

(8) Caldwell and Kornfeld, THIS JOURNAL, 64, 1695 (1942).

80° and the heating continued until the evolution of nitrogen ceased. A considerable amount of brownish resinous material separated at this stage.

The mixture was repeatedly extracted with ether (500 ml.). The ether solution was treated with aqueous sodium bicarbonate to neutralize the acetic acid. The ether extract was dried over anhydrous magnesium sulfate and the ether distilled. The reddish oily residue was distilled at 1 mm. and 0.35 g. of a light brown semi-solid was obtained; the rest was non-distillable. The semi-solid material was subjected to vacuum sublimation and 0.2 g. of a slightly waxy solid resulted. On recrystallization from toluene, 100 mg. of pure 2-methoxy-5-hydroxypyridine was obtained; m. p. 81°.

Anal. Calcd. for $C_8H_7O_2N$: C, 57.6; H, 5.64; N, 11.2. Found: C, 57.79; H, 5.66; N, 11.06.

2,5-Dihydroxypyridine (5-Hydroxy-2-pyridone).—A solution of 90 mg. of 2-methoxy-5-hydroxypyridine in 1 ml. of hydrobromic acid (sp. gr. 1.5) in an atmosphere of nitrogen was refluxed for four hours. The hydrobromic acid was then removed *in vacuo* and this residue carefully neutralized with dilute aqueous sodium carbonate keeping the end-point slightly on the acidic side. The solution was then evaporated to dryness over a steam-bath in a current of nitrogen. The residue was sublimed at 1 mm. pressure. The sublimation started at 150° and the temperature was gradually raised to 180° during three hours. A pale yellow solid was obtained, weighing 70 mg. On recrystallization from ethanol, it was obtained as clusters of thin colorless needles which darkened at 215° and decomposed between 240–250°. Additional recrystallizations gave product showing the same characteristics upon heating.

Anal. Calcd. for $C_5H_5O_2N$: C, 54.01; H, 4.54; N, 12.6. Found: C, 54.10; H, 4.46; N, 12.41.

Summary

1. 2,5-Dihydroxypyridine (5-hydroxy-2-pyridone) has been synthesized by the replacement of the amino group in 2-methoxy-5-aminopyridine by hydroxyl and hydrolysis of the product.

2. This compound was not identical with the dihydroxypyridine obtained by pyrolysis of leucenol.

URBANA, ILLINOIS RECEIVED MARCH 7, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Preparation of Highly Purified Mustard Gas and its Action on Yeast[1]

BY VINCENT DU VIGNEAUD AND CARL M. STEVENS[2]

In undertaking a study of the biochemistry of mustard-type vesicants[3] it was of importance to have available the highly purified compounds. Even though the vesicant activity of impure preparations could be shown to reside solely in the β-chloroethyl sulfide, there could be no assurance that another physiological property of the material might not be due, in part at least, to impurities. That active impurities are indeed present in certain preparations of mustard gas (H) was shown clearly by Hellerman[4] who reported that H prepared by the Levinstein process[5] rapidly "covered" the *a* sulfhydryl groups of urease, whereas H prepared from thiodiglycol did not enter into rapid reaction with these groups.

In this Laboratory, we had begun a study of the effect of H-type vesicants on the growth of yeast[6] in a chemically defined medium.[7] We had found that yeast growth was inhibited by the addition of H to the medium. Of further importance was the observation that aqueous solutions which had been allowed to stand for several hours and which did not contain a detectable amount of bis-(β-chloroethyl) sulfide were also active in inhibiting the growth of yeast. The degree of inhibition by aged aqueous solutions varied greatly with different samples of H. Furthermore, the inhibitory action of the aged solutions was largely removed by heating the solutions in an open flask at 100°, or by extracting the solutions with petroleum ether.

When the yeast growth data indicated conclusively that there was a physiologically active impurity (or impurities) in the purest available samples of H, we undertook the purification of the vesicant. Despite its low melting point (14°), crystallization from a suitable solvent seemed the most promising method. It was found that crystallization of the compound occurred readily from dilute solution in petroleum ether or absolute ethanol if the solutions were cooled to approximately −75°. In this way purification was readily effected. Freshly prepared aqueous solutions of the recrystallized compound inhibited yeast growth to an extent similar to that observed with the unrecrystallized compound, whereas aged solutions of recrystallized H showed no evidence of any impurity inhibiting yeast growth. It thus appears that bis-(β-chloroethyl) sulfide is itself growth inhibitory, but that other inhibitors contaminating the redistilled samples of H were removed by the recrystallization. The recrystallized material also showed a somewhat higher melting point (14.5°) than that of the purest

(1) The work described in this paper was carried out under Contract OEMsr-144 between the Office of Scientific Research and Development and Cornell University Medical College and is described in Progress Reports to Section B4C, January, 1942, to June, 1942.

(2) Present address: Department of Chemistry, State College of Washington, Pullman, Wash.

(3) We have considered that vesicant compounds of the type R—SCH_2CH_2Cl (where R is an alkyl or aryl group) may safely be considered as analogous to mustard gas (bis-(β-chloroethyl) sulfide or H) in mode of action. For convenience, therefore, we shall refer to these compounds as "mustard-type vesicants" or "H-type vesicants."

(4) L. Hellerman, final summarization of NDRC work, Contract OEMsr-94.

(5) Felsing and Arenson, *Ind. Eng. Chem.*, **12**, 1065 (1920).

(6) Magne and Remy (*Bull. soc. chim. biol.*, **19**, 1092 (1937)) have reported some effects of mustard gas on yeast. See also, Herriott, Anson, and Northrop (*J. Gen. Physiol.*, **30**, 185 (1946)) for some recent studies.

(7) Snell, Eakin and Williams, THIS JOURNAL, **62**, 175 (1940).