TABLE I

PHYSICAL DATA, ANALYSES, AND YIELDS OF DIMETHYLAMINOMETHYL DERIVATIVES

Compound		Analyses C	H	N	Cl	Neutral Equivalent	Color	M.P.[a]	Yield, %[b]
I	Found.[b]	77.00	6.43	4.70		299[b]	White	270–277[b]	72
	Calcd.:	77.14	6.13	4.74		296			
II[d]	Found.[e]	79.68	5.67	4.10	10.00	346[b]	Yellow	290–305[e]	50
	Calcd.:	79.90	5.79	4.05	10.25	346			
III	Found:[c]	75.30	6.67	5.33		275[b]	White	241–242[c]	81
	Calcd.:	75.12	6.67	5.15		272			
IV[d,f]	Found:[e]	77.62	6.19	4.24	10.30	319[e]	Yellow-brown	220–237[e]	61
	Calcd.:	78.36	6.27	4.35	11.02	322			

[a] All samples melted with decomposition. [b] Crude product. [c] After recrystallization from a mixture of concentrated hydrochloric acid and water (1:1) with the aid of charcoal. [d] After removal of the excess of dimethylformamide and formic acid a solid remained. Most of this solid was soluble in a very large amount of ethyl ether. A solid by-product, corresponding in weight to about 20% of the aldehyde used as starting material, was insoluble and was discarded. [e] The sample had been purified by dissolving the material in a very large amount of hot water, filtration, and addition of hydrochloric acid to the filtrate. [f] The analytical data indicate the presence of an impurity. However, there is little doubt that the product has predominantly the assigned structure.

cipitated 10.7 g. (72%) of white *N,N*-dimethyl-1-pyrenemethylamine hydrochloride, m.p. 270–277° with decomposition; it had a neutral equivalent of 299 (Calcd. 296).

RESEARCH DEPARTMENT
UNION CARBIDE CHEMICALS COMPANY
SOUTH CHARLESTON, W. VA.

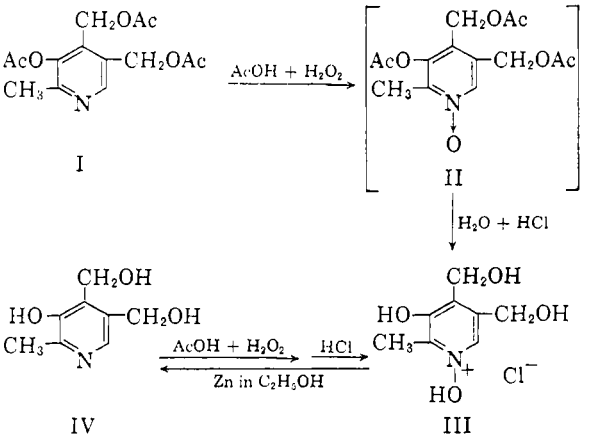
Pyridoxine *N*-Oxide[1]

TAKETAMI SAKURAGI AND FRED A. KUMMEROW

Received February 12, 1959

Upon treatment with acetic acid–hydrogen peroxide, a variety of pyridine derivatives form *N*-oxides.[2] It seemed possible, in a similar manner, to convert pyridoxine (IV) to pyridoxine *N*-oxide (III), which is of interest because of the following two possibilities: (a) pyridoxine *N*-oxide (III) may act as an antimetabolite, or (b) pyridoxine *N*-oxide (III) may serve as a source of vitamin B_6 *in vivo*. It has been reported that pyridine *N*-oxide was reduced to pyridine by baker's yeast, although an analogous reaction did not occur with 4-picoline *N*-oxide.[3]

In the present study, 3,4,5-triacetylpyridoxine (I) was treated with a mixture of glacial acetic acid and hydrogen peroxide at 37° for 72 hr., or at 60–70° for 8 hr. The intermediate, possibly 3,4,5-triacetylpyridoxine *N*-oxide (II), was hydrolyzed by refluxing in 65% ethanol containing 4.5% hydrogen chloride. The resulting product contained a considerable amount of pyridoxine. A longer reaction time and the use of an oxidation mixture which consisted of acetic acid–acetic anhydride–hydrogen peroxide failed to complete the oxidation. The *N*-oxide hydrochloride (III), however, was far more soluble than pyridoxine hydrochloride in 1-propanol, 2-propanol, and 1-butanol and could thus be purified by solvent fractionation. Pyridoxine (IV) was regenerated from pyridoxine *N*-oxide (III) upon refluxing in 95% ethanol in the presence of zinc dust. The identity of the reduced material was established by mixed melting point with an authentic specimen of pyridoxine, paper chromatography, and the biological activity to support growth of *Saccharomyces carlsbergensis*. For paper chromatography, the solvent systems reported by Rodwell *et al.*[4] and Snyder *et al.*[5] were satisfactory.



I II III IV

Free pyridoxine base (IV) also formed pyridoxine *N*-oxide (III), although the yield was considerably lower. It has been shown that under certain conditions, a portion of pyridoxine is oxidized to pyri-

(1) This work was supported by research grant No. A-257 from the National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare.
(2) E. Ochiai, *J. Org. Chem.*, **18**, 534 (1953).
(3) A. May, *Enzymologia*, **18**, 142 (1957).
(4) V. W. Rodwell, B. E. Volcani, M. Ikawa, and E. E. Snell, *J. Biol. Chem.*, **233**, 1548 (1958).
(5) J. Q. Snyder and S. H. Wender, *Arch. Biochem. Biophys.*, **46**, 465 (1953).

doxal when treated with hydrogen peroxide,[6] and that pyridoxal is readily reduced to pyridoxine upon treatment with zinc in boiling ethanol.[7] When the pyridoxine *N*-oxide (III) as prepared was treated with 1% phenylhydrazine in acetic acid, no yellow color developed, indicating the absence of possible contamination with pyridoxal. The mixture of acetic acid–hydrogen peroxide containing free pyridoxine base (IV) was also tested from time to time during the course of oxidation by paper chromatography. The only spots detected were that of pyridoxine (IV) and pyridoxine *N*-oxide (III), and thus the possibility of pyridoxal formation was excluded. On the other hand, pyridoxine hydrochloride failed to form the *N*-oxide (III) under the oxidation conditions, and in two cases, the recovery of pure pyridoxine hydrochloride was 65–70%.

The results of the bioautography and microbiological assay using *S. carlsbergensis* indicated that pyridoxine *N*-oxide (III) was not growth inhibitory. It retained 15% of the vitamin B_6 activity as compared with equimolar amounts of pyridoxine.

The preparation of pyridoxine *N*-oxide (III) (2–3 mg.) was refluxed in 0.5 ml. of distilled water, 5*N* hydrochloric acid or 5*N* aqueous sodium hydroxide for 1 hr., and the resulting solution was applied for paper chromatography. In all cases, the chromatographic patterns were identical with that of the original pyridoxine *N*-oxide (III).

EXPERIMENTAL[8]

Paper chromatography. The following two solvent systems were used: Solvent A, a mixture of water, acetone, *tert*-butanol, and diethylamine (20:35:40:5, *v/v*),[4] and Solvent B, the upper layer of a mixture of water, isoamyl alcohol, and pyridine (40:40:20, *v/v*).[5] Throughout this investigation, a descending system on Whatman No. 1 filter paper was employed at room temperature. The spots were detected by spraying a 0.1% benzene solution of *N*,2,6-trichloro-*p*-quinoneimine followed by exposure to ammonia vapor, or by spraying a 4% ethanolic solution of ferric chloride. The spots were also detectable on the papergram under ultraviolet light. Typical R_f values for pyridoxine (IV) and pyridoxine *N*-oxide (III) were 0.48 and 0.30, respectively, with Solvent A, and were 0.40 and 0.11, respectively, with Solvent B. The R_f value of pyridoxal with Solvent A was 0.67.

Assay with Saccharomyces carlsbergensis (A.T.C.C. 4228). The method reported by Atkin *et al.*[9] was employed. For bioautography, a papergram which had been developed with Solvent A was applied.

Pyridoxine N-oxide hydrochloride (III) *from 3,4,5-triacetylpyridoxine* (I). Two grams of triacetylpyridoxine (I)[10] was dissolved in a mixture of 25 ml. glacial acetic acid and 10 ml. of 30% hydrogen peroxide. After maintaining the solution at 37° for 72 hr., the volatile portion was removed *in vacuo* as much as possible. The residual oil was refluxed in 50 ml. of 65% ethanol containing 4.5% hydrochloric acid for 30 min., and the solvent was removed until dryness under diminished pressure. The product was taken up in 5 ml. of ethanol and ether was added. The white precipitate was collected, dissolved in 10 ml. of warm 1-propanol, and kept at −5° for 20 hr. The solid portion was removed by filtration and thoroughly washed with cold 1-butanol. The filtrate and the butanol washings were combined, and the product separated upon addition of ether. The compound thus isolated was essentially free from pyridoxine. The crystalline product was again shaken in 10 ml. of 1-butanol at room temperature for approximately 5 min., and a small amount of insoluble matter was removed by filtration. Upon addition of ether, pyridoxine *N*-oxide hydrochloride (III) crystallized. Recrystallization was effected from 1-butanol–ethyl acetate. Yield 0.45 g. (33%). M.p. 145.5–146.0°.

Anal. Calcd. for $C_8H_{11}NO_4 \cdot HCl$: C, 43.35; H, 5.00; N, 6.32; Cl, 16.02. Found: C, 43.11; H, 5.20; N, 6.40; Cl, 16.44.

The precipitate, which had been separated from 1-propanol and washed with 1-butanol as described, was recrystallized from 95% ethanol. One recrystallization was sufficient to recover pure pyridoxine hydrochloride (0.1 g.).

Comparable results were also obtained by carrying out the oxidation at 60–70° for 8 hr.

Pyridoxine N-oxide (III) *from pyridoxine* (IV). Free pyrodixine base (IV) (2.1 g.) was dissolved in a mixture of 30 ml. of glacial acetic acid and 12 ml. of 30% hydrogen peroxide, and allowed to stand at 37° for 144 hr. The volatile portion was removed *in vacuo*, and the residue was taken up in 10 ml. of ethanol containing dry hydrogen chloride. Upon addition of ether, a mixture of pyridoxine hydrochloride and pyridoxine *N*-oxide hydrochloride (III) separated. The product thus isolated was shaken, at room temperature, in 15 ml. of 1-propanol, and cooled at −5° for 5 hr. The insoluble portion (0.4 g.), which mainly consisted of pyridoxine hydrochloride, was removed by filtration. The crystals which had been separated after addition of ether, were shaken in 10 ml. of 1-butanol at room temperature for 5 min. A small amount of insoluble material was removed, and pyridoxine *N*-oxide hydrochloride (III) precipitated by adding ether. Recrystallization was effected from 1-propanol–ethyl acetate. Yield 0.25 g. (9%). M.p. 145.0–145.5° Mixed melting point with the pyridoxine *N*-oxide hydrochloride (III) obtained from triacetylpyridoxine (I) was 145.0–145.5° C. The identity of these two preparations was also recognized by paper chromatography.

Similar results were obtained by conducting the oxidation at 60–70° for 8 hr.

Pyridoxine hydrochloride (IV) *from pyridoxine N-oxide hydrochloride* (III). One hundred twenty mg. of pyridoxine *N*-oxide hydrochloride (III) was dissolved in 10 ml. of 95% ethanol and refluxed for 2 hr. in the presence of 500 mg. zinc dust. The reaction mixture was filtered, and the filtrate acidified with dry hydrogen chloride. The product crystallized upon addition of ether, and was recrystallized from methanol–ether. Yield 45 mg. (40%). M.p. 206.0–208.0° (dec.). Mixed melting point with an authentic specimen of pyridoxine hydrochloride was 208.0–208.5° C. (dec.).

This product was free from pyridoxine *N*-oxide (III) as proved by paper chromatography and was as active as an authentic sample of pyridoxine hydrochloride in supporting growth of *Saccharomyces carlsbergensis*.

DEPARTMENT OF FOOD TECHNOLOGY
UNIVERSITY OF ILLINOIS
URBANA, ILL.

(6) L. E. Carpenter and F. M. Strong, *Arch. Biochem.*, **3**, 375 (1944).

(7) T. Sakuragi and F. A. Kummerow, *J. Am. Chem. Soc.*, **78**, 839 (1956).

(8) A portion of pyridoxine hydrochloride used in the present study was kindly supplied by Merck & Co., through the courtesy of Dr. S. A. Harris.

(9) L. Atkin, A. S. Schultz, W. L. Williams, and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, **15**, 141 (1943).

(10) A. Ichiba and K. Michi, *Sci. Papers Inst. Phys. Chem. Res. (Tokyo)*, **35**, 73 (1938).