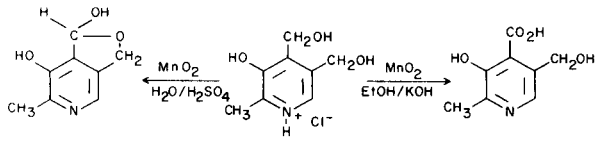
Department of Experimental Therapeutics Roswell Park Memorial Institute

A Direct Method for Preparing Pyridoxal and 4-Pyridoxic Acid (1)

H. Ahrens and W. Korytnyk

Despite many studies of the oxidation of pyridoxol (I) (2-8) and its derivatives (9,10), there has been no practical method for the direct conversion of pyridoxol to pyridoxal (II) and 4-pyridoxic acid (III). Although the two metabolites have been obtained by the oxidation of



II I III

pyridoxol, (2,7), low yields and considerable amounts of inorganic material in the reaction mixture have precluded direct isolation. The method of choice (11) for the synthesis of 4-pyridoxic acid (III) has been a multistep procedure developed by Heyl (3), a procedure that utilizes the not very readily accessible oxime of pyridoxal as the starting material.

In the course of studies of the selective conversion of the 4-hydroxymethyl group in pyridoxol analogs to 4-formyl or 4-carboxyl, we investigated the optimum conditions that would give either pyridoxal or pyridoxic acid isolatable by simple procedures. Treatment of pyridoxol with an active form of manganese dioxide (12) in acidic solution for a very short time gave pyridoxal (II), which could be isolated in good yield as either the free base or the oxime. When the oxidation of pyridoxol was carried out in ethanolic potassium hydroxide with manganese dioxide, very pure 4-pyridoxic acid could be isolated in 82% yield. Generally, use of ethanol as solvent for oxidations is unusual. Although manganese dioxide can be expected to be somewhat inactivated by ethanol, this solvent appears to give superior results in this reaction.

The reactions were conveniently followed by TLC, which permitted separating all of the products (see Experimental). The only spots that could be detected in the two experiments were the desired products II and III. Deviations from the reaction procedures mentioned, such as different reaction times, temperatures, or solvents, generally gave mixtures of products.

EXPERIMENTAL

Thin-Layer Chromatography.

Plates were coated with a 0.2 mm layer of silica gel (Merck HF_{254}), and were activated for 30 minutes at 110°. After activation, some plates were streaked with 2.5% boric acid in ethanol approximately 2 cm. from the starting point. For a plate 5 cm. wide, 50 μl of boric acid solution was required. The plates were developed with chloroform-methanol (1:1 by volume).

TABLE I

Rf Values of Some Pyridoxol Derivatives

	Untreated	H_3BO_3-treated	Gibb's test
Pyridoxol	0.63	In the strip	strong
Pyridoxal	0.68	0.33	strong
Pyridoxal ethyl acetal	0.83	0.82	strong
4-Pyridoxic acid	0.51	0.46	weak
4-Pyridoxic acid lactone	0.31	0.32	medium

Pyridoxol is completely retarded by the boric acid strip, but pyridoxal is retarded only partially, and derivatives lacking hydroxyl in the 4-position pass through the boric acid strip unhindered.

Preparation of Pyridoxal.

Pyridoxol hydrochloride (0.794 g.) was dissolved in water (30 ml.). Concentrated sulfuric acid (0.25 ml.) was added, and the mixture was shaken at room temperature with active manganese dioxide (12) (5 g.) for 75 seconds. The manganese dioxide was filtered off immediately, and was washed rapidly with several portions of water (total 40 ml.). From this solution, either pyridoxal oxime (a) or free pyridoxal base (b) was isolated as follows:

(a) Pyridoxal oxime.

Filtrate and washings were concentrated to 10 ml. *in vacuo*, and the solution was heated on a steam bath. Then sodium acetate (4.0 g.) and hydroxylamine hydrochloride (0.50 g.) were added. After the solution was kept on steam for 5 minutes, the oxime crystallized. The reaction mixture was allowed to reach room temperature, and was kept in a refrigerator for 12 hours. Filtration, washing with water, and drying at 60° and 1 mm Hg over phosphorus pentoxide yielded 0.540 g. (77%), m.p. 212° dec. Recrystallization from ethanol raised the melting point to 225° dec. (Lit. (2,3) m.p. 225-226°). The oxime was identical with authentic pyridoxal oxime on the basis of TLC and IR spectroscopy.

(b) Pyridoxal.

Filtrate and washings were concentrated to 5 ml. when crystallization of pyridoxal (free base) occurred. Water (5 ml.) was added to keep manganous sulfate in solution and the crude base was filtered and was washed with a few drops of water. After drying at 60° and 1 mm Hg over phosphorus pentoxide, the yield was 0.432 g. Addition of sodium acetate (4.0 g.) to the mother liquor and keeping in the refrigerator for 60 hours permitted isolation of another 0.066 g. of very pure material, making a total yield of 0.498 g. (77%). Both fractions migrate as a single spot on TLC, and have IR and UV spectra identical with those of authentic material.

Preparation of 4-Pyridoxic Acid.

Pyridoxol hydrochloride (0.991 g.) was added to alcoholic potassium hydroxide (1.00 g. of potassium hydroxide in 100 ml. of absolute ethyl alcohol) and was stirred magnetically with 10.0 g. of active manganese dioxide (12) for 3 hours at room temperature, moisture being excluded. Manganous salts were reoxidized by the addition of 5.0 ml. of 30% hydrogen peroxide. The solution then became hot, and was immediately filtered. The residue was washed with hot 0.1 *N* alcoholic potassium hydroxide, the wash liquid was combined with the filtrate, and the solution was neutralized to *p*H 7 with concentrated hydrochloric acid. The small amount of precipitated manganese dioxide was filtered off. Further acidification with concentrated hydrochloric acid to *p*H 4 precipitated 4-pyridoxic acid, which, after standing in a refrigerator for 6 hours, was filtered and was then washed with ice-water, ethanol, and ether. After thorough drying, the yield was 0.727 g. (82%); m.p. 242° dec., raised to 256° dec., after recrystallization from boiling water. The product was found to be identical with an authentic sample of pyridoxic acid on the basis of a mixture melting point, TLC and nmr (13) and IR spectra. Treatment with 1 *N* hydrochloric acid readily converted it to 4-pyridoxic acid lactone (m.p. 263-265°) (7).

Acknowledgment.

This study was supported in part by a research grant (CA-08793) from the National Cancer Institute, U. S. Public Health Service. We wish to thank Dr. Charles A. Nichol for his encouragement and interest.

REFERENCES

(1) Pyridoxine Chemistry XVI. Previous papers in this series: W. Korytnyk and B. Paul, *J. Org. Chem.*, (in press) and ref. 10.

(2) S. A. Harris, D. Heyl, and K. Folkers, *J. Am. Chem. Soc.*, 66, 2088 (1944).

(3) D. Heyl *ibid.*, 70, 3434 (1948).

(4) M. Viscontini, C. Ebnöther, and P. Karrer, *Helv. Chim. Acta*, 34, 1834 (1951).

(5a) Y. Sakurai, M. Shiroishi, and T. Masuhara, *Rept. Food Research Inst.* (Tokyo), 5, 63 (1951); *Chem. Abstr.*, 49, 16104f (1955). (b) M. Shiroishi, C. Fukai, T. Masuhara, and Y. Sakurai, *ibid.*, 6, 135 (1952); *Chem. Abstr.*, 49, 16104h (1955).

(6) E. Merck and Co. British Patent, 852, 398 (1960); *Chem. Abstr.*, 55, 10477g (1961).

(7) J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.*, 155, 345 (1944).

(8) M. M. Polansky, R. T. Camarra, and E. W. Toepfer, *J. Assoc. Offic. Agr. Chem.*, 47, 827 (1964).

(9) E. T. Stiller, J. C. Keresztesy, and J. R. Stevens, *J. Am. Chem. Soc.*, 61, 1237 (1939).

(10) B. Paul and W. Korytnyk, *Chem. & Ind.*, 230 (1967).

(11) C. J. Argoudelis and F. A. Kummerow, *Biochemistry*, 5, 1 (1966).

(12) O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

(13) W. Korytnyk, E. J. Kris, and R. P. Singh, *J. Org. Chem.*, 29, 574 (1964).

Received August 7, 1967 Buffalo, N. Y. 14203