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# Studies on the lactonization of 5 and 4-pyridoxic acid

The lactones of 5- and 4-pyridoxic acids (I, III*) have recently been isolated from growth media of Pseudomonas sp. IA and MA when pyridoxine[1] or pyridoxamine[2] was used as the sole or predominant source of carbon and nitrogen respectively. The possibility of interconversion between the acids and their lactones was not excluded.

In the present work, the lactonization of 5- and 4-pyridoxic acids was studied under conditions similar to those previously used for the isolation of the oxidation products of pyridoxine[1] and pyridoxamine[2].

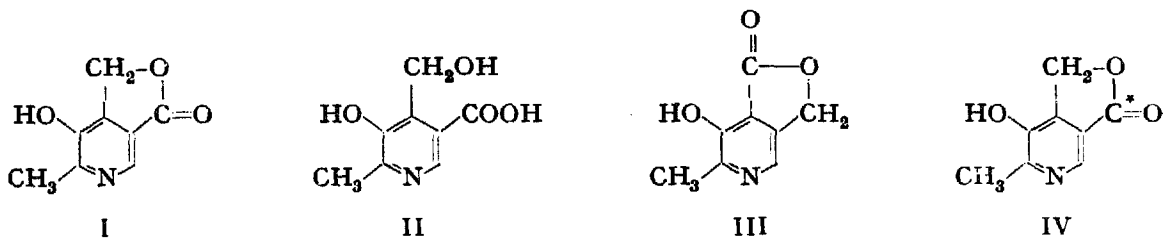


Fig. 1.

The lactone of 5-pyridoxic acid (I) was synthesized according to the method of BADDILEY AND MATHIAS[3] and the 5-pyridoxic acid (II) was obtained from its lactone by heating with 0.1 N KOH on a steam bath for 2 h and then acidifying the solution with dilute HCl. The precipitated acid was recrystallized from absolute ethanol, m.p. 280–283° decomp.

A 100-mg sample of 5-pyridoxic acid was dissolved in 1 l of buffer solution $KH_2PO_4$–KOH (pH 5.8) and placed in an oven at 37° for 3 days. The solution was concentrated under reduced pressure (bath temperature, 50°) to about one-eighth of its volume and applied to a column (4.5 × 37 cm) of Dowex-1-formate. The column was eluted with 3 l of distilled water and then with 0.1 M formic acid at a flow rate of about 3 ml/min. Care was taken to analyze the fractions (ultraviolet spectra) as soon as they emerged from the column. Two fractions could be isolated. The first fraction was the lactone of 5-pyridoxic acid and the second the starting material, 5-pyridoxic acid. For identification of the two fractions advantage was taken of the different ultraviolet spectra of the acid (II) and its lactone (I) in 0.1 N HCl. The lactone had a maximum at 251 mμ while the acid showed no absorption at that region[1]. Under these conditions 12% of the original acid was transformed into its lactone. When the fraction which contained the 5-pyridoxic acid was left overnight in the 0.1 M formic acid solution some lactone was formed as shown by ultraviolet spectra and by thin-layer chromatography. Silica Gel-G was used for thin-layer chromatography, the developing solvent was methanol–ether (1:1). Detection of the spots was possible by ultraviolet light or by spraying with 1% (w/v) 2,6-dichloroquinonechlorimide in toluene, followed by dilute ammonium hydroxide solution[1]. There was indication (ultraviolet spectrum) that some traces of lactone were formed even before application of the concentrate to the column.

4-Pyridoxic acid was synthesized according to the method of HEYL[4] and purified by dissolving it in aq. NaOH and reprecipitating by dilute HCl, m.p. 258–259° decomp.

* The Roman numerals refer to the formulae given in Fig. 1.

As for 5-pyridoxic acid, a 100-mg sample of 4-pyridoxic acid was dissolved in 1 l of buffer solution and placed in an oven at 37°. However, the buffer solution was placed in the oven for two days instead of three. Only 4-pyridoxic acid could be isolated from the Dowex-1-formate column indicating that no lactonization had occurred. When 4-pyridoxic acid was dissolved in 0.1 M formic acid and kept at room temperature only traces of lactone could be detected (ultraviolet spectrum) after one week. An appreciable amount of the lactone of 4-pyridoxic acid was formed after one month of storage at room temperature.

The change of pH accompanying the growth of Pseudomonas sp. MA was not given[2] so the behavior of 4-pyridoxic acid at a pH lower than 5.8 was not determined. It is probable that the growth medium reached a lower pH with Pseudomonas sp. MA than with sp. IA due to more acidic oxidation products. However, it seems unlikely that any lactonization takes place in short periods of time as only traces of lactone could be detected when the acid was suspended in 0.1 M formic acid (pH 2.4) for one week. Whether the lactone of 5-pyridoxic acid is an intermediate in the oxidation of pyridoxine by Pseudomonas sp. IA, is presently being studied in our laboratory with the aid of the $^{14}C$-labeled lactone of 5-pyridoxic acid (IV). The synthesis of the $^{14}C$-labeled lactone as well as the synthesis of $^{14}C$-labeled pyridoxine will be published elsewhere.

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## On the formation of estroprotein

Liver catalysis in the binding of [$^{14}C$]estrogens to albumin has been the subject of several reports in the last decade[1–6]. In contrast, the experiments described herein demonstrate that the metabolism *in vitro* of estrogens by liver slices does not lead to significant enzymatic binding to protein.

Rat-liver slices (250 mg) were incubated with 11 $\mu$g ($4 \cdot 10^5$ counts/min) of [16-$^{14}C$]estrone in 0.1 M phosphate buffer (pH 7.4) for 4 h as previously described[6]. The incubation mixture from a typical experiment, on centrifugation (900 $\times$ *g* for 5 min at 0°), afforded a supernatant fraction containing 75% of the added radioactivity. The liver pellet was rinsed with normal saline, removing another 3% of the radioactivity, then extracted with acetone for 24 h in a Soxhlet apparatus, and