839. *Synthetical Experiments in the B Group of Vitamins. Part V.* Novel Derivatives of Pyridoxine.*

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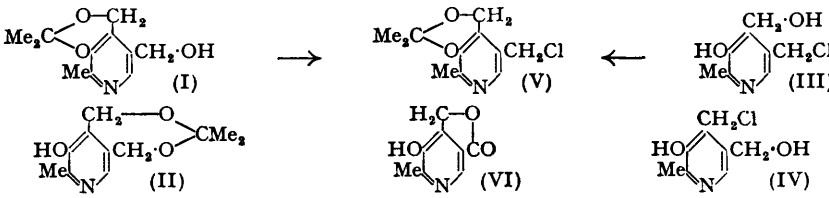
Pyridoxine hydrochloride yields an *iso*propylidene derivative (I) which provides a novel means of protection for the phenolic and 4-hydroxymethyl groups. A by-product in the conversion of 4 : 5-bisaminomethyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine into pyridoxine hydrochloride is 5-chloromethyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine (III). The structural relation between (I) and (III) is demonstrated by the chlorination of the former to a product (V), identical with the *iso*propylidene derivative of (III).

PYRIDOXINE HYDROCHLORIDE and acetone in the presence of sulphuric acid form an *iso*propylidene derivative, isolated as a hydrochloride $C_{11}H_{15}O_3N$,HCl. Of the possible structures (I) and (II) for the free base, the former was to be preferred since, apart from the less likely seven-membered ring in (II), involvement of the phenolic group is shown by a negative test with the 2 : 6-dichloroquinone-chloroimide reagent for a phenol with a free *para*-position; the test rapidly becomes positive when the compound is warmed with aqueous acid, which regenerates pyridoxine hydrochloride. In alkaline solution, *iso*propylidenepyridoxine is stable, in analogy with *iso*propylidene derivatives of sugars. The structure (I) has been confirmed by alkaline permanganate oxidation which yields, after acid hydrolysis, the known lactone (VI) of 3-hydroxy-4-hydroxymethyl-2-methylpyridine-5-carboxylic acid (cf. Harris, Stiller, and Folkers, *J. Amer. Chem. Soc.*, 1939, **61**, 1242).



This preparation of (I) is believed to be the first instance of the simultaneous protection of phenolic and alcoholic hydroxyl groups by cyclic ketal formation commonly used in carbohydrate chemistry, and should be more generally applicable to other aromatic compounds with these substituent groups similarly located. The *cyclo*hexylidene derivative of pyridoxine has also been prepared. It leaves the 5-hydroxymethyl group free for further reactions such as oxidation and esterification. Phosphorylation of this group is described by Baddiley and Mathias (*J.*, 1952, 2583) in the course of a synthesis of codecarboxylase, and further experiments relating to similar work will be reported later.

When 4 : 5-bisaminomethyl-3-hydroxy-2-methylpyridine is treated with sodium nitrite in hydrochloric acid solution for conversion into pyridoxine hydrochloride (preceding paper), the latter is extracted from the dry residue of the evaporated solution by absolute alcohol; sodium chloride is filtered off and pyridoxine hydrochloride crystallises. From the alcoholic mother-liquors there has been isolated a by-product in the form of an alcohol-soluble hydrochloride, $C_8H_{10}O_2NCl$,HCl, m. p. 170—171°. The base is 5-chloromethyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine (III), since it is a free phenol, and the alternative 4-chloromethyl compound (IV) is excluded by the ready formation of an *iso*propylidene derivative (V). Moreover, this *iso*propylidene derivative is identical with the chlorination product of (I). The structures of (I) and (III) are thus mutually confirmatory. It is not known whether the formation of (III) is due to a side reaction in which hydrochloric acid reacts with pyridoxine, or one in which some nitrosyl chloride is formed which chlorinates the 5-hydroxymethyl group. It has been found difficult to demonstrate

* Part IV, preceding paper.

unequivocally the formation of (III) by treatment of pyridoxine with even concentrated hydrochloric acid.

In microbiological assay with *Neurospora sitophila* (mutant 299) (III) has about one quarter of the activity of pyridoxine hydrochloride.

When treated with excess of diethylamine, (III) is converted into 5-diethylaminomethyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine, characterised as a dipicrate and dihydrochloride. The latter salt has little or no spasmolytic activity against histamine on isolated guinea-pig gut.

Experimental

iso*Propylidenepyridoxine*.—Pyridoxine hydrochloride (2 g.) was stirred with acetone (40 ml.) containing concentrated sulphuric acid (4 ml.) until dissolved. The solution was kept at room temperature overnight and treated with a solution of sodium (5·9 g.) in ethanol (100 ml.). The precipitated sodium sulphate was filtered off and washed with ethanol, and the filtrate evaporated to dryness under reduced pressure. The residue was extracted with ethanol and treated with anhydrous alcoholic hydrogen chloride and acetone, yielding iso*propylidenepyridoxine hydrochloride* (1·6 g.) which, crystallised from alcohol–ether, had m. p. 217—218° (decomp.) (Found: C, 53·0; H, 6·4; N, 5·6. $C_{11}H_{16}O_3NCl$ requires C, 53·6; H, 6·5; N, 5·7%). The base was liberated from the hydrochloride by aqueous sodium hydrogen carbonate and recrystallised from faintly ammoniacal water in colourless needles, m. p. 113—115° (Found: C, 64·0; H, 6·4; N, 7·1. $C_{11}H_{15}O_3N$ requires C, 63·2; H, 7·2; N, 6·7%). Pure *iso*propylidenepyridoxine gives no colour reaction with 2 : 6 dichloroquinone-chloroimide in an alkaline buffer, but the characteristic blue colour for a phenol is shown if the compound is previously hydrolysed by mere dissolution in dilute acid for a few seconds. The rate of acid hydrolysis has, however, not been studied quantitatively, and the phenol test is extremely sensitive.

cyclo*Hexylidenepyridoxine*.—Pyridoxine hydrochloride (3 g.) was stirred with *cyclo*hexanone (60 ml.) containing concentrated sulphuric acid (6 ml.) for 2½ hours. After 60 hours at room temperature the solution was treated with a solution of sodium (5·15 g.) in absolute alcohol, and the solvent and excess of *cyclo*hexanone were removed by distillation. The residue was extracted with chloroform, and, after removal of chloroform, the extract was treated with anhydrous alcoholic hydrogen chloride which yielded cyclo*hexylidenepyridoxine hydrochloride*, colourless crystals (from alcohol), m. p. 224—225° (decomp.) (Found: C, 59·0; H, 6·8; N, 5·4. $C_{14}H_{20}O_3NCl$ requires C, 58·8; H, 7·0; N, 4·9%).

5-Chloromethyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine Hydrochloride.—The alcoholic mother-liquors remaining from the isolation of pyridoxine hydrochloride after nitrous acid treatment of 4 : 5-bisaminomethyl-3-hydroxy-2-methylpyridine (preceding paper) were concentrated under reduced pressure and treated with anhydrous ether, which precipitated a hydrochloride, melting range 150—160°. Repeated crystallisation from absolute alcohol yielded cream-coloured flat needles, m. p. 170—171° (decomp.) (Found: C, 43·1; H, 5·2; N, 6·0; Cl, 31·3. $C_8H_{11}O_2NCl_2$ requires C, 42·9; H, 4·9; N, 6·25; Cl, 31·7%).

A solution of this compound (2·24 g.) in dry acetone (25 ml.) containing sulphuric acid (2·5 g.) was kept at room temperature for 24 hours after being warmed for a short time at 40°. It was filtered and most of the acetone removed under reduced pressure. The concentrate was poured into chloroform and an excess of sodium hydrogen carbonate (5 g.). Chloroform-extraction was repeated three times. The extract was dried and evaporated, and the residual oil treated in a small amount of ethanol with anhydrous ethereal hydrogen chloride. The iso*propylidene* derivative *hydrochloride* crystallised and was collected, washed with acetone, and dried (1·2 g.). Recrystallised from alcohol–ether, it formed colourless fine needles, m. p. 190—191° (decomp.) (Found: N, 5·55. $C_{11}H_{15}O_2NCl_2$ requires N, 5·3%), readily soluble in water, the solution giving a negative 2 : 6-dichloroquinone-chloroimide test. Liberation of the phenol group by brief heating with dilute acid is shown by the rapid appearance of the characteristic blue colour reaction with the above reagent.

The compound was identical in m. p. and mixed m. p. with material prepared as follows, from *iso*propylidenepyridoxine. The latter was gently refluxed with excess of thionyl chloride under anhydrous conditions. After volatilisation of excess of reagent, the residue was crystallised from alcohol–anhydrous ether, forming colourless needles of the *iso*propylidene derivative hydrochloride, m. p. 190° (decomp.).

5-Diethylaminomethyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine Hydrochloride.—A mixture of the 5-chloromethyl compound (2·24 g., 0·01 mole) and diethylamine (7 g.) was heated at 60° in a sealed tube for 16 hours. After addition of N-sodium hydroxide (20 ml.) the mixture

was evaporated to dryness and the oily residue extracted with boiling absolute alcohol and filtered from sodium chloride. The alcohol was removed under reduced pressure and the residual brown viscous oil (2·45 g.) dissolved in water (25 ml.) and made slightly acid with dilute hydrochloric acid. Addition of excess of picric acid solution precipitated the *dipicrate* (2·4 g.) which, crystallised from acetic acid, had m. p. *ca.* 174° (Found: N, 16·55. $C_{24}H_{26}O_{16}N_8$ requires N, 16·4%). This was treated with dilute hydrochloric acid and exhaustively extracted with ether. The remaining aqueous solution was evaporated to dryness under reduced pressure, and the residue, on crystallisation from methanol–ether, yielded *5-diethylaminomethyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine hydrochloride*, decomp. 212—214° (Found: N, 9·2; Cl, 24·6. $C_{12}H_{22}O_2N_2Cl_2$ requires N, 9·4; Cl, 23·9%).

3-Hydroxy-4-hydroxymethyl-2-methylpyridine-5-carboxylic Lactone.—A solution of *iso*-propylidenepyridoxine (3 g.) in water (600 ml.) was stirred at room temperature while a solution of potassium permanganate (3·02 g.) in water (120 ml.) was run in during 3½ hours. Stirring was continued for a further 1½ hours, and manganese dioxide was removed and washed with water in the centrifuge. The combined supernatant liquid was just acidified to litmus with 2N-hydrochloric acid. A further precipitation of manganese dioxide was removed and the clear solution evaporated to dryness under reduced pressure. The residue was heated on a steam-bath for 1 hour with water (10 ml.) and concentrated hydrochloric acid (5 ml.) to effect hydrolysis and lactonisation. After evaporation to dryness the residue was redissolved in water and the product precipitated by addition of sodium acetate. Acetic acid was removed under reduced pressure and the solid collected after cooling, washed with a little cold water, and dried [0·8 g.; m. p. 270—280° (decomp.)]. The m. p. of material recrystallised from alcohol varied somewhat with heating conditions, the same specimen giving values from 273° (decomp.) (after softening from 270°) to 282—283° (Found: C, 58·1, 58·2; H, 4·4, 4·2; N, 9·0, 8·85. Calc. for $C_8H_7O_3N$: C, 58·2; H, 4·2; N, 8·5%).

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