Department of Biochemistry and Biophysics, Iowa State University

Synthesis of Vitamin B_6 Derivatives. II

3-Hydroxy-4-Hydroxymethyl-2-Methyl-5-Pyridine Acetic Acid and Related Substances (1)

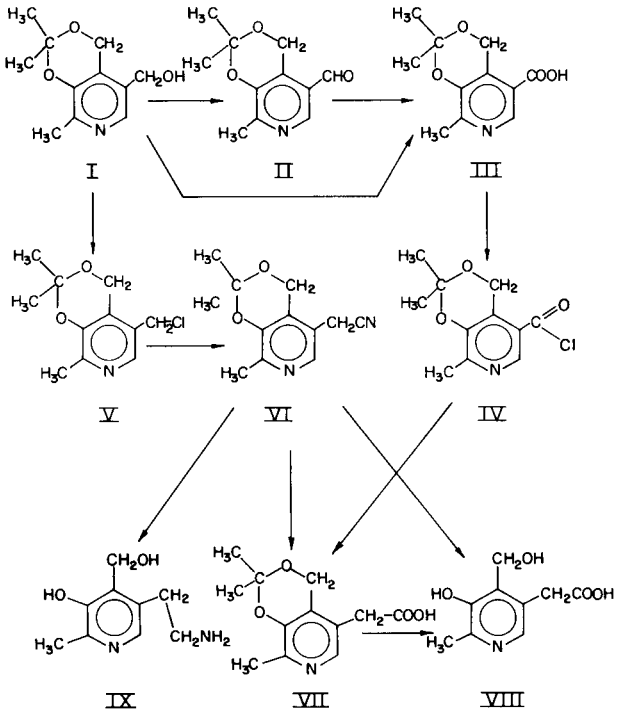
Isao Tomita, Houston G. Brooks and David E. Metzler

The syntheses of 3-hydroxy-4-hydroxymethyl-2-methyl-5-pyridineacetic and -5-pyridinepropionic acids and several related compounds are described. Although acid hydrolysis of α^4,3-*O*-isopropylidene-5-pyridoxic acid gives 5-pyridoxic acid lactone (α-pyracin), its higher homolog α^4,3-*O*-isopropylidene-pyridoxylformic acid gave a corresponding free alcohol whose carboxylic acid proton was shown to be exchanged rapidly with the 3-phenolic and -4-alcoholic -OH protons in nuclear magnetic resonance studies. Intermolecular hydrogen bonding between the side chain carboxylic acid and pyridine nitrogen atoms is suggested in the solid state.

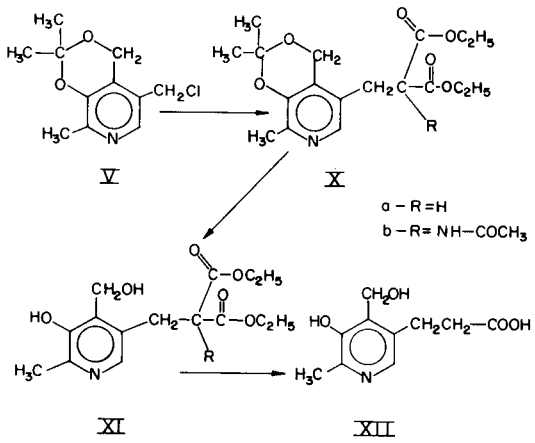
Analogs of pyridoxal, in which the 5-hydroxymethyl group has been replaced by carboxylic acid-bearing side chains, would be of considerable interest in studies of both enzymic and non-enzymic catalysis of reactions of amino acids (2). One approach to the synthesis of such aldehydes is to prepare the corresponding pyridoxol derivatives which may be subsequently oxidized to the aldehydes. In this paper we wish to report two different routes of synthesis of a homolog of 5-pyridoxic acid in which the 5-hydroxymethyl group of pyridoxol has been replaced by a two-carbon carboxylic acid side-chain (α^5-pyridoxylformic acid, VIII), as well as the synthesis of the related propionic acid homolog (α^5-pyridoxylacetic acid, XII). The latter compound has recently been prepared in a different way by Korytnyk (3). Preliminary studies have shown that this latter compound, α^5-pyridoxylacetic acid may be oxidized to the corresponding 4-aldehyde, a close analog of the coenzyme, pyridoxal phosphate. This new aldehyde readily forms Schiff bases with amino acids and its carboxylic acid side chain seems to act as an intramolecular catalyst in subsequent reactions (4). The starting material for these syntheses is α^4,3-*O*-isopropylidene pyridoxine (I), easily obtainable from pyridoxine hydrochloride (5). It was converted to the desired substances as shown in Schemes I and II.

One method of synthesis of α^5-pyridoxylformic acid, (VIII), is through the application of the Arndt-Eistert reaction to the isopropylidene derivative of 5-pyridoxic acid (III). This is similar to the preparation of pyridine-3-acetic acid from the 2-methyl ester of quinolinic acid (6).

Several methods of synthesis of 5-pyridoxic acid or its lactone (α-pyracin) have been described previously (7-12). Since the oxidation of pyridoxol takes place preferentially in its 4-hydroxymethyl side chain, these methods have all employed pyridoxol as the starting material in a form affording



SCHEME I



SCHEME II

protection of the 3-hydroxy and 4-hydroxymethyl groups. The most convenient compound of this type is α^4,3-*O*-isopropylidenepyridoxol (I). The oxidation of I to the isopropylidene derivative of 5-pyridoxic acid (III) was attempted using nickel peroxide (13) in an amount equivalent to 2.4 gram-atoms of oxygen per mole of I. No reaction took place, either in an alkaline water solution or in dioxane. (Although benzyl alcohol was completely oxidized to benzoic acid under these conditions.) It was possible to oxidize I to the desired carboxylic acid with potassium permanganate at room temperature in 43% yield. A more satisfactory method, however, was to oxidize I to the 5-aldehyde, isopropylideneisopyridoxal (II) with manganese dioxide (14) or with chromic acid-pyridine complex (7) and, in a second step, to oxidize II to III with hydrogen peroxide.

Hydrogen peroxide has been employed previously in the preparation of carboxylic acids of quinoline or isoquinoline from the aldehydes (15,16). In the present case, upon refluxing Compound II in acetone with an excess of hydrogen peroxide, the carboxylic acid (III) was formed gradually without any evident side products. In marked contrast to this result is the observation of Harris, *et al.* that oxidation of pyridoxal or pyridoxal phosphate leads to production of the corresponding 4-hydroxy compounds (17). This result can be explained by assuming the formation of a 4-*O*-formyl compound followed by hydrolysis (Dakin's reaction) under the alkaline (*p*H 10) conditions employed. In fact, no oxidation of pyridoxal by hydrogen peroxide occurs under the conditions that were used for our preparation of α^4,3-*O*-isopropylidene-5-pyridoxic acid. This lack of reactivity of pyridoxal presumably reflects the masking of the free aldehyde group of pyridoxal through internal hemiacetal formation.

The compound obtained by mild acid hydrolysis of α^4,3-*O*-isopropylidene-5-pyridoxic acid (III) was always the lactone (α-pyracin), a known product of bacterial metabolism of pyridoxal (18). This lactone was changed into the free carboxylic acid (19) under the same conditions as those employed by Heyl (20) for making 4-pyridoxic acid from its lactone.

Making use of the Arndt-Eistert reaction, compound III was converted to the pyridineacetic acid derivative (VII) via the carbonyl chloride, (IV), in

TABLE I

P.M.R. Spectra of Pyridoxol in Analogs in Dimethyl Sulfoxide (a)

Compound	2-CH_3	4-CH_2	5-CH_2	6-H	Others
α^4,3-*O*-Isopropylidene-pyridoxol (I) (b)	-135	-291	-265	-475	(5-OH)-307 (Isopropylidene)-89
5-Pyridoxic acid-lactone	-148	-320	----	-504	(3-OH)-629
α^4,3-*O*-Isopropylidene-pyridoxylformic acid (VII)	-138	-289	-210	-472	(Isopropylidene)-90 (Acid)-676
Pyridoxylformic acid (VIII)	-139	-277	-215	-464	(Acid)-517 (c)
Pyridoxylacetic acid (XII) (d)	-155	-286	-174	-484	(Acid) Broad (c)
5-Deoxypyridoxol	-138	-281	----	-464	(5-CH_3)-130 (e) (3-OH, α^4-OH)-403

(a) Expressed in units of c.p.s. at 60 Mc. The internal standard was TMS. All spectra were obtained using a Varian A-60 instrument. Either dimethyl sulfoxide or deuteriodimethyl sulfoxide was used as the solvent. (b) Compare with results reported in reference 25. (c) 3-OH and α^4-OH are included. (d) See Fig. 1 for complete spectrum. (e) It is not certain which proton peak belongs to the 2-CH_3 and which to the 5-CH_3.

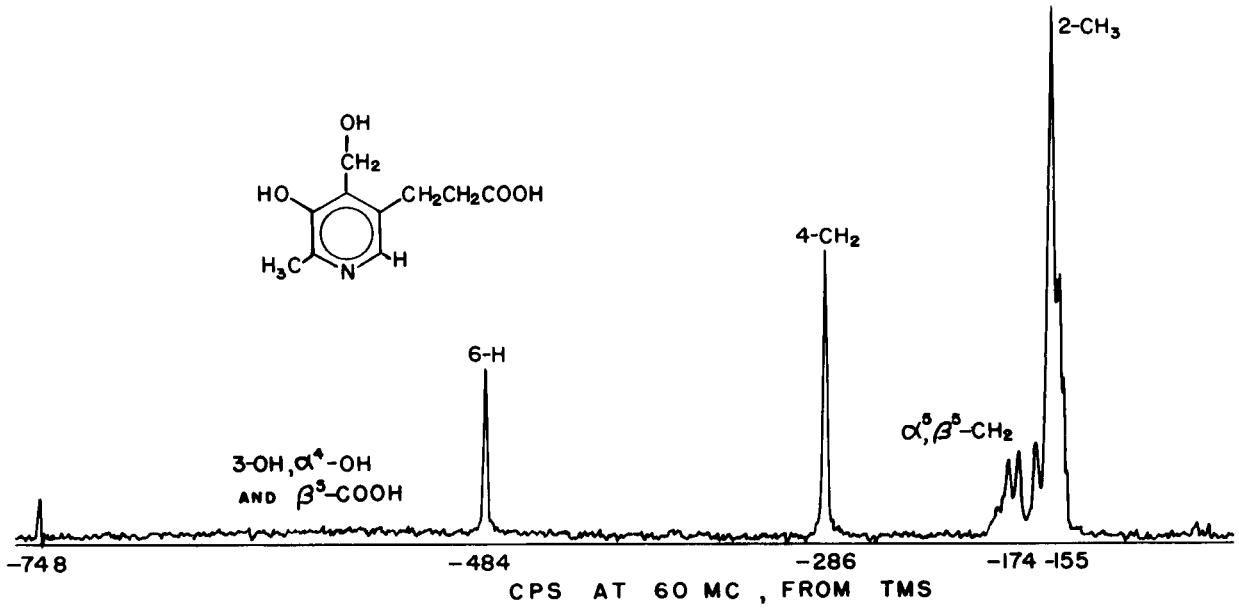


Figure 1. NMR spectrum of 5-pyridoxylacetic acid (XII) in deuterated DMSO. The very broad, low band which is centered at about -560 c.p.s. is attributed to the three protons of the 3-OH, α^4-OH and β^5-carboxyl groups. The sharp peak at -748 c.p.s. is a TMS audio frequency calibration side-band.

an overall yield of 22%, and the isopropylidene group was removed by acid hydrolysis with 8% formic acid to produce the 5-pyridoxic acid homolog, α^5-pyridoxylformic acid (VIII). Although it is not the best synthetic route to compound VIII, the Arndt-Eistert reaction does provide a convenient and general possible method for further extension of the side chain.

It is of interest to note that the hydrolysis of the isopropylidene derivative (VII) gave a free carboxylic acid, (as was proven by the infrared and proton magnetic resonance spectra and elemental analysis) rather than a lactone such as is obtained by hydrolysis of the isopropylidene derivative of 5-pyridoxic acid.

Compounds VII and VIII were also made by a second route. Isopropylidenepyridoxol (I) with thionyl chloride in benzene gave the 5-chloromethyl derivative (V) in excellent yield. This was converted to the cyanomethyl compound VI by reaction with potassium cyanide. The nitrile was hydrolyzed to the isopropylidene derivative (VII) of the desired carboxylic acid with sodium hydroxide, or to α^5-pyridoxylformic acid (VIII) directly with concentrated hydrochloric acid. The nitrile (VI) was also converted to the amino compound (IX), a homolog of isopyridoxamine, by reduction with lithium aluminum hydride.

In another series of reactions, the 5-chloromethyl compound (V) was condensed with malonic ester and acetamidomalonic ester to yield compounds Xa, Xb, XIa, and XIb. Acid hydrolysis of XIa gave the propionic acid homolog of 5-pyridoxic acid (α^5-pyridoxylacetic acid, XII). The same compound has been prepared in almost the same yield by a Knoevenagel condensation reaction between the 5-aldehyde (II) and the malonic acid ester followed by hydrogenation and acid hydrolysis (3).

As has been reported by Yoshida and Asai (21), and as has been discussed by Korytnyk (3), the infrared spectra of pyridine carboxylic acids in potassium bromide show two characteristic absorption bands which differ greatly from those of ordinary acids with dimeric carboxyl groups. Broad bands with peaks at 2448 cm^{-1} and 1850 cm^{-1} in α^4,3-*O*-isopropylidene-5-pyridoxic acid (III) must be related to its carboxyl group because of their absence in the methyl ester. Similar bands with peaks at 2500 cm^{-1} and 2083 cm^{-1} in pyridoxylformic acid (VIII) and at 2439 cm^{-1} (3) in the isopyropylidenepyridoxylformic acid (VII) likewise represent the characteristic carboxylic acid absorption. The presence of all these bands at lower frequencies than those of ordinary carboxylic acids may be attributed to the existence of intermolecular hydrogen bonding of the O-H--N type similar to that found in nicotinic acid (22). The higher frequency in VIII compared to that in VII may be explained on the basis of some intramolecular interaction between the 5-carboxyl and 4-hydroxymethyl group in addition to the intermolecular interaction.

Crystalline pyridoxal shows an intermolecular -O-H--N frequency at 2735 cm^{-1} instead of the usual free -OH frequency (23), and the same effect was

also clearly seen in the spectrum of 5-pyridoxic acid lactone. Clearly, the same type of interaction can also occur in α^5-pyridoxylformic acid.

Proton magnetic resonance spectroscopy has also been used in the elucidation of structures of vitamin B_6 derivatives (3,24,25). We have assigned the proton peaks of pyridoxylformic acid (VIII) and pyridoxylacetic acid (XII) on the basis of comparison with several analogs of pyridoxol as shown in Table I. Because of the presence in our deuteriodimethylsulfoxide of a small amount of dimethylsulfoxide-D_5, the proton signals of the methyl group at position 2 of these compounds show a little more than 3 protons in their integrals. In the case of pyridoxylacetic acid, the peaks of the two pairs of protons in the 5-α and 5-β positions are split into 2 triplets with a sharp singlet for the methyl of position 2 and with 5 lines which are attributed to the dimethylsulfoxide-D_5 (Fig. 1).

It is interesting to note that the proton resonance of the phenolic OH appears at a very low field as can be seen for 5-pyridoxic acid lactone (-629 c.p.s.). The same is true for 3-hydroxypyridine (26). This result may be explained on the basis of the zwitterionic structure which is known to occur in aqueous solutions (27). When the carboxyl group and aliphatic OH are added to the 3-hydroxypyridine structure as in compounds VIII and XII, the signal for the OH is moved upfield and a singlet (or a very broad peak) representing the 3 protons of these groups is observed. This doubtless arises as a result of a rapid proton exchange among these groups, as has been suggested by Korytnyk and Paul (25).

Pyridoxylformic acid shows a rather sharp singlet at -517 c.p.s. and pyridoxylacetic acid a very broad peak which rises only slightly above the base line (Fig. 1). The 3 protons in pyridoxol and the 2 protons in 5-deoxypyridoxol appear at -393 c.p.s. and at -403 c.p.s. respectively as rather broad peaks.

EXPERIMENTAL

All melting points were obtained in a capillary tube or on a Fisher-Johns block and are corrected. The elemental analyses were done by Schwarzkopf Microanalytical Lab., Woodside, N. Y.

α^4,3-*O*-Isopropylidenepyridoxol hydrochloride was prepared according to Korytnyk and Wiedeman (5). It was converted to the free base form by placing in an excess of a saturated solution of sodium bicarbonate. After all reaction had ceased the mixture was placed on a Buchner filter and the crystals were washed with cold water until the *p*H of the washings fell to about 6.

α^4,3-*O*-Isopropylidene-5-pyridoxic Acid (III).

(1) To a stirred solution of 24 g. of α^4,3-*O*-isopropylidenepyridoxol (I) (0.115 mole) in 200 ml. of acetone and 500 ml. of water, there was added, dropwise with good stirring, a solution of 36 g. (0.23 mole, equivalent to 3.0 gram-atoms of oxygen per mole of isopropylidenepyridoxol) of potassium permanganate in 1 l. of water during a period of 8 hours. After the addition was complete, stirring was continued for an additional 3 hours, the mixture was kept at room temperature overnight, and the manganese dioxide was removed by filtration with the aid of "celite". The clear filtrate was evaporated to dryness under reduced pressure 3 times with water to remove ammonia.

To the viscous residue, 200 ml. of cold water was added and the resulting solution was kept in the refrigerator. The crystals which appeared were proved to be starting material and were removed by suction filtration.

Subsequent to extraction of this filtrate with 200 ml. each of ether and chloroform, this alkaline solution was carefully neutralized to *p*H 6.0 with 10% hydrochloric acid and was treated with charcoal.

The clear slightly yellow solution was then evaporated to dryness under reduced pressure. To the residue was added 200 ml. of cold formic acid (8%). The white crystals were collected, washed with cold 8% formic acid and acetone, and were recrystallized from a mixture of cyclohexane and ethanol or from large amounts of acetone (11.1 g., 43%, m.p. 219-220° with decomposition). The recovery of the starting material was 6.4 g. (27%).

Anal. Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28. Found: C, 59.29; H, 5.74; N, 6.55.

Following essentially the same procedure described above, the oxidation of isopropylidenepyridoxol was carried out with potassium permanganate equivalent to 2.5 gram-atoms and 5.0 gram-atoms of oxygen, respectively. With the smaller amount of permanganate, the recovery of starting material was 60.0% and the yield of isopropylidene-5-pyridoxic acid was 3.1%, while with the larger amount of oxidant, the yields were 0 and 15.6% respectively.

(2) Eighteen g. of crude isopropylideneisopyridoxal (II) (0.087 mole) which was prepared by manganese dioxide oxidation of isopropylidenepyridoxol (12) was refluxed in a mixture of 100 ml. of anhydrous acetone and 50 ml. of 30% hydrogen peroxide for 7 hours. After standing at room temperature overnight, white crystals (13.2 g.) separated. These were washed with anhydrous acetone, and the combined filtrate was again refluxed for 3 hours with an additional 10 ml. of 30% hydrogen peroxide. The change of the aldehyde into the carboxylic acid was conveniently checked by thin-layer chromatography, (The Rf values of the aldehyde and carboxylic acid were 0.88, 0, respectively in ethyl acetate (alumina) and 0.65, 0.57 in the solvent system of normal butanol: acetic acid: water=4:1:5 (silica gel). Dragendorff reagent (28) was used for detecting these substances).

Careful evaporation of the solvent yielded a thick syrup to which 50 ml. of ethanol was added. The evaporation of alcohol, followed by acidification with 100 ml. of cold 8% formic acid, gave white crystals (4.7 g.) which decomposed at 217-219° and showed no melting point depression when mixed with the substance obtained by procedure 1.

α^4,3-*O*-Isopropylidene-α^5-pyridoxylformic Acid (VII).

Isopropylidene-5-pyridoxic acid (III) (4.4 g.) (2.0×10^{-2} mole) was suspended in 100 ml. of dry benzene and 6 g. (5.0×10^{-2} mole) of thionyl chloride was added. The reaction mixture was warmed at 60° for 30 minutes, and the precipitate was collected and washed with acetone, (m.p. 228-230° with decomposition). This material was suspended in 200 ml. of anhydrous ether, excess of an ethereal solution of diazomethane (made from 14.4 g. of DuPont EXR-101 diazomethane precursor) was added, and the solution was held at room temperature overnight. Evaporation of the ether left a residue which was washed with benzene. The resulting light yellow solid was next suspended in 100 ml. of dioxane. This dioxane solution of diazoketone was added dropwise at 50-60° to a mechanically stirred mixture of 1 g. of silver oxide, 2.5 g. of anhydrous sodium carbonate and 1.5 g. of sodium thiosulfate in 100 ml. of water. The mixture was stirred further for 3 hours after the final addition of diazoketone, while the temperature was raised to 90-100° in an oil bath where it was held for 30 minutes. The solution was then evaporated to near dryness, and was carefully neutralized with normal hydrochloric acid. The clear yellow solution which was obtained by treatment with charcoal (Norit) was evaporated to dryness, and the evaporation was repeated twice again with the addition of methanol. The residue was extracted with ethanol and the extract was again evaporated to dryness, the minimum amount of water required to dissolve the residue was added. Trituration of this solution in an ice bath gave white crystals which were collected after the solution had been stored in a refrigerator overnight, yield, 1.0 g. (22%), decomposition at 181-184°.

After several recrystallizations from absolute alcohol, the compound melted at 186-187°, dec.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.63; H, 6.56; N, 5.70.

α^4,3-*O*-Isopropylidene-α^5-pyridoxyl chloride Hydrochloride (V) (29-31).

To a solution of 10.5 g. (5.9 x 0.05 mole) of isopropylidenepyridoxol (I) in 200 ml. of anhydrous benzene, there was added dropwise with stirring a solution of 6.5 g. (5.0 x 10^{-2} mole) of thionyl chloride in 20 ml. of dry benzene. The product precipitated almost immediately. The mixture was heated just to the boiling point of the solvent, cooled and filtered with suction. The yield of the vacuum dried crude product was 13.0 g. (98%), m.p. 188-190°, dec.

Recrystallization from a large volume of anhydrous acetone afforded 12.6 g. (95%) m.p. 191-192°. The compound was usually only washed with hot acetone once before use in subsequent reactions rather than being recrystallized.

Anal. Calcd. for $C_{11}H_{15}Cl_2NO_2$: C, 50.01; H, 5.72; N, 5.30. Found: C, 49.99; H, 5.61; N, 5.31.

α^4,3-*O*-Isopropylidene-α^5-pyridoxyl Nitrile (VI).

To a mechanically stirred suspension of 2.64 g. (0.01 mole) of isopropylidene-α^5-pyridoxyl chloride hydrochloride (V) in 50 ml. of acetone there was added a solution of 4.55 g. (0.07 mole) of potassium cyanide in 17 ml. of water and the stirred mixture was refluxed for 16 hours. The solvent then was removed by distillation under reduced pressure on a water bath and the oily residue partially solidified on standing at room temperature for 2 hours. Recrystallization of this semi-solid first from water and then from petroleum ether (Skelly B, b.p. 60-71.2°) afforded 1.88 g. (86%) of the pure nitrile, m.p. 90-91°.

Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.46; N, 12.85. Found: C, 66.25; H, 6.47; N, 12.69.

α^4,3-*O*-Isopropylidene-α^5-pyridoxylformic acid (VII) from (VI).

Three g. of isopropylidene-α^5-pyridoxyl nitrile (VI) (1.4 x 10^{-2} mole) and 4 g. of sodium hydroxide in 40 ml. of 70% ethanol were refluxed for 3 hours. After evaporation of the solvent, the residue was dissolved in a small amount of water and was neutralized carefully with 10% hydrochloric acid. The clear solution obtained by treatment with charcoal was completely dried in a vacuum desiccator. Following extraction of this material with several portions of hot ethanol and evaporation of the extracts to dryness, the compound was crystallized from absolute alcohol. The white crystals decomposed at 186-187°. A mixed melting point of this substance with the compound obtained from the 5-carboxylic acid (III) showed no depression. The yield of the pure product was 2.70 g. (83%).

α^5-Pyridoxyl Formic Acid (3-Hydroxy-4-(hydroxymethyl)-2-methyl-5-pyridine Acetic Acid) (VIII).

(1) A solution of 2.18 g. (1.0 x 10^{-2} mole) of isopropylidene-α^5-pyridoxyl nitrile (VI) in 5 ml. of concentrated hydrochloric acid was heated at 40° on a water bath for 45 minutes. A white crystalline precipitate of inorganic material separated when the solution was placed in a refrigerator. The precipitate was discarded and the acidic supernatant was carefully neutralized to *p*H 6.0 with 10% aqueous sodium hydroxide. White crystals came out upon trituration and cooling in an ice bath. An additional amount of the compound was obtained by evaporation of the filtrate to dryness under reduced pressure, addition of a small amount of water to dissolve the residue and seeding. The compound was recrystallized from water several times.

The total yield was 0.85 g. (43%); m.p. 217-218°, dec.

Anal. Calcd. for $C_9H_{11}NO_4$: C, 54.82; H, 5.62; N, 7.10. Found: C, 54.61; H, 5.62; N, 6.69; λ max 290 mμ (ϵ = 9.0 x 10^3) in 0.1 *N* hydrochloric acid; λ max 244 mμ (ϵ = 7.1 x 10^3), 310 (ϵ = 7.8 x 10^3) in 0.1 *N* sodium hydroxide.

(2) α^4,3-*O*-Isopropylidenepyridoxyl formic acid (III) (1.2 g.) (5.0 x 10^{-3} mole) was added to 20 ml. of 8% formic acid and heated on a steam bath for 15 minutes. After treatment with charcoal, this solution was evaporated to half its volume and was neutralized to about *p*H 6 with 5% sodium hydroxide. The white crystalline product which soon separated was collected by filtration and was dried over calcium chloride, yield, 0.87 g. (87%); m.p. 217-218°, dec. The mixed melting point with the substance obtained by Method 1 was not depressed. The Rf value of this compound in TLC (Silica gel G) was 0.22 (the solvent system; acetic acid; butanol; water = 1:4:5).

In another experiment, a compound of m.p. 118-121° (which then decomposed above 210°) was isolated. It showed the same Rf value in TLC and showed the following elementary analysis. C: 54.35; H: 5.43.

α^5-Pyridoxylethylamine Dihydrochloride (5-(2-Aminoethyl)-3-hydroxy-4-hydroxymethyl-2-methylpyridine Dihydrochloride) (IX).

To a stirred solution of 0.57 g. (0.015 mole) of lithium aluminum hydride in 50 ml. of anhydrous ether (0°) there was added dropwise, during a period of 30 minutes a solution of 3.27 g. (0.015 mole) of α^4,3-*O*-isopropylidenepyridoxyl nitrile (VI) in 70 ml. of anhydrous ether and the mixture was stirred for an additional hour at 0°. With continued cooling and stirring, 2 ml. of ice water was added cautiously, and after 10 minutes 2 ml. of 20% aqueous sodium hydroxide was added, followed by 5 ml. of water. The ether layer was decanted and the granular inorganic hydroxides were washed with two 50 ml. portions of ether. The washings were combined with the decanted ether layer and dried over anhydrous sodium sulfate. Removal of the drying agent and evaporation of the ether under reduced pressure left an oily residue which crystallized partially upon standing. The semi-solid material was dissolved in 40 ml. of 1 *N* hydrochloric acid, and the resulting solution was heated on a steam bath for 15 minutes and evaporated to dryness under reduced pressure. This residue then was dissolved in 150 ml. of hot (75°) absolute ethanol and the solution was concentrated by distillation under reduced pressure to 50 ml. Upon standing in a refrigerator overnight (14 hours) crystalline product separated. The concentration process was repeated until 1.5 g. (45%) of the product was obtained, m.p. 190-191°.

Anal. Calcd. for $C_9H_{16}Cl_2N_2O_2$: C, 42.37; H, 6.32; N, 10.98. Found: C, 42.52; H, 6.43; N, 11.34.

α^4,3-*O*-Isopropylidene-α^5-pyridoxylmalonic Acid Diethyl Ester Hydrochloride (Xa).

To a stirred solution of 0.92 g. (0.02 g. at. wt.) of sodium in 50 ml. of absolute ethanol there was added 6.4 g. (0.04 mole) of diethyl malonate, and after a period of 15 minutes, there was added 5 g. (0.019 mole) of α^4,3-*O*-isopropylidene-α^5-pyridoxyl chloride hydrochloride (V) and 1 g. of potassium iodide. The mixture was stirred at room temperature (25-30° for 48 hours). The solvent was removed by distillation under reduced pressure, and the oily residue was treated with a solution of 2 g. of sodium bicarbonate in 50 ml. of water, then extracted with three 75 ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate. The drying agent was removed by filtration, and anhydrous hydrogen chloride was bubbled through the ethereal solution for a period of 30 minutes. An oil, which initially separated, slowly crystallized on standing. The solid was removed by filtration, and after three recrystallizations from ethyl acetate, the yield of the pure product was 2.5 g. (34%), m.p. 148-149°.

Anal. Calcd. for $C_{18}H_{26}ClNO_8$: C, 55.81; H, 6.76; N, 3.61. Found: C, 56.01; H, 6.76; N, 3.58.

α^4,3-*O*-Isopropylidene-α^5-pyridoxyl-(acetamidomalonic Acid) Diethyl Ester (Xb).

To a stirred solution of 0.92 g. (0.04 g. at. wt.) of sodium dissolved in 50 ml. of absolute ethanol, there was added 8.7 g. (0.04 mole) of diethyl acetamidomalonate, and, after a period of 15 minutes, there was added 5 g. (0.019 mole) of α^4,3-*O*-isopropylidene pyridoxyl chloride hydrochloride (V) and 1 g. of potassium iodide. The mixture was stirred at room temperature (25-30° for 48 hours). The solvent was removed by distillation under reduced pressure, and the oily residue was treated with 50 ml. of 2% aqueous sodium hydroxide, then extracted with four 50 ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The solid residue was recrystallized from petroleum ether (Skelly B, b.p. 60-71.2°), yielding 5 g. (65%) of pure air-dried product, m.p. 122-123°.

Anal. Calcd. for $C_{20}H_{28}N_2O_7$: C, 58.81; H, 6.91; N, 6.86. Found: C, 58.83; H, 6.75; N, 6.86.

α^5-Pyridoxylmalonic Acid Diethyl Ester (XIa).

A solution of 1.9 g. (0.005 mole) of (Xa) in 20 ml. of 1 *N* hydrochloric acid was heated on a steam bath for 15 minutes, then cooled and neutralized to *p*H 8 with 20% aqueous sodium hydroxide. The precipitated crystalline product was air-dried and recrystallized from 80% ethanol. The yield of the desired compound was 1.2 g. (80%), m.p. 134-135°.

Anal. Calcd. for $C_{15}H_{21}NO_6$: C, 57.87; H, 6.80; N, 4.50. Found: C, 57.76; H, 6.67; N, 4.43.

α^5-Pyridoxyl-(acetamidomalonic Acid) Diethyl Ester (XIb).

A solution of 3 g. (0.007 mole) of (Xb) in 15 ml. of hot (70°) 1 *N* hydrochloric acid was heated on a steam bath for 15 minutes, cooled and neutralized to *p*H 8 with 20% aqueous sodium hydroxide. The precipitated crystalline air-dried product was recrystallized from 1-propanol, yielding 2.5 g. (93%), m.p. 187-188°.

Anal. Calcd. for $C_{17}H_{24}N_2O_7$: C, 55.79; H, 6.51; N, 7.54. Found: C, 55.13; H, 6.20; N, 7.47.

α^5-Pyridoxylacetic Acid Hydrochloride (XII).

(1) A solution of 1 g. (0.003 mole) of (XIa) in 10 ml. of concentrated hydrochloric acid was heated under reflux for 4.5 hours, then evaporated to dryness under reduced pressure on a water bath. The residue was recrystallized from a mixture of ethylene glycol monoethyl ether and ethyl acetate, yielding 0.5 g. (68%) of the product, m.p. 213-215°, dec.

This substance (1.8 g., a 25% yield) was obtained also by the acid hydrolysis of the crude compound (Xa) without isolation of the intermediates. A mixed melting point of the materials obtained from the two procedures showed no depression (mixed m.p. 213-215°).

Anal. Calcd. for $C_{10}H_{14}ClNO_4$: C, 48.49; H, 5.70; N, 5.66. Found: C, 48.76; H, 5.69; N, 5.86.

(2) A 10 g. portion (0.038 mole) of compound V was treated as described above for the preparation of compound Xa through the step of extraction with ether. Three 100 ml. portions of ether were used for this purpose. After drying over anhydrous sodium sulfate the combined ether extracts were evaporated to yield a dark brown oil. This oil was refluxed for 4.5 hours with 20 ml. of concentrated hydrochloric acid, after which the solution was treated with Norit and evaporated to dryness under reduced pressure. Addition of 200 ml. of anhydrous acetone to the brownish, viscous oil gave dark yellow crystals which decomposed at 200-206°, yield 4.2 g. After several recrystallizations from anhydrous alcohol, the compound showed the decomposition point of 213-216°. The Rf value in TLC (Silica gel G) with the solvent system: acetic acid: butanol: water (1:4:5 by volume) was 0.28-0.30 and in the system: ethanol: water: butanol (1:4:5 by volume) it was 0.12.

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