amine (170 g, 2.2 moles) was added over 30 min. After 2 hr at 25°, the oil layer was separated, washed with water, dried over calcium sulfate, and concentrated under reduced pressure to a residue weight of 400 g. The latter was cooled to −40° and filtered to obtain 190 g of colorless, crystalline IV. This was a quantitative yield based on the acid chloride. An analytical sample was recrystallized from heptane. The infrared absorption spectra of IV and VI were compared to show that VI has characteristic absorption bands for carbamate C=O at 5.74 and 5.84 μ while IV has characteristic absorption bands for conjugated ketone C=O at 5.99 and for secondary amide C=O at 6.05 μ. The nmr spectra for VI and IV were compared to show that the resonance position of the methyl group on the aromatic substrate (VI) was at 2.3 ppm while the methyl group attached to the nonaromatic substrate (IV) appeared at 1.5 ppm. The melting points of VI and IV were 199–200° and 98–99°, respectively.

Anal. Calcd for $C_{17}H_{27}NO_2$: C, 73.70; H, 9.81; N, 5.18. Found: C, 73.63; H, 9.56; N, 5.85.

Hydrolysis of IV with potassium hydroxide, concentrated and dilute sulfuric acid, and concentrated hydrochloric acid produced the phenol (I) as the only product isolated.

3,5-Di-*t*-butyl-1-methyl-2,5-cyclohexadien-4-one-N,N-dimethylcarboxamide (V).—A toluene solution of the acid chloride was prepared as previously described except that the washing with water was omitted. After the excess phosgene had been removed under vacuum at 25°, 25% aqueous dimethylamine (149 g, 0.83 mole) was fed at 25°. After 1 hr of stirring at 25° water (300 ml) was added and the oil layer was separated, washed again with water, and dried over Drierite. The solution was concentrated under reduced pressure and crystallized to obtain 122 g of crystalline V. This corresponded to an over-all yield of 84% based on I. An analytical sample was crystallized from acetonitrile to obtain V having mp 84–86°.

Anal. Calcd for $C_{18}H_{29}NO_2$: C, 74.20; H, 10.03; N, 4.81. Found: C, 74.96; H, 9.96; N, 5.16.

The infrared absorption spectrum showed bands at 6.04 μ (tertiary amide C=O).

Summary

Phosgene reacted only under special conditions with 2,6-di-*t*-butyl-4-methylphenol. The product was 3,5-di-*t*-butyl-1-methyl-2,5-cyclohexadien-4-onecarboxylic acid chloride rather than the expected chloroformate of the phenol.

Registry No.—III, 7492-84-4; IV, 7492-85-5; V, 7492-86-6.

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Synthesis and Decarboxylation of Pyridine Carboxylic Acids from Pyridoxol[1]

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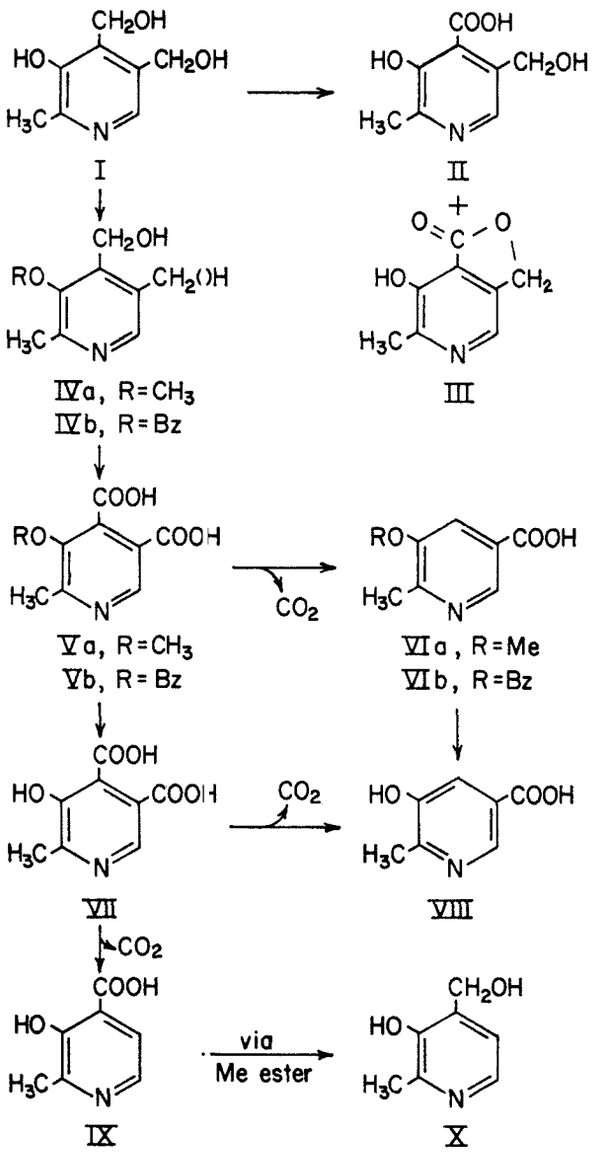
Bacterial oxidation of pyridoxol (I, Chart I) gives rise to several unusual pyridine derivatives.[4-6] These include 4-pyridoxic acid (II) and its lactone (III),[6] 5-pyridoxic acid,[4] 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylic acid,[6] 2-methyl-3-hydroxypyridine-4,5-dicarboxylic acid (VII),[6] and 2-methyl-3-hydroxypyridine-5-carboxylic acid (VIII).[6] Compound VII is rapidly decarboxylated to VIII by an enzyme isolated from these bacteria.[7] For further study of enzymatic steps in the degradation of vitamin B_6, a source of several of these compounds for use as substrates was required. This paper describes convenient procedures for obtaining VII and VIII from pyridoxol (I), which is commercially available. Both VII[8,9] and VIII[10] have been prepared previously by longer procedures from acyclic precursors. The formation and decarboxylation of VII were also of interest as a possible method for specifically degrading labeled pyridoxol in studies of its biosynthesis.

CHART I



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(4) V. W. Rodwell, B. E. Volcani, M. Ikawa, and E. E. Snell, *J. Biol. Chem.*, **233**, 1548 (1958).

(5) M. Ikawa, V. W. Rodwell, and E. E. Snell, *ibid.*, **233**, 1555 (1958).

(6) R. W. Burg, V. W. Rodwell, and E. E. Snell, *ibid.*, **235**, 1164 (1960).

(7) E. E. Snell, A. A. Smucker, A. Ringelmann, and F. Lynen, *Biochem. Z.*, **341**, 109 (1964).

(8) R. G. Jones and E. C. Kornfeld, *J. Am. Chem. Soc.*, **73**, 107 (1951).

(9) A. Itiba and S. Emoto, *Sci. Papers Inst. Phys. Chem. Res. (Tokyo)*, **38**, 347 (1941); *Chem. Abstr.*, **35**, 6960 (1941).

(10) C. J. Argoudelis and F. A. Kummerow, *J. Org. Chem.*, **26**, 3420 (1961).

Attempts to obtain VII by direct oxidation with 8 equiv of permanganate failed. Chromatography of the product on Dowex-1 formate[6] showed fairly large amounts of II and III,[11] but only a 2.5% yield of VII. Previous studies[12,13] showed that oxidation of IVa to Va was feasible. The yield was improved by carrying out the reaction under slightly alkaline conditions. Va was easily separated from by-products by chromatography on Dowex-1 formate.[6]

The possibility that the benzyl group might be superior to the methyl group for protecting the phenolic group of pyridoxol[14] was investigated. By reaction of pyridoxol with dimethylbenzylphenylammonium chloride, 3-O-benzylpyridoxol (Vb) was prepared in higher yields than the corresponding O-methyl ether (IVa) can be obtained with diazomethane. Oxidation of IVb with permanganate, although incomplete in water because of its insolubility, proceeded smoothly in aqueous pyridine to give 2-methyl-3-benzyloxypyridine-4,5-dicarboxylic acid (Vb), from which 2-methyl-3-hydroxypyridine-4,5-dicarboxylic acid (VII) was readily obtained by hydrogenolysis.

Compounds Va, Vb, and VII all lost up to 1 equiv of carbon dioxide on heating the free acid in quinoline or nitrobenzene at 180–200°. Approximately equal amounts of the two monocarboxylic acids VIII and IX were formed in this way from VII, together with minor amounts of four additional unidentified compounds. The previously unknown compound (IX) was identified by conversion *via* its methyl ester and reduction to 2-methyl-3-hydroxy-4-hydroxymethylpyridine (X). This unexpected lack of specificity for one position in the decarboxylation of VII largely disappears when the phenolic ethers Va and Vb are decarboxylated. At 180–200° Va was converted nearly quantitatively to 2-methyl-3-methoxypyridine-5-carboxylic acid (VIa), which upon refluxing with HBr gave 2-methyl-3-hydroxypyridine-5-carboxylic acid (VIII) in high yield. Under similar conditions decarboxylation of the benzyl ether (Vb) gave VIb (70% yield) which on hydrogenolysis yielded VIII.

The low over-all yields from pyridoxol limit the usefulness of these reactions for the stepwise degradation of isotopically labeled pyridoxol, but the reactions are useful for preparing substrates VII and VIII for enzymatic studies. In general, the benzylated derivatives gave over-all yields as good or better than the methylated derivatives, and their reactions were more convenient in most cases.

Experimental Section[15]

Oxidation of 3-O-Methylpyridoxol (IVa) to 2-Methyl-3-methoxypyridine-4,5-dicarboxylic Acid (Va).—Compound IVa was prepared according to Stiller, *et al.*,[12] with an average yield of 45%. When more than 2 g of pyridoxol was used, the yield of product was reduced. Potassium permanganate (6.35 g, 40 mmoles) in 400 ml of water was mixed with 7 ml of 1.0 *M* sodium hydroxide and added during 3 hr to a stirred, aqueous solution of 2.75 g (15 mmoles) of IVa at room temperature. After an additional 10 hr, the residual permanganate was destroyed with methanol at 60–70°, and the solution was filtered. The combined filtrate and washings were concentrated under vacuum to 200 ml and applied to a Dowex-1 formate column (2.6 × 30 cm). The column was washed with 0.5 l. of 0.25 *M* formic acid to elute 3-O-methyl-4-pyridoxic acid lactone, a minor by-product, which was discarded. The column was then eluted with 5 *M* formic acid until the ultraviolet absorption at 280 mμ reached blank values. The eluate was evaporated to dryness under vacuum and the residue crystallized from water. The product, Va monohydrate, was dried at 110°: yield 2.05 g (63%); mp 222° dec (lit.[8] mp 209–210°)[8]; λ_{max} (0.1 *M* HCl) 283 mμ (a_m 7200), (0.1 *M* NaOH) 278 mμ (a_m 4400), (0.1 *M* phosphate, pH 7.0) 288 mμ (a_m 4400).

3-O-Benzylpyridoxol (IVb).—The benzylation was carried out as described by Cohen, *et al.*,[14] with some modifications. To 20.56 g (100 mmoles) of pyridoxol hydrochloride in water was added 100 mmoles of sodium hydroxide. The solution was evaporated under vacuum, and the residue was dried overnight at room temperature, then extracted with hot absolute methanol until all the pyridoxol had been extracted, as shown by absence of ultraviolet absorption in the extract. To the combined extracts at room temperature were added 100 mmoles of sodium methoxide and 24.75 g (100 mmoles) of dimethylbenzylphenylammonium chloride, each in 50 ml of absolute methanol. After 10 min, the red-brown solution was decanted from the sodium chloride into a separatory funnel, and added over a period of 0.5 hr to 1500 ml of hot xylene, stirred by nitrogen, and arranged to distil off the methanol and a little xylene, the distillate coming off at 65–80°. After an additional 10 min, when all the methanol had been distilled off, the solution was decanted from the reaction flask, and cooled first to room temperature, then in ice. The pale tan, crystalline precipitate was filtered, washed with ice-cold ether, and dried in air, yield 15.77 g. By extracting the tarry residue in the reaction flask with boiling benzene and evaporating this extract with the mother liquors from the first crystallization to about 20 ml, a dark brown precipitate was obtained on cooling which was recrystallized from 50 ml of benzene to yield 1.51 g of product for a total yield of 67%. Recrystallization from ether (relatively large volumes are required to dissolve the product) and then from benzene, yields white needles of pure material. 3-O-Benzylpyridoxol hydrochloride, prepared by adding hydrogen chloride in ether to a solution of IVb in ethanol, precipitates as small rhombic crystals: mp 187° (lit.[14] mp 178°); λ_{max} (0.1 *M* HCl) 280 mμ (a_m 8100), (0.1 *M* NaOH) 273 mμ (a_m 5600), (0.1 *M* phosphate, pH 7.0) 274 mμ (a_m 5000).

Anal. Calcd for $C_{15}H_{17}NO_3 \cdot HCl$: C, 60.90; H, 6.13; N, 4.74. Found: C, 60. 56; H, 6.26; N, 4.91.

In a second preparation in which toluene was used in place of xylene, the product before recrystallization was less colored and the yield was increased to almost 90%. Presumably the lower boiling point of toluene causes less decomposition. Benzene might possibly be an even better solvent.

Oxidation of 3-O-Benzylpyridoxol (IVb) to 2-Methyl-3-benzyloxypyridine-4,5-dicarboxylic Acid (Vb).—To 15.8 g (60.8 mmoles) of IVb in 100 ml of pyridine was added with stirring a mixture of 25.6 g (162.2 mmoles) of potassium permanganate partially dissolved in 1480 ml of pyridine, and 608 ml of 0.2 *M* sodium hydroxide. Undissolved permanganate was rinsed in with a minimum amount of water. The mixture was stirred at room temperature for 2 hr, at 70° for 1 hr, then allowed to stand at room temperature overnight, by which time the permanganate color was completely discharged. The solution was filtered, the precipitate was extracted with three 200-ml portions of 0.01 *M* sodium hydroxide, and the combined filtrate and extracts were evaporated at 40° to about 300 ml, cooled in ice, acidified with 6 *M* HCl to about pH 2, and filtered with suction. The air-dried lemon yellow precipitate (yield, 15.85 g, 80%) containing 1 to 3 water of crystallization, was recrystallized from ethanol or water to give rhombic rods: mp 217° dec; λ_{max} (0.1 *M* HCl) 285 mμ (a_m 6900), (0.1 *M* NaOH) 277 mμ (a_m 4900), (0.1 *M* phosphate pH 7.0) 278 mμ (a_m 4500).

2-Methyl-3-hydroxypyridine-4,5-dicarboxylic Acid (VII).—A solution of 5.75 g (18.8 mmoles) of Vb in 600 ml of methanol–water (10:1, v/v) was added to 400 mg of palladium–charcoal catalyst (previously equilibrated with hydrogen) in 100 ml of the same solvent and hydrogenated at room temperature and pressure until hydrogen uptake ceased (18.8 mmoles was used).

(11) J. W. Huff and W. A. Perlzweig [*J. Biol. Chem.*, **155**, 345 (1944)] showed that II and III were major products of partial oxidation of pyridoxine with permanganate.

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

(13) R. Kuhn and G. Wendt, *Ber.*, **72**, 305 (1939).

(14) A. Cohen, J. W. Haworth, and E. G. Hughes, *J. Chem. Soc.*, 4374 (1952).

(15) All melting points are uncorrected. Ultraviolet spectra were determined with a Beckman DU or Cary 14 spectrophotometer.

The suspension was filtered, and the residue was extracted repeatedly[16] with hot water until ultraviolet absorption indicated completion of the extraction. The filtrate and extracts on evaporation to dryness at 40°, yielded 3.12 g (15.8 mmoles, 84%) of crude dicarboxylic acid. On purification by chromatography on Dowex-1 formate[6] followed by recrystallization from water, the product agreed fully in melting point, ultraviolet spectrum, and enzymological properties with the product isolated from bacterial culture media.[5]

Decarboxylation of 2-Methyl-3-hydroxypyridine-4,5-dicarboxylic Acid (VII).—Compound VII (197 mg, 1 mmole) was suspended in 10 ml of nitrobenzene in a small flask fitted with a nitrogen inlet tube and reflux condenser, with a carbon dioxide absorber connected to the condenser. Under a slow stream of nitrogen, the suspension was heated to 180–200° (oil bath) and maintained at this temperature until no more carbon dioxide was evolved (about 1 hr). The yield of carbon dioxide was 0.97 equiv. Most of the reaction products separated from the cooled nitrobenzene solution. The balance was obtained by extraction of the filtrate with 1 *M* aqueous ammonia in the presence of a little carbon tetrachloride to aid in the separation of the phases. The excess ammonia was evaporated, and the products were precipitated by adjusting the solution to pH 3.

The crude mixture was applied to a Dowex-1 formate column (2.2 × 28 cm) and eluted by increasing formic acid concentrations. The three major components, in order of elution, were VIII (eluted by 0.1 *M* formic acid, 35% yield), IX (eluted by 2 *M* formic acid, 33% yield), and unreacted starting material, VII (eluted by 5 *M* formic acid, 8%). Compound IX crystallized from water to give fine rods: mp 302–308° dec; λ_{max} (0.1 *M* HCl) 312 mμ (a_m 7200), (0.1 *M* NaOH) 307 mμ (a_m 5900), (0.1 *M* phosphate, pH 7.0) 307 mμ (a_m 5900).

Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.77; H, 4.56; N, 9.38.

Methyl 2-Methyl-3-hydroxypyridine-4-carboxylate (IX Methyl Ester).—Compound IX (200 mg, 1.3 mmoles) in 15 ml of methanol was saturated with hydrogen chloride and refluxed for 14 hr. The solvent was removed below 40°, 10 ml of water was added, and excess sodium bicarbonate was added. The product was extracted into three 25-ml portions of ethyl acetate, and the extract was dried with sodium sulfate. Upon partial evaporation and cooling, 145 mg (0.87 mmole, 67%) of the ester crystallized out. The ester sublimed at 100° and 14 mm (oil bath), forming colorless platelets on the seeded condenser: mp 48.5°; λ_{max} (0.1 *M* HCl) 315 mμ (a_m 7000), (0.1 *M* NaOH) 343 mμ (a_m 5500), (0.1 *M* phosphate, pH 7.0) λ_{max} 322 mμ (shoulder at 350 mμ) (a_m 4300).

Anal. Calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.15; H, 5.27; N, 7.92.

2-Methyl-3-hydroxy-4-hydroxymethylpyridine Hydrochloride (X HCl). Compound IX methyl ester (150 mg, 1.1 mmoles) in 15 ml of dry ether was added dropwise to 40 mg of lithium aluminum hydride in 25 ml of ether. The solution was refluxed for 20 min; excess reductant was destroyed by addition of ethyl acetate. After evaporation to dryness, the residue was dissolved in water, applied to a Dowex-1 formate column, and eluted with 0.2 *M* formic acid. The eluate was evaporated to dryness and the residue was dissolved in water and applied to a Dowex-50 column (acid form). After washing with water the column was eluted with 5 *M* hydrochloric acid. The first ultraviolet-absorbing fraction (λ_{max} 283 mμ) was collected, concentrated to a small volume, decolorized with charcoal, and evaporated to dryness. After washing in ethanol, the crystalline product melted at 165–168° (lit.[17] mp 165–166°); λ_{max} (0.1 *M* HCl) 233 and 283 mμ (a_m 3000 and 6900, respectively), (0.1 *M* NaOH) λ_{max} 260 and 319 mμ (a_m 8000 and 5800, respectively), (0.1 *M* phosphate, pH 7.0) 248 and 312 mμ (a_m 6500 and 7600). The product is easily distinguished by chromotography and spectrum from the isomeric 2-methyl-3-hydroxy-5-hydroxymethylpyridine.[6]

Decarboxylation of 2-Methyl-3-methoxypyridine-4,5-dicarboxylic Acid (Va).—A suspension of Va (211 mg, 1 mmole) in 15 ml of nitrobenzene was heated under nitrogen to 180–190° for 0.5–1 hr as described earlier for VII. The compound dissolved completely, and the yield of carbon dioxide was quantitative. The decarboxylation product, which crystallized on cooling, was washed with benzene and ether, then sublimed at 180° (14 mm) to give a 90% yield of VIa as small, colorless prisms: mp 224°; λ_{max} (0.1 *M* HCl) 242 and 298 mμ (a_m 5100 and 9970, respectively), (0.1 *M* NaOH) 227 and 228 mμ (a_m 6700 and 7400), (0.1 *M* phosphate, pH 7.0), 227 and 288 mμ (a_m 6500 and 7400).

Anal. Calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.79; H, 5.61; N, 8.64.

Decarboxylation of 2-Methyl-3-benzyloxypyridine-4,5-dicarboxylic Acid (Vb).—A suspension of Vb (2.87 g, 10 mmoles) in 40 ml of nitrobenzene was heated at 180–190° as described earlier. The compound dissolved completely; after 10 min CO_2 evolution ceased and the flask was cooled. The crude products were separated by filtration and ammonia extraction of the nitrobenzene layer as described earlier. The crude product (1 g) was suspended in 2 *M* hydrochloric acid (170 ml), heated, cooled, and filtered. The unreacted Vb remained on the filter. On adjusting the filtrate to pH 3, VIb crystallized as small plates: mp 230–231°; yield 43%; λ_{max} (0.1 *M* HCl) 242 and 297 mμ (a_m 7000 and 8900, respectively), (0.1 *M* NaOH) 287 mμ (a_m 6600), (0.1 *M* phosphate, pH 7.0) 287 mμ (a_m 6600).

Anal. Calcd for $C_{14}H_{13}NO_3$: C, 68.94; H, 5.32; N, 5.67. Found: C, 69.12; H, 5.38; N, 5.76.

2-Methyl-3-hydroxypyridine-5-carboxylic Acid (VIII). A. From VIa.—One millimole (167 mg) of VIa was gently refluxed in 5–10 ml of 48% HBr for 4 hr, then evaporated to dryness under reduced pressure. The residue was dissolved in a little water, and the product was precipitated by adjusting to pH 3 and recrystallized from water (yield 70%). The compound was identical both in spectral and chromatographic properties with the product from natural sources;[6] like the latter, it sublimed at 280–300°, but on rapid heating underwent modification at about 305° and sublimed at 325–330°.

B. From VIb.—Crude VIb (2.3 g) in 1 l. of methanol–water (1:1, v/v) was hydrogenated at room temperature and pressure over 400 mg of palladium–charcoal catalyst. About 10.5 mmoles of hydrogen was absorbed. The filtrate was evaporated to 200 ml at 40°, applied to a column of Dowex-1 formate (4 × 42 cm), and eluted with formic acid. A total of 933 mg (6.1 mmoles) of VIII was obtained by evaporation of the appropriate fractions. The over-all yield (from Vb *via* VIb to VIII) was 36%.

Registry No.—III, 4753-19-9; IVb, 7442-21-9; Va, 7442-22-0; Vb, 7442-23-1; VIa, 7442-24-2; VIb, 7442-25-3; VIII, 7428-22-0; IX, 4328-92-1; X, 7442-27-5; IX methyl ester, 7442-72-0; X HCl, 7442-73-1.

(16) During the reaction a gray precipitate containing much of the product formed. This was prevented in later experiments by doubling the amount of solvent and increasing the water to 20–50% by volume.

(17) G. J. Martin, S. Avakian, and J. Moss, *J. Biol. Chem.*, **174**, 495 (1948).

Synthesis and Reactions of 5-Bromoskatole and 5-Bromo-1,3-dimethylindole[1a]

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Skatole (3-methylindole) and 1,3-dimethylindole are appropriate model compounds for the more complex 3-alkylindoles, such as those derived biogenetically from tryptophan and tryptamine. We desired the benzene ring (*bz*) monobromoskatoles and 1,3-dimethylindoles as reference compounds for bromination studies and as synthetic intermediates. The synthesis of 5-bromoskatole (**3**) and 5-bromo-1,3-dimethylindole (**8**) is described here.

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