The Synthesis of 4-Deoxypyridoxine Phosphates.

By R. F. Long and A. L. Morrison.

[Reprint Order No. 5473.]

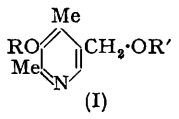
The preparation of the two crystalline dihydrogen phosphates of 4-deoxypyridoxine is described.

Early phosphorylations of 4-deoxypyridoxine (I; R = R′ = H) gave products which inhibited dissociated pyridoxal phosphate dependent enzymes (Beiler and Martin, *J. Biol. Chem.*, **1947**, **169**, 345; Umbreit and Waddell, *Proc. Soc. Exp. Biol. Med.*, 1949, **70**, 293) but were not specified nor fully described. Recently, pure 4-deoxypyridoxine 5-(dihydrogen phosphate) [I; R = H, R′ = $PO(OH)_2$] has been prepared (Peterson and Sober, *J. Amer. Chem. Soc.*, 1954, **76**, 169). We have prepared this compound in lower yield by a less direct route and also the isomeric 4-deoxypyridoxine 3-(dihydrogen phosphate) [I; R = $PO(OH)_2$, R′ = H].

Reaction of the potassium salt of (I; R = R′ = H) with tetrabenzyl pyrophosphate in *tert.*-butanol (Atherton and Todd, B.P. 674,089) gave dibenzyl 5-hydroxymethyl-2 : 4-dimethyl-3-pyridyl phosphate [I; R = $PO(O{\cdot}CH_2Ph)_2$, R′ = H], the hydrochloride of which was hydrogenolysed to give the crystalline 5-hydroxymethyl-2 : 4-dimethyl-3-pyridyl dihydrogen phosphate [I; R = $PO(OH)_2$, R′ = H]. This compound gave no

colour with ferric chloride and no reaction with Gibbs reagent, 2 : 6-dichloroquinone chloroimide, indicating that the phenol group was no longer free.

Phosphorolysis of aneurin acetate with anhydrous phosphoric acid had been used to prepare aneurin monophosphate (Morrison, Atherton, and Avison, B.P. 687,674).



(I)

Phosphorolysis of 5-acetoxymethyl-3-hydroxy-2 : 4-dimethylpyridine [I; R = H, R′ = AcO] therefore offered a route to the required dihydrogen phosphate [I; R = H, R′ = $PO(OH)_2$]. The acetate [I; R = CH_2Ph, R′ = AcO] was made by benzylating 4-deoxypyridoxine with benzyldimethylphenylammonium chloride, and acetylating the 3-benzyloxypyridine to give the 5-acetoxymethyl-compound [I; R = CH_2Ph, R′ = AcO] from which the benzyl group was hydrogenolysed. It was found to be unnecessary to carry out the last step, however, because, when the 3-benzyloxy-pyridine was heated with anhydrous phosphoric acid the phosphate [I; R = H, R′ = $PO(OH)_2$] was obtained, and was isolated, by means of its water-soluble barium salt, as the crystalline free acid. Benzyl phenyl ether gave phenol and benzyl dihydrogen phosphate when heated with anhydrous phosphoric acid under these conditions.

The deoxypyridoxine 5-phosphate [I; R = H, R′ = $PO(OH)_2$] was a moderately water-soluble, crystalline compound, giving a strongly positive Gibbs test at a dilution of 1 : 100,000 and a crimson colour with ferric chloride solution. Both phosphates inhibited dissociated tyrosine decarboxylase (cf. Umbreit and Waddell, *loc. cit.*), the 3-phosphate being about half as active as the 5-phosphate (Hawkins, unpublished work; Gale, personal communication).

Ultra-violet absorption measurements on some of the compounds described in this paper have been carried out by Lunn and Morton (*Analyst*, 1952, **77**, 718).

Experimental

Dibenzyl 5-Hydroxymethyl-2 : 4-dimethyl-3-pyridyl Phosphate [I; R = $PO(O{\cdot}CH_2Ph)_2$, R′ = H].—To 3-hydroxy-5-hydroxymethyl-2 : 4-dimethylpyridine hydrochloride (3·77 g.) in *tert.*-butanol containing 10% v/v of dry benzene (50 ml.), was added potassium *tert.*-butoxide (0·04 mole) in the same solvent. Tetrabenzyl pyrophosphate (10·7 g.) was added to the stirred mixture at 0° and stirring continued for 4 hr. Potassium chloride and potassium dibenzyl phosphate were filtered off and the filtrate was evaporated to dryness under reduced pressure. The residue was partitioned between chloroform and water. The chloroform layer was washed with ice-cold N-sodium hydroxide and distilled water, dried (Na_2SO_4), and evaporated under reduced pressure to give the dibenzyl phosphate as an oil (6·2 g.). The oil was dissolved in ether, the solution treated with charcoal and filtered, and the ester converted into its *hydrochloride*, which had m. p. 114—116° (from ethanol–ether) (6·2 g., 72%) (Found: C, 57·7; H, 5·9; N, 3·25; P, 7·2. $C_{22}H_{24}O_5NP,HCl$ requires C, 58·6; H, 5·6; N, 3·1; P, 6·9%).

5-Hydroxymethyl-2 : 4-dimethyl-3-pyridyl Dihydrogen Phosphate [I; R = $PO(OH)_2$, R′ = H].—The hydrochloride (4·5 g.) of the dibenzyl ester in 50% aqueous ethanol (50 ml.) was hydrogenolysed, a palladium–carbon catalyst (0·4 g.) being used (0·021 mole of hydrogen was taken up rapidly). The catalyst was filtered off and washed with distilled water, and the filtrate was concentrated to 25 ml. Acetone (200 ml.) was added and the precipitated crude *dihydrogen phosphate* crystallised from water–acetone, giving needles, m. p. 205—207° (1·4 g., 56%) (Found: C, 40·0; H, 5·0; N, 6·3; P, 13·0%; *M*, by titration, 237. $C_8H_{12}O_5NP$ requires C, 41·0; H, 5·1; N, 6·0; P, 13·4%; *M*, 233).

3-Benzyloxy-5-hydroxymethyl-2 : 4-dimethylpyridine (I; R = CH_2Ph, R′ = H).—3-Hydroxy-5-hydroxymethyl-2 : 4-dimethylpyridine hydrochloride (18·95 g.) was suspended in dry methanol. Benzyldimethylphenylammonium chloride (23·35 g.) and sodium methoxide (10·8 g.) in dry methanol were added, sodium chloride was filtered off, and the methanol solution was added dropwise during 1 hr. to boiling dry xylene (200 ml.). The methanol and some xylene distilled off during the addition, after which heating was continued for a further 1½ hr. The solution was cooled to room temperature and chloroform (*ca.* 300 ml.) was added. The solvent was then washed successively with distilled water, N-sodium hydroxide solution, and distilled water. Drying (Na_2SO_4), evaporation, and distillation at 0·3 mm. left a pinkish-grey solid which was recrystallised from benzene–light petroleum (b. p. 40—60°), to give the 3-*benzyloxy*-compound, m. p. 72—73° (14·1 g., 58%) (Found: N, 5·5. $C_{15}H_{17}O_2N$ requires

N, 5·7%). The *hydrochloride*, formed quantitatively, had m. p. 183—185° (Found : C, 63·8; H, 6·6; N, 5·2; Cl^-, 12·6. $C_{15}H_{17}O_2N,HCl$ requires C, 64·2; H, 6·4; N, 5·0; Cl^-, 12·7%).

5-Acetoxymethyl-3-benzyloxy-2 : 4-dimethylpyridine (I; R = CH_2Ph, R′ = OAc).—The 3-benzyloxy-compound (I; R = CH_2Ph, R′ = H) (12·2 g.) was treated with acetic anhydride (30 ml.) in pyridine (30 ml.) for 24 hr. at room temperature. Pyridine and acetic anhydride were evaporated under reduced pressure; the residue was dissolved in water, the solution made alkaline with aqueous sodium hydrogen carbonate, and the product extracted into chloroform. The chloroform solution was dried, the solvent evaporated, and the *acetate* distilled *in vacuo*; it had b. p. 160—163°/0·3 mm. (11·4 g., 80%) (Found : C, 71·7; H, 7·0; N, 5·3; Ac, 14·5. $C_{17}H_{19}O_3N$ requires C, 71·6; H, 6·7; N, 4·9; Ac, 15·1%). The *hydrochloride*, recrystallised from ethyl acetate containing 2% of ethanol, had m. p. 161—163° (Found : N, 4·7; Cl^-, 11·6; Ac, 12·7. $C_{17}H_{19}O_3N,HCl$ requires N, 4·4; Cl^-, 11·1; Ac, 13·4%).

5-Acetoxymethyl-3-hydroxy-2 : 4-dimethylpyridine Hydrochloride.—The 3-benzyloxy-hydrochloride (2·7 g.) was hydrogenolysed in ethyl acetate–ethanol, a palladium–carbon catalyst being used. The catalyst was filtered off and ether added; the precipitated *3-hydroxy-hydrochloride*, crystallised from cold ethanol–ether, had m. p. 180—181° (1·2 g., 62%) (Found : C, 51·2; H, 5·95; N, 6·6; Cl^-, 16·3; Ac, 17·4. $C_{10}H_{13}O_3N,HCl$ requires C, 51·8; H, 6·1; N, 6·1; Cl^-, 15·3; Ac, 18·5%).

5-Hydroxy-4 : 6-dimethyl-3-pyridylmethyl Dihydrogen Phosphate [I; R = H, R′ = $PO(OH)_2$]. —5-Acetoxymethyl-3-benzyloxy-2 : 4-dimethylpyridine (10·0 g.) was mixed with anhydrous phosphoric acid (34 g.), prepared by distilling the calculated amount of water from commercial 85% phosphoric acid at 120°/0·1 mm. The mixture was stirred under boiling dry toluene (500 ml.), which was distilled off at 60 ml./hr. The acetic acid which distilled over with the toluene was estimated by titration. After 4½ hr., 82% of the acetyl content of the starting material had been evolved as acetic acid. Heating was continued for a further 1½ hr. The mixture was cooled and the toluene decanted from the oily phosphoric acid layer which was dissolved in ice-cold water (100 ml.) and made alkaline (phenolphthalein) with saturated baryta solution. The barium phosphate precipitate was filtered off and thoroughly washed with distilled water (10 × 100 ml.). The combined aqueous filtrates were concentrated to 200 ml. under reduced pressure, filtered, and brought to pH 4·2 with sulphuric acid. Barium sulphate was removed on the centrifuge, and the supernatant liquor, and washings, concentrated to 100 ml., treated with charcoal (0·5 g.), and filtered. Acetone (600 ml.) was added to the filtrate and the crude dihydrogen phosphate filtered off after 24 hr. at 0°; recrystallisation from a mixture of water (50 ml.) and acetone (200 ml.) at 40° gave the crystalline dihydrogen phosphate, m. p. 251—252° (3·5 g., 43%) (Found : C, 40·3; H, 5·2; N, 6·1; P, 13·6%; *M*, by titration, 234. Calc. for $C_8H_{12}O_5NP$: C, 41·0; H, 5·1; N, 6·0; P, 13·4%; *M*, 233).

Cleavage of Benzyl Phenyl Ether with Anhydrous Phosphoric Acid.—Benzyl phenyl ether (3·5 g.) was heated at 110° with anhydrous phosphoric acid (35 g.) for 4 hr. The mixture was dissolved in ice–water, and the insoluble portion (1·9 g.) filtered off and recrystallised from ethanol [giving unchanged material (1·4 g.)]. From one portion of the acidic aqueous filtrate tribromophenol, m. p. 95° (1·1 g.), was prepared by the addition of bromine water. The second portion was made just alkaline (phenolphthalein) with barium hydroxide solution, and the barium salt of benzyl dihydrogen phosphate isolated (0·6 g.) after removal of barium phosphate on the centrifuge (Found : P, 9·8. Calc. for $C_7H_7O_4PBa$: P, 9·6%).

RESEARCH DEPARTMENT, ROCHE PRODUCTS LIMITED,
WELWYN GARDEN CITY, HERTS.

[*Received, June 16th*, 1954.]