840. *Synthetical Experiments in the B Group of Vitamins. Part VI.* Synthesis of the Pyridoxine Analogue, 2-Benzyl-3-hydroxy-4 : 5-bishydroxymethylpyridine.*

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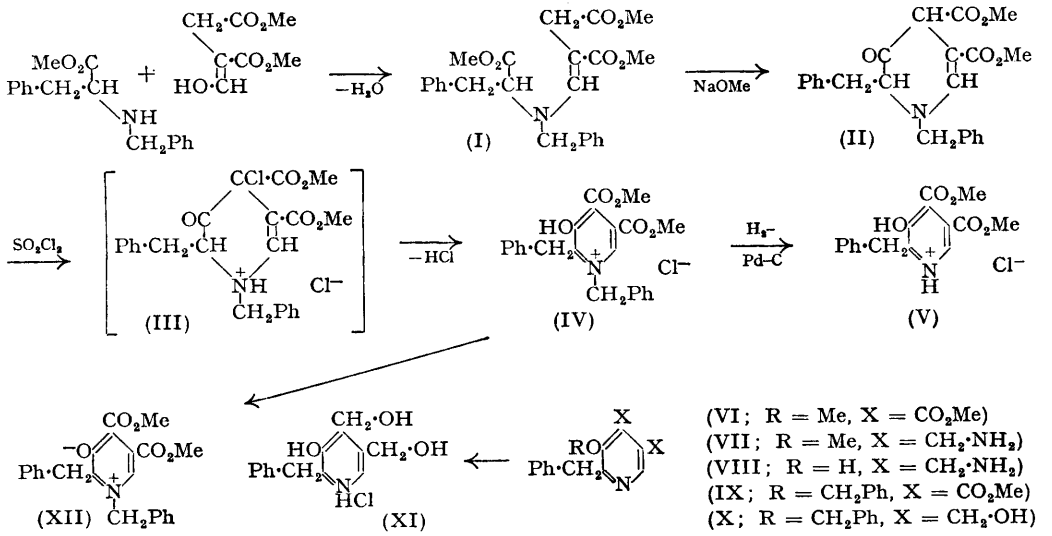
2-Benzyl-3-hydroxy-4 : 5-bishydroxymethylpyridine hydrochloride, a 2-benzyl analogue of pyridoxine, has been synthesised. It possesses little or no pyridoxine-like or antipyridoxine activity in microbiological tests.

Although a considerable number of compounds structurally related to pyridoxine have been examined for vitamin B_6 activity (for references see Williams, Eakin, Beerstecher, and Shive, "Biochemistry of B Vitamins," Reinhold, New York, 1950, p. 652), there has been comparatively little study of analogues in which the 2-substituent of the pyridine nucleus has been varied. The 2-ethyl analogue synthesised by Harris and Wilson (*J. Amer. Chem. Soc.*, 1941, **63**, 2526) showed less than 2% of pyridoxine activity in rats depleted of vitamin B_6. A synthesis of pyridoxine from an alanine derivative described in Part IV (*J.*, 1952, 4374) offers a flexible method of providing analogues with different 2-substituents, and 2-benzyl-3-hydroxy-4 : 5-bishydroxymethylpyridine hydrochloride (XI) has now been synthesised from a phenylalanine derivative. The synthesis of other analogues by this route has been undertaken by Dr. F. B. Kipping and his colleagues at Cambridge.

By the methods described in Part IV (*loc. cit.*), methyl α-benzylamino-β-phenylpropionate was condensed with methyl α-formylsuccinate, to yield methyl *N*-benzyl-*N*-

* Part V, preceding paper.

(1-carbomethoxy-2-phenylethyl)aminomethylenesuccinate (I) which was cyclised by sodium methoxide (cf. II). The crude cyclic keto-ester was directly treated with sulphuryl chloride, furnishing, presumably, through the intermediate chloroketone hydrochloride (III), the fully aromatic 1 : 2-dibenzyl-4 : 5-dicarbomethoxy-3-hydroxypyridinium chloride (IV). In spite of its quaternary salt character this compound is hydrolysed to such an extent in water, that the corresponding sparingly soluble phenol-betaine (XII) may be readily



isolated. The strength of the pyridinium base is no doubt considerably weakened by the neighbouring electron-attracting phenyl groups.

Hydrogenolysis of the *N*-benzyl group of (IV) led to methyl 2-benzyl-3-hydroxypyridine-4 : 5-dicarboxylate hydrochloride (V), the free base of which was methylated by diazomethane to methyl 2-benzyl-3-methoxypyridine-4 : 5-dicarboxylate (VI). This was converted by standard procedures *via* the diamide and dinitrile to the 4 : 5-bisaminomethyl compound (VII) which was demethylated by concentrated hydrobromic acid to 4 : 5-bisaminomethyl-2-benzyl-3-hydroxypyridine trihydrobromide (VIII). Exchange of hydrobromide for hydrochloride and then nitrous acid treatment furnished the desired 2-benzyl-3-hydroxy-4 : 5-bishydroxymethylpyridine hydrochloride (XI).

Alternatively, benzylation of the phenolic group of (V) with benzyldimethylphenylammonium hydroxide yielded methyl 2-benzyl-3-benzyloxypyridine-4 : 5-dicarboxylate (IX) which could be reduced by lithium aluminium hydride to 2-benzyl-3-benzyloxy-4 : 5-bishydroxymethylpyridine (X). Hydrogenolysis of the 3-benzyloxy-group then led to the 2-benzyl analogue (XI), a more convenient procedure than that outlined above.

Microbiological tests of the 2-benzyl analogue of pyridoxine by Miss J. Ward, B.Sc., of these laboratories, show that the compound only possesses slightly inhibitory action on *Staphylococcus aureus* and *B. coli* at a dilution of 1 : 2000. When tested alone it has no pyridoxine activity for *Neurospora sitophila* but, in the presence of 0·5 μg. of pyridoxine, 0·5 mg. of the analogue had a growth-promoting effect equivalent to that of 0·6 μg. of pyridoxine, indicating "activity" five thousand times less than that of pyridoxine. In conjunction with the results of animal tests on the ethyl homologue (Harris and Wilson, *loc. cit.*), it therefore appears that some degree of specificity is associated with the 2-methyl group for pyridoxine activity. This is rather surprising since the coenzyme function of pyridoxine and its congeners involves only the 4- and 5-hydroxymethyl groups, the former by its conversion into an aldehyde (pyridoxal) or aminomethyl (pyridoxamine) group, and the latter in esterification with phosphoric acid, as in pyridoxal phosphate. The influence of different 2-substituents on the nitrogen atom and the 3-hydroxy-group may contribute to the biological selectivity.

Experimental

α-Benzylamino-β-phenylpropionic acid was prepared in 77% yield by the method of Fischer and von Mechel (*Ber.*, 1916, **49**, 1355) from benzylamine and α-bromo-β-phenylpropionic acid (Marvel, *Org. Synth.*, **21**, 100).

Methyl α-Benzylamino-β-phenylpropionate.—α-Benzylamino-β-phenylpropionic acid (39 g.) was partly dissolved in concentrated sulphuric acid (50 ml.), mixed with methanol (50 ml.), and heated on a boiling-water bath under reflux for 2½ hours. Further quantities of methanol (10 ml.) and concentrated sulphuric acid (2 ml.) were added, and this repeated twice at hourly intervals during the heating. The mixture was cooled, poured on ice, and made alkaline with sodium hydrogen carbonate. The *ester* was extracted with ether, washed with water, dried, and distilled at 137—141°/0·3 mm. (yield, 32 g., 68%) (Found: C, 75·6; H, 7·3; N, 5·5. $C_{17}H_{19}O_2N$ requires C, 75·9; H, 7·1; N, 5·2%).

Esterification with methanol and hydrogen chloride gave an inferior yield. The reaction between benzylamine and methyl α-bromo-β-phenylpropionate at 100° (cf. Bischoff, *Ber.*, 1897, **30**, 3170) was also employed for preparing the α-benzylamino-ester but a considerable amount of *N-benzyl α-benzylamino-β-phenylpropionamide*, b. p. *ca.* 220°/0·4 mm., was also formed (Found: N, 7·95. $C_{23}H_{24}ON_2$ requires N, 8·15%).

Methyl N-Benzyl-N-(1-carbomethoxy-2-phenylethyl)aminomethylenesuccinate (I).—Methyl α-benzylamino-β-phenylpropionate (23·9 g.) and methyl α-formylsuccinate (18 g., 20% excess) were mixed, with development of heat, and then heated at 100° for 3 hours. The cooled viscous mixture was dissolved in anhydrous ether (80 ml.) from which the product separated as a colourless crystalline powder (23·5 g.). A further crop of 6·1 g. (total yield 78·4%) was obtained by evaporating the mother-liquor, removing unchanged formylsuccinate at 130°/oil-pump, and digesting the residue with ether. The *product*, crystallised from aqueous methanol, had m. p. 85—86° (Found: C, 67·7; H, 6·55; N, 2·9. $C_{24}H_{27}O_6N$ requires C, 67·8; H, 6·35; N, 3·3%).

1 : 2-*Dibenzyl*-4 : 5-*dicarbomethoxy*-3-*hydroxypyridinium Chloride* (IV).—A solution of the above itaconate (24·6 g.) in absolute methanol (130 ml.) was added to a solution of sodium (2·0 g.) in absolute methanol (50 ml.) and refluxed for 1¼ hours under nitrogen. The methanol was removed under reduced pressure and the residue dissolved in benzene and poured into a mixture of ice and sufficient acetic acid containing a little dilute sulphuric acid to render the mixture just acid to litmus. The aqueous portion was separated and re-extracted twice with benzene. The combined benzene extracts were washed with water, sodium hydrogen carbonate, and water again, and dried (Na_2SO_4). Evaporation of part of the solvent completed the drying, and the benzene solution was treated, with cooling, with sulphuryl chloride (4·0 ml.) added dropwise until no further precipitation of brown oil occurred. This heavy oil was allowed to settle, separated, and warmed at 40—45° under reduced pressure (water-pump). The residual frothy gum was dissolved in warm dry acetone (15 ml.), and a little dry ether added. On cooling, 1 : 2-*dibenzyl*-4 : 5-*dicarbomethoxy*-3-*hydroxypyridinium chloride*, m. p. 135—137° (decomp.), separated (19·4 g., 79%) (Found, on material recrystallised from methanol–ether: C, 64·5; H, 5·15; N, 3·45. $C_{23}H_{22}O_5NCl$ requires C, 64·5; H, 5·15; N, 3·3%). It gives a deep red colour with ferric chloride; its aqueous solution is acid to Congo-red, and deposits the corresponging *phenol-betaine* (XII), as a pale yellow powder, which, on being dried and recrystallised from methanol–ether, has m. p. 168° (decomp.) (Found: C, 69·6; H, 5·5. $C_{23}H_{21}O_5N$ requires C, 70·5; H, 5·35%).

Methyl 2-*Benzyl*-3-*hydroxypyridine*-4 : 5-*dicarboxylate Hydrochloride* (V).—A solution of the above pyridinium salt (17·7 g.) in methanol (50 ml.) was shaken in hydrogen with 10% palladised charcoal (1·9 g.). Absorption was rapid and almost quantitative (940 ml.). The filtered solution was evaporated to dryness under reduced pressure and the residue digested with dry ether and warmed with a little dry acetone which induced crystallisation (13·3 g., 95%). Crystallisation from methanol–ether gave colourless plates of *methyl* 2-*benzyl*-3-*hydroxypyridine*-4 : 5-*dicarboxylate hydrochloride*, m. p. 148—150° (decomp.) (Found: C, 57·3; H, 4·8; N, 4·2; Cl, 10·2. $C_{16}H_{16}O_5NCl$ requires C, 56·9; H, 4·7; N, 4·15; Cl, 10·5%). Hydrolysis with 10% sodium hydroxide solution furnished the 4 : 5-*dicarboxylic acid*, m. p. 241° (Found: C, 61·2; H, 4·2; N, 5·5. $C_{14}H_{11}O_5N$ requires C, 61·2; H, 4·0; N, 5·15%).

2-*Benzyl*-3-*methoxypyridine*-4 : 5-*dicarboxyamide*.—The above hydrochloride was converted into the free base by concentrated aqueous sodium hydrogen carbonate or acetate. Dried crude methyl 2-benzyl-3-hydroxypyridine-4 : 5-dicarboxylate so obtained (7·5 g.) was added portionwise to ethereal diazomethane (80 ml.) [from nitrosomethylurethane (12·5 ml.)]. After a few days at room temperature, excess of diazomethane and the solvent were removed. The

residual oily crude methyl 2-benzyl-3-methoxypyridine-4 : 5-dicarboxylate (VI) (negative test for phenol with dichloroquinone-imide reagent) was kept in liquid ammonia (25 ml.) in a sealed tube for 40 hours. Evaporation then left a yellow solid which was triturated with cold methanol, filtered off, and dried (5·0 g., 70%). *2-Benzyl-3-methoxypyridine-4 : 5-dicarboxyamide* crystallised from methanol in colourless plates, m. p. 194° (decomp. to imide and ammonia) (Found : C, 63·0; H, 5·2; N, 14·4; OMe, 11·1. $C_{15}H_{15}O_3N_3$ requires C, 63·1; H, 5·3; N, 14·7; OMe, 10·9%).

2-Benzyl-4 : 5-dicyano-3-methoxypyridine.—A cold suspension of the diamide (5·05 g.) in dry pyridine (30 ml.) and dry benzene (12 ml.) was stirred and treated with phosphoryl chloride (2·5 ml.) and warmed gradually; the temperature of the mixture suddenly rose spontaneously to about 40°. The mixture was stirred at about 65° for 1 hour, cooled, and decomposed with ice-water. The whole was evaporated to a small volume under reduced pressure and extracted with benzene. The benzene solution was washed with aqueous sodium hydrogen carbonate, dried, and freed from solvent. The residue was best purified by sublimation in a high vacuum, yielding the *dinitrile* as a white crystalline solid, m. p. 64° (3·23 g., 73%) (Found : N, 16·6. $C_{15}H_{11}ON_3$ requires N, 16·9%).

4 : 5-Bisaminomethyl-2-benzyl-3-methoxypyridine Trihydrochloride (cf. VII).—A solution of 2-benzyl-4 : 5-dicyano-3-methoxypyridine (3·23 g.) in methanol (95 ml.) and concentrated hydrochloric acid (5 ml.) was added in 3-ml. portions to a suspension of palladised charcoal (3·8 g.; cf. Alexander and Cope, *J. Amer. Chem. Soc.*, 1944, **66**, 888, note 7), and suspended in methanol (25 ml.) and water (5 ml.), the whole being shaken in hydrogen at approx. 1 atmosphere. Hydrogenation was complete in about 6 hours. The catalyst was filtered off, and the filtrate evaporated to dryness under reduced pressure. The residue was digested with absolute alcohol (5 ml.), filtered, and washed with alcohol (yield 3·35 g.); a further 0·5 g. was obtained by addition of dry ether to the alcoholic liquor. Recrystallisation from methanol–ether yielded *4 : 5-bisaminomethyl-2-benzyl-3-methoxypyridine trihydrochloride monohydrate*, m. p. 193° (Found : C, 47·3; H, 6·2; N, 11·0; Cl, 27·2. $C_{15}H_{22}ON_3Cl_3,H_2O$ requires C, 46·9; H, 6·2; N, 10·9; Cl, 27·7%).

4 : 5-Bisaminomethyl-2-benzyl-3-hydroxypyridine Trihydrobromide (cf. VIII).—A solution of the above methoxy-diamine trihydrochloride (2·25 g.) in hydrobromic acid (*d* 1·5; 20 ml.) was boiled under reflux for 2 hours. The solution was slightly diluted with water, treated with charcoal, and filtered hot. The filtrate was evaporated under reduced pressure until much crystalline product had separated. After cooling in ice, this was filtered off and washed with acetone, yielding 2·55 g. of the trihydrobromide which crystallised from methanol–ether as a *dihydrate*, m. p. 255—260° followed by decomposition (Found : Br, 46·2. $C_{14}H_{20}ON_3Br_3,2H_2O$ requires Br, 46·0%).

Methyl 2-Benzyl-3-benzyloxypyridine-4 : 5-dicarboxylate (IX).—A cold solution of methyl 2-benzyl-3-hydroxypyridine-4 : 5-dicarboxylate hydrochloride (12·3 g.) in absolute methanol (25 ml.) was treated with a solution of sodium (1·7 g.; 2 equivs.) in absolute methanol (25 ml.). A solution of benzyldimethylphenylammonium chloride (9 g.) in absolute methanol (20 ml.) was added and the mixture run into boiling xylene (100 ml.) stirred under nitrogen in an apparatus arranged for the distillation of the methanol and some of the xylene. Stirring was continued for 2½ hours while the mixture was heated in an oil-bath at about 150°. The sodium chloride was filtered off from the xylene solution which was cooled and washed with 0·5N-sodium hydroxide at 0° to remove any phenolic material, washed with water and dried (Na_2SO_4). The solvent and dimethylaniline were removed under reduced pressure, and the residual oil digested with warm light petroleum (b. p. 40—60°) which induced crystallisation. The dried product (10 g.) had m. p. 78° after recrystallisation from concentrated methanolic solution in colourless prisms (Found : C, 70·8; H, 5·55. $C_{23}H_{21}O_5N$ requires C, 70·6; H, 5·37%).

2-Benzyl-3-benzyloxy-4 : 5-bishydroxymethylpyridine Hydrochloride (cf. X).—Lithium aluminium hydride (0·6 g.) was stirred with sodium-dried ether (50 ml.) in nitrogen. A solution of methyl 2-benzyl-3-benzyloxypyridine-4 : 5-dicarboxylate (4·5 g.) in dry ether (75 ml.) was slowly added at 0°. The yellow precipitate which formed was stirred for 1½ hours, and decomposed by 5N-hydrochloric acid (18 ml.) with ice-cooling. The insoluble hydrochloride remaining was filtered off, washed with a little cold water, and dried *in vacuo* (yield 3·8 g., 88·5%; m. p. 170°). Recrystallisation from methanol–ether gave colourless needles of *2-benzyl-3-benzyloxy-4 : 5-bishydroxymethylpyridine hydrochloride*, m. p. 179—180° (Found : C, 68·0; H, 6·0; N, 4·1. $C_{21}H_{22}O_3NCl$ requires C, 67·8; H, 5·9; N, 3·8%).

2-Benzyl-3-hydroxy-4 : 5-bishydroxymethylpyridine Hydrochloride (XI).—(*a*) The 3-benzyloxy-derivative (1·86 g. of hydrochloride) and 20% palladised charcoal (0·6 g.) in alcohol (70 ml.)

and water (11 ml.) were shaken in hydrogen until the rapid uptake was complete. After filtration, the solution was evaporated to dryness under reduced pressure and the residue recrystallised from alcohol, yielding *2-benzyl-3-hydroxy-4 : 5-bishydroxymethylpyridine hydrochloride* as colourless feathery needles, m. p. 202° (decomp.) (Found: C, 59·8; H, 5·8; N, 5·1; Cl, 12·44. $C_{14}H_{16}O_3NCl$ requires C, 59·7; H, 5·7; N, 5·0; Cl, 12·6%).

(*b*) A solution of 2-benzyl-3-hydroxy-4 : 5-bisaminomethylpyridine trihydrobromide (1·0 g.) in water (8 ml.) was stirred with freshly prepared silver chloride (from 2·2 g. of silver nitrate) for 45 minutes on the hot-water bath, to convert the hydrobromide into hydrochloride. The mixture was filtered, and the silver halide washed with a little warm water. The solution was further clarified with the aid of kieselguhr, and mixed with 2N-hydrochloric acid (16 ml.). The whole was treated with sodium nitrite solution (10%; 3·4 ml.) on the water-bath for 30 minutes. The solution was evaporated to dryness under reduced pressure (bath *ca.* 50—60°) and the dry residue extracted with boiling absolute alcohol. Concentration of the filtered extract led to crystallisation of 2-benzyl-3-hydroxy-4 : 5-bishydroxymethylpyridine hydrochloride (0·235 g., 41%), identical with that described above.

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