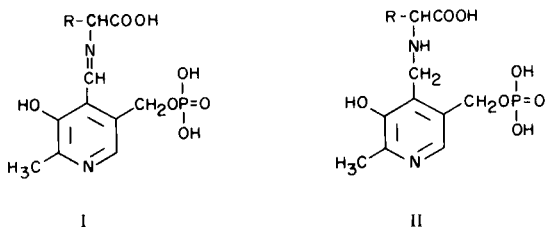


Vitamin B₆ Derivatives. Synthesis of *N*-[2-Methyl-3-Hydroxy-5-(2-Carboxyethyl)-4-pyridylmethyl]phenylalanine and Related Compounds

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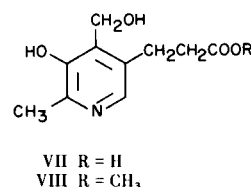
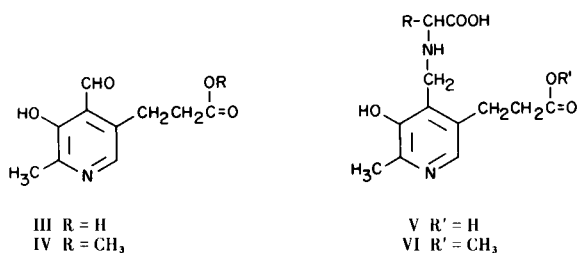
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Pyridoxal phosphate acts as a coenzyme in transamination reactions and in decarboxylation of α -amino acids. Most of the metabolic transformations involving pyridoxal phosphate and α -amino acids have intermediates of structure I. It is possible that the reduced products (II) would be inhibitors of the transaminases and other enzymes requiring pyridoxal phosphate as coenzyme.

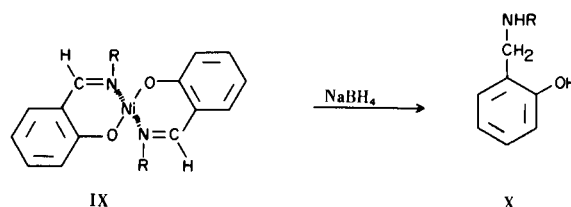


In an effort to prepare pyridoxal phosphate analogs (I) for testing as inhibitors of pyridoxal phosphate-requiring transaminases, we have prepared compounds of type V. Such structures bear a formal resemblance to the transamination intermediate (I) and its reduced counterpart (II) but perhaps would be more cell permeable than the latter.

The intermediate aldehydo-ester (IV) was prepared in 77% yield from VII (2,3) by esterification with diazomethane followed by active manganese dioxide oxidation. Compound IV was then converted to compounds of type V by the successive steps of condensation with phenylalanine or γ -aminobutyric acid, catalytic hydrogenation and alkaline saponification.



In connection with the reduction step, model studies were carried out using nickel complexes of salicylaldehyde Schiff bases (4). It was considered that in such complexes the C=N bond might be more susceptible to nucleophilic attack by sodium borohydride. It was subsequently found, however, that direct borohydride reduction was possible and that, in the case of the pyridoxal derivatives, catalytic hydrogenation (5) was, in fact, even more satisfactory. It is quite possible that in certain systems borohydride reduction of complexed Schiff bases might offer synthetic and stereochemical advantages.



EXPERIMENTAL

Melting points were taken in open pyrex capillaries and are uncorrected. Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Indiana. Structures of all compounds were supported by infrared and nmr data.

Methyl 3-(4-Hydroxymethyl-3-hydroxy-2-methyl-5-pyridyl)propanoate (VIII).

To 30 ml. of absolute methanol cooled in an ice bath was added 1.5 g. of the hydrochloride of VII. Diazomethane in ether was slowly added to the suspension with stirring. The disappearance of the yellow color of diazomethane, the bubbling due to nitrogen

and methyl chloride formation, and the dissolving of the suspended material indicated that the reaction was proceeding. When the reaction was completed, the solvents were removed on the rotary evaporator. The solid that remained was crystallized from absolute methanol to yield 1.2 g. (85%) of VIII, m.p. 145-146.5°.

Anal. Calcd. for $C_{11}H_{15}NO_4$: C, 58.7; H, 6.67. Found: C, 58.96; H, 6.62.

Methyl 3-(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)propanoate (IV).

To 450 ml. of chloroform in a flask was added 1.0 g. of VIII. The chloroform was warmed to dissolve the material. Stirring was begun and 7.0 g. of "active manganese dioxide" (6) was added. Stirring was continued at room temperature for 27 hours. After removing the manganese dioxide by filtration using a sintered-glass funnel and celite, the chloroform was evaporated. The crude product (0.87 g.) was purified by sublimation at 70° (0.2 mm) to yield 0.77 g. (77%) of yellow compound (IV), m.p. 95-97°.

Anal. Calcd. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.83. Found: C, 59.11; H, 6.06.

Imine from IV and L-Phenylalanine.

To 25 ml. of methanol cooled in an ice bath were added 0.6 g. (2.5 mmoles) of IV and 0.41 g. (2.5 mmoles) of L-phenylalanine. The aldehyde dissolved at once, but the amino acid went into solution slowly. The mixture was stirred at 0° for 5.5 hours, during which time a yellow compound precipitated. The yellow precipitate was dissolved in hot methanol, the solution was filtered to remove the few mg. of undissolved L-phenylalanine, and the yellow compound crystallized to yield 0.28 g. (30%) of the product, m.p. 156-158°.

Anal. Calcd. for $C_{20}H_{22}N_2O_5$: C, 64.90; H, 5.95. Found: C, 65.04; H, 6.23.

N-[2-Methyl-3-hydroxy-5-(2-carbomethoxyethyl)-4-pyridylmethyl]-L-phenylalanine (VI, R = $C_6H_5CH_2$).

To 25 ml. of methanol cooled in an ice bath were added 0.67 g. (3 mmoles) of IV and 0.495 g. (3 mmoles) of L-phenylalanine. The pale yellow aldehyde dissolved at once, but the amino acid went into solution slowly, during which time the solution became a darker yellow. After stirring at 0° for 3 hours, the dark yellow solution was filtered to remove the few mg. of undissolved L-phenylalanine, and was then hydrogenated with 100 mg. of 82.60% platinum oxide as catalyst in a Paar apparatus at 50 psi for 8 hours. After hydrogenation the reduced compound had precipitated; the mixture was heated to dissolve the precipitate, and the catalyst was removed by filtration with a sintered-glass funnel. The yellow-green solution was concentrated to a few ml. of the rotary evaporator, and left overnight at 5° to crystallize. The precipitate was then recrystallized twice from methanol to yield 0.430 g. (39%) of the coupled product, m.p. 180-181.5° dec. The same product could be obtained by reduction of the isolated imine reported above.

The hydrochloride of VI was prepared, m.p. 230-235°.

Anal. Calcd. for $C_{20}H_{25}ClN_2O_5$: C, 58.78; H, 6.12. Found: C, 59.12; H, 6.27.

N-[2-Methyl-3-hydroxy-5-(2-carboxyethyl)-4-pyridylmethyl]-L-phenylalanine (V, R = $C_6H_5CH_2$).

To 15 ml. of 0.0835 *N* sodium hydroxide (1.25 meq.) was added 175 mg. of VI (R = $C_6H_5CH_2$). The solution was stirred at 40° for 24 hours and then 12.85 ml. of 0.0975 *N* hydrochloric acid (1.25 meq.) was added. The solution was left standing at room temperature until crystals began to form (within an hour),

and then left for 4 hours at 5°. Filtration of the crystals and washing with methanol yielded 85.5 mg. (50.8%) of the product, m.p. 237-238.5° dec.

Anal. Calcd. for $C_{19}H_{22}N_2O_5$: C, 63.75; H, 6.15. Found: C, 63.86; H, 6.33.

Imine from IV and γ -Aminobutyric Acid.

To 25 ml. of methanol cooled in an ice bath were added 0.3345 g. (1.5 mmoles) of IV and 0.1545 g. (1.5 mmoles) of γ -aminobutyric acid. The aldehyde and amino acid both dissolved at once. The solution was stirred for 3 hours at 0°, during which time a yellow compound precipitated. The precipitate was collected on a filter to yield 0.120 g. (25.9%) of the product, m.p. 117-118° dec. An analytical sample was recrystallized from methanol.

Anal. Calcd. for $C_{15}H_{20}N_2O_5$: C, 58.45; H, 6.50. Found: C, 58.53; H, 6.75.

N-[2-Methyl-3-hydroxy-5-(2-carbomethoxyethyl)-4-pyridylmethyl]-4-aminobutanoic Acid.

The yellow filtrate obtained from the above preparation of the imine was hydrogenated in a Paar apparatus at 50 psi for 6 hours using 28 mg. of platinum oxide as catalyst. After workup an oil was obtained which crystallized from methanol-ether. Recrystallization from ethanol yielded 84 mg. of the product, m.p. 135-136° dec.

Anal. Calcd. for $C_{15}H_{22}N_2O_5$: C, 58.13; H, 7.10. Found: C, 57.89; H, 7.33.

bis(*N*-phenylsalicylaldehyde)nickel (II) (IX, R = C_6H_5).

This dark, olive green complex was prepared by the method of Sacconi, Paoletti, and Del Re (4), m.p. 269-270.5°.

Anal. Calcd. for $C_{26}H_{20}NiN_2O_2$: C, 69.22; H, 4.47. Found: C, 69.11; H, 4.49.

N-(2-Hydroxybenzyl)aniline (X, R = C_6H_5).

The above nickel complex was suspended in methanol. Excess sodium borohydride was slowly added with stirring. After stirring for 2 days, the solution was filtered, neutralized with acetic acid, and concentrated. Crude product precipitated on addition of water. The product was recrystallized from methanol-water, m.p. 110-111°.

Anal. Calcd. for $C_{13}H_{13}NO$: C, 78.36; H, 6.57. Found: C, 78.44; H, 6.87.

Nickel(II) Complex of the Imine from Salicylaldehyde and Phenylalanine.

The nickel salt of salicylaldehyde (0.92 g.) was suspended in water to which was added, with stirring, a hot saturated solution of phenylalanine (0.66 g.) in water. The mixture was allowed to stir for 2 days. The product was collected and washed with water. As in the case of a number of other compounds in this series, analysis indicated one molecule of water of hydration.

Anal. Calcd. for $C_{32}H_{28}NiN_2O_6 \cdot H_2O$: C, 62.67; H, 4.93. Found: C, 62.70; H, 5.00.

N-(2-Hydroxybenzyl)phenylalanine.

The above nickel complex (0.6 g.) was suspended in absolute ethanol. Sodium borohydride (1.2 g.) was added in small amounts. The mixture was allowed to stand for several hours. Methanol was added and the mixture was filtered and neutralized with acetic acid. After concentration and addition of water, the white crystalline product precipitated, 90%, m.p. 234-235° dec.

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.81; H, 6.32. Found: C, 70.54; H, 6.39.

Phenylalanine was dissolved in methanol and an equivalent

amount of 0.1 *M* methanolic potassium hydroxide was added. The solution was cooled and an equivalent of salicylaldehyde was added. The resulting Schiff base was reduced directly by the addition of sodium borohydride. The product, obtained in 72% yield, was identical in all respects to that obtained above.

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