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A Model Reaction Demonstrating Alkylating Properties of Pyridoxol, Involving an o-Quinone Methide Intermediate

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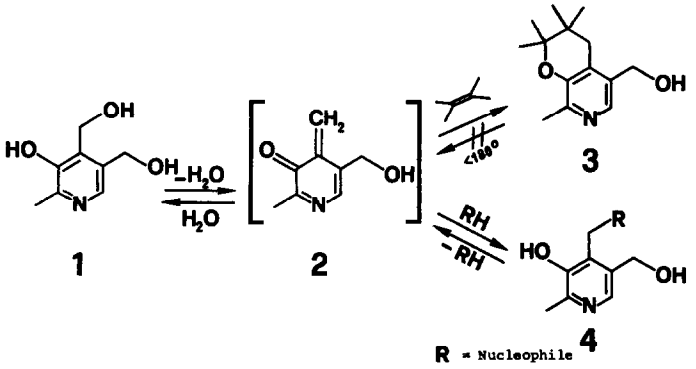
The synthesis of several specifically substituted pyridoxine derivatives **5**, **6**, **7**, **8**, **9**, **10**, **11**, and **12**, is described. A pyridoxol derived *o*-quinone methide, postulated as the common reaction intermediate, gives rise to the specific pyridoxol substitution encountered in these products. The mechanism is discussed as a model for a new type of pyridoxine transformation, either in known vitamin-B_6-dependent enzymatic reactions or in toxicological reactions induced by pyridoxine.

INTRODUCTION

In connection with toxicological studies concerning neuropathy induced by pyridoxine (HCl salt of **1**) (*1*, *2*), a number of highly lipophilic and specifically substituted analogs of pyridoxol **1** were synthesized. The synthetic procedure used to prepare these compounds demonstrated that pyridoxol can undergo an alkylating type of condensation with various nucleophilic or unsaturated compounds. Analogous to other α-hydroxymethylphenols (*3*), pyridoxol was expected to decompose *in vitro* to the unstable quinone–methide intermediate **2**, which might be trapped either by an olefin furnishing **3** or by a nucleophile through a Michael-type addition furnishing **4** (Scheme 1). Indeed, the anticipated reactions yielded the pyridoxol analogs reported in this



SCHEME 1

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paper. The possible significance of the postulated reactive intermediate **2** for the biochemical and toxicological effects of pyridoxine will be discussed.

EXPERIMENTAL

The following analytical instruments were used: Beckman DB GT for ultraviolet spectra; Beckman IR-12 for infrared spectra; Varian NMR 60 MHz, for nuclear magnetic resonance spectra, nmr data given in δ (ppm) from TMS = 0; mass spectrometer CEC 21-110B (at 70 eV) for mass spectra. As pyridoxol and some of the products were very light-sensitive, we performed all reactions and tlc analysis under exclusion of light. All reactions were run in a nitrogen atmosphere. The solvent system methanol–chloroform (ratio varying between 3:7 and 1:9) on silica gel was used for tlc analysis. Melting points were determined in open capillary tubes and were uncorrected.

4(4'-N,N-Dimethylamin)-Benzyl-3-Hydroxy-5-Hydroxymethyl-2-Methyl-Pyridin-dihydrochlorid D (**5**) *and 4(2'-N,N-Dimethylamino)-Benzyl-3-Hydroxy-5-Hydroxy-methyl-2-Methyl-Pyridin* (**6**)

Thirty grams of pyridoxol were refluxed 24 hr in *N,N*-dimethylaniline (abs.). Cooling and addition of about 300 ml of diethyl ether gave a precipitate which mainly consisted of the *p-N,N*-dimethylaniline derivative (**5**) and some orthosubstituted *N,N*-dimethylaniline derivative (**6**). The remaining solution was evaporated and diluted with pentane. The pentane-insoluble part consisted mainly of **6**. Column chromatography of the two fractions on silica gel with methanol–chloroform (15:85 changing to 20:80) gave, first, compound **6**, which was recrystallized from acetone–hexane to give 3.5 g (7%) of pure **6**, mp 160°C.

Physical properties of **6**. Nmr ($CDCl_3$): 7.71 (s; HC-6), 7.67–6.84 (m; 4 aromatic protons), 4.80 (s; CH_2–C-5), 4.07 (s; CH_2–C-4), 2.80 (s; $2 \times CH_3$–N=), 2.38 (s; CH_3–C-2). Ir (KBr, cm^{-1}): 3230, 1600, 1496, 1450, 1360, 1232, 1015. Uv (C_2H_5OH, 96%): λ_{max} 284 nm ($\varepsilon = 6700$), 212 ($\varepsilon = 22\,600$). Ms (*m/e*): 272 (M^+; 61%), 239 (16%), 228 (68%), 210 (33%), 135 (28%), 134 (67%), 122 (23%), 121 (C_6H_5–$N(CH_3)_2$; 100%), 120 (18%), 91 (18%). *Anal.* Calcd for $C_{16}H_{20}O_2N_2$: C, 70.55; H, 7.40; N, 10.28. Found: C, 70.47; H, 7.29; N, 10.50.

The second product which was eluted was treated with HCl in methanol and precipitated with ether. Subsequent recrystallization from methanol–ether furnished 17.3 g (35%) of **5**; mp 194°C (decomp.). Some unreacted pyridoxol ($\sim$1%) is eluted as the last component of the chromatographic separation.

Physical properties of **5** *and its free base, respectively.* Nmr (d_6-DMSO) of the free base: 7.96 (s; HC-6), 6.99 and 6.63 (AA' BB'-system of the four aromatic protons $J \sim 8$ Hz), 4.46 (s; CH_2–C-5), 3.96 (s; CH_2–C-4), 2.85 (s; $2 \times CH_3$–N=), 2.42 (s; CH_3–C-2). Ir (KBr, cm^{-1}) of the free base: 3300–3000, 3000–2740, 1610, 1515, 1360, 1230. Uv (C_2H_5OH, 96%) of the free base: λ_{max} 254 nm ($\varepsilon = 13\,700$). Ms (*m/e*) of the free base: 272 (M^+; 38%), 253 (20%), 210 (8%), 151 (9%), 134 (15%), 122 (30%), 121 (C_6H_5–$N(CH_3)_2$; 100%), 120 (34%). *Anal.* Calcd for $C_{16}H_{22}O_2Cl_2$: C, 55.66; H, 6.41; N, 8.11; Cl, 20.53. Found: C, 55.85, H, 6.71; N, 8.11; Cl, 20.34.

4'-o-Butylpyridoxol Hydrochloride (**7**)

Two hundred milliliters of tetralin were added to a solution of 20 g of pyridoxol in 200 ml of butanol-1. The solution refluxed at 128°C (instead of 117°C, which is the boiling point for pure butanol-1) for 24 hr. The solution which still contained a small amount of starting material was evaporated first at 14 mmHg, then at 1 mmHg to remove, first, butanol-1 and, then, tetralin. Column chromatography on silica gel, with chloroform–methanol (9:1), separated base **7** from residual tetralin, pyridoxol, and an unidentified byproduct. This yielded 16.5 g of base **7**, which was treated with HCl in methanol, precipitated with ether to give the hydrochloride salt. Recrystallization from methanol–ether furnished 15.0 g (49%) of **7**, mp 128°C.

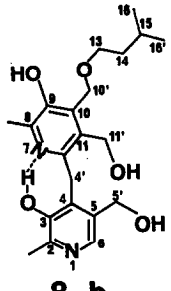
Physical properties of **7** *and its free base, respectively.* Nmr (CD_3OD): 8.18 (s; CH-6), 4.86 and 4.78 (s; CH_2–C-4 and s; CH_2–C-5), 3.60 (t; CH_2-7; $J \sim 6$ Hz), 2.63 (s; CH_3–C-2), 1.80–0.68 (m; CH_2-8, CH_2-9, CH_3-10). Ir (KBr, cm^{-1}): 3350, 3160, 2700, 1542, 1286, 1275, 1098, 1075, 1040. Uv (C_2H_5OH, 96%): λ_{max} 288 nm ($\varepsilon = 7400$). Ms (*m/e*) of the free base: 225 (M^+; 8%), 151 (29%), 122 (18%), 106 (61%), 94 (100%), 80 (21%), 66 (19%), 53 (44%), 51 (44%). *Anal.* Calcd for $C_{12}H_{19}O_3NCl$: C, 55.27; H, 7.34; N, 5.37; Cl, 13.59. Found: C, 54.94; H, 7.53; N, 5.45; Cl, 14.00.

4'-o-Isoamylpyridoxol Hydrochloride (**8**)

Twenty-five grams of pyridoxol were refluxed 20 hr in 250 ml of isoamyl alcohol. After evaporating the surplus isoamyl alcohol, column chromatography on silica gel with chloroform–methanol (9:1) separated the oily residue in three products. The main product, 16.2 g of base **8** was eluted first, mp 84°C. The second fraction contained a byproduct, **8-b**[1] and was followed by some unreacted pyridoxol. Base **8** was treated with HCl in methanol, precipitated with ether and recrystallized from methanol–ether to give 14.7 g (36%) of **8**, mp 150°C.

Physical properties of **8** *and its free base, respectively.* Nmr ($CDCl_3$) of the free base: 7.57 (s; CH-6), 4.88 and 4.48 (s; CH_2–C-4 and s; CH_2–C-5), 3.76–3.28 (m; CH_2-7), 2.34 (s; CH_3–C-2), 1.76–1.20 (m; CH_2-8 and CH-9), 0.89 (d; $(CH_3)_2$ C-9; $J \sim 7$ Hz).

[1] Product **8-b** could be recrystallized from methanol–pentane to give 1.2 g (4%), mp 175°. Mechanistic considerations in connection with the nmr and ms data, reported below, strongly indicated structure **8-b**. Physical properties of **8-b**: nmr (d6-DMSO): 7.86 (s; CH-6), 5.65 (s (broad); OH-3'), 4.90–4.50 (3 × s; OCH_2-5', OCH_2-10', OCH_2-11'), 4.20 (s; CH_2-4'), 3.50 (t; OCH_2-13), 2.40 (s; CH_2–C-2, CH_3–C-7), 1.45 (qi; CH_2-14, CH-15), 0.84 (d; 2 × CH_3-16). Uv (C_2H_5OH 96%): λ_{max} 284 nm (ε = 11 000), 210 ($\varepsilon = 24\,000$). ms (*m/e*): 390 (M^+).



8-b

Ir ($CHCl_3$, cm^{-1}) of the free base: 3600, 3500–3040, 2960, 1380. Uv (C_2H_5OH 96%): λ_{max} 284 ($\varepsilon = 6480$). Ms (*m/e*) of the free base: 239 (M^+; 7%) 151 (46%), 136 (15%), 123 (21%), 122 (21%). 107 (83%), 94 (100%), 80 (15%). *Anal.* Calcd for $C_{13}H_{23}O_3NCl$: C, 56.20; H, 8.34; N, 5.05; Cl, 12.76. Found: C, 56.05; H, 8.17; N, 5.34; Cl, 12.87.

4'-N,N-Dibutylaminpyridoxol Hydrochloride (**9**)

Twenty grams of pyridoxol were dissolved in 200 ml of dibutylamin and heated to 140°C for 20 hr. After removing surplus dibutylamin by distillation at 1 mmHg, the residue was dissolved in chloroform and extracted three times with water to remove a small amount of unreacted pyridoxol and some water-soluble, decomposed material. The chloroform phase was dried, evaporated, and the residual oil was dissolved in diethylether. Subsequent addition of ether saturated with HCl, until the solution remained weakly acidic, furnished **9**. Recrystallization from methanol–dimethoxyethane (1:3) gave 25 g (76%) of **9**, mp 141°C.

Physical properties of **9** *and its free base, respectively.* Nmr (CD_3OD): 8.36 (s; CH-6), 5.00 and 4.80 (2 × s; NCH_2–C-4, OCH_2–C-5), 3.38 (t; 2 × NCH_2-7), 2.80 (s; CH_3–C-2), 2.20–0.80 (m; 2 × $CH_2CH_2CH_3$-8, 9, 10). Ir (KBr, cm^{-1}): 3440, 3235, 1658, 1560, 1390, 1236. Uv (C_2H_5OH 96%): λ_{max} 283 nm ($\varepsilon = 3900$), 219 ($\varepsilon = 4100$). Ms (*m/e*) of the free base: 280 (M^+; 0.5%), 237 (2%), 205 (3%), 129 ($NH(CH_2CH_2CH_2CH_3)_2$, 11%), 86 (100%). *Anal.* Calcd for $C_{16}H_{29}N_2O_2Cl$: C, 60.64; H, 9.22; N, 8.84; Cl, 11.18. Found: C, 60.87; H, 9.51; N, 8.12; C, 11.44.

4'-N(1-Imidazole) Pyridoxol Dihydrochloride (**10**)

Two grams of pyridoxol and 10 g of imidazole were dissolved in 8 ml of H_2O and heated for 3½ hr at 145°C in a sealed evacuated glass tube. Tlc analysis of the resulting solution developed in the solvent system isopropanol–10% NH_3 (9:1) on silica gel plates, showed that pyridoxol was completely converted, mainly to the blue fluorescent (at 366 nm) imidazole derivative, **10**. This product was isolated after evaporating the reaction mixture to dryness and extracting the resulting crystalline mass in a continuous fashion with dry ether during 24 hr to remove excess imidazole. The remaining crystals were dissolved in methanol and treated with HCl in ether. Precipitation with more ether and recrystallization from methanol–ether yielded 3.9 g (57%) of **10**, mp 210°C (decomposition).

Physical properties of **10** *and its free base, respectively.* Nmr (d6-DMSO) of the free base: 8.05 (s; CH-6), 7.76 (s; CH-8), 7.20 and 6.91 (2 × s; CH-10, CH-11), 5.34 and 4.62 (2 × s; OCH_2–C-5, NHC_2–C-4), 2.47 (s; CH_3–C-2). Ir (KBr, cm^{-1}): 3440–2720, 1640, 1620, 1550, 1375, 1230, 1004. Uv (C_2H_5OH, 96%): λ_{max} 334 ($\varepsilon = 1900$), 286 ($\varepsilon = 4690$). Ms (*m/e*) of the free base: 219 (M^+; 32%), 151 (M^+–imidazole; 72%), 123 (16%), 106 (13%), 94 (16%), 68 (imidazole$^+$; 100%), 41 (66%). *Anal.* Calcd for $C_{11}H_{15}O_2N_3Cl_2$: C_1 45.01; H, 5.14; N, 14.40; Cl, 24.02. Found: C, 44.72; H, 5.44; N, 14.13; Cl, 23.81.

7,8 Dihydro-4-Hydroxymethyl-3-Methyl-6H-(1)-Benzopyrano(2,3-c)-Pyridin Hydrochloride (**12**), *and its 9a, 9-Morpholinadduct* (**11b**)

Ten grams of pyridoxol (dry) were heated in an oil bath of 190–200°C with 40 ml of morpholinocyclohexene. Water and morpholine which are formed simultaneously were distilled off as the reaction progressed. The morpholine formation was nearly

complete after 2 hr. Addition of some toluene and subsequent distillation removed the remainder of morpholine and water. Surplus morpholinocyclohexene was removed under high vacuum distillation (0.1 mmHg). The residual oil was treated with petroleum ether–ethyl acetate (2:1) until yellow crystals started precipitating. The precipitate was washed with petroleum ether–ethyl acetate (3:1), and petroleum ether, respectively, to furnish 5.8 g of the free base of **12**, mp 146–149°C. The unpurified base was treated with HCl in methanol. Dark yellow crystals precipitated with ether and were recrystallized from methanol–ether to give 5.2 g (33%) of **12**, mp 238°C (decomposition).

Physical properties of **12** *and its free base, respectively.* 100 MHz nmr ($CDCl_3$) of the free base: 7.70 (s; CH-3), 6.31 (s (broad); CH-5), 5.28 (d × t; CH-9; $J \sim 2$ Hz) 4.56 (s; CH_2–C-4), 4.63 (s broad); C4–C–OH, disappears after D_2O-addition), 2.60–2.15 (2 × t (broad); CH_2-6, CH_2-8), 2.36 (s; CH_3–C-1), 1.72 (qi; CH_2-7).

The interpretation of the nmr data was verified by the following decoupling experiments using a 100 MHz—nmr apparatus (ppm): 1.72 (site of irradiation)→2.60–2.15 (site of observation), (change); 2.60–2.15→1.72 (change); 5.28→6.31 (qi) and 2.30 (change); 6.31→5.28 (t; $J \sim 2$ Hz has disappeared) and 2.50 (t; sharper).

Ir ($CHCl_3$, cm^{-1}) *of the free base.* 3600, 3500–3040, 2940, 1620, 1596, 1398, 1304, 1126. Uv (C_2H_5OH 96%): λ_{max} 250 nm ($\varepsilon = 25\,000$), 382 ($\varepsilon = 5100$), Ms (*m/e*) of the free base: 229 (M^+; 100%), 214 (25%), 210 (8%), 201 (5%), 167 (5%), 128 (10%), 115 (8%), 91 (5%), 77 (10%). *Anal.* Calcd for $C_{14}H_{16}O_2NCl$: C, 63.27; H, 6.06; N, 5.27; Cl, 13.89. Found: C, 63.44; H, 6.00; N, 4.97; Cl, 13.75.

When the reaction for the synthesis of **12** was run omitting the removal of water and morpholine, besides **12** an intermediate product **11b** was detected in the thin-layer system: acetonitrile-methylene chloride–methanol (4:6:0.5) on silica gel plates. Using fractionated crystallization with pentane-ethyl acetate, we were able to separate a small quantity of the intermediate product **11b**. Colorless crystals were obtained and could be recrystallized from ethyl acetate to give **11b**, mp 170°C.

Physical properties of **11b**. Nmr ($CDCl_3$): 7.68 (s; CH-3), 6.68 (s (broad); CH-5), 4.60 (s; CH_2–C-4), 3.64 (t; 2 × CH_2–O in morpholine substituent), 3.00–1.40 (m; 2 × CH_2–N in morpholine substituent, CH_2-6, CH_2-7, CH_2-8, CH_2-9), 2.36 (s; CH_3–C-1). Ir (KBr, cm^{-1}): 3160, 2940, 2850, 1592, 1410, 1290, 1175, 1120. Uv (C_2H_5OH 96%): λ_{max} 286 nm ($\varepsilon = 9650$), 324 ($\varepsilon = 4200$); (0.1 *N* HCl): 212 ($\varepsilon = 21\,600$), 290 ($\varepsilon = 14\,300$). Ms (*m/e*): 316 (M^+; 2%), 230 (100%, M^+–morpholine radical), 214 (12%), 128 (5%), 115 (5%), 87 (18%, morpholine), 77 (6%), 57 (24%). *Anal.* Calcd for $C_{18}H_{24}O_3N_2$: C, 68.32; H, 7.64; N, 8.85. Found: C, 68.09; H, 7.73; N, 8.80.

Instead of separating more of **11b**, it was synthesized unambiguously. Eight grams of the free base of **12**, dissolved in 100 ml of morpholine, 100 ml of toluene, and 2 g of *para*-toluene sulfonic acid, were refluxed for 70 hr. The addition of morpholine was then nearly quantitative. Morpholine and toluene were evaporated. The residue was taken up in water and extracted with ether. The combined ether phases were washed with water to remove salts of sulfonic acid. The ether phase was dried and concentrated to allow for crystallization of **11b**. Recrystallization from ethylacetate gave 7 g (64%) of **11b**; mp 170°C.

All physical properties are identical to **11b**, which was isolated as an intermediate from the synthesis of **12**. The mixed melting point of unambiguously synthesized **11b** and **11b** previously isolated gave no depression.

Pyrolysis of **7**

To test the reversibility of the reaction described in Scheme 1, three times 100 mg of **7** were introduced into three glass tubes. Each was dissolved in 4 ml of water (dest.). The tubes were evacuated, sealed, and heated to 130°C for 1½, 3, and 20 hr, respectively. The decomposition of **7**, with simultaneous formation of pyridoxine, was observed on the thin-layer system, chloroform–methanol (8:2) on silica gel. The tubes yielded, in increasing chronological order, 5–10%, 50–60%, and 90–100% of pyridoxine, estimated in a semiquantitative fashion from the size of the thin-layer spots.

Pyrolysis of Pyridoxamindihydrochloride **18**

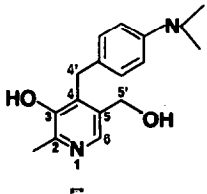
Similarly, as described under *Pyrolysis of* **7**, the pyridoxine formation from **18** was investigated. The decomposition temperature was 180°C. Reaction times of 1½, 3, and 20 hr, respectively, were employed; 1–5%, 5–10%, and 10–20% of pyridoxine was obtained, respectively. Longer reaction times or higher temperatures did not increase the yield of **1** but provided more unidentified decomposition products.

Pyrolysis of **5** *and* **12**

Prepared in a similar way to that described under *Pyrolysis of* **7**, the samples were heated at 130, 150, and 180°C for different times up to 20 hr. No pyridoxine formation occurred for either **5** or **12**.

RESULTS

Pyridoxol reacted at temperatures between 130 and 190°C with *N,N*-dimethylaniline, butanol-1, isoamyl alcohol, dibutylamine, and imidazole to give compounds **5**, **6**, **7**, **8**, **9**, and **10** isolated as HCl salts.



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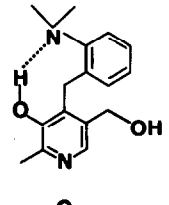

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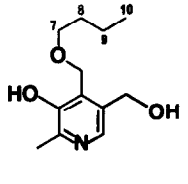

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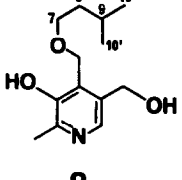

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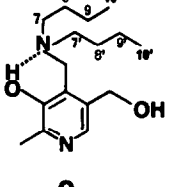

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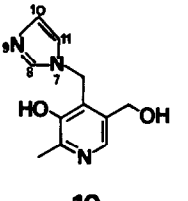

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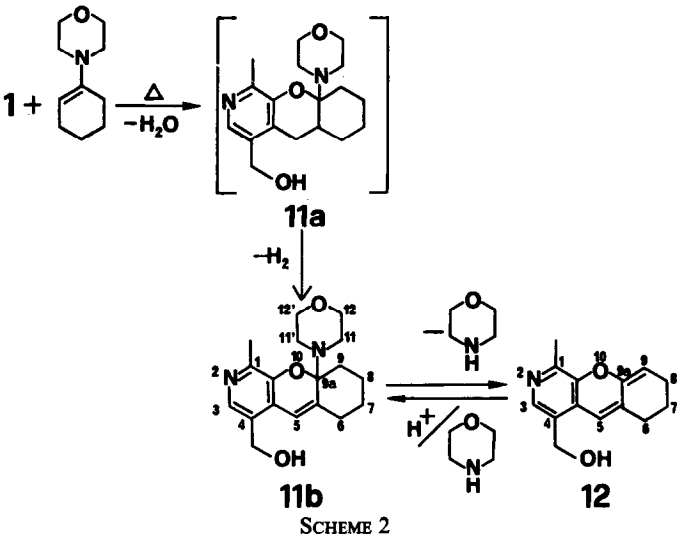
As expected from the postulated polar quinone methide intermediate **2**, and as reported for similar reactions (*4*, *5*), substitution only occurred at C-4′. In the case of

1-morpholinocyclohexene addition, the quinone methide was trapped to give derivatives **11b** and **12** containing the pyranoid ring system (Scheme 2).



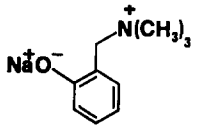
SCHEME 2

In order to achieve complete conversion of **1** to **12**, continuous removal of the reaction products water and morpholine was necessary. If the reaction was run omitting the removal of water and morpholine, products **11b** and **12** could be isolated from the reaction mixture. In analogy to the enamine addition products reported in Ref. (*6*), formation of primary addition product **11a** was also expected. We were not able to detect any **11a** in the course of the reaction. As the reaction took place in a 99.99% nitrogen atmosphere, it seems likely that the unexpected facile oxidation of **11a** to **11b** occurred during work-up. As we were interested in a useful synthetic procedure to make **11b** and because the separation of **11b** from the reaction mixture mentioned above proved tedious and incomplete, **11b** was synthesized by acid catalyzed addition of morpholine to **12**.

The reversibility of the reaction in Scheme 1 was demonstrated by heating 4′-*o*-butylpyridoxolhydrochloride (**7**) dissolved in water to 130°C in a sealed tube. The complete decomposition of **7** in 20 hr was observed by following the disappearance of **7** and appearance of **1**, respectively, on thin-layer plates. These results show that when RH = butanol-1 in Scheme 1, complete reversibility is possible under conditions that are very similar to those employed for the reaction itself. Cavitt et al. (*7*) report that the gas phase decomposition of *o*-methoxymethylphenol to a quinone methide trimer proceeds from 750 to 850°C. The very high decomposition temperature shows that quinone methide formation, which is the rate-determining step, is much slower here than in the case of pyridoxol or pyridoxol derivative **7** decomposition. This difference in reactivity is explained by the fact that the polar, protic solvents, alcohol and water, respectively, accelerate formation of the polar intermediate **2**. Analogous to the decomposition of 1-*N*,*N*-dimethylnaphthol-2, which is reported to proceed at 180°C in toluene (*8*), one can expect a facile retroquinone methide formation from 4-*N*,*N*-dibutylpyridoxamine (**9**).

DISCUSSION

Quinone methide formation resulting from phenolic Mannich base methiodide (**13**) decomposition in strong base at 35°C has been reported by Gardner et al. (**9**).



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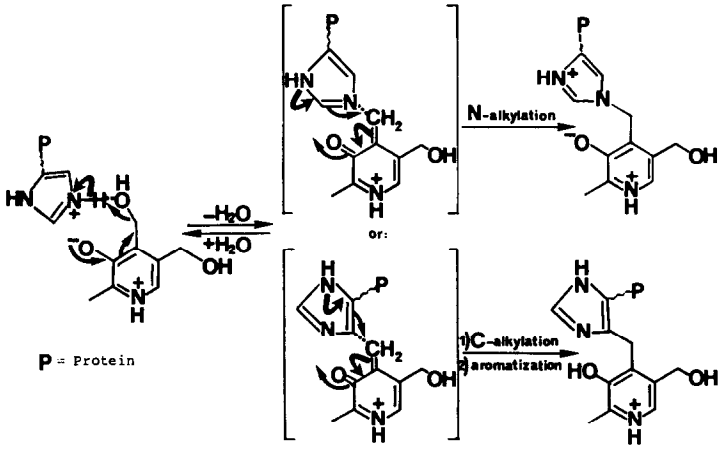
The ease with which this reaction occurs, compared to quinone methide formation under neutral conditions, is explained by these authors on the basis of electron release by the initially formed phenolate ion in connection with the good leaving group properties of the quarternary nitrogen atom. Under physiologic conditions (aqueous buffer of pH 7.4), pyridoxine is mainly in the zwitterionic form (*10*). As in **13**, there is a negative charge on the 3-O group of pyridoxine. In connection with a good leaving group, such as can be formed by protonation of the C4′-OH group by an enzyme-attached protonated histidine group as envisaged in Scheme 3, quinone methide formation from pyridoxine should be possible *in vivo*.

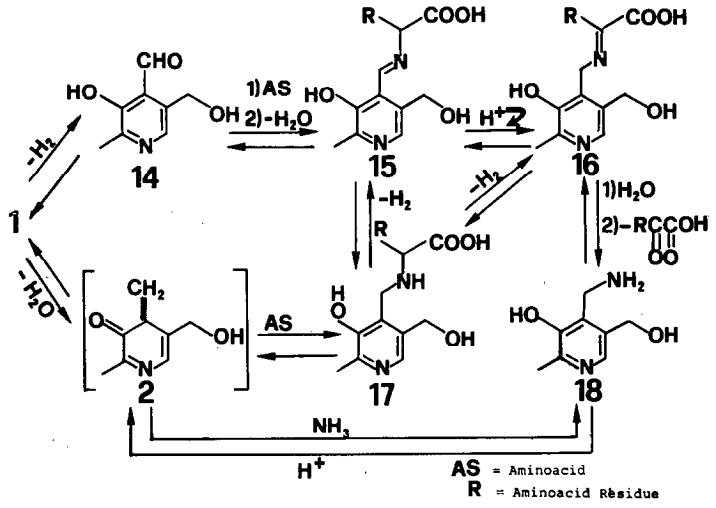


SCHEME 3

N- or C-alkylation of the histidine group seems a likely sequel to quinone methide formation in Scheme 3. The *in vitro* synthesis described in Experimental, where unsubstituted imidazole was used as a model compound for the histidine-containing protein in Scheme 3, actually yielded the *N*-alkylated imidazole derivative **10** as the main reaction product.

Keeping in mind the *in vitro* reactions described in this paper, we can consider a new possible reaction pathway for pyridoxine metabolism. It is postulated that vitamin-B_6-dependent enzyme systems might cause pyridoxine to decompose to a quinone methide. In contrast to the well-documented mechanism concerning vitamin-B_6 transformations (**1**→**14**→**15**→**16**→**18** in Scheme 4), where we are always confronted with the need for

pyridoxal (**14**) as the essential cofactor, we can also conceive a transformation of pyridoxine to pyridoxamine (**18**) and *vice versa*, without intermediate conversion to pyridoxal (**1**→**2**→**18** in Scheme 4).



SCHEME 4

This assumption is supported by two *in vitro* experiments. We observed pyridoxine formation after heating a pyridoxamine dihydrochloride water solution in an evacuated tube (see experimental section). Secondly, De Jongh et al. (*11*) in their work on the mass spectra of pyridoxol and derivatives found an identical major fragment (*m/e* 151, $C_8H_9NO_2$) for pyridoxol and pyridoxamine, which corresponds to the ion of quinone methide **2**. Aminoacid addition to **2** should give **17**, which after dehydrogenation should give either imine **15** or **16** (Scheme 4). The enzyme pyridoxine (pyridoxamine)-5′-phosphate oxidase, which can be isolated from rabbit liver, is reported (*12*) to be a very efficient dehydrogenating system yielding **15** from *N*-(phosphopyridoxyl)-amines of type **17**. This shows that the body is equipped to cope with the type of reaction which is necessary for the biotransformation of **17**→**15**. In this way, it is conceivable that pyridoxal is not always necessarily a precursor of **15**. We may even regard pyridoxal as a metabolite of **15**. A different system, where quinone methide intermediates are postulated to play an important role, is reported by Shamma and Jones (*13*). They described an *in vitro* reaction modeling the biogenesis of the alkaloid ochotensimine.

Many polar and unsaturated groups can react with the quinone methide **2** once it is formed, either at elevated temperatures, as demonstrated by the model reactions described in this paper, or at body temperature under enzymatic conditions, as postulated in Scheme 3. This means that most polar groups on biopolymers are eligible for quinone methide attack. If very large amounts of pyridoxine are present in the body, as in the case of experimentally induced nerve damage (*1*, *2*) a quinone methide-initiated alkylation of biological material becomes more likely. For example, essential SH groups might be trapped, leading to inactivation of enzymes. As a consequence, metabolic disturbances might cause nerve cell damage and other toxic reactions. This

theoretical concept will be examined by biological testing of various pyridoxol analogs. These will be selected with regard to their ability to form a quinone methide intermediate.

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