Table II. Helix–Coil Transitions

Polypeptide	Line width: Coil	Line width: Helix	Approximate pH of transition nmr (frozen)	pH midpoint solution studies
Asp	(150)	≥1,500[a]	6.0	
Glu	(100)	≥1,500[a]	5.3	5.4,[b] 5.6[c]
Tyr	200	≥360[a]	11.3	11.5[d]
Arg	(2,500)	(7,600)	≥8.5[a]	
Lys	750	1,900[a]	9.5	10[b]
DL-Lys	370	(2,900)	9.5	
Orn	800	(3,200)	≥8.5[a]	
LysGlu (4:6)	220	2,200	7 (broad)	5.5[e]
LysPhe (1:1)	800	≥1,450[a]	≥8.5[a]	9[f]

[a] Transition may not be complete. [b] Reference 8, p 550. [c] G. Barone, V. Crescenzi, and F. Quadrifoglio, *Biopolymers*, **4**, 529 (1966). [d] Reference 8, p 553. [e] Reference 7. [f] E. Peggion, A. S. Verdini, A. Cosani, and E. Scoffone, *Macromolecules*, **3**, 194 (1970).

copolymers. Both the lysine–phenylalanine and lysine–glutamic acid systems show line widths in agreement with the appropriate structure rather than composition. Thus, the latter polymer is thought to be helical at low pH (as polyglutamic acid) and random coil at high pH (as polyglutamic acid, but unlike polylysine).[7] The line widths at the limiting pH values parallel the glutamic acid results. In a similar way, the lysine–phenylalanine line widths and temperature dependences follow those of polylysine. This is really quite a different result from the hydration of the copolymers. The hydrations were essentially weighted averages of the appropriate homopolypeptide values.[1]

If we apply this rule (that a change in line width indicates a change in conformation) our results require us to assign a conformation transition to poly-DL-lysine at high pH. This is presumably a normal coil–helix transformation which can occur in such polymers because they are not truly optically random. That is, short runs of pure "L" or pure "D" isomer occur and are of sufficient length to assume helical structures under appropriate conditions.[8]

We can make a tentative effort to extend these ideas to the nonionic polymers. It is not easy to demonstrate conformational changes within these systems because their cooperative structures are independent of salt, pH, and temperature perturbations generally accessible in aqueous solutions. One useful point of reference, however, is the polyvaline polymer, which is considered to be sterically limited to the random coil form in solution.[9] As noted above, polyvaline shows a very narrow water line, with distinct side-chain resonances detectable as well. By contrast, poly-DL-alanine, polyproline, and polyglycine show considerable broadening of the water signal and no side-chain resonances below about −10°. We are thus tempted to suggest that these last three polymers assume some nonrandom structures in solution [presumably α helix, polyproline II, and β structure (?), respectively[8]] which serve to restrict the freedom of motion of the water protons in their immediate vicinity.

In sum, we feel there is considerable direct and indirect evidence to suggest that macromolecular conformation can influence the mobility and hence the nmr line width of "bound" water. There are important qualifications. (1) We cannot use the sign and/or magnitude of the line width change either to identify the conformation change involved or to determine the extent of the change from nmr data alone. In fact, we observe that the Arrhenius plots all show points of intersection at finite temperatures suggesting that line-broadening effects in one temperature region will become line-narrowing effects in some other temperature range. (2) There are no simple rules to predict line widths from compositions because we cannot predict conformation knowing only composition. Nor is it known how various structural components will interact with each other. Thus it is not possible to calculate, for example, the line widths of the water associated with globular proteins simply from their amino acid composition.

We hope that further experimentation will remove some of these restrictions, permitting fuller use of the large sensitivity of the nmr line widths for conformational studies.

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(8) G. D. Fasman in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N.Y., 1967, Chapter 11.
(9) Reference 8, but see R. F. Epand and H. A. Scheraga, *Biopolymers*, **6**, 1551 (1968).

Biosynthesis of Pyridoxine[1]

Sir:

Recently we presented evidence concerning the primary precursors of pyridoxine (= pyridoxol), one of the forms of vitamin B_6.[2] We demonstrated by Kuhn–Roth degradation of labeled pyridoxol obtained from cultures of the *Escherichia coli* B mutant WG2 (B_6-2),[3] which had been incubated with various radiomers of pyruvic acid, that the two-carbon fragment, C-2′,2, of pyridoxol is derived specifically from a C_2 unit, corresponding to the methyl and the carbonyl carbon atoms of pyruvate. The carboxyl carbon of pyruvate was not incorporated into the product. We suggested that pyridoxol was derived from three glycerol units, one of which was incorporated *via* pyruvate as a two-carbon fragment at the oxidation level of acetaldehyde, while the other two entered pyridoxol intact, possibly *via* triose phosphate (Scheme I). We now offer further evidence in support of this view.

(1) This investigation was supported by a grant from the National Research Council of Canada.
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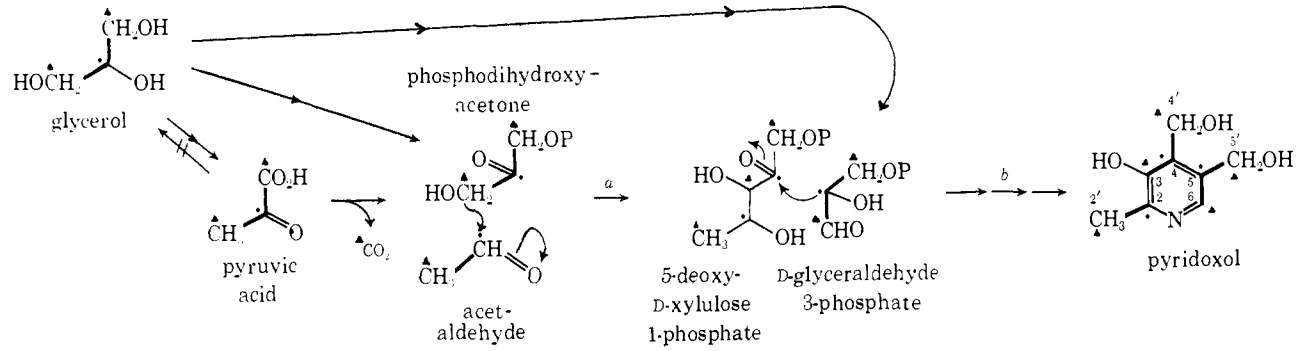
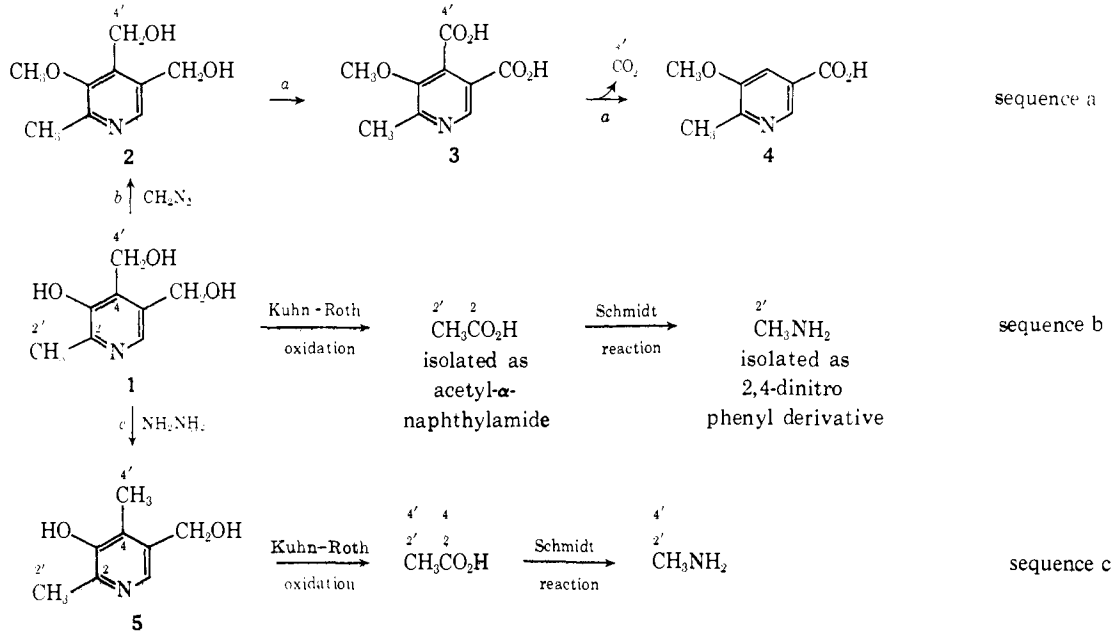
Scheme I. Biosynthesis of Pyridoxol



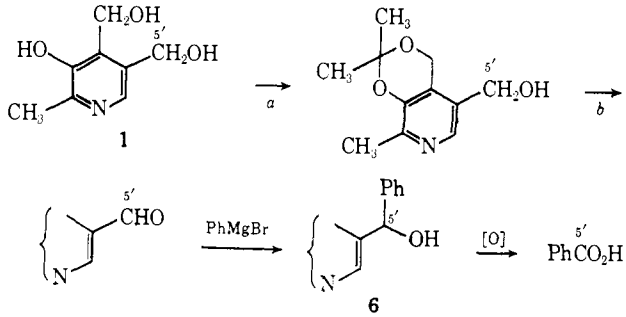
[a] See ref 5. [b] See ref 2.

Scheme II. Degradation Sequences, Permitting Assay of Activity at C-2, C-2′, C-4, and C-4′



[a] D. Palm, A. A. Smucker, and E. E. Snell, *J. Org. Chem.*, **32**, 826 (1967). [b] E. T. Stiller, J. C. Keresztesy, and J. R. Stevens, *J. Amer. Chem. Soc.*, **61**, 1237 (1937). [c] R. G. Taborsky, *J. Org. Chem.*, **26**, 596 (1961).

Scheme III. Degradation Sequence Permitting Assay of Activity at C-5′



[a] W. Korytnyk and W. Wiedeman, *J. Chem. Soc.*, 2531 (1962). [b] W. Korytnyk, E. J. Kris, and P. R. Singh, *J. Org. Chem.*, **29**, 574 (1964).

Samples of pyridoxol, isolated by carrier dilution from *E. coli* B-WG2 cultures, which had been incubated with [*1*-^{14}*C*]glycerol (1.0 mCi; Amersham/Searle) and with [*2*-^{14}C]glycerol (1.0 mCi; Amersham/Searle) as the sole carbon source, were partially degraded by the reactions shown in Schemes II and III, which permit assay of label at C-2 and C-2′ (Scheme II, sequence b), C-4′ (Scheme II, sequence a), C-5′ (Scheme III), and, somewhat indirectly,[4] at C-4 (Scheme II, sequences c and b). In Table I the distribution of activity detected within the labeled samples of pyridoxol is compared with predicted values. Pyridoxol, derived from [*1*-^{14}*C*]glycerol, bears approximately one-fifth of its label at each of C-2′, C-4′, and C-5′, with little, if any, activity present at C-2 and at C-4. Pyridoxol from [*2*-^{14}*C*]glycerol contains approximately one-third of its radioactivity at C-2 and at C-4, while C-2′, C-4′, and C-5′ are essentially free of

(4) The yield of acetate on Kuhn–Roth oxidation of **5** was found to be 1.2–1.4 equiv/mol. It follows that at least a small fraction of this acetate must be derived from the C_2 fragment, C-4′,4. The actual contribution made by the fragment, C-4′,4, to the isolated acetate can be deduced from Table III. Since virtually all activity of serine-derived pyridoxol resides in the C_2 unit, C-2′,2 (as shown by Kuhn–Roth degradation of this pyridoxol), the C_2 unit, C-4′,4, of this sample contains little, if any, activity. Since the specific activity of the acetate isolated by degradation of the 2,4-dimethyl derivative, **5**, obtained from serine-derived pyridoxol, was less than 66% of that of the intact vitamin (Table III), at least one-third of the acetate, obtained in this way, must have been derived from the C_2 unit, C-4′,4. It is evident that if the labeling pattern in the C_2 unit, C-4′,4, of a given pyridoxal sample is significantly different from that of the C_2 unit, C-2′,2, the specific activities of the samples of acetate and methylamine obtained from it by Scheme II, sequence b, and by Scheme II, sequence c, must differ. Conversely, if these specific activities do not differ significantly, the labeling patterns of the C_2 units, C-4′,4 and C-2′,2, must be similar. Since degradations are available permitting individual assay of activity at C-2′, C-2, and C-4′, it follows that activity at C-4 can be deduced.

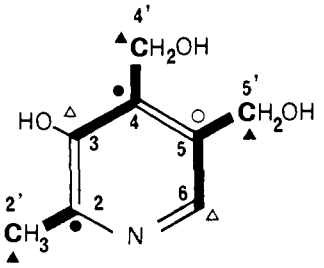


Figure 1. The distribution of label in pyridoxol. Sites of activity derived from [1-^{14}C]glycerol (triangles) (relative specific activity ~20%) and from [2-^{14}C]glycerol (circles) (relative specific activity ~33%), demonstrated by degradation (▲, ●) (Table I) or inferred (△, ○).

radiocarbon. The pattern of labeling observed within glycerol-derived pyridoxol (Figure 1) is exactly as predicted by, and therefore supports, the hypothesis outlined in Scheme I.

Table I. Observed and Predicted Distribution of Activity within Pyridoxol Derived from [^{14}C]Glycerol

Product (C atoms of pyridoxol)	Rel spec act. (%) (pyridoxol = 100) of the product derived from [1-^{14}C]Glycerol Obsd	Calcd	[2-^{14}C]Glycerol Obsd	Calcd
Pyridoxol (all)	100 ± 3	100	100 ± 3	100
Scheme II, sequence a				
3-*O*-Methylpyridoxol (**2**) (all)	99 ± 2	100		
Dicarboxylic acid (**3**) (all)	100 ± 3	100		
Monocarboxylic acid (**4**) (all but C-4′)	78 ± 2	80	103 ± 6	100
∴ C-4′	22 ± 2	20	0	0
Scheme III				
Phenylcarbinol (**6**) (all)	98 ± 2	100	99 ± 5	100
Benzoic acid (C-5′)	22 ± 1	20	0.5 ± 0.2	0
Scheme II, sequence b				
Acetic acid (C-2′,2)	22 ± 1	20	35 ± 1	33
Methylamine (C-2′)	18 ± 1	20	2.4 ± 0.4	0
Scheme II, sequence c				
2,4-Dimethylpyridine derivative **5** (all)	98 ± 1	100	101 ± 4	100
Acetic acid (C-2′,4′,2,4)	22 ± 1	20	32 ± 1	33
Methylamine (C-2′,4′)	19 ± 1	20		

A further experiment, with [1-^{14}C]glucose (0.5 mCi; Amersham/Searle) as the sole carbon source, was intended to throw light on the mode of incorporation of triose phosphate. Glycolytic breakdown of [1-^{14}C]-glucose leads to *in situ* formation of [1-^{14}C]dihydroxyacetone 1-phosphate and thence of [3-^{14}C]D-glyceraldehyde 3-phosphate. According to Scheme I, label from the latter should enter C-5′ and, *via* pyruvate and acetaldehyde, C-2′ of pyridoxol. Activity from the former might be found either at C-3 or at C-4′, depending on the mode of the postulated combination of acetaldehyde with phosphodihydroxyacetone, leading to the intermediacy of the 3-phosphate or the 1-phosphate, respectively, of 5-deoxy-D-xylulose.

Decarboxylation of the dicarboxylic acid (**3**) obtained from the [^{14}C]glucose-derived pyridoxol gave the monocarboxylic acid (**4**) containing only 60% of the total activity (Table II), demonstrating the presence of label at C-4′. This result is consistent with the latter of the two alternatives, *i.e.*, condensation of acetaldehyde and phosphodihydroxyacetone to form the 1-phosphate of 5-deoxy-D-xylose,[5] as shown in Scheme I.

Table II. Partial Degradation of Pyridoxol Derived from [1-^{14}C]Glucose

Product (C atoms of pyridoxol)	Rel spec act.
Scheme II, sequence a	
3-*O*-Methylpyridoxol (**2**) (all)	100 ± 4
Dicarboxylic acid (**3**) (all)	100 ± 5
Monocarboxylic acid (**4**) (all but C-4′)	62 ± 3
∴ C-4′	38 ± 4

In a fourth experiment, the role of serine in the biosynthesis of pyridoxol[6] was explored. Pyridoxol, isolated from a culture which had been incubated with [3-^{14}C]DL-serine (0.2 mCi; Amersham/Searle), on Kuhn–Roth oxidation yielded acetate (C-2′,2), containing almost the entire activity of the intact vitamin (Table III).

Table III. Partial Degradation of Pyridoxol Derived from [3-^{14}C]Serine

Product (C atoms of pyridoxol)	Rel spec act.
Pyridoxol (all)	100 ± 3
Scheme II, sequence b	
Acetic acid (C-2′,2)	92 ± 4
Scheme II, sequence c	
2,4-Dimethylpyridine derivative **5** (all)	100 ± 5
Acetic acid (C-2′,4′,2,4)	63 ± 3

Since this is the labeling pattern which was observed when pyruvate serves as the precursor of pyridoxol,[2] and since conversion of serine into pyruvate, a well-documented catabolic reaction,[7,8] is irreversible,[9] it follows that incorporation of serine into pyridoxol takes place *via* pyruvic acid.

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A Pseudo-Atom–Molecular Orbital Approach to Substituent Effects in Organic Compounds. I. Spin–Spin Coupling in Substituted Methanes[1]

Sir:

We report here a promising new approach to representing trends of substituent effects in organic

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