Tetrahedron. Vol. 25, pp. 1071 to 1087. Pergamon Press 1969. Printed in Great Britain

SELECTIVE ESTERIFICATIONS AND ACYL REARRANGEMENTS IN VITAMIN B_6[1, 2]

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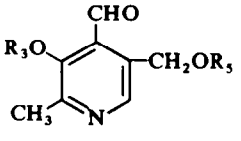
(*Received in USA* 5 *August* 1968; *Received in the UK for publication* 14 *October* 1968)

Abstract—After our work involving the acyl migration and selective esterification of pyridoxol, we investigated similar phenomena in pyridoxal and pyridoxamine. Esterification of pyridoxal under various conditions gave esters with the hemiacetal structure. In pyridoxal, acyl migration could not be detected, possibly because of inability to form the ortho acid intermediate, hence permitting selective esterification of the phenolic hydroxyl. In pyridoxamine, O → N acyl migration takes place very readily from both the 3-O and α^5-O positions, but the reverse migration could not be observed. 3-O, α^4-N and α^4-N, α^5-O diesters of pyridoxamine have been prepared. Thus it is now possible to obtain selectively esterified derivatives of pyridoxal and pyridoxamine by taking advantage of either the absence or the presence of acyl migration. 5-Thiol esters of pyridoxol have been obtained, but no acyl migration in the direction 5-S → 4-O could be detected. Factors that determine acyl migration are discussed.

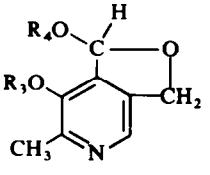
FATTY acid esters of the vitamin B_6 group are important as intermediates in chemical modifications of the various forms of vitamin B_6,[4] as analogs in enzymatic and pharmacological studies,[5,6] in gas chromatography,[7,8] and in studies regarding the active sites of certain enzymes.[9] Nevertheless, some important chemical properties of these derivatives have not been investigated, and some structures are still in doubt. Recently we reported acyl migration and selective esterification of pyridoxol.[10] The present paper is concerned with esters of pyridoxal and pyridoxamine, which are two other forms of the vitamin, and with esters of some thio analogs of pyridoxol.

Pyridoxal

Two basic structural types of esters can be derived from pyridoxal: straight-chain (I, R_3 and R_5 = acyl) and hemiacetal (II, R_3 and R_4 = acyl). In the literature, both



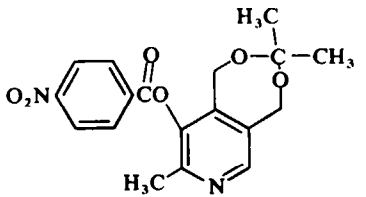
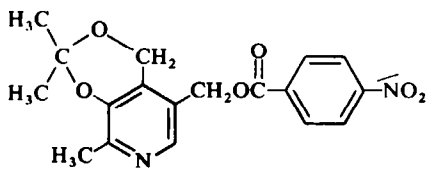
I

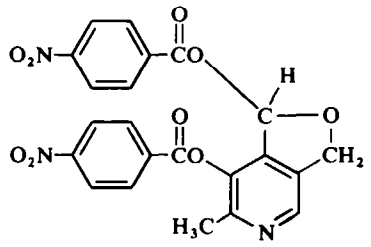
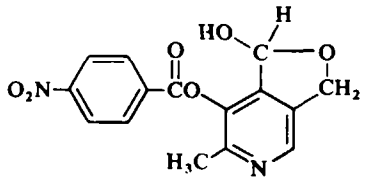
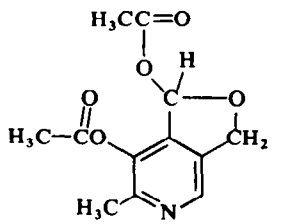


II

structural types of esters have been reported to arise from direct interaction of acylating reagents with pyridoxal. It was of considerable interest to investigate the structures of the reported products as well as to determine conditions under which they are formed.

TABLE 1. NMR SPECTRA OF SOME PYRIDOXAL ESTERS AND REFERENCE COMPOUNDS[a]

Structure	Solvent	4-CH_2 or hemiacetal H	5-CH_2	C_6-H	Other
	DMSO	−288	−293	−494	−509 3-O-*p*COPhNO_2
	DMSO	−309	−323	−490	−496 −499 α^5-O-*p*COPhNO_2

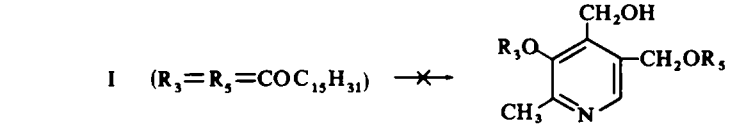
	DMSO	−452 (d)	−319 (b)	−513	−490 3-O-*p*COPhNO$_2$ −465 −474 AB quadruplet −484 α^4-*p*COPhNO$_2$ −493
	$CDCl_3$	−457 (d)	−327 (q)	−517	−498 (s) 3-O-*p*COPhNO$_2$ −476 −487 AB quadruplet −492 α^4-*p*COPhNO$_2$ −503
	DMSO	−372 −380	−302 (b)	−533	−500 −423 3-O-*p*COPhNO$_2$ −431 secondary OH
	$CDCl_3$	−431 −433	−315 (q)	−495	Three Me peaks at −113, −128, −138

* Positions of peaks are expressed in cycles per second from Me_4Si as an internal standard. Abbreviations: s = singlet; b = broad peak (unresolved quadruplet); q = quadruplet; d = doublet.

The first fully esterified ester of pyridoxal was obtained by Viscontini *et al.* in 1951 through the interaction of *p*-nitrobenzoyl chloride with pyridoxal in pyridine solution.[11] The structure of the product was given as the hemiacetal without any proof. On repeating Viscontini *et al.'s* preparation, we found that the product had a m.p. of 171–172°, and not the 99–102° reported by the original authors. The NMR (*vide infra*) and IR spectra, and the analysis of the product, were consistent with the hemiacetal structure. On the basis of this and other evidence reported later in the present paper, we have no doubt that the product obtained by Viscontini *et al.* and by us is the same, even though the nature of the discrepancy in the melting points is not clear. A corresponding benzoyl derivative (II, $R_3 = R_4 =$ COPh) could also be prepared.

In the NMR spectrum of bis(*p*-nitrobenzoyl)pyridoxal (Table 1), the 5-methylene protons appear as a broadened peak (in DMSO) or as an AB quadruplet (in $CDCl_3$), and the hemiacetal proton is shifted to the aromatic region by the electron-withdrawing effect of the *p*-nitrobenzoyl groups. Different types of *p*-nitrobenzoyl esters can be distinguished according to the appearance of their aromatic protons. Such protons of *p*-nitrobenzoyl esters derived from the phenolic 3-OH of pyridoxol invariably appear as singlets (Table 1); those of esters derived from the alcoholic α^5-OH appear as closely spaced doublets; those on the hemiacetal OH group appear as distinct AB quadruplets.

Interaction of pyridoxal with aliphatic acylating reagents has also been studied by various groups of investigators. Sakuragi and Kummerow[6] obtained a fully esterified ester by interaction of pyridoxal with palmitoyl chloride in pyridine. The structure of the product was formulated as "aldehydo" (I), since, according to the authors, the product could be reduced to the corresponding alcohol (3-O,α^5-dipalmitoylpyridoxol, III). We have repeated the preparation of dipalmitoylpyridoxal under conditions identical with those described, and the m.p. of our product was very similar to that reported by Sakuragi and Kummerow. The NMR and IR spectra indicate a hemiacetal structure (II) for the product, and we attribute the slightly different m.p. and



III

UV on attempted reduction of the product II to the formation of impurities. Reduction of dipalmitoylpyridoxal in the hemiacetal form is not to be anticipated under any conditions as long as the hemiacetal ring remains closed. The possibility of the occurrence of 3-O-acylpyridoxols of the general type III, in which the 4-hydroxymethyl group is unsubstituted, has been scrutinized in some of the work about to be described.

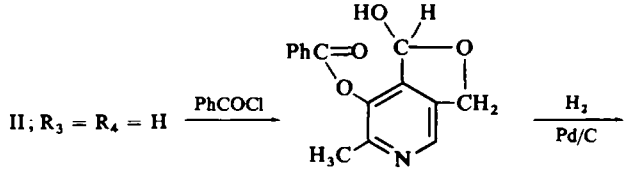
More recently, acetylation of pyridoxal with acetic anhydride has been studied in connection with gas chromatography of vitamin B_6 compounds by Prosser *et al.*[8] and by us.[7] Although the methods of synthesis were similar, different conclusions as

to the structure of the acetylation product were reached. Prosser *et al.*[8] have proposed the straight-chain structure (I, $R_3 = R_5 = COCH_3$), and we have postulated the hemiacetal structure (II, $R_3 = R_4 = COCH_3$).[7]

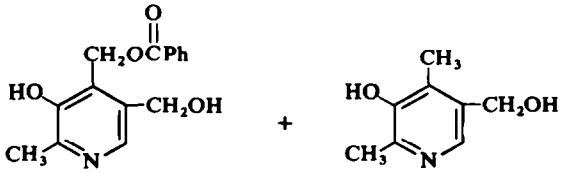
We have subjected pyridoxal to acetylating conditions exactly as described by Prosser *et al.* and have found that the yellow oil initially obtained underwent crystallization and was identical in all respects to the product described by us previously. The peak observed by Prosser *et al.* at −617 c/s and assigned by them to the aldehyde function is probably due to an impurity, and the hemiacetal proton may have been mistaken for a chloroform peak. We have also eliminated the possibility of a rearrangement during the handling of the compound by recording the NMR spectrum immediately after the acetylation reaction had been completed and most of the acylating reagent had been removed *in vacuo*. The crude reaction product had an NMR spectrum identical with that of the purified product.*† Thus quite diverse acylating conditions give rise to the hemiacetal structure II, a situation closely paralleling that found in carbohydrate chemistry when peracylation gives rise to cyclic furanose or pyranose derivatives.

Monosubstituted esters of pyridoxal have been of some interest as intermediates. Viscontini *et al.*[11] have obtained 3-O-(*p*-nitrobenzoyl)pyridoxal (II, $R_3 = p$-$COPhNO_2$, $R_4 = H$) by selective hydrolysis of bis(*p*-nitrobenzoyl)pyridoxal (II. $R_2 = R_4 = p$-$COPhNO_2$) with hydrobromic acid. Because of the great ease of migration from 3-O to α^4-O found in pyridoxol,[10] we have subjected the originally assigned structure to some scrutiny. The NMR spectrum in DMSO-d_6 confirms the original structure assignment: the hemiacetal proton is split by the α^4-OH proton, and the latter proton appears as a doublet (Table 1). It was established earlier[12] that splitting of the OH proton in DMSO is indicative of substitution on the phenolic OH group in vitamin B_6 compounds. Also, the fact that the four aromatic protons of the *p*-nitrobenzoyl ester appear as a singlet is consistent with attachment of *p*-nitrobenzoyl group to the phenolic hydroxyls (*vide supra*).

The absence of acyl migration opened up the possibility of obtaining pyridoxals



IV



V VI

* We are indebted to Dr. Hanns Ahrens for this experiment.

† L. T. Sennello and C. J. Argoudelis, independently of us, have reached the same conclusions with respect to the structures of fully acylated pyridoxals *J. Org. Chem.* **33**, 3983 (1968).

selectively esterified in the 3-O position by direct esterification. Indeed, when pyridoxal was reacted with an equimolar quantity of benzoyl, *p*-nitrobenzoyl, or palmitoyl chloride, an excellent yield of the corresponding 3-O esters of the general type IV was obtained. Earlier, an attempt to obtain 3-O-benzoylpyridoxal (IV) indirectly by hydrolysis of its ethyl hemiacetal was not successful.[13] A similar attempt to obtain 3-O-palmitoylpyridoxal by selective hydrolysis of its ethyl hemiacetal was reported to give the desired product.[6]

Catalytic reduction of 3-O-benzoylpyridoxal (IV) under mild conditions (Pd on charcoal) gave α^4-O-benzoylpyridoxol (V) and 4-deoxypyridoxol (VI). Despite the mildness of the conditions used in the reduction, no 3-O-benzoylpyridoxol could be isolated. This indicated that the 3-O $\rightarrow$ α^4-O rearrangement takes place under extremely mild conditions, and that structures of the type III (R_3 = acyl, R_5 = H) can be expected to be very unstable. 4-Deoxypyridoxol can be formed from α^4-O-benzoylpyridoxol very readily, indicating that further hydrogenolysis takes place after the initial rearrangement during the reduction of IV. The mechanism of this rearrangement has been discussed, and involves the formation of an ortho acid intermediate across the 3-O and α^4-O positions of pyridoxol.[10]

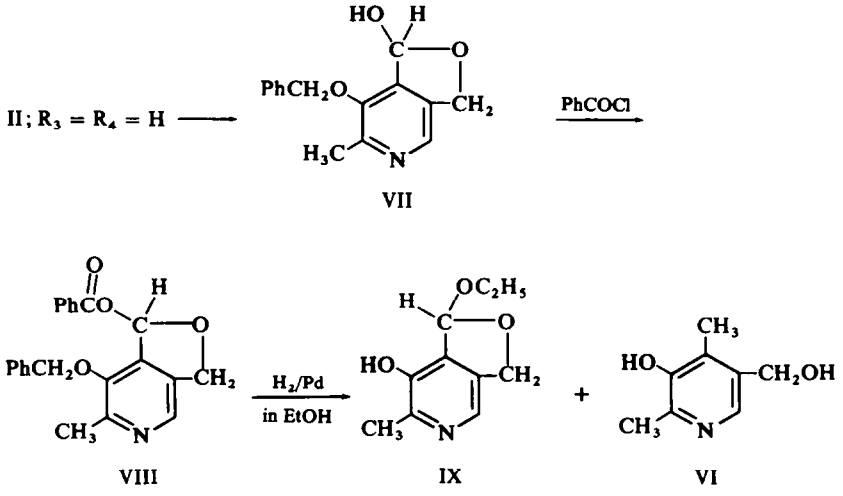
The absence of a similar rearrangement in pyridoxal may be explained by the difficulty of forming the intermediate ortho acid from the 3-O esters of pyridoxal. The freedom of rotation of the α^4-OH in pyridoxol permits the group to assume a conformation favorable for occurrence of the cyclization reaction, whereas rotation is restricted in the bicyclic pyridoxal. This difference between the two compounds is also evident in the resistance of pyridoxal to undergoing reactions that require cyclization between the 3- and α^4-OH groups. Thus pyridoxol gives a boric acid complex that exhibits characteristic shifts in the UV spectrum of pyridoxol and can be titrated,[14] no such properties being observed with pyridoxal. Although complexation could not be detected by these two criteria, weak complex formation with pyridoxal could be inferred by depression of the R_f value in TLC on plates containing boric acid,[15] and by the direction of migration in paper electrophoresis with borate buffer.* †

The possibility of a reverse migration (α^4-O $\rightarrow$ 3-O) in pyridoxal could not be disregarded. Accordingly, 3-O-benzyl-α^4-O-benzoylpyridoxal (VIII) was prepared and was subjected to hydrolysis and hydrogenolysis. Hydrolysis did not provide the desired selectivity, and hydrogenolysis gave only 4-deoxypyridoxol (VI) and pyridoxal ethyl acetal (IX). It is significant that no α^4-O-benzoylpyridoxol could be isolated. Since the hydrogenolysis conditions were mild, it could be expected that if any α^4-O-benzoylpyridoxol was formed, some would survive; but none was found in the

* We are indebted to Dr. U. Roze for the electrophoresis experiments. Pyridoxol, pyridoxal, and 4-deoxypyridoxol were subjected to paper electrophoresis (2,500 v for 25 min) in 0·01 M pH 9 pyrophosphate and in 0·05 M pH 9 borate. In pyrophosphate, all three substances moved towards the anode (4, 2, and 20 mm). In borate, only 4-deoxypyridoxol moved towards the anode (20 mm), but pyridoxol and pyridoxal moved towards the cathode (54 and 25 mm, respectively).

† It has been shown that pyridoxal chelates with metal ions.[16] Although the structure of these complexes has not been determined, the phenolic hydroxyl and the potential aldehyde group of pyridoxal have been invoked in explaining the complexation reaction.[17] In view of our experiments that indicate different strengths of boric acid complexes, it would be of some interest to compare complexation of various metal ions with the various forms of the vitamin.

reaction mixture. Thus migration from α^4-O to 3-O, which would ultimately yield α^4-O-benzoylpyridoxol, can probably be ruled out.



Esters of pyridoxal and some of their derivatives are summarized in Table 2.

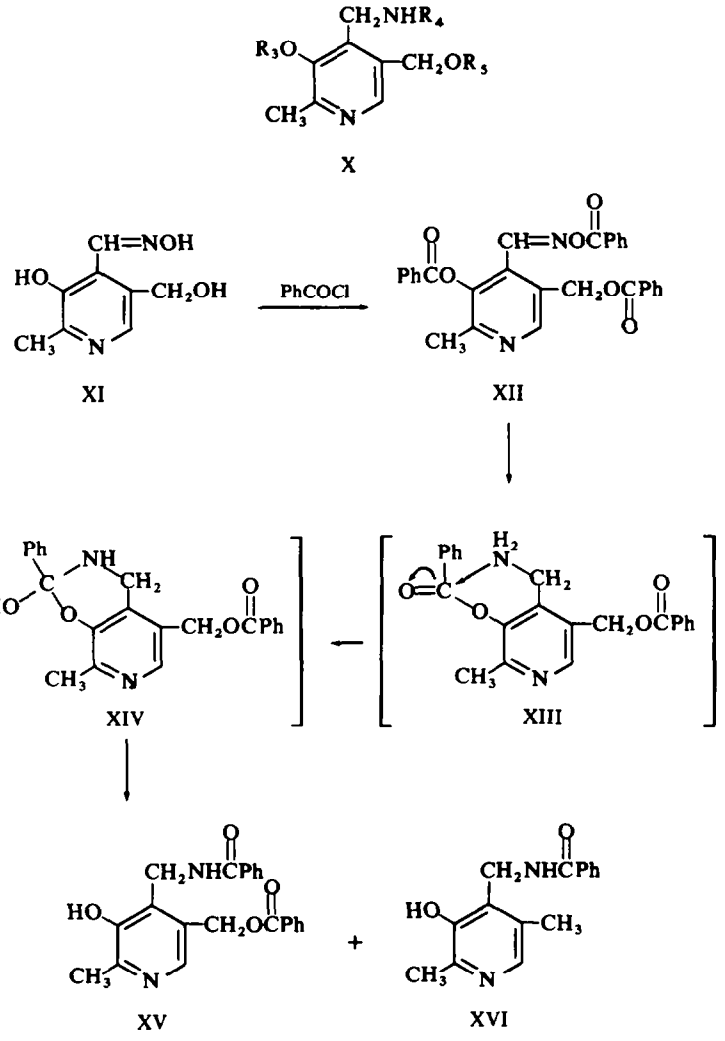
TABLE 2. ESTERS OF PYRIDOXAL (SEE STRUCTURE II)

Degree of Substitution	Nature of R substituent		References
	R_3	R_4	
Monosubstituted 3-O-esters	COPh	H	This study
	$COPhNO_2(p)$	H	This study and Ref. 10
	$COC_{15}H_{31}$	H	This study
	$COC_{15}H_{31}$	OC_2H_5	Ref. 6
	COPh	OC_2H_5	Ref. 12
	$COCH_3$	OC_2H_5	Ref. 12
Monosubstituted 4-O-esters	CH_2Ph	COPh	This study
Disubstituted esters	$COCH_3$	$COCH_3$	Ref. 7
	$COPhNO_2(p)$	$COPhNO_2(p)$	Ref. 10
	$COC_{15}H_{31}$	$COC_{15}H_{31}$	This study and Ref. 6
	COPh	COPh	This study

Pyridoxamine

In contrast to pyridoxal and pyridoxol, pyridoxamine (X, $R_3 = R_4 = R_5 = H$) has relatively few esters that are known, and we are not aware of any structural problems in this area. Esters of pyridoxamine are summarized in Table 3. Nevertheless, the possibility of an N → O acyl migration has been discussed by McCasland *et al.* in connection with the synthesis of some N-acyl derivatives of pyridoxamine.[18] Although no evidence of such a rearrangement was found, the reverse migration (O → N) remained a possibility.

In order to study acyl migration from the 3-OH to the α^4-amino groups, pyridoxal hydrochloride was converted to pyridoxal oxime (XI) and was benzoylated. The tribenzoyl derivative (XII) was reduced catalytically with H_2 in the presence of Pd/C until the required amount of H_2 was absorbed.



By using preparative TLC, the reduction product was separated into two components, both of which gave a positive Gibbs test, indicating that the phenolic OH was free. The compound corresponding to the higher R_f was α^4-N,α^5-O-dibenzoylpyridoxamine (XV), as indicated by its NMR and IR spectra. It could arise from the benzoyloxyoxime (XII) only by reduction of the oxime to the amine (XIII) and subsequent 3-O → N migration, most likely through formation of a six-member hydroxyoxazolidine ring, as in the presumed intermediate (XIV). The compound

corresponding to the lower R_f was identified as α^4-N-benzoyl-5-deoxypyridoxamine (XVI). The formation of α^4-N-benzoyl-5-deoxypyridoxamine is due to further reduction of the rearranged product. It is also formed by catalytic reduction of α^4-N,α^5-O-dibenzoylpyridoxamine under similar conditions.

In order to explore the transfer of the acyl from the α^5-OH to the α^4-amino group, α^5-O-acetylpyridoxamine hydrochloride (XVII) was prepared from pyridoxamine and a large excess of acetic acid in the presence of HCl. The hydrochloride thus obtained gave an IR spectrum characteristic of an O-acetyl group (5·74 μ or 1742 cm^{-1}). Liberation of the free base from XVII gave α^4-N-acetylpyridoxamine (XX), the carbonyl absorption of which changed to 6·07 μ (1647 cm^{-1}), which is characteristic of the acetyl-amide group. Thus an α^5-O → α^4-N acyl migration can be readily achieved, at least with the acetyl group, and most likely involves the 7-member hydroxyoxazolidine ring of the intermediate XIX. This rearrangement could not be reversed by treatment of α^4-N-acetylpyridoxamine with acid.

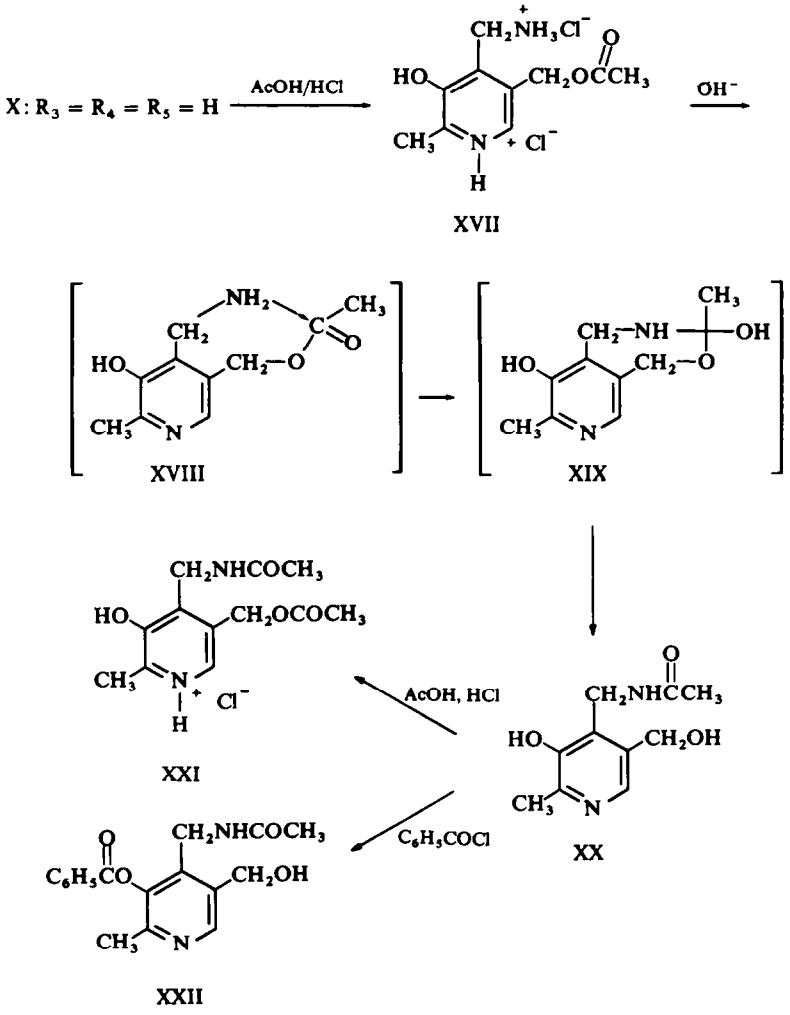


TABLE 3. ESTERS OF PYRIDOXAMINE (SEE STRUCTURE X)

Degree of Substitution	Nature of R substituent			References
	R_3	R_4	R_5	
Trisubstituted	$COCH_3$	$COCH_3$	$COCH_3$	This study and footnote[a]
	COC_6H_5	COC_6H_5	COC_6H_5	Footnote[a]
	$COC_6H_4NO_2(p)$	$COC_6H_4NO_2(p)$	$COC_6H_4NO_2(p)$	Footnote[a]
	COC_9H_{19}	COC_9H_{19}	COC_9H_{19}	Footnote[a]
	$COC_{15}H_{31}$	$COC_{15}H_{31}$	$COC_{15}H_{31}$	Ref. 6
(Trifuroyl)	COC_4H_3O	COC_4H_3O	COC_4H_3O	Ref. 18
Disubstituted	H	$COCH_3$	$COCH_3$	This study
	H	COPh	COPh	This study
	COPh	$COCH_3$	H	This study
Monosubstituted	H	COC_4H_3O	H	Ref. 18
	H	H	$COCH_3$	This study
	H	$COCH_3$	H	This study

[a] T. Sakuragi and F. A. Kummerow, *J. Org. Chem.* **22**, 825 (1957).

α^4-N-Acetylpyridoxamine (XX), which can now be obtained very readily, could be acetylated further in the α^5-position to yield α^4-N,α^5-O-diacetylpyridoxamine. Treatment of α^4-N-acetylpyridoxamine with benzoyl chloride gave 3-O-benzoyl-α^4-N-acetylpyridoxamine (XXII). There is no further acyl migration from the phenolic 3-O-OH to the α^4-N-amino group, a possibility that could be considered in view of the facile O → N migration in salicylamide.[19]

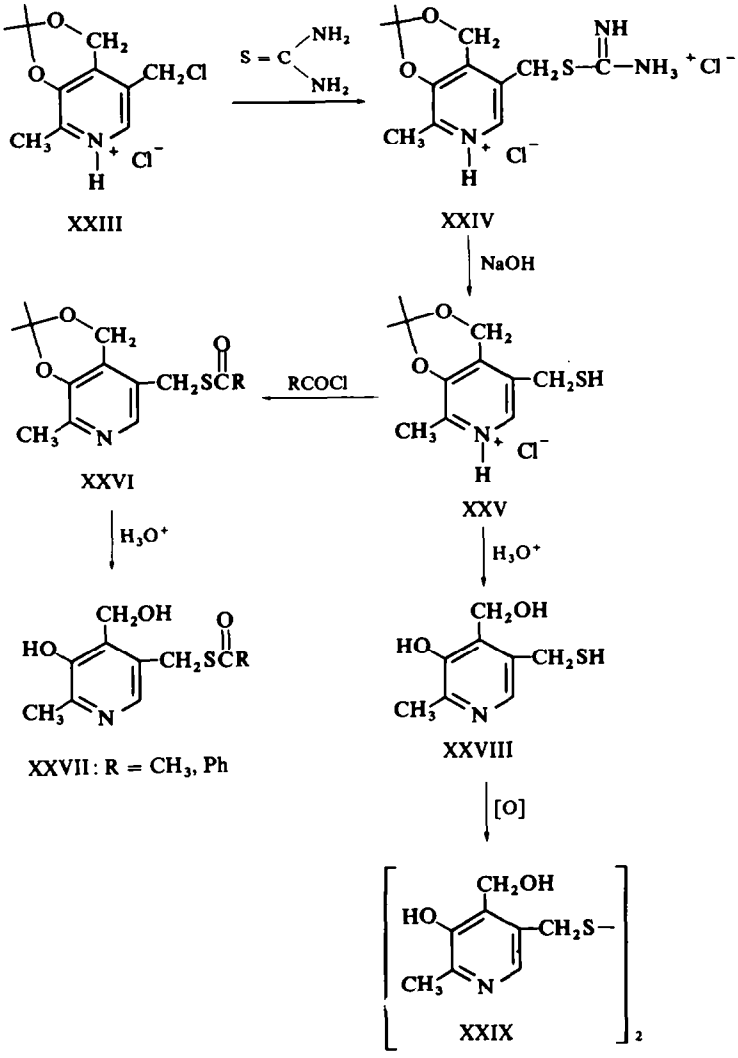
Thioanalogs

The chemistry of the thioanalogs of pyridoxol has received some attention because of the occurrence of such compounds in condensed milk[20] and the discovery of their radioprotective[21] and hypnotic[22] properties. Schmidt and Gieselmann reported an acyl migration converting 3-O,α^4-S-diacetyl-4-pyridoxthiol to the corresponding α^4-S,α^5-O-diacetyl derivative.[23] A direct transesterification (3-O → α^5-O) appeared unlikely because of the strain involved in the formation of the required intermediate, but a two-step acyl migration could account for the product formed. The rearranged product could be formed by the initial migration of the acyl group on the α^4 sulfur to the alcoholic α^5 oxygen, followed by migration of the acyl group on the phenolic oxygen in the 3-position to the α^4 sulfur.

We have now investigated the possibility of α^5-S → α^4-O migration by preparing the α^5-S-benzoyl and α^5-S-acetyl esters of pyridox-5-thiol, as indicated in Scheme I. We have not observed any acyl migration in these esters.

It can be safely assumed that most, if not all, acyl migration takes place through the formation of an appropriate ring intermediate. The cyclic ortho acid that is formed in O → O migrations is most likely derived from addition reactions involving the OH group on the carbonyl, as has been shown previously for the 3-O → α^4-O migration in pyridoxol.[10] An analogous mechanism can be assumed to take place in cases of O ⇋ S and O ⇋ N migrations.

SCHEME 1



Of the factors that favor acyl migration, the most important is the facility with which the intermediate ring can be formed. Lemieux[24] has pointed this out in regard to acyl migrations in carbohydrates. He says that "if two positions in a carbohydrate structure can be expected to be spanned readily by an acetal bridge . . . , then it can be expected that the two positions can be involved in an acyl group migration."

Although this factor goes a long way in explaining why facile 3-O → α^4-O migrations occur in pyridoxol but not in pyridoxal, it fails to explain the direction of the migration, or the nonoccurrence of O → O migration between the α^4 and α^5 positions.

The α^4-O and α^5-O positions in pyridoxol can be readily spanned by an acetal bridge,[25] and yet no acyl migration could be observed.[10] Not only ring strain, but also ring size and the natures of the groups to which the acyl is attached initially and finally, are likely to have a decisive influence on the ability of the acyl group to migrate.

(1) *Ring size.* Generally an intermediate with a 6-member ring is formed much more readily than one with a 7-member ring. Indeed, acyl migrations that require the formation of 7-member rings are very rare. In vitamin B_6 chemistry, 7-member rings would have to be formed in α^5-O → α^4-N acyl rearrangements and in the presumed α^4-S → α^5-O acyl migration,[23] but no corresponding α^4-O ⇄ α^5-O acyl migration has been observed. The formation of intermediates with 7-member rings in this type of migration is apparently associated with the presence of factors favoring acyl rearrangements, which are discussed in connection with the next two topics. Other rearrangements involve intermediates with 6-member rings.

(2) *Attachment of the migrating group.* The tendency to migrate from 3-O to α^4-O is enhanced by the lability of the phenolic acyl linkage. Thus 3-O-acetylpyridine has been found to be a good acylating agent.[26] Similarly, the lability of the S-acyl linkage might be a determining factor in the presumed α^4-S → α^5-O migration in the sulfur analog. The well-authenticated migration from N to O under mild conditions in other systems might be due to labilization of the otherwise stable amide linkage by quaternarization of the nitrogen.

(3) *Nature of the migration terminus.* As Lemieux[24] points out, steric effects may be of importance in explaining migrations from secondary (more hindered) to primary hydroxyls in carbohydrates. In the vitamin B_6 area, such effects appear to be of less importance than the nucleophilicity of the migration terminus. Thus the high nucleophilicity of the amino group induces migration of the α^5-O acetyl to the α^4-N position in spite of requiring formation of an unfavorable seven-member ring. Migration to the analogous but less nucleophilic α^4-O position does not take place.

(4) Finally, *after* the ring intermediate has been formed, it can open up in either of two directions. Factors that determine the direction of the ring opening may be quite subtle and are not completely understood. Thus we have postulated an α^4-S → α^5-O migration, but no migration has been observed to take place in the opposite direction, even though, as we have demonstrated in this study, reverse migration would involve a very similar ring intermediate.

EXPERIMENTAL

TLC was used routinely as described earlier.[10] IR spectra were determined with a Perkin–Elmer 137B spectrophotometer. NMR spectra were determined with a Varian A-60A instrument. The solvent was either DMSO or $CDCl_3$, and the soln contained 7–15% of the compound; positions of peaks are expressed in cycles per second from TMS as an internal standard. Assignments of peaks were made on the basis of previous work.[28]

3,α^4-*Di*-O-*palmitoylpyridoxal* (II, $R_3 = R_4 = COC_{15}H_{31}$). Pyridoxal hydrochloride (0·56 g, dried over P_2O_5) was dissolved in 50 ml dry pyridine, and palmitoyl chloride (4 ml) was added drop by drop with stirring and ice-cooling. The reaction mixture was stirred for 1½ hr in the cold, and was then shaken on a "wrist action" shaker for 2 days. The pyridine soln was next evaporated to dryness under reduced pressure, and the residue was dissolved in $CHCl_3$ (125 ml). The $CHCl_3$ soln was washed with 0·5N HCl followed by 5% Na_2CO_3aq and water, was dried over $CaSO_4$, and was filtered. The filtrate was completely evaporated under reduced pressure, and the residue was crystallized from MeOH, yielding 1·45 g (80·1%),

m.p. 76–77° (lit.[6] m.p. 74°); λ_{max}^{Nujol} 5·76 μ (C = O). Its NMR spectrum has been published in a previous communication.[29] (Found: C, 74·35; H, 10·68; N, 2·22. Calc. for $C_{40}H_{69}NO_5$: C, 74·60; H, 10·80; N, 2·19%).

Attempted reduction of 3,α⁴-*di-O-palmitoylpyridoxal with powdered zinc and ethanol.* The procedure described by Sakuragi and Kummerow[6] was followed. After working up, the final material melted at 76–77°. It was found to be identical with the starting material, pyridoxal dipalmitate, by determination of the mixed m.p. and by comparison of the IR spectra.

3,α⁴-*Di-O-p-nitrobenzoylpyridoxal* (II, $R_3 = R_4$ = p-$COPhNO_2$) was prepared from pyridoxal hydrochloride and *p*-nitrobenzoyl chloride in dry pyridine according to the method described by Viscontini *et al.*;[11] m.p. 170–171° (lit.[11] 99–102°) after crystallization from an acetone–water mixture; λ_{max}^{Nujol} 5·73, 5·81 μ (C=O); NMR spectra are given in Table 1. (Found: C, 56·93; H, 3·49; N, 8·87. Calc. for $C_{22}H_{15}N_3O_9$: C, 56·77; H, 3·25; N, 9·03%).

3α⁴-*Di-O-benzoylpyridoxal hydrochloride* (II, $R_3 = R_4$ = COPh) was prepared from pyridoxal hydrochloride (200 mg) and benzoyl chloride (0·6 ml) in dry pyridine (5 ml) by the usual procedure. The product was characterized as the hydrochloride, m.p. 129–130°, yield 350 mg (82%); λ_{max}^{Nujol} 5·74 μ (C=O); NMR ($CDCl_3$): 2-CH_3, −176 (s); 5-CH_2, −399 (s); C_6—H, −552. (Found: C, 64·43; H, 4·30; N, 3·56. Calc. for $C_{22}H_{18}NO_5Cl$: C, 64·15; H, 4·37; N, 3·42%).

3-O-p-*Nitrobenzoylpyridoxal* (II, R_3 = *p*-$COPhNO_4$ R_4 = H)

A. *By selective hydrolysis.* 3,α⁴-Di-O-*p*-nitrobenzoylpyridoxal was treated with hydrobromic acid in glacial AcOH according to the procedure of Viscontini *et al.*[11]; m.p. 209–210° (lit.[11] 210–211°); λ_{max}^{Nujol} 5·72 μ (C=O). Its NMR spectrum is given in Table 1. (Found: C, 57·22; H, 3·95; N, 8·59. Calc. for $C_{15}H_{12}N_2O_6$: C, 57·00; H, 3·82; N, 8·86%).

B. *By selective acylation.* Pyridoxal hydrochloride (470 mg) was dissolved in dry pyridine (10 ml) and cooled with ice, and *p*-nitrobenzoyl chloride (450 mg) in 20 ml of anhyd ether was added slowly drop by drop with stirring for 10 min. The reaction mixture was stirred at room temp for 8 hr and was then evaporated completely *in vacuo*. The residue was cooled with ice, and on addition of ice water a white ppt formed. It was filtered, washed several times with cold water, and dried. The product (0·70 g, 96%) was crystallized from acetone; m.p. 209–210°. Mixed m.p., IR spectra, and TLC (EtOAc) showed that the compound was identical with 3-O-*p*-nitrobenzoylpyridoxal.

3-O-*Benzoylpyridoxal* (IV). Benzoyl chloride (0·32 ml) in 5 ml of anhyd ether was added slowly, with stirring, to pyridoxal hydrochloride (500 mg) in 8 ml dry pyridine, and was stirred at room temp for 4 hr under the conditions as described for the *p*-nitrobenzoyl derivative (Method B). After working up as usual, the compound showed one major spot on TLC with EtOAc; yield 664 mg (94·4%), m.p. 171–172° (from acetone); λ_{max}^{Nujol} 5·75 μ (C=O); NMR (DMSO): 4C—H, −373, −381: 4C—OH, −417, −425; 5-CH_2, −303 (broad); C_6—H, −503. Benzoylation of this compound (benzoyl chloride in pyridine) gave 3,α⁴-di-O-benzoylpyridoxal (*vide supra*) in 82% yield. (Found: C, 66·20; H, 4·76; N, 5·02. Calc. for $C_{15}H_{13}NO_4$: C, 66·42; H, 4·79; N, 5·16%).

3-O-*Palmitoylpyridoxal* (II, $R_3 = COC_{15}H_{31}$, R_4 = H). Palmitoyl chloride (0·68 ml) in 10 ml of anhyd ether was added slowly to pyridoxal hydrochloride (500 mg) in 15 ml dry pyridine, and was stirred at room temp for 4 hr under the conditions described for the benzoyl derivative (see above). The crude material showed one major spot on TLC with EtOAc. The yield was 950 mg (95·7%), and the material was crystallized from ether; m.p. 118–120°, λ_{max}^{Nujol} 5·70 μ (C=O); NMR ($CDCl_3$): 2-CH_3, −146; 4-CH, −382, −384; 5-CH_2, −304, −308 (part of an AB quadruplet); C_6-H, −494; palmitoyl, −78 (multiplet). (Found: C, 70·81; H, 9·46; N, 3·49. Calc. for $C_{24}H_{39}NO_4$; C, 71·11; H, 9·62; N, 3·45%).

Catalytic hydrogenation of 3-O-*benzoylpyridoxal* (IV)

Formation of α⁴-O-*benzoylpyridoxol* (V) *and* 4-*deoxypyridoxol* (VI). Compound IV (100 mg) in EtOH (5 ml) containing two drops water was added to prereduced Pd/C (5%, 30 mg) in alcohol (5 ml), and was hydrogenated at room temp and atm press for approximately 30 min, until the calculated amount of H_2 was absorbed. TLC of the oily reduction product gave three spots, one of them being starting material. The oil was further reduced with an additional 3·3 ml of H_2, leaving no detectable starting material, and the catalyst was removed by filtration. After evaporation of the filtrate, the two reaction products were isolated by preparative TLC with EtOAc. The compound corresponding to R_f 0·07 was isolated as the hydrochloride, m.p. 258–259 (dec), and was found to be identical with 4-deoxypyridoxol hydrochloride by mixed m.p. and IR spectra. The compound corresponding to R_f 0·25 had an m.p. of 147–148° and was identical with α⁴-O-benzoylpyridoxol[10] by mixed m.p. and IR spectra.

Both compounds could also be separated by dissolving the reduced product in water and extracting it first with ether to remove benzoic acid, and then with EtOAc. Evaporation of the EtOAc extract gave α^4-O-benzoylpyridoxol, and 4-deoxypyridoxol could be isolated from the aqueous solution.

Catalytic hydrogenation of α^4-O-*benzoylpyridoxol* (V)

Formation of 4-deoxypyridoxol (VI). α^4-O-Benzoylpyridoxol was reduced as described above for ca. 30 min. 4-Deoxypyridoxol was isolated as the hydrochloride, m.p. 258–259° (dec), identical with 4-deoxypyridoxol on the basis of mixed m.p. and IR spectra.

3-O-*Benzyl*-α^4-O-*benzoylpyridoxol* (VIII). Compound VII[27] (200 mg) in toluene (dry, 5 ml) was added to a suspension of NaH (80 mg of a 53% suspension in mineral oil, which was removed by washing with pet. ether) in toluene, and the mixture was stirred at room temp overnight. The mixture was then cooled with ice, and benzoyl chloride (0·4 ml) in dry toluene (10 ml) was added drop by drop with stirring under dry N_2 for 10 min, stirring being continued for 1 hr. After cooling with ice, 0·05N NaOH (15 ml) was added carefully, and the toluene layer was washed three times with water until free from alkali, dried ($MgSO_4$), and evaporated. The oily residue was extracted several times with pet. ether (b.p. 37–54°), and evaporation of the ether extracts gave crystalline material, which was further recrystallized from pet. ether, giving 170 mg (64%) of pure compound, m.p. 95–96°; λ_{max}^{Nujol} 5·78 μ (C=O). (Found: C, 72·88; H, 5·17; N, 3·99. Calc. for $C_{22}H_{19}NO_4$: C, 73·13; H, 5·26; N, 3·87%).

Hydrogenolysis of 3-O-*benzyl*-α^4-O-*benzoylpyridoxal* (VIII). Compound VIII (22 mg) in EtOH (5 ml) was added to pre-reduced Pd–C (15 mg, 5% Pd) in 5 ml EtOH, and was subjected to hydrogenation at room temp and atm press until 1·5 ml of H_2 was taken up. TLC with EtOAc showed two spots, R_f = 0·03 and 0·21 (Gibbs-positive). The lower spot was identified as 4-deoxypyridoxol, and the higher spot as the ethyl acetal of pyridoxal (m.p. 130–132°, crude) by isolation and comparison with an authentic sample.

Acid hydrolysis of 3-O-*benzyl*-α^4-O-*benzoylpyridoxal* (VIII). 3-O-Benzyl-α^4-O-benzoylpyridoxal was added to hot 1N HCl, and the soln was heated on a steam bath and was spotted on the plate every 10 min. Initially the hydrolysis mixture consisted of the starting material, 3-O-benzylpyridoxal (VII), and pyridoxal. After 90 min on the steam bath, only pyridoxal could be detected.

Tri-O-*benzoylpyridoxal oxime* (XII). To ice-cooled XI (200 mg) in pyridine (5 ml), benzoyl chloride (0·43 ml) in ether (5 ml) was added drop by drop with stirring for 10 min, and the stirring was continued at room temp for 4 hr. After evaporation *in vacuo*, the residue was cooled in ice, ice water was added, and the mixture was allowed to stand overnight in a refrigerator. After filtration, washing with water, and drying, 460 mg (85%) of material was obtained. Following a single crystallization from a mixture of EtOAc and ether, the m.p. was 145–146°; λ_{max}^{Nujol} 5·69, 5·75, and 5·81 μ (C=O); NMR: 2-CH_3, −151; 5-CH_2, −344; C_6—H, −524; 4-CH protons, obscured by aromatic resonances at −436 to −490. (Found: C, 70·14; H, 4·18; N, 5·77. Calc. for $C_{29}H_{22}N_2O_6$: C, 70·44; H, 4·45; N, 5·66%).

Hydrogenation of tri-O-*benzoylpyridoxal oxime* (XII)

Formation of α^4-N,α^5-O-*dibenzoylpyridoxamine* (XV) *and* α^4-N-*benzoyl-5-deoxypyridoxamine* (XVI). Compound XII (80 mg) was dissolved in warm alcohol (20 ml) and was added to pre-reduced Pd on charcoal (30 mg, 10% Pd) in alcohol (10 ml). Reduction was continued after the theoretical amount of H_2 was absorbed and the uptake had slowed down considerably. TLC with EtOAc indicated the absence of starting material, and provided two new Gibbs-positive spots, R_f = 0·53 and 0·2. The compound giving R_f = 0·53 was isolated by preparative TLC, and was crystallized from a mixture of EtOAc and ether. The pure XV weighed 33 mg (54%) and melted at 181–182°; λ_{max}^{Nujol} 3·02 μ (NH), 5·83 μ (C=O, ester), 6·06 μ (C=O, amide); NMR ($CDCl_3$): 2-CH_3, −152; 4-CH_2, −279, −285 (d); 5-CH_2, −329; C_6—H, obscured by an aromatic region at −436 to −494.

The lower R_f (0·2) compound was extracted from the preparative TLC plate with EtOAc as a solvent. The extract was evaporated *in vacuo*, crystallized from EtOAc, filtered, and washed with ether. The pure XVI weighed 10 mg (24%) and melted at 189–190°; λ_{max}^{Nujol} 6·15 μ (C=O, amide); NMR (C_5D_5N): 2-CH_3, −163; 5-CH_3, −134; 4-CH_2, −282, −297 (doublet); C_6—H, obscured by aromatic multiplets at −429 to −491; OH, NH, −599. (Found: C, 69·96; H, 6·11; N, 10·56. Calc. for $C_{15}H_{16}N_2O_2$: C, 70·31; H, 6·25; N, 10·93%).

Compound XVI could also be obtained by the hydrogenolysis of α^4-N,α^5-O-dibenzoylpyridoxamine under similar reducing conditions.

Tribenzoylpyridoxamine. Pyridoxamine dihydrochloride (200 mg) was dissolved in pyridine (3 ml), the soln was cooled with ice, and benzoyl chloride (0·8 ml) was added. The reaction mixture was shaken for

1 hr, and was then left at 4° overnight. After complete evaporation *in vacuo*, it was cooled, and ice-water was added, when a white ppt separated out. Dilute Na_2CO_3 aq was added until slight alkalinity (pH 8), to remove benzoic acid. The ppt was filtered off, washed with water until free from alkali, and dried. The pure compound weighed 280 mg (70%) and melted at 139–140° after crystallization from alcohol); λ_{max}^{Nujol} 3·01 (NH), 5·79 μ (C=O, ester), 6·06 μ (C=O, amide); NMR ($CDCl_3$): 2-CH_3, −149; 4-CH_2, −282, −290 (doublet); 5-CH_2, −333; C_6—H, −518; NH, −403, aromatic protons from −437 to −497.

α^5-O-*Acetylpyridoxamine dihydrochloride* (XVII)

Method A. A 2·44M soln of anhyd HCl in AcOH was prepared as described by McCasland *et al.*[30] by dropwise addition of 11·8 ml Ac_2O to 3 ml 12N HCl with stirring and cooling in ice. Pyridoxamine dihydrochloride monohydrate (500 mg) was added, and was stirred at room temp overnight, giving a clear soln. The product crystallized on cooling with ice and scratching, was filtered, and was washed with ether. The acetate (490 mg, 90%) was recrystallized from MeOH–ether; m.p. 214–215° (dec); λ_{max}^{Nujol} 5·74 μ (C=O); NMR (10% HCl in D_2O): 2-CH_3, −168; CH_3 (acetyl), −132; 4-CH_2, −272; 5-CH_2, −326; C_6—H, −506. (Found: C, 41·96; H, 5·40; N, 9·47. Calc. $C_{10}H_{16}Cl_2N_2O_3$: C, 42·40; H, 5·65; N, 9·89%).

Method B. Pyridoxamine dihydrochloride monohydrate (500 mg) was dissolved in glacial AcOH (15 ml), and HCl was passed in till the soln was saturated. The reaction mixture was stirred at room temp overnight until a clear soln was obtained. On cooling and scratching, a white ppt separated out, which was filtered and was washed with anhyd ether. The product (510 mg, 93%) was recrystallized from MeOH–ether, and was identical with α^5-O-acetylpyridoxamine dihydrochloride on the basis of mixed m.p. (214–215° dec), IR spectra, and TLC.

α^4-N-*Acetylpyridoxamine* (XX) *from* α^5-O-*acetylpyridoxamine dihydrochloride* (XVII). Compound XVII (50 mg) was dissolved in 50% aqueous pyridine (10 ml), and was heated on a steam bath for 1 hr. The pyridine soln was evaporated *in vacuo*. EtOH was added to the crystalline residue and was then evaporated. The material was taken up in a small quantity of alcohol, cooled in ice, and filtered, and the residue was washed with cold EtOH. The N-acetate (38 mg, 88%), recrystallized from EtOH, melted at 223–224°. The compound gave positive Gibbs and silver nitrate tests, but a negative Ninhydrin test; λ_{max}^{Nujol} 2·94 (OH); 3·07 (NH); 6·07 (C=O, amide); NMR (10% HCl in D_2O): 2-CH_3, −166; CH_3 (acetyl), −130; 4-CH_2, −280; 5-CH_2, −301; C_6—H, −500. (Found: C, 48·81; H, 6·07; N, 11·42. Calc. for $C_{10}H_{15}ClN_2O_3$: C, 48·68; H, 6·08; N, 11·35%).

Method B. Compound XVII (50 mg) was dissolved in 5% $NaHCO_3$ aq (10 ml), and was heated on a steam bath for 30 min. The soln was evaporated *in vacuo*. The residue gave one major spot on TLC (1 : 1 MeOH–$CHCl_3$), identical with that of α^4-N-acetylpyridoxamine, obtained by treatment with 50% aqueous pyridine solution by Method A.

Time study of the rearrangement reaction with TLC indicated completion of the reaction in ca. 10 min in $NaHCO_3$ aq, but ca. 40 min in a mixture of water and pyridine.

α^4-N,α^5-O-*Diacetylpyridoxamine hydrochloride* (XXI). Compound XX (40 mg) was dissolved in a 2·44M soln of anhyd HCl in AcOH (14·8 ml), and was stirred at room temp for 28 hr. The reaction mixture was evaporated *in vacuo*, and was crystallized from a MeOH–ether mixture (charcoal treatment), yielding 36 mg (78%) of the diacetate, m.p. 181–183°; λ_{max}^{Nujol} 3·09 (NH), 5·69 (C=O, ester), 6·09 μ (C=O, amide); NMR (10% HCl in D_2O): 2-CH_3, −166; CH_3 (O- and N-acetyl), −130, −135; 4-CH_2, −281; 5-CH_2, −329; C_6—H, −502. (Found: C, 49·84; H, 5·94; N, 9·68. Calc. for $C_{12}H_{17}ClN_2O_4$: C, 49·91; H, 5·89; N, 9·70%).

3-O-*Benzoyl*-α^4-N-*acetylpyridoxamine* (XXII). Compound XX (100 mg) was dissolved in pyridine (10 mg) and cooled with ice, and benzoyl chloride (0·05 ml) in pyridine (2 ml) was added drop by drop for 5 min. Stirring was continued for 1 hr in a cold room and at room temp overnight, and then pyridine was removed *in vacuo*. The residue was taken up in EtOAc (25 ml), was washed with dil Na_2CO_3 aq followed by water, and was dried ($CaSO_4$). The ethyl acetate extract was evaporated *in vacuo*, when white crystalline material separated out. The crystals were filtered, washed with ether, and dried, yielding 88 mg (71%) of the benzoate, m.p. 182–183°; λ_{max}^{Nujol} 2·97 (NH), 5·73 (C=O, ester), 6·10 μ (C=O, amide). (Found: C, 64·74; H, 5·74; N, 8·64. Calc. for $C_{17}H_{18}N_2O_4$: C, 64·96; H, 5·73; N, 8·91%).

α^4,3-O-*Isopropylidenepyridoxylisothiouronium dihydrochloride* (XXIV). Thiourea (0·6 g) was added to a soln of XXIII[31, 32] (2 g) in EtOH (24 ml) and was refluxed for 12 hr. Evaporation and cooling gave crystalline material, which was washed with cold EtOH and recrystallized from MeOH. The yield was 1·8 g (70%), m.p. 204–205° (dec). (Found: C, 42·11; H, 5·50; N, 12·32; S, 9·61. Calc. for $C_{12}H_{19}Cl_2N_3O_2S$: C, 42·35; H, 5·58; N, 12·35; S, 9·51%).

α^4,3-O-*Isopropylidene-5-pyridoxthiol hydrochloride* (XXV). To a soln of XXIV (400 mg) in EtOH (1 ml) was added NaOH (0·23 g) in EtOH (2·3 ml), and the mixture was refluxed for 3 hr. After evaporation of ethanol *in vacuo*, the residue was dissolved in water (5 ml), cooled in ice, and gradually acidified with AcOH. The gummy material that separated out was extracted with ether, and the combined ether extracts were washed with water (3 ×), dried ($CaSO_4$), and evaporated. The gummy residue was then extracted with pet. ether (b.p. 371–54·3°), and the combined extract was evaporated to a small volume and was treated with HCl in ether. A white ppt separated out, was filtered off, and washed. The material (210 mg, 70%) had an m.p. of 175–176° (with frothing). (Found: C, 50·72; H, 6·14; N, 5·54. Calc. for $C_{11}H_{16}NO_2ClS$: C, 50·47; H, 6·11; N, 5·35%).

α^5-S-*Benzoyl*-α^4,3-O-*isopropylidenepyridoxthiol hydrochloride* (XXVI, R = Ph). XXV (100 mg) in pyridine (3 ml) was cooled with ice, and benzoyl chloride (0·12 ml) in ether (3 ml) was added slowly with stirring, stirring being continued at room temp for 6 hr. The reaction mixture was evaporated completely *in vacuo*, and ice water was added. The gummy material that separated out was extracted with ether, and the combined ether extracts were washed with cold 0·1N NaOH and water, and were dried ($MgSO_4$). Treatment with ethereal HCl precipitated the hydrochloride (105 mg, 75%), which was recrystallized from MeOH–ether mixture; m.p. 193–194° (dec); λ_{max}^{Nujol} 6·00 μ (C=O). (Found: C, 59·17; H, 5·51; N, 3·81. Calc. for $C_{18}H_{20}ClNO_3S$: C, 59·09; H, 5·47; N, 3·83%).

α^5-S-*Acetyl*-α^4,3-O-*isopropylidenepyridoxthiol hydrochloride* (XXVI, R = CH_3). Compound XXV (200 mg) in pyridine (5 ml) was reacted with acetyl chloride (0·1 ml) in ether (3 ml) under conditions described for the α-S-benzoyl derivative. The compound was isolated as the hydrochloride (180 mg, 77·5%), which is very hygroscopic. It shrinks at 90° and melts at 155–156°; λ_{max}^{Nujol} 5·91 (C=O). (Found: C, 50·80; H, 5·99; N, 4·83. Calc. for $C_{13}H_{18}ClNO_3S$: C, 51·07; H, 5·93; N, 4·61%).

α^5-S-*Benzoylpyridoxthiol* (XXVII, R = Ph). Compound XXVI (R = Ph; 100 mg) in 50% aqueous EtOH (15 ml) was heated on a steam bath for 1·5 hr. After evaporation *in vacuo*, the residue was found to be hygroscopic, and could not be isolated as the hydrochloride. The free base crystallized (35 mg, 44%), m.p. 172–173° (after recrystallization from EtOAc); λ_{max}^{Nujol} 6·00 μ (C=O). (Found: C, 62·40; H, 5·32; N, 4·74. Calc. for $C_{15}H_{15}NO_3S$: C, 62·21; H, 5·19; N, 4·84%).

α^5-S-*Acetylpyridoxthiol* (XXVII, R = CH_3). Compound XXVI (R = CH_3; 100 mg) was heated in 50% aqueous EtOH for 1 hr on a steam bath, and was worked up as described for the α^5-S-benzoyl derivative. The free base (38 mg, 51%) had an m.p. of 145–146°; λ_{max}^{Nujol} 5·91 (C=O). (Found: C, 52·84; H, 5·74; N, 5·96. Calc. for $C_{10}H_{13}NO_3S$: C, 52·96; H, 5·72; N, 6·16%).

5-*Pyridoxthiol* (XXVII). Compound XXV, (200 mg) in 50% aqueous EtOH (25 ml) was heated on a steam bath under N_2 for 90 min. The soln was evaporated *in vacuo*, and the residue was dissolved in water (10 ml), neutralized with $NaHCO_3$, and extracted several times with EtOAc. The combined EtOAc extracts were washed with water, dried ($CaSO_4$), and evaporated to dryness *in vacuo*. The residue (120 mg, 82%) was crystallized from EtOAc; m.p. 180–181°. (Found: C, 51·47; H, 6·38; N, 7·54. Calc. for $C_8H_{11}NO_2S$: C, 51·89; H, 5·94; N, 7·54%).

Bis(5-*pyridoxyl*) *disulphide* (XVIII). Compound XXVII (100 mg) was dissolved in water (10 ml) and the pH was adjusted to 9 with 2N NH_4OH, giving a clear soln. A stream of air was bubbled through the soln for 24 hr, and a white ppt separated out (92 mg, 92%). The product was crystallized from MeOH–EtOAc; m.p. 216–217° (dec) (lit.[33] 188–190°). The product gives a negative nitroprusside test. (Found: C, 52·44; H, 5·63; N, 7·31. Calc. for $C_{16}H_{20}O_4N_2S_2$: C, 52·17; H, 5·43; N, 7·60%).

Acknowledgement—This study was supported in part by a research grant (CA-08793) from the National Cancer Institute, U.S. Public Health Service. The Varian A-60-A instrument was purchased from funds provided by this grant as well as funds from the National Science Foundation (GB-6238). We would like to thank Dr. Charles A. Nichol for his encouragement and interest.

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