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CONDENSATION OF OXAZOLES WITH DIENOPHILES
SYNTHESIS OF VITAMIN B_6 ANALOGUES

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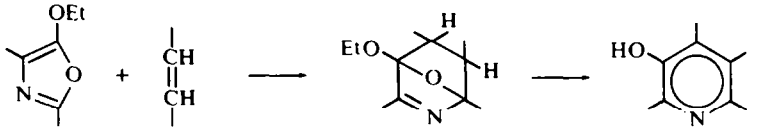
Abstract—Pyridoxine analogues have been synthesized via a Diels–Alder reaction of 5-ethoxyoxazoles with dimethyl maleate followed by reduction. The products on oxidation with manganese dioxide yielded pyridoxal analogues. Pyridoxamine derivatives were obtained by reduction of the pyridoxal oximes. Phosphorylation of the pyridoxamine analogues and the Schiff bases of pyridoxal compounds followed by hydrolysis afforded pyridoxamine phosphate and pyridoxal phosphate analogues, respectively. The mechanism of the Diels–Alder condensation of 5-ethoxyoxazoles is discussed on the basis of calculation of the π-electron charge distributions for diene and dienophile molecules according to the Hückel MO method. PMR, IR and UV spectra of the compounds synthesized and their TLC chromatography have been studied.

THE Diels–Alder condensation of oxazoles with dienophiles,[1] has been applied extensively to the synthesis of various pyridinic bases. The reaction of 5-alkoxyoxazoles with dienophiles (Chart I) provides an appropriate route for the preparation of substituted 3-hydroxypyridines.

CHART 1.



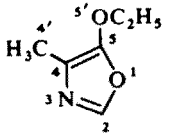
This route has been used by Harris *et al.*[2] for the synthesis of pyridoxine (2-methyl-3-hydroxy-4,5-bis-(hydroxymethyl)pyridine, PN) but the method has since been improved.[3,4]

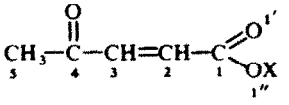
The present paper deals with some aspects of the mechanism of this interesting reaction and with its application to the synthesis of 2- and 6-alkyl analogues of B_6 vitamins.

It follows from Chart I that construction of the pyridine ring involves at least two stages: the Diels–Alder condensation, which leads to unstable adducts, and isomerization of the adducts into 3-hydroxypyridines.

Since the azadiene system is strongly polar, the first stage probably proceeds by a two-step mechanism with charge separation at the transitional state. If this hypothesis is valid, the spatial orientation of 5-ethoxyoxazoles towards ansymmetrical dienophiles

TABLE 1. π-ELECTRON CHARGE DISTRIBUTION AND BOND ORDERS* OF 4-METHYL-5-ETHOXYOXYZOLE, ETHYL ESTER AND ANION OF β-ACETYLACRYLIC ACID





	π-Electron charge densities on atoms:								
	1	1′	1″	2	3	4	4′	5	5′
4-Methyl-5-ethoxyoxazole	1·7327	—	—	1·0203	1·1956	1·0421	1·0705	1·0951	1·9211
4-Methyl-5-ethoxyoxazolium	1·6633	—	—	1·7810	1·6089	0·9937	1·0699	1·0547	1·9146
Ethyl ester of β-acetylacrylic acid	0·8154	1·3208	1·9219	0·8500	0·9376	0·7630	1·3979	1·0747	—
Anion of β-acetylacrylic acid	0·8230	1·3969	1·8253	0·9223	0·9622	0·7636	1·3226	1·0747	—

	Orders of bonds:								
	1–1′	1–1″	1–2	2–3	3–4	4–4′	4–5	5–5′	5–1
4-Methyl-5-ethoxyoxazole	—	—	0·4417	0·7695	0·5492	0·2906	0·7460	0·2660	0·4075
4-Methyl-5-ethoxyoxazolium	—	—	0·5656	0·6104	0·4444	0·2228	0·7657	0·2829	0·4127
Ethyl ester of β-acetylacrylic acid	0·8696	0·2994	0·3219	0·8927	0·3217	0·8836	0·1884	—	—
Anion of β-actylacrylic acid	0·8190	0·4334	0·3127	0·8947	0·3265	0·8836	0·1882	—	—

* These data were calculated by the Hückel molecular orbitals method.

will depend on the π-electron charge distribution on the C atoms which take part in bond formation.

Ethyl β-acetylacrylate (I) was chosen as a dienophile for experimental verification. According to the calculation (Table 1), the major product of reaction of I with 4-methyl-5-ethoxyoxazoles (II) should be ethyl 6-methyl-5-hydroxy-4-acetylnicotinate (III). Actually, the substance isolated from the reaction mixture and TLC chromatography of the mother liquor showed the presence of only trace amounts of other pyridine derivatives. The IR spectrum of the compound isolated indicated the presence of two CO bands at 1728 and 1660 cm^{-1}. The former was assigned to the 5-CO_2Et group not involved in a H-bond, the latter was ascribed to the 4-COMe group, shifted to lower wave numbers owing to participation in a chelate H-bond with the 3-OH group. To determine the structure unequivocally, this compound was reduced with LAH and the PMR spectrum of the prepared diol IV was compared with those of 2-methyl-3-hydroxy-4-hydroxymethyl-5-α-hydroxyethylpyridine (V) and PN. According to Korytnyk *et al.*,[5] the resonance signals of 4-and-5-hydroxymethyl groups of PN are situated at 4·99 and 4·76 δ, respectively. On the basis of this comparison, (Fig. 1) the structure of the compound under discussion may be depicted as III.

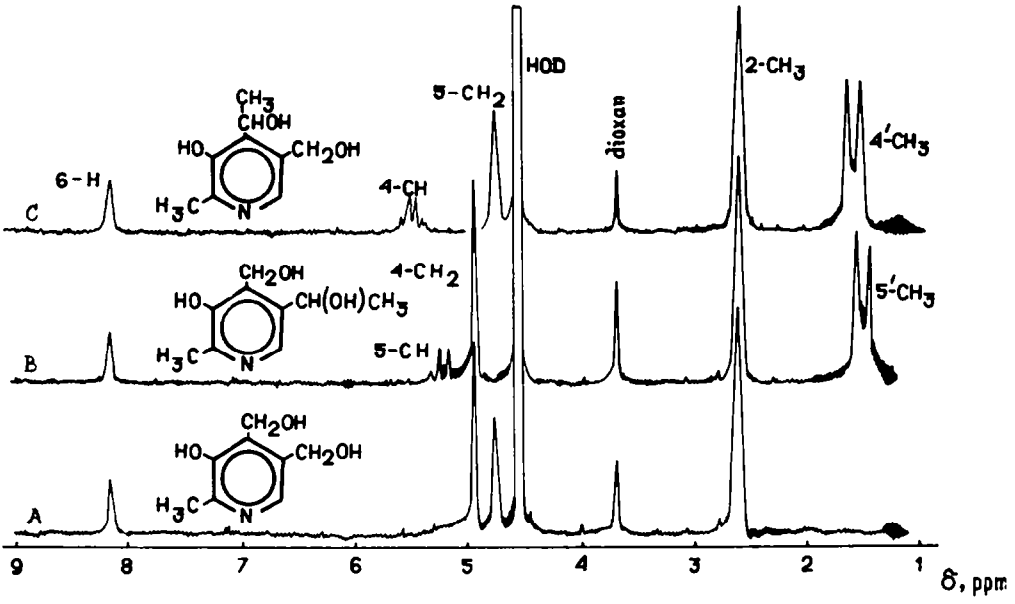
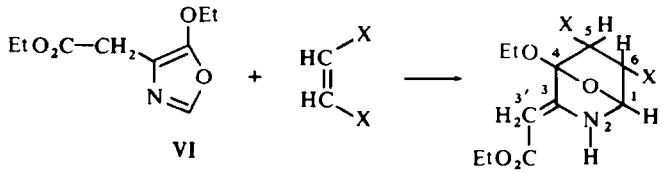


FIG. 1 NMR spectra in D_2O of hydrochlorides of 2-methyl-3-hydroxy-4,5-bis(hydroxymethyl) pyridine (A); 2-methyl-3-hydroxy-4-hydroxymethyl-5-α-hydroxyethylpyridine (B) and 2-methyl-3-hydroxy-4-α-hydroxyethyl-5-hydroxymethylpyridine (C).

Similarly, cyclopenten-3-one was shown to react with II to yield only one product which was proved by its IR and PMR spectra, to be 5-methyl-4-hydroxy-6-aza-hydrinden-3-one.

The mechanism of this condensation has been investigated[3,6,7] but we obtained additional information.

The C=N double bond of the adducts, which is formed on interaction of ethyl 5-ethoxy-4-oxazoleacetate (VI) with dienophiles should migrate to the 3–3′ position



VI

These intermediates with an exocyclic double bond appear to be more stable than the "normal" adducts, due to conjugation of the double bond with the ethoxycarbonyl group. Recently, such an "exocyclic adduct" structure was ascribed by Miki and Matsuo[8] to the compound prepared by reaction of VI with diethyl fumarate, but subsequently[9, 10] these authors reported a "normal" structure for similar compounds.

We investigated the reaction of VI with maleic anhydride. A colourless crystalline product was obtained in 80% yield, the IR spectrum of which shows two strong bands at 1868 and 1792 cm^{-1}, assignable to CO groups of a 5-membered cyclic anhydride (for example, succinic anhydride[11] has bands at 1871 and 1793 cm^{-1}). The strong band at 1681 cm^{-1} is ascribed to a 3′-ethoxycarbonyl group, which appears to participate in a H-bond and to be conjugated with the 3–3′ exocyclic double bond. Recently, it was shown[12, 13] that endocyclic double bonds exhibit a weak band in the range of 1695–1710 cm^{-1}, while exocyclic double bonds show strong peaks at 1649–1662 cm^{-1}. On this basis, the strong band at 1644 cm^{-1} exhibited by the compound under consideration suggests the presence of an exocyclic double bond. The PMR

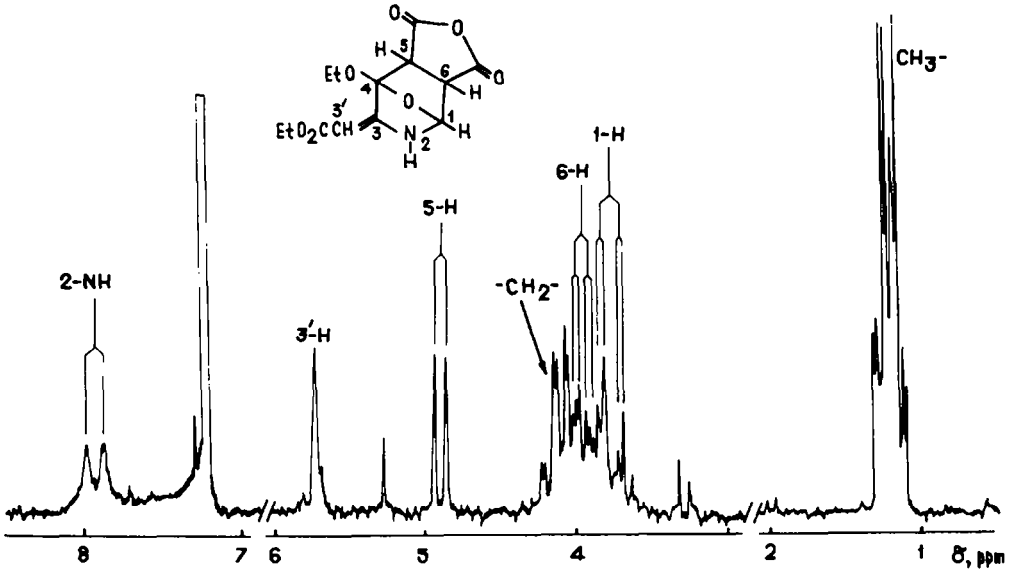


FIG. 2 NMR spectrum in $CDCl_3$ of anhydride of 3-carbethoxymethylene-4-ethoxy-7-oxa-2-azabicyclo-[2.2.1]-heptane-5,6-dicarboxylic acid.

spectrum of this substance (Fig. 2) shows a pair of triplets at 1·2 δ (J = 7 c/s), attributable to the Me components of Et groups, and a corresponding pair of quadruplets of the methylene components at 4 δ. The doublet at 7·9 δ (J_{12} = 12 c/s) is assigned to 2-H. This signal is broad owing to quadrupole interaction with the N^{14} atom and is shifted to lower field. It is reasonable to interpret this shift as resulting from participation of the proton in intramolecular H-bonding. The pair of quadruplets centred at 3·74 and 3·9 and the doublet centred at 4·86 δ (J_{12} = 12; J_{16} = 3·7; J_{56} = 7 c/s) are assigned to 1-H, 6-H and 5-H, respectively. Finally, the 3′-H proton exhibits a singlet signal at 5·69 δ.

All these factors mentioned are in good agreement with the structure of the anhydride of 3-carbethoxymethylene-4-ethoxy-7-oxa-2-azabicyclo-[2.2.1]-heptane-5,6-dicarboxylic acid (VII), shown in Fig. 3.

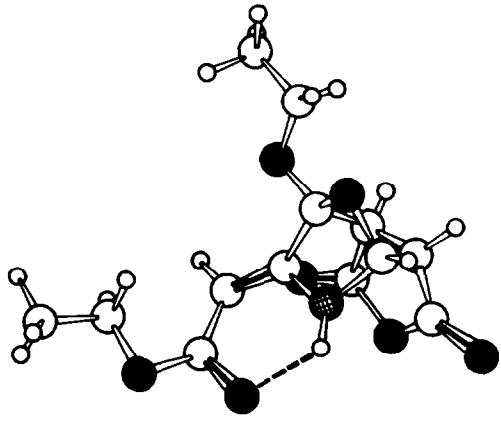


FIG. 3

Isomerization of the adducts in acid media, (Chart I), in our opinion, is initiated by scission of the 1-C—O bond, the transition state being stabilized by ethylate anion elimination from position 5 of the adduct.

This conclusion is in accordance with the following main assumptions:

(a) The 1-C—O bond is weakest, owing to δ-π-conjugation with the 3-C=N double bond. Protonation of the N atom in acid media further facilitates heterolysis of this bond.

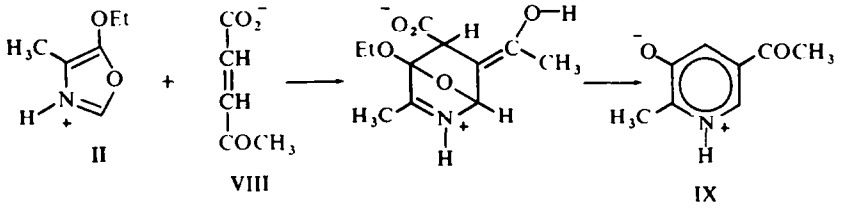
(b) Scission of the 4-C—O bond followed by hydrolysis is unlikely, since both the Diels–Alder reaction and isomerization of the adduct are carried out in anhydrous media. Validity of this assumption is confirmed by consideration of the reaction of 4-methyl-5-ethoxythiazole with dienophiles,[14] which always yields 3-ethoxypyridines as the final product, although the conditions for isomerization (heating with conc. hydrochloric acid) promote hydrolysis.

(c) Finally, if the 4-C—O bond had been broken, the formation of 3-hydroxypyridines on condensation of dienophiles with 5-cyanoxazoles[15] would be difficult to explain.

The mechanism suggested for the isomerization accounts for the fact that the reaction of 5-unsubstituted oxazoles is facilitated by the presence of oxidizing

agents.[16,17] In this case, isomerization of adducts, according to our mechanism, may be connected with the elimination of an hydride ion. Indeed, this reaction proceeds more effectively in the presence of hydride ion acceptors, such as nitrobenzene or hydrogen peroxide.

Experimental proof of the proposed mechanism, was provided by the condensation of II with β-acetylacrylic acid (VIII). In accordance with π-electron charge distribution (Table 1), the carboxyl and acetyl groups should occupy the 5 and 6 positions of the adduct respectively. The isomerization presumably will be accompanied by decarboxylation, since proton removal from the enol will be thermodynamically favourable:



II VIII IX

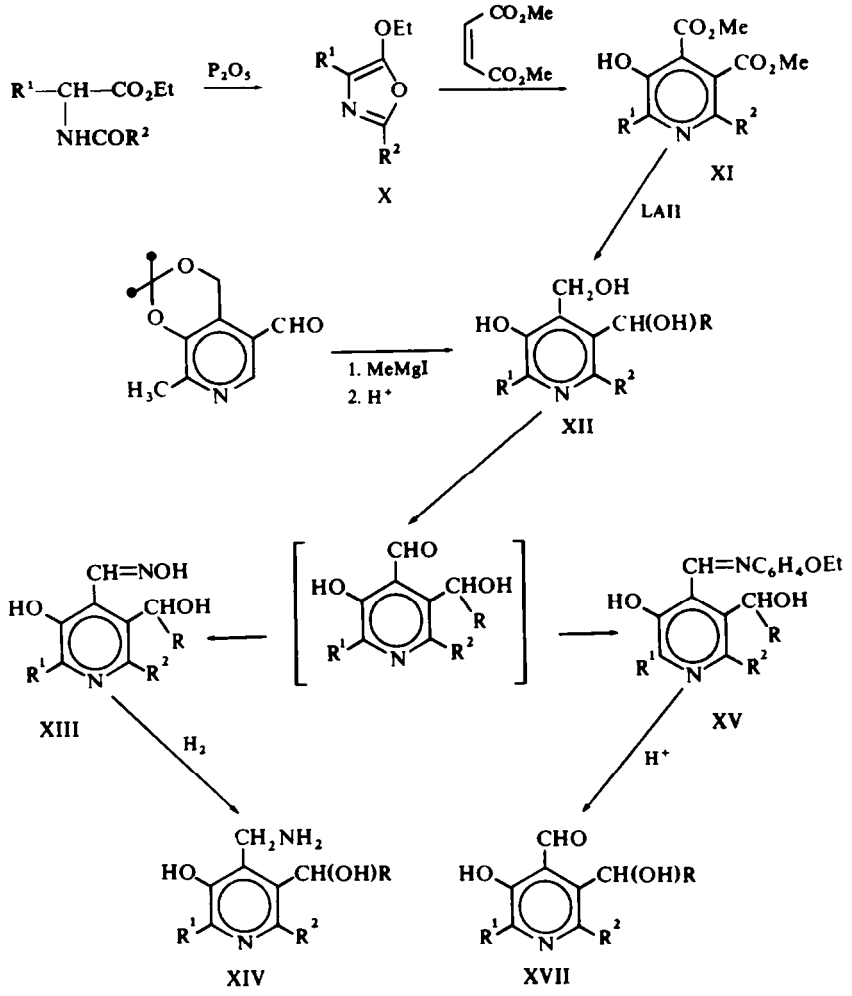
The same product was isolated from the reaction mixture both at room temperature and under heating at 80°. The PMR spectrum provides important information for the structure of the prepared compound. The singlets at 2·86 and 2·89 δ are assigned to 2-Me and 5-COMe groups. In addition, a pair of low-field doublets (J = 2·4 c/s) centred at 8·81 and 8·57 δ, are assigned to α- and γ-protons of the pyridine cycle respectively. Recently, it was shown that the J-value for interaction between *ortho*-protons is 6–10 c/s, between *meta*-protons—1–4 c/s and between *para*-protons—0–1 c/s.[18]

The data supports the structure of 2-methyl-3-hydroxy-5-acetylpyridine (IX) for the compound prepared. Thus, the experimental data agree with the postulated mechanism of the Diels–Alder reaction of 5-ethoxyoxazoles.

This condensation was used for the synthesis of 2- and 6-alkyl analogues of vitamins B_6:

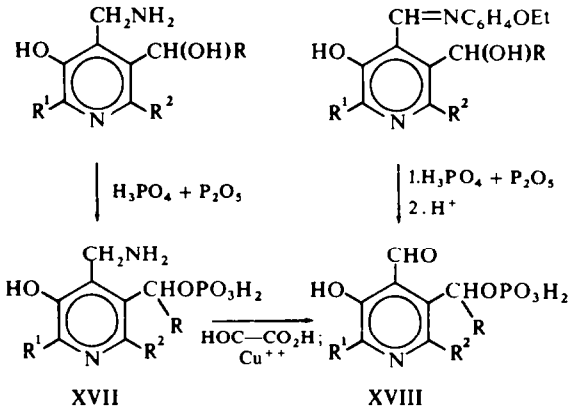
The starting 5-ethoxyoxazoles (X), were obtained by cyclization of ethyl esters of N-acylamino acids with phosphorus pentoxide. The interaction between X and dimethyl maleate gave dimethyl esters of 5-hydroxycinchomeronic acids (XI), which were converted to PN analogues (XII) by reduction with LAH. Analogues of two other forms of vitamin B_6-pyridoxamine (2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine, PM) and pyridoxal (2-methyl-3-hydroxy-4-formyl-5-hydromethylpyridine, PL), were prepared by oxidation of diols (XII) with active manganese dioxide. The aldehydes thus prepared were isolated from the reaction mixtures either in the form of their oximes (XIII), which were reduced to PM analogues (XIV), or in the form of Schiff bases with *p*-phenetidine (XV), which, upon hydrolysis on ion-exchange resin, yielded PL analogues (XVI).

CHART 2.



a: $R^1 = R^2 = R = H$; d: R^1 = iso-C_3H_7, $R^2 = R = H$;
b: $R^1 = R = H$, $R^2 = CH_3$; e: $R^1 = C_4H_9$, $R^2 = R = H$;
c: $R^1 = R^2 = CH_3$, $R = H$; f: $R^1 = R = CH_3$, $R^2 = H$.

The routes for the synthesis of 5′-phosphoric esters of PM and PL analogs were as follows:



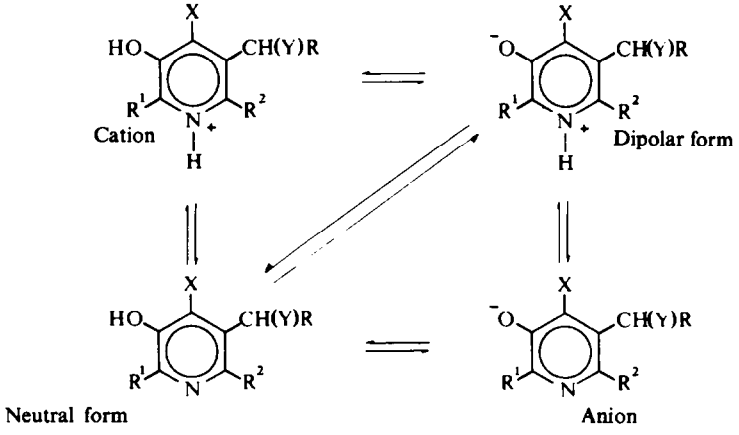
XVII XVIII

Analogues of pyridoxamine-5′-phosphate (PMP) were prepared by the method previously described for PMP itself.[19]

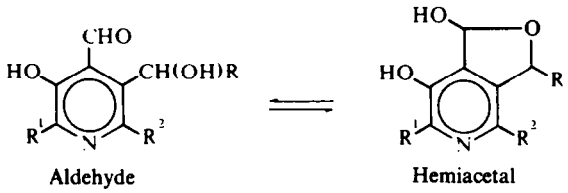
Recently, procedures were published for the synthesis of PLP by way of phosphorylation of Schiff bases of PL.[20, 21] This was applied in developing a method for the preparation of PLP analogues. Good yields were obtained for all substances investigated except 2-nor-6-methyl PLP (XVIIIb). The Schiff bases of the latter compounds were obtained only in poor yield, but preparative transamination of the corresponding PMP analogues, previously applied to the synthesis of PLP itself,[22] was used successfully.

The UV, IR and PMR spectral data of the compounds and also TLC chromatography on silica gel are listed in Tables 2 and 3. Only the principal features of UV spectra are reported, since they characterize the behaviour of the products obtained in aqueous solutions, which is of importance for further investigations with enzyme systems.

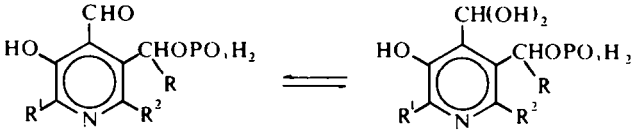
Since all the compounds investigated are 3-hydroxypyridines, equilibria between the following principal ionic forms should exist in aqueous solutions:



At the same time, the occurrence of equilibria between tautomeric forms of PL and PLP analogues should be borne in mind:



Aldehyde Hemiacetal



Aldehyde Hydrate

Since ionization of the 5′-phosphate group was recently shown[23] not to affect the absorption spectrum, assumption of the existence of such ionic and tautomeric forms as indicated above is sufficient for interpretation of variatious pH-dependent changes of UV spectra.

Two basic conclusions can be drawn from examination of UV spectra:

1. Whereas the 2-substituted analogues of B_6 vitamin exist mainly as dipolar forms in the pH range from 5 to 8, considerable amounts of the neutral form are present in the solution of analogues without a substituent in position 2 of the pyridine cycle (Fig. 4).

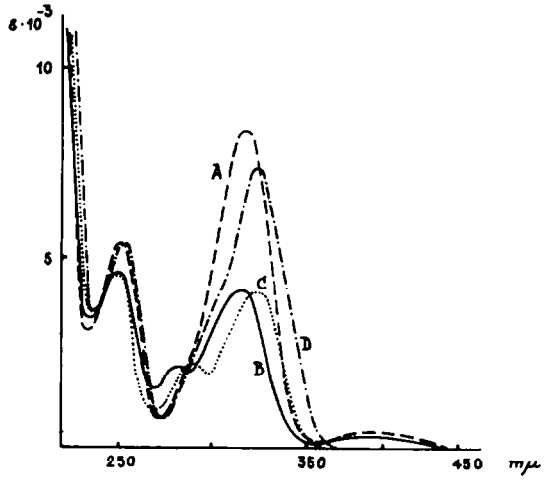


FIG. 4 UV spectra at pH 7. A: 3-hydroxy-4-formyl-5-hydroxymethylpyridine; B: 2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine; C: 6-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine; D: 2.6-dimethyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine.

TABLE 2. PMR SPECTRA OF VITAMIN B_6 ANALOGUES AND RELATED COMPOUNDS

Substances	δ ppm*									
	2-CH_3	6-CH_3	2-*iso*C_3H_7	2-n-C_4H_9	2-H	6-H	4-CH_2—	5-CH_2—	4′-H	CO_2CH_3
Dimethyl esters of 5-hydroxy-cinchomeronic acids, hydrochlorides inD_2O	2·66	2·63	1·36	0·87 1·33 1·69 3·06	8·56	8·66– 8·78	—	—	—	3·93– 3·94 3·97– 3·99
PN analogs, hydrochlorides in D_2O	2·60– 2·61	2·46– 2·48	1·35	0·86 1·29 1·68 2·98	.....	8·12– 8·14	4·96– 4·99	4·75– 4·76	— —	—
Oximes of PL analogues in deutero-pyridine	2·52	2·55– 2·56	1·32	0·76 1·25 1·78 3·00	8·42	8·30– 8·31	—	4·88– 4·91	9·20– 9·25	—

Schiff base of PL analogues with *p*-phenetidine in deuteropyridine	2·59	2·63	1·36	0·81 1·33 1·84 3·04	8·64	8·26– 8·37		4·99– 5·04	9·39– 9·46	—
PL analogues, hydrochlorides in D_2O	2·59	2·52	1·35	0·86 1·36 1·69 3·01	.....	8·14– 8·15	—	5·16– 5·22	6·68– 6·72	
PM analogues, dihydrochlorides in D_2O	2·65	2·68– 2·70	1·35	0·89 1·34 1·64 3·05	8·18	8·21	4·42 4·46	4·81– 4·84	—	—
PLP analogues, monohydrates in D_2O	2·58	2·68	1·35	0·85 1·29 1·82 3·00	.....	8·19	—	5·22– 5·31 J = 5·5 c/s	6·48– 6·49	—

*In the case of *iso*-propyl and n-butyl groups, the centres of multiplets are shown.

TABLE 3. IR SPECTRA OF VITAMIN B_6 ANALOGUES AND RELATED COMPOUNDS

Nos	Substances	ν cm^{-1}				
		—O—H ··· H—O—	OH...N	OH...O=C-chelate	C=C pyridine	C=C pyridine
1	Dimethyl esters of 5-hydroxycinchomeronic acids, hydrochlorides	3480–3300	2900–2570	—	1564–1541 m	1512–1509 m
2	PN analogues, hydrochlorides	3300–3200	3000–2730	—	1554–1547 m	1510–1493 m
3	Oximes of PL analogues	3510–3440	2700–2470	—	1547–1531 w	1513–1499 m
4	Schiff base of PL analogues with *p*-phenetidine	—	—	3280–3160 s	1568–1558 w	1522–1512 m
5	PL analogues, hydrochlorides	3950–3290	2770–2620	—	1583–1560 s	1509 m
6	PM analogues, dihydrochlorides	3470–3220	2980–2620	—	1596–1572 m	1510 m
7	PLP analogues, monohydrates	3430–3410	2920–2550		1581–1560 m	1524–1520 m
8	PMP analogues, dihydrates	3440–3400	3100–2650	—	.....	.....

Nos	ν, cm^{-1}								
	C=C pyridine	C=N⁺—H pyridinium	C=N pyridine	C=O aldehyd	C=O ester	C=N	$—NH_3^+$	P=O phosphate	C—O phosphate
1	1422–1404 m	1648–1630 m	—	—	1658–1652 s	—	—	—	—
2	1421–1400 m	1645–1631 m	—	—	—	—	—	—	—
3	1422–1400 m	—	1580–1571 m	—	—	1654–1640 m	—	—	—
4	1436–1410 m	—	1567–1558 s	—	—	1638–1626 s	—	—	—
5	1439–1420 m	1662–1635 m	—	—	—	—	—	—	—
6	1406–1400 s	1642–1623 w	—	—	—	—	1563–1540 s 1513–1488 s	—	—
7	1415–1410 m	1660–1646 m	—	1720 m	—	—	—	1290–1274 m 1185–1182 m	1070–1061 s
8	1412–1400 m	1653–1643 m	—	—	—	—	1547–1529 s	1294–1290 m 1174–1142 s	1084–1062 s

TABLE 4. ASSIGNMENT OF ABSORPTION MAXIMA TO IONIC FORMS IN UV SPECTRA OF VITAMINS B_6 ANALOGUES

Ionic forms	Solvent	λ_{max} mμ (ε . 10^{-3})					
		$R^1 = R^2 = R = H$	$R_1 = R = H$ $R^2 = CH_3$	$R^1 = R^2 = CH_3$ $R = H$	$R^1 = isoC_3H_7$ $R^2 = R = H$	$R^1 = nC_4H_9$ $R^2 = R = H$	$R^1 = R = CH^3$ $R^2 = H$
PN analogues: Cation	0·1N HCl	289 (6·4)	297 (6·2)	298 (10·8)	291 (8·7)	293 (9·7)	293 (8·4)
Dipolar form	pH 6·9[a]	251 (2·5)[b] 324 (2·8)	256 (3·6)[b] 331 (3·3)	257 (6·3) 332 (8·4)	256 (4·0) 328 (8·0)	254 (3·6) 327 (7·5)	254 (3·8) 325 (7·4)
Neutral form	pH 6·9[a]	286 (3·0)[b]	294 (2·7)[b]	—	—	—	—
Anion	0·1N KOH	242 (7·2) 310 (5·2)	247 (7·2) 317 (5·2)	248 (8·5) 317 (7·9)	246 (6·5) 310 (7·6)	245 (6·6) 311 (7·9)	246 (8·6) 310 (7·2)
PM analogues: Cation (4′-NH_3^+)	0·1N HCl	292 (6·8)	299 (7·4)	302 (8·8)	296 (9·2)	297 (9·2)	295 (8·6)
Dipolar form (4′-NH_3^+)	pH 6·9[a]	251 (2·6)[b] 324 (3·2)	253 (4·8) 328 (4·1)	255 (6·3) 333 (8·7)	254 (4·5) 329 (8·1)	253 (4·9) 328 (8·3)	253 (4·4) 328 (8·0)
Neutral form (4′-NH_3^+)	pH 6·9[a]	287 (3·0)[b]	297 (2·9)[b]	—	—	—	—
Anion	0·1N KOH	243 (7·5) 307 (5·9)	245 (7·8) 312 (5·8)	248 (7·2) 314 (7·0)	246 (7·5) 316 (8·1)	246 (6·7) 309 (8·1)	246 (6·5) 310 (7·1)
PL analogues: Cation, hemiacetal	0·1N HCl	284 (6·6)	292 (6·4)	295 (8·3)	289 (9·2)	292 (9·0)	289 (8·7)
Dipolar form, hemiacetal	pH 6·9[a]	249 (4·6)[b] 314 (4·2)	247 (4·7)[b] 323 (4·0)	250 (5·3) 324 (7·3)	253 (5·1)[b] 319 (8·3)	254 (5·8)[b] 321 (8·2)	256 (5·3)[b] 321 (8·1)
Dipolar form, aldehyde	pH 6·9[a]	390 (0·08)[b]	—	—	390 (0·6)[b]	390 (0·2)[b]	390 (0·05)[b]
Neutral form, hemiacetal	pH 6·9[a]	280 (2·1)[b]	290 (2·1)[b]	—	—	—	—
Anion, hemiacetal	0·1N KOH	240 (8·6)[b] 300 (5·0)	240 (9·0) 307 (5·6)	242 (8·4) 310 (7·8)	238 (8·0)[b] 302 (6·3)	243 (8·4)[b] 304 (6·5)	246 (7·2)[b] 304 (6·3)

Anion, aldehyde	0·1N KOH	390 (0·6)[b]	—	—	390 (2·1)[b]	390 (1·5)[b]	390 (0·3)[b]
PLP analogues:							
Cation, hydrate (5′-OPO_3H_2)	0·1N HCl	292 (6·0)[b]	298 (6·4)[b]	302 (1·1)[b]	297 (8·0)[b]	298 (7·9)[b]	296 (6·2)[b]
Cation, aldehyde (5′-OPO_3H_2)	0·1N HCl	332 (1·1)[b]	340 (1·1)[b]	350 (1·0)[b]	340 (1·2)[b]	350 (1·0)[b]	340 (1·0)[b]
Dipolar form, hydrate (5′-OPO_3H^-)	pH 6·9[a]	328 (2·4)[b]	331 (4·3)[b]	332 (4·8)[b]	329 (2·3)[b]	326 (2·3)[b]	325 (2·3)[b]
Dipolar form, aldehyde (5′-OPO_3H^-)	pH 6·9[a]	384 (3·2)[b]	380 (1·4)[b]	390 (2·5)[b]	390 (4·2)[b]	393 (4·3)[b]	390 (3·3)[b]
Neutral form, hydrate (5′-OPO_3H^-)	pH 6·9[a]	284 (1·5)[b]	290 (2·0)[b]	—	—	—	—
Anion, hydrate (5′-$OPO_3^{=}$)	0·1N KOH	3·5 (1·0)[b]	312 (3·9)[b]	313 (3·6)[b]	304 (1·8)[b]	302 (2·7)[b]	300 (1·0)[b]
Anion, aldehyde (5′-$OPO_3^{=}$)	0·1N KOH	386 (5·6)[b]	394 (2·9)[b]	398 (4·3)[b]	393 (5·7)[b]	395 (4·2)[b]	390 (4·4)[b]
PMP analogues:							
Cation (5′-OPO_3H_2)	0·1N HCl	292 (7·1)	299 (8·1)	302 (9·7)	294 (9·2)	293 (9·3)	294 (9·0)
Dipolar form (5′-OPO_3H^-)	pH 6·9[a]	248 (3·7)[b] 324 (3·6)	252 (4·9)[b] 333 (4·6)	252 (6·3) 336 (8·9)	252 (5·1) 327 (8·1)	253 (5·0) 327 (8·2)	253 (4·9) 325 (8·5)
Neutral form, (5′-OPO_3H^-)	pH 6·9[a]	233 (3·4)[b]	297 (2·8)[b]	—	—	—	—
Anion (5′-$OPO_3^{=}$)	0·1N KOH	243 (7·3) 307 (5·2)	246 (8·6) 313 (6·2)	248 (7·7) 314 (3·0)	244 (6·9) 310 (7·9)	245 (6·9) 309 (8·1)	245 (6·6) 309 (7·9)

[a] 0·1M phosphate buffer.

[b] In these cases, the figures in brackets are not the true molar absorbances, and show the absorbance of the given ionic form at equilibrium concentration, when the total concentration of all ionic forms is 1M.

2. Introduction of an alkyl substituent into position 6 of the pyridine ring shifts the equilibrium between aldehyde and hydrate (hemiacetal) in favour of the latter. The UV spectra of 6-methyl PL and 2-nor-6-methyl PL (Fig. 4) do not display absorption maxima of the aldehyde form at 390 mμ.

Similarly, dependence on the type of substitution is observed in the UV spectra of PLP analogues. The absorption maximum of the aldehyde at 390 mμ is decreased, while the maximum at 330 mμ assigned to the hydrate form is correspondingly increased. The assignment of absorption maxima to the ionic forms in UV spectra of vitamin B_6 analogues and their 5'-phosphoric esters is presented in Table 4.

EXPERIMENTAL

UV spectra were taken on a SF-4 "Optica Milano" spectrophotometer. Absorbancy in acid phenylhydrazine was determined by the procedure of Wada and Snell.[24] IR spectra were obtained on an UR-10 spectrophotometer for solid substances (pellets with KBr). PMR spectra were determined on an 100 Mc/s "Jeol" instrument. The chemical shifts are reported in δ values in ppm with the TMS signal (0 ppm) as internal standard. The compounds synthesized were subjected to chromatography on slides (2·5 × 7·5 cm) with a silica gel layer, according to reported procedure.[25] The following solvent systems were used:

A—EtOAc–acetone–25% NH_4OH (20:10:1·5);
B—n-BuOH–25%–NH_4OH–water (40:9:1);
C—n-BuOH–EtOH–5% NH_4OH–glacial AcOH (10:10:10:1).

1. *5-Ethoxyoxazoles*

To a suspension of 142 g (1 mole) P_2O_5 in 300 ml dry, alcohol-free $CHCl_3$, a soln of α-N-acylamino acid ester (0·5 mole) in 200 ml dry $CHCl_3$ was added dropwise with stirring. The mixture was gently boiled on a steam bath (see Table 5). To the cooled mixture, 750 ml 20% KOH aq was added with vigorous stirring. The mixture was then stirred at room temp for 30 min. The organic layer was separated and the aqueous layer was extracted with two 200-ml portions $CHCl_3$. The combined extracts were washed with 100 ml water and dried. The solvent was removed and the residue was distilled *in vacuo*. The compounds prepared are listed in Table 5.

2. *Diels–Alder reaction of 5-ethoxyoxazoles*

Dimethyl 5-hydroxycinchomerates (XIa–e). A mixture of dimethyl maleate (28·8 g; 0·2 mole) and X (0·1 mole) was heated at 110–115° (see Table 6). The reaction mixture was then cooled and 20 ml of 25% soln of dry HCl in abs MeOH was added. The hydrochlorides of dimethyl cinchomerates were isolated by one of two methods (A or B):

A. The resulting mixture was kept in a refrigerator for 2 hr. The crystalline product was filtered off, washed with cold MeOH and then with ether.

B. The mixture was shaken with 300 ml ether and allowed to crystallize in the refrigerator overnight. The crystalline product was filtered off and washed with ether.

The free bases XIa–e were prepared as follows:

To a soln of hydrochloride XIa–e in a minimum volume of water solid $NaHCO_3$ was added to pH 6·5–7. The suspension was extracted with $CHCl_3$. The combined extracts were dried and solvent was removed *in vacuo*. The residue was dried in a vacuum desiccator over P_2O_5 and paraffin.

The compounds thus prepared are presented in Table 6.

Ethyl 6-methyl-5-hydroxy-4-acetylnicotinate (III). To a soln of ethyl β-acetylacrylate[26] (17·9 g; 0·14 mole) in 20 ml ether, 8·87 g (0·07 mole) of 4-methyl-5-ethoxyoxazole, 1 ml glacial AcOH and 200 mg hydroquinone were added. The resulting mixture was kept in the dark at room temp during one week. The ppt was filtered off, washed with ether, and recrystallized from heptane, yield 8 g (51·2%), m.p. 151–152°; UV $\lambda_{max}^{0\cdot1N\,KOH}$ (ε): 250 (5800) and 326 mμ (56,500); PMR δ ppm ($CDCl_3$ as solvent): 6-H as singlet at 8·41; 2-Me and COMe as singlets at 2·47 and 2·51; CO_2Et as quadruplet at 4·30 and triplet at 1·34 (J = 7 c/s). TLC: in system A—R_f 0·11; in system B—R_f 0·75. (Found: C, 59·09; H, 6·00. Calc: for $C_{11}H_{13}NO_4$: C, 59·18; H, 5·87%).

TABLE 5. SYNTHESIS OF 5-ETHOXYOXAZOLES

Prepared substances	Ethyl ester of	Time of heating, hrs.	Yield, %	B.p.	Analysis					Lit.
					Formula	Calc:		Found·		
						C	H	C	H	
5-Ethoxyoxazole (Xa)	N-formylglycine	4	14–16	74–76° at 35 mm	$C_5H_7NO_2$	63·09	7·22	63·41	6·03	—
4-Methyl-5-ethoxyoxazole	N-formylalanine	4	56–60	86–88° at 62 mm	—	—	—	—	—	B.p. 80° at 50 mm[28]
2-Methyl-5-ethoxy-oxazole (Xb)	N-acetylglycine	8	60–64	83–84° at 37 mm	—	—	—	—	—	B.p. 60–62° at 12 mm[29]
2.4-Dimethyl-5-ethoxy-oxazole (Xc)	N-acetylalanine	6	56–60	89–90° at 40 mm	—	—	—	—	—	B.p. 60° at 12 mm[30]
4-*iso*-Propyl-5-ethoxy-oxazole (Xd)	N-formylvaline	6	49–55	90–92° at 40 mm	$C_8H_{13}NO_2$	61·91	8·44	61·63	8·57	—
4-n-Butyl-5-ethoxyoxazole (Xe)	N-formylnorleucine	6	44–49	94–96° at 18 mm	$C_9H_{15}NO_2$	63·88	8·94	63·94	8·77	—
Ethyl 5-ethoxy-4-oxazole-acetate (VI)	N-formylaspartate	6	42–47	106–108° at 2 mm	—	—	—	—	—	B.p. 93° at 0·7 mm[31]

TABLE 6. SYNTHESIS OF DIMETHYL 5-HYDROXYCINCHOMERATES

Prepared dimethyl ester of	Time of heating hrs	Method of isolation	Yield of hydrochloride %	M.p. of hydrochloride °C	M.p. of free base, °C	Analysis					Lit.
						Formula	Calc:		Found:		
							C	H	C	H	
5-Hydroxycinchomeronic acid (XIa)	2	A	43–44	200–201	136–137	—	—	—	—	—	M.p. of hydrochloride, 195–197°; of free base, 129–133°[32]
2-Methyl-5-hydroxy-cinchomeronic acid (XIb)	6	B	28–30	123–124	57–58	$C_{10}H_{12}ClNO_5$	45·90	4·62	45·63	4·38	—
2,6-Dimethyl-5-hydroxy-cinchomeronic acid (XIc)	4	B	41–43	166–167	56–67	$C_{11}H_{14}ClNO_5$	47·92	5·12	47·66	4·98	—
6-Isopropyl-5-hydroxy-cinchomeronic acid (XId)	2	B	72–76	144–145	66–67	—	—	—	—	—	M.p. of free base, 68°[33]
6-n-Butyl-5-hydroxy-cinchomeronic acid (XIe)	2	B	75–77	64–65	oil	$C_{13}H_{18}ClNO_5$	51·40	5·97	51·27	5·79	—

TABLE 7. SYNTHESIS OF PYRIDOXINE ANALOGUES

Prepared substances	Solvent	Method of isolation	Yield' %	M.p., °C	R_f in system A	Analysis					Lit.
						Formula	Calc:		Found:		
							C	H	C	H	
3-Hydroxy-4,5-bis(hydroxy-methyl) pyridine, hydrochloride (XIIa)	Tetra-hydro-furane	A	69–71	125–126	0·15	—	—	—	—	—	M.p. 124–125°[32]
2-Methyl-5-hydroxy-4,5-bis-(hydroxymethyl) pyridine (XIIb)	Ether	B	56–61	197–199 (decomp.)	0·27	$C_8H_{11}NO_3$	56·77	6·58	56·49	6·81	—
2,6-Dimethyl-3-hydroxy-4,5-bis (hydroxymethyl)pyridine (XIIc)	Ether	B	60–64	174–177 (decomp.)	0·52	$C_9H_{13}NO_3$	58·99	7·16	59·02	7·40	—
2-iso-Propyl-3-hydroxy-4,5-bis (hydroxymethyl)pyridine, hydro-chloride (XIId)	Ether	A	72	190–191	0·54	—	—	—	—	—	M.p. 192°[33]
2-n-Butyl-3-hydroxy-4,5-bis-(hydroxymethyl) pyridine, hydrochloride (XIIe)	Ether	A	74	187–188	0·50	$C_{11}H_{18}ClNO_3$	53·34	7·33	53·27	7·41	—
2-Methyl-3-hydroxy-4-α-hydroxyethyl-5-hydroxymethyl pyridine, hydrochloride (IV)	Tetra-hydro-furane	A	79	173–174	0·11	$C_9H_{14}ClNO_3$	49·20	6·42	48·85	6·53	—

2-Methyl-3-hydroxy-5-acetylpyridine (IX). A soln of 4-methyl-5-ethoxyoxazole (2·5 g; 20 mmoles) was poured into an Erlenmeyer flask fitted with a drying tube, and β-acetylacrylic acid[26] (3·42 g; 30 mmoles) in 40 ml ether was added. This mixture was allowed to stand in the dark at room temp for 5 days. The crystalline product was filtered off, washed with acetone and recrystallized from MeOH; yield 0·64 g (21·2%), m.p. 253–254° (dec.); UV $\lambda_{max}^{0\cdot NK\,OH}$ (ε): 265 (5000) and 345 mμ (5500); PMR δ ppm (CF_3COOH as solvent): 6-H and 4-H as doublets (J = 2·4 c/s) at 8·81 and 8·57, respectively, 2-Me and COMe as singlets at 2·86 and 2·89. TLC: in system A—R_f 0·47; in system B—R_f 0·77. (Found: C, 63·58; H, 5·98. Calc. for $C_8H_9NO_2$: C, 63·56; H, 6·01%).

5-Methyl-3-hydroxy-6-azahydrinden-3-one. A mixture of 18 g (0·22 mole) cyclopenten-3-one[27] and 28 g (0·22 mole) 4-methyl-5-ethoxyoxazole 100 mg hydroquinone was kept at room temp for 10 days. The resulting oil was shaken with 50 ml 5N dry HCl in abs MeOH and allowed to crystallize in the refrigerator for 3 hr. The ppt of hydrochloride (8 g) was filtered off. From the mother liquor a further crop, (2·6 g) was collected; total yield, 10·6 (24·5%). The free base was obtained by treatment of the hydrochloride soln with NaOAc. After recrystallization from 50% aqueous MeOH, m.p. 138–139° (dec.); UV $\lambda_{max}^{0\ NKOH}$ (ε): 380 mμ (7400): IR ν_{max}^{KBr}: 1700 cm^{-1} (C=O); PMR δ ppm ($CDCl_3$ as solvent): 7-H as singlet at 8·22, 1- and 2-CH_2 as triplets (J = 7 c/s) at 2·69 and 3·10, 5-Me as singlet at 2·46. (Found: C, 66·62; H, 5·55. Calc. for $C_9H_9NO_2$: C, 66·24; H, 5·56%).

Anhydride of 3-carbethoxymethylene-4-ethoxy-7-oxa-2-azabicyclo-[2.2.1] *heptane-5,6-dicarboxylic acid* (VII). To a saturated soln of maleic anhydride (1·96 g; 20 mmoles) in ether, 3·98 g (20 mmoles) ethyl 5-ethoxy-4-oxazole acetate was added and the resulting mixture was allowed to stand in the dark at room temp for 2 days. Filtration and washing with ether yielded 4·8 g (81%) of crystalline product, which after recrystallization from benzene melted at 137–138°. This substance is unstable and decomposes at room temp in a few days. (Found: C, 52·87; H, 4·73. Calc. for $C_{13}H_{15}NO_7$: C, 52·52; H, 5·09%).

3. *Reduction of the pyridine–dicarboxylic acid esters*

To a suspension of LAH (1·14 g, 30 mmoles) in 50 ml ether (or THF), 10 mmoles of the free base of pyridinedicarboxylic acid esters in 50 ml ether (or THF) was added dropwise with stirring and cooling.

TABLE 8. SYNTHESIS OF DERIVATIVES OF PYRIDOXAL ANALOGUES BY OXIDATION OF PYRIDOXINE ANALOGUES

Compound prepared	Yield, calculated on diol, %	M.p., °C
Oxime XIIIa	75	201–203 (decomp.)
Schiff base XVa	79	192–194 (decomp.)
Oxime XIIIb	59	185–187 (decomp.)
Schiff base XVb	38	179–183 (decomp.)
Oxime XIIIc	69	209–212 (decomp.)
Schiff base XVc	66	177–180 (decomp.)
Oxime XIIId	69	186–187 (decomp.)
Schiff base XVd	74	125–126
Oxime XIIIe	71	173–175 (decomp.)
Schiff base XVe	76	126–127
Oxime XIIIf	44	206–208 (decomp.)
Schiff base XVf	60	135–136

The mixture was gently refluxed for 6 hr and kept at room temp overnight. The resulting mixture was cooled and 100 ml water was added dropwise with stirring. CO_2 was passed through the mixture for 30 min, and the solid was collected, stirred with 100 ml water–ethanol (1 : 1) and CO_2 was passed again through this suspension. After filtration the solid was extracted 2 × 50 ml portions boiling EtOH. The combined filtrates were evaporated *in vacuo* to dryness. The residue was extracted with five 25-ml portions boiling

TABLE 9. PREPARATION OF PYRIDOXAL ANALOGUES BY HYDROLYSIS OF SCHIFF BASES

Prepared hydrochloride of	Amount of Schiff base mg	Solvent and its volume	Size of column, cm	Volume of void fractions, ml	Volume of fractions, containing XVI, ml	M.p. °C	R_f in system A*	Formula	Analysis			
									Calc:		Found:	
									C	H	C	H
3-Hydroxy-5-hydroxymethyl-4-formylpyridine (XVIa)	200	3 ml of 1N HCl	1·4 × 40	250	250	144–147 (dec.)	0·29	$C_7H_8ClNO_3$	44·35	4·25	44·15	4·17
2-methyl-5-hydroxy-3-hydroxymethyl-4-formylpyridine (XVIb)	20	0·3 ml of 1N HCl	1 × 20	250	100	decomp. above 170	0·44	$C_8H_{10}ClNO_3$	47·20	4·95	47·04	5·12
2,6-dimethyl-3-hydroxy-5-hydroxymethyl-4-formylpyridine (XVIc)	450	7·5 ml of 1N HCl	1·6 × 40	400	500	decomp. above 170	0·65	$C_9H_{12}ClNO_3$	49·66	5·56	49·39	5·67
2-iso-propyl-3-hydroxy-5-hydroxymethyl-4-formylpyridine (XVId)	200	4 ml of EtOH	1·4 × 40	1300	900	119–121 (dec.)	0·78	$C_{10}H_{14}ClNO_3$	51·83	6·09	51·69	5·93
2-n-butyl-3-hydroxy-5-hydroxymethyl-4-formylpyridine (XVIe)	470	15 ml of EtOH	1·5 × 42	800	600	63–64	0·77	$C_{11}H_{16}ClNO_3$	53·76	6·56	53·64	6·41
2-methyl-3-hydroxy-5-α-hydroxyethyl-4-formylpyridine (XVIf)	50	1 ml of 1N HCl	1·4 × 30	450	250	106–107	—	$C_9H_{12}ClNO_3$	49·66	5·56	49·50	5·39

* These aldehydes were chromatographed in the form of their methyl acetals easily prepared by heating of solutions of the aldehydes in absolute methanol at 50° for 1 hour.

TABLE 10. SYNTHESIS OF PYRIDOXAMINE ANALOGUES

Prepared dihydrochloride of	M.p. °C	R_f in system A	Formula	Analysis			
				Calc:		Found:	
				C	H	C	H
3-Hydroxy-4-aminomethyl-5-hydroxymethylpyridine (XIVa)	166–168 (dec.)	0·67	$C_7H_{12}Cl_2N_2O_2$	37·02	5·53	37·26	5·25
2-Methyl-5-hydroxy-4-aminomethyl-3-hydroxymethyl-pyridine (XIVb)	234–240 (dec.)	0·74	$C_8H_{14}Cl_2N_2O_2$	39·86	5·85	39·61	5·77
2,6-Dimethyl-3-hydroxy-4-aminomethyl-5-hydroxy-methylpyridine (XIVc)	182–184 (dec.)	0·85	$C_9H_{16}Cl_2N_2O_2$	42·37	6·32	42·12	6·61
2-Isopropyl-3-hydroxy-4-aminomethyl-5-hydroxy-methylpyridine (XIVd)	169–170	0·85	$C_{10}H_{18}Cl_2N_2O_2$	44·61	6·74	44·48	6·63
2-n-Butyl-3-hydroxy-4-aminomethyl-5-hydroxymethyl-pyridine (XIVe)	163–164	0·82	$C_{11}H_{20}Cl_2N_2O_2$	46·65	7·12	46·82	7·03
2-Methyl-3-hydroxy-4-aminomethyl-5-α-hydroxy-ethylpyridine (XIVf)	188–191 (dec.)		$C_9H_{16}Cl_2N_2O_2$	42·37	6·32	42·21	6·18

EtOH. The combined extracts were filtered and evaporated to dryness *in vacuo* at 40–50°. Two alternative methods were used for isolation of pyridoxine analogues (XIIa–e):

A. The residue was mixed with 10 ml acetone; the solid was filtered off, washed with acetone and dried. Free bases XII were obtained.

B. The residue was dissolved in 4 ml 12% dry HCl in abs EtOH; ether was added to this soln until crystals began to precipitate. The mixture was kept in a refrigerator overnight. The hydrochlorides were filtered off, washed with ether and dried. The substances obtained by this procedure are listed in Table 7.

4. *2-Methyl-3-hydroxy-4-hydroxymethyl-5-α-hydroxyethylpyridine* (V)

To a soln of Grignard reagent prepared from 0·5 g Mg and 2·92 g MeI in 20 ml ether, a soln of α^4-3-O-isopropylideneisopyridoxal[34] (4 g, 0·193 mole) in 20 ml ether was added dropwise with stirring and cooling. The mixture was then heated under reflux for 2 hr, cooled and the complex was destroyed by cautious addition, with stirring, of 10 g ice. The organic layer was collected and the aqueous layer was extracted with three 10 ml portions ether. The combined extracts were dried and evaporated to dryness. The residue was dissolved in 100 ml 10% HCl and this soln was heated under reflux for 30 min and evaporated *in vacuo* to dryness. The residue was carefully mixed with acetone and filtered, yield of hydrochloride V, 2·3 g (54·4%); m.p., 159–160° (from mixture ether–ethanol), (Korytnyk and Paul,[34] reported 160°).

5. *Oxidation of pyridoxine analogues*

To a soln of 3 mmoles of diol XII in 0·3M H_2SO_4 (10 ml in the case of hydrochlorides or 15 ml in the case of free bases) 270 mg of MnO_2 "B"[35] was added and this mixture was stirred at room temp for 3·5 hr. Near the end of this period, the MnO_2 dissolved almost completely, and the pH of the mixture was 5·5–6. The aldehydes were isolated in the form either of oximes or of Schiff bases by following procedures:

A. To the mixture, obtained as above, 310 mg NH_2OH·HCl was added and the soln was heated at 70° for 10 min. Then 820 mg anhyd NaOEt was added, the mixture was heated again at 70° for 10 min and then allowed to stand in the refrigerator for 2 hr. The ppt was filtered off, washed with cold water, dried and recrystallized from water–EtOH. The oximes obtained are listed in Table 8.

B. Unreacted MnO_2 was removed by filtration and washed with 2 ml water. To the combined filtrates, 8 ml 0·5M *p*-phenetidine hydrochloride was added, followed immediately by 12 ml 2N NaOAc. The mixture was kept in the refrigerator for 2 hr. The orange needles of Schiff base were filtered off, washed with water, dried and recrystallized from MeOH. The substances thus prepared are listed in Table 8.

Analogues of pyridoxal (XVIa–f)

The carefully purified Schiff bases were dissolved in 1N HCl or EtOH (the solvent and its volume are indicated in Table 9). The resulting soln was applied to the top of a column of Dowex 50Wx4 in the acid form. The column was eluted with 1N HCl at the rate of 50 ml/hr. The effluent, containing aldehyde* was evaporated to dryness *in vacuo* at 40–45°. The oily residue was dried in a vacuum desiccator over KOH. The hydrochlorides of XVI were analytically and chromatographically pure. The yield was nearly quantitative. The prepared substances are presented in Table 9.

Analogues of pyridoxamine (XIVa–f)

To a solution of 1 mmole oxime in 20 ml water and 1 ml conc HCl, 150 mg Pd–C catalyst. Hydrogenation was carried out at room temp and atm press and continued until about a 10% excess of the theoretical amount of H_2 (2 mmoles) had been absorbed. This required about 30 min. The catalyst was removed by filtration, using a hot water wash, and the filtrate was evaporated to dryness *in vacuo* at a temp below 45°. The residue was dried in a vacuum desiccator over KOH. The yields of dihydrochloride were nearly quantitative. Purification of XIV was performed by recrystallization from EtOH–ether. The compounds obtained are listed in Table 10.

Analogues of pyridoxamine phosphate (XVIIa–f)

To a mixture of 85% H_3PO_4 (0·52 g) and P_2O_5 (0·4 g), 0·46 mmole pyridoxamine analogue hydrochloride was added and the mixture kept until evolution of HCl was complete. The resulting mass was heated at 60° for 2 hr, cooled and mixed carefully with 3 ml EtOH, followed by 8 ml ether. After refrigeration for 1 hr,

* The effluent was controlled in a flow by measurement of its absorption at 295 mμ and of conductivity.

Table 11. Synthesis of pyridoxamine phosphate analogues

Dihydrate of 5'-phosphoric ester of	Purification				Yield %	R_f in system C	Analysis				
	Amount of amine XIII, mg	Size of column cm	Volume of empty fractions, ml	Volume of fractions containing XVII, ml			Formula	Calc:		Found:	
								C	H	C	H
3-Hydroxy-4-aminomethyl-5-hydroxymethylpyridine (XVIIa)	100	1·6 × 50	400	600	65	0·48	$C_7H_{11}N_2O_3P\cdot 2H_2O$	31·12	5·60	30·88	5·69
6-Methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine (XVIIb)	290	1·7 × 65	1000	900	51	0·49	$C_8H_{13}N_2O_5P\cdot 2H_2O$	33·82	6·03	33·39	5·84
2,6-Dimethyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine (XVIIc)	250	1·7 × 65	1100	900	53	0·56	$C_9H_{15}N_2O_5P\cdot 2H_2O$	36·24	6·42	35·95	6·12
2-Isopropyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine (XVIId)	400	2·1 × 50	2300	1400	63	—	$C_{10}H_{17}N_2O_5P\cdot 2H_2O$	38·45	6·78	38·21	6·63
2-n-Butyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine (XVIIe)	350	2·1 × 50	1250	1000	64	—	$C_{11}H_{19}N_2O_5P\cdot 2H_2O$	40·49	7·10	40·34	6·96
2-Methyl-3-hydroxy-4-aminomethyl-5-α-hydroxyethylpyridine (XVIIf)	100	1·6 × 50	500	500	58	—	$C_9H_{15}N_2O_5P\cdot 2H_2O$	36·24	6·42	36·12	6·28

TABLE 12. SYNTHESIS OF ANALOGUES OF PYRIDOXAL PHOSPHATE

Monohydrate of 5'phosphoric ester of	Method of preparation	Reaction conditions	Purification				Yield %	R_f in system C	Absorption in acid phenyl-hydrazine	Analysis				
			Amount of Schiff base g	Size of column, cm	Volume of void fractions, ml	Volume of fractions, containing XVII, ml				Formula	Calc:		Found:	
											C	H	C	H
3-Hydroxy-4-formyl-5-hydroxy-methylpyridine (XVIIIa)	A	4 hr 40°	1·0	1·6 × 50	1000	1500	73	0·59	18,900	$C_7H_8NO_6P·H_2O$	33·48	4·02	33·22	4·26
6-Methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine (XVIIIb)	B	—	—	1·4 × 40	350	450	67	—	20,300	$C_8H_{10}NO_6P·H_2O$	36·24	4·56	35·87	4·28
2,6-Dimethyl-3-hydroxy-4-formyl-5-hydroxymethyl-pyridine (XVIIIc)	A	6 hr, 60°	0·25	1·4 × 35	500	500	53	0·70	21,000	$C_9H_{12}NO_6P·H_2O$	38·72	5·05	38·44	4·92
2-Iso-propyl-3-hydroxy 4-formyl-5-hydromethylpyridine (XVIIId)	A	5 hr, 60°	0·5	1·4 × 40	400	600	69	—	21,200	$C_{10}H_{14}NO_6P·H_2O$	40·96	5·50	40·63	5·38
2-n-Butyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine (XVIIIe)	A	5 hr, 60°	1·0	1·6 × 50	2500	3000	71	—	20,900	$C_{11}H_{16}NO_6P·H_2O$	43·00	5·90	42·81	6·07
2-Methyl-3-hydroxy-4-formyl-5-α-hydroxyethylpyridine (XVIIIf)	A	6 hr, 50°	0·2	1·4 × 35	400	400	32	—	21,100	$C_9H_{12}NO_6P·H_2O$	38·72	5·05	38·54	4·82

the liquid was decanted off and the residue was dissolved in 5 ml 1N HCl. This soln was heated on a steam bath for 20 min and evaporated *in vacuo* at 35–45° to a volume of 1 ml, pH of the mixture was brought to 5 with 25% aqueous ammonia and the resulting soln was applied to the top of a column of Amberlite CG-50 in the acid form. This column was eluted with water at the rate of 50 ml/hr. The effluent containing the amine phosphate was evaporated to a small volume *in vacuo* at 35–45° and mixed with EtOH until crystals began to precipitate. After refrigeration for 2 hr, the solid was filtered off, washed with EtOH and dried. The compounds obtained (see Table 11) were purified by recrystallization from water heated to 80°.

Analogues of pyridoxal phosphate (XVIIIa–f)

Method A. To a mixture of 85% H_3PO_4 (3·72 g) and P_2O_5 (2·86 g), 2 mmoles of Schiff base was added. This was cautiously mixed and then, heated as indicated in Table 12. After heating the mixture was cooled and carefully mixed with 6 ml 0·1N HCl, until a homogeneous soln was obtained. The resulting mixture was heated at 60° for 15 min, cooled and applied to the top of a column of Dowex 50Wx4 in the acid form. The column was eluted with O_2-free water at the rate of 50 ml/hr. The effluent containing aldehyde phosphate was concentrated *in vacuo** to a volume of 10 ml and applied on the same column of Dowex 50Wx4. Chromatography was repeated as above. The new effluent was concentrated *in vacuo* to a volume of 30–40 ml and lyophilized.

Method B. To a soln prepared from 1 mmole pyridoxamine phosphate analogue, 7 ml water and 1 ml 2N NaOH, 240 mg (2·5 mmoles) sodium glyoxylate was added and this mixture was stirred at room temp during 10 min. The soln was brought to pH 5 with glacial AcOH and stirred for another 10 min. To this was added dropwise and with stirring 3 ml 0·25M soln of cupric acetate under a slow stream of N_2. Stirring was continued under a stream of N_2 for 30 min, and the resulting mixture was applied to the top of a column of Dowex 50Wx4 in the acid form and eluted with O_2-free water at a rate of 50 ml/hr. Further operations are carried out as in method A. The compounds obtained are listed in Table 12.

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REFERENCES

[1] G. Ya. Kondratjeva, *Khim. Nauka i Prom.* **2**, 666 (1957).
[2] E. E. Harris, R. A. Firestone, K. Pfister, R. R. Boettcher, F. J. Cross, R. B. Currie, M. Monaco, E. R. Peterson and W. Reuter, *J. Org. Chem.* **27**, 2705 (1962).
[3] R. A. Firestone, E. E. Harris and W. Reuter, *Tetrahedron* **23**, 943 (1967).
[4] F. Hoffmann–La Roche and Co., A.G., Neth. Appl. 6, 506, 703 (1965); *Chem. Abstr.*, **64**, 15851 (1966).
[5] W. Korytnyk and B. Paul, *J. Heterocycl. Chem.* **2**, 481 (1965).
[6] T. Naito, K. Ueno, M. Sano, Y. Omura, I. Itoh and F. Ishikawa, *Tetrahedron Letters* 5767 (1968).
[7] T. Naito and T. Yoshikawa, *Chem. Pharm. Bull.* **14**, 918 (1966).
[8] T. Miki and T. Matsuo, *The Kansai Branch Meeting of Pharm. Soc. of Japan.* September 17 (1966).
[9] T. Miki and T. Matsuo, *Yakugaku Zasshi* **87**, 323 (1967).
[10] T. Miki and T. Matsuo, Neth. Appl 6, 607, 005 (1967); *Chem. Abstr.* **67**, 32593 (1967).
[11] K. Nakanishi, *Infrared Absorption Spectroscopy.* Holden-Day, San Francisco (1962).
[12] E. A. Lukyanetz, N. P. Sgusherina, E. G. Treshchova, L. N. Kazitzina and R. Ya. Levina, *Zh. Organicheskoi Khim.* **1**, 1194 (1965).
[13] H. Rosenkrantz and M. Gut, *Helv. Chim. Acta* **36**, 1000 (1953).
[14] Takeda Chemical Industries, Ltd., Fr. Pat. 1 400 843 (1965); *Chem. Abstr.* **63**, 9922 (1965).
[15] W. Kimmel and W. Leimgruber, Fr. Pat. 1 384 099 (1965); *Chem. Abstr.* **63**, 4263 (1965).
[16] T. Yoshikawa, F. Ishikawa, Y. Omura and T. Naito, *Chem. Pharm. Bull.* **13**, 873 (1965).
[17] J. M. Osbond, Fr. Pat. 1 343 270 (1963); *Chem. Abstr.* **60**, 11991 (1964).
[18] H. J. Bernstein and W. G. Schneider, *J. Chem. Phys.* **24**, 465 (1956).
[19] E. A. Peterson and H. Sober, *J. Am. Chem. Soc.* **76**, 169 (1954).
[20] Yamanouchi Pharmaceutical Co. Ltd., Japan. Pat. 21786 (1965); *Chem. Abstr.* **64**, 2067 (1966).
[21] Yamanouchi Pharmaceutical Co. Ltd., Japan Pat. 25340 (1965); *Chem. Abstr.* **64**, 11181 (1966).

* Since the pyridoxal phosphate analogues are unstable, all concentration procedures were performed at a temperature not exceeding 30–35°.

[22] Kuroda Toshio, *Bitamin, Kyoto* **28**, 211 (1963).
[23] Yu. V. Morosoff, N. P. Bazhulina, L. P. Cherkashina and M. Ya. Karpeisky, *Biofizika* **12**, 397 (1967).
[24] H. Wada and E. E. Snell, *J. Biol. Chem.* **237**, 127 (1962).
[25] E. N. Dementieva, N. A. Drobinskaja, L. V. Ionova, M. Ya. Karpeisky and V. L. Florentiev, *Biokhimija* **33**, 350 (1968).
[26] W. G. Overend, L. M. Murton and L. F. Wiggins, *J. Chem. Soc.* 3500 (1950).
[27] A. V. Zakharychev and I. V. Torgov. *Isvestiya Akad. Nauk SSSR, Ser. Khim.* 1682 (1962).
[28] Merck and Co. Inc., Belg. Pat. 617 499 (1962); *Chem. Abstr.* **59**, 634 (1963).
[29] P. Karrer and C. Granacher, *Helv. Chim. Acta* **7**, 763 (1924).
[30] P. Karrer, E. Miyamichi, H. C. Storm and R. Widmer, *Ibid.* **8**, 205 (1925).
[31] M. Grifantini, M. L. Stein and A. Temperilli, *Ann. Chim. Rome* **56**, 946 (1966).
[32] S. M. Gadekar, I. L. Frederick and E. S. De Renzo, *J. Med. Pharm. Chem.* **5**, 531 (1962).
[33] H. Davoll and F. B. Kipping, *J. Chem. Soc.* 4386 (1952).
[34] W. Korytnyk, B. Paul, A. Bloch and C. A. Nichol, *J. Med. Chem.* **10**, 345 (1967).
[35] M. Harnfest, A. Bavely and W. A. Lazier, *J. Org. Chem.* **19**, 1608 (1954).