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SYNTHESIS OF PYRIDOXINE BY DIELS–ALDER REACTIONS WITH 4-METHYL-5-ALKOXY OXAZOLES

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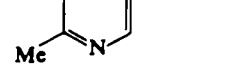
Abstract–Pyridoxine has been synthesized by the Diels–Alder reaction of 4-methyl-5-alkoxy oxazoles with maleic anhydride, diethyl maleate, diethyl fumarate and fumaronitrile, followed by reduction. At higher temperatures, 2-butene-1,4-diol, its dimethyl ether and 2,5-dihydrofuran afforded pyridoxine and its corresponding ethers directly. The reative reactivities of dienophiles and also the failure to isolate 3-alkoxy-pyridines are briefly discussed.

THE synthesis of pyridoxine I (Vitamin B_6) continues to arouse strong interest, despite its having already been accomplished in several different ways.[1] None of



I

these prior syntheses utilizes the Diels–Alder reaction to construct the pyridine ring, although the size of the ring and the symmetry about the 4- and 5-positions would seem to favor this approach. The hindrance here has doubtless been the lack of a suitable diene.

Such a diene exists in stable form as part of the oxazole ring, and the discovery that alkyl-substituted oxazoles readily undergo the Diels–Alder reaction with maleic anhydride to form cinchomeronic acids[2] thus opened the door to a new, short synthesis of vitamin B_6.[3]

It seemed the simplest approach to place all the necessary functional groups into the oxazole at the outset, so that the diene synthesis would produce directly pyridoxine or a 4,5-derivative. This suggested the preparation of a 4-methyl-5-alkoxy-oxazole II. Similar compounds had been reported before, although none with unsubstituted 2-positions. The usual route to 5-alkoxy oxazoles[4] is *via* cyclization of

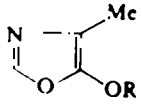
[1] [a] S. A. Harris and K. Folkers, *J. Amer. Chem. Soc.* **61**, 1245 (1939); [b] R. Kuhn, K. Westphal, G. Wendt and O. Westphal, *Naturwiss.* **27**, 469 (1939); [c] R. G. Jones, *J. Amer. Chem. Soc.* **73**, 5244 (1951); [d] A Cohen, J. W. Haworth and E. G. Hughes, *J. Chem. Soc.* 4374 (1952); [e] N. Elming and N. Clauson-Kaas, *Acta Chem. Scand.* **9**, 23 (1955).

[2] [a] G. Ya. Kondrat'yeva, *Khim. Nauk i Prom.* **2**, 666 (1957); *Izvest. Akad. Nauk SSSR, Otd. Khim. Nauk* 484 (1959); [b] G. Ya. Kondrat'yeva and C. N. Huang, *Dokl. Akad. Nauk SSSR* **141**, 628, 861 (1961).

[3] [a] C. N. Huang and G. Ya. Kondrat'yeva, *Izvest. Akad. Nauk SSSR, Otd. Khim. Nauk* 525 (1962); [b] E. E. Harris, R. A. Firestone, K. Pfister III, R. R. Boettcher, F. J. Cross, R. B. Currie, M. Monaco, E. R. Peterson and W. Reuter, *J. Org. Chem.* **27**, 2705 (1962).

[4] J. W. Cornforth, *The Chemistry of Penicillin* p. 688. Princeton University Press, Princeton, New Jersey (1949).

α-N-acylamino acid esters with reagents such as PCl_5, P_2O_5, arylsulfonyl chlorides, etc.; for example, 2-methyl-5-ethoxy oxazole[5] and 2,4-dimethyl-5-ethoxyoxazole[6] are readily prepared with PCl_5 from ethyl N-acetyl glycinate and alaninate, respectively.

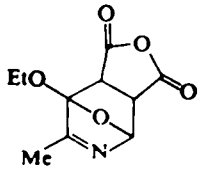


a, R = Me
b, R = Et
c, R = i-Pr

II

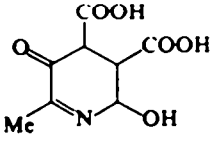
We found that ethyl N-formyl alaninate, on treatment with P_2O_5, afforded 4-methyl-5-ethoxy oxazole IIb. It was expected that this substance would undergo the Diels–Alder reaction at least as readily as did the 5-alkyl and 5-unsubstituted oxazoles, since 1-alkoxy butadienes react more readily than unsubstituted ones,[7] and this was found to be the case. Reaction of IIb with maleic anhydride at room temperature was vigorous unless moderated by the presence of a solvent, and went very well even at about 0° in ether overnight.

The product of this reaction was a clear, viscous, unstable oil which could not be

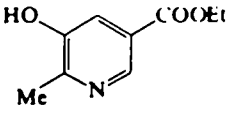


III

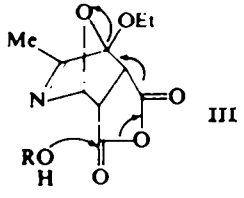
crystallized. Its spectral properties (Experimental) indicated a mixture, although the average mol wt was correct for the desired product III. This oil resinified on treatment with water but could be dissolved in aqueous sodium bicarbonate solution and then acidified without discoloration. When the non-volatile material from this treatment or, better still, the untreated Diels–Alder adduct was esterified in absolute EtOH–HCl, two new compounds were obtained. The first of these was identified as ethyl 2-methyl-3-hydroxypyridine-5-carboxylate V, apparently formed either by decarboxylation of



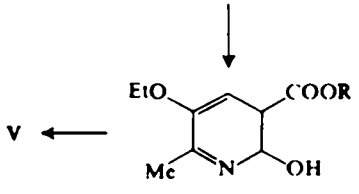
IV



V



III

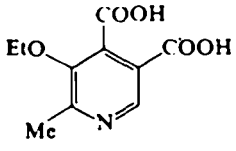


[5] P. Karrer and C. Granacher, *Helv. Chim. Acta* 7, 763 (1924).
[6] P. Karrer, E. Miyamichi, H. C. Storm and R. Widmer, *Helv. Chim. Acta* 8, 205 (1925).
[7] D. Craig, J. J. Shipman and R. B. Fowler, *J. Amer. Chem. Soc.* 83, 2885 (1961).

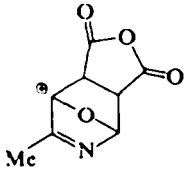
the β-ketoacid function in the first hydrolysis product IV, or perhaps via direct loss of CO_2 from the Diels–Alder adduct itself during hydrolysis.

The other substance, which was first obtained by the direct EtOH–HCl procedure, gave a correct analysis for a monoethylated 5-hydroxy-6-methyl cinchomeronic acid derivative, and was therefore taken to be the 3-ethoxy VI on these grounds:

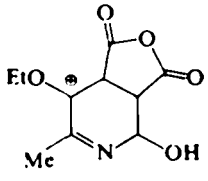
Attack of acid on the ketal function in the adduct III would be expected to provide



VI

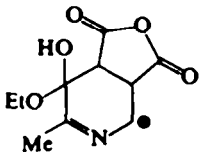


VII

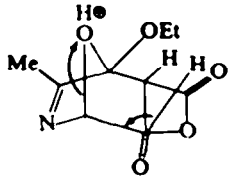


VIII

one of the carbonium ions VII, VIII or IX. Intermediate IX, however, seemed a poor choice next to VIII.[8] (Concerted elimination of the oxide bridge, III′ → X is not likely in view of the stereochemistry of III expected, by Alder's Rule, for the predominant isomer.) The first of these, VII, possessing a carbonium carbon atom at



IX



III′

X

the bridge-head of a small bicyclic system, violates the generally accepted rule that bridgehead carbonium ions in small systems are high-energy forms,[9] and therefore VIII is the expected primary intermediate. This could react with the solvent either to form the diethyl ketal XIa or to lose a proton; the eventual product on either event is VI. It soon became apparent that this was not the case, however, since the

a, R = Et b, R = H

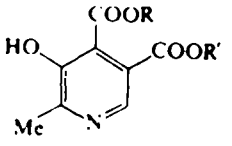
XI

carbonyl band in the IR spectrum of the product did not disappear in morpholine. The ethyl group, then, was present as an ester, not an ether, and this was firmly established by saponification to the diacid XIIa. The position of the ester function

[8] C. K. Ingold, *Structure and Mechanism in Organic Chemistry* p. 334. Cornell University Press, Ithaca, New York (1953); [b] A. M. Wenthe and E. C. Cordes, *J. Amer. Chem. Soc.* **87**, 3173 (1965).

[9] P. D. Bartlett and L. H. Knox, *J. Amer. Chem. Soc.* **61**, 3184 (1939); [b] W. von E. Doering, M. Levitz, A. Sayigh, M. Sprecher and W. P. Whelan, Jr., *J. Amer. Chem. Soc.* **75**, 1008 (1953); [c] Zefirov, *Zh. Obsch. Khim.* **34**, 2468 (1964) has recently extended the rule to a 7-oxa-2,2,1-bicyclohexane deriv.

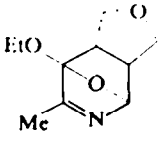
was not established, although we tentatively propose the structure XIIb. The other monoethyl ester and the diethyl ester XIId were also subsequently obtained.



a, R = R' = H
b, R = Et, R' = H
c, R = H, R' = Et
d, R = R' = Et

XII

The exclusive formation of 3-hydroxypyridine derivatives turned out to be the rule[3b] for every subsequent reaction as well; no 3-alkoxy-pyridines were ever isolated despite their ample stability to the conditions employed. Although it is possible that those adducts which were opened in "anhydrous" alcoholic HCl reacted so rapidly with traces of water relative to the solvent that the hemiketals, such as XIb, were always the exclusive initial products from the favored carbonium ion of type VIII, this explanation, a poor one in any event,[8b] is altogether untenable in the cases of 2,5-dihydrofuran and diethyl maleate (*vide infra*). The Diels-Alder adduct XIII from the former dienophile opened readily to the inner ether XIV in dihydrofuran itself as



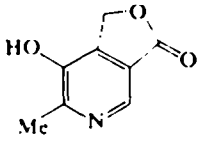
XIII

XIV

solvent using either traces of mineral acid or 20 mole % of trichloroacetic acid[10] as catalysts. When the adduct from the latter was treated with ethanolic HCl containing H_2O^{18}, no O^{18} was incorporated in the product XIId.

It appears, then, either that intermediate IX is actually favored over VIII despite our prediction to the contrary, or else that concerted cis-elimination III' → X is exceptionally facile.

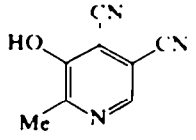
The synthesis of vitamin B_6 was completed by reduction in two ways. First, the known reduction of the diester XIId to pyridoxine with lithium aluminium hydride[1d] was carried out as a final structure proof. The diacid XIIa was then also reduced, using diborane generated *in situ* from sodium borohydride and boron trifluoride in diglyme.[11] In addition to pyridoxine (18%), the major product (70%) was the lactone XV, which had previously been reported from the diester with the similar combination sodium borohydride–aluminium chloride.[12]



XV

[10] This experiment was performed by Messrs. R. B. Currie and R. R. Boettcher.
[11] H. C. Brown and B. C. Subba Rao, *J. Amer. Chem. Soc.* **82**, 681 (1960).
[12] H. M. Wuest, J. A. Bigot, T. J. DeBoer, B.v.d. Wal and J. P. Wibaut, *Rec. Trav. Chim.* **78**, 244 (1959).

The loss in yield from decarboxylation of the maleic anhydride adduct was, of course, easy to avoid; both diethyl maleate and fumarate, while very much less reactive than maleic anhydride, provided the diester XIId in high yield as the hydrochloride when the adducts were treated with ethanolic HCl at room temperature.[13] Another derivative of the diacid that proved successful was the dinitrile; furaronitrile's reactivity toward the oxazole IIb was in a class with that of maleic anhydride itself, but lacking the drawback of the latter, it afforded the dinitrile XVI in high yield.



XVI

This compound was converted to pyridoxine *via* a known sequence of reactions.[14]

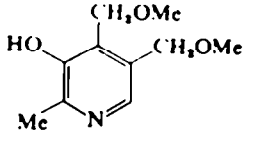
We next turned our attention to dienophiles whose reaction products might be convertible to vitamin B_6 by means other than reduction. The most obvious candidates were 2-butene-1,4-diol and its derivatives XVII. These compounds are comparatively unactivated dienophiles and therefore required

$$Y—CH_2—C=CH—CH_2—Y \qquad \text{XVII} \qquad \begin{matrix} \text{a, } Y = OH \\ \text{b, } Y = OMe \end{matrix}$$

much more vigorous conditions for the Diels–Alder reaction than those used previously.

The preferred olefin was butene diol XVIIa itself, since it leads directly to pyridoxine. This product was indeed formed, but isolation was severely hampered by the excess diol, usually used as solvent, and its by-products formed at the high temperatures employed, up to 200°. Identification was made by bioassay, paper chromatography, and the UV spectrum in both acid and base of paper-chromatographed material. Furthermore, it was possible to obtain the crystalline borate complex of pyridoxine in 6·75% yield. In addition to experiments at atmospheric pressure and in sealed tubes, a few runs were made at very high pressure, about 10,000 atmospheres; these conditions worked about as well as the others with respect to yield, but not any better.

Having established that butene diol could be made to undergo the diene synthesis, we turned to some simple derivatives. The dimethyl ether XVIIb reacted very well at



XVIII

100–200°, affording the 4,5-dimethyl ether of pyridoxine XVIII which was identified both by isolation and by paperstrip-UV in yields ranging up to 60% (Table 1).

[13] These experiments were performed by Mr. E. R. Peterson.

[14] [a] K. Makino, S. Morii, F. S. Chang and Y. Tagami, *Bull. Chem. Soc. Japan* **19**, 1 (1944); [b] A. Cohen and E. G. Hughes, Brit. Patent 625,997 (1949).

A well-known feature of the Diels–Alder reaction is the gulf in reactivity that separates maleic esters from the anhydride and imides.[15,16] The same phenomenon has recently been observed with azodicarboxylic acid derivatives.[16] Since this situation held good with our oxazole also, we were led to predict that the corresponding cyclized form of butene diol, 2,5-dihydrofuran XIX, would show greatly enhanced dienophilic reactivity.

This compound quickly established itself as the best dienophile in the butene diol

XIX

series, with yields to 65%. The product, pyridoxine inner ether XIV, is much more stable and easily crystallized than the dimethyl ether XVIII.

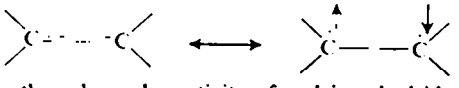
We were surprised, however, to note that 2,5-dihydrofuran was not markedly reactive *vis-a-vis* its noncyclic relatives; if anything, it was slower than the diol XVIIa, although a trifle (ca. 6X) faster than the dimethyl ether XVIId. It is therefore apparent that the enhanced reactivity of cyclic olefins relative to acyclic ones in the Diels–Alder reaction is not a general phenomenon, but is rather restricted to those olefins flanked by carbonyl groups or the like.[17]

Another observation made with these last two dienophiles was that yields of pyridoxine derivatives were very low when catalytic amounts of strong acid were not present, but that normal yields could be restored by subsequent acid treatment under conditions much too mild to effect the Diels–Alder reactions themselves. Thus, the opening of the intermediate adducts were acid-catalyzed reactions although the diene syntheses themselves were not.

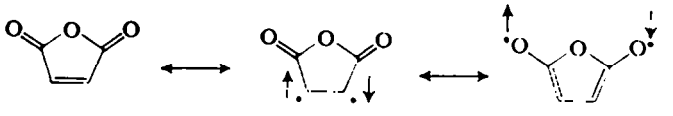
[15] W. R. Vaughan and K. S. Andersen, *J. Org. Chem.* **21**, 673 (1956).

[16] R. C. Cookson, S. S. H. Gilani and I. D. R. Stevens, *Tetrahedron Letters* 615 (1962).

[17] It may be noted that this phenomenon has bearing on the mechanism of the Diels–Alder reaction, since it finds no ready explanation in the one-step "no-mechanism" theory, but fits in uniquely with a two-step mechanism with a discrete intermediate, such as a spin-paired diradical,[18] in which two double bonds have been already consumed but only one of the two new single bonds has been formed. Thus, the formation of such an intermediate would be facilitated by any factor in the dienophile's structure which promoted separation of the pi-electrons, i.e., favored the diradical canonical form of the double bond:



From this point of view, the enhanced reactivity of maleic anhydride, maleimides and the like, relative to their acyclic counterparts, results from stabilization of the diradical resonance form above through participation of still another diradical canonical form which possesses aromatic



character.[19] One would not then expect cyclic structures *per se* to be especially powerful dienophiles.

[18] [a] G. B. Kistiakowsky *et al.*, *J. Amer. Chem. Soc.* **58**, 123 (1936); *J. Chem. Phys.* **5**, 682 (1937); *Ibid.* **7**, 725 (1939); [b] C. Walling and J. Peisach, *J. Amer. Chem. Soc.* **80**, 5819 (1958).

[19] Some other interesting cases from the literature which can be fitted to this hypothesis are described by R. A. Clement, *J. Org. Chem.* **25**, 1724 (1960); *Ibid.* **27**, 1115 (1962); T. J. Kealy, *J. Amer. Chem. Soc.* **84**, 966 (1962); E. F. Ullman and E. A. Barktus, *Chem. & Ind.* 93 (1962).

A number of reactions with several dienophiles were subjected to kinetic analysis. The general method was to seal samples from a stock mixture into capillary tubes, which were withdrawn at intervals from the heated bath and analyzed, either for the product by quantitative paperstrip-UV, or for oxazole by VPC. The dienophile was always present in large excess, so that reactions were pseudo first order in oxazole. Rate constants were not calculated because temperature control was maintained only to $\pm$ca. 1°, but considerable information was obtained nevertheless.

Since the general appearance of the data was alike in all cases, one example will suffice for all. In Fig. 1 are portrayed the data from the reaction at 175° between

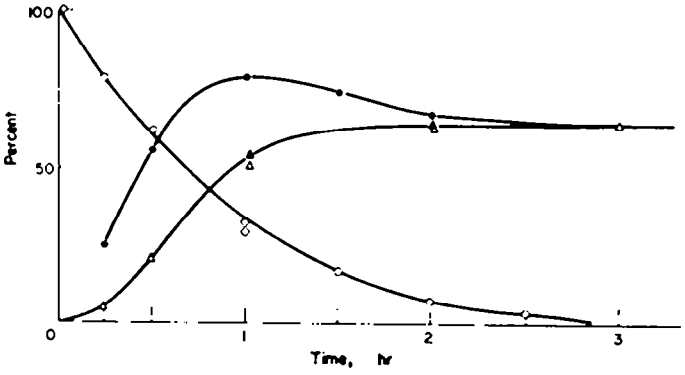


FIG. 1

△ Inner Ether
○ Oxazole
● Adjusted Yield

4-methyl-5-ethoxy oxazole and 2,5-dihydrofuran. The latter was present in twentyfold excess, and a trace of mineral acid was present to catalyze the opening of the Diels–Alder adduct. It may be noted that without the acid the direct yields of product were greatly reduced, but were quantitatively restored after brief refluxing with ethanolic HCl.

The data permit these conclusions: (1) The Diels–Alder reaction, depicted by the oxazole disappearance curve, proceeds normally. The slope of this curve, divided by remaining oxazole, is initially constant with time, although rising somewhat towards the end. (2) The appearance of the product lags behind the diene synthesis, corresponding to the temporary build-up of an unstable intermediate.

The following table presents yield data for some butene diol derivatives at various temperatures with 4-methyl-5-ethoxy oxazole.

TABLE 1[a]

Dienophile	Temp	Yield, %
Dihydrofuran	150°	65
Dihydrofuran	175°	64
Dihydrofuran	200°	64
Dimethoxybutene	100°	60
Dimethoxybutene	125°	53
Dimethoxybutene	150°	44
Dimethoxybutene	200°	22

[a] Dienophile in twentyfold excess.

In addition to the 5-ethoxyoxazole, the methoxy and isopropoxyoxazoles IIa and c were also prepared and reacted with dihydrofuran. The kinetic picture for these derivatives was about the same as that depicted above, with yield maxima a few per-cent lower than for the ethoxy. The relative rates for methoxy:ethoxy:isoproxy were approximately 1·8:1:1, showing that the steric effect in the 5-position, if any, is a slight one.

EXPERIMENTAL[20]

4-Methyl-5-ethoxyoxazole IIb. To a mixture of 25 ml alcohol-free chf and 11·36 g (0·08 mole) P_2O_5 was added, over 20 min at 25–30° with cooling, a soln of 5·81 g (0·04 mole) ethyl N-formyl alaninate[20] in the same solvent. Vigorous stirring was maintained throughout. The reaction mixture was heated slowly under a reflux condenser to 55°, at which point the heating mantle was removed and an exothermic reaction set in. When the exotherm subsided, the mass was too thick to stir, but with gentle external heating reflux was maintained for one hr.

After cooling, the chf was decanted and the hard mass chipped out on the flask, adding it to a stirred solution of 27·0 g (0·48 mole) KOH in 27 ml water and 34 ml MeOH with ice cooling. After cautious warming to room temp, the mixture was refluxed 1 hr, cooled and extracted with 10 × 15 ml CH_2Cl_2. The combined extracts were distilled through a 4″ helix-packed column to 60°, and then, with the column removed, at 50 mm; b.p. 75–80°, yield 1·57 g, 31%. (Found: C, 56·61; H, 7·20; N, 10·65. $C_6H_9O_2N$ requires: C, 56·68; H, 7·14; N, 11·02%.) UV 225 mμ (E% 296); IR and NMR spectra correct.

4-Methyl-5-methoxyoxazole IIa *and 4-methyl-5-isopropoxyoxazole* IIc. These were prepared by procedures similar to that given above. Their purity was less than perfect, but satisfactory for our purpose. Compound IIa had b.p. 142–143°, UV 230 mμ (E% 327), IR spectrum correct. (Found: C, 52·89; H, 5·88; N, 11·70. $C_5H_7O_2N$ requires: C, 53·09; H, 6·24; N, 12·38%). Compound IIc had b.p. 100° at 100 mm, UV 227 mμ (E% 321), IR correct. (Found: C, 58·97; H, 7·40; N, 9·52. $C_7H_{11}O_2N$ requires: C, 59·55; H, 7·86; N, 9·92%.)

Diels–Alder reactions between IIb *and Maleic anhydride.* Typically 1·27 g oxazole (0·01 mole) and 0·98 g maleic anhydride (0·01 mole) were allowed to react at 0–80° in about 10 ml ether or benzene. A transient yellow colour was observed, lasting, at room temp about 10–20 min. In smaller volumes of solvent, considerable heat evolution was noticeable. After evaporation of the solvent, the same crude adduct was obtained in all cases.

The adduct was a clear, colorless oil which could not be crystallized. It darkened and resinified, slowly in air and rapidly with water. It could be distilled, apparently unchanged, at 0·1 mm, oil-bath temp 70–110°. A sample, prepared in benzene and pumped down strongly, exhibited the correct IR, NMR and UV spectra for the desired product III (contaminated with benzene and maleic anhydride), in particular: anhydride bands at 5·37 and 5·61 μ in the IR, with oxazole absent; no UV maximum above 220 mμ; ethoxy and allylic methyl groups present by NMR but no oxazole. The mol. wt (determined osmometrically) was 243 (structure III requires 225). (Found: C, 52·41; H, 5·56; N, 5·63. $C_{10}H_{11}NO_6$ requires: C, 53·33; H, 4·92; N, 6·22%.)

A 0·01-mole sample of adduct was dissolved in 35 ml abs EtOH, saturated with anhydrous HCl in the cold, and refluxed overnight. The reaction mixture was stripped *in vacuo*, taken up in ether, and treated with $NaHCO_3$aq. The water layer was separated and washed three times more with ether; both phases were worked up separately.

The aqueous portion was acidified to Congo Red, and deposited on standing 0·2662 g (11·8%) of colorless crystals, m.p. 252°d., whose analysis corresponded to monoethyl 5-hydroxy-6-methyl-cinchomeronic acid. (Found: C, 52·95; H, 4·89; N, 6·49. $C_{10}H_{11}NO_6$ requires: C, 53·3; H, 4·89; N, 6·22%.) The UV spectrum showed maxima at 302 mμ in 0·1N HCl sol and 229, 265sh and 331 mμ in 0·1N KOH. The IR spectrum in morpholine soln showed a strong band at 5·8 μ. A sample was heated for 20 min with NaOHaq and acidified to Congo Red, producing a copious ppt of XIIa,

[20] M.ps are uncorrected. Microanalyses by Mr. R. N. Boos and associates; NMR spectra were measured and interpreted for us by Drs. B. Arison and N. R. Trenner; bioassays by Mr. H. Wallick and associates; mass spectra by Dr. N. R. Trenner and Mr. J. Beck.

m.p. 270–272°d. (reported, 259°;[14] 258°;[21] 265°[22]). The IR carbonyl absorption was at 5·75 μ in Nujol, shifted to 6·32 μ in morpholine. UV maxima were at 302 mμ in 0·1N HCl, and 254sh and 318 mμ in 0·1N NaOH. (Found: C, 48·64; H, 3·79. Calc. for $C_8H_7NO_5$: C, 48·74; H, 3·58%.) The 3-acetoxy anhydride was prepared in Ac_2O–AcCl and recrystallized from ether–Skelly B; m.p. 84–86° (lit. 84–86).[23]

The ether soln above was dried with $MgSO_4$, filtered and reduced to a very small volume. Crystals appeared, which were filtered and washed with ether. These were V, 0·1742 g (9·6%), m.p. 190–200°. After recrystallization from EtOH the m.p. was 200–202°, UV maxima ar 241, 298 mμ in 0·1N HCl, 258, 322 mμ in 0·1N NaOH; IR spectrum correct. The NMR showed 2 meta-disposed hydrogens at 2·08 and 1·04 τ, J = 2 c/s; O—H at 0τ (broad); $=\overset{|}{C}—CH_3$ at 7·23 τ; and —OEt as a quartet and triplet at 5·69 and 8·80 τ respectively, J = 7 c/s. (Found: C, 59·41; H, 5·92; N, 7·61. $C_9H_{11}O_5N$ requires: C, 59·66; H, 6·11; N, 7·74%.) The 3-acetate was prepared with Ac_2O–pyridine and purified by distillation (oil-bath 95°, 0·1 mm) and recrystallization from EtOH; m.p. 201–202°, IR spectrum correct. The NMR showed the two aromatic hydrogens at 2·12 and 1·13 τ, J = 2 c/s; $=\overset{|}{C}—CH_3$ at 7·53 τ; the acetyl methyl at 7·66 τ; and —OEt as a quartet and triplet at 5·69 and 8·80 τ respectively, J = 7 c/s. (Found: C, 59·44; H, 5·37; N, 6·43. $C_{11}H_{13}O_6N$ requires: C, 59·20; H, 5·87; N, 6·28%.)

The ether filtrate was distilled at 0·15 mm. At 70–90° (oil-bath) diethyl fumarate, 0·3238 g (18·8%), came over; it was identified by IR comparison. Then at 140–150° XIId distilled, 0·6453 g. 25·5% yield. This was converted to the hydrochloride with anhydrous HCl in EtOH and recrystallized from EtOH–ether; m.p. 140–144° (lit. 144–145°).[14] (Found: C, 49·96; H, 5·47; N, 5·24; ionic Cl, 12·39. Calc. for $C_{13}H_{16}O_5NCl$: C, 49·72; H, 5·57; N, 4·84; Cl, 12·24%.)

When XIId–HCl was treated 5 min at 50° with dilute alkali and acidified to Congo Red, a mono-ethyl ester XIIb or XIIc, m.p. 203–205°d., different from the one described above, was obtained. More vigorous treatment of XIId–HCl produced XIIa. Reduction of XIId with LAH produced pyridoxine I hydrochloride, identical by mixed m.p., IR, UV and paper chromatography (n-BuOH-pH 7 phosphate buffer) with an authentic sample.

Although the Diels–Alder adduct III was unstable to water, it could be dissolved in $NaHCO_3$aq and then acidified without darkening. Subsequent workup as described above produced the same set of products.

Reduction of XIIa *with* $NaBH_4$–BF_3. The reaction was run in carefully dried apparatus under N. To a soln of 0·0424 g (0·00112 mole) $NaBH_4$ in 1·5 ml diglyme was added 0·0985 g XIIa (0·000500 mole). A mild reaction occurred as the acid dissolved, with gas and heat liberated. After 3–4 min, a soln of 0·189 ml (0·00150 mole) redistilled BF_3-etherate in 1·0 ml diglyme was added and rinsed in with $\frac{1}{2}$ ml more solvent. An immediate reaction occurred, affording a clear soln. After stirring 1$\frac{1}{2}$ hr, a little EtOH was added, then 20 ml water. The mixture was now stripped to dryness *in vacuo* and 3 times boiled to dryness on the steam bath with 80 ml MeOH and 8 ml conc. ethanolic HCl, in order to cleave the B_6-borate complex. Paper chromatography (n–BuOH-pH 7 phosphate) showed two strong fluorescent spots, one of them pyridoxine. One-eighth of the sample was sent out for bioassay, which confirmed the presence of vitamin B_6.

The remainder was neutralized to pH 7 with $NaHCO_3$aq, evaporated to dryness, and extracted with hot EtOH. This was removed, leaving a white solid, which was washed twice with cold EtOH, leaving 0·0504 g (70% yield) of the lactone XV, m.p. 270°d. (lit. 269–270°).[13] Recrystallization from EtOH raised the m.p. to 278°, unchanged on admixture with authentic material. The IR spectrum was also identical with that of authentic XV.

The cold EtOH wash was spotted quantitatively on a paper chromatogram, and the UV of the eluted B_6 spot indicated a yield of 18%. From the EtOH soln, with anhydrous HCl, was isolated 0·0129 g (14% yield) of pyridoxine–HCl.

Diels–Alder reactions with diethyl maleate and fumarate.[18] A mixture of 1·3 g IIb (0·010 mole) and 3·5 g diethyl maleate (0·020 mole) was heated for 64 hr at 50–60°. After cooling to 0°, 10 ml abs

[21] A. Itiba and S. Emoto, *Sci. Papers Inst. Phys. Chem. Res. Tokyo* **38**, 347 (1941); *Chem. Abstr.* **35**, 6960 (1941).

[22] H. M. Wuest, J. A. Bigot, T. J. DeBoer, B. v. d. Wal and J. P. Wibaut, *Rec. Trav. Chim.* **78**, 226 (1959).

EtOH and 2 ml abs ethanolic HCl were added. The solvent was removed *in vacuo* and the residue slurried with ether, filtered, washed, and dried, yield 2·55 g (88%) XIId–HCl, m.p. 132–138°.

The same procedure was followed using diethyl fumarate, affording 2·5 g XIId–HCl, m.p. 133–138°.

Diels–Alder reaction with diethyl maleate using EtOH–H_2O^{18} *workup.* All glassware for this experiment was rinsed with $NaHCO_3$aq washed, and dried at 110°. Atm moisture was excluded throughout.

A mixture of 1·27 g redistilled IIb (0·01 mole) and 1·72 g redistilled diethyl maleate (0·01 mole), containing a trace of inhibitor [Shell's Ionox(R) 330], was heated at 60–61° for 23 hr. The IR spectrum progressively changed in the expected way. The reaction mixture was cooled and examined by IR, UV and NMR. No oxazole could be seen by IR or UV, with possibly a small amount visible in the NMR. The unopened Diels–Alder adduct consistuted about 70% of the mixture by NMR, a mixture of the two isomers in comparable amounts. Most significant was the exclusion of XIId by NMR and also by UV, which showed essentially no absorption above 210 mμ.

To 5·0 ml abs EtOH (0·06% water) was added 0·10 ml water containing 30% O^{18}, and then anhydrous HCl was passed in for a few seconds. The crude adduct 0·2912 g. was treated in the cold with 1·0 ml of this EtOH–H_2O^{18}–HCl soln, and allowed to stand for 2 hr at room temp. The reaction mixture was then twice diluted with abs EtOH and stripped *in vacuo*. Upon addition of ether and a trace of EtOH, the resulting oil crystallized. The product was washed with ether and dried, 0·0986 g, 35% overall yield. The IR spectrum was identical with that of authentic pure XIId–HCl.

The free base was liberated with $NaHCO_3$aq, extracted with ether, dried with $MgSO_4$, and analyzed for O^{18} content by mass spectroscopy on a Consolidated instrument. A sizeable parent peak was observed at mass 253, and no O^{18} was observed. Authentic XIId gave the same mass spectrum.

Diels–Alder reaction with fumaronitrile. Fumaronitrile 7·81 g (0·10 mole), 12·71 g (0·10 mole) oxazole IIb and a trace of hydroquinone were mixed together and warmed to melt the nitrile. An exothermic reaction set in which was controlled with ice cooling. The mixture was then heated 5 min on the steam bath; it was yellow during the reaction, but set to a viscous colorless oil after cooling. The IR spectrum now showed the absence of both starting materials, and absorbed *inter alia* at 4·44 μ (C≡N) and at 6·13 μ (C N).

The crude adduct was taken up in 100 ml MeOH, cooled in ice, and treated with 4 ml conc. HClaq. An exothermic reaction ensued, producing an instantaneous orange color; vigorous cooling was required for 5 min. (Careless treatment at this point resulted in rapid decomposition.) The solvent was removed rapidly on the rotating evaporator at the lowest practicable temp, leaving a thick slurry of XVI, which crystallized with one mole MeOH. This was chilled, filtered, washed twice with ice–cold MeOH, and vacuum dried at 30°, yield 10·50 g, 55%. The product can be recrystallized from MeOH, chf or benzene, m.p. 187–188°. The IR and NMR spectra are in accord with the structure XVI. Slow heating to ca. 130° dries the methanolate. (Found: C, 60·4; H, 2·9; N, 26·5. Calc. for $C_9H_6ON_2$: C, 60·4; H, 3·2; N, 26·4%.) *Benzoate*, m.p. 140–141° from ether. (Found: C, 68·39; H, 3·65; N, 15·68. $C_{16}H_{10}O_2N_2$ requires: C, 68·45; H, 3·42; N, 15·97%.)

A pure sample 0·0180 g, was heated with 2 ml conc. HCl for 4 hr at 100°, evaporated to dryness, dissolved in 1 ml water with just enough Na_2CO_3 for complete soln, and acidified with the minimum amount of HClaq. A ppt appeared, and after standing 2 hr it was filtered, washed with water, and dried, affording 0·0180 g (85·7%) of XIIa, identical with an authentic sample.

Hydrogenation of XVI[14] produced the known 2-methyl-3-hydroxy-4,5-bis-aminomethylpyridine trihydrochloride in 63% yield, m.p. 294° (reported[14b] 296°). (Found: C, 34·80; H, 6·20; N, 15·60; Cl, 38·49. Calc. for $C_9H_{14}ON_2Cl_3$: C, 34·7; H, 5·83; N, 15·18; Cl, 38·4%.) Treatment of this substance with nitrous acid[14] converted it to pyridoxine.

Diels–Alder reactions with butene diol. A mixture of 88·11 g (1 mole) butene diol, 6·357 g (0·05 mole) IIb and 0·250 g hydroquinone was heated in a sealed tube for 7 hr at 125°. After removal *in vacuo* of unreacted oxazole and excess diol, the residue was made up to 40·0 ml with abs EtOH and spotted (2 λ) on a paper chromatogram, using the system n-butanol–pH 7 phosphate buffer. The single fluorescent band corresponding to known B_6 was eluted with 4·00 ml of 0·1N HCl and assayed by UV; the OD at 291 mμ was 1·25, yield 22·8%. Pyridoxine HCl absorbs in acid at 291 mμ, E% 425. All attempts to isolate crystalline pyridoxine from this reaction mixture failed.

A portion of the reaction mixture containing 1·80 g pyridoxine by assay was stripped and dissolved in 3 ml boiling water. The soln was neutralized with Na_2CO_3 to pH 7–7·5, filtered hot and treated

with 0·270 g boric acid. Upon 3–4 days' standing, 0·450 g B_6-borate complex was obtained, 6·75% overall yield. After recrystallization from EtOH, the IR spectrum was the same as that of authentic material.

The borate complex (total sample) was distilled to dryness several times with methanolic HCl, until no more trimethyl borate was evolved (green flame test). Crystallization of the residue from EtOH afforded 0·485 g pure pyridoxine HCl (90% from borate or 6·1% overall) identical with authentic.

When the Diels–Alder reaction was run in sealed capillary tubes, following the assay yield with time, a maximum of 34% was reached in 5 hr ar 125°, falling off thereafter.

Some early reactions at very high press were tried, of which one example will be described. A mixture of 0·97 g butene diol (0·11 mole) 1·27 g IIb (0·010 mole), 58 ml 1,2-dimethoxyethane and a trace of hydroquinone was heated at 100° under 145,000 psi for 65 hr. The mixture was filtered, stripped, taken up again in 200 ml glyme and refiltered. The filtrate was stripped again and treated with 15 ml 0·1N HCl for 30 min on the steam bath. A bioassay, using *Saccharomyces carlsbergenesis*, was positive, and the paper-strip—UV assay described above showed 4·3% yield of vitamin B_6. When the UV soln was made alkaline, it exhibited max at 309 and 246 mμ, corresponding again to 4·3% yield. Pyridoxine absorbs at 309 and 244 mμ in base.

Diels–Alder reactions with 2,5-*dihydrofuran.* In a rubber-capped serum bottle was prepared a soln of 0·254 g IIb (0·0020 mole), 2·804 g dihydrofuran[23] (0·040 mole) containing a catalytic quantity of mineral acid and 0·010 g hydroquinone. The same results were obtained when 0·0005 mole of trichloroacetic acid was substituted for the mineral acid.

For the kinetic runs, about 20 resealed and weighed m.p. capillaries (Kimble) were each filled with 50 λ of soln, sealed at the other end and weighed again. All acceptable tubes contained 0·0491 ± 0·0003 g. Each tube, upon withdrawal from the oil-bath, was chilled and worked up either for the product, B_6 inner ether XIV, or for unreacted oxazole.

For determination of inner ether, each sample was transferred to a 2-ml volumetric flask, filled to the mark with 95% EtOH, and paper chromatographed in duplicate (25 λ), using chf–formamide. The R_f of inner ether is about 0·16 in this system but varies with the weather, being retarded when the humidity is high. Cleanly separated fluorescent spots were always obtained, which were cut out after drying at 50° *in vacuo*, stirred with 4·00 ml 0·1N HCl, and the optical density read at 283 mμ. Duplications were within 1%. In the reference cell was a similar soln made with a blank part of the paper. The E% of XIV under these conditions is 541 as the free base, so, as an example, an OD of 0·531 signifies a yield of 64·3%. Full curves were run from time to time to insure the purity of the product. The UV data for inner ether are: at pH 1, 283 mμ (E% 541); at pH 7, 252 (293), 309 mμ (587), 224sh; at pH 13, 243 (533), 293 mμ (415).

For determination of oxazole a sample of about 10 λ was withdrawn from the reaction tube into a hypodermic syringe immediately after opening, cushioning it with air in the syringe on both ends. After wiping the needle, the sample was injected into the gas chromatograph, weighing the syringe before and after the injection. The oxazole peak areas were read with a planimeter. In this way, duplications of identical samples were within 2%. Standards were frequently interspersed, using unpyrolyzed sample tubes.

A typical set of data, obtained at 175°, is given below.

Time, min	Yield, %	Oxazole, %
15	5·56	77·8
30	21·8	61·0
60	53·4	32·3
	50·2	29·2
90		16·9
120	63·8	
	63·0	7·1
150		3·0
180	63·7	

[23] A. J. Weinheimer, S. W. Kantor and C. R. Hauser, *J. Org. Chem.* **18**, 801 (1953).

The picture was about the same at the other temps, and with the other oxazoles; more yield data are given elsewhere in this paper.

The yield of XIV (3 hr at 175°) was 63·9% in a 4x larger tube; with a 3x increase in the hydroquinone, 65·4% and 66·4% were obtained.

In the absence of acid during the pyrolysis, the appearance of inner ether was very sluggish by the normal workup, but when this was preceded by heating the reaction mixture with ethanolic HCl on the steam bath for 5 min, normal yields were restored. These data for 175° are presented below.

Time, min	Yield, % Before and After HCl Treatment	
30	3	
60	11	49
80	16	58
120	28	
180	34	
375	50	

From one of the small reaction tubes that had received 6 hr. at 150°, a duplicate of which showed a 62·6% yield, the product, which crystallized on cooling, was isolated, 0·0026 g (54%). Larger samples of the same reaction mixture was treated in the same way to obtain enough XIV for proof of identity. Both the inner ether, m.p. 255–257°d, and its hydrochloride, m.p. 231–239°d (lit.[84] 239–240°), were identical with authentic samples by IR and UV: mixed m.ps were undepressed.

Diels–Alder reactions with methoxy oxazole IIa *and isopropoxy oxazole* IIc. The dienophile was dihydrofuran, the technique used was the same as that described above for the ethoxy oxazole, and the results were also of a similar nature.

Diels–Alder reactions with 1,4-*dimethoxybutene*-2. Sealed tubes were prepared in the same manner as those described for dihydrofuran, using a reaction mixture of 2·3299 g dimethoxybutene, 0·1277 g IIb, and 0·0051 g hydroquinone. Oxazole concentration was determined by VPC as before.

The reaction product, 2-*methyl*-3-*hydroxy*-4,5-*bis*-*methoxymethyl*-*pyridine* XVIII, was determined by paper-strip–UV, chromatographing the hydrochloride in the system CCl_4-formamide. Full UV curves were identical with those of authentic material, which shows maxima at 292 mμ (E% 386 as the hydrochloride) in 0·1N HCl, 314 (333) and 247 mμ (305) in 0·1N NaOH; the IR spectrum of isolated product was also identical with that of XVIII, which has been prepared[85] from 2-methyl-3-hydroxy-4-methoxymethyl-5-chloromethylpyridine by refluxing in MeOH with MeONa, acidification with HCl, evaporation of the solvent, extraction with hot isopropyl alcohol and recrystallization from isopropyl alcohol (4x) and water; m.p. 146·5–149°. (Found: C, 51·35; H, 6·77; N, 5·81; Cl, 14·92. $C_{10}H_{16}O_3NCl$ requires: C, 51·36; H, 6·92; N, 5·99; Cl, 15·21%.)

As with the inner ether, when no acid catalyst was present during the diene synthesis, it was found necessary to subject the reaction mixture to acid treatment before chromatography. The method

Dihydrofuran*			Dimethoxybutene†		
Time, min	Yield, %	Oxazole, %	Time	Yield, %	Oxazole, %
5	4·6		15 min	7·7	80·0‡
10	17·9	59·8	30 min	15·2	
20	50·4	31·7	1 hr	19·1	59·7
40	64·1	7·7	2 hr	22·1	31·5
			4·7 hr	9·3	0·0

* Catalytic acid present.

† Workup in 0·1N HCl.

‡ This point lay off the smooth graph

[84] S. A. Harris and K. Folkers, *J. Amer. Chem. Soc.* **61**, 3307 (1939).

[85] This preparation was performed by Dr. S. J. Etheredge.

used was 2 min's reflux in abs EtOH containing 1 drop/ml of conc HClaq. Of the several runs made at 100–200° the one described below is typical.

Relative reactivities of dihydrofuran and dimethoxybutene. Data were obtained at 200° for both dienophiles with IIb, using the method described previously.

These figures were plotted, and approximate pseudo-first order rate constants were obtained graphically from the oxazole disappearance curves by dividing the slope (in % ÷ time) by percent oxazole remaining. The results for dihydrofuran and dimethoxy butene were 330 and 56 hr^{-1}, respectively, approximately a sixfold ratio.

A semilog plot of the data yielded the same figure for the ratio of the slopes.