Tetrahedron. Vol. 26, pp. 5415 to 5425. Pergamon Press 1970. Printed in Great Britain

SCHIFF BASES OF PYRIDOXAL

THEIR STRUCTURE AND THE STABILIZATION OF THEIR RING-CHAIN TAUTOMERIC FORMS BY ACYLATION[1, 2]

W. KORYTNYK, H. AHRENS and N. ANGELINO

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203

(*Received in the USA* 13 *July* 1970; *Received in the UK for publication* 20 *July* 1970)

Abstract—Various tautomeric forms have been postulated for pyridoxal Schiff bases, including the ring-chain tautomers aldimine ⇌ aminoacetal (Ia ⇌ Ib). We have found that acylation stabilizes either the aldimine or the aminoacetal form, depending on the structure of the Schiff base and the acylating agent used. Thus pyridoxylidenebenzylamine gave a derivative of the aminoacetal form on benzoylation, but acetylation with acetic anhydride gave a tetraacetyl derivative of the aldimine form. Variation of the amine moiety of the Schiff base had a profound effect on product distribution, even though NMR revealed only the aldimine structure in the starting materials. Thus acetylation of pyridoxylidene-*p*-methoxybenzylamine gave a mixture of "ring" and "chain" derivatives, whereas pyridoxylidenepropylamine gave the "ring" form exclusively.

These results have been rationalized by assuming an initial attack of the acylating agent on the N atom of the aldimine. Intramolecular nucleophilic attack by the 5-CH_2OH gives rise to the "ring" derivative, whereas intermolecular reaction yields the "chain" compound.

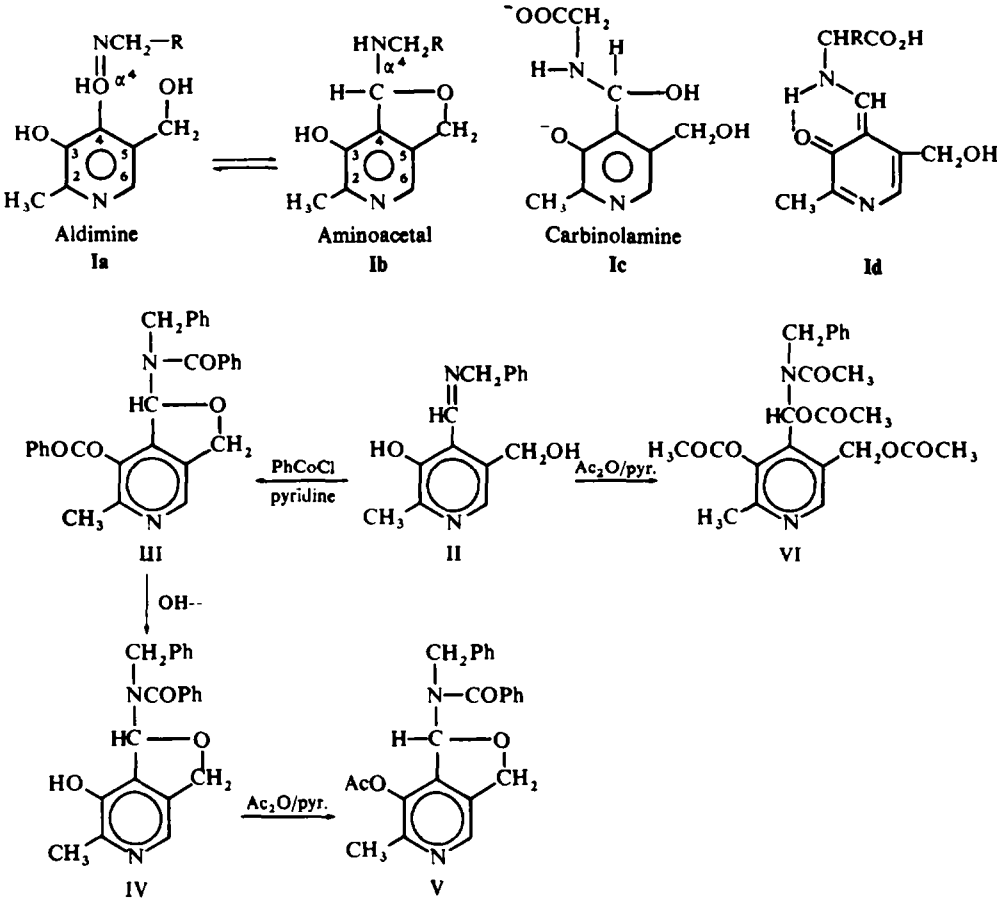
SCHIFF bases (aldimines) of pyridoxal or of pyridoxal phosphate with amino acids and certain amines are reaction intermediates in a number of enzymatic reactions, and their properties have been investigated extensively. Soon after the importance of these compounds became apparent, several model compounds were prepared by Witkop and Beiler,[3] and their ring-chain tautomerism (Ia ⇌ Ib, Scheme I) was scrutinized by means of IR and UV spectral techniques. In addition to these tautomers, a carbinol-amine form (Ic) has been considered to be formed in aqueous solution by the addition of the elements of water to the aldimine linkage.[4] More recently, another tautomeric form (Id) involving an O → N proton shift has been proposed.[5]

We had hoped that by examining the NMR spectra of some stable imines of pyridoxal in various solvents, a firmer basis for assignment of structures to the various tautomeric forms could be obtained. Acylation of these imines was also studied, as it was anticipated that it would provide insight into the reactive centers of these molecules, and possibly stabilize some unstable structures.* In addition, acylation studies may provide a useful model system, since enzymatic acylation of Schiff bases derived from pyridoxal phosphate and amino acids has been suggested as one of the steps in the biosynthesis of sphingolipid bases.[7]

Pyridoxylidenebenzylamine (II)[3] was examined first with respect to its structure and its products of acylation. Its NMR spectrum in pyridine-d_5 (Fig. 1) indicates that it exists as a single aldimine tautomer. The α^4-proton is quite deshielded, and

* Hypothetical acylation products from pyridoxal Schiff bases and their possible biological role have been considered.[6]

SCHEME I



appears as a very closely spaced triplet (J = 1·25 cs) coupled to the N–CH_2 protons of the benzylamine moiety. These methylene protons appear as a doublet (coupled to the α^4-proton, as can easily be shown by a decoupling experiment), and can thus be readily distinguished from the α^5-methylene protons, which appear as a singlet. A similar spectrum for II has been obtained in $CDCl_3$, but the splitting pattern was not so sharply defined as in pyridine. This splitting of the benzyl methylene protons by the α^4-protons has not been observed in the aminoacetal derivatives, as will be shown below.

Benzoylation of II gave the dibenzoyl derivative III. The structure of this compound has been established by its NMR spectrum and that of its derivatives, which were obtained as follows (Scheme I). Selective debenzoylation with alkali gave the phenolic compound IV, which was subsequently acetylated to V and mesylated. NMR spectra of the parent compounds and their derivatives were similar, and confirmed the structures indicated. The NMR spectrum of the 3-O-acetyl derivative V in $CDCl_3$ is shown in Fig 2. The two Me peaks are due to the 2-Me and 3-acetyl, respectively.

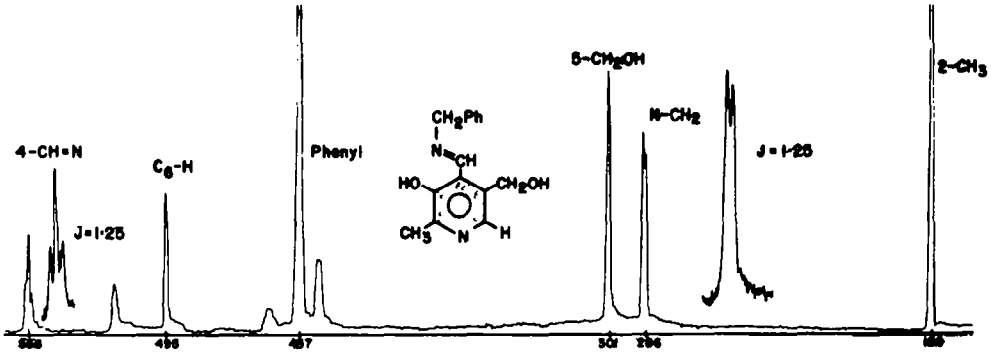


FIG 1. NMR spectrum of pyridoxylidenebenzylamine (II) in pyridine-d_5. Two peaks (286 and 556 Hz) have been expanded and inserted.

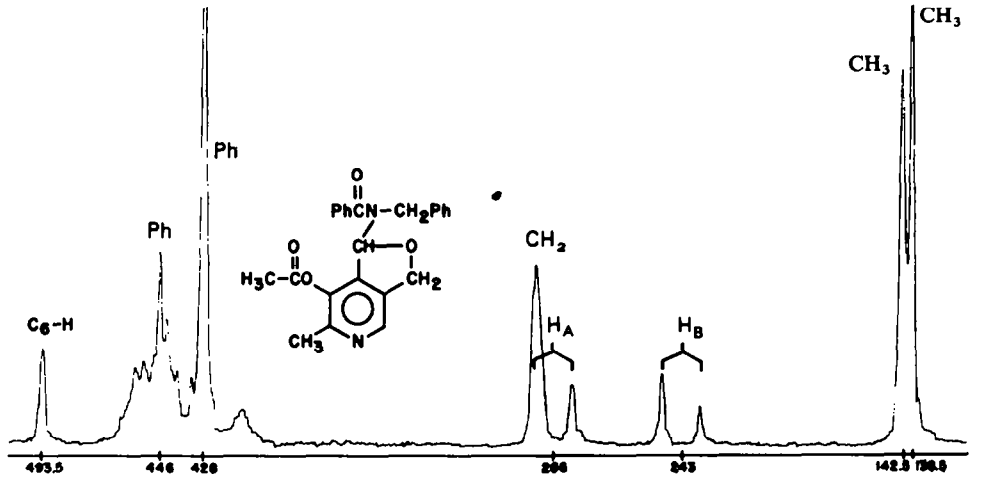


FIG 2. NMR spectrum of compound V in $CDCl_3$. Definite assignment could not be made for the methyl and methylene peaks, but the spectrum confirms the indicated structure. Part of the quartet of 286 cs is obscured by the CH_2 singlet.

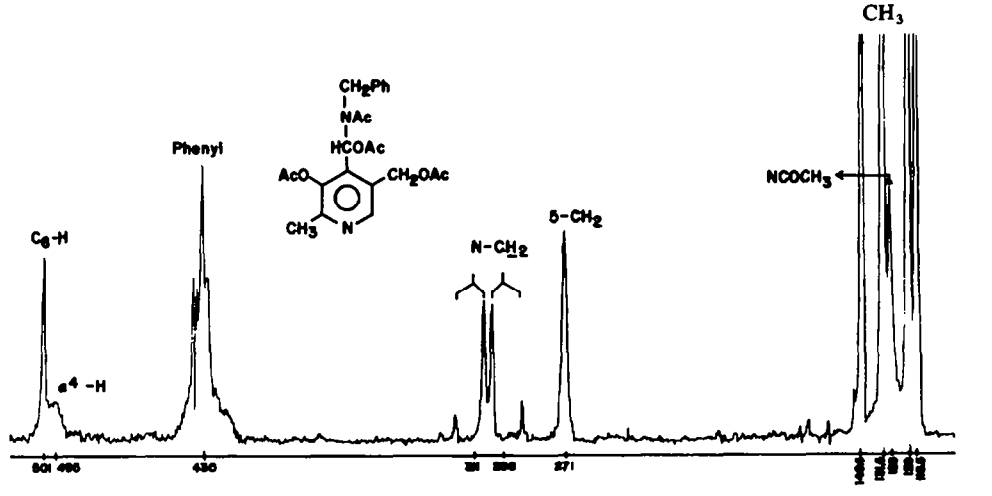


FIG 3. NMR spectrum of the tetraacetyl derivative VI.

Two types of methylene groups can be recognized, one giving rise to a broadened singlet, and the other giving a well-spaced AB quadruplet.* The α^4 proton could not be identified with certainty, but the C_6 proton at 493·5 c/s and the two Ph groups at 428 and 446 c/s could be distinguished. The IR and UV spectra of these compounds were also consistent with the proposed structure (Experimental).

Acetylation of II with acetic anhydride in pyridine gave an entirely different result (Scheme I). Four acetyl groups were introduced into the molecule, as was indicated by direct acetyl determination. Structure VI has been assigned to the acetylation product primarily on the basis of NMR spectroscopy (Fig 3). The four sharp Me peaks are due to the three O-acetyl groups and the 2-Me group, respectively. The broad Me peak at 128 c/s has been assigned to the N-acetyl on the basis of comparison with a model compound.† One of the methylene groups appears as an AB quadruplet because of the restriction of rotation, the creation of an asymmetric center, or both.‡ The molecular model of the tetraacetate (VI) shows considerable crowding at the 4-position, with severe restriction of free rotation around both the 4-C-α^4-C and N-CH_2 bonds. This crowding may explain the considerable width of the α^4-H peak in the spectrum. Structure VI has been confirmed by a high-resolution mass spectrum; IR and UV spectra were also consistent with this structure.

The present results indicate that the two hypothetical structural forms of pyridoxal Schiff bases (Ib and Ic) can be obtained as stable and crystalline acyl derivatives such as III and VI, respectively, under mild conditions and in high yield. It was of interest to determine the effects of the structure of the Schiff base and the acylating agent used on the composition of the product.

Acylation of pyridoxylidenebenzylamine (II) with *p*-nitrobenzoyl chloride proceeded in a manner analogous to benzoylation, giving the *p*-nitro analog of III.

Acylation of other Schiff bases derived from pyridoxal and substituted benzylamines with *p*-fluoro (an example of an electron-withdrawing substituent) and *p*-OMe (an example of an electron-donating substituent) has been examined with acetic anhydride and benzoyl chloride. Benzoylation of the *p*-OMe and *p*-F analogs proceeded as in the case of unsubstituted compounds, yielding the cyclic (amino acetal) derivatives exclusively. On acetylation, however, the *p*-F analog also gave only the straight-chain derivative, but the *p*-OMe analog VII gave a mixture of the two isomers VIII and IX (Scheme II), which could be separated by preparative TLC. Acetylation of pyridoxylidenepropylamine (X) gave only the cyclic derivative XI (Scheme II).

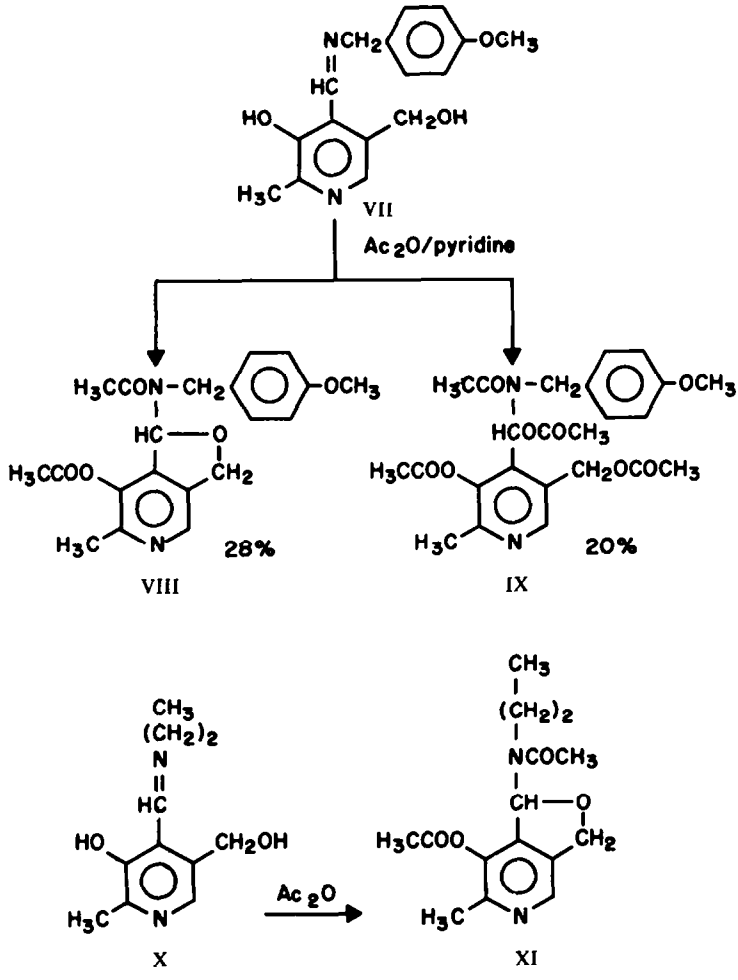
It is apparent from these results that the outcome of the acylation reaction depends

* α^5-Methylene protons in pyridoxal and its derivatives give rise to an AB quadruplet; but because compound V has an asymetric carbon, we could expect an AB quadruplet from the methylene protons of the N-benzyl group.

† The model compounds were $PhCH(CH_3)NHCH_AH_BPh$ and its N-acetyl derivative.[8] The NMR spectrum of its hydrochloride in D_2O exhibited one AB quadruplet centered at 244 c/s due to H_A and H_B, and a one-proton quadruplet due to α-CH. The Me peak was split by α-CH into a doublet (103 and 109 c/s). In the N-acetyl derivative, we observe the same Me doublet at 84 and 91 c/s, but the N-acetyl group appears as a broad peak at 127 c/s. This compares well with the shape and position of the Me group at 128 c/s assigned to N-acetyl in Fig 3.

‡ Examples of non-equivalence of methylene protons due to an asymmetric center are given in the preceding footnote, as well as by Siddal.[9]

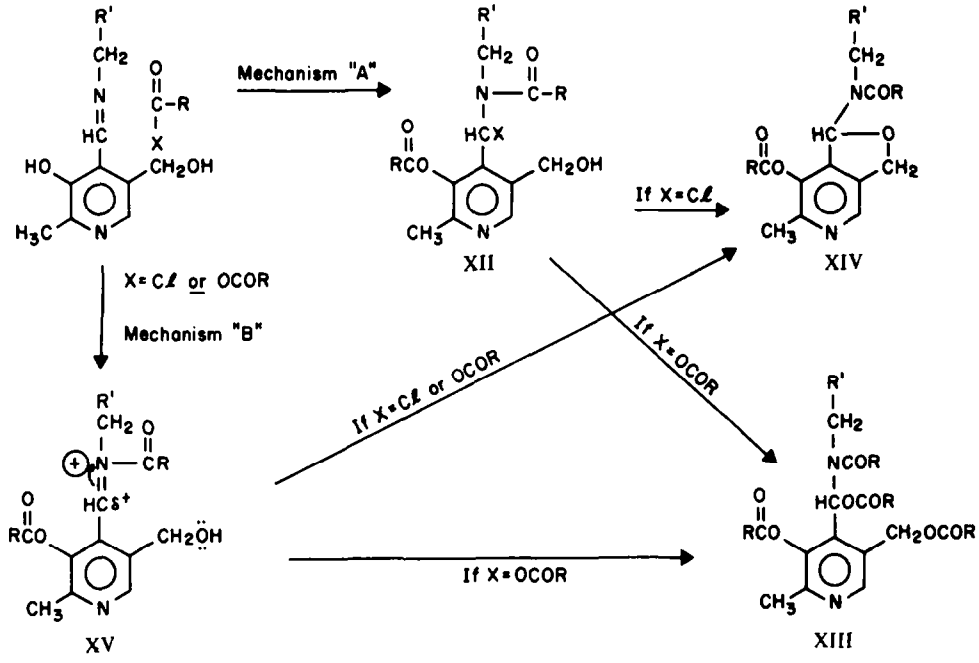
SCHEME II



upon both the nature of the Schiff base (with electronic effects probably playing a major role) and the nature of the acylating reagent. In all of the cases that we have examined, the starting Schiff bases were found to exist as aldimines exclusively, as shown by NMR spectroscopy. The NMR spectra of the pyridoxylidene *p*-fluoro and *p*-methoxybenzylamines were analogous to that of the unsubstituted analog II (Fig 1). Accordingly, the cyclic derivatives arise from interaction with the acylating agent, and this assumption is implied in the proposed reaction mechanism indicated in Scheme III.

In this scheme, the reagent could be either an acid chloride (X = Cl) or an anhydride ($X = OCOCH_3$). Initial acylation of the phenolic OH is assumed in both mechanisms "A" and "B". In mechanism "A", simple addition across the C=N bond of either of the reagents is postulated.

SCHEME III



With an acid anhydride, the initial product (XII, X = OCOMe) is acetylated in the α^5-position, giving the final product XIII. If the reagent is an acid chloride, however, the intermediate will be an α^4-chloro derivative (XII, X = Cl).* Chlorine being a good leaving group, an intramolecular nucleophilic attack by the α^5-O can be expected, creating the cyclic derivative XIV.

In mechanism "B", we assume that the reagent attacks the N of the aldimine first, forming the intermediate XV, in which the α^4-C atom can be attacked either intramolecularly by α^5-O to give XIV, or else intermolecularly by the reagent, which acylates both the α^5-O and the α^4-C to give XIII. Mechanism "B" would appear to be more consistent with the experimental facts, since we sometimes get mixtures of the cyclic and acylic forms on acid anhydride treatment (as when R^1 = p-C_6H_4OMe), whereas from mechanism "A" we predict the acyclic form as the sole product.

The greater probability of mechanism "B" notwithstanding, the operation of both mechanisms could be invoked to account for the products obtained. Perhaps mechanism "A" operates whenever an acylic product is obtained, and mechanism "B" leads to the formation of a cyclic derivative. Although initial acylation of the phenolic OH is assumed for both mechanisms and is reasonable on the basis of our previous work,[11] we cannot rule out the possibility of an initial attack on the imine nitrogen. Even so, this possibility would modify the proposed mechanisms only slightly.

* An analogous reaction has been described:[10] $C_6H_5CH = NC_6H_5 + C_6H_5COCl \rightarrow C_6H_5CH(Cl)N(COC_6H_5)C_6H_5$.

Electron-donating substituents on the phenyl ($R^1 = p\text{-}C_6H_4OMe$) and aliphatic side-chains ($R^1 = Et$) favor the formation of cyclic forms, whereas electron-withdrawing substituents ($R^1 = p\text{-}C_6H_4F$) and phenyl (R = Ph) yield only the acylic forms. It appears that the competition between the inter- and intramolecular reactions depends primarily on electronic factors, since changes in substituents far removed from the reaction center, as in the case of the *p*-OMe analog VII, have dramatic effects on the distribution of the products.

In many reactions, imines resemble carbonyl compounds,[12] as is also evident on comparing pyridoxal with its Schiff bases in the transamination and addition reactions that have been studied.[13] Nevertheless, there are some interesting differences. Whereas pyridoxal exists primarily in the hemiacetal form,[14] aldimines of pyridoxal exist in the extended form, as shown here. It has been shown previously that acylation of pyridoxal gives rise to hemiacetal derivatives, irrespective of the type of acylating agent used.[11] This can be regarded as simple acylation of the *preexisting* hemiacetal structure. In contrast, the cyclic aminoacetal structure, such as III, is formed from the aldimine under the influence of the acylating agent.*

Of fundamental importance in the ring-chain and hydration equilibria of pyridoxal and its aldamines is the electrophilicity of the α^4 carbon. The greater that electrophilicity, the more the appropriate double bond is destabilized, and the greater is its propensity for attack by a nucleophile to form addition products (hemiacetal or hydrate in the case of aldehydes; aminoacetal or carbinolamine in the case of aldimines). In the aldimines, the basic nitrogen atom decreases the electrophilicity of the neighboring α^4 carbon, and hence the tendency to form any addition products, including ring compounds. On the other hand, acylation of this nitrogen to give XV (Scheme III) increases the electrophilicity of the carbon, and consequently the reaction leading to XIII or XIV proceeds with relative ease.

EXPERIMENTAL

TLC was used routinely as described earlier.[15] IR spectra were determined with a Perkin–Elmer 457 spectrometer, UV spectra with a Perkin–Elmer 202 spectrometer, and NMR spectra with a Varian A-60A instrument. Positions of peaks are expressed in c/s from TMS as in internal standard. Assignments of peaks were made on the basis of previous work.[14,16]

Pyridoxylidenebenzylamine (II). Pyridoxal hydrochloride (500 mg) was suspended in abs EtOH (25 ml) and cooled in ice, and a soln of benzylamine (0·8 ml) in abs alcohol (10 ml) was added slowly, with stirring. The resulting soln was stirred at room temp for 4 hr, and was then evaporated completely *in vacuo*. The residue was taken up in EtOAc. Benzylamine hydrochloride, which separated out, was filtered off, and the filtrate was evaporated *in vacuo*. The residue was crystallized from ether, yielding 560 mg (89%) of II, m.p. 112–114° (D. Heyl *et al.*[17] reported a 35% yield, m.p. 114–115°; Witkop and Beiler[3] carried the reaction out in water, and obtained a 90% yield, m.p. 112·5°); UV: λ_{max}^{EtOH} 252 mμ (ε 12,600), 347 mμ (ε 5,300); IR, λ_{max}^{Nujol} 3270 (OH), 1625 (C=N) cm^{-1}.

Benzoylation of pyridoxylidenebenzylamine

7-Benzoyloxy-1-(N-benzylbenzamido)-6-methylfuro[3,4-c]pyridine (III). To an ice-cooled soln of pyridoxylidenebenzylamine (250 mg) in pyridine (dry, 8 ml), a soln of benzoyl chloride (0·3 ml) in anhyd ether (10 ml) was added during 10 min, dropwise. After the addition, the reaction mixture was stirred for 1 hr in the cold, and then for 8 hr at room temp. Volatile material was removed *in vacuo*, the residue was cooled,

* It can be argued that cyclic compounds can arise from the presence of a very small, undetectable amount of the ring tautomer (Ib). Although this possibility cannot be rigidly ruled out, it is nevertheless unlikely, since we obtain exclusively the cyclic derivatives on benzoylation.

ice-cold water (25 ml) was added, and the soln was brought to pH 6 with 1 N NaOH. The aqueous soln was extracted several times with EtOAc, and the combined EtOAc extract was washed with water, dried, and evaporated *in vacuo*. The residue was crystallized from ether, yielding 380 mg (83%), m.p. 136–138°; NMR (in $CDCl_3$): 2-CH_3 −139, CH_2Ph −243, −259, −267, −282 (AB quadruplet), 5-CH_2 −295 (broad), 4-CH −408, C_6-H −495·5, 3 × C_6H_5 −426·5 and multiplet −420 to −490 c/s; UV, λ_{max}^{EtOH} 275 mμ (ε 7450); IR, λ_{max}^{Nujol} 1720 (O-benzoyl), 1635, 1600 cm^{-1} (N-benzoyl). Found: C, 74·75; H, 5·34; N, 6·05. Calc. for $C_{29}H_{24}N_2O_4$: C, 75·00; H, 5·17; N, 6·03%).

Selective debenzoylation of III

1-(N-*Benzylbenzamido*)-7-*hydroxy*-6-*methylfuro*-[3,4-c]-*pyridine* (IV). Compound III (115 mg) was dissolved in EtOH (5 ml), 1 N NaOH (1 ml) was added, and the mixture was stirred for 1 hr at room temp. The soln was neutralized with AcOH to pH 6, evaporated, taken up in water (15 ml), and extracted with EtOAc (3 × 20 ml). The dried soln was evaporated to an oil, and was crystallized from ether-light petroleum; yield 69 mg (78%). The compound does not have a sharp m.p., but shrinks at 83°, with some frothing; NMR (DMSO-d_6): −140·5 (2-CH_3); −408 (broad, α^4-H), −238·5, 272 (AB quadruplet, J = 15, N-CH_2), 287·5 (5-CH_2) −426 (phenyl), −449·5 (benzoyl); UV λ_{max}^{EtOH} 280·5 mμ (ε 4700); IR. λ_{max}^{Nujol} 1625 (broad. N-benzoyl). (Found: C, 73·16; H, 5·77; N, 7·56. Calc. for $C_{22}H_{20}N_2O_3$: C, 73·40; H, 5·60; N, 7·48%).

Mesylation of IV

1-(N-*Benzylbenzamido*)-6-*methyl*-7-(*methylsulfoxy*)*furo*-[3,4-c]-*pyridine*. A soln of methanesulfonyl chloride (25 mg) in ether (0·8 ml) was added to a stirred and ice-cooled soln of IV (45·0 mg) in dry pyridine (4 ml). The mixture was stirred at room temp for 2 hr, and was evaporated to dryness. Next 2% aq Na_2CO_3 (*ca* 25 ml) was added, and the mixture was extracted with ether (3 × 10 ml). The combined ether solns were washed with water, dried ($CaSO_4$), and evaporated to *ca* 1 ml. Addition of light petroleum induced crystallization. The yield was 36·4 mg (67%), m.p. 182·5°; NMR ($CDCl_3$): −151 (2-CH_3), −242·5, −283·5 (AB quadruplet, J = 15·0), −496 (C_6-H), −292 (N-CH_2), −425·5 (Ph), −446 (m, benzoyl), −193 (3-OSO_2CH_3); UV, λ_{max}^{EtOH} 265 (ε 4000); λ_{max}^{Nujol} 1645 cm^{-1} (*N*-benzoyl). (Found: C, 63·19; H, 5·02; N, 6·70. Calc. for $C_{23}H_{22}N_2O_5S$: C, 63·00; H, 5·06; N, 6·39%).

Acetylation of IV

7-*Acetoxy*-1-(N-*benzylbenzamido*)-6-*methylfuro*[3,4-c]*pyridine* (V). Compound IV was prepared from III, and was subjected to acetylation. Compound III (115 mg) was dissolved in EtOH (5 ml), 1 N NaOH (1 ml) was added, and the mixture was stirred for 1 hr at room temp. The soln was neutralized with AcOH to pH 6, evaporated *in vacuo*, taken up in water (15 ml), and extracted with EtOAc (3 × 20 ml). The dried soln was evaporated to an oil, which was taken up in dry pyridine (10 ml), and Ac_2O (0·5 ml) was added. After 1·5 ml of 5% aq $NaHCO_3$ (*ca* 25 ml) was added, the mixture was evaporated to dryness. The residue was taken up in water (25 ml) and extracted with EtOAc (3 × 20 ml), and the EtOAc extracts were washed with water (25 ml), dried ($MgSO_4$), and evaporated to a small volume, yield, 70·9 mg (71%), m.p. 137° (from pyridine-water); UV, λ_{max}^{EtOH} 267 mμ (ε 4450); IR, λ_{max}^{Nujol} 1780 cm^{-1} (O-acetyl), 1645 cm^{-1} (*N*-benzoyl). (Found: C, 71·57; H, 5·58; N, 7·01. Calc. for $C_{24}H_{22}N_2O_4$: C, 71·63; H, 5·51; N, 6·96%).

p-*Nitrobenzoylation of pyridoxylidenebenzylamine*

1-(N-*Benzyl*-p-*nitrobenzamido*)-6-*methyl*-7-(p-*nitrobenzoyloxy*)*furo*[3,4-c]*pyridine*. Pyridoxylidenebenzylamine (210 mg) and *p*-nitrobenzoyl chloride were stirred overnight in dry pyridine (5 ml). An additional 210 mg of *p*-nitrobenzoyl chloride was added, and the reaction was continued for another 5 hr. The pyridine was now evaporated off, 10% aq Na_2CO_3 (20 ml) was added, and the mixture was shaken with EtOAc. At this stage, 51 mg of material (m.p. 197–198°) crystallized, and was removed by filtration. The filtrate was extracted with EtOAc, and the EtOAc layer was dried and partially evaporated. On adding light petroleum and cooling, 115 mg of an identical compound (m.p. 195–196°) was obtained. Mother liquors yielded more material, but it was contaminated with another compound. Recrystallization from chloroform-light petroleum raised the m.p. to 197°; NMR (in $CDCl_3$): 2-CH_3 −144, 5-CH_2 (AB quadruplet: H_A −255, H_B −273, J = 14·5), CH_2Ph −304, phenyl −434, α^4-H-408 (broad), *p*-nitrobenzoyl −435, −497·5 (centers of multiplets); UV, λ_{max}^{EtOH} 261 mμ (ε 27,000); IR, λ_{max}^{Nujol} 1740 (O-*p*-nitrobenzoyl), 1645 cm^{-1} (N-*p*-nitrobenzoyl). (Found: C, 62·48; H, 3·92; N, 9·80. Calc. for $C_{29}H_{22}N_4O_8$: C, 62·81; H, 4·00; N, 10·10%).

Acetylation of pyridoxylidenebenzylamine

N-(α⁴-*Acetoxy*-3,O⁵-*diacetylpyridoxyl*)-N-*acetylbenzylamine* (VI). To a stirred and cooled (0°) soln of pyridoxylidenebenzylamine (310 mg) in pyridine (7·5 ml), Ac_2O (1·5 ml) was added. On stirring for 12 hr, the formerly yellow soln became colorless. This soln was evaporated giving a thick oil. The oil was shaken with a mixture of 25 ml 5% aq Na_2CO_3 abd 25 ml of EtOAc. The aqueous layer was extracted again twice with EtOAc. The combined EtOAc extracts were dried ($MgSO_4$), evaporated, and triturated with light petroleum, and then the oil crystallized. After the isolation of crystalline material (298 mg, m.p. 128·5°), two additional fractions were obtained from the mother liquors, resulting in a total yield of 375 mg (70%). The m.p. increased to 130° after crystallization from pyridine-water; NMR (Fig 3); UV λ_{max}^{EtOH} 273 mμ (ε 5050); IR, λ_{max}^{Nujol} 1740 cm⁻¹ (broad, O-acetyl), 1670 cm⁻¹ (N-acetyl); mass spectrum: *m/e* 442 (molecular ion), 400, 399, 383, 382, 341, 340, 298, 297, 280, 255, 238, 210, 193, 150, 91 ($C_6H_5CH_2^+$), 55, 43 (CH_3CO^+). (Found: C, 62·72; H, 5·86; N, 6·68. Ac; 39·0. Calc. for $C_{23}H_{26}N_2O_7$: C, 62·43; H, 5·92; N, 6·33; Ac, 38·2%).

Pyridoxylidene-p-*fluorobenzylamine*

Pyridoxal (167 mg, 1 mmole) and *p*-fluorobenzylamine (135 mg, 1·1 mmoles) in dry EtOH (20 ml) were stirred for 2 hr at room temp. The yellow soln was evaporated, dissolved in ether (10 ml), and dried. After evaporation of the ether, the yellow oily residue crystallized when heated with a few drops of light petroleum. The yield amounted to 270 mg (98%), m.p. 109–110°. Recrystallization from EtOH-water and from ether-light petroleum gave yellow crystals, m.p. 121–122°; TLC, 1:1 MeOH-$CHCl_3$, *Rf* 0·88; NMR (in $CDCl_3$): −146 (s, 3, 2-CH_3), −284 (s, 5-CH_2), −290 (broad, 2, CH_2), −430 (m, 4, aromatic), −461 (s, 1, C_6-H), −541·5 (tr. J = 1·2, 1, α⁴-H). (Found: C, 66·06; H, 5·56; N, 9·73. Calc. for $C_{15}H_{15}N_2O_2F$: C, 65·69; H, 5·52; N, 10·21%).

Benzoylation of pyridoxylidene-p-*fluorobenzylamine*

7-*Benzoyloxy*-1-(N-*benzyl*-p-*fluorobenzamido*)-6-*methylfuro*[3,4-c]*pyridine*. To an ice-cooled soln of pyridoxylidene-*p*-fluorobenzylamine (100 mg) in pyridine (dry, 5 ml), a soln of benzoyl chloride (0·25 ml) in ether (anhydrous, 5 ml) was added drop by drop, with vigorous stirring. After the benzoyl chloride was washed into the flask with ether (2 ml), the reaction mixture was stirred overnight at room temp, moisture being excluded. Volatile material was evaporated *in vacuo*, and the resulting oily residue was treated with water and extracted 3 times with chloroform. The combined extracts were dried, and were evaporated to an oil. The oil was taken up in ether, to which anhyd ethereal HCl was added until precipitation was complete. Recrystallization from abs alcohol gave 141 mg (74·6%), m.p. 139–141·5° (dec); IR, λ_{max}^{KBr} 1761 (O-benzoyl), 1637 cm⁻¹ (N-benzoyl). (Found: C, 67·40; H, 4·89. Calc. for $C_{29}H_{24}O_4N_2ClF$: C, 67·11; H, 4·66%).

Acetylation of pyridoxylidene-p-*fluorobenzylamine*

N-(α⁴-*Acetoxy*-3,O⁵-*diacetylpyridoxyl*)-N-*acetyl*-p-*fluorobenzylamine*. To an ice-cooled soln of pyridoxylidene-*p*-fluorobenzylamine (100 mg) in pyridine (dry, 5 ml), Ac_2O (5 ml) was added slowly with rapid stirring. After stirring for 16 hr, the volatile material was evaporated *in vacuo*, water was added, and the aqueous soln was extracted with chloroform, dried, and evaporated to an oil. The compound was crystallized from ether-light petroleum, and yield 101 mg (60%) of the tetraacetyl derivative, m.p. 139–141·5; the NMR spectrum was very similar to that of VI (Fig 3); IR, λ_{max}^{KBr} 1742 (O-acetyl), 1667 cm⁻¹ (N-acetyl). (Found: C, 59·94; H, 5·45. Calc. for $C_{23}H_{25}FN_2O_5$: C, 59·99; H, 5·47%).

Pyridoxylidene-p-*methoxybenzylamine* (VII)

Clear solns of pyridoxal hydrochloride (1·0 g, 5 mmole) in pyridine (dry, 25 ml) and of *p*-methoxybenzylamine (1·35 g, 10 mmoles) in pyridine (5 ml) were combined, and the flask was protected from moisture. The soln turned yellow and *p*-methoxybenzylamine hydrochloride started precipitating after 30 sec. After stirring at room temp for 1 hr, *p*-methoxybenzylamine hydrochloride was filtered off, and the filtrate was evaporated, giving a yellow oil. The oil was dissolved in EtOAc (5 ml), and light petroleum was added dropwise. The first crop of crystals (0·17 g) was slightly contaminated with *p*-methoxybenzylamine hydrochloride, but an additional two crops (0·61 g, m.p. 96°, and 0·24 g, m.p. 97°, respectively) were pure (total yield 1·02 g, or 73%). Recrystallization from EtOH-water and from chloroform-light petroleum raised the m.p. to 103–105°.

When the reaction was carried out in EtOH, as in the case of pyridoxylidenebenzylamine, the yield was 65%; NMR: −147 (s, 2-CH_3), −227·5 (s, 3, OCH_3), −284·5 (s, 5-CH_2), −287·5 (broad, 2, CH_2), −414 (d, J = 9, 2, aromatic) −436·5 (d, J = 9, 2, aromatic), −462 (s, 1, C_6-H), −539·5 (tr. J = 1·2, 1, α⁴-H). (Found: C, 67·35; H, 6·38; N, 9·67. Calc. for $C_{16}H_{18}N_2O_3$: C, 67·13; H, 6·34; N, 9·87%).

Acetylation of pyridoxylidene-p-methoxybenzylamine

7-Acetoxy-1[N-(p-methoxybenzyl)acetamido]-6-methylfuro[3,4-c]pyridine (VIII) and *N-(α^4-acetoxy-3,O^5-diacetylpyridoxyl)-N-acetyl-p-methoxybenzylamine* (IX). Pyridoxylidene-*p*-methoxybenzylamine (300 mg) was dissolved in pyridine (10 ml), and Ac_2O (1·2 ml) was added dropwise, the reaction mixture being cooled with ice and stirred. After 5 hr at room temp. volatile material was removed *in vacuo*. and the oil that remained was dissolved in EtOAc and shaken with 5% $NaHCO_3$ (40 ml). The $NaHCO_3$ soln. was extracted with EtOAc, and the EtOAc layers were combined and dried ($MgSO_4$). TLC (EtOAc) showed two spots (R_f 0·48 and 0·57). On evaporation and addition of light petroleum, 58 mg of low-R_f material (diacetate) crystallized; m.p. 172°. Addition of more light petroleum gave a second crystalline fraction (52 mg), contaminated with a trace of the high-R_f material. The diacetyl derivative was recrystallized from chloroform-light petroleum; m.p. 175–176°, yield 110 mg (28%); IR, λ_{max}^{KBr} 1760 (O-acetyl), 1655 cm^{-1} (N-acetyl); NMR (in $CDCl_3$): −125, −137·5, −146, −227·5 (singlets, CH_3), −243, −301·5 (singlets, CH_2), −410·5 (s, ph), α^4-H obscured by phenyl peak, −501 (C_6-H). (Found: C, 64·87; H, 5·90; N, 7·77. Calc. for $C_{20}H_{22}N_2O_2$: C, 64·85; H, 5·99; N, 7·56.)

Addition of more light petroleum to the mother liquors resulted in crystallization of the tetraacetyl derivative, which was contaminated with some diacetate. It was purified by preparative TLC (silica gel, EtOAc) and recrystallized (light petroleum), giving the pure compound (yield 92 mg, 20%), m.p. 175·5–176°; IR, λ_{max}^{KBr} 1735 and 1755 (O-acetyl), 1667 cm^{-1} (N-acetyl). The NMR spectrum was very similar to that of compound VI (Fig 3). (Found: C, 60·73; H, 6·23; N, 6·14. Calc. for $C_{24}H_{28}N_2O_8$: C, 60·00; H, 5·98; N, 5·93.)

Benzoylation of pyridoxylidene-p-methoxybenzylamine

7-Benzoyloxy-1-[N-(p-methoxybenzyl)benzamido]-6-methylfuro[3,4-c]pyridine. Pyridoxylidene-*p*-methoxybenzylamine (50 mg) was suspended in dry pyridine (2·5 ml) and cooled (ice water), and benzoyl chloride (0·1 ml) in ether (4 ml) was added slowly to the vigorously stirred soln. After stirring overnight, the solvents were evaporated *in vacuo*, and the product was taken up in chloroform and extracted with 5% $NaHCO_3$. The bicarbonate soln was extracted with chloroform, and the combined chloroform extracts were evaporated and subjected to preparative TLC (ethyl ether, R_f 0·35–0·45). The material was eluted with EtOH, evaporated, and chromatographed on Sephadex LH-20 (column 1·4 × 15 cm). After elution with EtOAc, the compound was pure, but did not crystallize. The hydrochloride, obtained by passing HCl into a soln of the free base in ether, was recrystallized from EtOH and ether, yielding 40 mg (41·6%) of crystalline material, which turned dark on heating, and decomposed above 163°; IR, λ_{max}^{KBr} 1745 (broad, O-benzoyl), 1658 cm^{-1} (N-benzoyl). (Found: C, 68·14; H, 5·05. Calc. for $C_{30}H_{27}O_5N_2Cl$: C, 67·85; H, 5.13.)

Acetylation of pyridoxylidenepropylamine

7-Acetoxy-6-methyl-1-[N-(n-propylacetamido)]furo[3,4-c]pyridine (XI). In this case, the Schiff base was not isolated, but was subjected to acetylation *in situ*. Pyridoxal hydrochloride (407 mg, 2 mmoles) and n-propylamine (130 mg, 2·2 mmoles) were stirred in pyridine (dry, 20 ml) for 30 min, giving a yellow soln of the Schiff base X. Ac_2O (0·367 ml, 510 mg, 5 mmoles) was added dropwise during 1 hr, and then 0·6 ml was added at once. After stirring for 60 hr, TLC indicated two spots, one (approx 10%) of which could be identified as pyridoxal acetate. Volatile material was removed with an oil pump, and the residue was taken up in EtOAc. The resulting soln was shaken with 5% $NaHCO_3$, and the organic phase was washed with water and dried ($MgSO_4$). The extract was evaporated to a small volume, some light petroleum was added, and 176 mg of the compound (m.p. 102–105°) was obtained; on addition of more light petroleum, an additional 88 mg (m.p. 94–97°) was isolated; the total yield was 284 mg (45%).

The compound was recrystallized from chloroform-light petroleum; m.p. 108–109°. IR, λ_{max}^{KBr} 1760 (O-acetyl), 1650 cm^{-1} (N-acetyl); NMR (in $CDCl_3$): −46·5 (tr, J = 7, ω-methyl in propylamine), −133, −136·5 (N- and O-acetyl methyls), −148·5 (2-CH_3), −75 and −150 (broad methylene multiplets of the propyl group), −312 (broad, 5-CH_2), −505·5 (C_6-H); α^4-proton could not be detected. (Found: C, 61·36; H, 7·09; N, 9·43. Calc. for $C_{15}H_{20}N_2O_4$: C, 61·64; H, 6·90; N, 9·58.).

Acknowledgement—This study was supported in part by a research grant (CA-08793) from the National Cancer Institute, U.S. Public Health Service. We would like to thank Dr. Don C. DeJongh for the mass spectrum of compound VI. Initial acylation experiments were carried out by Dr. B. Paul.

REFERENCES

[1] Pyridoxine Chemistry XXIII. Previous papers in this series:
[a] W. Korytnyk and H. Ahrens, *J. Heterocyclic Chem.* in press;
[b] W. Korytnyk and B. Paul, *J. Med. Chem.* **13**, 187 (1970)

[2] Preliminary reports of this study have appeared: *Abstracts, 158th National Meeting, Am. Chem. Soc.*, MEDI 48, New York, N.Y. (1969); *Abstracts, 7th International Symposium on the Chemistry of Natural Products*, E124. Riga, Latvia (1970).

[3] B. Witkop and T. W. Beiler, *J. Am. Chem. Soc.* **76**, 5589 (1954)

[4] [a] H. N. Christensen and T. R. Riggs, *J. Biol. Chem.* **220**, 265 (1956)
[b] D. E. Metzler, *J. Am. Chem. Soc.* **79**, 485 (1957); H. N. Christensen, *Ibid.* **80**, 99 (1958)

[5] D. Heinert and A. E. Martell, *J. Am. Chem. Soc.* **85**, 183, 188, 1334 (1963)

[6] R. G. Hiskey and J. M. June, *J. Am. Chem. Soc.* **85**, 578 (1963)

[7] P. E. Braun, Ph.D. Thesis, p. 109, Department of Biochemistry, University of California, Berkeley (1968); see also P. E. Braun and E. E. Snell, *Proc. Natl. Acad. Sciences* **58**, 298 (1967)

[8] K. Parck, *J. prakt. Chem.* **2**, 86, 284 (1912)

[9] T. H. Siddal, *J. Phys. Chem.* **70**, 2249 (1966)

[10] T. C. James and C. W. Judd, *J. Chem. Soc.* **105**, 1427 (1914); see also H. Breederveld, *Rec. Trav. Chim.* **79**, 1197 (1960)

[11] B. Paul and W. Korytnyk, *Tetrahedron* **25**, 1071 (1969)

[12] R. W. Layer, *Chem. Rev.* **63**, 489 (1963)

[13] E. E. Snell in *Chemical and Biological Aspects of Pyridoxal Calalysis* (Edited by E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi Fanelli), p. 1, Macmillan, New York (1963)

[14] W. Korytnyk and R. P. Singh, *J. Am. Chem. Soc.* **85**, 2813 (1963)

[15] H. Ahrens and W. Korytnyk, *Anal. Biochem.* **30**, 413 (1969)

[16] W. Korytnyk and B. Paul, *J. Heterocyclic Chem.* **2**, 481 (1965)

[17] D. Heyl, E. Luz, S. A. Harris, and K. Folkers, *J. Am. Chem. Soc.* **70**, 3669 (1948)