[CONTRIBUTION OF THE DEPARTMENT OF NEUROLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

Oximes of the Pyridine Series[1]

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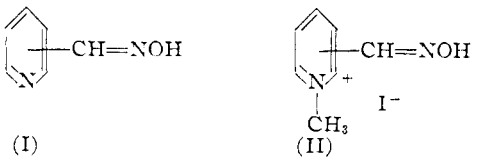
A number of oximes containing the pyridine and quaternary pyridine nucleus are described. The tertiary oximes have the *syn* configuration, but it appears that both isomers can in general be obtained in the quaternary series. One isomer is unstable and readily converts to the other.

In the course of our investigations of enzyme mechanisms, particularly the reactivation of alkylphosphate inhibited acetylcholinesterase,[2] we became interested in oximes containing the pyridine (I) and quaternary pyridine nucleus (II).



(I) (II)

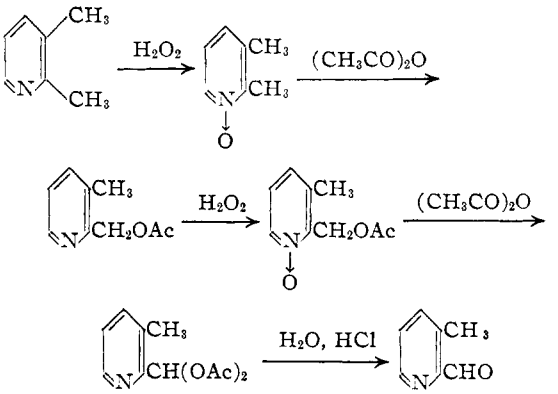
Since no quaternary oxime has been hitherto reported in the literature and only two pyridine aldoximes[3,4] have been described previously, it seemed of interest to report on these compounds.

In general it appears that both stereoisomers can be obtained for the quaternary oxime derivatives. Since we have not yet been able to assign the configurations with sufficient probability, we will refer to them as the A and B stereoisomers. The A series is obtained by treating the aldehyde or ketone methiodides with alcoholic hydroxylamine at −5° or somewhat lower. They are white to light yellow solids, with the exception of pyridine-3-aldoxime methiodide which was obtained as an oil. The B series is obtained by reaction as for the A series, but at room temperature or somewhat higher, or by methylation of the tertiary oxime. They are yellow solids having higher melting points and are stronger acids than the corresponding A series members. Members of the A series in the solid state convert slowly to the B series at room temperature but can be stored at −20°. In alkaline aqueous solution the conversion is extremely rapid. The anions of the B series are yellow, but since the A series anions rapidly convert to the B series, we cannot judge whether their anions may not also be yellow. The two series are so clearly recognizable that the assignment of configuration to any one pair would suffice to identify all the derivatives. There is a precedent for this procedure of obtaining one isomer by reaction in the cold and the other in warm media in the cases of furan-2-aldehyde and pyrrole-2-aldehyde where the *anti* configurations were obtained at a higher temperature.[5,6]

The tertiary aldoximes of pyridine-2-aldehyde yielded only one stereoisomer—the isomer with the *syn* configuration. The acetates of these oximes were obtained from acetic anhydride and their reconversion to oximes in alkali was the basis for assigning the *syn* configurations to both the acetates and the oximes.[5,7,8] The tertiary aldoximes here described with the exception of 6-methylpyridine-2-aldoxime react with methyl iodide to yield the B series quaternary oximes. This fact does not indicate that the B-series has the *syn* configuration because methylation need not occur with retention of configuration. Indeed, it is reported that quaternary ions catalyze the isomerization of oximes.[9]

Methylation of 6-methylpyridine-2-aldoxime with methyl iodide yielded the hydroiodide of the methyl ether. This is in accordance with the general observation that 2,6-disubstituted pyridines do not readily form quaternary salts.[10] Similarly with quinoline derivatives: while quinoline-4-aldoxime methiodide was easily formed, quinoline-2-aldoxime methiodide was not obtained.

Pyridine-2-aldoximes with substituents in the 3-position were prepared from the corresponding aldehydes which in turn were synthesized according



B.p. 83–84° (12–13 mm.)

(1) This work was supported in part by the Medical Research and Development Board, Department of the Army, Office of the Surgeon General, Contract No. DA-49-007-MD-37, and in part by the Division of Research Grants and Fellowships of the National Institutes of Health, Grant No. B-573, United States Public Health Service.

(2) I. B. Wilson and S. Ginsburg, *Biochim. Biophys. Acta*, **18**, 168 (1955).

(3) G. Lenart, *Ber.*, **47**, 808 (1914).

(4) S. G. Angyal, G. B. Barlin and P. C. Warles, *J. Chem. Soc.*, 1740 (1953).

(5) O. L. Brady, *et al.*, *ibid.*, (a) **117**, 1040 (1920); (b) **125**, 1418 (1924); (c) 1959 (1927).

(6) A. P. Terent'ev and A. N. Makarova, *J. Gen. Chem. USSR*, **21**, 270 (1951); *C. A.*, **45**, 7105 (1951).

(7) A. Hantzsch, *Ber.*, **24**, 21 (1891).

(8) C. R. Hauser and C. T. Sullivan, THIS JOURNAL, (a) **55**, 4611 (1933); (b) C. R. Hauser and E. Jordan, *ibid.*, **57**, 2450 (1935); (c) **58**, 1419 (1936); (d) **58**, 1772 (1936).

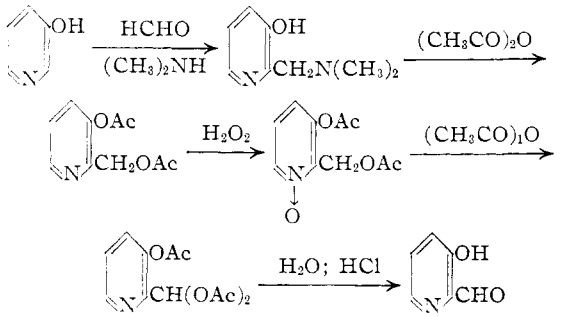
(9) N. V. Sidgwick, "The Organic Chemistry of Nitrogen," Clarendon Press, Oxford, 1942, p. 192.

(10) R. C. Elderfield, "Heterocyclic Compounds," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1950, p. 572.

to the procedure of Boekelheide and Linn.[11] Substituents in the 3-position are not affected in this procedure.

3-Methylpyridine-2-aldehyde was prepared from commercially available 2,3-lutidine as shown above. Applying the same method to the diacetate of 3-hydroxy-2 pyridine–methanol (which in turn was obtained according to the method of Stempel and Buzzi[12]), we obtained 3-hydroxypyridine-2-aldehyde.

The reaction sequence, starting with commercial 3-pyridol, is



3-Methoxypyridine-2-aldehyde was obtained by methylating the hydroxy aldehyde in alkaline solution according to the method of Kanewskaya[13] for the methoxyaldehydes of the benzene series.

Pyridine-3-aldoxime acetate and pyridine-4-aldoxime acetate react with methyl iodide to yield quaternary oxime acetates. In aqueous alkali these acetates readily hydrolyze to quaternary oximes of the B series and the quaternary acetates may therefore be assigned the *syn* configuration, but again no inference may be made concerning the configuration of the B series oximes. Attempts to methylate pyridine-2-aldoxime acetate yielded the quaternary nitrile, presumably because long times and slightly elevated temperature are required to produce reaction.

We have not yet been able to obtain acetates directly from either the A or B series quaternary aldoximes nor have we been able to obtain the Beckmann rearrangement from quaternary ketoximes, probably because the quaternary compounds are not soluble in the usual media for the Beckmann rearrangement. Our attempts to establish configuration of the quaternary oximes have thus been unsuccessful. Treatment of both isomers of pyridine-2-aldoxime methiodide with cold acetic anhydride leaves the oximes unaltered—warm acetic anhydride yields the nitrile in both cases. It could thus be argued with some logic that the B series has the *anti* configuration because it is much more probable that A converts to B and the latter then forms the nitrile since A to B, as already described, is the normal direction of conversion. Obviously, an assignment on this basis leaves much to be desired and further attempts will therefore be made.

In this work qualitative oxime and acetate tests were used to confirm the functional groups. In addition potentiometric titration of the oximes as acids was applied to determine the neutral equivalents and the approximate acid dissociation constants. The dissociation constants given are the *p*H values of a half neutralized solution to a concentration of somewhat less than 0.01 *M* at 25° as measured by a Beckman model G glass electrode *p*H meter. The acetates were first hydrolyzed with excess standardized alkali and then back titrated. Conversion to the oximes was quantitative. The calculated saponification equivalent is taken as the molecular weight; the first break occurs at neutralization of excess alkali, further addition of acid titrates the oxime anion and still further addition acetate ion so that a complete picture is obtained.

The higher acidities of the B series members would suggest that they have *anti* configurations because this configuration brings the hydroxyl group closer to the positive center and to the ring. However, there are complications arising from questions of resonance and possible steric inhibition of resonance.

The acidity order of the 2-, 3-, and 4-aldoximes and ketoximes of the quaternary B series appears to reflect the importance of resonance, since it would be expected that if resonance occurs, those forms which neutralize the positive charge of the ring nitrogen would be more important than those which do not. In the absence of resonance we should expect the acidity to decline with the distance of the functional group from the positive ring nitrogen, *i.e.*, in the order 2, 3, 4. Actually the 3 derivatives (for which resonance forms having a neutral ring nitrogen cannot be written) are the weakest acids.

The A and B series quaternary oximes can be compared with the *syn* and *anti* series of benzene and pyrrole derivatives to which there is a close parallel in behavior. Our A series in its lower melting point and greater solubility corresponds to the *syn* series of benzene and pyrrole oximes.[6] In its lesser acidity it again resembles the *syn* benzene series[14] but for the pyrrole series no acidity data are available. It would thus appear by analogy that our A series has the *syn* and our B series the *anti* configurations.

Experimental

Qualitative oxime[15] and acyl[16] tests were used wherever pertinent to identify the compounds. The described oximes were prepared from commercially available aldehydes and ketones, unless specified in the text or tables.

General Procedures. Method I.—Tertiary pyridine oximes were prepared in the usual manner by heating a neutralized aqueous solution of $NH_2OH \cdot HCl$ with the aldehyde or ketone on a steam-bath for 10–20 minutes. On cooling the oxime crystallized. Purification was achieved by recrystallization from water or water–alcohol.

Method II.—Tertiary O-acetyloximes were prepared by gently heating the oxime with an excess of acetic anhydride. The clear solution was kept 20 minutes at room temperature, poured into ice-water, neutralized with Na_2CO_3 and extracted with ether. The ether residue was recrystallized from benzene–hexane. The reactions of these acetyloximes with amines and NaOH were the basis for assigning their configurations. Those tertiary acetates which were tested gave the starting material with pyridine at room

(11) V. Boekelheide and W. J. Linn, THIS JOURNAL, **76**, 1286 (1954).

(12) A. Stempel and E. C. Buzzi, *ibid.*, **71**, 2969 (1949).

(13) S. I. Kanewskaya, *Arch. Pharm.*, **271**, 462 (1933).

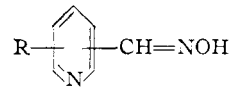
(14) N. K. Patwardhan and S. S. Deshpande, *J. Ind. Chem. Soc.*, **21**, 135 (1944); *C. A.*, **39**, 1390 (1945).

(15) F. Feigl, "Spot-Tests," Vol. II, Elsevier Press, New York, N.Y., 1954, p. 161.

(16) Ref. 15, p. 171.

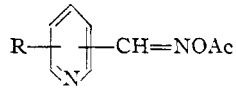
TABLE I

TERTIARY PYRIDINE ALDOXIMES



Substituents —CH= NOH	R	Conf.	Prep. method	Yield, %	Cryst. color	M.p.,[h] °C.	*p*Ka	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Ref.	Footnotes
2	.....	*syn*	I	90	White	114	10.4	..	...	..	..	..	..	3, 11	*a*
2	6-CH_3	*syn*	I	92	White	170–171	10.0	61.8	61.9	5.8	5.8	20.6	20.6	...	*b*
2	3-CH_3	..	I	70	White	152–154	10.5	..	...	..	..	..	..	11	*c*
2	3-OH	..	I	60	White	173–175	8.1(OH)	52.2	52.3	4.34	4.26	20.3	20.1	11, 12	*c*
2	3-OCH_3	..	I	40	White	198–199	..	..	...	..	..	..	..	11–13	*c, d*
3	.....	*syn*	I	88	White	150–151	10.2	59.0	59.7	4.9	4.95	22.9	22.8	4, 17	*e*
4	.....	*syn*	I	97	White	132	10.2	59.0	59.75	4.9	4.8	22.9	22.8	11	*a*
4	5,6-C_6H_4	..	I	96	White	180–181	..	..	..	..	..	..	..	...	*f*

TERTIARY PYRIDINE O-ACETYLALDOXIMES



Substituents —CH= NOAc	R	Conf.	Prep. Method	Yield, %	Cryst. color	M.p. °C.	Sapon. equiv. Calcd.	Sapon. equiv. Found	Footnotes
2	...	*syn*	II	55	White	51–53	164	167	...
2	6-CH_3	*syn*	II	..	White	58–60	..	..	...
3	...	*syn*	II	40	White	Room temp.	164	169	*g*
4	...	*syn*	II	70	White	101–102	164	157	*g*

[a] Pyridine 2- and 4-aldehydes were prepared according to the method[11] starting with picoline N-oxides. Subsequently these aldehydes became commercially available. [b] Attempts to prepare the quaternary oxime failed. Treating the tertiary oxime with methyl iodide gave apparently the hydroiodide of the O-methylated oxime, as judged by the negative oxime test; yellow powder, m.p. 164°. *Anal.* Calcd. C, 34.5; H, 3.95; N, 10.1; I, 45.7. Found: C, 34.6; H, 3.5; N, 9.9; I, 45.8. [c] Preparation of aldehyde described under Special Compounds. [d] Yield from crude aldehyde. [e] Aldehyde prepared according to the procedure.[17] Meanwhile it became commercially available. [f] Quinoline-4-aldoxime. Oxime formation in methanol. [g] Very difficult to purify. However, quaternary salts prepared from the tertiary acetyl compounds gave pure substances. [h] All m.p. are uncorrected.

temperature but with diethylamine and warm 2 *N* NaOH produced the oxime in high yields. The oximes subsequently have the *syn* configuration.

Method III. Preparation of Quaternary Oximes (B Form) (from Quaternary Aldehydes and Hydroxylamine).—Quaternary aldehydes and ketones were prepared by treating the pyridine aldehydes and ketones dissolved in nitrobenzene with an excess of CH_3I at room temperature for several days. The quaternary salts which precipitated were filtered and washed with acetone.

These aldehydes and ketones were treated with a 50% excess of aqueous $NH_2OH \cdot HCl$ neutralized with NaOH to *p*H 6 to 7. After heating for 15 min. on a steam-bath, the water was evaporated under reduced pressure and the almost dry residue extracted with hot absolute ethanol to eliminate mineral salts. On cooling, the oxime crystallized. Recrystallization was from methanol or ethanol.

Method IV. Preparation of Quaternary Oximes (B Form) (from Tertiary Oximes and Methyl Iodide).—The tertiary oximes dissolved in nitrobenzene were refluxed for 3 hr. with an excess of methyl iodide, the precipitated salt washed extensively with acetone and dried at 75° to expel the last traces of nitrobenzene. Excellent yields of practically pure compounds were obtained.

Method V. Preparation of Quaternary Oximes (A Form) (from Quaternary Aldehydes and Hydroxylamine below −5°).—A methanolic solution of hydroxylamine was prepared by dissolving $NH_2OH \cdot HCl$ (30% excess) in a minimum amount of warm methanol and neutralized with methanolic KOH. After cooling the KCl was filtered off, the solution was cooled to −5° with an ice–NaCl mixture and the pyridine aldehyde (or ketone) methiodide was added in small portions under vigorous stirring and cooling. The orange-yellow salt gradually dissolved and white precipitate appeared. The mixture was kept at −10 to −12° for 20 minutes, then filtered on a well cooled funnel and washed quickly with cold absolute ether. Where the compound was too soluble in cold methanol, cold absolute ether was added to precipitate the product. The product can be kept intact in the deep freezer.

These oximes are unstable and even at room temperature slowly converted to the B isomers. The isomerization is quite rapid in alkaline aqueous solution.

Method VI. Preparation of Heat Sensitive Quaternary Compounds.—The 3- and 4-oxime methiodides (particularly the aldoximes) and their derivatives are sensitive to heat. On the other hand they are more easily formed than the 2-derivatives. They were prepared by dissolving the tertiary material (oximes or O-acetyloximes) in acetone at room temperature with an excess of methyl iodide; a practically pure quaternary salt was obtained after 1–3 hr.

Special Compounds

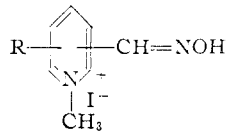
A. 3-Methylpyridine-2-aldehyde, 2,3-Lutidine-N-oxide and 3-Methylpyridine-2-methanol Acetate.—To 2,3-lutidine (21.4 g., 0.2 mole) in glacial acetic acid (80 ml.) was added 12 ml. of H_2O_2 (30%) and the solution heated at 80–90° for 3 hr. Another portion of H_2O_2 (6 ml.) was added and the solution was again heated for 3 hr. The solvents were distilled under reduced pressure (water pump vacuum) until the temperature inside the flask reached 140°. The residue solidified on standing in the refrigerator. Except for a small portion which was taken off for analysis, the crude 2,3-lutidine-N-oxide was dissolved in 50 ml. of acetic anhydride and heated under reflux. At 90–100° a quite exothermic reaction took place. After the reaction subsided the mixture was refluxed for 2 hr. longer, after which the solvents were removed under reduced pressure and the residue fractionally distilled; b.p. 118–124° (12–14 mm.); yellowish oil; yield 23 g. (70% of theory).

The crude 2,3-lutidine-N-oxide was recrystallized from benzene–ether; white crystals, m.p. 85–93°.

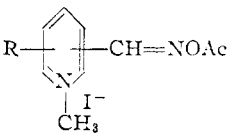
3-Methylpyridine-2-methanol Acetate N-Oxide and 3-Methylpyridine-2-aldehyde.—3-Methylpyridine-2-methanol acetate (16.5 g., 0.1 mole) dissolved in glacial acetic acid (70 ml.) and treated, as above, with H_2O_2 (16 ml.) in two portions. The solvents are removed *in vacuo*

(17) L. Panizzon, *Helv. Chim. Acta*, **24**, 24E (1941).

TABLE II

QUATERNARY PYRIDINE ALDOXIMES (IODIDES) 

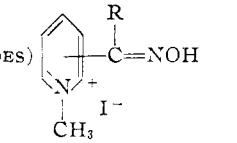
Substituents —CH=NOH	R	Series or conf.	Prep. method	Yield, %	Cryst. color	M.p.,[h] °C.	pK_a	Analyses Iodine, % Calcd.	Iodine, % Found	Neut. equiv. Calcd.	Neut. equiv. Found	Ref.	Footnotes
2	.....	A	V	80	Wh.	105–106	9.9	48.1	47.9	264	261	..	*a*
2	.....	B	III, IV	62	Yel.	224–225	8.0	48.1	48.5	264	261	..	*a*
				88				(Cl %)					
2	(Chloride)	B	..	87	L. yel.	226–227	8.0	20.58	20.52	..	..	..	*b*
2	3-CH_3	B	IV	72	L. yel.	201	8.4	45.7	45.0	278	284	..	..
2	3-OH	B	IV	23	Yel.	214	4.8(OH)	...	...	280	279	..	..
2	3-OCH_3	B	IV	82	Yel.	178–179	8.6	43.2	42.9	294	296	..	..
3	.....	A	V	..	....	Oil	...	...	...	..	..	17	*c*
3	.....	B	VI	91	L. yel.	154–155	9.2	48.1	47.7	264	261	..	..
4	.....	A	V	50	L. yel.	65–70	9.3	48.1	47.7	..	..	..	*d*
4	.....	B	VI	99	Yel.	181–183	8.6	48.1	47.9	264	262	..	..
4	5,6-C_6H_4	B	VI	46	Yel.	249–250	8.3	40.4	40.2	314	315	..	*e*

QUATERNARY PYRIDINE O-ACETYLALDOXIME 

Substituents —CH=NOAc	Conf.	Prep. method	Yield, %	Cryst. color	M.p., °C.	Analyses Iodine, % Calcd.	Iodine, % Found	Sapon. equiv. Calcd.	Sapon. equiv. Found	Footnotes
3	*syn*	VI	95	Yel.	159–161	41.5	41.6	306	302	*f*
4	*syn*	VI	97	Yel.	156	41.5	41.1	306	306	*f*

[a] Pyridine-2-aldehyde methiodide; yellow crystals, m.p. 174° (Lenart, ref. 3, described it as a liquid, his product presumably was impure.) Yield 56% of theory. [b] Pyridine-2-aldoxime methochloride prepared by shaking an aqueous solution of the iodide with AgCl. [c] Pyridine-3-aldehyde methiodide, m.p. 173° in acetone at room temperature. [d] Less stable than the 2-isomer. Pyridine-4-aldehyde methiodide, a low melting impure solid. [e] Quinoline-4-aldoxime methiodide. [f] Aqueous alkali quantitatively converted the acetate to the oxime. The oxime was not separated but identified by its potentiometric titration curve. On this basis the *syn* configuration is assigned to the acetate. [h] All m.p. are uncorrected.

TABLE III

QUATERNARY PYRIDINE KETOXIMES (IODIDES) 

Substituents —CH=NOH	R	Series or conf.	Prep. method	Yield, %	Cryst. color	M.p., °C.	pK_a	Analyses Iodine, % Calcd.	Iodine, % Found	Neut. equiv. Calcd.	Neut. equiv. Found	Ref.	Footnotes
2	CH_3	A	V	43	L. yel.	101–103	11	45.7	45.0	..	..	18	*a*
2	CH_3	B	IV	46	Wh.	185–196	9.0	45.7	45.7	278	279	18	*b*
2	C_6H_5	B	IV	57	Yel.	195	8.7	37.4	37.4	340	335	19	*b, c*
3	CH_3	A	V	86	L. yel.	103–106	11	45.7	46.0	..	..	..	*c, d*
3	CH_3	B	III	45	L. yel.	213–214	10.2	45.7	45.9	..	..	20	*c, d, e*
4	CH_3	A	V	40	Green yel.	104–109	11	45.7	45.4	..	..	21	*c, f, g*
4	CH_3	B	VI	50	Yel.	191–193	9.5	45.7	45.7	278	277	21, 22	*b, c, f*
4	C_6H_5	B	VI	63	L. yel.	215–217	9.3	37.4	37.1	340	340	19	*b, c*

[a] Methyl-2-pyridyl ketone methiodide in nitrobenzene at room temperature, dark yellow crystals, m.p. 159–160°. [b] Tertiary pyridine ketoximes were described in the literature. Prepared according to Method I or in aqueous alcohol solution if necessary, because of poor solubility of starting material. [c] Over-all yields calculated on ketone. [d] Methyl-3-pyridyl ketone methiodide, in acetone at room temperature, faint yellow crystals, m.p. 163–164°. [e] Could also be prepared from tertiary ketoxime according to Method IV. [f] Methyl-4-pyridyl ketone prepared from methyl isonicotinate by Claisen condensation. [g] Methyl 4-pyridyl ketone methiodide, in acetone at room temperature, red-brown crystals, m.p. 177–178°.

and the crude orange colored oil was heated with acetic anhydride (50 ml.) on steam-bath for 3 hr. and under reflux for 30 minutes longer. Solvents were removed under reduced pressure and the residue directly transformed into the aldehyde by heating on steam-bath with 70 ml. of 6 *N* HCl for 1 hr. After distilling off the major part of the acid, the residue was dissolved in water, neutralized with NaOH and extracted exhaustively with ether, care being taken to extract the gums which contain a large amount of the poorly water-soluble aldehyde. After evaporation of the ether, the aldehyde was distilled under reduced pressure; b.p. 83–84° (12–13 mm.); yield 2.8 g., (23% of theory); colorless oil, strong aldehyde smell.

(18) C. Engler and R. Rasumow, *Ber.*, **24**, 2528 (1891); G. R. Clemo, T. Holmes and G. C. Leitsch, *J. Chem. Soc.*, 754 (1910).

(19) A. Tschitschibabin, *Chem. Zentr.*, **73**, **I**, 206 (1902).

(20) C. Engler and W. Kiby, *Ber.*, **22**, 598 (1889); F. B. La Forge, THIS JOURNAL, **50**, 2480 (1928).

(21) A. Pinner, *Ber.*, **34**, 4250 (1901); B. Emmert and A. Wolpert, *ibid.*, **74 B**, 1018 (1941).

(22) R. C. Elderfield, ref. 10, p. 590.

B. 3-Hydroxypyridine-2-aldehyde.—Diacetate of 3-hydroxypyridine-2-methanol (42 g., 0.2 mole), prepared according to Stempel and Buzzi,[12] was dissolved in glacial acetic acid (140 ml.) and treated with H_2O_2 (32 ml.) in the same way as in the above procedure. The crude N-oxide was treated with acetic anhydride (140 ml.) on steam-bath for 6 hr. and the solvents eliminated under reduced pressure. Attempts to distil the triacetate under a vacuum of 2 mm. failed as the product decomposed. Another part of the crude triacetate was treated with 6 *N* HCl on steam-bath and a crude oil with strong aldehyde smell was obtained; yield (crude) 3 g., (12% of theory) (better yields could certainly be worked out). A small amount was distilled; b.p. 72–74° (12–14 mm.).

C. 3-Methoxypyridine-2-aldehyde.—3-Hydroxypyridine-2-aldehyde (crude) (2 g.), dissolved in 2 *N* KOH (8 ml.), was added to 10 ml. of methanol and 3.2 g. of methyl *p*-toluenesulfonate and refluxed on steam-bath for 1 hr. Very soon precipitation of sodium *p*-toluenesulfonate starts. The reaction mixture was poured in 100 ml. of distilled water and extracted with $CHCl_3$. The residue, dark oil, weighing about 0.5 g. (22%) was not purified but transformed directly into the oxime.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Equilibria between Pyridoxal and Amino Acids and their Imines[1]

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Spectrophotometry of aqueous solutions of pyridoxal with amino acids shows that extensive imine formation occurs over a wide *p*H range. The imines formed in the neutral *p*H range are yellow, absorb light maximally at about 414 mμ and are weak acids of *pK* about 10.5. The conjugate base forms which exist at high *p*H values have an absorption band at about 365 mμ. The *p*H dependence of the apparent equilibrium constants for the formation of the imines with valine and with glycine is analyzed quantitatively. Hydrogen bonding apparently increases the stability of the imines in the neutral *p*H range. Glycine is shown to yield a small amount of aminoacetal or carbinolamine as well as the imine. Apparent equilibrium constants for imine formation with 22 amines and amino acids are compared. The presence of a β-methyl group in the side chain of the amino acid increases the stability of the imine, whereas α-substitution decreases the stability. These and other factors affecting the stability and the ionization constants of the imines are discussed.

Imines (Schiff bases) have frequently been suggested as intermediates in the reactions of pyridoxal (vitamin B_6 aldehyde) with amino acids, both in the presence and absence of enzymes.[2,3] A number of such imines have been prepared,[4–6] but little information is available on their stability in aqueous media. The present study provides a quantitative description of the extent of formation of such imines in aqueous buffered solutions.

Valine–Pyridoxal Imine.—Valine was selected for a detailed study because of the favorable equilibrium constants. When this amino acid is mixed with pyridoxal in dilute aqueous solution, a marked change in the ultraviolet absorption spectrum occurs. This change is clearly displayed (Fig. 1) at *p*H 7.4, where pyridoxal has a very low absorption in the 400 mμ region. In the presence of valine, strong absorption bands appear with maxima at about 280 and 414 mμ. The latter band extends into the blue end of the visible spectrum and the solutions are consequently intensely yellow. These bands increase in intensity as the valine concentration increases, while the 317 mμ absorption band of the internal hemiacetal form of pyridoxal (Ia) decreases correspondingly. Equilibrium with respect to this change is achieved within 10 minutes or less. When the absorption spectra at various valine concentrations (and constant *p*H) are compared, sharp isosbestic points are observed at 256, 296 and 338 mμ (Fig. 1). The presence of these points of constant absorption indicates that the reaction can be treated in terms of a single equilibrium between pyridoxal and the product of its reaction with valine.

The position of the absorption maximum at 414 mμ indicates that the product is almost certainly the imine, IIa-IId (R = isopropyl). Pyridine derivatives which lack a double bond in conjugation with the aromatic ring absorb at wave lengths below 330 mμ,[7] whereas the free aldehyde form of pyridoxal (Ib) and pyridoxal phosphate which contain an additional double bond have an absorption peak at about 390 mμ.[7] Comparison with the spectra of salicylaldimines[8–10] further confirms the identity of the interaction product.

Conversion to the imine is incomplete at *p*H 7.4, even in near-saturated 0.6 *M* valine solutions, as shown by the small amount of residual pyridoxal absorption (Fig. 1) and by the equilibrium constant calculated later. However, between *p*H 8.3 and 12, the conversion is estimated to be over 95% complete in 0.5–0.6 *M* valine. The spectra of these solutions show little evidence of any unreacted pyridoxal (Fig. 2) and can be taken as approximating those of the pyridoxal–valine imine.

The spectrum of the pyridoxal–valine imine undergoes a marked change as the *p*H is raised (Fig. 2). The 414 mμ peak is shifted to 367 mμ, the rest of the spectrum shifting correspondingly. The high *p*H spectrum is still unmistakably differ-

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