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Studies on Schiff Bases in Connection with the Mechanism of Transamination

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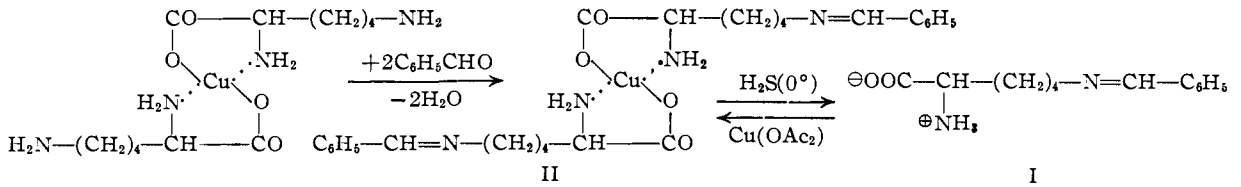
As evidenced by chemical and spectrophotometric observations dibasic amino acids such as lysine and ornithine condense with aromatic aldehydes (1 mole) at the *terminal* amino group reminiscent of the behavior of these amino acids in enzymatic transamination reactions. The influence of phenolic *ortho* and *para* hydroxy groups on the ring-chain tautomerism of arylidene-*o*-aminobenzyl alcohols in stabilizing the open Schiff base tautomer is demonstrated spectrophotometrically. A similar effect is operative in stabilizing (aromatic) Schiff bases derived from pyridoxal and pyridoxamine of which three isomeric pairs have been prepared and subjected to a base-catalyzed isomerization reaction. In the equilibration mixture starting with pyridoxylidene compounds there was found in all cases studied a greater amount of the azomethine in which the >C=N— double bond has moved out of conjugation with the pyridine system. Pyridoxylidene-*p*-hydroxybenzylamine (XV) has been obtained in two, possibly *cis–trans*, isomeric forms. Pyridoxylidene-*o*-hydroxybenzylamine (XIII) on warming in alkaline solution yielded salicylal-*o*-hydroxybenzylamine, a reaction involving isomerization (XIII → XIV) transamination as well as "*trans*-Schiff-ization."

Schiff bases play an important part in the transamination equilibrium—enzymatic or non-enzymatic—of keto and amino acids.[1] In this connection we investigated the behavior of certain Schiff bases in order to find the answers to the following three questions: (A) What is the reactive amino group forming azomethines with carbonyl compounds in dibasic amino acids? (B) Are model Schiff bases with neighboring phenolic and alcoholic hydroxyl groups such as in pyridoxal capable of undergoing internal ring closure reactions? (C) Can isomeric pyridoxylidene Schiff bases be prepared, differing only in the position of the >C=N— double bond, and do they undergo a base-catalyzed equilibration reaction?



A.—The conversion of lysine into α-aminoadipic acid,[2] as Meister[3] suggests, is reminiscent of the reaction sequence observed in the transamination of ornithine, starting with exchange of the *terminal* amino group, formation of the ω-semialdehyde and subsequent oxidation of the aldehyde to a carboxyl group.[4a]

The chemical proof for the preponderant reactivity of the terminal amino group with one mole of carbonyl reagent started with benzylidene-L-lysine[4] of unassigned structure. This Schiff base was converted to the characteristic and rather insoluble cupric N^{ϵ}-benzylidene-L-lysinate (II, m.p. 238–243°) which proved to be identical with the compound prepared by the condensation of benzaldehyde with the copper salt of lysine. Additional proof was provided by the decomposition of II[5] with hydrogen sulfide in the cold (the reaction at elevated temperature led to extensive destruction of the molecule) yielding the same benzylidene-L-lysine (I) as was obtained by the direct interaction of lysine with benzaldehyde. Since the reactivity of the α-amino group, subdued already in the free zwitterionic lysine, is suppressed in the copper complex,[6,7] the position of the benzal group in I and II is very probably at the terminal amino group.

It becomes now understandable that lysine fails to participate in transamination reactions[1,8] or shows an atypical behavior.[1,9,10] Our attempts to effect condensation of ω-carbobenzoxylysine with benz- or salicylaldehyde under a variety of conditions have not yet been successful. The behavior of lysine methyl ester toward aldehydes is described in the experimental section. So far only the stable copper salts of $N^{\alpha\epsilon}$-disalicylidene derivatives of free lysine and its ethyl ester have been prepared.[11]

Table I shows that the infrared absorption characteristics of the Schiff bases of lysine and ornithine are in better agreement with the significant bands of the zwitterions of α-amino rather than ε-aminocaproic acid. There is practically no difference between the infrared spectra of the Schiff bases of racemic and natural ornithine and, thus, no tangible indication for the existence of an azomethine *cis–trans* isomerism or equilibrium, possibly influenced by steric factors at the α-carbon of the amino

(1) For a discussion of the problem as well as an extensive bibliography, *cf.*: E. E. Snell, *Physiol. Revs.*, **33**, 516 (1953); R. M. Herbst, *Advances in Enzymology*, **4**, 75 (1944). See also (added in proof): D. E. Metzler, M. Ikawa and E. E. Snell, THIS JOURNAL, **76**, 648 (1954); A. E. Braunshtein and M. M. Shemiakin, *Biokhimiya*, **18**, (4), 393 (1953).

(2) H. Borsook, C. L. Deasy, A. J. Haagen-Smit, C. Keighley and P. H. Lowy, *J. Biol. Chem.*, **176**, 1383, 1395 (1948); **179**, 689 (1949).

(3) A. Meister, *ibid.*, **206**, 587 (1954).

(4) M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **152**, 282 (1926).

(4a) A different pathway for the conversion of lysine to α-aminoadipic acid *via* pipecolic acid involving intramolecular transamination of α-keto-ε-aminocaproic acid has been proposed in the meantime by M. Rothstein and L. L. Miller, THIS JOURNAL, **76**, 1459 (1954).

(5) F. Turba, *ibid.*, **283**, 19 (1948).

(6) A. C. Kurtz, *J. Biol. Chem.*, **140**, 705 (1941).

(7) A. Neuberger and F. Sanger, *Biochem. J.*, **37**, 515 (1943).

(8) S. E. Aqvist, *Acta Chem. Scand.*, **5**, 1046 (1951).

(9) D. E. Metzler and E. E. Snell, THIS JOURNAL, **74**, 979 (1952).

(10) A. Meister, *J. Biol. Chem.*, **195**, 813 (1952).

(11) P. Pfeiffer, W. Offermann and H. Werner, *J. prakt. Chem.*, **159**, 313 (1942).

TABLE I

INFRARED AND ULTRAVIOLET CHARACTERISTICS OF SCHIFF BASES OF DIBASIC AMINO ACIDS

All infrared spectra were taken as Nujol mulls, all ultraviolet spectra were measured in abs. methanol.

	M.p., °C.	Infrared absorption R—$C_6H_4C{=}N{-}$[a]	Phenyl	$COO^{\ominus}$	NH_3		Ultraviolet absorption λ_{max}	log ϵ
N^{δ}-Benzylidene-L-ornithine	198–201	6.06[sh]	6.21[sh]	6.31[sh]	6.61[s]		247	4.19[d]
N^{δ}-Benzylidene-D,L-ornithine	187	6.08[sh]	6.19[m]	6.32[s]	6.63[s]		247	4.12[d]
N^{ϵ}-Benzylidene-L-lysine	205–207	6.06[m]	6.23[sh]	6.31[s]	6.60[s]			
Cupric N^{ϵ}-benzylidene-L-lysinate	240–241	6.06[m]	6.18[s]	6.36[m]	...			
N^{δ}-Salicylidene-L-ornithine	202 dec.	6.09[s]	6.23[s]	6.32[s]	6.63[s]	6.67[s]	256[e]	3.94[e]
N^{δ}-Salicylidene-DL-ornithine	199 dec.	6.11[sh]	6.15; 6.23[m]	6.33[s]	6.63[s]	6.67[s]	255	3.95
N^{ϵ}-Salicylidene-L-lysine	205–207	6.09[s]	6.19[s]	6.31[s]	...	6.67[s]	259	3.87[f]
N^{ϵ}-Carbobenzyloxy-L-lysine	230	...	6.20[m]	6.32[m]	6.62[s]			
α-Aminocaproic acid		...	...	6.30[s]	6.58[sb]			
L-Arginine	244	5.95[s]	...	6.36[s]	6.58			
N^{α}-Benzylidene-L-arginine[c]	208	6.04–6.08[s]	...	6.41[s]	...			
ϵ-Aminocaproic acid		...	...	6.38[s]	6.51[sb]			

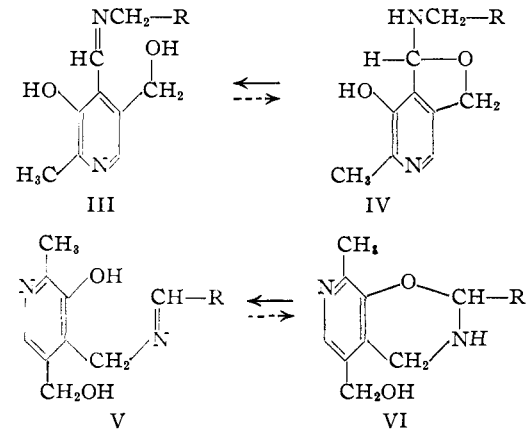
[a] B. Witkop, J. B. Patrick and H. Kissman, *Ber.*, **85**, 949 (1952); E. D. Bergmann, E. Zimkin and S. Pinchas, *Rec. trav. chim.*, 71, 168 (1952); E. D. Bergmann, E. Gil-Av and S. Pinchas, THIS JOURNAL, **75**, 358 (1953). [b] H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., New York, N. Y., 1949, p. 120. Whereas the band designated there Amino I falls together with the azomethine band in the benzylidene derivatives, it is clearly present in the salicylidene compounds

	Amino I		
α-aminocaproic acid	6.03	salicylidene-DL-ornithine	6.03
salicylidene-L-lysine	6.03	ϵ-aminocaproic acid	6.13

See also: J. Despas, J. Khaladji and R. Vergoz, *Bull. soc. chim.*, 1105 (1953). [c] *Cf.* "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 49. [d] Benzylideneaminoethanol has λ_{max} (log ϵ) 247 (4.11); G. E. McCasland and E. C. Horswill, THIS JOURNAL, **73**, 3923 (1951). [e] Salicylideneaminoethanol, a true Schiff base, shows λ_{max} (log ϵ) 259 (3.91), ref. 14, footnote 9. [f] The spectrum of this compound, measured in water (c 1.19 $\times$ 10^{-4} mole/l.) showed a second maximum at 380 mμ (3.54) and a shoulder at 275 (3.73).

acid.[12] The ultraviolet absorption spectra (Table I) show the molar extinction coefficients at 247 mμ typical of Schiff bases containing the structure C_6H_5–CH=N– and rule out the unlikely participation of both the α- and ϵ-amino group, as has been observed with 1,2- and 1,3-diamines in reactions with carbonyl compounds.[13]

B.—The reactive azomethine element in pyridoxal Schiff bases is capable of adding internally anionic groups as evidence by the formation of thiazolidine compounds from cysteine and penicillamine,[14] the condensation reactions with the reactive 4(5)-position of the imidazole nucleus in histidine and histamine, with the α-indole in tryptophan and with the activated 6-position of 3,4-dihydroxyphenylalanine.[15] In the same way the hydroxymethyl group of pyridoxal adjacent to the aldehyde group is capable of adding internally to form a cyclic hemiacetal.[16,17]



Similar interactions of the neighboring hydroxymethyl or phenolic hydroxyl with the reactive >C=N– element of a Schiff base might lead to equilibria between ring-chain tautomers such as III $\rightleftarrows$ IV, and V $\rightleftarrows$ VI.[18] With simple model compounds, such as arylidene-*o*-aminobenzyl alcohols it was found in extension of previous work[19,20] that the Schiff base dihydroöxazine tautomerism was greatly affected by substituents in the ring of the aromatic aldehyde components, confirming similar observations by Bergmann and collaborat-

(12) C. Wiegand and E. Merkel, *Ann.*, **550**, 175 (1942), discard the possibility of an azomethine *cis–trans* isomerism in the simple case of benzalaniline. On the basis of its easy dehydrogenation to 2,3,5,6-tetraphenylpyrazine the *cis*-structure of the >C=N– bonds in N,N'-dibenzylidenestilbenediamine has been suggested [I. J. Krems and P. E. Spoerri, *Chem. Revs.*, **40**, 279 (1947)]. It seems that *cis–trans* isomerism in simple Schiff bases or azines (*e.g.*, benzalazine) has not yet been clearly demonstrated (*cf.* the two isomers of *p*-hydroxybenzylidene pyridoxamine below), whereas highly conjugated systems (*e.g.*, cinnamalazine) are capable of existing in various *cis* and *trans* forms [J. Dale and L. Zechmeister, THIS JOURNAL, **75**, 2379 (1953)].

(13) E. D. Bergmann, E. Meeron, Y. Hirshberg and S. Pinchas, *Rec. trav. chim.*, **71**, 200 (1952). *Cf.* H.-W. Wanzlick and W. Löchel, *Chem. Ber.*, **86**, 1463 (1953).

(14) D. Heyl, S. A. Harris and K. Folkers, THIS JOURNAL, **70**, 3429 (1948).

(15) H. F. Schott and W. G. Clark, *J. Biol. Chem.*, **196**, 449 (1952).

(16) D. Heyl, E. Luz, S. A. Harris and K. Folkers, THIS JOURNAL, **73**, 3430 (1951).

(17) *Cf.* the tautomerism: *o*-hydroxymethylbenzaldehyde $\rightleftarrows$ hydrophthalide, *e.g.*, in dihydrogladiolic acid [L. A. Duncanson, J. F. Grove and J. Zealley, *J. Chem. Soc.*, 3637 (1953)]. The base-catalyzed interchange of aldehyde and hydroxymethyl functions is noteworthy; such an isomer of pyridoxal has been obtained synthetically and found to be inactive [S. A. Harris, D. Heyl and K. Folkers, THIS JOURNAL, **66**, 2088 (1944)].

(18) *Cf.* O,O-Isopropylidene pyridoxine, J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2583 (1952).

(19) F. W. Holly and A. C. Cope, THIS JOURNAL, **66**, 1875 (1944).

(20) B. Witkop, J. B. Patrick and H. M. Kissman, *Ber.*, **85**, 949 (1952).

ors.[21,22] Only the condensation product of unsubstituted benzaldehyde with *o*-aminobenzyl alcohol forms a cyclic internal addition product stable as the free base as well as the hydrochloride. The *p*-methoxy analog forms a colorless cyclic oxazine which passes into the yellow open Schiff base on salt formation. The reaction product between *p*-hydroxybenzaldehyde and *o*-aminobenzyl alcohol could be obtained as colorless crystals. Rubbing the white powder with a spatula produced a bright yellow form which reverted to the white modification in a matter of seconds. On mulling the white form in Nujol it also became yellow and, under pressure between the salt plates, stayed yellow long enough to record the infrared spectrum and to compare it with the spectrum of the white form to which the same sample returned on standing. Both spectra were identical, both showing a band at 6.13 μ(>C=N–),which however has the weakest intensity compared with the other Schiff bases in the same series. It seems, therefore, that the "tribochromoisomerism" is not the result of a change from the colorless cyclic form to the yellow Schiff base. The presence of small amounts of the dihydroöxazine would not be detectable by spectrophotometric studies. The low extinction of the >C=N– band in the infrared must be interpreted with caution, since *p*-hydroxybenzalaniline in which no ring closure can occur even lacks this band.[23] The chromoisomerism is also brought about by warming at 110°; in this case the product stays yellow permanently, but still shows the same infrared spectrum as the transitory yellow or the colorless modification. Prolonged warming markedly changes the infrared spectrum (Table II, footnote *c*); the resulting compound has not yet been obtained crystalline.

Salicylidene-*o*-aminobenzyl alcohol exists as the azomethine both in the free Schiff base as well as in the hydrochloride. The stabilizing influence of *ortho*-phenolic hydroxyl on azomethines has been noticed in a similar connection and been rationalized by the assumption of *o*-quinoid contributions[2] or hydrogen bonding.[24] The spectral shift on salt formation (hydrochloride in ethanol) is hypsochromic and may be due to partial hydrolysis rather than to the formation of the cyclic tautomer: the hydrochloride (mulled in Nujol) still shows a very strong immonium (>C=$\overset{\oplus}{N}$H—) band at 6.06 μ (Table II).

C.—The salient feature of all of the various transamination schemes favored at the present time[1] is the migration of an azomethine unsaturation in the pyridoxylidene chelate complex IX[2,25] to yield an isomeric complex X derived from pyridox-

(21) E. D. Bergmann, E. Zimkin and S. Pinchas, *Rec. trav. chem.*, **71**, 168 (1952).

(22) E. D. Bergmann, *Chem. Revs.*, **53**, 309 (1953).

(23) Reference 20, p. 962, Table 5.

(24) *Cf.* H. Hoyer, *Z. Elektrochem.*, **47**, 451 (1941); A. Kiss and G. Auer, *Z. physik. Chem.*, **A189**, 344 (1941). See also the discussion of the anomalous effects of *o*-hydroxy groups on the ultraviolet absorption of azomethines and azines, L. N. Ferguson and E. K. Branch, THIS JOURNAL, **66**, 1467 (1944). The participation of a phenolic group in a ring chain tautomerism of type V ⇄ VI has also been considered for the similarly constituted *o*-benzalaminophenol on the basis of infrared spectra [Rodebush, Buswell and Roy quoted by H. R. Snyder, R. H. Levin and P. F. Wiley, *ibid.*, **60**, 2025 (1938)].

(25) *Cf.* J. Baddiley, *Nature*, **170**, 710 (1952).

TABLE II

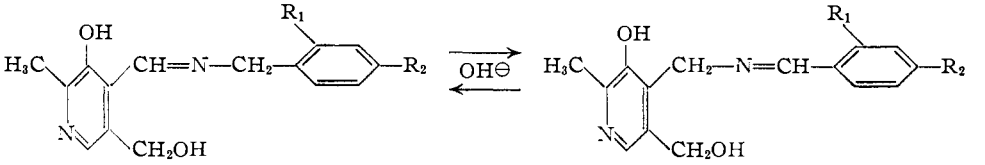
THE INFLUENCE OF SUBSTITUENTS ON THE RING CHAIN TAUTOMERISM OF ARYLIDENE SCHIFF BASES DERIVED FROM *o*-AMINOBENZYL ALCOHOL AS EVIDENCED BY SPECTROPHOTOMETRIC DATA

Compound	Ultraviolet absorption λ_{max} (log ϵ) (in EtOH) at pH 7	Ultraviolet absorption λ_{max} (log ϵ) (in EtOH) at pH 11	Infrared absorption Free base (in $CHCl_3$) OH or NH	Infrared absorption Free base (in $CHCl_3$) >C=N–	Infrared absorption Hydrochloride (in Nujol) OH or NH	Infrared absorption Hydrochloride (in Nujol) >C=N–	Tautomerism Free base present as	Tautomerism Salt present as
1,2-Dihydro-2-phenyl-3,1,4a-benzoxazine	295(3.30)[a] 246(3.95)	295(3.30) 246(3.95) stable	2.91[a] (NH) (CCl_4)	No band	2.96[w] (NH)	No band	Colorless dihydroöxazine	Colorless dihydroöxazine
1,2-Dihydro-2-(*p*-methoxyphenyl)-3,1,4a-benzoxazine	277 (3.25)	Hydrolyzed	280(3.99)	.....	No band	3.05	6.04[s]	Colorless cyclic dihydroöxazine	Yellow Schiff base
p-Hydroxybenzylidene-*o*-aminobenzyl alcohol	300–340 (4.14–3.90) 284(4.17) 224(4.19)	Hydrolyzed: 286(4.18) 240(3.05)	337(4.36) 240(4.01)	No NH	6.13[m] In Nujol		6.04[s]	Colorless Schiff base becoming yellow on pressure or warming[c]	Yellow Schiff base
Salicylidene-*o*-aminobenzyl alcohol	340(4.01) 268(4.09) 228(4.26)	330(3.65) 260(3.80) (hydrochloride in EtOH)	.....	2.75[b] (In $CHCl_3$)	6.15[t]	3.01	6.06[s]	Yellow Schiff base	Yellow Schiff base

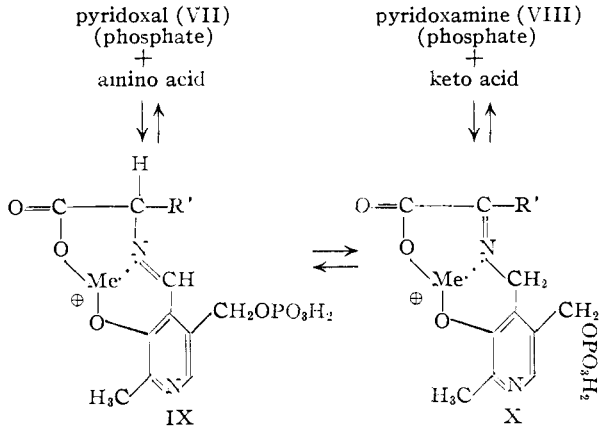
[a] Reported (ref. 19): λ_{max} log ϵ 245 (3.93) and an inflection point at 287.5 (3.38). [b] *o*-Aminobenzyl alcohol shows absorption bands 2.77 (OH); 2.90, 2.96 (NH_2) in chloroform. [c] On heating for several hours at 120° the character of a Schiff base apparently disappears: 6.02–608[w]; 6.23–6.35[s]; 6.62[s] (in Nujol).

TABLE III

PROPERTIES AND EQUILIBRIUM RATIOS OF ISOMERIC SCHIFF BASES DERIVED FROM PYRIDOXAL AND PYRIDOXAMINE



No.	R_1	R_2	M.p., °C.		No.	R_1	R_2	M.p., °C.	Equilibrium ratio on refluxing in 0.1 *N* NaOEt after 1 hr.	2 hr.	3 hr.	6 hr.	7.5 hr.
XI	H	H	112.5	⇄	XII	H	H	135–141	84:16	45:55	39:61	37:63	36:64
XIII	OH	H	169–170	⇄	XIV	OH	H	172–173	55:45	48:52	34:66	44:56	50:50
XVa	H	OH	145–148 (isomer I)	⇄	XVI	H	OH	154–158	43:57	35:65	34:66	24:76	29:71
XVb	H	OH	160–161 (isomer II)										
XVII	H	OCH_3	104–105	⇄	XVIII	H	OCH_3	(Gum)	52:48	48:52	49:51	...	...



amine. Such an isomerization would be difficult to formulate with the hydrated Schiff bases or carbinolamines theoretically capable of existence within the *p*H range (7–9) of (enzymatic) transamination reactions. The phosphate group seems to be dispensable in non-enzymatic, but not in enzymatic transamination reactions: the transaminase from frog heart muscle fails to utilize pyridoxylidene glutamic acid.[26]

We have prepared for the first time three pairs of isomeric Schiff bases (XI–XVI) derived from pyridoxal and pyridoxamine (Table III). The condensation of pyridoxal with benzylamine in absolute methyl alcohol has been reported previously by Folkers,[27] who obtained XI in 35% yield. In aqueous solution we obtained the same compound in 90% yield. The preparation of the isomeric benzalpyridoxamine was difficult and gave yellow Schiff bases containing either 0.5 mole of benzaldehyde or 1.5 moles of water. The introduction of *ortho* or *para* phenolic hydroxyl groups facilitated the preparation and increased the stability of these Schiff bases in accordance with the observations made in the series of phenolic arylidene-*o*-aminobenzyl alcohols. An interesting non-enzymatic transamination reaction was observed in the preparation (yield 60%) of pyridoxal-*o*-hydroxybenzylamine (XIV), the basic mother liquor of which gave a 30% yield of a compound having the composition of salicylal-*o*-hydroxybenzylamine (m.p. 183–184.5°). Apparently isomerization of XIII to XIV and subsequent "*trans*-Schiff-ization"[28] with unchanged *o*-hydroxybenzylamine must have occurred.

The ultraviolet absorption spectra (Table IV) of the isomeric pair of Schiff bases in the salicyl series (XIII and XIV) were measured in various solvents at neutral and basic *p*H in order to find out whether the isomerization XIII ⇄ XIV might be followed spectrophotometrically. However, the differences in the spectra of isomeric pairs are too small and uncharacteristic to make spectrophotometric methods useful. The method outlined below had to be used in the studies on isomerization and equilibrium.

The stabilizing effect of phenolic hydroxyls was also noticed in the *p*-hydroxybenzaldehydebenzylamine series: there were obtained two different isomeric compounds in the condensation of pyridoxal with *p*-hydroxybenzylamine, neither of which was *p*-hydroxybenzylidene pyridoxamine (XVI). Table V, listing the major infrared absorption bands of these two isomers, shows that there are only minor differences in the "finger print region." A ring-chain tautomerism of the type discussed above is, therefore, ruled out. It may be possible that we are dealing here with a pair of geometric *cis–trans* azomethine isomers, though the possibility of polymorphism has not been ruled out.[29]

The triad prototropic systems present in methyleneazomethines are comparatively immobile,[30] but are isomerized by strong nucleophilic agents.[31,32] Ossorio and Hughes[33] found the initial rates of racemization and hydrogen exchange in optically active methyleneazomethines equal and accordingly favored a Lowry type of synchronous bimolecular mechanism of bond formation and bond fission.

The results of base-catalyzed equilibration reactions using 0.1 *N* sodium ethoxide in refluxing alcohol are shown in Table III. After the proper reflux time the equilibrium was "frozen" by cooling

(26) H. Brandenberger and P. P. Cohen, *Helv. Chim. Acta*, **36**, 549 (1953).

(27) D. Heyl, E. Luz, S. A. Harris and K. Folkers, *ibid.*, **70**, 3669 (1948).

(28) *Cf.* R. Kuhn, F. Zilliken and H. Trischmann, *Ber.*, **83**, 304 (1950).

(29) The related salicylideneaniline exists in two modifications which become identical in solution: V. de Gaouck and R. J. W. Le Fevre, *J. Chem. Soc.*, 741 (1938).

(30) C. K. Ingold and H. A. Piggott, *J. Chem. Soc.*, **121**, 2381 (1922).

(31) C. K. Ingold, C. W. Shoppee and J. F. Thorpe, *ibid.*, 1477 (1926).

(32) C. K. Ingold and C. W. Shoppee, *ibid.*, 1199 (1929).

(33) R. P. Ossorio and E. D. Hughes, *ibid.*, 426 (1952).

TABLE IV

ULTRAVIOLET AND INFRARED ABSORPTION CHARACTERISTICS OF THE PAIR OF ISOMERIC SCHIFF BASES, SALICYLIDENE PYRIDOXAMINE (XIV) AND PYRIDOXYLIDENE *o*-HYDROXYBENZYLAMINE (XIII)

Compound	Ultraviolet absorption: In abs. EtOH	In 0.01 *N* NaOH	In 0.01 *N* NaOEt: After 3 min.	In 0.01 *N* NaOEt: After 13 hr.	Infrared (in Nujol): −C=N−	Infrared (in Nujol): Aromatic
Salicylidenepyridoxamine (XIV)	.....	.....	380 (3.80)	380 (3.69)		6.21[m]
	320 (3.85)	.....	315 (3.81)	315 (3.88)	6.07[s]	6.29
	286 (4.07)	.....	.....	.....		6.54
	257 (4.33)	.....	[250 (4,15)]	[232 (4.32)]		
Pyridoxylidene-*o*-hydroxybenzylamine[b] (XIII)		~390 (3.18)	388 (3.79)	385 (3.93)		
	335 (3.61)[a]	330 (2.58)	.....	.....	6.12[s]	6.29[s]
	[278 (3.71)]	267 (3.39)	296 (3.64)	295 (4.08)		
	253 (3.97)	.....	.....	.....		
	217 (4.38)	224 (4.08)	236–239 (4.30)	238 (4.27)		
Pyridoxylidenebenzylamine (XI)	.....	383 (3.88)		380 (3.62)		
	237 (3.63)	.....		300 (3.46)	6.11[s] (in $CHCl_3$)	.....
	253 (4.01)	.....				
	.....	236 (4.23)		237 (4.07)[c]		

[a] The following absorption peaks are observed in water (*c* 2.92 × 10^{-4} mole/l.): 390 (2.78); 310 (3.63); [282–286 (3.38)]; [255–260 (3.48)]; 241 (3.72). On going to half the concentration these peaks move to longer wave lengths and higher extinction. [b] In 0.1 *N* hydrochloric acid λ_{max} (log ϵ) 284 (3.99) is almost identical with λ_{max} of pyridoxal hydrochloride: 287 (3.96), ref. 16; *cf.* E. A. Peterson and H. A. Sober, THIS JOURNAL, **76**, 174 (1954). This ease of hydrolysis of the Schiff base was utilized in determining the ether-soluble aldehyde component in equilibration mixtures (see below). [c] These values were obtained in 0.01 *N* aqueous sodium hydroxide solution.

TABLE V

INFRARED ABSORPTION PEAKS OF ISOMER I (M.P. 145–148°) AND ISOMER II (M.P. 160–161°) OF PYRIDOXYLIDENE-*p*-HYDROXYBENZYLAMINE (XV)

Pyridoxylidene-*p*-hydroxybenzylamine: Isomer I	Isomer II	Isomer I	Isomer II
6.14[m]	6.14[m]	8.54[m]	8.54[m]
6.19[m]	6.19[m]	9.05[w]	9.05[m]
6.26[m]	6.26[m]	9.21[w]	9.23[m]
(6.46)	(6.42)	....	9.48[s]
6.61[s]	6.60[s]	9.84[m]	9.80[s]
6.79[s]	6.83[s]	10.03[w]	....
6.93[s]	...	....	10.16[m]
7.13[s]	7.08[s]	10.34[m]	....
7.26[s]	7.26[s]	....	10.49[w]
7.52[w]	7.51[m]	....	10.68[w]
7.73[m]	7.70[m]	10.82[w]	....
7.88[m]	7.89[m]	....	10.98[w]
7.94[m]	...	11.31[m]	11.21[m]
....	8.00[m]	(11.73)	11.72[m]
8.08[m]	8.07[m]	12.04[w]	12.03[m]
(8.24)	...	12.27[s]	....
....	8.29[m]	....	12.52[w]

and the mixture of Schiff bases was hydrolyzed in the cold by acidifying the solution with hydrochloric acid. From the pure pyridoxylidene Schiff bases (XI, XIII, XV or XVII) ether extraction of the acidified equilibration mixture removed only benzaldehyde, salicylaldehyde, *p*-hydroxy- or *p*-methoxybenzaldehyde. Pyridoxal, neither as the free base nor the hydrochloride, is extractable by ether. The respective aldehydes were converted into the 2,4-dinitrophenylhydrazones, which were then dried and weighed. On prolonged refluxing secondary changes other than mere isomerization occur under these conditions. The maximum yields of dinitrophenylhydrazones were obtained after 3–4 hours, and drops of 10–18% were noticed after 5–8 hours (Fig. 1). Only the initial rates follow approximately first-order kinetics (Fig. 1). The pair of Schiff bases with phenolic *o*-hydroxyl groups (XIII, XIV) was the least stable to the prolonged action of ethanolic ethoxide. The influence of the pyridine ring and the nature and position of a substituent on the approximate rate and ratio of the equilibration is in principle comparable to previous observations by Shoppee.[34]

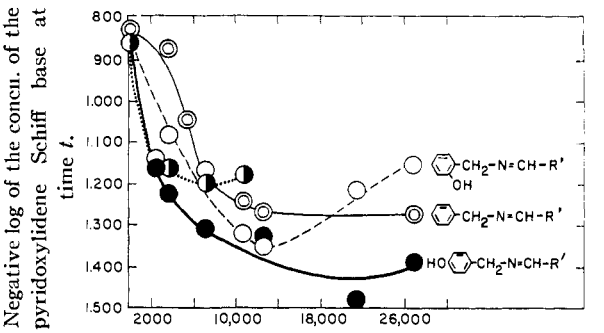


Fig. 1.—Isomerization and equilibration of pyridoxylideneamines to arylidenepyridoxamines on refluxing with 0.1 *N* sodium ethoxide in absolute ethanol. (The dotted line (half solid circles) shows the isomerization starting with pyridoxylidene-*p*-methoxybenzylamine (XVII).)

Ortho and *p*-hydroxylated Schiff bases have not been equilibrated heretofore. Both substituents have an activating effect in this connection, the *p*-hydroxyl group more so than the *o*-hydroxyl group; the activation of the latter is about equal to that of a *p*-methoxy group. The pyridine ring of pyridoxal, neglecting substituents, is comparable to nitrobenzene[34a] and allows an initial anion XIX to exist in forms XX and XXI. The postulation of a separate anion going to a mesomeric anion, recombining with a proton, to form either the one or the other component of the tautomeric systems is part

(34) C. W. Shoppee, *J. Chem. Soc.*, 1225 (1931); 696 (1932); 1847 (1935).

(34a) *Cf.* the reactions of *p*-nitrosalicylaldehyde, the benzene analog of pyridoxal: M. Ikawa and E. E. Snell, THIS JOURNAL, **76**, 653 (1954).

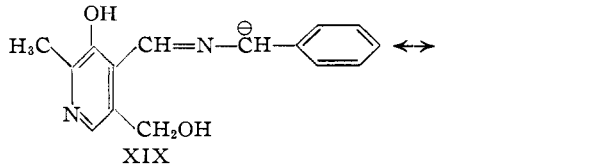
TABLE VI

	M.p., °C.	Formula	Yield, %	Notes	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
N^{δ}-Benzylidene-L-ornithine	198–201	$C_{12}H_{16}N_2O_2$	41	Colorless needles[a]	65.43	64.35	7.32	7.13	12.72	12.42
N^{δ}-Benzylidene-D,L-ornithine	187	$C_{12}H_{16}N_2O_2$	55	Silvery flakes[b]	65.43	65.08	7.32	7.34	12.72	12.76
N^{ϵ}-Salicylidene-L-lysine	205–207	$C_{13}H_{18}N_2O_3$	36	Yellow plates	62.38	61.91	7.25	7.11	11.20	11.19
N^{δ}-Salicylidene-L-ornithine	202 dec.	$C_{12}H_{16}N_2O_3$	56	Yellow plates[c]	61.00	61.10	6.83	6.82	11.86	11.67
N^{δ}-Salicylidene-D,L-ornithine	199 dec.	$C_{12}H_{16}N_2O_3$	71	Yellow plates[c]	61.00	60.98	6.83	6.74	11.86	12.11
N-Salicylidene-*o*-aminobenzyl alcohol	121.5–122	$C_{14}H_{13}NO_2$	93	Long yellow needles[d]	73.99	73.92	5.77	5.63	6.16	6.09
N-Salicylidene-*o*-aminobenzyl alc. hydrochloride	146–148	$C_{14}H_{13}NO_2 \cdot HCl$	90	Yellow powder	63.76	63.80	5.35	5.41	5.31	5.50
N-*p*-Hydroxybenzylidene-*o*-aminobenzyl alcohol	137–139	$C_{14}H_{13}NO_2$	84	Colorless → yellow[e]	73.99	74.22	5.77	5.88	6.16	5.98
N-*p*-Hydroxybenzylidene-*o*-aminobenzyl alc. HCl	>150 dec.	$C_{14}H_{13}NO_2 \cdot HCl \cdot H_2O$	90	Orange feathers[g]	59.78	60.29	5.68	4.97	4.97	5.24
Benzylidenepyridoxamine (XII) (A)	135–145	$C_{15}H_{16}N_2O_2 \cdot {}^1/_2C_6H_5CHO$	43	Yellow powder[h]	71.82	72.07	6.19	6.04	9.05	8.79
Benzylidenepyridoxamine (XII) (B)	183–210	$C_{15}H_{16}N_2O_2 \cdot {}^1/_2H_2O$	48	Yellow powder[i]	63.60	63.48	6.72	5.97	9.90	9.51
N-Salicylidenepyridoxamine (XIV)	172–173	$C_{15}H_{16}N_2O_3$	52,[k] 91[l]	Yellow needles	66.16	66.23	5.92	6.16	10.29	10.39
N-Pyridoxylidene-*o*-hydroxybenzylamine (XIII)	169–170	$C_{15}H_{16}N_2O_3$	68	Bright orange-yellow cryst. powd.	66.16	66.35	5.92	5.68	10.29	10.06
p-Hydroxybenzylidenepyridoxamine (XVI)	154–158	$C_{15}H_{16}N_2O_3$	65	Microcrystalline yellow powder	66.16	65.97	5.92	6.01	10.29	10.44
Pyridoxylidene-*p*-hydroxybenzylamine, isomer I	145–148	$C_{15}H_{16}N_2O_3$	62	Bright yellow needles[m]	66.16	66.09	5.92	5.84	10.29	10.26
Pyridoxylidene-*p*-hydroxybenzylamine, isomer II	160–161	$C_{15}H_{16}N_2O_3$	66	Rhombic yellow plates[n]	66.16	66.60	5.92	6.00	10.29	9.65
Pyridoxylidene-*p*-methoxybenzylamine (XVII)[o]	104–105	$C_{16}H_{18}N_2O_3$	98	Pale-yellow needles[p]	67.11	67.20	6.34	6.22	9.79	9.87

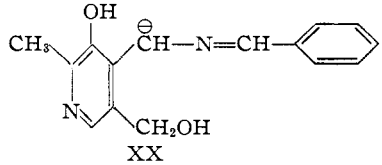
[a] The product was heat labile and could not be recrystallized satisfactorily. [b] Recrystallization from water was accompanied by decomposition. [c] Insoluble in ethanol, recrystallizable from hot water; soluble in alkali. [d] The compound was prepared by refluxing the components in benzene and recrystallized from benzene. [e] A solution of 500 mg. of *p*-hydroxybenzaldehyde and 502 mg. (one equivalent) of *o*-aminobenzyl alcohol in 20 ml. of dry benzene was boiled gently until 20 ml. of benzene had distilled off and the benzene was replaced as it distilled. The yellow solution was then filtered and allowed to cool. A precipitate formed which was pink and amounted to 756 mg. (81.5%), melting at 139–140°. It began to yellow above 100°, becoming quite yellow by 125°. On dissolving the compound in benzene with constant swirling and gentle heating, followed by slow cooling, a pure white form was obtained, melting at 137–139° to a bright yellow melt. Yellowing commenced below 120°. The white powder, upon being rubbed with a silver spatula, became quite yellow, but reverted on standing 10 or 20 seconds to the original white. When attempts were made to dissolve the substance quickly in hot benzene, an orange gum formed which was insoluble in benzene. Moderately fast heating produced yellow solutions from which many different shades of yellow-white crystals were precipitated. The melting points of these yellow-white crystals were always in the temperature range 137–140°. [f] A solution of pure *p*-hydroxybenzylidene-*o*-aminobenzyl alcohol in dry benzene was filtered into a flask containing 15 ml. of dry benzene saturated with hydrogen chloride. A yellow emulsion resulted which settled as an oil. After standing in the cold for three days a bed of yellow-orange feathers had formed. [g] To a solution of 76 mg. of pyridoxamine dihydrochloride in 1 ml. of water was added 0.5 ml. of benzaldehyde in 0.5 ml. of ethanol, after which two equivalents of potassium hydroxide was added dropwise. A yellow gummy deposit slowly formed, which was rubbed for an hour to bring it to a nearly solid consistency. The mother liquor was decanted and the gum was triturated for 15 minutes with 4 ml. of ether, which was then discarded, after which the mother liquor was returned and rubbing continued. The mass quickly crystallized. The yield was 34 mg. (43%) of a yellow powder melting at 135–141°. The product was soluble in ethanol and insoluble in water but could not be crystallized satisfactorily. [h] To a solution of 303 mg. of pyridoxamine dihydrochloride in 2 ml. of water was added 0.13 ml. of redistilled benzaldehyde and 0.3 ml. of ethanol. Two equivalents of potassium hydroxide in 1.5 ml. of water were added and the solution shaken. A yellow gum settled which crystallized in large part on standing for three days. After some rubbing, the gum was triturated in ether and then taken up in methanol, until only a few crystals remained. Slow addition of water to the stirred solution deposited a floeculent yellow precipitate and some gum. The mixture was rubbed briskly until it seemed to be a homogeneous solid and was then filtered, yielding 155 mg. (48%) of a slight yellow solid, m.p. 183–210°. It could not be recrystallized. [i] Prepared in aqueous methanol. [k] Prepared in aqueous base. [l] A warm solution of 0.852 g. of *p*-hydroxybenzylamine (m.p. 114–115°) in 3 ml. of ethanol plus 4 ml. of water was added to a solution of 1.41 g. of pyridoxal hydrochloride in 5 ml. of water. One equivalent of aqueous potassium hydroxide (1.6 g. in 25 ml. of water—6.06 ml. is one equivalent) was added slowly with stirring and intermittent warming. After about 4 ml. had been added the solution became cloudy yellow, and though it cleared upon being warmed it did so only by separation of an oil layer at the bottom. In subsequent runs it was found advantageous to stop adding potassium hydroxide after about half an equivalent had been added in order to allow crystallization to proceed before adding the remainder of the base. From the warm solution crystals separated rapidly and the precipitated oil solidified to a yellow crystalline cake. The remainder of the base (2 ml.) was added slowly, with stirring. The bright yellow needles were collected and washed with water. The yield was 1.16 g. (62%), m.p. 145–148°. The product was recrystallized readily from hot ethanol upon the addition of 4 to 5 parts of water. It was very soluble in methanol and soluble in ethanol. It was insoluble in hot ethyl acetate and water. It could not be recrystallized from methyl or ethyl alcohol–ethyl acetate mixtures and proved to be hygroscopic. [n] A warm solution of 606 mg. of *p*-hydroxybenzylamine in 2 ml. of alcohol plus 3 ml. of water was mixed with 1.0 g. of pyridoxal hydrochloride in 5 ml. of water. The bright yellow solution gave no precipitate on standing. From a potassium hydroxide solution (633 mg. in 10 ml.), 2.18 ml. (half an equivalent) was slowly added to the warmed reaction mixture. A thin oily film settled from the cloudy solution. This was rubbed with a rod and induced to crystallize, after which another 2.18 ml. of the potassium hydroxide solution was slowly stirred in. After standing at room temperature for a few hours, the bright yellow, hard crystals were filtered off. [o] In the attempted preparation of the isomeric *p*-methoxybenzylidenepyridoxamine (XVIII), only deep yellow solutions but no crystals were obtained

from the reaction of the free components in alcohol. The reaction in aqueous alcohol gave a yellow, cloudy solution which afforded only gums. [p] A solution of 0.70 ml. of *p*-methoxybenzylamine [C. K. Ingold and C. W. Shoppee, *J. Chem. Soc.*, 1202 (1929)] in not more than 5 ml. of alcohol was slowly added dropwise, with vigorous stirring, to a solution of 1.0 g. (one equivalent) of pyridoxal hydrochloride dissolved in 20 ml. of water. Yellow crystals formed very slowly. When the amine was added too rapidly an oil separated which slowly crystallized. When the separation of crystals seemed complete, 4.36 ml. of potassium hydroxide solution (1.581 g. dissolved in 25 ml. H_2O) was added dropwise and with stirring. This produced a yellow crystalline mush. After standing for one hour the precipitate was collected and washed with water. The yield of pale yellow, cottony needles was 1.38 g. (98%), m.p. 104–105°. The substance was readily soluble in cold alcohol and chloroform; it could be recrystallized by the slow addition of water to a stirred alcoholic solution.

of the bimolecular mechanism of Ingold, Shoppee and Thorpe.[31] This older mechanism explains



XIX



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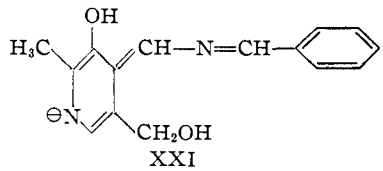


XXI

only in part the influence of various substituents on the equilibration rate and on the position of the equilibrium of azomethines. The fact that all pyridoxylidene compounds of Table III on equilibration form more of the isomeric azomethine is noteworthy in considering transamination mechanisms. The pyridoxylidene compounds, because of additional substituents, cannot be compared directly with *o*- and *p*-nitrobenzylidene Schiff bases, the methyleneazomethine system of which is known to be a non-mobile one.[32]

The influence of chelating metal ions on the equilibration and hydrolysis[26,35] of pyridoxylidene compounds derived from aromatic amines as well as amino acids using homogeneous and heterogeneous (ion-exchange resins) catalysis will be investigated.

Experimental[36]

The Schiff bases derived from amino acids were generally prepared following the procedure of Bergmann[4] with minor modifications. The following preparation is a representative example.

N$^\epsilon$-Benzylidene-L-lysine. A. By the Method of Bergmann and Zervas.[4]—A solution of 3.0 g. of L-lysine in 14 ml. of 2 *N* sodium hydroxide was chilled in ice and mixed with 3.5 ml. of redistilled benzaldehyde. The solution froze to a solid mass in half a minute of rapid shaking. The residue from filtration was washed with 10 ml. and then 20 ml. of water, pressed dry, and rewashed with methanol and ether. The yield was 5.89 g. (91.5%) of white powder which melted with decomposition at 203–206°. A product melting at 205–207° was obtained by solution of the crude product in alkali and subsequent slow addition of dilute hydrochloric acid. Precipitation commenced at about *p*H 9. The product was insoluble in chloroform and alcohol but slightly soluble in water.

An attempted esterification with one equivalent of diazomethane in methanolic solution was unsuccessful. Saturation of a solution of the Schiff base in methanol with dry hydrogen chloride, followed by standing at 25°, gave L-lysine methyl ester dihydrochloride, as did addition of benzylidene-lysine to cold saturated methanolic HCl and subsequent standing at room temperature. In one case, after addition of sodium carbonate and extraction with $CHCl_3$ and ethyl acetate, a crystalline highly hygroscopic mass was obtained that melted at 30 to 55°.

B. By the Decomposition of Cupric N$^\epsilon$-Benzylidene-L-lysine with Hydrogen Sulfide.—A suspension of cupric N$^\epsilon$-benzylidene-L-lysinate[5] in 80 ml. of water and 10 ml. of ethanol was mixed with a stream of H_2S in the cold for an hour and then left overnight in ice. The black sulfide was removed by filtration and the clear filtrate was evaporated to dryness in a vacuum desiccator. The yellow waxy residue was triturated with 10 ml. of ethanol and filtered. The white residue was a mixture of lysine and N$^\epsilon$-benzylidene-L-lysine. The filtrate on concentration afforded first a white precipitate which melted with decomposition at 201–202°, giving also the characteristic burning hair odor of N$^\epsilon$-benzylidene-L-lysine, and on further concentration a white precipitate melting with decomposition from 202–204°. A mixed melting point with authentic N$^\epsilon$-benzylidene-L-lysine, m.p. 205–208° dec., was 203.5–204.5°.

The use of sodium sulfide instead of hydrogen sulfide precipitated copper sulfide, but the free Schiff base was not obtained, and benzaldehyde was detected. An aqueous solution of potassium ferricyanide failed to decompose a suspension of the Schiff base copper complex in water.

Cupric N$^\epsilon$-Benzylidene-L-lysinate. A. From L-Lysine–Copper Complex.[6]—One gram of L-lysine monohydrochloride was dissolved in 150 ml. of water, brought to *p*H 8.8 with sodium hydroxide, and then shaken with 0.397 g. of copper phosphate for 25 minutes. After filtration the filtrate was diluted with an equal volume of methanol, then mixed with 6 ml. of redistilled benzaldehyde and again brought to *p*H 8.5–8.8. A pale blue, flocculent precipitate formed. It was washed with 50% methanol containing a trace of benzaldehyde and finally with methanol alone; yield 1.04 g. (72%). The compound becomes gray at 230° and melts at 240–241° dec. The product was very slightly soluble in hot water and in aqueous alcohol.

B. From N$^\epsilon$-Benzylidene-L-lysine.—To a solution of N$^\epsilon$-benzylidene-L-lysine was added an aqueous solution of cupric acetate monohydrate dropwise with vigorous shaking, until there was no more precipitation. After standing for half an hour the pale blue precipitate was filtered off and washed well with methanol–water; yield 62% of the theoretical. The complex melted, with decomposition, at 238–243°. The infrared spectrum was identical with that of the Schiff base prepared by method A.

L-Lysine Methyl Ester Dihydrochloride.[37]—A mixture of 5.0 g. of L-lysine hydrochloride in 200 ml. of absolute methanol was saturated with dry hydrogen chloride, during which the lysine dissolved. After standing about two hours crystallization commenced. The solution was chilled for three hours and filtered. The mother liquor was slowly diluted with an equal volume of ether, which caused fresh crystallization. After chilling for an hour this crop was filtered off. The combined yield was 6.26 g. (97.5%) of white crystals, m.p. 207–208°, dec. with frothing.

Anal. Calcd. for $C_7H_{18}N_2O_2Cl_2$: C, 36.06; H, 7.78; N, 12.02. Found: C, 36.26; H, 7.75; N, 12.08.

Saturation with dry HCl does not seem to be necessary.

(35) G. L. Eichhorn and J. C. Bailar, Jr., THIS JOURNAL, **75**, 2905 (1953).

(36) All melting points are corrected, all boiling points are uncorrected. The analyses were performed by Dr. William C. Alford and associates, Analytical Service Laboratory, National Institutes of Health.

(37) *Cf.* preparation of D,L-lysine methyl ester dihydrochloride (m.p. 218°), E. Fischer and U. Suzuki, *Ber.*, **38**, 4180 (1905).

The product can be recrystallized from methanol alone as clear, oblong prisms, which steam at about 190° and decompose to a froth sharply at 207–208°.

L-Lysine Methyl Ester.—Extraction from a sodium hydroxide solution of the dihydrochloride with $CHCl_3$ gave the free ester in only 25% yield as yellowish gum and polymer-like material. The best preparation was to mix equivalent amounts of concentrated solutions of the dihydrochloride and sodium bicarbonate and then to take the mixture to dryness *in vacuo*. The bed of clear gum and salt was triturated with dry methanol, filtered and the filtrate taken to dryness. Trituration of this residue with a little 1:2 methanol–chloroform separated more salt, but the filtrate yielded the free ester as a clear gum in approximately 60% yield.

Reaction of the ester dihydrochloride with two equivalents of sodium hydroxide and one equivalent of benzaldehyde in water at room temperature gave a gelatinous solution and eventually a gum which could not be crystallized and was soluble in $CHCl_3$. When duplicated, but using two equivalents of benzaldehyde, a precipitate formed rather quickly, but proved to be N-benzylidene-L-lysine. Experiments in aqueous solution at *p*H 7 and 8 failed. Refluxing the ester dihydrochloride with benzaldehyde in methanol gave back unchanged starting material in 88% yield, as did reaction of the free ester with benzaldehyde in chloroform. Prolonged refluxing with the crude free ester in alcohol, using benzene–water separation, yielded benzylidenelysine, and the mother liquor, with HCl, gave lysine hydrochloride. Reaction in concentrated cold HCl was fruitless, as was reaction in absolute methanolic HCl. However, by refluxing the free ester with benzaldehyde in 2:1 chloroform–methanol a white gum was obtained (by concentration *in vacuo* and ether treatment) the infrared spectrum of which (in chloroform) was identical neither with lysine, nor lysine ester, nor benzylidene lysine. It showed the following bands: no NH, OH between 2.75–3.25; 5.77^s; 5.86^s; 6.06^s; 6.24^m; 6.30^m; 6.88^s; 7.29^s; 7.64^s; 8.61^s; 9.13^w.[38] A concentrated solution of lysine methyl ester dihydrochloride, an equivalent of salicylaldehyde, and two equivalents of potassium hydroxide were mixed, giving a bright yellow solution which deposited a yellow gum, not yet crystallized.

***p*-Hydroxybenzylamine.**—A solution of 6.1 g. of *p*-hydroxybenzaldoxime (m.p. 114–115°)[39] in 85 cc. of 50% alcohol was reduced with freshly prepared 2.5% sodium amalgam in the same manner as was *o*-hydroxybenzaldoxime (see below). However, a vast excess of ammonia was added before crystallization could be induced. The solution was probably about 3 *N* NH_4OH. The yield of colorless crystals was 3.2 g. (58.5%). m.p. 114–115°.[40] A mixed m.p. with the oxime (m.p. 114–115°) was 80°. The product was soluble in hot water but insoluble in cold water or cold dilute hydrochloric acid. It gave a yellow precipitate with pyridoxal and formed a picrate, m.p. 208.5–209.5°. A second product was obtained once, insoluble in methanol, m.p. 236–237° dec.

Salicylamine (*o*-Hydroxybenzylamine).[41]—Two and a half per cent. sodium amalgam was added to a well-agitated solution of 5 g. of salicylaldoxime (m.p. 54–56°)[42] in 45 ml. of water and 45 ml. of ethanol. The solution was kept between *p*H 8 and 9 by the constant addition of concentrated hydrochloric acid and care was taken to keep the temperature below 50°. At the end the solution was made slightly acidic and was concentrated *in vacuo* until salt precipitated. More water was added and the solution reconcentrated. During the concentration process or later during the initial addition of ammonia, a dark, viscous oil separated. This had to be removed and discarded before proceeding further. The solution was then diluted with water to about 90 ml. and 5 ml. of ether was added. Ammonia was added dropwise and with stirring until it was in slight excess. The brown ether layer was separated. The aqueous phase, on prolonged rubbing with a glass rod, deposited crystals. After a few hours the crystalline solid was filtered off; yield 3.04 g. (68%), m.p. 125–130° (reported 126–129°[42]). The product could be recrystallized with appreciable losses by slow cooling of an alcohol solution and the cautious addition of water, or from alcohol–pentane.

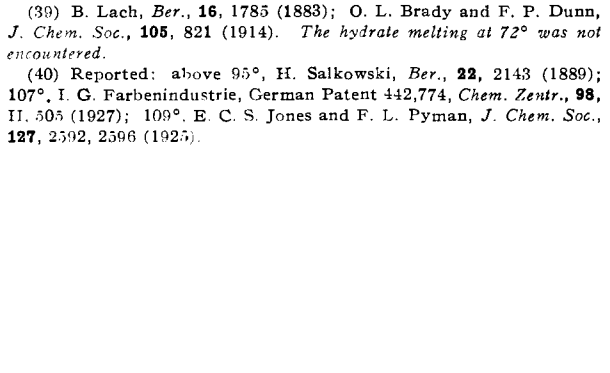
Bis-*o*-hydroxybenzylamine.—A solution of 6.16 g. of *o*-salicylaldoxime (m.p. 54–56°) in about 70 ml. of ethanol was mixed with 700 mg. of PtO_2 and reduced with hydrogen at atmospheric pressure. After 18 hours the uptake had been a little more than two moles of hydrogen so the reduction was discontinued, the platinum was filtered off and the filtrate, strongly smelling of ammonia, was concentrated. The concentrated oil crystallized. Recrystallization from ethanol afforded 3.6 g. (70%) of white needles which melted at 172–173°.[43] The basic material did not form a Schiff base but readily formed a yellow picrate. Reduction in acetic acid at 26 lb. pressure likewise afforded the same product in about the same yield.

Transamination Reaction of Pyridoxal with *o*-Hydroxybenzylamine. Salicylidene-*o*-hydroxybenzylamine.—In one case, after reaction in ethanol and addition of about an equal volume of water, the pyridoxylidene derivative slowly crystallized out in 60% yield. The basic mother liquor was then heated and diluted with a large volume of water. Upon cooling yellow crystals separated (30% yield), melting at 182–184°. This product (485 mg.) was recrystallized from 10 ml. of ethanol as transparent, yellow, elongated hexahedra, melting at 183–184.5°.

Anal. Calcd. for $C_{14}H_{13}NO_2$: C, 74.00; H, 5.73; N, 6.17. Found: C, 74.52; H, 5.69; N, 6.00.

Isomerization and Equilibration of Pyridoxylidene Amines to Arylidene Pyridoxamines.—A 300-mg. sample of the respective pyridoxylidene Schiff base was dissolved under nitrogen in 8 ml. of freshly prepared 0.1 *N* sodium ethoxide. The solution was heated in a nitrogen atmosphere under reflux for the stated time (Table III). At the end of the heating period, 6 ml. of 1 *N* hydrochloric acid was added and the mixture left for 15 minutes. Then 6 ml. of water was added and the solution was extracted with three 15-ml. portions of ether. The combined ether extracts were dried over sodium sulfate and concentrated to 5 ml. or less. To this was added 5 ml. of 2,4-dinitrophenylhydrazine reagent prepared from 1.0 g. of dinitrophenylhydrazine, 0.5 ml. of concentrated sulfuric acid and 20 ml. of methanol. The hydrazone mixture was allowed to stand for several hours at room temperature. After filtration the hydrazone was washed with 2 ml. of methanol followed by 2 ml. of ether. The hydrazone was then dried *in vacuo* over sulfuric acid and potassium hydroxide for a day and weighed. Pyridoxal, present after the hydrolysis, is insoluble in ether. Correction was not made for the solubility of the hydrazones, but

(38) The infrared spectrum rules out C, a compound which would



not be unexpected in these reactions, *cf.* the analogous ring closure of 1-allylamino-2-hydroxy-4,5-epoxido-pentane to 3,5-dihydroxy-N-allylpiperidine: R. Paul, *Angew. Chem.*, **63**, 305 (1951). The scheme to use such a ring-closure reaction for the detection of an azomethine equilibrium A ⇄ B (*cf.* ref. 21) is more likely to succeed with aliphatic residues R'.

(39) B. Lach, *Ber.*, **16**, 1785 (1883); O. L. Brady and F. P. Dunn, *J. Chem. Soc.*, **105**, 821 (1914). *The hydrate melting at 72° was not encountered.*

(40) Reported: above 95°, H. Salkowski, *Ber.*, **22**, 2143 (1889); 107°, I. G. Farbenindustrie, German Patent 442,774, *Chem. Zentr.*, **98**, II, 505 (1927); 109°, E. C. S. Jones and F. L. Pyman, *J. Chem. Soc.*, **127**, 2592, 2596 (1925).

(41) The use of lithium aluminum hydride in the reduction of salicylaldoxime [D. R. Smith, M. Maienthal and J. Tipton, *J. Org. Chem.*, **17**, 294 (1952)] gave unsatisfactory yields (31% of amine).

(42) L. C. Raiford and E. P. Clark, THIS JOURNAL, **45**, 1740 (1923).

(43) Reported m.p. 170°, O. Emmerich, *Ann.*, **241**, 350 (1887); 168°, C. Paal and H. Senninger, *Ber.*, **27**, 1801 (1894); 162°, H. Rupe and A. Metzger, *Helv. Chim. Acta*, **8**, 847 (1925); 168°, G. Zemplén and A. Kunz, *Ber.*, **55**, 983 (1922).

solubility tests indicated that *p*-hydroxybenzaldehyde 2,4-dinitrophenylhydrazone was the most soluble, and this is of the order of only 2–3 mg./ml. MeOH. The percentage isomerized was calculated as

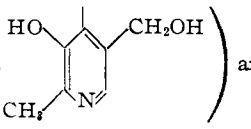
$$\% = \frac{\text{Formula wt. (I)} \times \text{wt. of III in mg.} \times 100}{\text{Formula wt. (III)} \times \text{wt. of sample of I in mg.}}$$

For R—CH_2—N=CH—R′ ⟶ R—CH=N—CH_2—R′ ⟶

I Ia

R—CHO ⟶ R—CH=N—NH—$Ph(NO_2)_2$

II III

The results (R′ always ) are listed in Table III and Fig. 1.

BETHESDA, MARYLAND

[CONTRIBUTION FROM THE NATIONAL INSTITUTES OF HEALTH]

Infrared Diagnosis of the Hydrochlorides of Organic Bases. II.[1] The Structure of Myosmine

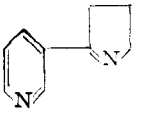
BY BERNHARD WITKOP

RECEIVED FEBRUARY 25, 1954

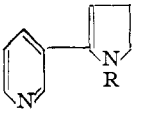
The alkaloid myosmine is particularly suited for the demonstration of *ammonium* and *immonium* bands observed in the infrared spectrum on stepwise salt formation, which makes possible the exact assignment of these bands to the Δ^1-pyrroline and pyridine part of the molecule. The structure of myosmine as the free base or in the form of the two hydrochlorides is that of a Δ^1-pyrroline (I).

It has been shown in the preceding paper that hydrochlorides (or other salts) of tertiary bases, containing the element >C=N— isolated or in an aromatic system, in all simple cases display *immonium* bands in the region 4.5–5.5 μ which can be utilized for the characterization and structural elucidation of dibasic alkaloids.

The application of this method to the location of the unsaturation in the pyrrolidine moiety of the tobacco alkaloid myosmine is an instructive example of the scope and usefulness of the method. Myosmine has hitherto been formulated as a Δ^2-pyrroline (IIb)[2] analogous to IIa, the structure favored for dihydronicotyrine ("N-methylmyosmine") by Wibaut and Beyerman.[3,4] It has now been found

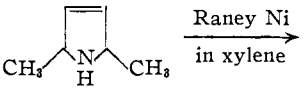


I

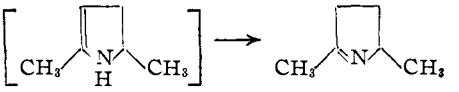


IIa, R = CH_3
IIb, R = H

that myosmine as the free compound in the solid state, in solution,[5] and in the form of its mono- and dihydrochlorides is best expressed by the Δ^1-pyrroline structure (I). As Eddy and Eisner observed independently,[5] there is *no band in the NH region* in the infrared spectrum of myosmine (Fig. 1A). A strong band at 6.15 μ is characteristic of a >C=N— element conjugated with an aromatic system, thus ruling out structure II and the non-conjugated Δ^1-pyrroline. Δ^1-Pyrrolines with an unconjugated azomethine group show a strong band at 6.00 μ, Δ^2-pyrrolines exhibit the narrow and shorter band typical of >C=C< at approximately the same wave length.[6]



III, picrate, m.p. 107°



IV V, picrate 136°

The preferred position of the double bond in pyrrolines may depend largely on the position and nature of substituents. According to the report of the Raney nickel-catalyzed isomerization of a Δ^3- into a Δ^1-pyrroline (III ⟶ V), the Δ^2-tautomer (IV) in this case is very labile and not observed[6]; likewise, there is no evidence for the existence of a Δ^2-tautomer of myosmine. Other substituents apparently facilitate the reverse migration to a Δ^3-pyrroline.[7] There are probably no authentic secondary Δ^2-pyrrolines. In fact the alleged 2-methyl-[6] and 2-phenyl-Δ^2-pyrrolines[8] were shown to be Δ^1-pyrrolines by Zerewitinoff determinations.[9] The infrared diagnosis of such bases and their hydrochlorides should prove useful in further establishing their structure and any possible tautomerism.

The tertiary pyrrolidine nitrogen in nicotine (pK_B 6.15) and the tertiary pyrroline nitrogen in myosmine

(1) Paper I in this series, B. Witkop, *Experientia*, **10**, Oct. (1954).

(2) *Cf.* L. Marion in "The Alkaloids," by R. H. F. Manske and H. L. Holmes, Academic Press, Inc., New York, N. Y., 1950, Vol. 1, p. 255.

(3) J. P. Wibaut and H. C. Beyerman, *Rec. trav. chim.*, **70**, 977 (1951).

(4) M. L. Swain, A. Eisner, C. F. Woodward and B. A. Brice, THIS JOURNAL, **71**, 1341 (1949).

(5) C. R. Eddy and A. Eisner, *Anal. Chem.*, in press.

(6) G. G. Evans, THIS JOURNAL, **73**, 5230 (1951).

(7) J. W. Cornforth and A. J. Henry, *J. Chem. Soc.*, 597 (1952).

(8) S. Gabriel and J. Colman, *Ber.*, **41**, 519 (1908).

(9) P. M. Maginnity and J. B. Cloke, THIS JOURNAL, **73**, 49 (1951). It is probably safe to conclude that the homologous six-membered tetrahydropyridines, such as γ-coniceine and tetrahydro-α-picoline [A. Lipp and E. Widnmann, *Ber.*, **38**, 2471 (1905)] have to be formulated as Δ^1- and not as Δ^2-piperidines.