to equation (2) through the use of α's established from the intrinsic viscosity in toluene, Θ being assumed equal to zero. Substituting the resulting quantities along with $K_{14.5^\circ} = 1.2 \times 10^{-3}$ into equation (6), the maximum change in K would amount to 12% in 100°; a similar result is obtained if Θ is taken to be 100°K. and $d[\eta]/dT = 0$. Thus, $(\overline{r_0^2}/M)^{1/2}$ for rubber must be approximately independent of temperature, from which it follows that conversion of the K and $(\overline{r_0^2}/M)^{1/2}$ values for rubber to 60°, the temperature at which determinations were carried out on gutta percha, would not affect the conclusions reached above.

Thermodynamic Parameters.—By computing $(\alpha^5 - \alpha^3)/M^{1/2}$ from the α's listed in Table II for rubber in methyl *n*-propyl ketone and plotting this function against $1/T$ (see equation (2)), it is found that $2C_M\psi_1 = 0.0049$. Employing equation (7), $C_M = 0.0288$, giving $\psi_1 = 0.085$. This value for ψ_1 is comparable to those found for polyisobutylene; since the temperature range is quite limited, the uncertainty probably is of the order of ± 0.05, however. The gutta percha data are inadequate for such calculations.

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[CONTRIBUTION FROM THE LABORATORY OF THE AMERICAN PHARMACEUTICAL CO.]

Acetic Acid—Ammonium Acetate Reactions. An Improved Chichibabin Pyridine Synthesis[1]

BY MARVIN WEISS

Acetic acid containing varying concentrations of ammonium acetate is examined as a medium for the synthesis of nitrogen heterocycles. These conditions have previously been found useful in the formation of substituted imidazoles, pyrazines, pyrroles, oxazoles and piperidones. The method has now been extended to include arylated pyridines, acetic acid being replaced by acetamide in the more sluggish reactions. A mechanism is proposed for pyridine ring formation in the Chichibabin synthesis.

In a series of papers by Davidson, Weiss and Jelling,[2a,b,c] the rather complex reactions of ammonia on benzil and benzoin were elucidated. The unusual approach to this problem was in the use of ammonium acetate in acetic acid as a source of ammonia.[3a,b,4] It was found that under simple reflux in this medium, excellent yields of single products were isolated, with minor amounts of other products. This procedure, which led to convenient methods of synthesizing imidazoles (Table I) (A), pyrazines (B), oxazoles (C) and pyrroles (D), stood in sharp contrast to that of the early investigators, who used alcoholic ammonia or fused ammonium salts in sealed tubes. Noller and Baliah[5] later applied this reaction medium in a modification of the Petrenko–Kritschenko piperidone synthesis (E). The success of these conditions probably depends upon the fact that such base-catalyzed condensations are sometimes facilitated by the inclusion of acids.[6]

TABLE I

APPLICATIONS OF THE ACETIC ACID–AMMONIUM ACETATE METHOD TO THE SYNTHESIS OF N-HETEROCYCLES

	Reactants	Ring type
A[2a]	Benzil + aldehydes	Imidazoles
B[2b]	Benzoins	Pyrazines
C[2b]	Benzoin esters	Oxazoles
D[2c]	Benzoin + $RCOCH_2R'$	Pyrroles
E[5]	$RNH_2 + R'CHO + R^2CH_2COCHR^3R^4$	Piperidones
F	$RCHO + C_6H_5COCH_2R'$	Pyridines

The most useful aspect of this method is that competitive reactions may be almost entirely eliminated by varying the ammonium acetate concentration. The more reactive donors require less ammonium acetate. However, the apparent sluggishness of methyl ketones as donors in the Chichibabin synthesis (F) allows aliphatic aldehydes to self-condense.[7]

The history of the Chichibabin pyridine synthesis is similar to the ammonia reactions mentioned previously. Frank and Seven[8] revealed in a recent careful study that there were but few instances of yields higher than 20%. Sealed tubes, high temperatures and vapor reactions over heated catalysts were necessary. While Frank and Seven modified the synthesis by employing aqueous ammonia and catalytic amounts of ammonium acetate and so produced favorable yields of single products, they still required the use of a steel autoclave at temperatures of 250° with pressures up to 1450 p.s.i.

The present investigation deals with the reaction of aromatic aldehydes with aryl alkyl ketones containing a methylene group, the reaction being conducted in glacial acetic acid in the presence of ammonium acetate. Under these conditions it was found that the aldehyde appears as a substituent in

(1) Presented at the Meeting-in-Miniature, Metropolitan Long Island Sub-Section, March, 1951.

(2) (a) Davidson, Weiss and Jelling, *J. Org. Chem.*, **2**, 319 (1937); (b) *ibid.*, **2**, 328 (1937); (c) Davidson, *ibid.*, **3**, 361 (1938).

(3) (a) Davidson, THIS JOURNAL, **58**, 1821 (1936); (b) Davidson and Epstein, *J. Org. Chem.*, **1**, 305 (1936).

(4) In an early isolated case Angelico and Calvello, *Gazz. chim. ital.*, **31**, II, 7 (1901), used this reaction medium to produce 2,3,5-triphenylpyrrole from ω-desylacetophenone.

(5) Noller and Baliah, THIS JOURNAL, **70**, 3853 (1948).

(6) E. R. Alexander, "Principles of Ionic Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 180.

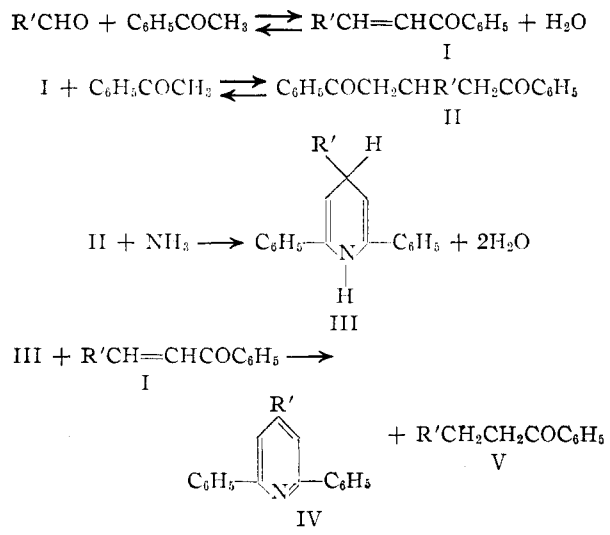
(7) Frank and Riener, THIS JOURNAL, **72**, 4182 (1950).

(8) Frank and Seven, *ibid.*, **71**, 2629 (1949).

the 4-position with the methylene groups oriented to positions 3 and 5.[8,9]

When benzaldehyde reacts with acetophenone, a simple picture of the mechanism (Table II)

TABLE II



involves an aldol condensation (I), followed by a Michael-type reaction to form a 1,5-diketone (II). This then condenses with ammonia to form a dihydropyridine (III), which, in turn, is dehydrogenated to a pyridine (IV).

closure of 1,5-diketones with ammonia is well known.[12] In this case stoichiometry demands the formation of a dihydropyridine which appears to undergo dehydrogenation by transfer of hydrogen to benzalacetophenone.[13,14]

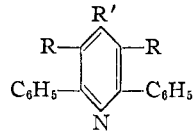
This of course is an explanation for the apparent low yields. For each mole of pyridine formed, a mole of benzalacetophenone may be reduced. In harmony with this, reactions using a ratio of two moles of aldehyde to three of ketone gave the same yield as a 2:4 ratio. The addition of cupric acetate or nitrobenzene or bubbling air through the reaction mixture was without effect. The low yield of benzylacetophenone (25%) may be explained by pointing out that it, too, is a methylene ketone capable of reacting with benzaldehyde.

To form pentaphenylpyridines it is necessary to use ammonium acetate in acetamide. However, the yield was unexpectedly low, despite the fact that desoxybenzoin has a more reactive methylene group than acetophenone.[2c] This may be reconciled by suggesting that the higher temperature of this medium required for the formation of pentaphenylpyridines, results, as well, in extensive side reactions.

Experimental (Table III)

Triphenylpyridines.—A mixture of 3.2 g. (0.03 mole) of benzaldehyde, 7.2 g. (0.06 mole) of acetophenone, 30 g. of ammonium acetate and 75 ml. of glacial acetic acid was refluxed for one hour. Upon cooling, an oil separated which gradually crystallized (seeding helps at this point). Crystals were filtered and washed with 70% acetic acid. Combining filtrate and washings yielded additional crystals; total yield 3.1 g., recrystallized from ethanol. Other pyridines were obtained in similar manner, with the exception of the 4-(2-methoxy)-phenyl derivative. This was obtained

TABLE III



Triphenylpyridines: R = H R′	Yield, %[a]	Cor. m.p. °C.,	Formula	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C[b]	Found H	Found N[c]
Phenyl	68	139[e]	$C_{23}H_{17}N$			4.56			4.57
4-Methoxyphenyl	68	102	$C_{24}H_{19}NO$			4.15			4.20
3,4-Methylenedioxyphenyl	68	156	$C_{24}H_{17}NO_2$	82.03	4.88	3.99	82.03	5.06	3.81
4-Dimethylaminophenyl	64	138	$C_{25}H_{22}N_2$	85.68	6.33	8.00	85.68[d]	6.37	7.98
2-Methoxyphenyl	60	119	$C_{24}H_{19}NO$	85.43	5.68	4.15	85.63	5.36	4.06
Pentaphenylpyridines: R = C_6H_5 R′									
Phenyl	30	247[f]	$C_{35}H_{25}N$			3.05			3.09
3,4-Methylenedioxyphenyl	24	271	$C_{36}H_{25}NO_2$	85.86	5.00	2.78	85.58	5.10	2.85

[a] Based upon production of one mole of pyridine for two moles of aldehyde. [b] C and H analyses by Schwartzkopf Analytical Laboratories, Middle Village, L. I. [c] Kjeldahl nitrogen analyses by Mr. Erwin Sommer of this Laboratory. [d] This C and H analysis by Mrs. Mildred Libowitz, Cornell University. [e] Reference 7, m.p. 137–138°. [f] Reference 7, m.p. 245–247°.

The following observations are pertinent to this proposed mechanism. In a reaction of benzaldehyde with desoxybenzoin in the presence of ammonium acetate and acetic acid, conditions which do not form a pyridine, benzaldesoxybenzoin was isolated.[10a,b] Frank and others have shown that the aldol and Michael steps are reversible.[8,11] Ring

(9) This same orientation occurs in the Hantzsch pyridine synthesis, where the increased activation of the methylene donors of ketonecarboxylic esters makes a reaction with aliphatic aldehydes possible.

(10) (a) Cope, THIS JOURNAL, **59**, 2327 (1937); (b) Cope, Hofman, Wyckoff and Hardenbergh, *ibid.*, **63**, 3452 (1941), in a study of the Knoevenagel condensation found that only catalytic amounts of ammonium acetate in acetic acid were necessary to effect condensation between ketones and cyanoacetic esters.

(11) Sawdey, Ruoff and Vittum, *ibid.*, **72**, 4947 (1950), demonstrated that anisylidenepyrazolones and anisylidenebispyrazolones are interconvertible, reacting with a pyrazolone and anisaldehyde, respectively, in the presence of sodium acetate in acetic acid.

(12) Merz and Richter, *Arch. Pharm.*, **275**, 294 (1937).

(13) Frank[8] isolated benzylacetophenone (V) as a reduction product in a reaction between benzalacetophenone and acetone, but did not indicate the cause of reduction, nor isolate a pyridine. A similarity is noticed in the Döbner–Miller quinaldine synthesis. Mills, Harris and Lambourne, *J. Chem. Soc.*, **119**, 1294 (1921), reacted aniline and acetaldehyde and as by-products obtained ethylaniline and butylaniline.

(14) As pointed out by a referee, spontaneous dehydrogenation is a possibility. However, no gas was obtained when the reaction was run in a closed system under slightly reduced pressure.

by the addition of 50 ml. of ammonia water to the cooled refluxed solution followed by several chloroform extractions. The chloroform extract was then steam distilled. After all the volatile material was removed, the oily residue was dissolved in methanol, water added to cause an incipient precipitate and allowed to crystallize overnight; yield 3.0 g., recrystallized from methanol.

Isolation of Benzylacetophenone.—To the filtrate and washings of the triphenylpyridine experiment 75 ml. of ammonia water was added, the solution extracted with chloroform and the chloroform extract was steam distilled. After the chloroform and acetophenone have distilled, the benzylacetophenone steam distils more slowly and 0.8 g. appears as a slowly crystallizable oil. Upon recrystallization from dilute acetic acid brilliant platelets appear, m.p. 71° (cor.), oxime m.p. 82–84° (cor.).

Anal. Calcd. for oxime: N, 6.22. Found: N, 6.14.

Pentaphenylpyridines.—A mixture of 2 g. (0.01 mole) of desoxybenzoin, 0.55 g. (0.005 mole) of benzaldehyde, 15 g. of ammonium acetate and 30 g. of acetamide was heated gently with stirring. At a gentle reflux temperature, small bubbles developed, smelling strongly of ammonia. After 2 hours, the reaction mixture was cooled slowly, 20 cc. of methanol added and the solution heated to boiling. Upon cooling crystals appeared gradually along with an oily solid. The crystals were filtered, redissolved in glacial acetic acid and methanol added to precipitate the pentaphenylpyridine; yield 0.35 g. recrystallized from acetic acid–methanol. **The 4-(3,4-methylenedioxy)-phenyl** derivative was obtained in a similar manner using a ratio of 1:1.5 for the aldehyde and ketone. Recrystallized from acetic acid in fine white needles.

Acknowledgment.—The author is grateful to Professor David Davidson for the many stimulating discussions, his encouragement and the interest shown in this work.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Structural Specificities in the Interactions of Some Organic Ions with Serum Albumin

BY IRVING M. KLOTZ, R. K. BURKHARD[1] AND JEAN M. URQUHART

Spectrophotometric investigations indicate that above *p*H 7 human albumin interacts in a specific fashion with anions of the structure $(CH_3)_2N$—⟨⟩—N=N—⟨⟩—X^-, where $-X^-$ may be $-SO_3^-$, $-CO_2^-$, $-PO_3H^-$ or $-AsO_3H^-$. If $-X^-$ is ortho, however, the specific interaction disappears. Studies with compounds containing additional substituents which block the azo or the dimethylamino nitrogen show that the latter nitrogen is involved in a linkage to the protein. Experiments with modified proteins suggest that tyrosine residues from human albumin complete this link. A bond is also formed between $-X^-$ and cationic sites of the protein. In view of the dependence of the interaction on the position of $-X^-$, it is suggested that the distance between the $(CH_3)_2N-$ and $-X^-$ substituents is the determining factor. Then at least some side chains from tyrosine residues must be about 12–13 Å. away from certain cationic loci on albumin. A method thus seems available for establishing distances between specific side chains in a protein molecule.

Introduction

Optical studies of the interactions of some azobenzene anions with bovine and human albumin, respectively, have shown[2,3] marked differences in the nature of the effects produced by these proteins. In aqueous solutions below about *p*H 7, both albumins change the spectrum (Fig. 1) of methyl orange (I) to that in organic solvents,[3] in which the

$(CH_3)_2N$—⟨⟩—N=N—⟨⟩—$SO_3^-Na^+$

I

salt does not ionize appreciably. Such optical behavior is in accord with chemical evidence that the anion is bound to the albumin largely through electrostatic interaction between the $-SO_3^-$ substituent and cationic loci of the protein. Bovine albumin behaves in essentially the same manner between *p*H 7–11 also.

On the other hand, human albumin produces an effect on the spectrum of methyl orange (Fig. 1) near and above *p*H 7 markedly different than at lower *p*H's. Apparently human albumin undergoes some configurational change in a *p*H region above neutrality, and in this region an arrangement of residues becomes available which is markedly different from that in bovine albumin. To obtain some insight into these differences studies have been carried out with isomers and analogs of methyl orange and with several chemically modified proteins. It has been possible to demonstrate that both substituents on the azobenzene skeleton of methyl orange are involved in interactions with human albumin at high *p*H's. These studies indicate further that it may be possible to establish the distances between certain side chains in native albumins from an examination of optical aspects of interactions with small molecules.

(1) Junior Fellow of the National Institutes of Health, 1948–1950. Present address: Department of Chemistry, Kansas State College, Manhattan, Kansas.

(2) I. M. Klotz and F. M. Walker, *J. Phys. & Colloid Chem.*, **51**, 666 (1947).

(3) I. M. Klotz, R. K. Burkhard and J. M. Urquhart, in press.

Experimental

Absorption Spectra.—The absorption of light was measured with the Beckman spectrophotometer, model DU, at approximately 25°. One-cm. cells were used. Extinction coefficients, ϵ, were calculated from the equation

$$\epsilon = \frac{1}{cd} \log_{10} (I_0/I)$$

where I_0 is the intensity of the light emerging from the solvent, I the intensity of the light emerging from the solution, c the molar concentration of the solute and d the thickness of the absorption cell in centimeters.

At *p*H 9, the following extinction coefficients were calculated at the corresponding absorption maxima.

Anion	λ_{max}, mμ	ϵ
Methyl orange	465	26,800
4′-Dimethylaminoazobenzene-3-sulfonate	460	25,500
4′-Dimethylaminoazobenzene-2-sulfonate	450	21,200
Methyl red	430	21,200
4′-Dimethylaminoazobenzene-3-carboxylate	455	23,200
4′-Dimethylaminoazobenzene-4-carboxylate	465	26,400
4′-Dimethylaminoazobenzene-3-phosphonate	450	19,800
4′-Dimethylaminoazobenzene-4-phosphonate	455	21,500
4′-Dimethylaminoazobenzene-4-arsonate	460	19,400