697. *Organic Reactions in Aqueous Solution at Room Temperature. Part I. The Influence of* pH *on Condensations involving the Linking of Carbon to Nitrogen and of Carbon to Carbon.*

By C. A. C. Haley and P. Maitland.

The object of this series of papers is to broaden the field initiated by Robinson and Schöpf and usually termed " syntheses under physiological (or ' cell-possible ') conditions," in relation to both biochemical problems and general organic synthetical methods. Extensive (rather than intensive) investigations have shown that water at room temperature is an effective medium for some very simple condensations involving substances containing the naturally-occurring groups CHO, CO, NH_2, $CO{\cdot}NH_2$, $NH_2{\cdot}C{:}NH$, $CH_2{\cdot}CN$, $CH_2{\cdot}CO$, $CO{\cdot}CH_2{\cdot}CO$, $CO{\cdot}CH_2{\cdot}CH_2{\cdot}CO$ (and $H{\cdot}CO_2H$), leading to well-known examples of Schiff bases, and quinoxaline, diazepine, pyrimidine glyoxaline, pyrrole, and pyridine derivatives. Some failures have suggested that in this type of work a methylene group requires activation from both sides for successful condensation. In two cases of Claisen–Knoevenagel condensations, glycine has been shown to be a useful catalyst. As found by Schöpf in other cases, variation of the pH has striking effects on the yields. Our reaction conditions differed from those used by Robinson and Schöpf in that, while they usually had to isolate their products from solution, we chose water-soluble reactants which produced very insoluble products. A considerable part of the driving force for the reactions is therefore the displacement of equilibria by precipitation. The products in most cases are obtained in reasonable, and sometimes very high, yields after a reaction time of a few days, and are isolated pure direct from the reaction mixture, the usual losses thus being eliminated. Several of the reactions may have preparative value, or may serve for future kinetic investigations. Some of the experimental results support the theory that some reactions, normally considered to be base-catalysed, may also take place under acid-catalysis.

The metabolic routes by which living cells elaborate their multifarious products are only known with certainty in comparatively few instances; but with the introduction into biochemistry of isotopic tracers and organisms with different blocked enzyme systems, and with the general development of spectrographic and chromatographic methods, which offer greater hope of detecting and possibly isolating very labile intermediates, some striking advances in this field of biogenesis may be expected. Although the final proof of any proposed biogenetic origin must always come from the biochemical side, the purely organic chemist has also played a part in trying to elucidate these mechanisms, and contributions to such studies have come from two directions. The first approach, difficult to credit to any single author, concerns the development of the idea that the cell, in addition to its many enzyme-controlled reactions, might produce during the course of its metabolism units which were so reactive that they condensed together without requiring enzymes: the second approach, developed almost entirely by Robinson and closely interlinked with the first, involves architectural analysis of a molecule with a view to detecting the building units.

The earliest ideas regarding non-enzymic cell reactions were first discussed in connection with alkaloid syntheses during 1900—1910 by Pictet, Willstätter, and Winterstein and Trier, and summarised by the last two authors (" Die Alkaloide," Borntraeger, Berlin, 1910, pp. 263—317); but the main theoretical and practical efforts to gain evidence in support of the general theory, by synthesising some natural products under very mild conditions from simple, known (or easily derived) cell units in the absence of enzymes, are associated principally with the work of three authors: Collie during the years 1893—1907 (see Stewart, " Recent Advances in Organic Chemistry," Longmans Green, London, 1927, Vol. I, p. 105) on the conversion of reactive aliphatic compounds (polyketides), which contain repeating $CO{\cdot}CH_2$ groupings, into aromatic compounds; Robinson, with his elegant synthesis of tropinone in aqueous solution at room temperature (*J.*, 1917, **111**, 762), and many outstanding theoretical contributions (*J.*, 1917, **111**, 876; Madrid Lecture, IX Congreso Internacional de Química Pura y Aplicada, 1934; *J.*, 1936, 1079; *J. Roy. Soc. Arts*, 1948, **96**, 795; First International

Congress of Biochemistry, Cambridge, 1949, Report, 1950, p. 32); and Schöpf, with many striking advances in the field of alkaloid syntheses in aqueous solution at 20° or 25° under varying pH conditions, which affect both the yield and the nature of the product (*Angew. Chem.*, 1937, **50**, 779, 797; F.I.A.T. Review of German Science 1939—1946, Preparative Organic Chemistry, Part II, 1948, p. 117; *Chimia*, 1948, **2**, 206). A convincing demonstration both of the soundness of Robinson's original theoretical conceptions of over 30 years ago, and of the importance of Schöpf's controlled pH conditions, has recently been given by Anet, Hughes, and Ritchie (*Nature*, 1949, **163**, 289; 1949, **164**, 501; 1950, **165**, 35), especially in their elegant synthesis of the complex sparteine ring system. The latest contributions to the alkaloid field, which include Woodward's important new concept, have been summarised by Johnson (*Ann. Reports*, 1949, **46**, 195).

In spite of all the above studies and of occasional references in the literature to investigations of this nature, which have come to be known as syntheses under physiological (or " cell-possible ") conditions, we consider that only a beginning in this valuable type of synthesis has been made. The immediate object of the present series of papers is therefore to carry out a broad study of organic reactions in aqueous solution at room temperature, using only mild reagents, simple catalysts, and a wide range of pH conditions, in order to discover what types of reaction are possible under these conditions. It is hoped that this will be of value, first, for the bearing it might possibly have on general problems of biogenesis and on specific attempts at biosynthesis of important cell substances, and, secondly, as a contribution to general synthetic methods. The emphasis has been placed on the discovery of as many types as possible, and the whole study has therefore aimed at being extensive rather than intensive. The model reactions selected are all exceedingly simple, leading to well-known products, but the information gained should enable us to embark later upon more ambitious syntheses. High yields of the products, although very desirable from the preparative aspect and in fact often obtained in the present work, have not been a principal aim from the biogenetic standpoint, because the cell, unlike the chemical models, probably possesses directional control mechanisms which prevent side-reactions: nor has the temperature of the experiments, about 18°, been subject to accurate control, because kinetic investigations were not part of the object.

The main objection to water as a solvent for organic reactions is the sparing solubility of the majority of organic compounds in the solvent, and it is for this reason that it has been comparatively neglected by organic chemists. It must be noted, however, that many organic compounds are loosely stated to be insoluble in water when in fact their solubilities are quite appreciable. In the present series of investigations the solubility at room temperature of the initial reactants has varied from complete miscibility to as low as 0·1%. Solubilities of the order of this low figure, which may still be very much higher than many cell concentrations, were nevertheless found to be experimentally quite practicable.

No limitation will be fixed for pH in these preliminary experiments, and the whole or any suitable part of the range will be examined; but in this connexion the pH values found in living cells are of interest. Concerning the physiologically permissible pH limits, Schöpf and Lehmann (*Annalen*, 1935, **518**, 4) state simply without any supporting evidence that the physiological pH range is 5—9. Small (" pH and Plants," Bailliere, Tindall and Cox, London, 1946) points out the serious difficulties attending the determination of pH in living cells, and the most acceptable method at present is the special indicator method elaborated by Small himself. The normal pH figures for plant sap are 5·0—6·2 but for plants with exceptionally acid metabolism the pH may be as low as 1·7. The figures given for the outer limits of the pH optima for enzymes are instructive. Small (*op. cit.*, p. 78) has compiled a table from various sources which shows that the general range for plant enzymes is 3·5—7·0, but with some extensions on either side—to 2·5 and 10·0. Since direct evidence for the existence of alkaline plant pH's is scanty, it is of interest that Cromwell (*Biochem. J.*, 1950, **46**, 578) has recently recorded a pH of 8·2 in the epidermal glands of the leaves of *Chenopodium vulvaria*. Baldwin (" Dynamic Aspects of Biochemistry," Cambridge Univ. Press, 1947, p. 51), quoting from Haldane's Tables (1930), gives the outer limits for enzymes generally as 1·5—11. Thus two-thirds of the whole pH scale is covered by some supporting evidence from the physiological side. The remaining pH ranges, which so far have never been detected in a living cell, are therefore the very strongly acid and alkaline regions 0—1·5 and 11—14. It is worth mention here that organic reactions found to proceed at pH's higher than 11 may be induced to take place at a lower pH by adopting a device on the lines of that elaborated by Schöpf and Lehmann (*Annalen*, 1932, **497**, 7). These authors have found that the Friedländer quinoline synthesis, which requires a pH of 11—12 for a ketone $R{\cdot}CO{\cdot}CH_3$, can be induced to proceed at pH 7—9,

and thus be brought within the physiological pH range, by using a compound with a more active methylene group, the keto-acid $R{\cdot}CO{\cdot}CH_2{\cdot}CO_2H$, which is decarboxylated during the reaction.

In the present work, the reaction conditions employed differ from those of Robinson and Schöpf, who usually had to extract their products from solution, or precipitate them as derivatives. In our first model experiments, readily accessible reactants have been selected, not necessarily naturally-occurring, but containing reactive groups such as CHO, CO, NH_2, $CO{\cdot}NH_2$, $NH_2{\cdot}C{:}NH$, $CH_2{\cdot}CN$, $CH_2{\cdot}CO$, $CO{\cdot}CH_2{\cdot}CO$, $CO{\cdot}CH_2CH_2{\cdot}CO$ (and $H{\cdot}CO_2H$) which are either found in Nature or can be derived easily from known natural products. The group $CH_2{\cdot}CN$ has been used in two instances as a convenient source of an active methylene group. The CN group itself is of course found in the widely occurring plant cyanophoric glycosides, but only rare instances of naturally occurring compounds containing the $CH_2{\cdot}CN$ group have been reported (Mowry, *Chem. Reviews*, 1948, **42**, 190; Forss, *Nature*, 1951, **167**, 733). All these groups are polar and confer, on the compounds of which they form part, water solubility varying in degree according to the nature of the rest of the molecule. In order to supply what must be a considerable part of the driving force needed to cause interaction of these groups, and also to have a simple means of detecting that it has taken place and of isolating the product, saturated (or strong) aqueous solutions of the polar reactants, either untreated, or with the pH's approximately adjusted, have been so chosen that when allowed to react together at room temperature the polar groups destroy one another and the expected product, a known solid product of high crystallising power and sharp melting point, if formed, is precipitated. In three cases this particular driving force was absent since the product, formed mainly on the acid side and soluble in acid, had to be isolated by making the mixture alkaline. No attempt was made to examine the residual filtrates, but in every case for our own satisfaction the solubility in water of the product was determined in order to obtain some idea of the loss due to this factor. These solubilities are given in the Experimental section only in the few cases where they were appreciable. Since in the majority of cases buffer solutions were used, this figure for pure water could only be applied roughly. It is realised that, under the conditions chosen for these experiments, the equilibrium in a reversible reaction will be shifted in favour of the insoluble product, but all that is desired at present is to show that formation of the product is possible. The living cell may alter the equilibrium by precipitation of the product when a certain concentration is reached, as in the model experiments, or by removing it whilst still in solution by means of a further reaction. The choice of a product with a sharp melting point (when pure) is considered advisable at this stage of the investigations, so that if there is any observed unsteadiness in the melting point of the product obtained it will be an indication that it is due to impurities and not to any inherent instability in the molecule of the product itself. In the present work this rule has been observed in every case except two. The appropriate pH range was obtained by noting the natural pH's of the untreated solutions, or after addition of acid or alkali and, if the results were favourable, a series of more accurate experiments over a wide pH range, using buffer solutions, was carried out in each case, except where the product was too soluble. Difficulties, presumably enhanced by the precipitation of the product, were experienced with buffer capacities, and a large excess of the buffer solution often had to be used with consequent diminished yields of the products.

The products, obtained in good and sometimes very high yield, were isolated by filtration, and after simple washing with water were found in every case but two to be analytically pure, without recrystallisation. They were identified in the usual way by mixed melting points with authentic specimens, confirmed by analysis. The general accuracy of the experiments described in the tables which follow is within 1—2%.

The work is described below in four sections, according to the type of condensation employed, namely, C–N, N–C–N, C–C, and a combination of C–C and C–N. When applicable, the sections are introduced by brief summaries of what is known regarding the biogenetic origin of the system under investigation.

(a) C–N *Condensations leading to Aromatic Schiff Bases, and Quinoxaline, Diazepine, Pyrimidine, and Pyrrole Derivatives.*

Aromatic Schiff Bases.—The first type of reaction selected for study was the interaction between CHO and NH_2 groups to form Schiff bases. In the alkaloid field, in which the attempts to imitate biosynthesis have been so outstandingly successful, Schiff bases have been postulated as intermediates in the reactions involving aldehydo- and amino-groups, although the weight

of evidence obtained appears to favour the previous stage, the hydroxy-amine, as the reactive intermediate (Robinson, *loc. cit.*, 1936; Schöpf and Salzer, *Annalen*, 1940, **544**, 11).

In the reaction between an aldehyde and an amine in general, the hydroxy-amine or Schiff base first formed may subsequently condense with one or other of the reactants, but, when both the aldehyde and the amine are aromatic, the highly crystalline stable Schiff bases are readily formed in high yield, and show no tendency towards the further reaction (Sprung, *Chem. Reviews*, 1940, **26**, 297).

The general conditions for the preparation of Schiff bases normally involve heating alone or in an anhydrous organic solvent, the presence of water usually being regarded as disadvantageous. There are, however, instances where water has been present and the conditions milder (Pyl, *Ber.*, 1927, **60**, 287; Werner, *Sci. Proc. Roy. Dublin Soc.*, 1944, **23**, 214; Cook, Heilbron, and Levy, *J.*, 1947, 1603; Morley and Simpson, *J.*, 1948, 2026; Lutz, *et al.*, *J. Org. Chem.*, 1947, **12**, 763).

In the present paper a representative number of Schiff bases have been prepared from aromatic aldehydes and aromatic amines (including benzylamine) (see Table XX). Several aromatic aldehydes are found free in Nature, but natural occurrence of aromatic amines possessing a free amino-group directly attached to the ring has been reported only in the cases of methyl anthranilate and *p* (and possibly *o*)-aminobenzoic acid. An example from Nature of an aromatic compound containing a free amino-group in the side-chain is tyramine. All the Schiff bases examined are readily formed at room temperature, in yields varying from 70 to 91%, by mixing in equimolecular proportions saturated aqueous solution of the two reactants without interfering with the pH developed. No attempt was made to examine the residual filtrates, which presumably on the large scale could be used again for a further preparation.

It was found that the general reaction could be extended to a reactive ketone: acetylacetone and aniline or *m*-aminophenol in aqueous solution gave the respective pure monoanils in 74% and 84% yield.

All the experiments so far described were very rough and no attempt was made to control the pH developed. Since the reaction commenced so quickly after mixing, no value of the pH during the condensation could be obtained, and only the limits of the probable pH prevailing were recorded. Since the starting materials were so readily accessible, a considerable number of experiments in this one case were performed (see Table XX) in order to establish that the unusual practical conditions were of general application in the field of aromatic Schiff bases, since they may be of preparative value.

The preparation of benzylideneaniline was selected for an accurate study of pH (see Table I). The highest yield (80%) of pure product resulted when precipitation occurred from

TABLE I.

Benzylideneaniline from benzaldehyde (1·06 *g.*) *in buffer solution* (300 *c.c.*) *and aniline* (0·93 *g.*) *in buffer solution* (30 *c.c.*); 2 *days at room temp.* *M. p.* 51—52°, *pure.*

Buffer	N-HCl–N-NaOAc				0·2N-AcOH–0·2N-NaOAc		0·2N-KH_2PO_4–0·2N-NaOH					
pH, initial and final	1·0 [a]	1·3 [a]	1·6 [a]	3·8 [a]	4·1 [b]	4·9 [b]	5·9	7·0	7·5	7·9	8·2	11·0
Yield, g.	0	0	0	trace	0·79	1·23	1·38	1·45	1·45	1·45	1·34	1·13
Yield, %	0	0	0	trace	44	68	76	80	80	80	74	62
M. p.	←						51—52°					→

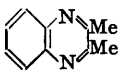
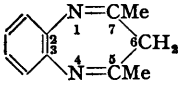
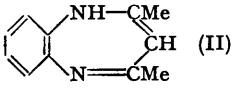
[a] Aniline in 200 c.c. of buffer } to maintain the pH.
[b] ,, 140 c.c. ,, }

solution at pH 7—7·9, with falling off on either side until on the acid side, at about pH 3·8 and less, no precipitation took place, the most probable explanation being that the equilibrium under these conditions lies on the side of the two reactants.

Quinoxaline.—The general reaction could be extended to include a quinoxaline derivative. *o*-Phenylenediamine and diacetyl were selected as the reactants. Although, as mentioned above, three aromatic compounds containing a free amino-group directly attached to the ring are known in Nature, no naturally occurring aromatic diamine has yet been reported. Diacetyl itself and two of its reduction products play some part in the metabolism of numerous cells (Neuberg, *Adv. Carbohydrate Chem.*, 1949, **4**, 86).

When saturated solutions of the two reactants were mixed at room temperature the solution

deposited directly, without forming an intermediate emulsion, fine needles of pure 2 : 3-dimethylquinoxaline (I) in 93% yield (see Table XX). Gabriel and Sonn (*Ber.*, 1907, **40**, 4850) state that the free base and ketone condense easily in aqueous solution but no details or

(I)   or  (II)

yield are given (they used *o*-phenylenediamine acetate and diacetyl monoxime in warm water). There are many recorded cases, based on Hinsberg's observations (*Annalen*, 1887, **237**, 327) in which *o*-diamines have been condensed in aqueous solution with reactive substances such as glyoxal, phenylglyoxal, pyruvic acid, and certain sugar derivatives, but only occasionally is the reaction carried out at room temperature (*idem, ibid.*, 1896, **292**, 245; Ohle, *Ber.*, 1934, **67**, 155; Erlbach and Ohle, *ibid.*, p. 555; see also Lanning and Cohen, *J. Biol. Chem.*, 1951, **189**, 109).

The results of an accurate pH study of the preparation of the quinoxaline (I) are shown

TABLE II.

2 : 3-*Dimethylquinoxaline* (I) *from* o-*phenylenediamine* (1·08 g.) *in buffer solution* (45 *c.c.*) *and diacetyl* (0·86 *g.*) *in buffer* (5 *c.c.*); 1 *day at room temp.* *M. p.* 105—107° (*pure*).

Buffer	N-HCl–N-NaOAc	N-NaOAc–N-AcOH		N-KH_2PO_4–N-NaOH			
pH (initial and final) ...	3·0 [a]	4·0	5·5	7·1	8·5	9·0	11·6
Yield, g.	0·70	1·28	1·55	1·55	1·50	1·46	1·30
Yield, %	44	81	98	98	95	92	82
M. p.	← 105—107° →						97—103°

[a] Final pH 1·7, even with 100 c.c. of buffer.

in Table II. Exceptionally high yields (maximum, 98%) of pure product were obtained over the pH range 4·0—9·0. At pH 11·6 the product was impure, owing very probably to self-condensation of the diacetyl (cf. von Pechmann, *Ber.*, 1888, **21**, 1420; von Pechmann and Wedekind, *Ber.*, 1895, **28**, 1846; Diels, Blanchard, and von der Heyden, *Ber.*, 1914, **47**, 2355).

Diazepine.—The reaction between *o*-phenylenediamine and acetylacetone, to give the seven-membered ring compound, 5 : 7-dimethyl-2 : 3-benzo-1 : 4-diazepine (II) (Thiele and Steimmig, *Ber.*, 1907, **40**, 955; Witter, Snyder, and Stotz, *J. Biol. Chem.*, 1948, **176**, 493), was next investigated. Acetylacetone itself has not been reported in Nature, but the β-diketonic grouping occurs occasionally. When saturated aqueous solutions of the two reactants were mixed at room temperature, the pure product crystallised out overnight in 36% yield. Our analyses and molecular-weight determinations support the formula suggested by Thiele and Steimmig.

An accurate pH study is reported in Table III below. Owing to the appreciable solubility

TABLE III.

5 : 7-*Dimethyl*-2 : 3-*benzo*-1 : 4-*diazepine* (II) *from* o-*phenylenediamine* (1·08 *g.*) *in water* (10 *c.c.*) (*approx.* pH *attained by addition of* AcOH), *and buffer solution* (25 *c.c.*), *and acetylacetone* (1·0 *g.*) *in buffer* (20 *c.c.*); 2 *hours at room temp.* *M. p.* 131—133° (*pure*).

Buffer	N-AcOH–N-NaOAc		0·2N-KH_2PO_4–0·2N-NaOH			
pH (initial and final)	3·8	4·8	5·8	6·8	7·7	8·2
Yield, g.[a]	0·79	1·04	0·97	0·85	0·21	0
Yield, % [a]	46	60	56	49	12	0
M. p.	← 130—132° →		← 131—133° →			

[a] Anhydrous.

of the product on the acid side the conditions employed here were not the normal. After the reaction had taken place during two hours at the pH indicated, the product was isolated by the addition of excess of dilute aqueous sodium hydroxide. Although there is no condensation above pH 7·7, this treatment might have invalidated the results of the accurate pH study, but in fact a considerable difference in the yields at various pH s was actually observed. Table III shows the maximum yield (56%) of pure product at pH 5·8. At pH values less than 3·8, the deep violet hydrochloride was precipitated.

Pyrimidines.—The evidence for the biological origin of the pyrimidine nucleus in simple pyrimidines (Mitchell and Houlahan, *Fed. Proc.*, 1947, **6**, 506; Heinrich and Wilson, *J. Biol. Chem.*, 1950, **186**, 447; Wright *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 1898) or in purines (Bentley, *Ann. Reports*, 1948, **45**, 250; Schlenk, *Adv. Enzymol.*, 1949, **9**, 460) is so exceedingly slender that no definite conclusions can be made. The most widely used chemical method of synthesis involves condensation of a N–C–N and a three-carbon unit. Since this type of synthesis offers experimental advantages (readily accessible materials and known reference compounds) it was chosen for investigation. From the many possible units used in this method three amidines and a β-diketone were selected.

(III)

The normal experimental conditions for this type of pyrimidine synthesis are condensation under anhydrous conditions with alcoholic sodium ethoxide, usually under reflux but sometimes in the cold. Acid conditions have been used occasionally (Rose and Swain, *J.*, 1945, 689; Curd, Graham, and Rose, *J.*, 1948, 594; Roblin and English, U.S.P. 2,309,739; *Chem. Abs.*, 1943, **37**, 3768). There are, however, a few cases in which the conditions reported were milder and water was present. In Pinner's orginal method (*Ber.*, 1893, **26**, 2122) aqueous potassium carbonate solution was used, but few details and no yields were given. In the special case where a very reactive β-dialdehyde replaces the β-diketone, as in the condensation of sodium nitromalondialdehyde with benzamidine hydrochloride or guanidine carbonate in water at room temperature (Hale and Brill, *J. Amer. Chem. Soc.*, 1912, **34**, 91) the pyrimidines were obtained almost immediately on mixing the solutions of the reactants. As mentioned below in the pyrrole section, sodium nitromalondialdehyde is not suitable for use in a pH study.

The compounds selected for a pH study were 2-amino-4 : 6-dimethyl- (III; R = NH_2), 4 : 6-dimethyl-2-phenyl- (III; R = Ph), and 2 : 4 : 6-trimethyl-pyrimidine (III; R = Me), prepared by condensing acetylacetone with guanidine, benzamidine, and acetamidine respectively. Difficulties were experienced here with buffering action, even when large amounts of the buffer solutions were used. In addition, the appreciable solubility in water of two of the products and the very slow speed of all the condensations made it advisable to work in as concentrated solution as possible. The more approximate method of adding potassium carbonate to the aqueous mixture of acetylacetone and the requisite amidine salt and determining initial and final pH's had therefore to be adopted.

The results for guanidine and benzamidine are given in Tables IV and V. Although both

TABLE IV.

2-*Amino*-4 : 6-*dimethylpyrimidine* (III; R = NH_2) *from guanidine carbonate* (0·90 *g.*), *acetylacetone* (1·0 *g.*) *and* K_2CO_3 (*or* HCl) *in water* (10 *c.c.*); 20 *days at room temp.* *M. p.* 152—154° (*pure*).

K_2CO_3, g.	[b]	0	0·7	2·8
Initial pH	8·5	9·0	9·2	10·0
Final pH	7·3	7·7	8·0	8·6
Yield, g.[a]	0	0·44	0·63	0·76
Yield, %[a]	0	36	51	62
M. p.	←	152—154°		→

[a] Anhydrous. [b] 2 c.c. of N-HCl.

TABLE V.

4 : 6-*Dimethyl*-2-*phenylpyrimidine* (III; R = Ph) *from benzamidine hydrochloride dihydrate* (1·93 *g.*), *acetylacetone* (1·0 *g.*), *and* K_2CO_3 *in water* (10 *c.c.*); 19 *days at room temp.* *M. p.* 81—83° (*pure*).

K_2CO_3, g.	0	0·05	0·3	0·5	0·7	1·0	2·8
Initial pH	4·0	7·5	8·7	8·8	8·9	9·1	9·6[a]
Final pH	4·0	6·5	7·9	8·1	8·2	8·3	8·9
Yield, g.	0	0	0·15	0·34	0·47	0·66	1·17
Yield, %	0	0	8	18	26	36	64
M. p.			←		81—83°		→

[a] At pH >10, benzamidine was hydrolysed to benzamide.

pyrimidines started to come out of solution after 1 day, the necessary duration of the experiments was considerable (19—20 days), especially when compared with the speed of the analogous condensation, under comparable conditions, with a β-ketonic ester (Cook and Reed, *J.*, 1945, 399). The maximum yields of pure products obtained were 62% (2-amino) and 64% (2-phenyl). No details are given regarding the preparation of 2 : 4 : 6-trimethylpyrimidine dihydrate, from acetamidine, except to record that, although examined over a pH range, only under Bowman's conditions (*J.*, 1937, 494), which we found to correspond to pH 9·6, was any product precipitated.

Since the 2-phenyl derivative is very insoluble in water and does not dissolve in acid buffers until pH 1—2, the pH range was in this case extended to pH 4, the natural pH of an aqueous solution of the reactants, but no product was precipitated.

It would thus appear that this type of condensation between an amidine and a β-diketone proceeds only on the alkaline side, with the optimum range at pH 9—10.

Pyrroles.—One of the methods described below in section (d) (method B) should properly be described now as it is an example of a C–N–C condensation. For convenience in having the three pyrrole methods together, however, it has been placed in section (d).

(b) N–C–N *Condensations to give Glyoxaline Derivatives.*

Only two approaches have been made to the biogenesis of the widely occurring glyoxaline nucleus; the first was by Robinson (*locc. cit.*, 1934, 1936); and the second [summarised by Bentley (*loc. cit.*, p. 248) and by Schlenk (*loc. cit.*; see also Heinrich and Wilson, *loc. cit.*)] is supported by biological tracer experiments. The evidence suggests that the glyoxaline ring in purines may be built up from the C–C–N atoms of glycine, a C atom from the carboxyl group of formic or acetic acid, and a N atom from a non-specific nitrogen source. It is not known what the sequence is. If the route required attachment of the nitrogen first to the glycine residue, it might involve a final condensation between a diamino-compound and formic (or acetic) acid. Since formic acid has also been shown to take part with glycine in a metabolic process in a rat (Swanson and Clark, *Ann. Rev. Biochem.*, 1950, **19**, 251; Bentley, *loc. cit.*, p. 243), and since it is so reactive chemically compared with acetic acid, it was selected for our studies.

Benziminazole.—The first reaction chosen for an accurate pH study was the condensation of *o*-phenylenediamine and formic acid to give benziminazole. This condensation is usually carried out under vigorous conditions (*Org. Synth., Coll. Vol.* II, p. 65; Phillips, *J.*, 1928, 2395). Since benziminazoles are readily soluble in mineral acids and in excess of caustic alkalis, they are isolated from strongly acid or alkaline solution by final treatment with ammonia to pH 8·5—9.

The results of an accurate pH study of the preparation of benziminazole are given in Table VI. The product remained in solution during the experiments and, after removal of an

TABLE VI.

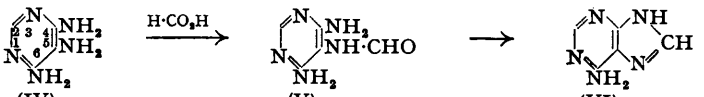
Benziminazole from o-phenylenediamine (1·08 *g.*), *formic acid* (90%; 1 *c.c.* ≡ 2 *mols.*), *and buffer solution* (30 *c.c.*); 5 *days at room temp. M. p.* 169—171° (*pure*).

Buffer	3N-HCl	N-NaOAc–N-HCl				
pH, initial and final	0·2	0·5	1·8	2·3	2·7	3·3
Yield, g.	0·79	0·98	0·60	0·29	0·08	0
Yield, %	67	83	51	25	7	0
M. p.	←	169—171°				→

impurity described in the Experimental section, was precipitated by adjustment to pH 8·5—9 with ammonia. It will be seen that pure benziminazole is formed under these mild conditions only between pH 0·2 and 2·7, the highest yield (83%) being obtained at pH 0·5.

Adenine (Attempts).—The second glyoxaline ring synthesis attempted was the condensation of 4 : 5 : 6-triaminopyrimidine (IV) with formic acid, in the hope of obtaining adenine (VI), under mild conditions. Although in the biosynthesis of purine derivatives some facts have been presented in support of the view that the pyrimidine ring is formed last (Bentley, *loc. cit.*; Schlenk, *loc. cit.*), the evidence is not conclusive as yet. Synthesis of this important purine was first accomplished by Traube (*Annalen*, 1904, **331**, 64; cf. Hoffer, Emil Barell Jubilee Vol., Basle, 1946, p. 428) whose cyclisation procedure involved heating a formamido-derivative at 230°. This method was too vigorous to be employed by Todd *et al.* in the synthesis of purine derivatives of biological importance, and the milder method elaborated consisted of heating the

more reactive thioformamido-derivatives with basic reagents (Baddiley, Lythgoe, McNeil, and Todd, *J.*, 1943, 383; Howard, Lythgoe, and Todd, *J.*, 1945, 560; Kenner and Todd, *J.*, 1946, 852; Andrews, Kenner, and Todd, *J.*, 1949, 2302). In a recent synthesis of theophylline



(IV) (V) (VI)

which involved a final cyclisation of a formamido-compound, the conditions employed were short heating in aqueous sodium hydroxide (Gepner and Kreps, *J. Gen. Chem. Russia*, 1946, **16**, 179; *Chem. Abs.*, 1947, **41**, 96; see also Bobranski and Synowiedski, *J. Amer. Pharm. Assoc., Sci. Edn.*, 1948, **37**, 62).

Although it is well-known that in the pyrimidine (IV) only the amino-group in position 5 shows normal properties (Lythgoe, *Quart. Reviews*, 1949, **3**, 201; for ultra-violet light absorption studies see Marshall and Walker, *J.*, 1951, 1004) it seemed just possible that at some definite pH an amino-group in position 4 or 6, instead of being involved in tautomerism with the adjacent ring-nitrogen atom, might react normally. In experiments, at a buffered pH range, with 4 : 5 : 6-triaminopyrimidine and formic acid in water at room temperature, the product was isolated at the end by bringing the pH to 8·5—9·0 with ammonia. In every case, however, the product, which had an indefinite m. p., gave analytical results for the uncyclised 5-formamido-compound (V) (see Table VII). The product was formed only at pH 0·3—2·8, the highest yield (61%)

TABLE VII.

4 : 6-*Diamino-5-formamidopyrimidine* (V) *from* 4 : 5 : 6-*triaminopyrimidine* (0·31 *g.*), *formic acid* (90%; 0·2 *c.c.*, ≡ *ca.* 2 *mols.*), *and buffer solution* (10 *c.c.*); 4 *days at room temp. M. p.*[a] *ca.* 300—360°.

Buffer	1½N-HCl	N-NaOAc–N-HCl					
pH (initial and final)	0·3	1·1	2·2	2·8	3·0	3·3	3·8
Yield, g.	0·17	0·23	0·17	0·01	0	0	0
Yield, %	45	61	45	3	0	0	0
M. p.[a]	←	About 300—360°			→		

[a] Melting commences at about 300°, followed by slow resolidification and final melting at about 360° (the m. p. of adenine). If plunged into the m. p. apparatus at 360°, the substance melts immediately, resolidifies, and melts again at once.

being obtained at pH 1·1. Since there was a possibility that the synthesis of adenine itself under comparatively mild conditions might proceed in two stages (formylation and cyclisation) necessitating different pH conditions, the formamido-derivative, formed at acid pH and known by its method of isolation to be stable at pH 9, was treated with sodium hydroxide solutions of pH 11, 12, 13, and 14 in the cold, and then adenine searched for by Kossel's test (*Z. physiol. Chem.*, 1888, **12**, 252), but without success. At pH 14 the formyl group was removed, and the original triaminopyrimidine was obtained. In order to prove the constitution of the formamido-derivative it was finally cyclised to adenine at 230° [it has since been made by Cavalieri *et al.*, (*J. Amer. Chem. Soc.*, 1949, **71**, 533) by a vigorous method, and by Clark and Kalckar (*J.*, 1950, 1029) in cold aqueous solution].

(c) C–C *Condensations of the Claisen–Knoevenagel Type* (*also Glycine-catalysed*).

The Claisen and the Knoevenagel reactions, which were originally easily distinguished, are now difficult to define separately. They are at present considered to belong to the larger group of reactions which have been classified until recently as base-catalysed reactions. Modern work on reactions of the Claisen–Knoevenagel type has shown that in certain cases these may be catalysed by either bases or acids (Watson, " Modern Theories of Organic Chemistry," Oxford Univ. Press, 1941, pp. 152—158; *Trans. Faraday Soc.*, 1941, **37**, 707; Hammett, " Physical Organic Chemistry," McGraw-Hill, New York, 1940, pp. 343—348; Dewar, " The Electronic Theory of Organic Chemistry," Oxford Univ. Press, 1949, pp. 107, 123—125). The essential features of both of these reactions, which were first recognised by Lapworth nearly 50 years ago (*J.*, 1901, **79**, 1269; Lapworth and Hann, *J.*, 1902, **81**, 1508; Hann and Lapworth,

J., 1904, **85**, 46) may best be expressed by the following tentatively accepted equations, where an aldehyde is being condensed with a compound containing an active methylene group :

Base-catalysed :

$$B + CH_2R'\cdot COR'' \rightleftharpoons BH^{\oplus} + {}^{\ominus}CHR'\cdot COR''$$

$$R\cdot CHO + {}^{\ominus}CHR'\cdot COR'' \rightleftharpoons R\cdot CH(O^{\ominus})\cdot CHR'\cdot COR''$$

$$R\cdot CH(O^{\ominus})\cdot CHR'\cdot COR'' + BH^{\oplus} \rightleftharpoons R\cdot CH(OH)\cdot CHR'\cdot COR''$$

$$R\cdot CH(OH)\cdot CHR'\cdot COR'' \longrightarrow R\cdot CH{:}CR'\cdot COR'' + H_2O + B$$

Acid-catalysed :

$$R\cdot CHO + H^{\oplus} \rightleftharpoons R\cdot CH(\overset{\oplus}{O}H)\cdot OH \xrightleftharpoons{CH_2R'\cdot COR''} R\cdot CH(OH)\cdot CHR'\cdot COR'' + H^{\oplus}$$

$$R\cdot CH(OH)\cdot CHR'\cdot COR'' \longrightarrow R\cdot CH{:}CR'\cdot COR'' + H_2O + H^{\oplus}$$

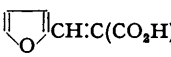
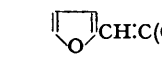
In the first case the reaction starts by the base pulling off a proton, and in the second by the aldehydonium cation pushing off a proton, from the activated methylene group (cf. Swain, *J. Amer. Chem. Soc.*, 1950, **72**, 4578, who has suggested that for polar reactions in water a concerted push–pull mechanism may be operating). Both mechanisms postulate an intermediate aldol stage, and in some cases this has been isolated. From the equations it can be inferred that the more activated the methylene group, the weaker will be the basic or acidic catalyst required to cause the removal of the essential proton, and the milder the conditions necessary for the reaction to take place. With a suitable choice of reactants these condensations might be induced to proceed under very mild conditions indeed in aqueous solution; and with a very reactive methylene compound it is possible that it would be sufficiently ionised in water to proceed without a catalyst—unless the solvent itself is considered to be the catalyst.

There are occasional references, spread over many years, to the Claisen–Knoevenagel type of reaction being carried out in aqueous solution in the cold in presence of alkali, but only recently has the importance of pH control been stressed (Schöpf and Thierfelder, *Annalen*, 1935, **518**, 127; D.R.-P. 702,894, 703,952; Hinz, Meyer, and Schücking, *Ber.*, 1943, **76**,676; Schechter, Green, and LaForge, *J. Amer. Chem. Soc.*, 1949, **71**, 1517, 3165; cf. Schöpf, *ibid.*, 1950, **72**, 2816). The most systematic study is that of Schöpf and Thierfelder, who examined the condensation between aldehydes and β-keto-acids in buffered aqueous solution at 25° and discussed the possible rôle of this type of condensation in biosynthesis.

In our work, the first example was the preparation of benzylideneacetophenone from benzaldehyde and acetophenone, both of which occur naturally. No precipitation took place at acid pH, but at pH 13 a 96% yield of the pure product resulted; at pH 12, the yield was 52% of a slightly impure product, and at pH 11 no product was obtained. Thus it appeared that, if this type of condensation is to take place at a pH less than 12, compounds containing a more active methylene group must be sought. This led to a consideration of reactions of the Knoevenagel type.

As far back as 1909, Dakin (*J. Biol. Chem.*, **7**, 49) had carried out a number of Knoevenagel reactions in aqueous alcohol at 37° or 100°, using amino-acids as catalysts, the object being the same as ours—to find out if reactions of this type, catalysed by well-known cell constituents, could have any place in cell metabolism. The catalytic effect of amino-acids on condensations of this general type has been noted in more recent work (Fischer and Marschall, *Ber.*, 1931, **64**, 2825; Blanchard, Klein, and MacDonald, *J. Amer. Chem. Soc.*, 1931, **53**, 2809; Kuzin and Nevraeva, *Biochimia*, 1939, **4**, 142; 1941, **6**, 261). These workers, like Dakin, stressed the possible biochemical importance of this activating influence of glycine and other amino-acids.

It was therefore decided to study the influence both of pH and of glycine on condensations of the Knoevenagel type. Furfuraldehyde, which occurs naturally, was chosen on account of its very active aldehyde group and its convenient solubility in water, and was condensed with two compounds containing methylene groups activated on both sides, namely, malonic acid, which is found in some plants, and acetylacetone. Aqueous solutions of furfuraldehyde and malonic acid reacted only in the presence of glycine; furfurylidenemalonic acid (VII) was then

(VII)  (VIII) 

precipitated in 61% yield. It was, however, unsuitable for an accurate pH and catalytic study, owing to its indefinite melting point.

The second product, furfurylideneacetylacetone (VIII), proved suitable for more accurate examination (its constitution is assumed by analogy with other acetylacetone condensation

products). When saturated aqueous solutions of furfuraldehyde and acetylacetone are mixed alone, the natural pH of the solution is 4·1. Overnight an emulsion starts to form, and this slowly crystallises over a period of days. The oil may be the intermediate aldol. The variation of the yield with the duration of the experiment at the natural pH 4·1 is shown in Table VIII.

TABLE VIII.

Furfurylideneacetylacetone (VIII) *from furfuraldehyde* (0·96 *g.*) *and acetylacetone* (1·0 *g.*) *in water* (20 *c.c.*) *at the natural* pH (4·1), *at room temp. M. p.* 55—57° (*pure*).

Duration, days	3	6	10	14
Yield, g.	0·31	0·78	1·06	1·20
Yield, %	17	44	60	67
M. p.	55—57°	←	54—57°	→

The effect of pH is shown in Table IX. The product was obtained in 60—76% yield in the pH range 3·6—6·5, but was not pure. Two points emerge from these results. (i) Formation of the product, without added reagent or ions, supports the view stated above that such a con-

TABLE IX.

Furfurylideneacetylacetone (VIII) *from furfuraldehyde* (0·96 *g.*), *and acetylacetone* (1·0 *g.*) *in buffer solution* (20 *c.c.*); 6 *days at room temp. M. p.* 55—57° (*pure*).

Buffer	N-NaOAc–N-HCl		N-NaOAc–N-AcOH			N-KH_2PO_4–N-NaOH		
Initial pH	2·0	3·6	3·7	4·1	4·7	6·5	7·7	8·5
Final pH	1·0	3·4	3·7	4·1	4·7	6·5	7·4	8·2
Yield, g.	oil	1·11	1·06	1·18	1·35	1·23	oil	oil
Yield, %	—	62	60	66	76	69	—	—
M. p.	←	54—57° →		← 52—55° →		50—53°		

densation might occur if the aldehyde and methylene groups are sufficiently reactive. (ii) This particular Claisen–Knoevenagel condensation to give (VIII) takes place only under acid conditions (pH 3·6—6·5), supporting the acid-catalysed mechanism.

The effect of glycine as a catalyst on the reaction is recorded in Table X, no buffer being

TABLE X.

Furfurylideneacetylacetone (VIII) *from furfuraldehyde* (0·96 *g.*), *acetylacetone* (1·0 *g.*) *and glycine, in water* (20 *c.c.*); 3 *days at room temp. M. p.* 55—57° (*pure*).

Glycine, g.	0	0·1	0·15	0·25	0·5	1·0	1·5	2·0 [a]
pH	4·1	4·3	4·3	4·4	4·5	4·5	4·6	4·7
Yield, g.	0·31	1·0	1·05	1·07	1·20	1·47	1·50	1·63
Yield, %	17	56	59	60	67	83	84	92
M. p.	←			55—57°				→

[a] 25 C.c. of water used.

used. The emulsion first precipitated started to crystallise within a few hours, compared with days when glycine was omitted, and the product was always pure. The slight gradual changes observed in the pH from the natural value of 4·1 must be due to the presence of the comparatively large amounts of glycine. From Table IX it can be deduced that the slight increase in pH in Table X from 4·1 to 4·7 cannot be responsible for the large observed increase in the yield; that it must thus be due to the glycine, the catalytic effect of which is best seen by comparing the first two columns in Table X. In the later columns, with the larger amounts of glycine, a salting-out effect may also be operating. No satisfactory explanation of the glycine catalysis can be offered. The glycine may act by combining with the aldehyde to form a loose, reversible addition product (cf. Gulland and Mead, *J.*, 1935, 210), the regenerated aldehyde molecules being suitably activated (cf. Kuzin and Nevraeva, *locc. cit.*).

(d) *Combination of* C–N *and* C–C *Condensations to give Pyrrole and Pyridine Derivatives.*

Pyrroles.—Robinson (*locc. cit.*) has produced some convincing evidence for the origin of the pyrrole nucleus in a large number of alkaloids from the skeleton of the amino-acid ornithine.

The origin of the pyrrole nuclei present in pyrrole pigments has recently been the subject of many reviews, the latest by Granick (*Ann. Rev. Plant Physiol.*, 1951, **2**, 115). Conclusive evidence from biological tracer experiments has been produced that the nitrogen and the methylene-carbon atoms of glycine, but not the carboxyl-carbon atom, are involved in the

biosynthesis and appear in the pyrrole rings intact in the 1 : 2-positions, and below the propionic acid or vinyl side-chains. From present knowledge of the general metabolic activity of glycine (Bentley, *Ann. Reports*, 1948, **45**, 241; Swanson and Clark, *loc. cit.*), no clue can be obtained regarding the entity employed in the biosynthesis of the pyrrole nuclei—whether glycine itself reacts, directly or through some active derivative. Concerning the nature of the other unit or units immediately involved with glycine in the biosynthesis, there is at present no direct experimental evidence; but in theoretical approaches designed to form the postulated common pyrrole precursor, α-ketoglutaric acid and the semialdehyde of succinic acid have been suggested, the interactions being assumed to be brought about in part by oxidative condensations.

This biological work with glycine has brought into prominence three chemical investigations in which glycine under mild conditions is said to give pyrrole-type derivatives, but in no case was a conclusive result obtained (Errera and Greenstein, *Arch. Biochem.*, 1947, **14**, 477; 1947, **15**, 445; *J. Nat. Cancer Inst.*, 1947, **8**, 39; Fu, Price, and Greenstein, *Arch. Biochem. Biophys.*, 1951, **31**, 83; Kuzin and Guseva, *Biochimia*, 1948, **13**, 27; Fischer and Fink, *Z. physiol. Chem.*, 1944, **280**, 123). It is relevant that a successful pyrrole synthesis under comparatively mild conditions, but using the more reactive α-aminoacetoacetic ester in place of glycine, was carried out by Kondo, Ono, and Sato (*J. Pharm. Soc. Japan*, 1937, **57**, 1).

In the present search for milder conditions three sets of starting units (A, B, and C) were

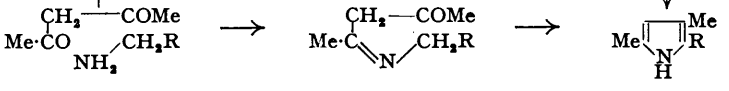

Method A.


Method B.


Method C.

examined : the [Method B, as already mentioned, properly belongs to section (a) but is placed in the present section for convenience].

Method A. The work of Fischer and Fink (*Z. physiol Chem.*, 1944, **280**, 123) with glycine and formylacetone was repeated, and similar inconclusive results obtained. Glycine analogues containing a more active methylene group were therefore selected, *viz.* : aminoacetonitrile and glycine ester hydrochloride. In both of these, unlike glycine, the amino-group is not involved in zwitterion formation with the β-carbon group, and the latter is therefore available to activate the α-methylene group. Acetylacetone was chosen instead of formylacetone, since as it is a symmetrical compound only one product can result, and because the expected derivatives, which if formed would be precipitated from solution, could be synthesised more easily by conventional methods for comparison. The condensation of the two reactants to give a pyrrole involves a combination of a Schiff base and a Claisen–Knoevenagel condensation. The pyrrole might be formed by simultaneous condensation or through the intermediate Schiff base, thus :


(IX) (X) (XI)

The reaction between aminoacetonitrile and acetylacetone was first studied over an approximate pH range (see Table XI). The product was precipitated within the pH range ~5·6—8·7, the maximum yield (69%) of slightly impure product being obtained at pH ~8·0—8·7. It was, however, the Schiff base (X; R = CN). At pH above 9·3, a feeble Ehrlich test

TABLE XI.

4-Cyanomethyliminopentan-2-one (X; R = CN) *from aminoacetonitrile hydrogen sulphate* (1·54 *g.*) *and acetylacetone* (1·0 *g.*) *in aq.* K_2CO_3 (20 *c.c.*); 8 *days at room temp. M. p.* 112—113° (*pure*).

K_2CO_3, g.	0·2	0·3	0·5	0·7	1·0	1·4	2·0	3·0	5·0	6·0
Initial pH	4·4	5·6	5·8	6·9	7·2	8·0	8·7	9·3	10	>10
Final pH	4·4	4·5	4·6	5·8	8·0	8·7	8·6	9·2	10	>10
Yield, g.	0	0·19	0·52	0·92	0·94	0·96	0·86	•	•	•
Yield, %	0	14	38	67	68	69	62	—	—	—
M. p.		← 108—111° →			← 110—112° →					

• Trace of high-melting compound.

was obtained on warming. When kept, these solutions deposited a trace of a high-melting compound which did not give the Ehrlich test. The Schiff base being slightly impure, no further examination of the reaction was made.

Glycine ethyl ester hydrochloride was condensed with acetylacetone in buffer solutionnot be account of the appreciable solubility of the product in water, excess of the buffer could . On

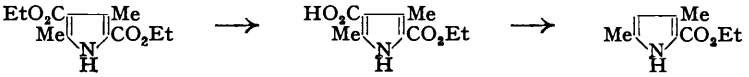
TABLE XII.

4-Carbethoxymethyliminopentan-2-one (X; R = CO_2Et) *from glycine ethyl ester hydrochloride* (1·40 *g.*), *and acetylacetone* (1·0 *g.*) *in buffer solution* (N-KH_2PO_4–N-NaOH) (20 *c.c.*); 2 *days at room temp. M. p.* 66—68° (*pure*).

Initial pH	7·1	7·5	7·7	8·3	8·7	9·0
Final pH	4·5	5·8	6·0	6·5	7·4	8·3 [a]
Yield, g.	0	0·17	0·39	0·88	0·85	0
Yield, %	0	9	21	48	46	0
M. p.		←	66—68°		→	

[a] Feeble Ehrlich reaction obtained.

used in this case and accurate buffering was not achieved. Results are given in Table XII. As in the first example, the compound was found to be a Schiff base (X; R = CO_2Et) and not the pyrrole. It was formed only within the limited pH range ~6—8·7 and in every case was obtained pure. The maximum yield at pH 8·3 (falling to 6·5) was 48%; when allowance is made for the considerable solubility of the product in water, this represents the maximum possible. Neither the product itself, nor any of the filtrates from which it was obtained, responded to the Ehrlich test. At pH 9·0, however, no Schiff base was precipitated

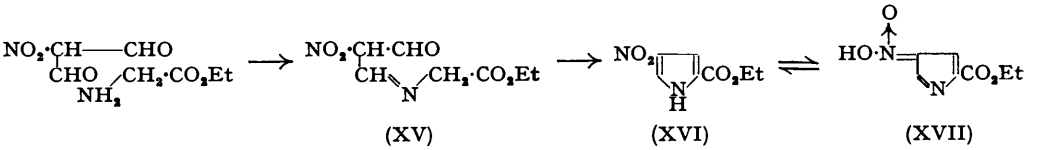


(XII) (XIII) (XIV)

and the solution gave a feeble Ehrlich reaction. In order to study the properties of the expected pyrrole (XIV), it was prepared by use of the normal drastic reagents: (*a*) by ring closure (X ⟶ XI; R = CO_2Et) with boiling alcoholic sodium ethoxide in 20% yield; and (*b*) by the known route from Knorr's widely used starting material in the pyrrole series (XII), by partial hydrolysis with concentrated sulphuric acid to (XIII), followed by distillation to give (XIV). This crystalline pyrrole was found to have a solubility in water of 0·04 g. per 100 c.c. Comparison of the Ehrlich colour given by a saturated solution of (XIV) and the reactant solution (pH 9·0) above showed that, if the Ehrlich colour in the latter is due to the expected pyrrole, it cannot be formed in greater amount than 0·5%. The Ehrlich test is not absolutely specific for pyrroles, but is given by other types of compounds, among which are some phenols, but the possibility that the positive test obtained was due to a self-condensation product of acetylacetone itself was eliminated by a separate experiment.

Since it was possible that formation of the pyrrole ring under mild conditions would require treatment at two different pH's, the crystalline Schiff base itself was shaken with buffer solutions at pH between 9 and 13. The substance dissolved rapidly but no Ehrlich test was obtained, even on long storage.

Attention was next directed to the activation of the ketonic rather than of the glycine component, sodionitromalondialdehyde being selected. Its condensation with glycine ethyl ester hydrochloride, to give the pyrrole (XVI), either directly or through the intermediate Schiff base (XV), was effected by Hale and Hoyt (*J. Amer. Chem. Soc.*, 1915, **37**, 2546; see also Hale and Honan, *ibid.*, 1919, **41**, 770; Hale and Britton, *ibid.*, p. 1020), but under relatively

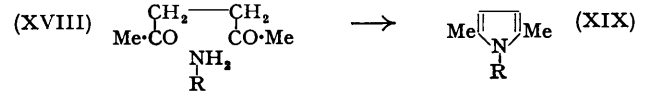


(XV) (XVI) (XVII)

drastic conditions. This work was repeated by us under mild aqueous conditions. The Schiff base was obtained in 65% yield (crude) by mixing the aqueous solutions of the reactants in the cold and leaving them overnight. The pyrrole was obtained in 32% yield (crude) by

immediately bringing the slightly acid solution of the reactants to pH 9·6. As sodionitromalondialdehyde is very difficult to obtain pure and the free aldehyde is unstable, this reaction was unsuitable for accurate study. In addition, nitropyrroles such as (XVI), although they contain a free α-position, do not respond to the Ehrlich test, probably because (Fischer and Orth, " Die Chemie des Pyrrols," Akad. Verlagsges., Leipzig, 1934, Vol. I, p. 108) they can exist in the *aci*-form (XVII); the absence of this useful test removes a valuable and quick method of determining whether or not ring-closure to a pyrrole has taken place. Enough encouragement has been obtained, however, from these successful rough experiments to indicate that the methylene group in glycine ethyl ester is sufficiently active to condense with the carbonyl group of an aldehyde group under mild aqueous conditions. Search is now being made for a suitably active, stable, symmetrical 1 : 3-diketo-compound for more accurate study.

Method B. The widely used Knorr–Paal method for preparing pyrroles, by condensing primary amines with 1 : 4-diketones (XVIII ⟶ XIX) usually requires vigorous conditions.



The crude products, although sometimes obtained in good yield, often require repeated recrystallisation. One case has however been recorded where the conditions employed were milder: diethyl 3 : 6-diketo-octanedioate, when warmed with aqueous methylamine, gives 1-methylpyrrole-2 : 5-diacetic ester in good yield (Willstätter and Pfannenstiel, *Annalen*, 1921, **422**, 14).

It has now been found that the Knorr–Paal reaction proceeds smoothly in aqueous solution at room temperature with reactants chosen to give crystalline products. The 1 : 4-diketone selected was acetonylacetone; this has never been isolated from a natural source and it has been found difficult to trace naturally occurring open-chain compounds containing the grouping $CO{\cdot}CH_2{\cdot}CH_2{\cdot}CO$ in the form of a diketone, dialdehyde, or keto-aldehyde. Although octane-3 : 6-dione has been isolated from wood spirit (Pringsheim and Leibowitz, *Ber.*, 1923, **56**, 2036) it is probably only a product of the destructive distillation. Succindialdehyde, used by

TABLE XIII.

1-*Benzyl*-2 : 5-*dimethylpyrrole* (XIX; R = CH_2Ph) *from benzylamine* (1·07 *g.*) *and acetonylacetone* (1·14 *g.*) *in water* (*approx.* pH *attained by* AcOH *or* NaOH), *then made up to* 200 *c.c. with buffer solution*; 7 *days at room temp.* *M. p.* 46—48° (*pure*).

Buffer	N-NaOAc–N-AcOH		0·2N-KH_2PO_4–0·2N-NaOH					
pH, initial and final	4·4	5·1	5·8	7·1	8·2	9·2	10·9	11·5
Yield, g.	0	0	0	0·02	0·09	0·28	1·30	1·25
Yield, %	0	0	0	1	5	15	70	68
M. p.			⟵			46—48°		⟶

TABLE XIV.

2 : 5-*Dimethyl*-1-*phenylpyrrole* (XIX; R = Ph) *from aniline* (0·93 *g.*) *and acetonylacetone* (1·14 *g.*) *in water* (*approx.* pH *attained by* AcOH *or* NaOH), *then made up to* 100 *c.c. with buffer solution*; 8 *days at room temp.* *M. p.* 51—52° (*pure*).

Buffer	N-NaOAc–N-HCl		N-NaOAc–N-AcOH		0·2N-KH_2PO_4–0·2N-NaOH					
pH, initial and final	3·5	4·0	4·4	5·5	6·4	7·2	8·2	9·3	11·0	11·6
Yield, g.	0·88	1·07	1·19	1·19	0·46	0·19	0	0	0	0
Yield, %	51	63	70	70	27	11	0	0	0	0
M. p.	⟵			51—52°			⟶			

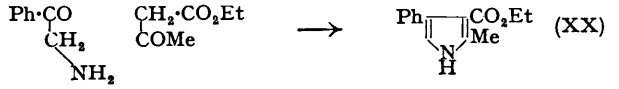
Robinson in his mild chemical synthesis of tropinone, has been postulated (*locc. cit.*) by him to be derived in the cell from ornithine or 1 : 4-diaminoadipic acid. Neither the latter acid nor αα′-diketoadipic acid has been detected in biological material, although the diketo-acid has received consideration as an intermediate in pyruvic ⟶ succinic acid metabolism (Bennet-Clark, *Ann. Rev. Biochem.*, 1937, **6**, 583; Wille, *Annalen*, 1939, **538**, 237).

When a 10% solution of acetonylacetone (pH 5·5) was mixed with either 10% benzylamine (pH 11·3) or saturated aniline solution (with acetic acid added to pH *ca.* 4), the reaction commenced almost immediately and the pyrroles (XIX; R = CH_2Ph and Ph) were deposited

as an emulsion, which rapidly crystallised. The pure products were obtained directly and the yields were respectively 62% and 55% after 1 day, and 92% and 91% after 14 days. The results of an accurate study using buffer solutions are given in Tables XIII and XIV. With the aliphatic amino-group of benzylamine the formation of the pyrrole occurs only in alkaline solution (maximum yield, 70% of pure product at pH 10·9). This is in striking contrast to the results obtained with the aromatic amino-group of aniline, where pyrrole formation takes place best in acid solution (maximum yield, 70% of pure product at pH 4·4—5·5). No satisfactory explanation can be offered for this observation.

Method C. Knorr's extensively used second method for synthesising pyrroles consists in the condensation of an α-amino-ketone, prepared *in situ* by reduction of a hydroxyimino-ketone with zinc and acetic acid, with a β-diketone or a β-keto-ester. Better yields are obtained if the amino-ketone itself, in the form of a salt, is used. Normally the reactants are heated with sodium acetate in 75% acetic acid. It was shown later that strong aqueous alkali could also be employed as the condensing agent (Piloty and Blömer, *Ber.*, 1912, **45**, 3749; Piloty and Hirsch, *Annalen*, 1913, **395**, 63): the reaction mixtures were either warmed gently or kept at room temperature for several days or even weeks. The yields obtained on use of alkali were poor, which is not surprising since it is now well known that two molecules of an α-amino-ketone cyclise in neutral or alkaline solution to give a dihydropyrazine, which oxidises when kept to a pyrazine derivative. Fischer and Orth (*op. cit.*, Vol. I, p. 178) have drawn attention to the fact that, in certain cases where two units can condense in two different ways, Knorr's method gives different pyrroles according to whether acid or alkaline conditions are used; and they describe a condensation in which the pH was kept at 6. A recent application of this modification of Knorr's method has been reported by Corwin and Krieble (*J. Amer. Chem. Soc.*, 1941, **63** 1831).

Since both acid and alkali appear to catalyse this type of condensation, it was considered that a study of such a reaction in aqueous solution over a pH range might be of interest. The reaction between ω-aminoacetophenone (as hydrobromide) and ethyl acetoacetate, to give the known compound ethyl 2-methyl-4-phenylpyrrole-3-carboxylate (XX), was selected. No naturally occurring α-amino-ketones can be traced, but the system might well be formed in the cell as an intermediate by a route similar to Knorr's, *e.g.*, $CO{\cdot}CH_2 \xrightarrow{\text{nitrite}} CO{\cdot}C{:}N{\cdot}OH \xrightarrow{\text{reduction}} CO{\cdot}CH{\cdot}NH_2$. Ethyl acetoacetate is not known in Nature, but the free acid is a well-known metabolite. As in method A, this condensation could be regarded as a combination of a Schiff base and a Claisen–



(XX)

Knoevenagel reaction. Since the methylene group involved in the present case is activated on both sides, the Claisen–Knoevenagel part of the reaction might be expected to proceed more easily, and in fact the double condensation to give the expected pyrrole readily took place. The results of an accurate pH study with buffer solutions are shown in Table XV. It is of interest that the pyrrole is formed over the wide pH range 3·9—8·2, the highest yield of the slightly

TABLE XV.

Ethyl 2-methyl-4-phenylpyrrole-3-carboxylate (XX) *from ω-aminoacetophenone hydrobromide* (0·60 *g.*) *and ethyl acetoacetate* (0·36 *g.*) *in buffer solution* (50 *c.c.*); **3** *days at room temp. M. p.* 104—106° (*pure*).

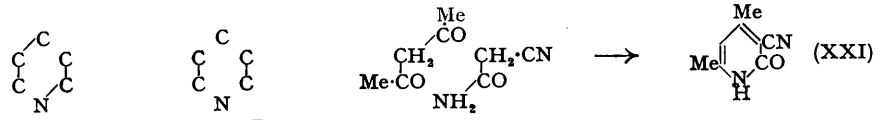
Buffer	N-NaOAc–N-AcOH				N-KH_2PO_4–N-NaOH					
pH, initial and final ...	3·9	4·5	5·5	6·1	6·3	6·6	6·9	7·2	7·8	8·2
Yield, g.	0·01	0·02	0·08	0·09	0·18	0·24	0·40	0·40	0·40	0·40
Yield, %	2	3	13	14	28	38	63	63	63	63
M. p.	← 103—105° →							← 102—105° →		

impure product (63%) being obtained at pH 6·9. On the alkaline side (pH 7·2—8·2), the product is probably contaminated by some dihydropyrazine.

Pyridines.—Approaches to the biosynthesis of pyridine derivatives have been confined to some pyridine alkaloids, and the widely distributed vitamins nicotinamide and pyridoxine. Robinson (*locc. cit.*) has produced arguments for the biological origin of the pyridine nucleus of some alkoloids from lysine, and successful alkaloid syntheses under mild conditions, carried out

by Robinson, and by Schöpf (*locc. cit.*) and Anet, Hughes, and Ritchie (*locc. cit.*), strongly support the theory. The present evidence for the origin of nicotinamide favours 3-hydroxyanthranilic acid as the probable immediate precursor (Stokstad and Jukes, *Ann. Rev. Biochem.*, **1949**, **18**, 444; Mitchell, " Vitamins and Hormones," 1950, Vol. VIII, p. 140). For pyridoxine the only evidence so far presented is that neither alanine (Holden and Snell, *J. Biol. Chem.*, **1949**, **178**, 799) nor α-formiminopropionic acid (Shive and Shive, *J. Amer. Chem. Soc.*, **1946**, **68**, 117) is a precursor.

In the present work it has been shown that the pyridine ring system can be synthesised under mild aqueous conditions at room temperature from two units (method A), or from four units (method B).



Method A. Method B.

Method A. One of the most fruitful methods for synthesising pyridine compounds is that involving the condensation of cyanoacetamide with a 1 : 3-diketone, -keto-ester, or -keto-aldehyde. Normally the reactants are heated in alcohol with piperidine, but refluxing in water with piperidine acetate has been used (Mariella and Kvinge, *J. Amer. Chem. Soc.*, **1948**, **70**, 3126). Milder conditions were used by Sen-Gupta (*J.*, **1915**, **107**, 1347). Condensation of acetylacetone and cyanoacetamide to 3-cyano-4 : 6-dimethyl-2-pyridone (XXI) (Wagtendonk and Wibaut, *Rec. Trav. chim.*, **1942**, **61**, 728) was selected for a pH study. Difficulties were experienced here with buffering action on the alkaline side, and a large volume of the buffer, with consequent reduction in yield, had to be used at alkaline pH. At nearly neutral or slightly acid pH, it was necessary to employ smaller volumes of buffer in order to obtain the product. Although accurate buffering was achieved in every case, no exact picture of the influence of pH on the yield could be obtained by this method, owing to the varying dilution. A second series of experiments was therefore carried out with rough adjustment of the pH by means of different quantities of potassium carbonate, the volume being kept constant. The results of the two series are given in Tables XVI and XVII. The highest yield obtained by the more accurate

TABLE XVI.

3-Cyano-4 : 6-dimethyl-2-pyridone (XXI) *from cyanoacetamide* (1·68 *g.*) *and acetylacetone* (2·0 *g.*) *in buffer solution* (700 *c.c.*) (N-KH_2PO_4–N-NaOH); 1 *day at room temp.* *M. p.* 288—289° (*pure*).

pH, initial and final	6·4 [a]	7·4 [b]	8·4	9·1	10·5
Yield, g.	0·60	0·85	1·18	2·19	1·23
Yield, %	20	29	40	74	42
M. p.	←		288—289°		→

[a] 40 c.c. of buffer used; no precipitate in 700 c.c. [b] 300 c.c. of buffer used; trace of precipitate in 700 c.c.

TABLE XVII.

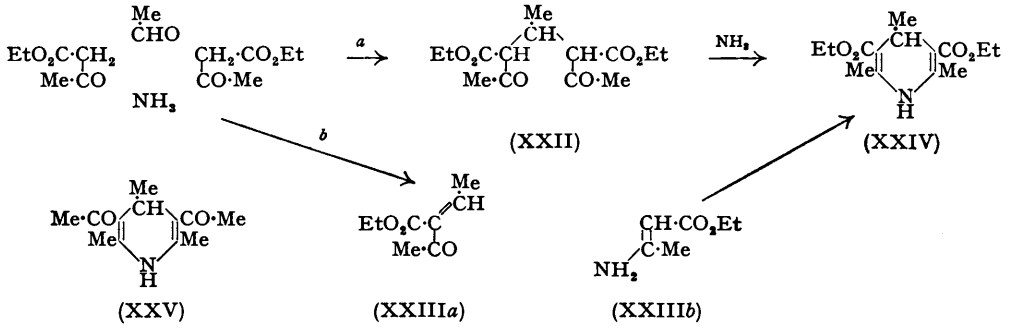
3-Cyano-4 : 6-dimethyl-2-pyridone (XXI) *from cyanoacetamide* (1·68 *g.*) *and acetylacetone* (2·0 *g.*), *and* K_2CO_3 *in water* (40 *c.c.*); 1 *day at room temp.* *M. p.* 288—289° (*pure*).

K_2CO_3, g.	0	0·04	0·06	0·1	0·4	0·8	1·2	1·5	2
Initial pH	4·4	6·4	7·1	7·7	8·5	8·6	8·9	9·0	9·1
Final pH	4·4	6·6	7·2	8·5	10	9·8	10	10	10
Yield, g.	0	0·80	1·35	2·48	2·83	2·89	2·84	2·83	2·79
Yield, %	0	27	46	84	96	98	96	96	94
	←				288—289°				→

buffering method (Table XVI) was 74% at pH 9·1. By the rough method (Table XVII), reasonable control of pH was attained; the highest yield, rising to 98%, was obtained at pH *ca.* 8·5—10. In both cases the product is formed in 20—27% yield on the acid side, at pH about 6·4. In all experiments it was obtained pure directly.

Method B. *The Hantzsch dihydropyridine synthesis.* The reactions so far described have in every case been concerned with the condensation between two molecules only. For study of reactions involving more than two molecules, the Hantzsch dihydropyridine synthesis was

selected (Hantzsch, *Annalen*, 1882, **215**, 8); its exact mechanism is still in doubt. For the condensation between two molecules of ethyl acetoacetate and one of aldehyde-ammonia, to give diethyl 1 : 4-dihydrocollidine-3 : 5-dicarboxylate (XXIV), there is supporting evidence for either mechanism *a* or *b*. The first stage of route *a*, the formation of the 1 : 5-diketone (XXII), is a well-known type of condensation which may proceed in one stage or by a preliminary Claisen–Knoevenagel condensation between one molecule each of acetaldehyde and ethyl acetoacetate to give ethyl α-ethylideneacetoacetate (XXIII*a*), followed by a Michael condensation of this product with a second molecule of the β-keto-ester: the second stage—



the action of ammonia on the 1 : 5-diketone to give the dihydropyridine (XXIV)—although well-supported by examples, led to much controversy in the older literature in cases like the present, owing to the possibility of internal condensation in the diketone in presence of basic reagents to give *cyclo*hexane instead of (or as well as) pyridine derivatives. In the case under discussion Rabe and Elze (*Annalen*, 1904, **332**, 19) stated that treatment of the diketone (XXII) with alcoholic ammonia resulted in both types being formed, although they only described the isolation of the dihydropyridine. In favour of mechanism *b*, it is known that, at low temperature in presence of piperidine, acetaldehyde and ethyl acetoacetate can form (XXIII*a*) (Knoevenagel, *Ber.*, 1898, **31**, 735; Auwers and Eisenlohr, *J. pr. Chem.*, 1911, **84**, 100), and also that ammonia and ethyl acetoacetate under certain conditions can give ethyl β-aminocrotonate (XXIII*b*). Although neither of these intermediates has been isolated from the Hantzsch synthesis reaction mixture, it was shown by Beyer (*Ber.*, 1891, **24**, 1662; cf. Knoevenagel, *loc. cit.*, p. 738) that, when prepared separately and then mixed, they readily gave the dihydropyridine (XXIV).

In the preparation of (XXIV) Hantzsch heated the aldehyde-ammonia and the keto-ester without a solvent to the boiling point. Phillips (*J. Amer. Chem. Soc.*, 1949, **71**, 4003) has recently carried out some syntheses by mixing one molecule of various aromatic aldehydes with two of ethyl acetacetate in an equal volume of ethanol, adding two volumes of concentrated aqueous ammonia, and then heating; he states that the yields obtained in this way are better than those obtained by treating the aldehyde with two molecules of ethyl β-aminocrotonate or with one of ethyl β-aminocrotonate and one of ethyl acetoacetate.

As two typical examples of the Hantzsch synthesis, the preparation of diethyl 1 : 4-dihydrocollidine-3 : 5-dicarboxylate (XXIV) and of 3 : 5-diacetyl-1 : 4-dihydrocollidine (XXV) was selected for pH study in aqueous solution. The results for compound (XXIV) prepared from acetaldehyde, ethyl acetoacetate, and ammonia are shown in Table XVIII. A large volume of buffer solution was required for satisfactory buffering. With the reactants in theoretical amounts the pure compound (XXIV) was formed within the pH range 6—10, the highest yield (43%) being obtained at pH 8·5. Since this pH is close to that of 10% aqueous ammonium carbonate

TABLE XVIII.

Diethyl 1 : 4-*dihydrocollidine*-3 : 5-*dicarboxylate* (XXIV) *from acetaldehyde* (0·44 *g.*), *ethyl acetoacetate* (2·60 *g.*) *and ammonia* (d 0·88; 0·17 *g.*) *in buffer solution* (N-KH_2PO_4–N-NaOH) (500 *c.c.*); 4 *days at room temp.* *M. p.* 129—130° (*pure*).

pH, initial and final	5·5	6·0	7·1	7·5	8·5	10 [a]
Yield, g.	0	0·08	0·47	0·89	1·15	0·55
Yield, %	0	3	18	33	43	21
M. p.		←	129—130°		→	125—127°

[a] Fell to 8·9.

(9·2), the condensation was also performed in a different way, with a view to its possible use as a preparative method. The reactants were dissolved in the minimum amount of 10% ammonium carbonate solution, thus ensuring a large excess of ammonia (pH during the experiment 8·5). The slightly yellow product was washed with acid to remove a yellow basic impurity, and yielded 70% of nearly pure pyridine derivative.

The results for the second example, the condensation, in buffer solutions, of the theoretical amounts of acetaldehyde, acetylacetone, and ammonia to give (XXV), are shown in Table XIX. The pure product was formed within the pH range 5·5—9·3, with a maximum yield of 29% over the range 6·6—8·5. In this case, a reasonable volume of buffer sufficed to maintain the pH within fairly accurate limits.

TABLE XIX.

3 : 5-*Diacetyl*-1 : 4-*dihydrocollidine* (XXV) *from acetaldehyde* (0·44 *g.*), *acetylacetone* (2·0 *g.*), *and ammonia* (d 0·88; 0·17 *g.*) *in buffer solution* (100 *c.c.*); 4 *days at room temp.* *M. p.* 152—153° (*pure*).

Buffer	N-NaOAc–N-AcOH	N-KH_2PO_4–N-NaOH							
Initial pH	5·5	6·0	6·3	6·6	7·1	8·0	8·5	9·0	9·3	10
Final pH	5·5	6·0	6·3	6·3	7·0	7·5	8·1	8·6	8·6	9·0
Yield, g.	0·02	0·24	0·50	0·59	0·59	0·59	0·60	0·47	0·23	0
Yield, %	1	12	24	29	29	29	29	23	11	0
M. p.	←	152—153°								→

The experiment was repeated with 10% aqueous ammonium carbonate solution replacing the theoretical amount of ammonia, with the object of seeking a useful preparative method as before. The condensation proceeded at pH 8·1 more slowly than in the corresponding preparation of (XXIV), but a 51% yield of pure (XXV) was obtained. In contrast to the first example performed under these conditions, no by-product contaminated the product.

In view of the number of different ways in which the four molecules taking part in these Hantzsch syntheses could react with themselves or with one another, it is perhaps surprising that the reaction proceeds so smoothly in aqueous solution and gives such good yields.

Since the suggested mechanism for both types of condensation (methods A and B) involves reactions which are usually considered to be of the base-catalysed type, it is of interest that the products are formed at acid as well as alkaline pH.

EXPERIMENTAL.

General.—Experiments recorded in Tables I—XX were performed in stoppered flasks at room temperature (average 18°). The starting materials were purified by distillation or crystallisation just before use. After the times stated, which were found after a number of trials, the products were separated by filtration, washed with a small amount of water, and dried for 1—2 days at room temperature in a vacuum-desiccator. The filtrates were set aside for a further period, in order to ensure that no appreciable additional amounts of the products were deposited. Yields and m. p.s, unless stated otherwise, are for these initial untreated products. Each product was also prepared by the usual methods described in the literature, and the two samples were proved to be identical by mixed m. p. All m. p.s are corrected.

Analyses.—All our products were analysed, the specimen used being obtained from a mixture of all the pure samples from the various ranges of pH. Before analysis, each specimen was finely powdered and dried at room temperature in a vacuum-desiccator for 5 days.

Solubilities of Reactants in Water at Room Temperature (*Average* 18°).—Most of these were already known. Solubilities not listed or uncertain were, in g. per 100 c.c. of water at room temperature, as follows :

p-Anisaldehyde	0·33	*α*-Naphthylamine	0·14
p-Hydroxybenzaldehyde	0·81	*o*-Phenylenediamine	2·16
Cinnamaldehyde	0·14		

Buffer Solutions.—The buffer solutions used are those given by Britton (" Hydrogen Ions," 3rd Edn., Chapman and Hall, London, 1942); 0·2N-solutions were used at first, but later N-solutions were found to be more suitable. In each case the reactants were dissolved separately in suitable volumes of the buffer solution, and the pH was measured to ensure that no change had occurred. The reactant solutions were then mixed, and the pH was at once determined again, unless precipitation of the product prevented this. When the reaction was complete, the pH of the filtrate was finally determined yet again.

The Muirhead pH meter, with the usual glass electrode supplied, was used throughout for measurements up to pH 10. In the later work, for pH's >10, the new " Alkacid " glass electrode (Doran Instrument Co., Ltd., Stroud) was substituted.

Schiff Bases.—Details are given in the Table XX. The smaller aqueous solution was added to the larger. After a short interval, normally less than 1 minute, an emulsion usually appeared suddenly and, after occasional shaking, crystallisation commenced. After 1 day (for the second case in the Table, 5 days' storage or 15 hours' shaking after seeding; for the fourth case, 2 days) the product

TABLE XX.

Preparation of Schiff bases, quinoxaline, and diazepine derivatives from saturated aqueous solutions of the reactants.

Amine in water, g./c.c.	Ketone or aldehyde in water, g./c.c.	Limits of pH[a]	Yield, %	M. p.	Formula	Found, % C	Found, % H	Found, % N	Calc., % C	Calc., % H	Calc., % N
$Ph{\cdot}NH_2$ 9·3/273	$Ph{\cdot}CHO$ 10·6/3000	4·2—7·6	74	51—52°[b]	$C_{13}H_{11}N$	86·1	6·3	8·0	86·2	6·1	7·7
α-$C_{10}H_7{\cdot}NH_2$ 2·86/2000	$Ph{\cdot}CHO$ 2·12/600	4·2—7·1	84	72—73	$C_{17}H_{13}N$	88·2	5·5	6·3	88·3	5·6	6·1
$Ph{\cdot}NH_2$ 2·79/82	p-$HO{\cdot}C_6H_4{\cdot}CHO$ 3·66/450	4·9—7·6	91	192—193	$C_{13}H_{11}ON$	79·0	5·9	7·2	79·2	5·6	7·1
$Ph{\cdot}CH_2{\cdot}NH_2$ 3·21/140[c]	,,	4·9—11·3	81	205—206	$C_{14}H_{13}ON$	79·3	6·6	6·6	79·6	6·2	6·6
$Ph{\cdot}NH_2$ 2·79/82	p-$MeO{\cdot}C_6H_4{\cdot}CHO$ 4·08/1250	4·7—7·6	70	61—63	,,	79·3	6·0	6·6	,,	,,	,,
$Ph{\cdot}CH_2{\cdot}NH_2$ 5·35/233[c]	p-$MeO{\cdot}C_6H_4{\cdot}CHO$ 6·8/2083	4·7—11·3	87	40—42	$C_{15}H_{15}ON$	79·7	6·9	6·5	80·0	6·7	6·2
$Ph{\cdot}NH_2$ 2·79/82	$Ph{\cdot}CH{:}CH{\cdot}CHO$ 3·96/2800	4·9—7·6	85	108—109	$C_{15}H_{13}N$	86·8	6·5	7·0	87·0	6·3	6·8
$Ph{\cdot}NH_2$ 3·72/109	$CH_2(COMe)_2$ 4/32	3·3—7·6	74	51—53[d]	$C_{11}H_{13}ON$	75·6	7·5	8·0	75·4	7·4	8·0
m-$NH_2{\cdot}C_6H_4{\cdot}OH$ 4·36/168	,,	3·3—7·4	84	132—134[d]	$C_{11}H_{13}O_2N$	68·9	6·8	7·5	69·1	6·8	7·3
o-$C_6H_4(NH_2)_2$ 3·24/150	$Me{\cdot}CO{\cdot}COMe$ 2·58/10·3	3·5—7·4	93[e]	105—107	$C_{10}H_{10}N_2$	76·2	6·3	17·7	76·0	6·3	17·7
o-$C_6H_4(NH_2)_2$ 5·4/250	$CH_2(COMe)_2$ 5/40	3·3—7·4	36[f]	131—133	$C_{11}H_{12}N_2$[g]	76·8	6·9	16·0	76·7	7·0	16·3

[a] pH of the aldehyde (or ketone) solution given first, followed by pH of the base solution. [b] This is the usual figure given in the literature. Pyl (*Ber.*, 1927, **60**, 287) gives 53·5°, but, using his method, we obtained 51—52°. [c] Large excess of water. [d] Monoanil. [e] Mixture warmed by 4°; 2 : 3-dimethylquinoxaline collected after 5 mins. [f] Colourless hydrate of 5 : 7-dimethyl-2 : 3-benzo-1 : 4-diazepine deposited from violet solution; easily dehydrated in a vacuum-desiccator. Solubility, 0·74 g. per 100 c.c. of water at room temperature. [g] Found: *M* (cryoscopic in C_6H_6), 164. Reqd.: *M*, 172.

was collected. The literature on Schiff bases contains many references to the existence of individuals in two forms, possessing different m. p.s and sometimes different colours. In the older work these were thought to be *cis-trans*-isomers but the later investigations (de Gaouck and Le Fevre, *J.*, 1939, 1392; Jensen and Bang, *Annalen*, 1941, **548**, 106; van Alphen, *Rec. Trav. chim.*, 1942, **61**, 875) support the view that they are simply different cyrstalline modifications.

2-*Amino*-4 : 6-*dimethylpyrimidine* (III; R = NH_2).—When prepared by Combes and Combes's method (*Bull. Soc. chim.*, 1892, **7**, 788) this melted 152—154° (anhyd.). Our sample (Table IV) was analysed (Found: C, 57·9; H, 7·1; N, 34·2. Calc. for $C_6H_9N_3$: C, 58·5; H, 7·3; N, 34·2%). Solubility, 4·0 g. per 100 c.c. of water at room temperature.

4 : 6-*Dimethyl*-2-*phenylpyrimidine* (III; R = Ph).—When prepared by Pinner's (*loc. cit.*) method this melted at 81—83°. Our sample (Table V) was analysed (Found: C, 78·2; H, 6·0; N, 15·7. Calc. for $C_{12}H_{12}N_2$: C, 78·3; H, 6·5; N, 15·2%).

Benziminazole.—The sample prepared by the method described in *Org. Synth., Coll. Vol.* II, p. 65 had m. p. 169—171°. In our experiments (Table VI), traces of a high-melting mauve substance were deposited, and this was filtered off and discarded before isolation of the benziminazole, by adjustment to pH 8·5—9·0 with ammonia and storage for 4 hours before collection. This substance, which was not investigated further, was not formed when the experiments were conducted in an atmosphere of nitrogen, and is probably an oxidation product of *o*-phenylenediamine. Our sample of benziminazole (Table VI), m. p. 169—171°, was analysed (Found: C, 71·6; H, 5·4; N, 23·4. Calc. for $C_7H_6N_2$: C, 71·2; H, 5·1; N, 23·7%).

4 : 6-*Diamino*-5-*formamidopyrimidine* (V).—Details are given in Table VII. Since this compound had an indefinite m. p., no attempt was made to obtain it by another method for comparison. It was isolated at the conclusion of the experiment by adjustment to pH 8·5—9 with ammonia, and filtering after 4 hours. The products from experiments at various pH's were mixed, dried *in vacuo* at 130° overnight, and analysed (Found: C, 39·2; H, 4·4; N, 45·8. Calc. for $C_5H_7ON_5$: C, 39·2; H, 4·6; N, 45·8%).

When 4 : 5 : 6-triaminopyrimidine (3·1 g.) was dissolved in N-hydrochloric acid (50 c.c.), formic acid (90%, 2 c.c.) added, and the whole kept for 2 days, stout colourless needles of the hydrochloride (1·84 g., 40%) were deposited (Found : C, 32·1; H, 4·1; N, 36·5. Calc. for $C_5H_8ON_4Cl$: C, 31·6; H, 4·2; N, 36·9%). The filtrate, treated with ammonia to pH 8·5—9, gave 1·0 g. (21%) of the free formamido-compound. In connection with the experiments carried out to cause cylisation to adenine, described below, it should be noted that the formamido-compound did not respond to Kossel's test (*loc. cit.*) for adenine.

Adenine.—The above formamido-compound (0·3 g.) was heated at 230° for 4 hours. The product was dissolved in dilute hydrochloric acid, then filtered, and aqueous ammonia added to the filtrate to bring the pH to 8·5—9. The precipitate (0·2 g.) was collected, dried in a vacuum at 130° overnight, and analysed (Found : C, 44·3; H, 3·5; N, 52·5. Calc. for $C_5H_5N_5$: C, 44·5; H, 3·7; N, 51·9%). The identity of this product with adenine was confirmed from its m. p. [351—353° (decomp.)], its response to Kossel's test (*loc. cit.*), and its ultra-violet absorption spectrum in N/20-hydrochloric acid ($\lambda_{max.}$ 2615—2635 Å; $\varepsilon_{max.}$ = 13,150) (cf. Baddiley, Lythgoe, and Todd, *J.*, 1943, 387; Clark and Kalckar, *J.*, 1950, 1030). Attempts were made to cyclise the formamido-compound (0·1 g.) by storing, at room temperature for 7 days, sodium hydroxide solutions of it at pH 14 (2 c.c.), pH 13 (5 c.c.), pH 12 (9 c.c.), and pH 11 (10 c.c.), and then searching for adenine by Kossel's test (*loc. cit.*), but with negative results. At pH 14, with the formamido-derivative (0·50 g.) in 3N-sodium hydroxide (5 c.c.), the original triamino-pyrimidine (0·26 g.), proved by m. p. and mixed m. p. 254—258° (decomp.) (inserted at 245°), was deposited.

Benzylideneacetophenone.—(*a*) Saturated aqueous solutions of benzaldehyde (1·06 g. in 300 c.c.) and acetophenone (1·2 g. in 200 c.c.) were mixed, and a rough pH range 1—11 made up by the addition of acid or alkali. Although on storage for several weeks, the solutions of pH 8—11 became cloudy, no crystalline compound was obtained even after addition of a nucleus of benzylideneacetophenone. Owing to the dilution used, the product could not have been the intermediate aldol, a known crystalline compound (Schöpf and Thierfelder, *loc. cit.*, p. 149). In any case the amount obtained was so small that it did not invite further investigation.

(*b*) Benzaldehyde (1·06 g.) in 0·1N-sodium hydroxide (300 c.c.) was mixed with acetophenone (1·2 g.) in the same solvent (200 c.c.). The mixture (pH 13) rapidly became cloudy, and crystallisation occurred on storage. After 7 days, the product was collected and washed with water. The yield was 2·0 g. (96%), and the m. p. 55—57° (pure) (Found : C, 86·3; H, 6·1. Calc. for $C_{15}H_{12}O$: C, 86·5; H, 5·8%). Repetition of the experiment, but with 0·01N-sodium hydroxide (pH 12), gave 1·08 g. (52%) of low m. p. (53—55°) With 0·001N-sodium hydroxide (pH 11), only a faint cloudiness was obtained after 21 days' storage. The authentic specimen (*Org. Synth., Coll. Vol.* I, p. 78) after crystallisation has m. p. 55—57°.

In attempts to catalyse the condensation with glycine alone, mixed aqueous solutions of benzaldehyde and acetophenone were treated with various amounts of glycine, but no product was precipitated after several weeks' storage.

Furfurylidenemalonic Acid (VII).—To malonic acid (2·1 g.) and glycine (1 g.) in water (15 c.c.) was added furfuraldehyde (1·9 g.). On shaking, the aldehyde went into solution (pH 2·7). Crystallisation commenced overnight, and after 2 days the product (1·6 g., 44%) was collected. After 5 days more, a further yield of 0·63 g. (17%) was obtained. The product was also prepared by Liebermann's method (*Ber.*, 1894, **27**, 285). All samples, alone or admixed, had m. p. 190—205° (decomp.), with darkening from 180° onwards. The sample prepared in aqueous solution was analysed (Found : C, 52·7; H, 3·4. Calc. for $C_8H_6O_5$: C, 52·8; H, 3·3%). On repetition of the experiment, but without glycine, at the natural pH (2·1) of the solution, no precipitation took place, even after 7 days in presence of a nucleus.

Furfurylideneacetylacetone (3-*Acetyl*-4-*furylbut*-3-*en*-2-*one*) (VIII).—Details sufficing for the preparation of this *compound* are given in Tables VIII, IX, and X. The material crystallises in small colourless prisms. The m. p. was not raised by recrystallisation from methanol [Found, for non-recrystallised material : C, 67·2; H, 5·5%; *M* (Rast, and cryoscopic in benzene), 187. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·6%; *M*, 178].

4-*Cyanomethyliminopentan*-2-*one* (X; R = CN).—Most of the details regarding this preparation are given in Table XI. Aminoacetonitrile hydrogen sulphate was obtained by the method described in *Org. Synth., Coll. Vol.* I, p. 298. For analysis the *ketone* was recrystallised from ethanol, the m. p. of the colourless plates being thus raised from 110—112° to 112—113° [Found : C, 60·5; H, 7·1; N, 20·4%; *M* (Rast), 145. $C_7H_{10}ON_2$ requires C, 60·9; H, 7·3; N, 20·3%; *M*, 138].

4-*Carbethoxymethyliminopentan*-2-*one* (X; R = CO_2Et).—Details will be found in Table XII [Found : C, 58·1; H, 8·2; N, 7·8%; *M* (Rast), 190. Calc. for $C_9H_{15}O_3N$: C, 58·4; H, 8·1; N, 7·6%; *M*, 185]. The compound was also prepared by Fischer's method (*Ber.*, 1901, **34**, 438). Solubility, 4·8 g. per 100 c.c. of water at room temperature.

Ethyl 3 : 5-*Dimethylpyrrole*-2-*carboxylate* (XIV).—The Schiff base (X; R = CO_2Et) (2·0 g.) was cyclised by heating it under reflux with sodium (0·25 g.) in ethanol (10 c.c.) for 3 hours (cf. Fischer and Fink, *Z. physiol. Chem.*, 1948, **283**, 152). The solution was poured into water (100 c.c.), and the product filtered off, washed with water, and dried. The yield was 0·35 g. (20%), and the m. p. 124—126° without recrystallisation (Found : C, 64·4; H, 7·4; N, 8·7. Calc. for $C_9H_{13}O_2N$: C, 64·7; H, 7·8; N, 8·4%). The sample prepared by the known method (Fischer and Walach, *Ber.*, 1925, **58**, 2820; see also Corwin and Quattlebaum, *J. Amer. Chem. Soc.*, 1936, **58**, 1083) melted at 122—125° after repeated crystallisation from ethanol. A mixed m. p. confirmed the identity. Solubility, 0·04 g. per 100 c.c. of water at room temperature.

3-*Carbethoxymethylimino*-2-*nitropropaldehyde* (XV).—Sodionitromalondialdehyde (Hill and Torrey, *Amer. Chem. J.*, 1899, **22**, 89) (0·31 g.) in water (3 c.c.) was added at room temperature to glycine ethyl

ester hydrochloride (0·28 g.) in water (1 c.c.). Within 5 minutes, the product commenced to crystallise. After 1 hour 0·24 g. (m. p. 97—100°) and after 1 day an additional 0·02 g. (m. p. 98—101°) was obtained (total yield, 65%). Recrystallisation from ethanol (5 c.c.) gave the pure product, m. p. 100—102° alone or mixed with an authentic specimen prepared by the method of Hale and Hoyt, *loc. cit.*, who give m. p. 104° (Found : C, 41·6; H, 4·9; N, 13·9. Calc. for $C_7H_{10}O_5N_2$: C, 41·6; H, 5·0; N, 13·9%).

Ethyl 4-Nitropyrrole-2-carboxylate (XVI).—The same quantities as above were used but, immediately after mixing, the slightly acid solution was brought to pH 9·6 by the addition of 20% aqueous potassium carbonate (5 c.c.). Precipitation of an oil started almost immediately, followed later by crystallisation. Next morning the nitropyrrole (0·12 g., 32%; m. p. 165—170°) was filtered off. Recrystallisation from ethanol (1 c.c.), or water (32 c.c.), gave the pure product, m. p. 170—172° alone or mixed with an authentic specimen prepared by the method of Hale and Hoyt, *loc. cit.*, who give m. p. 174°), not raised by further recrystallisation (Found : C, 46·1; H, 4·3; N, 15·3. Calc. for $C_7H_8O_4N_2$: C, 45·7; H, 4·3; N, 15·2%).

1-Benzyl-2 : 5-dimethylpyrrole (XIX; R = CH_2Ph).—A solution (pH 5·5) of acetonylacetone (5·7 g.) in water (57 c.c.) was added to a solution (pH 11·3) of benzylamine (5·35 g.) in water (214 c.c.). After a few minutes, an emulsion suddenly appeared, which rapidly crystallised. The solution was protected from light. After 1 day, the collected precipitate weighed 5·7 g. (62%) and, in a separate experiment, after 14 days 8·5 g. (92%). The m. p. alone or admixed with an authentic specimen prepared by the method of Hazlewood *et al.* (*J. Roy. Soc. N.S. Wales*, 1937, **71**, 92) who give 48° was 46—48° (Found : C, 83·8; H, 8·5; N, 7·8. Calc. for $C_{13}H_{15}N$: C, 84·3; H, 8·1; N, 7·6%). See also Table XIII.

2 : 5-Dimethyl-1-phenylpyrrole (XIX; R = Ph).—After acetonylacetone (3·42 g.) in water (34 c.c.) and aniline (2·79 g.) in water (82 c.c.) had been mixed, sufficient acetic acid (*ca.* 10 c.c.) was added to bring the pH to *ca.* 4. The solution was protected from light. After 1 day the yield was 2·8 g. (55%), and after 14 days 4·6 g. (91%). The m. p. alone or admixed with an authentic specimen prepared by the method of Hazlewood *et al.* (*loc. cit.*) was 51—52° (Found : C, 84·1; H, 7·5; N, 8·2. Calc. for $C_{12}H_{13}N$: C, 84·2; H, 7·6; N, 8·2%). See also Table XIV.

ω-Aminoacetophenone Hydrobromide.—This was prepared from phenacyl bromide (24 g.) (*Org. Synth., Coll. Vol.* II, p. 480) by Mannich and Hahn's method (*Ber.*, 1911, **44**, 1545 : see also Slotta and Heller, *Ber.*, 1930, **63**, 1027, and Lutz *et al.*, *J. Org. Chem.*, 1947, **12**, 106). The crude mixture of ω-aminoacetophenone hydrobromide and ammonium bromide obtained (35·5 g.) was repeatedly crystallised from ethanol, and the product (12·5 g.), m. p. 217° (decomp.), which probably still contained ammonium bromide, was separated.

Ethyl 2-Methyl-4-phenylpyrrole-3-carboxylate (XX).—Prepared by Knorr and Lange's method (*Ber.*, 1902, **35**, 3002) and crystallised from methanol, this had m. p. 104—106°. Our sample, m. p. 103—105° (Table XV), was crystallised once from methanol, and the m. p. after this treatment, alone or admixed with the authentic specimen, was 104—106° (Found : C, 73·8; H, 6·4; N, 6·4. Calc. for $C_{14}H_{15}O_2N$: C, 73·4; H, 6·6; N, 6·1%).

3-Cyano-4 : 6-dimethyl-2-pyridone (XXI).—A specimen prepared by Wagtendonk and Wibaut's method (*loc. cit.*) melted 288—289°. Our sample (Tables XVI and XVII) was analysed (Found : C, 65·2; H, 5·4; N, 19·1. Calc. for $C_8H_8ON_2$: C, 64·9; H, 5·4; N, 18·9%).

Diethyl 1 : 4-Dihydrocollidine-3 : 5-dicarboxylate (XXIV).—The crude product prepared by Hantzsch's method (*loc. cit.*) required repeated crystallisation from ethanol–water (1 : 2) to raise the m. p. to 129—130°. The sample described in Table (XVIII) was analysed (Found : C, 62·6; H, 7·9; N, 5·6. Calc. for $C_{14}H_{21}O_4N$: C, 62·9; H, 7·9; N, 5·2%). For our more approximate method, ethyl acetoacetate (5·2 c.c.) and acetaldehyde (1·2 c.c.) were dissolved in aqueous ammonium carbonate (10%; 100 c.c.; pH 9·2), and left for a day. The initial and the final pH during the experiment were 8·5. The yellow crystalline product was collected and shaken with dilute hydrochloric acid for 5 hours to remove a yellow basic impurity. The resulting colourless material obtained after filtration and washing with water weighed 3·75 g. (70%) and melted at 127—129°.

3 : 5-Diacetyl-1 : 4-dihydrocollidine (XXV).—This was prepared as described by Combes and Combes (*Bull. Soc. chim.*, 1889, **1**, 15; see also Knoevenagel and Ruschhaupt, *Ber.*, 1898, **31**, 1029; Mumm and Petzold, *Annalen*, 1938, **536**, 18); it melted 152—153°. The sample described in Table XIX was analysed (Found : C, 69·0; H, 8·2; N, 6·8. Calc. for $C_{12}H_{17}O_2N$: C, 69·6; H, 8·2; N, 6·8%). For our more approximate method, acetylacetone (2·2 c.c.) and acetaldehyde (0·6 c.c.) were dissolved in aqueous ammonium carbonate solution (10%; 20 c.c.; pH 9·2) and left for 4 days. The initial and the final pH during the experiment were 8·1. The product was collected and washed with water, the yield being 1·0 g. (51%) and the m. p. 152—153° (pure).

One of us (C. A. C. H.) thanks the Department of Scientific and Industrial Research for a maintenance grant.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, June 14th*, 1951.]