1030. *Organic Reactions in Aqueous Solution at Room Temperature. Part II.* The Influence of* pH *on Condensations involving the Linking of Carbon to Carbon, of Carbon to Nitrogen, and of Carbon to Sulphur.*

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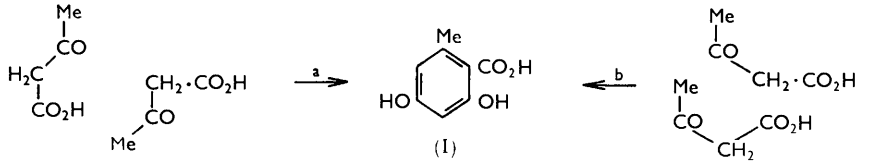
Further simple reactions under " physiological " (" cell-possible ") conditions are described, leading to the formation of the benzene, the pyrazole, and the thiazole ring system. Two examples of the Michael reaction under mild conditions are given.

In continuation of our investigations designed to broaden the field known as " syntheses under physiological (or ' cell-possible ') conditions " in relation to both biogenetic problems and general organic synthetical methods, some further simple model condensations in aqueous solution at room temperature over pH ranges have been examined. The reaction conditions employed were similar to those used before. Water-soluble reactants were chosen so that, on reaction, water-insoluble crystalline products were precipitated in a pure or nearly pure state, and were isolated directly from the reaction mixture by filtration, the usual losses thus being avoided. The permissible physiological pH range was discussed in Part I, and the conclusion reached that it might be as wide as 1·5—11. All the reactions investigated in this present work were found to proceed somewhere within this range.

The work is described below in four sections, according to the type of condensation employed, namely, C–C ⟶ a benzene derivative, C–C + C–N (Michael reaction), C–N ⟶ a pyrazole derivative, and C–S and C–N ⟶ a thiazole derivative. No evidence has been adduced in any of the sections for an exact scheme of biogenesis, which must come ultimately from specific biochemical investigations: but, with the exception of one reactant, all the condensations were carried out with substances either already known in Nature or containing naturally occurring groups.

(a) *Double* C–C *Claisen–Knoevenagel Condensation of Two* C_3 *Aliphatic Units, to give a Benzene Derivative.*—Robinson has long held the opinion that the readily formed, stable, benzene nucleus is very unlikely to be formed in Nature by only a single route.[1] He noted [2] the occurrence of erythritol in certain lichens as an ester of orsellinic acid (I), and suggested for the latter an origin from the condensation of two C_4 units, which might



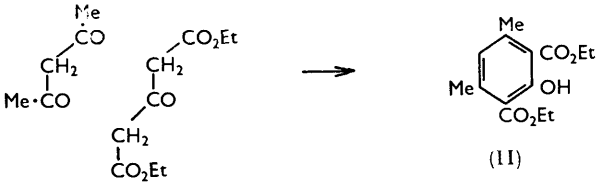
be acetoacetic acid, by route a or b. We have investigated a condensation of type (Ia). However, acetoacetic acid is too unstable and the ethyl ester has never been shown to undergo self-condensation in this manner. In Part I it was shown that furfuraldehyde, with its very active aldehyde group, condensed with the very active methylene group of acetylacetone to give furfurylideneacetylacetone in 60—76% yield over the pH range 3·6—6·5, supporting the acid-catalysed mechanism for Claisen–Knoevenagel reactions. The present model, which might be formed by a double Claisen–Knoevenagel condensation of the symmetrical C_3–C_3 type as in (Ia), was diethyl 2-hydroxy-4,6-dimethylisophthalate

* Part I, *J.*, 1951, 3155.

[1] Sir Robert Robinson, " The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, p. 30.

[2] Madrid Lecture, IX Congr. Internat. Union Pure Appl. Chem., 1934, p. 6; ref. 1, p. 8.

(II), prepared by condensing acetylacetone with diethyl acetonedicarboxylate. This reaction was first described by Prelog *et al.*,[3] who used alcoholic sodium ethoxide at room temperature. Similar condensations have been performed by using piperidine in boiling alcohol,[4] or an aqueous-alcoholic glycine–piperidine buffer at pH 7 at room temperature.[5] The original condensation, to give the ester (II), was repeated but in saturated buffered aqueous solution, and Table 1 shows that the phenol was formed in the pH range 5·6—9·2, the highest yield (86%) of pure product being precipitated at pH 7·3—7·6.



(II)

Unlike the simpler example of furfurylideneacetylacetone above, this condensation can therefore proceed by the acid- or base-catalysed mechanisms; and the fact that it takes place within the physiological pH range supports the possible origin of some natural benzene derivatives by a $C_3 + C_3$ route of this type.

TABLE 1.

Diethyl 2-hydroxy-4,6-dimethylisophthalate (II) from acetylacetone (1·00 g.) in buffer solution (N-KH_2PO_4–N-NaOH) (20 ml.) and diethyl acetonedicarboxylate (2·02 g.) in buffer solution (180 ml.); 5 and 12 days at room temp. M. p. 43—44° (pure).

Initial pH	4·5[a]	5·6	6·2	6·8	7·3	7·6[b]	8·2	8·6	9·2	11·0
Final pH[c]	4·5	5·7	6·3	6·8	7·3	7·7	8·2	8·5	8·7	10·0
Yield (g.) (5 days)	0	0·15	0·85	1·72	2·16	2·20	2·08	1·71	1·01	0·0
Yield (g.) (12 days)	0	0·88	1·55	2·10	2·29	2·28	2·08	1·71	1·01	0·0
Yield (%) (12 days)	0	26	58	79	86	86	78	64	38	0
M. p.		42—43°		←		43—44°			→	

[a] Natural pH of the mixed aqueous solutions. [b] As a quicker preparative method, at this optimum pH a yield of 77% may be obtained after 2 days. [c] pH measured after five days.

(b) *The Michael Reaction (Cyanoethylation).* C–C *Condensation of Methyl Acetoacetate and Vinyl Cyanide, and* C–N *Condensation of Aniline and Vinyl Cyanide.*—In the Michael reaction [6,7] the usual conditions are to heat the reactants in anhydrous solvents with sodium ethoxide or similar catalysts.[8] Only occasionally have acidic catalysts been employed, and usually under anhydrous conditions. In some cases neither solvent nor heat is used.[9] Only a few examples of the use of aqueous solvents are known.[10,11] Kamlet [11] pointed out that if the ionisation constant of the addendum was high, then the reaction should take place without the usual basic catalyst, and he showed that barbituric acid added to β-nitrostyrene at room temperature in aqueous mixtures with dioxan, methanol, or acetic acid. Since the Michael reaction, if it could be shown to

[3] Prelog, Metzler, and Jeger, *Helv. Chim. Acta*, 1947, **30**, 675.
[4] *J. Org. Chem.*, 1958, **23**, 34.
[5] Mühlemann, Festshrift Paul Casparis, 1949, p. 159; *Chem. Abs.*, 1951, **45**, 596.
[6] (*a*) Henecka, "Chemie der Beta-dicarbonylverbindungen," Springer-Verlag, 1950, p. 243; (*b*) *Org. Reactions*, 1959, **10**, 179.
[7] Arndt, Scholz, and Frobel, *Annalen*, 1935, **521**, 111.
[8] Hickinbottom, "Reactions of Organic Compounds," Longmans Green, 3rd edn., 1957, p. 48; ref. 6(*b*), p. 264.
[9] Kloetzel, *J. Amer. Chem. Soc.*, 1947, **69**, 2271; Leonard and Shoemaker, *ibid.*, 1949, **71**, 1876; Dreiding and Tomasewski, *ibid.*, 1955, **77**, 411.
[10] Ikawa, Stahmann, and Link, *J. Amer. Chem. Soc.*, 1944, **66**, 902; McKinney *et al.*, *ibid.*, 1950, **72**, 2599; 1951, **73**, 1643; Herzog, Gold, and Geckler, *ibid.*, 1951, **73**, 749; Shechter and Zeldin, *ibid.*, 1951, **73**, 1276; Jung and Cordier, *Compt. rend.*, 1959. **249**, 711.
[11] Kamlet, *J. Amer. Chem. Soc.*, 1955, **77**, 4896; *ibid.*, 1956, **78**, 4556.

proceed under sufficiently mild conditions, might merit consideration in biogenetic problems, it was decided to study the influence of pH on two examples of cyanoethylation.[12]

(i) *Methyl acetoacetate and vinyl cyanide.* Bruson and Riener condensed one mol. of methyl acetoacetate with two mol. of vinyl cyanide, obtaining methyl αα-di-(2-cyanoethyl)-acetoacetate (III) by reaction in dioxan containing Triton B at 30—40°; they obtained a yield (crude) of 50%. We also used methyl acetoacetate since it is much more soluble

$$2CH_2{:}CH{\cdot}CN + CH_3{\cdot}CO{\cdot}CH_2{\cdot}CO_2Me \longrightarrow CH_3{\cdot}CO{\cdot}C(CH_2{\cdot}CH_2{\cdot}CN)_2{\cdot}CO_2Me \quad (III)$$

in water (38%) than the ethyl ester (14·3%), and the solubility of vinyl cyanide in water (7·3%) made it also suitable. Our study (Table 2) shows that the ester (III) was obtained within the approximate pH range 7·7—11·5. It was impossible, even when large amounts of buffer were used, to keep the pH constant during the 6-day period; this was probably due to hydrolysis. The product was precipitated in analytically pure state from the

TABLE 2.

Diethyl αα-di-(2-cyanoethyl)acetoacetate (III) from methyl acetoacetate (2·32 g.) in buffer solution (N-KH_2PO_4–N-NaOH) (10 ml.) and vinyl cyanide (2·12 g.) in buffer solution (40 ml.); 6 days at room temp. M. p. 154—156° (pure; all products).

Initial pH	7·7	9·0	9·5	10·0	10·5	10·7	10·9	11·5	12·0
Final pH	7·3	7·6	7·8	7·9	8·0	9·7	10·0	10·3	10·0
Yield (%)	Trace	7	13	23	44	46	45	26	—

reaction mixture; the maximum yield (46%) was obtained at pH 10·7, falling to 9·7, *i.e.*, still within the physiological pH range. There was no trace of the monocyanoethylated product. No product was obtained in acid solution, which shows that this Michael reaction can only proceed by the base-catalysed mechanism.

(ii) *Aniline and vinyl cyanide.* Ammonia and many amines have been added to vinyl cyanide, but the conditions for addition vary from room temperature without a catalyst to an autoclave at high temperatures in presence of catalysts. In the present example, aniline was chosen as it has appreciable solubility (3·4%) in water and was known to give crystalline mono- and di-adducts, *N*-2-cyanoethyl- and *NN*-di-(2-cyanoethyl)-aniline. Cookson and Mann [14] found that aniline does not combine appreciably with boiling vinyl cyanide in the presence of sodium methoxide or acetic acid, but in acetic acid at 150° (autoclave) a mixture of mono- and di-adduct was produced. Bekhli and Serebrennikov [15] obtained a 98% yield of monoadduct by heating aniline, aniline acetate, and vinyl cyanide at 120—140°. Cymerman-Craig and Moyle [16] heated aniline hydrochloride and vinyl cyanide with diethylamine at 180° and obtained the monoadduct in 72—78% yield. Heininger [17] heated the reactants with cupric acetate monohydrate and obtained a 73% yield of the same product.

When saturated aqueous buffered solutions of aniline and vinyl cyanide were mixed, an oil was first deposited, which slowly crystallised to give the monoadduct uncontaminated with diadduct even in presence of an excess of vinyl cyanide. More dilute solutions favoured more rapid crystallisation but the yields decreased, so an optimum dilution with both factors in mind had to be employed. Table 3 shows that pure product was precipitated over the pH range 5·1—11·6, with a maximum yield of 44—45% in the pH range 7·5—10, except around pH 9·2 where there was a repeatable but unaccountable decrease of 5—6%.

Although the duration of the actual experiment was 20 days (to allow for complete precipitation), equimolar quantities of aniline and vinyl cyanide in the buffer solution

[12] Bruson, *Org. Reactions*, 1954, **5**, 79.
[13] Bruson and Riener, *J. Amer. Chem. Soc.*, 1942, **64**, 2850.
[14] Cookson and Mann, *J.*, 1949, 67.
[15] Bekhli and Serebrennikov, *Zhur. obschei Khim.*, 1949, **19**, 1553; *Chem. Abs.*, 1950, **44**, 3448.
[16] *Org. Synth.*, 1956, **36**, 6.
[17] Heininger, *J. Org. Chem.*, 1957, **22**, 1213.

TABLE 3.

N-2-Cyanoethylaniline from vinyl cyanide (1·06 g.) in buffer solution (50 ml.) and aniline (1·86 g.) in buffer solution (150 ml.); 20 days at room temperature. M. p. 50—51° (pure).

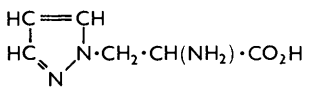
Buffer	N-AcOH–N-NaOAc		N-KH_2PO_4–N-NaOH					
Initial and final pH ...	4·2	5·1	6·1	6·8	7·5	8·0	8·3	8·8
Yield (%) [a]	—	23	38	42	44	44	45	44

Buffer	N-KH_2PO_4–N-NaOH							
Initial and final pH ...	9·2	9·7	10·0	10·4	10·8	11·0	11·6	11·9
Yield (%) [a]	39	45	45	43	43	39	17	—

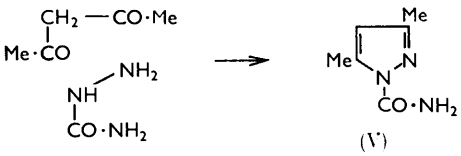
[a] All products were slightly coloured, even when the experiments were performed in nitrogen, but they melted at 50—51° and gave correct analyses.

at pH 8·8 gave a 29% yield after 7 days; using double the quantity of vinyl cyanide under the same conditions gave a 59% yield (calculated on aniline).

(c) C–N *Condensation to give a Pyrazole Derivative.*—Only one naturally occurring pyrazole derivative (IV) is known.[18] There has been as yet no approach to the biogenesis of the pyrazole ring system. Most of the methods already known for synthesising the system [19] involve intermediates and conditions unlikely to obtain in a cell. One method which offered the possibility of mild conditions was the condensation of β-dicarbonyl compounds with hydrazines; the latter have not been reported in Nature, but seem not unlikely cell intermediates. 3,5-Dimethylpyrazole-1-carboxyamide (V), prepared by



(IV)



(V)

condensation of acetylacetone and semicarbazide,[20] proved to be suitable for a pH study (Table 4). The pyrazole (V) was formed in the pH range 4·1—8·2, the highest yield of pure product (86%) being precipitated at pH 4·1 and diminishing to 17% at pH 8·2 and

TABLE 4.

3,5-Dimethylpyrazole-1-carboxyamide (V) from semicarbazide hydrochloride (2·20 g.) and acetylacetone (2·00 g.) in buffer solution (70 c.c.) (N-KH_2PO_4–N-NaOH). M. p. 111—113° (pure; all products).

Initial pH ...	4·1	4·4	4·8	5·3	5·8	6·4	7·1	7·5	8·1	8·2	10
Final pH ...	4·6	4·6	4·9	5·3	5·6	6·2	6·7	6·9	7·4	7·3	—
Duration: *	2h	2h	3h	6h	1d	2d	2d	5d	12d	12d	7d
Yield (%) ...	86	85	83	80	76	64	49	40	31	17	0

* h = hours; d = days.

nil at pH 10. The duration of the experiments to obtain the maximum yield at each pH varied greatly, from 2 hours at pH 4·1 to 12 days at pH 8·2. Below pH 4, hydrolysis and decarboxylation to 3,5-dimethylpyrazole was observed.

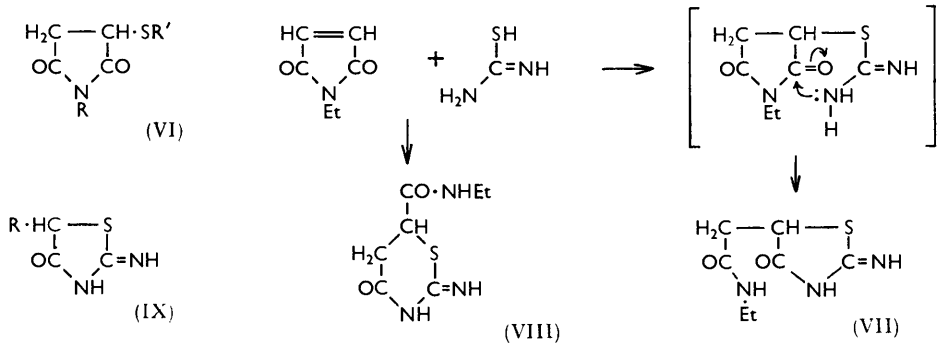
(d) *Combination of* C–S *and* C–N *Condensations to give a Thiazole Derivative.*—Only

[18] Fowden, Noe, Ridd, and White, *Proc. Chem. Soc.*, 1959, 131; Noe and Fowden, *Nature*, 1959, **184**, B.A. Suppl., 69; *Biochem. J.*, 1960, **77**, 543; Sugimoto, Watanabe, and Ide, *Tetrahedron*, 1960, **11**, 231.

[19] Thorpe's "Dictionary of Applied Chemistry," 1950, Vol. X, p. 291; Rodd, "Chemistry of the Carbon Compounds," Elsevier, 1957, Vol. IVA, p. 245; Elderfield, "Heterocyclic Compounds," John Wiley and Sons, 1957, Vol. V, p. 47.

[20] Posner, *Ber.*, 1901, **34**, 3973.

a few of the many syntheses of the thiazole ring system [21] might be of biogenetic significance. An unexpected synthesis was discovered by Marrian,[22] who referred to the possible biological importance of the addition of certain thiols to maleimides, and later [23] investigated the action of these imides with the incipient thiol group of thioureas. The normal thiols yielded products of the type (VI), in which simple addition takes place across the double bond. This was proved by treatment with Raney nickel, which caused



fission across the S–C bond, resulting in a succinimide derivative. In contrast to this, thioureas produced compounds which he showed might contain a five-membered (VII) or a six-membered ring (VIII). He prepared the compound in 69·5% yield in aqueous alcohol at room temperature. This reaction was found to be suitable for a pH study.

When buffer solutions of *N*-ethylmaleimide and thiourea were mixed, the product, proved below to be the thiazolidone (VII), was precipitated in pure, or nearly pure state, over the pH range 2·6—7·9, the highest yield (74%) of pure product being obtained at pH 5·6 (see Table 5). The rapid decline in the yield on the alkaline side is probably due to hydrolysis of the imide.[24]

TABLE 5.

N-Ethyl-α-(2-imino-4-oxothiazolidin-5-yl)acetamide (VII) from *N*-ethylmaleimide (0·625 g.) in buffer solution (35 c.c.) and thiourea (0·38 g.) in buffer solution (5 c.c.); 2 days at room temperature. M. p. 210—212° (pure).

Buffer	N-HCl–N-NaOAc	N-AcOH–N-NaOAc			N-KH_2PO_4–N-NaOH		
Initial pH	2·6	3·8	4·5	5·6	6·8	7·9	8·8
Final pH	2·6	3·8	4·5	5·6	6·7	7·6	7·9
Yield (%) [a]	37	59	72	74	63	14	Trace
M. p.[b]	209—210°		210—212°		209—211°	207—208°	

[a] Monohydrate. [b] Inserted at 190° and heated rapidly. Slow heating gives m. p. 195°, as Marrian noted.

For a structural study of the product, 2-imino-4-oxothiazolidin-5-ylacetic acid [25] (IX; R = CH_2·CO_2H) and 2-iminothiazolid-4-one (IX; R = H) [26] were prepared and their infrared spectra compared with that of our product. For the acid there was some masking in the vital region, but for the compound (IX; R = H) and our product the CO·NH frequencies were identical (1670 $cm.^{-1}$), proving that the five-membered ring structure (VII) is correct.

It is difficult to find a satisfactory mechanism for the formation of this product. It

[21] (*a*) Sprague and Land in Elderfield's "Heterocyclic Compounds," John Wiley and Sons, New York, 1957, Vol. V, pp. 484 *et seq.*; (*b*) Wiley, England, and Behr, *Org. Reactions*, 1955, **6**, 367.
[22] Marrian, *J.*, 1949, 1515.
[23] Marrian, *J.*, 1949, 1797.
[24] Cf. Gregory, *J. Amer. Chem. Soc.*, 1955, **77**, 3922.
[25] Andreasch, *Monatsh.*, 1895, **16**, 789; 1897, **18**, 56.
[26] Andreasch, *Monatsh.*, 1887, **8**, 407.

was formed, not only in aqueous alcohol (Marrian) and in aqueous buffers, but also in absolute alcohol. This rules out the possibility of the opening of the maleimide ring by addition of the elements of water, and subsequent elimination of water by a reaction involving the amino-group of thiourea. Two reactions would seem to be necessary: addition of the thiol form of thiourea across the double bond of the maleimide followed by nucleophilic attack by the more basic thiourea amino-group on the adjacent carbonyl group, with subsequent proton shift, as shown in the formulæ.

Experimental.—The general experimental conditions adopted were those described in Part I.

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