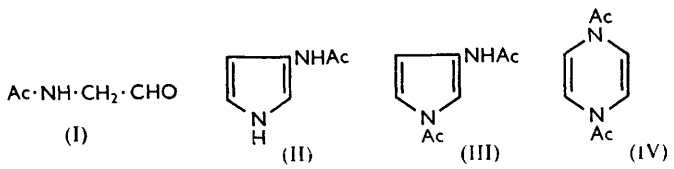
231. *Self-condensation of Acetamidoacetaldehyde and of Aminoacetone.*

By J. W. Cornforth.

Acetamidoacetaldehyde in hot, slightly alkaline, aqueous solution gives 3-acetamido-1-acetylpyrrole which is then hydrolysed to 3-acetamidopyrrole. The liquid base $C_6H_{10}N_2$ obtained by Gabriel and Colman from aminoacetone is shown to be a dihydro-2 : 5-dimethylpyrazine.

N-Acetylhexosamines, on being heated with mild aqueous alkali, give solutions which become purple on addition of Ehrlich's reagent;[1] the principal biochemical method for analysis of *N*-acetylhexosamines[2] depends on this property. In 1936 Professor W. T. J. Morgan, F.R.S., found that a solution of acetamidoacetaldehyde (I), prepared *in situ* by acid hydrolysis of the acetal, behaved similarly and was therefore interesting as a simple model. A crystalline product, m. p. 175—176° (corr.), was isolated from the heated alkaline solution, but its structure was not determined at the time. In 1950, Professor Morgan kindly made a personal communication of his results and the reaction was examined further. Sodium acetate was found to be a suitable base for generating chromogen from the aldehyde (I), and this afforded a crystalline product, m. p. 91—92°, intensely chromogenic to Ehrlich's reagent. Its properties (including a sensitivity to oxidation which defeated attempts to recrystallize it from solvents) and its composition, $C_6H_8ON_2$, pointed to 3-acetamidopyrrole (II) as a probable structure. A Curtius degradation of the known methyl pyrrole-3-carboxylate,[3] the azide being decomposed by boiling acetic acid, gave an identical substance and proved that structure (II) was correct.



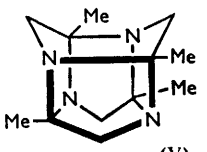
A Knorr synthesis from aminoacetaldehyde and acetamidoacetaldehyde could give the pyrrole (II), but it seemed unlikely that significant hydrolysis of the acetyl group in the aldehyde would occur under such mild conditions. 1-Acetylpyrroles, on the other hand, seem to be hydrolysed readily by alkalis,[4] so that a more likely precursor of the pyrrole (II) was 3-acetamido-1-acetylpyrrole (III). When acetamidoacetaldehyde was heated with aqueous sodium acetate and the solution was extracted every few minutes with ethyl acetate, a crystalline product, m. p. 171°, of the expected composition was isolated, to which the formula (III) is ascribed. It is probably identical with the product obtained in the early experiment. In keeping with its formulation as a 1-acylpyrrole it was relatively stable to air; it gave no colour with Ehrlich's reagent alone, but an intense purple colour was produced when the reagent was added after the substance had been warmed briefly with dilute sodium carbonate solution. The infrared spectrum was of the expected type; in particular, the presence of a band in the N–H stretching region excluded the slight possibility that the substance was an *NN*-diacetyldihydropyrazine (IV).

By these observations the production of a chromogen from acetamidoacetaldehyde (I) is explained; but the value of the latter as a model of the more complex *N*-acetylhexosamines has been nullified by Kuhn's work[5] indicating that furans, not pyrroles, are the chromogens in the Morgan–Elson analysis.

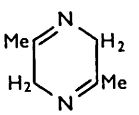
[1] Zuckerkandl and Messiner-Klebermass, *Biochem. Z.*, 1931, **236**, 19.
[2] Morgan and Elson, *Biochem. J.*, 1934, **28**, 988.
[3] Rinkes, *Rec. Trav. chim.*, 1938, **57**, 423.
[4] Ciamician and Dennstedt, *Ber.*, 1883, **16**, 2354.
[5] Kuhn, "Festschrift Prof. Dr. Arthur Stoll," Birkhauser, Basel, 1957, p. 845; and earlier papers there cited.

At one stage of this investigation we were interested to know whether simple dihydropyrazines would give coloured solutions with Ehrlich's reagent. Gabriel and Colman [6] described a base $C_6H_{10}N_2$ obtained from aminoacetone hydrochloride and potassium hydroxide. Two forms were obtained, an unstable liquid boiling near 100°/50 mm. and a more stable crystalline solid, m. p. 115—116°, isolated *via* the oxalate. Both forms, the liquid at 139° and the solid at 182°, had vapour densities corresponding to $C_6H_{10}N_2$. Because the liquid base was so unstable, the solid base and its salts were chiefly studied. It gave aminoacetone on acid hydrolysis; but since it could not be directly oxidized to dimethylpyrazine or reduced to dimethylpiperazine Gabriel and Colman were doubtful whether to describe it as a dihydropyrazine and they enumerated five other possible structures.

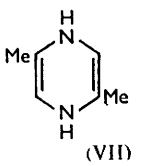
From the evidence, it seemed likely that the solid base was a polymer, dissociated to monomer when heated, of dihydrodimethylpyrazine. An interesting possibility for a reversible dimerization peculiar to dihydropyrazines is shown in structure (V). The liquid base would then be the monomer, as its boiling point suggests.



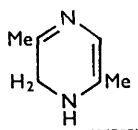
(V)



(VI)



(VII)



(VIII)

The present investigation was limited to an examination of the liquid base. The report of its instability was fully confirmed. When it was hydrogenated immediately after preparation, *trans*-2 : 5-dimethylpiperazine was formed and was characterized as its dibenzoyl derivative, thus substantiating the presence of dihydro-2 : 5-dimethylpyrazine in the liquid $C_6H_{10}N_2$. Three structures for the dihydropyrazine are possible: (VI), (VII), and (VIII). The infrared spectrum of the base showed a band at 3260 $cm.^{-1}$ (associated N–H) and a doublet in the double-bond region at 1665 and 1656 $cm.^{-1}$. If the base is substantially homogeneous, the unsymmetrical structure (VIII) is indicated; but a similar spectrum might be given by a mixture of (VI) and (VII). Dihydropyrazines from aminoketones are usually formulated as analogues of (VI) or (VII); yet the conjugated structure (VIII) seems more attractive. The liquid base gave no colour with Ehrlich's reagent.

Experimental

3-Acetamidopyrrole (II).—(i) Acetic anhydride (1·5 ml.) was added to aminoacetaldehyde diethyl acetal (2 g.) and pyridine (2 ml.) in dry ether (20 ml.). After 10 min. potassium carbonate (2·8 g.; freshly ignited) was added; the mixture was shaken occasionally for 2 hr., left overnight, and filtered. Distillation gave acetamidoacetal (2·4 g.), b. p. 72°/0·04 mm., m. p. 20°. This product (1·8 g.) was hydrolysed when heated for 5 min. with 0·1N-hydrochloric acid (50 ml.). The cooled solution was neutralized and saturated with sodium acetate trihydrate, then boiled gently (no reflux) for 2 hr., cooled, and extracted five times with half its volume of ethyl acetate. The evaporated extract was taken up in a little water, and aqueous mercuric chloride was added until precipitation was complete. The collected solid was suspended in water (10 ml.) and decomposed by hydrogen sulphide. The filtered, neutralized solution was extracted thrice with ether. After evaporation the crystalline residue (60 mg.) was purified by two sublimations at 75—90°/0·01 mm. *3-Acetamidopyrrole* (35 mg.) was a colourless crystalline powder, m. p. 91—92° (Found: C, 58·2; H, 6·3; N, 22·6. $C_6H_8ON_2$ requires C, 58·1; H, 6·5; N, 22·6%). The substance blackened after a few hours in air but a sample has been kept in nitrogen for six years without visible deterioration. A test with Ehrlich's reagent gave an intense purple colour.

(ii) Methyl pyrrole-3-carboxylate (500 mg.) was heated on a steam-bath with 90% hydrazine

[6] Gabriel and Colman, *Ber.*, 1902, **35**, 3805.

hydrate (0·23 ml.) and water (0·3 ml.) for 7 hr. The crystalline hydrazide (484 mg.), m. p. about 120°, obtained by evaporation *in vacuo* was dissolved in N-hydrochloric acid (4 ml.), cooled in ice, and treated with sodium nitrite (276 mg.) in water during 10 min. The dark mixture was extracted with ether; from the dried extract by treatment with charcoal, evaporation, and recrystallization from cold acetone by addition of water, 3-azidocarbonylpyrrole (317 mg.) was obtained as slightly discoloured irregular prisms, decomposing with decrepitation at 105°. Analysis was difficult (Found: N, 43·4. Calc. for $C_5H_4ON_4$: N, 41·2%). The azide (300 mg.) in acetic acid (3 ml.; purified) was boiled until gas evolution appeared complete (8 min.). The cooled solution was evaporated at low pressure and the product, after extraction by ethyl acetate from a considerable blue residue, was purified *via* the mercury complex as described above. Two sublimations gave colourless crystals (42 mg.) (Found: C, 58·1; H, 6·7%), m. p. 91—93° alone or mixed with the specimen obtained as in (i).

3-Acetamido-1-acetylpyrrole (III).—Acetamidoacetaldehyde diethyl acetal (2 g.) was hydrolysed for a few min. with hot 0·05N-hydrochloric acid (20 ml.). The solution was made faintly alkaline, saturated with sodium acetate, and heated in boiling water. After a few min. the mixture was cooled and extracted twice with ethyl acetate; heating was then continued and the sequence was repeated some ten times. The extracts, on evaporation, left a partly crystalline residue which was sublimed, with rejection of the first sublimate (100°/0·05 mm.) and collection at 110—170°. The product on recrystallization from methanol–ether gave *3-acetamido-1-acetylpyrrole* (50—60 mg.) as colourless leaflets, m. p. 171° (Found: C, 57·6; H, 6·3; N, 17·1. $C_8H_{10}O_2N_2$ requires C, 57·8; H, 6·0; N, 16·9%); ν (in KCl) 3300 (N–H), 1695 (1-acetyl C=O), 1660 and 1570 cm.$^{-1}$ (NH·CO).

Experiments with the Liquid Base $C_6H_{10}N_2$.—The freshly prepared, twice-distilled base [6] (384 mg.) was hydrogenated in ethanol over platinum (from 50 mg. of oxide) at room temperature and pressure. Hydrogen corresponding to 1·6 mols. was taken up overnight. The filtered solution was acidified with hydrochloric acid and evaporated; on treatment with ethanol an insoluble hydrochloride remained. A portion, on being heated with a pellet of potassium hydroxide, gave an iridescent cloud of sublimed four-sided plates, m. p. 117° (*trans*-2 : 5-dimethylpiperazine,[7] m. p. 117°). Another portion gave 1 : 4-dibenzoyl-*trans*-2 : 5-dimethylpiperazine, m. p. 225° (lit.,[7] 224—225°) (Found: C, 74·5; H, 7·0; N, 8·6. Calc. for $C_{20}H_{22}O_2N_2$: C, 74·5; H, 6·8; N, 8·7%).

In another experiment the base was redistilled (b. p. 35°/0·4 mm.) as a colourless mobile liquid which crystallized and melted at about 10°. An infrared spectrum (liquid film) was taken immediately (see above for description). The ultraviolet absorption spectrum (in ethanol) showed maxima at 310 mμ (log ε 2·44) and 275 mμ (log ε 2·97) but since oxygen was not excluded the spectrum may not be that of the original base. The base very rapidly became yellow and viscous on exposure to air. No solvent could be found for a low-temperature crystallization.

I am much indebted to Professor W. T. J. Morgan, F.R.S., for directing attention to this problem.

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[7] Bamberger and Einhorn, *Ber.*, 1897, **30**, 227.