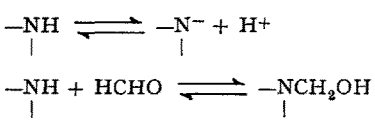
Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

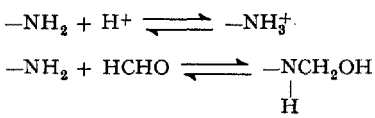
The Reaction of Inosine with Formaldehyde

It has been postulated that as inosine does not possess a basic amino group it does not react with formaldehyde[1,2]. This thesis neglected, however, to take into consideration the possibility that formaldehyde may react with the acid imino group of inosine. We shall advance here evidence in favour of the concept that inosine possesses an acid imino group and that this group is recognizable by its reaction with formaldehyde.

We have previously shown[3–5] that the existence of a formaldehyde reaction with an acid imino group can be demonstrated, over the ionization range of the latter, by a pH elevation according to the scheme

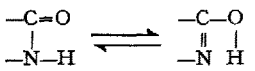


in contrast with the pH depression resulting from the formaldehyde reaction with basic amino groups, thus

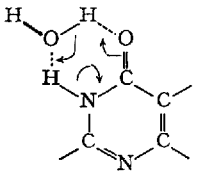


We have also shown spectrophotometrically that the reaction of formaldehyde with the $-N_9H$ group in purine[5] results in hypochromicity whereas that with uracil[5] results in bathochromicity, and that with hypoxanthine[6] is accompanied partly by decrease in optical density over the range of ca. 230 to 260 mμ and partly by an increase in optical density over the range of ca. 260 mμ to 290 mμ.

The inosine pK value of ca. 8.8 has been originally allocated to the C_6-OH group[7–10]. However, recently evidence has been accumulated to show that in purines and pyrimidines any tautomeric equilibrium



exists predominantly in the keto form[11–16]. A possible integration of the above may be achieved by postulating a resonating hydrated form, thus (uracil[5]).



The following work on inosine and on inosine-formaldehyde solutions demonstrates the existence of reaction with the acid imino group:

(a) *pH elevation.* In Figure 1 are given the titration curves of inosine in the pH region of pK 8.8 in absence and presence of formaldehyde. Greater pH elevations have been obtained with stronger formaldehyde concentrations.

The significant pH elevations accompanying the formaldehyde addition to inosine are parallel to those noted on formaldehyde reaction with succinimide, hydantoin, uracil[4,5], xanthine[4,6], hypoxanthine[6], purine[5,17] and adenine in the slightly alkaline pH range[3,4] and can therefore be reasonably attributed to the existence of an acid imino group.

(b) *Spectrophotometry.* The presence of formaldehyde results in a decrease in optical density over the range 225 mμ to 263 mμ accompanied by an increase over the higher wavelengths (see Figure 2). This result is similar to that noted in the formaldehyde reaction with hypoxanthine[6].

The conclusion that acid imino groups do react with formaldehyde signifies that formaldehyde reaction is likely to take place not merely with the basic amino

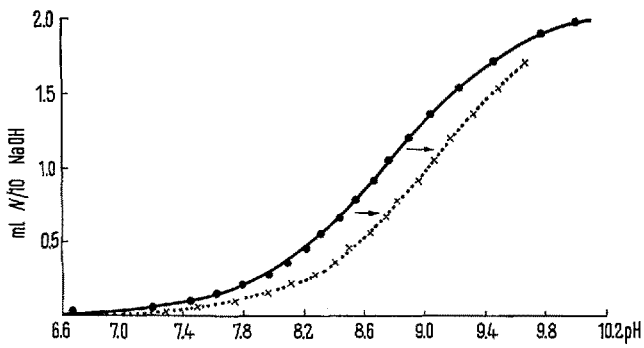


Fig. 1. Titration of inosine with and without formaldehyde at 30°C. 20 ml, 0.01 *M* inosine titrated with 0.1 *N* NaOH under pure nitrogen. Continuous line, inosine only; cross-dot line, inosine in presence of 0.5 *N* formaldehyde.

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groups – as has been suggested previously[1,18–20] – but also with any free acid imino groups present in nucleic acids. This aspect will be considered separately in further detail[21,22].

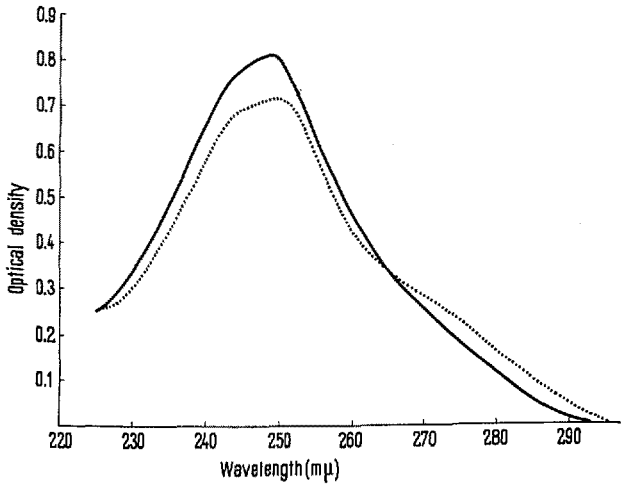

Fig. 2. Variation of the absorption spectrum of inosine on reaction with formaldehyde, at 30°C. $6.67 \cdot 10^{-5} M$ inosine; $0.05 M$ acetate buffer pH 4.70. 1.0 cm optical path length. Continuous line, inosine only; dotted line, inosine in presence of $1.0 M$ formaldehyde.

Zusammenfassung. Die Existenz einer Reaktion zwischen Formaldehyd und der sauren Iminogruppe von Inosin wird an Hand der dabei auftretenden pH-Erhöhung sowie den Veränderungen der UV-Absorption gezeigt.

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Physical Biochemistry Laboratory, South-West Essex Technical College, London (England), June 15, 1964.

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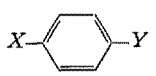
[21] All chemicals used were of Analytical Grade. Formaldehyde was purified by passage through Deacidite FF (Permutit Company, London, England), assayed by the bisulphite method and checked by the peroxide method and by titration. Spectrophotometric measurements were carried out and checked on the calibrated Beckman DK-2 recording, Perkin Elmer U-V/137 and manual Unicam SP/500 spectrophotometers.
[22] I should like to thank Mr. R. EDMONDS for technical assistance in re-checking several of the measurements involved.

Chemical Structure and Biological Activity of *p*-Disubstituted Derivatives of Benzene

Several papers deal with quantitative relationships between chemical structure of organic compounds and the magnitude of their biological effect[1–9]. This communication is an attempt to work out a mathematical model which would express these relationships in the group of compounds



(X, Y = H, CH_3, Cl, OH, NO_2, NH_2). The chosen series includes all possible combinations of groups X and Y.

The papers cited and the experiments from our laboratory[10] show that satisfactory correlations of biological activity with Hammett constants can be found in some cases. However, often this is not so. For example, attempts to correlate LD_{50} of substituted thiophenols with Hammett constants were not successful[11]. We are of the opinion that, whilst during the study of chemical reactivity reactions take place at the chosen reaction centre (secured by an appropriate choice of the reaction mixture), this fact cannot be guaranteed with reactions taking place *in vivo*. In other words, it is not possible to force the reaction centre upon the biological system. For example, with disubstituted derivatives of benzene both functional groups must be taken into account. When interpreting the results, it cannot be assumed that the effect-controlling reaction, taking place at a certain reaction centre, is influenced by the *unchanged* original substituent. Accordingly, even if substituent effects on the reaction *in vitro* are fitted by the Hammett equation, the order of the substituents, which expresses their effects *in vivo*, may be different. Therefore, we have selected a group of compounds which contains all combinations of the chosen substituents. It proved advantageous to arrange the values of the experimental activities into a triangle matrix, rows and columns corresponding to the individual substituents arranged in the same order. This simplifies the finding of mathematical models for statistical treatment[12]. Altogether, four equations were tested.

$$1 \quad \log \frac{[LD_{50}]_{HH}}{[LD_{50}]_{XY}} = a_X + a_Y \qquad \text{additive model}$$

$$2 \quad \log \frac{[LD_{50}]_{HH}}{[LD_{50}]_{XY}} = d_X\, d_Y \qquad \text{product model}$$

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[12] *Acknowledgment.* We should like to thank Mr. Z. ROTH for the statistical evaluation of our results.