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].

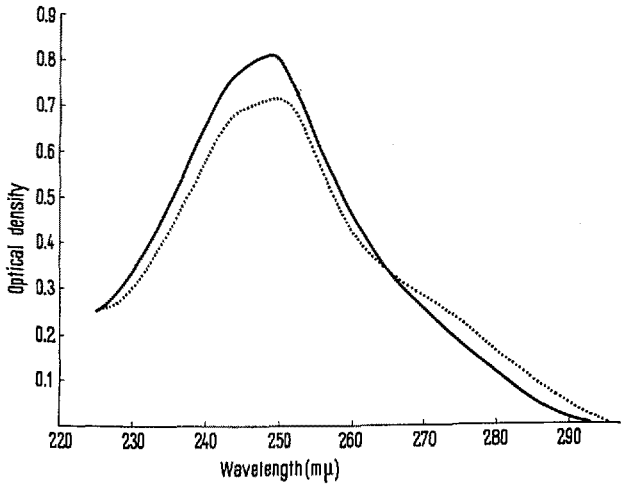


Fig. 2. Variation of the absorption spectrum of inosine on reaction with formaldehyde, at 30°C. $6.67 \cdot 10^{-5} M$ inosine; $0.05 M$ acetate buffer pH 4.70. 1.0 cm optical path length. Continuous line, inosine only; dotted line, inosine in presence of $1.0 M$ formaldehyde.

*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.

S. LEWIN

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

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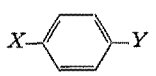
[19] L. GROSSMAN, S. S. LEVINE, and W. S. ALLISON, J. mol. Biol. *3*, 47 (1961).
[20] M. STAEHELIN, Biochim. biophys. Acta. *29*, 410 (1958).
[21] All chemicals used were of Analytical Grade. Formaldehyde was purified by passage through Deacidite FF (Permutit Company, London, England), assayed by the 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.

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

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

[1] R. ZAHRADNÍK and M. CHVAPIL, Exper. *16*, 511 (1960).
[2] R. ZAHRADNÍK, Exper. *18*, 534 (1962).
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[10] D. VLACHOVÁ, Dissertation Thesis, Charles University (1962).
[11] M. KRIVUCOVÁ, J. KOPECKÝ, D. VLACHOVÁ, and K. BOČEK, unpublished results.
[12] *Acknowledgment.* We should like to thank Mr. Z. ROTH for the statistical evaluation of our results.

$$3 \quad \log \frac{[LD_{50}]_{HH}}{[LD_{50}]_{XY}} = b_X + b_Y + e_X e_Y$$

$$4 \quad \log \frac{[LD_{50}]_{HH}}{[LD_{50}]_{XY}} = b_X + b_Y - e_X e_Y$$

combined models

Four sets of structural parameters, statistically elaborated with the help of the four equations, were used to work out the theoretical $LD_{50}$ and these were plotted against the experimental results. The evaluation of these correlations by the correlation coefficient ($r$) proved, for the group under study, that only equation 3 is appropriate ($r = 0.981$). This model was then checked by the $\psi^2$-test. Although the $\psi^2$-test on a 5% significance level is not satisfactory for the whole group, it is satisfactory if hydroquinone is disregarded (Figure). The correlation coefficient equals 0.999. The values of the substituent constants $b_i$ and $e_i$ are as follows:

| | $NO_2$ | Cl | OH | $CH_3$ | H | $NH_2$ |
|---|---|---|---|---|---|---|
| $b_i$ | 0.565 | 0.328 | 0.318 | 0.217 | 0.005 | −0.026 |
| $e_i$ | 0.59 | −0.07 | 0.53 | 0.04 | −0.04 | −0.87 |

We plan to investigate the meaning of constants $b_i$ and $e_i$. It is possible that a relation can be found between the mathematical model mentioned and the L.F.E.R. constants[13,14]. The latter have already been applied to biological problems, especially in papers by ZAHRADNÍK[1-3] and lately HANSCH[8,9].

At present the *m*- and *o*-disubstituted derivates are being studied in the same way, and we are also working with additional substituents.

*Zusammenfassung.* Es wurden die i.v. $LD_{50}$ einer Gruppe *p*-disubstituierter Benzolderivate, welche alle Kombinationen der erwähnten Substituenten enthielten, in Polyvinylpyrrolidonlösungen bestimmt. Der Zusammenhang zwischen der chemischen Struktur dieser Verbindungsklasse und ihrer biologischen Aktivität konnte mit einer vorgeschlagenen Gleichung beschrieben werden.

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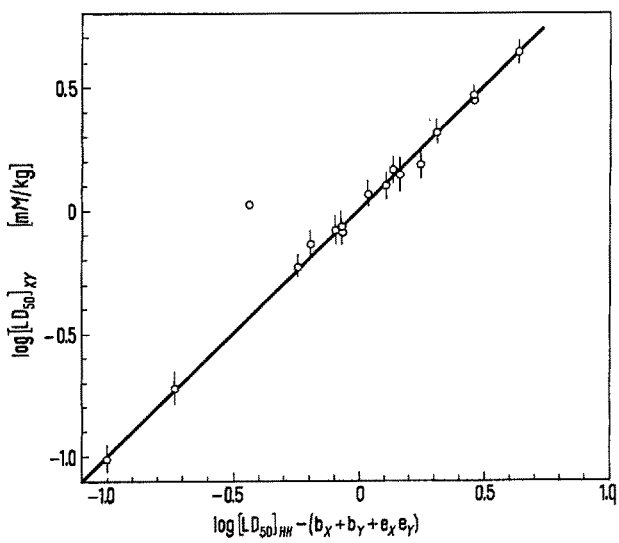
*Institute of Industrial Hygiene and Occupational Diseases, Prague (Czechoslovakia), July 21, 1964.*



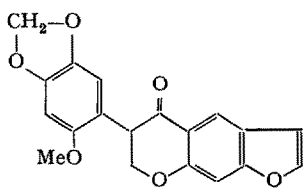

$LD_{50}$'s were determined on white mice with weight 20 ± 2 g by the Thompson method. The substances were administered intravenously in a 20% aqueous polyvinylpyrrolidone solution.

[13] L. P. HAMMETT, *Physical Organic Chemistry* (McGraw-Hill Book Co. Inc., New York 1940).
[14] R. W. TAFT JR., J. Am. chem. Soc. *75*, 4231 (1953).

## Synthesis of Dehydroneotenone[1]

CROMBIE and WHITING have isolated (±)-neotenone (I) and dolineone (II) along with other compounds from the root of *Neorautanenia pseudopachyrrhiza* Harms[2]. This is the first plant in which both an isoflavanone and its corresponding rotenoid have been shown to occur together. They have also shown that (I) was easily dehydrogenated to dehydroneotenone (III) and the former could be reconstituted from the latter. We wish to report the synthesis of dehydrocompound (III) by a method used earlier[3]. Hoesch condensation of 6-hydroxy-2,3-

[1] Presented in part at the IUPAC Symposium on the Chemistry of Natural Products, Kyoto (Japan), April 1964.
[2] L. CROMBIE and D. A. WHITING, Tetrahedron Letters No. 18, 801 (1962); J. chem. Soc. *1963*, 1569.
[3] K. FUKUI and M. NAKAYAMA, Exper. *19*, 621 (1963).



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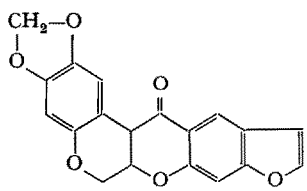

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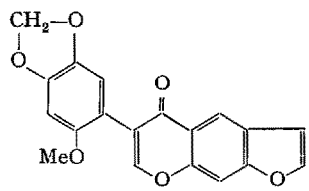

III