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Hammett Series with Biological Activity

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The possibility of relation between biological activity and Hammett's sigma values in appropriate series of benzene derivatives is discussed. A "biological Hammett equation" is set up, based on a number of simplifying conditions, and the limitations of this equation are discussed. Finally, on the basis of data from the literature examples are given of series to which the Hammett equation is or can be applied, and it is mentioned that special deviations occur when certain substituents are used.

Scientists engaged in the production of biologically active substances have always desired to be able to predict the action of a given substance by virtue of its chemical constitution. Many more or less successful theories have been advanced, and still more *ad hoc* explanations have been given, but no fairly general theory is available and may probably not be advanced since biological action cannot be related to one single type of mechanism.

If it is assumed that many substances exert their actions by reacting with cell components, there would be every reason to apply the Hammett equation [1] to appropriate series of benzene derivatives where the action is supposed to be due to a reaction with the side-chain.

The Hammett equation

$$\log k_\sigma - \log k_0 = \varrho\sigma \qquad (1)$$

describes the effect of *meta* and *para* substituents on the rates of side-chain reactions of benzene derivatives and is a linear relationship for the logarithms to the rate constants (and therefore also equilibrium constants) to the σ-values, which are substituent constants independent of the reaction. ϱ is a proportionality constant dependent upon the nature of the reaction and the conditions. k_σ is the rate constant of the member which has a substituent with the value σ, and k_0 is the rate of the unsubstituted member. The degree of biological activity in groups of related compounds is generally believed to depend on the degree of chemical reactivity. It is therefore worth while to plot biological activity against σ-values in appropriate series and try to find simple relationships.

In a review on the Hammett equation [2] Jaffé says that in the literature he has not been able to find a series of substances the biological action of which

has followed the Hammett equation, but on the other hand, he does not consider it inconceivable that such series may be found. Since it is generally assumed that biological action is due to an interaction with enzymes, it is of interest that the velocity constants for hydrolysis of substituted benzoyl cholines with cholinesterase has been found to fit into the Hammett equation [3]. Moreover, Fukuto has shown that the inhibition of cholinesterase with phenyl N-methylcarbamates and with diethyl-phenyl phosphates follows the Hammett equation, and that the insecticide action of the latter with certain exceptions follows the cholinesterase inhibition [4]. As an example of a series of substances with pharmacological effect to which the Hammett equation can be applied, phenylethers of choline with a nicotine-like activity may be mentioned [5].

For the purpose of finding precise expressions of biological activity as a function of σ-values we will regard the growth inhibition. Everywhere in the following, temperature and other environmental conditions other than inhibitors are assumed to be kept constant.

Growth of living organisms is supposed to be an extremely complicated but exactly balanced production of substances. Almost without exception the synthetic activity is proceeding by means of more or less specific enzymes, and an inhibition of one or more of these enzymes will influence the process of growth. Thus, if we affect an organism with a substance which is able to penetrate to an enzyme and to react with it, causing loss of its catalytic ability, we will see that this has an effect on the growth. No effect will generally be seen by adequate low concentrations of the substance because the amount of enzyme may be reduced somewhat before a noticeable decline in the capacity occurs. Increasing concentrations of the inhibitory substance will then show that the rate of growth decreases for at last to become zero. The organism will possibly die when the process of growth has reached a certain low rate. In a living organism proteins and consequently also enzymes are incessantly decomposed and rebuilt, and we assume that an enzyme E_n during the growth is formed at the rate dE_n/dt. If, however, an inhibitory substance is present which reacts with the enzyme by a bimolecular reaction, the enzyme will be destroyed at the rate

$$\left(\frac{dE_n}{dt}\right)_{dest} = k\, E_n\, [I] \qquad (2)$$

where k is the rate constant, and [I] is the concentration of the inhibitor just around the enzyme. The net production of E_n will be reduced partly because some of the output is destroyed and partly because the production may be less than before. The other enzymes of the organism are dependent on E_n which in its turn is dependent on the other enzymes, and by means of this complicated feed back mechanism the organism assumes a lower growth rate.

If the inhibitory substance is a member of a Hammett series (H) we have by analogy with (2)

$$\left(\frac{dE_n}{dt}\right)_{dest} = k_\sigma E_n [H_\sigma] \qquad (3)$$

We now use such a concentration that the growth is inhibited, *e.g.* to q % of uninhibited growth, and we have

$$k_\sigma[H_\sigma]_q = K(q) \tag{4}$$

where $K(q)$ is a constant when q is fixed.

Eqn. (4) must apply to the whole series independent on σ as it is seen from eqn. (3) that the rate of destruction which determines the growth rate is the same when eqn. (4) is satisfied.

From eqn. (4) we get

$$\log\ k_\sigma + \log\ [H_\sigma]_q = \log\ K(q) \tag{5}$$

Specifically applying to the unsubstituted member of the series we have

$$\log\ k_o + \log\ [H_o]_q = \log\ K(q) \tag{6}$$

And from eqns. (5) and (6) we obtain

$$\log\ k_\sigma - \log\ k_o = -\log\ [H_\sigma]_q + \log\ [H_o]_q \tag{7}$$

If the Hammett eqn. (1) is substituted in eqn. (7), we get

$$-\log\ [H_\sigma]_q + \log\ [H_o]_q = \varrho\sigma \tag{8}$$

Eqn. (8) is derived on the assumption of q being a growth inhibition in per cent, but q may generally be interpreted as *equal effect*.

The inhibition of bacterial growth can easily be measured with a reasonable accuracy and can therefore with advantage be used for the verification of the biological Hammett eqn.

When bacteria are transferred to a fresh culture medium, a short time — "the lag" — will often pass before division takes place. If like Hinshelwood [6] we imagine this lag to be a period used for production of substances and reorganization in the cell, we have

$$\int_0^L R\mathrm{d}t = \text{const.} \tag{9}$$

where L is the lag and R the rate of production.

If the culture medium contains an inhibitor reducing R to R' we have

$$\int_0^{L+\Delta L} R'\mathrm{d}t = \text{const.} \tag{10}$$

where ΔL is the increase of lag necessary for the bacterium to reach the same state as the uninhibited bacterium reaches at the time L.

Thus a fixed value of ΔL can be used as a measure of equal effect.

The lag is succeeded by a growth phase in which the bacteria are dividing. This often takes place according to an exponential law

$$N_t/N_o = \exp(kt) \tag{11}$$

where N_t is the number of bacteria at the time t, N_o is the original number of bacteria, and k is a constant. k is a measure of the rate of production in the single cell, and at last k decreases due to the culture medium being consumed at the same time as toxic metabolic products are accumulated.

If as before an inhibitor is added, k is reduced to k' and N_t to N_t'.

Here we can make use of fixed values of N'_t/N_t or of k'/k as a measure of equal effect. In the latter case it is necessary to know the growth curve which moreover has the advantage that ΔL as well as k'/k may be determined independent of each other.

As a measure of inhibition of plants, reduction of the growth in length or of the production of dry metter may, *e.g.*, be used, and in pharmacology the respective responses may be applied.

As appears from eqn. (8) we may plot the negative logarithms of the isotoxic molarities as a function of σ. If the theory holds good, a straight line could be drawn through the points. The slope of this line will be ϱ, and the ϱ-value thus determined appears to be identical with the ϱ-value of the damaging reaction in the cell.

It will, however, hardly be possible to find a series of substances with a considerable number of substituents of different types to which the theory fully applies. The reasons are many, and some of the presumably most important ones are set out below:

1. The reaction was assumed to be "bimolecular". Even though this will often be the case, side-chain reactions of a more complicated nature may well be imagined. The effect may thus be due to a catalytic action in which, *e.g.*, the side-chain is acetylated by active acetyl, whereupon the acetyl group is liberated by hydrolysis. Velocity and equilibrium constants in this cycle will presumably follow the Hammett equation, but the catalytic and consequently the biological effect will not necessarily obey the expressions deduced.

2. In order to enter the cell, the substances must pass the cell-wall, but if the molecule is associated to more than a few molecules of water there will be no free passage, and in order to enable it to pass through, the cell must perform an active transport. Two substances almost similar both in respect of their partition coefficients and their σ-values may therefore be treated quite differently with a consequential difference in biological activity. This fact will be particularly pronounced in more complicated organisms, higher plants and animals where discrimination due to the partition coefficients and permeability is apt to obscure every trace of linearity in the relation between σ-values and activities.

3. One of the conditions of the Hammett equation is that the substituents of the benzene ring only exert their actions *via* the electronic density in the side-chain. In the case of reactions between small molecules this condition will in general be fulfilled. But where it is a question of enzyme reactions in which the reacting molecule as a step in the reaction will be oriented on the enzyme surface, the substituents may take part in the orientation and make irregular contributions to the entropy of reaction. Thus in the above-mentioned diethyl-phenyl phosphate series, the *m*-dimethylamino and *m-tert*-butyl members are *in vitro* much stronger than would appear from the equation. The two groups are far more sterically than chemically alike, and deviations of this type provide valuable information as to the topography of the enzyme surface. As regards the phenyl N-methylcarbamates there are two parallel curves applying to *m*- and *p*-derivatives, respectively.

4. The deduction has been made on the assumption that the inhibitory substance would attack one enzyme only, an assumption which is not always

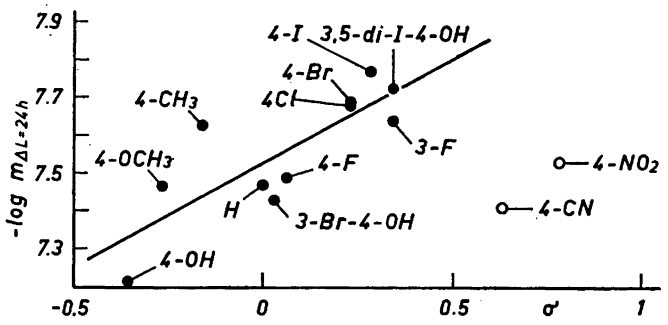

Fig. 1. Activity of substituted G-penicillins plotted against the σ-values of the substituents. The ordinate is the negative logarithm of the molarity which gives a lag of 24 h. A sensitive strain of *Staphylococcus aureus* is used as test organism. Data from Burger [7].

true. Substances reacting with SH-groups must thus be able to attack numerous enzymes simultaneously, and the ϱ-values may vary from one enzyme to another. However, if the reactions with the various enzymes are of the same type, the ϱ-values will not vary much, and what will happen is simply that the "isotoxic curve" will have a curvilinear course.

5. We have used molarity instead of activity, but this error is negligible in comparison with those mentioned above. Very dilute solutions of the inhibitor in the culture medium do, however, present a problem in that the substance will more or less be adsorbed to peptide molecules and to glass surfaces.

No good accordance with eqn. (8) is thus to be expected, at most that rejection of obvious deviations will make it possible to draw a regression line with

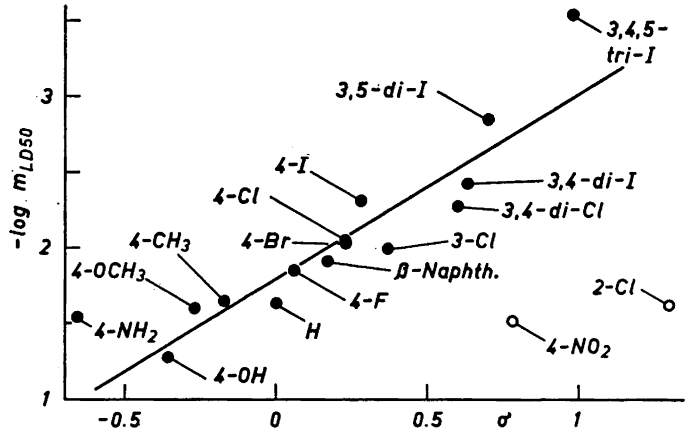

Fig. 2. Activity of substituted benzoic acids plotted against the σ-values of the substituents. The ordinate is the negative logarithm of the molarity which kills 50 % of the larvae of the mosquito *Aedes aegypti* in 24 h. This mortality (LD50) is supposed to be reached when the processes of growth attain a certain low mean value. Data from Casida [8].

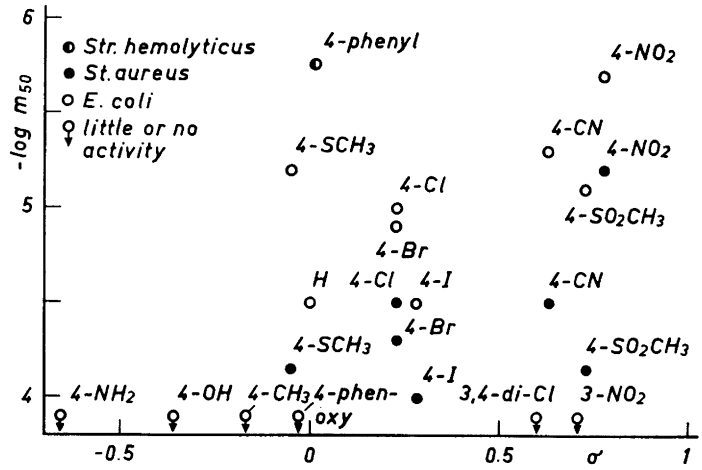

Fig. 3. Activity of chloramphenicol analogues plotted against the σ-values of the substituents. The ordinate is the negative logarithm of the molarity giving 50 % growth of the bacteria *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus hemolyticus*. Data are taken from [13-22], and the values obtained by van der Meer *et al.*[16] have been used as standard values.

reasonable "accidental" deviations. Although such rejection is a difficult and also somewhat questionable matter, it is necessary in order to obtain a ϱ-value which applies to most members of the series and in addition represents the ϱ-value of the enzyme reaction in the cell. In I and II below, the principles for rejection are exemplified.

Biological Hammett series have already been referred to, and some additional series shall be mentioned here:

I. *Substituted G-penicillins* (Fig. 1). The *p*-nitro and *p*-cyano derivatives have not been included in the computation of the regression line. The material is somewhat scanty, and the appearance of a larger material may alter the picture considerably.

II. *Substituted benzoic acids* (Fig. 2). Also in this case it is justifiable to ignore the *p*-nitro derivative. $\varrho = 1.2$ which is approximately the ϱ-value for K_A (1.0). That the effect is not merely a matter of acid strength is seen from

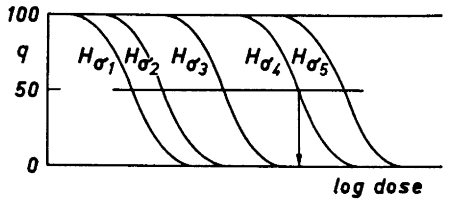

Fig. 4. A curve family showing per cent growth as a function of log dose for five different members of a Hammett series. For the fourth member it is demonstrated how the concentration giving 50 % growth can be estimated.

the fact that the *o*-chlorobenzoic acid, marked in the diagram after acid strength, is far too weak.

These two curves are based primarily on halogen derivatives. In a number of series of substances with antibacterial effect or inhibitory effect on germinating seeds, synthesized in this laboratory for the purpose of investigating the relation between biological activity and σ-values, we have in most instances found such relation by the application of amino, alkyl, hydroxyl, alkoxyl, and halogen groups. Nitro and methylsulfonyl groups, however, as a rule give products with a much lower activity than calculated [9].

It is common procedure to introduce halogen, particularly chlorine, into benzene rings in biologically active substances, this often resulting in more active substances. The trifluoro-methyl group has lately been added to this class of activating substituents. In two antimetabolite series, sulphanilic acid anilides [10] and pantoyltauryl anilides [11], the strongest members are the 3,5-dibromo derivatives, and this has been called a "3,5-dibromoaniline activity". We have synthesized the series in question and furthermore the 3,4,5-trihalogen derivatives which have proved to be stronger than the 3,5-dibromo derivatives [9]. In the present case it would be more reasonable to speak of Hammett effect.

III. *Chloramphenicol analogues* (Fig. 3). This material is difficult to evaluate since it derives from many sources and has been tested on different bacteria. The series has been thoroughly discussed on about the same lines as presented here, and correlation has been found only for the *p*-substituted members, the known *m*-substituted ones being entirely inactive [12]. If the activity of this series is postulated to obey the Hammett equation, it must be added that *m*-substituents prevent the benzene ring from being attached correctly to the enzyme surface. Furthermore, *p*-nitro, *p*-cyano, and *p*-methylsulfonyl are the most activating substituents, contrary to what is found in most other series.

APPENDIX

The deduction of eqn. (8) has been based on a fixed inhibition level of q. A 50 % level is often used, but any other value would have given a straight line parallel to the 50 % line, *i.e.* with the same slope, ϱ.

The isotoxic concentration is obtained by determining the q's for a number of concentrations of each H_σ and plotting them as a function of log $[H_\sigma]$. These curves are parallel because at every value of σ we have eqn. (4)

$$k_\sigma[H_\sigma] = K(q)$$

or

$$\log k_\sigma + \log [H_\sigma] = \log K(q) \tag{12}$$

from which we obtain the slope of each curve in the curve family:

$$\left(\frac{\partial q}{\partial \log [H_\sigma]}\right)_\sigma = 2.30 \frac{K(q)}{K'(q)} \tag{13}$$

i.e. that the slope of all curves is identical for any given q. By intersecting the curve family (Fig. 4) with $q = 50$ we get $\log [H_\sigma]_{50}$ for each substance. The

negative values of these logarithms are plotted against the σ-values, and we get the "isotoxic curve" with the slope ϱ. When choosing another value of q we get the corresponding log $[H_\sigma]_q$ for each substance and a new "isotoxic curve" with the same slope.

The law of the parallel log dose-response curve applies, of course, whether the k's satisfy the Hammett equation or not. It is, indeed, a general assumption within pharmacology that related substances with the same mechanism of action give parallel log dose-response curves. We have here deduced the rule applying to "bimolecular" processes, but conversely, no safe conclusion as to the type of reaction can be drawn from parallel curves.

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