Our present results from the $^{14}CO_2$ feedings to *N. glutinosa* show an early and high activity in the pyridine portion of nicotine (Fig. 3). This rapid appearance of label in the pyridine ring certainly is consistent with the hypothesis that the pyridine ring arises from the simple precursors mentioned above. These compounds, with origins close to the fixation of carbon dioxide, would acquire an early and high label which would be reflected in the pyridine ring.

The relatively slow incorporation of carbon into the pyrrolidine ring is consistent with entry of carbon *via* an amino acid, since amino acids would be expected to become labeled more slowly than the simpler precursors of the pyridine ring. However, it is very interesting to note the consistently low activity of carbon-2′ (Table V) and to attempt to reconcile these data with the currently accepted hypothesis for biosynthesis of the pyrrolidine ring. This hypothesis[8,9,28b,38] invokes glutamic acid through several possible pathways, all involving a symmetrical intermediate. Applying the glutamate-symmetrical intermediate hypothesis to our data would require equal labeling in C-2′ and C-5′. As a result, by difference, C-3′ and C-4′ would contain significantly larger amounts of activity. Such a requirement, that the methylene carbons of glutamate be the more highly labeled, is contrary to all current ideas on the biosynthesis of glutamate.[59-62] Therefore, either the glutamate-symmetrical intermediate hypothesis does not apply in the present case or a new, as yet unsuspected, mechanism exists for glutamate biosynthesis.

(59) R. B. Roberts, D. B. Cowie, R. Britten, E. Bolton, and P. H. Abelson, *Proc. Natl. Acad. Sci. U. S.*, **39**, 1013 (1953).
(60) N. Tomlinson, *J. Biol. Chem.*, **209**, 605 (1954).
(61) V. Moses, O. Holm-Hansen, J. A. Bassham, and M. Calvin, *J. Mol. Biol.*, 1, 21 (1959).
(62) D. S. Hoare, *Biochem. J.*, **87**, 284 (1963).

---

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POMONA COLLEGE, CLAREMONT, CALIFORNIA]

# $\rho$–$\sigma$–$\pi$ Analysis. A Method for the Correlation of Biological Activity and Chemical Structure

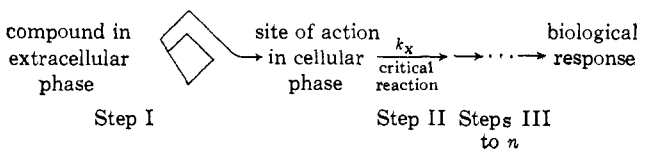
BY CORWIN HANSCH AND TOSHIO FUJITA[1]

RECEIVED AUGUST 19, 1963

Using the substituent constant, $\sigma$, and a substituent constant, $\pi$, defined as $\pi = \log P_X - \log P_H$ ($P_H$ is the partition coefficient of a parent compound and $P_X$ that of a derivative), regression analyses have been made of the effect of substituents on the biological activity of benzoic acids on mosquito larvae, phenols on gram-positive and gram-negative bacteria, phenyl ethyl phosphate insecticides on houseflies, thyroxine derivatives on rodents, diethylaminoethyl benzoates on guinea pigs, and carcinogenic compounds on mice.

Recently[2,3] we have shown the advantage of using partition coefficients in connection with the Hammett equation to rationalize the substituent effect on the growth-promoting activity of the phenoxyacetic acids and the bactericidal action of chloromycetin derivatives on various bacteria. In particular, it was found that a substituent constant, $\pi$, patterned after the Hammett $\sigma$-constant was useful in evaluating the lipo–hydrophilic character of a molecule upon which biological activity is highly dependent. $\pi$ is defined as: $\pi = \log (P_X/P_H)$ where $P_H$ is the partition coefficient of a parent compound and $P_X$ is the value for a derivative. The reference system is octanol–water, and all of the work reported in this paper refers to this pair of solvents. The purpose of this report is to show that our previously employed expression appears to have general applicability.

We have assumed that the rate-limiting conditions for many biological responses to chemicals can be defined in the simplest and most general way as follows.




The first step in the above reaction scheme is pictured as a random walk process in which the molecule in question makes its way from a very dilute solution outside the cell to a particular site in the cell which may be within an organelle. This is visualized as being a relatively slow process, the rate of which is highly dependent on the molecular structure of the compound in question. It is assumed, as a first approximation, for many types of biologically active molecules there will be one key rate-controlling reaction at the active sites. This could be formulated as in eq. 1. $A$ is the probability of a molecule reaching a site of action in a given time interval and $C$ is the extracellular molar concentration of the compound being tested. The product $AC$ represents the "effective" concentration at the sites of action. The constant $k_X$ might be either an equilibrium or rate constant. It is assumed that a relatively large number of reaction sites are available so that these remain essentially constant during the test interval. In certain instances, it may be that new sites are being constantly generated. It also is considered that the many reactions which may occur subsequently to the one critical one (Steps III to $n$) before the visible response is elicited can be neglected for a first approximation. Thus the two parameters $A$ and $k_X$ will be the important determinates governing the relative effectiveness of the members of a given series of biologically active compounds. Our model, then, is a steady-state one in contrast to the equilibrium model of Ferguson.[4,5]

$$\text{rate of biological response} = \frac{\text{d(response)}}{\text{d}t} = ACk_X \quad (1)$$

(1) On leave from Kyoto University, Kyoto, Japan.
(2) C. Hansch, P. P. Maloney, T. Fujita, and R. M. Muir, *Nature*, **194**, 178 (1962).
(3) C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, C. F. Geiger, and M. J. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963).
(4) A. Burger, "Medicinal Chemistry," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1960, p. 49.
(5) J. Ferguson, *Proc. Roy. Soc.* (London), **127B**, 387 (1939).

In trying to relate $A$ to an experimental quantity, we have chosen the partition coefficient ($P$). This was suggested by the classical work of Meyer and Overton and finds support in the work of Collander[6] who has shown that for *Nitella* cells the rate of movement of a great variety of organic compounds through cellular material is approximately proportional to the logarithm of their partition coefficients between an organic solvent and water. Instead of using $P$, we have developed[3] a comparative substituent constant, $\pi$. $\pi$ is a free energy-related constant like the Hammett function, $\sigma$, and is a measure of the relative free energy change resulting in moving a derivative from one phase to another. It is expected that a molecule within a cell would have maximum freedom of movement when $P_X$ for the cell phases is 1. The chance of its finding the site of action by a random walk process would then be maximum. There will be an ideal $\pi$-value ($\pi_0$) for substituents such that $P_X$ will be 1 for the various cellular phases. This, of course, will be an idealized average value since it is now well known[7] that the internal cellular structure is very complex. A molecule would have to make many partitionings between "aqueous" phases and a variety of different more or less fixed "organic" phases in going through the wall–membrane section, then the endoplasmic reticulum, and, finally (in many but not all cases), the membrane structure of a particular organelle. The process will be more complex than simple partitioning, since accompanying this there will be many adsorption–desorption steps at solid surfaces. It is expected that the relative polarity of the molecules will play a role here parallel to that in the partitioning process. That molecule for which the sum of the free energy changes is minimum for the many boundary crossings of the two types will be the one with the ideal lipo–hydrophilic character; that is, the one where $\pi$ is such that the idealized $P_X$ is 1. Any increase or decrease from $\pi_0$ will result in a slower rate of movement of the molecule in the partitioning–diffusion process by which the site of action is attained.

While Collander's work indicated that the rate of movement of organic compounds through cellular material was linear with respect to log $P$, he unfortunately did not investigate compounds with high partition coefficients. The highest values he studied were about 8.5 for the ether–water system. It has long been known[8] that as one increases the partition coefficient of a biologically active function, activity often rises, but after a certain point falls off and eventually reaches zero. Thus there is abundant evidence to indicate that there is often an optimum partition coefficient (or value of $\pi$ in a group of derivatives) for a biologically active series such as the phenols or chloromycetins.[3] In our model we have assumed, in contrast to Ferguson, that this falloff in activity with increase in $P$ is due to the longer time needed to find the site of action as $P_X$ increases beyond the ideal value.

Eventually, the rate would become so slow that it is not possible during the test interval to build a sufficient concentration at the reaction sites to bring about the particular biological response under consideration. Insufficient evidence is at hand to know whether the biological activity would follow a normal gaussian type distribution with respect to log $P$ or $\pi$, other factors being held constant. As a working hypothesis we have assumed that the distribution would be normal. The coefficient $A$ would then be related to $\pi$ as follows.

$$A = f(\pi) = ae^{-(\pi-\pi_0)^2/b} \quad (2)$$

In eq. 2, $a$ and $b$ are constants. Substitution of eq. 2 into eq. 1 yields eq. 3.

$$\frac{d(\text{response})}{dt} = ae^{-(\pi-\pi_0)^2/b}Ck_X \quad (3)$$

Most biological results are reported in terms of a constant equivalent response (*e.g.*, isotoxic concentration, $LD_{50}$, $ED_{50}$, % growth, etc.) obtained in a definite time interval. This is usually expressed as the concentration necessary to cause a particular rate of response. For these conditions we can replace d(response)/d$t$ with a constant. Doing this, taking the logarithm of eq. 3 and collecting constants, we obtain eq. 4.

$$\log \frac{1}{C} = -k\pi^2 + k'\pi\pi_0 - k''\pi_0^2 + \log k_X + k''' \quad (4)$$

The Hammett equation[9] which applies to either equilibrium or rate constants is formulated as

$$\log \frac{k_X}{k_H} = \rho\sigma \quad (5)$$

$\sigma$ is a constant dependent upon the electronic characteristics of the substituent X relative to H, and $\rho$ is a constant related to the reaction the parent molecule is undergoing. Substitution of eq. 5 into eq. 4 yields eq. 6.

$$\log \frac{1}{C} = -k\pi^2 + k'\pi\pi_0 - k''\pi_0^2 + \rho\sigma + k'''' \quad (6)$$

It must be borne in mind that we have used octanol and water to approximate the cellular phases. The original impetus for this came from the Meyer–Overton work. Collander[10] has provided more concrete justification by showing that the partition coefficient of a compound between water and a polar organic solvent can be related to $P$ obtained in a second polar solvent and water as follows.

$$\log P_1 = a \log P_2 + b \quad (7)$$

Thus the constants $a$ and $b$ from eq. 7 must be contained in those of eq. 6. In eq. 6, $\pi_0$ is the ideal value for a substituent such that the sum of the many free energy changes in the penetration process is a minimum. Since this is a constant for a given type of parent molecule in a particular biological system, eq. 6 reduces to eq. 8. The constants in eq. 8 are, of course, different

$$\log \frac{1}{C} = -k\pi^2 + k'\pi + \rho\sigma + k'' \quad (8)$$

from those in eq. 6.

(6) R. Collander, *Physiol. Plantarum.*, **7**, 420 (1954).
(7) J. Brachet and A. E. Mirsky, "The Cell," Vol. II, Academic Press, New York, N. Y., 1961.
(8) Numerous examples can be found in ref. 4.
(9) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).
(10) R. Collander, *Acta Chem. Scand.*, **5**, 774 (1954).

While eq. 6 rationalizes the results previously reported,[3] there are a number of situations in which eq. 6 reduces to simpler forms capable of describing the results observed in a variety of systems. We have attempted to categorize (see below) a number of different biologically active series into types according to the importance of the terms in eq. 6. These are types which one might, *a priori*, expect to find.

One should not attach too much importance to this typing for two reasons. In the first place, in most of the work we have for consideration, relatively few molecules have been investigated. Further investigation of compounds with much higher partition coefficients might show, for example, that a series was not Type I but Type II. Secondly, the uncertainty in the biological results is usually high and, since standard deviations are not usually reported, one cannot be certain that better testing would not result in retyping. For example, in a number of instances $\sigma$ appears to play no part. Better testing might reveal a very small, yet for the purposes of reaction mechanism quite significant, role for $\sigma$. Although one cannot place great reliance on the assignments we have made for the various series of compounds considered in this paper, the typing is, nevertheless, quite helpful for mechanistic discussions as well as for planning further work.

**Type I.**—In the simplest case, where $\pi_0$ is large with respect to $\pi$ and $\sigma$ is very small or zero, then eq. 6 reduces to 9.

$$\log \frac{1}{C} = a\pi + b \qquad (9)$$

Activity conforming to this expression has been recognized since the classical work of Meyer and Overton and has been considered in general terms by Ferguson.[4,5] Ferguson has assumed that for "nonspecific toxicity" or narcosis an equilibrium between the drug outside the cell and that within the cell is established very rapidly. Since the site of action in the interior is more lipophilic than the exobiophase, an increase in log $P$ (or $\pi$ for a substituent) results in a higher concentration at the site of action and linearity between log $P$ and biological response is observed. It is well known that as log $P$ increases (*i.e.*, $\Sigma\pi$ increases) for a given type of molecule, a point is invariably reached where activity falls off from what would be expected from eq. 9. Ferguson has suggested that this is due to a drop in the chemical potential of derivatives which have reached the point where $\Sigma\pi$ makes them very much less soluble in the hydrophilic phase; that is, the concentration ($S$) necessary for an equivalent biological response approaches the limiting solubility of the derivative in the hydrophilic phase ($So$). As $S/So$ approaches 1, the chemical potential is assumed to drop until it reaches zero at 1. While this mechanism is attractive in situations where the falloff from linearity occurs at high concentrations (*e.g.*, action of general anesthetics), it seems an unlikely explanation where the falloff occurs at concentrations below 0.001 $M$ (*e.g.*, the plant growth regulators). A general explanation for the departure from linearity of activity with $\Sigma\pi$ in a series of derivatives is contained in eq. 6. As $\pi$ approaches $\pi_0$, departure from linearity is to be expected. This falloff phenomenon results in Type II activity. A special case of the Type I situation arises when the action of a series of derivatives occurs on or in the cell wall or membrane. Here a one-step partitioning from the hydrophilic to lipophilic phase occurs, and eq. 9 describes the expected result.

The toxicity of benzoic acids to mosquito larvae and the bactericidal action of phenols to *Micrococcus pyogenes var. aureus* (*vide infra*) represent examples of the Type I relationship. Type I activity will, of course, be limited in any series of derivatives. As one increases the fat solubility, sooner or later a falloff from linearity will occur for the reason cited by Ferguson.

**Type II.**—The next order of complexity is represented in the situation where $\sigma$ is zero and $\pi$ and $\pi_0$ are relatively close in value. Now eq. 6 takes the form

$$\log \frac{1}{C} = -a\pi^2 + b\pi + c \qquad (10)$$

Examples of Type II activity are considered below in the action of phenols on *Salmonella typhosa* and the carcinogenic activity of the dimethylaminoazobenzenes.

**Type III.**—It is possible to imagine that $\pi$ could be of no importance (at least over a rather large range) in determining the activity of a series of compounds, in which case eq. 6 reduces to a Hammett expression.

$$\log \frac{1}{C} = \rho\sigma + c \qquad (11)$$

While such would be the normal case *in vitro*, it is harder to visualize for *in vivo* reactions. A close approximation, however, appears to occur in the action of phenyl phosphate esters as insecticides (see below). It might also occur with a very highly polar molecule such as a quaternary ammonium salt acting on the outside surface of the cell.

**Type IV.**—When $\pi_0$ is large compared to $\pi$ and when $\sigma$ is significant, then eq. 6 simplifies to eq. 12.

$$\log \frac{1}{C} = a\pi + \rho\sigma + c \qquad (12)$$

Two examples of this situation are considered below in the action of insecticides on houseflies and that of local anesthetics on guinea pigs. Again this is possible only for a limited range of derivatives as in the above case for Type I activity.

**Type V.**—The most complex situation is that described by eq. 8. In this report, further examples are considered in the action of thyroxine analogs and in the carcinogenic potency of aromatic hydrocarbons and benzacridines.

**Toxicity of Benzoic Acids to Mosquito Larvae.**—In order to show the value of the substituent constant, $\pi$, for the analysis of "nonspecific toxicity" (Type I), we have selected the excellent data of Casida[11] on the toxicity of substituted benzoic acids to mosquito larvae. Using the method of least squares with the 14 derivatives listed in Table I for which the necessary substituent constants are available, eq. 13–15 are obtained. (In this example $C$ represents $LD_{50}$.)

It is thus apparent that the role of $\sigma$, and, hence, the relative ionization of the acids under the conditions of the test is of minor importance. Since the isotoxic concentrations vary from about $5 \times 10^{-2}$ to $3 \times 10^{-4}$

(11) J. E. Casida, *Biochem. J.*, **59**, 216 (1955).

| | $r^2$ | $r$ | $s$[12] | |
|---|---|---|---|---|
| $\log \frac{1}{C} = 0.519\pi + 1.540;$ | 0.955 | 0.977 | 0.130 | (13) |
| $\log \frac{1}{C} = 0.015\pi^2 + 0.469\pi + 1.556;$ | 0.956 | 0.978 | 0.134 | (14) |
| $\log \frac{1}{C} = 0.016\pi^2 + 0.449\pi + 0.072\sigma + 1.557;$ | 0.958 | 0.979 | 0.138 | (15) |

$M$, it would appear that changes in the ionization of benzoic acid due to substituents are unimportant compared to changes in lipo–hydrophilic character. Of special interest is the good agreement obtained with the polysubstituted derivatives. This provides evidence for the additive character of $\pi$ when strong group interaction is absent. The calculated values in Table I were obtained with eq. 13.

TABLE I

TOXICITY OF BENZOIC ACIDS TO MOSQUITO LARVAE

| Functions | $\Sigma\sigma$ | $\Sigma\pi$[a] | log $C$[b] Calcd. | log $C$[b] Obsd. | $\Delta$ log $C$ |
|---|---|---|---|---|---|
| 3,4,5-Tri-I | 0.98 | 3.73 | 3.476 | 3.540 | 0.064 |
| 3,5-Di-I | 0.70 | 2.56 | 2.869 | 2.850 | 0.019 |
| 4-I | 0.28 | 1.17 | 2.147 | 2.310 | 0.163 |
| 3,4-Di-Cl | 0.60 | 1.61 | 2.376 | 2.280 | 0.096 |
| 4-Cl | 0.23 | 0.80 | 1.955 | 2.060 | 0.105 |
| 4-Br | 0.23 | 1.01 | 2.064 | 2.030 | 0.034 |
| 3-Cl | 0.37 | 0.81 | 1.960 | 2.000 | 0.040 |
| 3,4-(CH)$_4$ | 0.17 | 1.27[c] | 2.199 | 1.920 | 0.279 |
| 4-F | 0.06 | 0.22 | 1.654 | 1.850 | 0.196 |
| 4-CH$_3$ | −0.17 | 0.43 | 1.763 | 1.660 | 0.103 |
| H | 0.00 | 0.00 | 1.540 | 1.640 | 0.100 |
| 4-OCH$_3$ | −0.27 | 0.11 | 1.597 | 1.600 | 0.003 |
| 4-NO$_2$ | 0.78 | 0.04 | 1.561 | 1.520 | 0.041 |
| 4-OH | −0.36 | −0.27 | 1.400 | 1.290 | 0.110 |

[a] The $\pi$-values are those obtained with the monosubstituted benzoic acids. These were simply summed to get the figure for the polysubstituted derivatives. [b] $-\log C$ was calculated from the molar $LD_{50}$ value reported in ref. 7. [c] This value was obtained from naphthoxyacetic acid.

**Toxicity of Phenols to *M. pyogenes var. aureus* and *S. typhosa*.**—A more complicated situation arises from the study of the toxicity of phenols to gram-positive and gram-negative bacteria. The examples with gram-positive *M. pyogenes var. aureus* and gram-negative *S. typhosa* shown in Table II are taken from the extensive studies of E. G. Klarmann and associates.[13] Although they have not reported on a group of substituents for which $\sigma$ varies greatly, it would appear that $\sigma$ is of little importance in determining the activity of the phenol as measured by the phenol coefficient.

Equations 16–18 result from the least-squares fits with the data on the gram-positive organism, *M. pyogenes var. aureus*.

| | $r^2$ | $r$ | $s$ | |
|---|---|---|---|---|
| $\log PC' = 0.951\pi + 0.144;$ | | | | |
| $\log PC' = -0.003\pi^2 +$ | 0.954 | 0.977 | 0.224 | (16) |
| $0.959\pi + 0.141;$ | 0.953 | 0.976 | 0.228 | (17) |
| $\log PC' = -0.001\pi^2 +$ | | | | |
| $0.953\pi - 0.201\sigma +$ | | | | |
| $0.134;$ | 0.954 | 0.977 | 0.230 | (18) |

$PC'$ is the molar phenol coefficient. Comparison of eq. 16, 17, and 18 shows that nothing is to be gained by the introduction of a squared term for a $\pi$- or $\sigma$-term. The result is like that obtained with the benzoic acids and their action on mosquito larvae and may be rationalized by Ferguson's hypothesis or by the hypothesis that the primary action occurs on the "outside" of the organism (cell wall and/or membrane). The calculated values in Table II come from eq. 16. It may be that more precise data would reveal a small role for $\sigma$.

TABLE II

THE TOXIC ACTION OF PHENOLS ON GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA[a]

| Functions | $\sigma$[b] | $\pi$ | log $PC'$ *M. pyogenes var. aureus* Calcd. | log $PC'$ *M. pyogenes var. aureus* Obsd. | log $PC'$ *S. typhosa* Calcd. | log $PC'$ *S. typhosa* Obsd. |
|---|---|---|---|---|---|---|
| H | 0.00 | 0.00[c] | 0.144 | 0.00 | 0.139 | 0.00 |
| 3-OH | 0.00 | −0.70[c] | −0.522 | −0.33 | −0.920 | −0.33 |
| 3-OCH$_3$ | 0.12 | 0.08[c] | 0.220 | 0.20 | 0.242 | 0.23 |
| 3-OEt | 0.15 | 0.52 | 0.639 | 1.64 | 0.743 | 0.72 |
| 3-O-*n*-Pr | 0.15 | 0.96 | 1.057 | 0.94 | 1.133 | 1.05 |
| 3-O-*n*-Bu | 0.15 | 1.40 | 1.475 | 1.50 | 1.412 | 1.55 |
| 3-O-*n*-Amyl | 0.15 | 1.84 | 1.894 | 1.84 | 1.578 | 1.86 |
| 3-O-*sec*-Amyl | 0.15 | 1.76 | 1.818 | 1.77 | 1.556 | 1.70 |
| 3-O-*n*-Hexyl | 0.15 | 2.28 | 2.312 | 2.41 | 1.633 | 1.98 |
| 3-O-*n*-Heptyl | 0.15 | 2.72 | 2.731 | 2.86 | 1.577 | 1.67 |
| 3-O-*n*-Octyl | 0.15 | 3.16 | 3.149 | 3.14 | 1.409 | 0.74 |
| 3-O-*n*-Nonyl | 0.15 | 3.60 | 3.568 | 3.21 | 1.130 | 0.93 |
| 3-CH$_3$ | −0.07 | 0.46[c] | 0.581 | 0.42 | 0.682 | 0.42 |
| 4-OCH$_3$ | −0.27 | −0.16[c] | −0.008 | 0.03 | −0.078 | 0.12 |
| 4-OEt | −0.25 | 0.28 | 0.410 | 0.34 | 0.484 | 0.34 |
| 4-O-*n*-Pr | −0.25 | 0.72 | 0.829 | 0.82 | 0.935 | 0.94 |
| 4-O-*n*-Bu | −0.25 | 1.16 | 1.247 | 1.22 | 1.273 | 1.39 |
| 4-O-*n*-Amyl | −0.25 | 1.60 | 1.666 | 1.74 | 1.501 | 1.75 |
| 4-O-*sec*-Amyl | −0.25 | 1.52 | 1.590 | 1.70 | 1.468 | 1.56 |
| 4-O-*n*-Hexyl | −0.25 | 2.04 | 2.084 | 2.32 | 1.616 | 1.57 |
| 4-O-*n*-Heptyl | −0.25 | 2.48 | 2.502 | 2.65 | 1.622 | 1.58 |
| 4-O-*n*-Octyl | −0.25 | 2.92 | 2.921 | 2.93 | | |
| 4-CH$_3$ | −0.17 | 0.44[c] | 0.562 | 0.42 | 0.660 | 0.42 |
| 4-Et | −0.15 | 0.88 | 0.981 | 0.91 | 1.071 | 0.91 |
| 4-*n*-Pr | −0.15 | 1.32 | 1.399 | 1.37 | 1.369 | 1.42 |
| 4-*n*-Bu | −0.15 | 1.76 | 1.818 | 1.84 | 1.556 | 1.87 |
| 4-*n*-Amyl | −0.15 | 2.20 | 2.236 | 2.34 | 1.631 | 1.97 |
| 4-*t*-Amyl | −0.20 | 2.13 | 2.170 | 2.21 | 1.627 | 1.72 |
| 4-*n*-Hexyl | −0.15 | 2.64 | 2.655 | 2.77 | 1.596 | 1.80 |
| 4-*n*-Heptyl | −0.15 | 3.08 | 3.073 | 3.11 | 1.448 | 1.53 |
| 3,4-DiCH$_3$ | −0.24 | 0.90 | 1.000 | 0.69 | 1.087 | 0.81 |
| 2-Cl | 0.23 | 0.65[c] | 0.762 | 0.60 | 0.870 | 0.53 |
| 4-Cl | 0.23 | 0.89[c] | 0.990 | 0.77 | 1.079 | 0.77 |
| 2-Br | 0.23 | 0.85[c] | 0.952 | 0.76 | 1.046 | 0.79 |
| 4-Br | 0.23 | 1.09[c] | 1.181 | 0.96 | 1.227 | 1.04 |

[a] The phenol coefficients were taken from the work of Klarmann and associates, ref. 9. They are normally reported on a per cent basis. $PC'$ represents the molar phenol coefficient. [b] $\sigma$-values have not been determined for alkoxy groups above ethyl. It has been assumed that these would not differ significantly from ethoxy. The same assumption has been made for the higher *n*-alkyl functions. [c] These are primary values, all others are derived; see text for discussion.

The results in the case of the gram-negative *S. typhosa*, however, are more complex. Equations 19–21 result from the data in Table II.

The calculated values in Table II were obtained with eq. 20. An F test (see ref. 12, p. 108) reveals that the $\sigma$-term in eq. 21 is not significant at the 0.90 confidence level. These organisms are much less sensitive to the

(12) $r^2_{1\cdot23\cdots} = 1 - \frac{(n-k)s^2_{1\cdot23\cdots}}{(n-1)s_1^2}$ where $k$ is the number of variables being considered. For a discussion of correlation in multiple regression analysis see, for example, C. A. Bennett and N. L. Franklin, "Statistical Analysis in Chemistry and the Chemical Industry," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 286; $r$ is the correlation coefficient and $s$, the standard deviation.

(13) Ref. 4, p. 1123.

$$\log PC' = 0.454\pi + 0.477; \quad r^2\ 0.538\ \ r\ 0.733\ \ s\ 0.439 \quad (19)$$

$$\log PC' = -0.288\pi^2 + 1.312\pi + 0.139; \quad 0.845\ 0.919\ 0.259 \quad (20)$$

$$\log PC' = -0.283\pi^2 + 1.300\pi - 0.328\sigma + 0.129; \quad 0.855\ 0.925\ 0.255 \quad (21)$$

toxic action of the phenols. Moreover, although $\sigma$ plays a very minor role, the biological activity is not linearly related to $\pi$. Comparison of the coefficients of determination for eq. 19 and 20 shows the great importance of the squared term. A plot of observed *vs.* calculated values from eq. 20 suggests conformity to a normal distribution for those phenols with supraoptimal values of $\pi$. Unfortunately, relatively few such molecules were tested. However, considering that the cell wall in gram-negative bacteria is much more complex than for gram-positive bacteria,[14] it is not surprising that the rate of penetration of the phenols is indicated to be a slower process in the former. Since the phenol coefficient is determined in a rather short time interval (normally 5, 10, or 15 min.), the equilibrium situation envisaged by Ferguson might never be reached. In this connection, it is instructive to consider the effect of hydroquinone on the two bacteria. It was not included in Table II because of its great ease of oxidation which Klarmann noted makes it unusually toxic to *S. typhosa*. The phenol coefficient for hydroquinone with *S. typhosa* is 12, while the value for *M. pyogenes var. aureus* is 0.4. The latter value is moderately well predicted by eq. 16, while the former is over 100 times as great as eq. 20 would indicate ($\pi$ for 4-OH is $-0.88$). While the difference in sensitivity of the two microorganisms may be due to differences in the susceptibility of their metabolic machinery, it could also be rationalized by the hypothesis that hydroquinone (and other phenols) exerts its action on *S. typhosa* internally and on *M. pyogenes var. aureus* externally. Thus, phenols moving through the complex fat–protein matrix of the cell wall and membrane would do so by a multistep partitioning, adsorption–desorption process in which the minimum free energy change for the sum of the steps would occur in the case of *S. typhosa* when $\Sigma\pi \sim 2$. Whether the action of a particular series of organic compounds on a given organism would be a linear or a parabolic function of $\pi$ might, therefore, depend more on the nature of the biological material through which the compounds must pass to attain the site at which the primary action occurs, rather than on the type of action (*i.e.*, specific or nonspecific).

The additive character of $\pi$ is clearly illustrated by the good correlations obtained from the action of phenols on the gram-positive and gram-negative organisms. $\pi$-Constants were measured directly on only those 10 of the 35 phenols in Table II; the other values were derived indirectly. The values for normal alkyl groups longer than ethyl were obtained by adding 0.44 for each $CH_2$ unit. This figure represents the difference between log $P$ for 3-methyl- and 3-ethylphenols. The value for the *sec*-O-amyl function was obtained by subtracting 0.08 from the *n*-O-amyl function. The figure of 0.08 represents the difference between $\pi$ for the *n*-propyl and isopropyl groups in the phenoxyacetic acid series. The value for the *t*-amyl group was obtained by adding 0.44 to the value found experimentally for the *t*-butyl function using phenoxyacetic acids.

(14) M. Frobisher, "Microbiology," 7th Ed., W. B. Saunders Co., Philadelphia, Pa., 1962, p. 116.

**Phenyl Ethyl Phosphate Insecticides.**—The elegant work of Metcalf and Fukuto[15,16] on the toxicity toward houseflies of the phenyl phosphate esters presents a different example of how substituent character as measured by $\sigma$ and $\pi$ affect the biological activity of a series of derivatives. Using the first eight examples in Table III, the following equations were derived.

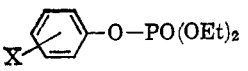
$$\log \frac{1}{C} = 2.282\sigma - 0.348; \quad r^2\ 0.952\ \ r\ 0.976\ \ s\ 0.286 \quad (22)$$

$$\log \frac{1}{C} = 2.420\sigma + 0.256\pi - 0.600; \quad 0.975\ 0.987\ 0.228 \quad (23)$$

$LD_{50}$ is represented by $C$; the calculated values in Table III were obtained with eq. 23. In formulating eq. 22 and 23 we have used $\sigma^-$ as did Metcalf and Fukuto.

TABLE III

TOXICITY OF DIETHYL PHENYL PHOSPHATES TO HOUSEFLIES



| No. | Substituent | $\sigma^-$ | $\pi^a$ | $\log \frac{1}{C}$ Calcd. | $\log \frac{1}{C}$ Obsd. | $\Delta \log \frac{1}{C}$ |
|---|---|---|---|---|---|---|
| 1 | 4-$NO_2$ | 1.27 | 0.46 | 2.59 | 2.40 | 0.19 |
| 2 | 4-$SO_2CH_3$ | 1.05 | −0.03[b,c] | 1.93 | 2.10 | 0.17 |
| 3 | 4-CN | 1.00 | 0.10 | 1.85 | 1.89 | 0.04 |
| 4 | 3-$NO_2$ | 0.71 | 0.50 | 1.25 | 1.46 | 0.21 |
| 5 | 3-$SF_5$ | 0.68 | 1.92[b] | 1.54 | 1.38 | 0.16 |
| 6 | 4-Cl | 0.23 | 0.89 | 0.19 | 0.04 | 0.15 |
| 7 | 3-*t*-Bu | −0.12 | 1.60[b] | −0.48 | −0.24 | 0.24 |
| 8 | H | 0.00 | 0.00 | −0.60 | −0.82 | 0.22 |
| 9 | 3-$N(CH_3)_2$ | −0.21 | 0.06 | −1.09 | 1.04 | 2.13 |
| 10 | 4-COOH | 0.73 | 0.08 | 1.19 | 0.25 | 0.94 |
| 11 | 4-*t*-Bu | −0.20 | 1.55[b,c] | −0.61 | $<-0.24$ | |
| 12 | 3-$OCH_3$ | 0.12 | 0.08 | −0.29 | $<-0.28$ | |
| 13 | 4-$OCH_3$ | −0.27 | −0.16 | −1.29 | $<-0.28$ | |
| 14 | 4-$CH_3$ | −0.17 | 0.44 | −0.90 | $<-1.32$ | |

[a] $\pi$-Constants were obtained directly from phenols except where indicated. [b] These values were derived from $\pi$ obtained *via* the phenoxyacetic acids. $\pi$-Values for substituents obtained from two different series of compounds (*e.g.*, phenols and phenoxyacetic acids) differ depending on the $\sigma$-values of the groups. $\Delta\pi$ for the two series is related as follows: $\Delta\pi = k\sigma$. If $\pi$ for a few members of each series is known, then $k$ can be determined and the values for other groups calculated. A study of $\Delta\pi$ is in progress. [c] The value for the 3-phenoxyacetic acid derivative was used.

The values for $\pi$ obtained from phenols were also used. Compound 9 was not used in the curve fitting because the unusual activity of this compound would indicate a different mode of action. As usual, it was expected and found that the carboxyl group would produce a derivative of lower than calculated activity since no correction was made for its degree of dissociation. Compounds 11 through 14 agree well with what would be expected from eq. 23, although the activities were

(15) R. L. Metcalf and T. R. Fukuto, *J. Econ. Entomol.*, **55**, 340 (1962).
(16) T. R. Fukuto and R. L. Metcalf, *J. Agr. Food Chem.*, **4**, 930 (1956).

not precisely determined. The correlation coefficient of 0.987 highlights the unusual precision of the biological test developed by these authors. An F test reveals that the $\pi$ term in eq. 23 is significant at the 0.90 level of confidence; however, the improvement in correlation using $\pi$ is slight. Of more than a dozen cases of structure–activity relationship which we have studied, this is the only instance yet encountered in which $\pi$ has such a minor influence. The low dependence on $\pi$ for these antiacetylcholinesterase insecticides is highly significant and may be a natural consequence of their extracellular action; that is, the toxic action appears to result from their inactivation of the enzyme acetylcholinesterase located in the synaptic junction of the nerve cells. The biological data in Table III was obtained by applying a drop of an acetone solution of the compound to be tested to the thorax of a housefly. The low significance of $\pi$ in eq. 23 indicates that these insecticides make their way from the thorax to the critical sites of action without crossing any fatty membranes. Or, alternatively, the acetone solvent may pave their way by disrupting fatty barriers. Further work to elucidate this lack of high dependence on $\pi$ is in order to complete our understanding of the mechanism of action of the phenyl phosphate insecticides.

In addition to measuring the toxicity of the phenyl phosphates toward insects, Metcalf and Fukuto determined the $I_{50}$ concentrations against cholinesterase obtained from homogenizing fly brains. In the *in vitro* process, they assumed that the phenyl phosphates were able to react directly with the enzyme involved. From this data we have derived eq. 24 for the relation between $I_{50}(C)$ and $\sigma$.

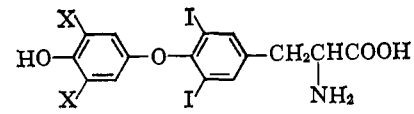
$$\log \frac{1}{C} = 2.59\sigma + 4.29; \quad r^2\ 0.821 \quad r\ 0.906 \quad s\ 0.620 \quad (24)$$

In deriving the above expression, we used all of the 3- and 4-monosubstituted derivatives (12) reported in ref. 16 for which definite values were obtained, except 3-*t*-$C_4H_9$ and 3-$N(CH_3)_2$. Although the correlation between observed and expected activity with eq. 24 is not nearly as good as with eq. 23, the two values for $\rho$ (2.59 and 2.42) are in good agreement. The value of $\rho$ from eq. 23 is closer to that of $\rho$ from eq. 24 than is the value from eq. 22, again indicating significance for the $\pi$-term. It is indeed unfortunate that the activity of these insecticides depends to such a small extent on $\pi$; otherwise, this would be a unique test of the correspondence of $\rho$ in eq. 6 with the Hammett $\rho$.

**Thyroxine Analogs.**—The correlation of thyroxine-like activity with chemical structure is a far more complex problem then those we have just considered. However, the partial success of the ingenious approach of Bruice, Kharasch, and Winzler[17] encouraged us to attempt a $\rho$–$\sigma$–$\pi$ analysis on this class of compounds. The chances for success in this analysis are particularly unfavorable since it is necessary to use biological test results from several different investigators. Also, the substituents involved are in *ortho* positions and, thus, the uncertainty of the $\sigma$-values is a serious handicap. In order to simplify the problem, we have considered from the data assembled by Bruice, *et al.*, only those molecules in which the second ring was modified. Using $\sigma_p$ (it is assumed that the substituent effect on the *ortho* position will be comparable to that on the *para*) and $\pi$ obtained from phenols, eq. 25–28 were derived from the results in Table IV.

TABLE IV

ACTION OF THYROXINE ANALOGS ON RODENTS




| —Functions— | | | | log A | | |
|---|---|---|---|---|---|---|
| X | X | $\Sigma\sigma$ | $\Sigma\pi$ | Calcd. | Obsd. | $\Delta$ log A |
| H | I | 0.28 | 1.15 | 2.201 | 2.74 | 0.539 |
| H | Br | 0.23 | 0.85 | 1.463 | 2.30 | 0.837 |
| I | I | 0.56 | 2.30 | 1.705 | 2.00 | 0.295 |
| F | I | 0.34 | 1.36 | 2.188 | 1.45 | 0.738 |
| Br | Br | 0.46 | 1.70 | 1.585 | 1.00 | 0.585 |
| H | F | 0.06 | 0.21 | 0.243 | 0.38 | 0.137 |
| F | F | 0.12 | 0.42 | 0.678 | 0.26 | 0.418 |
| Cl | Cl | 0.46 | 1.30 | −0.035 | −0.05 | 0.015 |
| H | H | 0.00 | 0.00 | −0.290 | −0.43 | 0.140 |

$$\log A = 2.339\sigma + 0.420; \quad r^2\ 0.168 \quad r\ 0.410 \quad s\ 1.088 \quad (25)$$

$$\log A = -0.553\pi^2 + 2.010\pi - 0.143; \quad 0.360 \quad 0.600 \quad 1.031 \quad (26)$$

$$\log A = 3.602\pi - 10.978\sigma + 0.416; \quad 0.516 \quad 0.718 \quad 0.896 \quad (27)$$

$$\log A = -1.134\pi^2 + 7.435\pi - 16.323\sigma - 0.287; \quad 0.781 \quad 0.884 \quad 0.660 \quad (28)$$

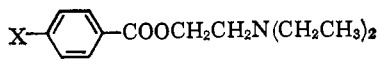
Although the correlation between calculated and observed activity is not high, it is not bad considering the above-mentioned uncertainties. An F test indicates that the exponential term in eq. 28 is justified at better than the 0.90 confidence level when compared to eq. 27. The calculated values in Table IV were obtained using eq. 28. The very low correlation obtained with $\pi$ or $\sigma$ alone highlights the importance of the combined effect of these two parameters on the structure–activity relationship. Equation 28 adds to our understanding of the substituent effects on thyroxine analogs.[18] Most interesting is the large negative value for $\rho$, indicating the importance of the electron-releasing groups for activity. The reliability of this coefficient is very probably not high because of the unreliability of the biological tests and the relatively small differences in the $\sigma$-constants. Nevertheless, the importance of this term is well illustrated in the example of the dinitro analog. Although this compound has a high enough $\Sigma\pi$ to be active (0.58), its $\Sigma\sigma$ renders it inactive.[18] The coefficients for $\pi$ in eq. 28 are such that the optimum value for $\Sigma\pi$ on this ring is in the range 3.0–3.5. This fact, plus the negative value for $\rho$, indicates that the halogens are not the best functions for this ring. Allyl, propyl, or butyl groups should be more effective than iodine. The ideal group for increasing activity (assuming steric effects to be absent) would be the *t*-butyl. Although such compounds have not been tested on rodents, it is interesting to note that for amphibia,[17]

(17) T. C. Bruice, N. Kharasch, and R. J. Winzler, *Arch. Biochem. Biophys.*, **62**, 305 (1956).

(18) H. A. Selenkow and S. P. Asper, *Physiol. Rev.*, **35**, 426 (1955).

TABLE V

LOCAL ANESTHETIC ACTION OF 2-DIETHYLAMINOETHYL BENZOATES ON GUINEA PIGS



| Function | $\sigma$ | $\pi$[a] | $\log \frac{1}{C}$ Calcd. | $\log \frac{1}{C}$ Obsd. | $\Delta \log \frac{1}{C}$ |
|---|---|---|---|---|---|
| 4-OEt | −0.25 | 0.54 | 1.590 | 1.92 | 0.330 |
| 4-N(CH$_3$)$_2$ | −0.60 | −0.08[b] | 1.672 | 1.72 | 0.048 |
| 4-OCH$_3$ | −0.27 | 0.11 | 1.366 | 1.22 | 0.146 |
| 4-NH$_2$ | −0.66 | −1.52[b] | 0.914 | 1.13 | 0.216 |
| 4-Cl | 0.23 | 0.80 | 1.134 | 1.05 | 0.084 |
| 4-OH | −0.36 | −0.27 | 1.259 | 0.90 | 0.359 |
| 4-NHCOCH$_3$ | −0.02 | −0.98[b] | 0.419 | 0.28 | 0.139 |
| 4-NO$_2$ | 0.78 | 0.04 | 0.000 | 0.13 | 0.130 |

[a] Except where noted, the values of $\pi$ obtained from benzoic acids were used. [b] These values were derived from the benzene analogs by the procedure described in footnote *b*, Table III.

replacing iodine with methyl groups gives a compound of greater activity than thyroxine itself.

**Local Anesthetic Action of Diethylaminoethyl Benzoates.**—The recent work[19] on comparative potencies of 4-substituted 2-diethylaminoethyl benzoates as local anesthetics provides another test of eq. 8. The relative activity of the compounds was determined using guinea pigs. Galinsky, *et al.*, noted there was a correlation between the substituent and its infrared absorption frequency which is, of course, related to the Hammett $\sigma$-constant. They noted that the more electron-releasing groups gave the most pronounced increase in activity. The following equations result from the data in Table V.

$$\log \frac{1}{C} = -0.882\sigma + 0.917; \quad r^2\ 0.448\ \ r\ 0.669\ \ s\ 0.498 \quad (29)$$

$$\log \frac{1}{C} = 0.579\pi - 1.262\sigma + 0.961; \quad 0.871\ 0.933\ 0.265 \quad (30)$$

$$\log \frac{1}{C} = 0.180\pi^2 + 0.711\pi - 1.247\sigma + 0.889; \quad 0.897\ 0.947\ 0.265 \quad (31)$$

The calculated values in Table V are those obtained with eq. 30. $C$ is the $ED_{50}$ concentration in mmole/100 ml. Considering the difficulties in quantitatively testing for local anesthetic action, the correlation obtained with eq. 30 is satisfyingly high. The low significance of the exponential term in eq. 31 is noteworthy.

With the knowledge of the influence of $\pi$ indicated in eq. 30 and the excellent quantitative test of Galinsky, *et al.*, it should be possible to construct much more active drugs of this type. For example, the addition of alkyl groups onto the 4-amino group or onto the ring should result in higher activity.

**Carcinogenic Compounds.**—Of the various classes of carcinogenic molecules which have been studied, the class which is most susceptible to analysis of substituent effects is the dimethylaminoazobenzenes (DAB). In particular, the extensive studies of Miller and Miller[20,21] and Badger[22] provide a series of derivatives for which activities are placed on a rough quantitative scale. Table VI summarizes the results of a $\rho$–$\sigma$–$\pi$ analysis of those derivatives of DAB in which substitution is limited to the second ring. Unfortunately, little data is available for activities of derivatives of the ring to which the dimethylamino group is attached. The Millers have come to the conclusion that the important step in carcinogenesis of the liver is the reaction of the first ring at a 2- or 6-position with a protein. Their conclusions have been reinforced by the quantum mechanical calculations of Fukui and co-workers,[23] who conclude that a nucleophilic substitution at one of these positions is most likely. Our results indicate that *electronically* the substituents on the second ring have no discernible affect on carcinogenic activity, and thus indirectly support the above conclusions. Using the 14 points in Table VI for which definite activity was specified (4-ethyl was omitted), eq. 32 and 33 were obtained *via* least-squares fits.

$$\log A = -2.02\pi^2 + 0.93\pi + 0.81; \quad r^2\ 0.489\ \ r\ 0.699\ \ s\ 0.241 \quad (32)$$

$$\log A = -2.06\pi^2 + 0.94\pi - 0.12\sigma + 0.83; \quad 0.504\ 0.710\ 0.249 \quad (33)$$

Although the correlation coefficients are low, these values refer only to the 14 active molecules used in the derivation of the equations. Equation 32 nicely delineates the cut-off points in activity which occur when $\Sigma\pi$ is too high or too low, and it clearly brings out the fact that the function of substituents in the second ring is limited to modifying the lipo–hydrophilic character of the parent molecule. Assuming that the ring of prime importance is the one carrying the dimethylamino group, it is not surprising that substituents in the second ring have a small electronic effect. It has been known for some time that, even in solution, electronic effects are very poorly transmitted from one ring to the other in, for example, the biphenyls.[24] In a reaction occurring on a protein, it might easily be that a twisting of the second ring and/or the azo-linkage could block resonance interaction between the two rings leaving only the inductive effect which would be slight, considering the distance involved. The narrow range set for $\pi$ by eq. 32 attracts attention. Most other biologically active series which we have studied have shown activity over a wider range of $\pi$-values. It would appear with the DAB derivatives, which for testing purposes are fed to mice, that the barriers to leaving the digestive system and arriving at the site of action in the liver are very great. It seems highly probable that if the more lipophilic compounds in Table VI were introduced directly into the liver, carcinomas would result. Several interesting exceptions are evident in Table VI.

Most difficult to rationalize is the complete inactivity of the 4′-NO$_2$ derivative. All of the inactive compounds except this one and 4′-N(CH$_3$)$_2$ have negative

(19) A. M. Galinsky, J. E. Gearien, A. J. Perking, and S. V. Susina, *J. Med. Chem.*, **6**, 320 (1963).

(20) J. A. Miller and E. C. Miller, *Advan. Cancer Res.*, 1, 339 (1953).

(21) J. A. Miller, E. C. Miller, and G. C. Finger, *Cancer Res.*, **17**, 387 (1957).

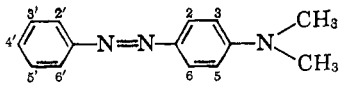
(22) G. M. Badger, *Advan. Cancer Res.*, **2**, 73 (1954).

(23) K. Fukui, C. Nagata, T. Yonezawa, Y. Inamoto, and A. Imamura, *Gann*, **51**, 67 (1960).

(24) V. P. Kreiter, W. A. Bonner, and R. H. Eastman, *J. Am. Chem. Soc.* **76**, 5770 (1954).

TABLE VI

CARCINOGENIC ACTIVITY OF DERIVATIVES OF DIMETHYLAMINOAZOBENZENE



| No. | Function | $\Sigma\sigma$[a] | $\Sigma\pi$[b] | $A$[c] | —log $A$— Obsd. | Calcd. | $\Delta$log $A$ |
|---|---|---|---|---|---|---|---|
| 1 | 3′-$CH_3$ | −0.07 | 0.51 | 10–12 | 1.04 | 0.76 | 0.28 |
| 2 | 4′-F | 0.06 | 0.14 | 10–12 | 1.04 | 0.90 | 0.14 |
| 3 | 3′-F | 0.34 | 0.13 | 10–12 | 1.04 | 0.89 | 0.15 |
| 4 | 3′-$OCH_3$ | 0.12 | 0.11 | 10–12 | 1.04 | 0.88 | 0.16 |
| 5 | 2′,4′-Di-F | 0.12 | 0.13 | >10 | 1.00+ | 0.89 | |
| 6 | 3′,4′-Di-F | 0.40 | 0.27 | >10 | 1.00+ | 0.91 | |
| 7 | 2′,5′-Di-F | 0.40 | 0.12 | >10 | 1.00+ | 0.89 | |
| 8 | 3′,5′-Di-F | 0.68 | 0.26 | >10 | 1.00+ | 0.91 | |
| 9 | 2′,4′,6′-Tri-F | 0.18 | 0.12 | >10 | 1.00+ | 0.89 | |
| 10 | 4′-Et | −0.15 | 0.97[d] | 10 | 1.00 | −0.19 | 1.19 |
| 11 | 2′-F | 0.06 | −0.01 | 7 | 0.85 | 0.80 | 0.05 |
| 12 | H | 0.00 | 0.00 | 6 | 0.78 | 0.81 | 0.03 |
| 13 | 3′-Cl | 0.37 | 0.76 | 5–6 | 0.74 | 0.35 | 0.39 |
| 14 | 3′-$NO_2$ | 0.71 | 0.10 | 5 | 0.70 | 0.88 | 0.18 |
| 15 | 2′-$NO_2$ | 0.78 | −0.23 | 3 | 0.48 | 0.48 | 0.00 |
| 16 | 4′-$OCH_3$ | 0.27 | −0.04 | 3 | 0.48 | 0.76 | 0.28 |
| 17 | 2′-$CH_3$ | −0.17 | 0.68 | 2–3 | 0.40 | 0.51 | 0.11 |
| 18 | 2′-Cl | 0.23 | 0.59 | 2 | 0.30 | 0.65 | 0.35 |
| 19 | 2′-$OCH_3$ | −0.27 | −0.34 | 2 | 0.30 | 0.25 | 0.05 |
| 20 | 4′-Cl | 0.23 | 0.70 | 1–2 | 0.18 | 0.47 | 0.29 |
| 21 | 4′-$CH_3$ | −0.17 | 0.52 | <1 | <0 | 0.74 | |
| 22 | 3′-OEt | 0.15 | 0.62[e] | <1 | <0 | 0.61 | |
| 23 | 4′-$NO_2$ | 0.78 | 0.06 | Inactive | | 0.85 | |
| 24 | 3′-Br | 0.39 | 0.95 | Inactive | | −0.14 | |
| 25 | 2′-OH | −0.36 | −0.59 | Inactive | | −0.45 | |
| 26 | 3′-OH | 0.00 | −0.51 | Inactive | | −0.20 | |
| 27 | 4′-OH | −0.36 | −0.62 | Inactive | | −0.55 | |
| 28 | 3′-$CF_3$ | 0.42 | 1.09 | Inactive | | −0.58 | |
| 29 | 4′-$CF_3$ | 0.55 | 1.09[d] | Inactive | | −0.58 | |
| 30 | 4′-$NH_2$ | −0.66 | −1.55[f] | Inactive | | −5.48 | |
| 31 | 4′-$N(CH_3)_2$ | −0.60 | −0.12[f] | Inactive | | 0.67 | |
| 32 | 4′-$C_6H_5$ | | 1.91[d] | Inactive | | −4.78 | |
| 33 | 4′-$NHCOCH_3$ | −0.02 | −0.79[d] | Inactive | | −1.19 | |
| 34 | 3′,4′-$(CH)_4$ | 0.17 | 1.27 | Inactive | | −1.27 | |
| 35 | 3′,4′-Di-$CH_3$ | −0.24 | 1.03 | <1 | <0 | −0.38 | |
| 36 | 3′,5′-Di-$CH_3$ | −0.14 | 1.02 | Inactive | | −0.35 | |
| 37 | 2′,4′-Di-$CH_3$ | −0.34 | 1.20 | Inactive | | −0.99 | |
| 38 | 2′,5′-Di-$CH_3$ | −0.24 | 1.19 | Inactive | | −0.95 | |
| 39 | 2′,5′-Di-Cl | 0.60 | 1.35 | Inactive | | −1.62 | |
| 40 | 2′,4′,6′-Tri-Cl | 0.68 | 1.88 | Inactive | | −4.59 | |
| 41 | 2′,4′,6′-Tri-Br | 0.70 | 2.52 | Inactive | | −9.68 | |

[a] $\sigma_p$ was used for *ortho* substituents. [b] $\pi$-Values were those obtained from the phenoxyacetic acids. [c] Activity is that reported by Miller, *et al.*, ref. 17. [d] These figures were obtained from 3-substituted phenoxyacetic acids. [e] This value was calculated by the addition of 3-$CH_3$ and 3-$CH_3O$–. [f] The reliability of these values is not high; see discussion.

predicted values of log $A$. It seems unlikely that this inactivity is to be attributed to steric factors since the 4′-Et derivative is active. It may be that the conjugative power of this group is so great that it is sufficient to hold the two ring systems and the azo bridge in a planar conformation and in this way greatly reduce the electron density on the amino group in the other ring. The inactivity of the nitro group might also be caused by its reduction to the amino group in the liver, before it reaches the site of action. This highly polar group then would prevent its reaching the lipophilic site of action. The nitro group appears to be the only function of this type to have been tested in the 4′-position. It would be of interest to check this hypothesis by testing the 4′-CN function. In this instance, it would be necessary to balance the low $\pi$-value of the CN function (−0.31) with a methyl group in the 3′-position. Alternatively, one could offset this low $\pi$-value by using the diethylamino instead of the dimethylamino group. Although both diethylamino and dipropylaminoazobenzenes have been reported inactive, this is probably due to the supraoptimal lipophilic character of the molecules rather than to steric effects.

Strangely, the 4′-$CH_3$ has much lower activity than expected, while the 4′-Et is predicted to be inactive and is actually quite active. Since this difference cannot be rationalized by a comparison of $\sigma$- or $\pi$-values or steric effect, the only alternative is to consider metabolic modification of these two alkyl groups. Oxidation of these would give rise to substances with lower $\pi$-values which, in the case of the 4′-$CH_3$, would result in almost complete loss of activity but would, in the case of the 4′-Et, bring the $\pi$-value of the resulting molecule into the ideal range. No explanation comes to mind easily for the unexpectedly low activity found for 3-OEt.

In the above correlation we have used $\pi$-constants derived from phenoxyacetic acids. It is quite clear from work under way with a variety of systems (*e.g.*, phenols, anilines, nitrobenzenes, benzenes, etc.) that $\pi$-constants vary from system to system, depending on $\sigma$. The change in $\pi$ with $\sigma$ is particularly important in the case of the amino group in changing from one series of compounds to another. However, since all of the values for $\pi$ employed in Table VI, except two, were obtained from phenoxyacetic acids, the treatment is self-consistent, and we would not expect an essential difference in results had the constants been obtained from dimethylaminoazobenzenes.

For the two exceptions, $NH_2$ and $N(CH_3)_2$, the constants of which were derived from benzene, considerable uncertainty exists. For the 4-$NH_2$ function, even a considerable difference in $\pi$ would still result in a value indicating inactivity for this derivative. The $(CH_3)_2$-N function is a borderline case and the fact that it is predicted active by eq. 32 is not very significant.

Equations 32–34 arise from eq. 3 in a slightly different way from the previous examples, where equivalent biological responses were involved. With the carcinogenic azobenzenes, as well as with the aromatic hydrocarbons and benzacridines, a constant amount of test compound is applied to the mouse and a variable response is obtained. The rate of cancer incidence with respect to time determines the relative activity. Thus, $C$ in eq. 3 is, under these conditions, a constant and d(response)/d$t$ $\propto$ relative biological response. In eq. 4, log $(1/C)$ now becomes log $A$, where $A$ represents relative biological response.

The cancer-producing activity of the polynuclear aromatic hydrocarbons and benzacridines has been the subject of an enormous amount of work undertaken to solve the structure–activity relationship. Despite this great effort and the brilliant work of the French school centering around the Pullmans and the Daudels[25–27] the present understanding of the mode of action of these compounds is not complete.[22] From our work with better defined systems it seemed that, even though great uncertainty exists as to the relative activity of these substances, some insight into the possible role of lipo–hydrophilic character and its relation to activity might be gained through the application of eq. 8. Because of the crudeness of the biological assay, our main interest was not in the degree of variance accounted for,

(25) C. A. Coulson, *Advan. Cancer Res.*, **1**, 1 (1953).
(26) A. Pullman and B. Pullman, *ibid.*, **3**, 117 (1955).
(27) A. Lacassagne, N. P. Buu-Hoï, R. Daudel, and F. Zajdela, *ibid.*, **4**, 316 (1956).

TABLE VII
CARCINOGENICITY OF AROMATIC HYDROCARBONS AND BENZACRIDINES

| No. | Substance | Total charge[a] | log $P$ − 5[b] | log $A$ Obsd.[c] | log $A$ Calcd. | Δ log $A$ |
|---|---|---|---|---|---|---|
| 1 | Naphthacene | 1.258 | 0.91 | Inactive | 0.226 | |
| 2 | Anthracene | 1.259 | −0.36 | Inactive | −0.053 | |
| 3 | Triphenylene | 1.260 | 0.91 | Inactive | 0.282 | |
| 4 | Benz[*a*]acridine | 1.260 | −0.33 | Inactive | −0.012 | |
| 5 | Benz[*c*]acridine | 1.270 | −0.33 | Inactive | 0.269 | |
| 6 | Chrysene | 1.272 | 0.91 | 1.10 | 0.619 | 0.481 |
| 7 | 5-Methylbenz[*a*]acridine | 1.273 | 0.17 | Inactive | 0.527 | |
| 8 | Naphthalene | 1.274 | −1.63 | Inactive | −0.420 | |
| 9 | 1,2-Benzanthracene | 1.283 | 0.91 | 1.10 | 0.928 | 0.172 |
| 10 | 5,8-Dimethylbenz[*a*]acridine | 1.284 | 0.67 | 1.10 | 0.935 | 0.165 |
| 11 | 5,7-Dimethylbenz[*a*]acridine | 1.285 | 0.67 | Inactive | 0.963 | |
| 12 | 5,9-Dimethylbenz[*a*]acridine | 1.286 | 0.67 | 1.10 | 0.991 | 0.109 |
| 13 | Phenanthrene | 1.291 | −0.36 | Inactive | 0.845 | |
| 14 | 8-Methyl-1,2-benzanthracene | 1.292 | 1.41 | 1.10 | 1.170 | 0.070 |
| 15 | 3,4-Benzphenanthrene | 1.293 | 0.91 | 1.10 | 1.209 | 0.109 |
| 16 | 7-Methyl-1,2-benzanthracene | 1.294 | 1.41 | 1.40 | 1.226 | 0.174 |
| 17 | 6-Methyl-1,2-benzanthracene | 1.294 | 1.41 | 1.40 | 1.226 | 0.174 |
| 18 | 5-Methyl-1,2-benzanthracene | 1.296 | 1.41 | 1.70 | 1.282 | 0.418 |
| 19 | 9-Methyl-1,2-benzanthracene | 1.296 | 1.41 | 1.80 | 1.282 | 0.518 |
| 20 | 3-Methyl-1,2-benzanthracene | 1.298 | 1.41 | 1.57 | 1.338 | 0.232 |
| 21 | 4-Methyl-1,2-benzanthracene | 1.298 | 1.41 | 1.57 | 1.338 | 0.232 |
| 22 | 5,7,9-Trimethylbenz[*a*]acridine | 1.298 | 1.17 | 1.57 | 1.353 | 0.217 |
| 23 | 5,9-Dimethylbenz[*c*]acridine | 1.302 | 0.67 | 1.88 | 1.440 | 0.440 |
| 24 | 5,7-Dimethylbenz[*c*]acridine | 1.304 | 0.67 | 1.94 | 1.496 | 0.444 |
| 25 | 5,8-Dimethylbenz[*c*]acridine | 1.304 | 0.67 | 1.94 | 1.496 | 0.444 |
| 26 | 10-Methyl-1,2-benzanthracene | 1.306 | 1.41 | 1.94 | 1.563 | 0.377 |
| 27 | 5,6-Dimethyl-1,2-benzanthracene | 1.307 | 1.91 | 1.88 | 1.505 | 0.375 |
| 28 | 5,9-Dimethyl-1,2-benzanthracene | 1.309 | 1.91 | 2.00 | 1.561 | 0.439 |
| 29 | 8-Methyl-3,4-benzphenanthrene | 1.309 | 1.41 | 1.10 | 1.647 | 0.547 |
| 30 | 6-Methyl-3,4-benzphenanthrene | 1.310 | 1.41 | 1.40 | 1.675 | 0.275 |
| 31 | 4,9-Dimethyl-1,2-benzanthracene | 1.311 | 1.91 | 1.88 | 1.617 | 0.263 |
| 32 | 1-Methyl-3,4-benzphenthrene | 1.312 | 1.41 | 1.40 | 1.731 | 0.331 |
| 33 | 2-Methyl-3,4-benzphenthrene | 1.312 | 1.41 | 1.70 | 1.731 | 0.031 |
| 34 | 5,7,9-Trimethylbenz[*c*]acridine | 1.312 | 1.17 | 1.80 | 1.746 | 0.054 |
| 35 | 7-Methyl-3,4-benzphenanthrene | 1.313 | 1.41 | 1.10 | 1.760 | 0.660 |
| 36 | 5,10-Dimethyl-1,2-benzanthracene | 1.317 | 1.91 | 2.00 | 1.786 | 0.214 |
| 37 | 9,10-Dimethyl-1,2-benzanthracene | 1.319 | 1.91 | 1.94 | 1.842 | 0.098 |
| 38 | 4,10-Dimethyl-1,2-benzanthracene | 1.321 | 1.91 | 1.70 | 1.898 | 0.198 |
| 39 | 6,9,10-Trimethyl-1,2-benzanthracene | 1.330 | 2.41 | 1.88 | 1.990 | 0.110 |
| 40 | 5,9,10-Trimethyl-1,2-benzanthracene | 1.332 | 2.41 | 1.94 | 2.047 | 0.107 |
| 41 | 5,6,9,10-Tetramethyl-1,2-benzanthracene | 1.343 | 2.91 | 1.70 | 2.121 | 0.421 |
| 42 | 5-Ethyl-1,2-benzanthracene | 1.296 | 1.91 | Active | 1.196 | |
| 43 | 5-Isopropyl-1,2-benzanthracene | 1.296 | 2.29 | Active | 1.081 | |
| 44 | 5-Propyl-1,2-benzanthracene | 1.296 | 2.41 | Active | 1.036 | |
| 45 | 5-Butyl-1,2-benzanthracene | 1.296 | 2.91 | Inactive | 0.801 | |
| 46 | 5-Hexyl-1,2-benzanthracene | 1.296 | 3.91 | Inactive | 0.108 | |
| 47 | 5-Heptyl-1,2-benzanthracene | 1.296 | 4.41 | Inactive | −0.351 | |

[a] Total charge on the K region as calculated by Pullman, see ref. 5, p. 66. [b] We have used log $P$ for the whole molecule; see discussion. [c] All of the data is that of Pullman except compounds 42–47, which come from the work of Badger, ref. 18. We have assumed the same total charge for compounds 42–47 as found for 18.

but in finding an explanation for the lack of carcinogenicity which is so apparent in the larger polycyclic compounds. Although a rationalization has been advanced[26] in the concept of an L region competing with the K region, this is not of help in explaining why, as one goes to higher alkyl derivatives of the 1,2-benzanthracenes for example, activity decreases. Since we are dealing with acridines as well as hydrocarbons, we have elected to use log $P$ rather than $\pi$ for the regression analysis. Because of the extreme insolubility of the polycyclic aromatic hydrocarbons with more than four rings, we have not been able to measure log $P$ except for the first few members of the series. We have instead taken advantage of the additive character of $\pi$ to estimate log $P$ for the larger molecules. Using octanol and water, the following values were obtained.

| | log $P$ |
|---|---|
| Benzene | 2.13 ± 0.01 |
| Napthalene | 3.37 ± 0.01 |
| Acridine | 3.40 ± 0.01 |
| Anthracene | 4.45 ± 0.05 |
| Phenanthrene | 4.46 ± 0.04 |

The additive character of $\pi$ or log $P$ can be illustrated as follows

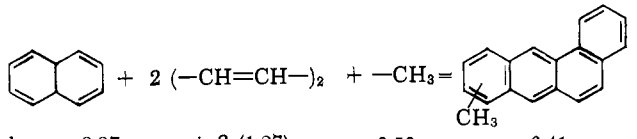
$$\log P_{\text{2-naphthoxyacetic acid}} - \log P_{\text{phenoxyacetic acid}} = \pi_{-CH=CHCH=CH-} = 1.27$$

$\pi$ for the $(CH)_4$ can also be calculated from the hydrocarbons.

$$\log P_{\text{naphthalene}} - \log P_{\text{benzene}} = \pi_{(CH)_4} = 1.24$$

Anthracene and phenanthrene result from the addition of a $(CH)_4$ unit to naphthalene, and hence, both should have the same value for log $P$, *i.e.*, 3.37 + 1.27 = 4.64. This value is in fair agreement with that determined experimentally. The experimental values for anthracene and phenanthrene are not as reliable (the standard deviation is 4–5 times as great) as those for the smaller molecules because of the experimental difficulties in working with such insoluble substances. Also, there may be a small systematic error in the values for the two three-ring hydrocarbons due to a slight, but persistent, cloudiness in the aqueous phases which could not be eliminated even by long centrifuging and standing. This would mean that log $P$ values reported above for anthracene and phenanthrene are slightly low.

The following example for methylbenzanthracene illustrates how log $P$ values in Table VII are calculated.



log $P$ = 3.37 + 2 (1.27) + 0.50 = 6.41

The constants in eq. 34 were obtained using all of the molecules in Table VII except 42, 43, and 44, the relative activities of which we were not certain. In eq. 34,

$$\log A = -0.14(\log P - 5)^2 + 0.32(\log P - 5) + 28.07\epsilon - 35.26; \quad r^2\ 0.729 \quad r\ 0.865 \quad s\ 0.400 \quad (34)$$

$\epsilon$ is the total charge on the K region as calculated by Pullman, rather than $\sigma$. The relative activity of carcinogenic compounds is exceedingly hard to establish and is normally reported as 1, 2, 3, or 4 +-units. To convert these pluses to a numerical scale we have arbitrarily assigned each + a value of 25 to place the compounds on a scale of 100. For curve fitting purposes, the inactive molecules were given a value of log $P$ = 0 since the logarithm of zero would be meaningless. This implies that their activity on the scale of 100 is 1. All of the compounds in Table VII except numbers 42–47 have been taken from the work of Pullman.[25] In this compilation, data from two tests for carcinogenicity are often given. Where the two results differ, we have used an average. We have omitted 5-methylacridine which seems to be an error, since subsequent work has shown that acridines without a benz unit are inactive. Also, later work[22] has shown 1,2-benzanthracene to be inactive in the skin test although active in subcutaneous tissue so that we have used a value of 12.5 for this compound rather than 25. Agreement is not complete[26] on chrysene so that here, too, we have used a value of 12.5 rather than 25 called for by the original table of Pullman. We have used the total charge on the K region (or highest total charge in molecules lacking a normal K region) as calculated by Pullman,[25] rather than $\sigma$. The use of total charge in place of $\sigma$ seems justified, since it has been shown[28] that a close correlation exists between $\sigma$ and a variety of quantum mechanical parameters which have been used in the investigation of aromatic substitution reactions.

(28) A. Streitwieser, "Molecular Orbital Theory," John Wiley and Sons, Inc., New York, N. Y., 1961, Chapter 11.

As imprecise as this analysis must be because of the difficulty in ascertaining the relative biological activities, it nevertheless affords new insight into the mechanism of carcinogenicity of the polycyclic aromatic compounds. Of greatest significance is the simple explanation for inactivity of the large molecules of six or more rings and the higher alkyl derivatives of 1,2-benzanthracene.

Badger[22] has pointed out the falloff in activity which results when the alkyl group in either the 5-position or the 10-position in 1,2-benzanthracene is increased in size. Comparison of the calculated values for compounds 18 and 42–47 in Table VII shows that this is to be expected as a natural consequence of increase in log $P$, and that it is unnecessary to attribute this to steric factors. The fact that polycyclic aromatic hydrocarbons with six or more rings have been found to be inactive[26] (except the condensed type such as dibenzpyrene which have lower log $P$ values) is also well accommodated by eq. 34.

Again, in the case of the 20-alkylcholanthrenes it has been noted[22] that activity falls off greatly in going from methyl (calcd. log $P$, 7.36) to isopropyl (calcd. log $P$, 7.86) to $t$-butyl (calcd. log $P$, 8.25). This, too, is in line with expectations from eq. 34, although in these two examples the electronic contribution from three alkyl groups would have to be considered.

The very interesting study of Badger[22] of the effect of a wide variety of substituents in the 10-position of 1,2-benzanthracenes deserves more attention. Unfortunately, the necessary electronic data as well as $\pi$-values are not available so that an analysis of this group of derivatives cannot be made at present. From preliminary crude attempts it would seem that the relative activities of these compounds would be hard to rationalize except on the basis of a radical-type mechanism.

Another advantage of using the parameter $\pi$ is that it is not necessary to invoke the concepts of L and K regions to rationalize the inactivity of certain classes of compounds. Those ascribed as being inactive because of the importance of the L region[26] (usually compounds of five or more rings) are generally too lipophilic. This would always be true for those with six or more rings. Some five-membered ring compounds are active; others are inactive. This is to be expected since log $P$ for five-ring compounds would be 7.00. This is the point at which the $\pi$-terms in eq. 34 make zero contribution to log $A$. Above 7, the contribution becomes negative, while the ideal value is about 6.1. Thus, for the five-ring aromatics, the total charge as well as small steric factors becomes quite critical.

Small molecules said to be inactive because the K region is missing (naphthalene, anthracene, triphenylene) are simply not lipophilic enough, considering their low "highest total charge." When log $P$ is increased, activity results as, for example, with 9,10-dimethylanthracene[22] (calcd. log $P$, 5.64). Another striking example is the carcinogenic 1,2-diethyl-1,2-diphenylethene,[22] which also lacks a typical K region. The work of Tilak[29] also indicates that a K region is unessential.

It has been suggested[25] that an upper as well as a lower limit to total charge density may be necessary to explain why a compound such as 9,10-dimethyl-1,2,5,6-dibenzanthracene (calcd. log $P$, 7.89) is inactive or very

(29) B. D. Tilak, *Tetrahedron*, **9**, 76 (1960).

slightly active while the 9-methyl derivative (calcd. log $P$, 7.48) is active. Equation 34 avoids this difficulty. From Table VII it is seen that the cut off in activity is to be expected at about log $P = 8$. This is the point where the carcinogen is 100 million times more soluble in the lipophilic phase than in the aqueous—an enormous differential. For aromatics with six rings, the differential would be over 600 million. Considering the great insolubility of such compounds in either phase, it seems quite natural to expect a small aqueous barrier to isolate the cellular site of action from these insoluble substances.

While the results from the $\rho$–$\sigma$–$\pi$ analysis with carcinogenic hydrocarbons are not as sharp as those we have found for the other systems, they are convincing enough to show that the lipo–hydrophilic character of these molecules must be considered in attempts to rationalize structure–activity relationships.

From the results obtained with the $\rho$–$\sigma$–$\pi$ analysis for the eight examples reported herein, plus the two previously considered cases,[3] the view emerges that, although eq. 8 was developed to rationalize structure–activity relationships of the plant growth regulators, it now appears to have a general use in a wide variety of pharmacological systems. Our basic hypothesis that, as a first approximation, biologically active compounds appear to exert a rate-controlling effect on one chemical or physical process and that biological effects resulting from structural changes can be correlated by means of regression analyses with two parameters, $\sigma$ and $\pi$, seems well worth further study.

If $\rho$ in eq. 6 is indeed the same as that in the Hammett equation as we have assumed, then eq. 6 (or its simpler forms) should be useful in estimating electronic and steric demands of enzymes without the necessity of isolating and studying them *in vitro*.

The constants associated with $\pi$ provide insight into the nature of the cellular material through which a molecule must make its way before reaching the site of action. The additive character of $\pi$ or log $P$ should be of considerable help in making gross modifications in the structure of biologically active compounds in the quest for more active or more selective drugs. For example, in the synthesis of the so-called nonclassical antimetabolites,[30] where the attachment of a very large moiety to a basic structure is desired, one must, in addition to holding electron distribution constant, achieve an isolipotropic character in the derivative if one expects to find equivalent biological response. Work in progress indicates that when strong electronic interaction is absent, $\pi$ is constant and additive in character. This should make the construction of isolipotropic molecules relatively easy.

**Acknowledgment.**—This work was supported under Research Grant GM-07492-04 from the National Institutes of Health. We wish to thank Prof. R. Nelson Smith for several valuable discussions. We are indebted to Miss Margaret Streich for help with curve fitting of the data on the carcinogenic compounds.

(30) R. B. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, *J. Med. Chem.*, **2**, 633 (1960).

---

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY OF THE UNIVERSITY OF BRITISH COLUMBIA AND THE TECHNOLOGICAL RESEARCH LABORATORY, FISHERIES RESEARCH BOARD OF CANADA, VANCOUVER 8, B. C., CANADA]

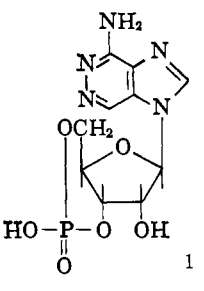
# Deoxyribonucleoside-3′,5′ Cyclic Phosphates. Synthesis and Acid-Catalyzed and Enzymic Hydrolysis[1]

BY G. I. DRUMMOND,[2] M. W. GILGAN,[2] E. J. REINER,[3] AND MICHAEL SMITH[3]

RECEIVED NOVEMBER 16, 1963

Methods previously developed for the synthesis of ribonucleoside-3′,5′ cyclic phosphates[4] have been utilized for the synthesis of cyclic phosphates derived from deoxyadenosine, deoxycytidine, deoxyguanosine, deoxyinosine, deoxyuridine, and thymidine. The glycosidic bonds in these nucleotides have unusual stabilities in the presence of acid, analogous to the stabilities found in ribonucleoside-3′,5′ cyclic phosphates. A nucleoside-3′,5′ cyclic phosphate diesterase from beef brain, which converts adenosine-3′,5′ cyclic phosphate to adenosine-5′ phosphate, hydrolyzes purine deoxynucleoside-3′,5′ cyclic phosphates.

The discovery[5] and characterization[6] of adenosine-3′,5′ cyclic phosphate (1) as a factor in the activation of hepatic glycogen phosphorylase has been followed by a rapidly increasing appreciation of the widespread occurrence[7,8] of the nucleotide and the multiplicity of its



1

biological functions, especially as an intermediate in the action of hormones.[9] Consequently, it is of interest to

(1) This work has been supported by a grant from the Division of General Medical Sciences, National Institutes of Health, U. S. Public Health Service (GM 09241), and by grants from the Life Insurance Medical Research Fund, Philadelphia, Pa., and the B. C. Heart Foundation.

(2) Department of Pharmacology, University of British Columbia, Vancouver 8, B. C.

(3) Technological Research Laboratory, Fisheries Research Board of Canada, Vancouver 8, B. C.

(4) M. Smith, G. I. Drummond, and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 698 (1961).

(5) E. W. Sutherland and T. W. Rall, *ibid.*, **79**, 3608 (1957); T. W. Rall, E. W. Sutherland, and J. Berthet, *J. Biol. Chem.*, **224**, 463 (1957); E. W. Sutherland and T. W. Rall, *ibid.*, **232**, 1077 (1958).

(6) W. H. Cook, D. Lipkin, and R. Markham, *J. Am. Chem. Soc.*, **79**, 3607 (1957); D. Lipkin, W. H. Cook, and R. Markham, *ibid.*, **81**, 6198 (1959).

(7) R. W. Butcher and E. W. Sutherland, *J. Biol. Chem.*, **237**, 1244 (1962).

(8) A. Kobata, M. Kida, and S. Ziro, *J. Biochem.* (Tokyo), **50**, 275 (1961); A. Kobata, S. Ziro, and M. Kida, *ibid.*, **51**, 277 (1962); T. Okabayashi, M. Ide, and A. Yoshimoto, *Arch. Biochem. Biophys.*, **100**, 158 (1962).

(9) E. W. Sutherland and T. W. Rall, *Pharmacol. Rev.*, **12**, 265 (1960);