index and the main component, is positive and may be larger than any of the correlation coefficients calculated between pairs of nontransformed pharmacological activity indices. The dependent variable was not included into the data subset subjected to the decomposition procedure.

There is virtually no correlation between the dependent variable and component PR_2^X, extracted from four variate data subsets, although PR_2^X explains after PR_1^X the next highest part of the total SV of the subsets. The factors accounting for the secondary effects appear in PR_3^X, if four variate subsets are investigated. The factors accounting for the secondary effects may also appear in PR_2^X, if three variate subsets are considered.

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Notes

Mutagenicity of Substituted (*o*-Phenylenediamine)platinum Dichloride in the Ames Test. A Quantitative Structure–Activity Analysis

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A set of 13 substituted (*o*-phenylenediamine)platinum dichlorides has been studied in the Ames test using *Salmonella typhimurium* (TA-92). These *cis*-platinum compounds are mutagenic without activation by microsomes. The following correlation equation shows that the most important determinant of mutagenicity by substituents (X) is electron withdrawal via through resonance: $\log 1/C = 2.23\sum\sigma^- + 5.78$. C in this expression is the molar concentration of compound producing 30 mutations/10^8 bacteria initially delivered above background mutation, and σ^- is the Hammett constant obtained from substituted anilines.

Despite the huge amount of work being undertaken studying the mutagenicity of organic compounds with the Ames test, very little has been reported on quantitative structure–activity relationships (QSAR). Sugiura et al.[1] have shown a linear relationship between the mutagenicity of *Salmonella typhimurium* (TA-100) acting on four styrene oxides (I) and the Hammett σ constant. We have formulated eq 1 from their data, where C is the molar

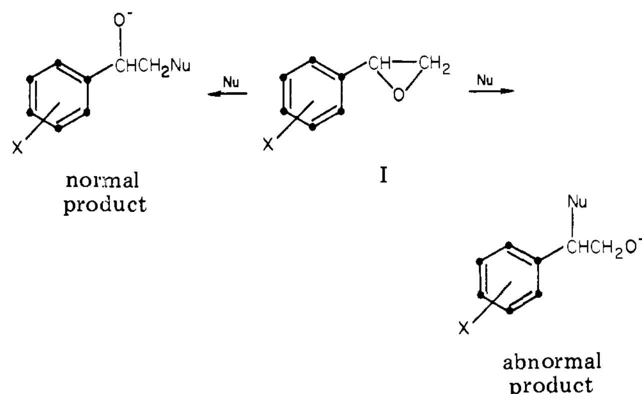
$$\log 1/C = -1.56(\pm 1.2)\sigma + 6.18(\pm 0.27) \quad (1)$$

$$n = 4;\ r = 0.971;\ s = 0.113$$

concentration of styrene oxide producing 200 mutants/10^9 survivors, the figures in parentheses are the 95% confidence limits, n represents the number of data points, r is the correlation coefficient, and s is the standard deviation from regression. Equation 1 is statistically significant ($F_{1,2} = 32.1$; $F_{1,2;\alpha=0.05} = 18.5$). The negative slope of eq 1 shows that electron release by substituents increases mutagenicity. Sugiura et al. concluded that there was little, if any, dependence of activity on the hydrophobic parameter π.[2] Using π in place of σ, we find a poor correlation ($r = 0.492$; $s = 0.411$). However, there are not enough data points to test the linear combination of π and σ so that hydrophobic effects cannot be completely ruled out.

Sugiura et al. noted that there are two ways styrene oxides might react with nucleophiles (Scheme I). They show that ρ (slope of Hammett equation) is 0.87 for the "normal" reaction of styrene oxide with a typical nucleophilic reagent, benzylamine, while ρ for the "abnormal" reaction has been shown to be negative (−1.15 for reaction with benzylamine and −1.6 for reduction with lithium borohydride). Thus, the negative slope of eq 1 suggests that the abnormal (possibly S_N1) type of reaction in the bacterial cell is apparently causing the mutation. While the results of Sugiura et al. are most interesting and highly suggestive, one cannot place much weight on a biological QSAR based on only four data points which, moreover, are not widely separated in data space. It would be interesting to test substituents such as OH, NH_2, and OCH_3 to see if σ^+ might give a better correlation than σ. If so, this would establish a role for through resonance and an S_N1-type mechanism. It should be noted that the styrene oxides are mutagenic as such and do not require microsomal activation.

Scheme I



In a recent study of the mutagenic potency of nitroimidazoles, nitrobenzenes, and nitrofurans, Chin et al.[3]

(1) Sugiura, K.; Kumura, T.; Goto, M. *Mutat. Res.* **1978**, *58*, 159.
(2) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.
(3) Chin, J. B.; Sheinin, D. M. K.; Rauth, A. M. *Mutat. Res.* **1978**, *58*, 1.

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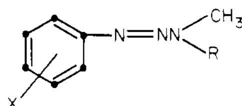
noted some correlation between electron affinity and mutagenic potency. Their compounds were also mutagenic in the absence of microsomes. Unfortunately from the point of view of QSAR, the substituent changes were complex and not easily parameterizable by the usual QSAR techniques.[4] In addition, they have defined activity in terms of revertants/nanomole of drug rather than log $1/C$, producing a standard response. This also makes QSAR more difficult. Nevertheless, their results do show a significant dependence on electron affinity and little, if any, dependence on octanol/water log P values.

In contrast to the above two studies where microsomal oxidation is not involved, we have developed[5] a QSAR (eq 2) for triazenes of type II acting on *Salmonella typhimurium* (TA-92) where microsomal activation is necessary for mutagenicity.



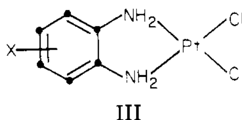
II

$$\log 1/C = 1.09 \log P - 1.63\sigma^+ + 5.58 \quad (2)$$

$$n = 17;\ r = 0.974;\ s = 0.315$$

In eq 2, C is the molar concentration producing 30 mutations/10^8 bacteria initially delivered above background. This low mutation rate was necessary to avoid toxicity of some of the triazenes to the bacteria. At this mutation rate it was possible to find log $1/C$ values ranging from 3.46 to 7.67. On this same scale, aflatoxin B_1 has a log $1/C$ of 9.5. Of the two terms in eq 2, the log P term is the more significant. This stands in marked contrast to the results of the two studies discussed above. Equation 2, like eq 1, shows that electron-releasing substituents (X) increase mutagenicity. It was surmised in the triazene study that the log P term might be accounting for the effect of microsomal activation.

With this limited background in view, we decided to undertake the testing of a series of platinum compounds (III) with *Salmonella typhimurium* in the Ames assay.



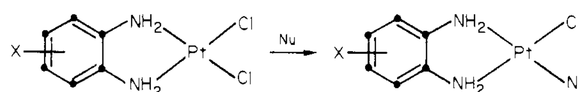
III

The triazenes (II), as well as the platinum compounds,[6-13] are important antitumor drugs. It is unfortunate from the point of view of chemotherapy that both of these classes of compounds are highly mutagenic. One of the reasons for studying such compounds in the Ames test is to see whether or not mutagenicity can be separated from antitumor activity by suitable structural modification of the parent compound. Some evidence was found for this possibility in the case of the triazenes.[5] The platinum compounds were found to be quite mutagenic with TA-92 in the absence of microsomes; hence, they behave more like the styrene oxides and nitro compounds than the triazenes. Using the same definition of activity as in eq 2, we have formulated the QSAR of eq 3. Although microsomes are not essential for the mutagenic activity of the platinum compounds, they were included in the test medium just as with the triazenes to make the experimental conditions comparable. This is especially necessary if one is expected to understand the role of hydrophobic character of organic molecules in mutagenicity.

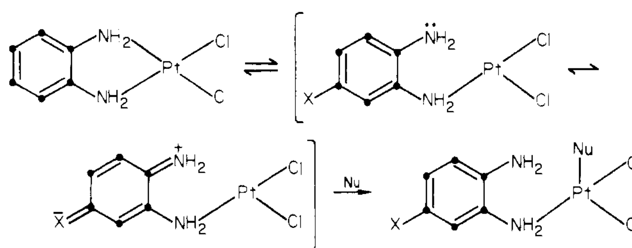
Table I. Parameters Used in the Derivation of Equation 3

no.	X	log $1/C$ obsd	log $1/C$ calcd	|Δ log $1/C$|	σ^-
1	4,5-$(OCH_3)_2$	4.64	5.07	0.43	−0.32
2	4,5-$(CH_3)_2$	4.99	5.11	0.12	−0.30
3	4-CH_3	5.60	5.45	0.15	−0.15
4	4-OC_2H_5	5.60	5.43	0.17	−0.16
5	4-OCH_3	5.65	5.43	0.22	−0.16
6	H	5.63	5.78	0.15	0.00
7	4,5-$(CH)_4$	5.92	5.87	0.05	0.04
8	4-Cl	6.94	6.41	0.53	0.28
9	4,5-Cl_2	6.89	7.03	0.14	0.56
10	4-$COOCH_3$	7.00	7.21	0.21	0.64
11	4-COC_6H_5	7.52	7.75	0.23	0.88
12	4,5,6-Cl_3	7.73	7.66	0.07	0.84
13	4-NO_2	8.65	8.55	0.10	1.24

Scheme II



Scheme III



Results and Discussion

We have derived eq 3 from the data in Table I. In this

$$\log 1/C = 2.23(\pm 0.32)\sum\sigma^- + 5.78(\pm 0.18) \quad (3)$$

$$n = 13;\ r = 0.977;\ s = 0.260$$

equation, σ is the suitable parameter when substituents are in conjugation with a reaction center where delocalization of a developing negative charge in the transition state is crucial in the rate-limiting step.[14] If the normal σ constant is used in place of σ^-, a poorer correlation is

(4) Martin, Y. C. "Quantitative Drug Design", Marcel Dekker: New York, 1978.
(5) Venger, B. H.; Hansch, C.; Hatheway, G. J.; Amrein, Y. U. *J. Med. Chem.* **1979**, *22*, 473.
(6) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature (London)* **1969**, *222*, 385.
(7) Leh, K. V.; Wolf, W. *J. Pharm. Sci.* **1976**, *65*, 315.
(8) Connors, T. A.; Jones, M.; Ross, W. C. J.; Braddock, P. D.; Khokhar, A. R.; Tobe, M. L. *Chem.-Biol. Interact.* **1972**, *5*, 415.
(9) Schwartz, P.; Meischen, S. J.; Gale, G. R.; Atkins, L. M.; Smith, A. B.; Walker, E. M., Jr. *Cancer Treat. Rep.* **1977**, *61*, 1519.
(10) Tobe, M. L.; Khokhar, A. R. *J. Clin. Hematol. Oncol.* **1977**, *7*, 114.
(11) Gale, G. R.; Atkins, L. M.; Meischen, S. J.; Smith, A. B.; Walker, E. M., Jr. *J. Natl. Cancer Inst.* **1976**, *57*, 1363.
(12) Kidani, Y.; Noji, M.; Tsukagoshi, S.; Tashiro, T. *Gann* **1978**, *69*, 263.
(13) Simon, Z.; Mracec, M.; Maurer, A.; Policec, S.; Dragulescu, C. *Rev. Roum. Biochim.* **1977**, *14*, 117.
(14) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley-Interscience, New York, 1979.

found (r = 0.967; s = 0.313).

It should be noted that no matter where substituents are placed on the benzene ring of III, they will be conjugated with one or the other of the two amino moieties. For this reason, we have used σ_p^- in every instance.

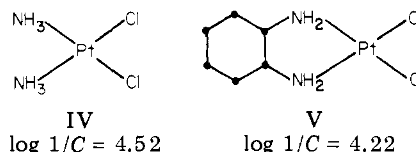
The positive coefficient with $\sum\sigma^-$ in eq 3 brings out the fact that electron-withdrawing groups promote mutagenic activity. What this means mechanistically is not unambivalent. It is generally assumed[7] that platinum complexes such as III act as alkylating agents of nucleophilic sites in DNA (Scheme II). If alkylation of DNA-producing mutagenesis occurs as in Scheme II, one would not expect such a large value for ρ in eq 3, since a saturated amino group (no lone-pair electrons) insulates the Pt atom from conjugation with the aromatic ring. Moreover, if attack were directly by the nucleophile on Pt, one would expect σ rather than σ^- to be the relevant parameter. Hence, it may be that breaking of the N–Pt bond occurs with NH_2 acting as the leaving group rather than Cl^- (Scheme III). This might occur by either an S_N1 or S_N2 process. Actually, there is only one congener in Table I (NO_2) whose σ is much different from its σ^-. Other analogues of this type (e.g., CN, SO_2CH_3, etc.) must be tested before much weight can be placed on the mechanism depicted in Scheme III.

Adding a term in π for eq 3 does not improve the correlation significantly nor does adding two terms, π and π^2, result in improvement. Hence, we must conclude that at least in the range of $\sum\pi$ studied (-0.04 to 2.13), hydrophobic effects are not significant. This was surprising to us, since we have found in many hundreds of QSAR in biological systems that it is very rare indeed that hydrophobic constants can be omitted from a biological QSAR. The fact that π or log P is not significant in eq 3 (or, possibly, eq 1) implies that there is not a significant lipophilic barrier between the mutagen in solution and its point of action in the bacterium. Since the bacterium does not contain a nucleus, the genetic material may be much more readily approached by hydrophilic agents than in the case of mammalian cells or whole animals. This is a point we plan to explore by using the sister chromatid exchange test for mutagenic activity. This test is based on mammalian cells. The information in hand does suggest that bacterial cells may be better suited to uncover mutagenicity of hydrophilic compounds than are mammalian cells; however, the structure–activity relationship between the especially sensitive cells developed by Ames and normal bacteria or mammalian cells may be quite different. There is abundant evidence that log P and π play dominant roles in the toxicity of a wide variety of organic compounds to many different kinds of bacteria.[15,16]

Two congeners in Table I [4-Cl and 4,5-$(OCH_3)_2$] are rather poorly fit. The 4-Cl analogue is about three times more potent than predicted, while the dimethoxy derivative is about 2.5 times less active than expected. The reasons for these deviations are not apparent.

(15) Lien, E. J.; Hansch, C.; Anderson, S. M. *J. Med. Chem.* **1968**, *11*, 430.
(16) Hansch, C.; Dunn, W. J., III *J. Pharm. Sci*, **1976**, *61*, 1.
(17) Gale, G. R.; Atkins, L. M.; Walker, E. M., Jr.; Smith, A. B.; Meischen, S. J. *Proc. Soc. Exp. Biol. Med.* **1973**, *142*, 1349.

We have also determined mutagenic potency for two platinum complexes (IV and V) which cannot, because of



IV log $1/C$ = 4.52 V log $1/C$ = 4.22

the lack of suitable parameters, be correlated using eq 3. Both IV and V are relatively nonmutagenic compared to the compounds in Table I. This is in line with eq 3, since both of the bases, NH_3 and 1,2-diaminocyclohexane, are stronger than the phenylenediamines of Table I. Compound IV is now widely used in the treatment of a variety of different types of tumors. Compound V and similar analogues are quite potent against L-1210 leukemia and are under extensive preclinical study.

One must bear in mind that eq 1–3 apply only to especially sensitive bacteria in vitro. Whether the same compounds acting on mammalian cells in vivo will show the same sensitivity to substituent effects is an open question which urgently calls for investigation.

In summary, we can say that the results of eq 1–3 provide encouraging evidence that QSAR can be formulated for congeneric sets of organic compounds acting in the Ames test. Such QSAR are of great value in the design of less mutagenic (and presumably less carcinogenic) drugs, as well as other industrial compounds. The results in Table I show that mutagenicity has been varied by a factor of 10 000 simply by manipulation of the electronic properties of substituents. Equation 3 implies that a congener with two nitro groups on the aromatic ring would be about 1 000 000 times more potent than a congener with two methoxy groups.

Experimental Section

(*o*-Phenylenediamine)dichloroplatinum (II) Complexes. Potassium tetrachloroplatinate was obtained commercially. Most of the *o*-phenylenediamines were obtained commercially; the others were prepared by reduction of the nitro compounds. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

The complexes were prepared by previously reported procedures.[8,17] Potassium tetrachloroplatinate was dissolved in sufficient 1 N HCl and filtered to obtain a clear solution. To this was added an equimolar amount of the *o*-phenylenediamine. The mixture was protected from light and kept at 50 °C for 20 h. The resulting precipitate was removed by filtration and washed successively with water, acetone, methanol, and ether, after which it was vacuum dried over P_2O_5. All the complexes were high melting (>360 °C) and were insoluble in the usual solvents. They dissolved in DMF, producing characteristic colors.

Substituent Constants. Substituent constants have been taken from our recent compilation.[14]

Biological Testing. Our previously reported[5] experimental procedure was followed.

Acknowledgment. The biological testing of the platinum complexes and the structure–activity analysis were supported by Grant CA-11110 from the National Cancer Institute. The preparation of the platinum complexes was carried out under Contract NO1-CM-67062 from the National Cancer Institute.