at present a better case for radical effect, but also for other molecular configurations containing C–H bonds adjacent to functions having high radical stabilizing ability.

Recently, further evidence for the toxophoric character of the benzylic H in chloramphenicol comes from an elegant study by Kutter and Garrett.[40] The α-deutero analog of chloramphenicol was synthesized in Kutter's laboratory, and then its antibacterial activity tested by the microbial kinetic method[5] in Garrett's laboratory. An isotope effect of 1.4 was found; that is, the D analog was considerably less active than the parent H-containing drug. This establishes the involvement of the α-H atom in the toxicity of these compounds to *E. coli* as predicted.[4]

Craig and McMahon have followed up their studies on the microsomal oxidation of α-deuteroethylbenzene and have shown[34] that there is an isotope effect in this process of 1.8. It is extremely interesting that the size of the isotope effect is comparable in the two similar systems. Even if identical enzymes were involved (this is most likely not the case), one would expect a smaller isotope effect with the more highly activated α position of chloramphenicol. The isotope effect found for chloramphenicol and the comparable effect in the PhEt system strongly support the structure–activity concept[4] deduced from eq 1 as well as the special character of benzyl and allyl moieties in medicinal chemistry.

Another large class of compounds containing benzylic hydrogens is the psychotomimetic amines in which there is presently great interest in structure–activity correlations.[41–44] The most active and interesting of these drugs is LSD. This compound has both benzylic hydrogens (those on C attached to the indole ring) and two allylic hydrogens (one on each side of the 9,10-double bond). Homolytic abstraction of either type of H should yield quite a stable radical extensively delocalized over the extended π-electron system. It has been shown[44] that there is some correlation between electronic effects of substituents and activity in such drugs.

With the exception of the chloramphenicol data, none of the data at hand is from sets of congeners ideally designed to isolate and characterize a radical delocalizing role for substituent effects. Taken as a whole, however, it points up the importance of carefully designing sets of congeners to assay such effects. It seems most likely that such effects will be most evident in the high energy-requiring processes of rapidly growing cells and the high oxygen-consuming processes of the central nervous system.

It must be remembered in undertaking such studies that the particular oxidative processes perturbed by benzylic or allylic H abstraction will also be highly dependent on the relative lipophilic character as well as the geometry of a given drug.

Acknowledgment.—We wish to thank Miss Catherine Church for measuring the partition coefficient of allyl alcohol.

(40) Personal communication from E. Kutter, Dr. Karl Thomae GmbH Company, Biberach, Germany.

(41) A. T. Shulgin, T. Sargent, and C. Naranjo, *Nature (London)*, **221**, 537 (1969).

(42) C. Chothia and P. Pauling, *Proc. Nat. Acad. Sci. U.S.*, **63**, 1063 (1969).

(43) S. H. Snyder and E. Richelson, *ibid.*, **60**, 206 (1968).

(44) S. H. Snyder and C. R. Merril, *ibid.*, **54**, 258 (1965).

Notes

The Use of σ^+ in Structure–Activity Correlations[1]

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The use of substituent constants and computerized regression analysis for correlating chemical structure with biological activity is beginning to yield fascinating results.[2] However, since our knowledge of receptor sites and enzymic mechanisms is so very limited, we are still in the embryonic stages of developing systematic procedures for disentangling substituent effects in structure–activity relationships. In the present state of the art, one must try all reasonable parameters before making a choice of the "best" regression equation. Of course, in deciding on the "final" equation, all other knowledge about the system must also be considered. As the physical–chemical parameters become more refined, and as our use of them becomes more skillful, greater insight into biomedical reaction mechanisms will certainly ensue. The purpose of this report is to reconsider studies which we now find can be better defined by a more judicious choice of parameters.

In the correlation[3] of relative sweetness of compounds of structure 1 from the work of Blanksma and Hoegen,[4] eq 1 was formulated. Equation 1 is a satisfying result

	n	r	s	
$\log RS = 1.610\pi - 1.831\sigma + 1.729$	9	0.936	0.282	(1)

and a comparatively simple expression showing that relative sweetness (RS) depends on the hydrophobic

(1) This work was supported by Grant CA 11110 from the National Institutes of Health.

(2) (a) C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969); (b) C. Hansch, *J. Org. Chem.*, **35**, 620 (1970).

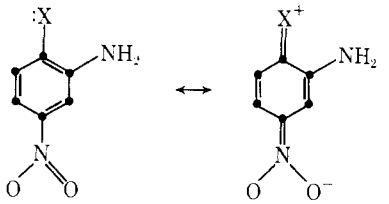
(3) E. W. Deutsch and C. Hansch, *Nature (London)*, **211**, 75 (1966).

(4) J. J. Blanksma and D. Hoegen, *Rec. Trav. Chim. Pays-Bas*, **65**, 333 (1946).

TABLE I
DATA FOR REDUCTION OF ACETOPHENONES BY RABBIT KIDNEY REDUCTASE

Substituent	σ[a]	σ^+[a]	π[b]	E_s[c]	Log k_0 Obsd[d]	Log k_0 Calcd[e]	$\lvert\Delta$ Log $k_0\rvert$
H	0.00	0.00	0.00	1.24	1.068	1.205	0.14
4-$COCH_3$	0.50	0.50	−0.37	−1.28[f]	2.597	2.580	0.02
4-NO_2	0.78	0.79	0.24	−1.28	2.944	2.931	0.01
4-$NHCOCH_3$	0.00	−0.60	−0.79	0.63[f]	0.591	0.667	0.08
3-OCH_3	0.12	0.05	0.12	1.24	1.631	1.266	0.37
3-NO_2	0.71	0.67	0.11	1.24	1.965	2.015	0.05
4-CH_3	−0.17	−0.31	0.52	0.00	1.628	1.210	0.42
4-OCH_3	−0.27	−0.78	−0.04	0.69	0.204	0.431	0.23
4-CF_3	0.54	0.61	1.07	−1.16	2.500	2.676	0.18
3-CH_3	−0.07	−0.07	0.51	1.24	0.973	1.121	0.15

[a] From Leffler and Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, p 204. [b] Phenoxyacetic acid values from T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964). [c] From E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969). [d] From ref 7. [e] Calculated using eq 5. [f] Estimated values; see text for discussion.



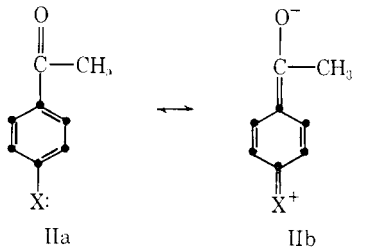
Ia Ib

character of X as well as its electron-releasing ability. Reconsidering the problem and the possibility of a direct resonance interaction between the substituent X and the NO_2 function suggested that σ^+ should be a better parameter than σ to correlate such a set of congeners. Equation 2 shows this to be true. Not only is

$$\log RS = 1.434(\pm 0.39)\pi - 1.026(\pm 0.41)\sigma^+ + 1.584(\pm 0.30) \quad n = 9,\ r = 0.972,\ s = 0.190 \quad (2)$$

eq 2 a much better correlation than eq 1 (compare values of s), it makes more mechanistic sense in the light of the resonance interaction shown in I. Moreover, the lower coefficient with π in eq 2 is more in line with results from many other systems.[2] Values of the coefficient of π beyond 1.2 are extremely rare. It has been pointed out[5] that differences in dipole moment of the various congeners may be responsible for the variation in sweetness. If indeed this is true, structures such as Ib would be most important in determining biological response. The correlation of dipole moments with σ constants is of course well known.[6]

A situation similar to that of the sweet compounds considered above comes from the studies of McMahon and his colleagues.[7] They have made an extensive study of the enzymic reduction of substituted acetophenones. Here, too, as shown by II, we find the possibility for direct resonance interaction between substituent X and the reaction center. From their data on the purified form of rabbit kidney reductase in Table I the following equations result.



IIa IIb

$$\log k_0 = 2.042(\pm 0.98)\sigma + 1.173(\pm 0.41) \quad n = 10,\ r = 0.862,\ s = 0.487 \quad (3)$$

$$\log k_0 = 1.514(\pm 0.55)\sigma^+ + 1.480(\pm 0.29) \quad n = 10,\ r = 0.914,\ s = 0.390 \quad (4)$$

Again considerable improvement is found in the use of σ^+ instead of σ (the figures in parentheses are the 95% confidence intervals). The small differences between eq 3 and the corresponding equation reported by McMahon, *et al.*, are due to computer rounding errors. Using CNDO/2 method for the calculation of molecular orbital indices, McMahon, *et al.*, found that LEMO values yielded a linear relation almost as good as eq 3 ($r^2 = 0.72$, $s = 0.504$). The positive slope of eq 4 indicates that electron-releasing functions retard the rate of reduction and that functions such as 4-OCH_3 and 4-$NHCOCH_3$ have particularly bad effects. The more positive the carbonyl carbon atom, the faster the rate of reduction. This, of course, supports the suggestion of McMahon, *et al.*, that the first step in the reduction is hydride transfer.

A variety of attempts to improve eq 4 by addition of hydrophobic terms (π) were unsuccessful as McMahon's group had also found. However, it was found that the addition of a steric constant for the 4 substituent did significantly improve the correlation as shown in eq 5. The negative coefficient with the E_s term for the 4-substituent indicates that large substituents in this position are effective in increasing the rate of reaction. In comparing eq 5 with eq 4, we find $F_{1,7} = 12.4$, indicating significance at $\alpha \leqq 0.01$. However, eq 5 must be accepted with caution since E_s values for 4-$COCH_3$ and 4-NHAc are not known and were estimated. The 4-$COCH_3$ function was assigned the same value as 4-NO_2. E_s for 4-NHAc was taken as equivalent to E_s for N; that is, it is assumed that this function can rotate to a position of minimum repulsion so that only the steric effect of the nitrogen atom is significant. This same assumption has been used in the calculation of E_s

$$\log k_0 = -0.306(\pm 0.21)E_s - 4 + 1.208(\pm 0.42)\sigma^+ + 1.585(\pm 0.20) \quad n = 10,\ r = 0.970,\ s = 0.251 \quad (5)$$

(5) J. W. McFarland, unpublished results.
(6) M. Charton, *J. Org. Chem.*, **30**, 552 (1965).
(7) R. B. Herman, H. W. Culp, R. E. McMahon, and M. M. Marsh, *J. Med. Chem.*, **12**, 749 (1969).

values for -OMe (see footnote c, Table 1). Evidence for the validity of this assumption comes from a study of the steric effects in hapten–antibody interactions.[8] In this study, the value of E_s was found to be 0.40. This is close to that of the calculated value used in Table 1. Use of 0.40 actually gives a slightly better fit with eq 5. It is of particular interest that E_s as estimated for -OMe and -NHAc gives quite satisfactory results in two very different biological systems.

The negative coefficient with the E_s terms means that the rate of reduction is increased by bulky groups in the 4 position. Mechanistically, this is probably best rationalized in terms of Koshland's induced-fit hypothesis.[9] The rigid positioning of the substituent in the 4 position appears to be quite important. A small effect is produced by groups such as -OMe and -NHAc which can adjust to positions of minimum repulsion while large effects are brought about by CF_3 and NO_2. The use of a polarizability term in eq 5 in place of $E_s - 4$ results in a much poorer correlation, indicating volume alone is not the determining factor.

(8) E. Kutter and C. Hansch, *Arch. Biochem. Biophys.*, **135**, 125 (1969).
(9) D. E. Koshland, Jr. and K. E. Neet, *Annu. Rev. Biochem.*, **37**, 359 (1968).

Metal Chelate Steroid Analog. [7-Amino-3-(1,5-dimethylhexyl)dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-6-methylamine]bis(ethylenediamine)cobalt(3 +) Trichloride[1]

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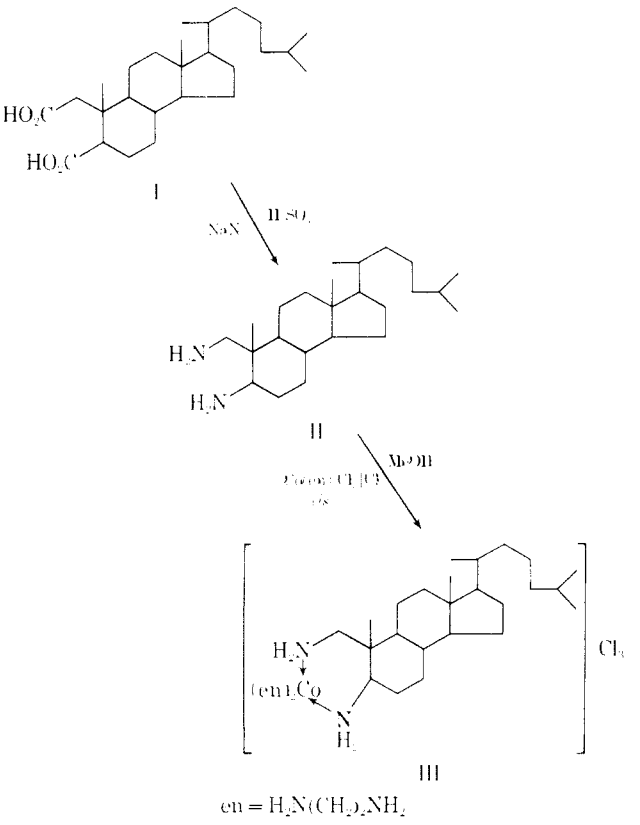
Over the last decade, much work has been done concerning the synthesis of steroid analog systems wherein various ring carbon atoms have been replaced with N, O, or S. Some of the aza, oxa, and thia steriods possess biological activity. We wondered if it might be possible to synthesize a molecule which contained a metal chelate ring within a reasonable facsimile of a steroid ring system. Scheme I shows the reaction employed to prepare the requisite ligand, 7-amino-3-(1,5-dimethylhexyl)dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-6-methylamine (II).

Compound II was prepared in 86% yield by reaction of A-nor-2,3-secocholestan-2,3-dioic acid (I)[2] in $CHCl_3$ mixed with concentrated H_2SO_4 and NaN_3. The ir spectrum was in agreement with structure II in all respects. All elemental analyses of bis derivatives, α-naphthylurea, benzensulfonamide, *p*-chlorobenzamide, prepared from II by standard procedures[3] were within experimental error (±0.3%), and the ir spectra of the derivatives were consistent with the expected structures. The dihydrochloride of II(IIa) was prepared by precipitation from C_6H_6 with dry HCl. Compound II was added to *cis*-dichlorobisethylenediaminecobalt(3+) chloride in MeOH to yield 96% of golden crystalline [7-amino-3-(1,5-dimethylhexyl)dodecahydro-3a,6-dimethyl-1*H*-benz[e]indene-6-methylamine]bis(ethylenediamine)cobalt(3+) trichloride (III). The ir spectrum was consistent with the assigned structure. Ir maxima reported to be characteristic of Co–N bonds were present.[4] Finally, III, which is completely water soluble, gave a 4 particle depression of the freezing point in aqueous solutions and was diamagnetic. Thus, all the data confirm the structures assigned for II and III.

Scheme I



The preparation of other metal chelate steroid analogs is underway, and the complete conformational characterization of II and III by X-ray diffraction methods will soon be initiated. It is interesting to note that models of III show that the normal shape of the "A" ring is not completely distorted, and the two external rings present due to the bidentate ethylenediamine ligands might be said to be structurally somewhat similar to compounds such as the ethylene ketal of cholestanone.

Biological Activity.—The capacity of the test compound to interfere with the incorporation of labeled acetate and/or mevalonate into cholesterol by rat liver homogenate was determined *in vitro* by the method of Dvornik, *et al.*[5] Any test compound producing 40% inhibition of cholesterol synthesis at 1×10^{-4} *M* is considered active and a lead worth further work. III was biologically active as a hepatic cholesterogenesis inhibitor displaying 58% inhibition of cholesterol synthesis. II also was put through the same screens as III, but III was more active than II. II showed only

(1) Taken in part from the thesis submitted by Patricia U. Flath in partial fulfillment of the requirements for the M.S. degree.
(2) A. Windaus and C. Ubrig, *Ber.*, **47**, 2387 (1914).
(3) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds", 5th ed, Wiley, New York, N. Y., 1964.
(4) E. P. Bertin, I. Nakagawa, S. Misushima, T. J. Lane, and J. V. Quagliano, *J. Amer. Chem. Soc.*, **80**, 525 (1958).
(5) D. Dvornik, M. Kraml, and J. Dulnic, *Proc. Soc. Exp. Biol. Med.*, **116**, 537 (1964).