Role of the Benzyl Moiety in Biochemical and Pharmacological Processes[1]

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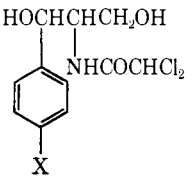
The structure–activity relationship is examined for several sets of drugs containing the benzyl moiety. The results indicate that substituents with radical delocalizing ability have the most pronounced effect on activity. This suggests that radical abstraction of benzylic or allylic hydrogens yields radicals which are quite toxic to a variety of oxidative biochemical processes.

In extending our studies of extrathermodynamic biochemical structure–activity correlations, interest has developed in reactions which may be influenced by substituents which are effective stabilizers of free radicals.[2-4] The radical parameter E_R in linear combination with the hydrophobic parameter π was found[4] to give a quite good structure–activity relationship for chloramphenicols of the type:



The quality of the correlation is seen in eq 1. In

	n	r	s	
$\log A = 3.07E_R + 0.23\pi + 0.77$	8	0.954	0.140	(1)

eq 1, A represents relative activity as determined by the microbial kinetic method by Garrett.[5] Using σ in place of E_R in eq 1 gave a much poorer correlation with $r = 0.441$. The chloramphenicol data, because of the good choice of substituents, are particularly well suited to bring out the differences between the two parameters, E_R and σ. For many of the functions commonly used in medicinal chemistry such as the halogens, alkyl, and alkoxy, there is little difference in the values of E_R and σ.

In a previous study of bactericidal action of benzyl alcohols,[6] good correlation of structure with activity was obtained using log P and σ. In reexamining[4] these data, E_R was found to give a higher correlation than σ. In reexamining the data the assumption was made that E_R for ortho substituents would be the same as for para substituents. This allowed the use of 18 data points in the correlation. For a more rigorous test we have omitted 5 derivatives with ortho substituents to obtain eq 2. Although the correlation with log P alone was good ($r = 0.946$), adding the term in E_R is statistically significant ($F_{1,10} = 3.32$; $F_{1,10\ \alpha 0.10} = 3.28$). For a limited set of substituents containing mostly halogens and alkyl derivatives there may be a close parallel between E_R and σ_I. Using σ_I in eq 2 in place of E_R does not result in a significant reduction in the variance.

Benzyl alcohols *vs. Staphylococcus aureus* + *S. albus* + *S. faecalis*

	n	r	s	
$\log \frac{1}{C} = 1.234(\pm 1.5)E_R + 0.709(\pm 0.15) \log P + 0.774(\pm 0.39)$	13	0.960	0.244	(2)

Using some of the same benzyl alcohols[6] against a different set of microorganisms yielded data correlated by eq 3. Log P alone gave a good correlation ($r = 0.942$); however, addition of the term in E_R resulted in a significant reduction in variance ($F_{1,8} = 11.3$; $F_{1,8\ \alpha 0.01} = 11.3$). The use of σ in place of E_R in eq 3 does not result in a significant reduction in variance ($F_{1,8} = 2.1$).

Benzyl alcohols *vs. P. vulgaris* + *Escherichia coli* + *Pseudomonas sp.*

	n	r	s	
$\log \frac{1}{C} = 1.555(\pm 1.1)E_R + 0.578(\pm 0.11)\log P + 0.800(\pm 0.28)$	11	0.976	0.164	(3)

Another example of the exceptional toxicity of benzyl alcohol turns up in the study of the action of a set of 7 aliphatic alcohols and $PhCH_2OH$. In our first attempt to correlate the toxicity of these alcohols to *Salmonella typhosa*,[7] a poor linear relation between log $1/C$ and log P ($r = 0.826$) was obtained. At the time the correlation was made there was no reason to assume that $PhCH_2OH$ operated in a different mode and therefore drop it from the correlation. Doing so now, we obtain the much better correlation:

	n	r	s	
$\log \frac{1}{C} = 0.818 \log P - 1.301$	7	0.934	0.098	(4)

$PhCH_2OH$ is about 3 times as toxic as eq 4 predicts.

The allylic group is also well known for its ability to stabilize a radical: $CH_2{=}CH{-}CH_2 \cdot \rightleftharpoons \cdot CH_2{-}CH{=}CH_2$. One might look for exceptional toxicity in this function. If its toxicity is of the chloramphenicol type, this should be most apparent in rapidly dividing cells where the rate of protein synthesis is

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

(2) C. Hansch, *J. Med. Chem.*, **11**, 920 (1968).

(3) C. Hansch and E. J. Lien, unpublished results.

(4) C. Hansch, E. Kutter, and A. Leo, *J. Med. Chem.*, **12**, 746 (1969).

(5) E. R. Garrett, O. K. Wright, G. H. Miller, and K. L. Smith, *ibid.*, **9**, 203 (1966).

(6) E. J. Lien, C. Hansch, and S. M. Anderson, *ibid.*, **11**, 430 (1968). In converting the original experimental data used in deriving eq 23 and 61 into units of log $1/C$ where C = moles/kg, a systematic error of 3 log units was made. This does not affect the correlation coefficient or the coefficients with π or σ in these equations. It does lower the value of the intercept by 3.

(7) J. M. Schaffer and F. W. Tilley, *J. Bacteriol.*, **14**, 259 (1927).

most important; this indeed appears to be true. Recently, the toxicity of a group of miscellaneous compounds to developing chick embryos was reported.[8] The structure–activity relationship is contained[4] in:

$$\log \frac{1}{C} = -0.16(\log P)^2 + 0.76 \log P + 2.08 \qquad n \quad r \quad s$$
$$10 \quad 0.982 \quad 0.112 \qquad (5)$$

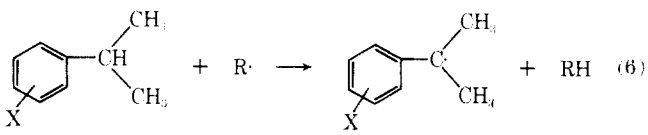
Because of its extremely poor fit the log $1/C$ for allyl alcohol was not used in deriving eq 5. Using the value of 0.17 for log P of allyl alcohol in eq 5, it is seen that allyl alcohol is 100 times as toxic as predicted by simple lipophilic character alone. This same exceptional toxicity for allyl alcohol was first noted by Picaud.[9] He observed that allyl alcohol was more toxic than amyl alcohol to goldfish. From the linear relation of eq 17 one would expect AmOH (log P 1.34) to be more than 10 times as narcotic as allyl alcohol.

In considering other drugs which contain active benzylic hydrogens, the antimalarials come to mind. Quinine itself falls into this category. More interesting is the fact that some of the most potent antimalarials are the 2-phenylquinolinemethanols.[10] The enhanced activity of the 2-phenylquinoline derivatives over various other functions at this position may of course be due to a variety of reasons; however, it is of interest to note that while σ for the Ph group is close to zero, Ph is one of the more effective substituents in the delocalization of radicals.[11] The phototoxicity of these drugs may also have a dependence on radical stabilization. The recent evidence[12] that quinine inhibits many of the same processes that chloramphenicol does also points to the fact that both molecules are derivatives of $PhCH_2OH$ and that the benzylic hydrogens may well be the critical toxophoric group.

The above observations as well as a number of less significant ones initiated the search for the examples considered in this paper.

Method

The radical parameter E_R is analogous to the Hammett-type parameter σ^+ formulated by Brown and is defined as follows:[13,14]



(6)

$$E_R = \log \left(\frac{C_X}{C_H}\right) - 0.7\sigma \qquad (7)$$

In eq 6, R· is a styrene polymer radical and in eq 7, C_X is the chain transfer constant of the substituted cumene, and C_H is that of the parent hydrocarbon. Chain transfer constants were defined by Mayo[15] as:

$$\frac{1}{P} = C\frac{[S]}{[M]} + \frac{1}{P_0} \qquad (8)$$

In eq 8, P_0 refers to the degree of polymerization (in the present instance of styrene) in the absence of cumene and P is the degree of polymerization in the presence of cumene under the same conditions. The symbol [S] refers to the concentration of solvent (cumene), and [M] to the concentration of monomer (styrene). The value of -0.7σ in eq 7 was chosen to place E_R on a basis similar to that of the two-parameter Q, e equation of Price and Alfrey[16] and the α,β equation of Bamford.[17] E_R then, is a measure of the ability of substituent X to facilitate radical abstraction of a benzylic hydrogen atom.

Otsu and his coworkers have shown that E_R may be employed to correlate radical reactions in an equation analogous to the Yukawa–Tsuno extension[18] of the Hammett equation:

$$\log \left(\frac{k_X}{k_H}\right) = aE_R + b\sigma \qquad (9)$$

In eq 9, a and b are disposable parameters evaluated by the method of least squares. In eq 9, a reflects the sensitivity of the reaction to resonance stabilization of a single electron, and b the sensitivity of the reaction of polar effects of substituents. By the definition of eq 6 and 7, E_R is not a pure resonance parameter such as $\mathcal{R}$, recently defined by Swain and Lupton.[19] E_R also contains a polar component in so far as it is involved in eq 6. It has been shown[20] that radical stabilization by substituents is quite different from substituent stabilization of charges; that is, the correlation between σ^+ and E_R is quite low (for 16 substituents, $r = 0.269$). Also, the correlation between E_R and 14 para substituents with the two Swain and Lupton parameters $\mathcal{R} + \mathcal{F}$ is quite low: $r = 0.690$. One therefore expects quite different substituent effects in radical processes.

If the radical process follows very closely that of eq 6, one expects the single-variable eq 10 to correlate the rates or equilibrium constants:

$$\log \left(\frac{k_X}{k_H}\right) = aE_R + b \qquad (10)$$

If polar effects are much different than in the reference system (eq 6), then eq 9 is essential. For work with the chloramphenicols and benzyl alcohols it was found that the addition of a term in σ did not improve the correlation although for these biochemical processes a term in π is necessary for good correlation. The fact that the σ term is not necessary in these correlations suggests the biochemical processes are very close in nature to those of eq 6.

While there is little correlation between E_R and σ^+, there is, unfortunately good correlation,[20] at least for a

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(9) M. Picaud, *C. R. Acad. Sci.*, **124**, 108 (1897).
(10) A. J. Saggiomo, K. Kato, and T. Kaiya, *J. Med. Chem.*, **11**, 277 (1968).
(11) G. H. Williams, *Chem. Ind. (London)*, 1286, (1961).
(12) K. A. Conklin, S. C. Chou, and S. Ramanathan, *Pharmacology*, **2**, 247 (1969).
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(14) T. Otsu, T. Ito, Y. Fuji, and M. Imoto, *Bull. Chem. Soc. Jap.*, **41**, 204 (1968).
(15) F. R. Mayo, *J. Amer. Chem. Soc.*, **65**, 2324 (1943).
(16) C. C. Price, *J. Polym. Sci.*, **3**, 772 (1948).
(17) C. H. Bamford, A. D. Jenkins, and R. Johnston, *Trans. Faraday Soc.*, **59**, 530 (1963).
(18) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, p 211.
(19) C. G. Swain and E. C. Lupton, *J. Amer. Chem. Soc.*, **90**, 4328 (1968).
(20) C. Hansch and R. Kerley, *Chem. Ind. (London)*, 294 (1969).

Table I

Bactericidal, Anesthetic, and Antispasmodic Action of Benzyl Alcohols

Derivative	Σ_{E_R}[a]	$\Sigma\pi$[b]	$\Sigma\sigma$[c]	$\Sigma\sigma_I$[d]	$\Sigma\sigma^+$[e]	Log relative bactericidal action[f] Obsd	Calcd[g]	Log relative anesthetic action[f] Obsd	Calcd[h]	Log relative antispasmodic action[f] Obsd	Calcd[i]
3,5-I_2-2-OH	0.41	1.76	0.33	1.16	−0.20	1.96	2.040	1.48	1.715	1.70	1.823
3-I-5-Br-2-OH	0.41	1.55	0.37	1.19	−0.16	1.72	1.908				
3,5-Br_2-2-OH	0.41	1.34	0.41	1.22	−0.12	1.66	1.775	1.18	1.157	1.40	1.528
3-Cl-5-Br-2-OH	0.37	1.16	0.39	1.19	−0.12	1.54	1.562				
3,5-Cl_2-2-OH	0.33	0.98	0.37	1.16	−0.12	1.19	1.349	0.88	0.967	0.43[j]	1.276
5-Pr-2-OH	0.20	0.96	−0.44	0.28	−0.98	1.20	1.011	1.30	1.410	1.00	1.262
5-I-2-OH	0.29	0.61	−0.02	0.74	−0.56	1.15	1.016	1.00	0.620	1.40	1.017
5-Br-2-OH	0.29	0.40	0.02	0.77	−0.52	0.99	0.884	0.70	0.341	1.22	0.870
5-Cl-2-OH	0.25	0.22	0.00	0.74	−0.52	0.88	0.671	0.31	0.246	0.78	0.744
5-Et-2-OH	0.20	0.46	−0.44	0.28	−0.98	0.69	0.697	0.53	0.746	0.70	0.912
5-Me-2-OH	0.20	−0.04	−0.44	0.29	−0.99	0.04	0.382	0.40	0.081	0.60	0.561
2-OH	0.17	−0.54	−0.37	0.32	−0.92	0.00	−0.008	0.00	−0.475	0.00	0.211
6-Me-2-OH	0.20	−0.04	−0.54	0.29	−0.99	0.34	0.382	−0.05	0.081	0.74	0.561
4-Me-2-OH	0.20	−0.04	−0.54	0.29	−1.23	0.34	0.382	0.26	0.081	0.78	0.561
6-Br-3-OH	0.29	0.40	−0.14	0.77	0.20	0.99	0.884	0.30	0.341	1.40	0.870
3-OH	0.17	−0.61	0.12	0.32	0.05	−0.30	−0.052	−0.70	−0.568	0.04	0.162
3-Br-4-OH	0.29	0.22	0.02	0.77	−0.52	1.36	0.771	−0.10	0.102	0.18	0.744
4-OH	0.17	−0.85	−0.37	0.32	−0.92	−0.30	−0.203	−1.52	0.887	−0.12	−0.006

[a] Ref 14. [b] See Table III. [c–e] C. D. Ritchie and W. F. Sager, *Progr. Phys. Org. Chem.*, **2**, 323 (1966). [f] Ref. 22. [g] Calculated using eq 13. [h] Calculated using eq 15. [i] Calculated using eq 16. [j] This point was not used in deriving eq 16.

normal selection of substituents, between eq 9 and 11, the Yukawa–Tsuno equation:

$$\log\left(\frac{k_X}{k_0}\right) = a\sigma^+ + b\sigma + c \qquad (11)$$

This means that one cannot use the Otsu–Yamamoto and Yukawa–Tsuno equations to unequivocally diagnose a reaction mechanism as being radical or polar in nature without additional information. However, if eq 10 is obeyed and the correlation is significantly superior to the corresponding equation using σ or σ^+, this constitutes good evidence for a radical reaction.

In correlating structure with activity, we have used the linear combination of free energy based substituent parameters[21] and regression analysis. In each example all reasonable combinations of electronic, hydrophobic, and steric parameters were tested. Hundreds of equations were considered; however, because of space limitations only a few equations, including the "best" equation for each case, are given. The "best" equation is considered to be the highest order equation justified by F tests ($\alpha \leq 0.10$). The biological data and constants used are listed in Tables I–IV.

Table II

Narcotic Action of Alcohols on Goldfish at 37°

	log P	Log $1/C$ Obsd[a]	Log $1/C$ Calcd[b]	$\lvert\Delta \text{Log } 1/C\rvert$
MeOH	−0.66	−0.30	−0.418	0.12
EtOH	−0.16	0.00	0.156	0.16
PrOH	0.34	0.82	0.729	0.09
2-PrOH	0.14	0.46	0.500	0.04
BuOH	0.84	1.40	1.303	0.10
2-Me-PrOH	0.64	1.10	1.073	0.03
2-BuOH	0.64	1.00	1.073	0.07
2-Me-2-PrOH	0.37	0.70	0.764	0.06
$PhCH_2OH$[c]	1.10	2.12	1.601	0.52

[a] H. C. Brill and A. K. Presnell, *Ohio J. Sci.*, **41**, 431 (1941). [b] Calculated using eq 17. [c] These data not used in deriving eq 17.

(21) C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969).

Results

Pharmacological Action of Benzyl Alcohols.—An interesting comparative study of the physiological action of a set of substituted hydroxybenzyl alcohols is that of Dunning, *et al.*,[22] (Table I) who compared bactericidal action, anesthetic efficiency, and antispasmodic action. Eighteen derivatives were tested as bactericides, but only 16 were used in the other two tests. Correlation of relative bactericidal biological response (RBR) with E_R gave a linear equation with correlation coefficient (r) of 0.899. Using σ gave a linear relation with $r = 0.682$, and σ_I one with $r = 0.837$. The correlation between σ_I and E_R for this set of substituents is rather high ($r^2 = 0.731$). The pertinent correlations to consider are:

	n	r	s	
$\log \text{RBR} = 0.886(\pm 0.17) \log P + 0.467(\pm 0.14)$	18	0.943	0.240	(12)
$\log \text{RBR} = 2.500(\pm 2.8)E_R + 0.629(\pm 0.32) \log P - 0.093(\pm 0.63)$	18	0.953	0.222	(13)

Equation 13 is an improvement over eq 12 ($F_{1,15} = 3.7$). However, this is not a great improvement and using σ_I in place of E_R gives essentially the same quality correlation ($s = 0.223$). A slight improvement ($s = 0.201$; $F_{1,14} = 4.2$) over eq 13 is obtained by the addition of a term in $(\log P)^2$. However, such a correlation is not good enough to place confidence intervals[23] on $\log P_0$ (the ideal partition coefficient for the series) and is therefore of little value. Although eq 12 and 13 are satisfactory in that the data are well correlated, a firm decision cannot be reached about the electronic role of the substituent other than to say that it is of some importance and that the

(22) B. Dunning, Jr., F. Dunning, and E. E. Reid, *J. Amer. Chem. Soc.*, **58**, 1565 (1936).

(23) C. Hansch, A. R. Steward, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, **11**, 1 (1968).

Table III
Oxidation of Phenylglycines by Hog Kidney d-Amino Acid Oxidase

Substituent	σ	E_R	σ^-	π^a	Log V_{max} Obsd[b]	Log V_{max} Calcd[c]	Δ Log V_{max}
4-$N(CH_3)_2$	−0.83	0.24	−1.70	0.18[d]	−3.52	−3.471	0.05
4-NH_2	−0.66	0.24[e]	−1.30	−1.63[d]	−2.80	−2.963	0.16
4-OH	−0.37	0.17	−0.92	−0.61	−1.82	−1.650	0.17
4-OCH_3	−0.27	0.11	−0.78	−0.04	−1.04	−0.968	0.07
4-CH_3	−0.17	0.03	−0.31	0.52	−0.60	−0.159	0.44
4-F	0.06	−0.07[f]	−0.07	0.15	0.83	1.167[f]	0.34
4-Cl	0.23	0.10	0.11	0.70	0.61	0.590	0.02
H	0.00	0.00	0.00	0.00	0.89	0.541	0.35
3-CH_3	−0.07	0.03	−0.07	0.51	0.54	0.140	0.40
3-OCH_3	0.12	0.11	0.05	0.12	0.28	0.197	0.08
3-F	0.34	−0.02[f]	0.35	0.13	0.86	1.684[f]	0.82
3-Cl	0.37	0.08	0.40	0.76	0.77	1.136	0.37
3-NO_2	0.71	0.35	0.67	0.11	0.51	0.428	0.08

[a] From the phenoxyacetic acid system (T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5174 (1964). [b] From ref 26. These workers also reported on the derivatives having the functions 4-COO^-, 4-$N^+(CH_3)_3$, 3-NH_2, and 3-OH which we have not included because of the lack of E_R values. [c] Calculated using eq 20. [d] From the benzene system. [e] We have assumed the same value of E_R for NH_2 as reported for $N(CH_3)_2$. [f] These points not included in the regression.

radical stabilizing character of the substituent *may* be involved.

The anesthetic action of the benzyl alcohols was measured by testing them on frog skin. The results are summarized as follows:

$$\log \text{RBR} = 0.984(\pm 0.26) \log P + 0.051(\pm 0.20) \quad n = 16,\ r = 0.905,\ s = 0.337 \quad (14)$$

$$\log \text{RBR} = -3.607 (\pm 4.0)E_R + 1.329 (\pm 0.45) \log P + 0.086 (\pm 0.92) \quad n = 16,\ r = 0.927,\ s = 0.308 \quad (15)$$

The additional term in eq 15 is significant with respect to eq 14 ($F_{1,13} = 3.8$). Although the confidence intervals on the E_R terms in eq 13 and 15 are broad, it seems safe to say that the coefficient in eq 13 is very likely positive while that in eq 15 is very likely negative. Thus, while radical delocalization by the substituent appears to improve bactericidal action, it tends to decrease anesthetic action in frog skin. This might be the result of greater ease of oxidation in the frog skin. Adding a term in $(\log P)^2$ to eq 15 results in a further improvement in the correlation ($r = 0.942$; $s = 0.287$); however, again the correlation is not good enough to place confidence limits on $\log P_0$. The use of σ_I in eq 15 instead of E_R results in a slightly poorer correlation ($s = 0.317$).

The coefficients with the $\log P$ terms in eq 13 and 15 are quite different. The values in eq 14 and 15 are near 1, which is what is usually found with nonspecific hypnotic activity.[24] The value of 0.63 of eq 13 compares with the coefficient of 0.73 found for a number of equations correlating antibacterial activity for a variety of drugs with Gram-positive organisms.[25]

Sixteen of the benzyl alcohols were also tested on cat intestine for antispasmodic action. One derivative (3,5-Cl_2-2-OH) was very poorly correlated and was omitted in the derivation of eq 16:

$$\log \text{RBR} = 0.701(\pm 0.25)\log P + 0.589(\pm 0.18) \quad n = 15,\ r = 0.864,\ s = 0.300 \quad (16)$$

(24) C. Hansch and S. M. Anderson, *J. Med. Chem.*, **10**, 745 (1967).
(25) E. J. Lien, C. Hansch, and S. M. Anderson, *ibid.*, **11**, 430 (1968).

No improvement in correlation was obtained by adding electronic terms or a term in $(\log P)^2$ to eq 16. This may be due to the noise in the data. Equation 16 is a poorer correlation than those obtained for bactericidal or anesthetic action.

While the above correlations are for quantitative structure–activity work a satisfaction in themselves, they are only partial support for the special role of the benzyl moiety in biochemical systems. The expected effect is seen in the bactericidal action; however, essentially the same rationalization of the data can be obtained with σ_I. One would not expect the same effect to be present in anesthetic and antispasmodic action and, in fact, it is not.

One of the reasons that the role of E_R is not more clearly seen in the bactericidal activity of the benzyl alcohols is that *all* of the molecules contain a ring OH which means that as a group they are quite activated to begin with; that is, abstraction of an α-H is only mildly rate-limiting. By far the more rate-limiting factor is relative hydrophobic character. Also, the selection of substituents was very poor for the purpose of making a decision between the relative merits of E_R and σ_I.

Another example of the possible exceptional toxicity of $PhCH_2OH$ is seen in the work of Brill and Presnell on the toxic action of alcohols to goldfish. The following equation, derived from the data of Table II, gives an excellent correlation of 8 alcohols, not including $PhCH_2OH$. Benzyl alcohol is more than three times

$$\log \frac{1}{C} = 1.148(\pm 0.20)\log P + 0.339(\pm 0.11) \quad n = 8,\ r = 0.985,\ s = 0.106 \quad (17)$$

as effective as eq 17 would predict. While the primary narcotic activity of the alcohols is undoubtedly due to a physical mechanism, it may well be that relatively high concentrations of radical-forming molecules could contribute to the narcotic action by interfering with oxidative processes on which the transmission of nerve impulses in the CNS depend. In the case of the anesthetic action of the hydroxybenzyl alcohols correlated by eq 14 and 15, we find little or no evidence for the

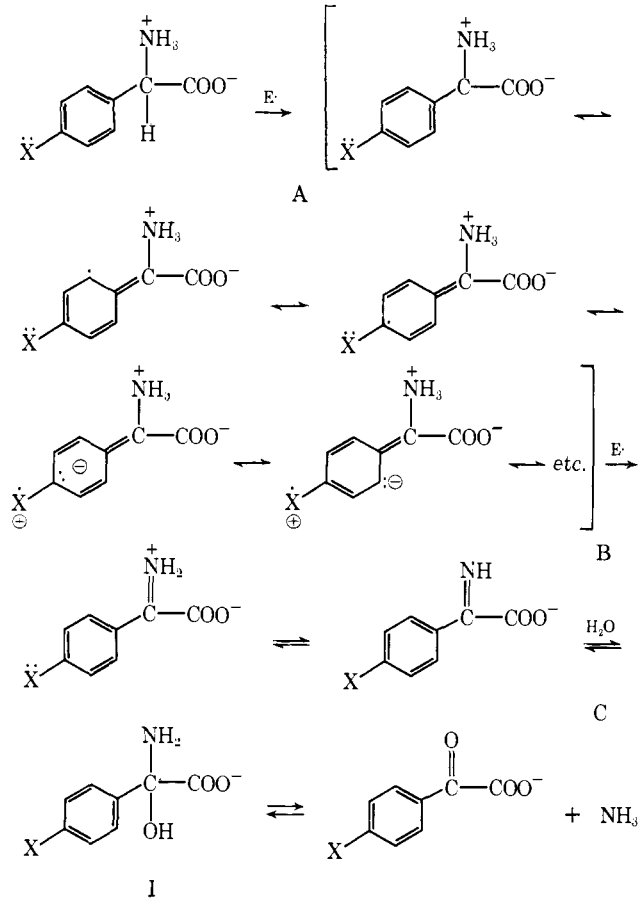
radical substituent effect. Presumably because the benzylic hydrogens are already so active, it is difficult to discriminate between small substituent effects (no groups with large E_R values were employed). However, in eq 17, where one is comparing very active benzylic hydrogens with those of the aliphatic alcohols, a clear difference can be seen.

Enzymic Action of Amino Acid Oxidases.—A structure containing benzylic H atoms similar to the benzyl alcohols are the phenylglycines. Several studies of the enzymic oxidation of such compounds have been made. One of the most extensive is that of Neims, *et al.*,[26] in which hog kidney D-amino acid oxidase was employed. In studying the correlation of their data, the following parameters were studied singly and in all reasonable combinations: π, σ, σ^+, σ_R, E_R, $\mathcal{R}$, $\mathcal{F}$. Of the large number of equations considered, the following are essential for consideration. The highest single variable

	n	r	s	
$\log V_{max} = 2.000(\pm0.49)\sigma^+ + 0.138(\pm0.38)$	11	0.952	0.499	(18)
$\log V_{max} = -3.287(\pm0.279)\sigma + 3.946(\pm1.69)\sigma^+ + 0.539(\pm0.45)$	11	0.975	0.382	(19)
$\log V_{max} = 2.988(\pm0.48)\sigma - 6.383(\pm1.99)E_R + 0.541(\pm0.34)$	11	0.986	0.293	(20)
$\log V_{max} = -2.705\ (\pm1.43)\sigma^2 + 2.592\ (\pm0.77)\sigma + 0.170\ (\pm0.43)$	11	0.966	0.446	(21)

correlations were obtained using σ (r = 0.880) or σ^+ (r = 0.952). The resonance parameter $\mathcal{R}$ (r = 0.865) was less successful. The addition of the hydrophobic parameter π did not improve the above correlations. Equations 19 and 20 represent the extended Hammett equation developed by Yukawa and Tsuno[27] and applied by Otsu and Yamamoto to radical reactions *via* E_R.[13] Equation 20 yields the best correlation and indicates that radical effects appear to be most important. However, as we have pointed out,[20] it is hazardous to use only equations 19 and 20 to diagnose a reaction mechanism as radical or polar. Equation 21 was included partly because Cammarata[28] has recently shown that there is a high correlation between σ^2 and E_R and partly because Neims, *et al.*, emphasized the parabolic dependence of oxidation on σ. Other two-variable equations such as $\mathcal{R} + \mathcal{F}$, $\sigma + \sigma_R$, etc. gave much poorer correlations than eq 19–21. The high correlations with eq 18–20 emphasize the probability that direct resonance interaction between substituent and reaction center is involved in the transition state. Equation 21 can be interpreted to mean that there is an optimum electron density at the point of side chain attachment as Neims, *et al.*, emphasized, or that a radical effect is involved because of the relationship between σ^2 and E_R. This set of data constitutes an excellent example of the complex set of possibilities which one encounters in interpreting reaction mechanisms and underlines the importance of carefully considering the interrelationships between the various substituent constants now in use. Of eq 18–20, there are several reasons for selecting eq 20 as most likely describing the situation besides the fact that it gives the highest correlation. It fits in with the other examples discussed earlier in this paper that benzyl hydrogens are implicated in biochemical processes. A most important fact supporting eq 20 is that considerable evidence has been found that radicals are involved in the oxidations of amino acid oxidases.[29–31]

If we accept eq 20 as most likely, we must then ask about the significance of the signs of the coefficients associated with the two variables, σ and E_R. The positive coefficient with σ indicates electron withdrawal by the substituent promotes oxidation and the negative coefficient with E_R indicates that delocalization of an odd electron by substituents inhibits hydrogen abstraction of a benzylic hydrogen atom. Oxidation could be formulated as



In the above sequence, E· represents a free radical containing enzyme or a radical generated by such an enzyme.

The general process has been formulated by Massey and Curti.[30]

(26) H. H. Neims, D. C. DeLuca, and L. Hellerman, *Biochemistry*, **5**, 203 (1966).
(27) Y. Yukawa and Y. Tsuno, *Bull. Chem. Soc. Jap.*, **32**, 965, 971 (1959).
(28) A. Cammarata, S. J. Yau, J. A. Collett, and A. N. Martin, *Mol. Pharmacol.*, **6**, 61 (1970).
(29) H. Beinert, *J. Biol. Chem.*, **225**, 465 (1957).
(30) V. Massey and B. Curti, *ibid.*, **242**, 1259 (1967).
(31) J. L. Fox and G. Tollin, *Biochemistry*, **5**, 3873 (1966).

Table IV
Oxidation of Phenylglycines by Sheep Kidney

Substituent	σ	E_R	σ^-	σ^+	Log V_{max} Obsd[b]	Log V_{max} Calcd[c]	Δ Log V_{max}
4-N$(CH_3)_2$	−0.83	0.24	−1.70	0.18	−2.70	−2.915	0.22
4-OH	−0.37	0.17	−0.92	−0.61	−1.78	−1.732	0.05
4-OCH_3	−0.27	0.11	−0.78	−0.04	−1.75	−1.244	0.51
4-Cl	0.23	0.10	0.11	0.70	−0.34	−0.300	0.04
H	0.00	0.00	0.00	0.00	0.14	−0.193	0.33
3-NO_2	0.71	0.35	0.67	−0.05	−0.69	−0.737	0.05

[a] See Table I. [b] From ref 26. [c] Calculated using eq 26.

$$\text{I} \quad -\text{FAD} \underset{+\text{AA}}{\rightleftharpoons} \underset{-\text{AA}}{-\text{FAD}} \rightleftharpoons -\text{FADH}\cdot \rightleftharpoons \underset{-\text{IA}}{-\text{FADH}_2} \rightleftharpoons \text{II} \; -\text{FAD}_2 \; \underset{}{+\text{IA}}$$

In this symbolism FAD = flavin adenine dinucleotide, AA = amino acid, and IA = imino acid. In the first step, the amino acid is bound to the enzyme complex; then 2 H atoms are removed stepwise to yield the imino acid which is then desorbed and hydrolyzed to the keto acid.

In sequence I, step A would be promoted by $+E_R$ substituents and step B would be hindered by such substituents. It seems unlikely that step A would be so critical that radical substituent effects could be easily detected in this process. Both the benzene ring and $RCOO^-$ are present to stabilize a radical on the α-C. However, once such a radical is formed, extensive delocalization of it would hinder step B, the abstraction of the second H·. Polar effects implicated by the positive coefficient with σ would probably be less important in steps A and B than in the hydration step C.

The interrelationship between E_R, σ^-, and σ has been established[29] as shown in eq 22. The relationship between eq 19 and 20 is very similar to eq 22. This

$$\sigma^+ = -1.32E_R + 1.60\sigma - 0.02 \qquad n = 16 \quad r = 0.989 \quad s = 0.104 \quad (22)$$

can be shown by equating eq 19 and 20 and solving for σ^+:

$$-3.29\sigma + 3.95\sigma^+ + 0.54 = 2.99\sigma - 6.38E_R + 0.54\sigma^+ = -1.62E_R + 1.59\sigma \quad (23)$$
$$\sigma^+ = -1.62E_R + 1.59\sigma$$

This fortuitous relationship between σ^+, E_R, and σ means that the Yukawa–Tsuno extension of the Hammett equation cannot discriminate between radical and polar charge delocalization. Although eq 18–20 all point to the importance of resonance interactions in the transition state, in singling out eq 20 as most likely representing the true state of affairs we must rely most heavily on the esr studies[29–31] of the mechanism of action of the amino acid oxidases.

The two fluoro derivatives of Table III were not included in deriving eq 18–21. This was because E_R values for these derivatives have not been measured. E_R values estimated from the work of Hey and Williams[11] are listed in Table III so that rough calculations can be compared with those observed. That E_R values so calculated do not give very good results is apparent. If eq 20 is used to calculate E_R for F, one obtains E_R m-F = 0.11 and E_R p-F = −0.02. These are not unreasonable values for E_R for this function.

Results similar to eq 18–21 are obtained from the data in Table IV on the oxidation of phenylglycines by sheep kidney:

$$\log V_{max} = 1.088(\pm 0.84)\sigma^+ - 0.712(\pm 0.75) \qquad n = 6 \quad r = 0.873 \quad s = 0.581 \quad (24)$$

$$\log V_{max} = 4.238(\pm 1.05)\sigma^+ - 5.170(\pm 1.7)\sigma + 0.208(\pm 0.35) \qquad n = 6 \quad r = 0.996 \quad s = 0.118 \quad (25)$$

$$\log V_{max} = -5.172(\pm 4.5)E_R + 1.784(\pm 1.02)\sigma - 0.193(\pm 0.89) \qquad n = 6 \quad r = 0.962 \quad s = 0.374 \quad (26)$$

In this instance the equation $\sigma + \sigma^2$ yields a quite poor correlation (r = 0.884). So few data points are available that much less confidence can be placed in eq 25 and 26 than in eq 19 and 20. Qualitatively, the results are the same.

Discussion

The overall import of the examples presented above clearly points to the special character of the benzylic and allylic moieties in biochemical processes. This seems best explained in terms of the special reactivity of H atoms so situated that the radical generated in the process of their removal is readily delocalized by the π electron system. Unfortunately, with the exception of the chloramphenicol case, none of the data sets provide an ideal test of the superiority of E_R over σ. To clearly establish the importance of radical delocalization by substituents, one needs a set of derivatives in which functions such as CN, NO_2, SR, SO_2R, NR_2, etc. constitute a large fraction of the population of the derivatives rather than functions such as OR and the halogens which have little spread in E_R or σ values.

In 2 of the above examples $PhCH_2OH$ has stood out in toxicity from a group of saturated aliphatic alcohols. One might interpret this as being due to some special character of the aromatic ring (having nothing to do with radical delocalization) such as greater polarizability, charge transfer complex formation, etc. A search of the literature did not uncover any data with which this point could be settled in solid quantitative terms; however, a study by Lester[32] shows that in a small series of arylalkyl alcohols (Table V), $PhCH_2OH$ is again more toxic than lipophilic character alone would predict.

(32) G. Lester, *J. Bacteriol.*, **90**, 29 (1965).

Table V
Inhibition of *Neurospora crassa* by 0.05 *M* Arylalkyl Alcohols

Compd	% inhibition	Log *P*
$C_6H_5CH_2OH$	36	1.10
$C_6H_5(CH_2)_2OH$	18	1.36
$C_6H_5(CH_2)_3OH$	64	1.88

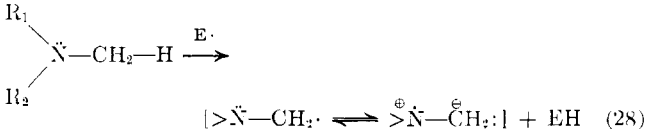
From Table V it is seen that $PhCH_2OH$ is twice as effective as the next higher homolog which has a higher log *P* value. Unfortunately, only 3 homologs in the series were tested so that it is not possible to derive an equation to show with much certainty how far out of line benzyl alcohol is. However, the two points of the higher homologs do determine the following equation:

$$\log \% \text{ inhibition} = 1.05 \log P - 0.16 \quad (27)$$

The slope of this equation is reasonably close to what one might expect from eq 4 and 17. The intercept means little since this is so highly dependent on the test system. From eq 27 we can calculate that $PhCH_2OH$ is 3 times as active as its log *P* alone would predict. While one cannot place confidence in a linear relationship determined by two points, qualitatively the answer is in line with the results obtained by comparing $PhCH_2OH$ with simple aliphatic alcohols (eq 4 and 17).

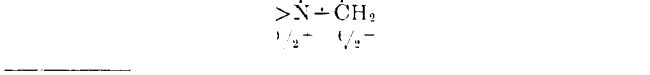
The unusual activity of $PhCH_2OH$ toward alcohol dehydrogenase has recently been demonstarated.[33] When $PhCH_2OH$ is placed on a scale of 100, $Ph(CH_2)_2OH$ has activity of only 25 and hexanol has activity of only 19.

The most elegant work revealing the special nature of benzylic H atoms is that of McMahon and coworkers.[34] This group showed that the α-H's of PhEt are stereospecifically attacked by microsomal enzymes. In fact, they were able to establish that oxidation occurred with retention of configuration and suggested that this was due to frontal attack with displacement occurring on H or insertion of O between H and C. The displacement on H mechanism for microsomal oxidation has been advanced[35] for radical microsomal oxidation involved in demethylation of amines:



$$|>\dot{N}—CH_2\cdot \rightleftharpoons >\overset{\oplus}{\dot{N}}—\overset{\ominus}{C}H_2:| + EH \quad (28)$$

The first step in such an oxidation is depicted in eq 28. It is well known in organic chemistry that C–H bonds adjacent to O and N are especially labile to radical attack.[36] The unusual stabilizing effect of elements such as O, N, Cl, etc. on adjacent radicals is possibly best pictured in terms of Linnett's double quartet symbolism:[36]

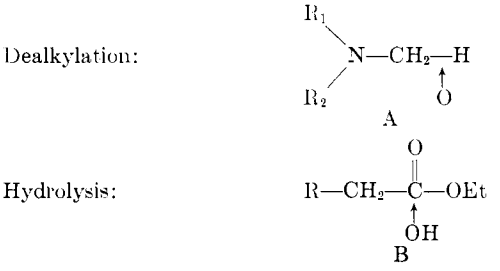


Demethylation of aliphatic amines was shown[35] to be quantitatively correlated by eq 29:

$$\log K_{rel} = 0.47 \log P - 0.27(pK_a - 9.5) - 1.31 \quad n = 18 \quad r = 0.890 \quad s = 0.222 \quad (29)$$

In eq 29, K_{rel} is the relative demethylation rate. The negative coefficient with the pK_a term indicates that the higher the electron density on N, the more rapid the rate of demethylation. One of the most surprising aspects of the correlation by eq 29 is that the steric effects of the R groups of eq 28 have little or no affect on the rate of demethylation. Even derivatives as sterically hindered as *tert*-amyl-*tert*-butylmethylamine are reasonably well accommodated by eq 29. One might argue that the pK_a term contains some steric information. However, this now appears not to be true. The base strengths of aliphatic amines are correlated by the inductive effects and hydrogen bonding potentials alone.[37] Attempts to improve correlations using steric parameters were unsuccessful. No doubt this is because the proton is so small its binding to the lone pair electrons of nitrogen is little hindered by groups even as large as *tert*-amyl. It was this lack of steric effect seen in eq 29 which led to the idea that displacement on hydrogen was involved in the dealkylation reaction.

It is worthwhile to analyze the suggestion of McMahon, *et al.*,[34] that insertion of O between the C and H atom might occur and to compare this with reaction in which the steric parameter E_s was derived. The



use of E_s constants obtained from reaction B in eq 29 did not improve the correlation. Although E_s constants were derived from system B and thus might not be expected to apply to other systems, it has in fact been shown that these constants are quite meaningful[38] in a variety of systems quite different from B. Therefore, it is valid to compare A with B. In reaction A, the R groups are one atom further removed from the reaction center than in B. One might, at first glance, be tempted to say that reaction A would be less subject to steric effects. However, this is not true as consideration of Newman's rule of 6 will show.[39] Therefore, it seems most likely that H abstraction by enzyme processes is a displacement on H. Since microsomal oxidases play such an important role in drug metabolism, their inhibition by stable radicals formed from the drugs themselves must be carefully considered in drug design. This would appear to be true not only for benzylic- and allylic-type radicals for which substituent constants provide

(33) M. Katagiri, S. Takemori, K. Nakazawa, H. Suzuki, and K. Akagi, *Biochim. Biophys. Acta*, **139**, 173 (1967).
(34) R. E. McMahon, H. R. Sullivan, J. Cymerman Craig, and W. E. Pereira, *Arch. Biochem. Biophys.*, **132**, 575 (1969).
(35) C. Hansch, A. R. Steward, and J. Iwasa, *J. Med. Chem.*, **8**, 868 (1965).
(36) R. A. Firestone, *J. Org. Chem.*, **34**, 2621 (1969).
(37) C. Hansch and E. J. Lien, *Biochem. Pharmacol.*, **17**, 709 (1968).
(38) E. Kutter and C. Hansch, *Arch. Biochem. Biophys.*, **135**, 126 (1969).
(39) M. S. Newman, "Steric Effects in Organic Chemistry," Wiley, New York, N. Y., 1956, p 203.

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).

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(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 I 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).