TABLE IV

P.M.R. SPECTRA OF PYRIDOXAL AND PYRIDOXAL PHOSPHATE

Compound	2-CH_3 Acid	2-CH_3 Neutral	2-CH_3 Alkaline	Aldehyde or hemiacetal H Acid	Aldehyde or hemiacetal H Neutral	Aldehyde or hemiacetal H Alkaline	5-CH_2OR Acid	5-CH_2OR Neutral	5-CH_2OR Alkaline	C_6-H Acid	C_6-H Neutral	C_6-H Alkaline	Others Group	Others Acid	Others Neutral	Others Alkaline
Pyridoxal phosphate	−156	−144	−140.5	[a]	−624	−622	−303	−300	−297	−492	−463	−457				
							−306	−305	−303							
4-Deoxypyridoxol phosphate	−155	−143	−138				−296	−285	−283	−488	−457	−454	4-CH_3	−142	−131	−130
							−303	−291	−287							
Pyridoxal	−159	−145	−139	−402	−392[c]	−425	−315[b]	−304	−289	−492	−457	−442				
				−404				−307								
Pyridoxal ethylacetal	−159	−145	−140	−404	−378	−376	−314[b]	−304[b]	−301	−492	−455	−448				
				−405		−378			−305							

[a] A small peak at −390 c.p.s. is probably due to an impurity in the sample. [b] Broad peak. [c] Split by 1 c.p.s.

c.p.s., similar to that of other compounds in which the 5-hydroxymethyl side chain is unsubstituted (Table I). This would indicate that in alkaline solution the aldehyde group of pyridoxal is modified in a way which does not involve hemiacetal formation with the 5-hydroxymethyl side chain. The one-proton peak at −425 c.p.s. is probably associated with the modified aldehyde proton.

From the work described in this paper, it should be apparent that p.m.r. spectroscopy is potentially a valuable tool in such studies as the elucidation of reaction mechanisms catalyzed by pyridoxal phosphate, and the determination of the exact nature of the involvement of the aldehyde group in the binding of pyridoxal phosphate on various apoenzyme surfaces.[17]

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The Correlation of Biological Activity of Plant Growth Regulators and Chloromycetin Derivatives with Hammett Constants and Partition Coefficients

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An equation using two experimentally based variables, σ and π, has been developed for correlating the effect of a given substituent on the biological activity of a parent compound; σ is the Hammett substituent constant and π is an analogous constant representing the difference in the logarithms of the partition coefficients of the substituted and unsubstituted compounds ($\pi = \log P_X - \log P_H$). The value of this equation has been tested on two systems of biologically active molecules: the phenoxyacetic acids and chloromycetin analogs. Using π and σ it becomes possible to disentangle three of the most important parameters governing the biological activity of organic compounds: steric, electronic, and rate of penetration.

Since the classic paper by Koepfli, Thimann, and Went[2] pointing out that a variety of acids of quite different gross structure function as plant growth regulators in the cell elongation process, an enormous amount of work has been done on the chemical and/or physical properties responsible for the biological activity and common to the great assortment of compounds which will produce this effect. The theories which have been developed have been summarized and analyzed from various points of view.[3a,b]

In our "two point attachment" theory[3a] to rationalize chemical structure and biological activity, we have assumed that auxins react *via* two points, one on the side chain and one on the ring, with a plant substrate. The fact that a ring of considerable aromatic character seems essential for auxin activity[4] has caused us to focus our attention on the nature of the substituent effect. It was early apparent[5] that the electronegative groups such as nitro and halogen were more effective in increasing biological activity when substituted onto the ring than electron-releasing groups such as alkyl, OH, etc. However, our attempts to find any quantitative relationship between the biological activating ability of functional groups and their relative electronegativity were unsuccessful. The molecular orbital calculations of Fukui[6,7] and others[8,9] attempting to correlate activity with π-electron delocalizability at various points on the ring, while quite suggestive and of qualitative value, leave much to be desired. In setting up a more exact model to test our two-point reaction hypothesis, we have made the following assumptions: 1. Consideration of three critical steps I, II, and III in the movement of auxin from solution to the site of action followed by a two point attachment to a plant substrate would be sufficient to rationalize growth rates caused by the different monosubstituted phenoxyacetic acids. Considering the vast number of molecules of such

(1) On leave from Kyoto University, Kyoto, Japan.

(2) J. B. Koepfli, K. V. Thimann, and F. W. Went, *J. Biol. Chem.*, **122**, 763 (1938).

(3) (a) R. M. Muir and C. Hansch, *Ann. Rev. Plant Physiol.*, **6**, 157 (1955); (b) H. Veldstra, *ibid.*, **4**, 151 (1953).

(4) C. Hansch and R. M. Muir, "Plant Growth Regulation," Iowa State University Press, Ames, Iowa, 1961, p. 431.

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(6) K. Fukui, C. Nagata, and T. Yonezawa, *J. Am. Chem. Soc.*, **80**, 2267 (1958).

(7) R. M. Muir and C. Hansch, ref. 4, p. 249.

(8) K. Koshimizu, T. Fujita, and T. Mitsui, *J. Am. Chem. Soc.*, **82**, 4041 (1960).

(9) T. Fujita, T. Komazawa, K. Koshimizu, and T. Mitsui, *Agr. Biol. Chem.*, **25**, 719 (1961).

$$\text{auxin in solution} \xrightarrow[\text{step I}]{k_1} \text{site of action} \xrightarrow[\text{step II}]{k_2} \text{one point attachment} \xrightarrow[\text{step III}]{k_3}$$

$$\text{two point attachment} \xrightarrow[\text{steps IV} \rightarrow n]{k_4 \rightarrow k_n} \text{growth}$$

greatly different structure which so effectively promote cell elongation, it seems likely that auxins act to initiate the growth process, but that they are not involved in steps IV to n. Thus, a first approximation of the rate of growth could be formulated as in 1 where A is a probability factor. Steps I and II are equilibrium processes, while step III might or might not be.

$$\text{growth rate} = A(k_2)(k_3) \qquad (1)$$

2. We have assumed that auxins penetrate to the site of action (step I) by a random walk process with many partitionings between "organic phases" (*e.g.*, cell membrane) and "aqueous phases" of the plant cell. We have chosen octanol and water as a model system to approximate the effect of step I on the growth reaction in much the same fashion as the classical work of Meyer and Overton[10] rationalized the relative activities of various anesthetics. This assumption is expressed in 2 where P is the partition coefficient (octanol–water) of the auxin.

$$A = \text{f}(P) \qquad (2)$$

Collander[11] has shown that the partition coefficients for a given compound in two different solvent systems (*e.g.*, ether–water, octanol–water) are related as in 3.

$$\log P_1 = a \log P_2 + b \qquad (3)$$

This would also indicate, as does the Meyer–Overton work, that it is not unreasonable to use the results from one set of solvents to predict results in a second set. Of course, the complexity of the biophases is well appreciated; therefore, one cannot hope for very high precision with the oversimplifying assumption that they can be treated as two simple phases.

3. After penetration to the site of action, the next critical step is considered to be the attachment of the auxin to a plant substrate *via* the carboxyl group of the side chain. The fact that all α,α-disubstituted phenoxyacetic acids are completely inactive[12] and that great differences in the activity of the optical antipods of the α-substituted phenoxyacetic acids and their analogs are apparent from the extensive studies of Fredga[13] and co-workers indicates that interference with the carboxyl group may be rate controlling. It is assumed that for phenoxyacetic acids with a single function in the 3-position, steric effects could be ignored and electronic effects on the carboxyl group would be slight (and possibly parallel) in comparison to those on the aromatic ring. Hence, k_2 is presumed to be constant for the auxins considered in this study.

4. After attachment through the side chain, it is assumed a second point of attachment takes place through an *o*-position of the ring. Previous work[3a,4] has indicated this position to be most suitable stereoelectronically. Also, the kinetic studies of Bonner and co-workers,[14,15] molecular orbital calculations,[6–9] and the metabolic work of Klämbt[16] all point to the importance of the *o*-positions. The potential rate-controlling character of the *o*-positions has been shown by the studies of activities of 33 trisubstituted phenoxyacetic acids.[17] In the absence of substituents in the *o*-positions, it is assumed that the rate of this second point attachment as reflected in growth is proportional to the electron density as measured by the Hammett σ-function[18,19] using σ_p for 3-substituted phenoxyacetic acids and σ_m for the 4-derivatives.

$$\log k_3 = \rho\sigma \qquad (4)$$

5. Since Åberg[20] has shown that large groups in the 4-position of the phenoxyacetic acids destroy auxin activity, such molecules have not been used in testing our model.

6. It is assumed that changes in activity due to metabolic modification of the phenoxyacetic acids employed in this work can be ignored (except where noted).

With the above assumptions in mind, expression 5 follows from 1, 2, 3, and 4.

$$\log (\text{growth rate}) = \log \text{f}(P) + \rho\sigma + \text{constant} \qquad (5)$$

Since the movement of auxin between phases is an equilibrium process as illustrated in 6

$$\text{auxin in "aqueous phase" } (H_2O) \underset{k_b}{\overset{k_a}{\rightleftharpoons}} \text{auxin in "organic phase" (octanol)} \qquad (6)$$

the partition coefficient, P, can be defined as an equilibrium constant: $P = k_a/k_b$. This being so, it is reasonable to express the effect of a given function on the partition coefficient of a parent molecule in terms of the so-called Hammett "linear free-energy relationship" which has proved to be so useful in the analysis of electronic effects of substituents on organic reactions.

$$\log (P_X/P_H) = \pi \text{ (for octanol–water)} \qquad (7)$$

From 3, the general formulation would be $\log (P_X/P_H) = k_p\pi$ (7). The left-hand side of 7 is proportional to the difference in the free energy changes involved in moving unsubstituted and substituted molecules from one phase to another; P_X represents the partition coefficient of the substituted phenoxyacetic acid and P_H that of the parent compound. In 7, k_p will be a constant dependent on the nature of the phases employed in the measurement of P. By definition, it is 1 for octanol–water.

Ferguson[21] appears to be the first to appreciate the general correlation between the biological activity of various series of organic compounds and the logarithm of a number of physical constants, including the partition coefficient. Later, Collander[22] showed that the rate of movement of a variety of organic molecules

(10) A. Burger, "Medicinal Chemistry," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1960, p. 48.

(11) R. Collander, *Acta Chem. Scand.*, **5**, 774 (1951).

(12) C. H. Fawcett, R. L. Wain, and F. Wightman, *Ann. Appl. Biol.*, **43**, 342 (1955).

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(16) H. D. Klämbt, *Planta*, **57**, 391 (1961).

(17) (a) C. H. Fawcett, R. M. Pascal, M. P. Pybus, H. F. Taylor, R. L. Wain, and F. Wightman, *Proc. Roy. Soc.* (London), **150B**, 95 (1959); (b) J. M. F. Leaper and J. R. Bishop, *Bot. Gaz.*, **112**, 250 (1951); (c) C. Wolfe, J. W. Wood, L. W. Klipp, T. D. Fontaine, and J. W. Mitchell, *J. Org. Chem.*, **14**, 900 (1949); (d) R. M. Muir and C. Hansch, *Plant Physiol.*, **28**, 218 (1953); (e) G. W. K. Cavill and D. L. Ford, *J. Chem. Soc.*, 565 (1954); (f) D. J. Osborne, G. E. Blackman, S. Novoa, F. Sudzuki, and R. G. Powell, *J. Exptl. Bot.*, **6**, 392 (1955); (g) J. Toothill, R. L. Wain, and F. Wightman, *Ann. Appl. Biol.*, **44**, 547 (1956); (h) M. P. Pybus, R. L. Wain, and F. Wightman, *Nature*, **182**, 1094 (1958); (i) K. H. Klassens and C. J. Schoot, *Rec. trav. chim.*, **75**, 186 (1956); (j) M. P. Pybus, M. S. Smith, R. L. Wain, and F. Wightman, *Ann. Appl. Biol.*, **47**, 173 (1959).

Of the 15 having an unsubstituted *o*-position, 14 are active in both the avena and pea tests. The 4-chloro-2-isopropyl-5-methyl derivative is inactive.[17d] Of the 18 substituted on the 2,4- and 6-positions (*i.e.*, both *ortho* positions), 11 are inactive in both the avena and pea tests. Two have been tested only in the tomato test in which both were inactive. Of the other 5, all but two (2,4-diCl, 6-F, and 2,4-diBr, 6-F)[17a] are inactive in the avena test and show low to doubtful activity in the split pea test.

(18) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940.

(19) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).

(20) B. Åberg, "The Chemistry and Mode of Action of Plant Growth Substances," Butterworths, London, 1956, p. 93.

(21) J. Ferguson, *Proc. Roy. Soc.* (London), **127B**, 387 (1939).

(22) R. Collander, *Physiol. Plant.*, **7**, 420 (1954).

through plant tissue was linear with respect to the logarithm of the partition coefficients. However, the compounds investigated by Collander had low partition coefficients, the highest being 8.5 in the system ether–water. In view of the examples just cited, we have elected to use the logarithm of the partition coefficient in the form of the comparative variable π rather than the specific variable P. Accordingly, we replaced $f(P)$ by $f(\pi)$ in eq. 5.

Since it is most convenient to make one measurement of growth (*i.e.*, total increase in a 24-hr. period), we have chosen to use $\log (1/C)$ in eq. 5 rather than a more typical rate expression, where C is the concentration of auxin in moles/liter causing a 10% elongation in 24 hr. Using π, we have modified 5 to 8.

$$\log (1/C) = \log f(\pi) + \rho\sigma + \text{constant} \qquad (8)$$

π, then, is a constant for a given functional group representing its effect on the relative free energy changes as indicated in 9.

$$\Delta\Delta F^0 = \Delta F_X{}^0 - \Delta F_H{}^0 = -RT \ln P_X + RT \ln P_H$$
$$\ln P_X - \ln P_H = -\Delta\Delta F^0/RT$$
$$\log (P_X/P_H) = k(-\Delta\Delta F^0) = k_p\pi \qquad (9)$$

The maximum probability of an auxin molecule finding an active site by a random walk partitioning process between two phases will occur when the maximum freedom of movement is possible; that is, when the free energy change in moving from one phase to another is a minimum. For octanol this can be expressed as in 10

$$\log P_X = \log P_H + \pi \qquad (10)$$

where, when $\log P_H + \pi$ is zero, the free energy change in moving from one phase to the other will be zero. For the situation in the cell there will be an ideal value for π such that the sum of the free energy changes of many boundary crossings by a molecule will be a minimum. If we assume a normal probability distribution such that the probability of movement to the site of action decreases exponentially with the square of the differences between π and a constant ideal value, b, then

$$f(\pi) = K \exp\left[-\frac{(\pi - b)^2}{a}\right] \qquad (11)$$

(a and b in 11 are constants and, of course, different from those in eq. 3). Taking the logarithm of both sides and combining constants, we get

$$\log f(\pi) = k\pi - k'\pi^2 + k'' \qquad (12)$$

For the general case, it must be borne in mind that the constants a and b from eq. 3 are contained in the constants of eq. 12. Substituting 12 for $\log f(\pi)$ in 8, we get 13.

$$\log (1/C) = k'\pi - k\pi^2 + \rho\sigma + k'' \qquad (13)$$

This equation could be expected to hold if the rate of transport of the organic compound to the site of action is slow compared to the rate of combination at the active site. Equation 13 will, of course, be subject to the recognized limits of the Hammett linear free energy treatment of equilibria and reaction rates.

An equation similar to 13 might fortuitously arise, and apply in certain instances, from the steady-state hypothesis of Ferguson.[21] To rationalize the fact that the activity of a given series of biologically active molecules first rises and then falls as the partition coefficient of the parent molecule is increased by the introduction of substituents, Ferguson has suggested that a decrease in thermodynamic activity follows the increase in the partition coefficient. He has assumed that the compound in solution quickly establishes an equilibrium with that at the site of action inside the cell and that, since the active site would be more lipophilic than the surrounding solution, an increase in the fat–water partition coefficient should result (other factors being held constant) in an increase in the concentration at the site of action and hence in greater activity. Ferguson has said that the relative thermodynamic activity of a given molecule will decrease as its partition coefficient is increased because its solubility in the aqueous phase will approach the limiting value. That is, as S/S_0 (S = concentration under investigation and S_0 = solubility of the compound in the aqueous phase) approaches unity, the chemical potential will drop. When this drops to the point where the concentration necessary to produce the biological response reaches S_0, the molecule is completely inactive. If, by chance, the term $-k'\pi^2$ in eq. 13 would, in the examples reported in this paper, be a measure of this fall in chemical potential, then our results could be interpreted by Ferguson's model. This seems unlikely, but further work is in order to settle this point.

To evaluate this hypothesis, we have measured the partition coefficients of a variety of monosubstituted phenoxyacetic acids between octanol and water. These values, together with corresponding σ-functions, are given in Table I.

Results and Discussion

Using the data in Table I, we have obtained the "best set" of constants for eq. 13, shown in 14.

$$\log 1/C = -1.97\pi^2 + 3.24\pi + 1.86\sigma + 4.16 \qquad (14)$$

In evaluating the constants by the least squares method, values for all of the substituents producing active molecules were used except the 2-fluoro, 3-SO_2CF_3, 3-OH, and 3-COOH. The over-all agreement between observed and predicted activity for the 34 compounds in Table I, with partition coefficients of 1 to 1500, σ-values of -0.67 to $+0.93$, and active concentration ranges of 3×10^{-7} to 1×10^{-3} M, is surprisingly good in view of the assumptions necessary in correlating a process as complex as growth with a simple physicochemical model. The correlation coefficient for all of the active 3- and 4-derivatives in Table I, except 3-OH, 3-SO_2CF_3, and 3-COOH, and using π' for 3-SO_2CH_3, is 0.881. The carboxyl group was not included because of the uncertainty in its degree of ionization. Using the same points to derive a 3-constant equation *via* a least squares fit, one obtains: $0.88\pi + 0.74\sigma + 4.37 = \log (1/C)$. The coefficient of determination for this equation is only 0.391 while that for 14 is 0.777. The constants in 14 are slightly different from those previously published[23] because of additional and improved data used in their evaluation.

The typical relationship of auxin concentration and biological effect is an increase in growth rate up to an optimum concentration and then a decrease in growth rate at higher concentrations until actual inhibition of growth occurs. Our experience indicates that most auxins become inhibitory at concentrations of about 10^{-3} M. For a few compounds which produce inhibition at lower concentrations, it has not been possible to demonstrate any growth-promoting effect. For example, with 3-*n*-butylphenoxyacetic acid, inhibition occurs at a concentration of only 5×10^{-5} M, while the predicted concentration for activity is $\sim 1 \times 10^{-3}$ M. Similarly, the active concentrations predicted for 3,4-$(CH_2)_4$, 3-C_6H_5, and 3,4-$(CH_2)_3$ are so high that, except for the latter molecule, inhibition occurs well below the concentration at which an increase in growth rate is expected. The 3-C_6H_5 deriva-

(23) C. Hansch, P. P. Maloney, T. Fujita, and R. M. Muir, *Nature*, **194**, 178 (1962).

TABLE I

COMPARISON OF CALCULATED AND OBSERVED ACTIVITIES OF PHENOXYACETIC ACIDS

POA	$K \times 10^4$[a]	σ[b]	$\overline{P}$	π	log (1/C) obsd.	log (1/C) calcd.	Δ log (1/C)
3-CF_3	8.90*	0.55	229	1.09 ± 0.03	6.5	6.38	0.12
4-Cl	7.89	.37	94	0.70 ± .03	6.4	6.15	.25
3-I	7.44	.28	272	1.17 ± .02	6.3	5.78	.52
4-F	7.42	.34	26	0.14 ± .02	6.3	5.21	1.09
3-Br	8.03	.23	165	0.95 ± .02	6.0	5.89	0.11
3-SF_5	10.20*	.68[d]	598	1.51 ± .02	5.7	5.83	.13
3-Cl	8.51	.23	107	0.76 ± .03	5.7	5.91	.21
3-NO_2	11.20	.78	24	.10 ± .02	5.3	5.92	.62
3-SCH_3	7.40*	− .05	78	.62 ± .02	5.3	5.32	.02
3-C_2H_5	6.55*	− .15	174	.97 ± .03	5.3	5.17	.13
3-SCF_3	8.90*	.51[c]	717	1.59 ± .02	5.2	5.28	.08
3-SO_2CF_3	12.50*	.93[d]	152	0.92 ± .02	5.2	7.20	2.00
3,4-$(CH)_4$[e]	7.50*	.17	347	1.27 ± .02	5.0	5.41	0.41
3-OCH_3	7.22	− .27	24	0.11 ± .02	4.7	3.99	.71
3-CH_3	6.27	− .17	60	.51 ± .02	4.5	4.98	.48
3-CN	9.24	.63	8.9	− .32 ± .02	4.5	4.09	.41
3-n-C_3H_7	6.55*	− .15[f]	510	1.44 ± .03	4.5	4.46	.04
4-OCH_3	6.13	.12	17	−0.04 ± .02	4.2	4.25	.05
3-$COCH_3$	8.30*	.52	9.5	− .29 ± .03	4.0	4.02	.02
3-F	8.28	.06	25	.13 ± .02	4.0	4.66	.66
H	6.75	.00	18.5	.00	3.5	4.16	.66
3-OH	6.70*	− .36	5.7	− .51 ± .03	3.5	1.33	2.17
3-SO_2CH_3	10.50*	.73	1	−1.26 ± .05	3.5	−1.69	5.19
3-SO_2CH_3	10.50*	.73		−0.47(π')	3.5	3.56	0.06
2-F	8.22	.34	18	− .01 ± .02	3.3	4.76	1.46
3-COOH[g]	8.50*	.27	13	− .16 ± .03	3.0	4.09	1.09
3,4-$(CH_2)_4$[h]	4.80*	− .48	470	1.40 ± .02	Inactive	3.94	0[k,l]
3,4-$(CH_2)_3$[i]	5.60*	− .26	214	1.06 ± .03	Inactive	4.90	0[k]
3-$NHCOCH_3$	7.20	− .02[j]	3.0	−0.79 ± .02	Inactive	0.33	0[l]
3-n-C_4H_9	6.55*	− .15[f]	1500	1.91 ± .03	Inactive	2.88	0[l]
3-$NHCOC_6H_5$	7.80*	.08	97	0.72 ± .03	Inactive	5.62	5.62
3-C_6H_5	7.80*	.01	1500	1.91 ± .03	Inactive	3.18	0[l]
2-OCH_3	5.88	.12	8.4	−0.34 ± .02	Inactive	3.05	0[l]
4-OH	5.10	− .002	4.5	−0.62 ± .02	Inactive	1.39	0[l]
3-OCF_3	8.80*	.35[c]	300	1.21 ± .02	Inactive	5.85	0[m]

* These values were estimated from a plot of the known ionization constants against σ-values. [a] Unless otherwise indicated, values of the ionization constants used in the calculation of P were taken from the work of Hayes and Branch, ref. 32. [b] Unless otherwise noted, the values for σ were taken from the compilation of Jaffé, ref. 19. [c] Reported by W. A. Sheppard, *J. Am. Chem. Soc.*, **83,** 4860 (1961). [d] Private communication, W. A. Sheppard. [e] 2-Naphthoxyacetic acid. [f] Since a σ-constant for this function has not been reported, we have used the value for ethyl. [g] The ionization of only the oxyacetic carboxyl was considered in the calculation of P. [h] 5,6,7,8-Tetrahydro-2-naphthoxyacetic acid. [i] Indane-5-oxyacetic acid. [j] Reported by W. N. White, R. Schlitt, and D. Gwyn, *J. Org. Chem.*, **26,** 3613 (1961). [k] log $1/C$ is hard to estimate since it is suspected that steric factors may be involved with each of these compounds (see Discussion). [l] As indicated in the Discussion, it is not expected that molecules having predicted values of log $1/C$ near 3 will be active since most auxins are inhibitory at about 10^{-3} M. [m] Inhibitory effects overshadow growth-promoting activity (see Discussion).

tive causes inhibition at 5×10^{-5} M, while the 3,4-$(CH_2)_4$ and 3,4-$(CH_2)_3$ derivatives become inhibitory at 1×10^{-4} M. For these latter two substances, the CH_2 group in the 4-position probably has a sterically depressing effect on auxin activity.

The fact that the 3-OCF_3 function produced an inactive molecule was quite surprising considering the favorable values for σ and π. The 3- and 4-OCF_3 derivatives were the most inhibitory molecules of those we have yet tested, showing inhibitory effects at concentrations almost as low as 1×10^{-6} M. This is the concentration at which eq. 14 predicts elongation should begin to occur; however, it would appear that this is masked by the inhibitory effect, the nature of which is not apparent.

The most difficult and perplexing aspect of studying substituent effects in biologically active molecules is that of disentangling steric, electronic, and penetration characteristics conferred on a parent molecule by a given function. Equation 14 provides a new approach to the problem. Preliminary qualitative studies indicated the 3-position in the monosubstituted phenoxyacetic acids to be insensitive to the substitution of rather large groups. Quantitative support for this is apparent from a comparison of the 3-alkyl derivatives: methyl, ethyl, n-propyl, and n-butyl. Electronic effects for these groups differ so slightly that σ can be assumed to be constant. Since the results obtained from eq. 14 agree so well with the experimental values, steric effects from the 3-position can be ruled out. It may well be that the angular attachment of the alkyl groups plays a role in relieving steric hindrance of activity by these groups. Again, the rather good agreement between experimental and calculated values for 3-I, 3-SF_5, and 3-SCF_3 indicates steric factors to be absent for functions of moderate size.

The most interesting of the points in Table I are those which are very poorly accounted for by eq. 14. Foremost among these is the 3-OH derivative, the activity of which is underestimated by a factor of more than 100. The first thought, that the octanol–water model simply is a poor one to account for the effect of the OH on the penetration of the phenoxyacetic acid, does not seem to be true. The 4-OH derivative is expected and found to be inactive. More convincing, however, are the data from the chloromycetin analogs; each of the equations, 15, 16, 17, 18, predicts a value for the hydroxy analog of chloromycetin very close to that observed for each of

the four organisms. The unexpectedly great activity for 3-hydroxyphenoxyacetic acid suggests that metabolic change is involved (steric acceleration of this order of magnitude is out of the question). In this connection, the hypothesis of Leaper and Bishop[17b] that auxins may be converted to quinones deserves further investigation. Also of interest is Klämbt's[16] work showing that auxins are converted to glucosides. Substitution of an OH unit may be the first step in glucoside formation. The same phenomenon is observed with the 3- and 4-aminophenoxyacetic acids. The latter is inactive while the former is active (log $1/C = 3.3$). The π-value for the amino group would be expected to be even lower than that for the hydroxyl group. Unfortunately, it was not possible to obtain a reliable π-value because of the very low solubility of the aminophenoxyacetic acid in octanol. The amino group is also well known for its ease of quinone formation and it, too, may be involved in this kind of metabolic modification. Whether this type of metabolic change would account for the higher than expected value of the 3-OCH_3 function is questionable.

Also of particular interest is the very much greater than predicted value for 3-SO_2CH_3. During the course of this work it has occurred to us that the octanol–water model might not be ideal, but that by using suitable activity coefficients a practical and useful set of π' constants might be derived for pharmacological work. Our preliminary experience with the data in Tables I and III, as well as other work in progress, indicates that, with the exception of the 3-SO_2CH_3 group, no consistent variation of the various π-values is observed. However, if a π' value of -0.47 is assigned to this function, good agreement is obtained with the chloromycetin analog as well as the phenoxyacetic acid derivatives. Also, in work with the auxin activity of 3-methylsulfonylphenylacetic acid, good agreement is obtained between observed and predicted activity using -0.47 for π'.

That the 3-methylsulfonylphenoxyacetic acid is active is of special interest since molecules of the type $ArSO_2CH_2COOH$ have invariably been found inactive.[24–26] It is now apparent that at least one reason for the inactivity of these sulfones is the high water solubility conferred by the very polar and hydrogen-bonding sulfone group; π for the $-SO_2CH_3$ function is the lowest of all the functions we have checked. That there is nothing inherently inactivating (other than a low π-value) about the sulfone group is evident from the relatively high activity of the 3-SO_2CF_3 compound. Decreasing the solubility and the hydrogen-bonding power of the 3-SO_2CH_3 group by the introduction of the three fluorine atoms gives a much more active molecule in the case of 3-trifluoromethylsulfonylphenoxyacetic acid, although the activity falls below that expected on the basis of its high σ-value and ideal π-value. This less-than-expected activity appears to be due to the instability of the substance in aqueous solution. Solutions of this compound tend to lose activity on standing; the ultraviolet absorption spectra changed markedly when the compound stood at room temperature overnight in basic solution.

Another interesting exception in Table III is the 3-benzamido group. Although predicted to be of moderate activity, it is completely inactive. This would appear to be a steric effect and it shows that a group of sufficient size will sterically destroy activity even in the 3-position.

(24) H. Erdtman and G. Nilsson, *Acta Chem. Scand.*, **3**, 901 (1949).
(25) C. Wilske and H. Burström, *Physiol. Plant.*, **3**, 58 (1950).
(26) J. Kato, *Mem. Coll. Sci. Univ. Kyoto*, **21,B** 77 (1954).

Even though the data in Table I do not represent a perfect correlation, a number of important substituent effects up to now poorly understood come clearly into focus. Foremost in importance is the electron-withdrawing character of the substituent. The high positive value of ρ in 14 clarifies the importance of the electron-attracting role of the substituent. Highly active molecules are obtained only with substituents having positive σ-values. Although the 3-C_2H_5 function (σ -0.15) produces an auxin of moderate activity, this is nicely accounted for by its ideal π-value. The constants in eq. 14 are such that optimum mobility is conferred on the phenoxyacetic acid molecule by those functions having values of π between 0.5 and 1.2. Increasing π above 0.8 increases lipophilic character until at $\pi = 1.7$ the contribution of π to activity in 14 becomes negative.

The uncertain role of the methyl group[27] now becomes evident. Its activity-increasing effect (when its steric action can be eliminated) is satisfactorily accounted for by its favorable π-value.

The relatively weak activating power of the strongly electron-attracting nitro group has been a difficult point to rationalize. Such an easily reduced group has been suggested to undergo reduction in plant tissue. Our analysis would indicate normal activity for this function; its high σ-value is simply offset by a low value for π.

A particularly interesting group is the recently discovered[28] $-SF_5$ function. Its high σ- and π-values should make this group very useful for increasing the biological activity of molecules having low lipophilic character. The reasonable correspondence between observed and predicted activity of this phenoxyacetic acid implies that the $-SF_5$ group must resist metabolic change and that it has no special toxic nature. These observations indicate that this function is worthy of further pharmacological study.

Even though it was not expected that 2- and 4-substituted phenoxyacetic acids would be very helpful in developing a model for structure–activity study, a number of these derivatives were investigated, partly to learn the effect of position on the substituent constant, π, and partly to substantiate Åberg's[20] hypothesis that large groups in the 4-position destroy auxin activity in the phenoxyacetic acids. The results are summarized in Table II. Activity resulting from 2-substitution is quite unpredictable. For example, the rather large nitro group increases activity over that of the parent molecule, while the ethyl group, which is just as effective as the nitro group in the 3-position, gives an inactive molecule when placed in the 2-position. The 2-F and 2-OCH_3 derivatives are listed in Table I, since it was considered that steric effects with these groups would be small and that eq. 14 could be expected to hold. This expectation seems justified with 2-OCH_3, although 2-F does not correlate as well.

The assumption that substitution of groups larger than bromine in the 4-position completely destroys activity is confirmed by a comparison of the data in Tables I and II. The eight large groups I, NO_2, COOH, CH_3CO, CH_3, C_2H_5, n-C_3H_7, and CN all give active auxins when placed in the 3-position, but completely inactive compounds when set in the 4-position. The inactivity of the 4-CN derivative is particularly interesting and would imply that the linear dimensions of a substituent in this position are more important than sheer bulk. This is also borne out by the activity of 4-OCH_3. Since it is not possible to rationalize this substituent effect of large groups in the 4-position

(27) K. V. Thimann, ref. 4, p. 444.
(28) W. A. Sheppard, *J. Am. Chem. Soc.*, **82**, 4751 (1960).

TABLE II

ACTIVITIES AND PARTITION COEFFICIENTS OF 2- AND 4-SUBSTITUTED PHENOXYACETIC ACIDS

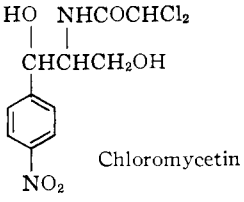
Substituent	$K \times 10^4$[a]	$\bar{P}$[b]	π	Auxin activity[c]
H	6.75	27	0.00	1.0
2-Cl	8.90	104	.59 ± 0.03	6.0
2-Br	7.53	150	.75 ± .06	6.0
2-I	6.72	230	.92 ± .02	6.0
2-NO_2	12.70	16	− .23 ± .05	10.0
2-CH_3	5.93	130	.68 ± .05	15.0
2-C_2H_5	5.93[d]	450	1.22 ± .03	0
2-C_6H_5				0
2-CH_3CO	9.60[d]	29	0.03 ± 0.07	30
4-Br	7.37	285	1.02 ± .05	100
4-I	6.94	488	1.26 ± .03	0
4-NO_2	12.80	31	0.06 ± .05	0
4-CH_3	6.09	90	0.52 ± .05	0
4-C_2H_5				0
4-*n*-C_3H_7				0[e]
4-C_6H_5				0
4-CH_3CO	9.60[f]	12	−0.35 ± 0.06	0
4-COOH				0
4-CN	11.70	13	−0.31 ± 0.03	0

[a] Unless otherwise noted, ionization constants are those of Hayes and Branch, ref. 32. [b] Determined coulometrically. [c] Activity compared to phenoxyacetic acid = 1. [d] Estimated K-value. [e] Taken from the work of Åberg, ref. 20. [f] This value estimated using the Hammett relationship.

in terms of π or σ, steric interference with orientation at the active site appears to be the best explanation. It is noteworthy that methoxyl gives an active compound when in the 4-position, but when placed in the 2-position an inactive compound results. This difference is rationalized by the difference in π-values highlighting the critical potential of this parameter in structure–activity considerations.

The function π appears to be very close to constant for 3- and 4-positions. Placing a group in the 2-position results in a considerable change for π. It would seem from the limited results so far obtained that, as a substituent constant, π would be subject to the same limitations as σ.

The over-all surprisingly good correlation of the observed auxin activity of the phenoxyacetic acids with the values predicted from eq. 14 suggests that eq. 13 might be applicable in a general sense. Results of a number of examples under current study indicate this to be true. Unfortunately, the number of examples in the literature where a particular compound has been modified with a wide variety of substituents, and these derivatives tested on a single organism under a standard set of conditions to yield quantitative results, is severely limited. An outstanding exception is the Russian[29] work on chloromycetin analogs in which the nitro group in the 4-position was replaced by a wide variety of substituents.



Chloromycetin

In addition to testing their compounds on *Staphylococcus aureus*, a most widely used organism, three other bacteria were also studied. From a least squares fit to eq. 13, using all of the points of definite activity (some were reported as less than a certain figure) except the carboxyl group, the following equations result for each of the four organisms

Staphylococcus aureus, gram pos., $-0.54\pi^2 + 0.48\pi + 2.14\sigma + 0.22 = \log A$, r 0.945 (15)

Bacillus subtilis, gram pos., $-0.67\pi^2 + 0.85\pi + 1.39\sigma + 0.55 = \log A$, r 0.882 (16)

Coli, gram negative, $-0.74\pi^2 + 0.36\pi + 1.82\sigma + 0.62 = \log A$, r 0.824 (17)

Vibrio fluoresens, gram negative, $-0.81\pi^2 + 0.61\pi + 2.04\sigma + 0.33 = \log A$, r 0.880 (18)

The individual values for *Staphylococcus aureus* are given in Table III. Unfortunately, σ- and π-constants are available for only eleven of the twenty-two analogs investigated. The carboxyl group was not used in evaluating the constants in eq. 13 because this molecule would be highly dissociated under the testing conditions. This, of course, would greatly affect its rate of penetration relative to the other analogs. As expected, the observed activity of this analog is, with each of the four organisms, 10–15 times less active than predicted by eq. 15–18. The Hammett constant σ_m was finally chosen rather than σ_p because a better correlation was obtained with its use. The correlation coefficient r for the above equations is almost as good as for the phenoxyacetic acids. From a consideration of eq. 15–18 the great difficulty in finding a chloromycetin analog more active than the natural product becomes obvious. Although activity could be increased by introducing a stronger electron-withdrawing group than nitro, there is little in practice from which to choose. The large parent molecule has such an ideal partition coefficient that almost nothing is to be gained by its modification by substituents. Significantly, the maximum contribution of π for the two gram positive organisms comes with a value of 0.4 in eq. 15 and 0.6 in eq. 16. Even then the contribution to log A is very small, being 0.11 in 15 and 0.27 in 16. For the two gram negative bacteria, optimum π is 0.2 for *Coli* and 0.4 for *Vibrio fluoresens*. With the gram negative organisms the contribution of the π-character of substituents to log A is insignificant, being 0.04 for the former and 0.11 for the latter. The above results do indicate that to reach the site of action the drugs

TABLE III

ACTION OF CHLOROMYCETIN ANALOGS ON *Staphylococcus aureus*

Substituent in 4-position	σ_m	π[a]	log A calcd.	log A obsd.[b]	Δ log A
—NO_2 (chloromycetin)	0.71	0.06	1.77	2.00	0.23
—CN	.68	− .31	1.47	1.40	.07
—SO_2CH_3	.65	− .47[c]	1.27	1.04	.23
—$COOCH_3$	.32	− .04[d]	0.89	1.00	.11
—Cl	.37	.70	1.08	1.00	.08
—N=NC_6H_5	.58[e]	1.72	0.69	0.78	.09
—OCH_3	.12	−0.04	.46	.74	.28
—$NHCOC_6H_5$	.22	.72[f]	.76	.40	.36
—$NHCOCH_3$	.10	− .79[f]	− .28	− .30	.02
—OH	.00	− .62	− .29	< − .40	
—COOH	.36	− .16[f]	.90	< − .40	

[a] π is that for the substituent in the 4-position of phenoxyacetic acid except where noted. [b] This is simply the log of activity relative to chloromycetin (in mole %) from ref. 29. [c] π'. [d] Determined with monomethyl ester of isophthalic acid. Work in progress with benzoic acids indicates π to be constant for phenoxyacetic and benzoic acids. [e] No value for *m*-N=NC_6H_5 has been reported. The value for *para* is 0.64.[19] In calculating this value we have assumed that the change for this function would be in the same proportion as that for 3- and 4-NO_2 substituents. [f] π for the *m*-substituent was used. Comparison of data in Table I and II shows little difference in π in the 3- and 4-positions.

(29) M. M. Shemyakin, M. N. Kolosov, M. M. Levitov, K. I. Germanova, M. G. Karapetyan, Yu. B. Shvetsov, and E. M. Bamdas, *J. Gen. Chem. USSR*, **26**, 885 (1956) (English translation).

must travel through biological material of different composition in gram positive and gram negative bacteria, and that in the former this material is more lipophilic in character. Of particular interest in eq. 15–18 is the fact that the reaction constant ρ is, for practical purposes, the same for three of the four organisms. This indicates that the stereoelectronic demands of the substrate with which the chloromycetin reacts is the same. Also of interest is the fact that a better fit with eq. 13 is obtained if one uses σ_m instead of σ_p. This would imply that it is the electron density in the *o*-positions which is most important.

The great difficulty in preparing more active analogs of chloromycetin becomes quite clear from eq. 15–18, especially when it is known that 3-substitution destroys activity. None of the 28 carefully tested analogs reported[29] showed more activity than chloromycetin itself; nevertheless, it may be possible to prepare more active derivatives. For instance, keeping in mind that $\Sigma\pi$ for the added substituents must not exceed 0.5 and that 3-substitution must be avoided, the 2-fluoro-4-nitro analog would seem worthy of investigation. In attempting to modify the side chain, the extreme importance of the partition coefficient must be kept in mind. Assuming stereoelectronic factors could be kept constant, any increase or decrease in the lipophilic nature of the side chain would have to be carefully compensated for by appropriate changes on the ring. Since the 4-position would appear to be the only completely free position as far as steric hindrance goes, this leaves little room for maneuver.

The results expressed in Tables I and III support the assumptions upon which eq. 13 rests; in particular, the idea that π, like σ, can be used to measure the substituent effect on "rate," as well as equilibrium processes, seems a worthwhile hypothesis upon which to base further work. It appears, then, that the rate at which a molecule crosses an interface depends on π, and that when many such crossings occur, the rate of movement of a molecule through a cell can be greatly affected by substituents.

Experimental

Plant Growth Test.—The activity of the auxins was determined by using the Avena cylinder straight growth technique with 3-mm. sections. Conditions of Bonner and co-workers[14,15] were used, since under these we have found that a linear growth rate prevails for at least 24 hr.

Partition Coefficients.—A 20–40-mg. sample of the acid was dissolved in the organic solvent which had previously been purified by washing with sodium hydroxide solution followed by distillation. The organic solvent was saturated with carbon dioxide-free distilled water, and the water phase was always saturated with organic solvent before partitioning was commenced. Usually, 20–50-ml. portions of octanol were used with 200 to 400-ml. portions of water. For some acids of very low *P*-values, 150 ml. of octanol and 50 ml. of water were used. The mixtures were shaken vigorously on a mechanical shaker for 1.5 hr., after which the water layer was drawn off and centrifuged at 2500 r.p.m. for 1.5 hr. The amount of auxin in the water layer was determined coulometrically.[30] A 40-ml. sample, in which 200 mg. of KBr was dissolved just before "titration," was used. A Beckman model M pH meter was employed to determine the end point. Because of the difficulty in determining the end point at such low concentrations, the accuracy of the *P*-values is probably not high. Centrifuging was extremely important in obtaining reproducible results even in those instances where no visible emulsification occurred. All operations were carried out under nitrogen to avoid contamination by carbon dioxide. Each determination was done in at least duplicate at two different volume ratios and an average value is reported. Because of the limited solubility of the phenoxyacetic acids in water[31] (our determination showed 3-*n*-butylphenoxyacetic acid to be soluble in water saturated with octanol at 30° only to the extent of 2.3×10^{-3} *M*), it was not possible to determine *P*-values for all of the compounds at two or more greatly different concentrations. For two of the more soluble acids a series of partitions were made of a 10-fold concentration range. This permitted the calculation of the degree of association in the octanol layer. For phenoxyacetic and 3-nitrophenoxyacetic acids only about 10% association occurred at 1×10^{-2} *M*. For the concentrations used in our experiments (on the order of 1×10^{-3} *M*), the association would not be more than a few per cent. Therefore, the association in the organic layer was ignored and the expression used for the partition coefficient was $P = C_{octanol}/C_{H_2O}(1-\alpha)$. It is expected that at the very low concentrations (10^{-3}–10^{-7} *M*) used in testing, association in the biophases will also be unimportant. The degree of disocciation (α) in the water was calculated from ionization constants.[32] For those acids for which experimental values were lacking, a value calculated from the Hammett relationship was used. It was assumed that the very small amount of octanol dissolved in the water would have no effect on α.

During the course of this work a Cary model 14 spectrophotometer became available with which much more accurate determination of the partition coefficient was possible. In this work it was found that small changes in temperature did not have a measurable effect on π. The temperature during this work was 27 ± 5°. Although difference in *P* obtained by the two methods are considerable because of a systematic error in the coulometric method, the differences in π for the two methods are small when compared to the error inherent in the bioassays. Shemyakin, *et al.*, estimate an accuracy of ±25% in testing the chloromycetin analogs. The work with the phenoxyacetic acids is of this same order of accuracy.

Preparation of Compounds. 3-Methylthiophenoxyacetic Acid.—The 3-methylthiophenol was prepared by the method of Bordwell and Boutan.[33] This was converted to the phenoxyacetic acid which was purified by recrystallization from water and vacuum sublimation; m.p. 98°.

Anal. Calcd. for $C_9H_{10}O_3S$: C, 54.53; H, 5.08. Found: C, 54.75; H, 5.46.

3-Methylsulfonylphenoxyacetic acid was made in the usual way from the phenol.[33] Upon crystallization from water, it formed a hydrate, m.p. 110–113°.

Anal. Calcd. for $C_9H_{10}O_5S \cdot H_2O$: C, 43.54; H, 5.83. Found: C, 43.21; H, 4.79.

On vacuum sublimation, the water was eliminated to give the anhydrous phenoxyacetic acid, m.p. 132–134°.

Anal. Calcd. for $C_9H_{10}O_5S$: C, 46.95; H, 4.37. Found: C, 46.41; H, 4.46.

3-Pentafluorosulfurphenoxyacetic Acid.—A sample of 3-pentafluorosulfurphenol was generously supplied by Dr. W. A. Sheppard of the du Pont Company. Conversion of this to the phenoxyacetic acid gave a substance of m.p. 113–115° after recrystallization from water and vacuum sublimation.

Anal. Calcd. for $C_8H_7F_5O_3S$: C, 34.54; H, 2.54. Found: C, 34.31; H, 2.88.

3-Cyanophenoxyacetic acid was prepared from a sample of 3-cyanophenol purchased from Columbia Chemicals Co. After two recrystallizations from water and vacuum sublimation, our product melted at 117–119°. Hayes and Branch[32] have reported a melting point of 192°.

Anal. Calcd. for $C_9H_7NO_3$: C, 61.01; H, 3.98. Found: C, 61.47; H, 4.07.

3-Phenylphenoxyacetic Acid.—3-Phenylphenol was made from 3-nitrobiphenyl.[34] The nitro compound was reduced catalytically with palladium-on-charcoal in benzene. The amine was converted to the phenol by usual diazotization technique. The phenoxyacetic acid made from the phenol was crystallized from water, then from benzene–ligroin and vacuum sublimed; m.p. 108–109.5°.

Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.67; H, 5.31. Found: C, 73.66; H, 5.13.

3-Benzamidophenoxyacetic acid was made from 3-aminophenoxyacetic acid by the Schotten–Bauman technique.[35] It was recrystallized from benzene–ethanol; m.p. 186–187°.

Anal. Calcd. for $C_{15}H_{13}NO_4$: C, 66.41; H, 4.63. Found: C, 66.61; H, 5.14.

3-Trifluoromethylthiophenoxyacetic Acid.—3-Trifluoromethylthioaniline was prepared according to Yagupolsky and Marenets[36] To a solution at 0° of the above amine, 10 ml. of water and 3 ml. of concentrated sulfuric acid was added 1.4 g. of sodium nitrite in 4 ml. of water. The diazotized amine was then slowly

(30) J. J. Lingane, "Electroanalytical Chemistry," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1958, p. 608.

(31) V. H. Freed, F. J. Reithel, and L. F. Remmert, ref. 4, p. 294.

(32) V. H. Hayes and G. E. K. Branch, *J. Am. Chem. Soc.*, **65**, 1555 (1943).

(33) F. G. Bordwell and P. J. Boutan, *ibid.*, **79**, 720 (1957).

(34) J. Elks, J. W. Haworth, and D. H. Hey, *J. Chem. Soc.*, 1284 (1940).

(35) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 3rd Ed., John Wiley and Sons, Inc., New York, N. Y., p. 98.

(36) L. M. Yagupolsky and M. S. Marenets, *J. Gen. Chem. USSR*, **26**, 99 (1956).

added to a boiling solution of 22 g. of sodium sulfate in 15 ml. of water and 15 ml. of concentrated sulfuric acid. This solution was steam distilled and the distillate made alkaline with sodium hydroxide and extracted with ether. The aqueous fraction was then acidified and extracted with ether. Evaporation of the ether and vacuum distillation of the residue gave 1.2 g. of phenol, b.p. 76–80° (2.5 mm.). The product solidified on cooling and was recrystallized from benzene–ligroin; m.p. 63.5–64.5°.

Anal. Calcd. for $C_7H_5F_3OS$: C, 43.30; H, 2.60. Found: C, 43.15; H, 2.55.

The phenol was converted to the phenoxyacetic acid as usual. After recrystallization from water and vacuum sublimation, it melted at 91.5–92.5°.

Anal. Calcd. for $C_9H_7F_3O_3S$: C, 42.85; H, 2.80. Found: C, 42.56; H, 2.74.

3-Trifluoromethylsulfonylphenoxyacetic Acid.—Our attempts to oxidize 3-trifluoromethylthionitrobenzene to the sulfone according to Yagupolsky and Marenets[36] gave negligible yields. If, however, enough glacial acetic acid was used to increase the solubility of the sulfide in the chromic acid solution, good yields were obtained. In a typical run, 21 g. of 3-trifluoromethylthionitrobenzene was placed in a solution of 16 g. of concentrated sulfuric acid, 25 ml. of water, 10 ml. of glacial acetic acid, and 16 g. of CrO_3. The mixture was refluxed for 50 hr., then poured into ice water and extracted with ether. Evaporation of the ether and vacuum distillation of the residue gave 22.5 g., b.p. 108–112° (1 mm.). After two recrystallizations from ethanol, 16 g. of sulfone of melting point 55–56° was obtained. The 3-trifluoromethylsulfonylnitrobenzene was reduced to the amine and this in turn converted to the phenol. In a solution of 6.3 g. of concentrated sulfuric acid and 22 ml. of water, 6.3 g. of 3-trifluoromethylsulfonylaniline was dissolved. Diazotization was completed with 2.3 g. of sodium nitrite in 7 ml. of water. The diazonium solution was then added dropwise to a boiling solution of 6 g. of sodium sulfate in 45 ml. of water and 45 ml. of concentrated sulfuric acid. The mixture was steam distilled and the distillate extracted with ether. The ether was extracted with sodium hydroxide solution which was then treated with Norit. The alkaline solution was acidified and extracted with ether. Evaporation of the ether and distillation of the residue gave 5.5 g., b.p. 118° (1 mm.). After crystallization from benzene–hexane, the product melted at 72–73°.

Anal. Calcd. for $C_7H_5F_3O_3S$: C, 37.18; H, 2.23. Found: C, 36.90; H, 2.61.

The phenol was converted to the phenoxyacetic acid, which, after recrystallization from water, melted at 101–102°. The yield was very low because of hydrolysis of the $-SO_2CF_3$ function.

Anal. Calcd. for $C_9H_7F_3O_5S$: C, 38.03; H, 2.54. Found: C, 38.33; H, 2.73.

3-Trifluoromethylphenoxyacetic acid was made from a commercial sample of the phenol. After recrystallization from water, it melted at 94.5–95.5°.

Anal. Calcd. for $C_9H_7F_3O_3$: C, 49.10; H, 3.21. Found: C, 48.89; H, 3.46.

3-Trifluoromethoxyphenoxyacetic Acid.—A sample of 3-trifluoromethoxyphenol was kindly supplied by Dr. W. A. Sheppard. This was converted as usual to the phenoxyacetic acid. After recrystallization from water, this substance melted at 89–90°.

Anal. Calcd. for $C_9H_7O_4F_3$: C, 45.77; H, 2.99. Found: C, 46.07; H, 3.27.

4-Trifluoromethoxyacetic acid was prepared by Dr. P. E. Aldrich of du Pont. Its melting point was 87–89°.

3-*n*-Propylphenoxyacetic Acid.—3-*n*-Propylphenol was converted as usual to the phenoxyacetic acid. After recrystallization from water and sublimation, this substance melted at 70.5–72°.

Anal. Calcd. for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.85; H, 7.23.

3-*n*-Butylphenoxyacetic Acid.—3-*n*-Butylphenol was converted as usual to the phenoxyacetic acid. After recrystallization from hexane and subsequent sublimation, the substance melted at 76.5–77.5°.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 68.90; H, 7.78.

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Isolation, Configuration, and Synthesis of Natural *cis*- and *trans*-3-Hydroxyprolines

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3-Hydroxy-L-proline has been isolated from Mediterranean sponge and identified in hydrolysates of collagen of various sources. In addition, a diastereoisomeric 3-hydroxy-L-proline has been found in the antibiotic telomycin. Hydroboration and oxidation of the boron complex of N-carbobenzyloxy-3,4-dehydro-DL-proline methyl ester gave, after saponification and hydrogenolysis, about 70% uniform 3-hydroxy-DL-proline besides 10% *trans*-4-hydroxy-DL-proline. From the absence of 4-allohydroxy-DL-proline and the homogeneity of the synthetic 3-hydroxy-DL-proline, it is concluded that the hydroboration is stereospecific and *trans* with respect to the carboxyl function. The N-tosylmethyl esters of the synthetic *cis*- and *trans*-3-hydroxy-DL-proline and of the natural amino acids from sponge and telomycin, respectively, had identical infrared spectra. *cis*-3-Hydroxy-DL-proline was prepared *via* the 3-ketoproline derivative IV by reduction with sodium borohydride and was found to be identical with 3-hydroxy-L-proline from telomycin with regard to column, paper, and gas chromatographic analysis. This procedure was also used for the preparation of selectively tritiated *cis*-3-hydroxy-DL-proline-3-H^3. Enzymatic studies with D-amino acid oxidase and n.m.r. data confirmed these assignments.

Previous preliminary reports[3,4] from this Laboratory have described the isolation, characterization, and synthesis of the two diastereoisomers of 3-hydroxyproline. The *trans* isomer was isolated from dried Mediterranean sponge and from the antibiotic telomycin. The *cis* isomer was obtained only from telomycin. Independent reports[5,6] from other laboratories have also described the isolation and synthesis of 3-hydroxyprolines. No assignments of configuration have been made.

This paper gives detailed account of the work previously reported and presents additional information on new synthetic approaches.

The key step in the isolation from acid hydrolysates of sponge was the separation of the cyclic amino acids from the primary amino acids by either nitrosation and hydrolysis[7,8] or by treatment of the hydrolysates with 2,4,6-trinitrobenzenesulfonic acid (TNBS)[9] and ion-exchange chromatography[10] on IR-120 resin of the fraction containing the cyclic amino acids. The *trans*- and *cis*-3-hydroxyprolines were separated quantitatively by this method and were easily further purified by recrystallization from aqueous ethanol.

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