residues were crystd from isopropyl alcohol or hexane to yield the *p*-nitrobenzoates, D-1a–f, listed in Table IV.

Preparation of *p*-Aminobenzoates D-2a–f. Solns containing 0.05 mole of the *p*-nitrobenzoates, D-1a–f, in 100 ml of EtOH were hydrogenated over 0.2 g of platinum oxide catalyst at room temperature at 15 psi for 30 min. After filtration of the catalyst, the solvent was removed and the residue recrystd from PhH–hexane or CCl_4 to yield the *p*-aminobenzoates listed in Table IV in over 95% yield.

Biological Results. The biological activities of the compounds prepared in this study are summarized in Table V. Cholinergic agonist, cholinergic antagonist, and antihistamine activities were determined using the cumulative dose–response technique of van Rossum.[12]

Compounds A-7a–f were tested for cholinergic agonist activity on rat jejunum using acetylcholine as the reference agonist.

Compounds B-2a–f were tested for postganglionic cholinergic blocking activity on rat jejunum using furtrethonium as the reference agonist and atropine as the reference antagonist.

Compounds C-1a–f were tested for antihistamine activity on guinea pig ileum using histamine as the reference agonist and papaverine as the reference antagonist.

Compounds D-2a–f were tested for corneal anesthetic activity in rabbits. Each compound was tested at 3 different concentrations, both eyes of a single rabbit being used for each concentration. *The concentration which produced anesthesia of 5-min duration was obtained from a dose–response curve of anesthesia against log concentration.* This concentration is the "threshold anesthetic concentration" or TAC_5.[13] The cocaine anesthetic ratio is the TAC_5 of cocaine divided by the TAC_5 of the compound being tested.

While many of the compounds tested (particularly the antagonists) exhibited activities comparable to the reference drugs, we were unable to detect progressive changes in activity which could be related to the nature of the *gem*-dialkyl substituents. Apparently, small differences in the distance between the oxygen and nitrogen functions attributable to the *gem*-dialkyl effect are not important in the systems under scrutiny.

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References

(1) M. S. Newman and R. J. Harper, Jr., *J. Amer. Chem. Soc.*, 80, 6350 (1958).
(2) S. W. Smith and M. S. Newman, *ibid.*, 90, 1249 (1968).
(3) S. W. Smith and M. S. Newman, *ibid.*, 90, 1253 (1960).
(4) (a) M. Martin-Smith, G. A. Smail, and J. B. Stenlake, *J. Pharm. Pharmacol.*, 19, 561 (1967); (b) R. B. Barlow, "Introduction to Chemical Pharmacology," 2nd ed, Wiley, New York, N. Y., 1964.
(5) A. D. Mitchell and J. F. Thorpe, *J. Chem. Soc.*, 97, 997 (1910).
(6) W. J. Bailey and J. J. Daly, Jr., *J. Amer. Chem. Soc.*, 81, 5397 (1959).
(7) H. Najer, R. Giudicelli, J. Sette, and J. Menin, *Bull. Soc. Chim. Fr.*, 204 (1965).
(8) C. Mannich, B. Lesser, and F. Silten, *Ber.*, 65, 378 (1932).
(9) F. Nerdel, D. Frank, and H. J. Lengert, *ibid.*, 98, 728 (1965).
(10) V. J. Traynelis and J. G. Dadura, *J. Org. Chem.*, 26, 686 (1961).
(11) M. L. Moore, *Org. React.*, 5, 323 (1949).
(12) J. D. Van Rossum, *Arch. Int. Pharmacodyn.*, 143, 299 (1963).
(13) A. H. Afifi, Ph.D. Thesis, The Ohio State University, Columbus, Ohio, 1961.
(14) A. Blankart, *Festschr. Emil Barell, 1936*, 284 (1936).

Utilization of Operational Schemes for Analog Synthesis in Drug Design†

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Some proposals are presented for the stepwise selection for synthesis of new analogs of an active lead compound which are designed to maximize the chances of synthesizing the most potent compounds in the series as early as possible. The schemes are based on a fundamental assumption of the Hansch method that a particular substituent may modify activity relative to the parent compound by virtue of resulting changes in hydrophobic, electronic, and steric effects. Some examples of how this approach might have fared if it had been applied to a number of existing series have been analyzed.

A very common problem in drug design is to find the optimum substitution on a benzene ring or on the benzenoid portion of a fused ring system in an active lead compound for maximization of drug potency. Since there are many possible substituents and several different ring positions, the number of possible compounds to consider containing up to say two substituents is very large. Thus, it would be highly advantageous to determine at an early stage which of these compounds might really be worth synthesizing.

Historically, approaches to this problem have been rather haphazard, depending for the most part on the particular experience and intuition of the medicinal chemist involved and the relative availability of the starting materials required for synthesis. With the advent[1] and subsequent development[2] of the Hansch method for structure-activity correlations a more rational approach to this problem became possible. Thus, a limited group of substituents which will give good discrimination between π, σ, and E_s can be selected[3] and an initial group of 6–12 compounds synthesized. After performing a regression analysis and assuming a worthwhile correlation is obtained, it should be possible to determine which parameters are influencing activity and to what relative degree. Knowing this, and having available a comprehensive list of possible substituents and their respective parameters, those compounds can be selected for synthesis with the highest indicated potency values commensurate with synthetic accessibility.

Since the regression analysis has been carried out with a minimum number of observations, the reliability of the correlation will not be high, but nevertheless the analysis will identify those compounds with the highest probability of enhanced potency based on the available data. When data on the second group of compounds become available, they can be combined with the first group so that the correlations can be continuously refined as new data become available.

This procedure is suitable when the compounds are relatively easy to synthesize and a considerable time lag is encountered in obtaining activity data. However, it is less satisfactory under circumstances where synthesis is more difficult and test results are more readily forthcoming. In the latter case it would be desirable to proceed with every compound synthesized in the most probable direction toward greater potency. This maximizes the chances of

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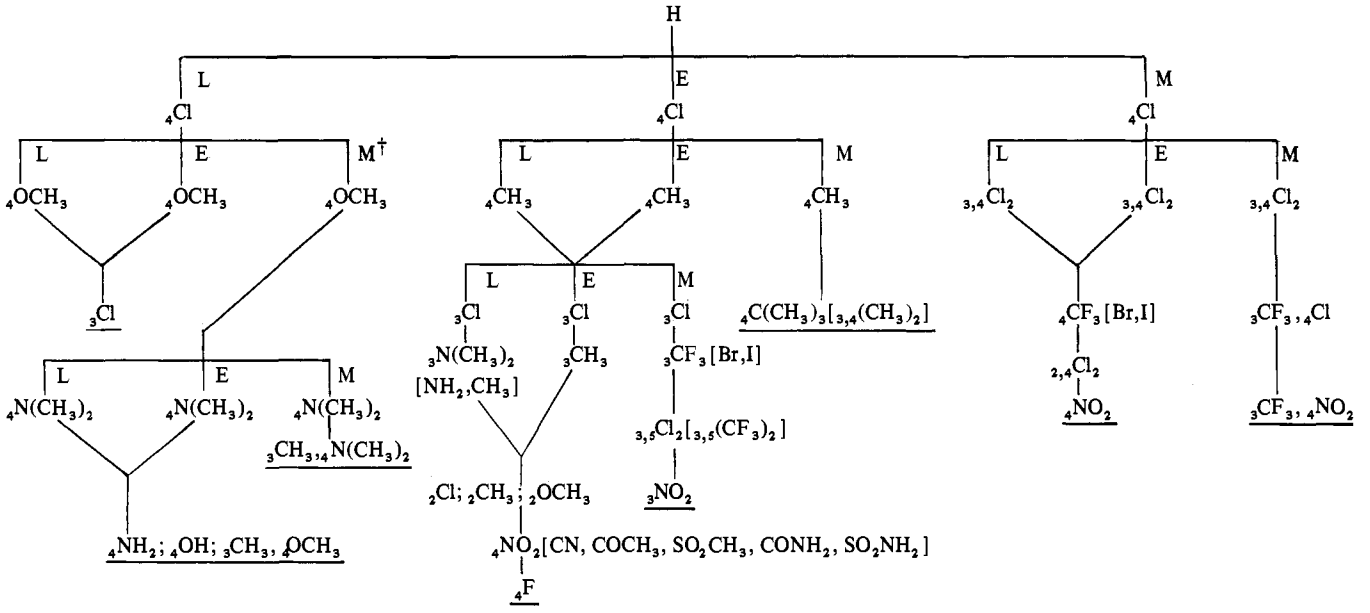
Scheme I. Operational Scheme. Aromatic Substitution



M = More active, E = equiactive, L = less active. Descending lines indicate sequence. Square brackets indicate alternates. †Compared to 4-H compound.

finding the most potent compounds as early as possible.

Another problem in the utilization of the standard Hansch method is the reluctance on the part of some medicinal chemists to become involved with mathematics, statistical procedures, and computers. For these individuals a nonmathematical utilization of the Hansch approach might be of considerable interest.

In the context of the foregoing discussion the following operational scheme for aromatic substitution may be considered (Scheme I). The π, σ (σ *), and E_S values of the substituents mentioned are listed in Table I.‡ The assumption is made that the starting compound is the unsubstituted phenyl compound and that its biological activity has been measured. Since many systems are $+\pi$ dependent, *i.e.*, activity increases with increasing π values, the *p*-chloro analog is a good first choice, particularly since the ease of synthesis, relative to other substituted phenyl compounds, is generally favorable.

For the purposes of this analysis the potency of the 4-chloro compound can be classified as greater than, equal to, or less than the activity of the parent compound. If the potency is increased, this can be attributed most probably to a $+\pi$ effect, a $+\sigma$ effect (activity increases with increasing σ values), or to a combination of $+\pi$ and $+\sigma$. In this event the 3,4-dichloro compound would be selected for synthesis next since this would result in both larger $+\pi$ and $+\sigma$ values when summed for the two substituents. Again this particular substituent combination should be highly favorable from a synthetic standpoint. Moving to the stage where the compound has been synthesized and tested, the compound can be classified as more potent, equipotent, or less potent than the 4-chloro analog. If, as seemed most probable, potency did increase, then the 3-CF_3,4-Cl analog would be the next choice for synthesis since again both $\Sigma\pi$ and $\Sigma\sigma$ would be larger. Assuming again a favorable outcome it would be desirable at this stage to proceed to the 3-CF_3,4-NO_2 compound since if activity was principally $+\sigma$ controlled a further substantial enhancement of potency should result.

If, however, the potency of the 3,4-dichloro compound turned out to be about the same as that of the 4-chloro compound, this might be ascribed to either an unfavorable steric effect of meta substitution or to exceeding the optimum lipophilic value of the substituents. In either event, the 4-CF_3 analog would then be a good candidate since there is no meta substitution and it would be less lipophilic than the 3,4-dichloro compound but more lipophilic than the 4-Cl compound.

Essentially the same arguments can be applied when the 3,4-dichloro analog is less potent than the 4-chloro analog. The 4-bromo and 4-iodo compounds represent alternates to the 4-CF_3 compound. The 2,4-Cl_2 analog might prove interesting particularly if a meta steric effect adversely affected the activity of the 3,4-dichloro analog. The 2,4 isomer should also be slightly less lipophilic than the 3,4 isomer. At the next stage in the sequence the 4-NO_2 should be checked since this could enhance potency if there is an important $+\sigma$ dependency and an optimum π value less than that for the CF_3 group.

Returning now to the first analog synthesized, the 4-chloro compound, in the event this was found to be about equipotent with the parent compound it could be reasoned that this most probably results from a favorable $+\pi$ effect counterbalanced by an unfavorable $-\sigma$ dependency. If this is correct, then the 4-CH_3 analog should show enhanced potency since this is a $+\pi-\sigma$ type substituent. Assuming a favorable result the next selection would be the 4-$C(CH_3)_3$ compound with increased $+\pi$ and $-\sigma$ values. An alternate here would be the 3,4-$(CH_3)_2$ analog which might prove advantageous if the high steric requirement of the $C(CH_3)_3$ proved to be a significant negative factor.

In the situation where the 4-CH_3 compound is equi- or less potent than the 4-Cl compound it would seem reasonable to assume either an unfavorable steric effect from para substitution or a $-\pi$ effect. Since $-\pi$ effects, *i.e.*, decreasing

‡The σ values relate to an electronic effect at the point of attachment of the phenyl group to some other moiety. It is possible that in some situations the electronic effect of a substituent may be important with respect to another position on the phenyl group. However, for the most part the arguments presented will not be materially affected.

activity with increasing π values are uncommon, the steric explanation seems more probable, hence the next move to 3-Cl in the operational scheme.

Assuming that activity increases with this substituent then a sequence is followed, essentially analogous in choice of substituents and reasoning with that commencing with the 4-CF_3 compound (already discussed) and located on the right-hand side of the scheme. On the other hand, if there is no change in activity with the 3-Cl substituent this could be ascribed to a $+\pi-\sigma$ effect which points to 3-methyl as the next substituent choice. If there is still no potency enhancement the next step would be to examine 2 substituents, chloro, methyl, and methoxy.

Lack of success in this direction now would prompt the synthesis of the 4-NO_2 analog on the premise that a $+\sigma$ effect is operating but that something less than the π value for Cl is optimal. It will be noted that this is a basically opposite premise from the $+\pi-\sigma$ concept with which the analysis of this central part of the chart commenced. The relative orders of these in the sequence represents a judgment of relative probabilities. Alternatives to 4-NO_2 are 4-CN, $COCH_3$, CH_3SO_2, $CONH_2$, and SO_2NH_2. Assuming that this direction proves favorable there is a good chance that one of these substituents will provide the optimal $+\sigma-\pi$ balance.

The remaining choice in the sequence for this branch of the scheme is the 4-F analog which provides the minimal change in π and σ effects compared to the unsubstituted compound. This should prove advantageous in the event that the latter is essentially optimal in terms of π and σ but is subject to rather rapid metabolic transformation by 4-hydroxylation.

Returning at this point to the 3-Cl analog, if the potency of this is less than the 4-CH_3 compound, which would be consistent with a dominant $-\sigma$ effect, the next substituent choice would be 3-$N(CH_3)_2$ (with 3-NH_2 and 3-CH_3 as alternatives). Lack of success in this direction would then lead to an examination of 2-substituents as shown in Scheme I.

The remaining segment of the scheme is concerned with the synthetic sequence to be followed in the case where the 4-chloro analog was found to be significantly less potent than the parent compound. One may conclude that either there is an unfavorable effect from any kind of para substitution for steric reasons, or activity is $-\sigma$ or $-\pi$ controlled. Assuming that the $-\sigma$ effect is the most probable explanation the 4-OCH_3 analog ought to be a favorable selection for synthesis. Confirmation of this in the form of increased activity for the 4-OCH_3 compound would lead to the next choice of the 4-$N(CH_3)_2$ analog where there would be an even greater $-\sigma$ effect. A further trend in the right direction would prompt the synthesis of the 3-CH_3,4-$N(CH_3)_2$ compound where the $-\sigma$ effect would be further reinforced.

No improvement or a drop in activity for the $N(CH_3)_2$ analog would perhaps signal some $-\pi$ effect which would suggest synthesis of the 4-NH_2 and 4-OH compounds. The synthesis of the 3-CH_3,4-OCH_3 analog would be desirable at this stage in the event that the basic character of the 4-$N(CH_3)_2$ function proved to be a negative factor either through ionization or a change in receptor site interaction.

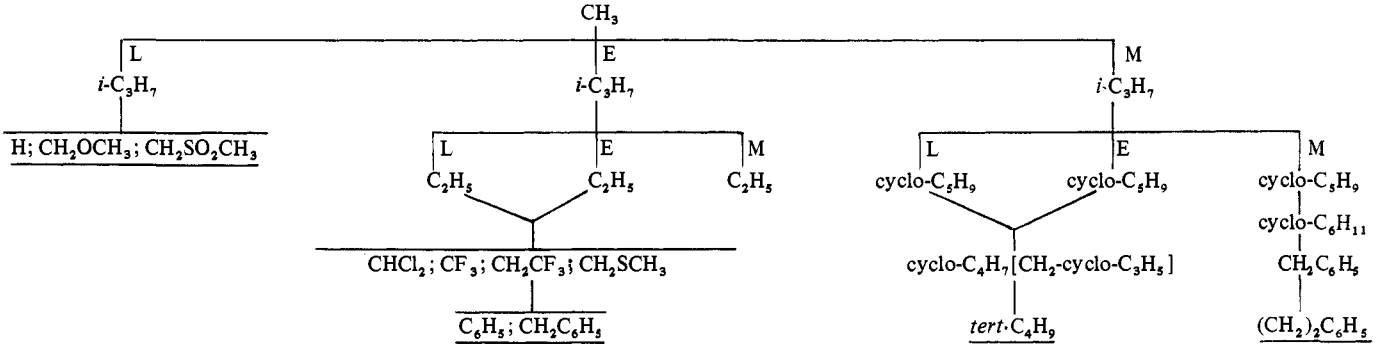
Returning to the 4-OCH_3 compound, if this has the same or less activity than the 4-Cl analog, this would indicate unfavorable prospects for para substitution in general and would suggest the synthesis of the 3-Cl compound as the next step. Subsequently, the sequence would proceed as for the 3-Cl compound in the center branch of the chart.

A similar operational scheme may be drawn up for side-chain problems (Scheme II). This type of situation arises when groups adjacent to a carbonyl, amino, or amide function, for example, are varied. Such situations may be represented by $-\overset{O}{\overset{\|}{C}}R$, $-NHR$, $-\overset{O}{\overset{\|}{C}}NHR$, $-NH\overset{O}{\overset{\|}{C}}R$ where R is the variable substituent. Many other types may, of course, be described. By and large the cases covered are all those other than direct substitution on an aromatic nucleus.

Starting with methyl (Scheme II) as the base compound, the isopropyl substituent would be a good first choice on the premise that a $+\pi$ effect is most probable. Assuming an increase of activity is obtained, cyclopentyl would be the next selection on account of its larger π value with minimal change in E_s, the steric factor (Table I). Specific regional hydrophobic bonding is often a positive factor in drug activity while steric requirements if exceeded may prove to be a negative factor. Cycloalkyl groups have the advantage of maximizing the possibility of hydrophobic bonding while minimizing unfavorable steric influences. If enhanced potency is noted with the cyclopentyl compound, the cyclohexyl, benzyl, and phenethyl analogs, in sequence, would be prime candidates for synthesis with their progressively larger π values and moderate E_s values.

The failure of cyclopentyl to show a potency increase would indicate that the optimum π value had been exceeded thus suggesting cyclobutyl which has the advantage of a very low E_s value in addition to having about the right π value. A suitable alternative choice would be cyclopropylmethyl. A second possibility is that activity is increasing with increasing $-\sigma^*$ values and π is not as important, in which case *tert*-butyl should be a favorable substituent.

Scheme II. Operational Scheme. Side Chain



M = More active, E = equiactive, L = less active. Descending lines indicate sequence. Square brackets indicate alternates.

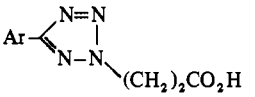
Table I. Substituent Constant Values

Substituent	Aromatic π^a	Aromatic σ^b	$E_s{}^c$
H	0.00	0.00	1.28
4-Cl	0.70	0.23	0.27
3-Cl	0.76	0.37	0.27
3-CF_3	1.21	0.43	−0.98
4-NO_2	0.24	0.78	−1.28
4-CF_3	1.07	0.54	−0.98
4-Br	1.19	0.23	0.08
4-I	1.43	0.28	−0.16
2-Cl	0.76	0.23	0.27
4-CH_3	0.60	−0.17	−0.14
4-$C(CH_3)_3$	1.68	−0.20	−1.65
3-CH_3	0.54	−0.07	−0.14
3-Br	0.94	0.39	0.08
3-I	1.15	0.35	−0.16
3-NO_2	0.11	0.71	−1.28
2-CH_3	0.68	−0.17	−0.14
2-OCH_3	−0.33	−0.27	0.69
4-CN	−0.32	0.66	
4-$COCH_3$	−0.37	0.38	
4-SO_2CH_3	−1.26	0.72	
4-$CONH_2$	−1.49	0.40	
4-SO_2NH_2	−1.82	0.57	
4-F	0.15	0.06	0.78
3-$N(CH_3)_2$	0.18	−0.21	
3-NH_2	−1.23	−0.16	0.63
4-OCH_3	−0.04	−0.27	0.69
4-NH_2	−1.23	−0.66	0.63
4-OH	−0.61	−0.37	0.69

Substituent	Side chain π^a	Side chain σ^{*d}	$E_s{}^d$
CH_3	0.50	0.00	0.00
i-C_3H_7	1.30	−0.19	−0.47
cyclo-C_5H_9	2.14	−0.20	−0.51
cyclo-C_6H_{11}	2.51	−0.15	−0.79
$CH_2C_6H_5$	2.63	0.22	−0.38
$(CH_2)_2C_6H_5$	3.13	0.08	−0.38
cyclo-C_4H_7	1.80[e]	−0.20[e]	−0.06
CH_2-cyclo-C_3H_5	1.80[e]	−0.13[e]	
tert-C_4H_9	1.98[f]	−0.30	−1.54
C_2H_5	1.00	−0.10	−0.07
$CHCl_2$	1.15[e]	1.92	−1.54
CF_3	1.07[e]	2.76[e]	−1.16
CH_2CF_3	1.57[e]	0.92	
CH_2SCH_3	0.77[e]	0.44[e]	−0.34
C_6H_5	2.13	0.60	
H	0.00	0.49	1.24
CH_2OCH_3	0.02[e]	0.64	−0.19
$CH_2SO_2CH_3$	−0.76[e]	1.32	

[a]Ref 8. [b]Ref 9. [c]Ref 10. [d]Ref 11. [e]Estimated. [f]Ref 12.

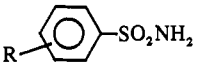
Table II. Antiinflammatory Activity of Some 5-Aryltetrazolylpropionic Acids



No.[a]	No.[b]	Ar	AI[c]
1	5	C_6H_5	8.2
2	8	4-ClC_6H_4	5.9
3	23	4-$CH_3OC_6H_4$	4.9
4	9	3-ClC_6H_4	11.2
5	10	3-$CF_3C_6H_4$	7.9
6	14	3-BrC_6H_4	11.2
7	16	3-IC_6H_4	8.4
8	12	3,5-$Cl_2C_6H_3$	11.1

[a]Order of compound synthesis according to operational scheme (Scheme I). [b]Compound no. as given in tabulation by Buckler, *et al.*[4] [c]Activity index as defined by Buckler, *et al.*[4]

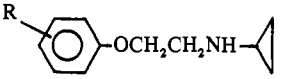
Table III. Natriuretic Activity of Some Substituted Benzenesulfonamides



No.[a]	No.[b]	R	Log $1/C^c$
1	6	H	0.155
2	7	4-Cl	0.301
3	14	3,4-Cl_2	0.267
4	8	4-Br	0.267
5	13	4-NO_2	0.845
6	11	4-CN	1.020

[a]Order of compound synthesis according to operational scheme (Scheme I). [b]Compound number as given in tabulation by Kakeya, *et al.*[5] [c]Activity value as reported by Kakeya, *et al.*[5]

Table IV. Inhibition of Monoamine Oxidase by *N*-(Phenoxyethyl)cyclopropylamines



No.[a]	R	Log $1/C^b$
1	H	5.93
2	4-Br	6.64
3	3,4-Cl_2	6.30
4	4-CF_3	6.99[c]
5	4-N=N-C_6H_5	7.56
6	4-SO_2CF_3	7.56[c]

[a]Order of compound synthesis according to operational scheme (Scheme I). [b]Activity value as reported by Fuller, *et al.*[6] [c]Calculated from equation based on Table II as reported in ref 6.

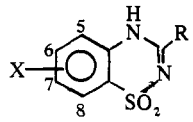
Returning now to further consideration of the isopropyl substituent, if there is no potency increase over methyl, it is likely that the π value for maximum activity corresponds to ethyl so that synthesis of the ethyl analog is prompted. If potency decreases or stays the same with ethyl, it could result from an adverse electronic effect which suggests examination of the dichloromethyl, trifluoromethyl, trifluoroethyl, and methylthiomethyl substituents which all have $+\sigma^*$ values. As a subsequent step it would be desirable to check the phenyl and benzyl substituents which have higher π values and electronic effects in the desired direction of electron withdrawal relative to ethyl.

A loss of potency with the isopropyl relative to the methyl substituent would suggest that either the π or σ^* or perhaps both of these values were trending in the wrong direction. In this event the hydrogen, methoxymethyl, and methylsulfonylmethyl analogs would be favored selections for further synthetic effort.

It is interesting to examine how these operational schemes might have worked out in practice. The first example is based on the work of Buckler, *et al.*,[4] concerning the antiinflammatory activity of a series of substituted aryltetrazolylalkanoic acids. Of some 28 compounds synthesized which differed only in the type of substitution of the 5-aryl function, 8 are listed in Table II in the order indicated for synthesis by following the operational scheme given in Scheme I. The fourth, sixth, and eighth compounds in the sequence constitute three of the four most active of the 28 compounds.

Another example comes from the publication of Kakeya, *et al.*,[5] on the natriuretic activity of some substituted benzenesulfonamides (Table III). Of the 19 compounds which were actually synthesized in this series the two most active would be the fifth and sixth to be synthesized following the sequence indicated by Scheme I. By use of regres-

Table V. Effect of Substituted Benzothiadiazines on Vascular Reactivity of Rat Aorta



No.[a]	X	R	Activity[b]
1	H	CH_3	0.41
2	7-Cl	CH_3	1.79
3	6,7-Cl_2	CH_3	2.64
4	6-CF_3,7-Cl	CH_3	2.80
5	6-CF_3,7-Cl	$CH(CH_3)_2$	3.96[c]
6	6-CF_3,7-Cl	cyclo-C_5H_9	4.07[c]

[a]Order of compound synthesis according to operational schemes (Schemes I and II). [b]Activity value as reported by Topliss and Yudis.[7] [c]Topliss and Wohl, unpublished data.

sion analysis on 16 of the 19 compounds (3 ortho compounds were omitted) Kakeya, *et al.*, obtained an excellent correlation between activity and a combination of $-\pi$, $-\pi^2$, and $+\sigma$ terms. The $+\sigma$ term was dominant; the optimum π value was -0.303.

A further illustration of the potential operation of the scheme is provided by an examination of the activities of 16 *N*-(1-phenoxyethyl)cyclopropylamines as monoamine oxidase inhibitors reported by Fuller, *et al.*[6] (Table IV). These authors obtained a good correlation between activity and $+\sigma$, $+\pi$, and γ terms, where γ is a steric parameter. On the basis of this equation the activity of a more active compound, the phenyldiazo analog (Table IV, **5**) was well predicted. Using the operational scheme (Scheme I) and commencing with the unsubstituted phenyl compound which has an activity of 5.93, the first target for synthesis is the 4-bromo compound (Fuller did not report a 4-Cl compound) which results in a substantial increase in activity (6.64). According to the scheme the 3,4-Cl_2 compound would be synthesized next which Fuller reports as having an activity value of 6.30 which is lower than the 4-bromo compound. This result may be attributed to an adverse steric effect from meta substitution which then prompts the synthesis of the 4-CF_3 compound. This was not actually synthesized by Fuller but from his equation its activity may be calculated as 6.99 which represents an improvement in activity over the 4-bromo analog. The indicated direction, then, is for other substituents in the 4 position with high $+\pi$ $+\sigma$ values. One such compound is the 4-phenyldiazo which was found to be the most active compound in the series. Another substituent with high $+\pi+\sigma$ values is trifluoromethylsulfonyl. This compound was not actually synthesized but has a computed activity value of 7.56 based on Fuller's equation, the same as the observed value for the phenyldiazo compound. Thus, using the scheme described, this simulation shows that one would have very quickly found the most active compounds in the series. It may be observed that Fuller, *et al.*, used 16 compounds as the basis for their calculations and subsequent predictions.

An illustration of the application of both operational schemes, for aromatic substitution and side chain, is provided by analyzing some extensive studies reported[7] for the effects of substituted benzothiadiazines on the vascular reactivity of rat aorta (Table V). Starting with the parent compound **1** and proceeding with benzenoid ring substitution first, the 6-CF_3,7-Cl substituent pattern is arrived at as the fourth compound in the sequence. Holding this constant and now varying the side-chain group R according to Scheme II yields sequentially compounds **5** and **6** with R substituents isopropyl and cyclopentyl, respectively. It can be seen that in this manner compounds very close to maximum potency in a very large series have been arrived at in very short order. In actual experience when this work was done some years ago, it was only after making many compounds that these extremely potent analogs were found.

Although there are a large number of compound series with attendant biological data recorded in the literature few of these contain a sufficient number of the analogs required by the operational schemes to achieve complete or nearly complete simulations of the technique. However, in addition to the examples cited above a number of other series allowed partial simulations of the application of the operational schemes. In most cases the results were favorable in that they appeared to chart an efficient course to the more potent analogs in the series. It is realized that retrospective testing of this kind has inherent limitations in assessing the utility of the method. Nevertheless, this test must be passed before application in a prospective manner is attempted.

Many possible substituents have not been included in the operational schemes. These schemes as drawn are not unique and it is possible to devise others, probably better ones. Tailor-made schemes may also be constructed for special situations. Also, some allowance will have to be made in any scheme used, for the degree of lipophilicity of the parent compound. The main intent has been to illustrate the principles involved in this type of approach and this is facilitated by avoiding a high degree of complexity. When the most favorable type of substituent has been identified by working through operational schemes such as those discussed, a detailed examination of similar substituents, including more unusual and difficultly accessible ones, would be a logical procedure. It should also be pointed out that when data on enough compounds have been obtained the option to perform a multiple regression analysis as in the standard Hansch method is open.

Since the approach is based on the fundamental assumption of the Hansch method that a particular substituent may modify activity relative to the parent compound by virtue of resulting changes in hydrophobic, electronic, and steric effects it would not be advantageous if some other unrecognized effects were determining with respect to activity. However, in cases such as these no disadvantage should be incurred relative to any other way of proceeding; particularly since the more accessible substituents have been emphasized. If the objective is to obtain the best compound more efficiently, the judgment of whether to synthesize a particular analog or a different one should be made on the basis of the twin factors of probability of improved activity and the resources required for the synthesis. In the current proposals probabilities have been estimated based on experience with the Hansch method, and analog synthesis has been concentrated on the simplest analogs commensurate with estimated probability levels.

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References

(1) C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, 86, 1616 (1964).
(2) C. Hansch, "Drug Design," Vol I, E. J. Ariens, Ed., Academic Press, New York, N. Y., 1971, p 271.
(3) P. N. Craig, *J. Med. Chem.*, 14, 680 (1971).

(4) R. T. Buckler, S. Hayao, O. J. Lorenzetti, L. F. Sancilio, H. E. Hartzler, and W. G. Strycker, *ibid.*, 13, 725 (1970).
(5) N. Kakeya, N. Yata, A. Kamada, and M. Aoki, *Chem. Pharm. Bull.*, 18, 191 (1970).
(6) R. W. Fuller, M. M. Marsh, and J. Mills, *J. Med. Chem.*, 11, 397 (1968).
(7) J. G. Topliss and M. D. Yudis, *ibid.*, 15, 394 (1972).
(8) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, 86, 5175 (1964); J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, 8, 150 (1965). E. Miller and C. Hansch, *J. Pharm. Sci.*, 56, 92 (1967). A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 551 (1971).
(9) J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1962, p 87; H. H. Jaffé, *Chem. Rev.*, 53, 191 (1953).
(10) E. Kutter and C. Hansch, *J. Med. Chem.*, 12, 647 (1969); *Arch. Biochem. Biophys.*, 135, 126 (1969).
(11) R. W. Taft, Jr., "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, Chapter 13.
(12) C. Hansch and E. Coats, *J. Pharm. Sci.*, 59, 731 (1970).

Relationship of Structures and Microbiological Activities of the 16-Membered Macrolides

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The 16-membered macrolide antibiotics were divided into three types depending on the number and position of carbonyl groups in the aglycone. The role of the carbonyl groups in the microbial activity of the macrolides was presented.

During the past 15 years, the structures of a number of macrolide antibiotics have been elucidated. Of particular interest to us is the group of macrolides containing the 16-membered lactone ring which include such compounds as leucomycins,[1-4] YL-704 antibiotics,[5,6] espinomycin,[7] SF-837 antibiotics,[8,9] spiramycins,[10] niddamycin,[11,12] magnamycins,[10,13,14] cirramycin,[15] B-58941,[16] tylosin,[17] chalcomycin,[18] and neutramycin.[19] While these antibiotics have many similar structural features, there are some marked structural differences which appear, however, to be of little microbiological consequence. All these antibiotics have about the same antibacterial spectra exhibiting pronounced activity against Gram-positive bacteria, Gram-negative cocci, and some species of mycoplasma. It is for this reason that we felt it appropriate and timely to ascertain which functional groups are essential for microbiological activity and which are not.

The 16-membered macrolide antibiotics may be divided into three classes depending on the number and position of carbonyl groups in the molecule. The first class represented by leucomycins, spiramycins, etc. (Figure 1) possesses an aldehyde group attached to the six position of the ring structure through a methylene group. The second class includes the magnamycins, cirramycin, tylosin, etc. (Figure 2) and is similarly characterized by the aldehyde group attached to carbon 6 through a methylene group but also by a carbonyl group in position 9. The antibiotics of the third class, illustrated by chalcomycin and neutramycin, have only one carbonyl group, the keto function at position 9 (Figure 3).

In previous publications it was shown that the aldehyde group in leucomycins[20] and spiramycins[21] is important for antibiotic activity. In the case of chalcomycin, it had been assumed that the carbonyl group at position 9 may be important for microbial activity but no data were presented. The present report offers new data which demonstrate further the importance of the carbonyl groups in the microbial activity of the 16-membered macrolides and also summarizes on a broader basis the relationship of microbial activities of these antibiotics with chemical structures.

Results and Discussion

The importance of the 9-keto group in chalcomycin is evident from a comparison of the microbial activity of chalcomycin with its hexahydro derivative **2** and its octahydro derivative **3** (Scheme I), both derivatives having been prepared from the parent by catalytic hydrogenation.[18] As seen in Table I, in which is listed our microbial data of compounds discussed in this report, compound **2**, which still possesses the keto group, showed activity close to that of its parent whereas the octahydro compound **3**, with no keto group, possesses negligible microbial activity. The high activity of the hexahydro compound **2** also demonstrates the unimportance of the conjugated double bonds between carbons 10 and 13 for microbiological activity. The same conclusion was reached previously for leucomycin A_3.[20]

The 9-keto group does not appear to be important in those antibiotics of the magnamycin type so long as the aldehyde group remains intact. While leucomycin A_3 (**4**) and its 9-dehydro derivative, magnamycin B (**5**), prepared from **4** by oxidation with MnO_2 (Scheme II), showed about the same activity, 18-dihydroleucomycin (**6**), prepared from **4** by reduction with $NaBH_4$, possesses very little microbial activity. The contribution of the 9-keto group was noted further from the microbial activity of 9-dehydro-18-dihydroleucomycin (**7**) prepared from **6** by allylic oxidation. Compound **7** with its 9-keto group but without the aldehyde group shows a low order of microbial activity, although greater than its parent **6**.

A similar structure-activity relationship obtains with isoleucomycin A_3 (**8**), an isomer of **4** prepared by rearrangement[2] of the 9-hydroxyl group to the C-13 position. While **8** has almost the same activity as **4**, its 18-dihydro derivative (**9**) has very little or no activity. Similarly, 13-dehydro-18-dihydroisoleucomycin A_3 (**10**), prepared from **9** by oxidation with MnO_2, shows, in contrast to **9**, small but definite activity.

Tylosin (**11**) which like magnamycins has carbonyl functions at C_9 and C_{20} also loses its antimicrobial activity by reduction of these groups with $NaBH_4$ to the tetrahydro