Prediction of the *in Vitro* Activity of Sulfonamides Synthesized from Simple Amines by Use of Electronic Data Obtained from the Simple Amines

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Received October 5, 1970

Nmr spectroscopy has been used to obtain the chemical shift (in ppm) of amino group protons of simple amines (anilines and pyridines) which are precursor substances in sulfonamide (SA) synthesis. These chemical shift values have been found to be related to the *in vitro* activity of the corresponding SAs. A correlation coefficient between amine chemical shift in $CDCl_3$ and sulfonamide activity of 0.992 (n = 19) is obtained, when meta- and para-substituted amines are used. When DMSO is used as solvent for nmr measurements, intramolecular hydrogen bonding decreases and the inhibitory activities of some ortho-substituted sulfonamides can also be correlated (r = 0.976, n = 25). Disubstituted anilines can be included without changing the correlation (r = 0.96, n = 32). The chemical shift values obtained are correlated to the Hammett σ values of the substituents of the amine component. A comparison of the correlation of the electronic data of the amines to sulfonamide activities determined by either an MIC method and/or by a bacterial growth kinetics method is given.

Previous reports from this laboratory[1-3] have demonstrated correlations between physicochemical parameters of precursor amines used in SA synthesis, and *in vitro* antibacterial activity of the corresponding sulfonamides. Correlations have been shown to exist between the minimum inhibitory concentration (MIC) of a sulfanilamide drug and the following physicochemical parameters of the corresponding anilines: b values of the amino group obtained from ir measurements, Hammett σ values,[1,2] and some nmr chemical shift values of amino group protons.[3]

The success of our previous attempts indicates that the SAs offer an excellent model system for the application of physicochemical approaches to precursor substances (prior to the actual synthesis of a complete pharmacologic entity) in a rational approach to the design of new chemotherapeutic agents. In this paper we wish to report: (1) the application of our approach to several additional SA compounds and their precursor anilines or aminopyridines; (2) bacterial inhibition data for two bacterial strains (Gram-negative and Gram-positive); (3) the regression generated by this data; (4) a comparison of activities obtained in a dilution test (MIC), with changes in kinetically determined bacterial growth rate constants.[4]

Experimental Section

Synthesis.—Sulfanilamides were prepared according to previously published methods.[5,6]

Nmr Measurements.—The majority of the anilines and aminopyridines used in this investigation were available from commercial sources and were purified, if necessary, by recrystn in the usual manner. Others were synthesized by methods described in the literature.[5,6] The nmr spectra of these compounds were in accordance with their reported structures.

All spectra were detd on a Varian HA-100 spectrometer; solvents used were $CDCl_3$ and DMSO(UVASOL grade, Merck, Darmstadt). Amino group proton signals were reproducible to 0.2 Hz (temp, 23°), and showed no dependence on amine concn in the concn range used (50–100 μmoles/l.). The chemical shift values of amino group protons are expressed in parts per million downfield (TMS) and are listed in Tables I and II.

Acid Dissociation Constants (pK_{a_2}).—The pK_{a_2} values of the N^1 atom were detd spectrophotometrically according to the method outlined by Albert[7] and Yoshioka,[8] and are listed in Tables I and II.

Minimum Inhibitory Concentrations.—The methods for MIC determination have been previously described.[1,9] The MIC values are included in Tables I and II.

Bacterial Growth Kinetic Experiments. Culture Medium.—The culture medium used was that described by Anton[10] and Garrett[11] and was sterilized by sterile filtration.

Growth Determination.—Log growth phase *Escherichia coli* cultures were obtained by a subculturing procedure similar to that described by Garrett.[11]

Results and Discussion

Structure-Activity Relationships of Substituted Phenylsulfanilamides.—A linear relationship between MIC and the chemical shift (ppm) in $CDCl_3$ of the corresponding monosubstituted anilines has been demonstrated previously.[3]

Regression analysis of the data—excluding the ortho-substituted compounds—results in a regression coefficient of 0.99 (s = 0.041) for 19 compounds. The deviation of ortho-substituted compounds does not mean that a different mode of action (or different correlation) must be considered. The deviations may in part be explained by the occurrence of intramolecular H bonding in the amines, which causes intramolecular magnetic reciprocal actions, observed as a larger chemical shift than expected. Nmr measurements utilizing DMSO, a highly polar solvent system capable of disrupting intramolecular H bonding, were therefore undertaken. The new chemical shift values obtained for the anilines correlate well with the MIC values of most of the ortho-substituted compounds. Regression analysis results in a regression coefficient of 0.97 (s = 0.04) (Figure 1). The *o*-chloro and *o*-iodo compounds however, still deviate from the line. From examination of the chemical shift values in DMSO compared to the corresponding antibacterial activities

(1) (a) J. K. Seydel, *Mol. Pharmacol.*, **2**, 259 (1966); (b) J. K. Seydel, *Int. Congr. Chemother., Proc.*, **1969**, (1970); *Int. Symp. Drug Design*, **1969** (1970).

(2) J. K. Seydel, *Arzneim.-Forsch.*, **16**, 1447 (1966).

(3) J. K. Seydel, *Proc. Int. Pharmacol. Meeting, 3rd, 1966*, **7**, 169 (1968).

(4) E. R. Garrett, J. B. Mielck, J. K. Seydel, and H. J. Kessler, *J. Med. Chem.*, **12**, 740 (1969).

(5) M. L. Crosley, E. H. Northey, and M. E. Hultquist, *J. Amer. Chem. Soc.*, **62**, 374 (1940).

(6) L. Knorr and P. Rössler, *Ber. Deut. Chem. Ges.*, **36**, 1279 (1903).

(7) A. Albert and A. B. Sergeant, "Ionization Constants of Acids and Bases," Methuen and Co., London, 1962.

(8) M. Yoshioka, K. Hamamoto, and T. Kubota, *Nippon Kagaku Zasshi*, **84**, 412 (1963).

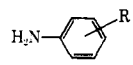
(9) E. Krüger-Thiemer, E. Wempe, and M. Töpfer, *Arzneim.-Forsch.*, **15**, 1309 (1965).

(10) A. H. Anton, *J. Pharmacol. Exp. Ther.*, **129**, 282 (1960).

(11) E. R. Garrett and O. K. Wright, *J. Pharm. Sci.*, **56**, 1576 (1967).

TABLE I

PHYSICOCHEMICAL AND BACTERIOLOGICAL PARAMETERS OF SOME SUBSTITUTED PHENYLSULFONAMIDES AND THEIR PRECURSOR SUBSTANCES IN SYNTHESIS (SUBSTITUTED ANILINES)

No.		Chem shift of the amino group protons, ppm (TMS)		Hammett σ	pK_a'	MIC, μmoles/l.	
		$CDCl_3$	DMSO			*E. coli*	*M. smegmatis*
1	4-$N(CH_3)_2$	3.15	4.50	−0.270	9.46	45.00	28.80
2	2-OCH_3	3.56	4.64	−0.268	9.43	35.00	31.00
3	2-OC_2H_5	3.55	4.60	−0.240	9.60	45.00	33.70
4	4-OCH_3	3.18	4.56	−0.268	9.34	34.50	32.00
5	4-OC_2H_5	3.10	4.55	−0.240	9.21	32.00	31.00
6	4-CH_3	3.27	4.77	−0.170	9.25	21.80	23.60
7	2-CH_3		4.74	−0.170	9.34	32.00	32.00
8	3-CH_3	3.18	4.90	−0.069	9.05	25.00	23.70
9	H	3.30	4.96	0.000	8.97	16.00	16.00
10	3-OC_2H_5	3.61	5.00	0.120	8.46	16.00	16.00
11	3-OCH_3	3.26	5.02	0.115	8.72	16.00	16.00
12	4-Cl	3.41	5.18	0.227	8.56	13.00	13.00
13	4-Br	3.43	5.22	0.232	8.24	12.80	12.80
14	2-Br		4.94	0.232	8.02	10.15	11.80
15	4-I	3.57	5.24	0.276	8.17	11.25	11.20
16	3-I	3.58	5.27	0.352	8.37	9.10	11.20
17	3-Cl	3.61	5.35	0.373	8.28	8.00	9.10
18	3-Br	3.54	5.33	0.391	8.31	11.25	11.70
19	3-NO_2	3.97	5.70	0.710	7.67	2.50	2.50
20	3-CF_3	3.70	5.48	0.450	7.98	5.60	9.60
21	4-CF_3	3.92	5.70	0.700	7.80	4.00	2.80
22	4-CN	4.16	6.10	1.000	7.36	1.00	1.20
23	4-$COCH_3$	4.06	5.99	0.874	7.52	2.00	2.00
24	4-NO_2	4.36	6.60	1.270	6.97	1.00	1.00
25	4-SO_2CH_3	4.16	6.07	0.874	7.30	1.40	1.70
26	2,3-CH_3		4.66		9.72	48.00	32.00
27	2-CH_3, 5-Cl		4.97		9.00	16.00	16.00
28	2-CH_3, 6-Cl		5.14		8.78	16.00	16.00
29	3,4-Cl		5.40			4.00	4.00
30	3,5-Cl		5.64		7.62	2.80	2.80
31	2-Cl, 4-OCH_3		4.86		8.81	16.87	13.60
32	2-OCH_3, 4-Cl		4.98		8.67	8.00	8.00
33	2-Cl, 4-NO_2		6.89		6.17	8.00	1.80
34	2-CH_3, 4-NO_2		6.32		6.98	2.80	0.70
35	2-NO_2, 4-Cl		7.54		6.79	3.90	1.20
36	2-Br, 4-NO_2		6.83		5.70	2.30	0.15
37	2-NO_2, 4-CF_3		7.92		6.10	4.80	0.50
38	4-NO_2, 2-CF_3		7.10		5.90	3.80	2.40
39	2-Cl		5.26		8.08	2.80	2.80
40	2-I		5.12		7.99	2.10	4.20

(see Table I, **39–40**), it is apparent that the deviation cannot be attributed to intramolecular hydrogen bonding since the chemical shift is too small compared to the activity.

In order to test further the reliability of the observed relationship, several disubstituted anilines were studied. Utilization of disubstituted anilines (r = 0.92, s = 0.21, n = 7), increased the total number of compounds studied to 32 and did not change the observed correlation (r = 0.96, s = 0.046) (Figure 1). The same electronic dependency was found for inhibitory activities obtained using two different bacterial strains (*E. coli* and *Mycobacterium smegmatis*) as shown in Figure 2. The regression lines are essentially identical, with a regression coefficient of 0.97, and standard deviations of 0.040 and 0.042, respectively. We have therefore not found a difference between the action of sulfonamides on a Gram-positive strain (*M. smegmatis*) and on a Gram-negative (*E. coli*) strain as was stated to exist between pneumococcus and Friedländer bacillus by Fujita and Hansch.[12] The data[13] used in their study give only a range of minimum inhibitory concn and the culture medium utilized contained proteins (which are excluded in our system).

Some of the same substituted sulfanilamides and MIC data obtained in our laboratories were used by Hansch and coworkers,[12] in the Hansch approach to structure–activity relationships (SAR), using partition coefficients, Hammett σ values, and the pK_a values of the sulfonamides.

The following equation was derived[12]

$$\log\frac{1}{C} + \log\frac{K_A + [H^+]}{[H^+]} = 1.298\sigma - 0.141\pi - 1.204$$

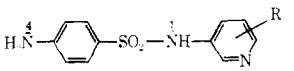
$$r = 0.987,\ s = 0.094,\ n = 17$$

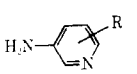
(12) T. Fujita and C. Hansch, *J. Med. Chem.*, **10**, 991 (1967).

(13) L. H. Schmidt and L. Sesler, *J. Pharmacol. Exp. Ther.*, **87**, 313 (1946).

TABLE II

PHYSICOCHEMICAL AND BACTERIOLOGICAL PARAMETERS OF SOME SUBSTITUTED 3-SULFAPYRIDINES AND THEIR PRECURSOR SUBSTANCES IN SYNTHESIS (SUBSTITUTED 3-AMINOPYRIDINES)





	Chem shift of the amino group protons, ppm (TMS)			MIC, μmoles/l.	
	$CDCl_3$	DMSO	pK_a'	*E. coli*	*M. smegmatis*
6-N(C_2H_5)$_2$		4.30	9.00	77.00	17.9
6-OCH_3	2.95	4.72	8.37	32.00	27.2
2-Cl-6-OCH_3		5.05	7.95	80.00	10.8
2-Cl	3.64	5.52	6.76	0.70	0.6
6-S-CH_3	3.48	5.17	7.66	5.60	6.8
3-NH_2	3.02	5.23	7.57	4.00	4.0
6-Cl	3.53	5.46	7.07	32.00	27.2
6-N(CH_3)$_2$		4.65		32.00	54.5

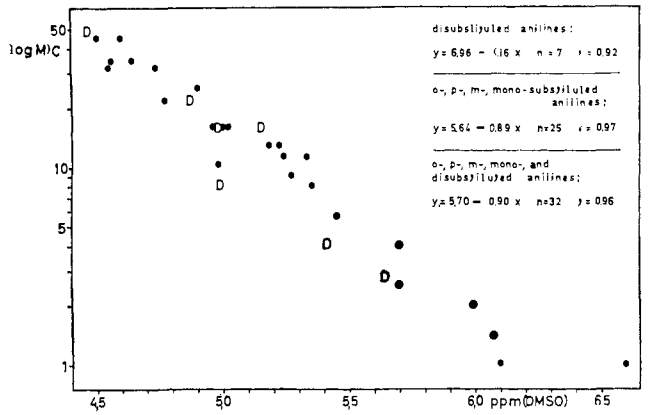


Figure 1.—Correlation between chemical shift (ppm) of anilines including ortho-, meta-, para-, mono-, and disubstituted anilines and the log MIC of the corresponding sulfanilamides.

The regression coefficient obtained is the same as that obtained in our study, using only the chemical shift value of the amines.

$$\log C = 5.638 - 1.315 \text{ ppm}$$

$$r = 0.992 \quad n = 19 \quad s = 0.041$$

As seen from the Hansch equation, lipid partitioning plays no important role and can be neglected. This is not very surprising in this type of *in vitro* study, where often equilibrium between the different phases does not seem to play a rate-determining role.† This is also in agreement with our finding that there is no significant difference in the activity of these drugs against the tested Gram-positive and Gram-negative bacteria in spite of the large difference in the lipid character of the membranes. Differences can however be observed for certain substituted NO_2 derivatives, and the reason for this is not known.

It is not surprising that σ, pK_a, and chemical shift give equally good correlations, since, as functions of electronic distribution, they are all interrelated. This

† Lipid partitioning undoubtedly plays an important role in the *in vivo* properties of sulfonamides. Lipid partitioning, pK_a, and solubility are highly correlated to protein binding, plasma tissue distribution, elimination, tubular reabsorption, and other pharmacokinetic properties of sulfa drugs. These relations have recently been discussed.[14,15]

(14) J. K. Seydel, *Int. Symp. Rational Develop. Appl. Drugs*, **1969**, in press.

(15) J. K. Seydel, "Progress Antimicrobial Anticancer Chemotherapy," University of Tokyo Press, Vol. II, Tokyo, 1970, p 881.

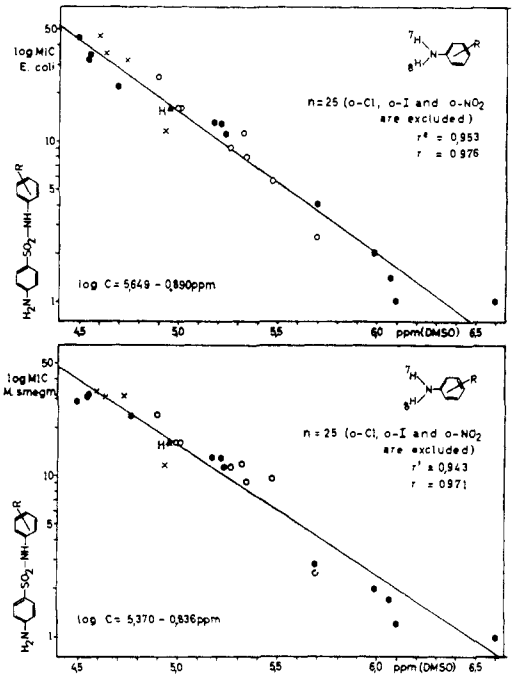


Figure 2.—Correlation between the chemical shift (ppm) of the substituted anilines and the logarithm of the minimum inhibition concentration (MIC) of the corresponding sulfanilanilides against: (a) *E. coli*, (b) *M. smegmatis*; (●) para substituted, (○) meta substituted, (×) ortho substituted.

has previously been substantiated in our laboratory.[3,14] It is, however, more convenient in this case, to use a correlation with chemical shift. This parameter may be measured for all the compounds and in addition has the advantage of being applicable to the precursor substances. This gives one the opportunity to predict the activity before the sulfonamide's synthesis.

Structure–Activity Relationships of Other Sulfonamides.—The general relationship between electronic data of the precursor amines and the *in vitro* activity of the corresponding sulfonamides is not limited to anilines. Studies on 2-sulfapyridines, 3-sulfapyridines, and sulfapyrimidines show the same correlation. It is much more difficult, however, to obtain a large enough number of compounds with different biological activity for a statistical treatment. From the data available, the same general picture is observed for these other homologous series. In the case of the 3-sulfapyridines (3-aminopyridines) (Figure 3), less shielding of the amino group protons results in a sulfanilamide with high activity (r = 0.986, s = 0.105, n = 8). The slope of the line, and therefore the pK_a of the highest activity, is, however, different than that in the substituted phenyl series (Figure 3) (Table II). A plot of log antibacterial activity *vs.* chemical shift shows that the quantitative electronic dependency is different (different slopes) for the various homologous series. This difference, undoubtedly is related to the different electronic nature of the ring systems involved, and may lead to different binding affinities at the active site, or, may be related to differences in penetration ability. The only type of experiment which may result in a clear answer to this question is a study in a cell-free system where penetration steps are excluded. These studies are in

progress[16] and seem to indicate that these differences are not related to penetration factors.

Occurrence of Optimum Values in SAR.—In many cases where relationships between physicochemical parameters and biological activities are observed, an optimum physicochemical parameter is often seen.[17] This phenomenon is well known for physical constants such as lipid–water partition, or for change in activity with the number of carbon atoms in aliphatic chains.[18,19] It has been described before for sulfonamides by Bell and Roblin.[20] A bell-shaped curve was obtained when the pK_a values of sulfonamides were plotted against the *in vitro* activities.

This curve was interpreted in terms of two factors which influence the activity: penetration, which usually occurs through the unionized species, and biological activity, which is thought to be a result of the ionized species.

Therefore, it was very unlikely that a similar phenomenon would occur in the "linear" relation between electronic data of the precursor amines and the biological activity of the corresponding sulfonamides. As observed from the data presented in this paper, an optimal value (either chemical shift or pK_a) is reached. Increasing the activity of the SA group (manifested by a chemical shift value $\geqq$ 6.5 ppm for the parent amine, or a $pK_a \leqq 7$ for the SA itself (Figure 4, Table I) results in a decrease in activity (compounds **33–38**). Compounds with low pK_a values would be highly ionized at the pH of the test medium. Under these conditions it is logical to assume that the concentration of unionized form available for permeation should be the limiting factor in their biological activity. Previous optima in sulfonamide structure–activity relationships have all consisted of the combination of several sulfonamide series.[20] The present results with the homologous sulfanilanilide series offer a convincing confirmation of the existence of such an optimum. Preliminary experiments in a cell-free system inhibited by sulfonamides indicate that the compounds with low pK_a values do indeed possess high activity when the need for cell-wall permeation is eliminated.[16]

Utilization of MIC Data in Structure–Activity Relationships.—It is generally recognized that Hammett σ constants, or other parameters related to these constants, describe linear free energy relationships. The MIC determination, however, involves the determination of the lowest concentration of a sulfa drug that shows total inhibition of bacterial growth after a fixed time interval. It is essentially a one-point method and initially one cannot be certain that it is valid to use this type of data in a free energy relationship. Garrett and coworkers[4] have recently determined the apparent first-order generation rate constants, k_{app}, for steady-state growth of *E. coli* in the presence of graded concentrations, S, of some of the same substituted sulfanilanilides.

From the expression $k_{app} = k_0 - k_0 k_b S/(1 + k_b S)$, where k_0 is the determined generation rate constant in the absence of drug, bacteriostatic activity parameters, k_b, were calculated for several compounds. It would seem valid to employ such kinetic rate constants in linear free energy relationships. As expected, a good linear relationship between log k_{b_i}/k_{b_0} and Hammett σ values was obtained whereas correlation with π was poor.[4] It was of interest to see how these kinetic parameters compared to the MIC data of the anilines. As already pointed out by Garrett and coworkers[4] the activity parameters, k_b, of two drugs can be related to their MIC values when the ideal condition $k_{app} = 0$ is considered and where S_i might be equal to the MIC. The ratio of the activity parameters can be shown to be of the form $k_{b_i}/k_{b_0} = MIC_0/MIC_i$. As shown in Figure 5 very similar lines are obtained when log MIC_i/log MIC_0 or log k_{b_i}/k_{b_0}, respectively, are plotted against either the Hammett σ value ($r = 0.978$, $s = 0.04$, $n = 25$ and $r = 0.948$, $s = 0.096$, $n = 11$) or the chemical shift of substituted anilines or sulfanilides. In the above mentioned kinetic study[4] the various sulfa drugs are added to a culture in the logarithmic growth phase, whereas in the MIC test the sulfa drug is added to a culture before growth is started. We therefore studied the influence of drug addition time on growth rates. Figure 6 shows an experiment in which SA was added to cultures in the lag phase and also in

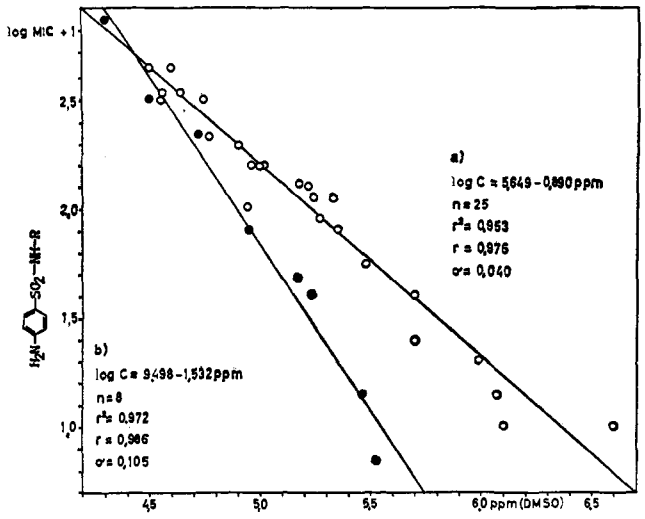


Figure 3.—Comparison of the correlation between log MIC of sulfanilamides and the chemical shift (ppm) of the corresponding amines, (a) anilines, and (b) 3-aminopyridines.

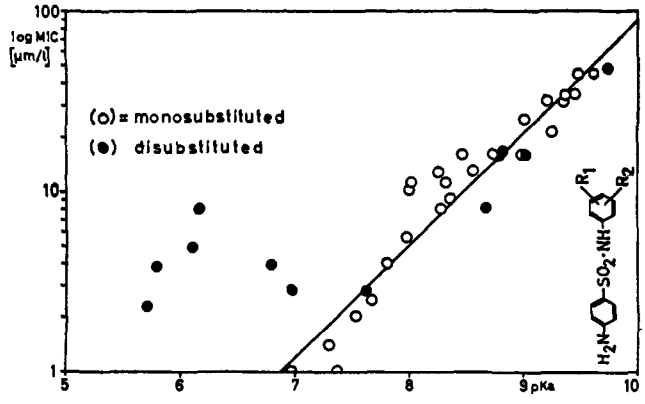


Figure 4.—Correlation between the acid pK_a of substituted sulfanilanilides and the log of the corresponding MIC (*E. coli*).

(16) G. H. Miller, P. H. Doukas, and J. K. Seydel, *Mol. Pharmacol.*, in press.
(17) E. J. Ariens, *Farmaco Ed. Sci.*, **24**, 3 (1969).
(18) H. G. Bray, *Biochem. J.*, **47**, 294 (1950).
(19) G. Malcolm-Dyson and P. May, New York, N. Y., "Chemistry of Synthetic Drugs," P. May, Ed., Longmans, Green and Co., Ltd., 1959, p 526.
(20) P. H. Bell and R. O. Roblin, *J. Amer. Chem. Soc.*, **64**, 2905 (1942).

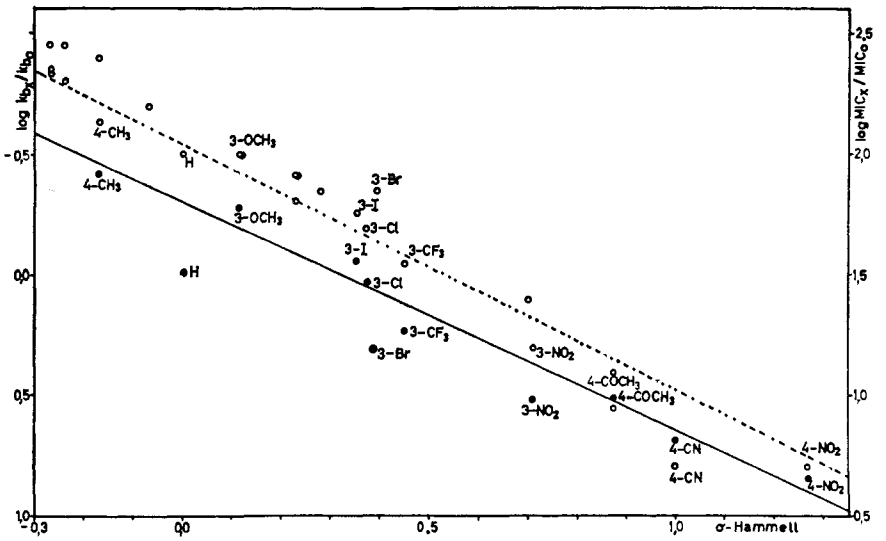


Figure 5.—Comparison of the correlation of Hammett σ (a) with log MIC_x/MIC_o, $n = 25$, $r = 0.978$ (○), and (b) with log k_{bx}/k_{bo}, $n = 11$, $r = 0.948$ (●), data from Garrett, *et al.*[4]

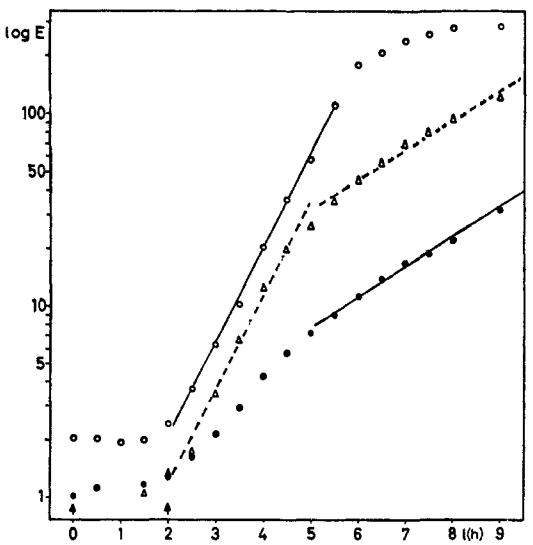


Figure 6.—Growth rate of *E. coli* (synthetic culture medium, $t = 37°$: (a) control (○), (b) sulfonamide added immediately (●), (c) sulfonamide added after log growth phase of the control is obtained (2 hr) (Δ).

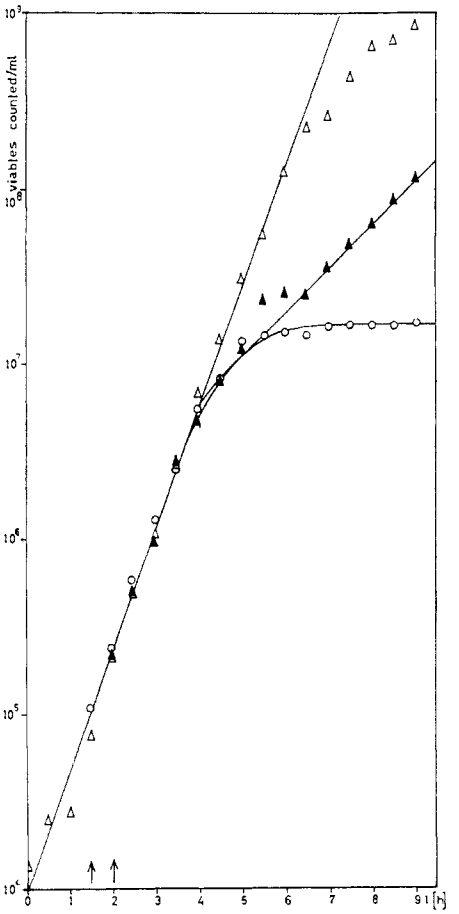


Figure 7.—Growth rate of *E. coli* (control Δ) in presence of graded sulfonamide concentrations (2-sulfa-3-methoxypyrazin) (a) 20 μmoles/l. (▲), (b) 200 μmoles/l. (○). Addition after 1.5, 2 hr, resp (colony count).

the log phase. It can be seen that after steady-state growth is attained, the slopes, and therefore generation rate constants, are the same for both cultures. There is also no large difference in the time observed for onset of the logarithmic growth phase between the control culture and the culture containing SA during the lag phase. For this reason the MIC data can be considered a reflection of the generation rate constants. Another objection to the MIC might be the possibility of simultaneous bacteriostatic and bactericidal action by the drug, which cannot easily be detected in an MIC experiment. The general opinion is, that sulfonamides act only bacteriostatically.[21] However, Garrett has shown, in a kinetic experiment, that there was a kill rate at higher concentrations of sulfisoxazole.[11] We have restudied this phenomenon and could not find support for the results of Garrett[11] using sulfisoxazole with the bacterial strain *E. coli* mutaflor. Figure 7 demonstrates that there is no kill of *E. coli* by this sulfonamide even at very high concentrations. Studies with other SAs in our laboratory, have yielded similar results. This supports the general validity of the assumption that the activity parameters k_b, obtained from a kinetic approach in growing cultures, and the activity parameters, obtained from a minimum inhibition experiment (MIC) are comparable. This conclusion may be limited to activity studies of sul-

(21) J. Hirsch., *Schweiz. Med. Wochenschr.*, **73**, 1470 (1943).

fonamides or similar compounds which are solely bacteriostatic. Under these circumstances the MIC method for the determination of the activity seems preferable because it is less expensive and less time consuming than the kinetic method. Kinetic experiments, however, may give a better indication of the type of antibacterial action.

Acknowledgment.—The author is greatly indebted to Miss E. Wempe for biological activity determinations, to Miss H. Henke and Mr. W. Diller for physicochemical measurements, and to Dr. P. H. Doukas of Temple University, School of Pharmacy, Philadelphia, Pa. 19140, for assistence in the preparation of the manuscript.

Synthesis of Compounds Structurally Related to Poison Ivy Urushiol. 3.[1a-c] 3-*n*-Pentadecylcatechol and 3-*n*-Alkylcatechols of Varying Side-Chain Length

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Received July 3, 1970

The allergenic components of poison ivy urushiol have the carbon skeleton of 3-*n*-pentadecylcatechol. An improved synthetic route to 3-*n*-pentadecylcatechol and related 3-*n*-alkylcatechols has been developed incorporating pyridinium chloride cleavage of the corresponding veratrole(s). The route has been shown to be of general utility through its application to the synthesis of a series of 3-*n*-alkylcatechols bearing saturated side chains of varying length: C_1, C_3, C_5, C_8, C_{11}, C_{15}, C_{17}, C_{19}. Biological evaluation of these compounds has demonstrated that antigenic specificity is sensitive to changes in side-chain length in the function of these agents as sensitizers and elicitors of delayed contact dermatitis.

Poison ivy urushiol, the dermatitis-producing principle of the poison ivy plant, is an oil composed of four 3-*n*-alkylcatechols, each having the C skeleton of 3-*n*-pentadecylcatechol (3-PDC, **1g**) and each varying in the degree of unsaturation of the alkyl side chain.[2] 3-PDC, the saturated component of the urushiol of poison ivy and many related plants,[3] is itself a compound increasingly in demand for diagnostic patch testing for Rhus hypersensitivity.[4] As part of a continuing systematic investigation into the structure–activity relationships involved in the function of 3-alkylcatechols as allergic agents,[1] an improved synthesis of 3-PDC has been developed and applied to the synthesis of its analogs having varying side-chain length.

Since poison ivy dermatitis, in the great majority of instances, is an allergic phenomenon (delayed contact hypersensitivity),[5] an interest in a possible antigenic specificity for side-chain length prompted the synthesis of a series of analogs of 3-*n*-pentadecylcatechol varying only in the length of the saturated side chain.[1,6] The structures of the alkylcatechols involved, and their synthesis precursors, are indicated and labeled as shown in Scheme I.

Chemistry.[7a]—Veratrole precursors to the alkylcatechols (**5b–5g, 5i**) were obtained routinely as described in the Experimental Section and outlined in Scheme I by direct hydrogenolysis[8] of the product of the reaction of the appropriate Grignard reagent with *o*-veratraldehyde (**3**). 3-Methylveratrole (**5a**) was obtained by direct hydrogenolysis of **3**, while 3-*n*-propylveratrole (**5b**) was obtained by purification of an available sample. Each veratrole was brought to a state of high purity by fractional distillation *in vacuo*. Data and physical properties[9] pertinent to these preparations are presented in Table I. Data for the preparation of 3-*n*-pentadecylveratrole (**5g**), precursor to the naturally occurring 3-PDC, are presented in Table I.

(1) (a) Previous paper in the series (2): J. S. Byck and C. R. Dawson, *J. Org. Chem.*, **33**, 2451 (1968); (b) accompanying paper (4): A. P. Kurtz and C. R. Dawson, *J. Med. Chem.*, **14**, 733 (1971), describes the synthesis of analogs varying in side-chain shape and flexibility; (c) taken from the Ph.D. Dissertation of A. P. Kurtz, Columbia University, 1968; these investigations were supported by Contract PH-43-64-76 with the Division of Biologics Standards of the National Institutes of Health; (d) National Institutes of Health Predoctoral Fellow, 1965–1968.

(2) (a) W. F. Symes and C. R. Dawson, *J. Amer. Chem. Soc.*, **76**, 2959 (1954); (b) K. H. Markiewitz and C. R. Dawson, *J. Org. Chem.*, **30**, 1610 (1965).

(3) (a) G. A. Hill, V. Mattacotti, and W. D. Graham, *J. Amer. Chem. Soc.*, **56**, 2736 (1934); (b) R. Majima and J. Tahara, *Chem. Ber.*, **48**, 1606 (1915).

(4) (a) H. Keil, D. Wasserman, and C. R. Dawson, *J. Allergy*, **16**, 275 (1945); (b) H. Keil, D. Wasserman, and C. R. Dawson, U. S. Patent No. 2,451,955 (Oct 19, 1948); (c) A. M. Kligman, *Arch. Dermatol.*, **77**, 149 (1958); (d) R. Auerbach and H. Baer, *J. Allergy*, **35**, 201 (1964).

(5) A. J. Crowle, "Delayed Hypersensitivity in Health and Disease," C. T. Thomas, Springfield, Ill., 1962, Chapter IV.

(6) (a) Although the synthesis of a series of 3-*n*-alkylcatechols (C_5, mp 34–35°; C_6, mp 30–31°; C_7, solid at 5°; C_8, liquid; C_{15}, mp 57–59°; C_{17}, mp 59°) was reported in 1946 (see ref 6b), characterization data for the synthetic products was inconclusive by present standards and yield data for the preparations were conspicuously absent. See footnote *k* to Table II. (b) R. D. Haworth and D. Woodcock *J. Chem. Soc.*, 999 (1946).

(7) (a) A review of the literature[7b-7e] indicates that the least satisfactory step in the prior syntheses of 3-PDC and alkylcatechols has been the last step, *i.e.*, the conversion of the precursor veratrole to the catechol. Halogen acid sealed-tube cleavages give poor yields of product difficult to purify.[3a,7d] The optimum method of cleavage reported prior to the present study incorporates $AlCl_3$–chlorobenzene. This reagent system gives 3-PDC in about 75% yield[7c,7e] after distn and several recrystns of the crude product but is not practical for large scale preps. After experimentally investigating all of the routes previously reported, an exploratory study of pyridinium chloride (PyrCl)[7f] as cleavage reagent for 3-alkylveratroles was made. Use of a PyrCl cleavage procedure analogous to that given in the literature (procedure A) or the more convenient procedure (procedure B) given in the Experimental Section affords easily purifiable 3-alkylcatechols in high yields and is practical for large scale work. (b) H. J. Backer and N. H. Haack, *Recl. Trav. Chim. Pays-Bas*, **57**, 225 (1938); (c) H. Keil, D. Wasserman, and C. R. Dawson, *J. Amer. Chem. Soc.*, **68**, 534 (1946). (d) H. S. Mason, *ibid.*, **67**, 1538 (1945); (e) B. Loev and C. R. Dawson, *ibid.*, **78**, 4083 (1956); (f) this reagent had previously been used for the cleavage of unsubstituted mono- and diphenol ethers [V. Prey, *Chem. Ber.*, **74**, 1219 (1941); *ibid.*, **75**, 350 (1942)] and long-chain alkylresorcinol dimethyl ethers (E. Wenkert, E. M. Loeser, S. N. Mahapatra, F. Schenker, and E. M. Wilson, *J. Org. Chem.*, **29**, 435 (1964).

(8) The direct hydrogenolysis (**4** to **5**) is essentially quantitative yielding a stable distillable product free of degraded material often characteristic of routes employing prior carbinol dehydration.

(9) As described in detail elsewhere,[10] the boiling points of the veratroles, when adjusted to a common pressure, and the logarithms of the vpc retention times for the C_{11}–C_{19} members of the series form a linear plot when graphed *vs.* chain length.

(10) Details on these and other studies are presented in the Ph.D. Diserstation of A. P. Kurtz, Columbia University, New York, N. Y., 1968.