Electronic Aspects of the Antibacterial Action of Sulfanilamides

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Intramolecular interactions in N^1-substituted sulfanilamides (SA) can rationalize the trend of their antibacterial powers with the use of a resonance scheme, derived from d orbital symmetry and tested with an extensive spectroscopic investigation on amidic, imidic, and anionic SA. On quantitative grounds, a good relationship is presented between the antibacterial power and the proton chemical shift of the *p*-amino group. The electronic features for high activity are described.

Very recently,[1] extensive spectroscopic data were reported for the different forms (amidic, imidic, and anionic) of a fairly large group of sulfanilamides (SA) with a wide variability of N^1 substituents and were discussed in terms of local electronic changes induced by the substituents on the common moiety *p*-NH_2-C_6H_4-SO_2- via intramolecular interactions. In this context the spectroscopic values assume the role of indices representative of the electronic features in the series of related compounds.

On the other side, SA are well-known antibacterial agents exerting their action by a competitive inhibition of *p*-aminobenzoate utilization in the enzymatic synthesis of folate,[2] and the relative antibacterial potencies of an enormous number of substances have been measured by means of standardized experiments of bacterial growth inhibition. In spite of the fact that, following the pioneering work of Woods,[3] Fildes,[4] and Bell and Roblin,[5] the problem of relating the in vitro potencies of SA to their physicochemical properties and of finding out the relationship between the molecular structure and the activity has received the greatest attention, satisfactory solutions have not yet been reached.[6] In particular, all of the many attempts to correlate the spectral properties of SA with their observed antibacterial activities have met with depressing failure.[7-10] Only recently[11,12] did it become apparent that failure was linked with improper approaches to the two following problems.

(a) Although it has been widely accepted that the antibacterial measure reflects, in general, the effectiveness of a mixture of forms in tautomeric and dissociation equilibria in the active solution, the condition that for extended structure–activity relationships a specific activity value (as well as a specific structural index) is needed for each individual form has been usually ignored.[11,12] Subject to the assumption that the contributions of the different forms are additive and to the approximation that, in equilibrium with less active species—the neutral forms—the anionic fraction is responsible for the overall activity, a set of activity parameters for individually active species has been calculated from the observed potencies.[11]

(b) The mechanisms of intramolecular interactions in SA are rather complicated, so that the role of N^1 substituents in modulating the electronic features of the common part, NH_2-C_6H_4-SO_2-, was not understood satisfactorily. Now a qualitative understanding of the spectroscopic behavior of SA has been obtained[1] on the basis of Moffitt's analysis of the different kinds and degrees of conjugation in unsaturated and aromatic sulfones,[13,14] and we are able to show that the antibacterial potency of SA can be rationalized in that scheme as well. The experimental data needed for our analysis are collected in Table I.

A plot (Figure 1) of the activity parameters vs. the *p*-amino proton chemical shifts demonstrates that they are strictly related. Some scatter is justified by experimental uncertainties in the biological measurements (they are claimed to be rather large and they are even larger in this case since the antibacterial data are taken from different sources), in the determination of pK_a used for calculating the activity parameters from the observed potencies, and in the proton chemical shifts. Only two points (**14**, **17**) fall clearly outside the correlation. The fairly close relationship of Figure 1, extended over all the three forms and the complete range of spectroscopic and biological data at present available, contributes significantly to the view that, in this class of compounds, the antibacterical potency of any active species is modulated by the electronic changes induced by the substituent X on the common moiety as they are reflected by the values of the *p*-amino proton chemical shifts and that X does not participate directly in the chemical process determining the biological yield; direct participation of X could be, in general, one of the causes of exceptions.

A few aspects of Table I and Figure 1 deserve some comments. The anions of sulfanilamide and N^1-methylsulfanilamide (**1** and **1b**, respectively) appear to be the most active inhibitors in the whole series of compounds, in full agreement with the Bell and Roblin's hypothesis that the less acidic the sulfonamide, the higher the antibacterial activity of either form (ionic and molecular forms). Actually, the activity parameters of the anions **1** and **1b** could be somewhat overestimated by the approximation which ignores the antibacterial contributions of the respective molecular forms and assigns the whole activity of the mixture of forms to the anionic forms whose fractions are less than 5×10^{-4}; however, the ratio of the bacteriostatic concentration of an anionic form to that of a molecular form can be so small as 10^{-3}–10^{-4} so that, in the limit conditions of sulfanilamide and N^1-methylsulfanilamide, one can easily realize that both the molecular form and the ionic one reach the order of magnitude of their respective bacteriostatic concentrations. If we assume, for example, that the two forms, in their actual ratio in the active solution, give equal contributions to bacteriostasis, the activity parameters of the ionic forms pass from 1.29 to 0.99, where the difference (log 2) does not exceed the error of MIC determinations (a factor of 2); in regard to the plot of Figure 1, points **1** and **1b** still follow the correlation, in the limit of its normal dispersion. It is obvious that, in spite of the high potency of these anions, there is no practical interest to design acidic sulfonamides whose anions possess these features because of their very low fraction in aqueous solutions; however, for a more general problem of designing PAB antimeta-

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Table I. Activity Parameters and *p*-Amino Proton Chemical Shifts of Sulfanilamides in Their Anionic, Imidic, and Amidic Forms

no.	X	pK_a	MIC, μmol/L	ap[a]	chemical shift of the amino group protons, ppm (Me_4Si) in Me_2SO
		anions: *p*-NH_2-C_6H_4-SO_2-NX			
1	H	10.43[b]	128[c]	1.29	5.25
1b[i]	CH_3	10.77[b]	300[b]	1.29	5.31
2	4-methoxyphenyl	9.34[d]	34.5[d]	0.80	5.31
3	phenyl	8.97[d]	16[d]	0.77	5.34
4	2-pyridinyl	8.56[e]	6.1[c]	0.79	5.36
5	3-pyridinyl	7.89[b]	2[b]	0.64	5.42
6	2-(4-methylthiazolyl)	7.79[b]	2[b]	0.55	5.42
7	2-(4,6-dimethylpyrimidinyl)	7.51[f]	1.78[c]	0.38	5.35
8	2-thiazolyl	7.23[e]	1.5[c]	0.25	5.42
9	6-(3-methoxypyridazinyl)	7.17[e]	1[c]	0.39	5.37
10	2-(6-methylpyrimidinyl)	6.85[e]	0.93[c]	0.26	5.37
11	2-pyrimidinyl	6.37[e]	1.02[c]	0.08	5.37
12	2-(3-methoxypyrazinyl)	6.1[d]	1.59[c]	−0.15	5.37
13	6-(2,4-dimethoxypyrimidinyl)	5.98[e]	0.77[c]	0.15	5.44
14	$CONH_2$	5.42[b]	32[c]	−1.49	5.40
15	$COCH_3$	5.38[b]	3.3[c]	−0.51	5.47
16	2-(5-methyl-1,3,4-thiadiazolyl)	5.22[e]	2[b]	−0.29	5.51
17	1,2,4-triazolyl	4.66[b]	>800[b]	<−2.90	5.50
17b[i]	COPh	4.57[b]	5.6[g]	−0.75	5.48
		imides: *p*-NH_2-C_6H_4-SO_2-N=X			
18	$-N=C(NH_2)_2$		64[c]	−1.81	5.71
19	2-(*N*-methylpyridinyl)		24.4[h]	−1.39	5.74
20	2-(*N*-methylthiazolyl)		24[h]	−1.38	5.89
		amides: *p*-NH_2-C_6H_4-SO_2-N(CH_3)X			
21	CH_3		300[b]	−2.48	6.07
22	2-pyridinyl		780[h]	−2.89	6.14
23	2-thiazolyl		1540[h]	−3.19	6.31

[a] Activity parameters for anions were calculated from MIC and pK_a values by ignoring the contribution of neutral forms. [b] Reference 5. [c] E. Krüger-Thiemer, E. Wempe, and M. Töpfer, *Arzneim.-Forsch.*, **15**, 1309 (1965). [d] J. K. Seydel, *ibid.*, **16**, 1447 (1966). [e] M. Yoshioka, K. Hamamoto, and T. Kubota, *Yakugaku Zasshi*, **84**, 90 (1964). [f] A. V. Willi and W. Meier, *Helv. Chim. Acta*, **39**, 54 (1956). [g] J. K. Seydel and E. Wempe, *Arzneim.-Forsch.*, **14**, 705 (1964). [h] Calculated from the activity ratios [R. G. Shepherd et al., *J. Am. Chem. Soc.*, **64**, 2532 (1942)] and from MIC values of the parent compounds (see also ref 11). [i] Compounds 1b and 17b have been added to the collection studied in previous work[12] and so labeled as to maintain the same numbering.

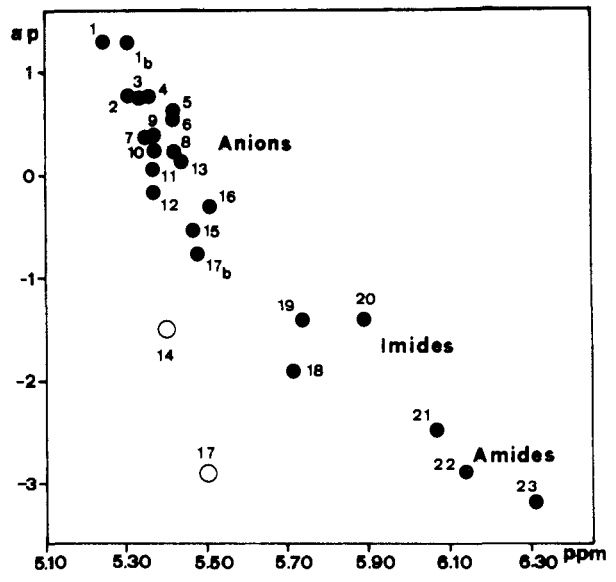

Figure 1. Relation between the activity parameter and the *p*-amino proton chemical shift. Least-squares linear equation: ap = −4.201 ppm + 22.960 (N = 23, r = 0.96), 14 and 17 omitted.

bolites, the electronic features linked to high activity, although not exclusive, should be important.

As a consequence of the observations made in ref 1 that different interaction mechanisms between the substituent X and the common framework appeared to operate in amidic and in anionic forms (in amidic forms the interaction bypasses the SO_2 group, whereas in anionic—and probably in imidic—forms both the SO_2 group and the NH_2–C_6H_4– group feel the effects of X, and these effects are correlated), the *p*-amino proton chemical shift, although representative of a part of a conjugated system, cannot be taken as rigorously representative of the electronic changes of the common moiety as a whole, but for subsets of substances pertaining to the same form, either amidic or anionic. However, in spite of the fact that *p*-NH_2 proton chemical shifts, SO_2 stretching frequencies, and maximum wavelengths of the conjugation band are not related in *single* relationships extended over all the forms,[1] at a qualitative level, low chemical shifts are related to low stretching frequencies, low wavelengths, and, according to Figure 1, to *high* potencies.

In terms of the commonly used concepts of structural organic chemistry, high potency is a concomitant factor of (a) high polarization of the S–O bond (i.e., high negative charges on the oxygens) and (b) low conjugation within the common moiety due to (c) low engagement of the *p*-NH_2 *lone pair* to the aromatic system. Quantum chemical calculations are now in progress which appear to substantiate these findings.

NMR Measurements. The spectra were recorded on a Jeol C60-HL; the solvent used was Me_2SO (Uvasol, Merck, minimum deuterium grade 99.8%). Product concentrations were in the range 5×10^{-2}–1×10^{-1}, except for compound **15**, where saturation was reached at a lower concentration. The amino group proton signals were reproducible to less than 0.03 ppm (25 °C) and showed no

concentration dependence in the concentration range 5×10^{-1}–1.25×10^{-2}. The chemical shift values are expressed in parts per million (ppm) downfield (Me_4Si).

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Antiallergic Activity of Some 9*H*-Xanthen-9-one-2-carboxylic Acids

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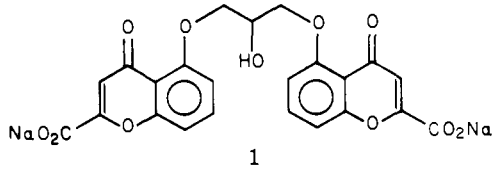
The synthesis and antiallergic activity of a new series of 9*H*-xanthen-9-one-2-carboxylic acids are described. Antiallergic activity was evaluated in the rat passive cutaneous anaphylaxis (PCA) screen. Biological results were analyzed using regression analysis techniques, and the antiallergic activity of the compounds in the series was found to be highly correlated with substituent size.

Following the discovery,[1] in 1967, of the novel antiallergic properties of disodium cromoglycate (1), research



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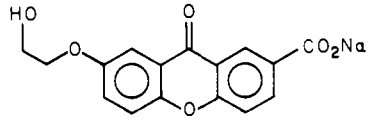

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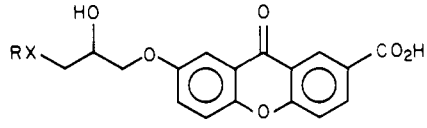
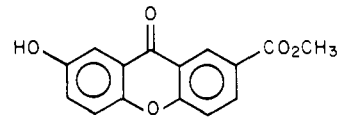


3, X = S
4, X = O
5, X = S^+-O^-

on prophylactic inhibitors of the release of mediators of immediate hypersensitivity has expanded rapidly. Many different classes of chemical compounds have been claimed to exhibit varying degrees of antiallergic activity.[2] Included among these are three reports of the antiallergic activity of 9*H*-xanthen-9-onecarboxylic acids.[3-5] AH7725 (**2**) was the first 9*H*-xanthen-9-one-2-carboxylic acid to undergo clinical investigation.[6] We report the preparation and antiallergic activity of a new series of 9*H*-xanthen-9-one-2-carboxylic acids of general structures **3**–**5** which have thiomethyl, oxomethyl, and sulfinylmethyl substituents appended to the terminal methylene group of **2**.

Chemistry. Alkylation of methyl 7-hydroxy-9*H*-xanthen-9-one-2-carboxylate (**6**)[3] with epichlorohydrin gave the epoxide **7** which served as a common intermediate to



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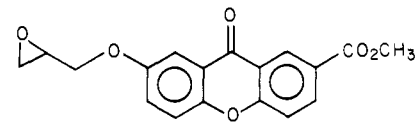

7

the target compounds **3**–**5**. Under basic conditions, reaction of the requisite nucleophile (mercaptide or alkoxide) at the terminus of the epoxide, followed by saponification of the ester, provided **3** and **4**. $NaIO_4$ oxidation of the thioether **3** gave the sulfoxide **5**.

Results and Discussion

The antiallergic activity of the 9*H*-xanthen-9-one-2-carboxylic acids reported herein was assessed in the standard rat PCA screen (see Experimental Section for test details). Compounds were initially screened by intraperitoneal (ip) administration at 5 or 10 mg/kg. Representative compounds which provided greater than 50% inhibition of the rat PCA reaction by ip dosing were subsequently tested by oral administration.

The biological results described in Table I present no obvious structure–activity relationships. In order to provide further insight into the factors influencing the antiallergic activity of this series of compounds, the biological data were studied by regression analysis. Biological activities were expressed as log (percent inhibition) instead of the usual molar scale, since antiallergic activity was measured only at a single dose. Expression of the biological activity as log (molecular weight × percent inhibition) was not warranted since a high correlation ($r = 0.99$) existed between this and log (percent inhibition).

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