Notes

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Protein Bindings. IV.[1] Relations of an Index for Electronic Structure to Binding Constant with Serum Albumin and Bacteriostatic Activities of Sulfonamides

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The preceding work[1] has shown that there are some relations between binding constant with serum albumin and other physicochemical and biological properties of sulfonamides. These properties may directly or indirectly related to the electronic structure of the sulfonamides. In the present paper an index for the electronic structure—"Q–value," the π–electron net change at the carbon atom adjacent to the N^1–nitrogen atom of simplified models in which the conjugation of the sulfanilamido group was omitted—was calculated using the Hückel molecular orbital (HMO) method[3] to find a clue to elucidate relations of the electronic structure to binding constant with serum albumin and bacteriostatic activities of sulfonamides.

Estimation of HMO Parameters used in the Calculation

The application of the HMO method to a molecule involving a heteroatom X leads to a secular determinant which contains in the terms α_X, the Coulomb integral for the p–orbital of X, and β_{CX}, the resonance integral for the carbon–X bond. The heteroatom integrals are expressed in terms of the carbon integrals of benzene, α_C, and β_{CC}, by putting[3]

$$\alpha_X = \alpha_C + h_X\beta_{CC}$$
$$\beta_{CX} = k_{CX}\beta_{CC}$$

where h_X and k_{CX} are dimensionless parameters for a particular heteroatom in a given molecular environment. Since these integrals are defined in terms of a monoelectronic effective Hamiltonian which is assumed to include the electronic interactions,[4] it may be desirable to evaluate the parameters, h_X and k_{CX}, using a model structurally similar to the compounds whose indices are to be calculated. As the models for this work, furan for Ö, pyrrole for N̈, pyridine for Ṅ, thiophene for S̈, and toluene for methyl were used for evaluation of the parameters.

Table I. k–Values from Proportionality to Overlap Integrals (S)

Bond	Model	Bond Length (Å)	S_{CX}/S_O[a]	k_{CX}[b]
C–O	Furan	1.37	0.65	0.7
C–N	Pyrrole	1.42	0.75	0.8
C=N	Pyridine	1.34	0.87	0.9

a) Overlap integral of carbon–carbon bond of benzene.
b) Rounded value of S_{CX}/S_O was adopted for k_{CX}.

1) Part III: I. Moriguchi, S. Wada, and T. Nishizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 601 (1968).
2) Location: *Takadaminami-cho, Toshima-ku, Tokyo*; *a*) Present address: *School of Pharmaceuitcal Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo.*
3) See for example, A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemists," John Wiley & Sons, Inc., New York, 1961.
4) E. Hückel, *Z. Physik. Chem.* (Frankfurt), **76**, 628 (1932).

Wheland[5] has suggested that the assumption of the proportionality of resonance integral to overlap integral be extended to heteroatom bonds as well as conjugated carbon–carbon bonds. Values of k_{CX} derived with this assumption are listed in Table I.

Overlap integrals were calculated using the tables of Mulliken, *et al.*[6] based on Slater orbitals. Beacuse the assumption was not appropriate with a change in principal quantum number,[7] k_{C-S} value was estimated 0.4 for 1.47 Å in thiophene by Sandorfy's method.[8]

Variations in Coulomb integrals are known to sensitive to the charge distribution.[9] Hence values of h_X were estimated so as to reproduce well the π–electron distribution given by advanced SCF (self–consistent field) calculations. Values of the parameters so obtained are shown in Table II. The inductive model[10] was adopted for methyl group. For the

Table II. Parameters Estimated and Their Reproductivities of SCF π–Electron–Density Distributions (q_r)

X	h_X	k_{CX}	Model	HMO q_r				Average SCF q_r				Ref.
				1	2	3	4	1	2	3	4	
Ö	2.1	0.7	Furan	1.841	1.008	1.071	—	1.821	1.033	1.056	—	*a,b,c)*
N̈	1.7	0.8	Pyrrole	1.752	1.026	1.099	—	1.766	1.056	1.062	—	*b,c)*
S̈	1.2	0.4	Thiophene	1.880	1.017	1.043	—	1.892	1.017	1.037	—	*a,b,c)*
Ṅ	0.4	0.9	Pyridine	1.176	0.932	1.004	0.953	1.166	0.910	1.030	0.959	*d,e)*
CH_3	($h_{C_\alpha}=-0.1$)		Toluene	0.960	1.016	0.999	1.010	0.984	1.014	1.002	1.010	*f)*

a) D.S. Sappenfield and M. Kreevoy, *Tetrahedron*, **19**, Suppl. 2, 157 (1963).
b) R.D. Brown, through *a*).
c) N. Solony, F.W. Birss, and J.B. Greenshields, *Canad. J. Chem.*, **43**, 1569 (1965).
d) R.D. Brown and B. Coller, Personal Communication (1960), through D.J. Brown, "The Pyrimidines," Interscience Publishers, New York, 1962, p, 27.
e) O.W. Adams and P.C. Lykos, Private Communication, through A.H. Gawer, B.P. Dailey, *J. Chem. Phys.*, **42**, 2658 (1965).
f) L.B. Kier, *Tetrahedron Letters*, **1965**, 3273

parameters of Ö, because there was not an appropriate model for this work whose π–electron density was calculated by advanced SCF method, 1.2 for $h_{\ddot{O}}$ and 2.0 for $k_{C=O}$ suggested by Pullman and Pullman[11] were used.

Results and Discussions

The Q–values calculated by using the parameters so estimated are listed in Table III.

These values are correlated with log K, logarithm of intrinsic binding constant for sulfonamides with bovine serum albumin as shown in Fig. 1. Coefficient of the correlation was 0.491 (19 samples); the correlation was significant at the 5–percent level.

When sulfonamides are bound to serum albumin, N^1–nitrogen of the sulfonamide anions[12] may slightly increase its Coulomb integral by accept of electronic charge from cationic sites on the albumin molecule through the bond. The change of total π–electronic energy, E, due to the change of Coulomb integral of the r–th atom, α_r, is expressed as[13]

5) G.W. Wheland, *J. Am. Chem. Soc.*, **64**, 900 (1942).
6) R.S. Mulliken, C.A. Rieke, D. Orloff, and H. Orloff, *J. Chem. Phys.*, **17**, 1248 (1949).
7) Ref. 3) page 119.
8) C. Sandorfy, *Bull. Soc. Chim. France*, **1949**, 615.
9) Ref. 3) page 121.
10) A. Streitwieser Jr. and P.M. Nair, *Tetrahedron*, **5**, 149 (1959).
11) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, New York, 1963, p. 108.
12) That anions combine more strongly with proteins than do the corresponding non–ionized acids has been evident from the work by Klotz (I.M. Klotz, *J. Am. Chem. Soc.*, **68**, 2299 (1946)).
13) C.A. Coulson and H.C. Longuet–Higgins, *Proc. Roy. Soc.* (London),Ser. A, **192**, 16 (1947).

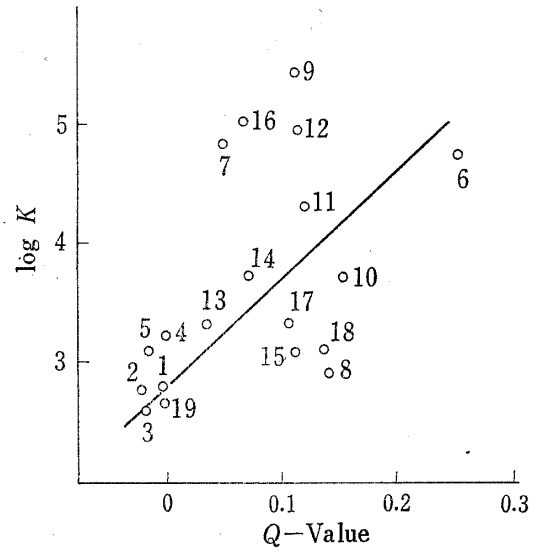

Fig. 1. Correlation of the Q–Value with log K[1]

For numbering, see Table III.

$$\delta E = q_r \delta\alpha_r + (1/2)\pi_{rr}(\delta\alpha_r)^2 + \cdots$$

where q_r represents the π–electron density, and π_{rr} the self polarizability. When $\delta\alpha_r$ is small, δE may be proportional to q_r. Because $\delta\alpha_r > 0$ in the sulfonamide–albumin binding, δE may be smaller and consequently the binding may be easier with the smaller q_r–value at the N^1–nitrogen of sulfonamides. It may be considered that π–electron net charge, $2-q_r$, at the N^1–nitrogen is approximately correlated with the Q–value for the adjacent carbon, and that δE is linearly related to $-\log K$ assuming that differences of the entropy changes for the binding are negligible with many sulfonamides. Hence log K may eventually bear a linear relation to the Q–value with rough approximation. In Fig. 1, log K values for sulfamethoxypyridazine, sulfadimethoxine, sulfamethomidine, and sulfisoxazole are indicated extraordinary high. In case of sulfisoxazole, the method or the parameters used for calculation of the Q–value seem inappropriate for this compound judging from its pK_a value.[14] In other three cases, methoxy group seems to play a non–negligible part for the binding. Probably hydrophobic binding or the forces of dispersion contributes to the stronger binding forces,[15] but the details are not cleared.

Table III. Q–Values for Sulfonamides

No.	Sulfonamides	Q–Value	No.	Sulfonamides	Q–Value
1	N^1–Phenylsulfanilamide	0.000	11	Sulfamethizole	0.122
2	N^1–*p*–Methoxyphenylsulfanilamide	−0.023	12	Sulfamethomidine	0.114
3	N^1–*o*–Tolylsulfanilamide	−0.016	13	Sulfaphenazole	0.038
4	N^1–*m*–Tolylsulfanilamide	0.001	14	Sulfisomezole	0.074
5	N^1–*p*–Tolylsulfnailamide	−0.010	15	Sulfisomidine	0.114
6	Xyloylsulfanilamide	0.253	16	Sulfisoxazole	0.067
7	Sulfamethoxypyridazine	0.050	17	Sulfamonomethoxine	0.113
8	Sulfadiazine	0.140	18	Sulfathiazole	0.136
9	Sulfadimethoxine	0.114	19	Sulfanilamide	(0.000)
10	Sulfamerazine	0.154			

It was previously reported[1] that relationships between log K and *in vitro* bacteriostatic activities of sulfonamides against *Escherichia coli* 0–55 and *Staphylococcus aureus* 209P were expressed with parabolic curves. Because the log K is correlated with the Q–value, there may be the similar relations between bacteriostatic activities and the Q–value. The relationships are actually recognized in Fig. 2, which indicates that the optimum Q–value for the antibacterial activities is about 0.10 to 0.15.

14) The value of pK_a for sulfisoxazole is 4.8. Since this value is close to that for xyloylsulfanilamide (pK_a = 4.4), Q–value for sulfisoxazole is also expected near to that for xyloylsulfanilamide.

15) W. Scholtan, *Arzneimittel–Forsch.*, **14**, 469 (1964).

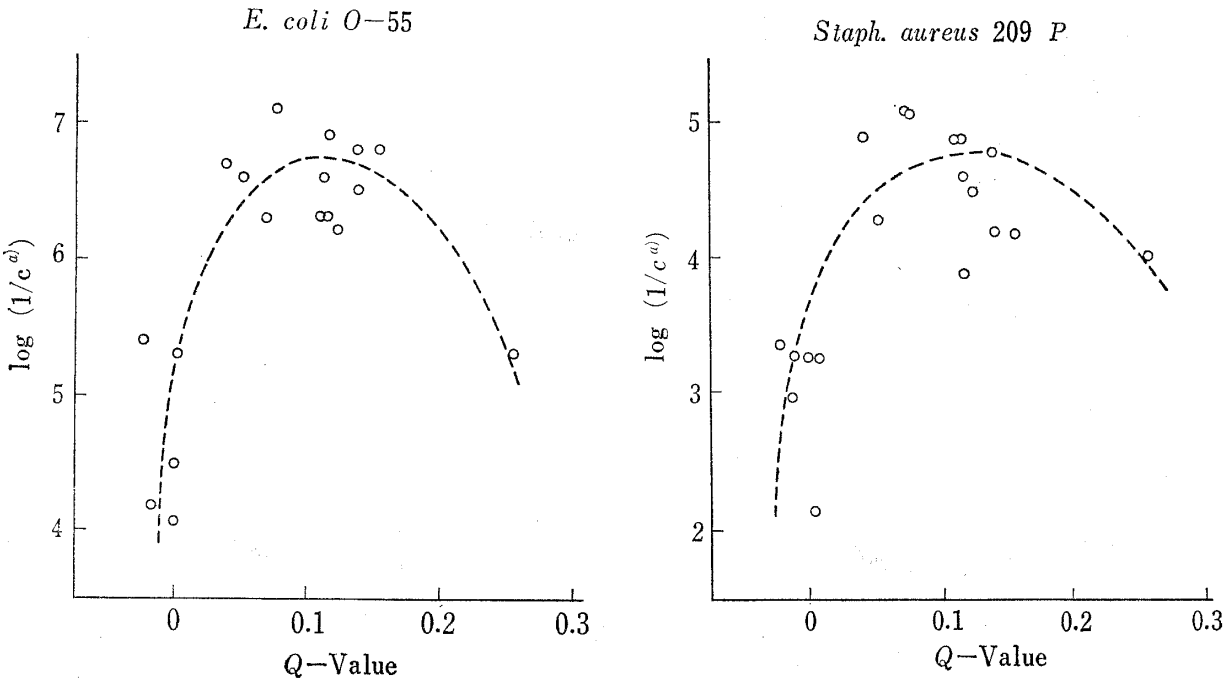

Fig. 2. Relationships between the Q–Value and Baceteriostatic Activites[a)]

a) c is the minimum bacteriostatic concentration in M.

On the mechanism of sulfonamide action, many possible explanations have been presented by Bell and Roblin,[16] Kumler and associates,[17,18] Klotz and Bordwell,[19,20] Seydel, *et al.*,[21] and Jardetzky, *et al.*,[22] but they are all based on the original suggestion of Woods[23] and Fildes[24] that sulfonamides interfere with the utilization of *p*–aminobenzoic acid (PABA) in enzyme systems through competitive enzyme inhibition. Chart 1 shows π–electron net charge distribution of PABA and sulfanilamide calculated by Pullman, *et al.*[25] and by Martin, *et al.*,[26] respectively. The net charge on N^4–nitrogen atom of sulfanilamide substantially equals that of PABA, and this may be approximately true in case of many N^1–substituted sulfanilamides. On the other hand, the π–electron distribution on sulfanylamide group of sulfanilamide seems considerably different from that on carboxyl group of PABA. This may be maintained by the fact that pK_b is 11.58 and 11.64, and that pK_a is 4.68 and 10.43, for PABA and sulfanilamide, respectively.[16] From this

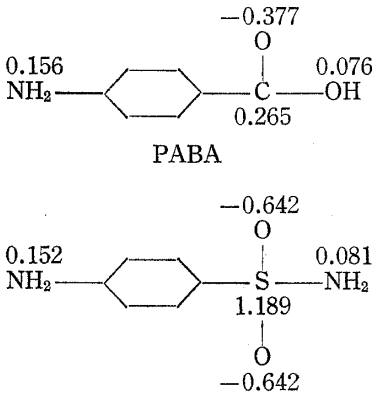

Chart 1. π–Electron Net Charge Distribution[a)] of *p*–Aminobenzoic Acid[25] and Sulfanilamide[26]

a) Calculated by HMO method using parameters of $h_{\ddot{N}}=1.0$, $k_{C-N}=0.9$, $h_{\dot{O}}=1.2$, $k_{C=O}=2.0$, $h_{\dot{S}}=0$, $k_{C=S}=0.6$, $k_{O=S}=2.0$, and $k_{N-S}=0.9$ for the both compounds.

16) P.H. Bell and R.O. Roblin, Jr., *J. Am. Chem. Soc.*, **64**, 2905 (1942).
17) W.D. Kumler and T.C. Daniels, *J. Am. Chem. Soc.*, **65**, 2190 (1943).
18) W.D. Kumler and J.J. Eiler, *J. Am. Chem. Soc.*, **65**, 2355 (1943).
19) I.M. Klotz, *J. Am. Chem. Soc.*, **66**, 459 (1944).
20) F.G. Bordwell and I.M. Klotz, *J. Am. Chem. Soc.*, **66**, 660 (1944).
21) J. Seydel, E. Krüger–Thiemer, and E. Wempe, *Z. Naturforsch.*, **15b**, 628 (1960).
22) O. Jardetzky and N.G. Wade–Jardetzky, *Mol. Pharmacol.*, **1**, 214 (1965).
23) D.D. Woods, *Brit. J. Exp. Pathol.*, **21**, 74 (1940).
24) P. Fildes, *Lancet*, **1940, I**, 955.
25) Page 802 of Ref. 11).
26) R.S. Schnaare and A.N. Martin, *J. Pharm. Sci.*, **54**, 1707 (1965).

and a number of theories[16-20,27] proposed on the mode of action of sulfonamides, it may be followed that there are two binding sites essential to the action located apart about 6.5 to 7 Å each other on the exzyme receptor: a site for specific binding of N^4–nitrogen and a cationic site for non–specific and electrostatic binding of the acidic group of PABA or sulfonamides. Besides these major bindings, there may possibly be other non–specific and non–essential bindings somewhat contributing to the increasing action such as hydrophobic binding, hydrogen binding, charge transfer forces, and π–hydrogen binding between the substituents on N^1 of sulfonamides and the receptor protein. The strength of the specific binding of N^4–nitrogen to the receptor may be approximately same with PABA and many sulfonamides, while the strength may be diverse in case of non–specific bindings. It was reported that the ability of non–specific binding of sulfonamides to a number of different proteins and macromolecular substances increased approximately in the same sequence as to serum albumin.[15] Accordingly the explanation on the relationship between bacteriostatic activities and ability of protein bindings of sulfonamides proposed in the previous paper[1] may be reasonable.

From the general point of view, however, the activity of sulfonamides seems to be related to many other properties such as solubility, permeability to biological membranes, and metabolization rate. Most of these properties may be more or less dependent on the electronic structure of the molecules. Consequently, it may be a matter of course that the Q–value, one of the indices for the electronic structure, is approximately related to the bacteriostatic activities as well as the protein–binding properties of sulfonamides. A better correlation is probably expected by a more complete calculation procedure.

Anyway, it is of great interest to note that some biological properties can be predicted by the calculation of an electronic index, and furthermore that the index may play the part of a pilot for creation of new potent sulfonamides.

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27) M. Tsuruoka, *Yakugaku Zasshi*, **71**, 336, 347, 350, 354 (1951).

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Release of Lipids from Red Cell Membrane by Surface-active Agents

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In due course of studies on the hemolytic action of homologous surface–active electroytes, a marked difference in the action between the surface–active cations and anions has been found[2]. Thus, it has been demonstrated that the cations with shorter alkyl chain than octyl radical still keep the hemolytic activity, while the corresponding members of the anions are not

1) Location: *12 Funakawara–machi, Ichigaya, Shinjuku–ku, Tokyo.*
2) T. Kondo and M. Tomizawa, *J. Colloid & Interface Sci.*, **21**, 224 (1966).