# Biological Implications of Molecular and Crystal Structures of Sulfadimethoxine, Sulfadoxine, and Sulfisoxazole

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**Abstract** □ X-ray crystallographic methods were used to determine the structures of sulfadimethoxine, sulfadoxine, and sulfisoxazole. The molecules have similar conformations about their sulfonamide linkage (S—N1) but have markedly different orientations of their respective heterocyclic rings relative to the sulfanilamide portion of the molecule. There appears to be a resemblance of these structures to a molecular model constructed of aminobenzoic acid and glutamic acid.

**Keyphrases** □ Sulfadimethoxine—crystallography, relationship to biological activity □ Sulfadoxine—crystallography, relationship to biological activity □ Sulfisoxazole—crystallography, relationship to biological activity □ Sulfa drugs—crystallography, relationship to biological activity □ Structure-activity relationships—sulfonamides □ Sulfonamides—crystallography, relationship to biological activity □ X-ray crystallography—sulfonamides, structure-activity relationships

Numerous attempts have been made to correlate the antibacterial activity of sulfonamides with various physical and pharmacological properties such as pKa, protein binding, electronic charge distribution, and urine and lipid solubilities (1–3). However, despite the great deal of information available on sulfonamides, no structure–activity relationships with general applicability appear to have been formulated. The present study was undertaken to obtain detailed structural information on three sulfa drugs whose properties have been widely studied: sulfadimethoxine, sulfadoxine, and sulfisoxazole[1]. By furnishing information about the conformations and the hydrogen-bonding capabilities of these three sulfonamides, it was hoped that an understanding of their biological characteristics on a molecular level could be derived.

## EXPERIMENTAL

The crystal data are as follows:

| | sulfadimethoxine | sulfadoxine | sulfisoxazole |
|---|---|---|---|
| $a$ | 10.411 (2) Å | 8.873 (2) Å | 11.578 (7) Å |
| $b$ | 9.309 (2) Å | 8.784 (1) Å | 14.151 (11) Å |
| $c$ | 7.951 (1) Å | 18.938 (5) Å | 14.811 (12) Å |
| $\alpha$ | 114.11 (2)° | 90.00° | 90.00° |
| $\beta$ | 95.43 (2)° | 107.64 (2)° | 90.00° |
| $\gamma$ | 93.38 (3)° | 90.00° | 90.00° |
| $d_0$ | 1.51 g./cm.$^3$ | 1.47 g./cm.$^3$ | 1.45 g./cm.$^3$ |
| $d_c$ | 1.48 g./cm.$^3$ | 1.47 g./cm.$^3$ | 1.42 g./cm.$^3$ |
| Z | 2 | 4 | 8 |
| Space group | $P\bar{1}$ | $P2_1/c$ | Pcab |

Intensity data for both sulfadimethoxine and sulfadoxine were collected by the stationary counter-stationary crystal method on a quarter-circle diffractometer[2], using nickel-filtered Cu$K_\alpha$ radiation, out to a maximum $2\theta$ value of 110° for each. For sulfisoxazole, the moving crystal–moving detector method on a diffractometer[3] was used for data collection (maximum $2\theta$ was 140°). The data in each case were corrected for absorption, Lorentz-polarization effects, and $\alpha_1$–$\alpha_2$ splitting where appropriate.

The structure of sulfadimethoxine was solved by direct methods through application of the Sayre (4) relationship, while the structures of sulfadoxine and sulfisoxazole were solved by use of Patterson and Fourier syntheses. In each case, all hydrogen atoms were located in difference electron density maps, calculated near the conclusion of least-squares refinement. The final unweighted residual factors calculated for each structure at the conclusion of least-squares refinement (block diagonal approximations used) are:

| | |
|---|---|
| sulfadimethoxine: | $R_1$ = 0.047 for 1577 independent observed reflections |
| sulfadoxine: | $R_1$ = 0.071 for 1774 independent observed reflections |
| sulfisoxazole: | $R_1$ = 0.048 for 1379 independent observed reflections |

The estimated standard deviations for bond distances and angles are, in general, for the three structures 0.004 Å and 0.2° for those bonds involving sulfur and 0.007 Å and 0.5° for those involving the other nonhydrogen atoms. The errors in the hydrogen parameters are about 10 times as great as those of the nonhydrogen atoms.

The final atomic positional and thermal parameters of each structure are given in Tables I and II. Tabulations of the structure factor amplitudes for the various structures may be obtained from the authors.

## RESULTS AND DISCUSSION

The intramolecular bond distances and angles of the three structures are shown in Fig. 1. In each case the amido tautomer (hydrogen atom attached to N1) is the stable form in the solid. Of the sulfonamide structures capable of attaining the imido configuration (hydrogen on N2), only sulfathiazole was reported to prefer this tautomer in the crystal (5, 6).

Kumler and Halverstadt (7) and Kumler and Strait (8) suggested that a factor which might influence the activity of a sulfonamide is the ability of the sulfanilamide portion of the molecule to form the resonance quinoid structure. Their dipole moment measurements indicated that there is a very small but significant contribution of this resonance form to a number of sulfonamide derivatives. The sulfadoxine and sulfadimethoxine structures both show that their phenyl rings have a small amount of quinoidal character (*i.e.*, length of C5—C6 and C2—C3 bonds are shorter than the other phenyl ring bonds). In the sulfisoxazole structure, the C2—C3 and C1—C6 bonds are found to be shorter than the benzene C—C bond (1.392 ± 0.004 Å) (9). The phenyl ring of sulfanilamide (10) as well as other aminophenyl analogs (11) has also been shown to contain analogous shortening.

In the sulfanilamide portion of each molecule, the principal structural differences observed are:

1. The C4—N4 distance in sulfadoxine is shorter than the corresponding length in sulfadimethoxine which, in turn, is slightly shorter than that in sulfisoxazole. This appears to be a function of the hydrogen bonds emanating from N4. The two protons of sul-

[1] Sulfadimethoxine, sulfadoxine, and sulfisoxazole are the active ingredients in Madribon, Fanzil, and Gantrisin, respectively (Hoffmann–La Roche Inc.).

[2] General Electric XRD-6.

[3] Hilger-Watts model Y 290.

Table I—Positional and Thermal Parameters for Nonhydrogen Atoms together with Their Estimated Standard Deviations (in Parentheses) × $10^4$[a]

| Atom | $x$ | $y$ | $z$ | $b_{11}$ | $b_{22}$ | $b_{33}$ | $b_{12}$ | $b_{13}$ | $b_{23}$ |
|---|---|---|---|---|---|---|---|---|---|
| **Sulfisoxazole** | | | | | | | | | |
| S | 5407(1) | 1659(1) | 981(1) | 93(1) | 38(0.3) | 52(1) | 8(1) | −7(1) | −6(1) |
| O(1) | 4640(2) | 1225(1) | 698(2) | 136(3) | 59(2) | 79(2) | 68(4) | 10(4) | −6(3) |
| O(2) | 6755(2) | 1156(2) | 830(2) | 129(2) | 47(1) | 71(2) | −45(4) | −30(4) | −4(3) |
| O(3) | 3733(2) | 2244(1) | 2264(1) | 77(2) | 61(3) | 67(3) | −18(3) | −12(3) | −16(3) |
| N(1) | 5669(3) | 1930(2) | 1998(2) | 76(2) | 48(2) | 52(3) | 16(4) | −14(4) | 10(3) |
| N(2) | 3036(2) | 2927(2) | 2680(2) | 70(2) | 72(2) | 65(2) | −10(4) | 1(4) | −20(3) |
| N(4) | 6097(5) | 5079(3) | −1191(2) | 149(5) | 69(3) | 66(2) | −44(6) | −16(6) | 45(4) |
| C(1) | 5791(3) | 2680(2) | 315(2) | 80(3) | 46(2) | 39(1) | −4(4) | −5(4) | −9(3) |
| C(2) | 6802(3) | 3216(2) | 364(2) | 80(3) | 62(2) | 48(2) | 2(5) | −5(5) | −1(3) |
| C(3) | 6884(4) | 4004(2) | −127(2) | 86(3) | 62(3) | 52(2) | −38(5) | 8(5) | 4(3) |
| C(4) | 5983(3) | 4294(2) | −677(2) | 96(4) | 56(2) | 41(3) | −18(4) | −1(4) | 4(3) |
| C(5) | 4989(4) | 3761(3) | −715(2) | 101(4) | 66(3) | 58(2) | −6(6) | −27(5) | 27(4) |
| C(6) | 4894(3) | 2955(3) | −209(2) | 83(3) | 64(2) | 57(2) | −38(6) | −30(5) | 6(4) |
| C(7) | 4843(3) | 2537(2) | 2328(2) | 66(3) | 53(2) | 39(2) | −14(4) | −21(4) | 22(3) |
| C(8) | 4913(3) | 3350(2) | 2765(2) | 66(3) | 49(2) | 47(2) | −14(4) | −9(4) | 12(3) |
| C(9) | 3753(3) | 3551(2) | 2981(2) | 81(3) | 59(2) | 42(2) | 1(4) | −18(4) | 0(3) |
| C(10) | 5964(5) | 3906(4) | 2984(4) | 88(4) | 70(3) | 99(4) | −24(7) | −16(7) | −18(6) |
| C(11) | 3306(5) | 4376(4) | 3460(3) | 112(5) | 75(3) | 71(3) | 12(6) | 7(6) | −20(5) |
| **Sulfadimethoxine** | | | | | | | | | |
| S | −1642(1) | −1959(1) | 4129(1) | 86(1) | 123(1) | 149(2) | 43(2) | 20(2) | 131(2) |
| O(1) | −2626(2) | −2832(3) | 4572(3) | 115(3) | 160(4) | 190(6) | 47(5) | 87(6) | 200(8) |
| O(2) | −374(2) | −1615(3) | 5186(3) | 95(3) | 154(4) | 189(5) | 53(5) | −40(6) | 157(8) |
| O(3) | −4089(2) | 3451(3) | 3263(4) | 94(3) | 120(4) | 275(7) | 36(5) | −36(7) | 159(8) |
| O(4) | −6372(2) | −1427(3) | 1357(4) | 72(3) | 163(4) | 268(7) | −10(5) | −19(6) | 193(9) |
| N(1) | −2071(3) | −195(3) | 4546(4) | 74(3) | 121(4) | 156(5) | 12(5) | −33(7) | 93(8) |
| N(2) | −3146(3) | 1619(3) | 3895(4) | 75(3) | 117(4) | 159(6) | 40(5) | 11(7) | 95(8) |
| N(3) | −5288(2) | 1042(3) | 2278(4) | 67(3) | 133(4) | 143(6) | 42(5) | 29(6) | 102(8) |
| N(4) | −1230(3) | −5162(4) | −3866(4) | 147(4) | 137(5) | 161(6) | 32(7) | 42(8) | 115(9) |
| C(1) | −1548(3) | −2889(4) | 1748(4) | 76(3) | 104(5) | 134(7) | 28(6) | 29(7) | 93(9) |
| C(2) | −961(3) | −2070(4) | 861(5) | 93(4) | 108(5) | 166(7) | −4(7) | 14(8) | 94(10) |
| C(3) | −865(3) | −2822(4) | −998(5) | 91(4) | 131(6) | 187(8) | 3(7) | 52(9) | 162(11) |
| C(4) | −1351(3) | −4403(4) | −2019(4) | 71(3) | 122(5) | 148(7) | 48(6) | 16(8) | 100(10) |
| C(5) | −1928(4) | −5205(4) | −1093(5) | 112(4) | 96(5) | 200(8) | 1(7) | 39(9) | 93(10) |
| C(6) | −2037(4) | −4458(4) | 762(5) | 108(4) | 116(5) | 186(8) | 11(7) | 68(9) | 135(11) |
| C(7) | −3208(3) | 137(4) | 3748(4) | 72(3) | 118(5) | 115(6) | 47(6) | 44(7) | 78(9) |
| C(8) | −4204(3) | 1978(4) | 3132(4) | 87(4) | 111(5) | 148(7) | 55(6) | 49(8) | 91(10) |
| C(9) | −8309(3) | −406(4) | 2191(4) | 67(3) | 135(5) | 142(7) | 15(6) | 49(8) | 99(10) |
| C(10) | −4283(3) | −944(4) | 2914(5) | 81(3) | 117(5) | 174(7) | 31(7) | 40(8) | 129(10) |
| C(11) | −5219(4) | 3958(5) | 2526(6) | 116(5) | 139(6) | 294(11) | 79(8) | −25(11) | 183(13) |
| C(12) | −7463(4) | −851(5) | 676(6) | 72(4) | 242(8) | 263(10) | 11(9) | 4(10) | 237(15) |
| **Sulfadoxine** | | | | | | | | | |
| S | 916(1) | 1456(1) | 1014(1) | 120(2) | 97(2) | 16(0.3) | −34(3) | 19(1) | −11(1) |
| O(1) | 726(4) | 2490(4) | 1566(2) | 143(5) | 125(5) | 20(1) | −16(9) | 31(4) | −29(4) |
| O(2) | −219(4) | 252(4) | 778(2) | 135(5) | 116(5) | 22(1) | −82(9) | 28(4) | −10(4) |
| O(3) | 2674(4) | 5562(4) | −1296(2) | 198(7) | 124(5) | 27(1) | −48(10) | 59(5) | 3(4) |
| O(4) | 1016(4) | 2983(4) | −1114(2) | 185(6) | 93(5) | 17(1) | −18(9) | 15(4) | −15(4) |
| N(1) | 757(5) | 2389(4) | 243(2) | 141(6) | 101(6) | 15(1) | −39(10) | 9(4) | −12(4) |
| N(2) | 2415(5) | 4468(5) | 749(2) | 178(7) | 110(6) | 20(1) | −72(11) | 16(5) | −10(5) |
| N(3) | 3292(5) | 6118(5) | −50(2) | 194(8) | 107(6) | 25(2) | −66(12) | 32(5) | −5(5) |
| N(4) | 7317(5) | −1179(6) | 1980(2) | 168(8) | 182(8) | 26(2) | 128(13) | 19(6) | 13(6) |
| C(1) | 2825(5) | 697(5) | 1301(2) | 128(7) | 91(7) | 15(1) | −38(11) | 23(5) | −14(5) |
| C(2) | 3439(6) | 27(6) | 786(2) | 138(8) | 109(7) | 15(2) | −3(12) | 5(5) | −16(5) |
| C(3) | 4926(6) | −609(6) | 1012(3) | 163(9) | 109(7) | 19(2) | 10(13) | 35(6) | −23(6) |
| C(4) | 5829(6) | −605(5) | 1761(3) | 157(8) | 87(7) | 19(2) | 3(12) | 27(6) | 9(5) |
| C(5) | 5146(6) | 13(7) | 2270(3) | 171(9) | 146(8) | 16(2) | 17(4) | 6(6) | −1(6) |
| C(6) | 3677(6) | 671(6) | 2052(3) | 155(9) | 128(8) | 16(2) | 31(13) | 27(6) | −3(6) |
| C(7) | 1663(5) | 3634(5) | 156(2) | 113(7) | 68(6) | 19(1) | 1(11) | 8(5) | 4(5) |
| C(8) | 3178(7) | 5666(6) | 598(3) | 216(11) | 117(8) | 24(2) | −99(5) | 10(7) | −23(6) |
| C(9) | 2579(6) | 5249(6) | −618(3) | 133(8) | 107(7) | 23(2) | 29(12) | 29(6) | 13(6) |
| C(10) | 1711(5) | 3960(5) | −546(3) | 126(7) | 77(6) | 20(2) | 15(11) | 22(5) | 3(5) |
| C(11) | 3346(7) | 7021(7) | −1386(3) | 209(11) | 148(9) | 36(2) | −71(16) | 76(8) | 37(8) |
| C(12) | −231(7) | 3592(8) | −1733(3) | 178(10) | 202(11) | 28(2) | 82(18) | −9(7) | −27(8) |

[a] Temperature coefficient = $\exp -(b_{11}h^2 + b_{22}k^2 + b_{33}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)$.

fadoxine are involved in hydrogen bonds, whereas one N4 proton of sulfadimethoxine is participating in such an interaction, and none in the sulfisoxazole crystal structure is involved.

2. The S—O bonds, where the oxygen is involved in an N—H hydrogen bond, are longer than those where such intermolecular bonding is absent. Such observations have been made with other sulfonamide structures (5, 6, 12).

3. The S—N1—C7 angle for sulfisoxazole is closer to a trigonal value than that found for the other compounds.

These sulfonamides are capable of a variety of conformational states by means of rotation about the three bonds C1—S, S—N1, and N1—C7 (5). The three dihedral angles [calculated in the manner prescribed by Klyne and Prelog (13)] describing these conformations are $\phi$ C1—S—N1—C7, $\phi$ S—N1—C7—N2 (O3), and $\phi$ C6—C1—S—N2 or $\phi$ C2—C1—S—N1, the choice being the acute angle. The choice of the smaller of the latter two angles resolves the twofold symmetry ambiguity of the *p*-aminophenyl system. In Table III the torsion angles about these bonds for the three sulfonamides are compared. It can be seen that the only significant conformational difference between sulfadimethoxine and sulfadoxine involves a rotation of about 180° about the N1—C7 bond. Therefore, in sulfadimethoxine the H(N1) is eclipsed with N2 [H(N1)...N2 distance

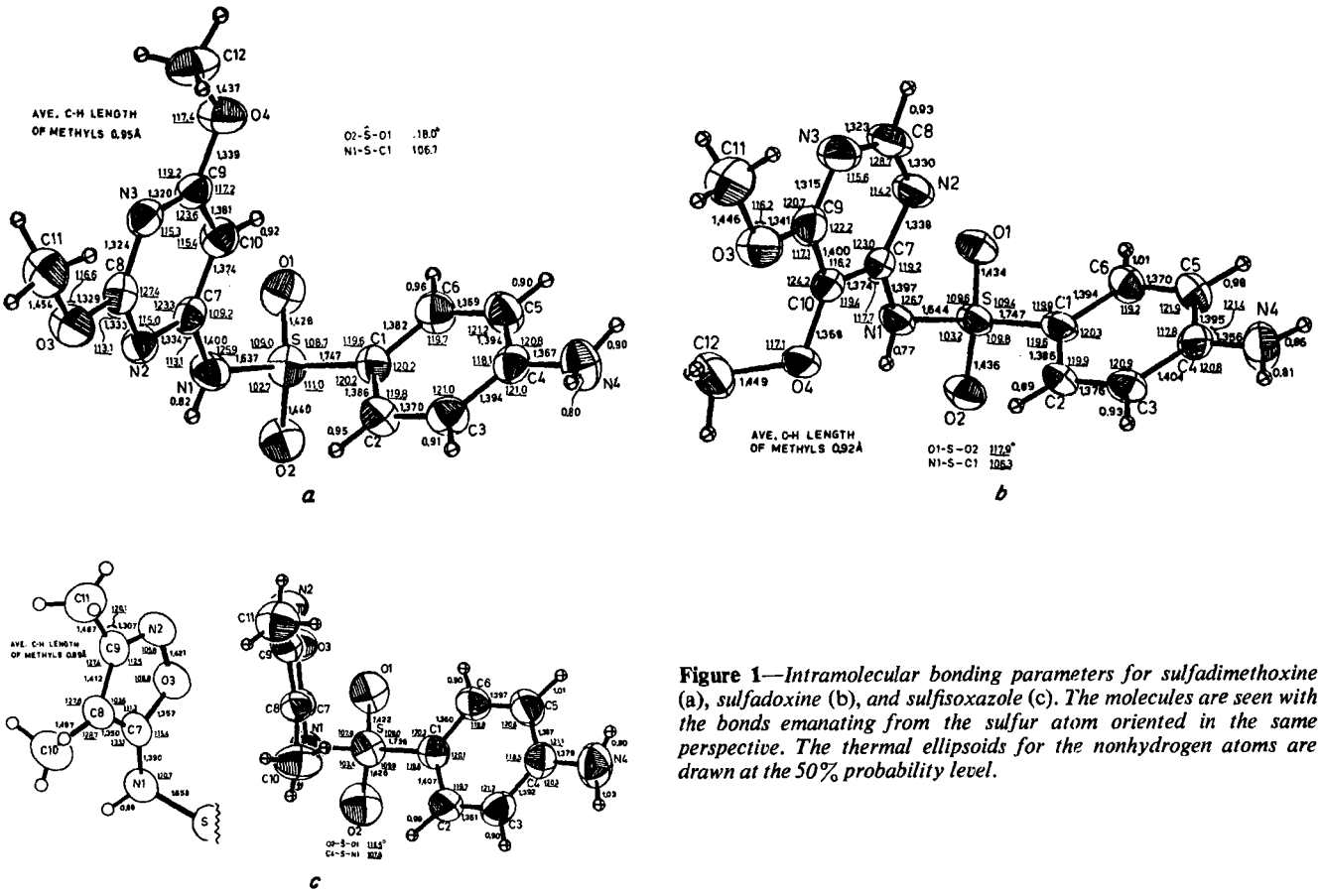

**Figure 1**—*Intramolecular bonding parameters for sulfadimethoxine* (a), *sulfadoxine* (b), *and sulfisoxazole* (c). *The molecules are seen with the bonds emanating from the sulfur atom oriented in the same perspective. The thermal ellipsoids for the nonhydrogen atoms are drawn at the 50% probability level.*

equals 2.29 Å] when projected down the N1—C7 bond, while in sulfadoxine they are *trans* to each other (Fig. 1).

The sulfonamide linkages (S—N1) are conformationally quite similar in the three structures. They do, however, differ substantially from the spatial arrangement found for the C1—S—N1—C7 backbone of sulfathiazole. Sulfathiazole, which prefers the imide configuration rather than the amide form present in these compounds, imparts the larger magnitude for this torsion angle.

The variability in torsion angles about the S—C1 bonds of the compounds of Table III arises from the degree of rotational freedom about this bond. Space-filling molecular models[4] of various sulfonamide structures show that there is indeed a great deal of rotational freedom for the phenyl ring to turn about the S—C1 bond. The ring system attached to N1 poses the only major barrier to complete rotational freedom, with apparently minor hindrance coming from the sulfur oxygens (O1 and O2). Dihedral angles between 40 and 90° represent the most sterically preferred values. The actual angle found in any particular case is no doubt influenced to a certain extent by crystal packing forces.

The molecular models of these sulfonamides show that rotation about the N1—C7 bond is quite restricted. Although the models indicate that the conformational flexibility about this bond is steric in nature (the phenyl ring restricts the torsional mobility of the heterocyclic ring system attached to N1), the small amount of double-bond character in the N1—C7 bond of each structure may have a further limiting effect.

The oxazole and phenyl rings of sulfisoxazole are folded closer toward each other than the pyrimidine and phenyl rings of the other two sulfonamides. The dihedral angle between the two rings is 68° for sulfisoxazole, 98° for sulfadoxine, and 106° for sulfadimethoxine. This folding effect can be seen clearly in Fig. 1.

In sulfadimethoxine the two methoxy substituents are located at positions 2 and 4 of the pyrimidine ring; *i.e.*, they are *ortho* and *para* with respect to the N2 position. Both of these groups are coplanar within $3\sigma$ with the heterocyclic ring (Table IV). The O3—C8 and O4—C9 distances (1.329 and 1.339 Å, respectively) indicate that substantial double-bond character exists in these bonds (14). As electron donors, the two methoxy groups should increase the basicity of N2 of the pyrimidine ring.

On the other hand, the two methoxy groups of sulfadoxine are *para* and *meta* to the N2 position, but only the *para* group can increase the basicity of N2 by resonance. Although the methyl of the *para*-methoxy group on C9 is 10° from coplanarity with the pyrimidine ring, the methyl of the *meta*-methoxy group is bent 52° out of the plane of the heterocyclic ring. Moreover, the O4—C10 bond distance of 1.370 Å is significantly longer (about 0.03 Å) than the

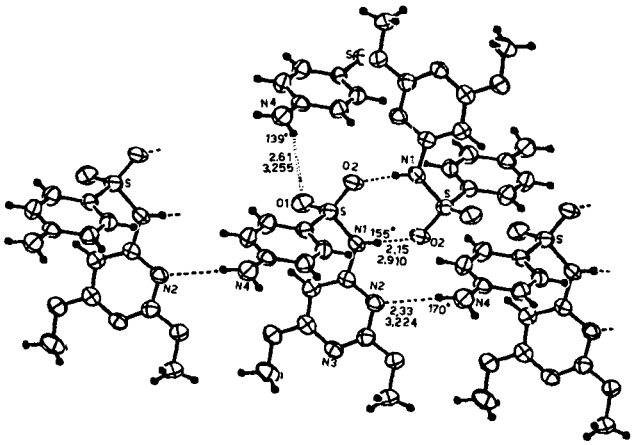

**Figure 2**—*A general view showing the intermolecular hydrogen bonds* (*dashed lines*) *in the sulfadimethoxine structure. The dotted line represents a contact that is only slightly longer than an acceptable hydrogen-bond length. The smaller length is that between the proton and the acceptor atom, while the larger is the distance between the nonhydrogen atoms.*

[4] CPK molecular models, Ealing Corp., Cambridge, Mass.

**Table II**—Positional and Thermal Parameters for Hydrogens

| Atom | $x \cdot 10^3$ | $y \cdot 10^3$ | $z \cdot 10^3$ | Biso (Å²) |
|---|---|---|---|---|
| | | **Sulfisoxazole** | | |
| H(N1) | 636(3) | 204(2) | 222(2) | 4.1(0.9) |
| H(C2) | 736(4) | 293(3) | 78(3) | 6.9(1.2) |
| H(C3) | 757(3) | 429(2) | −10(2) | 4.4(0.9) |
| H(C5) | 439(4) | 403(3) | −114(3) | 8.4(1.4) |
| H(C6) | 422(3) | 260(2) | −22(2) | 3.5(0.7) |
| H1(N4) | 684(4) | 545(3) | −108(3) | 6.3(1.1) |
| H2(N4) | 547(5) | 521(4) | −138(4) | 10.4(2.2) |
| H1(C10) | 569(5) | 447(4) | 279(4) | 8.9(1.7) |
| H2(C10) | 588(6) | 419(5) | 362(5) | 12.4(2.4) |
| H3(C10) | 659(5) | 352(4) | 295(4) | 9.7(1.8) |
| H1(C11) | 372(6) | 448(5) | 400(5) | 14.1(2.4) |
| H2(C11) | 255(6) | 448(4) | 342(4) | 10.3(1.8) |
| H3(C11) | 337(6) | 510(6) | 317(5) | 16.3(2.4) |
| | | **Sulfadimethoxine** | | |
| H(N1) | — | — | — | — |
| H(C2) | −66(3) | −98(4) | 152(4) | 3.9(0.7) |
| H(C5) | −224(3) | −623(4) | 175(5) | 3.9(0.7) |
| H(C3) | −49(4) | −228(4) | −159(5) | 5.4(0.9) |
| H(C6) | −247(3) | −506(4) | 131(5) | 5.2(0.9) |
| H1(N4) | −168(4) | −613(4) | −451(5) | 5.6(0.9) |
| H2(N4) | −116(4) | −453(5) | −430(6) | 6.9(1.0) |
| H(C10) | −430(3) | −198(4) | 273(4) | 3.6(0.7) |
| H1(C11) | −606(4) | 383(5) | 314(6) | 6.6(1.0) |
| H2(C11) | −542(4) | 333(5) | 125(6) | 7.3(1.1) |
| H3(C11) | −500 | 496(5) | 284(6) | 6.8(1.0) |
| H1(C12) | −811(4) | −178(5) | 13(6) | 7.0(1.0) |
| H2(C12) | −726(4) | −43(5) | −16(6) | 6.3(1.0) |
| H3(C12) | −782(5) | −16(6) | 162(7) | 8.8(1.3) |
| | | **Sulfadoxine** | | |
| H(N1) | 48(5) | 180(5) | −7(2) | 2.3(0.8) |
| H(C2) | 291(5) | 6(5) | 30(2) | 2.9(0.9) |
| H(C3) | 529(5) | −108(6) | 65(3) | 3.9(1.1) |
| H(C5) | 578(6) | −2(7) | 279(3) | 4.8(1.2) |
| H(C6) | 316(5) | 110(6) | 242(3) | 3.6(1.0) |
| H1(N4) | 769(6) | −160(6) | 169(3) | 4.7(1.2) |
| H2(N4) | 771(6) | −129(6) | 245(3) | 5.1(1.3) |
| H(C8) | 379(5) | 632(5) | 98(3) | 3.6(1.0) |
| H1(C11) | 444(6) | 714(6) | −110(3) | 5.4(1.3) |
| H2(C11) | 330(6) | 710(6) | −182(3) | 4.6(1.2) |
| H3(C11) | 280(8) | 787(8) | −122(4) | 8.0(1.8) |
| H1(C12) | −65(8) | 270(8) | −204(4) | 8.6(1.9) |
| H2(C12) | 33(11) | 402(11) | −210(5) | 12.4(2.7) |
| H3(C12) | −95(9) | 375(9) | −165(4) | 9.8(2.0) |

O3—C9 bond and the two corresponding distances in sulfadimethoxine. It thus appears that the *meta*-methoxy group has less conjugation with the pyrimidine ring than the *para* one. As a result, N2 of sulfadoxine should not be as basic as N2 in sulfadimethoxine; moreover, the sulfadoxine O4 atom is more basic than the other methoxy oxygens, as evidenced by its participation in an intermolecular hydrogen bond.

The molecular composition of the heterocyclic ring in sulfisoxazole is quite different from that of both sulfadimethoxine and sulfadoxine. The N2—C9 bond distance of 1.307 Å indicates a double bond (14). Despite the fact that methyl groups are usually poor hydrogen donors, both methyl groups in sulfisoxazole appear to be involved in short intermolecular hydrogen interactions. This might suggest that the electronic distribution of the isoxazole ring makes the hydrogens of the two methyl groups more acidic than usual.

In Figs. 2-4, the intermolecular hydrogen-bonding schemes of the three sulfonamides are shown. For sulfadimethoxine, the dominant intermolecular binding consists of a pair of centrosymmetrically related hydrogen bonds between N1—H and O2. In addition, there is another hydrogen bridge between N2 and H(N4). Sulfadoxine also has cyclic centrosymmetric hydrogen bonds between N1—H and O2. The ring nitrogen N2, however, is not involved in hydrogen bonding. The O1 atom of the sulfone moiety and the methoxy oxygen O4 are hydrogen bonded to the two protons on the anilino nitrogen N4 in this structure. The O1 of sulfadimethoxine does not appear to be involved in any significant intermolecular interaction.

Each sulfisoxazole molecule is involved in several short interactions, in which a carbon hydrogen appears to be the donor atom

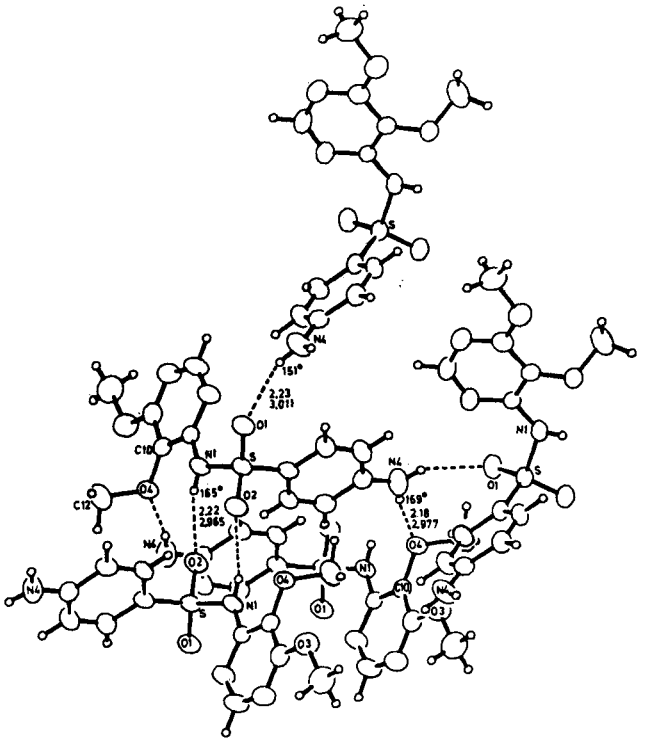

**Figure 3**—*Intermolecular hydrogen bonds observed in the sulfadoxine crystal.*

(Fig. 4). Interactions of this type are, in general, weaker than those of the normal hydrogen bond, in which an oxygen or nitrogen proton is the donor atom[5]. Of particular interest is the fact that N2 interacts weakly with three different protons of two adjacent molecules: H(C2), H(N1), and H(C10). A sulfone oxygen (O2) apparently acts as a hydrogen-bonding acceptor for a proton from the C11 methyl group.

As indicated previously, attempts to correlate biological activities of the sulfonamides with various parameters such as pKa, solubilities, and electronic charge distribution have been proposed. Included among these are studies involving protein binding, primarily those associated with serum albumin. The pharmacokinetics and activity of a sulfonamide are greatly influenced by the extent of its involvement in protein binding. Extensive experimentation (3)

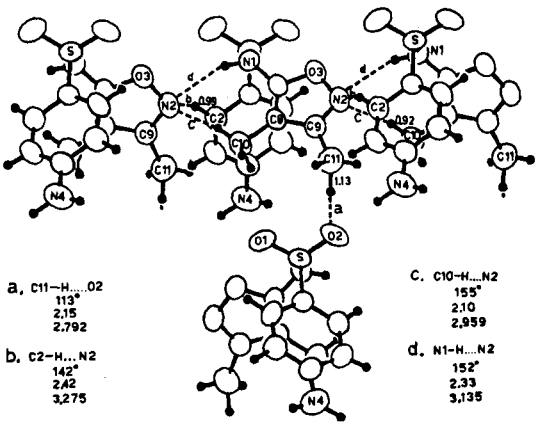


**Figure 4**—*The short intermolecular hydrogen contacts observed for sulfisoxazole are denoted.*

[5] Although the existence of C—H hydrogen bonds are well documented in the literature, much discussion has appeared relative to what distance between the proton and the acceptor atom results in an intermolecular bond (see *Reference 20*).

**Table III**—Conformational and Biological Parameters for a Number of Sulfonamides

| Compound | N1SC1C6[C2] | C1SN1C7 | SN1C7N2[O3] | Serum Albumin Binding | | Concentration Effective against *Escherichia coli* (*In Vitro*)[a], μmoles/l. |
|---|---|---|---|---|---|---|
| | | | | % Bound[a] | log $K$[b] | |
| Sulfadimethoxine[c] | 44° | 59° | −162° | 99 | 5.4 | 0.7 |
| Sulfadoxine[c] | 43 | 61 | 21 | 95 | | 0.8 |
| Sulfisoxazole[c] | 68 | −55 | −64 | 86 | 5.0 | 2.2 |
| Sulfathiazole | | | | | | |
| –theophylline complex[d] | 87 | 85 | −173 | 77 | 3.1 | 1.6 |
| –sulfanilamide complex[d] | 57 | 80 | −174 | — | — | — |
| Sulfanilamide | 59–90° | — | — | 12 | 2.7 | 128 |

[a] *Reference 2* is the source of data. [b] Average binding constant from *Reference 15*. [c] Values given are for the chiral isomer of the molecule whose coordinates are published. For other enantiomers, the sign of the angle is changed only. [d] *Reference 5*.

showed that, in general, the sulfonamides have a greater affinity for serum albumin over other blood proteins. High resolution NMR studies (16) clearly indicated that the primary binding site on albumin pertains to the *p*-aminobenzenesulfonamide moiety. However, the various substituents attached to N1 of the basic sulfanilamide molecule markedly influence the extent of the binding (Table III). The question as to the specific structural requirements of these N1 substituents which are important to binding does not appear to have been clearly demonstrated.

On studying the intermolecular bonding parameters of the various sulfonamide structures, it appears that the characteristics of certain hydrogen bonds could possibly be important determinants for serum albumin binding. Both sulfadimethoxine and sulfadoxine have three hydrogen interactions evolving from their structures in a similar spatial arrangement. The hydrogen bonds are nearly coparallel (closer to being parallel in the sulfadimethoxine structure) and involve O2, H(N1), and either a methoxy oxygen (sulfadoxine) or the pyrimidine N2 (sulfadimethoxine). The arrangement of these interactions may be important for the increased protein binding found for these molecules, due to a hydrogen-bonded interaction to a secondary site on the albumin molecule.

Sulfonamides are known to be effective inhibitors of the enzymatic

**Table IV**—Least-Squares Planes[a]

**Sulfadimethoxine**

| Benzene Ring Atom | Benzene Ring Displacement, Å | Pyrimidine Ring Atom | Pyrimidine Ring Displacement, Å |
|---|---|---|---|
| *C1 | 0.000 | *C7 | −0.007 |
| *C2 | 0.001 | *C8 | 0.000 |
| *C3 | 0.000 | *C9 | 0.003 |
| *C4 | −0.004 | *C10 | 0.003 |
| *C5 | 0.005 | *N2 | 0.005 |
| *C6 | −0.004 | *N3 | −0.004 |
| S | 0.043 | O3 | −0.024 |
| N4 | 0.023 | O4 | 0.005 |

Equations[b]: Benzene $0.8895X - 0.4039Y + 0.2137Z - 0.1761 = 0$
Pyrimidine $-0.4840X - 0.0701Y + 0.8723Z - 4.1938 = 0$

**Sulfadoxine**

| Benzene Ring Atom | Benzene Ring Displacement, Å | Pyrimidine Ring Atom | Pyrimidine Ring Displacement, Å |
|---|---|---|---|
| *C1 | −0.018 | *C7 | −0.011 |
| *C2 | 0.013 | *C8 | 0.002 |
| *C3 | 0.005 | *C9 | 0.010 |
| *C4 | −0.019 | *C10 | 0.001 |
| *C5 | 0.015 | *N2 | 0.009 |
| *C6 | 0.003 | *N3 | −0.011 |
| S | 0.039 | O3 | 0.057 |
| N4 | −0.079 | O4 | 0.088 |
| | | N1 | −0.072 |

Equations: Benzene $-0.4587X - 0.8817Y + 0.1103Z + 1.0705 = 0$
Pyrimidine $0.8154X - 0.5588Y + 0.1514Z + 0.6004 = 0$

**Sulfisoxazole**

| Benzene Ring Atom | Benzene Ring Displacement, Å | Isoxazole Ring Atom | Isoxazole Ring Displacement, Å |
|---|---|---|---|
| *C1 | −0.006 | *C7 | −0.002 |
| *C2 | 0.002 | *C8 | −0.005 |
| *C3 | 0.002 | *C9 | 0.010 |
| *C4 | −0.002 | *O3 | 0.007 |
| *C5 | −0.002 | *N2 | −0.011 |
| *C6 | 0.006 | N1 | 0.090 |
| S | −0.052 | C10 | −0.006 |
| N4 | −0.045 | C11 | −0.001 |

Equations: Benzene $-0.3861X + 0.5198Y + 0.7620Z + 0.2033 = 0$
Oxazole $0.0903X - 0.4889Y + 0.8677Z - 1.7195 = 0$

[a] Atoms preceded by * are those for which the least-squares planes are calculated. [b] $X$, $Y$, and $Z$ correspond to the orthogonalized coordinates (in Angströms) parallel to $a$, $b$, and $c^*$.

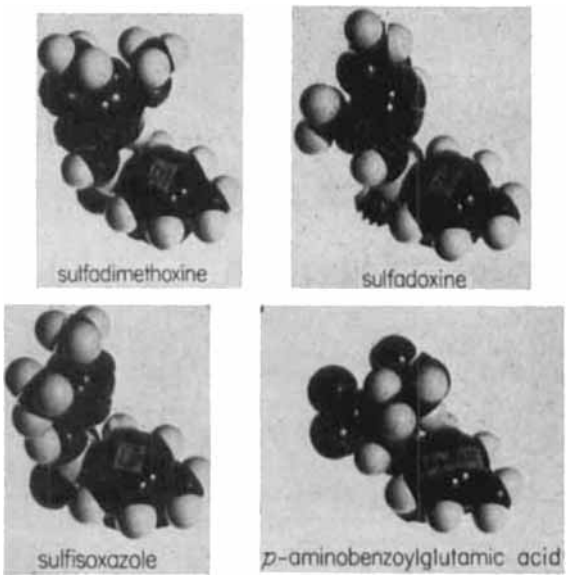


**Figure 5**—*Comparison of space-filling molecular models of the three sulfonamide structures with* p-*aminobenzoylglutamic acid.*

synthesis of folic acid compounds (17). Two bacterial enzyme systems capable of this synthesis were reported (18). One of these involves the formation of dihydropteroic acid from the condensation of aminobenzoic acid and a pteridine, with its subsequent addition to glutamic acid to complete the synthesis of the folate compounds. Alternatively, the folates are formed by direct coupling of *p*-aminobenzoyl glutamate with pteridines. Brown (19) demonstrated that sulfonamides are capable of antagonizing the enzymatic synthesis of folic acid by both routes.

Molecular models were constructed for the various sulfonamides studied, based on the structural data already presented. In addition, space-filling models of a number of possible conformers of aminobenzoic acid–glutamic acid were built. One of these has a certain degree of similarity with the constructed models of sulfadimethoxine, sulfadoxine, and sulfisoxazole (Fig. 5). As can be seen, in addition to the planar *p*-aminophenyl system common to all these structures, there is a basic atom found in both sulfadimethoxine and sulfadoxine, which corresponds to the position occupied by the α-carboxyl group of the glutamate residue. For sulfadoxine it is O4, and for sulfadimethoxine it is N2. The nitrogen of the heterocyclic ring of sulfathiazole (5, 6) is also present at a similar position in space. The other glutamate carboxyl group has a spatial orientation which corresponds with the O4 of sulfadimethoxine, the N2 of sulfisoxazole, and the exposed N3 of sulfadoxine. The position of the C10 methyl group of sulfisoxazole is in close spatial proximity to the methylene atoms of the glutamate residue. Common to each of these molecules is a hydrophobic region (a methyl group) extending upward from the heterocyclic rings. The C11 methyl group in each of these structures lies in a plane above the glutamate residue of the aminobenzoic acid–glutamic acid molecule.

The observed structural similarities between the sulfonamides and aminobenzoic acid–glutamate, as described here, may be responsible for their high biological activity. Therefore, the N1 substituent may function by competing for a site on the enzyme surface reserved for the glutamate residue. This could be accomplished by competing directly in the linking of aminobenzoic acid–glutamate with a pteridine or influencing the coupling of glutamate to the dihydropteroic acid.

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