After this period, a known volume of the dialyzate was evaporated to dryness, dissolved in a known volume of absolute ethanol, and spotted on a fluorescent thin-layer plate. Following multiple-pass, discontinuous developing in a developer consisting of ethyl acetate–absolute alcohol–10% aqueous ammonia (200:5:2), the air-dried plates were scanned using a thin-film scanning attachment for a spectrophotofluorometer[1]. An activation wavelength of 280 nm. and a fluorescent wavelength of 530 nm. were used. The scan was quantitated by comparing areas of unknowns to those of known standard compounds.

The data show that the exchange reaction did take place for all the nucleosides tried. Furthermore, for optimum conversion of 5-fluorouracil to 5-fluoro-2′-deoxyuridine, an excess of the nucleoside is necessary.

The implication for cancer chemotherapy is that one can, by using an excess of a relatively inexpensive, possibly nontoxic substance like deoxyuridine, obtain within the body at desired levels the more effective 5-fluoro-2′-deoxyuridine from 5-fluorouracil. Obtaining 5-fluoro-2′-deoxyuridine this way may provide an antitumor agent for such forms of cancer as rectal cancer, for which 5-fluoro-2′-deoxyuridine seems by far superior to 5-fluorouracil. The hope that such a conversion may in fact take place is supported by the results of the extensive study of inhibitors referred to earlier in this report (4). In light of the present results, Birnie and Heidelberger's observation of great toxicity in the use of uridine with 5-fluoro-2′-deoxyuridine may be explained in terms of the exchange reaction, where one of the products is the very toxic 5-fluorouridine. As an extension of this, it may be suggested that the effect which has up to now been attributed to inhibition of the nucleoside phosphorylase may be due to a contribution from the exchange reaction noted earlier.

Experiments to test this speculation of the increased efficacy of 5-fluorouracil and 5-fluoro-2′-deoxyuridine are now underway in mice[2]. Preliminary data on both solid and liquid tumors appear to bear out the initial findings. These results will be published.

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[2] Collaborative studies now being carried out in the laboratory of Dr. C. Heidelberger, University of Wisconsin.

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Conformational Stabilization by *o*-Methyl Groups of a Sulfanilamide

Keyphrases ☐ Sulfanilamides—conformational stabilization by *o*-methyl groups, NMR, PMR spectroscopy ☐ Conformation, sulfanilamides—NMR evidence for stabilization by *o*-methyl groups ☐ NMR spectroscopy—stabilization of sulfanilamides ☐ PMR spectroscopy—stabilization of sulfanilamides

Sir:

The conformational attributes of drug molecules are frequently alluded to in order to account for variations in drug response among members of a noncongeneric or pseudocongeneric series. Conformationally distinct molecules might be expected to differ in their physical properties, *e.g.*, partition coefficient (1) or pKa (2), and in this way differences between the magnitudes of their biological responses could be "explained." Alternatively, the conformational distinction between molecules could be translated at the receptor level as providing differing degrees of stimulus which, in turn, become elicited as variations in the relative responses. The relative importance of each of these alternatives most probably will have to be established for each drug system of interest, but studies having this intent are notably lacking in the literature. In view of recent progress in the study of sulfonamide action (3, 4), it seems appropriate to point out certain of our experimental findings which have significance in relation to the conformational attributes of sulfanilamides in solution.

Table I—Calculated Chemical Shifts for the Sulfanilyl Ring Protons of Some N^1-Phenylsulfanilamides

Anilyl Ring Substituent	Absorbance of N^4-Acetyl Derivative	$\bar{\nu}$, Hz.[a]	Jobs, Hz.	*Ortho* δ_A, Hz.	*Meta* δ_B, Hz.
2-CH_3O	S	45.8	8.8	18.0	73.6
2-CH_3; 6-CH_3	Q	47.5	8.8	23.6	71.4
2-CH_3	Q	46.7	9.0	21.9	71.6
2-Cl	S	44.2	8.9	16.6	71.9
2-Br	S	44.8	8.8	17.2	72.4
2-I	S	44.0	8.9	16.8	71.2
2-NO_2	S	39.5	9.0	9.7	69.4

[a] Chemical shift measured to center of pattern relative to the sulfanilyl ring absorption of N^4-acetylsulfanilamide (singlet, τ 2.30).

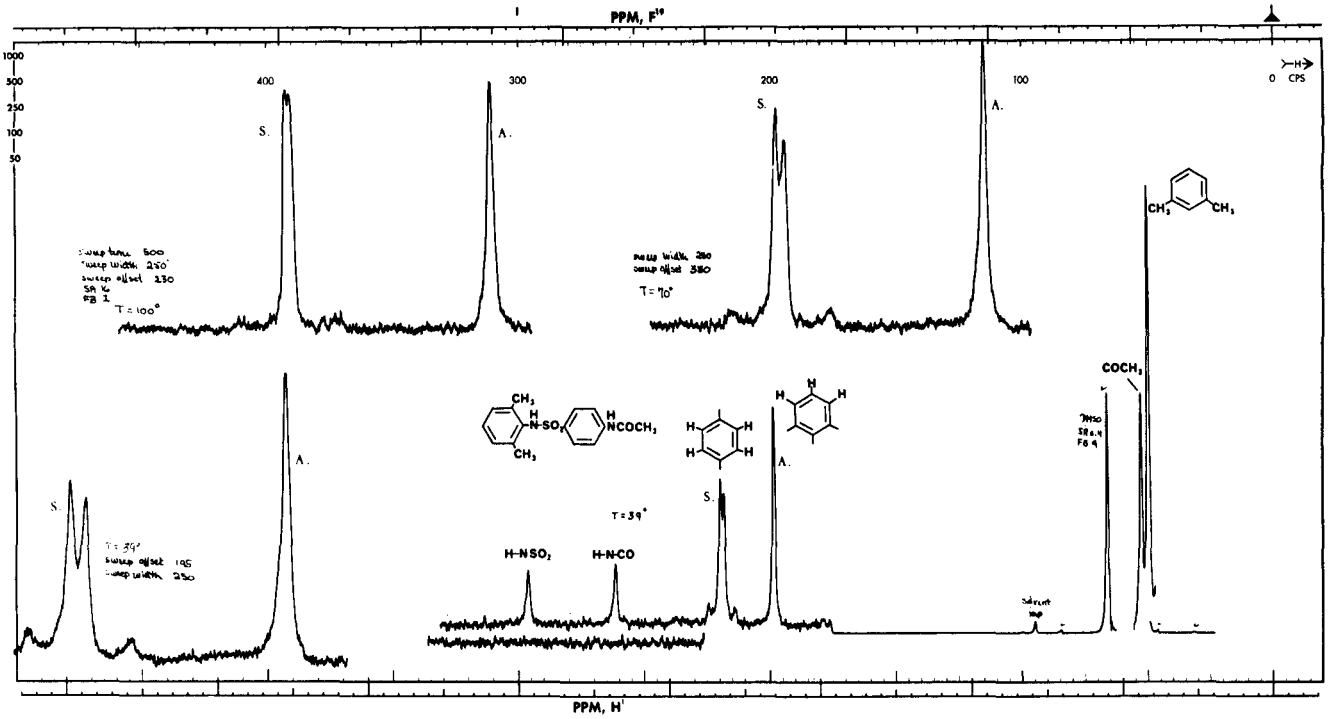

Figure 1—*PMR spectra of* N⁴-*acetyl*-N¹-(*2,6-dimethylphenyl*)*sulfanilamide.*

In this communication, we present NMR evidence which suggests that an N^1-substituent on sulfanilamide that has a suitably placed methyl group can lead to conformational stabilization. Our findings are for sulfanilamides derived from 2-methylaniline and 2,6-dimethylaniline, but analogous results may also be anticipated for other similarly substituted sulfonamides, *e.g.*, sulfisoxazole.

The basis for this study stems from our earlier observation (5) that, of a series of N^4-acetyl-N^1-phenylsulfanilamides, only the compound derived from 2-methylaniline had a PMR spectrum (60 MHz.) exhibiting a quartet for the sulfanilyl ring (S-ring) absorbance. The corresponding absorbance for all other compounds in the series was a singlet. This spectral behavior is consistent with a situation where, in solution, there is relatively unrestricted rotation about the SO_2—N bond of a sulfanilamide, except when a stabilizing Coulombic interaction is possible between the sulfonyl (SO_2) group and a substituent on the N^1-moiety. In this latter instance, an additional partial rotational mode about the amido C—N bond is possible, which would change the population distribution of the various rotomers and could lead to the seemingly anomalous S-ring absorbance for the 2-methyl-substituted compound.

If such an interaction is assumed to occur, it might be predicted that increasing the temperature of a solution of N^4-acetyl-N^1-(2,6-dimethylphenyl)sulfanilamide (I) would increase the probability for rotation about the sulfonamido (SO_2—N) bond at the expense of any conformation stabilized by sulfonyl–methyl (SO_2–CH_3) interactions. Conformational stabilization by the interaction of a methyl group with electron-rich bonds, such as a carbon–carbon double bond (C=C) or carbonyl (C=O), is well documented (6), and an analogous type of interaction involving the sulfonyl and methyl groups of appropriately substituted sulfanilamides is a distinct possibility. Thus, the S-ring quartet observed in the PMR spectrum of I is expected to collapse to a singlet as the temperature of the solution is increased.

Figure 1 shows the collapse of the S-ring quartet for I as the temperature is increased from 39 to 70° and finally to 100°. For this determination, dimethyl sulfoxide was used as the solvent and the spectra were obtained using an NMR spectrometer[1].

In efforts to substantiate further a sulfonyl–methyl group interaction in sulfanilamides, the PMR spectra for a series of N^1-phenylsulfanilamides derived from *o*-substituted anilines were determined using a spectrometer[2] at the normal operating temperature and with the compounds in 2–5% (w/v) tetrahydrofuran solution. Chemical shifts for the S-ring protons that are *ortho* (δ_A) and *meta* (δ_B) to the sulfonyl group were estimated under the assumption that the S-ring quartet observed for these compounds fit the simple *AB* case (7, 8). Table I summarizes the results of these calculations. The chemical shift δ_A estimated for the 2-methyl and 2,6-dimethyl derivatives indicates that protons *ortho* to the sulfonyl group in the S-ring are more shielded than the corresponding protons in the other derivatives. This increased shielding could be viewed as indicating increased polarity of the sulfur–oxygen (S—O) bond due to an interaction with a methyl group.

More direct measurements on the sulfur–oxygen bond character in these same N^1-phenylsulfanilamides were made in the IR spectral region (9–11). This study was done using a grating IR spectrophotometer[3] with

[1] Varian A56-60. We are indebted to Dr. Frank B. Mallory of Bryn Mawr College, Bryn Mawr, Pa., for the determination of these spectra.
[2] Varian A60.
[3] Perkin-Elmer model 257.

Table II—Force Constants for the Sulfur–Oxygen (S—O) Bond of Some N^1-Phenylsulfanilamides

Anilyl Ring Substituent	ν_{sym}, cm.$^{-1}$	ν_{asym}, cm.$^{-1}$	$k_{SO} \times 10^{-5}$, dynes/cm.
2-CH_3O	1153.6	1342.5	9.78
2-CH_3; 6-CH_3	1152.6	1335.7	9.72
2-CH_3	1153.0	1337.0	9.73
2-Cl	1153.8	1341.0	9.77
2-Br	1153.8	1341.0	9.77
2-I	1153.6	1338.9	9.75
2-NO_2	1147.6	1348.6	9.78

the compounds in tetrahydrofuran solution. Beer's law plots for the symmetric and asymmetric sulfonyl absorptions were constructed to determine the concentration range within which intermolecular association effects could be considered negligible. At concentrations of 0.1 *M* or less, the position of the asymmetric band was not found to vary with concentration. However, a slight concentration dependence was noted for the symmetric band position. Consequently, the positions of the sulfonyl absorptions in 0.132, 0.100, 0.067, 0.050, and 0.025 *M* solutions of the compounds were measured relative to the 1181.4 cm.$^{-1}$ band of polystyrene, and band positions were assigned as the intercept at zero concentration in a plot of absorption frequency against molar concentration. Absorption frequencies assigned in this manner may be considered accurate to within ± 0.5 cm.$^{-1}$. Force constants for the sulfur–oxygen bond were subsequently calculated (12) using the relation in Eq. 1:

$$k_{SO} = 0.1570\,(\nu_{sym} + \nu_{asym})^2 \qquad \text{(Eq. 1)}$$

Based on the error associated with the assignment of band position, the calculated force constants (Table II) are subject to a variation of $\pm 0.02 \times 10^5$ dynes/cm. Inspection of Table II shows the force constants for the sulfur–oxygen bond in the 2-methyl and 2,6-dimethyl derivatives to be essentially equal in magnitude but slightly lower in value than the force constants for the other derivatives. It could be inferred, therefore, that the sulfur–oxygen bond in the methyl derivatives is induced to be more polar as a result of an interaction with a methyl group.

Because of the small difference in the force constant between the methyl and the alternatively substituted compounds, the force constants for a series of benzenesulfonanilides were also determined. The intent here was to establish the optimum range in k_{SO} for our experimental conditions, when a conjugative interaction is possible between the sulfonyl group and a group situated *para* to it. Table III presents these findings. In this instance the force constant was relatively insensitive to the electronic nature of the substituent, except for N^1-phenylsulfanilamide. Conjugation between the sulfonyl group and a *p*-amino group was cited previously (13–15) so this finding was not unexpected. Based on the values for k_{SO} given in Table III, a range of about 0.10×10^5 dynes/cm. is as large as could be expected under the most ideal conjugative conditions involving the sulfonyl group. From this, the reduction in k_{SO} by about 0.05×10^5 dynes/cm. due to a methyl group substitution on the compounds in Table II may be considered an appreciable difference.

Table III—Force Constants for the Sulfur–Oxygen (S—O) Bond of Some Benzenesulfonanilides

Sulfanilyl Ring, *p*-Substituent	ν_{sym}, cm.$^{-1}$	ν_{asym}, cm.$^{-1}$	$k_{SO} \times 10^{-5}$, dynes/cm.
NH_2	1151.5	1346.8	9.80
CH_3	1158.7	1350.3	9.89
H	1159.5	1350.8	9.89
Cl	1161.2	1353.2	9.93
NO_2	1161.5	1349.0	9.90

In conclusion, the NMR spectral evidence presented suggests that N^4-acetyl-N^1-(2,6-dimethylphenyl)sulfanilamide adopts a preferred conformation in solution which can be equilibrated with other conformations upon heating. Information gained by supporting NMR and IR studies tends to suggest that an interaction between sulfonyl and methyl groups stabilizes this preferred conformation. This interaction is best described as Coulombic in origin and perhaps may resemble a type of hydrogen bond. Conformational studies that make use of molecular orbital methods may allow an estimate of the three-dimensional structure for such conformationally stabilized sulfanilamides and might also contribute to a description of the sulfonyl–methyl group interaction.

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