Polymorphism in Sulfanilamide-d_4

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Abstract □ Thermal behaviors of four crystalline forms of sulfanilamide and of sulfanilamide-d_4 have been examined. Heats of transition and fusion have been determined. The deuterated modifications exhibit smaller heats of transition and heats of fusion than the corresponding undeuterated forms. There is, however, a difference in the magnitude of this decrease in the case of the heats of transition.

Keyphrases □ Sulfanilamide-d_4—polymorphism □ Polymorphic forms—sulfanilamide-d_4 □ Differential thermal analysis—identity □ IR spectrophotometry—identity □ NMR spectroscopy—identity

Polymorphic forms of the same compound exhibit a number of divergent physical properties including crystal structure, rate of dissolution, density, solubility, and refractive index. Differences in dissolution rate have been noted in such widely used drugs as aspirin (1) and chloramphenicol palmitate (2). These differences in solution properties can be expected to result in variations in therapeutic blood levels and therapeutic efficacy.

In spite of the fact that polymorphism is a widespread phenomenon among organic compounds, in general, and among pharmaceuticals, in particular, very little progress has been made toward an understanding of the factors that permit some compounds to exhibit and prevent others from exhibiting polymorphism. The availability of commercial instrumentation which makes thermodynamic data concerning heats of transition and fusion more readily accessible encouraged the authors to study a group of structurally related compounds, the sulfonamides (3). Polymorphism has been detected in some sulfonamides but not in others.

One sulfonamide that has been studied extensively is sulfanilamide. The existence of polymorphic forms of the compound was first reported by Zyp in 1938 (4). He observed that sulfanilamide appeared in several crystal forms when crystallized from a drop of water and examined under the microscope. Watanabe (5, 6) conducted an X-ray diffraction study of sulfanilamide crystallized from various solvents, and he determined that the compound could crystallize in at least three polymorphic modifications designated as α-, β-, and γ-forms. Yakowitz (7), in his report, confirmed Watanabe's discovery that sulfanilamide is polymorphic, with at least three phases, and reported refractive indexes and heats of solution of these forms. McLachlan (8), in his book on X-ray crystal structure, mentions four polymorphic forms of sulfanilamide, designated as β-, γ-, δ-, and ϵ-forms.

The techniques of neutron diffraction and X-ray diffraction have been employed in the elucidation of the crystal structures of some polymorphic forms of sulfanilamide. O'Connor and Maslen have determined the structures of α-sulfanilamide (9) and of β-sulfanilamide (10), and Alleaume and Decap have determined the structures of β- and of γ-sulfanilamide (11–14). The present investigation was undertaken to obtain thermodynamic data on heats of transition and fusion of sulfanilamide and sulfanilamide-d_4.

EXPERIMENTAL

Materials—Sulfanilamide-d_4 was prepared by repeated recrystallization of sulfanilamide from monodeuteroethanol, monodeuteromethanol, or deuterium oxide. The progressive exchange of D for H in the amino and amide groups of sulfanilamide was observed in the IR spectrum of the material. After five or six recrystallizations, the exchange was virtually complete. The isotopic purity of sulfanilamide-d_4 was further confirmed by NMR spectroscopy using dimethyl-d_6-sulfoxide as the solvent. Monodeuteromethanol and monodeuteroethanol were prepared by the method described by Greive and Sporek (15). Monodeuterobutanol was obtained by repeated isotopic exchange of *n*-butanol with deuterium oxide (Merck, AG, 99.75% isotopic purity) in the presence of deuterium chloride as the catalyst. Acetone-d_6 (Mallinckrodt) had a stated isotopic purity of not less than 99.5%.

Methods and Instrumentation—Polymorphic forms of sulfanilamide and sulfanilamide-d_4 were prepared by recrystallization from appropriate solvents. Distilled water, ethanol, methanol, butanol, and acetone were used as solvents in the recrystallization of sulfanilamide, while deuterium oxide, monodeuteroethanol, monodeuteromethanol, monodeuterobutanol, and acetone-d_6 were used in the recrystallization of sulfanilamide-d_4, which had been prepared as described. The α-, β-, and γ-forms of sulfanilamide were obtained by methods described by Watanabe and Kamio (5). The δ-form was obtained by recrystallization of sulfanilamide from hot, saturated butyl alcohol. Deuterated solvents, analogous to those used for obtaining the four forms of sulfanilamide, were employed in the preparation of the four deuterated modifications.

The Du Pont 900 differential thermal analyzer (DTA), equipped with a standard cell, was employed to detect polymorphic transitions and melting in the various forms of sulfanilamide and sulfanilamide-d_4. Thermograms, plots of differential temperature as a function of the sample temperature, were obtained. In each case the sample was heated from room temperature to a temperature several degrees above fusion, at a uniform rate of 10°/min. Samples of approximately 2 mg. were employed in ordinary glass capillary tubes, 2 mm. in diameter. Nitrogen gas was flushed through the standard cell during the heating procedure to minimize oxidative decomposition. The reference used in these experiments was glass beads. Temperatures of phase transitions were obtained from the thermograms and were corrected for nonlinear temperature response of the chromel/alumel thermocouple.

Quantitative measurements of the heats of transition and fusion of the compounds were obtained using the Du Pont 900 differential thermal analyzer equipped with a calorimeter cell. While the Du Pont standard cell provides useful qualitative information concerning polymorphic transitions, the geometry of the cell precludes its use for quantitative determinations of heats of transition and fusion. The calorimeter cell was designed for this purpose. Samples of approximately 5 mg. were employed in measurements of heats of fusion, and samples of approximately 7 mg. were employed in measurements of heats of transition. These were weighed on a Cahn electrobalance to the nearest 0.002 mg. The samples were placed in cups fashioned from aluminum foil, and the cups were fitted into the silver sample holder of the calorimeter cell in such a way as to obtain good contact with the bottom of the sample holder. Neither aluminum liner nor reference material was used in the reference cup. Thermograms incorporating the temperature ranges of interest were recorded using the chart recorder of the

Table I—Thermal Behaviors of Sulfanilamide and Sulfanilamide-d_4

Polymorphic Forms	Solvents Used for Recrystallization	Phase Transition Temperature	Heat of Transition, cal./mole × 10^{-2}	Fusion Temperature	Heat of Fusion, cal./mole × 10^{-3}
Form I (α-form)	Ethanol, water	108°	3.58 ± 0.15	166°	5.22 ± 0.07
	Monodeuteroethanol, heavy water	101–105°	3.45 ± 0.02	165°	5.09 ± 0.05
Form II (β-form)	Ethanol, water, methanol, acetone	131–141°	3.47 ± 0.16	166°	5.32 ± 0.03
	Monodeuteroethanol, monodeuteromethanol, heavy water, acetone-d_6	120–122°	2.66 ± 0.02	165°	5.04 ± 0.05
Form III (γ-form)	*n*-Pentanol			166°	5.24 ± 0.05
	Monodeuteropentanol			165°	5.07 ± 0.05
Form IV (δ-form)	*n*-Butanol	108°	3.86 ± 0.07	166°	5.27 ± 0.05
	Monodeuterobutanol	105°	3.60 ± 0.05	165°	5.04 ± 0.03

DTA instrument. The areas of the transition and fusion peaks were obtained by drawing a line from the point at which the thermogram first departed from the baseline to the point at which the baseline was reestablished. Area measurements were performed using a polar planimeter (Keuffel & Esser No. 62005). The area of the peaks obtained in DTA calorimetry is proportional to the heat of transition, and the value of ΔH can be obtained by calculation based on the use of a calibration curve. This curve is constructed from data on the areas of the fusion peaks of materials with known heats of fusion, including gallium, tin, indium, and zinc. The procedure is described in more detail in a paper by Guillory (16). The calibration was found to be accurate to within 2.1% when checked with samples of silver nitrate and benzoic acid. Heats of transition and fusion reported in Table I are average values of six or more determinations. The average deviations from the mean for these measurements are also given.

IR spectra of crystals of sulfanilamide and sulfanilamide-d_4 were taken as potassium bromide pellets. The pellets were prepared using a Beckman potassium bromide dye and a Pasadena Hydraulics, Inc., press at a pressure of 20 tons. The sample–potassium bromide ratio employed was 1:200. Some samples were obtained using the Wilks Mini-Press with a sample–potassium bromide ratio of 0.7:120. Both methods resulted in identical IR patterns. Spectra were obtained on a Beckman IR-10 IR spectrophotometer. The instrument employs two precision replica gratings in a single monochromator. The first operates from 4000 to 600 $cm.^{-1}$, and the second grating operates from 650 to 300 $cm.^{-1}$. Polystyrene film was used to calibrate the wavelength axis.

As an independent check, observations of phase transitions were carried out on the Koefler micro hot stage. A small amount of sample was placed between a slide and cover glass, and the slide was placed on the hot stage. The sample was observed through a microscope as it was heated at a constant rate (10°/min.) up to fusion.

X-ray diffractograms of the polymorphic forms were recorded by the powder diffraction method.[1] These diffractograms are reproduced elsewhere (17).

RESULTS AND DISCUSSION

Four polymorphic modifications of sulfanilamide and sulfanilamide-d_4 were obtained by recrystallization from the solvents listed in Table I. These modifications were subjected to DTA, IR spectroscopy, and DTA calorimetry. Forms I, II, and III of undeuterated sulfanilamide are analogous to the α-, β-, and γ-forms of Watanabe and Kamio (5). The deuterated polymorphic forms are named by analogy to the corresponding undeuterated forms.

The commercially available form of sulfanilamide is the β-form. The three other forms of sulfanilamide also remain stable at room temperature. DTA reveals that the α-, β-, and δ-forms are transformed to the γ-form prior to melting. The γ-form, then, is the only polymorph that melts without undergoing a polymorphic transformation. The deuterated forms of these four polymorphs behave in a precisely analogous fashion.

[1] National Spectrographic Laboratories, Inc., Cleveland, Ohio.

In 1961, Inoue and Saito (18) reported the use of DTA in the study of polymorphic transitions occurring in sulfanilamide. Two thermograms appear in this paper, and an explanation of the thermograms is given by the authors. Two transition peaks are observed in the thermogram of what the authors refer to as the α-form of sulfanilamide. They associate these two peaks with transformations from the α- to the β-form and from the β- to the γ-form, respectively. The latter peak is substantially smaller in area than the former. In the second thermogram, the transition peak is identified as being associated with the β- to γ-transition.

In the present investigation (Figs. 1 and 2), repeated experiments have failed to produce two transition peaks when the α-form is heated from room temperature to fusion. An examination of the data obtained from DTA calorimetry (Table I) reveals that the magnitude of the heat of transition in the case of these two forms is virtually the same. If the α-form did indeed transform to the β-modification at 108°, one would expect to find a transition peak of approximately the same area at 131–141°, the transition temperature range of the β-form. The fact that Inoue and Saito (18) found only a small peak in this range indicates that their sample of the α-form was contaminated with some β-form. The authors of this study believe

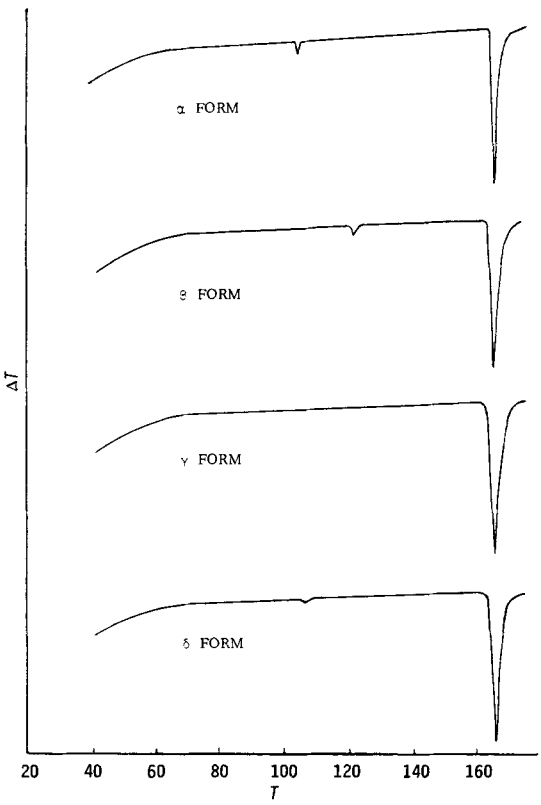

Figure 1—*DTA thermograms of four polymorphic forms of sulfanilamide.*

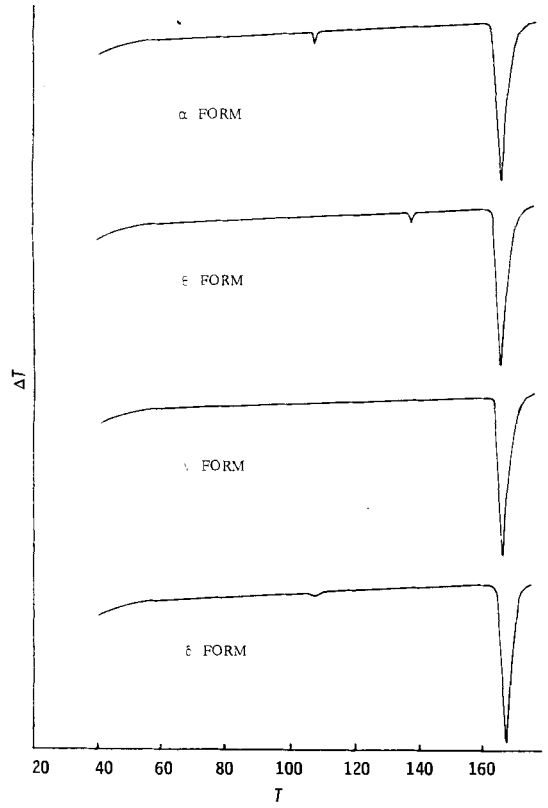

Figure 2—*DTA thermograms of four polymorphic forms of sulfanilamide-d_4.*

that Watanabe and Kamio (5), who reported from thermomicroscopic observations a transition from α to β occurring at temperatures near 90°, made a similar error. According to their account, when suction was used to dry crystals of the α-form, they were converted to the β-form. The authors have found that a similar transition occurs on trituration of the α-modification. Inoue and Saito (18) do not describe the δ- to γ-transition which was detected in this study, nor did they estimate the heats of transition of the polymorphic changes.

Data obtained from DTA calorimetry are listed in Table I. Heats of transition are reported for the α-, β-, and δ-forms. These three forms are converted to the γ-form, which melts without undergoing polymorphic transition. Since three forms are converted to the γ-form prior to fusion, all four forms are expected to exhibit the same heat of fusion. This is found to be true, within the limits of experimental error, for both the nondeuterated and the deuterated forms.

An examination of the heats of transition for the three polymorphs which undergo transition reveals that the δ-form requires the greatest amount of energy to affect its transition to the γ-form. The α- and β-modifications apparently have similar internal energies. This may explain why conversion from the α-form to the β-form occurs so readily. Simple trituration will bring about this change (although heating from room temperature to fusion will not, as pointed out previously).

Hydrogen bonding plays an important role in the crystalline structure of sulfanilamide. Apparently the two hydrogens of each amide group are engaged in NH---O bonding with oxygens on two adjacent sulfanilamide molecules. Similarly, the two hydrogens on each amine function participate in similar hydrogen-bonding interactions. It is interesting to note that X-ray diffraction studies (9–14) suggest that the hydrogen bonds in the α- and β-forms are, on the average, very nearly the same length, but those of the β-modification are slightly longer. This observation is in line with the heats of transition determined in this study (*i.e.*, higher heat of transition for the form with shorter bonds).

The γ-form, which does not exhibit a polymorphic transition, forms hydrogen bonds that are, on the average, somewhat longer than those of the α- and β-forms. Data are not available for the lengths of the hydrogen bonds in the δ-form, but results of this investigation suggest that, on the average, the hydrogen bond lengths in this form will be considerably shorter than those of the α- and β-forms.

The heat of fusion of the deuterated γ-form is approximately 5% less than that of the undeuterated modification, although the melting point is decreased only about 1° by deuteration. Substitution of deuterium for hydrogen apparently lengthens the hydrogen bonds in the crystal lattice (19), and this accounts for the lower heat of fusion.

In the case of the three modifications that exhibit phase transitions prior to fusion, deuteration diminishes the magnitude of the energy required for conversion to the γ-form. The degree of reduction of the heat of transition is approximately the same for the α- and δ-forms as the reduction in the heat of fusion measurements. The β-form, however, shows anomalous behavior, with a reduction in heat of transition of approximately 25%. Apparently, isotopic substitution affects the bond lengths and bond strengths of the hydrogen bonds of these crystals to a different degree.

When the X-ray diffraction patterns obtained for the four modifications of sulfanilamide and sulfanilamide-d_4 are compared (17), it is seen that the crystal structures appear to have similar molecular arrangements in the case of the deuterated and undeuterated β- and δ-forms. There are, however, significant differences in the molecular arrangements in the α- and γ-forms following deuteration. This implies that, at least in some cases, substitution of deuterium for hydrogen in sulfanilamide does more than simply expand the crystal lattice. Deuteration may, or may not, affect the lattice structure.

Figures 3 and 4 show IR spectra of sulfanilamide and sulfanilamide-d_4, respectively. In Fig. 3 it can be seen that the spectra for the α- and β-forms are virtually identical. In preparing samples for IR analysis, grinding and pressing operations are employed which can be expected to bring about a transformation from the α-form to the β-form. Therefore, the first two spectra shown are both of the β-modification. A comparison of the spectra for the deuterated sulfanilamides reveals that three of the forms exhibit essentially identical spectra. Ito and Sekiguchi (20), in a paper on the formation of a molecular compound of sulfanilamide and sulfathiazole, reported that they had examined three deuterated forms of sul-

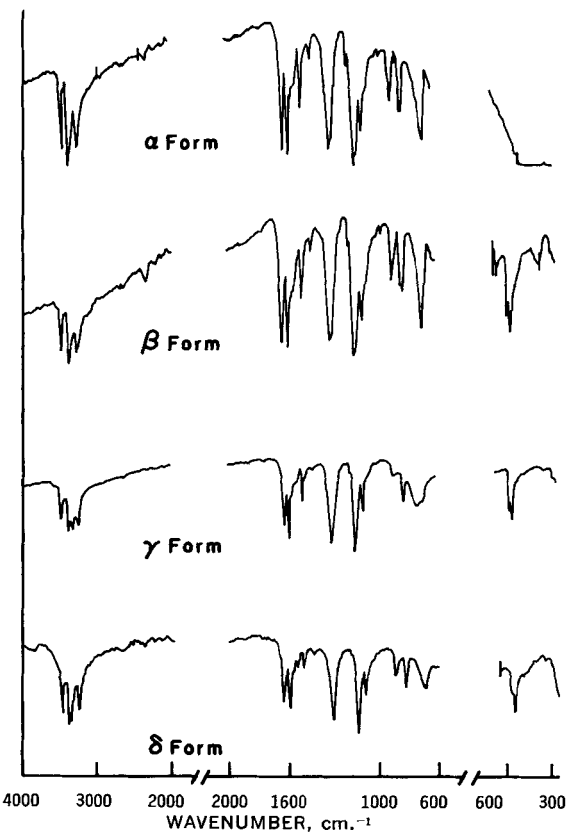

Figure 3—*IR spectra of four polymorphic forms of sulfanilamide.*

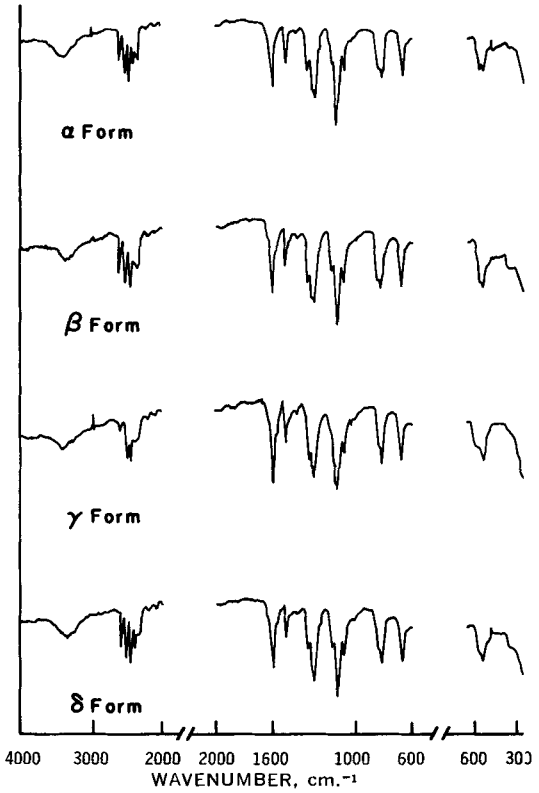

Figure 4—*IR spectra of four polymorphic forms of sulfanilamide-d_4.*

fanilamide and found that: "spectral differences between the three polymorphic forms . . . (were) comparatively few." In the present investigation, minor differences in the spectra were noted in the $-NH_2$ stretching regions but not in the $-ND_2$ stretching regions. Similar differences can be seen in spectra published by Novak *et al.* (21).

The study of polymorphism in sulfanilamide is complicated by the large number of possible hydrogen bonding sites in the molecule; by the formation of hydrogen bonds of various lengths; by the fact that in some forms the unit cell is made up of eight, and in others, of four molecules; and by the fact that the crystal structure of the δ-form has not yet been elucidated. Additional information is required before the mechanisms of the polymorphic transitions occurring in this compound can be understood.

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