0.5% chloroform on the callus is not surprising in light of the findings that solvents such as alcohol in concentration of 0.2 to 0.3% enhanced Avena coleoptile elongation (28).

There is a decrease in the constituents in the callus harvested from the media treated with cholesterol as compared to the callus obtained from its chloroform control. However, in case of the media tincture, the condition is reversed. Because of fluctuation in temperature during experiment *C*, it is not possible to draw any definite conclusions.

From the over-all data of experiment *A*, it seems reasonable to recommend 0.5% sterol *A* as an extra adjunct to the media for the cultivation of static callus cultures of *D. mertonensis*.

SUMMARY

A method is described for the growth of callus tissue from the seedlings of *D. mertonensis* in tissue culture. Certain sterols were also studied for their effects on callus growth and digitalis-like glycoside production.

Increases in callus growth and constituents positive to the Baljet test were observed when a solubilized lanolin fraction sterol *A* was added to the nutrient media in amounts of 0.5%.

Cholesterol, as an adjunct to the culture media in quantities of 0.1%, stimulated callus growth. However, 0.024% of an ethoxylated derivative of cholesterol sterol *B* with a 24-molecule ethylene oxide side chain and a polymer of ethylene oxide (polyethylene glycol 1000) in quantities of 0.018% inhibited callus growth.

Constituents in the callus, positive to the Baljet test increased slightly in the presence of cholesterol and polyethylene glycol 1000. There was no significant change in the callus constituents when sterol *B* was added to the media.

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Heats of Transition of Methylprednisolone and Sulfathiazole by a Differential Thermal Analysis Method

By J. KEITH GUILLORY

Heats of transition and fusion have been determined for methylprednisolone and sulfathiazole by differential thermal analysis calorimetry. Methylprednisolone undergoes an endothermic transition at 209°, with heat of transition, 1380 ± 50 cal. mole^{-1}. Fusion occurs at 240°, with heat of fusion, 5350 ± 200 cal. mole^{-1}. Sulfathiazole undergoes an endothermic transition at 161°, with heat of transition, 1420 ± 40 cal. mole^{-1}. Fusion occurs at 200°, with heat of fusion, 5960 ±210 cal. mole^{-1}.

As part of a program investigating the phenomenon of polymorphism in pharmaceuticals it was desirable to obtain reliable data on the heats of transition and fusion for a large number of compounds. Adiabatic calorimetry (1), solubility measurements (2–4), and differential thermal analysis were among the techniques considered for use in these determinations. Calorimetry was rejected as being too time consuming for routine analyses. There are a num-

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ber of factors which tend to reduce the utility of the solubility method. Practically speaking, its use is restricted to systems exhibiting only two polymorphic forms. It is further limited to those systems in which transitions occur within a temperature range for which liquids having appropriate solvent properties are available if one is to obtain data that can be extrapolated with confidence.

Differential thermal analysis (DTA) is a technique in which the heat loss or gain resulting from physical or chemical changes occurring in a sample is recorded as a function of temperature or of time as the substance is heated at a uniform rate. Heat or enthalpic changes, either endothermic or exothermic, are caused by phase transitions—such as fusion, crystalline structure inversions, boiling, sublimation, and vaporization. Solid–solid transitions, fusion, and dehydration generally produce endothermic effects, whereas crystallization and oxidation produce exothermic effects (5).

In DTA a sample substance and a thermally inert reference substance are heated at a fixed rate by an external furnace. The junctions of a differential thermocouple are embedded in or near the sample and inert reference. As the temperature increases the electromotive forces developed by the junctions are amplified and compared by displaying on a recorder. Exothermic or endothermic changes are consequently recorded as deviations from a base line, and appear as peaks on one side of the base line or the other (6).

Although primarily a tool for qualitative analysis, DTA has been employed for estimating heats of reaction, fusion, and transition. These thermodynamic functions can be evaluated by integrating the endothermic or exothermic peak produced as the sample reacts, melts, or undergoes a solid-state transition. The area of the peak is proportional to the heat absorbed or evolved in the sample transformation. The heat involved in the process, ΔH, is given by

$$\Delta H = \psi \int_{t_1}^{t_2} \theta \, dt$$

where ψ is the proportionality constant, usually evaluated experimentally, or (rarely) calculated from instrument parameters; t_2 and t_1 are the times at the end and beginning of the peak; θ is the differential temperature; and t is the time.

Kung and Goddard (7) used this technique to obtain an estimate of the heat effects associated with the formation of a complex between sodium lauryl sulfate or myristyl sulfate and lauryl alcohol or myristyl alcohol. They pointed out that although the area of the DTA peak is primarily controlled by the enthalpy change involved, it is, at constant heating rate and sample size, also a function of the heat capacity and thermal conductivity of the sample. The latter property for solid substances is affected by the state of packing of the sample.

Varma (8) determined the heats of fusion for a group of organic compounds, including catechol and naphthalene. Care was taken to maintain the quantity of material employed in each determination exactly the same. Effects of thermal diffusivity of the different materials used in the analyses were ignored, however.

Sakurai and Yabe (9) have calculated the heat absorbed in the solid phase transition and fusion of *n*-hexadecanamide. Other quantitative applications of DTA have been reviewed periodically by Murphy (10).

There are a number of shortcomings associated with the use of conventional DTA equipment for the quantitative evaluation of thermodynamic functions, and several of these shortcomings have been pointed out by Speros and Woodhouse (6). In an attempt to overcome these objections, various design modifications have been proposed from time to time to improve the precision and accuracy of DTA instruments. The equipment used in this investigation incorporates modifications which were first proposed by Boersma (11). Holders for sample and reference are located in separate cavities in a nickel heating block. A resistance heating element is wound around this block, and chromel–alumel thermocouples contact the bottoms of the holders. This construction affords high resistance to heat transfer. When a sample undergoes transition or reaction, the heat developed is retained by the sample and its holder. As a result, the heat values obtained are independent of sample size, specific heat, and packing density.

The primary purpose of this investigation was to determine whether DTA calorimetry could be employed routinely, conveniently, and with confidence in the determination of thermodynamic parameters for organic crystalline materials undergoing polymorphic transformations. Two substances whose heats of transition had previously been determined by an independent method, methylprednisolone (3) and sulfathiazole (4), were selected for examination by DTA calorimetry.

EXPERIMENTAL

Materials.—Gallium, indium, tin, and zinc were

Fisher certified reagents. Ethanol U.S.P. was employed as recrystallization solvent for sulfathiazole, and Merck and Co. reagent grade acetone was employed as recrystallization solvent for methylprednisolone. Sulfathiazole N.F. XI was obtained from Merck and Co. Methylprednisolone[1] N.F. was supplied by Upjohn Laboratories.

Calibration of the Calorimeter Cell.—The instrument employed in these determinations was the Dupont 900 differential thermal analyzer equipped with a Dupont modular calorimeter cell. The cell was calibrated with gallium, indium, tin, and zinc. Six samples of each of these substances were weighed into aluminum sample liner holders fashioned with the aid of a dowel pin. The aluminum foil used for this purpose was 0.001 in. thick. No liner was employed in the reference holder.

The samples were weighed on a Cahn Electrobalance to ±0.002 mg. Sample sizes ranged from approximately 4 mg. for gallium to as much as 13 mg. for indium, the sample size being selected to produce a fusion peak whose area could be measured with an error of not more than 2%.

Samples of indium, tin, and zinc were each heated in the calorimeter cell from room temperature to 20–50° above the melting point. Samples of gallium were cooled to −40° with the aid of a dry ice–acetone mixture, then heated to 20° above the melting point. The heating rate used was 10° min.$^{-1}$.

In each case a graph of differential temperature as a function of reference temperature gave an essentially straight base line, with a single endothermic peak corresponding to fusion. The areas of these endothermic peaks were determined by use of a Keuffel and Esser Compensating Polar Planimeter. The area to be measured was found by drawing a line from the point where the thermogram first departed from the base line to the point where the base line was resumed following fusion.

The fusion temperature for each material was taken as the extrapolated onset temperature of the endothermic peak. The low temperature side of the fusion peak was extrapolated to the prefusion base line. Since the temperature response of the chromel-alumel thermocouples employed in the calorimeter cell is nonlinear, a small temperature correction (never more than 4°) had to be applied to obtain the corrected fusion temperature.

Calibration coefficients were calculated from the following equation:

$$E = \frac{\Delta H\ M\ a}{A\ T_s \Delta T_s}$$

where

E = calibration coefficient, mcal. °C.$^{-1}$ min.$^{-1}$,
ΔH = heat of fusion, mcal. mg.$^{-1}$,
M = sample mass, mg.,
a = heating rate, °C. min.$^{-1}$, 10° min.$^{-1}$,
A = peak area, in.^{2},
T_s = x-axis sensitivity, °C. in.$^{-1}$, 20° in.$^{-1}$, except for zinc, 50° in.$^{-1}$,
ΔT_s = y-axis sensitivity, °C. in.$^{-1}$, 0.5° in.$^{-1}$.

Heats of fusion employed in the above equation were as follows: gallium, 19.9 cal. Gm.$^{-1}$; indium, 6.79 cal. Gm.$^{-1}$; tin, 14.2 cal. Gm.$^{-1}$; and zinc, 27.0 cal. Gm.$^{-1}$ (12). Calibration coefficients and fusion temperatures obtained were as follows: gallium, 7.40 mcal. °C.$^{-1}$ in.$^{-1}$ and 29.8°; indium, 9.37 mcal. °C.$^{-1}$ in.$^{-1}$ and 156°; tin, 11.0 mcal. °C.$^{-1}$ in.$^{-1}$ and 231°; and zinc, 16.0 mcal. °C.$^{-1}$ in.$^{-1}$ and 418°. These data are plotted in Fig. 1.

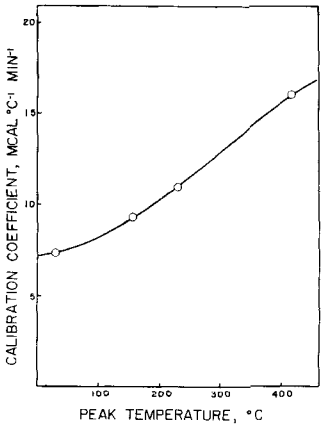

Fig. 1.—Calibration curve for the DTA calorimeter cell.

Measurement of Heats of Fusion and Transition.—Two crystal forms of sulfathiazole were obtained by the methods described by Milosovich (4). Form I, obtained by slow crystallization from warm alcohol consisted of hexagonal plates. Observed on a Kofler hotstage, these crystals underwent a transition to prisms at 155–162°, the exact temperature of the transition being dependent upon the heating rate employed. If a rapid heating rate was employed the crystals melted at 175° without undergoing a polymorphic transition. The melting point of the prismatic crystals was 201°, with decomposition. Form II was obtained by heating form I to 180° in an oven.

Form I of methylprednisolone was prepared by recrystallization from cold acetone. Form II was obtained by heating form I to 210° in an oven. Observed on a Kofler hotstage, form I was transformed to form II over the range 209–213°, with final melting occurring at 238–242°, with decomposition.

Infrared spectra of the polymorphs were taken as Nujol mulls and as potassium bromide pellets on a Beckman IR-10 spectrophotometer. Spectra of the two forms of methylprednisolone were identical with those reported by Higuchi *et al.* (3). The spectrum of sulfathiazole reproduced in the "Sadtler Standard Spectra" catalog is apparently that of form I (13).

Six samples of each polymorphic form were weighed into aluminum sample holder liners, and thermograms, graphs of differential temperature as a function of reference temperature, were obtained. The sulfathiazole samples employed weighed approximately 4.9 mg., and the methylprednisolone samples, approximately 6 mg. Typical thermograms for the four polymorphic forms investigated are shown in Figs. 2 and 3. In these thermograms, however, the y-axis sensitivity setting employed was 0.5° in.$^{-1}$ in the case of sulfathiazole and 0.2° in.$^{-1}$ in the case of methylprednisolone, whereas in the case of the thermograms from which area measurements were taken, only the fusion peaks

[1] The author is indebted to Dr. A. J. Taraszka for a gift of the methylprednisolone used in this study.

were determined at these settings. The transition peaks were determined at a higher sensitivity setting (0.2° in.$^{-1}$ for sulfathiazole and 0.1° in.$^{-1}$ for methylprednisolone) to facilitate measurement of the areas.

The temperatures of transition and fusion were determined by extrapolating the fusion endotherms to the prefusion base line and correcting this temperature for the nonlinear temperature response of the chromel–alumel thermocouples. The values of the calibration coefficients corresponding to each fusion and transition temperature were determined from Fig. 1.

Since, in the case of both sulfathiazole and methylprednisolone, transition from form I to form II occurs prior to fusion, the heat of fusion which is measured is the heat of fusion of form II. A separate determination of the heat of fusion of form II was made in each case, however, to determine whether there might be a kinetic effect which could influence the magnitude of the heat of fusion of a compound determined immediately following transition to a metastable form. Values of heats of fusion determined immediately following transition from form I to form II are listed under "Heat of Fusion, DTA, cal. mole^{-1}," while heats of fusion determined by heating form II from room temperature to fusion are listed separately. The values of the heats of transition and fusion are based on molecular weights 255.32 and 374.46 Gm. mole^{-1}, respectively, for sulfathiazole and methylprednisolone. (Table I.)

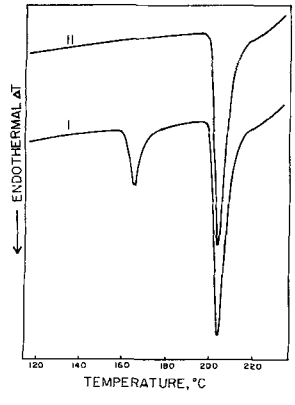

Fig. 2.—Thermograms for forms I and II of sulfathiazole.

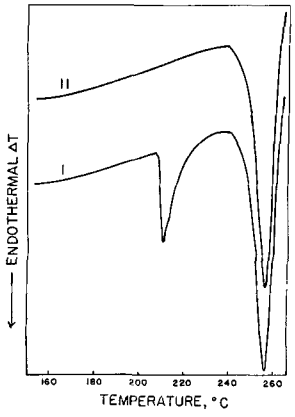

Fig. 3.—Thermograms for forms I and II of methylprednisolone.

TABLE I.—COMPARISON OF DTA CALORIMETRY AND SOLUBILITY ANALYSIS RESULTS

	Sulfathiazole	Methylprednisolone
Calibration coefficient, transition, mcal. °C.$^{-1}$ min.$^{-1}$	9.51	10.5
Calibration coefficient, fusion, mcal. °C.$^{-1}$ min.$^{-1}$	10.2	11.0
Heat of transition, DTA, cal. mole^{-1}	1420 ± 40	1380 ± 50
Temp. of transition, DTA, °C.	161	209
Heat of fusion, DTA, cal. mole^{-1}	5960 ± 210	5350 ± 200
Heat of fusion, DTA, form II, heated from room temp., cal. mole^{-1}	5970 ± 230	6020 ± 240
Fusion temp., °C.	200	240
Heat of transition, lit., cal. mole^{-1}	1744[a]	1600[b]
Temp. of transition, lit., °C.	94.5 ± 2.7[a]	118[b]

[a] *Reference 4.* [b] *Reference 3.*

RESULTS AND DISCUSSION

Clearly there is a large discrepancy in the transition temperatures obtained by the DTA calorimetry method and those obtained by the solubility method. If a heating rate of 10° min.$^{-1}$ is employed and crystals of sulfathiazole and methylprednisolone are observed on a Kofler hotstage, transition temperatures identical with those obtained by the DTA method are observed. The transition temperature of sulfathiazole is particularly sensitive to changes in the rate of heating, as has been noted by Miyazaki (14). Inoue and Saito determined the transition temperature of sulfathiazole by a qualitative DTA method. At a heating rate of 1° min.$^{-1}$ they obtained a transition temperature of 140° (15). Milosovich (4) demonstrated that form I is converted to II at 100° in glycerin suspension, and form II is converted to I at 90°. It appears that crystals of a polymorph uncontaminated with a second form, and heated in the solid state, can be subjected to considerable overheating or underheating before phase transition occurs. In spite of these large differences in transition temperatures, the values of the heats of transition for the two compounds are not far removed from those determined by the solubility method.

One of the advantages of the DTA calorimetry method is that it also affords a means of obtaining data on the heats of fusion of the compounds investigated. The two values of the heat of fusion for sulfathiazole are clearly within experimental error. There is, however, a considerable difference in the values obtained for methylprednisolone. The heat of fusion is significantly larger for form II heated from room temperature to fusion than for the same polymorph when the heat of fusion measurement is taken immediately following transition from form I to form II. This discrepancy may be due, in part, to the fact that methylprednisolone undergoes a highly exothermal decomposition reaction

immediately following fusion. This makes the precise determination of the area of the fusion peak difficult. It is possible, however, that the difference in the measured heats of fusion may indicate that some time is required following transition from form I to form II for orientation of the molecules of the crystal to occur. Since intermolecular attractive forces are not yet completely established when the fusion temperature is reached, less energy may be required to destroy the crystal lattice.

In their discussion of the mechanism of the phase transition occurring in methylprednisolone, Higuchi *et al.* speculate that the entropy difference between the two forms is a result of greater localization of the functional groups in the side chain in form I, resulting either from intermolecular or intramolecular interactions. A comparison of the infrared spectra of the two crystal forms as potassium bromide pellets reveals that there are large differences in the spectra in the regions designated as methylene stretching and bending regions (16). It seems likely, then, that there may be significant differences in the degree of participation of the methylene groups in the ring structure in intramolecular van der Waals' interactions.

In the case of sulfathiazole, the infrared spectra of forms I and II are strikingly different in the NH— stretching and bending regions. There are strong peaks in the spectrum of II corresponding to the NH— stretching vibration of the amino group (3360, 3480 $cm.^{-1}$) (17). These peaks are shifted to lower frequencies of vibration in form I. Such a shift is indicative of an increase in intermolecular hydrogen bonding (18). In addition, the intense NH_2— bending vibration at 1625 $cm.^{-1}$ in form II is reduced to a shoulder in form I. The SO_2— asymmetric stretching band is quite pronounced in form I (1320 $cm.^{-1}$), but is shifted to a lower frequency and is less intense in form II. The symmetric stretching band at 1135 $cm.^{-1}$ is little affected by the polymorphic transition.

These differences in infrared spectra provide a basis for explaining the greater solubility of form II. There is apparently a greater tendency for intermolecular hydrogen bonding in form I than in form II. Hence, there is less tendency for the molecules of sulfathiazole in form I crystals to form hydrogen bonds with the solvent. Similar interpretations have been suggested by other workers for the differences in solubility for two forms of chloroacetamide (19), and for the spectral shifts observed to occur in the various forms of estradiol (20).

SUMMARY

DTA calorimetry is a convenient method for obtaining heats of transition and fusion of pharmaceuticals. It should be particularly useful for obtaining these data when compounds which undergo several phase transitions prior to fusion must be examined. The method can also be recommended for those compounds that do not form ideal solutions with solvents appropriate for use in the solubility method. The transition temperatures observed in this method may, however, differ appreciably from those observed in the solubility method.

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