Thermodynamics of Mercaptopurine Dehydration

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Abstract □ The hydrate form of mercaptopurine was shown to undergo peritectic decomposition of its water molecule, localized dissolution, and dehydration around 125°. The anhydrate form was prepared by a thermal method, whose effectiveness was confirmed by X-ray diffraction, NMR spectroscopy, and differential scanning calorimetry. The activation energy for mercaptopurine dehydration calculated by various methods ranged from 45.74 to 63.04 kcal/mole. The dehydration enthalpy was calculated to be 8.27 kcal/mole by differential scanning calorimetry. The solution enthalpy for the hydrate was calculated to be 4.85 kcal/mole from its saturation solubility and differential scanning calorimetry. Anhydrate solubility in water was calculated based on initial dissolution rate data since the anhydrate converts to hydrate in aqueous media. The high degree of stability against interconversion of the hydrate and anhydrate forms and the higher solubility of the anhydrate suggest that use of the anhydrate might improve mercaptopurine bioavailability.

Keyphrases □ Mercaptopurine—thermodynamics of dehydration, stability of hydrate and anhydrate forms under various thermal conditions □ Thermodynamics—mercaptopurine dehydration, stability of hydrate and anhydrate forms under various thermal conditions □ Dehydration—thermodynamics, mercaptopurine, stability of hydrate and anhydrate under various thermal conditions □ Stability—mercaptopurine, hydrate and anhydrate forms, various thermal conditions □ Antineoplastic agents—mercaptopurine, thermodynamics of dehydration, stability of hydrate and anhydrate forms under various thermal conditions

Mercaptopurine, a potent purine inhibitor (1), is an effective anticancer drug. The monohydrate form generally gives erratic and low bioavailability (2). Mercaptopurine dehydration, resulting in a complete change in its polymorphic form, was recently reported (3). The anhydrate form also shows a higher dissolution rate, and its use has been recommended to improve bioavailability (3).

In this investigation, the thermodynamic parameters related to mercaptopurine dehydration were calculated and the relative stability of each form under various thermal conditions was estimated.

EXPERIMENTAL

Identification of Crystal Structure Modification and Dehydration—The purity of mercaptopurine[1] was confirmed by differential thermal analysis[2]. Repeat thermograms on mercaptopurine suggested an endothermic response and elevation of the thermodynamic energy at around 125°. This high energy compound was prepared by incubating mercaptopurine in an aluminum foil sack at 200° for 20 min. This treatment resulted in the complete transformation of mercaptopurine to the high energy form without decomposition, as evidenced by differential scanning calorimetry (3).

X-ray diffraction studies were performed on the two forms of mercaptopurine by smearing identical amounts (5 mg) on a double-stick tape fixed on a glass slide. The X-ray diffractometer[3] was run at 1.27 cm/min and 1°/2.54 cm with scanning from 10 to 35° (2θ) under the following conditions: Cu radiation, filtration through a nickel filter, 40 kv, 13 mamp, time constant 3, range 300, and scale expansion 21.

The loss of the water molecule upon heat treatment was also confirmed by NMR[4] studies. An excess of the anhydrate form was shaken in dimethyl sulfoxide[5] in the sample tube, and 100 scans were accumulated at 10-sec intervals with dimethyl sulfoxide set at 151.2 Hz. The aromatic peaks at 503.03 and 491.77 Hz were set equal to 2.0. Similar conditions were used for the hydrate, except for a higher sensitivity to pick up the water molecules in the organic phase released from mercaptopurine upon dissolution. In all instances, the amount of mercaptopurine dissolved was very small because of its solubility characteristics.

Conversion of Hydrate to Anhydrate as a Function of Heating Rate—Estimation of activation energies for the transition of the hydrate to the anhydrate form requires calculation of the amount of the hydrate remaining at various temperatures as a function of heating rates. Accurately weighed amounts (~8–9 mg) of mercaptopurine were transferred to differential scanning calorimetry pans, which were heated at 20, 5, or 2°/min. The sides of the cover were not crimped so that water could evaporate freely. The percent of the hydrate form remaining was calculated from the relative areas under the transition peaks obtained by using a planimeter[6].

Enthalpies of Dehydration and Fusion—Enthalpies were calculated by comparing the areas under the peaks with benzoic acid as the standard. All calculations were performed using a programmable desk-top computer[7].

RESULTS AND DISCUSSION

Purity of Mercaptopurine Forms—The drug supplied by the manufacturer was the monohydrate and showed the loss of the water molecule around 125° (Fig. 1). The purity of the compound was ascertained from the sharpness of the final melting point, since the reported melting points vary from 300 to 315° (4–10). Thermogram reruns showed a high degree of reproducibility, suggesting a lack of significant decomposition upon fusion. However, heating the compound a few degrees above its fusion resulted in decomposition.

Based on these thermal stability considerations, an incubation method was developed (3) for the preparation of the anhydrate. As expected, the

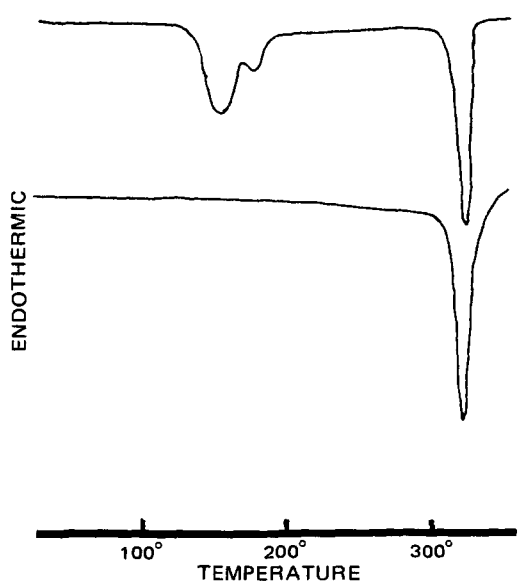

Figure 1—*Differential scanning calorimetry thermograms of mercaptopurine. Key: top, original drug; and bottom, heat-treated sample or rerun of sample in the top thermogram.*

[1] Supplied by Burroughs Wellcome Co., Research Triangle Park, N.C.
[2] A 990 thermal analyzer, E. I. du Pont de Nemours & Co., Wilmington, Del.
[3] Picker X-ray powder diffractometer.
[4] Varian T60A NMR spectrometer, Varian Instruments, Palo Alto, Calif.
[5] Dimethyl sulfoxide-d_6, Aldrich Chemicals, Milwaukee, Wis.
[6] Hruden, Germany.
[7] HP-97 calculator, Hewlett-Packard, Cupertino, Calif.

Table I—Main X-Ray Diffraction Peaks of Mercaptopurine Hydrates and Anhydrates

Original Compound (Hydrate)		Heat-Treated Compound (Anhydrate)	
Peak, $2\theta°$	Peak Height, cm	Peak, $2\theta°$	Peak Height, cm
11.8	3.4	10.7	6.9
12.8	1.2	14.3	3.9
14.8	17.7	15.9	5.0
15.4	1.5	20.0	3.4
23.6	2.3	20.3	2.0
25.3	4.6	21.5	1.8
26.0	6.9	24.8	5.1
27.7	9.1	28.5	8.4
29.6	12.3		
30.5	3.7		
34.2	2.7		

anhydrate showed an identical melting point and no endothermic response around 125°, suggesting complete conversion to the high energy form. The purity of the two forms was further established by X-ray diffraction studies, which showed a complete transition of the lattice structure upon dehydration. Table I reports the characteristic X-ray diffraction peaks for identification purposes.

The NMR studies proved exceedingly fruitful in detecting even traces of water in dimethyl sulfoxide from the hydrate. Both forms showed the characteristic aromatic peaks (Fig. 2) along with NH peaks around 819.74 Hz, equal to 1.74 and 1.95 for the anhydrate and hydrate, respectively. The aromatic peaks were set equal to 2.0. The dimethyl sulfoxide peak at 151.2 Hz was accompanied by the water peak at 1.5 from the hydrate, confirming the complete loss of water molecules upon the heat treatment described.

Dehydration Mechanism—The mechanism of mercaptopurine dehydration can be ascertained partly by differential scanning calorimetry (Fig. 1). The endothermic response occurring around 125° was composed of two peaks, attributed to the release and evaporation of water molecules since the sample pans were open to the atmosphere (*i.e.*, not crimped). The mechanism suggested here involved peritectic decomposition of the water molecule and formation of a solution, followed by the evaporation of the water molecule. This mechanism was suggested for various other compounds (10).

Support for this argument comes from the observation that, upon dehydration, mercaptopurine attains an entirely different lattice structure, which is more feasible if local dissolution is involved in the transition of structure. The reaction order for the transition process is most likely to be first order since higher order reactions occur only with difficulty in the solid state. As discussed later, this hypothesis was confirmed by thermodynamic measurements.

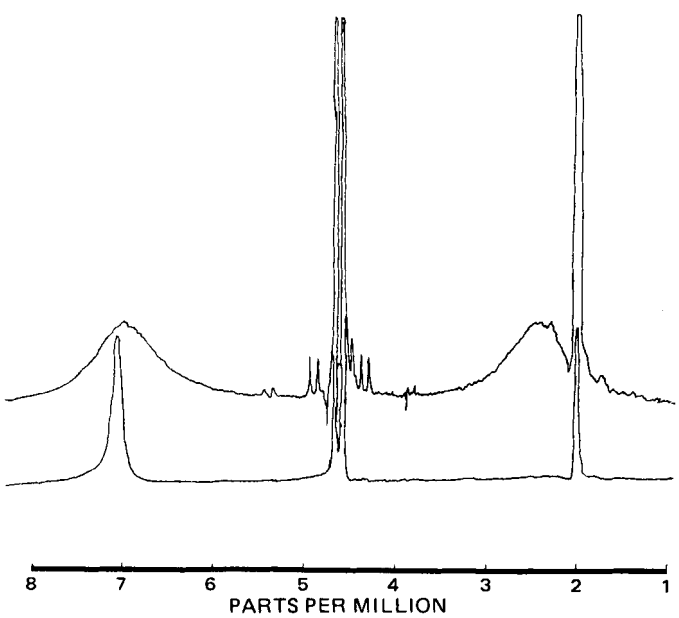

Figure 2—*NMR spectra of mercaptopurine hydrate (top) and anhydrate (bottom) in dimethyl sulfoxide.*

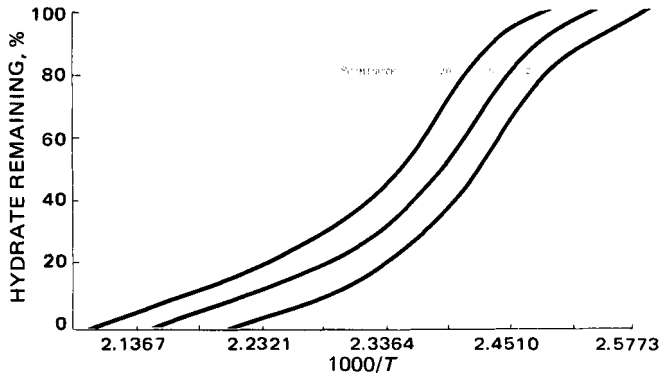

Figure 3—*Ozawa method for the calculation of temperature for a percent dehydration as a function of heating rates.*

Activation Energy of Dehydration—Although some work has been reported regarding the heat of solution of mercaptopurine (11), no study has reported the thermodynamic constants for the transition of mercaptopurine hydrate to the anhydrate.

The activation energy for mercaptopurine transition can be calculated by several methods (12–14). According to Ozawa (12), the kinetic equation for the nth-order reaction can be expressed as:

$$-\frac{dW}{dt} = W^n A e^{(-\Delta E)/RT} \qquad \text{(Eq. 1)}$$

where W is the fractional residual amount of solvate, T is the absolute temperature, R is the gas constant, t is time, ΔE is the activation energy, A is the frequency factor, and n is the order of reaction. A function, P, introduced by Doyle (15), is:

$$\left(\frac{\Delta E}{R}\right) P\left(\frac{\Delta E}{RT}\right) = \int_0^T e^{-(\Delta E)/RT}\, dt \qquad \text{(Eq. 2)}$$

If $\Delta E/RT$ is larger than 20, an approximation (16) can be made:

$$\log P\left(\frac{\Delta E}{RT}\right) \simeq -2.315 - 0.4567\frac{\Delta E}{RT} \qquad \text{(Eq. 3)}$$

Thus, if the weight of the solvate decreases to a given fraction at temperature T_i for the heating rate a_i:

$$\frac{A}{a_i R}\frac{\Delta E}{P}\frac{(\Delta E)}{RT_1} = \frac{A}{a_2 R}\frac{\Delta E}{P}\frac{(\Delta E)}{RT_2} = \ldots \qquad \text{(Eq. 4)}$$

By using Eq. 3, the following linear relations can be easily derived (12):

$$-\log a_1 - 0.4567\frac{\Delta E}{RT_1} = -\log a_2 - 0.4567\frac{\Delta E}{RT_2} = \ldots \qquad \text{(Eq. 5)}$$

Thus, the plots of log a *versus* the reciprocal absolute temperature for a given value of W must give a straight line, the slope of which gives the activation energy:

$$\Delta E = \frac{\text{slope} \times R}{0.4567} \qquad \text{(Eq. 6)}$$

The fraction of the hydrate form converted to the anhydrous form as a function of temperature can be calculated from the corresponding areas under the transition endothermic peak at different heating rates and plotted (Fig. 3). Similar profiles have been reported for the dehydration of other compounds (10). From Fig. 3, a constant fraction remaining for different heating rates can be determined as a function of temperature (Fig. 4). An excellent linear relationship ($r^2 > 0.99$) between the log of the heating rate and the reciprocal temperature suggests good application of Eq. 5 for this system. The activation energy calculated from the slopes ranged from 50.53 to 63.04 kcal/mole.

Another method for the calculation of activation energy, suggested by Kofstad (13), involves approximations of the orders of reaction:

$$(n+1)\ln(ANH) + \ln\frac{d\ln(ANH)}{dt} = \ln A - \frac{\Delta E}{RT} \qquad \text{(Eq. 7)}$$

Calculations using Eq. 7 for zero-, first-, and second-order reactions are plotted in Fig. 5. The calculated activation energies were 24.85–26.60 kcal/mole (zero order), 49.69–52.01 (first order), and 74.54–78.01 (second order). Although the energies calculated from this method for the first-order reaction were somewhat lower than the previous method if the reaction order is assumed to be the same, such approximations can be re-

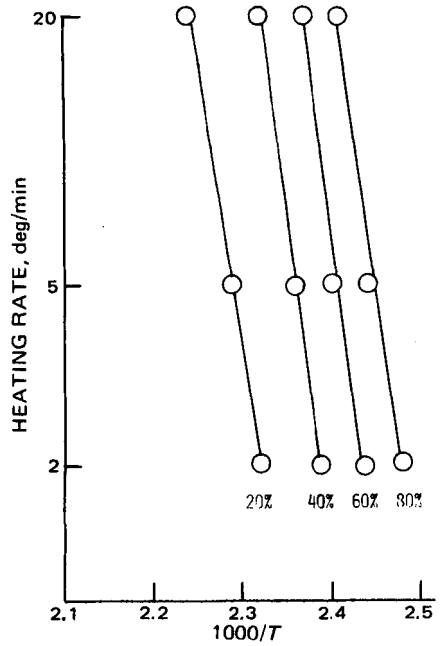

Figure 4—*Calculation of activation energy using Ozawa method. (See text for details.)*

garded as good correlation in view of the larger numbers of parameters and assumptions involved and the possibility of mixed orders of reactions. The order of reactions other than first yielded very low or high energies, excluding them as possible mechanisms.

A simpler method for the calculation of the activation energy, proposed by Kissinger (14), takes into account only the peak of temperature, Tp_1, of the endothermic response as a function of the heating rate:

$$\frac{\Delta Ea}{RTp^2} = \left[A + A(n-1)\frac{2RTp}{\Delta E}\right] e^{-\Delta E, RTp} \qquad \text{(Eq. 8)}$$

$$\frac{d \ln (a/Tp^2)}{d(1/T)} = -\frac{\Delta E}{R} \qquad \text{(Eq. 9)}$$

Figure 6 shows the Kissinger plot; the activation energy calculated from the slope was 45.74 kcal/mole. However, it was suggested (17) that slight measurement errors in this method result in significantly large errors in the activation energy. This method gave lower values for the activation energies than were calculated by the other two methods.

Discounting any significant errors inherent in the Kissinger method (14), the low activation energy can be explained mechanistically as suggested for mercaptopurine dehydration. As shown in Fig. 1, the peritectic decomposition of the water molecule is followed by energy absorption to evaporate it. Whereas the Ozawa (12) and Kofstad (13) methods

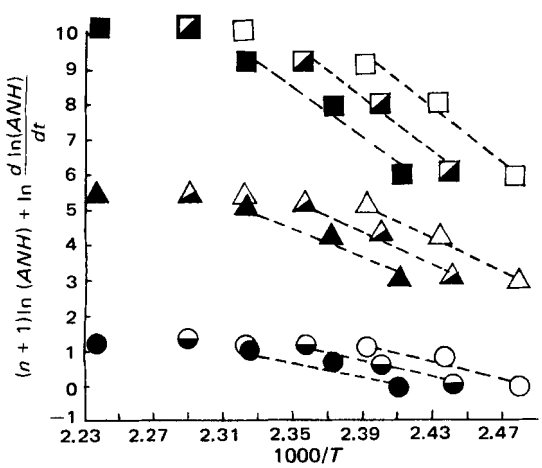

Figure 5—*Kofstad's method for the calculation of activation energies assuming second (squares), first (triangles), and zero (circles) orders for the reaction. Key: open symbols, 2°/min; half-closed symbols, 5°/min; and closed symbols, 20°/min.*

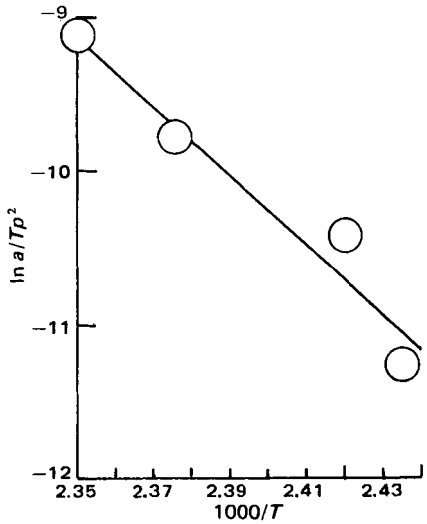

Figure 6—*Kissinger plot for the calculation of activation energy of mercaptopurine dehydration.*

consider the total process of dehydration, the Kissinger method (14) does not; the peak temperature (Eq. 8) represents the time at which the water molecule has not evaporated and thus refers only to peritectic decomposition.

The activation energies calculated for the transition of mercaptopurine by the three methods appear to be relatively high compared with reactions of a similar nature. The high activation energy would, therefore, mean a higher stability of both forms of mercaptopurine (18).

Enthalpy and Stability of Transition—The enthalpy of transition was calculated by comparing the areas under the endothermic peaks of mercaptopurine and the standard, benzoic acid, and was calculated to be 8.27 kcal/mole. This value is comparable to literature reports for glutethimide, theophylline, and succinylsulfathiazole (18). Since the transition enthalpy is much smaller compared to the activation energy, reasonable stability of the anhydrous form can be assumed.

This premise was confirmed by storing the anhydrate at room temperature for over 6 months without any significant conversion to the hydrate. Further support can be drawn from the calculation of the frequency factor for this reaction. With the data already given, a plot of the percent of the hydrate remaining against log $[(\Delta E/aR)P(\Delta E/RT)]$ (12) will give the plot whose lateral shift will be equal to the logarithm of the frequency factor (Fig. 7).

The lateral shift can be easily calculated since the reaction order is confirmed to be first. Thus (12):

$$\log A = \ln W - \log\left(\frac{\Delta E}{aR} P \frac{\Delta E}{RT}\right) \qquad \text{(Eq. 10)}$$

Table II reports the calculation of A using activation energies obtained from Kofstad's method for illustration. In a first-order reaction, the re-

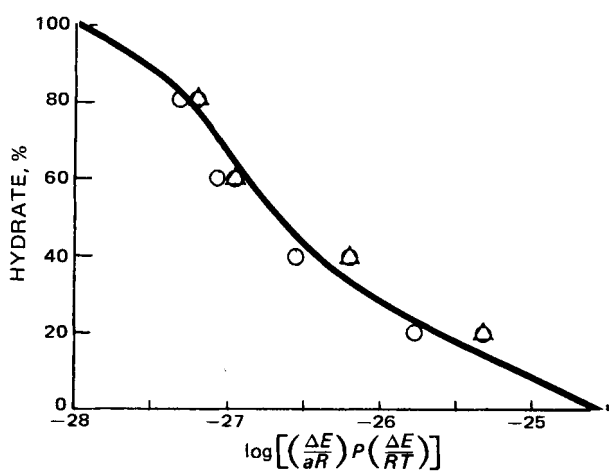

Figure 7—*Lateral shift for the calculation of frequency factor (Table II).*

Table II—Calculation of Frequency Factor for Mercaptopurine Dehydration

Heating Rate, deg/min	W	ΔE, cal	T, °K	$P\frac{\Delta E_a}{RT}$	log A[b]
20	0.8	49,690	415	1.168×10^{-30}	26.61
20	0.6	49,690	422	3.353×10^{-30}	25.87
20	0.4	49,690	420.5	1.1527×10^{-29}	24.92
20	0.2	49,690	447	1.1078×10^{-28}	23.25
5	0.8	52,010	409.5	2.453×10^{-32}	27.67
5	0.6	52,010	416.5	7.621×10^{-32}	26.89
5	0.4	52,010	424	2.462×10^{-31}	25.97
5	0.2	52,010	436.5	1.591×10^{-30}	24.47
2	0.8	50,520	403.5	6.394×10^{-32}	26.87
2	0.6	50,520	410.5	1.987×10^{-31}	26.08
2	0.4	50,520	418	6.420×10^{-31}	25.17
2	0.2	50,520	430.5	4.140×10^{-30}	23.66

[a] $P(\Delta E/RT)$ = antilog $[-2.315 - 0.4567(\Delta E/RT)]$. [b] Equation 9.

action rate constant can be expressed as:

$$K = Ae^{-\Delta E/RT} \quad \text{(Eq. 11)}$$

Table III reports the half-lives of mercaptopurine dehydration as a function of temperature. The high activation energy for dehydration makes this reaction highly susceptible to temperature changes; although it can be considered absolutely stable at room temperature, the half-life decreased to 3.4 min at 150°.

Heat of Solution and Solubilities—The heat of solution of mercaptopurine in water was recently reported to be 9.3 kcal/mole (11). However, when using the solubility determination developed in this laboratory, the heat of solution was determined by the following equation with the assumption of an ideal solution:

$$\Delta H(\text{kcal/mole}) = \ln \frac{Cs(\mu\text{g/ml})}{152.19} \frac{RT_{mp}T}{T_{mp} - T} \quad \text{(Eq. 12)}$$

The values of solution enthalpy thus calculated based on the melting-point range of 298–319° range from 4.75 to 4.95 kcal/mole. A direct measurement of fusion enthalpy made by comparing the areas under the curve to a standard compound gave a value of 5.5 kcal/mole, which is slightly higher than the calculated value since the ideality of solution may not be applicable here. Disagreement between the present values and those reported (11) may be due to the solubility determination used in the present study being more sensitive and relatively error free.

A direct determination of the solubility of the anhydrate cannot be made since it converts to the hydrate in an aqueous medium (3). However, based on the initial dissolution rates (3) of the anhydrate and the hydrate, the solubility can be correlated:

$$\frac{dC_h}{dt} \alpha\, Cs_h \quad \text{(Eq. 13)}$$

$$\frac{dC_{anh}}{dt} \alpha\, Cs_{anh} \quad \text{(Eq. 14)}$$

$$\frac{Cs_h}{Cs_{anh}} = \frac{dC_h/dt}{dC_{anh}/dt} \quad \text{(Eq. 15)}$$

where Cs_h is the solubility of the hydrate, and Cs_{anh} is the solubility of the anhydrate.

Based on the reported (3) dissolution rate constants of 0.25 and 0.16 min^{-1} from similar surface area particles, the anhydrate solubility is 404.68 μg/ml.

The presented data show that the mercaptopurine anhydrate is highly stable at room temperature and will dissolve faster in an aqueous medium in spite of its conversion to the hydrate. It is suggested that the anhydrate can result in improved bioavailability, and these studies will be reported.

Table III—Half-Lives of Mercaptopurine Dehydration as a Function of Temperature

Temperature	Half-Life, min
25°	3.69×10^{11}
50°	4.75×10^{8}
75°	1.59×10^{6}
100°	1.14×10^{4}
125°	1.53×10^{2}
150°	3.4×10^{0}
200°	5.62×10^{-3}

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