

## **SOLUTION THERMODYNAMICS OF SOME POTENTIALLY LONG-ACTING NORETHINDRONE DERIVATIVES I. VARIATION OF ENTHALPIES AND ENTROPIES OF FUSION WITH TEMPERATURE**

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### **SUMMARY**

Differential scanning calorimetry was used to determine the thermodynamic parameters of fusion of a number of potentially long-acting derivatives of the progestational steroid norethindrone from 260°K to their melting points. The differences in molar heat capacities of the crystals and supercooled liquid states were measured, and the enthalpies and entropies of fusion were shown to increase significantly with temperature. Entropies of fusion were correlated with the molecular structures of the derivatives. The value of such data in preliminary investigations of the physicochemical properties of new compounds is discussed.

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### **INTRODUCTION**

Norethindrone is a progestational steroid that is a more potent inhibitor of ovulation than progesterone itself. The drug is given in a 350 µg daily dose continuous regimen as an oral contraceptive (Extra Pharmacopoeia, 1977). There continues to be a demand for long-acting systemic agents for control of fertility (WHO, 1977), and attempts are being made to produce delivery devices that release norethindrone over prolonged periods of time (Anderson et al., 1976). Another approach to the problem has been to synthesize derivatives of norethindrone that exhibit prolonged activity possibly by virtue of their low aqueous solubility, slow rate of regeneration of the parent steroid or their high affinity for the fatty tissues of the body. A number of norethindrone derivatives have been made available to us for investigation of their physicochemical properties. One aspect of such a study entails evaluation of their solution properties and, as a first step, ideal solubilities are being determined from a knowledge of the enthalpies and entropies of fusion of the compounds.

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In correlating and predicting solubilities of a solid, it is necessary to obtain a reliable value for the free energy of fusion at the temperature of interest from experimental measurements. The free energy of fusion is dependent on the crystal structure as well as molecular structure in a complex manner (Bondi, 1967), whereas the relationship between the free energy of solution of the liquid solute and the molecular structure of solute and solvent is simpler and more amenable to theoretical treatment.

In estimating the free energy of fusion at a given temperature,  $T_0$ , if one assumes that the enthalpy of fusion,  $\Delta H^f$ , is constant with temperature, then the free energy of fusion,  $\Delta G^f$ , is

$$\Delta G^f = \Delta H^f \left( 1 - \frac{T_0}{T_m} \right) \quad (1)$$

where  $T_m$  is the melting temperature (Yalkowsky et al., 1972).

The molar heat capacity of a liquid is normally higher than that of a crystal. The enthalpy and free energy of fusion are related to the difference in heat capacity,  $\Delta C_p$ , as follows:

$$\Delta H_m^f - \Delta H^f = \int_{T_0}^{T_m} \Delta C_p \cdot dT \quad (2)$$

and

$$\Delta G^f = \Delta H_m^f \left( 1 - \frac{T_0}{T_m} \right) - \int_{T_0}^{T_m} \Delta C_p dT + T_0 \int_{T_0}^{T_m} \frac{\Delta C_p}{T} dT \quad (3)$$

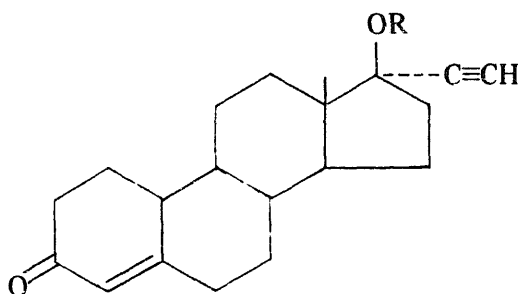
where  $\Delta H_m^f$  is the enthalpy of fusion at  $T_m$ .

The correct value of  $\Delta G^f$  obtained from Eqn. 3 is thus less than that given by Eqn. 1. Although the error in  $\Delta G^f$  in assuming  $\Delta H^f$  constant is much less than the error in  $\Delta H^f$  itself, it still may be significant, and one must therefore know the heat capacity of the liquid. This may either be obtained above the melting point of the solid, assumed to be constant and to have the same value below the melting point, or may be obtained by extrapolation of values at temperatures above the melting point to the temperature of interest (James and Roberts, 1968) or be measured directly. The first two methods are unreliable, and direct measurement is only possible where the liquids supercool (Young and Hildebrand, 1942).

Where the solid dissolves to give regular solutions and  $\Delta C_p$  cannot be measured directly, it is possible to obtain consistent values of the thermodynamic activity of the liquid and of the solubility parameter of the solute from the solubilities in a number of solvents of known solubility parameters using the extrapolated value of  $\Delta C_p$  for an initial estimate (Hildebrand et al., 1970).

We report here the direct measurement of the  $\Delta C_p$  values for 9 derivatives of nor-ethindrone and the calculated values of enthalpies, entropies and free energies of fusion together with estimates of the ideal solubilities of these compounds.

## MATERIALS AND METHODS


*Materials*

I Norethindrone [17 $\alpha$ -ethynyl-17-hydroxy-4-estrene-3-one] R = H

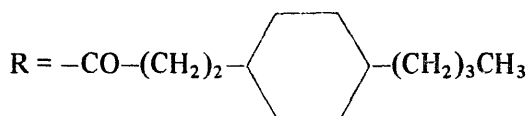
II Norethindrone acetate R =  $-\text{CO}-\text{CH}_3$

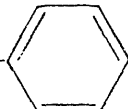
III Norethindrone heptanoate R =  $-\text{CO}-(\text{CH}_2)_5\text{CH}_3$

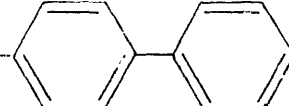
IV Norethindrone dimethylpropionate R =  $-\text{CO}-\text{C}(\text{CH}_3)_2-\text{CH}_3$

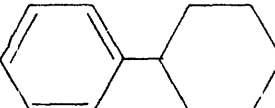
V Norethindrone trans-4-hexylcyclohexylcarboxylate R =  $-\text{CO}$    $-(\text{CH}_2)_5\text{CH}_3$

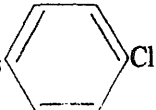
VI Norethindrone trans-3-(4-butylcyclohexyl)propionate

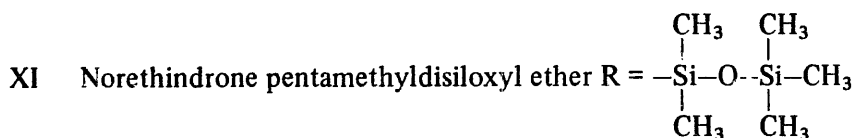


VII Norethindrone benzoate R =  $-\text{CO}$  

VIII Norethindrone biphenyl-4-carboxylate R =  $-\text{CO}$  

IX Norethindrone 4-cyclohexylbenzoate R =  $-\text{CO}$  

X Norethindrone-6-(4-chlorophenyl)-hexanoate R =  $-\text{CO}-(\text{CH}_2)_5$  



Compounds I and II were obtained from Serva Feinbiochemica and compound III from Schering A.G., Berlin. The remaining compounds were obtained through the World Health Organisation from the following principal investigators, who synthesized them:

Dr. A. Shafiee, College of Pharmacy, Tehran, Iran.	Compounds V, VI, VIII and IX
Professor P.N. Natarajan, Department of Pharmacy, University of Singapore	VII
Dr. J.E. Herz, Instituto Politecnico Nacional, Mexico 14	IV (Hertz et al., 1977), XI
Dr. G. Krakower, Department of Chemistry, Bar Ilan University, Ramat-Gan, Israel.	X

### *Differential scanning calorimetry*

Molar heat capacities of the crystals were measured using a Perkin-Elmer Differential Scanning Calorimeter DSC-1B over the temperature range 260°K to the melting point of each compound with a rate of temperature increase of 32 degrees per minute. This rapid rate was considered desirable to avoid decomposition of the sample. The temperature scale was calibrated by using substances of known melting points; i.e. water, benzoic acid, indium and tin.

After the steroid sample had melted it was cooled rapidly to 260°K and the experiment repeated to obtain the molar heat capacity of the liquid using the same sample. A typical trace is shown in Fig. 1.  $\Delta C_p$  was calculated by comparison with a sapphire standard whose specific heat capacity is known accurately over a wide range of temperature (Ginnings and Furakawa, 1953).

The enthalpy of fusion was calculated from the area under the melting peak using the sapphire standard for comparison. This was considered a more reliable method than using a standard of known enthalpy of fusion such as indium. The calibration can thus be carried out at the exact melting temperature of the steroid samples; it is not necessary to compare steroid and standard which may melt at considerably different temperatures.

### RESULTS AND DISCUSSION

All of the fused steroids supercooled with the exception of norethindrone (I) and the hexylcyclohexylcarboxylate ester (V). A peak was noted in most of the  $\Delta C_p$  plots against temperature marking the second-order transition of glass to liquid. An example is shown in Fig. 2. A second-order transition was not observed with the pentamethyldisiloxyl ether (XI), presumably because this lies below 260°K.

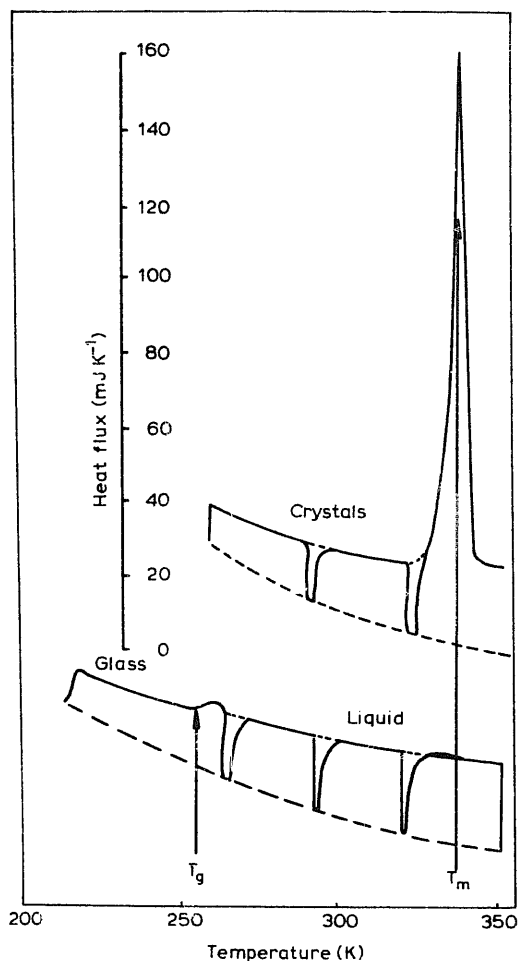


Fig. 1. Differential scanning calorimeter traces of norethindrone heptanoate (III). The upper trace shows the fusion endotherm of the crystals, and the lower one the transition of glass to liquid. (Breaks in the curve indicate where the temperature was held constant with the chart recorder stopped in order to establish the base line.)

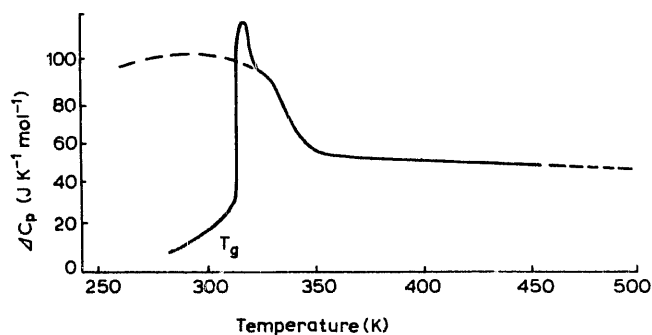


Fig. 2 The variation of  $\Delta C_p$  with temperature for norethindrone dimethylpropionate (IV). The plot shows the glass transition point and the extrapolation of  $\Delta C_p$  below  $T_g$ .

TABLE 1

MELTING POINTS,  $T_m$ , GLASS TRANSITION TEMPERATURES,  $T_g$ , AND ENTHALPIES OF FUSION AT THE MELTING TEMPERATURE,  $H_m^f$ , FOR NORETHINDRONE AND ITS DERIVATIVES

Steroid	$\Delta H_m^f$ (kJ mol <sup>-1</sup> )	$T_m$ (°K)	$T_g$ (°K)
Norethindrone (I)	39.6	479	—
Norethindrone acetate (II)	27.3	480	334
Norethindrone heptanoate (III)	21.6	340	262
Norethindrone dimethylpropionate (IV)	37.8	500	311
Norethindrone hexylcyclohexylcarboxylate (V)	22.6	398	—
Norethindrone butylcyclohexylpropionate (VI)	22.5	374	282
Norethindrone benzoate (VII)	41.5	531	340
Norethindrone biphenylcarboxylate (VIII)	31.6	462	356
Norethindrone cyclohexylbenzoate (IX)	36.8	482	350
Norethindrone chlorophenylhexanoate (X)	28.8	413	280
Norethindrone pentamethyldisiloxy ether (XI)	22.9	355	Below 260

Experimentally determined enthalpies of fusion and melting temperatures are listed in Table 1 with the observed glass transition temperatures. However, the glass transition temperature as normally measured is a function of the rate of heating (in this case 32°K/min) and is not an equilibrium quantity (Kovaks, 1958). The true glass transition temperature would be determined by extrapolation of data obtained at several heating rates to zero rate of temperature increase.

Enthalpies, entropies and free energies of fusion at temperatures below the melting point were calculated using Eqns. 2 and 3. The results obtained for norethindrone dimethylpropionate (IV) are shown in Fig. 3.

The enthalpy and entropy of fusion depend on the nature of the forces between the molecules and on the geometry of the crystal lattice, and there is no simple accurate relationship between the molecular structure and the thermodynamic properties of the crystal. Norethindrone itself is presumably hydrogen bonded in the crystal whereas its derivatives form molecular crystals without hydrogen bonding. The strengths of van der Waals forces depend on the size of the molecules and on their polarisabilities — aromatic side-chains will cause such intermolecular forces to be slightly stronger. The effect of bulky side-chains also inhibits close packing and this lowers the melting temperature and enthalpy of fusion.

As expected, therefore, compounds with higher melting points are those with compact side-chains allowing close packing and stronger intermolecular forces — norethindrone (I), and its acetate (II) and dimethylpropionate (IV), and those with aromatic side chains: the benzoate (VII), biphenylcarboxylate (VIII), cyclohexylbenzoate (IX) and chlorophenylhexanoate (X). These compounds, although supercooling, crystallized easily above their glass transition temperature and in some cases repeated experiments were necessary before the heat capacities of the supercooled liquids could be determined. The remaining compounds, with more flexible side-chains, formed more stable supercooled liquids. Crystallization required the presence of a crystal nucleus. A sample of liquid nor-

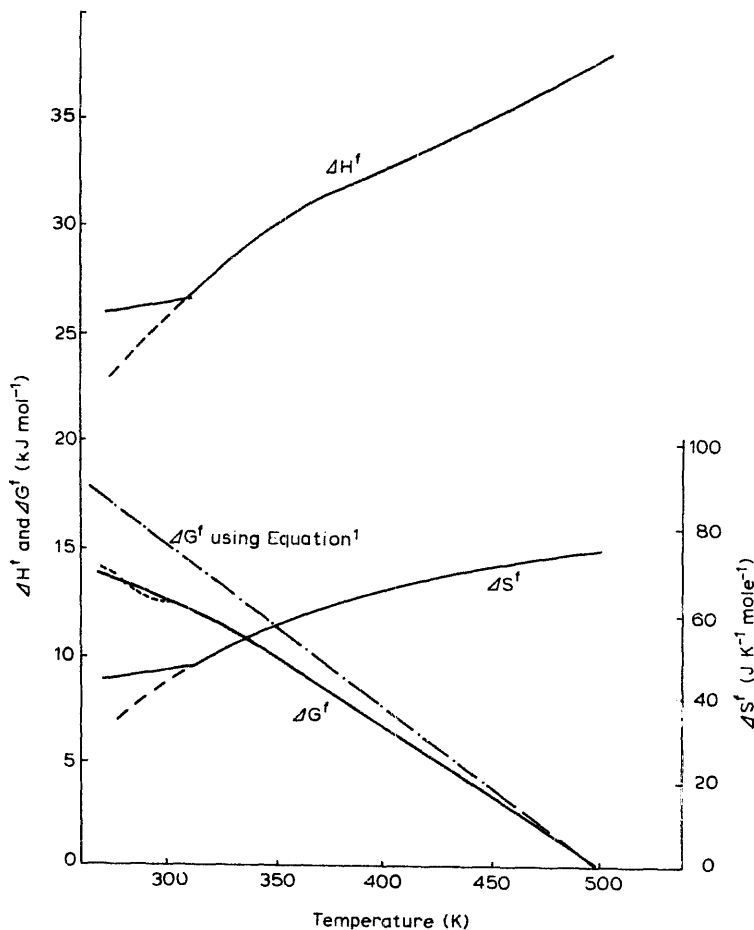


Fig. 3. Variation of enthalpy, entropy and free energy of fusion with temperature for norethindrone dimethylpropionate (IV). - - - -  $\Delta H^f$ ,  $\Delta S^f$ , and  $\Delta G^f$  calculated using extrapolation of the  $\Delta C_p$  values below  $T_g$  shown in Fig. 2. · · · ·  $\Delta G^f$  calculated using Eqn. 1.

ethindrone pentamethyldisiloxy ether (XI) was kept for 4 weeks without any sign of crystallization.

The calculated values of enthalpies and entropies at room temperature, when compared with the experimentally determined values at the melting point, show clearly that the error in assuming constant enthalpy and entropy is very great. The error is greater the larger and more flexible the molecule. The corresponding errors in the Gibbs free energy of fusion, although not as great, since changes in enthalpy and entropy tend to compensate one another (Hildebrand and Scott, 1950), are still considerable and should be taken into account wherever possible.

The thermodynamic functions of melting of related compounds are normally compared at the melting temperature of each compound because of an absence of data for  $\Delta H^f$  (T). Approximately constant increments have been observed, for example, in entropy and enthalpy, for the addition of methylene groups to alkyl *p*-aminobenzoates (Yalkowsky et al., 1972), a series in which the melting temperatures are all similar. For

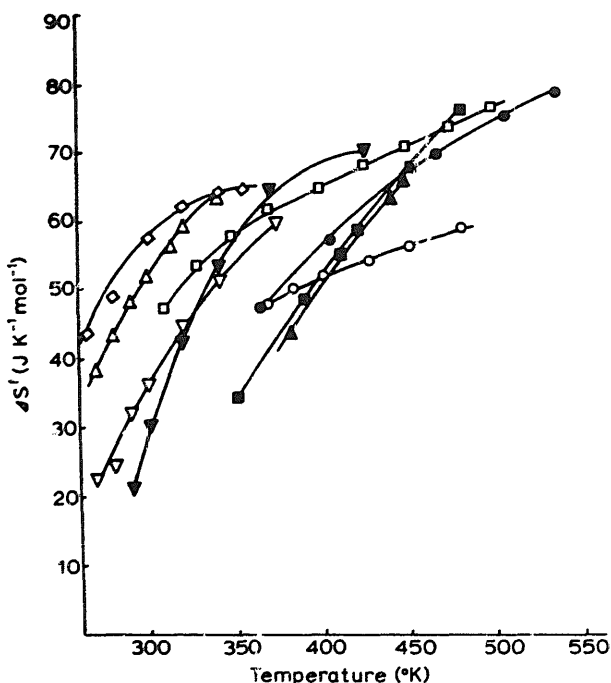


Fig. 4. Variation of molar entropies of fusion,  $\Delta S^f$ , with temperature above the glass transition temperature. Key:  $\circ$ , norethindrone acetate (II);  $\triangle$ , norethindrone heptanoate (III);  $\square$ , norethindrone dimethylpropionate (IV);  $\nabla$ , norethindrone trans-3-(4-butylcyclohexyl)-propionate (VI);  $\diamond$ , norethindrone pentamethyldisiloxyl ether (XI);  $\bullet$ , norethindrone benzoate (VIII);  $\blacktriangle$ , norethindrone biphenyl-4-carboxylate (VIII);  $\blacksquare$ , norethindrone 4-cyclohexylbenzoate (IX);  $\blacktriangledown$ , norethindrone-6-(4-chlorophenyl)-hexanoate (X).

those compounds not clearly related and melting at different temperatures, the enthalpies and entropies of fusion are better compared at the same temperature. Fig. 4 shows the entropies of fusion from  $T_g$  to  $T_m$  and Fig. 5 the enthalpies of fusion. Whereas the enthalpy of fusion is a complex function dependent on the form of the crystal lattice, the entropy of fusion depends more on molecular structure (Bondi, 1967) and the plots allow a number of generalizations to be made.

(a) At a given temperature the entropy of fusion depends upon the difference in degrees of freedom available to the molecule between crystal and liquid, which in turn depends on the size and flexibility of the molecule. At 350°K the heptanoate ester (III) and the pentamethyldisiloxyl ester (XI) have the highest entropies. The side-chain, although frozen in the solid in an equilibrium conformation, can exhibit internal rotation in the liquid to a variety of conformations thus increasing the entropy.

The entropies of the molecules with equally bulky but stiffer aromatic side-chains (benzoate (VII), biphenylcarboxylate (VIII), cyclohexylbenzoate (IX)) are lower. The values of  $\Delta S^f$  for the biphenylcarboxylate are very similar to those of the cyclohexylcarboxylate. For both of these groups the gain in entropy would be by rotation of the ring and by changes in the vibrational entropy (Ubbelohde, 1965a). Conformational changes of the cyclohexyl group have little effect on entropy as the conformation with



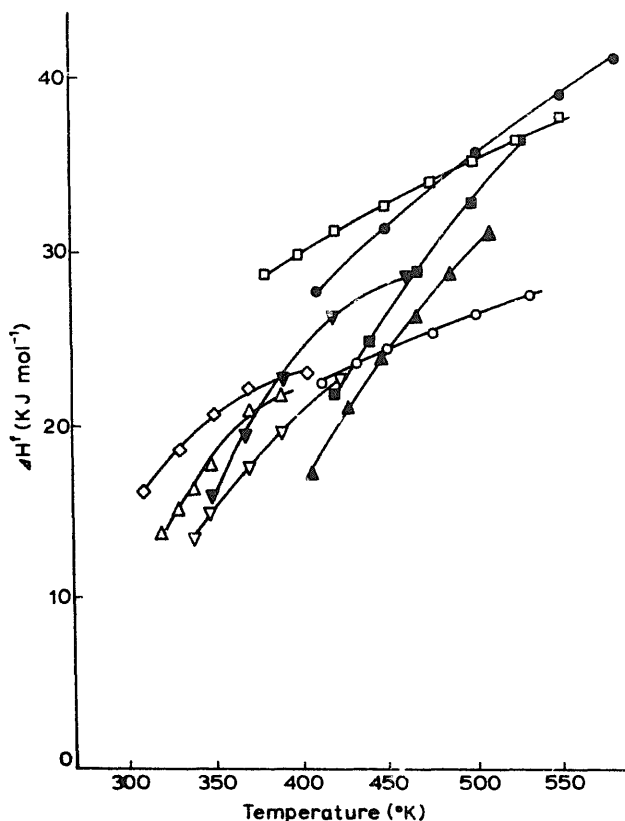


Fig. 5. Variation of molar enthalpies of fusion,  $\Delta H^f$ , with temperature above the glass transition temperature. Key as in Fig. 4.

the aromatic group equatorial must be considerably more stable than the axial conformation.

The data for the chlorophenylhexanoate ester (X) and the butylcyclohexylpropionate ester (VI) indicate that addition of a cyclohexyl or aromatic group to a flexible chain leads to a decrease of  $\Delta S^f$  at a given temperature. However, because  $T_m$  is normally increased, the entropy at the melting point  $\Delta S_m^f$  is increased (Bondi, 1967).

(b) The entropies of fusion of the large molecules decrease rapidly with decreasing temperature as conformational changes, and perhaps rotation of the whole molecule, become restricted by intermolecular and intramolecular interactions. The entropies of the molecules with simpler side-chains decrease less rapidly as internal rotation of these groups is relatively unhindered. It is unlikely that the methyl group of the acetyl side-chain of norethindrone acetate (II) adds much to  $\Delta S^f$  as hindrance to internal rotation might be expected to be similar in both liquid and solid. Thus it has a lower entropy of fusion than the other molecules. Norethindrone dimethylpropionate (IV) has a higher entropy of fusion than the acetate (approximately 2R). It may be that rotations of the tertiary butyl group and of the methyl group comprising it are more hindered in the solid than in the liquid but it may also be a result of changes in the modes of vibration (Ubbelohde, 1965a).

(c) The slopes of the curves for  $\Delta H^f(T)$  (Fig. 5) follow related patterns because

$$\Delta S^f = \frac{\Delta H^f}{T_0} + \int_{T_0}^{T_m} \frac{\Delta H^f}{T^2} dT$$

at constant pressure. The acetate (II), dimethylpropionate (IV) and benzoate (VII) esters have enthalpies of fusion which decrease relatively slowly with decreasing temperature, whereas the enthalpies of fusion of the remaining compounds decrease more rapidly. The influence of the different groups on the enthalpy of fusion and the melting point has been discussed above.

Below  $T_g$  the entropies are much more constant (Fig. 6) as the conformations are frozen (Ubbelohde, 1965b). Changes with temperature will reflect differences in the modes of vibration of glass and solid. The glass of the biphenylcarboxylate ester (VIII) is remarkable in that the entropy and enthalpy for the transition of crystal to glass decrease with increasing temperature. The benzoate (VII) behaves in a similar manner although entropy does not increase below  $T_g$  to the same extent. In these two substances  $\Delta C_p$  below  $T_g$  is negative. The remaining compounds have  $\Delta C_p$  close to zero below  $T_g$ .

The ideal solubility is given by

$$\ln x_2 = \frac{-\Delta G^f}{RT}$$

where  $x_2$  is the mole fraction concentration of solute in the saturated solution. Values of  $\Delta G^f$  are plotted in Fig. 7. There is a discontinuity at  $T_g$  and one would postulate that the

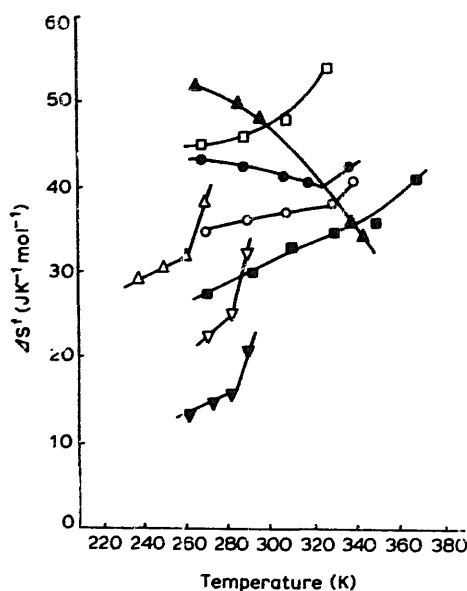


Fig. 6. Variation of molar entropies of fusion,  $\Delta S^f$ , with temperature below glass transition temperature. Key as in Fig. 4.

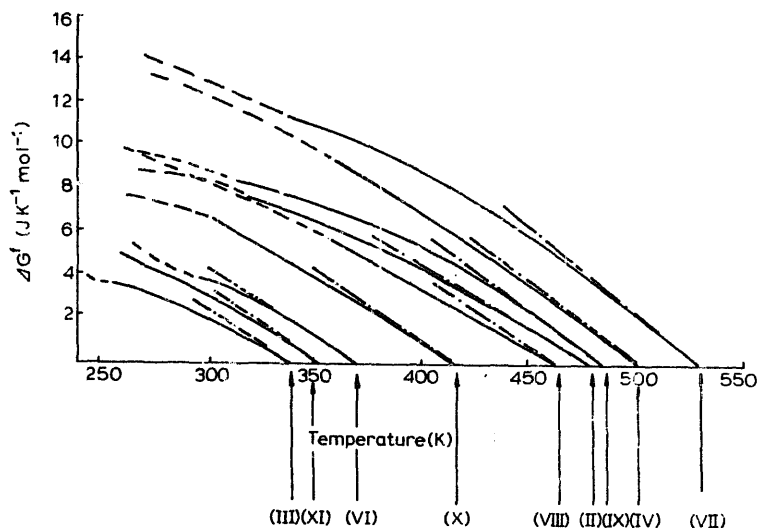


Fig. 7. Variation of free energies of fusion with temperature of norethindrone derivatives. —, above  $T_g$ ; - - - - -, below  $T_g$ ; · - · - ·, relationship calculated from Eqn. 1.

ideal solubility should be obtained by extrapolation of  $\Delta C_p$  below  $T_g$  and calculation of  $\Delta G^f$  using Eqn. 3, (Figs. 2 and 3). As has been stated in the Introduction, long extrapolations of  $\Delta C_p$  should be avoided. It appears that, using Eqn. 3, reliable values of  $\Delta G^f$  can be obtained as the extrapolated line always lies very close to the experimental line for the glass for 60–70 degrees below  $T_g$ . This is not true for  $\Delta H^f$  and  $\Delta S^f$  for the liquid below  $T_g$  and extrapolations of more than 30–50 degrees must be suspect. It will thus not be possible to obtain reliable values of the excess entropy and enthalpy of solution of the cyclohexylbenzoate (IX) and biphenylcarboxylate (VIII) below 300°K, although the values of  $\Delta G^f$ , and thus the excess free energy of solution, should be fairly close to the 'true' values down to 270°K.

The actual solubility of the compound can be obtained with a knowledge of the ideal solubility and the excess free energy of the solute–solvent interaction. A high  $\Delta G^f$  value for a compound is indicative of strong crystal lattice forces and will result in relatively low solubility in both water and organic solvents. This is in contrast to the low aqueous solubilities and high solubilities in organic solvents exhibited by very hydrophobic compounds with low melting points, such as norethindrone heptanoate (I). The heptanoate is more hydrophobic than the dimethylpropionate (IV) as there are two more carbon atoms in the alkyl group of (III), and the side-chain of (IV) is branched, thus lowering its hydrophobicity. The dimethylpropionate has the greater free energy of fusion and this compensates for its less hydrophobic nature. Both compounds would be expected to have similar aqueous solubilities, and this has been found to be the case (Lewis and Enever, unpublished data, 1978). The heptanoate has been found to be considerably more soluble than the dimethylpropionate in a hydrocarbon solvent. These considerations can influence the choice of compounds for biological testing and the subsequent approach to formulation.

Certain of the glasses and liquids were found to be stable at room temperature, and it

may prove possible to prepare formulations based on these states, when an increased solubility and/or rate of solution is desired. The supercooled liquid pentamethyldisiloxyl ether (XI), for example, would have twice the solubility of its crystalline form at 37°C, and, as stated above, it is very stable at room temperature.

Investigations of new compounds can be limited in many cases by the amounts available for use. Each sample has, in the first instance, taken a considerable amount of time and effort to prepare and purify, and its cost is therefore high. Experimental methods which enable one to approximately predict the results of subsequent experiments and which use only a small amount of sample are accordingly valuable. As has been demonstrated here, differential scanning calorimetry comes into this category and the results obtained assist in making an initial prediction of solubility in organic solvents and in the choice of formulation, as well as being of great utility in determining the relationships which govern solubility in water and other solvents.

#### ACKNOWLEDGEMENT

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