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The solubilities of the lower testosterone esters

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The solubilities of the formate to valerate esters of testosterone have been determined in water and several organic solvents. The aqueous solubilities decrease logarithmically as the homologous series is ascended, but the acetate is less soluble than anticipated in the organic solvents. The variation in solubility from ester to ester can be predicted in the organic solvents from thermodynamic data, and is a reflection of the differences in melting point. The melting point differences are explained from the space group dimensions and the area of α to α face contact in the crystals.

IT is well established that the intensity and duration of biological action of testosterone are enhanced by esterification, and vary from ester to ester (Miescher, Wettstein & Tschopp, 1936; Parkes, 1936). The postulate that this is due to slow release of testosterone by hydrolysis has been tested by Pesez & Bartos (1962) and Schenck & Junkmann (1955) with limited success, but no attempt has been made to relate the changes in biological activity to solubility. The solubilities of the lower testosterone esters from formate to valerate have therefore been determined as a preliminary to such an investigation.

Experimental and results

Materials. The testosterone esters were from British Drug Houses Ltd. Organic solvents were fractionally distilled and their purity checked by refractive index.

Determination of solubility in water. An excess of ester was stirred with water at 25° until a saturated solution was obtained. About 500 ml was filtered off, weighed and continuously extracted with a small volume of hexane, which was subsequently adjusted to 50 or 100 ml. The concentration of the saturated aqueous solution was then calculated from the extinction of the hexane extract measured at 229·5 mμ. This procedure was necessary because the aqueous solutions were too dilute to yield reliable spectrophotometer readings. Results are given in Table 1. The presence of colloidal dispersed material was eliminated by subjecting saturated solutions to high speed centrifugation. The extinctions, measured at 244 mμ in a 40 mm cell, did not change after 4 hr at 100,000 *g*.

Determination of solubilities in organic solvents. Saturated solutions were prepared at 25° by percolating about 0·25 ml of solvent through a column of about 500 mg of solute, supported on cotton wool. The eluate was returned to the top of the column until a saturated solution was obtained. An aliquot of the solution was assayed by weighing the residue after evaporation at 80°; vacuum was necessary to remove nitrobenzene. Results are given in Table 1.

Calorimetric determinations. Heat capacities and heats of fusion were measured on a Perkin Elmer D.S.C.1 differential scanning calorimeter.

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TABLE 1. SOLUBILITIES OF THE TESTOSTERONE ESTERS IN VARIOUS SOLVENTS (25°)

Ester	Mole fraction solubility						
	Water	Ethanol	Toluene	Benzene	Chloroform	1,2-Dichloroethane	Nitrobenzene
Formate	$2{\cdot}50 \times 10^{-7}$	$2{\cdot}31 \times 10^{-2}$	0·211	0·283	0·381	0·315	0·245
Acetate	$1{\cdot}28 \times 10^{-7}$	$1{\cdot}70 \times 10^{-2}$	0·133	0·165	0·290	0·227	0·147
Propionate ..	$7{\cdot}74 \times 10^{-8}$	$2{\cdot}79 \times 10^{-2}$	0·199	0·260	0·354	0·294	0·220
Butyrate	$2{\cdot}53 \times 10^{-8}$	$1{\cdot}60 \times 10^{-2}$	0·197	0·237	0·339	0·250	0·158
Valerate	$1{\cdot}40 \times 10^{-8}$	$6{\cdot}85 \times 10^{-3}$	0·124	0·169	0·278	0·188	0·124

TABLE 2. THERMAL DATA FOR TESTOSTERONE ESTERS

Ester	m.p. °C	ΔH_M^F kcal mole^{-1}	$\Delta Cp(T_M - T)$ kcal mole^{-1}	$\Delta H_{25°}^F$ kcal mole^{-1}	Ideal mole fraction solubility (25°)	
					Integrated method	By mean heat of fusion
Formate ..	125	4·33	3·31	1·02	0·182	0·322
Acetate ..	140	5·38	4·06	1·32	0·087	0·204
Propionate ..	120	5·29	3·85	1·44	0·149	0·254
Butyrate ..	109	6·05	3·03	3·02	0·172	0·192
Valerate ..	107	7·40	2·25	5·15	0·127	0·102

The technique has been described elsewhere (Watson, O'Neill & others, 1964). Calibration curves were constructed for heat of fusion with metallic tin and for heat capacity with sapphire. The instrument is claimed by its manufacturer to yield heat capacity results within ±2% of those obtained by conventional means. Heat capacities were measured to about 50° above the melting point. Results are shown in Table 2.

Determination of densities of saturated solutions. A drop of saturated solution in chloroform was added to a concentrated solution of cadmium chloride in water, saturated with chloroform and the relevant testosterone ester. The concentration of the aqueous solution was adjusted until the drop neither rose nor fell, and the density of the aqueous phase then determined by hydrometer and density bottle.

Discussion

The solubilities of members of an homologous series normally decrease logarithmically with the addition of each successive methylene group (Butler & Ranchandani, 1935). The testosterone esters examined here behaved in this way in water, but in all the other solvents, minimal solubility was found with the acetate. The validity of this observation was first tested by calculating ideal solubilities from thermodynamic data. Ideal solubility assumes uniform intermolecular attraction involving no energy change when the components are brought together, except that necessary to liquify the solute, and can be calculated from,

$$\frac{d}{dT} \ln a_2 = \frac{\Delta H^F}{RT^2} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where a is the ideal solubility, and the subscript 2 represents solute. If ΔH^F, the molar heat of fusion, is considered constant, integration gives,

$$-\ln.a_2 = \frac{\Delta H^F}{R}\left[\frac{T_M - T}{T_M T}\right] \quad .. \quad .. \quad .. \quad (2)$$

where T_M is melting point. ΔH^F, the mean molar heat of fusion over the range T to T_M, is given by the equations.

$$\Delta H^F = \tfrac{1}{2}(\Delta H^F_T + \Delta H^F_M) \quad .. \quad .. \quad .. \quad (3)$$

where ΔH^F_T and ΔH^F_M are molar heats of fusion at the temperature of interest and the melting point respectively, and,

$$\Delta H^F_T = \Delta H^F_M - \Delta C_p\,(T_M - T) \quad .. \quad .. \quad (4)$$

ΔC_p is the difference between the heat capacities of the solid and supercooled liquid. ΔC_p is not necessarily independent of temperature, and the calorimeter results indicated that ΔC_p varied with temperature. The correction equivalent to $\Delta C_p\,(T_M - T)$ was therefore obtained by extrapolating the liquid enthalpy line from above the melting point at 25° and measuring the area between this line and that for the solid. This is illustrated in Fig. 1.

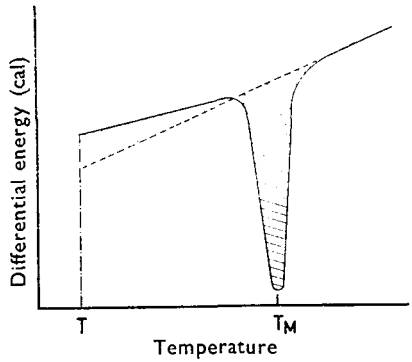

FIG. 1. Determination of heat capacity and heat of fusion. Vertical hatching, $\Delta Cp\Delta T$. Diagonal shading, ΔH^F_M.

Solubilities were also calculated using the integrated value of ΔH^F between T_M and T. Heat capacity can be expressed as,

$$\Delta C_p = a + b\,(T_M - T) \quad .. \quad .. \quad .. \quad (5)$$

where a and b are constants, evaluated by plotting ΔC_p against $(T_M - T)$.

Since,

$$\frac{d}{dT}\Delta H^F = \Delta C_p \quad .. \quad .. \quad .. \quad .. \quad (6)$$

substitution for ΔC_p from equation (5) and integration between T_M and T gives,

$$\Delta H^F = \Delta H^F_M - a\,(T_M - T) - b/2\,(T_M - T)^2 \quad .. \quad (7)$$

which, when substituted in equation (1), yields, on integration between the same limits,

$$\ln.a_2 = \frac{1}{R}\left[\frac{(-\Delta H^F_M + aT_M + b/2\,T_M^2)\,(T_M - T)}{T_M T} - (a + bT_M)\ln.\frac{T_M}{T} + b/2\,(T_M - T)\right] \quad .. \quad .. \quad .. \quad (8)$$

Heats of fusion and calculated solubilities are shown in Table 2. Both sets of results confirm that the solubility should pass through a minimum at the acetate, and indicate that it is a reflection of the high melting point of this ester.

At the melting point vibrational energy exceeds the intermolecular attraction, which in testosterone esters results from London forces. These are significant only with those groups which are close to neighbouring molecules, since the forces decrease in proportion to the sixth power of distance. Intermolecular forces can be estimated from the space group dimensions, reproduced in Table 3 from Griffiths, James & Rees (1965). Courtauld models were fitted into scaled up space groups of these dimensions, and indicated that the alkyl chains of the acetate and propionate continue along the b axis, but those of the butyrate and valerate are folded back over the molecule. This produced a shortening of the b axis and an increase in the c axis due to the alkyl chain acting as a wedge. The inference from this is that the acetate should have a similar melting point to the propionate, and the butyrate a similar melting point to the valerate, those of the former pair being higher than those of the latter pair.

TABLE 3. CRYSTALOGRAPHIC DATA FOR TESTOSTERONE ESTERS

Ester	Angstrom units			Area of α face adjacent to those of neighbouring molecules (Å^2)	Area / m.p.
	a	b	c		
Acetate	12·6	18·1	7·8	77	0·55
Propionate ..	12·6	20·3	7·6	63	0·53
Butyrate	12·3	16·3	10·3	88	0·81
Valerate	12·3	16·7	10·3	89	0·83

The difference between the melting points of acetate and propionate is probably due to α to α face attraction. The testosterone molecule is essentially planar. One side of the plane, the β face, has two angular methyl groups projecting from it, while the other side, the α face, is substituted entirely with hydrogen atoms. The α face therefore presents a large comparatively flat surface, and the area of α to α face contact is probably the largest contributing factor towards the melting points. The β face would be of lesser importance because the projecting methyl groups allow fewer points of contact. This theory is supported by the fact that the ratio of the area per molecule of α face adjacent to the α face of a neighbouring molecule, measured from the models, to the melting point, is the same for acetate and propionate. A similar relationship obtains with butyrate and valerate. These are shown in Table 3. The formate could not be considered in this way because it belongs to a different space group.

Restaino & Martin (1964) have shown that benzoate esters form regular solutions (Hildebrand & Scott, 1962) in low polarity solvents, and therefore testosterone esters were expected to behave in the same

way. Ideal solubility is the product of the regular solubility and the activity coefficient (γ), which can be calculated from the equation,

$$\ln\gamma_2 = \frac{\phi_1^2 V_2 (\delta_1 - \delta_2)^2}{RT} \quad .. \quad .. \quad .. \quad (9)$$

where ϕ is the volume fraction, and V the molar volume. The subscript 1 denotes solvent and 2 solute. δ is the solubility parameter and is a measure of escaping tendency. When $\delta_1 = \delta_2$ solute and solvent have the highest affinity for each other, the activity coefficient becomes unity, and the solution is said to be ideal.

The solubility parameter of a solute can be determined by plotting solubilities in a series of solvents against solvent solubility parameter (Chertkoff & Martin, 1960). Equation (9) predicts that solubility is maximal when $\delta_1 = \delta_2$ so that the peak of the graph coincides with the solubility parameter of the solute. The method has the disadvantages that the maximum is not sharp and the solute solubility parameter can not be fixed accurately. More precise results were obtained here, by plotting the logarithms of the solubilities against the solubility parameters of the solvents. Two intersecting straight lines were obtained near the solubility maximum, so that the solubility parameter could be determined to within 0·1 $cal^{\frac{1}{2}}$ $cm^{-3/2}$. A typical plot is shown in Fig. 2.

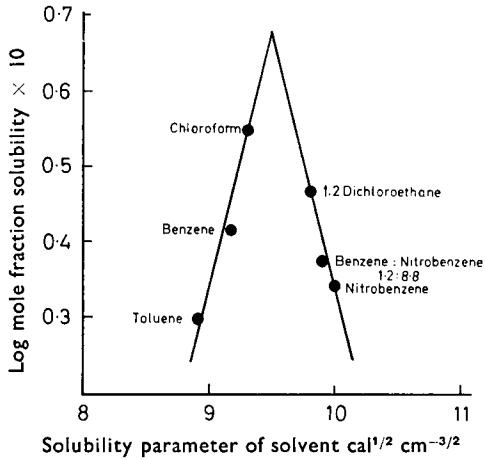

FIG. 2. Determination of solubility parameters.

All 5 esters, and testosterone, gave a solubility parameter of 9·5 $cal^{\frac{1}{2}}$ $cm^{-3/2}$. A difference between the solubility parameters of testosterone and its esters would be anticipated if the polarities of ester and hydroxy groups had a significant effect on intermolecular attraction. Hildebrand & Scott (1962) consider that regular solutions are formed by esters, only "when the dipole is reasonably well buried within the molecule". It appears that the bulky steroid nucleus in the testosterone esters has caused such an effect.

Solutions in chloroform, because its solubility parameter is close to 9·5 $cal^{\frac{1}{2}}\ cm^{-3/2}$ can, as an approximation, be assumed to be ideal. Evaluation of the activity coefficient gave a value close to unity, confirming that this was a reasonable simplification. V_2 was calculated from,

$$\frac{x_1M_1 + x_2M_2}{\text{Density of saturated solution}} = x_1V_1 + x_2V_2 \quad .. \quad (10)$$

where M is molecular weight, and ϕ_1 from,

$$\phi_1 = \frac{x_1V_1}{x_1V_1 + x_2V_2} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

Thus for testosterone acetate, which was found to give a saturated solution of density 1·28, calculation yielded a molar volume of 286 and an activity coefficient of 1·003. The other esters gave similar results. The observed solubilities in chloroform, shown in Table 1, should therefore agree with the ideal solubilities, calculated from thermodynamic data, and shown in Table 2. The poor correlation could be due to the long extrapolation in obtaining $\Delta C_p\ \Delta T$, or the large difference in molar volumes of solute and solvent which does not permit the random distribution assumed in regular solution theory. These possibilities are being investigated.

The fact that the aqueous solubilities did not follow the same pattern as in the organic solvents can be attributed to deviation from regular solution behaviour.

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