J. Pharm. Pharmacol. 1981, 33: 9–13
Received May 15, 1980

0022–3573/81/010009–05 $02.50/0

Some solute-solvent complexes involving testosterone and testosterone propionate

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Solute-solvent interactions have been studied by observing the influences of carbon tetrachloride, carbon disulphide, ethyl oleate, isopropyl myristate and octanol on the solubilities of testosterone and testosterone propionate in an inert solvent (cyclohexane). Complexation with testosterone was detected, but there was no evidence of complexation between testosterone propionate and these solvents. The solvent-induced infrared shifts were due to non-specific solvent effects. The existence of solute-solvent complexes between the two solutes and chloroform, previously detected by infrared absorption, was confirmed. The solubility technique, which has previously only been used with solid complexing agents, is adaptable to liquid complexing agents, but has limitations.

Testosterone esters are known to complex with solvents possessing electron deficient groups (James & Noyce 1970, 1971). The situation is more complicated with steroid alcohols (James & Ramgoolam 1975), since both solute–solute and solute–solvent complexation occurs, and solvents with either electron-rich or electron-deficient centres can be involved. Carbon disulphide and carbon tetrachloride gave ambiguous results (James & Noyce 1970). Continuous variation studies of i.r. spectra of solutions in these solvents showed no isosbestic points, but there were shifts in hydroxyl and carbonyl stretching frequencies, relative to those in cyclohexane solutions, suggesting some form of interaction. Three pharmaceutically important solvents were not examined, because their structures imposed limitations on the technique, which involved absorption spectra in the carbonyl and/or hydroxyl stretching regions. Two of these solvents were ethyl oleate and isopropyl myristate, which are useful solvents for lipophilic drugs, and are, in fact, recommended for steroid injections in the British Pharmacopoeia. The third was octanol, which is extensively used in quantitative structure activity relationships.

Complexation can be studied by following the effect of the complexing agent on the solubility of the substrate (Higuchi & Connors 1965), and has the advantage over i.r. methods that association constants are easily calculated from the results. We have used this technique to test the conclusions derived from i.r. data, and to investigate the behaviour of ethyl oleate, isopropyl myristate and octanol.

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MATERIALS AND METHODS

Materials

Testosterone and testosterone propionate were gifts from Organon Laboratories Ltd. Purities were checked by melting point.

All solvents except isopropyl myristate, which came from Fulka AG, were purchased from BDH Ltd. Carbon tetrachloride, chloroform and cyclohexane were 'spectroscopy' grade. The chloroform was first washed with M sodium carbonate, followed by distilled water, dried over calcium chloride and fractionally distilled. The fraction collected at 61 °C was passed through a column of alumina. 'Specially purified' n-octanol was purified in the same way, except that the fraction distilling at 194 ± 0·5 °C was collected. Carbon disulphide was 'analytical reagent' grade, and isopropyl myristate and ethyl oleate of pharmaceutical quality. All were used without further purification.

Solubility determinations

Saturated solutions were prepared by percolation (James & Roberts 1968), and concentrations determined from the extinction at 231 nm.

Infrared spectra

Spectra were run on a Perkin Elmer 521 grating spectrophotometer, using a linear scale expanded by a factor of 10. Sodium chloride cells (0·1 mm) were

used, with the solvent placed in the reference beam. Binary solvent systems were chosen to cover the concentration range from pure cyclohexane to the pure solvent under investigation.

DISCUSSION

The formation of a 1:1 complex between a substrate (A) and complexing agent (B) can be represented by equation (1). K_{AB} is the equilibrium constant

$$A + B \overset{K_{AB}}{\rightleftharpoons} AB \quad .. \quad .. \quad (1)$$

and AB the complex. Any increase in the apparent solubility of A brought about by addition of complexing agent is considered to be due to complex formation, so that the concentration of uncomplexed solute can be represented as $[A_0]$, the solubility in the pure solvent. Since this is constant, the equilibrium can be represented by equation (2). K'_{AB} is termed the interaction constant, and is defined by equation (3). [AB] can be expressed as $[A_t] - [A_0]$ and [B] as

$$K'_{AB} = \frac{[AB]}{[B]} \quad .. \quad .. \quad (2)$$

$$K'_{AB} = K_{AB}[A_0] \quad .. \quad .. \quad (3)$$

$[B_t] - ([A_t] - [A_0])$, where $[A_t]$ and $[B_t]$ are total substrate and total complexing agent concentrations respectively. Equation (2) can then be rearranged to give equation (4), so that a plot of total solute concentration against total complexing agent concentration should be rectilinear, from which K'_{AB} can be evaluated, and the true equilibrium constant then calculated from equation (3).

$$[A_t] = \frac{K'_{AB}}{1 + K'_{AB}} . [B_t] + [A_0] \quad .. \quad (4)$$

This treatment is suitable for solid complexing agents, because they have no effect on the solubility of the unassociated substrate, except at high complexing agent concentrations. However, liquid complexing agents are solvents in their own right, and also contribute significantly to the total volume of the equilibrium mixture. $[A_0]$ cannot therefore be assumed to be independent of complexing agent concentration.

Despite this, the plot of total solubility of testosterone in cyclohexane–octanol blends at 25 °C was approximately rectilinear from zero to 100% v/v octanol (Fig. 1). Similar plots were obtained with ethyl oleate and with isopropyl myristate, as complexing agents suggesting that 1:1 solute–solvent complexation occurs between testosterone and these solvents, and that $[A_0]$ remains reasonably

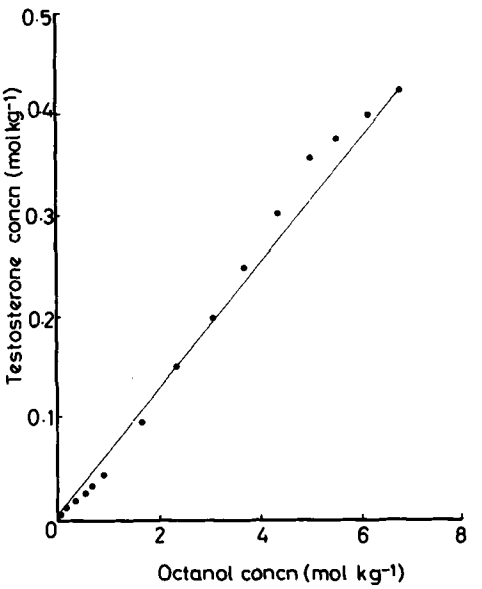

FIG. 1. Solubilities of testosterone in cyclohexane–octanol mixtures at 25 °C.

constant. In subsequent work, apparent solubilities were determined over a limited range of solvent concentrations (0 to 10% v/v in cyclohexane) because of the uncertainty of $[A_0]$ at high concentrations. Table 1 shows the 1:1 association constants obtained at various temperatures for testosterone in octanol. The results yielded a reasonable rectilinear plot ($r = 0{\cdot}954$) of $\ln K_{11}$ against 1/T, giving a heat of association of $-15{\cdot}6$ kJ mol^{-1}. The corresponding figures for ethyl oleate and isopropyl myristate were $-22{\cdot}4$ and $-41{\cdot}6$ kJ mol^{-1} respectively.

Testosterone propionate gave a parabolic plot of apparent solubility against octanol concentration, in cyclohexane–octanol mixtures. This is shown in Fig. 2. The failure to follow a rectilinear plot could be a consequence of testosterone propionate having a much higher solubility than testosterone in cyclohexane, so that variations in $[A_0]$ are significant in comparison with the total concentrations of steroid and complexing solvent. An alternative explanation is that no complexation occurs, but the parabolic

Table 1. Association constants for 1 : 1 complexes between testosterone and solvents.

	Temperature (°C)				
	20	25	30	35	40
Octanol	19·5	16·1	15·3	14·1	—
Ethyloleate	—	14·6	10·9	9·5	—
Isopropyl myristate	5·8	—	3·4	—	1·9

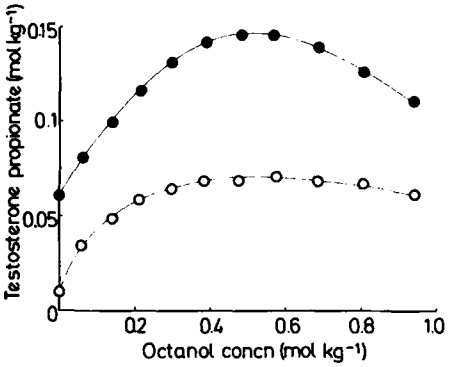

FIG. 2. Solubilities of testosterone propionate in cyclohexane—octanol mixtures at 25 °C. ○, observed; ●, predicted.

plot represents regular solubility behaviour. The solubility parameters of testosterone propionate and cyclohexane are 9·5 (James et al 1976) and 8·2 (Hildebrand et al 1970) respectively. Rheineck & Lin (1968) have published a list of group contributions to molar volumes and attractions, from which a solubility parameter of 10·3 can be calculated for n-octanol. These solvent solubility parameters lie on either side of that of testosterone propionate, so that its calculated regular solubilities in blends of cyclohexane and n-octanol pass through a maximum. This is shown in Fig. 2. The similarity of the two plots suggests that variations in the solubility of testosterone propionate with blend composition can be explained in terms of regular solution theory. Complexation is further contraindicated because the observed solubilities are lower than predicted. Solute–solvent complexation would be expected to result in a higher apparent solubility than that predicted by regular solution theory.

Testosterone propionate behaved in a similar way in ethyl oleate and in isopropyl myristate, although the plots were not parabolic, a consequence of the solubility parameters of cyclohexane, ethyl oleate and isopropyl myristate all lying on the same side of 9·5. Both observed and calculated plots were curved, and similar in form; the calculated solubilities were higher than the observed values. Testosterone propionate, ethyl oleate and isopropyl myristate contain electron-donating groups, but no electron acceptors. Solute–solvent interaction between testosterone propionate and either ethyl oleate or isopropyl myristate would not therefore be anticipated. The fact that testosterone propionate behaves in a similar way in octanol as in these two solvents, lends support to the conclusion that solute–solvent complexation does not occur between testosterone propionate and octanol.

A solubility parameter of 10·9 was calculated for testosterone with Rheineck & Lin (1968) substituent constants, and used to obtain regular solubilities in cyclohexane–octanol blends. The plot of calculated solubility against octanol concentration was curved, in contrast to the rectilinear plot obtained with the observed solubilities (Fig. 1) indicating that regular solution theory does not explain the behaviour of testosterone in cyclohexane–octanol blends.

A plot of apparent solubilities of testosterone propionate against carbon tetrachloride concentration, in cyclohexane–carbon tetrachloride mixtures, at 25 °C, was rectilinear and yielded a low 1:1 apparent association constant of 0·49. A similar result was obtained with testosterone propionate in cyclohexane–carbon disulphide mixtures. Both plots ran parallel to and below the calculated regular solubilities.

Formation of solute–solvent complexes is therefore unlikely in these solutions, an opinion which is supported by the absence of isosbestic points from the i.r. spectra (James & Noyce 1970). It is probable that the i.r. shifts observed by these workers are due to non-specific interactions. Non-specific solvent effects on i.r. frequencies have been explained in terms of an oscillating dipole in a spherical cavity within the solution, and related to the dielectric constant (ϵ) through equation (5).

$$\frac{\Delta\nu}{\nu} = \frac{C(\epsilon - 1)}{(2\epsilon + 1)} \quad \ldots \quad \ldots \quad (5)$$

ν represents the frequency of the pure solute, $\Delta\nu$ the frequency shift and C a constant. Noyce (1972) has determined the ester and ketone stretching frequencies of testosterone propionate in a range of saturated hydrocarbon solvents, and shown that both vibrations follow equation (5). Linear regression analysis of his results yielded equations (6) and (7), which predict frequency maxima for carbon disulphide and carbon tetrachloride solutions in good agreement with the observed values, as shown in Table 2. The frequency shifts are therefore due to

Table 2. Observed and predicted carbonyl stretching frequencies of testosterone propionate

Solvent	Ketone ν cm^{-1}		Ester ν cm^{-1}	
	obs.	calc.	obs.	calc.
Carbon disulphide	1679	1678	1739	1739
Carbon tetrachloride	1681	1683	1739	1743

non-specific solvent effects, rather than complexation. The relationships were not improved by adding terms involving refractive index, as suggested by Buckingham (1958).

Ketone

$$\nu = -126{\cdot}4\frac{\epsilon - 1}{2\epsilon + 2} + 1711{\cdot}2$$

n	r
9	0·959

.. (6)

Ester

$$\nu = -91{\cdot}84\frac{\epsilon - 1}{2\epsilon + 2} + 1763{\cdot}4$$

9	0·978

.. (7)

n = number of results; r = correlation coefficient.

The i.r. spectra of testosterone dissolved in cyclohexane–octanol blends, were typical of a system which undergoes solute–solvent complexation involving a carbonyl group. Octanol caused a decrease of about 20 cm^{-1} in the carbonyl stretching frequency, and the spectra passed through an isosbestic point. Examples are shown in Fig. 3. Testosterone propionate exhibited a similar shift with octanol, but the spectra did not pass through an isosbestic point (Fig. 4). Substitution of a dielectric constant of 10·3 for octanol (Weast 1970) into equation (6) gave an absorption maximum of 1659 cm^{-1}, in excellent agreement with Fig. 4. It is therefore concluded that the shift is due to a non-specific interaction of the same type as that observed with carbon disulphide.

Infra red evidence could not be obtained for solutions in ethyl oleate or isopropyl myristate, because the carbonyl stretching absorption of the 3-keto group was swamped by the ester group of the solvent. Similarly, the hydroxyl stretching of testosterone was oblitered by the stretching overtone of the solvent ester carbonyl.

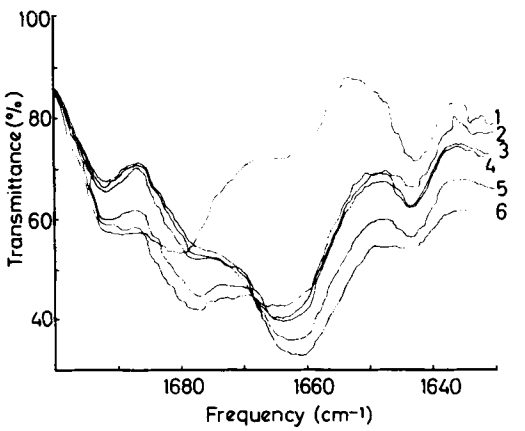

FIG. 3. I.r. spectra of testosterone in cyclohexane—octanol mixtures. 1. Cyclohexane. 2. 10% 3, 25% 4, 50% 5, 75% octanol in cyclohexane. 6. Octanol.

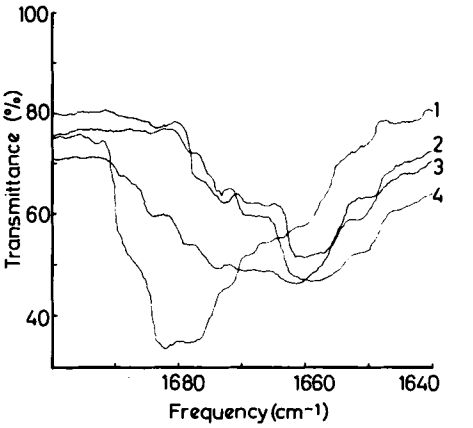

FIG. 4. I.r. spectra of testosterone propionate in cyclohexane—octanol mixtures. 1. Cyclohexane. 2, 20% 3, 60% octanol in cyclohexane. 4. Octanol.

Solubilities of testosterone in a range of cyclohexane–chloroform blends are plotted against chloroform concentration in Fig. 5. The initial part of the graph appears to be rectilinear, and typical of 1:1 complexation, but closer inspection and regression analysis revealed that it formed a continuous curve with the later results, culminating in an inflection at around 4·5 mol g^{-1} chloroform. This levelling-out process has been observed with other systems, and attributed to the limit of solubility of the complexes (Higuchi & Lach 1954). Infrared absorption studies indicate that testosterone complexes with chloroform, which hydrogen-bonds to the 3-keto and 17-hydroxyl groups, and the process occurs at low chloroform concentrations (James & Ramgoolam 1975). It is therefore probable that the

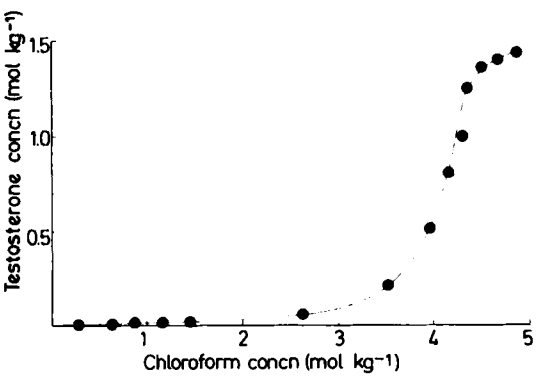

FIG. 5. Solubility of testosterone in chloroform—cyclohexane systems.

initial part of Fig. 5 represents a mixture of 1:1 and 1:2 complexation, as represented by equations (1) and (8).

$$A + 2B \overset{K_{12}}{\rightleftharpoons} AB_2 \quad .. \quad .. \quad (8)$$

Higuchi et al (1969) showed that when 1:1 and 1:2 complexation occurred simultaneously, the composition of the equilibrium mixture can be expressed by equation (9). K'_{12} is the interaction constant for the 1:2 complex,

$$K'_{11}(\text{apparent}) = K'_{11} + K'_{12}[B] \quad .. \quad (9)$$

$$K'_{12} = \frac{[AB_2]}{[B]^2} \quad .. \quad .. \quad (10)$$

as defined by equation (10). Hence a plot of K'_{11} (apparent) against B should give a rectilinear plot, with intercept K'_{11} and slope K'_{12}, [B] was calculated as $[B_t] - [A_t] + [A_0]$ and K'_{11}(apparent) as $([A_t] - [A_0])/([B_t] - [A_t] + [A_0])$.

Equation (9) was followed by results up to 10% v/v chloroform (1·1 mol kg^{-1}), giving a correlation coefficient of 0·992 and association constants of 2·07 kg mol^{-1} and 0·98 for 1:2 and 1:1 complexation respectively. The derivation of equation (9) assumes that complexation is mainly 1:1. It also fits if the assumption is that complexation is mainly 1:2, but [B] is then represented by $[B_t] - 2([A_t] - [A_0])$. This too gave a good correlation ($r = 0{\cdot}991$), and yielded similar association constants ($K_{12} = 2{\cdot}12$ kg mol^{-1}; $K_{11} = 0{\cdot}97$). A third alternative assumes that complexation is exclusively 1:2, which leads to equation (11). This relationship

$$[A_t] - [A_0] = \{[B_t] - 2([A_t] - [A_0])\}^2 K'_{12} \quad .. \quad (11)$$

also fitted the results ($r = 0{\cdot}998$) and gave a 1:2 association constant of 2·81 kg mol^{-1}. None of the equations fitted results corresponding to chloroform concentrations exceeding 10% v/v, probably because of formation of higher complexes. James & Noyce (1971) have identified 1:3 complexes between testosterone propionate and chloroform from i.r. spectra.

Testosterone propionate solubilities in chloroform differed from Fig. 5 only in that the curvature was more pronounced, and was obvious even at low chloroform concentrations. The results did not fit equations (9) or (11) with any precision, but the general shape of the plot was characteristic of a system containing solute–solvent complexes higher than 1:1. The failure to quantitate the results was probably due to the higher solute concentrations involved and to formation of 1:3 complexes (James & Noyce 1971).

Closer scrutiny of Fig. 1 reveals that the points follow a sigmoid pattern, of similar form to Fig. 5, but shallower. This suggests that one molecule of testosterone is able to complex with more than one molecule of octanol. Infrared spectra show that the 3-keto group interacts with octanol, and complexation between 17-hydroxyl and octanol is also probable, although demonstration by i.r. absorption measurements was impracticable. The increase in slope of Fig. 1, following an initial rectilinear plot, indicates the presence of complexes higher than 1:1 but attempts to fit equations (9) and (11) to the results failed, even when only parts of Fig. 1 were considered. There is therefore qualitative evidence of the existence of 1:2 complexes, but quantitative measurements are not possible, and a probable consequence of the complexing agent being a liquid. It can therefore be concluded that the solubility method can be used with liquid complexing agents, provided the concentration of the reacting solvent is kept low. Solubility results in medium and high concentrations of complexing agents are suitable for qualitative impressions only.

Acknowledgement

We are grateful to Organon Laboratories Ltd. for gifts of testosterone and testosterone propionate.

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