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The properties of testosterone and related androgens crystallized from normal alkanols

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Summary

X-Ray diffraction and IR spectrophotometry showed that testosterone crystallized from octanol had a different form from that crystallized from the other C_5 to C_{10} alkanols. Differential scanning calorimetry thermograms and melting points were similar for all crystallizates, but the heat of fusion of the octanol product was greater than the others. Purity determinations by DSC revealed that the samples were at least 0.99 mole fraction pure, indicating they were polymorphs, rather than solvates. This was confirmed by thermogravimetric and elemental analysis. Methyltestosterone behaved in a similar manner, but the properties of methandienone, mestanolone and nandrolone were not affected by the nature of the crystallizing solvent.

Introduction

The work described below originated as part of a bioavailability study of methyltestosterone (17β-hydroxy-17α-methylandrost-4-en-3-one) from soft gelatin capsules (James et al., 1980). A selection of water-immiscible solutions covering a wide range of solubilities was required, and as part of the search for suitable solvents, the homologous series of normal alkanols from C_5 to C_{10} was examined. In these solvents, mole fraction solubilities increased with carbon number of solvent to C_8, and then decreased. Methandienone (17β-hydroxy-17α-methylandrosta-1,4-dien-

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3-one), testosterone (17β-hydroxyandrost-4-en-3-one) and mestanolone (17β-hydroxy-17α-methyl-5α-androstan-3-one) followed similar patterns. but the solubilities of nandrolone (17β-hydroxy-oestr-4-en-3-one) decreased uniformly as the solvent homologous series was ascended. Flyn et al. (1979) observed that the solubilities of cholesterol in *n*-alkanols behaved in a similar manner to that observed here with methyltestosterone, and associated irregularities in the solubility profile with solvate formation. The possibility that this occurred with methyltestosterone and related androgens was therefore investigated.

Materials and methods

Mestanolone, methandienone, methyltestosterone, nandrolone and testosterone of British Pharmacopoeia or Pharmaceutical Codex standard were recrystallized from ethanol. Melting points agreed with published values. Alkanols were obtained from BDH and were used without further purification. All were claimed to be at least 98% pure, and had boiling points which agreed with the literature. 80 mg of each steroid was dissolved in 10 ml of each alkanol, and the resulting solutions allowed to evaporate in the open until crystals separated. These were removed and dried in a vacuum oven at 50°C for 48 h.

X-Ray powder diffraction photographs were taken with a Debye-Scherer, 57.3 mm diameter camera, using a Raymax-60 source unit with $Cu_{K\alpha}$ filter. Samples were exposed for 6 h, and 5 replicates carried out on each. Mean d values, calculated from the spacings, yielded confidence limits of less than 1% ($P = 0.01$). IR absorption spectra were obtained with a Perkin Elmer 357 grating spectrophotometer, using potassium bromide discs. Thermogravimetric analysis was carried out on a Stanton Redcroft TG 750 thermobalance. Samples were heated from ambient temperature to the melting points. Thermograms were obtained with a Perkin Elmer DSC 2c differential scanning calorimeter, and calibrated using ultra-pure tin. Elemental analyses were carried out at The School of Pharmacy, University of London.

Results and discussion

The X-ray powder diffraction pattern for testosterone which had been crystallized from octanol was different from those of the other testosterone samples, which gave identical patterns, irrespective of the alkanol from which they had been crystallized. Spacings calculated from the Bragg equation are given in Table 1, those for the octanol crystallizate were significantly different from the remainder, and are shown separately. Crystals of testosterone which separated from alkanols other than octanol gave identical spacings, and are presented together as the arithmetic means and confidence limits ($P = 0.01$). It is therefore assumed that the end products from all the solvents, except octanol, are the same. Methyltestosterone gave the same pattern of results, octanol yielding a different product from the remainder. However, with methandienone, mestanolone and nandrolone, the diffraction patterns for the

TABLE 1

CRYSTAL SPACINGS (Å) FOR ANDROGENS CRYSTALLIZED FROM ALKANOLS

Carbon number of alkanol	Line			
	1	2	3	4
Testosterone				
8	4.37	4.82	5.26	5.94
5, 6, 7, 9, 10	4.32 ± 0.03	4.90 ± 0.01	5.78 ± 0.01	6.78 ± 0.00
Methyltestosterone				
8	4.37	4.76	5.79	6.04
5, 6, 7, 9, 10	4.49 ± 0.04	5.30 ± 0.03	5.64 ± 0.03	6.18 ± 0.00
Methylandrostanolone				
5, 6, 7, 8, 9, 10	4.87 ± 0.07	5.33 ± 0.03	5.84 ± 0.07	10.63 ± 0.02
Nandrolone				
5, 6, 7, 8, 9, 10	5.26 ± 0.01	5.49 ± 0.07	5.99 ± 0.03	7.37 ± 0.03
Methandienone				
6, 7, 8	6.77 ± 0.06	7.56 ± 0.09	7.75 ± 0.17	12.54 ± 0.03

Values following the means are standard errors.

samples crystallized from octanol were the same as those obtained from the other alkanols.

Similar conclusions follow from the IR absorption results. Testosterone which had been crystallized from octanol gave a maximum at 3410 cm^{-1} in the hydroxyl stretching region, with a minor peak at 3540 cm^{-1}, while the other samples gave two major peaks, one at 3370 and the other at 3520 cm^{-1}. Similarly, all samples of testosterone, except that which was crystallized from octanol gave a doublet in the carbonyl stretching region between 1657 and 1664 cm^{-1}. The material obtained from octanol gave only one maximum. The relevant parts of the spectra are shown in Fig. 1. Mesley (1966) quoted these hydroxyl stretching features when distinguishing between two polymorphs of testosterone, but he did not use alkanols as crystallizing solvents. He attributed the two hydroxyl stretching maxima to free and OH---OH hydrogen-bonded hydroxyl, but the doublet observed in the carbonyl frequency, suggests that hydroxyl–carbonyl hydrogen-bonding is more probable. Intramolecular hydrogen-bonding between carbonyl and hydroxyl has been established in testosterone and methyltestosterone solutions (James and Ramgoolam, 1975).

Methyltestosterone which had been crystallized from octanol did not exhibit any gross differences from the other methyltestosterone samples, but minor differences were observed in the fingerprint region.

Flynn et al. (1979) used differential thermal analysis, and obtained different thermograms and melting points for cholesterol, depending on the alkanol used as crystallizing solvent. The steroids investigated here gave simple, single-peaked differential scanning thermograms, and melting points which were independent of the solvent used. Heats of fusion were also independent of solvent, except for testosterone which had been crystallized from octanol, and which gave a heat of fusion significantly higher than for the samples crystallized from other alkanols. The thermo-

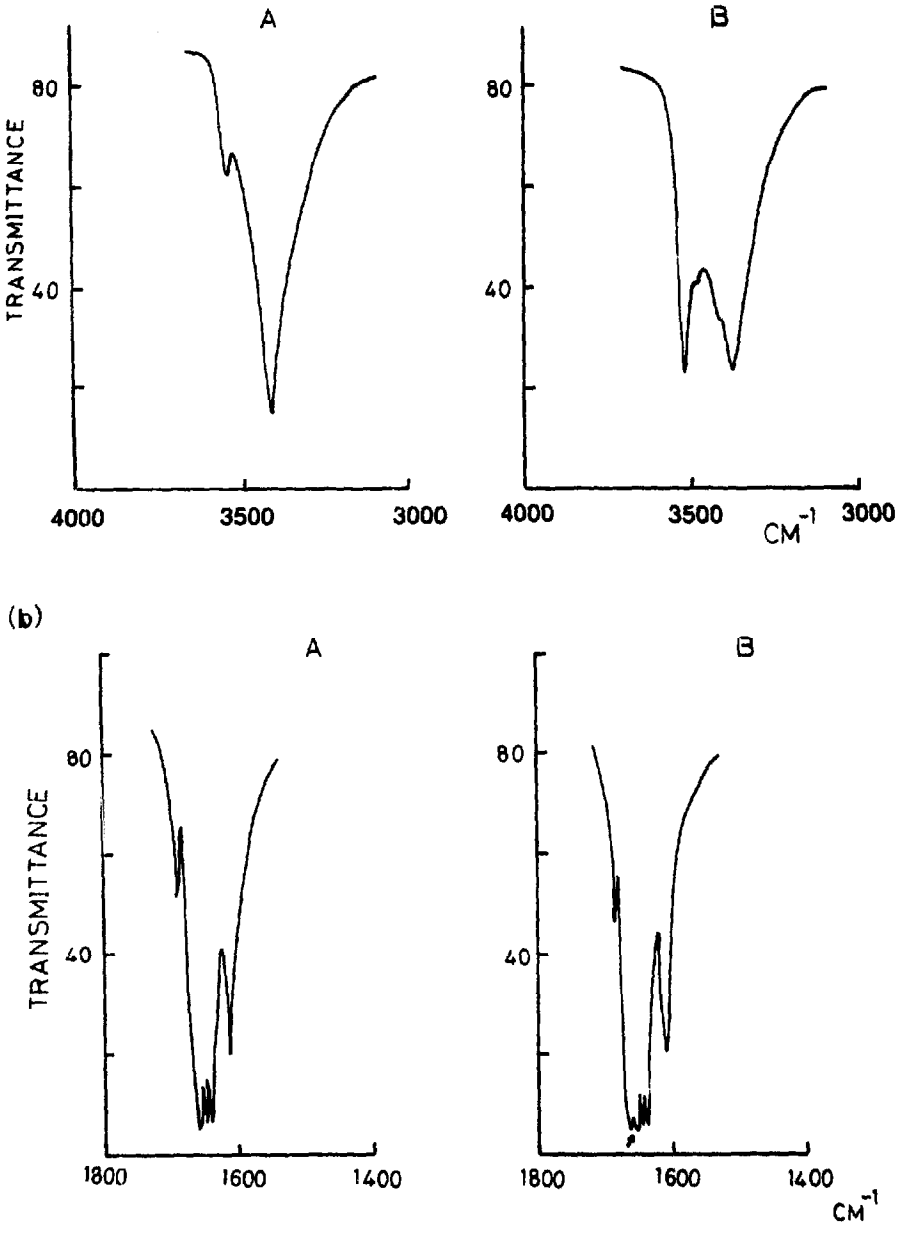

Fig. 1. Infrared spectra of bromide discs of testosterone crystallized from (A) octanol and (B) decanol. (a) Hydroxyl stretching region. (b) Carbonyl stretching region.

grams for methyltestosterone were similar for all solvents, including octanol. Results are shown in Table 2.

Three techniques, IR absorption, calorimetry and X-ray diffraction, thus all indicate that testosterone exists in one form when it is crystallized from octanol, and in another when it is crystallized from the other alkanols. The IR spectrum of the octanol crystallizate corresponds to Mesley's polymorph B(II) and the remainder to his polymorph A(I). The evidence therefore suggests that testosterone which has

TABLE 2

ENTHALPIES OF FUSION OF TESTOSTERONE AND METHYLTESTOSTERONE CRYSTALLIZATES

Solvent	Enthalpy of fusion ($kJ \cdot mol^{-1}$)	
	Testosterone	Methyltestosterone
Pentanol	20.8	22.5
Hexanol	20.9	22.3
Heptanol	21.3	22.7
Octanol	23.0	22.2
Nonanol	22.1	-

been crystallized from octanol is a different polymorph, rather than a different solvate, since each solvent would be expected to give its own individual solvate. However, it is possible that octanol forms a solvate and the remainder do not. Evidence favouring polymorphism came from thermogravimetric analysis, which could not detect any loss in weight when the samples were heated. If the materials were solvates, one would expect the solvents to be volatilized off when their boiling points were approached.

Confirmation was obtained when the purities of the samples were determined from the differential scanning calorimetry results. Thermograms were divided into segments by drawing lines parallel to the leading edge of the fusion peak, as shown in Fig. 2. The fraction of the total mass melted at temperature T, expressed as the

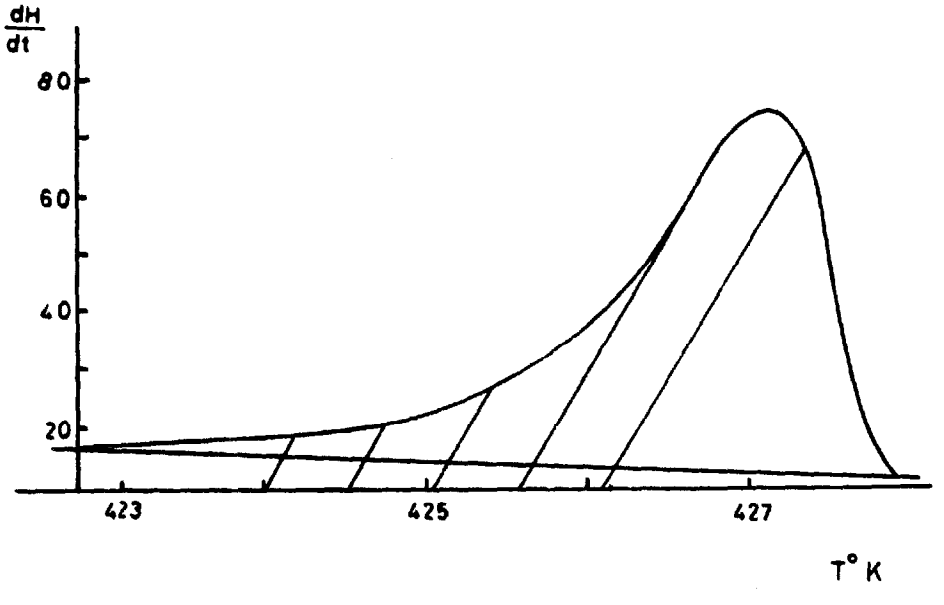

Fig. 2. Diagram of thermogram, split into segments for purity determinations.

point at which the outer edge of each segment crossed the baseline was calculated as,

$$F = \frac{\text{cumulative area of segments}}{\text{total area of thermogram}} \quad (1)$$

which is given in terms of temperatures in Eqn. 2.

$$F = \frac{T_0 - T_m}{T_0 - T} \quad (2)$$

T_0 and T_m are melting points of the pure compound and of the sample, respectively. Rearrangement shows that a plot of T against $1/F$ should give a straight line with a slope equal to the depression of melting point $(T_0 - T_m)$. The mole fraction of impurity (X_2) is then given by Eqn. 3.

$$T_0 - T_m = \frac{RT_0^2 X_2}{\Delta H_f} \quad (3)$$

ΔH_f is the enthalpy of fusion, and R the gas constant. The procedure is described in detail by Reubke and Mollica (1967). Segment areas were corrected using a calculation suggested by Sondack (1972). All the samples of testosterone and methyltestosterone had mole fraction purities in excess of 0.99.

Carbon and hydrogen contents were obtained by elemental analysis for the samples of testosterone and methyltestosterone which had been crystallized from octanol. The results corresponded with the theoretical composition of the pure steroids. From this and the purities obtained by differential scanning calorimetry, it can be concluded that the samples obtained by crystallization from octanol are not solvates.

The evidence presented above suggests that testosterone forms a different polymorph when crystallized from octanol, compared to that obtained from the remaining C_5 to C_{10} alkanols, but the morphologies of methandienone, mestanolone and nandrolone are not influenced by the nature of the crystallizing solvent. The situation with methyltestosterone is not so definite, except to say that solvates are not formed. The X-ray diffraction data strongly suggest the existence of a polymorph peculiar to the octanol crystallizate, but there is no support from differential calorimetry, and that from the IR evidence is slender. However, the X-ray evidence cannot be ignored, and it is probable that a different polymorph separates from octanol solution, but its properties must be similar to the one obtained from the other alkanols.

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