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An investigation of the distribution coefficients of some androgen esters using paper chromatography

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R_M values have been determined for four homologous series of androgen esters, using a Bush paper chromatography system, to assess the value of the ratio of the solubilities in the individual solvents (solubility ratio), as an estimate of distribution coefficient. The distribution coefficients were proportional to R_M values in all four series, and for three of these the proportionality constant was the same. Limitations in the use of R_M values to predict biological effects have been pointed out.

James, Nicholls & Roberts (1969) have observed a relation between ethyl oleate-water distribution coefficients and biological half life for testosterone esters. Distribution coefficients were quoted as the ratio of the solubilities in the individual solvents (solubility ratio). This is valid provided both saturated solutions are dilute and the mutual solubilities of the solvents are negligible, but it is questionable whether the distribution coefficients of steroid esters, between organic solvents and water would satisfy these requirements. It was therefore considered necessary to confirm the distribution coefficients by another method.

Paper chromatography is essentially a liquid–liquid distribution process. Bate-Smith & Westall (1950) have shown that

$$\log \alpha = R_M + k \qquad (1)$$

where

$$R_M = \log\left(\frac{1}{R_f} - 1\right) \qquad (2)$$

α is the distribution coefficient between moving and stationary phases and k is a constant, dependent on the ratio of the volumes of stationary and moving phases. R_M values have been used to estimate distribution coefficients by Iwasa, Fujita & Hansch (1965), and Boyce & Milborrow (1965) correlated R_M values of *N*-alkyltritylamines with moluscicidal activity, which is dependent on distribution coefficients.

A further objection to the use of solubility ratios to estimate distribution coefficients is the difficulty of determining aqueous solubilities of steroids, which are frequently too low to give reliable spectrophotometer readings. We have measured the solubilities and R_M values of a series of steroid esters on paper to assess the value of the ratio of the solubilities as an estimate of their distribution coefficients, and to develop a simpler method of determining the distribution coefficients of steroids.

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EXPERIMENTAL

Preparation of esters

Formate esters were prepared according to Ringold, Loken & others (1957) (preparation of Δ^5-androsten-3β-ol-17-one formate) and recrystallized from n-hexane. The remainder were obtained by refluxing with the anhydride in the presence of pyridine, and recrystallized from aqueous ethanol. Melting points, where published, agreed with the literature.

5,6-*Dehydroisoandrosterone* (3β-hydroxyandrost-5-ene-17-one) *formate.* $[\alpha]_D^{20}$ + 10·5 (*c* 3·6 in EtOH). M.p. 140°; Fajkos (1959) gave 140–141°.

5,6-*Dehydroisoandrosterone acetate.* $[\alpha_D^{20}]$ + 14·9 (*c* 1·9 in EtOH). M.p. 167°; De Ruggieri & Ferrari (1959) gave 167–170°.

5,6-*Dehydroisoandrosterone propionate.* White crystals, $[\alpha]_D^{20}$ + 14·7 (*c* 1·6 in EtOH). M.p. 197°; Dr J. L. Marsh, in a personal communication, gave near 198°. Found: C, 76·4; H, 9·8. $C_{22}H_{32}O_3$ requires: C, 76·7; H, 9·3.

5,6-*Dehydroisoandrosterone butyrate.* White crystals, $[\alpha_D^{20}]$ + 13·1 (*c* 1·8 in EtOH). M.p. 163°. Found: C, 76·6; H, 9·9. $C_{23}H_{34}O_3$ requires: C, 77·05; H, 9·6.

Oxime. White crystals, m.p.150°. Found: C, 73·3; H, 9·25; N, 3·8. $C_{23}H_{35}NO_3$ requires: C, 74·0; H, 9·4; N, 3·75.

5,6-*Dehydroisoandrosterone valerate.* White crystals, $[\alpha]_D^{20}$ + 13·1 (*c* 1·8 in EtOH). M.p. 120°. Found: C, 78·3; H, 9·0. $C_{24}H_{36}O_3$ requires: C, 77·4; H, 9·7.

Oxime. White crystals, m.p. 160°. Found: C, 75·0; H, 9·55; N, 3·35. $C_{24}H_{37}NO_3$ requires: C, 74·4; H, 9·6; N, 3·6.

Nuclear magnetic resonance spectra for propionate, butyrate and valerate gave peaks at τ 4·55, equivalent to one olefinic proton which would therefore be in position 4, 6, 7 or 11. Klyne (1957) has shown that the optical rotations of steroids are affected by the presence and position of olefinic bonds, and quoted the following increments for substitution of a double bond for a single bond: Δ^4 + 194°; Δ^5 − 298°; 5α,Δ^7 − 68°; 5β,Δ^7 + 119°; 5α,$\Delta^{9(11)}$ + 109°; 5β,$\Delta^{9(11)}$ + 49°. The specific rotations found for propionate, butyrate and valerate agree with those for formate and acetate, which are known compounds, and with $[\alpha]_D^{20}$ + 10·0° (in EtOH) quoted for the parent alcohol (Lang, 1961). It is therefore concluded that the double bond is in the 5,6 position in all the esters, since a movement of the unsaturation to another position would bring about a large change in specific rotation.

Testosterone decanoate was a gift from Organon Laboratories Ltd.

Chromatography

Preliminary experiments established that the Bush (1961) system, formic acid–methanol–light petroleum (85–90°) (100:90:10) was suitable, yielding Rf values between 0·15 and 0·75 with most of the compounds examined, and gave measurable differences between esters without streaking. Whatman No. 1 paper was spotted with 1·0 μl of a 5% solution of the pure steroid, using a microlitre syringe, and equilibrated with the stationary phase and moving phase in a tank placed in a constant temperature cupboard at 25° for at least 3 h, usually overnight. The length of run, the position of the starting line and volume of mobile phase added were kept constant. Development was carried out using the descending technique and the spots detected by spraying with 2,4-dinitrophenylhydrazine, except those of

the 5,6-dehydroisoandrosterone esters which were detected with alkaline *m*-dinitrobenzene.

Solubility determinations

The solubilities of the testosterone esters in water and organic solvents have been reported previously (James & Roberts, 1968). The same techniques were used to prepare saturated solutions of the remaining compounds and to determine their solubilities in cyclohexane. Saturated solutions in water, of androstanolone and its esters were extracted with n-hexane and assayed colorimetrically using 2,4-dinitrophenylhydrazine (Jordan & Veatch, 1964).

RESULTS AND DISCUSSION

The solubilities of the formate to valerate esters of testosterone in organic solvents change irregularly as the series is ascended, the acetate appearing particularly anomalous. This behaviour has been linked to the melting points by James & Roberts (1968) and reasons suggested for the changes in solubility. The analogous androstanolone esters behave differently. Both profiles are shown in Fig. 1A. Aqueous solubilities increased logarithmically with addition of each CH_2 in both

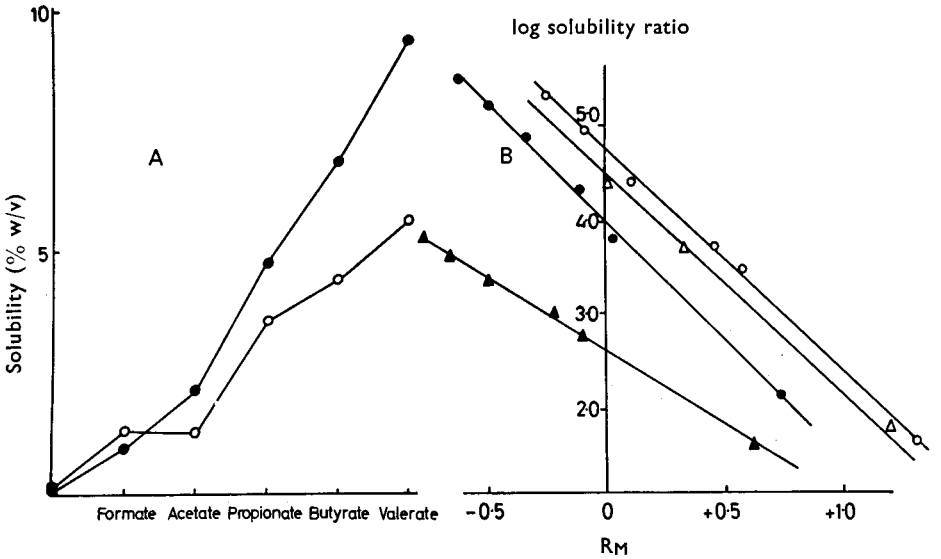

FIG. 1A. Solubilities of testosterone and androstanolone esters in cyclohexane. ●, Androstanolone series; ○, testosterone series. B. Relations between R_M value and log solubility ratio (ordinate). ○, Testosterone series; △, methyltestosterone series; ●, androstanolone series; ▲, 5,6-dehydroandrostanolone series.

series. Solubilities in cyclohexane and water are given in Table 1, together with R_M values for the formic acid–methanol–light petroleum system. Plots of solubility ratio cyclohexane-water against R_M were linear for both series. These are shown in Fig. 1B, and are typical of all solvents examined. The slopes of the two lines are not significantly different ($P = 0{\cdot}99$), having a mean slope of $-2{\cdot}40$, despite the fact that the testosterone and androstanolone series give different solubility profiles in

Table 1. *Solubilities and R_M values*

Compound	Solubility, % w/v Cyclohexane	Water × 10^5	R_M value
Testosterone	0·088	196	1·31*
Formate	1·24	44·0	0·58 ± 0·06
Acetate	1·18	23·5	0·46 ± 0·04
Propionate	3·55	14·8	0·11 ± 0·03
Butyrate	4·41	5·03	—0·09 ± 0·03
Valerate	5·71	2·91	—0·26 ± 0·02
Androstanolone	0·069	52·5	0·74*
Formate	0·891	14·9	0·03 ± 0·04
Acetate	0·210	9·75	—0·11 ± 0·04
Propionate	0·479	6·20	—0·34 ± 0·03
Butyrate	0·694	4·40	—0·50 ± 0·03
Valerate	0·949	3·05	—0·63 ± 0·03
17α-Methyltestosterone	0·145	226	1·20 ± 0·03
Acetate	0·881	17·9	0·33 ± 0·02
Propionate	2·448	10·2	0·01 ± 0·03
5,6-Dehydroisoandrostorone	0·106	249	—
Formate	0·758	140	—0·10 ± 0·03
Acetate	1·10	115	—0·22 ± 0·03
Propionate	1·83	83·2	—0·50 ± 0·04
Butyrate	3·17	79·0	—0·67 ± 0·03
Valerate	4·79	76·8	—0·78 ± 0·04

* Calculated by Socziwinski's method.

organic solvents. This does not prove that the solubility ratio gives the true distribution coefficient, but it can be inferred that it varies from ester to ester in the same way as the distribution coefficients. A third plot, for 17α-methyltestosterone and its acetate and propionate is also shown and suggests that this series parallels the other two. The use of solubility ratios in comparing distribution coefficients with biological activity therefore appears to be justified.

Collander (1951) has shown that, for two solvent systems A and B,

$$\log \alpha_A = a \log \alpha_B + b \qquad (3)$$

where a and b are constants characteristic of the solvent systems used. The constant slope for the androstanolone, testosterone and methyltestosterone series in Fig. 1B supports equation (3) and suggests that, given the distribution coefficient of one ester, those of its homologues can be estimated from R_M values using the equation

$$\log (\text{unknown } \alpha) = \log (\text{known } \alpha) - 2{\cdot}4 \times (R_{M\,(\text{known})} - R_{M\,(\text{unknown})}) \qquad (4)$$

The equation is limited in its scope, however, thus testosterone decanoate gave a point above the line in the plot of R_M against solubility ratio, indicating that equation (4) does not extend indefinitely up the homologous series. Deviations have been observed with the higher members of other homologous series, by Trzaska & Kowkabany (1967), and attributed to the increasing influence of adsorption as the size of the alkyl chain increased.

The R_M values for androstanolone and testosterone did not agree with those predicted from Fig. 1B, but were identical with those for the corresponding formate esters. The spots were eluted from the paper with ethanol, and the residues, obtained after evaporating off the ethanol, taken up in carbon tetrachloride. The infrared spectra of these solutions were characteristic of esters, neither absorbed in the –OH stretching region, but both gave an ester C=O peak at 1740 cm^{-1}, suggesting

that formylation had occurred on the paper. Methyltestosterone was not formylated, probably because of the hindrance of the 17α-methyl group.

Theoretical R_M values for testosterone and androstanolone were estimated by Socziwinski's method (1965), using acetic acid–light petroleum. This did not acetylate the steroid alcohols, but was otherwise less satisfactory than the formic acid system, as marked streaking occurred. The calculated R_M values fitted the results in Fig. 1B.

A further limitation of equation (4) can be seen from the results for the esters of 5,6-dehydroisoandrosterone, given in Fig. 1B. R_M values are linearly related to solubility ratios, but the slope is significantly less than that for the other three series. Application of equation (4) would therefore lead to incorrect conclusions if applied to the 5,6-dehydroisoandrosterone series. If A represents the solvent system in a chromatographic process and B the solvent system with which it is compared, equations (1) and (3) can be combined to give

$$\log \alpha_B = a.R_{MA} + ak + b \quad .. \quad .. \quad .. \quad .. \quad (5)$$

a, b and k are responsible for slopes and intercepts in Fig. 1B. If these were constant, as required by equations (1) and (3), there would be no variation between homologous series and the plots in Fig. 1B would be superimposable. A dependence of a and b on the nature of the solute was noted by Collander (1951) and attributed to the number of hydrophilic groups in the solute molecule and their effect on hydrogen bonding. The deviations noted here must be due to structural differences, since testosterone and 5,6-dehydroisoandrosterone have the same functional groups in the steroid nucleus but while 17α-methyltestostosterone and androstanolone are 17β-hydroxy-3-one steroids, 5,6-dehydroandrosterone is a 3β-hydroxy-17-one steroid. Several workers, notably Hansch, Muir & others (1963) have based their conclusions on equation (3), assuming that changes in the distribution coefficients in the solvent system used *in vitro* would be the same as those *in vivo*. It appears that this assumption must be treated with caution, particularly when applied to steroid molecules.

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