dance with instructions for the standard gas chromatographic method (Pharmacopoea Nordica, 1973). The peak 6A is shown in Fig. 3 and is identified as a compound with a bromo substituent instead of a chloroatom as in clofibrate. Fig. 4 shows a mass spectrum of component 10 with a probable parent ion at *m/e* 328. If the area of peaks 6A, 11 and 12 had been added to the area of other by-products found in each product of clofibrate the differences in the amount would be much decreased. The major impurities in Fig. 1 were found at shorter retention times than clofibrate and the quantity of 4-chlorophenol, for instance is about 0·4‰ of the drug. This corresponds to an oral administration of 0·8 mg per day for a daily dosage of 2 g of clofibrate. The corresponding dosage for component 10 is about 0·6 mg per day. Due to the possible toxicity of different impurities in drugs the use of gas chromatography to identify these impurities is insufficient. Therefore it is expected that the g.c.-m.s. method will supplement the gas chromatographic method for such work which requires quantitative and qualitative analysis.

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Free energies of solution in water, of some androstanolone, nandrolone and testosterone esters

K. C. JAMES, *Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cathays Park, Cardiff, U.K.*

The effects of the methylene group on the solubilities of homologues in various series have been the subject of numerous investigations (see Davis, Higuchi & Rytting, 1974). The evidence from these suggests that free energies of solution change by a constant increment for each additional methylene group, but the supporting results are mainly for volatile, liquid solutes, for which vapour pressures are readily measured. A similar approach to solid solutes is more difficult because they are rarely sufficiently volatile for vapour pressures to be determined, and because the energy required to liquify the solid, which does not always change uniformly as the series is ascended, must be considered. Thus, for example, James & Roberts (1968) showed that the solubilities in organic solvents of a range of testosterone esters changed irregularly as the series was ascended, and that the pattern took the same form as the ideal solubilities calculated from heats of fusion and melting points. Similar observations and conclusions have been made on solutions of alkyl *p*-aminobenzoates in n-hexane and in silicone oil (Yalkowski, Flynn & Slunick, 1972). In contrast solubilities in water do not follow the organic solubility pattern, e.g. solubilities of n-alkanols and n-fatty acids have been shown to decrease logarithmically with the numbers of methylene groups in the molecules (Robb, 1966) and a similar relationship has been observed with *p*-aminobenzoates (Yalkowski & others, 1972). The aqueous solubilities of numerous steroid esters have been determined in these laboratories over recent years, and it has invariably been found that while solubilities in organic solvents change irregularly as a homologous series is ascended, aqueous solubilities decrease logarithmically with a constant decrement for each additional methylene group. This information has been used to calculate some group contributions for aqueous solubilities of steroid esters.

Solubilities were obtained from the following sources: androstanolone esters (Bowen, James & Roberts, 1970; Ng, 1974), methyltestosterone esters (Roberts, 1969), nandrolone esters (Chaudry & James, 1974; Ng, 1974) and testosterone esters (Chaudry & James, 1974; James & Roberts, 1968; Ng, 1974). Ideal solubilities and heats of fusion of formate to valerate esters of testosterone were taken from James & Roberts (1968) and of the same esters of androstanolone and nandrolone from Ng (1974). The remainder were obtained from Chaudry (1972).

The concept of group contributions to physical properties assumes that a given substituent will add a constant, characteristic increment of free energy to that of the molecule to which it becomes attached. Group contributions to solubility are usually expressed in terms of ΔG^E, the free energy in excess of that required for ideal mixing, which can be calculated from equation (1). γ^∞ is the activity coefficient, relative to the pure liquid solute, at infinite dilution. The activity a_2 of a solid non-electrolyte in its saturated solution, is equal to its ideal solubility X_2^i, calculated from equation 2 (Hildebrand, Prausnitz & Scott, 1970).

ΔH^f is the heat fusion, and Tm the melting point. Equation 3 therefore follows.

$$\Delta G^E = RT \ln \gamma^\infty \quad .. \quad .. \quad .. \quad (1)$$

$$\ln X_2^1 = \frac{\Delta H^f}{R}\left[\frac{Tm - T}{TmT}\right] \quad .. \quad .. \quad (2)$$

$$\Delta G^E = RT \ln \frac{X_2^1}{X_2} \quad .. \quad .. \quad .. \quad (3)$$

Excess free energies of solution for androstanolone, testosterone and nandrolone esters, calculated from equation (3), are shown in Fig. 1. By plotting the total number of carbon atoms, rather than the number in the ester side chain, the nandrolone series has been made to coincide with the plots for the other two series, to give a rectilinear relationship up to about C = 28. Above this, the plot tends to flatten out, a trend which has been observed with other systems (Nelson & De Ligny, 1968) and attributed to coiling of the alkyl chain in the solution. Davis, Higuchi & Rytting (1972) have examined a wide range of aqueous solutions of liquids and found a mean excess free energy contribution for the methylene group of 3·6 kJ mol^{-1}. The slope of Fig. 1 (1·80 kJ mol^{-1}) is somewhat lower. Robb (1966) determined the aqueous solubilities and heats of fusion of three long chain fatty acids. These results followed a straight line when $\ln X_2^1/X_2$ was plotted against the number of methylene groups in the alkyl chain, and gave an excess free energy increment of 2·18 kJ mol^{-1}, in good agreement with that for the steroid esters. In contrast, the same treatment of similar data for alkyl *p*-aminobenzoates (Yalkowsky & others, 1972), although it yielded an extremely good rectilinear plot, gave an excess free energy increment of 5·40 kJ mol^{-1} per CH_2, much greater than any of the other values.

The method of calculation thus appears to give consistent answers within closely related groups of compounds, but varies widely from group to group. Furthermore, the results are not sufficiently sensitive to distinguish side chain methylene from 19-methyl in the steroid esters, or to detect 4,5-unsaturation. The procedure is also demanding in that it requires thermal data, and that the calculation of precise ideal solubilities require a more exacting process than is indicated by equation (2) (James & Roberts, 1968). A simpler treatment is possible for sparingly soluble solids, if the the convention that the activity coefficient of the solute in an infinitely dilute solution is unity, is adopted. The concentrations of the saturated aqueous solutions of the steroid esters, which are very small, can then be considered as equal to their activities and substituted in equation (4) to give free energies of solution.

$$\Delta G^\circ = -RT \ln X_2 \quad .. \quad .. \quad (4)$$

Fig. 2 shows plots of $RT \ln X_2$ against number of carbon atoms in the alkyl side chain for androstanolone, nandrolone and testosterone series. They form three parallel straight lines which yield incremental free energies of solution ($\Delta G_{2(CH_2)}$) (of 1·13 ± 0·01 kJ mol^{-1} for androstanolone, −1·17 ± 0·18 kJ mol^{-1} for testosterone and 1·10 ± 0·09 kJ mol^{-1} for nandrolone, giving a mean value of 1·1 kJ mol^{-1} ($P' = 0·01$).

The procedure thus yields a methylene group contribution which can be used for calculating the aqueous solubilities of steroid esters. It has been shown to apply to three series of compounds and appears to be valid for ester alkyl groups up to at least $[CH_2]_9$. The

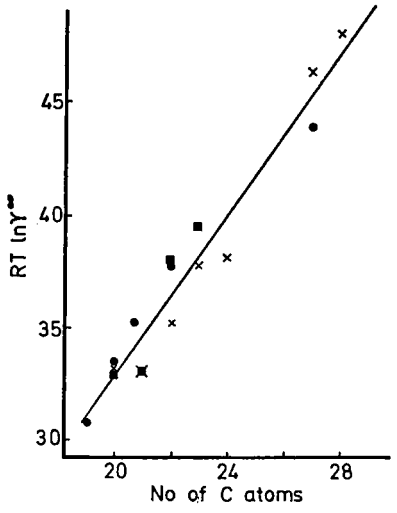

FIG. 1. Excess free energies (kJ mol^{-1}) of solution of steroid esters, in water, ■ androstanolone series, ● nandrolone series, × testosterone series.

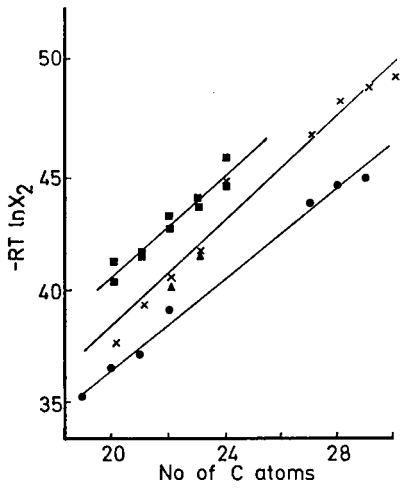

FIG. 2. Free energies of solution (kJ mol^{-1}) of steroid esters in water. ■ androstanolone series, ● nandrolone series, × testosterone series, ▲ methyltestosterone series.

method requires that the solubilities are low. This can be demonstrated with Yalkowsky & others (1972) aqueous solubilities of alkyl *p*-aminobenzoates, which give a curved plot when treated in the same way, because their solubilities are high in comparison with the steroids examined above, and their solutions are too concentrated for an activity coefficient of unity to be assumed. Comparison of the graphs in Fig. 2 suggest other group contributions. Thus, a free energy change of 2·0 ± 0·6 kJ mol^{-1} is indicated for saturation of the 4,5-double bond. Similarly a value of 2 to 4 kJ mol^{-1}, increasing from formate to undecanoate, can be deduced for substitution of a methyl group for hydrogen in position 19. Both results apply to significant ranges of the homologous series in question, but are based on only one pair of steroid nuclei in either case. On the limited evidence of two methyltestosterone esters, 17α-methylation has surprisingly little effect (about 0·5 kJ mol^{-1}) on the free energy of solution.

Plots of log solubility against carbon number for homologous series of liquid solutes are rectilinear because the methylene group makes a characteristic, constant contribution to free energy of mixing (Davis & others, 1972). For solid solutes, the energy required for liquefaction must be considered in assessing solubility, and is expressed in terms of ideal solubility, calculated from equation (2). This can cause deviation from the logarithmic change in solubility observed in ascending homologous series of liquid solutes. Thus for example, the melting points and heats of fusion of the lower testosterone esters do not change uniformly as the homologous series is ascended, with the result that the ideal solubilities vary irregularly with carbon number, and since each methylene group gives a constant increment to free energy of mixing, the observed solubilities in many organic solvents follow a similar pattern to the ideal solubilities. Water is exceptional because solubilities in this solvent decrease logarithmically, in the manner which would be anticipated if the testosterone esters were all liquids. Other steroid ester series behave in the same way, as shown above.

Real solutions deviate from ideality for one or both of two reasons: there may be a finite heat of mixing or the entropy of mixing ΔS^m may be different from that predicted by equation (5). When the heat of mixing is the sole cause,

$$\Delta S^m = - R \ln X_2 \quad .. \quad .. \quad .. \quad (5)$$

the solution is said to be regular (Hildebrand & others, 1970). Nelson & De Ligny (1968) have reviewed the literature on the solubilities of non-polar solutes in water. They showed that the heats of mixing are small and sometimes even negative, and that the low solubilities are a consequence of the high entropy change involved in reordering solute and solvent during the mixing process. It therefore seems that while testosterone ester solutions in organic solvents are usually regular, the non-ideality of aqueous solutions is a consequence of the large entropy of mixing, in considerable excess of that predicted by equation (5). The entropy of fusion ΔS^f of a solid at its melting point is given by equation (6).

$$\Delta S^f = \frac{\Delta H^f}{T_m} \quad .. \quad .. \quad .. \quad (6)$$

In contrast to the melting points and heats of fusion, entropies of fusion of the testosterone esters, calculated from equation (6), increase reasonably uniformly as the series is ascended. It is suggested that the small variation from linearity of the entropies of fusion in ascending the homologous series, is masked by the large entropy of mixing term, accounting for the rectilinear nature of the plots in Figs 1 and 2.

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