CCX. FURTHER EXPERIMENTS ON SURFACE FILMS OF STEROLS AND THEIR DERIVATIVES.

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MOST of the compounds of the sterol group form stable unimolecular films when spread on a water surface. As with the long-chain aliphatic substances, the two principal stabilising forces for such films are (a) the anchorage to the water afforded by the strong attraction perpendicular to the surface between the water-soluble or "polar" groups and the underlying water, and (b) the lateral adhesion between the molecules of the film, which is largely, but not wholly, due to the hydrocarbon part of the molecules. In the great majority of cases, surface films of sterols are of the "coherent" type, *i.e.* they show a very small surface vapour pressure, or tendency of the molecules to escape along the surface to form a film of the gaseous or vapour type.

Diagram I.

Sterols containing a single hydroxyl group in the position occupied by that in cholesterol, *i.e.* at 3 on the modern formula, frequently form very incompressible films with areas per molecule near to 40 sq. Å. This has been shown by Leathes [1923], Adam [1928], Knight [1928], Adam and Rosenheim [1929], Fosbinder [1933], and Danielli and Adam [1934]. In such films there is little doubt that the molecules are standing very closely packed and perpendicular (or very nearly perpendicular) to the surface, the hydroxyl groups at 3 being at the lowest point of the molecules. Measurements on models show that the area of cross-section of a sterol ring system perpendicular to its longest axis is of this order of magnitude; moreover, in the vertical position it is the carbon atom at 3 which is at the lowest point of the molecule, hence as the water-attracting group always attempts to approach the water as closely as possible, a vertical position is more probable with a soluble group at position 3 than at any other. If the molecules are vertical, a low compressibility is to be expected, as no reduction of area can be obtained by altering the tilt of the molecules to the surface. Slight differences in area have been found between various sterols

giving this type of film [Knight, 1928; Adam and Rosenheim, 1929]; the nature of these will be discussed further in this paper.

There are numerous cases, however, where sterols or their derivatives with a single hydroxyl or other water-soluble group at position 3 give a film of much greater area than 40 sq. Å.; such films always have a very much greater compressibility than the type just described. $\Delta^{4:5}$ -Coprostone-3-one ("cholestene") [Adam and Rosenheim, 1929, p. 27] and the various irradiation products of ergosterol [Rosenheim and Adam, 1929; Danielli and Adam, 1934] are well known cases of this. Such films are coherent and have a small surface vapour pressure. There is little doubt that the molecules are considerably tilted to the vertical, especially at low compressions; the tilt is reduced on lateral compression with a corresponding decrease in area, this giving to the film its high compressibility. Temperature variation has little, if any, effect on the area in these cases.

When the water-soluble groups are in other positions, in other rings than ring I, areas considerably larger than 40 sq. Å. have always been obtained, the compressibility being also high. It may be difficult to distinguish such films from those substances with polar groups at 3, which give films with tilted molecules; they are usually coherent films.

In a few cases, where there are two or more water-attracting groups, situated far apart in the molecule, so that the molecules might be expected to lie flat in the surface, films of the gaseous type have been found; such include a few substances of the oestrin group [Adam *et al.*, 1932; Danielli *et al.*, 1933].

It was shown by Leathes [1923] and later in more detail by Adam [1928] that cholesterol molecules present in a film of an aliphatic substance which normally forms "liquid expanded" films, have a strong tendency to reduce these films to the condensed state. This is probably due to the bulky, vertically orientated molecules of cholesterol obstructing the oscillatory motions of the hydrocarbon chains which are the cause of the "liquid expanded" state of the films. This obstructive effect is evidence of the rigidity of the greater part of the cholesterol molecules, a property necessitated by their constitutional formulae.

The present study of about thirty substances has been undertaken in order to survey more completely the various types of films obtainable from these substances, in the hope that surface film experiments, which are comparatively easy and rapid to carry out, may afford assistance in elucidating the structure of new members of the group, or in ascertaining rapidly whether any compound contains the sterol type of skeleton or not.

METHODS.

The surface pressure has been measured by the instrument of Adam and Jessop [1926], as described by Adam [1930]. The surface potential, *i.e.* the change in contact potential between the water and air caused by the presence of the surface film, was measured by the now well-known technique of using an insulated metal wire, connected with an electrometer, the lower end of the wire being coated with a small amount of polonium and being supported about 1 mm. above the water surface. A valve electrometer was used to measure the potentials, exactly as described by Harding and Adam [1933]. In order to compare the surface potentials for equal numbers of molecules per sq. cm. of the film, the quantity μ given by Helmholtz's equation

$$\Delta V = 4\pi n\mu$$

(ΔV = surface potential in millivolts, n the number of molecules per sq. cm. in the film) has been calculated and is shown in the figures.

In all cases where collapse or imperfect spreading of the film was suspected, with consequent rendering of the measurement of the area per molecule deceptive, the films were examined with a high power dark ground illuminator fixed in the bottom of the trough and focused on the surface, according to the method of Zocher and Stiebel [1930], the details being as described by Adam [1932]. A fully spread film appears dark, but collapsed film or unspread material appears as bright patches.

The substances were dissolved in carefully purified benzene, or, in a few cases, in a benzene-alcohol mixture (5:2 by volume) and the amounts placed on the surface measured by a micrometer syringe [Trevan, 1925]; occasionally a calibrated dropping pipette was used. They were spread, except where otherwise stated, on $N/50$ HCl at room temperature.

Models of the molecules were frequently made to obtain a clear picture of their probable size, shape and orientation on the surface. They were constructed of spheres of hard wood, those for carbon and oxygen being 1.5 in. diameter and bored with 4 holes arranged tetrahedrally; hydrogens were represented by 1-in. diameter spheres. This gives a scale of approx. 1 in. to 1 Å. Short pegs permitting the spheres to touch each other were always used.

Experience with such models of substances of known constitution indicates that the molecules in surface films practically never pack into areas smaller than the smallest rectangular figures required to surround the models; also that in the surface films there is always a strong tendency for the molecules to orient so that the water-attracting groups are at, or very near to, the lowest point of the molecules as indicated by the models. Considerably larger areas than those indicated by models packed in this way are not uncommon among aliphatic compounds, but with the sterols they are not often found, except through tilting, which is also indicated by a high compressibility of the films. Such models are often very useful in deciding between possible constitutions; they show, for instance, that the modern formula for the sterols is compatible with the area of about 40 sq. Å., but that the older formula would require at least 54 sq. Å. in any orientation with the hydroxyl group near the water. Measurements on the models can be trusted to give the minimum area which the molecules can occupy within 10%.

The figures show the areas per molecule as abscissae, and as ordinates the surface pressure F , in dynes per cm., the surface potential ΔV in millivolts, and μ in E.S.U. $\times 10^{-21}$.

NEW RESULTS AND DISCUSSION.

Sterols with a hydroxyl group in position 3. Fig. 1 shows the data for 6 sterols with the above constitution. The surface pressure curves are generally similar but the limiting areas vary from 37.5 to 44 sq. Å., the compressibilities being small. The *epi*-isomerides tend to occupy slightly more space on the surface than the normal form, though the differences in area at a given surface pressure are not great. There are, however, very marked differences in surface potential between the different sterols, the *epi*-isomerides having a much smaller surface potential than the normal; while the difference between the normal and the *epi*-forms is much greater in cholestanol than in coprostanol.

The above data are in good accord with the now generally accepted theory that the difference between the cholestanol and the coprostanol series lies in the steric arrangement of the linkage between rings I and II, well summarised

Fig. 1. I. Ergosterol. II. Cholestane-3-ol. III. $\Delta^{5,6}$ -Cholestene-3-ol (cholesterol). IV. *epi*Cholestane-3-ol. V. Coprostan-3-ol (coprosterol). VI. *epi*Coprostan-3-ol.

Diagram II. I. Cholestane-3-ol. II. *epi*Cholestane-3-ol. III. Coprostan-3-ol. IV. *epi*Coprostan-3-ol.

by Rosenheim and King [1934, 3] and Ruzicka *et al.* [1933, 1, 2]. Constructing models with the linkages between rings II and III, and between III and IV of the *trans* type, but the linkage between I and II *cis* for coprostanol and *trans* for cholesterol (the ring I being of the "chair" configuration with cholesterol and "boat-shaped" with coprostanol), diagrammatically represented as above (Diagram II), we have the following data for the models:

	Cholesterol	Coprostanol
Horizontal cross-section of minimum enclosing rectangular parallelepiped	36 sq. Å.	40 sq. Å.

These area measurements on the models are uncertain to about 2 sq. Å., and there is a considerably greater uncertainty in comparing them with the areas actually measured in the films, the molecules may not pack exactly into the area of the enclosing rectangular figure. They do indicate, however, that even with fully saturated sterols, the difference in stereochemical linkage between rings I and II is likely to cause the coprostanol films to occupy an area decidedly greater than the cholesterol. The smaller area occupied by ergosterol, and by one or two of the other unsaturated sterols reported by Adam and Rosenheim [1929], may be due either to the presence of unsaturated linkages in the molecules, or, less probably, to closer packing through some degree of interlocking of the projecting hydrogen atoms.

The large differences in the surface potential and μ are probably due to differences in the orientation of the dipoles present in the hydroxyl groups. The relation of μ to the dipole moment of the molecules in the film has been much discussed [*cf.* Frumkin and Williams, 1929; Schulman and Rideal, 1931; Adam and Harding, 1932; Adam *et al.*, 1934] without a full interpretation being yet possible. If $\bar{\mu}$ is the dipole moment of the film molecule, and θ the inclination of its axis to the vertical, then $K\mu = \bar{\mu} \cos \theta$,

i.e. for a given value of the dipole moment, μ depends on the angle of tilt of the axis of the dipole and on K , the dielectric constant of the surface film. The magnitude of the "dielectric constant" of the surface film is very uncertain; there is some evidence, however, that it does not change much even with quite large changes in packing of the films, and it will be assumed that K is practically the same for all these very similar films.

Examination of models of cholesterol and coprostanol shows that in both these compounds, of the two carbon valencies available for the —C—O— linkage, one is nearly parallel to the long axis of the molecule, and the other makes approximately a right angle with the axis (109° from the first; unfortunately, owing to some flexibility in this ring, a closer definition of these angles does not appear to be possible). The difference between the normal and the *epi*-forms of these compounds is supposed to be due to the hydroxyl being attached to one or other of these two valencies (the principal qualitative difference between the normal and the *epi*-forms of the sterols being that the latter do not form insoluble compounds with digitonin [*v.* Rosenheim and King, 1934, 1, 2]). This is in agreement with the observed facts that the *epi*-derivatives of cholesterol and coprostanol give very small values of surface potential and μ , if we assume that in the normal derivatives the —C—O— link of the hydroxyl is attached to the valency which is approximately in line with the axis of the molecule, and hence will have a maximum, or nearly maximum, vertical component when the molecule is vertical, and in the *epi*-derivatives to the other valency, which, being almost at right angles to the molecule, would have only a small vertical component when the molecule is upright. The negative values

of ΔV and μ observed for *epicholesterol* suggest that when molecules of this compound are upright in the surface film the dipole is inclined upwards, *i.e.* the negative end is uppermost. Observation of models of this compound show that the —C—O— link can actually have a small upward angle.

For the reasons explained above, these arguments can at present only be qualitative, but if they hold, they afford confirmation of the hypothesis, based on the non-precipitability of these substances by digitonin, that the early irradiation products of ergosterol have their hydroxyls epimerised [Rosenheim and King, 1934, 2]; for example, the value of ΔV falls from about 400 mv. for ergosterol to about 200 mv. for lumisterol, the first product of irradiation, this change being accompanied by only a small change in area [Danielli and Adam, 1934].

Ketones with one carbonyl group at 3. Fig. 2 shows the data for some compounds of this type. In general the surface pressure-area curves tend to considerably larger areas than those for the corresponding 3-hydroxy-compounds, and the films are much more compressible. The surface potentials and values of μ are also considerably greater, and the values of μ tend to constant values as the area is diminished.

Fig. 2. 3-Keto-compounds. I. Cholestane-3-one. II. Coprostane-3-one. III. $\Delta^{4:5}$ -Coprostene-3-one ("cholestenone"). IV. Oxime of "cholestenone." V. "Cholestenone" on 0.1% neutral KMnO_4 solution.

Cholestane-3-one, the ketone corresponding to cholestane-3-ol (cholestanol) and *epicholestane*-3-ol, occupies an area a few sq. Å. larger than either of these compounds. The molecule is thus probably slightly tilted at low compressions, being brought upright as the pressure is increased.

As in the hydroxyl compounds there is an increase in area corresponding to the more bulky *cis*-linking of rings I and II in passing from the ketone derived from cholestane to that derived from coprostane, but with the ketones the difference is much more marked, coprostane-3-one occupying some 6–8 sq. Å. more than cholestane-3-one.

The unsaturated compound $\Delta^{4:5}$ -coprostene-3-one (formerly called cholestenone) occupies still larger areas, reaching 59 sq. Å. at zero compression, and

the value of μ rises to 900×10^{-21} E.S.U. The large area occupied by this compound was observed by Adam and Rosenheim [1929], who further found that the area occupied by the corresponding oxime was much smaller, as shown in Fig. 2. The increase in the minimum area of the molecule, as measured on models, caused by the *cis*-linkage of rings I and II is insufficient in itself to account for the large areas shown by these ketones; the limiting area observed in the film (59 sq. Å. for coprostenone) is nearly 50% greater than the minimum area required by the models (about 40 sq. Å.). That this increase in area is almost certainly due to a tilt of the molecules is shown by the following experiments (Fig. 3). Mixtures of coprostenone ("cholestenone") and cholesterol in known proportions were spread on a water surface, and the areas occupied by the coprostenone molecules calculated from the experimental figures, assuming that the cholesterol occupied the same areas in these films as when in a film alone. From the curves in Fig. 3 it is seen that the area occupied by coprostenone in such mixtures is considerably reduced, and that in the 1:1 mixture, under 15 dynes per cm. pressure, its area is similar to that of cholesterol itself, indicating that in these films the coprostenone molecule is being held upright by the upright cholesterol molecules, and hence that in the films of coprostenone alone the large areas are due to tilt of the molecules. The surface potentials of these mixed films are plotted against the mean area per molecule, of either kind, and are much closer to the surface potential of coprostenone itself than to that of cholesterol; the meaning of this is, however, not easy to see.

Fig. 3. Cholesterol-coprostenone mixed films. I. Cholesterol. II. Coprostenone. III. Mixed films, ratio $\frac{\text{one}}{\text{ol}} = 1$. IV. Mixed films, ratio $\frac{\text{one}}{\text{ol}} = 3$.

The reason why the ketones should be so much tilted remains to be explained. If the carbonyl group is not in line with the axis of the molecule (as a first approximation one would expect it to be attached at an angle midway between those of the —C—O— links of coprostanol and *epicoprostanol* since it is attached by both these valencies, *i.e.* making an angle approaching 45° with the axis of the molecule), this may account for some of the increase in area, since under zero compression one would expect the molecules to orient themselves with the

dipole vertical. On this view, however, the molecules should be brought upright as the pressure is increased and the dipoles tilted; the values of μ do not support this, since they tend to give constant values on compression. Other observed facts in connection with coprostenone for which explanation is at present lacking are the reversion of the oxime to a comparatively small area and the observation that on solutions of neutral KMnO_4 this compound again shows a diminished area (tending to 56 sq. Å. on 0.02 %, to 51 sq. Å. on 0.1 % and 0.5 %, Fig. 2).

Monohydroxy- and monoketo-compounds having the polar groups in positions 6 or 7. The data are given in Fig. 4 for cholestane-6-ol, $\Delta^{4:5}$ -coprostene-7-ol ("ψ-cholesterol"), and cholestane-6-one. The surface pressure-area curve of cholestane-6-ol shows some resemblance to those of the 3-hydroxy-compounds described above, but the film occupies a larger area and is considerably more compressible. Models of this compound show that here the hydroxyl group can only be in contact with the water surface if the molecule is considerably tilted, and the limiting area given by such models, 48 sq. Å., is in good agreement with the experimentally found limiting area of about 50 sq. Å. The values of ΔV and μ are lower than for the 3-hydroxy-compounds in which the dipole is nearly

Fig. 4. I. Cholestane-6-ol. II. Cholestane-6-one. III. $\Delta^{4:5}$ -Coprostene-7-ol ("ψ-cholesterol").

perpendicular, and the value of μ diminishes on compression; these data suggest that the hydroxyl group is inclined to the surface even under low compressions and is tilted still further as the molecules are crowded together. Variation of temperature has a slight effect on the areas occupied by the films of this compound, the limiting area being increased by about 1.5 sq. Å., when the temperature of the underlying solution was raised from 17° to 30°, and decreased by a similar amount by cooling to 3°. Experiments were also carried out, similar to those described above in connection with coprostenone, in which mixtures of cholestane-6-ol and cholesterol were spread on a surface. In this case however, in contrast to the behaviour of coprostenone, cholestane-6-ol retained its normal area in the mixed films. This is to be expected, since, though the larger area is again due to a tilt of the molecule, the tilt in this case is due to the water-attracting group being attached in a position removed from the end of the

molecule, and to bring the molecules upright the hydroxyl group would have to be removed from the water.

The corresponding keto-compound, cholestane-6-one, occupies a much larger area at low compressions than the hydroxyl compound, and is much more compressible. Collapse at constant pressure occurs at about 16 dynes per cm. and 42 sq. Å. The larger area and compressibility of the keto-compound compared with the corresponding alcohol is a similar effect to that observed above with the 3-substituted compounds. Here the compressibility is much higher, however, and the value of μ , unlike that of the 3-keto-compounds, falls as the compression rises. Models of this compound show that, tilted with the keto-group oriented nearly vertically, the compound would occupy about 60 sq. Å., and that tilting the molecule, still keeping the keto-group in contact with the surface, will allow this area to be reduced to approximately 50 sq. Å.; further reduction of area could only be obtained by partial removal of the keto-group from contact with the surface. This accounts qualitatively for the high compressibility of the film, and also for the fall in μ which takes place on compression, since the keto-group is tilted to make a larger angle with the vertical.

$\Delta^{4,5}$ -Coprostone-7-ol (" ψ -cholesterol") gives a surface pressure-area curve quite different from those of any of the hydroxy-compounds previously discussed. The area at 0.5 dyne per cm. pressure is about 80 sq. Å., and the film is extremely compressible; above 80 sq. Å. the curve falls off gradually to about 0.02 dyne per cm. at 120 sq. Å. Over this latter region, the fluctuations in ΔV over the surface become much larger, of the order of 50 mv. In mixed films with cholesterol, the area of this compound was only slightly diminished, and the compressibility almost unaffected, indicating that, as with cholestane-6-ol, if the molecules are tilted, the tilt is not one which can be overcome by mechanical support from other molecules. The area under 0.5 dyne occupied by this coprostone-7-ol is dependent to some extent on temperature, being decreased by about 10 sq. Å. by a 15° fall of temperature, but there was no significant change in the form of the curve either on warming to 45° or cooling to 3°.

The cholestane ring system with the anchorage to the water at 7 must lie with the long axis of the molecule practically horizontal; the ring system may be on edge or flat. The side-chain may be stretched out flat or be perpendicular to the surface or be oscillating, as with the "expanded" films of aliphatic compounds. The models give an area for the flat ring-system with fully extended chain of about 130 sq. Å. We think it very unlikely, however, that many of the molecules are in this position even at the lowest pressures, since previous experience with many other types of molecules, some of which (*e.g.* apocholic acid below) would have more lateral adhesion when flat than ψ -cholesterol, shows that such molecules lying flat always give gaseous films; the film shows cohesion of a rather curious type, being heterogeneous according to the surface potential measurements above 80 sq. Å., but yet falling in surface pressure from 0.5 to 0.02 dyne between 80 and 120 sq. Å. We suggest that the films consist of aggregates of the ring systems adhering together, standing on edge with the long axis horizontal, and the chains oscillating in much the same way as they do in liquid expanded films. The cohesion is thus due to the adhering ring-systems, supplemented by the adhesion between the oscillating chains [v. Langmuir, 1933].

Dihydroxy-compounds and their acetates. Fig. 5 shows the data for the isomeric α - and β -diols, whose structure [Rosenheim and Starling, 1933] is probably $\Delta^{5,6}$ -cholestene-3:4-diol, the difference between the two compounds being probably due to one of the hydroxyls, most probably that in position 4, being normal in one isomeride and *epi* in the other.

α -Diol and its 3-acetate give identical surface pressure-area curves; the surface potential curves are similar, but that of the acetate is about 25 mv. higher. The film of β -diol occupies a larger area, is much more compressible and has a lower surface potential. Its 3:4-diacetate occupies a much larger area than the diol and has a considerably higher surface potential.

Fig. 5. Dihydroxy-compounds and their acetates. I. $\Delta^{5:6}$ -Cholestene-3:4-diol (α -diol). II. 3-Acetate of α -diol. III. $\Delta^{5:6}$ -Cholestene-3:4-diol (β -diol). IV. 3:4-Diacetate of β -diol.

Models show that, in order to bring both the hydroxyl groups to the lowest point of the molecule, different degrees of tilt are required with the four possible stereoisomerides of 3:4-diol. With the centres of the oxygen atoms in the same horizontal plane, the areas of the rectangles enclosing the tilted molecules are approximately as follows:

	Hydroxyl 3	Hydroxyl 4	Areas of tilted molecules (sq. Å.)
(a)	Normal	Normal	48.5
(b)	Normal	<i>Epi</i>	ca. 60
(c)	<i>Epi</i>	Normal	50.5
(d)	<i>Epi</i>	<i>Epi</i>	51

Configuration (a) is the smallest, (c) and (d) slightly larger, and (b) much larger, the tilt being larger, the greater the area given above—the area of all these models packed vertically being under 40 sq. Å. The observed areas are less than these, hence the molecules do not tilt to the extreme angle at which the centres of the oxygen atoms are in the same horizontal plane, but there is about the difference in area required on the supposition that the α -diol has configuration (a) and the β -diol has (b). Further, the hydroxyl at 3 projects laterally if *epi* and continues the general line of the molecule if *normal*, so the fact that the 3-monoacetate of α -diol has exactly the same area as the diol indicates that the 3-hydroxyl is normal. In the β -diol the *epi*hydroxyl at 4 projects sideways, so that the acetate occupies a considerably larger area than the diol. The surface pressure results agree with the configuration (a) for the α -diol and (b) for the β -diol.

Trihydroxy-compounds and their acetates. Data for the following compounds are given in Fig. 6: cholestane-3:5:6-triol ("α-cholestanetriol"), cholestane-3:5:6-triol-3-acetate, cholestane-3:5:6-triol-3:6-diacetate and "β-ergostadienetriol".

The surface pressure-area curve for the parent cholestanetriol shows a limiting area for this substance of about 56 sq. Å. A rather smaller value for this substance was given by Adam and Rosenheim [1929]; the present value was obtained with three specimens spread from benzene solution (other, variable values were obtained if benzene-alcohol mixtures were used as the solvent; the reason for this is probably that the substance, having three hydroxyl groups, is near the limit of insolubility required by the surface pressure technique, and part of the solute tends to be carried below the surface when a solvent partially miscible with water is used. Using benzene as a solvent, there is little chance of this occurring, and the value given is probably correct).

Fig. 6. Trihydroxy-compounds and their acetates. I. Cholestane-3:5:6-triol. II. Cholestane-3:5:6-triol-3-acetate. III. Cholestane-3:5:6-triol-3:6-diacetate. IV. "β-Ergostadienetriol".

Measurement of a model of this compound shows that two of the hydroxyls, those on carbon atoms 3 and 6, can readily reach the water surface if the molecule is tilted to about 45° from the vertical, the main plane of the molecule remaining approximately at right angles to the surface; in this position the area occupied is approximately 52 sq. Å. To enable the third hydroxyl, that on carbon atom 5, to reach the water surface, the plane of the molecule must be tilted as well, the area then occupied being larger, about 66 sq. Å. The limiting area observed, 56 sq. Å., for the parent triol suggests that in this compound only the hydroxyls on 3 and 6 actually reach the surface. This is on the assumption that the hydroxyl on 6 is in the more favourable of the two possible positions, that in which it is approximately in the plane of the molecule; if it were in the alternative position, a much larger limiting area would be required. It is difficult to explain the very small surface potential shown by this compound; the possible explanation that might be advanced, that the hydroxyls are so arranged that their moments approximately cancel one another, does not appear to hold, since examination of a model shows that the resultant of the —C—O— linkages, at any rate, should have a moment of the same order of magnitude as that of one such

linkage; the explanation of this difficulty may lie in the unknown orientation of the hydroxyls round the —C—O— linkage.

The acetates of this triol, like the acetate of β -diol described above, give surface pressure curves having much larger limiting areas and compressibilities than the parent triol. Two factors probably contributing to this change are (a) the extra space taken up by the acetyl groups themselves, (b) the known weakening effect of acetylation on the water-attracting power of a hydroxyl group. In the monoacetate, which is acetylated in the 3-position, the weakening of the attraction of the 3-group for water may cause the molecule to tilt over to enable the 5-hydroxyl to reach the water; this would account for the limiting area observed, about 67 sq. Å. The diacetate, in which the 3:6-hydroxyls are acetylated, gives a film which is practically of the gaseous type, in which the molecule is probably lying flat on the surface. In these two acetylated compounds, the surface potentials and values of μ are much larger than for the parent triol, a fact similar to that observed with the diacetate of β -diol. The value of μ decreases considerably on compression, indicating that considerable tilt of the molecules takes place on compression.

The data for " β -ergostadienetriol" are also given in Fig. 6. This is also considered to be a 3:5:6-triol [Rosenheim and King, 1934, 2; Windaus *et al.*, 1934]. The position here is complicated by the presence of the double bonds, but it does not appear, from the small value of the limiting area, that the molecule can be held in the surface by hydroxyls at 3 and 6. That the arrangement of the hydroxyls is different from that obtaining in cholestanetriol is also shown by the very much larger value of the surface potential. A possible, though admittedly very tentative, explanation would lie in the suggestion that whilst the compound is a 3:5:6-triol, the 6-hydroxyl is in the *epi*-position to that assumed in cholestanetriol. In this case it would, like the 5-hydroxyl, project sideways, but in the opposite direction. The fields of these two hydroxyls being thus to some extent opposed, the molecule would probably be more ready to assume positions in which only the 3-hydroxyl actually reaches the water. The value of μ offers some support for this, in that it is of the same order as that of compounds containing a single hydroxyl orientated vertically.

Diketones. Two diketones of the cholestane series are shown in Fig. 7, cholestane-3:6-dione, and $\Delta^{4:5}$ -cholestene-3:6-dione ("oxycholestenone").

Cholestane-3:6-dione (Fig. 7) shows a similar surface pressure curve to cholestane-6-one (Fig. 4), but the areas occupied are some 6 sq. Å. larger. In connection with the 6-keto-compound it was shown that the area and compressibility could be accounted for if the molecule tilts from a position in which the keto-group is nearly upright to one in which the molecule is as nearly upright as is consistent with the keto-group remaining attached to the water. Similar considerations hold in the case of the 3:6-diketo-compound, if we make the reasonable assumption that the second keto-group tends to keep the molecule rather more firmly attached to the surface, and since the 6-keto-group is tilted away from the vertical, as the 3-keto-group is tilted towards the vertical, we can account for the comparatively small change in μ .

The corresponding unsaturated compound, $\Delta^{4:5}$ -cholestene-3:6-dione gives rather similar surface pressure and surface potential curves, except in that they correspond to areas some 10 sq. Å. larger; the surface pressure curve of the cholestenedione is not unlike that of 7-substituted compounds, where the long axis of the molecule is lying extended along the surface. This behaviour of the unsaturated compared with the saturated ketone is to some extent parallel with that of the 3-keto-compounds (Fig. 2).

Fig. 7. Diketones. I. Cholestane-3:6-dione. II. $\Delta^4:5$ -Cholestene-3:6-dione ("oxycholestenone").

Compounds with two or three different groups, at 3, 5, 6 or 7. Data for five compounds are shown in Fig. 8. Cholestane-3-ol-6-one gives a surface pressure curve, I, intermediate between that of cholestane-3-ol and cholestane-6-one. Its acetate, curve II, occupies a decidedly larger area, the molecules being more

Fig. 8. Compounds with two or more different water-attracting groups. I. Cholestane-3-ol-6-one. II. Cholestane-3-ol-6-one acetate. III. Cholestane-3:6-dione-5-ol. IV. Cholestene-3-ol-7-one acetate ("β-oxycholestenol acetate"). V. Cholestane-3-chloro-6-one.

tilted to the vertical. The tilt of the molecules is probably decided by the relative intensities of the attraction of the hydroxyl or acetate group at 3, and of the keto-group at 6, for the water. The attraction of the hydroxyl group is stronger than that of the keto-group, so that in the 3-ol-6-one compound the molecule is pulled rather more upright through the hydroxyl group becoming further immersed in the water than the keto-group. When the attraction of the

hydroxyl group for the water is weakened, though not destroyed, by acetylation, the two groups become more equally immersed in the water, tilting the molecule rather more.

Cholestane-3:6-dione-5-ol shows areas intermediate between those of the 3:6-dione and the 3:5:6-triol, the hydroxyl group pulling the molecule somewhat nearer the vertical position than with the 3:6-dione. $\Delta^{5:6}$ -Cholestene-3-ol-7-one acetate (" β -oxycholestenol acetate") is much less tilted than other compounds with a water-attracting group at 7, exerting practically no surface pressure at areas greater than 62 sq. Å. This result is difficult to reconcile with the constitution assigned on chemical grounds, but would be consistent with a constitution in which the keto-group was at 6.

Cholestane-3-chloro-6-one (from nitrocholesteryl chloride) occupies an area much greater than other compounds with a water-attracting group at 6. It is known [Adam, 1930, p. 84] that the chlorine in alkyl chlorides has very little attraction for water. Probably the molecule is anchored by the 6-keto-group alone, and possibly the size of the chlorine atom causes the area to be greater than that of the other compounds with one anchorage to the water at 6.

The apocholic acids. Fig. 9 shows the data for *apocholic* and β -*apocholic* acids, which crystallise with half a molecule of xylene; this probably evaporates when the films are spread. Both films are gaseous, and surface pressure and

Fig. 9. *Apocholic* acid and β -*apocholic* acid.

surface potential are identical for the two isomerides, within experimental error. There are hydroxyl groups at 3 and 12 and a carboxyl at the end of the side-chain, in both acids; also one double bond which may be at 8-9 in *apocholic* [Wieland and Dane, 1932] but may be observed in the β -acid recently isolated by Callow (private communication). As usual, the three widely separated water-attracting groups cause the molecules to lie flat and the films are gaseous; similar gaseous films, with compounds containing the sterol skeleton and two or more water-attracting groups far apart in the molecule, have been previously found with oestriol, pregnandiol *etc.* [Adam *et al.*, 1932; Danielli *et al.*, 1933]. As the films are gaseous, with the molecules lying flat and relatively far apart, the small difference in the form of the molecules, if any, caused by the change in position of the double bond, does not produce any difference in the surface pressure curves of the two isomerides. The identity of the surface potential curves indicates that this isomerism does not involve any serious change in the orientation of the polar groups relatively to the ring system.

SUMMARY.

Surface pressure and surface potential measurements of about 30 compounds related to the sterols are described. All the data are consistent with the modern view of the constitution of the sterol skeleton.

Most of the compounds show coherent condensed films.

Sterols with one hydroxyl at 3 and with not more than one double bond stand practically vertical in the surface; their area per molecule varies from 37 to 44 sq. Å., according to the stereochemical configurations of the ring systems. Highly unsaturated sterols are sometimes, but not always, tilted.

Ketones with one carbonyl at 3 tilt, sometimes far, from the vertical, but can be brought more nearly upright by admixture with sterols which normally stand upright in the surface.

One hydroxy- or keto-group at 6 or 7 causes the molecules to tilt considerably, in order that the group may come close to the water. This tilt is not altered by admixture with sterols standing upright. Keto-groups usually cause more tilt than hydroxy-groups. The molecules may tilt so that their long axis is practically horizontal, without the films losing coherence and becoming gaseous; the molecules may, however, be standing on edge, permitting much lateral adhesion between ring systems of adjacent molecules, in addition to that due to the hydrocarbon chains.

Compounds with two or three water-attracting groups in rings I and II always tilt. The amount of tilt depends on the position in the molecule and the relative attraction for water of the various groups.

Surface potential measurements sometimes indicate whether a hydroxyl group has the "normal" or the "epi" stereochemical configuration.

The apocholic acids form gaseous films with the molecules lying flat.

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