CCX. SURFACE FILMS OF ERGOSTEROL AND ITS IRRADIATION PRODUCTS.

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(Received June 30th, 1934.)

THIS paper describes a continuation of the work of Rosenheim and Adam [1929] on unimolecular films of derivatives of ergosterol, spread on a water surface. Its object is to obtain information as to the properties of the molecules in these films, in the hope of throwing fresh light on the constitution of the various products. While some progress has been made, there are still many points in the interpretation of the curves presented in this paper whose meaning is obscure; it seems desirable at this stage however to record the results obtained.

Surface pressure and surface potential measurements were made; surface pressure according to the technique of Adam and Jessop [1926, 1; *cf.* Adam, 1930]; surface potential with one of the instruments described by Adam and Harding [1932; 1933], the method being essentially the same as that used by Guyot [1924], Frumkin [1925], Schulman and Rideal [1931] and other workers. The completeness of spreading of the films was occasionally verified by examining them with a cardioid dark-ground condenser in the bottom of the trough, using the method of Zocher and Stiebel [1930], as modified by Adam [1933]. The solidity or otherwise of the films was tested by sprinkling a little talc on the surface and noting whether the particles could be blown about freely on the surface, or whether the surface behaved like a solid film.

The films have been investigated on *N*/100 hydrochloric acid and on various strengths of permanganate. The substances investigated were ergosterol, lumisterol, tachysterol, calciferol and two "suprasterols," the last five being formed successively during the irradiation of ergosterol; pyrocalciferol, formed by the action of heat on calciferol; α -dihydroergosterol, in which one of the double bonds in the ring system of ergosterol is hydrogenated; α - and β -ergostenols, in which the double bond in the side-chain is hydrogenated in addition to one of those in the ring system; β - and γ -ergostadienetriols, formed by the action of perbenzoic acid on ergosterol at low and high temperatures respectively. Our warmest thanks are due to Drs Rosenheim and Callow, of the National Institute for Medical Research, to Prof. Windaus and Dr Lüttringhaus, of Göttingen, and to Mr A. L. Bacharach, of Messrs Glaxo, Ltd., for specimens of these compounds.

In the figures, areas per molecule are plotted horizontally, the scale of surface pressures in dynes per cm. being indicated vertically on the left of the diagrams; on the right, the scale of the surface potentials ΔV (the change in contact potential between liquid and air caused by the presence of the films) in mv. and the values of μ , in e.s.u. $\times 10^{-21}$ per molecule calculated from Helmholtz' equation

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

n being the number of film molecules per cm^2 μ is undoubtedly related to the vertical component of the dipole moment of the molecules in the surface films, but contains also terms of unknown magnitude depending on the degree of re-orientation of the molecules of water near the surface and of re-arrangement of the ions, which occur when the film is put on the surface.

Films on N/100 hydrochloric acid.

Fig. 1 shows the results obtained with ergosterol and its various irradiation products. Ergosterol has a smaller area (about $37.5 \text{ sq. \AA.}$ at no compression) than that given by Rosenheim and Adam [1929] and by Fosbinder [1933], who gave $40.5 \text{ sq. \AA.}$ within $0.5 \text{ sq. \AA.}$ as the area at no compression. We now find that if the surface pressure curves are taken rapidly, an area of about $40.5 \text{ sq. \AA.}$ is obtained, but that the film is exceedingly rigid, and probably consists of islands of rigid film, not quite closely packed. It appears to collapse under moderate pressure until an area of $37.5 \text{ sq. \AA.}$ is reached, when the film can be

Fig. 1.

compressed and decompressed several times without further apparent collapse. At this stage we think that the rigid islands of film have been so deformed that the space between them is negligible, and the area is nearly that of closely packed molecules. On several occasions a bridge across the full width of the trough (14 cm.) was formed, which resisted a pressure of 4 dynes per cm. on one side only; the thickness of the film is so small that this corresponds to a pressure of the order of 20 atmospheres.

There is the possibility that the film was slightly collapsed at $37.5 \text{ sq. \AA.}$ Examination with the cardioid dark-ground condenser showed very slight signs of collapse; but forcible reduction of area by a further 10 % caused a very great increase in the amount of visible material expelled from the film (which is itself invisible); we think that the amount of unspread material, if any, in the film at $37.5 \text{ sq. \AA.}$ is less than would occupy an additional $1 \text{ sq. \AA.}$ when spread. The larger areas reported previously are probably due to the films being imperfectly packed owing to the rigidity of the islands of film first deposited.

The changes of area with successive irradiation products are very remarkable. The area increases with the progress of irradiation up to tachysterol, and then decreases. Rosenheim and Adam [1929] found a steady increase in area on irradiation, but not this diminution of area in the later stages of irradiation; we think that the substances here used are of much greater purity.

The surface potential became constant, within about 10 mv. over the whole films, when the pressure exceeded about 1.5 dynes. μ lay between 3 and 4×10^{-19} e.s.u. for most of the substances, but was decidedly lower, about 1.9×10^{-19} , for lumisterol. μ rises considerably when ergosterol is compressed, although the area diminution is small.

Ergosterol and lumisterol formed solid films; the other irradiation products liquid films. α -Dihydroergosterol gave a surface pressure curve almost indistinguishable from that of ergosterol; it is solid and shows a slight tendency to

Fig. 2. Dinitrobenzoates.

I. Ergosteryl	} on HCl.	III. Calciferyl	} on permanganate.
II. Lumisteryl and calciferyl		IV. Ergosteryl	

collapse. This substance is believed to have one of the double bonds in the ring system hydrogenated. Two ergosterols, α and β , which have one double bond only in the ring system, gave a rather larger area, approaching 45 sq. Å. at no compression; α -ergosterol films were much more compressible than β , the latter having a compressibility not much larger than that of ergosterol itself. The β -ergosterol was probably a complex mixture.

Fig. 2, curves I and II, III and IV, show the results obtained with the 3:5-dinitrobenzoates of ergosterol, lumisterol and calciferol. In spite of the large size of the dinitrobenzoyl radical, there is not any large increase in the area of the films. Indeed calciferol dinitrobenzoate actually packs into a smaller area than calciferol itself. As however the areas are all over 37 sq. Å., this result is not surprising, for the benzene ring itself is known to have a cross section, packed closely and upright in the films, of only 24 sq. Å., and an additional 12 sq. Å. are likely to be sufficient to accommodate the two nitro-groups. But the results show that the dinitrobenzoate molecules are so orientated on the

surface that the dinitrobenzoyl residue forms approximately a continuation of the general direction of the sterol ring system down into the water.

The esterification with the dinitrobenzoyl group increases the surface potential and μ , but not equally in the three cases. The increase in μ is much greater in the case of ergosterol than with the other two and is least with lumisterol. A curious feature of the $\Delta V-A$ and $\mu-A$ curves is the abrupt change in direction at a certain stage of the compression, as if some part of the molecule were being re-orientated so as to alter the vertical component of its dipole moment, as the film is compressed to a certain area beyond which the re-orientation with decrease in area either ceases or changes abruptly in direction.

Oxidation of the films by permanganate solutions.

It was found by Adam and Jessop [1926, 2] that the oxidation of surface films of unsaturated compounds often causes a drastic change in the orientation of the molecules, through changing the not very water-attractive group, $-\text{CH}=\text{CH}-$ into $-\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})-$, which is strongly attracted to the water. In particular, if the double bond is present in a part of the molecule of an aliphatic compound, remote from the polar end group providing the first anchorage, the molecules lie flat on oxidation and the films usually become gaseous instead of coherent. Hughes and Rideal [1933] have shown that the velocity of oxidation depends on the ease of access of the double bonds in the molecule to the surface of the permanganate solution; thus when oleic acid films were compressed so much that the molecules were inclined at a steep angle to the surface, the double bond was oxidised much more slowly than when there was more room on the surface, the motions of the hydrocarbon chains in the film under small compression (the film is of the liquid expanded type) permitting the double bond to come into more frequent contact with the underlying liquid. It was hoped that by careful investigation of the rates of oxidation of compounds of the sterol group, on oxidising solutions of different strengths, information might be gained as to the position and accessibility to the water surface of the double bonds. Other considerations besides simple accessibility to the underlying solution are, however, likely to influence the rate of oxidation; it is well known that conjugated double bonds are more readily oxidised than isolated ones.

Potassium permanganate solutions varying in strength from 2 % downwards were employed. For the surface potential measurements, since a silver-silver chloride electrode was used to make the connection between the solution and the rest of the electrical circuit, the presence of some chloride ion in the solution was desirable, in order to maintain a constant and reproducible potential at this junction. The permanganate solutions were therefore made up with equal weights of potassium chloride and potassium permanganate; the addition did not appear to affect the manner of oxidation of the films.

Strong solutions were usually found rather unsatisfactory, the oxidation proceeding in an uncontrolled manner to a stage at which the films either collapsed or dissolved. Most of the substances discussed in this paper contain two or more double bonds; complete oxidation to the stage where one molecule of hydrogen peroxide is added to each double bond would therefore result in the presence of five or more water-soluble groups in the molecule, a state of affairs which would almost certainly result in dissolution of the film. It was, however, noticed that calciferol was the most readily oxidised to the stage of dissolution, while α -dihydroergosterol remained unattacked even on 1 % permanganate

containing $N/10$ sulphuric acid. Cholesterol was oxidised to an initial stage, on this solution, in about 10 minutes. Except where otherwise stated, the permanganate solutions were made up with the addition of potassium chloride but without adding acid.

On more dilute solutions (0.1–0.001 %) it was often possible to recognise a fairly definite end to an initial stage of oxidation, which presumably corresponds to the conversion of one of the double bonds into the dihydroxylated stage. Further changes in the films occurred but were slow, so that the characteristics of the films in this stage of oxidation could be approximately ascertained. Often the same initial stage of oxidation was found over a wide range of dilutions of the permanganate.

The curves of oxidised ergosterol, lumisterol and suprasterol I (curves IV, III and II respectively of Fig. 3) are so nearly the same that the differences

Fig. 3.

I. Suprasterol II.	II. Suprasterol I.	III. Lumisterol.
IV. Ergosterol.	V. Pyrocalciferol.	VI. Calciferol.

are probably experimental errors. They end at about 60 sq. Å. at no compression and occupy about 51 at 10 dynes. The oxidised films of calciferol occupy a decidedly larger area (ca. 70 sq. Å. at low compressions), those of pyrocalciferol a slightly larger area and of suprasterol II a slightly smaller area. Surface potentials were measured with ergosterol, lumisterol and calciferol; μ changed very little on compressing the films and was always a good deal higher than with the unoxidised films, especially with lumisterol, where the increase of μ is approximately threefold on oxidation. The films of ergosterol and lumisterol, after oxidation to this stage, are practically indistinguishable from each other, either in surface pressure or surface potential, although the unoxidised films are very different, especially in surface potential. Tachysterol was not oxidised by 0.1 % permanganate.

The dinitrobenzoates of ergosterol and calciferol were also examined; they were decidedly more resistant to oxidation than the sterols themselves but were attacked by 0.25 and 0.1 % solutions, on which nearly the same initial stage of oxidation was obtained. On stronger solutions no definite stage of

oxidation could be found; on weaker the rate of oxidation was inconveniently slow. The areas of these oxidised films are very much greater than those of the same substances on non-oxidising solutions. It is probable that the change in orientation of the molecule, caused by the introduction of additional points of anchorage to the water on oxidation at the double bonds, is drastic enough to cause the large dinitrobenzoyl group to spread out along the water surface more or less horizontally, for the areas at no compression reach 100 sq. Å. for ergosteryl dinitrobenzoate and 85 for calciferyl dinitrobenzoate. μ is diminished on oxidation, a further indication that the orientation of the dinitrobenzoyl group, which provides the greater part of the value of μ in the unoxidised films and is not likely to be particularly easily oxidised, is radically changed in orientation on oxidation of the rest of the molecule. The slower rate of oxidation of the dinitrobenzoates is presumably due to the obstruction of the large dinitrobenzoyl group.

Rates of oxidation on permanganate solutions.

The different substances were found to vary very much in the rate at which the area increased on a given strength of permanganate, *i.e.* in their ease of oxidation. In three cases, cholesterol, pyrocalciferol and lumisterol, the course of the reaction was followed and found to be approximately unimolecular. For the purpose of comparing the rates of oxidation, the times required for the area to increase by one-half of the total increase of area found when the initial stage of oxidation is completed were measured. The figures in Table I are for 0.001 % potassium permanganate under 2 dynes pressure at room temperature. The quantitative aspect of these figures should not be emphasised, but we think that considerable differences in the rate of oxidation, in the order shown above, certainly exist.

Taking the formula of ergosterol provisionally as

[Windaus *et al.*, 1934], and constructing a model with the diameter of the carbon atom 1.5 Å., and that of the hydrogen atom 1.0 Å., the cross section of the molecule placed vertically, with the flexible side-chain above the ring system, is about 37 sq. Å. This is in good agreement with the experimentally observed area of ergosterol; it is the smallest area that can be obtained on the above formula for the ring system of the sterols. Such measurements are only approximate, as it is not possible to estimate how much, if any, interlocking of the projecting hydrogen atoms is permissible between adjacent molecules, or on the other hand, whether repulsive forces keep the molecules further apart in the films than corresponds to these crude models in contact without inter-

locking. In the case of long aliphatic chains, closely packed models of this type give areas some 6.5 sq. Å. smaller than the smallest found in films or crystal packing. For ring structures, *e.g.* long chain derivatives of phenol or of cyclohexanol, the models measured with no allowance for interlocking or for intermolecular repulsive forces keeping the molecules apart gave areas about 3 sq. Å. smaller than those found experimentally in the films. These models probably give a minimum cross section for the molecules, which approaches more nearly to the area occupied in natural packing, the more complex the ring systems. The area of 37 sq. Å. is the smallest measured area found with sterols and probably corresponds to the sterol ring system vertically and closely packed.

Table I. *Times of half oxidation on 0.001 % permanganate.*

Pyrocalciferol	...	...	...	20 seconds
Lumisterol	...	...	...	45 "
Calciferol	...	...	...	200 "
Suprasterol I	...	...	...	ca. 200 "
Ergosterol	...	...	...	330 "
Tachysterol	}	...	...	Very large
Cholesterol				
α-Dihydroergosterol				

The models show that only one carbon atom, numbered 3, is at the bottom of the molecule if the molecule is vertical. Any other position but 3 for the hydroxyl would require the molecules to be considerably tilted, unless the hydroxyl group is somewhat removed from the water, which is unlikely, as the hydroxyl group provides the anchorage for the water, and the rest of the molecule has a much smaller attraction for water. Model measurements give an area of about 42 sq. Å. for a sterol ring system with a hydroxyl at 4, tilted so as to permit the hydroxyl to be the lowest point of the molecule. We think that the area to be expected, if the hydroxyl were in position 4, would be considerably larger than that observed; and also, since the molecules would be tilted, lateral compression of the film would probably diminish their tilt, so as to produce a greater compressibility than that observed. The evidence from the surface films appears decidedly unfavourable to the position 4 for the hydroxyl, suggested by Rosenheim and King [1934], following Heilbron *et al.* [1933]. Position 2 is improbable for similar reasons, and position 1 still less probable. In constructing the sterols, Nature appears to prefer that constitution which permits the molecules to stand upright in the interfaces.

It is worth noting also that the areas found for the sterols which give only slightly compressible films, mostly between 37 and 41 sq. Å., are much too small for the older formulae for sterols. These would require at least 50 sq. Å. from measurements on models; the original formulae of Rosenheim and King (with hydroxyl at 3) will fit into 37 sq. Å.

The larger areas of the irradiation products may be due to one or more of three possible causes: (a) the stereochemical configuration of the carbon ring system may be changed so that the molecules, packed closely and in a vertical orientation to the water surface, would occupy more space; (b) the molecules may become tilted through a change in position of the hydroxyl group, *e.g.* to positions 4, 5 or 6; (c) they may tilt because of much smaller changes in the molecule, such as stereochemical alteration in the angle of the hydroxyl group to the ring system (epimerisation). The first type of change (a) may occur, but would not be likely, alone, to make more than a few sq. Å. difference to the area; it is probably the cause of the differences in area between ergosterol,

sitosterol, cholesterol and coprosterol; change (b), an actual migration of the hydroxyl group, is unlikely on chemical grounds and need not be assumed, because a change of the type (c) is already known, in one case, to cause a large change in the tilt of the molecules and in the area and compressibility of the films. Adam and Rosenheim [1929] found that cholestenone gave a film of nearly as large an area as tachysterol, while cholesterol occupies 40.7 sq. Å.

The manner in which a change in the nature of a water-attracting group, or a change in its orientation to the rest of the molecule without change in nature, may alter the orientation of the whole molecule may be as follows. The group is a dipole, and the surface potential measurements show that it is nearly always oriented with the positive end uppermost in the surface. There are probably two antagonistic forces tending to orient these dipoles in different ways on the surface. A single, isolated dipole would probably stand vertically on the surface on account of the symmetry of the field of force of the water molecules. But when a large number of these dipoles are packed close together, as in a surface film, their mutual interaction will tend to make the dipoles lie end to end with their axes parallel to the surface. The Van der Waals attraction between the non-polar parts of the molecules will tend to pack the whole molecules side by side vertically, and thermal agitation tends to destroy any orientation. It is doubtful, however, if thermal agitation is an important factor in the case of these very heavy sterol molecules; evidence has been found in certain cases that change of temperature does not appreciably influence the structure of the films.

A change in tilt without shift in the position of the water-attracting group and without change in its nature, may therefore be achieved by so altering the stereochemical configuration of the skeleton that the angle of tilt of the hydroxyl group to the rest of the molecule is changed. Even without changing the arrangement of the carbon atoms, in a saturated ring a hydroxyl group can alter its angle to the ring by 109.5° by simply changing places with the hydrogen on the same carbon atom. We think it likely that the greater part of the change in the form of the curves observed with the successive irradiation products of ergosterol is due to quite simple changes of this nature in the molecule. The changes in the value of μ indicate some considerable change in the orientation of the hydroxyl group to the ring system during irradiation. The non-precipitation of the irradiation products by digitonin is usually ascribed to such epimerisation of the hydroxyl group. The changes in the case of oxidation of the films also indicate that the double bonds change in position; in pyrocalciferol and in lumisterol they appear to be much more accessible to the water than in the other compounds. It would be impossible to say at present, however, from the results on surface films, precisely what are the changes in position undergone by the double bonds. Stereochemical changes in ring I may be caused, in part, by movements of the double bonds in ring II.

An interesting, if minor, point is the effect of the dinitrobenzoyl group of the esters on the films. This group does not increase the area of the molecules on dilute hydrochloric acid, but very greatly increases it on permanganate, the initial stage of oxidation. The fairly large dinitrobenzoyl group must therefore continue the general direction of the ring system. This is nearly vertical on the non-oxidising solutions, but at a considerable angle of tilt (though probably not horizontal) when the sterol part of the molecule is tilted on the oxidising solutions. The link between the sterol and the esterifying dinitrobenzoyl group cannot be very flexible, unless the dinitrobenzoyl group is itself oxidised.

SUMMARY.

Surface pressure and surface potential measurements on unimolecular films of ergosterol and its irradiation products, and other derivatives, show considerable changes in tilt of the molecules in the films as irradiation proceeds. It is probable that stereochemical changes occur in the carbon skeleton, resulting in changes in the angle of tilt of the hydroxyl group to the ring system during irradiation. The results also afford evidence that the position of the double bonds changes during irradiation.

The results with ergosterol favour position 3 for the hydroxyl group.

One of us (J. F. D.) thanks the Department of Scientific and Industrial Research, and the Surrey County Council, for personal grants held during the progress of this work; we wish again to thank those who have so kindly supplied the material for this work. Part of the cost of the apparatus was met by a grant from the Royal Society.

REFERENCES.

- Adam (1930). The physics and chemistry of surfaces, pp. 37–42.
— (1933). *Trans. Faraday Soc.* **29**, 91.
— and Harding (1932). *Proc. Roy. Soc. Lond. A* **138**, 411.
— (1933). *Trans. Faraday Soc.* **29**, 837.
— and Jessop (1926, 1). *Proc. Roy. Soc. Lond. A* **110**, 423.
— (1926, 2). *Proc. Roy. Soc. Lond. A* **112**, 371.
— and Rosenheim (1929). *Proc. Roy. Soc. Lond. A* **125**, 25.
Fosbinder (1933). *Proc. Roy. Soc. Lond. A* **139**, 93.
Frumkin (1925). *Z. physikal. Chem.* **116**, 486.
Guyot (1924). *Ann. Physique*, **10**, 506.
Heilbron, Samant and Simpson (1933). *J. Chem. Soc.* 1410.
Hughes and Rideal (1933). *Proc. Roy. Soc. Lond. A* **140**, 253.
Rosenheim and Adam (1929). *Proc. Roy. Soc. Lond. B* **105**, 422.
— and King (1934). *J. Soc. Chem. Ind.* **53**, 196.
Schulman and Rideal (1931). *Proc. Roy. Soc. Lond. A* **130**, 259.
Windaus, Inhoffen and v. Reichel (1934). *Liebig's Ann.* **510**, 248.
Zocher and Stiebel (1930). *Z. physikal. Chem.* **147 A**, 401.