CXLVI. SURFACE FILMS OF OESTRIN DERIVATIVES.

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INTRODUCTION.

THIS paper describes the results of surface pressure and surface potential measurements on various derivatives of oestrin, spread in the form of uni-molecular films on water surfaces. The work was undertaken in the hope of throwing light on the constitution of the molecules, as well as for the intrinsic interest attaching to the surface properties of substances of such biochemical importance as the oestrous hormone.

The recent work of Butenandt, Marrian, Doisy, and their collaborators [*cf.* Marrian, 1932] has shown that at least two oestrus-producing substances are present in the urine of pregnant women, namely (a) trihydroxyoestrin $C_{18}H_{21}(OH)_3$ and (b) ketohydroxyoestrin $C_{17}H_{21}CO(OH)$. The former compound contains two hydroxyl groups one of which is phenolic; the latter one phenolic hydroxyl and one ketonic group. The fact that trihydroxyoestrin may be converted into ketohydroxyoestrin by distillation with sodium bisulphate clearly shows the close chemical relation between the two compounds and suggests that the two alcoholic hydroxyl groups in the former compound, from which water is eliminated to form the hydroxyketone, are attached to adjacent carbon atoms.

Little so far is known with certainty concerning the constitution of these compounds. It is evident that at least one benzene nucleus is present, while the work of Doisy and his colleagues [Thayer, Levin and Doisy, 1931, 1, 2] suggests the presence of one double bond which reacts readily with halogens. Butenandt and co-workers [1932] have recently discussed the question of the presence or absence of a reactive double bond. They suggest tentatively that an "enolic" double bond is present in the molecule in addition to the three double linkages in the benzene ring.

If it is assumed that a double bond is present, then it is probable that the carbon skeleton is a substituted partially reduced three benzene ring system.

Such a compound might belong to the group of (a) diphenylbenzene, (b) phenanthrene or anthracene, (c) phenylnaphthalene. If the double bond were not present these structures would still be possible if a bridged ring were present.

With the assumption that no double bond or bridged ring is present a condensed four benzene ring structure seems most probable. Another possibility is a structure based on the Windaus-Wieland formula for sterols. The well known facts that, during pregnancy, there is some disturbance of sterol metabolism, and that the sterol-like compound, pregnandiol, is present together with the hormone in the urine of pregnant women, might suggest a connection between oestrin and the sterols. On the basis of Rosenheim and King's [1932] recently suggested chrysene structure for sterols, the condensed four ring structures are again supported. In this connection, however, if the chrysene suggestion proves to be incorrect, other four ringed structures containing one or more five membered rings are possible. Certain of these skeletons appear less probable on consideration of the chemical evidence. The diphenylbenzenes and phenylnaphthalenes ought easily to yield aromatic acids of lower molecular weight on fusion with potash, but in numerous experiments by two of us (G. A. D. H. and G. F. M.) no indication of such products has been found in brief fusions. The main product of potash fusion has been found to be (unpublished experiments by the above authors) an eighteen carbon atom dicarboxylic acid. Since this is extremely stable to potash fusion it is more probable that a condensed ring structure is present. It will be seen that the surface film results are in accordance with this view, as also are the crystallographic measurements of which Bernal [1932] has given a preliminary account.

Butenandt and his collaborators [1932] have come to the conclusion that a phenanthrene structure is probable. While the findings in this paper provide support for this view, the possibility of four ring structures is equally probable.

EXPERIMENTAL.

The surface pressure (the outward force on a barrier in the surface dividing the film-covered surface from a clean water surface) was measured by the apparatus of Adam and Jessop [1926, 1] and Adam [1930, p. 37]. The substances were weighed out in small stoppered bottles, in quantities of 1-2 mg.; sufficient alcohol was added to dissolve the substance, and the solution was diluted to about 2 cc. with benzene. This solution was dropped on to the surface from a calibrated dropping pipette, employing the corrections for evaporation of the solvent described by Adam and Jessop [1926, 2]. The error in the measurement of the amount put on the surface is estimated as of the order of 3 %.

The films were examined for collapse by the method of Zocher and Stiebel [1930], in which a cardioid condenser is inserted through the bottom of the trough, giving intense dark-ground illumination of the surface. Collapsed aggregates show up in this way as bright spots on a dark ground, whereas perfect unimolecular films show nothing.

The change in the electrical potential at the air-liquid surface was measured by a modification of the method recently published by Schulman and Rideal [1931] an account of which will be published shortly. We are greatly indebted to Mr J. B. Harding for assistance in making the surface potential measurements.

EXPERIMENTAL RESULTS.

Trihydroxyoestrin, and ketohydroxyoestrin, also the acetyl derivative and the oxime of the latter, dissolved too rapidly in the water for any measurements to be made on the films. The methyl ether of the ketone and the trimethyl ether of trihydroxyoestrin formed unstable, rapidly collapsing films, on which no measurements could be made. The triacetyl derivative and the diacetyl derivative of the methyl ether of the trihydroxyoestrin, in which the two alcoholic hydroxyls are acetylated and the phenolic hydroxyl is methylated, were, however, found to give films stable up to about 7 dynes per cm., and collapse was slow even at higher pressures, so that fairly accurate measurements could be taken above 10 dynes. The results are shown in Figs. 1 and 2.

Fig. 1.

Fig. 2.

Both substances give, at large areas, gaseous films with only moderately large corrections to the perfect gas laws. On compression to about 100 sq. Å., per molecule, a sharp break occurs in the surface pressure area curves, which become nearly, but not quite, horizontal; there is only a slight increase in pressure down to 35 sq. Å. These breaks occur at 81 sq. Å. with the triacetyl compound, and at 109 sq. Å. with the diacetyl methyl ether. The surface pressures at the beginning of this transition region are about 3.6 and 1.5 dynes per cm. respectively; the curves were taken on distilled water and on *N*/100 potassium chloride solution. At the left-hand end of the transition region, the pressure rises sharply, giving a typical curve of a condensed film of the "close-packed head" type, which tends to an area 32.5 sq. Å. at no compression, with a probable error of about 1 sq. Å., for both substances.

These curves resemble, at first sight, the curves for aliphatic substances which give both gaseous and condensed films, but there is a very important difference; in the present case temperature has no detectable influence on the surface pressure at which the transition from gaseous to condensed film takes place, whereas with long-chain substances the surface pressure in the transition region rises steadily with temperature. The change in surface pressure with temperature is less than 0.05 dyne, per 10°, from 0° to 45°.

Fig. 2 shows the changes in surface potential ΔV , due to the film, and also the values of μ , calculated from Helmholtz's equation

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

n being the number of molecules per sq. cm., and μ the average vertical component of the change in dipole moment of the part of the surface occupied by a molecule of the film, including the dipole moment of the film molecules themselves, and all effects due to redistribution of water molecules and of ions in the underlying solution. The breaks at the points where the gaseous films begin to change into the condensed films are clearly marked on the surface potential curves, at exactly the same areas as on the surface pressure curves. At large areas, although naturally ΔV decreases as the number of molecules in the film becomes smaller, μ increases and becomes practically constant, at 9×10^{-19} e.s.u. per molecule for the triacetyl compound, and 7.5×10^{-19} e.s.u. for the diacetyl methyl ether. There is a slight decrease in the gaseous film, as the area is reduced, before the discontinuity is reached; at smaller areas the values of μ decrease more rapidly, converging in the case of both compounds to a value about 4×10^{-19} e.s.u. for the condensed film. The surfaces were carefully searched at all areas to see if the surface consisted of two surface phases, the presence of which would be indicated by large fluctuations in ΔV . No fluctuations greater than the experimental error were found, except in the condensed films at pressures where the films were obviously collapsing.

Ultramicroscopic examination disclosed no sign of collapse of the films above about 35 sq. Å.; at smaller areas typical collapse aggregates were seen, forming slowly unless the pressure was above 7 dynes per cm.

The remaining curves in Fig. 1 are inserted for comparison, and include the surface pressure area curves for long-chain phenols [Adam, 1923], cholesterol and ergosterol [Adam and Danielli, unpublished], long-chain *cyclohexanols* obtained by reduction of the phenols, and the dihydroxylic sterol pregnandiol recently isolated by Marrian [1929] and identified by Butenandt [1931], and for fatty acids on distilled water and other compounds giving the typical curve of closely packed long chains.

The *p*-dodecyl- and hexadecyl-*cyclohexanols* were specially prepared for this work, in order to obtain an idea of the size of the *cyclohexane* ring, standing upright and closely packed in the surface. The unreduced benzene ring had earlier been shown to pack to an area of 24 sq. Å. [Adam, 1923]; it was probable that the fully reduced *cyclohexane* ring would occupy a good deal more space,

partly because of the bulk of the additional hydrogen atoms, and partly because it is a "puckered," not a plane ring. Both the dodecyl- and hexadecyl-*cyclohexanols* formed condensed films of the "close-packed heads" type, having an area of about 30 sq. Å. at no compression, and being only slightly compressible. The cross section of the oestrin molecule, as packed in the condensed films, is thus about 8 % larger than that of the *cyclohexane* group. As the temperature was raised, dodecyl*cyclohexanol* appeared to pass into a liquid expanded film, having an area only slightly greater than that of the condensed film, about 35 sq. Å. at no compression. The investigation of this change was difficult, on account of the unusually small difference in area between the condensed and expanded films; the half expansion temperature under 1.5 dynes compression was $28^{\circ} \pm 5^{\circ}$. At high temperatures, the film of hexadecyl*cyclohexanol* collapsed too rapidly for any expansion to be observed.

Pregnandiol gives a gaseous film at all pressures; the oestrin films resemble those of the *cyclohexanol* derivatives much more closely than any of the others.

DISCUSSION.

There can be little doubt that the molecules are lying flat, and moving about in the surface more or less independently in the gaseous regions of the films, *i.e.* at areas over 110 sq. Å. per molecule. Previous work has indicated that the molecules are always lying flat in the gaseous state of the films at large areas, though some degree of tilt may be caused by lateral compression. It is equally clear that the molecules are standing on end, closely packed, in the condensed regions of the curves, and therefore the cross-sectional area of the oestrin molecules, in their closest packing, is about 32.5 sq. Å., decidedly less than that of the sterols, and slightly greater than that of *cyclohexanol*.

It is also certain that the vertical component of the electric moment per molecule is greater when the molecules are lying flat than when they are upright. This must indicate that the direction of the resultant dipole of the various polar groups in the molecule is inclined at a sharp angle to the plane of the molecule as a whole. In all probability this means that the two hydroxyl groups which have an alcoholic character are side by side, on the same side of the ring —*i.e.* they are in *cis*, not *trans*, relation to each other. If they were on opposite sides of the ring, they would neutralise one another. Further, since the dipole component perpendicular to the surface decreases very greatly as the molecules stand upright, it appears that these two hydroxyl groups are arranged with the bond between hydrogen and oxygen, or between oxygen and carbon, at a sharp angle to the plane of the ring.

The transition regions between the condensed and fully gaseous regions of the films indicate varying degrees of tilt in the molecules as the lateral compression is increased. The fact that the vertical dipole component decreases appreciably at areas greater than those corresponding with the discontinuities at 81 and 109 sq. Å., indicates that there is some tilting of the molecules away from the flat position before these areas are reached. There is, however, at the

areas where the discontinuities occur, a sudden decrease in the stability of the molecules lying flat; a comparatively slight increase in lateral compression has a much greater tilting effect here than at greater areas.

In interpreting the results of surface potential measurements, it must be remembered that μ is not simply the vertical component of the dipole moment of the film molecules, but includes terms of unknown amounts due to redistribution of ions and re-orientation of water molecules, as Schulman and Rideal [1931] have pointed out. We are not in entire agreement with these authors as to the method of eliminating these secondary effects, and the subject will be discussed by Harding and one of us in another paper; in fact we do not see at the moment any satisfactory way of disentangling the contributions to μ made by the dipole moment of the film molecules themselves and by the re-orientation of the ions and water molecules. This difficulty does not appear, however, to invalidate the above arguments as to the orientation of the dipoles in the molecules; for it is clear that, since the re-orientation of water and redistribution of ions are secondary effects consequent on the introduction of the dipoles of the film molecules at the surface, the total value of μ , including the secondary effects, will increase as the vertical component of the dipole moment of the molecule itself increases.

Confirmatory evidence of the view that the molecules are lying flat in the gaseous state and are tilted at smaller areas, is found in the fact that the pressure required to bring about the transition from gaseous to condensed film is greater with the triacetyl compound than with the diacetyl methyl ether. It is known that acetyl groups have a stronger adhesion to water than methyl ether groups; the acetates of long-chain alcohols form stable films, while the methyl ethers do not [Adam and Dyer, 1924]. The second anchorage, that of the phenolic hydroxyl, would therefore be expected to leave the water surface more easily when methylated than when acetylated. Such a re-orientation as that described above would require the phenolic polar group to leave the water surface. A re-orientation of molecules lying flat, under lateral compression, so that one of two anchorages to the water is broken, is not unknown; it has already been found with several of the long-chain dibasic acids and esters, which have two polar groups, one at each end of the molecule [Adam and Jessop, 1926, 2].

It is clear, since the molecules so easily assume the flat position, that the phenolic hydroxyl is situated near the opposite end of the molecule from the two alcoholic hydroxyls. Butenandt *et al.* [1932] favour the structure (1) or (2). These two structures seem quite impossible when the evidence of surface films is considered, since both (1) and (2) at their areas of closest packing would have a limiting area of about 70 sq. Å. instead of the 32.5 actually found. This small area of 32.5 sq. Å. could only be given by such a structure as (3), with the adjacent alcoholic hydroxyls well to the end of the molecule.

The absence of any effect of temperature on the surface pressure necessary to change the gaseous into the condensed film may perhaps mean that the

molecule is not flexible. One of us has advanced the theory [Adam, 1930, p. 75] that the change from the condensed to the expanded state, in long-chain aliphatic compounds, is due to whip-like oscillations in the flexible hydrocarbon chains. In all cases, where expansion (to either gaseous, vapour expanded, or liquid expanded films) takes place in the aliphatic series, there is a

(1)

(2)

(3)

(4)

(5)

(6)

large temperature coefficient of the pressure in the transition region. The possibility of oscillations in the chains, becoming more violent as the temperature rises, naturally renders the condensed state less stable at higher temperatures. In the case of the oestrin derivatives, we have a change apparently very similar to the expansion of condensed films of aliphatic substances, but without this temperature coefficient. The most probable explanation is that the molecules are not flexible.

Evidence as to the constitution of the molecules.

Skeletons of the phenanthrene, anthracene, naphthacene, chrysene and 2 : 3-benzphenanthrene (4) types among the non-flexible structures appear admissible on the grounds of the area occupied when standing upright. The steroid skeleton of Windaus and Wieland (6) occupies about 36 sq. Å. as a minimum, as is indicated by models, and is therefore excluded. 3 : 4-Benzphenanthrene (5) is probably also too large for an area of 32.5 sq. Å. The structures of a phenanthrene type, in which the lowest ring is standing on one corner, with one of the upper ones slightly to one side, would naturally occupy an area slightly larger than that of the alkyl *cyclohexanols*; also the anthracene and naphthacene skeletons, in which the rings are all in one line, but the lowest one is lying with a side, instead of a corner, on the water, would be somewhat larger than the *cyclohexanols*. The inflexibility probably excludes the diphenylbenzene and phenylnaphthalene structures.

The ease with which the molecules lie flat, or stand up, as the lateral compression is changed, indicates that the phenolic hydroxyl is in a remote part

of the molecules from the two alcoholic hydroxyls. Both sets of hydroxyl groups are probably situated near the ends of the molecules. The evidence that the alcoholic hydroxyls are probably in the *cis* position, and inclined at a steep angle to the plane of the rings, has been detailed above.

PREPARATION OF MATERIAL.

Ketohydroxyoestrin monoacetate. Ketohydroxyoestrin prepared by dehydration of trihydroxyoestrin was heated to 100° for 3 hours with excess of acetic anhydride; excess of water was then added, and the precipitated acetate filtered, washed and dried. It was decolorised by boiling with charcoal in alcoholic solution, and finally recrystallised twice from aqueous methyl alcohol; m.p. 118–121°.

Trihydroxyoestrin monomethyl ether diacetate. Trihydroxyoestrin monomethyl ether was made by treating trihydroxyoestrin in alkaline solution with methyl sulphate in the cold [cf. Marrian and Haslewood, 1932]. 30.9 mg. of the methyl ether were heated with 5 cc. acetic anhydride at 100° for 3 hours; excess water was added, the diacetate filtered, washed and dried. It was decolorised with charcoal and crystallised once from aqueous methyl alcohol; m.p. 140–142°.

Trihydroxyoestrin triacetate. Trihydroxyoestrin was heated with excess of acetic anhydride for 3 hours at 100°; excess of water was added, and the acetate filtered off, washed and dried. The product was crystallised twice from aqueous ethyl alcohol; m.p. 119–121°.

p-Dodecylcyclohexanol and *p*-hexadecylcyclohexanol were obtained by reducing the corresponding phenols [cf. Adam, 1923] in alcoholic solution with hydrogen, under about 2½ atmospheres pressure, using the platinum oxide catalyst of Adams *et al.* [1928]. The products were recrystallised from alcohol.

Dodecylcyclohexanol. m.p. 50–55° (phenol melts at 66°). Iodine value 0. (Found: C, 80.4; H, 13.2 %; calc.: C, 80.6; H, 13.4 %.)

Hexadecylcyclohexanol. m.p. 65–66° (phenol melts at 79°). Iodine value 10. (Found: C, 82.3; H, 13.0 %; calc.: C, 81.6; H, 13.6 %.) The hexadecyl compound may not have been quite fully reduced.

SUMMARY.

Surface pressure and surface potential measurements on diacetyltrihydroxyoestrin methyl ether, and triacetyltrihydroxyoestrin, show that these compounds can orient themselves at a water-air interface, either flat, or perpendicularly to the surface. The vertical component of the change in dipole moment of the surface, per molecule, due to the film, is much greater when the molecules are lying flat than when they are upright. The conclusion is drawn that the molecules probably have an anthracene or phenanthrene skeleton, or a system of four condensed rings, with two alcoholic hydroxyl groups near one end of the molecule, and one phenolic group near the other end. The

alcoholic groups are probably in the *cis* position relative to each other, on adjacent carbon atoms, and are inclined at a steep angle to the plane of the ring in which they are situated.

Cyclohexanols with long chains in the *para*-position to the hydroxyl group give condensed films of area about 30 sq. Å. per molecule, slightly smaller than the cross section of the oestrin molecules.

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Note added August 17th. Since this paper was written, Marrian and Haslewood [*Lancet*, 1932, ii, 282] have shown that no double linkage other than those in the benzene nucleus is present in the oestrin molecule. This indicates that the molecule must have a four-ringed structure. Butenandt (*Nature*, August 13th) has now advanced similar views.