381. *Monolayers of Porphyrins and Related Compounds.*

By A. E. Alexander.

A number of porphyrins and closely related compounds, some containing a central co-ordinated metal atom (chlorophyll-*a* and -*b*, hæmin, magnesium naphthalocyanine, magnesium and iron phthalocyanines), and some without (protoporphyrin, bilirubin, mesoporphyrin dimethyl ester, and hæmatoporphyrin), have been examined in the form of monolayers on aqueous substrates. With the exception of the chlorophylls and hæmatoporphyrin, the compounds formed very unstable solid-condensed films with the conjugated ring systems vertically orientated and close-packed. Owing to the phytol side chains, the chlorophylls gave liquid-expanded films which could be condensed by increase of pressure to the close-packed form with the dihydroporphin rings vertically orientated. The hæmatoporphyrin molecule was unique in that it lay flat on the surface and formed a vapour-expanded type of film.

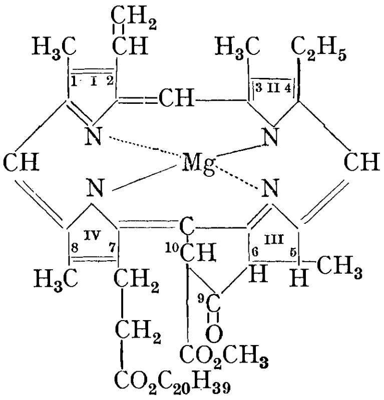
Solutions of all these compounds were intensely coloured, and with the metallic phthalocyanines, particularly magnesium naphthalocyanine, the colour was still visible in the monolayer.

Owing to the biological importance of substances containing the porphin and other similar ring structures, it seemed desirable to examine the nature and properties of such compounds when orientated as monolayers on aqueous substrates. Those examined were: chlorophyll (*a* and *b*), hæmin, magnesium naphthalocyanine, magnesium and iron phthalocyanines, protoporphyrin, bilirubin, mesoporphyrin dimethyl ester, and hæmatoporphyrin. With the exception of bilirubin, which is an open-chain compound, they all possess a fully conjugated ring system of the porphin or closely related phthalocyanine type, the last four differing from the others in having no co-ordinated metal atom at the centre of this system. The metal-containing compounds were originally investigated by Hughes (*Proc. Roy. Soc.*, *A*, 1936, **155**, 710).

Experimental.

The films were examined by the usual methods for surface pressures and surface potentials, and in addition by the dark-field ultramicroscopic method of Zocher and Stiebel (*Z. physikal. Chem.*, 1930, *A*, **147**, 401) in cases of doubtful homogeneity.

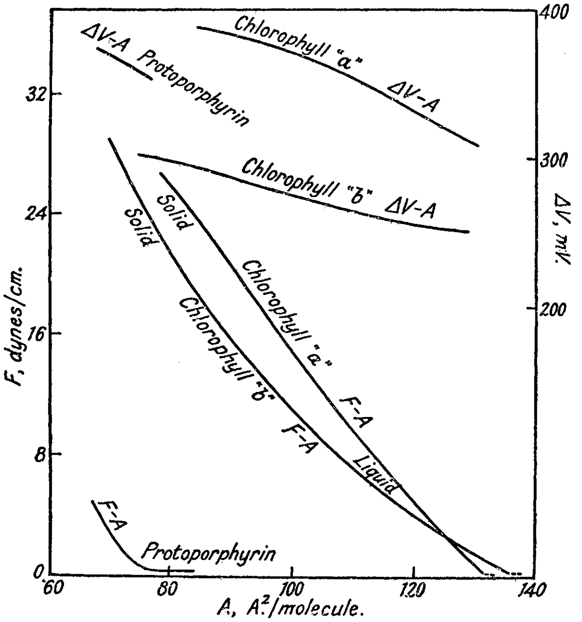
Chlorophyll-a *and* -b.—These gave the most stable films of all the compounds examined. The *F–A* and ΔV–*A* curves for films on a phosphate buffer of p_H 7·3 at room temperature are shown in the figure. Both forms gave easily compressible liquid-expanded films, differing but slightly in the limiting area (132 and 137 A.^{2} respectively). The areas since found by Sjoerdsma (*Nature*, 1936, **138**, 405) and by Hanson (*Proc. K. Akad. Wetensch. Amsterdam*, 1937, **40**, 281) agree well with these. The surface potentials were rather different, the range being 305—385 mv. for the *a* form and 250—300 mv. for the *b*. Since the latter contains one oxygen atom more than the former (and therefore an extra polar group), the lower moment found for the *b*-form would indicate that the position of this extra polar group is such that its moment acts in the opposite sense to the other polar groups in the molecule. In the formulæ proposed by Fischer (*Chem. Rev.*, 1937, **20**, 41) the *b*- differs from the *a*-form only in that the methyl group in position 3 has been replaced by a formyl group. Since it is extremely probable that the molecules are orientated in the surface with the ketonic and the two ester groups as the main anchorage, the moment of the formyl group which is attached to the opposite side of the molecule would act in opposition to these, thus reducing the apparent moment. These results from the films are thus seen to be in accord with Fischer's proposed structures (*loc. cit.*).



Chlorophyll-*a*.

The difference between these liquid-expanded films and the condensed films given by the other porphin derivatives must be due to the phytol side chains, which would tend to lie on the surface and so expand the film. On compression, these phytol chains would be removed from the surface, and the change from liquid to solid at areas of about 90—80 A.^{2} would indicate the formation of a close-packed layer of vertically orientated dihydroporphin rings. Evidence for this type of packing is available from a study of films of hæmin and protoporphyrin (see below).

On alkaline solutions the films were stable, but for those of $p_H < 6$ a sharp rise in the surface potential occurred, probably owing to the elimination of magnesium from the molecule. A slow fall in potential of some 50—80 mv. took place with the *b*-form over about 20 minutes. This fall was arrested by the presence of 0·5% of quinol in the substrate, suggesting that it was due to autoxidation.



Examination of the films at large areas by means of the dark-field ultramicroscope showed them to be homogeneous. On compression to the solid state, break lines appeared in the film with no previous point-structure and no fluorescence (cf. the phthalocyanines below).

Hæmin.—The solution for spreading purposes was made up in benzene–pyridine (*ca.* 4 : 1 by vol.). On a phosphate buffer, p_H 7·3, at room temperature, the films obtained were solid and unstable, collapsing very readily on compression at an area of about 70 A.^{2}. If the hæmin rings were lying flat on the surface, the area per molecule would be approx. 125 A.^{2}, and if vertically close-packed, about 68 A.^{2}. The proximity of the limiting area to the latter figure showed that the molecules were close-packed vertically, owing to the much stronger hydrophilic nature of the carboxyl than of the vinyl groups. The surface potentials showed that the films were never really homogeneous, the value of ΔV at the limiting area being about 500 mv.

Protoporphyrin, Bilirubin, Mesoporphyrin Dimethyl Ester, and Hæmatoporphyrin.—Solutions were made up by warming the solid with pyridine (*ca.* 1 c.c./mg.) and then diluting with benzene to a suitable volume: unless this procedure was followed, there was a tendency for the solid to be precipitated on standing. A preliminary examination with the dark-field ultramicroscope showed that spreading occurred, but that the films collapsed very readily on compression.

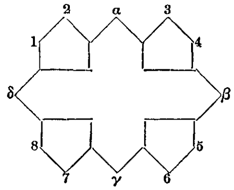
Protoporphyrin (*i.e.*, hæmin minus FeCl) gave the most stable films of these four compounds, although even in this case there was always a quite rapid collapse in the condensed state. The figure gives the *F–A* and ΔV–*A* curves for films on N/100-sulphuric acid at 20°. The films were inhomogeneous at large areas, but formed homogeneous films of the solid-condensed type at areas less than about 76 A.^{2}. With increasing p_H the surface potential decreased owing to ionisation of the carboxyl groups. A comparison of the surface potentials of this compound and of hæmin at the same area showed that the latter was the higher by some 350 mv. both at p_H 2 and at p_H 7·8, which must be due to the central co-ordinated metal atom. The area for the solid-condensed films showed that the molecules were orientated vertically in a similar manner to those of hæmin. On these substrates there was no evidence for the molecules ever lying flat—the cohesion between the molecules evidently being greater than the adhesion to the water. It was thought that by spreading on bromine-water or permanganate solutions the adhesion of the vinyl groups would be increased sufficiently to indicate this second limiting area. No effect was observed with bromine-water, but when N/100-sulphuric acid containing 0·5% of potassium permanganate was used as substrate and the film allowed to remain spread at large areas for about 15 minutes, a pronounced effect was observed on the film characteristics. The first definite sign of surface pressure occurred at about 120 A.^{2}, and on compression the film condensed to a liquid and not a solid film as before. This, in conjunction with the results for hæmatoporphyrin given below, suggests that on permanganate solutions at any rate the molecules do lie flat at the largest areas.

Bilirubin differs from the other compounds in not containing the complete porphin nucleus, since one of the $=CH-$links has been removed. It formed extremely unstable films of the weak solid or viscous liquid type. On N/100-sulphuric acid at room temperature, the limiting area (*i.e.*, the area at which the first sign of pressure was noted) was about 140 A.^{2} at a surface potential of approximately 145 mv. The fission of the porphyrin nucleus would explain the much greater area occupied as compared with the other compounds.

Mesoporphyrin dimethyl ester resembled protoporphyrin closely, giving a solid-condensed film of limiting area about 75 A.^{2}, and a surface potential of about 425 mv. (at p_H 2), although the films were much less stable owing to the less polar nature of the molecule.

Hæmatoporphyrin only differs from protoporphyrin in possessing secondary alcoholic groups $-CH(OH)\cdot CH_3$ instead of the vinyl groups $-CH=CH_2$. This would increase the polarity of the molecule considerably, and owing to the more symmetrical distribution of the polar groups around the porphin nucleus as compared with protoporphyrin, it would be expected that the molecules would lie flat on the water surface. Actually, this was found to be so. The films were spread from an alcohol–benzene solution of the hydrochloride, and were found to be of the vapour-expanded type, remaining homogeneous out to areas of over 400 A.^{2} (p_H 2 and 7·8). Compression gave a liquid film, as would be expected from the presence of the hydroxyl groups. The films were very readily compressible, but this was due in part to an appreciable dissolution of the film, and owing to this effect it was impossible to determine the limiting area in the condensed state.

Magnesium Naphthalocyanine, Magnesium and Iron Phthalocyanines.—The solutions for spreading were made up in pyridine–benzene as above. The films given by these phthalocyanines were even less stable than those of hæmin, since these compounds contain no polar side chains at all. All formed very weak solid-condensed films, which even at the lowest compressions were never really homogeneous, surface-potential fluctuations of 10—20 mv. existing over different parts of the film. The *F–A* measurements gave areas at zero compression of about 50 A.^{2}, indicating that the molecules were vertically orientated as in the hæmin and protoporphyrin films, since if they were lying flat the area occupied would be at least 130 A.^{2} (Crowfoot, *Ann. Reports*, 1936, **33**, 222). One property of these films was highly remarkable, and was shown particularly well by magnesium naphthalocyanine. A film of this compound of mean thickness certainly less than 30 A. could be seen with the unaided eye to possess the greenish-blue colour of the original compound in bulk. By means of a comparative test with an alcoholic solution, it was shown that the remarkably high molecular extinction coefficient possessed by this compound was of the same order in the film as in the bulk solution.



The dark-field examination confirmed the instability of the films. All three compounds gave an unusual fluorescent point structure, the fluorescence decreasing on compression and break lines then appearing.

Summary of Results.—For simplicity, the substituents in the molecular structures are denoted by reference to the inset skeleton.

Compound.	C_2.	C_3.	C_4.	C_6.	C_7.	Type of film.
Hæmin.	$CH{:}CH_2$	CH_3	$CH{:}CH_2$	$[CH_2]_2\cdot CO_2H$	$[CH_2]_2\cdot CO_2H(+FeCl)$	Solid.
Protoporphyrin.	,,	,,	,,	,,	,,	,,
Hæmatoporphyrin.	$CH(OH)\cdot CH_3$	,,	$CH(OH)\cdot CH_3$	,,	,,	Vapour expanded.
Mesoporphyrin dimethyl ester.	$CH_2\cdot CH_3$	,,	$CH_2\cdot CH_3$	$[CH_2]_2\cdot CO_2CH_3$	$[CH_2]_2\cdot CO_2CH_3$	Solid.
Chlorophyll-*a*.	$CH{:}CH_2$	,,	$CH_2\cdot CH_3$	$CO\cdot \overset{*}{C}H\cdot CO_2CH_3$	$[CH_2]_2\cdot CO_2C_{20}H_{39}(+Mg)$	Liquid expanded.
,, -*b*.	,,	CHO	,,	,,	,, ,,	,,

* Cyclised to C_γ.

Discussion of Results.

It appears that the presence or absence of a central metallic atom in the porphin ring system has a pronounced effect on the electric moment of the molecule, but has little effect on the intermolecular attractions or on the adhesion of the molecules to an aqueous substrate, the latter being governed largely by the nature of the various side chains. Thus hæmin and protoporphyrin both give close-packed solid films differing only in electric moment. On the other hand, a striking difference is shown between these compounds and hæmatoporphyrin, in which the vinyl groups have been hydrated to $-CH(OH)\cdot CH_3$ groups. The adhesion to water has been increased sufficiently to overcome the intermolecular cohesion, so the film is vapour-expanded and forms no close-packed solid film. With mesoporphyrin dimethyl ester, where ethyl groups replace the vinyl, and the carboxyl groups have been esterified, we find once more the low adhesion to water and a high cohesion resulting in a solid film.

Examination of bilirubin shows the effect of free rotation in loss of cohesion and thus of molecular orientation.

Again, in the allied chlorophyll group it seems that removal of the metal by acids causes a change in electric moment without marked effect on the mechanical properties of the film. These properties are conditioned by the presence of the long phytol side chain, which ensures secure anchorage at an oil–water interface, at the same time permitting a large range of surface density of the molecules from $0{\cdot}75$—$1{\cdot}25 \times 10^{14}$ molecules/cm.^{2} (80—135 A.^{2}) in a liquid film, a consideration possibly of some significance in the action of chlorophyll at the surface of the chloroplasts.

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