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MONOLAYER STUDIES ON DERIVATIVES OF SPHINGANINE AND 4t-SPHINGENINE

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The pressure-area isotherms (π-A-isotherms) at temperatures between 4° and 47°C of the following derivatives of sphinganine and 4 *t*-sphingenine have been determined.

N-palmitoyl-D,L-sphingenine (I), N-palmitoyl-diacetyl-D,L-sphingenine (II), N-acetyl-sphingenine (III), N-acetyl-D,L-erythro-sphinganine (IV), N-acetyl-D,L-threo-sphinganine (V), triacetyl-D,L-erythro-sphinganine (VI), N-acetyl-3-dehydrosphingenine (VII), N-acetyl-3-dehydro-D,L-sphinganine (VIII) and diacetyl-3-dehydro-D,L-sphinganine (IX).

The phases of the monolayer films have been discussed. Compounds III, V, VI, VIII and IX form trilayers when their monolayers are compressed beyond the collapse point. The folding to a trilayer is only possible from the liquid expanded state of the monolayer. In addition the formation of trilayer films occurs only when the area of the hydrophilic group exceeds that of the alkane chain of the long chain base.

I. Introduction

The long chain amino alcohols 4*t*-sphingenine and sphinganine are the common basic structures of the naturally occuring sphingolipids, which include sphingophospholipids and sphingoglycolipids. The long chain bases are amphiphilic molecules. Their three functional groups at C–1 to C–3 resemble similarities to the glycerol moiety of the glycerophospholipids.

Stimulated by Danielli and Davson's membrane model [1] with the lipid bilayer as backbone, numerous studies on the monolayer properties of fatty acids, cholesterol, triglycerides and phospholipids have been performed with the Langmuir technique [2–7]. In comparison monolayer-studies with sphingolipids have been neglected for a long period of time. The properties of biological membranes again led Shah and Shulman [8–10] to compare the surface potential and Ca^{2+}-binding of monolayers of beef heart sphingomyelin, hydrogenated sphingomyelin and dipalmitoyllecithin. Colaccio et al. [11] measured pressure-area isotherms of synthetic dihydro-ceramide-lactoside with different fatty acyl residues. Particular care was taken regarding the influence of the hydrophobic part of the molecules on the phase properties of the monolayers. Since these measurements have been carried out discontinously below the collapse point essential information on film stability and molecular areas at the collapse point are missing.

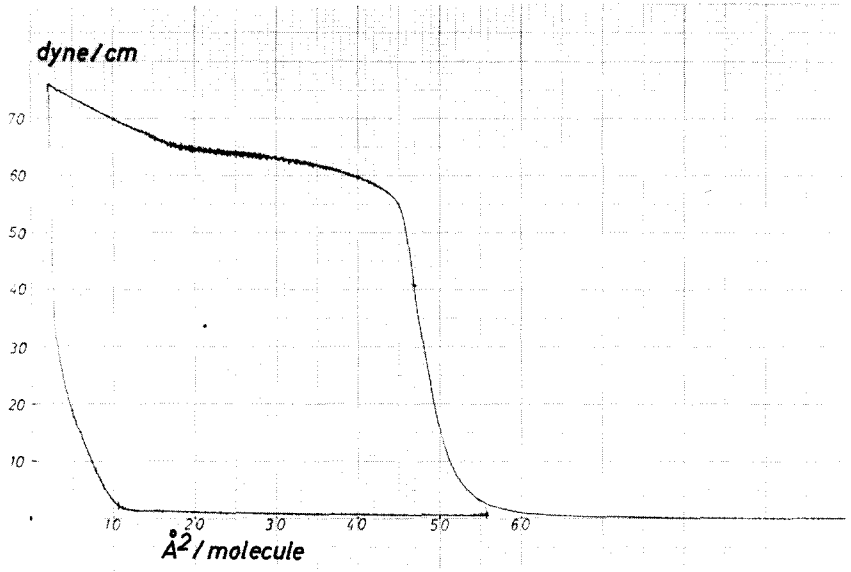

Fig. 1. 25° C π-A-isotherm of D,L-erythro-N-palmitoylsphingenine.

In our previous study [12] we described the influence of the fatty acid structures of 1,2-diglycerides, phosphatidylethanolamines and phosphatidylcholines on the phase transitions, film compressibility and compression energy. A model lipid compound which allows many chemical and stereochemical variations of the hydrophilic part of the molecule is sphinganine (dihydrosphingosine). Its derivatives presumably will form condensed states as compared to the unsaturated 4*t*-sphingenine (sphingosine) and therefore should give insight into the structural requirements of the hydrophilic group for the formation of liquid expanded and liquid condensed states of monolayers.

II. Methods

The properties of the 4*t*-sphingenine and sphinganine monolayers were studied with a surface film balance of the horizontal Langmuir-type (13). The π-A-isotherms were recorded continuously and automatically on the dyne cm^{-1} $Å^2$ $molecule^{-1}$ scale.

A teflon coated thermostated trough (700 X 150 X 6 mm) was used. The subphase was kept at constant temperature within ± 0.1°C. Benzene/methanol (9 : 1) was used as solvent for spreading the monolayer. The solutions were spread on a whiped surface of quarz distilled water. The film was compressed at a rate of 50 $Å^2$ $molecule^{-1}$ min down to 2 $Å^2$ $molecule^{-1}$ and then expanded at a rate of 70 $Å^2$ $molecule^{-1}$.

The compounds used in these studies were synthesized in this laboratory. The

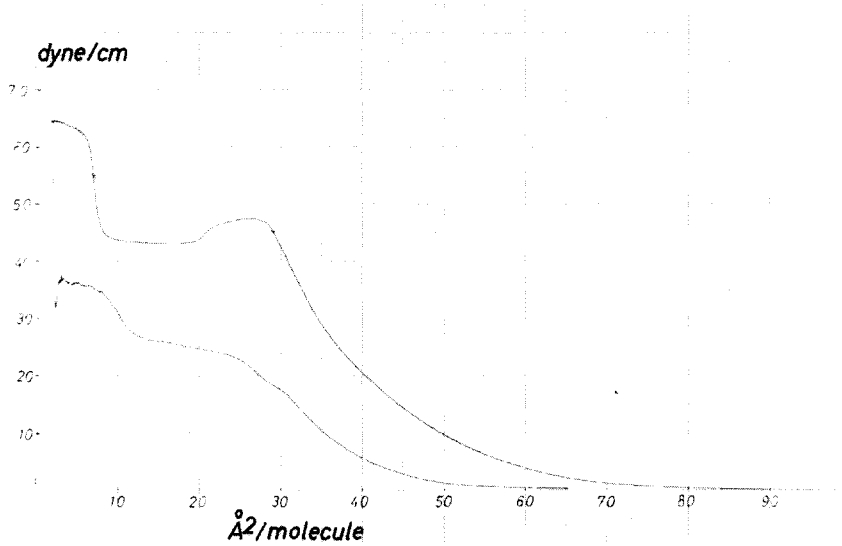

Fig. 2. 25°C π-A-isotherm of D-erythro-N-acetylsphingenine.

details of their synthesis, their analytical data (elementary analysis, mass spectra, melting points, optical rotation etc.), their physical properties and their chromatographic behaviour have been described in a previous paper [14].

III. Results

A. N-Palmitoyl-D,L-erythro-sphingenine (I)

Pressure π-A-area isotherms were recorded between 10 and 40°C. The monolayer remains in this temperature interval in a liquid condensed state. The minimum of compressibility is 0.002–0.003 cm/dyne. The film collapses at a pressure of 54–57 dyne cm^{-1} with a molecular area of 43.5 ± 1.5 $Å^2$ at the collapse point. There is no reversible expansion of the fully compressed film (fig. 1).

B. N-Palmitoyl-D,L-erythro-diacetyl-sphingenine (II)

The π-A-isotherms were recorded in this case with a compression rate of 125 $Å^2$ $molecule^{-1}$ between 10 and 40°C. Again the monolayer remains in a liquid condensed state. A minimum of compressibility can be calculated of 0.005–0.006 cm/dyne. At higher temperatures the collapse point becomes ill-defined. The collapse pressure decreases from 62 dyne cm^{-1} at 10°C to about 52 dyne cm^{-1} at 40°C. The molecular area at the collapse point ranges between 60–65 $Å^2$ $molecule^{-1}$. No reversible expansion of the completely compressed film can be achieved.

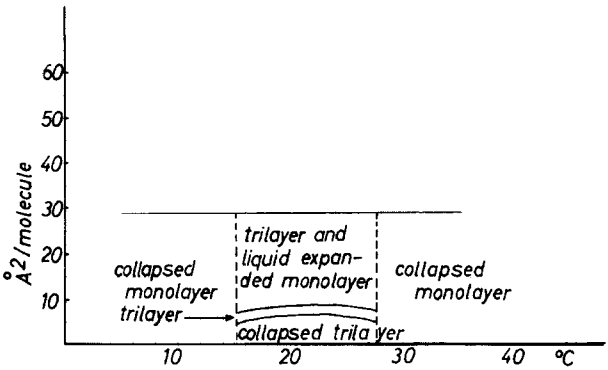

Fig. 3. Characteristic molecular areas in monolayers and trilayer of D-erythro-N-acetylsphingenine.

C. D-Erythro-N-acetylsphingenine (III)

The monolayer of this compound remains liquid expanded between 100 $Å^2$ to 2 $Å^2$ molecule^{-1} at temperatures between 5 and 35°C. The area requirement is 28 to 29 $Å^2$ molecule^{-1}. The pressure at the collapse point increases with the temperature from 40.5 dyne cm^{-1} at 5°C to 48 dyne cm^{-1} at 35°C. The monolayer does not collapse between film temperatures of 15 to 27.5°C but folds to a trilayer at a pressure of 43 to 46 dyne cm^{-1} (figs. 2 and 3).

The energy required for this folding process $F_{l\,\mathrm{exp} \rightarrow \mathrm{tril}}$ is 1430 cal mol^{-1} at 25° The trilayer is in a liquid expanded state and reaches its highest stability at 25°C. At this temperature the collapse pressure is 66 dyne cm^{-1}.

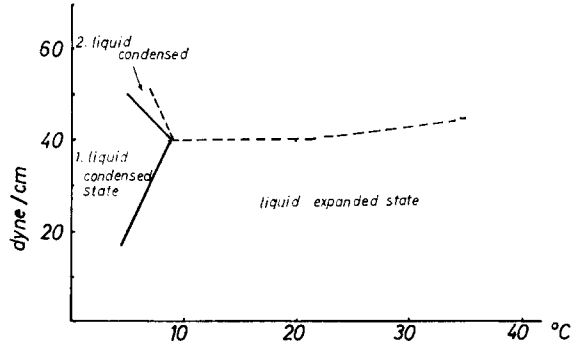

Fig. 4. π-T-phase diagram for D,L-erythro-N-acetylsphinganine. Phase transitions are shown by fully drawn lines and monolayer collapse pressures by dashed lines.

D. D,L-Erythro-N-acetyl-sphinganine (IV)

Isotherms between 5 and 35°C indicate three states of the monolayers. As visualized in the π-T-phase diagram, Fig. 4, there are two liquid condensed states below 10°C,

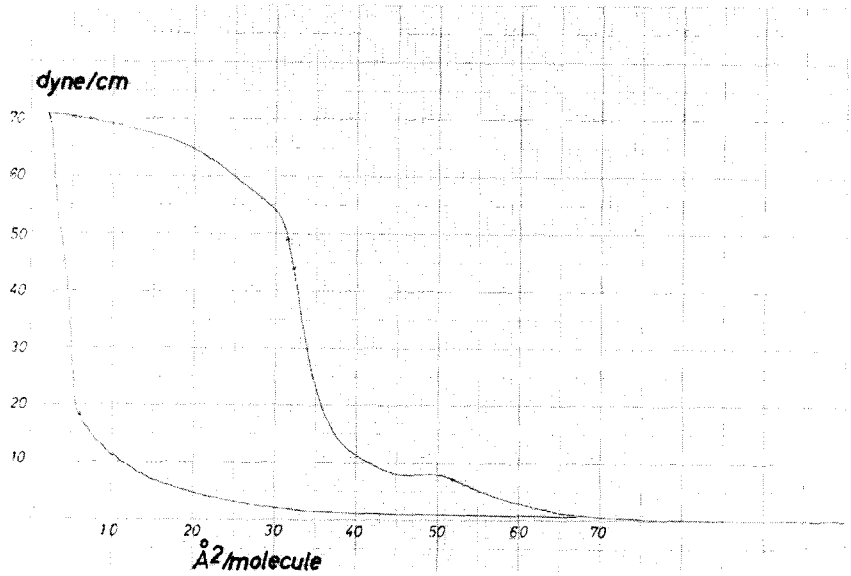

Fig. 5. π-A-isotherms of D,L-erythro-N-acetylsphinganine. (a) 3,5°C; (b) 9°C; (c) 13°C; (d) 30°C.

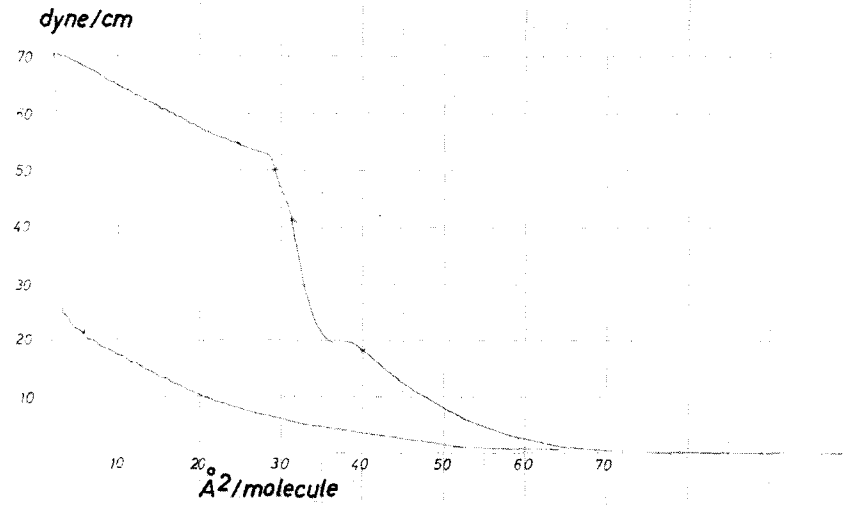

Fig. 5b.

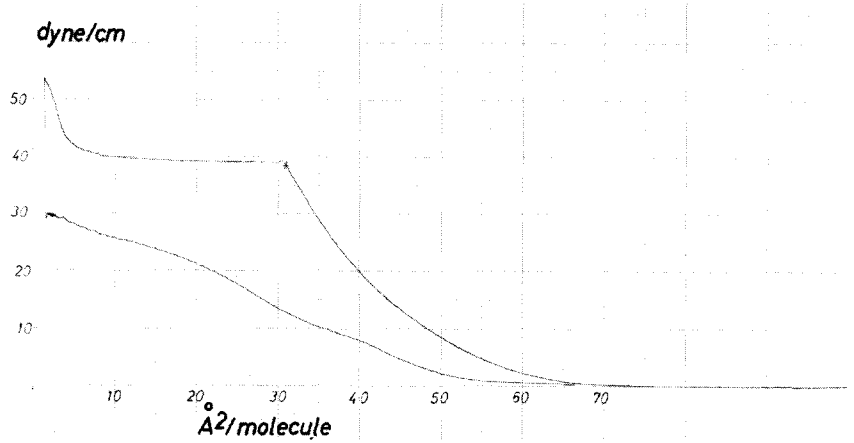

Fig. 5c.

whereas above 10°C completely liquid expanded films are formed with highest packing around 20°C. The collapse pressure at this temperature is 40 dyne cm^{-1} and a molecular area of 31 $Å^2$ is required.

E. D,L-Threo-N-acetylsphinganine (V)

Fig. 5. summarizes four isotherms at 4, 9, 13 and 30°C. The film is liquid expanded above 13°C but shows two phase transitions between 4 and 13°C (fig. 6);

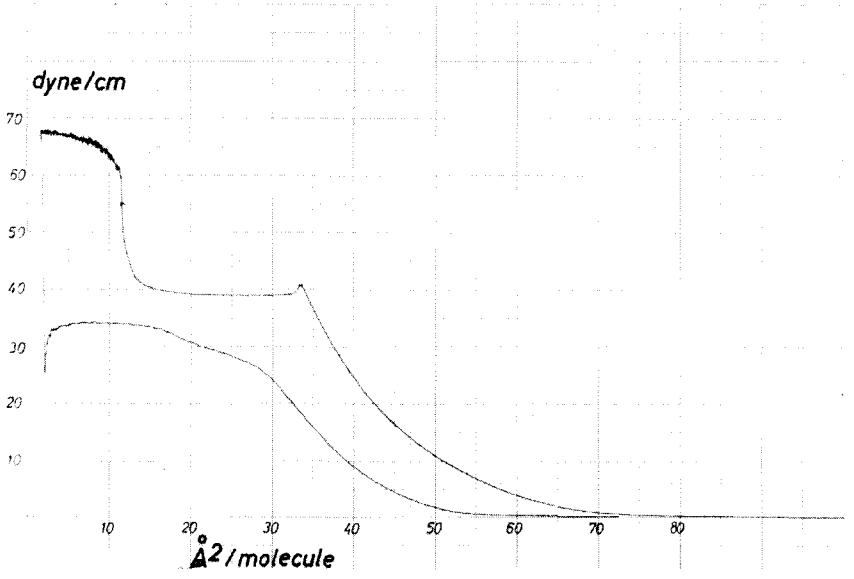

Fig. 5d.

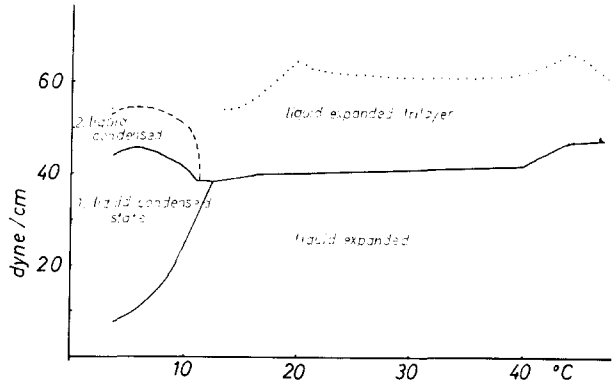

Fig. 6. π-T-phasediagram for D,L-threo-N-acetylsphinganine. Phase transitions are shown by fully drawn lines, monolayer collapse pressures by dashed lines and trilayer collapse pressures by a dotted line.

above 13°C the liquid expanded film is stable up to a pressure of 40 dyne cm^{-1} and a molecular area of 30 $Å^2$ $molecule^{-1}$ (fig. 7). Further compression folds the monolayer to a trilayer which collapses at a pressure larger than 60 dyne cm^{-1}. The energy required for this process is temperature dependent as demonstrated in table 1. The trilayer remains in the liquid expanded state and exhibits great stability and a low compressibility characteristic for liquid expanded films.

F. D,L-Erythro-triacetylsphinganine (VI)

Monolayers of this derivatives of the long chain base form liquid expanded films in the temperature range between 10 and 40°C. The collapse point is reached at

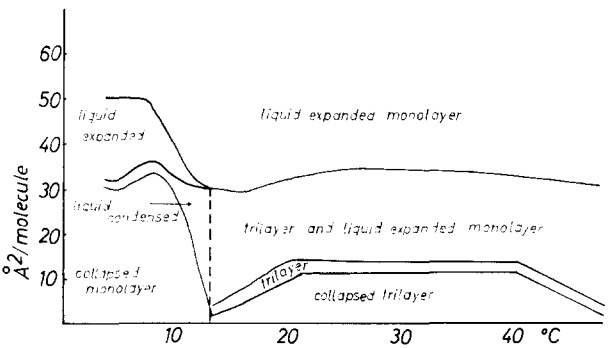

Fig. 7. Characteristic molecular areas in monolayers and trilayer of D,L-threo-N-acetylsphinganine as a function of temperature.

Table 1
Temperature dependence of transition from monolayer to trilayer structure.

T(°C)	20	25	30	35	40
$F\frac{\text{cal}}{\text{mole}}$	1150	1170	1230	1260	1300

42–44 dyne cm^{-1} and each molecule requires a surface area of 42–44 $Å^2$. At 10°C the molecular area at 42 dyne cm^{-1} pressure (collapse pressure of the film) is approximately 15 $Å^2$ molecule^{-1} (fig. 8).

G. N-Acetyl-3-oxo-sphingenine (VII)

The phase diagram (fig. 9) derived from π-A-isotherms between 4 and 45°C indicates that the monolayers of this compound above 15°C are in the liquid expanded state, below 15°C the film passes through a liquid condensed phase and collapses at a pressure of 50–51 dyne cm^{-1} (fig. 10). The molecule requires an area of 29 to 31 $Å^2$ at this collapse pressure.

H. N-Acetyl-D,L-3-oxo-sphinganine (VIII)

The films of this saturated derivative exhibit four different phase transitions and a trilayer between 30 and 40°C between 100 and 2 $Å^2$ molecule^{-1} (fig. 11 a–d).

On compression there is a phase transition from the liquid expanded to the liquid condensed state between 12 and 30°C. The collapse points of these isotherms

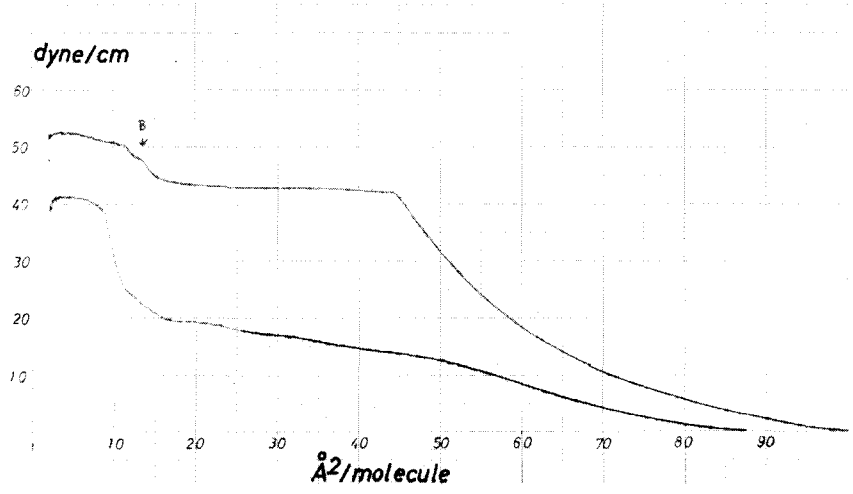

Fig. 8. 10°C π-A-isotherm of D,L-erythro-triacetylsphinganine.

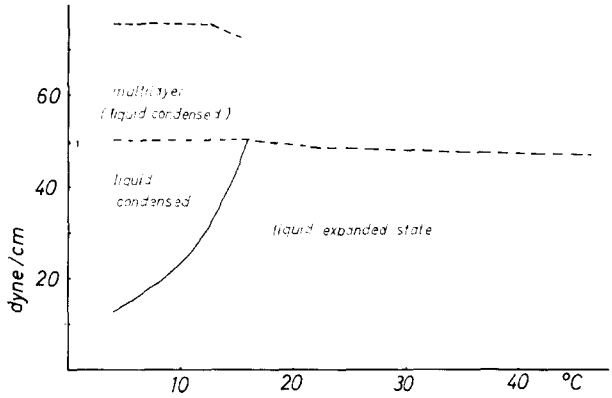

Fig. 9. π-T-phase diagram for N-acetyl-3-oxo-sphingenine. Phase transitions are shown by fully drawn lines, monolayer collapse pressures by dashed lines and multilayer top pressures by a dotted line.

are characterized by a pressure of 49–67 dyne cm^{-1} and a molecular area of 24–28 $Å^2$ as shown in the π-T-phase diagram (fig. 12a and the A-T-diagram in fig. 12b).

The monolayers at temperatures below 12°C exhibit two additional phase transitions and are very stable up to a collapse pressure of 73–74 dyne cm^{-1}. Above 30°C the liquid expanded monolayers are stable up to a pressure of 50 dyne cm^{-1} with a molecular area of 28–30 $Å^2$ but on further lateral compression are folded

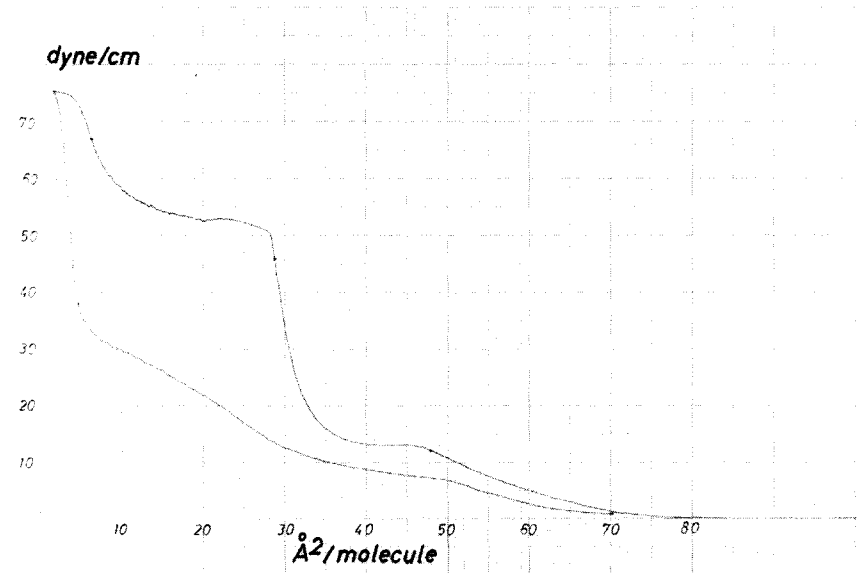

Fig. 10. 4°C π-A-isotherm of N-acetyl-3-oxo-sphingenine.

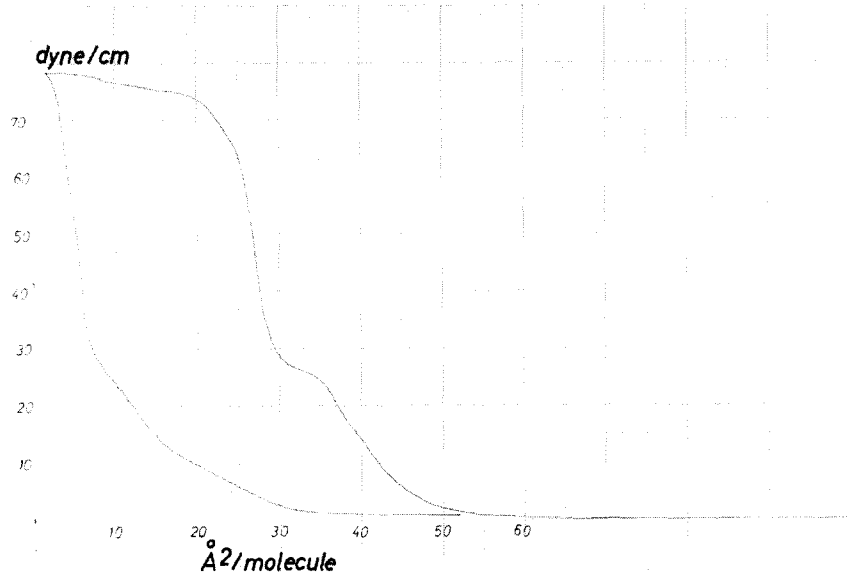

Fig. 11a. π-A-isotherms of D,L-N-acetyl-3-oxo-sphinganine (a) 5°C; (b) 11°C; (c) 15°C; (d) 35°C.

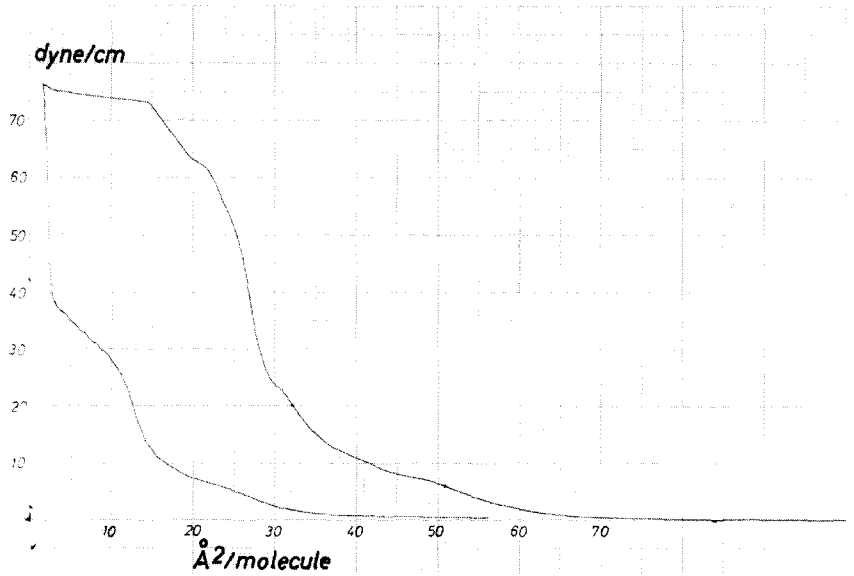

Fig. 11b.

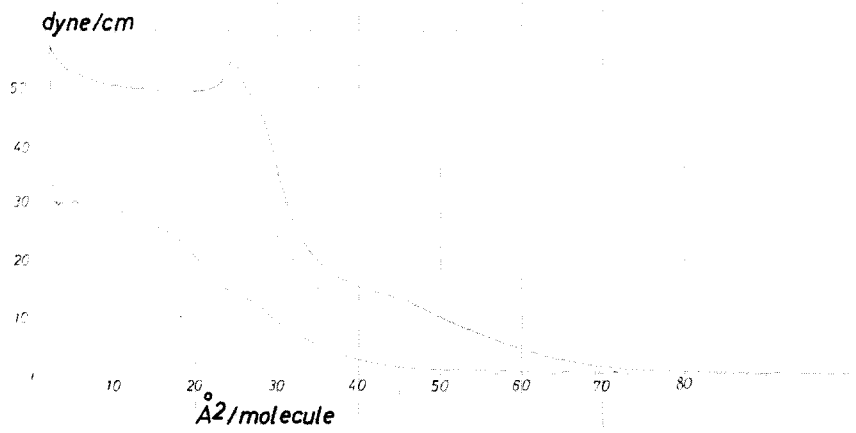

Fig. 11c.

to a trilayer which collapses at 71–75 dyne cm^{-1}. The energy which is required for the transition from the monolayer into the trilayer is temperature dependent. At 30°C 1160 cal/mole and at 40°C 1350 cal/mole are needed. The compressibility of the trilayer is 0.4×10^{-2} cm $dyne^{-1}$, which is exactly one third of the liquid expanded monolayer between 30 and 40°C.

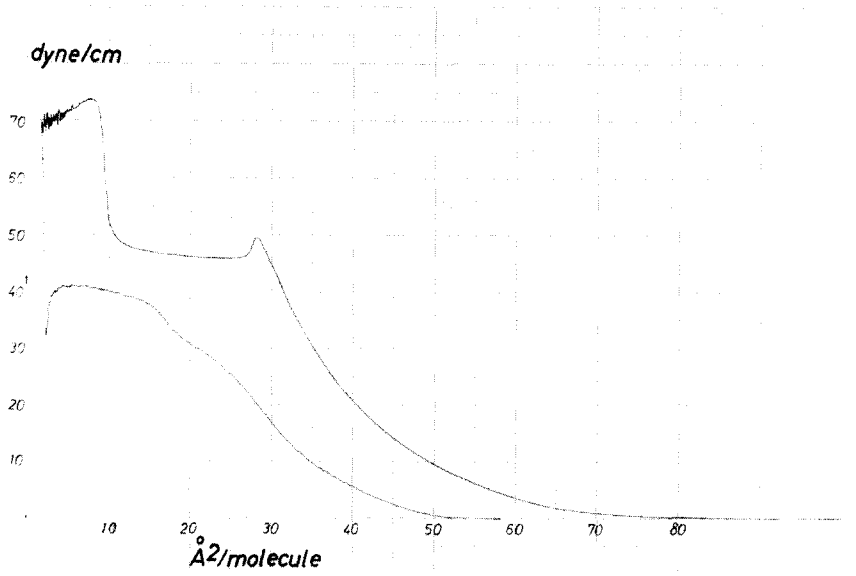

Fig. 11d.

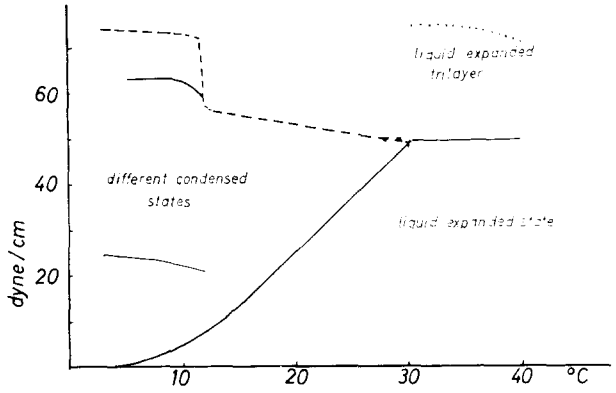

Fig. 12a. Phase transitions are shown by fully drawn lines, monolayer collapse pressures by dashed lines and trilayer collapse pressures by a dotted line.

I. Diacetyl-D,L-oxo-sphinganine (IX)

This derivative also forms trilayers between 10 and 40°C, when the monolayers are compressed beyond a molecular area of 37 Å^2 at pressures between 37 and 43 dyne cm^{-1}. The most complete trilayer formation is observed between 20 and 30°C (fig. 13).

The virtual molecular area of the trilayer at its collapse point is 13 Å^2, which is one third of the molecular area of the monomolecular film at the same pressure. The folding process requires 1360 cal/mole^{-1}.

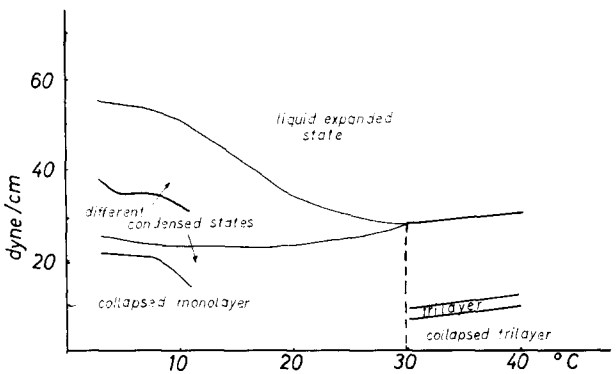

Fig. 12b. Characteristic molecular areas in monolayers and trilayer of D,L-N-acetyl-3-oxo-sphinganine.

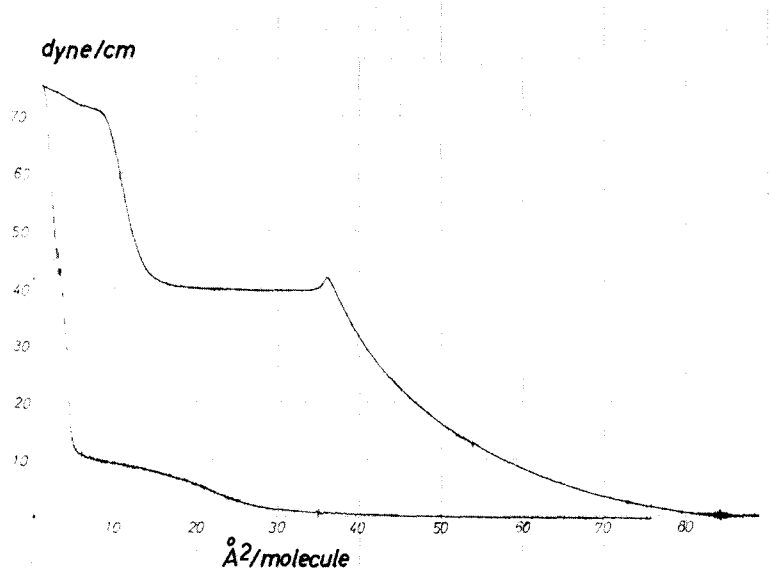

Fig. 13. π-A-isotherm of diacetyl-D,L-oxo-sphinganine at 30°C.

IV. Discussion

Ceramides and diglycerides have structural similarities in common. This is also expressed in their monolayer properties. Ceramide (I) and 1,2-distearoylglycerol form liquid condensed films between 10 and 40°C [12]. Their compressibility at the inversion point of the π-A-isotherms ranges between 0.2×10^{-2} and 0.3×10^{-2} cm dyne^{-1}. The molecular area of the ceramide however exceeds that of the distearoylglycerol at the collapse point by 7 Å^2 molecule^{-1}. This could express a larger hydration shell of the hydrophilic part of the ceramide due to the additional secondary alcoholic group at C–3 of the base. Its diacetylderivatives (II) on the other hand require a molecular area 20 Å^2 molecule^{-1} larger than the ceramide. The area of ceramide corresponds to the hexagonal closest packing of two alkylchains, whereas its diacetylderivative demands the area of a hexagonal closest packing of three alkylchains. This and the large compressibility with a broad collapse area of the monolayer of diacetylceramide suggests that only one acetyl group of diacetylceramide is elevated out of the water surface.

The most remarkable property of monolayers of derivatives of sphingenine and sphinganine is their frequent folding to trilayers with rather stable structures. So far these structures have been observed only rarely. Ekwall et al. [15] reported on the trilayer formation of lithocholic and glycolithocholic acid. They interpret their results by assuming the association of two molecules with their hydrophylic groups. The formation of a trilayer of these compounds would simply require the folding

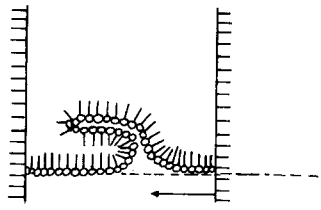

Fig. 14. Schematic illustration of the way proposed for the folding of monolayers to trilayer structures.

of the monolayer. If however the bilayer has tuning fork conformation and forms an overlay on top of the monolayer a rearrangement of the alkylchain would be required. Bursh et al. [16] postulated trilayers of this structure.

The multilayers of poly-ε-benzyloxycarbonyllysine observed by Malcolm [17] and of long chain fatty acid esters described by Larsson et al. [18] presumably arose from the piling up of monolayers and not from a folding process since these authors found even and odd numbered multilayers.

The sphingenine and sphinganine derivatives, which form trilayers yielded neither bi- nor multilayers. The trilayers are folded directly from the liquid expanded monolayer. Reaching the collapse point on compression of the film the pressure decreases by 1 to 5 dyne cm^{-1} and remains constant until the complete trilayer has been formed. The trilayer collapses on further compression after a rapid increase of the film pressure by 20 dyne cm^{-1}. No additional phase transition can be observed during this process. N-Acetyl-D,L-threo-sphinganine (V) does not form trilayers at temperatures below 13°C. Above this temperature which kept the monolayer of this compound in a liquid expanded state up to the collapse point, trilayer formation again occurs. The liquid expanded state is one prerequisite for the trilayer formation.

Other factors may be involved, e.g. N-acetyl-sphinganine forms liquid expanded films between 5 and 35°C, but although no changes of the molecular area or compressibility occur, this compound yields trilayers only between 15 and 27.5°C. We have no interpretation so far of this observation. Steric effects are of great importance on the properties of the monolayer films. Whereas erythro-N-acetyl sphinganine (IV) as liquid expanded film above 10°C forms no trilayer, its threo-diastereomer can be compressed to a stable trilayer. This trilayer can be expanded even beyond the collapse point by more than 50% reversibility. This lends additional support to a simple folding process of the monolayer as indicated in fig. 14.

Rather small changes in the hydrophobic part of the molecule exert a completely different behaviour of the monolayer in the trilayer formation. N-Acetyl-3-oxo-sphingenine (VII) and N-acetyl-3-oxo-D,L-sphinganine (VIII) differ chemically only by a trans–double bond in (VII). The unsaturated compound cannot be folded from its liquid expanded state into a trilayer. The saturated derivative however regularly yields a trilayer structure when the temperature guarantees a liquid ex-

panded film up to the collapse point. We refer this difference to the trans double bond in (VII), which leads to a more spacious arrangement of the alkenylchain.

It appears to be essential for the formation of trilayers that the area demanded by the hydrophilic part is distinctly larger than that of the hydrophobic part of the molecule. This interpretation is supported by the observations that diacetyl- and triacetyl-derivatives of erythro-sphinganine and 3-oxo-sphinganine also form trilayer structures.

Acknowledgements

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