

Chemistry. — “*On substances, which possess more than one stable liquid state, and on the phenomena observed in anisotropic liquids.*” By DR. F. M. JAEGER. (Communicated by Prof. FRANCHIMONT).

§ 1. The compounds now investigated belong to the series of fatty cholesterol-esters, which were the subject of a recent communication¹⁾. They are intended to supplement the number of the synthetic esters, studied previously and include: *Cholesterol-Heptylate*, *Nonylate*, *Laurate*, *Myristate*, *Palmitate* and *Stearate*. The *Palmitic* ester, as is well known, is also important from a physiological point of view, as it occurs constantly in blood-serum accompanied by the *Oleate* m.p. (43° C.)²⁾.

I have prepared these compounds by melting together equal parts by weight of pure cholesterol and fatty acid, and purifying by fractional crystallisation from mixtures of ether and alcohol, or ethyl acetate and ether. The details will be published later on in a more elaborate paper in the “Recueil”. The substances were regarded as pure, when their characteristic temperature-limits and the typical transformations occurring therein, remained the same in every particular, even after another recrystallisation, whilst also the solid phase, when examined microscopically, did not appear to contain any heterogenous components.

Most of these esters were obtained in the form of very flexible, tabular crystals of great lustre and resembling fish-scales; some of them, such as the *heptylate* and the *laurate*, crystallise in long, hard needles.

The investigation showed, that most of these esters of the higher fatty acids possess *three stable liquid phases*. Whereas, in the first terms of the series one at least of these anisotropic phases was *labile* in regard to the isotropic fusion, all three are now *stable* under the existing circumstances, although sometimes definite, irreversible transitions may still occur. It is a remarkable fact, that the stearate again exhibits an analogy with the lower terms, as it appears that only labile liquid-anisotropic phases may occur, or else none at all. A relation and similarity between the initial and final terms

¹⁾ F. M. JAEGER, These Proc. 1906; Rec. d. Trav. d. Chim. d. Pays-Bas, T. XXIV, p. 334—351.

²⁾ K. HÜRTHLE, Z. f. physiol. Chem. 21. 331. (1895); The blood serums of: man, horse, ox, sheep, hog and dog were investigated.

of the homologous series is plainly visible here. In what follows there will be described, firstly, the thermic, and then the microscopic behaviour of these substances.

§ 2. The Thermometric Behaviour of these Substances.

Cholesterol-Laurate exhibits the following phenomena: The isotropous fusion L of this substance has still, at 100° the consistency of glycerol, and gradually thickens on cooling. At $87^\circ.8$ C. ($= t_2$) there suddenly occurs a peculiar violet and green opalescence of the phase, which commencing at the surface, soon embraces the whole phase. The still transparent thin-jelly-like mass quite resembles a coagulating colloidal solution: the opalescence is analogous to that often noticed in the separation of two liquid layers.

As the cooling proceeds, the opalescence colours disappear and the mass gradually becomes less transparent and also more liquid. It is then even thinner than the isotropous fusion L . This doubly-refracting liquid A now solidifies at $82^\circ.2$ C. ($= t_1$) to a crystalline mass S , accompanied by a distinct heat effect.

If, however we start with the solid phase S and subject the same to fusion, the behaviour is apparently quite different. The substance softens and yields after some time a thick doubly-refracting mass, which will prove to be identical with the phase A . On heating further the viscosity decreases, and at about 86° it becomes very slight. There is, however, no sign of opalescence this time. The turbid mass may be heated to over 90° , without becoming clear and now and then A seems as if solid particles are floating in the liquid phase. At $90^\circ.6$ C. ($= t_3$) everything passes into the isotropous fused mass L . The microscopical investigation shows, that between A and L another stable, less powerfully refracting liquid phase B is now traversed, and that, owing to retardation occurring, the phase S may be kept for a few moments adjacent to L , when A and B have already disappeared.

This is therefore, a case where a substance may be heated a few degrees above its actual melting point without melting.

It should, however, be observed that the order of the temperatures is here quite irreconcilable with the phenomena considered possible up to the present, with homogenous substances; the temperature of $90^\circ.6$, at which these crystals disappear in contact with L finds no place in the p - t -diagram of Fig. 1. Such a position of the said temperatures might be possible, when the system could be regarded as containing two components, for instance, if there was question of *tautomeric* forms which are transformed into each other with finite velocities. I think it highly probable that in all these substances,

“phenomena of retardation” play a great rôle; moreover the enormous undercooling which the phase *A* can undergo without transformation, proves this satisfactorily in the majority of these esters.

The different behaviour of the laurate on melting and on cooling the fused mass is so characteristic, that no doubt can be entertained as to the irreversibility of each series of transformations. Fuller details will be given below in the micro-physical investigation.

§ 3. *Cholesterol-Nonylate* forms at 90° an isotropous fused mass of the consistency of paraffin oil: on cooling to $89^{\circ}5$ a stable, greyish, doubly-refracting liquid *B* appears which, gradually thickening, passes into a second strongly doubly-refracting liquid phase *A*, — which transformation is accompanied with a brilliant display of colours. All three liquids are, however, quite stable within each specific temperature-traject. On melting, as well as on cooling the substance, they succeed each other in the proper order.

The viscous, strongly doubly-refracting, liquid phase *A* now becomes more viscous on cooling, and is finally transformed into a horny, transparent mass which exhibits no trace of crystallisation. Even after some hours, the often still very tenacious mass has not got crystallised. In the case of this substance it is therefore impossible to give the solidifying point or the exact temperature at which the heated mass begins to melt. The reason of this is, that the doubly refracting liquid *A* can be undercooled enormously and passes gradually into the solid condition without crystallising.

As the micro-physical research has shown, a spherulite-formation occurs afterwards suddenly in the mass, which ultimately leads to the complete crystallisation of the substance.

The velocity, with which such spherulites are formed appeared in some cases not to exceed 0.000035—0.000070 m.m. per second!

§ 4. *Cholesterol-Myristate*, at 80° , is still an isotropous, paraffin oil-like liquid. On cooling, it gradually becomes viscous: at about $82^{\circ}6$ the glycerol-like phase then turns, with violet-blue opalescence, into a thick, strongly doubly-refracting mass *A* which, gradually assuming a thicker consistency, is finally converted into a horny mass, without any indication of a definite solidifying point. In this respect the substance is quite analogous to the previous one. On the other hand, on being melted, it behaves more like the laurate, in so far as it is converted into a double-refracting liquid *B*, before passing completely into *L*. The transition temperature cannot be determined sharply, but I estimate it at about 80° .

§ 5. *Cholesterol-Palmitate* at 80° is a clear, isotropous liquid as thick as simple syrup. On cooling, the isotropous phase is converted at 80° with green opalescence into a fairly clear, transparent, doubly-refracting jelly *A*, which rapidly assumes a thinner consistency, and becomes at the same time more turbid, and finally solidifies at 77.92 , with a perceptible caloric effect, to a crystalline mass *S*. In this case also, a doubly-refracting phase *B* appears to be traversed when the mass is being melted, before the occurrence of the isotropous fusion *L*; I estimate the transition temperature at about 78° .

§ 6. With *Cholesterol-Stearate*, I did not succeed in demonstrating the occurrence of a doubly refracting liquid. The isotropous, thick-fluid fusion solidifies at 81° to well-formed crystals *S*.

§ 7. *Cholesterol-Heptylate* exhibits, in undercooled fusion only, one doubly-refracting liquid phase which is labile in regard to the solid phase *S*. The compound behaves, thermically, analogously to the caprylate. The temperature of solidification is at 110.55 , the transition-temperature of the labile doubly-refracting phase lies a little lower.

Of *Cholesterol-Arachate*, I could only obtain an impure product on which no further communications will be made. The ester could not be purified properly as it is not soluble to any extent in the ordinary solvents. The crude substance obtained does not seem to exhibit any anisotropous liquid phases.

§ 8. **Micro-physical behaviour of these substances.** If a little of the pure solid *cholesterol-laurate* is melted on an object glass to an isotropous, clear liquid *L*, and the same is allowed to cool very slowly, there is formed, usually, a very strongly doubly-refracting, liquid phase, gleaming with lucid interference colours. It consists of large, globular drops, which exhibit the black axial cross and, on alternate heating and cooling, readily amalgamate to a syrupy, highly coloured, but mainly yellowish-white liquid. This phase will be called *A* in future. On cooling, it gradually thickens, until no more movement of the mass is noticed, which continues to exhibit a granular structure. Around this mass an isotropous border liquid is found. At first I felt inclined to look upon this tenacious, isotropous mass, which is visibly different from the fusion *L*, as a distinct phase differing from the fusion *A*. But on using a covering glass and pressing the same with a pair of pincers, or by stirring with a very thin platinum wire, I found that this border liquid is only

“pseudo-isotropic” (LEHMANN) and is, in reality, not different from A ; only, the optical axes of the liquid crystals are all directed perpendicularly to the glass-surface. The other cholesterol-esters also exhibit this phenomenon. On further cooling, this phase A crystallises like the pseudo-isotropic border to a similar mostly spherulitic crystalline mass S .

Between the spherulites one often sees currents of the pseudo-isotropic border liquid.

If now the entire mass is allowed to solidify to S , and then again is melted carefully, it is at once transformed into the liquid A , recognisable by its high interference colours and its slow currents. Then, there appears suddenly a new, greyish liquid B , consisting of smaller individuals with a less powerful double refraction, which after a short time is replaced suddenly by the isotropic fusion L . If L is now cooled again, it is A which appears at once and *not* the phase B .

Only a very feeble, greyish flash of light, lasting only for a moment, points to a rapid passing of the phase B ; it cannot, however, be completely realised now. On further cooling, S is formed suddenly, sometimes in plate-like crystals. When once crystallisation has set in, S will not melt when the mass is heated, as might have been expected, but actually increase in size of the crystals occurs, and the velocity of crystallisation is now many times increased. It must be remarked that the growing flat needles of S drive before them, at their borders, the liquid phase A amid violent currents. If the heating is now continued a little longer, we may notice sometimes, that whilst the little plates of S remain partly in existence, A passes first into the grey phase B , which then is converted into the isotropic mass L . We then have adjacent to L the solid phase S , *which therefore, may be heated above its melting point, before disappearing finally into the isotropic fusion L .*

All this shows, that the *laurate* possesses *three stable liquid phases* and also that the isotropic fusion being cooled, B is always passed over, but is realised when the solid phase is heated. All this is represented in the annexed p - t -diagram; the arrows, therefore, indicate the order of the phases traversed on melting and on cooling. The phase A in its quasi-immovable period may be kept a long time solid at the temperature of the room, and may be considerably undercooled before it passes into S . Notwithstanding its apparently solid appearance in that undercooled condition, A is still a tenacious, thick liquid, as I could prove by stirring the mass with a thin platinum wire.

The point (t_3) agrees with the *opalescence* which occurs when

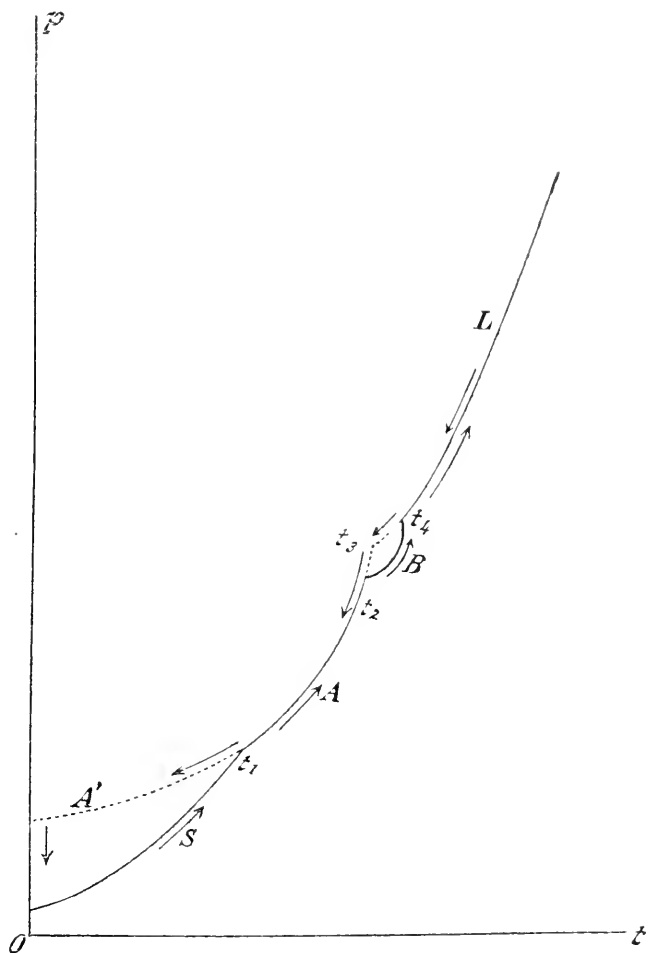


Fig. 1. Schematic p - t -diagram for Cholesterol-Laurate.

the isotropous fusion is cooled; this indicates, therefore, the moment where the stable phase B is replaced by the as yet still less stable phase A , which will soon afterwards be the more stable one: a fact which may be perhaps important in the future for the explanation of the analogous phenomena observed in the separation of two liquid layers and the coagulation of a colloidal solution.

Indeed, the transition at (t_3) presents quite the aspect of a gelatinising colloidal solution. The temperature of this transition point may be determined, but not sharply, at $87^{\circ}.8$. The temperature at which, when the solid substance melts, the liquid may be still kept turbid, owing probably to the presence of the meta-stable plate-like crystals of S , was determined at $90^{\circ}.6$; the solidifying temperature (t_1) lies at $82^{\circ}.2$.

That the border liquid, obtained by cooling the isotropous fusion L , differed from L itself, could be demonstrated in more way than one. By heating and cooling we may get so far that, apparently, nothing more of A is visible, but that we have only the border liquid, which on cooling, crystallises immediately to S . Occasionally, the doubly-refracting individuals of A turn up in the mass for a moment to disappear again immediately. However, that isotropous liquid thus obtained is nothing else but A itself, when owing to the temperature variations, all individuals have, like magnets, placed themselves parallel with their (optical) axes and the whole has, consequently, become pseudo-isotropous. This same phenomenon also occurs with the other esters, for instance very beautifully with the nonylate and the myristate. The difference between these pseudo-isotropous phases and the isotropous fused masses L of these substances, is shown by the fact that the pseudo-isomorphous mass of A , and also the doubly-refracting portion of the same has a very thick-fluid consistency; the isotropous fusion L of the laurate has a consistency more like that of glycerol.

As regards the solid phase and its transformation into the liquid condition, it cannot be proved in this case that there exists a continuous transformation between the last solid particle and the first anisotropous ones. From the velocity, with which the diverse phases usually make room for each other in the microscopic examination, one would feel inclined to believe just the opposite. The thermic observation of the transformation, which generally exhibit only insignificant caloric effects, would, however, make the observer feel more inclined to look upon the matter as an uninterrupted concatenation of more or less stable intermediate conditions, which I have observed previously with *cholesterol-cinnamylate*. A somewhat considerable heat effect occurs in some cases in the crystallisation of the solid phase only: in all other phases the exact transition temperature cannot be determined accurately by the thermic method.

§ 9. *Cholesterol-Nonylate* exhibits microscopically the following phenomena:

Starting from the crystallised substance, this was fused first on an object glass to an isotropous liquid L . On cooling a greyish doubly-refracting liquid phase B appears, which, at a lower temperature, makes room for a very tenacious, strongly doubly-refracting, mostly yellowish-white phase, A . This phase A is often surrounded by an isotropous border: if pressure is applied to the covering glass or if the mass is stirred with a very thin platinum wire, this isotropous

liquid appears to be identical with *A*, and to be pseudo-isotropic by homocotropism only. The optical axes of the doubly-refracting modification *A* again place themselves perpendicularly on the surface of the covering glass. On continued cooling *A* becomes increasingly thicker: at last a movement in the mass can be seen only on stirring. After a longer time there are formed from numerous centres in this tenacious mass thin, radiated spherulites, whose velocity of growth is but very small. When a number of these spherulites have formed and the mass is then heated carefully, the spherulites do not melt, but actually *increase* owing to the greater crystallisation-velocity. Soon afterwards — however, they melt, on further heating, to the doubly-refracting phase *A*, where the *circumferences of the spherulites and the black axial crosses are preserved for some time*; so that the whole much resembles a liquid mozaic. Subsequently the phase *B* reappears and afterwards the isotropous fusion *L*. The whole series of phases is traversed in a *reversible* manner; the liquid phase *A*, however may be so much undercooled, that a proper melting or solidifying point of the substance cannot be given. In larger quantities of the substance, the crystallisation does not set in till after some hours, and the substance turns first to a horny mass, which always remains doubly-refracting to finally exhibit local, white spots, from which the spherulite-formation slowly spreads through the entire mass. One would feel inclined to call this transformation of liquid-anisotropous into crystallised substance a *continuous one*, if it were only possible to observe, even for a moment, the intermediate conditions in that transition. As the matter cannot be settled by direct experiment, the transition must be put down, provisionally as a *discontinuous one*.

In this case also, and the same applies to the other cholesterol-esters as well, the *spherulite-structure* of the solid phase is of great importance for this entire transformation of undercooled, anisotropous-liquid condition into the solid one. At the end of this communication I will allude briefly to a few cases from which the particular significance of the spherulite-structure in the transitions between anisotropous-liquid and anisotropous solid phases is shown also plainly in a different manner.

§ 10. *Cholesterol-Palmitate* behaves in quite an analogous manner: I observed one solid phase and three liquid conditions *A*, *B* and *L*: as in the case of the laurate, *B* is generally observed only on warming. The succession of the liquid and solid phases takes place, however comparatively rapidly, so that a real solidifying point may

be observed, which has also been proved by the thermic research. The solid phase crystallises in broad flat needles, when fused and then solidified in conglomerated spherulites. On melting, the thick, doubly-refracting liquid *A* is mostly orientated in regard to the previous solid spherulites.

§ 11. *Cholesterol-Stearate* could not be obtained in a doubly-refracting liquid form: the isotropous fusion always crystallises immediately amid rapid, rotating movements, to small needles, which often consist of a conglomeration of rosettes. It is possible that labile anisotropous phases are formed, owing to strong undercooling or by addition of some admixture¹⁾.

§ 12. *Cholesterol-Myristate* lends itself splendidly to the experiment. It behaves mainly in the same manner as the *laurate*; the phase *B* can only be observed on heating, but not on cooling the isotropous fusion *L*. Most brilliant is the formation of large, globular crystal-drops of the modification *A*, also the colour-zone which precedes the formation of *A* from *L*, on cooling. This phase *A* also exhibits the phenomenon of pseudo-isotropism in a particularly distinct form. On the other hand, an important difference between this compound and the *laurate* is the much smaller velocity with which, on cooling, the spherulites *S* are formed from *A*; in this respect the compound exhibits more similarity with the *nonylate*. Sometimes it may be observed readily how in the phase *A*, which consists of an enormous number of linked, globular crystal-drops, which all exhibit the black cross of the spherulite crystals, centrifugal current-lines are developed from a number of points in the mass, along which the crystal-drops range themselves. After the lapse of some time those doubly-refracting globules are seen to disappear, while the current-lines have now become rays of the spherulite. Here again, the question arises whether the transformation of the doubly-refracting liquid globules, which are orientated along the current-lines, into the true spherulite form, does not take place continuously, and whether we do not speak of a *sudden* transformation merely because we are not able to observe the stadia traversed in this transformation.

The liquid globules of the phase *A* themselves exhibit much similarity with a kind of liquid spherulites; a few times, I have even been able to observe such "liquid spherulites" of greater dimens-

¹⁾ Prof. LEHMANN informed me recently that the *stearate* possesses indeed two labile, anisotropous liquid phases.

ions, which rapidly solidified to solid spherulite crystals. In the case of this compound also, one feels convinced that there must exist a very intimate relation between the spherulite-formation of a substance and its power of forming anisotropic liquid phases; on the nature of this relation, I hope to make a communication later on.

It may, however, be observed, provisionably that in all transformations: liquid \rightleftharpoons solid, where serious "phenomena of retardation" may occur, the undercooling, or superfusion, for instance is generally abrogated amid a differentiation of the phase into spherulites. All the cholesterol-derivatives, mentioned in this paper, exhibit this spherulite-formation. In the case of *α -phytosterol-propionate*, I have been able to show, that a complex of a large number of doubly-refracting microscopic spherulites may imitate the optical peculiarities of the liquid phases in process of separation and of the colloidal opalescence. This might lead to the strengthening of the previous conception of the colloidal solidification as a separation-phenomenon of labile liquids.

§ 13. *Cholesterol-Heptylate* contains only *labile* liquid anisotropic phases. It exhibits great similarity with the caprylate described previously: I have only a few times been able to obtain one single thick-fluid phase *A* from the undercooled isotropous fusion *L*. The solid phase crystallises rapidly and in beautiful flat needles, which exhibit high interference colours. On warming, the substance readily migrates towards the colder parts of the object glass.

§ 14. In conclusion, I will communicate a few more points as regards some phenomena, which prove plainly the significance of the spherulite structure for with these questions.

Some time ago, I published a research on the fatty esters from *Phytosterol* from Calabar-fat and stated how they all are wont to crystallise in the spherulite-form from their cooled, isotropous fused mass, while anisotropic liquid phases are *not* observed therein, with the exception of the *normal valerate* which possesses a thick-fluid anisotropic modification, and exhibits the phenomenon of the changeable melting point, which again becomes normal on long keeping: a fact also observed in the case of a few fatty glycerol-esters. Since then, WINDAUS has proved that the phytosterol, extracted from Calabar fat is a mixture of two isomorphous phytosterols, which cannot be separated by crystallisation. Being engaged in preparing the pure fatty esters from the principal of those two phytosterols, namely the α -compound (m.p. 136°), I discovered that the fused *propionate* of *α -phytosterol* (m.p. 108°), when cooled rapidly in cold water,

exhibited the most brilliant interference-colours, which is also the case with the cholesterol esters (acetate for instance), which possess *labile* anisotropic liquid phases. The thought naturally at once occurred, to attribute these phenomena to the appearance of liquid crystals in the now *pure* α -phytosterol-ester. A similar behaviour was also shown by perfectly pure α -*phytosterol-acetate*, but with a much less display of colours. It was, however, a remarkable fact, that α -*phytosterol-propionate* even after complete solidification *still retained those colours for an indefinite length of time*, particularly at those sides of the testtube, where the layer of the substance was thinner and had cooled rapidly.

The microscopic investigation now showed that these two substances exhibit extremely rapidly disappearing anisotropic liquid phases or, more probably, *none at all*¹⁾; but that *the said colour-phenomenon is caused by a very peculiar spherulite-structure*.

In what follows, I have given the description of the solidifying phenomena of the α -*propionate*, and also a figure representing the typical structure of the fused and then cooled compound, such as is present at the coloured sides of the tube.



Fig. 2.

If a little of the solid substance is fused on a slide to an isotropous liquid the following will be noticed on cooling. The mass solidifies completely to spherulites, namely to a conglomeration of circular, concentrically grouped figures, which appear connected with a series of girdles. When three spherulites meet, they are joined by means of straight lines which inclose angles of about 120° .

The mass is slightly doubly-refracting and of a greyish colour; the rings and girdles are light greyish on a darker back-ground. Each spherulite exhibits besides a concentric structure, the black cross, but generally very faint. The whole resembles a drawing of polished malachite from the Oeral, or of some polished agates.

¹⁾ Whereas the *phytosterol*-esters from Calabar fat which, of course, contain a definite amount of the β -homologue, exhibit *no* liquid crystals, the pure α -esters commencing with the butyrate [or perhaps the propionate] did show this phenomenon. This discovery is a powerful argument against the remarks often made in regard to the *cholesterol*-esters, that the remarkable phenomena described are attributable to an admixture of homologous cholesterol. Foreign admixtures prevent as a rule these phenomena altogether: in any case they are rather spoiled than improved.

The walls of the test-tube or the object-glass, which exhibit the said colour-phenomena, have that same structure, but with this difference, that the globular, concentrically deposited spherulites have much smaller dimensions and lie much closer together. Each little spherulite has also a cross; this however, is not dark, but coloured with yellow and violet arms. The spherulite is also coloured in the alternate circle-quadrants.

This ensemble of small, coloured spherulites is the cause of the said brilliant colour-phenomena; they are quite analogous to those which are wont to appear in the case of liquid crystals and remain in existence for an indefinite period. Each of them exhibits one or generally two luminous points in the centre; they exhibit a strong circular polarisation and are left-handed. The whole appears between crossed nicols as a splendid variegated mozaic of coloured cellular parts. The size of each individual is 0.5—1 micron.

The acetate also exhibits something similar, but the spherulites are built more radial and the whole is not at all so distinct.

I hope to contribute more particulars as to these remarkable phytosterol-compounds shortly. I have mentioned them here merely to show the importance of this structure-form for the optical phenomena, observed in the anisotropic phases.

Zaandam, 14 November 1906.

Chemistry. — “*On irreversible phase-transitions in substances which may exhibit more than one liquid condition.*” By Dr. F. M. JAEGER. (Communicated by Prof. FRANCHIMONT).

(Communicated in the meeting of November 24, 1906).

§ 1. The fatty esters of *α-Phytosterol* from *Calabar-fat*, which the Phytosterol mostly occurring in the vegetable kingdom, and which has also been isolated from rye and wheat under the name of “sitosterol”, exhibit very remarkable properties in more than one respect.

In my previous communication, I alluded briefly to the colour phenomena and the spherulite-structure in the *propionate* and the *acetate*. In the latter I could not observe anisotropic liquid phases: in the former a doubly-refracting phase is discernible just before melting, but it lasts too short a time to allow the accurate measurement of the temperature-traject.

With the following four terms of the series, however, these phenomena are more distinct, and occur under conditions so favourable as