that really has a vortex i.e. a rotation vector everywhere in space. We can say namely:

If of a force field in each point the divergence (a scalar) and the rotation (a planivector) are given, then it is the $\nabla$ of a potential: $\int \frac{div.\, dv}{k_n(n-2)r^{n-2}} + \int \frac{rot.\, dv}{k_n(n-2)r^{n-2}}$; this formula takes the field as an integral of fictitious fields of agens points and of single vortices.

**Crystallography.** — "*On the fatty esters of Cholesterol and Phytosterol, and on the anisotropous liquid phases of the Cholesterol-derivatives.*" By Dr. F. M. JAEGER. (Communicated by Prof. A. P. N. FRANCHIMONT.)

(Communicated in the meeting of May 26, 1906).

§ 1. Several years ago I observed that phytosterol obtained from rape-seed-oil suffers an *elevation* of the melting point by a small addition of cholesterol. The small quantity of the first named substance at my disposal and other circumstances prevented me from going further into the matter.

My attention was again called to this subject by some very meritorious publications of BÖMER [1]) on the meltingpoint-elevations of *phytoterol* by *cholesterol* and also of *cholesterol-acetate* by *phytosterol-acetate*. Apart from the fact that the crystallographic data from O. MÜGGE led me to the conclusion, that there existed here an uninterrupted miscibility between heterosymmetric components, a further investigation of the binary meltingpoint-line of the two acetates appeared to me very desirable, as the ideas of BÖMER on this point are not always clear; this is all the more important, as we know that BÖMER based on these melting point elevations a method for detecting the adulteration of animal with vegetable fats. My further object was to ascertain in how far the introduction of fatty acid-residues into the molecule of *cholesterol* would modify the behaviour of the esters in regard to the phenomenon of the *optically-anisotropous liquid phases*, first noticed with the *acetate, propionate* and *benzoate*, with an increasing carbon-content of the acids. Finally I wished to ascertain whether there was question of a similar meltingpoint-elevation as with the acetates in the other terms of the series too.

[1]) BÖMER, Zeit. Nahr. u. Genussm. (1898). 21, 81; (1901). 865, 1070; the last paper (with WINTER) contains a complete literature reference to which I refer.

§ 2. In the first place the esters of cholesterol and phytosterol had to be prepared.

The cholesterol used, after being repeatedly recrystallised from absolute alcohol + ether, melted sharply at 149°.2. The phytosterol was prepared by MERCK, by HESSE's[1]) method from Calabar fat, and also recrystallised. It melted at 137°. A microscopic test did not reveal in either specimen any inhomogeneous parts.

First of all, I undertook the crystallographic investigation of the two substances. The result agrees completely with the data given by MÜGGE, to which I refer. I have not, up to the present, obtained any measurable crystals; on account of the optical properties, cholesterol can possess only triclinic, and phytosterol only monoclinic symmetry.

Although an expert crystallographer will have no difficulty in microscopically distinguishing between the two substances, the crystals deposited from solvents are, however, so much alike that a less experienced analyst may easily make a mistake. I, therefore, thought it of practical importance to find a better way for their identification with the microscope.

This was found to be a very simple matter, if the crystals are allowed to form on the object-glass by fusion and solidification, instead of being deposited from solvents. Figs. 1 and 2 show the way in which the solidification of the two substances takes place.

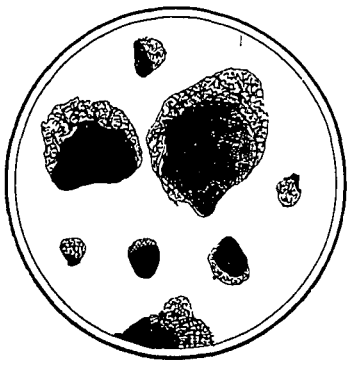

Fig. 1.
*Cholesterol,*
fused and then solidified.

Fig. 2.
*Phytosterol,*
fused and solidified by cooling.

Phytosterol crystallises in conglomerate spherolites. Between crossed nicols they exhibit a vivid display of colours and each of them is

[1]) HESSE, Annal. der Chemie, **192**. 175.

traversed by a dark cross, so that the whole conveys the impression of adjacent interference images of monaxial crystals, viewed perpendicularly to the axis and without circular polarisation. The character of the apparently simple crystals is optically negative.

Cholesterol, however, presents a quite different image. When melted on an object-glass, the substance contracts and forms small droplets, which are scattered sporadically and, on solidification, look like little nuggets with scaly edges, which mostly exhibit the white of the higher order.

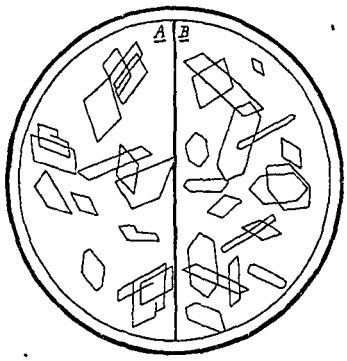


Fig. 3.
Phytosterol and Cholesterol from 95% Alcohol.

That the microscopical distinction in this manner is much safer than by MÜGGE's method, may be seen from fig. 3 where phytosterol and cholesterol are represented as seen under the microscope, after being crystallised from alcohol. *A* is cholesterol, *B* phytosterol.

§ 3. Of the fatty esters, I have prepared the *acetates, propionates, butyrates* and *isobutyrates* by heating the two alcohols with the pure acid-anhydride in a reflux apparatus. A two or three hours heating with a small flame, and in the case of the cholesterol, preferably in a dark room, gives a very good yield. When cold, the mass was freed from excess of acid by means of sodium hydrocarbonate, and then recrystallised from alcohol + ether, afterwards from ethyl acetate + ligroin, or a mixture of acetone and ligroin, until the meltingpoint was constant. Generally, I used equal parts by weight of the alcohol and the acidanhydride.

The *formiates, valerates, isovalerates, capronates, caprylates* and *caprinates* were prepared by means of the pure anhydrous acids. These (valeric, caprylic and capric acids) were prepared synthetically by KAHLBAUM; the isovaleric acid and also the anhydrous formic acid were sold commercially as pure acids "KAHLBAUM". Generally, a six hours heating of the alcohol with a little more than its own weight of the acid sufficed to obtain a fairly good yield. Owing, however, to the many recrystallisations required the loss in substance is much greater than with the above described method of preparing.

Both series of esters crystallise well. The phytosterol-esters in soft, flexible, glittering scales; the formiate and the valerates present some difficulties in the crystallisation, as they obstinately retain a trace of

an adhesive by-product which it is difficult to remove. The cholesterol-esters give much nicer crystals; the formiate, acetate and benzoate have been measured macroscopically; the other derivatives crystallise in delicate needles or very thin scaly crystals which are not measurable; I hope yet to be able to obtain the butyrate in a measurable form [1]). In the case of the caprylate, the purification was much assisted by the great tendency of the product to crystallise. The purification of the capric ester was, however, much more difficult; at last, this has also been obtained in a pure state even in beautiful, colourless, plate-shaped crystals, from boiling ligroïn [2]).

The phytosterol-esters retain their white colour on exposure to the light; the cholesterol-esters gradually turn yellowish but may be bleached again by recrystallisation.

The determination of the melting points, and in the case of the cholesterol-esters, also that of the transition-temperatures: solid → anisotropous-liquid, was always executed in such manner, that the thermometer was placed in the substance, which entirely surrounded the mercury-reservoir. Not having at my disposal a thermostat, I have not used the graphic construction of the cooling-curve, in the determinations, but simply determined the temperature at which the new phases first occur when the outer bath gets gradually warmer.

As regards the analysis of the esters, nothing or little can be learned from an elementary analysis in this case, where the formulae of cholesterol and phytosterol are still doubtful, and where the molecules contain from 28 to 37 carbon-atoms. I have therefore rested content with saponifying a small quantity of the esters with alcoholic potassium hydroxide, which each time liberated the cholesterol or phytosterol with the known melting points. On acidifying the alkaline solution with hydrochloric acid, the fatty acids could be identified by their characteristic odour.

The esters were called pure, when the melting points, and in the case of cholesterol-esters, *both* temperatures, remained constant on further recrystallisation.

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[1]) I have even succeeded lately in obtaining the formiate in large transparent crystals from a mixture of ligroïn, ethyl acetate and a little alcohol.

[2]) The crystals of the *caprinate* are long, flat needles. They form monoclinic individuals, which are elongated parallel to the *b*-axis, and flattened towards {001} The angle $\beta$ is 88° à 89°; there are also the forms: {100} and {$\bar{1}$01}; measured: $(\bar{1}00):(\bar{1}01) = \pm 20.°$. The optic axial plane is {010}; inclined dispersion: $\rho > \nu$ round the first bissectria. Negative double refraction. On {001} there is one optical axis visible about the limits of the field. The crystals are curved-plane

§ 4. I give in the following tables the temperatures observed etc.[1]) Next to my data are placed those of BÖMER as far as he has published them. The temperatures in [ ] will be discussed more in detail later on.

I. FATTY ESTERS OF CHOLESTEROL.

| | $t_1$ | $t_2$ | $t_3$ | BOMER's data: | |
|---|---|---|---|---|---|
| Chol Formiate | — | [± 90°] | 96°.5 | — | 96°. |
| » Acetate | — | [80 à 90°][1]) | 112°.8 | — | 113°.5 |
| » Propionate | 93°.0 | 107°.2 | — | 96° | 111° |
| » -*n*-Butyrate | 96°.4 | 107°.3 | — | 96° | 108° |
| » Isobutyrate | — | — | 126°.5 | — | — |
| » -*n*-Valerate | 91°.8 | 99°.2 | — | — | — |
| » Iso valerate | — | [± 109°] | 110°.6 | — | — |
| » Capronate | 91o.2 | 100°.1 | — | — | — |
| » Caprylate | — | [± 101°] | 106°.4 | — | — |
| » Caprinte | 82°.2 | 90° 6 | — | — | — |
| » Benzoate | 145° 5 | 178°.5 | — | 146° | 178°.5 |
| » Phtalate [2]) | — | — | — | — | 182°.5 |
| » Stearinate [3]) | — | — | — | 65° | |

Benzoates and phthalates although not being fatty esters, have nevertheless been included.

[1]) According to SCHONBECK, Diss. Marburg. (1900).
[2]) According to BÖMER loco cit.
[3]) According to BERTHELOT. It is as yet undecided, whether liquid crystals are present here; perhaps this case is analogous with that of the caprylate.
The temperatures in [] cannot be determined accurately; see text.

§ 5. Most striking with these remarkable substances are the splen-

[1]) It should be observed that in these substances *three* temperatures should be considered, namely 1. transition: solid → anisotropous-liquid; 2. transition: anisotropous-liquid → isotropous-liquid; 3. transition: solid → isotropous-liquid.
This distinction has been retained, particularly on account of the cases of labile, liquid crystals discovered here.

did colour-phenomena observed during the cooling of the clear, isotropous, fused mass to its temperature of solidification, and also during the heating in the reverse way. These colour phenomena are caused by interference of the incident light, every time the turbid anisotropous liquid-phase occurs, or passes into the isotropous liquid. During this last transition we notice while stirring with the thermometer, the "oily slides" formerly described by REINITZER, until the temperature $t_2$ has been exceeded. These colours also occur when the solid phase deposits from the anisotropous liquid, therefore below $t_1$. The most brilliant, unrivalled violet and blue colour display is shown by the butyrate and normal valerate, also very well by the capronate and caprinate.

The temperatures in [ ] $t_2$, answer to anisotropous liquid phases which are *labile* in regard to the isotropous liquid, and *which double-refracting liquids are, therefore, only realisable in undercooled fused material.* Of this case, which is comparable with the monotropism, as distinguished by LEHMANN from the case of enantiotropous transformations, the acetate is the only known example up to the present. Now the number of cases is increased by *three,* namely the *formiate,* the *caprylate* and without any doubt also the *isovalerate,* to which I will refer presently. Cholesterol-formiate and caprylate melt therefore, *perfectly sharply* to a clear liquid at, respectively $96^1/_2°$ and 106.°2. If, however, the clear liquid is suddenly cooled in cold water, one notices the appearance of the turbid, anisotropous, more-labile phase, accompanied by the said colour phenomena. The acetate in particular exhibits them with great splendour. It is quite possible that many organic compounds which are described as "melting sharply", belong to this category and on being cooled suddenly possess a double-refracting liquid phase, even although this may last only a moment. The phenomenon of liquid crystals would then be more general than is usually believed.

Prof. LEHMANN, to whom I have forwarded a little of the cholesterol-esters, has been able to fully verify my observations. This investigator has, in addition, also found that *cholesterol-caprinate may probably exhibit two anisotropous liquid phases.* Although, personally, I never noticed more than one single phase, and Prof. LEHMANN's determinations are only given provisionally, this case would certainly have to be regarded as one of the most remarkable phenomena which may be expected in a homogeneous body, particularly because the perceptibility of those *two* phases implies that they would *not* be miscible in all proportions with each other.

§ 6. The behaviour of *cholesterol-isobutyrate* is a very remarkable one. Microscopic and macroscopic investigation shows absolutely nothing of an anisotropous liquid phase, not even on sudden cooling and this in spite of the fact that the normal butyrate gives the phenomenon with great splendour. This differently-behaving ester has been prepared from the same bulk of cholesterol as was used for preparing the other esters. The cause of the difference can, therefore, be found only *in the structure of the fatty acid-residue*, which contrary to that of the other esters, is branched.

All this induced me, to prepare the analogous ester of isovaleric acid; perhaps it might be shown also here that the branching of the carbon-chain of the acid destroys the phenomenon of the anisotropous liquid phase. At first I thought this was indeed the case, but a more accurate observation showed that in the rapid cooling there occurs, if only for an indivisible moment, a *labile* anisotropous liquid; the duration, however, is so short that, for a long time, I was in doubt whether this phase ought to be called stable or labile as in the case of the formiate and caprylate! Even though the carbon-branching does not cause a total abrogation of the phenomenon of liquid crystals, the realisable traject appears to become so much smaller by that branching, that it almost approaches to *zero*, and the expected phase is, moreover, even still labile. From all this I think we may conclude, as has been stated more than once by others, that the occurrence of the liquid phases is indeed a *inherent* property of the matter, which cannot be explained by the presence of foreign admixtures etc. (TAMMANN c. s.).

§ 7. We now give the melting points of the analogous phytosterol-esters which, with one exception, do not exhibit the phenomenon of the double-refracting liquids. As the phytosterols from different vegetable fats seem to differ from each other, and as BÖMER does not mention any phytosterol esters from Calabar-fat in particular, I have indicated in the second column only the *limits* within which the melting points of the various esters prepared by him from diverse oils, vary. (See table following page.)

From a comparison of the two tables it will be seen that the lowering of the melting point of phytosterol by the introduction of fatty acid-residues of increasing carbon-content, takes place *much more rapidly* than with cholesterol. On the other hand, the succession of the melting points of the acetate, propionate, butyrate and *n*-valerate is *more regular* than with the cholesterol-derivates.

All phytosterol-esters share with phytosterol itself the great ten-

II. FATTY ESTERS OF PHYTOSTEROL.

| | | Limits according to BÖMER: |
|---|---|---|
| Phytosterol-Formiate | 110° | 103°—113° |
| Phytosterol-Acetate | 129°.1 | 123°—135° |
| Phytosterol-Propionate | 105°.5 | 104°—116° |
| Phytosterol-Butyrate | 91°.2 | 85°— 90° |
| Phytosterol-Isobutyrate | 117° | — |
| Phytosterol-norm.-Valerate | $t_3 = 67°$; $t'_3 = 30°$ | — |
| Phytosterol-Isovalerate | 100°.1 | — |

dency to crystallise from the melted mass in *sphaerolites;* with an increasing carbon-content of the fatty acid-residue, these seem generally to become smaller in circumference.

The formiate crystallises particularly beautifully; this substance possesses, moreover, two solid modifications, as has been also stated by Prof. LEHMANN, who is of opinion that these two correspond with the two solid phases of the cholesterol-derivative. In the phytosterol-ester the sphaerolite-form is the *more-labile one.*

On the other hand, when recrystallised from monobromonaphthalene or almond-oil, they form under the microscope well-formed needle-shaped crystals which, however, are always minute. Probably, we are dealing in all these cases with polymorphism. I have also often observed whimsical groroths and dendritics.

A difficulty occurred in the determination of the melting point of the normal valerate. It melts, over a range of temperature at about 67°.1, but if the mass is allowed. to cool until solidified, the ester fuses to a clear liquid when heated to 30°. This behaviour is quite analogous to that observed with a few glycerides of the higher fatty acids, for instance with *Trilaurin* and *Trimyristin* by SCHEY. [1])

After half an hour the melting point had risen again to $53^1/_2°$ and after 24 hours to 67°. After 24 hours, small white *sphaerolites* had deposited in the previously coherent, scaly and slightly double-refracting layer on the object glass, which exhibited the dark cross of the phytosterol. In order to explain this phenomenon, I think I must assume a *dimorphism* of the solid substance. Moreover, liquid crystals are formed here, as has also been observed by Prof. LEHMANN.

[1]) SCHEY. Dissertatie, Leiden (1899) p. 51, 54.

According to Prof. LEHMANN, normal phytosterol-valerate forms very beautiful liquid crystals, which are analogous to those of cholesterol-oleate; like these they are not formed until the fused mass is undercooled. Consequently, the anisotropous liquid phase is here also *labile* in regard to the isotropous one.

I do not think it at all improbable that the changes in the melting points observed by SCHEY with his higher trygliceride also owe their origin to the occurrence of labile, double-refracting liquid phases. A further investigation is certainly desirable.

§ 8. We now arrive at the discussion of the mutual behaviour of both series of fatty esters in regard to each other.

It has been sufficiently proved by BÖMER that the meltingpoint-line of *cholesterol* and of *phytosterol* is a *rising* line. In connection with MÜGGE's and my own crystal determinations we should have here indeed a gradual mixing between heterosymmetric components! In mixtures which contain about 3 parts of cholesterol to 1 part of phytosterol, the microscopical research appears to point to a new solid phase, which seems to crystallise in trigonal prisms. This compound (?) also occurs with a larger proportion of cholesterol [1]). Whether we must conclude that there is a miscibility of this new kind of crystal with both components, or whether an eventual transformation in the solid mixing phases proceeds so slowly that a transition point in the meltingpoint-line escapes observation, cannot be decided at present.

The matter is of more interest with the esters of both substances. According to BÖMER [2]) the formiates give a meltingpoint-line with a eutectic point; the acetates, however, a continuously rising melting point-line.

The method of experimenting and the theoretical interpretation is, however, rather ambiguous, as BÖMER prepares mixed solutions of the components, allows these to crystallise and determines the meltingpoint of the solid phase first deposited. By his statement of the proportion of the components in the solution used, he also gives an incomplete and confusing idea of the connection between the meltingpoint and the concentration.

Although a *rising* of the binary meltingpoint-line may, of course, be ascertained in this manner quite as well as by other means — and BÖMER's merit certainly lies in the discovery of the fact

[1]) Compare BÖMER, Z. f. Nahr. u. Gen. M. (1901) 546.

[2]) BÖMER, Z. f. Nahr. u. Gen. Mitt. (1901) 1070. In connection with the dimorphism of the formiates, a mixing series with a blank is however very probable in this case.

itself — the determination of the binary meltingpoint-line must be reckoned faulty as soon as it is to render *quantitative* services, which is of importance for the analysis of butter; for if the meltingpoint-curve is accurately known, the quantity of phytosterol added may be calculated from the elevation of the melting point of the cholesterol acetate. I have, therefore, now determined the binary melting point line in the proper manner. (Fig. 4).

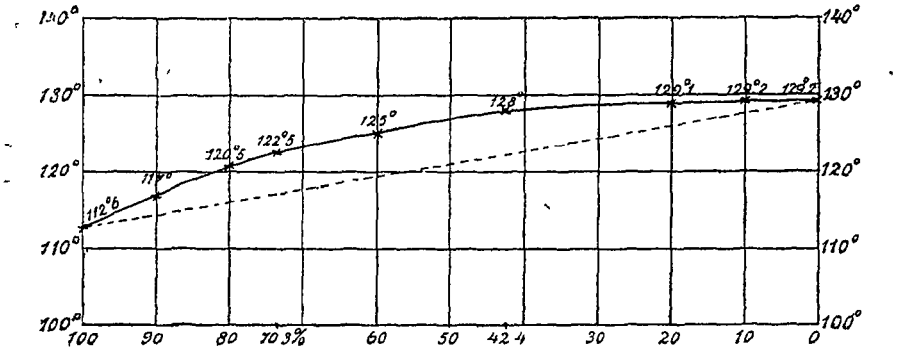


Fig. 4.
Cholesterol-, and Phytosterol-Acetate.

Although the curve takes an upward course it still deviates considerably, from the straight line which connects the two melting-points. As the course of the curve from 40 % cholesterol-acetate to 0 % is nearly *horizontal*, it follows that the composition of mixtures can be verified by the melting-point, when the admixture of phytosterol in the animal fat does not exceed 60 %. The results are the most accurate when the quantity of phytosterol-ester[1]) amounts to 2%—40%. In practice, this method is therefore applicable in most cases. The cholesterol-acetate used in these experiments melted at 112.°8; the phytosterol-acetate at 129.°2.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A mixture of | 90 | % | Chol. | Acet. | + | 10 | % | Phyt. | Acet. | melts | at | 117° |
| » | » | » | 80 | » | » | » | + | 20 | » | » | » | » | » | 120.°5 |
| » | » | » | 73.3 | » | » | » | + | 26.7 | » | » | » | » | » | 122.°5 |
| » | » | » | 60 | » | » | » | + | 40 | » | » | » | » | » | 125° |
| » | » | » | 42.4 | » | » | » | + | 57.6 | » | » | » | » | » | 128° |
| » | » | » | 20 | » | » | » | + | 80 | » | » | » | » | » | 129.°1 |
| » | » | » | 10 | » | » | » | + | 90 | » | » | » | » | » | 129.°2 |

[1]) It should be observed that although Bömer, in several parts of his paper, recommends the said method for qualitative purposes only, it is plain enough in other parts that he considers the process suitable for quantitative determinations in the case of small concentrations. In his interpretation of the melting point line this is, however *not* the case, for his experiments give no explanation as to the mixing proportion of the components in mixtures of definite observed melting point. Quantitative determinations are only rendered possible by a complete knowledge of the binary melting point line. When the concentration of cholesterol-acetate is 0,5 — 1%, the meltingpoint is practically not altered; when it is 2% however, the amount is easy to determine.

Probably, a case of isomorphotropous relation occurs here with the acetates; both esters are, probably, monoclinic, although this is not quite certain for the cholesterol-ester. This is pseudotetragonal and according to VON ZEPHAROVICH: *monoclinic*, with $\beta = 73°38'$; according to OBERMAYER: *triclinic*, with $\beta = 106°17'$, $\alpha = 90°20'$, $\gamma = 90°6'$, while the axial relations are 1,85 : 1 : 1,75.

The phytosterol-ester has been approximately measured microscopically by BEYKIRCH and seems to possess a monoclinic or at least a triclinic symmetry with monoclinic limit-value. In my opinion both compounds are certainly *not* isomorphous. In any case it might be possible that even though a direct isomorphism does not exist in the two ester-series, there are other terms which exhibit isomorphotropous miscibility in an analogous manner, as found for the acetates by BÖMER. I have extended the research so as to include the isovalerates; the result however is negative and the case of the acetic esters seems to be the only one in this series.

The following instance may be quoted:

31.8% cholesterol-butyrate + 68,2% phytosterol-butyrate indicate for $t_1$ 81° and for $t_2$ 83° etc. etc.

For the formiates, the lowering had been already observed by BÖMER; other esters, also those of the iso-acids behave in an analogous manner: at both sides of the melting-diagram occurs a lowering of the initial melting points. It is, however, highly probable that in some, perhaps in all cases, there exists an iso*di*morphotropous mixing with a blank in the series of the mixed crystals.

The anisotropous liquid phase of cholesterol-esters gives rise in this case to anisotropous liquid mixed crystals. I just wish to observe that for some of the lower-melting esters, such as the butyrate, capronate, caprinate, normal valerate, etc., the temperature $t_1$ for these mixed crystals may be brought to about 50° or 60° or lower and this creates an opportunity for studying liquid mixed crystals at such temperatures, which greatly facilitates microscopical experiments.

In all probability, I shall shortly undertake such a study of these substances. Of theoretical importance is also the possibility, to which Prof. BAKHUIS ROOZEBOOM called my attention, that in those substances where $t_2$ answers to the more-labile condition, the at first more labile liquid mixed crystals, on being mixed with a foreign substance, become, finally, stable in regard to the isotropous fused mass. Experiments with these preparations, in this sense, will be undertaken elsewhere. Perhaps, a study of the low-melting derivatives or else a similar study of the low-melting liquid mixed crystals by means of the *ultra-microscope* might yield something of importance.

*Zaandam*, May 1906.