and as the two conjugated lines of a ray s lie likewise on the complex cone of the focus P, they themselves are again rays s.

The complex curve lying in a plane α we find by regarding the two poles A_1 and A_2 of α. The conjugated lines l_1, l_2 of the lines l of α pass respectively through A_1 and A_2, and are conjugated by the rays l one by one to each other, so that two projective nets of rays are formed; the locus of the points of intersection of rays conjugated to each other is a twisted cubic through A_1 and A_2, and furthermore through the four vertices of the cones $T_1 \ldots T_4$; for, the two conjugated lines of the line of intersection of α with $T_2T_3T_4$ are A_1T_1, A_2T_1. The rays s conjugated to the points P of that twisted cubic as foci lie in α and envelope the complex curve; and as each line of the plane $T_2T_3T_4$ can be taken as a ray s conjugated to e.g. T_1, so also the line of intersection with α, the complex conic will touch the four surfaces of the tetrahedron.

Botany. — "*On the demonstration of carotinoids in plants*" (First communication): *Separation of carotinoids in crystalline form*. By Prof. C. VAN WISSELINGH. (Communicated by Prof. MOLL).

(Communicated in the meeting of September 28, 1912).

Many of the chemical, physical, and microscopical investigations on the yellow and red colouring matters of the vegetable kingdom which are grouped under the name carotins [1]) or carotinoids [2]) bear witness to great care and originality. They have, however, not all led to similar results. Especially the microscopical investigation has led to very divergent results which sometimes seriously conflict with those obtained by chemical and physical means. The investigators might be divided into two groups; one is inclined to consider all carotinoids identical; believing that the differences observed are not of a chemical nature. The other group distinguishes several carotinoids.

T. TAMMES [3]) is especially a representative of the first group. After investigating a fairly large number of plants, she comes to the conclusion that the yellow to red colouring matter of plastids, in green, yellow variegated and etiolated leaves, in autumn leaves, in flowers, fruits and seeds, in diatoms, green, blue, brown and red

[1]) CZAPEK, Biochemie der Pflanzen, I. p. 172.

[2]) M. TSWETT, Über den makro- und mikrochemischen Nachweis des Carotins, Ber. d. d. bot. Ges. 29. Jahrg., Heft 9, 1911, p. 630.

[3]) T. TAMMES, Über die Verbreitung des Carotins im Pflanzenreiche, Flora, 1900, 87. Bd. 2. Heft, p. 244.

algae, completely agrees in chemical and physical properties with the carotin from the root of Daucus Carota.

The most recent macrochemical investigations of carotinoids, namely that by WILLSTÄTTER and his pupils have not confirmed TAMMES' results. WILLSTÄTTER and MIEG [1]) isolated two carotinoids from the leaves of the stinging nettle, namely, carotin ($C_{40}H_{56}$) which substance was found to be identical with the carotin from the root of Daucus Carota and xanthophyll ($C_{40}H_{56}O_2$), whilst WILLSTÄTTER and ESCHER [2]) obtain from tomatoes another carotinoid, lycopin ($C_{40}H_{56}$) isomeric with Daucus-carotin. From two objects three different carotinoids were thus obtained, namely, two hydrocarbons and one oxygenated substance.

The great difference between the results of microscopical and macro-chemical investigations determined me to try various methods which have been recommended for the demonstration of carotinoids by microscopical means.

These methods are sometimes divided into direct and indirect ones. The direct ones depend on the addition of reagents which bring about colorations, such as, for example, the beautiful blue coloration with sulphuric acid; the indirect methods are based on the separation of the carotinoids in crystalline form in the cells or tissues. Only in a few cases do the carotinoids occur as crystals in the cells; generally they are combined with, or dissolved in a substance that is fluid, fatty and saponifiable by alkalies [3]). This substance occurs in the plastids, or forms, as in the case of lower organisms, oily drops in the cells [4]). The aim of the indirect methods is to free the carotinoids and to crystallize them out. The following methods belong to this category.

Potash Method.

This method invented by MOLISCH [5]) was used originally for the demonstration of xanthophyll or carotin in green leaves. Fresh leaves

[1]) R. WILLSTÄTTER, Untersuchungen über Chlorophyll, IV. RICHARD WILLSTÄTTER und WALTER MIEG, Über die gelben Begleiter des Chlorophylls, Justus Liebig's Annalen der Chemie, 355. Bd. 1907, p. 1.

[2]) RICHARD WILLSTÄTTER und HEINR. H. ESCHER, Über den Farbstoff der Tomate, Hoppe-Seyler's Zeitschrift für Physiol. Chemie, 64. Bd. 1910, p. 47.

[3]) F. G. KOHL, Untersuchungen über das Carotin und seine physiol. Bedeutung in der Pflanze, 1902, p. 118 et seq.

[4]) W. ZOPF, Zur Kenntnis der Färbungsursachen niederer Organismen, Erste Mitteilung, Beiträge zur Physiol. und Morphol. niederer Organismen, Erstes Heft, 1892, p. 35. Zweite Mitteilung, l. c. Zweites Heft, 1892, p. 5.

[5]) HANS MOLISCH, Die Krystallisation und der Nachweis des Xanthophylls (Carotins) im Blatte, Ber. d. d. bot. Ges. Bd. XIV, 1896, p. 19.

or parts of them are placed in alcoholic potash containing 40% by volume of alcohol and 20% by weight of potassium hydroxyde; they are left in the mixture for several days while light is excluded, until all the chlorophyll has been extracted. With green leaves the potash method gives good results, but also in many other cases, for example, with etiolated, autumn, and variegated leaves, flowers, fruits, algae, etc. We may assume that generally the carotinoids are separated in those cells in which they occur in the living plant. Sometimes the carotinoids wander and form aggregations of crystals in apparently arbitrary places or outside the objects. As a rule the method gives positive results; only in a few cases does it fail.

In order to obtain an idea of the way in which the crystal-formation takes place, I have in a few cases studied the effect of MOLISCH's reagent on the living material under the microscope. The crystallisation will be illustrated by a few examples.

Large yellow plastids are found in the corolla of Calceolaria rugosa. The carotinoid occurs dissolved in a substance, fluid and easily saponifiable, which forms a yellow peripheral layer in the plastids. On the addition of MOLISCH's reagent the plastids sometimes form yellow globules with a sharp edge, which quickly change into globules or masses which show a less well-defined contour and are products of saponification. Often saponification proceeds still more rapidly, so that globules with sharp outline are no longer seen, but the saponification-products appear immediately. They dissolve and out of the solution there grow in a few minutes orange-yellow acicular or narrow band-shaped crystals, often very long and strongly curved and sometimes fissured.

In the ligulate florets of Gazania splendens large orange-coloured plastids occur in which can be distinguished globules that are in constant movement. When MOLISCH's reagent is added they rapidly form orange balls with sharp outline. These arise out of the union of the globules described above. The phenomenon is not the result of saponification, as KOHL [1]) assumes, for warming in water or a stay in dilute spirit (70%) has the same effect. In my opinion it is caused by the cells dying and the plastids losing their structure. In Gazania splendens saponification of the globules formed proceeds very slowly. After being in MOLISCH's reagent for 20 days (in vitro), I saw only orange globules in the cells which were coloured dark-blue by sulphuric acid. When I investigated the flowers after 24 days, I again found many orange globules, but at the same time there

[1]) F. G. KOHL, l. c. p. 122.

were also many well-formed crystals, orange crystal-plates with rounded ends and aggregates of the same crystal-plates. The crystals give the various colour-reactions of carotinoids and the same is the case with the orange globules, in proof, that all the carotinoid has not yet crystallized out.

The formation of crystals by the potash method is easily explained. In the living plant the carotinoids occur in solution. They are dissolved in a fluid, fatty substance. When MOLISCH's reagent is added the plastids are destroyed and the fluid substance forms globules, which are coloured orange-yellow or orange by the carotinoid. MOLISCH's reagent farther brings about saponification and solution. The oily substance is saponified and the cells are filled with a solution of the saponification-product in which the carotinoid is soluble. This solution is diluted by the reagent in which the objects are placed and the carotinoids, which are not soluble in MOLISCH's reagent, separate in the cells.

By reason of the above facts, I assumed that the carotinoids must be soluble in soap-solutions. This was indeed found to be the case. If, for example, after being washed out with water, preparations, in which carotinoids occur in the form of crystals, are placed in soap-spirit (Spiritus saponatus Pharm. Nederl. Ed IV without oil of lavender) the crystals dissolve.

As is evident from the examples described above, the saponification of the fatty substance and the separation of crystals sometimes takes place rapidly and sometimes very slowly. According to the nature of the object minutes, hours, days, weeks, or months are required for the separation of the crystals. Among objects which require much patience are the following in addition to the ligulate florets of Gazania splendens those of Hiëracium aurantiacum, Doronicum Pardalianches and Taraxacum officinale, in which crystals were observed after 24, 48 and 74 days respectively. In the ligulate florets of Hiëracium murorum and Inula Helenium and in the petals of Viola cornuta no crystals were perceived after 60, 39 and 29 days respectively. That the carotinoids do not separate out in these last examples, must be attributed to the fact that the oily substance is not saponified and holds the carotinoid in solution. The yellow or orange-yellow globules, which are seen in the cells, are coloured blue by sulphuric acid, transient blue by bromine water and green by iodine in potassium iodide; this proves that the carotinoid is still present.

I do not think that the long continued action of MOLISCH's reagent is accompanied by any disadvantage. I have no indication that the carotinoids are destroyed by it and often fine crystallisations are finally obtained. I have tried MOLISCH's potash method in about 40 cases and

only in the 3 last-mentioned was there no crystallisation of carotinoid. It is however possible that in these cases also a still more prolonged action might have had the desired result.

TAMMES [1]) and KOHL [2]) maintain that all the crystals, which are obtained by the potash method consist of carotin, however they may differ in colour and shape. The colour would only depend on the thickness of the crystals and of the angle at which they are seen. BECKE [3]) however, considers as a result of crystallographic investigations that the crystals obtained by MOLISCH's method are not identical. I myself have come to the following conclusions. Before proceeding I wish to remark that the names of the colours which I use are in agreement with those of KLINCKSIECK et VALETTE's Code des Couleurs, 1908. Often the numbers, given to the colours in the book, have also been mentioned.

In many cases the crystals differ greatly in colour and shape. In general, with respect to the colours the crystals can be arranged in two groups, namely, orange-red and red (Kl. et V. 91, 76, 51, 46) to which the violet-red (Kl. et V. 581) of the fruit of Solanum Lycopersicum are allied and a second group composed of orange-yellow and orange crystals (Kl. et V. 176, 151, 126, 101). The colour is also influenced by the thickness of the crystals. Red is always present in the first group, but not in the second.

However different the shape of the crystals may be, it is still true that colour and form are often connected. Among the red crystals, as a rule well-developed plates are found which have the shape of small parallelograms and sometimes also of rhombs. Generally small plates are formed which are several times more long than broad or narrower ones which resemble pointed needles. The parallelograms and rhombs are often imperfect. Parts may be wanting, angles and sides may be rounded. Very often the red crystals form aggregates. The root of Daucus carota and the fruit of Solanum Lycopersicum belong to the objects in which carotinoids occur in the form of crystals. In Daucus the carotin has formed in addition to well-developed red parallelograms and rhombs all sorts of other crystals which are even curved band shaped. In the tomato lycopin is found in the form of red-violet needles.

The orange-yellow and orange crystals are also very varied. Especially in those cases in which the carotinoids crystallize out slowly, little plates of crystal are often found which are generally

[1]) l. c. p. 242, 244.
[2]) l. c. p. 33 et seq. and p. 67.
[3]) HANS MOLISCH, l. c. p. 24.

several times more long than broad, rarely about as long as broad. The ends are for the most part rounded, occasionally pointed, irregular or more or less straight. Often oval and whetstone-shaped crystals are found. Once they were seen as rhombs with rounded sides. In a few cases the crystals showed large surfaces of indefinite shape, in other cases the surfaces were narrow, long and slightly curved. The multifarious ribbon- and needle-shaped crystals that occur are allied to this last-mentioned form. These are generally much curved. Straight needles are rare. The ribbon-shaped crystals are often branched or split up into separate curved needles. Finally connected with the curved, needle-shaped crystals there are filamentous ones, which may be very much twisted and often form clews. The crystal plates often form aggregates.

When leaves are treated with MOLISCH's reagent aggregates of crystals are generally formed in the cells which contain chlorophyll; they are composed of orange-yellow plates and red crystals resembling needles.

The shape of the orange-yellow and orange crystals often differs in one and the same object. In the flower of Dendrobium thyrsiflorum I found orange-yellow (Kl. et V. 151) whetstone-shaped plates and orange-yellow (151) thread-like crystals, also aggregates of fine needle-shaped crystals coloured bright orange (101) and to some extent orange-red (81). In the flower of Cucurbita melanosperma I found thread-like crystals and very thick, almost completely straight, flat needles in the hairs.

The shape of the crystals is sometimes very dependent on external circumstances, as for example, on the quantity of MOLISCH's reagent into which the object is put. In the petals of Chelidonium majus, for example, I got thread-shaped crystals whenever I placed them in a flask with a large quantity of MOLISCH's reagent, and crystal plates when a petal was placed in a small quantity of MOLISCH's reagent between a cover-slip and a slide.

However diverse the crystals may be there is an important point of difference between the red and orange-red on the one hand and the orange-yellow and orange crystals on the other hand, namely, that when the carotinoids have separated out in the form of plates, among the former well-shaped parallelograms are nearly always formed and these are not met with among the orange-yellow and orange crystals.

In the leaves of Urtica dioica I was able to observe that the quantity of the reagent may influence not only the shape of the crystals but also the place where they are formed. By using much

of MOLISCH's reagent a small aggregate of orange-yellow and red crystals appears in every cell; with little of the reagent I got large red and yellow aggregates of crystals in various places in the tissue or outside it. This last result need cause no surprise. The carotinoids are soluble in the solution of the saponification-products formed and not in MOLISCH's reagent. When a small quantity of the reagent is used no quick dilution of the soap-solution occurs and the carotinoids will have the chance of changing their place in the tissue. In general it is therefore preferable not to use too small a quantity of the reagent, unless for any reason large aggregates of crystals are desired.

I have applied the potash method of MOLISCH to the following objects.

Flowers: Trollius caucasicus Stev., Nuphar luteum Sm., Chelidonium majus L., Meconopsis cambrica Vig., Corydalis lutea DC., Erysimum Perofskianum Fisch. et Mey., Sinapis alba L., Isatis tinctoria L., Viola cornuta L. var. Daldowie Yellow, Cytisus sagittalis Koch (Genista sagittalis L.), Cytisus Laburnum L., Spartium junceum L., Thermopsis lanceolata R. Br., Cucurbita melanosperma A.Br., Ferula sp., Inula Helenium L., Doronicum Pardalianches L., Doronicum plantagineum L. excelsum, Calendula arvensis L., Taraxacum officinale Wigg., Hiëracium aurantiacum L., Hiëracium murorum L., Gazania splendens Hort., Asclepias curassavica L., Calceolaria rugosa Hook., Dendrobium thyrsiflorum Rchb. fil., Iris Pseudacorus L., Narcissus Pseudonarcissus L., Clivia miniata Regel, Tulipa hortensis Gaertn., Fritillaria imperialis L., Lilium croceum Chaix, Hemerocallis Middendorffii Trautv. et Mey.

Green leaves: Chelidonium majus L., Taraxacum officinale Wigg., Urtica dioica L., Triticum repens L., Selaginella Kraussiana A.Br.

Yellow variegated leaves: Sambucus nigra L. fol. var., Graptophyllum pictum Griff., Croton ovalifolius Vahl.

Fruits: Sorbus aucuparia L., Solanum Lycopersicum Trn.

Root of Daucus Carota L.

Algae: Cladophora sp., Spirogyra maxima (Hass.) Wittr., Haematococcus pluvialis Flot.

It must be noted that in four of the above-named objects in their natural state carotinoids already occur in the form of crystals, namely in the root of Daucus Carota, the fruits of Sorbus aucuparia and of Solanum Lycopersicum, and in the flower of Clivia miniata.

Acid method.

By treating parts of green plants with dilute acids FRANK [1]) observed

[1]) A. TSCHIRCH, Untersuchungen über das Chlorophyll, Landwirtsch. Jahrbücher, XIII. Bd., 1884, p. 490. HANS MOLISCH, l.c. p. 26.

the formation of red or reddish yellow crystals, especially in the stomata. MOLISCH [1]) repeated the experiment with the leaves of *Elodea* and also observed such crystals which according to him, correspond to the crystals he obtained by his potash method. TAMMES [2]) experimented on a great number of plants and various parts of plants with dilute acids, as, for example, hydrochloric acid, oxalic acid, tartaric acid, chromic acid, picric acid, acetic acid, and hydrofluoric acid. Picric acid was used in a solution of alcohol, the other acids in aqueous solutions of various strengths. The investigation yielded positive results in the case of leaves, and other green parts of plants, flowers, green algae and Fucoideae. In all the cases investigated, over 30, crystals were obtained after some hours or days which, according to the above-mentioned writer, agreed completely with the crystals which had been obtained by the potash method and were found to consist of carotin. With yellow variegated, yellow autumn and etiolated leaves the experiment was without result, a fact which TAMMES [3]) is unable to explain.

When plants or parts of plants which contain chlorophyll are investigated with dilute acids allowance must be made for the action of the acids on the chlorophyll. When MOLISCH's reagent is used the chlorophyll dissolves with saponification of the ester, separation of phytol and formation of chlorophyllin potassium [4]), but the action of acids on the chlorophyll produces insoluble derivatives. WILLSTÄTTER, who treated alcoholic extracts obtained in the cold from dried plants with acids, obtained, when the magnesium had been eliminated, phaeophytin [5]), which like chlorophyll [6]) consists of two component parts, namely, phaeophytin a (phytylphaeophorbide a) and phaeophytine b (phytylphaeophorbide b). Earlier investigators also already

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[1]) l. c. p. 26.

[2]) l. c. p. 216 et seq. and p. 242 et seq.

[3]) l. c. p. 220.

[4]) RICHARD WILLSTÄTTER, (Untersuchungen über Chlorophyll), II. Zur Kenntnis der Zusammensetzung des Chlorophylls, Justus Liebig's Annalen der Chemie, 350. Bd. 1906, p. 48.

RICHARD WILLSTÄTTER und FERDINAND HOCHEDER, III. Uber die Einwirkung von Säuren und Alkalien auf Chlorophyll, l. c. Bd. 354, 1907, p. 205.

[5]) R. WILLSTÄTTER, II. Zur Kenntnis der Zusammensetzung des Chlorophylls, l c.

R. WILLSTÄTTER und F. HOCHEDER, l. c.

RICHARD WILLSTÄTTER und MAX ISLER, XX. Über die zwei Komponenten des Chlorophylls, l. c. Bd 390, Heft 3, 1912, p. 269.

[6]) RICHARD WILLSTÄTTER und MAX UTZINGER, XVI. Über die ersten Umwandlungen des Chlorophylls, l. c. 382. Bd. p. 129.

R. WILLSTÄTTER und M. ISLER, l. c.

obtained products produced by the action of acids on chlorophyll. HOPPE-SEYLER [1]) obtained from grass by extraction with boiling alcohol a crystalline chlorophyll derivative, which he subjected to a number of operations in order to separate it from other substances and to purify it. He named it chorophyllan. TSCHIRCH [2]) states that when parts of plants that contain chlorophyll are treated with acids, chlorophyllan crystallizes out in the cells. WILLSTÄTTER, ISLER, and HUG [3]) have after further investigation compared the chlorophyllan of HOPPE-SEYLER to phaeophytin. In the opinion of these investigators it is not a pure compound but chlorophyll more or less decomposed by plant acids and allomerised by treatment with solvents. For this reason they consider the name chlorophyllan unsuitable for the chlorophyll derivative obtained by means of acids.

TAMMES [4]) also discussed the action of acids on chlorophyll and comes to the conclusion that the formation of chlorophyllan offers no hindrance to the demonstration of carotin, because, although it must be admitted that the crystals obtained may perhaps be contaminated by some chlorophyllan, yet in the main they are composed of carotin. KOHL [5]) evidently agrees with TAMMES. He writes: "Mehr oder minder unbewusst ist die Säuremethode schon früher von einigen Forschern angewandt worden, unbewusst insofern, als das auskrystallisirende Carotin irrtümlich für Chlorophyllan gehalten und nur in einzelnen Fällen als solches erkannt wurde." I consider TAMMES' reasoning inconclusive, whilst KOHL does not further explain his views. A simple investigation of the crystals shows that they are very different from carotin-crystals and there is even no reason to assume that they contain any carotin.

I exposed fresh plants and parts of plants containing chlorophyll to the action of acids at the ordinary temperature, oxalic acid from 1% to 10%, hydrochloric acid of 5%, tartaric acid of 10% and hydrofluoric acid of 2%. Without exception after a day crystals had separated out. They form small aggregates attached to the chromatophores. The crystal aggregates resemble spherical bodies, but with high magnification the constituent crystal plates can be distinguished. Only in one case, namely in Cladophora, did I see long whip-shaped crystals projecting from the crystal aggregates. The crystal aggregates are not yellow, orange yellow, or red, but brown. In acetone they

1) F. HOPPE-SEYLER, Zeitschr. f. physiol. Chemie 3, 1879, p. 339.
2) A. TSCHIRCH, Untersuchungen über das Chlorophyll, l. c. p. 441.
3) R. WILLSTÄTTER und M. ISLER, l. c. p. 287 et seq. and p. 337.
4) l. c. p. 217 and 218.
5) l. c. p. 47.

are easily soluble, slowly in glacial acetic acid. With concentrated or somewhat dilute sulphuric acid, for example $66^1/_2\%$, they are not coloured blue, but green (KL. et V. 326). The colour never resembles the blue colour which the crystals of carotinoids assume with sulphuric acid, and which never shows a green, but sometimes a slightly violet tint. The green-coloured crystal aggregates are soluble in sulphuric acid. The brown crystal aggregates are also coloured green by concentrated hydrochloric acid (specific gravity $= 1.19$); afterwards they dissolve slowly. With concentrated nitric acid they are not, as is the case of carotinoids, temporarily coloured blue; they deliquesce and form globules, which when gently warmed, gradually become colourless and presumably consist of phytol. Nor do they, like carotinoids, become temporarily blue in bromine water; the brown colour at first remains unchanged. Towards caustic potash the brown aggregates of crystals also behave quite differently from the crystals of carotinoids; they are entirely soluble in it; they also are completely soluble in dilute alcoholic caustic potash, as, for example, in MOLISCH's reagent, in which the crystals of carotinoids are of course insoluble. Since they leave nothing behind on solution there is no reason for thinking that they contain carotinoids.

The behaviour of the brown aggregates towards reagents shows that they are composed of a chlorophyll derivative. Phaeophytin [1]) gives the same reactions, and sometimes more or less clearly shows crystalline structure. TAMMES and KOHL have confused carotin with a chlorophyll derivative. TAMMES' drawing N°. 22 of Elodea canadensis in particular clearly shows that such a confusion has taken place. In each cell a number of brown, round crystal aggregates are figured attached to and on the chromatophores. The crystalline structure is not indicated in the figure, but is not always easily distinguished in the full cells. Besides these crystal aggregates, I found in many cells, though not in all, red aggregates of crystals which resemble carotin and which are coloured blue by concentrated or somewhat dilute sulphuric acid, namely of 76%. These crystal aggregates are however not figured by TAMMES, nor are they mentioned.

Now it is somewhat explicable why TAMMES [2]) obtained negative results with yellow variegated, yellow autumn and etiolated leaves. These objects or the yellow parts of them contain no chlorophyll and are therefore unable to produce brown crystal aggregates of a chlorophyll derivative. But this does not, however, clear up every-

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[1]) R. WILLSTÄTTER und F. HOCHEDER, l. c. p. 222 and 223.

[2]) l. c. p. 220.

thing. For the non-green leaves and the parts which are not green, yet contain substances which belong to the carotinoids. How is it that these were not found by TAMMES, whilst in other non-green parts of plants such as flowers, TAMMES obtained after some days in all the eight cases investigated well-formed crystals which with reagents showed the reactions proper to carotin. I am convinced by the use of MOLISCH's reagent that carotinoids exist in the yellow parts of yellow variegated leaves. Sometimes I obtained separation of orange-yellow crystals, in other cases they were orange-yellow and red, but all gave the reaction proper to carotinoids. KOHL [1]), with etiolated leaves, arrived at a different conclusion from that of TAMMES. I cannot refrain from remarking that KOHL does not always correctly reproduce the results of TAMMES, with whom he is in entire agreement. The following is quoted from TAMMES [2]): Ich habe auch gelbbunte, herbstlich gelbe und etiolirte Blätter in verdünnte Säurelösungen gebracht, aber stets mit negativen Resultaten. And from KOHL [3]): Durch die neueren Untersuchungen der etiolirten Pflanzen mit Säuren, welche T. TAMMES in grosser Zahl ausführte und welche ich, um in die unsicheren Anschauungen einige Klarheit zu bringen, planmässig fortgesetzt habe, ist es nun mit Sicherheit erwiesen, dass in allen etiolirten Pflanzenteilen, so weit sie gelb gefärbt, mit verdünnten Säuren Carotin-Krystalle zur Ausscheidung gebracht werden können.

I treated objects, both with and without chlorophyll, such as green and yellow variegated leaves, yellow, orange-yellow, and orange flowers, and algae, with dilute acids at the ordinary temperature, namely, with 1%, 2% and 10% oxalic acid, 1% and 5% hydrochloric acid, 10% tartaric acid and 2% hydrofluoric acid solutions. The treatment often lasted one or two months. The objets which were subjected to this investigation, were the same as those investigated by the potash method of MOLISCH.

In the case of green leaves I obtained with the dilute acids the above mentioned brown crystalline aggregates of a chlorophyll derivative which were formed in each cell containing chlorophyll, and here and there in the tissue red crystals, loose plates or aggregates. In the case of flowers, of which I investigated about 25, I generally obtained no crystals with dilute acids. Only in two cases was there a positive result, namely, in Asclepias curassavica, where red crystals separated and in Calceolaria rugosa where orange-

[1]) l. c. p. 48.
[2]) l. c. p. 220.
[3]) l. c. p. 48.

yellow ones appeared. In one of the yellow variegated leaves, namely, of Graptophyllum pictum I obtained the separation of small orange-yellow crystals in the yellow portion of the leaf. The crystals which had separated behaved towards reagents and solvents exactly as did the corresponding crystals obtained by the potash method.

With regard to the investigation of flowers with dilute acids, TAMMES' [1]) results and mine differ. Whilst she obtained well formed crystals in all cases, I obtained them only exceptionally. Our investigations were however mostly concerned with different flowers. I propose if possible to examine with acids those flowers which have been studied by TAMMES, but not yet by myself, in order to reach greater certainty on this point of difference. Whatever the ultimate results, I nevertheless already venture to state, that the method of inducing crystallisation of the carotinoids in plants by means of acids cannot in general be recommended. Often the yellow carotinoids in particular do not crystallize. Red crystals very often form in the tissue but not in all cases in which they can be obtained by the use of the potash method. This is the case, for example, in the flowers of Nuphar luteum, Isatis tinctoria, Cytisus Laburnum and Thermopsis lanceolata as also in the peduncles of Trollius caucasicus. In these many oranje-yellow and a few red crystals were obtained by MOLISCH's reagent, whilst in the flower of Asclepias curassavica, in which, as stated above, red crystals had been separated out by means of acids, MOLISCH's reagent produced many red as well as a few orange-yellow crystals. When the carotinoids which yield red crystals are present in great quantity, they can therefore be demonstrated by acids, but when they are present in small quantity, they escape observation.

Another drawback to the acid method is that the carotinoids which yield orange-yellow crystals are very liable to decompose. Continuous treatment with acids as is necessary with the acid method, often is very harmful and may lead to complete decomposition of the carotinoids. They are much more liable to decomposition by acids, while they are still in solution in the fatty substance of the plastids, than when they have been separated as crystals by some other method. According to HUSEMANN [2]) WACKENRODER pointed out this decomposition so far back as 1832. In the treatment with acids I have sometimes found decomposition to occur even in the first few days. The colour of the flowers becomes paler and the

[1]) l. c. p. 248.

[2]) A. HUSEMANN, Über Carotin und Hydrocarotin, Ann. der Chem. u. Pharm. Bd. CXVII, 1861, p. 200.

yellow or orange oily globules and masses, which have been formed in the cells and which contain the carotinoid, also lose more or less of their colour. Sulphuric acid then no longer colours them blue or much more feebly than at the beginning of the experiment. The carotinoid decomposes without crystallising out. This decomposition is easily confirmed in Chelidonium majus, Narcissus Pseudonarcissus, Doronicum Pardalianches and Tulipa hortensis, for instance.

Resorcinol Method.

TSWETT [1]) has described a method of crystallising the carotinoids from plants and parts of plants under the microscope. The objects are placed on the microscope slide in a concentrated solution of resorcinol, containing 10 to 12 parts of resorcinol in 10 parts of water. I have used this method in eight cases, namely, in the leaves of Urtica dioica, in the flowers of Chelidonium majus, Erysimum Perofskianum, Gazania splendens, Calceolaria rugosa and Narcissus Pseudonarcissus, in Cladophora sp. and in Haematococcus pluvialis. In five cases, namely, in Urtica, Chelidonium, Calceolaria, Narcissus and Cladophora crystals separated rather quickly. In Chelidonium, Calceolaria and Cladophora crystals appeared in the cells, in the other two cases in and around the preparations. Erysimum, Gazania and Haematococcus which had given positive results with the potash method, gave negative results with the resorcinol solution. In the case of Haematococcus pluvialis JACOBSEN [2]) was also unable to obtain separation of crystals.

The shape of the crystals differs greatly. When with MOLISCH's reagent red and orange-yellow crystals were obtained, crystals of the same colour were formed with TSWETT's reagent in those cases in which the experiment gave a positive result. With respect to reagents the crystals behave in the same way as the carotinoid crystals obtained by the potash method.

TSWETT [3]) has also pointed out the variation in the crystals and has shown in Lamium by his adsorption method that different chemical bodies are present, carotin and xanthophyll. It seems to me that TSWETT's method will be applicable with success to many cases.

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[1]) M. TSWETT, Über den makro- und mikrochemischen Nachweis des Carotins, Ber. d. d. bot. Ges. 29. Jahrg. Heft 9, 1911, p. 630.

[2]) H. C. JACOBSEN, Die Kulturbedingungen von Haematococcus pluvialis, Folia Microbiologica I, 1912, p. 25.

[3]) l. c.

Other methods.

KOHL [1]) has remarked that possibly other substances also might be used to bring about the crystallisation of carotin. He surmises that chloralhydrate might be used for the purpose and intends to investigate this possibility. When the solvent action of chloralhydrate upon the various constituents of cells is considered and it is seen that carotin crystals in contrast to those of xanthophyll are fairly resistant, then it is natural to suppose that chloralhydrate may offer a suitable means of separating carotin as crystals. I have experimented with the leaves of Urtica dioica in a concentrated aqueous solution (7 in 10) of chloralhydrate. We know from the investigations of WILLSTÄTTER and MIEG [2]) that these leaves contain carotin and xanthophyll. When I placed a small piece of the tissue containing chlorophyll in a solution of chloralhydrate and observed it under the microscope, I could soon detect changes in the chromatophores and the formation of a globule in each cell, which gradually dissolved and left behind a small aggregate of red carotin crystals. Orange-yellow crystals of xanthophyll were not separated. As was to be expected therefore the method is of no use for the separation of xanthophyll because decomposition takes place. I cannot moreover recommend it for the separation of carotin-crystals, because carotin is also attacked by chloralhydrate and small quantities therefore may escape observation.

According to WILLSTÄTTER and MIEG [3]) xanthophyll is "spielend löslich" in phenol. Having in mind the solubility of many substances in liquefied phenol and having confirmed the fact that carotin dissolves much more slowly than xanthophyll, it occurred to me to try liquefied phenol for the separation of carotin or allied carotinoids. I used two mixtures, one of 10 parts by weight of phenol in loose crystals and 1 part by weight of water, the other consisting of 3 parts by weight of phenol in loose crystals and 1 part by weight of glycerine. I prefer the latter mixture, because it mixes more quickly with the water contained in the objects. I examined the flowers of Erysimum Perofskianum, Asclepias curassavica, the leaves of Urtica dioica and the ligulate florets of Taraxacum officinale. With petals of Erysimum Perofskianum the potash method yielded no beautiful result, and the acid method a negative one. I placed parts of the petals in the above mixtures between a miscroscope slide and a cover-slip. Under the

[1]) l. c. p. 124.
[2]) l. c. p. 10.
[3]) l. c.

microscope I saw that the brightly coloured orange-yellow plastids quickly formed orange-yellow globules; crystals soon appeared in these globules. While the globules dissolve the crystals remain behind. These are orange-red plates and aggregates which very slowly dissolve in the phenol mixtures. To investigate these, the preparations can be washed out successively with dilute alcohol (70 %) and with water. With reagents they give the reactions characteristic of carotinoids.

When parts of the flower of Asclepias curassavica are placed in the mixture of phenol and glycerine, there quickly appear in all the cells numerous light and dark red or orange-red (Kl. et V. 11, 46, 51, 71, 91) crystals, in the same way as in Erysimum Perofskianum, among which were many plates and aggregates. They do not dissolve in the phenol solution; at any rate after three days they were still unchanged. When investigated with reagents in the way indicated above, they show the reactions proper to carotinoids. In Urtica dioica orange-red (81) crystal aggregates are formed here and there in the tissue, which after three days are still present in the mixture of phenol and glycerine. In the ligulate florets of Taraxacum officinale yellow globules soon arise; in this case no crystals occur; the globules completely dissolve. Clearly in these four objects carotinoids occur, which differ greatly with respect to their solubility in a mixture of phenol and glycerine (3 to 1), and are either insoluble or dissolve slowly or readily. In the last case the carotinoids do not separate.

Willstätter and Mieg [1]) have dealt with the question whether, in addition to carotin, xanthophyll is also present as such in the living plant and have answered it affirmatively. Both substances, can indeed be separated with simple solvents, carotin from dried leaves with petroleum ether, xanthophyll from alcoholic extracts of fresh leaves according to the "Entmischungsmethoden" of G. Stokes, G. Kraus, H. C. Sorby and R. Sachsse [2]). It is therefore reasonable to assume that in some cases the use of simple solvents in which the carotinoids themselves are but little or not soluble, might lead to the crystallisation of these substances. In a few cases I have indeed succeeded in doing this.

With the ligulate florets of Taraxacum officinale and Doronicum Pardalianches I did at first not succeed in crystallising even a part of the carotinoid by means of the potash method. It remained in solution in the yellow or orange-yellow globules which had formed in the cells. When I had treated the ligulate florets for a very short time with absolute alcohol or a certain quantity with very

[1]) l. c. p. 10.

[2]) See Willstätter und Isler, l. c. p. 275 et seq.

little absolute alcohol, I ascertained, that the oily substance which retained the carotinoid, was dissolved and that part of the latter had separated more or less crystalline and gave the reactions characteristic of carotinoids. Direct treatment of the florets with absolute alcohol led to similar results. When the treatment with absolute alcohol is prolonged or when too much of it is taken, the carotinoid dissolves completely.

In a few cases I succeeded in obtaining even with dilute spirit the separation of carotinoids in crystalline form. After being placed for one day in 70 % spirit the corolla of Calceolaria rugosa was seen to contain orange-yellow crystals, loose plates and aggregates. The petals of Chelidonium majus when soaked for a month in 20 % spirit are found to contain not only orange-yellow and yellow drops and globules but also orange-yellow needle and thread-shaped crystals, some straight and some very much curved. They are often attached to the globules and give the impression of having grown out of them. In the flower of Narcissus Pseudonarcissus crystallisation of the carotinoid took place already after one day in 20 % spirit. Long continued treatment with dilute spirit may cause the complete decomposition of the carotinoid; this was already the case in Narcissus Pseudonarcissus after a few days.

Finally I wish to point out that on account of ARNAUD's [1]) investigations it must be assumed that the results sometimes depend greatly on the season of the year. ARNAUD found, for instance, that the leaves of the chestnut and the stinging nettle contain most carotin during the flowering time (May). I also found that the separation of crystals in one and the same species was not always the same. This was especially the case in Cladophora, in which treatment with MOLISCH's reagent sometimes resulted in the separation of many orange-yellow and a few red crystals, and at other times yielded many red and a few orange-yellow ones. It is desirable to point out this difference. When these experiments are repeated by other investigators it must be taken into account.

It must be admitted that the results of the above crystallisation experiments point strongly to the frequent occurrence of several distinct carotinoids in a plant. In a subsequent communication the behaviour of carotinoids with respect to reagents and solvents will be dealt with and the results of the direct and indirect methods will be summarised.

[1]) A. ARNAUD, Recherches sur la carotine; son rôle physiol. probable dans la feuille. Compt. rend. CIX, 1889, 2, p. 911.

Astronomy. — "*Determination of the geographical latitude and longitude of Mecca and Jidda executed in* 1910—'11." By Mr. N. SCHELTEMA. Part I. (Communicated by Prof. E. F. VAN DE SANDE BAKHUYZEN).

(Communicated in the meeting of May 25, 1912).

I. *Introduction.*

Mecca as we know is the holy city and the meeting-place for Mohammedan believers. Yearly some 200.000 gather there from different parts of the world in order to make their pilgrimages and many of them stay there for a couple of years to gain a thorough knowledge of the doctrines of their religion.

From an economical and political point of view as well as for the history of religion Mecca is a place of great significance. Moreover it forms an important starting-point for the geography of the interior of Arabia. Hence it is not surprising that constant efforts have been made to obtain closer and the most accurate possible knowledge about this centre of the Islam; but great and peculiar difficulties are connected with these endeavours on account of the fact that entrance into the "holy domain" is strictly prohibited to non-Mohammedans. Only now and again a few Eurepeans succeeded in stealthily penetrating into it and spending there some time.

It is well known that among these stands first our compatriot the present professor Dr. C. SNOUCK HURGRONJE, who spent some eight months in Mecca and put down his exhaustive researches in his standardwork about this town. It stands to reason that my position as Consul of the Netherlands at Jidda, the harbour of Mecca, often brought me into contact with this scholar, and it was he who in the course of our talks drew my attention to the fact that so much scientific work might be done in the Hedjaz. In particular he pointed out that even the geographical position of Mecca was not accurately known and he raised the question if I might not supply this deficiency.

Others had succeeded in making fairly accurate plans of the town but its absolute position had not yet been determined with sufficient exactness. Lack of good instruments, which are not easily transportable and the necessity of taking care that no attention was drawn in the vicinity had generally prevented astronomical observations.

The only person by whom direct determinations of the latitude and the longitude of Mecca have been published is ALI BEY EL ABASSI, or at any rate the man who under that name travelled in many