Botany. — "*Dipsacan and Dipsacotin, a new chromogen and a new colouring-matter of Dipsaceae*". By Miss T. Tammes. (Communicated by Prof. J. W. Moll).

If leaves of *Dipsacus sylvestris* are heated for a few hours in a moist space to a temperature of 60° C., they acquire a fine dark-blue coloration. I have more closely investigated this phenomenon, which once accidentally came to my notice, and have studied the conditions of the formation of the blue colouring-matter *dipsacotin*, its properties and those of the chromogen *dipsacan*, the localisation of the latter and its distribution in the vegetable kingdom. At the same time I have traced the occurrence of *dipsacase*, the enzyme which splits the chromogen.

Here I wish briefly to communicate the chief results of the investigation; a more detailed paper on this subject will be published in Recueil des Trav. bot. Néerl. Vol. V, 1908.

The investigation, which was chiefly carried out with radical leaves of *Dipsacus sylvestris* and *fullonum*, has shown that for the formation of the blue colouring-matter a temperature of at least 35° C. and the presence of water and oxygen are necessary.

Between 35° and 100° C. the rate of formation of dipsacotin increases with the temperature. It is only formed after the death of the leaf. No blue colouring-matter is formed in the living plant, even when exposed for several days to a temperature of 35°—40° C.; the pigment only appears in the dead leaves, when the plant is dying off.

If leaves are dried very rapidly at a temperature above 35° C., no dipsacotin is formed, or only a very small quantity; if, however, during the warming, the leaves are in a moist atmosphere,t hey are coloured blue.

Neither does the blue coloration occur when oxygen is absent. Since it is extremely difficult to free the leaves completely from air, I have proved in another way, that oxygen is necessary. The chromogen can be extracted by warm water, and if the extract is warmed in a space completely shut off from the air, no dipsacotin is formed, even on heating for days together. As soon as the extract is warmed in contact with the air, the blue colour rapidly appears.

The formation of dipsacus-blue is therefore accompanied by an oxidation. Experiments have shown, however, that the colouring-matter does not result directly from dipsacan by oxidation. An intermediate product is first formed, as is shown by the fact that the light yellow extract becomes yellowish red on being heated in a space shut off from the air, and that the yellowish red solution has acquired the property of turning blue even without being heated. In the formation of dipsacotin from dipsacan a chemical transformation, which can only occur on warming, evidently takes place first; the subsequent oxidation can also proceed at the ordinary temperature, although it is greatly accelerated by warming.

Of the properties of dipsacotin I only propose to mention, that this colouring matter is soluble in water, that it is decomposed by sulphuric acid with the formation of a yellowish red product, and that it is decomposed by light; three points in which it differs from indigo.

The chromogen dipsacan is decomposed by acids and by alkalies, and can only exist in a feebly acid solution, such as that of the extract. Acids and alkalies do not, however, even on heating, produce the transformation-product which by oxidation forms dipsacotin. This is formed from dipsacan, not only by warming above 35° C., but also at the ordinary temperature, through the agency of dipsacase, the enzyme occurring in the plant. This perhaps explains an observation made long ago by DE VRIES [1]), that the press-juice of *Dipsacus fullonum* becomes black after a few days' exposure to the air. Probably the juice contains both the chromogen and the enzyme, and the former is decomposed by the latter. That the colour, after oxidation, is black and not blue, may perhaps be attributed to the presence of other substances, or to other chemical reactions taking place simultaneously.

Dipsacan occurs in all organs, even including the flower and the seed, and all tissues, except the pith of the stem, contain it. The cellwall is probably free from dipsacan, as it does not become coloured blue.

The quantity of the chromogen, present in the various organs, depends on internal and external causes Young parts growing vigorously, contain most. Under favourable conditions of life the quantity is larger than under unfavourable; at temperatures which approach the limits of life of the plant, the quantity of dipsacan is less. Light exercises no direct influence on the presence of the chromogen. In the dark the dipsacan does not disappear from the leaves, but it is formed in new, completely etiolated ones. Dipsacan is therefore not directly related to carbon-assimilation. More probably the chromogen takes part in metabolism, and as it occurs in the plant in such large

[1]) HUGO DE VRIES, Een middel tegen het bruin worden van plantendeelen bij het vervaardigen van praeparaten op spiritus. Maandbl. v. Natuurw. 1886, No. 1.

quantity, and especially in parts growing vigorously, it must indeed be an important substance to the plant. I imagine that dipsacan is continually formed and continually decomposed in the plant, and that the product of transformation, most probably that product which yields dipsacotin on oxidation, is used in various vital processes. In those places, where it is required, it is formed by the enzyme from the dipsacan present, and since it is not oxidized in the living plant to dipsacus-blue, we must conclude, that it is used up at once. Probably therefore dipsacan is the form under which the product used in metabolism, is stored up by the plant. This view not only explains the presence of the enzyme, but also the fact, that no dipsacus-blue is formed during life.

Besides in *Dipsacus sylvestris* and *fullonum*, I have been able to demonstrate dipsacan in several other species of *Dipsacus*, and in various species of the genera *Succisa*, *Scabiosa*, *Knautia*, *Asterocephalus*, *Pterocephalus*, *Trichera* and *Cephalaria*. It is not wanting in any of the members of the order *Dipsaceae* which I have examined, so that I conclude, that it is characteristic of this order. It does not occur in other plants, as was shown by an examination of about 80 species, belonging to widely different orders. Only in the three species of the genus *Scaevola* of the order *Goodeniaceae*, which were at my disposal, I found, after warming parts of the plants in a moist space, that a blue colouring-matter occurs which is doubtless dipsacotin. The occurrence of dipsacan is therefore limited to two closely related natural orders, and a certain systematic value must undoubtedly be attached to it.

Groningen, Botanical Laboratory, Nov. 23rd, 1908.

Chemistry. — "*On the bromation of toluol*" and "*On the sulfonisation of benzol sulfonic acid.*" By Prof. A. F. HOLLEMAN and Dr. J. J. POLAK.

(These communications will not be published in this Proceedings).

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