tion in concentrated sulphuric acid the nitration does not go further than the metamononitrocompound, so that the entry of the second nitro group must occur after the admixture of water.

I pointed out previously (loc. cit) that the two dinitrocompounds may also be prepared by dissolving the metanitrocompound in an excess of very dilute nitric acid.

I now found that by dissolving 1 gram of metanitrodimethylaniline in a mixture of 26 grams of sulphuric acid and 50 grams of water, cooled to 30°, and adding 0.85 grams nitric acid of 50 pCt, a paste of the yellow dinitro-compound melting at 176° is obtained whilst, by means of sodium carbonate, the red isomeric is separated from the filtrate. These relative quantities are exactly those found in the liquid obtained by pouring the nitration mixture into the quantity of water prescribed.

The small quantity of the dinitrodimethylaniline of MERTENS which is produced shows that in nitrating dimethylaniline by the method of GROLL the meta-compound is formed almost exclusively in the concentrated sulphuric acid solution and that the para-compound is most probably formed in the liquid after dilution with water by the action of unused nitric acid on dimethylaniline which has escaped nitration.

When para-nitrodimethylaniline (1 mol.) is dissolved in concentrated sulphuric acid and 1 mol. of nitric acid is added to the solution, the dinitro-compound of MERTENS is found alone after pouring the mixture into water; no nitro-group has taken up the meta position with regard to the amino-group.

Chemistry — "*On the formation of Indigo from Indigoferas a nd from Marsdenia tinctoria*". By Dr. P. VAN ROMBURGH. (Communicated by Prof. A. P. N. FRANCHIMONT).

The interesting communication which Prof. BEYERINCK made to the meeting of Sept. 30th last, from which it appears that the indigo yielding plants belong to two, physiologically quite distinct groups induce me to invite attention to some observations which I made some years ago during an investigation of indigo-yielding Indigoferas and of Marsdenia tinctoria which was published in the "Verslagen van 's Lands Plantentuin". I would add one remark. Owing to my other affairs I was unable to devote as much time to these researches as I could have wished and they are therefore of a more or less preliminary nature. When I found, on the occasion of a meeting with Mr. HAZEWINKEL, Director of the Indigo Experimental Station

at Klaten, that our researches were tending in many ways in the same direction, I terminated mine for the time being and I am postponing the publication of various results until Mr. HAZEWINKEL shall have ended his researches, which are in many respects of importance, and published the results of them.

In the "Verslag" for 1891/2 it is stated that preliminary investigations into the preparation of indigo showed that the extraction of the leaves with water at the temperature prevailing here is not accompanied by evolution of gas during the time which in Java is considered needful to extract the constituents which yield the colouring matter from the plant viz: 6—7 hours. Later, for example in a day, this does occur. The gas evolved consists of carbon dioxyde and a gas which burns with a colourless flame, very probably hydrogen.

What compound exists in the aqueous extract of the indigoleaves is not yet made out with certainty. It had a distinctly acid reaction [1]) and shows the so called indicanreaction very beautifully when it is shaken with hydrochloric acid, chloroform and air.

The filtrate obtained after treating the extract with excess of lead acetate gives a yellow precipitate [2]) with ammonia, stated by SCHUNCK to be characteristic of indican.

Since indigowhite is said to be insoluble in acid liquids, it is not very probable that this substance is present in the aqueous extract of the indigoleaves. A dilute solution of indigowhite in lime water behaves also in many respects quite differently from the extract.

If the indigoleaves are extracted with dilute acetic acid (1 pCt.) instead of with water, the extract yields indigoblue abundantly when shaken with air, especially if ammonia is added."

According to Mr. v. LOOKEREN CAMPAGNE [3]) the liquid produced by the so called fermentation is alkaline and contains indigowhite in solution. In the "Jaarverslag van 's Lands Plantentuin" for 1893, the following is to be found:

"The solution obtained by extracting indigoleaves with water for 7 hours has again been the subject of an investigation, a few of the results of which will be indicated here. The liquid contained free

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[1]) That the extract of the indigo-leaves in acid is very easily shown by running it into a solution of potash coloured red by phenolphthaleïn. The reaction is also successful with a solution of blue litmus. (Note of 1892).

[2]) I have found, subsequently, that an extract of the leaves of *Indigofera galegoïdes*, which contains a substances resembling amygdalin, also gives a yellow coloured precipitate in which, however, the glucoside has no part.

[3]) Verslag omtrent onderzoekingen over Indigo, 1893, 16.

or very loosely combined carbon dioxide [1]) in very large quantity, which may be driven out not only by warming but also by a current of gas free from oxygen [2]).

The substance which yields indigoblue on oxidation may be extracted from the solution by means of chloroform either with or without addition of acetic acid. When the chloroform solution, which has a light greenish yellow colour, is evaporated by blowing a current of air into it, a greenish coloured residue is obtained which is partially soluble in water. The aqueous solution, which possesses a splendid fluorescence, gives indigoblue at once when shaken with air and ammonia; indigoblue is also formed by exposure to the air, and very rapidly when warmed.

An extract of the leaves of *Marsdenia tinctoria*, which is also very distinctly acid to litmus, behaves in a similar way. It has not yet been possible to obtain the substance which yields the indigoblue in a state fit for analysis, nor to prepare crystallized derivatives of it."

The fact that the indigo-yielding substance is formed from the leaves by dilute organic acids was confirmed by Messrs. VAN LOOKEREN CAMPAGNE and VAN DER VEEN in 1895 [3]); notwithstanding the insolubility in acids it is still taken for indigowhite. The solubility of the indigowhite in the extract is explained, by these investigators, by the formation of an unstable compound with substances which yield indigored and indigorubin on oxidation. The ready oxydation of the substance which is extracted by chloroform, in presence of mineral acids or of alkaline carbonates, shows, according to the same authors, that we are not dealing with indoxyl.

In 1897 I again took up my researches, and in the "Verslag" for that year the following is to be found:

"If Indigoleaves (Guatemala or Natal) or leaves of *Marsdenia tinctoria* are placed in an atmosphere of chloroform or carbondioxide they retain their green colour. If, after some time, they are brought in contact with the air, they quickly become bluish, proving that the indican in the dead leaves, which had probably escaped from the cells in the form of a solution, had been decomposed.

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[1]) If carbon dioxide is passed into a solution of indigowhite in lime water until the lime is converted into bicarbonate, the whole of the indigo is precipitated and no indigoblue can be obtained from the filtrate by means of air and ammonia. (Note of 1898.).

[2]) If the current of gas is passed through the liquid for a long time, it is well to mix it with chloroform vapour in order to render living organisms, which might give rise to carbon dioxide during the experiment, inactive.

[3]) Landwirtsch. Versuchsstationen. XLVI, 249.

The presence of a soluble enzyme, capable of decomposing the glucoside, has not so far been proved with certainty [1]).

The many attempts which have been made to separate such a soluble compound have so far given no result. On the other hand it was possible to show that the leaves contain, either a substance of this kind which is so firmly retained that it may be regarded as practically insoluble, or else they carry an insoluble substance which has the power of decomposing a solution of indican so that the substance which on oxidation yields indigo, is set free.

If indigoleaves are steeped in water for 7 hours and the sherry-coloured liquid, which contains the decompositionproduct which yields indigo on oxydation, is then displaced by distilled water containing chloroform, it is found that after several repetitions of this treatment, the displaced liquid yields no more indigo. The dried leaves are again washed several times with water containing chloroform and then placed in contact with a solution of indican [2]) to which chloroform is added; after two hours this gives, when shaken with air, an abundant separation of indigo. If the leaves are now again thoroughly washed, they are still able to decompose a sterilized solution of indican. This may be repeated several times with the same leaves, even when they have been in contact with chloroform water for more than a month.

The washed leaves may be dried over sulphuric acid without losing this property.

The property of decomposing a solution of indican in an hour is retained after extracting the fresh leaves with ether, alcohol, acetone or chloroform.

By treating the leaves, dried in this way, with dilute acids or bases or with glycerine, it has not sofar been possible to obtain

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[1]) VAN LOOKEREN CAMPAGNE, Verslag omtrent onderzoekingen over Indigo, p. 13. contented himself with a *reductio ad absurdum*, a kind of proof which is somewhat unusual in researches of this nature.

[2]) Such a solution of indican is prepared, according to ALVAREZ (C. R. 105, 287) by placing indigoleaves in small quantities at a time in boiling water. Quantitative determinations show that the decomposition of indican is very small when the time of contact is short. Mr. LOHMANN, assistent for the examination of tea, found that such a solution of indican yields indigo in contact with emulsin and air; this agrees with older, vague statements that indican is decomposed by enzymes. The specimens of emulsin in the laboratory were insoluble in water. In the mean time it appeared from a publication of the Indigo-experimental-station at Klaten that Mr. HAZEWINKEL was experimenting with soluble enzymes in the preparation of indigo, this observation was therefore not followed further. (Note of 1897).

a solution of the substance which decomposes the indican. The dried leaves of *Indigofera galegoïdes*, as also some other kinds of leaves with which experiments were made, were incapable of producing the decomposition, so that it appears to be a specific property of some indigo yielding plants. These researches, which proceed slowly, will be continued, as also those on the substance which on oxidation gives rise to indigo; this substance may also be extracted by carbon tetrachloride."

Finally in the recent "Verslag" for 1898:

"Investigations on the composition and properties of a red compound, which is obtained by evaporation of the chloroform solution of the liquid decompositionproduct of indican from Indigoferas which yields indigo, progress but little owing to lack of time. MARCHLEWSKI and RADCLIFFE (Chem. Centralbl. 1898, II, 204), consider indican to be the glucoside of indoxyl. The properties of the decomposition product which yields indigo on oxidation, and which has already been shown here not to be identical with indigowhite, agree, to some extent, well with those of indoxyl. Since Mr. HAZEWINKEL, Director of the Experimental Station at Klaten is occupied with this matter, I have not followed it further."

Physics. — Dr. E. VAN EVERDINGEN JR.: "*The* HALL-*effect and the increase of resistance of bismuth in the magnetic field at very low temperatures*" I (continued). (Communication N°. 53 (cont.) from the Physical Laboratory at Leiden, by Prof. H. KAMERLINGH ONNES.)

5. In the Proceedings of October 28, 1899, p. 221, I expressed the hope that the measurement of the HALL-effect at the boiling-point of liquid oxygen would yield a more decisive answer to the question as to whether or no this phenomenon has a maximum at low temperatures. This measurement has now been made, though as yet only for one strength of field, and the answer is certainly a decided negative, as will appear from § 7.

6. *The liquid oxygen bath.* For pouring out the liquid oxygen we used the vessel without a vacuum-wall, described and drawn in § 2 of this communication, but somewhat altered for this purpose after the manner of Prof. KAMERLINGH ONNES' cryostat [1]). Besides

[1]) See Communication N°. 51, Proc. 30 Sept. '99. p. 126. Comm. Phys. Lab. Leiden N°. 51, p. 3.

Fig. 2.