

VIII.—*On the Preparation of Pure Chlorophyll.*

By Dr. A. TSCHIRCH.

ALL attempts hitherto made to prepare pure chlorophyll must be regarded as failures, their authors having started with the idea that chlorophyll is a comparatively stable substance, not altered by treatment with hydrochloric acid, &c. But an exact study of the alterations produced in the very characteristic spectrum of living leaves and of alcoholic chlorophyll-solutions, by the action of various reagents, shows that pure chlorophyll is an extremely unstable body, which is decomposed even on treating the leaves with alcohol, even though no

alteration of colour is thereby produced. Hence it follows that the substances hitherto described as "chlorophyll" or "crystallised chlorophyll," and obtained either by treating chlorophyll extracts with strong hydrochloric acid, and precipitating the resulting blue solution (phyllocyanin) with excess of water* or with marble†—i.e., by energetic chemical actions—or by absorbing the chlorophyll from its alcoholic solution with animal charcoal and washing it out with ether‡—must be regarded as products of decomposition more or less remote from the original substance.

This conclusion is confirmed by spectroscopic examination, which shows that the crystallised chlorophyll of Gautier and Rogalski is identical with the chlorophyllan of Hoppe-Seyler (*Zeitschr. Physiol. Chem.*, **3**, 347), which, as I have shown (*Ber. der deutsch. botan. Gesellsch.* **1**, 145), is a product of the oxidation of chlorophyll, and that the pure chlorophyll of Berzelius, Mulder, and Pfaundler is identical with Frémy's phyllocyanic acid.§ Here then are two bodies which agree perfectly in their absorption-spectra, but—as shown by their behaviour to caustic alkalis, which dissolve phyllocyanic acid, but not chlorophyllan—are nevertheless totally distinct one from the other.||

But it is not only towards concentrated acids that chlorophyll is so sensitive, for it is quickly decomposed even by weak acids,¶ always with formation of chlorophyllan. The constant presence of vegetable acids in the cells of the leaf explains therefore the rapid decomposition of chlorophyll-tinctures, which, as may be shown spectroscopically, takes place even during the preparation of the solution, and goes on till the whole of the chlorophyll is converted into chlorophyllan, as evidenced by the change of colour of the liquid from green to yellow. Hence all attempts to obtain the colouring-matter in the pure state from chlorophyll solutions, either by precipitation with saline solutions, as I formerly proposed (*loc. cit.*, p. 181), or by

* Berzelius (*Annalen*, **27**, 298).—Harting (*Pogg. Ann.*, **96**, 547).—Pfaundler (*Annalen*, **115**, 43).

† Mulder (*J. pr. Chem.*, **33**, 479).

‡ Gautier (*Compt. rend.*, **89**, 862).—Rogalski (*Compt. rend.*, **90**, 881), and Rôle de la chlorophylle dans l'assimilation.—*Inauguraldissertation*, Krakau.

§ (*Compt. rend.*, **61**, 191).—I here designated as *Phyllocyanic acid* only the body formed by decomposition of phyllocyanin, which is not identical with that which Frémy obtained by treating chlorophyll with barium hydroxide, magnesia, or alumina, and decomposing the resulting salts with acids.

|| The spectroscopic behaviour of these two bodies shows therefore that a body may undergo chemical alterations not recognisable by spectroscopic observation.

¶ Compare also Kraus, "Zur Kenntniss der Chlorophyllfarbstoffe und ihrer Verwandten."—Stuttgart, 1872. Sachsse, "Die Farbstoffe, Kohlehydrate und Protein-substanzen."—Leipzig, 1877.

separation with benzene, carbon sulphide, &c., fail in their object, inasmuch as the colouring-matter is decomposed by the accompanying substances, even during the process of extracting it from the leaves.

Equally unavailing have been the attempts made to prepare the pure colouring-matter by saponification of chlorophyll extracts. Chautard (*Compt. rend.*, **76**, 570) has drawn attention to the differences between the spectroscopic characters of these alkaline solutions of chlorophyll and those of chlorophyll tincture. I myself have also further studied the action of alkalis, and have found that this treatment always yields products of decomposition, recognisable as such by their spectroscopic characters.

According to the present state of our knowledge, we must regard as pure chlorophyll the product whose absorption-spectrum agrees with that of living leaves, as regards both the positions of the individual bands and likewise their breadth and intensity. Such a body I have obtained by reduction of chlorophyllan, a substance easily obtained in the crystalline state by the action of zinc-dust on alcoholic solution of chlorophyllan at the heat of the water-bath. The alcoholic solution of this splendid emerald-green body exhibits the following absorption-spectrum:—

| Band. | I. | II. | III. |
|-------------------|-----------------------|-----------------|-----------|
| Thin layers | $\lambda = 68-63$ | 62—59·5 | 58·3—55·7 |
| Thick layers | $\lambda =$ | 68·5 | 55·5 |
| Band. | IV. | End-absorption. | |
| Thin layers .. | $\lambda = 54·0-52·5$ | 50 to the end* | |
| Thick layers.. | $\lambda = 53·5-52$ | 51 to the end | |

The absorption-spectrum of living leaves exhibits the following bands:—

| Band. | I. | II. | III. |
|---------------|-------------------|-----------------|-------|
| 2 Leaves..... | $\lambda = 70-65$ | 63—61 | 60—57 |
| 3 Leaves..... | $\lambda =$ | 70·5 | 57 |
| Band. | IV. | End-absorption. | |
| 2 Leaves.. | $\lambda = 55-54$ | 52 to the end† | |
| 3 Leaves.. | $\lambda = 55-54$ | 52 to the end | |

Disregarding the shifting of all the bands towards the red, which is apparent in the spectrum of living leaves, and, according to Kundt (*Pogg. Ann.*, 1874, *Jubelband*, 615), is due to the shifting influence of

* The measurements are made, according to Ångström's scale, in wave-lengths of $\frac{100000}{\lambda}$ mm.

† The sun-light employed was highly concentrated by a large double convex lens, the invisible heat-rays being intercepted by three alum cells.

the solvent, or of accompanying substances in the grain of chlorophyll, the bands of the two spectra agree perfectly in breadth and intensity. The order of intensity of these bands, starting from the darkest, is in both spectra—

I II III IV.

Especially characteristic are the relative breadth and darkness of III, which in the chlorophyll solutions always appears narrower;* also the remarkable narrowness and brilliancy of IV:† even in very thick layers, by which the bands I—III are fused into a single broad band, band IV is in both cases still dull and indistinct.

In the end-absorption, the pure chlorophyll prepared by reduction of chlorophyllan does not show any perceptible bands. On diluting the solution, or diminishing the thickness of the layer traversed by the light, the end-absorption gradually recedes towards the blue. The band between *b* and *F* observed in the living leaf belongs to the accompanying yellow colouring matters.

Pure chlorophyll prepared as above forms blackish-green drops, which have not yet been made to crystallise. It dissolves with great facility in alcohol, ether, and benzene, easily also in oils both fatty and volatile, sparingly in fused paraffin, not at all in water. It is converted by dilute acids into yellow chlorophyllan, by strong hydrochloric acid into blue phyllocyanin, and is resolved by potash-lye into an emerald-green substance which dissolves readily in water, forming an emerald-green strongly fluorescing liquid, externally very much like chlorophyll solutions, and a yellow body which may be extracted by ether from the aqueous solution. The alcoholic solution of pure chlorophyll is much less sensitive to light than ordinary tincture of chlorophyll. I regard this pure chlorophyll as identical with the chlorophyll of living plants, and reserve to myself the right of examining it further.

The following is a contribution to the synonymy of certain bodies of the chlorophyll-group:—

Chlorophyll (Pelletier and Handbooks) = crude chlorophyll (Wiesner).

Cyanophyll + Xanthophyll (G. Kraus).

Cyanophyll (G. Kraus) = chlorophyll (Wiesner).

Blue chlorophyll (Sorby).

Pure chlorophyll (Tschirch) + some α -Xanthophyll.

* Compare the representation of the chlorophyll spectrum by Pringsheim (*Berlin Akad. Monatsberichte*, 1874, October), to whom we are indebted for the latest exact representation of the spectrum.

† In the solutions band IV is relatively dark (*vide* Kraus, Hagenbach, &c.).

Chlorophyllan (Hoppe-Seyler) = Modified chlorophyll (Stokes).

Acid chlorophyll of the Handbooks.

Acidoxanthin (C. Kraus).

Filhol's precipitate (obtained on adding organic acids to chlorophyll tincture).

Crystallised chlorophyll (Gautier and Rogalski).

Pure chlorophyll (Jaudin).

Yellow chlorophyll (Sorby).

α -Hypochlorin (Pringsheim's hypochlorin).

(?) Borodin's chlorophyll crystals.

Colouring-matter which produces the winter fading of certain evergreen plants (Haberlandt, G. Kraus, Askenasy).

Colouring-matter which produces the discoloration of strongly acid leaves in the dark.

Phylloxanthin (Frémy?) (Tschirch) = xanthophyll (Berzelius) ex part.

? Chlorophyllic acid (Liebermann).

Xanthin (C. Kraus).

Phylloxantheïn (Weiss) = alkali phyllocyanate (Tschirch).

Frémy's etiolin.

Phyllocyanic acid (Frémy, ex part) = pure chlorophyll (Berzelius, Mulder, Pfaundler, Harting).

Body precipitated by water from solution of phyllocyanin (Tschirch).

(?) Chlorophyllanic acid (Hoppe-Seyler).

Potassium chlorophyllinate (Tschirch) = Chlorinkali (C. Kraus).

Sachsse's precipitate formed by potassium or sodium in solution of cyanophyll.

α -Xanthophyll* = xanthophyll (G. Kraus).

β -Xanthophyll = xanthophyll (Pringsheim). Yellow colouring-matter of autumn leaves (perhaps identical with α).

γ -Xanthophyll = yellow colouring-matter soluble in ether; precipitated by potash from cyanophyll.

Xanthin (Dippel).

Xanthin (C. Kraus) ex part.

δ -Xanthophyll = Frémy's phylloxanthin, separated by barium hydroxide from cyanophyll.

ϵ -Xanthophyll = yellow colouring-matter formed in Sachsse's reaction (treatment of cyanophyll solution with sodium); permanent in benzene solution (perhaps identical with γ).

* The xanthophylls here enumerated are perhaps identical; but till the point is definitely established, they may be conveniently distinguished by Greek letters.

Xanthophyll (G. Kraus) = etiolin (G. Kraus).

Xanthophyll (Sorby) ex part.

Erythrophyll (Bougarel ?) = chrysophyll (Hartsen).

Borodin's yellow crystals.

ε-Xanthophyll (Tschirch).

Anthoxanthin (Marquardt) = anthoxanthin (Pringsheim).

Xanthin and Xanthein (Frémy and Cloëz).

Yellow colouring-matters of flowers.
