

III.—*On the existence of a Second Crystallizable Fluorescent Substance (Paviin) in the Bark of the Horse-Chestnut.*

BY G. G. STOKES, M A., SEC. R. S., &c.

ON examining, a good while ago, infusions of the barks of various species of *Æsculus*, and the closely allied genus *Pavia*, I found that the remarkably strong fluorescence shown by the horse-chestnut ran through the whole family. The tint of the fluorescent light was, however, different in different cases, being as a general rule blue throughout the genus *Æsculus*, and a blue-green throughout *Pavia*. This alone rendered it evident, either that there were at least two fluorescent substances present, one in one bark and another in another, or, which appeared more probable, that

there were two (or possibly more) fluorescent substances present in different proportions in different barks.

On examining, under a deep violet glass, a freshly cut section of a young shoot, of at least two years' growth, of these various trees, the sap which oozed out from different parts of the bark or pith was found to emit a differently coloured fluorescent light. Hence, even the same bark must have contained more than one fluorescent substance; and as the existence of two would account for the fluorescent tints of the whole family, a family so closely allied botanically, the second of the suppositions mentioned above appeared by far the more probable.

I happened to put some small pieces of horse-chestnut bark with a little ether into a bottle, which was laid aside, imperfectly corked. On examining the bottle after some time, the ether was found to have evaporated, and had left behind a substance crystallized in delicate radiating crystals. This substance, which I will call paviin, when dissolved in water, yields, like æsculin, a highly fluorescent solution, and the fluorescence is in both cases destroyed (comparatively speaking) by acids, and restored by alkalies. The tint, however, of the fluorescent light is decidedly different from that given by pure æsculin, for a specimen of which I am indebted to the kindness of the Prince of Salm-Horstmar, being a blue-green in place of a sky-blue. The fluorescent tint of an infusion of horse-chestnut bark is intermediate between the two, but much nearer to æsculin than to paviin.

In all probability, the fluorescence of the infusions of barks from the closely allied genera *Æsculus* and *Pavia*, is due to æsculin and paviin present in different proportions, æsculin predominating generally in the genus *Æsculus*, and paviin in *Pavia*.

Æsculin and paviin are extremely similar in their properties, so far as they have yet been observed. They are most easily distinguished by the different colour of the fluorescent light of their solutions, a character which is especially trustworthy, as it does not require for its observation that the solutions should be pure. Paviin, as appears from the way in which it was first obtained, must be much more soluble than æsculin in ether. Æsculin is indeed described as insoluble in ether, but it is sufficiently soluble to render the ether fluorescent. Paviin, like æsculin, is withdrawn from its ethereal solution by agitation with water. Though of feeble affinities, it is rather more disposed than æsculin to combine with oxide of lead. If a decoction of horse-chestnut bark be

purified by adding a sufficient quantity of a salt of peroxide of iron or of alumina, precipitating by ammonia, and filtering, and the ammoniacal filtrate be partially precipitated by very dilute acetate of lead, the whole redissolved by acetic acid, reprecipitated by ammonia, and filtered, the fluorescent tint of the filtrate will be found to be a deeper blue than that of the original solution; while, if the fluorescent substances combined with oxide of lead (the compound itself is not fluorescent) be again obtained in alkaline solution, the tint, as compared with the original, will be found to verge towards green. The required solution is most easily obtained from the lead-compounds by means of an alkaline bicarbonate, which plays the double part of an acid and an alkali, yielding carbonic acid to the oxide of lead, and ensuring the alkalinity of the filtrate from carbonate of lead. It is very easy in this way, by repeating the process, if necessary, on the filtrate from the first precipitate, to obtain a solution which will serve as a standard for the fluorescent tint of pure *æsculin*. A solution, serving nearly enough as a standard of comparison in this respect for pure *paviin*, may be had by making a decoction of a little ash bark, adding a considerable quantity of a salt of alumina, precipitating by ammonia, and filtering. By partial precipitation in the manner explained, it is very easy to prove a mixture of *æsculin* and *paviin* to be a mixture, even when operating on extremely small quantities.

It must be carefully borne in mind, that the characteristic fluorescent tint of a solution is that of the fluorescent light coming from the solution *directly* to the eye. Even should a solution of the pure substance be nearly colourless by transmitted light, though strong enough to develop the fluorescence to perfection, if the solution be impure it is liable to be coloured, most commonly yellow of some kind, which would make a blue seen through it appear green. To depend upon the fluorescent tint, as seen through and modified by a coloured solution, would be like depending on the analysis, not of the substance to be investigated, but of a mixture containing it. Yet in solutions obtained from the horse-chestnut, and in similar cases, the true fluorescent tint can be observed very well, in spite of considerable colour in the solution.

The best method of observing the true fluorescent tint is to dilute the fluid greatly, and to pass into it a beam of sunlight, condensed by a lens fixed in a board, in such a manner that as

small a thickness of the fluid as may be shall intervene between the fluorescent beam and the eye. If a stratum of this thickness of the dilute solution be sensibly colourless, the tint of the fluorescent light will not be sensibly modified by subsequent absorption. This, however, requires sunlight, which is not always to be had. Another excellent method, requiring only daylight, and capable of practically superseding the former in the examination of horse-chestnut bark, is the following, in using which it is best that the solutions should be pretty strong, or at least not extremely dilute.

A glass vessel with water is placed at a window, the vessel being blackened internally at the bottom by sinking a piece of black cloth or velvet in the water, or otherwise. The solutions to be compared as to their fluorescent tint are placed in two test tubes, which are held nearly vertically in the water, their tops slightly inclining from the window, and the observer regards the fluorescent light from above, looking outside the test tubes. Since by far the greater part of the fluorescent light comes from a very thin stratum of fluid next the surface by which the light enters, the fluorescent rays have mostly to traverse only a very small thickness of the coloured fluid before reaching the eye; the water permits the escape of those fluorescent rays which would otherwise be internally reflected at the external surface of the test tubes; and the intensity of the light of which the tint is to be observed is increased by foreshortening. The observer would do well to practice with a fluorescent fluid purposely made yellow by introducing some non-fluorescent indifferent substance; thus, a portion of the standard solution of *æsculin* mentioned above may be rendered yellow by ferrid-cyanide of potassium. The more completely the fluorescent tints of the yellow and the nearly colourless solution agree, the more nearly perfect is the method of observation. If ferro-cyanide of potassium be used in the experiment suggested, instead of ferridcyanide, the most marked effect is a diminution in the intensity of the fluorescent light, the cause of which is that the absorption by this salt takes places more upon the active, or fluorogenic, than upon the fluorescent rays. Since substances of a similar character may be present in an impure solution, the observer must not always infer poverty with regard to fluorescent substances from a want of brilliancy in the fluorescent light.

The existence of paviin may perhaps account for the discrepancies between the analyses of *æsculin* given by different chemists.

I should mention, however, that I have met with three specimens of æsculin, and they all appeared to be free from paviin. The reason why æsculin was obtained pure from a decoction containing paviin also, is probably that the former greatly preponderates over the latter in the bark of the horse-chestnut. A decoction of this bark yielded to me a copious crop of crystals of æsculin, while the paviin, together with a quantity of æsculin still apparently in excess, remained in the mother liquor. I may, perhaps, on some future occasion communicate to the Society the method employed, when I have leisure to examine it further; I will merely state for the present that it enabled me to obtain crystallized æsculin in a few hours, without employing any other solvent than water. In the method commonly employed, the first crystallization of æsculin is described as requiring some fourteen days.

On account of the small quantity, apparently, of paviin, as compared with æsculin, present in the bark of the horse-chestnut, a chemist who wished to obtain the substance for analysis would probably do well to examine a bark from the genus *Pavia*, if such could be procured. The richness of the bark in paviin, as compared with æsculin, may be judged of by boiling a small portion with water in a test tube; those barks in which the substance presumed to be paviin abounds yield a decoction having almost exactly the same fluorescent tint as that of a decoction of ash bark.

A crystallizable substance, giving a highly fluorescent solution, has been discovered in the bark of the ash, by the Prince of Salm-Horstmar,* who has favoured me with a specimen. This substance, which has been named *fraxin* by its discoverer, is so similar in its optical characters to paviin that the two can hardly, if at all, be distinguished thereby; but as *fraxin* is stated to be insoluble in ether, it can hardly be identical with paviin, which was left in a crystallized state by that solvent. I find, however, that *fraxin* is sufficiently soluble in ether to render the fluid fluorescent, so that after all it is only a question of degree, which, cannot be satisfactorily settled till paviin shall have been prepared in greater quantity.

* Poggendorff's *Annalen*, vol. 100 (1857), p. 607.