XXXV. COLOURING MATTER CONTAINED IN THE SEED-COATS OF *ABRUS PRECATORIUS*.

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(Received May 2nd, 1914)

The toxic principle contained in the seeds of *Abrus precatorius* has been the subject of elaborate investigations by many workers, including Klein, De Wecker, Sattler, Warden, Waddell etc. But it appears that none has thought it worth his while to investigate the chemical nature or the properties of the colouring matters contained in the coatings of the seeds of *Abrus precatorius*.

Dr D. D. Cunningham, however, has given a description of the microscopical structure of the seeds of *Abrus precatorius* [1882], which I quote below so that the description may be recorded in a journal which is readily available for reference.

“The integument of the seed consists of no less than nine distinct layers of cells differing considerably from one another in their characters.

Proceeding from without inwards we find :—

(1) A layer of considerable thickness but containing only a single stratum of cells. They are thick-walled, columnar cells containing the colouring matter to which the seed owes its bright scarlet hue, and are arranged in radiant fashion around the deeper portions of the integument. Each cell is dilated peripherally and in many cases a slight basal bulbosity is also present. The peripheral dilated portion is cut up into a number of more or less cuneate portions which are closely adapted to one another. In many cases in the mature seeds the central point where the apices of the segment come into relation to one another really consist of a narrow canal leading directly into the cell-cavity. The surface of the seed-coat due to the close apposition of the cells, necessarily comes to present the appearance of being composed of a layer of cuneate facets arranged in rosetted fashion around central points—each rosette representing the extremity of one of the columnar cells, and the central points in many instances being openings, leading directly into the cell-cavities.

(2) A thick stratum of small cells with thick walls and irregular sinuous outlines.

(3) A thick stratum of small, also thin-walled cells.

(4) A thin stratum of small, also thin-walled cells.

(5) A stratum of elongated thin-walled cells.

(6) A stratum of thickened cells two or three layers deep.

(7) A single row of minute thin-walled cells of more or less cubical character.

(8) A stratum of thick-walled cells with dense yellowish granular contents.

(9) A stratum of thickened more or less parenchymatous cells with mere traces of cavities or contents.

The entire thickness of this stratified coating may be broken up in various ways, but apparently the line which may be taken as representing the transition from testa to tegumen runs through the thin-walled cells of the 7th stratum. The cells save in the 1st and 8th stratum appear to be almost or quite devoid of contents."

The above description throws some light on the following peculiar behaviour of these seeds towards certain reagents.

A few entire *Abrus precatorius* seeds are kept in a corked test-tube immersed in chlorine water, along with seeds broken into pieces and with those which have cracks or holes on the surfaces of the testa. The scarlet material contained in the testa of those seeds which are broken or which have cracks or holes in their surfaces is found to be decolourised, while the entire seeds are perfectly unaffected, though some of these were kept in the solution of chlorine water for several months.

When broken *Abrus precatorius* seeds or those which have cracks or holes on their surfaces are kept immersed in a dilute alkaline solution, e.g. of caustic potash or ammonia or simply water, the solutions become coloured owing to the dissolving out of the colouring matter contained in the testa of the seeds, which gradually lose their scarlet hue. The entire seeds without any hole or cracks on their surface, are not in the least affected when kept immersed in these solutions.

The above phenomenon is not due to the presence of any waxy material in the superficial part of the seed-coat, for, if the seeds having the seed-coats entire, i.e. having no cracks or holes in them, are kept immersed in a solution of ether, previously to their treatment with caustic soda solution etc., the seeds are not decolourised as before. The above experiments prove that

(1) there is a structure on the surface of the seeds which is perfectly impermeable to fluids;

(2) the colouring matter which gives a scarlet hue to the seeds is situated beneath this superficial structure ;

(3) when any fluid can make its way, at any point, through the superficial impermeable structure this can readily find access to every part of the structure which contains the scarlet colouring matter of the seeds.

These phenomena can be readily understood by taking into account the fact that the superficial layer of cells on the coating of the seeds are not only elongated structures with thick walls, but really consist of two parts, viz. the peripheral portion and the central portion. The peripheral portion is dilated and cut up into cuneate portions, which are so closely applied to one another as to be perfectly impermeable to fluid.

To make the description quoted above consistent with the experiments described we are led to suppose that the openings, which according to Dr Cunningham lead directly into the cell-cavities, are merely blind pouches. In case of an entire seed being kept immersed in a fluid, which has the power of dissolving out the colouring matter contained in the seeds, no colouring matter comes out into the solution because the fluid cannot penetrate through these blind pouches into the portions of the cells which contain the colouring matter.

This property of impermeability of the superficial layer of cells to fluids and its power of thus protecting the internal layers of the seed, is evidently meant by nature for protection of the seeds so that the propagation of the species of the plant may be kept up. It is necessary for the propagation of the species of this plant that the seeds should be able to keep themselves intact for a long time without suffering any disintegration. For the plants die seasonally every year scattering their seeds all round and these germinate fresh plants next year. The above is also in agreement with the views expressed by many observers that the seeds are distributed by being eaten by birds, and passing out unchanged with their excreta. In the *Pharmaceutical Journal*, July 7, 1883, it has been stated that Dr Noura of Brazil, who, while a member of the government commission appointed to regulate the boundary between Brazil and Bolivia, found it in virgin forests where the husbandmen had never penetrated and where the seeds had very probably been carried by birds. C. Owatari in his article on "Botanical Excursion to Formosa (Taiwan)" published in *Botanical Magazine*, XI. Tokyo, 1897, holds however the opposite view. He considers that the attractive colouration of the seed in *Abrus precatorius* might be accounted for as imitating some beetles which are offensive to birds and are thus protected from being devoured." Extended observations are required to decide which

of these views are really correct. I carried on experiments with pigeons, by giving them entire *Abrus precatorius* seeds which they avoided taking while they greedily devoured paddy-grains. So this experimental evidence is in favour of the Japanese observer.

According to Dr Cunningham, as will be seen from the description quoted above, there are two kinds of colouring matter present in the seed-coats. The cells of the 1st layer contain the colouring matter which gives the scarlet hue to the seed-coats. The cells of the 8th layer contain a yellowish colouring matter. I have found by microscopical examination of the section of the seed-coats, that besides the dyes mentioned by Dr Cunningham a kind of black granule is present at the black spot of the seed-coat. This is however insoluble in acids and alkalis and is not a soluble dye but is in the nature of a pigment and so may be omitted from consideration.

For examination of the colouring matters contained in the seed-coats, a quantity of *Abrus* seeds are taken and the coatings are separated from the cotyledons and are then soaked in water for about four hours and the coloured solution filtered. The filtrate is shaken with ether which extracts traces of yellow colouring matter, which can be identified by the microscope with the yellow colouring matter contained in the 8th layer of the cell. This was obtained in very minute quantities and was not chemically examined any further. The aqueous extract on evaporation leaves a fine pink coloured residue. This is again dissolved in water and copper acetate solution is added in sufficient quantity to cause complete precipitation of the colouring matter. The mixture is left standing for a day and then filtered. The precipitate is repeatedly washed with distilled water, is dissolved in dilute hydrochloric acid, and decomposed by sulphuretted hydrogen. The filtrate from the copper sulphide is then evaporated to dryness in a steam-bath and the residue is extracted with water and filtered. This filtrate contains the colouring matter which gives the scarlet hue to the seed-coats.

The following reactions show that the colouring matter is a tannin substance.

(1) Dilute ferric chloride solution gives a blue black precipitate, the precipitation becoming complete in 24 hours. This reaction with iron salts is not only an evidence that the solution under the examination is a tannin substance, but it also gives indication as to the nature of the chemical composition of this tannin substance. For regarding tannin substances it may be stated as a general truth, that those which give blue-black colourations with ferric salts are derivatives of pyrogallol and gallic acid, while those which afford a green colouration are derived from catechol and protocatechuic acid.

(2) Lead acetate gives a copious blue coloured precipitate.

(3) Allen in his book on commercial analysis says:— “A delicate reaction for tannins (first noticed by the author and apparently general) is the deep red colour produced on treating a solution with potassium ferricyanide mixed with ammonia.” When solutions of the colouring matter and of tannic acid are treated side by side with ammoniacal ferricyanide solution for comparison, exactly the same shade of colour is noticed in both cases.

(4) The solution is found to be gradually decolourised by strips of isinglass, which are found to acquire a reddish violet shade. This reaction proves that the reactions of tannin noticed in the solution are not due to any colourless tannin substance, but to the presence of the colouring matter, for it is the colouring matter which is attracted by isinglass.

(5) The colouring matter is precipitated by a solution of gelatin.

(6) On adding stannous chloride solution to the solution, it acquires a pinkish violet shade and after about 12 hours a copious precipitate having the same colour is formed.

(7) The residue obtained by evaporating the solution has an astringent taste like that of tannic acid, and is insoluble in ether, chloroform, benzene and carbon disulphide as is the case with tannins generally.

I should note here that I have carried on the above set of experiments as well as the experiments which will be described below, not only in test-tubes, but where possible by micro-chemical methods also. That is to say I have taken microscopical sections of the seed-coats of *Abrus precatorius*, have treated these with chemical reagents such as solutions of ferric chloride, stannous chloride, lead acetate etc., and have observed under the microscope changes in the colouring matter contained in the 1st layer of cells corresponding with those noticed in the test-tubes. For example, by adding ferric chloride solution in sufficient dilution, we can readily stain this colouring matter violet, leaving other portions of the seed-coats unaffected.

All these experiments, I think, conclusively prove that the colouring matter contained in the 1st layer of the seed-coats of *Abrus precatorius*, is a definite chemical substance of the nature of a tannin.

The reactions of this tannin substance were further studied with the view of finding out whether it can be identified with any known tannin or whether the substance is to be regarded as an altogether new tannin. Some of these reactions are stated below.

(1) Copper sulphate gives a bluish coloured precipitate, which dissolves in ammonia giving a brown coloured solution.

(2) On dissolving crystals of sodium nitrite in the solution on a

porcelain tile, the liquid is coloured from yellowish brown to reddish brown.

- (3) With hydrochloric acid a red colour is produced.
- (4) Addition of ammonia produces a brown colouration.
- (5) Crystals of sodium sulphate added to the solution on a porcelain plate, after some time give yellowish red colour near the edges.
- (6) Addition of lime-water causes a bluish coloured precipitate.
- (7) Addition of ammonia after addition of ferric chloride solution, causes a violet coloured solution with a precipitate of the same colour.
- (8) Tartar emetic solution slowly produces a yellowish brown coloured precipitate.
- (9) Tartar emetic and ammonium chloride yield a clay-coloured precipitate.
- (10) Nitrous fumes passed into the hot solution, give a yellow colouration and a yellow dye is left on evaporation.

Comparing the reactions stated above, with the reactions of the known tannins one will find that the colouring matter of *Abrus precatorius* seeds appears to be a new tannin substance.

In conclusion I beg to thank the authorities of the Indian Association for the Cultivation of Science for kindly allowing me to work at the laboratory of the Association.

REFERENCES.

Cunningham (1882), *Indian Medical Gazette*, 288.