THE MORPHOLOGY AND HISTOLOGY OF SEEDS OF *DATURA CORNIGERA* HOOK

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The macroscopical and microscopical characters of the seeds of *Datura cornigera* Hook. are described and illustrated. A most striking feature is the peculiar spongy parenchyma which constitutes the major part of the testa. The location of alkaloids is surprising, only the collapsed inner part of the testa showed no content of alkaloids. In the outer layers of the endosperm, many cells contained regular crystals of a substance which dissolved in strong sulphuric acid giving an intense orange colour.

THE systematics of the section *Brugmansia* are rather complicated, one of the main difficulties being the hybrid nature of most forms. The plants are very often only cultivated as garden ornamentals in their native home, the tropical parts of South America and in greenhouses in temperate countries. A reason making study difficult is the limited ability of many forms to produce mature fruits; according to Lagerheim (1895), even in South America, specimens with fruits are rare, and for several species the fruits are still unknown. The only comprehensive records of South American brugmansias are the monograph by Lagerheim (1895) and the synopsis by Safford (1921).

The general features of the seeds from the section *Brugmansia* have been mentioned by Lagerheim (1895), but no detailed illustrations given. According to him most brugmansias have flattened seeds which on maturity are loose within the fruit. Both *Datura aurea* Lagerh. and *D. arborea* Linn. have seeds which are irregular and thick and not loose within the fruit; *D. aurea* seeds have a smooth testa. Certain similarities exist between the seed structure of *D. stramonium* Linn. and that of the seeds of brugmansias but two characteristic features are different: in the brugmansia seeds the inner walls of the epidermal cells are not thickened to the same degree as in stramonium seeds and a thick middle layer is developed in the testa of several brugmansia seeds. This layer also found in the seeds here in question is composed of a very spongy tissue which is responsible for the surprisingly low weight of these large seeds.

The anatomy of seeds of different *Datura* species has been thoroughly investigated by Timmerman (1927) and Moll and Janssonius (1923) but only the seeds of commerce, i.e., seeds of *D. stramonium*, *D. innoxia* and *D. metel* were included in these studies.

The position of alkaloids in the seeds of *D. stramonium* has always been shown to be in the inner part of the testa only (Siim, 1900; James, 1946).

PLANT MATERIAL

The seeds of *D. cornigera* Hook. (Fig. 1) used in this investigation are samples from Nottingham University. The origin of the seeds and the

identity of the plants have been described by Evans and Pe Than (1962). Both the original seeds from Cochabamba, Bolivia and seeds from plants cross pollinated in Nottingham were investigated, and showed identity.

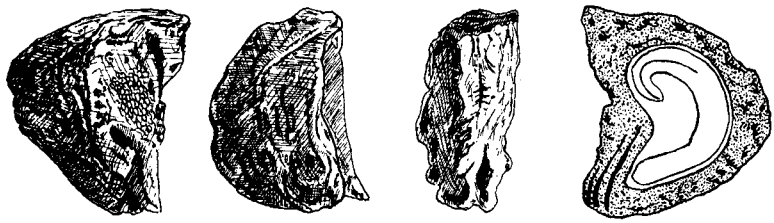

FIG. 1. Seed of *Datura cornigera* Hook. The seeds seen from different visual angles. One seed cut longitudinally, shows the curved embryo in the endosperm and the large spongy parenchyma in the testa. × 2.

The 100-seed weight was calculated to 12·40 g. and average measurements were length, 1·3 cm. width, 1·1 cm. and thickness, 0·6 cm. The greyish-brown seeds are flattened and from the side the outline is triangular to half orbicular. The two faces are quite smooth with only a few wrinkles and furrows, the convex side is deeply furrowed. The hilum is present as a small cavity at one end of the acute edge. The interior is composed of the spongy brown part of the testa which surrounds the oily endosperm embedded within which is a cylindrical embryo, about 2 mm. in diameter. The hypocotyle-radicle is directed towards the hilum; the embryo is coiled but not as strongly as in stramonium seeds.

HISTOLOGICAL CHARACTERS (Fig. 2)

The testa of the *D. cornigera* seed consists of three distinct parts: outer epidermis, a spongy parenchyma and an inner epidermis. The structure of the cells of the outer epidermis may be elucidated by comparing transverse sections, surface preparations and isolated cells. As Timmerman (1927) points out it is rather difficult to macerate the epidermis. In this case the usual Schultze method (nitric acid and potassium chlorate) was too drastic but very fine preparations were obtained with Franklin's method (1937), using glacial acetic acid and 30 per cent hydrogen peroxide at 100° for ½ hr. In transverse section the epidermal cells are about 135 μ in height on the faces but when the testa is wrinkled the height may increase up to 270 μ; the width of the cells is up to 135 μ; the lumen is more or less isodiametric. The outer wall of the epidermal cells consists of three distinct layers. An outer lamella (cuticle) which stains deep red with ruthenium-red, a middle layer of cellulose which stains blue with iodine and 60 per cent sulphuric acid, and an inner part which gives a lignin reaction with phloroglucinol and concentrated hydrochloric acid. Sudan III does not stain any layer. The outer lamella must therefore consist of pectin or a mucilagineous substance. An isolated epidermal cell shows several rounded processes in the upper end and the base shows a similar structure due to folds in the inner part of the lateral walls. The

lignified portion of the wall shows a fine striation due to alternating lignified and cellulose layers. After staining with iodine-sulphuric acid it is difficult to see the blue colour due to the strong brownish colour of the wall but after bleaching with hypochlorite, extremely thin sections show blue coloured lamellae within the cell wall.

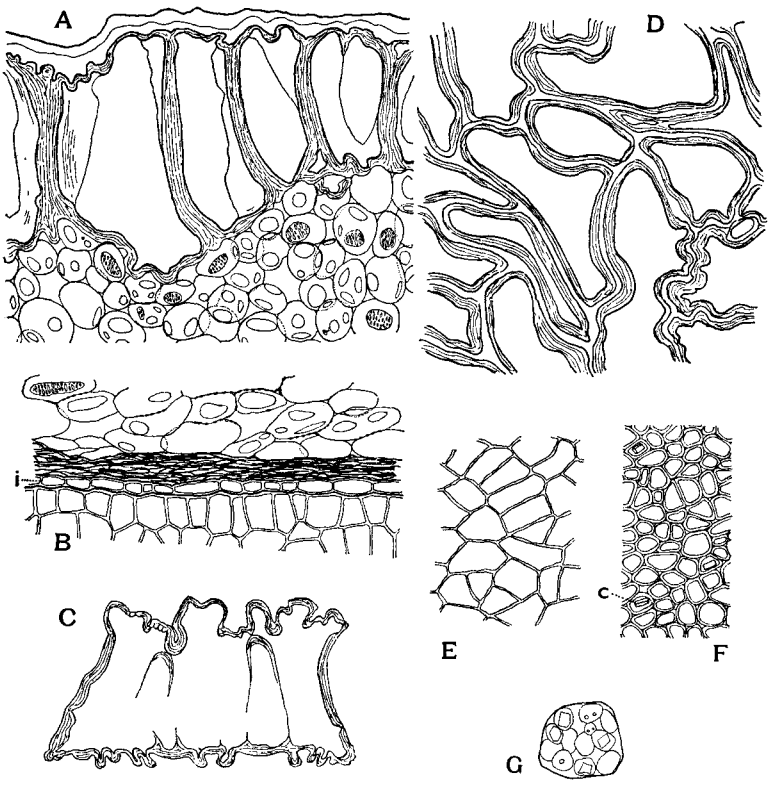

FIG. 2. Seed of *Datura cornigera* Hook. A, transverse section through the outer epidermis and the outer part of the spongy parenchyma. B, transverse section through the inner part of the testa and the endosperm. C, isolated epidermal cell (from a maceration). D, surface view of the outer epidermis. E, surface view of the inner epidermis. F, surface view of the outer layer of the endosperm. G, single cell with aleurone grains from the endosperm. All figures × 105 except G: × 275. i = inner epidermis of the testa; c = crystal in the endosperm. A, B and D from preparations cleared with chloral hydrate reagent.

A surface view of the epidermis shows very sinuous lateral walls and below the surface the middle lamella is quite distinct. Numerous processes are seen, often looking like very small single cells.

The dominant tissue in the testa is the enormously thick lignified layer which occupies all the space between the outer and the inner epidermis. The single cells are sphaeric in outline and joined to one another by numerous poral connections. These connections are very easy to observe

due to the large single pores between two neighbouring cells: the pores show reticulated membranes. Close to the inner epidermis the cells become more closely packed and the cell-walls show many small simple pores. The last 3–10 layers outside the inner epidermis are completely collapsed. The inner epidermis is formed by a 15–20 μ high layer of, in transverse section, rectangular cells. In surface view they are polygonal in shape, with somewhat uneven thickened walls. The reason this layer should be called "inner epidermis" of the seed testa and not "perisperm" as stated by many earlier authors (see Moll and Janssonius, 1923) is the existence of a cuticularised membrane on the inner walls of this layer. In unripe seeds of *D. stramonium* a similar cuticle separates the inner epidermis of the integument from the tissue in the nucellus (see Netolitzky, 1929).

The cells of the endosperm have hyaline walls and contain abundant protein and globules of oil. The aleurone grains are of variable size, especially small grains are found in the outer cell-layers which also contain single crystals of a colourless highly refractive substance soluble in concentrated sulphuric acid with an intense orange colour. The embryo also contains protein and oil.

Location of Alkaloids

A fresh seed is very easy to divide into the different tissues. The epidermis scales off and the spongy parenchyma splits away exposing the collapsed cells on the surface of the endosperm. These few cell-layers may then be scraped off with a fine knife and the whitish-grey endosperm split open to remove the white embryo. The five "seed-fractions" are then each extracted with a few ml. ethanol and a few drops of strong ammonia. The liquid extracts are filtered, evaporated to dryness and the residues each dissolved in a few drops of chloroform. Spotted on to filter-paper and sprayed with Dragendorff's reagent only four extracts gave a strong positive reaction; the collapsed cell-layer showed no reaction for alkaloids. Submitted to paper chromatography the four extracts gave spots with the same R_F value as hyoscine (see Evans and Pe Than, 1962). The amount of seeds in my possession did not permit further chemical investigations.

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