THE NON-ARTICULATED LATICIFERS AND LATEX OF *TABERNAEMONTANA CORONARIA* WILLD.

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Two varieties of *Tabernaemontana coronaria* Willd. have been examined and they both contain non-articulated, unbranched and branched laticifers. The embryos could not be examined, as the plant does not set seeds, but younger stages of the laticifers have been traced by growing the cuttings of the plant and examining them at intervals of five to nine days. A preliminary qualitative analysis of the latex of the two varieties shows that it contains proteolytic enzymes, carbohydrates, dehydrogenases, lysozyme, apart from the organic particles present in it.

Introduction

A detailed account of latex-cells and a brief chemical analysis of the latex of *Jatropha* (Rao and Malaviya 1964) and observations on the latex of two members of Asclepiadaceae (Rao and Malaviya 1966) have already been reported from this laboratory. The present paper is a continuation of the same line of work on a commonly grown plant, *Tabernaemontana coronaria* Willd., a member of the Apocynaceae. Some of the earlier authors like Desch (1934) gave an account of latex-canals in the family Apocynaceae. More recently Mahlberg (1963, 1961) studied the origin and structure of laticifers in *Nerium oleander*. The chemical nature of the latex of *Tabernaemontana coronaria* has been studied in detail by Murti and Kidwai (1963) but the latex of the other members of the family or the ontogeny and structure of the laticifers has not been studied, so far as we are aware.

An account of the structure, development and distribution of laticifers in two varieties of *Tabernaemontana coronaria* and a qualitative chemical analysis of its latex form the subject-matter of this paper. Both the single-flowered and the double-flowered varieties were examined.

Some preliminary chemical tests were performed, with the help of paper chromatography, to find out the presence of some acids, enzymes and carbohydrates, apart from the solid component of it.

Material and Methods

The material investigated was collected from the departmental garden and was fixed in formalin-propiono alcohol (Johansen 1940). Both young

TABLE I

A qualitative analysis of the latex of Tabernaemontana coronaria

Name of the plant	Colour of latex	Amino acids (ninhydrin positive spots)	Carbo-hydrates	Amylase activity	Proteolytic activity	Milk-clotting enzyme activity	Lytic activity	Peroxidase activity	Dehydro-genase activity
Tabernae-montana coronaria single-flowered variety	Milky white	9	Three spots; two corre-sponding to galactose, one corre-sponding to glucose	nil	Optical density 50	Activity slow, 20 sec. are taken for clotting of the milk	Present as reported earlier by Krishna Murti and Kidwai (1963)	nil	The colour comes after five to eight hours, acti-vity slow
Tabernae-montana coronaria double-flowered variety	Milky white	9	Two spots; both correspond-ing to galactose	nil	Optical density 44	Activity very slow, 30 sec. are taken for clotting of the milk	Present	nil	Same

Optical density—It is the Klett reading. This refers to the colour intensity due to formation of tyrosine liberated from casein by the action of proteolytic enzyme present in the latex.

and old flowers, fruits, growing stem-tips and young and old leaves were examined by cleared mounts, macerations, hand sections and microtome sections of 6–15 μ thickness. Safranin-light green and haematoxylin-orange-G were the most suitable stains. Cotton-blue was also used as a temporary stain.

A qualitative analysis of the latex of Tabernaemontana coronaria

The latex was collected usually between 8 a.m. and 10 a.m. by cutting the tips of young branches. The latex was centrifuged at 5,000 r.p.m. after being diluted five or six times its volume with glass-distilled water. The pellet was discarded and the supernatant was used for analysis. The procedure for the various tests is given below and the results are summarized in Table I.

Detection of the free amino acids

Five ml of latex were taken from all the parts. In order to precipitate the protein, about 10 times its volume of alcohol was added, and then centrifuged. The chromatograms were developed using butanol-acetic acid-water (4 : 1 : 1) and phenol water (80 : 20) as the two solvents. The chromatograms after being dried were sprayed with a 0·2% ninhydrin solution in acetone for the detection of the amino-acid spots. The spots were counted in each case. These amino-acid spots are being identified as a quantitative study. Further work is under progress.

Detection of the carbohydrates

The chromatograms, after being developed in butanol-acetic acid-water, were sprayed with a solution of anilin phthalate. Reference spots of glucose, galactose and fructose were made. In each case one or the other carbohydrate spots were found as shown in the table.

Analysis of the enzymes present in the latex

Detection of proteolytic activity.—This enzyme seems to be the most common component of the latices and is present in abundance. The method of Anson (1938) was followed to detect the proteolytic enzyme using casein 1 mg/ml as the substrate and detecting the tyrosine released during the reaction. The activity in all the cases was compared by finding out the optical density in a *Klett Summerson* photo-electric colorimeter.

Detection of amylase activity.—The activity was observed following Bernfield method (as cited in Sidney and Nathan 1955) using soluble starch solution as the substrate and dinitrosalicylic acid as the reagent. The optical density was observed in order to compare the activity.

Detection of peroxidase activity.—Pyrogallol test (Sidney and Nathan 1955) was performed to find out the activity of peroxidase using hydrogen

peroxide (H_2O_2) as the substrate. This enzyme had to be tested immediately after the collection of the latex as it is denatured very soon.

Detection of dehydrogenase activity.—Tetrazonium test (Sidney and Nathan 1955) was carried out using succinate as the substrate. The difference in the enzyme activity was noted by the colour changes. The colour, after it has developed, could be extracted by adding equal volumes of acetic acid and toluene. After shaking well, all the colour comes in the topmost layer.

Detection of lytic activity.—The presence of lysozyme was tested by the method of Murti and Kidwai (1963) using cell-suspensions of *Micrococcus lysodeikticus.*

Detection of milk-clotting enzyme.—Fresh latex was tested immediately, as the enzyme is denatured very soon. To 2 ml of fresh milk, 1 ml of latex was added. The time required for clotting of the milk was noted.

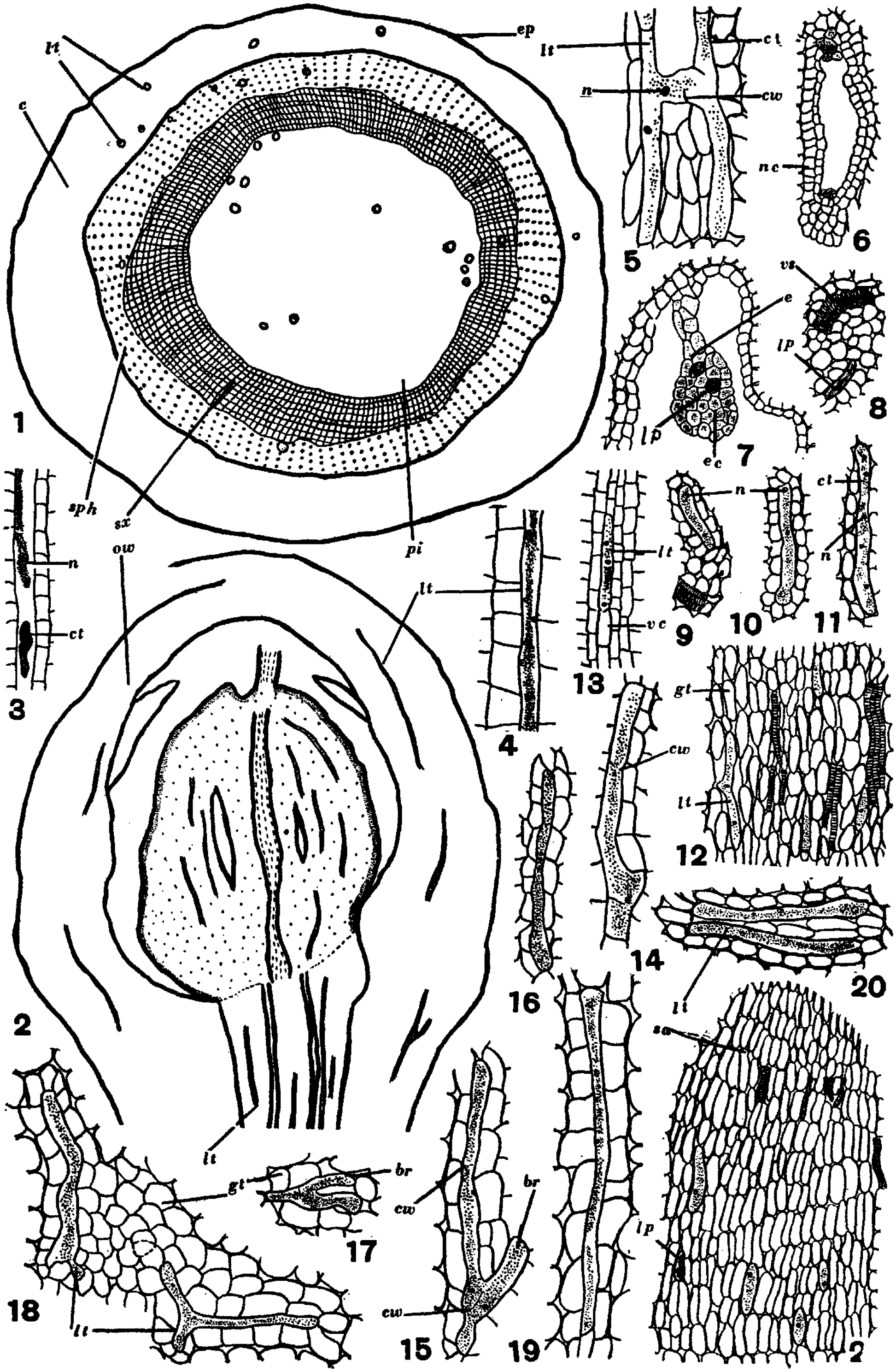
Distribution and structure of laticifers

The laticifers occur in all parts of the plant. They occur in very large numbers in the cortex, pith, xylem and phloem regions of the stem (Figs. 1, 22). The number is very great in the region of the pith of very old stems. In leaf they occur mostly in the midrib region and near the veinlets. The laticifers are more concentrated near the vascular elements.

The laticifers also run in the pedicel, perianth tube and ovary of the flower. The mature ovaries show many branched and unbranched latex-tubes (Figs. 2, 23). In general the laticifers are distributed all over the plant body at all the stages of its development. The young and old roots also show laticifers in the cortex.

The laticifers of *Tabernaemontana* are of the *non-articulated* type (Esau 1962) full of contents which may be scattered or concentrated at places and show several nuclei (Figs. 3–5, 24, 25). The multinucleate condition of the latex-tubes is a characteristic feature of the laticifers in *Tabernaemontana*. The tubes are rarely branched (Figs. 5, 25); the branching takes place in the later stages only. The wall of the tube is uniform, slightly thick, bears simple pits, and is probably made up of cellulose. The breadth of the latex-tubes varies in different parts of the plant. In old stems they are about 20–33 μ in thickness and in perianth lobes the latex-tubes are reduced to 16·8–25·2 μ.

FIGS. 1–21. (*br*—branch; *c*—cortex; *ct*—cell contents; *cw*—cell wall; *e*—embryo; *ec*—embryonic cortex; *ep*—epidermis; *gt*—ground tissue; *lp*—latex-primordia; *lt*—latex-tube; *n*—nucleus; *nc*—nucellus; *ow*—ovary wall; *pi*—pith; *sa*—stem apex; *sph*—secondary phloem; *sx*—secondary xylem; *vc*—vegetative cell; *vs*—vascular supply). 1, diagrammatic representation of transection of a stem showing the latex-tubes. ×32. 2, diagrammatic representation of a longitudinal section of the ovary. ×32. 3–5, showing the structure, branching and contents of the latex-tubes. ×140. 6–7, longitudinal sections of the mature ovary showing the suspected embryo. ×140. 8–12, stages in the development of the latex-tubes. ×140. 13–20, various aspects of the growth and branching of the latex-tubes. ×140. 21, a part of the longitudinal section of the growing tip of the stem showing some latex-primordia. ×140.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Course of development of the laticifers in the plant

Earlier authors (DeBary 1884; Haberlandt 1914; Foster 1949; Esau 1962) have already shown that in different latex-bearing families the non-articulated type of laticifers differentiate in the embryonic stage of the plant, and these later on grow, elongate and penetrate into the different plant organs. We could not fully confirm this in *Tabernaemontana* because it does not set seeds and fruits at Lucknow. However, some ovules develop a short-lived embryonic tissue and it has been possible to study it in serial sections, some of which are illustrated here. Figure 6 represents a part of a vertical section of an ovary with an ovule at the mature embryo-sac stage. Later on, an embryo-like structure develops in some ovules. Although the tissue of this structure shows no laticifer initials in younger stages (Fig. 6) yet, at a slightly later stage, they can be made out in the embryonic cortex (Fig. 7). These initial cells look very different from other surrounding cells. These embryonic laticifers do not develop further except in some cases where they are seen as very small tubes. They disorganize, without further development, along with the embryo-like development. The latter breaks contact with the surrounding tissue as it shrivels and aborts.

In the ovary wall and the perianth portion which has fused with it, laticifer primordia are visible near the vascular elements as elongated cells with very dense protoplasmic contents and a prominent nucleus (Fig. 8). This very soon elongates and it seems that the nucleus also divides into two. Thus, two distinct nuclei are seen in a slightly elongated young laticifer (Fig. 9) even before it has branched or fused with a neighbouring vegetative cell. There is further elongation followed by repeated divisions of the nuclei which persist till a late stage of development (Figs. 10, 11).

Figure 12 shows a few laticifers in various stages of development. Branching occurs in a few laticifers only. The elongating young latex-tube may either fuse with a neighbouring vegetative cell (Fig. 13) or may show at one point a small projection (Fig. 12) which after further elongation (Fig. 14) may fuse with the neighbouring vegetative cell to form a branched laticifer (Fig. 15). The partly-dissolved cell wall or septum may be seen sometimes in the developing latex-tube (Figs. 13–16). This union of a developing laticifer with a vegetative cell may be mistaken for articulation and the laticifer may even be recorded as an *articulated laticifer*. But according to Haberlandt (1914), Foster (1949) and Esau (1962) laticifers are regarded as articulated only when several laticifer initials, or rows of meristematic cells, fuse with one another in the embryonic tissue itself and produce branched laticifers. In the few cases of fusion noticed by us the union is between an elongated laticifer and an adjoining vegetative cell. This, too, happens only when the laticifer is unequally branched. Occasionally, at a very early stage of development, the

latex-tube may show a typical dichotomous type of branching (Figs. 17, 18). In some cases there are no branches and the latex-tube simply elongates (Fig. 19). Two or more latex-tubes (Fig. 20) may elongate side by side during their development but they never fuse.

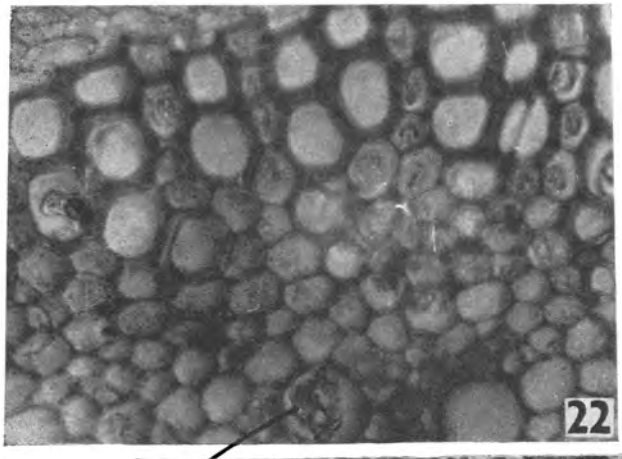
The growing tips of the stem which were raised by cuttings were examined periodically. They also show exactly similar types of laticifer primordia (Fig. 20). Sometimes branching occurs when the adjacent cells fuse with an elongating laticifer. In a very long non-articulated latex-tube, the contents may be so disposed as to suggest that the tube is formed by the union of a number of cells but actually this is not the case. As already stated, laticifer initials are no doubt found in the short-lived embryonic tissue but this does not persist. The laticifers developed in the very young parts of the plant ultimately form a branched system of laticifers in the adult plant. The laticifer primordia seen in the stem-apex of the plants obtained from the cuttings are not different from those found in the short-lived embryos.

At what particular stage the latex is produced in these laticifers is not very clear but it is evident from the study that very young parts show the latex. It is also found that when very old plant parts are injured, no latex comes out but the laticifers can be drawn out as fibres from older parts. This is due to the fact that in these laticifers there are no milky contents, and the wall has become thick. However, from the younger parts, such as growing stem-tips and leaves, latex comes out very freely.

Conclusion and Discussion

Two varieties of *Tabernaemontana coronaria* were examined. Only non-articulated laticifers occur and have probably a double origin. In the older plants the laticifers are developed from the primordia located in the shoot-apex. Laticifers are also probably differentiated in the embryonic tissue. This is very difficult to trace as the plant does not set seeds. A careful study of the development of the ovary showed that the wall has both branched and unbranched laticifers derived from the primordia differentiated by the stem-apex. Young ovules do not have any laticifers. Later stages of the ovules show, in the centre of the embryo sac, a tissue which by its position and mode of development seems to be embryonic. This may indicate completion of fertilization but in that case the factors responsible for the abortion of seeds and fruits of the plant remain unknown. A few unusual cells and some short tubular structures seen in the embryonic tissue are regarded here as laticifer initials. It was not possible to confirm the true nature of these cells because they abort along with other cells of the suspected embryo.

Describing the non-articulate laticifers of *Nerium oleander* Mahlberg (1961) has referred to the work of Milanez and Neto (1956) on the non-articulated laticifers of *Euphorbia pulcherrima* Willd. and remarks, 'Rather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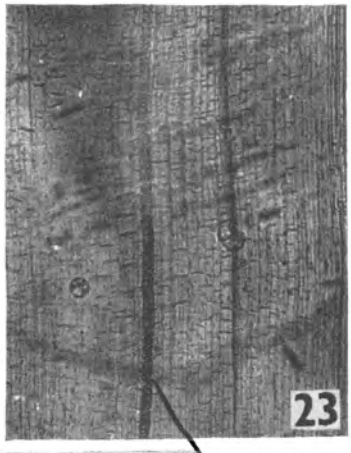
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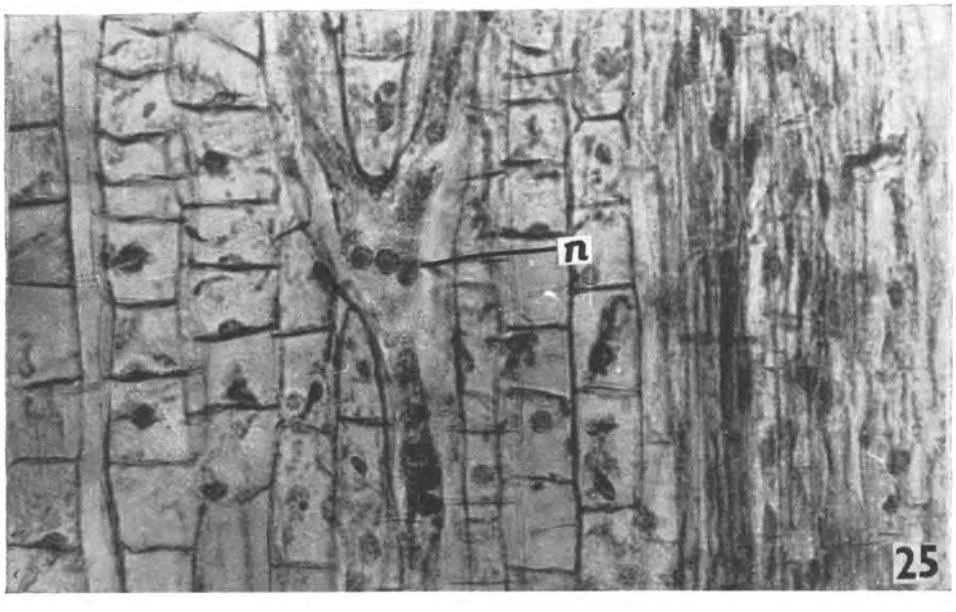
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origin of the laticiferous system within the young embryo was ascribed to occurrence of cellular fusion, which result in a series of "foci" at the cotyledonary node. These would correspond to laticifer initials described in the classical investigations.' These foci or laticifer initials further fuse with the adjoining vegetative cells followed by protoplasmic mixing. It thus appears that the fusion of laticifer initials with neighbouring vegetative cells is just an occasional deviation in the general pattern of development of the non-articulated laticifers. Accordingly the laticifers of *Tabernaemontana coronaria* studied in this paper would also come under the category of non-articulated laticifers. This view is further supported by the fact that other features of development of the laticifers conform to the non-articulate type. Indeed it appears from the literature so far studied by us that the laticifers are wholly of the non-articulate type in the Apocynaceae and Euphorbiaceae (Esau 1962).

Two functions are attributed to the laticifers. The long-believed view was that the latex constitutes the waste product of the plant and at best is useful to it by attracting insects that may help in fertilization. The second view, a more recent one to which we subscribe on the basis of biochemical studies of the latex of *Tabernaemontana coronaria*, is that the laticifers are not merely the carriers of waste products but are also secretory areas where enzymes, amino acids, etc., are produced. Studies on laticifers in older parts of the plants have shown that even though the latex is less in quantity yet the enzymatic and amino-acid contents do not altogether diminish. Older laticifers, like these, develop a very thick wall and in some cases look almost like sclereids and were even mistaken for them (Rao and Tewari 1960; Rao 1951).

From a brief biochemical qualitative analysis of the latex collected from the younger parts of the plant, mostly the growing tips, it was found that the latex has a few amino acids, carbohydrates and some very important enzymes, namely the proteolases and bacteriolytic enzymes. In view of this study, the laticifers assume great importance. This study has, of course, its industrial significance as a source of enzymes and amino acids. This will have to be assessed properly by quantitative estimations which are gradually being studied by us. But the more important aspect which interests the botanists is how far these enzymes and amino acids contribute to the vital activities of the plant itself.

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FIGS. 22–25. (*ct*—cell-contents; *gt*—ground tissue; *lt*—latex-tube; *n*—nucleus). 22, a transection of the stem showing the pith with the latex-tubes cut transversely. ×17. 23, longitudinal section of the ovary wall showing the latex-tubes. ×64·5. 24, some unbranched latex-tubes full of contents. ×50. 25, a branched latex-tube showing the nuclei. ×127·3.

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* Not seen by the authors.