SCLEREIDS IN *CRYPTOMERIA JAPONICA* D. DON

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The sclereids occurring in the male and female cones and in the stems of *Cryptomeria japonica*, but absent from the leaves, may, on their structure and form, be placed under the categories brachysclereids (Tschirch 1889; Foster 1949) and fusiform sclereids (T. A. Rao 1957). The presence of a massive deposit of tannin in the lumen of young as well as very old sclereids is a peculiarity not commonly seen. The sclereids develop in the cones as well as in the stems by the 'secondary sclerosis' of parenchyma cells. Their ontogenetic development proceeds by intrusive growth involving many important visible protoplasmic changes and sclerosis. The sclereids present in *Cryptomeria japonica* seem to constitute an important feature for the identification of the fertile parts of this genus from the allied genus *Taxodium*.

Introduction

The presence of peculiar sclerenchyma cells in some forms like *Welwitchia* (Bower 1881; Chamberlain 1955), *Araucaria* (Seward 1906) and a few other gymnosperms has been mentioned by various authors. But the systematic study of gymnosperm sclereids has not been done as thoroughly as in angiosperms. Recently T. A. Rao (1959) showed the presence of well-defined sclereid forms in sterile parts of a few gymnosperms. A. R. Rao and Tewari (1961) have given an account of foliar sclereids in *Taxodium distichum* Rich. So far as we are aware, the presence of these sclereids has been reported in sterile parts of gymnosperms only. In *Cryptomeria japonica*, a member of the Taxodiaceae, we have found numerous sclereids in both the male and female cones. In addition to these fertile parts, a few sclereids are also found in the cortex and the pith of stems. But none could be found in the leaves. Cross (1941) has given an account of histogenetic features in the shoot of *Cryptomeria japonica*, but without taking into consideration the presence of the sclereids. In the present paper an attempt has been made to study the ontogeny of sclereids in the cones as well as the stems of this species of conifer.

Material and Methods

The material under investigation was collected from some localities in Darjeeling and Kurseong at altitudes of 7,157 ft. and 6,000 ft. respectively above sea-level. It was fixed in formalin-acetic alcohol. Male cones were examined from herbarium specimens. They were boiled in 50 per cent glycerine to make them swell. Microtome sections were cut at a thickness between 8 and 15 μ for ontogenetic and structural studies. Iron-haematoxylin orange-G and safranin light green were found useful in permanent preparations and phloroglucin conc. HCl for temporary preparations. It was not possible to get cleared mounts of the cones, so slightly thicker free-hand sections of the entire cone were made and these were cleared by keeping them in conc. HNO_3 usually for 48 hours.

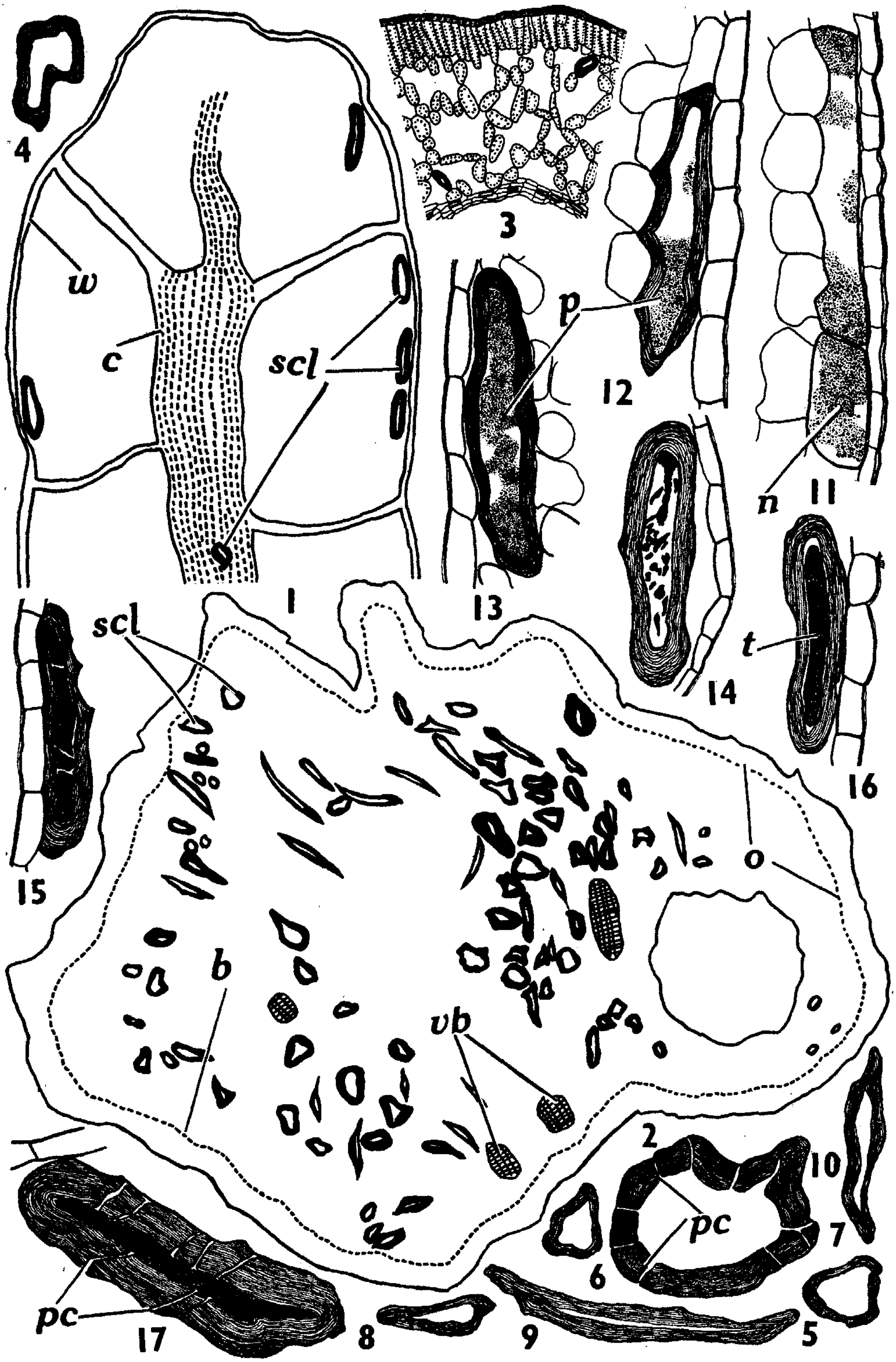
Distribution of Sclereids

The sclereids occur abundantly in a diffuse manner in the female cones (Fig. 30), in lesser number in the male cones, still lesser in the stems and are altogether absent in the leaves. In the male cone very few sclereids are found occurring in the sterile part of the cone. They are chiefly confined to the walls of the sporangia, a few also occur in the cone-axis (Fig. 1). In the female cones they occur in both sterile and fertile parts since the bract scale and the fertile scale are fused completely in the adult cone. The sclereids number more in the region of this fusion (Fig. 2). They also occur in greater numbers near the central region of the ovule (Fig. 31). But so far as the stems are concerned, they have a very small number of sclereids in the cortex (Fig. 3) and pith region (Fig. 32).

Structural Characteristics of the Adult Sclereid

The sclereids found in the stems and cones of *Cryptomeria japonica* can be classified into two kinds. The majority of them are rounded or oval-shaped, showing slight variations in their outlines (Figs. 4–6). These can be placed under the category of brachysclereids (Tschirch 1889; Foster 1949) or according to the more recent classification of T. A. Rao (1957) under *Spheroidal sclereids*. The second type, fewer in number, elongated in form, without much variation in shape but showing variation in size, can be more conveniently placed under the *fusiform sclereids* of T. A. Rao (1957) (Figs. 7–9). This type occurs in the female cone only, not in the male cone and stem.

Figs. 1–17 (*b*, bract scale; *c*, cone-axis; *n*, nucleus; *o*, ovuliferous scale; *p*, protoplasm; *pc*, pit canals; *scl*, sclereid; *t*, tannin; *vb*, vascular bundle; *w*, wall of microsporangia). Fig. 1. A part of longitudinal section through the male cone showing the distribution of sclereids. ×40. Fig. 2. A cleared mount of a fertile scale from a female cone. ×32. Fig. 3. A sector of transverse section of stem. ×40. Figs. 4–9. Various forms of sclereids. ×40. Fig. 10. A sclereid magnified to show the wall and pit canals. ×408. Figs. 11–13. The earlier stages in the ontogeny of the sclereid in the male cone. ×260. Fig. 14. A stage in the sclereid ontogeny showing the fusion of small granules in the lumen. ×240. Figs. 15–17. Later stages in the development when the sclereid has nearly matured. ×240.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The fusiform sclereids may have pointed, dilated or blunt ends (Figs. 8, 9). The wall of the adult sclereid is very thick, highly lignified, lamellated, with simple and ramiform pits, and having pit canals running horizontally up to the lumen (Fig. 10). The wall, when stained with either phloroglucin conc. HCl or with orange-G, under very high magnification shows concentric lamellations. The lignin layer seems to alternate with cellulose layers. These concentric layers are sometimes slightly irregular and are very deeply penetrated by the pit canals (Fig. 10).

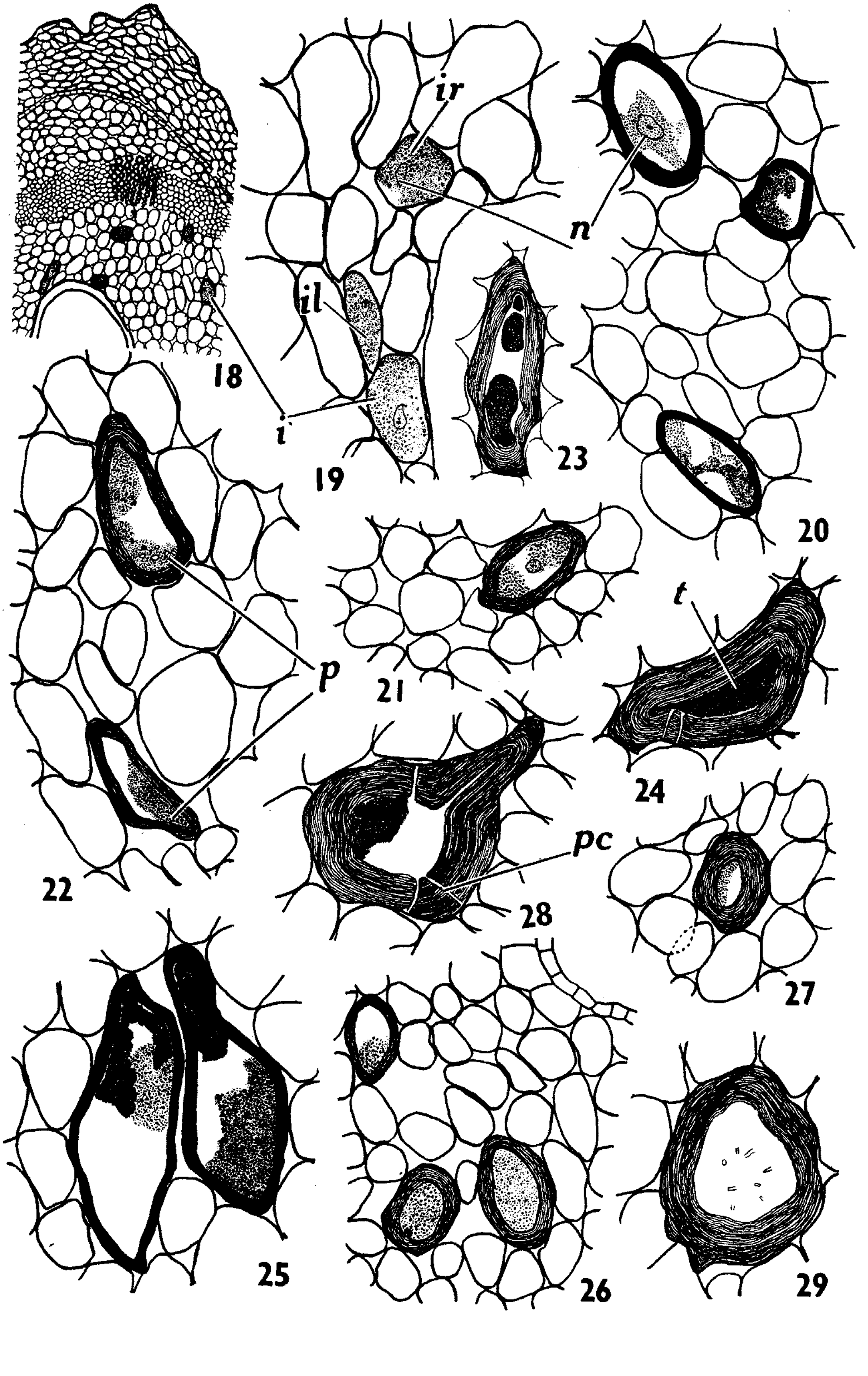
Unlike many sclereids reported in various genera where the lumen is more or less empty at maturity, in *Cryptomeria japonica* even the very old sclereids have the entire lumen filled with some deposit (Fig. 33). When following the various ontogenetic stages and studying the adult sclereids, some qualitative staining tests were also performed. It was thus possible for us to find out the nature of this deposition. The protoplasm itself during ontogeny undergoes many changes which are brought out clearly by staining. The substance present in the lumen is a yellowish and more or less homogeneous mass at maturity, but in the younger stages the entire substance is granular. It was found that the protoplasm has numerous granules, or small bodies, seen clearly under the high power of the microscope. These granules answer to the tannin test and they all fuse in the ultimate stages forming a homogeneous mass as has also been noticed by Esau (1958). She referred to this coloured form of tannin as *phlobaphenes*. Of course, more confirmatory quantitative estimations and specific chemical tests are needed to prove the exact nature of the tannin present in the sclereid. We were unable to do these tests due to some laboratory difficulties.

The below-mentioned qualitative staining tests for tannin were done as follows:—All the tests were done with the material which was fixed in formalin-acetic alcohol (fresh material was not available) and slightly thick free-hand sections of young and old cones were made.

1. The sections were treated with neutral iron salts like ether solution of anhydrous ferric chloride ($FeCl_3$) or concentrated aqueous solution of ferrous suplhate ($FeSO_4$) (Gatenby and Painter 1937). The same technique was modified slightly by Johansen (1940). A 10 per cent aqueous solution of ferric chloride was used, mixed with a little sodium carbonate (Na_2CO_3).

It was then noticed that the deposits inside the lumen become bluish-black. In the younger stages the granules inside the cytoplasm turn blue-black

FIGS. 18–29 (*i*, sclereid initials; *il*, elongated initials; *ir*, rounded initials; *n*, nucleus; *p*, pit; *pc*, pit canals; *t*, tannin). Fig. 18. A sector of a transverse section of a fertile scale showing the region of the ovule with sclereid initials. ×64. Fig. 19. A magnified view of the same showing rounded and elongated initials. ×240. Figs. 20–24. Various stages in ontogeny of fusiform sclereid. ×240. Fig. 25. Two peculiar bottle-shaped sclereids in the developmental stage. ×240. Figs. 26–29. Various stages in the ontogeny of brachysclereids. ×240.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in colour, as the rest of the cytoplasm remains colourless. The reaction was not so quick when ferrous sulphate was used. The sections were left in the solution of ferrous sulphate overnight. Then the colour became bluish-black inside the cell lumen.

2. The sections were treated with an aqueous solution of iodine and potassium iodide mixed with a 10 per cent solution of ammonia. It was observed that the deposits in the lumen turn red from the original yellowish-red. This change in colour is not very sharp or easily detected. It had to be observed very carefully under the microscope and by comparing with the control.

3. Another test performed was treatment of sections with a small amount of dilute ammoniacal solution of potassium ferricyanide. Here the deposits turn reddish-brown in colour more sharply than in the reaction (2).

Lastly, the sections were treated for more than two to three hours in excess of the same reagent. The reaction was very sharp, showing a rapid disappearance of the reddish-brown colour.

The only inference that can be drawn from these tests is that tannin is present in the lumen of the sclereids in *Cryptomeria japonica.*

Ontogeny of Sclereids in the Male Cone

In the male cones, the sclereids develop when the cones have become a little old, about 1-2 mm long. The initials first appear all along the walls of sporangia just below the outermost epidermal layer in the sterile tissue (Fig. 11). It is one of the ordinary ground parenchyma cells which becomes converted into the sclereid. The initial is usually bigger in size than the surrounding cells, and has a prominent cell-wall. The nucleus generally occupies a peripheral position in the beginning, although, very rarely, it may be centrally placed in some. It is more or less oval or slightly irregular in outline, with a single distinct nucleolus. The protoplasm is very dense and granular from the very beginning. The sclereid develops by the 'secondary sclerosis' (Foster 1949) of this parenchyma cell, involving many other protoplasmic changes. The sclereid grows in size by intrusive growth, pushing the other cells aside. In the sporangium after the pollen grains have developed, the surrounding sterile tissue is very soft, so that the sclereid can very easily grow and enlarge. Lignification and sclerization of the wall sets in very soon. In fact, after a slight increase in the size of the initial, the primary wall more or less disappears, being replaced by a thick secondary wall (Figs. 12, 13). The protoplasm undergoes some remarkable changes. In the earlier stages it is usually granular as shown in figures above. These small granules gradually start coalescing with each other and becoming larger in size (Fig. 14). Very soon the entire protoplasmic contents fuse, forming either two or three solid lumps (Fig. 15), or more or less a homogeneous mass (Fig. 16).

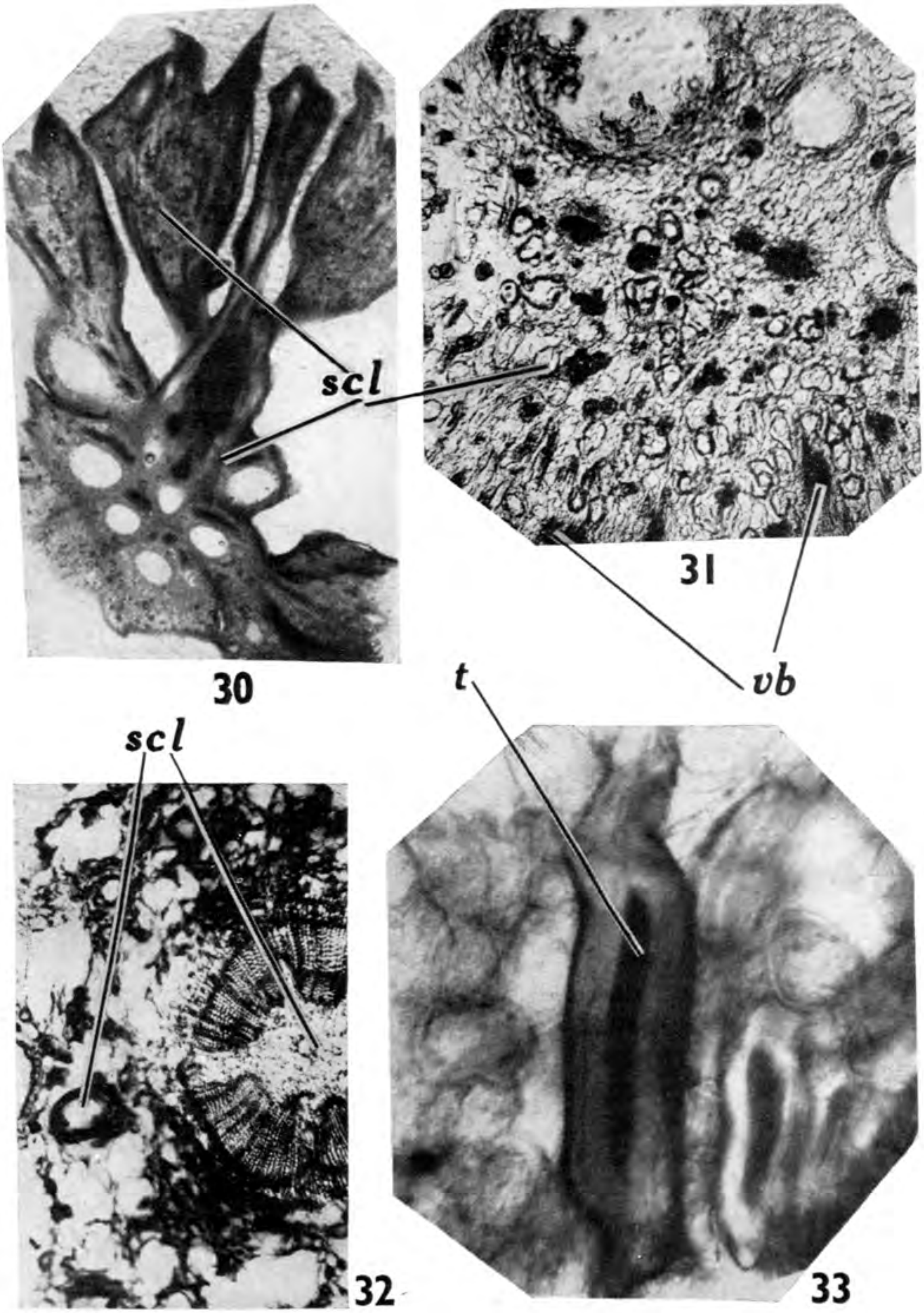
The pits now begin to appear in the secondary wall. The pits are either simple or ramiform, their pit-canals running through the entire thickness of the wall (Fig. 17) till the opposite end. The sclereids develop either singly or in groups of two or three (Fig. 1). The nucleus does not persist in the later stages when the lignification and sclerization have set in. This is an important feature seen also in the sclereid ontogeny of many angiospermous genera.

Ontogeny of Sclereids in the Female Cones

The sclereid development in the female cones is in some respects like the development in the male cones. But there are certain notable differences, too. The initials are first seen when the cones have become about 2–4 mm long. It is a parenchyma cell which is first recognizable at an early stage when the bract scale and ovuliferous scales have fused. The initials first appear towards the side of the bract scale, near the region of the ovule (Fig. 18). They also have a very dense granular protoplasm and an elliptical-shaped nucleus which, unlike the male cone, has usually two clear nucleoli (Fig. 19). The development is, however, on the same lines, by 'secondary sclerosis' (Foster 1949).

The initials in the female cones are of two types, either they are perfectly rounded (*ir*) or they are more or less elongated (*il*) in the longitudinal axis of the cone scale (Fig. 19). The primary wall does not persist for a long time and is soon replaced by a thick secondary wall (Fig. 20). The former type of initials develop into the brachysclereids and later ones into the fusiform sclereids. In the case of fusiform sclereids one end of the initial usually becomes more prominent and slightly projecting or sometimes even both the ends become prominent (Figs. 21, 22). These ends remain meristematic and grow through the surrounding tissues by intrusive growth. The contents migrate in this growing region as in *Dendrophthoe falcata* (Rao and Malaviya 1962), concentrating at this end and helping the cell in further elongation. The protoplasm generally shows similar changes as in the male cone (Figs. 23, 24). In these fusiform sclereids, one or sometimes both the ends become very elongated during the development, giving the sclereid a peculiar bottle-like shape (Fig. 25).

There are not many changes involved in the development of brachysclereids. The contents from the very beginning are distributed in the entire lumen. The shape of brachysclereids here is slightly different from those of the male cone where the sclereids are intermediate between the typical brachysclereids and fusiform sclereids. As the development proceeds, the lamellations start becoming distinct (Fig. 26) and the nucleus degenerates (Fig. 27). In the case of one brachysclereid it was found that the nucleus was seen in one corner



Figs. 30–33 (*scl*, sclereid; *t*, tannin; *vb*, vascular bundle). Fig. 30. A longitudinal section of cone showing the cone axis with the sclereids distributed all over. ×0·33. Fig. 31. A transverse section of the fertile scale showing the sclereids and vascular bundles. ×122. Fig. 32. A portion of transverse section of stem. ×55. Fig. 33. Two sclereids magnified to show the inner tannin contents. ×159.

of the lumen along with other cell contents (Fig. 28). But this was only an exception; in all other cases it disappears. Pits appear all over the wall. The apertures of these pits are seen as either oval or rounded or slit-like openings (Fig. 29).

Ontogeny of the sclereid in the stem follows more or less the same course as in the cones. A parenchyma cell either from the cortex or in the pith region gets transformed into the sclereid. The initial undergoes the usual lignification and sclerization. However, the protoplasmic changes are not so conspicuous as in the cones.

Discussion

The above-discussed sclereids present in *Cryptomeria japonica*, specially in the female cones, constitute an important feature from the anatomical as well as taxonomic point of view. In the allied genus *Taxodium* no sclereids are present in the cones. Their presence in the cones of *Cryptomeria* would thus constitute a clear distinction between the exceedingly similar fertile parts of the two closely related genera of the Taxodiaceae. It might also be pointed out that the leaves of *Cryptomeria* do not possess sclereids while those of *Taxodium* do. In fact, the presence or absence of sclereids, their form and distribution, if fully and properly studied, may furnish some reliable criteria in conjunction with other anatomical characters for the identification of fragmentary material of living as well as fossil conifers.

One important feature of the sclereids of *Cryptomeria* is the presence of tannin in the lumen of young as well as adult sclereids. As regards the function of these tannin granules present in the cytoplasm, we cannot be very definite. In the ontogenetic stages these are small droplets or granules and in the final stages these unite and form a solid lump (Esau 1958). Gortner and Gortner (1949) have given the views of many authors regarding the functions of tannin. One suggestion of Moore (*quoted by* Gortner and Gortner 1949) is that the presence of tannin may help in the cell-wall formation. As far as we have observed the structure and also the ontogeny of these sclereids, the occurrence of tannin in these isolated cell structures is to some extent correlated with the lignification as expressed by Moore.

Acknowledgement

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