On the Siliceous Deposit in the Cortex of Certain Species of Selaginella, Spr.

BY

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With Plate XVIII.

THE existence of a mineral incrustation on the cortical wall of the lacuna of *Selaginella Martensii*, Spr. is well known to botanists; but, so far as I am aware, no account has been published of the exact nature, distribution and mode of origin of this mineralization, nor has its presence in any other species of the genus been noted. In discussing the structure of the stele in *S. Martensii*, Strasburger[1] casually mentions the presence of the deposit, but gives no details.

Whilst engaged last winter on a research into the comparative anatomy of the genus *Selaginella* in the Botanical Laboratory of the University of Strassburg, Graf zu Solms suggested to me that it might be worth while to examine the deposit in some detail, and to determine its exact chemical character, distribution, and mode of origin in the species, and also to investigate whether similar deposits were to be met with in other species of the genus. I have examined in all fifty-two species of *Selaginella*, for material of which I am indebted to

[1] Ueber d. Bau. u. d. Verricht. d. Leitungsbahnen in d. Pflanzen: 'Hingegen sind an letzteren oft unregelmässige, spröde, farblose Belege zu sehen, die Kieselsäure zu sein scheinen.'

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Graf zu Solms; to the Director of the Royal Gardens, Kew; and to the Curator of the Botanic Gardens, Glasnevin, Dublin. Of these species I found sixteen to contain a siliceous deposit, viz.: *S. Martensii*, Spr. (both the type species and the varieties *flexuosa*, *compacta*, *stolonifera* and *variegata*); *S. grandis*, Moore; *S. Griffithii*, Spr.; *S. inaequalifolia*, Spr.; *S. Lobbii*, Moore; *S. haematodes*, Spr.; *S. suberosa*, Spr.; *S. atroviridis*, Spr.; *S. erythropus*, Spr.; *S. bakeriana*, Bail.; *S. stenophylla*, A. Br.; *S. involvens*, Spr.; *S. gracilis*, Moore; *S. flabellata*, Spr.; *S. caulescens*, Spr. var. *amoena*, and *S. emiliana*.

In all these the deposit presents the same essential characters; but there are individual differences, more especially in the distribution and amount of mineralization. I purpose selecting for detailed description and analysis *S. Martensii*, Spr., var. *compacta*, A. Br. (a form kindly named for me by Professor Dr. Kuhn), and giving a brief account of the deposit as seen in the other species.

S. Martensii, Spr., var. **compacta**, A. Br.

Without going into the minute histology of the stem, which will be treated of fully in a future paper, it will be sufficient to say that the single stele is suspended in a well-developed lacuna by trabeculae stretching from the innermost layer of the cortex to the pericycle. The external surface of the pericycle is covered by a cuticle. The trabeculae are of two kinds, simple and compound. The simple type consists of usually one, at most three cells; the compound type, which is by far the more numerous, of an attaching cell on the one side to the pericycle—endodermal cell—on the other to the cortex, and between these a cluster of thin-walled swollen cells, containing protoplasm, chlorophyll and starch. In the fully-developed stem these swollen cells completely fill the lacuna, and give it the appearance of a layer of loosely-arranged parenchyma, with numerous intercellular spaces. The cortical wall of the lacuna is covered by a plentiful deposit of mineral matter, laid down apparently in quite irregular colourless plates. If a portion of a stout stem be longitudinally sectionized so as to expose the inner surface of the cortex, and if the section be cleared, one gets an appearance similar to that represented at Fig. 1, Pl. XVIII. The vertical walls of the innermost cortical cells are observed more or less clearly through thin glassy plates of quite irregular size and shape. They have occasionally smooth, but more commonly very ragged edges, from which shorter or longer cracks extend into the body of the plate. Frequently a large plate becomes cracked into several pieces, the cracks widening into narrow channels. The edges of these channels are as a rule sharply defined, but occasionally at their inner extremities they become very faintly marked, as though the plates at that point were of extreme thinness. This feature is represented in the central plate of Fig. 1. Here and there areas occur which are quite uncovered by the deposit, but it is possible that in these situations the plates have been displaced in the preparation of the section. In a longitudinal section of the stem, prepared to show the edge of the plates, one finds that the above interpretation of the surface-view is the correct one, for the plates are seen to be of irregular thickness, in some places being as much as three times the thickness of the cell-wall they cover, in other places lessening in thickness very considerably and finally ending in an exceedingly delicate film, only distinguishable by its difference in refractive index from the cell-wall below. Fig. 2 shows a portion of such a section. Through gaps left in the deposit the trabecular cells arise from the cortex; the base of one of these is shown in the figure.

In addition to the deposit to be found on the outer lacunar wall, there occurs what seems to be a similar mineralization both in the walls of the innermost cortical cells and on the swollen cells of the compound trabeculae. If a transverse section be prepared cleared and mounted in balsam, the deposit may be quite easily made out in both situations. In Fig. 6 one of the compound trabeculae of *S. Martensii*, var. *stolonifera*, is represented. (The lower end of the figure is that next to the cortex, the upper is that next to the pericycle.) The mineral deposit in such a trabecula occurs in the small intercellular spaces between the constituent cells of the median cluster. It fills up these spaces completely and spreads as a very delicate film over the walls which are adjacent to each other. I can find no evidence of mineralization on the outer sides of these cells, facing the lacuna proper. Moreover, if the trabeculae be destroyed by concentrated sulphuric acid, the mineralization remains, and has the form of a number of concave shells or hemispheres united by their bases. Sometimes the basal cell next the cortex has a mineral deposit running up its external wall for a short distance, more commonly the deposit ceases abruptly at the base. I have never found any deposit on the pericycle nor on the cuticularized trabecular cells arising from it. Further, the cortical cells have a mineral deposit in their walls continuous with the surface layer in the lacuna. The small intercellular spaces between the cells of the innermost cortex are filled with the same deposit. As a rule, in var. *compacta*, only the three cell-layers next the lacuna are so mineralized; the cell-walls of the outer cortex contain no mineralization (Fig. 5).

As one would expect, the younger branches have considerably less development of the mineral, although it can be traced right up the stem almost to the merismatic region, being coincident in its appearance with the lacunar space. Fig. 3 shows a surface-view of the cortex lining the lacuna of a young branch about a quarter of an inch from its apex. Here it will be seen that the plates appear to arise at first close to or immediately over the vertical walls of the cortical cells. Two types of plates may be distinguished at this stage: (1) those which arise immediately over the vertical walls and spread out equally to either side, and (2) those which arise to one side of the vertical wall and develop over the cell-surface on that side only. The former are generally oblong and rather narrow, thickest just over the vertical wall and thinning off to either side. In sectional outline they appear as extremely obtuse-angled triangles. The second type of plate is thick at one (the outer) side, and thins off towards the centre of the cell. These plates have the appearance of razor-blades.

Cracks very soon appear on the thickened edge, passing inwards towards the centre of the adjoining cell, often bifurcating or branching irregularly as they go. The thin edge very often, even in the young condition, extends quite across the cell. If a development of the second type of plate occurs on the same cell from both or all sides, the deposits may become continuous in the centre of the cell area. The very youngest condition of the deposit I have been able to detect give the appearance of exceedingly delicate rods lying just over the vertical cell-walls. I have never seen the plates begin from the centre of the cell-area.

An examination of many sections taken from different parts of the stem leads one to the conclusion that the cracks are secondary in origin, and that the differential growth of the cortex obliterates in time the evidence of the mode of origin of the plates. It will be seen that in Fig. 1 the plates bear no relation whatever to the vertical cell-walls, whilst in Fig. 3 the relationship is obvious. A comparison of the size of the superficial cortical cells renders this still more apparent.

If a suitable section be boiled in concentrated sulphuric acid the organic matter is destroyed, but the plates remain quite uninjured. Figs. 4 and 7 show several forms of plates—Fig. 4 from a young stem, Fig. 7 from a mature stem. The trabeculae must of necessity be formed before the commencement of the deposition. The deposit, indeed, completely surrounds the base of the trabeculae, but does not, at least as a general rule, run up them. Fig. 7 represents a large plate from a mature stem, showing two apertures through which trabeculae passed in the fresh condition.

With regard to the chemical nature of the deposit there cannot be a difference of opinion, although it is not quite so easy to feel certain as to its mode of origin and deposition. The plates are undestroyed by concentrated hydrochloric, nitric, or sulphuric acids, hot or cold. They are unaffected by heating to redness. They cannot be stained, and they remain after the sections have been treated with cupric ammonium hydrate. All these negative reactions point to the mineral being what it has always been stated to be, viz. silica.

The amount of silica present in measured lengths of the stem was next determined. Portions of stem were dried for twenty-four hours over strong sulphuric acid. The dried material was then fused over a blowpipe and the weight of ash determined. It was found to amount to 9 per cent. of the dry weight.

The ash was then fused over the blowpipe with excess of sodium carbonate. The fusion was boiled with water, and after the addition of a little hydrochloric acid the whole mass was evaporated just to dryness on a water bath. Hydrochloric acid was again added, and the mixture taken again just to dryness on the water bath.

Water and a few drops of hydrochloric acid were then added, the whole slightly warmed and allowed to stand for an hour. The separated $Si\,O_2$ was filtered off and weighed. It was found to amount to 30 per cent. of the ash.

To the hot filtrate, ammonium chloride, ammonium hydrate and ammonium oxalate were added. After twenty-four hours the precipitate was filtered off, and, to ensure separation of the calcium and magnesium, the precipitate was redissolved in hydrochloric acid preparatory to a second precipitation with ammonium oxalate. A small quantity of $Si\,O_2$ (3 per cent. of the ash) remained here undissolved. The calcium was reprecipitated by ammonium hydrate and ammonium oxalate and filtered off after twenty-four hours. The calcium oxalate was then transformed into calcium oxide by ignition and weighed as such. It was found to amount to 18 per cent. of the ash.

The filtrate was then treated with ammonium hydrate and ammonium phosphate and allowed to stand for forty-eight hours. The magnesium was thus precipitated as $Mg\,NH_4\,PO_4$. The precipitate was filtered off and the magnesia determined from the weight of $Mg_2\,P_2\,O_7$, obtained by heating. It was found that the magnesia amounted to 18·7 per cent. of the ash.

The other ash constituents were not determined. It may be concluded from this analysis that $Si\,O_2$ is taken up by the plant as a soluble silicate of magnesia or of lime, or possibly as a double silicate of these bases. (I am greatly indebted to my colleague Dr. T. L. Bailey for his aid in this part of my work.)

S. Martensii, var. flexuosa.

In this variety the deposit on the lacunar wall is very well marked and the plates are of considerable thickness. The superficial cortical cell-layer alone, however, is well mineralized, although here and there the mineral may be detected in the minute intercellular spaces between the second and third layer of cortical cells. On the other hand, the trabeculae are plentifully supplied with $Si\,O_2$, the plates lying loosely round the basal cells next the cortex, and the clustered cells of the compound trabeculae have a large amount of $Si\,O_2$ in their intercellular spaces.

S. Martensii, var. variegata.

In this form the trabeculae are not so much silicified. The plates on the lacunar walls are more uniform and thinner, but the mineral can be traced into the cortex to the third, or even, in some places, the fourth cell-layer.

S. Martensii, var. stolonifera.

This variety resembles in all respects, so far as regards the siliceous deposit, var. *flexuosa.*

S. grandis, Moore.

I am not aware of any published account of the occurrence of $Si\,O_2$ in any species of *Selaginella* save *S. Martensii.* In examining the fine collection in cultivation at the Royal Gardens, Kew, I found that a deposit of the same mineral occurred in the cortex of *S. grandis*, as well as in the other species named above. I propose to give a brief account of the essential features shown by the deposit in that form.

In the first place the deposit is relatively much greater in amount than in *S. Martensii* or any of its varieties.

The plates are thicker and more regular in form, but still have the same characters so far as regards their mode of origin, viz. the first appearance of the deposit is over the vertical walls of the cells of the innermost layer of the cortex. The knife-blade form of plate is by far the most frequent, though occasionally the double-wedge form is to be met with.

In older stems the deposit is on the whole extremely regular, and consists of ragged, much-elongated bands, which anastomose frequently, leaving gaps between for the exit of trabeculae. Fig. 8 shows this feature in the siliceous lining in the main stem after treatment with concentrated sulphuric acid.

The full-grown plant has a well-marked primary axis, quite unbranched for a considerable distance, finally deliquescing in a flabellate manner into a number of secondary axes. At the point of origin of a branch the mineralization is much more irregular. Fig. 9 shows a portion of the deposit in such a situation after treatment with sulphuric acid. The silica runs up the trabecular cells and is deposited also between the cells of the compound trabeculae.

The cells of the inner cortex are long and sclerotized, have narrow lumina and run longitudinally. The cortical cells of the trabeculae also run in a creeping manner along the cortical wall before crossing the lacuna. In section the silica is found to penetrate between these cells, so that the trabecular cell as it leaves the cortex is encased by the mineral. Fig. 10 shows a section of the cortex next to the lacuna, where it will be seen that the silica is in places pierced by apertures through which run the creeping cortical cells. Silica may also be distinguished in the minute intercellular spaces between the cortical cells.

S. Griffithii, Spr.

The siliceous deposit in this species has similar characters to that in *S. grandis*, although it is much smaller in amount. The plates in the adult stem are not so regular, but the cortical trabecular cells are, at their origin from the cortex, encased in $Si\,O_2$.

S. inaequalifolia, Spr.

In young stems the deposit is small in amount and can scarcely be said to do more than form a thin lining to the cortical wall of the lacuna, though here and there it penetrates between the cells of the innermost layer of the cortex. In old stems, however, the appearance presented by the deposit is very similar to that seen in *S. grandis*. As this species is very commonly used for laboratory study, perhaps I may add that the deposit is very clearly seen in sections mounted in balsam. The figure from Sachs' Text-book, quoted in De Bary's Comparative Anatomy of Phanerogams and Ferns, and indeed in most other text-books treating of the genus, represents no such deposit. As a matter of fact the figure is more fatally erroneous in other respects—for instance, three steles are represented, each with two protoxylem-masses, one at either margin; whilst in reality there are twelve to fifteen protoxylems (in a full-grown stem), each stele having at least four. The two lateral steles are also occasionally double, two within one lacuna. I need not, however, discuss the anatomy further in the present paper.

S. Lobbii, Moore.

In the large suberect stems of this species a small amount of $Si\ O_2$ may be distinguished lying on and round the innermost cortical cells. The quantity is very small compared to that in some of the other species described. There are no special features of the deposit in this form that require description.

S. haematodes, Spr.

The thick-walled sclerotic cells lining the cortical wall of the lacuna have a small incrustation of $Si\ O_2$, which penetrates between the cells, but is not traceable beyond the third layer. There is no deposit on the swollen parenchyma, which partially fills the lacuna.

S. suberosa, Spr.

The deposit in this species is also small in amount and generally consists of a simple coating of thin irregular plates over the parenchyma forming the cortical wall of the lacuna.

S. atroviridis, Spr.

The features of the deposit in this species are precisely similar to those of *S. Martensii*, var. *compacta*.

S. erythropus, Spr.; **S. bakeriana**, Bailey; and **S. stenophylla**, A. Br.

A thin deposit occurs in these species on the innermost cell-layer of the cortex, penetrating between its constituent cells.

S. involvens, Spr.

The cortex of this species shows considerable variation from the type condition. The epidermis encloses several layers of sclerotized elongated cells with minute intercellular spaces, showing a gradual transition to the loosely arranged inner-layer and finally to what corresponds to the trabecular tissue. In fact, the entire conjunctive tissue outside the pericycle and endodermis cannot be differentiated into layers such as one sees in other forms. The intercellular spaces are numerous and large and either filled or lined by a large amount of siliceous deposit. The silica is most abundant in the middle layers, but can be traced almost to the epidermal layer outwards, and to the endoderm inwards. In the latter case the deposit begins in the angles formed by the juxtaposition of the cylindrical cells, of which the cortex is uniformly composed.

S. gracilis, Moore.

The deposit in this species resembles in all respects that in *S. Lobbii*.

S. flabellata, Spr.

The silica is confined to the minute intercellular spaces between the two innermost layers of the cortex and to the depressions between the free cells of the superficial layer facing the lacuna.

S. caulescens, Spr. var. **amoena**, and **S. emiliana.**

The very faintest trace of silica is to be found in the minute intercellular spaces between and on the innermost cortical cells. I could detect none, however, in *S. caulescens* itself.

Before any definite conclusions can be arrived at as to the part played by the siliceous deposit in the economy of the species that possess it, it will be necessary to cultivate specimens in an artificial soil from which silicates have been carefully excluded. In the absence of any experimental data on this aspect of the question, I can only at present suggest that the $Si\,O_2$ is an excreted product, and that calcium and magnesium are absorbed, at least in great part, in the form of soluble silicates, the silica being eliminated in the insoluble form. What the agent in the decomposition is would also form an interesting question. I have endeavoured to artificially produce decomposition of the silicate by hermetically sealing branches of *S. Martensii* in a glass tube filled with water saturated with carbon dioxide. After a week's exposure, however, I could detect no trace of silica in any situation save where it is to be found in the fresh condition.

EXPLANATION OF FIGURES IN PLATE XVIII.

Illustrating Mr. Harvey Gibson's paper on Siliceous Deposit in *Selaginella.*

All the figures are drawn under a magnification of 350.

Figs. 1–5. *S. Martensii,* Spr. var. *compacta.*

Fig. 1. Surface view of the siliceous plates from the cortical wall of the lacuna of a fully-developed stem.

Fig. 2. Siliceous plates on the cortical wall of the lacuna in section.

Fig. 3. Young stages in the development of the plates, taken from the stem about a quarter of an inch from the meristem region.

Fig. 4. Isolated plates from the apex of a young stem after treatment with concentrated sulphuric acid.

Fig. 5. Transverse section though the cortex after clearing in eau de Javelle and mounting in balsam. (The dark shading indicates the siliceous deposit.)

Fig. 6. Trabecula of *S. Martensii,* var. *stolonifera,* showing distribution of silica between the median cells of the trabecula.

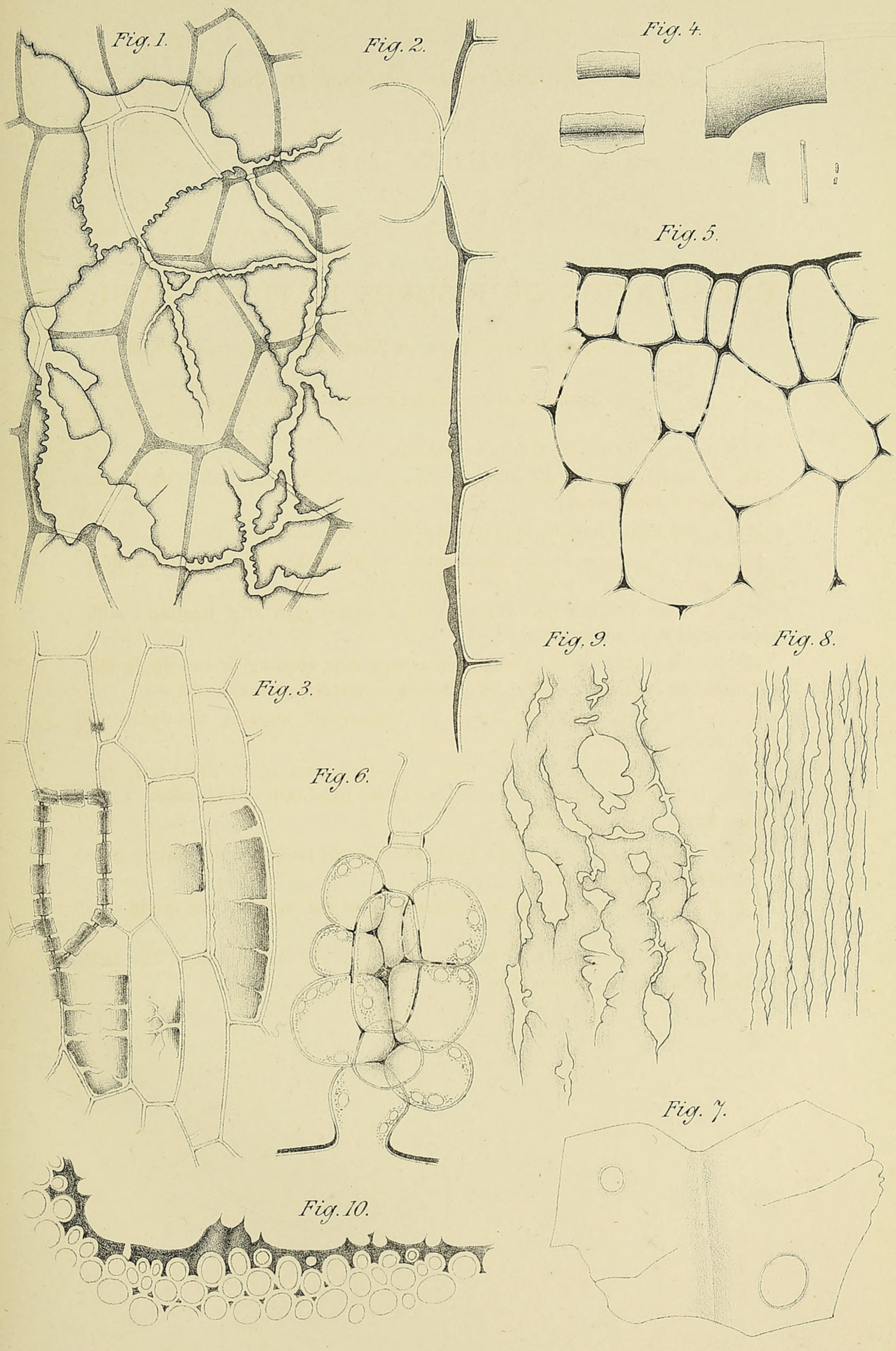
Fig. 7. Isolated siliceous plate from a mature stem of *S. Martensii,* var. *flexuosa,* showing two apertures for the exit of the basal cells of trabeculae.

Figs. 8–10. *S. grandis,* Moore.

Fig. 8. Siliceous plates from the cortical wall of the lacuna of the mature stem at the level of an internode.

Fig. 9. Siliceous plates from the cortical wall of the lacuna at the origin of a branch.

Fig. 10. Transverse section of the inner cortex of the mature stem. The dark shading indicates the siliceous deposit.



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