exposure is sufficient to kill spores of even extraordinary resistance.

7. The new method requires only small quantities of an inexpensive chemical and can be used in locations and under conditions where the bulky and heavy autoclave cannot be considered. The apparatus

needed for this method is very simple, easy and safe to handle, and requires little attendance.

8. This new method may be of special importance for the armed forces, when their mobile hospitals are unable to use autoclave sterilization.

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A Histological Study of Eriodictyon californicum*

By L. David Hiner and Kenneth J. Merrillt

Criticism of the United States Pharmacopæial description of Eriodictyon californicum (Hydrophyllaceæ) has led the Committee on Botany and Pharmacognosy to request that a further study of the drug be made. In accordance with this suggestion, an investigation of some of the histological features of the leaves was undertaken by Mr. Merrill during the past year. It was deemed advisable, however, to continue the study this summer, so additional samples were obtained for corroborative work as well

† Advanced Senior, Ohio State University, College of Pharmacy.

as for checking additional points which were still in question. The study was based chiefly on a review of the older descriptions, and also on the newly proposed monograph resulting from the work of Drs. Youngken, Wirth and Goodrich of the present Committee.

Since the first histological studies of this drug which were made by Ritter (1), it appears that very little interest has been shown in the leaves until this Committee expressed its disapproval of the existing monograph. Some definite changes are suggested herewith as a result of their proposal to revive the study of *Eriodictyon californicum*, and ultimately to revise the monograph.

^{*}From the Pharmacognosy Research Laboratory, Ohio State University, College of Pharmacy, Columbus, Ohio.

Presented to the Scientific Section of the A. Ph. A., Detroit meeting, 1941.

HISTOLOGY OF THE LEAF

The following characteristics have been noted in the study of the leaf structure.

Transverse Section.—The upper epidermis is composed of large, clear cells with thick walls, oval or rectangular shaped, with the surface edges wrinkled. Glandular hairs with 1- to 3-celled stalks and multicellular heads occur abundantly on this surface. They are deep seated and have very short stalks, which is in agreement with the findings reported by other investigators (see Fig. 1).

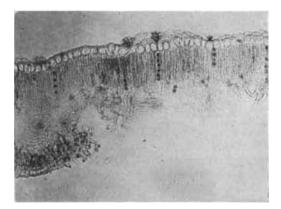


Fig. 1.—Deep-Seated Trichomes and Wrinkled Epidermis.

In a transverse and surface view of this same upper epidermis the cells are polygonal or hexagonal shaped and appear to be deeply striated due to the wrinkled surface of the epidermis. The striations are more prominent around the base of the glandular hairs and therefore appear to be formed by the epidermis pushing against the stalks of the trichomes (see Fig. 2). The tendency to wrinkle was more noticeable in the turgid cells.

Many glandular hairs are present on the upper epidermis, seeming to form at the junction of the epidermal cells rather than to develop from any one particular cell. The heads of these hairs are multicellular, occasionally containing a yellowish brown, granular secretion. Preliminary cell counts of these multicellular heads raised another question, when it was found that they were not always in agreement with the "up to 8-celled heads," as given in the official description of the newly

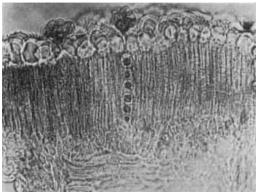


Fig. 2.—Epidermal Striations and Glandular Hairs.

proposed monograph. Therefore it was deemed advisable to make additional studies of this point.

Samples of Eriodictyon californicum were obtained from several reliable laboratories and crude drug dealers, and a total of 137 leaves selected at random from 11 commercial samples of the drug were prepared and examined. A Howe ocular counting chamber was used in the procedure, and the investigations were carried out on the surface sections using the oil immersion lenses. Unfortunately it was impossible to record the findings photographically inasmuch as the cells of the glandular heads did not all come into focus at the same level. They were frequently piled on top of each other to a depth of three distinct layers of cells.

Trichomes containing as high as 16-celled heads were discovered, although such an occurrence was rare indeed. A record of a typical count made from equal area surface sections of 11 different Eriodictyon samples is contained in Table I.

It is easily evident that the "up to 8-celled heads" predominate, but it is also noteworthy that trichomes with heads containing cells in excess of that number are present. This is not in agreement with the now proposed monograph, and needs additional study (2).

In preparing the leaves for making these surface section studies, they were macerated in chloral hydrate solution for at least 24 hrs. Prior to cutting the sections, the leaves were washed with fresh chloral hydrate solution to remove the dissolved resinous material. The glandular hairs showed

TABLE I.—CELL COUNT OF GLANDULAR HAIRS

Sample	Number of Head Cells												
	4	5	6	7	8	9	10	11	12	13	14	15	16
M-1	8	5	5	15	36	15	12	2	3	1	0	1	1
S-0	Ó	2	3	15	40	3	2	0	1	0	1	0	0
F-2	6	3	12	7	26	4	5	2	1	0	1	0	0
S-a	3	2	0	19	22	4	1	1	0	0	0	0	0
L-o	5	10	5	11	31	3	4	0	1	0	0	0	0
L-n	23	12	18	30	50	24	8	4	2	0	0	1	0
P-g	15	0	0	0	2 3	0	2	1	0	0	0	0	1
O-291	6	5	0	8	15	0	5	1	0	0	0	0	0
W-m	3	5	5	27	85	17	12	1	2	0	0	0	0
W-s	7	6	9	1	24	0	3	0	0	0	0	0	0
I-1	1	2	4	5	17	0	0	0	0	0	0	0	0

well in sections prepared in this way, when they were mounted in the same solution. Washing or mounting the sections in water, alcohol or glycerin alone collapsed the heads so that the number of cells present could not be distinguished.

The palisade layer consists of very narrow cells, 2 to 6 rows deep, and filled with chloroplastids. Viewed from above through the epidermal cells they appeared as masses of very small cells, sometimes as many as 10 appearing within the boundaries of a single epidermal cell. The palisade cells thin out at the edge of the leaf to a single layer of cells which may extend to the lower epidermis.

Merrill found that between the palisade cells at more or less regular intervals there are perpendicular rows of large parenchyma cells bearing rosette crystals of calcium oxalate. These cells are larger than the palisade tissue, have thicker walls, and do not contain chloroplastids. They vary mostly from 4 to 9 cells in the rows, are very conspicuous, and appear to be a very diagnostic feature of *Eriodictyon californicum*. They are best observed when the tissue has been cleared with chloral hydrate (see Fig. 3).

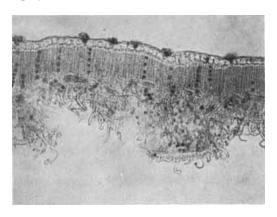


Fig. 3.—Rosette-Bearing Parenchyma.

Cross sections through the midrib of the leaf show the lower epidermis bearing occasional glandular hairs with multicellular heads. The number of cells in these heads vary from 4 to 8, with the 8celled heads occurring most frequently. No nonglandular hairs were found growing on the lower epidermis of the midrib; however, this contingency must be taken into consideration. Radial longitudinal sections through the midrib may show the epidermis appearing to bear nonglandular hairs if the cut ends adjacent to the surface of the lamina. Nonglandular hairs abound in this sinus, and in the isolated sections may appear to have come from the lower epidermis of the midrib proper (see Fig. 4). No nonglandular hairs were observed on the upper epidermis of the midrib structure. All other histological features of the midrib structure were found to be in complete agreement with the findings as reported by other investigators. Calcium oxalate crystals were present.



Fig. 4.—Nonglandular Hairs in Lamina-Midrib Sinus.

Spongy parenchyma is conspicuous, increasing in depth around the more prominent veins. The vascular system through the spongy parenchyma is composed chiefly of spiral trachea. Pitted trachea were not numerous, and were found mostly in the older leaves.

The lower epidermis is composed of thin-walled cells and masses of unicellular nonglandular hairs. These hairs are undulate and fill the sinuses formed by the more prominent veins.

Sections showing the stomata reveal them as nearly round, and having an oval-shaped opening between the guard cells (see Fig. 5). Rarely multi-

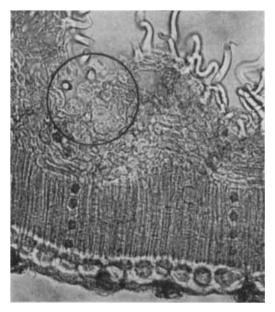


Fig. 5.—Round Stomata.

cellular glandular hairs are found on the lower epidermis. These were mostly with 2- to 4-celled heads.

SUMMARY

Glandular hairs were found to have 1- to 2-celled stalks as reported in the Committee Bulletin; 3-celled stalks also were found.

Glandular hairs containing multicellular heads varying from 4 to 16 cells were found; 9- to 10-celled heads were frequent.

Calcium oxalate rosette aggregates occur perfectly aligned in perpendicular rows of parenchyma cells interspersed in the palisade region. These cells are mostly from 4 to 9 cells deep, have thicker walls, and are larger than the palisade cells (see Fig. 6). These were observed in all leaves which were examined.

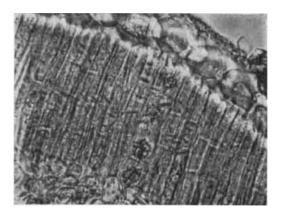


Fig. 6.—Rosettes of Calcium Oxalate.

Calcium oxalate crystals in rosette aggregates extend throughout the length of the midrib. The crystals may be present in two rows of cells that are adjacent to each other, but usually only one row of parenchyma cells holds the aggregates.

CONCLUSIONS

It is recommended that the following changes as indicated be incorporated in the official monograph of Eriodictyon.

Histology: Upper epidermis with large

cells, the outer walls very uneven in cross sections, owing to indentations which appear as striations in surface view, and with numerous deep-seated glandular hairs, possessing a 1- to 3-celled stalk and a multicellular glandular head with up to 8 cells predominating, but nonglandular hairs absent on this epidermis. Palisade cells very narrow, from 2 to 6 rows deep, in which are interspersed regularly arranged perpendicular rows of parenchyma cells, nearly each one of which contains a calcium oxalate rosette aggregate. Cells of spongy parenchyma forming but a few layers; vascular tissues not strongly developed except in the midrib and more prominent veins; lower surface in cross section showing numerous sinuses lined by the lower epidermis from which occasional deep-seated glandular hairs and many curved nonglandular hairs emanate and nearly fill the sinuses, frequently concealing the stomata.

Powdered Eriodictyon: Greenish yellow; nonglandular hairs 1-celled, undulate with thick walls, up to 250 μ in length and up to about 10 μ in width; glandular hairs with 1- to 3-celled stalks and multicellular heads with up to 8 cells predominating, the latter up to 120 μ in diameter; fragments of palisade tissue containing regularly arranged columnar parenchyma cells, nearly each one of which contains a rosette aggregate of calcium oxalate; tracheæ with spiral thickenings or simple pores and associated with lignified fibers; calcium oxalate in rosette aggregates numerous and from 5 to 30 μ in diameter.

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