

## CRYSTALS IN THE LEAF OF *LOBELIA INFLATA* LINN.

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The leaves of *Lobelia inflata* Linn. have been said either to contain crystals of calcium oxalate or to have no crystals of any kind. Other descriptions note the presence of droplets of oil in the mesophyll. This anomalous situation needs clarifying. It is shown that the crystals are fat.

THE presence or absence of crystals in the leaf of *Lobelia inflata* Linn. has been variously recorded by the writers of textbooks of pharmacognosy. Some authors give no description of the histology of the leaf, others state that calcium oxalate or crystals are absent, e.g., Zörnig<sup>1</sup> 1925; Gilg, Brandt and Schürhoff<sup>2</sup> (although crystals appear to be shown in their figure) 1927; Karsten and Bencke<sup>3</sup> 1928; Brandt and Wasicky (in Thoms)<sup>4</sup> 1931; Gathercoal and Wirth<sup>5</sup> 1956; Trease<sup>6</sup> 1952. Others again state that calcium oxalate in microcrystals is present, e.g., Koch<sup>7</sup> 1914; Flück, Schlumpf and Siegfried<sup>8</sup> 1935. Wasicky<sup>9</sup> 1936 makes the rather curious statement that "in cold chloral hydrate there crystallise out in the mesophyll after some time large needles, rods and sphaerites."

For some years past I have noticed in some sections of leaves of *Lobelia inflata* the presence of small crystals often almost filling certain cells of the palisade and spongy parenchyma of the mesophyll; in other similar sections, crystals appeared to be absent. When checking the details of microscopical structure for inclusion in the British Pharmaceutical Codex, I decided that this anomaly ought to be investigated.

The occurrence of crystals was reported in 1914 and 1935 (see above); the presence of droplets of oil was first recorded in the British Pharmacopoeia 1932<sup>10</sup>. When crystals were described they were said to be calcium oxalate either by direct statement or by inference. Wasicky in 1936 (see above) appears to refer to some decomposition product formed on standing with chloral hydrate.

The crystals present are small slender prisms about 10 to 15 $\mu$  long and 2 $\mu$  thick, often arranged in fan-shaped groups of about 2 to 6; they occur in many cells of the palisade tissue and in cells scattered throughout the spongy parenchyma; the cells containing them are often almost completely filled with the crystals (see Fig. 1). They are well seen in sections mounted in glycerol or in cold chloral hydrate solution and they polarise brightly. When these preparations are warmed to the boiling point, the crystals are no longer seen, but abundant droplets of oil are present and are stained deeply with Soudan red III. These facts and observations suggested to me that the globules of oil and the crystals might have some relationship to each other and that the crystals might possibly be regarded as crystals of fat which become globules of oil when the preparations are warmed.

To test the proposition that the crystals and globules of oil are the same substance, a rough sketch of a section was made superimposed by an eyepiece-scale and the positions of the crystals were marked; after warming droplets of oil were found in the same positions and no polarisable particles could be found, thus confirming the suggestion that the crystals are fat.

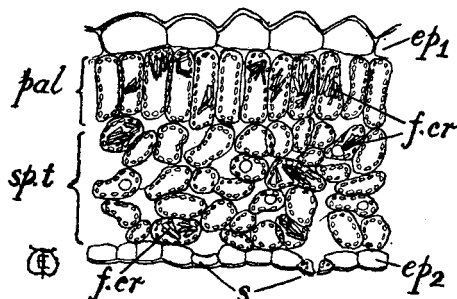


FIG. 1. Transverse section of the lamina of a leaf of *Lobelia inflata* Linn.  $\times 200$ ; *ep*<sub>1</sub>, upper epidermis; *ep*<sub>2</sub>, lower epidermis; *f.cr*, fat crystal; *pal*, palisade tissue; *s*, stoma; *sp.t*, spongy tissue.

Since the presence of crystals of calcium oxalate has been recorded by Koch and others, experiments were made to show the absence of calcium oxalate. All the preparations made in the course of this work were examined in polarised light as well as by ordinary illumination; this was done because the crystals are not always clearly visible in ordinary light.

#### EXPERIMENTS TO SHOW THE ABSENCE OF CALCIUM OXALATE

1. A transverse section was mounted in strong hydrochloric acid (s.g. 1.16) and after standing for ten days, the crystals showed no change, whereas calcium oxalate is rapidly dissolved under these conditions.

2. A transverse section was mounted in sulphuric acid 20 per cent v/v and no apparent change was observed during four days and no formation of the characteristic crystals of calcium sulphate was seen, showing an absence of calcium salts.

It is therefore evident that calcium oxalate is absent from the leaf.

#### FURTHER EXPERIMENTS TO CONFIRM THE FATTY NATURE OF THE CRYSTALS

1. A piece of leaf was soaked in chloroform for a few hours, a transverse section was then cut and mounted in chloroform with a little glycerol and crystals were absent.

2. A piece of leaf was soaked in alcohol, 95 per cent, for 24 hours after which a transverse section was cut and mounted in glycerol, when only a very few small bright specks were visible in polarised light, showing that the crystals dissolve in alcohol.

3. A transverse section was mounted in caustic potash, 5 per cent, and numerous crystals were seen similar to those seen in mounts in glycerol

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or cold chloral hydrate. On warming to the boiling point, the crystals were replaced by globules of oil which stained deeply with Souden red III.

Hence the crystals present must be fat.

### *Conclusion*

It is therefore evident that: (i) crystals are abundantly present in the mesophyll of the leaf of *Lobelia inflata*; (ii) calcium oxalate is absent from the leaves; (iii) the crystals present are composed of fat.

### REFERENCES

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8. Flück, Schlumpf and Siegfried, *Pharmakognostischer Atlas*, Basle, 1935, p. 260.
9. Wasicky, *Leitfaden für die pharmakognostischen Untersuchungen*, vol. II, Leipzig, 1936, p. 341.
10. *British Pharmacopoeia*, 1932, p. 275.