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INULIN CRYSTALS IN LIVING SPECIMENS OF *CENTAUREA RUPESTRIS* L. (*ASTERACEAE*)

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During anatomical investigations of *Centaurea rupestris* L., an Illyrian-Adriatic endemic species, the presence of inulin crystals was established not only in plant material preserved in fixing fluid FAA, but unexpectedly also in tissues of living plants. The latter phenomenon was rather rare, because the crystals were observed only in some of the samples of *C. rupestris*. In addition, inulin was found only in the fresh stem but not in the leaf tissue. In those stems, crystallization of inulin in the form of grains, of larger irregular crystals or spherocrystals, was noticed in the pith and pith ray cells, and inside the tracheary elements. The appearance of inulin crystals in some of the living specimens could be explained by desiccation of the plants growing in particularly dry places inside the habitat under the Mediterranean climate and soil conditions. In the investigated Balkan endemic *C. fritschii* Hayek, with area distribution in continental climate, inulin crystals were never detected in living plants.

Introduction

One of the most distinctive features in biochemistry of the family *Asteraceae* is the production of inulin as a storage polysaccharide. A large number of perennial, but not annual species of *Asteraceae* forms inulin as a reserve material, especially in the rhizomes and roots, but it is also found in stems, leaves, inflorescences and even in seeds (Meier and Reid 1982).

It is well known that inulin appears in the cell vacuoles only in solution, and with any dehydration of cells it precipitates, mostly in the form of spherocrystals. However, during the anatomical investigations of the endemic species *Centaurea rupestris* L. and *C. fritschii* Hayek (Rusak et al. 1992), we found crystals of inulin in fresh plant material of *C. rupestris,* too. This paper deals with the finding of inulin crystals in living *C. rupestris* plants.

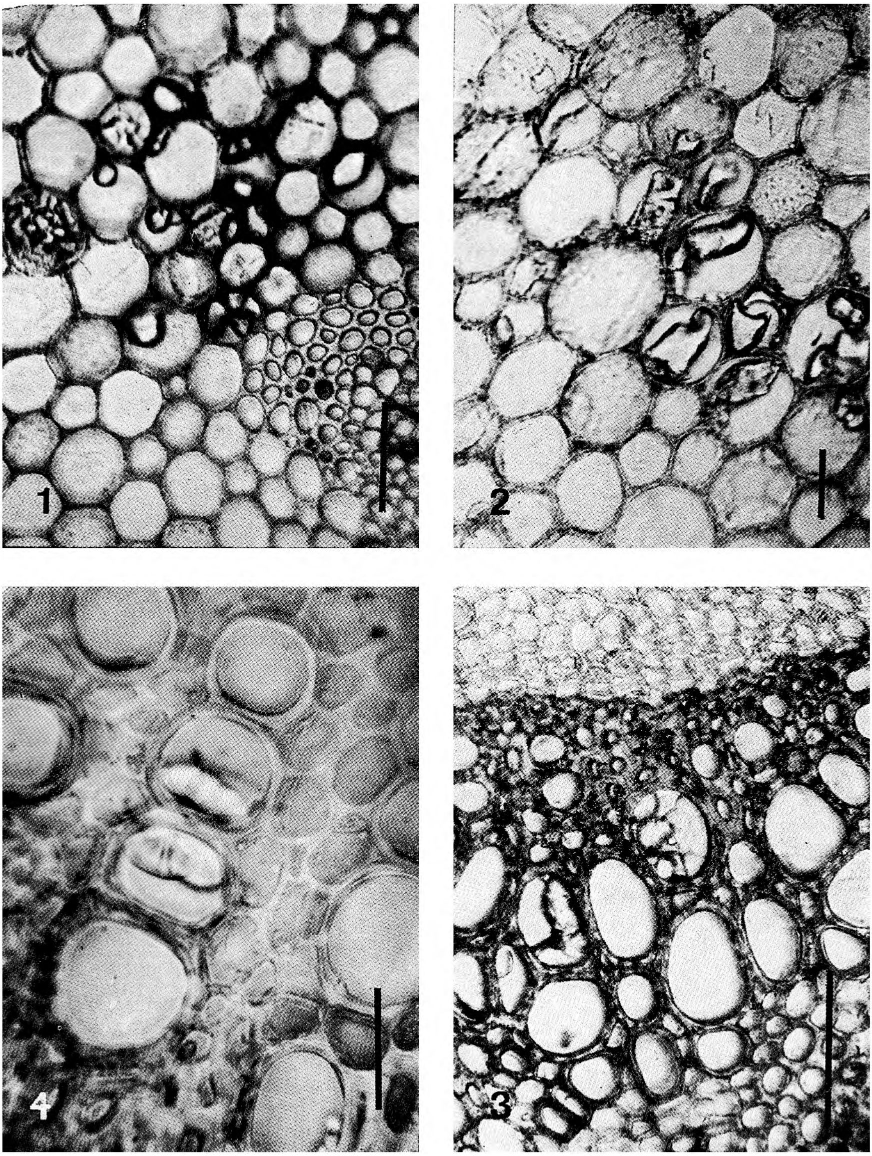
Material and Methods

The Illyrian-Adriatic endemic species *C. rupestris* was collected in an Adriatic coastal region (Uvala Scott) and the Balkan endemic plant *C. fritschii* in a continental district (Stojdraga) near Zagreb. The specimens of both species were sampled within two successive years during the flowering. The anatomy of the stem of these species was studied on material preserved in standard fixing fluid FAA containing 70% ethanol (Johansen 1940: 41), and on sections through live material which were immersed in tap water. The stem was analysed mainly in cross sections made manually by means of a razor blade and examined by light microscopy. The root and leaves were analysed only in fixed state. The identification of inulin crystals in fresh unfixed tissue was accomplished by incubating the crystals containing sections in warm water up to 50°—60° C (Behrens 1908: 167) as well as by exposing the crystals to reaction with thymol (Johansen 1940: 188).

Results and Discussion

In the specimens of *C. rupestris* and *C. fritschii* preserved in fixing fluid FAA inulin crystals were observed in all organs of the vegetative body, except the leaf blade. They were located inside previously living cells as well as in dead spaces such as tracheary elements (Fig. 4), and in large intercellular spaces including the pith hollow. In these spaces the crystals undoubtedly appeared secondarily as a result of diffusion of inulin from killed cells.

The dehydration effect of ethanol (present in FAA) which led to the crystallization of inulin mainly in the form of larger or smaller spherocrystals is well known. On the other hand, the same process of inulin crystallization takes place during desiccation of plant material for drugs or herbarium (Solereder 1899: 932, Frohne 1985: 130). Therefore, it was especially surprising and unexpected to find inulin crystals in fresh stem tissue of *C. rupestris.* It is necessary to emphasize that inulin crystals were not noticed in all the living samples of *C. rupestris.* They were present in specimens collected in both years, but only in some of the fresh stems investigated, i. e. in approximately one of about ten of the stems analysed. The crystals of inulin in living sections appeared in several forms: as small grains or rounded bodies in pith and pith ray parenchyma cells (Fig. 1) and inside the tracheary elements, in the form of larger irregular to nearly spherical crystals in pith parenchyma (Fig. 2), and as true spherocrystals attached to the wall inside the tracheary elements (Fig. 3). The nature of the crystals was proved by positive reaction with thymol and by their solubility in warm water.



Figs 1—4. Cross sections through the stem of Centaurea rupestris L. Inulin crystals in fresh stem: in the form of small grains and rounded bodies (Fig. 1) or irregularly shaped (Fig. 2) in the pith parenchyma cells, and in the form of spherocrystals (Fig. 3) in tracheary elements of the vascular bundle. Inulin spherocrystals in tracheary elements of the vascular bundle in stem preserved in FAA fixing fluid (Fig. 4).

Bar = 60 μm (Figs 1—3) and 20 μm (Fig. 4).

The presence of inulin crystals in living tissue of *C. rupestris* is very probably due to climate and soil conditions in the Mediterranean area of distribution of the species. In this region the high insolation and porosity of soil can lead to a pronounced loss of water from the cells, especially in hot summer, and consequently to the crystallization of inulin. Since the living plants containing inulin crystals were rather rare, we have come to the conclusion that such plant samples grew in the most unfavourable places where the water deficit in the habitat of *C. rupestris* was marked more than in the surrounding places. The explanation given is supported by the fact that the inulin crystals were never found in the living stem tissue of *C. fritschii,* whose area of distribution includes a district with continental climate. It seems that differently shaped inulin crystals in living cells are a consequence of the various rate of dehydration of single cells (Haberlandt 1924: 384). The formation of spherocrystals inside the tracheary elements seems to be a result of alterations in the permeability of some cells injured by excessive desiccation. The age of the plant can also play a role in the appearance of inulin crystals in living plant material.

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SAŽETAK

INULINSKI KRISTALI U ŽIVIM PRIMJERCIMA BILJKE *CENTAUREA RUPESTRIS* L. (*ASTERACEAE*)

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Pri anatomskim istraživanjima ilirsko-jadranske endemične vrste *Centaurea rupestris* L. prisutnost inulinskih kristala utvrđena je ne samo u fiksiranom (konzerviranom) biljnom materijalu nego također i u tkivu živih biljaka. Pojava kristala inulina u živim primjercima vrste *C. ru-*

pestris bila je prilično rijetka. Naime, kristali inulina zapaženi su samo u nekim svježim stabljikama analiziranih primjeraka biljke *C. rupestris*, a dolazili su u stanicama srčike i zraka srčike, te unutar provodnih elemenata ksilema. Inulin je kristalizirao u obliku zrnaca, većih nepravilnih kristala i u obliku sferokristala. Kristali inulina nisu zapaženi u svježim listovima biljke.

Pojava inulinskih kristala u nekim živim primjercima vrste *C. rupestris* može se objasniti znatnim isušivanjem onih biljaka koje su rasle na osobito suhim staništima a pod mediteranskim klimatskim i edafskim uvjetima. U istraživanog balkanskog endema *C. fritschii* Hayek, koji ima areal rasprostranjenja u područjima s kontinentalnom klimom, inulinski kristali nisu nikad zapaženi u živom biljnom materijalu.

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