Localization of Chromenes and Benzofurans in the Genus *Encelia* (Asteraceae)

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Phytochemical and microscopial analysis of leaves and stems of various species of *Encelia* showed a strict correlation between the presence of resin ducts and the accumulation of benzopyrans and benzofurans. Fluorescence microscopy of *Encelia farinosa* proved unambigously that these compounds are stored exclusivly in the resin ducts and the surrounding cells.

Introduction

Benzopyrans (chromenes) and benzofurans are characteristic and important natural products of the Asteraceae. Up to date more than 250 different compounds have been isolated from many species of this family [1, 2]. Several benzopyrans and benzofurans are biologically active. Some compounds, called precocenes, act as anti-juvenile hormones that accelerate the metamorphosis of many species of insects, causing nonviable precocious adults [3]. Other compounds that are more common throughout the family act as strong feeding repellents and are also directly toxic against herbivorous insects [4]. Among the various other bioactivities reported for this class of compounds [1], the phototoxicity of several compounds against microorganisms is especially noteworthy [5]. In previous studies we have established that benzopyrans and benzofurans are among the dominant natural products from many species of the North American genus *Encelia* Adans. [5, 6, 7, 8]. In organ specific analysis conducted with many species of *Encelia* we could show that benzopyrans and benzofurans are present in roots, stems, leaves, and capitula [6]. Nothing however has been known so far on the cellular localization of this class of natural products, an important aspect in regard to their possible biological role. In this present study we have investigated the localization of benzopyrans and benzofurans in *Encelia farinosa* Gray, a dominant species of the southwestern United States and Mexico [9] as well as in other benzopyran and benzofuran producing species of *Encelia* using phytochemical and microscopical techniques.

Results and Discussion

Microscopic studies of fixed and stained stems, petioles, and leaves of *Encelia farinosa* revealed a well developed system of resin ducts (Fig. 1). A typical stem cross section showed the ducts to be present in the bark as well as in the pit, whereas in the petioles and leaves they are embedded in the parenchymal cells. The diameters of the ducts were large (50 μm–100 μm in the leaves, 50 μm–150 μm in the petioles and 70 μm–340 μm in the stems) accommodating the copious amounts of resin produced by the plant. Subsequent fluorescence microscopic studies using fresh stems of *Encelia farinosa* proved unambigiously that the benzopyrans and benzofurans accumulate exclusively in the resin ducts. The natural fluorescence of the benzopyrans and benzofurans from *Encelia* triggered by the irridation of long wave UV-light was observed only in the lumina of the resin ducts and the surrounding secretory cells (Fig. 2).

Collecting *Encelia farinosa* in the field we made the observation that upon injury of the leaves or stems copious amounts of resin oozed out of the wounds. Subsequent chemical analysis revealed that 10–20% of the resin consisted of benzopyrans and

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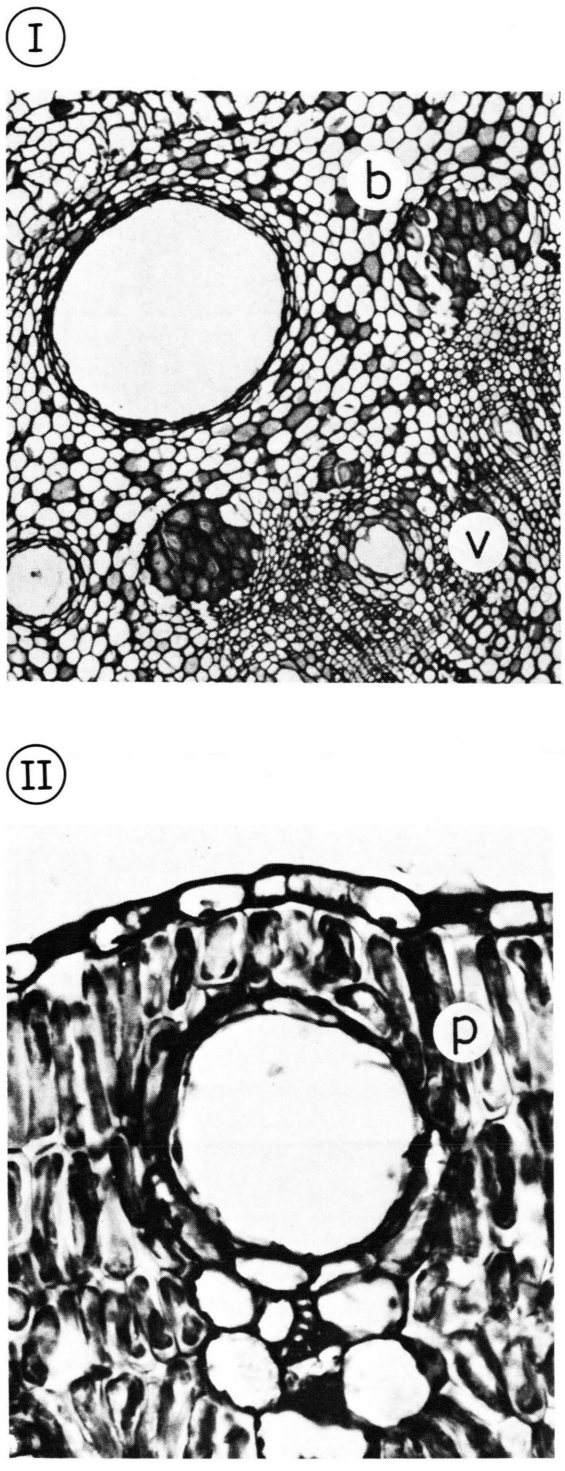

Fig. 1. Cross sections through stems (I) and leaves (II) of *Encelia farinosa* showing resin ducts to be embedded in the bark (b) of the stem and in the parenchymal cells (p) of the leaves respectively (v = vascular bundle).

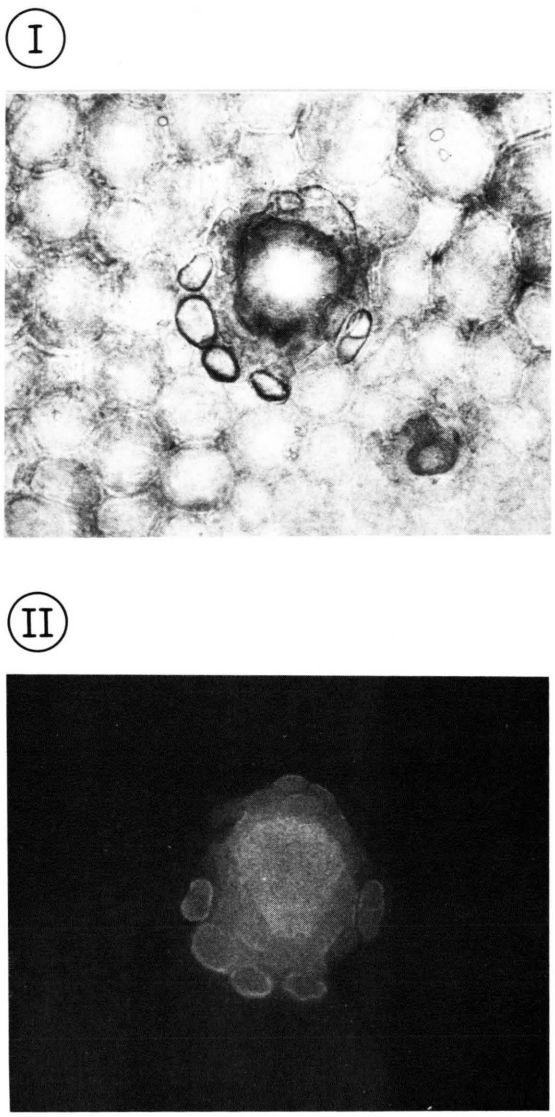

Fig. 2. Resin duct in the stem (I), exhibiting natural fluorescence after irridiation with long range UV-light (II), due to the presence of chromenes and benzofurans.

benzofurans. The major compounds that were identified include three benzopyrans, two benzofurans, and a new benzopyran-benzofuran dimer (Fig. 3). Also the fingerprint of the whole set of the resin compounds as elucidated by HPLC was virtually identical to the one obtained from the whole plant extract (Fig. 4).

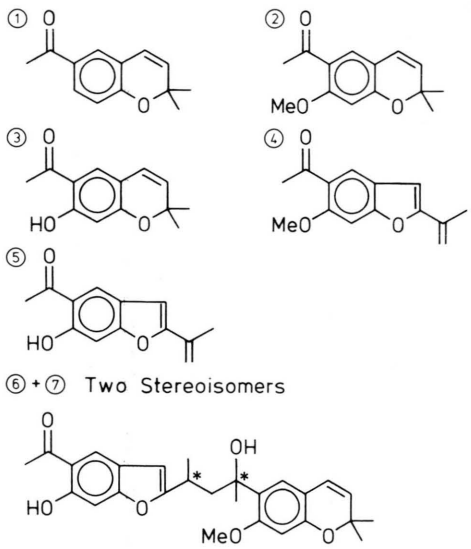

Fig. 3. Chromenes and benzofurans identified in the resin of *Encelia farinosa*.

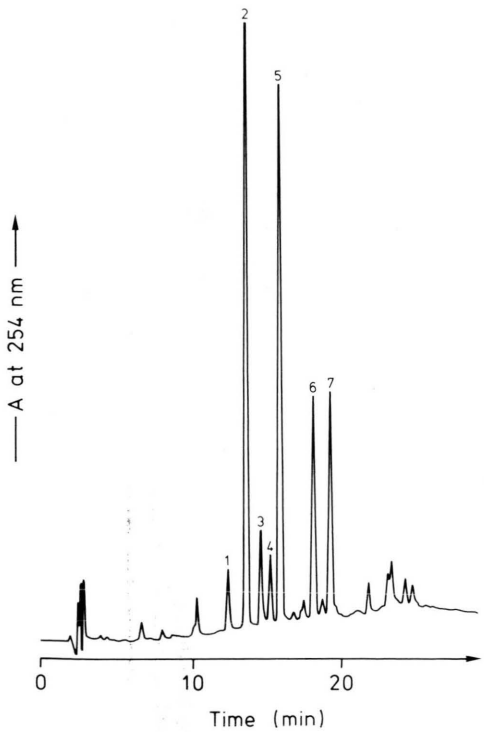

Fig. 4. HPLC chromatogramm of chromenes and benzofurans identified in the resin of *Encelia farinosa*. Numbers of compounds refer to Fig. 3.

Subsequent studies showed the resin ducts to be also present in all other species of *Encelia* that accumulate benzopyrans and benzofurans (Proksch, P. in preparation).

This new finding also bears several important chemoecological implications. Several of the benzopyrans and benzofurans from *Encelia* are toxic. Insecticidal and cytotoxic effects against microorganisms have been shown [4, 5]. Storing the compounds inside the resin ducts would therefore help in avoiding also possible autotoxicity. In bioassays we could demonstrate that the insecticidal activities of these compounds are most pronounced by direct contact via the cuticle of the insect, a phenomenon which in nature can easily be achieved by the resin oozing out from the resin ducts upon injury of the plant by an herbivorous insect.

Experimental

Encelia farinosa was collected in June 1984 in the Mohave Desert, California.

For light microscopy the plant material was prepared by glutaraldehyde fixation, followed by a dehydration series in tertiary butanol and paraffin embedding [10]. The sections were stained with toluidine blue. Fresh plant material was used for fluorescence microscopy. The natural fluorescence of benzofurans and benzopyrans was observed after irridiation with UV-light of 350 nm.

Resin oozing out from freshly cut leaves and stems of *E. farinosa* was directly collected into vials containing acetone. The phytochemical analysis of the resin by HPLC was performed as described previously [7].

Light microscopy of the following species was performed with dried plant material: *E. californica, E. canescens, E. conspersa, E. densifolia, E. halimifolia, E. laciniata, E. farinosa var. radians, E. palmeri, E. stenophylla, E. tenuifolia, E. ventorum.*

The dried plant material (stems) was allowed to soak 6 hrs in 2 N KOH, then thoroughly rinsed with water and used for light microscopy.

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