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DISTRIBUTION OF ALKALOIDS IN *CHELIDONIUM MAJUS* AND FACTORS AFFECTING THEIR ACCUMULATION

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Key Word Index—*Chelidonium majus*; Papaveraceae; isoquinoline alkaloids; accumulation, environmental factors.

Abstract—The alkaloid content in *Chelidonium majus* has been examined during the day, both in summer and in winter. Sanguinarine, chelidonine, chelerythrine, berberine and coptisine were detected in all parts of the plant. While the berberine content was rather stable, the other alkaloids undergo remarkable daily variations probably due to the combined effect of light and temperature.

INTRODUCTION

Chelidonium majus L., a hemicryptophyte with basal leaves partially overwintering, is a medicinal plant employed from time immemorial owing to its chemically and pharmacologically interesting isoquinoline alkaloids. It is a well known source of benzophenanthridine (sanguinarine, chelidonine, chelerythrine), protoberberine (berberine) and protopine (coptisine) alkaloids [1, 2]. Cytotoxic, antiviral, antifungal and anti-inflammatory activities have also been found in crude extracts of this species. Furthermore, chelidonine, the main alkaloid from the aerial parts of *C. majus*, is used for the preparation of a carcinostatic agent (thio-phosphoramide) covered by several patents [3, 4].

The aim of the present work was to evaluate the influence of environmental factors, such as daylight and temperature, on the accumulation of alkaloids in the various parts of *C. majus*.

RESULTS AND DISCUSSION

Five isoquinoline alkaloids (sanguinarine, chelidonine, chelerythrine, berberine and coptisine) were detected in extracts from 2–3 g of green parts or roots and 20–30 mg of latex (recovered from the collar zone). In order to evaluate the influence of daylight and temperature we analysed the alkaloid content in the course of the day both in winter, in the shortest days and with a temperature near to 0°, and during summer when the temperature and the day length reach a maximum. Plant material was collected for two consecutive days, early in the morning, at mid-day and in the evening.

In the latex (Fig. 1) during summer, the accumulation of chelidonine, coptisine, chelerythrine and sanguinarine increased throughout the day reaching a maximum content in the evening, i.e. accumulation continues during the whole light period. The increase is remarkably high during the morning and is maximal for sanguinarine (from 1.5% fr. wt early in the morning to 3% fr. wt in the evening) and coptisine (from 6.0% to 10.0% fr. wt). During the night, the alkaloid content decreases so that every morning it is nearly the same.

During the winter, the alkaloid content is highest in the central hours of the day, when light and temperature are at maximum levels. From 12.00 to 17.00, a slight decrease occurs, perhaps due to low temperature the reduced light in the afternoon is not sufficient to influence the accumulation of alkaloids. The decrease becomes very sharp in the subsequent first hours of darkness (from 17.00 to 20.00) and no more decrease is detectable during the night. Only the berberine content appears to be rather constant under all conditions.

To clarify whether the observed decrease in alkaloids during the dark period is due to degradation rather than to translocation, the alkaloid content was examined in the same conditions in the roots and in epigeous parts of the plant. The alkaloid accumulation patterns during the day in roots (Fig. 2) were similar to that in the latex, with a higher increase, in summer, during the afternoon. In the epigeous parts of the plant (Fig. 3) during summer, the alkaloids begin to accumulate only in the afternoon, reach a maximum in the evening and diminish during the night and, to a lesser extent, until late in the morning. During the winter, the alkaloid content is low throughout the day and appears to be stable, but during this period the basal leaves are becoming senescent and presumably have a reduced metabolism.

From our results it appears that only the berberine content is rather stable, while the other alkaloids (sanguinarine, chelidonine, chelerythrine and coptisine) undergo remarkable daily variations. Considerable changes in

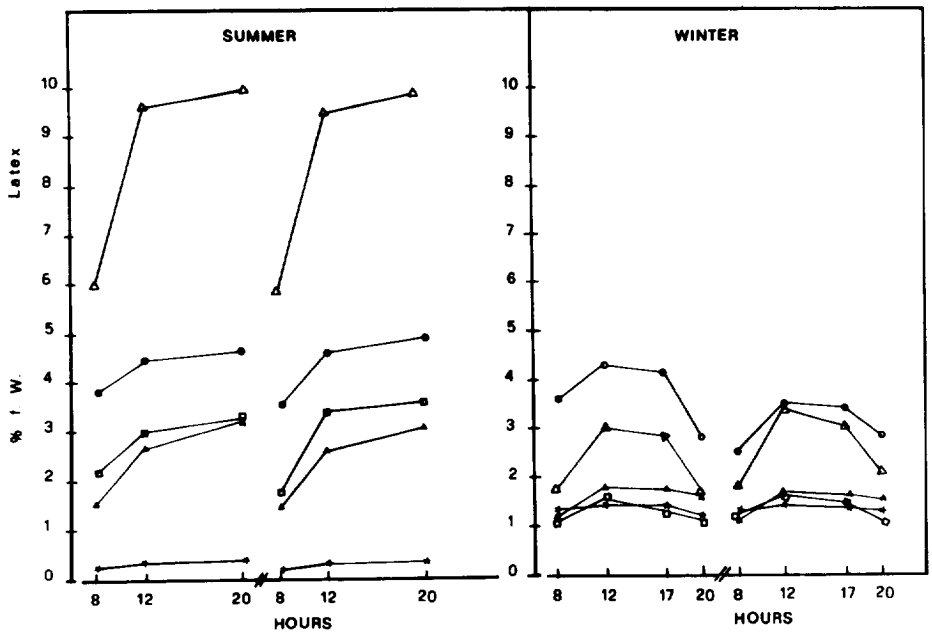

Fig. 1. Pattern of alkaloid accumulation in latex of *C. majus* during summer and winter (▲ sanguinarine, ○ chelidonine, □ chelerythrine, ★ berberine, △ coptisine).

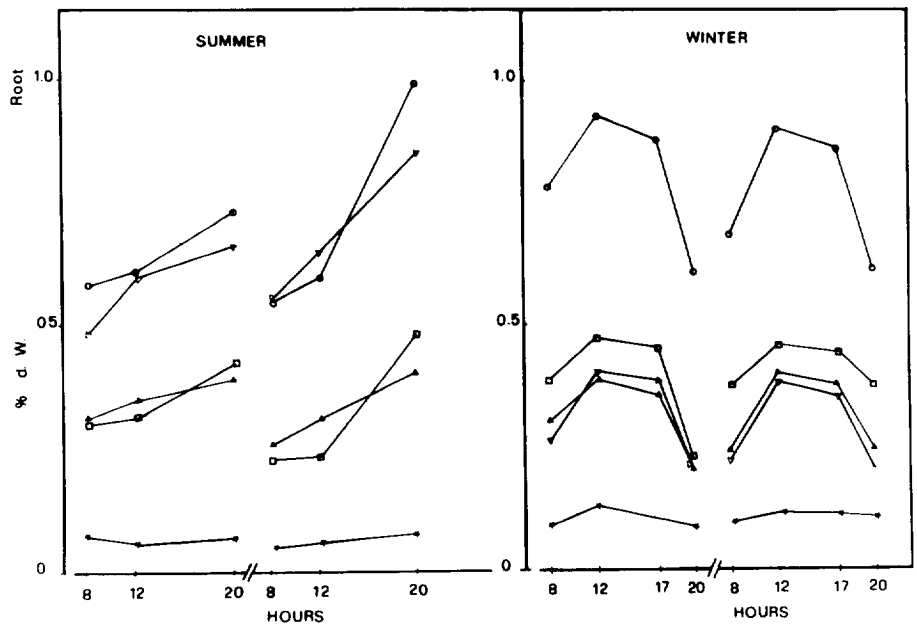

Fig. 2. Pattern of alkaloid accumulation in roots of *C. majus* during summer and winter (▲ sanguinarine, ○ chelidonine, □ chelerythrine, ★ berberine, △ coptisine).

alkaloid content in the course of the day have also been described in other Papaveraceae, like *Papaver somniferum* [5], suggesting a possible metabolic role for these compounds. The fluctuations in intervals of only a few hours detected by us in *C. majus* are probably due mainly to synthesis and subsequent catabolism rather than to translocation, because the time-course is similar in all parts of the plant. These rapid changes are in agreement with the hypothesis that alkaloids have a dynamic role in plants and, furthermore, that in *C. majus* an extensive alkaloid metabolism occurs.

The time-course of alkaloid accumulation shows an increase during the day and a net decrease in the night. It is well known that environmental conditions, such as

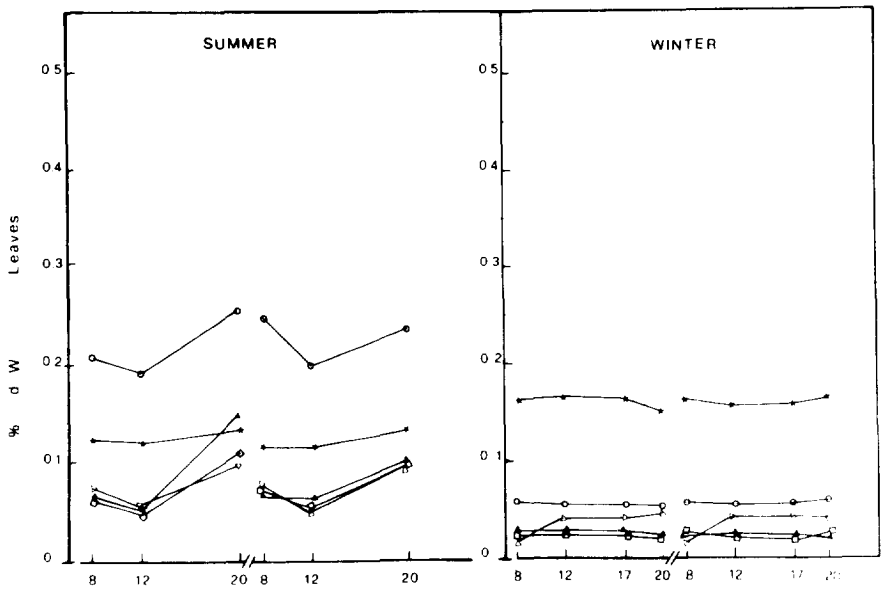

Fig. 3. Pattern of alkaloid accumulation in leaves of *C. majus* during summer and winter (▲ sanguinarine, chelidonine, chelerythrine, ★ berberine, coptisine).

light and temperature, may affect alkaloid formation and, in particular, that the presence of light is, in some cases, necessary for the onset of alkaloid accumulation [6]. It is noteworthy, that in *C. majus*, light conditions affect the alkaloid accumulation pattern mainly in hypogeous rather than in epigeous parts of the plant.

In summer, this accumulation goes on as long as there is day light, in winter, only for a part of the day, until 12.00. Thus, it appears that the quantitative variations are due to the combined effect of light and temperature: light is essential to reach a level of alkaloid content. To exceed this level it seems that the temperature should be high enough, i.e. it becomes a limiting factor.

EXPERIMENTAL

Fresh material (aerial parts, roots and latex, separately) were exhaustively extracted in a Soxhlet apparatus with 70% EtOH. 1-Octanesulphonic acid Na salt (0.4%) was added to the concd and acidified (pH 1, HCl 0.1 N) alcoholic extract. The alkylammonium compounds were extracted with $CHCl_3$, the organic layer concd and the residue diluted in a volumetric flask with MeOH [7]. Alkaloids were analysed by HPLC and detected using a photo diode-array detector. A silica gel column (250 mm × 4.6 mm i.d., 10 μm particle size) was used, eluting with 0.005 M NaOAc in MeOH–1,4-dioxane–HOAc (44:5:1). Flow rate was 1.5 ml min^{-1} [8].

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