# DIURNAL AND DEVELOPMENTAL VARIATION OF ALKALOID ACCUMULATION IN *ATROPA BELLADONNA*

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Abstract

The production and distribution of tropane alkaloids in organs of wild *Atropa belladonna* plants was studied by HPLC. Two different growth stages were examined in June and July and in addition berries and seeds in September 1991. A seasonal variation in total alkaloid content and alkaloid patterns of these plant parts was observed. Hyoscyamine was the main product throughout. A diurnal variation of tropane alkaloid content could be determined. Two peaks were significant: maximal alkaloid yields were detected at early night and at early morning. In maturing seeds, the alkaloid content was highest in the afternoon.

## 1. Introduction

Tropane alkaloids are constituents of several genera of the Solanaceae such as *Atropa, Datura, Duboisia, Hyoscyamus* and *Scopolia.* Their main alkaloids are the medicinally important hyoscyamine and scopolamine, which were often accompanied by their biosynthetic intermediates 6ß-hydroxyhyoscyamine, 7ß-hydroxyhyoscyamine and littorine (Figure 1). The biosynthesis of the tropane alkaloids is well established (Leete 1990) and it is commonly assumed that the alkaloids are synthesized in the root system of plants. From there they are transported via the xylem to the aerial part of the plant (Mothes et al., 1985), where further modifications occur. Alkaloid metabolism is often dynamic and substantial turnover rates have been recorded in some species (Wink and Witte 1984). A preliminary report indicated that also tropane alkaloids in *A. belladonna* display diurnal fluctuation (Fairbairn and Wassel 1967 in Waller and Nowacki 1978).

In this study we have analyzed the diurnal rhythmicity of tropane alkaloid formation in *Atropa belladonna* in more detail. We report on the diurnal fluctuations of tropane alkaloids in several organs of the plant and especially in maturing seeds.

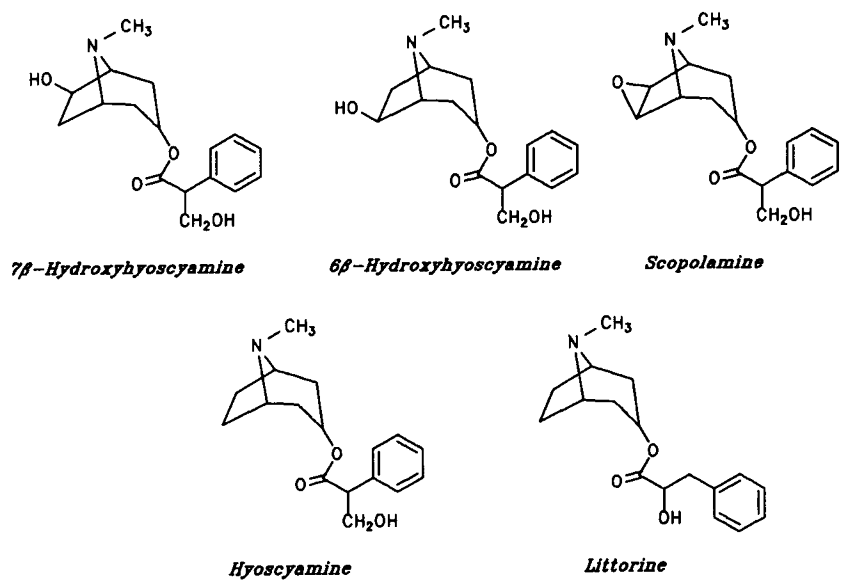


Figure 1: Tropane alkaloids in *Atropa belladonna*

## 2. Materials and methods

### 2.1 Plant material

Three 2-year-old plants of *A. belladonna* which were growing wild near Heidelberg were used for the experiments. The plant material (1 g fresh wt.) was collected from the plants every 3 h during a period of 24 h and immediately frozen in liquid nitrogen. Two sets of experiments were carried out, one with flowering plants on June 25, one with fruiting plants on July 25, 1991. The following tissues were analyzed: Flower buds, young leaves (< 3 cm in diameter, "leaf 1"), young stems ("stem 1"), old leaves (> 6 cm in diameter, "leaf 2"), stem bark ("stem 2") and roots. In addition three berries were harvested every 2 h during a period of 24 h in September 1991. The maturing seeds were isolated, washed with water and frozen in liquid nitrogen.

### 2.2 Sample preparation and HPLC analysis

After lyophilization the milled plant material (50 mg) was extracted with 5 ml $CHCl_3$/ MeOH/ $NH_4OH$ (15:5:1) using sonication (10 min). Further sample preparation followed a standard protocol (Sauerwein and Shimomura 1991). The alkaloid extracts were dissolved in 100 µl MeOH and 5 µl was analyzed by HPLC. A

TOSOH ODS-120A column (4.6 I.D. x 250 mm) was used, kept at 40° and eluted isocratically with $CH_3CN$ / 10 mM SDS (pH 3.3) 2:3. The flow rate was 1.1 ml / min throughout. The effluent was monitored by a photo diode array detector at 215 nm. External standards of authentic alkaloids were for quantification.

## 3. Results and discussion

### 3.1. Alkaloid pattern in different tissues of *A. belladonna*

The alkaloid pattern was quite similar in all plants and in both sets of experiments. Hyoscyamine was the main alkaloid in all tissues at any time, followed by 6ß-hydroxyhyoscyamine, scopolamine and 7ß-hydroxyhyoscyamine (Figure 2).

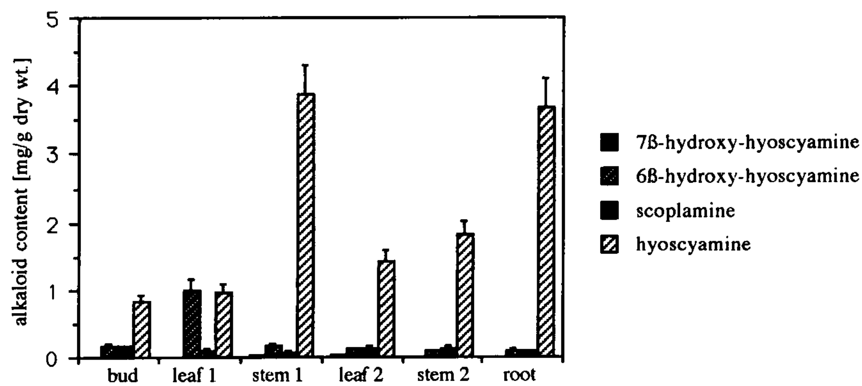


Figure 2. Distribution of alkaloids in *A. belladonna* in different organs in June at 18°° (Bars represent standard deviation; measurement of 3 independent plants)

Littorine was found in trace amounts only in the upper part of the roots. Total alkaloids were two times as high in July as compared to June (Figure 3). In June, the highest alkaloid content was found in the younger parts of the stems, here the alkaloids were predominantly located in the bark. Another main storage site for tropane alkaloids were the roots, whereas leaves and flower buds contained less alkaloid. In July, however, the highest alkaloid content was detected in leaves.

### 3.2. Diurnal rhythm of alkaloid accumulation in plants

All plants showed their maximal alkaloid content during the early evening, mainly between 18-21°°. Another optimum was observed early in the morning (6-9°°). Two minima of alkaloid content were observed, one at noon and one late at night (Figure 3). Maxima were about 3h earlier in the June experiments as compared to the July data, which might be related either to development stage and / or to changing light periods. As discussed in 3.1 already, the distribution of alkaloids in the tissues differed in the two sets of experiments. Whereas in June the alkaloids were mainly located in the bark and in the root, in July, the leaves were the main storage site of alkaloids.

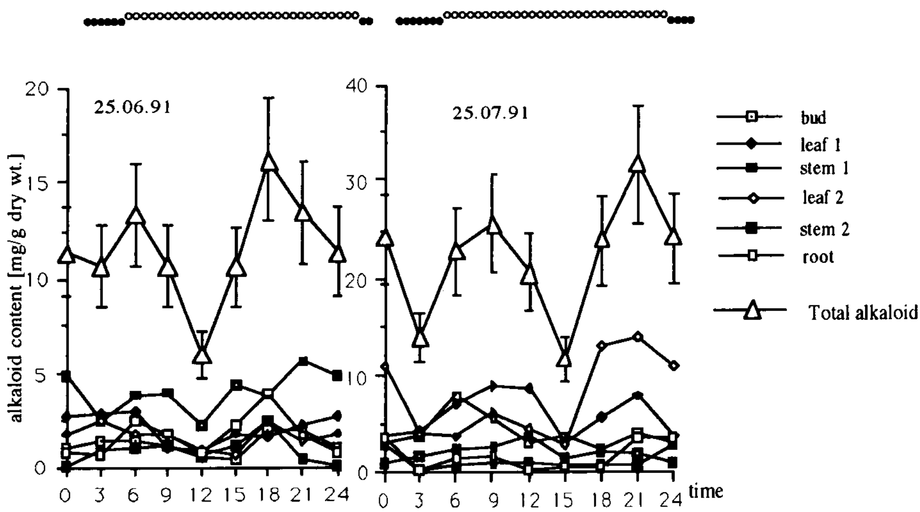


Figure 3. Diurnal alkaloid fluctuation in organs of *A. belladonna* in June and July (Bars represent standard deviation; measurement of 3 independent plants)

When we consider total alkaloid contents (setting minimal level = 100%) maximal contents reach 300%. In the June experiments the diurnal variation was clearly visible in roots and stems, whereas in July fluctuations were high in leaves (Figure 3). Since the amplitude and time of maximal and minimal alkaloid content were somewhat different in both experiments they might be influenced by the weather conditions, or the light period. These data are in good agreement with earlier observations by Fairbairn and Wassel (1967), who found a diurnal fluctuation with maxima at 18°° and 6°°.

3.3 Diurnal alkaloid fluctuation in seeds

Also in maturing seeds of *A. belladonna* a fluctuation of alkaloid content was observed. However alkaloid yields were maximal at 18°° and minimal during the night (Figure 4). The main alkaloid again was hyoscyamine, whereas the other tropane alkaloids occurred in traces only. The fruit itself only contained small amounts of alkaloids. Fairbairn and Wassel (1967) came to similar conclusions with a single maximum at 16°°.

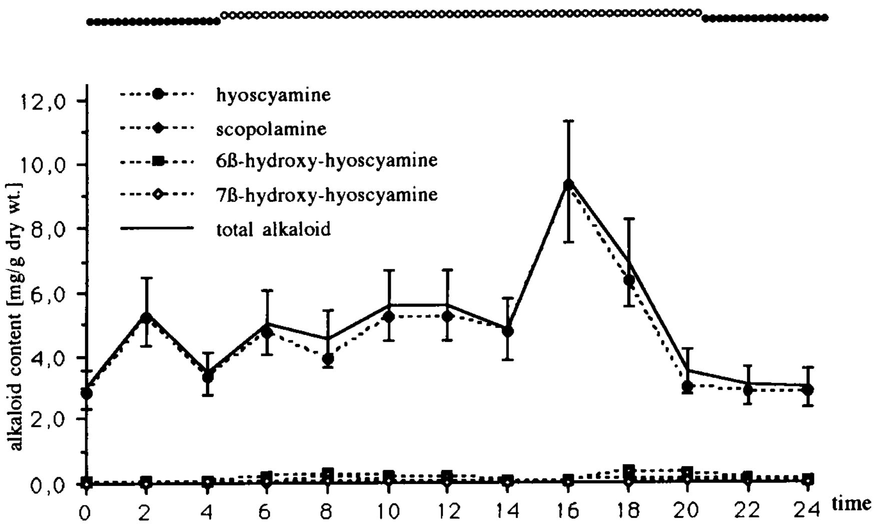


Figure 4. Diurnal alkaloid fluctuation in seeds of *A. belladonna.*
(Bars represent standard deviation; measurement of 3 individual berries)

The diurnal variation of tropane alkaloids in the tissues and organs of *A. belladonna* shows that the tropanes are metabolically dynamic compounds. The phenomenon of diurnal fluctuations is not restricted to tropane alkaloids, but has been observed in a number of other plants: Quinolizidine alkaloids fluctuate diurnally in taxa of the genera *Lupinus, Baptisia, Laburnum* and *Cytisus* (Wink and Hartmann 1982, Wink and Witte 1984). Furthermore fluctuations were observed in alkaloids of *Conium* and *Papaver, Datura, Nicotiana* (Waller and Nowacki 1978) and in *Duboisia* (Kitamura et al. 1991) and in addition in cyanogenic glycosides, cardiacglycosides, flavonoids and essential oils (cited in Wink and Witte 1984). Most of the latter experiments were performed using crude methods of phytochemical analysis and need to be reanalyzed with modern methods.

What is the functional significance of this rhythmicity? It is well established that L-hyoscyamine inhibits the muscarinergic receptor which results in a total paralysis when applied at higher concentrations. Only L-hyoscyamine is active, whereas the D-form is inactive. Tropane alkaloids have been selected during evolution as potent chemical defense compounds against herbivores. In fact, only few animals are known which can feed on plant material rich in tropane alkaloids. Hyoscyamine is not a stable alkaloid, but it is converted easily to its less active racemate, atropine. If tropane alkaloids would not show any turnover, a plant would loose its chemical defense by and by, since the degradation products do not influence the acetylcholine-receptor any longer. We postulate, that the diurnal turnover is a means to avoid this problem, since the stereochemically needed L-hyoscyamine is generated *de novo* all the time, whereas the inactive forms are continuously degraded (Wink 1992).

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