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SHORT REPORTS

CAPROLACTAM, A LIGHT-PROMOTED GROWTH INHIBITOR IN SUNFLOWER SEEDLINGS

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Key Word Index—*Helianthus annuus*; Compositae; hexahydro-2H-azepin-2-one; caprolactam; growth inhibitor.

Abstract—A neutral growth inhibitor, isolated from methanolic extracts of sunflower seedlings, was characterized by spectral data as caprolactam. Light-grown seedlings had 1.7 times the caprolactam content of etiolated ones. Above concentrations of 100 mg/l., caprolactam inhibited hypocotyl growth in the etiolated cress seedling test.

INTRODUCTION

Earlier studies showed that the phototropic curvature of light-grown sunflower seedlings is not brought about by asymmetric auxin distribution but is accompanied by an increased xanthoxin content at the illuminated side [1, 2]. Thus phototropism may be caused by uneven distribution of growth-inhibiting rather than growth-promoting substance(s). Recently, improved purification of these neutral inhibitor(s) led to an amount of xanthoxin one order of magnitude lower than reported before [3]. The search for other neutral inhibitors was therefore intensified. We now wish to describe the chemical structure, occurrence and biological activity of an inhibiting substance not reported earlier to occur in the plant kingdom.

RESULTS AND DISCUSSION

The growth inhibitor, at first isolated from light-grown sunflower seedlings, had the molecular formula $C_6H_{11}NO$ (MS: m/z 113 $[M]^+$). The mass spectrum was identical with that of caprolactam, hexahydro-2H-azepin-2-one [4]. The IR and ^1H NMR spectra proved beyond doubt that the substance was caprolactam [5, 6].

Caprolactam, a precursor in the fabrication of 6-nylon (perlon L), had not yet been found in nature [7]. It was therefore checked whether its occurrence could be ascribed to contamination of the plant extracts. Vermiculite, treated as under the conditions of seedling growth, was extracted with water; the extract contained no caprolactam. A blank experiment including all purification techniques was run parallel to an isolation experiment; no trace of caprolactam could be detected except in the plant extracts. Because also the extracts from light- and dark-grown plants showed consistent differences in their contents of caprolactam, this can only be a genuine, native plant substance.

The contents of caprolactam in dark-grown and in de-etiolated seedlings are shown in Table 1. One-day white light, which strongly inhibits hypocotyl growth, increased the caprolactam content 70% on a fresh wt basis and 60% on a per plant basis.

The biological activities of isolated and synthetic caprolactam were tested in a bioassay with etiolated cress seedlings. Figure 1 shows that the activities were identical and that hypocotyl growth was reduced at exogenous concentrations from 100 mg/l. Although this concentration is much higher than that of endogenous caprolactam, this does not rule out the possibility of a physiological role of the latter, the penetration of exogenous caprolactam

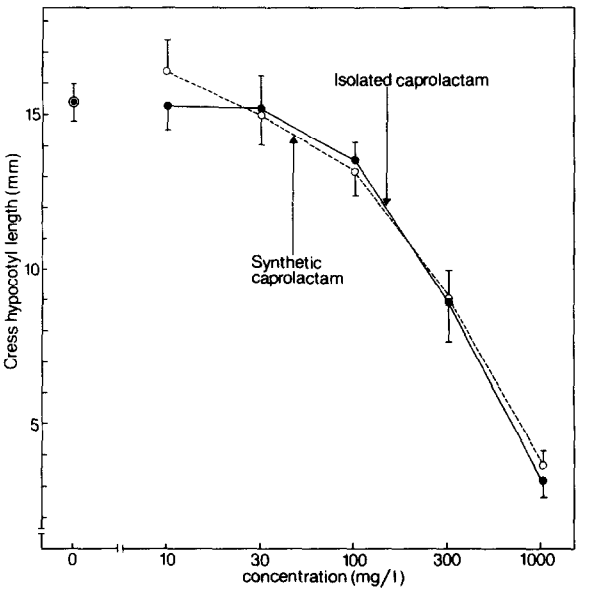

Fig. 1. Effects on isolated and synthetic caprolactam on hypocotyl growth of etiolated cress seedlings. Each value is the average of 10 measurements; bars indicate s.e.

Table 1. Amounts of caprolactam in light- and dark-grown sunflower seedlings

	Light	Dark
Hypocotyl length (mm ± s.e.)	75.9 ± 3.5	108.1 ± 3.6
Caprolactam, μg/g fr. wt	5.1	3.0
Caprolactam, μg/seedling	2.4	1.5

and the distribution of endogenous caprolactam in the plant (cells) being unknown.

The results demonstrate the occurrence of caprolactam as a natural plant substance with growth-inhibiting activity, which might contribute to light-induced growth responses of plants.

EXPERIMENTAL

Extraction and isolation. Sunflower seeds (*Helianthus annuus* L., var. Giganteus) were sterilized in a 1.3% NaOCl soln for 1 hr, then rinsed with H_2O for 1.5 hr. The rinsed seeds were spread evenly on wet double-layered Whatman No. 2 filter paper in large trays. The seeds were then covered with two layers of filter papers moistened with H_2O and the trays covered with a pane and placed in the dark at 25°. About 20 hr later, germinated seeds were transplanted under dim green light into large trays containing vermiculite moistened with H_2O, and were kept in the dark at 25° for 3 days. Etiolated seedlings (hypocotyl length *ca* 5 cm) were cultured under a high-pressure mercury vapour lamp (Philips HP/T 400 W) for 1 day at 25°. Ten kg of green overground parts were harvested, rinsed with H_2O and frozen at −20°. The frozen materials were homogenized in 30 l. of 70% cold MeOH with a homogenizer. The filtered extract was reduced at 35° *in vacuo* to give an aq. concentrate. The aq. soln was adjusted to pH 7.5 with 1 M Pi buffer and extracted × 3 with equal vols. of EtOAc. The neutral fraction was dried (Na_2SO_4) and then evapd to dryness *in vacuo* to give 35 g crude material. The crude material was chromatographed on 10 columns (4 × 58.5 cm) of silica gel (Kieselgel 60 reinst, 70–230 mesh, Merck) with a C_6H_6–EtOAc solvent system by increasing the EtOAc concn in a series of 10% steps (500 ml/step), and finally with MeOH (1500 ml). Biological activities of fractions were determined with a cress root and hypocotyl growth test. Cress seeds were spread on a wet filter paper in a tray and cultured in the dark for 10 hr. Ten uniform, germinated seeds were placed in a Petri dish (4 cm) containing 1 ml of test soln, and the lengths of their roots and hypocotyls were measured after 2 days. The inhibitory activities were detected in 30% and 60–80% EtOAc in C_6H_6, and in MeOH fractions. The MeOH fraction was evapd to dryness *in vacuo* to give 12 g crude material. It was chromatographed on four columns of the same silica gel with a $CHCl_3$–MeOH solvent system by increasing the MeOH concn in a series of 5% steps (500 ml/step). The most active fraction was found in the 0–5% MeOH in $CHCl_3$. This was evapd to dryness *in vacuo* at 35° to yield 510 mg still crude material. The sample was further chromatographed on two 2.5 × 32 cm Sephadex LH-20 columns with a H_2O–MeOH solvent system by increasing the MeOH concn in a series of 20% steps (200 ml/step). The active fraction was detected in 20% MeOH in H_2O and evapd to dryness *in vacuo* at 35° to give 100 mg of a light yellowish oil. This was purified on TLC (Kieselgel 60 GF_{254} prewashed with MeOH, 0.5 mm thickness) with $CHCl_3$–MeOH (5:1). The active zone (R_f 0.57–0.77) was scraped off and eluted with MeOH, giving after evapn 60 mg light yellowish oil. This was finally purified by HPLC (Chrompack 10 RP 18, 25 × 1.2 cm, H_2O–MeOH, 3:2, 6 ml/min). An active eluate (retention time 6–10 min) was evapd to dryness *in vacuo* at 35°, giving 13.4 mg colourless crystals.

Determination of endogenous caprolactam in light- and dark-grown sunflower seedlings. Uniform sunflower seedlings (hypocotyl length *ca* 7 cm) grown in vermiculite in the dark for 4 days at 25° were either irradiated under a high-pressure mercury vapour lamp or kept in the dark at 25° for 1 day. Light- and dark-grown overground parts (150 g fr. wt) were harvested and frozen at −20°. Frozen materials were extracted and fractionated under dim room light as described above to obtain neutral fractions. They were chromatographed on a column (2.5 × 32 cm, silica gel) with a C_6H_6–EtOAc–MeOH solvent system as described above. The MeOH fractions were purified on TLC (Kieselgel 60 GF_{254} prewashed with MeOH, 0.5 mm thickness) with $CHCl_3$–MeOH (5:1). The MeOH eluate from R_f 0.57–0.77 was subjected to HPLC (Chrompack 10 RP 18, H_2O–MeOH, 2:1, 6 ml/min, 222 nm detector, retention time of caprolactam 9.24 min). Endogenous caprolactam was determined by measurement of the peak area (retention time 9.10–9.50 min).

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