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GROWTH INHIBITORS, *cis,trans-* and *trans,trans-*XANTHOXINS, FROM SHOOTS OF A DWARF CULTIVAR OF *PHASEOLUS VULGARIS*

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Two growth inhibitors were isolated from light-grown dwarf bean shoots (*Phaseolus vulgaris* L. cv. Morocco) and their structure were determined by spectral data as *cis,trans-* and *trans,trans-*xanthoxins. *Cis,trans-* and *trans,trans-*xanthoxins inhibited the growth of hypocotyl segments of dwarf bean seedlings at concentrations greater than 0.1 and 1 μM, respectively.

Key words : dwarf bean, growth inhibitor, *Phaseolus vulgaris*, Legume, xanthoxin

Introduction

In a study of the occurrence of native growth inhibitors in seedlings of dwarf and tall bean cultivars, three inhibitors were separated from both cultivars by silica-gel column chromatography using stepwise elution with benzene with an increasing concentration of ethyl acetate[1]. Among them an inhibitor eluted by 50 - 60% ethyl acetate in benzene was isolated from a large amount of light-grown bean seedlings and identified by spectral data as *R*-(-)-3-hydroxy-β-ionone[2]. It was shown that the growth inhibitor might be involved in light-induced growth inhibition of dwarf bean seedlings[3]. This paper described isolation, identification and biological activity of more polar inhibitors eluted by 90 - 95% ethyl acetate in benzene.

Materials and Mehtods

Plant material. Dwarf bean plants (*Phaseolus vulgaris* L. cv. Morocco) were grown from seeds in soil under natural daylight conditions for 20 days. At harvest, the shoots were on the average 14 cm tall, weighing 4.3 g fresh weight each and having 4 internodes. The green shoots (10 kg fresh weight) were rinsed with distilled water, immediately frozen on dry ice and stored at −40℃ prior to extraction of inhibitors.

Extraction and isolation. Plant materials were homogenized in 100 L of cold acetone and the homogenate was filtered through filter paper (No. 1, Toyo) in a Buchner funnel. The residue was homogenized again with 80% cold aqueous acetone, using the same volume as used in the first extraction, and filtered. The two filtrates were combined and evaporated *in vacuo* at 35℃ to give an aqueous residue. The residue was adjusted to pH 7.5 with 1M phosphate buffer and partitioned three times against an equal volume of ethyl acetate. The

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ethyl acetate phase was evaporated to dryness after drying over anhydrous Na_2SO_4.

The crude material (32 g) was chromatographed on a column (6 cm i.d. × 40 cm) of silica gel (500 g; kieselgel 60, 70-230 mesh; Merck) and eluted stepwise with 500 mL per step of benzene that included increasing (5% per step, v/v) concentrations of ethyl acetate. The biological activity of the fractions was determined using a bean bioassay as described below. Fractions obtained by elution with 85-95% ethyl acetate in benzene gave a growth-active, greenish oil (1.8 g). The residue was further purified by column chromatography (2 cm i.d. × 60 cm, 100 g silica gel; benzene containing increasing amounts of acetone in 5% steps, v/v; 100 mL for each step), yielding 85 mg of a yellowish active residue that was eluted by 30-40% acetone in benzene. The residue (38 mg) obtained after passing the active material through a reverse-phase Sep-Pak cartridge (Waters Associate) with 50% aqueous methanol was subjected to reverse-phase HPLC (0.8 cm i.d. × 30 cm; TSK Gel ODS-120T; Tokyo; eluted at a flow rate of 2 mL min^{-1} with 40% aqueous methanol, v/v; detected at 280 nm), yielding two active components, compound β (1.2 mg) and α (0.8 mg), which eluted with retention times of 36.5 and 44.8 min, respectively.

Bean bioassay. Ten-mm segments were excised below the hook of dark-grown 4-day-old seedlings of the dwarf cultivar (hypocotyl length, about 40 mm) and used for the bioassay. Xanthoxins isolated as described above were dissolved in a small volume of acetone, added to a sheet of filter paper (No.2, Toyo) in a 4.5-cm Petri dish and dried. The filter paper in the Petri dish was moistened with 1.5 mL of a 0.05% (v/v) aqueous solution of Tween 20, and 10 segments of the bean seedlings were arranged on it and allowed to grow in the dark at 25℃ for 24 h. The length of the segments was then measured by use of a photographic enlarger, and the percentage elongation of growth was calculated by reference to the elongation of control segments.

Results and Discussion

Growth inhibitors were purified from the neutral fraction (32 g) obtained from 10 kg fresh weight of light-grown shoots of the dwarf cultivar, and it was separated by HPLC into two active substances, compound α and β, corresponding to 0.8 and 1.2 mg of colorless resin, respectively, which were named in the reverse order of elution on reverse phase HPLC. The peak heights of α and β as determined at 280 nm on HPLC were variable, suggesting that these two compounds are interconvertible. Compounds α and β had UV spectra with peaks of absorbance in methanol at 282 and 284 nm, respectively. High-resolution mass spectroscopy (70 eV) yielded the following data (relative intensity, element composition): M^+, 250.1569 (12, $C_{15}H_{22}O_3$), 168.1158 (39, $C_{10}H_{16}O_2$) and 149.0968 (100, $C_{10}H_{13}O_1$) for α; and M^+, 250.1570 (17, $C_{15}H_{22}O_3$), 168.1159 (41, $C_{10}H_{16}O_2$) and 149.0969 (100, $C_{10}H_{13}O_1$) for β, respectively. These data indicate that both compound were xanthoxins[4,5]. Protone magnetic resonance (200 NHz, TMS, $CDCl_3$) signals at δ 2.12 (3H, *s*) for compound α and at δ 2.30 (3H, *s*) for compound β (methyl group at C-3) discriminated α and β as *cis,trans-* and *trans,trans*-xanthoxin, respectively[6].

The results of bioassay of xanthoxins are shown in Fig. 1. *Cis,trans-* and *trans,trans*

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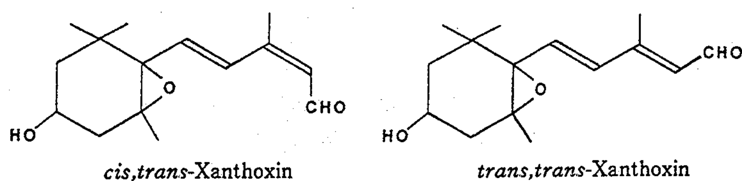
-xanthoxins inhibited the growth of hypocotyl segments of dwarf bean seedlings at concentrations greater than 0.1 and 1 μM, respectively. The doses required for 50% inhibition that were interpolated from the dose-response curves (Fig. 1) were 0.93 and 16 μM for *cis,trans*- and *trans,trans*-xanthoxin, respectively.



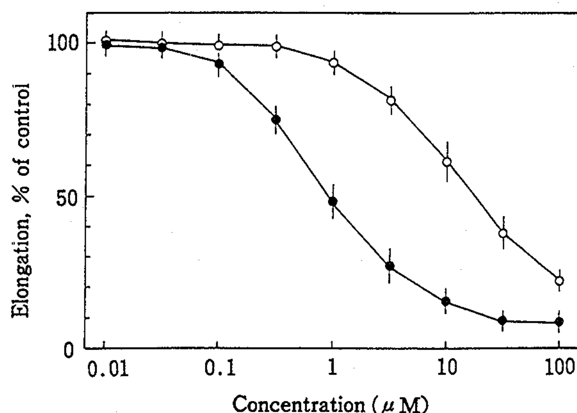


Fig. 1 Effects of *cis,trans*-xanthoxin (●) and *trans,trans*-xanthoxin (○) on the growth of hypocotyl segments of bean seedlings. Values are means of results from 10 plants ± s.e. Elongation of control segments was 4.7 ± 0.5 mm.

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矮性インゲンのシュートから単離された生長抑制物質，シス，トランス型とトランス，トランス型キサントキシン

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２種の生長抑制物質が，明所で育てた矮性インゲン（*Phaseolus vulgaris* L. cv. Morocco）のシュートより単離された．機器分析の結果，それらの化合物の化学構造はシス，トランス型とトランス，トランス型キサントキシンであることが解った．シス，トランス型とトランス，トランス型キサントキシンは，それぞれ0.1と１μM以上の濃度で矮性インゲン芽生えの下胚軸切片の生長を抑制した．