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A NEW AUXIN-INHIBITING SUBSTANCE, 4-Cl-6,7-DIMETHOXY-2-BENZOXAZOLINONE, FROM LIGHT-GROWN MAIZE SHOOTS

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Key Word Index—*Zea mays*; Gramineae; maize shoots; auxin-inhibiting substance, 4-Cl-6,7-dimethoxy-2-benzoxazolinone.

Abstract—Two auxin-inhibiting substances were isolated from light-grown maize shoots. One was a new compound and determined from its spectral data as 4-Cl-6,7-dimethoxy-2-benzoxazolinone (Cl-DMBOA). Another was identified from its spectral data as a known compound, 6,7-dimethoxy-2-benzoxazolinone (DMBOA). Above concentrations of 10^{-5} M, Cl-DMBOA inhibited the auxin-inducing elongation in the *Avena* coleoptile section test. Its inhibitory activity was almost comparable to that of DMBOA and higher than that of 6-methoxy-2-benzoxazolinone (MBOA) which we previously isolated.

INTRODUCTION

During the last century, many studies on phototropism have been carried out using natural and synthetic phytohormones. A famous model is the Cholodny-Went theory, in which phototropic curvature is caused by asymmetric auxin distribution [1, 2]. However, some groups have shown that endogenous auxin, indole-3-acetic acid (IAA), did not accumulate asymmetrically on unilaterally illuminated seedlings and that the levels of light-induced growth inhibitors increased in the illuminated side of phototropic seedlings [3–12]. One of these inhibitors, raphanusanin, caused de-polarization of IAA-mediated microtubule organization [13]. We have isolated an auxin-inhibiting substance, MBOA, from light-grown maize shoots [14] and reported the structure-activity relationships of benzoxazolinones concerning auxin-induced growth and membrane-bound auxin-binding protein(s) [15]. In our recent studies, other substances with anti-auxin activity were found in the extract of maize shoots, and these compounds were purified. In this study, we describe the characterization of these growth inhibitors and their biological activities.

RESULTS AND DISCUSSION

Two benzoxazolinones were isolated from light-grown maize shoots as auxin-inhibiting substances. One was identified as a known substance, DMBOA, on the base of spectral data. DMBOA had previously been isolated from maize [16]. A second substance was a new natural product.

On the bases of HR-MS spectrometry, the molecular formula of the newly isolated compound **1** was assigned as $C_9H_8NO_4Cl$. The ^1H NMR spectrum (Table 1) showed signals for two methoxyl protons (δ 3.38, 4.11), one methine proton (δ 6.66) and one proton of NH (δ 7.62). The ^{13}C NMR spectrum (Table 1) allowed the assignments of OMe (δ 57.66, C-8), OMe (δ 61.21, C-9), =CH– (δ 109.90, C-5), >C= (δ 107.98, C-4), >C= (δ 124.96, C-3a), >C= (δ 134.20, C-7), >C= (δ 136.78, C-7a), >C= (δ 149.37, C-6), >C= (δ 156.26, C-2). In NOE experiments on **1**, irradiation at δ 3.83 (OMe-8) increased the intensity of H at C-5 by 12.0%, while irradiation at δ 4.11 (OMe-9) did not

Table 1. ^1H and ^{13}C NMR chemical shifts (δ values from TMS) and multiplicities of compound **1**

Atom	^1H (in $CDCl_3$)	^{13}C (in CD_3OD)
1	—	—
2	—	156.26 *s*
3	7.62 *br s*	—
3a	—	124.96 *s*
4	—	107.98 *s*
5	6.66 *s*	109.90 *d*
6	—	149.37 *s*
7	—	134.20 *s*
7a	—	136.78 *s*
8	3.83 *s*	57.66 *q*
9	4.11 *s*	61.21 *q*

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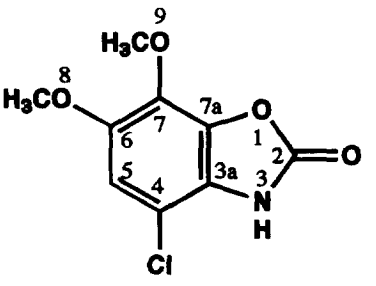

1

increase the intensity of H at N-3, C-5 and OMe-8. On the basis of these studies, we assign the structure 4-Cl-6,7-dimethoxy-2-benzoxazolinone for **1**.

Biological activities of two isolated benzoxazolinones together with natural benzoxazolinones, 2-benzoxazolinone (BOA) and MBOA were tested in the *Avena* coleoptile section test (Fig. 1). Benzoxazolinones having methoxy group(s) at C-6 or C-6 and C-7 showed an inhibiting activity in the *Avena* coleoptile section test, whereas BOA having no methoxy group did not show an inhibitory activity. Therefore, the presence of methoxy group(s) attached to the 2-benzoxazolinone is necessary for an inhibitory activity. Moreover, the activity of DMBOA was about 10-fold higher than that of MBOA, suggesting that the presence of a methoxy group at C-7 leads to an increase in activity. On the other hand, the activity of Cl-DMBOA was almost the same as that of DMBOA. It was thus clarified that the attachment of Cl at the C-4 position leads to neither an increase nor a reduction in activity. It was reported that many benzoxazolinones were induced by wounding or pathogenic attack and act as anti-fungal substances [17]; however, the benzoxazolinone MBOA seems to be a light-induced anti-auxin [15]. Whether Cl-DMBOA production is also induced by wounding or pathogenic attack has not yet been examined.

EXPERIMENTAL

Plant materials. Maize (*Zea mays* L. cv Canadian Rocky Bantam 85, Kaneko Seed Co., Japan) seeds were soaked in tap H_2O for 24 hr in darkness at 25°. Seeds were transferred on a moist filter paper in a tray under red light (0.35 mmol m^{-2} s^{-1}, λ_{max} 665 nm) for the first 24 hr and then in darkness for next 2 days. These seedlings were de-etiolated under white light (4W m^{-2}, Plantlux, Toshiba Co., Japan) for 24 hr just before harvesting.

Extraction and isolation. 5-Day-old de-etiolated maize shoots (250 g, FW.) were homogenized in 1 l H_2O at room temp. After incubation for 30 min at room temp, the homogenate was boiled for 1 hr and filtered through one layer of filter paper (type No. 1, Toyo

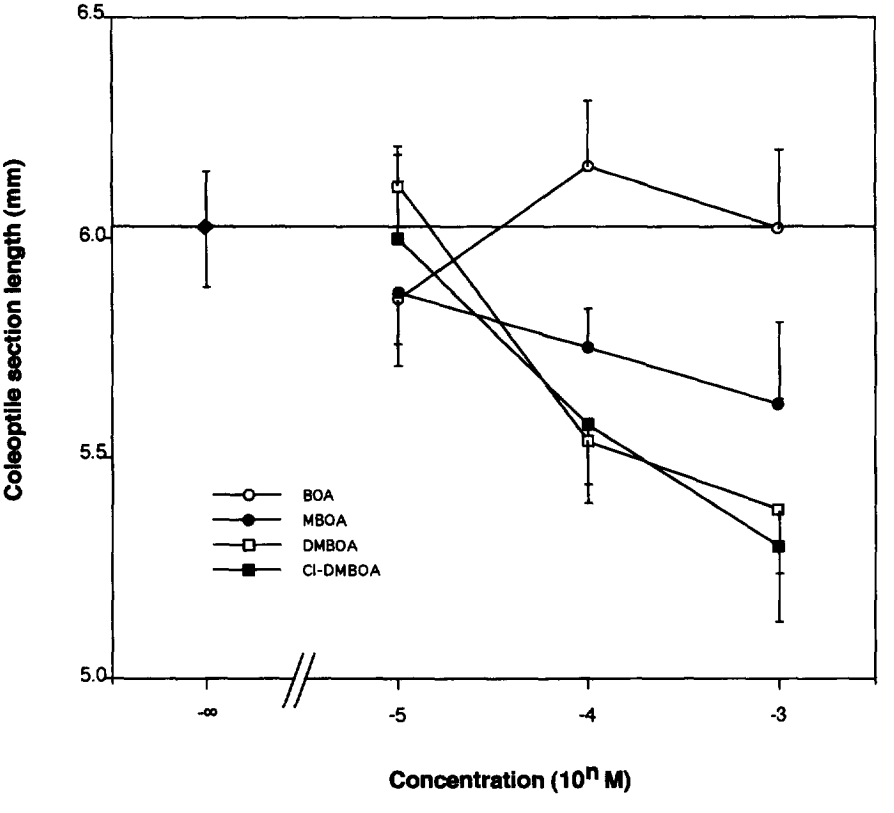

Fig. 1. Effect of benzoxazolinones on the IAA-induced growth of *Avena* coleoptile sections. Each value is the average of 10 measurements; bars indicate s.e.

Roshi Co., Japan). The filtrate was concd to 300 ml *in vacuo* at 40° and partitioned three times with equal vols of CH_2Cl_2. The organic fr. was evapd to dryness *in vacuo* at 40° and partitioned between 50 ml EtOAc and 3×50 ml 0.5 M Na_2CO_3. The aq. layer was adjusted to pH 5.5 with H_3PO_4 and partitioned into CH_2Cl_2. The organic fraction was dried (Na_2SO_4) and evapd to dryness *in vacuo* at 40°. The crude extract was partially purified on TLC (Silicagel 70 F254, Wako Co., Japan) with toluene–EtOAc (1:1). Inhibition of shoot elongation was tested in the *Avena* coleoptile section test. The active zone (R_f 0.3–0.7) was scraped off and eluted with $CHCl_3$–MeOH (1:1). After evapn, the sample was purified by HPLC (TSKgel ODS-80Ts, 21.5 mm × 30 cm, Tosoh Co., Japan; H_2O–MeCN, 3:2, 5 ml min^{-1}). Two peak frs (R_t 34 and 39 min) showed auxin-inhibitory activities and one fr. (R_t 39 min) contained a single compound that was identified as 6-methoxy-2-benzoxazolinone that we previously isolated from de-etiolated maize shoots; the second fr. (R_t 34 min) was a mixture. The latter was separated by TLC (Silicagel F254, Merck) with *n*-hexane–EtOAc (3:2) into 3 compounds. Of these, the mass and ^1H NMR spectra of 2 compounds (R_f 0.21, 2.7 mg, R_f 0.27, 0.2 mg) coincided with those of authentic 6,7-dimethoxy-2-benzoxazolinone and 2-benzoxazolinone, respectively. The spectral data of the third compound were as follows.

4-Cl-6,7-methoxy-2-benzoxazolinone (**1**). IR ν_{max} (film): 3000, 1770 cm^{-1}; ^1H and ^{13}C NMR: see Table 1; HR-MS: calcd for $C_9H_8NO_4Cl$, *m/z* 229.0141 $[M]^+$, found 229.0153. EI-MS *m/z* (rel. int.): 229 $[M]^+$ (100), 214 $[M - Me]^+$ (70).

Avena section test. Oat (*Avena sativa* L. cv Victory) seeds were grown on a moist filter paper in a tray under red light (0.35 μmol m^{-2} s^{-1}, λ_{max} 665 nm) for first 24 hr and in darkness for next 3 days. The 4-day-old seedlings were used for *Avena* section test. 10 4-mm sections of *Avena* coleoptiles were incubated in 1% sucrose soln (pH 5.4) containing 0.005% Tween 20, 10^{-6} M IAA and various concns of benzoxazolinones for 8 hr at 25° in the dark. After incubation, section lengths were measured using a photographic enlarger.

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