Isolation and Characterization of 4-Chloro-6,7-dimethoxybenzoxazolin-2-one, A New Auxin-inhibiting Benzoxazolinone from *Zea mays*

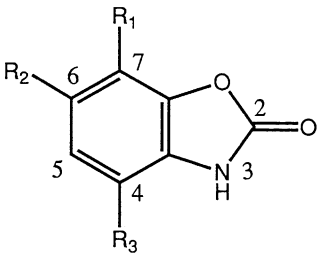
Seiji Kosemura, Hideyuki Emori, Shosuke Yamamura,* Toyoaki Anai,† Hiroko Aizawa,† Nobuyuki Ohtake,† and Koji Hasegawa*†
Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223
†*Institute of Applied Biochemistry, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305*

(Received August 16, 1995)

A new auxin-inhibiting substance was isolated from light-grown maize shoots. The structural determination was performed by spectroscopic methods and synthesis of 4-chloro-6,7-dimethoxybenzoxazolin-2-one.

During the past century a number of physiological and chemical studies have been done to elucidate the mechanism of phototropic curvature.[1] Recently, we have isolated an auxin-inhibiting substance, 6-methoxybenzoxazolin-2-one (MBOA, **1**) from light-grown maize (*Zea mays* L.) shoots as a potent antiauxin,[2,3] and we reported the structure-activity relationships of benzoxazolinones with respect to auxin-induced growth and membrane-bound auxin-binding protein(s).[4] In this communication we wish to report the isolation and structural determination of a new auxin-inhibiting substance, 4-chloro-6,7-dimethoxybenzoxazolin-2-one (4-Cl-DMBOA, **2**).

Five-days-old de-etiolated maize (*Zea mays* L. cv Canadian Rocky Bantam 85, Kaneko seed Co., Japan) shoots (250 g, FW) were homogenized in 1 *l* distilled water at room temperature. After incubation for 30 min at room temperature, the homogenate was boiled for 1 h. The filtrate was concentrated to 300 ml and partitioned three times with equal volumes of CH_2Cl_2. The organic extract was concentrated to dryness *in vacuo* and partitioned between 50 ml of EtOAc and 3 x 50 ml of 0.5 M Na_2CO_3. The aqueous layer was adjusted to pH 5.5 using H_3PO_4 and extracted with CH_2Cl_2. The crude extract was separated by TLC (Silica gel 70 F_{254}) with toluene-EtOAc (1:1) followed by HPLC (TSK gel ODS-80Ts; H_2O-MeCN) and preparative TLC (Silica gel F_{254}) with hexane-EtOAc (3:2) to give **2**[5] and two known benzoxazolin-2-ones (DMBOA, **3**[6] and BOA, **4**[7]) in 0.00032, 0.0011, and 0.00008% yields, respectively.



	R_1	R_2	R_3
1: (MBOA)	H	OMe	H
2: (4-Cl-DMBOA)	OMe	OMe	Cl
3: (DMBOA)	OMe	OMe	H
4: (BOA)	H	H	H
5: (4-ABOA)	H	H	COMe
6: (6-ABOA)	H	COMe	H

Compound **2** was obtained as an amorphous powder; its molecular formula, $C_9H_8NO_4Cl$ (M^+, calcd, *m/z* 229.0141 : found, *m/z* 229.0153), was confirmed by HR-EIMS. The IR absorptions at 3000 and 1770 cm^{-1} suggested the presence of a carbamate group. The ^1H NMR spectrum of **2** also showed the presence of a carbamate proton (δ 7.62), an aromatic proton (δ 6.66), and two MeO groups (δ 4.11 and 3.83). The structure of **2** was determined on the basis of spectral data and NOE experiments[8] compared with those of **3**, and unambiguously established by synthesis; by chlorination with surfuryl chloride in benzene at room temperature for 10 min., compound **3**[9] prepared from 2,3-dimethoxyphenol, was readily converted into **2** in 86% yield. The ^1H NMR spectrum of synthetic sample **2** was compatible with that of natural one. Above concentrations of 10^{-5} M, compound **2** inhibited the auxin inhibiting elongation in the *Avena* coleoptile section test. Inhibitory activity of **2** was almost comparable to that of **3** and higher than that of **1**.[2] Interestingly, compound **6** and its analogues have been synthesized and exhibited antiinflammatory properties,[10] however, inhibitory activities of **4**, **5**[11] isolated from kernels of a *Fusarium*-resistant hybrid of *Zea mays* (Funks 4106; Ciba-Geigy seeds) and **6**[12] were lower than that of **1**. It is clear that 2β-glucosides of 1,4-benzoxazin-3-ones are initially hydrolyzed with endogeneous glucosidase to afford 1,4-benzoxazin-3-ones at the light exposure side, further 1,4-benzoxazin-3-ones immediately afford benzoxazolin-2-ones which interestingly inhibit the coleoptile growth of maize. These observations strongly suggest the possibility that compound **2** is formed from the corresponding 4-chloro-1,4-benzoxazin-3-one. Studies are now in progress to isolate both the glycoside and its aglycon.

The authors wish to thank the Ministry of Education, Science and Culture (Japan) for Grant-in-Aid for Scientific Research on Priority Areas No. 06240103.

References and Notes

1 J. Bruinsma, C. M. Karssen, M. Benschop, and J. B. Van Dort, *J. Exp. Botany*, **26**, 411 (1975); J. M. Franssen and J. Bruinsma, *Planta*, **151**, 365(1981); H. Noguchi, K. Nishitani, J. Bruinsma, and K. Hasegawa, *Plant Physiol.*, **81**, 980(1986); K. Hasegawa, M. Sakoda, and J. Bruinsma, *Planta*, **178**, 540(1989); S. Kosemura, S. Yamamura, and K. Hasegawa, *Tetrahedron Lett.*, **34**, 481 (1993).
2 K. Hasegawa, S. Togo, M. Urasima, J. Mizutani, S. Kosemura, and S. Yamamura, *Phytochemistry*, **31**, 3673 (1992).
3 S. Kosemura, S. Yamamura, T. Anai, and K. Hasegawa, *Tetrahedron Lett.*, **35**, 8221(1994).
4 M. Hoshi-Sakoda, K. Usui, K. Ishizuka, S. Kosemura, S.

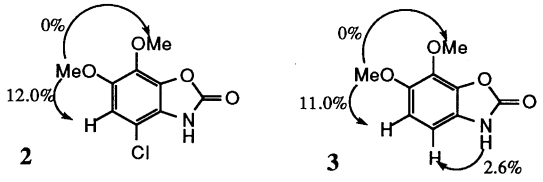
Yamamura, and K. Hasegawa, *Phytochemistry*, **37**, 297 (1994).

5 Physical data for **2**: $C_9H_8O_4NCl$ [*m/z* 229.0153 (M^+)]; ν_{max}(film) 3250 and 1770 cm^{-1}; δH($CDCl_3$) 3.83(3H, s) (MeO-C_6), 4.11(3H, s)(MeO-C_7), 6.66(1H, s)(H-C_5) and 7.62(1H, br.s)(H-N_3); δC(CD_3OD) 57.66(q), 61.21(q), 107.98(s), 109.90(d), 124.96(s), 136.78(s), 149.37(s), and 156.26(s).

6 Physical data for **3**: $C_9H_9O_4N$ [*m/z* 195.0510 (M^+)]; ν_{max}(film) 3250 and 1760 cm^{-1}; δH($CDCl_3$) 3.84(3H, s) (MeO-C_6), 4.14(3H, s)(MeO-C_7), 6.61(1H, d, J=8.4 Hz) (H-C_4), 6.69(1H, d, J=8.4 Hz)(H-C_5), 8.70(1H, br.s)(H-N_3); δC($CDCl_3$) 57.02(q), 60.61(q), 101.94(d), 108.07(d), 124.42(s), 134.14(s), 134.39(s), 147.39(s), and 155.39(s).

7 Physical data for **4**: $C_7H_5O_2N$ [*m/z* 135.0311 (M^+)]; ν_{max}(film) 3200, 1775, and 1735 cm^{-1}; δH($CDCl_3$) 7.02 - 7.16(4H, complex) and 7.88(1H,br.s).

8 NOE experiments of **2** and **3**



9 R. J. Nachman, *J. Heterocyclic Chem.*, **19**, 1545 (1992).

10 C. Safak, H. Erdogan, E. Palaska, R. Sunal, and S. Duru, *J. Med. Chem.*, **35**, 1296(1992).

11 D. A. Fielder, F. W. Collins, B. A. Blackwell, C. Bensimon, and J. W. ApSimon, *Tetrahedron Lett.*, **35**, 521(1994).

12 S. Ishida, Y. Hashida, H. Shizuka, and K. Matsui, *Bull. Chem. Soc. Jpn.*, **52**, 1135 (1979).