Pergamon

Tetrahedron Letters, Vol. 38, No. 12, pp. 2125-2128, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00323-7

Design of Photoaffinity Reagents for Labeling the Auxin Receptor in Maize

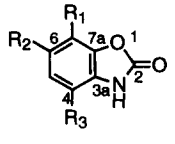
Seiji Kosemura,[a*] Hideyuki Emori,[a] Shosuke Yamamura,[a*]
Toyoaki Anai,[b] Kaori Tomita,[b] and Koji Hasegawa[b]

[a]Department of Chemistry, Faculty of Science and Technology,
Keio University, Hiyoshi, Yokohama 223, Japan

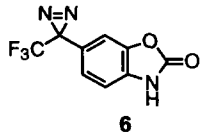
[b]Institute of Applied Biochemistry,
University of Tsukuba, Tennodai 1-1-1,Tsukuba 305, Japan

Abstract: In order to isolate the auxin receptor, we have successfully synthesized two analogues of benzoxazolinones with a trifluoromethyldiazirine group as a photoaffinity probe. These compounds inhibited the auxin-induced growth of etiolated *Avena* coleoptile segments. Photolyses of these compounds in methanol gave intermolecular O-H insertion products in moderate yields, respectively.

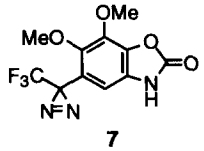
During the past century a number of physiological and chemical studies have been carried out to elucidate the mechanism of phototropic curvature. Recently, we have isolated auxin-inhibitory benzoxazolinones (**1**, **2**, **3**) from light-grown maize (*Zea mays* L.) shoots as potent antiauxins,[1] and we reported the structure-activity relationships of benzoxazolinones (**4**, **5**, and others.) with respect to auxin-induced growth and membrane-bound auxin-binding protein(s).[2] The precise mechanism by which cell elongation is evoked by auxin remains unknown, although presumably it involves a complex of auxin and auxin receptor(s). The identity of the auxin receptor that mediated cell elongation is also unknown. Recently, indirect methods have been used to identify an auxin-binding protein (ABP)[3] which has some of the expected characteristics of the receptor that mediates cell elongation.[4] Photoaffinity labeling has been successful



	R1	R_2	R_3
1 =	H	OMe	H
2 =	OMe	OMe	H
3 =	OMe	OMe	Cl
4 =	H	OCH_2CHMe_2	H
5 =	H	Ac	H



6



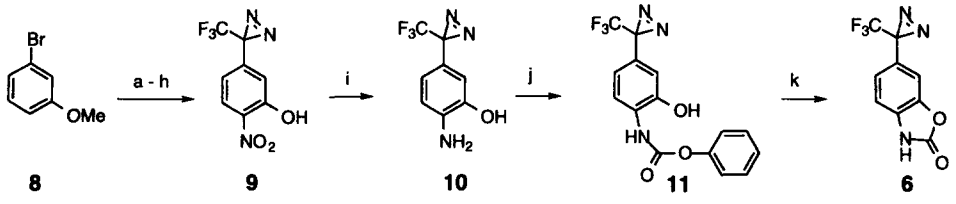
7

in identifing and characterizing receptors from animals and plants.[5] In this communication we wish to report the results of studies on the synthesis and photolysis of two photolabile benzoxazolinone analogues with a

trifluoromethyldiazirine group[6] which has been proved to be a useful photoaffinity probe for direct labeling of indole-3-acetic acid (IAA) receptor(s) or IAA binding proteins in maize.

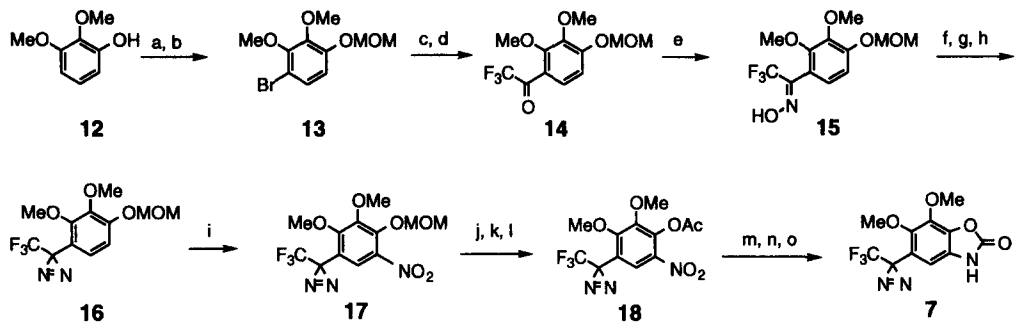
The photoaffinity labeling technique requires the synthesis of a photolabile reagent(s) that can bind noncovalently to its receptor or active site in the absence of light. Ideally, photolysis transforms the reagent into a highly chemically reactive species, which binds covalently to the receptor before diffusion away from the active site can occur. Based on the results of structure-activity relationships of benzoxazolinones and investigation of the properties of various photogenerated reaction intermediates and its precursors, two photolabile benzoxazolinone analogues (**6**, **7**) were designed with a trifluoromethyldiazirine group in place of the methoxy group at the C-6 position of 6-methoxy-benzoxazolin-2-one (MBOA, **1**) and the proton at the C-5 position of 6,7-dimethoxy-benzoxazolin-2-one (DMBOA, **2**).

The synthetic procedure of the photolabile benzoxazolinone analogues **6** and **7** are summarized in Schemes 1 and 2. The photoreactive analogue **6** was synthesized by the following procedure. Diazirine **9** was synthesized according to the procedure of Hatanaka et al.[7] 2-Nitro-5-trifluoromethyldiazirnyl phenol **9** was reduced with sodium hydrosulfite[8] to amine **10**. The amine **10**, which is extremely susceptible to air oxidation, was treated with phenyl chloroformate, followed by intramolecular cyclization of the carbamate **11** in the presence of triethylamine to give the desired diazirine **6**[9] (14.0% in 3 steps). The photoreactive analogue **7** was synthesized by the following procedure. Protection of 2,3-dimethoxy phenol **12** with MOMCl followed by bromination with NBS afforded **13**. The compound **13** was treated with BuLi, followed by trifluoroacetylation with ethyl trifluoroacetate, to give trifluoroacetate **14**. Treatment of **14** with hydroxylamine hydrochloride gave oxime **15**. Tosylation of **15** with *p*-TosCl, followed successively by formation of diaziridine with liq. NH_3, then oxidation with *tert*-butyl hypochloride afforded trifluoromethyl diazirine **16**. Nitration of diazirine **16** with fuming nitric acid gave nitro compound **17**. This compound **17** was deprotected upon acidic hydrolysis and then protected again with acetic anhydride to give acetate **18**. Reduction of the ester **18** was reduced with sodium hydrosulfite followed by carbamation with phenyl chloroformate, then treated with sodium hydroxide to give the desired diazirine **7** (32.0% in 3 steps).[10]



a) Mg, THF ; b) N-trifluoracetylpiperidine, THF, Ar, r.t., 1 h (91.0% in 2 steps) ; c) $NH_2OH \cdot HCl$, EtOH-Pyr. 55 °C, 15 h ; d) p-TosCl, DMAP, Et_3N, CH_2Cl_2, r.t., 20 min (86.7% in 2 steps); e) liq. NH_3 CH_2Cl_2, -78 °C - r.t., 13 h ; f) tBuOCl, Et_3N, tBuOH, EtOH, 0 °C - r.t., 4 h , then $Na_2S_2O_5$aq. (48.0% in 2 steps) ; g) HNO_3, AC_2O, 0 °C - r.t., 30 min ; h) BBr_3, CH_2Cl_2 , Ar, 0 °C - r.t., 4 h (42.9% in 2 steps); i) $Na_2S_2O_4$, THF-H_2O, r.t., 5 min ; j) PhOCOCl, THF-H_2O, r.t., 5 min ; k) Et_3N, r.t.,40 min (14.0% in 3 steps).

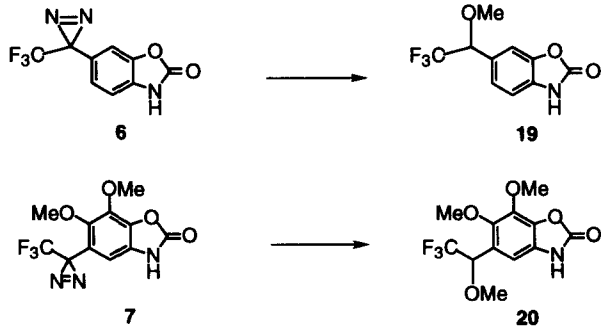
Scheme 1



a) MOMCl, $^{i}Pr_2EtN$, THF, r.t., 3 h (quant.) ; b) NBS, DMF, r.t., 12 h (39.5%) ; c) nBuLi, THF, Ar, -78 °C, 10 min ; d) CF_3CO_2Et, -78 °C - r.t., 1.5 h (62.3% in 2 steps) ; e) NH_2OH · HCl, EtOH, Pyr., 55 °C, 16 h (74.8%) ; f) *p*-TosCl, Et_3N, DMAP, CH_2Cl_2, r.t., 1 h ; g) liq.NH_3, CH_2Cl_2, -78 °C - r.t., 16 h (68.3% in 2 steps) ; h) tBuOCl, Et_3N, tBuOH, EtOH, 0 °C, 5 h, then $Na_2S_2O_5$ (86.0% in 2 steps); i) fuming HNO_3, Ac_2O, -72 °C, 10 min, then $Na_2S_2O_5$ r.t., 2 h (72.2% in 2 steps) ; j) 1N HCl , AcOH, r.t., 12 h (95.6%); k) Ac_2O, Pyr., r.t., 30 min (quant.) ; l) $Na_2S_2O_4$, THF - H_2O, r.t., 5 min ; m) PhOCOCl, THF-H_2O, r.t., 5 min ; o) 1N NaOH, THF, r.t., 1 h (32.0%).

Scheme 2.

Photolysis of the benzoxazolinones **6** and **7** was performed to examine the photoreactivities of these new diazirines. The irradiation was carried out until all the diazirines (1 mM solution in methanol) were consumed, with a 12 W UV lamp (365 nm, at a distance of 1 cm) or a 500 W high-pressure mercury lamp (at a distance of 5 cm), monitoring by reverse phase HPLC. In methanol, the formal O-H insertion products (**19**, **20**) were obtained in moderate yields, respectively. The diazirine **6** (half-life time is about 16 min with a 12 W UV lamp) was found to be photolyzed much more rapidly than the diazirine **7** (half-life time is about 6 min with a 500 W UV lamp).



Scheme 3

These two benzoxazolinones with a trifluoromethyldiazirine group inhibited the elongation of *Avena* coleoptile sections in the presence of auxin demonstrate that **6** and **7** show growth inhibitory activities almost identical with those of native benzoxazolinone **1**, **2** and **3**.[1,2]

In summary, we have successfully synthesized photolabile benzoxazolinone analogues **6** and **7**. The present studies on inhibitory activities and photoreactivity experiments are proved to be a potential photoaffinity probe for labeling the auxin receptor(s) or auxin binding proteins. Studies to isolate the auxin receptor using these photolabile analogues are in progress.

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. Hasegawa, K.; Togo, S.; Urashima, M.; Mizutani, J.; Kosemura, S.; Yamamura, S. *Phytochemistry*, **1992**, *31*, 3673.
2. Hoshi-Sakoda, M.; Usui, K.; Ishizuka, K.; Kosemura, S.; Yamamura, S.; Hasegawa, K. *Phytochemistry*, **1994**, *37*, 297.
3. a) Shimomura, S.; Sotobayashi, S.; Futani, M.; Futani, T. *J. Biochem.*, **1986**, *99*, 1513. b) Napier, R. M.; Venis, M. A.; Bolton, M. A.; Richardson, L. I.; Butcher; *Planta*, **1988**, *176*, 519. c) Shimomura, S.; Inohara, N.; Fukui, T.; Futai, M. *Planta*, **1988**, *175*, 558.
4. a) Löbler, M.; Klämbt, D. *J. Biol. Chem.*, **1985**, *260*, 9854. b) Barbier-Brygoo, H.; Ephritikhine, G.; Klämbt, D.; Ghislain, M.; Guern, J. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 891.
5. a) Chowdry, V.; Westheimer, F. *Annu, Rev. Biochem*, **1979**, *48*, 293. b) Zhang, H.; Lerro, K. A.; Yamamoto, T.; Lien, T. H.; Sastry, L.; Gawinowiez, M. A.; Nakanishi, K. *J. Am. Chem. Soc.*, **1994**, *116*, 10165.
6. a) Smith, R. A. G.; Knowles, J. R. *J. Am. Chem. Soc.*, **1973**, *95*, 5072. b) Bayley, H. "Laboratory Techniques in Biochemistry and Molecular Biology: Photogenerated reagents in Biochemistry and Molecular Biology"; Work, T. S.; Burdon, H. R. Ed., Elsevier, Amsterdam, 1983, Chapter 3.
7. a) Hatanaka, Y.; Hashimoto, M.; Nakayama, H.; Kanaoka, Y. *Chem. Pharm. Bull.*, **1994**, *42*, 826. b) Hatanaka, Y.; Hashimoto, M.; Kurihara, H.; Nakayama, H.; Kanaoka, Y. *J. Org. Chem.*, **1994**, *59*, 383.
8. a) Richey, J. D.; Scism, A. J.; Caskey, A. L.; BeMiller, J. N. *Agr. Biol. Chem.*, **1975**, *39*, 683. b) Richey, J. D.; Caskey, A. L.; BeMiller, J. N.; *Agr. Biol. Chem.*, **1976**, *40*, 2414.
9. Physical data for **6**: $C_9H_4F_3NO_2$ [*m/z* 215.0115 (M^+-N_2)]; IR (film) νmax 3220, 1790, 1740, and 1610 cm^{-1}; UV (MeOH) λmax(ε) 360 (560), 280 (3020), 237 (6900) nm; ^{1}H NMR (δ, $CDCl_3$) 9.03 (1H,br.s, NH), 7.17 (1H, d, J= 1.64 Hz, H-7), 7.11 (1H, d, J= 8.23 Hz, H-4), and 7.01 (1H, dd, J= 8.23, 1.64 Hz, H-5); ^{13}C NMR (δ, $CDCl_3$) 156.7 (s,C-2), 145.6 (s, C-1a), 133.6 (s, C-3a), 124.1 (d, C-5), 123.8 (s, C-6), 123.5 (q, $^1J_{C\text{-}F}$=273.4 Hz, CF_3), 111.4 (d, C-7), 109.4 (d, C-4), and 29.5 (q,$^2J_{C\text{-}F}$= 40.3 Hz).
10. Physical data for **7**: $C_{11}H_8F_3N_3O_4$ [*m/z* 303.0544 (M^+)], $C_{11}H_8F_3NO_4$ [*m/z* 275.0300 (M^+-N_2)]; IR (film) νmax 3260, 1820, 1780, and 1625 cm^{-1}; UV (MeOH) λmax(ε) 287 (4100), 215 (61000) nm; ^{1}H NMR (δ $CDCl_3$) 8.85 (1H, br.s, NH-3), 6,82 (1H, s, H-4), 4.17 (3H, s, OMe-7), and 3.98 (s, 3H, OMe-6); ^{13}C NMR (δ, $CDCl_3$) 155.2 (s, C-2), 148.4 (s, C-6), 138.2 (s, C-7), 135.8 (s, C-3a), 127.0 (s, C-1a), 122.4 (s, C-5), 121.8 (q, $^1J_{C\text{-}F}$= 274.3 Hz, CF_3), 103.5 (d, C-4), 61.9 (q, OMe-6), 60.5 (q, OMe-7), and 26.6 (q, $^2J_{C\text{-}F}$= 43.0 Hz, diazirine).

(*Received in Japan* 20 *January* 1997; *revised* 10 *February* 1997; *accepted* 14 *February* 1997)