Pergamon

Tetrahedron Letters, Vol. 38, No. 48, pp. 8327-8330, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10213-1

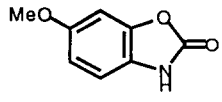
Light-induced Auxin-inhibiting Substance from Cabbage (*Brassica oleacea* L.) Shoots

Seiji Kosemura,*[a] Kazuki Niwa,[a] Hideyuki Emori,[a]
Kaori Yokotani-Tomita,[b] Koji Hasegawa[b] and Shosuke Yamamura*[a]

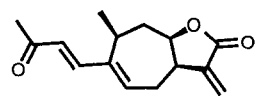
[a]Department of Chemistry, Faculty of Science and Technology,
Keio University, Hiyoshi, Yokohama 223, Japan
[b]Institute of Applied Biochemistry, University of Tsukuba, Ibaragi, 305 Japan

Abstract : A light-induced auxin-inhibitory substance, indolylacetonitrile (IAN), was isolated from light-grown cabbage (*Brassica olearea* L.) shoots. The IAN content in cabbage shoots increased in response to light. Cabbage hypocotyl elongation is inhibited by 10^{-5}M of IAN in 90 min but promoted after 48 hr. Light-induced growth inhibition is caused by the degradation of 3-indolylmethylglucosnolathe (IMG) to IAN which is, in turn, converted to the growth hormone indole-3-acetic acid (IAA). © 1997 Elsevier Science Ltd.

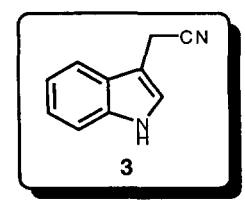
Most people are familiar with the sight of a young seedling bending towards a window or the brightest source of light to which it is exposed. This directional growth response is known as phototropism. Went (1928) proposed that phototropism is caused by a lateral gradient of growth-promoting auxin in the bending organ.[1] This led to the Cholodny-Went theory of photo- and geotropic curvatures (1937).[2] Brinsma and Hasegawa recently found that the shaded half did not contain more auxin than the illuminated half as required by the C.-W. theory.[3] Instead, it was found that the even distribution of auxin was accompained by a lateral gradient of growth-inhibitory substances during phototropic curvature.



1



2



3

We have succeeded in isolating some Light-induced growth inhibitory substances related to phototropism, raphanusanins from radish (*Raphanus sativus* var. *hortensis* f. *gigantissimus* Makino)

hypocotyls, 6-methoxy-2-benzoxazolinone (MBOA, **1**) from light-grown maize (*Zea mays* L.) shoots,[4] 3-hydroxy-β-ionone from light-grown dwarf bean (*Phaseolus vulgaris*) shoots,[5] 8-epixanthatin (**2**) from light-grown de-etiolated sunflower (*Helianthus annuus* L.) seedlings,[6] and carried out some chemical studies on these precursors, 4-methylthio-3-butenyl-isothiocyanate (MTB-ITC),[7] 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzozazin-3(4*H*)-one (DIMBOA) and related compounds.[8] In this study we wish to report the isolation and the mechanism of the accumulation of the photo-induced auxin-inhibiting substance from light-grown cabbage (*Brassica oleracea* L.) shoots.

Cabbage seeds were germinated on moist filter paper and grown in the dark for 2d at 25°C. Etiolated seedlings were irradiated with white fluorescent light for 7 days at 25°C. 2.4 kg of green seedlings were homogenized with a blender, and then directly pressed out and shaken with EtOAc. The EtOAc extract (2.4 g) was directly chromatograhed on silica gel. After elution of higher fatty acids with benzene, further elution with benzene-acetone (4 : 1) afforded a greenish oil, which was further separated by repeated preparative TLC (Kieselgel PF254) using benzene, hexane-AcOEt (3 : 1), and then benzene-acetone (1 : 1) to afford the photo-induced growth inhibitor , which has been identified as 3-indolylacetonitrile (**3**, IAN) in 0.00015% yields.[9]

The light-grown seedlings were observed to contain a higher level of IAN (10^{-5} M) than the dark control (10^{-6} M) determined by measurement of the peak area on HPLC (ODS-HG-5, ϕ 4.6 x 250 mm, 70% MeOH, 0.5ml/min, 254 nm detecter, Rt., 12.5 min), and the difference in the IAN levels was noticeable before the growth inhibition by light appeared. Exogenous concentration (10^{-5} M) of IAN required to inhibit auxin-induced growth of cabbage sections, is equal to the endogenous level in light-grown cabbage hypocotyls. Furthermore, in the endogeneous level (10^{-5} M) of IAN in light-grown cabbage hypocotyls, 4.0 mm sections of 4-day-old etiolated cabbage hypocotyls were incubated in 0.05% Tween 20 soln containing 10^{-6}M indole-3-acetic acid (IAA) in the dark at 25 °C; after 90 min and 48 h of incubation, the lengths of cabbage hypocotyls sections were measured. The resulting cabbage hypocotyl elongation is inhibited (3.0 ± 1.7%) by IAN in 90 min but promoted (4.0 ± 2.4%) after 48 h because of conversion of IAN to the auxin (IAA) by nitrilase.[10]

As shown in Figure 1, the growth inhibitory activity of IAN toward the cabbage seedling is enough to explain the phototropic bending when it is illuminated unilaterally. 4-Day-old etiolated cabbage hypocotyl is 50.7 ± 0.61 mm length and its diametor is 1.02 ± 0.022 mm (center of hypocotyl, Fig 1a). Figure 1c illustrates that the growth rate of the difference between the shaded side (50.0 mm) and illuminated side (48.5 mm) is 3.0% and the radius of curvature is 32.3 mm. Moreover, Figure 1b and 1d show that it is enough that the growth inhibitory activity is only 1.0% to bend the cabbege hypocothyl, if the diametor is 1.0 mm. From these results, it is suggested that IAN may play an important role in light inhibition of hypocotyl growth of cabbage seedlings.

Glucosinolates[11] are sulphur-containing compounds that occur naturally in the Brassicaceae and which, during processing, may undergo enzymatic hydrolysis by the enzyme myrosinase (thioglucoside glucohydrolase) to yield a variety of biologically active products, including isothiocyanates, nitriles and oxazolidine-2-thiones. Cabbage which accumulates 3-indolylmetylglucosinolate (IMG) also contains myrosinase which under light condition can convert IMG to the auxin precursor IAN which is, in turn,

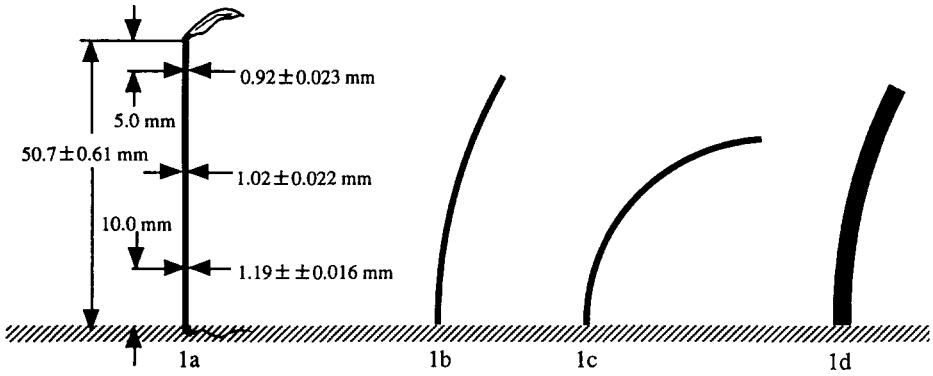
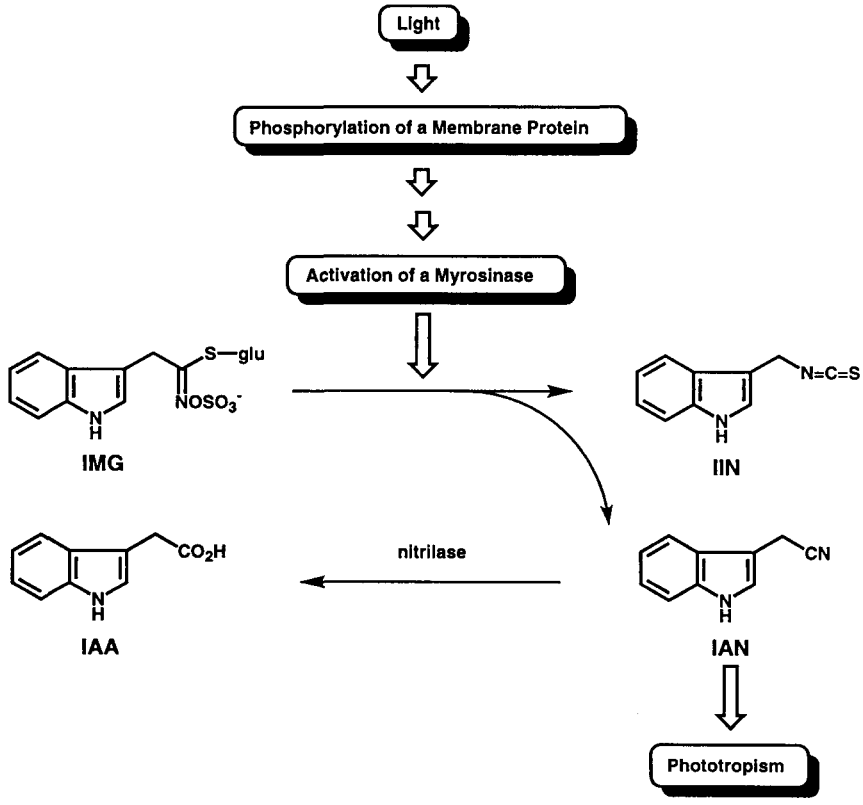


Figure 1 . The relationships between a curvature and a diameter in 1% and 3% of growth inhivitory activities. 1a: 4-day-old etiolated cabbage hypocotyl length and diametors, 1b:1% inhibition, Φ= 1 mm, radius of curvature (r)= 97 mm, 1c: 3% inhibition, Φ=1 mm, r= 32.3 mm, 1d: 3% inhibition, Φ= 3 mm, r= 97 mm.



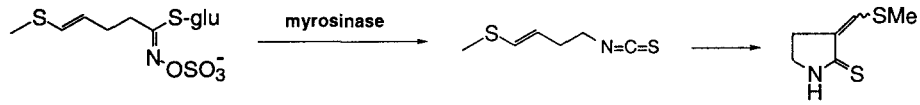
Scheme 1. Postulated Mechanism of Formation of IAN from IMG in Cabbage Tissue

converted to the growth hormone IAA.[12] As results of our present study and Briggs' report,[13] that light-induced phosphorylation of a membrane protein plays an early role in the signal transduction chain for phototropism in higher plants, we proposed that light induces the activity of myrosinase resulting in an increase of IAN, which inhibit auxin-induced growth of cabbage hypocotyls as shown in Scheme 1. Consequently, phototropic response is caused by the local accumulation of growth inhibitor(s) at the lighted side, so that as regulating factor(s) in phototropism growth inhibitor(s) are to be considered rather than auxin. Further studies on the detailed mechanism of formation of IAN from IMG in cabbage tissue are in progress.

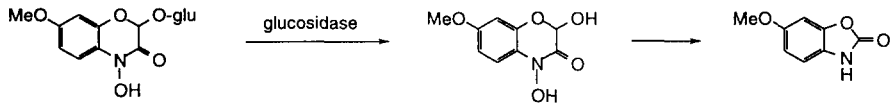
The authors wish to thank the Ministry of Education, Science and Culture (Japan) for Grant-in-Aid for Scientic Research on Priority Areas No 06240103.

References and Notes

1. Went. F. W. Rec. Trav. Bot. Neerl., **1928**, *15*, 1.
2. Went, F. W.; Thimann, K. V.: "Photohormones". MacMillan, New York, **1937**.
3. Hasegawa, K.; Knegt, E.; Bruinsma, J. *Phytochemistry*, **1983**, *22*, 2611.
4. Hasegawa, K.; Togo, S.; Urashima, M.; Mizutani, J.; Kosemura, S.; Yamamura, S. *Phytochemistry*, **1992**, *31*, 3673.
5. Kato-Noguchi, H.; Kosemura, S.; Yamamura, S.; Hasegawa, K. *Phytochemistry*, **1993**, *33*, 553.
6. Yokotani-Tomita, K.; Kato, J.; Kosemura, S.; Yamamura, S.; Kushima, M.; Kakuta, H.; Hasegawa, K. Phytochemistry, **1997**, in press.
7. Kosemura, S.; Yamamura, S.; Hasegawa, K. *Tetrahedron Letters*, **1993**, *34*, 481.



8. Kosemura, S.; Yamamura, S.; Anai, T.; Hasegawa, K. *Tetrahedron Letters*, **1994**, *35*, 8221.



9. Physical data for IAN (**3**): a colorless oil; $C_{10}H_8N_2$ [m/z 156 (M^+)]; IR(film) 3450 and 2255 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.35 (1H, d, J = 8 Hz), 7.2 (1H, m), 7.15 (1H, d, J = 8 Hz), 6.99 (1H, d, J = 8 Hz), 6.56 (1H, bs), 6.48 (1H, s), and 2.94 (2H, s); ^{13}C-NMR ($CDCl_3$) δ 136.5 (s), 126.3 (s), 123.0 (d), 122.5 (d), 120.2 (d), 118.4 (d), 118.0 (s), 111.5 (d), 105.3 (s), and 13.8 (t).
4-Methoxyindole-3-acetonitrile has also been isolated but has no growth inhibitory activity.
10. McMeekin, D. *Plant Growth Regulation, 1996*, 18, 183.
11 Fenwick, G. R.; Heaney, R. K.; Mulin, D. J. *Crit. Rev. Food Sci. Nutr.*, **1983**, *18*, 123.
12. In undamaged cells this conversion is considered to be limited because the enzyme and substrate are physically separated in different cell compartments. We proposed that this compartmentalization breaks down in hypocotyls irradiated by light, resulting in an increase of IAN.
13. Reymond, P.; Short, T. W.; Briggs, W. R.; Poff, K. L. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 4718.

(*Received in Japan* 1 *September* 1997; *accepted* 17 *September* 1997)