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Research Articles

(S)-(+)- 4,4,4-Trifluoro-3-(indole-3-)butyric acid, a novel fluorinated plant growth regulator

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Abstract. Racemic 4,4,4-trifluoro-3-(indole-3-)butyric acid (TFIBA) has been synthesized and shown to inhibit *Avena* coleoptile elongation. (S)-(+)-TFIBA (fig. 1), which was prepared by an enzymatic method and markedly promotes root growth of Chinese cabbage, lettuce and rice plants, is a novel fluorinated plant growth regulator. Activity of the (S)-(+)-enantiomer of TFIBA was 10-fold greater than that of the (R)-(−)-enantiomer in the first two plant species and 5-fold greater in rice.
Key words. Plant growth regulator; trifluoro-3-(indole-3-)butyric acid; fluorinated PGR; root growth promotion; *Avena* coleoptile elongation.

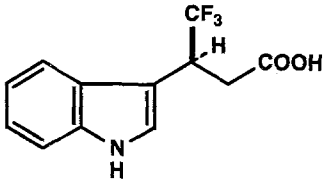

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Figure 1. Structure of (S)-(+)-4,4,4-trifluoro-3-(indole-3-)-butyric acid.

Biologically active substances that contain fluorine, e.g., 5-fluorouracil, ofloxacin, diflubenzuron, fluazifop-butyl, have been synthesized by medical and agricultural researchers[1–4]. The introduction of the fluorine atom(s) to biologically important molecules has dramatic effects on the properties of those molecules. In our studies on the syntheses of plant growth regulators which contain fluorine atom(s) and which promote biomass growth, we synthesized 4,4,4-trifluoro-3-(indole-3-)butyric acid (TFIBA) by introducing one trifluoromethyl and one methylene group to the side chain of indole-3-acetic acid, the first natural plant hormone identified. We here report the synthesis and biological activities of racemic and opitcally active TFIBA, a novel fluorinated plant growth regulator.

Methods

Racemic TFIBA was synthesized as follows: 2,2,2-trifluoro-1-(indole-3-)ethanol[5], obtained from the condensation of indole with an excess of trifluoroacetaldehyde ethyl hemiacetal, was coupled with the sodium salt of diethyl malonate in toluene at 100°C, giving a diester. The ester was hydrolyzed and decarboxylated with potassium carbonate in methanol-water under refluxing conditions, after which methanol was removed *in vacuo*. The resulting aqueous solution was acidified with hydrochloric acid, giving crude TFIBA which was chromatographed on silica gel with ethyl acetate-*n*-hexane to give pure racemic TFIBA in high yield. TFIBA: m.p. 117–119 °C; ^{1}H-NMR spectrum (360 MHz, acetone-d_6, TMS, ppm) 3.04 (1H, dd, J = 16.1, 9.3 Hz), 3.13 (1H, dd, J = 16.1, 5.2 Hz), 4.36 (1H, ddq, J = 5.2, 9.3, 9.4 Hz), 7.09 (1H, ddd, J = 7.8, 6.8, 1.1 Hz), 7.14 (1H, ddd, J = 8.0, 6.8, 1.1 Hz), 7.41 (1H, d, J = 8.0 Hz), 7.47 (1H, s), 7.70 (1H, d, J = 7.8 Hz), 10.39 (1H, br.s); ^{19}F-NMR spectrum (84.7 MHz, acetone-d_6, TFA, ppm) 6.65 (d, J = 9.4 Hz); mass spectrum (70 eV, m/z, relative intensity(%)) 257 (M^+, 86), 237(31), 198(100), 188(22); IR spectrum ν_{max}(KBr)(cm^{-1}) 3430, 1722, 1460, 1438, 1422, 1380, 1326, 1313, 1296, 1280, 1155, 1113, 962, 823, 745, 664, 618.

Results

Biological activities of racemic TFIBA were compared with those of racemic 3-(indole-3-)butyric acid (3-IBA), synthesized according to refs 6 and 7, 4-(indole-3)-butyric acid (4-IBA), 3-(indole-3-)propionic acid (IPA) and indole-3-acetic acid (IAA), using two kinds of auxin bioassays. The result of the bioassay using elongation of *Avena sativa* coleoptiles is shown in figure 2. Although IAA, IPA and 4-IBA showed the elongation activity of *Avena* coleoptiles, both TFIBA and 3-IBA produced no coleoptile elongation for the entire

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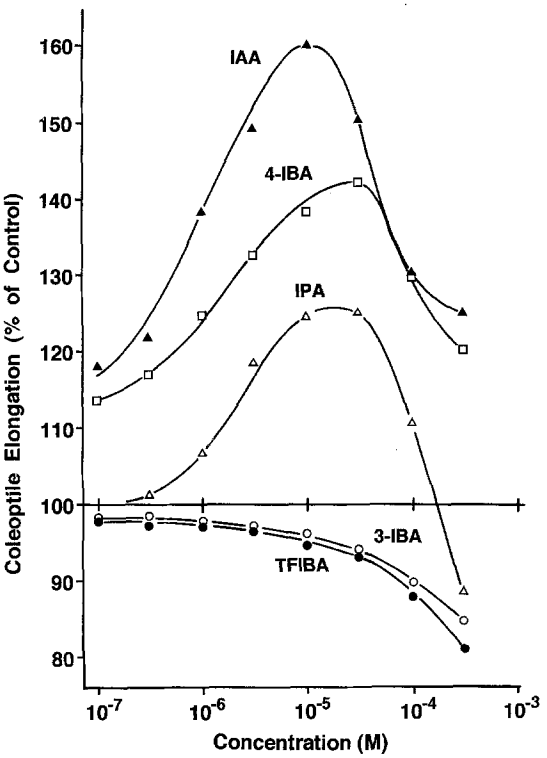

Figure 2. Effects of TFIBA, 3-IBA, IAA, IPA and 4-IBA on *Avena* coleoptiles.
Coleoptile segments (5 mm length) that were cut from 2 mm below the tip of seedlings of *Avena sativa* L. cv. Almighty, grown under red light for one day and then in the dark for two days, were incubated in an aqueous solution (2 ml) of the various compounds in the dark at 25 °C for 20 h, and the length of coleoptiles was measured. The experiment was repeated twice. TFIBA = 4,4,4-trifluoro-3-(indole-3-)butyric acid; 3-IBA = 3-(indole-3-)butyric acid; IAA = indole-3-acetic acid; IPA = 3-(indole-3-)propionic acid; 4-IBA = 4-(indole-3-)butyric acid.

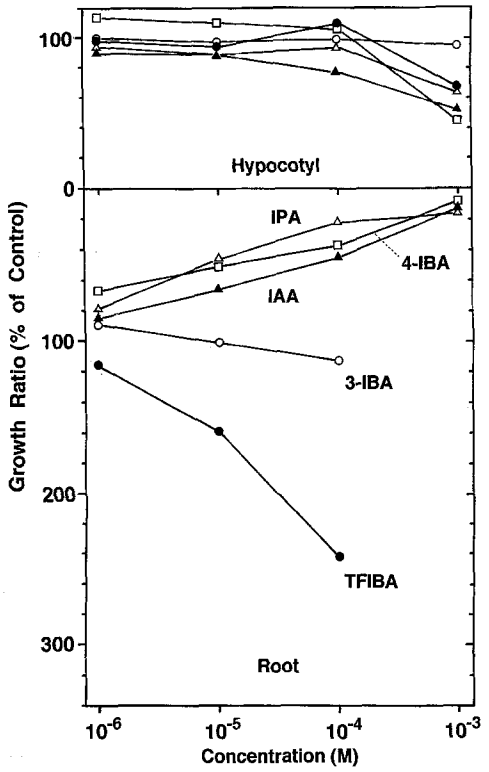

Figure 3. Effects of TFIBA, 3-IBA, IAA, IPA and 4-IBA on Chinese cabbage seedlings.
Chinese cabbage (*Brassica pekinensis* cv. Kinshu) seeds were first placed on wet cotton in a Petri dish (diameter 15 cm) in darkness at 25 °C for 1 day in a growth chamber. Ten germinated seeds were then cultured in a Petri dish (diameter 6 cm) containing an aqueous solution (4 ml) of the test substance under a cycle of 16 h of light and 8 h of darkness at 25 °C for 3 days in the growth chamber, after which the root and hypocotyl lengths were measured. The experiment was repeated three times. Root necrosis occurred at the concentration of 1×10^{-3} M of TFIBA and 3-IBA. Abbreviation of chemicals: see figure 2.

range of concentrations tested and the inhibitory activity of TFIBA was comparable to that of 3-IBA. Inhibitory activity of TFIBA and 3-IBA increased as their concentration increased. In a test using Chinese cabbage grown under 8 h light-16 h dark photoperiod conditions, TFIBA showed strong root growth-promoting activity (about 2.5-fold that of the control at 1×10^{-4} M) but 3-IBA showed very weak promoting activity of root growth only at a concentration of 1×10^{-4} M (fig. 3). Other compounds (IAA, IPA, 4-IBA) showed only inhibitory activity of root growth in Chinese cabbage. This indicates that the introduction of a methyl substituent into the β-position of the side chain in 3-(indole-3-)-propionic acid changes the elongation activity in *Avena* coleoptiles to an inhibitory one, and further suggests that the presence of fluorines in the methyl substituent is very important for strong enhancement of root growth-promoting activity.

In the preliminary bioassay, racemic TFIBA also promoted root growth in lettuce and rice plants. To clarify the relationship between its stereochemistry and root growth-promoting activity, both enantiomers of TFIBA were prepared by an enzymatic method[8]. Racemic TFIBA ethyl ester was treated with lipase AK in acetate buffer containing 10% t-butanol at 55 °C to give (+)-TFIBA and (−)-TFIBA ethyl ester in 98% enantiomeric excess (ee). (+)-TFIBA was converted to its ethyl ester with lipase AK in heptane containing ethanol in >99.9%ee. (−)-TFIBA ethyl ester was purified with lipase AK in buffer containing 10% t-butanol, giving the ester in >99.9%ee. Both esters were easily hydrolyzed to the free acids with esterase PLE-A or 40% aqueous potassium hydroxide solution. The enantiomeric purities of the TFIBAs were determined by high-performance liquid chromatography on a chiral column (CHIRALCEL OD, 4.6 ID × 250 mm, DAICEL Chem. Ind. LTD.) with a solvent system of *n*-hexane-2-propanol-tri-fluoroacetic acid (9:1:0.05). The less polar enantiomer with a specific rotation of minus was eluted at 10.0 min. The other, polar, enantiomer with a specific rotation of plus was eluated at 12.0 min. The enantiomeric excess for both optically active TFIBAs was >99.9%. The absolute configurations of the enan-

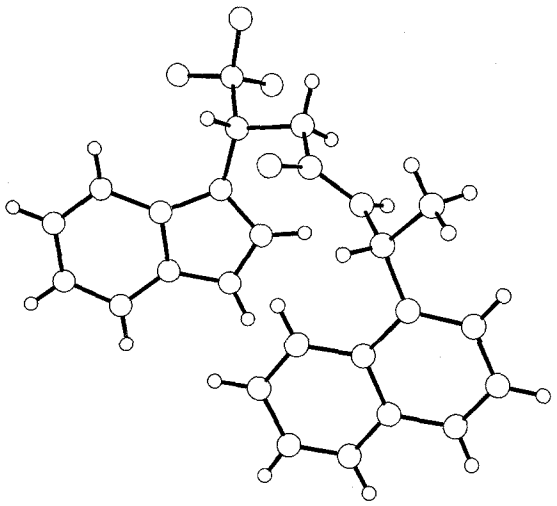

Figure 4. Crystal structure of the amide from (−)-TFIBA and (R)-(+)-1-(1-naphthyl)ethylamine.

tiomers were determined from the results of X-ray analysis of an amide prepared from the (−)enantiomer and (R)-(+)-1-(1-naphthyl)ethylamine (fig. 4). The amide, obtained by the coupling reaction in dichloromethane in the presence of dicyclohexylcarbodiimide, was purified by silica gel column chromatography and recrystallization from 30% ethyl acetate in *n*-hexane. The crystals are monoclinic, a = 4.860(1), b = 16.226(1), c = 13.001(1) Å, $\beta = 96.71(2)°$, space group $P2_1$, z = 2, dc = 1.339 g/cm^3. The final R-factor, based on 1335 observed reflections, was 0.042. X-ray analysis of the amide showed that (+)-TFIBA($[\alpha]_D^{20}$ 10.4° (c 2.0, EtOH)) has the S configuration and (−)-TFIBA($[\alpha]_D^{20}$-10.4° (c 2.0, EtOH)) the R one.

The biological activities of optically active TFIBA were studied in seedlings of Chinese cabbage, lettuce and rice. Results of the bioassays showed that (S)-(+)-TFIBA had strong activity for root growth in all of these plants. The results for seedlings of Chinese cabbage and lettuce are shown in tables 1 and 2, respectively. The growth-promoting activity of (S)-(+)-TFIBA in Chinese cabbage and lettuce was much higher than that of (R)-(−)-TFIBA, but neither enantiomer affected hypocotyl growth in either plant, except at the high concentration of 1×10^{-3} M in lettuce. At 1×10^{-4} M, (S)-(+)-TFIBA promoted root growth of the Chinese cabbage control 3-fold and that of the lettuce control 2-fold. (S)-(+)-TFIBA had significant effects on root growth in the seedlings of both species at 1×10^{-6} M. The growth-promoting activity of (S)-(+)-TFIBA was about 10-fold that of (R)-(−)-TFIBA on Chinese cabbage and lettuce. At the high concentration of 1×10^{-3} M, however, the TFIBAs completely inhibited root growth in both species and hypocotyl growth in lettuce. Necrosis was particularly severe in the roots. In rice seedlings (table 3), (S)-(+)-TFIBA at 1×10^{-5} M promoted root growth 3.8-fold the value for the control. This activity of (S)-(+)-TFIBA was about 5-fold that of (R)-(−)-TFIBA in rice; but at 1×10^{-3} M root necrosis occurred. These results show that the stereochemistry at C-3 is very important for the promotion of root growth in these three plant species.

Table 1. Effects of (S)-(+)-, (R)-(−)- and (RS)-TFIBA on Chinese cabbage seedlings.

TFIBA Concentration (M)	(+)		(−)		(±)	
	Root	Hypocotyl	Root	Hypocotyl	Root	Hypocotyl
	% of Control					
1×10^{-3}	N	121.1	N	94.7	N	96.5
1×10^{-4}	304.2	108.8	175.9	91.2	250.9	98.2
1×10^{-5}	195.8	94.7	134.0	91.2	166.0	93.0
1×10^{-6}	134.9	96.5	101.4	98.2	118.9	94.7

N = necrosis.
Bioassay with Chinese cabbage seedlings was carried out according to the method used in figure 3.

Table 2. Effects of (S)-(+)-, (R)-(−)- and (RS)-TFIBA on lettuce seedlings.

TFIBA Concentration (M)	(+)		(−)		(±)	
	Root	Hypocotyl	Root	Hypocotyl	Root	Hypocotyl
	% of Control					
1×10^{-3}	N	33.3	N	15.2	N	25.8
1×10^{-4}	229.0	93.9	162.9	77.3	193.8	83.3
1×10^{-5}	183.7	109.1	133.9	104.5	165.1	106.1
1×10^{-6}	129.6	107.6	99.7	100.0	119.9	95.5

N = necrosis.
Lettuce (*Lactuca sativa* cv. Gokuwase-CISCO) seeds were grown by the procedure used in the Chinese cabbage seedling test (fig. 3). The experiment was repeated three times.

Table 3. Effects of (S)-(+)-, (R)-(−)- and (RS)-TFIBA on rice seedlings.

TFIBA Concentration (M)	(+) Root	(+) Shoot	(−) Root	(−) Shoot	(±) Root	(±) Shoot
	% of Control					
1×10^{-3}	N	56.3	N	53.5	N	49.0
1×10^{-4}	233.6	82.4	101.0	87.5	115.2	78.1
1×10^{-5}	384.5	102.8	257.5	92.7	308.4	93.5
1×10^{-6}	232.3	101.5	120.5	96.3	156.4	98.3

N = necrosis.
Rice (*Oryza sativa* cv. Tan-ginbozu) seeds were placed in a Petri dish (diameter 18 cm) on cotton that had been soaked with water, then cultured for 3 days at 28 °C in darkness in a growth chamber. Six germinated seeds were then placed on filter paper soaked in the enantiomer test solution (4 ml) in a test tube (∅ 4 × 12 cm). The plants were cultured at 28 °C under a cycle of 16 h of light and 8 h of darkness in the growth chamber. After 7 days, root and shoot lengths were measured. The experiment was repeated three times.

Field tests showed further TFIBA activities: 1) stimulation of potato tuber growth; 2) enhancement of the number of ears in wheat; 3) stimulation of radish tuber growth; 4) enhancement of ripening and enlargement of tomato fruits; 5) stimulation of tillering and increases in fresh weights of rice plants; and 6) enhancement of differentiation in female cucumber flowers[9]. Treatment of corn with racemic TFIBA strongly promoted root growth, which should increase yields and/or advance the date of harvest. This is important because corn is a major source of ethanol for fuel. Field tests on the use of racemic TFIBA to enhance other biomasses are in progress.

1 Filler, R., and Kobayashi, Y., Biomedical Aspects of Fluorine Chemistry. Kodansha Ltd., Tokyo 1982.
2 Filler, R., Organofluorine Chemicals and their Industrial Application, pp. 123–153. Ed. E. Banks. Ellis Horwood Ltd., Chichester 1979.
3 Newbold, G. T., Organofluorine Chemicals and their Industrial Application, pp. 167–187. Ed. E. Banks. Ellis Horwood Ltd., Chichester 1979.
4 Kitazume, T., Ishihara, T., and Taguchi, T., in: Fluorine Chemistry (in Japanese). Kodansha Scientific Co., Tokyo 1993.
5 Maki, Y., Kimoto, H., Fujii, S., Senga, M., and Cohen, L. A., J. fluorine Chem. *39* (1988) 47.
6 Preobranzhenskaya, M. N., Balashova, E. G., Turchin, K. F., Padeiskaya, E. N., Uvarova, N. V., Pershin, G. N., and Suvorov, N. N., Tetrahedron, *24* (1968) 6131.
7 Oikawa, Y., Hirasawa, H., and Yonemitsu, O., Tetrahedron Lett. (1978) 1759.
8 Kato, K., Katayama, M., Gautam, R. K., Fujii, S., and Kimoto, H., J. Ferment. Bioeng. *76* (1993) 178.
9 Kato, K., Katayama, M., Kimoto, H., Fujii, S., Gautam, R. K., and Kamuro, Y., Reports of the Government Industrial Research Institute, Nagoya (in Japanese with English abstract) *42* (1993) 214.

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