Biosci. Biotechnol. Biochem., **72** (8), 2025–2033, 2008

Synthesis and Biological Activities of 4-Trifluoromethylindole-3-acetic Acid: A New Fluorinated Indole Auxin

Masato KATAYAMA,[1,†] Yuko MASUI,[2] Eiji KAGEYAMA,[2]
Youichi KAWABATA,[3] and Kozo KANAYAMA[4]

[1]*Cooperative Research Laboratory for Plant Growth Regulator Development, National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-8560, Japan*
[2]*Tokai-Kasei Company Ltd., 66 Sodai, Mino, Gifu 501-3714, Japan*
[3]*Nippon Pharmaceutical Chemicals Company Ltd., 2-8-18 Choda, Higashiosaka, Osaka 577-0056, Japan*
[4]*Advanced Wood-based Material Technology Group, Materials Resaerch Institute for Sustainable Development, AIST, 2266-98 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-8560, Japan*

Received March 5, 2008; Accepted May 19, 2008; Online Publication, August 7, 2008
[doi:10.1271/bbb.80138]

In our studies on the development of new promoters for the root formation of tree cuttings, 4-trifluoromethylindole-3-acetic acid (4-CF_3-IAA), a new fluorinated auxin, was synthesized *via* 4-trifluoromethylindole and 4-trifluoromethylindole-3-acetonitrile by using 2-methyl-3-nitrobenzotrifluoride as the starting material. As a control compound for comparing biological activities, 4-methylindole-3-acetic acid (4-CH_3-IAA) was also synthesized by using 2,3-dimethylnitrobenzene as the starting material. The biological activities of these compounds were compared by three bioassays with those of indole-3-acetic acid and 4-chloroindole-3-acetic acid (4-Cl-IAA), which, like 4-CF_3-IAA and 4-CH_3-IAA, has a substituent at the 4-position of the indole nucleus. 4-CF_3-IAA showed strong root formation-promoting activity with black gram cuttings which was 1.5 times higher than that of 4-(3-indole)butyric acid at 1×10^{-4} M. 4-CH_3-IAA, however, only weakly promoted root formation in spite of its strong inhibition of hypocotyl growth in Chinese cabbage and promotion of hypocotyl swelling and lateral root formation in black gram. On the other hand, 4-CF_3-IAA demonstrated weaker activities than 4-CH_3-IAA and 4-Cl-IAA in these two bioassays.

Key words: 4-trifluoromethylindole-3-acetic acid; 4-methylindole-3-acetic acid; fluoroindole auxin; 4-chloroindole-3-acetic acid; root formation-promoting activity

Our past studies of halogenated plant growth regulators, especially of root formation promoters, originated from the initial isolation of 4-chloroindole-3-acetic acid (4-Cl-IAA), a natural auxin, from immature seeds of *Pisum sativum*.[1,2] Subsequently, 5,6-dichloroindole-3-acetic acid (5,6-Cl_2-IAA), the most active of the known natural and synthetic auxins, was synthesized.[3] The synthesis and biological activities of 4-Cl-IAA and its esters have also been reported.[4,5] Soaking and spraying methods employing these compounds are potentially useful for the mass production of tree saplings in reforestation.[6,7] In a recent report describing the synthesis and auxin activities of the L-lactic acid derivatives of 4-Cl-IAA, the root formation activities of some esters with black gram cuttings were greater than or the same as that of 4-Cl-IAA.[8]

Biologically active substances that contain fluorine have been previously synthesized in the medical and agricultural fields.[9–12] The introduction of fluorine atoms into biologically important molecules has a dramatic effect on the properties of those molecules. In studies of fluorinated plant growth regulators, the fluoroindole auxins, 5,6-difluoroindole-3-acetic acid (5,6-F_2-IAA) and 2-(5,7-difluoro-3-indolyl)propionic acid (5,7-F_2-IPA), as well as the fluoroindole antiauxin, 2-(5,7-difluoro-3-indolyl)isobutyric acid (5,7-F_2-IIBA), have been synthesized.[13,14] The introduction of fluorine atom(s) into the benzene moiety of indole-3-acetic acid (IAA) resulted in an increase in the auxin activity and enhanced the growth of the lateral roots of mung beans. Furthermore, we have reported that such fluorinated root growth promoters as the nonsubstituted and substituted 4,4,4-trifluoro-3-(3-indolyl)butyric acids (TFIBAs) showed strong root growth-promoting activities in Chinese cabbage, lettuce, and rice seedlings, whereas the nonfluorinated compound, 3-(3-indolyl)butyric acid, only showed weak promoting activity.[15–17] Thus, replacement of the methyl moiety of 3-(3-indolyl)bu-

† To whom correspondence should be addressed. Fax: +81-52-736-7611; E-mail: m.katayama@aist.go.jp

tyric acid with a trifluoromethyl group caused a dramatic increase in root growth promotion in these three plants. Some other interesting activities of TFIBAs have also been recently reported.[18–22] We are continuously developing new compounds that induce roots in the cuttings of trees that often do not easily produce roots. 4-Methylindole-3-acetic acid (4-CH_3-IAA) has been reported to have strong growth promoting activity toward pea pericarp as well as 4-Cl-IAA.[23] We therefore focused on the synthesis of the new fluorinated auxin, 4-trifluoromethylindole-3-acetic acid (4-CF_3-IAA, **1**), replacing the methyl group of 4-CH_3-IAA with a trifluoromethyl group for the development of new compounds with strong root formation-promoting activity. We report here the synthesis and biological activities of 4-CF_3-IAA (**1**).

Materials and Methods

Instrumentation. Infrared spectra were measured with a Shimadzu FTIR-8600PC spectrometer. ^{1}H-NMR spectra were recorded with a Varian INOVA-300 spectrometer, with tetramethylsilane in acetone-d_6 used as the internal standard. Mass spectra were recorded with a Jeol DX-705L spectrometer. Elemental analyses were conducted with a Perkin-Elmer 2400 CHN elemental analyzer.

*Synthesis of 4-trifluoromethylindole-3-acetic acid (**1**) 4-trifluoromethylindole (**3**).* A mixture of 2-methyl-3-nitrobenzotrifluoride (**2**, 93.3 g, 0.455 mol), dimethylformamide dimethyl acetal (108 g, 0.91 mol) and dimethylformamide (149 ml) was heated at 110 °C under nitrogen as methanol produced in the reaction was removed with Dean-Stark apparatus. After stirring for 22 h, the reaction mixture was cooled to room temperature, and the solvent was removed *in vacuo* to give a dark brown oil (139 g) which was dissolved in diisopropyl ether (300 ml), the ether layer being washed with water and the aqueous layer treated twice with diisopropyl ether. The combined ether layer was successively washed with water and saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give 128 g of a styrene derivative as a dark brown oil which was used in the next reaction without purification.

Raney-nickel (50 ml) was added to a stirred solution of the styrene (128 g) in methanol while cooling, and the styrene was reduced under a pressure of 3 kgf/cm^2 of hydrogen as the temperature was raised to room temperature. To complete the reduction, additional Raney-nickel (5 ml) was added to the solution while cooling. After this reductive cyclization, the reaction solution was filtered through Celite, and the filtrate was evaporated *in vacuo* to give a brown oil which was dissolved in a mixture (300 ml) of *n*-hexane and ethyl acetate, before the mixture was treated with water. The aqueous layer was treated with the mixed solvent (200 ml). The combined organic layer was successively washed with a 0.1 N hydrochloric acid solution, water, a saturated sodium bicarbonate solution and saturated brine, dried over anhydrous sodium sulfate, decolorized with activated charcoal (1 g), and evaporated *in vacuo* to give 76.8 g of a brown oil. This oil was distilled under reduced pressure (0.5 mm of Hg at 63–68 °C) to afford 56.4 g (67.0% yield from the trifluoride) of 4-trifluoromethylindole (**3**) as a pale purple oil. NMR (300 MHz) δ_H (acetone-d_6): 6.64 (1H, dq, $J = 3.2$, 1.5 Hz), 7.26 (1H, ddq, $J = 8.1$, 7.4, 0.8 Hz), 7.40 (1H, dq, $J = 7.4$, 0.9 Hz), 7.57 (1H, dd, $J = 3.2$, 1.4 Hz), 7.73 (1H, dq, $J = 8.1$, 0.8 Hz), 10.80 (1H, broad s); MS (relative intensity, %) *m/z*: 185 (M$^+$, 100), 166 (29), 158 (7), 138 (10), 135 (29), 116 (5), 107 (6), 89 (12), 68 (9), 63 (11). IR ν_{max}(KBr) cm^{-1}: 3442, 1624, 1586, 1508, 1442, 1419, 1368, 1346, 1318, 1272, 1191, 1164, 1117, 1078, 1053, 939, 897, 830, 800, 755, 726, 609, 487. Found: C, 58.39; H, 3.06; N, 7.68%. Calcd. for $C_9H_6F_3N$: C, 58.38; H, 3.27; N, 7.57%.

*3-Dimethylaminomethyl-4-trifluoromethylindole (**4**).* A portion of 37% aqueous formaldehyde (26.7 g, 0.32 mol) was added dropwise at below 7 °C to a mixture of 31.3 g (0.35 mol) of a 50% dimethylamine solution and 40 ml of acetic acid that had been prepared below 20 °C. A solution of 4-trifluoromethylindole (**3**, 55.4 g, 0.299 mol) in acetic acid (15 ml) was added to the mixture over about 1.5 h at 5 °C, and the mixture was stirred overnight at room temperature. The reaction mixture was acidified with a 4 N-hydrochloric acid solution and treated three times with ethyl acetate. Water (330 ml) was added to the reaction mixture, and the mixture was treated twice with a mixed solvent (110 ml) of *n*-hexane and *tert*-butyl methyl ether (MTBE; 1:1). The aqueous layer was alkalized with a 24% sodium hydroxide solution and treated twice with MTBE. The combined organic layer was successively washed with water and saturated brine, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to give pale brown crystals. These crystals were disperse-washed with a mixed solvent (100 ml) of *n*-hexane-MTBE (2:1) to give 57.0 g (78.6% yield) of 3-dimethylaminomethyl-4-trifluoromethylindole (**4**). Mp 134–135 °C; NMR δ_H (acetone-d_6): 2.27 (6H, s), 3.59 (2H, dq, $J = 1.1$, 1.1 Hz), 7.23 (1H, ddq, $J = 8.1$, 7.4, 0.8 Hz), 7.44 (1H, dq, $J = 7.4$, 0.8 Hz), 7.53 (1H, s), 7.72 (1H, dq, $J = 8.1$, 0.8 Hz), 10.73 (1H, broad s); IR ν_{max}(KBr) cm^{-1}: 3313, 2954, 2830, 1468, 1429, 1373, 1318, 1280, 1199, 1162, 1112, 1073, 996, 942, 860, 821, 796, 763, 725; MS (relative intensity, %) *m/z*: 242 (M$^+$, 43), 198 (100), 178 (11), 169 (13), 151 (20), 147 (12), 129 (6), 101 (4), 75 (6), 58 (7). Found: C, 59.51; H, 5.28; N, 11.64%. Calcd. for $C_{12}H_{13}F_3N_2$: C, 59.50; H, 5.41; N, 11.56%.

*4-Trifluoromethylindole-3-acetonitrile (**5**).* A solution of **4** (57.0 g, 0.235 mol) in methanol (740 ml) was added to a solution of potassium cyanide (33.0 g, 0.51 mol) in water (57 ml). Methyl iodide (110.5 g, 0.78 mol) was

added dropwise to the solution while cooling with ice, and the mixture was stirred overnight as the temperature was raised to room temperature. Additional potassium cyanide (3.3 g, 0.051 mol) was added to the mixture which was then stirred overnight. Water (1 liter) was added to the reaction solution, before treating with MTBE (600 ml) and a mixed solvent (400 ml) of MTBE and *n*-hexane (1:1). The combined organic layer was successively washed water and saturated brine, treated with activated charcoal (1 g) and sodium sulfate, and evaporated *in vacuo* to give a nitrile which was recrystallized from ethyl acetate-*n*-hexane to afford 51.3 g (97.6% yield) of 4-trifluoromethylindole-3-acetonitrile (**5**). Mp 121–122 °C; NMR δ_H (acetone-d_6): 4.01 (2H, dq, $J = 1.4$, 0.6 Hz), 7.33 (1H, ddq, $J = 8.2$, 7.6, 0.8 Hz), 7.53 (1H, dq, $J = 7.6$, 0.8 Hz), 7.72 (1H, s), 7.81 (1H, dq, $J = 8.2$, 0.8 Hz), 11.01 (1H, broad s); IR ν_{max}(KBr) cm^{-1}: 3346, 2256, 1430, 1365, 1314, 1212, 1188, 1158, 1133, 1113, 1069, 946, 823, 796, 755, 721, 629, 605, 577, 486; MS (relative intensity, %) *m/z*: 224 (M^+, 100), 223 (79), 205 (11), 198 (67), 176 (17), 155 (47), 151 (9), 127 (6), 77 (7), 75 (6), 60 (6). Found: C, 58.83; H, 3.02; N, 12.58%. Calcd. for $C_{11}H_7F_3N_2$: C, 58.93; H, 3.15; N, 12.50%.

*4-Trifluoromethylindole-3-acetic acid (**1**).* Dry hydrogen chloride gas, which was produced from concentrated sulfuric acid (500 ml) and hydrochloric acid (280 ml) and then passed through concentrated sulfuric acid, was slowly bubbled into a solution of nitrile **5** (51.3 g, 0.229 mol) in dry methanol (205 ml) below 5 °C. After stirring for 5 h while raising the temperature to room temperature, the reaction mixture was poured into a mixture of methanol (820 ml) and water (4.1 g, 0.23 mol), and the resulting mixture was stirred overnight at room temperature. After refluxing for 1 h, methanol was removed *in vacuo*, and the residue was poured into water (650 ml). The resulting crystals were cooled, filtered, and washed with water to give 87.8 g of a wet and pale yellow methyl ester (**6**) which was recrystallized from ethyl acetate-*n*-hexane to give methyl 4-trifluoromethylindole-3-acetate (**6**). Mp 121–122 °C; NMR δ_H (acetone-d_6): 3.65 (3H, s), 3.87 (2H, s), 7.25 (1H, ddq, $J = 8.2$, 7.4, 0.8 Hz), 7.45 (1H, dq, $J = 7.4$, 0.8 Hz), 7.55 (1H, s), 7.75 (1H, dq, $J = 8.2$, 0.8 Hz), 10.81 (1H, broad s); IR ν_{max}(KBr) cm^{-1}: 3301, 1728, 1440, 1364, 1346, 1305, 1218, 1201, 1159, 1138, 1108, 1072, 1052, 995, 948, 765, 746; MS (relative intensity, %) *m/z*: 257 (M^+, 72), 210 (8), 198 (100), 178 (12), 169 (7), 151 (28), 147 (10), 129 (12), 101 (6), 73 (8), 60 (9). Found: C, 55.84; H, 3.78; N, 5.43%. Calcd. for $C_{12}H_{10}F_3NO_2$: C, 56.04; H, 3.92; N, 5.45%.

A 24% sodium hydroxide solution (38.1 g, 0.23 mol) was added to a solution of wet **6** in methanol (510 ml). After stirring overnight at room temperature, the reaction mixture was condensed to an aqueous solution and water was added. The solution was treated twice with a mixed solvent (100 ml and 50 ml) of MTBE and *n*-hexane (1:1). The aqueous layer was treated with activated charcoal (2.5 g) and then stirred for half an hour, before the charcoal was filtered off. The pH value of the aqueous filtrate was adjusted to 8–9, and activated charcoal (2.5 g) was again added to the solution which was then stirred for half an hour. After removing the charcoal by filtration, the aqueous solution was acidified with a hydrochloric acid solution, and the resulting powder was collected by filtration. This powder was thoroughly washed with water and then dried to give an indoleacetic acid which was recrystallized from IPA-H_2O and dried at 60 °C to yield 43.1 g (77.5% yield from the nitrile) of 4-trifluoromethylindole-3-acetic acid. Mp 206–208 °C; NMR δ_H (acetone-d_6): 3.86 (2H, dq, $J = 1.0$, 1.0 Hz), 7.24 (1H, ddq, $J = 8.2$, 7.4, 0.8 Hz), 7.45 (1H, dq, $J = 7.4$, 0.8 Hz), 7.57 (1H, s), 7.74 (1H, dq, $J = 8.2$, 0.8 Hz), 10.80 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3357, 1708, 1426, 1312, 1226, 1208, 1185, 1158, 1131, 1100, 1074, 778, 745, 630; MS (relative intensity, %) *m/z*: 243 (M^+, 100), 224 (4), 198 (100), 178 (36), 176 (18), 151 (72), 147 (19), 129 (23), 128 (13), 101 (12), 75 (12), 60 (10). Found: C, 54.33; H, 3.10; N, 5.83%. Calcd. for $C_{11}H_8F_3NO_2$: C, 54.33; H, 3.32; N, 5.76%.

*Synthesis of 4-methylindole-3-acetic acid (**11**)*

*4-methylindole (**8**).* A mixture of 2, 3-dimethylnitrobenzene (**7**, 500 g, 3.31 mol), dimethylformamide dimethyl acetal (477 g, 4.00 mol), pyrrolidine (205 g, 2.88 mol) and dimethylformamide (488 ml) was heated at 95 °C for 3 h under nitrogen, the methanol produced in the reaction being removed with Dean-Stark apparatus. After a further addition of dimethylformamide dimethyl acetal (79 g, 0.66 mol), the reaction temperature was gradually raised from 95 °C to 125 °C, and the mixture was heated. After 30 h, the reaction mixture was cooled to room temperature, and the solvent was removed *in vacuo* to give a dark red oil (866 g) of a styrene which was used in the next reaction without purification.

Raney-nickel (100 g) was added to a stirred solution of this styrene (736 g) in tetrahydrofuran (THF, 1.7-liter) while cooling, before a hydrazine solution (666 g, 13.3 mol) was added dropwise to the THF solution and an additional hydrazine solution (15 g, 0.3 mol) was added at 40 °C, and this mixture then being heated at 60 °C. The reaction solution was filtered through Celite, and the filtrate was condensed *in vacuo* to two-thirds of its volume, before being treated with *n*-hexane (700 ml) and water. The aqueous layer was treated with *n*-hexane (300 ml). The combine organic layer was successively washed with water, a hydrochloric acid solution, water, a saturated sodium hydrogen bicarbonate solution and saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a dark brown oil (318.0 g) which was distilled under reduced pressure (0.4 mm of Hg at 94 °C) to yield 280.4 g (76.0% yield from **7**) of 4-methylindole (**8**) as a pale yellow oil. NMR (300 MHz) δ_H (acetone-d_6): 2.50 (3H, s), 6.49–6.51 (1H,

m), 6.80 (1H, ddq, $J = 7.1$, 0.9, 0.9 Hz), 6.99 (1H, dd, $J = 8.1$, 0.9 Hz), 7.24 (1H, dd, $J = 8.1$, 0.9 Hz), 7.27–7.31 (1H, m), 10.20 (1H, broad s); IR ν_{max}(KBr) cm^{-1}: 3408, 3053, 2918, 1608, 1583, 1500, 1456, 1410, 1377, 1354, 1342, 1281, 1238, 1157, 1115, 1080, 955, 895, 835, 752, 721, 619, 517; MS (relative intensity, %) *m/z*: 131 (M^+, 75), 130 (100), 103 (11), 89 (2), 77 (17), 65 (19), 51 (14). Found: C, 82.35; H, 6.73; N, 10.86%. Calcd. for C_9H_9N: C, 82.40; H, 6.92; N, 10.68%.

*3-Dimethylaminomethyl-4-methylindole (**9**).* A portion of 37% aqueous formaldehyde (187 g, 2.30 mol) was added dropwise to a mixture of 218 g (2.41 mol) of a 50% dimethylamine solution and 224 ml of acetic acid at below 5 °C, and the mixture was stirred for half an hour. Fifty-six ml of THF was added to the Shiff base solution, and a mixed solution of 4-methylindole (**8**, 280 g, 2.13 mol) and methanol (56 ml) was then added to the mixture over 2.5 h at 5 °C, before the temperature of the reaction solution was raised to room temperature and the solution stirred overnight. The reaction mixture was filtered, and the filtrate was evaporated *in vacuo*, before water (840 ml) was added to the residue, and the aqueous solution was treated three times with a mixed solvent of MTBE-*n*-hexane (1:1). After the aqueous solution had been evaporated again *in vacuo* to remove the materials with a low boiling point, the resulting solution was alkalized with a 24% sodium hydroxide solution to give a dimethylaminomethylindole which was thoroughly washed with water. The resulting wet powder was dissolved in ethyl acetate. The ethyl acetate solution was washed with saturated brine, dried over anhydrous sodium sulfate, and evaporated to give a crude dimethylaminomethylindole which was recrystallized from ethyl acetate-*n*-hexane to give 296.7 g (73.8% yield) of 3-dimethylaminomethyl-4-methylindole (**9**). Mp 134–135 °C; ^{1}H-NMR δ_H (acetone-d_6): 2.18 (6H, s), 2.74 (3H, s), 3.51 (2H, d, $J = 0.6$ Hz), 6.74 (1H, ddq, $J = 7.1$, 1.0, 1.0 Hz), 6.95 (1H, dd, $J = 8.1$, 7.1 Hz), 7.14 (1H, broad s), 7.18 (1H, d, $J = 8.1$ Hz), 10.02 (1H, broad s); IR ν_{max}(KBr) cm^{-1}: 3168, 3119, 2997, 2964, 2936, 2855, 2816, 2772, 1541, 1508, 1472, 1439, 1414, 1340, 1275, 1234, 1167, 1126, 1069, 1036, 993, 961, 853, 820, 741, 579; MS (relative intensity, %) *m/z*: 188 (M^+, 13), 144 (52), 143 (100), 142 (40), 129 (11), 115 (48), 89 (12), 73 (16), 60 (17). Found: C, 76.42; H, 8.48; N, 15.03%. Calcd. for $C_{12}H_{16}N_2$: C, 76.56; H, 8.57; N, 14.88%.

*4-Methylindole-3-acetonitrile (**10**).* Dimethyl sulfate (241 g, 1.91 mol) was added to dimethylformamide (450 ml), before the solution was cooled and 3-dimethylaminomethyl-4-methylindole (**9**, 300 g, 1.59 mol) was added at below −10 °C. After the mixture had been stirred for 1.5 h, a solution of potassium cyanide (125 g, 1.92 mol) in water (210 ml) was added, and the mixture stirred for 1 h under slightly reduced pressure. After cooling the solution, additional dimethyl sulfate (30 g, 0.24 mol) was added to it at below 10 °C. The solution was stirred for 40 min, and additional potassium cyanide (15.6 g, 0.24 mol) was added. After heating at 55 °C, water (800 ml) was added to the reaction mixture, and the resulting nitrile was collected, washed with water and dispersed into a 0.01 N hydrochloric acid solution (1 liter). The nitrile was filtered, washed with water, and dried *in vacuo* at 60 °C to give 254.0 g (93.6% yield) of 4-methylindole-3-acetonitrile (**10**). Mp 104–105 °C; ^{1}H-NMR δ_H (acetone-d_6): 2.73 (3H, s), 4.17 (2H, d, $J = 1.0$ Hz), 6.81 (1H, ddq, $J = 7.1$, 1.0, 1.0 Hz), 7.01 (1H, dd, $J = 8.1$, 7.1 Hz), 7.26 (1H, d, $J = 8.1$ Hz), 7.35 (1H, broad s), 10.30 (1H, broad s); IR ν_{max}(KBr) cm^{-1}: 3396, 3055, 2966, 2932, 2249, 1614, 1545, 1504, 1460, 1427, 1416, 1340, 1252, 1159, 1123, 1059, 1026, 961, 918, 827, 781, 768. 758, 746, 638, 581, 529, 498; MS (relative intensity, %) *m/z*: 170 (M^+, 100), 169 (77), 155 (25), 144 (43), 143 (80), 142 (25), 130 (22), 115 (47), 89 (8), 63 (9). Found: C, 77.60; H, 5.84; N, 16.61%. Calcd. for $C_{11}H_{10}N_2$: C, 77.62; H, 5.92; N, 16.46%.

*4-Methylindole-3-acetic acid (**11**).* 4-Methylindole-3-acetonitrile (**10**, 247 g, 1.45 ml) was added to a mixed solution of a 48% sodium hydroxide solution (300 g, 3.6 mol), methanol (370 ml) and water (210 ml). After refluxing for 15.5 h, the methanol was removed *in vacuo*, water (600 ml) was added to the residual solution, and the aqueous solution was treated with activated charcoal (2.5 g). After filtration, the filtrate was acidified with hydrochloric acid, and the resulting crystals were filtered and washed with water. The wet crude crystals were dissolved in ethyl acetate (1.2-liter), and the ethyl acetate layer was washed with saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a crude indoleacetic acid. This acid was dispersed in a mixed solvent of ethyl acetate and *n*-hexane (1:1), cooled, filtered and dried *in vacuo* at 60 °C to give 261.9 g (95.4% yield) of 4-methylindole-3-acetic acid (**11**). Mp 158–159 °C; NMR δ_H (acetone-d_6): 2.65 (3H, s), 3.91 (2H, d, $J = 0.8$ Hz), 6.73 (1H, ddq, $J = 7.0$, 1.0, 1.0 Hz), 6.95 (1H, dd, $J = 8.0$, 7.0 Hz), 7.21 (1H, d, $J = 8.0$ Hz), 7.22 (1H, broad s), 10.08 (1H, broad s); IR ν_{max}(KBr) cm^{-1}: 3414, 3391, 2914, 1701, 1578, 1504, 1460, 1441, 1414, 1400, 1346, 1333, 1298, 1261, 1240, 1219, 1159, 1115, 1067, 934, 770, 748, 689, 656, 625, 527, 496; MS (relative intensity, %) *m/z*: 189 (M^+, 88), 145 (29), 144 (100), 143 (37), 142 (21), 130 (7), 115 (38), 91 (8), 89 (8), 72 (6), 63 (7). Found: C, 69.72 H; 5.72; N, 7.51%. Calcd. for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40%.

High-performance liquid chromatography (HPLC). A lipophilicity analysis of 4-CF_3-IAA, 4-CH_3-IAA, 4-Cl-IAA, IAA, and 4-(3-indole)butyric acid (IBA) was performed in a reversed-phase HPLC column containing Develosil C30-UG-5 (Nomura Chemical Co., Japan) with a solvent of CH_3CN:CH_3OH:H_2O:AcOH (5:5:10:0.1) at a flow rate of 1.0 ml/min and a UV detection wavelength of 280 nm.

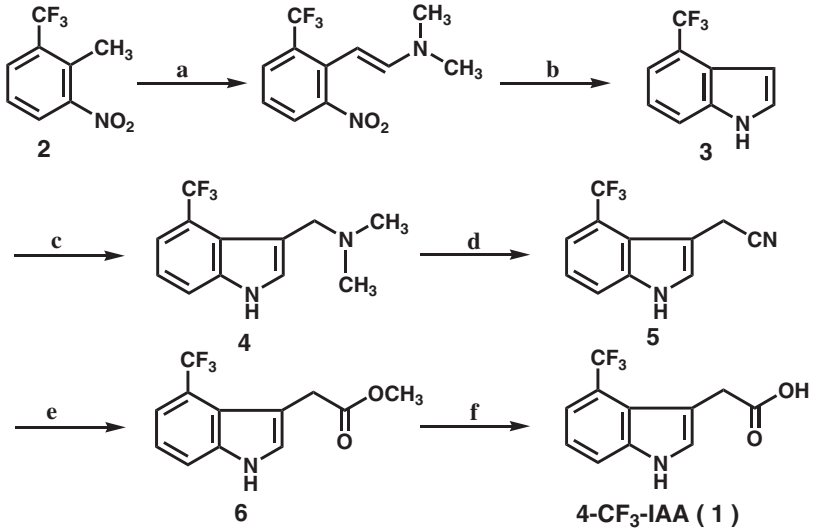

Fig. 1. Synthetic Scheme for 4-CF_3-IAA.
a, DMFDMA/DMF, 110 °C; b, Raney-nickel, H_2 (3 kgf/cm^2)/MeOH, THF; c, HCHO, Me_2NH, AcOH; d, CH_3I; KCN, MeOH, H_2O; e, CH_3OH, *p*-TsOH, H_2O; f, NaOH, MeOH, H_2O; H_3O^+.

Plant materials and bioassay. Chinese cabbage (*Brassica pekinensis* cv. Kinshu; Takii Seed Co., Ltd., Japan) and black gram (*Vigna mungo* (L.) Hepper; Sakata Seed Co., Japan) seeds, were stored at 5 °C and used in the auxin activity tests.

Hypocotyl growth inhibition test with Chinese cabbage, and hypocotyl swelling and lateral root formation tests with black gram. These tests were as described in a previous study.[4] Duplicate bioassays were conducted five times.

Root formation-promoting activity test with black gram cuttings. An ethanol solution (500 µl) of the sample (2×10^{-2} M) was placed in a deep Petri dish (6 cm × 6 cm i.d.) and evaporated *in vacuo* in a desiccator, after which 100 ml of distilled water containing 20 µl of a spreading agent (Dain; Sumika-Takeda Garden Co., Japan) was added. The aqueous solution was then sonicated for 10 min. The test procedure was conducted as described in a previous study.[8] Duplicate bioassays were conducted five times.

IBA and IAA were purchased from Kanto Kagaku Co. (Tokyo, Japan) and Merck Co., respectively, for use as standards.

Results and Discussion

The new fluoroindole auxin, 4-CF_3-IAA (**1**), was synthesized from 2-methyl-3-nitrobenzotrifluoride (**2**) by a procedure similar to that employed for the synthesis of 4-Cl-IAA and its ester;[4] the reactant was heated with *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) in dimethylformamide (DMF) to produce a dimethylaminostyrene derivative (Fig. 1). The product was subjected to reductive cyclization with Raney nickel-hydrogen in methanol to afford a trifluoromethylindole (**3**) which was converted to 4-trifluoromethylindole-3-acetonitrile (5) in a good yield. Since direct hydrolysis of the trifluoromethylindole-3-acetonitrile (**5**) to 4-CF_3-IAA (**1**) with an aqueous 40% potassium hydroxide solution gave overreacted products (not identified), the methyl ester (**6**) was prepared by methanolysis of the nitrile under acidic conditions and finally converted to 4-CF_3-IAA (**1**) by alkaline hydrolysis with 24% sodium hydroxide. The overall yield from six steps was approximately 40%. 4-CH_3-IAA was also synthesized by a procedure similar to that used in the synthesis of 4-CF_3-IAA to allow comparisons of the biological activities of 4-CF_3-IAA and 4-CH_3-IAA. Although 4-CH_3-IAA had already been synthesized from 4-methylindole as a starting material under severe conditions by Reinecke *et al.*, the overall yield was very low (<32%). We synthesized it in five steps from 2,3-dimethylnitrobenzene in about a 50% overall yield (66% from 4-methylindole by slightly improved procedure) (Fig. 2). The only difference in the synthetic procedure between 4-CF_3-IAA and 4-CH_3-IAA was that, for 4-CH_3-IAA, the final acid was obtained by alkaline hydrolysis of the nitrile.

The biological activities of 4-CF_3-IAA were determined by three bioassays: Chinese cabbage hypocotyl growth inhibition, black gram hypocotyl swelling and lateral root formation, and root formation with black gram cuttings. The activities of 4-CF_3-IAA were then compared to those of 4-CH_3-IAA, 4-Cl-IAA, IAA and IBA.

The results of the assays for hypocotyl growth inhibition using intact Chinese cabbage are shown in Fig. 3. 4-CF_3-IAA strongly inhibited the hypocotyl growth in Chinese cabbage. The inhibition activity of 4-CF_3-IAA was six times higher than that of IAA,

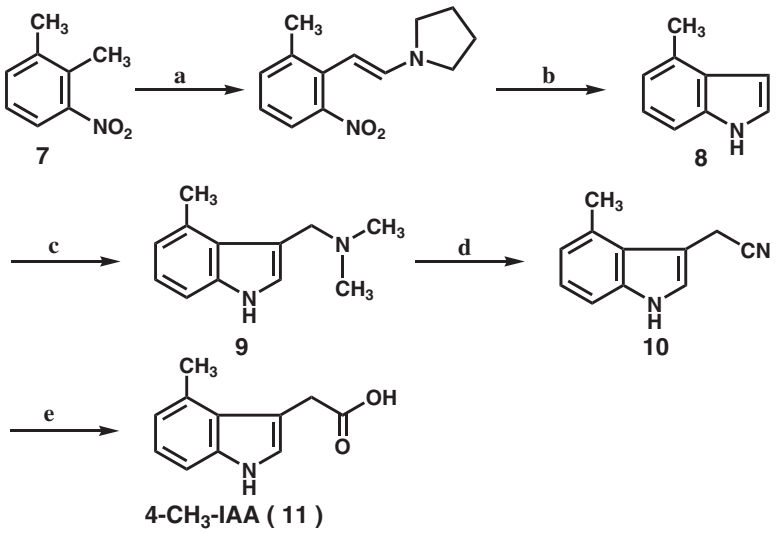

Fig. 2. Synthetic Scheme for 4-CH_3-IAA.
a, DMFDMA, pyrrolidine/DMF, 95 °C → 125 °C; b, Raney-nickel, H_2NNH_2-H_2O/THF; c, HCHO, Me_2NH, AcOH, THF/MeOH; d, $(CH_3)_2SO_4$/DMF; KCN/H_2O; e, NaOH, MeOH, H_2O; H_3O^+.

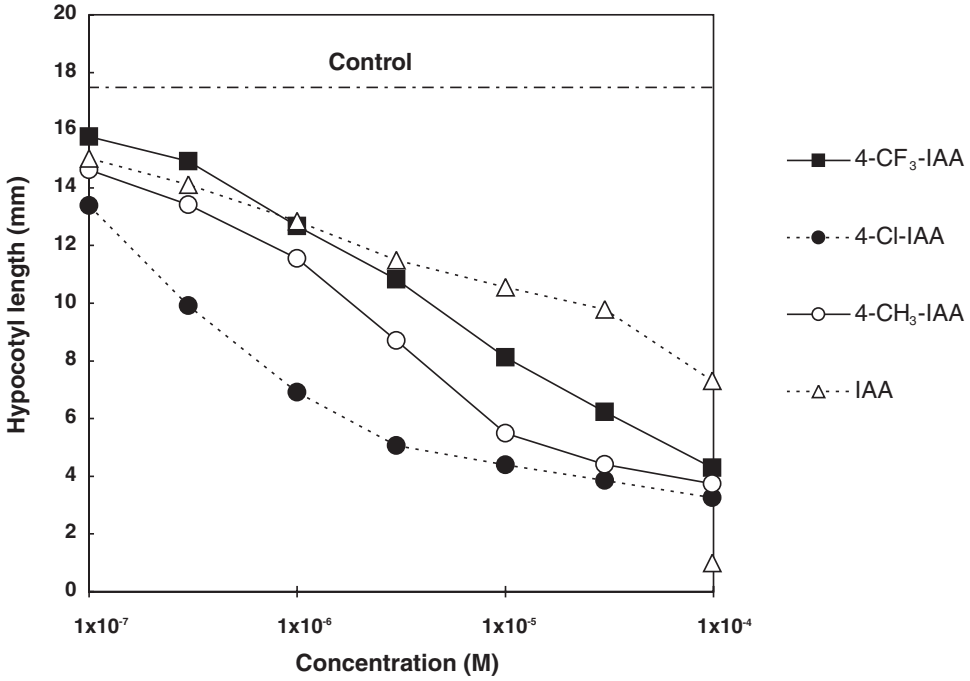

Fig. 3. Hypocotyl Growth Inhibition in Chinese Cabbage by 4-CF_3-IAA, 4-CH_3-IAA, 4-Cl-IAA and IAA.
Each value is shown as the mean ± SD, n = 5.

whereas it was 40% and 6% of that of 4-CH_3-IAA and 4-Cl-IAA, respectively. Thus, replacement of the methyl group in 4-CH_3-IAA with a trifluoromethyl moiety significantly decreased the inhibition activity observed with Chinese cabbage. Reinecke *et al.* have explained that the weaker activity of 4-ethylindole-3-acetic acid (4-Et-IAA) than the activity of 4-Cl-IAA was caused by steric problems entering the active site of the receptor in the elongation activity of pea pericarp.[23] The van der Waals volume of the trifluoromethylgroup is similar to that of the ethyl group, although of significantly different shape.[24,25] In the case of hypocotyl growth inhibition, the decrease in inhibition activity by replacement of the methyl group in 4-CH_3-IAA with a trifluoromethyl moiety may have been caused by the increase in steric hindrance to entering the active site of the receptor. The increased lipophilicity may benefit the inhibition activity of 4-Cl-IAA and 4-CH_3-IAA which can fit smoothly into the receptor site. The relative lipophilicity was determined from the retention time in a reversed-phase HPLC column as shown in Table 1; that is, the HPLC analysis showed that the lipophilicity was

Table 1. Retention Times for 4-CF_3-IAA, 4-CH_3-IAA, 4-Cl-IAA, IAA and IBA in the HPLC Analysis[a]

Compound	Retention time (min)
IAA[b]	6.15
4-CH_3-IAA	8.12
4-Cl-IAA	9.34
IBA[c]	11.07
4-CF_3-IAA	12.17

[a]The analytical conditions for HPLC were as follows: column, Develosil C30-UG-5, 250 mm × 4.6 mm i.d.; eluent, CH_3CN:CH_3OH:H_2O:AcOH = 5:5:10:0.1; flow rate, 1.0 ml/min; Detector, UV at 280 nm
[b]IAA, indole-3-acetic acid
[c]IBA, 4-(3-indole)butyric acid

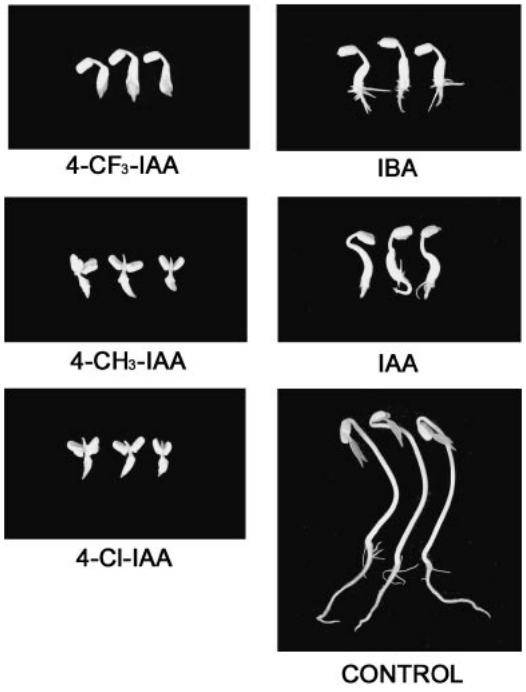

Fig. 4. Hypocotyl Swelling and Lateral Root Formation in Black Gram by 4-CF_3-IAA, 4-CH_3-IAA, 4-Cl-IAA and IAA (1×10^{-4} M concentration).

4-CF_3-IAA > IBA > 4-Cl-IAA > 4-CH_3-IAA > IAA. 4-CF_3-IAA is more lipophilic than IBA which has two methylene groups in the side chain of IAA. Furthermore, it shows that 4-Cl-IAA was more liphophilic than 4-CH_3-IAA as indicated in Table 1, although the increase of only the lipophilicity did not maximize the inhibition activity, since 4-CF_3-IAA had the highest lipophilicity and moderate inhibition activity. On the other hand the electron-withdrawing inductive effect may not be as important in the inhibition activity.

The results from the bioassay of hypocotyl swelling and lateral root formation in intact black gram seedlings are shown in Fig. 4. 4-CF_3-IAA enhanced hypocotyl swelling and lateral root formation. The activity of 4-CF_3-IAA was slightly higher than that of IAA, but much weaker than the activities of 4-Cl-IAA and 4-CH_3-IAA, for which the activities were about 300 and 100 times

Table 2. Root Formation-Promoting Activities of 4-CF_3-IAA, 4-CH_3-IAA, 4-Cl-IAA, IBA and IAA in Black Gram Cuttings[a]

Compound	Root number/cutting	Ratio to IBA (%)
4-CF_3-IAA (**1**)	21.5 ± 2.5	156 ± 12
4-CH_3-IAA (**11**)	9.7 ± 1.1	70 ± 11
4-Cl-IAA	53.0 ± 7.8	384 ± 15
IBA[b]	13.8 ± 1.4	100 ± 10
IAA[c]	4.9 ± 0.4	36 ± 8
Control	4.8 ± 0.4	35 ± 8

[a]All compounds were used at 1×10^{-4} M. IBA was used as a standard promoter of root formation. Each value is presented as the mean ± standard error of the mean.
[b]IBA, 4-(3-indole)butyric acid
[c]IAA, indole-3-acetic acid

greater than the activity of 4-CF_3-IAA, respectively; that is, equivalent degrees of hypocotyl swelling and lateral root formation were observed with 3×10^{-4} M 4-CF_3-IAA, 1×10^{-6} M 4-Cl-IAA, and 3×10^{-6} M 4-CH_3-IAA. 4-CF_3-IAA induced calluses to form on the black gram seedlings at concentrations of more than 3×10^{-3} M after only three days, whereas 4-Cl-IAA and 4-CH_3-IAA caused callus formation at a concentration threshold of 3×10^{-5} M. The activity strengths of these four compounds in black gram seedlings were similar to those observed for the inhibition of hypocotyl growth in Chinese cabbage. The difference in the hypocotyl swelling and lateral root formation activities of these auxins in intact black gram seedlings may have been for the same reason as that of the hypocotyl growth inhibition of Chinese cabbage.

The adventitious root formation-promoting activity of 4-CF_3-IAA in black gram cuttings is detailed in Table 2. 4-CF_3-IAA strongly promoted root formation at a concentration of 1×10^{-4} M. Its activity was 1.5 times greater than that of IBA, the active ingredient in commercially available root formation agents such as Seradix and Hormodin. It was also twice greater than the activity of 4-CH_3-IAA. Interestingly, however, 4-CH_3-IAA weakly promoted root formation, even though it strongly inhibited hypocotyl growth in Chinese cabbage, and induced hypocotyl swelling and lateral root formation in black gram; this contrasts with the other compounds that showed strong activity toward hypocotyl growth inhibition in Chinese cabbage, like the hypocotyl swelling and lateral root formation in black gram seedlings and root formation in black gram cuttings.[4,8] At 1×10^{-4} M, 4-CF_3-IAA and 4-Cl-IAA induced a number of adventitious roots all over the cutting stems that were soaked in solutions of these compounds, whereas IBA induced root formation only on the lower portions of the cutting stems; in particular, among the roots induced by 4-CF_3-IAA, a few adhered to each other to create a single intertwined root structure. 4-CF_3-IAA appeared to strongly stimulate the induction of root primordia all over the soaked cuttings, as has been observed for 4-Cl-IAA in cuttings

of gardenia, cosmos, *Rhododendron oomurasaki*, and *Serissa japonica*.[12,14] In regard to the root formation in black gram, the introduction of fluorine atoms onto the methyl group of 4-CH_3-IAA caused a dramatic increase in the promoting activity. It has been reported that the high auxin activity of 4-Cl-IAA may be ascribed to its low reactivity to peroxidase.[26] The electron-withdrawing effect caused a decrease of electron density in the indole nucleus to resist oxidative degradation of indole-3-acetic acid by the reaction at the 2 or 3 position of the indole nucleus with electrophilic oxgen and IAA oxidase or peroxidase.[27] Indeed, 4-CF_3-IAA was extremely stable to exposure to air on a silica gel thin layer in darkness at room temperature for 20 h, but most of 4-CH_3-IAA easily decomposed and was coloured dark yellow, the same as IAA did under the same conditions used in 4-CF_3-IAA (data not shown). 4-Cl-IAA was also stable to oxidation by air. The introduction of fluorine atoms and a chlorine atom strongly increased the stability to oxidation. The increase in the root formation-promoting activity in black gram cuttings by the introduction of fluorine atoms may have been due to the stability to oxidation that was brought by the electron-withdrawing inductive effect, but it did not maximize the promoting activity, since 4-CF_3-IAA had the highest electron-withdrawing group and moderate root formation-promoting activity. 4-Cl-IAA, which could fit smoothly into the active site of the receptor and also had the electron-withdrawing substituent, has the strongest root formation activity. On the other hand, 4-CH_3-IAA with weak root formation activity did not have the electron-withdrawing group, and reversely had the electron-donating one. To strengthen the root formation activity, the smooth fit into the active site of the receptor and the increased resistance to plant oxidation enzymes by the group with an electron-withdrawing inductive effect may have been important. The auxin activity of these compounds can also be influenced by such factors as the chemical stability and facility for transport and uptake, as well as the metabolic stability.

Acknowledgment

We thank Mr. Shigeyuki Kitamura of the Graduate School of Bioagricultural Sciences at Nagoya University for his help with the mass spectral measurements and elemental analyses. This research was partially supported by grant-aid from Japan Science and Technology Agency.

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