Biosci. Biotechnol. Biochem., **64** (4), 808–815, 2000

Synthesis and Biological Activities of 4-Chloroindole-3-acetic Acid and Its Esters

Masato KATAYAMA†

Laboratory of Bioorganic Chemistry, Department of Chemistry, National Industrial Research Institute of Nagoya, 1-1 Hirate-cho, Kita-ku, Nagoya 462-8510, Japan

Received November 18, 1999; Accepted December 15, 1999

4-Chloroindole-3-acetic acid (4-Cl-IAA) and its esters were synthesized from 2-chloro-6-nitrotoluene as the starting material. The biological activities of 4-Cl-IAA and its esters were determined by four bioassays. Except for the *tert*-butyl ester, 4-Cl-IAA and its esters had stronger elongation activity toward *Avena* coleoptiles than had indole-3-acetic acid. The biological activities of the methyl, ethyl and allyl esters were as strong as the activity of the free acid. All the esters, except for the *tert*-butyl, inhibited Chinese cabbage hypocotyl growth more than the free acid did, and all the esters induced severe swelling and formation of numerous lateral roots in black gram seedlings even at a low concentration. Furthermore, adventitious root formation was strongly promoted in *Serissa japonica* cuttings by all the esters. The root formation-promoting activities of the ethyl and allyl esters were about three times the value for indole-3-butyric acid which is used to promote and accelerate root formation in plant cuttings.

Key words: 4-chloroindole-3-acetic acid esters; chlorinated auxins; *Avena* coleoptile elongation; hypocotyl growth inhibition; adventitious root formation

Methyl 4-chloroindole-3-acetate, a chlorinated auxin, was initially isolated from immature *Pisum sativum* seeds.[1,2] 4-Chloroindole-3-acetic acid (4-Cl-IAA, **1**), the free acid, was later isolated from pea seeds.[3] 4-Cl-IAA has been detected in immature seeds of many species of *Vicieae* besides *Pisum sativum*.[4] The natural occurrence of 4-Cl-IAA was assumed to be limited to the *Vicieae*, but Ernstsen and Sandberg[5] identified it in both immature and mature seeds of *Pinus sylvestris*, a gymnosperm taxonomically distant from the *Vicieae* of Leguminosae.

4-Cl-IAA is characterized by its exceptionally strong biological activity as compared to indole-3-acetic acid (IAA), producing a ten-fold increase in *Avena* coleoptile elongation, a 1000-fold inhibition of Chinese cabbage hypocotyl growth, and more than a 100-fold increase in mung bean hypocotyl swelling and lateral root formation.[6–8] The high biological activities of 4-Cl-IAA relative to those of IAA have been attributed to its resistance to peroxidase oxidation.[6]

4-Cl-IAA has been synthesized by Fischer indolization,[9] as a product of research on novel synthetic methodologies for substituted indole compounds by using the Sandmeyer or Schiemann reaction,[10] and *via* directed lithiation of a gramine compound.[11] However, neither the practical synthesis of 4-Cl-IAA and its derivatives nor their biological activities toward important plant species have been reported. A practical method for the synthesis of 4-Cl-IAA and its esters is reported here, and an examination of their biological activities is discussed.

Materials and Methods

Instrumentation. Melting point (mp) data were determined with a Büchi 535 melting point apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded with a Bruker AMX360Wb spectrometer (360 MHz). Low-resolution electron-impact mass spectra were recorded by a JEOL DX-705L instrument with direct probe insertion. Infrared (IR) spectra were recorded by a JASCO IR-810 spectrometer as wave numbers (cm^{-1}). Elemental analyses were conducted with a Perkin-Elmer 2400 CHN elemental analyzer.

Bioassay. Seeds of *Avena* (*Avena sativa* cv. Victory-1), Chinese cabbage (*Brassica pekinensis* cv. Kinshu) and black gram (*Vigna mungo*, Sakata Seed Co. Japan) which had been stored at 5°C in a refrigerator were used in the subsequent tests.

Avena elongation test. Coleoptile segments (5 mm long) were excised 2 mm below the tip of *Avena sativa* cv. Victory-1 seedlings, grown under red light for two days and then in the dark for one day, after which they were incubated in the dark at 25°C with slow agitation in a buffer solution (2 ml) containing the test compound and 2% sucrose. After 20 hr, the elongated coleoptiles were copied by using a Xerox

† To whom correspondence should be addressed. Tel: +81-52-911-3059; Fax: +81-52-911-2428; E-mail: katayama@nirin.go.jp

machine on a glass plate with 2-fold expansion. The increase in length of the copied coleoptiles was measured with an elongation-length data analyzer equipped with a CCD monochrome video camera module. As the control, segments were incubated in the buffer solution without a test compound, and treated by the same procedure as that used with the test compound.

Hypocotyl growth inhibition test on Chinese cabbage. Chinese cabbage seeds washed thoroughly in running water were placed on cotton soaked with distilled water in a Petri dish (ϕ 15×3 cm) and kept for one day in the dark at 25°C in a growth chamber (Shimadzu BITEC-400L). Ten of the germinated seeds were incubated on filter paper (ϕ 5.5 cm) soaked in an aqueous solution of the test compound (this had been prepared by removing the solvent from the ethanol solution (500 μl) of the test compound and then adding 5 ml of distilled water) in a Petri dish (ϕ 6×1.8 cm) at 25°C in the dark. After 72 hr, the hypocotyl length was measured. As the control, the germinated seeds were incubated on filter paper soaked with distilled water after removing the solvent from the ethanol solution and without a test compound, as in the procedure used with the test compound.

Hypocotyl swelling and lateral root formation test on black gram. Black gram seeds washed thoroughly in running water were placed on cotton soaked with distilled water in a Petri dish (ϕ 15×3 cm) and kept for one day in the dark at 25°C in a growth chamber. Ten of the germinated seeds were incubated on filter paper (ϕ 5.5 cm) soaked with an aqueous solution (5 ml) of the test compound (this had been prepared by removing the solvent from the ethanol solution (500 μl) of the test compound and then adding 5 ml of distilled water) in a Petri dish (ϕ 6×1.8 cm) at 25°C in the dark. After 72 hr, hypocotyl swelling and lateral root formation were evident. As the control, the germinated seeds were incubated on filter paper soaked with distilled water after removing the solvent from the ethanol solution and without the test compound, and treated in the same way as that used for the test compound.

Adventitious root formation-promotion test on Serissa japonica. Twenty-two-centimeter-long cuttings with pairs of leaves at only three nodes of the newly grown part of *Serissa japonica* seedlings were prepared. The stems, including five nodes, were soaked to 4 cm from the cut end in an ethanol solution for exactly ten seconds, after which the ethanol was removed by a stream of cool air from a dryer. The cuttings were planted on a bed of 'Akadama' soil (Fujimi Gardening Materials Company, Shizuoka, Japan) which had been thoroughly dampened with distilled water. After receiving water every fourth day for 68 days and being kept at 23°C in the day time and at 15°C during the night in a greenhouse, the number of generated roots was counted, and their weight measured after drying the roots in a dessicator for 2 weeks. As the control, cuttings were soaked in an ethanol solution without the test compound for exactly ten seconds, after which the procedure used for the test compounds was followed.

Synthetic procedure.

(E)-2-Chloro-β-dimethylamino-6-nitrostyrene (**3**). A 267.3 g (2.24 mol) portion of *N,N*-dimethylformamide dimethyl acetal was added to a solution of 296.1 g (1.72 mol) of 2-chloro-6-nitrotoluene (**2**) in 500 ml of dimethylformamide (DMF). The mixture was heated at reflux (110°C) for 80 hr under nitrogen and then allowed to cool to room temperature, after which it was concentrated *in vacuo* to 200 ml of a DMF solution. This crude styrene was dissolved in diethyl ether. The ethereal solution was successively washed with distilled water and saturated brine, dried over anhydrous sodium sulfate, and then evaporated *in vacuo* to dryness to give 388.4 g of crude **3**. This styrene was used in the next reaction without purification.

Chloroindole (**4**). Raney nickel (34 ml) was added to a stirred solution of 385.0 g (1.70 mol) of **3** in 1 l of tetrahydrofuran (THF) and 1.2 l of methanol at 5°C under nitrogen, before adding 320 ml of 85% hydrazine hydrate while cooling in an ice-bath. During this addition, the temperature was maintained below 30°C by cooling with an ice-water mixture. After standing at 10–15°C for 2 hr and at room temperature overnight, the reaction mixture was filtered through Celite, giving a dark brown filtrate. This filtrate was concentrated *in vacuo*, giving 275.0 g of crude dark-brown oil which was chromatographed in a silica gel column with *n*-hexane as the eluting solvent to afford 243.3 g (93.8% from 2-chloro-6-nitrotoluene) of **4** as pale light yellow-green oil. NMR δ_H (An-d_6): 6.58 (1H, ddd, J=3.2, 2.1, 0.9 Hz), 7.07 (1H, d, J=3.2 Hz), 7.08 (1H, dd, J=9.9, 7.6 Hz), 7.38–7.44 (2H, m), 10.57 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3430, 3110, 1615, 1570, 1500, 1485, 1430, 1410, 1335, 1265, 1190, 1150, 1105, 1075, 1055, 915, 900, 825, 785, 750, 725, 620, 580, 520; MS (relative intensity, %) m/z: 153 (41), 151 (M^+, 100), 124 (8), 123 (4), 116 (13), 115 (4), 89 (20), 76 (3). *Anal.* Found: C, 63.37; H, 4.13; N, 9.37%. Calcd. for C_8H_6ClN: C, 63.37; H, 3.96; N, 9.24%.

4-Chloro-3-diethylaminomethylindole (**5**). A portion of 37% aqueous formaldehyde (105.4 g, 1.30 mol) was added to a mixture of 95.8 g (99%) of diethylamine and 350 ml of acetic acid, that had been prepared below 5°C. This mixture was poured into **4** (178.6 g, 1.18 mol) in an ice-bath. After stirring at 5°C for 10 min and then at room temperature for 4

hr, the reaction mixture was acidified with a 4 N-hydrochloric acid solution and treated three times with ethyl acetate. The aqueous layer was made alkaline with a 4 N-sodium hydroxide solution and then treated three times with ethyl acetate. The ethyl acetate layer was successively washed with water and saturated brine, dried over anhyrous sodium sulfate, and then evaporated *in vacuo*, giving a white powder which was recrystallized from ethyl acetate-*n*-hexane affording 201.3 g (72.2% yield) of **5**. Mp 130–131°C; NMR δ_H (An-d_6): 1.03 (6H, t, J=7.1 Hz), 2.60 (4H, q, J=7.1 Hz), 3.96 (2H, d, J=1.0 Hz), 6.98 (1H, dd, J=7.6, 1.3 Hz), 7.03 (1H, ddd, J=7.9, 7.6, 0.2 Hz), 7.33 (1H, broad d, J=2.4 Hz), 7.35 (1H, dd, J=7.9, 1.3 Hz), 10.40 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3430, 2970, 2820, 1615, 1570, 1550, 1490, 1470, 1430, 1375, 1350, 1340, 1265, 1190, 1155, 1120, 1080, 1055, 1030, 975, 935, 850, 815, 760, 735, 580; MS (relative intensity, %) m/z: 238 (7), 236 (M^+, 22), 223 (3), 221 (8), 195 (4), 166 (39), 164 (100), 128 (15), 101 (10), 72 (12). *Anal.* Found: C, 65.95; H, 7.29; N, 12.02%. Calcd. for $C_{13}H_{17}ClN_2$: C, 65.96; H, 7.19; N, 11.84%.

4-Chloroindole-3-acetonitrile (**6**). A solution of **5** (123.0 g, 0.52 mol) in dimethylformamide (1100 ml) was added to a solution of potassium cyanide (101.4 g, 1.56 mol) in water (550 ml), and the mixture refluxed for 6 hr while stirring. After being cooled to room temperature and water added, the mixture was treated three times with diethyl ether. The ethereal layer was successively washed with water and saturated brine, dried over anhyrous sodium sulfate, and evaporated *in vacuo* to yield a crude nitrile which was purified in a silica gel column with an eluting solvent of 20% ethyl acetate in *n*-hexane, before being recrystallized from ethyl acetate-*n*-hexane to 89.5 g (87.3% yield) of **6**. Mp 135–136°C; NMR δ_H (An-d_6): 4.20 (2H, d, J=0.9 Hz), 7.08 (1H, dd, J=7.6, 1.1 Hz), 7.13 (1H, dd, J=7.9, 7.6 Hz), 7.42 (1H, dd, J=7.9, 1.1 Hz), 7.50 (1H, dt, J=2.6, 0.9 Hz), 10.67 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3400, 3120, 2920, 2240, 1645, 1610, 1575, 1545, 1485, 1435, 1335, 1310, 1200, 1190, 1100, 1040, 935, 910, 825, 780, 750, 730, 650, 615, 555; MS (relative intensity, %) m/z: 192 (31), 190 (M^+, 100), 166 (14), 164 (43), 155 (66), 128 (14), 127 (16), 126 (14), 101 (12), 100 (11), 99 (10), 77 (16), 75 (14). *Anal.* Found: C, 62.98; H, 3.65; N, 14.82%. Calcd. for $C_{10}H_7ClN_2$: C, 62.99; H, 3.67; N, 14.70%.

4-Chloroindole-3-acetic acid (**1**). A 300-ml portion of a 40% potassium hydroxide solution was added to a solution of **6** (70.9 g, 0.36 mol) in methanol (500 ml). After being refluxed for 16 hr, the reaction mixture was condensed to an aqueous solution. This solution was treated three times with diethyl ether. The aqueous layer was acidified with a 6 N-hydrochloric acid solution and treated four times with ethyl acetate. The ethyl acetate layer was successively washed with distilled water and saturated brine, dried over anhydrous sodium sulfate, and then evaporated *in vacuo* to give a crude yellow powder which was purified in a silica gel column and recrystallized from ethyl acetate-*n*-hexane to yield 70.8 g (94.1% yield) of **1**. Mp 179–180°C; NMR δ_H (An-d_6): 4.00 (2H, d, J=0.8 Hz), 6.99 (1H, dd, J=7.6, 1.1 Hz), 7.05 (1H, dd, J=7.9, 7.6 Hz), 7.35 (1H, broad d, J=2.4 Hz), 7.36 (1H, dd, J=7.9, 1.1 Hz), 10.43 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3375, 2920, 1700, 1620, 1490, 1425, 1400, 1335, 1300, 1260, 1215, 1205, 1180, 1080, 1050, 930, 900, 815, 810, 765, 735, 590, 570; MS (relative intensity, %) m/z: 211 (16), 209 (M^+, 52), 166 (47), 164 (100), 129 (14), 128 (21), 102 (16), 101 (19), 75 (13). *Anal.* Found: C, 57.29; H, 3.60; N, 6.72%. Calcd. for $C_{10}H_8ClNO_2$: C, 57.28; H, 3.82; N, 6.68%.

Methyl 4-chloroindole-3-acetate (**7a**). Methanol (2.3 g, 71.8 mmol), *N*,*N*′-dicyclohexylcarbidiimide (7.4 g, 35.9 mmol) and *N*,*N*-dimethylaminopyridine (0.3 g, 2.5 mmol) were added to a solution of **1** (5.0 g, 23.8 mmol) in methylene chloride (100 ml), and the mixture stirred for 2 hr. After filtrating off the precipitated urea, the solvent was removed *in vacuo* to give a crude ester which was purified in a silica gel column with methylene chloride as the eluting solvent and then recrystallized from ethyl acetate-*n*-hexane to afford 3.9 g (73.5% yield) of **7a**. Mp 120–121°C; NMR δ_H (An-d_6): 3.65 (3H, s), 3.99 (2H, d, J=0.7 Hz), 6.99 (1H, dd, J=7.6, 1.1 Hz), 7.06 (1H, ddd, J=8.0, 7.6, 0.2 Hz), 7.34 (1H, broad d, J=2.4 Hz), 7.37 (1H, dd, J=8.0, 1.1 Hz), 10.46 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3325, 2940, 1720, 1620, 1570, 1550, 1490, 1440, 1345, 1290, 1270, 1250, 1185, 1050, 1015, 940, 845, 820, 760, 740, 670, 590, 425; MS (relative intensity, %) m/z: 225 (13), 223 (M^+, 40), 166 (41), 164 (100), 129 (9), 128 (16), 102 (11), 101 (14), 75 (8). *Anal.* Found: C, 58.99; H, 4.47; N, 6.26%. Calcd. for $C_{11}H_{10}ClNO_2$: C, 59.06; H, 4.47; N, 6.26%.

Ethyl 4-chloroindole-3-acetate (**7b**, Typical procedure). Ethanol (0.66 g, 14.31 mmol), *N*,*N*′-dicyclohexylcarbidiimide (1.47 g, 7.15 mmol) and *N*,*N*-dimethylaminopyridine (60 mg, 0.5 mmol) were added to a solution of **1** (1.00 g, 4.77 mmol) in methylene chloride (20 ml). The resulting mixture was stirred for 4 hr, and after filtrating off the precipitated urea, the solvent was removed *in vacuo* to give a crude ester which was purified in a silica gel column with methylene chloride as the eluting solvent. The ester was recrystallized from ethyl acetate-*n*-hexane, affording 840 mg (74.4% yield) of **7b**. Mp 79–80°C; NMR δ_H (An-d_6): 1.12 (3H, t, J=7.1 Hz), 3.98 (2H, d, J=0.8 Hz), 6.99 (1H, dd, J=7.6, 1.1 Hz), 7.05 (1H, ddd, J=8.0, 7.6, 0.2 Hz), 7.34 (1H, broad d, J=2.5 Hz), 7.37 (1H, dd, J=8.0, 1.1 Hz), 10.44 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3325, 2975, 1735, 1620, 1570, 1555, 1485, 1435, 1405, 1370, 1340, 1250, 1210, 1185, 1140, 1110, 1080, 1045, 1015, 935,

875, 815, 760, 735, 680, 595; MS (relative intensity, %) m/z: 239 (12), 237 (M^+, 37), 166 (44), 164 (100), 129 (9), 128 (16), 102 (12), 101 (13), 75 (8). *Anal.* Found: C, 60.63; H, 5.00; N, 5.92%. Calcd. for $C_{12}H_{12}ClNO_2$: C, 60.63; H, 5.05; N, 5.89%.

The synthetic procedure used for the following esters was the same as that used for the synthesis of **7b**.

1-Propyl 4-chloroindole-3-acetate (**7c**). 65.5% yield; mp 83–84°C; NMR δ_H (An-d_6): 0.90 (3H, t, J=7.4 Hz), 1.63 (2H, qt, J=7.4, 6.6 Hz), 3.99 (2H, d, J=0.7 Hz), 4.04 (2H, t, J=6.6 Hz), 6.99 (1H, dd, J=7.6, 1.0 Hz), 7.05 (1H, dd, J=8.0, 7.6 Hz), 7.34 (1H, broad d, J=2.5 Hz), 7.37 (1H, dd, J=8.0, 1.0 Hz), 10.45 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3290, 2980, 2970, 2925, 1730, 1620, 1570, 1490, 1440, 1410, 1395, 1340, 1255, 1220, 1195, 1175, 1155, 1145, 1080, 1055, 1045, 965, 940, 920, 820, 780, 760, 740; MS (relative intensity, %) m/z: 253 (12), 251 (M^+, 37), 223 (4), 166 (47), 164 (100), 129 (7), 128 (12), 102 (8), 101 (9), 75 (4). *Anal.* Found: C, 62.00; H, 5.32; N, 5.64%. Calcd. for $C_{13}H_{14}ClNO_2$: C, 62.03; H, 5.57; N, 5.57%.

2-Propyl 4-chloroindole-3-acetate (**7d**). 80.1% yield; mp 100–101°C; NMR δ_H (An-d_6): 1.22 (6H, d, J=6.3 Hz), 3.94 (2H, d, J=0.8 Hz), 4.99 (1H, septet, J=6.3 Hz), 6.99 (1H, J=7.6, 1.1 Hz), 7.05 (1H, ddd, J=8.0, 7.6, 0.2 Hz), 7.33 (1H, broad d, J=2.4 Hz), 7.37 (1H, dd, J=8.0, 1.1 Hz), 10.44 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3300, 2980, 1715, 1615, 1565, 1485, 1430, 1365, 1340, 1325, 1250, 1200, 1170, 1140, 1100, 955, 930, 810, 760, 730, 600; MS (relative intensity, %) m/z: 253 (8), 251 (M^+, 25), 166 (31), 164 (100), 129 (5), 128 (9), 102 (6), 101 (7), 75 (4). *Anal.* Found: C, 61.98; H, 5.57; N, 5.57%. Calcd. for $C_{13}H_{14}ClNO_2$: C, 62.03; H, 5.57; N, 5.57%.

Allyl 4-chloroindole-3-acetate (**7e**). 78.7% yield; mp 84–85°C; NMR δ_H (An-d_6): 4.04 (2H, d, J=0.8 Hz), 4.60 (2H, ddd, J=5.5, 1.7, 1.4 Hz), 5.17 (1H, ddt, J=10.5, 1.7, 1.4 Hz), 5.29 (1H, ddt, J=17.2, 1.7, 1.7 Hz), 5.95 (1H, ddt, J=17.2, 10.5, 5.5 Hz), 7.00 (1H, dd, J=7.6, 1.1 Hz), 7.06 (1H, ddd, J=8.0, 7.6, 0.2 Hz), 7.35 (1H, broad d, J=2.5 Hz), 7.37 (1H, dd, J=8.0, 1.1 Hz), 10.46 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3320, 1730, 1645, 1620, 1565, 1550, 1495, 1435, 1405, 1370, 1335, 1250, 1185, 1140, 1110, 1045, 975, 935, 910, 815, 765, 735, 690, 590; MS (relative intensity, %) m/z: 251 (15), 249 (M^+, 45), 166 (47), 164 (100), 129 (8), 128 (13), 102 (9), 101 (11), 75 (5). *Anal.* Found: C, 62.51; H, 4.83; N, 5.52%. Calcd. for $C_{13}H_{12}ClNO_2$: C, 62.53; H, 4.81; N, 5.61%.

1-Butyl 4-chloroindole-3-acetate (**7f**). 73.8% yield; mp 75–76°C; NMR δ_H (An-d_6): 0.89 (3H, t, J=7.4 Hz), 1.30–1.41 (2H, m), 1.55–1.64 (2H, m), 3.98 (2H, d, J=0.8 Hz), 4.09 (2H, t, J=6.6 Hz), 6.99 (1H, dd, J=7.6, 1.1 Hz), 7.05 (1H, ddd, J=8.0, 7.6, 0.2 Hz), 7.33 (1H, broad d, J=2.5 Hz), 7.37 (1H, dd, J=8.0, 1.1 Hz), 10.44 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3310, 2940, 2850, 1720, 1615, 1565, 1545, 1480, 1465, 1430, 1390, 1355, 1335, 1180, 1105, 1080, 1020, 970, 930, 805, 750, 730, 675, 585; MS (relative intensity, %) m/z: 267 (11), 265 (M^+, 35), 166 (40), 164 (100), 129 (5), 128 (9), 102 (6), 101 (7), 75 (3). *Anal.* Found: C, 63.28; H, 6.11; N, 5.26%. Calcd. for $C_{14}H_{16}ClNO_2$: C, 63.28; H, 6.03; N, 5.27%.

2-Butyl 4-chloroindole-3-acetate (**7g**). 76.3% yield; mp 114–115°C; NMR δ_H (An-d_6): 0.87 (3H, t, J=7.5 Hz), 1.19 (3H, d, J=6.3 Hz), 1.48–1.65 (2H, m), 3.96 (1H, dd, J=17.4, 0.8 Hz), 3.97 (1H, dd, J=17.4, 0.8 Hz), 4.83 (1H, tq, J=5.7, 6.3 Hz), 6.99 (1H, dd, J=7.6, 1.1 Hz), 7.05 (1H, ddd, J=8.0, 7.6, 0.2 Hz), 7.33 (1H, broad d, J=2.5 Hz), 7.37 (1H, dd, J=8.0, 1.1 Hz), 10.43 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3280, 2970, 2920, 1725, 1620, 1570, 1490, 1435, 1405, 1375, 1335, 1255, 1215, 1195, 1110, 1080, 1045, 995, 945, 885, 815, 775, 750, 735; MS (relative intensity, %) m/z: 267 (11), 265 (M^+, 34), 166 (41), 164 (100), 129 (6), 128 (10), 102 (6), 101 (8), 75 (3). *Anal.* Found: C, 63.44; H, 6.11; N, 5.29%. Calcd. for $C_{14}H_{16}ClNO_2$: C, 63.28; H, 6.03; N, 5.27%.

Isobutyl 4-chloroindole-3-acetate (**7h**). 69.8% yield; mp 109–110°C; NMR δ_H (An-d_6): 0.89 (6H, d, J=6.7 Hz), 1.91 (1H, t of septet, J=6.6, 6.7 Hz), 3.87 (2H, d, J=6.6 Hz), 4.00 (2H, d, J=0.8 Hz), 6.99 (1H, dd, J=7.6, 1.1 Hz), 7.05 (1H, ddd, J=8.0, 7.6, 0.2 Hz), 7.34 (1H, broad d, J=2.5 Hz), 7.37 (1H, dd, J=8.0, 1.1 Hz), 10.44 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3300, 2960, 1720, 1620, 1570, 1485, 1470, 1430, 1375, 1355, 1340, 1250, 1205, 1170, 1140, 1045, 1000, 935, 810, 765, 740, 590; MS (relative intensity, %) m/z: 267 (8), 265 (M^+, 25), 166 (31), 164 (100), 129 (5), 128 (9), 102 (6), 101 (7), 75 (3). *Anal.* Found: C, 63.29; H, 6.13; N, 5.29%. Calcd. for $C_{14}H_{16}ClNO_2$: C, 63.28; H, 6.03; N, 5.27%.

tert-Butyl 4-chloroindole-3-acetate (**7i**). 21.1% yield; mp 142–143°C; NMR δ_H (An-d_6): 1.44 (9H, s), 3.89 (2H, d, J=0.8 Hz), 6.99 (1H, dd, J=7.6, 1.1 Hz), 7.05 (1H, ddd, J=7.9, 7.6, 0.2 Hz), 7.31 (1H, broad d, J=2.3 Hz), 7.36 (1H, dd, J=7.9, 1.1 Hz), 10.42 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3335, 3000, 2985, 1725, 1620, 1560, 1490, 1440, 1410, 1370, 1360, 1340, 1300, 1260, 1220, 1160, 1120, 1080, 1050, 950, 935, 865, 820, 755, 735, 600, 570; MS (relative intensity, %) m/z: 267 (13), 265 (M^+, 40), 209 (9), 166 (53), 164 (100), 129 (10), 128 (17), 102 (10), 101 (12), 57 (40); HRMS m/z (M^+): Calcd. for $C_{14}H_{16}ClNO_2$: 265.0870, Found: 265.0871.

In the process of synthesizing this ester, by-products were produced, and the ester and by-products gradually decomposed under the reaction conditions after standing for several hours.

1-Pentyl 4-chloroindole-3-acetate (**7j**). 72.8% yield; mp 70–71°C; NMR δ_H (An-d_6): 0.86 (3H, t, J=7.0 Hz), 1.25–1.35 (4H, m), 1.55–1.67 (2H, m), 3.98 (2H, d, J=0.8 Hz), 4.08 (2H, t, J=6.6 Hz),

6.99 (1H, dd, $J=7.6$, 1.1 Hz), 7.05 (1H, ddd, $J=8.0$, 7.6, 0.2 Hz), 7.33 (1H, broad d, $J=2.4$ Hz), 7.37 (1H, dd, $J=8.0$, 1.1 Hz), 10.45 (1H, broad s); IR ν_{max} (KBr) cm^{-1}: 3330, 2950, 2925, 1715, 1620, 1570, 1490, 1435, 1410, 1390, 1355, 1335, 1300, 1255, 1200, 1180, 1045, 975, 935, 835, 815, 760, 735, 590, 570; MS (relative intensity, %) m/z: 281 (12), 279 (M^+, 35), 209 (2), 166 (37), 164 (100), 129 (5), 128 (8), 102 (5), 101 (6), 75 (2). *Anal.* Found: C, 64.31; H, 6.43; N, 4.97%. Calcd. for $C_{15}H_{18}ClNO_2$: C, 64.40; H, 6.44; N, 5.01%.

Results and Discussion

2-Chloro-6-nitrotoluene (**2**) was used as the starting material for 4-Cl-IAA synthesis (Fig. 1). It was heated with *N*,*N*-dimethylformamide dimethyl acetal in DMF to give **3**, which was then subjected to reductive cyclization, according to Batcho and Leimgruber,[12] with Raney nickel-hydrazine in a mixture of tetrahydrofuran and methanol, affording a high yield of **4** (94% from 2-chloro-6-nitrotoluene). Compound **4** was converted to 4-Cl-IAA (**1**) *via* **6** in a good yield (59% in 3 steps). With the exception of the *tert*-butyl ester, all the 4-Cl-IAA esters (**7a–7j**) were easily prepared in good yields (65–80%) by coupling 4-Cl-IAA with the appropriate alcohol in the presence of *N*,*N*′-dicyclohexylcarbodiimide and *N*,*N*-dimethylaminopyridine.

The biological activities of synthetic 4-Cl-IAA and its esters were determined by four bioassays: *Avena* coleptile elongation, Chinese cabbage hypocotyl growth inhibition, black gram hypocotyl swelling and lateral root formation, and *Serissa japonica* adventitious root formation. The biological activities of the esters were compared to those of IAA and indole-3-butyric acid (IBA). Untreated seedlings and cuttings comprised the control group.

The bioassay results for *Avena sativa* coleoptile elongation are shown in Fig. 2. The methyl, ethyl, allyl, and 2-propyl esters had maximal activity at 3×10^{-7} M, the lowest concentration tested, as did the original free acid, 4-Cl-IAA. The methyl, ethyl, and allyl esters, in particular, had more potent elongation activities than 4-Cl-IAA, being approximately 10 times that of IAA. The elongation activities of the 1-propyl, 1-butyl, 2-butyl, isobutyl, and 1-pentyl esters were between those of 4-Cl-IAA and IAA. The *tert*-butyl ester, which had bulkiness, had inhibitory activity. This inhibition may have been caused by the degraded product of this ester (see the Materials and Methods section). The esters with a short alkyl chain at the alcohol end were more potent for *Avena* coleoptile elongation than those with longer end chains, indicative that an increase in the length of the carbon chain at the alcohol end decreased the activity of the 4-Cl-IAA esters for *Avena* coleoptile elongation.

All the esters, except the *tert*-butyl one, showed strong inhibitory activity toward the hypocotyl growth of intact seedlings of Chinese cabbage (Fig. 3), ranging from about 300 to 1000 times that of IAA, and all, except the *tert*-butyl one, showed more potent inhibition than the original free acid, 4-Cl-IAA. Five esters (methyl, ethyl, 1-propyl, allyl, and 1-butyl) had particularly strong inhibitory activities of similar magnitude toward the Chinese cabbage seedlings. The inhibitory activity of the 1-propyl ester

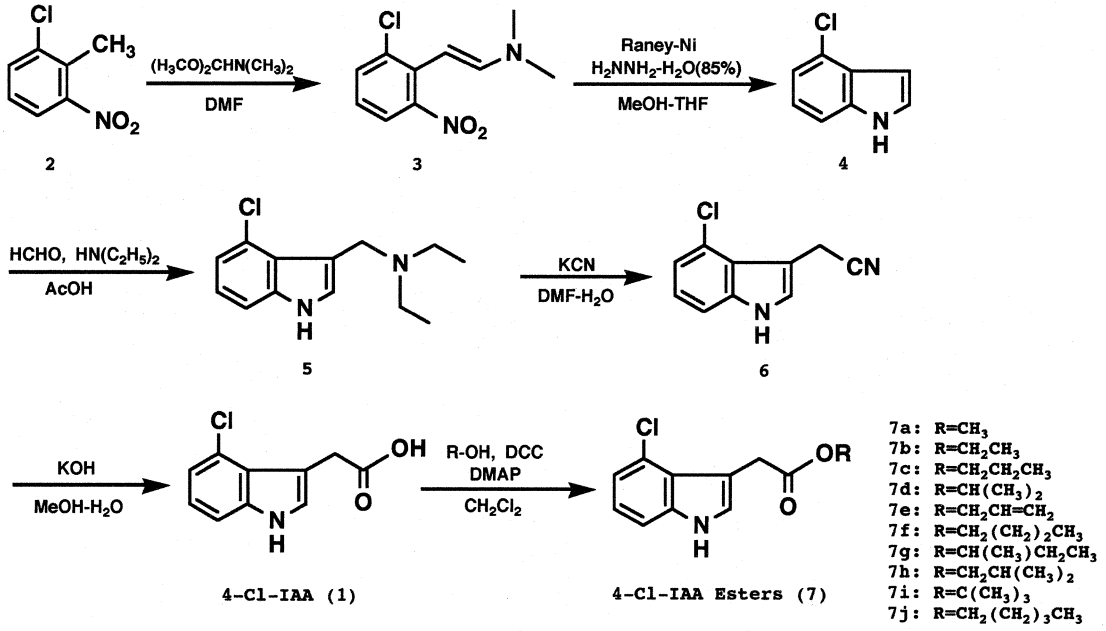

Fig. 1. Synthesis of 4-Cl-IAA and Its Esters.

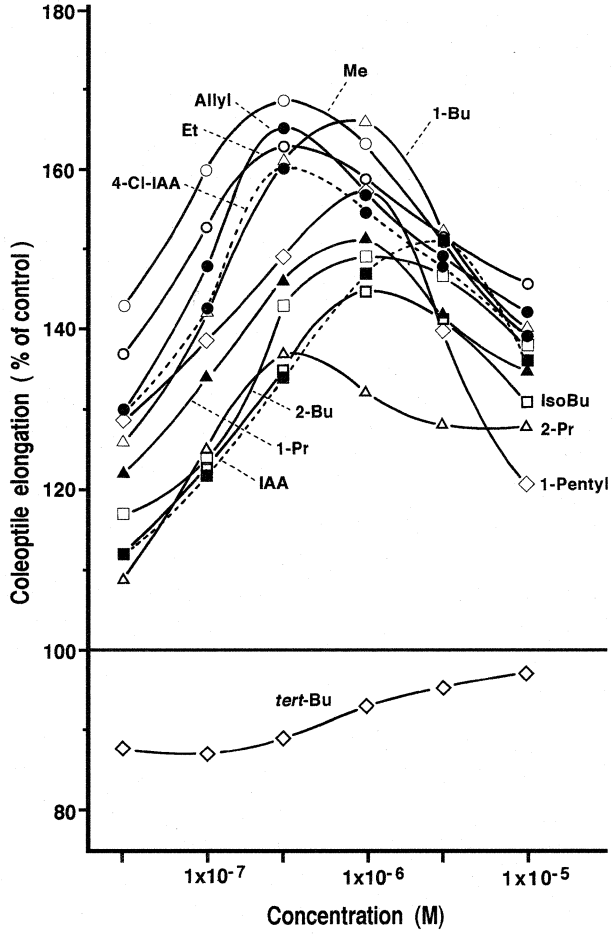

Fig. 2. *Avena* Coleoptile Elongation by 4-Cl-IAA, Its Esters and IAA.

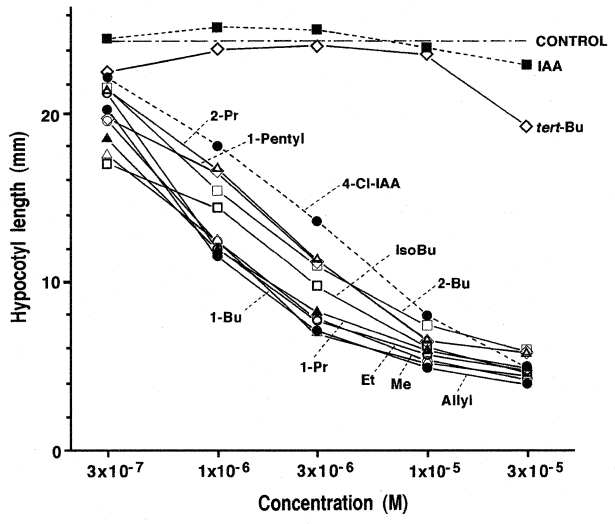

Fig. 3. Chinese Cabbage Hypocotyl Growth Inhibition by 4-Cl-IAA, Its Esters and IAA.

with a straight chain was slightly greater than that of the 2-propyl ester with a small bulky chain. The activity of the 1-butyl ester was also somewhat greater than that of other butyl esters (2-butyl and isobutyl) that had more bulky or branched end chains. The esters with a straight chain at the alcohol end inhibited hypocotyl growth more than did those with a branched chain. This indicates that esterification of 4-Cl-IAA increased the inhibitory activity, as did the presence of a short straight carbon chain at the alcohol end. All the esters, except the *tert*-butyl one, induced hypocotyl swelling in Chinese cabbage seedlings at a concentration of more than 1×10^{-6} M. The potent inhibitory activities of these esters may have been due to the increase of their permeability into the plant by the added lipophilicity of the alkyl chains in the esters to 4-Cl-IAA and their high resistance to *in vivo* decomposition by such enzymes as peroxidase.[6,13]

Furthermore, all the esters, except the *tert*-butyl one, induced severe hypocotyl swelling and the formation of numerous lateral roots in the black gram seedlings (Fig. 4), the ethyl and allyl esters producing the greatest hypocotyl swelling and lateral root formation. Their biological activities were about three times that of 4-Cl-IAA, and about 100 times that of IBA, and both had activity 1000 times that of IAA. The biological activities of the 1-propyl, 2-propyl, and 1-pentyl esters were intermediate between those of the ethyl and allyl esters and the activity of 4-Cl-IAA. IAA induced weak hypocotyl swelling and lateral root formation, even at 3×10^{-5} M, the highest concentration tested. The methyl, 1-butyl, 2-butyl, and isobutyl esters induced hypocotyl swelling and

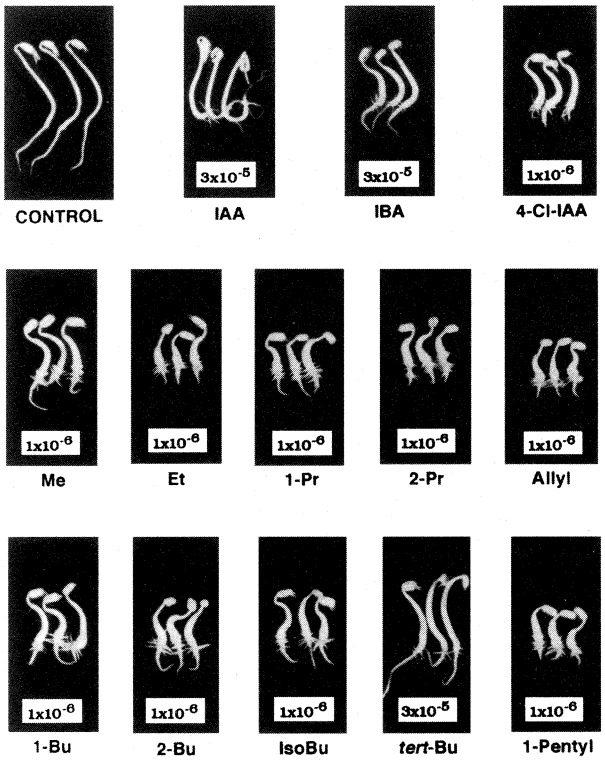

Fig. 4. Black Gram Hypocotyl Swelling and Lateral Root Formation Induced by 4-Cl-IAA, Its Esters, IAA and IBA.

lateral root formation of a similar magnitude to that of 4-Cl-IAA in black gram seedlings. IBA had activity somewhat higher than the *tert*-butyl ester and IAA. Callus was induced on the black gram seedlings by all except the *tert*-butyl ester at a concentration of more than 1×10^{-5} M. 4-Cl-IAA also induced callus above this concentration in only three days (not shown in Fig. 4). The high biological activities of these esters compared to that of 4-Cl-IAA toward the intact black gram seedlings may reflect the increase in their permeability. Their high activity may also have been due to their higher resistance to enzymatic decomposition than that of IAA.[6,13]

The biological activities of 4-Cl-IAA and its esters in promoting adventitious root formation in *Serissa japonica* are summarized in Table 1. The ethyl and allyl esters had the strongest activity, being respectively 10.9 and 10.3 times the control value, 5.8 and 5.5 times that of IAA, and 3.5 and 3.3 times that of IBA. IBA is the active ingredient sold under the commercial names of Hormodin, Rhizopon-AA and Seradix, and is used to promote and accelerate root formation in plant cuttings. The methyl ester also showed very potent activity, being 9.7 times the control value, 5.2 times that of IAA, and 3.1 times that of IBA. The remaining esters all had strong adventitious root formation-promoting activity. All the esters greatly promoted adventitious root formation at the first node on the basal side of *Serissa* cuttings, whereas IAA and IBA only weakly promoted root formation at that node. Interestingly, all the esters promoted marked adventitious root formation at the second and third nodes of the cuttings. IAA and IBA, however, weakly promoted nodal adventitious root formation at those nodes. The root dry weight ratios were similar to the root number ratios when the adventitious roots induced by the esters were compared to those of the control, indicative that the adventious roots induced by the esters were normal ones. The number of adventitious roots induced by the ethyl and allyl esters on the second through fifth nodes was similar to the number induced by IBA on the first node. Clearly, the ethyl, allyl, and other 4-Cl-IAA esters, as well as 4-Cl-IAA itself, can be used as root formation-promoting agents.

The high biological activities of the 4-Cl-IAA esters in these experiments may have been due to their increased permeability into the plants as well as to their high resistance to enzymatic oxidation. There also is the possibility that 4-Cl-IAA produced from its esters by translocation and subsequent hydrolysis by enzymes in the plant may function as an active principle.

In summary, the esters of 4-Cl-IAA had very potent hormonal activities in the four bioassays. Field application tests of these esters, as well as of 4-Cl-IAA, on agronomically important plant species are in progress.

Acknowledgments

I thank Miss Kuniko Tani for the biological activity measurements, Miss Yumiko Makino of the National Institute for Basic Biology at Okazaki for the 360 MHz ^{1}H-NMR spectral measurements, and Mr. Shigeyuki Kitamura of the Graduate School of Bioagricultural Sciences at Nagoya University for the mass spectral measurements and elemental analyses.

References

1) Gandar, J.-C. and Nitsch, C., Isoement de l'ester méthylique d'un acide chloro-3-indolylacétique à partir de graines immatures de pois, *Pisum sativum*. *C.R. Hebd. Seances Acad. Sci., Ser. D*, **265**, 1795–1798 (1967).

Table 1. *Serissa japonica* Adventitious Root Formation-promoting Activity by 4-Cl-IAA, Its Esters, IAA and IBA[a]

Sample	Root number/node Node number from cut end					Root number/plant (ratio to control)		Root dry weight (mg)/plant[b] (ratio to control)	
	1	2	3	4	5				
Control	7.0	—	—	—	—	7.0	(1.0)	2.4	(1.0)
IAA	12.3	0.6	0.2	—	—	13.1	(1.9)	7.0	(2.9)
IBA	22.5	0.4	0.2	—	—	21.6	(3.1)	8.8	(3.7)
4-Cl-IAA	55.1	10.9	4.1	0.3	—	70.4	(10.1)	22.2	(9.3)
4-Cl-IAA Me	55.7	9.0	3.4	—	—	68.1	(9.7)	22.9	(9.5)
4-Cl-IAA Et	57.5	11.3	5.1	2.3	0.4	76.6	(10.9)	27.1	(11.3)
4-Cl-IAA 1-Pr	50.5	7.7	3.0	0.5	—	61.7	(8.8)	16.3	(6.8)
4-Cl-IAA 2-Pr	36.1	7.3	3.4	—	—	46.8	(6.7)	12.3	(5.1)
4-Cl-IAA Allyl	51.9	16.8	3.7	0.1	—	72.5	(10.4)	28.0	(11.7)
4-Cl-IAA 1-Bu	40.5	6.6	4.4	1.0	0.1	52.6	(7.5)	20.2	(8.4)
4-Cl-IAA 2-Bu	38.9	4.8	1.8	0.2	—	45.7	(6.5)	16.4	(6.8)
4-Cl-IAA IsoBu	40.0	9.7	9.0	0.9	1.1	60.7	(8.7)	19.8	(8.3)
4-Cl-IAA 1-Pentyl	40.5	9.2	4.9	0.6	—	55.2	(7.9)	20.4	(8.5)

a) The *tert*-butyl ester was not tested.
b) Dried in a desiccator for 2 weeks.

2) Marumo, S., Abe, H., Hattori, H., and Munakata, K., Isolation of a novel auxin, methyl 4-chloroindoleacetate from immature seeds of *Pisum sativum*. *Agric. Biol. Chem.*, **32**, 117–118 (1968).
3) Marumo, S., Hattori, H., Abe, H., and Munakata, K., Isolation of 4-chloroindolyl-3-acetic acid from immature seeds of *Pisum sativum*. *Nature*, **214**, 959–960 (1968).
4) Reinecke, D. M., 4-Chloroindole-3-acetic acid and plant growth. *Plant Growth Regulation*, **27**, 3–13 (1999).
5) Ernstsen, A. and Sandberg, G., Identification of 4-chloroindole-3-acetic acid and indole-3-aldehyde in seeds of *Pinus sylvestris*. *Physiol. Plant.*, **68**, 511–518 (1986).
6) Marumo, S., Hattori, H., and Yamamoto, A., Biological activity of 4-chloroindolyl-3-acetic acid. *In* "Plant Growth Substances 1973", Hirokawa Publishing Company Inc., Tokyo, pp. 419–428 (1974).
7) Böttger, M., Engvild, K. C., and Soll, H., Growth of *Avena* coleoptile and pH drop of protoplast suspensions induced by chlorinated indoleacetic acid. *Planta*, **140**, 89–92 (1978).
8) Katayama, M. and Futatsuya, F., Synthesis and biological activities of chlorinated indole auxins and antiauxins. *Plant Cell Technology* (in Japanese), **2**, 487–502 (1990).
9) Engvild, K. C., Preparation of chlorinated 3-indolylacetic acids. *Acta Chem. Scand.*, **B31**, 338–339 (1977).
10) Somei, M., Kizu, K., Kunimoto, M., and Yamada, F., The chemistry of indoles. XXIV. Syntheses of 3-indoleacetic acid and 3-indoleacetonitrile having a halogeno group and a carbon functional group at the 4-position. *Chem. Pharm. Bull.*, **33**, 3696–3708 (1985).
11) Iwao, M. and Motoi, O., Methodology for the efficient synthesis of 3,4-differentially substituted indoles. Fluoride ion-induced elimination-addition reaction of 1-triisopropylsilylgramine methiodides. *Tetrahedron Lett.*, **36**, 5929–5932 (1995).
12) Batcho, A. D. and Leimgruber, W., Indoles from 2-methylnitrobenzenes by condensation with formamide acetals followed by reduction: 4-Benzyloxyindole. *Org. Syn.*, **63**, 214–225 (1985).
13) Hatano, T., Katayama, M., and Marumo, S., 5,6-Dichloroindole-3-acetic acid as a potent auxin: its synthesis and biological activity. *Experientia*, **43**, 1237–1239 (1987).