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Synthesis, Absolute Configuration and Biological Activity of Both Enantiomers of 2-(5,6-Dichloro-3-indolyl)propionic Acid: New Dichloroindole Auxins

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Racemic 2-(5,6-dichloro-3-indolyl)propionic acid (5,6-Cl_2-2-IPA) was synthesized from 5,6-dichloroindole-3-acetic acid (5,6-Cl_2-IAA) by successive esterification, methoxycarbonylation, methylation, and double hydrolysis. The racemate was converted to the diastereomeric esters of (*S*)-(−)-1-phenylethyl alcohol. These were separated by HPLC into two optically active diastereomers and then hydrolyzed with *p*-TsOH to the optically active enantiomers of 5,6-Cl_2-2-IPA. The absolute configurations of both the 5,6-Cl_2-2-IPA enantiomers were determined by comparing the ^{1}H-NMR spectra of their diastereomeric (*S*)-(−)-1-phenylethyl esters with those of the diastereomeric (*S*)-(−)-1-phenylethyl esters of 2-(3-indolyl)propionic acid (2-IPA) whose absolute configurations are already known.

There was no essential difference between (*S*)-(+)- and (*R*)-(−)-5,6-Cl_2-2-IPA in hypocotyl growth-inhibiting activity toward Chinese cabbage, but their inhibitory activities were stronger than that of the potent mother auxin, 5,6-Cl_2-IAA. No essential difference in the coleoptile elongating activity of *Avena sativa* was apparent for the enantiomers, this activity being about one-third that of 5,6-Cl_2-IAA.

Key words: (*R*)-(−)-2-(5,6-dichloro-3-indolyl)propionic acid; (*S*)-(+)-2-(5,6-dichloro-3-indolyl)propionic acid; dichloroindole auxin; *Avena* coleoptile elongation; hypocotyl growth inhibition

In past plant growth regulator studies, we have reported the synthesis and biological activities of 5,6-dichloroindole-3-acetic acid (5,6-Cl_2-IAA, **3a**), the most active of the natural and synthetic auxins known so far,[1-3] and of α-(5,7-dichloroindole-3-) isobutyric acid (5,7-Cl_2-IIBA), a potent antiauxin.[4] The new fluoroindole auxin, 5,6-difluoroindole-3-acetic acid (5,6-F_2-IAA), was also synthesized.[5] These studies followed from our original isolation of 4-chloroindole-3-acetic acid (4-Cl-IAA) from immature seeds of *Pisum sativum*.[6,7] We have also synthesized non-substituted and substituted 4,4,4-trifluoro-3-(3-indolyl)butyric acids (TFIBAs), which were novel fluorinated indolic plant growth regulators, and have shown that they had strong root growth-promoting activity toward Chinese cabbage, lettuce, and rice seedlings.[8-10]

2-(3-Indolyl)propionic acid (2-IPA), which has a methyl group at the α-position of the side chain of indole-3-acetic acid, was assayed for its auxin activity to clarify the effect of this α substituent. Several findings on the activity by various bioassays showed that it often equaled or surpassed that of IAA. Its 2-IPA activity in the *Avena* straight growth test was markedly stronger than that of IAA,[11] and both the (+)- and (−)-antipodes of 2-IPA have been confirmed to have the same activity.[11-13] Kögl, however, has reported the (+)-form of 2-IPA to be about 30 times as active as the (−)-form in the *Avena* curvature test.[14] In the wheat root growth inhibition test, the (+)-antipode was about 6 times more active than the (−)-antipode,[11] whereas in the flax root test, the (−)-antipode was about twice as active as the (+)-antipode.[11,13] To clarify the relationship between the auxin activity and stereochemistry of the α-substituted side chain, we synthesized both enantiomers of 2-(5,6-dichloro-3-indolyl)propionic acid (5,6-Cl_2-2-IPA) basaed on our previous research into 5,6-Cl_2-IAA (**3a**). We report here the synthesis, determina-

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Abbreviations: 5,6-Cl_2-2-IPA, 2-(5,6-dichloro-3-indolyl)propionic acid; 2-IPA, 2-(3-indolyl)propionic acid; 5,6-Cl_2-IAA, 5,6-dichloroindole-3-acetic acid

tion of the absolute configuration by a ^{1}H-NMR spectral analysis, and biological activity of (*S*)-(+)- and (*R*)-(−)-5,6-Cl_2-2-IPAs [(+)- and (−)-**1**].

Materials and Methods

Instrumentation. Optical rotation values were measured with a Jasco DIP-370 polarimeter. ^{1}H-NMR spectra were recorded with a Jeol FX-200 spectrometer, using tetramethylsilane in acetone-d_6 (An-d_6) or $CDCl_3$ as the internal standard, and low- and high-resolution mass spectra were recorded with a Jeol DX-705L spectrometer.

Synthesis of (±)-2-(5,6-Dichloro-3-indolyl) propionic acid [(±)-5,6-Cl_2-2-IPA, (±)-1].

Methyl 5,6-dichloro-1-methoxycarbonylindole-3-acetate (5a). Methyl chloroformate (0.3 ml, 3.88 mmol) was added dropwise over 15 min to a rapidly stirred mixture of methyl 5,6-dichloroindole-3-acetate (**4a**) [prepared from 5,6-Cl_2-IAA (**3a**, 570 mg, 2.33 mmol) by methylating with diazomethane] and benzyltriethylammonium bromide (5.0 mg, 18.4 μmol) in dichloromethane (10 ml) and a 30% sodium hydroxide solution (10 ml) at 0°C. After being stirred for 1 hr at 0°C, the two layers were separated, and the aqueous layer was extracted three times with dichloromethane. The combined dichloromethane layer was successively washed with water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give a crude carbamate. This carbamate was purified by chromatography in a short silica gel column, affording 668 mg (90.5% yield from **3a**) of **5a**. ^{1}H-NMR (200 MHz) δ_H (An-d_6): 3.68 (3H, s), 3.83 (2H, d, J=1.0 Hz), 4.07 (3H, s), 7.75 (1H, d, J=1.0 Hz), 7.82 (1H, s), 8.31 (1H, s); MS (70 eV, relative intensity, %) *m/z*: 319 (13), 317 (67), 315 (M^+, 100), 260 (11), 258 (60), 256 (92), 216 (8), 214 (31), 212 (45).

Methyl (±)-2-(5,6-dichloro-1-methoxycarbonyl-3-indolyl)propionate (6a). A solution of **5a** (460 mg, 1.46 mmol) in dry THF (20 ml) was injected with a syringe through the silicon rubber septum, and a dry THF solution of lithium diisopropylamide (LDA, 3.5 ml, 1.5 equiv) was then added dropwise with a syringe at −78°C. After being stirred for 1 hr, a solution of methyl iodide (311 mg, 2.19 mmol) in THF (4 ml) was added dropwise to the THF solution with a syringe at −78°C, and the mixture stirred for 2 hr. The reaction mixture was poured into a mixture of diethyl ether and an aqueous ammonium chloride solution. The aqueous layer was extracted three times with diethyl ether. The combined diethyl ether layer was successively washed with water and saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a crude oil. This oil was purified by preparative thin-layer chromatography on silica gel, affording 136 mg (28.3% yield) of (±)-**6a**. ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.57 (3H, d, J=7.0 Hz), 3.66 (3H, s), 4.06 (1H, dq, J=1.0, 7.0 Hz), 4.07 (3H, s), 7.68 (1H, d, J=1.0 Hz), 7.84 (1H, s), 8.32 (1H, s); MS (70 eV, relative intensity, %) *m/z*: 333 (4), 331 (27), 329 (M^+, 40), 274 (12), 272 (62), 270 (100), 214 (10), 212 (17).

(±)-2-(5,6-Dichloro-3-indolyl)propionic acid [(±)-1]. A solution of potassium hydroxide (85%, 1.8 g, 27.3 mmol) in water (10 ml) was added to a solution of **6a** (136 mg, 0.41 μmol) in methanol (40 ml), and the mixture stirred at 70°C for 1 hr. The reaction mixture was cooled to room temperature and methanol removed *in vacuo*, giving an aqueous solution. This solution was acidified with a 1 N HCl solution and extracted three times with ethyl acetate. The combined ethyl acetate layer was successively washed with water and saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo*, giving crude propionic acid which was purified by preparative thin-layer chromatography on silica gel to afford 99 mg (93.1% yield) of (±)-**1**. ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.56 (3H, d, J=7.1 Hz), 4.00 (1H, q, J=7.1 Hz), 7.39 (1H, d, J=3.3 Hz), 7.61 (1H, s), 7.85 (1H, s); MS (70 eV, relative intensity, %) *m/z*: 261 (3), 259 (19), 257 (M^+, 29), 216 (12), 214 (66), 212 (100); HRMS *m/z* (M^+): calcd. for $C_{11}H_9Cl_2NO_2$, 257.0010; Found, 257.0014.

Preparation of (S)-(+)-2-(5,6-dichloro-3-indolyl) propionic acid [(S)-(+)-5,6-Cl_2-2-IPA, (+)-1] and (R)-(−)-2-(5,6-dichloro-3-indolyl)propionic acid [(R)-(−)-5,6-Cl_2-2-IPA, (−)-1].

(S)-(−)-1-Phenylethyl 2-(5,6-dichloro-3-indolyl) propionate (9a). A solution of *N,N'*-dicyclohexylcarbodiimide (DCC, 8.8 mg, 43.1 μmol) in dichloromethane (5 ml) was added to a solution of racemic **3a** (10.0 mg, 38.8 μmol), (*S*)-(−)-1-phenylethyl alcohol (5.2 mg, 42.6 μmol) and 4-(*N,N*-dimethylamino)pyridine (DMAP, 5.0 mg, 41.0 μmol) in dichloromethane (20 ml). After the reaction mixture had been stirred at 25°C for 5 hr, the resulting *N,N'*-dicyclohexylurea (DCU) was filtered off, and the filtrate was evaporated to dryness. Ethyl acetate was added to the residue, the ethyl acetate solution was cooled, and insoluble DCU was removed by filtration. The filtrate was successively washed with a 1 M NH_4Cl solution, water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*, giving a crude ester which was purified by preparative thin-layer chromatography on silica gel to afford 10.0 mg (71.3% yield) of **7a**. This diastereomeric propionate was then separated by high-performance liquid chromatography (HPLC).

HPLC separation of diastereomeric 1-phenylethyl 2-(5,6-dichloro-3-indolyl)propionate (7a) into (+)-1-phenylethyl 2-(5,6-dichloro-3-indolyl)propionate (8a) and (−)-1-phenylethyl 2-(5,6-dichloro-3-indolyl) propionate (9a). The diasteromeric mixture of **7a**

(10.0 mg) was separated by HPLC in a column of Develosil 60-5 ($\phi 8 \times 500$ mm), using a solvent of 15% EtOAc in *n*-hexane at a flow rate of 3.0 ml/min to give 6.4 mg of **8a** and 3.6 mg of **9a**. The less-polar and more-polar diastereomers were respectively eluted at retention times of 51.0 and 54.2 min in a ratio of 1.8:1. The diastereomeric excess of the respective diastereomers was 98.6% and 96.7%. **8a** (less polar): $[\alpha]_D^{25}$ +49° (*c* 0.18, benzene); ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.484 (3H, d, J=6.6 Hz), 1.563 (3H, d, J=7.1 Hz), 4.071 (1H, q, J=7.1 Hz), 5.836 (1H, q, J=6.6 Hz), 7.187 (5H, m), 7.324 (1H, d, J=1.7 Hz), 7.598 (1H, s), 7.780 (1H, s); MS (70 eV, relative intensity, %) *m/z*: 363 (12), 361 (M^+, 17), 216 (11), 214 (63), 212 (100), 105 (66). **9a** (more polar): $[\alpha]_D^{25}$ −98° (*c* 0.10, benzene); ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.398 (3H, d, J=6.6 Hz), 1.548 (3H, d, J=7.1 Hz), 4.066 (1H, d, J=7.1 Hz), 5.844 (1H, q, J=6.6 Hz), 7.287–7.390 (5H, m), 7.402 (1H, d, J=1.7 Hz), 7.619 (1H, s), 7.858 (1H, s); MS (70 eV, relative intensity, %) *m/z*: 363 (12), 361 (M^+, 17), 216 (11), 214 (63), 212 (100), 105 (66).

(S)-(+)-2-(5,6-Dichloro-3-indolyl)propionic acid [(+)-1]. A solution of **8a** (2.5 mg, 6.9 μmol) and 1 N *p*-toluenesulfonic acid (*p*-TsOH, 0.4 ml) in methanol (0.1 ml) was placed in a 5-ml round flask fitted with reflux condenser and magnetic stirrer. The mixture was heated at 80°C for 6 hr. The reaction mixture was cooled to room temperature, adjusted to pH 3 with a 1 N NaOH solution and extracted three times with ethyl acetate. The combined ethyl acetate layer was successively washed with water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo*, giving a crude oil which was purified by preparative thin-layer chromatography on silica gel to afford 0.62 mg (34.8% yield) of (+)-**1**. $[\alpha]_D^{25}$ +119° (*c* 0.04, benzene); ^{1}H-NMR (200 MHz) δ_H ($CDCl_3$): 1.56 (3H, d, J=7.1 Hz), 4.00 (1H, q, J=7.1 Hz), 7.39 (1H, d, J=3.3 Hz), 7.61 (1H, s), 7.85 (1H, s); MS (70 eV, relative intensity, %) *m/z*: 261 (4), 259 (19), 257 (M^+, 24), 216 (10), 214 (66), 212 (100); HRMS *m/z* (M^+): calcd. for $C_{11}H_9Cl_2NO_2$, 257.0010; found, 256.9991.

(R)-(−)-2-(5,6-Dichloro-3-indolyl)propionic acid [(−)-1]. **9a** (2.5 mg) was treated by the procedure used for the hydrolysis of (+)-**8a**, giving 0.5 mg (28.1% yield) of (−)-**1**. $[\alpha]_D^{25}$ −121° (*c* 0.06, benzene); ^{1}H-NMR (200 MHz) δ_H ($CDCl_3$): 1.56 (3H, d, J=7.1 Hz), 4.00 (1H, q, J=7.1 Hz), 7.39 (1H, d, J=3.3 Hz), 7.61 (1H, s), 7.85 (1H, s); MS (70 eV, relative intensity, %) *m/z*: 261 (5), 259 (21), 257 (M^+, 32), 216 (12), 214 (67), 212 (100); HRMS *m/z* (M^+): calcd. for $C_{11}H_9Cl_2NO_2$, 257.0010; found, 257.0011. The enantiomeric purity of each enantiomer was measured by a chiral column of Sumipak OA-2000A ($\phi 4 \times 250$ mm), using a solvent system of CH_3CN:H_2O:AcOH (40:60:0.5). The (*S*)-(+)- and (*R*)-(−)-enantiomers had a 93.3% and 93.0% enantiomeric excess and retention times of 57.5 and 52.9 min, respectively (flow rate of 1.0 ml/min, detection by UV at 280 nm).

Preparation of (±)-2-(3-Indolyl)propionic acid [(±)-2].

Methyl 1-methoxycarbonylindole-3-acetate (5b). **3b** (2.00 g) was treated by the procedure used for the synthesis of **5a**, giving 2.73 g (96.7% yield from IAA) of **5b**. ^{1}H-NMR (200 MHz) δ_H (An-d_6): 3.67 (3H, s), 3.77 (2H, d, J=1.0 Hz), 4.03 (3H, s), 7.25 (1H, ddd, J=7.3, 7.3, 1.2 Hz), 7.34 (1H, ddd, J=7.3, 7.3, 1.2 Hz), 7.58 (1H, dd, J=7.3, 1.2 Hz), 7.64 (1H, brs), 8.15 (1H, dd, J=7.3, 1.2 Hz); MS (70 eV, relative intensity, %) *m/z*: 247 (M^+, 53), 188 (100), 144 (70), 129 (32).

Methyl (±)-2-(1-methoxycarbonyl-3-indolyl) propionate (6b). **5b** (1.00 g) was treated by the procedure used for the synthesis of **6a**, giving 0.96 g (90.8% yield) of **6b**. ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.57 (3H, d, J=7.0 Hz), 3.64 (3H, s), 4.03 (1H, q, J=7.0 Hz), 4.04 (3H, s), 7.26 (1H, ddd, J=7.3, 7.3, 1.2 Hz), 7.35 (1H, ddd, J=7.3, 7.3, 1.2 Hz), 7.59 (1H, s), 7.64 (1H, dd, J=7.3, 1.2 Hz), 8.16 (1H, dd, J=7.3, 1.2 Hz); MS (70 eV, relative intensity, %) *m/z*: 261 (M^+, 31), 202 (100), 158 (18), 143 (26).

(±)-2-(3-Indolyl)propionic acid [(±)-2]. **6b** (333 mg) was treated by the procedure used for the synthesis of (±)-**1**, giving 220 mg (91.2% yield) of (±)-**2**. ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.58 (3H, d, J=7.1 Hz), 4.00 (1H, q, J=7.1 Hz), 7.01 (1H, dd, J=7.1, 7.1 Hz), 7.09 (1H, dd, J=7.1, 7.1 Hz), 7.24 (1H, d, J=2.4 Hz), 7.35 (1H, brd, J=7.1 Hz), 7.66 (1H, brd, J=7.1 Hz), 8.04 (1H, brs); MS (70 eV, relative intensity, %) *m/z*: 189 (M^+, 14), 144 (100); HRMS *m/z* (M^+): calcd. for $C_{11}H_{11}NO_2$, 189.0790; found, 189.0781.

Preparation of (S)-(+)-2-(3-indolyl)propionic acid [(+)-2] and (R)-(−)-2-(3-indolyl)propionic acid [(−)-2].

1-Phenylethyl 2-(3-indolyl)propionate (7b). (±)-**2** (50.0 mg) was treated by the same procedure as that used for the synthesis of **7a**, giving 30.8 mg (40% yield) of **7b**. This diastereomeric propionate was then separated by HPLC.

HPLC separation of diastereomeric (S)-1-phenylethyl 2-(3-indolyl)propionate (7b) into (S)-1-phenylethyl (+)-2-(3-indolyl)propionate (8b) and (S)-1-phenylethyl (−)-2-(3-indolyl)propionate (9b). The diasteromeric mixture of **9b** (10.0 mg) was separated by HPLC in a column of Develosil 60-5 ($\phi 8 \times$ 500 mm), using a solvent of 15% EtOAc in *n*-hexane (flow rate of 3 ml/min) to give 6.1 mg of **8b** and 3.4 mg of **9b**. The less-polar and more-polar diastereomers were respectively eluted at retention times of 21.3

min and 22.6 min in a ratio of 1.8:1. **8b** (less polar): $[\alpha]_D^{25}$ +94° (*c* 0.04, benzene); ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.478 (3H, d, J=6.6 Hz), 1.571 (3H, d, J=7.1 Hz), 4.067 (1H, q, J=7.1 Hz), 5.841 (1H, q, J=6.6 Hz), 6.969 (1H, ddd, J=8.1, 8.1, 1.2 Hz), 7.083 (1H, ddd, J=8.1, 8.1, 1.2 Hz), 7.169 (6H, m), 7.365 (1H, dd, J=8.1, 1.2 Hz), 7.606 (1H, dd, J=8.1, 1.2 Hz); MS (70 eV, relative intensity, %) *m/z*: 293 (M^+, 8), 144 (100), 105 (30). **9b** (more polar): $[\alpha]_D^{25}$ −100° (*c* 0.04, benzene); ^{1}H-NMR (200 MHz) δ_H (An-d_6): 1.391 (3H, d, J=6.6 Hz, H-2′), 1.549 (3H, d, J=7.3 Hz, H-10), 4.065 (1H, q, J=7.3 Hz, H-8), 5.851 (1H, q, J=6.6 Hz, H-1′), 7.011 (1H, ddd, J=7.3, 7.3, 1.2 Hz, H-5), 7.104 (1H, ddd, J=7.3, 7.3, 1.2 Hz, H-6), 7.250–7.344 (6H, m, H-2, H-2″–H-6″), 7.384 (1H, dd, J=7.3, 1.2 Hz, H-7), 7.665 (1H, dd, J=7.3, 1.2 Hz, H-4); MS (70 eV, relative intensity, %) *m/z*: 293 (M^+, 18), 144 (100), 105 (35).

(S)-(+)-2-(3-indolyl)propionic acid [(+)-2]. **8b** (3.0 mg) was treated by the procedure used for the hydrolysis of (+)-**8a**, giving 0.65 mg (33.6% yield) of (+)-**2**. $[\alpha]_D^{25}$ +94° (*c* 0.04, benzene); ^{1}H-NMR (200 MHz) δ_H ($CDCl_3$): 1.57 (3H, d, J=7.1 Hz), 4.00 (1H, q, J=7.1 Hz), 7.01 (1H, dd, J=7.1, 7.1 Hz), 7.09 (1H, dd, J=7.1, 7.1 Hz), 7.24 (1H, d, J=2.4 Hz), 7.36 (1H, brd, J=7.1 Hz), 7.66 (1H, brd, J=7.1 Hz); MS (70 eV, relative intensity, %) *m/z*: 189 (M^+, 30), 144 (100); HRMS *m/z* (M^+): calcd. for $C_{11}H_{11}NO_2$, 189.0790; found, 189.0761.

(R)-(−)-2-(3-indolyl)propionic acid [(−)-2]. **9b** (3.1 mg) was treated by the procedure used for the hydrolysis of (+)-**8a**, giving 0.55 mg (27.5% yield) of (−)-**2**. $[\alpha]_D^{25}$ −100° (*c* 0.04, benzene); ^{1}H-NMR (200 MHz) δ_H ($CDCl_3$): 1.57 (3H, d, J=7.1 Hz), 4.00 (1H, q, J=7.1 Hz), 7.01 (1H, dd, J=7.1, 7.1 Hz), 7.09 (1H, dd, J=7.1, 7.1 Hz), 7.24 (1H, d, J=2.4 Hz), 7.36 (1H, brd, J=7.1 Hz), 7.66 (1H, brd, J=7.1 Hz); MS (70 eV, relative intensity, %) *m/z*: 189 (M^+, 17), 144 (100); HRMS *m/z* (M^+): calcd. for $C_{11}H_{11}NO_2$, 189.0790; found, 189.0788.

Plant materials and bioassays. The plants (Chinese cabbage and *Avena sativa*) used for the bioassays were the same as those used in a previous study, and the bioassays were conducted as reported there.[1] Duplicate experiments were conducted twice in the two bioassays.

Results and Discussion

The carbamate (**5a**) was readily prepared by protecting an amino group of methyl 5,6-dichloroindole-3-acetate [**4a**, obtained by methylation of 5,6-Cl_2-IAA (**3a**) with diazomethane] with methyl chloroformate in the presence of a phase-transfer catalyst (benzyltriethylammonium bromide). Lithiation of the carbamate (**5a**) with LDA and subsequent methylation with methyl iodide produced a methylated carbamate [(±)-**6a**] which was doubly hydrolyzed to racemic (±)-5,6-Cl_2-2-IPA [(±)-**1**] (Fig. 1).

Racemic 1 reacted with (*S*)-(−)-1-phenylethyl alcohol and DCC, giving diastereomeric esters (**7a**) in the presence of DMAP in dichloromethane. These esters (**7a**) were separated by HPLC to yield a pair of diastereomers. Interestingly, the production ratio of the less-polar (**8a**) to the more-polar diastereomer (**9a**) was 1.8:1. The predominant formation of **8a** was caused by the interaction between an indole and phenyl group in the esterification with DCC. The respective diastereomeric excess of the less-polar and more-polar diastereomers was 98.6% and 96.7%. They finally were respectively converted to optically active (+)-**1** and (−)-**1** by acidic hydrolysis with *p*-TsOH. The respective enantiomeric excess of (+)-**1** and (−)-**1** was 93.3% and 93.0% by an HPLC analysis. The slight decrease in the enantiomeric excess of both enantiomers [(+)-**1** and (−)-**1**] resulted from epimerization at an asymmetric carbon by the acidic hydrolysis.

The optically active 2-(3-indolyl)propionic acids [(+)-**2** and (−)-**2**] were also synthesized by the procedure used to synthesize optically active (+)-**1** and (−)-**1**, and were used as standards in the determination of the absolute configuration of the 5,6-Cl_2-2-IPA enantiomers (Fig. 1). The production ratio of the less-polar and more-polar diastereomers in this case was also 1.8:1. The less-polar diastereomer produced the enantiomer that had a (+) specific rotation by acidic hydrolysis, and the more-polar one was hydrolyzed to the (−)-enantiomer. Sjöberg has reported that the absolute configuration of the enantiomer of 2-IPA with the (+) specific rotation was *S* and that the (−)-antipode had the *R*-configuration.[11] Consequently, the (+)-enantiomer of 2-IPA derived from the less-polar diastereomer (**8b**) had the *S* configuration, and the (−)-enantiomer, the *R* configuration.

The absolute configuration of (+)-**1** and (−)-**1** was determined by comparing the ^{1}H-NMR spectra of both (*S*)-(−)-1-phenylethyl diastereomers of (+)-**1** and (−)-**1** with those of the diastereomers of (+)-**2** and (−)-**2**. There was no essential difference in the chemical shifts of H-8 and H-1′ in the ^{1}H-NMR spectra of the (*S*)-(−)-1-phenylethyl diastereomeric esters of (+)-**2** and (−)-**2**, whereas the chemical shifts of the protons on the indole and benzene rings and of two methyl protons significantly differed (Table 1). All the protons on the indole and benzene rings of diastereomer **8b** were in a higher field than those of diastereomer **9b**; for example, H-4 and H-5 on the indole ring of diastereomer **8b** were respectively 0.059 and 0.042 ppm higher field than those of diastereomer **9b**. H-10 and H-2′ of diastereomer **9b**, however, appeared respectively 0.022 and 0.087 ppm higher field than those of diastereomer **8b**. These high-field

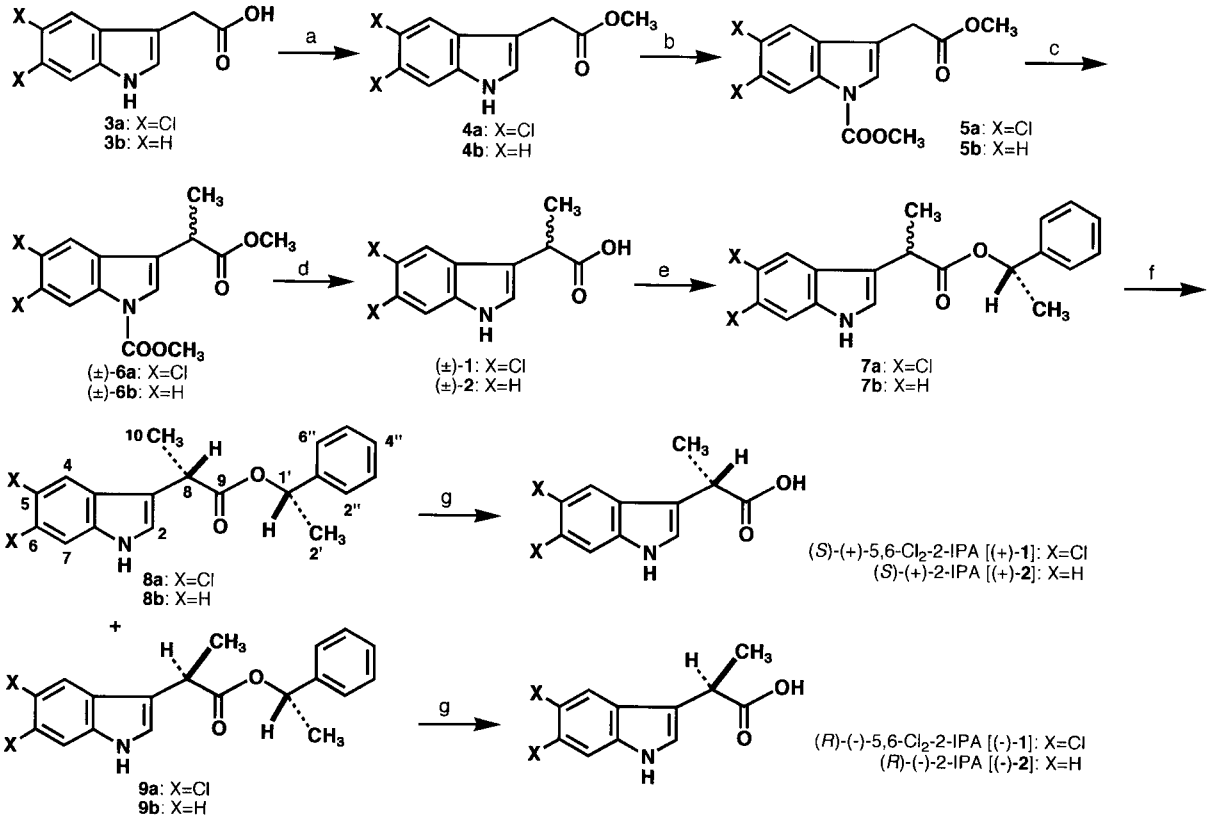

Fig. 1. Synthetic Scheme for Optically Active 5,6-Cl_2-2-IPA (**1**) and 2-IPA (**2**).
(a) CH_2N_2, Et_2O; (b) $ClCOOCH_3$, $C_6H_5CH_2N^+Et_3Br^-$, 30%NaOH, CH_2Cl_2, 0°C; (c) LDA, THF, −78°C; CH_3I, THF; (d) KOH, MeOH-H_2O, 70°C; (e) (*S*)-(−)-1-phenylethyl alcohol, DCC, DMAP, CH_2Cl_2; (f) HPLC separation; (g) *p*-TsOH, MeOH.

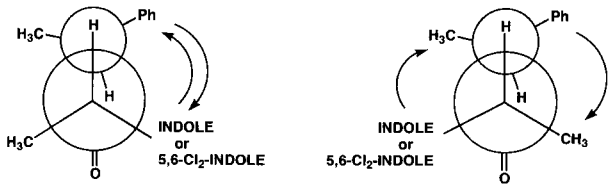

Fig. 2. Conformation of Both Diastereomeric (*S*)-(−)-1-Phenylethyl Esters of **1** and **2**.

shifts were the result of the anisotropy effect of the indole and benzene rings. These findings indicate that, in the less-polar (**8b**) diastereomer converted to (*S*)-(+)-**2**, the indole ring was located on the same side of the benzene ring, and that, in the more-polar (**9b**) diastereomer of (*R*)-(−)-**2**, these rings were located on opposite sides, as shown by the conformation in Fig. 2.

Based on the results of ^{1}H-NMR spectral analyses of diastereomers **8b** and **9b**, the ^{1}H-NMR spectra of diastereomers **8a** and **9a** were analyzed. ^{1}H-NMR spectral data are shown in Table 1. All the protons on the indole and benzene rings of diastereomer **8a** appeared in a higher field than those of diastereomer **9a**; for example, H-4 on the indole ring of diastereomer **8a** was 0.078 ppm higher field than that of diastereomer **9a**, as was the case in the ^{1}H-NMR spec-

Table 1. Chemical Shifts and Their Differences in the ^{1}H-NMR Spectra of Diastereomeric (*S*)-1-Phenylethyl Esters **8a**, **9a**, **8b** and **9b**

Position of proton	Chemical shift (ppm)		Difference in chemical shift (ppm)
	8a (**8b**)	**9a** (**9b**)	**8a-9a** (**8b-9b**)
2	7.324	7.402	−0.078
	(*)	(*)	(—)
4	7.780	7.858	−0.078
	(7.606)	(7.665)	(−0.059)
5	—	—	—
	(6.969)	(7.011)	(−0.042)
6	—	—	—
	(7.083)	(7.104)	(−0.021)
7	7.598	7.619	−0.021
	(7.365)	(7.384)	(−0.019)
8	4.071	4.066	+0.005
	(4.067)	(4.065)	(+0.002)
10	1.563	1.548	+0.015
	(1.571)	(1.549)	(+0.022)
1′	5.836	5.844	−0.008
	(5.841)	(5.851)	(−0.010)
2′	1.484	1.398	+0.086
	(1.478)	(1.391)	(+0.087)
2″-6″	7.187	7.287 ~ 7.390	−0.100 ~ −0.203
	(7.169)	(7.250 ~ 7.344)	(−0.081 ~ −0.156)

* not separated from the phenyl protons.

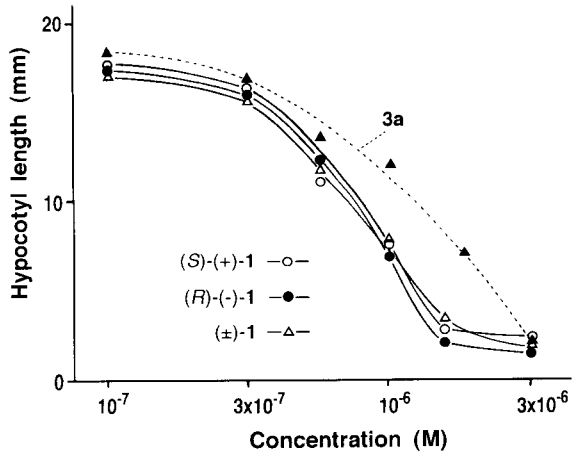

Fig. 3. Hypocotyl Growth Inhibition of Chinese Cabbage by (*S*)-(+)-**1**, (*R*)-(−)-**1**, (±)-**1** and **3a**.

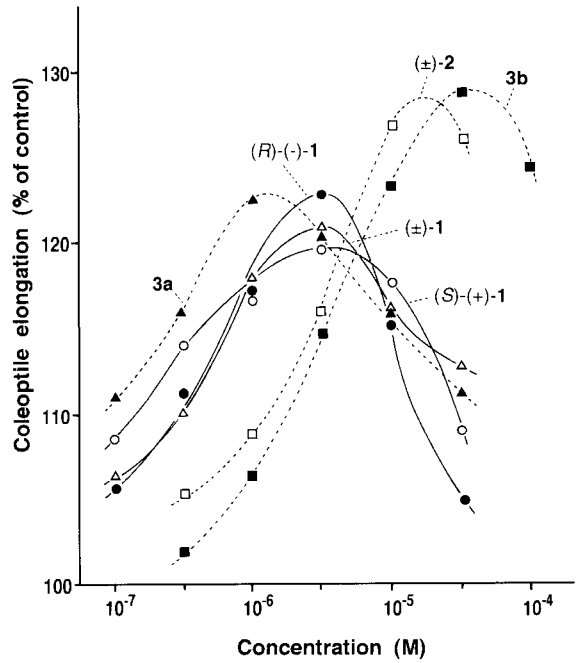

Fig. 4. Elongation of *Avena* Coleoptiles by (*S*)-(+)-**1**, (*R*)-(−)-**1**, (±)-**1**, **3a**, (±)-**2** and **3b**.

trum of diastereomer **8b**. Furthermore, H-10 and H-2′ of diastereomer **9a** were respectively 0.015 and 0.086 ppm higher field than those of diastereomer **8a**. This means that, in less-polar diastereomer **8a** converted to (+)-**1**, the indole ring was located on the same side as the benzene ring, and that, in more-polar diastereomer **9a** of the (−)-enantiomer, the rings were on opposite sides (Fig. 2). These findings coincided with those of the ^{1}H-NMR spectral analyses of diasteromers **8b** and **9b**. Consequently, the absolute configuration of (+)-**1** derived from the less-polar diastereomer was determined to be *S*, and that of the (−)-enantiomer to be *R*.

The biological activities of (*S*)-(+)-**1** and (*R*)-(−)-**1** were measured in two auxin bioassays (hypocotyl growth inhibition of Chinese cabbage and elongation of *Avena* coleoptiles) and compared with those of **3a**. The results of the hypocotyl growth inhibition of Chinese cabbage are shown in Fig. 3. All the compounds tested strongly inhibited the hypocotyl growth of Chinese cabbage. Interestingly, there was no essential difference in the inhibition activities of (*S*)-(+)-**1** and (*R*)-(−)-**1**, the activity being equal to that of racemic **1**. All **1** produced stronger inhibition than **3a**, the most active of the known natural and synthetic auxins so far examined.[1] This indicates that the absolute configuration of 5,6-Cl_2-2-IPA had no effect on the hypocotyl inhibition of Chinese cabbage.

The results of the *Avena sativa* coleoptile elongation bioassay are shown in Fig. 4. There was no essential difference in the elongation activities of (*S*)-(+)-**1** and (*R*)-(−)-**1**, and the elongation activity was equal to that of racemic **1**. This lack of an essential difference between the (+)- and (−)-antipode in *Avena* coleoptile elongation agrees with the findings for optically active 2-IPA reported by Sjöberg[11,12] and by Kögl and Verkaaik.[13] The elongation activities of all **1** were about one-third of the activity of **3a**, but much stronger than that of IAA. All **1** had 3 times the elongation activity of racemic **2** with no chlorine atoms in its molecule that had stonger activity than IAA. This indicates that the introduction of a methyl group to the side chain of indole-3-acetic acids does not always increase the elongation activity. The decrease in the elongation activity of 5,6-Cl_2-IAA produced by methylation at the a position of the acetic acid part can probably be ascribed to a decrease in the stability, affinity for the receptor, or permeability.

Acknowledgment

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