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Note

Synthesis of 2-(5,7-Difluoro-3-indolyl)propionic Acid (5,7-F_2-IPA) and 2-(5,7-Difluoro-3-indolyl)isobutyric Acid (5,7-F_2-IIBA) and Their Respective Auxin and Antiauxin Activities

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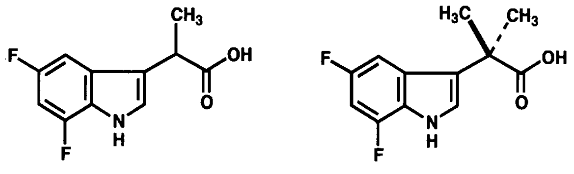
INTRODUCTION

In our research on new plant growth regulators we earlier reported a promising indolic auxin, 5,6-dichloroindole-3-acetic acid (5,6-Cl_2-IAA), the most potent of all the known natural and synthetic auxins.[1] An antiauxin, 2-(5,7-dichloro-3-indolyl)isobutyric acid (5,7-Cl_2-IIBA), was synthesized. It inhibited auxin-mediated elongation of *Avena* coleoptiles and stimulated root growth of rice seedlings.[2] A fluorinated auxin, 5,6-difluoroindole-3-acetic acid (5,6-F_2-IAA) also was synthesized which, interestingly, promoted growth of the lateral roots of mung bean.[3] The synthesis and biological activities of non-substituted and substituted 4,4,4-trifluoro-3-(3-indolyl)butyric acids (TFIBAs), novel fluorinated plant gowth regulators which promote the root growth of Chinese cabbage, lettuce and rice plants, have been reported.[4-6] The introduction of fluorine atom(s) to biologically active molecules sometimes has dramatic effects on the biological activities.[7,8] To develop novel fluorinated plant growth regulators, we focused on the syntheses of fluorinated 2-(3-indolyl)propionic acid (IPA) and 2-(3-indolyl)isobutyric acid (IIBA); *i.e.*, 5,7-difluoro-IPA (5,7-F_2-IPA, **1**) and 5,7-difluoro-IIBA (5,7-F_2-IIBA, **2**) (Fig. 1). We here report the syntheses of 5,7-F_2-IPA (**1**) and 5,7-F_2-IIBA (**2**) and their respective auxin and antiauxin activities.



5,7-F_2-IPA (1) 5,7-F_2-IIBA (2)

Fig. 1 Structures of 2-(5,7-difluoro-3-indolyl)propionic acid (5,7-F_2-IPA, **1**) and 2-(5,7-difluoro-3-indolyl)isobutyric acid (5,7-F_2-IIBA, **2**).

MATERIALS AND METHODS

1. *Instrumentation*

^{1}H-NMR spectra were recorded with a JEOL FX-200 spectrometer with tetramethylsilane in acetone-d_6 (An-d_6) as the internal standard. Mass spectra were recorded with a JEOL DX-705L spectrometer.

2. *Synthesis*

2.1 *2-(5,7-Difluoro-3-indolyl)propionic acid (5,7-F_2-IPA, 1)*

2.1.1 *2,4-Difluorophenylhydrazine (5)*

2,4-Difluoroaniline (**4**, 25.0 g, 193.8 mmol) was added to concentrated hydrochloric acid (100 ml) at 0°C. The mixture was stirred for 20 min and a solution of sodium nitrite (21.0 g, 304.3 mmol) in water (30 ml) was added dropwise at 0°C. After 30 min, stannous chloride (90.0 g, 474.7 mmol) in concentrated hydrochloric acid (100 ml) was added. The mixture was stirred for 1 hr and adjusted to pH 10 with 4 N NaOH solution, and extracted three times with ethyl acetate. The combined ethyl acetate layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a crude hydrazine. The hydrazine was recrystallized from ethyl acetate-*n*-hexane to afford 12.6 g (45.2% yield) of 2,4-difluorophenylhydrazine (**5**). ^{1}H-NMR (200 MHz) δH (An-d_6): 6.89 (2H, m), 7.43 (1H, m), 8.25 (1H, brs); MS (relative intensity, %) *m/z*: 144 (M$^+$, 100), 128 (13), 127 (65).

2.1.2 *3-Carboxypropionaldehyde 2,4-difluorophenylhydrazone (6)*

In a 500 ml-round-bottomed flask, fitted with a reflux condenser and magnetic stirrer, were placed **5** (1.0 g, 6.9 mmol) and 4,4-dimethoxybutyric acid (1.2 g, 8.1 mmol) with a mixture of benzene (200 ml) and water (50 ml), and the mixture was heated at 90°C. After 1 hr, the reaction mixture was cooled to room temperature and extracted three times with ethyl acetate. The combined ethyl acetate layer was washed with water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give a crude hydrazone. The hydrazone was recrystallized from ethyl acetate-*n*-hexane to afford 1.4 g (88.4% yield) of 3-carboxypropionaldehyde 2,4-difluorophenylhydrazone (**6**). ^{1}H-NMR (200 MHz) δH

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(An-d_6): 2.59 (4H, m), 6.99 (2H, m), 7.42 (1H, m), 7.48 (1H, brt, $J=3.9$ Hz), 8.72 (1H, brs); MS (relative intensity, %) m/z: 228 (M$^+$, 18), 172 (13), 127 (100).

2.1.3 5,7-Difluoroindole-3-acetic acid (3)

In a dry 200 ml-round-bottomed flask, fitted with a drying tube, were placed **6** (950.0 mg, 4.1 mmol) and zinc chloride (6.8 g, 50.0 mmol) with xylene (60 ml) and mixture was heated at 130°C for 1 hr. The reaction mixture was cooled to room temperature, and ethyl acetate and water were added to it. The ethyl acetate layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a crude acetic acid. The acetic acid was purified by column chromatography on silica gel to afford 274.2 mg (31.2% yield) of 5,7-difluoroindole-3-acetic acid (**3**). ^{1}H-NMR (200 MHz) δH (An-d_6): 3.75 (2H, s), 6.80 (1H, ddd, $J=11.7, 9.5, 2.2$ Hz), 7.18 (1H, dd, $J=9.3, 2.2$ Hz), 7.45 (1H, d, $J=2.4$ Hz); MS (relative intensity, %) m/z: 211 (M$^+$, 32), 166 (100).

2.1.4 Methyl 5,7-difluoro-1-methoxycarbonylindole-3-acetate (7)

Methyl chloroformate (0.14 ml, 1.8 mmol) was added dropwise for 15 min at 0°C to a rapidly stirred mixture of methyl 5,7-difluoroindole-3-acetate (250.0 mg, 1.1 mmol, prepared from 5,7-difluoroindole-3-acetic acid (**3**)* by methylation with diazomethane), benzyltriethylammonium bromide (20.0 mg) in dichloromethane (5 ml) and a 30% sodium hydroxide solution (5 ml). After this mixture was stirred for 1 hr at 0°C, two layers formed. The aqueous layer was treated three times with dichloromethane. The combined dichloromethane layer was washed successively with water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give the crude ester. It was purified by preparative thin layer chromatography on silica gel to give 198.0 mg (63.0% yield) of methyl 5,7-difluoro-1-methoxycarbonylindole-3-acetate (**7**). ^{1}H-NMR (200 MHz) δH (An-d_6): 3.68 (3H, s), 3.79 (2H, s), 4.02 (3H, s), 6.99 (1H, ddd, $J=12.0, 9.7, 2.2$ Hz), 7.21 (1H, dd, $J=8.4, 2.2$ Hz), 7.80 (1H, s); MS (relative intensity, %) m/z: 283 (M$^+$, 38), 224 (52), 180 (100).

2.1.5 Methyl 2-(5,7-difluoro-1-methoxycarbonyl-3-indolyl)-propionate (8)

A two-neck, round-bottom flask was flame-dried with a Bunsen burner. A solution of **7** (140.0 mg, 0.49 mmol) in dry tetrahydrofuran (THF, 4.0 ml) was injected by syringe through the rubber septum to the flask, after which a THF solution of lithium diisopropylamide (LDA, 1.2 ml, 1.3 equiv.) was added dropwise at −78°C by syringe. After the mixture had been stirred for 1 hr, a solution of methyl iodide (1.0 ml, from a solution prepared by the addition of dry alumina to a solution of 0.2 ml of methyl iodide in 4.0 ml of dry THF) was added dropwise at −78°C by syringe, and the whole stirred for 2 hr. The reaction mixture then was poured into a mixture of diethyl ether and aqueous ammonium chloride solution. The ether layer was washed with water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give the crude propionate. It was purified by preparative thin layer chromatography on silica gel giving 40.0 mg (27.2% yield) of methyl 2-(5,7-difluoro-1-methoxycarbonyl-3-indolyl)propionate (**8**). ^{1}H-NMR (200 MHz) δH (An-d_6): 1.57 (3H, d, $J=7.1$ Hz), 3.66 (3H, s), 4.02 (1H, q, $J=7.1$ Hz), 4.03 (3H, s), 7.00 (1H, ddd, $J=12.0, 9.7, 2.2$ Hz), 7.25 (1H, dd, $J=8.5, 2.2$ Hz), 7.73 (1H, s); MS (relative intensity, %) m/z: 297 (M$^+$, 33), 238 (100), 194 (70).

2.1.6 2-(5,7-Difluoro-3-indolyl)propionic acid (5,7-F$_2$-IPA, 1)

A 40% solution (2 ml) of potassium hydroxide in water was added to a solution of **8** (20.0 mg, 67.3 μmol) in methanol (10 ml), and the whole stirred at 70°C for 1 hr. The reaction mixture was cooled to room temperature and then condensed *in vacuo* to an aqueous solution. This solution was acidified with 1 N HCl solution and treated three times with ethyl acetate. The combined ethyl acetate layer was washed with water and saturated brine, dried over anhydrous sodium sulfate and then evaporated *in vacuo* giving the crude acid. It was purified by preparative thin layer chromatography on a silica gel yielding 14.5 mg (95.7% yield) of 2-(5,7-difluoro-3-indolyl)propionic acid (5,7-F$_2$-IPA, **1**). ^{1}H-NMR (200 MHz) δH (An-d_6): 1.57 (3H, d, $J=7.1$ Hz), 4.00 (1H, q, $J=7.1$ Hz), 6.80 (1H, ddd, $J=11.0, 9.5, 2.2$ Hz), 7.20 (1H, dd, $J=9.3, 2.2$ Hz), 7.42 (1H, d, $J=1.8$Hz); MS (relative intensity, %) m/z: 225 (M$^+$, 33), 180 (100).

2.2 2-(5,7-Difluoro-3-indolyl)isobutyric acid (5,7-F$_2$-IIBA, 2)

2.2.1 Methyl 2-(5,7-difluoro-1-methoxycarbonyl-3-indolyl)-isobutyrate (9)

A solution of **8** (20.0 mg, 67.3 $\mu\mu$ol) in THF (2.0 ml) was injected by syringe through the rubber septum to a two-neck, round-bottom flask. A THF solution of LDA (0.3 ml, 1.5 equiv) was added dropwise by syringe at −78°C to the propionate solution. After the mixture had been stirred for 1 hr, a solution of methyl iodide (0.2 ml) in THF was added dropwise by syringe at −78°C, and the whole stirred for 2 hr. This reaction mixture was poured into a mixture of diethyl ether and aqueous ammonium chloride solution. The aqueous layer was treated three times with diethyl ether. The ether layer was washed with water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give the crude ester. It was purified by preparative thin layer chromatography on silica gel giving 2.5 mg (11.9% yield) of methyl 2-(5,7-difluoro-1-methoxycarbonyl-3-indolyl) isobutyrate (**9**). ^{1}H-NMR (200 MHz) δH (An-d_6): 1.64 (6H, s), 3.65 (3H, s), 4.02 (3H, s), 6.96 (1H, ddd, $J=11.3, 9.5, 2.2$ Hz), 7.23 (1H, dd, $J=10.0, 2.2$ Hz), 7.75 (1H, s); MS (relative intensity, %) m/z: 311 (M$^+$, 15), 252 (100), 208 (85).

2.2.2 2-(5,7-Difluoro-3-indolyl)isobutyric acid (2)

A solution of potassium hydroxide (300 mg, 5.2 mmol) in water (1 ml) was added to a solution of **9** (2.5 mg, 8 μmol) in methanol (4 ml), and the whole stirred at 70°C for 1 hr. The reaction mixture was cooled to room temperature and condensed *in vacuo* to an aqueous solution which was acidified with

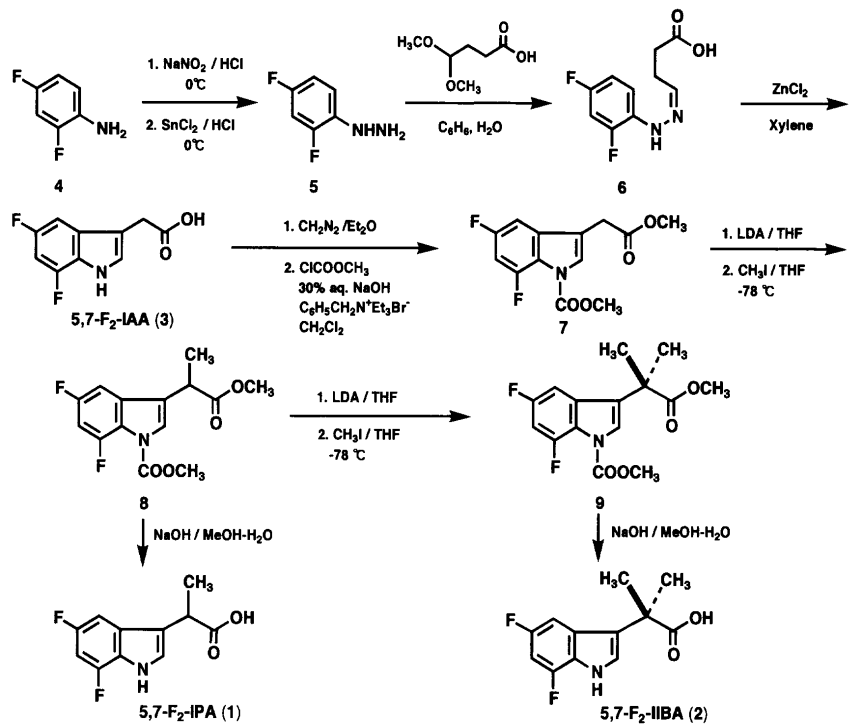

Fig. 2 Synthetic scheme for 5,7-F$_2$-IPA (**1**) and 5,7-F$_2$-IIBA (**2**).

1 N HCl solution and treated three times with ethyl acetate. The combined ethyl acetate layer was washed with water and saturated brine, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give the crude acid. It was purified by preparative thin layer chromatography on silica gel giving 1.7 mg (88.5% yield) of 2-(5,7-difluoro-3-indolyl)isobutyric acid (5,7-F$_2$-IIBA, **2**). ^{1}H-NMR (200 MHz) δH (An-d_6): 1.65 (6H, s), 6.79 (1H, ddd, J=9.5, 9.5, 2.0 Hz), 7.20 (1H, ddd, J=10.0, 2.0, 2.0 Hz), 7.41 (1H, d, J=1.8 Hz); MS (relative intensity, %) m/z: 239 (M$^+$, 32), 194 (100).

2.3 2-(5,7-Dichloro-3-indolyl)propionic acid

Synthesis of this compound will be reported elsewhere.

3. Plant Material and Bioassay

The plant species (*Avena sativa* L. cv. Victory-1) used for the bioassays was the same as that used in previous studies, and the bioassays were as reported there.[1,2] Two duplicate experiments were done in each bioassay.

RESULTS AND DISCUSSION

2-(5,7-Difluoro-3-indolyl)propionic acid (5,7-F$_2$-IPA, **1**) and 2-(5,7-difluoro-3-indolyl)isobutyric acid (5,7-F$_2$-IIBA, **2**) were synthesized from 5,7-difluoroindole-3-acetic acid (5,7-F$_2$-IAA, **3**) which was prepared from 2,4-difluoroaniline (**4**) as a starting material[2] (Fig. 2). *N*-Protected 5,7-F$_2$-IAA methyl ester was lithiated followed by monomethylation with methyl iodide which gave the carbamate ester (**8**). Direct double hydrolysis of **8** produced 5,7-F$_2$-IPA (**1**), and remethylation and subsequent double hydrolysis of **8** produced 5,7-F$_2$-IIBA (**2**). Yields of the methylated compounds in the two methylation steps were very low, possibly because of the semi-dried conditions used in the small scale synthesis.

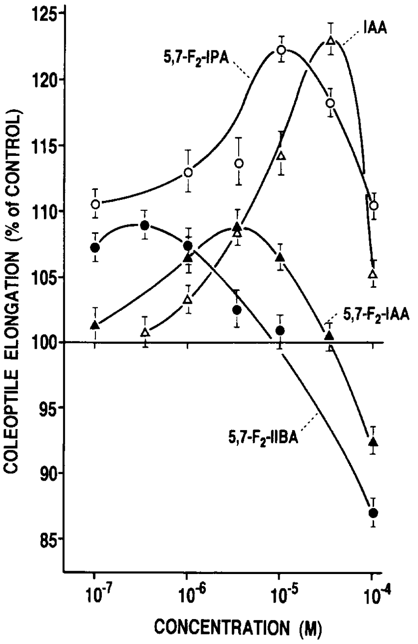

Fig. 3 Elongation of *Avena* coleoptiles by 5,7-F$_2$-IPA (**1**), 5,7-F$_2$-IIBA (**2**), 5,7-F$_2$-IAA and IAA.

The biological activities of synthetic 5,7-F$_2$-IPA (**1**) and 5,7-F$_2$-IIBA (**2**) were measured by the *Avena* coleoptile elongation test and compared with those of IAA and 5,7-F$_2$-IAA. In that test (Fig. 3), 5,7-F$_2$-IPA (**1**) had stronger auxin activity than IAA but unlike 5,7-F$_2$-IAA, did not have coleoptile growth inhibition activity at any concentration tested. The activity of 5,7-F$_2$-IPA (**1**) differed slightly from that of 5,7-F$_2$-IAA which had the coleoptile growth-inhibition activity

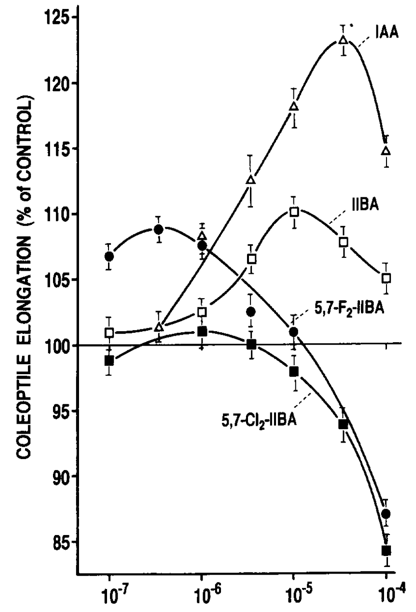

Fig. 4 Elongation of *Avena* coleoptiles by 5,7-F_2-IIBA (**2**), 5,7-Cl_2-IIBA, IIBA and IAA.

at a high concentration (1×10^{-4} M). The activity of 5,7-F_2-IPA also differed markedly from that of 2-(5,7-dichloro-3-indolyl)propionic acid (5,7-Cl_2-IPA) which has two chlorine instead of fluorine atoms and had very weak elongation activity at 3×10^{-6} M, but strong coleoptile growth-inhibition activity at the high concentration of 1×10^{-4} M (not shown in Fig. 3).[9] In contrast, at a high concentration (1×10^{-4} M), 5,7-F_2-IIBA had coleoptile growth-inhibition activity, but interestingly had weakly positive auxin activity at a low concentration of less than 3×10^{-6} M. These findings suggest that the introduction of a single methyl group to the α-position of the side chain of 5,7-F_2-IAA enhances *Avena* coleoptile elongation activity of 5,7-F_2-IAA but that the further introduction of a methyl group to the same position decreases the activity.

The activity of 5,7-F_2-IIBA (**2**) was compared with that of IIBA and 5,7-Cl_2-IIBA for *Avena* coleoptile elongation. Results are shown in Fig. 4. IIBA, which has no halogen atom in its molecule, had coleoptile elongation activity which was weaker than that of IAA, but did not have the elongation-inhibitory activity. Unlike 5,7-F_2-IIBA, 5,7-Cl_2-IIBA showed no coleoptile elongation activity over the entire range of concentrations tested. This indicates that the introduction of fluorine rather than chlorine atoms to the 5 and 7 positions of the indole nucleus results in a weak auxin activity at the low concentration less than 3×10^{-6} M and an antiauxin activity at high concentration more than 1×10^{-5} M. The difference in the activities of 5,7-F_2-IIBA and 5,7-Cl_2-IIBA may be due to a difference in the electronegativities or lipophilicities of fluorine and chlorine.

5,7-Cl_2-IIBA acts as an antiauxin because it inhibits auxin-mediated elongation of the *Avena* coleoptile.[2] Its inhibition activity in auxin-mediated elongation was measured by an addition of 5,7-F_2-IIBA (10^{-5} to 10^{-4} M) to bioassay media containing 10^{-5} M of IAA and the activities of antiauxins, 5,7-Cl_2-IIBA and 2-(*p*-chlorophenoxy)isobutyric acid (PCIB) were compared. Results are shown in Fig. 5. At 10^{-5} M, 5,7-F_2-IIBA had about 80% of the elongation of *Avena* coleoptiles induced by IAA. The promotive effect of exogeneous IAA was reversed at 3×10^{-5} M (about three times the molar concentration of exogeneous IAA), but at more than 5.6×10^{-5} M there was no elongation. The inhibitory activity of 5,7-F_2-IIBA was slightly weaker than that of 5,7-Cl_2-IIBA for IAA-induced elongation of *Avena* coleoptiles. These results demonstrate that 5,7-F_2-IIBA is a new fluorinated antiauxin with activity slightly weaker than that of 5,7-Cl_2-IIBA but much stronger than that of PCIB.

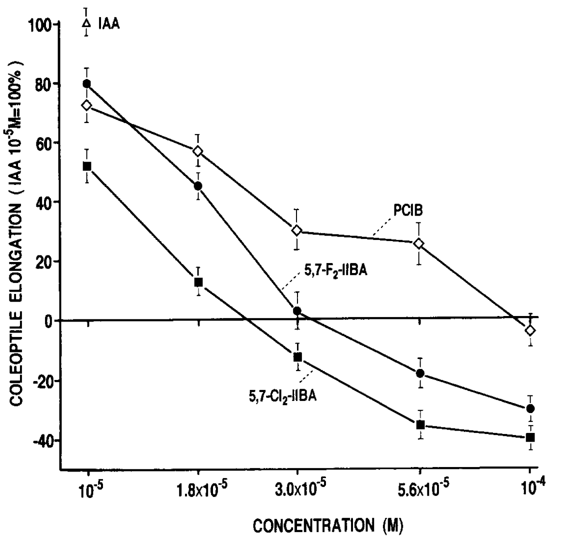

Fig. 5 Inhibitory activity of 5,7-F_2-IIBA (**2**), 5,7-Cl_2-IIBA and PCIB in IAA-mediated elongation of *Avena* coleoptiles.

5,7-F_2-IIBA and 5,7-Cl_2-IIBA are new antiauxins that have the indole nucleus. In contrast, 4-Cl-IAA, 5,6-Cl_2-IAA, 5,6-F_2-IAA, 5,7-F_2-IPA and IAA, which also have indole nucleus, are indolic auxins. The combined use of these 5,7-dihalogenated indolic antiauxins and indolic auxins in physiological studies of plants may prove very useful.

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要　　約

2-(5,7-ジフルオロ-3-インドリル)プロピオン酸（5,7-F_2-IPA）および 2-(5,7-ジフルオロ-3-インドリル)イソ酪酸（5,7-F_2-IIBA）の合成とそれら各々のオーキシンおよびアンチオーキシン活性

加藤康仁，片山正人，丸茂晋吾

2-(5,7-ジフルオロ-3-インドリル) プロピオン酸 (5,7-F_2-IPA, **1**) および 2-(5,7-ジフルオロ-3-インドリル) イソ酪酸 (5,7-F_2-IIBA, **2**) を 5,7-ジフルオロインドール-3-酢酸 (5,7-F_2-IAA) から官能基の保護，メチル化および脱保護化を行って合成した．アベナ子葉鞘伸長試験の結果，5,7-F_2-IPA は IAA より強い伸長活性を示したが，子葉鞘伸長阻害活性を示さなかった．5,7-F_2-IIBA は 1×10^{-4} M で子葉鞘伸長阻害活性を示したが，興味あることに 3×10^{-6} M 以下の濃度では弱いポジティブなオーキシン活性を示した．更に 5,7-F_2-IIBA は IAA 誘導のアベナ子葉鞘伸長において 5,7-Cl_2-IIBA より若干弱い活性を示す新規なアンチオーキシンであることが示された．