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5,6-Dichloroindole-3-acetic acid as a potent auxin: its synthesis and biological activity

T. Hatano, M. Katayama and S. Marumo

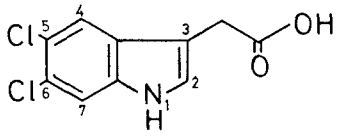
Department of Agricultural Chemistry, Nagoya University, Chikusa-ku, Nagoya 464 (Japan), 28 April 1987

Summary. 5,6-Dichloroindole-3-acetic acid (**1**), a new auxin, has been synthesized by Fischer's indolization. It showed the strongest auxin activity among all the known natural and synthetic auxins in three bioassays (elongation of *Avena* coleoptiles, hypocotyl growth inhibition of Chinese cabbage, and hypocotyl swelling of mung bean seedlings). It induced many lateral roots in mung bean seedlings, and resisted peroxidase-catalyzed decomposition.
Key words. 5,6-Dichloroindole-3-acetic acid; indole-auxin; *Avena* elongation; hypocotyl swelling; lateral root formation; peroxidase oxidation.

Our idea for the synthesis of chloro-derivatives of indole-3-acetic acid originated from our previous isolation of 4-chloroindole-3-acetic acid(4-Cl-IAA) and its methyl ester from immature seeds of *Pisum sativum*[1,2]. The isolated 4-Cl-IAA showed strong auxin activity as compared with that of IAA, e.g., 10-fold for the elongation of *Avena* coleoptiles[3,9], 100-fold for the root growth inhibition of Chinese cabbage[3], and more than 100-fold for the hypocotyl swelling of mung bean[3]. The high biological activity of 4-Cl-IAA was partly ascribed to its relative lack of susceptibility to peroxidase oxidation[3]. Fox and Bullock synthesized monochloro-derivatives of IAA including 4-Cl-IAA[4], and Porter and Thimann found that 4- and 6-Cl-IAAs were more active than 5-, 7- and 2-Cl-IAAs[5]. Biological activity of monochloro-IAAs were also investigated by Hoffmann et al.[6], Sell et al.[7], Engvild[8], Böttger et al.[9] and Katekar and Geissler[9,10]. Engvild synthesized dichloro-IAAs, e.g., 4,6-, 4,7-, 5,7- and 6,7-Cl_2-IAAs[12]. Auxin activity of his synthesized dichloro-IAAs was, however, shown to be rather weak as compared with that of IAA[8-11]. Cohen and co-workers reported synthesis of a mixture of 5,6- and 4,5-Cl_2-IAAs by Fischer's indolization, but they did not separate the mixture into the isomers[13]. Therefore, the physicochemical properties as well as the biological activities of these two dichloro-IAAs remained unclear.

We have now synthesized all isomers of dichloro-IAAs in a pure form, and measured their auxin activity. As described below, 5,6-Cl_2-IAA (**1**) showed much stronger activity than any of the other dichloro-isomers and of typical auxins such as IAA, 4-Cl-IAA, 2,4-D and NAA.

5,6-Cl_2-IAA (**1**) was synthesized by Fischer's indolization. 3,4-Dichlorophenylhydrazine hydrochloride was coupled with 4,4-dimethoxybutyric acid (prepared from 4,4-dimethoxybutyronitrile by alkaline hydrolysis followed by acidification) in a benzene-water (v/v = 4/1) solution under reflux for 1.5 h to afford the hydrazone in 89% yield. The hydrazone was then subjected to Fischer's indolization by heating at 100 °C with anhydrous zinc chloride in dry xylene for 2 h. This gave a mixture of 5,6- and 4,5-Cl_2-IAAs in 37% yield. The mixture was separated by column chromatography on silica gel using a solvent system of ethyl acetate in *n*-hexane to give pure 5,6- and 4,5-Cl_2-IAAs in a ratio of 5:4. 5,6-Cl_2-IAA: m.p. 189–191 °C; ^{1}H NMR spectrum (acetone-d_6, TMS, ppm) 3.74(2H, doublet, J = 1Hz), 7.40 (1H, broad singlet), 7.60 (1H, singlet), 7.77(1H, singlet); mass spectrum



(1)

Figure 1. The structure of 5,6-dichloroindole-3-acetic acid.

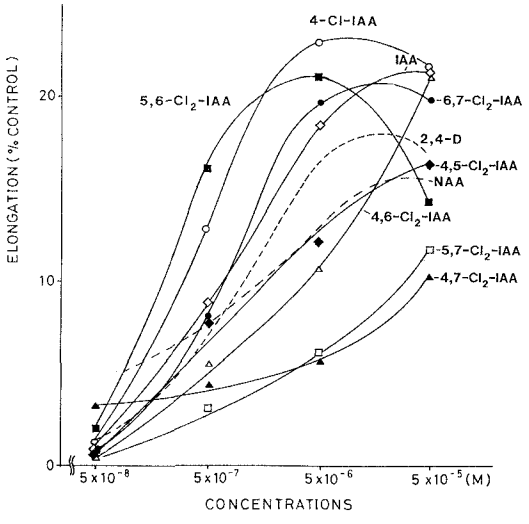


Figure 2. Elongation of *Avena* coleoptiles with dichloro-IAAs, 4-Cl-IAA, IAA, 2,4-D and NAA. Coleoptile segments (5 mm length) that were cut from 2 mm below the tip of seedlings of *Avena sativa* cv. Victory-1, grown under red light for 2 days and then in the dark for 1 day, were incubated in a sample-containing aqueous solution (2 ml) in the dark at 25 °C for 16 h, and the increased length of coleoptiles was measured[16].

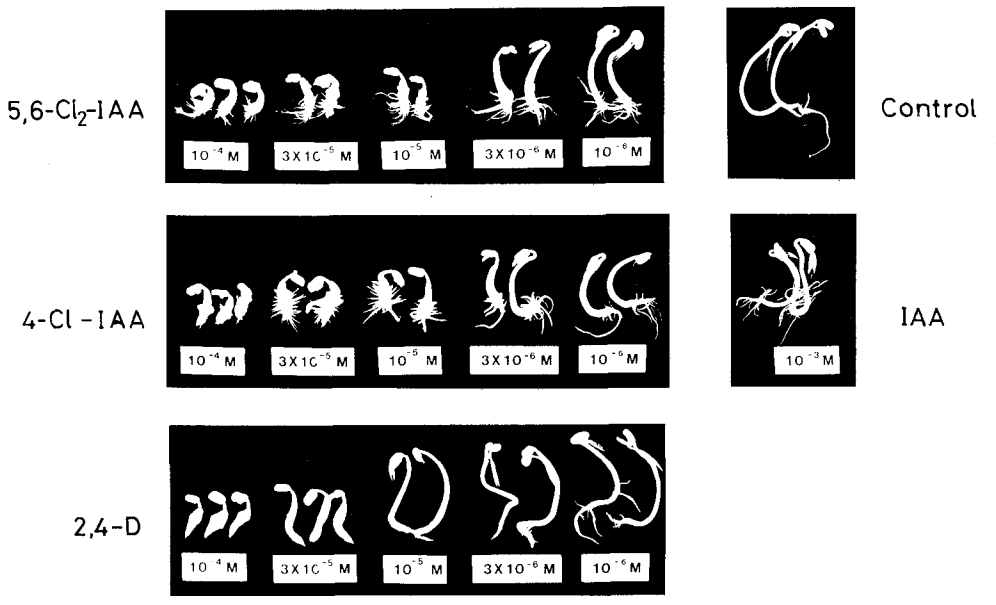


Figure 3. Hypocotyl swelling and lateral root formation of mung bean by 5,6-Cl_2-IAA, 4-Cl-IAA and 2,4-D. Mung bean seeds preliminarily grown on a wet cotton sheet in a Petri dish (diameter 6 cm) for 1 day, were incubated in a sample-containing aqueous solution (3 ml) in the dark at 25 °C for 72 h, and the hypocotyl swelling and lateral root formation were observed[3].

(75ev, relative intensity (%)) m/z 243 (M^+, 37), 245 (M^++2, 25), 247 (M^++4, 6), 198 (100), 200 (70), 202 (14); IR spectrum ν_{max} (KBr) (cm^{-1}) 3420, 2950, 1703, 1455, 1400, 1300, 1245, 1225, 1210, 1100 and 860.

4,5-Cl_2-IAA: m.p. 200–203 °C; ^1H NMR spectrum (acetone-d_6, TMS, ppm) 3.98 (2H, singlet), 7.21 (1H, doublet, J = 8.4 Hz), 7.39 (1H, doublet, J = 8.4 Hz), 7.41 (1H, singlet); mass spectrum (75 ev, relative intensity (%)) 243 (M^+, 39), 245 (M^++2, 26), 247 (M^++4, 5), 198 (100), 200 (65), 202 (13); IR spectrum ν_{max}(KBr) (cm^{-1}) 3440, 2900, 1695, 1460, 1425, 1400, 1315, 1225, 1210, 1160, 955, 850 and 780.

Other dichloro-IAAs, i.e., 4,6-, 4,7-, 5,7- and 6,7-Cl_2-IAAs, were similarly synthesized by coupling each of corresponding dichloro-stubstituted phenylhydrazines with 4,4-dimethoxybutyric acid under the reaction conditions employed in the synthesis of 5,6-Cl_2-IAA.

Biological activities of 5,6-Cl_2-IAA and of the other dichloro-IAAs were measured using three kinds of auxin bioassays. The result of the bioassay with the elongation of coleoptiles of *Avena sativa* is shown in figure 2. 5,6-Cl_2-IAA reached maximal activity at 5×10^{-6} M, the lowest concentration of all compounds tested. 4-Cl-IAA was also very active in this bioassay. 6,7-Cl_2-IAA was comparable in activity to IAA, but 4,6- and 4,5-Cl_2-IAAs were weaker than IAA. 5,7- and 4,7-Cl_2-IAAs showed very weak activity. It has been suggested that these compounds are anti-auxins[9]. We also observed that 5,7-Cl_2-IAA induced negative geotropism in growing roots of rice seedlings, one of the typical anti-auxin activities.

5,6-Cl_2-IAA induced both severe swelling and formation of numerous lateral roots in seedlings of mung bean, as shown in figure 3. The activity was quite similar to that induced by 4-Cl-IAA and also by IAA. 5,6-Cl_2-IAA was more than one thousand-fold more effective than IAA. Interestingly, 2,4-D

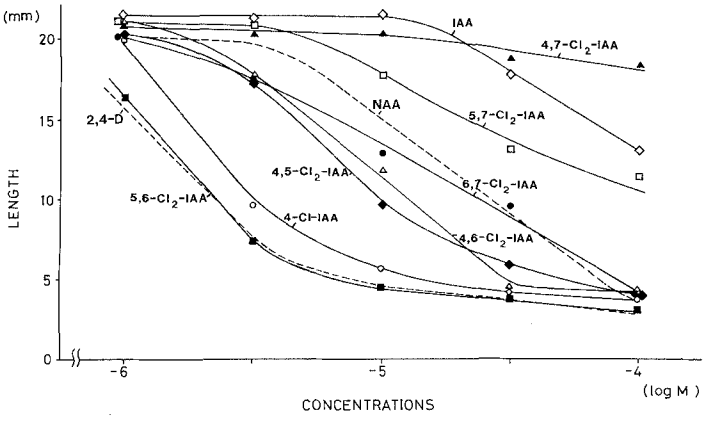


Figure 4. Inhibition of the hypocotyl growth of Chinese cabbage by dichloro-IAAs, 4-Cl-IAA, IAA, 2,4-D and NAA. Chinese cabbage seeds, preliminarily grown on a filter paper in Petri dish (diameter 6 cm) for 1 day, were incubated in a sample-containing aqueous solution (3 ml) in the dark at 25 °C for 72 h, and the lengths of the hypocotyls were measured[17].

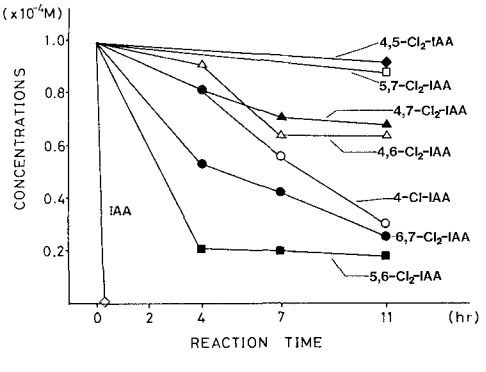


Figure 5. The rate of decomposition of 5,6-Cl_2-IAA, 4-Cl-IAA and other dichloro-isomers as a substrate at 1.0×10^{-4} M with horseradish peroxidase at 4.0×10^{-8} M.

behaved differently in this bioassay since it induced only swelling but no formation of lateral roots. 4,5-, 4,6- and 6,7-Cl_2-IAAs also induced lateral root formation, but their activity was much weaker than that of 5,6-Cl_2-IAA. 5,6-Cl_2-IAA also exhibited the strongest activity with regard to the inhibition of the hypocotyl growth of intact seedlings of Chinese cabbage, as shown in figure 4. Its activity was about 100-fold stronger than that of IAA and comparable to that of 2,4-D.

We examined the susceptibility of 5,6-Cl_2-IAA to peroxidase. Peroxidase is considered to be the major enzyme for the decomposition of endogenous auxin in plants[14], and we have previously shown that the potent activity of 4-Cl-IAA may be partly ascribed to its resistance to peroxidase decomposition[3]. The decomposition rate of 5,6-Cl_2-IAA and other dichloro-IAAs with horseradish peroxidase was measured by Meudt's procedure[15]. When the auxins at 1.0×10^{-4} M were treated with 1.3×10^{-10} M peroxidase, all of the dichloro-IAAs tested survived an 11-h incubation, in contrast to IAA which was rapidly degraded within 2 h. When the auxins at 1.0×10^{-4} M were treated with 4.0×10^{-8} M peroxidase, six isomers of dichloro-IAAs and 4-Cl-IAA decomposed, as shown in figure 5. 5,6-Cl_2- and 4-Cl-IAAs with strong auxin activity decomposed more rapidly than other dichloro-IAAs with weaker activity. These data indicate that the position and number of chlorine atoms on the indole nucleus is more important for differences in auxin activity than the resistance to peroxidase decomposition.

5,6-Cl_2-IAA has thus been shown in three kinds of bioassay to be the most active auxin among all of the known natural and synthetic auxins so far examined. It contains an indole nucleus just like endogenous auxin. Because of its increased resistance to peroxidase degradation, as compared to IAA, 5,6-Cl_2-IAA might be useful in long term experiments with plant tissue culture or with intact plants. In particular, it may be promising for the regeneration of plants from cultured cells that have been difficult to regenerate with the hitherto known auxins such as NAA or 2,4-D and phenoxyacetic acid derivatives.

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17 The bioassy has been routinely conducted in our laboratory in order to examine the long term activity of auxins on the growing intact plants. An extent of the hypocotyl inhibition is in proportion to the amount of 2,4-D and 4-Cl-IAA in a concentration range of 10^{-6} to 10^{-4} M.

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Announcements

Italy

Vth International Symposium on Bioluminescence and Chemiluminescence

Florence, 25–29 September 1988

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The Ernest Witebsky Center for Immunology will present this symposium in its regular biennial series at the Hyatt Regency Buffalo Hotel. Closed plenary sessions will focus on the topics: Basic immunologic considerations; Immunologically responsive tissue cells; Immunopathologic conditions (dental caries; periodontal disease; inflammatory bowel disease; celiac disease; gastrointestinal infections and infestations); Immune response in oral and gastrointestinal neoplasms; Nutritional effects on the immune response; and Development of vaccines. Open poster sessions for free contributions on the theme will be offered.

For further information contact: Dr. James F. Mohn, Director, The Ernest Witebsky Center for Immunology, 240 Sherman Hall, State University of New York at Buffalo, Buffalo, New York 14214 (Telephone: 716-831-2848).