

ruptive cryptic colouration to minimize predation. This hypothesis (6) has the highest and most significant partial correlation with the generalized pattern and all individual characters, except for the number of blue blotches. Lower, but significant, partial correlations are found between hypothesis 7 and the size and number of yellow cross bars and also the generalized pattern, so one cannot reject the hypothesis of substantial introgression (or a more gradual transition in the ecotones) in these cases. The rejected allopatry hypotheses (1–4) are also unlikely on other grounds. The time factor for hypotheses 1–3 is gross in relation to the relatively subtle variation²; the populations are in fact not separated into allopatric altitudinal categories (hyp. 4); the degree of incongruence between character systems is unlikely for allopatric (phylogenetic) causation^{1, 2, 5}; and the latitudinal variation in the herpetofauna of adjacent islands cannot be due to a common historical event as each island has its own geological history.

This study of geographic variation within an island provides an example of how complex phenotypic features may be a compromise between specific selective forces, and raises, for the first time, the possibility of climatic factors influencing the balance between sexual selection and crypsis. The balance between sexual selection and natural selection is also thought to cause geographic variation in the colour pattern of guppies^{9, 22–24}. In the guppy case multiple alternative hypotheses have been tested, but not simultaneously⁹. It can be particularly difficult to distinguish between causative factors in geo-

graphic variation analysis^{1, 5} but the relatively simple procedure used in this study proved to be very effective for simultaneously testing multiple hypotheses when the patterns are unidimensional.

Acknowledgment. We wish to thank the Royal Society, the Consejo Superior Investigaciones Científicas and the Carnegie Trust for grant support (to R.S.T.), and Marcos Baez for his assistance.

- 1 Thorpe, R. S., *Evolution* 38 (1984) 233.
- 2 Thorpe, R. S., and Baez, M., *Evolution* 41 (1987) 256.
- 3 Pasteur, G., and Salvidio, S., *Bonn. zool. Beitr.* 36 (1985) 553.
- 4 Bischoff, W., *Bonn. zool. Beitr.* 33 (1982) 362.
- 5 Thorpe, R. S., *Boll. Zool.* 54 (1987) 3.
- 6 Thorpe, R. S., *Biol. Revs* 51 (1976) 407.
- 7 Thorpe, R. S., *Syst. Zool.* 34 (1985) 127.
- 8 Thorpe, R. S., *Biol. J. Linn. Soc.* 13 (1980) 7.
- 9 Douglas, M. E., and Endler, J. A., *J. theor. Biol.* 99 (1982) 777.
- 10 McKechnie, S. W., et al., *Genetics* 81 (1975) 571.
- 11 Dillon, R. T., *Syst. Zool.* 33 (1984) 69.
- 12 Sokal, R. R., *Syst. Zool.* 28 (1979) 227.
- 13 Dietz, E. J., *Syst. Zool.* 32 (1983) 21.
- 14 Manly, B. F. J., *Res. Popul. Ecol.* 28 (1986) 201.
- 15 Borley, G. D., *Proc. geol. Assoc.* 85 (1974) 259.
- 16 Thorpe, R. S., *Proc. R. Soc. Edin.* 78 B (1979) 1.
- 17 Thorpe, R. S., *Z. zool. Syst. Evolut. Forsch.* 25 (1987) 161.
- 18 Szymura, J. M., et al., *Experientia* 41 (1985) 1469.
- 19 Huetz de Lemps, A., *Le climat des Iles Canaries*, vol. 54. *Facul. Lett. Sci. Hum. Paris-Sorbonne, France* 1969.
- 20 Kammer, F., *Scr. Geobot.* 7 (1974) 1.
- 21 Endler, J. A., *Biol. J. Linn. Soc.* 22 (1984) 187.
- 22 Endler, J. A., *Evol. Biol.* 11 (1978) 319.
- 23 Endler, J. A., *Evolution* 34 (1980) 76.
- 24 Endler, J. A., *Envir. Biol. Fish.* 9 (1983) 173.

0014-4754/89/040397-04\$1.50 + 0.20/0

© Birkhäuser Verlag Basel, 1989

A new indolic antiauxin, α -(5,7-dichloroindole-3)-isobutyric acid: its chemical synthesis and biological activity

T. Hatano, Y. Kato, M. Katayama and S. Marumo*

Department of Agricultural Chemistry, Nagoya University, Nagoya 464-01 (Japan)

Received 23 August 1988; accepted 29 November 1988

Summary. A new potent antiauxin, α -(5,7-dichloroindole-3)-isobutyric acid has been synthesized and shown to inhibit auxin-mediated elongation of *Avena* coleoptiles and to stimulate root growth of rice seedlings. Its activity is stronger than α -(*p*-chlorophenoxy)isobutyric acid and is comparable to that of 2,3,5-triodobenzoic acid, which are typical antiauxins.

Key words. α -(5,7-Dichloroindole-3)-isobutyric acid; antiauxin; inhibition of *Avena* coleoptile elongation; stimulation of root growth of rice seedlings.

We have recently shown that 5,6-dichloroindole-3-acetic acid (5,6-Cl₂-IAA, **1**) is the most potent of all the known natural and synthetic auxins¹. Our synthetic studies on chlorinated indole auxins have been based on our previous isolation of 4-chloroindole-3-acetic acid (4-Cl-IAA) and its methyl ester from immature seeds of *Pisum sativum*^{2, 3}. All the regioisomers of ten dichlorinated and

five monochlorinated indole-3-acetic acid derivatives have been synthesized, and their auxin activities bioassayed by their effect on the elongation of *Avena* coleoptiles.

One of the notable results of this bioassay was that the 5,7- and 4,7-dichloroindole-3-acetic acids (5,7-Cl₂-IAA and 4,7-Cl₂-IAA) showed much weaker activity than

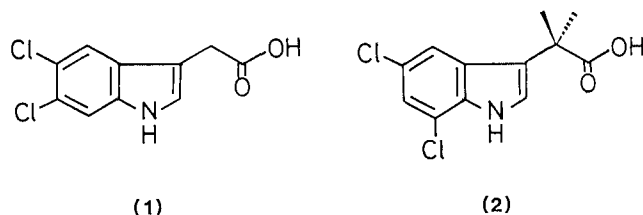


Figure 1. Structures of 5,6-dichloroindole-3-acetic acid (1) and α -(5,7-dichloroindole-3-yl)isobutyric acid (2).

the other chlorinated indole derivatives¹. Interestingly, when wheat and rice seeds were germinated with 5,7-Cl₂-IAA their roots curled markedly upwards. This negative geotropism⁴ induced in growing roots by the action of 5,7-Cl₂-IAA is clearly an antiauxin activity. For the elongation of *Avena* coleoptiles, 5,7-Cl₂-IAA showed weak stimulating activities at low concentrations. It appears to be unique in having both auxin-like and antiauxin activities.

In an attempt to eliminate its auxin-like activity, we have structurally modified the 5,7-Cl₂-IAA molecule. Changing the side chain from the acetyl to the isobutyryl group is known to change the biological activity of some synthetic auxins to antiauxin, as in the change of 2,4-dichlorophenoxyacetic acid (2,4-D) to α -(*p*-chlorophenoxy)isobutyric acid (PCIB)⁵. We chemically converted 5,7-Cl₂-IAA to α -(5,7-dichloroindole-3-yl)isobutyric acid (5,7-Cl₂-IIBA, 2).

5,7-Cl₂-IAA was synthesized as described previously by coupling 2,4-dichlorophenylhydrazine with 4,4-dimethoxybutyric acid; then subjecting the product to the Fischer indole synthesis¹. 5,7-Cl₂-IAA was methylated with diazomethane to the methyl ester, the indole-nitrogen of which was then protected as methylcarbamate by treatment with methyl chloroformate in the presence of benzyltriethylammonium bromide (phase transfer catalyst) in a mixed solution of methylene chloride and 30% aqueous sodium hydroxide; yield 96%. The acetyl side chain of the ester-carbamate was double-methylated by treatment with lithium diisopropylamide in THF followed by the addition of CH₃I at -78°C. This gave a monomethylated product that was similarly methylated, and the dimethylated product in 48% yield. The protecting ester and carbamate groups were removed by alkaline hydrolysis which left the desired 5,7-Cl₂-IIBA in 78% yield. 5,7-Cl₂-IIBA: m.p. 145–147°C; ¹H-NMR spectrum (acetone-d₆, TMS, ppm) 1.66 (1 H, singlet), 7.19 (1 H, double doublet, *J* = 0.4, 1.7 Hz), 7.42 (1 H, doublet, *J* = 2.6 Hz), 7.63 (1 H, double doublet, *J* = 0.6, 1.7 Hz); mass spectrum (75 eV, relative intensity (%)) *m/z* 271 (*M*⁺, 24), 273 (*M*⁺ + 2, 15), 275 (*M*⁺ + 4, 3), 226 (100), 228 (67), 230 (12), 191 (32), 193 (12); IR spectrum ν_{\max} (KBr) (cm⁻¹) 3480, 2980, 1700, 1560, 1470, 1280, 1260, 1160, 1100 and 860.

The biological activity of synthetic 5,7-Cl₂-IIBA was measured with two auxin bioassays: the elongation test

using *Avena* coleoptiles and the root growth-inhibition test using rice seedlings. In the elongation test with *Avena* coleoptiles (fig. 2), 5,7-Cl₂-IIBA produced no coleoptile elongation for the entire range of concentrations tested. This indicates that it had completely lost auxin activity, even at low concentrations. At high concentrations it severely inhibited the growth of coleoptiles; at 10⁻⁴ M they remained the original length of the excised segments. In the test on rice seedlings grown under 8 h light-16 h dark photoperiod conditions, 5,7-Cl₂-IIBA released the suppression of growth of roots caused by endogenous IAA. At 10⁻⁶ M it enhanced both the weight (136% as dry matter) and length (126%) of roots, as compared with values (100%) for control plants without chemicals. A slight but significant stimulating effect was also observed on the growth of shoots; an increase of the weight (113%) and height (108%) were obtained at 10⁻⁶ M. This enhancing effect may be similar to that of PCIB, which has been reported to stimulate root growth of wheat seedlings considerably⁵. In contrast, 2,3,5-triiodobenzoic acid (TIBA), another antiauxin, did not stimulate root growth but rather induced upcurling of roots as did 5,7-Cl₂-IAA in our experiments. Neither 5,7-Cl₂-IIBA nor PCIB showed a clear tendency to cause upcurling during root growth of rice seedlings.

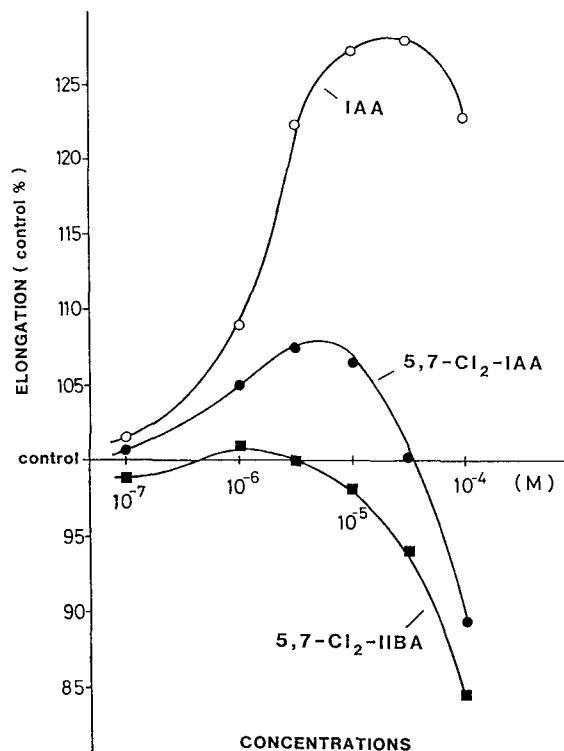


Figure 2. Elongation of *Avena* coleoptiles with 5,7-Cl₂-IIBA, 5,7-Cl₂-IAA and IAA. Coleoptile segments (5 mm length) that were cut from 2 mm below the tip of seedlings of *Avena sativa* L. cv. Victory-1, grown under red light for two days and then in the dark for 1 day, were incubated in an aqueous solution (2 ml) of the various compounds in the dark at 25°C for 16 h, and the increased length of coleoptiles was measured.

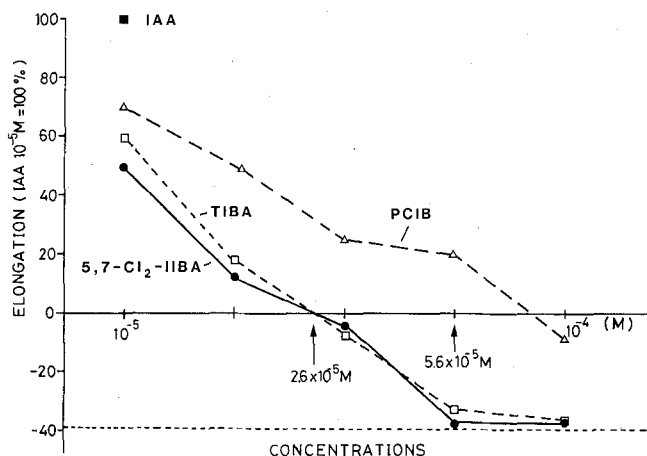


Figure 3. Antiauxin activity of 5,7-Cl₂-IIBA, PCIB and TIBA. Coleoptile segments prepared as described in the legend of figure 2 were incubated in a solution of IAA (10⁻⁵ M) containing the different antiauxins in the dark at 25°C. After 16 h incubation, the inhibition of IAA-stimulated coleoptile elongation was measured.

5,7-Cl₂-IIBA acted as antiauxin since it inhibited auxin-mediated elongation of *Avena* coleoptiles. 5,7-Cl₂-IIBA (10⁻⁵ to 10⁻⁴ M) was added to bioassay media containing 10⁻⁵ M of IAA. Its activity was compared to those of PCIB and TIBA. Results are shown in figure 3. At 10⁻⁵ M, 5,7-Cl₂-IIBA decreased IAA-induced elongation of *Avena* coleoptiles by ca 50%. At 2.6 × 10⁻⁵ M (about 3 times the molar concentration of IAA), all the promotive effect of exogenous IAA was reversed. At 5.6 × 10⁻⁵ M, none of the excised coleoptile segments

grew. 5,7-Cl₂-IIBA is thus shown to be a new antiauxin, with an activity stronger than that of PCIB and comparable to TIBA in the *Avena* coleoptile inhibition test. The two latter compounds are typical antiauxins^{5,6}.

5,7-Cl₂-IAA and 5,7-Cl₂-IIBA are structurally similar in having an indole skeleton in which the two chlorine atoms are substituted. Interestingly, a shift of one chlorine atom from the 6- to 7- position of the indole reversed the compound's activity from auxin to antiauxin, but weak auxin-like activity remained at low concentrations. No similar effect of the shift of substituents has ever been reported for other types of chlorinated auxin-like compounds, such as chlorophenoxyacetic acid and chloronaphthaleneacetic acid derivatives.

The use of 5,7-Cl₂-IIBA as an antiauxin in addition to IAA, 4-Cl-IAA and 5,6-Cl₂-IAA in physiological studies of plants may prove to be a very useful tool for mapping auxin action in plants; in particular, in investigating the intrinsic target to which endogenous auxins bind as they develop hormonal activity.

- 1 Hatano, T., Katayama, M., and Marumo, S., *Experientia* 43 (1987) 1237.
- 2 Marumo, S., Abe, H., Hattori, H., and Munakata, K., *Agric. biol. Chem.* 32 (1968) 117.
- 3 Marumo, S., Hattori, H., Abe, H., and Munakata, K., *Nature* 219 (1968) 959.
- 4 Moore, R., and Evans, M. L., *Am. J. Bot.* 73 (1986) 574.
- 5 Bruström, H., *Physiol. Plant.* 3 (1950) 277.
- 6 Caiston, A. W., *Am. J. Bot.* 34 (1947) 356.

0014-4754/89/040400-03\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1989

Statement of Ownership, Management and Circulation (Required by 39 U.S.C. 3685).

(1) Title of publication: EXPERIENTIA. A. Publication No.: 0014-4754. (2) Date of filing: 10/1/88. (3) Frequency of issue: monthly. A. No. of issues published annually, 13. B. Annual subscription price, \$398.00. (4) Location of known office of publication: 44 Hartz Way, Secaucus, NJ 07094. (5) Location of the headquarters of general business offices of the publishers: Ringstrasse 39, CH-4106 Therwil/Basel, Switzerland. (6) Names and addresses of publisher, editor, and managing editor: Publisher: Birkhäuser Verlag AG, P.O. Box 133, CH-4010 Basel, Switzerland. Editor: Experientia, P.O. Box 133, CH-4010 Basel. Managing Editor: Dr. H. P. von Hahn, P.O. Box 133, CH-4010 Basel, Switzerland. (7) Owner: Birkhäuser Verlag AG, P.O. Box 133, CH-4010 Basel, Switzerland. (8) Known bondholders, mortgagees, and other security holders owning or holding 1 percent or more of total of bonds, mortgages or other securities: none. (9) The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes has not changed during preceding 12 months. (10) Extent and nature of circulation. A. Total no. copies printed (net press run): Average no. copies each issue during the preceding 12 months, 2000; no. copies single issue nearest to filing date, 1800. B. Paid circulation: 1. Sales through dealers and carriers, street vendors, and counter sales: Average no. copies each issue during preceding 12 months, none; no. copies single issue nearest to filing date, none. 2. Mail subscriptions: average no. copies each issue

during preceding 12 months, 532; no. copies single issue nearest to filing date, 516. C. Total paid circulation: average no. copies each issue during preceding 12 months, 532; no. copies single issue nearest to filing date, 516. D. Free distribution by airmail, carrier, or other means. 1. Samples, complimentary, and other free copies: average no. copies each issue during preceding 12 months, none; no. copies single issue nearest to filing date, none. 2. Copies distributed to news agents but not sold, none. E. Total distribution: average no. copies each issue during the preceding 12 months, 532; no. copies single issue nearest to filing date, 516. F. Office use, left-over, unaccounted, spoiled after printing: average no. copies each issue during preceding 12 months, none; no. copies single issue nearest to filing date, none. G. Total: average no. copies each issue during preceding 12 months, 532; no. copies single issue nearest to filing date, 516. I certify that the statements made by me above are correct and complete.

Matthauer

Edi Mazenauer
Director of Production