Concluding remarks

By providing a straightforward method for classifying the hydrogen-bond patterns is crystal structures, graph-set analysis permits a very simple means for comparing the structures of polymorphic systems. Differences in structural patterns may be readily identified. The application of graph-set analysis to the LGA system yielded significant differences between the hydrogen-bonding patterns only when rather high-order networks were considered. It also yielded a structural rationale for the significant molecular conformation differences between the two polymorphs and the relationship of those conformational differences to the crystal packing. The hydrogen-bond patterns, clearly defined and hierarchically ordered with the aid of graph theory, can also aid in the interpretation of the crystallization chemistry, including relative rates of growth and the influence of impurities. We consider this to be a very general approach to studying the packing patterns of hydrogen-bonded crystals, and look forward to its application to many additional systems.

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Structural Studies on 5-(*n*-Alkyl)-Substituted Derivatives of the Plant Hormone Indole-3-acetic Acid

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Abstract

The biological properties of the plant hormone (auxin) indole-3-acetic acid (IAA) and its ring-substituted derivatives have so far been rationalized by a number of contradictory hypotheses based on incomplete structural data deduced mainly by inspection of molecular models. To permit a more detailed insight into structure–activity correlations, we here compare the molecular structures of IAA and 5-(*n*-alkyl)indole-3-acetic acids (alkyl = methyl, ethyl,

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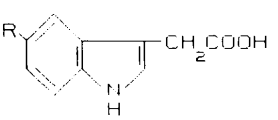
propyl, butyl), as revealed by X-ray crystallography and molecular-mechanics calculations, and their growth-promoting activity in a standard bioassay, the *Avena* mesocotyl test. The following molecular properties were identical for the compounds studied: The benzene part of the indole nucleus was slightly distorted around C7 (bond length C6—C7 = 1·37 Å; bond angle C6—C7—C71 = 117·4°), as previously observed for many other indole derivatives (statistical data included). The 5-(*n*-alkyl) residue was in an extended zigzag conformation with the C_α—C_β bond perpendicular to, and the C_β—C_γ bond pointing away from, the aromatic nucleus. Conformational differences were observed with respect to the orientation of the CH_2—carboxyl bond in the crystalline state: perpendicular to the indole ring plane for 5-methylindole-3-acetic acid (as previously found for IAA), and approximately in that plane for the other IAA derivatives studied. Crystal-packing influences appear to be responsible for this effect, as indicated by the results of molecular-mechanics calculations. The potential-energy-minimized conformations in the crystal lattice environment corresponded closely to those observed by X-ray analysis while, for the isolated molecules in the gas phase, perpendicular orientation of the carboxyl group was always predominant. The plant-growth-promoting properties of the compounds studied were not dramatically different. In particular, 5-methyl- and 5-(*n*-butyl)indole-3-acetic acids showed very similar activity. This indicates that a 5-substituent as bulky as *n*-butyl, in the orientation detailed above, does not sterically obstruct interaction with the auxin-binding protein(s) involved in the growth response. Crystal data at 297 K, using Mo $K\alpha$ radiation (λ = 0·7107 Å) are as follows: 5-methylindole-3-acetic acid, $C_{11}H_{11}NO_2$, monoclinic, $P2_1/a$, a = 10·048 (8), b = 5·382 (2), c = 17·525 (13) Å, β = 95·63 (4)°, V = 943 (1) Å^3, Z = 4, D_x = 1·333 g cm^{-3}, μ = 0·863 cm^{-1}, $F(000)$ = 400, R = 0·041 for 1162 symmetry-independent [$I \geq 3\sigma(I)$] reflections; 5-ethylindole-3-acetic acid, $C_{12}H_{13}NO_2$, monoclinic, $C2/c$, a = 41·986 (13), b = 7·954 (2), c = 6·347 (2) Å, β = 97·45 (2)°, V = 2102 (1) Å^3, Z = 8, D_x = 1·285 g cm^{-3}, μ = 0·820 cm^{-1}, $F(000)$ = 864, R = 0·044 for 930 symmetry-independent [$I \geq 3\sigma(I)$] reflections; 5-(*n*-propyl)indole-3-acetic acid, $C_{13}H_{15}NO_2$, monoclinic, $P2_1/a$, a = 6·281 (2), b = 8·022 (1), c = 23·205 (6) Å, β = 91·26 (1)°, V = 1169·0 (5) Å^3, Z = 4, D_x = 1·235 g cm^{-3}, μ = 0·778 cm^{-1}, $F(000)$ = 464, R = 0·052 for 726 symmetry-independent [$I \geq 2\sigma(I)$] reflections; 5-(*n*-butyl)indole-3-acetic acid, $C_{14}H_{17}NO_2$, monoclinic, $P2_1/a$, a = 6·332 (6), b = 8·116 (4), c = 24·924 (21) Å, β = 93·33 (4)°, V = 1279 (2) Å^3, Z = 4, D_x = 1·202 g cm^{-3}, μ = 0·749 cm^{-1}, $F(000)$ = 496, R = 0·058 for 757 symmetry-independent [$I \geq 3\sigma(I)$] reflections.

Introduction

A variety of derivatives and analogues of the plant hormone (auxin), indole-3-acetic acid (IAA), have been studied (Jönsson, 1961; Thimann, 1977; Schneider & Wightman, 1978), and a number of them have found application as herbicides and growth regulators in crop management (Gianfagna, 1987). To elucidate their mode of action, some of the macromolecular cell components involved, like the auxin-binding proteins, have been isolated and rapid progress has been made in their characterization (Jones, Lamerson & Venis, 1989; Hesse, Feldwisch, Balshüsemann, Bauw, Puype, Vandekerckhove, Löbler, Klämbt, Schell & Palme, 1989; Klämbt, 1990). It is thus unfortunate that for the auxins to be tested for interaction with those proteins only fragmentary structural data are available. The widely different biological activities of ring-substituted indole-3-acetic acids have been rationalized in terms of: (*a*) metabolic stability, (*b*) lipophilicity, (*c*) electron densities in the indole nucleus, and (*d*) stereochemistry. While factors (*a*) and (*b*) have been checked experimentally, it has been difficult, with the IAA derivatives so far available, to distinguish the effects of factors (*c*) and (*d*). This prompted us to prepare a series of 5-(*n*-alkyl)indole-3-acetic acids (alkyl = Me, Et, Pr, Bu), see scheme below, which



R = CH_3- 5-Me-IAA

R = CH_3CH_2- 5-Et-IAA

R = $CH_3(CH_2)_2$- 5-*n*-Pr-IAA

R = $CH_3(CH_2)_3$- 5-*n*-Bu-IAA

were expected to show near-identical electron densities in the indole nucleus, but different stereochemical properties. Indeed, while comparison of their UV and NMR spectra to those of unsubstituted IAA indicated minor electron redistribution in the indole ring as a result of 5-alkylation, no specific effects related to the length of the alkyl chain were detected (Ilić, Klaić, Magnus, Vikić-Topić & Gàcs-Baitz, 1991). Here we report on its stereochemistry and, in particular, on its influence on the orientation of the carboxyl group with respect to the indole ring plane. The results of X-ray crystallographic analysis are supplemented by molecular-mechanics calculations to assess possible, energetically more favorable, conformations. These data, and observations on the plant-growth-promoting properties of 5-(*n*-alkyl)indole-3-acetic acids, suggest reevaluation of some of the published theories on auxin structure–activity correlations (Kaethner, 1977; Katekar, 1979; Thimann, 1977) which were based on incomplete structural information deduced by inspection of molecular models.

Table 1. *Crystal data and summary of experimental details*

	5-Me-IAA	5-Et-IAA	5-*n*-Pr-IAA	5-*n*-Bu-IAA
Molecular formula	$C_{11}H_{11}NO_2$	$C_{12}H_{13}NO_2$	$C_{13}H_{15}NO_2$	$C_{14}H_{17}NO_2$
M_r	189·21	203·24	217·27	231·29
Crystal size (mm)	0·5 × 0·4 × 0·07	0·4 × 0·4 × 0·03	0·4 × 0·4 × 0·05	0·3 × 0·2 × 0·1
a (Å)	10·048 (8)	41·986 (13)	6·281 (2)	6·332 (6)
b (Å)	5·382 (2)	7·954 (2)	8·022 (1)	8·116 (4)
c (Å)	17·525 (13)	6·347 (2)	23·205 (6)	24·924 (21)
β ( )	95·63 (4)	97·45 (2)	91·26 (1)	93·33 (4)
V (Å^3)	943 (1)	2102 (1)	1169·0 (5)	1279 (2)
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/a$	$C2/c$	$P2_1/a$	$P2_1/a$
D_x (g cm^{-3})	1·333	1·285	1·235	1·202
Z	4	8	4	4
μ(Mo $K\alpha$) (cm^{-1})	0·863	0·820	0·778	0·749
F(000)	400	864	464	496
T (K)	297 (2)	297 (2)	297 (2)	297 (2)
No. of reflections used for cell parameters, θ range ( )	25, 8–15	25, 5 13	25, 5 16	25, 5 12
θ range for intensity measurement ( )	2 27	2 25	2 20	2 25
hkl range	0,12; 0,6; 22,22	0,50; 0,9; 7,7	0,6; 0,7; 24,24	0,7; 0,9; 29,29
ω scan	$\Delta\omega = 0{\cdot}8 + 0{\cdot}35\tan\theta$	$\Delta\omega = 0{\cdot}5 + 0{\cdot}35\tan\theta$	$\Delta\omega = 1 + 0{\cdot}35\tan\theta$	$\Delta\omega = 0{\cdot}5 + 0{\cdot}35\tan\theta$
No. of measured reflections	2405	2035	1333	2661
No. of symmetry independent reflections	1162 $I \geq 3\sigma(I)$	930 $I \geq 3\sigma(I)$	726 $I \geq 2\sigma(I)$	757 $I \geq 3\sigma(I)$
R	0·041	0·044	0·052	0·058
wR, $w^{-1} = k(\sigma F_o^2 + gF)$	0·048	0·043	0·051	0·064
Final shift/e.s.d.	0·53 (C5, z)	0·42 (C11, y)	0·10 (C11, z)	0·19 (C13, z)
Residual electron density $(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (e Å^{-3})	0·18, −0·17	0·13, −0·18	0·16, −0·20	0·16, −0·19
No. of parameters	171	186	192	199

X-ray analysis

Crystals of the 5-alkylindole-3-acetic acids, in the shape of very thin plates, were prepared by slow evaporation of chloroform solution, at about 275 K. A summary of crystal data and experimental details is presented in Table 1. Data were collected on an Enraf–Nonius CAD-4 diffractometer with graphite-monochromated Mo $K\alpha$ radiation and rescaled for decay on the basis of intensity reduction of standard reflections. Lorentz and polarization corrections were applied. Structures were solved by direct methods using the program *SHELX*86 (Sheldrick, 1986) and refined by *SHELX*76 (Sheldrick, 1976) using a full-matrix least-squares procedure. Difference Fourier maps were used to locate the H atoms, except for the 5-alkyl H atoms which were generated according to stereochemical criteria. The terminal methyl groups of 5-*n*-Pr-IAA and 5-*n*-Bu-IAA were, because of high thermal vibration, treated as rigid groups during refinement. The coordinates of the other H atoms and isotropic temperature factors were refined. The structure factors were those included in *SHELX*76 (Sheldrick, 1976). Molecular geometry was calculated using *PARST* (Nardelli, 1983). Plots of the molecules were prepared with *ORTEP*II (Johnson, 1976), packing diagrams with *PLUTON*90 (Spek, 1982), and overlap diagrams with *FIT* (Horvatić, 1990). The final atomic coordinates and equivalent isotropic thermal parameters are listed in Tables 2–5.* Calculations were performed on Apple Macintosh (*FIT*) and MicroVAX II (other programs listed above) computers.

Molecular structures in the crystalline state

Interatomic distances, bond and selected torsion angles for 5-alkylindole-3-acetic acids are listed in Tables 6, 7 and 8. The molecular structures are shown in Fig. 1. The data presented permit comparison of the molecular geometry of the native plant hormone, indole-3-acetic acid (Karle, Britts & Gum, 1964; Chandrasekhar & Raghunathan, 1982) with that of its 5-(*n*-alkyl)-substituted derivatives. For the latter, the geometry of the benzene part of the indole nucleus significantly deviates from that of a six-membered aromatic ring. Shortening of the C6—C7 bond [⟨1·366 (8)⟩ Å, mean vlaue for the four structures presented in Table 6] and shrinkage of the C6—C7—C71 angle [⟨117·4 (5)⟩°, Table 7] were observed. Low- and room-temperature diffraction data for IAA (Kojić-Prodić, Puntarec & Nigović, to be published; Chandrasekhar & Raghunathan, 1982,

* Lists of structural factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 54421 (27 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for* 5-Me-IAA

$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j$.

	x	y	z	U_{eq} (Å^2)
N1	0.0942 (2)	−0·2593 (5)	0·7463 (1)	641 (8)
C2	0·1006 (3)	0·1268 (5)	0·8130 (1)	617 (10)
C3	0·1947 (2)	0·0536 (5)	0·8128 (1)	492 (7)
C31	0·2483 (2)	0·0375 (4)	0·7402 (1)	408 (7)
C4	0·3403 (2)	0·1779 (4)	0·7036 (1)	437 (7)
C5	0·3658 (2)	0·1192 (4)	0·6297 (1)	453 (7)
C6	0·3013 (3)	0·0842 (5)	0·5930 (1)	527 (8)
C7	0·2116 (3)	−0·2263 (5)	0·6269 (1)	561 (9)
C71	0·1842 (2)	−0·1626 (4)	0·7003 (1)	479 (8)
C8	0·2386 (3)	0·2240 (6)	0·8773 (1)	557 (10)
C9	0·3547 (2)	0·1277 (5)	0·9290 (1)	497 (8)
O1	0·4008 (2)	0·2877 (4)	0·9812 (1)	762 (8)
O2	0·4014 (2)	−0·0784 (4)	0·9250 (1)	773 (8)
C10	0·4606 (3)	0·2741 (7)	0·5881 (2)	641 (11)

Table 3. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for* 5-Et-IAA

$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j$.

	x	y	z	U_{eq} (Å^2)
N1	0·3346 (1)	0·1295 (4)	0·7887 (5)	504 (12)
C2	0·3150 (1)	0·1837 (4)	0·6117 (5)	436 (13)
C3	0·3329 (1)	0·2700 (4)	0·4828 (5)	370 (11)
C31	0·3653 (1)	0·2669 (4)	0·5819 (5)	358 (11)
C4	0·3943 (1)	0·3280 (5)	0·5277 (6)	434 (13)
C5	0·4225 (1)	0·2979 (5)	0·6557 (5)	467 (13)
C6	0·4217 (1)	0·2066 (5)	0·8456 (6)	557 (15)
C7	0·3940 (1)	0·1472 (5)	0·9062 (6)	542 (16)
C71	0·3658 (1)	0·1750 (4)	0·7727 (5)	399 (13)
C8	0·3220 (1)	0·3599 (5)	0·2778 (6)	439 (15)
C9	0·2899 (1)	0·3111 (5)	0·1661 (5)	442 (13)
O1	0·2763 (1)	0·4261 (3)	0·0393 (4)	658 (11)
O2	0·2778 (1)	0·1741 (3)	0·1839 (4)	689 (11)
C10	0·4542 (1)	0·3537 (6)	0·5917 (8)	649 (18)
C11	0·4700 (1)	0·2195 (10)	0·4741 (9)	999 (27)

Table 4. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for* 5-*n*-Pr-IAA

$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j$.

	x	y	z	U_{eq} (Å^2)
N1	0·7358 (9)	0·1188 (6)	0·1524 (2)	597 (23)
C2	0·5695 (10)	0·0654 (6)	0·1169 (3)	503 (23)
C3	0·4289 (9)	0·0193 (6)	0·1489 (2)	425 (21)
C31	0·5037 (8)	−0·0147 (6)	0·2072 (2)	426 (22)
C4	0·4247 (11)	−0·0744 (7)	0·2594 (3)	528 (25)
C5	0·5336 (10)	−0·0428 (7)	0·3101 (3)	571 (25)
C6	0·7283 (10)	0·0414 (8)	0·3094 (3)	663 (29)
C7	0·8126 (12)	0·1004 (8)	0·2587 (3)	662 (32)
C71	0·6971 (9)	0·0725 (7)	0·2081 (3)	497 (27)
C8	0·2290 (11)	−0·1056 (9)	0·1291 (3)	523 (29)
C9	0·1386 (9)	−0·0594 (9)	0·0712 (2)	518 (23)
O1	0·0235 (7)	−0·1755 (5)	0·0472 (2)	731 (19)
O2	0·1649 (7)	0·0765 (5)	0·0499 (2)	766 (19)
C10	0·4399 (15)	0·0926 (11)	0·3670 (3)	824 (35)
C11	0·3222 (18)	0·0431 (13)	0·3952 (4)	1122 (46)
C12	0·2213 (13)	0·0047 (11)	0·4503 (3)	1259 (39)

respectively) revealed the same effects. The values from low- and room-temperature experiments are, respectively: C6—C7 = 1·378 (3), 1·373 (5) Å, and C6—C7—C71 = 117·4 (2), 117·2 (3)°. Inspection of the Cambridge Structural Database (Version 4, 1991) yielded 144 structures containing an indole moiety,

Table 5. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for* 5-*n*-Bu-IAA

$U_{eq} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j$.

	x	y	z	U_{eq} (Å^2)
N1	0·2638 (11)	0·3784 (9)	0·1360 (3)	649 (29)
C2	0·0928 (12)	0·4358 (9)	0·1051 (4)	556 (30)
C3	−0·0412 (10)	0·5192 (8)	0·1356 (3)	439 (23)
C31	0·0537 (10)	0·5154 (7)	0·1895 (3)	448 (25)
C4	−0·0084 (12)	0·5756 (10)	0·2391 (3)	539 (29)
C5	0·1169 (13)	0·5424 (10)	0·2856 (3)	638 (31)
C6	0·3040 (14)	0·4536 (11)	0·2815 (4)	698 (35)
C7	0·3715 (14)	0·3930 (11)	0·2345 (4)	699 (36)
C71	0·2431 (11)	0·4239 (9)	0·1890 (3)	558 (32)
C8	0·2422 (12)	0·6072 (10)	0·1188 (3)	499 (30)
C9	−0·3436 (11)	0·5606 (10)	0·0662 (3)	500 (27)
O1	−0·4622 (9)	0·6745 (7)	0·0429 (2)	729 (23)
O2	0·3253 (9)	0·4243 (7)	0·0457 (2)	744 (23)
C10	0·0470 (18)	0·6003 (13)	0·3393 (4)	885 (41)
C11	−0·0943 (19)	0·4796 (16)	0·3655 (4)	1051 (52)
C12	−0·1425 (20)	0·5299 (17)	0·4210 (4)	1289 (61)
C13	−0·3031 (24)	0·4243 (22)	0·4454 (5)	2037 (99)

Table 6. *Bond lengths* (Å) *for* 5-(*n-alkyl*)*indole*-3-*acetic acids*

	5-Me-IAA	5-Et-IAA	5-*n*-Pr-IAA	5-*n*-Bu-IAA
N1—C2	1·365 (3)	1·373 (5)	1·384 (8)	1·373 (11)
N1—C71	1·371 (3)	1·376 (6)	1·373 (8)	1·385 (11)
C2—C3	1·355 (4)	1·365 (5)	1·349 (8)	1·353 (11)
C3—C31	1·431 (3)	1·424 (6)	1·424 (7)	1·440 (10)
C3—C8	1·488 (3)	1·502 (5)	1·496 (9)	1·498 (10)
C31—C4	1·397 (3)	1·395 (6)	1·404 (8)	1·406 (11)
C31—C71	1·405 (3)	1·411 (5)	1·400 (8)	1·411 (9)
C4—C5	1·381 (3)	1·368 (5)	1·373 (10)	1·392 (11)
C5—C6	1·396 (3)	1·410 (5)	1·396 (9)	1·395 (12)
C5—C10	1·506 (4)	1·508 (6)	1·512 (10)	1·509 (13)
C6—C7	1·362 (4)	1·356 (6)	1·385 (10)	1·362 (14)
C7—C71	1·384 (3)	1·382 (5)	1·386 (10)	1·379 (12)
C8—C9	1·498 (3)	1·492 (6)	1·495 (9)	1·475 (10)
C9—O1	1·307 (3)	1·301 (5)	1·297 (8)	1·306 (9)
C9—O2	1·209 (3)	1·214 (5)	1·210 (8)	1·227 (10)
C10—C11		1·505 (8)	1·476 (14)	1·501 (16)
C11—C12			1·472 (12)	1·491 (15)
C12—C13				1·487 (20)

Table 7. *Bond angles* (°) *for* 5-(*n-alkyl*)*indole*-3-*acetic acids*

	5-Me-IAA	5-Et-IAA	5-*n*-Pr-IAA	5-*n*-Bu-IAA
C2—N1—C71	109·0 (2)	109·5 (3)	109·4 (5)	109·2 (7)
N1—C2—C3	110·3 (2)	109·2 (9)	108·7 (5)	110·8 (8)
C2—C3—C8	126·5 (2)	128·9 (4)	128·1 (5)	129·2 (7)
C2—C3—C31	106·4 (2)	107·2 (3)	107·8 (5)	105·8 (6)
C31—C3—C8	127·1 (2)	123·9 (4)	124·2 (5)	124·9 (6)
C3—C31—C4	134·3 (2)	134·5 (3)	134·2 (5)	133·6 (6)
C3—C31—C71	107·1 (2)	107·2 (3)	107·2 (5)	108·2 (6)
C4—C31—C71	118·5 (2)	118·3 (4)	118·6 (5)	118·1 (7)
C31—C4—C5	119·9 (2)	120·7 (4)	120·0 (6)	119·3 (7)
C4—C5—C6	119·4 (2)	188·9 (4)	119·9 (6)	118·9 (7)
C4—C5—C10	120·7 (2)	121·1 (3)	120·4 (6)	119·7 (8)
C6—C5—C10	119·9 (2)	120·0 (3)	119·6 (6)	121·3 (8)
C5—C6—C7	122·4 (2)	122·4 (4)	121·7 (6)	124·0 (8)
C6—C7—C71	117·8 (2)	118·0 (4)	117·7 (6)	116·2 (8)
C31—C71—C7	121·9 (2)	121·7 (3)	122·0 (6)	123·3 (7)
N1—C71—C7	130·9 (2)	131·5 (3)	131·0 (6)	130·7 (7)
N1—C71—C31	107·2 (2)	106·8 (3)	106·9 (6)	106·0 (6)
C3—C8—C9	113·7 (2)	116·3 (3)	117·5 (5)	116·4 (7)
C8—C9—O1	113·4 (2)	114·1 (3)	113·9 (5)	115·0 (7)
C8—C9—O2	124·5 (2)	123·6 (3)	122·6 (5)	123·7 (7)
O1—C9—O2	122·1 (2)	122·2 (4)	123·4 (5)	121·3 (7)
C5—C10—C11		112·7 (4)	113·6 (7)	113·3 (9)
C10—C11—C12			117·4 (8)	112·6 (9)
C11—C12—C13				113·8 (9)

Table 8. *Selected torsion angles (°) for 5-(n-alkyl)indole-3-acetic acids*

	5-Me-IAA	5-Et-IAA	5-*n*-Pr-IAA	5-*n*-Bu-IAA
C2—C3—C8—C9	91·1 (3)	−19·3 (6)	17·7 (9)	20 (1)
C31—C3—C8—C9	−85·0 (3)	163·2 (3)	162·7 (5)	163·9 (7)
C31—C4—C5—C10	177·6 (2)	176·0 (4)	174·3 (6)	177·4 (7)
C3—C8—C9—O1	173·9 (2)	156·3 (3)	154·6 (5)	−154·3 (6)
C3—C8—C9—O2	−7·4 (4)	−26·5 (6)	−27·9 (9)	28 (1)
C4—C5—C10—C11		−92·5 (5)	−95·1 (9)	88 (1)
C6—C5—C10—C11		84·6 (5)	82·5 (9)	−91 (1)
C5—C10—C11—C12			178·7 (7)	173·7 (9)
C10—C11—C12—C13				173 (1)

73 of which, with R values smaller than 0·07, were selected for statistical analysis by *GSTAT*89 (Motherwell, Murray-Rust, Raftery, Allen & Doyle, 1989). The results are summarized in the histograms shown in Figs. 2 and 3. Arithmetic means calculated from the data presented are 1·371 (13) Å for the length of the C6—C7 bond and 117·2 (7)° for the angle C6—C7—C71. It is evident, therefore, that these changes have not been introduced by alkylation of the system, but rather are characteristics of the indole moiety.

Concerning the orientation of the —CH_2COOH group, two structural patterns were observed. In 5-Me-IAA, the carboxyl group is perpendicular to the indole ring plane, *i.e.* torsion angle C2—C3—C8—C9 is 91·1 (3)°, which is almost identical to the value observed for unsubstituted IAA [95·5 (3)°]. However, in the other 5-alkyl homologues, the carboxyl group is almost coplanar with the aromatic nucleus [C2—C3—C8—C9 = −19·3 (6), −17·7 (9), 20 (1)° and C3—C8—C9—O(carbonyl) = −26·5 (6), −27·9 (9), 28 (1)° for the Et, *n*-Pr and *n*-Bu derivatives, respectively]. The 5-alkyl chain is, starting at C_β, nearly perpendicular to the indole ring plane [torsion angle C4—C5—C10—C11 = −92·5 (5), −95·1 (9), 88·0 (1)° for the Et, *n*-Pr and *n*-Bu derivatives, respectively]. An overlap diagram (Fig. 4) illustrates the discussed similarities and differences in the conformations of the 5-alkylindole-3-acetic acids studied. However, as reported in detail below, these conformational variations are not reflected in the biological activity (Fig. 9).

To check for general tendencies in the conformation of the C2—C3—C8—C9 bond sequence for 3,5-substituted indoles, the Cambridge Structural Database (Version 4, 1991) was searched. Twelve structures of interest were found, with equal distribution of the folded and the extended conformations. For instance, the molecule of 5-methoxyindole-3-acetic acid is almost planar (Sakaki, Wakahara,

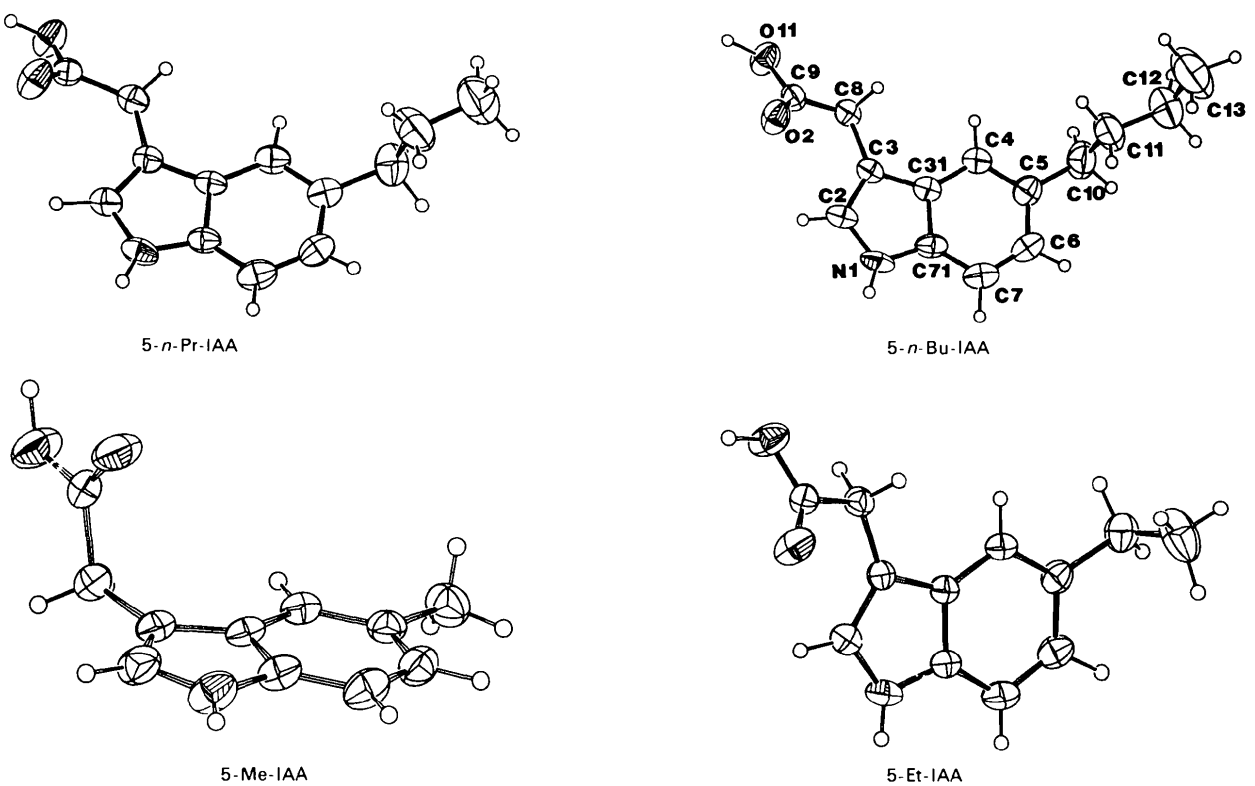


Fig. 1. Molecular structures (*ORTEP* drawings) of 5-(*n*-alkyl)indole-3-acetic acids. Atom numbering is shown for the *n*-butyl derivative as the highest homologue.

Fujiwara & Tomita, 1975), with torsion angles of 5·9° about the C3—C8 (—CH_2COOH moiety) and −1·1° about the O—CH_3 bonds.

Crystal packing

Diagrams illustrating the packing of 5-Me-IAA, 5-Et-IAA and 5-*n*-Pr-IAA in the crystal lattices *via* intramolecular hydrogen bonds are given in Figs. 5, 6 and 7. The crystal structure of 5-Et-IAA is the only representative of $C2/c$ symmetry, but the essential structural pattern remains the same as for 5-*n*-Pr-IAA and 5-*n*-Bu-IAA. The molecules are packed in head-to-head orientation using about 18 Å^3 per atom. The structure of 5-Me-IAA has the highest density, that of 5-*n*-Bu-IAA with its bulky butyl group has the lowest.

The hydrogen bonds in the crystal lattices are of the O—H···O type involving the carboxyl groups. The inversion symmetry operating between the molecules results in the formation of hydrogen-bonded dimers (Figs. 5, 6 and 7 and Table 9). The same type of packing was observed for unsubstituted IAA (Karle, Britts & Gum, 1964; Chandrasekhar & Raghunathan, 1982) and for its following amino-acid conjugates: *N*-(IAA)-DL-aspartic, *N*-(IAA)-δ-aminovaleric and *N*-(IAA)-ε-aminohexanoic acids (Kojić-Prodić *et al.*, 1991). This pattern is not associated with the particular space group ($P2_1/c$), but rather with the carboxyl groups oriented around the inversion center. In these crystal structures, hydrophobic regions comprising the benzene rings and the alkyl chains alternate with hydrophilic channels formed of the carboxyl groups. The indole ring nitrogen is not involved in hydrogen bonds (Table 9).

Molecular-mechanics calculations

To develop at least preliminary ideas about the biologically active conformation of the 5-alkylindole-3-acetic acids studied, molecular-mechanics calculations were carried out. The results are summarized in Table 10 and Fig. 8. The consistent valence force field approach of Lifson & Warshel (1969), Hagler, Huler & Lifson (1974) and Hagler, Lifson & Dauber (1979) incorporated in the program *DISCOVER* (Biosym Technologies, 1990) was used. Steepest descent and conjugate gradients and, in some cases, modified Newton–Raphson algorithms were used.

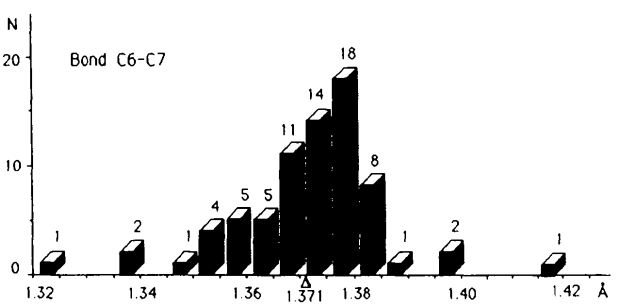


Fig. 2. Histogram showing the distribution of the C6—C7 (sp^2) bond lengths observed in a sample of 73 indole derivatives extracted from the Cambridge Structural Database. *N* gives the number of structures. The sample mean is indicated by a small triangle.

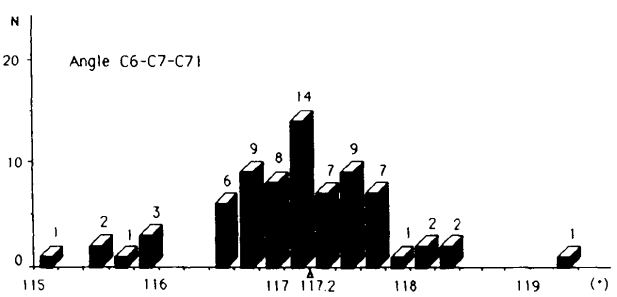


Fig. 3. Histogram showing the distribution of the C6—C7—C71 bond angles observed in the same sample of indole derivatives as in Fig. 2. *N* gives the number of structures. The sample mean is indicated by a small triangle.

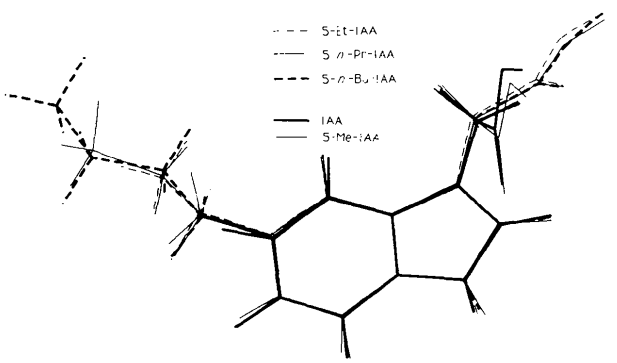


Fig. 4. Overlap of crystallographically observed conformations of IAA and 5-(*n*-alkyl)indole-3-acetic acids.

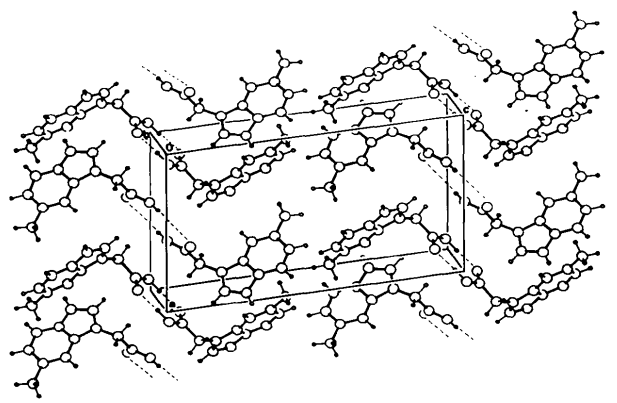


Fig. 5. Molecular packing of 5-Me-IAA with O—H···O hydrogen bonds which create dimers around the inversion centre.

Energy minimization was performed using the atomic coordinates from X-ray analysis: (*a*) considering the molecule in the crystal lattice, *i.e.* using periodic boundary conditions; (*b*) treating it as an isolated molecule '*in vacuo*' (gas phase). The same calculations as in (*b*) were carried out using 'ideal' molecules generated from templates (*c*), program *INSIGHT*II (Version 1.1.0).

In an alternative approach, the *MMPMI* program (Burkert & Allinger, 1982) was utilized with: (*a*) molecules generated from templates; (*b*) the atomic coordinates obtained by X-ray diffraction analysis (with and without constraints on the indole ring). The results obtained by *MMPMI* were consistent with those from *DISCOVER*.

The above results suggest two possible conformations for the carboxyl group: near-coplanar with the indole ring (torsion angles C2—C3—C8—C9 = 11 to 20°) or perpendicular to the plane of that ring (above torsion angle about 90°). Both are energetically feasible, as the respective calculations did not reveal a single global energy minimum. A Boltzmann distribution (at room temperature) for these two conformations gave nearly the same populations.

To check the feasibility of the extended conformation for 5-Me-IAA, rotation around the C3—C8 bond was performed to bring the carboxyl group into the indole ring plane, and energy minimization in the crystal environment was carried out. The conformation obtained was very close to that found for the other 5-alkyl homologues in the crystalline state (C2—C3—C8—C9 = −28·2°). The respective energy of that conformation in the crystal lattice is about 15% higher than the energy minimized for the crystallographically determined conformation (cut-off parameter *ca* 10 Å). Energy minimization of the 5-Me-IAA molecule in an environment of dielectric constant 80 (simulates aqueous solution) ended in a conformation (C2—C3—C8—C9 = 101°) close to that experimentally determined for the crystalline state (91·1°) and calculated for the isolated molecule in the gas phase (95°).

Concerning the 5-alkyl residue, in the energy minimized conformations, the C_α—C_β bond is perpendicular to the indole ring plane (C4—C5—C10—C11 *ca* 90°) as in the crystal structures, but depending on the initial atomic coordinates used it either appeared on the same side of the aromatic nucleus as the CH_2COOH moiety, or on the opposite side (Table 10). There is no potential energy difference for these two conformations.

Among the conformations listed in Table 10, energetical preferences were not detected. Only the following conservative explanation can be offered for the conformations found in the crystalline state: for homologues bearing bulky 5-alkyl substituents, packing influence brings the carboxyl groups into the indole ring plane.

Auxin activity of 5-(*n*-alkyl)indole-3-acetic acids

The growth response to IAA and its 5-*n*-alkyl derivatives in the *Avena* mesocotyl test (Nitsch & Nitsch, 1956, with slight modifications) is presented in Fig. 9. Optimal concentrations ranged from about 3×10^{-6} to 1×10^{-5} mol l^{-1} and optimal increase in length from 310 to 410% over the increase in no-auxin controls. Clear correlations between those two parameters and the size of the 5-alkyl substituent were not observed. However, such correlations appear to exist in the range of half-optimal concentrations. Their ratio for IAA:5-Me-IAA:5-Et-IAA:5-*n*-Pr-IAA:5-*n*-Bu-IAA was 1·0:1·6:9·0:17:3·5, a ratio indicating significant, but not dramatic, differences in auxin activity.

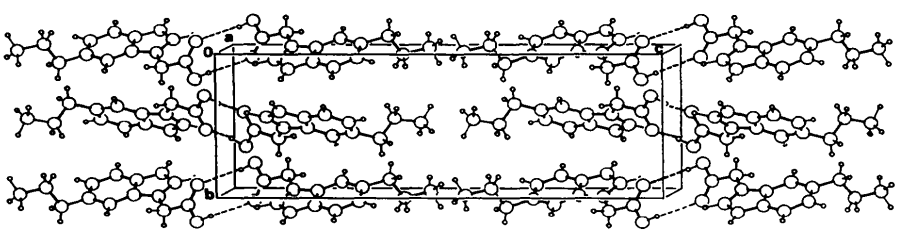


Fig. 6. Molecular packing of 5-Et-IAA with O—H···O hydrogen bonds.

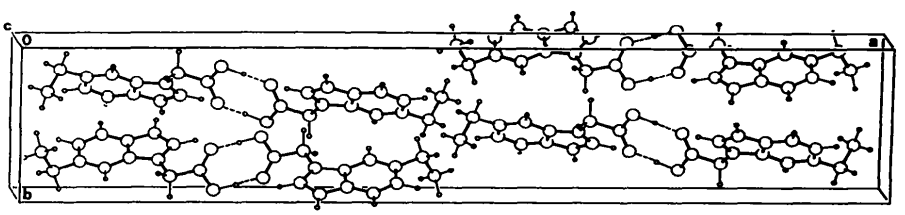


Fig. 7. Molecular packing of 5-*n*-Pr-IAA with O—H···O hydrogen bonds.

Table 9. *Hydrogen bonds in the structures of IAA and 5-(n-alkyl)indole-3-acetic acids*

		D—H···*A* (Å)	*D*—H (Å)	H···*A* (Å)	*D*—H···*A* ( )	Symmetry operations on *A*
Indole-3-acetic acid (IAA)*	O—H···O	2·653 (4)	0·79 (5)	1·87 (5)	160 (4)	$-x, -y, -z$
5-Me-IAA		2·698 (3)	0·97 (3)	1·73 (3)	179 (3)	$x+1, y, -z+2$
5-Et-IAA		2·641 (5)	0·98 (4)	1·66 (4)	174 (4)	$-x+\frac{1}{2}, y+\frac{1}{2}, z$
5-*n*-Pr-IAA		2·646 (6)	0·97 (7)	1·69 (8)	170 (6)	$-x, -y, -z$
5-*n*-Bu-IAA		2·643 (7)	0·98 (9)	1·68 (9)	164 (9)	$-x+1, -y+1, -z$

* Coordinates were taken from Chandrasekhar & Raghunathan (1982).

Table 10. *Conformation analysis about* C3—C8 *and* C5—C10 *bonds*

Torsion angles ( ) refer to: C2—C3—C8—C9, first entry; C4—C5—C10—C11, second entry. The molecules studied are achiral and the sign of the torsion angles is relevant in the comparison of relative orientations of alkyl and carboxylic groups only.

	5-Me-IAA	5-Et-IAA		5-*n*-Pr-IAA		5-*n*-Bu-IAA	
X-ray analysis	91·1	19·3	−92·5	−17·7	−95·1	19·6	87·6
Molecular mechanics							
Input atomic coordinates							
(1) X-ray data							
(*a*) Molecule in crystal lattice	89	11	95	19	92	20	83
(*b*) Molecule in gas phase	95	100	90	103	90	107	89
(2) Template							
(*c*) Molecule in gas phase	94	94	90	94	90	−94	102

Discussion and conclusions

Growth-promoting activity in a system such as the *Avena* mesocotyl is based on a complex response which not only depends on the affinity of – possibly multiple – cellular auxin-binding sites to the compounds tested, and thus on their physico-chemical properties (see *Introduction* for details), but is also a function of the substrate concentration available at those binding sites (metabolic stability, partition

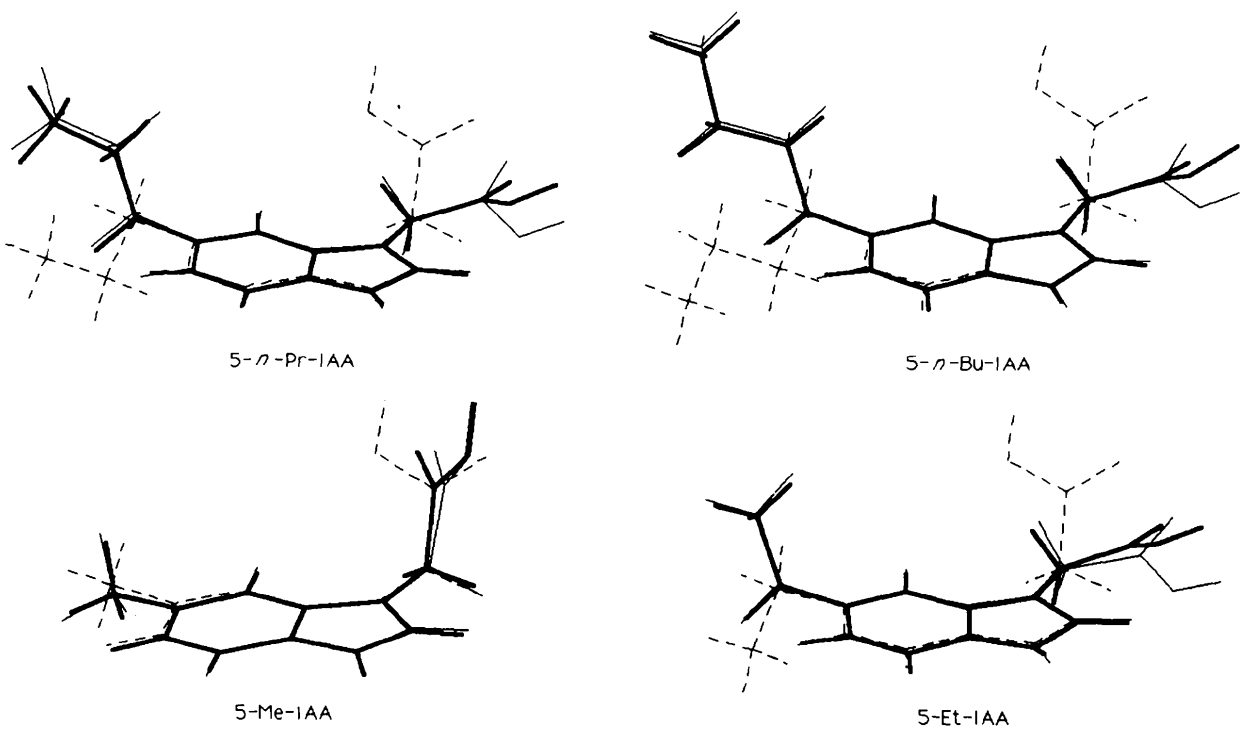


Fig. 8. Overlap of crystallographically determined conformations (heavy lines), conformations of the minimized X-ray models (crystal lattice environment) (light lines), and conformations of the minimized template models (gas phase) (dashed lines).

between cell compartments) and may be further modified by the functional state of the enzymatic mechanisms converting the hormonal stimulus into measurable cell elongation. In principle, all these parameters should be defined before reliable structure–activity correlations can be derived. This is not possible at present, because the cell proteins involved have not been completely characterized as yet. However, for a series of homologues, such as the 5-(*n*-alkyl)indole-3-acetic acids studied in this work, physical and biochemical properties should generally change in a systematic fashion. Discontinuous changes are unlikely to involve more than one parameter at the same time, and this should distinctly reflect on cell elongation. The effect of bulky substituents on the interaction with auxin-binding sites appears to be close to such an all-or-none response. At least this would be a plausible explanation for observations on the growth-promoting properties of 2-alkylindole-3-acetic acids (Kögl & Kostermans, 1935): the 2-methyl derivative is active, its 2-ethyl homologue completely inactive. No such abrupt decline in activity was found in the series of 5-(*n*-alkyl)indole-3-acetic acids; in fact, the half-optimal concentration was *smaller* (means *higher* activity) for 5-*n*-Bu-IAA than for its ethyl and *n*-propyl homologues. Katekar (1979) postulated a zone of 'steric obstruction' of auxin activity between positions 4 and 5 of the indole nucleus. Our bioassay, crystallographic and molecular-mechanics data suggest rather convincingly that a substituent as bulky as *n*-butyl has no major steric effect on growth-promoting activity, if pointing away from the aromatic nucleus and located in a plane perpendicular to the indole ring and bisecting the bond angle C4—C5—C6.

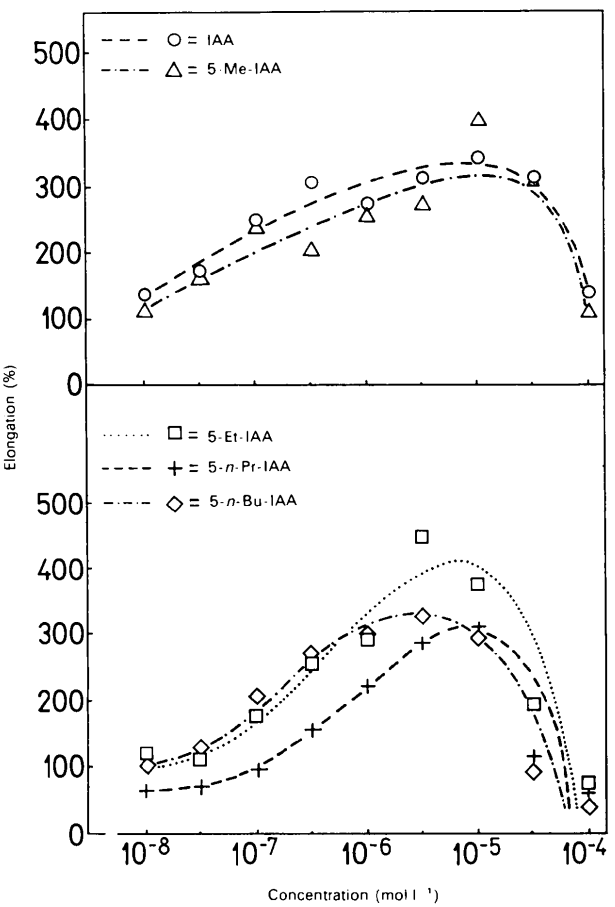


Fig. 9. Growth-promoting properties of IAA and 5-(*n*-alkyl)indole-3-acetic acids in the *Avena* mesocotyl test. The experimental conditions were as given by Nitsch & Nitsch (1956), except the auxin solutions were buffered at pH 5·4. Elongation is expressed as the increase in length (final mean length of mesocotyl sections − length immediately after cutting) over the control calculated as 100 × (increase in length of auxin-treated sections)/(increase in length in no-auxin controls). Fiducial limits (5% level) calculated from the standard error of the mean increase in length of the control sections were ±40%. This implies that an increase in length over the control of 140% or more indicates statistically significant growth stimulation by the auxin used.

It has also been claimed that the conformation of the CH_2COOH moiety drastically affects auxin activity. This concept, originally proposed by Veldstra (1944), was extended by Kaethner (1977) who postulated that the side chains containing the carboxyl groups of any plant-growth-promoting arylaliphatic acid must be able to rotate freely to assume two characteristic positions: (*a*) the *recognition conformation* with the COOH moiety coplanar with, and (*b*) the *modulation conformation* with that group perpendicular to the indole nucleus. While our results on the molecular mechanics of 5-(*n*-alkyl)indole-3-acetic acids do indicate that there are no major energy barriers impeding rotation of the CH_2—COOH moiety, data on a much larger number of auxins would be necessary to justify Kaethner's claim that this is a molecular property *essential* for biological activity. The relatively unconstrained rotation of the 3-side chain makes it particularly difficult to predict the conformation of IAA and its 5-*n*-alkyl homologues attached to auxin-binding sites, the modulation conformation in Kaethner's terms. We suggest that further discussions should be postponed until quantities of auxin-binding proteins sufficient for experimental studies on the structures of their substrate complexes become available.

Such investigations will also permit a final evaluation of Kaethner's hypothesis that auxin-binding proteins can only recognize their substrates in a conformation with the carboxyl group coplanar with the aromatic nucleus. So far there are no convincing arguments in favor of this assumption. The CH_2-COOH side chain of 5(*n*-alkyl)indole-3-acetic acids is

flexible, and, in the energetically preferred conformation, the carboxyl group is perpendicular to the indole ring plane.

Regardless of the discussed orientations of the carboxyl groups, the intramolecular distances between its oxygens and the pyrrole nitrogen remain about the same (*ca* 5·5 Å). These distances have been postulated to be essential for auxin activity (Thimann, 1977; Farrimond, Elliot & Clack, 1978). Although the original concept is based on the separation of charge between the electropositive pyrrole nitrogen and the electronegative carboxyl oxygens (equivalent when dissociated at physiological pH), which may be an oversimplification, the essence of this theory would perfectly correlate our structural data and the fact that all the compounds studied have similar growth-promoting properties. Speculating on the interaction of IAA and its 5-(*n*-alkyl) derivatives with cellular auxin-binding proteins, assuming at least two recognition sites, for the carboxyl group and for the pyrrole NH, located on different loops of the polypeptide chain, appears to be both plausible and in accord with the results of this work.

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