IYER, R. P., LYGA, J. W., SECRIST, J. A. III, DAUB, G. H. & SLAGA, T. J. (1980). *Cancer Res.* **41**, 3441–3447.
JONES, P. G. (1984). *Chem. Soc. Rev.* **13**, 157–172.
KASHINO, S., ZACHARIAS, D. E., PROUT, C. K., CARRELL, H. L., GLUSKER, J. P., HECHT, S. S. & HARVEY, R. G. (1984). *Acta Cryst.* C**40**, 536–540.
MAIN, P., FISKE, S. J., HULL, S. E., LESSINGER, L., GERMAIN, G., DECLERCQ, J.-P. & WOOLFSON, M. M. (1980). *MULTAN*80. *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data.* Univs. of York, England, and Louvain, Belgium.
MAYOH, B. & PROUT, C. K. (1972). *J. Chem. Soc. Faraday Trans.* 2, **68**, 1072–1082.
NASIPURI, D. & ROY, D. N. (1963). *J. Indian Chem. Soc.* **40**, 327–338.
OCHIAI, E., OKAMOTO, T., SEKIJIMA, M., NISHIKAWA, M. & SHONO, K. (1957). *Pharm. Bull. (Tokyo)*, **5**, 48–52.
PROUT, C. K., DAUB, G. H., ZACHARIAS, D. E. & GLUSKER, J. P. (1989). In preparation.
PROUT, C. K. & KAMENAR, B. (1973). *Mol. Complexes*, **1**, 151–207.
SIMS, P., GROVER, P. L., SWAISLAND, A., PAL, K. & HEWER, A. (1974). *Nature (London)*, **256**, 326–328.
WASER, J. (1963). *Acta Cryst.* **16**, 1091–1094.
WATKIN, D. J., CARRUTHERS, J. P. & BETTERIDGE, P. W. (1986). *CRYSTALS User Guide.* Chemical Crystallography Laboratory, Univ. of Oxford, England.
WESSELY, F. & WANG, S. (1940). *Chem. Ber.* **73B**, 19–24.
WISLOCKI, P. G., FIORENTINI, K. M., FU, P. P., YANG, S. K. & LU, A. Y. H. (1982). *Carcinogenesis*, **3**, 215–217.
ZACHARIAS, D. E., KASHINO, S., GLUSKER, J. P., HARVEY, R. G., AMIN, S. & HECHT, S. S. (1984). *Carcinogenesis*, **5**, 1421–1430.
ZALKIN, A. & WARD, D. (1974). *INFORM.* Lawrence Berkeley Laboratory, Berkeley, CA, and Chemistry Department, Michigan State Univ., Lansing, MI, USA.

Acta Cryst. (1991). B**47**, 107–115

Comparison of the Structures of the Plant Growth Hormone Indole-3-acetic Acid, and Six of its Amino-Acid Conjugates

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Abstract

The crystal structures of six biologically active conjugates of the plant growth hormone, indole-3-acetic acid (IAA = auxin), with the amino acids L-alanine (1), α-amino-L-butyric acid (2), L-norvaline (3), DL-aspartic acid (4), L-isoleucine (5), and δ-aminovaleric acid (6) were determined. (1) $C_{13}H_{14}N_2O_3$, $M_r = 246{\cdot}26$, monoclinic, $P2_1$, $a = 6{\cdot}777\ (2)$, $b = 9{\cdot}611\ (2)$, $c = 10{\cdot}003\ (1)$ Å, $\beta = 106{\cdot}24\ (1)^\circ$, $V = 625{\cdot}1\ (2)$ Å^3, $Z = 2$, $D_x = 1{\cdot}308$ g cm^{-3}, Mo $K\alpha$ radiation, $\lambda = 0{\cdot}71073$ Å, $\mu = 0{\cdot}88$ cm^{-1}, $F(000) = 260$, $T = 293\ (1)$ K, $R = 0{\cdot}048$, $wR = 0{\cdot}053$ for 1313 reflections with $I \geq 3\sigma(I)$. (2) $C_{14}H_{16}N_2O_3$, $M_r = 260{\cdot}30$, monoclinic, $P2_1$, $a = 7{\cdot}380\ (1)$, $b = 9{\cdot}727\ (1)$, $c = 9{\cdot}741\ (1)$ Å, $\beta = 105{\cdot}08\ (1)^\circ$, $V = 675{\cdot}2\ (1)$ Å^3, $Z = 2$, $D_x = 1{\cdot}280$ g cm^{-3}, Mo $K\alpha$ radiation, $\lambda = 0{\cdot}71073$ Å, $\mu = 0{\cdot}85$ cm^{-1}, $F(000) = 276$, $T = 293\ (1)$ K, $R = 0{\cdot}045$, $wR = 0{\cdot}043$ for 1281 reflections with $I \geq 3\sigma(I)$. (3) $C_{15}H_{18}N_2O_3$, $M_r = 274{\cdot}32$, monoclinic, $P2_1$, $a = 8{\cdot}165\ (4)$, $b = 9{\cdot}635\ (4)$, $c = 9{\cdot}792\ (3)$ Å, $\beta = 106{\cdot}33\ (3)^\circ$, $V = 739{\cdot}3\ (2)$ Å^3, $Z = 2$, $D_x = 1{\cdot}232$ g cm^{-3}, Mo $K\alpha$ radiation, $\lambda = 0{\cdot}71073$ Å, $\mu = 0{\cdot}81$ cm^{-1}, $F(000) = 292$, $T = 293\ (1)$ K, $R = 0{\cdot}065$, $wR = 0{\cdot}053$ for 1502 reflections with $I \geq 3\sigma(I)$. (4) $C_{14}H_{14}N_2O_5$, $M_r = 290{\cdot}28$, monoclinic, $P2_1/n$ (nonstandard, No. 14), $a = 7{\cdot}577\ (1)$, $b = 18{\cdot}939\ (3)$, $c = 9{\cdot}442\ (4)$ Å, $\beta = 97{\cdot}30\ (1)^\circ$, $V = 1343{\cdot}9\ (6)$ Å^3, $Z = 4$, $D_x = 1{\cdot}434$ g cm^{-3}, Cu $K\alpha$ radiation, $\lambda = 1{\cdot}5418$ Å, $\mu = 8{\cdot}88$ cm^{-1}, $F(000) = 608$, $T =$

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293 (1) K, $R = 0.078$, $wR = 0.089$ for 1988 reflections with $I \geq 3\sigma(I)$. (5) $C_{16}H_{20}N_2O_3$, $M_r = 288.35$, orthorhombic, $P2_12_12_1$, $a = 8.859$ (1), $b = 11.679$ (1), $c = 14.889$ (2) Å, $V = 1540.5$ (3) Å^3, $Z = 4$, $D_x = 1.243$ g cm^{-3}, Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å, $\mu = 0.81$ cm^{-1}, $F(000) = 616$, $T = 293$ (1) K, $R = 0.077$, $wR = 0.065$ for 1866 reflections with $I \geq 1.5\sigma(I)$. (6) $C_{15}H_{18}N_2O_3$, $M_r = 274.32$, monoclinic, $P2_1/a$ (nonstandard, No. 14), $a = 10.066$ (3), $b = 4.892$ (1), $c = 28.250$ (9) Å, $\beta = 99.47$ (2)°, $V = 1372.2$ (3) Å^3, $Z = 4$, $D_x = 1.328$ g cm^{-3}, Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å, $\mu = 0.87$ cm^{-1}, $F(000) = 584$, $T = 293$ (1) K, $R = 0.054$, $wR = 0.050$ for 1235 reflections with $I \geq 3\sigma(I)$. In these conjugates the conformations of the indol-3-ylacetyl moieties are very similar to that observed in free IAA, as are the values of bond lengths and intramolecular contact distances within the IAA moiety. The indole ring system and the C atom of the adjacent methylene group are coplanar, whereas the —COOH or —CON*R* residues, respectively, adopt a folded conformation. The carbonyl group of the free hormone points towards the indole ring; however, in the amino-acid conjugates it points away from the ring system. The orientation of the amino-acid side chains with respect to the aromatic ring varies in compounds (1)–(6). Consistently, however, only the region of the IAA moiety in immediate proximity to the —CO group is sterically blocked by the conjugant. The rest of the indole nucleus, which appears to include the —NH group, remains potentially available for binding competively (with free IAA) to proteins such as auxin receptors and enzymes regulating intracellular levels of growth hormones.

Introduction

Indole-3-acetic acid (IAA) is a long-known plant hormone: an 'auxin' which regulates physiological functions such as cell division and enlargement, developmental differentiation, and the synthesis of specific proteins (Thimann, 1977; Davies, 1987). A *master mechanism* underlying the various aspects of auxin action has so far not been agreed upon, nor is it fully understood how the hormone level in a growing tissue is optimized. A special regulatory function has been attributed to the bound auxins, or auxin conjugates (Cohen & Bandurski, 1982; Magnus, 1987). They appear to be involved in hormone transport and as long- and short-term storage forms, and are frequently more abundant in plants than the free hormone. Although not all naturally occurring bound auxins have been characterized chemically, a number of IAA esters, such as those containing monosaccharide, inositol, inositol glycoside, or glucan residues, and of IAA amides involving amino acids and peptides have been identified. We focus here on the *N*-(indol-3-yl-acetyl) L-amino acids. The glutamic (Epstein, Baldi & Cohen, 1985; Percival, 1986; Sonner & Purves, 1985) and, in particular, the aspartic acid conjugate (Cohen, 1982; Andersson & Sandberg, 1982) appear to be fairly common in plants (Sembdner, Gross, Liebisch & Schneider, 1980). In addition to these compounds, crown gall callus of *Parthenocissus tricuspidata* cultured in the presence of IAA accumulation has been reported in the glycine, alanine and valine conjugates (Feung, Hamilton & Mumma, 1976). N_ε-(Indol-3-ylacetyl)-L-lysine (Hutzinger & Kosuge, 1968) and its N_α-acetyl derivative (Evidente, Surico, Iacobellis & Randazzo, 1986) are formed by the pathogen *Pseudomonas syringae* pv. *savastanoi*. Other *N*-(indol-3-ylacetyl) amino acids which so far have not been found to occur naturally, have been synthesized and used as sources of auxin in plant tissue culture (*e.g.* Hangarter, Peterson & Good, 1980; Feung, Hamilton & Mumma, 1977). Their widely varying activities and morphogenetic effects in *in vitro* systems have been difficult to rationalize. One of the reasons is lack of knowledge concerning the physico-chemical and structural properties of IAA conjugates which could be used as a basis as from which their biological effects could be correlated. Therefore we have, for a number of representative examples, performed NOE measure-

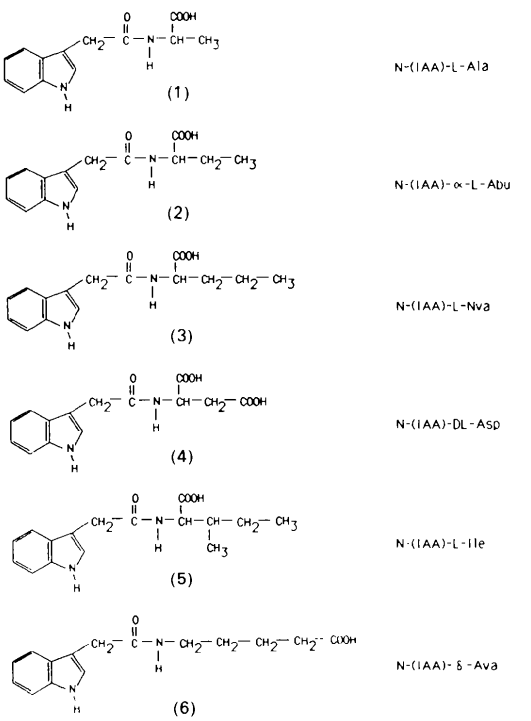

Fig. 1. Structural formulae for compounds (1)–(6) and abbreviations used in the paper.

Table 1. *Details of data collection and refinement*

	(1)	(2)	(3)	(4)	(5)	(6)
Size of crystals (mm)	0·35 × 0·15 × 0·50	0·20 × 0·15 × 0·40	0·25 × 0·15 × 0·30	0·30 × 0·20 × 0·25	0·35 × 0·15 × 0·90	0·30 × 0·20 × 0·50
$\omega/2\theta$ (°), $\Delta\omega$	1·2 + 0·35tanθ	1·1 + 0·35tanθ	1·0 + 0·35tanθ	1·2 + 0·35tanθ	1·1 + 0·35tanθ	1·0 + 0·35tanθ
θ_{min}, θ_{max} (°)	3·0, 28·0	2·1, 30·0	2·1, 32·5	2·0, 60·0	3·0, 28·0	1·5, 27·5
No. of reflections for unit-cell parameters	25	25	25	25	25	16
θ_{min}, θ_{max} (°)	12·1, 20·4	10·1, 17·6	13·0, 17·4	19·9, 29·6	9·8, 18·4	11·4, 13·6
No. of measured reflections	1677	2087	2818	2750	2141	3535
No. of symmetry-independent reflections	1313	1281	1502	1988	1866	1235
	$I > 3\sigma(I)$	$I > 3\sigma(I)$	$I > 3\sigma(I)$	$I > 3\sigma(I)$	$I > 1{\cdot}5\sigma(I)$	$I > 3\sigma(I)$
Minimized function	$\sum w(\lvert F_o\rvert - \lvert F_c\rvert)^2$, $w = k/[\sigma^2(F_o) + gF_o^2]$					
R, wR, S	0·048, 0·053, 0·29	0·045, 0·043, 0·30	0·065, 0·053, 0·34	0·078, 0·089, 0·77	0·077, 0·065, 0·70	0·054, 0·050, 1·00
$(\Delta/\sigma)_{max}$	0·085 (C9, y)	0·048 (N1, z)	0·172 (C5, z)	0·729 (C31, y)	0·103 (C7, x)	0·038 (C22, z)
$(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (e Å^{-3})	0·28, −0·42	0·18, −0·17	0·36, −0·29	0·35, −0·37	0·29, −0·33	0·20, −0·23

ments in order to evaluate their conformations in solution (Duddeck, Hiegemann, Simeonov, Kojić-Prodić, Nigović & Magnus, 1989). Here we present the crystal structures of six *N*-(indol-3-yl-acetyl) amino acids (see Fig. 1) and compare our data with those obtained previously for free IAA (Karle, Britts & Gum, 1964; Chandrasekhar & Raghunathan, 1982). The results should help in understanding the structure–activity relationships for *N*-(indol-3-yl-acetyl) amino acids *in vitro*. More importantly, comparing structural parameters and the experimental effects on growth and differentiation for the naturally occurring conjugates and a series of synthetic analogues should eventually permit insight into the function of IAA conjugation *in vivo*. Also, IAA binds reversibly to certain plant proteins, some of which have been proposed to be receptors mediating the auxin effect (Davies, 1987). IAA conjugates may be used as preliminary models to gain an understanding of the interaction of the hormone with the amino acids at the active sites of such binding proteins, which are not available thus far in sufficient quantity and purity for direct structural studies.

Experimental

Crystals of (1), (3), (4) and (5) were obtained from a mixture of 2-propanol (30%) and water (70% vol.) after 1 to 2 days at 275 (2) K. Crystals of (2) were grown from the same solvent mixture, but in a ratio of 2:3, over 12 days at 275 (2) K. Crystals of (6) were prepared from a mixture of ethyl acetate and benzene (1:1) over 3 days. Crystal data are given in the *Abstract*.

The molecules *N*-(IAA)-L-Ala (1), *N*-(IAA)-α-L-Abu (2), and *N*-(IAA)-L-Nva (3) are chiral; space group $P2_1$ was confirmed during refinement. The L-amino acids were used for the syntheses and enantiomers with *S* configuration were selected for structure determination; the signs of torsion angles are in accord with this assignment. The same argument applies to *N*-(IAA)-L-Ile(5). The synthesis of the aspartic acid conjugate, *N*-(IAA)-DL-Asp (4), employed the DL-amino acid and the product is racemic; both enantiomers are required in a 1:1 ratio by the centrosymmetric space group ($P2_1/n$). Final atomic coordinates of the *S* enantiomer are given (Table 5) to simplify comparison with the other L-amino-acid conjugates examined. The *N*-(IAA)-δ-Ava (6) molecule is achiral and its crystals appear in monoclinic holohedry ($P2_1/a$).

Intensity data were collected on Enraf–Nonius CAD-4F [for (1), (4) and (5)] and Nicolet P3F [for (2), (3) and (6)] diffractometers with Mo $K\alpha$ radiation [(1), (2), (3), (5) and (6)] and Cu $K\alpha$ radiation (4) at 293 (1) K. Details of data collection are given in Table 1. No significant intensity variation for standard reflections was observed. Data were corrected for Lorentz and polarization effects, but not for absorption. Structures were solved by *SHELX*86 (Sheldrick, 1985) and refinements performed using the *SHELX*77 system of programs (Sheldrick, 1983). The H-atom coordinates of the indole moiety (with the exception of the pyrrole N—H) were introduced at calculated positions. Others were located from the difference Fourier syntheses. The positions of the H atoms for indole N—H [in compounds (1), (2), (4) and (6)], the peptide N—H [in (2), (3), (4) and (6)], and the carboxylic O—H [in (2), (3), (4) and (6)] were normalized to the values obtained by neutron diffraction (N—H 1·009, O—H 0·983 Å) using the program *GSTAT*89 included in the Cambridge Structural Database (Motherwell, Murray-Rust, Raftery, Allen & Doyle, 1989). The non-H atoms were refined anisotropically; details of the refinement procedure are listed in Table 1. Scattering factors are those included in *SHELX*77 (Sheldrick, 1983). Interatomic distances, bond and torsion angles were calculated using a program for analysis of molecular geometry (Nardelli, 1983).

Calculations were carried out at the University Computing Centre in Zagreb on an IBM 4341 computer. Illustrations of molecular structure were by the *BALL & STICK* program (Mueller & Falk, 1986) and packing diagrams by *MOL* (Horvatić, 1986) using an Apple Macintosh computer. Final atomic coordinates of the non-H atoms with equivalent

Table 2. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for compound* (1)

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

	x	y	z	U_{eq}(Å^2)
N1	0·2397 (4)	0·0550 (4)	0·3525 (3)	595 (5)
C2	0·3686 (5)	0·0977 (4)	0·4774 (3)	561 (5)
C3	0·2687 (5)	0·1879 (4)	0·5422 (3)	456 (5)
C31	0·0635 (4)	0·2010 (3)	0·4509 (3)	429 (4)
C4	−0·1091 (5)	0·2794 (4)	0·4577 (3)	534 (5)
C5	−0·2856 (5)	0·2694 (4)	0·3473 (4)	634 (5)
C6	−0·2931 (5)	0·1815 (4)	0·2334 (4)	645 (5)
C7	−0·1269 (5)	0·1046 (4)	0·2249 (3)	589 (5)
C71	0·0519 (5)	0·1164 (4)	0·3344 (3)	501 (5)
C8	0·3533 (4)	0·2611 (4)	0·6784 (3)	501 (5)
N22	0·1455 (3)	0·1046 (3)	0·7783 (2)	390 (4)
C9	0·2705 (4)	0·2150 (3)	0·7976 (3)	394 (5)
O9	0·3155 (3)	0·2785 (0)	0·9091 (2)	495 (4)
C23	−0·1194 (4)	−0·0400 (4)	0·8321 (3)	524 (5)
C22	0·0742 (4)	0·0469 (4)	0·8896 (3)	379 (5)
C21	0·2401 (4)	−0·0389 (4)	0·9922 (3)	403 (4)
O211	0·4155 (4)	−0·0442 (4)	0·9628 (3)	489 (5)
O212	0·2075 (4)	−0·0946 (4)	1·0918 (2)	657 (5)

Table 3. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for compound* (2)

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

	x	y	z	U_{eq}(Å^2)
N1	0·2940 (6)	0·1873 (5)	1·1594 (4)	631 (15)
C2	0·1698 (6)	0·2335 (6)	1·0383 (4)	555 (15)
C3	0·2558 (5)	0·3185 (5)	0·9666 (3)	431 (12)
C31	0·4476 (5)	0·3281 (5)	1·0470 (3)	423 (11)
C4	0·6042 (5)	0·4001 (5)	1·0303 (4)	543 (14)
C5	0·7727 (6)	0·3870 (6)	1·1338 (5)	695 (17)
C6	0·7883 (7)	0·3008 (6)	1·2516 (5)	729 (19)
C7	0·6364 (8)	0·2291 (6)	1·2704 (4)	692 (18)
C71	0·4672 (6)	0·2439 (5)	1·1680 (3)	509 (16)
C8	0·1706 (5)	0·3916 (5)	0·8274 (3)	463 (14)
C9	0·2275 (4)	0·3401 (4)	0·6983 (3)	370 (10)
O9	0·1861 (4)	0·4027 (0)	0·5847 (3)	525 (9)
N22	0·3266 (4)	0·2242 (4)	0·7119 (3)	382 (9)
C24	0·7259 (6)	0·1530 (7)	0·7236 (6)	826 (20)
C23	0·5536 (5)	0·0717 (5)	0·6450 (4)	490 (13)
C22	0·3799 (5)	0·1612 (4)	0·5928 (3)	373 (11)
C21	0·2213 (5)	0·0793 (5)	0·4975 (3)	402 (11)
O211	0·0691 (4)	0·0723 (4)	0·5415 (3)	513 (9)
O212	0·2381 (4)	0·0262 (4)	0·3889 (2)	632 (11)

isotropic thermal parameters are listed in Tables 2–7 [for (1) to (6)].*

Results and discussion

Interatomic distances, bond and selected torsion angles for (1)–(6) are listed in Tables 8, 9 and 10. The molecular structures of (1)–(6) are shown in Figs. 2–7. Diagrams illustrating the packing of molecules in the crystal lattices *via* hydrogen bonds are given in Figs. 8–11; hydrogen-bonding geometry is displayed in Table 11. Comparative illustrations of the overall molecular conformations as space-filling models are presented in Fig. 12.

* Lists of structure factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53481 (56 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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

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

	x	y	z	U_{eq}(Å^2)
N1	0·6905 (5)	0·3479 (4)	0·3362 (3)	634 (14)
C2	0·8090 (5)	0·3949 (5)	0·4558 (4)	608 (16)
C3	0·7352 (4)	0·4789 (5)	0·5332 (3)	479 (12)
C31	0·5576 (4)	0·4837 (5)	0·4563 (3)	432 (11)
C4	0·4169 (4)	0·5525 (5)	0·4793 (4)	580 (14)
C5	0·2594 (5)	0·5359 (5)	0·3809 (5)	766 (18)
C6	0·2385 (7)	0·4524 (6)	0·2613 (5)	820 (19)
C7	0·3762 (7)	0·3836 (5)	0·2370 (4)	728 (18)
C71	0·5346 (5)	0·4017 (5)	0·3347 (3)	512 (15)
C8	0·8214 (4)	0·5541 (5)	0·6698 (3)	534 (15)
C9	0·7810 (4)	0·5040 (4)	0·8022 (3)	423 (12)
O9	0·8279 (3)	0·5673 (4)	0·9154 (2)	636 (10)
N22	0·6897 (3)	0·3877 (0)	0·7944 (3)	412 (10)
C25	0·1796 (5)	0·2243 (7)	0·7480 (5)	895 (20)
C24	0·3293 (4)	0·3183 (6)	0·8001 (5)	772 (18)
C23	0·4925 (4)	0·2350 (5)	0·8690 (3)	475 (11)
C22	0·6505 (4)	0·3267 (5)	0·9168 (3)	407 (11)
C21	0·8000 (4)	0·2465 (4)	1·0116 (3)	412 (11)
O211	0·9281 (3)	0·2305 (4)	0·9603 (2)	609 (9)
O212	0·7947 (3)	0·1993 (4)	1·1251 (2)	707 (11)

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

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

	x	y	z	U_{eq}(Å^2)
N1	0·2018 (5)	0·5111 (2)	0·4508 (4)	619 (13)
C2	0·2797 (6)	0·4460 (3)	0·4562 (4)	591 (14)
C3	0·3662 (5)	0·4334 (2)	0·5897 (4)	459 (10)
C31	0·3384 (5)	0·4953 (2)	0·6728 (4)	437 (10)
C4	0·3941 (6)	0·5154 (2)	0·8143 (4)	554 (13)
C5	0·3401 (7)	0·5795 (2)	0·8609 (5)	626 (15)
C6	0·2352 (6)	0·6258 (2)	0·7705 (5)	626 (15)
C7	0·1794 (6)	0·6085 (2)	0·6297 (5)	574 (14)
C71	0·2332 (5)	0·5432 (2)	0·5825 (4)	484 (13)
C8	0·4748 (5)	0·3704 (2)	0·6403 (4)	534 (14)
C9	0·3981 (5)	0·3246 (2)	0·7498 (4)	446 (10)
O9	0·4910 (3)	0·2831 (1)	0·8289 (3)	614 (11)
N22	0·2247 (4)	0·3300 (2)	0·7551 (3)	480 (10)
C22	0·1298 (5)	0·2951 (2)	0·8597 (4)	432 (11)
C21	−0·0643 (5)	0·2904 (2)	0·7997 (4)	465 (11)
O211	−0·1657 (4)	0·2694 (2)	0·8952 (3)	591 (10)
O212	−0·1199 (4)	0·3037 (2)	0·6770 (3)	658 (11)
C23	0·1605 (5)	0·3328 (2)	1·0048 (4)	462 (11)
C24	0·0916 (5)	0·4061 (2)	0·9979 (3)	464 (11)
O241	0·1480 (4)	0·4443 (2)	1·1103 (3)	626 (11)
O242	−0·0105 (4)	0·4289 (1)	0·8964 (3)	561 (8)

Table 6. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for compound* (5)

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

	x	y	z	U_{eq}(Å^2)
N1	−0·2503 (3)	0·1620 (3)	0·2650 (2)	542 (8)
C2	−0·1300 (4)	0·0949 (3)	0·2390 (3)	480 (8)
C3	−0·0028 (4)	0·1284 (3)	0·2831 (2)	374 (7)
C4	0·0312 (4)	0·2919 (4)	0·4011 (3)	543 (8)
C5	−0·0467 (6)	0·3736 (4)	0·4482 (3)	687 (9)
C6	−0·2012 (5)	0·3903 (4)	0·4339 (3)	683 (8)
C7	−0·2806 (4)	0·3248 (4)	0·3731 (3)	615 (8)
C31	−0·0448 (4)	0·2231 (3)	0·3400 (2)	373 (7)
C71	−0·2018 (4)	0·2411 (3)	0·3273 (3)	431 (8)
C8	0·1480 (4)	0·0708 (3)	0·2805 (3)	432 (8)
C9	0·2736 (4)	0·1340 (3)	0·2323 (2)	332 (7)
O9	0·4083 (3)	0·1070 (2)	0·2447 (2)	476 (7)
N22	0·2344 (3)	0·2157 (2)	0·1759 (2)	323 (6)
C22	0·3461 (3)	0·2850 (3)	0·1290 (2)	288 (6)
C21	0·4079 (3)	0·3760 (3)	0·1919 (2)	319 (7)
O211	0·5154 (3)	0·4367 (2)	0·1527 (2)	497 (6)
O212	0·3640 (3)	0·3906 (2)	0·2675 (2)	482 (6)
C23	0·2831 (4)	0·3350 (3)	0·0415 (2)	314 (7)
C24	0·1579 (4)	0·4226 (3)	0·0583 (2)	450 (8)
C25	0·1130 (5)	0·4926 (4)	−0·0225 (3)	569 (8)
C26	0·2344 (5)	0·2383 (3)	−0·0220 (2)	490 (7)

Table 7. *Final atomic coordinates and equivalent isotropic thermal parameters* ($\times 10^4$) *for compound* (6)

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

	x	y	z	U_{eq}(Å^2)
N1	0·9978 (4)	0·3068 (10)	0·3778 (2)	480 (16)
C2	0·9874 (5)	0·2615 (11)	0·3292 (2)	445 (18)
C3	0·8968 (4)	0·0569 (10)	0·3159 (2)	382 (15)
C31	0·8481 (4)	−0·0276 (9)	0·3586 (2)	356 (15)
C4	0·7585 (5)	−0·2300 (11)	0·3686 (2)	479 (19)
C5	0·7373 (5)	−0·2627 (12)	0·4153 (2)	615 (22)
C6	0·8019 (6)	−0·0975 (13)	0·4521 (2)	594 (22)
C7	0·8907 (6)	0·1015 (13)	0·4434 (2)	550 (21)
C71	0·9137 (5)	0·1343 (10)	0·3967 (2)	417 (19)
C8	0·8587 (5)	−0·0567 (12)	0·2666 (2)	440 (20)
C9	0·7355 (5)	0·0763 (10)	0·2377 (2)	386 (15)
O9	0·7205 (4)	0·3250 (7)	0·2348 (1)	632 (16)
N22	0·6449 (4)	−0·0923 (10)	0·2132 (2)	449 (16)
C25	0·5258 (6)	0·0138 (12)	0·1828 (2)	524 (21)
C24	0·4606 (5)	−0·1867 (12)	0·1461 (2)	444 (19)
C23	0·3400 (5)	−0·0622 (13)	0·1136 (2)	457 (18)
C22	0·2765 (6)	−0·2494 (13)	0·0743 (2)	503 (21)
C21	0·1563 (5)	−0·1346 (11)	0·0423 (2)	398 (17)
O211	0·1147 (4)	−0·2847 (8)	0·0046 (1)	585 (14)
O212	0·1008 (3)	0·0775 (8)	0·0512 (1)	533 (13)

Table 8. *Bond lengths* (Å) *for conjugates* (1)–(6)

	(1)	(2)	(3)	(4)	(5)	(6)
N1—C2	1·372 (4)	1·368 (5)	1·370 (5)	1·365 (7)	1·378 (5)	1·378 (8)
N1—C71	1·367 (5)	1·374 (6)	1·371 (6)	1·377 (5)	1·378 (5)	1·365 (7)
C2—C3	1·369 (5)	1·343 (7)	1·360 (6)	1·366 (5)	1·362 (5)	1·365 (7)
C3—C31	1·439 (4)	1·431 (5)	1·434 (4)	1·441 (5)	1·442 (5)	1·436 (8)
C3—C8	1·498 (4)	1·515 (5)	1·510 (5)	1·493 (5)	1·496 (5)	1·490 (8)
C31—C4	1·408 (5)	1·397 (6)	1·398 (6)	1·402 (5)	1·388 (6)	1·399 (7)
C31—C71	1·405 (5)	1·411 (5)	1·397 (5)	1·419 (5)	1·419 (5)	1·410 (7)
C4—C5	1·385 (4)	1·388 (5)	1·382 (5)	1·371 (6)	1·371 (7)	1·380 (8)
C5—C6	1·408 (6)	1·401 (7)	1·391 (7)	1·399 (6)	1·399 (7)	1·391 (8)
C6—C7	1·369 (5)	1·372 (8)	1·382 (8)	1·382 (6)	1·378 (6)	1·371 (9)
C7—C71	1·392 (4)	1·388 (6)	1·387 (6)	1·393 (6)	1·381 (6)	1·386 (8)
C8—C9	1·519 (5)	1·511 (5)	1·504 (5)	1·521 (6)	1·516 (5)	1·515 (7)
C9—O9	1·233 (3)	1·230 (4)	1·228 (4)	1·240 (4)	1·248 (4)	1·227 (6)
C9—N22	1·337 (4)	1·332 (5)	1·336 (4)	1·325 (5)	1·318 (4)	1·335 (7)
N22—C22	1·443 (4)	1·454 (5)	1·449 (5)	1·453 (5)	1·457 (4)	
N22—C25						1·451 (7)
C21—O211	1·302 (4)	1·304 (5)	1·291 (4)	1·318 (5)	1·323 (4)	1·305 (6)
C21—O212	1·205 (4)	1·212 (4)	1·213 (4)	1·208 (5)	1·203 (4)	1·224 (7)
C21—C22	1·533 (4)	1·517 (5)	1·520 (5)	1·510 (5)	1·519 (5)	1·495 (7)
C22—C23	1·525 (4)	1·523 (5)	1·525 (5)	1·536 (5)	1·533 (4)	1·499 (8)
C23—C24		1·524 (6)	1·539 (5)	1·482 (5)	1·530 (5)	1·523 (7)
C24—C25			1·491 (7)		1·508 (6)	1·498 (8)
C24—O241				1·310 (4)		
C24—O242				1·231 (4)		
C23—C26					1·535 (5)	

Table 9. *Bond angles* (°) *for conjugates* (1)–(6)

	(1)	(2)	(3)	(4)	(5)	(6)
C2—N1—C71	108·8 (3)	109·0 (4)	108·4 (3)	109·9 (3)	109·2 (3)	109·9 (5)
N1—C2—C3	110·4 (3)	110·4 (4)	110·9 (4)	110·3 (4)	109·9 (3)	109·3 (5)
C2—C3—C8	127·9 (3)	127·7 (4)	127·6 (3)	128·0 (4)	126·7 (3)	126·1 (4)
C2—C3—C31	105·8 (3)	106·8 (3)	105·5 (3)	105·9 (4)	106·9 (3)	106·6 (4)
C31—C3—C8	126·3 (3)	125·5 (3)	126·9 (3)	126·1 (3)	126·2 (3)	127·4 (4)
C3—C31—C71	107·2 (3)	106·9 (3)	107·6 (3)	107·6 (3)	106·8 (3)	107·3 (4)
C3—C31—C4	133·1 (3)	134·4 (3)	133·3 (3)	133·9 (4)	134·7 (3)	133·9 (5)
C4—C31—C71	119·7 (3)	118·7 (3)	119·1 (3)	118·5 (3)	118·5 (3)	118·7 (5)
C31—C4—C5	117·9 (3)	118·8 (4)	118·6 (4)	118·7 (4)	119·6 (4)	118·8 (5)
C4—C5—C6	121·0 (3)	121·1 (4)	121·6 (4)	121·9 (4)	120·8 (4)	121·4 (5)
C5—C6—C7	122·0 (3)	121·1 (4)	120·6 (4)	121·3 (4)	121·5 (4)	121·1 (5)
C6—C7—C71	117·2 (3)	117·7 (4)	117·8 (4)	116·9 (4)	117·3 (4)	118·0 (6)
C31—C71—C7	122·3 (3)	122·5 (4)	122·3 (4)	122·7 (4)	122·3 (3)	122·1 (5)
N1—C71—C7	129·9 (3)	130·6 (4)	130·0 (4)	131·1 (4)	130·5 (3)	131·0 (5)
N1—C71—C31	107·8 (3)	106·9 (4)	107·6 (3)	106·2 (3)	107·2 (3)	106·9 (5)
C3—C8—C9	116·5 (3)	116·7 (3)	116·7 (3)	115·5 (3)	116·7 (3)	113·9 (4)
C8—C9—O9	120·9 (3)	121·8 (3)	122·0 (3)	122·3 (3)	120·6 (3)	122·9 (5)
C8—C9—N22	118·4 (3)	117·5 (3)	118·3 (3)	116·6 (3)	117·4 (3)	116·3 (5)
O9—C9—N22	120·6 (3)	120·7 (3)	119·7 (3)	121·1 (3)	122·0 (3)	120·8 (5)
C9—N22—C22	121·9 (2)	122·2 (3)	122·8 (2)	124·7 (3)	121·9 (3)	
C9—N22—C25						120·9 (5)
O211—C21—O212	124·0 (3)	123·9 (4)	123·6 (3)	123·8 (4)	124·7 (3)	123·3 (5)
C22—C21—O211	114·4 (3)	114·8 (3)	115·0 (3)	112·7 (3)	111·3 (3)	113·6 (5)
C22—C21—O212	121·6 (3)	121·3 (3)	121·4 (3)	123·5 (4)	124·0 (3)	123·1 (5)
N22—C22—C21	112·5 (2)	112·6 (3)	112·6 (2)	108·3 (3)	109·8 (2)	
N22—C22—C23	110·9 (2)	110·7 (3)	110·2 (2)	111·3 (3)	111·8 (3)	
N22—C25—C24						113·6 (5)
C23—C22—C21	110·5 (3)	110·6 (3)	111·0 (3)	113·1 (3)	112·9 (3)	115·0 (5)
C24—C23—C22		113·0 (4)	112·7 (4)	112·6 (3)	112·3 (3)	113·7 (5)
C25—C24—C23			111·0 (4)		115·1 (3)	111·7 (5)
O241—C24—O242				123·0 (3)		
C23—C24—O241				113·9 (3)		
C23—C24—O242				123·1 (3)		
C26—C23—C22					110·2 (3)	
C26—C23—C24					112·9 (3)	

Table 10. *Torsion angles* (°) *for conjugates* (1)–(6)

	(1)	(2)	(3)	(4)	(5)	(6)
C2—C3—C8—C9	−111·1 (4)	−108·6 (5)	−111·0 (5)	−113·4 (5)	110·5 (4)	−93·5 (6)
C31—C3—C8—C9	70·3 (4)	71·4 (5)	70·3 (5)	69·6 (5)	−76·3 (5)	87·5 (6)
C3—C8—C9—O9	−172·7 (3)	−170·5 (3)	−171·4 (3)	−160·0 (3)	162·9 (3)	47·6 (7)
C3—C8—C9—N22	6·6 (4)	8·9 (5)	7·9 (5)	21·3 (5)	−18·7 (5)	−135·5 (3)
C8—C9—N22—C22	174·7 (3)	176·5 (3)	177·0 (3)	−174·8 (3)	177·3 (3)	
O9—C9—N22—C22	−6·1 (4)	−4·1 (5)	−3·7 (5)	6·6 (6)	−4·3 (5)	
C9—N22—C22—C21	−76·2 (4)	−79·9 (4)	−78·3 (4)	−158·4 (3)	−78·4 (4)	
C9—N22—C22—C23	159·6 (3)	155·7 (3)	157·1 (3)	76·7 (4)	155·5 (3)	
N22—C22—C21—O211	0·6 (4)	−3·7 (5)	−8·3 (4)	−170·2 (3)	176·7 (3)	
N22—C22—C21—O212	179·9 (3)	175·9 (3)	173·3 (3)	10·6 (5)	−3·4 (4)	
C23—C22—C21—O211	125·2 (3)	120·8 (4)	115·8 (3)	−46·4 (4)	−57·8 (4)	170·8 (5)
C23—C22—C21—O212	−55·6 (4)	−59·7 (5)	−62·6 (4)	134·4 (4)	122·1 (3)	−10·5 (8)
N22—C22—C23—C24		−62·8 (5)	−66·7 (4)	62·8 (4)	66·6 (3)	
C21—C22—C23—C24		171·7 (4)	167·8 (3)	−59·4 (4)	−57·7 (3)	179·0 (5)
C22—C23—C24—C25			177·0 (4)		168·0 (3)	176·4 (5)
C22—C23—C24—O241				−166·3 (3)		
C22—C23—C24—O242				14·3 (5)		
N22—C22—C23—C26					−60·2 (3)	
C21—C22—C23—C26					175·5 (3)	
C26—C23—C24—C25					−66·6 (4)	
C8—C9—N22—C25						−177·5 (5)
O9—C9—N22—C25						−0·5 (8)
C9—N22—C25—C24						160·6 (5)
N22—C25—C24—C23						−177·4 (5)

Molecular structure

The molecular geometry of the indol-3-ylacetyl moiety of the free hormone (Karle *et al.*, 1964; Chandrasekhar & Raghunathan, 1982) has been compared with the analogous moiety in the six conjugates examined (Fig. 12). Bond lengths and angles do not reveal any anomalies (Tables 8 and 9). In the conjugates, lengthening of the C9—O9 bond to a mean value of 1·234 (4) Å in contrast to the value of 1·210 (4) Å in the free auxin (Chandrasekhar & Raghunathan, 1982) was observed. This is a normal consequence of peptide bond formation.

The flexibility of the side chain and its orientation with respect to the indole ring might play a role in relation to biological or biochemical function (Davies, 1987; Schneider & Wightmann, 1978). ^{1}H NOE measurements in solution (Duddeck *et al.*, 1989) and X-ray structure determinations (Kojić-Prodić, Nigović, Ružić-Toroš & Magnus, 1988; Kojić-Prodić, Magnus, Nigović & Ružić-Toroš, 1989) revealed that the C8—C9 bond is nearly perpendicular to the indole-ring plane with a slight tilt towards the benzene ring (Table 10). The NMR measurements (^{1}H NOE) (Duddeck *et al.*, 1989) for (1), (2), (4) and (5) show two conformers in equilibrium about the C8—C9 bond with torsion angles, C3—C8—C9—N22, of 0 and 180°. In the crystalline state the torsion angles range from −18·7 (5) to

21·3 (5)° (Table 10). In the crystalline state and in solution (1), (2) and (5) have nearly the same conformation along the N22—C22 bond but in solution the terminal groups of the side chain are the most flexible part of the molecules.

The part of the side chain closer to the indole moiety is not much affected by packing forces. For five of the six conjugates the torsion angle C2—C3—C8—C9 lies in the range −93·5 (6) (6) to −113·4 (5)° (4); in (5) it is +110·5 (4)° (Fig. 12) which brings the amino-acid aliphatic backbone closer to the pyrrole part of the indole moiety rather than to the benzene ring [as in (1) to (4)]. The terminal methyl group of (5) is 7·1 Å from the closest atom of the benzene ring, whereas the closest contacts between the terminal part of the aliphatic chain and the benzene ring are 4·5 in (2), 4·8 in (3), 5·4 in (1) and 5·5 Å in (4).

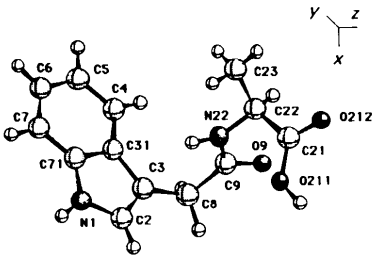

Fig. 2. Molecular structure of *N*-(IAA)-L-Ala with atom numbering.

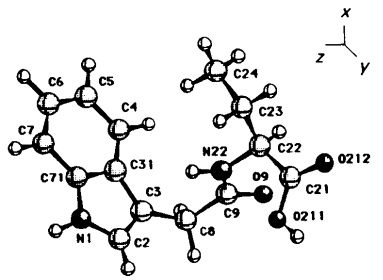

Fig. 3. Molecular structure of *N*-(IAA)-α-L-Abu with atom numbering.

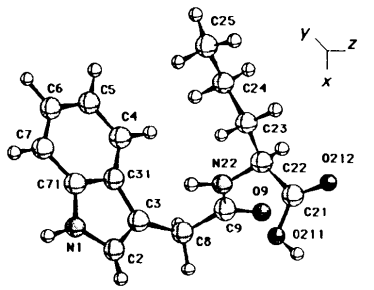

Fig. 4. Molecular structure of *N*-(IAA)-L-Nva with atom numbering.

In (6) the aliphatic chain is in the fully extended conformation (Table 10). For molecules (1)–(5), the amide nitrogen N22—H is oriented towards the indole ring. The conformation of the peptide bond is *trans* for all the conjugates, as is common for peptides (Karle, 1981). In the crystals of (1) and (4) the conformation at N22—C22 is stabilized by intramolecular hydrogen bonds, of the O=C—OH···O=C type in (1) and of the N—H···O=C—OH type (involving the peptide nitrogen) in (4) (Table 11).

In (1)–(3), the hydrophilic negative carboxyl groups stick out from the main body of the molecules (Fig. 12). In (4), which has two carboxylic groups, the separation of hydrophilic and hydrophobic groups is less pronounced than in structures (1)–(3). Branching of the aliphatic chain of isoleucine affects the overall molecular conformation of (5) in such a fashion that the carboxylic group is oriented towards C4 of the benzene ring.

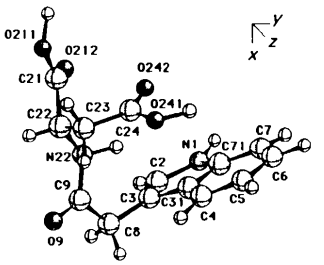

Fig. 5. Molecular structure of *N*-(IAA)-DL-Asp with atom numbering.

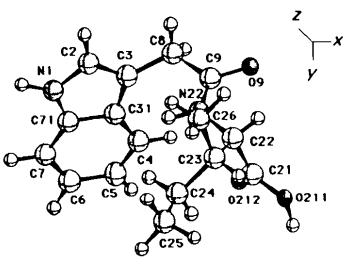

Fig. 6. Molecular structure of *N*-(IAA)-L-Ile with atom numbering.

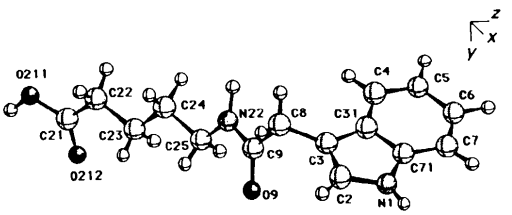

Fig. 7. Molecular structure of *N*-(IAA)-δ-Ava with atom numbering.

Table 11. *Hydrogen bonds*

		D—H···*A* (Å)	*D*—H (Å)	H···*A* (Å)	*D*—H···*A* (°)	Symmetry operations on *A*
(1)	N1—H···O212	2·932 (4)	1·01 (1)	2·037 (8)	146·7 (5)	$x,y,z-1$
	O211—H···O9	2·561 (4)	0·98 (9)	1·605 (8)	162·8 (6)	$-x+1,y-\frac{1}{2},-z+2$
(2)	N1—H···O212	2·847 (5)	1·01 (5)	1·84 (5)	180 (5)	$x,y,z+1$
	O211—H···O9	2·564 (4)	0·98 (5)	1·60 (5)	166 (5)	$-x,y-\frac{1}{2},-z+1$
(3)	N1—H···O212	2·835 (5)	1·06 (4)	1·85 (4)	154 (3)	$x,y,z-1$
	O211—H···O9	2·561 (4)	0·98 (4)	1·61 (4)	162 (4)	$-x+2,y-\frac{1}{2},-z+2$
(4)	N22—H···O242	3·011 (4)	1·01 (6)	2·51 (6)	110 (4)	x,y,z
	O211—H···O9	2·610 (4)	0·98 (6)	1·65 (6)	165 (5)	$x-1,y,z$
	O241—H···O242	2·615 (4)	1·07 (5)	1·55 (5)	177 (4)	$-x,-y+1,-z+2$
(5)	N1—H···O9	3·107 (4)	1·02 (1)	2·14 (1)	157 (2)	$x-1,y,z$
	O211—H···O9	2·597 (4)	1·02 (2)	1·58 (2)	176 (1)	$-x+1,y+\frac{1}{2},-z+\frac{1}{2}$
(6)	N22—H···O9	2·987 (6)	1·01 (5)	1·99 (5)	168 (5)	$x,y-1,z$
	O211—H···O212	2·663 (5)	0·98 (8)	1·69 (8)	172 (8)	$-x,-y,-z$

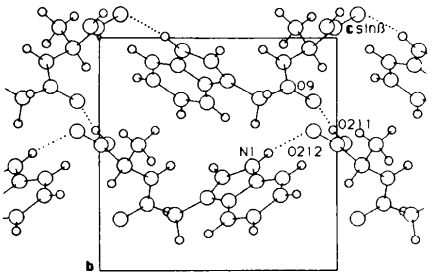

Fig. 8. View along **a** of *N*-(IAA)-L-Ala; dotted lines illustrate hydrogen bonds.

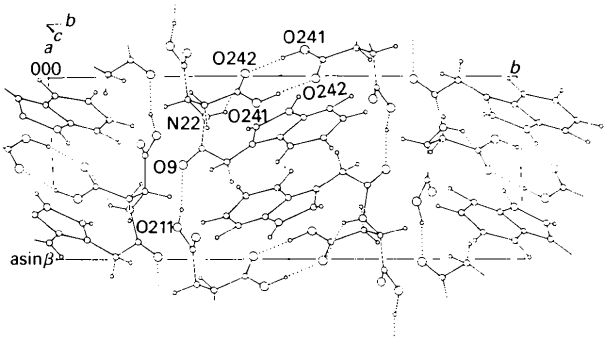

Fig. 9. View along **c** of (IAA)-DL-Asp.

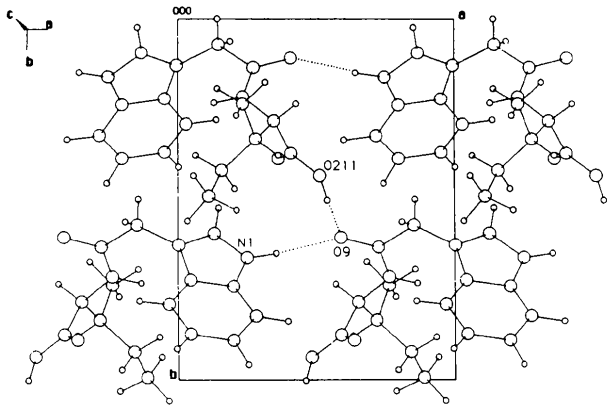

Fig. 10. View along **c** of *N*-(IAA)-L-Ile.

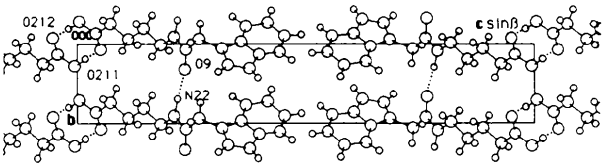

Fig. 11. View along **a** of *N*-(IAA)-δ-Ava.

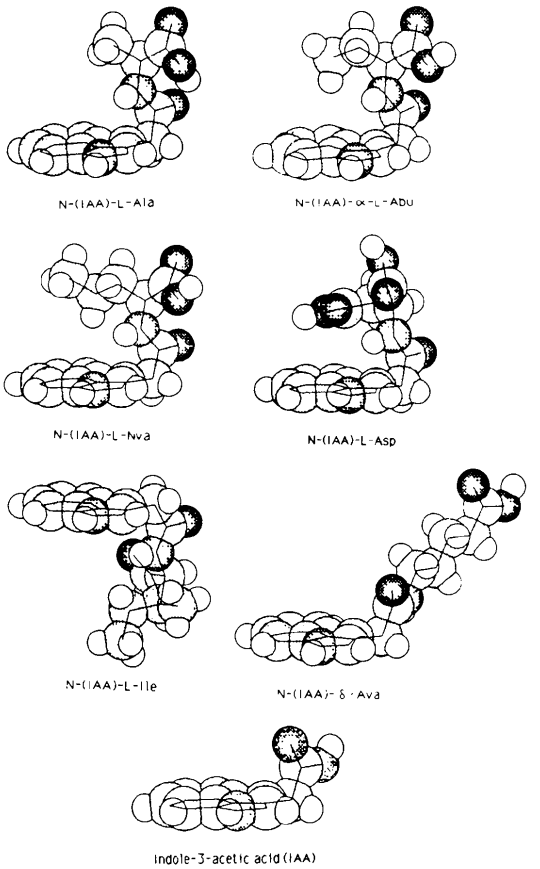

Fig. 12. Space-filling models of conjugates (1)–(6) and of the free hormone (IAA) illustrating the overall molecular shape and the separation of hydrophilic (polar) and hydrophobic regions at the molecular surface.

Crystal packing

The crystal packing of these conjugates is predominantly determined by intermolecular hydrogen bonds (Table 11). The molecular conformations and crystal packing of (1)–(3), whose amino-acid moieties are members of a series of straight-chain homologs, exhibit the same pattern of hydrogen bonds. [Their chemical formulae differ by $(CH_2)_{1,2}$. The space group symmetry is the same and the unit-cell volumes are in relation to their chemical formulae.]

In the crystal structure of (4) (Fig. 9) the pattern of intermolecular hydrogen bonds is similar to that in IAA (Karle *et al.*, 1964; Chandrasekhar & Raghunathan, 1982) and in (6). In (4) the basic pattern is a tetrameric unit formed by hydrogen bonds between molecules along **a** and those around the centers of inversion.

In these structures the hydrophobic regions, which are comprised of the benzene ring and the aliphatic backbone of the amino acids, appear around the centers of symmetry at (0,0,0) and $(\frac{1}{2},\frac{1}{2},0)$ and hydrophilic channels are pronounced in the area around the centers of inversion at $(0,\frac{1}{2},0)$ and $(\frac{1}{2},0,0)$. In the crystal structure of (5) (Fig. 10) the indole nitrogen acts as as donor to the peptide oxygen O9 so as to form an infinite chain along **a** by means of an N1—H···O9 hydrogen bond. This kind of hydrogen-bond network is not found in any of the other structures.

Concluding remarks

Any physiological interpretation of the above structural data must be preliminary, since only selected *N*-(indol-3-ylacetyl) amino acids have been examined thus far.

The ratio of molar concentrations which causes the same (half-optimal) growth stimulation in *Solanum nigrum* callus for IAA-Ala:IAA-Abu:IAA-δ-Ava:IAA-Nva:IAA-Ile is approximately 1:9:32:37:90 (V. Magnus, R. P. Hangarter & N. E. Good, unpublished work). IAA-Asp was not examined in this system; its activity in tomato hypocotyl explants (Hangarter *et al.*, 1980) and soybean cotyledon callus (Feung *et al.*, 1977) was roughly 10–100 times less than that of IAA-L-Ala. The growth-promoting activity of IAA conjugates has been correlated to the rates of hydrolysis of the free hormone (Bialek, Meudt & Cohen, 1983; Hangarter & Good, 1981). Enzymatic activity capable of cleaving IAA-Ala has been detected in buffer extracts of *Phaseolus vulgaris* internodes (Bialek & Cohen, 1984), but not fully characterized. Known amidases such as papain, acylase I, carboxypeptidase Y from Baker's yeast, and a protease from *Streptomyces griseus* were unable to hydrolyze IAA-alanine, IAA-L-valine, IAA-glycine and IAA-L-phenylalanine (Hangarter, 1981). The amide bond in the conjugates examined here has the same conformation as in most peptides (Mutter & Vuilleumier, 1989) and it is freely exposed at the hydrophilic pole of the molecules, so there are no obvious structural reasons for general resistance to amidases. The notorious sensitivity of individual representatives of this group of enzymes to inhibition by factors such as the buffer system used in assays, metal ions and the products of hydrolysis may, however, explain the so far limited success in the search for IAA-amino acid hydrolases.

Discussion of IAA-δ-Ava, regarding the growth-promoting activity of the conjugates examined here in terms of their rates of hydrolysis, should be postponed until a more representative sample of conjugates of the same structural type has been analysed. While the remaining compounds studied have roughly the same molecular shape, IAA-Asp may (in this context) represent another particular case, since amide bonds at aminodicarboxylic acids are usually cleaved by special enzymes. For the conjugates of the α-amino monocarboxylic acids, which may well be hydrolyzed by the same enzyme, activity appears to be correlated with the size of the molecule, or to the hydrophobic pole, which is optimal in magnitude in the case of IAA-Ala (IAA-glycine is less active).

The conformation of the indol-3-ylacetyl moiety in the conjugates proved to be highly conservative. Only in one case (IAA-L-Ile) was there any significant deviation from the geometry observed in free IAA: a 180° rotation around the C3—C8 bond with no effect on the respective intramolecular contact distances, particularly N1···O9 (5·0 Å). However, the minimum energy calculations for *N*-IAA-L-Ile, based on the atomic coordinates from X-ray analysis (Fig. 12), revealed a lower value of the total molecular energy than for the molecule rotated by 180° about C3—C8 (101·7, 215·6 kJ mol^{-1}, respectively). The total molecular energies for the molecules presented in this paper range from 26·8 (*N*-IAA-Asp) to 259·2 kJ mol^{-1} (*N*-IAA-α-Abu). For the whole series, conformations along the C3—C8 bond obtained from a molecular-mechanics approach fit those observed in the crystals (B. Kojić-Prodić, S. Tomić & B. Nigović, in preparation).

In conjugates (1)–(5), the amide group (substituting the IAA carboxyl —OH) assumes the position above the indole ring occupied by the carbonyl oxygen in crystalline free IAA. Its structure in solution still requires detailed study, although it would appear that, at physiological pH, both oxygens of the dissociated carboxyl group are equivalent owing to resonance effects, so the sign of torsion angle C3—C8—C9—O9 becomes irrelevant. A change in

its numerical value, as observed in the δ-Ava conjugate (6) may, however, deserve further attention. The aliphatic residues of the amino acids approach only the part of the indole ring in the immediate proximity of the CH_2—CO— side chain closely enough for steric shielding and, possibly, van der Waals interactions. A similar situation should be expected at the site of an auxin-binding protein which recognizes the carboxyl group of IAA (although not necessarily by forming a covalent bond). The NH group and the neighbouring part of the benzene ring remain free for interaction with other recognition sites. Indeed, an auxin-binding protein must, in addition to the carboxyl group, recognize other topological elements of IAA to discriminate against the large number of different carboxylic acids present in a plant cell.

These results pose the question: can an IAA amino-acid conjugate, which is structurally identical to the free hormone except for a restricted area on the molecule surface, attach to part of recognition sites in an auxin-binding protein, and thus compete with free IAA? Although against common dogma, such an assumption could help to explain the complex interaction of free and conjugated IAA in some *in vitro* systems (Wodzicki, Pharis & Wodzikcki, 1987; V. Magnus, R. P. Hangarter & N. E. Good, unpublished work). As far as the stereochemistry of the amino-acid conjugates examined in this work is concerned, there appear to be no reasons against competition of free and bound IAA for at least some auxin-binding sites (*i.e.* those not specific for the free IAA carboxyl group).

References

ANDERSSON, B. & SANDBERG, G. (1982). *J. Chromatogr.* **238**, 151–156.

BIALEK, K. & COHEN, J. D. (1984). *Plant Physiol.* **75**(Suppl.), 108.

BIALEK, K., MEUDT, W. J. & COHEN, J. D. (1983). *Plant Physiol.* **73**, 130–134.

CHANDRASEKHAR, K. & RAGHUNATHAN, S. (1982). *Acta Cryst.* **B38**, 2534–2535.

COHEN, J. D. (1982). *Plant Physiol.* **70**, 749–753.

COHEN, J. D. & BANDURSKI, R. S. (1982). *Annu. Rev. Plant Physiol.* **33**, 403–430.

DAVIES, P. J. (1987). *Plant Hormones and Their Role in Plant Growth and Development*. Dordrecht: Martinus Nijhoff.

DUDDECK, H., HIEGEMANN, M., SIMEONOV, M. F., KOJIĆ-PRODIĆ, B., NIGOVIĆ, B. & MAGNUS, V. (1989). *Z. Naturforsch. Teil C*, **44**, 543–554.

EPSTEIN, E., BALDI, B. G. & COHEN, J. D. (1985). *Plant Physiol.* **80**, 256–258.

EVIDENTE, A., SURICO, G., IACOBELLIS, N. S. & RANDAZZO, G. (1986). *Phytochemistry*, **25**, 125–128.

FEUNG, C.-S., HAMILTON, R. H. & MUMMA, R. O. (1976). *Plant Physiol.* **58**, 666–669.

FEUNG, C.-S., HAMILTON, R. H. & MUMMA, R. O. (1977). *Plant Physiol.* **59**, 91–93.

HANGARTER, R. P. (1981). PhD Thesis, Michigan State Univ., USA.

HANGARTER, R. P. & GOOD, N. E. (1981). *Plant Physiol.* **68**, 1424–1427.

HANGARTER, R. P., PETERSON, M. D. & GOOD, N. E. (1980). *Plant Physiol.* **65**, 761–767.

HORVATIĆ D. (1986). *MOL*. An interactive program for graphical visualization and manipulation of molecules including structural parameter calculations. Rudjer Bošković Institute, Zagreb, Yugoslavia.

HUTZINGER, O. & KOSUGE, T. (1968). *Biochemistry*, **7**, 601–605.

KARLE, I. L. (1981). In *Peptides*, Vol. 4, *X-ray Analysis: Conformation of Peptides in the Crystalline State*. New York: Academic Press.

KARLE, I. L., BRITTS, K. & GUM, P. (1964). *Acta Cryst.* **17**, 496–499.

KOJIĆ-PRODIĆ, B., MAGNUS, V., NIGOVIĆ, B. & RUŽIĆ-TOROŠ, Ž. (1989). *Plant Physiol.* **89**(Suppl.), 110.

KOJIĆ-PRODIĆ, B., NIGOVIĆ, B., RUŽIĆ-TOROŠ, Ž. & MAGNUS, V. (1988). *Z. Kristallogr.* **185**, M47–M57 MO.

MAGNUS, V. (1987). *Conjugated Plant Hormones. Structure, Metabolism and Function*, edited by K. SCHREIBER, H. R. SCHUETTE & G. SEMBDNER, pp. 31–40. Berlin: VEB Deutscher Verlag der Wissenschaften.

MOTHERWELL, S., MURRAY-RUST, P., RAFTERY, J., ALLEN, F. & DOYLE, M. (1989). *GSTAT*89. Cambridge Structural Database integrated program for molecular geometry parameter calculations. CCDC, Cambridge, England.

MUELLER, N. & FALK, A. (1986). *BALL & STICK* 1.78. Three-dimensional visualization and manipulation of molecular structure. Johannes Kepler Univ., Linz, Austria.

MUTTER, M. & VUILLEUMIER, S. (1989). *Angew. Chem. Int. Ed. Engl.* **28**, 535–554.

NARDELLI, M. (1983). *Comput. Chem.* **7**, 95–98.

PERCIVAL, F. W. (1986). *Plant Physiol.* **80**, 259–263.

SCHNEIDER, E. A. & WIGHTMANN (1978). *Phytohormones and Related Compounds – A Comprehensive Treatise*, Vol. 1, pp. 29–105. Amsterdam: Elsevier.

SEMBDNER, G., GROSS, D., LIEBISCH, H.-W. & SCHNEIDER, G. (1980). *Encyclopedia of Plant Physiology*, Vol. 9, edited by J. MACMILLAN, pp. 281–444. Berlin: Springer.

SHELDRICK, G. M. (1983). *SHELX*77. Program for crystal structure determination. Univ. of Cambridge, England.

SHELDRICK, G. M. (1985). In *Crystallographic Computing* 3, edited by G. M. SHELDRICK, C. KRUEGER & R. GODDARD. Oxford Univ. Press.

SONNER, J. M. & PURVES, W. K. (1985). *Plant Physiol.* **77**, 784–785.

THIMANN, K. V. (1977). *Hormone Action in the Whole Life of Plants*. Amherst: Univ. of Massachusetts Press.

WODZICKI, T. J., PHARIS, R. P. & WODZICKI, A. B. (1987) *Plant Physiol.* **84**, 1139–1142.