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Structure of a Biologically Active Conjugate of Auxin: *N*-Indol-3-ylacetyl-L-norleucine at 297 and 133 K

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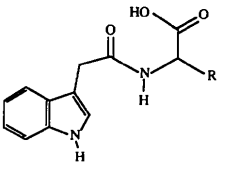
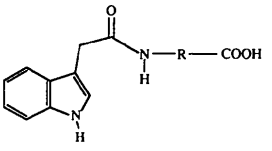
Abstract. $C_{16}H_{20}N_2O_3$, $M_r = 288.35$, monoclinic, $P2_1$, $Z = 2$, Mo $K\alpha$, $\lambda = 0.71073$ Å, $F(000) = 308$. At 297 K: $a = 9.350$ (6), $b = 9.271$ (2), $c = 9.695$ (12), $\beta = 108.38$ (3)°, $V = 798$ (1) Å^3, $D_x = 1.201$ g cm^{-3}, $\mu = 0.782$ cm^{-1}, $R = 0.072$, $wR = 0.070$ for 851 reflections with $I > 1.5\sigma(I)$. At 133 K: $a = 9.474$ (6), $b = 9.163$ (4), $c = 9.524$ (6) Å, $\beta = 107.83$ (3)°, $V = 787.1$ (8) Å^3, $D_x = 1.217$ g cm^{-3}, $\mu = 0.792$ cm^{-1}, $R = 0.053$, $wR = 0.046$ for 986 reflections with $I > 1.5\sigma(I)$. The indole ring system and the C atom of the adjacent methylene group are coplanar, whereas the CON*R* residue adopts a folded conformation. The peptide H is orientated towards the aromatic nucleus. The crystal packing involves hydrogen bonds, N—H···O between the indole N atom and the carboxylic acid group, and O—H···O between the carboxylic acid group and the peptide O atom.

Introduction. Indole-3-acetic acid (IAA, auxin) is a plant growth hormone which regulates many physiological functions such as cell division and enlargement, development differentiation, and the synthesis of specific proteins (Thimann, 1977; Davies, 1987). Biogenesis and metabolism of the plant hormones are, as yet, incompletely understood and there is no general agreement on the molecular mechanism of their action. A special regulatory function has been attributed to the bound auxins, or auxin conjugates (Cohen & Bandurski, 1982; Magnus, 1987). They are involved in hormone transport and serve as long- and short-term storage forms of the hormone. In addition, some of amino-acid conjugates are biologically active *per se* (Magnus, Hangarter & Good, 1992). Investigations of molecular recognition of auxins have so far been based on a few structural and physico-chemical parameters of the compounds studied: relative orientation of side chains towards the indole nucleus, intramolecular distance between the active sites (indole nitrogen and O=C), distribution of hydrophobic and hydrophilic regions, and electronic properies of the aromatic system. In order to recognize essential parameters of the auxin system more accurate structural data are required. However, L-amino-acid conjugates are rather difficult to crystallize and poor crystal quality is a limiting factor. In order to reduce the effect of thermal vibrations of the alkyl chain and to increase the number of reflections recorded data were also collected at low temperature.

In studying the structure–activity relationships of the auxins and related compounds, we have performed a systematic analysis of their molecular structures by X-ray diffraction and spectroscopic methods, and by molecular mechanics and dynamics (Kojić-Prodić *et al.*, 1991; Duddeck, Hiegemann, Simeonov, Kojić-Prodić, Nigović & Magnus, 1989). The present structure determination is a part of the studies on amino-acid conjugates of indole-3-acetic acid; the compounds studied are listed in Fig.1.

Experimental. *N*-(IAA)-L-Nle was synthesized by the aminolysis of the mixed anhydride formed from indole-3-acetic acid and ethyl chloroformate (Wieland & Hoerlein, 1955; Magnus *et al.*, 1992) using the L-amino acid and leading to an enantiomer with the *S* configuration; the nature of the chemical reaction precludes the introduction of a chiral centre. After many crystallization trials, crystals of *N*-(IAA)-L-Nle were obtained from ethanol (95% vol.) after 14 days at 275 (2) K.





(1) *N*-(IAA)-Gly	$R = H$	(11) *N*-(IAA)-β-Ala	$R = (CH_2)_2$
(2) *N*-(IAA)-L-Ala	$R = CH_3$	(12) *N*-(IAA)-γ-Abu	$R = (CH_2)_3$
(3) *N*-(IAA)-α-Aib	$R = (CH_3)_2$	(13) *N*-(IAA)-δ-Ava	$R = (CH_2)_4$
(4) *N*-(IAA)-α-L-Abu	$R = CH_2CH_3$	(14) *N*-(IAA)-ε-Ahx	$R = (CH_2)_6$
(5) *N*-(IAA)-L-Nva	$R = (CH_2)_2CH_3$		
(6) *N*-(IAA)-L-Nle	$R = (CH_2)_3CH_3$		
(7) *N*-(IAA)-L-Val	$R = CH(CH_3)_2$		
(8) *N*-(IAA)-L-Leu	$R = CH_2CH(CH_3)_2$		
(9) *N*-(IAA)-L-Ile	$R = CH(CH_3)CH_2CH_3$		
(10) *N*-(IAA)-DL-Asp	$R = CH_2COOH$		

Fig. 1. Structural formulae of amino-acid conjugates of indole-3-acetic acid.

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Intensity data were collected on an Enraf–Nonius CAD-4F diffractometer (Table 1). Measurements were carried out at room temperature (297 K) and 133 K using the same sample. The crystal size was 0.25 × 0.14 mm × 0.10 mm. No significant intensity variation was observed for the standard reflections. Data were corrected for Lorentz and polarization effects (but not for absorption) using the Enraf–Nonius *SDP/VAX* package (Frenz, 1978). Structures were solved by *SHELXS*86 (Sheldrick, 1985), and refinement performed using the *SHELX*77 system of programs (Sheldrick, 1983). The H-atom coordinates were determined from successive difference Fourier syntheses for the low-temperature data. H coordinates for the terminal methyl group were calculated on stereochemical grounds and refined under conditions of defined tetrahedral C—H geometry. In the structure at 297 K the H atoms attached to C6, C8, C23, C24, C25 and C26 were treated analogously. In both sets of data the N1—H and N22—H distances were normalized to an N—H value of 1.009 Å (Allen, Kennard & Watson, 1987). The non-H atoms were refined anisotropically; details of the refinement procedures are listed in Table 1. In both data sets the *y* coordinates of O9 were used to fix the origin in $P2_1$. In both refinements, the poor crystal quality and weak diffraction data led to low observation-to-parameter ratios and adversely affected the refinements. However, high thermal vibrations of the terminal part of alkyl chain (Fig. 2*a*) were reduced using the low-temperature data and an *R* value of 0.053 was achieved. Scattering factors are those included in *SHELX*77 (Sheldrick, 1983). Molecular geometry was calculated by the program package *EUCLID* (Spek, 1982). Drawings were prepared by *PLUTON* incorporated into *EUCLID* and *ORTEP*II (Johnson, 1976). The final atomic coordinates and equivalent isotropic thermal parameters are listed in Table 2.* Calculations were performed on MicroVAX II and IRIS-4D25G computers of the X-ray Laboratory, Ruder Bošković Institute, Zagreb.

Discussion. Interatomic distances, bond angles and selected torsion angles of both sets are listed in Table 3. The *ORTEP* plots with the atom numbering for the molecules at 297 and 133 K are shown in Figs. 2(*a*) and (2*b*), respectively. The molecular packing including hydrogen bonds is illustrated in Fig. 3 and details of the intermolecular hydrogen bonds are listed in Table 4.

* 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 54800 (13 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England. [CIF reference: LI0095]

Table 1. *Details of data collection and refinement*

	297 K	133 K
No. of reflections for cell parameters	25	25
θ range (°)	6–14	4–17
θ range for intensity measurement (°)	2.5–25	2–25
hkl range	0,11; 0,11; −11,11	0,11; 0,10; −11,11
ω scan (°)	0.8 + 0.35tanθ	1.0 + 0.35tanθ
No. of measured reflections	1591	1575
No. of symmetry-independent reflections	851	986
	[$I > 1.5\sigma(I)$]	[$I > 1.5\sigma(I)$]
No. of parameters	224	242
R	0.072	0.053
wR, $w^{-1} = k(\sigma F_o^2 + gF)$	0.070	0.046
Final shift/e.s.d.	0.599 (C25, *y*)	0.073 (N22, *y*)
$(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (e Å^{-3})	0.39, −0.31	0.29, −0.29

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

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

	x	*y*	*z*	U_{eq} (Å^2)
297 K				
N1	0.6735 (13)	0.7894 (15)	−0.1772 (9)	779 (50)
C2	0.7916 (14)	0.8390 (17)	−0.0587 (11)	764 (53)
C3	0.7262 (12)	0.9281 (15)	0.0186 (9)	608 (44)
C31	0.5679 (13)	0.9327 (15)	−0.0549 (10)	560 (43)
C4	0.4474 (14)	1.0032 (16)	−0.0328 (13)	651 (52)
C5	0.3039 (15)	0.9838 (17)	−0.1278 (13)	842 (65)
C6	0.2796 (18)	0.8938 (17)	−0.2468 (15)	900 (69)
C7	0.3949 (17)	0.8218 (17)	−0.2743 (13)	829 (63)
C71	0.5389 (14)	0.8438 (16)	−0.1766 (10)	608 (52)
C8	0.8119 (11)	1.0117 (16)	0.1554 (10)	619 (51)
C9	0.7915 (10)	0.9631 (14)	0.2946 (10)	464 (36)
O9	0.8493 (8)	1.0277	0.4086 (7)	708 (33)
N22	0.7063 (10)	0.8480 (14)	0.2942 (9)	490 (35)
C21	0.8132 (11)	0.7000 (15)	0.5177 (11)	587 (43)
C22	0.6817 (11)	0.7869 (15)	0.4241 (10)	535 (41)
C23	0.5342 (9)	0.7068 (17)	0.3858 (10)	678 (46)
C24	0.3984 (11)	0.7975 (21)	0.3147 (14)	1101 (70)
C25	0.2697 (16)	0.6965 (33)	0.2978 (17)	1699 (112)
C26	0.1256 (26)	0.7597 (37)	0.2079 (21)	2806 (227)
O211	0.9213 (9)	0.6808 (13)	0.4614 (7)	700 (32)
O212	0.8122 (9)	0.6517 (13)	0.6323 (7)	952 (38)
133 K				
N1	0.3301 (6)	0.3186 (8)	1.1828 (6)	260 (22)
C2	0.2210 (8)	0.3737 (9)	1.0645 (7)	231 (25)
C3	0.2804 (7)	0.4637 (9)	0.9849 (7)	209 (23)
C31	0.4386 (7)	0.4667 (9)	1.0599 (6)	175 (22)
C4	0.5584 (7)	0.5391 (9)	1.0338 (7)	189 (22)
C5	0.6984 (7)	0.5171 (9)	1.1311 (7)	234 (25)
C6	0.7227 (8)	0.4228 (9)	1.2526 (7)	266 (28)
C7	0.6081 (8)	0.3513 (9)	1.2803 (7)	251 (27)
C71	0.4647 (8)	0.3750 (9)	1.1844 (6)	215 (26)
C8	0.1996 (8)	0.5492 (10)	0.8507 (7)	210 (26)
C9	0.2134 (7)	0.4981 (8)	0.7039 (7)	186 (23)
O9	0.1536 (5)	0.5628	0.5877 (4)	237 (17)
N22	0.2955 (6)	0.3793 (8)	0.7066 (5)	178 (19)
C21	0.1808 (7)	0.2320 (9)	0.4797 (7)	201 (22)
C22	0.3141 (7)	0.3176 (9)	0.5718 (6)	184 (22)
C23	0.4551 (7)	0.2274 (10)	0.6058 (7)	207 (24)
C24	0.5973 (7)	0.3135 (11)	0.6774 (8)	292 (27)
C25	0.7343 (7)	0.2191 (10)	0.7056 (7)	266 (26)
C26	0.8744 (8)	0.2995 (12)	0.7867 (9)	533 (34)
O211	0.0732 (5)	0.2191 (8)	0.5404 (5)	250 (16)
O212	0.1768 (5)	0.1797 (7)	0.3609 (5)	270 (16)

Molecular recognition of the auxin and its biologically active conjugates has been studied previously (Kojić-Prodić *et al.*, 1991) and the crucial aspect appears to be the overall molecular conformation (Davies, 1987). The indole moiety and the C atom of the adjacent methylene group are coplanar but the CONR residue can be either extended or folded. Structural studies of *N*-(IAA)-amino-acid

Table 3. *Bond lengths* (Å), *bond angles* (°) *and selected torsion angles* (°) *for N-(IAA)-L-norleucine*

	297 K	133 K
N1—C2	1.397 (14)	1.371 (8)
N1—C71	1.357 (19)	1.372 (10)
C2—C3	1.380 (19)	1.354 (11)
C3—C31	1.427 (15)	1.449 (8)
C3—C8	1.527 (14)	1.495 (10)
C31—C4	1.376 (19)	1.401 (11)
C31—C71	1.393 (16)	1.412 (9)
C4—C5	1.379 (16)	1.380 (8)
C5—C6	1.383 (20)	1.405 (10)
C6—C7	1.363 (24)	1.361 (12)
C7—C71	1.396 (17)	1.402 (9)
C8—C9	1.490 (15)	1.518 (10)
C9—O9	1.223 (11)	1.230 (7)
C9—N22	1.331 (17)	1.333 (10)
N22—C22	1.463 (15)	1.462 (9)
C21—C22	1.510 (15)	1.516 (9)
C22—C23	1.506 (15)	1.519 (10)
C23—C24	1.497 (17)	1.529 (10)
C24—C25	1.492 (27)	1.513 (11)
C25—C26	1.476 (29)	1.507 (10)
C21—O211	1.303 (15)	1.322 (9)
C21—O212	1.200 (14)	1.219 (9)
C2—N1—C71	112 (1)	109.6 (6)
N1—C2—C3	106 (1)	110.3 (6)
C2—C3—C8	125 (1)	127.3 (7)
C2—C3—C31	108.3 (8)	106.3 (6)
C31—C3—C8	126.6 (9)	126.4 (6)
C3—C31—C71	107.4 (9)	106.9 (6)
C3—C31—C4	135.1 (9)	133.9 (6)
C4—C31—C71	118 (1)	119.2 (6)
C31—C4—C5	120 (1)	118.4 (6)
C4—C5—C6	120 (1)	121.6 (7)
C5—C6—C7	122 (1)	121.1 (6)
C6—C7—C71	117 (1)	118.1 (7)
C31—C71—C7	123 (1)	121.6 (7)
N1—C71—C7	130 (1)	131.4 (7)
N1—C71—C31	107 (1)	107.0 (6)
C3—C8—C9	116.8 (8)	117.4 (7)
C8—C9—N22	119.1 (9)	116.6 (6)
C8—C9—O9	121.6 (9)	122.3 (6)
O9—C9—N22	119.3 (8)	121.0 (5)
C9—N22—C22	124.3 (9)	121.7 (6)
N22—C22—C21	113.8 (9)	113.9 (5)
N22—C22—C23	111.0 (8)	111.0 (5)
C21—C22—C23	113.2 (9)	111.3 (6)
C22—C23—C24	115 (1)	114.3 (7)
C23—C24—C25	104 (1)	112.0 (7)
C24—C25—C26	112 (2)	112.6 (7)
C22—C21—O211	114.4 (9)	114.2 (6)
C22—C21—O212	120.8 (9)	121.6 (6)
O211—C21—O212	124.7 (9)	124.2 (7)
C2—C3—C8—C9	−111 (1)	−107.9 (9)
C3—C8—C9—O9	−176 (1)	−178.5 (6)
C3—C8—C9—N22	3 (2)	0.8 (9)
C8—C9—N22—C22	178 (1)	178.5 (6)
C9—N22—C22—C21	−77 1)	−76.7 (8)
C9—N22—C22—C23	154 (1)	156.7 (6)
O9—C9—N22—C22	−3 (2)	−2.2 (9)
N22—C22—C21—O211	−7 (1)	−4.1 (9)
N22—C22—C21—O212	175 (1)	176.6 (7)
N22—C22—C23—C24	−60 (1)	−61.1 (8)
C23—C22—C21—O211	122 (1)	122.3 (7)
C23—C22—C21—O212	−57 (1)	−57.1 (9)
C21—C22—C23—C24	170 (1)	171.0 (6)
C22—C23—C24—C25	−176 (1)	−178.7 (6)
C23—C24—C25—C26	−171 (2)	−175.6 (7)

conjugates indicate that the folded conformation predominates (Kojić-Prodić *et al.*, 1991). The torsion angle C2—C3—C8—C9 is −107.9 (9)° (133 K) [−111 (1)°, 297 K] so that the amino-acid aliphatic backbone (with a zigzag shape) is brought closer to the benzene ring of the indole moiety. The hydrophobic pocket so formed accommodates the peptide NH group in its base (Fig. 1). On the other hand the

Table 4. *Hydrogen-bond geometry* (Å, °)

D—H···*A*	*D*···*A*	*D*—H	H···*A*	∠*D*—H···*A*	Symmetry operations on *A*
N1—H···O212					
297 K	2.87 (2)	1.0 (1)	1.90 (13)	152 (11)	$x, y, z-1$
133 K	2.849 (9)	1.01 (7)	1.96 (8)	145 (6)	$x, y, z+1$
O211—H···O9					
297 K	2.55 (1)	1.0 (1)	1.58 (13)	175 (13)	$-x+2, y-\frac{1}{2}, -z+1$
133 K	2.56 (6)	1.11 (7)	1.53 (8)	151 (6)	$-x, y-\frac{1}{2}, -z+1$

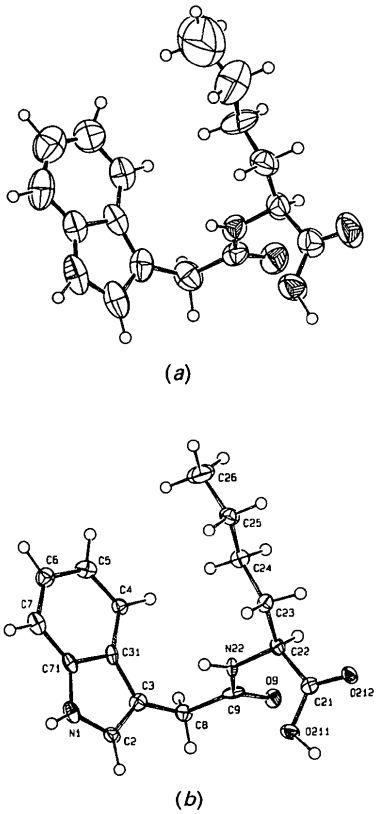

Fig. 2. Molecular structure and atom numbering: (*a*) at 297, (*b*) at 133 K. The thermal ellipsoids are scaled at the 50% probability level.

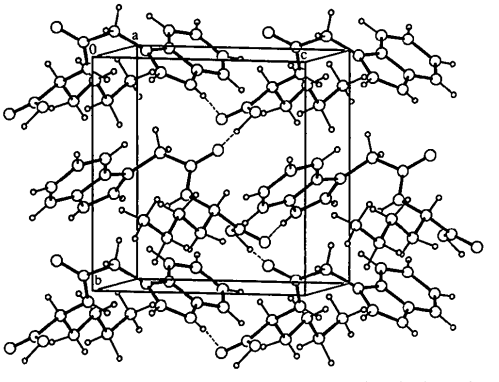

Fig. 3. Crystal packing with hydrogen bonds (dashed lines).

carboxyl group and the peptide O atom protrude from the main body of the molecule and the indole NH is exposed at the outer region of the molecule; this emphasizes the amphipatic character of the molecule. The conformation of the peptide bond is *trans*, and there is no intramolecular hydrogen bond.

The crystal packing of *N*-IAA-L-Nle is determined by intermolecular hydrogen bonds (Table 4, Fig. 3). The molecular conformations and crystal packing of *N*-(IAA)-L-Nva (Kojić-Prodić *et al.*, 1991) and the title compound *N*-(IAA)-L-Nle, the amino-acid moieties of which are members of a series of straight-chain homologues (they differ by a CH_2 group), exhibit a similar pattern of hydrogen bonds. The indole NH acts as a donor to the carboxylic O212 group joining molecules along **c**. The carboxylic group is a donor to the peptide O atom forming the hydrogen bonds between molecules related by a 2_1 axis.

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Structure of Lansimide 2, a Product from *Clausena lansium*

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Abstract. The natural product lansimide 2 is a 1:1 mixture of two different cyclic amides, $C_{18}H_{17}NO_2.C_{18}H_{19}NO_3$. The mixture crystallizes as a molecular pair in the centrosymmetric space group $P2_1/n$. $M_r = 576.69$, monoclinic, $a = 20.151$ (2), $b = 6.2984$ (4), $c = 24.051$ (2) Å, $\beta = 104.339$ (8)°, $V = 2957.4$ Å^3, $Z = 4$, $D_x = 1.30$ g cm^{-3}, Cu $K\alpha$, $\lambda = 1.54178$ Å, $\mu = 6.1$ cm^{-1}, $F(000) = 1224$, $T = 163$ (1) K, $R = 0.033$, $wR = 0.034$ for 5002 observed reflections.

Introduction. A number of amides have been isolated from the roots and leaves of *Clausena lansium*. In folk medicine the leaf extract has been used for the treatment of dermatological diseases, viral hepatitis, asthma and gastro-intestinal diseases. The molecular structures of a number of these amides have been determined. These are lansimide 1 (Prakash, Raj, Kapil & Popli, 1980), the isomeric clausenamide and neoclausenamide together with cycloclausenamide (Yang, Chen & Huang, 1988), several cinnamamides (Lin, 1989), and lansimide 3, which is identical to molecule (II) in lansimide 2 (Lakshmi & Kapil, 1992). The present communication reports the structure of lansimide 2 which proves to be a 1:1 mixture of two cyclic amides. The lansimides have been

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