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Pseudophomins A and B, a class of cyclic lipodepsipeptides isolated from a *Pseudomonas* species

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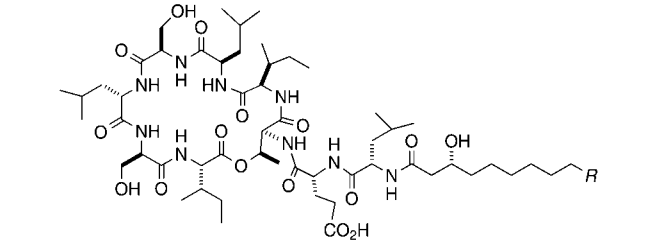
The crystal structures of pseudophomins A and B, with primary structures β-hydroxydecanoyl–L-Leu–D-Glu–D-*allo*-Thr–D-Ile–D-Leu–D-Ser–L-Leu–D-Ser–L-Ile monohydrate, $C_{55}H_{97}N_9O_{16}\cdot H_2O$, and β-hydroxydodecanoyl–L-Leu–D-Glu–D-*allo*-Thr–D-Ile–D-Leu–D-Ser–L-Leu–D-Ser–L-Ile monohydrate, $C_{57}H_{101}N_9O_{16}\cdot H_2O$, new cyclic lipodepsipeptides isolated from *Pseudomonas fluorescens* strain BRG100, have been solved. The absolute configuration of pseudophomin A has been determined from anomalous dispersion and the stereochemistry of the β-hydroxy acid group is *R*.

Comment

As a result of their general ability to produce potent antifungal and herbicidal metabolites, bacteria of the genus *Pseudomonas* comprise a large group of potential biocontrol agents (Balkovec, 1994; Dowling & O'Gara, 1994; Faull & Powell, 1995). Microorganisms, such as soil bacteria, can be used for the biological control of weeds and plant pathogens to avoid the continuous use of chemical herbicides and fungicides. However, the successful application of such agents presupposes a good understanding of their chemistry and mechanism of action. Integrated in a program to discover and apply new biological control agents, we investigated the production of antifungal and herbicidal metabolites by the bacterium *P. fluorescens* strain BRG100. We established that the major metabolites, named pseudophomin A, (I), and B, (II), are a pair of new cyclic depsipeptides active against some plant pathogens. The chemical structure of these compounds was established by a combination of spectroscopic techniques, chemical derivatization and degradation, and X-ray crystallography. We describe here the crystal structure determinations of (I) and (II).

X-ray crystallographic analysis allowed us to assign the following primary structure to the peptide moiety, *i.e.* Leu–Glu–*allo*-Thr–Ile–Leu–Ser–Leu–Ser–Ile, with the terminal carboxy group closing a macrocyclic ring on the hydroxy group of the *allo*-threonine residue. The N-terminus is, in turn, acylated by 3-hydroxydecanoate in pseudophomin A and by 3-hydroxydodecanoate in pseudophomin B. The configuration of ten non-centrosymmetric centers in the ring and lipid side chain are given in the *Abstract* in terms of D and L nomenclature. The configurations of all 13 non-centrosymmetric sites are given in *R*/*S* nomenclature in the *Scheme* below.



(I) Pseudophomin A, R = Me, hydrate
(II) Pseudophomin B, R = Pr, hydrate

The few crystal structures of cyclic lipodepsipeptides determined to date include WLIP (Han *et al.*, 1992), tensin (Henriksen *et al.*, 2000), and amphisin (Sørensen *et al.*, 2001). A literature search showed pseudophomin A to be a diastereomer of massetolide A and pseudophomin B to be a diastereomer of massetolide C (Gerard *et al.*, 1997). Interestingly, the chemical structure of pseudophomin A is very similar to that of WLIP, except for the D-Ile-4 residue,

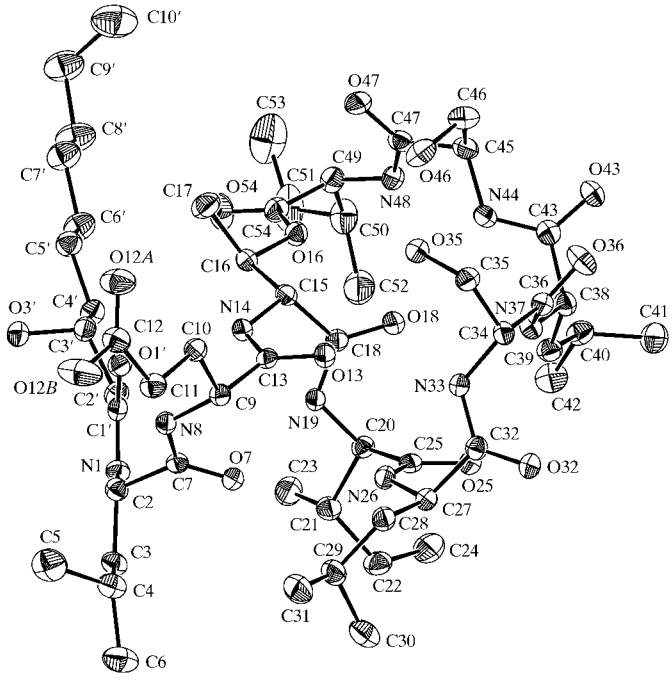


Figure 1
A general view (Hall *et al.*, 2000) of pseudophomin A with non-H-atom displacement ellipsoids drawn at the 50% probability level. For clarity, the H atoms have been omitted.

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which in WLIP is replaced by a D-Val residue (Mortishire-Smith *et al.*, 1991; Han *et al.*, 1992). Furthermore, pseudophomin A has the absolute configuration of each stereogenic center identical to the equivalent stereogenic center of WLIP (Bateman *et al.*, 1990; Baldwin *et al.*, 1990; Abraham & Podell, 1981).

Because (I) and (II) are produced in the same bacteria and possess almost identical sequences of amino acids, we have presumed that they must have the same stereochemistry. Thus, for pseudophomin B, the primary structure is β-hydroxydodecanoyl–L-Leu–D-Glu–D-*allo*-Thr–D-Ile–D-Leu–D-Ser–L-Leu–D-Ser–L-Ile. The superimposition of all atoms of (II), except for the disordered regions but including the solvent water molecule, on the equivalent atoms of (I), using the program *PROFIT* (Smith, 1983), gave an r.m.s. deviation of 0.225 Å, with the largest deviation being 0.378 Å for C5. When the superimposition was limited to the backbone atoms of (II), starting at atom O3′ and including the O atoms in the side chains and the solvent water molecule, the r.m.s. deviation was 0.070 Å, with the largest deviation being 0.225 Å for the solvent water molecule. Examination of the ellipsoid plots and coordinates reported for WLIP (Han *et al.*, 1992) indicated that the authors had deposited the mirror-image coordinates of the reported isomer and used these coordinates to draw the diagrams. The coordinates of WLIP were inverted to fit the structure to the reported stereochemistry. The superimposition of the inverted coordinates of the WLIP atoms, including the solvent water molecule, on the backbone atoms of (I) (leaving out the methyl group which is not in WLIP) using *PROFIT* gave an r.m.s. deviation of 0.042 Å, with the largest deviation being 0.171 Å. When the comparison was limited to the backbone plus side-chain O atoms and the water molecule, the r.m.s. deviation was 0.027 Å, with the maximum deviation being 0.150 Å for atom O12*B*.

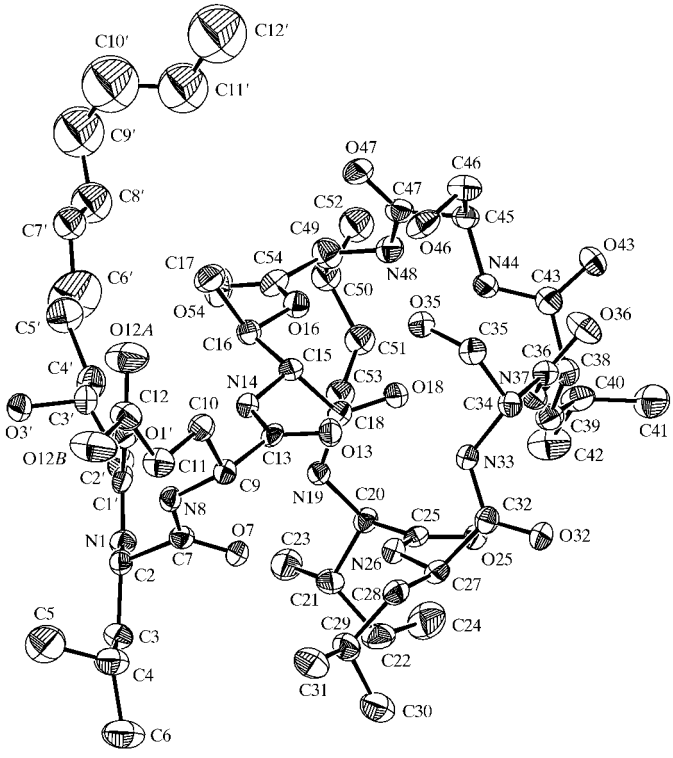

Figure 2
A general view (Hall *et al.*, 2000) of pseudophomin B with non-H-atom displacement ellipsoids drawn at the 50% probability level. For clarity, the H atoms have been omitted. In the disordered parts of the molecule, the segment with the largest population parameter is displayed.

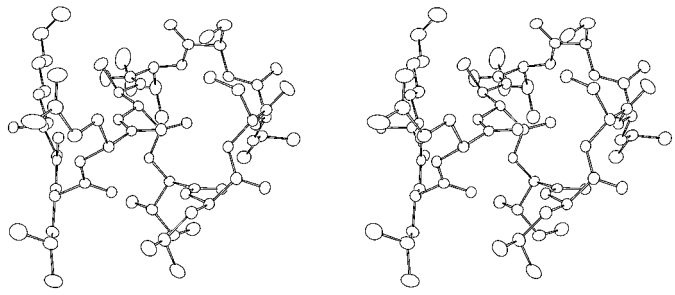

Figure 3
A stereoview of pseudophomin A (Hall *et al.*, 2000).

The structures of (I) and (II) are shown in the same orientation in Figs. 1 and 2, respectively. A stereoview of (I) in the same orientation is shown in Fig. 3. Compounds (I), (II) and WLIP are isostructural, and have the same space group and very similar cell dimensions. Given the remarkable similarities in the structures, it is not suprising that the hydrogen-bonding schemes are essentially identical for (I), (II) and WLIP. 17 separate hydrogen bonds (both inter- and intramolecular) were identified per asymmetric unit in (I) and (II), and are described in Tables 1 and 2, respectively. Only 11 hydrogen bonds per asymmetric unit were identified in WLIP (Han *et al.*, 1992), but these authors limited their list to $D\cdots A$ distances of less than 3 Å. They also included two hydrogen bonds with bond angles of less than 100° that we have not selected.

The crystal structure of (I) showed a solvent-accessible void of about 35 Å^3. In the structures of (I) and (II), there is no evidence of electron density from further solvent water molecules. The void volume is smaller than required for a water molecule (40 Å^3 according to *checkCIF*). It is likely that the shape of the void is not consistent with the shape required by a water molecule. There was a low-occupancy water molecule reported by Han *et al.* (1992) in WLIP. The crystallization conditions may have also reduced the ability of a water molecule to fill the void in (I) and (II).

Experimental

Pseudophomins A and B were isolated, guided by bioassay, from EtOAc extracts obtained from culture supernatants of *P. fluorescens* strain BRG100, following multiple column chromatography utilizing both normal and reverse-phase silica gel. Pseudophomin A was crystallized twice from dichloromethane–methanol (10:90), whereas pseudophomin B was crystallized from aqueous acetonitrile. The specific optical rotations at 299 K and λ = 589 nm for the two lipodepsipeptides in ethanol were found to be $[\alpha]^D = -23$ for (I) and $[\alpha]^D = -15$ for (II).

Table 1
Hydrogen-bonding geometry (Å, °) for (I).

D—H···A	D—H	H···A	D···A	D—H···A
O12B—H12B···O25^{i}	0.84	1.79	2.6215 (17)	169
O35—H35···O46	0.84	1.93	2.6824 (17)	148
O46—H46···O1′^{ii}	0.84	2.01	2.8126 (16)	161
O3′—H3′O···O35^{iii}	0.84	2.23	2.8402 (16)	129
O3′—H3′O···O13^{iii}	0.84	2.49	3.0925 (16)	129
O99—H99A···O43^{iv}	0.85	2.05	2.8215 (17)	152
O99—H99B···O36^{iv}	0.86	1.93	2.7791 (18)	170
N1—H1···O99	0.88	1.97	2.8304 (18)	166
N8—H8···O47^{iii}	0.88	2.00	2.8432 (17)	161
N14—H14···O1′	0.88	2.15	2.9516 (18)	152
N19—H19···O7	0.88	2.44	2.9720 (17)	119
N26—H26A···O7	0.88	2.26	3.1237 (17)	166
N33—H33···O13	0.88	2.03	2.8307 (17)	150
N37—H37···O25	0.88	2.36	3.1608 (17)	151
N37—H37···O18	0.88	2.53	3.0220 (18)	116
N44—H44···O18	0.88	2.06	2.9252 (18)	169
N48—H48···O32^{v}	0.88	2.18	2.9008 (17)	139

Symmetry codes: (i) $\frac{1}{2}-x, 1-y, \frac{1}{2}+z$; (ii) $x-\frac{1}{2}, \frac{1}{2}-y, 2-z$; (iii) $\frac{1}{2}+x, \frac{1}{2}-y, 2-z$; (iv) $1+x, y, z$; (v) $-x, y-\frac{1}{2}, \frac{3}{2}-z$.

Table 2
Hydrogen-bonding geometry (Å, °) for (II).

D—H···A	D—H	H···A	D···A	D—H···A
O12B—H12B···O25^{i}	0.84	1.81	2.645 (3)	170
O35—H35···O46	0.84	1.91	2.677 (3)	151
O46—H46···O1′^{ii}	0.84	1.94	2.785 (3)	179
O3′—H3′O···O35^{iii}	0.84	2.00	2.827 (3)	170
O3′—H3′O···O13^{iii}	0.84	2.73	3.176 (3)	115
O99—H99A···O43^{iv}	0.85	2.06	2.832 (3)	151
O99—H99B···O36^{iv}	0.87	1.90	2.766 (3)	170
N1—H1···O99	0.88	1.96	2.834 (3)	173
N8—H8···O47^{iii}	0.88	1.99	2.844 (3)	163
N14—H14···O1′	0.88	2.14	2.941 (3)	152
N19—H19···O7	0.88	2.39	2.955 (3)	123
N26—H26···O7	0.88	2.35	3.204 (3)	165
N33—H33···O13	0.88	2.08	2.863 (3)	148
N37—H37···O25	0.88	2.43	3.231 (3)	151
N37—H37···O18	0.88	2.51	2.995 (3)	115
N44—H44···O18	0.88	2.08	2.949 (3)	167
N48—H48···O32^{v}	0.88	2.38	3.057 (3)	134

Symmetry codes: (i) $\frac{1}{2}-x, 1-y, \frac{1}{2}+z$; (ii) $x-\frac{1}{2}, \frac{1}{2}-y, 2-z$; (iii) $\frac{1}{2}+x, \frac{1}{2}-y, 2-z$; (iv) $1+x, y, z$; (v) $-x, y-\frac{1}{2}, \frac{3}{2}-z$.

Compound (I)

Crystal data

$C_{55}H_{97}N_9O_{16}\cdot H_2O$
M_r = 1158.43
Orthorhombic, $P2_12_12_1$
a = 14.139 (1) Å
b = 18.739 (1) Å
c = 24.473 (2) Å
V = 6484.1 (8) Å^3
Z = 4
D_x = 1.187 Mg m^{-3}
Cu $K\alpha$ radiation
Cell parameters from 6097 reflections
θ = 3.0–65.1°
μ = 0.72 mm^{-1}
T = 100 (2) K
Block, colorless
0.22 × 0.17 × 0.15 mm

Data collection

Nonius KappaCCD 2000 diffractometer
φ and ω scans
Absorption correction: multi-scan (Otwinowski & Minor, 1997)
T_{min} = 0.857, T_{max} = 0.899
61 093 measured reflections
11 039 independent reflections
10 778 reflections with I > 2σ(I)
R_{int} = 0.050
θ_{max} = 65.7°
$h = -16 \rightarrow 16$
$k = -21 \rightarrow 22$
$l = -28 \rightarrow 28$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.035
$wR(F^2)$ = 0.092
S = 1.09
11 039 reflections
742 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0590P)^2 + 1.1181P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.30 e Å^{-3}
$\Delta\rho_{min}$ = −0.26 e Å^{-3}
Extinction correction: *SHELXL*97
Extinction coefficient: 0.00210 (9)
Absolute structure: Flack (1983), 4944 Friedel reflections
Flack parameter = 0.02 (9)

Compound (II)

Crystal data

$C_{57}H_{101}N_9O_{16}\cdot H_2O$
M_r = 1186.48
Orthorhombic, $P2_12_12_1$
a = 14.245 (2) Å
b = 18.870 (3) Å
c = 24.558 (4) Å
V = 6601.7 (18) Å^3
Z = 4
D_x = 1.194 Mg m^{-3}
Cu $K\alpha$ radiation
Cell parameters from 5508 reflections
θ = 3.0–65.1°
μ = 0.72 mm^{-1}
T = 100 (2) K
Rod, colorless
0.46 × 0.04 × 0.04 mm

Data collection

Bruker SMART 6000 diffractometer
ω scans
Absorption correction: multi-scan (Blessing, 1995)
T_{min} = 0.735, T_{max} = 0.975
30 846 measured reflections
11 751 independent reflections
9543 reflections with $I > 2\sigma(I)$
R_{int} = 0.049
θ_{max} = 71.3°
$h = -16 \rightarrow 12$
$k = -22 \rightarrow 22$
$l = -30 \rightarrow 28$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.050
$wR(F^2)$ = 0.134
S = 1.07
11751 reflections
798 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0655P)^2 + 1.5509P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.54 e Å^{-3}
$\Delta\rho_{min}$ = −0.31 e Å^{-3}
Extinction correction: *SHELXL*97
Extinction coefficient: 0.00052 (7)
Absolute structure: Flack (1983), 4927 Friedel reflections
Flack parameter = 0.10 (17)

All H atoms were placed in calculated positions on their parent atoms (C—H = 0.99 Å on aliphatic C atoms, 0.95 Å on aromatic C atoms, 0.84 Å on sp^2 N atoms, and 0.85 Å on O atoms). The U_{iso} value of each H atom was assigned as equal to 1.2 times the U_{eq} of the attached atom. Methyl groups were staggered relative to the bonds of the attached atom. The H atoms on the solvent water molecules (O99) for (I) and (II) were constrained to have O—H distances of 0.85 Å and H···H distances of 1.34 Å. U_{iso} values for the water H atoms were allowed to refine for (I) and were set to 1.2U_{eq} of the water O atom for (II). For (I), the *TWIN* and *BASF* commands were used in the refinement to determine the Flack parameter (Flack, 1983; Flack & Bernardinelli, 1999, 2000), whose value of 0.02 (9) indicated that the correct configuration had been assigned. The refinement of (II) showed two regions of disorder. The last six C atoms of the lipid chain are disordered and L-Ile-9 is also disordered. The lipid region disorder was modeled by three chains having isotropic displacement parameters and riding H atoms. The isoleucine was modeled with two separate isoleucine units modeled with isotropic displacement parameters and riding H atoms. The methyl group of each ethyl unit on the two Ile components was modeled with two disordered methyl positions. The *TWIN* and *BASF* commands were used in the refinement to determine the Flack parameter (Flack, 1983; Flack & Bernardinelli, 1999, 2000), which was found to be

0.10 (17); the s.u. value is too large to give certainty in the determination of the absolute configuration. Since the two molecules have similar specific optical rotations and come from the same biological source, it is probable that (I) and (II) have the same absolute configuration.

For compound (I), data collection: *COLLECT* (Nonius, 2000); cell refinement: *HKL SCALEPACK* (Otwinowski & Minor, 1997); data reduction: *HKL DENZO* (Otwinowski & Minor, 1997) and *SCALEPACK*. For compound (II), data collection: *SMART* (Bruker, 1997); cell refinement: *SMART*; data reduction: *SAINT* (Bruker, 1997). For both compounds, program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *Xtal*3.7 (Hall *et al.*, 2000); software used to prepare material for publication: *SHELXL*97.

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: BK1644). Services for accessing these data are described at the back of the journal.

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