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Structure of the White-Line-Inducing Principle Isolated from *Pseudomonas Reactans*

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Abstract. $C_{54}H_{93}N_9O_{16}.H_2O$, $M_r = 1142.40$, orthorhombic, $P2_12_12_1$, $a = 14.230\ (1)$, $b = 24.370\ (5)$, $c = 18.780\ (2)$ Å, $V = 6512.6\ (11)$ Å^3, $Z = 4$, $D_x = 1.16$ g cm^{-3}, λ(Cu $K\alpha$) = 1.5418 Å, $\mu = 6.8$ cm^{-1}, $F(000) = 2472$, $T = 158\ (2)$ K, $R = 0.073$ for 6299 unique reflections. The structure of this cycle peptide is that of β-hydroxydecanoyl-L-Leu-D-Glu-D-*allo*-Thr-D-Val-D-Leu-D-Ser-D-Ser-L-Ile. Ring closure is through an ester linkage between the carboxyl of L-Ile and the hydroxyl of D-*allo*-Thr, leaving the γ-carboxyl of D-Glu free. The stereochemistry of the β-hydroxy acid is shown to be *R*.

Introduction. Members of the genus *Pseudomonas* characteristically produce a wide variety of extracellular compounds including siderophores, antibiotics, toxins and enzymes. A range of fluorescent *Pseudomonas* species are commonly found in association with the sporophore of the edible mushroom, *Agaricus bisporus* (Lange) Imbach (Olivier, Guillames & Martin, 1978; Zarkower, Wuest, Royse & Myers, 1983; Goor, Vantomme, Swings, Gillis, Kersters & deLey, 1986). Of particular interest is the taxonomically diverse group of saprophytic *Pseudomonas* species, collectively referred to as *Pseudomonas reactans* (Wong & Preece, 1979), which are capable of forming a white line in agar when grown in association with *Pseudomonas tolaasii* Paine, the causal organism of the economically significant brown blotch disease of *A. bisporus* (Tolaas, 1915). This white-line reaction is a rapid and reliable means of identifying *P. tolaasii* isolates (Zarkower, Wuest, Royse & Myers, 1983; Wong & Preece, 1979) and is the result of a specific interaction between a diffusible peptide (Mortishire-Smith, Nutkins, Packman, Brodey, Rainey, Johnstone & Williams, 1991) produced by *P. reactans* (called the 'white-line-inducing principle' or WLIP) and the water-soluble peptide toxin (tolaasin) produced by *P. tolaasii* (Peng, 1986; Nutkins, Mortishire-Smith, Packman, Brodey, Rainey, Johnstone & Williams, 1991; Mortishire-Smith, Drake, Nutkins & Williams, 1991). Neither the mechanism whereby the two peptides give rise to

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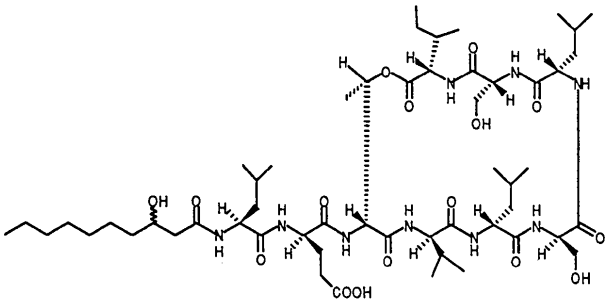
the white line nor the biological significance (if any) of the interaction are understood.



We have recently determined the primary structure of WLIP (1) by a combination of degradative and spectroscopic techniques (Mortishire-Smith, Nutkins, Packman, Prodey, Rainey, Johnstone & Williams, 1991) and report here the crystal structure of WLIP. Crystals of sufficient quality for an X-ray structure determination were obtained by the diffusion of water into a saturated solution of WLIP in methanol.

Experimental. A clear chunky crystal, 0.18 × 0.18 × 0.21 mm, was used for data collection on a Nicolet $P2_1$ diffractometer controlled by a Harris computer. All 6299 measured reflections were unique (5613 had intensities $> 2\sigma$) and were collected using graphite-monochromated Cu $K\alpha$ radiation at low temperature (~158 K) to $2\theta_{max} = 138°$ at 4° min^{-1} using $\theta/2\theta$-step scans with scan widths > 3.4°. Ten periodically monitored reflections showed no trend towards deterioration. $\sigma^2(I)$ was approximated by $\sigma^2(I)$ from counting statistics plus $(0.012I)^2$, where the coefficient of I was calculated from the variations in intensities of the monitored reflections. Accurate cell parameters were determined by least-squares fit of $K\alpha_1$ 2θ values [$\lambda(K\alpha_1) = 1.5402$ Å] for 25 high 2θ reflections (Duchamp, 1977). Lorentz and polarization corrections appropriate for a monochromator with 50% perfect character were made; however, there was no absorption correction. A partial structure from NMR, as a randomly positioned fragment, was used as input to the direct methods program *RANTAN*81 (Yao, 1980); this was carried out in order to optimize the structure factor normalization procedure. The best figure of merit trial solution set from *RANTAN* yielded a 41-atom partial structure containing the frame of the ring. The successive Fourier syntheses located the remaining 38 non-H atoms as well as two partially occupied water molecules. H atoms were found in difference maps very close to positions generated using planar or tetrahedral geometry. Least-squares refinement included coordinates and anisotropic thermal parameters for the non-H atoms; H-atom parameters were included

Table 1. *Fractional coordinates* ($\times 10^4$) *and equivalent isotropic thermal parameters* (Å^2)

$B_{eq} = (4/3)(a^2B_{11} + b^2B_{22} + c^2B_{33})$.

	x	y	z	B_{eq}
C(1)	4849 (3)	3158 (2)	485 (3)	2.0 (2)
C(1A)	4687 (5)	2546 (2)	567 (3)	3.3 (3)
C(2)	4535 (3)	3366 (2)	−250 (3)	1.8 (2)
N(2A)	5162 (3)	3133 (2)	−792 (2)	1.9 (2)
C(2B)	4959 (3)	3221 (2)	−1484 (3)	1.9 (2)
O(2C)	4243 (2)	3446 (1)	−1678 (2)	2.2 (2)
C(2D)	5624 (3)	2980 (2)	−2037 (3)	1.9 (2)
C(2E)	5294 (3)	2409 (2)	−2266 (3)	2.0 (2)
C(2F)	5872 (4)	2174 (2)	−2880 (3)	2.5 (2)
C(2G)	5705 (4)	1570 (2)	−2998 (3)	2.9 (3)
O(2H)	6170 (4)	1386 (2)	−3554 (3)	5.4 (3)
O(2I)	5224 (3)	1295 (2)	−2624 (3)	4.5 (2)
N(2J)	6584 (3)	2959 (2)	−1791 (2)	1.8 (2)
C(2K)	6997 (3)	3416 (2)	−1551 (3)	2.1 (2)
O(2L)	6601 (2)	3873 (1)	−1549 (2)	2.1 (2)
C(2M)	8014 (3)	3360 (2)	−1309 (3)	2.0 (2)
C(2N)	8657 (4)	3679 (2)	−1789 (3)	2.5 (2)
C(2O)	8632 (4)	3513 (2)	−2573 (3)	2.8 (3)
C(2P)	9170 (5)	3921 (3)	−3036 (4)	4.6 (3)
C(2Q)	8984 (6)	2929 (3)	−2689 (4)	5.0 (3)
N(2R)	8111 (3)	3542 (2)	−570 (2)	2.3 (2)
C(3)	4493 (3)	3995 (2)	−281 (3)	2.1 (2)
O(3A)	3757 (2)	4235 (1)	−182 (2)	2.4 (2)
N(4)	5309 (3)	4249 (2)	−400 (2)	2.0 (2)
C(5)	5294 (4)	4840 (2)	−513 (3)	2.2 (2)
C(5A)	6298 (4)	5075 (2)	−532 (3)	2.9 (3)
C(5B)	6297 (5)	5670 (3)	−767 (4)	4.7 (3)
C(5C)	6768 (5)	5008 (3)	193 (4)	4.5 (3)
C(6)	4733 (3)	4989 (2)	−1176 (3)	2.3 (2)
O(6A)	4130 (3)	5352 (2)	−1173 (2)	2.7 (2)
N(7)	4915 (3)	4687 (2)	−1764 (2)	2.0 (2)
C(8)	4462 (4)	4823 (2)	−2441 (3)	2.4 (2)
C(8A)	4890 (4)	4467 (2)	−3037 (3)	2.4 (2)
C(8B)	5969 (4)	4518 (3)	−3127 (3)	2.9 (3)
C(8C)	6273 (4)	5105 (3)	−3235 (4)	3.7 (3)
C(8D)	6264 (4)	4162 (3)	−3756 (4)	4.3 (3)
C(9)	3399 (4)	4758 (2)	−2427 (3)	2.0 (2)
O(9A)	2902 (2)	5040 (2)	−2802 (2)	2.7 (2)
N(10)	3046 (3)	4345 (2)	−2003 (2)	1.9 (2)
C(11)	2047 (3)	4246 (2)	−1962 (3)	2.2 (2)
C(11A)	1826 (4)	3634 (2)	−1957 (3)	2.4 (2)
O(11B)	2245 (3)	3346 (1)	−1384 (2)	2.5 (2)
C(12)	1571 (4)	4512 (2)	−1315 (3)	2.2 (2)
O(12A)	725 (3)	4452 (2)	−1224 (2)	3.7 (2)
N(13)	2113 (3)	4814 (2)	−885 (2)	2.5 (2)
C(14)	1748 (4)	5092 (2)	−260 (3)	2.5 (2)
C(14A)	2511 (4)	5464 (2)	33 (3)	2.7 (2)
C(14B)	2277 (5)	5804 (3)	693 (3)	3.4 (3)
C(14C)	1527 (5)	6234 (2)	539 (4)	4.0 (3)
C(14D)	3176 (5)	6069 (3)	961 (4)	4.3 (3)
C(15)	1353 (4)	4709 (2)	309 (3)	2.3 (2)
O(15A)	575 (2)	4773 (2)	568 (2)	2.8 (2)
N(16)	1916 (3)	4282 (2)	498 (2)	2.5 (2)
C(17)	1540 (4)	3843 (2)	950 (3)	2.4 (2)
C(17A)	1054 (4)	3422 (2)	493 (3)	3.0 (3)
O(17B)	1670 (3)	3201 (2)	−36 (2)	3.2 (2)
C(18)	2316 (4)	3590 (2)	1407 (3)	2.3 (2)
O(18A)	2376 (3)	3090 (2)	1497 (2)	3.0 (2)
N(19)	2920 (3)	3940 (2)	1704 (2)	2.3 (2)
C(20)	3733 (4)	3755 (3)	2103 (3)	3.0 (3)
C(20A)	4161 (5)	4242 (3)	2502 (4)	4.3 (3)
C(20B)	4579 (6)	4643 (3)	1996 (6)	6.7 (4)
C(20C)	4880 (7)	4103 (4)	3068 (5)	7.8 (5)
C(20D)	4527 (10)	3720 (5)	3619 (5)	11.9 (8)
C(21)	4445 (4)	3454 (2)	1657 (3)	2.7 (2)
O(21A)	5140 (3)	3236 (2)	1894 (2)	3.9 (2)
O(22)	4240 (2)	3460 (1)	961 (2)	2.1 (2)
C(A)	7615 (4)	3288 (2)	−56 (3)	2.4 (2)
O(A1)	7041 (2)	2916 (2)	−193 (2)	2.4 (2)
C(B)	7781 (4)	3495 (2)	703 (3)	2.2 (2)
C(C)	7606 (4)	3039 (2)	1259 (3)	2.4 (2)
O(C1)	8294 (2)	2622 (1)	1152 (2)	2.4 (2)
C(D)	7663 (4)	3280 (2)	2007 (3)	2.5 (2)
C(E)	7388 (4)	2869 (2)	2584 (3)	2.7 (3)
C(F)	7414 (6)	3125 (3)	3326 (3)	4.1 (3)
C(G)	7006 (7)	2733 (3)	3896 (4)	6.2 (4)
C(H)	6971 (9)	2971 (3)	4619 (4)	7.9 (5)
C(I)	6513 (9)	2601 (5)	5176 (5)	9.5 (7)
C(J)	5477 (11)	2524 (6)	5073 (7)	11.8 (8)
O(W1)	−890 (3)	4529 (2)	−368 (3)	3.9 (2)
O(W2)	−1141 (23)	5036 (14)	−729 (18)	2.0 (6)

Table 2. *Bond lengths* (Å) *and angles* (°)

C(1)—C(1*A*)	1.516 (8)	N(10)—C(11)	1.444 (6)
C(1)—C(2)	1.537 (7)	C(11)—C(11*A*)	1.523 (8)
C(1)—O(22)	1.446 (6)	C(11)—C(12)	1.536 (7)
C(2)—N(2*A*)	1.467 (6)	C(11*A*)—O(11*B*)	1.417 (7)
C(2)—C(3)	1.537 (8)	C(12)—O(12*A*)	1.225 (6)
N(2*A*)—C(2*B*)	1.349 (7)	C(12)—N(13)	1.336 (7)
C(2*B*)—O(2*C*)	1.213 (6)	N(13)—C(14)	1.452 (7)
C(2*B*)—C(2*D*)	1.522 (7)	C(14)—C(14*A*)	1.518 (8)
C(2*D*)—C(2*E*)	1.530 (7)	C(14)—C(15)	1.524 (8)
C(2*D*)—N(2*J*)	1.443 (6)	C(14*A*)—C(14*B*)	1.527 (8)
C(2*E*)—C(2*F*)	1.528 (7)	C(14*B*)—C(14*C*)	1.523 (9)
C(2*F*)—C(2*G*)	1.507 (8)	C(14*B*)—C(14*D*)	1.518 (10)
C(2*G*)—O(2*H*)	1.316 (8)	C(15)—O(15*A*)	1.220 (6)
C(2*G*)—O(2*I*)	1.188 (7)	C(15)—N(16)	1.360 (7)
N(2*J*)—C(2*K*)	1.388 (7)	N(16)—C(17)	1.467 (7)
C(2*K*)—O(2*L*)	1.247 (6)	C(17)—C(17*A*)	1.506 (8)
C(2*K*)—C(2*M*)	1.524 (7)	C(17)—C(18)	1.529 (8)
C(2*M*)—C(2*N*)	1.500 (7)	C(17*A*)—O(17*B*)	1.430 (7)
C(2*M*)—N(2*R*)	1.464 (7)	C(18)—O(18*A*)	1.233 (6)
C(2*N*)—C(20)	1.527 (8)	C(18)—N(19)	1.334 (7)
C(2*O*—C(2*P*)	1.527 (9)	N(19)—C(20)	1.451 (7)
C(2*O*)—C(2*Q*)	1.524 (10)	C(20)—C(20*A*)	1.532 (9)
N(2*R*)—C(*A*)	1.347 (7)	C(20)—C(21)	1.505 (8)
C(3)—O(3*A*)	1.213 (6)	C(20*A*)—C(20*B*)	1.487 (11)
C(3)—N(4)	1.334 (6)	C(20*A*)—C(20*C*)	1.514 (12)
N(4)—C(5)	1.457 (7)	C(20*C*)—C(20*D*)	1.480 (14)
C(5)—C(5*A*)	1.539 (8)	C(21)—O(21*A*)	1.206 (7)
C(5)—C(6)	1.523 (7)	C(21)—O(22)	1.341 (6)
C(5*A*)—C(5*B*)	1.517 (9)	C(*A*)—O(*A*1)	1.248 (7)
C(5*A*)—C(5*C*)	1.525 (9)	C(*A*)—C(*B*)	1.530 (8)
C(6)—O(6*A*)	1.231 (6)	C(*B*)—C(*C*)	1.545 (8)
C(6)—N(7)	1.351 (7)	C(*C*)—O(*C*1)	1.426 (6)
N(7)—C(8)	1.465 (7)	C(*C*)—C(*D*)	1.526 (8)
C(8)—C(8*A*)	1.543 (8)	C(*D*)—C(*E*)	1.526 (8)
C(8)—C(9)	1.522 (7)	C(*E*)—C(*F*)	1.527 (8)
C(8*A*)—C(8*B*)	1.551 (8)	C(*F*)—C(*G*)	1.547 (10)
C(8*B*)—C(8*C*)	1.508 (9)	C(*G*)—C(*H*)	1.478 (11)
C(8*B*)—C(8*D*)	1.525 (9)	C(*H*)—C(*I*)	1.527 (14)
C(9)—O(9*A*)	1.211 (6)	C(*I*)—C(*J*)	1.500 (20)
C(9)—N(10)	1.378 (6)		

C(1*A*)—C(1)—C(2)	111.8 (4)	O(9*A*)—C(9)—N(10)	122.5 (5)
C(1*A*)—C(1)—O(22)	110.3 (4)	C(9)—N(10)—C(11)	120.8 (4)
C(2)—C(1)—O(22)	102.3 (4)	N(10)—C(11)—C(11*A*)	111.6 (4)
C(1)—C(2)—N(2*A*)	108.6 (4)	N(10)—C(11)—C(12)	114.0 (4)
C(1)—C(2)—C(3)	112.0 (4)	C(11*A*)—C(11)—C(12)	108.6 (4)
N(2*A*)—C(2)—C(3)	112.6 (4)	C(11)—C(11*A*)—O(11*B*)	113.7 (4)
C(2)—N(2*A*)—C(2*B*)	118.5 (4)	C(11)—C(12)—O(12*A*)	119.5 (5)
N(2*A*)—C(2*B*)—O(2*C*)	122.8 (5)	C(11)—C(12)—N(13)	117.1 (4)
N(2*A*)—C(2*B*)—C(2*D*)	117.6 (4)	O(12*A*)—C(12)—N(13)	123.3 (5)
O(2*C*)—C(2*B*)—C(2*D*)	119.4 (5)	C(12)—N(13)—C(14)	122.6 (4)
C(2*B*)—C(2*D*)—C(2*E*)	110.7 (4)	N(13)—C(14)—C(14*A*)	108.4 (4)
C(2*B*)—C(2*D*)—N(2*J*)	112.6 (4)	N(13)—C(14)—C(15)	114.4 (5)
C(2*E*)—C(2*D*)—N(2*J*)	110.3 (4)	C(14*A*)—C(14)—C(15)	112.0 (4)
C(2*D*)—C(2*E*)—C(2*F*)	112.9 (4)	C(14)—C(14*A*)—C(14*B*)	117.5 (5)
C(2*E*)—C(2*F*)—C(2*G*)	113.1 (5)	C(14*A*)—C(14*B*)—C(14*C*)	111.9 (5)
C(2*F*)—C(2*G*)—O(2*H*)	111.7 (5)	C(14*A*)—C(14*B*)—C(14*D*)	108.4 (5)
C(2*F*)—C(2*G*)—O(2*I*)	123.8 (5)	C(14*C*)—C(14*B*)—C(14*D*)	111.2 (5)
O(2*H*)—C(2*G*)—O(2*I*)	124.5 (6)	C(14)—C(15)—O(15*A*)	122.4 (5)
C(2*D*)—N(2*J*)—C(2*K*)	119.6 (4)	C(14)—C(15)—N(16)	115.7 (4)
N(2*J*)—C(2*K*)—O(2*L*)	123.0 (4)	O(15*A*)—C(15)—N(16)	121.8 (5)
N(2*J*)—C(2*K*)—C(2*M*)	116.3 (4)	C(15)—N(16)—C(17)	119.7 (4)
O(2*L*)—C(2*K*)—C(2*M*)	120.6 (5)	N(16)—C(17)—C(17*A*)	109.6 (4)
C(2*K*)—C(2*M*)—C(2*N*)	110.7 (4)	N(16)—C(17)—C(18)	110.8 (4)
C(2*K*)—C(2*M*)—N(2*R*)	110.2 (4)	C(17*A*)—C(17)—C(18)	112.1 (4)
C(2*N*)—C(2*M*)—N(2*R*)	110.9 (4)	C(17)—C(17*A*)—O(17*B*)	111.7 (5)
C(2*M*)—C(2*N*)—C(2*O*)	115.4 (5)	C(17)—C(18)—O(18*A*)	121.7 (5)
C(2*N*)—C(2*O*)—C(2*P*)	111.4 (5)	C(17)—C(18)—N(19)	116.2 (4)
C(2*N*)—C(2*O*)—C(2*Q*)	112.1 (5)	O(18*A*)—C(18)—N(19)	122.1 (5)
C(2*P*)—C(2*O*)—C(2*Q*)	111.3 (5)	C(18)—N(19)—C(20)	122.0 (5)
C(2*M*)—N(2*R*)—C(*A*)	119.4 (4)	N(19)—C(20)—C(20*A*)	109.1 (5)
C(2)—C(3)—O(3*A*)	120.6 (4)	N(19)—C(20)—C(21)	113.7 (4)
C(2)—C(3)—N(4)	115.7 (4)	C(20*A*)—C(20)—C(21)	112.5 (5)
O(3*A*)—C(3)—N(4)	123.7 (5)	C(20)—C(20*A*)—C(20*B*)	110.9 (6)
C(3)—N(4)—C(5)	117.9 (4)	C(20)—C(20*A*)—C(20*C*)	116.0 (6)
N(4)—C(5)—C(5*A*)	110.9 (4)	C(20*B*)—C(20*A*)—C(20*C*)	109.0 (7)
N(4)—C(5)—C(6)	111.3 (4)	C(20*A*)—C(20*C*)—C(20*D*)	113.8 (9)
C(5*A*)—C(5)—C(6)	112.3 (4)	C(2)—C(21)—O(21*A*)	124.2 (5)
C(5)—C(5*A*)—C(5*B*)	111.2 (5)	C(20)—C(21)—O(22)	112.9 (5)
C(5)—C(5*A*)—C(5*C*)	110.3 (5)	O(21*A*)—C(21)—O(22)	122.9 (5)
C(5*B*)—C(5*A*)—C(5*C*)	111.2 (5)	C(1)—O(22)—C(21)	117.8 (4)
C(5)—C(6)—O(6*A*)	122.1 (5)	N(2*R*)—C(*A*)—O(*A*1)	122.0 (5)
C(5)—C(6)—N(7)	115.9 (4)	N(2*R*)—C(*A*)—C(*B*)	115.8 (5)
O(6*A*)—C(6)—N(7)	121.9 (5)	O(*A*1)—C(*A*)—C(*B*)	122.2 (5)
C(6)—N(7)—C(8)	120.1 (4)	C(*A*)—C(*B*)—C(*C*)	111.5 (4)
N(7)—C(8)—C(8*A*)	109.2 (4)	C(*B*)—C(*C*)—O(*C*1)	107.9 (4)
N(7)—C(8)—C(9)	113.5 (4)	C(*B*)—C(*C*)—C(*D*)	109.7 (4)

Table 2 (*cont.*)

C(8*A*)—C(8)—C(9)	110.3 (4)	O(*C*1)—C(*C*)—C(*D*)	111.6 (4)
C(8)—C(8*A*)—C(8*B*)	115.1 (4)	C(*C*)—C(*D*)—C(*E*)	112.8 (5)
C(8*A*)—C(8*B*)—C(8*C*)	112.1 (5)	C(*D*)—C(*E*)—C(*F*)	111.9 (5)
C(8*A*)—C(8*B*)—C(8*D*)	108.0 (5)	C(*E*)—C(*F*)—C(*G*)	111.7 (5)
C(8*C*)—C(8*B*)—C(8*D*)	111.0 (5)	C(*F*)—C(*G*)—C(*H*)	114.0 (6)
C(8)—C(9)—O(9*A*)	120.7 (5)	C(*G*)—C(*H*)—C(*I*)	114.4 (8)
C(8)—C(9)—N(10)	116.6 (4)	C(*H*)—C(*I*)—C(*J*)	113.9 (10)

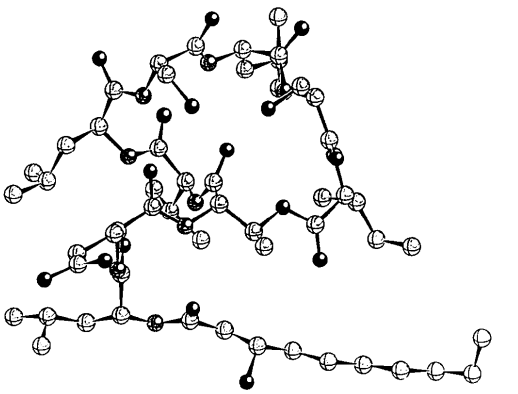


Fig. 1. Crystal structure of WLIP (1).

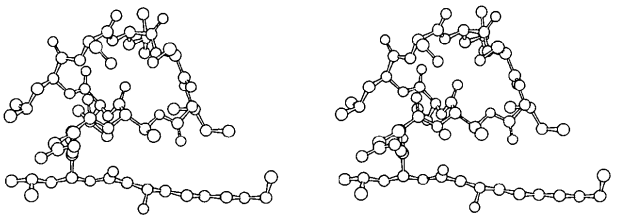


Fig. 2. Stereoview of WLIP (1).

in the calculations but were not refined. Population factors, 0.9 for O(*w*1) and 0.1 for O(*w*2), were assigned to the two water molecules during the refinement. The function minimized in the refinement was $\sum w(F_o^2 - F_c^2)^2$, where weights w were $1/\sigma^2(F_o^2)$. Atomic form factors were from Doyle & Turner (1968) and for H atoms, from Stewart, Davidson & Simpson (1965). In the final refinement cycle, with block-matrix least-squares method, all shifts were $< 1.54\sigma$, $R = 0.073$, $S = 5.08$. These values are slightly higher than normal because of the strong thermal motion of atoms [C(*H*), C(*I*) and C(*J*)] which are at the end of the β-hydroxydecanoic acid chain. The final difference Fourier peaks were < 0.39 e Å^{-3}. The *CRYM* system of computer programs was used (Duchamp, 1984). Final atomic coordinates can be found in Table 1.* All bond distances and angles are within the expected ranges (Table 2).

* Lists of structure factors, anisotropic thermal parameters and H-atom coordinates have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 55144 (47 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England. [CIF reference: CR0373]

Table 3. *Close intermolecular contacts between non-*H *atoms* (Å)

Symmetry operations listed were performed on the first atom.

C(2*N*)...O(*W*1)	$x-1, y, z$	3.441 (7)
N(2*R*)...O(*W*1)	$x-1, y, z$	2.821 (7)
C(5*C*)...O(*W*2)	$x-1, y, z$	3.443 (34)
O(15*A*)...C(8*A*)	$\frac{1}{2}-x, 1-y, z-\frac{1}{2}$	3.275 (7)
O(15*A*)...C(8*C*)	$\frac{1}{2}-x, 1-y, z-\frac{1}{2}$	3.473 (7)
N(19)...O(9*A*)	$\frac{1}{2}-x, 1-y, z-\frac{1}{2}$	2.899 (6)
C(20*A*)...O(9*A*)	$\frac{1}{2}-x, 1-y, z-\frac{1}{2}$	3.465 (8)
O(6*A*)...C(2*G*)	$1-x, y-\frac{1}{2}, -z-\frac{1}{2}$	3.362 (7)
O(6*A*)...O(2*H*)	$1-x, y-\frac{1}{2}, -z-\frac{1}{2}$	2.608 (6)
O(6*A*)...O(2*I*)	$1-x, y-\frac{1}{2}, -z-\frac{1}{2}$	3.351 (6)
C(2*F*)...O(18*A*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.427 (7)
N(2*J*)...O(18*A*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	2.849 (5)
N(2*R*)...C(1*A*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.473 (8)
O(*A*1)...O(17*B*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	2.805 (6)
O(*A*1)...O(18*A*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.497 (5)
C(*C*)...O(11*B*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.422 (7)
O(*C*1)...C(2)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.432 (6)
O(*C*1)...N(2*A*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.302 (5)
O(*C*1)...C(2*B*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.197 (6)
O(*C*1)...O(2*C*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	3.094 (5)
O(*C*1)...O(11*B*)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	2.824 (5)

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

D	*A*		*D*···*A*	H···*A*	*D*—H···*A*
N(10)	O(2*C*)	x, y, z	2.842 (5)	1.94	146
N(4)	O(2*L*)	x, y, z	2.979 (5)	2.39	116
N(16)	O(3*A*)	x, y, z	2.917 (5)	1.92	168
N(10)	N(7)	x, y, z	2.823 (6)	2.47	99
N(13)	N(10)	x, y, z	2.735 (6)	2.29	105
O(*W*1)	O(12*A*)	x, y, z	2.811 (6)	1.79	168
O(*W*1)	O(15*A*)	x, y, z	2.791 (6)	1.94	171
N(19)	N(16)	x, y, z	2.804 (6)	2.50	96
N(2*R*)	O(*W*1)	$x+1, y, z$	2.821 (7)	1.85	162
N(19)	O(9*A*)	$\frac{1}{2}-x, 1-y, z+\frac{1}{2}$	2.899 (6)	2.09	134
N(2*J*)	O(18*A*)	$x+\frac{1}{2}, \frac{1}{2}-y, -z$	2.849 (5)	1.86	160

Discussion. The crystal structure of WLIP (Figs. 1 and 2) is significantly different from that deduced for a solution in DMSO-d_6. In a series of conformers generated by distance geometry and molecular dynamics calculations using distance constraints obtained from two-dimensional NMR experiments in DMSO-d_6 (Mortishire-Smith, Nutkins, Packman, Brodey, Rainey, Johnstone & Williams, 1991), residues Ser6 to Ile9 appear to adopt a type II β-turn conformation. Although several hydrogen-bonding interactions are evident in the crystalline conformation, no β-turn is present between Ser6 and Ile9, and an NOE identified between Ser6α and Ile9γ is not satisfied by the crystal structure. Amide exchange-rate measurements demonstrate that in DMSO, Ile9NH is solvent inaccessible; in the crystal structure, Ile9NH is intramolecularly hydrogen bonded (Tables 3 and 4).

In the crystal structure (Figs. 1 and 2), the *N*-terminal blocking group and residues 1 to 3 adopt a β-turn conformation in which the carbonyl group of the fatty acid is hydrogen bonded to Thr3NH. The stereochemistry of the β-turn residues (OHDec-L-Leu-D-Glu-D-*a*-Thr) prevents an unfavorable 1,3 steric interaction between the $i+1$CO and the $i+2$ side chain. Glu2CO forms a hydrogen bond to Ser6NH, and Ser8NH is hydrogen bonded to Thr3CO across the peptide macrocycle. The stereochemistry of the β-hydroxy acid is shown to be *R*.

It is noteworthy that the utilization of the side-chain hydroxyl of threonine to form a macrocyclic lactone, while the amine group of this amino acid is used for side-chain attachment, is a feature found in numerous secondary metabolites [*e.g.* virginiamycin S (Vanderhaeghe & Parmentier, 1960), mikamycin B (also known as ostreogryein B3) (Cox, Eastwood, Snell & Todd, 1970), and didemnin (Rinehart *et al.*, 1982)].

Knowledge of the three-dimensional structures of WLIP and tolaasinl (Mortishire-Smith & Williams, 1991) will aid our investigation of the white-line complex by NMR spectroscopy.

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