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Omphalotins B, C and D, nematicidal cyclopeptides from *Omphalotus olearius*. Absolute configuration of omphalotin A.

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Abstract: The isolation and structure determination by spectroscopic methods of omphalotins B (**2a**), C (**2b**) and D (**2c**), containing oxidised glycine, valine, isoleucine and tryptophan, from extracts of the basidiomycete *Omphalotus olearius* is described. All amino acids of omphalotin A (**1**) were shown to have L configuration.

Omphalotin A[1] (**1**) is a modified cyclic dodecapeptide consisting of 5 valine, 3 glycine, 3 isoleucine and 1 tryptophan, with 9 α-nitrogens methylated, produced by the basidiomycete *Omphalotus olearius*.[2,3] It was isolated because of its potent and selective nematicidal activity. Omphalotin A (**1**) is highly toxic (LD_{90} 0.76 μM) towards the economically important plant parasite *Meloidogyne incognita* while the saprophytic nematode *Caenorhabditis elegans* is approximately 50 times less sensitive (LD_{90} 38 μM).[2] The corresponding LD_{90} values for the commercially available nematicide ivermectin is 4.6 μM (towards *M. incognita*) and 0.46 μM (towards *C. elegans*).[2] Omphalotin A (**1**) lacks antimicrobial and phytotoxic activity, and only shows weak cytotoxic activity at 100 μg/ml,[2] making it a potentially useful nematicide. During preparative fermentations of various strains of *O. olearius*, it became evident that the fungus also produces several omphalotin derivatives, and here we report the isolation and structure determination by spectroscopic techniques of omphalotins B (**2a**), C (**2b**) and D (**2c**). In addition, the absolute configuration of the amino acids of omphalotin A (**1**) was determined by chemical methods.

The latter was achieved by hydrolysing omphalotin A (**1**) in 6 M HCl and derivatising the free amino acids with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate as described previously.[4,5] By comparing the RP-HPLC retention times of the resulting thiourea derivatives with samples prepared in the same way from the corresponding D- and L-amino acids, it could be shown that omphalotin A (**1**) is composed of L-amino acids and that the structure shown in Figure 1 represents its absolute configuration. Although this was not demonstrated, it is reasonable to believe that omphalotins B (**2a**), C (**2b**) and D (**2c**) are formed from **1** as they appear later during the fermentation. Their absolute configuration should therefore be the same.

Being relatively lipophilic cyclopeptide derivatives, the omphalotins are soluble in organic solvents

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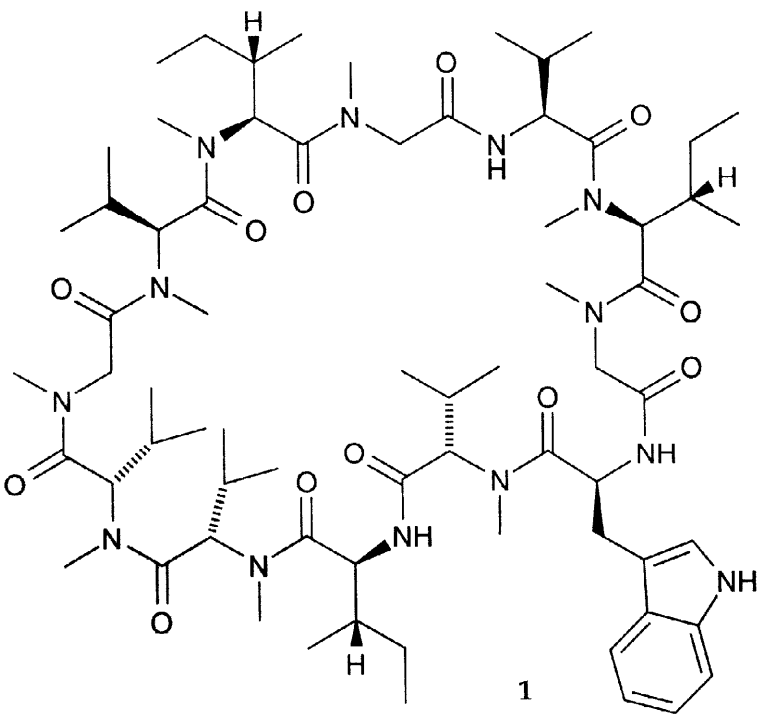

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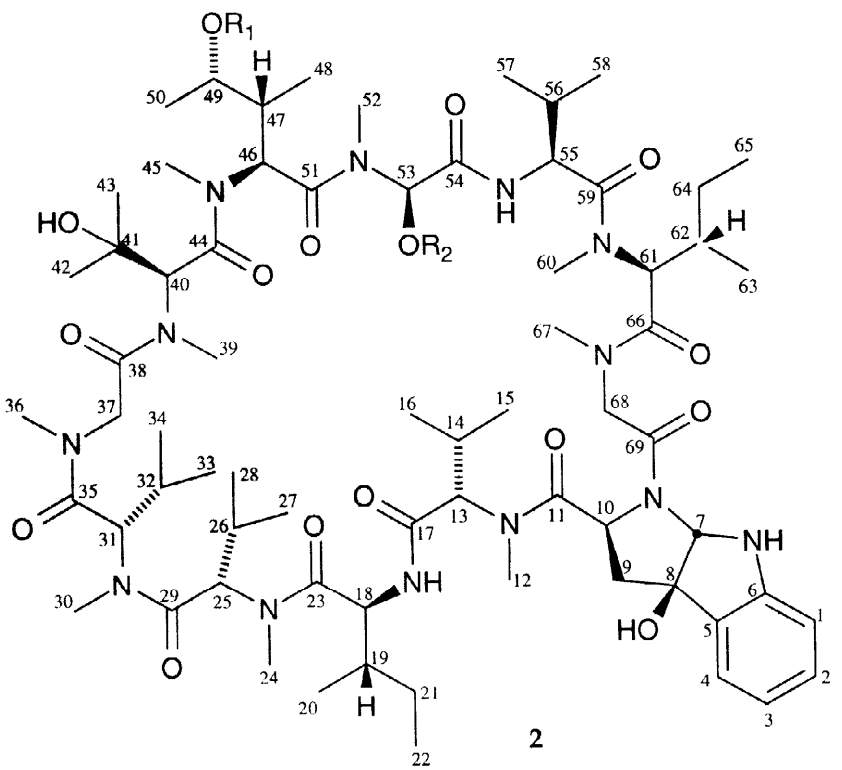

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Figure 1. Structures of the omphalotins A (**1**), B (**2a**), C (**2b**) and D (**2c**).
a: R_1 = 3-hydroxy-3-methylbutanoyl, R_2 = H.
b: R_1 = 3-hydroxy-3-methylbutanoyl, R_2 = acetyl.
c: R_1 = R_2 = acetyl.

and NMR measurements were carried out in several deuterated solvents. The data obtained in CD_3OD were most suitable for the elucidation of the structures (^1H and ^{13}C NMR data in CD_3OD are reported in Tables 1 and 2), although data obtained in $(CD_3)_2SO$, in which signals for exchangeable protons can be observed, were important for the determination of the stereochemistry (*vide infra*). As with many macrocyclic compounds, the stability of various conformers is affected by the solvent. While omphalotin B (**2a**) is present essentially as one conformer in methanol, omphalotins C (**2b**) and D (**2c**) are approximately 1:1 mixtures of 2 major conformers. On the other hand, in DMSO **2a** is a 1:1 mixture while **2c** is essentially a single conformer, and correlations observed in the ROESY spectra of **2a** in $(CD_3)_2SO$ and **2b** as well as **2c** in CD_3OD indicate that the two co-existing conformers are substantially different. For example, 58-H_3 has an unusual chemical shift (approximately 0.30 ppm) in **2a** as well as in one of the conformers of **2b** and **2c** in CD_3OD, and in one of the conformers of **2a** in $(CD_3)_2SO$. In the conformers with this peculiar chemical shift, 58-H_3 gives strong ROESY correlations to both 10-H and 13-H indicating the presence of a folding that brings these groups close together in space.

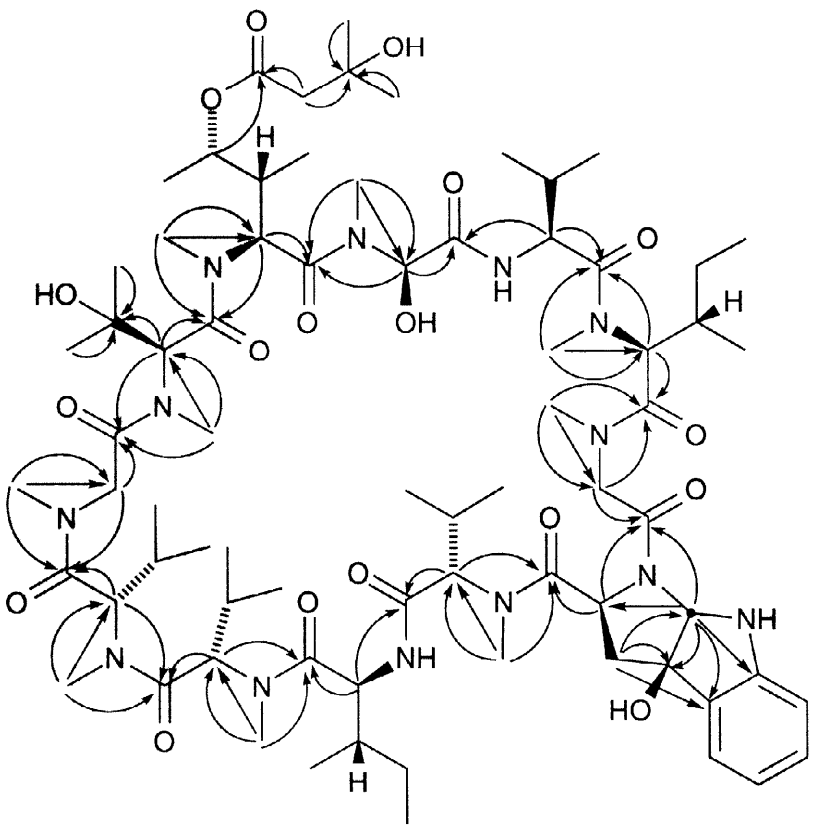

Figure 2. Long range ^1H-^1H (straight arrows) and ^1H-^{13}C (curved arrows) correlations observed in COSY and HMBC spectra of omphalotin B (**2a**) in CD_3OD.

The elemental composition of omphalotin A (**1**) is $C_{69}H_{115}N_{13}O_{12}$, and results from high resolution FAB mass specrometry indicated that the new omphalotins are slightly bigger ($C_{74}H_{123}N_{13}O_{18}$ for **2a**, $C_{76}H_{125}N_{13}O_{19}$ for **2b** and $C_{73}H_{119}N_{13}O_{18}$ for **2c**). The similar number of nitrogens in all omphalotins suggest that they consist of the same amino acids, although the NMR data revealed that the indole system of

tryptophan in **1** no longer was part of **2a**, **2b** or **2c**. The structures of the new omphalotins could be determined by the analysis of long range correlations observed in both COSY (^{1}H-^{1}H) and HMBC (^{1}H- ^{13}C) 2D NMR spectra, as indicated for omphalotin B (**2a**) in Figure 2. After realising that omphalotin C (**2b**) is 53-0-acetylomphalotin B, this could be confirmed by acetylating omphalotin B (**2a**) with acetic anhydride in pyridine. The product obtained was identical in all respects with the isolated omphalotin C (**2b**).

The ^{1}H-^{1}H coupling between the N-methyls and the corresponding α-protons is weak, but clearly visible in COSY experiments. The HMBC correlations from the N-methyls to both the α-carbons and the carbonyl carbons in the peptide bond, together with the correlations between the α-hydrogens and the carbonyl carbon on both sides, determine the backbone of the omphalotins. The side chains in the unmodified 4 valines and 2 isoleucines could be determined by COSY correlations and comparison with the data of omphalotin A (**1**), while the presence of a hydroxylated valine was shown by the HMBC correlations shown in Figure 2. The structure of the oxidised tryptophan in omphalotins **2a**, **2b** and **2c** could be determined by the HMBC correlations indicated in Figure 1, and also by the HMBC correlations observed from 6-NH to C-6 and C-7 as well as from 8-OH to C-5, C-7, C-8 and C-9, with **2a** and **2c** in $(CD_3)_2SO$. The stereochemistry of this moiety could be determined by the ROESY correlations indicated in Figure 3.

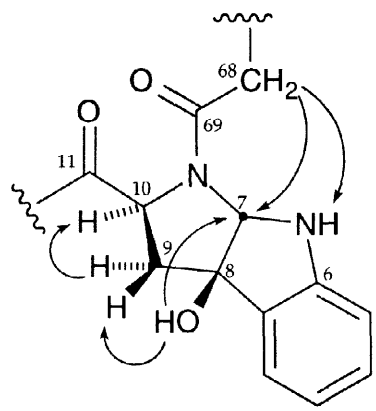

Figure 3. The stereochemistry of the modified tryptophan, as suggested by correlations observed in the ROESY spectra of omphalotins B (**2a**) and D (**2c**) in $(CD_3)_2SO$.

As 8-OH gives strong ROESY correlation to both 7-H and 9-Hβ, while 10-H correlates to 9-Hα, the conclusion is that 7-H and 8-OH are *cis*, and on the opposite side of the ring system compared to 10-H. 68-H_2 correlate to both 7-H and 6-NH, but not to 10-H, indicating that the 68/69 glycine is positioned over this part of the modified tryptophan. The free amino acid has previously been shown to be formed from dye-sensitized photooxidation of L-tryptophan,[6] but the fact that omphalotin A (**1**) is stable during isolation and storage indicates that the oxidation of tryptophan in *O. olearius* is enzymatic. Bioactive compounds containing a similar hydroxylated tricyclic system have been reported from nature.[7] The configuration of the oxidised isoleucine could also be determined from the NMR data recorded in $(CD_3)_2SO$. A very strong ROESY correlation is observed between 52-H_3 and 46-H, indicating that the N-methyl group and α-proton of the oxidised isoleucine are close in space as shown in Figure 4. 52-H_3 gives a weak ROESY correlation to 53-H, which in turn correlates to 48-H_3, suggesting that the 47-methyl group is oriented towards the oxidised glycine and that 53-H is situated as shown in Figure 4. The large ^{1}H-^{1}H coupling constant between 46-H and

47-H (10.8 Hz) and the weak ROESY correlation between the two protons show that the oxidised isoleucine has retained the staggered conformation that normally is observed for valine and isoleucine. ROESY correlations can be obseved between 46-H and 48-H_3, 49-H as well as 50-H_3, between 45-H_3 and 47-H as well as 49-H, and between 48-H_3 and 50-H_3, all supporting the structure and conformation presented in Figure 4. The dihedral angle 47-H/C-47/C-49/49-H should be small according to this model, and this is confirmed by the small (1.4 Hz) coupling constant observed between the two protons in the ^1H spectrum of **2c** in $(CD_3)_2SO$. 53-H gives its strongest ROESY correlation to 55-NH, which in addition correlates to the neighbouring isopropyl group but not at all to 52-H_3. This makes the suggested configuration of the hydroxylated glycine most likely, as the opposite would place the 55-NH rather close to 52-H_3 and a notable ROESY correlation should be expected.

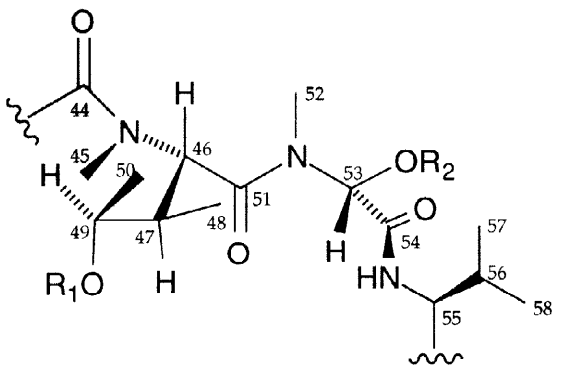

Figure 4. The stereochemistry and preferred conformation of the 4-hydroxy-L-isoleucine/2-hydroxyglycine part, as suggested by correlations observed in the ROESY spectra of omphalotins B (**2a**) and D (**2c**) in $(CD_3)_2SO$.

The absolute configuration of 4-hydroxyisoleucine in omphalotins B (**2a**), C (**2b**) and D (**2c**) is 2*S*, 3*R*, 4*S*, and the identical amino acid has been reported from fungi (e.g. in the cyclopeptide γ-amanitin isolated from several *Amanita* species[8]) and a plant (existing as a free amino acid in *Trigonella foenum-graecum*[9]). The new omphalotins possess similar nematicidal activity as omphalotin A (**1**), which together with their other bioactivities will be reported elsewhere.

EXPERIMENTAL

Extraction and isolation: The characteristics and fermentation of *Omphalotus olearius* strain TA90170 have been described.[2] A 50 l fermentation yielded after filtration and lyophilisation 240 g mycelium which was extracted with 5 l methanol for 5 h at room temperature. After evaporation of the solvent and partition chromatography (EtOAc-H_2O), the resulting residue (12 g) from the organic layer was subjected to gelfiltration on Sephadex LH 20 with methanol as mobile phase. The fractions possessing nematicidal activity[2] were combined (1.2 g) and further fractionated on a column with modified silica (diol) eluted with mixtures of cyclohexane and EtOAc (0, 25, 50, 75 and 100 % EtOAc). The fraction obtained with 100 % EtOAc (80 mg) contained omphalotins A (**1**) and B (**2a**), while the 75 % EtOAc fraction (120 mg) contained omphalotins C (**2b**) and D (**2c**). Purification by reversed phase HPLC [RP 18, 7 μm, H_2O-acetonitrile (2:3)] yielded 11 mg omphalotin A (**1**), 11 mg omphalotin B (**2a**), 7 mg omphalotin C (**2b**) and 7 mg omphalotin D (**2c**).

Spectroscopy: ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with shielded gradient

Table 1. ^1H (500 MHz) NMR data (δ; multiplicity; *J*) for omphalotins B (**2a**), C (**2b**) and D (**2c**), in CD_3OD with the solvent signal (3.31 ppm) as reference. The coupling constants *J* are given in Hz. **2b** and **2c** are mixtures of two conformers (1:1) in methanol.

H	**2a**	**2b**	**2c**	H	**2a**	**2b**	**2c**
1	6.95; d; 7.8	6.72/6.93; d; 7.9	6.72/6.93; d; 7.9	39	3.17; s	3.17/3.16; s	3.15/3.14; s
2	7.22; dd; 7.8, 8	7.22/7.17; dd; 8, 8	7.22/7.17; dd; 8, 8	40	5.11; s	5.13/5.15; s	5.12/5.13; s
3	6.87; dd; 7.5, 8	6.82/6.87; dd; 8, 8	6.82/6.87; dd; 8, 8	42	1.01; s	1.02/1.21; s	1.02/1.20; s
4	7.28; d; 7.5	7.26/7.28; d; 7.5	7.26/7.28; d; 7.5	43	1.20; s	1.08/1.25; s	1.08/1.25; s
7	5.53; s	5.56/5.65; s	5.56/5.65; s	45	2.78; s	2.83/2.76; s	2.82/2.76; s
9a	2.67; m	2.66/2.48; m	2.65/2.48; m	46	5.20; d; 11	5.53/5.20; d; 10.9	5.55/5.22; d; 10.9
9b	2.52; m	2.53/2.33; m	2.52/2.32; m	47	2.24; m	2.25/2.28; m	2.24/2.27; m
10	4.39; dd; 7.4, 8.6	4.42/5.15; m	4.41/5.16; m	48	1.10; d; 6.5	1.00/1.07; m	0.99/1.05; m
12	2.70; s	2.70/2.78; s	2.70/2.78; s	49	4.73; m	4.68/4.69; m	4.62/4.65; m
13	3.83; d; 10.8	4.41/3.89; d; 10.8	4.42/3.88; d; 10.8	50	1.30; d; 6.5	1.31/1.29; m	1.30/1.28; m
14	2.30; m	2.33/2.30; m	2.32/2.29; m	52	2.67; s	2.70/3.06; s	2.70/3.06; s
15	0.78; m	0.77/0.82; m	0.78/0.82; m	53	6.36; s	7.01/7.23; s	7.02/7.24; s
16	0.78; m	0.89/ 0.78; m	0.89/ 0.78; m	55	4.79; m	4.81/4.55; d; 7.8	4.81/4.55; d; 7.8
18	4.67; dd; 8, 10	4.67/4.77; m	4.68/4.78; m	56	1.91; m	2.00/2.08; m	2.00/2.08; m
19	1.99; m	1.99/2.07; m	1.99/2.06; m	57	0.74; m	0.76/1.01; m	0.76/1.01; m
20	0.84; m	0.89/0.89; m	0.87/0.90; m	58	0.29; d; 6.5	0.32/0.96; d; 6.5	0.32/0.97; d; 6.5
21a	1.49; m	1.47/1.46; m	1.46/1.46; m	60	3.10; s	3.06/3.18; s	3.06/3.18; s
21b	1.35; m	1.23/1.36; m	1.22/1.37; m	61	5.18; d; 11	5.43/5.16; d; 10.8	5.42/5.15; d; 10.8
22	0.87; m	0.88/0.87; m	0.87/0.85; m	62	2.18; m	2.16/2.18; m	2.16/2.18; m
24	3.17; s	3.15/3.09; s	3.15/3.09; s	63	0.86; m	0.85/1.02; m	0.85/1.02; m
25	5.32; d; 10.8	5.29/5.23; d; 10.8	5.29/5.23; d; 10.8	64a	1.28; m	1.25/1.07; m	1.24/1.07; m
26	2.27; m	2.28/2.33; m	2.29/2.33; m	64b	1.28; m	1.25/1.35; m	1.24/1.35; m
27	0.70; d; 6.8	0.68/0.73; d; 6.8	0.68/0.73; d; 6.8	65	0.95; m	0.86/0.83; m	0.86/0.83; m
28	0.89; m	0.90/0.85; m	0.93/0.85; m	67	2.83; s	2.83/3.38; s	2.82/3.38; s
30	2.94; s	2.91/2.94; s	2.91/2.94; s	68a	5.15; d; 17.3	5.05/5.14; d; 17	5.04/5.14; d; 17
31	5.30; d; 10.8	5.29/5.10; d; 10.8	5.28/5.08; d; 10.8	68b	4.25; d; 17.3	3.77/4.26; d; 17	3.77/4.25; d; 17
32	2.23; m	2.25/2.31; m	2.24/2.31; m	$R_1/2\text{-}H_2$	2.52; m	2.52/2.52; m	-
33	0.74; m	0.78/0.75; m	0.77/0.75; m	$R_1/3\text{-}CH_3$	1.32; s	1.32/1.33; s	-
34	0.88; m	0.90/0.93; m	0.90/0.93; m	$R_1/4\text{-}H_3$	1.32; s	1.32/1.33; s	-
36	3.12; s	3.02/3.12; s	3.02/3.12; s	$R_1/2\text{-}CH_3$	-	-	2.04/2.02; m
37a	4.87; d; 16.5	4.90/4.84; d; 17	4.88/4.83; d; 17	$R_2/2\text{-}CH_3$	-	2.17/2.27; s	2.17/2.27; s
37b	3.68; d; 16.5	3.68/3.67; d; 17	3.67/3.65; d; 17	17-NH	8.17; d; 7.9	8.40; d; 9.0/ 8.18; d; 7.9	8.40; d; 9.0/ 8.18; d; 7.8

Table 2. ^{13}C (125 MHz) NMR data (δ; multiplicity) for omphalotins B (**2a**), C (**2b**) and D (**2c**) in CD_3OD, with the solvent signal (49.15 ppm) as reference. **2b** and **2c** are mixtures of two conformers (1:1) in methanol.

C	2a	2b	2c	C	2a	2b	2c
1	113.4; d	112.2/113.2; d	112.2/113.2; d	39	33.2; q	33.2/33.4; q	33.3/33.4; q
2	131.4; d	131.2/131.4; d	131.2/131.4; d	40	60.4; d	59.3/60.3; d	59.2/60.4; d
3	121.2; d	120.8/121.2; d	120.8/121.2; d	41	75.5; s	75.4/75.5; s	75.3/75.5; s
4	124.3; d	124.0/124.3; d	124.0/124.3; d	42	26.2; q	26.1/26.2; q	26.1/26.2; q
5	131.5; s	131.5/131.8; s	131.5/131.8; s	43	29.2; q	29.3/29.4; q	29.3/29.4; q
6	150.9; s	150.0/150.8; s	150.0/150.8; s	44	172.8; s	172.9/173.2; s	172.9/173.2; s
7	83.3; d	83.6/86.6; d	83.6/86.6; d	45	31.7; q	30.9[b]/31.3; q	30.9[e]/31.3; q
8	88.8; s	88.9/90.2; s	88.9/90.2; s	46	59.4; q	55.6/58.8; d	55.6/58.8; d
9	41.6; t	41.9/43.2; t	41.9/43.2; t	47	37.6; d	37.9/38.3; d	37.6/37.9; d
10	57.7; d	57.9/58.3; d	57.9/58.4; d	48	11.2; q	10.4/11.2; q	10.4/11.1; q
11	174.7; s	175.0/176.2; s	175.0/176.2; s	49	70.4; d	70.3/70.3; d	70.4/70.4; d
12	30.2; q	30.2[a]/30.3; q	30.3[d]/30.4; q	50	18.2; q	17.9/18.2; q	17.9/18.1; q
13	67.9; d	67.8/67.9; d	67.9/67.9; d	51	170.8; s	170.4/171.0; s	170.6/171.1; s
14	27.2; d	27.2/27.4; d	27.2/27.4; d	52	28.2; q	29.4[a]/31.0[c]; q	29.4[d]/31.0[f]; q
15	19.8; q	18.1/19.4; q	18.1/19.4; q	53	77.0; d	76.8/77.0; d	76.9/77.0; d
16	20.6; q	20.1/20.5; q	20.1/20.5; q	54	169.3; q	166.6/167.4; s	166.5/167.4; s
17	169.8; s	169.9/170.1; s	169.9/170.1; s	55	56.1; d	56.5/56.8; d	56.5/56.7; d
18	54.3; d	54.4/54.6; d	54.4/54.6; d	56	31.2; d	30.9/31.9; d	30.9/31.9; d
19	35.9; d	36.0/37.2; d	36.0/37.2; d	57	18.7; q	18.1/18.6; q	18.1/18.6; q
20	15.3; q	15.7/16.3; q	15.3/15.8; q	58	18.9; q	19.1/19.7; q	19.1/19.7; q
21	26.4; t	26.1/26.2; t	26.1/26.2; t	59	174.6; s	174.2/174.2; s	174.1/174.2; s
22	9.6; q	9.8/11.4; q	9.8/11.4; q	60	31.6; q	31.4[c]/31.5; q	31.4[f]/31.5; q
23	174.7; s	174.3/174.6; s	174.3/174.6; s	61	59.0; d	56.7/58.8; d	56.8/59.2; d
24	31.1; q	30.9/31.1; q	30.9/31.1; q	62	35.4; d	34.4/35.1; d	34.4/34.5; d
25	59.6; d	59.8/60.0; d	59.8/60.0; d	63	16.4; q	16.4/17.2; q	16.4/17.2; q
26	28.7; d	28.4/28.6; d	28.4/28.6; d	64	24.7; t	25.8/25.8; t	25.4/25.8; t
27	19.1; q	18.3/18.9; q	18.3/18.9; q	65	11.4; q	10.6/11.3; q	10.6/11.3; q
28	20.1; q	19.8/20.3; q	20.2/20.6; q	66	171.9; s	171.9/173.2; s	171.9/173.2; s
29	172.4; s	171.3/172.2; s	171.3/172.2; s	67	35.1; q	35.0[b]/38.9; q	35.1[e]/38.9; q
30	31.2; q	31.0/31.3; q	31.0/31.3; q	68	53.2; t	51.7/53.2; t	51.7/53.2; t
31	58.8; d	58.6/60.2; d	58.8/60.2; d	69	170.2; s	170.2/170.9; s	170.2/170.9; s
32	27.6; d	27.6/27.8; d	27.6/27.8; d	R_1/C-1	172.9; s	172.6/173.2; s	172.7/172.9; s
33	18.3; q	18.1/18.6; q	18.1/18.6; q	R_1/C-2	49.1; t	49.0/49.0; t	21.5/21.6; q
34	19.8; q	19.8/20.7; q	19.8/20.7; q	R_1/C-3	70.4; s	70.3/70.4; s	-
35	172.8; s	171.4/172.6; s	171.4/172.6; s	R_1/C-4	29.5; q	29.6/29.6; q	-
36	38.4; q	37.6/38.3; q	37.6/38.3; q	R_1/3-CH_3	29.6; q	29.6/29.6; q	-
37	51.3; t	51.2/51.6; t	51.2/51.6; t	R_2/C-1	-	170.3/170.6; s	170.3/170.6; s
38	169.9; s	170.0/170.4	170.0/170.4	R_2/C-2	-	20.4/20.6; q	20.5/20.7; q

[a,b,c,d,e,f] Interchangable

coil. The spectra were recorded in CD_3OD and DMSO-d_6, and the solvent signals (δH 3.31 and δC 49.2 in CD_3OD; δH 2.50 and δC 39.5 in DMSO-d_6) were used as references. The chemical shifts (δ) are given in ppm, and the coupling constants (*J*) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}$=145 Hz and $^nJ_{CH}$=10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). FAB mass spectra (direct inlet, positive ions) were recorded with a Jeol SX102 spectrometer. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22 °C.

Omphalotin B (2a) was obtained as white crystals, m.p. 180-183 °C. [α]D_{22} -246 ° (c 1.0 in CH_3OH). MS (FAB), m/z: 1504.8990 (M + Na^+, $C_{74}H_{123}N_{13}O_{18}Na$ requires 1504.9006), 1482.9155 (M + H^+, $C_{74}H_{124}N_{13}O_{18}$ requires 1482.9186). See Tables 1 and 2 for NMR data.

Omphalotin C (2b) was obtained as white crystals, m.p. 173-178 °C. [α]D_{22} -242 ° (c 1.0 in CH_3OH). MS (FAB), m/z: 1524.9269 (M + H^+, $C_{76}H_{126}N_{13}O_{19}$ requires 1524.9292). See Tables 1 and 2 for NMR data.

Omphalotin D (2c) was obtained as white crystals, m.p. 165-170 °C. [α]D_{22} -254 ° (c 1.0 in CH_3OH). MS (FAB), m/z: 1466.8851 (M + H^+, $C_{73}H_{120}N_{13}O_{18}$ requires 1466.8874). See Tables 1 and 2 for NMR data.

Hydrolysis of omphalotin A: About 100 μg of omphalotin A (**1**) was hydrolysed with 60 μl 6 M HCl in a sealed ring cap for 1 h at 155°C. After evaporation to dryness, the residue was dissolved in aqueous triethylamine (0.2 %, v:v). To this solution 20 μl of 0.2 % GITC in acetonitrile (w:v) were added. After a reaction time of 10 minutes at room temperature, 5 μl were injected on a RP 18 column using a mixture of acetonitrile, methanol and 10 mM aqueous phosphate buffer (pH = 4.2) as mobile phase.[5] Each HPLC peak was identified by comparison with authentic samples.

Acetylation of omphalotin B: 5 mg of omphalotin B (**2a**) was dissolved in 500 μl pyridine, 100 μl acetic anhydride was added and the solution was left at room temperature overnight. Evaporation to dryness with a stream of nitrogen gas yielded omphalotin C (**2c**), identical in all respects with the isolated compound, as the only product.

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