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# Omphalotins E–I, Five Oxidatively Modified Nematicidal Cyclopeptides from *Omphalotus olearius*

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Omphalotins E–I, oxidatively modified cyclic dodecapeptides, were isolated from mycelial extracts of the basidiomycete *Omphalotus olearius*, and their structures were determined by NMR spectroscopic and MS methods. Four of the five omphalotins contained an unprecedented *N*-hydroxylated tricyclic tryptophan derivative. All compounds exhibited strong and selective nematicidal activity against the plant pathogen *Meloidogyne incognita* with $LD_{90}$ values between 2 and 5 $\mu g\,mL^{-1}$. Cytotoxic activities were not detected up to 50 $\mu g\,mL^{-1}$.


## Introduction

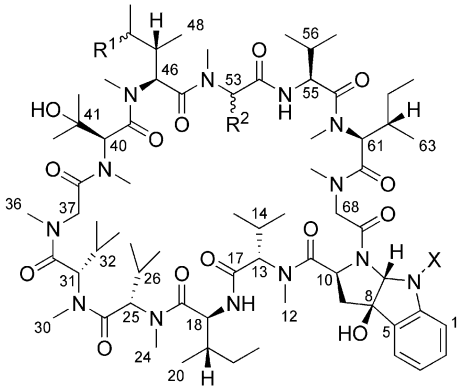
Extracts from mycelial cultures of the basidiomycete *Omphalotus olearius* (DC ex Fr.) Sing. (jack o'lantern mushroom) exhibited strong nematicidal activity against the notorious plant pathogenic root-knot nematode *Meloidogyne incognita* while being much less active against the saprophytic nematode *Caenorhabditis elegans*. In 1997 and 1998, Sterner, Anke and coworkers reported the isolation of the omphalotins A–D, cyclic dodecapeptides with partially modified valine, glycine, isoleucine and tryptophan residues, which were identified as the active agents.[1–4] Broad evaluation of their effectiveness as novel nematicides was hampered by the low production in the range of 1 to 5 $mg\,L^{-1}$. All strains of *O. olearius* produced the compounds regardless of the origin of the fruiting bodies from which they were obtained (e.g., Africa, Australia, America or Europe). During our efforts to obtain strains with higher production rates, a monokaryotic strain was generated, whose crude extracts contained not only higher amounts of the known omphalotins but also novel derivatives. In the following, we wish to report the production, isolation, structure elucidation and biological activities of these novel cyclopeptides.

## Results and Discussion

### Fermentation and Isolation

From protoplasts prepared from mycelia of *O. olearius*, eight monokaryotic strains were obtained. Most of them showed faster growth than the parental dikaryotic strain, and all of them produced omphalotins in submerged culture. HPLC–MS and HPLC–DAD analyses revealed that the crude extracts contained novel compounds similar to the omphalotins. One of these strains was selected for the isolation of the compounds, which were located in the mycelia.

From fermentation in a 200 L batch harvested at a late stage (after 9 d), omphalotin A (18 mg)[1,2] and compounds **1** (10 mg), **2** (23 mg) and **3** (10 mg) were obtained. In extracts taken earlier during the fermentation, for example, days 5 to 7, omphalotins A, B, C and D were detected.



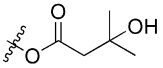
**1** Omphalotin E: $R^1$, $R^2$, X = H
**2** Omphalotin F: $R^1$, $R^2$ = H, X = OH
**3** Omphalotin G: $R^1$ = H, $R^2$, X = OH
**4** Omphalotin H: $R^1$, $R^2$ = OAc, X = OH
**5** Omphalotin I: $R^1$ = 
$R^2$ = OAc, X = OH

Figure 1. Structures of omphalotins E–I.

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These derivatives disappeared, and the novel compounds seemed to be produced at the expense of the known omphalotins including omphalotin A, the amount of which decreased to one fifth of the original value. Three 20 L fermentations, also harvested at a late stage, yielded 32 mg of compound **4** and 15 mg of compound **5** (Figure 1).

## Structure Elucidation

The isolated compounds were analyzed by APCI-MS in the positive and negative ionization modes and nominal masses of 1349 for **1**, 1365 for **2**, 1381 for **3**, 1481 for **4** and 1539 for **5** were found. Analysis of the compounds by ESI-HRMS provided elemental compositions of $C_{69}H_{115}N_{13}O_{14}$ for **1**, $C_{69}H_{115}N_{13}O_{15}$ for **2**, $C_{69}H_{115}N_{13}O_{16}$ for **3**, $C_{73}H_{119}N_{13}O_{19}$ for **4** and $C_{76}H_{120}N_{13}O_{20}$ for **5**. As all compounds contained a modified tryptophan unit, the presence of 13 nitrogen atoms was in agreement with the dodecapeptide backbone found in omphalotins A–D.

The rather lipophilic nature of the isolated peptides allowed NMR spectroscopic experiments to be carried out in $CDCl_3$, where compounds **3**–**5** were apparently present as a single conformer, whereas one minor conformer was detected in the NMR spectra of **1** (ca. 15%) and **2** (ca. 25%). In both cases, chemical exchange could be observed in the NOESY spectra. The $^{13}C$ NMR spectra showed the presence of 12 amide carbonyl groups. Additionally, 12 $C_\alpha$ atoms, 9 methine and 3 methylene carbon atoms for **1** and **2**, or 10 methine and 2 methylene carbon atoms for **3**–**5**, were found between 49 and 68 ppm. Assignment of the $C_\alpha$ atoms to the respective $H_\alpha$ resonances and the corresponding side chain spin systems by HSQC and TOCSY experiments in **1** showed the presence of three glycine, four valine and three isoleucine residues; one valine moiety and the tryptophan residue were modified by oxidation.

In all cyclopeptides, 9 amino acids were *N*-methylated. Assignment of the backbone resonances was performed by HMBC and NOESY, mostly relying on the $^3J$ correlations between $C_\alpha$ and $NCH_3$ as well as between CO and $NCH_3$ where available. NOE contacts were found from most α-protons to the *N*-methyl group of the adjacent amino acid, further confirming the sequence. The sequence of **1** corresponded to that of omphalotin B without the oxidized glycine and isoleucine fragments found in the latter compound. The tricyclic tryptophan derivative contained an aminal moiety and a tertiary alcohol that was also present in omphalotins B–D;[3] **1** was therefore named omphalotin E.

The structure of compound **2** was almost identical to that of **1**, so **2** was named omphalotin F, accordingly. However, an additional oxygen atom was present in **2**, and the chemical shifts of the tryptophan derivative differed significantly. In **1**, the aminal proton at $\delta$ = 5.76 ppm exhibited $^3J$ coupling to the NH proton at $\delta$ = 5.35 ppm, both signals being broad doublets in the $^1H$ NMR spectrum, whereas the corresponding aminal signal in **2** was a sharp singlet at $\delta$ = 5.59 ppm, which only gave a weak long-range COSY correlation to a singlet at $\delta$ = 9.03 ppm. The corresponding aminal carbon atom had a rather unusual chemical shift of 98.8 ppm. The now overlapping $^1H$ NMR resonances of the benzene ring were shifted to lower field. Further analysis revealed a $^1H$–$^{15}N$ HMBC correlation between the proton at $\delta$ = 9.03 ppm and the nitrogen at $\delta$ = 165.6 ppm, but neither $^1H$–$^{15}N$ nor $^1H$–$^{13}C$ HSQC correlations, suggesting the presence of an *N*-hydroxy group and ruling out the possibility of an endocyclic NO fragment. The chemical shifts of the *N*-hydroxylated aminal unit were in agreement with those reported for related structures.[5–7] Representative HMBC correlations are summarized in Figure 2. The long-wave UV absorption maximum is hypsochromically shifted by about 10 nm in *N*-hydroxylated compound **2**, which was also reported for *N*-hydroxylated anilines.[8]

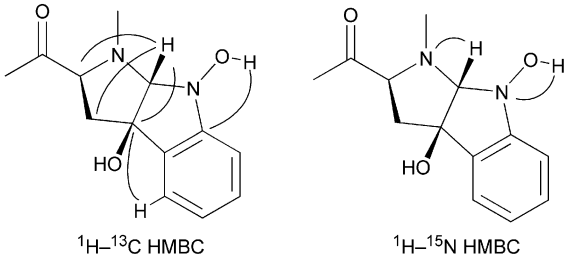


Figure 2. HMBC correlations of the modified tryptophan fragment in **2**.

To substantiate the hypothesis, **1** and **2** were treated with alkaline 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) solution on TLC plates.[9] Whereas neither omphalotin A, tryptophan nor **1** gave any reaction, **2** produced the same vermillion colour as hydroxylamine hydrochloride even at low concentrations. When an analytical sample of **2** was reduced with $TiCl_3$ in methanolic solution,[10] the formation of **1** could be detected by TLC,[11] $^1H$ NMR spectroscopy and HPLC–MS, further confirming the presence of the *N*-hydroxylated tryptophan derivative in omphalotin F (**2**).

Compound **3** contained one more oxygen atom compared to **2**. Its structure was established by NMR spectroscopic analysis to be similar to the previously known omphalotins; hence, **3** was named omphalotin G. The chemical shifts of the oxidized tryptophan fragment were highly consistent with those of **2**, as was the positive TTC test. In omphalotin G, one of the glycine residues was hydroxylated in the α-position (C-53).

Compounds **4** (omphalotin H) and **5** (omphalotin I) contained the same oxidized glycine unit; however, the α-hydroxy group was acetylated in both cases. In addition, one of the three isoleucine residues was oxidized and acylated at C-49 in both compounds to form an acetate in **4** and a 3-hydroxy-3-methylbutanoate in **5**. Both compounds gave a colour reaction with TTC, and the chemical shifts of the tryptophan fragment were consistent with those of **2** and **3**, and so **4** and **5** can be regarded as *N*-hydroxylated analogues of the omphalotins B and C.[3] Compounds **3**–**5** decomposed slowly in methanolic solution, presumably due to the presence of the N,O-acetalic moiety.

  

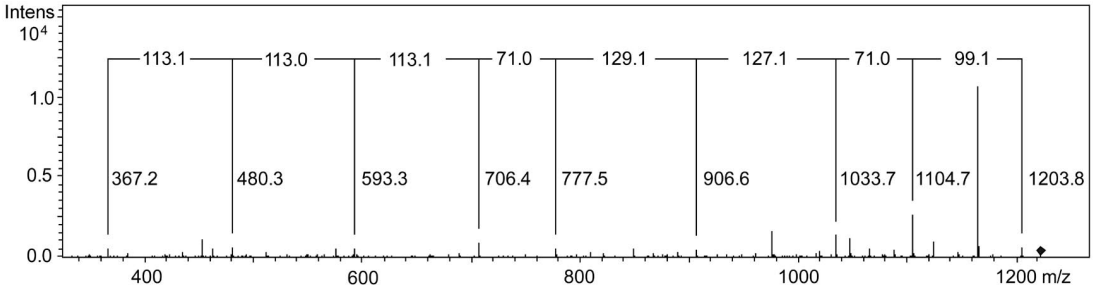


Figure 4. $MS^3$ analysis (positive ionization mode, precursor ions 1349.0→1221.8) of compound **2**.

The relative stereochemistry of the modified tryptophan residue in **5** was examined by NOESY spectroscopy (Figure 3) and turned out to be identical to that proposed by Sterner et al. for omphalotins B–D.[3] Two-dimensional NMR spectra showed only weak scalar coupling between $H_\alpha$ and the pro-(*S*)-methylene proton so that a dihedral torsion angle of approximately 90° was expected,[12] which was in agreement with the suggested relative configuration. Considering the nearly identical chemical shifts of the modified tryptophan units, omphalotins F–H (**2**–**4**) should have the same relative configuration. Hydrolysis and HPLC analysis of the FDAA-derivatized amino acids confirmed the presence of sarcosine, L-valine, *N*-methyl-L-valine, L-isoleucine and *N*-methyl-L-isoleucine for all compounds. As omphalotins E–I are metabolites ultimately derived from omphalotin A, it can be assumed that the modified tryptophan, valine and isoleucine residues have the L configuration.[3] The configuration of C-53 in **3**–**5** and that of C-49 in **4** and **5** was not determined; however, the scalar coupling between H-47 and H-49 is in accordance with the data reported for omphalotins B–D.[3]

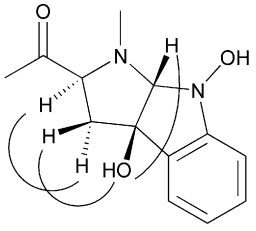


Figure 3. Configuration of the modified tryptophan residue as found by analysis of NOESY correlations in **5**.

The amino acid sequences of the omphalotins could be partially confirmed by $MS^n$ analysis. As shown for **2** in Figure 4, differences of fragment masses would correlate (from low to high mass) to isoleucine (113), *N*-methylvaline (113), *N*-methylvaline (113), sarcosine (71), 3-hydroxy-*N*-methylvaline (129), *N*-methylisoleucine (127), sarcosine (71) and valine (99), supporting the structures determined by NMR spectroscopy.

### Biological Activities

As shown in Table 1, compounds **1**–**5** exhibited similar nematicidal activities against *M. incognita* (Kofoid & White) as omphalotin A. Antibacterial or antifungal activities were not detected and none of the compounds showed cytotoxic effects towards mouse leukaemia cells (L1012 cells) or human colon adenocarcinoma cells (Colo 320 cells) at concentrations up to 50 $\mu g\,mL^{-1}$.

Table 1. Nematicidal activity of omphalotins A, E–I towards *Meloidogyne incognita* larvae. Ivermectin was used as a positive control.

| Compound | Nematicidal activity $LD_{50}$ $\mu g\,mL^{-1}$ | Nematicidal activity $LD_{90}$ $\mu g\,mL^{-1}$ |
|---|---|---|
| Omphalotin A | 2 | 5 |
| Omphalotin E (**1**) | 5 | 10 |
| Omphalotin F (**2**) | 2 | 5 |
| Omphalotin G (**3**) | 1 | 2 |
| Omphalotin H (**4**) | 0.5–1 | 2 |
| Omphalotin I (**5**) | 1 | 2 |
| Ivermectin | 2 | 5 |

## Experimental Section

**Producing Organism:** The monokaryotic strain was obtained from protoplasts prepared from the mycelium of the dikaryotic strain *O. olearius* (DC ex Fr.) Sing. TA90170. The protoplasts were plated on agar plates and the mycelia obtained were evaluated under the microscope. Dikaryotic mycelia with clamp connections were discarded. In submerged culture, all monokaryoric strains produced omphalotins; therefore, the fastest growing strain was selected for further studies.

**Fermentation and Isolation:** Fermentations were carried out in yeast malt glucose medium in 20 L and 200 L fermentors as described earlier.[1,3,13] The medium was composed of yeast extract (4 $g\,L^{-1}$, Hartge Ingredients, Hamburg, Germany), malt extract (10 $g\,L^{-1}$, Difco Laboratories, Detroit, USA), glucose (4 $g\,L^{-1}$, Stockmeier, Herford, Germany). Cultures were harvested after 7 d, when the concentration of the omphalotins (analyzed by HPLC–MS) had reached its maximum. The compounds were extracted from lyophilized mycelia with methanol. Isolation was achieved by chromatography on Sephadex LH 20 in MeOH, followed by preparative HPLC on LiChroSpher 100 RP18, 5 µm particle size (Merck, Darmstadt, Germany, column size 25 × 250 mm, flow 30 $mL\,min^{-1}$, isocratic elution with 80% acetonitrile in $H_2O$) and Zorbax Eclipse XDB-Phenyl, particle size 5 µm (Agilent, Waldbronn, column size 9.4 × 250 mm, flow 7 $mL\,min^{-1}$ with 60% acetonitrile in $H_2O$), by using a Jasco modular HPLC system (Groß-Umstadt, Germany) consisting of two pumps PU-1586 and a UV-1570M detector.

  

**Biological Assays:** Cultivation and testing of *Meloidogyne incognita* has been described previously.[1,13] Cytotoxicity was assayed as described previously. Colo-320 cells (DSMZ ACC 144) and L-1210 cells (DSMZ ACC 123) were grown in RPMI 1640 medium (Invitrogen). All media were supplemented with 10% heat inactivated fetal calf serum (Invitrogen), 65 μg mL$^{-1}$ of penicillin G and 100 μg mL$^{-1}$ of streptomycin sulfate.

**Spectroscopy:** NMR spectra were recorded at 25 °C with a Bruker Avance-II spectrometer equipped with an inverse multinuclear 5 mm probehead and a *z*-gradient coil (400 MHz) or with a Bruker DRX-500 spectrometer (500 MHz). $^{15}$N NMR spectra were recorded with a Bruker Avance 600 spectrometer at 61 MHz. The spectra were measured in $CDCl_3$ and the chemical shifts were referenced to the residual solvent signal ($CDCl_3$: $\delta_H$ = 7.26 ppm, $\delta_C$ = 77.16 ppm).[14] For $^{15}$N NMR spectra, $NH_3$ was used as external reference ($\delta_N$ = 0 ppm). Standard pulse sequences for gs-COSY, gs-TOCSY, gs-HSQC, gs-HMBC and gs-NOESY experiments were used. The refocusing delays for the inverse heterocorrelations were set to 3.45 and 62.5 ms, corresponding to $^1J_{C,H}$ = 145 Hz, $^nJ_{C,H}$ = 8 Hz and $^nJ_{N,H}$ = 8 Hz, respectively. The mixing times were 800 ms for the gs-NOESY and 80 ms for the gs-TOCSY. Processing of the data was performed with the MestReNova Software (Mestrelab Research). ESI-HRMS spectra were recorded with a MicroMass/Waters ESI Q-TOF mass spectrometer equipped with a LockSpray interface using NaI/CsI or trialkylamines as external reference. HPLC–MS analyses were performed with a Hewlett–Packard Series 1100LC-MSD instrument operating at a fragmentor voltage of 140 V in the APCI-positive (PI) and -negative ionisation (NI) modes. The gas temperature was 350 °C, with the vapourizer set to 400 °C; drying gas was supplied at 6 L min$^{-1}$, with a nebulizing pressure of 50 psi. Chromatographic separation was achieved with a LiChroCART Superspher 100 RP-18 column (4 μm; 125 × 2 mm; Merck, Darmstadt) at 40 °C in a gradient from 1 to 99% acetonitrile in 14 min at a flow rate of 0.45 mL min$^{-1}$. MS$^n$ analysis was performed with a LC1200/MSD Trap XCT (Agilent, Waldbronn) equipped with a Zorbax XDB-C18 column (3.5 μm;2.1 × 30 mm). Electrospray ionization in positive mode was used (nebulizer pressure 50 psi, 10 L min$^{-1}$ drying gas at 350 °C, capillary voltage 3.5 kV, capillary extractor 140 V). Other fragmentation parameters were optimized for every ion in order to obtain a homogeneous fragmentation pattern. IR and UV spectra were measured with a Bruker IFS48 FTIR spectrometer and a Perkin–Elmer Lambda-16 spectrophotometer, respectively. Optical rotations were measured with a P8000 polarimeter (Krüss) at 589 nm. Melting points were determined with a Dr. Tottoli apparatus and are uncorrected.

**Chemical Methods:** TLC experiments were carried out on Merck glass plates coated with silica gel 60 $F_{254}$. After elution, the chromatograms were developed with the respective reagent and heated. 2,3,5-Triphenyl-2*H*-tetrazolium chloride (TTC), commercially available from Fluka, was employed as solution (0.2%) in *n*-butyl alcohol saturated with water. TLC plates were dipped in the solution, dried in a stream of nitrogen, treated with a mixture of 1 M aqueous NaOH/*n*-butyl alcohol/ethanol (1:1:1) and heated.[9] $TiCl_3$ solution (12%) in aqueous HCl (free acid 5–10%) was purchased from Sigma Aldrich. Phosphomolybdic acid reagent was prepared by dissolving phosphomolybdic acid (25 g), $Ce(SO_4)_2{\cdot}4H_2O$ (10 g) and $H_2SO_4$ (60 mL) in water (940 mL).[15]

**Acid Hydrolysis:** About 100 μg of all compounds were hydrolyzed with 50 μL 6 M HCl in a sealed glass capillary for 2 h at 120 °C. After evaporation to dryness, samples were treated with Marfey's reagent as described elsewhere.[16] After addition of 0.2 N HCl (10 μL) to the cooled reaction, samples were injected on a RP column (Zorbax Eclipse XDB-C8, 5 μm, 4.6 × 150 mm, Agilent) by using a gradient of acetonitrile and 0.1% aqueous TFA at 1.5 mL min$^{-1}$ as mobile phase (*t* / min, % MeCN: 0, 30; 12.5, 30; 13, 40; 16.5, 40; 18, 100; 19, 100; 20, 30), detection by UV at 340 nm and LCMS. Identification of amino acids was performed by comparison with standards (sarcosine $t_R$ = 2.7 min, L-valine $t_R$ = 7.1 min, *N*-methyl-L-valine $t_R$ = 11.1 min, L-isoleucine $t_R$ = 11.6 min, *N*-methyl-L-isoleucine $t_R$ = 15.5 min).

**Omphalotin E (1):** Colourless solid (9.9 mg), m.p. 192–195 °C, $R_f$ = 0.33 (ethyl acetate), $[a]_D^{25}$ = –143.1 (*c* = 0.83, $CDCl_3$). UV (MeOH): λ (log ε) = 204 (4.58), 294 (3.05) nm. IR (KBr): ṽ = 3436, 2968, 1641, 1472, 1410, 1103 cm$^{-1}$. NMR spectroscopic data see Tables 2–4. APCI-MS (+): *m*/*z* (%) = 1332.7 (25) [M – OH]$^+$, 1350.7 (100) [M + H]$^+$. APCI-MS (–): *m*/*z* (%) = 1348.7 (100) [M – H]$^-$. ESI-HRMS: calcd. for $C_{69}H_{115}N_{13}O_{14}$ + $Na^+$ 1372.8584; found 1372.8555.

**Omphalotin F (2):** Colourless solid (14.9 mg), m.p. 195–198 °C, $R_f$ = 0.51 (ethyl acetate), $[a]_D^{25}$ = –147.0 (*c* = 0.41, $CDCl_3$). UV (MeOH): λ (log ε) = 204 (4.87), 283 (3.32) nm. IR (KBr): ṽ = 3380, 2974, 1647, 1457, 1411, 1091, 1050, 882 cm$^{-1}$. NMR spectroscopic data see Tables 2–4. APCI-MS (+): *m*/*z* (%) = 1348.8 (25) [M – OH]$^+$, 1366.8 (100) [M + H]$^+$. APCI-MS (–): *m*/*z* (%) = 1364.7 (100) [M – H]$^-$. ESI-HRMS: calcd. for $C_{69}H_{115}N_{13}O_{15}$ + $Na^+$ 1388.8533; found 1388.8528.

**Omphalotin G (3):** Colourless solid (8.2 mg), m.p. 160–161 °C, $R_f$ = 0.63 (ethyl acetate), $[a]_D^{25}$ = –109.0 (*c* = 0.68, $CDCl_3$). UV (MeOH): λ (log ε) = 205 (4.88), 283 (3.31) nm. IR (KBr): ṽ = 3436, 2967, 1642, 1467, 1409, 1100, 1046, 644 cm$^{-1}$. NMR spectroscopic data see Tables 2 and 3. APCI-MS (–): *m*/*z* (%) = 1380.7 (100) [M – H]$^-$. ESI-HRMS: calcd. for $C_{69}H_{115}N_{13}O_{16}$ + $Na^+$ 1404.8482; found 1404.8466.

**Omphalotin H (4):** Colourless solid (14.0 mg), m.p. 194–197 °C, $R_f$ = 0.54 (ethyl acetate), $[a]_D^{25}$ = –114.0 (*c* = 1.17, $CDCl_3$). UV (MeOH): λ (log ε) = 205 (4.87), 283 (3.26) nm. IR (KBr): ṽ = 3436, 2965, 1739, 1643, 1468, 1409, 1100, 1027 cm$^{-1}$. NMR spectroscopic data see Tables 2 and 3. APCI-MS (+): *m*/*z* (%) = 1464.8 (100) [M – OH]$^+$, 1482.8 (21) [M + H]$^+$. APCI-MS (–): *m*/*z* (%) = 1421.7 (100), 1462.7 (10) [M – $H_3O$]$^-$, 1480.8 (40) [M – H]$^-$. ESI-HRMS calcd. for $C_{73}H_{119}N_{13}O_{19}$ + $Na^+$ 1504.8643; found 1504.8667.

**Omphalotin I (5):** Colourless solid (10.3 mg), m.p. 210–215 °C, $R_f$ = 0.46 (ethyl acetate), $[a]_D^{25}$ = –150.3 (*c* = 0.86, $CDCl_3$). UV (MeOH): λ (log ε) = 205 (4.87), 284 (3.23) nm. IR (KBr): ṽ = 3437, 2969, 1638, 1468, 1410, 1103, 1029 cm$^{-1}$. NMR spectroscopic data see Tables 2, 3 and 4. APCI-MS (+): *m*/*z* (%) = 1522.9 (100) [M – OH]$^+$, 1540.9 (15) [M + H]$^+$. APCI-MS (–): *m*/*z* (%) = 1478.8 (100), 1520.9 (10) [M – $H_3O$]$^-$, 1538.9 (30) [M – H]$^-$. ESI-HRMS: calcd. for $C_{76}H_{125}N_{13}O_{20}$ + $H^+$ 1540.9237; found 1540.9205.

**Reduction of 2:** A sample of **2** (0.1 mg) was dissolved in MeOH (1 mL), and a solution of $TiCl_3$ (12%) in aqueous HCl was added (300 μL). The mixture was stirred for 5 min, then saturated aqueous $Na_2CO_3$ (1 mL) was added, and the mixture was extracted with ethyl acetate (2 × 2 mL). The combined organic extract was dried with $Na_2SO_4$ and the solvent was evaporated in vacuo. The residue (approx 0.1 mg) was dissolved in $CH_2Cl_2$ (0.5 mL) and analyzed by TLC (ethyl acetate) to find the conversion of **2** ($R_f$ = 0.51) into **1** ($R_f$ = 0.33). $^1$H NMR (500 MHz, $CDCl_3$) showed *N*-methyl signals at 3.41, 3.22, 3.13, 3.08, 3.07, 3.02, 2.91, 2.84, 2.83 ppm. These data as well as the mass and the retention time determined by HPLC–MS were in accordance with those of genuine **1**.

**Supporting Information** (see also the footnote on the first page of this article): NMR and MS$^n$ spectra of the omphalotins.

  

Table 2. $^1$H NMR (400 or 500 MHz) data for **1**–**5** in $CDCl_3$. The residual solvent signal ($\delta$ = 7.26 ppm)[14] was used as reference.

| Position | Omphalotin E (**1**) $\delta_H$ / ppm, mult. ($J$ / Hz) | Omphalotin F (**2**) $\delta_H$ / ppm, mult. ($J$ / Hz) | Omphalotin G (**3**) $\delta_H$ / ppm, mult. ($J$ / Hz) | Omphalotin H (**4**) $\delta_H$ / ppm, mult. ($J$ / Hz) | Omphalotin I (**5**) $\delta_H$ / ppm, mult. ($J$ / Hz) |
|---|---|---|---|---|---|
| 1 | 6.64, d (7.8) | 7.06, m | 7.04, m | 7.04, m | 7.04, m |
| 2 | 7.13, dd (7.8, 7.4) | 7.29, m | 7.31, m | 7.30, m | 7.30, m |
| 3 | 6.80, t (7.4) | 7.03, m | 7.03, m | 7.03, m | 7.03, m |
| 4 | 7.29, d (7.4) | 7.34, m | 7.34, m | 7.34, m | 7.34, m |
| 7 | 5.76, br. d (3.3) | 5.59, s | 5.59, s | 5.59, s | 5.59, s |
| 9 | 2.41, 2.23, m | 2.29, 2.18, m | 2.26, 2.20, m | 2.25, 2.22, m | 2.27, 2.20, m |
| 10 | 5.18, m | 5.29, m | 5.28, br. d (8.7) | 5.28, m | 5.28, br. d (8.6) |
| 12 | 2.84, s | 2.84, s | 2.84, s | 2.84, s | 2.84, s |
| 13 | 4.35, d (10.9) | 4.31, d (10.9) | 4.33, d (10.9) | 4.31, d (10.9) | 4.30, d (10.9) |
| 14 | 2.41, m | 2.40, m | 2.40, m | 2.41, m | 2.40, m |
| 15 | 0.77, d (6.9) | 0.75, d (7.4) | 0.75, d (7.0) | 0.74, d (7.0) | 0.75, d (7.1) |
| 16 | 0.89, m | 0.87, m | 0.87, m | 0.87, m | 0.87, m |
| 18 | 4.86, pseudo t (9.6) | 4.87, pseudo t (9.6) | 4.89, pseudo t (9.6) | 4.87, dd (9.6, 9.3) | 4.87, pseudo t (9.6) |
| 19 | 1.90, m | 1.87, m | 1.84, m | 1.87, m | 1.87, m |
| 20 | 0.87, m | 0.85, m | 0.85, m | 0.85, m | 0.85, m |
| 21 | 1.40, 1.10, m | 1.40, 1.10, m | ≈1.40, m | 1.41, 1.09, m | 1.40, 1.10, m |
| 22 | 0.85, m | 0.82, m | 0.83, m | 0.83, m | 0.83, m |
| 24 | 3.06, s | 3.07, s | 3.07, s | 3.07, s | 3.07, s |
| 25 | 5.20, m | 5.20, d (9.5) | 5.19, d (11.0) | 5.18, d (10.7) | 5.19, d (10.7) |
| 26 | 2.30, m | 2.34, m | 2.34, m | 2.36, m | 2.36, m |
| 27 | 0.67, d (6.7) | 0.66, d (6.7) | 0.66, d (6.8) | 0.65, d (6.8) | 0.65, d (6.8) |
| 28 | 0.86, m | 0.84, m | 0.84, m | 0.83, m | 0.83, m |
| 30 | 2.90, s | 2.92, s | 2.93, s | 2.93, s | 2.99, s |
| 31 | 5.08, d (10.4) | 5.09, d (10.4) | 5.09, d (10.5) | 5.08, d (10.4) | 5.08, d (10.5) |
| 32 | 2.33, m | 2.33, m | 2.34, m | 2.32, m | 2.32, m |
| 33 | 0.73, d (6.8) | 0.72, d (6.9) | 0.73, d (6.9) | 0.72, d (6.9) | 0.72, d (6.9) |
| 34 | 0.91, m | 0.90, m | 0.90, m | 0.90, m | 0.90, m |
| 36 | 3.02, s | 3.01, s | 3.00, s | 2.97, s | 2.99, s |
| 37 | 4.96, d (15.8) | 4.97, d (16.0) | 4.99, d (16.1) | 5.01, d (16.3) | 4.99, d (16.2) |
| | 3.21, d (15.8) | 3.22, d (16.0) | 3.22, d (16.1) | 3.24, d (16.3) | 3.25, d (16.2) |
| 39 | 3.13, s | 3.10, s | 3.11, s | 3.07, s | 3.14, s |
| 40 | 5.28, s | 5.25, s | 5.24, s | 5.16, s | 5.17, s |
| 42 | 1.06, s | 1.04, s | 1.03, s | 1.02, s | 1.03, s |
| 43 | 1.19, s | 1.18, s | 1.13, s | 1.10, s | 1.11, s |
| 45 | 2.85, s | 2.83, s | 2.85, s | 2.85, s | 2.86, s |
| 46 | 5.22, m | 5.22, d (9.7) | 5.19, d (10.6) | 5.49, d (10.9) | 5.42, d (10.9) |
| 47 | 2.30, m | 2.31, m | 2.18, m | 2.31, m | 2.30, m |
| 48 | 0.92, m | 0.84, m | 0.88, m | 0.97, d (6.6) | 0.99, d (6.6) |
| 49 | ≈1.35, m | 1.36, 0.97, m | ≈1.37, m | 4.50, qd (6.3, 1.5) | 4.57, qd (6.0, 0.9) |
| 50 | 0.82, m | 0.91, m | 0.91, m | 1.29, d (6.3) | 1.32, d (6.0) |
| 52 | 3.22, s | 3.23, s | 2.91, s | 3.06, s | 3.02, s |
| 53 | 4.82 d (14.1) | 4.82, d (13.7) | 6.04, d (6.7) | 6.85, s | 6.84, s |
| | 3.17 d (14.1) | 3.16, d (13.7) | | | |
| 55 | 4.80, m | 4.80, t (9.3) | 4.93, dd (8.7, 3.6) | 4.86, m | 4.85, dd (8.4, 3.7) |
| 56 | 1.99, m | 1.97, m | 1.97, m | 1.97, m | 1.97, m |
| 57 | 0.90, m | 0.86, m | 0.83, m | 0.84, m | 0.84, m |
| 58 | 0.97, m | 0.94, d (7.1) | 0.98, d (6.8) | 0.98, d (6.8) | 0.98, d (6.8) |
| 60 | 3.08, s | 3.07, s | 3.04, s | 3.04, s | 3.04, s |
| 61 | 5.45, d (10.9) | 5.48, d (11.0) | 5.50, d (11.1) | 5.51, d (11.1) | 5.51, (11.1) |
| 62 | 2.15, m | 2.33, m | 2.19, m | 2.22, m | 2.20, m |
| 63 | 1.03, d (6.5) | 1.04, d (6.2) | 1.05, d (6.6) | 1.05, d (6.6) | 1.05, d (6.6) |
| 64 | ≈1.30, m | 1.46, 1.03, m | ≈1.28, m | 1.29, 1.26, m | ≈1.27, m |
| 65 | 0.85, m | 0.92, m | 0.92, m | 0.91, m | 0.91, m |
| 67 | 3.41, s | 3.50, s | 3.49, s | 3.52, s | 3.52, s |
| 68 | 4.93, d (15.4) | 5.41, d (15.5) | 5.41, d (15.7) | 5.43, d (15.7) | 5.44, d (15.7) |
| | 3.41, d (15.4) | 3.46, d (15.5) | 3.47, d (15.7) | 3.47, d (15.7) | 3.47, d (15.7) |
| 6-N(O)H | 5.35, br. d (3.3) | 9.03, s | 9.02, s | 9.05, s | 9.03, s |
| 8-OH | 6.68, br. s | 6.90, br. s | 6.96, br. s | 6.92, s | 6.92, s |
| 41-OH | 5.41, br. s | 5.41, br. s | 5.41, br. s | 5.28, br. s | 5.06, |
| 53-OH | – | – | 4.64, d (6.7) | – | – |
| 18-NH | 8.51, d (9.6) | 8.51, d (9.6) | 8.55, d (9.6) | 8.52, d (9.6) | 8.53, d (9.6) |
| 55-NH | 7.53, br. d (≈7) | ≈7.6, br. s | 7.99, d (8.7) | 7.31, d (8.6) | 7.29, d (8.4) |
| $R^1$/H-2′ | – | – | – | 2.02, s | 2.54, d (14.5) |
| | | | | | 2.42, d (14.5) |
| $R^1$/H-4′ | – | – | – | – | 1.31, s |
| $R^1$/3′-OH | – | – | – | – | 3.74, s |
| $R^2$/H-2′ | – | – | – | 2.22, s | 2.22, s |

  

Table 3. $^{13}C$ NMR (101 MHz) data for **1**–**5** in $CDCl_3$. The solvent signal ($\delta$ = 77.16 ppm)[14] was used as reference.

| Position | Omphalotin E (**1**) $\delta_C$ / ppm, mult. | Omphalotin F (**2**) $\delta_C$ / ppm, mult. | Omphalotin G (**3**) $\delta_C$ / ppm, mult. | Omphalotin H (**4**) $\delta_C$ / ppm, mult. | Omphalotin I (**5**) $\delta_C$ / ppm, mult. |
|---|---|---|---|---|---|
| 1 | 110.3, CH | 114.0, CH | 114.0, CH | 114.1, CH | 114.1, CH |
| 2 | 129.8, CH | 129.9, CH | 130.0, CH | 130.1, CH | 129.94, CH |
| 3 | 119.8, CH | 123.3, CH | 123.4, CH | 123.3, CH | 123.4, CH |
| 4 | 122.6, CH | 121.9, CH | 122.0, CH | 121.9, CH | 121.9, CH |
| 5 | 130.4, qC | 129.6, qC | 129.7, qC | 129.7, qC | 129.7, qC |
| 6 | 146.2, qC | 147.7, qC | 147.7, qC | 147.8, qC | 147.7, qC |
| 7 | 87.2, CH | 98.8, CH | 98.9, CH | 98.9, CH | 98.8, CH |
| 8 | 89.5, qC | 85.9, qC | 86.0, qC | 86.0, qC | 86.0, qC |
| 9 | 42.2, $CH_2$ | 41.6, $CH_2$ | 41.6, $CH_2$ | 41.6, $CH_2$ | 41.6, $CH_2$ |
| 10 | 57.5, CH | 58.5, CH | 58.6, CH | 58.6, CH | 58.6, CH |
| 11 | 174.5, qC | 174.1, qC | 174.1, qC | 174.1, qC | 174.1, qC |
| 12 | 29.9, $CH_3$ | 30.0, $CH_3$ | 30.1, $CH_3$ | 30.0, $CH_3$ | 30.0, $CH_3$ |
| 13 | 67.1, CH | 67.2, CH | 67.2, CH | 67.2, CH | 67.3, CH |
| 14 | 26.1, CH | 26.1, CH | 26.1, CH | 26.1, CH | 26.1, CH |
| 15 | 18.9, $CH_3$ | 18.9, $CH_3$ | 18.9, $CH_3$ | 18.9, $CH_3$ | 18.9, $CH_3$ |
| 16 | 20.0, $CH_3$ | 19.9, $CH_3$ | 20.0, $CH_3$ | 20.0, $CH_3$ | 20.0, $CH_3$ |
| 17 | 167.5, qC | 167.4, qC | 167.4, qC | 167.4, qC | 167.4, qC |
| 18 | 53.1, CH | 52.9, CH | 52.9, CH | 52.9, CH | 52.9, CH |
| 19 | 36.6, CH | 36.7, CH | 36.9, CH | 36.8, CH | 36.8, CH |
| 20 | 15.8, $CH_3$ | 15.7, $CH_3$ | 15.7, $CH_3$ | 15.6, $CH_3$ | 15.6, $CH_3$ |
| 21 | 24.4, $CH_2$ | 24.5, $CH_2$ | 24.4, $CH_2$ | 24.5, $CH_2$ | 24.5, $CH_2$ |
| 22 | 10.9, $CH_3$ | 10.9, $CH_3$ | 10.9, $CH_3$ | 10.9, $CH_3$ | 10.9, $CH_3$ |
| 23 | 172.2, qC | 172.2, qC | 172.3, qC | 172.3, qC | 172.3, qC |
| 24 | 30.2, $CH_3$ | 30.7, $CH_3$ | 30.4, $CH_3$ | 30.6, $CH_3$ | 30.4, $CH_3$ |
| 25 | 58.1, CH | 58.1, CH | 58.1, CH | 58.2, CH | 58.2, CH |
| 26 | 27.2, CH | 27.1, CH | 27.1, CH | 27.2, CH | 27.2, CH |
| 27 | 17.4, $CH_3$ | 17.6, $CH_3$ | 17.5, $CH_3$ | 17.5, $CH_3$ | 17.5, $CH_3$ |
| 28 | 19.7, $CH_3$ | 19.6, $CH_3$ | 19.7, $CH_3$ | 19.7, $CH_3$ | 19.7, $CH_3$ |
| 29 | 169.7, qC | 169.7, qC | 169.7, qC | 169.6, qC | 169.6, qC |
| 30 | 30.5, $CH_3$ | 30.2, $CH_3$ | 30.2, $CH_3$ | 30.2, $CH_3$ | 30.3, $CH_3$ |
| 31 | 58.4, CH | 58.3, CH | 58.3, CH | 58.4, CH | 58.4, CH |
| 32 | 26.7, CH | 26.7, CH | 26.6, CH | 26.6, CH | 26.7, CH |
| 33 | 17.6, $CH_3$ | 17.3, $CH_3$ | 17.3, $CH_3$ | 17.3, $CH_3$ | 17.3, $CH_3$ |
| 34 | 20.3, $CH_3$ | 20.2, $CH_3$ | 20.2, $CH_3$ | 20.2, $CH_3$ | 20.2, $CH_3$ |
| 35 | 169.7, qC | 169.7, qC | 169.8, qC | 169.7, qC | 169.8, qC |
| 36 | 37.0, $CH_3$ | 36.8, $CH_3$ | 36.8, $CH_3$ | 36.8, $CH_3$ | 36.9, $CH_3$ |
| 37 | 50.0, $CH_2$ | 49.9, $CH_2$ | 50.0, $CH_2$ | 50.3, $CH_2$ | 50.3, $CH_2$ |
| 38 | 168.7, qC | 168.4, qC | 168.4, qC | 168.3, qC | 168.4, qC |
| 39 | 32.2, $CH_3$ | 32.1, $CH_3$ | 32.1, $CH_3$ | 32.4, $CH_3$ | 32.6, $CH_3$ |
| 40 | 55.6, CH | 55.6, CH | 55.5, CH | 56.2, CH | 56.3, CH |
| 41 | 73.5, qC | 73.5, qC | 73.5, qC | 73.4, qC | 73.5, qC |
| 42 | 25.7, $CH_3$ | 25.7, $CH_3$ | 25.7, $CH_3$ | 25.7, $CH_3$ | 25.7, $CH_3$ |
| 43 | 29.0, $CH_3$ | 29.0, $CH_3$ | 28.9, $CH_3$ | 29.0, $CH_3$ | 29.0, $CH_3$ |
| 44 | 171.5, qC | 171.5, qC | 171.6, qC | 172.0, qC | 172.2, qC |
| 45 | 30.4, $CH_3$ | 30.4, $CH_3$ | 30.4, $CH_3$ | 30.3, $CH_3$ | 30.4, $CH_3$ |
| 46 | 56.9, CH | 56.7, CH | 57.7, CH | 54.1, CH | 54.1, CH |
| 47 | 33.0, CH | 32.9, CH | 32.8, CH | 36.8, CH | 36.7, CH |
| 48 | 15.9, $CH_3$ | 15.8, $CH_3$ | 15.8, $CH_3$ | 10.1, $CH_3$ | 10.2, $CH_3$ |
| 49 | 23.5, $CH_2$ | 23.5, $CH_2$ | 23.5, $CH_2$ | 68.8, CH | 69.4, CH |
| 50 | 10.9, $CH_3$ | 10.9, $CH_3$ | 10.9, $CH_3$ | 17.9, $CH_3$ | 17.9, $CH_3$ |
| 51 | 170.4, qC | 170.2, qC | 170.8, qC | 170.1, qC | 169.7, qC |
| 52 | 37.0, $CH_3$ | 37.0, $CH_3$ | 27.9, $CH_3$ | 30.1, $CH_3$ | 30.2, $CH_3$ |
| 53 | 54.1, $CH_2$ | 54.1, $CH_2$ | 74.5, CH | 76.1, CH | 76.0, CH |
| 54 | 168.8, qC | 168.8, qC | 169.5, qC | 165.0, qC | 164.9, qC |
| 55 | 54.1, CH | 54.1, CH | 54.7, CH | 54.0, CH | 54.1, CH |
| 56 | 31.9, CH | 31.9, CH | 32.4, CH | 32.0, CH | 32.0, CH |
| 57 | 17.1, $CH_3$ | 17.0, $CH_3$ | 16.4, $CH_3$ | 16.1, $CH_3$ | 16.0, $CH_3$ |
| 58 | 20.0, $CH_3$ | 20.0, $CH_3$ | 20.3, $CH_3$ | 20.2, $CH_3$ | 20.2, $CH_3$ |
| 59 | 171.9, qC | 172.0, qC | 171.2, qC | 171.5, qC | 171.5, qC |
| 60 | 30.8, $CH_3$ | 30.4, $CH_3$ | 30.7, $CH_3$ | 30.5, $CH_3$ | 30.6, $CH_3$ |
| 61 | 55.5, CH | 55.3, CH | 55.5, CH | 55.3, CH | 55.3, CH |
| 62 | 33.1, CH | 32.8, CH | 32.7, CH | 36.6, CH | 32.6, CH |
| 63 | 16.1, $CH_3$ | 16.1, $CH_3$ | 16.0, $CH_3$ | 16.0, $CH_3$ | 16.1, $CH_3$ |
| 64 | 24.6, $CH_2$ | 24.4, $CH_2$ | 24.5, $CH_2$ | 24.4, $CH_2$ | 24.4, $CH_2$ |
| 65 | 10.9, $CH_3$ | 10.6, $CH_3$ | 10.5, $CH_3$ | 10.3, $CH_3$ | 10.3, $CH_3$ |
| 66 | 171.9, qC | 173.2, qC | 173.0, qC | 173.1, qC | 173.0, qC |
| 67 | 38.7, $CH_3$ | 40.4, $CH_3$ | 40.4, $CH_3$ | 40.4, $CH_3$ | 40.4, $CH_3$ |
| 68 | 50.4, $CH_2$ | 52.3, $CH_2$ | 52.4, $CH_2$ | 52.3, $CH_2$ | 52.3, $CH_2$ |
| 69 | 169.2, qC | 169.9, qC | 170.0, qC | 170.0, qC | 170.0, qC |
| $R^1$/C-1′ | – | – | – | 170.7, qC | 172.0, qC |
| $R^1$/C-2′ | – | – | – | 21.5, $CH_3$ | 47.6, $CH_2$ |
| $R^1$/C-3′ | – | – | – | – | 69.3, qC |
| $R^1$/C-4′ | – | – | – | – | 29.0, $CH_3$ |
| $R^2$/C-1" | – | – | – | 169.1, qC | 169.1, qC |
| $R^2$/C-2" | – | – | – | 20.8, $CH_3$ | 20.8, $CH_3$ |

  

Table 4. $^{15}N$ NMR (61 MHz) data for **1**, **2** and **5** in $CDCl_3$. $NH_3$ ($\delta$ = 0 ppm) was used as an external reference.

| Position | Omphalotin E (**1**) $\delta_N$ / ppm | Omphalotin F (**2**) $\delta_N$ / ppm | Omphalotin I (**5**) $\delta_N$ / ppm |
|---|---|---|---|
| N-(O)H | –[a] | 165.6 | 165.5 |
| 10 | 148.7 | 146.5 | 146.5 |
| 13 | 124.2 | 122.4 | 124.7 |
| 18 | 126.2 | 127.8 | 126.8 |
| 25 | 112.0 | 112.8 | 112.5 |
| 31 | 116.7 | 117.5 | 116.9 |
| 37 | 108.3 | 109.0 | 108.4 |
| 40 | 108.5 | 108.2 | 107.4 |
| 46 | 121.1 | 122.4 | 118.9 |
| 53 | 112.4 | 112.9 | 121.0 |
| 55 | 117.3 | –[a] | 112.6 |
| 61 | 111.6 | 111.0 | 111.5 |
| 68 | 108.3 | 112.7 | 113.3 |

[a] Obscured.

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