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Identification and synthesis of three cyclodidepsipeptides as potential precursors of enniatin B in *Fusarium sporotrichioides*

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ABSTRACT

A pathogenic fungus, *Fusarium sporotrichioides* Sherb., was isolated from *Hypericum barbatum* Jacq. The volatile compounds of broth and mycelium were analyzed using GC–MS and three cyclodidepsipeptides (dioxomorpholines), 3,6-di(propan-2-yl)-4-methyl-morpholine-2,5-dione, 3-(2-methylpropyl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione and 3-(butan-2-yl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione, were found for the first time in the natural products. The structures of the compounds were confirmed by comparison of the analytical data for the natural products with samples obtained via synthetic methods. The conformational features and vibrational spectra of the three cyclodidepsipeptides were characterized by density functional theory (DFT) calculations and IR spectroscopy. The cyclic hexadepsipeptide enniatin B was identified by a LC–MS/MS analysis of the non-volatile products of broth and mycelium. The above-mentioned three cyclodidepsipeptides are probably synthesized using similar biosynthetic ways to enniatin B involving a nonribosomal mechanism.

1. Introduction

The family of cyclodepsipeptides comprises natural products and synthetic peptide lactones with at least one ester bond in their skeleton. The 18-membered cyclodepsipeptides, the so-called enniatins, typically possess an alternating sequence of D-2-hydroxy-3-methylbutanoic and L-configured *N*-methylamino acid residues. This class of compounds have been known to exhibit anthelmintic [1–3], insecticidal [4,5] and phytotoxic activities [6], as well as inhibitory activity towards acyl-CoA:cholesterol acyltransferase [7]. Dornetshuber et al. [8] demonstrated that enniatin B also exerts profound cytotoxic activity against several human tumour cells. The identification of enniatins is sometimes accompanied by the identification of cyclodidepsipeptides, which are potential precursor in the biosynthesis of the former. The reports about the biological activities of the cyclodidepsipeptides [9,10] inspired attempts of total synthesis of some of these compounds [11]. A number of other publications deal with the synthesis of the non-*N*-methylated derivatives which are useful building blocks for the preparation of biodegradable polymers [12–14].

Endophytes are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in wide variety of medical, agricultural, and industrial areas [15]. The number of studies on *Hypericum* species has increased rapidly over the years, mainly due to the pharmaceutical importance of dianthrone derivatives isolated from *Hypericum perforatum* [16]. In this work, we report the identification and synthesis of three cyclodidepsipeptides (morpholine-2,5-diones) as potential precursors of enniatin B in the pathogenic fungi *Fusarium sporotrichioides*, isolated from the stem of fresh *Hypericum barbatum* Jacq.

2. Results and discussion

The isolated fungus, *F. sporotrichioides* Sherb. [W&R,G,B,J], was cultured in a potato dextrose agar medium. After fermentation the volatile compounds of broth and mycelium were analyzed using GC–MS. In both, broth and mycelium ethyl acetate extracts three cyclodidepsipeptides were found, namely: 3,6-di(propan-2-yl)-4-methyl-morpholine-2,5-dione (**1a**), 3-(2-methylpropyl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione (**1b**) and 3-(butan-2-yl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione (**1c**) (Scheme 1). To the best of our knowledge in the present work compounds

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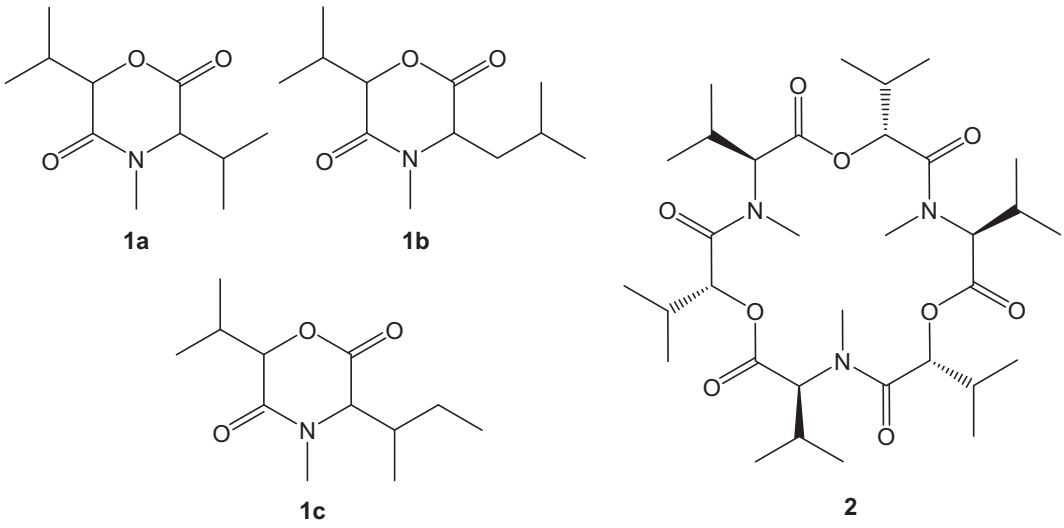


Scheme 1. Chemical structures of the compounds under study.

1a–**c** were found for the first time in natural products. However, due to the complexity of the studied mixture of the fungus products, compounds **1a**–**c** could not be isolated. The mass spectra of **1a**–**c** were compared to the mass fragmentation of a series of synthetic cyclodepsipeptides, reported by Shemyakin et al., and a perfect match was observed [17]. Therefore, the absolute configuration of **1a**–**c** can be predicted with very high probability to be (*3S*,*6R*).

The ethyl acetate extracts of broth and mycelium were analyzed by mycotoxin LC–MS/MS multi-method [18,19]. The cyclic hexadepsipeptide enniatin B ((3S,6R,9S,12R,15S,18R)-4,10,16-trimethyl-3,6,9,12,15,18-hexa(propan-2-yl)-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone; **2**, Scheme 1) could be unambiguously identified. We did not find any of the other mycotoxins integrated in the LC–MS/MS method (neosolaniol, 15-acetyldeoxynival, monoacetoxyscirpenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, verrucarin A, deoxynivalenol, nivalenol, 3-acetyldeoxynivalenol, fusarenon-X, zearalenone, α-zearalenol, alfatoxin-B1, alfatoxin-B2, alfatoxin-G1, alfatoxin-G2, ochratoxin A, ochratoxin B, fumonisin B1, fumonisin B2, beauvericin, altenuene, alternariol, alternariolmethylether, moniliformin, ergocornine, ergotamine, citrinin, patulin and gibberellic acid). An HPLC–ESI-MS/MS chromatogram of enniatin B is depicted in Fig. 1. The mechanism of enniatin biosynthesis was described [20] as step-wise condensation of dipeptidol blocks, terminated by cyclization reaction of the formed linear hexapeptide. The above-mentioned three cyclodidepsipeptides (**1a**–**c**) are probably synthesized as side products of cyclization in this process using similar nonribosomal biosynthetic mechanism to **2**. According to the suggested scheme [20] the main precursor for the formation of **2** would be a dipeptide block, containing *N*-methyl-L-valine and D-2-hydroxy-3-methylbutanoic acid residues, which could explain the fact that **1a** was found in prevailing amount in the broth and the mycelium. The production of the other two cyclodidepsipeptides, **1b** and **1c**, presumably is due to low substrate specificity of the enniatin synthetase for L-amino acids [20,21].

For identification and confirmation of the compounds the cyclodepsipeptides were synthesized. The synthesis of 4-methylmorpholine-2,5-diones was previously reported as a coupling of *N*-methylamino acids and 2-hydroxy-3-methylbutanoic acid [11] or 2-bromo-3-methylbutanoyl chloride [22]. Other authors explored also the condensation of esters of secondary amines with 2-chloropropionyl chloride [23] for the preparation of morpholine-2,5-diones with larger *N*-substituents. In this work the experimental conditions were adjusted in order to implement the method described by Cook and Cox [22]. Namely, the synthesis of cyclodidepsipeptides was performed via *N*-(α-bromoacyl)-α-amino acids as non-cyclic intermediate products (Scheme 2). The generic 2-bromo-3-methylbutanoyl chloride **4** was obtained by a sequence of condensation reactions using the experimental protocols of Harpp et al. [24]. Treatment of isovaleric acid (3-methylbutanoic acid; **3**) with thionylchloride, and a subsequent *in situ* reaction with *N*-bromosuccinimide (NBS; 1-bromopyrrolidine-2,5-dion) readily afforded **4**. This one-pot synthesis is straightforward and the yields are acceptable for a variety of primary and secondary acid chlorides and diacid chlorides. The amidation of **4** was carried out with large excess of the respective *N*-methylamino acids **5a**–**c** and gave the non-cyclic products **6a**–**c**. In principle, three general approaches might be envisioned for the cyclization of the linear intermediate products [14]. In our case we converted the 2-(2-bromo-3-methylbutanoyl(methyl)amino) acids **6a**–**c** into K^+ salts **7a**–**c** and after acidification and extraction procedures at room temperature, we isolated the corresponding 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones **1a**–**c**. Compounds **1a**–**c** were characterized by FTIR, NMR and MS spectra. Retention times in GC and mass spectra of synthesized and fungus-produced compounds are identical.

The most probable structures of **1a**–**c** were further characterized by computational tools [25–27]. Evaluation of the molecular structure and conformational isomerism of **1a**–**c** was done by DFT methods. The conformational flexibility of the heterocycle and the presence of two stereogenic centers (C_3 and C_6) give rise to a wide number of possible structures for **1a**–**c**. Additionally, compound **1c** contains a third chiralic center in the 3-alkyl substituent, which increases further the number of the possible structures. In order to determine the preferred geometry we constructed the most probable diastereomers of **1a**–**c** and then optimized their geometries at the B3LYP/6-311G** level of theory. For **1a** and **1b** molecule, 16 diastereomers were considered for optimization, taking into account all relevant combinations of boat and chair cycle conformations and axial and equatorial substituents positions. As a result, we found that in all cases the morpholine-2,5-dione ring adopts boat conformation and thus the number of the possible isomers was reduced to 8. The eight different minima found are doubly degenerated by symmetry states and correspond to four enantiomeric pairs of molecules. In the case of **1c** the analysis followed the same reasoning with larger number of diastereoisomer included in order to account for the third stereogenic center.

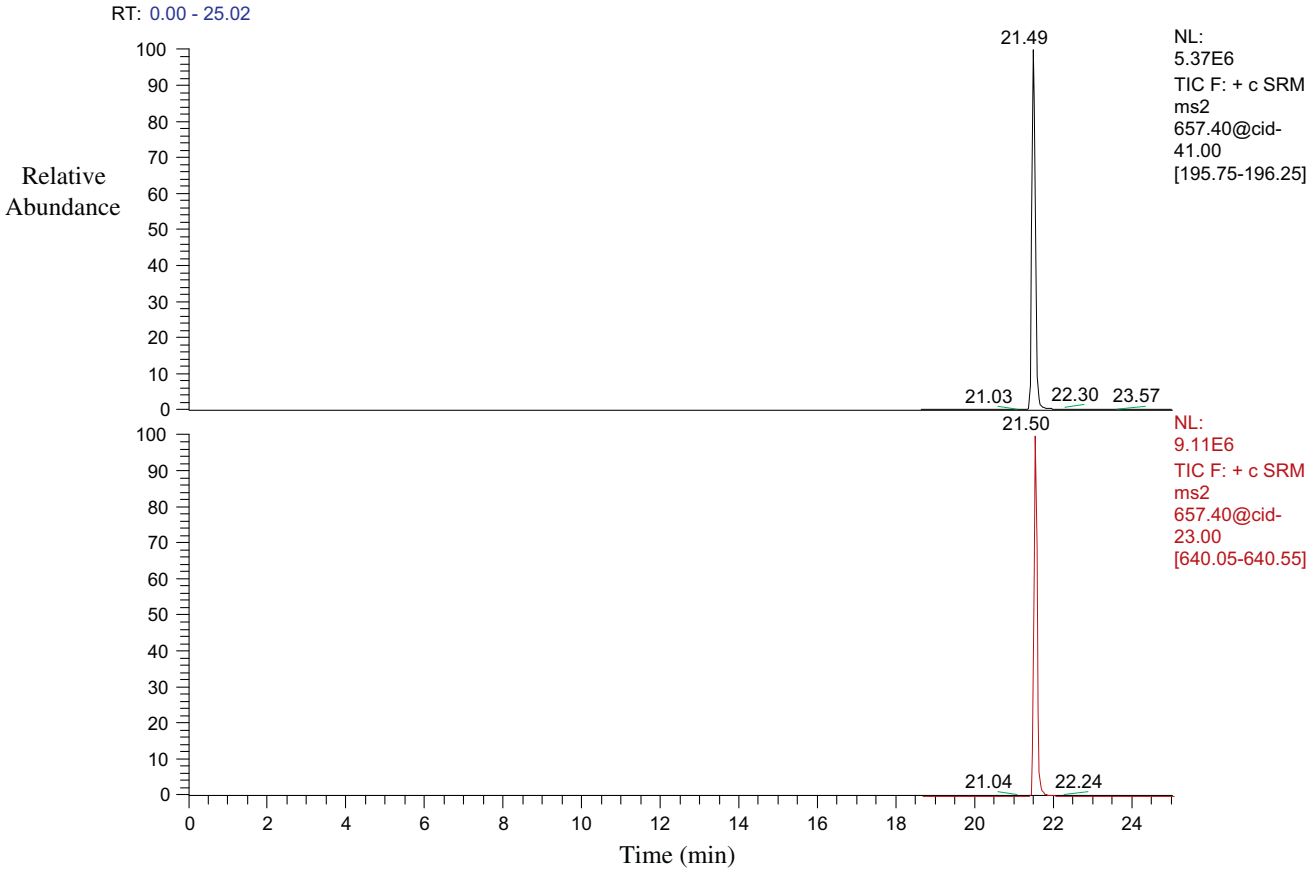
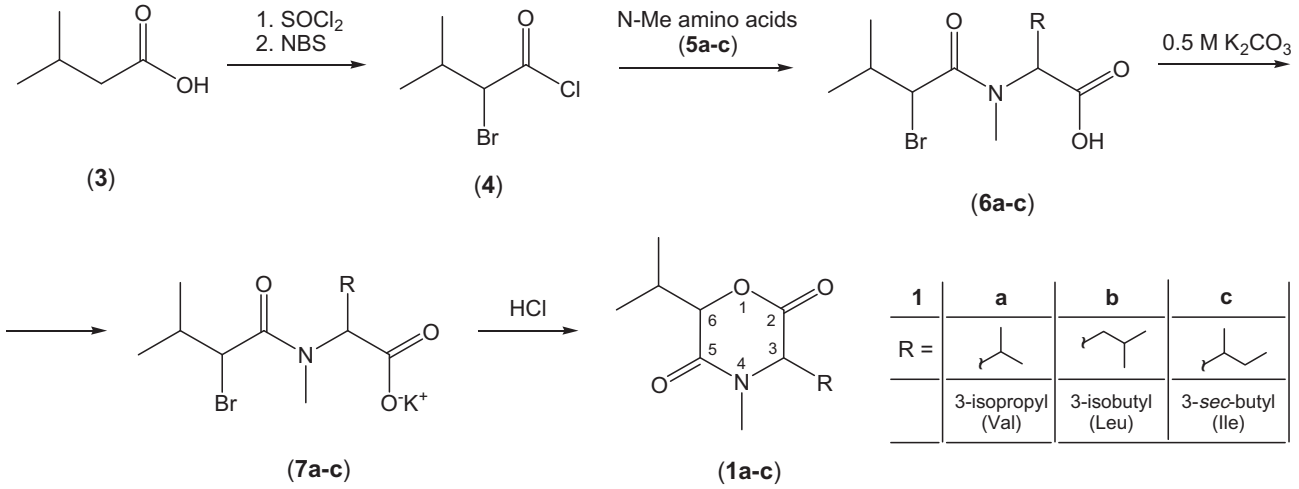


Fig. 1. HPLC–ESI(pos)-MS/MS chromatogram of enniatin B (*m/z* 657, $[M + NH_4]^+$) and its corresponding product ions (*m/z* 196/CID 41 eV and *m/z* 640/CID 23 eV).



Scheme 2. Synthesis of 6-(propan-2-yl)-4-methyl-morpholine-2,5-diones **1a**–**c**.

For illustration of the relative stability of the diastereomers studied, we focused our attention only on the molecules with (*3S*) configuration as inherent for the naturally occurring amino acids. The optimized energies (E_{corr}), vibrational zero-point energies (VZPE), and relative energies (E_{rel}) with respect to the most stable forms are summarized in Table 1. The two different boat conformations are denoted "*a*" and "*b*". Each of the diastereoisomers of **1c** included in Table 1 corresponds to an enantiomeric pair.

As it can be seen from the values listed in Table 1, the stability of the diastereomers decreases in the following order: *a*(*3S,6R*) > *a*(*3S,6S*) > *b*(*3S,6S*) > *b*(*3S,6R*). The energy difference between the most stable and the least stable diastereoisomers of **1a** and **1c** falls within the range 30.1–32.9 kJ mol^{-1}. For **1b** this value is much lower (12.8 kJ mol^{-1}) due to the smaller sterical hindrance between the isobutyl group and the morpholine-2,5-dione ring in its isomers. According to the calculations, the most stable molecular structures of **1a–c** are those with equatorial isopropyl group and axial 3-alkyl substituent (Fig. 2). These conformations reflect the stereostructure expected according to the possible mechanism for the biosynthesis of **1a–c**, i.e. the cycles are formed by D-2-hy-

Table 1
Calculated total energies E_{corr} and relative energies E_{rel} of (3*S*) diastereoisomers of **1a–c**.

Entry	E_{corr}[a] (a.u.)	VZPE (kJ mol^{-1})	E_{rel} (kJ mol^{-1})
1a			
a(3*S*,6*R*)	−710.962654	769.515	0.00
a(3*S*,6*S*)	−710.960431	769.862	5.836
b(3*S*,6*S*)	−710.954504	769.589	21.397
b(3*S*,6*R*)	−710.951171	770.613	30.148
1b			
a(3*S*,6*R*)	−750.259985	843.378	0.00
a(3*S*,6*S*)	−750.259249	843.596	1.932
b(3*S*,6*S*)	−750.255984	844.400	10.504
b(3*S*,6*R*)	−750.255080	844.426	12.878
1c			
a(3*S*,6*R*)[b]	−750.256677	844.198	0.0
a(3*S*,6*S*)[b]	−750.254642	843.904	5.342
b(3*S*,6*S*)[b]	−750.247871	844.746	23.120
b(3*S*,6*R*)[b]	−750.244145	844.830	32.902

[a] Corrected with the zero-point vibrational energy (ZPVE).
[b] Corresponds to an enantiomeric pair of diastereoisomers.

droxy-3-methylbutanoic acid and L-*N*-methylamino acid residues. In molecules **1a** and **1c** the sp^3 C-atoms of the heterocycle (C_3 and C_6) are displaced by 33° and 40° out of the plane formed by atoms O_1, C_2, N_4 and C_5. The boat in **1b** is slightly more flat and the corresponding values are 29° and 38° respectively. The experimentally determined X-ray structure of the *b*(3*R*,6*R*) diastereoisomer of **1a** [28] shows an envelope conformation much closer to flatness – C_2, C_3, N_4, C_5 and C_6 lying in one plane and only O_1 being displaced by 18° out of the plane formed by the other atoms. However, this should be regarded rather as an exception since all other X-ray studies on related morpholine-2,5-diones [10,29–32] report boat conformations similar to those found in our molecular modelling study.

The vibrational spectra of **1a–c** closely resemble each other due to the structural similarity of the compounds. The most characteristic IR region (3500–900 cm^{-1}) is presented in Fig. 3 with the experimental IR spectrum of **1a**. The most prominent feature of the IR spectra of **1a–c** is the appearance of two very strong bands of equal intensity between 1740 and 1620 cm^{-1}. They correspond to the stretching vibrations of the carbonyl groups in the morpholine-2,5-dione ring.

The higher frequency band, at 1738 cm^{-1} in solid state, is assigned to the ester ν(C=O). It has the typical value for an aliphatic ester carbonyl group, but somewhat lower than the data reported for *N*-methyl cyclodidepsipeptides, containing valine and phenylalanine residues [10,11,33]. The second band in this region, at 1622 cm^{-1}, is attributed to the stretching vibration of the amide group. The band is also downshifted in comparison to the frequencies reported for similar *N*-substituted and unsubstituted cyclodidepsipeptides [11,13,33,34]. The literature IR data are based only on a few compounds of this type and suggest a broad frequency range

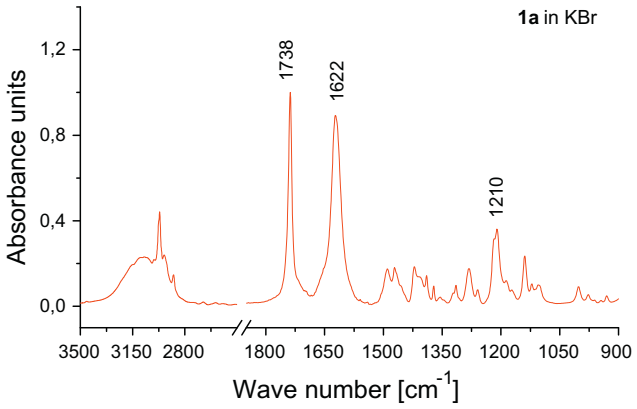


Fig. 3. Experimental IR spectrum of **1a** in solid state.

for both carbonyl stretching vibrations depending on the substitution and the stereostructure of the compounds [11]. From that perspective, and given the absence of strong electrostatic interactions in solid state, the registered band shift for **1a–c** (Fig. 3) provides further support that the position of the two bands is very sensitive to substitution at 3-, 4- and 6-position and the cycle conformation. In **1a–c** only alkyl substituents are present and their *N*-methyl group prevents hydrogen bonding. The stretching and deformation C–H vibrations of the aliphatic part are not affected by the formation of the heterocycle and appear in their usual ranges. The characteristic ν(C_2–O) and ν(N–CH_3) vibrations give rise to intensive and strongly overlapped bands at 1217 and 1210 cm^{-1} in KBr.

In conclusion, **1a–c** were found for the first time in the natural products. For identification and confirmation, the compounds were synthesized through *N*-(α-bromoacyl)-α-amino acids as non-cyclic intermediate products. Biosynthetic ways of **1a–c** are probably similar with those of enniatin B, which was also identified in the broth and mycelium. The stereostructure of **1a–c**, expected according to the assumed biosynthetic mechanism, was characterized by quantum chemical calculations. Based on them, the preferred conformations of **1a–c** are those with equatorial isopropyl group and axial 3-alkyl substituent. The role of **1a–c** for the producing organism is questionable as well as for enniatins and other mycotoxins.

3. Experimental

3.1. Plant material

H. barbatum Jacq. was collected at bloom stage in August 2005 on Rtanj mountain (Southeast Serbia). Plant material was identified by Prof. Dr. N. Randjelovic (Faculty of Occupation Safety, Nis, Serbia) and a voucher specimen was deposited in the Herbarium Moesicum Doljevac (Serbia) under the accession number 735.

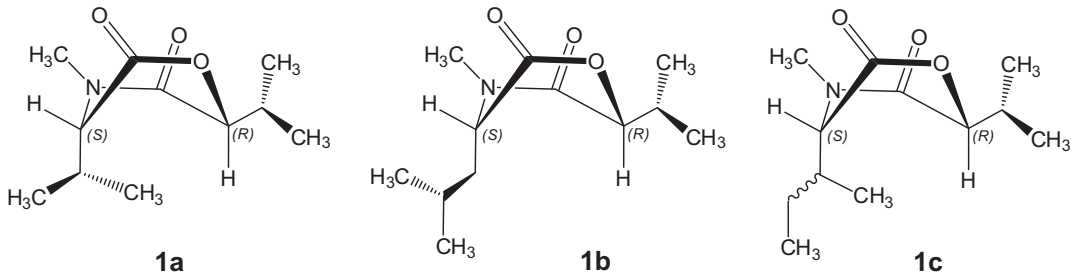


Fig. 2. Lowest energy (3*S*) diastereoisomers of **1a–c**.

3.2. Isolation, identification and fermentation of the fungi

The isolation of the endophytic fungi was carried out following the method of Strobel et al. [15]. Briefly, in the laboratory, fresh plant materials were thoroughly surface treated with 70% ethanol. The stems were used for the isolation of endophytic fungi. Then, with a sterile knife blade, outer tissues were removed from the samples and the inner tissues carefully excised and placed on the water agar plates (WA; DIFCO, Cat. No. 214530). After 8 days of incubation at 28 ± 2 °C hyphal tips of the fungi were removed and transported to potato dextrose agar (PDA; DIFCO, Cat. No. 213400). The pure cultures, thus obtained, were preserved by cryopreservation at 70 °C. Fungus used in the present study was identified as *F. sporotrichioides* Sherb. [W&R,G,B,J] according to the protocol of Nelson et al. [35]. Shake flask fermentation was carried out for a period of 5 days at 28 °C. After fermentation mycelium and broth were separated by centrifugation.

3.3. GC–MS analysis

GC–MS analyses of the broth and mycelium ethyl acetate extracts were performed on a Thermo Finnigan Trace Gas Chromatograph and Trace MS^{PLUS} detector, equipped with a fused silica column (DB-5 30 m × 0.25 mm × 0.25 μm); carrier gas helium (1 ml/min). The operating conditions were: temperature program, 60–320 °C at 10 °C/min and 320 °C (4 min); injector temperature, 310 °C; detector temperature, 320 °C. Ionization was performed at 70 eV.

3.4. Mycotoxin analysis

The analysis of mycotoxins from ethyl acetate extracts of the broth and mycelium was performed using a LC–MS/MS multimethod. The detailed procedure has been reported earlier [18,19] and is briefly described in this work. A Thermo-Finnigan Surveyor HPLC system was interfaced to the mass spectrometer for automated LC–MS and LC–MS/MS analyses. The Surveyor HPLC system included a quaternary, low-pressure mixing pump with vacuum degassing, an autosampler with temperature-controlled tray (10 °C), a column oven (25 °C). A C18(2) Luna Phenomenex column (150 mm × 2 mm × 3 μm) attached to a Phenomenex C18 Guard column held at 25 °C was used with a solvent flow rate of 0.2 ml/min and an injection volume of 10 μl. The mobile phase for ESI-pos consisted of eluent A containing methanol/water/acetic acid (97:2:1 v/v/v), and eluent C consisting of methanol/water/acetic acid (10:89:1 v/v/v) containing 10 mM ammonium acetate. The chromatographic conditions were as follows: 0–2 min, 10% A; 2–5 min, 10–60% A; 5–20 min, 60–100% A; followed by a hold time of 8 min (20–28 min, 100% A). Finally, column re-equilibration was carried out at 10% A for 5 min. Mobile phase for ESI-neg consisted of eluent A containing methanol/water/acetic acid (97:2:1 v/v/v), and eluent B composed of water/acetic acid (99:1 v/v). The solvent gradient was as follows: 0–1.5 min, 5% A; 1.5–5.5 min, 5–70% A; 5.5–13 min, 70–100% A; The final hold time was set at 10 min (13–23 min 100% A) and column re-equilibration was carried out at 5% A for 5 min. Identification and quantification, in which ammonium adduct of enniatin B $[M+NH_4]^+$ (*m*/*z* 657) was fragmented in the collision cell to the product ions (*m*/*z* 196 at 41 eV as quantifier ion and *m*/*z* 640 at 23 eV as qualifier ion) were performed.

3.5. Synthesis

2-Bromo-3-methylbutanoyl chloride (**4**): 21.8 ml (35.7 g; 0.3 mol) of thionyl chloride were added dropwise to a stirred solution of 11 ml (10.2 g; 0.1 mol) isovaleric acid in 20 ml tetrachloromethane. The reaction mixture was heated for 40 min at 80 °C, then cooled down and diluted with 15 ml of tetrachloromethane. 23.9 g (0.12 mol) of *N*-bromosuccinimide and 15 drops of 40% aqueous solution of HBr were applied. After 10 min of heating at 70 °C, and 90 min at 85 °C, the solvent and the excess of $SOCl_2$ were removed under reduced pressure. The residue was suction filtered, the solid was washed several times with tetrachloromethane and the solvent was removed from the combined filtrate as before. The obtained 2-bromo-3-methylbutanoyl chloride was used in the next step of the reaction without further purification.

(**4**): GC–MS (4 min), *m*/*z*: 201, 199, 197 (M^+); IR (CCl_4, 0.61 mm CaF_2 cell), cm^{-1}: 2972, 2939, 2871, 1794, 1559, 1464, 1391, 1373, 1225, 1191, 1127; ^1H NMR (250 MHz; $CDCl_3$): 1.92 s, 6 H ($2CH_3$); 2.26–2.36 m, 1 H (CH); 4.30–4.33 d, 1 H, *J* = 7.2 (CH); ^{13}C NMR (250 MHz; $CDCl_3$): 19.9, 20.1 (2 CH_3), 30.6 (CH), 62.3 (CH), 173.5 (CO).

6-(Propan-2-yl)-4-methyl-morpholine-2,5-diones: 0.006 mol of *N*-methylamino acid was suspended in 15 ml dry chloroform cooled in ice, and 0.003 mol of 2-bromoisovaleryl chloride was added. The mixture was stirred for 1 h, and then the temperature was allowed to rise to 20 °C. The chloroform was removed under reduced pressure, the residue was dissolved in 20 ml ether and the solution was filtered to remove the solid. The filtrate was extracted with 30 ml of 0.5 M aqueous solution of potassium carbonate (three portions of 10 ml) and washed with water (10 ml). The combined alkaline solution was immediately acidified with HCl (pH ∼ 2) and twice re-extracted with 30 ml ether (three portions of 10 ml). The combined ether layers from the re-extraction were dried over sodium sulfate and the solvent was removed under reduced pressure. The evaporation yielded small amount of light yellow oil which crystallized on being kept. The residual crude material was purified by column chromatography (*d* = 1 cm; *l* = 7 cm) on silica gel eluting with mixture of cyclohexane and ethyl acetate (1:1) to afford the pure title compound. After recrystallization in mixture of diethyl ether and petroleum ether colourless crystals as needles were obtained;

3,6-Di(propan-2-yl)-4-methyl-morpholine-2,5-dione (**1a**): $C_{11}H_{19}NO_3$, M = 213.27; yield = 35%; RI (retention index) 1558; MS [60–500 *m*/*z*]: 213 (M^+), 171, 142, 100, 83, 71, 69; IR (KBr), cm^{-1}: 2969, 2938, 2876, 1737, 1622, 1489, 1471, 1420, 1389, 1371, 1355, 1314, 1281, 1259, 1209, 1139, 1121, 1105, 1001, 977, 930, 861, 847, 770, 752, 661, 636, 548; ^1H NMR (600 MHz; $CDCl_3$): 0.91–0.94 m, 3 H (CH_3); 0.97–1.01 m, 3 H (CH_3); 1.07–1.12 m, 3 H (CH_3); 1.17–1.19 dd, 3 H, *J* = 3.5, 3.6 (CH_3); 2.09–2.11 t, 1 H, *J* = 5.1, 4.8 (CH); 2.39–2.42 m, 1 H (CH); 3.10 s, 3 H (N–CH_3); 4.17–4.18 d, 1 H, *J* = 9.5 (CH); 4.85–4.86 d, 1 H, *J* = 10.4 (CH); ^{13}C NMR (600 MHz; $CDCl_3$): 18.8, 19.1, 20.1, 21.1 (4 CH_3), 27.9 (CH), 29.7 (CH), 31.9 (N–CH_3), 64.4 (CH), 70.0 (CH), 170.5 (COO), 171.4 (CON).

3-(2-Methylpropyl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione (**1b**): $C_{12}H_{21}NO_3$; M = 227.30; yield = 32%; RI 1631; MS [60–500 *m*/*z*]: 227 (M^+), 185, 170, 142, 129, 112, 100, 83, 69; IR (film), cm^{-1}: 2964, 2934, 2874, 1730, 1645, 1472, 1410, 1388, 1370, 1323, 1258, 1187, 1135, 1097, 1058, 928, 851, 651; ^1H NMR (600 MHz; $CDCl_3$): 0.90–0.99 m, 6 H ($2CH_3$); 1.06–1.17 m, 6 H ($2CH_3$); 1.79–1.80 m, 1 H (CH); 2.08–2.10 m, 2 H (CH_2); 2.36–2.42 m, 1 H (CH); 3.03 s, 3H (N–CH_3); 4.18–4.20 d, 1 H, *J* = 9.6 (CH); 5.36–5.39 dd, 1 H, *J* = 7.1, 6.7 (CH); ^{13}C NMR (600 MHz; $CDCl_3$): 19.7, 20.1, 20.9, 21.3 ($4CH_3$), 23.5 (CH), 24.8 (CH), 31.8 (N–CH_3), 36.8 (CH_2), 51.0 (CH), 69.5 (CH), 170.1 (COO), 176.2 (CON).

3-(Butan-2-yl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione (**1c**): $C_{12}H_{21}NO_3$; M = 227.30; yield = 36%; RI 1645; MS [60–500 *m*/*z*]: 227 (M^+), 185, 171, 156, 142, 129, 126, 100, 88, 83, 71, 69; IR (film), cm^{-1}: 2969, 2935, 2877, 1733, 1650, 1464, 1436, 1418, 1387, 1258, 1220, 1160, 1104, 1064, 960, 930, 899, 851, 776, 669.

3.6. Computational details

The molecular structure and vibrational spectra of the compounds were studied by theoretical and computational methods. All density functional theory (DFT) computations were performed with the Gaussian 98 program package [25]. The geometries of the most probable conformational isomers were fully optimized using DFT. The DFT methodology employed in the present study was based on the application of Becke's three-parameter adiabatic connection exchange functional (B3) [26] in combination with the Lee–Yang–Parr correlation functional (LYP) [27] – BLYP. Subsequent vibrational analysis together with the eigenvalues of the Hessian matrix served as test for the character of the stationary point found on the potential energy hypersurface, and showed, that it corresponds to a real minimum.

3.7. IR spectra measurements

The IR spectra were measured on a Bruker Tensor 27 FTIR spectrometer. In all cases the spectra were recorded at a resolution of 2 cm^{-1} (64 scans).

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