# Two New Fusidilactones from the Fungal Endophyte *Fusidium* sp.[‡]

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The new bicyclic fusidilactones D (**1**) and E (**2**), along with the known and related fusidilactone B (**3**), have been isolated from the fungal endophyte *Fusidium* sp. The structures of the new compounds were elucidated by detailed spectroscopic analysis and the relative configurations were confirmed by an X-ray diffraction study of a single crystal of **3**. The abso-

lute configurations were determined by TDDFT calculations of CD spectra using the solid-state CD/TDDFT approach. The metabolites exhibited moderate antifungal, antibacterial, and antialgal activities.

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## Introduction

Fungal endophytes are widespread in nature and attract considerable attention as prolific sources of new natural products with diverse structures and biological properties, including antibacterial, antifungal, anti-inflammatory, and especially antitumor activities.[2–7] This is probably due to their metabolic interactions with their environments.[5,8] As part of our ongoing screening for biologically active secondary metabolites from fungi,[1] we have reinvestigated the metabolites produced by the endophytic *Fusidium* sp., isolated from the leaves of *Mentha arvensis* growing in a meadow near Hahausen, Lower Saxony, Germany. Our previous chemical research on the extracts of this fungus resulted in the isolation of a new group of polycyclic lactones,[9] the fusidilactones B (**3**), A (**4**), and C (**5**) (Figure 1), the last possessing an unusual oxoadamantane skeleton. The fungus was cultivated on the biomalt semi-solid agar medium. The crude diethyl acetate extracts showed good antifungal activity against *Eurotium repens* and *Fusarium oxysporum*, weak antibacterial activity against *Escherichia coli* and *Bacillus megaterium*, and antialgal activity against

*Chlorella fusca*.[9] In the reinvestigation of a second fermentation, two new fusidilactones D (**1**) and E (**2**), together with the known fusidilactone B (**3**), were obtained from the crude diethyl acetate extract (Figure 1). Herein we report the isolation and structural elucidation (including the relative and absolute configurations) of these new fusidilactones. In particular, the stereochemistries of **1** and **2** were deduced by analogy with the parent fusidilactone B (**3**), the configuration of which has been established in this work for the first time by analysis of its circular dichroism (CD) spectrum by the solid-state CD/TDDFT (time-dependent density functional theory) approach.
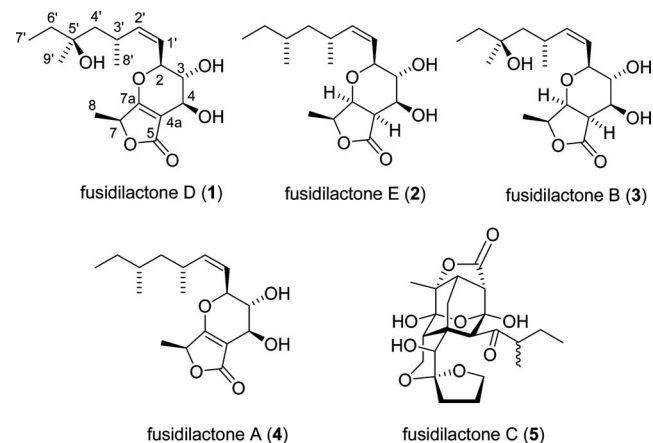


Figure 1. Structures of fusidilactones D (**1**), E (**2**), B (**3**), A (**4**), and C (**5**).

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## Results and Discussion

The fungus *Fusidium* sp., which had been cultivated on the biomalt agar medium for 4 weeks, was extracted with

Table 1. $^1H$ and $^{13}C$ NMR spectroscopic data for compounds **1** and **2** and $^{13}C$ NMR spectroscopic data for **3**.[a]

| Pos. | 1 | | 2 | | 3 |
|---|---|---|---|---|---|
| | $\delta(H)$ | $\delta(C)$ | $\delta(H)$ | $\delta(C)$ | $\delta(C)$ |
| 2 | 5.09 (m) | 79.2 (d) | 3.89 (t, 9.0) | 74.4 (d) | 75.1 (d) |
| 3 | 3.83 (dt, 7.2, 5.5) | 71.9 (d) | 3.33 (t, 9.1) | 73.7 (d) | 74.6 (d) |
| 4 | 4.56 (d, 5.5) | 65.2 (d) | 3.90 (dd, 6.7, 9.1) | 71.6 (d) | 72.3 (d) |
| 4a | | 100.8 (s) | 3.16 (dd, 6.7, 2.6) | 46.4 (d) | 47.7 (d) |
| 5 | | 177.1 (s) | | 176.8 (s) | 177.9 (s) |
| 7 | 4.84 (d, 6.7) | 74.0 (d) | 4.50 (dq, 2.6, 6.6) | 79.4 (d) | 79.6 (d) |
| 7a | | 171.2 (s) | 4.16 (t, 2.8) | 75.6 (d) | 76.8 (d) |
| 8 | 1.46 (d, 6.8) | 17.2 (q) | 1.43 (d, 6.7) | 13.3 (q) | 13.8 (q) |
| 1′ | 5.53 (t, 10.5) | 120.8 (d) | 5.30 (dd, 9.0, 11.1) | 123.9 (d) | 125.3 (d) |
| 2′ | 5.81 (dt, 11.5, 11.1) | 146.5 (d) | 5.47 (t, 11.1) | 143.5 (d) | 144.1 (d) |
| 3′ | 2.82 (m) | 28.9 (d) | 2.56 (m) | 30.5 (d) | 29.3 (d) |
| 4′a | 1.57 (m) | 48.3 (t) | 1.26 (m) | 44.4 (t) | 49.4 (t) |
| 4′b | 1.53 (m) | | 1.08 (m) | | |
| 5′ | | 73.2 (s) | 1.27 (m) | 30.7 (d) | 72.7 (d) |
| 6′a | 1.49 (m) | 35.3 (t) | 1.26 (m) | 31.9 (t) | 35.1 (t) |
| 6′b | 1.49 (m) | | 1.26 (m) | | |
| 7′ | 0.88 (t, 6.9) | 8.1 (q) | 0.83 (t, 6.9) | 11.3 (q) | 8.5 (q) |
| 8′ | 1.05 (d, 6.7) | 23.0 (q) | 0.98 (d, 6.6) | 21.8 (q) | 23.5 (q) |
| 9′ | 1.16 (s) | 26.6 (q) | 0.80 (d, 6.3) | 19.0 (q) | 27.8 (q) |

[a] Assignments made by DEPT, $^1H$–$^1H$ COSY, HMQC, HMBC, and ROESY experiments. Multiplicity and coupling constants are given in parentheses.

ethyl acetate. The crude extract was fractionated on silica gel and then subjected to Sephadex LH-20 column chromatography to yield a crude mixture of fusidilactone B (**3**), D (**1**), and E (**2**), which was further purified by preparative TLC.

Compound **1** was isolated as an amorphous powder and the pseudomolecular ion peak was observed at $m/z = 327.2$ [M + H]$^+$ in its EIMS. Its molecular formula, $C_{17}H_{26}O_6$, established by HREIMS [$m/z = 327.1795$, [M + H]$^+$, $\Delta = +6.6$ mmu] in conjunction with NMR spectroscopy (Table 1), indicated five degrees of unsaturation. In the $^{13}C$ NMR spectrum (Table 1), signals for four primary, two secondary, one tertiary, four oxygenated tertiary, two sp$^2$-hybridized tertiary, three sp$^2$-hybridized quaternary, and one oxygenated quaternary carbon atoms were detected. In the $^1H$ NMR spectrum, signals for two olefinic, four heterosubstituted, and 17 aliphatic protons were seen. In addition to a broad band at $\tilde{\nu}_{max} = 3356$ cm$^{-1}$ corresponding to hydroxy groups, typical bands at $\tilde{\nu}_{max} = 1734$ and $1667$ cm$^{-1}$ for $\gamma$-lactones and at $\tilde{\nu}_{max} = 1061$ cm$^{-1}$ for alkyl ethers were observed in the IR spectrum in agreement with signals at $\delta = 177.1$ ppm for an ester carbonyl and at $\delta = 65$–$80$ ppm for oxygen substitution in the $^{13}C$ NMR spectrum. Thus, the molecular skeleton of compound **1** must comprise two rings on the basis of the subtraction of three double bond equivalents for one carbonyl group and two double bonds. The $Z$ configuration of the double bond can be deduced from the coupling constant of 10.5 Hz between the two olefinic protons at $\delta = 5.53$ and $5.81$ ppm.

Interpretation of the $^1H$–$^1H$ COSY, HMQC, and HMBC data (Figure 2) suggest that **1** is a compound with the fusidilactone B-like skeleton. Observation of a series of diagnostic HMBC correlations from H–C(1′) [$\delta(H) = 5.53$ ppm, t, $J = 10.5$ Hz] to C(3) [$\delta(C) = 71.9$ ppm, d], from H–C(4) [$\delta(H) = 4.56$ ppm, d, $J = 5.5$ Hz] to C(5) [$\delta(C) = 177.1$ ppm,

d], and from H$_3$–C(7′) [$\delta(H) = 8.1$ ppm, t, $J = 6.9$ Hz] to C(5′) [$\delta(C) = 73.2$ ppm, s] permitted the assignment of hydroxy groups at C(3), C(4), and C(5′), respectively.
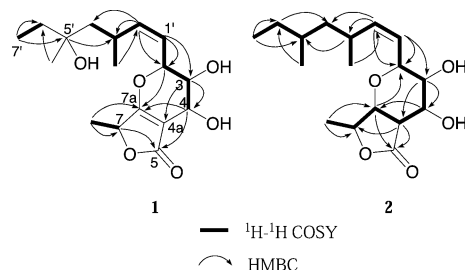


Figure 2. Key HMBC and $^1H$–$^1H$ COSY correlations of fusidilactone D (**1**) and E (**2**).

The NMR spectroscopic data reported above are strongly reminiscent of the co-occurring bicyclic lactone, fusidilactone B (**3**). A comparison of the overall $^1H$ and $^{13}C$ NMR spectroscopic data (Table 1) revealed that **1** differs from **3** only by the double bond at C(4a) and C(7a) in **1**, in agreement with the molecular weight difference of 2 mass units observed between **1** and **3**. Thus, the planar structure of **1** was established and the compound named fusidilactone D (**1**).

In the previous research,[9] the relative configuration of **3** was tentatively determined by analysis of coupling constants and NOE difference spectra. In this study, we obtained prismatic crystals of **3** from methanol that were suitable for single-crystal X-ray analysis (Figure 3). The structure confirmed the previously assigned relative configuration of the stereocenters of the cycles. In addition, the relative configuration of the stereogenic centers in the sidechain was now also available and the complete relative configuration of the molecule was assigned to

$2R*,3S*,4R*,7R*,3'S*,5'R*$ (Figure 3). The same relative configuration has recently been established for a synthetic compound with the ring system of fusidilactone B.[10] Considering the close biogenetic relationship between **1** and **3** and the resemblance of their NMR spectra, the relative $2R*,3'S*,5'R*$ configuration of **3**, unambiguously established now by X-ray analysis, is also assumed for **1**, as shown in Figure 1.
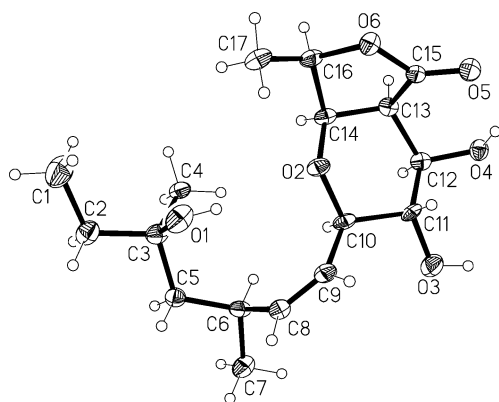


Figure 3. X-ray crystal structure of **3** (ORTEP drawing showing ellipsoids at the 50% probability level).

Fusidilactone E (**2**) was obtained as colorless optically active needles with $[a]_D^{25} = +1.2$ ($c = 1.3$ mg/mL, CHCl$_3$). The molecular formula of **2** was determined to be C$_{17}$H$_{28}$O$_5$ from the HREIMS data with the molecular ion at $m/z = 312.2146$ ([M + H]$^+$), which indicates four degrees of unsaturation. Its NMR spectroscopic data (Table 1) are similar to those of **1** and **3**, which suggests that **2** is also a member of the fusidilactone family. A careful analysis of the 2D NMR data (Figure 2) and comparison with that of **3** revealed that both **2** and **3** share the same partial structure of bicyclic rings and differ from each other at C(5′) in the side-chain; in **2** the oxygenated quaternary carbon atom was hydrogenated. As suggested above, the relative configuration of **2** should be the same as that established for **1** and **3**. Accordingly, the structure of **2** was determined to be ($2R*,3S*,4R*,4aS*,7R*,7aR*,3'S*,5'S*,Z$)-2-(3,5-dimethylhept-1-enyl)-3,4-dihydroxy-7-methyl-4,4a,7,7a-tetrahydro-2H-furo[3,4-b]pyran-5(3H)-one, and named fusidilactone E.

The absolute stereochemistries of the natural fusidilactones have, thus far, remained unknown. In this work we determined the absolute configuration of fusidilactone B (**3**) from an analysis of its CD spectra by using the solid-state CD/TDDFT method.[11–20] The solution CD spectrum of **3** (Figure 4) showed a weak negative CD band between 250 and 195 nm, conceivably allied with the lactone chromophore and hence determined by the chirality of the γ-lactone ring.[21,22] The flexibility of the side-chain would render calculation[23,24] of the solution CD with the TDDFT method[25,26] impracticable due to the large number of low-energy conformers populated at room temperature. A conformational search with MMFF revealed the presence of nine minima with a Boltzmann population >1% at 300 K and six further minima with populations

>0.5%. The problem of a more detailed conformational analysis and of costly calculations was avoided by using the X-ray structure as an input for TDDFT calculation of the CD spectrum, which was subsequently compared with the microcrystalline solid-state experimental one.
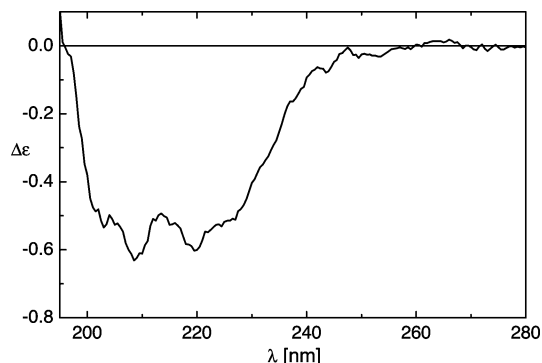


Figure 4. CD spectrum of fusidilactone B (**3**) in acetonitrile.

Similar to the CD spectrum in solution, the solid-state CD spectrum (Figure 5) has a negative band below 240 nm. However, the solid-state CD spectra recorded by rotating the KCl disc around the z axis (direction of light) by 0, 90, 180, and 360° showed periodic changes; the 0° rotation was nearly identical to the 180° rotation, whereas the 90° rotation spectrum was drifted and coincident with the 270° rotation spectrum (Figure 5). This change in the CD spectrum with rotation indicated that macroscopic anisotropies contributed to the CD spectrum, namely, the contribution of linear birefringence (LB) was not negligible.[27] The drift of the rotated spectra could not be eliminated by thorough grinding and homogenization of KCl with the sample and repeated experiments showed similar rotation dependence. In spite of the apparent macroscopic anisotropy contribution, the solid-state CD spectra consistently had a negative band below 240 nm, as was also observed in the solution CD spectrum.

According to the chirality rule for γ-lactones,[21,22] the first CD band (at lower energies) that can be assigned to a n–π* transition has a negative sign for positive ring chirality of the lactone ring [i.e., a positive O–C(O)–Cα–Cβ dihedral]. In the X-ray structure, the lactone ring lies in a Cβ envelope conformation with an oxygen atom O(1) attached to the β-carbon C(7a) occupying an axial position (Figure 5). A positive value for the dihedral $\omega_{O6-C5-C4a-C7a}$ (+21.3°) would be obtained for a 4aR,7S,7aS configuration of stereogenic centers on the lactone ring. In fact, TDDFT calculations on the solid-state geometry of **3** [after reoptimization of the hydrogen atoms at the B3LYP/6-31G(d) level of theory] with a 2S,3R,4S,4aR,7S,7aS,3'R,5'S absolute configuration led to a first negative CD band regardless of the functional and basis set employed (Figure 6). In all cases, such a band is dominated by the lactone n–π* transition, computed at 219 nm with B3LYP/TZVP, and the alkene π–π* transition (203 nm), both of which are associated with a negative rotational strength. Comparison of the experimental CD spectra with TDDFT-calculated ones and consider-
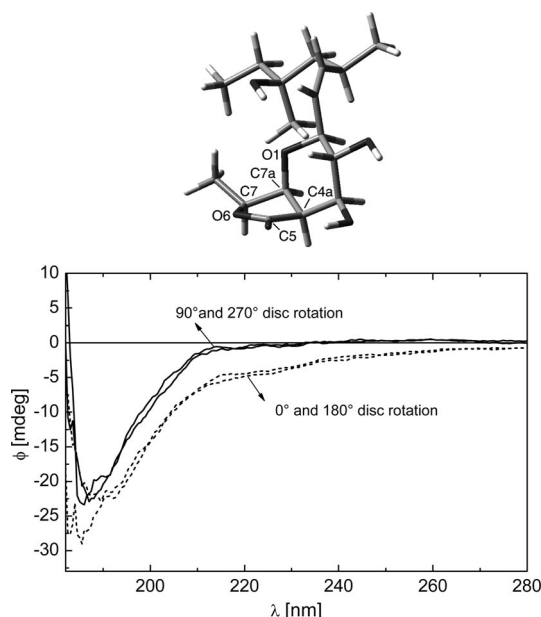
Figure 5. Bottom: solid-state CD spectra of fusidilactone B (**3**) obtained by rotation of the KCl disc around the *z* axis (direction of light). Top: lactone ring conformation found in the X-ray structure of **3** assuming 4a*R*,7*S*,7a*S* absolute configuration.

ation of the lactone chirality rule allowed us to assign the absolute structure of fusidilactone B as (2*S*,3*R*,4*S*,4a*R*, 7*S*,7a*S*,3′*R*,5′*S*)-**3**. It is important to stress that although the CD spectrum of **3** is weak and not very structured, TDDFT CD calculations of other compounds containing only a lactone chromophore have been successfully employed for absolute configurational assignments.[12,28] On the basis of biogenetic considerations, we may similarly assign the absolute configurations of fusidilactones D and E as (2*S*,3*R*,4*S*,7*S*,3′*R*,5′*S*)-**1** and (2*S*,3*R*,4*S*,4a*R*,7*S*,7a*S*,3′*R*,5′*R*)-**2**. The above configurations are in keeping with those of other natural products containing analogous ring systems.[10]
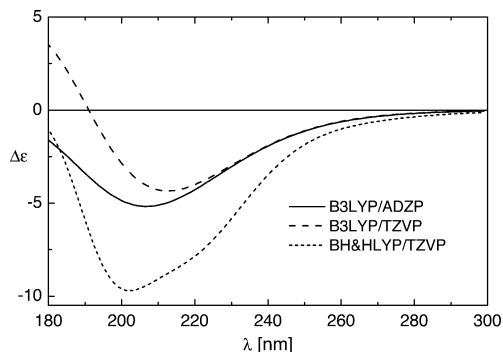


Figure 6. TDDFT-calculated CD spectra of (2*S*,3*R*,4*S*,4a*R*,7-*S*,7a*S*,3′*R*,5′*S*)-fusidilactone B (**3**) with various functionals and basis sets. The spectrum calculated with BH&HLYP is shifted by 50 nm to the right for better comparison.

## Bioactivity − Agar Diffusion Assay

The fusidilactones D and E were tested for algicidal, antibacterial, and antifungal activities (Table 2). Both metabolites had only moderate antibacterial, antifungal, and algicidal activities, as had previously been found for fusidilactone B.[29] The tested compounds were dissolved in acetone at a concentration of 1 mg/mL. Then 50 µL of the solution were pipetted onto a sterile filter disc (50 µg/disc), which was placed on an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism.[31] The test organisms were the Gram negative bacterium *Bacillus megaterium* and the Gram positive bacterium *Escherichia coli* (both on an NB medium), the fungus *Microbotryum violaceum*, and the alga *Chlorella fusca* (both on an MPY medium). The radius of the zone of inhibition was measured in mm commencing at the center of the disc.

Table 2. Biological activity of the pure compounds in an agar diffusion assay (n.t.: not tested; gi = growth inhibition).

| Compound (50 µg) | Algicidal Chl[a] | Antifungal Mb | Antibacterial | |
|---|---|---|---|---|
| | | | Bm | Ec |
| Fusidilactone D (**1**) | 6 gi | 7 gi | 10 gi | 0 |
| Fusidilactone E (**2**) | 11 gi | 10 gi | 7 gi | 6 gi |
| Penicillin | 0 | 0 | 18 | n.t. |
| Tetracycline | 10 gi | 0 | 18 | n.t. |
| Nystatin | 0 | 20 | 0 | n.t. |
| Actidione | 35 | 50 | 0 | n.t. |
| Acetone | 50 | 0 | 0 | n.t. |

[a] *Chlorella fusca* (Chl), *Microbotryum violaceum* (Mb), *Bacillus megaterium* (Bm) and *Escherichia coli* (Ec). The radius of the zone of inhibition was measured in mm from the center of the filter disc.

## Experimental Section

**General Experimental Procedures:** Commercial silica gel (Merck, 0.040–0.063 mm) was used for column chromatography. Precoated silica gel plates (Merck, G60 F-254 or G50 UV-254) were used for analytical and preparative thin-layer chromatography (TLC), respectively. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde (0.5 mL) in HOAc (50 mL) and $H_2SO_4$ (1 mL). TLC $R_f$ values are reported. The NMR spectra were recoded at 293 K with a Bruker Avance 500 (11.7 T) spectrometer. Chemical shifts are reported in parts per million (ppm, $\delta$ values) with use of the residual $CHCl_3$ signal ($\delta_H$ = 7.26 ppm) as an internal standard for ^{1}H NMR and $CDCl_3$ ($\delta_C$ = 77.0 ppm) for ^{13}C NMR; coupling constants (*J*) are given in Hz. ^{1}H and ^{13}C NMR assignments were supported by ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY experiments. The following abbreviations have been used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplet. Optical rotations were measured with a Perkin–Elmer 241 MC polarimeter at the sodium D line. Infrared spectra were recorded with a Nicolet-510P spectrometer; peaks are reported in cm^{−1}. UV absorption spectra were recorded with a UV-2101PC spectrophotometer; peak wavelengths are reported in nm. CD spectra were recorded with a J-810 spectropolarimeter, and the concentrations are given in mol/dm³. Solid-state CD spectra were measured on microcrystals of **3** dispersed in a glassy KCl matrix; for the experimental protocol, see ref.[12]

**Culture, Extraction, and Isolation:** The fungal endophyte, *Fusidium* sp. (internal strain 2072), was isolated following surface steriliza-

tion from the leaves of *Mentha arvensis* and was cultivated for 91 d on the biomalt (5% w/v; Villa Natura, Gesundprodukte GmbH, Kirn, Germany) semi-solid agar medium at room temperature or alternatively in a liquid biomalt culture (5% w/v; 120 rpm) for 11 d at 20 °C.[30,31] The cultures were homogenized in a Waring blender after dilution of the semi-solid agar culture (5 L) with crushed ice (2 kg). The resulting homogenates were extracted four times with ethyl acetate (0.5 L each) and the organic phase was dried (Na$_2$SO$_4$), filtered, and the solvent removed at reduced pressure to afford a crude extract (1100 mg). The crude material was purified by chromatography on a silica gel column using eluents of increasing polarity, from petroleum ester to acetone to MeOH. The fractions eluted with petroleum ether/acetone (8:2) were recrystallized from CH$_2$Cl$_2$/MeOH (1:1) and were then filtered to yield compound **2** (7.3 mg). The fractions eluted with petroleum ester/acetone (7:3) were further purified by a Sephadex LH-20 column chromatography using CHCl$_3$/MeOH (1:1) as eluent to afford compound **1** (6.7 mg) and **3** (12.7 mg).

**Fusidilactone D (1):** Amorphous powder. $[a]_D^{25}$ = +37.3 ($c$ = 2.2 mg/ mL, CHCl$_3$). UV (MeOH): $\lambda$ (log$\varepsilon$) = 280 (3.48) nm. IR (KBr): $\tilde{v}$ = 3356 (OH), 2962, 2923, 1734, 1667 (C=O), 1465, 1061 (*O*-alkyl) cm$^{-1}$. For $^1$H and $^{13}$C NMR spectroscopic data, see Table 1. MS (EI, 230 °C): $m/z$ (%) = 327.2 (10) [M + H]$^+$, 276.2 (7), 184.1 (70) [M + H – C$_5$H$_{11}$ – C$_3$H$_4$O$_2$]$^+$, 143.1 (3), 93.0 (100), 45.0 (23). HRMS (EI): calcd. for C$_{17}$H$_{26}$O$_6$ [M + H]$^+$ 327.1729; found 327.1795.

**Fusidilactone E (2):** Colorless needles. $[a]_D^{25}$ = +1.3 ($c$ = 1.3 mg/mL, CHCl$_3$). UV (MeOH): $\lambda$ (log$\varepsilon$) = 280 (3.48) nm. IR (KBr): $\tilde{v}$ = 3432 (OH), 2918, 2851, 1707 (C=O), 1460, 1041 (*O*-alkyl) cm$^{-1}$. For $^1$H and $^{13}$C NMR spectroscopic data, see Table 1. MS (EI, 230 °C): $m/z$ (%) = 312.2 (8) [M]$^+$, 292.2 (13) [M – H$_2$O]$^+$, 213.2 (31) [M – C$_7$H$_{15}$]$^+$, 167.1 (37) [M – C$_5$H$_{11}$ – C$_3$H$_6$O$_2$]$^+$, 149.1 (72), 97.1 (60), 73.0 (95), 53.0 (100). HRMS (EI): calcd. for C$_{17}$H$_{26}$O$_6$ [M]$^+$ 312.1937; found 312.2146.

**Crystal Structure Determination of Fusidilactone B (3):** Colorless prismatic crystals of **3** were obtained by recrystallization from MeOH. C$_{17}$H$_{28}$O$_6$ ($M_r$ = 328.39), monoclinic, space group $P2_1$, $a$ = 5.735(3), $b$ = 7.628(4), $c$ = 20.161(9) Å, $\beta$ = 92.461(10)°, $V$ = 881.3(7) Å$^3$, $Z$ = 2, $D_{calcd.}$ = 1.238 g cm$^{-3}$, $\lambda$ = 0.71073 Å. Intensity data were measured with a Bruker-AXS SMART APEX CCD diffractometer. A total of 7841 reflections were collected to a maximum $2\theta$ value of 27.9° at 120(2) K. Data reduction and absorption correction from equivalents were performed with the Bruker software packages.[32,33] The structure was solved by direct methods[34] and refined by full-matrix least-squares procedures.[34] Fusidilactone **3** crystallizes in the non-centrosymmetric space group $P2_1$, however, in the absence of significant anomalous scattering effects, the Flack parameter is essentially meaningless. Accordingly, Friedel pairs were merged. All non-hydrogen atoms were given anisotropic thermal parameters; hydrogen atoms were located from difference Fourier maps and refined at idealized positions riding on their parent atoms. The refinement converged at $R1$ [$I > 2\sigma(I)$] = 0.0589, $wR2$ (all data) = 0.1046 for 2258 independent reflections and 212 variables. Min/max height in the final $\Delta F$ map = –0.21/0.27 e Å$^{-3}$.

CCDC-719932 (for **3**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam. ac.uk/data_request/cif.

**CD of Fusidilactone B (3):** CD (CH$_3$CN, $c$ = 3.46 × 10$^{-4}$): $\lambda$ ($\Delta\varepsilon$) = 220 (–0.60), 208 (–0.63) nm. CD (KCl): $\lambda$ ($\phi$) = 202 (–7.29), 187 (–22.92 mdeg) nm from the 0° rotation CD spectrum.

**Bioactivity. Agar Diffusion Assay:** The tested compounds were dissolved in acetone at a concentration of 1 mg/mL. Then 50 µL of the solution were pipetted onto a sterile filter disc (50 µg/disc), which was placed on an appropriate agar growth medium for the corresponding test organism and subsequently sprayed with a suspension of the test organism.[31] The test organisms were the Gram negative bacterium *Bacillus megaterium* and the Gram positive bacterium *Escherichia coli* (both NB medium), the fungus *Microbotryum violaceum*, and the alga *Chlorella fusca* (both on MPY medium). The radius of the zone of inhibition was measured in mm.

**Computational Section:** Conformational searches (MMFF method) and geometry optimizations [DFT method, B3LYP/6-31G(d) level of theory] were performed with Spartan 06[35] (Wavefunction, Inc., Irvine CA) with default parameters and convergence criteria. TDDFT calculations were performed with Gaussian 03[36] using various functionals (B3LYP, BH&HLYP) and basis sets (TZVP, ADZP).[37] CD spectra were generated as the sum of Gaussians with 5500 cm$^{-1}$ half-height width (ca. 25 nm at 210 nm), using dipole-velocity computed rotational strengths. The first excited states, including lactone n–π* and alkene π–π* transitions, which contribute to the observed CD band, had transition energies below the estimated ionization potential (7.1 eV, B3LYP/TZVP) and involved virtual orbitals with negative eigenvalues.[38]

## Acknowledgments

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