Fusidilactones, a New Group of Polycyclic Lactones from an Endophyte, *Fusidium sp.*[‡]

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Dedicated to Professor Dr. Lutz Friedjan Tietze on the occasion of his 60th birthday

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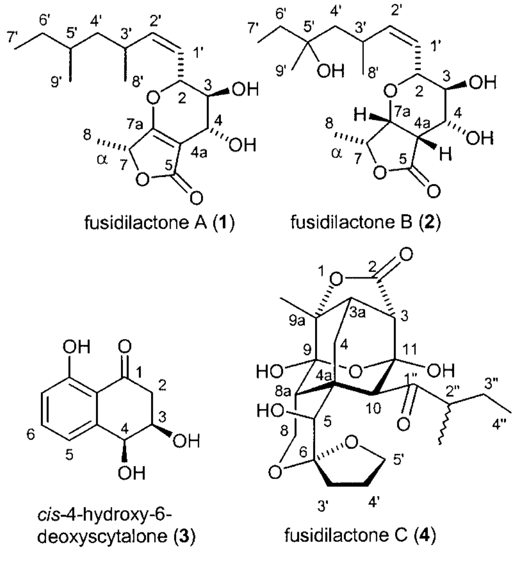
The bicyclic fusidilactones A (1) and B (2) and the structurally unusual fusidilactone C (4) with an oxoadamantane skeleton, a spiro acetal structure and two ether-bridged hemiacetals were isolated from the fungal endophyte *Fusidium sp.*

In connection with our ongoing screening for biologically active secondary metabolites from fungi, we investigated an endophytic *Fusidium sp.* isolated from the leaves of *Mentha arvensis*, growing in a meadow near Hahausen, Lower Saxony, Germany. The genus *Fusidium* belongs to the Mitosporic fungi; the teleomorphic form is *Biostictus.* The fungus was cultivated on biomalt semi-solid agar medium and alternatively in biomalt liquid culture. The crude diethyl ether extracts of both cultures showed good antifungal activity against *Eurotium repens* and *Fusarium oxysporum*, weak antibacterial activity against *Escherichia coli* and *Bacillus megaterium* and inhibited the alga *Chlorella fus*ca.

Since the two crude extracts also showed similar patterns on TLC analysis, they were combined and then separated by column chromatography on silica gel into five fractions that were further purified by PTLC (preparative TLC) and HPLC (for details see Exp. Sect.). Four pure compounds were isolated and their structures are shown in Scheme 1 (with increasing polarity). Compound 3 was identified as *cis*-4-hydroxy-6-deoxyscytalone (**3**), first isolated in 1981 from the fungus *Phialophora lagerbergii*.[2] The other three compounds are structurally new natural products and were named fusidilactones A (**1**), B (**2**), and C (**4**).



Scheme 1. Structures of 4-hydroxy-6-deoxyscytalone (**3**) and fusidilactones A (**1**), B (**2**), and C (**4**)

Structure Elucidation

Compound **1** was isolated as an oil. The molecular composition was determined by HRMS (m/z = 311.1906 [M + H^+]) in conjunction with the NMR spectra (see below) as $C_{17}H_{26}O_5$. In the ^{13}C NMR spectrum, signals for four primary, two secondary, two tertiary, four oxygenated tertiary, two sp^2-hybridized tertiary, and three sp^2-hybridized quaternary carbon atoms can be detected. In the ^1H NMR spectrum, signals for two olefinic, four hetero-substituted,

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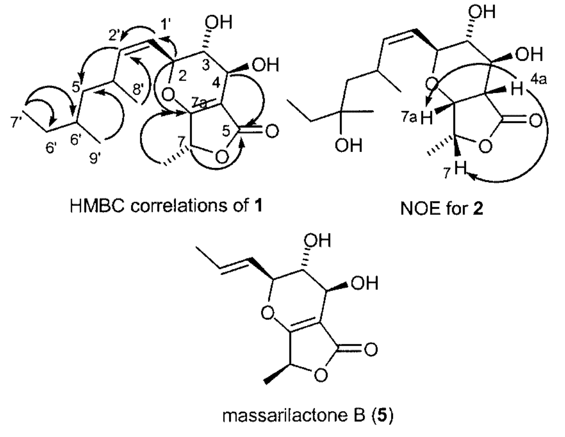
and eighteen aliphatic protons were seen. In addition to a broad band at $\tilde{\nu} = 3428$ cm^{-1} for hydroxyl groups, typical bands at $\tilde{\nu} = 1780$ cm^{-1} for γ-lactones and at $\tilde{\nu} = 1068$ cm^{-1} for alkyl ethers appear in the IR spectrum in agreement with signals at $\delta = 172.3$ ppm for an ester carbonyl and at $\delta = 65-80$ for oxygen substitution in the ^{13}C NMR spectrum. The molecular formula $C_{17}H_{26}O_5$ suggested five double bond equivalents. Thus, two rings must form the molecular skeleton of compound **1**, considering 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 10.3 Hz from the two olefinic protons at $\delta = 5.52$ and 5.66 ppm. The relatively low field signals at $\delta = 3.78$, 4.58, 4.84, and 4.96 ppm, directly bound to the respective carbon atoms resonating at $\delta = 65.3$, 72.3, 74.6 and 79.7 ppm, prove the attachment to oxygen atoms. In addition, the presence of four methyl groups can be deduced from the integration of three doublets and one triplet.

Molecular fragments were deduced from the H,H COSY and HMQC spectra. The connection of these fragments was inferred from the HMBC spectrum; the correlations are shown in Figure 1. The hydroxyl groups proved to be located at C-3 and C-4 by H/D exchange, shifting the relevant signals for C-3 and C-4 by a statistically significant 0.21 and 0.17 ppm to lower field in the ^{13}C NMR spectrum with respect to the shifts of the non-deuterated compound.



HMBC correlations of **1**

NOE for **2**

massarilactone B (**5**)

Figure 1. HMBC correlation of fusidilactone A (**1**), NOE correlations of fusidilactone B (**2**) and structure of massarilactone B (**5**)

The relative configuration was established by analysis of the coupling constants and NOE difference spectra. The coupling constant of $J = 7.1$ Hz for 2-H and 3-H proves the *trans*-diaxial position of these protons, as does the coupling constant of $J = 5.6$ Hz for 3-H and 4-H. The relative configuration of the methyl groups of the side chain could not be assigned by NOE. The α-position of the methyl group at C-7 could also not be deduced from the NMR spectroscopic data of **1**, but was tentatively based on the stereochemistry of the related hydrogenated metabolite fusidilactone B (**2**) (see below).

Metabolite **2** was isolated as the second compound in order of increasing polarity, crystallizing as colorless long needles with m.p. 156 °C. The spectroscopic data are similar to those of fusidilactone A (**1**) and the structure elucidation can be mainly restricted to the differences with respect to **1**. The molecular formula of $C_{17}H_{28}O_6$ can be deduced from the mass spectra in conjunction with the NMR spectroscopic data. Thus, one oxygen atom and two hydrogen atoms have to be added to the molecular formula of **1**. In the IR spectrum, bands at $\tilde{\nu} = 3498$, 1776, and 1191 cm^{-1} indicated the presence of hydroxyl groups, a lactone carbonyl and ether groups, respectively. In the ^{13}C NMR spectrum, a signal at $\delta = 72.7$ ppm was significant for a new oxygenated quaternary carbon atom. Instead of the two low-field signals at $\delta = 101.0$ and 178.0 ppm for C-4a and C-7a in **1**, two signals at $\delta = 47.7$ and 76.8 ppm appeared for these carbon atoms, indicating a saturation of the 4a-7a bond. This is supported by signals at $\delta = 3.38$ and 4.40 ppm for 4a-H and 7a-H in the ^{1}H NMR spectrum. The HMBC correlations confirmed the location of the new hydroxyl group at C-5′ as well as of the other connectivities.

In the elucidation of the relative stereochemistry, a comparison with the related metabolite massarilactone B (**5**), recently isolated by Gloer et al. from cultures of the aquatic fungus *Massarina tunicata*,[3] was instructive. The *E* double bond of the side chain in **5** ($J_{1',2'} = 15.0$ Hz) contrasts with the *Z* double bond of the side chains in **1** ($J_{1',2'} = 10.3$ Hz) and **2** ($J_{1',2'} = 10.8$ Hz). The all-*trans* equatorial position of the substituents at C-2, C-3, and C-4 in **1**, **2** and **5**, and also that for C-4 and C-4a in **2** were confirmed by the typical large coupling constants for *trans*-diaxial protons (**2**: $J_{2,3} = J_{3,4} = 9.0$, $J_{4,4a} = 6.7$ Hz). Interestingly, these couplings are larger than the corresponding values in fusidilactone A (**1**) or massarilactone B (**5**), confirming the increase of the respective torsional angles caused by the saturation of the 4a-7a double bond in **2**. The orientation of the C-7 methyl group in **2** with respect to the substituents of the pyran ring was unambiguously established by NOE experiments as shown in Figure 1. Irradiation of 4a-H resulted in enhancement (4.5%) of the 7a-H signal, clearly demonstrating the *cis* fusion of the two rings, as well as the signal for 7-H (3.2%) showing the α-configuration of the C-7 methyl group. Based on the biosynthetic relationship of **1** and **2** and occurrence in the same fungus, we also propose this configuration for the methyl group in fusidilactone A (**1**), as shown in Scheme 1. Again, the stereochemical assignment of the side-chain methyl groups in **2** was not possible by analysis of the NMR spectra.

The third metabolite crystallized as colorless needles with m.p. 82 °C (ref.[2] m.p: 83−85 °C) and $[\alpha]_D^{20} = +6.7$ and was identified as the known *cis*-4-hydroxy-6-deoxyscytalone (**3**).[2] The positive rotation is in agreement with a sample isolated from *Tubakia dryina*.[4a] The absolute configuration as shown in Scheme 1 is based on the assignment of *S*-chirality to (+)-isosclerone by interpretation of the optical rotatory dispersion of isosclerone dibenzoate.[4b] However, the validity of that assignment has been called into question and **3** may be the enantiomer.[4c]

Table 1. ^{1}H and ^{13}C NMR spectroscopic data and the corresponding H,H-COSY and HMBC correlations of fusidilactone C (**4**)

	^{13}C	^{1}H [*J*]	H,H-COSY	HMBC
2	174.4	–		–
3	50.0	2.86 (d) [5.2]	3a	2, 3a, 9a, 11
3a	44.2	2.73 (m)	3, 4	
4	25.4	1.90 (dd) [14.3; 3.7]	3, 4α	4a, 8a, 9a
		2.31 (dd) [14.3; 3.5]	3, 4β	
4a	43.2	–		–
5	70.8	3.08 (d) [7.7]	5-OH	4a, 6, 8a, 10
6	108.6	–		–
8	56.6	3.91 (dd) [11.6; 4.6]	8α, 8a	4a, 6, 8a
		4.01, t [11.6]	8β, 8a	
8a	38.6	2.15 (dd) [4.6; 11.6]	8α, 8β	8, 9, 10
9	98.5	–		–
9a	85.5	–		–
9a-CH$_3$	17.0	1.46 (s)		3a, 9, 9a
10	54.5	4.28 (s)		3, 4, 4a, 11, 1′′, 2′′
11	95.7	–		–
3′	37.3	1.80–1.89 (m)	3′α, 4′α, 4′β	4′, 6
		1.91–2.08 (m)	3′β, 4′α, 4′β	
4′	22.9	1.80–1.89 (m)	3′α, 3′β, 4′α, 5′	3′, 6
		1.91–2.08 (m)	3′α, 3′β, 4′β, 5′	
5′	68.8	3.93–4.07 (m)	4′α, 4′β	4′, 6
1′′	216.7	–		–
2′′	51.5	3.01 (m)	2′′-CH$_3$, 3′′	1′′, 2′′-CH$_3$
2′′-CH$_3$	15.7	1.10 (d) [6.9]	2′′	1′′, 2′′, 3′′
3′′	24.9	1.20 (m); 1.68 (m)	2′′, 4′′	1′′, 2′′, 2′′-CH$_3$, 4′′
4′′	11.9	0.85 (t) [7.3]	3′′	2′′, 3′′
5-OH	–	4.19 (d) [7.7]	5	–
9-OH	–	5.51 (s)		8a, 9
11-OH	–	5.45 (s)		3, 10, 11

Only 2 mg of the metabolite **4** were initially isolated from the first culture broth of *Fusidium sp.* as a colorless solid, m.p. 197 °C. However, the production was reproducible and in a second fermentation a further 11 mg of pure fusidilactone C (**4**) were obtained. The molecular composition was determined by HRMS (CI [M$^+$] at *m/z* = 438.1881) in accordance with the NMR spectroscopic data (see below) as $C_{22}H_{30}O_9$. The IR spectrum showed bands for hydroxyl ($\tilde{\nu}$ = 3458 cm^{-1}) and ether groups [$\tilde{\nu}$ = 1195 and 1062 cm^{-1}). Two characteristic bands at $\tilde{\nu}$ = 1775 and 1701 cm^{-1} appeared for a γ-lactone and a ketone, respectively. The ^{1}H and ^{13}C NMR spectroscopic data and their corresponding HMBC correlations are listed in Table 1.

The unprecedented structure of **4** and the inability to get suitable crystals for X-ray structure analysis necessitated a very detailed analysis of the NMR spectroscopic data. In the ^{1}H NMR spectrum, three signals were exchangeable with deuterium [$\delta_{9\text{-OH}}$ = 5.51 (s); $\delta_{11\text{-OH}}$ = 5.45 (s); $\delta_{5\text{-OH}}$ = 4.19 (d) ppm]. Several spin systems can be identified from the ^{1}H NMR, the H,H-COSY spectra, and the most important HMBC correlations shown in Figure 2. This leads to four independent fragments of the molecule **4** (Figure 2). The first fragment **A** is a 2-methylbutanoyl fragment, which can be deduced from the H,H-COSY and HMBC spectra.

Another partial structure **B** consists of three methylene groups and one ketalic carbon atom. The connectivity could be deduced directly from the H,H-COSY spectrum. The low field resonances for 5′-H (δ = 3.93–4.07 ppm) and C-5′ (δ = 68.8 ppm) suggested an oxygen substitution at C-5′, which was attached to the tertiary carbon C-6. The low-field resonance for C-6 at δ = 108.6 ppm is characteristic of a ketalic carbon as shown in Fragment **B** of Figure 2.

A further fragment **C** (Figure 2), involving the protons 3-H, 3a-H, and 4-H, could also be inferred directly from the H,H-COSY spectrum. All free valences of the carbons attached to 3-H, 3a-H, and 4-H were further connected to quaternary carbon atoms. Fragment **D** (Figure 3) was deduced from the signals in the H,H-COSY spectrum. It consists of an oxygen-bonded methylene group with geminal coupling and a methyne group, attached to two quaternary carbon atoms. One of the quaternary atoms must be C-9 as inferred from the HMBC spectrum. The proton of the hydroxyl group at C-9 shows long-range correlations to C-9 and C-8a. The proton of the hydroxyl group δ = 4.19 ppm couples to 5-H. Thus, this hydroxyl group can be assigned to C-5. Because of the correlations to C-6 and the quaternary C-4a, fragment **B** can be extended to **E**.

The remaining structural elucidation required the correct attachment and extension of fragments **A**–**E**. Fragments **D** and **E** must be connected via the free ketal oxygen and a methylene bridge because 8-H shows a strong correlation to C-6 in the HMBC spectrum. In addition, C-4a and C-8a must be bound to each other because 5-H shows a strong correlation to C-8a. This connectivity was further confirmed by couplings of 5-H and 8a-H to C-4a. The correla-

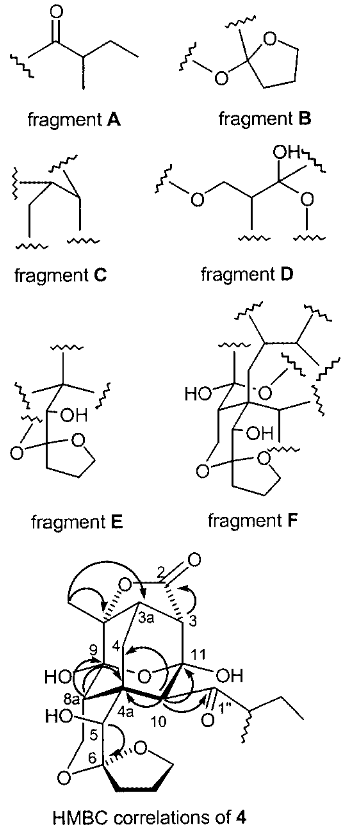


Figure 2. Independent fragments **A**−**D**, joined fragments **E** and **F**, and selected HMBC correlations of fusidilactone C (**4**)

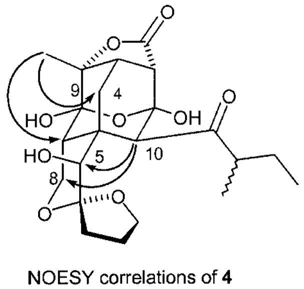


Figure 3. Selected NOE correlations of **4** deduced from the NOESY spectrum

tions of 8a-H and 5-H to the carbon atoms C-4 and C-10 demonstrated the connectivity of C-4a to C-4 and C-10. Thus fragment **F** results, adding this information to that known for fragments **C**, **D**, and **E** (Figure 3).

The molecular structure could be extended further by the attachment of the side chain of fragment **A** to C-10, because 10-H is the only proton showing long-range correlations to the carbonyl group at C-1′′ and to C-2′′. Since 10-H appears as a singlet, a further quaternary carbon atom must be attached to C-10. This has to be the hemiacetalic C-11, because the hydroxyl proton correlates to C-10, C-11, and C-3, directly bound to C-11. The remaining atoms, not yet assigned, are one carbonyl group, the quaternary oxygen-substituted carbon atom C-9a, and a methyl group, which is attached to C-9a. The only cross signal in the HMBC spectrum to the carbon atom of this methyl group results from 3a-H and the protons of the methyl group show resonances to the quaternary carbon C-3a, C-9 and C-9a. Thus, C-9a must form the bridge between C-3a and C-9. The lactone carbonyl is connected to C-9a by an oxygen atom similar to the connection of the two hemiacetalic centers at C-9 and C-11. The lactone carbonyl group C-2 could be connected to the last free position at C-3 due to correlations from 3-H to the lactone. The relative configurations of the substituents at C-9a and C-3 of the oxoadamantane skeleton are fixed by the lactone bridge, which cannot be forced into a *trans* fusion on an oxoadamantane skeleton. Only the stereochemistry at C-5, C-8a, C-9a, C-10, and that of the spiro center remained to be elucidated.

Figure 3 shows NOE correlations from the NOESY spectrum of fusidilactone C (**4**). In particular, NOE cross peaks from 10-H to 5-H and to 8-H clearly demonstrate the axial orientation of the hydroxy group at C-5. The absence of correlations of 10-H with the C-3′ methylene protons of the spiroketal suggests the proximity of the oxygen to 10-H as shown in **4**. In addition, crosspeaks of the methyl group proved their proximity to the protons on the oxoadamantane skeleton at C-4 and C-8a. The NOE correlations confirm the relative configuration shown in **4** with the exception of the methyl group at C-2′′. In addition, the relative configuration is supported by several W-couplings of equatorial protons at C-3, C-3a, C-4, and C-10 on the oxoadamantane skeleton, similar to those observed in the recently isolated sampsonions with adamantane skeleton.[5] Even more importantly, unusual $^{4}J_{C,H}$ correlations can be detected between C-2 and the protons at C-3, C-4, C-10 and the methyl group at C-9a. These $^{4}J_{C,H}$ correlations are normally not observable but can be detected when the coupling pathway has a planar (*w*-coupling) configuration[6] as, for instance, observed in the rigid oxoadamantane skeleton of fusidilactone C (**4**).

Fusidilactone C (**4**) has several very unusual structural features. First, with the exception of the alkaloid 5,15-oxidolycpodan,[7] it is the only oxoadamantane structure found in nature, comparable in structural complexity perhaps to tetrodotoxin, the poison of the Japanese fish *Spheroides rubripes*.[8] Also, the spiroacetal structure is present in less then 2% of known natural products. Last but not least, the most unusual structural elements are the two ether-bridged hemiacetals, present in only 35 out of more than 150000 natural products and stabilized by the rigid oxoadamantane structure, similar to that in a bridged triquinacene prepared by Woodward et al.[9]

The biological properties of the fusidilactones are presently under investigation.

Experimental Section

For general methodology, instrumentation and culture conditions see ref.[10] NMR spectra were recorded on a Bruker AMX-600 spectrometer (^{1}H: 600 MHz, ^{13}C: 150 MHz) or AMX-300 (^{1}H: 300 MHz, ^{13}C: 75 MHz).

The fungal endophyte (*Fusidium sp.*) was isolated following surface sterilization from *Mentha arvense* and was cultivated for 91 days on biomalt (5% w/v; Villa Natura, Gesundprodukte GmbH, Kirn, Germany) semi-solid agar medium at room temperature or alternatively in liquid biomalt culture (5% w/v; 120 rpm) for 11 days at 20° C. 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), the organic phase was dried (Na_2SO_4), filtered, and the solvent removed at reduced pressure to afford a crude extract (1200 mg). The crude material was separated into five fractions by flash chromatography on silica gel using petroleum ether/diethyl ether (2:1) and petroleum ether/diethyl ether (1:1) as the eluent. The main fractions were then further separated by PTLC and HPLC. Tests for antimicrobial activity were performed according to Schulz et al.[11]

Metabolite **1** (fusidilactone A, 6 mg) was isolated as a colorless oil from the least polar fraction by PTCL using dichloromethane/methanol (98:2) as the eluent (fraction with R_f = 0.5 to 0.6). Metabolite **2** (fusidilactone B, 6 mg) was isolated from the second fraction from the flash chromatography by repeated PTLC (CH_2Cl_2/5% MeOH) as colorless needles. Metabolite **3** (*cis*-4-hydroxy-6-deoxyscytalone, 2 mg) was isolated from the same fraction, however from a more polar fraction of the PLC. Further purification by column chromatography on Sephadex LH 20 (10 cm × 0.5 cm, CH_2Cl_2/5%MeOH) afforded the pure crystalline material **3**.

Metabolite **4** (fusidilactone C, 2 mg) was isolated from the most polar fraction of the flash chromatography followed by PLC purification on silica gel (CH_2Cl_2/4% MeOH) to afford 8 mg of almost pure material. On attempted further purification on Sephadex LH 20 (10 cm × 0.5 cm, CH_2Cl_2/5% MeOH) only 2 mg of pure metabolite **4** was recovered. Therefore, in a second fermentation, the purification procedure was changed, omitting the chromatography on Sephadex. Thus, the total extract from the second fermentation (1.19 g) was flash-chromatographed on silica gel using a Biotage Flash 40 S column with CH_2Cl_2/3% MeOH (820 ml), followed by CH_2Cl_2/10% MeOH (400 ml). Fraction C (125 mg, R_f = 0.16−0.35 with CH_2Cl_2/6% MeOH) was chromatographed on a Merck Hibar RT 250-25 Lichrosorb Si 60 (5 µm) HPLC column with methyl *tert*-butyl ether as the eluent. From this chromatography, 11 mg of pure fusidilactone C (**4**) was obtained (R_f = 0.52 with pure methyl *tert*-butyl ether as the eluent on Si 60 TLC).

2-(′-*cis*-3′,5′-Dimethylhept-1′-enyl)-3,4-dihydroxy-7-methyl-2,3,4,4a,7,7a-tetrahydrofuro[3,4-*b*]pyran-5-one (Fusidilactone A, 1): $[\alpha]_D^{20}$ = +8.2 (c = 1.2 mg/ml, CH_2Cl_2). UV (methanol): λ_{max} (lg ε) = 217 (3.122). IR (KBr): ṽ = 3428 cm^{-} (OH), 2961, 2920, 1780 (C=O), 1761, 1358, 1188 (O−alkyl), 1115, 1068 (O−alkyl). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.80 (d, $J_{9',5'}$ = 6.1 Hz, 3 H, 9′-H), 0.86 (t, $J_{7',6'}$ = 6.9 Hz, 3 H, 7′-H), 1.01 (d, $J_{8',3'}$ = 6.8 Hz, 3 H, 8′-H), 1.40−1.06 (m, 6 H, 3′-H, 4′-H, 5′-H, 6′-H), 1.47 (d, $J_{8,7}$ = 6.6 Hz, 3 H, 8-H), 2.61 (m, 1 H, 3′-H), 3.78 (dd, $J_{3,2}$ = 7.1 Hz, $J_{3,4}$ = 5.6 Hz, 1 H, 3-H), 4.58 (dd, $J_{3,4}$ = 5.6 Hz, $J_{4,7}$ = 1.8 Hz, 1 H, 4-H), 4.84 (dq, $J_{7,8}$ = 6.9 Hz, $J_{7,4}$ = 1.8 Hz, 1 H, 7-H), 4.96 (t, $J_{2,1'}$ = 7.9 Hz, $J_{2,3}$ = 7.1 Hz, 1 H, 2-H), 5.52 (dd, $J_{1',2'}$ = 10.3 Hz, $J_{1',2}$ = 7.9 Hz, 1 H, 1′-H), 5.66 (t, $J_{2',1'}$ = 10.3 Hz, $J_{2',3'}$ = 6.8 Hz, 1 H, 2′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.7 (q, C-7′), 17.6 (q, C-8), 19.4 (q, C-9′), 22.2 (q, C-8′), 30.5 (t, C-5′), 30.8 (d, C-5′), 32.5 (d, C-3′), 44.7 (t, C-4′), 65.3 (d, C-3), 72.3 (d, C-4), 74.6 (d, C-7), 79.7 (d, C-2), 101.0 (s, C-4a), 121.7 (d, C-1′), 146.0 (d, C-2′), 172.3 (s, C-5), 178.0 (s, C-7a) ppm. MS (EI, 230 °C): m/z (%) = 311 (1) [M + H^+], 240 (1), 221 (21), 168 (31) [M^+ + H^+ − C_5H_{11} − $C_3H_4O_2$], 137 (18), 124 (22), 97 (100) ppm. MS (CI, isobutane): m/z (%) = 311 (100) [M + H^+]. HRMS ($C_{17}H_{27}O_5$): calcd. 311.1859 [M + H^+]; found: 311.1906 ± 3 ppm

3,4-Dihydroxy-2-(1′-*cis*-5′-hydroxy-3′,5′-hept-1′-enyl)-7-methyl-2,3,4,4a,7,7a-hexahydrofuro[3,4-*b*]pyran-5-one (Fusidilactone B, 2): M.p. 156 °C. $[\alpha]_D^{20}$ = +10.2 (c = 2.1 mg/ml, CH_2Cl_2). IR (KBr): ṽ = 3498 cm^{-1} (OH), 2956, 2924, 2903, 1776 (C=O), 1460, 1191 (*O*−alkyl), 1036 (*O*−alkyl). UV (methanol): λ_{max} (lg ε) = 208 (2.720). ^1H NMR (300 MHz, $[D_6]$acetone): δ = 0.85 (t, $J_{7',6'}$ = 7.4 Hz, 3 H, 7′-H), 0.99 (d, $J_{9',3'}$ = 6.4 Hz, 3 H, 9′-H), 1.12 (s, 3 H, 8′-H), 1.35 (d, $J_{8,7}$ = 6.6 Hz, 3 H, 8-H), 1.46 (q, $J_{7',6'}$ = 7.4 Hz, 2 H, 6′-H), 1.50 (d, $J_{4',3'}$ = 5.8 Hz, 2 H, 4′-H), 2.83 (m, 1 H, 3′-H), 3.20 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1 H, 3-H), 3.38 (dd, $J_{4a,7a}$ = 3.2 Hz, $J_{4a,4}$ = 6.7 Hz, 1 H, 4a-H), 3.91 (dd, $J_{4,4a}$ = 6.7 Hz, $J_{4,3}$ = 9.0 Hz, 1 H, 4-H), 4.10 (t, $J_{2,1'}$ = $J_{2,3}$ = 9.0 Hz, 1 H, 2-H), 4.40 (t, $J_{7a,4a}$ = 3.2 Hz, $J_{7a,7}$ = 2.9 Hz, 1 H, 7a-H), 4.71 (dq, $J_{7,7a}$ = 2.9 Hz, $J_{7,8}$ = 6.6 Hz, 1 H, 7-H), 5.28 (dd, $J_{1',2'}$ = 10.8 Hz, $J_{1',2}$ = 9.0, 1 H, 1′-H), 5.57 (pt, $J_{2',1'}$ = 10.8 Hz, $J_{2',3'}$ = 10.6 Hz, 1 H, 2′-H) ppm. ^{13}C NMR (75 MHz, $[D_6]$acetone): δ = 8.5 (q, C-7′), 13.8 (q, C-8), 23.5 (q, C-8′), 27.8 (q, C-9′), 29.3 (d, C-3′), 35.1 (t, C-6′), 47.7 (d, C-4a), 49.4 (t, C-4′), 72.3 (d, C-4), 72.7 (s, C-5′), 74.6 (d, C-3), 75.1 (d, C-2), 76.8 (d, C-7a), 79.6 (d, C-7), 125.3 (d, C-1′), 144.1 (d, C-2′), 177.9 (s, C-5) ppm. MS (EI, 230 °C): m/z (%) = 328 (8) [M^+], 310 (24) [M^+ − H_2O], 240 (19), 223 (25) [M^+ − H_2O − C_5H_{11}], 213 (7) [M^+ − C_7H_{15}], 195 (22) [M^+ − H_2O −C_7H_{15}], 174 (19) [M^+ − CH_3 − C_9H_{15}],167 (60) [M^+ − C_5H_{11} − $C_3H_4O_2$], 137 (40), 89 (99) 97 (100). HRMS ($C_{17}H_{28}O_6$): calcd. 328.1886; found 328.1900 ± 3 ppm.

***cis*-4-Hydroxy-6-deoxyscytalone (3):** M.p. 82 °C. (ref.[2] m.p: 83−85 °C) $[\alpha]_D^{20}$ = +6.7 (c = 0.51 mg/ml, CH_2Cl_2). ^1H NMR ($[D_6]$acetone): δ = 3.05 (m, 2 H, 2-H), 4.12 (m, 1 H, 3-H), 4.68 (d, $J_{4,3}$ = 2.9 Hz, 4-H), 6.80 (dd, $J_{7,6}$ = 8.9 Hz, $J_{7,5}$ = 2.2 Hz, 1 H, 7-H), 7.10 (dd, $J_{5,6}$ = 9.0 Hz, $J_{5,7}$ = 2.2 Hz, 1 H, 5-H), 7.49 (t, $J_{6,7}$ = 8.9 Hz, $J_{6,5}$ = 9.0 Hz, 1 H, 6-H), 12.40 (s, 1 H, OH), ppm. ^{13}C NMR ($[D_6]$acetone): δ = 47.1 (t, C-2), 71.8 (d, C-4), 73.1 (d, C-3), 117.2 (d, C-6), 119.9 (d, C-7), 127.6 (s, 8a), 137.9 (s, C-5), 147.6 (s, C-4a), 164.3 (s, C-8), 206.5 (s, C-1).

Fusidilactone C (4): M.p. 197 °C. $[\alpha]_D^{20}$ = +26.2 (c = 0.21 mg/ml, CH_2Cl_2). IR (KBr): ṽ = 3458 cm^{-1} (OH), 2961, 2920, 1775 (C=O), 1701 (C=O), 1195 (*O*−alkyl), 1062 (*O*−alkyl). UV (methanol): λ_{max} (lg ε) = 216 (2.463). ^1H NMR (600 MHz, $[D_6]$acetone): δ = 0.85 (t, $J_{4'',3''}$ = 7.3 Hz, 3 H, 4′′-H), 1.10 (d, $J_{2''\text{-}CH3,5''}$ = 6.9 Hz, 3 H, 2′′-CH_3), 1.20 (m, 1 H, 3′′β-H), 1.46 (s, 3 H, 9a-CH_3), 1.68 (m, 1 H, 3′′α-H), 1.80−1.89 (m, 2 H, 3′β-H, 4′β-H), 1.90 (dd, $J_{4\beta,4\alpha}$ = 14.3 Hz, $J_{4\beta,3a}$ = 3.7 Hz, 1 H, 4β-H), 1.90−2.08 (m 2 H, 3′α-H, 4′α-H), 2.15 (dd, $J_{8a,8\alpha}$ = 4.7 Hz, $J_{8a,8\beta}$ = 11.5 Hz, 1 H, 8a-H), 2.31 (dd, $J_{4\alpha,4\beta}$ = 14.3 Hz, $J_{4\alpha,3a}$ = 3.5 Hz, 1 H, 4α-H), 2.73 (m, 1 H, 3a-H), 2.86 (d, $J_{3,3a}$ = 5.2 Hz, 3-H), 3.01 (m, 1 H, 2′′-H), 3.08 (d, $J_{5,5\text{-}OH}$ = 7.7 Hz, 1 H, 5-H), 3.91 (dd, $J_{8\beta,8\alpha}$ = 11.6 Hz, $J_{8\beta,8a}$ = 4.6 Hz, 1 H, 8β-H), 3.93−4.07 (m, 2 H, 5′α-H, 5′β-H), 4.01 (t, $J_{8\alpha,8a}$ = $J_{8\alpha,8\beta}$ = 11.6 Hz, 1 H, 8α-H), 4.19 (d, $J_{5\text{-}OH,5}$ = 7.7 Hz, 1 H, 5-OH), 4.28 (s, 1 H, 10-H), 5.45 (s, 1 H, 11-OH), 5.51 (s, 1 H, 9-OH) ppm. ^{13}C NMR (150 MHz, $[D_6]$acetone): δ = 11.9 (q, C-4′′), 15.7 (q, 2′-CH_3), 17.0 (q, 9a-CH_3), 22.9 (t, C-4′), 24.9 (t, C-3′′), 25.4 (t, C-4), 37.3 (t, C-3′), 38.6 (d, C-8a), 43.2 (s, C-4a), 44.2 (d, C-3a), 50.0 (s, C-3), 53.5 (s, C-2′′), 54.5 (d, C-10), 56.6 (t, C-8), 68.8 (t, C-5′), 70.8 (d, C-5), 85.5 (s, C-9a), 95.7 (s, C-11), 98.5 (s, C-9), 108.6 (s, C-6), 174.4 (s, C-2), 216.7 (s, C-1′′). MS (CI,NH_3): m/z (%) = 456 (100) [M + NH_4^+]. HRMS ($C_{22}H_{30}O_9$): calcd. 438.1890; found 438.1881 ± 4 ppm.

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