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NOTE

Three novel polyene macrolides isolated from cultures of *Streptomyces lavenduligriseus*

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Actinomycetes have evolved in the process of ecological interactions between animals, plants and microorganisms in the environment to produce new classes of secondary metabolites with novel structures,[1–3] of which polyene macrolides are very interesting bioactive compounds with a wide range of biological activities and subject to a relatively low incidence of resistance.[4,5] Many of these molecules have been successfully isolated and turned into useful drugs and other organic chemicals. In our study aimed at the discovering of novel antifungals, the soil actinomycete, *Streptomyces lavenduligriseus*, was found to produce strong antifungal components. Bioactivity-guided isolation and purification yielded filipin III **1** and three novel polyene macrolides, compound **2**, **3** and **4** (Figure 1). Details of the isolation, structure elucidation and the antifungal activities of these compounds are presented below.

The strain of *S. lavenduligriseus* was cultivated in 250-ml Erlenmeyer flasks containing 30 ml of seed medium (2% glucose, 3% soybean meal, 2% soluble starch, 2% glycerol, 0.02% $MgSO_4{\cdot}7H_2O$ and 0.02% KH_2PO_4, pH 7.5). The flasks were shaken at 220 r.p.m. on a rotary shaker at 28 °C for 2 days. The seed culture (8%) was transferred into 500-ml Erlenmeyer flasks containing 50 ml of production medium (4% cornstarch, 0.8% glucose, 2.2% soybean meal, 0.1% $MgSO_4{\cdot}7H_2O$, 0.02% KH_2PO_4 and 0.2% NaCl). The flasks were incubated at 220 r.p.m. on a rotary shaker at 28 °C for 7 days. The cells were lysed with EtOH, which was then removed by concentration *in vacuo*. The resulting aqueous concentrate was partitioned successively with EtOAc (3 × 3 l) and BuOH (3 × 3 l). The combined extracts were concentrated under reduced pressure to yield 68 g of brown gum. This material was subjected to silica gel chromatography using a gradient mixture of $CHCl_3$-MeOH (from 100:0 to 0:100), yielding nine fractions (A–I). A fraction (5.65 g) was identified as containing polyene macrolides by assaying for bioactivity against *Candida albicans* and by a diode array detector (DAD)/UV spectra with three characteristic λ_{max} at 289, 303, 319 nm.[6] The fraction was further fractionated by successive preparative HPLC (YMC-Pack RP-C18 (YMC, Tokyo, Japan), 30 × 250 mm^2, 35% MeCN in H_2O, 10 ml-min^{-1}), yielding fractions 1–8. Fraction 3 (26.5 mg) was further purified by semi-prep RP-HPLC (YMC-Pack RP-C18, 20 × 250 mm^2, 35% MeCN in H_2O, 7 ml min^{-1}) to attain compound **4** (23.6 mg; t_R = 20.1 min). Using the same procedure, purification of fraction 6 (56.5 mg) yielded compounds **3** (8.3 mg; t_R = 72.6 min) and **2** (7.3 mg; t_R = 81.2 min), fraction 8 (69.5 mg) afforded compound **1** (12.6 mg; t_R = 63.5 min).

Compound **1** was identified as filipin III by comparing its NMR and MS data with those previously reported.[7]

The molecular formula of compound **2** was determined to be $C_{38}H_{64}O_{13}$ (seven degrees of unsaturation) on the basis of its positive HR-ESI-MS at the *m*/*z* value = 751.4226 $[M+Na]^+$, 74 a.m.u. higher than that of compound **1**, in agreement with the presence of a glycidyl group in **2** as evidenced by NMR and DEPT spectra (δ_H = 3.50, 3.90 and 3.65–3.75; δ_C = 70.3, 72.3 and 64.6)[8]. The ^1H–^1H COSY connections of H-30/H-31/H-32 and the HMBC correlation from H-30 to C-15 established the linkage of C-30 and C-15 via an ether bond (Figure 2). This was supported by the downfield shift of C-15 (δ_C = 84.3) in **2** compared with that of C-15 (δ_C = 73.6) in **1** (Table 1). The fine structured UV band at 380–210 nm with maxima at 320, 336 and 356 nm exhibited the same UV features for both compounds, and the CD spectra of compound **2** and **1** in methanol display the same pattern of Cotton effects (Figure 3), indicating that they possess identical basis skeletons (Figure 1). The H-17/H-19, H-18/H-20, H-19/H-21 and H-23/H-25 ROEs combined with $J_{(17,18)}$ of 15.6 Hz and $J_{(24,25)}$ of 15.1 Hz suggest that the pentaene chain has a fully extended planar *trans* configuration in solution. The UV band at 380–210 nm with maxima at 320, 336 and 356 nm is characteristic of a rigid, all-*trans* pentaene chromophore. Based on the rigid all-*trans* polyene chain and the geometric requirements for closing the macrolactone ring, only a fully extended conformation of the C-1′–C-11 fragment meets the steric requirements imposed by an all-*trans* pentaene chromophore.[9,10] The regular alignment of the

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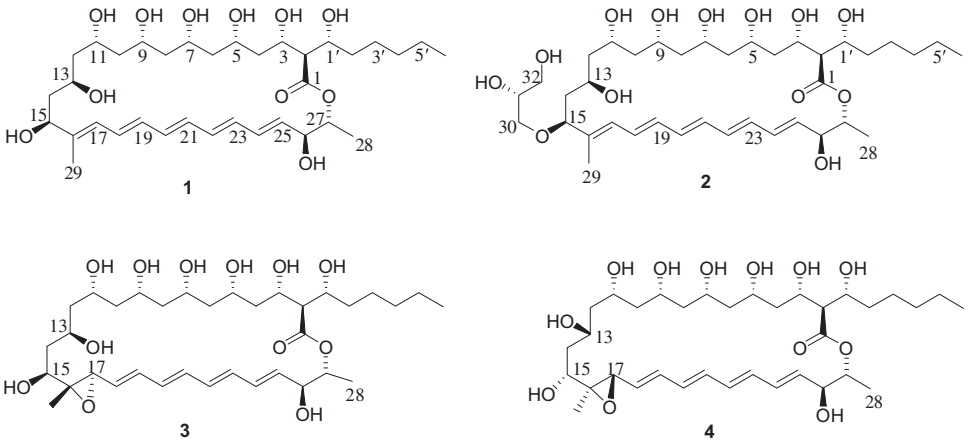

Figure 1 The chemical structures of compounds **1**–**4**.

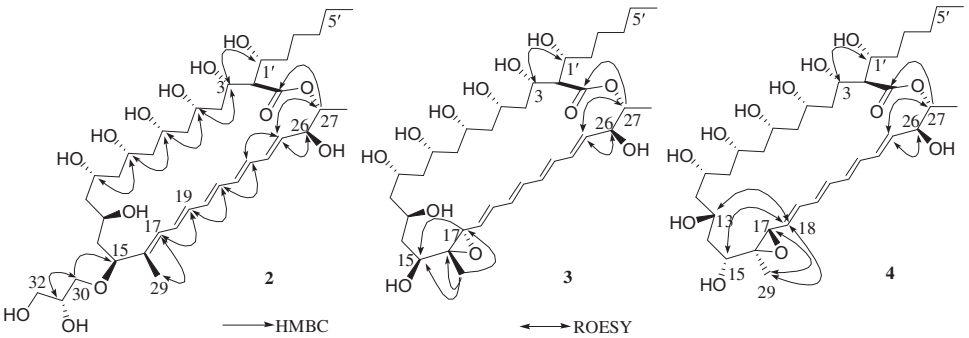

Figure 2 Key HMBC and ROESY correlations of **2**–**4**.

hydroxymethine protons, as indicated by the 1′-H/3-H, 3-H/5-H, 5-H/7-H, 7-H/9-H and 9-H/11-H ROE's, strongly suggests a fully extended conformation of the C-1′–C-11 fragment (Figure 2 and Supplementary Table 1). In addition, the conformationally relevant proton H-17 shows dipolar coupling with protons 19 and 29 but not with protons 13 and 15. This fixes the configuration of the C-13 OH group to be facing the inner side of the turn and also places the H-15 in the equatorial position in the plane of the macrocycle.[11] The fully extended C-1′–C-11 fragment is positioned above the pentaene chromophore plane. Therefore, C-27 must move above the chromophore plane in order to close the macrolactone ring. This condition is only met by a single enantiomer of the C-26–C-27 fragment with a relative configuration on the same face of the polyene macrolide ring system, as indicated by vicinal coupling constants $J_{(25,26)}$ of 6 Hz, $J_{(26,27)}$ of 6 Hz and the H-25/H-26 and H-25/H-27 ROEs (Figure 2 and Supplementary Table 1). Based on these results, the structure of **2** represents a new compound named 15-glycidylfilipin III (Figure 1).

Compound **3** was determined to have the molecular formula $C_{35}H_{58}O_{12}$ (seven degrees of unsaturation) on the basis of its HR-ESI-MS *m/z* value of 671.4035 $[M+H]^+$. Comparison of the ^1H and ^{13}C NMR spectroscopic data of compound **3** with the spectra of compound **1** revealed resonances for one more oxygenated sp^3 quaternary carbon ($\delta_C = 75.0$) and one more oxymethine ($\delta_H = 3.68$, $\delta_C = 82.3$). The comparison also revealed the absence of signal for an olefin unit ($\delta_C = 138.2$, $\delta_H = 6.25$, $\delta_C = 130.7$), which suggested that the C-16/C-17 double bond carbons in compound **1** should be oxidized to an epoxy unit in compound **3**. This oxidation was suggested by the unsaturation data and the sum of the data regarding the oxygen atom.[8] The COSY interactions of H-17/H-18/H-19 and strong HMBC correlations between H-29 and C-15, C-16 and C-17 as well as correlations between H-17 and C-15, C-16, C-18 and C-29 indicate that the oxygen atom is positioned at C-16 and C-17 and is vicinal to C-18 (Figure 2). H-17 was assigned to the axial position based on cross peaks between H-17 and H-18 in the ROESY spectrum. The lack of H-13/H-18 and H-15/H-18 ROEs in the ROESY spectrum also indicates that the C13 OH group is facing the inner side of the turn and that H-15 is in the equatorial position in the plane of the macrocycle (Figure 2). The CD spectra of compound **3** and **1** in methanol display similar Cotton effects,[11] indicating that there is no configurational differences between these two compounds (Figure 3). Based on these results, compound **3** is a new compound named 16α, 17α-epoxyfilipin V (Figure 1). Compound **4** was assigned the same molecular formula ($C_{35}H_{58}O_{12}$) as compound **3** using HR-ESI-TOF MS with $m/z = 671.4033$ $[M+H]^+$. The UV absorption spectrum of **4** was also almost identical to that of compound **3**, suggesting that **4** possesses the same chromophore as **3**. 2D NMR (^1H–^1H COSY, HMQC and HMBC) experiments for **4** allowed for the complete assignment of all proton and carbon signals as well as determination of the planar structure for **4**. Unlike compound **3**, the ^1H and ^{13}C NMR data for **4** display different chemical shifts for C-15 ($\delta_H/\delta_C = 3.65/76.8$ in **3**; 3.69/71.0 in **4**), C-16 ($\delta_C = 75.0$ in **3**; 72.7 in **4**) and C-17 ($\delta_H/\delta_C = 3.68/82.3$ in **3**; 4.09/83.6 in **4**; Table 1). The downfield shift of H-17 suggests that it adopts an equatorial configuration in **4**. In the ROESY spectrum, the H-13/H-18 and H-15/H-18 ROEs indicate that the C13 OH group faces the outer side of the turn and that H-15 is in an axial position to the plane of the macrocycle (Figure 2). The positive CD bands of compound **4** at 289 nm (+4.7), 303 nm (+6.8) and 319 nm (+6.1) are in contrast to the negative ones of compound **3**

Table 1 ^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectra data of 2–4 (δ in p.p.m., J in Hz)

	2		*3*		*4*	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		172.9		173.0		173.0
2	2.60, t(8.2)	60.4	2.44, t(8.0)	59.6	2.48, t(8.1)	59.9
3	4.21–4.24, m	73.2	4.21–4.24, m	72.7	4.15–4.18, m	72.2
4	1.51–1.58, m	41.3	1.37–1.40, m	42.5	1.46–1.54, m	42.9
5	4.00–4.07, m	73.8	4.06–4.10, m	72.3	3.95–3.99, m	71.3
6	1.51–1.58, m	45.1	1.61–1.68, m	44.7	1.46–1.54, m	44.9
7	4.00–4.07, m	74.1	4.06–4.10, m	73.0	4.03–4.06, m	71.7
8	1.51–1.58, m	45.3	1.46–1.50, m	45.1	1.46–1.54, m	45.0
9	4.00–4.07, m	73.9	4.06–4.10, m	73.5	4.03–4.06, m	72.9
10	1.51–1.58, m	44.1	1.75–1.83, m	44.0	1.65–1.69, m	42.0
	1.36–1.41, m					
11	4.00–4.07, m	71.1	4.06–4.10, m	68.9	4.03–4.06, m	70.4
12a	1.73–1.79, m	45.2	1.75–1.83, m	42.8	1.87–1.92, m	44.4
12b	1.36–1.41, m		1.46–1.50, m		1.39–1.44, m	
13	3.25–3.29	67.1	3.76	73.1	3.74–3.77, m	67.9
14	1.93–1.97, m	41.3	1.75–1.83, m	40.1	1.65–1.69, m	37.8
	1.80–1.83, m		1.37–1.40, m			
15	3.84, dd(10.9,4.4)	84.3	3.65, dd(11.6,4.8)	76.8	3.69, dd(9.2,6.8)	71.0
16		138.1		75.0		72.7
17	6.12, d(11.2)	130.7	3.68, d(7.6)	82.3	4.09, d(7.5)	83.6
18	6.54, dd(15.6,11.7)	128.9	5.92, dd(14.8, 3.3)	130.2	6.00, dd(14.8,7.6)	130.2
19	6.39–6.43, m	134.9	6.20–6.42, m	132.8	6.39–6.43, m	137.9
20	6.33–6.37, m	133.7	6.20–6.42, m	133.8	6.28–6.33, m	133.8
21	6.39–6.43, m	135.3	6.20–6.42, m	134.5	6.28–6.33, m	134.5
22	6.39–6.43, m	134.1	6.20–6.42, m	134.4	6.28–6.33, m	134.7
23	6.39–6.43, m	134.4	6.20–6.42, m	133.4	6.28–6.33, m	134.6
24	6.48, dd(10.4,6.6)	132.0	6.20–6.42, m	134.0	6.28–6.33, m	135.4
25	6.05, dd(15.1,4.9)	134.3	5.67, dd(14.8, 7.6)	135.0	5.66, dd(14.8, 7.6)	135.3
26	4.13, t(6.0)	73.3	3.89–3.94, m	76.0	3.88, t(8.3)	76.3
27	5.01, dd(6,12)	75.2	4.80, t(6)	73.9	4.83, t(6)	74.1
28	1.33, d(6.5)	17.9	1.29, d(6.3)	18.0	1.29, d(6.2)	18.0
29	1.77, d(5.1)	10.8	0.99, s	14.2	1.14, s	22.1
30a	3.45, dd(10.0, 4.7)	70.6				
30b	3.35–3.38, m					
31	3.77, dt(5.2)	72.3				
32a	3.60–3.62, m	64.6				
32b	3.54–3.57, m					
1′	3.88–3.90, m	72.5	3.89–3.94, m	72.8	3.90–3.93, m	72.8
2′a	1.36–1.41, m	36.2	1.46–1.50, m	35.8	1.46–1.54 m	35.8
2′b			1.37–1.40, m		1.39–1.44, m	
3′a	1.51–1.58, m	26.1	1.46–1.50, m	26.1	1.31–1.34, m	26.1
3′b	1.36–1.41, m		1.37–1.40, m			
4′	1.36–1.34, m	32.9	1.29–1.35, m	32.9	1.31–1.34, m	33.0
5′	1.36–1.41, m	23.7	1.29–1.35, m	23.7	1.31–1.34, m	23.7
6′	0.95, t(6.8)	14.4	0.90, t(6.2)	14.1	0.91, t(6.7)	14.4

(Figure 3). Based on these results, the configurations at C16 and C17 of **4** are assigned opposite to those of **3**. Therefore, **4** is named as 16β, 17β-epoxyfilipin V (Figure 1).

Physicochemical properties of compounds **2**, **3** and **4** are listed in Supplementary Table 2. Antifungal activities were evaluated by using a serial twofold dilution method in 96-well sterilized microplates. The MICs ($\mu g\,ml^{-1}$) of compounds **1**–**4** against *C. albicans* were 6.25, 6.25, 200 and 200 $\mu g\,ml^{-1}$, respectively. The MIC of the control, nystatin, was 3.13 $\mu g\,ml^{-1}$. Compounds **1** and **2** exhibited strong inhibitory effects on mycelia growth of *C. albicans*. However, compounds **3** and **4** showed considerably weaker antifungal activity. This result was unexpected because it had been previously reported that microbial secondary metabolites with epoxide function are more toxic, or have a stronger cytostatic effect, than analogs lacking the epoxide function.[12] Regarding compounds **3** and **4**, it must be considered that one double bond in the hydrophobic polyene region in both compounds is replaced by an epoxy group. It is possible that the epoxy group causes steric hindrance, interfering with the polyene macrolide–sterol

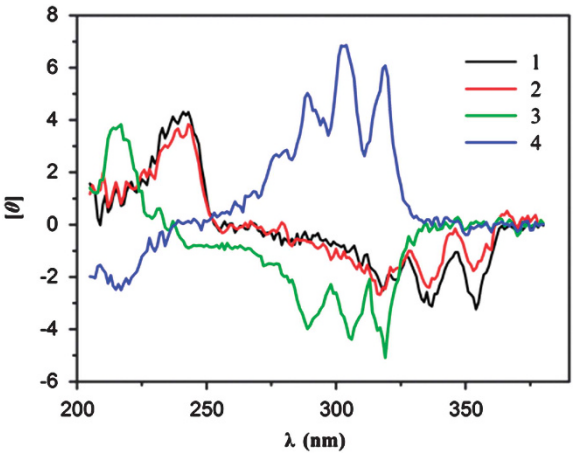

Figure 3 The CD of compounds **1**–**4** in methanol.

interaction. Steric hindrance may be due to the new oxygen atom being significantly larger than the native sole hydrogen atom. This difference in atomic size may lead to even more flexibility and structural disorder. Moreover, the hydrophobic/hydrophilic balance is significantly perturbed in derivatives **3** and **4**, as a more rigid polyene region is a crucial factor contributing to fungicidal action. This region is related to the ability of the molecules to interact with highly hydrophobic sterols and form channels.[13] The data obtained here provide new insights into the structure–function relationship of polyene macrolides, and may facilitate the engineered biosynthesis of new antifungals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)