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# Aliphatic acid amides of the fruits of *Zanthoxylum piperitum*

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**Abstract**

Six aliphatic acid amides (**1**–**6**) were isolated from the pericarp of *Zanthoxylum piperitum* fruits. MS and NMR spectroscopic investigation revealed that these compounds have a ketone and/or hydroxyl group(s) in the unsaturated aliphatic acid moiety of the structure of the amides. Combinations **3**–**4** and **5**–**6** are stereoisomers in terms of the relative configurations of their two asymmetric carbons.


*Keywords:* *Zanthoxylum piperitum*; Rutaceae; Fruit; Aliphatic acid amide; Stereoisomer

## 1. Introduction

The pericarp of the fruits of *Zanthoxylum piperitum* DC (=*Xanthoxylum piperitum*; Rutaceae) and related species has been used as an anthelmintic and for the treatments of disorders of the digestive organs in Asia (Perry, 1980; Xie and Huang, 1984). The fruit and leaves of *Z. piperitum* have been reported to contain terpenoids (Sakai et al., 1968; Jiang et al., 2001), aliphatic acid amides (Aihara, 1951; Yasuda et al., 1982), an alkaloid, flavonoids, and other phenolics (Hisatomi et al., 2000; Cho et al., 2003; Hur et al., 2003). Further investigation of the constituents of the pericarp of *Z. piperitum* has led to the isolation of several amides, including novel amides. In the present study, we investigated the structures of the amides.

## 2. Results and discussion

### 2.1. Isolation of the aliphatic acid amides

The pericarp of *Z. piperitum* fruits was homogenized in MeOH, and the EtOAc-soluble portion of the homogenate was subjected to Toyopearl HW-40C, MCI-gel CHP-20P, and YMC-gel ODS chromatographic seperations. The fractions that contained the amides were purified by preparative HPLC to obtain six compounds, which were designated as ZP-amide A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**).

### 2.2. ZP-amide A

ZP-amide A (**1**) was obtained as an unstable colorless syrup. The electrospray-ionization mass spectrometry (ESI-MS) showed ion peaks corresponding to the molecular formula $C_{16}H_{25}NO_4$, and this was confirmed by high-resolution (HR) ESI-MS. The $^1$H NMR spectrum [in $(CD_3)_2CO + D_2O$] showed signals of three pairs of protons on *trans*-olefinic carbons [$\delta$ 6.04 (1H, *dt*, $J = 15.5$, 1 Hz, H-2), 6.78 (1H, *dt*, $J = 15.5$, 7 Hz, H-3); 6.28 (1H, *dd*, $J = 6$, 15 Hz, H-7), 6.43 (1H, *br dd*, $J = 11$, 15 Hz, H-8); 7.22 (1H, *dd*, $J = 11$, 15.5 Hz, H-9), 6.10 (1H, *d*, $J = 15.5$ Hz, H-10)], protons of one methine [$\delta$ 4.23 (1H, *br q*, $J = 6$ Hz, H-6)], three methylene [$\delta$ 2.27 (2H, *m*, H-4), 1.65 (2H, *m*, H-5), and 3.22 (2H, *d*, $J = 6$ Hz, H-1′)] and three methyl [$\delta$ 1.11 (6H, *s*, H-3′/H-4′) and 2.21 (3H, *s*, H-12)] carbons, and an NH proton [$\delta$ 7.42 (1H, *br t*, $J = 6$ Hz)]. The correlations H-2–H-3–H-4–H-5–H-6–H-7–H-8–H-9–H-10, and

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H-1′–NH among these signals were revealed by vicinal correlations that were observed in the $^1$H–$^1$H COSY spectrum. The spectrum also showed the presence of the $^1$H–$^1$H long-range correlations H-10–H-12 and H-1′–H-3′/H-4′. The $^{13}$C NMR spectrum [in $(CD_3)_2CO$ + $D_2O$] showed an amide carbon at $\delta$ 167.5 (C-1) and a ketonic carbon at $\delta$ 199.4 (C-11), along with six olefinic carbons [$\delta$ 124.9 (C-2), 128.1 (C-8), 130.7 (C-10), 144.2 (C-9), 144.3 (C-3), 147.9 (C-7)], one quaternary carbon with a hydroxyl group [$\delta$ 70.81 (C-2′)], one methine carbon with a hydroxyl group [$\delta$ 70.77 (C-6)], three methylene carbons [$\delta$ 28.3 (C-4), 36.1 (C-5), 50.6 (C-1′)], and three methyl carbons [$\delta$ 27.0 (C-12), 27.2 (C-3′/C-4′)]. The $^1$H–$^{13}$C heteronuclear multiple-bond connectivity (HMBC) spectrum showed correlations concerning the amide carbonyl carbon with H-1′, H-2, and H-3, and the ketone carbon with H-9, H-10, and H-12, indicating the locations of these carbons to be at C-1 and C-11, respectively. Although ZP-amide A (**1**) has an asymmetric carbon at C-6, the optical inactivity of this compound indicated that this was a racemic mixture. The structure of (6*RS*)-(2*E*,7*E*,9*E*)-6-hydroxy-*N*-(2-hydroxy-2-methylpropyl)-11-oxo-2,7,9-dodecatrienamide (**1**), was therefore assigned to this compound.

### 2.3. ZP-amide B

ZP-amide B (**2**) was obtained as an unstable colorless syrup. The molecular formula $C_{16}H_{25}NO_4$ for this compound was indicated by ESI-MS. The $^1$H NMR spectrum [in $(CD_3)_2CO$] showed signals of three pairs of *trans*-olefinic protons [$\delta$ 6.04 (1H, *dt*, $J$ = 15.5, 1 Hz, H-2), 6.76 (1H, *dt*, $J$ = 15.5, 7 Hz, H-3); 6.18 (1H, *d*, $J$ = 15.5 Hz, H-7), 7.25 (1H, *dd*, $J$ = 11, 15.5 Hz, H-8); 6.41 (1H, *br dd*, $J$ = 11, 15 Hz, H-9), 6.29 (1H, *dd*, $J$ = 5.5, 15 Hz, H-10)], an aliphatic methine proton [$\delta$ 4.38 (1H, *m*, H-11)] that couples with a hydroxyl proton at $\delta$ 4.00 (1H, *br d*, $J$ = 5 Hz), three pairs of methylene protons [$\delta$ 2.43 (2H, *dq*, $J$ = 1, 7 Hz, H-4), 2.76 (2H, *t*, $J$ = 7 Hz, H-5), 3.23 (2H, *d*, $J$ = 6 Hz, H-1′)], three sets of methyl protons [$\delta$ 1.12 (6H, *s*, H-3′/H-4′), 1.23 (3H, *d*, $J$ = 6 Hz, H-12)], and an amide proton [$\delta$ 7.13 (1H, *br*)]. The $^1$H–$^1$H COSY spectrum indicated vicinal correlations of H-2–H-3–H-4–H-5, H-7–H-8–H-9–H-10–H-11–H-12, and H-1′–NH, and also the long-range correlation H-1′–H-3′/H-4′. Although the $^{13}$C signal pattern of **2** is similar to that of **1**, the correlations in the COSY spectrum suggested that **2** has a structure in which the sequence C-6 → C-11 has been changed to C-11 → C-6 in **1**. The assigned structure **2** was verified by $^1$H–$^{13}$C long-range correlations of C-6 with H-4, H-5, H-7, and H-8 in the HMBC spectrum. The HMBC spectrum also showed correlations of C-1 with H-1′, H-2 and H-3, which substantiated the connectivity around the amide bond. The optical inactivity of this compound indicated that **2** was a racemic mixture, and therefore this compound was represented as (11*RS*)-(2*E*,7*E*,9*E*)-11-hydroxy-*N*-(2-hydroxy-2-methyl-propyl)-6-oxo-2,7,9-dodecatrienamide.

### 2.4. ZP-amides C and D

ZP-amide C (**3**) was obtained as an unstable colorless syrup. The molecular formula $C_{16}H_{27}NO_4$ was indicated by ESI-MS. The $^1$H NMR spectrum (in $CDCl_3$) showed signals of a series of conjugated *cis*- and *trans*-olefinic protons [$\delta$ 5.40 (1H, *dt*, $J$ = 11, 7 Hz, H-6), 6.04 (1H, *t*, $J$ = 11 Hz, H-7), 6.50 (1H, *dd*, $J$ = 11, 15 Hz, H-8), and 5.70 (1H, *dd*, $J$ = 6.5, 15 Hz, H-9)] along with a pair of isolated *trans*-olefinic protons [$\delta$ 5.81 (1H, *d*, $J$ = 15 Hz, H-2) and 6.78 (1H, *dt*, $J$ = 15, 6.5 Hz, H-3)]. The $^1$H NMR spectrum also showed the protons of two aliphatic methine [$\delta$ 4.08 (1H, *dd*, $J$ = 3.5, 6.5 Hz, H-10), 3.85 (1H, *dq*, $J$ = 3.5, 6 Hz, H-11)], three methylene [$\delta$ 2.2 – 2.4 (4H, *m*, H-4, H-5), 3.27, 3.30 (1H each, *dd*, $J$ = 6, 14.5 Hz, H-1′)], and three methyl [$\delta$ 1.12 (3H, *d*, $J$ = 6 Hz, H-12), 1.19 (6H, *s*, H-3′/H-4′)] carbons, and an amide proton [$\delta$ 6.26 (1H, *br t*, $J$ = 6 Hz)]. The $^1$H–$^1$H COSY spectrum indicated correlations of H-2–H-3–H-4–H-5–H-6–H-7–H-8–H-9–H-10–H-11–H-12 and NH–H-1′. The COSY spectrum also showed the $^1$H–$^1$H long-range correlations H-1′–H-3′/H-4′. These correlations and the molecular formula indicated that the structure of ZP-amide C is compound **3**. The HMBC spectrum showed correlations of the amide carbon C-1 with H-3, H-2, NH, and H-1′, which substantiated the connectivity C-2–CO–NH–C-1′. Although the optical inactivity indicated that this compound was a racemic mixture, the relative configuration at C-10 and C-11 remains to be analyzed.

ZP-amide D (**4**) was obtained as an unstable colorless syrup. The molecular formula $C_{16}H_{27}NO_4$ was indicated by ESI- and FAB-MS. Although the $^1$H NMR signal pattern (see Section 4) resembled that of **3**, the $J_{10,11}$ (coupling constant between H-10 and H-11) for **4** (6.5 Hz) differed from that of **3** (3.5 Hz), and the signals of H-9, H-10, and H-11 were shifted upfield, relative to the corresponding protons of **3**. Because **4** was optically inactive, it was a racemic mixture. These data suggest that **4** has the same overall structure as that of **3**, but the relative stereochemistry concerning the asymmetric carbons C-10 and C-11 is different. The $^{13}$C NMR spectrum of **4** was similar to that of **3**, except for the following changes in the chemical shifts of C-9–C-12 carbons: $\delta$ 131.5 (**3**) → $\delta$ 132.7 (**4**) (C-9); $\delta$ 76.0 (**3**) → 77.1 (**4**) (C-10); $\delta$ 70.2 (**3**) → 70.7 (**4**) (C-11); and $\delta$ 17.8 (3) → 18.9 (**4**) (C-12).

Compound **4** was much less stable than **3**, and treatment with acetone resulted in **4a**, which had signals in the $^1$H and $^{13}$C NMR spectra that suggested the existence of a newly formed dimethyl ketal residue [$\delta_H$ 1.42 and 1.43 (3H each, *s*, *gem*-dimethyl); $\delta_C$ 27.0, 27.3 (C-

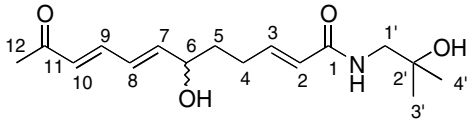
2″ × 2), 108.4 (C-1″)]. Downfield shifts of C-10 and C-11 [δ 77.1 (**4**) → 83.7 (**4a**) (C-10); δ 70.7 (**4**) → 76.8 (**4a**) (C-11)] in the $^{13}$C NMR spectrum further substantiated the presence of vicinal hydroxyl groups in **4**. Rotating-frame Overhauser spectroscopy (ROESY) indicated spatial proximity of H-2″a (one of the *gem*-dimethyl, δ 1.42) with H-11 and H-9. Protons on the other side of the newly formed five-membered ring showed ROE correlations H-2″b (δ 1.43) – H-10–H-12. The H-10 proton also showed ROE with H-8. These data indicate that the stereochemistry around the five-membered ring was that shown in Fig. 1, or its mirror image. The 10*R**, 11*R** configuration was therefore assigned to **4a**. Based on these arguments, the configurations at C-10 and C-11 in **3** and **4** were assigned as (10*R**, 11*S**) and (10*R**,11*R**), respectively. A compound with the same overall structure as those of **3** and **4** was recently reported (Higashi and Komatsu, 2002), although the stereostructure was not given. Since these two were racemic, compounds **3** and **4** were represented as (10*RS*,11*SR*)- and (10*RS*,11*RS*)-(2*E*,6*Z*,8*E*)-10,11-dihydroxy-*N*-(2-hydroxy-2-methylpropyl)-2,6,8-dodecatrienamide, respectively.

### 2.5. ZP-amides E and F

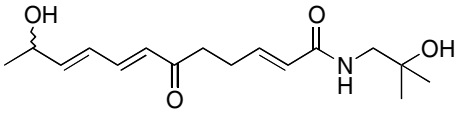
ZP-amide E (**5**) was obtained as unstable colorless syrup. The ESI-MS indicated the molecular formula $C_{16}H_{27}NO_4$. The $^1$H NMR spectrum [in $(CD_3)_2CO$] showed signals of three pairs of protons on double bonds [δ 6.04 (1H, *dt*, *J* = 15, 1 Hz, H-2), 6.78 (1H, *dt*, *J* = 15, 7 Hz, H-3); δ 5.68 (1H, *br dd*, *J* = 6.5, 15 Hz, H-7), 6.20 (1H, *ddd*, *J* = 10, 15, 1 Hz, H-8); δ 6.18 (1H, *ddd*, *J* = 10, 15, 1 Hz, H-9), 5.70 (1H, *br dd*, *J* = 6.5, 15 Hz, H-10)]. The same spectrum showed signals of protons on two methine carbons, each of which bears a hydroxyl group [δ 4.12 (1H, *m*, H-6) that couples with a hydroxyl proton at δ 3.84 (1H, *br d*, *J* = 4 Hz); δ 4.26 (1H, *m*, H-11), which couples with a hydroxyl proton at δ 3.72 (1H, *br d*, *J* = 4 Hz)], three sets of methylene protons [δ 1.62 (2H, *m*, H-5), 2.26 (2H, *m*, H-4), 3.24 (2H, *d*, *J* = 6 Hz, H-1′)], three sets of methyl protons [δ 1.12 (6H, *s*, H-3′/H-4′), 1.18 (3H, *d*, *J* = 6.5 Hz, H-12)], and an NH proton [δ 7.15 (1H, *br*)]. The $^1$H–$^1$H COSY spectrum showed a series of connectivity from H-2 to H-12, along with a correlation H-1′–NH and a long-range correlation H-1′–H-3′/H-4′. The HMBC spectrum showed correlations of H-2, H-3 and H-1′ to the amide carbonyl carbon at δ 167.1, thus indicating connectivity between the aliphatic acid and the amine moieties. The plain structure of ZP-amide E was therefore represented by formula **5**. The optical inactivity of this compound indicated that it was a racemic mixture. However, the relative configuration of the two asymmetric carbons still remains to be determined.

ZP-amide F (**6**) was obtained as an unstable colorless syrup. The ESI-MS indicated the molecular formula $C_{16}H_{27}NO_4$. Although the $^1$H and $^{13}$C NMR spectra of **6** was almost indistinguishable from those of **5**, $^1$H chemical shifts of the OH and NH protons, and the HPLC retention times (*Rts*) of **5** and **6** were different (*Rt* 8.8 and 10.4 min for **5** and **6**, respectively; HPLC conditions are presented in the Section 4). Therefore, ZP-amide F is the stereoisomer of **5** with respect to the relative configuration at the two asymmetric carbons, C-6 and C-11, although the optical inactivity of **6** again indicated that this was a racemate.

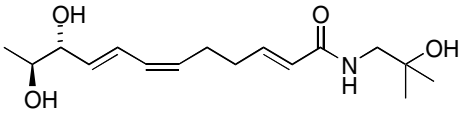
Therefore, compounds **5** and **6** were (6*RS*,11*SR*)- and (6*RS*,11*RS*)-(2*E*,7*E*,9*E*)-6,11-dihydroxy-*N*-(2-hydroxy-2-methylpropyl)-2,7,9-dodecatrienamides



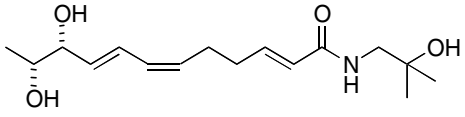

**1**



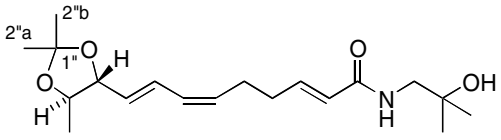

**2**



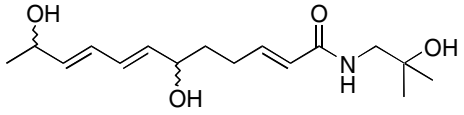

**3** (relative configuration)




**4** (relative configuration)




**4a** (relative configuration)




**5** and **6** (diastereoisomer)

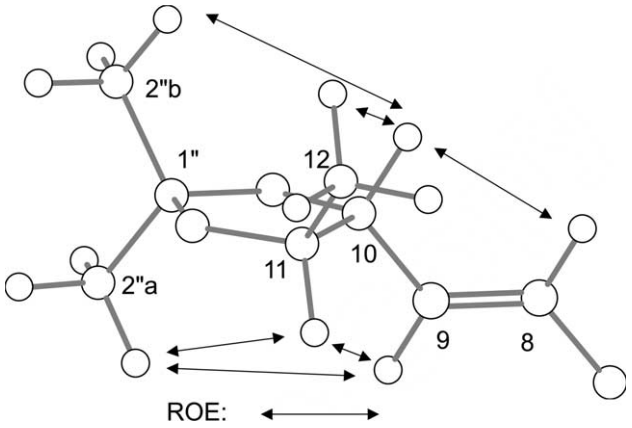



Fig. 1. Observed rotating frame Overhauser effects (ROEs) and stereostructure around the five-membered ring of compound **4a**.

## 3. Conclusion

Unsaturated fatty acid amides are constituents characteristic to *Zanthoxylum* species (Yasuda et al., 1982; Xiong et al., 1997; Chen et al., 1999). Present investigation revealed presence of the aliphatic acid amides with oxygen functions, **1**–**6**, in the fruit pericarps of *Z. piperitum*. These compounds were assignable to be formed from the corresponding unsaturated fatty acid amides by oxidation (and hydration). The optical inactivity of all of them suggested that the oxidation is non-enzymatic. Although it is unclear whether the oxidation proceeds in living organism of plants or after harvesting, these products suggest that unsaturated fatty acid amides are labile to oxidation. The compounds shown in the present study should therefore be taken into account when results of quantitative analyses using the fatty acid amides will be correlated to taxonomic significance.

Because the contribution of unsaturated fatty acid amide constituents to pharmacological properties is known (Hashimoto et al., 2001), the amides that have been identified in the present study may be related to the medicinal properties of *Z. piperitum*.

## 4. Experimental

$^1$H and $^{13}$C NMR spectra were measured on a Varian Mercury 300, VXR-500, or INOVA AS600 instrument in $(CD_3)_2CO$ (with or without $D_2O$) or $CDCl_3$. Chemical shifts were presented in $\delta$ (ppm), based on the signals of the solvents [$\delta_H$ 2.04 and $\delta_C$ 29.8 for $(CD_3)_2CO$; $\delta_H$ 7.26 and $\delta_C$ 77.0 for $CDCl_3$]. ESI-MS was performed on a Micromass Autospec OA-Tof instrument, and the solvent was 50% MeOH (aq.) that contained 0.1% ammonium acetate. Fast-atom bombardment mass spectroscopy (FAB-MS) was conducted on a VG-70SE instrument, and glycerol was used for the matrix. Analytical HPLC was performed at 40 °C on a YMC ODS A302 column, with aqueous MeOH as the mobile phase. For the analysis of **5** and **6**, MeOH–$H_2O$ (3:7) was used as the mobile phase, and the flow rate was 1.0 ml/min. Detection was effected by UV absorption at 230 nm.

### 4.1. Isolation of amides from pericarp of Zanthoxylum piperitum fruit

Dried fruits of *Z. piperitum* were purchased from Tochimoto-tenkai-do (Osaka, Japan) (No. of the specimen, 070301), and seeds were removed from the fruit. The pericarp that was thus obtained (420 g) was homogenized in MeOH, and the concentrated filtrate of the homogenate was extracted successively with *n*-hexane, $Et_2O$, and EtOAc successively. The EtOAc extract (4.2 g) was then subjected to CC (Toyopearl HW-40C) in 70% EtOH. The fraction that contained amides was successively applied to a MCI-gel CHP-20P column, and thereafter on a YMC-gel ODS AQ120-50S, using MeOH–$H_2O$ (3:7) as the mobile phase. The fractions were purified further with preparative HPLC on a YMC A324 column, which resulted in the isolation of amides **1** (5.4 mg), **2** (4.8 mg), **3** (10.1 mg), **4** (4.0 mg), **5** (17.2 mg), and **6** (9.5 mg).

### 4.2. ZP-amide A (*1*)

Unstable colorless syrup. $[\alpha]_D \pm 0°$ (*c* 2, acetone). ESI-MS *m/z*: 296 ($[M + H]^+$), 318 ($[M + Na]^+$). HR-ESI-MS *m/z*: 296.1818 ($[M + H]^+$) (Calculated for $C_{16}H_{25}NO_4 + H$, 296.1862). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$: 270 (4.63). For $^1$H and $^{13}$C NMR: see Section 2.2.

### 4.3. ZP-amide B (*2*)

Unstable colorless syrup. $[\alpha]_D \pm 0°$ (*c* 1, MeOH). ESI-MS *m/z*: 296 ($[M + H]^+$), 318 ($[M + Na]^+$). HR-ESI-MS *m/z*: 296.1901 ($[M + H]^+$) (Calculated for $C_{16}H_{25}NO_4 + H$, 296.1862). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 271 (4.66). $^1$H NMR: see Section 2.3 $^{13}$C NMR [in $(CD_3)_2CO$]: $\delta$ 23.6 (C-12), 26.9 (C-4), 27.6 (C-3′/C-4′), 39.1 (C-5), 51.2 (C-1′), 67.7 (C-11), 70.9 (C-2′), 125.5 (C-2), 127.2 (C-9), 130.0 (C-7), 143.0 (C-8), 143.1 (C-3), 149.4 (C-10), 166.9 (C-1), 198.9 (C-6).

### 4.4. ZP-amide C (*3*)

Unstable colorless syrup. $[\alpha]_D \pm 0°$ (*c* 1, MeOH). ESI-MS *m/z*: 298 ($[M + H]^+$), 320 ($[M + Na]^+$). HR-ESI-MS *m/z*: 298.2018 ($[M + H]^+$) (Calculated for $C_{16}H_{27}NO_4 + H$, 298.2018). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 233

(4.69). $^1$H NMR: see Section 2.4 $^{13}$C NMR (in $CDCl_3$): $\delta$ 17.8 (C-12), 26.3 (C-5), 27.2, 27.3 (C-3′/C-4′), 31.7 (C-4), 50.3 (C-1′), 70.2 (C-11), 71.1 (C-2′), 76.0 (C-10), 123.9 (C-2), 127.9 (C-8), 129.2 (C-7), 130.4 (C-6), 131.5 (C-9), 144.3 (C-3), 167.2 (C-1).

### 4.5. ZP-amide D (*4*)

Unstable colorless syrup. $[\alpha]_D \pm 0°$ (*c* 1, MeOH). ESI-MS *m/z*: 298 ($[M + H]^+$), 320 ($[M + Na]^+$). FAB-MS *m/z*: 298 ($[M + H]^+$). HR-FAB-MS *m/z*: 298.1985 ($[M + H]^+$) (Calculated for $C_{16}H_{27}NO_4$ + H, 298.2018). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 230 (4.71). $^1$H NMR ($CDCl_3$): $\delta$ 1.15 (3H, *d*, *J* = 6 Hz, H-12), 1.21, 1.22 (3H each, *s*, H-3′/H-4′), 2.2 - 2.4 (4H, *m*, H-4, 5), 3.30 (2H, *br d*, *J* = 6.5 Hz, H-1′), 3.62 (1H, quintet, *J* = 6.5 Hz, H-11), 3.87 (1H, *br t*, *J* = 6.5 Hz, H-10), 5.43 (1H, *br dt*, *J* = 11, 7 Hz, H-6), 5.63 (1H, *dd*, *J* = 6.5, 15 Hz, H-9), 5.79 (1H, *dt*, *J* = 15, 1 Hz, H-2), 5.97 (1H, *br*, NH), 6.04 (1H, *t*, *J* = 11 Hz, H-7), 6.51 (1H, *ddt*, *J* = 11, 15, 1 Hz, H-8), 6.79 (1H, *dt*, *J* = 15, 7 Hz, H-3). $^{13}$C NMR ($CDCl_3$): $\delta$ 18.9 (C-12), 26.4 (C-5), 27.3, 27.4 (C-3′/C-4′), 31.7 (C-4), 50.2 (C-1′), 70.7 (C-11), 71.2 (C-2′), 77.1 (C-10), 123.9 (C-2), 127.8 (C-8), 129.2 (C-7), 130.5 (C-6), 132.7 (C-9), 144.5 (C-3), 167.1 (C-1). Treatment of **4** (1 mg) in acetone (1 ml) in the presence of HCl (0.004%) (2 h) gave **4a**, $[\alpha]_D \pm 0°$ (*c* 1, MeOH). ESI-MS *m/z*: 338 ($[M + H]^+$). FAB-MS *m/z*: 338 ($[M + H]^+$). HR-FAB-MS *m/z*: 338.2295 ($[M + H]^+$) (Calculated for $C_{19}H_{31}NO_4$ + H, 338.2331). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 231 (4.83). $^1$H NMR ($CDCl_3$): $\delta$ 1.235, 1.237 (3H each, *s*, H-3′/H-4′), 1.26 (1H, *d*, *J* = 6.5 Hz, H-12), 1.42, 1.43 (3H each, *s*, H-2″), 2.29 (2H, *m*, H-4), 2.36 (2H, *m*, H-5), 3.33 (2H, *d*, *J* = 5.5 Hz, H-1′), 3.79 (1H, *dq*, *J* = 8, 6.5 Hz, H-11), 3.98 (1H, *br t*, *J* = 8 Hz, H-10), 5.47 (1H, *br dt*, *J* = 11, 8 Hz, H-6), 5.61 (1H, *dd*, *J* = 8, 15 Hz, H-9), 5.85 (1H, *dt*, *J* = 15, 1 Hz, H-2), 5.96 (1H, *br*, NH), 6.04 (1H, *br t*, *J* = 11 Hz, H-7), 6.57 (1H, *ddt*, *J* = 11, 15, 1 Hz, H-8), 6.83 (1H, *dt*, *J* = 15, 6.5 Hz, H-3). $^{13}$C NMR ($CDCl_3$): $\delta$ 16.5 (C-12), 26.4 (C-5), 27.0, 27.3 (C-2″ × 2), 27.4 (C-3′/C-4′), 31.8 (C-4), 50.4 (C-1′), 71.0 (C-2′), 76.8 (C-11), 83.7 (C-10), 108.4 (C-1″), 124.0 (C-2), 128.4 (C-7), 129.1 (C-8), 129.7 (C-9), 131.7 (C-6), 143.9 (C-3), 166.9 (C-1).

### 4.6. ZP-amide E (*5*)

Unstable colorless syrup. $[\alpha]_D \pm 0°$ (*c* 1, MeOH). ESI-MS *m/z*: 298 ($[M + H]^+$). HR-ESI-MS *m/z*: 298.2040 ($[M + H]^+$) (Calculated for $C_{16}H_{27}NO_4$ + H, 298.2018). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 230 (5.04). $^1$H NMR: see Section 2.5 $^{13}$C NMR [in $(CD_3)_2CO$]: $\delta$ 24.0 (C-12), 27.6 (C-3′/C-4′), 28.6 (C-4), 37.0 (C-5), 51.2 (C-1′), 68.0 (C-11), 70.9 (C-2′), 71.5 (C-6), 125.1 (C-2), 128.8 (C-9), 130.1 (C-8), 137.3 (C-7), 139.1 (C-10), 144.0 (C-3), 167.1 (C-1).

### 4.7. ZP-amide F (*6*)

Unstable colorless syrup, $[\alpha]_D \pm 0°$ (*c* 1, MeOH). ESI-MS *m/z*: 298 ($[M + H]^+$). HR-ESI-MS *m/z*: 298.2010 ($[M + H]^+$) (Calcd. for $C_{16}H_{27}NO_4$ + H, 298.2018). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 230 (5.00). $^1$H NMR [in $(CD_3)_2CO$]: $\delta$ 1.12 (6H, *s*, H-3′/H-4′), 1.18 (3H, *d*, *J* = 6.5 Hz, H-12), 1.62 (2H, *m*, H-5), 2.26 (2H, *m*, H-4), 3.24 (2H, *d*, *J* = 6 Hz, H-1′), 3.70 (1H, *br d*, *J* = 4 Hz, OH at C-11), 3.81 (1H, *br d*, *J* = 4 Hz, OH at C-6), 4.12 (1H, *m*, H-6), 4.26 (1H, *m*, H-11), 5.68 (1H, *br dd*, *J* = 6.5, 15 Hz, H-7), 5.70 (1H, *br dd*, *J* = 6.5, 15 Hz, H-10), 6.04 (1H, *dt*, *J* = 15, 1 Hz, H-2), 6.18 (1H, *ddd*, *J* = 1, 10, 15 Hz, H-9), 6.20 (1H, *ddd*, *J* = 1, 10, 15 Hz, H-8), 6.78 (1H, *dt*, *J* = 15, 7 Hz, H-3), 7.12 (1H, *br*, NH). $^{13}$C NMR [in $(CD_3)_2CO$]: $\delta$ 24.0 (C-12), 27.6 (C-3′/C-4′), 28.6 (C-4), 37.0 (C-5), 51.2 (C-1′), 68.0 (C-11), 70.9 (C-2′), 71.5 (C-6), 125.2 (C-2), 128.8 (C-9), 130.0 (C-8), 137.3 (C-7), 139.1 (C-10), 144.0 (C-3), 167.1 (C-1).

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