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Osirisynes A-F, Highly Oxygenated Polyacetylenes from the Sponge *Haliclona osiris*

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Abstract: Osirisynes A-F(**1-6**), highly oxygenated C_{47} polyacetylenes have been isolated from the sponge *Haliclona osiris* collected from Guam. These compounds possess a diacetylenic carbinol and an α-acetylenic carboxylic acid as common structural features. The structures of osirisynes have been determined by combined spectroscopic methods. These compounds exhibited moderate cytotoxicity and inhibitory activities against Na^+/K^+-ATPase and reverse transcriptase (RT). © 1998 Elsevier Science Ltd. All rights reserved.

Linear polyacetylenes and related compounds are a rapidly growing class of sponge metabolites.[1] Although these compounds are found in only a few families of sponges, sponge-derived polyacetylenes vary greatly in both chain-lengths and functional groups. Several sponge-derived polyacetylenes exhibit potent bioactivities including antimicrobial, cytotoxic, antiviral, RNA-cleaving, and enzyme-inhibitory activities as well as brine-shrimp lethality.[1-15] In addition, some of these compounds have important ecological roles: inducing metamorphosis of ascidian larvae, preventing fouling by barnacle larvae, and inhibiting fertilization of starfish gametes.[16,17]

In our continuing search for novel secondary metabolites of biomedical and ecological importances from tropical marine animals, we collected the sponge *Haliclona osiris* (de Laubenfels 1954) in Apra Harbor, Guam. The organic crude extract of this animal exhibited potent toxicity against brine-shrimp larvae (LC_{50} 52 ppm) as well as moderate inhibitory activity against Na^+/K^+-ATPase. Bioassay-guided partitioning and separation by chromatographic methods yielded several secondary metabolites. We report herein the structures and bioactivities of osirisynes A-F, highly oxigenated C_{47} linear polyacetylenes. These compounds possessed a diacetylenic carbinol and an α-acetylenic carboxylic acid group as common structural features that made osirisynes structurally comparable to petrosolic acid and nepheliosyne A, previously isolated from the sponges *Petrosia* sp. and *Xestospongia* sp., respectively.[5,7] However, the structures of these compounds differ significantly from each other in their functional groups and the locations of the functional groups in carbon skeletons. Osirisynes exhibited moderate cytotoxicity against a human leukemia cell-line as well as inhibitory activities against Na^+/K^+-ATPase and reverse transcriptase (RT).

The sponge was collected by hand while scuba diving in Apra Harbor, Guam.[18] The specimens were lyophilized, macerated, and exhaustively extracted with CH_2Cl_2 and MeOH. The combined crude extracts were

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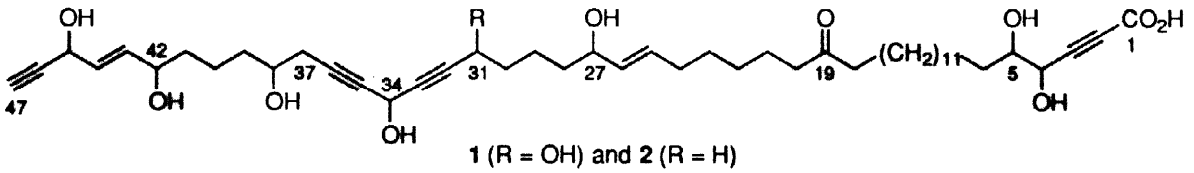
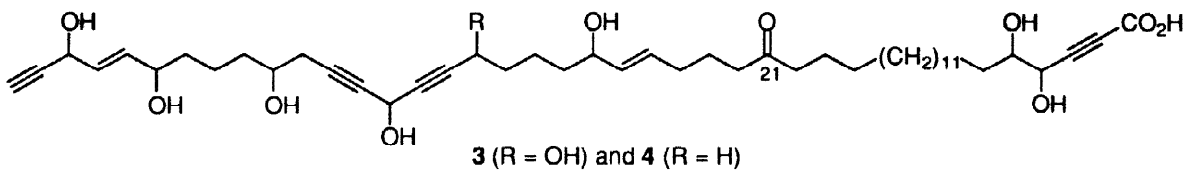


1 (R = OH) and 2 (R = H)



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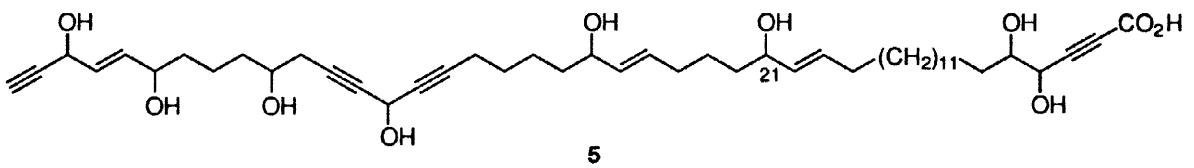

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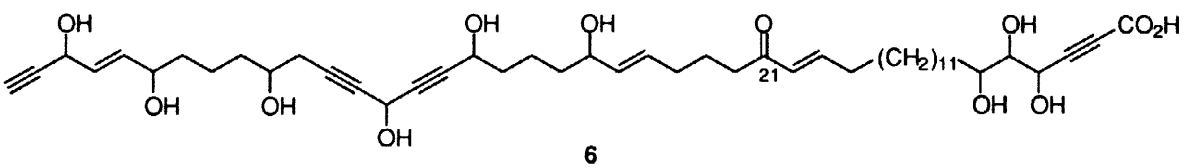

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partitioned between *n*-BuOH and water. The *n*-BuOH layer was dried and re-partitioned between hexane and aqueous MeOH. The polar organic materials were separated by reversed-phase vacuum flash chromatography using gradient mixtures of MeOH and water as eluents. Subsequently, repeated use of reversed-phase HPLC of the polar flash chromatographic fraction (40-20% aqueous MeOH) led to the isolation of osirisynes A-F(**1-6**) as pure compounds.

Osirisyne A(**1**) was isolated as a white amorphous solid. The molecular formula for this compound was deduced as $C_{47}H_{72}O_{11}$ by a combination of HRFABMS and ^{13}C NMR spectrometry. The presence of a ketone and an α,β-unsaturated carboxyl group were readily recognized by carbon signals at δ 214.4 and 161.2, respectively, in the ^{13}C NMR spectrum. This interpretation was supported by a strong absorption band at 1700 cm^{-1} in the IR spectrum and an absorption maximum at 209 nm in the UV spectrum. Similarly the presence of two double bonds, four acetylenes and several hydroxyl groups were indicated by characteristic carbon signals in the ^{13}C NMR spectrum (Table 1). The *E* olefin geometry was assigned for both double bonds on the basis of the 15.2 Hz vicinal coupling constants.

With the aid of this information, the structure of **1** was determined by a combination of 2-D NMR experiments. All of the proton-bearing carbons and their protons were precisely matched by HSQC and HETCOR experiments. Combined with ^1H COSY data, partial structures of **1** were elucidated as depicted in Figure 1. Connectivities among these as well as the structure of the entire molecule were established by

Table 1. ^{1}H and ^{13}C NMR Data for Osirisyne A(**1**).

No.	H[a]	C[b]	No.	H[a]	C[b]
1		161.2	31	4.33, 1H, br dd (7.9, 6.7)	62.7
2		80.1	32		85.5
3		83.7	33		83.3
4	4.18, 1H, d (4.4)	67.3	34	5.11, 1H, br s	52.5
5	3.55, 1H, dd (8.3, 4.4)	75.5	35		80.8
6	1.65, 1H, m; 1.45, 1H, m	33.4	36		82.1
7	1.53, 1H, m; 1.32, 1H, m	26.9	37	2.37, 2H, br dd (5.6, 1.8)	28.2
17	1.55, 2H, m	24.9	38	3.69, 1H, m	70.9
18	2.45, 2H, t (7.2)	43.5[c]	39	1.63, 1H, m; 1.46, 1H, m	37.0
19		214.4	40	1.53, 2H, m	22.6
20	2.45, 2H, t, (7.2)	43.4[c]	41	1.57, 1H, m; 1.49, 1H, m	38.1
21	1.55, 2H, m	24.7	42	4.09, 1H, m	72.4
22	1.32, 2H, m	29.8	43	5.88, 1H, ddt (15.2, 6.4, 1.1)	136.2
23	1.38, 2H, p (7.3)	30.2	44	5.75, 1H, ddt (15.2, 5.6, 1.1)	130.5
24	2.04, 2H, dt (7.1, 7.3)	33.1	45	4.81, 1H, br d (5.6)	62.7
25	5.62, 1H, dt (15.2, 7.1)	132.4	46		84.5
26	5.43, 1H, br dd (15.2, 7.1)	134.4	47	2.90, 1H, br s	74.8
27	3.97, 1H, m	73.6	others[d]	1.30 - 1.26, 18H, m	30.8
28	1.53, 1H, m; 1.46, 1H, m	38.1			30.7
29	1.46, 2H, m	22.4			30.6
30	1.68, 2H, m	38.7			30.3

[a,b] measured in a CD_3OD solution at 500 and 125 MHz, respectively. Assignments were aided by ^{1}H COSY, TOCSY, HSQC, HMBC, and DEPT experiments. [c] Interchangeable signals. [d] Positions at 8 - 16. Due to the overlapping of both proton and carbon signals, assignments for each position were not possible.

combined HMBC and TOCSY experiments. Long-range correlations of methylene carbon signals at δ 22.6 and 22.4, distinctively upfield-shifted by the γ-effect of hydroxyl groups, with neighboring protons were particularly helpful to determine the connectivities among **a**-**c**. That is, the carbon signal at δ 22.6 displayed long-range couplings with the methine proton signals at δ 4.09(**a**) and 3.69(**b**) connecting the partial structures **a** and **b**. This interpretation was confirmed by a TOCSY correlation containing both protons. Similarly the connection between **b** and **c** via a methylene was determined by three-bond correlations of the carbon signal at δ 22.4 with the methine proton signals at δ 4.33(**b**) and 3.97(**c**). The placement of an ethylene moiety between **c** and **d** was established by long-range couplings of the carbon signals at δ 29.8 and 24.7 with neighboring protons. This connection was ascertained by a series of TOCSY correlations containing the olefinic proton at δ 5.62(**c**) and an α-carbonyl proton at δ 2.45(**d**). Since the connectivities among four of five partial structures were confidently determined, all of the remaining eleven methylenes had to be located between **d** and **e**, forming a long chain. Thus, the structure of osirisyne A(**1**) was determined as a C_{47} tetraacetylenic carboxylic acid.

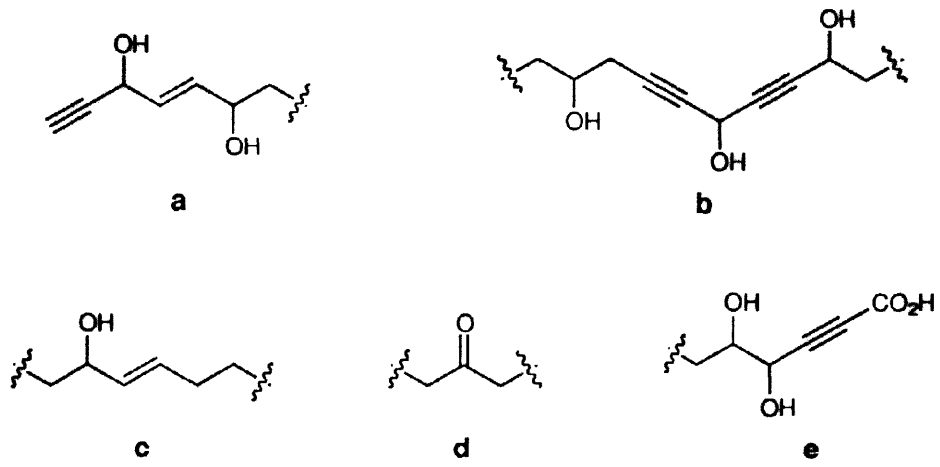

Figure 1. Partial structures of osirisyne A(**1**).

Long-chain polyacetylenes have been frequently isolated from sponges, making them as a representative group of natural products for these animals. However, highly functionalized ones such as **1** are only rarely found. Moreover, the α-acetylenic carboxylic acid moiety of this compound is particularly uncommon. A literature survey revealed that osirisyne A was structurally related only to petrosolic acid and nepheliosyne A, previously isolated from the sponges *Petrosia* sp. and *Xestospongia* sp., respectively.[5,7] However, the structures of these compounds differed considerably from each other. The chain-length (petrosolic acid) and functional groups as well as their locations in the carbon framework differed among the molecules. In addition to these compounds, to the best of our knowledge, corticatic acids from the sponge *P. corticata* are the only other examples of linear acetylenes containing the α-acetylenic carboxylic acid functionality.[8]

The molecular formula for osirisyne B(**2**), a white solid, was deduced as $C_{47}H_{72}O_{10}$ by combined HRFABMS and ^{13}C NMR analysis. The spectral data for this compound were similar to those derived from **1**. However, the ^{13}C NMR data showed that the signal of a hydroxyl-bearing methine carbon in **1** was replaced by that of a methylene carbon at δ 19.3 (Table 2). The upfield shift of this carbon indicated that a hydroxyl group attached to one of the α-acetylenic carbons (C-4, C-31, and C-45) was removed. In the ^1H NMR spectrum, the signal of the H-31 proton at δ 4.33 (1H, br dd, J = 7.9, 6.7 Hz) of **1** was replaced by that of a methylene at δ 2.22 (2H, td, J = 6.8, 2.0 Hz) in **2**. Thus, the structure of osirisyne B(**2**) was defined as the 31-dehydroxy derivative of osirisyne A(**1**), and this assignment was confirmed by detailed 2-D NMR experiments.

The molecular formula of osirisyne C(**3**) was analyzed for $C_{47}H_{72}O_{11}$, identical with that of **1**, by combined HRFABMS and ^{13}C NMR spectrometry. The spectral data for this compound were very similar to those obtained for **1**. However, careful examination of the ^{13}C NMR data revealed that chemical shifts of the carbons at C-20–C-26 of **1** were significantly changed in **3** (Table 2). Corresponding differences were also observed in the ^1H NMR spectrum in which the signal of the H-23 proton at δ 1.38 (2H, p, J = 7.3 Hz) of **1** was replaced by that of a methylene at δ 1.64 (2H, p, J = 7.3 Hz). In addition, signals of the α-carbonyl H-18 and H-20 protons, identically observed at δ 2.45 (4H, t, J = 7.2 Hz) in **1**, were slightly but noticeably shifted to δ 2.46 (2H, t, J = 7.3 Hz) and 2.44 (2H, t, J = 7.3 Hz). Accordingly the length of the methylene chain between the C-19 carbonyl and C-25 double bond of **1** must be changed in **3**. A combination of 2-D NMR experiments revealed that only three methylenes, instead of the five in **1**, were located between the carbonyl and double bond in **3**. Supporting evidence for this interpretation was provided by the HMBC data: several

Table 2. Carbon NMR Assignments for Compounds 2-6.

No.	2		3		4		5		6	
1	161.2	s	161.2	s	161.2	s	161.2	s	162.2	s
2	80.2	s	80.1	s	80.2	s	80.3	s	79.8	s
3	83.6	s	83.7	s	83.6	s	83.6	s	83.9	s
4	67.3	d	67.3	d	67.3	d	67.3	d	65.2	d
5	75.5	d	75.5	d	75.5	d	75.5	d	78.7	d
6	33.4	t	33.4	t	33.5	t	33.5	t	72.9	d
7	26.9	t	26.9	t	26.9	t	26.9	t	34.2	t
8	NA[a]		NA[a]		NA[a]		NA[a]		26.6	t
17	24.9	t	NA[a]		NA[a]		30.3	t	24.9	t
18	43.5	t[b]	30.3	t	30.3	t	33.3	t[c]	43.5	t
19	214.4	s	24.9	t	24.9	t	132.5	d	214.4	s
20	43.4	t[b]	43.6	t	43.6	t	134.6	d	43.5	t
21	24.7	t	214.2	s	214.2	s	73.6	d	24.7	t
22	29.8	t	42.7	t	42.6	t	37.9	t	29.8	t
23	30.2	t	24.4	t	24.3	t	26.4	t	30.2	t
24	33.1	t	32.6	t	32.6	t	33.2	t[c]	33.1	t
25	132.3	d	131.7	d	131.6	d	132.3	d	132.4	d
26	134.5	d	135.1	d	135.2	d	134.4	d	134.5	d
27	73.6	d	73.5	d	73.5	d	73.6	d	73.6	d
28	37.9	t	38.1	t	37.9	t	37.9	t	38.1	t
29	25.9	t	22.4	t	25.9	t	26.0	t	22.5	t
30	29.6	t	38.7	t	29.6	t	29.6	t	38.8	t
31	19.3	t	62.7	d	19.3	t	19.3	t	62.7	d
32	79.7	s	85.5	s	79.7	s	79.7	s	85.6	s
33	84.6	s	83.4	s	84.6	s	84.6	s	83.4	s
34	52.6	d	52.5	d	52.6	d	52.6	d	52.5	d
35	81.3	s	80.8	s	81.3	s	81.3	s	80.8	s
36	81.5	s	82.1	s	81.6	s	81.6	s	82.1	s
37	28.2	t	28.2	t	28.1	t	28.2	t	28.2	t
38	70.9	d	70.9	d	70.9	d	70.9	d	70.9	d
39	37.0	t	37.0	t	37.0	t	37.0	t	37.0	t
40	22.6	t	22.6	t	22.6	t	22.6	t	22.6	t
41	38.1	t	38.1	t	38.1	t	38.1	t	38.1	t
42	72.4	d	72.4	d	72.4	d	72.4	d	72.4	d
43	136.2	d	136.2	d	136.2	d	136.2	d	136.3	d
44	130.5	d	130.5	d	130.5	d	130.6	d	130.6	d
45	62.7	d	62.7	d	62.7	d	62.7	d	62.7	d
46	84.5	s	84.5	s	84.5	s	84.5	s	84.4	s
47	74.8	d	74.8	d	74.8	d	74.8	d	74.8	d

measured in CD_3OD solutions at 125 MHz. Assignments were aided by DEPT, HETCOR, HSQC, and HMBC experiments. [a] NA = not assigned. [b,c] Interchangeable signals.

correlations of the H-22~H-25 protons with neighboring carbons were observed. In addition, the TOCSY data showed a definite correlation containing all of the H-22~H-25 protons. Thus, the structure of osirisyne C(3) was defined as the 19-deoxo-21-oxo derivative of osirisyne A(1).

Another related compound, osirisyne D(4) was isolated as a white solid. The molecular formula of this compound was established as $C_{47}H_{72}O_{10}$ by combined HRFABMS and ^{13}C NMR analysis. As observed for compound 2, the NMR data for this compound indicated the replacement of an α-acetylenic hydroxyl group by a hydrogen atom; ^{13}C NMR δ 19.3 (CH_2). A combination of 2-D NMR experiments showed that it was the 31-hydroxyl group replaced by a hydrogen while the remaining portion including the methylene chain between the C-21 carbonyl and C-25 double bond was identical with that of 3. Thus, the structure of 4 was defined as the 31-dehydroxy derivative of 3, possessing the same structural relationship as found between 1 and 2.

The molecular formula for osirisyne E(5), a white solid, was deduced as $C_{47}H_{72}O_{10}$ by combined HRMS and ^{13}C NMR analysis. The spectral data for this compound were similar to those obtained for 2 and 4. However, the ^{13}C NMR spectrum of 5 indicated the disappearance of the carbonyl group located in the middle of molecule (C-19 of 2 or C-21 of 4). This observation was supported by loss of the carbonyl stretching band in the IR data. Instead, signals for a new double bond and a hydroxyl-bearing methine appeared at δ 134.6 (CH), 132.5 (CH), and 73.6 (CH), respectively (Table 2). Corresponding differences were also observed in the ^1H NMR spectrum: new signals appeared at δ 5.60 (1H, dt, J = 15.6, 6.8 Hz), 5.40 (1H, dd, J = 15.6, 6.8 Hz), and 3.96 (1H, m). The proton-decoupling and ^1H COSY experiments showed that these protons were directly connected to each other. Therefore, the spectral changes could be accommodated by a replacement of the carbonyl group at C-19 of 2 (or C-21 of 4) by an ene-ol functionality. This interpretation was verified by a combination of TOCSY, HSQC, and HMBC experiments. In addition, 2-D NMR data showed that only three methylenes were placed between two allylic-hydroxyl groups (Figure 2). Based upon a 15.6 Hz vicinal coupling constant, the E geometry was assigned for the newly formed C-19 double bond. Thus, the structure of osirisyne E(5) was defined as a highly functionalized C_{47} linear polyacetylene.

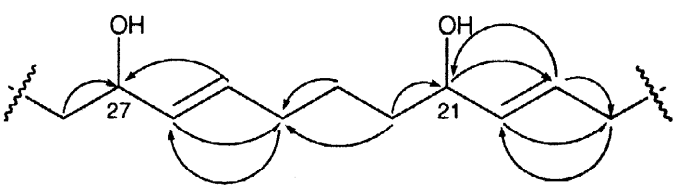

Figure 2. A partial structure of 5 and key HMBC correlations

The molecular formular of osirisyne F(6) was deduced as $C_{47}H_{72}O_{12}$ by HRFABMS and ^{13}C NMR analysis. Although the spectral data for this compound were very similar to those obtained for 3, the NMR data showed the presence of an additional hydroxyl group; ^{13}C δ 72.9 (CH), ^1H δ 3.61 (1H, m) (Table 2). A combination of the HSQC and ^1H COSY experiments allowed us to assign the attachment of a new hydroxyl group to C-6. This interpretation was confirmed by a HMBC correlation between the signal of the hydroxyl-bearing carbon at δ 72.9 and that of the H-5 proton at δ 3.42 (1H, dd, J = 8.3, 4.4 Hz). Thus, the structure of 6 was defined as the 6-hydroxy derivative of 3.

Sponge-derived polyacetylenes are widely recognized for their diverse and potent bioactivities. For

recent examples, Petrocortynes and petrosiacetylenes, isolated from *Petrosia* sp., exhibited significant RNA-cleaving and enzyme-inhivitory activities, and potent lethality against brine-shrimp larvae.[15] In our bioassay, osirisynes A-F exhibited moderate cytotoxicity against a human leukemia cell-line(K562); LC_{50} 25, 48, 52, 25, 20, and 22 μM for **1-6**, respectively. In addition, **3**, **5**, and **6** exhibited inhibitory activities against Na^+/K^+-ATPase and reverse transcriptase (RT) at concentrations of 1μg/10μl. Ecological roles for these metabolites are currently under investigation.

EXPERIMENTAL

General Experimental Procedures. NMR spectra were recorded in CD_3OD solutions on a Varian Unity-500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me_4Si. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. Mass spectra were obtained by using a VG ZAB-2FHF and a Jeol JMS-HX 110 high-resolution mass spectrometer and provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside and Korea Basic Science Institute, Taejeon, Korea, respectively. The optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. All solvents used were spectral grade or were distilled from glass prior to use.

Animal material. The specimens were collected by scuba diving at 20-25 m depth in July, 1996 at a site known as the Sponge Mound in Apra Harbor, Guam.[18] The sponge is found on many reefs in Apra Harbor, Guam. It is common in many parts of Micronesia and is pictured (p.39, under original name *Prianos osiris*) in the field guide "Tropical Pacific Invertebrates."[19] The sponge has been identified as *Haliclona osiris* (de Laubenfels 1954) (Order Haplosclerida, Family Chalinidae) (M. Kelly-Borges, personal communication). A voucher specimen has been deposited at The Natural History Museum, London (BMNH 1997.5.13.2).

Extraction and isolation. Freshly collected specimens were immediately frozen and stored at -25 °C. The defrosted animals were lyophilized (dry weight 130 g), macerated, and extracted with CH_2Cl_2 (1 L x 3) and MeOH (1 L x 3). The combined crude extracts (33.4 g) were partitioned between *n*-butanol and water. The *n*-butanol layer (8.05g) was dried under vacuum and re-partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (4.05g) was separated by C_{18} reversed-phase vacuum flash chromatography by using sequential mixtures of water and MeOH as eluents; elution order 50, 40, 30, 20, and 10% aqueous MeOH, and MeOH. Fractions eluted with 40 % aqueous MeOH (0.39g) were subjected to reversed-phase HPLC (Shiseido Capcell ODS column, 30% aqueous MeOH) to yield compounds **1**, **3**, and **6**. Final purification was made by reversed-phase HPLC (YMC ODS-AQ column, 65% aqueous MeCN) to afford pure compounds; 98.2 (0.07% of dry animal), 94.6 (0.07%), and 7.6 (0.005%) mg for **1**, **3**, and **6**, respectively. Fractions eluted with 30 and 20% aqueous MeOH were combined and separated by reversed-phase HPLC (YMC ODS-AQ column, 10% aqueous MeOH) to yield **2**, **4**, and **5**. Purification was made by reversed-phase HPLC (50% aqueous MeCN) using the same column; 36.2, 26.1, and 5.3 mg for **2** (0.03% of dry animal), **4** (0.02%), and **5** (0.007%), respectively.

Osirisyne A(**1**)-a white solid, mp 118-120 °C; $[\alpha]^{25}_D$ +11.8 ° (*c* 0.15, MeOH); UV (MeOH) λ max

(log ε) 209 (3.60) nm; IR (KBr) ν max 3400, 2920, 2850, 1700, 1600, 1385, 1070, 1020 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HMBC correlations (6 and 8 Hz) H-4/C-1, C-2, C-3, C-5, C-6; H-5/C-2, C-4; H-18/C-17, C-19; H-20/C-19, C-21, C-22; H-23/C-21, C-22, C-24, C-25; H-24/C-22, C-23, C-25, C-26; H-25/C-23, C-24, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-25, C-28, C-29; H-31/C-29, C-30, C-33, C-34; H-34/C-37; H-37/C-34, C-36, C-38, C-39; H-38/C-40; H-42/C-40, C-41, C-43, C-44; H-43/C-42, C-44, C-45; H-44/C-42, C-43, C-45, C-46; H-45/C-44, C-46, C-47; H-47/C-45; HRFABMS $[M+Na]^+$ *m/z* 835.4986; $C_{47}H_{72}O_{11}Na$ calculated 835.4972(Δ -1.4 mmu).

Osirisyne B(2)-a white solid, mp 123-124 °C; $[\alpha]^{25}_D$ +16.1 ° (*c* 0.12, MeOH); UV (MeOH) λ max (log ε) 207 (3.62) nm; IR (KBr) ν max 3450, 2920, 2850, 1700, 1630, 1365, 1070, 970 cm^{-1}; ^1H NMR (CD_3OD) δ 5.87 (1H, ddd, 15.6, 5.9, 2.0, H-43), 5.75 (1H, ddd, 15.6, 5.9, 2.0, H-44), 5.61 (1H, br dt, 15.6, 6.4, H-25), 5.42 (1H, ddt, 15.6, 6.8, 1.5, H-26), 5.03 (2H, tt, 2.0, 2.0, H-34), 4.81 (1H, br d, 5.9, H-45), 4.18 (1H, d, 5.1, H-4), 4.08 (1H, td, 6.4, 5.9, H-42), 3.96 (1H, m, H-27), 3.69 (1H, m, H-38), 3.54 (1H, ddd, 9.3, 5.1, 3.4, H-5), 2.89 (1H, d, 2.0, H-47), 2.43 (4H, t, 7.3, H-18, H-20), 2.36 (2H, dd, 5.6, 2.0, H-37), 2.22 (2H, td, 6.8, 2.0, H-31), 2.03 (2H, td, 7.3, 6.4, H-24), 1.66 (1H, m, H-6), 1.63 (1H, m, H-39), 1.57 (1H, m, H-41), 1.54 (4H, m, H-17, H-21), 1.52 (4H, m, H-7, H-28, H-30 (2H)), 1.49 (3H, m, H-40 (2H), H-41), 1.47 (4H, m, H-28, H-29 (2H), H-39), 1.45 (1H, m, H-6), 1.38 (2H, p, 7.3, H-23), 1.32 (3H, m, H-7, H-22 (2H)), 1.30-1.26 (18H, m, H-8~H-16); ^{13}C NMR, see Table 2; HMBC correlations (8 Hz) H-4/C-1, C-2, C-3, C-5, C-6; H-5/C-2, C-4, C-6; H-18/C-17, C-19; H-20/C-19, C-21, C-22; H-23/C-21, C-22, C-24, C-25; H-24/C-22, C-23, C-25, C-26; H-25/C-23, C-24, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-25, C-28, C-29; H-31/C-29, C-30, C-32, C-33; H-34/C-32, C-35; H-37/C-34, C-36, C-38, C-39; H-38/C-36; H-42/C-40, C-41, C-43, C-44; H-43/C-41, C-42, C-44, C-45; H-44/C-42, C-43, C-45, C-46, C-47; H-47/C-45; HRFABMS $[M+Na]^+$ *m/z* 819.5047; $C_{47}H_{72}O_{10}Na$ calculated 819.5023 (Δ -2.4 mmu).

Osirisyne C(3)-a white solid, mp 121-122 °C; $[\alpha]^{25}_D$ +13.4 ° (*c* 0.17, MeOH); UV (MeOH) λ max (log ε) 209 (3.56) nm; IR (KBr) ν max 3400, 2920, 2850, 1705, 1595, 1380, 1070, 1020 cm^{-1}; ^1H NMR (CD_3OD) δ 5.87 (1H, ddd, 15.1, 6.3, 1.2, H-43), 5.75 (1H, ddd, 15.1, 5.6, 1.0, H-44), 5.60 (1H, dt, 15.6, 6.8, H-25), 5.44 (1H, br dd, 15.6, 6.8, H-26), 5.10 (1H, br t, 1.5, H-34), 4.81 (1H, br d, 5.6, H-45), 4.33 (1H, ddd, 6.8, 6.4, 1.5, H-31), 4.18 (1H, d, 4.9, H-4), 4.09 (1H, m, H-42), 3.98 (1H, m, H-27), 3.68 (1H, m, H-38), 3.54 (1H, m, H-5), 2.90 (1H, d, 2.0, H-47), 2.46 (2H, t, 7.3, H-22), 2.44 (2H, t, 7.3, H-20), 2.36 (2H, dd, 6.4, 2.0, H-37), 2.03 (2H, td, 7.3, 6.8, H-24), 1.67 (2H, m, H-30),1.65 (1H, m, H-6), 1.64 (2H, p, 7.3, H-23), 1.63 (1H, m, H-39), 1.57 (1H, m, H-41), 1.55 (3H, m, H-7, H-19 (2H)), 1.52 (3H, m, H-28, H-40 (2H)), 1.49 (1H, m, H-41), 1.47 (4H, m, H-28, H-29 (2H), H-39), 1.43 (1H, m, H-6), 1.32 (1H, m, H-7), 1.30-1.27 (22H, m, H-8 ~ H-18); ^{13}C NMR, see Table 2; HMBC correlations (8 Hz) H-4/C-1, C-2, C-3, C-5, C-6; H-5/C-2, C-4; H-20/C-18, C-19, C-21; H-22/C-21, C-23, C-24; H-23/C-21, C-22, C-24, C-25; H-24/C-22, C-23, C-25, C-26; H-25/C-23, C-24, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-25, C-26, C-28, C-29; H-31/C-29, C-30, C-32, C-33; H-34/C-36; H-37/C-34, C-36, C-38, C-39; H-42/C-40, C-41, C-44; H-43/C-42, C-44, C-45; H-44/C-42, C-43, C-45, C-46; H-45/C-43, C-44, C-46, C-47; H-47/C-45; HRFABMS $[M+Na]^+$ *m/z* 835.4989; $C_{47}H_{72}O_{11}Na$ calculated 835.4972 (Δ -1.7 mmu).

Osirisyne D(4)-a white solid, mp 138-140 °C; $[\alpha]^{25}_D$ +10.3 ° (*c* 0.12, MeOH); UV (MeOH) λ max (log ε) 206 (3.72) nm; IR (KBr) ν max 3350, 2920, 2850, 1705, 1600, 1380, 1070, 1010 cm^{-1}; ^1H NMR (CD_3OD) δ 5.87 (1H, ddd, 15.6, 5.9, 1.5, H-43), 5.75 (1H, ddd, 15.6, 6.3, 1.0 Hz, H-44), 5.60 (1H, br dt, 15.6, 6.8, H-25), 5.43 (1H, br dd, 15.6, 6.8, H-26), 5.03 (2H, tt, 2.0, 2.0, H-34), 4.82 (1H, br d, 6.3, H-

45), 4.18 (1H, br d, 4.9, H-4), 4.08 (1H, td, 6.4, 5.9, H-42), 3.97 (1H, m, H-27), 3.68 (1H, m, H-38), 3.54 (1H, ddd, 9.3, 4.9, 3.4, H-5), 2.89 (1H, d, 2.0, H-47), 2.46 (2H, t, 7.3, H-22), 2.44 (2H, t, 7.3, H-20), 2.36 (2H, dd, 5.9, 2.0, H-37), 2.22 (2H, td, 6.8, 2.0, H-31), 2.03 (2H, td, 7.3, 6.8, H-24), 1.67 (1H, m, H-6), 1.65 (1H, m, H-39), 1.63 (2H, p, 7.3, H-23), 1.57 (1H, m, H-41), 1.55 (3H, m, H-7, H-19 (2H)), 1.52 (5H, m, H-28, H-30 (2H), H-40 (2H)), 1.49 (2H, m, H-39, H-41), 1.46 (4H, m, H-6, H-28, H-29 (2H)), 1.32 (1H, m, H-7), 1.30-1.27 (22H, m, H-8~H-18); ^{13}C NMR, see Table 2; HMBC correlations (8 Hz) H-4/C-1, C-2, C-3, C-5, C-6; H-5/C-3, C-4; H-20/C-19, C-21; H-22/C-21, C-23, C-24; H-23/C-21, C-22, C-24, C-25; H-24/C-22, C-23, C-25, C-26; H-25/C-23, C-24, C-26, C-27; H-26/C-24, C-25, C-27, C-28; H-27/C-25, C-28, C-29; H-31/C-29, C-30, C-32, C-33; H-34/C-36; H-37/C-34, C-36, C-38, C-39; H-42/C-40, C-41, C-44; H-43/C-42, C-44, C-45; H-44/C-42, C-43, C-45, C-46; H-45/C-43, C-44, C-46, C-47; H-47/C-45; HRFABMS $[M+Na]^+$ *m/z* 819.5012; $C_{47}H_{72}O_{10}Na$ calculated 819.5023 (Δ -1.4 mmu).

Osirisyne E(5)-a white solid, mp 126-128 °C; $[\alpha]^{25}_D$ +18.5 ° (*c* 0.10, MeOH); UV (MeOH) λ max (log ε) 207 (3.69) nm; IR (KBr) ν max 3400, 2920, 2850, 1600, 1470, 1360, 1070, 965 cm^{-1}; ^{1}H NMR (CD_3OD) δ 5.87 (1H, ddd, 15.6, 5.9, 1.5, H-43), 5.75 (1H, ddd, 15.6, 5.9, 1.5, H-44), 5.61 (1H, br dt, 15.6, 6.8, H-25), 5.60 (1H, dt, 15.6, 6.8, H-19), 5.42 (1H, br dd, 15.6, 6.8, H-26), 5.40 (1H, br dd, 15.6, 6.8, H-20), 5.03 (1H, tt, 2.0, 2.0, H-34), 4.81 (1H, br d, 5.9, H-45), 4.18 (1H, d, 4.9, H-4), 4.08 (1H, td, 6.4, 5.9, H-42), 3.96 (2H, m, H-21, H-27), 3.68 (1H, m, H-38), 3.54 (1H, ddd, 9.3, 4.9, 3.4, H-5), 2.89 (1H, d, 2.4, H-47), 2.37 (2H, dd, 5.9, 2.0, H-37), 2.21 (2H, td, 6.8, 2.0, H-31), 2.05 (2H, m, H-24), 2.03 (2H, m, H-18), 1.66 (1H, m, H-6), 1.63 (1H, m, H-39), 1.57 (1H, m, H-41), 1.53 (1H, m, H-7), 1.51 (6H, H-22, H-28, H-30 (2H), H-40 (2H)), 1.49 (2H, m, H-39, H-41), 1.46 (6H, m, H-22, H-23 (2H), H-28, H-29 (2H)), 1.44 (1H, m, H-6), 1.35 (1H, m, H-7), 1.30-1.27 (20H, m, H-8~H-17); ^{13}C NMR, see Table 2; HMBC correlations (8 Hz) H-4/C-1, C-2, C-3, C-5, C-6; H-5/C-3; H-18/C-19, C-20; H-19/C-18, C-20, C-21; H-20/C-18, C-19, C-21; H-21/C-19, C-22; H-24/C-22, C-23, C-25, C-26; H-25/C-24, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-25, C-28, C-29; H-31/C-29, C-30, C-32, C-33; H-34/C-35, C-36; H-37/C-36, C-38, C-39; H-42/C-40, C-41, C-44; H-43/C-42, C-44, C-45; H-44/C-42, C-43, C-45, C-46; H-45/C-43, C-44, C-46, C-47; H-47/C-45; HRFABMS $[M+Na]^+$ *m/z* 819.5042; $C_{47}H_{72}O_{10}Na$ calculated 819.5023 (Δ 1.1 mmu).

Osirisyne F(6)-a white solid, mp 138-140 °C; $[\alpha]^{25}_D$ +6.8 ° (*c* 0.09, MeOH); UV (MeOH) λ max (log ε) 207 (4.01); IR (KBr) ν max 3350, 2925, 2860, 1705, 1560, 1415, 1350, 1075, 1015 cm^{-1}; ^{1}H NMR (CD_3OD) δ 5.88 (1H, ddd, 15.1, 6.4, 1.0, H-43), 5.75 (1H, ddd, 15.1, 5.9, 1.0, H-44), 5.61 (1H, br dt, 15.1, 6.8, H-25), 5.42 (1H, br dd, 15.1, 6.8, H-26), 5.10 (1H, td, 2.0, 1.5, H-34), 4.81 (1H, br d, 5.9, H-45), 4.32 (1H, td, 6.8, 1.5, H-31), 4.18 (1H, d, 4.4, H-4), 4.09 (1H, m, H-42), 3.97 (1H, m, H-27), 3.68 (1H, tt, 6.3, 5.9, H-38), 3.61 (1H, m, H-6), 3.42 (1H, dd, 8.3, 4.4, H-5), 2.44 (2H, t, 7.3, H-18/H-20), 2.43 (2H, t, 7.3, H-18/H-20), 2.36 (2H, dd, 5.9, 2.0, H-37), 2.04 (2H, td, 7.3, 6.8, H-24), 1.76 (1H, m, H-7), 1.66 (2H, m, H-30), 1.63 (1H, m, H-39), 1.57 (2H, m, H-8, H-41), 1.54 (3H, m, H-17, H-21 (2H)), 1.52 (3H, m, H-28, H-40 (2H)), 1.49 (2H, m, H-39, H-40), 1.47 (3H, m, H-28, H-29 (2H)), 1.38 (2H, m, H-23), 1.36 (1H, m, H-7), 1.32 (3H, m, H-8, H-22 (2H)), 1.30-1.27 (16H, m, H-9~H-16); ^{13}C NMR, see Table 2; HMBC correlations (8 Hz) H-4/C-1, C-2, C-3; H-5/C-3, C-4, C-6, C-7; H-18/C-17, C-19; H-20/C-19, C-21, C-22; H-23/C-21, C-22, C-24; H-24/C-23, C-25, C-26; H-25/C-23, C-24, C-26, C-27; H-26/C-24, C-25, C-27; H-27/C-26, C-28, C-29; H-31/C-29, C-30, C-32, C-33; H-34/C-36; H-37/C-36, C-38, C-39; H-42/C-40, C-41; H-43/C-42, C-44, C-45; H-44/C-42, C-43, C-45, C-46; H-45/C-43, C-44, C-46; HRFABMS $[M+Na]^+$ *m/z* 851.4952; $C_{47}H_{72}O_{12}Na$ calculated 851.4921 (Δ -3.1 mmu).

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