Proisocrinins A−F, Brominated Anthraquinone Pigments from the Stalked Crinoid *Proisocrinus ruberrimus*

Klaus Wolkenstein,*,† Wolfgang Schoefberger,‡ Norbert Müller,§ and Tatsuo Oji⊥

Institutes of Analytical Chemistry, Inorganic Chemistry, and Organic Chemistry, Johannes Kepler University Linz, Altenbergerstrasse 69, 4040 Linz, Austria, and Department of Earth and Planetary Science, University of Tokyo, Hongo, Tokyo 113-0033, Japan
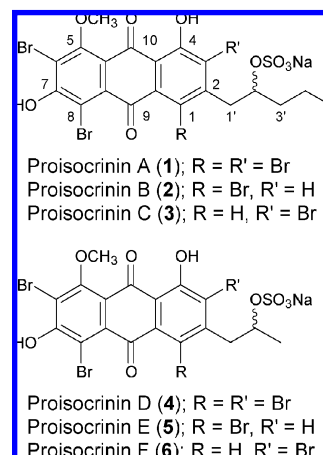
New brominated anthraquinone pigments, proisocrinins A−F (**1**−**6**), were isolated from the strikingly scarlet-colored stalked crinoid *Proisocrinus ruberrimus*, which had been collected in the deep sea of the Okinawa Trough, Japan. The structures of the compounds were elucidated by spectroscopic analysis including HRMS, 1D ^{1}H and ^{13}C NMR, and 2D NMR. CD spectroscopy revealed that all compounds are present as optically active enantiomers. This is the first report of tribromo and tetrabromo anthraquinones from a natural source.

Crinoids, like other members of the phylum Echinodermata, are often brightly colored. Quinones and related compounds are common pigments of comatulid crinoids,[1] and the presence of phenanthroperylene quinones in the living stalked crinoid *Gymnocrinus richeri* and in fossil crinoids from the Jurassic and Triassic shows that polyketide-derived pigments have been biosynthesized since ancient times.[2,3] Some polyketide pigments from crinoids are also known for their biological activity: the anthraquinone rhodoptilometrin-6-*O*-sulfate,[4] crinemodin-rhodoptilometrin bianthrone,[5] and several benzochromenones are constituents of comatulid crinoids,[6] and the phenanthroperylene quinones gymnochrome D and isogymnochrome D have been isolated from the stalked crinoid *Gymnocrinus*.[7] Moreover, many polyketide pigments in living crinoids are present as sulfate esters,[2,4,8,9] and antifeedant activity of polyketide sulfates on fish has been reported.[9,10] However, since living stalked crinoids are mainly restricted to the deep sea, previous studies on crinoidal metabolites almost exclusively focused on the more accessible comatulid crinoids. Although stalked crinoids are widespread, and the first attempts to characterize their pigments were made in the 19th century,[11] to date, no constituents of stalked crinoids with the exception of those from *Gymnocrinus* have been described.

The recent discovery of a new occurrence of the strikingly scarlet-colored stalked crinoid *Proisocrinus ruberrimus* Clark, 1910 (Proisocrinidae) in the Okinawa Trough, southwestern Japan,[12] gave opportunity for an investigation of its pigments. A few specimens of the crinoid were collected during a dive of the submersible Shinkai 6500 in a water depth of about 1800 m. In this study we report the isolation and structures of new water-soluble brominated anthraquinones from this rare species, which is well-known for its brilliant coloration.[12,13]

The aqueous extract of lyophilized crinoid material was subjected to solid-phase extraction to concentrate the pigments and to remove noncolored compounds. Pigments were eluted with MeOH followed by evaporation of the eluate to dryness. The intensely red-colored residue was dissolved in water and subjected to semipreparative HPLC to yield six fractions of the main pigments. Each fraction was then desalted by solid-phase extraction to give the new pigments proisocrinins A−F (**1**−**6**).

Proisocrinin A (**1**), the most abundant pigment, showed UV/vis absorption maxima at 267, 320, and 473 nm. The ESIMS spectrum

Proisocrinin A (**1**); R = R' = Br
Proisocrinin B (**2**); R = Br, R' = H
Proisocrinin C (**3**); R = H, R' = Br

Proisocrinin D (**4**); R = R' = Br
Proisocrinin E (**5**); R = Br, R' = H
Proisocrinin F (**6**); R = H, R' = Br

of **1** showed a complex multiplet with *m/z* 769, 771, 773, 775, and 777 (relative intensities 1:4.1:6.3:4.4:1.2) for the quasimolecular ion [M − H]$^-$, indicating the presence of four bromine atoms. HRESIMS data of **1** suggested the molecular formula $C_{20}H_{15}Br_4NaO_9S$ (*m/z* 768.6985 [M − H]$^-$). Furthermore, the characteristic fragment peaks (monoisotopic) at *m/z* 747 [M − Na]$^-$ and *m/z* 649 [M − H_2SO_4Na]$^-$ suggested the presence of a sodium sulfate group that is located in a side chain (for a sulfate group directly attached to an aromatic ring fragmentation of SO_3Na would be expected),[9] also corroborated by a strong absorption at 1230 cm^{-1} (sulfate) in the IR spectrum. The ^{1}H NMR spectrum (D_2O) of **1** (Table 1) showed signals for a methine proton at δ_H 4.61 (m, 1H), a methoxy group at δ_H 3.89 (s, 3H), two AB subsystems attributed to protons of two methylene groups at δ_H 3.19 (dd, 1H), 2.77 (dd, 1H) and δ_H 1.66 (m, 1H), 1.54 (m, 1H), a methylene group at δ_H 1.32 (m, 2H), and a methyl group at δ_H 0.91 (t, 3H). No aromatic proton signals were present. However, using DMSO-d_6 as the solvent an additional signal for a hydroxy group at δ_H 15.30 (s, 1H) was observed. The ^{13}C NMR spectrum (D_2O) (Table 1) revealed the presence of 20 carbon signals with chemical shifts consistent with a highly substituted anthraquinone system (12 quaternary aromatic signals and two carbonyl signals at δ_C 188.7 and 183.6). These shifts excluded the possibility of an *ortho*-phenanthrenequinone structure according to CSEARCH comparison.[14] Due to the lack of aromatic protons, the structure determination of **1** became possible only by comparison of its spectroscopic data with those of the closely related compound **2**.

Proisocrinin B (**2**) showed UV/vis absorption maxima at 264, 321, and 465 nm. The ESIMS spectrum of **2** showed a complex multiplet with *m/z* 691, 693, 695, and 697 (relative intensities 1:3.2:

* To whom correspondence should be addressed. Tel: +43-732-2468-8720. Fax: +43-732-2468-8679. E-mail: klaus.wolkenstein@jku.at.
† Institute of Analytical Chemistry, Johannes Kepler University Linz.
‡ Institute of Inorganic Chemistry, Johannes Kepler University Linz.
§ Institute of Organic Chemistry, Johannes Kepler University Linz.
⊥ University of Tokyo.

Table 1. NMR Spectroscopic Data for Proisocrinins A (**1**) and B (**2**)[a]

		1			2	
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[b]	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[b]
1	114.1, qC			113.3, qC		
2	146.5, qC			147.6, qC		
3	122.2, qC			126.4, CH	6.94, s	1, 4, 4a, 9a, 10, 1′
4	157.1, qC			159.4, qC		
4a	117.2, qC			117.7, qC		
5	160.0, qC			159.5, qC		
6	112.6, qC[c]			112.7, qC[c]		
7	170.4, qC			170.1, qC		
8	112.3, qC[c]			112.3, qC[c]		
8a	137.3, qC			137.1, qC		
9	188.7, qC			189.3, qC		
9a	133.0, qC			134.4, qC		
10	183.6, qC			184.4, qC		
10a	116.5, qC			116.5, qC		
1′	42.2, CH_2	3.19 br, dd; 2.77, dd (14.0, 3.4)	1, 2, 3, 2′, 3′	42.0, CH_2	2.86, dd (13.8, 3.5); 2.69, dd (13.8, 8.6)	1, 2, 3, 2′, 3′
2′	79.6, CH	4.61, m	2, 1′, 3′, 4′	80.0, CH	4.55, m	2, 1′, 3′, 4′
3′	38.1, CH_2	1,66, m; 1.54, m	1′, 2′, 4′, 5′	37.3, CH_2	1.66, m; 1.59, m	1′, 2′, 4′, 5′
4′	18.6, CH_2	1.32, m	2′, 3′, 5′	18.5, CH_2	1.37, m	2′, 3′, 5′
5′	14.3, CH_3	0.91, t (7.4)	3′, 4′	14.1, CH_3	0.90, t (7.4)	3′, 4′
5-OCH_3	61.8, CH_3	3.89, s	5	61.7, CH_3	3.86, s	5

[a] Spectra were recorded in D_2O at 500.13 MHz for ^{1}H NMR and 125.7 MHz for ^{13}C NMR. [b] HMBC correlations are from proton(s) stated to the indicated carbon. [c] Assignments may be interchangeable within the same column.
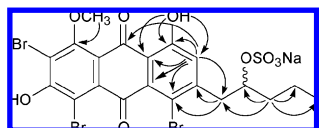
Figure 1. Key HMBC (H→C) correlations of proisocrinin B (**2**).

3.3:1.2) for [M − H]⁻, indicating the presence of three bromine atoms. HRESIMS data of **2** suggested the molecular formula $C_{20}H_{16}Br_3NaO_9S$ (*m/z* 690.7896 [M − H]⁻). In the ^{1}H NMR spectrum (D_2O) of **2** (Table 1) signals of the side chain and the methoxy group similar to **1** were present, but in addition an aromatic proton signal at δ_H 6.94 (s, 1H) could be observed. In the HMBC spectrum (D_2O) correlations from the only aromatic proton, H-3 (δ_H 6.94), to C-1 (δ_C 113.3), C-4 (δ_C 159.4), C-4a (δ_C 117.7), C-9a (δ_C 134.4), and C-10 (δ_C 184.4) as well as to the aliphatic carbon C-1′ (δ_C 42.0) were observed (Figure 1). Further correlations were identified from H-3′ (δ_H 1.66 and 1.59) to C-1′ (δ_C 42.0), C-2′ (δ_C 80.0), C-4′ (δ_C 18.5), and C-5′ (δ_C 14.1), suggesting the presence of a 2′-sulfoxypentyl side chain. A HSQC experiment confirmed the assignments of the side chain carbons. With the exception of the protonated C-3, the ^{13}C NMR shifts of **2** (Table 1) showed only minor differences to those of **1**. Using DMSO-d_6 as the solvent, in the ^{1}H NMR spectrum an additional signal for a hydroxy group at δ_H 13.93 (s, 1H) was observed, showing a large upfield shift in comparison to **1**. HMBC correlations from the hydroxy proton (δ_H 13.93) to C-3 (δ_C 124.5), C-4 (δ_C 159.1), and C-4a (δ_C 116.7) indicated that the hydroxy group is located at position 4 (Figure 1). Under the very likely assumption of a polyketide-based biosynthesis an alternating pattern of oxygen substituents is expected.[15] As the only observable hydroxy group was C-4-OH, the proton hydrogen-bonded to the carbonyl group at C-10, the remaining hydroxy group was assigned to position 7, where the lack of an intramolecular hydrogen bond allows exchange with the residual H_2O. The methoxy group was consequentially assigned to position 5. The position of the C-7-OH is also corroborated by the large chemical shift difference between the phenolic carbons C-4 (δ_C 159.4) and C-7 (δ_C 170.1), the latter being flanked by the two bromine atoms in positions 6 and 8. Assignments of the remaining aromatic carbons are based on comparison of the observed ^{13}C chemical shifts with published data of related anthraquinones and brominated phenanthroperylene quinones (gymnochromes)[2,4,9] and on C−C correlations obtained from covariance

processing of the HMBC spectrum.[16,17] Concluding from the very similar ^{13}C shifts (except for C-3) that were observed for **1** and **2**, together with the analogous HMBC correlations from H-1′ to the aromatic carbons C-1, C-2, and C-3, the NMR signal assignments of **1** could be deduced from those of **2**. The mass spectrometric data and the lack of the single aromatic proton signal H-3 observed in **2** indicated that **1** is the tetrabromo analogue of **2**.

HRESIMS of the minor pigment proisocrinin C (**3**) revealed that this compound is isomeric with proisocrinin B (**2**). ^{1}H NMR and HSQC data of **3** suggested a structural change in the vicinity of the aliphatic side chain. Assuming that the general biosynthetic pathway is conserved, an interchange of the hydrogen and bromine atoms in positions 1 and 3 seems most likely.

Proisocrinin D (**4**) is closely related to the proisocrinins described above and differs from proisocrinin A (**1**) only in the side chain. HRESIMS of **4** established the molecular formula $C_{18}H_{11}Br_4NaO_9S$ (*m/z* 740.6677 [M − H]⁻). The ^{1}H NMR spectrum of **4** (Table 2) showed signals for a methine proton at δ_H 4.67 (m, 1H), a methoxy group at δ_H 3.87 (s, 3H), an AB subsystem attributed to protons of a methylene group at δ_H 3.33 (dd, 1H), 2.94 (dd, 1H), and a methyl group at δ_H 1.36 (d, 3H). In the ^{13}C NMR spectrum of **4** virtually identical shifts for the quaternary carbon atoms and the methoxy group to those of proisocrinin A (**1**) were observed (Tables 1 and 2), suggesting the presence of analogous aromatic systems. On the basis of HMBC and HSQC experiments, the remaining three carbon signals could be assigned as a 2′-sulfoxypropyl side chain.

Proisocrinin E (**5**) was found to have the molecular formula $C_{18}H_{12}Br_3NaO_9S$ (*m/z* 662.7581 [M − H]⁻) as determined by HRESIMS. The ^{1}H NMR spectrum of **5** (Table 2) was similar to that of proisocrinin D (**4**); however, an aromatic proton signal was present at δ_H 7.00 (s, 1H) and an overlapping of the AB subsystem signals was observed. In the ^{13}C NMR spectrum of **5** nearly identical shifts for the quaternary carbon atoms and the methoxy group to those of proisocrinin B (**2**) and very similar shifts for the three carbons of the 2′-sulfoxypropyl side chain present in proisocrinin D (**4**) were observed (Tables 1 and 2).

HRESIMS of the minor pigment proisocrinin F (**6**) indicated that this compound is isomeric with proisocrinin E (**5**). ^{1}H NMR and HSQC data suggested an interchange of the hydrogen and bromine atoms in positions 1 and 3 analogous to compounds **2** and **3**.

The proisocrinins are chiral due to an asymmetric carbon atom at position 2′. Therefore chiroptical properties of **1**−**6** were

Table 2. NMR Spectroscopic Data for Proisocrinins D (**4**) and E (**5**)[a]

	4			5		
position	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[b]	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC[b]
1	113.7, qC			113.3, qC		
2	146.3, qC			147.3, qC		
3	122.0, qC			126.3, CH	7.00, s	1, 4, 4a, 10, 1′
4	157.0, qC			159.4, qC		
4a	117.3, qC			117.9, qC		
5	159.8, qC			159.6, qC		
6	112.5, qC[c]			112.7, qC[c]		
7	170.5, qC			169.9, qC		
8	112.2, qC[c]			not determined		
8a	137.1, qC			137.1, qC		
9	188.9, qC			189.8, qC		
9a	133.3, qC			134.8, qC		
10	183.7, qC			184.5, qC		
10a	116.5, qC			116.4, qC		
1′	44.1, CH₂	3.33, dd (14.0, 10.0); 2.94, dd (14.0, 3.7)	1, 2, 3, 2′, 3′	43.9, CH₂	2.94, two br dd	1, 2, 3, 2′, 3′
2′	76.6, CH	4.67, m[d]	2, 1′, 3′	76.9, CH	4.74, m[d]	2, 1′, 3′
3′	21.2, CH₃	1.36, d (6.4)	2, 1′, 2′	20.8, CH₃	1.36, d (6.3)	2, 1′, 2′
5-OCH₃	61.7, CH₃	3.87, s	5	61.7, CH₃	3.85, s	5

[a] Spectra were recorded in D_2O at 500.13 MHz for ¹H NMR and 125.7 MHz for ¹³C NMR. [b] HMBC correlations are from proton(s) stated to the indicated carbon. [c] Assignments may be interchangeable within the same column. [d] δ_H values determined from HSQC spectra.
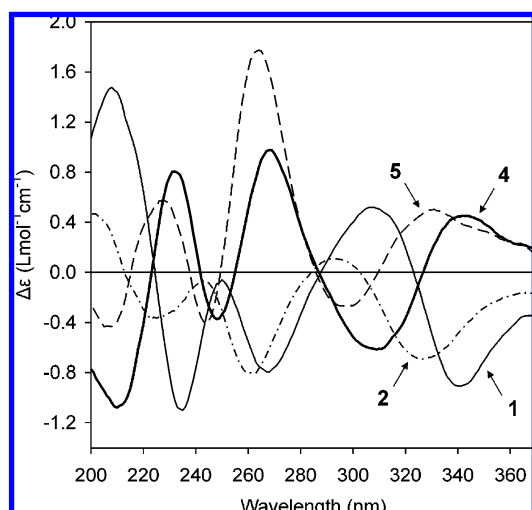
Figure 2. CD spectra (H_2O) of proisocrinins A (**1**), B (**2**), D (**4**), and E (**5**).

investigated. Circular dichroism (CD) spectroscopy revealed that all proisocrinins are present as optically active enantiomers. The CD spectra of proisocrinins A (**1**) and D (**4**) were opposite in sign, as was also the case for the spectra of proisocrinins B (**2**) and E (**5**) (Figure 2), suggesting that **1** and **4** have opposite chirality at C-2′ as well as **2** and **5**. The CD spectra of proisocrinins C (**3**) and F (**6**) exhibited more complex band shapes, which could not be unambiguously related to those of the other proisocrinins.

The proisocrinin pigments are closely related to the brominated phenanthroperylene quinones (gymnochromes) isolated from the stalked crinoid *Gymnocrinus*,[2] the latter derived by a formal dimerization of the polyketidic anthraquinone precursors. Furthermore, proisocrinins A (**1**), B (**2**), and C (**3**) exhibit a 2′-sulfoxypentyl side chain, which is also present in gymnochrome D and isogymnochrome D. Proisocrinins D (**4**), E (**5**), and F (**6**) exhibit a 2′-sulfoxypropyl side chain like gymnochrome C. In the gymnochromes the absolute configurations of all C₅ side chains are *R*, while the configurations of all C₃ side chains are *S*.[2] By analogy, the absolute configurations of **1** and **2** may be tentatively assigned as *R* and the configurations of **4** and **5** as *S*. However, the absolute configuration assignments of these compounds still remain speculative. Although there is a striking similarity between the proisocrinins and the gymnochromes, no direct comparison of CD spectra

between the two groups is possible, because the former constitute an inherently achiral chromophore, which is perturbed by a distant chiral center, whereas the latter possess an inherently chiral chromophore (helically twisted phenanthroperylene quinone system).

Brominated quinones are relatively rare in nature,[18] and as far as is known, the proisocrinin pigments represent the first multiply brominated anthraquinones from a natural source. The only other bromoanthraquinone that has been previously reported is the 3-bromo-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-carboxylic acid isolated from the stony coral *Tubastraea micrantha*.[19] Moreover, the proisocrinins are the first anthraquinone pigments from a stalked crinoid.

Although quinone pigments are widespread in comatulid crinoids, including anthraquinones with the same carbon skeletons as present in the proisocrinins,[20] the pigments from the stalked crinoids *Proisocrinus* and *Gymnocrinus* are the only known crinoidal pigments which are brominated. The sulfated side chains are also very characteristic for both pigment groups. Many other aromatic polyketides in crinoids are known to be present as sulfate esters;[2,4,8,9] however, in all these cases the sulfate group is directly attached to an aromatic ring. Thus, similar structural features can be observed in pigments from stalked crinoids, which are not known from comatulid crinoids. Because *Proisocrinus* occurs in a habitat without light, and antifeedant activity against predatory fish has been reported for anthraquinone pigments in the sulfate form,[9,10] it is likely that the proisocrinins are used by the animals as a chemical defense.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Varian CARY 100 Bio UV−visible spectrophotometer, CD spectra were recorded on a Jasco J-810 spectropolarimeter, and IR spectra were measured on a Bruker TENSOR 27 FT-IR spectrometer. 1D and 2D NMR spectra (500.13 MHz for ¹H and 125.7 MHz for ¹³C) were recorded in D_2O and DMSO-d_6 at 298 K on a Bruker DRX 500 spectrometer equipped with a cryogenically cooled triple resonance probe (TXI) with a z-gradient coil. Chemical shifts were referenced externally to the signals of CH_3OH in D_2O at δ_H 3.34 and δ_C 49.5 or internally to the solvent signals in DMSO-d_6 at δ_H 2.54 and δ_C 39.5, respectively. Standard pulse sequences for ¹H−¹³C gs-HSQC and ¹H−¹³C gs-HMBC experiments were used. No proton decoupling was applied during acquisition of the HMBC spectra. The refocusing delays for the inverse heterocorrelations were set to 3.57 and 71.4 ms, corresponding to ¹$J_{C,H}$ = 140 Hz and ⁿ$J_{C,H}$ = 7 Hz, respectively. High-resolution MS spectra were obtained using an Agilent 6520 Q-TOF

LC/MS mass spectrometer with electrospray ionization in the negative-ion mode. HPLC was performed on an Agilent 1100 Series system using an ODS Hypersil column (250 × 10 mm, 5 μm, Thermo).

Animal Material. Five specimens of *Proisocrinus ruberrimus* were collected in 2006 during a dive of the submersible Shinkai 6500 on the southern slope of Aguni Knoll, central Okinawa Trough, at a depth of about 1800 m.[12] A sample (four arms) from one of these specimens (UMUT RE 29475) was available for chemical analysis. Voucher samples were deposited in the University Museum of the University of Tokyo.

Extraction and Isolation. Lyophilized *P. ruberrimus* material (1.6 g) was extracted with distilled water and the extract purified and concentrated using solid-phase extraction (Bondesil C18, 40 μm). Pigments were washed with water and eluted with MeOH (without drying of the column) followed by evaporation of the eluate to dryness. The red-colored extract (7.8 mg) was subjected to semipreparative HPLC using a linear gradient of MeOH/20 mM aqueous ammonium acetate (40:60) to 65% MeOH. Fractions were concentrated and desalted by solid-phase extraction under the same conditions as described above to give the new compounds **1** to **6**.

Proisocrinin A (1): red solid (1.2 mg); UV (H$_2$O) λ_{max} (log ε) 267 (4.19), 320 (4.30), 473 (3.87) nm; CD (H$_2$O) λ ($\Delta\varepsilon$) 208 (+1.5), 235 (−1.1), 268 (−0.8), 308 (+0.5), 341 (−0.9); IR (KBr) ν_{max} 1614, 1523, 1420, 1355, 1230 cm^{-1}; ^{1}H NMR (D$_2$O, 500.13 MHz) and ^{13}C NMR (D$_2$O, 125.7 MHz), see Table 1; ^{1}H NMR (DMSO-d_6, 500.13 MHz) δ 15.30 (1H, s, 4-OH), 4.70 (1H, m, H-2′), 3.78 (3H, s, 5-OCH$_3$), 3.68 (1H, dd, J = 13.7, 7.8 Hz, H-1′a), 3.31 (1H, dd, J = 13.7, 7.0 Hz, H-1′b), 1.64 (1H, m, H-3′a), 1.45 (1H, m, H-3′b), 1.31 (2H, m, H-4′), 0.85 (3H, t, J = 7.3 Hz, H-5′); selected HMBC correlations (DMSO-d_6, H/C) 4-OH/3 (δ 120.5), 4-OH/4 (157.0), 4-OH/4a (116.9), 5-OCH$_3$/5 (159.4), 1′/1 (111.2), 1′/2 (145.4), 1′/3 (120.5), 1′/2′ (73.3), 1′/3′ (36.2); HRESIMS m/z 768.6985 [M − H]$^-$ (calcd for C$_{20}$H$_{14}$Br$_4$NaO$_9$S, 768.6995), 746.7170 [M − Na]$^-$ (calcd for C$_{20}$H$_{15}$Br$_4$O$_9$S, 746.7176), 648.7501 [M − H$_2$SO$_4$Na]$^-$ (calcd for C$_{20}$H$_{13}$Br$_4$O$_5$, 648.7502), 372.8545 [M − H − Na]$^{2-}$ (calcd for 0.5·C$_{20}$H$_{14}$Br$_4$O$_9$S, 372.8552).

Proisocrinin B (2): red solid (0.8 mg); UV (H$_2$O) λ_{max} (log ε) 264 (4.23), 321 (4.31), 465 (3.87); CD (H$_2$O) λ ($\Delta\varepsilon$) 201 (+0.5), 223 (−0.4), 262 (−0.8), 293 (+0.1), 327 (−0.7); IR (KBr) ν_{max} 1620, 1528, 1443, 1360, 1237 cm^{-1}; ^{1}H NMR (D$_2$O, 500.13 MHz) and ^{13}C NMR (D$_2$O, 125.7 MHz), see Table 1; ^{1}H NMR (DMSO-d_6, 500.13 MHz) δ 13.93 (1H, s, 4-OH), 7.21 (1H, s, H-3), 4.42 (1H, m, H-2′), 3.78 (3H, s, 5-OCH$_3$), 3.16 (1H, dd, J = 14.0, 5.8 Hz, H-1′a), 3.10 (1H, dd, J = 14.0, 6.0 Hz, H-1′b), 1.46 (1H, m, H-3′a), 1.43 (1H, m, H-3′b), 1.32 (2H, m, H-4′), 0.86 (3H, t, J = 7.0 Hz, H-5′); selected HMBC correlations (DMSO-d_6, H/C) 3/1 (δ 110.3), 3/4 (159.1), 3/4a (116.2), 3/1′ (39.8), 4-OH/3 (124.5), 4-OH/4 (159.1), 4-OH/4a (116.7), 5-OCH$_3$/5 (159.1), 1′/1 (110.3), 1′/2 (145.8), 1′/3 (124.5), 1′/2′ (75.1); HRESIMS m/z 690.7896 [M − H]$^-$ (calcd for C$_{20}$H$_{15}$Br$_3$NaO$_9$S, 690.7890), 668.8080 [M − Na]$^-$ (calcd for C$_{20}$H$_{16}$Br$_3$O$_9$S, 668.8071), 570.8406 [M − H$_2$SO$_4$Na]$^-$ (calcd for C$_{20}$H$_{14}$Br$_3$O$_5$, 570.8397), 333.8994 [M − H − Na]$^{2-}$ (calcd for 0.5·C$_{20}$H$_{15}$Br$_3$O$_9$S, 333.8996).

Proisocrinin C (3): red solid (0.2 mg); UV (H$_2$O) λ_{max} (log ε) 263 (4.21), 320 (4.35), 482 (3.80); CD (H$_2$O) λ ($\Delta\varepsilon$) 242 (−0.3), 261 (+0.5), 316 (−0.8); ^{1}H NMR (D$_2$O, 500.13 MHz) δ 7.30 (1H, s, H-3), 4.64 (1H, m, H-2′), 3.87 (3H, s, 5-OCH$_3$), 3.00 (2H, m, H-1′), 1.66 (2H, m, H-3′), 1.43 (2H, m, H-4′), 0.90 (3H, t, signal partially obscured, H-5′); ^{13}C NMR (D$_2$O, 125.7 MHz, from HSQC spectrum) δ 122.4 (CH, C-1), 80.4 (CH, C-2′), 61.9 (CH$_3$, 5-OCH$_3$), 42.5 (CH$_2$, C-1′), 37.5 (CH$_2$, C-3′), 19.3 (CH$_2$, C-4′), 14.3 (CH$_3$, C-5′); HRESIMS m/z 690.7902 [M − H]$^-$ (calcd for C$_{20}$H$_{15}$Br$_3$NaO$_9$S, 690.7890), 668.8083 [M − Na]$^-$ (calcd for C$_{20}$H$_{16}$Br$_3$O$_9$S, 668.8071), 570.8409 [M − H$_2$SO$_4$Na]$^-$ (calcd for C$_{20}$H$_{14}$Br$_3$O$_5$, 570.8397), 333.8997 [M − H − Na]$^{2-}$ (calcd for 0.5·C$_{20}$H$_{15}$Br$_3$O$_9$S, 333.8996).

Proisocrinin D (4): red solid (0.8 mg); UV (H$_2$O) λ_{max} (log ε) 266 (4.18), 319 (4.28), 473 (3.85); CD (H$_2$O) λ ($\Delta\varepsilon$) 210 (−1.1), 232 (+0.8), 248 (−0.4), 268 (+1.0), 309 (−0.6), 342 (+0.5); IR (KBr) ν_{max} 1613, 1523, 1420, 1359, 1235, 935 cm^{-1}; ^{1}H NMR (D$_2$O, 500.13 MHz) and ^{13}C NMR (D$_2$O, 125.7 MHz), see Table 2; ^{1}H NMR (DMSO-d_6, 500.13 MHz) δ 15.31 (1H, s, 4-OH), 4.80 (1H, m, H-2′), 3.78 (3H, s, 5-OCH$_3$), 3.55 (1H, dd, from HSQC spectrum, H-1′a), 3.30 (1H, dd, from HSQC spectrum, H-1′b), 1.19 (3H, d, J = 5.3 Hz, H-3′); HRESIMS m/z 740.6677 [M − H]$^-$ (calcd for C$_{18}$H$_{10}$Br$_4$NaO$_9$S, 740.6682), 718.6858 [M − Na]$^-$ (calcd for C$_{18}$H$_{11}$Br$_4$O$_9$S, 718.6863),

620.7180 [M − H$_2$SO$_4$Na]$^-$ (calcd for C$_{18}$H$_9$Br$_4$O$_5$, 620.7189), 358.8386 [M − H − Na]$^{2-}$ (calcd for 0.5·C$_{18}$H$_{10}$Br$_4$O$_9$S, 358.8392).

Proisocrinin E (5): red solid (0.5 mg); UV (H$_2$O) λ_{max} (log ε) 263 (4.17), 319 (4.25), 465 (3.81) nm; CD (H$_2$O) λ ($\Delta\varepsilon$) 205 (−0.4), 227 (+0.6), 244 (−0.4), 264 (+1.8), 297 (−0.3), 331 (+0.5); IR (KBr) ν_{max} 1620, 1529, 1443, 1362, 1238, 936 cm^{-1}; ^{1}H NMR (D$_2$O, 500.13 MHz) and ^{13}C NMR (D$_2$O, 125.7 MHz), see Table 2; ^{1}H NMR (DMSO-d_6, 500.13 MHz) δ 13.95 (1H, s, 4-OH), 7.21 (1H, s, H-3), 4.56 (1H, m, H-2′), 3.78 (3H, s, 5-OCH$_3$), 3.11 (1H, dd, J = 14.0, 7.0 Hz, H-1′a), 2.97 (1H, dd, J = 14.0, 5.9 Hz, H-1′b), 1.20 (3H, d, J = 6.3 Hz, H-3′); HRESIMS m/z 662.7581 [M − H]$^-$ (calcd for C$_{18}$H$_{11}$Br$_3$NaO$_9$S, 662.7577), 640.7763 [M − Na]$^-$ (calcd for C$_{18}$H$_{12}$Br$_3$O$_9$S, 640.7758), 542.8089 [M − H$_2$SO$_4$Na]$^-$ (calcd for C$_{18}$H$_{10}$Br$_3$O$_5$, 542.8084), 319.9838 [M − H − Na]$^{2-}$ (calcd for 0.5·C$_{18}$H$_{11}$Br$_3$O$_9$S, 319.8840).

Proisocrinin F (6): red solid (0.2 mg); UV (H$_2$O) λ_{max} (log ε) 261 (4.08), 319 (4.22), 477 (3.66) nm; CD (H$_2$O) λ ($\Delta\varepsilon$) 226 (−0.2), 244 (+0.1), 263 (−0.5), 342 (−0.2); ^{1}H NMR (D$_2$O, 500.13 MHz) δ 7.37 (1H, s, H-3), 4.76 (1H, m, H-2′), 3.87 (3H, s, 5-OCH$_3$), 3.09 (1H, dd, J = 13.8, 8.7 Hz, H-1′a), 3.05 (1H, dd, J = 13.8, 4.6 Hz, H-1′b), 1.37 (3H, d, J = 6.2 Hz, H-3′); ^{13}C NMR (D$_2$O, 125.7 MHz, from HSQC spectrum) δ 121.8 (CH, C-1), 77.1 (CH, C-2′), 61.8 (CH$_3$, 5-OCH$_3$), 44.4 (CH$_2$, C-1′), 21.2 (CH$_3$, C-3′); HRESIMS m/z 662.7564 [M − H]$^-$ (calcd for C$_{18}$H$_{11}$Br$_3$NaO$_9$S, 662.7577), 640.7749 [M − Na]$^-$ (calcd for C$_{18}$H$_{12}$Br$_3$O$_9$S, 640.7758), 542.8078 [M − H$_2$SO$_4$Na]$^-$ (calcd for C$_{18}$H$_{10}$Br$_3$O$_5$, 542.8084), 319.8830 [M − H − Na]$^{2-}$ (calcd for 0.5·C$_{18}$H$_{11}$Br$_3$O$_9$S, 319.8840).

Supporting Information Available: UV/vis spectrum of **1**; 1D and 2D NMR spectra of **1**−**6**; HRESIMS spectra of **1**, **2**, **4**, and **5**; CD spectra of **3** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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