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New isoquinolinequinone alkaloids from the South China Sea nudibranch *Jorunna funebris* and its possible sponge-prey *Xestospongia* sp.

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ABSTRACT

Two new renieramycin-type bistetrahydroisoquinolinequinone alkaloids, fennebricins A (1) and B (5), and one new isoquinolinequinone alkaloid, *N*-formyl-1,2-dihydrorenierol (7), were isolated from the skin of the South China Sea nudibranch *Jorunna funebris* and its possible sponge-prey *Xestospongia* sp., together with eight known metabolites, including three bistetrahydroisoquinolinequinones (2–4) and five isoquinolinequinones (8–12). Their structures were elucidated by analysis of spectroscopic data including 1D and 2D NMR and high-resolution electrospray ionization mass spectrometry (HRESIMS) and by comparison with data for related known compounds. All the metabolites except for 7 occurred simultaneously in the two animals, supporting recent ecological studies that the nudibranch *J. funebris* preys on the sponge of the genus *Xestospongia*.

1. Introduction

Dorid nudibranchs are a group of shell-less opisthobranch mollusks found throughout the world, mostly in shallow water tropical and subtropical marine habitats [1]. These dorids have proven to be a rich source of structurally unusual molecules, which in some instances apparently play a defensive role against potential predators and which also possess interesting biological activities [2,3]. Most of these allomones are accumulated from dietary sources, such as sponges, soft corals, and marine algae, but some of them are biosynthesized de novo or obtained by bio-transformation of dietary metabolites [4,5].

The genus *Jorunna* (Bergh, 1876) belongs to the family Discodorididae (Mollusca: Gastropoda: Opisthobranchia: Nudibranchia: Doridina: Kentrodorididae) and is represented by approximately 16 species [6]. These *Jorunna* nudibranchs are carnivorous and feed exclusively on a variety of sponges, including *Xestospongia* sp., *Haliclona* sp., *Euplacella* cf. *australis*, and *Oceanapia* sp. [7,8]. A literature search revealed that reports on the chemistry of the genus *Jorunna* are extremely rare, with only three ones found in the literature so far, and the secondary metabolites isolated from those *Jorunna* nudibranchs are structurally novel bistetrahydroisoquinolinequinone alkaloids, such as jorunnamycins A–C and jorumycin [8–10]. Interestingly, similar metabolites have also been isolated from blue sponges of the genus *Xestospongia* [11–14], suggesting the possible prey–predator relationship between the mollusks and the sponges.

As a part of our ongoing studies on bioactive products from marine organisms [15–18], we recently investigated the nudibranch *Jorunna funebris* (Kelaart, 1859) and the sponge *Xestospongia* sp., both collected in the South China Sea waters,

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resulting in the discovery of three new alkaloids, namely two new bistetrahydroisoquinolinequinones, fennebricins A (1) and B (5), and one new isoquinolinequinone, *N*-formyl-1,2-dihydrorenierol (7), together with eight known related metabolites (2–4 and 8–12). Their structures were elucidated on the basis of detailed spectroscopic analysis and by comparison with data for related known compounds. Fennebricins A (1) and B (5) are two new renieramycin—type dimeric isoquinolinequinones structurally related to the ecteinascidins and the saframycins, two well-known classes of promising antitumor and antimicrobial alkaloids. In this paper, we report the isolation and structural elucidation of these new compounds.

2. Experimental

2.1. General

Optical rotations were measured on a Jasco DIP 370 digital spectropolarimeter. UV spectra were recorded in MeOH on an Agilent 8453 spectrophotometer; peak wavelengths are reported in nm. IR spectra were recorded on a Bio-Rad FT-IR spectrometer; peaks are reported in cm^{-1}. 1D and 2D NMR spectra were measured on a Bruker Avance-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), using the residual $CHCl_3$ signal (δ_H 7.26 ppm) or CH_3OH (δ_H 3.31 ppm) as an internal standard for ^1H NMR and $CDCl_3$ (δ_C 77.00 ppm) or CD_3OD (δ_C 49.5 ppm) for ^{13}C NMR. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. ^1H and ^{13}C NMR assignments were supported by $^1H-^1H$ COSY, HSQC, HMBC, and ROESY experiments. ESI-MS and HR-ESI-MS spectra were recorded on a Micromass Q-TOF micro coupled with a HPLC Waters Alliance 2695. HPLC purification was carried out on a Waters liquid chromatograph equipped with a Waters R401 RI detector and with a reversed-phase Kromasil C_{18} column [5 μm, 25 cm × 10 mm (i.d.), Phenomenex]. Commercial silica gel (Merck, Kieselgel 60 0.063–0.200 mm) was used for column chromatography (CC), and precoated silica gel plates (Merck, KieselGel 60F254) were used for analytical TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde H_2SO_4 reagent. Sephadex LH-20 (Amersham Biosciences) was also used for CC. All solvents used for CC were of analytical grade, and solvents used for HPLC were of HPLC grade.

2.2. Animal material

Both the mollusk and the sponge were collected by hand using scuba at a depth of 20 m, and were frozen immediately after collection. The collections of the mollusk and the sponge were undertaken in January 2003 from Linshui Bay, Hainan Province, China, and in January 2006 from Yalong Bay, Hainan Province, China, respectively. The mollusks were subsequently identified by one of the authors (E. Mollo) as *J. funebris* and the sponge was identified as *Xestospongia* sp. by J.-H. Li of Institute of Oceanography, CAS. Voucher specimens of *J. funebris* (HN-45) and *Xestospongia* sp. (YAL-90) are available for inspection at the Shanghai Institute of Material Medica, CAS.

2.3. Extraction and isolation

The frozen specimens of *J. funebris* (10 individuals, dry weight 3.5 g) were sonicated in acetone (2 × 30 mL) to yield a surface mucus extract and then diced and sonicated repeatedly in acetone (3 × 15 mL) to give an acetone tissue extract. The tissue and mucus extracts were concentrated in vacuo, and the aqueous residues were individually partitioned with Et_2O (3 × 10 mL) and then with *n*-BuOH (3 × 10 mL). After evaporation of the solvent, the organic layers gave Et_2O- and *n*-BuOH-soluble extracts: 218 mg and 30 mg from the mucus and 120 mg and 11 mg from the tissue, respectively. The mucus Et_2O-soluble portion (218 mg) was subjected to silica gel column chromatography (CC) using gradient elution [light petroleum ether (PE)/Et_2O, 9:1 → 8:2 → 7:3 → 1:1, then $CHCl_3$/MeOH, 9:1 → 7:3 → 1:1], affording compounds 8 (8.2 mg) and 9 (7.4 mg), as well as nine fractions (A–I), which were combined based on TLC profiles. Fraction E (19 mg), eluted with PE/Et_2O (1:1), was further purified by C_{18} reversed-phase HPLC using a 5 μm Kromasil column (250 × 4.60 mm, Phenomenex) eluted at 1 mL/min with MeOH/H_2O (7:3) as the eluent to yield compound 4 (2.8 mg). Fraction G (43 mg), eluted with $CHCl_3$/MeOH (9:1), was further subjected to a Sephadex LH-20 column eluted with $CHCl_3$/MeOH (1:1), and then purified by C_{18} reversed-phase HPLC eluted with a gradient of MeOH/H_2O (4.5:5.5 to 1:0, 45 min; 1 mL/min) to afford compounds 1 (1.0 mg), 3 (3.2 mg), 5 (0.8 mg), 10 (2.0 mg), 11 (4.1 mg), and 12 (3.0 mg). Comparison of the Et_2O-soluble extract of the tissue by TLC with that of the mucus, in different system solvents, revealed the presence of similar spots in both extracts. All the compounds mentioned above were also isolated from the tissue Et_2O-soluble extract (120 mg) using the same chromatographic procedure as described for the mucus Et_2O-soluble extract, together with an additional metabolite (2, 3.5 mg).

The frozen sample of *Xestospongia* sp. (20 g, dry weight) was cut into small pieces and extracted ultrasonically with acetone (3 × 250 mL) at room temperature. The combined extracts were partitioned between Et_2O and H_2O. The Et_2O extract was concentrated under vacuum to give a dark residue (112 mg). The Et_2O extract was fractionated by silica gel CC with a gradient system as the eluant [PE/Et_2O, 9:1 → 8:2 →7:3 → 1:1 → 3:7] to afford five fractions (I–V). Fraction III (52 mg) was further subjected to a Sephadex LH-20 column eluted with $CHCl_3$/MeOH (1:1), and then repeatedly purified by silica gel CC to afford compounds 2 (5.6 mg), 3 (2.0 mg), 4 (2.2 mg), 8 (2.7 mg), 9 (4.5 mg), and 11 (5.8 mg). Fraction IV (18 mg) was further purified by a silica gel column eluted with $CHCl_3$/MeOH (9:1), yielding compounds 7 (1.1 mg), 10 (3.5 mg), and 12 (2.0 mg). Fraction V (16 mg) was further purified by a silica gel column eluted with $CHCl_3$/MeOH (8.5:1.5 → 8:2) to afford compounds 1 (0.6 mg) and 5 (1.0 mg).

Fennebricin A (1): pale yellow amorphous powder; $[\alpha]_D^{25}$ −96.2° (c = 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 269 (4.60), 372 (3.12) nm; IR ν_{max} (KBr) 3451, 2936, 1712, 1652, 1486, 1402, 1221, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 547 $[M + Na]^+$; HRESIMS m/z 547.2054 $[M + Na]^+$ (calcd for $C_{28}H_{32}N_2O_8Na$, 547.2056).

Table 1
^{1}H and ^{13}C NMR data for fennebricins A (1) and B (5) in $CDCl_3$ [a,b].

Position	Fennebricin A (1)			Fennebricin B (5)		
	δ_C [c]	δ_H (mult. J in Hz)	HMBC[d]	δ_C [c]	δ_H (mult. J in Hz)	HMBC[d]
1	54.5, CH	3.61, m	21, 22	55.0, CH	4.55, dd (4.9, 3.7)	21
3	51.5, CH	3.05, br d (11.8)	19	54.0, CH	3.30, br d (11.2)	19
4	23.7, CH_2	α 2.83, dd (16.7, 2.2)	3, 5, 9, 10, 11	25.9, CH_2	α 2.80, br d (17.5)	3, 5, 9, 10
		β 1.25, dd (16.7, 11.8)	3, 5, 10		β 1.60, dd (17.5, 11.2)	3, 5, 10
5	185.6, C	–		141.2, C	–	
6	128.5 C	–		108.0 C	–	
7	155.0, C	–		145.0, C	–	
8	180.8, C	–		140.6, C	–	
9	135.8, C	–		113.0, C	–	
10	142.5, C	–		120.2, C	–	
11	54.5, CH	3.97, overlapped	13, 18, 19, 20, N-CH_3	56.0, CH	3.90, overlapped	18, 19, 20, N-CH_3
13	55.3, CH	2.92, m	11, 20, N-CH_3	59.0, CH	3.20, m	20, N-CH_3
14	23.4, CH_2	α 2.85, dd (20.1, 7.3)	13, 15, 19, 20, 21	24.0, CH_2	α 2.70, dd (21.0, 7.5)	13, 15, 19, 21
		β 2.20, d (20.1)	15, 19, 20, 21		β 2.20, d (21.0)	15, 19, 21
15	185.5, C	–		186.0, C	–	
16	126.8, C	–		128.5, C	–	
17	155.0, C	–		155.0, C	–	
18	183.1, C	–		182.9, C	–	
19	135.0, C	–		135.5, C	–	
20	143.6, C	–		141.5, C	–	
21	58.5, CH	3.40, m	1, 3, 14, 24	84.0, C	4.50, d (10.5)	14, 24
22	63.7, CH_2	a 3.35, dd (11.0, 3.7)	1, 9	67.0, CH_2	a 3.80, dd (11.4, 6.0)	1, 9
		b 3.67, dd (11.0, 3.5)	1, 9		b 4.19, dd (11.4, 2.1)	1, 9
23	38.5, CH_2	a 2.42, d (16.5)	24, 25			
		b 3.48, d (16.5)	21, 24, 25			
24	208.0, C					
25	30.8, CH_3	2.18, s	23, 24			
6-CH_3	8.5, CH_3	1.92, s	5, 6, 7	9.6, CH_3	2.10, s	5, 6, 7
16-CH_3	8.5, CH_3	1.95, s	15, 16, 17	9.9, CH_3	1.99, s	15, 16, 17
7-OCH_3	61.5, CH_3	3.96, s	7			
17-OCH_3	61.5, CH_3	3.98, s	17	61.0, CH_3	4.03, s	17
N-CH_3	41.5, CH_3	2.19, s	11, 13	41.0, CH_3	2.19, s	11, 13
5-$OCOCH_3$				170.5, C	–	
5-$OCOCH_3$				20.8, CH_3	1.93, s	5-$OCOCH_3$
OCH_2O				98.5, CH_2	a 5.87, d (1.5)	7, 8
					b 5.96, d (1.5)	7, 8

[a] Spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[b] Chemical shifts (ppm) referred to either $CHCl_3$ (δ_H 7.26) or $CDCl_3$ (δ_C 77.0).
[c] By DEPT experiment.
[d] HMBC correlations are from proton(s) stated to the indicated carbon.

Fennebricin B (5): pale yellow amorphous powder; [α] 25 D −39.0° (c = 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 244 (4.01), 270 (4.03), 371 (2.98) nm; IR ν_{max} (KBr) 3455, 2921, 2226, 1717, 1650, 1450, 1228, 755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS m/z 527 $[M + H]^+$, 509 $[M-H_2O + H]^+$; HRESIMS m/z 527.5435 (calcd for $C_{27}H_{31}N_2O_9$, 527.5430).

N-formyl-1,2-dihydrorenierol (7): red amorphous powder; [α] 25 D −41.0° (c = 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.59), 265 (4.20), 340 (3.75), 512 (3.50) nm; IR ν_{max} (KBr) 3462, 2958, 1656, 1403, 1089, 935, 755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS m/z 286 $[M + Na]^+$; HRESIMS m/z 286.0691 $[M + Na]^+$ (calcd for $C_{13}H_{13}NO_5Na$, 286.0691).

3. Results and discussion

Fennebricin A (1) was obtained as an unstable pale-yellow amorphous powder, and its molecular formula, $C_{28}H_{32}N_2O_8$, was established by HRESIMS of its pseudomolecular ion $[M + Na]^+$ (m/z 547.2054, calcd 547.2056), implying 14 sites of unsaturation. An intense IR band at 1652 cm^{-1} (quinone carbonyl) and UV absorption maxima at 269 and 372 nm were characteristics for compounds having a bisquinone moiety [1]. The ^{1}H NMR spectrum of 1 revealed only few well-resolved signals, including six methyl groups and eight midfield resonances ranging from δ_H 2.92 to 3.97 (Table 1). The ^{13}C NMR and DEPT spectra showed 28 carbon resonances, most of which were assignable to aromatic or heteroatom-bearing carbons (Table 1). In particular, the ^{13}C NMR data suggested the presence of four quinone carbonyls (δ_C 185.6, 185.5, 183.1 and 180.8), two arylmethyl groups (δ_C 8.5), and two methoxy groups (δ_C 61.5), which were characteristic of two quinone rings of renieramycins.

The NMR data of 1 were very similar to those of the co-occurring known renieramycin, renieramycin J (2) [19]. In fact, careful comparison of the NMR data of 1 and 2 revealed that the only difference between them resided in replacement of the angelic acid ester group at C-22 in 2 by a hydroxyl group, in agreement with a mass difference of 82 units, while the rest

Table 2
^{1}H and ^{13}C NMR data for 7a and 7b in CD_3OD [a,b].

	7a		7b	
Position	δ_C [c]	δ_H (mult. *J* in Hz)	δ_C [c]	δ_H (mult. *J* in Hz)
1	49.8, CH	5.85, dd (4.5, 3.2)	52.4, CH	5.13, dd (9.8, 4.0)
3	133.6, CH	6.93, d (7.6)	129.0, CH	7.46, d (7.6)
4	101.2, CH	6.09, d (7.6)	102.4, CH	6.17, d (7.6)
5	185.2, C	–	184.6, C	–
6	127.9 C	–	126.4 C	–
7	156.2, C	–	156.4, C	–
8	180.9, C	–	180.8, C	–
9	135.1, C	–	no[d]	–
10	141.2, C	–	no[d]	–
11	162.6, CH	8.64, s	161.7, CH	8.27, s
12	62.7, CH_2	3.65, dd (11.4, 3.5) 3.72, dd (11.4, 3.7)	60.5, CH_2	3.47, dd (11.8, 3.6) 3.68, dd (11.8, 6.8)
6-CH_3	8.4, CH_3	1.93, s	8.4, CH_3	2.00, s
7-OCH_3	61.6, CH_3	4.05, s	61.6, CH_3	4.05, s

[a] Spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[b] Chemical shifts (ppm) referred to either CH_3OH (δ_H 3.31) or CD_3OD (δ_C 49.5).
[c] By DEPT experiment.
[d] The unobservable signals.

of the structure of 1 is the same as in 2. This was supported by the absence of the proton signals at δ_H 5.93 (1H, qq, $J = 7.3$, 1.6 Hz), 1.79 (3H, dq, $J = 7.3$, 1.6 Hz), and 1.59 (3H, dq, $J = 1.6$, 1.5 Hz) and the carbon resonances at δ_C 167.0, 139.4, 126.8, 20.3, and 15.5 in the NMR spectrum of 1.

There are five stereogenic centers (C-1, C-3, C-11, C-13, and C-21) in 1. The almost identical ^{13}C NMR spectroscopic data of 1 and 2 strongly suggested that the relative configuration of 1 is the same as that in 2. This assumption was further supported by a ROESY experiment (Fig. 2), in which the correlations from H-3 to H-1 and H-4α, from H-21 to H-14β, and from H-13 to H-11 and H-14α were observable. Detailed analysis of its 2D NMR spectra (^{1}H–^{1}H COSY, ROESY, HMQC, and HMBC) allowed unambiguous assignments of the ^{1}H and ^{13}C NMR data of 1 (Table 1). Therefore, the structure of 1 was determined as shown in Fig. 1.

Finally, from the biogenic point of view, the absolute configurations at C-1, C-3, C-11, C-13, and C-21 of 1 were tentatively suggested as *R*, *S*, *R*, *S*, and *S*, respectively, the same as those of renieramycin J (2), since the absolute configuration of 2 has been determined by synthetic methods [20,21].

Fennebricin B (5) was isolated as an unstable pale-yellow amorphous powder. The molecular formula was established as $C_{27}H_{30}N_2O_9$ by pseudomolecular ion peak $[M + H]^+$ at *m/z* 527.5435 (calcd 527.5430) and an intense peak at *m/z* 509.1930 due to the loss of water from 5 in the HRESIMS spectrum of 5, requiring 14 sites of unsaturation. Three carbonyls and 10 olefinic carbons accounted for only eight sites of unsaturation, and thus there should be a hexa-cyclic skeleton. The ^{1}H NMR spectrum contained a characteristic pair of AB-type doublets at δ_H 5.87 and 5.96 ($J = 1.5$ Hz) and the ^{13}C NMR spectrum displayed a downfield methylene carbon signal resonating at δ_C 98.5, clearly indicating the presence of a methylenedioxy group in 5. Moreover, the existence of an acetoxyl group in 5 was evident by the typical NMR signals (δ_H 1.93, 3H, s; δ_C 170.5). The molar extinction coefficient at maximum UV absorption was reduced to half in 5 compared to that in bisquinone-type renieramycins (such as 1), and only two carbonyl resonances (δ_C 186.0 and 182.9) of the quinone rings were observed in the ^{13}C NMR spectrum (Table 1). These data revealed that one of the quinone rings might have been reduced to form an aromatic ring in 5. The strong HMBC correlations from H-4α (δ_H 2.80, br d, $J = 17.5$ Hz) to C-5 (δ_C 141.2), C-9 (δ_C 113.0), and C-10 (δ_C 120.2), and from H-4β (δ_H 1.60, dd, $J = 17.5$, 11.2 Hz) to C-5 and C-10 in the HMBC spectrum revealed that 5 might have an aromatic ring only at the A ring with the methylenedioxy functionality, similar to acetylrenieramycin T (6), a synthetic product derived from renieramycin T [13].

The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) of 5 were almost the same as those for 6 except for the absence of several signals. A comparison of the NMR data of 5 and 6 indicated that the structure of 5 differs from 6 only by the different substituents at C-21 and C-22. The nitrile carbon signal (δ_C 117.3) present in the ^{13}C NMR spectrum of 6 was absent in that of 5. Furthermore, the H-21 signal at δ_H 4.50 of 5 was shifted downfield relative to the corresponding signal (δ_H 4.12) of 6, and a signal at δ_C 84.0 for the C-21 carbon was also significantly shifted downfield as compared to that of 6 (δ_C 59.0). These data suggested that 5 had a hydroxyl group at C-21 in place of the nitrile group of 6. In addition, like compound 1, the angelic acid ester group at C-22 in 6 was shown to be replaced by a hydroxyl group due to the absence of the characteristic signals for the angelic acid ester moiety in the NMR spectrum of 5. The relative configuration of 5 was suggested to be the same as that in 1 and 6 on the basis of almost identical ^{13}C NMR data, and unequivocally confirmed by a ROESY experiment as shown in Fig. 2. Hence, the structure of 5 was characterized as depicted in Fig. 1. Finally, analogously to 1, the absolute configurations at C-1, C-3, C-11, C-13, and C-21 in the molecule of 5 were tentatively suggested as *R*, *S*, *R*, *S*, and *R*, respectively, the same as those of acetylrenieramycin T (6).

N-formyl-1,2-dihydrorenierol (7) was isolated as a red amorphous powder. The molecular formula was established to be $C_{13}H_{13}NO_5$ by analysis of its HRESIMS spectrum in which the pseudomolecular ion at *m/z* 286.0691 $[M + Na]^+$ (calcd 286.0691) was observed. The IR spectrum of 7 displayed the intense absorption band at 1656 cm^{-1} due to

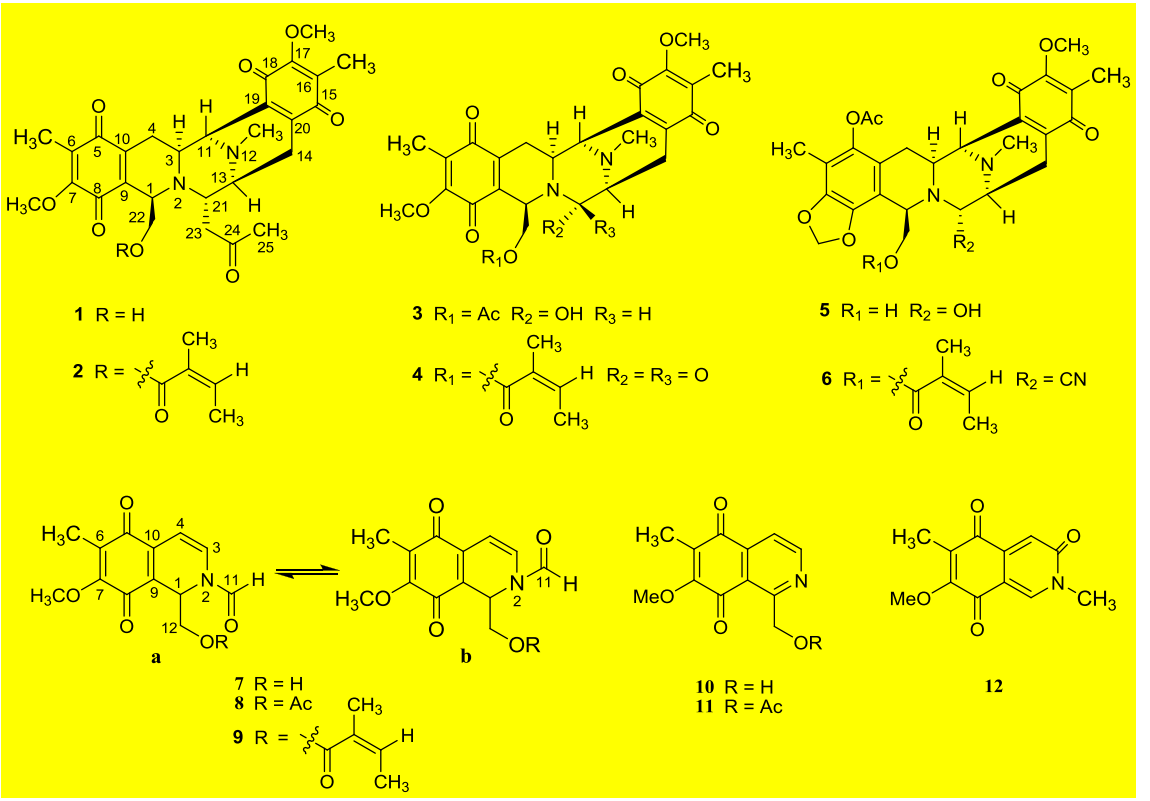

Fig. 1. Chemical structures of compounds 1–12.

both the formamide and quinone functionalities. The UV spectrum showed the same absorption maxima at 210, 265, 340, 512 nm as those of the two co-occurring known isoquinolinequinones, *N*-formyl-1,2-dihydrorenierol acetate (8) and *N*-formyl-1,2-dihydrorenierone (9) [22,23]. The ^{1}H and ^{13}C NMR spectral data for 7, however, contained a pair of well-separated signals with a ratio of approximately 2:1, indicating a mixture of two isomers. The compound must be a physically inseparable equilibrated mixture of 7a and 7b since TLC and HPLC analysis of 7 are homogeneous in a variety of solvent systems. Therefore, all the well-separated NMR signals for the major (7a) and minor (7b) rotamers except for the unobservable ^{13}C NMR resonances in 7b due to C-9 and C-10 were readily assigned by extensive NMR measurements (including $^{1}H-^{1}H$ COSY, HMQC, and HMBC techniques). The rotameric equilibration between 7a and 7b is driven by the partial double bond property of the *N*-formyl bond in the two molecules. This pattern of conversion was also observed in the co-occurrence of 8 and 9. The ^{1}H and ^{13}C NMR data of 7 were strongly reminiscent of those for 8 and 9 [22,23]. Comparison of the NMR data of 7, 8 and 9 revealed that the only difference between them resided in replacement of the substituent at C-12 (the acetoxyl group for 8; the angelic acid ester moiety for 9) by a hydroxyl group. The configuration of the rotamers 7a and 7b was determined by comparison of the chemical shifts of the NMR signals due to C-1 and C-3 with those for those for 8a/8b and 9a/9b. On the basis of the above evidence, the structures of the rotamers 7a and 7b were assigned as described. Like compounds 8 and 9, the stereochemistry at C-1 of 7 has not been determined yet.

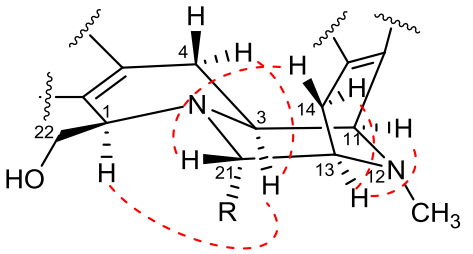

Fig. 2. Key ROESY correlations for fennebricins A (1) and B (5).

The known isoquinolinequinone alkaloids were readily identified as renieramycin J (2) [19], Jorumycin (3) [9], renieramycin G (4) [24], *N*-formyl-1,2-dihydrorenierol acetate (8) [23] and *N*-formyl-1,2-dihydrorenierone (9) [22], renierol (10) [25,26], renierol acetate (11) [25,27], and mimosamycin (12) [22], by comparing their NMR and MS spectroscopic data with those reported in the literature.

Isoquinolinequinone natural products and their reduced forms constitute an important class of secondary metabolites biosynthesized by a diverse range of marine organisms, including sponges, mollusks, tunicates, and bacteria. These metabolites have attracted considerable interest over the past 30 years due to their potent biological properties. The

new compounds, fennebricins A (1) and B (5), are two renieramycin-type dimeric isoquinolinequinones structurally related to the ecteinascidins and the saframycins, two groups of promising antimicrobial and antitumor alkaloids. However, since these two compounds were available in only trace amounts and were relatively unstable, decomposing during spectral measurements, we have been unable to determine whether they have antitumor and antimicrobial properties similar to those reported for the ecteinascidins and the saframycins. Further studies should be conducted to (semi) synthesize these two new compounds to evaluate their biological activities.

In a previous study, a series of isoquinolinequinone alkaloids, such as *N*-formyl-1,2-dihydrorenierol acetate (8) and mimosamycin (12), have been reported from the mollusk *J. funebris* and its associated prey, the sponge *Xestospongia* sp., suggesting the prey–predator relationship between the two animals [10]. Very interestingly, we have isolated simultaneously ten isoquinolinequinones (1–5 and 8–12) from both the nudibranch *J. funebris* and the sponge *Xestospongia* sp. This fact, together with the previous findings, lets us to suppose that these isoquinolinequinones might have an important ecological role as chemicals for self-defensive purpose in the nudibranch and the sponge. Moreover, all the metabolites except for 7 occurred simultaneously in the two animals, supporting recent ecological studies that the nudibranch *J. funebris* preys on the sponge of the genus *Xestospongia* [7,10]. It is worth noting that no trace of *N*-formyl-1,2-dihydrorenierol (7) was found in the nudibranch. This could be due to the ability of the nudibranch to accumulate dietary compounds selectively useful for its own protection.

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