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Remisporine B, a novel dimeric chromenone derived from spontaneous Diels–Alder reaction of remisporine A

Fangming Kong* and Guy T. Carter

Departments of Natural Products and Discovery Analytical Chemistry, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965, USA

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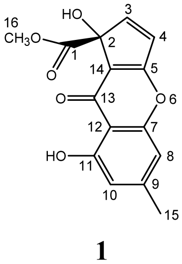
Abstract—A novel cyclopentachromenone, remisporine A, has been isolated from liquid culture of the marine fungus *Remispora maritima*. Remisporine A is not stable under normal conditions and autocatalytically dimerizes to give rise to a stereospecific product remisporine B via Diels–Alder reaction. The structure of remisporine B was elucidated by spectroscopic analyses including 2D-NMR and HRMS data. © 2003 Elsevier Science Ltd. All rights reserved.

A well-known synthetic method for formation of multi carbon–carbon or carbon–heteroatom bonds is the Diels–Alder reaction.[1] It also occurs in nature for organisms to produce spatially complex secondary metabolites. For example, a growing number of polycyclic alkaloids have been isolated from marine sponges. Their complex skeletal types are apparently formed through a key reaction, the biological equivalent of [4+2] cycloaddition.[2] In the course of searching for novel substances as therapeutic agents from microorganisms, a marine fungus identified as *Remispora maritima* was examined. Fractionation of the culture led to isolation of two major products, the known fungal metabolite citrinin[3] and a novel cyclopentachromenone, which we have named remisporine A (**1**). The isolated remisporine A autocatalytically dimerizes through Diels–Alder reaction to give rise to remisporine B (**2**).



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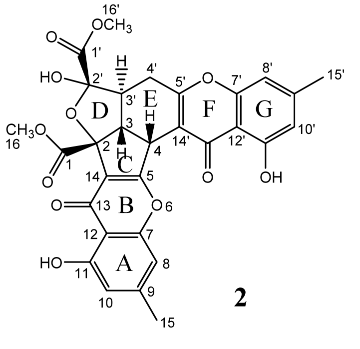

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The strain 22G295 of marine fungus, *R. maritima*, was isolated from a wood sample. The culture was grown in twenty 250-mL Erlenmeyer flasks containing 50 mL of potato-dextrose broth (Difco), shaken at 200 rpm at 22 °C for 7 days, and harvested. The frozen fermentation whole broth (1 L) of *R. maritima* was thawed and centrifuged. The supernatant was extracted with *n*-butanol (2×600 mL) and the pellets were extracted with methanol (2×200 mL). Both *n*-butanol and methanol extracts were combined on the basis of their similar LC–MS profiles, concentrated, and fractionated by reversed phase HPLC. The C-18 column (Metachem ODS-3, 250×20 mm) was washed with a linear gradient of 10 to 65% acetonitrile in 0.05% triflouroacetic acid (TFA) buffer over 70 min and isocratically thereafter at a flow rate of 7 mL/min. Major peaks eluted at 61 and 72 min. The most abundant component (72 min) was identified to be citrinin.[3] The peak eluted at 61 min was found to be a mixture as judged by ^{1}H NMR, which was further purified using the same HPLC column. A linear gradient of methanol/aqueous TFA (0.02%) with 40 to 90% methanol over 60 min followed by 90% methanol isocratic at 7 mL/min was used with UV detection at 260 nm. Remisporine A (0.7 mg) was eluted at 53 min and remisporine B (1.9 mg) at 98 min.

It was apparent that remisporine A was converted to remisporine B during the isolation and purification process. The LC–MS chromatogram recorded on the original culture broth exhibited only the remisporine A peak at 15.3 min.[4] However, the LC–MS traces of the *n*-butanol extract as well as the methanol extract showed not only the remisporine A peak but also a later peak of remisporine B at 23.0 min with a ratio of

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Keywords: marine fungus; cyclopentadiene; chromenone; natural product; cycloaddition; dimerization; Diels–Alder; retro Aldol.

* Corresponding author. Tel.: +1-845-602-4899; fax: +1-845-602-5687; e-mail: kongf@wyeth.com

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10:1. Moreover, an analysis of the preparative HPLC sample eluted at 61 min indicated a 1:1 mixture of remisporine A and remisporine B. The second round HPLC purification of this mixture gave mainly remisporine B.

Remisporine A is a novel cyclopentadiene derivative. It had UV absorption maxima at 270 and 344 nm. The LRESMS showed a $[M+H]^+$ peak at m/z 289, appropriate for a molecular formula of $C_{15}H_{12}O_6$. Although compound **1** could not be fully characterized using spectroscopic methods, the NMR data acquired on the freshly isolated sample clearly indicated the presence of a cyclopentadiene moiety (Table 1). In the ^1H, ^{13}C, and HMQC spectra, there were two sp^2 methines at δ 6.92/127.2 (C-4) and 6.98/149.4 (C-3). Both of the proton signals displayed HMBC correlations to a quaternary carbon resonance at δ 82.0 (C-2), while the dimerized compound **2** had the oxygenated quaternary carbon at δ 86.6. The proton spectrum also showed the coupling constant of 5.8 Hz for these two methine protons, typical for a cyclopentadiene compound.

The molecular formula of remisporine B was determined as $C_{30}H_{24}O_{12}$ by high-resolution FT-ICR mass spectrometry (measured: 576.1275, calcd: 576.1268), suggesting a dimeric structure composed of two $C_{15}H_{12}O_6$ subunits. The UV spectrum exhibited long-wavelength absorption maxima at 240 and 325 nm, and shoulder peaks at 228 and 260 nm. Inspection of the ^1H NMR spectrum of **2** revealed the dimeric nature of the molecule: two phenolic hydroxy protons (δ 12.19 and 12.50), four aromatic protons (δ 6.68, 6.72, 6.86, 6.91), two methoxy groups (δ 3.66 and 3.70), and two aryl C-methyl groups (δ 2.36 and 2.39). In the ^{13}C NMR spectrum, all 30 carbon resonances were observed. Most of those signals occurred as pairs, indicating the dimeric character of the molecule.

The COSY data identified two sets of two *meta*-coupled aromatic protons at δ 6.86/6.68 (H-8/H-10) and 6.91/6.72 (H-8′/H-10′), which also showed benzylic coupling to methyls at δ 2.36 (H-15) and 2.39 (H-15′), respectively. Two intra-molecularly hydrogen-bonded phenolic hydroxy protons (δ 12.19 and 12.50), which must be adjacent to carbonyls for their ^1H chemical shifts, showed HMBC correlations to C-10, C-11, C-12, and C-10′, C-11′, C-12′, respectively. Furthermore, both benzylic methyls showed HMBC correlations to C-8, C-9, C-10, and C-8′, C-9′, C-10′, respectively. Additional HMBC correlations from H-8 to C-7 and from H-8′ to C-7′ established the presence of two tetra-substituded benzenoid rings shown below (Fig. 1).

The most down field aliphatic methine proton doublet at δ 4.60 was assigned to H-4 to account for its doubly allylic position. In the COSY spectrum, H-4 was coupled to a methine proton at δ 3.29 (H-3), the latter

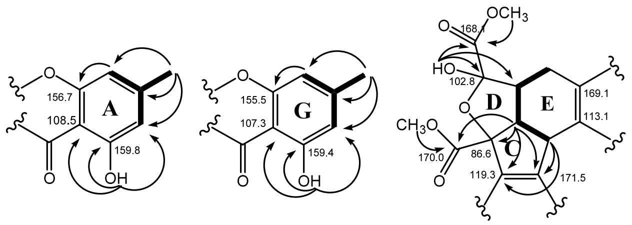

Figure 1. Partial structures of remisporine B (**2**) with selected COSY (—) and HMBC (⌒) correlations.

Table 1. NMR data for remisporine B (**2**) in DMSO-d_6[a]

Position	^1H[b]	^{13}C[c]	HMBC (^1H)	Position	^1H[b]	^{13}C[c]	HMBC (^1H)
1		170.0	3.29, 3.70	1′		168.1	3.66, 7.94
2		86.6	3.29	2′		102.8	7.94
				2′-OH	7.94 (bs)		
3	3.29 (dd, 6.0, 11.9)	47.2	2.63, 2.84, 2.91, 4.60	3′	2.63 (dt, 4.0, 11.9)	45.4	2.84, 2.91, 3.29, 4.60, 7.94
4	4.60 (d, 6.0 Hz)	39.1	2.63	4′	2.84 (dd, 4.3, 17.1) 2.91 (dd, 12.2, 17.1)	28.4	3.29
5		171.5	3.29, 4.60	5′		169.1	2.84, 2.91, 4.60
7		156.7	6.86	7′		155.5	6.91
8	6.86 (bs)	108.3	2.36, 6.68	8′	6.91 (bs)	107.4	6.72, 2.39
9		147.5	2.36	9′		147.7	2.39
10	6.68 (bs)	112.6	2.36, 6.86, 12.21	10′	6.72 (bs)	111.9	2.39, 6.91, 12.52
11		159.8	6.68, 12.21	11′		159.4	6.72, 12.52
11-OH	12.21 (s)			11′-OH	12.52 (s)		
12		108.5	6.68, 6.86, 12.21	12′		107.3	6.72, 6.91, 12.52
13		179.7		13′		181.4	4.60
14		119.3	3.29, 4.60	14′		113.1	2.84, 2.91, 4.60
15	2.36 (bs)	21.5	6.68, 6.86	15′	2.39 (bs)	21.8	6.72, 6.91
16	3.70 (s)	52.7		16′	3.66 (s)	52.4	

[a] Chemical shifts were referenced to residual solvent signals (2.49 or 39.5 ppm).

[b] Recorded at 400 MHz.

[c] Recorded at 100 MHz.

further correlated to another methine proton at δ 2.63 (H-3′) that was coupled to both methylene protons at δ 2.84 and 2.91 (H-4′a and H-4′b). A weak correlation between H-4 and H-4′b implied these two protons had a homo-allylic relationship. The presence of the homo-allylic substructure was confirmed by the HMBC data. Thus, both H-3 and H-4 displayed HMBC correlations to an sp^2 carbon resonance at δ 113.1 (C-14′) that also correlated to the methylene protons (H-4′a and H-4′b). Additional HMBC correlations observed between H-4′a/H-4′b and C-5′ at δ 169.1 identified the cyclohexaene moiety (E-ring). The HMBC correlations observed from both H-3 and H-4 to the only remaining pair of olefinic carbon resonances at δ 119.3 and 171.5 suggested the double bond location at C-14/C-5, and also implied the presence of a 5-membered ring system (C-ring). A two-bond HMBC correlation observed between H-3 and an oxygenated carbon signal at δ 86.6 (C-2) established the C-ring moiety. A proton singlet at δ 7.90 was assigned to aliphatic hydroxy proton (2′-OH) since it did not show correlation to any carbons in the HSQC experiment. This hydroxy proton showed a three-bond HMBC correlation to C-3′ and two-bond correlation to C-2′ at δ 102.8, which was assigned as a hemiketal carbon on the basis of its chemical shift. The ether linkage between C-2 and C-2′, as required by the molecular formula, comprised the D-ring. It was evident that there were two methoxy carbonyl groups that resonated at δ 170.0 and 168.1 attached at C-2 and C-2′, respectively, based on the HMBC correlations illustrated in Figure 1.

A carbon resonance at δ 181.4 was assigned to C-13′ since a three-bond HMBC correlation was observed to this carbon atom from H-4. No correlations were observed to the only remaining signal at δ 179.7 that must be assigned to C-13 to account for its chemical shift. Identification of ring fusion between B-ring and C-ring, as well as between E-ring and F-ring, was based on the ROESY data. Weak ROESY correlations observed between the hydroxy proton 11′-OH and H-4, and between H′-4 and H′-8, established the E-ring and F-ring connectivity. A weak ROESY correlation between H-4 and H-8 confirmed the B-ring and C-ring fusion.

The relative stereochemistry was established by analysis of the ROESY spectrum. Strong NOE observed between H-3 and H-4 indicated a *cis* relationship. And weak ROESY correlation between H-3 and H-3′ suggested that H-3′ was located on the other side. Both methoxy carbonyl groups were revealed as β-configuration as correlations were observed between the methoxy protons H-16 and H-3/H-4, and between the hydroxy proton 2′-OH and H-3′ as shown. This was consistent with the stereochemistry requirement by the proposed mechanism of dimerization of **1** below. The CD spectrum of remisporine B shows exciton coupling of the two chromenone chromophores.[5]

Remisporine B is presumably formed from remisporine A as outlined in Figure 2. An *endo* mode of Diels–Alder reaction of the cyclopentadiene portion of **1**, which adopts the less hindered orientation where the hydroxyl groups face each other rather than the bulky methoxy carbonyls, generates adduct **3**. A retro Aldol condensation of **3** affords intermediate **4**, which undergoes aromatization to come back to the chromenone moiety as shown in **5**. Final attack of the hydroxyl at C-2 to the keto group, forming the hemiketal, yields the dimer **2**.

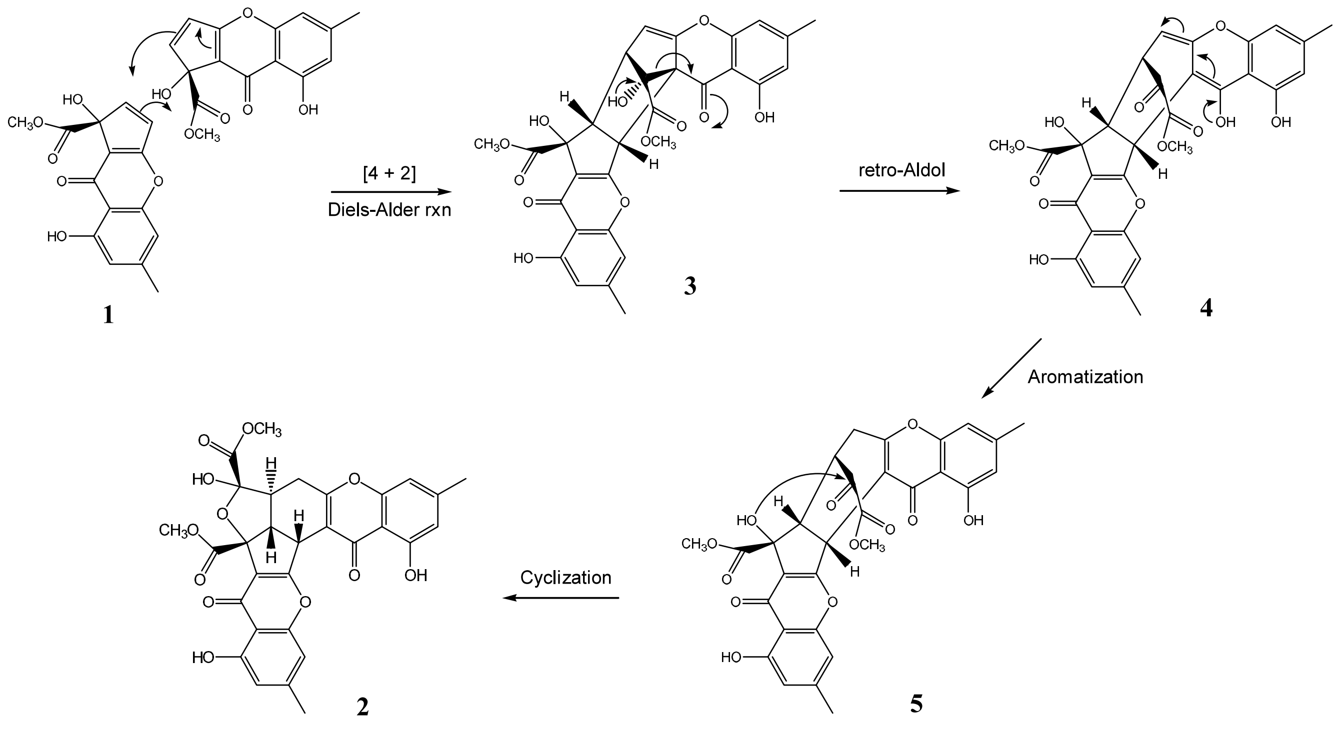

Figure 2. Proposed mechanism for the formation of remisporine B (**2**) from remisporine A (**1**).

Remisporine A is the first example of a cyclopentadiene derivative present in nature which spontanously dimerizes during the isolation process to give stereospecific product remisporine B. It is well known that cyclopentadienes undergo Diels–Alder cycloaddition reaction. In fact, commercial cyclopentadiene is only available in a dimeric form. Remisporine A is structurally related to coniochaetone A, a fungal product.[6] A small amount of coniochaetone A was also isolated from the *R. maritima* culture.

Remisporine B exhibited no in vitro antimicrobial activity against *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecium*, and *Escherichia coli* at 1 mg/mL when 20 μL was spotted per well.

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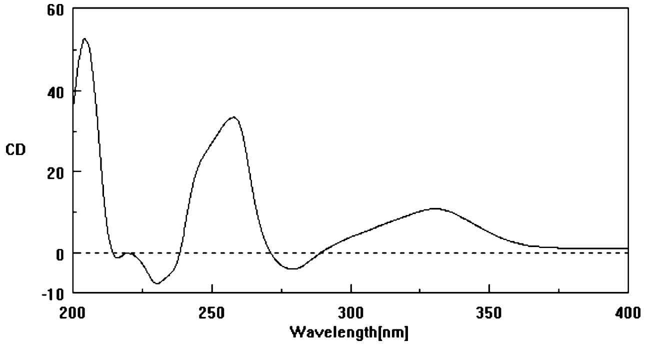
References

1. (a) Yli-Kauhaluoma, J. *Tetrahedron* **2001**, *57*, 7053–7071; (b) Tietze, L. F.; Kettschau, G. *Top. Curr. Chem.* **1997**, *189* (Stereoselective Heterocyclic Synthesis I), 1–120.
2. Andersen, R. J.; Van Soest, R. W. M.; Kong, F. In *Alkaloids*: *Chemical & Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon Press: New York, 1996; Vol. 10, pp. 301–355.
3. Sprenger, R. D.; Ruoff, P. M. *J. Org. Chem.* **1946**, *11*, 189–193.
4. HPLC conditions: YMC ODS-A column (150×4.6 mm), linear gradient gradient from 5 to 95% acetonitrile in 0.05% TFA buffer over 25 min, 0.8 mL/min on a Finnigan LCQ system.
5. CD spectrum of remisporine B (1.5 mg in 2 mL MeOH).



6. Wang, H.; Gloer, J. B.; Scott, J. A.; Malloch, D. *Tetrahedron Lett.* **1995**, *36*, 5847–5851.