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Structure Revision of Circumdatins A and B, Benzodiazepine Alkaloids Produced by Marine Fungus *Aspergillus ostianus*, by X-ray Crystallography

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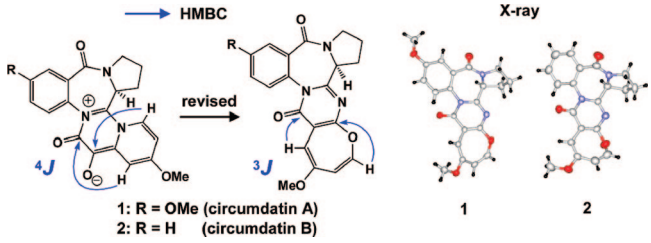
Received February 11, 2008



The structures of circumdatins A and B, pentacyclic alkaloids produced by *Aspergillus ostianus*, were revised from the previously reported betaine structures to unique oxepin ones by X-ray crystallography. The co-occurring known alkaloids, circumdatins D, E, and H, and a new compound reported here, circumdatin J, have a common framework.

Marine microorganisms are recognized as important sources of pharmacologically active metabolites, and a growing number of marine fungi have been reported to produce novel bioactive secondary metabolites.[1] We have been studying the metabolites of marine-derived fungus *Aspergillus ostianus* strain 01F313 isolated from an unidentified marine sponge collected at Pohnpei, the Federated States of Micronesia, and have already reported new chlorine-containing compounds.[2] The chlorine must have originated from the cultivation medium, which was composed of natural seawater. Expecting that bromine-containing compounds might be obtained by the use of a medium in which a bromide solution replaces seawater, we cultivated the same strain in a bromine-modified 1/2PD medium. Actually, we were able to isolate the corresponding brominated analogues,[3] and found, at the same time, that the other metabolites[4] present were considerably different from those obtained from the strain cultured in seawater medium. We also succeeded in isolating several benzodiazepine alkaloids that were not produced in seawater medium.

A group of unique benzodiazepine alkaloids, including circumdatins A (**1**),[5] B (**2**),[5] D (**3**),[6] E (**4**),[6] and H (**5**)[7] (Figure 1), have been reported from fungi of the genus *Aspergillus*. This group of compounds are considered to be useful chemotaxonomic markers.[5] Among the compounds, circumdatin H (**5**) was recently isolated from *A. ochraceus* as a new inhibitor of mitochondrial NADH oxidase.[7] Circumdatins D (**3**), E (**4**), and H (**5**) possess a typical circumdatin skeleton composed of an L-proline and two anthranilic acid moieties, which are condensed to form a pentacyclic framework. On the other hand, circumdatins A (**1**) and B (**2**) do not fall into this category; although they have a pentacyclic skeleton consisting of an L-proline and an anthranilic acid unit, the skeleton also includes a biogenetically unprecedented oxo-pyridin-2-yl-acetyl group. Another peculiar feature of **1** and **2** is that they have a betaine structure that is rarely encountered in natural products.

Chromatographic separation of the ethyl acetate extract of the filtered cultivation medium of *A. ostianus* strain 01F313 gave circumdatins **1**−**5**, together with a new compound, named circumdatin J (**6**) (Figure 1). This paper revises the structures of circumdatins A and B and elucidates the structure of circumdatin J.

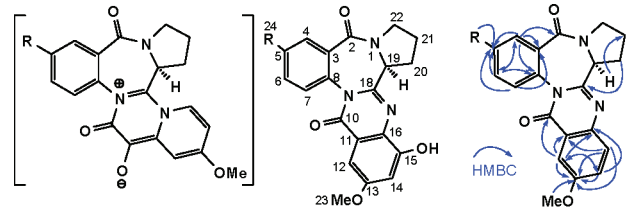


FIGURE 1. Structures of known circumdatins A (**1**), B (**2**), D (**3**), E (**4**), and H (**5**), and a new compound, circumdatin J (**6**). Structures **1** and **2** were revised to those depicted in Figure 2.

The *A. ostianus* strain was cultured in a bromine-modified 1/2PD (potato-dextrose) medium for 10 weeks, and the mycelial cake was removed by filtration. The filtrate was separated by solid-phase extraction with HP-20 SS and flash chromatography on silica gel, followed by reversed-phase (C_{18}) HPLC, giving **1**−**6**. The identities of compounds **1**−**5** with circumdatins A, B, D, E, and H, respectively, were corroborated by comparing their ^{1}H and ^{13}C NMR data with those reported in the literature.[5−7] The structure of new compound **6** was easily deduced by comparison of its ^{1}H and ^{13}C NMR data with those of circumdatin D (**3**),[6] which lacks a hydroxyl group at C-15. The HMBC spectrum of **6** showed the appropriate H/C correlation peaks ($^2J_{CH}$ and $^3J_{CH}$) (Figure 1) supporting the

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(1) Bungni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143.
(2) Namikoshi, M.; Kobayashi, H. *J. Antibiot.* **2003**, *56*, 755.
(3) Kito, K. Ph.D. Thesis, The University of Tokushima, 2008.
(4) Kito, K.; Ookura, R.; Yoshida, S.; Namikoshi, M.; Ooi, T.; Kusumi, T. *Org. Lett.* **2008**, *10*, 225.
(5) Rahbæk, L.; Breinholt, J.; Frisvad, J. C.; Christophersen, C. *J. Org. Chem.* **1999**, *64*, 1689.
(6) Rahbæk, L.; Breinholt, J. *J. Nat. Prod.* **1999**, *62*, 904.
(7) Lopez-Gresa, M. P.; Gonzalez, M. C.; Primo, J.; Moya, P.; Romero, V.; Estornell, E. *J. Antibiot.* **2005**, *58*, 416.

10.1021/jo800348d CCC: $40.75 © 2008 American Chemical Society
Published on Web 05/07/2008

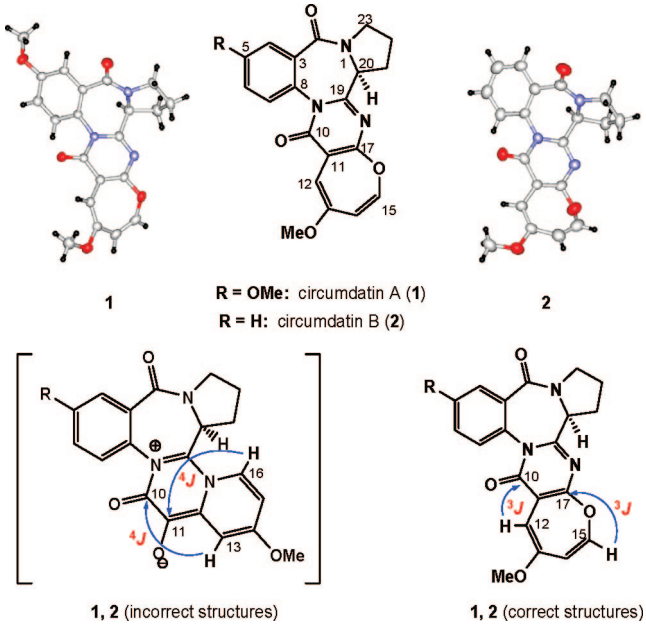

FIGURE 2. Above: Structures of circumdatins A (**1**) and B (**2**) determined by X-ray crystallography. Below: HMBC correlations assigned in the previous (incorrect) structures and correct structures of **1** and **2**.

structure. The absolute configurations of the circumdatins were previously established by obtaining L-proline from the hydrolysis products.[5] The 19(*S*)-configuration of **6** was confirmed by comparing the CD data of **3** ($\Delta\epsilon$ −4.1, 16, −22, λ_{max} 310, 266, 247 nm)[6] with those of **6** ($\Delta\epsilon$ −8, 20, −32, λ_{max} 300, 262, 230 nm).

In the course of the NMR study, we noticed unusually long-range H−C couplings between H-13/C-10 and H-16/C-11 ($^4J_{CH}$) in the HMBC spectra of circumdatins A (**1**) and B (**2**) (Figure 2). Cross peaks due to such $^4J_{CH}$ interactions may be observed, though rarely, in HMBC spectra, but the coupling constants are usually so small in magnitude that the H/C correlations normally appear only as weak cross peaks. In the present cases, however, **1** and **2** gave very intense HMBC cross peaks between the protons and carbons mentioned above. On the other hand, no $^4J_{CH}$ peaks were observed in the HMBC spectra of **3**−**6**. We therefore reexamined the structures of **1** and **2**. Their structures were originally deduced on the basis of INEPT2- and HMBC-INADEQUATE spectroscopy and on the hydrolytic products of **1**: L-proline, 4-methoxyanthranilic acid, and a tricyclic bislactam (upper-half of **1**).[5] The UV spectra of **1** and **2** are similar to those of **3**, **4**, and **6**, except that the bands at 340 nm observed for the latter ones are missing in the spectra of **1** and **2**.

Fortunately, we were able to obtain single crystals of **1** and **2**, and subjected them to X-ray crystallography. The results were surprising: both compounds had an oxepin framework [**1**, **2** (correct structures) in Figure 2] that we had not considered. On the basis of the X-ray structures the ^{1}H and ^{13}C NMR signals were reassigned. The aforementioned puzzling appearance of $^4J_{CH}$ values between H-13/C-10 and H-16/C-11 in **1**, **2** (incorrect structures) is now more reasonably interpretable as $^3J_{CH}$ between H-12/C-10 and H-15/C-17 in **1**, **2** (correct structures) (Figure 2).

(8) Cutler, H. G.; Arison, B. H.; Cole, P. D.; Roberts, R. G. *Agric. Biol. Chem.* **1988**, *52*, 1725.

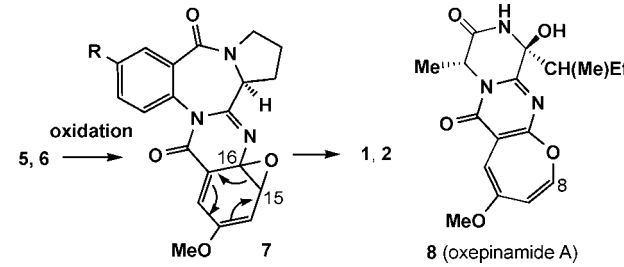

FIGURE 3. Benzene oxide−oxepin tautomerism of circumdatins.

A literature survey revealed that cinereain,[8] asperloxin,[9] and oxepinamides[10] have an oxepin framework. The structure of oxepinamide A (**8**) (Figure 3), a metabolite of *Aspergillus fumigatus*, a fungus obtained from the gastrointestinal tract of a marine fish, was determined by extensive 1D- and 2D-NMR studies including ^{15}N NMR spectroscopy together with computer calculations. Previous authors[10] noticed a large $^1J_{CH}$ value (198 Hz) between H-8 and C-8. In the case of circumdatins A (**1**) and B (**2**), the corresponding $^1J_{CH}$ (H-15/C-15) values determined by HMBC experiments were 197 and 196 Hz, respectively. This large $^1J_{CH}$ value, typical for the α-methine carbon of an enol moiety,[10] may serve as a good indication of an oxepin group, the presence of which can otherwise be difficult to deduce.

The reported INADEQUATE spectra of circumdatin A are compatible with the X-ray structure (**1**).

Biosynthetically, circumdatins H (**5**) and J (**6**) are likely to be the precursors of circumdatins A (**1**) and B (**2**), respectively: the electron-rich C_{15}−C_{16} bond could be oxidized to form a benzene oxide (**7**), in which a retro-pericyclic reaction (benzene oxide−oxepin tautomerism)[11] takes place, producing **1** or **2** (Figure 3).

The inhibitory activity against MRSA of circumdatins A (**1**), B (**2**), D (**3**), E (**4**), and H (**5**) was examined. Only circumdatin D showed weak activity in a paper disk assay [9 mm inhibitory zone around a filter paper (7 mm diameter) soaked with a 25.4 μM DMSO solution of **3** after 48 h]. None of the compounds showed cytotoxicity against A548 lung cancer cells.

Experimental Section

Circumdatin A (1). Yellow solid; $[\alpha]^{27}_D$ −135 (*c* 0.47, EtOH); UV λ_{max} (EtOH) nm (ϵ) 356 (6400), 286 (5000), 239 (19000); X-ray-quality crystals (yellow prisms) were obtained from MeOH at room temperature. The unit cell contained two molecule of **1** and two molecules of MeOH. Mp 131−132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 (1H, d, J = 9.6 Hz, H-7), 7.43 (1H, d, J = 3.0 Hz, H-4), 7.07 (1H, dd, J = 9.6, 3.0 Hz, H-6), 6.21 (1H, d, J = 8.0 Hz, H-15), 5.80 (1H, d, J = 1.6 Hz, H-12), 5.54 (1H, dd, J = 8.0, 1.6 Hz, H-14), 4.44 (1H, d, J = 7.4 Hz, H-20), 3.90 (3H, s, H-25), 3.79 (1H, m, H-23a), 3.70 (3H, s, H-24), 3.56 (1H, m, H-23b), 2.94 (1H, m, H-21a), 2.23 (1H, m, H-22a), 2.08 (1H, m, H-21b) (determined by HSQC), 2.03 (1H, m, H-22b) (determined by HSQC); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 163.9 (C-2), 162.3 (C-10), 159.4 (C-5), 158.5 (C-17), 157.0 (C-13), 155.9 (C-19), 144.3 (C-15), 133.1 (C-3), 129.2 (C-7), 125.2 (C-8), 117.8 (C-6), 115.6 (C-14), 112.8 (C-4), 111.0 (C-11), 95.0 (C-12), 58.1 (C-20), 55.7

(9) Bode, H. B.; Bethe, B.; Hofs, R.; Zeeck, A. *ChemBioChem.* **2002**, *3*, 619.

(10) Belofsky, G. N.; Anguera, M.; Jensen, P. R.; Fenical, W.; Kock, M. *Chem. Eur. J.* **2000**, *6*, 1355.

(11) Henderson, A. P.; Mutlu, E.; Leclercq, A.; Bleasdale, C.; Clegg, W.; Henderson, R. A.; Golding, B. T. *Chem. Commun.* **2002**, 1956.

(C-25), 55.1 (C-24), 46.5 (C-23), 26.6 (C-21), 23.7 (C-22); HRTOFMS *m/z* 394.1395 $[M + H]^+$ (calcd for $C_{21}H_{20}N_3O_5$ 394.1403).

Circumdatin B (2). Yellow solid; $[\alpha]^{27}_D$ −163 (*c* 0.04, EtOH); UV λ_{max} (EtOH) nm (ϵ) 357 (1800), 288 (2100), 240 (8800); X-ray-quality crystals (red platelets) were obtained from hexane/EtOAc mixture at room temperature. The unit cell contained two molecules of **2** and one molecule of water. Mp 198−199 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.96, (1H, ddd, J = 7.6, 1.6, 0.8 Hz, H-7), 7.55 (2H, m, H-5 or H-6), 7.50 (1H, m, H-7) (determined by HSQC), 6.20 (1H, d, J = 6.0 Hz, H-15), 5.80 (1H, d, J = 1.9 Hz, H-12), 5.44 (1H, dd, J = 6.0, 1.9 Hz, H-14), 4.42 (1H, dd, J = 8.0, 1.2 Hz, H-20), 3.79 (1H, m, H-23a), 3.70 (3H, s, H-24), 3.55 (1H, m, H-23b), 2.93 (1H, m, H-21a), 2.23 (1H, m, H-22a), 2.10 (1H, m, H-21b) (determined by HSQC), 2.03 (1H, m, H-22b) (determined by HSQC); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 163.9 (C-2), 162.1 (C-10), 158.5 (C-17), 157.2 (C-13), 155.9 (C-19), 144.2 (C-15), 132.4 (C-3 or C-8), 132.0 (C-8 or C-3), 130.5 (C-5 or C-6), 129.9 (C-4), 129.0 (C-7), 127.9 (C-6 or C-5), 115.6 (C-14), 111.2 (C-11), 95.0 (C-12), 58.1 (C-20), 55.1 (C-24), 46.4 (C-23), 26.6 (C-21), 23.7 (C-22); HRTOFMS *m/z* 364.1313 $[M + H]^+$ (calcd for $C_{20}H_{18}N_3O_4$ 364.1297).

Circumdatin J (6). Colorless solid; UV λ_{max} (EtOH) nm (ϵ) 343 (6000), 330 (7600), 287 (17000); CD, λ_{ext} (*c* 0.0014, MeOH) ($\Delta\epsilon$) 300 (−8.0), 262 (20), 230 (−32); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.66 (1H, d, J = 2.9 Hz, H-12), 7.62 (1H, d, J = 8.8 Hz, H-15), 7.46 (1H, d, J = 8.8 Hz, H-7), 7.45 (1H, d, J = 3.0 Hz, H-4), 7.36 (1H, dd, J = 8.8, 2.9 Hz, H-14), 7.09 (1H, dd, J = 8.8, 3.0 Hz, H-6), 4.45 (1H, d, J = 8.0 Hz, H-19), 3.91 (3H, s, H-24), 3.90 (3H, s, H-23), 3.76 (1H, m, H-22a), 3.60 (1H, m, H-22b), 3.15 (1H, m, H-20a), 2.29 (1H, m, H-21a), 2.14 (1H, m, H-20b), 2.05 (1H, m, H-21b); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 164.2 (C-2) (determined by HMBC), 161.5 (C-10) (determined by HMBC), 158.9 (C-5), 158.7 (C-13) (determined by HMBC), 151.6 (C-18), 140.4 (C-16) (determined by HMBC), 133.2 (C-3), 129.6 (C-7), 129.0 (C-15), 126.0 (C-8), 124.7 (C-14), 122.1 (C11) (determined by HMBC), 117.9 (C-6), 112.7 (C-4), 106.7 (C-12), 58.7 (C-19), 55.8 (C-24), 55.7 (C-23), 46.5 (C-22), 26.9 (C-20), 23.6 (C-21) (determined by HMBC); HRTOFMS *m/z* 364.1313 $[M + H]^+$ (calcd for $C_{20}H_{18}N_3O_4$ 364.1297).

Acknowledgment. The authors sincerely acknowledge Dr. Yoshikazu Shizuri and Dr. Kaneo Kanoh, Marine Biotechnology Institute Co., Ltd, and Dr. Hirofumi Shibata, Faculty of Pharmaceutical Sciences, The University of Tokushima, for their technical support with the bioassay.

Supporting Information Available: Copies of ^{1}H, ^{13}C NMR, ^{1}H,^{1}H-COSY, HMBC, HSQC, and NOESY spectra of compounds **1**, **2**, and **6** and crystallographic information files (CIF) of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO800348D