Circumdatins D, E, and F: Further Fungal Benzodiazepine Analogues from *Aspergillus ochraceus*

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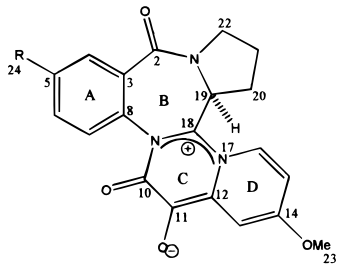
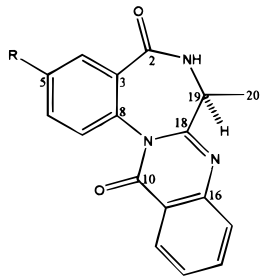
Three new benzodiazepine alkaloids belonging to the circumdatin series have been isolated as minor constituents of culture extracts of a terrestrial strain of the fungus *Aspergillus ochraceus*. Their structures were solved by MS and NMR comparison with previously reported circumdatin analogues.

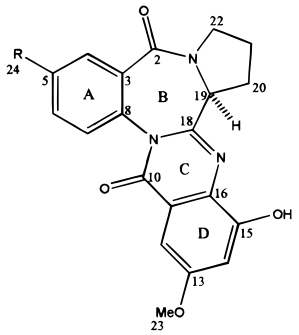
During our research aimed at the discovery of new secondary metabolites and guided by HPLC UV/vis analysis of crude extracts combined with chemotaxonomic knowledge within the genera *Aspergillus* and *Penicillium*, we recently uncovered the first naturally occurring zwitterionic benzodiazepines, circumdatins A (**1**) and B (**2**).[1] Compounds **1** and **2** were, together with circumdatin C (**3**),[1] the first benzodiazepines reported from *A. ochraceus*, although benzodiazepines have been reported as metabolic products from other *Aspergilli*.[2−5] Further detailed examination of the metabolite profile of the extract containing **1**, **2**, and **3** as major constituents implied that another three circumdatin analogues exhibiting UV characteristics similar to those of **1**, **2**, and **3** were present, albeit in minor amounts. We herein report the isolation and structure characterization of circumdatins D (**4**), E (**5**), and F (**6**) as minor constituents in culture extracts of a terrestrial isolate of *A. ochraceus* (*Aspergillus* subgenus Circumdati, section Circumdati, formerly the *A. ochraceus* group).



1: R = OMe
2: R = H



3: R=OH
6: R=H



4: R=OMe
5: R=H

The molecular formula of circumdatin D, $C_{21}H_{19}N_3O_5$, was established by NMR analysis in combination with HREIMS (M^+, 393.137, $C_{21}H_{19}N_3O_5$, Δ +12 ppm). Thus, the NMR analysis, including COSY, NOESY, HSQC, and HMBC experiments, provided evidence for the presence of a benzodiazepine structure derived from proline and 5-methoxyanthranilic acid as in **1**. The C−D ring system, however, was found to be identical to that in **3**, save for additional methoxy and hydroxy substituents in positions 13 and 15, respectively, leading to the structure **4** for circumdatin D. The assigned structure is supported by diagnostic long-range $^1H^{-13}C$ correlations (from C-10, C-13, C-14, and C-16 to H-12; from C-13 to H-23; and from C-16 to H-14) observed in the HMBC spectrum of **4**.

Comparison of the NMR spectra of circumdatin D (**4**) and circumdatin E suggested that their structures were identical except for substitution of the A ring methoxy group in **4** with a proton in circumdatin E, which accordingly is assigned the structure **5**. The limited amount of **5** available precluded complete assignment of the ^{13}C resonances, but the chemical shifts corresponding to the protonated carbon atoms were extracted from the HSQC spectrum. Comparing the chemical shift values for C-12 and C-14 in **4** (97.9 and 108.5 ppm, respectively) with those of **5** (98.1 and 108.7 ppm, respectively) further substantiated the identical substitution patterns of the D rings in **4** and **5**. Finally, the expected molecular composition of **5**, $C_{20}H_{17}N_3O_4$, was confirmed by HREIMS (M^+, 363.126, $C_{20}H_{17}N_3O_4$, Δ +11 ppm).

The molecular weight of circumdatin F (EIMS *m*/*z* 291 M^+), in combination with NMR data, indicated that its structure was identical with that of circumdatin C (**3**) except for the A-ring substituent, and circumdatin F is represented by the structure **6**. Again, HREIMS verified the molecular formula of **6**, $C_{17}H_{13}N_3O_2$ (M^+, 291.104, $C_{17}H_{13}N_3O_2$, Δ +11 ppm).

The characteristic A−B−C−D ring system of circumdatins C (**3**), D (**4**), E (**5**), and F (**6**) has previously been encountered in the asperlicins isolated from *A. alliaceus*[2] and the benzomalvins obtained from a *Penicillium* sp.,[6] of which asperlicin is used for treatment of gastrointestinal and CNS disorders. The benzodiazepine rings of **1**, **2**, **4**, and **5** are constructed from (substituted) anthranilic acid and L-proline, whereas L-alanine is incorporated in **3** and **6**.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in DMSO-d_6 solution on a Bruker DRX400 instrument equipped with a selective inverse 2.5-mm probe head

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Table 1. ^{1}H NMR Data for Circumdatins A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**)

no.	1	2	3	4	5	6
4	7.26 (d, 2.9)	7.82 (ddd, 7.0, 1.5, 0.5)	7.22 (d, 3.0)	7.27 (d, 2.9)	7.81 (dd, 7.4, 1.9)	7.92 (m)[b]
5		7.58 (ddd, 8, 8, 2)[c]			7.56[d]	7.65 (m)[e]
6	7.19 (dd, 9.2, 2.9)	7.64 (ddd, 7.5, 7.5, 2.0)[c]	7.05 (dd, 9, 3)	7.20 (dd, 8.9, 2.9)	7.63[d]	7.65 (m)[e]
7	7.49 (d, 9.2)	7.57 (dd, 8.0, 2.0)	7.43 (d, 9.0)	7.49 (d, 8.9)	7.58[d]	7.65 (m)[e]
12			8.24 (ddd, 8.0, 1.0, 0.5)	6.98 (d, 2.4)	7.07 (d, 2.5)	8.31 (dd, 8.0, 1.6)
13	5.73 (br d, 1.8)	5.74 (br d, 1.8)	7.56 (ddd, 8.0, 7.0, 1.0)			7.73 (ddd, 8.7, 7.0, 1.7)
14			7.84 (ddd, 8, 8, 2)	6.87 (d, 2.6)	6.87 (br s)	7.92 (m)[b]
15	5.68 (dd, 5.9, 2.0)	5.70 (dd, 5.9, 1.9)	7.76 (ddd, 8.0, 1.0, 0.5)			7.82 (d, 7.7)
16	6.40 (dd, 5.9, 0.4)	6.40 (d, 5.9)				
19	4.55 (dd, 6.6, 1.8)	4.53 (dd, 6.7, 2.0)	4.45 (q, 7.0)	4.60 (d, 6.4)	4.60 (d, 6.9)	4.48 (q, 6.6)
20	2.66 (m)	2.66 (m)	1.66 (d, 7.0)	3.20 (m)	3.21 (m)	1.71 (d, 6.6)
	2.0 (m)[e]	2.0 (m) [f]		2.06 (m)	2.04 (m)	
21	2.0 (m)[e]	2.0 (m) [f]		2.11 (m)	2.11 (m)	
	1.93 (m)	1.94 (m)		1.93 (m)	1.93 (m)	
22	3.6 (m) [f]	3.6 (m)[g]		3.57 (m)	3.58 (m)	
	3.39 (m)	3.41 (m)		3.43 (m)	3.44 (m)	
23	3.64 (s)	3.65 (s)		3.80 (s)	3.81 (s)	
24	3.86 (s)			3.87 (s)		

[a] NMR data for **1**, **2**, **4**, and **5** were recorded in DMSO-d_6. NMR data for **3** and **6** were recorded in MeOH-d_4. [b] Signals overlapping. [c] Assignments are interchangeable. [d] Strongly coupled, overlapping resonances. [e] Average value of unresolved signals from three protons. [f] Average value of unresolved signals from two protons. [g] Signal is overlapping with methoxy signal (C23).

with *z*-axis gradients. EIMS and HRMS data were recorded on a JEOL AX505W instrument.

Separation. Collection, fermentation, and extraction procedures have been described previously.[1] All separations were guided by UV spectra of the circumdatins using analytical or preparative HPLC systems coupled to a photodiode-array detector. A portion of the crude extract was fractionated by liquid chromatography using a Lobar LiChroprep column (Si 60, 40−63 μm, size B, Merck) eluted at a flowrate of 6 mL/min with EtOAc−EtOH−heptane 80:2.5:17.5, and employing UV detection at 270 nm. The fraction containing **4**, **5**, and **6** was subjected to three consecutive HPLC separation steps (1. LiChroCART LiChrospher Si 60 (10 μm) 250−10, EtOAc−EtOH−heptane 80:2.5:17.5, 270 nm, 6 mL/min; 2. Same conditions as first step, but with EtOAc−EtOH−heptane 60:5:35 as eluent; 3. Nova-Pak C_{18} 8 × 100 mm Radial-Pak Cartridge from Waters, CH_3CN-H_2O (3:7 for **4** and **5**, 1:4 for **6**), 2 mL/min, PDA detector) yielding **4** (1.1 mg), **5** (0.5 mg), and **6** (0.6 mg).

Circumdatin D (4): solid; $[\alpha]^{22}_D$ −129° (*c* 0.017, MeOH); UV (MeOH) λ_{max} (log ϵ) 339 (3.50), 285 (3.83), 247 (4.28); CD, λ_{ext} (*c* 0.023, MeOH) ($\Delta\epsilon$) 310 (−4.15), 266 (16.88), 247 (−22.50), 227 (27.53); EIMS *m*/*z* 393; $C_{21}H_{19}N_3O_5$, ^{1}H and ^{13}C NMR data see Tables 1 and 2, respectively. The following HMBC correlations were observed: from C-2 to H-4; from C-3 to H-7; from C-4 to H-6, from C-5 to H-4, H-6, H-7, and H-24; from C-6 to H-4; from C-8 to H-4, H-6, and H-7; from C-10 to H-12; from C-12 to H-14; from C-13 to H-14 and H-23; from C-14 to H-12; from C-15 to H-14; from C-16 to H-12 and H-14; from C-18 to H-19; and from C-20 and C-21 to H-19.

Circumdatin E (5): solid; $[\alpha]^{22}_D$ −90° (*c* 0.007, MeOH); UV (MeOH) λ_{max} (log ϵ) 339 (3.16), 244 (4.00); CD, λ_{ext} (*c* 0.007, MeOH) ($\Delta\epsilon$) 277 (−2.39), 254 (2.94), 234 (−3.80), 213 (8.30); EIMS *m*/*z* 363; $C_{20}H_{17}N_3O_4$, ^{1}H NMR data see Table 1. The following carbon chemical shift values were obtained from an HSQC experiment: δ_C C-4 (130); C-5 (130); C-6 (131), C-7 (130); C-12 (98.1); C-14 (108.7); C-19 (59.2); C-20 (27.1); C-21 (24.0); C-22 (46.8); and C-23 (56.2).

Circumdatin F (6): solid; EIMS 291 *m*/*z*, $C_{17}H_{13}N_3O_2$; ^{1}H NMR data, see Table 1.

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Table 2. ^{13}C NMR Data for Circumdatins A (**1**), B (**2**), C (**3**), and D (**4**)

no.	1	2	3	4
2	163.0	163.2	170.2	164.2
3	133.1	132.5[b]	133.3	134.3
4	112.4	130.5[c]	116.2	113.0
5	158.8	129.0[c]	160.6	159.4
6	117.0	128.7[c]	120.2	117.9
7	130.2	129.1[c]	131.1	131.2
8	125.3	131.9[b]	125.9	127.1
10	161.9	161.9	163.9	162.5
11	158.1	158.2	122.6	not obs.
12	109.8	110.0	128.1	97.9
13	95.2	95.1	128.7	159.8
14	156.6	156.7	136.2	108.5
15	115.3	115.4	128.8	156.0
16	145.1	145.2	148.0	130.9
18	157.2	157.3	157.9	151.3
19	58.0	58.0	51.4	59.5
20	26.2	26.2	15.4	26.9
21	23.2	23.3		24.0
22	46.2	46.2		46.8
23	55.0	55.1		56.2
24	55.7			56.5

[a] NMR data for **1**, **2** and **4** were recorded in DMSO-d_6 and **3** was recorded in MeOH-d_4. [b] Assignments are interchangeable. [c] Assignments are interchangeable.

References and Notes

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