Notes

CYCLOGREGATIN, A NEW METABOLITE FROM *ASPERGILLUS PANAMENSIS*

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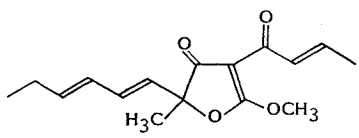
During the course of the isolation of gregatin A (**1**) from submerged cultures of *Aspergillus panamensis* a new tetronic acid derivative was obtained. According to its cyclic structure this compound was named cyclogregatin (**2**). In the following the structural elucidation and the biological activities of **2** in comparison with gregatin A will be described.

A. panamensis, CBS 120.45, was cultivated as described previously[1]. The crude extract from the culture filtrate was purified by means of chromatography on silica gel. With $CHCl_3$ - Me_2CO (98 : 2) gregatin A and cyclogregatin were eluted together. Separation of the two compounds was achieved by rechromatography on silica gel with CH_2Cl_2 as eluant. The isolation of cyclogregatin was monitored by TLC on silica gel with toluene - Me_2CO - AcOH (70 : 30 : 1) and spraying with vanillin - H_2SO_4 (orange color). Cyclogregatin was obtained as a colorless solid, mp 150~155°C, it is soluble in MeOH, Me_2CO and $CHCl_3$ and poorly soluble in water.

The physico-chemical data of cyclogregatin, $C_{15}H_{18}O_4$, are summarized in Table 1. Similar to the gregatins[1,2], its ^1H NMR spectrum shows signals for a 1,3-hexadienyl side chain and a quaternary methyl group, together with an ABX-system which can be ascribed to a $COCH_2CH(CH_3)$ moiety. These findings are confirmed by the ^{13}C NMR data and spin-decoupling experiments. Irradiation at δ 1.48 simplifies the multiplet at 4.77 ppm to a pair of doublets, and irradiation at δ 4.77 causes the methyl doublet at 1.48 ppm to collapse to a singlet and causes simplification in the multiplet centered at 3.07 ppm.

The NMR data are in accord with structures **2** or **3** for cyclogregatin. In order to differentiate between both possibilities, selective decouplings in the ^1H-coupled ^{13}C NMR spectrum were performed. Irradiation at the signal of 9-H leads to a simplification of the signal at δ 194.99 which therefore can be ascribed to C-3. When 6-H



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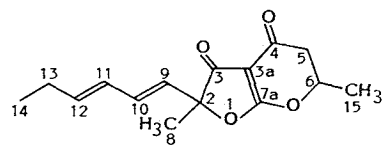

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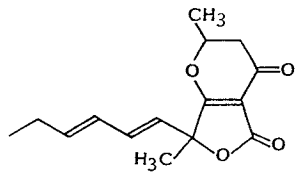

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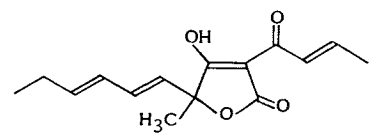

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Table 1. Physico-chemical data of cyclogregatin (2).

MS (DI, 180°C) *m/z*	262.1221 (M^+, 22.97%, calcd for $C_{15}H_{18}O_4$ 262.1205)
$[\alpha]_D$	+21.8° (*c* 0.17, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)	230 (4.14), 300 (sh, 3.45)
CD (EtOH)	$[\theta]_{315}$ −3,530, $[\theta]_{275}$ 0, $[\theta]_{267}$ +10,030, $[\theta]_{234}$ +20,350
IR (KBr) cm^{-1}	1750, 1690, 1585, 1465, 1330, 1028, 988
^1H NMR (200 MHz, acetone-d_6)[a] δ	0.98 (3H, t, *J*=6.5 Hz, 14-H), 1.48 (3H, d, *J*=6 Hz, 15-H), 1.56 (3H, s, 8-H), 2.10 (2H, pentet, *J*=6.5 Hz, 13-H), 3.07 (2H, m, 5-H), 4.77 (1H, ddq, *J*=9, 5.5, 6 Hz, 6-H), 5.61 (1H, br d, *J*=15 Hz, 9-H), 5.87 (1H, dt, *J*=15.4, 6.5 Hz, 12-H), 6.07 (1H, dd, *J*=15.4, 10 Hz, 11-H), 6.35 (1H, dd, *J*=15.4, 10 Hz, 10-H)
^{13}C NMR (100.2 MHz, acetone-d_6) δ	13.56 (Q, *J*=126 Hz, C-14), 20.79 (Q, *J*=127 Hz, C-8), 21.99 (Q, *J*=130 Hz, C-15), 26.21 (T, *J*=125 Hz, C-13), 33.18 (T, *J*=134 Hz, C-5), 73.34 (D, *J*=150 Hz, C-6), 94.39 (S, C-2), 104.30 (S, C-3a), 126.19 (D, *J*=160 Hz, C-9), 128.89 (D, *J*=152 Hz, C-10)[b], 133.24 (D, *J*=154 Hz, C-11)[b], 140.11 (D, *J*=150 Hz, C-12), 160.19 (S, C-7a), 194.99 (S, C-3), 196.56 (S, C-4)

[a] In a second isolation of **2** a doubling of the signals for H-8, 9, 10, 11 and 12 was observed which indicates the presence of two diastereomers.

[b] Signals interchangeable.

DI: Direct in let.

Q: Quadruplet, T: triplet, D: doublet, S: singlet.

Table 2. Antimicrobial activity of cyclogregatin and gregatin A in the serial dilution assay (MIC in μg/ml; for media composition see refs 4 and 5).

Test organism	Medium	Cyclogregatin	Gregatin A
Bacteria			
Acinetobacter calcoaceticus	NB	10	1
Bacillus brevis ATCC 9999	NB	5	0.5
B. subtilis ATCC 6633	NB	10	2
B. subtillis ATCC 6633	MM2	1	<0.5
Escherichia coli K-12	MM2	>50	>50
Micrococcus luteus	NB	10	2
Pseudomonas fluorescens	MM2	>50	>50
Proteus vulgaris	NB	50	10
P. vulgaris	MM2	2	<0.5
Staphylococcus aureus	NB	5	1
Yeasts			
Candida albicans	YMG	>50	50
Nematospora coryli	YMG	5	5
Rhodotorula glutinis	YMG	>50	>50
R. glutinis	MM4	>50	>50
Saccharomyces cerevisiae	YMG	20	20
Schizosaccharomyces pombe	YMG	10	5

is irradiated, a sharpening of the signals at δ 196.56 and 160.19 is observed which can only be explained if cyclogregatin possesses formula **2**.

On treatment with 0.1 N NaOH, **2** yielded the ring-opened compound **4** within a few seconds. **4** was characterized by its intense UV maximum at 308 nm (EtOH) and the appearance of the typical signals of the crotonyl side chain at δ 2.06 (3H, dd, *J*=6.5 and 1.0 Hz), 7.34 (1H, dq, *J*=16.0 and 6.5 Hz), and 7.38 (1H, dq, *J*=16.0 and 1.0 Hz) in the ^1H NMR spectrum ($CDCl_3$)[3].

Table 3. Antifungal activity of cyclogregatin and gregatin A (plate diffusion assay as described ref 5).

Test organism	Zones of inhibition (mm)		
	Cyclogregatin		Gregatin A
	10 μg	50 μg	10 μg
Absidia glauca (+)	—	—	10
A. glauca (−)	—	—	10
Ascochyta pisi	—	13	11
Aspergillus ochraceus	—	7	—
Alternaria porri	7	18	20
Botrytis cinerea	13	24	30
Cladosporium cladosporioides	—	—	—
Curvularia lunata	7	21	17
Epicoccum purureum	—	—	—
Eurotium cristatum	—	—	14
Fusarium fujikuroi	—	10	—
Mucor miehei	14	20	20
Neurospora crassa	—	14	10
Paecilomyces varioti	—	10	15
Penicillium islandicum	—	—	—
P. notatum	—	—	10
Phoma clematidina	13	20	20
Phytophthora infestans	—	—	12
Verticillium sp.	—	15	15
Venturia inaequalis	—	10	15
Zygorhynchus moelleri	—	11	12

—: No inhibition zone.

Table 4. Cytotoxic activity of cyclogregatin and gregatin A against Ehrlich carcinoma ascitic cells (test according to ref 6; 7×10^5 cells/ml).

Compound	IC_{50} (μg/ml)	Complete growth inhibition (μg/ml)
Cyclogregatin	10	25
Gregatin A	2	10

On prolonged hydrolysis, these signals disappear and the UV maximum is shifted to 274 nm. This may be explained by water addition to the enone group.

Since **2** can be formally derived from **1** *via* demethylation followed by cyclization, an attempt was made to convert gregatin A into cyclogregatin. However, under the chosen conditions (*p*-toluenesulfonic acid - Me_2CO; pyridinium tosylate - H_2O - Me_2CO) no formation of **2** was observed. This renders it improbable that **2** is an artefact formed from **1** during the isolation procedure. The relative and absolute configuration of cyclogregatin remain to be established.

The antimicrobial activity of cyclogregatin is shown in Tables 2 and 3, the cytotoxic activity in Table 4. In comparison with gregatin A the new compound showed weaker activity in all test systems. Most strains sensitive to gregatin A at low concentrations were also sensitive to cyclogregatin, the MIC being 5 to 10 times higher for the latter compound.

Aeknowledgment

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