Chlorotonil A, a Macrolide with a Unique *gem*-Dichloro-1,3-dione Functionality from *Sorangium cellulosum*, So ce1525**

*Klaus Gerth, Heinrich Steinmetz, Gerhard Höfle, and Rolf Jansen**

[*] Dr. R. Jansen
Arbeitsgruppe Mikrobielle Wirkstoffe
Helmholtz-Zentrum für Infektionsforschung
Inhoffenstrasse 7, 38124 Braunschweig (Germany)
Fax: (+49)531-6027-9499
E-mail: rolf.jansen@helmholtz-hzi.de
Homepage: www.helmholtz-hzi.de/en/research_groups/other_research_groups/microbial_drugs/

Dr. K. Gerth, Dipl.-Ing. H. Steinmetz
Arbeitsgruppe Mikrobielle Wirkstoffe
Helmholtz-Zentrum für Infektionsforschung
Inhoffenstrasse 7, 38124 Braunschweig (Germany)

Prof. G. Höfle
Helmholtz-Zentrum für Infektionsforschung
Inhoffenstrasse 7, 38124 Braunschweig (Germany)

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

In the course of our broad screening program for biologically active secondary metabolites from myxobacteria, strains belonging to the genus *Sorangium cellulosum* in particular were found to produce intriguing structures exhibiting multiple biological activities that are useful as drugs or leads for further development,[1] examples include highly potent antibiotics such as the antifungal soraphens[2] or the antibacterial sorangicins[3] and thuggacins[4] as well as anticancer agents such as the epothilones.[5]

Certain strains of *S. cellulosum*, such as strain So ce1525, are even able to produce several complex structural families belonging to different substance classes simultaneously. According to HPLC-MS analyses this strain not only produces sorangicins but also the macrolide carbonic acids sorangiolides,[6] and the group of oxazole bislactones, the disorazoles,[7] as well as new homologues of the chivosazoles,[8] oxazole containing macrolide glycosides. In addition, the HPLC-MS analyses of strain So ce1525 showed the presence of a novel chlorine containing metabolite. Herein isolation, spectroscopic structure elucidation and the X-ray analysis of chlorotonil A (**1**) are described.

Adsorbent resin Amberlite XAD 16 and cell mass (2.65 kg) were recovered from 70 L of fermentation broth[9] of *S. cellulosum*, strain So ce1525, by centrifugation and extracted batch-wise with methanol and acetone. All batches were evaporated and remaining aqueous oily mixtures were partitioned between water and CH_2Cl_2 in order to eliminate polar impurities. For the removal of lipophilic by-products each CH_2Cl_2 extract was partitioned between MeOH and heptane. During these partitions an off-white precipitate developed in the MeOH layers and was removed by filtration to give a total of 5.4 g of chlorotonil A (**1**), which represents an isolated yield of ~ 77 mg/L fermentation broth. For analytical purposes this material was purified by silica gel flash chromatography with gradient of CH_2Cl_2 in petroleum ether (PE). Finally, **1** was crystallized from CH_2Cl_2/MeOH, CH_2Cl_2/PE or pure CH_2Cl_2.

Scheme 1. Absolute configuration of chlorotonil A (**1**)

Chlorotonil A (**1**) was isolated as white crystals melting at 197-198°C. Its molecular formula $C_{26}H_{32}Cl_2O_4$ was derived from the HRESIMS of the molecular ion $[M + H]^+$ 479.1762 (calcd 479.1756) and its isotope pattern in the (+)-DCIMS spectrum[10] in accord with ^{13}C NMR and ^{13}C DEPT spectra. Accordingly, ten double bond equivalents were calculated for **1**. While the UV spectrum in MeOH only showed a broad absorption at 232 nm,

the IR spectrum in KBr clearly suggested the presence of ester or keto groups from three intense sharp bands at 1755, 1742, and 1714 cm^{-1}.

The solubility of **1** in most organic solvents was low. Since it was fairly soluble in chloroform, the NMR spectra for spectroscopic structure elucidation were recorded in $CDCl_3$. All 26 carbon signals appeared separately in the ^{13}C NMR spectrum. From their chemical shifts three signals, namely δ_C 196.8, 192.0, and 167.9, were assigned to two ketone groups and one ester or lactone group. The ^{13}C DEPT spectrum characterized seven of the eight signals resonating between 139.3 and 123.5 ppm as olefinic methine signals, the signal at δ_C 70.2 as oxymethine and only one signal δ_C 38.3 as a methylene group. Since a small ^{13}C signal δ_C 81.5 was not present in the DEPT spectrum it was confirmed to be a quaternary carbon. From the correlations in the HMQC NMR spectrum a further five methyl groups and seven aliphatic methine carbons were identified. Thirty protons of the elemental composition $C_{26}H_{32}Cl_2O_4$ of **1** could be assigned unambiguously to their corresponding carbons in the HMQC spectrum. Because of their overlap in the ^1H NMR spectrum at δ_H 2.15 – 2.17 the remaining two protons were interchangeable and assigned to the methine carbon signals at δ_C 36.8 and 33.3. The otherwise favorable separation of the ^1H NMR signals allowed identification of three main structural fragments **A** – **C** from strong vicinal and weaker long-range correlations in the COSY spectrum as depicted in Figure 1.

Figure 1. Structural units from ^1H–^1H COSY NMR spectra and selected interconnecting ^1H–^{13}C HMBC correlations of **1**.

All COSY-derived connectivities within structural fragments **A** – **C** were supported by the HMBC data (Table 1). Further the HMBC spectrum provided their interconnections (Figure 1). Fragments **A** and **B** are linked via methines-7 and -12. These have to be bound with each other because both carbons, C7 and C12 show a HMBC correlation with the H-7/H-12 signal centered between the symmetrically doublet signal of their direct $^1J_{C,H}$ couplings. The relative orientation of fragment **A** and **B** was inferred from HMBC correlations of C7 on the one hand with methyl-24, H-8 and H-9, and on the other hand with H-6. The combined HMBC correlations indicated the presence of an unsaturated decalin system in **1** as shown in Figure 1. The HMBC correlation of C5 (δ_C 196.7) with H-6 allowed connection of the ketone to methine-6, and thus provided an explanation for the chemical shift of the aliphatic H-6 at δ_H 3.77. Similarly, the carbonyl C3 (δ_C 192.0) had HMBC correlations with H-2 and H_3-23 connecting the second carbonyl group to structural unit **C**. Additionally, the ester or lactone C1 (δ_C 167.9) was correlated to H-2 and H_3-23 and presented a small HMBC signal with H-21,

indicating the ester/lactone linkage between structural fragments **A** and **C**. This was supported by the distinct acylation shift of the allylic H-21 at δ_H 5.70. The last unassigned C4 finally had to bear both chlorine atoms and was placed into the only possible position between the ketone groups, which unambiguously explains its chemical shift δ_C 81.6 and also the absence of any HMBC correlation.

Slow recrystallization of chlorotonil A (**1**) from CH_2Cl_2/MeOH furnished single crystals suitable for X-ray analysis. The perspective presentation of the final structure is shown in Figure 2. The X-ray analysis was refined to R_1 = 0.0497 (wR_2 = 0.0939). Accordingly, the absolute configurations of the eight stereocenters in **1** are 2*S*, 6*R*, 7*R*, 8*R*, 12*R*, 15*S*, 16*R*, 21*S*.[11]

Figure 2. Structure of chlorotonil A (**1**) from X-ray analysis

The stereochemical information from the NMR data was analyzed in order to compare the configuration in solution and in the solid state. Although H-7 and H-12 overlap in the 1D ^{1}H NMR spectrum the relative stereochemistry of the double unsaturated decalin system could be deduced from the 1D ^{1}H and 2D ^{1}H–^{1}H ROESY NMR data (Table 1). The strongest NOE of methyl-24 was observed with H-6 indicating both are on one side, the upper side of the system. Then H-7 is on the opposite side, which explains the *trans* axial coupling $J_{6,7}$ 11.8 Hz. The other strong ROESY correlations of both, H-6 and methyl-24, with the multiplet H-7/12 is thus due to H-12 only, signifying the 7,12-*trans* configuration, which fixes H-6, H-12 and methyl-24 axial in a triangle on the upper side. The vicinal coupling $J_{6,15}$ 6.7 Hz requires an axial-equatorial relation of H-6 with H-15. Thus the side chain at C15 adopts an axial position, pointing to the underside of the molecule. A ROESY correlation of the α-methyl-26 with H-7 is only possible due to its rotation towards the decaline system as found in the X-ray structure of **1** (Figure 2). As a consequence of this rotation and of the equatorial direction of the C5–C6 bond the major part of the lactone ring is nearly in plane with the *trans* decaline system. In the ^{1}H NMR spectrum the $\Delta^{17,18}$ *cis* and $\Delta^{19,20}$ *trans* configurations of the double bonds were implied from vicinal coupling constants of 10.4 and 15.3 Hz, respectively, while the unrestrained planar *s-trans* arrangement of the diene was apparent from the coupling constant $J_{18,19}$ = 11.2 Hz and further supported by a NOE between H-16 and H-19 indicating their cisoidal arrangement. Due to their spatial disposition no conclusion could be reached about the relative orientation of H-2 and methyl-23 from the NMR data. The X-ray structure shows the in-plane orientation of methyl-23. However, the chlorine atoms point to the upper

side and both ketogroups to the underside of the molecule. The unique *gem*-dichloro-1,3-dione functionality in chlorotonil A (**1**) is a novel structural feature among natural polyketides. Its biosynthetic origin from *S. cellulosum* again exemplifies the enormous genetic potential of myxobacteria as a source of novel secondary metabolites. Further, the position of the Δ^{13} double bond in **1** suggests an intramolecular Diels-Alder reaction of an α-keto-6-ene dienophile with a 12,14-diene intermediate as a probable biosynthetic one-step reaction establishing the unsaturated *trans* decaline system (Figure 3) in a stereo-specific manner.

Figure 3. Probable intermediate preceeding an intra-molecular Diels-Alder reaction leading to chlorotonil A (**1**).

Further work on biological properties and biosynthetic precursors of **1**, and the isolation of chlorotonil variants is in progress.

CCDC 658473 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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((References = Word endnotes are located at the end of the file))

Table

Table 1. NMR spectral data of chlorotonil A (**1**) in $CDCl_3$[a]. ((This caption and table are in a textbox, because their formatting is protected))

H	δ_H	m	*J* [Hz]	COSY[b]	ROESY[b, c]	C	δ_C	m	HMBC[d]
-						1	167.9	s	23, 2 >21
2	4.54	q	7.0	23	-	2	47.0	d	23
-						3	192.0	s	23, 2
-						4	81.5	s	-
-						5	196.7	s	6 >14
6	3.77	dd	11.8, 6.7 br.	12/7[e] > 15 > 11	(15) 12(/7) [e], 24	6	49.6	d	14, 12/7 > 15, 16
7	2.15	m[e]	-	6, 11 >8 > 14, 13	26, 24, 14, 8	7	36.7	d	24> 6, 11α > 13, 9 not 12!
8	2.36	m	-	24, 9 > 12/7 > 25	> 12/7	8	30.1	d	24 > 9
9	5.38	d	5.3, br.	25, 8, 11β >11α > 24	8, 25 >24	9	128.0	d	25, 24 > 11 > 8
-						10	132.3	s	25, 11αβ > 8
11α	2.03	dd	16.9, 4.1	12/7 > 6, 9 > 13	25, 13	11	38.3	t	25 > 13, 9 > 6 (14)
11β	1.75	dd	16.9, 9.5 br.	12/7, 6 > 9, 8 > 13	-				
12	2.17	m[e]	-	6, 11 >8 > 14, 13	26, 24, 14, 8	12	30.3	d	6, 13, 7, 11 > 8, 14
13	5.74	d	10.2 br.	14 > 15 > 12/7	11α, 12(/7)	13	133.1	d	15, 14, 6, 12/7, 11
14	5.50	ddd	10.2, 4.6, 1.9	13, 15 > 12/7	17 > 26	14	123.5	d	13, 16, 15 ((12/7))
15	3.02	m	-	6, 14 > 13, 16 > 12/7	19 (16, 6)	15	42.7	d	26 > 13, 14, 6 > 18, 16, 17
16	2.79	m	-	26, 17 > 15, 18	19 (17)	16	33.3	d	26 > 6, 18, 17 > 15, 13, 14
17	5.30	ddq	10.4, 8.4, 0.8	18, 16 > 19	14, 26	17	139.3	d	26, 19, 16 >> 21, 20, 15
18	5.87	dd	10.4, 11.2 br.	19, 17 > 20	20	18	125.4	d	20, 19 > 21, 16, 26
19	6.05	dddd	15.3, 11.2, 1.8, 1	18, 20 > 21, 17	16, 17 >21	19	123.9	d	18, 17, 21 >22, 20
20	5.50	dd	15.3, 2.4 br	19, 21 > 18, 17	18, 23	20	130.2	d	22, 18 > 19
21	5.60	ddq	2.2, 2.4, 6.7 br	22 > 20, 19	>19	21	70.3	d	22, 19, 20
22	1.32	d	6.5	21	20	22	20.9	q	21, 20 > 19
23	1.65	d	7.0	2	-	23	17.0	q	2
24	0.83	d	7.0	8 > 9	6, 12(/7), 9	24	14.8	q	25, 12/7 > 11, 6 > 8 > 9, 17
25	1.66	ddd	1, 1, 1	9, 11α, 8	9, 11α	25	23.2	q	9, 11α
26	0.95	d	6.5	16	17, (12/)7	26	15.6	q	17 > 15, 16

[a] ^{1}H 600 MHz; ^{13}C 75 MHz; ^{13}C multiplicities were obtained from a DEPT spectrum. [b] Proton numbers correlated to ^{1}H resonances are sorted by intensity (>). [c] some vicinal NOE correlations given in ( ). [d] Proton numbers correlated to carbon resonances are sorted by intensity (>) within ^{13}C rows. [e] H-7 and H-12 overlap.

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Structure elucidation

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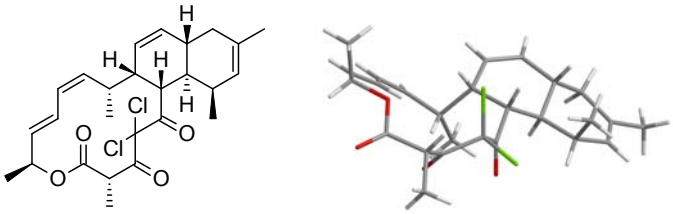
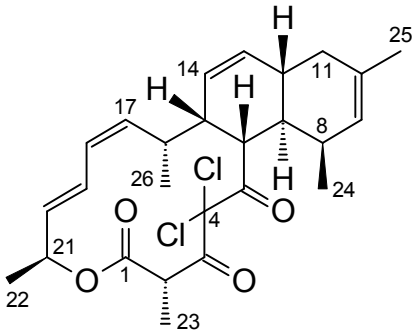


Unprecedented in nature: *gem*-Dichloro-1,3-diones are unknown as natural compounds. Chlorotonil A (**1**) features this functionality in a 14-membered lactone ring. **1** was isolated from the myxobacterium *Sorangium cellulosum* and the structure was elucidated by spectroscopic methods, including x-ray crystallography. The poor solubility of **1** in both organic solvents and water again raises the old question, why bacteria make an effort to create such complex metabolites.

Figures and Schemes



Scheme 1. Absolute configuration of chlorotonil A (**1**)

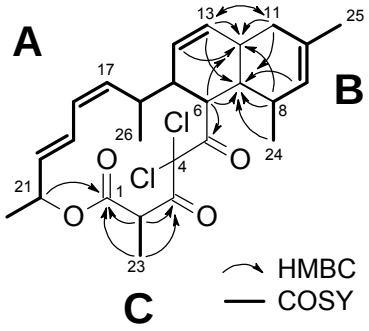


Figure 1. Structural units from ^{1}H–^{1}H COSY NMR spectra and selected interconnecting ^{1}H–^{13}C HMBC correlations of **1**.

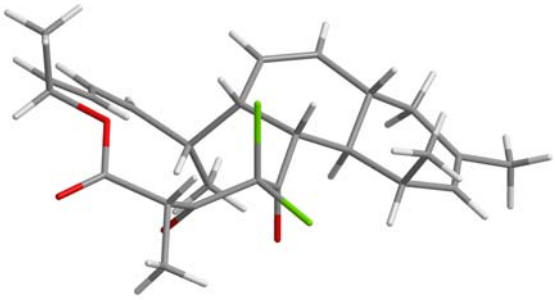


Figure 2. Structure of chlorotonil A (**1**) from X-ray analysis

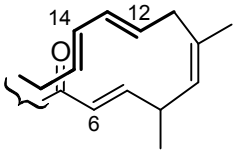


Figure 3. Probable intermediate preceding an intra-molecular Diels-Alder reaction leading to chlorotonil A (**1**).

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[10] (+)-DCIMS (isobutane): *m/z* (%) = 483 (11) 482 (14), 481 (62), 480 (23), 479 (100), 447 (17), 445 (71), 409 (4.9). Calculated for $[C_{26}H_{32}Cl_2O_4 + H]^+$: *m/z* (%) = 483 (13), 482 (19), 481 (69), 480 (29), 479 (100).

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