Structure Elucidation

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

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In the course of our broad screening program for biologically active secondary metabolites from myxobacteria, strains belonging to the genus *Sorangium cellulosum* 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] and 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**, Figure 1) are described.

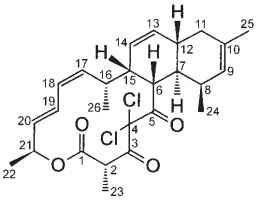

Figure 1. Absolute configuration of chlorotonil A (**1**).

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

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 batchwise with methanol and acetone. All batches were evaporated, and the 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 corresponds to a yield of isolated product of approximately 77 mg per liter of fermentation broth. For analytical purposes this material was purified by silica gel flash chromatography with a 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.

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 high-resolution (HR) ESIMS of the molecular ion $[M+H]^+$ (m/z: found 479.1762, calcd 479.1756) and its isotope pattern in the (+)-DCI mass spectrum[10] and is 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 showed only a broad absorption at 232 nm, the IR spectrum in KBr clearly suggested the presence of ester or keto groups based on 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 $\delta_C = 196.8$, 192.0, and 167.9 ppm—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 $\delta = 139.3$ and 123.5 ppm as olefinic methine signals, the signal at $\delta_C = 70.2$ ppm as an oxymethine group, and only one signal at $\delta_C = 38.3$ ppm as a methylene group. Since the small ^{13}C NMR signal $\delta_C = 81.5$ ppm was not present in the DEPT spectrum it was confirmed to correspond to a quaternary carbon. From the correlations in the heteronuclear multiple quantum coherence (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 $\delta_H =$ 2.15–2.17 ppm the remaining two protons were interchangeable and assigned to the methine carbon signals at $\delta_C = 36.8$

and 33.3 ppm. The otherwise favorable separation of the ^{1}H NMR signals allowed identification of the three main structural fragments **A**–**C** from strong vicinal and weaker long-range correlations in the COSY spectrum (Figure 2).

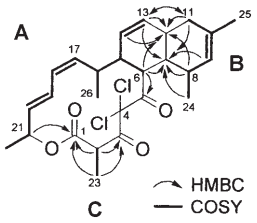

Figure 2. Structural units from ^{1}H–^{1}H COSY NMR spectra and selected ^{1}H–^{13}C HMBC correlations of **1**.

All COSY-derived connectivities within structural fragments **A**–**C** were supported by the heteronuclear multiple-bond correlation spectroscopy (HMBC) data (Table 1). Further the HMBC spectrum provided their interconnections (Figure 2). Fragments **A** and **B** are linked through methines 7 and 12. These have to be bound with each other because the two carbons, C-7 and C-12, show an HMBC correlation with the H-7/H-12 signals centered between the symmetrical doublet signal of their direct $^1J_{C,H}$ couplings. The relative orientation of fragment **A** and **B** was inferred from HMBC correlations of C-7 on the one hand with Me-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 2. The HMBC correlation of C-5 ($\delta_C = 196.7$ ppm) 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 $\delta_H = 3.77$ ppm. Similarly, the carbonyl C-3 ($\delta_C = 192.0$ ppm) had HMBC correlations with H-2 and H_3-23 connecting the second carbonyl group to structural unit **C**. Additionally, the ester or lactone C-1 ($\delta_C = 167.9$ ppm) 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 $\delta_H = 5.70$ ppm. The last unassigned C-4 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 $\delta_C = 81.6$ ppm 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 3. 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*, and 21*S*.[11,12]

The stereochemical information from the NMR data was analyzed in order to compare the configuration in solution with that in the solid state. Although H-7 and H-12 overlap in

Table 1: NMR spectroscopic data of chlorotonil A (**1**) in $CDCl_3$.[a]

H	δ_H	m	*J* [Hz]	COSY[b]	ROESY[b,c]	C	δ_C	m	HMBC[d]
–						1			23, 2 > 21
2	4.54	q	7.0	23	–	2	47.0	d	23
–						3			23, 2
–						4			–
–						5			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			–	6, 11 > 8 > 14, 13	26, 24, 14, 8	7	36.7	d	24 > 6, 11α > 13, 9 not 12!
8			–	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			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			–	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			-	6, 14 > 13, 16 > 12/7	19 (16, 6)	15	42.7	d	26 > 13, 14, 6 > 18, 16, 17
16			-	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 NMR (600 MHz), ^{13}C NMR (75 MHz); ^{13}C multiplicities were obtained from a DEPT spectrum. [b] The numbers of protons associated with the ^{1}H NMR signals are sorted by intensity (>). [c] Some vicinal NOE correlations are given in parentheses. [d] The numbers of protons correlated to ^{13}C NMR signals are sorted by intensity (>) within ^{13}C rows. [e] The signals of H-7 and H-12 overlap.

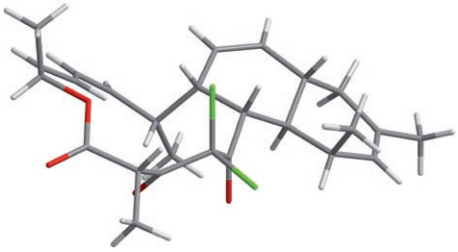

Figure 3. Structure of chlorotonil A (**1**) from X-ray crystal structure analysis. Red O, green Cl, gray C, white H.

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 Me-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* diaxial coupling constant, $J_{6,7} = 11.8$ Hz. The other strong ROESY correlations of H-6 and Me-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 Me-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 C-15 adopts an axial position, pointing to the underside of the molecule. A ROESY correlation of the α-Me-26 with H-7 is only possible owing to its rotation towards the decalin system as found in the X-ray structure of **1** (Figure 3). 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* decalin 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. Owing to their spatial disposition, no conclusion could be reached about the relative orientation of H-2 and Me-23 from the NMR data. The X-ray structure shows the in-plane orientation of Me-23. However, the chlorine atoms point to the upper side and both keto groups to the underside of the molecule.

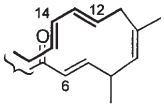

Figure 4. Probable intermediate preceding an intramolecular Diels–Alder reaction leading to chlorotonil A (**1**).

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 underlines the enormous 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* decalin system (Figure 4) in a stereospecific manner.[13] Further work on biological properties and biosynthetic precursors of **1**, and the isolation of chlorotonil variants is in progress.

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Keywords: halogenated compounds · myxobacteria · polyketides · structure elucidation

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