Chondrochloren A and B, New β-Amino Styrenes from *Chondromyces crocatus* (Myxobacteria)[‡]

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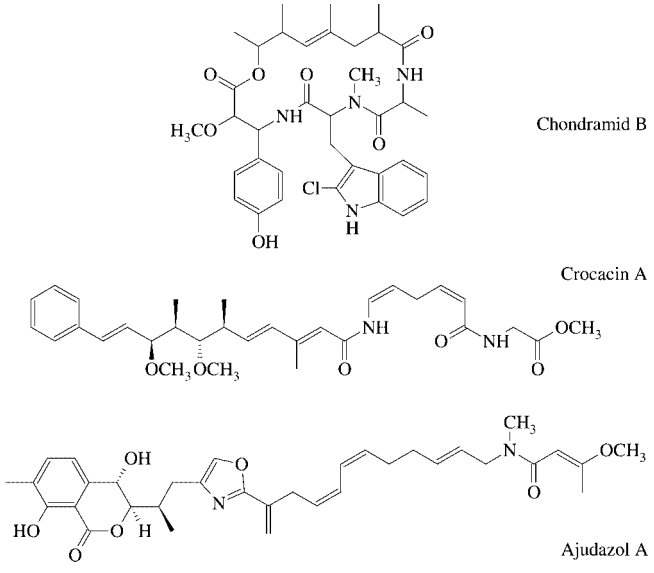
Keywords: Natural products / Myxobacteria / Structure elucidation / Absolute configuration

In a screening for biologically active metabolites of the genus *Chondromyces*, two novel metabolites, chondrochloren A (**1**) and B (**2**), were isolated from several strains of *C. crocatus*. Compounds **1** and **2** are unique chloro-hydroxy-styryl amides of a highly modified C_{14} carboxylic acid, which comprises an unsaturated ketone, two hydroxy, two methoxy and three methyl groups. After assignment of the absolute configuration of both carbinol stereocenters by Mosher's method, NMR spectroscopic data combined with MM2 calculations allowed the prediction of the preferred conformation in solution.

Introduction

In the course of a screening for biologically active metabolites from myxobacteria, several strains of *Chondromyces crocatus* were noticed for their high biological activity against fungi, yeasts and animal cell cultures.[2,3] On closer inspection these strains proved to be a rich source of novel compounds: for example strain Cm c5 simultaneously produces six entirely different groups of metabolites, namely chondramides, crocacins, ajudazoles, crocapeptins, thuggacins and chondrochlorens (Scheme 1). The chondramides A−D[4] are cytostatic cyclic depsipeptides inducing actin polymerization and formation of multinucleate cells.[5,6] Crocacin A, a complex *N*-acyl dipeptide, is an inhibitor of the cytochrome bc_1 segment (complex III) of the eukaryotic respiratory chain, and is highly toxic for mammalian cells, fungi and yeasts.[7,8] Besides the main biologically active compounds, this strain produces further metabolites that were detected by HPLC analysis of culture extracts (Figure 1) and were also isolated. The crocapeptins were identified as octapeptide lactones, the thuggacins as 17- and 18-membered polyketide lactones and the ajudazoles as iso-



Scheme 1. Structural diversity of secondary metabolites from *Chondromyces crocatus*, strain Cm c5

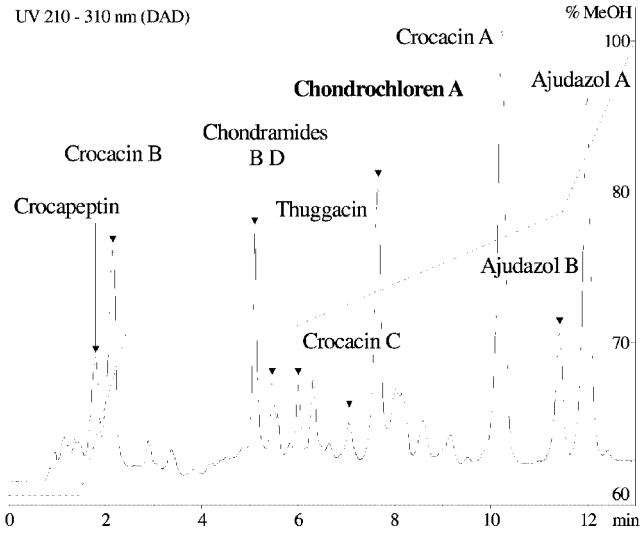


Figure 1. Typical RP-HPLC chromatogram of an extract of *Chondromyces crocatus*; column 125 × 2 mm and pre-column 11 mm, Nucleosil 120−5 C_{18}, solvent gradient water/methanol shown by the dashed line, flow rate 0.3 mL·min^{-1}; compound **2** does not appear as a separate peak

[‡] Antibiotics from Gliding Bacteria 93. Part 92: Ref.[1]

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  DOI: 10.1002/ejoc.200200699

chroman-1-ones with an unusual 3-methoxy-butenoic acid *N*-methyl amide residue.[9] Similarly, the chondrochlorens A (**1**) and B (**2**) attracted our attention by their characteristic UV spectrum during HPLC analysis.

Here we report the isolation of chondrochlorens A (**1**) and B (**2**) from *C. crocatus*, strain Cm c5, and their structure elucidation by means of NMR, UV and IR spectroscopy, and mass spectrometry. The absolute configuration of **1** was derived by Mosher's method. Finally, NMR spectroscopic data combined with MM2 calculations allowed the prediction of the preferred conformation in solution. As pure compounds, the chondrochlorens show weak antibiotic activity against *Micrococcus luteus*, *Schizosaccharomyces bombe*, *B. subtilis*, and *Staphylococcus aureus*.

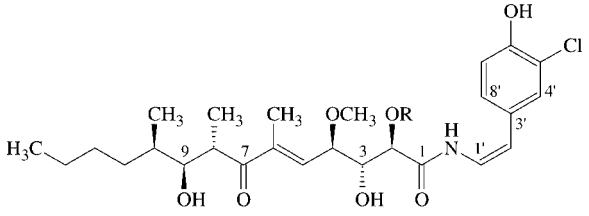
Results and Discussion

Like crocacins, chondramides and ajudazoles, the chondrochlorens are regularly found in the extracts of *C. crocatus* strains. As a typical example the analytical diode-array-detected (DAD) RP-HPLC of a cell extract from a large-scale fermentation of strain Cm c5 is shown in Figure 1. The production of the main chondrochloren **1** is in the region of 3 mg/L.

The chondrochlorens were isolated from the acetone extract of a wet cell mass of *C. crocatus* strain Cmc 5, by partition between methanol and heptane, which initially removes a good deal of the more lipophilic by-products. Further separation and purification of **1** and **2** was accomplished by consecutive chromatographic separations on silica gel and on RP-18 silica gel.

The structure elucidation of the chondrochlorens was mainly based on data obtained with the more abundant variant A (**1**). High-resolution EI MS of the molecular ion at m/z = 525, which was identified by EI and (+)- and (−)-DCI MS, furnished the elemental composition $C_{27}H_{40}ClNO_7$, which implies eight double-bond equivalents.

The UV spectrum shows three intense bands at 203, 224 and 278 nm, while the IR spectrum indicates an unsaturated ketone, an amide and hydroxy groups by bands at 1677, 1653, and 3403 cm^{-1}, respectively.



Chondrochloren A (**1**) R = Me
Chondrochloren B (**2**) R = Et

With the exception of three exchangeable protons, all ^1H NMR signals of **1** in $CDCl_3$ were correlated by $^1H,^{13}C$-HMQC NMR spectra with their corresponding carbon signals (Table 1). Three basic structure elements, designated as

Table 1. NMR spectroscopic data of chondrochloren A (**1**) in $CDCl_3$ (600 MHz/150 MHz)

H	δ_H	m	J	C	δ_C	m	H in HMBC[a]
8′	7.09	dd	8.4, 2.1	8′	128.07	d	4′, 2′
7′	7.00	d	8.4	7′	116.90	d	(8′)
–	–	–	–	6′	150.41	s	4′, 8′, (7′)
–	–	–	–	5′	120.48	s	7′, (4′)
4′	7.27	d	2.1	4′	128.43	d	8′, 2′
–	–	–	–	3′	129.06	s	1′, 7′
2′	5.78	d	8.0	2′	109.94	d	8′, 4′, 1′, NH
1′	6.87	d	11.4, 9.4	1′	120.83	d	2′, NH
NH	8.69	d	11.4	–	–	–	–
–	–	–	–	1	168.26	s	1′, 2 > 3, NH
2	3.93	d	4.0	2	82.09	d	2-OMe, 3, 4
2-OMe	3.50	s	–	2-OMe	59.58	q	2
3	3.99	dd	7.1, 4.0	3	73.97	d	2, 4 > 5, 6-Me
4	4.37	dd	9.1, 7.1	4	77.43	d	4-OMe, 2, 3, 6-Me
4-OMe	3.27	s	–	4-OMe	57.07	q	4
5	6.43	dd	9.2, 1.4	5	138.03	d	6-Me > 3, 4
–	–	–	–	6	142.46	s	6-Me, 4 (5)
6-Me	1.85	s	1.3	6-Me	12.67	q	5
–	–	–	–	7	206.84	s	8-Me, 6-Me, 8, 5 (9)
8	3.44	dq	8.3, 7.0	8	42.19	d	9, 8-Me
8-Me	1.04	d	7.0	8-Me	15.18	q	8 (9)
9	3.68	dd	8.3, 2.9	9	77.25	d	8, 10-Me
10	1.57	m	[b]	10	34.92	d	10-Me, 8 (9)
10-Me	0.86	d	6.8	10-Me	12.74	q	10, 9
11_a	1.32	m	–	11	33.82	t	10 (9), 10-Me
11_b	1.24	m	–				
12	1.24	m	–	12	29.53	t	11, 13, 10, 14
13	1.24	m	–	13	22.92	t	11, 12, 14
14	0.86	t	7.2	14	14.09	q	13

[a] Sorted by decreasing intensity, very weak correlations are given in bracket. [b] Among others 3 and 6.9 Hz visible.

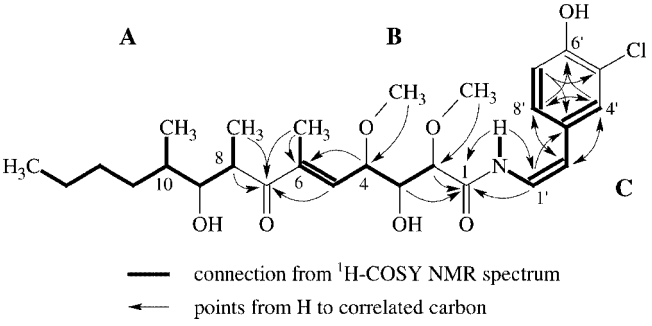


Figure 2. Structure elements of chondrochloren A (**1**) and selected correlations from NMR spectroscopy

A, **B** and **C** in Figure 2, were recognized by taking the vicinal and long-range correlations in the $^1H,^1H$-COSY NMR spectra into account. These were complemented by the remaining quaternary carbons and methoxy groups and their interconnections were determined by correlations derived from $^1H,^{13}C$-HMBC NMR spectra as shown in Figure 2.[10] Thus, elements **A** and **B** are linked via the C-7 ketone group (δ = 206.8 ppm), which has signals correlating with 8-H and 8-CH_3 as well as with 5-H and 6-CH_3. The complementary HMBC correlations indicate both methoxy groups are attached to the oxymethines at C-2 and C-4 in structure

element **B**.. Further correlations of the amide C-1 with 2- and 3-H as well as NH and 1′-H indicate the connection of structure element **B** with **C**. Additionally, the connection of the amide within element **C**, which was primarily derived from the coupling between NH and 1′-H, is supported by HMBC correlations between C-2′ and C-1′ with NH. The positions of the remaining two quaternary carbons, the phenolic carbon C-6′ (δ = 150.4 ppm) and the chlorine-substituted carbon C-5′ (δ = 120.48 ppm) within the aromatic ring, followed from ^{1}H,^{13}C-HMBC correlations that are also shown in Figure 2.

The remaining two protons are not directly visible in the ^{1}H NMR spectra. However, their positions as hydroxy protons on the oxymethines C-3 and C-9 were indicated by the sharpening of the 3-H and 9-H signals upon deuterium exchange.[11]

The *Z* configuration of the $\Delta^{1'}$-vinyl part in chondrochloren A (**1**; Table 1) was indicated by the vicinal ^{1}H coupling, $J_{1',2'}$ = 9.4 Hz, while the *anti* orientation of 1′-H and the amide proton follows from the coupling constant of 11.5 Hz in both well-resolved signals. The *E* configuration of the methyl-substituted Δ^5-double bond was evident from the nuclear-Overhauser-effects (NOE) observed in a ROESY NMR spectrum of **1** in $CDCl_3$: the 6-methyl group shows a strong correlation signal with 4-H but none with 5-H.

However, the strongest NOE correlation of the entire ROESY spectrum is observed between 5-H and 8-H. This effect indicates that, contrary to the streamlined structure drawing, in reality the structure of an α,β-unsaturated ketone adopts a *transoidal* conformation of the double bonds. This fixes in chondrochloren (**1**) 5-H and 8-H, the smallest substituent at C-8, in close proximity.

In fermentation extracts chondrochloren B (**2**) was present only in a small amount and was not visible as a separate peak during HPLC analysis. It was recognized during diode-array-detected HPLC analysis of chromatographic fractions from its typical UV maxima at 224 and 278 nm. High-resolution mass spectrometry gave a molecular ion at *m*/*z* = 539 that suggests the molecular formula $C_{28}H_{42}ClNO_7$ for **2**, which thus differs from **1** by an additional methylene group. Surprisingly, this group was detected as part of an ethoxy substituent, which replaces the methoxy group at C-2 of **1**. Its position was clearly indicated in the ^{1}H,^{13}C-HMBC NMR spectrum of **2** (Table 2) by mutual correlations between the oxymethine (C-2) and the oxymethylene group.

Since the six asymmetric centers of chondrochloren A (**1**) are grouped in two regions, with each three centered around a hydroxy function, the absolute configuration could be determined by Mosher's method, the NMR spectroscopic comparison of (*R*)- and (*S*)-α-methoxy-α-trifluoro-methylphenylacetic acid (MTPA) esters.[12] The MTPA esters of **1** were prepared using (*R*)- and (*S*)-MTPA chloride with DMAP in pyridine to give the corresponding 3,9,6′-tri-(*S*)-MTPA ester (**3**) and 3,9,6′-tri-(*R*)-MTPA ester (**4**), respectively. Equal amounts of the esters **3** and **4** were used to assign the relevant chondrochloren signals in $CDCl_3$ from ^{1}H,^{1}H-COSY and 1D ^{1}H NMR spectra. The NMR spectroscopic data of the protons close to the ester carbons C-3 and C-9 are given in Table 3. Nearly identical coupling constants of **1** and the esters **3** and **4** show that the original conformation of the chiral regions is retained in both esters. The observed shift differences are well defined: negative values are found for 2-H and 2-Me as well as for 9-H, 10-H and 10-Me, while all ^{1}H-shift differences between 4-H and 8-H are positive. According to Mosher's rule the shift differences $\Delta\delta = \delta_{(S)} - \delta_{(R)}$ of the protons on the right side of an O-MTPA group should be positive and on the left side be negative if looked upon as shown in Figure 3.[13] The ^{1}H-shift differences and the resulting absolute 3*R*, 9*S*-configuration of the alcohol positions in chondrochloren A (**1**) are shown in Figure 4.

Table 2. NMR spectroscopic data of chondrochloren B (**2**) in [D_6]-acetone (400 MHz/100 MHz)

H	δ_H	m	J	C	δ_C	m
1′	6.84	dd	11.5 9.5	1′[a]	121.60	d
2′	5.66	d	9.5	2′	109.00	d
–	–	–		3′	129.72	s
4′	7.36	d	2.2	4′	129.88	d
–	–	–		5′[a]	121.60	s
–	–	–		6′	152.56	s
7′	7.04	d	8.4	7′	118.06	d
8′	7.18	dd	2.2, 8.4	8′	128.54	d
NH	8.95	d	11.5 br.	–	–	–
–	–	–		1	168.80	s
2	4.10	d	2.8	2	82.28	d
3	4.03	d	7.9, 2.8 br.	3	74.88	d
4	4.41	dd	9.0, 7.9	4	78.26	d
4-OMe	3.22	s		4-OMe	56.81	q
5	6.48	dd	9.0, 1.3	5	139.32	d
–	–	–		6	142.39	s
6-Me	1.83	d	1.3	6-Me	12.93	q
–	–	–		7	206.87	s
8	3.52	dq	8.7, 7.0	8	43.47	s
8-Me	0.99	d	7.0	8-Me	15.44	q
9	3.72	m		9	76.40	d
10	1.61	dddq	6.8, 6.3, 2.7, 6.8	10	35.48	d
10-Me	0.89	d	6.8	10-Me	13.03	q
11_a	1.43	m		11	34.75	t
11_b	1.27	m				
12	1.32	m		12	30.32	t
13	1.32	m		13	23.60	t
14	0.88	t	7.1	14	14.37	q
$1''_a$	3.76	dq	9.2, 7.0	1"	67.79	t
$1''_b$	3.67	dq	9.2, 7.0			
2"	1.25	t	7.0	2"	15.80	q

[a] 1′- and 5′-C overlap according to HMBC data.

The presumption of one preferred conformation of **1** in solution is well founded on the high degree of substitution of the molecular backbone by hydroxy, methoxy and methyl groups and on the involvement of the planar *transoidal* unsaturated ketone. Thus, starting with the absolute configuration of the alcohol carbons C-3 and C-9, a model of the asymmetric part of chondrochloren was developed, which completely explains the NMR spectroscopic data. The conformation in Figure 5 was calculated with the MM2 module

Table 3. Selected NMR spectroscopic data of 3,9, 6′-tri-(*S*)- (**3**) and 3,9,6′-tri-(*R*)-MTPA ester (**4**) in $CDCl_3$

Group	*S*-Ester (**3**)			*R*-Ester (**4**)			$\Delta\delta = \delta_S - \delta_R$
	δ	m	*J*	δ	m	*J*	
2	3.976	d	3.3	4.052	d	3.5	−0.076
2-OMe	3.307	s		3.431	s		−0.125
3	5.651	dd	7.4, 3.1	5.578	dd	7.4, 3.5	(0.073)
4	4.556	dd	9.0, 7.4	4.525	dd	9.2, 7.5	0.031
4-OMe	3.212	s		3.180	s		0.031
5	6.383	dd	9.2, 1.0	6.246	dq	9.3, 1.4	0.137
6-Me	1.835	d	1.0	1.713	d	1.2	0.122
8	3.507	dq	9.0, 7.7	3.479	dq	9.5, 7.2	0.028
8-Me	1.044	d	7.2	1.032	d	7.2	0.012
9	5.588	dd	9.7, 1.8	5.586	dd	9.5, 2.0	(0.002)
10	1.751	m		1.784	ddq	13.5, 1.7, 7.0	−0.033
10-Me	0.813	d	6.9	0.899	d	6.9	−0.086

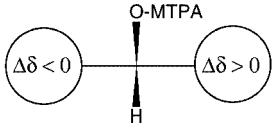


Figure 3. Expected algebraic sign of ^{1}H-shift differences ($\Delta\delta = \delta S - \delta R$) between (*S*)- and (*R*)-MTPA esters

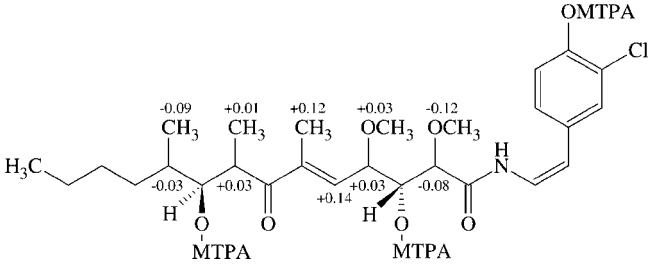


Figure 4. Chemical shift differences of the (*R*)- and (*S*)-MTPA esters **3** and **4** and the resulting absolute configuration of the carbinolgroups in chondrochloren A (**1**)

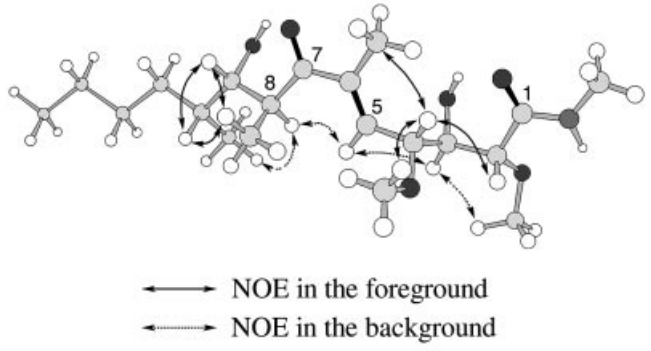


Figure 5. MM2-calculated model fragment of **1** with nuclear Overhauser effects from the ROESY NMR spectrum of **1**

of Chem3D Ultra (version 6.0). Each single bond was optimized starting from several different dihedral angles.

The steric arrangement of the substituents around the alcohol carbon C-3 was elucidated from the nuclear Overhauser effects (NOE) observed in the ROESY NMR spectrum of **1** in $CDCl_3$: as next neighbors of 3-H the 2-methoxy group and 5-H are indicated — but not 4-methoxy or 4-H. On the other side, 4-H is surrounded by 6-methyl, 2-H, and obviously by the 4-methoxy group. These requirements were met by the energy-minimized 2*R*,3*R*,4*R* configuration shown in Figure 5. In this structure element the conformation is partially stabilized by a hydrogen bond between 3-OH and the amide oxygen. The calculated H−C−C−H dihedral angles and their vicinal coupling constants are given in Table 4 and compared to estimated coupling constants.[14] The differences between calculated and observed coupling constants reflect the mobility of the chondrochloren molecular chain.

Table 4. Calculated dihedral angles, observed and estimated ^{1}H-coupling constants of **1**

	Protons/bonds				
	2−3	3−4	4−5	8−9	9−10
Calcd. angle [°]	−85	175	−158	176	−64
Observed *J* [Hz]	4.0	7.1	9.1	8.3	3.0
Estimated *J* [Hz][a]	>1	9.9	8.7	9.9	1.8

[a] Karplus equation with $J^{180} \approx 10$ Hz.

Similarly, the next neighbors of 9-H were recognized from the ROESY spectrum, which shows correlation signals of 9-H with 8-methyl, 10-H and with the 11-methylene group but none with 8-H or the 10-methyl group; 8-H has the above mentioned most intense correlation with 5-H and additionally with 10-methyl. According to these constraints the 8*S*,9*S*,10*R* configuration was chosen for the modeling, resulting in dihedral angles compatible with the vicinal coupling constants (Table 4). The configuration of this part of the molecule is also stabilized by a hydrogen bond between the 10-hydroxy proton and the ketone oxygen.

Overall, the carbon backbone from C-5 to C-14 of chondrochloren A (**1**) is nearly coplanar with the unsaturated ketone. At C-4 the molecular chain is slightly bent into the background while the 4-methoxy is pointing forward out of plane. The *transoidal* double bonds of the unsaturated ketone induce a pronounced kink of the molecular chain.

Conclusion

Halogen derivatives are rare among myxobacterial metabolites. All the more surprisingly, strains of *Chondromyces crocatus* produce two different types of compounds simultaneously — the chondramides B and D with a 2-chloroindole residue, and the chondrochlorens A (**1**) and B (**2**), with a 2-chlorophenol residue. Remarkably, the 2-chlorophenol residue has been previously found, among others, as part of some sponge depsipeptides, the geodiamolides (e.g. C, F,[15] K, and N[16]). In their polyketide-derived structure elements these, in turn, are closely related to the chondramides. Except for the chlorine, the amide part of **1** and **2** is similar to the formamide WF 5239, a platelet aggregation inhibitor isolated from *Aspergillus fumigatus* [17] and from

a marine *Penicillium* spec.[18] Remarkably, the 9-hydroxy-7-keto-8,10-methyl part of the acid residue in **1** and **2** is also present in the structures of the antibiotics family of leptomycins, though with different stereochemistry.

Although the molecular structure of the chondrochlorens may be looked upon as a flexible chain, the modeling-assisted conformation analysis showed that they adopt a distinctly preferred conformation due to their high degree of substitution.

In a screening for biological activity of chondrochloren A (**1**), a weak antibiotic effect was observed in agar diffusion tests. A sample of **1** (20 µg) on a 6 mm paper disk provided an inhibition zone of 13 mm diameter with *Micrococcus luteus*, of 10 mm with *Schizosaccharomyces bombe* and traces of inhibition with *B. subtilis* and *Staphylococcus aureus.*

Experimental Section

UV: Shimadzu UV/Vis scanning spectrometer UV-2102, solvent methanol [Uvasol, (Merck)]. IR: Nicolet FT-IR spectrometer 20 DXB. NMR: Bruker spectrometer DMX 600 (^{1}H: 600.1 MHz, ^{13}C: 150.9 MHz), Bruker spectrometer ARX 400 (^{1}H: 400.1 MHz; ^{13}C: 100.6 MHz) or Bruker spectrometer AM 300 (^{1}H: 300.1 MHz; ^{13}C: 75.5 MHz); internal standard was the solvent signal. Mass spectrometry: EI: Finnigan spectrometer MAT 95 (EI with 70 eV), resolution M/ΔM = 1000; high-resolution data from peak matching (M/ΔM = 10000); DCM = dichloromethane.

Isolation of Chondrochlorens A (1) and B (2): Pooled wet cell mass (6.3 kg) from several fermentations (about 700 L) of *Chondromyces crocatus*, strain Cm c5, was extracted with three portions of acetone (20 L). After evaporation of the organic solvent, the remaining aqueous phase was extracted three times with dichloromethane (DCM) (9 L). Drying of the organic phase with sodium sulfate and evaporation at reduced pressure of the solvent provided an oily residue (101 g). This was partitioned between methanol (3 L) containing 4% of water and heptane (three portions of 2.5 L). An oily residue (37.5 g) remained after evaporation of the aqueous methanol at reduced pressure. After thorough removal of traces of polar solvent, the residue was subjected to silica-gel flash chromatography (1.2 L silica gel, 63−200 µ, 60 Å) eluting sequentially with DCM (2.2 L), 95:5 DCM/acetone (2.8 L), 90:10 DCM/acetone (2.7 L), 90:9:1 DCM/acetone/methanol (4 L) and then with 90:5:5 DCM/acetone/methanol (2.7 L). First the chondrochlorens and later the chondramides were eluted. The chondrochloren-containing fraction was collected to give 2.15 g of a product enriched in **2**. This fraction was purified further by silica gel MPLC [column 420 × 37 mm; HD-Sil 15−60 (Kronlab); eluting solvent petroleum benzine/*tert*-butylmethyl ether (3:7), flow rate 22.5 mL/min, detection UV absorption at 227 nm]. The first peak contained a chondrochloren mixture (1.1 g), which was separated by RP-MPLC [column 340 × 60 mm, HD-Sil 18−20−60 (Kronlab), solvent gradient from 35−65% aqueous methanol in 30 min and then to 73% methanol in 30 min, 73% methanol for 200 min, at a flow rate of 25 mL/min; detection UV absorption at 226 nm]; the main peak (130−155 min) contained chondrochloren A (**1**) (162 mg) followed by a peak (160−185 min) identified as chondrochloren B (**2**) (113 mg). Further batches of chondrochloren A (**1**) could be acquired from side fractions of the separations mentioned above.

Properties of the Chondrochlorens: Compounds **1** and **2** were obtained as colorless, amorphous solids and found to be pure according to TLC and HPLC analysis. They are soluble in methanol, acetone, chloroform and ethyl acetate, sparingly soluble in diethyl ether and insoluble in hexane.

Analytical TLC: Aluminum sheets with a layer of 0.2 mm silica gel 60 F_{254}, (Merck); detection by UV quenching at 254 nm and color reaction: sprayed with vanillin/sulfuric acid reagent and heated to 120 °C, the chondrochlorens give brown spots; solvents: *tert*-butyl methyl ether **1** R_f = 0.5, **2** R_f = 0.58; DCM/acetone (9:1) **1** R_f = 0.09; DCM/methanol (9:1); **1** R_f = 0.54; **2** R_f = 0.58.

Analytical RP-HPLC: Column 125 × 2 mm and pre-column of 11 mm with Nucleosil 120−5 C18, 5 µm (Macherey−Nagel); solvent gradient with aqueous methanol, 60% methanol for 4 min, gradient to 70% in 6 min, to 90% in 5 min, and to 100% methanol in 2 min, flow rate = 0.3 mL/min; chondrochloren A (**1**) t_R = 13.7 min; chondrochloren B (**2**) t_R = 14.5 min;

Chondrochloren A (1): $C_{27}H_{40}ClNO_7$ M = 526.07. $[\alpha]_D^{21}$ = −82.4 (c = 0.9 in methanol). UV (methanol): λ_{max} (lg ε) = 205 (4.47), 224 (4.47), 278 (4.38). IR (KBr): $\tilde{\nu}$ = 3403 (s), 2958, 2930, 2858 (m), 1677 (s), 1653 (s), 1489 (s) cm^{-1}. NMR spectroscopic data see Table 1. EI MS (160 °C): *m/z* (%) = 525 (8) [M$^+$], 507 (3) [M − 18]$^+$, 411 (58), 241 (32), 169 (59), 142 (100). HR-EI MS: M$^+$ = $C_{27}H_{40}ClNO_7$: calcd. 525.2502; found 525.2509; [M − 18]$^+$ = $C_{27}H_{38}ClNO_6$: calcd. 507.2388; found 507.2369.

Chondrochloren B (2): $C_{28}H_{42}ClNO_7$ M = 540.10. $[\alpha]_D^{21}$ = −81.7 (c = 0.84 in methanol). UV (methanol): λ_{max} (lg ε) = 204 (4.40), 224 (4.42), 278 (4.32). NMR spectroscopic data see Table 2. EI MS (200 °C): *m/z* (%) = 539 (6) [M$^+$], 521 (2) [M − 18]$^+$, 425 (44), 255 (21), 169 (39), 142 (100). HR-EI MS: M$^+$ = $C_{28}H_{42}ClNO_7$: calcd. 539.2649; found 539.2628.

3,9,6′-Tri-MTPA-esters 3 and 4: Compound **1** (10 mg), MTPA chloride (24 µL), and DMAP (5 mg) were stirred in 0.4 mL of pyridine and DCM (3:1) for 3 days. Fifteen minutes after addition of some water the mixture was partitioned between DCM and water. The product was purified by preparative RP-HPLC (RP-18) eluting with 85% aqueous acetonitrile to give 11 mg of the pure ester. With (*R*)-MTPA chloride the 3,9,6′-(*S*)-MTPA ester (**3**) and with (*S*)-MTPA chloride the 3,9,6′-tri-(*R*)-MTPA ester (**4**) was obtained.
3,9,6′-Tri-(*S*)-MTPA ester (3): [M + H]$^+$ = [$C_{57}H_{61}ClF_9NO_{13}$+H]$^+$: found 1174.46, calcd. 1174.38 (ESI-MS). $[\alpha]_D^{21}$ = −44.7 (c = 0.55 in chloroform). NMR spectroscopic data in $CDCl_3$ see Table 3.
3,9,6′-Tri-(*R*)-MTPA ester (4): $[\alpha]_D^{21}$ = +10.3 (c = 1.4 in chloroform). NMR spectroscopic data in $CDCl_3$ see Table 3.

Acknowledgments

We thank S. Knöfel and N. Dankers for their skilful assistance in the isolation work, A. Roß, H. Schüler and R. Krützfeld and coworkers for their contribution in the large scale fermentation and downstream processing, B. Jaschok-Kentner and C. Kakoschke for recording the NMR spectra, and R. Christ for measuring the mass spectra.

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Received December 13, 2002

