Antibiotics from Gliding Bacteria, LXXXVI[‡]

Apicularen A and B, Cytotoxic 10-Membered Lactones with a Novel Mechanism of Action from *Chondromyces* Species (Myxobacteria): Isolation, Structure Elucidation, and Biosynthesis

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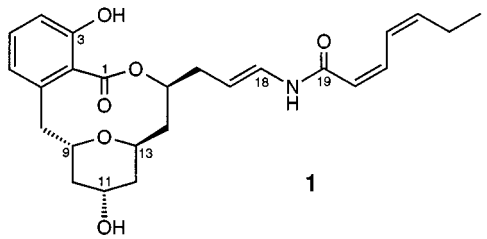
Keywords: Chondromyces spec. / Myxobacteria / Antibiotics / Cytotoxic / Structure elucidation

A novel highly cytotoxic metabolite, apicularen A (**1**), was isolated in a screening of the myxobacterial genus *Chondromyces*. The structure of **1** is characterized by a salicylic acid residue as part of a 10-membered lactone, which bears an acylenamine side chain. Compound **1** is an inhibitor of the proliferation of human cancer cell lines and induces apoptosis. Apicularen A (**1**) is present in nearly every strain of *C. apiculatus*, *C. pediculatus*, *C. lanuginosus* and *C. robustus*. Habitually **1** is accompanied by different amounts of a more polar variant, apicularen B (**2**), which was identified as 11-*O*-(2-*N*-acetamido-2-deoxy-β-D-glucopyranosyl)apicularen. According to feeding experiments with ^{13}C-labeled acetates, glycine, and methionine, apicularen A (**1**) is an acetate-derived polyketide containing a glycine residue as precursor of the enamine. Uncommonly, the C_3 starter unit is not assembled from propionate but from acetate and methionine.

Introduction

When the myxobacterial genus *Chondromyces* was introduced into our screening for biologically active metabolites, primarily strains of the species *C. crocatus* had been selected for further investigation because of the high antibiotic and cytotoxic activities of their extracts. These were caused by different metabolites, e.g. chondramides,[1] crocacins[2] and some others, which are usually produced by these strains simultaneously. Several other strains of *Chondromyces*, i.e. *C. apiculatus*, *C. lanuginosus, C. pediculatus* and *C. robustus*, do not produce any of the metabolites of *C. crocatus* but provide a common metabolite, apicularen A (**1,** Scheme 1)[3] which was found to be a powerful inhibitor of human cancer cells, including a multi-drug resistant line.[4]Herein the isolation, structure elucidation and biosynthesis of apicularen A (**1**) and its glycosylated co-metabolite apicularen B (**2**) are reported.



Scheme 1. Apicularen A (**1**)

[‡] Part LXXXV: B. Böhlendorf, M. Herrmann, H.-J. Hecht, F. Sasse, E. Forche, B. Kunze, H. Reichenbach, G. Höfle, *Eur. J. Org. Chem.* **1999**, 2601–2608.

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Isolation

For production, *Chondromyces robustus* was cultivated in a Probion (single-cell protein of the Hoechst company) liquid medium for 7–10 days at 30 °C in the presence of 1% of the neutral adsorber resin Amberlite XAD 16.[3] The

Table 1. NMR spectroscopic data of apicularen A (**1**) in [D_6]acetone (^1H at 600 MHz; ^{13}C at 150 MHz)

H	δ_H	m	*J* [Hz]	C	δ_C	m
1	–	–	–	1	169.28	s
2	–	–		2	125.44	s
3	–	–	–	3	154.23	s
4	6.77	dd	8.2, 1.2	4	114.39	d
5	7.10	dd	8.2, 7.6	5	130.20	d
6	6.69	dd	7.6, 1.2	6	122.24	d
7	–	–	–	7	140.15	s
8a	3.34	dd	14.8, 9.7	8	40.26	t
8b	2.44	dd	14.8, 1.8 (br.)			
9	3.87	dddd	9.7, 8.2, 4.8, 1.7	9	73.64	d
10a	1.93	ddd	12.8, 4.8, 4.2, 1.0	10	39.60	t
10b	1.48	ddd	12.8, 8.8, 8.5			
11	3.98	dddd	8.8, 7.6, 5.1, 4.1	11	64.87	d
12a	1.68	ddd	12.8, 7.1, 5.1, 1.0	12	39.88	t
12b	1.52	ddd	12.8, 7.6, 4.8			
13	4.25	dddd	10.8, 7.1, 4.8, 2.2	13	68.05	d
14a	1.83	ddd	14.7, 10.9, 10.8	14	38.81	t
14b	1.57	ddd	14.7, 2.3, 2.2			
15	5.42	ddt	10.9, 2.3, 6.3	15	74.21	d
16	2.34	ddd	7.5, 6.3, 1.5	16	36.37	t
17	5.26	dt	14.5, 7.5	17	108.09	d
18	6.89	ddt	14.5, 10.2, 1.5	18	126.24	d
19	–	–	–	19	163.64	s
20	5.74	ddd	11.6, 1.5, 1.5	20	120.84	d
21	6.84	ddd	11.6, 11.5, 1.3	21	136.77	d
22	7.51	dddt	11.5, 10.9, 1.6, 1.5	22	125.37	d
23	5.80	dddt	10.9, 1.3, 1.1, 7.7	23	141.48	d
24	2.27	ddq	7.7, 1.6, 7.6	24	21.00	t
25	1.00	t	7.6	25	14.30	q
3-OH	8.37	s	br.		–	–
11-OH	3.79	m	–		–	–
18-NH	9.08	d	10.2 (br.)		–	–

  1434–193X/00/0306–0913 $ 17.50+.50/0

main component **1** was isolated in four steps after simultaneous extraction of resin and cells with acetone by (1) partition of the crude extract between dichloromethane and water, (2) by partition of the dichloromethane-soluble products between methanol and heptane, followed by (3) silica gel flash chromatography of the polar fraction with an ascending gradient of methanol in dichloromethane and, finally, (4) by crystallization from methanol.

Because apicularen B (**2**) is very soluble in water, it was isolated from the water layer of step (1) mentioned above by re-extraction with *n*-butyl alcohol followed by RP-MPLC.

Structure Elucidation

The apicularens were analyzed in the screening extracts by UV and ESI-MS-detected HPLC, where the apicularen peaks are easily recognized by their common UV spectrum, a broad UV absorption band at λ_{max} = 278 nm. In the mass spectra apicularen A (**1**) generates a molecular ion $[M - H]^-$ at *m*/*z* = 440, while the more polar variant B (**2**) gives the molecular ion at *m*/*z* = 643. Characteristically, both molecular ions are accompanied by a highly abundant $[M - 108]^-$ fragment ion.

HREIMS of the molecular ion *m*/*z* 441 of apicularen A (**1**) furnished the elemental composition $C_{25}H_{31}NO_6$. Since the structure and relative configuration were verified by X-ray analysis, the structure elucidation is only summarized briefly. NMR spectra of **1** (Table 1) in [D$_6$]acetone allowed the elucidation of the complete structure: after ^{1}H,^{13}C-HMQC correlation of the twenty-eight protons directly bound to 20 carbon atoms, the correlations in the ^{1}H,^{1}H-COSY spectrum led to three structural elements, marked as **A**, **B** and **C** in Figure 1. Four correlations to α and β protons in the ^{1}H,^{13}C-HMBC spectrum linked elements "**C**" and "**B**" by the enamide carbonyl group C-19 (Figure 1), while another correlation connected the second carbonyl group to the oxygen of methine C-15 as part of an acyl residue, thus accounting for the downfield acyl shift of 15-H (δ = 3.87). Mutual ^{1}H,^{13}C-HMBC correlations between the oxygen-bearing methines C-9 and C-13 closed the pyran ring in "B". As indicated by a NOESY correlation to 4-H, one hydroxy group was attached to C-3 to give the phenol group demanded by its ^{1}H- and ^{13}C-chemical shifts (δ_{OH} = 7.1; δ_C = 154.2). The remaining carbons were required for the aromatic ring containing the structure element "A". The exact positions of carbons C-2, C-3 and C-7 within the aromatic ring and the connection with the lactone carbonyl were defined according to ^{1}H,^{13}C-HMBC correlations as shown in Figure 1: In particular, the correlations of C-6 and C-2 with methylene C-8 as well as the long-range correlations over four bonds between C-1 and both aromatic methines 4-H and 6-H should be emphasized here.

The relative configuration of all four stereogenic centers in **1** was unambiguously provided by an X-ray analysis of apicularen A (**1**) crystallized from methanol (Figure 2). However, the NMR spectroscopic data do not fully agree with the chair conformation of the pyran ring. As an alternative conformation in solution, a twisted pyran ring was found by MM+ calculations with HyperChem starting from differently distorted ring conformations. As shown in Figure 3, this twisted conformation meets the stereochemical restrictions set by the NMR spectroscopic data, i.e. vicinal coupling constants as well as nuclear Overhauser enhancements (NOEs) from ^{1}H,^{1}H-NOESY and from NOE difference spectra, significantly better than the chair con-

Table 2. NMR spectroscopic data of apicularens B (**2**) and (**1**) in [D$_4$]methanol

	2[a]				**1**			
H	δ_H	m	*J* [Hz]	C	δ_C	m	δ_C	$\Delta\delta_C$[b]
–	–	–	–	1	171.63	s	171.58	0.05
–	–	–	–	2	125.43	s	125.08	0.35
–	–	–	–	3	154.86	s	155.06	–0.20
4	6.75	d	8.3	4	114.57	d	114.59	–0.02
5	7.15	dd	8.3, 7.7	5	130.94	d	130.89	0.05
6	6.71	d	7.7	6	122.29	d	122.39	–0.10
–	–	–	–	7	140.23	s	139.75	0.48
8a	3.40	dd	15, 11, br	8	40.01	t	40.80	–0.79
8b	2.45	d	15, br					
9	3.95	ddd	10.4, 5.3, 5.3, br	9	75.08	d	74.06	1.02
10a	1.97	ddd	13.5, 5.4, 4.0	10	35.09	t	39.78	–4.69
10b	1.64	ddd	13.5, 6.6, 6.6					
11	4.31	m	–	11	72.28	d	65.43	6.85
12	1.73	m	– (2 H)	12	38.12	t	39.55	–1.43
13	4.31	m	–	13	67.85	d	69.68	–1.83
14a	1.84	ddd	14.7, 10.8, 10.7	14	39.44	t	38.51	0.93
14b	1.67	d	14.7, br					
15	5.52	m	– (br.)	15	75.29	d	75.34	–0.05
16	2.41	m	– (2H)	16	36.64	t	36.79	–0.15
17	5.38	ddd	14.3, 7.3, 7.3	17	110.12	d	110.10	0.02
18	6.87	d	14.3	18	126.32	d	126.37	–0.05
–	–	–	–	19	165.84	s	165.87	–0.03
20	5.74	d	11.4	20	120.42	d	120.40	0.02
21	6.91	ddd	11.8, 11.4, 1.1	21	137.66	d	137.68	–0.02
22	7.36	dddd	11.8, 10.9, 1.6, 1.3	22	125.36	d	125.36	0.00
23	5.87	dtt	10.9, 7.5, 1.5	23	142.57	d	142.57	0.00
24	2.33	ddq	7.5, 1.6, 7.5	24	21.50	t	21.51	–0.01
25	1.07	t	7.5	25	14.38	q	14.37	0.01
1′	4.65	d	8.1	1′	100.24	d		
2′	3.61	dd	10.5, 8.1	2′	57.87	d		
3′	3.57	dd	10.5, 8.1	3′	75.87	d		
4′	3.35	m	–[c]	4′	72.24	d		
5′	3.30	ddd	9.7, 5.6, 2.1	5′	77.97	d		
6′a	3.91	dd	11.9, 2.1	6′	62.85	t		
6′b	3.71	dd	11.9, 5.6	6′	62.85	t		
–	–	–	–	Ac	173.74	s		
Ac	2.00	s	–	Ac	23.15	q		

[a] Unlike **1,** variant B (**2**) was not sufficiently soluble in acetone. The data of **2** and **1** in [D$_4$]methanol were independently assigned by interpretation of 1D and 2D NMR spectra, respectively. – [b] $\Delta\delta = \delta_C(\mathbf{1}) - \delta_C(\mathbf{2})$. – [c] Obscured by methanol signal.

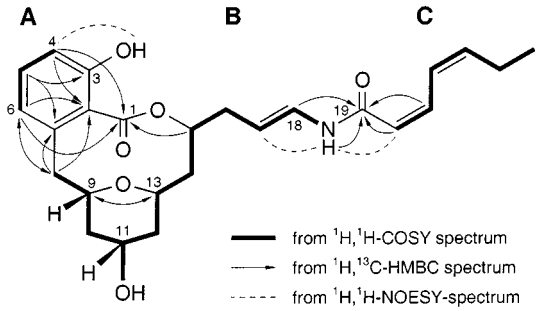


Figure 1. NMR spectroscopic evidence for the structure of apicularen A (**1**)

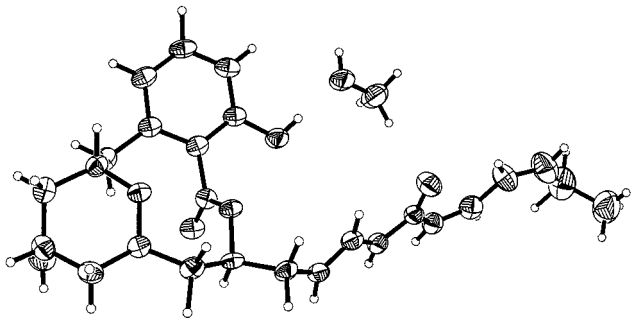


Figure 2. Crystal structure of apicularen A (**1**) with the hydrogen-bonded methanol molecule; thermal ellipsoids were drawn with the XP module of SHELXTL[31] at the 30% probability level; three disordered water molecules were omitted for clarity

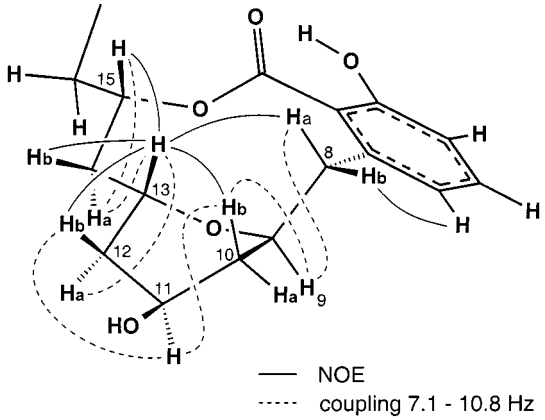
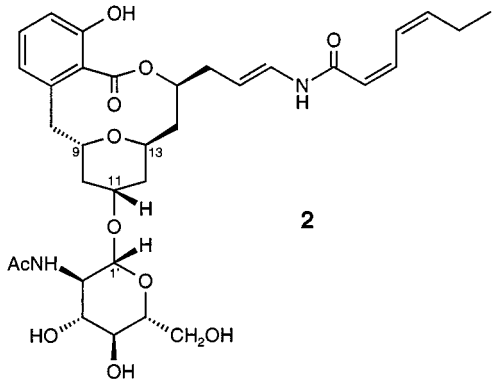


Figure 3. Selected nuclear Overhauser enhancements and proton couplings indicating the conformation of apicularen A (**1**) in solution

formation: a strong NOE between 13-H and 8-H_a confirms the biaxial position of 13-H and C-8 in the pyran ring. Further NOEs of 13-H with 15-H, 14-H_b, and 12-H_b require these protons to be on the same side of the lactone or pyran ring. An NOE of 13-H was not observed with 11-H because the latter resides on the opposite side of the pyran ring. This interpretation was supported by NOEs between 11-H and 10-$H_{a/b}$, 12-$H_{a/b}$, and even 9-H and 14-H_a. The latter long-range NOEs, although very weak, were absolutely inconsistent with the equatorial position of 11-H in crystalline **1**. Additionally, the chair conformation does not account for the NOE observed between 13-H and 10-H_b. The twisted conformation completely agrees with the restrictions set by the NOEs, and it further accounts for the vicinal coupling constants (Figure 3, Table 1) of this structural element. Thus, the twisted pyran ring is proposed as the most preferred conformation of **1** in solution.

The more polar variant, apicularen B (**2,** Scheme 2), showed a molecular ion at m/z = 644 in the DCI (–) MS and this corresponds to an elemental composition $C_{33}H_{44}N_2O_{11}$, which is 203 mass units (i.e. a fragment of $C_8H_{12}NO_5$) larger than **1**. Since the ^{1}H-NMR signals of the apicularen skeleton were largely preserved in **2**, the new fragment could easily be recognized as *N*-acetyl-β-D-glucosamine residue: starting from a typical 1′-H signal of a β-glycoside at δ = 4.65 with $J_{1,2}$ = 8.1 Hz a chain of vicinal



Scheme 2. Apicularen B (**2**)

trans couplings of 10.5 Hz for $J_{2,3}$, 8.1 Hz for $J_{3,4}$, and 9.7 Hz for $J_{4,5}$ provided the glucopyranose configuration, which in the case of *N*-acetyl-glucosamine should belong to the D series.[5] Its β-glycoside connection was verified by a $^1J_{C,H}$ coupling of 159 Hz for the anomeric methine.[6] A correlation in the ^{1}H,^{13}C-HMBC spectrum between the acetyl carbonyl and the 2′-H signal furnished the position of the acetamido group at C-2′, which was also indicated by the chemical shift δ_C = 55.9. Similarly, the mutual ^{1}H,^{13}C correlations through the glycoside bond – between the anomeric methine and the oxymethine group C-11 – allowed the determination of the attachment of the glycosyl residue at 11-O. Compared to **1**[7] the signal of C-11 is shifted by 6.8 ppm downfield, the typical glycosidation shift of an α carbon.

Additionally the glycosidation induces distinct β-shifts in the aglycon, i.e. –4.7 ppm for C-10 and –1.4 ppm for C-9 (Table 2). Since these β-shifts mainly reflect the chirality of the aglycon they provide a valuable tool for the determination of the absolute configuration of the glycosylated carbon.[8] Because they are independent of the configuration at C-2 of the carbohydrate,[9,10] the shifts caused by the *N*-acetyl-β-D-glucosamine moiety in **2** can be utilized. Favorably in the case of **2** the aglycon has an unhindered 11-O position owing to the spatially undemanding methylene groups in both β positions. As confirmed by a strong NOE between 11-H and 1′-H, glycoside **2** adopts the expected conformation (as shown in Figure 4), which is furthered by the exoanomeric effect.[11]

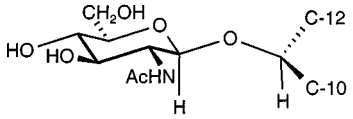


Figure 4. Partial view of the absolute configuration of apicularen B (**2**)

According to Seo et al.[8] the absolute value of $\Delta\delta_C = \delta_{glycoside} - \delta_{aglycon}$ for the β-CH_2 *anti* to the pyranose-ring oxygen is always larger (ca. –4 ppm) than that for the β-CH_2 *syn* to the oxygen (ca. –2 ppm). In the case of **2** a shift of –4.7 ppm indicates that C-10 is *anti* to the pyranose oxygen and thus the absolute configuration of C-11 is as shown in Figure 4. Since the relative configuration of **1** is

known, both compounds must have the (9*S*,11*R*,13*R*,15*S*)-configuration.

The absolute configuration of **1** and **2** was verified by Mosher's method, which is based on an NMR-spectroscopic analysis of the (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters.[12] These compounds were prepared by reacting **1** with (*R*)- and (*S*)-MTPA chloride in pyridine to give the 3,11-di-(*S*)-MTPA ester (**4**) and 3,11-di-(*R*)-MTPA ester (**5**), respectively. Equal amounts of esters **4** and **5** were used to reassign the relevant apicularen NMR signals in [D_6]acetone[13] as well as in CD_2Cl_2[14] from ^{1}H,^{1}H-COSY and 1D ^{1}H-NMR spectra. For both solvents the chemical shifts of the protons close to carbinol C-11[15] are given in Table 3. The shift differences $\Delta\delta = \delta_{(S)} - \delta_{(R)}$ of the protons on the right side of the MTPA ester in Figure 5 should be positive and on the left side be negative.[16] Since the values of protons 8 to 10 are positive and of protons 12 to 14 are negative without exception, and since their absolute values clearly reflect the correct stereochemical arrangement of the MTPA esters,[17] the (*R*)-configuration at C-11 and consequently the (9*S*,11*R*,13*R*,15*S*)-configuration of **1** is confirmed.

Table 3. Selected ^{1}H NMR spectroscopic data of (*S*)- and (*R*)-MTPA esters **4** and **5**

δ in [D_6]acetone[a][b]				δ in [D_2]dichloromethane[a][c]		
H	**4**	**5**	Δδ[d]	**4**	**5**	Δδ[d]
8a	3.364	3.283	0.081	3.382	3.334	0.048
8b	2.642	2.437	0.205	2.523	2.353	0.170
9	4.029	3.999	0.030	4.082	4.026	0.056
10a	2.226	2.149	0.077	2.202	2.138	0.064
10b	1.795	1.637	0.158	1.763	1.664	0.099
11	5.387	5.396	–0.009	5.366	5.361	0.005
12a	1.801	1.887	–0.086	1.818	1.876	–0.058
12b	1.628	1.812	–0.184	1.670	1.789	–0.119
13	4.086	4.272	–0.186	4.151	4.257	–0.106
14a	1.772	1.880	–0.108	1.866	1.904	–0.038
14b	1.541	1.675	–0.134	1.507	1.599	–0.092

[a] The chemical shifts were assigned from 1D and 2D NMR spectra. – [b] 600 MHz. – [c] 400 MHz. –

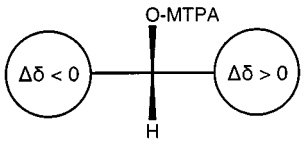


Figure 5. Expected ^{1}H-shift differences between (*S*)- and (*R*)-MTPA esters **4** and **5**

Biosynthesis of Apicularen

The biosynthetic origin of the apicularens A (**1**) and B (**2**) was studied by feeding experiments with ^{13}C-labeled precursors. After feeding [1-^{13}C]acetate the uneven numbered carbons from C-1 to C-15 and C-19 to C-23 of **1** (Figure 6) were found with ^{13}C-enrichments[18] between 3.3 and 7% above the natural ^{13}C content (Table 4). In a further

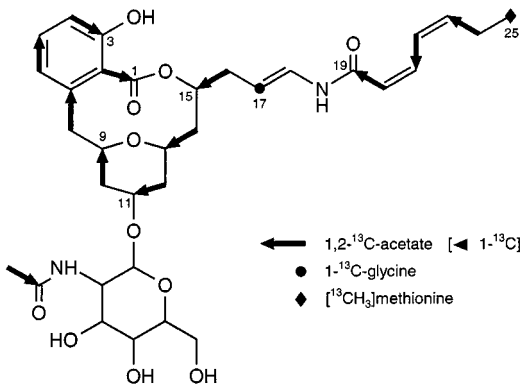


Figure 6. Biosynthetic precursors of the apicularens

feeding experiment with [1,2-^{13}C]acetate eleven acetate molecules were incorporated as intact units, which were identified by the C,C-coupling constants $^1J_{C,C}$ given in Table 4. This sample of **1** had an ^{13}C enrichment between 1.5 and 2.1%.[19] Because the carbon signals within the labeled regions were observed with distinct coupling to neighbor acetate units, the individual ^{13}C content of the labeled molecules in our sample was much higher.[20]

These feeding experiments left three carbons not labeled by acetate: the only methyl group (C-25) in the heptadienoic acid residue of **1** and the pair of C-17 and C-18 as part of the enamide group. As a precursor of the latter [1-^{13}C]glycine was incorporated with a ^{13}C enrichment of 14% at C-17. Since no label could be detected in **1** after feeding ^{13}DC-labeled propionate, the last carbon was traced back to originate from methionine by feeding the ^{13}C-methyl label. It was recovered in a sample of **1** with a very high ^{13}C enrichment of 52% in C-25.[21] The observed labeling of **1** is a rare example of the incorporation of a methyl from methionine into a supposed C_3 starter unit of the polyketide syntheses. Other examples of an acetate/methionine-derived C_3 starter unit are myxovirescin A_1[22] and borophycin[23] as well as asteltoxin,[24] where the alternative starter propionate was incorporated equally good. In the case of apicularens the otherwise purely acetate-based elongation of the polyketide chain is disturbed only once by the incorporation of a glycine unit. Thus, a modular polyketide synthase containing a nonribosomal peptide synthetase should be involved in the biosynthesis of **1**. Recently, combined gene clusters of polyketide synthases and peptide synthetases have been identified in *Sorangium cellusosum*[25] and *Stigmatella aurantiaca*.[26]

The glycoside Apicularen B (**2**) isolated from the acetate feeding experiments showed the same labeling pattern in its aglycon. Additionally, the *N*-acetyl group of the carbohydrate moiety was labeled with similar ^{13}C enrichment.

Biological Activity

The glycoside apicularen B (**2**) shows a weak antimicrobial activity against some Gram-positive bacteria, e.g. *Micrococcus luteus* (MIC 12.5 μg/mL) and *Corynebacterium fas-*

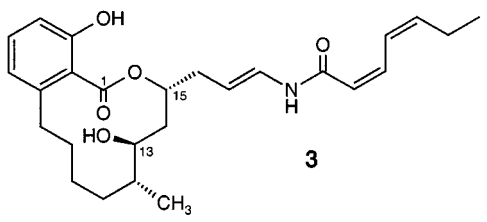
Table 4. ^{13}C Enrichment and C,C-coupling constants in ^{13}C-labeled apicularen A (**1**)

C	δ_C	m	[1-^{13}C]acetate enrichment [%]	[1,2-^{13}C]acetate enrichment [%]	$^1J_{C,C}$ [Hz]
1	169.28	s	4.9	1.5	75.3[d]
2	125.44	s	n. o.[a]	n.a.[b]	75.3[d]
3	154.23	s	3.3	n.a.	64.6
4	114.39	d	0.0	n.a.	64.6
5	130.20	d	5.5	n.a.	56.4
6	122.24	d	–0.1	n.a.	56.4
7	140.15	s	4.4	n.a.	44.2
8	40.26	t	–0.2	n.a.	44.2
9	73.64	d	5.1	n.a.	35.6[d]
10	39.60	t	0.5	n.a.	35.6[d]
11	64.87	d	7.0	n.a.	36.6[d]
12	39.88	t	–0.1	n.a.	36.6[d]
13	68.05	d	5.1	n.a.	38.6
14	38.81	t	0.0	n.a.	38.6
15	74.21	d	3.9	n.a.	39.2
16	36.37	t	0.0	2.1	39.2
17	108.09	d	0.4	n.e.[c]	–
18	126.24	d	0.3	n.e.	–
19	163.64	s	5.8	2.0	65.6
20	120.84	d	0.4	n.a.[b]	65.6
21	136.77	d	6.4	n.a.	52.9[d]
22	125.37	d	0.2	n.a.	52.9[d]
23	141.48	d	6.6	n.a.	41.7
24	21.00	t	0.1	2.1	41.7
25	14.30	q	0.2	n.e.[c]	–

[a] Signal not observed. – [b] n.a. = not analyzed signal group, see ref.[19] – [c] n.e. = not enriched singlet signal for unlabeled carbon observed with appropriate intensity.[d] Since the signals of C-10 and C-12 as well as those of C-22 and C-2 closely overlap, the corresponding values were determined from their coupled counterpart only.

cians (MIC 25 μg/mL) and a moderate cytotoxic activity with IC_{50} values between 0.2 and 1.2 μg/mL. The corresponding aglycon, apicularen A (**1**), though lacking any antibiotic activity against microorganisms, shows a very high cytostatic activity with IC_{50} values ranging between 0.3 and 3 ng/mL with nine different human cancer cell lines, including the multi-drug resistant line KB-V1.[3] Sasse et al.[4] observed several abnormal effects in tumor cells in the presence of **1**, e.g. mitotic spindles with multiple spindle poles, clusters of bundled actin from the cytoskeleton, and the induction of an apoptose-like cell death. The unusual combination of effects suggests a novel mode of action.

A search for related compounds identified the lobatamides A–D from the tunicate *Aplidium lobatum*,[27] and the more closely related salicylihalamide A (**3**, Scheme 3) and B from a marine sponge *Haliclona sp.*[28] Both groups of cytotoxic metabolites were recently isolated at the NCI. Together with the apicularens they contain the same salicylic acid structure element in their lactone ring and a similar enamide side chain.



3

Scheme 3. Salicylihalamide (**3**)

In the 60-cell line human tumor screen at the NCI the salicylihalamides and lobatamides gave a very similar, characteristic pattern of differential cytotoxicity, without any significant correlation to the profiles acquired for other antitumor compounds. This result of the pattern-recognition analysis is understood as an indication of a novel mechanism of action. In view of the structural similarity and the unique biological effects it seems reasonable to suppose a common, yet unexplained mode of action for lobatamides, salicylihalamides and apicularen A (**1**).

Besides the chondramides[1] the apicularens are a second group of biologically active metabolites from myxobacteria, which are closely related to compounds from marine sources. However, the apicularens have an antipodal configuration[29] at the lactone carbon C-15 in comparison to the salicylihalamides, whose absolute configuration has been determined as 12*R*,13*S*,15*R* using Mosher's ester method. Furthermore, the apicularens are another rare example of the structural relation between a myxobacterial metabolite and a product from another bacterial genus, since YM-75518, which was isolated from a *Pseudomonas* sp., is identical to lobatamide A.[30]

Experimental Section

General: Optical Rotation: Perkin–Elmer polarimeter 245. – UV: Shimadzu UV/Vis scanning spectrometer UV-2102, solvent methanol [Uvasol (Merck)]. – IR: Nicolet FT-IR spectrometer 20 DXB. – NMR: Bruker spectrometer DMX 600 (^1H: 600.1 MHz, ^{13}C: 150.9 MHz), Bruker spectrometer ARX 400 (^1H: 400.1 MHz; ^{13}C: 100.6 MHz) or Bruker spectrometer AM 300 (^1H: 300.1 MHz; ^{13}C: 75.5 MHz); internal standard was the solvent signal. – Mass spectrometry: EI or DCI: Finnigan spectrometer MAT 95 (EI with 70 eV) (DCI with isobutane), resolution $M/M = 1000$; high-resolution data from peak matching ($M/M = 10000$).

Isolation of Apicularens: At the end of a fermentation (90 L) of *Chondromyces robustus*, strain Cm r8, (as described in ref.[3]) wet cell mass and adsorber resin Amberlite XAD 16, which had been present during fermentation, were harvested by centrifugation and extracted with three portions of acetone (1.5 L each). The aqueous mixture, which remained after evaporation of the organic solvent, was adjusted to pH 7 and twice extracted with ethyl acetate (600 mL in all). The organic solution was dried with sodium sulfate and concentrated. To remove the lipophilic cell products the residue of about 16 g was partitioned between methanol containing 3% water (275 mL) and heptane (two portions of 200 mL each). Concentration of the polar layer yielded about 6 g of a raw product, which was separated by silica gel flash chromatography [25 g silica gel LiChroprep Si 100, 25–40 μ (Merck), solvents CH_2Cl_2 and CH_2Cl_2/methanol (98:2), 120 mL each]. After TLC analysis the fractions containing **1** were combined, conentrated to dryness (residue 390 mg) and crystallized with methanol to yield nearly white crystals of apicularen. Since the crystals contained varying amounts of methanol, they were dried in vacuo for several hours to give 156 mg apicularen A (**1**). Analytical samples were recrystallized from the same solvent.

The water layer, which remained after extraction with ethyl acetate, was again extracted with three portions (150 mL) of *n*-butyl alcohol. Before chromatography the solvent was thoroughly removed in vacuo. RP chromatography (ODS-AQ, 120 Å, 15 μ (YMC); solvent 45% methanol in water, detection UV absorption at 280 nm) furnished 85 mg of apicularen B (**2**) after concentration of the main fraction. An analytical sample can be purified further by LH-20 chromatography using methanol as solvent.

Apicularen A (1): $C_{25}H_{31}NO_6$ (441.52). – Analytical TLC: aluminum sheets with 0.2 mm silica gel 60 F_{254}, (Merck, Darmstadt); solvent CH_2Cl_2/methanol (9:1), R_f = 0.39, UV quenching at 254 nm, brown-rosa upon dying with vanillin/sulfuric acid reagent and heating to 120 °C. – Analytical HPLC: column 2 mm ID × 125 mm and pre-column of 11 mm, RP silica gel Nucleosil 120–5 C_{18}, 5 μ (Macherey–Nagel, Düren); solvent gradient with methanol/water, 50% methanol for 4 min, rising to 65% methanol in 6 min; flow = 0.3 mL/min; UV detection at 278 nm; R_t = 7.7 min. – M.p.: 139–141 °C (dec.). – $[\alpha]_D^{19}$ = –36.1 (*c* = 1, acetonitrile). – UV (methanol): λ_{max} (lg ε) = 278 (4.43). – IR (KBr): ν = 3417, 3288, 3194, 2960, 2932, 2917, 1715, 1678, 1654, 1610, 1522, cm^{-1}. – NMR see Table 1. – MS (EI); *m*/*z* (%): 442 (5.4), 441 (23) $[M]^+$, 423 (2) $[M – 18]^+$, 412 (5), 394 (2), 332 (9) $[M – 109]^+$, 272 (4), 233 (7), 215 (15), 207 (9), 191 (50), 109 (100); (+)-DCI (isobutane); *m*/*z* (%): 442 (100), 443 (26); (–)-DCI (isobutane); *m*/*z* (%): 441 (100), 442 (20). – HR-EI MS: $C_{25}H_{31}NO_6$: calcd. 441.2151; found 441.2130. $C_{18}H_{22}NO_5$: calcd. 332.1497; found 332.1510. – C_7H_9O: calcd. 109.0653; found 109.0647.

X-ray Crystal Structure of Apicularen A (1):[31] $C_{25}H_{31}NO_6$, orthorhombic $P2_12_12_1$, Z = 4, a = 9.610(5), b = 35.342(5), c = 8.465(5) Å, V = 2875(2), $\rho_{(calc)}$ = 1.205 g/cm^3, λ(Cu-K_α) = 1.54178.Å. Data collection was carried out by omega scan techniques ($2° < \theta < 57°$) using a Siemens P4 diffractometer. Three standard reflections were monitored periodically and used for correction of crystal decay (< 10%). Of the 2600 measured reflections 2419 had $I > 2\sigma(I)$. The structure was solved by direct methods using SIR97 and refined on F^2 for all reflections with positive F^2 using SHELXTL (Siemens).[32] Hydrogen atoms were generated with SHELXTL after anisotropic refinement of the nonhydrogen atoms and included in the full-matrix least-squares refinement restrained to the corresponding nonhydrogen atom and with isotropic temperature factors. The resulting R value for 332 variables and 2593 observations was R = 0.0782, based on F and using $w = 1/[s^2(F_o^2)+(0.1351P)^2 + 1.5368P]$ where $P = (F_o^2 + 2Fc^2)/3$ as weighting scheme. Determination of the absolute configuration using the Flack parameter was not possible due to the small crystal size and insufficient anomalous dispersion.[33] The structure includes one methanol molecule (hydrogen bonded to the aromatic hydroxyl group) and three disordered water molecules.

Apicularen B (2): $C_{33}H_{44}N_2O_{11}$, M = 644.71. – Analytical TLC: aluminum sheets with 0.2 mm silica gel 60 F_{254}, (Merck, Darmstadt); solvent CH_2Cl_2/methanol (9:1), R_f = 0.1, (8:2), R_f = 0.43, UV quenching at 254 nm, brown-rosa upon dying with vanillin/sulfuric acid reagent and heating to 120 °C. – Analytical HPLC: like **1** but R_t = 3.9 min. – $[\alpha]_D^{21}$ = –5.5 (*c* = 0.3, in methanol). – UV (methanol): λ_{max} (lg ε) = 280 nm (4.489). – NMR see Table 2. – DCI-MS [(+) (isobutane)]; *m*/*z* (%): 645 [M + H]; DCI-MS [(–) isobutane]; *m*/*z* (%): 644 [M]. – HR-DCI MS: $C_{33}H_{44}N_2O_{11}$: calcd. 644.2945; found 644.2985.

Preparation of Apicularen A Di-MTPA Esters: Compound **1** (10 mg) was stirred in 0.3 mL pyridine/CH_2Cl_2 (1:1) with 8 μL of α-methoxy-α-trifluoromethyl phenylacetyl chloride. After 6 h an additional 4 μL of the chloride was added. After standing overnight the mixture was diluted with CH_2Cl_2 and extracted with water three times. The dichloromethane layer was dried with anhydrous sodium sulfate and concentrated to dryness. The product was freed from residual pyridine by concentration with toluene. The MTPA-esters were purified by preparative silica gel TLC (0.5 mm) with ethyl acetate/petroleum ether (4:6), yield 11 mg. – $C_{45}H_{45}F_6NO_{10}$ (873.84). – EI-MS (200 °C); *m*/*z* (%): 873 (6.3) $[M]^+$, [874 (3), 875 (1)], 764 (2) $[M – 109]^+$, 189 (100). – HR-EI MS: $C_{45}H_{45}F_6NO_{10}$: calcd. 873.3054; found 873.2948.

Feeding Experiments With 13C-Labeled Precursors: For each feeding experiment 3 portions of 400 mL of *Chondromyces robustus*, strain Cm r8, were cultured for 5–8 days in Probion liquid medium [Probion 0.4%, soluble starch 0.3%, $MgSO_4 \cdot 7H_2O$ (0.2%), $CaCl_2 \cdot 2H_2O$ (0.05%), vitamin B_{12} 0.5 mg/L, 1 mL/L of the tenfold concentrated standard trace element solution,[34] pH 7.0, 30°C] in presence of 1% Amberlite XAD 16. Starting after 48 h the labeled precursors were added as three equal parts of an aqueous solution at intervals of 24 h. Cells and XAD were harvested after six days. Precursors added: [1-^{13}C]glycine (284 mg), L-[methyl-^{13}C]methionine (400 mg), sodium [1-^{13}C]acetate (400 mg), sodium [1,2-$^{13}C_2$]-acetate (400 mg) [Cambridge Isotope Laboratories (CIL)]. Because of the high ^{13}C content of the precursors (^{13}C: 98–99%) the enrichment in the labeled products is given and not converted into incorporation rates.

Acknowledgments

We thank N. Dankers for assistance in the isolation work, A. Roß, H. Schüler and co-workers for large scale fermentation, V. Wray, B. Jaschok-Kentner and C. Kakoschke for recording the NMR spectra, R. Christ for measuring the mass spectra, and H. J. Hecht for the crystal structure.

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[18] % enrichment = 1.1 (intensity$_{labeled}$/intensity$_{natural}$) – 1.1
[19] The ^{13}C enrichment for this experiment was calculated using those four carbons, where only a single coupling with a labeled neighbor carbon was visible: the carboxyl carbons C-1 and C-19 and the methylene carbons C-16 and C-24 Their values were calculated from the intensities of the doublet signal of the labeled carbon versus the intensity of the enclosed singlet signal of the unlabeled carbon. The remaining signals of ^{13}C-enriched carbons appeared as signal groups, each consisting of (1) a doublet (due to coupling within an acetate unit) and (2) a smaller doublet of doublet (occurring due to the less abundant coupling with a labeled neighbor acetate unit together with the coupling within a labeled acetate unit) for each labeled carbon and of (3) a singlet for the natural abundant, unlabeled carbon.
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Received June 16, 1999
[O99358]