The Ajudazols A and B, Novel Isochromanones from *Chondromyces crocatus* (Myxobacteria): Isolation and Structure Elucidation

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Keywords: Myxobacteria / Antibiotics / Structure elucidation / Natural products

Two novel metabolites, ajudazols A (**1**) and B (**2**), were isolated in a screening of the myxobacterial genus Chondromyces from several strains of *C. crocatus*. Both **1** and **2** are unique isochromanone derivatives with an extended side chain containing an oxazole, a Z,Z-diene, and a 3-methoxybutenoic acid amide as characteristic structural features.

Introduction

In the course of our screening for biologically active metabolites from myxobacteria, extracts of several strains of *Chondromyces crocatus* have been identified that are noteworthy for their high antibiotic activity against fungi, yeast and animal cell cultures.[2,3] This activity was subsequently ascribed to several groups of diversely active compounds produced simultaneously by these *Chondromyces* strains. The chondramides A−D[4] are structurally related to the sponge metabolite jaspamide (jasplakinolide).[5,6] These depsipeptides are only moderately active against yeasts but have proved to be highly cytostatic in mammalian cell cultures:[7] induction of actin polymerisation and the occurrence of multinucleate cells was observed in chondramide-treated cell cultures.[8] The biological activity of crocacin A, the main representative of a second group of metabolites, lies in its effective growth inhibition of fungi and yeasts, caused by inhibition of the electron flow within the cytochrome bc_1 segment (complex III) of the respiratory chain.[9,10] In this paper we report on the isolation and structural elucidation of the ajudazols A (**1**) and B (**2**), a further group of novel metabolites produced by *C. crocatus*. Even though the ajudazols failed to show any significant biological activity in our primary screening, their ease of access and their promising UV and NMR spectra prompted their isolation as promising candidates for secondary screenings.[11]

Results and Discussion

Like crocacins[10] and chondramides,[4] the ajudazols A (**1**) and B (**2**) are regularly found in 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 component ajudazol A (**1**) is usually about 3−4 mg/L.

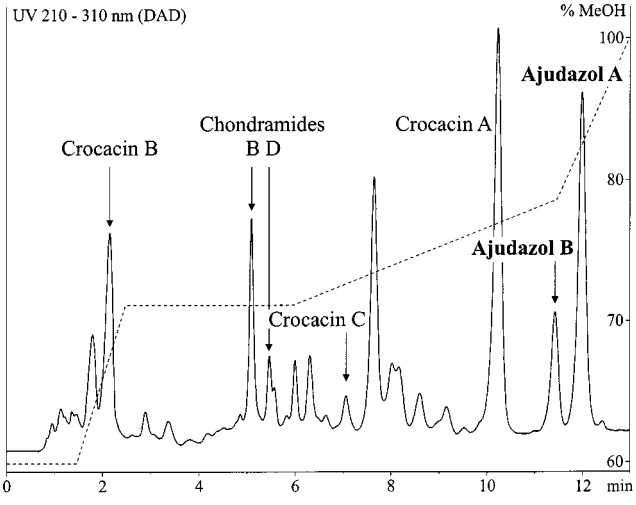

Figure 1. Typical RP-HPLC of an extract of *Chondromyces crocatus*; column 125×2 mm and pre-column 11 mm filled with Nucleosil 120−5 C_{18}, solvent gradient with solvent A = water and solvent B = MeOH as shown by dashed line, flow 0.3 mLmin^{-1}

The ajudazols were isolated from the acetone extract of the wet cell mass of *C. crocatus* by partition between methanol and heptane which initially removed most of the more lipophilic by-products. Separation and purification of **1** and **2** was accomplished by consecutive chromatographic separations on RP-18 silica gel and Sephadex LH-20. Due to a severe decomposition of ajudazols during normal-phase silica-gel chromatography, which was previously used for the isolation of the chondramides,[4] this step was avoided by the modified separation procedure.

[‡] Antibiotics from Gliding Bacteria, 89. Part 88: Ref.[1]

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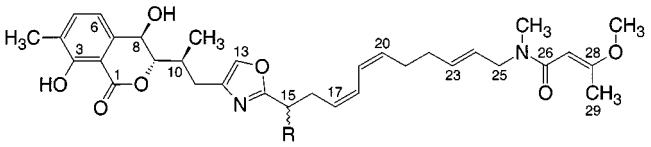

Ajudazol A (**1**) R = =CH_2
Ajudazol B (**2**) R = —CH_3, H

The structural elucidation of the ajudazols was based mainly on data obtained from ajudazol A (**1**). High resolution EI MS of the molecular ion at m/z = 590, which was identified by EI and (+)-DCI MS, furnished the elemental composition $C_{34}H_{42}N_2O_7$, which implies 15 double bond equivalents.

The UV spectrum showed two intense, broad bands at 213 and 235 nm and a weak band at 320 nm while the IR spectrum indicated lactone, amide and hydroxy groups by bands at 1710, 1674, 1650 and 3360 cm^{-1}.

The structure elucidation by NMR spectroscopy was hampered by a broadening and doubling of the signals, although this could be overcome by measuring the spectra at 80 °C in [D_6]DMSO. With the exception of two H/D-exchangeable protons, all ^{1}H NMR signals were correlated in the ^{1}H,^{13}C-HMQC spectra with their corresponding carbon signals (Table 1). Taking the direct and stronger long-range correlations in the ^{1}H,^{1}H-COSY NMR spectra into account three basic structural elements, designated as **A**, **B** and **C** in Figure 2, were recognized. These were complemented with the remaining quaternary carbons from the correlations derived from the ^{1}H,^{13}C-HMBC spectra.

As shown in Figure 2 five carbons — a lactone, a phenol and three further quaternary carbons — are required to complete structural element **A** and give an isochromanone substituted with a methyl group [δ_H = 2.17 (s)], a chelated phenol [δ_H = 11.1 (s)] and a secondary benzylic alcohol [δ_H = 6.05 (d, J = 6.5 Hz)], which both account for the H/D-exchangeable protons. The position of the exchangeable protons was assigned from the NMR spectroscopic data of **1** and was checked with a diacetylated sample, giving a downfield shift of $\Delta\delta$ = 1.1 ppm for 8-H and small shifts of about $\Delta\delta$ = 0.2 ppm for the aromatic 5-H and 6-H signals in $CDCl_3$. Additionally, the doublet of the 10-methyl group was shifted upfield by about 0.25 ppm due to the shielding effect of the neighbouring ester carbonyl group.

The extension of unit **A** with an oxazole residue was indicated by ^{1}H,^{13}C-HMBC correlations between methylene C-11 and the aromatic methine C-13 at δ = 135.75. The latter showed a ^{13}C-^{1}H coupling of $^1J_{C,H}$ = 209 Hz with the singlet at δ = 7.68, which requires methine C-13 to be part of an unsaturated heterocyclic ring. The oxazole is completed by an oxygen, a nitrogen and the quaternary carbon atoms C-12 and C-14, which had appropriate chemical shifts (δ = 139.39/161.02) and matching correlations in the ^{1}H,^{13}C-HMBC spectrum.

The linking of the oxazole and structural element **B** by a vinylidene group was indicated by ^{1}H,^{13}C-HMBC correlations (Figure 2) of the oxazole C-14 and a second quaternary carbon C-15 (δ = 135.7) with a broad doublet at δ_H = 3.27 of the saturated methylene group C-16. Both carbons showed additional correlations with the typical singlets of an *exo*-methylene group (δ = 5.85 and 5.35; 15-CH_2), thus providing the required double-bonded substituent for the intermediary carbon C-15. ^{1}H,^{1}H long-range correlations of these methylene protons support the assignment.

The aliphatic chain of unit **B** ends with the methylene group C-25, which at ambient temperature occurs as a double signal in both the ^{13}C NMR spectrum (δ = 48.1/51.3) and the ^{1}H NMR spectrum (δ = 2.84/2.74). Furthermore, a doubling of the ^{1}H and ^{13}C NMR signals with a nearly even ratio of 1:1.2 was observed for the *N*-methyl group and the carbonyl C-26 (Table 1) indicating a hindered rotation of an *N*-methyl amide in the remaining structural part of ajudazol A (**1**). A doubling of the ^{1}H NMR signals is also seen for the 28-methoxy group and methine C-27. Although these doubled ^{1}H NMR signals collapse to sharp signals at about 80 °C, the ^{13}C NMR signals of C-25, *N*-methyl and C-26 became lost in the baseline of the spectrum. Above 80 °C a rapid decomposition of **1** was also observed.

In spite of this handicap, the correlations shown in Figure 2 could be assigned from the ^{1}H,^{13}C-HMBC NMR spectra measured at ambient temperature and at 80 °C. These unambiguously provide the connections of the remaining carbon atoms with structural part **C** to give the terminal methoxybutenoic acid methyl amide residue of ajudazol A (**1**). These were supported by correlations based on nuclear Overhauser enhancement between the protons of methylene C-25, the *N*-methyl group and methine C-27 in the ^{1}H,^{1}H-ROESY spectrum. Additional NOE correlations prove the Z configuration of 27-H and the 28-methoxy group, while no NOE could be detected for the methyl group C-29.[12]

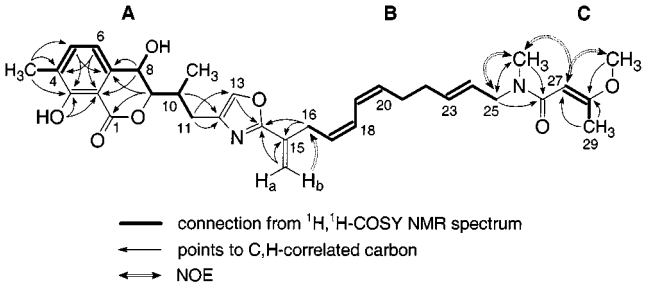

Figure 2. Structure elements of ajudazol A (**1**) and selected correlations from NMR spectroscopy

A vicinal coupling constant of $J_{23,24}$ = 15.4 Hz confirmed the E configuration of the Δ^{23} double bond, whereas overlapping signals obscured the coupling information of the Δ^{17} and Δ^{19} double bonds in the ^{1}H NMR spectrum in [D_6]DMSO. However, in $CDCl_3$ at 55 °C the 18- and 19-H signals were clearly separated (δ = 6.37 and 6.29) as doublets of doublets with coupling constants in the order of 10 to 12 Hz, proving the Z configuration of both double bonds of the conjugated diene (Table 1).

Table 1. NMR spectroscopic data of ajudazol A (**1**) and B (**2**)

Atom	Ajudazol A (1)[a] δ_H	m	*J*	δ_C	Atom	Ajudazol B (2)[b] δ_H	m	*J*	δ_C	m
1	–	–	–	168.62	1	–	–	–	170.25	s
2	–	–	–	106.26	2	–	–	–	107.20	s
3	–	–	–	158.63	3	–	–	–	160.61	s
3-OH	11.10	s	br	–	3-OH	11.28	s	–	–	–
4	–	–	–	125.11	4	–	–	–	126.13	s
4-CH_3	2.17	s	br	14.82	4-CH_3	2.22	s	–	15.40	q
5	7.44	dd	7.6, 0.8	137.24	5	7.47	d	7,5	137.94	d
6	6.95	d	7.6	116.63	6	7.08	dd	7.6, 1.1	116.54	d
7	–	–	–	140.21	7	–	–	–	142.05	s
8	4.79	d(d)	6.7 (6.5[c])	63.87	8	4.96	dd	8.0, 6.6	65.29	d
8-OH	6.01	d	6.5[c]	–	8-OH	5.51	d	–	–	–
9	4.39	dd	6.7, 5.6	87.26	9	4.43	dd	8.3, 4.1	88.08	d
10	2.20	m	–	33.29	10	2.47	m[d]	–	33.83	d
10-CH_3	0.94	d	6.9	15.78	10-CH_3	1.26	d	6,9	16.85	q
11_a	2.78	ddd	14.8, 4.7, 1.1	27.63	11_a	2.89	ddd	14.1, 4.3, 1.4	27.75	t
11_b	2.45	dd	14.9, 8.7	11_b	2.49	m[d]	–			
12	–	–	–	139.39	12	–	–	–	139.39	s
13	7.68	s	–	135.75	13	7.61	d	1.3	135.75	d
14	–	–	–	161.02	14	–	–	–	168.22	s
15	–	–	–	134.36	15	3.03	tq	7,0, 7,0	34.62	d
15-CH_a	5.85	s	br	117.42	15-CH_3	1.29	d	7.0	18.26	q
15-CH_b	5.35	s	br							
16_a, 16_b	3.27	d	7.8 br	30.03	16_a	2.62	ddd	14.4, 7.2, 1.4	33.48	t
					16_b	2.49	dd	14.0, 8.2, 1		
17	5.52	m	–	127.64	17	5.40	m[e]		128.98	d
18	6.29	m	–	125.20	18	6.29	ddd	11.5, 10.3, 1.5	126.28	d
19	6.28	m	–	123.64	19	6.24	ddd	11.6, 10.1, 1.5	124.62	d
20	5.45	m	–	131.97	20	5.45	m[e]		132.35	d
21	2.19	m	–	26.75	21	2.24	m[f]		27.84	t
22	2.08	m	–	31.36	22	2.11	m[g]		32.77	t
23	5.54	ddt	15.4, 6.7, 1.4	131.97	23	5.60	ddd	15.2, 6.7, 6.7, 1	132.69	d
24	5.39	dt	15.3, 5.7, br	125.70	24	5.48	m[e]		126.96	d
25	3.84	d[h]	5.7 br	50.05	25	3.92	ddt	5.8, 1.1, 1.0	52.47	t[i]
49.21										
N-CH_3	2.82	s[j]	–	35.01	N-CH_3	2.84	s	–	33.50	q[i]
35.00										
26	–	–	–	167.96	26	–	–	–	170.13	s
27	5.24	s[k]	br	91.84	27	5.33	s	br	92.17	d
28	–	–	–	166.96	28	–	–	–	170.13	s
29	2.04	s[l]	br	18.10	29	2.13	s	–	18.70	q
28-OCH_3	3.53	s[m]	–	54.76	28-OCH_3	3.60	s	br	55.25	q

[a] In [D_6]DMSO/D_2O at 80 °C (600/150 MHz). [b] In [D_6]acetone at 400/100 MHz. [c] Without H/D exchange. [d] Overlapping signals. [e] Overlapping signals. [f] With 4-CH_3. [g] With 29. [h] δ = 3.84/3.80 at room temp. [i] Two small broad signals. [j] δ = 2.84/2.74 at room temp. [k] δ = 5.26/5.21 at room temp. [l] Broad signal at δ = 2.02. [m] δ = 3.54/3.49 at room temp., integrated ratio 1:1.2.

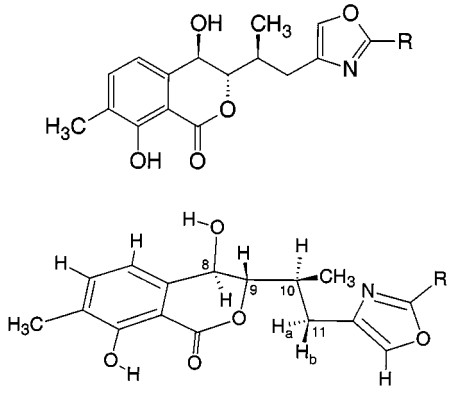

Figure 3. Relative configuration and preferred conformation of the isochromanone region of ajudazol A (**1**)

The relative configuration of C-8 and C-9 of the isochromanone residue was evident from the vicinal coupling $J_{8,9}$ = 6.7 Hz[13] clearly observed for the 8-H signal after H/D exchange. This vicinal coupling constant[14] indicates the *trans* configuration of the hydroxymethine and the lactone proton in **1**. This assignment was supported by comparison with the ^{1}H NMR spectroscopic data of benaphthamycin in DMSO,[15] which has a coupling constant of 7.6 Hz for an analogous *trans* configuration. The spatial arrangement was further illustrated by NOE correlations in the ^{1}H,^{1}H-ROESY spectrum at 80 °C and by NOE difference spectra at ambient temperature, where the hydroxymethine 8-H showed strong correlations with protons belonging to the side chain, especially with the methylene 11-H_a and, to a lesser extent, with the 10-methyl group. The

lactone 9-H gave similarly intense correlations with 10-H and the 10-methyl group as well. Besides NOEs to 8-, 9- and 10-H the 10-methyl group showed a prominent NOE correlation with the methylene proton 11-H_b and none with 11-H_a. The relative configuration and predominant conformation of ajudazol A (**1**) is depicted in Figure 3, reflecting the stereochemical requirements from the NMR spectroscopic data, i.e. 8-H and 9-H in *trans* positions, an equatorial orientation of the side chain, and 8-H and the 10-methyl group above and 9-H and 10-H below the molecular plane specified by the benzene ring. The arrangement shown in Figure 3 was derived by energy minimization of a structural fragment including the oxazole residue. The torsion angle between 9-H and 10-H was calculated as −70°, which is in good agreement with the vicinal coupling $J_{9,10}$ = 5.6 Hz observed for the signal of 9-H. The next minima for this torsion are at about 54° and about 175°. At least one of these conformations is also present in solution, since at 80 °C and at room temperature the NOEs between the 10-methyl group on one hand and 8-H and 9-H on the other are of almost equal intensity, which cannot be explained by the single rotamer shown.[16]

Ajudazol B (**2**) was recognized as a structural variant of **1** from its nearly identical UV spectrum. DCI and EI mass spectrometry provided the molecular ion at *m*/*z* = 592, which is two units more than **1**. The elemental composition of **2** was calculated from the HREI-MS as $C_{34}H_{44}N_2O_7$, i.e. a dihydrogenated ajudazol.

Since the UV spectrum is almost unaffected in spite of the additional hydrogens, the basic ajudazol chromophores must be preserved. In fact, in the ^{1}H NMR spectrum of **2** the majority of the signals are identical to **1**. However, the prominent singlets of the 15-*exo*-methylene group in **1** are replaced by a doublet of a new aliphatic methyl group at δ = 1.29 (*J* = 7.0 Hz). Its position at C-15 was confirmed by a COSY NMR spectrum from a correlation to the additional C-15 methine signal at δ_H = 3.03, which was further correlated to the structural element **B** in Figure 2. Due to the asymmetry at C-15 the ^{1}H NMR signals of the methylene group C-16 now appear as an AB system at δ = 2.62 and 2.49. With the exception of the hydrogenated carbons C-15 and C-16 only small shifts of the neighbouring carbon signals are observed in the ^{13}C NMR spectrum.

Conclusion

Two conspicuous structural features make the ajudazols stand out among natural products: a novel 4,8-dihydroxy-7-methyl-isochroman-1-one, which is related to 4-hydroxymellein, a 4,8-dihydroxy-3-methyl-isochroman-1-one that is widespread in plants and microorganisms[17] in all its stereochemical variants, and an unusual 3-methoxybutenoic acid methyl amide residue, which may be identical to a similar chain end of lyngbyapeptin A, a modified tetrapeptide from the cyanobacterium *Lyngbya boulloni*. Since the latter has only been isolated in trace amounts and decomposed during structure elucidation it has been described without assignment of the configuration at the double bond.[18]

The combination of at least four different, overlapping UV chromophores within the ajudazols prevents the use of optical methods for the direct determination of their absolute configuration.

Experimental Section

UV: Shimadzu UV/Vis scanning spectrometer UV-2102; solvent: methanol [Uvasol, (Merck)]. IR: Nicolet FT-IR spectrometer 20 DXB. NMR: Bruker DMX 600 (^{1}H: 600.1 MHz, ^{13}C: 150.9 MHz), Bruker ARX 400 (^{1}H: 400.1 MHz. ^{13}C: 100.6 MHz) or Bruker AM 300 (^{1}H: 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 *iso*-butane), resolution $M/\Delta M$ = 1000; high-resolution data from peak matching ($M/\Delta M$ = 10000).

Isolation of Ajudazols A (1) and B (2): From 800 L of fermentation broth of *Chondromyces crocatus*, strain Cm c5, 6.3 Kg of wet cell mass was harvested by centrifugation and extracted with 60 L of acetone in three portions of 30, 15 and 15 L. After the organic solvent had been removed from the combined extracts by evaporation, the remaining water-oil emulsion was extracted with dichloromethane (DCM) at pH 6.7. The extract was dried with sodium sulfate and concentrated to yield 92.4 g of a brown oil. This was dissolved in 1 L of methanol containing 3% of water and extracted with 2.4 L of heptane in three portions. After evaporation the methanol layer yielded 49.9 g of a brown oily residue, which was separated by gel chromatography in two portions [column Superformance 1000−100 (Merck), bed height 850 mm of Sephadex LH-20 (Pharmacia), solvent methanol, flow 18 mL/min; the fractions were taken according to TLC analysis]. A main fraction of 17.5 g, characterized by a number of TLC spots giving different colour reactions with vanillin/sulfuric acid spray reagent, contained the biologically active metabolites. This fraction was separated by RP-MPLC in two portions [column 300 × 60 mm (Kronlab); HD-Sil 18−30−60, RP-18, 20−45 μ, 60 Å; solvents: 70% aqueous methanol for 120 min, gradient to 80% methanol in 60 min, 80% methanol for 95 min, gradient to 100% methanol in 30 min, flow 24 mL/min; detection UV absorption at 254 nm]. A crude mixture of chondramides (3.5 g) eluted between 52 and 85 min, crude crocacin A (0.62 g) eluted between 190 and 218 min, and was followed by an intermediate fraction of 640 mg of enriched ajudazol B (**2**). Finally, a fraction eluted containing 3.3 g of crude ajudazol A (**1**). This was further purified by gel chromatography in DCM/methanol (1:1) [column 650 × 80 mm, Sephadex LH-20 (Pharmacia), flow 10 mL/min, detection UV absorption at 275 nm] to yield 2.58 g of ajudazol A (**1**). The intermediate fraction was separated by RP-MPLC [column 480 × 30 mm, ODS-AQ, 120 , 16 μ (Kronlab), flow 28 mL/min, solvent gradient: 70% methanol for 50 min, then rising to 80% methanol in 120 min, detection UV absorption at 254 nm]. The peak at 116 min was collected (358 mg from 1.1 g) and finally purified by gel chromatography in DCM/methanol (1:1) [column 860 × 40 mm, Sephadex LH-20 (Pharmacia), flow 2 mL/min, detection UV absorption at 256 nm]. The main peak of two runs was collected to yield 259 mg of ajudazol B (**2**).

Properties of the Ajudazols: The **1** and **2** were obtained as colourless 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 ether and insoluble in hexane.

Analytical TLC: Aluminium sheets with a layer of 0.2 mm silica gel 60 F_{254}, (Merck); solvent: DCM/methanol (9:1); detection by UV quenching at 254 nm; colour reaction: sprayed with vanillin/sulfuric acid reagent and heated to 120 °C, the ajudazols gave orange-brown spots; **1** $R_f = 0.71$; **2** $R_f = 0.68$.

Ajudazol A (1): $C_{34}H_{42}N_2O_7$, M = 590.72. $[\alpha]_D^{21} = -44.3$ ($c = 1.0$, in methanol). UV (methanol): λ_{max} (lg ε) = 213 (4.669), 235 (4.592), 320 (3.690). IR (KBr): $\tilde{\nu}$ = 3350 (m), 2928 (m), 1710 (w), 1675 (s), 1644 (s), 1622 (m), 1595 (s) cm^{-1}. EI MS (230 °C): *m*/*z* (%) = 590 (24) $[M^+]$, 575 (6) $[M - 15]^+$, 557 (7), 492, (7), 409 (12), 203 (43), 182 (73), 99 (100). (+) DCI MS (*i*-butane): *m*/*z* (%) = 591 (100). HR-EI MS: calcd. 590.2992; found 590.2984.

Ajudazol B (2): $C_{34}H_{44}N_2O_7$, M = 592.73. $[\alpha]_D^{21} = +6.1$ ($c = 1.34$, in methanol). UV (methanol): λ_{max} (lg ε) = 214 (4.58), 237 (4.54), 306 (sh), 319 (3.66). EI MS (230 °C): *m*/*z* (%) = 592 (11) $[M^+]$, 559 (6) $[M - 15]^+$, 553 (8), 493, (4), 410 (10), 392 (12), 331 (34), 313 (24), 262 (45), 203 (15), 182 (76), 99 (100). (+) DCI MS (*i*-butane): *m*/*z* (%) = 593 (100). HR-EI MS: calcd. 592.3149; found 592.3125.

Acknowledgments

We thank S. Knöfel and N. Dankers for their skilful assistance in the isolation work, A. Roß, H. Schüler and R. Krützfeld and co-workers 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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[13] $J_{8,9} = 10.4$ Hz in $CDCl_3$.
[14] Due to the larger residue at C-3 in **1** the stereochemical situation is different to the more flexible *trans*-4-hydroxymellein, which has a coupling of just 4 Hz while the *cis* variant has a coupling of 2 Hz: R. D. Hutchinson, P. S. Steyn, *Tetrahedron Lett.* **1971**, *12*, 4033−4036; D. C. Aldridge, S. Galt, D. Giles, W. B. Turner, *J. Chem. Soc. (C)* **1971**, 1623−27.
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[16] Of course, inversion of the asymmetric centre C-10 would bring the 10-methyl group close to 8- and 9-H and thus explain their mutual NOEs. However, it would never be possible to explain the highly predominant NOEs between 8-H and 11-H_a on one side and 10-methyl and 11-H_b on the other side.
[17] Dictionary of Natural Products on CD-ROM, Version 9:2, 2001, Chapman & Hall.
[18] D. Klein, J.-C. Braeckman, D. Daloze, L. Hoffmann, G. Castillo, V. Demoulin, *Tetrahedron Lett.* **1999**, *40*, 695−696.

Received September 17, 2001
[O01442]