New Triquinane-Type Sesquiterpenoids from *Macrocystidia cucumis* (Basidiomycetes)

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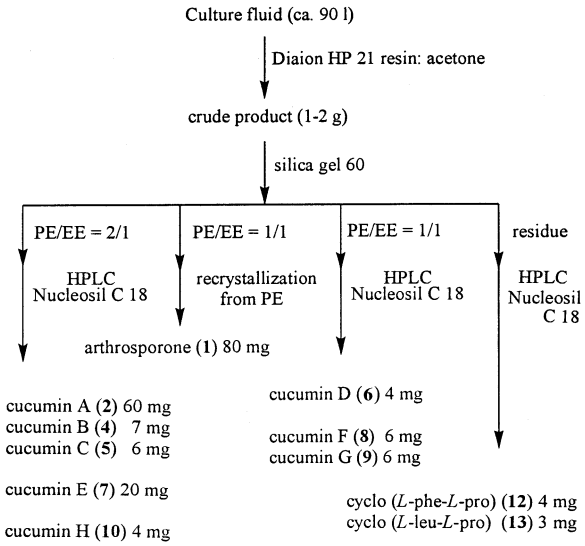
Arthrosporone (**1**) and eight new triquinane-type sesquiterpenoids have been isolated from mycelial cultures of the agaric *Macrocystidia cucumis*. The cucumins A−D (**2**, **4**−**6**) are highly unsaturated hirsutane derivatives, whereas the cucumins E−G (**7**−**9**) represent a new type of linear triquinanes. Cucumin H (**10**) is a new member of the ceratopicane group. The absolute configuration of cucumin F (**8**) was assigned by ^{1}H-NMR analysis of the corresponding Mosher esters. Two further metabolites were identified as *cyclo*(phenylalanylprolyl) (**12**) and *cyclo*(leucylprolyl) (**13**). Cucumin A (**2**) exhibits antibacterial and cytotoxic activities.

Culture extracts from fermentations of the agaric *Macrocystidia cucumis* (Pers. ex Fr.) Heim (German: Gurkenschnitzling) have previously been shown to exhibit antimicrobial activity and strong cytotoxic effects[1]. We now report on the isolation of the active principles, which were identified as a series of biosynthetically related triquinane-type sesquiterpenoids.

Fermentation of the Fungus and Isolation of the Metabolites

For production of the cucumins, fermentations of *Macrocystidia cucumis* were carried out in 100 l of YMG medium. When the antifungal activity had reached its maximum, the mycelia were separated from the culture fluid by filtration and discarded. The antibiotics were adsorbed onto HP 21 resin, washed with methanol, and eluted with acetone. The qualitative and quantitative composition of the culture filtrate varied with the fermentation time. The metabolites were isolated from the crude fermentation extract by flash chromatography on silica gel followed by HPLC on RP-18 material (Figure 1 and Experimental Section).

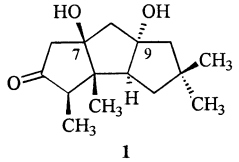
Figure 1. Work-up procedure (PE = petroleum ether; EE = ethyl acetate)



Hirsutane Derivatives 1, 2, 4−6

Silica gel chromatography of the crude extract yielded crystals of arthrosporone (**1**), a known hirsutane-type sesquiterpenoid, which had previously been isolated from cultures of an undefined arthroconidial fungus[2]. We confirmed its structure by X-ray crystallography (Figure 2) and assigned the ^{13}C-NMR signals by means of HETCOR and COLOCS experiments.

The least polar fraction obtained from the silica gel chromatography contained cucumin A, $C_{15}H_{16}O_2$, as the main constituent. Its IR spectrum (KBr) exhibits absorptions at



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$\tilde{\nu}$ = 1700, 1690 (two α,β-Δ-C=O), 1650, 1640, and 1590 cm^{-1} (C=C). The ^{13}C-NMR spectrum of **2** (Table 1) shows two carbonyl signals at δ_C = 206.73 and 195.63 and six olefinic carbon signals at δ_C = 187.70, 156.75, 151.31 (each C), 125.73 (CH; δ_H = 6.25), 124.60 (CH; δ_H = 7.03), and 114.50 (CH_2; δ_H = 5.99 and 5.30). The remaining aliphatic

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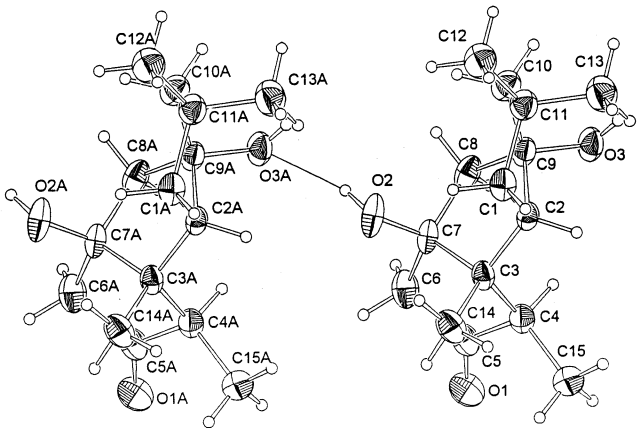
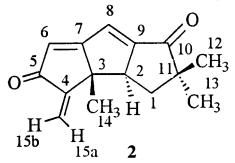
Figure 2. ZORTEP drawing of two molecules of arthrosporone (**1**) showing the intermolecular hydrogen bond between the hydroxy groups at C-7 and C-9′



signals can be assigned to $(CH_3)_2C$, CH_2CH, and $CH_3(C)$ fragments. From COLOC and INADEQUATE experiments (see Experimental Section), the hirsutane structure **2** can be deduced for cucumin A.

On irradiation at the resonance of 2-H, a nuclear Overhauser enhancement (NOE) is observed for 13-CH_3, but not for 12- or 14-CH_3, which establishes the *anti* orientation of 2-H and 14-CH_3. Cucumin A is the 2-deoxy derivative of incarnal (**3**), an antibacterial metabolite from cultures of the basidiomycete *Gloeostereum incarnatum*[3].



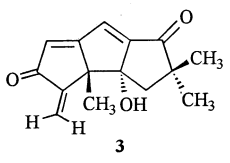


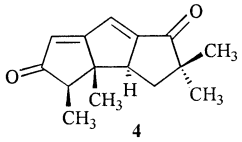
Table 1. ^{1}H- and ^{13}C-NMR data of cucumin A (**2**) in $CDCl_3$ (400 and 100 MHz, respectively)[a]

No.	δ_C	δ_H	No.	δ_C	δ_H
1	35.87	α: 1.97 (dd, 1 H)	9	156.75	
		β: 1.86 (dd, 1 H)	10	206.73	
2	50.49	3.28 (dddd, 1 H)	11	51.98	
3	61.81		12	24.08	1.20 (s, 3 H)
4	151.31		13	25.23	1.15 (s, 3 H)
5	195.63		14	24.52	1.26 (s, 3 H)
6	125.73	6.25 (s, br., 1 H)	15	114.50	α: 5.30 (s, 1 H)
7	187.70				β: 5.99 (s, 1 H)
8	124.60	7.03 (d, 1 H)			

[a] J[Hz]: 1α-1β = 12.0; 1α-2 = 7.8; 1β-2 = 12.0; 2-6 = 0.8; 2-8 = 3.2.

Cucumin B, $C_{15}H_{18}O_2$, contains two more hydrogen atoms than cucumin A (**2**). In its ^{1}H-NMR spectrum, the signals for the exocyclic methylene protons are missing and a doublet for a methyl group appears at δ_H = 1.18 (J = 7.5 Hz) instead. This is coupled with a quadruplet at δ_H = 2.40. In agreement with these findings, the ^{13}C-NMR spectrum (Table 2) shows signals for only two C=C double bonds and additional aliphatic signals at δ_C = 46.97 (CH) and 17.91 (CH_3). The remaining signals correspond to those of **2**. Consequently, structure **4** can be assigned to cucumin B.

Cucumin C, $C_{15}H_{16}O_3$, contains an additional oxygen atom that forms part of a trisubstituted epoxide with NMR signals at δ_C = 75.74 (C) and 64.84 (CH; δ_H = 3.86). Comparison of the NMR data (Table 2) with those of the hirsutanes **2** and **4** leads to formula **5** for cucumin C, which was confirmed by NOE experiments. Irradiation at the 1-H resonance enhances the signals of the 12-, 14-, and 15-CH_3 groups, whereas irradiation at 4-H affects only the 13-CH_3 signal.



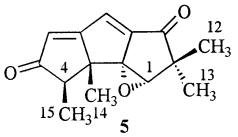


Table 2. ^{1}H- and ^{13}C-NMR data of cucumins B (**4**), C (**5**), and D (**6**) in $CDCl_3$ (**4**, **5**: 400 and 100 MHz, **6**: 600 and 150 MHz, respectively)[a]

No.	**4**		**5**		**6**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	35.98	α: 1.82 (dd, 1 H)	64.84	3.86 (s, 1 H)	74.88	4.56 (br. s, 1 H)
		β: 1.75 (dd, 1 H)				
2	52.87	3.31 (dddd, 1 H)	75.74		187.55	
3	62.81		52.32$^+$		56.99	
4	46.97	2.40 (q, 1 H)	49.83	2.72 (q, 1 H)	52.01	2.66 (q, 1 H)
5	212.91		208.88		209.47	
6	121.84$^\#$	5.95 (br. s, 1 H)	126.46$^\#$	6.22 (s, 1 H)	124.79	5.98 (br. s, 1 H)
7	191.78		187.28		182.15	
8	123.66$^\#$	6.95 (d, 1 H)	128.44$^\#$	7.25 (s, 1 H)	28.07	3.37 (br. s, 2 H)
9	158.54		150.03		143.33	
10	206.93		202.75		205.13	
11	51.84		51.60$^+$		53.81	
12	25.31*	1.20* (s, 3 H)	22.65*	1.25* (s, 3 H)	22.84	1.18 (s, 3 H)
13	24.18*	1.19* (s, 3 H)	21.49*	1.24* (s, 3 H)	20.01	1.06 (s, 3 H)
14	26.90*	1.18* (s, 3 H)	18.44*	1.10* (s, 3 H)	21.35	1.18 (s, 3 H)
15	17.91	1.18 (d, 3 H)	9.37	1.10 (d, 3 H)	9.29	1.23 (d, 3 H)

[a] *, $^\#$, $^+$ Assignments may be interchanged. – **4**: J[Hz]: 1α-1β = 12.0; 1α-2 = 8.0; 1β-2 = 12.0; 2-8 = 3.0; 2-6 = 0.5; 4-15 = 7.5. – **5**: J[Hz]: 4-15 = 7.0. – **6**: J[Hz]: 4-15 = 7.2.

Cucumin D, $C_{15}H_{18}O_3$, represents an unsaturated hirsutane derivative of higher polarity, with strong IR absorptions at $\tilde{\nu}$ = 1690 and 1630 cm^{-1}. According to the ^{13}C-NMR spectrum (Table 2), the compound contains two α,β-enone systems with signals at δ_C = 209.47 (C=O), 124.79 (α-CH; δ_H = 5.98), 182.15 (β-C), and δ_C = 205.13 (C=O), 143.33 (α-C), 187.55 (β-C), respectively. Furthermore, signals for $CH(CH_3)-C(CH_3)$, $(CH_3)_2C$, and CH(OH) fragments and an isolated CH_2 group can also be recognized. From this information and the HMBC correlations given in Figure 3, formula **6** can be deduced for cucumin D. NOESY experiments show a correlation between 6-H and 8-H, but not between 8-H and 1-H (δ_H = 4.56). Strong NOEs are also observed between 1-H and 12-H. Biosynthetically, cucumin D may be formed from cucumin C (**5**)

by opening of the oxirane ring after attack of a hydride equivalent at C-8 of the enone system.

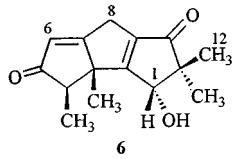


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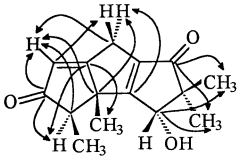
Figure 3. Selected HMBC correlations for cucumin D (**6**)



Cucumane Derivatives 7−9

Cucumin E, $C_{15}H_{20}O_2$, exhibits IR bands (KBr) at $\tilde{\nu}$ = 1745 (C=O), 1704 and 1664 cm^{-1} (α,β-Δ C=O). According to the ^{13}C-NMR spectrum (Table 3), the carbonyl groups can be assigned to a cyclopentanone (δ_C = 217.37) and a 2-cyclopentenone unit (δ_C = 207.78, 177.70, 134.69), the latter bearing a methyl group (δ_C = 8.29, δ_H = 1.72) at C-α of the double bond. Two multiplets at δ_H = 3.69 (ddd) and 3.11 (ddd) with $J \approx 10$ Hz couplings are typical for bridgehead protons of *cis*-fused triquinanes with adjacent CH_2 groups. The assignment of structure **7** to cucumin E is supported by the ^{1}H,^{1}H-COSY, and HMBC spectra (Figure 4). In the NOESY spectrum of **7**, 2-H and 9-H show correlation signals to each other and to 13-CH_3, consistent with a *cis* annelation of the five-membered rings. The protons 1β-H and 10β-H are correlated to the methyl groups C-12 and C-14, and the methyl group C-15 shows an NOE to the angular proton 2-H. Cucumin E possesses a novel carbon skeleton for which the name cucumane is proposed.

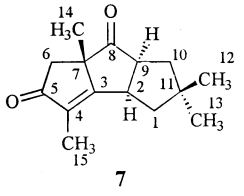


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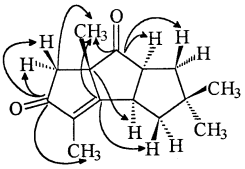
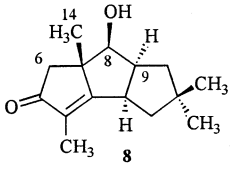
Figure 4. Selected HMBC correlations for cucumin E (**7**)



The more polar cucumins F (**8**) and G (**9**), $C_{15}H_{22}O_2$, are dihydro derivatives of **7**. Cucumin F exhibits IR bands at $\tilde{\nu}$ = 1690 and 1650 cm^{-1} (α,β-Δ C=O), whereas cucumin G shows a carbonyl absorption at $\tilde{\nu}$ = 1760 cm^{-1}. In cucumin F, the saturated carbonyl group at C-8 is reduced to the secondary alcohol, which causes the ^{1}H-NMR signal for 9-H at δ_H = 3.11 to become a dddd owing to the additional coupling (J = 9.5 Hz) with the proton at C-8 (Table 3). Important NOESY correlations are found between 8-H and the protons 6α-H and 9-H, as well as between 14-CH_3 and 6β-H. No correlation signal can be detected between 14-CH_3 and the protons 8-H or 9-H. This evidence leads to formula **8** for cucumin F.

In cucumin G (**9**), the enone carbonyl of **7** is reduced to the alcohol, which causes the protons at C-6 to form part of an ABX system. The remaining NMR data of cucumin G, as shown in Table 3, correspond to those of cucumin E (**7**). Important NOESY correlations between 6β-H and the protons 5-H and 14-CH_3, as well as between 14-CH_3 and 12-CH_3, are in accord with the stereochemistry indicated in formula **9**.



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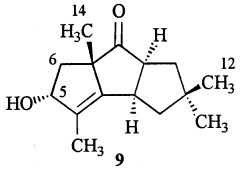

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Table 3. ^{1}H- and ^{13}C-NMR data of cucumins E (**7**), F (**8**) and G (**9**) in $CDCl_3$ (600 and 150 MHz, respectively)[a]

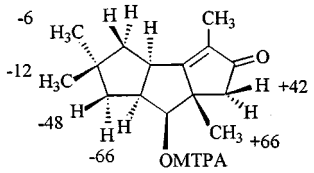
No.	**7** δ_C	δ_H	**8** δ_C	δ_H	**9** δ_C	δ_H
1	45.87*	α: 1.97 (ddd, 1 H) β: 1.82 (dd, 1 H)	45.68	α: 1.84 (dd, 1 H) β: 1.60 (m, 1 H)	46.22	α: 1.78 (ddd, 1 H) β: 1.50 (m, 1 H)
2	42.79	3.69 (ddd, 1 H)	42.62	3.33 (ddd, 1 H)	40.05	3.39 (ddd, 1 H)
3	177.70		183.92		144.82	
4	134.69		132.98		135.24	
5	207.78		209.87		80.42	4.95 (br. s, 1 H)
6	47.48	α: 2.55 (d, 1 H) β: 2.32 (d, 1 H)	53.03	α: 2.25 (d, 1 H) β: 2.32 (d, 1 H)	47.82	α: 1.60 (m, 1 H) (m, 1 H) β: 2.31 (dd, 1 H)
7	55.57		51.84		57.95	
8	217.37		78.34	3.97 (br. d, 1 H)	221.98	
9	56.33	3.11 (ddd, 1 H)	50.01	3.11 (dddd, 1 H)	55.84	3.00 (ddd, 1 H)
10	45.82*	α: 2.07 (ddd, 1 H) β: 1.71 (dd, 1 H)	41.73	α: 1.55 (m, 1 H) β: 1.87 (dd, 1 H)	45.44	α: 1.95 (ddd, 1 H) β: 1.55 (m, 1 H)
11	44.42		43.12		43.48	
12	28.19	1.17 (s, 3 H)	28.61	1.14 (s, 3 H)	28.40	1.09 (s, 3 H)
13	26.64	1.04 (s, 3 H)	26.90	1.01 (s, 3 H)	26.62	0.97 (s, 3 H)
14	27.88	1.47 (s, 3 H)	22.53	1.27 (s, 3 H)	25.63	1.27 (s, 3 H)
15	8.29	1.72 (s, 3 H)	8.25	1.65 (s, 3 H)	10.32	1.72 (s, 3 H)

[a] * Assignments may be interchanged. − **7**: J [Hz]: 1α-1β = 12.9; 1α-2 = 9.8; 1β-2 = 9.8; 1α-10α = 2.2; 2-9 = 9.8; 6α-6β = 17.6; 9-10α = 9.8; 9-10β = 9.8; 10α-10β = 13.0. − **8**: J [Hz]: 1α-1β = 12.1; 1α-2 = 9.5; 1β-2 = 9.5; 2-9 = 9.5; 6α-6β = 17.1; 8-9 = 9.5; 9-10α = 9.5; 9-10β = 9.5; 10α-10β = 12.5. − **9**: J [Hz]: 1α-1β = 12.7; 1α-2 = 9.6; 1β-2 = 9.6; 1α-10α = 2.2; 2-9 = 9.6; 5-6β = 5.9; 6α-6β = 12.4; 9-10α = 9.6; 9-10β = 9.6; 10α-10β = 12.9.

The absolute configuration of cucumin F (**8**) was determined by applying the high-field NMR modification of Mosher's method[4][5][6]. Treatment of **8** with either (*R*)-(−)- or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl

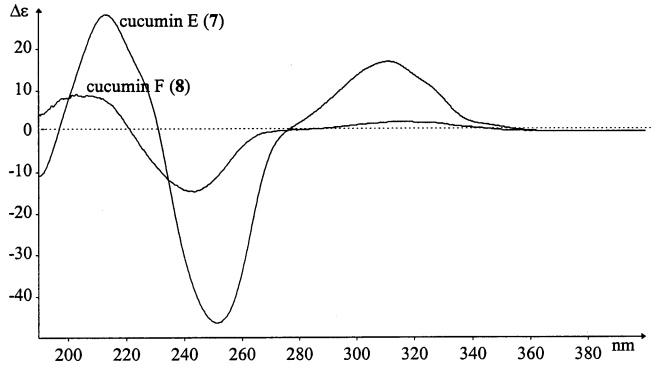
chloride (MTPA-Cl) and 4-(dimethylamino)pyridine (DMAP) yielded the (*S*)- and (*R*)-MTPA esters **8a** and **8b**, respectively. The characteristic shielding effects of the phenyl ring in the MTPA esters **8a** and **8b** were determined after complete assignment of the protons by ^{1}H,^{1}H-COSY, and NOESY experiments. 6β-H and the angular methyl group (14-CH_3) show positive values for $\Delta\delta = \delta(S) - \delta(R)$ and are therefore placed on the right-hand side of the MTPA plane, as shown in Figure 5. Both 10α- and 10β-H and the methyl groups 12-CH_3 and 13-CH_3 are placed on the left-hand side of the MTPA plane as a consequence of their negative Δδ values. This leads to the (2*R*,7*S*,8*S*,9*S*) configuration for cucumin F (**8**).

Figure 5. Δδ values in Hz obtained for the MTPA esters **8a** and **8b** [Δδ = δ(*S*-MTPA ester) − δ(*R*-MTPA ester)][4][5]



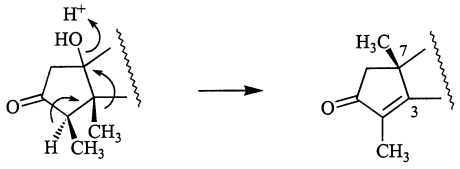
From the similarity of its CD spectrum (Figure 6), the same absolute configuration can be assigned to cucumin E (**7**). The configuration of the cucumanes **7** and **8** corresponds to that of hirsutanes with known absolute configuration, e.g. hirsutic acid[7] and complicatic acid[8].

Figure 6. CD spectra of cucumins E (**7**) and F (**8**) in CH_3CN



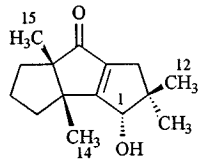
The cucumane derivatives **7**−**9** represent a new group of linear triquinane sesquiterpenoids with the usual *cis,anti,cis* arrangement of the three five-membered rings. The cucumanes differ from hirsutanes by the shift of a methyl group from C-3 to C-7, which may occur by a biogenetic Wagner-Meerwein rearrangement as indicated in Figure 7.

Figure 7. Proposed formation of the cucumane system from a hirsutane derivative by Wagner-Meerwein rearrangement



Ceratopicane Derivative 10

Cucumin H, $C_{15}H_{22}O_2$, is present in the culture extract only in minor amounts. The ^{13}C-NMR spectrum (Table 4) features signals for four CH_3, four CH_2, one CH, and six quaternary carbon atoms. IR absorptions at $\tilde{\nu}$ = 1680 and 1645 cm^{-1} and ^{13}C-NMR signals at δ_C = 212.76 (C=O), 190.64, and 145.53 indicate a 2-cyclopentenone moiety. The IR absorption (KBr) at $\tilde{\nu}$ = 3400 cm^{-1} and the ^{13}C-NMR signal at δ_C = 81.45 (CH; δ_H = 4.51) are in agreement with the presence of a secondary alcohol group. In contrast to the compounds with a hirsutane or cucumane skeleton, all the methyl groups in cucumin H are bound to quaternary carbon atoms and give rise to singlets in the ^{1}H-NMR spectrum. Formula **10** was deduced for cucumin H from ^{1}H,^{1}H-COSY and HMBC experiments (Figure 8). In a NOESY experiment, 1-H correlates with 12-CH_3 as well as with 14-CH_3. A NOESY correlation signal between 14-CH_3 and 15-CH_3 supports the *cis* connectivity of rings A and B (see also Figure 8)[9]. The absolute configuration of cucumin H is arbitrarily assigned and corresponds to that of the other metabolites from *M. cucumis*.



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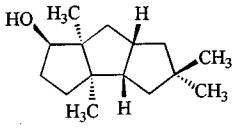
(relative configuration)



11

Figure 8. Selected HMBC (A) and NOESY (B) correlations for cucumin H (**10**)

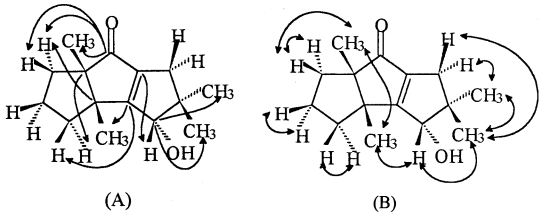


Table 4. ^{1}H- and ^{13}C-NMR data of cucumin H (**10**) in CD_3OD (600 and 150 MHz, respectively)[a]

No.	δ_C	δ_H	No.	δ_C	δ_H
1	81.45	4.51 (br. s, 1 H)	8	212.76	
2	190.64		9	145.53	
3	52.47#		10	38.55	α: 2.22 (dd, 1 H)
4	37.77	α: 2.33 (ddm, 1 H)			β: 2.09 (dd, 1 H)
		β: 1.40 (m, 1 H)	11	49.56	
5	23.85	α: 1.50 (m, 1 H)	12	27.75	1.18 (s, 3 H)
		β: 1.20 (m, 1 H)	13	22.91	1.07 (s, 3 H)
6	39.73	α: 1.95 (ddm, 1 H)	14	20.19*	1,25 (s, 3 H)
		β: 1.35 (m, 1 H)	15	19.65*	1.06 (s, 3 H)
7	64.12#				

[a] *, # Assignments may be interchanged. − *J* [Hz]: 1-10α = 2.2; 1-10β = 2.2; 4α-4β = 12.3; 6α-6β = 13.2; 10α-10β = 15.6.

Compound **10** is the second reported member of the ceratopicane group of triquinanes[10]. The prototype (+)-ceratopicanol (**11**) has been isolated previously from cultures of

the ascomycete *Ceratocystis piceae*[11]. Its absolute configuration was proven by a total synthesis[12]. The common occurrence of sesquiterpenoids of the hirsutane, cucumane, and ceratopicane groups in cultures of *Macrocystidia cucumis* is remarkable and points to a close biosynthetic relationship.

Identification of Other Compounds

Two further compounds were identified as the known fungal metabolites *cyclo*(L-phenylalanyl-L-prolyl) (**12**) and *cyclo*(L-leucyl-L-prolyl) (**13**) by their NMR and MS data[13].

Biological Activity

The antimicrobial activities of cucumins A (**2**), B (**4**), and C (**5**) are summarized in Table 5. All three compounds are highly cytotoxic with IC_{100} = 0.5−1 mg/ml for L1210 cells.

Table 5. Antimicrobial activities of cucumins A (**2**), B (**4**), and C (**5**) in the serial dilution assay (MIC: minimal inhibitory concentration)

Test organism	MIC [μg/ml]		
	2	**4**	**5**
Bacteria:			
Acinetobacter calcoaceticus	> 50	> 50	> 50
Escherichia coli	> 50	> 50	> 50
Bacillus brevis	1−10	≥ 20	> 50
Bacillus subtilis	20−50	20−50	> 50
Corynebacterium insiduosum	> 50	> 50	> 50
Mycobacterium phlei	> 50	20−50	> 50
Fungi:			
Nadsonia fulvescens	> 50	> 50	> 50
Nematospora coryli	1−10	20−50	1−10
Saccharomyces cerevisiae α S288c	> 50	> 50	> 50
S. cerevisiae is1	> 50	> 50	1−10
Paecilomyces variotii	> 50	> 50	> 50
Fusarium oxysporum	> 50	> 50	> 50
Penicillium notatum	10−20	10−20	> 50
Mucor miehei	1−10	> 50	> 50
Rhodotorula glutinis	> 50	> 50	10−50

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Experimental Section

General: Melting points: Reichert Thermovar hot-stage, uncorrected values. − Optical rotations: Perkin-Elmer 241. − IR spectra: Perkin-Elmer 1420 Ratio Recording Infrared Spectrometer (KBr or in $CHCl_3$ between NaCl plates). − UV/Vis and CD spectra: Jobin-Yvon Instruments S.A. CD-6 Dichrograph. − NMR: Bruker WM 400, Bruker ARX 300, Bruker AMX 600; chemical shifts δ in ppm are referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.24, δ_C = 77.0; [D_4]methanol: δ_H = 3.35, δ_C = 49.0). − MS: A.E.I. MS 50 instrument, Finnigan MAT 90, 95 Q (direct inlet, 70 eV). − TLC: Silica gel 60 F_{254} (0.25 mm) on aluminium foil (Merck). The R_f values were determined with toluene/acetone/acetic acid (70:30:1) as eluant. − Column chromatography: Silica gel 60 (40−63 μm). − HPLC separations: Waters-Millipore with gradient controller M680, two M 590 EF pumps and U 6K injector equipped with a Knauer variable-wavelength monitor with a super-preparative flow cell. A Nucleosil 100 C18 (7 mm) prepacked HPLC column 250 × 20 mm with a precolumn 30 × 20 mm (Macherey & Nagel) was used. − Fermentor: Biostat U equipped with an MFCS system. − *Macrocystidia cucumis* showed the characteristics of the genus and species. A herbarium specimen and mycelial cultures are deposited in the culture collection of the LB Biotechnologie, Kaiserslautern. − Bioactivity studies: L1210 cells (mouse lymphocytic leukaemia ATCC CCL 219) were grown in F 12 medium (Gibco) containing 20% horse serum, 20 mM HEPES buffer, 100 μg/ml streptomycin sulfate, and 65 μg/ml penicillin G/ml. Incubation was carried out at 37°C in a humidified atmosphere containing 5% CO_2. Cell growth and lysis were observed by means of a microscope at 24-h intervals for 3 d. For the plate diffusion assays, fungi were grown in YMG medium containing (g/l): malt extract, 10; glucose, 4; yeast extract, 4; agar 20. *Paecilomyces varioti* and *Mucor miehei* were grown at 37°C; *Penicillium notatum* and *Nematospora coryli* bacteria were grown in nutrient broth (Difco) containing 2% agar at 37°C.

Fermentation and Isolation (Figure 1): *Macrocystidia cucumis* strain 84092 was isolated from tissue plugs of a fruiting body collected in the vicinity of Kaiserslautern. For maintenance on agar slants, the fungus was grown on YMG medium (yeast extract 0.4%, malt extract 1.0%, glucose 0.4%, pH = 5.5). Fermentations were carried out in 100 l of YMG medium with stirring (150 rpm) and aeration (15 l air/min.) at 22°C. When the antifungal activity in the culture fluid had reached its maximum, the mycelia were separated from the culture fluid (90 l) by filtration and discarded. The culture fluid was adsorbed onto HP 21 resin (Mitsubishi, 10 × 20 cm) and the antibiotics were eluted with 4 l of methanol, then with 4 l of acetone. The acetone fraction contained the active compounds. After evaporation of the solvent, the crude oily product was subjected to flash chromatography on silica gel with petroleum ether/ethyl acetate gradients. The fraction eluted with petroleum ether/ethyl acetate (2:1) contained compounds **2** (60 mg), **4** (7 mg), **5** (6 mg), **7** (20 mg) and **10** (4 mg), which were purified by HPLC on RP-18 with water/acetonitrile (6:4). **1**, **6**, **8** and **9** were eluted from the column with petroleum ether/ethyl acetate (1:1). Arthrosporone (**1**) (80 mg) was recrystallized from petroleum ether to give colourless needles. **6** (4 mg), **8** (6 mg), and **9** (6 mg) were further purified by HPLC on RP-18 material with water/acetonitrile gradients.

Arthrosporone (**1**): Colourless needles. − R_f = 0.35. − M.p. 135°C (petroleum ether). − $[\alpha]_D^{18}$ = −112.1 (*c* = 1.14, $CHCl_3$). − UV/Vis (CH_3CN): λ_{max} (lg ε) = 232 nm (1.45), 283 (1.03). − CD (CH_3CN): λ_{max} (Δε) = 194 nm (−0.25), 208 (+0.27), 298 (−3.61), 340 (+0.08). − IR (KBr): $\tilde{\nu}$ = 3421 cm^{-1} (vst), 2954 (st), 2867 (m), 1730 (vst), 1459 (m), 1383 (m), 1192 (m), 1017 (m). − ^1H NMR (300 MHz, $CDCl_3$): δ = 2.65 (dd, *J* = 18.1, 0.9 Hz, 1 H, 6β-H), 2.51 (q, *J* = 7.0 Hz, 1 H, 4-H), 2.49 (dd, *J* = 11.8, 8.6 Hz, 1 H, 2-H), 2.36 (d, *J* = 15.4 Hz, 1 H, 8α-H), 2.17 (d, *J* = 18.1 Hz, 1 H, 6α-H), 2.17 (d, *J* = 15.4 Hz, 1 H, 8β-H), 1.92 (d, *J* = 13.7 Hz, 1 H, 10α-H), 1.76 (dd, *J* = 13.7, 2.7 Hz, 1 H, 10β-H), 1.65 (dd, *J* = 11.8, 11.8 Hz, 1 H, 1α-H), 1.53 (ddd, *J* = 11.8, 8.6, 2.7 Hz, 1 H, 1β-H), 1.11 (s, 3 H, 12-H), 1.04 (s, 3 H, 13-H), 1.00 (d, *J* = 7.0 Hz, 3 H, 15-H), 0.81 (s, 3 H, 14-H). − ^{13}C NMR (75 MHz, $CDCl_3$) δ = 216.76 (C-5), 90.81 (C-9), 86.80 (C-7), 60.41 (C-2), 58.66 (C-10), 56.72 (C-8), 55.36 (C-4), 54.43 (C-3), 49.75 (C-6), 44.55 (C-1), 40.02 (C-11), 29.48 (C-13), 26.73 (C-12), 11.19 (C-14), 8.16 (C-15). − MS (DE, 70°C); *m/z* (%): 252 (25), 234 (34), 219 (16), 206 (12), 193 (16), 192 (99), 191 (42), 177 (27), 164 (27), 150 (17), 135 (23), 125 (100), 110 (44), 95 (38), 83 (45), 69 (28), 55 (29). − $C_{15}H_{24}O_3$: calcd. 252.1725; found 252.1722.

Crystallographic Data of **1**: Arthrosporone (**1**) was recrystallized from petroleum ether. Crystallographic data: $C_{15}H_{24}O_3$, M_r = 252.24, space group $P2_1$, monoclinic with *a* = 6.3135(3), *b* =

22.5889(12), c = 10.1894(8) Å, β = 93.916(5), V = 1449.8(2) Å^3, Z = 4, d_c = 1.156 g/cm^3; Mo-K_α radiation (20°C); reflections collected 4256, unique reflections 4014, observed reflections 3380 [$I > 2\ \sigma(I)$], $R1$ index 0.0535 (all data). The X-ray diffraction analysis was carried out on an Enraf-Nonius CAD4 diffractometer at room temperature [293(2) K] using Mo-K_α (λ = 0.71073 Å) radiation. Programs used were SHELXS-86[14] for structure solution and SHELXL-93[15] for refinement, and ZORTEP for producing the drawing[16]. Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data (supplementary publication no. CCDC-100647) can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44 (0)1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

Cucumin A (**2**): Yellow oil. − R_f = 0.73. − $[\alpha]_D^{18}$ = −101 (c = 0.87, $CHCl_3$). − UV/Vis (CH_3OH): λ_{max} (lg ε) = 234 nm (3.75), 322 (3.65). − CD (CH_3OH): λ_{max} (θ) = 252 (−20260), 299 (−1350), 331 (+4050), 371 (−8510). − IR (KBr): $\tilde{\nu}$ = 3390 cm^{-1} (br., m), 2940 (st), 2910 (m), 1700 (vst), 1695 (vst, sh), 1690 (vst), 1650 (st), 1640 (st), 1590 (st), 1450 (m), 1445 (m), 1375 (m), 1155 (m), 1115 (st). − ^{1}H and ^{13}C NMR: See Table 1. − INADEQUATE experiment (90 mg **2** in $CDCl_3$; 100 MHz): $^1J_{cc}$: C-1/C-2, C-2/C-3, C-3/C-4, C-5/C-6, C-7/C-8, C-11/C-12, C-11/C-13; $^2J_{CC}$: C-4/C-6. − MS (DE, 180°C); *m/z* (%): 228 (100), 213 (15), 200 (11), 185 (26), 158 (29), 157 (24), 145 (11), 144 (32), 142 (18), 141 (10), 130 (12), 129 (21), 128 (11), 116 (17), 115 (39), 91 (15), 77 (13), 51 (11). − $C_{15}H_{16}O_2$: calcd. 228.1150; found 228.1154.

Cucumin B (**4**): Yellow oil. − R_f = 0.69. − $[\alpha]_D^{18}$ = −136 (c = 1.47, $CHCl_3$). − UV/Vis (CH_3OH): λ_{max} (lg ε) = 226 nm (3.31), 312 (3.26). − CD (CH_3OH): λ_{max} (θ) = < 230 (> +8800), 261 (−960), 274 (−390), 391 (−4820), 345 (−2700), 364 (−2120), 386 (−1410), 395 (−1160). − IR (KBr): $\tilde{\nu}$ = 1730 cm^{-1} (m), 1700 (st), 1685 (st), 1600 (m). − ^{1}H and ^{13}C NMR: See Table 2. − MS (DE, 180°C); *m/z* (%): 231 (12), 230 (100), 215 (19), 203 (12), 202 (12), 187 (65), 175 (10), 159 (39), 146 (28), 145 (30), 132 (24), 117 (24), 91 (31), 77 (19). − $C_{15}H_{18}O_2$: calcd. 230.1307; found 230.1308.

Cucumin C (**5**): Yellow oil. − R_f = 0.70. − $[\alpha]_D^{18}$ = −59 (c = 0.74, $CHCl_3$). − UV/Vis (CH_3OH): λ_{max} (lg ε) = 220 nm (3.51), 301 (3.67). − CD (CH_3OH): λ_{max} (θ) = < 230 (> +16500), 258 (−1290), 303 (+12470), 358 (−8810). − IR (KBr): $\tilde{\nu}$ = 1710 cm^{-1} (st), 1695 (vst), 1600 (st), 1580 (m), 1370 (m), 1170 (m), 1065 (m). − ^{1}H and ^{13}C NMR: See Table 2. − MS (DE, 180°C); *m/z* (%): 244 (13), 229 (12), 217 (5), 201 (8), 189 (5), 188 (21), 173 (7), 162 (9), 159 (10), 146 (9), 91 (15), 83 (100), 77 (12), 58 (12), 55 (15), 43 (90). − $C_{15}H_{16}O_3$: calcd. 244.1100; found 244.1096.

Cucumin D (**6**): Colourless oil. − R_f = 0.44. − $[\alpha]_D^{25}$ = +188.73 (c = 0.07, $CHCl_3$). − UV/Vis (CH_3CN): λ_{max} (lg ε) = 224 nm (3.21). − CD (CH_3CN): λ_{max} (Δε) = 196 nm (−20.79), 223 (+27.75), 246 (−20.25), 326 (+4.50). − IR (film): $\tilde{\nu}$ = 3460 cm^{-1} (m, br.), 2960 (m), 1690 (vst), 1630 (st), 1460 (m), 1210 (m), 1085 (m). − ^{1}H and ^{13}C NMR: See Table 2. − MS (DE, 120°C); *m/z* (%): 247 (16), 246 (76), 232 (16), 231 (100), 218 (37), 213 (29), 203 (53), 185 (12), 175 (24), 173 (21), 161 (71), 159 (42), 146 (25). − $C_{15}H_{18}O_3$: calcd. 246.1256; found 246.1234.

Cucumin E (**7**): Colourless solid. − R_f = 0.75. − M.p. 108°C. − $[\alpha]_D^{18}$ = +124.4 (c = 0.09, $CHCl_3$). − UV/Vis (CH_3OH): λ_{max} (lg ε) = 232 nm (3.00), 252 (3.11), 301 (1.91). − CD (CH_3CN): λ_{max} (Δε) = 213 nm (+28.24), 251 (−46.47), 311 (+18.24). − IR (KBr): $\tilde{\nu}$ = 3439 cm^{-1} (br., m), 2957 (m), 2933 (m), 1745 (st), 1704 (vst), 1664 (st), 1076 (m). − ^{1}H and ^{13}C NMR: See Table 3. − MS (DE, 60°C); *m/z* (%): = 232 (17), 205 (11), 204 (100), 189 (31), 161 (16), 148 (11), 133 (10), 119 (10), 105 (16), 91 (14), 85 (12), 83 (20), 79 (11), 77 (10). − $C_{15}H_{20}O_2$: calcd. 232.1463; found 232.1455.

Cucumin F (**8**): Colourless amorphous solid. − R_f = 0.38. − M.p. 120°C. − $[\alpha]_D^{33}$ = −48.5 (c = 0.2, $CHCl_3$). − UV/Vis (CH_3CN): λ_{max} (lg ε) = 240 nm (2.85). − CD (CH_3CN): λ_{max} (Δε) = 204 nm (+8.75), 243 (−14.57), 314 (+2.34). − IR (film): $\tilde{\nu}$ = 3440 cm^{-1} (br., m), 2940 (st), 2860 (m), 1690 (vst), 1650 (vst), 1030 (m). − ^{1}H and ^{13}C NMR: See Table 3. − MS (DE 40°C); *m/z* (%): 234 (38), 216 (6), 206 (58), 191 (100). − $C_{15}H_{22}O_2$: calcd. 234.1620; found 234.1618.

(S)-MTPA Ester of Cucumin F (**8a**): To a solution of **7** (1.2 mg) in CH_2Cl_2 (1.5 ml) were added NEt_3 (20 µl), DMAP and (*R*)-(−)-MTPA-Cl (10 µl) at 0°C. After stirring at room temperature for 2 d, all volatile components were evaporated in vacuo. The residue was chromatographed on silica gel. **8a** was eluted with petroleum ether/ethyl acetate (5:1) (2 mg). Colourless oil. − $[\alpha]_D^{25}$ = −25.7 (c = 0.12, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.51 (m, 2 H), 7.40 (m, 3 H), 4.94 (d, J = 9.0 Hz, 1 H, 8-H), 3.52 (s, 3 H, OCH_3), 3.40 (m, 2 H, 2-H and 9-H), 2.42 (d, J = 17.2 Hz, 1 H, 6α-H), 2.31 (d, J = 17.2 Hz, 1 H, 6β-H), 1.87 (m, 1 H, 1α-H), 1.70 (dd, J = 13.3, 10.0 Hz, 1 H, 10β-H), 1.68 (s, 3 H, 15-H), 1.57 (dd, J = 12.7, 9.7 Hz, 1 H, 1β-H), 1.39 (m, 1 H, 10α-H), 1.23 (s, 3 H, 14-H), 1.08 (s, 3 H, 12-H), 0.98 (s, 3 H, 13-H). − MS (DE 80°C); *m/z* (%): 450 (14), 233 (10), 217 (28), 216 (20), 215 (15), 190 (11), 189 (100). − $C_{25}H_{29}F_3O_4$: calcd. 450.2018; found 450.2005.

(R)-MTPA Ester of Cucumin F (**8b**): **8b** (4mg) was prepared from **8** (4 mg) with (*S*)-(+)-MTPA-Cl (20 µl) analogously to **8a**. Colourless oil. − $[\alpha]_D^{25}$ = +17.4 (c = 0.26, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.52 (m, 2 H), 7.40 (m, 3 H), 5.01 (d, J = 9.4 Hz, 1 H, 8-H), 3.55 (s, 3 H, OCH_3), 3.40 (ddd, J = 9.4, 9.4, 9.4 Hz, 1 H, 2-H), 3.32 (dddd, J = 9.4, 9.4, 9.4, 9.4 Hz, 1 H, 9-H) 2.50 (d, J = 17.4 Hz, 1 H, 6α-H), 2.24 (d, J = 17.4 Hz, 1 H, 6β-H), 1.87 (m, 1 H, 1α-H), 1.81 (dd, J = 12.8, 9.4 Hz, 1 H, 10β-H), 1.67 (s, 3 H, 15-H), 1.56 (dd, J = 12.4, 9.4 Hz, 1 H, 1β-H), 1.48 (m, 1 H, 10α-H), 1.12 (s, 3 H, 14-H), 1.10 (s, 3 H, 12-H), 0.99 (s, 3 H, 13-H). − MS (DE 80°C); *m/z* (%): 450 (23), 233 (10), 217 (43), 216 (26), 215 (15), 190 (10), 189 (100). − $C_{25}H_{29}F_3O_4$: calcd. 450.2018; found 450.2012.

Cucumin G (**9**): Colourless oil. − R_f = 0.50. − $[\alpha]_D^{33}$ = +227.3 (c = 0.3, $CHCl_3$). − UV/Vis (CH_3CN): λ_{max} (lg ε) = 212 nm (2.72), 3.13 (2.18). − CD (CH_3CN): λ_{max} (Δε) = 204 nm (−13.20), 314 (+6.25). − IR (film): $\tilde{\nu}$ = 3420 cm^{-1} (br., m), 2950 (st), 2860 (m), 1760 (vst), 1685 (w), 1440 (m), 1365 (m), 1070 (m), 1050 (m), 1025 (m). − ^{1}H and ^{13}C NMR: See Table 3. − MS (DE 40°C); *m/z* (%): 235 (13), 234 (75), 206 (49), 205 (32), 192 (21), 191 (100), 177 (15), 173 (14), 150 (10), 149 (18), 136 (15), 135 (28), 121 (15), 110 (23), 109 (52), 95 (14). − $C_{15}H_{22}O_2$: calcd. 234.1620; found 234.1629.

Cucumin H (**10**): Colourless amorphous solid. − R_f = 0.65. − m.p. 120−122°C. − $[\alpha]_D^{18}$ = −25 (c = 1.03, $CHCl_3$). − UV/Vis (CH_3CN): λ_{max} (lg ε) = 242 nm (2.77), 325 (2.37). − CD (CH_3CN): λ_{max} (Δε) = 218 nm (+7.14), 247 (−5.10), 338 (+0.30). − IR (KBr): $\tilde{\nu}$ = 3400 cm^{-1} (st, br.), 2965 (st), 2940 (st), 1680 (vst), 1645 (st), 1470 (m), 1445 (m), 1385 (m), 1375 (m), 1365 (m), 1250 (m), 1240 (m), 1210 (m), 1120 (m), 1085 (m), 1065 (m). − ^{1}H and ^{13}C NMR: See Table 4. − MS (DE, 70°C); *m/z* (%): 235 (66), 234 (100), 219 (17), 201 (19), 191 (22). − $C_{15}H_{22}O_2$: calcd. 234.1620; found 234.1663.

cyclo(L-Phenylalanyl-L-prolyl) (**12**): Colourless amorphous solid. − $[\alpha]_D^{30}$ = −78.5 (c = 0.25, $CHCl_3$). − IR (KBr): $\tilde{\nu}$ = 3436 cm^{-1} (st), 2925 (w), 1653 (m), 1455 (w). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32−7.15 (m, 5 H), 5.62 (s, br., 1 H), 4.25 (dd, J =

10.2, 2.8 Hz, 1 H), 4.06 (dd, J = 7.3, 7.3 Hz, 1 H), 3.6−3.5 (m, 3 H), 2.76 (dd, J = 14.4, 10.6 Hz, 1 H), 2.25 (m, 1 H), 2.0−1.8 (m, 3 H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.37, 165.06, 135.92, 129.28, 129.09, 127.56, 59.13, 56.17, 45.45, 36.78, 28.34, 22.54. − MS (DE, 140°C); *m/z* (%): 244 (100), 153 (56), 125 (81), 120 (13), 91 (32), 85 (11), 83 (17), 70 (31). − $C_{14}H_{16}N_2O_2$: calcd. 244.1212; found 244.1220. − FAB-MS (+ve; *m*-NBA); *m/z* (%): 245 (12.5) $[M + H]^+$.

cyclo(L-Leucyl-L-prolyl) (**13**): Colourless amorphous solid. − $[\alpha]_D^{30}$ = −125.4 (c = 0.35, $CHCl_3$). − IR (KBr): $\tilde{\nu}$ = 3536 cm^{-1} (st), 2957 (w), 2936 (w), 1654 (m), 1437 (w). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.67 (s, br., 1 H), 4.10 (dd, J = 8.1, 8.1 Hz, 1 H), 4.00 (dd, J = 9.5, 3.9 Hz, 1 H), 3.55 (m, 2 H), 2.34 (m, 1 H), 2.2−1.8 (m, 4 H), 1.7 (m, 1 H), 1.5 (m, 1 H), 0.99 (d, J = 6.7 Hz, 3 H), 0.94 (d, J = 6.5 Hz, 3 H). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.09, 166.13, 58.98, 53.38, 45.51, 38.63, 28.11, 24.73, 23.28, 22.74, 21.18. − MS (DE 100°C); *m/z* (%): 210 (0.3), 154 (100), 86 (13), 70 (35). − $C_{11}H_{18}N_2O_2$: calcd. 210.1368; found 210.1366. − FAB-MS (+ve; *m*-NBA); *m/z* (%): 211 (11) $[M + H]^+$.

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