Communesins G and H, New Alkaloids from the Psychrotolerant Fungus *Penicillium rivulum*

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Communesins G (**1**) and H (**2**), two new alkaloids, have been isolated from the psychrotolerant, new species *Penicillium rivulum* Frisvad. The compounds were isolated by high-speed countercurrent chromatography and preparative HPLC using UV-guided fractionation. The structures were determined from 1D and 2D NMR techniques and HRESIMS. In contrast to all other known communesins, **1** and **2** were found inactive in antimicrobial, antiviral, and anticancer assays.

The genus *Penicillium*, despite the large numbers of natural products identified, still proves to be a rich source of new compounds with high chemical diversity.[1] Especially extreme habitats such as cold and arctic environments that are still relatively unexplored harbor new species with new compounds, e.g., the psychrophilins and the cycloaspeptides.[2−4] We are currently engaged in the exploration of fungi from such habitats for chemotaxonomic markers and bioactive compounds. Using established de-replication procedures,[5] two new alkaloids, communesins G (**1**) and H (**2**), were detected and isolated from the psychrotolerant and new species *Penicillium rivulum* Frisvad. Their structures were elucidated by 1D and 2D NMR and HRESIMS. Furthermore, they were subjected to antiviral, antimicrobial, and anticancer activity tests.

An ethyl acetate extract of *P. rivulum* (IBT 24420) was fractionated by high-speed countercurrent chromatography (HSCCC) and further purified by preparative HPLC to afford communesins G (**1**) and H (**2**). Communesin G (**1**) was isolated as a white amorphous powder. The HRESIMS showed a pseudomolecular ion at *m/z* 471.2760 corresponding to the molecular formula $C_{29}H_{34}N_4O_2$ (15 degrees of unsaturations). In addition to the presence of a 1,2-disubstituted and a 1,2,3-trisubstituted benzene ring, which were deduced from examination of the ^{1}H NMR, ^{13}C NMR, and COSY spectra, an ethyl group, two $-CH_2-CH_2-$, a $-CH-CH-$ spin system, two singlet methines, an *N*-methyl group, an epoxide, and a geminal dimethyl system appeared in the NMR spectra. A combination of the CIGAR, HSQC, and 1D NMR studies allowed the assignment of its structure. Abundant information supporting this assignment was obtained from long-range ^{1}H−^{13}C correlations from the H-6, H-9, and H-11 methine resonances (δ 4.69, 5.05, and 4.10, respectively). These connectivities allowed the elucidation of the "west" and "east" regions of the molecule (Figure 1, only key CIGAR correlations are shown; see Table 1 for a full listing of ^{1}H−^{13}C correlations). From H-9 (δ 5.05) key correlations were observed to C-1″, C-18, C-8a, C-7, and C-20, which, when combined with chemical shift data and other ^{1}H−^{13}C correlations (see Table 1), defined the west region. Similarly, from H-11 (δ 4.10) key correlations were observed to C-22, C-12a, and C-7a, thus defining the east region. A number of $^3J_{C-H}$ correlations then served to link the west and east region. For example, $^3J_{C-H}$ correlations from H-11 to C-9, H-9 to C-11, and H-11 to C-20 connected the west and east regions, while correlations from H-6 to C-7, C-7a, C-8, C-14a, and C-4a unambiguously connected the rest of the molecule with the west and the east regions.

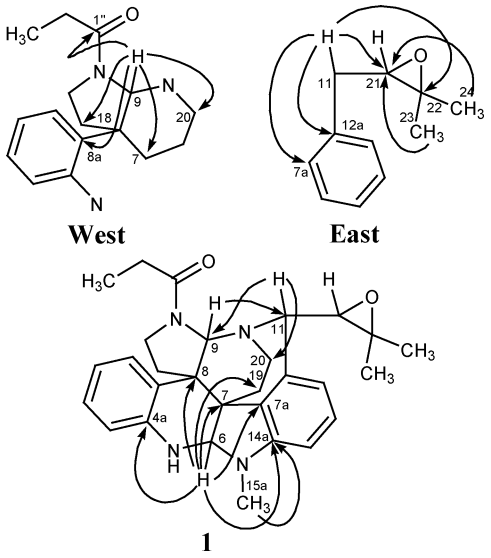

Figure 1. Selected CIGAR connectivities in the west and east regions and communesin G (**1**).

Communesin H (**2**) was isolated as a white amorphous solid. The HRESIMS revealed the $[M + H]^+$ ion at *m/z* 485.2910, indicating the molecular formula $C_{30}H_{36}N_4O_2$; this was substantiated by 30 signals in the ^{13}C NMR spectrum. The molecular formula requires 15 degrees of unsaturation. This information, in conjunction with the difference of 14 amu in the molecular weights of the two compounds, suggests that communesin H (**2**) has one additional methyl group. The similarity of the spectra of communesins G (**1**) and H (**2**) supported this theory. The COSY, NOESY, and CIGAR spectra indicated identical substitution patterns in the ring systems. In addition to these assignments, a TOCSY experiment (1D) highlighted the difference between **1** and **2**. An irradiation of the triplet methyl signal at δ 1.22 in communesin G (**1**) revealed a

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Table 1. NMR Data for Communesins G (**1**) and H (**2**) (500 MHz (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$)[a]

	communesin G (**1**)				communesin H (**2**)			
atom no.	δ_C	δ_H	mult., *J* (Hz)	CIGAR (δ_H to δ_C)	δ_C	δ_H	mult., *J* (Hz)	CIGAR (δ_H to δ_C)
1	123.3	6.66	m[b]	C-3, C-4a, C-8	123.2	6.66	m[b]	C-3, C-4a, C-8
2	117.0	6.68	m[b]	C-4, C-8a	116.9	6.68	m[b]	C-4, C-8a
3	127.4	6.99	td 8, 1.5	C-1, C-4a	127.3	6.99	td 7.5, 1.5	C-1, C-4a
4	120.6	6.69	m[b]	C-2, C-8a	120.5	6.70	m[b]	C-2, C-8a
4a	142.6				142.6			
6	82.5	4.69	S	C-4a, C-7a, C-8, C-14, C-14a, C-15a, C-19	82.4	4.69	s	C-4a, C-7a, C-8, C-14, C-14a, C-15a, C-19
7	51.7				51.9			
7a	132.4				132.3			
8	51.4				51.4			
8a	132.5				132.4			
9	78.9	5.05	S	C-1″, C-7, C-8a, C-11, C-17, C-18, C-20	78.9	5.04	s	C-1″, C-7, C-8a, C-11, C-17, C-18, C-20
11	65.1	4.10	d 9.5	C-7a, C-9, C-12, C-12a, C-20, C-21, C-22	65.2	4.10	d 9.0	C-7a, C-9, C-12, C-12a, C-20, C-21, C-22
12	113.2	6.06	d 7.5	C-7, C-7a, C-11, C-14, C-14a	113.2	6.07	d 7.7	C-7, C-7a, C-11, C-12a, C-14, C-14a
12a	136.7				136.9			
13	128.8	6.87	t 7.5	C-7a, C-12, C-12a, C-14, C-14a	128.8	6.89	t 7.7	C-7a, C-12, C-12a, C-14, C-14a
14	101.7	5.95	d 7.5	C-7a, C-12, C-12a	101.7	5.95	d 7.7	C-7a, C-12, C-12a
14a	150.5				150.5			
15a	29.6	2.82	S	C-6, C-14a	29.6	2.84	s	C-6, C-14a
17A	44.1	3.01	ddd 12.0, 11.7, 6.5	C-18	44.0	3.00	ddd 11.8, 11.5, 7.4	C-18
17B		3.88	dd 12.0, 8.8	C-8, C-9, C-18		3.88	dd 11.8, 8.6	C-8, C-9, C-18
18A	30.5	1.95	dd 13.0, 6.5	C-7, C-8a, C-9	30.8	1.96	dd 13.2, 7.4	C-7, C-8a, C-9
18B		2.73	ddd 13.0, 11.7, 8.8	C-7, C-17, C-8a		2.72	ddd 13.2, 11.5, 8.6	C-7, C-8a, C-17
19A	38.0	2.27	m[b]	C-7, C-7a, C-8, C-20	38.1	2.27	m[b]	C-6, C-7, C-7a, C-8, C-20
19B		2.35	m[b]	C-6, C-7a, C-8, C-20		2.35	m[b]	C-6, C-7a, C-8,
20A	36.3	3.35	M	C-9, C-11, C-19	36.3	3.36	m	C-9, C-11, C-19
20B		3.44	M	C-9		3.44	m	
21	64.0	2.85	m[b]	C-11, C-12a, C-22, C-23	63.9	2.86	m[b]	C11, C-12a, C-22, C-23
22	59.7				59.6			
23	24.6	1.37	s	C-21, C-22, C-24	24.8	1.39	s	C-21, C-22, C-24
24	20.4	1.54	s	C-21, C-22, C-23	20.4	1.54	s	C-21, C-22, C-23
1″	175.3				174.5			
2″A	27.6	2.42	m[b]	C-1″, C-3″	36.6	2.35	m[b]	C-1″, C-3″, C-4″
2″B		2.88	m[b]	C-1″, C-3″		2.84	m[b]	C-1″, C-3″, C-4″
3″	9.22	1.22	t 7.5	C-1″, C-2″	18.4	1.75	m[b]	C-1″, C-2″, C-4″
4″					14.2	1.00	t 7.4	C-2″, C-3″

[a] Reference: $CDCl_3$ ^{1}H 7.25 ppm, ^{13}C 77.01 ppm. [b] Signals overlapping.

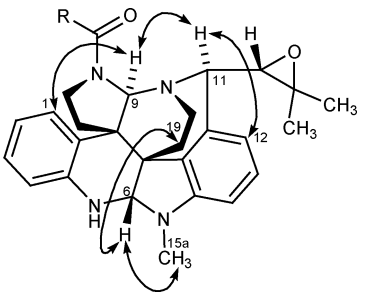


Figure 2. Important NOE connectivities found in communesins G (**1**) and H (**2**).

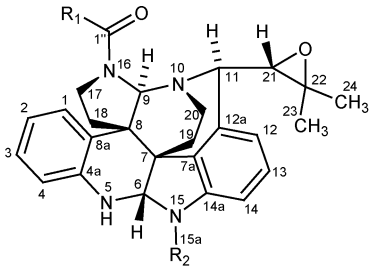
CH_3-CH_2-X spin system, and in the case of communesin H (**2**) a $CH_3-CH_2-CH_2-X$ spin system.

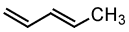
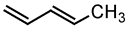
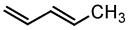
The relative stereochemistry of **1** and **2** was established from NOE experiments. Connectivities are shown in Figure 2. Cross-peaks were observed from H-1 to H-9; H-6 to H-19A and H-19B; H-9 to H-11; and H-12 to H-11. This confirms the $-CH_2-CH_2-$ bridges to be on the same side of the molecular plane.

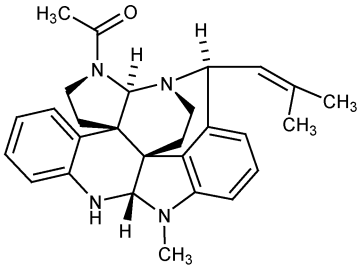
Murata's *J*-based method[6] was used to determine the relative stereochemistry at C-21. The protons H-21 and H-11 exhibited a coupling constant of approximately 9 Hz, leaving two options of *anti* arrangement. The NOE cross-peaks between the geminal methyl groups and H-12 established the epoxide oxygen is oriented *syn* to N-10.

The communesins were originally believed to originate from an isolate of *Penicillium commune* (hence the name communesin). Such isolates are now referred to as *P. marinum*.[7] However, we have never been able to detect these communesins in any *P. commune* strain.[1,8] The communesins are consistently produced by the common species *P. expansum* and also by the less common marine species *P. marinum*.[1,7,8] *P. expansum* and *P. marinum* consistently produce both communesins A and B,[1,8] in contrast to *P. rivulum*, which does not produce communesin B.

Several biological activities have been reported for the communesins.[9−12] Spurred by these findings, we tested communesins G (**1**) and H (**2**) in antimicrobial, antiviral, and anticancer assays. In the antimicrobial assay three bacteria (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*) and three fungi (*Candida albicans*, *Trichophyton mentagrophytes*, *Amorphotheca resinae*) were used. The paper disk assay (30 μL of a 1 mg/mL solution of **1** and **2**) did not show any inhibition zones. In the antiviral assay communesins G (**1**) and H (**2**) were tested against *Herpes simplex* type 1 virus (ATCC VR 733) and *Polio* virus type 1 (Pfizer vaccine strain) in infected African green monkey kidney cells (BSC-1). There was no inhibition of the viruses or alteration of the host cells. Communesins G (**1**) and H (**2**) were tested in a P388 murine leukemia cell assay and showed an ID_{50} value higher than 12.5 μg/mL and were therefore considered inactive.



$R_1 = CH_3$	$R_2 = CH_3$	communesin A
$R_1 =$ 	$R_2 = CH_3$	communesin B / nomofungin
$R_1 =$ 	$R_2 = H$	communesin C
$R_1 =$ 	$R_2 = CHO$	communesin D
$R_1 = CH_3$	$R_2 = H$	communesin E
$R_1 =$ 	$R_2 = CH_3$	communesin G (**1**)
$R_1 =$ 	$R_2 = CH_3$	communesin H (**2**)



communesin F

Communesin B/nomofungin[9−13] can still be considered the most active communesin. It is the only communesin that has been isolated in all the previous papers[9−12] and has been tested in various combinations with the other communesins for cytotoxic[9−11] and insecticidal[12] activity. Since the communesins only differ with respect to the epoxide group between C-21 and C-22, the group at 15a, and the acyl group at 2″, and communesin B has been found most active in 10 out of 11 assays,[9−12] it can be concluded that a methyl group at 15a, a 2,4-hexadienoyl at N-16, and an epoxide between C-21 and C-22 seem to be important features for activity. None of the previously isolated communesins have been tested in antibacterial, antifungal, or antiviral assays.[9−12] The communesins are hypothesized to be biosynthesized through the oxidative coupling of tryptamine with the ergot alkaloid aurantioclavine.[13]

Experimental Section

General Experimental Procedures. The circular dichroism (CD) spectrum was measured on a modified JASCO 710 spectropolarimeter. UV spectra were recorded on a Perkin-Elmer UV/vis lambda 2 spectrophotometer. Rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H and 2D NMR (^{1}H−^{1}H COSY, ^{1}H−^{1}H 1D NOESY, ^{1}H−^{1}H 1D TOCSY, ^{1}H−^{13}C HSQC-DEPT, ^{1}H−^{13}C CIGAR) spectra were recorded on a Varian INOVA 500 MHz NMR spectrometer. ^{13}C NMR was recorded on a Varian UNITY 300 MHz NMR spectrometer. HRESIMS analyses were performed using a Micromass TOF LCT mass spectrometer. Data were acquired and processed using the MassLynx program. Analytical HPLC data were obtained on an Agilent 1100 HPLC system using Chemstation software and a Phenomenex (Torrance, CA) Luna C_{18} II column (100 × 2 mm, 3 μm) and a Phenomenex Security-Guard C_{18} precolumn using a linear water−acetonitrile gradient starting with 85% water and 15% acetonitrile (MeCN), and going to 100% MeCN in 20 min, keeping 100% MeCN for 5 min at a flow of 0.4 mL/min at 40 °C. Both solvents contained 50 ppm TFA. HSCCC was carried out with a Model CCC-1000 high-speed counter-current chromatograph (Pharma-Tech Research, Baltimore, MD). The apparatus consisted of three coils, connected in series (total volume, ~850 mL). Solvents for extraction and chromatography were distilled prior to use. Culture media Czapek yeast autolysate (CYA).

Fungal Material and Fermentation. The isolate of *Penicillium rivulum* (IBT 24420) was obtained from the IBT Culture Collection at BioCentrum-DTU, Technical University of Denmark. Isolates were cultured on LECA[14] nuts covered by CYA (4 × 500 mL) at 20 °C for 19 days in the dark. A voucher specimen is located in the collection at the Danish Technical University as IBT 24420.

Extraction and Separations. All the mycelium and agar were extracted twice overnight with EtOAc. After filtration through a Whatman 1PS phase separation filter, the solvent was evaporated in vacuo, leaving the crude extract (2 g). A part of the crude extract was directly separated using a PharmaTech CCC-1000 HSCCC [*n*-heptane−EtOAc−MeOH−water (1:1:1:1), mobile phase: upper phase, tail to head, 850 mL coils, flow 5 mL/min] connected to a Waters pump and a Waters diode array detector. The crude extract (800 mg) was dissolved in mobile phase (*n*-heptane−EtOAc (1:1)) (30 mL). Twenty-four fractions (50 mL) were collected. Fractions 22−24 and fractions 17 and 18 were further purified by HPLC on a preparative Phenomenex Luna 5 μm C18(2) column (250 × 10.00 mm, 5 μm) (flow rate 5 mL/min). Fractions 22−24 was separated with MeCN−H_2O (1:1) to afford pure **1** (6.5 mg), and fractions 17 and 18 were separated with MeCN−H_2O (6:4) to afford pure **2** (4.5 mg).

Communesin G (1): white amorphous powder from MeCN−H_2O; mp 162−166 °C; $[\alpha]^{25}_D$ −157° (*c* 0.021, MeOH); CD λ_{ext} (*c* 0.0021, MeOH) ($\Delta\epsilon$) 214sh (−18.83), 230sh (6.23), 245 (19.8), 271 (−50.8), 294 (13.89), 319 (−2.21) nm; UV (MeOH) λ_{max} (log ϵ) 207.5 (4.59), 248 (3.92), 268 (3.94), 316 (3.39); ^{1}H and ^{13}C NMR data, see Table 1; CIGAR, see Table 1; HREISMS obsd $(M + H)^+$ at *m/z* 471.2747, calcd for $C_{29}H_{35}N_4O_2$ 471.2760.

Communesin H (2): white amorphous powder from MeCN−H_2O; mp 143−147 °C; $[\alpha]^{25}_D$ −167° (*c* 0.024, MeOH); CD λ_{ext} (*c* 0.0024, MeOH) ($\Delta\epsilon$) 214sh (−20.22), 230sh (7.25), 245 (21.9), 271 (−55.37), 294 (15.57), 318 (−2.11) nm; UV (MeOH) λ_{max} (log ϵ) 208 (4.68), 248 (4.00), 268 (4.02), 316 (3.48); ^{1}H and ^{13}C NMR data, see Table 1; CIGAR, see Table 1; HREISMS obsd $(M + H)^+$ at *m/z* 485.2910, calcd for $C_{30}H_{37}N_4O_2$ 485.2917.

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