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Quinocitrinines A and B, New Quinoline Alkaloids from *Penicillium citrinum* Thom 1910, a Permafrost Fungus

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In the course of our previous work we isolated alkaloids such as agroclavine-I and epoxy-agroclavine-I from *Penicillium citrinum* VKM FW-800 as a microorganism isolated from permafrost[1,2]. This fungus was shown to co-produce two other alkaloid compounds, which were extractable only under acidic conditions. Positive staining by Dragendorff's reagent but negative Ehrlich reaction suggested the occurrence of unusual alkaloid structures. Here we report isolation and structure elucidation of quinocitrinines A (**1**) and B (**2**) as new quinoline alkaloids from *Penicillium citrinum*.

Penicillium citrinum VKM FW-800 obtained from the permafrost region of Northern Russia was deposited in the All Russian Culture Collection of Microorganisms (VKM, Pushchino, Moscow region, Russia). Cultivation was carried out at 24°C on rotary shaker (220 r.p.m.) in Erlenmeyer flasks (750 ml) containing 150 ml medium. The medium was composed as follows (g/liter): mannitol 50, succinic acid 5.4, $MgSO_4 \cdot 7H_2O$ 0.3, KH_2PO_4 1.0 (pH adjusted to 5.2 by addition of 25% ammonia solution). Inoculation occurred with spore suspensions. After 11~13 days of cultivation the metabolites were extracted. The culture broth was adjusted to pH 3~4 by 2% tartaric acid and extracted three-times by chloroform. The residue of the dried and evaporated extract was subjected to column chromatography on silica gel 60 (Merck, 0.063~0.1 mm). Elution was done first by $CHCl_3$/MeOH/25% aqueous ammonia (90 : 10 : 0.1; v/v) and subsequently by the same solvent mixture but in ratio 80 : 20 : 0.2 (v/v).

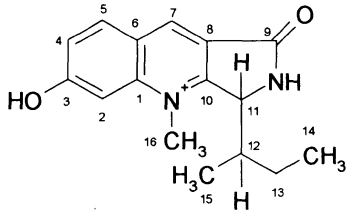
Thereby 15.2 mg of **1** and 40.1 mg of **2** were obtained as slightly brownish solids. The physico-chemical properties of **1** and **2** are shown in Table 1.

Elucidation of structure of **1** and **2** (Fig. 1) was carried out by optical spectroscopy (UV-VIS, IR, polarimetry), mass spectrometry (HREI-MS, ESI-MS) and 1D/2D NMR spectroscopy.

Quinocitrinines A (**1**) and B (**2**) differed by their chromatographic behaviour (Rf on TLC), melting points and optical rotations. However the UV-Vis spectra were identical but distinguishable from those of typical ergot alkaloids. Absorbances at 300~330 nm suggested the occurrence of a quinoline chromophore. **1** and **2** displayed different optical rotations (Table 1). The identical IR spectra of both alkaloids displayed absorbances (cm^{-1}) typical for carbonyl (1690 cm^{-1}), hydroxyl (3245 cm^{-1}) and double bonds (1605 cm^{-1}).

ESI-MS of **1** afforded *m/z* 293.3 ($[M+Na]^+$) and of **2** *m/z* 293.5 ($[M+Na]^+$). Additionally in both spectra *m/z* 563 ($[2M+Na]^+$) was visible. HREI-MS of **1** furnished *m/z* 270.1373 ($[M-H]^+$; calcd. 270.1379 for $C_{16}H_{18}N_2O_2$), and of **2** *m/z* 270.1374 ($[M-H]^+$; calcd. 270.1379 for $C_{16}H_{18}N_2O_2$). The appearance of $[M-H]^+$ ions in the HREI-MS of both compounds is in full accord with the

Fig. 1. Structure (relative stereochemistry tentative) of quinocitrinines A (**1**) and B (**2**).



1 11,12-trans-diastereoisomer

2 11,12-cis-diastereoisomer

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Table 1. Physico-chemical properties of quinocitrinine A (**1**) and quinocitrinine B (**2**).

	1	**2**
Appearance:	slightly brownish solid	slightly brownish solid
Mp (°C):	142-143	152-153
Molecular weight:	270	270
HREI-MS found:	270.1373	270.1374
calcd.:	270.1379	270.1379
Molecular formula:	$C_{16}H_{18}N_2O_2$	$C_{16}H_8N_2O_2$
UV-VIS MeOH, $\lambda_{max.}$(nm):		
ε (cm^2/mmol)	216(8108), 248(5285), 256(5430), 300 (2388), 300(2388), 314(2785), 328(2499)	216 (8108), 248 (5772), 256 (5831) 300 (2542), 314 (3074), 328 (1973)
IR film, $\nu_{max.}$ (cm^{-1}):	3263, 1689, 1604, 1545, 1523, 1462, 1420	3245, 1690,1605, 1545, 1524, 1462, 1420
$[\alpha]_D^{22}$ (c 0.32, MeOH):	+25.3°	--
$[\alpha]_D^{22}$ (c 0.62, MeOH):	--	-13.2°
R_f on TLC (silical gel aluminium sheets, Merck)		
Solvent 1: $CHCl_3$-MeOH-conc. NH_4OH: (80:20:0.2):	0.68	0.62
Solvent 2: $CHCl_3$-MeOH-conc. NH_4OH (90:10:0.1):	0.49	0.45
Solvent 3: $CHCl_3$-MeOH-conc. NH_4OH (90:10:1)	0.14	0.12

occurrence of quaternary ammonium structures. The chemical formulas of **1** and **2** suggested the presence of nine double bonds or rings in the molecules.

Conclusive evidence for the structure of **2** as shown in Fig. 1 was furnished by 1D and 2D NMR spectroscopy (^1H, ^{13}C, DEPT, HMQC, HMBC, NOESY, in pyridine-d_5). However using $CDCl_3$ as solvent several carbons and C,H long-range couplings in the spectra of both compounds were invisible. The NMR spectra of **1** and **2**, too, were fully identical supporting the view that both compounds are diastereomers. In the ^{13}C and DEPT NMR spectra (Table 2) 16 carbon atoms and their bonding types were visible (five CH or CH_3 groups, one methylene structure (21.3 ppm), 4 doubly bonded C–H groups, and six quaternary double bond carbons). Downfield shift of three of the latter carbons (C-9: 169.9 ppm, C-3: 172.5 ppm, C-10: 164.7 ppm) proposed the presence of carbon-heteroatom bonds. If the ^{13}C and 2D NMR spectra of **1** and **2** were recorded in chloroform the aromatic carbon signals of C-6, C-7 and C-8 were not seen in accord with the occurrence of tautomeric forms constituting the molecules under ambient conditions. In the same manner the unusual downfield shift of C-3 (172.5 ppm) was explainable.

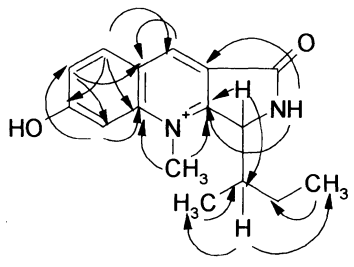
The ^1H NMR and ^1H,^1H-COSY NMR spectra showed the presence of two *ortho*-coupled (H-4, H-5) and two *meta*-coupled aromatic protons (H-2, H-7). The aliphatic side chain at C-11 was fully assignable due to the observable shift data and coupling pattern. Additional proton signals at

Table 2. Assignment of the identical ^{1}H and ^{13}C NMR spectra quinocitrinines A (**1**) and B.

position	δ_C	δ_H
1	141.7(s)	-
2	116.7 (d)	7.60 d, 1.5
3	172.5 (s)	-
4	124.8 (d)	7.38 dd, 1.5; 7.4
5	126.8 (d)	8.70 dd, 7.4, 1.0
6	128.9	-
7	132.7 (d)	7.60 d, 1.0
8	111.0 (s)	-
9	169.9 (s)	-
10	164.7 (s)	-
11	60.2 (d)	4.88 m
12	36.7 (d)	2.21 m
13	21.3 (t)	H_A 0,.82 m; H_B 0.90 m
14	11.5 (q)	0.49 t, 7.1
15	17.8 (q)	1.21 d, 6.9
16	36.8 (q)	3.60 s

In pyridine-d_5, chemical shifts (δ) in ppm; abbreviations: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad, coupling constants in Hz.

Fig. 2. Instructive C,H long-range couplings (HMBC, arrows at one side) and NOESY correlations (arrows at both sides) in the NMR spectra of **1**.



1

9.20 ppm (N–H, s; broad) and 3.60 ppm (s, 3H) were attributable to a nitrogen-bonded lactam proton and, respectively at methyl group (C-16) attached to the quaternary ring nitrogen.

For full structural assignment the 2D spectra such as HMQC and HMBC were of pivotal importance. C,H long-range couplings in the HMBC spectra of **1** and **2** (Fig. 2) confirmed doubtlessly the structures of **1** and **2**. Thus diagnostic couplings of H-16 with C-1 and C-11, of H-5 with C-7, of H-11 with C-8 and C-10, and of the nitrogen-bonded proton at 9.20 ppm with C-8 and C-11 (Fig. 2) supplied supporting evidence. Due to the identity of UV-VIS, IR and NMR spectra of **1** and **2**, and the differences in Rf values, optical rotation and melting point (Table 1) it can be suggested that both compounds possess diastereomeric structures at the two stereocenters (C-11, C-12). However the differences in optical rotation values will not enable the assignment of absolute configuration for one of the stereocenters. The relative stereochemistry (see Fig. 1) was based on the assumption that D- or L-isoleucine forms the precursors for the aliphatic part of the molecules. The quinoline ring and the attached carbonyl group (C-9) can be suggested to originate from tyrosine.

1 and **2** display moderate antibacterial activities against a spectrum of Gram-positive and Gram-negative bacteria, and fungi (Table 3). Moreover, moderate antiproliferative

Table 3. Antimicrobial activities of **1** and **2**.

Test organism	IZ (**1** ; mm)	IZ (**2** ; mm)
Bacillus subtilis ATCC 6633	13	12*
Staphylococcus aureus SG511	12*	13*
Escherichia coli SG 458	14	14*
Pseudomonas aeruginosa SG137	15	15*
Pseudomonas aeruginosa K599/61	0	0
Staphylococcus aureus 134/9	11*	11*
Enterococcus faecalis 1528	12*	12*
Mycobacterium smegmatis SG987	13	12*
Sporobolomyces almonicolor 549	0	0
Candida albicans Bay R.	12*	12*
Penicillium sp. JP 36	15*	15*

50 μl of 1 mg/ml solutions were added to agar wells, and the diameter of inhibition zones (IZ) was determined in mm (* means diffuse inhibition halo).

activity (IC_{50}; μg/ml) was found with L-929 cells (**1**: 33.1; **2**: 18.6), K-562-cells (**1**: 19.5; **2**: 7.8) and HeLa cells (**1**: >50; **2**: >50).

The physico-chemical data thus show quinocitrinines A (**1**) and B (**2**) as new bioactive representatives of the rare quinoline-type of fungal alkaloides.

Experimental

UV-VIS spectra were recorded on a Specord 2000 double beam instrument (Analytik Jena, Germany), FTIR spectra on a Mattson Satellite FTIR spectrometer equipped with ATR device (Mattson, Chicago, USA), optical rotation on a Propolis polarimeter (Dr. KERNCHEN OPTICS, Seelze, Germany), ESI mass spectra on a triple quadrupole mass spectrometer Quattro (VG Biotech, Altrincham England), EREI mass spectra on a Finnigan MAT 95XL (Finnigan Bremen, Germany) and NMR spectra on a Bruker Avance DRX 500 (Bruker, Karlsruhe, Germany).

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