Cottoquinazolines E and F from *Neosartorya fischeri* NRRL 181

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Two new norfumiquinazolines, cottoquinazolines E and F (**1** and **2**, resp.), together with pyripyropene A (**3**), were isolated from the fungus *Neosartorya fischeri* NRRL 181. The structures of the new isolates were established by spectroscopic methods, including UV, IR, HR-ESI-MS, and extensive 1D- and 2D-NMR techniques. To the best of our knowledge, this is the first report on norfumiquinazolines and pyripyropenes produced by the genus *Neosartorya*.

Introduction. – Fumiquinazolines (FQs), which possess a 2*H*-pyrazino[2,1-*b*]quinazoline-3,6(1*H*,4*H*)-dione scaffold, are a subclass of quinazoline alkaloids derived from the condensation of anthranilic acid with two or three additional amino acids [1]. Fumiquinazolines A – G, isolated from a symbiotic fungus, *Aspergillus fumigatus*, of the saltwater fish *Pseudolabrus japonicus*, represent the first reported FQs [2][3]. Subsequent studies have revealed that FQs could also be produced by other fungi, such as *Aspergillus clavatus* [4], *Aspergillus versicolor* [5][6], *Aspergillus flavipes* [7], *Acremonium* sp. [8], *Penicillium thymicola* [9], and *Neosartorya fischeri* [10], in addition to their wide occurance in *A. fumigatus* [11]. Although the ecological roles of these metabolites remain unknown, FQs have attracted attention as substance P inhibitors [7][10]. Herein, we report the isolation of two new FQs from the fungus *N. fischeri* NRRL 181, together with the known compound pyripyropene A, which was reported for the first time from *N. fischeri*.

Results and Discussion. – The 95%-EtOH extract of the solid culture media was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ extract was then subjected to extensive chromatography, which led to the isolation of cottoquinazolines E and F (**1** and **2**, resp.), and pyripyropene A (**3**; *Fig. 1*). The structures of the two new compounds were established by extensive spectroscopic methods, while pyripyropene A (**3**) was identified by comparing its spectroscopic data with those reported in the literature [12].

Compound **1** was isolated as pale-yellow amorphous powder. Its molecular formula was deduced as $C_{23}H_{19}N_5O_4$ on the basis of the $[M+H]^+$ peak at *m/z* 430.1509 ($C_{23}H_{20}N_5O_4^+$; calc. 430.1510) in the HR-ESI-MS spectrum, indicating 17 degrees of unsaturation. The IR absorption band at 1693 cm^{-1} suggested the presence of NC=O groups. The ^{1}H-NMR data (*Table*) of **1** revealed the presence of one Me group (δ(H) 1.36 (*d*, *J* = 6.5)) and eight aromatic H-atoms (8.19 (*dd*, *J* = 8.0, 1.5), 7.87 (*ddd*, *J* = 7.5,
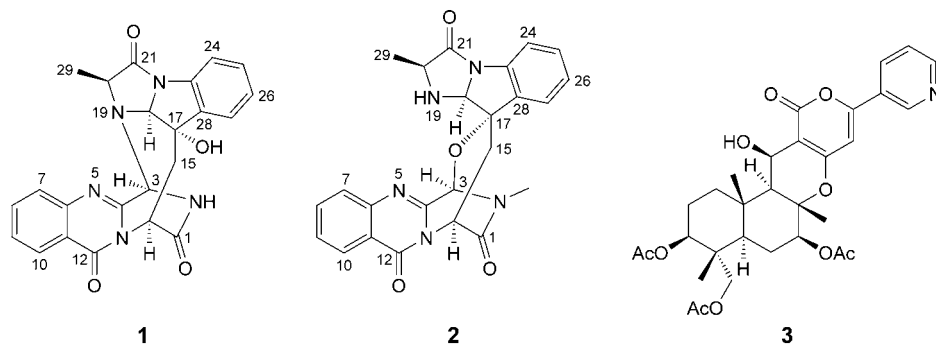
Fig. 1. *Structures of compounds* **1 – 3**

Table. *^{1}H- and ^{13}C-NMR Data for* **1** *and* **2** (500 and 125 MHz, resp.; in (D$_6$)DMSO). δ *in ppm,* J *in Hz. Atom numbering as indicated in Fig. 1.*

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	–	172.3	–	167.3
Me–N	–		3.24 (*s*)	33.0
2	9.42 (*s*)		–	
3	5.36 (*d*, *J* = 4.5)	67.8	6.10 (*s*)	85.8
4	–	148.9	–	149.8
6	–	146.6	–	147.1
7	7.77 (*d*, *J* = 8.0)	127.2	7.76 (*d*, *J* = 7.5)	127.6
8	7.87 (*ddd*, *J* = 7.5, 7.5, 1.5)	134.6	7.90 (*ddd*, *J* = 8.5, 8.5, 1.5)	134.7
9	7.60 (*ddd*, *J* = 8.0, 8.0, 1.0)	127.5	7.63 (*ddd*, *J* = 8.0, 8.0, 1.0)	127.7
10	8.19 (*dd*, *J* = 8.0, 1.5)	126.5	8.19 (*dd*, *J* = 8.0, 1.5)	126.4
11	–	120.4	–	120.8
12	–	160.0	–	158.5
14	5.54 (*d*, *J* = 11.5)	50.7	5.42 (*dd*, *J* = 6.0, 1.5)	51.9
15	2.12 (*dd*, *J* = 15.0, 2.0, H$_a$), 3.07 (*dd*, *J* = 15.0, 12.0, H$_b$)	37.2	2.04 (*dd*, *J* = 15.0, 1.0, H$_a$), 3.05 – 3.09 (*m*, H$_b$)	33.7
17	–	80.4	–	85.4
17-OH	3.49 (*s*)		–	
18	4.78 (*d*, *J* = 1.5)	86.8	5.39 (*dd*, *J* = 6.0, 1.0)	86.1
19	–		3.05 – 3.09 (*m*)	
20	4.16 (*dq*, *J* = 6.5, 1.5)	63.2	4.14 – 4.19 (*m*)	58.0
21	–	171.8	–	172.3
23	–	137.1	–	136.8
24	7.40 (*d*, *J* = 8.0)	114.0	7.38 (*dd*, *J* = 8.0, 1.0)	114.0
25	7.30 (*dd*, *J* = 7.5, 1.0)	129.4	7.31 – 7.34 (*m*)	130.0
26	7.12 (*ddd*, *J* = 7.5, 7.5, 1.0)	124.8	7.05 (*ddd*, *J* = 7.5, 7.5, 1.0)	125.0
27	7.40 (*d*, *J* = 8.0)	124.9	7.31 – 7.34 (*m*)	126.6
28	–	137.9	–	136.8
29	1.36 (*d*, *J* = 6.5)	16.0	1.20 (*d*, *J* = 6.5)	18.2

7.5, 1.5)), 7.77 (d, $J = 8.0$), 7.60 (ddd, $J = 8.0$, 8.0, 1.0), 7.40 (d, $J = 8.0$, 2 arom. H), 7.30 (dd, $J = 7.5$, 1.0), and 7.12 (ddd, $J = 7.5$, 7.5, 1.0)). The ^{13}C-NMR and DEPT spectra of **1** exhibited signals for three C=O groups (δ(C) 172.3, 171.8, and 160.0). In-depth examination of the ^{1}H- and ^{13}C-NMR data (*Table*) of **1** displayed resemblances with those of norfumiquinazolines, cottoquinazolines A–D [5][6]. Extensive analysis of 2D-NMR spectra (*Fig. 2*) indicated that **1** had the same constitution as cottoquinazoline A. The ring closure from N(19) to C(3) was confirmed by HMBCs H–C(3)/C(20), H–C(3)/C(21), and H–C(20)/C(3) (*Fig. 2*). The optical rotation of **1** ($[\alpha]_D^{25} = +378$ ($c = 0.32$, MeOH)) was quite different from that of cottoquinazoline A ($[\alpha]_D^{23} = +98$ ($c = 0.03$, MeOH)). The major differences were reflected in the NMR data of **1** and cottoquinazoline A (C(17): δ(C) 80.4 in **1** and 73.9 in cottoquinazoline A, C(18): 86.8 in **1** and 79.7 in cottoquinazoline A) [5]. These differences suggested that **1** was a *C(17)*-epimer of cottoquinazoline A. Considering that NOE correlation was used to define a *cis*-configuration of H–C(18) and HO–C(17) in fumiquinazoline D (very similar to cottoquinazoline A) [3], the NOESY experiment was also carried out for **1**. Differing from cottoquinazoline A [5], the NOE data exhibited the correlation H–C(18)/HO–C(17), indicating an *α*-configuration of the OH group at C(17) (*Fig. 3*). Furthermore, the NOESY correlations H–C(18)/H–C(3), H–C(18)/H–C(14), and HO–C(17)/H–C(14) were observed. Thus, **1** was determined to have the structure as depicted in *Fig. 1*.
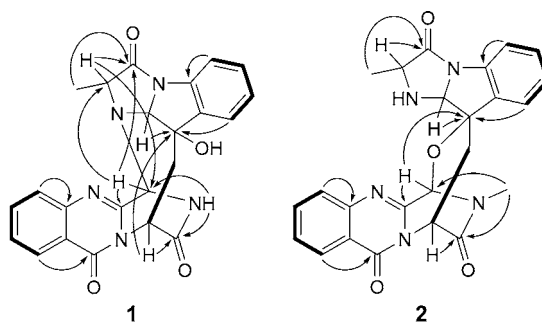

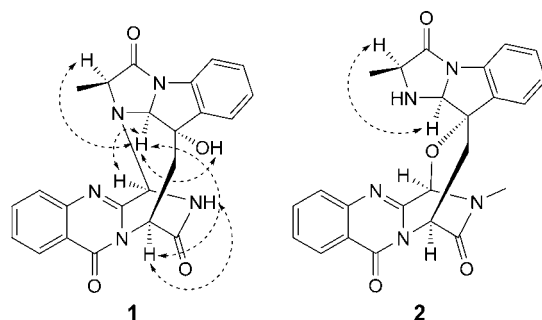Fig. 2. *Selected ^{1}H,^{1}H-COSY (—) and HMBC (H→C) features of **1** and **2***

Fig. 3. *Selected NOESY (H↔H) correlations of **1** and **2***

Compound **2** was also isolated as pale-yellow amorphous powder. The molecular formula $C_{24}H_{21}N_5O_4$ (17 degrees of unsaturation) was deduced from the $[M + H]^+$ peak at m/z 444.1664 ($C_{24}H_{22}N_5O_4^+$; calc. 444.1666) in the HR-ESI-MS spectrum. The IR spectrum of **2** displayed a strong absorption band at 1704 cm^{-1} for NC=O groups. The ^{1}H-NMR spectrum exhibited signals of eight aromatic H-atoms due to two 1,2-disubstituted benzene rings (δ(H) 8.19 (*dd*, $J = 8.0, 1.5$), 7.90 (*ddd*, $J = 8.5, 8.5, 1.5$), 7.76 (*d*, $J = 7.5$), 7.63 (*ddd*, $J = 8.0, 8.0, 1.0$), 7.38 (*dd*, $J = 8.0, 1.0$), 7.31 – 7.34 (*m*, 2 arom. H), and 7.05 (*ddd*, $J = 7.5, 7.5, 1.0$)), one Me *singlet* (3.24), and one Me *doublet* (1.20). The ^{13}C-NMR spectrum showed 24 C-atom resonances including signals of three C=O groups (δ(C) 172.3, 167.3, and 158.5). The NMR data indicated that **2** was also a norfumiquinazoline. The major differences between **1** and **2** were the chemical shifts of C(3), C(17), and C(20) (C(3): δ(C) 67.8 in **1** and 85.8 in **2**, C(17): 80.4 in **1** and 85.4 in **2**, C(20): 63.2 in **1** and 58.0 in **2**). Additionally, no OH signal was observed in **2**, but an additional H-atom signal (H–N(19) at δ(H) 3.05 – 3.09 (*m*)) appeared. These observations suggested that C(3) was connected to C(17) *via* an O-bridge in **2**, instead of ring closure directly from C(3) to N(19) as in **1**. The deduction was further confirmed by the HMBC H–C(3)/C(17) (*Fig. 2*). Another difference was the chemical shift of C(1) (δ(C) 172.3 in **1** and 167.3 in **2**) caused by the replacement of the H-atom at N(2) in **1** by a Me group in **2**, which was further confirmed by the HMBCs Me–N(2)/C(1) and Me–N(2)/C(3) (*Fig. 2*). The constitution of **2** was thus established. Further, the observation of a NOE correlation between H–C(18) and H–C(20) indicated that they were on the same side of the molecular plane (*Fig. 3*). However, all spectroscopic efforts for defining the relative configurations at C(3), C(14), and C(17) proved inconclusive. Attempts at recrystallizing **2** to confirm its absolute configuration were unsuccessful. Many investigations have been conducted on the FQs' biosynthetic pathway [1][13][14]. Fumiquinazoline A can be converted to seven-membered spirohemiaminal fumiquinazoline C by the flavoenzyme Af12070. Then, fumiquinazoline C can be slowly rearranged to the eight-membered aminal containing fumiquinazoline D *via* nonenzymatic equilibration, while the relative configurations at C(3), C(14), and C(17) remain unchanged during this process [13]. It was assumed that **1** and **2** are formed by a similar process. Thus, the relative configurations at C(3), C(14), and C(17) of **2** were assumed to be the same as in **1**. A compound with the CAS registry No. 1379769-71-7 was found to possess the same constitution as **2**, though, neither physiochemical data nor any reference were available. Therefore, we reported the physicochemical data and assigned complete NMR data of this compound.

Experimental Part

General. All solvents were of anal. grade and obtained from commercially available sources. TLC: Precoated silica-gel *GF$_{254}$* plates (SiO$_2$; *Qingdao Haiyang Chemical Co., Ltd.*). Prep. liquid chromatography (LC): *Waters 2767* and *Waters 2545*; *SunFire C18 OBD* prep. column (10 μm, 19 × 150 mm); visualization by UV light (at 254 and/or 365 nm) and 10% H$_2$SO$_4$/EtOH. Column chromatography (CC): SiO$_2$ (200 – 300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), RP-C$_{18}$ SiO$_2$ (50 μm; *Merck*), and *MCI CHP20P* gel (75 – 150 μm; *Mitsubishi Chemical Industries, Ltd.*). Optical rotations: *Rudolph Autopol IV* polarimeter. UV Spectra: *Shimadzu UV-2450* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Thermo Nicolet 6700* FT-IR microscope instrument (FT-IR microscope transmission); $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-500* spectrometer; δ in ppm rel. to Me$_4$Si as internal standard; J in Hz. HR-ESI-MS: *Agilent 6210 LC/TOF* mass spectrometer; in m/z.

Fungus and Culture Conditions. The fungus was purchased from *DSMZ* (DE-Braunschweig). Fermentation was carried out at 28° in 80 *Erlenmeyer* flasks (500 ml) containing 90.0 g of moist wheat bran for 30 d.

Extraction and Isolation. N. fischeri NRRL 181 cultivated in moist wheat bran was extracted three times with 95% EtOH (35.0 l, 7 d each) at r.t. The solvent was evaporated under reduced pressure to give a crude extract (432.0 g). The EtOH extract was suspended in 3.0 l of H_2O and partitioned with $CHCl_3$ (5 × 1.0 l). The $CHCl_3$ fraction (95.0 g) was then subjected to CC (SiO_2; petroleum ether (PE)/acetone, 20 : 1 → 1 : 1) to give eleven fractions, *Frs. 1 – 11. Fr. 10* (1.6 g) was subjected to CC (*RP-C$_{18}$*; $MeOH/H_2O$ 40 : 60 → 80 : 20) to afford three subfractions, *Frs. 10.1 – 10.3. Fr. 10.1* (127.0 mg) was further purified by CC (SiO_2; PE/acetone 2 : 1) to afford **1** (24.0 mg). Compound **3** (20.0 mg) was obtained from *Fr. 10.3* (137.0 mg) by CC (SiO_2; $CHCl_3/MeOH$ 50 : 1). *Fr. 9* (5.1 g) was subjected to CC (*MCI* gel; $MeOH/H_2O$ 50 : 50 → 100 : 0) to give nine subfractions, *Frs. 9.1 – 9.9. Fr. 9.7* (432.5 mg) was seperated by prep. LC ($MeOH/H_2O$ 60 : 40 → 63.3 : 36.7 in 20 min; flow rate, 17 ml min^{-1}; UV detection at 254 nm) to yield 61.0 mg of **2** (t_R 15.7 min).

Cottoquinazoline E (= *(1S,6bS,8S,15R,15bS)-6b,7,8,15-Tetrahydro-6b-hydroxy-15,8-(iminomethano)-1-methyl-2a,8a,14,15a-tetraazabenzo[2′,3′]pentaleno[1′,6′:5,6,7]cycloocta[1,2-b]naphthalene-2,9,17(1H,15H)-trione*; **1**). Pale-yellow amorphous powder. $[\alpha]_D^{25} = +378$ ($c = 0.32$, MeOH). UV (MeOH): 207 (4.55), 227 (4.46), 254 (4.15), 269 (4.13), 281 (4.10). IR: 2981, 2929, 2856, 1693, 1613, 1479, 1406, 1327. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 430.1509 ($[M + H]^+$, $C_{23}H_{20}N_5O_4^+$; calc. 430.1510).

Cottoquinazoline F (= *(1R,2′S,3S,5S,9a′S)-1′,9a′-Dihydro-2′,14-dimethyl-4H-spiro[1,5-(epiminomethano)[1,4]oxazepino[3,4-b]quinazoline-3,9′-imidazo[1,2-a]indole]-3′,7,13(1H,2′H,5H)-trione*; **2**). Pale-yellow amorphous powder. $[\alpha]_D^{25} = +71$ ($c = 0.38$, MeOH). UV (MeOH): 207 (4.59), 227 (4.51), 260 (4.17), 265 (4.17). IR: 3357, 2927, 2855, 1704, 1627, 1608, 1487, 1469, 1398, 1312, 1060. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 444.1664 ($[M + H]^+$, $C_{24}H_{22}N_5O_4^+$; calc. 444.1666).

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