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FUMIQUINAZOLINES, NOVEL METABOLITES OF A FUNGUS ISOLATED FROM A SALTFISH

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Summary: Fumiquinazolines A, B and C, exhibiting moderate cytotoxicity, were isolated from the mycelium of a strain of Aspergillus fumigatus which existed in the gastrointestinal tract of the saltwater fish Pseudolabrus japonicus. Their structures were elucidated by spectroscopic and X-ray diffraction analyses and chemical evidence.

Marine bacteria have been demonstrated to produce the toxic principles (tetrodotoxin, neosurugatoxin, prosurugatoxin and palytoxin) of marine animals[1] as well as cytotoxic maclorides.[2] This fact has evoked wide interest because of the potential for the development of new pharmaceutical agents and also the search for an origin of marine animal metabolites. We have initiated a screening program for antineoplastic and/or cytotoxic metabolites from microorganisms which inhabit the marine environment. As part of this program, we have found that a strain of Aspergillus fumigatus, isolated from the gastrointestinal tract of the saltwater fish Pseudolabrus japonicus, produces the novel metabolites fumiquinazolines (FQ) A (1), B (2) and C (3) which exhibit moderate cytotoxicity against the cultured P-388 lymphocytic leukemia cells.

The fungal strain was cultivated at 27°C for 14 days in a medium containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater, pH 7.4. The MeOH extract of the mycelium was purified by Sephadex LH-20 and silica gel column chromatographies and reverse phased HPLC to afford FQ-A (1), B(2), and C(3).

FQ-A (1), mp 178-182°C (CH_2Cl_2), $[\alpha]_D^{33}$ -214.5° (c=0.47, $CHCl_3$), analyzed for $C_{24}H_{23}N_5O_4$ by HREIMS (m/z 445.1769[M$^+$]). The ^{1}H- and ^{13}C-NMR spectra of **1** (Tables 1 and 2) indicated the presence of one secondary amine and one secondary and two tertiary amides. The coupling relationship between aromatic protons revealed the presence of two 1,2-disubstituted benzenes. The H-21 proton in one of them was shown to correlate with the hydroxy-bearing carbon (C-13) in the XCORFE spectrum (Table 2) which was a modified technique of long range ^{1}H-^{13}C HETCOR spectrum.[3] This indicates a partial structure representing C17-C22-C13. The H-8 proton in the other benzene showed a correlation with the C-10 conjugated amide carbonyl carbon in the XCORFE spectrum, and the C-4 quaternary carbon was deduced from its chemical shift to be linked to a nitrogen atom. In addition

Table 1. ^{1}H-NMR (300MHz) spectral data for fumiquinazolines in $CDCl_3$

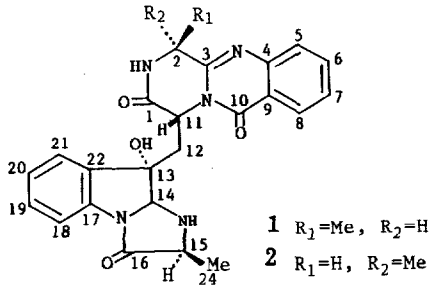
H		1 ppm	J (Hz)	2 ppm	J (Hz)	3 ppm	J (Hz)
2		4.88 q	7.1	4.72 qd	7.2, 4.0		
5		7.67 dd	8.2, 1.0	7.56 dd	8.0, 1.0	7.79 dd	7.4, 1.7
6		7.75 ddd	8.2, 7.0, 1.0	7.73 ddd	8.9, 7.0, 1.0	7.79 ddd	7.4, 6.3, 1.7
7		7.49 ddd	7.9, 7.0, 1.0	7.45 ddd	7.8, 7.0, 1.0	7.60 ddd	7.4, 6.3, 1.7
8		8.23 dd	7.9, 1.0	8.19 dd	7.8, 1.0	8.35 dd	7.4, 1.7
11		5.97 dd	10.9, 6.0	5.79 dd	11.2, 4.8	5.72 d	7.4
12	A	2.28 dd	13.7, 6.0	2.48 dd	13.3, 4.8	2.14 d	15.1
	B	2.51 dd	13.7, 10.9	2.61 dd	13.3, 11.2	2.98 dd	15.1, 7.4
14		5.49 s		5.42 br s		5.34 d	6.9
15		4.22 q	6.7	4.14 q	6.7	3.71 qd	6.9, 6.7
18		7.52 dd	7.5, 1.0	7.51 dd	7.5, 1.0	7.45 dd	7.4, 1.0
19		7.31 td	7.5, 1.0	7.30 td	7.5, 1.0	7.32 td	7.4, 1.0
20		7.16 ddd	7.5, 6.8, 1.0	7.17 td	7.5, 1.0	7.19 td	7.4, 1.0
21		7.61 dd	6.8, 1.0	7.61 dd	7.5, 1.0	7.37 dd	7.4, 1.0
23		1.79 d	7.1	1.83 d	7.2	2.06 s	
24		1.35 d	6.7	1.29 d	6.7	1.06 d	6.9
OH		4.89 s		5.47 s			
NH		2.79 br s		2.75 br s		1.04 dd	6.9, 6.7
		6.61 s		7.34 br d	4.0	8.04 br s	

Table 2. ^{13}C-NMR (75.4 MHz) spectral data and long range ^{1}H-^{13}C correlations of fumiquinazolines in $CDCl_3$

C	1	Long range ^{1}H-^{13}C-correlations	2	3
1	172.33 (q)[a]	H-11	170.69 (q)	171.01 (q)
2	49.11 (t)	H-23	52.73 (t)	84.16 (q)
3	150.75 (q)	H-2, H-23, H-11[b]	150.71 (q)	150.38 (q)
4	146.83 (q)	H-6, H-8	146.99 (q)	146.31 (q)
5	127.54 (t)	H-7	126.88 (t)	128.44 (t)
6	134.76 (t)	H-8	134.97 (t)	134.91 (t)
7	127.41 (t)	H-5	127.26 (t)	128.56 (t)
8	126.73 (t)	H-6	126.88 (t)	126.97 (t)
9	120.15 (q)	H-7	120.00 (q)	121.33 (q)
10	160.44 (q)	H-8, H-11[b]	160.30 (q)	159.52 (q)
11	52.94 (t)		52.00 (t)	51.39 (t)
12	36.68 (s)	H-11	38.97 (s)	31.35 (s)
13	80.16 (q)	H-21	80.19 (q)	87.07 (q)
14	86.24 (t)	H-12B	86.42 (t)	87.07 (t)
15	58.97 (t)	H-24	59.07 (t)	58.61 (t)
16	170.49 (q)	H-15, H-24	170.56 (q)	170.90 (q)
17	136.14 (q)	H-19, H-21	136.55 (q)	135.73 (q)
18	114.97 (t)	H-20	114.85 (t)	115.46 (t)
19	129.72 (t)	H-21	129.73 (t)	130.23 (t)
20	125.54 (t)	H-18	125.50 (t)	126.17 (t)
21	124.81 (t)	H-19	125.01 (t)	124.87 (t)
22	138.55 (q)	H-20	138.61 (q)	138.41 (q)
23	16.72 (p)	H-2	24.87 (p)	24.42 (p)
24	18.59 (p)		18.14 (p)	18.71 (p)

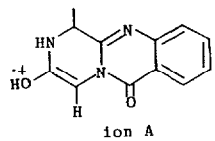
a Letters, p, s, t and q, indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

b These correlations were obtained from LSPD and other ones from XCORFE spectrum.

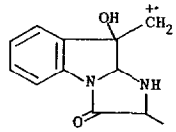


1 R_1=Me, R_2=H

2 R_1=H, R_2=Me



ion A
m/z : 229.0862



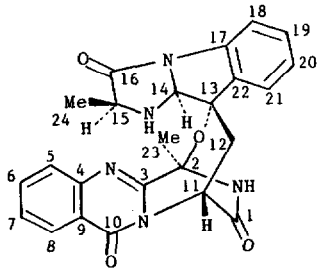
ion B
m/z : 217.0973

to this evidence, the UV absorption bands [λ_{max}^{EtOH} nm: 226, 233 sh, 256, 265 sh, 277, 305, 327] revealed a partial structure (quinazolone ring) representing C3-C10-N. The XCORFE spectrum indicated correlations between the C-1 amide carbonyl carbon and the H-11 methine proton coupled with the H-12 methylene protons, and between the C-16 amide carbonyl carbon and both the H-15 methine and H-24 methyl protons, coupled to each other. These results revealed partial structures representing N-C1-C11-C12 and N-C15(C24)-C16-N. The chemical shifts of the remaining ^{1}H- and ^{13}C-NMR signals as well as the proton coupling relationship elucidated partial structures representing C23-C2-N and N-C14-N. The connection of the partial structures thus obtained was determined on the basis of the two EIMS fragment ions (A and B) and the long range ^{1}H-^{13}C correlation, observed between H-23 and C-3, H-12 and C-14, and H-11 and both C-3 and C-10. Thus, the planar structure of FQ-A was assigned as **1**.

FQ-C (3), mp 244-246°C (acetone), $[\alpha]_D^{21}$ -193.7° (c=0.31, $CHCl_3$), had the molecular formula $C_{24}H_{21}N_5O_4$ being two protons less than 1 as deduced from HREIMS (m/z 443.1591 $[M]^+$). A close inspection of the ^{1}H- and ^{13}C-NMR signals of both 1 and 3 revealed that the H-2 methine and hydroxyl proton signals in 1 were missing from 3, and the H-23 methyl proton signal as a singlet and the downfield shifted C-2 carbon signal (δ 84.16) appeared in 3. This evidence allowed assignment of planar structure 3, which had the ether linkage between C-2 and C-13, for FQ-C. The absolute stereostructure of 3 was determined by X-ray crystallographic analysis and the production of L-(+)-alanine by its acidic hydrolysis. The X-ray crystallographic analysis was carried out on a single cubic crystal of 3 obtained from acetone.[4] A computer generated perspective drawing of the final X-ray model is given in Fig.1.

FQ-B (2), mp 174-176°C (acetone), $[\alpha]_D^{21}$-196.7° (c=0.38, $CHCl_3$), has the same molecular formula as that of 1. The general spectral features of 2 closely resembled those of 1 except for the signals of C-2, C-12 and C-23 in the ^{13}C-NMR spectrum, indicating 2 to be a stereoisomer of **1**.

Hydrogenation of 3 with $NaBH_4$ gave FQ-A (1). This shows the absolute configuration of 1 to be the same as 3 except for C-2. Compounds 1 and 2 were each treated by 0.1% KOH in MeOH at room temperature to displace the balance of the interconversion reaction in favor of 1 (1/2=4/1). Treatment of 1 with 0.1% KOD in CD_3OD afforded the mixture of 1 and 2, deuterated at both C-2 and C-11, whereas a small amount of 2 deuterated only at



3

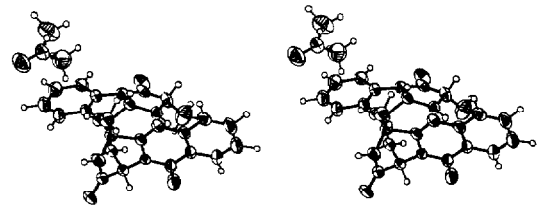


Fig. 1 ORTEP stereodiagram of **3**

C-2 was caught by treatment of 1 with 2 % DCl in CD_3OD. This evidence indicates that 1 and 2 are the stereoisomer at C-2, and 1 is thermodynamically more stable than 2. Thus, the C-2 methyl groups of 1 and 2 are expected to be equatorial and axial manners, respectively. These results led to assignment of stereostructures 1 and 2 for FQ-A and B, respectively.

Tryptoquivaline-related metabolites have been isolated from A. clavatus and A. fumigatus by each group of Büchi[5] and Yamazaki[6], respectively. The fungus isolated herein is supposed to be a diffferent strain from that isolated by Yamazaki et al. because any tryptoquivalines were not detected in this experiment. It has not been reported that a saltwater fish P. japonicus caused an outbreak of food poisoning.

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References and Notes

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4. The crystal data are as follows: $C_{24}H_{21}N_5O_4 \cdot C_3H_6O$, M=501.543, orthorhombic, space group $P2_12_12_1$, a=10.613 (6) Å, b=25.36 (1) Å, c=9.321 (3) Å, v=2508 (2) Å, z=4, Dx=1.3279 g.cm^{-3}. The reflectional intensities within 2θ =130° were collected on a Rigaku automatic four-circle diffractometer with graphite-monochromated Cu-K$_\alpha$ radiation (λ=1.5418 Å). The structure was finally solved by direct methods using MULTAN87 and the 2376 reflections were used in the refinement. Hydrogen atoms were located from a difference Fourier synthesis. The structure was refined by the block-diagonal least-squares procedure with anisotropic temperature factors for non-H atoms and isotropic ones for H atoms. The final R value was 0.086. Other crystallographic parameters have been deposited with the Cambridge Crystallographic Data Center.
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