Fumiquinazolines A–G, novel metabolites of a fungus separated from a *Pseudolabrus* marine fish

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Seven new fumiquinazolines (FQs) A–G have been isolated from a strain of *Aspergillus fumigatus* originally separated from the marine fish *Pseudolabrus japonicus*, and their stereostructures and conformations have been established on the basis of spectral and X-ray analyses and some chemical transformations. All the compounds exhibited moderate cytotoxicity against cultured P388 cells.

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria,[1–4] we have focused our attention on new antitumour materials from microorganisms inhabiting the marine environment. As part of this program, we previously reported that antitumour and cytotoxic compounds were produced by microorganisms originally isolated from the marine fish *Halichoeres bleeki*[5] and the marine algae *Enteromorpha intestinalis*[6] and *Sargassum tortile*[7–10] and that their structures had been established. In our continuing search for cytotoxic compounds from marine microorganisms, we have isolated seven metabolites designated fumiquinazolines (FQs) A–G **1–3** and **7–10** from a strain of *Aspergillus fumigatus* separated from the gastrointestinal tract of the marine fish *Pseudolabrus japonicus*. We report herein the isolation and structure determination of compounds **3**, **7**, **8** and **10** and the details of the structure elucidation of compounds **1**, **2** and **9**, already briefly reported in a preliminary form.[11]

Results and discussion

The fungal strain was cultured at 27 °C for 3 weeks in a medium containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater adjusted to pH 7.5. The MeOH extract of the mycelium was purified by a combination of Sephadex LH-20 and silica gel column chromatography and high-performance liquid chromatography (HPLC) to afford FQs A–G, **1–3** and **7–10**.

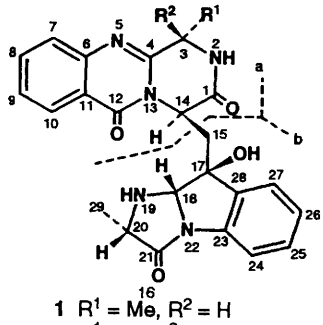
FQ A **1** had the molecular formula $C_{24}H_{23}N_5O_4$ established by high-resolution electron impact mass spectrometry (HREIMS). Its IR spectrum exhibited absorption at 3349, 1680 and 1608 cm^{-1}, characteristic of an alcohol, an amine, an amide and an aromatic ring. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) by distortionless enhancement by polarization transfer (DEPT) and ^1H-^1H and ^1H-^{13}C correlation spectroscopy (COSY) experiments revealed signals for the following functionalities: three amide carbonyls (C-1, C-12 and C-21) including one α,β-unsaturated amide (C-12), two NHs of a secondary amine (N-19) and a secondary amide (N-2), one tertiary alcohol (C-17), two ethylidene (C-3 to C-16 and C-20 to C-29) and one ethylene (C-14 and C-15) groups each bearing a nitrogen, two 1,2-disubstituted benzenes (C-6 to C-11 and C-23 to C-28), one methine (C-18) bearing two nitrogens and a quaternary sp^3-hybridized carbon, and one quaternary sp^2-carbon (C-4) linked to a sp^2-nitrogen as a double bond. The C-18 methine was assigned by comparison with the ^1H and ^{13}C chemical shift data for leptosins,[7–10] and assignment of C(4)=N(5) was based on the absence of any other sp^2-carbons to bond to C-4 and the appearance of the C-4 carbon signal at lower field (δ_C 150.75). The signal for one quaternary sp^2-carbon (C-6) of one aromatic ring was found shifted lowfield (δ_C 146.83), implying that the carbon is linked to a nitrogen atom.

Long-range ^1H–^{13}C COSY correlations (Table 1) for the functional groups thus established led to partial structures A, B and C (Fig. 1). Principal correlations are shown in Fig. 1. A ^1H–^{13}C long-range coupling, observed between 14-H and C-12 in partial structures A and B, respectively, in a long-range selective proton decoupling (LSPD) experiment, indicated the connection of N-13 to C-12 and consequently of N-5 to C-6. Since there are one secondary and two tertiary amides in the molecule of **1** as described above and the secondary amide (C-1) is included in partial structure A, the amide (C-21) in partial structure C should be tertiary. In addition, a long-range coupling between 20-H and 18-H was observed in the ^1H–^1H COSY experiment. This evidence implied that N-19 and N-22 in partial structure C are linked to C-18, and C-18 and C-23 in partial structure A, respectively, and hence led to planar structure **1** for FQ A. This structure was supported by the EIMS fragments at *m/z* 229 ($[a + H]^+$) and 217 (b^+), arising from cleavage of the C-14–C-15 bond in **1** as confirmed by HREIMS.

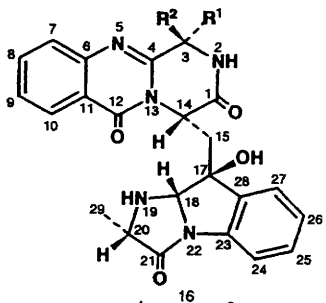
FQ C **9** was assigned a molecular formula which contained two proton atoms less than that of **1**. The general features of its ^1H and ^{13}C NMR spectra (Table 2) closely resembled those of **1** except that the C-3 methine and hydroxy proton signals in **1** disappeared from **9**, the C-16 protons of **9** resonated as a singlet signal, and the carbon signals for C-3, C-15, C-16 and C-17 in **9** revealed a chemical shift difference relative to those of **1**. This evidence led to planar structure **9** with an ether linkage between C-3 and C-17 for FQ C. This structure was supported by long range ^1H–^{13}C correlations such as C-4/2-H, C-4/14-H, C-12/14-H, C-17/14-H, *etc.* (Table 2).

The relative configuration of **9** was ascertained from analysis of nuclear Overhauser enhancement spectroscopy (NOESY) (Table 2). Because of the presence of the ether linkage between C-3 and C-17 in **9**, the C(3)–O and C(14)–C(15) bonds should have a *cis* configuration. An NOE correlation between 29-H and 15-H_B implied that the C(18)–N(19), N(22)–C(21) and C(17)–C(15) bonds are orientated *cis* to one another, and the 29-methyl group is on the same side as the C(17)–C(15) bond. Therefore, 18-H must be *cis* to the C(17)–O bond and 20-H (Fig. 2). The relative stereostructure of **9** thus expected was confirmed by X-ray crystallographic analysis.[11] The absolute configuration of **9** was based on production of L-(+)-alanine by its acidic hydrolysis.

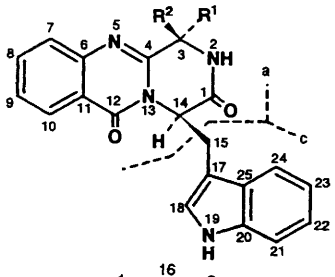
FQ B **2** had the same molecular formula as **1**. The general



1 R^1 = Me, R^2 = H
2 R^1 = H, R^2 = Me
3 R^1 = Me, R^2 = OMe
4 R^1 = Me, R^2 = OH



5 R^1 = Me, R^2 = H
6 R^1 = H, R^2 = Me



7 R^1 = Me, R^2 = H
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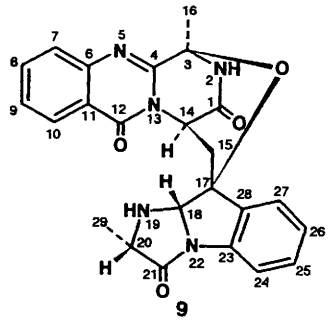

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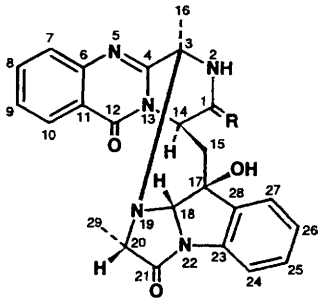
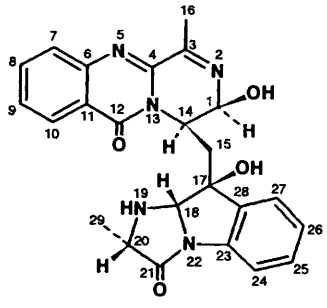


10 R = O
11 R = α-H, β-OH



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spectral features of **2** closely resembled those of **1** except for the signals of C-1, C-3, C-15 and C-16 in the ^{13}C NMR spectrum, implying that **2** is a stereoisomer of **1** at C-3, C-14 or both positions (Table 1). Compounds **1** and **2** were each treated with 0.4% KOH in MeOH at room temperature for 16 h to afford a mixture of **1**, **2**, **5** and **6** in a 4:2:2:1 ratio. Compounds **5** and **6** were assumed to be stereoisomers of **1** and **2** on the basis that their molecular formulae were identical with those of **1** and **2** and, moreover, their ^1H NMR spectra were closely similar to those of **1** and **2** (Table 1). Treatment of **1** with 0.4% KOD in CD_3OD afforded a mixture of [$^2H_2$]-**1**, [$^2H_2$]-**2**, [$^2H_2$]-**5** and [$^2H_2$]-**6**, deuteriated at both C-3 and C-14, whereas [$^2H_1$]-**1** and [$^2H_1$]-**2** deuteriated only at C-3 were obtained by treatment of **1** with 1% DCl in CD_3OD. This result implied that **1** and **2** are stereoisomers at C-3, **5** and **6** are stereoisomers of either **1** or **2** at C-14, and **1** and **5** are thermodynamically more stable than **2** and **6**, respectively. A suggested mechanism for epimerization of these compounds at C-3 is illustrated in Fig. 3.

In selected difference NOE experiments, **1** exhibited NOEs between 3-H and 15-H_B, 2-H and 16-H, and 2-H and 3-H, whereas **2** showed NOEs between 16-H and 15-H_B, and 2-H and 3-H. In addition, a W-type of long-range coupling between 14-H and 2-H was observed in the ^1H–^1H COSY experiments of both **1** and **2** as observed in that of **9**, in which the oxopiperazine ring [C(1)–C(4), N(13) and C(14)] exists in a twist boat conformation with 14-H in an equatorial arrangement. Analysis of respective coupling constants of *ca.* 0.3 and 4.9 Hz between 3-H and 2-H in **1** and **2**, using a Karplus relationship,[12] suggested that 3-H and 2-H dihedral angles in **1** and **2** are approximately 75 and 38°, respectively. These observations implied that the oxopiperazine rings [C(1)–C(4), N(13) and C(14)] in **1** and **2** exist in a twist boat conformation with 3-H and the C(14)–C(15) bond, and the 16-methyl group and the C(14)–C(15) bond, respectively, in coaxial arrangements. Furthermore, the observation of an NOE between 27-H and 14-H in both **1** and **2** indicated that the indoline rings are arranged on a nearly vertical plane to the oxopiperazine ring [C(1)–C(4), N(13) and C(14)] in the both compounds. Production of **1** by reduction of **9** with $NaBH_4$ revealed that the absolute configurations of **1** and consequently **2** are the same as **9** except for C-3. Based on the above evidence, the absolute stereostructures and conformations of **1** and **2** were established as shown in Fig. 4.

In the stereoisomers **5** and **6** of **1** or **2** at C-14, a cross peak for a W-type of long-range coupling between 14-H and 2-H was found in their ^1H–^1H COSY experiments as observed in **1**, **2** and **9**, but no NOE between 15-H and 16-H or 3-H was observed, implying that the oxopiperazine rings [C(1)–C(4), N(13) and C(14)] of **5** and **6** exist in a twist chair conformation with 14-H in an equatorial arrangement. Compound **5**

Table 1 ^1H and ^{13}C NMR spectral data of FQs A **1** and B **2** and derivatives **5** and **6** in $CDCl_3$

	1			**2**		**5**	**6**
Position	δ_H[a]	δ_C	LR ^1H-^{13}C COSY (H)	δ_H	δ_C	δ_H	δ_H
1		172.36 (q)[b]	14, 15_A, 15_B		170.69 (q)		
2	6.61dd (0.9, 0.3)			7.34dd (4.9, 0.9)		6.63br d (0.3)	7.01br d (4.2)
3	4.88qd (7.1, 0.3)	49.15 (t)	2, 16	4.72qd (7.2, 4.9)	52.73 (t)	4.81qd (7.0, 0.3)	4.77qd (7.0, 4.2)
4		150.78 (q)	2, 14, 16		150.72 (q)		
6		146.87 (q)	8, 10		147.00 (q)		
7	7.67dd (8.2, 1.0)	127.57 (t)	9	7.56dd (8.0, 1.0)	126.88 (t)	7.75dd (7.8, 1.0)	7.67dd (7.8, 1.0)
8	7.75ddd (8.2, 7.0, 1.0)	134.80 (t)	10	7.73ddd (8.0, 7.0, 1.0)	134.97 (t)	7.83td (7.8, 1.2)	7.81td (7.8, 1.2)
9	7.49ddd (7.9, 7.0, 1.0)	127.45 (t)	7	7.45ddd (7.8, 7.0, 1.0)	127.27 (t)	7.56td (7.8, 1.0)	7.55td (7.8, 1.0)
10	8.23dd (7.9, 1.0)	126.77 (t)	8	8.19dd (7.8, 1.0)	126.88 (t)	8.31dd (7.8, 1.2)	8.29dd (7.8, 1.2)
11		120.18 (q)	7, 9		120.01 (q)		
12		160.48 (q)	10		160.30 (q)		
14	5.97ddd (10.9, 6.0, 0.9)	52.98 (t)	2, 15_A, 15_B	5.79ddd (11.2, 4.8, 0.9)	52.01 (t)	5.91dd (9.8, 5.0)	5.97t (6.8)
15_A	2.28dd (13.7, 6.0)	36.72 (s)		2.48dd (13.3, 4.8)	38.97 (s)	2.14dd (14.8, 5.0)	2.18dd (14.8, 6.8)
15_B	2.51dd (13.7, 10.9)			2.61dd (13.3, 11.2)		2.67dd (14.8, 9.8)	2.68dd (14.8, 6.8)
16	1.79d (7.1)	16.75 (p)		1.83d (7.2)	24.88 (p)	1.78d (7.0)	1.81d (7.0)
17		80.20 (q)	15_B, 27, OH		80.20 (q)		
18	5.49s	86.28 (t)	15_A, OH	5.42br s	86.43 (t)	5.41d (4.8)	5.51br d (6.8)
19	2.79br s			2.75br s		2.22br s	2.88br t (6.8)
20	4.22q (6.7)	59.01 (t)	29	4.14q (6.7)	59.07 (t)	3.97 quintet (6.8)	4.07 quintet (6.8)
21		170.53 (q)	29		170.56 (q)		
23		136.17 (q)	25, 27		136.56 (q)		
24	7.52dd (7.5, 1.0)	115.01 (t)	26	7.51dd (7.5, 1.0)	114.86 (t)	7.51dd (7.8, 1.0)	7.43dd (7.8, 1.0)
25	7.31td (7.5, 1.0)	129.76 (t)	27	7.30td (7.5, 1.0)	129.73 (t)	7.30td (7.8, 1.0)	7.30td (7.8, 1.0)
26	7.16ddd (7.5, 6.8, 1.0)	125.58 (t)	24	7.17td (7.5, 1.0)	125.50 (t)	7.15td (7.8, 1.0)	7.13td (7.8, 1.0)
27	7.61dd (6.8, 1.0)	124.85 (t)	25	7.61dd (7.5, 1.0)	125.02 (t)	7.51dd (7.8, 1.0)	7.52dd (7.8, 1.0)
28		138.59 (q)	24, 26, OH		138.62 (q)		
29	1.35d (6.7)	18.63 (p)		1.29d (6.7)	18.14 (p)	1.19d (6.8)	1.31d (6.8)
OH	4.89s			5.47s		5.24s	5.27s

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (J/Hz) in parentheses. [b] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

Table 2 ^1H and ^{13}C NMR spectral data of FQs C **9** and D **10** in $CDCl_3$

	9				**10**			
Position	δ_H[a]	NOEs (H)[b]	δ_C	LR ^1H-^{13}C COSY (H)	δ_H	NOEs (H)[b]	δ_C	LR ^1H-^{13}C COSY (H)
1			171.02 (q)[c]	14			172.58 (q)	15_A
2	8.04br s				9.16br s	18, 20		
3			84.16 (q)	2, 16			70.84 (q)	16, 18
4			150.39 (q)	2, 14, 16			152.14 (q)	16
6			146.32 (q)	8, 10			146.34 (q)	8, 10
7	7.78dd (7.4, 1.7)		128.45 (t)	9	7.66dd (8.3, 1.2)		127.79 (t)	9
8	7.81ddd (7.4, 6.3, 1.7)		134.91 (t)	10	7.75ddd (8.3, 6.8, 1.2)		134.83 (t)	10
9	7.60ddd (7.4, 6.3, 1.7)		128.56 (t)	7	7.50ddd (7.9, 6.8, 1.2)		127.68 (t)	7
10	8.35dd (7.4, 1.7)		126.98 (t)	8	8.19dd (7.9, 1.2)		126.85 (t)	8
11			121.34 (q)	7, 9			120.35 (q)	7, 9
12			159.53 (q)	10, 14			160.86 (q)	10
14	5.72dd (10.9, 6.0)		51.39 (t)	2, 15_A, 15_B	5.65d (10.3)		52.76 (t)	15_A
15_A	2.14dd (13.7, 6.0)	27	31.36 (s)		2.27d (15.1)	27	43.44 (s)	
15_B	2.98dd (13.7, 10.9)	29			3.38dd (15.1, 10.3)	27		
16	2.06s	18	24.43 (p)		2.02s	20	18.77 (p)	
17			87.07 (q)	14, 27			84.13 (q)	15_B
18	5.34d (6.9)	16	87.07 (t)	15_A	5.52d (1.3)	2, 20, OH	85.59 (t)	15_B
19	1.04dd (6.9, 6.7)							
20	3.71qd (6.9, 6.7)		58.61 (t)	29	3.96qd (6.5, 1.3)	2, 18, 16	59.11 (t)	29
21			170.90 (q)	29			171.41 (q)	29
23			135.73 (q)	25, 27			137.60 (q)	25, 27
24	7.45dd (7.4, 1.0)		115.46 (t)	26	7.41dd (7.4, 1.0)		115.43 (t)	26
25	7.32td (7.4, 1.0)		130.23 (t)	27	7.23td (7.4, 1.0)		130.07 (t)	27
26	7.19td (7.4, 1.0)		126.17 (t)	24	7.05td (7.4, 1.0)		125.77 (t)	24
27	7.37dd (7.4, 1.0)	15_A	124.88 (t)	25	7.44dd (7.4, 1.0)	15_{AB}	124.31 (t)	25
28			138.41 (q)	15_A, 24, 26			137.38 (q)	24, 26
29	1.06d (6.9)	15_B	18.71 (p)		1.08d (6.5)		17.41 (p)	
OH					5.27br s	18		

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (J/Hz) in parentheses. [b] Observed in NOESY experiments. [c] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

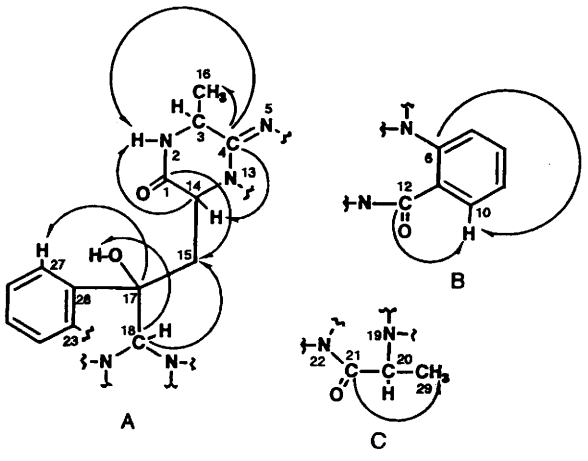

Fig. 1 Partial structures of compound **1** and long-range ^{1}H–^{13}C correlations

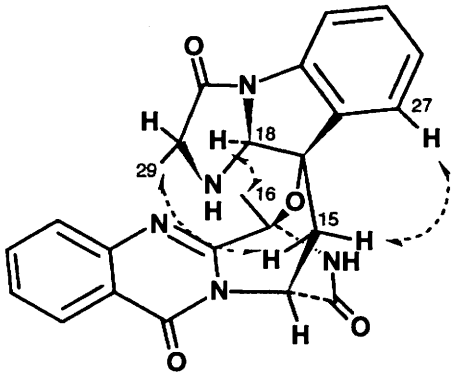

Fig. 2 Stereostructure of compound **9** and observed NOEs

exhibited a coupling constant of *ca.* 0.3 Hz between 3-H and 2-H, and NOEs between 2-H and 16-H, and 2-H and 3-H as observed in **1**, implying that the 16-methyl group in **5** is orientated equatorial as in **1**. On the other hand, **6** exhibited a coupling constant of 4.2 Hz between 3-H and 2-H, and an NOE only between 3-H and 2-H as observed in **2**, implying that the 16-methyl group in **6** is arranged axial as in **2**. In addition, both the compounds showed an NOE between 15-H_A and 27-H. The above evidence allowed assignments of stereostructures of **5** and **6**.

FQ E **3** was assigned the molecular formula $C_{25}H_{25}N_5O_5$. Its ^{1}H and ^{13}C NMR signals (Table 3) showed close correspondence with those of **1** except for the absence of the C-3 methine proton signal, appearance of a proton signal for one methoxy group and the C-16 proton signal as a singlet, and a chemical-shift difference of the carbon signals for C-3, C-4, C-15 and C-16. This finding suggested that 3-H in **1** was replaced by a methoxy group in **3**. As observed in **1**, **3** exhibited a cross peak for a W-type of long-range coupling between 14-H and 2-H in the ^{1}H–^{1}H COSY experiment, and NOEs between 16-H and 2-H, 16-H and OMe and 14-H and 27-H, implying that **3** exists in a twist boat conformation with the methoxy group and the C(14)–C(15) bond in a coaxial arrangement. Treatment of **9** with 2% HCl in MeOH afforded a mixture of compounds **3** and **4** at a ratio of 2:1. Formation of **3** from **9** in addition to the above mentioned fact led to absolute stereostructure **3** and the conformation, shown in Fig. 4, for FQ E. The molecular formula and the ^{1}H and ^{13}C NMR spectra of **4** revealed that the methoxy group in **3** was replaced by a hydroxy group in **4** (Table 3). Considering that the formation mechanism of **4** from **9** should be the same as that of **3** from **9**, the absolute

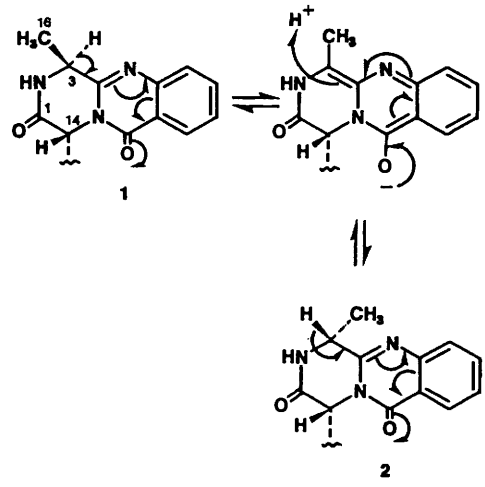

Fig. 3 Mechanism for epimerization of compounds **1** and **2** at C-3

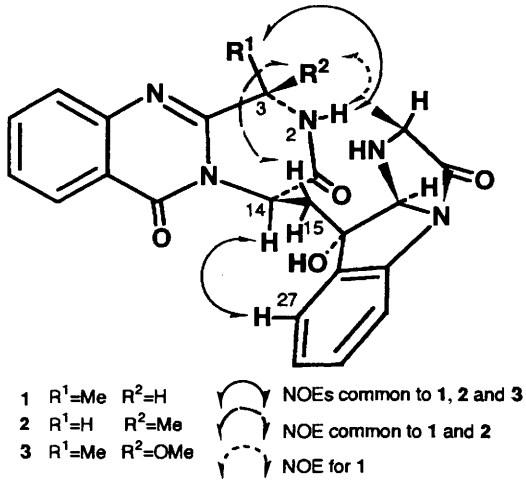

Fig. 4 Conformations of compounds **1**, **2** and **3**, and observed NOEs

stereostructure of the hydroxy derivative is represented as **4** with the hydroxy group in an axial arrangement.

FQ D **10** had the same molecular formula as **9**. Its spectral data showed similarities to those of **9**, and treatment of **10** with 2% HCl in MeOH afforded **3** and **4** at a ratio of 2:1. This finding seemed to suggest that **10** is a stereoisomer of **9** at C-17, but this was negated by detailed analysis for ^{1}H and ^{13}C NMR data of **10** (Table 2). The carbon signals for C-3, C-15 and C-16 in **10** revealed a large chemical-shift difference (5–13 ppm) relative to those of **9**. Especially the C-3 carbon signal (δ_C 70.85) appeared shifted upfield by 13.31 ppm. The proton signal for a secondary amine found at δ 1.04–2.85 in **1**–**3** and **9** was not observed in **10** and it appeared more likely that the proton signal at δ 5.26 in **10** is ascribed to the hydroxy proton because it resonated at δ 4.55–5.47 in **1**, **2** and **3**. Additionally, long-range ^{1}H–^{13}C COSY correlation between C-3 and 18-H (Table 2) required the connection of C-3 and N-19. The absolute stereochemistry of **10** was deduced from transformation of **10** into **3** as described above, the observation of NOESY cross peaks between 20-H and 16-H, 18-H and 2-H, 18-H and 20-H, 27-H and 15-H_A, and 18-H and OH (Table 2, Fig. 5), and production of L-(+)-alanine from **10**. The stereostructure of **10** thus expected was confirmed by X-ray structure analysis on a single crystal of **10** (Fig. 6). Incidentally, though **9** gave **1** on $NaBH_4$ reduction, **10** afforded **11** in the same reaction, which was transformed into **12** due to its unstability during purification by HPLC or on storage at room temperature.

Table 3 ^{1}H and ^{13}C NMR spectral data of FQ E **3** and derivatives **4**, **11** and **12** in $CDCl_3$

	3		**4**			**11**		**12**	
Position	δ_H[a]	δ_C	δ_H	δ_C	LR ^{1}H-^{13}C COSY (H)	δ_H	δ_C	δ_H	δ_C
1		172.70 (q)[b]		172.96 (q)		5.63dd (7.8, 2.0)	85.70 (t)	5.78dq (6.2, 2.5)	88.12 (t)
2	7.57br d (1.0)		5.56br d (0.9)			3.17br s			
3		84.83 (q)		80.98 (q)	16		69.35 (q)		137.52 (q)[e]
4		148.17 (q)		149.79 (q)	16		153.80 (q)		160.31 (q)[f]
6		146.18 (q)		146.56 (q)	8		146.38 (q)		146.59 (q)
7	7.74dd (7.7, 1.5)	127.96 (t)	7.70d (7.8)	127.92 (t)		7.67dd (7.8, 1.2)	127.57 (t)	7.83d (3.8)	128.82 (t)
8	7.79ddd (8.2, 7.7, 1.5)	134.82 (t)	7.76t (7.8)	134.87 (t)		7.75td (7.8, 1.2)	134.45 (t)	7.83d (4.8)	134.85 (t)
9	7.53ddd (8.2, 6.8, 1.5)	127.96 (t)	7.50t (7.8)	127.83 (t)	7	7.48td (7.8, 1.2)	127.01 (t)	7.60ddd (7.8, 4.8, 3.8)	128.97 (t)
10	8.25dd (6.8, 1.5)	126.95 (t)	8.23d (7.8)	126.81 (t)		8.28dd (7.8, 1.2)	126.89 (t)	8.31d (7.8)	126.96 (t)
11		120.60 (q)		120.46 (q)	7, 9		120.49 (q)		121.62 (q)
12		161.01 (q)		161.20 (q)			161.97 (q)		161.89 (q)[f]
14	5.93ddd (8.9, 5.2, 1.0)	53.38 (t)	5.92td (7.8, 0.9)	53.08 (t)		5.84dddd (11.0, 7.8, 2.5, 1.0)	49.43 (t)	5.52td (9.8, 6.2)	49.96 (t)
15_A	2.34dd (14.4, 5.2)	38.91 (s)	2.72d (7.8)	39.41 (s)		2.25dd (13.0, 11.0)	42.18 (s)	2.35dd (13.2, 9.8)	34.82 (s)
15_B	2.80dd (14.4, 8.9)		2.72d (7.8)			2.63dd (13.0, 2.5)		2.85dd (13.2, 9.8)	
16	1.97s	20.88 (p)	2.06s	26.76 (p)		1.89s	19.09 (p)	2.66d (2.5)	22.36 (p)
17		80.11 (q)		80.56 (q)			90.51 (q)		91.27 (q)
18	5.45s	86.29 (t)	5.45s	86.29 (t)		5.71d (1.8)	83.74 (t)	5.76s	83.54 (t)
19	2.85s		2.60br s					2.43br s	
20	4.16q (6.6)	59.21 (t)	4.15q (6.7)	59.51 (t)	29	4.16qd (6.5, 1.8)	61.15 (t)	4.26q (6.5)	59.37 (t)
21		171.28 (q)		169.35 (q)	29		168.35 (q)		170.51 (q)
23		136.78 (q)		135.94 (q)	27		133.64 (q)[d]		136.71 (q)[e]
24	7.56dd (7.2, 0.8)	115.09 (t)	7.52d (7.8)	114.69 (t)		7.47dd (7.8, 1.0)	115.80 (t)	7.55dd (7.8, 1.0)	115.38 (t)
25	7.33ddd (8.0, 7.2, 1.0)	129.74 (t)	7.31t (7.8)	129.94 (t)	27	7.34td (7.8, 1.2)	130.23 (t)	7.36td (7.8, 1.2)	130.02 (t)
26	7.16ddd (8.0, 7.2, 0.8)	125.16 (t)	7.16t (7.8)	125.68 (t)	24	7.18td (7.8, 1.0)	125.73 (t)	7.22td (7.8, 1.0)	125.84 (t)
27	7.57dd (7.2, 1.0)	124.69 (t)	7.59t (7.8)	124.90 (t)		7.38dd (7.8, 1.2)	124.50 (t)	7.45dd (7.8, 1.0)	124.36 (t)
28		138.61 (q)		138.22 (q)	24, 26		137.30 (q)[d]		138.93 (q)[e]
29	1.33d (6.6)	17.85 (p)	1.32d (6.7)	17.22 (p)		1.19d	17.03 (p)	1.25d (6.5)	18.83 (p)
OMe	3.33s	50.80 (p)							
1-OH						3.60br s		3.18br s	
3-OH			c						
17-OH	4.55s		5.36s			5.30s		5.58s	

[a] ^{1}H Chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (J/Hz) in parentheses. [b] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT. [c] Not detected. [d,e,f] Interchangeable.

Table 4 ^1H and ^{13}C NMR spectral data of FQs F **7** and G **8** in $CDCl_3$

Position	**7** δ_H[a]	**7** δ_C	**7** LR ^1H-^{13}C COSY (H)	**8** δ_H	**8** δ_C
1		169.33 (q)[b]	14, 15		167.17 (q)
2	6.60br s			6.60br s	
3	3.14q (6.8, 0.3)	49.18 (t)	16	4.46qd (6.0, 4.2)	51.73 (t)
4		151.68 (q)	16		151.43 (q)
6		147.08 (q)	10		146.80 (q)
7	7.60dd (7.8, 1.0)	127.30 (t)	9	7.59dd (8.6, 1.0)	126.89 (t)
8	7.77td (7.8, 1.8)	134.70 (t)		7.80ddd (8.6, 7.8, 1.5)	134.95 (t)
9	7.53td (7.8, 1.0)	127.12 (t)		7.55td (7.8, 1.0)	127.07 (t)
10	8.37dd (7.8, 1.8)	126.85 (t)		8.39dd (7.8, 1.5)	126.99 (t)
11		120.24 (q)	9		120.04 (q)
12		160.82 (q)			160.89 (q)
14	5.68dd (5.2, 3.6)	57.53 (t)	15	5.55dd (5.2, 3.6)	56.93 (t)
15_A	3.64dd (15.0, 5.2)	27.04 (s)		3.71dd (15.0, 3.6)	27.07 (s)
15_B	3.71dd (15.0, 3.6)			3.78dd (15.0, 5.2)	
16	1.37d (6.8)	19.08 (p)		0.58d (6.0)	22.76 (p)
17		109.39 (q)	19		109.32 (q)
18	6.71d (2.5)	123.55 (t)	15	6.74d (2.0)	123.70 (t)
19	8.26br s			8.15br s	
20		135.98 (q)	18, 22, 24		135.69 (q)
21	7.30dd (8.0, 0.8)	111.22 (t)		7.27dd (8.2, 0.8)	111.07 (t)
22	7.13td (8.0, 0.8)	122.57 (t)	24	7.08ddd (8.2, 7.0, 1.4)	122.31 (t)
23	6.92td (8.0, 0.8)	120.01 (t)	21	6.85ddd (8.2, 7.0, 0.8)	119.91 (t)
24	7.40dd (8.0, 0.8)	118.48 (t)	22	7.31dd (8.2, 1.4)	118.59 (t)
25		127.30 (q)	15, 18, 19, 21		127.83 (q)

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constant (J/Hz) in parentheses. [b] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

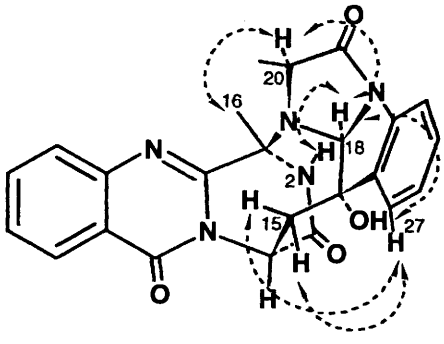

Fig. 5 Stereostructure of compound **10** and observed NOEs

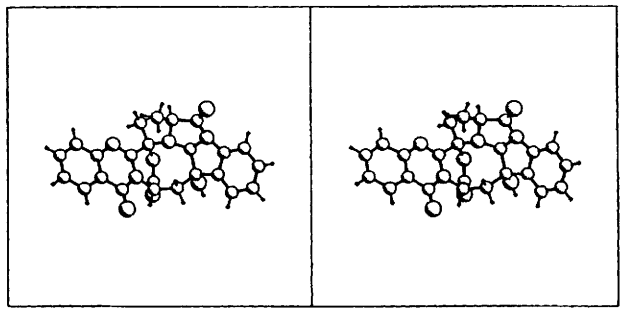

Fig. 6 X-Ray crystal structure for compound **10**

Comparison of the ^1H and ^{13}C NMR spectra of **11** with those of **10** revealed that the C-1 amide carbonyl signal (δ_C 172.69) in **10** was replaced by a hydroxymethine (δ_H 5.63, dd, J 7.8 and 2.0 Hz; δ_C 85.70) in **11** (Table 3). The coupling constants (7.8 and 2.0 Hz) between 1-H and 14-H, and 1-H and 2-H suggested that the hydroxy group is arranged equatorial. On the other hand, the ^1H and ^{13}C NMR spectra of **12** showed that the C-3 carbon signal (δ_C 137.52) and the C-16 proton signal (δ_H 2.66), having a long-range coupling (2.5 Hz) with 1-H, appeared shifted lowfield by 68.17 and 0.77 ppm relative to **11**, respectively, implying that a double bond, formed between C-3 and N-2 with cleavage of the C-3 and N-19 bond, exists in **12** (Table 3). The above evidence allowed assignments of structures **11** and **12**.

FQ F **7** and G **8** had the same molecular formula $C_{21}H_{18}N_4O_2$ established from HREIMS data. A close inspection of their ^1H and ^{13}C NMR spectra revealed that the partial structures [C(17)–C(29)] including the indoline skeletons of **1** and **2** were replaced by 3-substituted indoles in **7** and **8** (Table 4). This was supported by the EIMS fragments at m/z 228 (a^+) and 130 (c^+), arising from cleavage of the C(14)–C(15) bonds in **7** and **8**. When **7** and **8** were each treated with 0.4% KOH in MeOH, each of them underwent epimerization to afford a mixture of **7** and **8** in a 3:2 ratio as observed in the same reaction for **1** and **2**. In this case, other stereoisomers were not isolated. Treatment of **7** with 1% DCl in CD_3OD afforded a mixture of [$^2H_1$]-**7** and [$^2H_1$]-**8** deuteriated at C-3. This evidence implied that **7** and **8** are the stereoisomer at C-3 and **7** is thermodynamically more stable than **8**. The ^1H and ^{13}C NMR spectral differences between **7** and **8** showed close correspondence with those between **1** and **2** as follows. A chemical-shift difference of the carbon signals for C-1 and C-16 between **7** and **8**, and the coupling relationships (*ca.* 0.3 and 4.2 Hz) between 3-H and 2-H in **7** and **8** were similar to those of **1** and **2**. In addition, as observed in **1** and **2**, NOEs between 2-H and 3-H, and 2-H and 16-H were observed in **7**, while an NOE between 2-H and 3-H were observed in **8**. Also, a cross peak for a W-type of long-range coupling between 2-H and 14-H was found in their ^1H–^1H COSY experiments. Though not found between **1** and **2**, a quite large difference of the chemical shifts for 3-H and 16-H was observed between **7** and **8**. The 16-H signal in **8** was found shifted upfield by 0.79 ppm relative to that of **7**, while the 3-H signal in **7** appeared shifted upfield by 1.32 ppm relative to that of **8**. These quite large frequency shifts were considered to arise from long-range shielding by the indole ring, and hence suggested that 16-H in **8** and 3-H in **7** lie above the

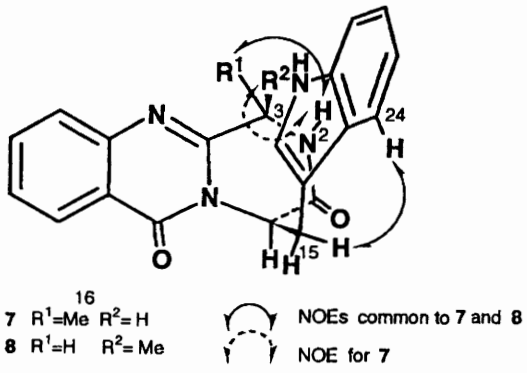

Fig. 7 Conformations of compounds **7** and **8** and observed NOEs

plane of the indole ring. In addition, NOEs between 24-H and 15-H_B were observed in both **7** and **8**. The evidence summarized above showed that the oxopiperazine rings [C(1)–C(4), N-13 and C-14] in **7** and **8** exist in a twist boat conformation with the 16-methyl group and the C(14)–C(15) bond, and 3-H and the C(14)–C(15) bond, respectively, in a coaxial arrangement; these results supported the relative stereostructures and conformations (Fig. 7) of **7** and **8** for FQs F and G.

The cytotoxic activities of the compounds obtained herein were examined in the P388 lymphocytic leukemia test system in cell culture, according to the method reported previously.[13] Compounds **1–3** and **7–10** exhibited moderate cytotoxicities (ED_{50} 6.1, 16.0, 52.0 and 13.5, 13.8, 14.6, 17.7 μg cm^{-3}, respectively).

The research group of Yamazaki have previously isolated tryptoquivaline related metabolites from a strain of *Aspergillus fumigatus* separated from rice.[14] Since no tryptoquivalines were produced by the fungal strain from the marine fish *P. japonicus*, this strain is supposed to be different from that separated by his group.

Experimental

General procedures

Mps were obtained on a Yanagimoto micromelting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin-Elmer FT-IR spectrometer 1720X. Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded on a JASCO J-500A spectrometer. NMR spectra were recorded at 27 °C on a Varian XL-300 spectrometer, operating at 300 and 75.4 MHz for ^1H and ^{13}C, respectively, in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The ^1H–^1H and ^1H–^{13}C COSY spectra were recorded on a Varian XL-300 spectrometer, and the NOESY spectra on a Bruker AM 400 spectrometer with the usual parameters. EIMS was determined using a Hitachi M-80 spectrometer. Liquid chromatography over silica gel (mesh 230–400) was performed in a medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401), using Shimpack PREP-ODS (25 cm × 20 mm i. d.) for separation of FQs and CROWNPAK CR for analysis of amino acids. Analytical TLC for amino acids was performed on HPTLC precoated plate CHIR with concentrating zone (Merck) with MeOH–water–MeCN (1 : 1 : 4).

Culturing and isolation of metabolites

A strain of *Aspergillus fumigatus* was initially separated from the gastrointestinal tract of the marine fish *Pseudolabrus japonicus*, collected in the Tanabe Bay of Japan. The content in the gastrointestinal tract was applied onto the surface of nutrient agar layered in a Petri dish. Serial transfers of one of the resulting colonies provided a pure strain of *A. fumigatus*. The fungal strain was grown in a liquid medium (40 dm^3) containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater adjusted to pH 7.5 for 2 weeks at 27 °C. The culture was filtered under suction and the mycelium collected was extracted thrice with MeOH. The combined extracts were evaporated under reduced pressure. The resulting extract (34 g) was partitioned between CH_2Cl_2 and water, and removal of solvents gave the CH_2Cl_2 fraction (16 g). This fraction was passed through Sephadex LH-20, using MeOH as the eluent. The third fraction (6.8 g) was chromatographed on a silica gel column with a CH_2Cl_2–MeOH gradient as the eluent and 3 fractions were collected. The CH_2Cl_2 eluate (2.0 g) yielded **9** (400 mg) after purification by HPLC [MeOH–water (7:3)]. The MeOH–CH_2Cl_2 (1:99) eluate (1.6 g) was purified by HPLC [MeOH–water (7:3)] to afford **1** (130 mg) and **10** (561 mg). The MeOH–CH_2Cl_2 (2:98) eluate (610 mg) gave **8** (17 mg), **7** (14 mg), **2** (39 mg) and **3** (21 mg) after purification by HPLC [MeOH–water (7:3)].

FQ A 1. This compound was obtained as a pale yellow powder, mp 178–182 °C, $[\alpha]_D$ −214.5 (*c* 0.47 in $CHCl_3$); λ_{max}(EtOH)/nm 208 (log ε 4.58), 226 (4.49), 233 (4.44), 256 (4.17), 265 (4.13), 277 (4.01), 305 (3.54) and 327 (3.44); ν_{max}(KBr)/cm^{-1} 3349 (OH, NH), 1680 (CON) and 1608 (Ar-C-C); *m/z* (EI) 445 (5%, M^+), 428 (36, M^+ − OH), 229 (13, aH^+), 228 (4, a^+), 217 (100, b^+), 199 (20, $b^+ - H_2O$) and 146 (22); *m/z* (HREI) 445.1769 (M^+; $C_{24}H_{23}N_5O_4$ requires 445.1750), 229.0862 (aH^+; $C_{12}H_{11}N_3O_2$ requires 229.0851), 228.0778 (a^+; $C_{12}H_{10}N_3O_2$ requires 228.0773) and 217.0974 (b^+; $C_{12}H_{13}N_2O_2$ requires 217.0977); CD λ (*c* 1.47 × 10^{-5} mol dm^{-3} in EtOH)/nm 230 (Δε −11.82), 245 (0), 251 (+2.11), 261 (0), 278 (−3.53), 284 (−2.99), 290 (−2.65), 297 (−2.31), 305 (−2.45), 312 (−1.90), 316 (−1.77), 330 (−0.20) and 334 (0). ^1H and ^{13}C NMR data are listed in Table 1.

FQ B 2. This compound was obtained as a pale yellow powder, mp 174–176 °C, $[\alpha]_D$ −196.6 (*c* 0.38 in $CHCl_3$); λ_{max}(EtOH)/nm 206 (log ε 4.77), 225 (4.69), 232 (4.65), 256 (4.38), 266 (4.32), 277 (4.24), 305 (3.79) and 317 (3.68); ν_{max}(KBr)/cm^{-1} 3350 (OH, NH), 1673 (CON) and 1604 (ArC-C); *m/z* (EI) 445 (5%, M^+), 229 (10, aH^+), 228 (2, a^+), 217 (100, b^+), 199 (6, $b^+ - H_2O$) and 146 (15); *m/z* (HREI) 445.1741 (M^+; $C_{24}H_{23}N_5O_4$ requires 445.1750); CD λ (*c* 2.22 × 10^{-5} mol dm^{-3} in EtOH)/nm 227 (Δε −11.04), 246 (0), 252 (+2.32), 259 (0), 280 (−7.36), 287 (−5.31), 297 (−4.36), 305 (−4.77), 318 (−3.13) and 329 (0). ^1H and ^{13}C NMR data are listed in Table 1.

FQ C 9. This compound was obtained as colourless prisms, mp 244–246 °C (from acetone), $[\alpha]_D$ −193.7 (*c* 0.31 in $CHCl_3$); λ_{max}(EtOH)/nm 207 (log ε 4.56), 225 (4.48), 260 (4.06), 271 (4.02), 282 (3.98), 304 (3.61) and 317 (3.50); ν_{max}(KBr)/cm^{-1} 3443, 3257 (NH), 1715 (CON) and 1611 (ArC-C); *m/z* (EI) 443 (70%, M^+), 372 (8), 256 (30), 228 (42, a^+), 227 (49, $a^+ - H$), 217 (20, b^+), 216 (31, $b^+ - H$), 199 (100, $b^+ - H_2O$) and 146 (86); *m/z* (HREI) 443.1591 (M^+; $C_{24}H_{21}N_5O_4$ requires 443.1594), 227.0697 ($a^+ - H$; $C_{12}H_9N_3O_2$ requires 227.0694) and 216.0904 ($b^+ - H$; $C_{12}H_{12}N_2O_2$ requires 216.0892); CD λ (*c* 4.02 × 10^{-5} mol dm^{-3} in EtOH)/nm 226 (Δε −15.84), 238 (0), 243 (+5.28), 247 (+3.77), 253 (+4.90), 269 (0), 303 (−13.58), 310 (−10.18) 314 (−10.56) and 328 (0). ^1H and ^{13}C NMR data are listed in Table 2.

FQ D 10. This compound was obtained as colourless prisms, mp 214–216 °C (from acetone), $[\alpha]_D$ +68.9 (*c* 0.27 in $CHCl_3$); λ_{max}(EtOH)/nm 205 (log ε 4.30), 225 (4.26), 232 (4.24), 254 (3.91), 265 (3.84), 276 (3.77), 304 (3.43) and 316 (3.54); ν_{max}(KBr)/cm^{-1} 3418 (OH, NH), 1703 (CON) and 1611 (ArC-C); *m/z* (EI) 443 (97%, M^+), 372 (10), 256 (40), 228 (53, a^+), 227 (78, $a^+ - H$), 217 (48, b^+), 199 (100, $b^+ - H_2O$) and

146 (76); *m/z* (HREI) 443.1588 (M^+) ($C_{24}H_{21}N_5O_4$ requires 443.1594); CD λ (*c* 4.33 × 10^{-5} mol dm^{-3} in EtOH)/nm 232 ($\Delta\varepsilon$ −1.75), 235 (0), 247 (+6.12), 258 (0), 268 (−4.90), 275 (−5.25), 294 (−2.45), 303 (−2.80), 310 (−2.10), 315 (−2,10) and 326 (0). ^1H and ^{13}C NMR data are listed in Table 2.

FQ E 3. This compound was obtained as a pale yellow powder, mp 168–172 °C, $[\alpha]_D$ −143.3 (*c* 0.18 in $CHCl_3$); λ_{max}(EtOH)/nm 210 (log ε 4.54), 226 (4.48), 233 (4.44), 256 (4.16), 278 (4.06), 304 (3.62) and 317 (3.52); ν_{max}(KBr)/cm^{-1} 3427 (OH, NH), 1685 (CON) and 1608 (Ar C-C); *m/z* (FAB) 476 (11 %, MH^+), 442 (8), 228 (18, a^+), 217 (38, b^+), 199 (34, $b^+ - H_2O$), 154 (100) and 136 (90); CD λ (*c* 2.59 × 10^{-5} mol dm^{-3} in EtOH)/nm 219 ($\Delta\varepsilon$ −2.69), 221 (−2.34), 233 (−7.73), 245 (0), 251 (+1.70), 259 (0), 278 (−3.98), 290 (−2.87), 296 (−2.34), 312 (−2.58), 310 (−2.05), 314 (−1.99) and 329 (0). ^1H and ^{13}C NMR data are listed in Table 3.

FQ F 7. This compound was obtained as a pale yellow powder, mp 88–90 °C, $[\alpha]_D$ −411.2 (*c* 1.36 in $CHCl_3$); λ_{max}(EtOH)/nm 207 (log ε 4.71), 219 (4.73), 270 (4.13), 277 (4.13), 289 (3.99), 306 (3.78) and 320 (3.66); ν_{max}(KBr)/cm^{-1} 3264 (NH), 1679 (CON) and 1610 (ArC-C); *m/z* (EI) 358 (3%, M^+), 229 (4, aH^+), 228 (1, a^+) and 130 (100, c^+); *m/z* (HREI) 358.1436 (M^+; $C_{21}H_{18}N_4O_2$ requires 358.1430), 228.0779 (a^+; $C_{12}H_{10}N_3O_2$ requires 228.0773) and 130.0656 (c^+; C_9H_8N requires 130.0657). ^1H and ^{13}C NMR data are listed in Table 4.

FQ G 8. This compound was obtained as a pale yellow powder, mp 119–121 °C, $[\alpha]_D$ −462.8 (*c* 0.61 in $CHCl_3$); λ_{max}(EtOH)/nm 208 (log ε 4.61), 220 (4.67), 273 (4.14), 278 (4.13), 288 (4.01), 307 (3.66) and 323 (3.49); ν_{max}(KBr)/cm^{-1} 3278 (NH), 1680 (CON) and 1610 (ArC-C); *m/z* (EI) 358 (11%, M^+), 229 (3, aH^+), 228 (1, a^+) and 130 (100, c^+); *m/z* (HREI) 358.1428 (M^+; $C_{21}H_{18}N_4O_2$ requires 358.1430), 228.0779 (a^+; $C_{12}H_{10}N_3O_2$ requires 228.0773) and 130.0656 (c^+; C_9H_8N requires 130.0657). ^1H and ^{13}C NMR data are listed in Table 4.

Epimerization of FQ A 1 and FQ B 2

FQA **1** (31 mg) was dissolved in a solution (5 cm^3) of 40% aqueous KOH–MeOH (1:99), and the reaction mixture left at room temperature for 16 h. The mixture was diluted with water, neutralized with HCl and extracted with CH_2Cl_2. Evaporation of the extract gave a 4:2:2:1 mixture of **1**, **2**, **5** and **6** as estimated by HPLC. The mixture yielded **1** (12 mg), **2** (5 mg), **5** (6 mg) and **6** (3 mg) after purification by HPLC [MeOH–water (6.5:3.5)]. Compound **5**: ν_{max}(KBr)/cm^{-1} 3347 (OH, NH), 1677 (CON) and 1608 (ArC-C); *m/z* (EI) 445 (5 %, M^+); *m/z* (HREI) 445.1735 (M^+; $C_{24}H_{23}N_5O_4$ requires 445.1750). Compound **6**: ν_{max}(KBr)/cm^{-1} 3352 (OH, NH), 1675 (CON) and 1605 (ArC-C); *m/z* (EI) 445 (4 %, M^+); *m/z* (HREI) 445.1740 (M^+; $C_{24}H_{23}N_5O_4$ requires 445.1750). ^1H and ^{13}C NMR data for compounds **5** and **6** are listed in Table 1.

The same reaction with FQ B **2** (5 mg) afforded a 4:2:2:1 mixture of **1**, **2**, **5** and **6** as estimated by HPLC.

Deuterium labelling of FQ A 1 and FQ B 2

(i) FQ A **1** (8 mg) was dissolved in a solution (1 cm^3) of 40% aqueous KOD–MeOD (1:99) and the reaction mixture left at room temperature for 16 h. The mixture was diluted with water, neutralized with HCl and extracted with CH_2Cl_2. Evaporation of the solvent gave a mixture of [$^2H_2$]-**1**, [$^2H_2$]-**2**, [$^2H_2$]-**5** and [$^2H_2$]-**6**, labelled at both C-3 and C-14 with deuterium, in a ratio of 4:2:2:1 as estimated by HPLC. Purification of the mixture by HPLC [MeOH–water (6.5:3.5)] afforded [$^2H_2$]-**1** (3 mg), [$^2H_2$]-**2** (1.3 mg), [$^2H_2$]-**5** (1.2 mg) and [$^2H_2$]-**6** (1 mg). The ^1H NMR spectra of [$^2H_2$]-**1**, [$^2H_2$]-**2**, [$^2H_2$]-**5** and [$^2H_2$]-**6** were, respectively, identical to those of **1**, **2**, **5** and **6** except that the 3-H and 14-H signals disappeared and the 16-H and 15-H signals appeared as a singlet and a pair of doublet, respectively. Compound [$^2H_2$]-**1**: *m/z* (EI) 447 (2%, M^+). Compound [$^2H_2$]-**2**: *m/z* 447 (4, M^+). Compound [$^2H_2$]-**5**: *m/z* 447 (3, M^+). Compound [$^2H_2$]-**6**: *m/z* 447 (3, M^+). (ii) FQ A **1** (54 mg) was dissolved in a solution (1.2 cm^3) of 10% aqueous DCl–CD_3OD (3:20) and the mixture was left at room temperature for 3 h. After this it was evaporated under reduced pressure, and the residue was diluted with water, neutralized with NH_4OH and extracted with CH_2Cl_2. Evaporation of the extract followed by purification of the residue by HPLC [MeOH–water (7:3)] afforded [$^2H_1$]-**2** (2 mg), labelled at C-3 with deuterium, and a mixture (30 mg) of **1** and [$^2H_2$]-labelled at C-3 with deuterium. The ^1H NMR spectrum of [$^2H_1$]-**2** was identical with that of **2** except for the absence of the 3-H signal and appearance of the 16-H signal as a singlet. Compound [$^2H_1$]-**2**: *m/z* (HREI) 446.1799 (M^+) ($C_{24}H_{22}DN_5O_4$ requires 446.1810).

Formation of FQ A 1 from FQ C 9

$NaBH_4$ (3 mg) was added to a solution of FQ C **9** (5 mg) in diglyme (0.2 cm^3). The reaction mixture was left at room temperature for 30 min and then concentrated under reduced pressure. The residue was diluted with water and extracted with CH_2Cl_2. The extract was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column with a CH_2Cl_2–MeOH gradient as the eluent. The MeOH–CH_2Cl_2 (1:99) eluate afforded **1** (3 mg), which was identified by comparison with an authentic material.

Treatment of FQ D 10 with $NaBH_4$

$NaBH_4$ (20 mg) was added to a solution of FQ D **10** (24 mg) in diglyme (0.5 cm^3). The mixture was left at room temperature for 2 h and then concentrated under reduced pressure. The residue was diluted with water and extracted with CH_2Cl_2. Evaporation of the extract afforded **11** (15 mg) as a crude solid, purification of which by HPLC [MeOH–water (7:3)] gave **12** (10 mg) as a pale yellow solid. Compound **12**: ν_{max}(KBr)/cm^{-1} 3442, 3346 (OH, NH), 1713, 1680 (CON), 1647 (C=N) and 1608 (ArC-C); *m/z* (FAB) 446 (3%, MH^+), 428 (100, $MH^+ - H_2O$), 227 (14, $a^+ - H$), 217 (20, b^+) and 199 (81, $b^+ - H_2O$); *m/z* (HREI) 427.1639 ($M^+ - H_2O$; $C_{24}H_{21}N_5O_3$ requires 427.1644). ^1H and ^{13}C NMR data for **11** and **12** are listed in Table 3.

Formation of L-(+)-alanine from FQs C 9 and D 10

A solution of FQ C **9** (5 mg) in 6 mol dm^{-3} HCl (1 cm^3) was heated at 100 °C for 3.5 h after which the reaction mixture was concentrated to dryness under reduced pressure. The water-soluble fraction of the residue was subjected to HPTLC pre-coated plates CHIR (Merck) [MeOH–water–Me_2CO (1:1:4)] and analytical HPLC [aqueous $HClO_4$, pH 1.5] to detect L-(+)-alanine.

The same reaction with FQ D **10** (5 mg) followed by HPTLC and HPLC analysis detected L-(+)-alanine.

Formation of FQ E 3 from FQs C 9 and D 10

FQ C **9** (10 mg) was dissolved in a solution of 10% aqueous HCl-MeOH (1:5; 0.6 cm^3). The reaction mixture was left at room temperature for 30 min, and then diluted with water, neutralized with NH_4OH and extracted with CH_2Cl_2. Evaporation of the extract followed by silica gel column chromatography using a CH_2Cl_2–MeOH gradient as the eluent afforded **3** (4 mg) and **4** (2 mg), the former being identified by comparison with an authentic sample. Compound **4**: ν_{max}(KBr)/cm^{-1} 3377 (OH, NH), 1675 (CON) and 1611 (ArC-C). ^1H and ^{13}C NMR data are listed in Table 3.

The same reaction with FQ D **10** (30 mg) gave **3** (15 mg) and **4** (7 mg).

Epimerization of FQs F 7 and G 8

Using the same procedure as above with **1** and **2**, FQ F **7** (10 mg) was treated with a solution of 40% aqueous KOH–MeOH (1:99; 1 cm^3) for 16 h to yield a 3:2 mixture of **7** and **8** as estimated by HPLC. The mixture was subjected to HPLC [MeOH–water (6.5:3.5)] to afford **7** (4 mg) and **8** (2 mg), identical with authentic samples.

The same reaction with FQ G **8** (2 mg) yielded a 3:2 mixture of **7** and **8** as estimated HPLC.

Deuterium labelling of FQs F 7 and G 8

FQ F **7** (6 mg) was dissolved in a solution of 10% aqueous DCl-CD_3OD (3:20; 1 cm^3) and the mixture was left at room temperature. Work-up by the manner described above with **1** gave a 2:1 mixture of [3-$^2H_1$]-**7** and [3-$^2H_1$]-**8**. The mixture was subjected to HPLC [MeOH–water (6.5:3.5)] to afford [3-$^2H_1$]-**7** (3 mg) and [3-$^2H_1$]-**8** (1 mg). Compound [3-$^2H_1$]-**7**: *m/z* (EI) 359 (24%, M^+). Compound [3-$^2H_1$]-**8**: *m/z* (EI) 359 (22, M^+). The ^1H NMR spectra of [3-$^2H_1$]-**7** and [3-$^2H_1$]-**8** were identical with those of **7** and **8** respectively except that the 3-H signal disappeared and the 16-H signal appeared as a singlet.

X-Ray crystallography of FQ D 10

FQ D **10** was crystallized from acetone–methanol by the vapour diffusion method. Crystal data: $2(C_{24}H_{21}N_5O_4 \cdot H_2O)$, M = 923.2, monoclinic, $P2_1$, a = 7.991(1), b = 28.414(3), c = 11.589(2) Å, β = 109.88(1)°, V = 2474.6(6) $Å^3$, Z = 4, d_x = 1.233 g cm^{-3}, $F(000)$ = 928, μ(Cu-Kα) = 6.87 cm^{-1}. Data collection was performed by a Rigaku AFC-5 using graphite-monochromated radiation (λ = 1.5418 Å). Total 4301 reflections were collected until θ = 62.1°, in which 3972 reflections were observed ($I > 2\sigma(I)$). The crystal structure was solved by the direct method using SHELXS-86.[15] The structure was refined by the full matrix least-squares method on F using SHELXL-93.[16] Water molecules were found from the difference Fourier map, and the molecule of **10** was finally monohydrated in the crystal. The chirality of C-20 was matched with the C_α atom of L-(+)-alanine. In the structure refinements, non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were calculated on the geometrically ideal positions by the 'ride on' method, and were included in the calculation of structure factors with isotropic temperature factors. In the final stage, R = 0.0838, R_w = 0.2288 and S = 1.145 were obtained.

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References

1 M. Yotsu, T. Yamazaki, Y. Meguro, A. Endo, M. Murata, H. Naoki and T. Yasumoto, *Toxicon*, 1987, **25**, 225; T. Yasumoto, D. Yasumura, M. Yotsu, T. Michishita, A. Endo and Y. Kotaki, *Agric. Biol. Chem.*, 1986, **50**, 793; T. Noguchi, J. K. Jeon, O. Arakawa, H. Sugita, Y. Deguchi, Y. Shida and K. Hashimoto, *J. Biochem. (Tokyo)*, 1986, **99**, 311.
2 T. Kosuge, K. Tsuji, K. Hirai and T. Fukuyama, *Chem. Pharm. Bull.*, 1985, **33**, 3059.
3 M. Kodama, T. Ogata and S. Sato, *Agric. Biol. Chem.*, 1988, **52**, 1075.
4 R. E. Moore, P. Helfrich and G. M. L. Patterson, *Oceanus*, 1989, **25**, 54.
5 C. Takahashi, T. Takada, T. Yamada, K. Minoura, K. Uchida, E. Matsumura and A. Numata, *Tetrahedron Lett.*, 1994, **35**, 5013.
6 A. Numata, C. Takahashi, Y. Ito, T. Takada, K. Kawai, Y. Usami, E. Matsumura, M. Imachi, T. Ito and T. Hasegawa, *Tetrahedron Lett.*, 1993, **34**, 2355.
7 C. Takahashi, A. Numata, Y. Ito, E. Matsumura, H. Araki, H. Iwaki and K. Kushida, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1859.
8 C. Takahashi, A. Numata, E. Matsumura, K. Minoura, H. Eto, T. Shingu, T. Ito and T. Hasegawa, *J. Antibiotics*, 1994, **47**, 1242.
9 C. Takahashi, Y. Takai, Y. Kimura, A. Numata, N. Shigematsu and H. Tanaka, *Phytochemistry*, 1995, **38**, 155.
10 C. Takahashi, K. Minoura, T. Yamada, A. Numata, K. Kushida, T. Shingu, S. Hagishita, H. Nakai, T. Sato and H. Harada, *Tetrahedron*, 1995, **51**, 3483.
11 A. Numata, C. Takahashi, T. Matsushita, T. Miyamoto, K. Kawai, Y. Usami, E. Matsumura, M. Inoue, H. Ohishi and T. Shingu, *Tetrahedron Lett.*, 1992, **33**, 1621.
12 M. Karplus, *J. Chem. Phys.*, 1953, **30**, 11; M. Karplus, *J. Am. Chem. Soc.*, 1963, **85**, 2870; A. A. Bothner-By, *Adv. Magn. Reson.*, 1965, **1**, 195.
13 A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae and E. Fujita, *Chem. Pharm. Bull.*, 1989, **37**, 648.
14 M. Yamazaki, H, Fujimoto and E. Okuyama, *Chem. Pharm. Bull.*, 1977, **25**, 2554; M. Yamazaki, H. Fujimoto and E. Okuyama, *Chem. Pharm. Bull.*, 1978, **26**, 111; M. Yamazaki, E. Okuyama and Y. Maebashi, *Chem. Pharm. Bull.*, 1979, **27**, 1611.
15 G. M. Sheldrick, SHELXS-86, Program for the Solution of Crystal Structures, University of Gottingen, Gottingen, 1986.
16 G. M. Sheldrick, SHELXS-93, Program for the Refinement of Crystal Structures from Diffraction Data, University of Gottingen, Gottingen, 1993.

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