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Chemistry of Tremorogenic Metabolites. I. Fumitremorgin A from *Aspergillus fumigatus*

MIKIO YAMAZAKI, HARUHIRO FUJIMOTO, and TAKAO KAWASAKI

Research Institute for Chemobiodynamics, Chiba University[1]

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Two new indolic metabolites, fumitremorgin A and B, were isolated from the fungus *Aspergillus fumigatus*. These two metabolites caused severe tremor and convulsion in experimental animals. The structure of fumitremorgin A, including the stereochemistry, was determined to be **1** mainly on the basis of the spectral data for the compound and some derivatives obtained from it. The stereochemical factors affecting the appearance of tremorogenic activity in fumitremorgin A and related compounds are discussed.

Keywords——fumitremorgin A; structure; fungal metabolite; *Aspergillus fumigatus* tremorogenic activity; carbon nuclear magnetic resonance spectrum (CMR)

We have previously reported the isolation of some toxigenic strains of *Aspergillus fumigatus* from rice and home-made miso (soybean paste). Upon intraperitoneal administration of an ethyl acetate extract of the fungus, experimental animals such as mice, rats and rabbits exhibited sustained tremor with intermittant convulsion.[2] Two major metabolites were isolated from the extract and confirmed to be the tremorogenic principles of the fungus. These metabolites were designated as fumitremorgin A (**1**) and B (**2**).[3] In this paper, we report in detail the isolation and structure determination of **1**.[4] The characteristic features of the tremorogenic activity of **1** and related compounds are also described.

A strain of *Aspergillus fumigatus* (strain 0011) was grown in stationary culture on sterilized rice. On silica gel column chromatography, crude crystals of fumitremorgin A and B were obtained from the ethyl acetate extract of the fungus, together with some other non-tremorogenic metabolites.[5] When the fungus was cultured in the modified Czapek-Dox solution, **1** was the major product, with only a trace of **2**. Purification of **1** and **2** was carried out by repeated recrystallization.

Fumitremorgin A (**1**), colorless needles, mp 206—209°, $[\alpha]_D^{10}$ +61° (c=0.80, acetone), $C_{32}H_{41}N_3O_7$ (M^+: m/e 579), caused sustained tremor with intermittant convulsion and was often fatal to mice, rats and rabbits after severe tonic-clonic convulsion. The pharmacological findings suggested that **1** and **2** have a significant motor-stimulating effect on the central nervous system.[6] The LD_{50} value of **1** to mice by intravenous administration was 185 μg/kg.[7] It was also found that administration of **1** caused a significant increase of the serotonin content and a decrease of the γ-aminobutyric acid (GABA) content of the brain in mice.[7]

Fumitremorgin A (**1**) exhibited ultraviolet (UV) and infrared (IR) absorption spectra very similar to those of fumitremorgin B (**2**), and the structure of **2** has already been shown

1) Location: *1-8-1, Inohana, Chiba, 280 Japan.*
2) M. Yamazaki, Y. Horie, S. Suzuki, H. Fujimoto, Y. Maebayashi, K. Miyaki, and S. Hatakeyama, *J. Food Hyg. Soc. Japan*, **12**, 370 (1971).
3) M. Yamazaki, S. Suzuki, and K. Miyaki, *Chem. Pharm. Bull.*, **19**, 1739 (1971).
4) The structure determination of fumitremorgin A has been reported in brief from our laboratory in *Tetrahedron Lett.*, **1975**, 1241.
5) These metabolites were demonstrated to be tryptoquivaline-related compounds and were found to be non-tremorogenic in our laboratory. See *Tetrahedron Lett.*, **1976**, 2861 and *Chem. Pharm. Bull.*, **25**, 2554 (1977); **26**, 111 (1978); **27**, 1611 (1979).
6) T. Kuga, Y. Watanabe, and J. Naito, *Japan J. Pharmacol.*, **24**, suppl., 62 (1974).
7) M. Yamazaki, S. Suzuki, and K. Kukita, *J. Pharm. Dyn.*, **2**, 119 (1979).

to contain a dioxopiperazine ring, consisting of tryptophan and proline.[8] Proline was actually obtained from **1** as well as from **2** as a product of acidic hydrolysis. The proton magnetic resonance (PMR) spectrum of **1** indicated that it contains one hydroxyl and three isopentenyl groups (Table I). The presence of an additional isopentenyl group in **1** compared with **2** was suggested by the difference in the molecular formulae between **1** and **2** *i.e.*, $C_5H_8O_2$.

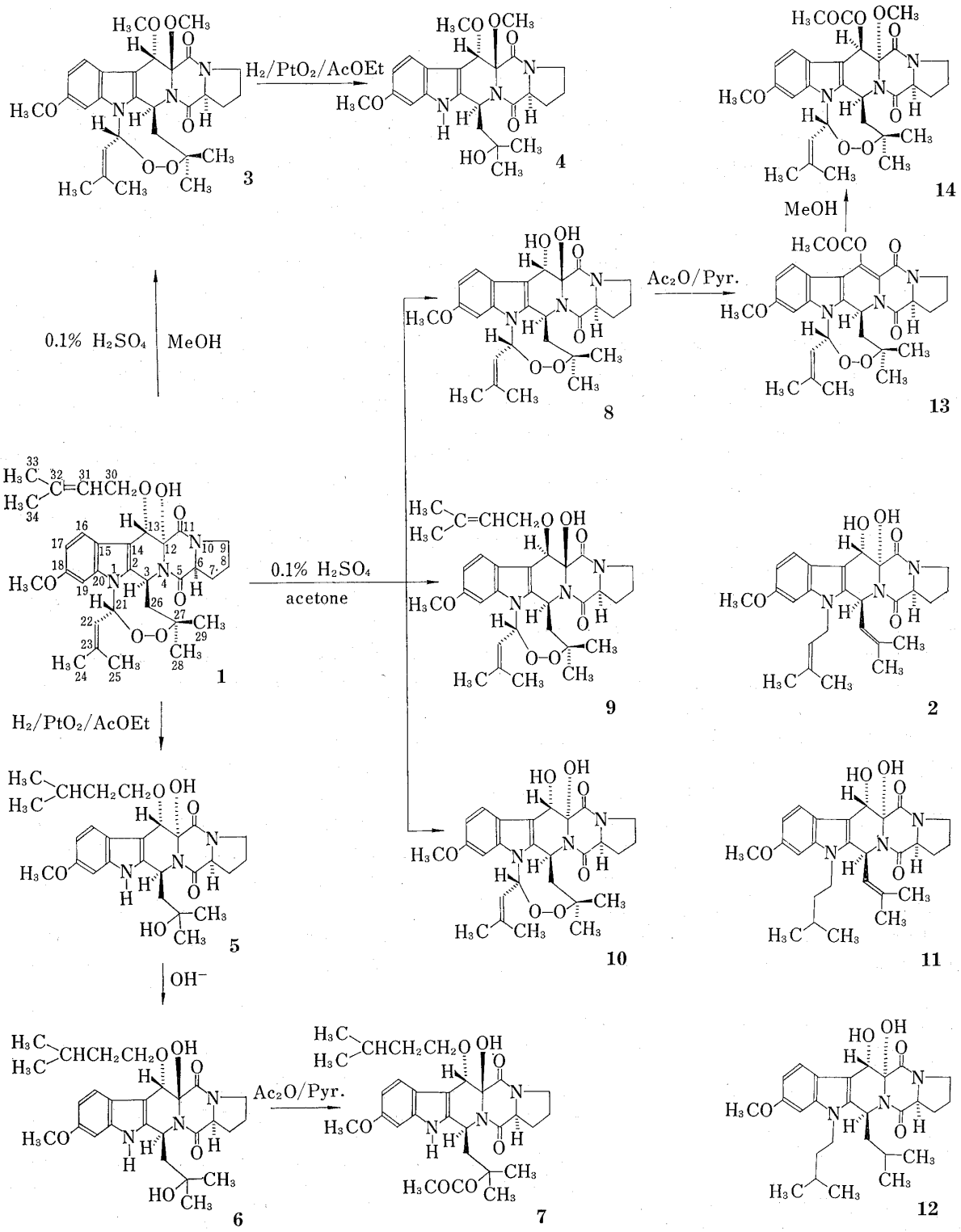

Chart 1

On refluxing **1** with 0.1% H_2SO_4 in methanol, a degradation product (**3**) was obtained (Chart 1). The UV and IR spectra of **3** were very similar to those of **1**, except for the absence of the hydroxyl absorption in the IR spectrum. In the PMR spectrum of **3**, the signals of two methoxyl groups appeared at 3.16 and 3.38 ppm, whereas the signals of one isopentenyl group observed in the spectrum of **1** were absent.

On hydrogenation with PtO_2, **3** gave a product (**4**) (Chart 1). The UV spectrum of **4** was slightly different from those of **1**, **2** and **3**, but was very similar to that of a model compound, 7-methoxytetrahydrocarbazole, indicating that **4** contains a 6-methoxyindole ring system having no substitution on the nitrogen atom (N-1). The disappearance of one isopentenyl group from **3** and the appearance of a tertiary OH (2.50 ppm) and an indolic NH (10.00 ppm) in **4** were noted in the PMR spectra during the hydrogenation of **3** to obtain **4**. The absorption bands of OH or NH appeared at about 3370 cm^{-1} in the IR spectrum of **4**, supporting the above findings. Hydrogenation of **1** itself gave a similar result and afforded a hydrogenolysis product (**5**). However, this product (**5**) was rather unstable and was gradually converted into another compound (**6**) on standing for a few days in an organic solvent or on a silica gel thin layer chromatography plate. On treatment with alkali in ethanol, **5** was converted into **6** more smoothly. A monoacetate (**7**) was formed from **6** by acetylation. The product (**6**) was considered to be a stereoisomer of **5** based on a comparison of the PMR, IR and UV spectral data of these compounds.

It is known that secondary-tertiary dialkyl peroxides generally afford secondary and tertiary alcohols on catalytic hydrogenation.[9] In fact, **1** afforded secondary and tertiary alcohols on catalytic hydrogenation. The secondary alcohol was subsequently removed from the nitrogen atom (N-1), perhaps due to the effect of the neighboring N-1. The structure of fumitremorgin A was thus proposed to be **1**, as shown in Chart 1.

The difference between the chemical shifts of two methyl groups (0.99 and 2.00 ppm) in the PMR spectrum of **1** could be explained by postulating that one methyl group protrudes upwards from the plane of the eight-membered peroxide-containing ring, and the other downwards, respectively.

On refluxing **1** with 0.1% H_2SO_4 in acetone, a major product (**8**) and two minor products (**9** and **10**) were obtained (Chart 1). The physicochemical and spectral properties of **10** were very similar to those of verruculogen.[10,11] Direct comparison of **10** with an authentic specimen of verruculogen confirmed their identity. From the spectral data, **8** was assumed to be a stereoisomer of **10**, and **9** to be an isomer of **1**. Clardy and his coworkers reported the results of an X-ray analysis of **1**,[12] and they were identical with our conclusion.

It is interesting that **5**, **9**, **10** and two hydrogenated derivatives of **2**(dihydrofumitremorgin B (**11**) and tetrahydrofumitremorgin B (**12**)[8]) are tremorogenic to experimental animals, while the others are not. A clear difference in the pattern of the circular dichroism (CD) spectra was seen between these two groups of the compounds. In the IR spectra, the amide-carbonyl bands of non-tremorogenic compounds (**3**, **4**, **6**, **7** and **8**) were observed at rather lower wave number, whereas those of tremorogenic compounds (**1**, **2**, **5**, **10**, **11** and **12**) were observed at higher wave number, as indicated in Table II.

The conformation of the 2,5-dioxopiperazine ring in **1**, **2** and **10** has already been shown to be "boat-form" by X-ray analysis.[8,11,12] Therefore, the appearance of the amide bands

8) The structure of **2** has been determined in our laboratory by X-ray analysis and was briefly reported in *J. C. S. Chem. Commun.*, **1974**, 408 and *Tetrahedron Lett.*, **1975**, 27. The results will be described in detail elsewhere.

9) *a*) G.D. Laubach, B.C. Schreiber, E.J. Agnello, and K.J. Brunings, *J. Am. Chem. Soc.*, **78**, 4746 (1956); *b*) E.J. Agnello, R. Pinson Jr., and G.D. Laubach, *J. Am. Chem. Soc.*, **78**, 4756 (1956).

10) R.J. Cole, and J.W. Kirksey, *J. Agr. Food Chem.*, **21**, 927 (1973).

11) J. Fayos, D. Lokensgard, J. Clardy, R.J. Cole, and J.W. Kirksey, *J. Am. Chem. Soc.*, **96**, 6785 (1974).

12) N. Eickman, J. Clardy, R.J. Cole, and J.W. Kirksey, *Tetrahedron Lett.*, **1975**, 1051.

Table I. PMR Data for Fumitremorgin A (1) and Its Derivatives;

Part. str.	Position No.	Compound 1	3	4	5	6
CH_3–CH(C)(C)	33, 34	—	—	—	0.99 (6H, d.)	0.77 (3H, d.) 0.83 (3H, d.)
CH_3–C(C)–O	28, 29	0.99 (3H, s.) 2.00 (3H, s.)	1.14 (3H, s.) 2.00 (3H, s.)	1.42 (3H, s.) 1.71 (3H, s.)	1.12 (3H, s.) 1.44 (3H, s.)	1.28 (3H, s.) 1.48 (3H, s.)
CH_3–C(=C)–C	24, 25 33, 34	1.71 (6H, s.) 1.81 (6H, s.)	1.68 (3H, s.) 1.76 (3H, s.)	—	—	—
CH_3–CO–O	13, 27	—	—	—	—	—
CH_3–O–C	12, 13	—	3.16 (3H, s.) 3.38 (3H, s.)	3.22 (3H, s.) 3.42 (3H, s.)	—	—
	18	3.84 (3H, s.)	3.85 (3H, s.)	3.86 (3H, s.)	3.82 (3H, s.)	3.80 (3H, s.)
C–CH_2–C C–CH(C)–C	7, 8, 26, 31, 32	1.60—2.20 (4H, m.) 2.40 (2H, m.)	1.60—2.30 (4H, m.) 2.50 (2H, m.)	1.60—2.30 (4H, m.) 2.44 (2H, m.)	1.60—2.20 (7H, m.) 2.40 (2H, m.)	1.20—2.30 (7H, m.) 2.44 (2H, m.)
C–CH_2–N	9	3.63 (2H, m.)	3.78 (2H, m.)	3.80 (2H, m.)	3.62 (2H, t.)	3.60 (2H, m.)
C–CH_2–O	30	4.71 (2H, br. q.)	—	—	4.10—4.70 (2H, m.)	3.60 (2H, m.)
C–CH=C	22	5.02 (H, br. d.)	4.74 (H, br. d.)	—	—	—
	31	5.60 (H, br. t.)	—	—	—	—
C–CH(N)–C	6	5.06 (H, t.)	4.26 (H, q.)	4.28 (H, q.)	4.10—4.70 (H, m.)	4.30 (H, m.)
	3	6.13 (H, br. d.)	6.61 (H, d.)	6.00 (H, br. d.)	5.51 (H, t.)	6.04 (H, br. q.)
C–CH(O)–C	13	5.50 (H, s.)	5.00 (H, s.)	5.03 (H, s.)	5.52 (H, s.)	4.77 (H, s.)
C–CH(O)–N	21	6.62 (H, d.)	6.74 (H, d.)	—	—	—
H–O–C	12, 13, 27	4.48 (H, s.)	—	2.50 (H, br. s.)	2.22 (H, br. s.) 4.53 (H, s.)	2.94 (H, br. s.) 4.64 (H, br. s.)
Aromatic H	19	6.59 (H, d.)	6.68 (H, d.)	6.84 (H, d.)	6.85 (H, br. s.)	6.85 (H, d.)
	17	6.82 (H, q.)	6.86 (H, q.)	6.79 (H, q.)	6.81 (H, q.)	6.76 (H, q.)
	16	7.68 (H, d.)	7.50 (H, d.)	7.44 (H, d.)	7.59 (H, d.)	7.38 (H, d.)
H–N(C)–C	1	—	—	10.00 (H, br. s.)	9.18 (H, br. s.)	9.72 (H, br. s.)

a) taken from reference 11).

δ (ppm) from Tetramethylsilane (TMS) in $CDCl_3$

7	8	9	10[a)]	13	14
0.81 (3H, d.) 0.87 (3H, d.)	—	—	—	—	—
1.35 (3H, s.) 1.69 (3H, s.)	1.13 (3H, s.) 2.00 (3H, s.)	1.01 (3H, s.) 2.00 (3H, s.)	1.01 (3H, s.) 1.99 (3H, s.)	1.07 (3H, s.) 1.96 (3H, s.)	1.13 (3H, s.) 2.02 (3H, s.)
—	1.66 (3H, s.) 1.69 (3H, s.)	1.26 (3H, s.) 1.73 (6H, s.) 1.84 (3H, s.)	1.72 (6H, s.)	1.65 (3H, s.) 1.69 (3H, s.)	1.70 (3H, s.) 1.75 (3H, s.)
1.92 (3H, s.)	—	—	—	1.92 (3H, s.)	1.96 (3H, s.)
—	—	—	—	—	3.45 (3H, s.)
3.85 (3H, s.)	3.80 (3H, s.)	3.87 (3H, s.)	3.82 (3H, s.)	3.79 (3H, s.)	3.86 (3H, s.)
1.28—2.30 (7H, m.) 2.37 (2H, m.)	1.60—2.20 (4H, m.) 2.48 (2H, m.)	1.60—2.20 (4H, m.) 2.40 (2H, m.)	1.80—2.60 (6H, m.)	1.60—2.20 (4H, m.) 2.40 (2H, m.)	1.60—2.20 (4H, m.) 2.42 (2H, m.)
3.70 (2H, m.)	3.60 (2H, m.)	3.61 (2H, t.)	3.61 (2H, t.)	3.50 (2H, m.)	3.50, 3.87 (2H, m.)
3.70 (2H, m.)	—	4.04 (2H, q.)	—	—	—
—	4.74 (H, br. d.)	5.00 (H. d.)	5.05 (H, d.)	4.68 (H, d.)	4.75 (H, br. d.)
—	—	3.10 (H, m.)	—	—	—
4.26 (H, m.) 5.91 (H, br. d.)	4.36 (H, q.) 6.60 (H, d.)	4.74 (H, t.) 6.20 (H, m.)	4.48 (H, m.) 6.05 (H, d.)	4.24 (H, m.) 6.44 (H, d.)	4.27 (H, q.) 6.45 (H, d.)
5.07 (H, s.)	5.08 (H, s.)	4.88 (H, s.)	5.64 (H, d.)	—	5.02 (H, s.)
—	6.74 (H, d.)	6.66 (H, d.)	6.63 (H, d.)	6.66 (H, d.)	6.70 (H, d.)
2.37 (H, br. peak)	2.54 (H, br. s.) 4.52 (H, s.)	4.10 (H, s.)	4.13 (H, s.) 4.79 (H, d.)	—	—
6.88 (H, br. s.) 6.83 (H, q.) 7.47 (H, d.)	6.56 (H, d.) 6.76 (H, q.) 7.42 (H, d.)	6.68 (H, d.) 6.85 (H, q.) 7.61 (H, d.)	6.58 (H, d.) 6.81 (H, q.) 7.89 (H, d.)	6.58 (H, br. s.) 6.80 (H, q.) 7.74 (H, d.)	6.64 (H, d.) 6.86 (H, q) 7.50 (H, d.)
10.00 (H, br. s.)	—	—	—	—	—

Table II. Amide-Carbonyl IR Data for Fumitremorgin A(**1**), B(**2**), and Their Derivarives; cm^{-1} in KBr tablets

a) 2,5-Dioxopiperazine Ring: Boat Conformation

position No.	compound **1**	**10**	**14**	**2**	**5**	**11**	**12**
5	1682	1683	1682	1688	1670[a]	1690	1690
11	1690	1663[a]	1686	1668[a]	1678	1665[a]	1665[a]

b) 2,5-Dioxopiperazine Ring: Chair Conformation

position No.	compound **3**	**8**	**9**	**4**	**6**	**7**
5	1655	1653	1657	1655[a]	1655[a]	1678
11	1678	1665[a]	1683	1665	1664	1668

a) hydrogen bonding.

in the lower wave number region in the IR spectra of non-tremorogenic compounds suggests that the conformation of the dioxopiperazine ring in these compounds may be "chair", releasing the strain in the ring.

The partial structure of the boat-form 2,5-dioxopiperazine ring may be illustrated as in (**A**) in Fig. 1. The stereochemical interrelationships among the carbonyl group at position 11, hydroxyl at 12 and isopentenyl ether at 13 are shown (R^1=H, R^2=isopentenyl). When both R^1 and R^2 are hydrogen in the **A**-type compounds (*e.g.* **2** or **10**), the presence of strong hydrogen bonding between the carbonyl at position 11 and hydroxyl at 13 and also between the two hydroxyls at position 12 and 13 is to be expected. The findings that **2** and **10** (**A**, R^1=R^2=H) were not acetylated with acetic anhydride in pyridine support this view. On the other hand, the structure of the chair-form dioxopiperazine in non-tremorogenic compounds may be as illustrated by (**C**) or (**D**) in Fig. 1.

The stereochemical situation around the carbonyl and two hydroxyl groups in the **D**-type compounds is thought to be the same as that in the **A**-type compounds, as shown in Fig. 1, so that the chemical and biological properties of the **D**-type compounds might be expected to

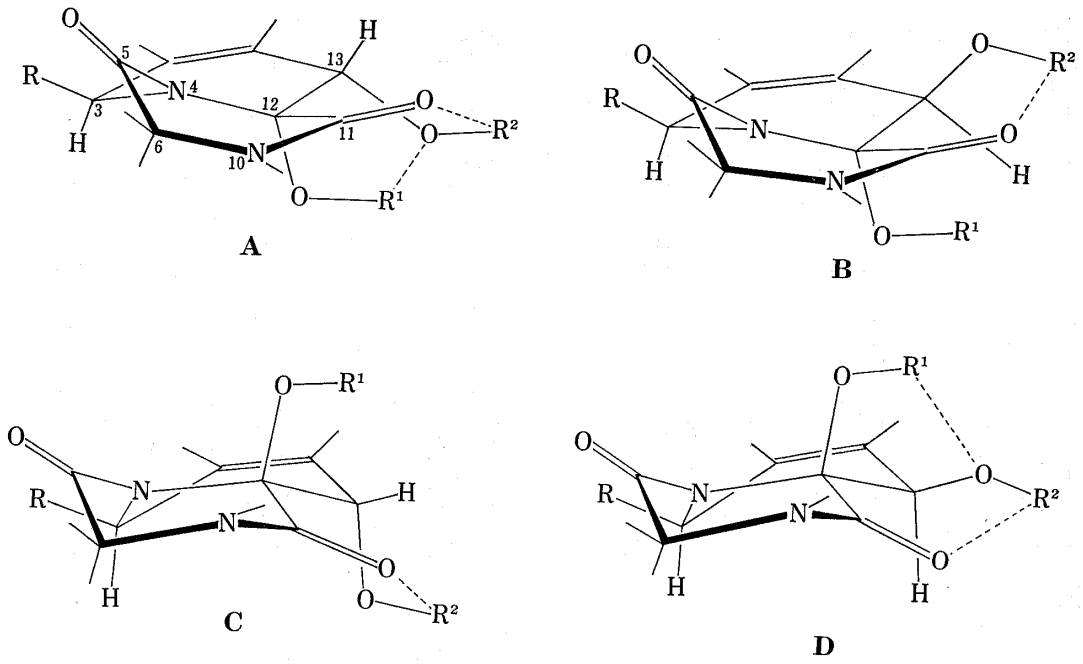

Fig. 1. Possible Stereochemistry of a Partial Structure Including the 2,5-Dioxopiperazine Ring in Fumitremorgin-Related Compounds

resemble those of the **A**-type compounds. In fact, the signal of H-3 of **9** appeared at almost the same magnetic field as that of **1** in the PMR spectrum, indicating that the interrelationships between H-3 and the carbonyl at position 5 in **9** and **1** are similar. The signal of H-13 of **9** appeared at rather higher magnetic field than that of **1**, indicating that the deshielding effect toward H-13 from the carbonyl at position 11 in **1** is a little larger than that in **9**. However, the IR spectrum of **9** exhibited two amide bands at 1683 and 1657 cm^{-1}, indicating that **9** contains a chair-form dioxopiperazine ring (see Table II). It is interesting that **9** retains tremorogenic activity. These findings can be explained in terms of the stereochemistry of **9** (**D**, R^1=H, R^2=isopentenyl), in which the stereochemical situation around the carbonyl at position 11 and the two hydroxyl groups at position 12 and 13 is similar to that of **1**, although the conformation of the dioxopiperazine ring in **9** should be "chair".

Another reaction product (**8**) obtained from **1** afforded a dehydroacetate (**13**) on acetylation with acetic anhydride in pyridine, whereas **2** and **10** did not. Accordingly, it can be assumed that **8** no longer contains such strong hydrogen bonds as **2** and **10**.

Comparing the PMR spectrum of **8** with that of **10**, the signal of H-16 in **8** appeared at higher magnetic field than that in **10**, indicating that the deshielding of the proton by the secondary hydroxyl group at position 13 in the latter was larger than in the former compound. The signal of H-13 in **8** also appeared at higher field than that in **10**, indicating that the deshielding of H-13 by the carbonyl group at position 11 was larger in **10** than in **8**. The signal of H-3 in **8** appeared at lower field on the other hand, indicating that deshielding of H-3 by the carbonyl group at position 5 was larger in **8** than in **10**. All these findings support the assumption that **8** has a **C**-type conformational structure and **10** has an **A**-type structure. The conformations of **3** and its hydrogenolysis product (**4**) appeared to be the same as that of **8** on the basis of the similarity in their spectral data.

As for the stereochemistry of **5**, which was obtained from **1** by catalytic hydrogenation, retention of the boat-form of the dioxopiperazine ring was suggested by the spectral data. In the IR spectrum of **5**, two amide bands were observed at 1678 and 1670 cm^{-1} (Table II), and the similarity of the PMR data of **5** and other **A**-type compounds was clear (Table I). On the other hand, the conformations of **6** and **7** are proposed to be "chair" (**C**-type).

The dehydroacetate (**13**) obtained from **8** afforded the methoxyacetate (**14**) on treatment with methanol. The IR spectrum of **14** indicated that this compound contains a boat-form dioxopiperazine ring, but differences were observed between the PMR data for **14** and those for other boat-form compounds. It the methoxyl group attacks the double bond between positions 12 and 13 from behind the molecule (to provide the boat-form dioxopiperazine ring), the acetoxyl group at position 13 may take the β-configuration. The stereochemistry of **14** was thus assigned as (**B**) in Fig. 1. The tremorogenic activity of **14** was not tested because only a small quantity was available.

On intravenous administration of **1** and **2** (0.34 mmol), mice exhibited tremor; tremor was also observed with 3.4 mmol of **9**, **10**, **11** and **12**, and with 34 mmol of **5**. In contrast, **3**, **4**, **6** and **8** were found to be non-tremorogenic. All the non-tremorogenic compounds were demonstrated to have **C**-type stereostructure,[13] in which there is no possibility of the presence of strong hydrogen bonds among the carbonyl and two hydroxl groups, even if R^1 and R^2 are both hydrogen. Interestingly, **8** (**C**, R^1=R^2=H), which was obtained from **1** by treatment with acid, did not exhibit tremorogenic activity, although the structure of **8** has been confirmed to be the same as that of verruculogen (**10**), excluding the stereochemistry. This suggests that the major structural factor in the tremorogenic activity is the stereochemical situation illustrated as (**A**) or (**D**) in Fig. 1, *i.e.*, in compounds **1**, **2**, **5**, **9**, **10**, **11** and **12**.

The carbon magnetic resonance (CMR) spectra of **1**, **2** and the related compounds **3** and

13) The presence of the C-type conformation in **3** has recently been demonstrated by X-ray analysis (private communication from Prof. J. Clardy of Cornel University).

4 have been measured and all of the signals have been assigned. The data for these compounds are listed in Table III.

Table III. CMR Data for Fumitremorgin A(**1**), B(**2**), and Compounds **3** and **4**; δ (ppm) from TMS in $CDCl_3$

Position No.	**1**	**2**	**3**	**4**
2	131.656 (s.)	131.170 (s.)	133.840 (s.)	134.447 (s.)
3	47.869 (d.)	49.082 (d.)	48.658 (d.)	48.294 (d.)
5	166.360 (s.)	166.360 (s.)	166.481 (s.)	167.088 (s.)
6	58.850 (d.)	58.790 (d.)	59.093 (d.)	58.972 (d.)
7	28.818 (t.)	28.940 (t.)	29.850 (t.)	29.850 (t.)
8	23.054 (t.)	22.630 (t.)	21.477 (t.)	21.598 (t.)
9	52.116 (t.)	45.260 (t.)	50.114 (t.)	48.294 (t.)
11	171.759 (s.)	170.607 (s.)	161.991 (s.)	162.598 (s.)
12	83.968 (s.)	83.119 (s.)	90.642 (s.)	90.885 (s.)
13	72.441 (d.)	68.982 (d.)	74.261 (d.)	75.110 (d.)
14	106.235 (s.)	104.657 (s.)	108.237 (s.)	103.747 (s.)
15	121.160 (s.)	120.674 (s.)	122.919 (s.)	122.191 (s.)
16	120.978 (d.)	121.463 (d.)	118.430 (d.)	118.308 (d.)
17	109.268 (d.)	109.268 (d.)	109.814 (d.)	109.450 (d.)
18	156.349 (s.)	156.228 (s.)	156.288 (s.)	155.924 (s.)
19	94.222 (d.)	93.918 (d.)	95.374 (d.)	95.374 (d.)
20	136.509 (s.)	137.966 (s.)	137.480 (s.)	136.267 (s.)
21	85.910 (d.)	41.802 (t.)	87.123 (d.)	
22	118.612 (d.)	120.432 (d.)	118.915 (d.)	
23	142.880 (s.)	134.507 (s.)	142.577 (s.)	
24 or	18.807 (q.)	18.201 (q.)	18.807 (q.)	
25	24.329 (q.)	25.542 (q.)	23.418 (q.)	
26	45.745 (t.)	123.101 (d.)	44.775 (t.)	44.775 (t.)
27	81.966 (s.)	135.114 (s.)	81.784 (s.)	71.227 (s.)
28 or	27.119 (q.)	25.724 (q.)	27.059 (q.)	32.641 (q.)
29	25.603 (q.)	18.383 (q.)	25.360 (q.)	28.394 (q.)
30	69.407 (t.)			
31	120.735 (d.)			
32	136.995 (s.)			
33 or	18.443 (q.)			
34	25.845 (q.)			
18-OCH_3	55.756 (q.)	55.696 (q.)	55.696 (q.)	55.696 (q.)
12-OCH_3 or			57.152 (q.)	56.666 (q.)
13-OCH_3			51.449 (q.)	51.570 (q.)

Experimental

Melting points are not corrected. Optical rotation was measured with a Yanagimoto OR-50 automatic polarimeter. CD: Japan Spectroscopic Manufac. Co. J-20 ORD/CD spectropolarimeter. Mass: Hitachi RMU-6E double-focusing mass spectrometer. UV: Hitachi 323 recording spectrophotometer. IR: Hitachi EPI-G3 grating infrared spectrophotometer. PMR and CMR: Japan Electron Optics Lab. JNM-PS-100 NMR spectrometer. Liquid chromatographic analyses were carried out with a Hitachi 034 liquid chromatograph.

Isolation of Fumitremorgin A(1)——a) *A. fumigatus* (strain 0011) was grown in stationary culture on sterilized rice (200 g×50 flasks) for 10 days at 25°. All of the moldy rice obtained was extracted with 10 l of AcOEt three times. After removal of the solvent under reduced pressure, 84 g of resinous residue was obtained. The extract was treated with *n*-hexane (1.25 l) to remove the *n*-hexane–soluble fraction. The *n*-hexane–insoluble fraction (53 g) was chromatographed on a silica gel (1 kg) column using benzene, and benzene–acetone mixture (50: 1), (25: 1), (10: 1) and (4: 1) as developing solvents. The fraction eluted with benzene–acetone (50: 1) afforded fumitremorgin A (**1**) (220 mg) as colorless needles on recrystallization from MeOH. Fumitremorgin B (**2**) was obtained (690 mg) as colorless needles from the fraction eluted with benzene–acetone (25: 1) on recrystallization from MeOH–CH_2Cl_2. Fumitremorgin A (**1**), mp 206—209°,

$[\alpha]_D^{10}$ +61° (c=0.80, acetone). *Anal.* Calcd for $C_{32}H_{41}N_3O_7$ (579.67), C, 66.30; H, 7.13; N, 7.25. Found: C, 66.39; H, 7.55; N, 7.40. MS *m/e* (%): 579(M^+, 22), 495(17), 342(22), 284(24), 258(49), 242(38), 202(73), 83(74), 70(100), 69(74), 55(49), 41(72). UV λ_{max}^{EtOH} nm (ε): 226(31700), 278(5300), 296(4900). IR ν_{max}^{KBr} cm^{-1}: 3480(OH), 1690(amide C=O), 1682(amide C=O). CD ($c=1.067\times10^{-4}$ g/ml, EtOH) [θ] (nm): −14110(207) (negative maximum), +29300(228) (positive maximum), −12370(244) (negative maximum), +14540(270) (positive maximum), +3830(287) (negative maximum), +5860(298) (positive maximum).

b) *A. fumigatus* (strain 0011) was grown in modified Czapek-Dox solution medium[14] (200 ml×200 flasks) in stationary culture for 14 days at 25°. After removing the culture filtrate, mycelia were dried at 60° (220 g), then extracted with ether (2 l) three times. The residual ether extract (32.5 g) was shaken with a mixture of *n*-hexane (1 l) and 90% MeOH (1 l). The lower layer was concentrated under reduced pressure. and kept at room temperature to afford a white precipitate (5 g). The precipitate was chromatographed on a silica gel (75 g) column as described above. From the fraction eluted with benzene–acetone (50: 1), **1** (510 mg) was obtained as colorless needles on recrystallization from MeOH. However, only a trace amount of **2** was obtained in this case.

Acid Hydrolysis of Fumitremorgin A(1)——A suspension of **1** (3 mg) in 6N HCl (3 ml) was refluxed for 5 hr. The reaction mixture was concentrated under reduced pressure to afford a resinous residue. The water-soluble fraction of the residue gave one spot which was identified with that of authentic proline on silica gel thin layer chromatography (TLC) developed with *n*-BuOH–AcOH–H_2O (4: 1: 1), $CHCl_3$–MeOH–17% NH_4OH (2: 2: 1), EtOH–H_2O (63: 37) and EtOH–34%NH_4OH (77: 23). The production of proline was confirmed by liquid chromatography (retention time, 78 min; buffer solution, citrate buffer (pH from 3.25 to 4.25); column temperature, 59°; reaction bath temperature, 115°).

Treatment of Fumitremorgin A(1) with 0.1% H_2SO_4 in MeOH——A solution of **1** (300 mg) in 12 ml of 0.1% H_2SO_4 in MeOH was refluxed for 30 min, neutralized with 0.5M Na_2CO_3 under ice-cooling and extracted with ether. The residue obtained after removal of the solvent was recrystallized with MeOH to give 168 mg of a degradation product (**3**), colorless prisms, mp 222—224° (decomp.), $[\alpha]_D^{11}$ +104° (c=0.26, $CHCl_3$). *Anal.* Calcd for $C_{29}H_{37}N_3O_7$ (539.61), C, 64.54, H, 6.91; N, 7.79. Found: C, 64.39; H, 6.97; N, 7.79. MS *m/e* (%): 539(M^+, 100), 476(8), 457(8), 424(8), 412(10), 397(10), 384(27), 382(21), 273(42), 258(31), 216(61), 215(96). UV λ_{max}^{EtOH} nm (ε): 227(25700), 275(4400), 297(4400), 303(shoulder, 3900). IR ν_{max}^{KBr} cm^{-1}: 1678 (amide C=O), 1655 (amide C=O). CD ($c=1.037\times10^{-4}$ g/ml, EtOH) [θ](nm): −140720(203) (negative maximum), +88100 (228) (positive maximum), +14680(252) (negative maximum), +19820(264) (positive maximum), +5140(289) (negative maximum), +6120(298) (positive maximum).

Hydrogenation of the Degradation Product 3——A solution of **3** (35 mg) in AcOEt (30 ml) was shaken with PtO_2 (42 mg) under H_2 gas for 1.5 hr, then filtrated to remove the catalyst. Removal of the solvent under reduced pressure afforded a residue, which was recrystallized from MeOH to give 21 mg of a hydrogenolysis product (**4**), colorless prisms, mp 218—219° (decomp.), $[\alpha]_D^{19.5}$ +86° (c=0.57, $CHCl_3$), *Anal.* Calcd for $C_{24}H_{31}N_3O_6$ (457.51), C, 63.00; H, 6.83; N, 9.19. Found: C, 62.73; H, 6.88; N, 8.94. MS *m/e* (%): 457(M^+, 28), 394(14), 275(25), 217(16), 216(100). UV λ_{max}^{EtOH} nm (ε): 223(41100), 265(4600), 296(6200), 302(shoulder, 5200). IR ν_{max}^{KBr} cm^{-1}: 3370(OH and NH), 1665(amide C=O), 1655(amide C=O). CD ($c=1.13\times10^{-4}$ g/ml, EtOH) [θ] (nm): −74100(204) (negative maximum), +20640(227) (positive maximum), +4880(244) (negative maximum), +18390(264) (positive maximum), +3190(283) (negative maximum), +4880(298) (positive maximum).

Hydrogenation of Fumitremorgin A (1)——A solution of **1** (100 mg) in AcOEt (25 ml) was shaken with PtO_2 (60 mg) under H_2 gas for 1.5 hr. Removal of the solvent afforded a residue, which was recrystallized from aqueous acetone to give 44 mg of the hydrogenolysis product (**5**), colorless needles, mp 118—121°, $[\alpha]_D^{12}$ +6.8° (c=0.44, acetone). UV λ_{max}^{EtOH} nm: 223, 267, 296. IR ν_{max}^{KBr} cm^{-1}: 3520—3350 (OH and NH), 1678(amide C=O), 1670(amide C=O). CD ($c=1.295\times10^{-4}$ g/ml, EtOH) [θ] (nm): −107730(231) (negative maximum), +14620(267) (positive maximum), +1080(287) (negative maximum), 2230(299) (positive maximum).

Conversion of the Hydrogenolysis Product 5 to Compound 6——a) A solution of NaOH (15 mg) in EtOH (6 ml) was added to a solution of **5** (100 mg) in EtOH (2 ml) under ice-cooling. The alkaline solution was kept at room temperature for 1.5 hr, neutralized with AcOH under ice-cooling, diluted with water and extracted with ether. Removal of the solvent afforded a residue, which was further purified by preparative TLC, developing with benzene–EtOH (10: 1), to give 65 mg of **6**, amorphous, UV λ_{max}^{EtOH} nm: 223, 265, 295. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH and NH), 1664 (amide C=O), 1655 (amide C=O). CD ($c=1.114\times10^{-4}$ g/ml, EtOH) [θ] (nm): −54440(203) (negative maximum), +10890(225) (positive maximum), +1090(242) (negative maximum), +11070(268) (positive maximum), +1540(285) (negative maximum), +2450(293) (positive maximum), −730(315) (negative maximum).

b) On standing for several days, a solution of **5** in MeOH or acetone afforded a mixture which gave

14) The medium consisted of D-glucose (25 g), ammonium succinate (1.6 g), $MgSO_4\cdot7H_2O$(1.02 g), KH_2PO_4 (0.5 g), yeast extract (Difco) (0.1 g), $FeSO_4\cdot7H_2O$ (1 mg), $ZnSO_4\cdot7H_2O$ (1 mg), $CuSO_4\cdot5H_2O$ (0.15 mg), $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (0.1 mg) and L-tryptophan (250 mg) dissolved in 1l of water.

two spots corresponding to **5** and **6** on TLC, developing with benzene–EtOH (10: 1). A similar result was obtained after standing on a silica gel thin-layer plate for several days.

Acetylation of Compound 6——A solution of **6** (20 mg) in Ac_2O (0.2 ml) and pyridine (0.4 ml) was kept at 36° for 7 days. The reaction mixture was poured into ice-water and extracted with ether. The residue obtained after removal of the solvent was purified by preparative TLC, developing with benzene–EtOH (10: 1), to give 8 mg of a monoacetate (**7**), amorphous, UV λ_{max}^{MeOH} nm: 222, 264, 295, 303(shoulder), IR ν_{max}^{KBr} cm^{-1}: 3340(OH and NH), 1747(acetyl C=O), 1678 (amide C=O), 1668 (amide C=O).

Treatment of Fumitremorgin A (1) with 0.1% H_2SO_4 in Acetone——A solution of **1** (300 mg) in 12 ml of 0.1% H_2SO_4 in acetone was refluxed for 30 min, neutralized with 0.5 M Na_2CO_3 under ice-cooling and extracted with ether. The residue (250 mg) obtained after removal of the solvent was chromatographed on a silica gel (6.5 g) column using benzene and benzene–acetone (50: 1), (30: 1) and (10: 1) as developing solvents. The fraction eluted with benzene–acetone (50: 1) was recrystallized from MeOH to afford 7 mg of colorless prisms (**9**), mp 232—233.5° $[\alpha]_D^{10.5}$ +45° (c=0.71, $CHCl_3$), *Anal.* Calcd for $C_{32}H_{41}N_3O_7$ (579.67), C, 66.30; H, 7.13; N, 7.25. Found: C, 66.16; H, 7.20; N, 7.15. MS *m/e* (%): 579 (M^+, 11), 506(10), 495(11), 424(53), 327(32), 281(32), 253(33), 239(35), 84(100), 83(82), 70(73), 55(86). UV λ_{max}^{EtOH} mn (ε): 229(42700), 277(8100), 295(7500). IR ν_{max}^{KBr} cm^{-1}: 3460(OH), 1683(amide C=O), 1657 (amide C=O). CD ($c=1.12\times10^{-4}$ g/ml, EtOH) [θ] (nm): −94 510(220) (negative maximum), −12460(240) (positive maximum), −18690 (245) (negative maximum), +20 770 (269) (positive maximum), +6650(290) (negative maximum), +7270 (295) (positive maximum).

The fraction eluted with benzene–acetone (30: 1) was further purified by preparative TLC, developing with benzene–acetone (10: 1), and recrystallized from MeOH to afford 2 mg of colorless needles. The product was identical with authentic verruculogen (**10**) on the basis of mixed melting point (mp 226—227°), IR spectra (KBr tablet) and TLC with benzene–acetone (10: 1), benzene–EtOH (10: 1), benzene–AcOEt (5: 1) and $CHCl_3$–acetone (95: 5). CD ($c=1.10\times10^{-4}$ g/ml, EtOH) [θ] (nm): −60,390(205) (negative maximum), +29730(230) (positive maximum), +370(248) (negative maximum), +11,150(269) (positive maximum), +1860(289) (negative maximum), +2790(299) (negative maximum).

The fraction eluted with benzene–acetone (10: 1) was recrystallized from acetone to afford 65 mg of colorless needles (**8**), mp 211—213°, $[\alpha]_D^{28}$ +57° (c=0.76, $CHCl_3$), *Anal.* Calcd for $C_{27}H_{33}N_3O_7$ (511.56), C, 63.39; H, 6.50; N, 8.21. Found: C, 63.07; H, 6.40; N, 7.92. UV λ_{max}^{EtOH} nm (ε): 227(31300), 274(5200), 296 (4900). IR; ν_{max}^{KBr} cm^{-1}: 3400(OH), 1665 (amide C=O), 1653 (amide C=O). CD ($c=1.139\times10^{-4}$ g/ml, EtOH) [θ] (nm): 123380(201) (negative maximum), +75370(228) (positive maximum), +11330(251) (negative maximum), +13640(263) (positive maximum), +3410(283) (negative maximum), +3770(297) (positive maximum).

Formation of Dehydroacetate 13——A solution of **8** (50 mg) in Ac_2O (0.7 ml) and pyridine (1.8 ml) was kept at room temperature for 26 hr. The reaction mixture was then poured into ice-water to give a white precipitate (after drying, 46 mg). The precipitate was subjected to preparative TLC using benzene–acetone (5: 1), then extracted with benzene–acetone (1: 1) to give a pure dehydroacetate (**13**), amorphous, UV λ_{max}^{EtOH} nm (ε): 223(36700), 273(7600), 294(6800), 301 (shoulder, 5800). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750 (acetyl C=O), 1688 (amide C=O).

Formation of Methoxyacetate 14——A solution of **8** (30 mg) in Ac_2O (0.4 ml) and pyridine (1.3 ml) was kept at room temperature for 28 hr. The white precipitate (after drying, 27 mg) obtained was recrystallized from MeOH to give 21 mg of the methoxyacetate (**14**), colorless crystals, mp 225—227° (decomp.), $[\alpha]_D^{28}$ +182° (c=0.12, $CHCl_3$), $C_{30}H_{37}N_3O_8$ (MS, M^+: *m/e* 567). UV λ_{max}^{EtOH} nm: 223, 272, 295. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (acetyl C=O), 1686 (amide C=O), 1682 (amide C=O).

Assignment of The CMR Signals——All the CMR signals were assigned with the aid of the off-resonance decoupling technique, by comparison of the data with those for verruculogen and its derivative,[15] and by reference to monographs on CMR spectroscopy.[16]

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