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Chemistry of Tremorogenic Metabolites. II.[1] Structure Determination of Fumitremorgin B, a Tremorogenic Metabolite from *Aspergillus fumigatus*

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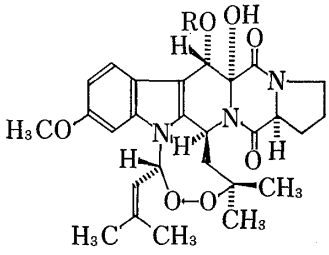
Fumitremorgin B (**2**), isolated from *Aspergillus fumigatus* together with fumitremorgin A (**1**), caused severe tremors and convulsion in experimental animals. The structure of fumitremorgin B was determined from the spectral data. The stereochemistry, including the absolute configuration, was determined by X-ray analysis and comparison of the ORD of proline obtained from this compound with that of authentic L-(−)-proline.

Keywords——fumitremorgin B; fungal metabolite; *Aspergillus fumigatus*; tremorogenic principle; structure determination; biosynthetic investigation; X-ray analysis

In the course of a survey of toxigenic fungi contaminating foodstuffs, thirteen new indolic metabolites have been isolated from a toxigenic fungus, *Aspergillus fumigatus*. Among these metabolites, fumitremorgin A and B were found to be the tremorogenic principles of the fungus.[3] These two compounds caused severe tremors and convulsion in experimental animals on oral and parenteral administration. The structure of fumitremorgin A (**1**) has been elucidated, as reported previously.[1] In this paper, the structure determination of fumitremorgin B (**2**) is described in detail.

The other metabolites isolated from this fungus, tentatively named FTC–FTN, were confirmed to be tryptoquivaline-related compounds, and were non-tremorogenic.[4]

Fumitremorgin B (**2**), colorless needles, mp 211—212°, $C_{27}H_{33}N_3O_5$ (M^+: *m/e* 479) was suspected to contain a 6-methoxyindole ring on the basis of a comparison of its ultraviolet (UV) spectrum with those of some model compounds such as 7-methoxytetrahydrocarbazole.[5] The presence of a 2,3,6-trisubstituted indole ring system was also indicated by the observation of one methoxyl group (3.84 ppm, 3H, singlet) and three atomatic protons (6.71 ppm, 1H, doublet, J=2 Hz; 6.81 ppm, 1H, doublet of doublets, J=9 and 2 Hz; 7.88 ppm, 1H, doublet, J=9 Hz) in the PMR spectrum. The infrared spectrum of **2** showed two amide carbonyl bands (1668 and 1688 cm^{-1}), indica ting the presence of a dioxopiperazine ring.[6] On hydroly-



1: R=$CH_2CH=C(CH_3)CH_3$

5: R=H

Fig. 1

1) A preliminary report on this work was presented at the XXIVth IUPAC Congress in Hamburg (1973) ,and in *J.C.S. Chem. Commun.*, **1974**, 408 and *Tetrahedron Lett.*, **1975**, 27. Part I: M. Yamazaki, H. Fujimoto, and T. Kawasaki, *Chem. Pharm. Bull.*, **28**, 245 (1980).
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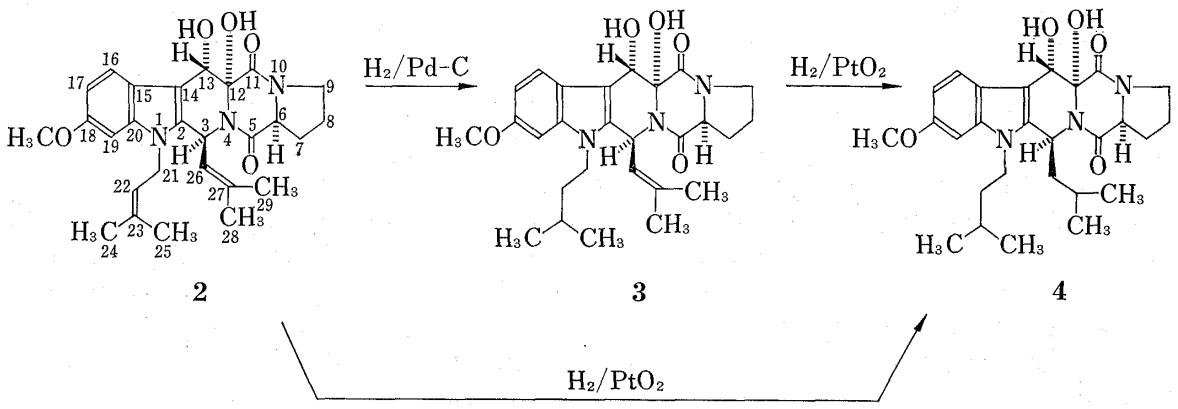
3) M. Yamazaki, S. Suzuki, and K. Miyaki, *Chem. Pharm. Bull.*, **19**, 1739 (1971).
4) M. Yamazaki, H. Fujimoto, and E. Okuyama, *Tetrahedron Lett.*, **1976**, 2861; *Idem*, *Chem. Pharm. Bull.*, **25**, 2554 (1978); **26**, 111 (1978); M. Yamazaki, E. Okuyama, and Y. Maebayashi, *ibid.*, **27**, 1611 (1979).
5) S. Sakai, A. Kubo, and J. Haginiwa, *Tetrahedron Lett.*, **1969**, 2031; S. Agurell, B. Holmstedt, and J.E. Lindgren, *Acta Chim. Scand.*, **23**, 903 (1969).
6) A.J. Birch and J.J. Wright, *Tetrahedron*, **26**, 2329 (1970); P.S. Steyn, *Tetrahedron Lett.*, **1971**, 3331.

sis with 6 N HCl, **2** afforded proline. The partial structure of **2** was thus assumed to contain a 2,5-dioxopiperazine moiety consisting of 6-methoxytryptophan and proline. A biosynthetic investigation showed that 3-^{14}C-tryptophan was well incorporated into **2**, as was U-^{14}C-proline, further supporting the above assumption.

The presence of isopentenyl groups in **2** was suggested by biosynthetic investigation (incorporation of mevalonolactone) and also by the PMR data. In the PMR spectrum, a signal due to methylene (4.54 ppm, 2H, doublet), which was assumed to be adjacent to the nitrogen atom, was confirmed to be coupled with that of an olefinic proton (5.06 ppm), indicating that one of the two isopentenyl groups was located on N-1 of the indole ring. A methine signal (6.00 ppm), which was coupled with the other olefinic proton signal (4.72 ppm), was also assigned to a position adjacent to the nitrogen. The low chemical shift of this methine proton (6.00 ppm) suggested that this methine was also located at an allyl position between two double bonds. These findings indicated that the other isopentenyl group should be located on the carbon at position 2 of the indole ring and linked to the nitrogen (N-4) of the dioxopiperazine ring.



2 **3** **4**

Chart 1

On catalytic hydrogenation using 5% palladium–carbon, **2** afforded a dihydro derivative (**3**), mp 179—180.5°, $C_{27}H_{35}N_3O_5$ (M^+ *m/e* 481). The PMR spectrum of **3** showed that a double bond in one isopentenyl group attached to N-1 had been hydrogenated in the reaction. On hydroganation of **2** using platinum dioxide, a tetrahydroderivative (**4**) was obtained, mp 97—100°, $C_{27}H_{37}N_3O_5$ (M^+ *m/e* 483). This compound (**4**) was also obtained from 3 by further hydrogenation using platinum dioxide. In the tetrahydroderivative (**4**), the double bonds in both isopentenyl groups are hydrogenated.

The PMR spectrum of **2** indicated the presence of two hydroxyl groups, but **2** was resistant to acetylation with acetic anhydride and pyridine. In the PMR spectrum of **2**, one hydroxyl group (4.73 ppm) was observed to be coupled with a methine proton (5.78 ppm) but the other hydroxyl (4.11 ppm) was not. Accordingly, the latter was assigned as tertiary and the former as secondary.

In the mass spectrum of **2**, the base peak appeared at *m/e* 311 ($C_{20}H_{25}NO_2$) owing to the elimination of the dioxopiperazine fragment. The appearance of the base peak at *m/e* 313 in the mass spectrum of **3** may be due to a similar process. The structure of fumitremorgin B was thus proposed to be **2**, excluding the stereochemistry.

The relative stereostructure of **2** was determined by the X-ray analysis of **2** itself, obtained as an orthorhombic crystal from methanol, having a space group $P2_12_12_1$ with 4 molecules in a unit cell of dimensions a=14.771, b=24.925, c=7.321 (A). A total of 2011 (1699 observed) independent structure factors was collected with a 4 circle diffractometer using CuKα radiation. The structure was solved using the MDKS and triple product formulae of

Hauptman,[7] and by application of the tangent formula. The E-map of the solution giving the lowest *R*-value (Karle) showed all the skeletal atoms. The structure was refined to obtain an *R*-factor of 0.11 with anisotropic thermal parameters for carbon, oxygen and nitrogen atoms.

The absolute configuration of **2** was determined based on the configuration of proline obtained by the hydrolysis of **2** with 6 N HCl. The optical rotatory dispersion (ORD) spectrum of proline thus obtained showed a negative plain curve, which was identical with that of authentic L-(−)-proline (*S* configuration). Therefore, the structure of fumitremorgin B, including the absolute configuration, was established as **2** as shown in Figure 2.

The resistance of the secondary hydroxyl group in **2** to acetylation may be explained by the presence of a strong hydrogen bond involving this hydroxyl group, as described in the previous report.[1]

In 1972, Moppett *et al.*[8] reported the isolation and structure determination of lanosulin from a fungus, *Penicillium lanosum*. A comparison of **2** with lanosulin showed that they were identical, although an alternative structure had been proposed for lanosulin.

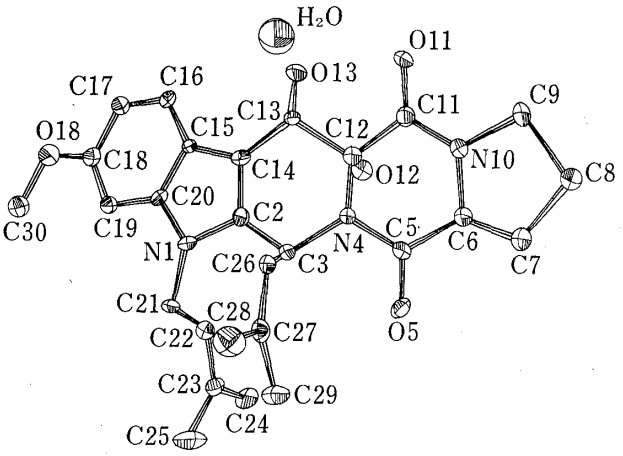


Fig. 2. Stereostructure of Fumitremorgin B(**2**) (C, N and O Atoms are depicted)

It has recently been found that **2** is produced by some fungi other than *Aspergillus fumigatus*, namely *A. caespitosus*,[9] and *Penicillium piscarium*.[10] These two fungi also produce verruculogen (**5**), which was isolated from *Penicillium verruculosum* as the tremorogenic principle.[11] It has been reported that *Penicillium janthinellum* produces fumitremorgin A (**1**) together with verruculogen (**5**),[12] and that *P. paraherquei* produces **5**.[13]

Experimental

Melting points are uncorrected. Optical rotation was measured with a Yanagimoto OR-50 automatic polarimeter. CD and ORD: Japan Spectroscopic Manufact. Co. J-20 ORD/CD spectropolarimeter. Mass spectra: Hitachi RMU-6E double-focusing mass spectrometer. UV: Hitachi 323 recording spectrophotometer. IR: Hitachi EPI-G3 grating infrared spectrophotometer. PMR: Japan Electron Optics Lab. JNM-PS-100 NMR spectrometer. Radioactivity was measured with a Beckman LS-150 liquid scintillation system. Liquid chromatographic analysis was carried out with a Hitachi 034 liquid chromatograph.

Isolation of Fumitremorgin B (2)——The method of isolation of fumitremorgin B (**2**) was as described in the preceeding report.[1] Fumitremorgin B (**2**), mp 211—212°, $[\alpha]_D^{13.5}$ +9° (c=0.68, $CHCl_3$), *Anal.* Calcd for $C_{27}H_{33}N_3O_5$ (479.56), C, 67.62; H, 6.94; N, 8.76. Found: C, 67.15; H, 7.03; N, 8.65. MS *m/e* (%): 479 (M^+, 87), 462 (13), 311 (100), 268 (78), 240 (29), 214 (32), 200 (31), 187 (17), 70 (20), 69 (27). UV λ_{max}^{EtOH} nm (ε): 226 (31700), 278 (7300), 295 (7900). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1688 (amide C=O), 1668 (amide C=O). CD ($c=1.01\times10^{-4}$ g/ml, EtOH) [θ] (nm): +14700 (211) (positive maximum), −19920 (231) (negative maximum),

7) H.A. Hauptman, "Crystal Structure Determination," Plenum Press, New York, 1972.
8) D.T. Dix, J. Martin, and C.E. Moppett, *J.C.S. Chem. Commun.*, **1972**, 1168.
9) H.W. Schroeder, R.J. Cole, H. Hein, Jr. and J.W. Kirksey, *Appl. Microbiol.*, **29**, 857 (1975).
10) R.T. Gallagher and G.C.M. Latch, *Appl. Environ. Microbiol.*, **33**, 730 (1977).
11) R.J. Cole, J.W. Kirksey, J.H. Moore, B.R. Blankenship, U.L. Diener, and N.D. Davis, *Appl. Microbiol.*, **24**, 248 (1972); J. Fayos, D. Lokensgard, J. Clardy, R.J. Cole, and J.W. Kirksey, *J. Am. Chem. Soc.*, **96**, 6785 (1974).
12) M.E. di Menna and P.G. Mantle, *Res. Vet. Sci.*, **24**, 347 (1978); G.W. Lanigan, A.L. Payne, and P.A. Cockrum, *Aust. J. Exp. Biol. Med. Sci.*, **57**, 31 (1979).
13) T. Yoshizawa, N. Morooka, Y. Sawada, and S. Udagawa, *Appl. Environ. Microbiol.*, **32**, 441 (1976).

+28650 (275) (positive maximum), +12330 (290) (negative maximum), +15180 (300) (positive maximum). PMR δ (ppm) from tetramethylsilane (TMS) in $CDCl_3$: 1.64 (3H, singlet(s), 28- or 29-C$\underline{H}_3$), 1.71 (3H, s, 24- or 25-C$\underline{H}_3$), 1.85 (3H, s, 24- or 25-C$\underline{H}_3$), 2.00 (3H, s, 28- or 29-C$\underline{H}_3$), 1.60—2.60 (4H, multiplet(m), 7- and 8-C$\underline{H}_2$), 3.60 (2H, deformed triplet (t), 9-C$\underline{H}_2$), 3.84 (3H, s, 18-OC$\underline{H}_3$), 4.11 (1H, broad s, 12-O$\underline{H}$), 4.45 (1H, doublet (d) of d, $J_1=10$, $J_2=8$ Hz, 6-C$\underline{H}$), 4.54 (2H, d, $J=6$, 21-C$\underline{H}_2$), 4.72 (1H, d, $J=10$, 26-C$\underline{H}$), 4.73 (1H, d, $J=2$, 13-O$\underline{H}$), 5.06 (1H, broad t, $J_1=J_2=6$, 22-CH), 5.78 (1H, broad d, $J=2$, 13-C$\underline{H}$), 6.00 (1H, d, $J=10$, 3-C$\underline{H}$), 6.71 (1H, d, $J=2$, 19-C$\underline{H}$), 6.81 (1H, d of d, $J_1=9$, $J_2=2$, 17-C$\underline{H}$), 7.88 (1H, d, $J=9$, 16-C$\underline{H}$).

Incorporation of 3-^{14}C-DL-Tryptophan into Fumitremorgin A (1) and B (2)——*A. fumigatus* strain 0011 was grown in a modified Czapek-Dox solution medium[1] (200 ml×5 flasks) under stationary culture for 2 days at 25°. 3-^{14}C-DL-Tryptophan (specific radioactivity, 8.35×10^9 dpm/mmol) amounting to 2.00×10^8 dpm (4.88 mg, 0.024 mmol) was added to the culture medium, and the fungus was further cultured for 11 days at 25°. After removing the culture filtrate, the mycelia were dried at 60°. The whole of the dried mycelia (8.9 g) was extracted with ether three times. The residual ether extract (510 mg) was chromatographed on a silica gel (15 g) column with benzene and benzene–acetone (50: 1), (25: 1), (10: 1), and (4: 1). On recrystallization from MeOH, the fraction eluted with benzene–acetone (50: 1) afforded radioactive fumitremorgin A (1) (9 mg) as colorless needles, which were crystallized repeatedly to afford fumitremorgin A having constant radioactivity. The radioactivity of fumitremorgin A (1) was measured in a toluene solution containing 0.5% 2,5-diphenyl oxazole (PPO) as a scintillator. The specific radioactivity of fumitremorgin A (1) was 6.90×10^7 dpm/mmol. The specific incorporation ratio of 3-^{14}C-DL-tryptophan into fumitremorgin A (1) was 0.83%.

The radioactivities of fumitremorgin A and B, which were separated from the ether extract by preparative thin layer chromatography (each gave a single spot on thin layer chromatography), were also measured with the liquid scintillation counter. The total radioactivities of fumitremorgin A and B were 3.28×10^5 dpm and 2.30×10^4 dpm, respectively.

Incorporation of 2-^{14}C-*dl*-Mevalonolactone into Fumitremorgin A(1) and B(2)——*A. fumigatus* starin 0011 was cultured as described above for 2 days (200 ml×5 flasks). 2-^{14}C-*dl*-Mevalonolactone (specific radioactivity, 1.41×10^{10} dpm/mmol) amounting to 2.22×10^8 dpm (2.05 mg, 0.016 mmol) was added to the medium, and the fungus was further cultured for 11 days. Chromatography of the ether extract (650 mg) obtained from the whole of the dried mycelia (8.60 g) gave a fraction which was crystallized repeatedly from MeOH to afford fumitremorgin A (1) as colorless needles (12 mg) having constant radioactivity. The radioactivity was meaeured as described above. The specific radioactivity of fumitremorgin A (1) was 8.92×10^7 dpm/mmol. The specific incorporation ratio of 2-^{14}C-*dl*-mevalonolactone into fumitremorgin A (1) was 0.63%.

The total radioactivities of fumitremorgin A and B, which were separated from the ether extract by preparative thin-layer chromatography (each gave a single spot on thin layer chromatography), were 2.76×10^5 dpm and 2.48×10^4 dpm, respectively.

Incorporation of U-^{14}C-L-Proline into Fumitremorgin A (1) and B (2)——*A. fumigatus* strain 0011 was cultured as described above for 2 days (200 ml×5 flasks). U-^{14}C-L-Proline (specific radioactivity, 5.13×10^{11} dpm/mmol) amounting to 5.55×10^7 dpm (0.0124 mg, 0.0001 mmol) was added to the medium, and the fungus was cultured for a further 11 days. Chromatography of the ether extract (550 mg) obtained from the whole of the dried mycelia (4.85 g) gave a fraction which was crystallized repeatedly from MeOH to afford fumitremorgin A (1) as colorless needles (11 mg) having constant radioactivity. The radioactivity was meaeured as described above. The specific radioactivity of fumitremorgin A (1) was 5.20×10^7 dpm/mmol. The specific incorporation ratio of U-^{14}C-L-proline into fumitremorgin A (1) was 0.01%.

The total radioactivites of fumitremorgin A and B, which were separated from the ether extract by preparative thin layer chromatography (each gave a single spot on thin layer chromatography), were 1.88×10^5 dpm and 2.73×10^4 dpm, respectively.

Formation of Dihydrofumitremorgin B (3)——A solution of fumitremorgin B (2) (50 mg) in EtOH (50 ml) was shaken with 5% palladium–carbon (100 mg) under a stream of hydrogen at room temperature for 4 hr. After filtration to remove the catalyst, the filtrate was concentrated under reduced pressure. The residue (45 mg) obtained by evaporation, which gave a single spot on thin layer chromatography with benzene–EtOH (10: 1), was recrystallized with EtOH to afford pure dihydrofumitremorgin B (3) as colorless needles, mp 179—180.5°, *Anal.* Calcd for $C_{27}H_{35}N_3O_5\cdot C_2H_5OH$ (527.64): C, 66.01; H, 7.83; N, 7.97. Found: C, 65.93; H, 7.72; N, 7.96. MS *m/e* (%): 481 (M^+, 34), 464 (7), 313 (100), 270 (69), 201 (52), 70 (41), 69 (27). UV λ_{max}^{EtOH} nm (ε): 227 (28000), 279.5 (5700), 297.5 (7700). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1690 (amide C=O), 1665 (amide C=O). PMR δ (ppm) from TMS in $CDCl_3$: 0.98 (3H, d, $J=4$, 24- or 25-C$\underline{H}_3$), 1.00 (3H, d, $J=4$, 24- or 25-C$\underline{H}_3$), 1.60 (3H, s, 28- or 29-C$\underline{H}_3$), 2.00 (3H, s, 28- or 29-C$\underline{H}_3$), 1.60—2.45 (7H, m, 7-, 8- and 22-C$\underline{H}_2$, and 23-C$\underline{H}$), 3.64 (2H, deformed t, 9-C$\underline{H}_2$), 3.85 (3H, s, 18-OC$\underline{H}_3$), 3.95 (3H, partially overlapped with the signal at 3.85 ppm, 21-C$\underline{H}_2$ and 12-O$\underline{H}$), 4.42 (1H, deformed t, 6-C$\underline{H}$), 4.68 (1H, d, $J=10$, 26-C$\underline{H}$), 4.72 (1H, d, $J=2$, 13-O$\underline{H}$), 5.72 (1H, broad d, $J=2$, 13-C$\underline{H}$), 5.97 (1H, d, $J=10$, 3-C$\underline{H}$), 6.74 (1H, d, $J=2$, 19-C$\underline{H}$), 6.80 (1H, d of d, $J_1=10$, $J_2=2$, 17-C$\underline{H}$), 7.87 (1H, d, $J=10$, 16-C$\underline{H}$).

Formation of Tetrahydrofumitremorgin B (4)——a) A solution of dihydrofumitremorgin B (3) (25 mg) in AcOEt–AcOH (4: 1) (10 ml) was shaken with PtO_2 (50 mg) under a stream of hydrogen at room tempera-

ture for 5 hr. After filtration to remove the catalyst, the filtrate was concentrated under reduced pressure. The residue (26 mg) obtained by evaporation, which gave one spot on thin layer chromatography with benzene-EtOH (10:1), was recrystallized with EtOH to afford pure tetrahydrofumitremorgin B (**4**) as colorless needles, mp 97—100°, *Anal.* Calcd for $C_{27}H_{37}N_3O_5$ (483.59) C, 67.05; H, 7.71; N, 8.69. Found: C, 66.86; H, 7.75; N, 8.64. MS *m/e* (%): 483 (M^+, 88), 426 (100), 408 (30), 392 (22), 380 (16), 314 (67), 411 (37), 272 (40), 70 (23). UV λ_{max}^{EtOH} nm (ε): 227 (37300), 278 (6300), 296 (6600). IR ν_{max}^{KBr} cm^{-1}: 3510 (OH), 3390 (OH), 1690 (shoulder, amide C=O), 1665 (amide C=O). PMR δ (ppm) from TMS in $CDCl_3$: 0.84 (3H, d, $J=4$), 0.90 (3H, d, $J=4$), 0.96 (3H, d, $J=3$) and 1.02 (3H, d, $J=3$) (these four signals were assigned to 24-, 25-, 28- and 29-C$\underline{H}_3$), 1.50—2.55 (10H, m, 7-, 8-, 22-, 26-C$\underline{H}_2$ and 23- and 27-C$\underline{H}$), 3.64 (2H, deformed t, $J=6$, 9-C$\underline{H}_2$), 3.88 (3H, s, 18-OC$\underline{H}_3$), 4.01 (3H, partially overlapped with the signal at 3.88 ppm, 21-C$\underline{H}_2$ and 12-O$\underline{H}$), 4.47 (1H, broad t, $J=9$, 6-C$\underline{H}$), 4.60 (1H, d, $J=2$, 13-O$\underline{H}$), 5.55 (1H, t, $J=5$, 3-C$\underline{H}$), 5.77 (1H, d, $J=2$, 13-C$\underline{H}$), 6.77 (1H, d, $J=2$, 19-C$\underline{H}$), 6.82 (1H, d of d, $J_1=10$, $J_2=2$, 17-C$\underline{H}$), 7.87 (1H, d, $J=10$, 16-C$\underline{H}$).

b) A solution of fumitremorgin B (**2**) (50 mg) in AcOEt (20 ml) was shaken with PtO_2 (50 mg) under a stream of hydrogen at room temperature for 8 hr. After treatment as described above, a residue was obtained, which was confirmed to be a mixture of dihydrofumitremorgin B (**3**) and tetrahydrofumitremorgin B (**4**) by thin layer chromatography. The residue was further hydrogenated with PtO_2 (50 mg) in AcOEt–AcOH (5:1) (18 ml) at room temperature for 1.5 hr. After treatment in the usual way, a residue which gave a single spot identical with that of tetrahydrofumitremorgin B (**4**) on thin layer chromatography was obtained. Recrystallization from EtOH afforded pure tetrahydrofumitremorgin B (**4**) (27 mg) as colorless needles.

Formation of Optically Active L-(−)-Proline by 6N HCl Hydrolysis of Fumitremorgin B (**2**)——A suspension of fumitremorgin B (**2**) (95.8 mg) in 6 N HCl (25 ml) was refluxed for 8 hr. A resinous residue obtained by removal of the solvent was purified by preparative paper chromatography on Toyo filter paper No. 50 with *n*-BuOH–AcOH–H_2O (4:1:1), and the material was extracted with EtOH to afford a product (17 mg). This product gave a single spot identical with that of authentic L-(−)-proline on thin layer chromatography with three different solvent systems and on liquid chromatography with citrate buffer (pH 3.25—4.25) at a column temperature of 59° and a reaction bath temperature of 115°. ORD of proline thus obtained ($c=0.204$, H_2O) $[\alpha]^{28}$ (nm): −27° (589), −39° (500), −74° (400), −147° (300), −270° (240). ORD of authentic L-(−)-proline(recrystallized twice from abs-EtOH) ($c=0.133$, H_2O) $[\alpha]^{30}$ (nm): −80° (589), −117° (500), −200° (400), −432° (300), −827° (240).

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