

Communications to the Editor

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Tremorgenic Toxins from *Aspergillus fumigatus* FRES.

During the investigation of the toxigenic food-borne fungi, we isolated eight strains of *Aspergillus fumigatus* Fres. having strong tremorgenic action on mice by the intraperitoneal injection of crude extract. From the soluble portion to hot *n*-hexane of the ethyl acetate extract of the toxic strains, two new crystalline compounds, named fumitremorgin A and B, were isolated by column chromatography on silica gel with *n*-hexane and benzene-acetone (4:1). The both compounds contain nitrogen and produce greenish purple color with an addition of Ehrlich's reagent but are negative to the coloring test with Dragendorff's reagent and FeCl₃ solution. The presence of sulphur and halogen atoms was excluded by the respective qualitative analysis. The both compounds are soluble in chloroform and ethylacetate but slightly soluble in methyl and ethyl alcohols. The resemblance of the pattern of ultraviolet (UV) spectra of those two compounds possibly indicates the presence of 2,3-substituted 6-O-methylindole system in those structures as an important chromophore.¹⁾ UV $\lambda_{\max}^{\text{EtOH}}$ m μ (ϵ): 225.5 (66900), 275.5 (12000), 295 (8500).

Fumitremorgin A was crystallized from methanol as colorless prisms, mp 202.5–203.5°. This has the composition C₃₃H₄₅O₆N₃ (Mass Spectrum *m/e*: 579 (M⁺), Elementary analysis Found: C, 68.34%; H, 7.05%; N, 6.70%). By the nuclear magnetic resonance (NMR) spectrum of A in CDCl₃, the substitution pattern in 2,3-substituted 6-O-methylindole was strongly supported. The fine signals due to the aromatic protons, δ (ppm): 7.68 (1H, doublet, *J*=2.0 Hz), 6.82 (1H, doublet of doublet, *J*=9 and 2 Hz), 6.67 (1H, doublet, *J*=9 Hz) and the protons of OCH₃, δ (ppm) 3.84 (3H, singlet) were observed. In infrared (IR) spectrum of fumitremorgin A, the following peaks were observed. IR (KBr) cm⁻¹: 3420, 2940, 1670, 1565, 1440, 1370, 1300, 1160, 1070, 1035.

Fumitremorgin B was purified by the preparative thin-layer chromatography and crystallized from methanol as colorless needles. The crystals melted sharply at 211–212° but this compound was unstable in some degree, especially in chloroform or acidic solutions. The chemical composition of B was assumed as C₂₆H₂₉O₆N₃ (Mass Spectrum *m/e*: 479 (M⁺), Elementary analysis Found: C, 65.37%; H, 6.92%; N, 7.66%). UV and IR spectra of B were completely resembled to that of A. The presence of three aromatic protons on methoxyindole system was also shown in NMR spectrum.

When *A. fumigatus* Fres. (IFM 4482) was cultured in a basal liquid medium²⁾ (glucose 25 g, ammonium succinate 1.6 g, KH₂PO₄ 0.5 g, MgSO₄ 0.5 g, yeast extract 0.1 g in 1000 ml of water and added 1 ml of minor element solution consisted of FeSO₄·7H₂O 100 mg, CuSO₄·5H₂O 15 mg, ZnSO₄·7H₂O 100 mg, MgSO₄·7H₂O 10 mg, (NH₄)₆Mo₇O₂₄·H₂O 10 mg in 100 ml

TABLE I. Effect of L-Tryptophan to the Production of Fumitremorgins

Medium	Final pH	Dry mycelia (g/l)	Crude toxins (mg/l)
Basic	4.8	8.3	trace
Basic + L-Try (125 mg)	4.8	9.1	19.2
Basic + L-Try (250 mg)	4.2	8.6	49.3

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- 2) J.R. Johnson, A.R. Kidwai, and J.S. Warner, *J. Am. Chem. Soc.*, 75, 2110 (1953).

of water), the production of fumitremorgins was almost of non-detectable. However, the addition of L-tryptophan to the medium caused abundant production of the toxins as shown in Table I.

Substantially, the efficient incorporation of the radioactivity to fumitremorgin A and B from DL-tryptophan-3-¹⁴C has been confirmed in a tracer experiment, and the result strongly supports that fumitremorgin A and B contain the indole ring in those structures as assumed above. As the metabolites of *A. fumigatus* Fres., some indolic compounds have been isolated, such as agroclavine, erymoclavine, festuclavine, chanoclavine, fumigaclavine³⁾ or sulphur containing gliotoxin.⁴⁾ However, fumitremorgin A and B are obviously differed in their spectral and chemical properties from the above metabolites.

The intraperitoneal injection of 1 mg of fumitremorgin A and B causes sustained trembling with intermittent convulsion to mouse. The tremor action on mice normally appears five minutes after injection and continued for several hours. The injection of fumitremorgin B causes usually more severe convulsion than that by A. No lethal examples are observed in a dose of 1 mg of the pure toxins, however the death of 70% of animals is observed within 96 hours by the administration of 5 mg.

The investigation on the chemical structure of these compounds is still going on and details of the study will be reported elsewhere in near future.

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On the Structure of Senegin-II of *Senegae Radix*

As we reported in the previous paper,¹⁾ four glycosides, namely senegin-I, -II, -III and -IV, were isolated from the *n*-BuOH soluble fraction of MeOH extract of *Senegae Radix* (root of *Polygala senega* LINNE var. *latifolia* TORRY et GRAY (Polygalaceae)).

Senegin-II (I), C₇₀H₁₀₄O₃₂·4H₂O, mp 247—248°, colorless needles from *n*-BuOH-AcOH-H₂O (4:1:5, upper layer), [α]_D²⁰ -6.2° (*c*=2.0, MeOH), IR ν_{max}^{Nujol} cm⁻¹: 3500—3300 (OH), 1750 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid), UV λ_{max}^{EtOH} μμ (log ε) 317 (4.28), is composed of presenegenin, 3,4-dimethoxycinnamic acid, glucose, galactose, rhamnose, fucose and xylose.

On methylation with CH₂N₂ in MeOH, I gave a monomethyl ester (II), which was acetylated with acetic anhydride and pyridine to afford senegin-II monomethyl ester tetradecaacetate (III), C₉₉H₁₃₄O₄₆·2H₂O, colorless powder, mp 164—166°. Acid hydrolysis of II

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