Malformin A₁ as a Mammalian Toxicant from *Aspergillus niger*

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The fungi that infect cereal grains are classified as field, storage, and advanced decay molds,[1] and much attention has been given to the storage and field fungi such as *Aspergillus*, *Penicillium* and *Fusarium* species. Little is known, however, on the microbiological and toxicological significance of these fungi in the air, despite that these fungi are possibly important in causing food contaminations and respiratory mycotoxicoses.[2] In this paper, we report the isolation, identification and mammalian toxicity of malformin A₁ from cultures of *Aspergillus niger* strain No. 203, isolated from the air.

Malformin A₁ and related compounds have been isolated from cultures of *A. ficum*, *A. niger*, *A. awamori*, and *A. phoenicis* which are closely related to the fungi used in the fermentation industry.[3~5] Curtis[6] reported that malformins are the plant growth regulators which induce malformation on stems and petioles of *Phaseolus vulgaris* and curvature in roots of *Zea mays*. However, very little is known on the mammalian toxicity of malformin.[7]

Microorganism and media. *Aspergillus niger* No. 203 was isolated from the air in Kochi city, Kochi. The toxin-producing ability was tested on the following media: the peptone-supplemented Czapek-Dox (PSC) medium, the Czapek-yeast extract, the Czapek-corn steep liquor, the modified Wickerham medium, the corn steep-dextrose medium, the yeast extract-sucrose, and the Sabouraud medium. After stationary cultivation at 25°C for 14 days, the culture filtrate was treated with charcoal, and charcoal adsorbent was eluted with methanol. The eluate was evaporated *in vacuo* to afford dry matter, and administered intraperitoneally to male mice for toxicity tests. The extracts from all of the media used except the Sabouraud medium showed toxicities to mice. For mass production of toxic principle, we used the PSC medium which provided the highest toxicity among the above media.

Isolation and identification of malformin A₁. The

fungus was surface-cultured on the PSC medium for 9 days at 25°C. The culture filtrate was treated by the procedure as mentioned above to give dry matter (2.4 g/liter of the filtrate). The dry matter was dissolved in chloroform-methanol (10: 1), and the insoluble material was discarded. The soluble fraction was evaporated *in vacuo*, and the resultant (1.1 g/liter of the filtrate) was chromatographed on a silica gel column using a solvent system of toluene-ethyl acetate-formic acid (5: 4: 1) to afford a chromatographically pure toxin (3.1 mg/liter of the filtrate). The toxin gave a single spot with Rf values of 0.44 and 0.39 (toluene-ethyl acetate-formic acid, 5: 4: 1 and 6: 3: 1, respectively), 0.17 (chloroform-methanol, 97: 3) on TLC using silica gel G, and showed positive color tests both for molybdophosphoric acid (blue) and Dragendorff's reagent (orange), and negative tests both for ninhydrin and nitroprusside. Melting point was over 300°C(decomp.), $[\alpha]_D^{20} -37.5°C$ ($c=1.4$, methyl cellosolve). UV λ_{max}^{MeOH} nm (ε): 220 (4100). IR ν_{max}^{KBr} cm⁻¹: 3260, 1660, 1540. The acid hydrolysate of the toxin contained leucine, isoleucine, valine, and cystine (1.00: 0.95: 1.06: 1.08). These results suggest that the toxin is a cyclic peptide related to malformin A.[8,9] The toxin was indistinguishable from an authentic sample of malformin A₁ on TLC using silica gel H. Therefore, the mammalian toxicant from *A. niger* was identified as malformin A₁: the disulfide form of cyclo-D-cysteinyl-D-cysteinyl-L-valyl-D-leucyl-L-isoleucyl.[10]

Acute toxicity of malformin A₁ to mice. The LD₅₀ value of malformin A₁ for male mice of DDY strain was 3.1 mg/kg intraperitoneally, but oral administration of the toxin at doses up to 50 mg/kg in male mice failed to give significant evidence of acute toxicity. In the fatal cases, the survival time of mice was within 4 days (Table I), and the acute symptom of them were marked dilatation with hemorrhage of gastointestinal tract and fading of liver and kidney. The toxin easily lost its activity during the isolation procedure to give a methanol insoluble compound, though infrared spectrum of

TABLE I. SURVIVAL OF MALE MICE (DDY Strain)
ADMINISTERED INTRAPERITONEALLY WITH
MALFORMIN A₁

Dose (mg/kg)	Survival (days)							Death rate	LD₅₀ (mg/kg)
	1	2	3	4	5	6	7~14		
1.59	0	0	0	0	0	0	0	0/8	3.1
2.07	0	1	0	0	0	0	0	1/8	(2.6~3.6)[a]
2.69	0	2	1	0	0	0	0	3/8	
3.50	1	3	1	1	0	0	0	6/8	
4.55	4	1	2	0	0	0	0	7/8	
5.92	7	0	0	0	0	0	0	7/8	
7.69	8	–	–	–	–	–	–	8/8	

[a] 95% confidence limits.

the insoluble compound was almost identical to that of malformin A_1. This suggests that the inactive compound is a conformational isomer of malformin A_1.[11]

When malformin A_1 was mixed with an excess of L-cysteine in an aqueous solution at room temperature, malformin disappeared completely on TLC after 24 hr, and formed a suspension of the malformin-cysteine (1:1) adduct. Intraperitoneal administration of the suspension in mice failed to show any evident toxicity. As shown in Table II, the similar result was obtained from the reaction of malformin A_1 and reduced glutathione or 2-mercapto-ethanol. On the other hand, a mixture of malformin and cystine showed only a slight decrease in toxicity. These results indicate the detoxification of malformin by the thiol-disulfide exchange reaction.

TABLE II. EFFECTS OF THIOL COMPOUNDS
ON THE TOXICITY OF MALFORMIN A_1

Combination (μg/mouse, 15 g)	Mortality (dead/treated)
Malformin A_1 (100)	5/5
Malformin A_1 (100) and cysteine (240)	0/5
Malformin A_1 (100) and cystine (240)	4/5
Malformin A_1 (100) and reduced glutathione (600)	0/5
Malformin A_1 (100) and 2-mercaptoethanol (1000)	0/5

Suda and Curtis[12] reported that a variety of sulfhydryl compounds, such as cysteine, glutathione, and 2,3-dimercapto-propanol, inhibit the promotion of corn root curvature caused by malformin. Moreover, Iriuchijima and Curtis[13] demonstrated that malformin reacts with cysteine or 2-mercapto-ethanol to form an equimolar addition product, which markedly decrease the plant-growth regulating activity.

Hofmann et al,[14] reported that the mycotoxin patulin rapidly reacts with glutathione at pH 7.4, and the reaction product is not toxic to chick embryoes, rabbit skins, and mice. Recently, the inhibition of thiol enzymes (alcohol dehydrogenase, lactic dehydrogenase, and muscle aldolase) by patulin and penicillic acid has been demonstrated.[15,16]

The present study demonstrates that the toxicity of malformin A_1 may be derived partially from the interac-

tion of its disulfide group with essential thiol compounds. Further studies are required for the inhibitory effect of malformin on enzyme systems as well as the interaction of the toxin with amino acid residues in enzyme active sites.

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