required a demonstration that the techniques used were adequate to detect Arthus phenomena i.e., that this result did not represent a false negative finding. The findings in experimentally immunized animals tended to exclude this possibility. In contrast to the findings in control animals, dermal inflammatory reactions with associated vasculitis and tissue necrosis were elicited in all actively immunized animals. The cells involved in the vascular inflammatory reaction were polymorphonuclear leukocytes. Challenge of passively immunized animals produced skin ulceration and association Arthus vasculitis as observed for actively immunized animals.

This inability to demonstrate Arthus reactivity to endotoxin is consistent with previous findings that this species does not appear to develop antibodies to *E. coli* antigens under natural conditions[18,19]. This lack of immune products to endotoxin possibly reflects the fact that guinea-pigs do not harbor *E. coli* among their natural bowel flora[20,21], and consequently are not normally exposed to *E. coli* antigens.

Bacteriological study of a variety of species ranging from cockroaches to elephants has shown that *E. coli* are present among the bowel flora of most animals; guinea-pigs, rabbits and gerbils represent isolated exceptions[20,21]. This pattern of flora appears to correlate with the development of circulating antibodies in most mammals studied[18]. Consequently the demonstration here that endotoxins may elicit Arthus reactions in immunized animals might have implications for the pathogenesis of at least a component of endotoxin toxicity in the majority of mammals. More importantly, however, it may be concluded from this study that there is an intrinsic toxicity mechanism activated by endotoxins which is independent of this major class of immune, hypersensitivity process. The combination of these findings and previous findings that Type I mechanisms are not involved[16] make it unlikely that immunoglobulin-dependent immune mechanisms play any role in endotoxin toxicity beyond potentiation of other, as yet undefined, toxic mechanisms.

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New antibiotics, trichopolyns A and B: Isolation and biological activity[1]

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Summary. Polypeptide antibiotics, trichopolyns A and B, were isolated from the culture broth of *Trichoderma polysporum* (Link ex Pers) Rifai (TMI 60146). Assessment of biological activity of the antibiotics against microorganisms was made.

Lentinus edodes, one of the most popular edible mushrooms in Japan, is often prevented from growing by *Hypocrea*, *Trichoderma*, *Gliocladium* and *Cephalosporium*, which causes serious damage for cultivators[5–8]. Especially, *Trichoderma polysporum* (Link ex Pers.) Rifai (strain TMI 60146) has been known to have a strong inhibitory action against *Lentinus edodes*. Recently, the novel cyclic oligopeptide antifungal metabolites, cyclosporins A and C have been isolated from strain number NRRL 8044 of the same species, and their structures have been determined by chemical investigations[9] and X-ray analysis[10]. Our investigations on *Trichoderma polysporum*, strain TMI 60146, resulted in the isolation of 2 new metabolites trichopolyns[11] A and B, which strongly inhibit growth of hymenomycetes as well as other organisms. In this communication, preliminary studies on the structure and the biological activity of trichopolyns are reported.

Material and methods. The filtrate of the culture broth of *Trichoderma polysporum* was concentrated to about $^1/_5$ in volume at 45–50 °C under reduced pressure, and extracted with ether. The ether layer was dried over Na_2SO_4 and concentrated to dryness followed by chromatography over silicic acid. Elution with 10% methanol-acetone gave fractions having a strong inhibitory activity against *Lentinus edodes*. Further fractionation by column chromatography and preparative thin-layer chromatography (TLC) resulted in the isolation of 2 new peptide antibiotics, trichopolyn A as an amorphous solid which was shown to be homogeneous on TLC over silicic acid (R_f 0.29; solvent system, 70% dichloromethane-15% acetone-15% methanol) and trichopolyn B as crystals (m.p. 114–116 °C from dichloromethane-ligroin) whose R_f-value was 0.27 under the same conditions as trichopolyn A. Maximum yields (3–4 mg/l) of trichopolyns A and B were obtained from the culture medium which consisted of glucose (25 g), ammonium tartarate (4 g), KH_2PO_4 (2 g), $MgSO_4$ (1 g) and $FeCl_3$ (0.1 g) in 1000 ml of tap water at initial pH 3.5 under artificial ventilation for 4–5 days.

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11 Tricholides A and B, the names adopted for these new antibiotics in the 94th annual meeting of Pharmaceutical Society of Japan at Sendai (Japan) in 1974 have been changed for trichopolyns A and B, since the term 'olide' is generally used for lactonic compounds.

Minimum inhibitory concentration (μg/ml) of trichopolyns A and B

Test organisms	Trichopolyn A	Trichopolyn B	Conditions
Lentinus edodes TMI 563	1.6	1.6	A
	1.6	3.2	B
Pholiota nameko TMI 30077	1.6	3.2	A
	3.2	3.2	B
Flammulina velutipes TMI 30076	3.2	3.2	A
	12.5	12.5	B
Pleurotus ostreatus TMI 30009	6.3	6.3	A
	6.3	6.3	B
Saccharomyces cerevisiae IFO 0209	25	12.5	C
Candida albicans IFO 0583	6.25	3.13	C
Candida utilis IFO 0619	0.78	0.78	C
Cryptococcus neoformans IFO 0410	0.78	0.78	C
Penicillium chrysogenum IFO 4626	3.13	3.13	C
Aspergillus niger IFO 4066	3.13	3.13	C
Aspergillus fumigatus IFO 5480	6.25	3.13	C
Trichophyton mentagrophytes IFO 7522	0.78	0.78	C
Trichophyton rubrum IFO 5467	3.13	1.56	C
Microsporum gypseum IFO 6078	100	25	C
Pyricularia oryzae IFO 6193	0.78	0.78	C
Staphylococcus aureus FDA 209P	6.25	6.25	D
Staphylococcus aureus 308A-1	6.25	6.25	D
Staphylococcus aureus 1840	6.25	6.25	D
Staphylococcus epidermidis FS 5019	3.13	6.25	D
Bacillus subtilis PCI 219	6.25	6.25	D
Bacillus cereus IFO 3466	6.25	6.25	D
Sarcina lutea PCI 1001	1.56	1.56	D
Micrococcus flavus IFO 3242	0.78	0.78	D
Mycobacterium sp. Takeo	6.25	6.25	C
Mycobacterium sp. ATCC 607	3.13	3.13	C
Mycobacterium phlei IFO 3158	1.56	3.13	C

A, Paper disk method. B, Agar dilution method. C, Medium; 1% glucose bouillon agar, incubation; 28 °C, 40 h. D, Medium; trypticase soy agar (BBL), incubation, 37 °C, 18 h, inoculum; a loopful of bacterial suspension (ca. 10^7 CFU/ml).

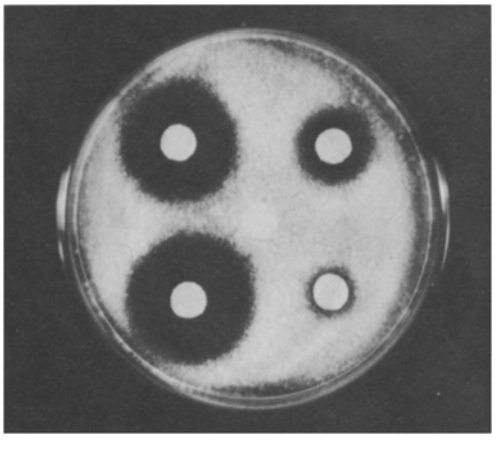
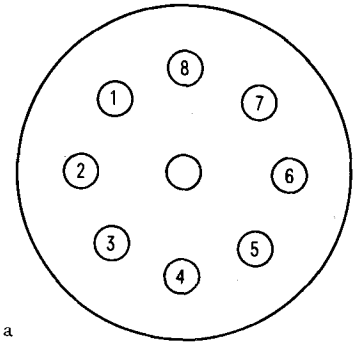


Fig. 1. The zones of inhibition by trichopolyn B against *Lentinus edodes*. Concentrations: Upper left, 25 μg/ml; bottom left, 12.5 μg/ml; bottom right, 6.25 μg/ml; upper right, 3.13 μg/ml; middle, control.

To the best of our knowledge, since no established methods of assessment of antagonistic activity against hymenomycetes have been reported, *minimum inhibitory concentration* (MIC) of trichopolyns A and B for hymenomycetes was determined by the similar method for mould fungi[12–14] and bacteria[15,16].

Paper disk method. Into a petri-dish (90 mm in diameter) 15 ml of medium (glucose, 20 g; yeast extract, 5 g; agar, 15 g; and tap water to make 1000 ml) was poured. After allowing the agar surface to dry, 0.3 ml of basidiospore suspension (ca. 18×10^5 spores/ml) of a test fungus was incubated and spread over the plate uniformly. After precultivation at 20 °C for 20 h, paper disks (8 mm in diameter) containing a given amount of trichopolyn A or B were placed on the agar plate. Clear circles resulting from inhibition of growth were formed as shown in figure 1 after cultivation at 25 °C for 10 days. Maximum concentration where the zone of inhibition was not formed, was regarded as MIC.

Agar dilution method. Into a petri-dish (60 mm in diameter) 6 ml of the same medium as described above with a given amount of trichopolyn A or B was poured to prepare an agar plate. Agar disks (10 mm in diameter) were taken out by a sterile cork borer and placed on a petri-dish as shown in figure 2a. After a drop of basidiospore suspension (ca. 18×10^4 spores/ml) of a test fungus had been inoculated in the middle of each disk by a micropipette, it was covered with another petri-dish with wet



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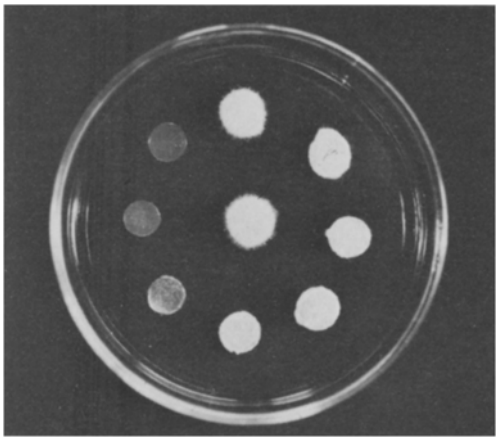

b

Fig. 2. Assessment of activity of trichopolyn A against *Pholiota nameko* by agar dilution method. *a* Diagram showing arrangement of agar disks on a petri-dish. 2fold successive dilution of initial concentration (3.13 μg/ml, No. 1) gave each agar disk containing a given concentration of the antibiotic. Final concentration; 0.024 μg/ml (No. 8). Control agar disk is placed in the middle. *b* Results obtained from cultivation at 25 °C for 7 days. Clear zone indicates flourishing mycelial growth.

filter paper to supply sufficient humidity. After 10 days cultivation at 25 °C, the extent of spore germination and mycelial growth was judged with the naked eye and under a microscope.

Results and discussion. Assessment of biological activity of trichopolyns A and B is summarized in the table. Trichopolyns were found to be ineffective on the following organisms up to a concentration of 100μg/ml: *Escherichia coli, Salmonella typhosa, Shigella flexneri, Shigella sonnei, Klebsiella pneumoniae, Proteus vulgaris, Proteus morganii, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Citrobacter freundii and Enterobacter aerogenes.* Trichopolyns have strong inhibitory activity against fungi, gram-positive bacteria and acid-fast bacteria, which is quite different from cyclosporins[17]. The maximal tolerated dose of a 1:1 mixture of trichopolyns A and B in mice was 5 mg/kg when administered i.p. It is noteworthy that MIC of trichopolyns A and B against *Flammulina velutips* is exceedingly high as compared to those against other hymenomycetes in the agar dilution method, while in the paper disk method it remains on nearly the same level as other hymenomycetes. The observed fact is well explained by assuming that trichopolyns inhibit rather the mycelial growth than spore germination of *Flammulina velutipes*.

Though the mol.wt of trichopolyns A and B have not yet been settled, determination by gel filtration gave a value of about 2000 for both compounds. Moreover, infrared spectra of these antibiotics showed very similar absorption bands characteristic of polypeptides (ν_{max}^{KBr}; 3300, 1670 and 1535 cm^{-1}). Amino acid composition of trichopolyns A and B was determined to be [(α-amino isobutyric acid)$_4$, (Ala)$_2$, (Ile)$_1$, (Pro)$_1$]n. It is interesting that cyclosporins contain a number of methylated amino acids which are missing in trichopolyns. In the hydrolysates of both trichopolyns A and B with 6 N HCl the presence of α-methyl capric acid, and a ninhydrin-positive compound whose structure has not been determined, were recognized. Trichopolyn A was easily converted to trichopolyn B by stirring its acetone solution with saturated aqueous sodium chloride solution, while trichopolyn B, on stirring its acetone solution with 10% aqueous silver nitrate, gave trichopolyn A. These interconversions clearly indicate that these 2 antibiotics have the same basic skeleton but only differ in counter anions, that is NO_3^- for trichopolyn A and Cl^- for trichopolyn B. A more detailed investigation on the structure of trichopolyns is currently underway.

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Quantitative studies on in vitro transformation of hamster chondrocytes[1]

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Summary. Hamster chondrocytes could be transformed in a quantitative assay system which used X-irradiated feeder layer cells. Morphological transformation occurred on addition of, 4NQO, but not in control cultures. Differentiation was classified into 3 types (good, poor and none); normal and transformed colonies contained similar proportions of the 3 types.

Since the 1st report of chemical carcinogenesis in vitro by Berwald and Sachs[3], various mammalian cells have been transformed by a number of chemical carcinogens. However, almost all studies have been done with fibroblastic cells which have no specific differentiated characters. Recently, several epithelial cell systems, such as rat liver parenchymal cells[4–6], rat submandibular gland cells[7], and rat urinary bladder cells[8], have been established, but they have not yet been used as model systems to investigate the relationship between carcinogenesis and differentiation. We reported previously that hamster chondrocytes, which originate from mesoderm but which have clearly differentiated characters, could be transformed into neoplastic cells with chemicals[9,10]. From our findings we concluded that carcinogenesis and differentiation are more or less incompatible. However, it was not clear whether transformation (or the initial step of carcinogenesis) and differentiation were incompatible, because we only observed a close relationship between malignancy and dedifferentiation. This paper reports the in vitro transformation of hamster chondrocytes with 4-nitroquinoline-1-oxide (4NQO) in a system for quantitative assay of transformation.

Chondrocytes were obtained from suckling hamsters as described previously[10]. Primary cultures of chondrocytes were trypsinized and stored in liquid nitrogen for use as target cells. The method for quantitative assay of transformation was essentially as reported previously[11]. The feeder layer cells used were cryopreserved hamster cells derived from 14 gestation days embryos. When the cells became confluent, they were irradiated with 5,000 R,

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