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## BIOACTIVE COMPOUNDS FROM MUSHROOMS

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**Abstract** – Anti-dementia compounds, anti-MRSA compounds, osteoclast-forming suppressing compounds, gastrointestinal toxins and sick-from-drinking toxins from mushrooms are explicated.

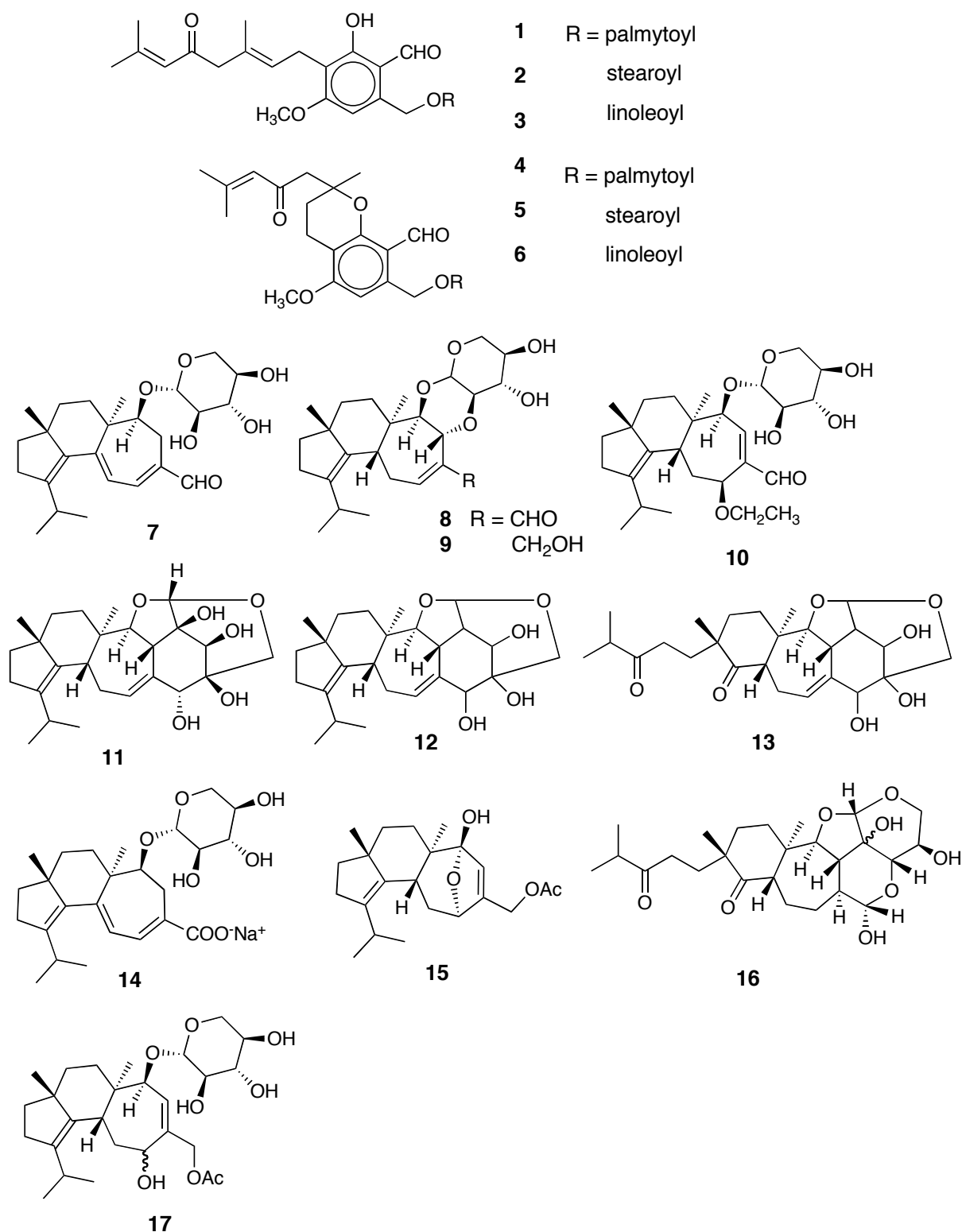
### INTRODUCTION

There is an expression which states that “the plants act as producer, animals as consumer, and fungi as restorer and decomposer”. In other words, the plants create organic compounds by means of photosynthesis and animals consume such plants. Then fungi, including mushrooms, play an important role in restoring the plants and animals back to the land. There are some differences in the structures of metabolic products by mushrooms compared to those by plants and animals. These differences sometimes indicate biological activities indigenous to mushrooms. The review presents some of our studies on bioactive substances isolated from various mushrooms.

### RESULTS AND DISCUSSION

#### Anti-dementia Compounds

We have been engaged in a study to search for nerve growth factor (NGF) synthesis-promoting compounds in medicinal mushrooms since 1991. We discovered a series of benzyl alcohol



**Figure 1.** Hericenones and erinacines isolated from *Hericium erinaceum*

derivatives named as hericenones C ~ H (**1 - 6**), as well as a series of diterpenoid derivatives named as erinacines A ~ I (**7 -15**) from *Hericium erinaceum* (Figure 1),<sup>1-10</sup> which evidently stimulate NGF production from mouse astroglial cells.

Recently, a study *in vivo* was conducted to examine the effect of erinacine A on the production of NGF in the various regions of central nervous system of rats.<sup>11</sup> Newborn rats were given erinacine A (8 mg/kg body weight) for four weeks. After the last administration, the rats were decapitated in the anesthetized condition. The NGF contents were measured in the following rat brain regions: olfactory bulb (OLB), locus coeruleus (LC), hippocampus (Hip), and cerebral cortex (CC). The effect of erinacine

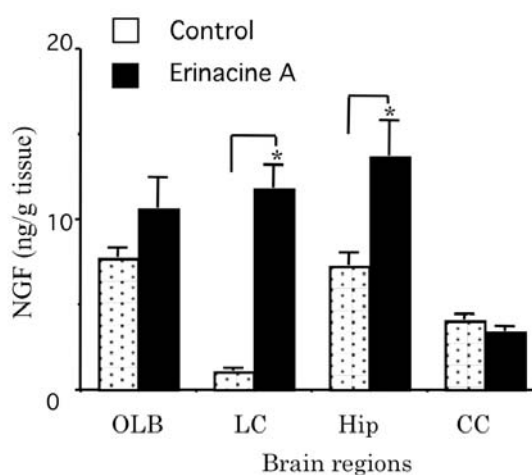


Figure 2. NGF content in the brain of rats fed with erinacine A  
\* $p < 0.05$

A on the production of NGF in various brain regions is shown in Figure 2. In LC and Hip, the NGF content of erinacine A-treated group was much higher than that of the control group. There was no significant difference in the NGF contents of OLB and CC between both groups.

Another study *in vivo* was done to investigate the effects of hericenone C and erinacine A on rats with ibotenic acid-induced dementia and rats with artificially-induced cerebrovascular dementia. The results demonstrated that these compounds were beneficial to maintaining memory and improving learning skills in these model rats (unpublished).

A clinical study using *H. erinaceum* was conducted to investigate its effectiveness against dementia in a rehabilitative hospital in Gunma prefecture in Japan, with 50 patients in an experimental group (average age 75.0) and 50 patients used as a control (average age 77.2).<sup>12</sup> All patients were suffering from cerebrovascular disease, degenerative orthopedic disease, Parkinson's disease, spinocerebellar degeneration, diabetic neuropathy, spinal cord injury, or disuse syndrome. Seven of the patients in the experimental group suffered from different types of dementia. The patients in this group received 5 g of dried fruiting body of *H. erinaceum* per day in their soup for a 6-month period. All patients were evaluated before and after the treatment period for their

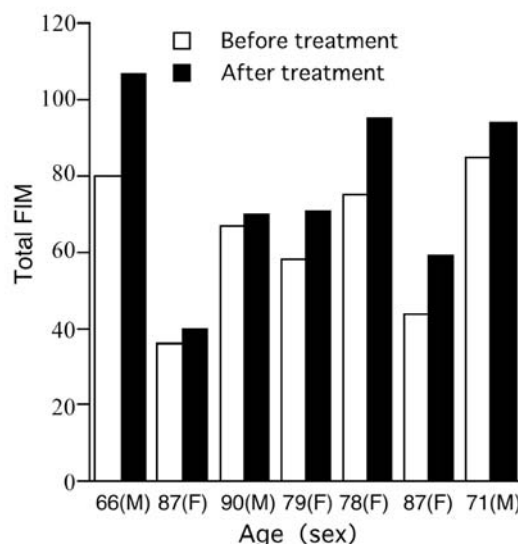


Figure 3. Effect of *Hericium erinaceum* treatment on total FIM

Functional Independence Measure (FIM) which is an international valuation standard of independence in physical capabilities (eating, dressing, evacuating, walking, bathing/showering etc.) and in perceptive capabilities (understanding, expression, communication, problem-solving, memory). The results showed that after six months of taking *H. erinaceum*, six out of seven dementia patients demonstrated improvements in their perceptual capacities, and all the seven had improvements in their overall FIM score (Figure 3). Particularly, three bedridden patients are able to get up to eat meals after the administration. More extensive clinical studies are currently underway at different hospitals.

### Anti-MRSA Compounds

Methicillin-resistant *Staphylococcus aureus* (MRSA), a type of *Staphylococcus* bacterium, has developed resistance to most antibiotics and is one of the most prevalent pathogen in nosocomial infections. *Staphylococci* bacteria are carried by healthy people without disease being present. However MRSA colonization may precede or lead to infection in persons with weakened immune systems or very ill from other medical conditions. In above clinical studies with aged and disabled patients, some patients with MRSA infection were healed during *Hericium erinaceum* treatment.<sup>13</sup> Due to the finding, we screened various extracts of *H. erinaceum*, and found that erinacines A (**7**), C (**9**), and novel erinacine K (**17**) showed evidently anti-MRSA activities (Figure 1).<sup>14</sup> Erinacine J (**16**) exhibited no activity, suggesting that the three-ring skeleton of the aglycon in the active compounds was indispensable to the anti-MRSA activity.

### Osteoclast-Forming Suppressing Compounds

Osteoclasts and osteoblasts are two basic types of bone regulating cells. The process of dissolving older bones by osteoclasts and forming new bone by osteoblasts requires hormonal guidance to properly function. Osteoporosis can occur when osteoclasts dissolve more bone than what the osteoblasts are able to replace due to the deficiency of

hormones with advancing age. Therefore, it may be possible to prevent and improve osteoporosis by properly suppressing the activities of osteoclasts. Because osteoclast-like multinucleated cells can be differentiated *in vitro* from co-cultures mouse bone marrow cells and osteoblastic cells by treatment with osteotropic factors,  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub> and prostaglandin E<sub>2</sub>, we screened osteoclast-forming

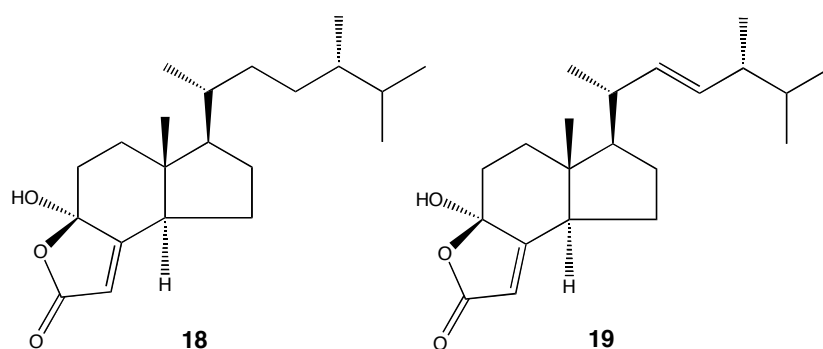


Figure 4. Osteoclast-forming suppressing compounds isolated from *Agrocybe chaxingu*

suppressing compounds from the extracts of various mushrooms by the assay, and isolated two active compounds (**18**, **19**) from *Agrocybe chaxingu* (Figure 4).<sup>15</sup> As shown in Figure 5, these two compounds specifically suppressed the formation of osteoclasts, and had no cytotoxicity. Now, a study *in vivo* on these active compounds is under consideration.

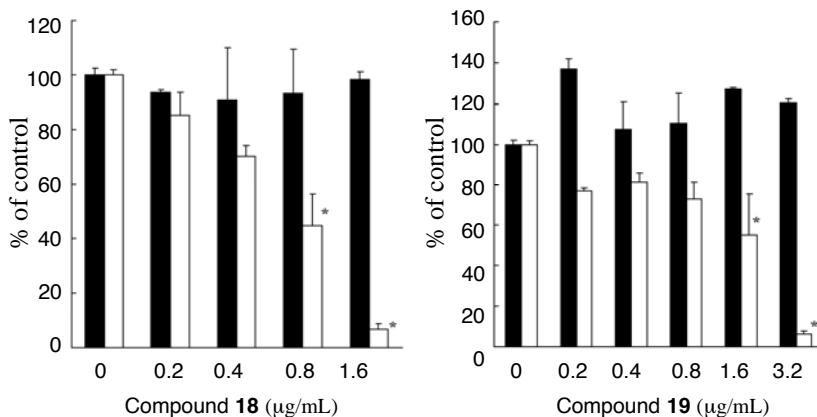


Figure 5. Activities of osteoclast-forming suppressing compounds from *Agrocybe chaxingu*. Black bar, cell viability; white bar, osteoclast-forming. \*  $p < 0.05$

### Gastrointestinal Toxins

In Japan, people enjoy collecting various kinds of wild mushrooms in the fall, and some of them get poisoned by accidentally eating toxic mushrooms. Every year the top three mushrooms responsible for most cases of poisoning are *Entoloma rhodopolium*, *Lampteromyces japonicus*, and *Tricholoma ustale* in Japan. It has been reported that the toxin of *E. rhodopolium* was a hemolytic protein and that of *L. japonicus* was illudin S (**20**) (Figure 6).<sup>16-22</sup> However, the toxin of *T. ustale*, which usually causes gastro-intestinal poisoning accompanied by vomiting and diarrhea, had remained unknown. Such toxin may be used as a new laxative, therefore, we conducted a study to investigate the toxin of this mushroom.

The screening of the toxins was guided by toxicities such as hesitancy to move, tremors, and death after mice were orally fed with fractions. According to the biological responses of various fractions, we isolated a novel toxin named as ustalic acid (**21**) and its derivatives (**22–25**) by various chromatography (Figure 6).<sup>23</sup> When ustalic acid was orally given to mice (body weight about 40 g) at three doses of 2, 5, and 10 mg per mouse, the mice fed with 2 mg showed lower locomotor activity, and the mice fed with 10 mg died. However no diarrhea was observed. We conducted the autopsy on mice with lower locomotor activity,

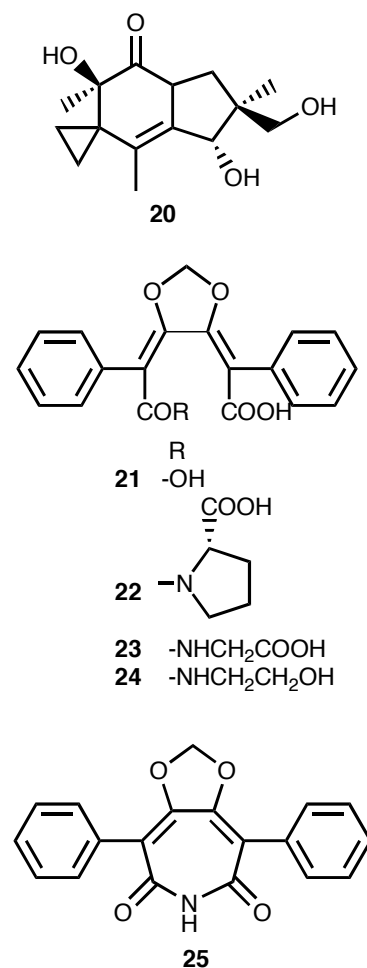


Figure 6. Gastrointestinal toxins isolated from *Lampteromyces japonicus* and *Tricholoma ustale*

and found the feed stayed in the stomach and nothing reached the intestine. Various experiments to estimate the mechanism suggest that ustalic acid may suppress the absorption of water from the intestines by inhibiting intestinal  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, and result in diarrhea.

### Sick-from-drinking Toxins

*Clitocybe clavipes* is a delicious mushroom and popular in Japan. However, when ethanol is consumed with this mushroom, the person may experience one or more of the following symptoms; profound flushing, metallic taste, palpitations, hyperventilation, hypertension, tachycardis, nausea, vomiting, and occasionally collapse. In addition, a study showed that the extract of *C. clavipes* increased the acetaldehyde concentration in the blood when it was orally given to mice. Therefore, the reason of disulfiram-like reaction induced by *C. clavipes* is considered to be that something inhibits the activity of enzyme, aldehyde dehydrogenase (ALDH), which catalyzes conversion of

acetaldehyde to acetic acid during the metabolism of alcohol, and results in acetaldehyde accumulation. As there are some side effects in ALDH inhibitors clinically used as an anti-alcoholic drug, researchers focus on discovering the lead compound of new drugs. We conducted a study to isolate ALDH inhibitors from *C. clavipes*. The screening of active fractions was guided by inhibitory effect on aldehyde dehydrogenase. Since the chloroform-soluble fraction showed inhibitory activity, the fraction was further fractionated by repeated chromatography. As a result, five active compounds (**26–30**) were isolated (Figure 7).<sup>24</sup> Compounds (**26**, **27**, and **30**) showed stronger inhibitory activity against the enzyme compared to compounds (**28** and **29**). Because there is cysteine groups in the active site of the enzyme, it is suggest that these active compounds from *C. clavipes* may inhibit the enzyme by forming a Michael addition adduct between the enone and the thiol group in the enzyme.

### CONCLUSIONS

This review introduces some bioactive substances, including toxins, from mushrooms. It is clear that there is only a fine line between medicinal compounds and toxins. It is said that there are more than 140,000 species of mushrooms on earth.<sup>25</sup> However so far the species which have been studied may be less than 1% of them. Nevertheless, we believe that unknown mushrooms are a vast source of new bioactive substances, and they will be found out one by one in future.

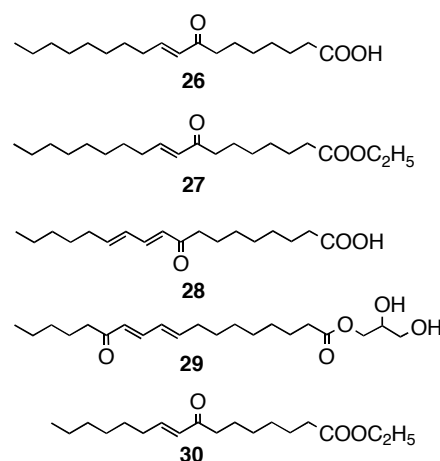


Figure 7. Sick-from-drinking toxins isolated from *Clitocybe clavipes*

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