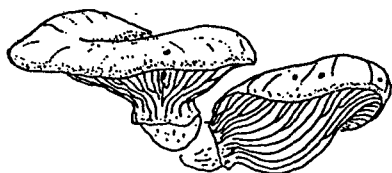


## The principles of poisonous mushrooms, their unexpected utilitie

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Abstract:: *Lampteromyces japonicus* is the most popular source of mushroom poisoning in Japan. We isolated poisonous substance, illudin S, 40 years ago, which showed strong anti-tumoric activity. Its analogs are now the most hopeful anti-cancer agent. *Clitocybe acromelalga* gives unique poisoning which exhibits symptoms similar to acromelalgia and erythromelalgia. We intended to isolate the toxin causing these poisoning but neuroexcitatory compounds, acromelic acids A and B, were isolated. They are now known as the most potent glutamate agonists and good reagents for the study of the glutamate receptor of a neurocell.

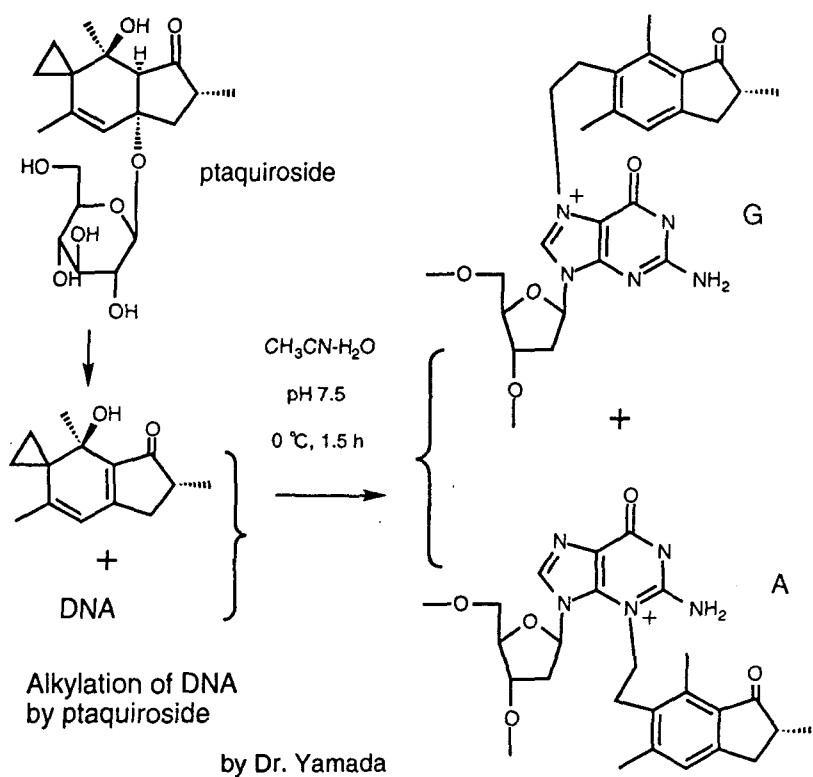
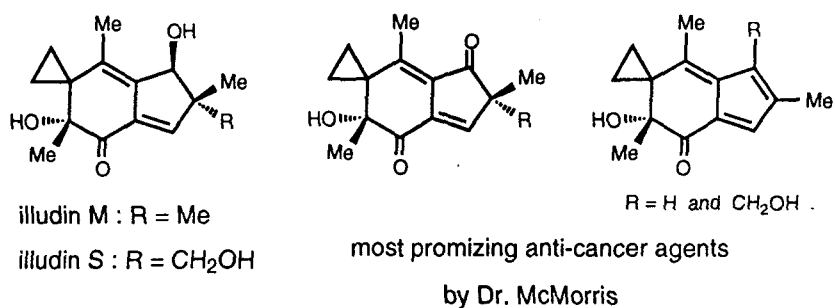


In every autumn the newspapers have reported mushroom-poisoning in my place. Among these poisonings *Lampteromyces japonicus* (Japanese name: Tsukiyotake, which means moon-light mushroom) is the most popular source in Japan.

The mushroom is fluorescent and a young and small one looks very delicious. Erroneous eating causes strong stomachache and diarrhea but the poisoning is not so serious that a fatal accident has not been recorded. I was quite interested in what a compound causes the poisoning and started an extraction of the principle of that mushroom in 1950's. Almost a ton of the mushroom was collected in several years and extracted with methanol-water. The separation was carried out monitoring lethal effect in mice and after many trials and errors a crystalline sesquiterpene was isolated as the toxin, after all. During the isolation, the screening of mushrooms through anti-cancer activity test was carried out in Tokyo University and this mushroom was found to have the most potent activity. I thought that the anti-cancer activity was showed by the toxin which I sought. Indeed, using our sample we found that the anti-cancer substance was the toxin itself. A mouse inoculated with 2,500,000 cells of Ehrlich ascites carcinoma was thoroughly cured by the 4 days treatment, an injection of 2.5  $\gamma$  of the toxin per day. I named the toxin lunamycin after Japanese name Tsukiyotake (moon-light mushroom) and reported its isolation at the meeting. Prof. Nakanishi (Tohoku Univeresity at that time; Columbia Univ. at present) reported also isolation of the toxin named lampterol after Latin name of mushroom, *Lampteromyces*, at the same meeting next to me. We

consulted and selected the name lampterol and we continued the study of the toxin independently.

Drs. Anchel and McMorris at New York Botanical Garden reported the isolation and structure determination of illudins M and S which were extracted from *Clitocybe illudens* (Jack O'lantern, also fluorescent mushroom) on an issue of *Journal of the American Chemical Society* a little bit after our presentation. Their structure explained so well our study results that we sent our sample to them to ask identification with illudins and we found that lampterol was identical with illudin S. Lunamycin changed again its name to illudin S. The structures of illudin S and M are very similar and the S has one more OH group at a methyl of gem-dimethyl group of M. We looked after the compound for

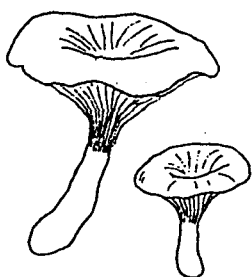


stomach poisoning and found anti-cancer compound after all. Though a Japanese pharmaceutical company made an efforts to make an anti-cancer agent from the illudin S, their work remained unsuccessful at that time. The stereochemistry of illudins was next studied. The relative configuration was analysed by observation of intramolecular H-bonds among their three OH groups and absolute configuration was determined by Horeau's method. Prof. Nakanishi proposed the alternative configuration to ours employing the same method. But we confirmed our formulae by X-ray crystallographic analysis and Prof. Nakanishi corrected their conclusion by newly developed their own method, exciton chirality method. Total syntheses of illudins S and M were also performed by us through an unique method which constructs the illudin from almost the same two fragments of the molecule recognizing the hidden symmetry of the illudin molecule. Our syntheses were introduced in several books and accounts.

We presumed a simple cyclic sesquiterpene, humulene, to be the biosynthetic precursor and studied the stereochemistry of this biogenesis using molecular mechanics calculations and the biogenetic conversion of humulene to cyclohumulanoids, to which illudin belongs as a member were achieved.

Prof. Yamada (Nagoya University) isolated a carcinogenic substance, ptaquiroside, from a fern and its structure was found to be very similar to the illudin's. Ptaquiroside was then revealed to add to guanine and adenine to break DNA. The illudin was also thought to break DNA in the similar manner because of its very similar chemical structure. We saw the very interesting fact that one of the very similar two compounds worked as a carcinogenic and the other did as an anti-cancer agent.

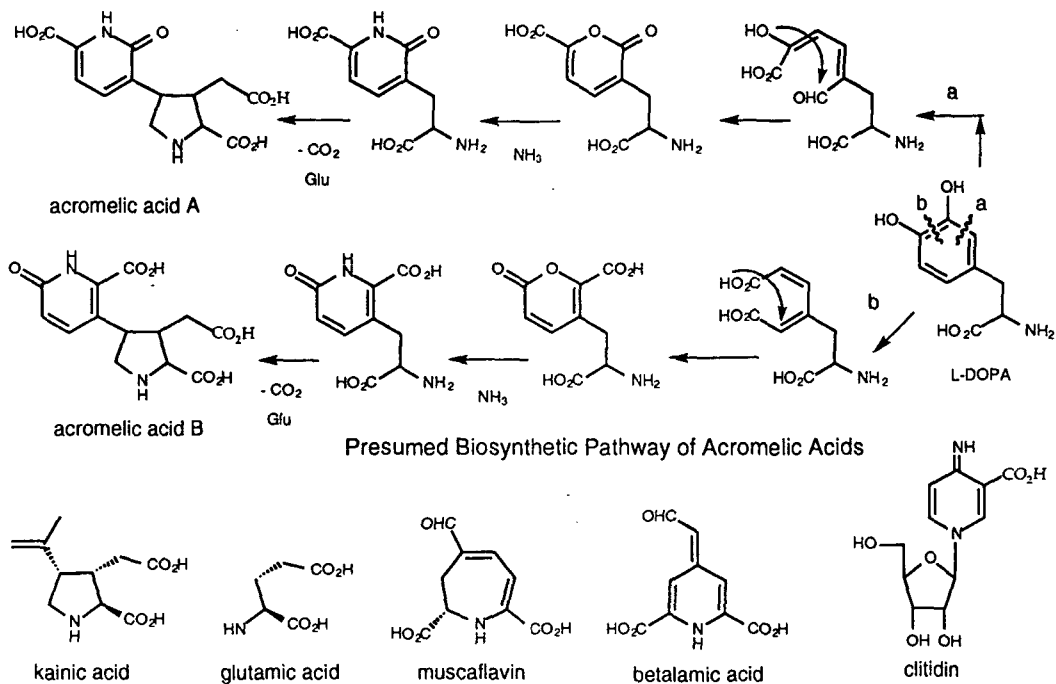
Very recently potently active anti-cancer agents were synthesized assuming illudin as a prototype by Dr. Mc Morris (Univ. California), who has eagerly studied to make an anti-cancer agent out of illudin for forty and some years. American Chemical Society reported recently that they are now the most promising anti-cancer agents. Efficient new syntheses were still reported one after another in chemistry journals.



*Clitocybe acromeralga* is a unique mushroom.

Erroneous ingestion of this mushroom causes a severe pain and a marked reddish edema in hand and foot after several days and it continues for about a month. Since symptom of poisoning appears a week or ten days after the ingestion, people have misunderstood it was endemic disease. The mushroom grows only in Tohoku and Hokuriku district of Japan. Before starting isolation of toxin, I asked doctors about any good bioassay for

such unique symptom and I checked several method but I could not find such symptom in animals. I began the isolation monitoring a lethal effect in mouse in 1970's after all. Severe fractionation using dialysis, a few sorts of chromatographies and electrophoresis gave a few principle. One of them, clitidine, is a weak toxin, whose toxicity is comparable to nicotine. Its molecule has very similar structure to nicotinamide nucleoside but having an imino group at 4 position of pyridine nucleus. If the enzyme might take this molecule in NAD which could not work as an oxidation and reduction reagent and so, clitidine might work as an anti-cancer agent. A company tested eagerly its anti-cancer activity but they could not find any good results. The other toxins, acromelic acids A and B were stronger toxins. They were obtained in very small amounts, 110  $\mu$  of A and 40  $\mu$  of B, from 16 Kg of fresh fruiting body which were the total quantity collected for several years in Niigata prefecture and we could not have any more sample at that time.



Structure determination using such a small amount of sample is very difficult even in present time. We could have no means to know what elements and how many that elements were contained in this molecule, because elemental analysis and mass spectrum could not be employed for such trace of sample. Only available NMR and UV spectrum suggested the kinic acid skeleton to the two acids A and B and the biogenesis of muscaflavin and betalamic acid, which were the red pigments of *Amanita muscaria*, gave

a support to our tentative structures. We imagined its stereochemistry also and achieved syntheses of acromelic acids A and B by chemical conversion of kainic acid and found that our suggested structure were really sound. Completion of the syntheses gave us a chance to have biological test. Since kainic acid has very strong depolarizing activity on a neuro-cell, acromelic acids must have the similar activity. Biological test showed actually very potent activity and it was the the strongest activity which was never observed in other reagents.

Acromelic acids were probably not the toxins which caused a pain and reddish edema in hand and foot but very interesting compounds they were. They worked on the glutamate receptor of a neuron, which was known to be involved with learning and memory of mammal. Senile dementia was also connected to this receptor and the study of its physiology was very much increased. Once I presented these results on a chemistry journal, so many requests for application of our toxin to studies of the glutamate receptor came to me. We have had to synthesize some quantities of the acids. Since the kainic acid was very expensive and a volume of it was not on market, we must have invent new and efficient synthetic methods. The development of several methods has been achieved and some quantity of the sample has been supplied to some physiologists and pharmacologists but it is still not enough to satisfy these requests. We are still making effort to find the better synthetic method to produce easily a volume of sample. In order to study the shape of the receptor and working mechanism, we synthesized several analogs and examined molecular orbital energy of the reagents. The proline ring of acromelic acid works to arrange the eligible conformation of its glutamaic acid residue and a C-4 substituent must have a  $\pi$  electron system to exhibit strong activity and further more, the higher HOMO energy of this  $\pi$  system is, the stronger the neuro-exciting activity is. Original aim of this study, namely, finding of the source of the poisoning is not advanced but the very interesting and important compounds were found. This study is again developed to the unexpected utilities.

#### Materials and methods:

*Lampteromyces japonicus* was collected in South Hokkaido in fall. They grow on a withered beech. The fresh fruiting bodies were soaked in methanol and the extracts were concentrated and subjected to several solvent extractions and chromatographies to give crystalline illudin S. The toxicity was examined by intraperitoneal injection to mice. The structure of illudin S was determined by spectroscopic analysis and some chemical conversions.

*Clitocybe acromelalga* was collected in Niigata prefecture. The frozen fruiting bodies

were extracted with water and the extract was concentrated. Acetone was added to precipitate the toxic matter which was dissolved in water and dialysed against water. The dialyzate was concentrated and fractionated by several chromatographies and paper electrophoresis. Fractionation monitoring by lethal effect in mice led to the isolation of two neurotoxic amino acids, acromelic acids A and B. Their structures were surmised by spectroscopic analysis and confirmed by the chemical conversion of kainic acid to acromelic acids A and B.

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