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STRUCTURES AND BIOLOGICAL ACTIVITIES OF SOME TOXIC NATURAL PRODUCTS

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Abstract - Concerning the relation of chemical structures and physiological activities, there are cases in which similar physiological actions are observed among compounds of similar structures, whilst, in contrast, some cases are known in which slight changes of the structure of compounds result in the remarkable alteration or complete loss of their physiological activities. A typical example of the latter case is toxicity, as illustrated with many compounds, for example, neurotoxins such as tetrodotoxin, saxitoxin and batrachotoxin, and convulsants, such as anisatin, dendrobine, picrotoxinine. With these toxic compounds it will be almost impossible to find a general and clear relationship between chemical structures and toxicity.

INTRODUCTION

In the field of natural products chemistry, attention has been paid in recent years mainly to the substances having biological activity. Chemical studies of such substances are expected not only to disclose unique structures and new reactions which would be extremely difficult to be deduced from the usual chemical knowledge, but also to make these compounds useful in human life by developing new and precious medicines, pesticides, etc. Indeed, from these studies there have been found a number of compounds that possess novel structures, reveal unexpected reactivities and show unusual physical properties, contributing much to the progress of organic chemistry.

We have been interested in the structure determination of natural products having a strong toxicity in the hope of finding unexpected structures and reactivities. Another reason of selecting toxicity as the biological activity is the ease of testing: we can test toxicity without the cooperation of biologists. Of course, structures of toxic compounds vary, and hence there are many modes of toxic action.

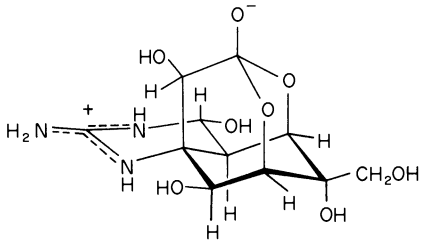
Toxic compounds are usually classified according to the organs suffering from the toxic action. There are neurotoxins, anesthetic poisons acting on central and peripheral nerves, convulsants, blood poisons, cardiac poisons, inflammatory poisons, etc. We are interested in compounds exhibiting toxic actions on well defined tissues.

STRUCTURE AND TOXICITY OF SOME COMPOUNDS

Tetrodotoxin

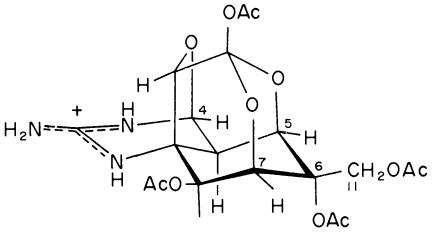
Apart from toxic proteins, one of the most poisonous natural products is tetrodotoxin, which is especially well-known in Japan since puffer fish in which the toxin is found is well esteemed as a delicious food on this country. History of tetrodotoxin poisoning goes way back to B.C., when the first Chinese Pharmacopea Pem-Tso Chin (the book of herbs, ca 2800-2700 B.C.) described the poisoning. Modern scientific research had started in Japan about 100 years ago; Dr. Tahara gave the name of tetrodotoxin to the crude toxin obtained from puffer fish. Later, professor Yokoo obtained the toxin in crystalline form in 1950. Chemical investigation of tetrodotoxin was initiated by professor Tsuda and his coworkers, and later we also started it. It is interesting to note that four groups [Tsuda et al., Goto et al. (our group), Woodward et al., and Mosher et al.] reached the same conclusion, reporting the structure of tetrodotoxin in April 1964 at the 3rd International Symposium on the Chemistry of Natural Products in Kyoto (Ref. 1).

Tetrodotoxin originally found in puffer fish has been shown to be distributed among various animals: a kind of octopus found in Australia, frogs in Costa Rica, California newt, and a kind of goby fish found near Okinawa. Puffer fish contains tetrodotoxin not only in eggs, but in skin and liver, and in the case of a certain kind of puffer fish, even in muscles. It is therefore interesting whether the toxin is produced by the fish itself or not, and how it is biosynthesized. It is puzzling that tetrodotoxin is not toxic towards puffer fish itself.



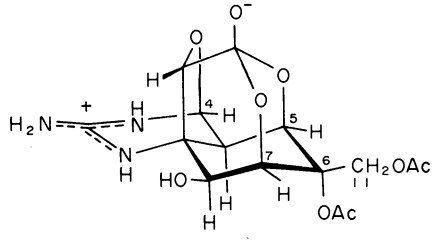
Tetrodotoxin
8 μg/kg mouse

Ac_2O
p-TSA



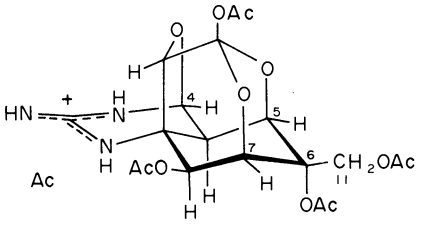
Tetraacetylanhydroepitetrodotoxin
non toxic

Ac_2O-Py



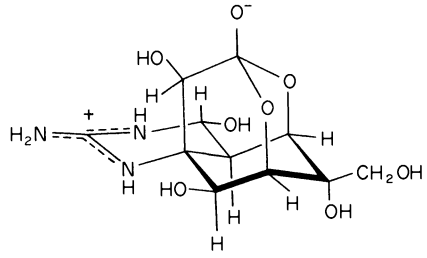
Diacetylanhydroepitetrodotoxin
non toxic

aq. NH_3



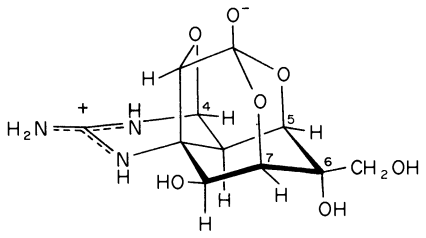
Pentaacetylanhydroepitetrodotoxin
non toxic

Tetrodotoxin has several unusual structural features: (i) many hydroxyl groups are present, which make this toxin insoluble in organic solvents; unexpectedly it is insoluble even in water unless acidic; (ii) a unique acidic ortho ester linkage is present between C-5 and C-7 hydroxyl groups; and (iii) the guanidinium group makes a hemiacetal-like structure with the aldehyde in the C-4 position. Preparations of the derivatives of this toxin were very difficult owing to the complexity of its structure, but during the structure elucidation of this compound, the following derivatives have been prepared: Acetylation of the toxin with acetic anhydride and p-toluenesulfonic acid gave tetraacetylanhydroepitetrodotoxin, which was further acetylated in the presence of pyridine to afford pentaacetylanhydroepitetrodotoxin. These acetates could be hydrolyzed with aqueous ammonia to diacetylanhydroepitetrodotoxin and then to anhydroepitetrodotoxin. Using concentrated ammonia we could obtain aminodesoxytetrodotoxin, in which only C-4 hydroxyl group of tetrodotoxin was replaced by amino group. All of these derivatives showed almost no toxicity, in spite of very minute change on the structure of the toxin.



Tetrodotoxin
toxicity 8μg/kg mouse

$H^{\oplus}$



Anhydroepitetrodoxin
almost no toxicity

Tetrodotoxin and anhydroepitetrodotoxin are interconvertible in acidic aqueous solutions, yet anhydroepitetrodotoxin shows almost no toxicity when injected into mice intraperitoneally.

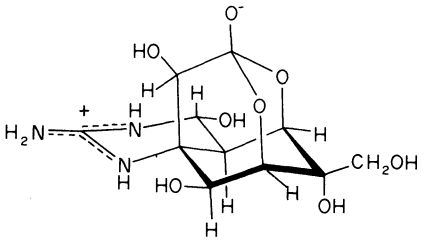
Tetrodotoxin was synthesized in 1972 by Kishi, Goto, Inoue and others (Ref. 2). None of the many intermediates synthesized during the total synthesis showed appreciable toxicity. Thus, the bioassay of the toxicity was used satisfactorily to detect the formation of tetrodotoxin itself in the final stage of the total synthesis.

In many cases the essential structure to produce a particular biological activity is deduced a posteriori from the observation of relation between structural modification and change of biological activity. For example, prostaglandis, β-lactams, ecdysones, juvenile hormones, etc. have been extensively studied in this manner with great success.

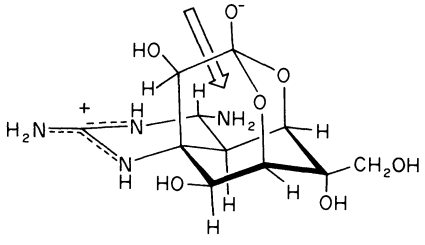
In the case of tetrodotoxin, however, the receptor site is so specific that even very minor change of the structure makes complete loss of its toxicity, thus reasoning a posteriori cannot be applied to obtain a minimum structure for development of the toxicity. This is true not only in the case of tetrodotoxin but also with many other natural toxic substances so far examined.

Anisatin and neoanisatin

Anisatin and neoanisatin have been isolated as toxic components from the ripe fruits of the plant Illicium anisatum L. (Japanese name: shikimi). We determined their structures in 1968 (Ref. 3). Very interestingly, they contain a β-lactone moiety, which was the first instance to have been found in natural products. The β-lactone in anisatin was unusually stable because of protection of the β-carbon atom from nucleophilic attack by the axially oriented δ-lactone ring. Intramolecular opening of the β-lactone

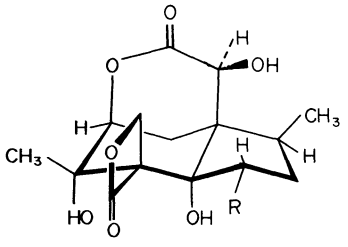


Tetrodotoxin
toxicity 8μg/kg mouse



Aminodesoxytetrodotoxin
almost non toxic

ring occured by heating or by treatment with alkali to produce anisatinic acid and isoanisatinic acid, respectively. Permanganate oxidation of anisatin in acetic acid afforded noranisatin which retains the β-lactone moiety. Toxicity of anisatin and neoanisatin are similar and about 1 mg/kg mouse, whereas the toxicity was completely abolished in the case of other derivatives, although the essential structural feature was almost retained. One might consider that the β-lactone is essential for development of the toxicity, but noranisatin which has the β-lactone moiety shows almost no toxicity. Very interestingly, the stereostructure of these compounds is quite similar to that of tetrodotoxin.

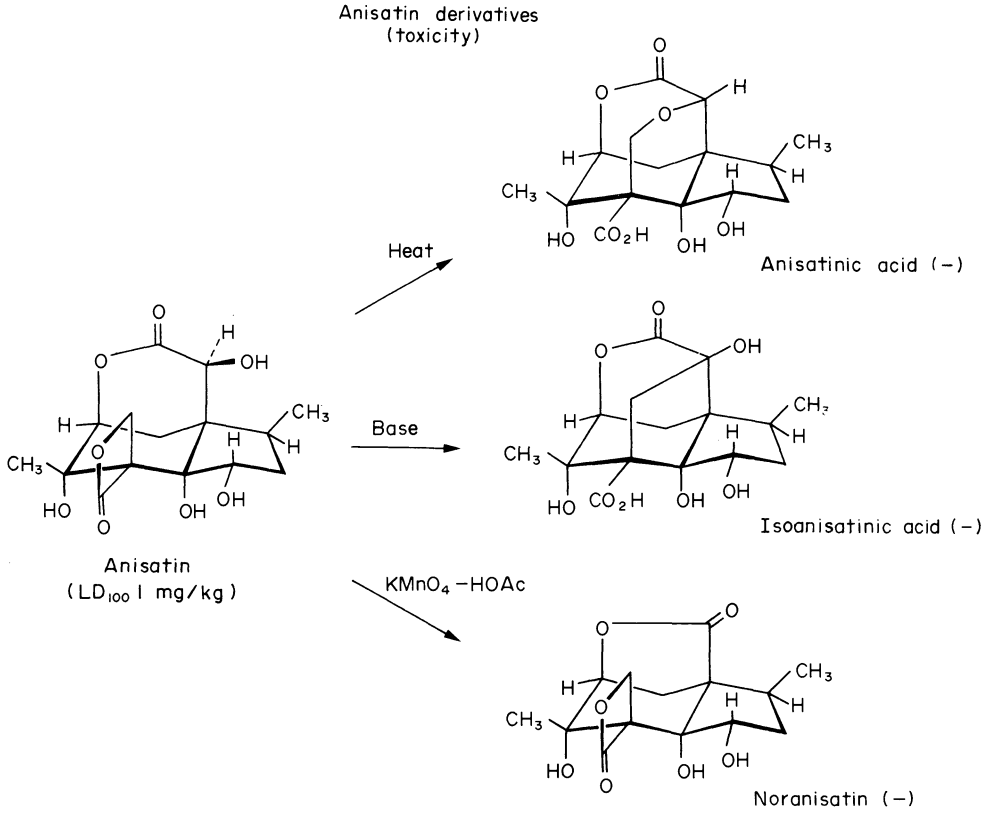


Toxicity

Anisatin	R=OH	ca.1 mg/kg mouse
Neoanisatin	R= H	ca.1 mg/kg mouse

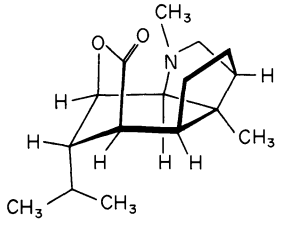
Dendrobine, picrotoxinin, coriamyrtin and tutin

Dendrobine is one of several plant convulsants. It is an alkaloid of ornamental orchid, Dendrobium nobile L. and we established its structure in 1964 (Ref. 4) and synthesized it in 1972 (Ref. 5). It has a strychnine-like toxicity. Picrotoxinin, coriamyrtin, and tutin have structures similar to dendrobine, although the former three have an oxygen bridge instead of the nitrogen in the latter. All of them are convulsant when administered to mammals, but there is evidence indicating that these substances distinctly differ in their modes of action. Dendrobine is a glycine antagonist and the others are γ-aminobutyric acid (GABA) antagonists in mammals (Ref. 6). Dendrobine has a pharmacological activity five to seven times stronger than picrotoxinin (Ref. 7), i.e. the epoxide group of the latter seems not to be responsible for toxicity.

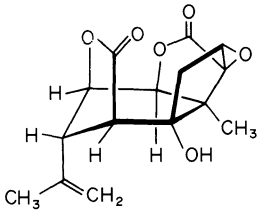


Milliamines

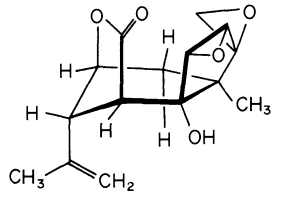
Euphorbia milli Ch. des Moulins has some toxicity. We have isolated from the roots of this plant three toxic constituents, milliamines A, B, and C, and elucidated their structures (Ref. 8). The basic skeleton of these toxic constituents is a diterpene, ingenol, which was found to be non-toxic. Milliamines A and C are toxic, but milliamine B, a positional isomer of the aromatic acid, is not toxic. Esterification of ingenol with appropriate fatty acids makes the products toxic as shown below. Similar observations were reported by Hecker in the case of phorbol, which is non-toxic itself but becomes toxic on esterification with appropriate fatty acids (Ref. 9).



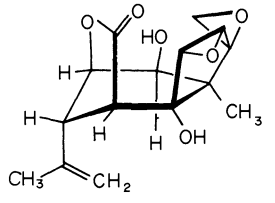
Dendrobine



Picrotoxinin



Coryamyrtin



Tutin

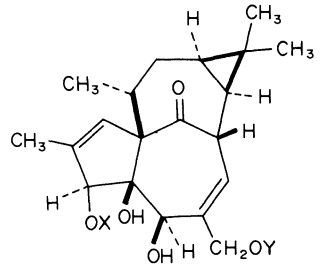
Petasitenine and otosenine

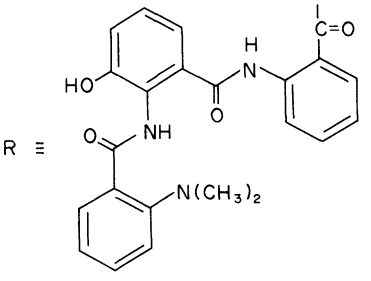
A new pyrrolidine alkaloid, petasitenine, was isolated by us from Petasites japonicus Maxim, and we determined its structure. Petasitenine was recently proved to be carcinogenic, whereas its stereoisomer at the epoxide ring, named otosenine, is not carcinogenic (Ref. 10). This difference in toxicity could be attributed to stereospecific reactivity of the epoxide ring of the former towards nucleic acids, but it might be simply a solubility problem, since the latter compound is extremely insoluble in water. This point must be clarified before discussion of the reactivity.

Teleocidin B, citreoviridin and aureothin

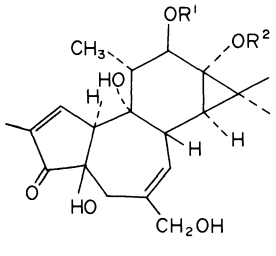
We have also determined the structure of some other poisons as follows:

Teleocidin B was isolated from the mycelia of several Streptomyces and it shows a strong toxicity against fish and also the human skin. The same Toxicity was found with dihydroteleocidin B, a catalytic hydrogenation product of teleocidin B (Ref. 11).

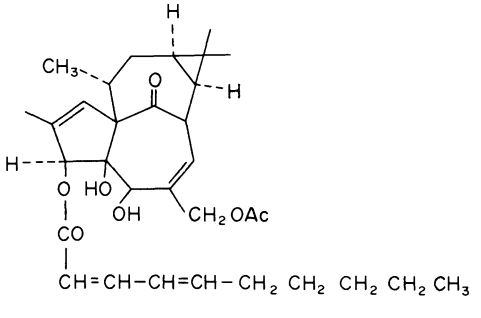




		Toxicity
X=Y=H	Ingenol	(-)
X=R, Y=Ac	Milliamine A	LD_{100} 0.64 mg/kg
X=H, Y=R	Milliamine B	(-)
X=R, Y=H	Milliamine C	(+)



	R^1	R^2
A_1	myristyl	acetyl
A_2	capryl	acetyl
A_3	lauryl	acetyl
A_4	palmityl	acetyl
B_3	tiglyl	acetyl
B_4	acetyl	lauryl
B_6	tiglyl	capryl
B_7	acetyl	capryl



Toxic phorbol ester
(Hecker *et al.*)

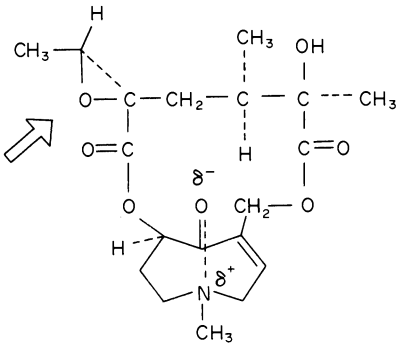
Toxic ingenol ester
LD_{100} 0.75 mg/kg

Citreoviridin is one of the toxic substances of so-called "yellowed rice", ocurrence of which in stored rice has been a serious problem in Japan. It is produced by a fungus, Penicillium citreoviride and has toxicity to liver (Ref. 12).

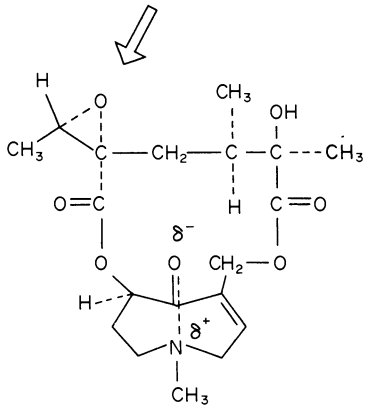
Aureothin is a yellow toxic compound produced by Streptomyces thioluteus. A characteristic feature of this compound is the nitro group attached to the benzene ring. It is however not known whether the nitro group is essential for its toxicity (Ref. 13).

ACTION OF TETRODOTOXIN, SAXITOXIN, AND BATRACHOTOXIN ON CELL MEMBRANES

So far it has been impossible to find a relationship between chemical structure and toxicity since the biological actions are dramatically altered by subtle changes in the structure. However, modes of action are now analyzed on the molecular level:

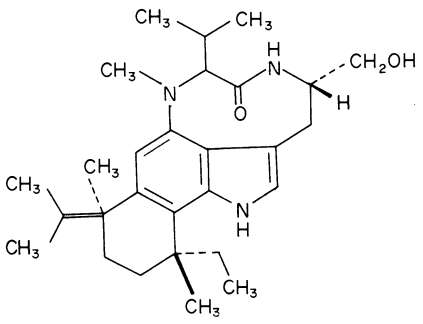


Petasitenine
(carcinogenic)

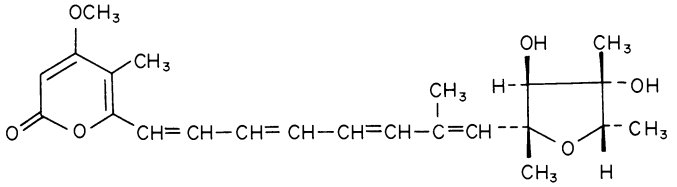


Otosenine
(non carcinogenic)

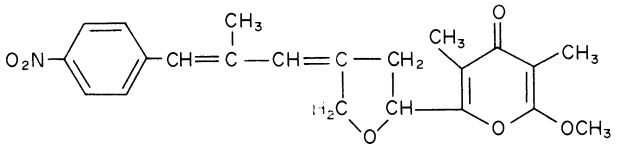
actions of tetrodotoxin, saxitoxin and batrachotoxin, towards nerve cells, in particular towards membranes, have been analyzed by Narahashi et al., Kao et al., and Albuquerque et al. (Ref.14).



Dihydroteleocidine B



Citreoviridin



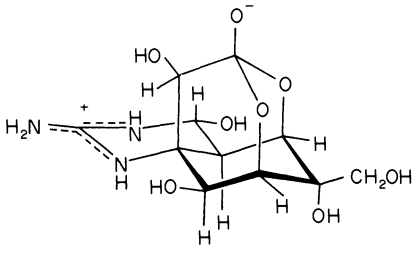
Aureothin

Tetrodotoxin and saxitoxin inhibit selectively the rapid influx of sodium ions into cells caused by excitation. These compounds, therefore, prevent the potential change in muscles and nerves, thus intercept the transmission of excitation. Permeation of potassium ions through membranes is not influenced by these compounds. Presumably the guanidinium group of the toxin molecule is attached near the sodium channel, making it difficult for sodium ions to approach and come in, although this is not experimentally confirmed (Ref. 15). Although the biological actions of tetrodotoxin and saxitoxin are very similar, some minor differences are noted: (i) hypotensive action of tetrodotoxin on animals is prominent, whereas that of saxitoxin is relatively weak; (ii) after the action on the membrame, the recovery is faster and more complete in the case of saxitoxin than that of tetrodotoxin; and (iii) these toxins act differently on the nerves of Taricha newt, puffer fish, and frogs, the newt and puffer fish being at least 1000 times and 30,000 times, respectively, more resistant toward tetrodotoxin than frog nerves, whereas the toxicity of saxitoxin toward these animal nerves is almost the same in each case.

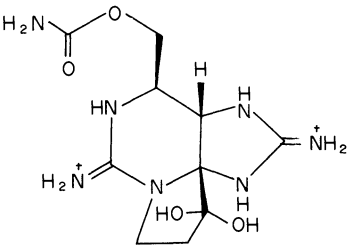
As already mentioned, tetrodotoxin loses its toxicity on subtle change of its structure. Similarly, dihydrosaxitoxin is non-toxic. Accordingly, these toxins should not be considered to bind with the membranes simply through their guanidinium group.

Actions of batrachotoxin on cell membranes are known to be antagonized by tetrodotoxin, although batrachotoxin bears no resemblance structurally to tetrodotoxin and saxitoxin. It causes a selective increase in sodium ion permeability in a variety of electrogenic membranes. It was reported that this action is believed to be caused by attaching the

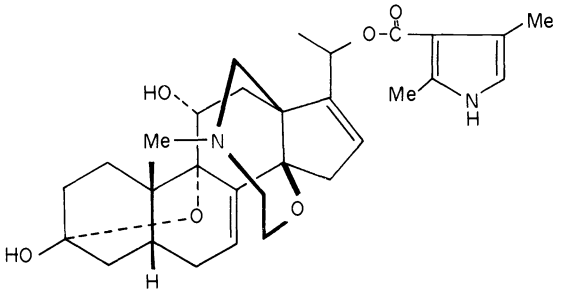
toxin directly to the sodium channels (Ref. 16), but it is also questionable as mentioned in the case of tetrodotoxin.



Tetrodotoxin



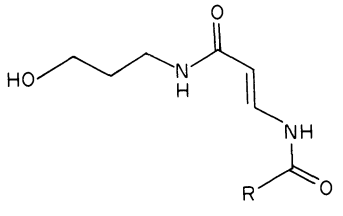
Saxitoxin



Batrachotoxin

PALYTOXIN

Palytoxin is the most toxic compound known. It is fairly big molecule (molecular weight 3300) and was first isolated by Scheuer and Moore in 1971 from the coelenterata Palythoa toxica found in Nuolea island (Ref. 17). The coelenterate is called "Limu-make-o-Hana" that means the dead seaweed of Hana. Its lethal does 50 is 0.5 μg/kg mouse by intraperitoneal injection, which is 16 times stronger than that of tetrodotoxin. Biological action of polytoxin to animals consists in producing powerful vasoconstriction and coronary spasms. Moore et al. reported that palytoxin has a UV absorption at 263 mm which was attributed to the β-amino-α, β-unsaturated acid amide chromophore shown below (Ref. 18).



We have collected Palythoa tuberculosa at the coast of Ishigaki Island, which locates very close to Formosa and is very famous with black pearl culture. The toxicity of Palythoa tuberculosa is confined to the female polyps, and the toxicity is the strongest in the mature eggs, which are kept from May to September. In order to obtain Palythoa tuberculosa with high toxicity, we have collected it in this season, and transported it by air to Nagoya in a frozen state with dry ice.

Our procedure of extraction and purification of palytoxin is as follows. The frozen animals were crushed by a mixer in 75% aqueous ethanol. After removal of the solid residue by filtration, the orange-colored filtrate was concentrated carefully under reduced pressure at a low temperature. The aqueous solution thus obtained was charged on a Toyo-Soda TSK G 3000 S polystyrene gel column. Inorganic salts were washed out with water from the column and the toxic fractions were eluted with 75% ethanol. The active fractions were chromatographed again on the same column using 30% and then 60% ethanol as eluants, the latter solvent eluted the toxin. A DEAE Sephadex A-25 column was equilibrated with a phosphate buffer of pH 6.9 and then charged with the toxin fraction, which was eluted in the solvent front without adsorption since the toxin is weakly basic substance. The toxin

fraction containing the inorganic salts used as the buffer was desalted by passing it through a TSK G 3000 S column, which was washed well with water and then eluted with 75% ethanol; the toxin fractions could be monitored by a UV detector. Further purification was carried out using a column of CM Sephadex C-25 buffered with pH 4.6 phosphate buffer. The toxin was eluted with the buffer solution; desalting was again done by the TSK G 3000 S column chromatography.

In order to confirm the purity of palytoxin, analysis was made by high pressure liquid chromatography on a column packed with Toyo-Soda TSK G 3000 SW gel. Elution of the toxin was carried out with phosphate buffer of pH 6.9; retention time of palytoxin is 29.0 min in a rate of 1.0 ml/min, and 12 times recycling showed no indication of impurities. This result suggested that palytoxin may be either a single compound or a mixture of structurally closely related compounds. The spectral data of palytoxin are: UV (H_2O) 233 (ε 47,000), 263 nm (ε 28,000); IR (KBr) 3600, 1655 cm^{-1}; PMR (DMSO-d_6, 100 MHz) δ 7.60 (1H, dd, J=10 and 14 Hz), 7.82 (1H, br.s), 10.2 (1H, d, J=10 Hz); and the lethal dose of 50 for mice was 0.5 μg/kg by intraperitoneal injection.

Catalytic hydrogenation of this toxin with platinum oxide in aqueous ethanol gave a perhydropalytoxin, in which the 263 nm chromophore and some other double bonds were reduced. Since palytoxin is a fairly large molecule having its molecular weight 3300 and molecular formula $C_{145}H_{264}N_4O_{78}$, the catalytic hydrogenation made only very minor change of the molecular structure of palytoxin, but yet its toxicity was completely lost; the perhydropalytoxin showed no toxicity by 9.2 mg/kg injection. The similar results were obtained with palytoxin by treatment with acids and bases. For example, treatment of the toxin with 0.1N NaOH at room temperature for 50 min or with 0.1N acetic acid at 80° for 24 hours afforded non-toxic products. Thus, very minor changes on the structure also lead to the complete loss of toxicity.

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