

## SIKIMITOXIN, THE TOXIC PRINCIPLE OF ILLICIUM RELIGIOSUM, SIEB. MANG-T'SAO

TSAN-QUO CHOU

(From the Department of Pharmacology, Peking Union Medical College, Peking)

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The fruit of the Bastard Anise, *Illicium religiosum*, Sieb., (Japanese Sikimi-no-ki or Chinese Mang-t'sao 莽草) has long been known to be poisonous. The chemical investigation of its toxic principle was made by Eykman in 1881 (2) who isolated a crystalline body from Japanese sikimi-no-ki which he called Sikimin and claimed to be one of the most powerful poisons. He pointed out, however, that this crystalline substance or Sikimin, was still somewhat impure, melting at about 175°C. It was soluble with difficulty in cold water and produced a faint turbidity with potassium mercuric iodide. No further progress was made in its purification and isolation by other workers, crude seeds or extracts, being generally used for the study of its toxic principles (1, 3, 4). The present work consists of a detailed investigation upon the chemical properties of this well known toxic substance. A white amorphous substance, definite in its physical and chemical character, has been isolated from the seeds of Mang-t'sao. It is highly poisonous, causing symptoms of poisoning similar to those produced by the crude seeds or extracts. Working with cats, 0.2 mg per kilogram of body weight was found to be the fatal dose. Its toxicity was greatly decreased by the action of boiling water and dilute acids, and rapidly destroyed by the action of caustic alkalies. It is a feebly acid nitrogen-free body, easily soluble in cold water and gives no precipitate with most metallic salts except lead acetate, with which it produces a slight turbidity. I propose that this poisonous principle should be named Sikimitoxin, as it is different from Eykman's sikimin in both its chemical and physical properties and also in its toxicity. As this principle is sensitive to the action of acids, it is questionable whether Eykman's sikimin was

the original substance present in the Japanese star anise, or the resulting product formed by the prolonged action of acetic and hydrochloric acids used in his process of isolation (1).

#### EXPERIMENTAL

##### *I. Isolation of Sikimitoxin.*

After a long series of preliminary experiments, the following process was found to give good results.

2500 gm of the seeds of Mang-t'sao are powdered and percolated with petroleum ether at ordinary temperature for two days. After filtering and washing the marc thoroughly with petroleum ether to get rid of any fatty matters present, the filtrate and washings are united and the solvent distilled. There remains in the distilling flask a yellow oil mixed with some essential oil from the seed covering and about 20 per cent of the total amount of the toxic principle present in the seeds. The latter is recovered from the oil by shaking several times with a sufficient quantity of cold water and working up the aqueous extract by the following method. The marc obtained as above and containing the greater part of the toxic principle, is dried at room temperature and percolated again with cold chloroform for three days, then filtered, washed with chloroform, and the filtrate is distilled. There remains in the distilling flask about 3 gm of a reddish gelatinous residue which, by the addition of an excess of petroleum ether, becomes a solid amorphous precipitate. When filtered and kept in a vacuum desiccator, it again becomes a gelatinous mass. It will be found to be highly poisonous, but in a very impure state, only a small part of which constitutes the toxic principle. Further purification is carried out as follows:

(1) Boiling with a sufficient quantity of ether over a water bath with reflux condenser completely removes the toxic principle together with some impurities soluble in ether, leaving behind a gelatinous residue which is found to be non-toxic to cats.

(2) The ethereal extract is distilled. The residue is taken up with 10 cc of cold water which extracts all the toxic principle present; a second warm water extract will be found to be inert.

(3) The aqueous extract is evaporated to dryness in a vacuum desiccator at room temperature, and the residue taken up again with chloroform in which a part does not dissolve. The insoluble part is

very soluble in water; its aqueous solution when allowed to evaporate slowly in a crystallising dish, gives rise to the formation of some prisms, which are organic but non-toxic.

(4) The chloroform extract, now containing the toxic principle in a concentrated form, is evaporated to dryness. The residue is treated with hot benzol by warming over water bath for about 10 minutes, the toxic principle dissolves, leaving behind an inert residue.

(5) The benzene solution is filtered and poured gradually into an equal volume of petroleum ether. The toxic principle or Sikimitoxin, separates out slowly as a snow-white amorphous powder. It refuses to crystallize in the many solvents tried, but its physical and chemical properties remain constant when subjected to various processes of further purification.

After the removal of Sikimitoxin from the seeds as above, the marc is again percolated with chloroform in the presence of a 5 per cent solution of  $\text{Na}_2\text{CO}_3$  at ordinary temperature. Instead of obtaining a basic substance as I had expected, a neutral crystalline body has been isolated from the chloroform extract. It crystallises from alcohol in long, yellowish, prismatic needles, melting at  $116^\circ\text{C}$ . It is soluble in chloroform, less so in alcohol and ether, and insoluble in water. It is very stable towards the action of dilute acids and alkalies at water bath temperature.

Its toxicity has not been determined.

## II. *Properties of Sikimitoxin.*

Sikimitoxin is a white amorphous powder having no definite melting point: when heated slowly it sinters at  $63^\circ\text{C}$ , then gradually increases in volume to an opaque creamy mass as the temperature rises, and finally becomes a clear liquid at  $135^\circ\text{C}$ . It is a feebly acid nitrogen-free body, easily soluble in water, chloroform and alcohol, less so in ether and hot benzene, and insoluble in petroleum ether. When a small quantity of the substance is brought into contact with a drop of cold water, it becomes at once converted into oily drops as seen under the microscope, and which dissolves after adding more water. Its aqueous solution is faintly acid to litmus paper and gives a slight turbidity with lead acetate, but not with the following reagents:— $\text{HgCl}_2$ ,  $\text{CuSO}_4$ ,  $\text{AuCl}_3$ ,  $\text{Am}_2\text{SO}_4$ , picric acid, phosphotungstic acid, Mayer's reagent and bromine water. Once melted, it becomes a yellowish hard mass which dissolves with difficulty in cold water. When exposed to the

action of dry heat at 100°C for 8 hours, its toxicity does not seem to undergo any change when tested on cats.

### III. Toxicity of Sikimitoxin.

Working with crude seeds or aqueous extracts, the symptoms observed on cats were chiefly salivation, repeated vomiting, defecation, strong convulsion and death. Sikimitoxin produces a similar train of symptoms. In the following experiments, sikimitoxin was administered subcutaneously:—

(1) A cat weighing 2200 gm received 0.2 mg of sikimitoxin in 2 cc of water. Salivation and vomiting took place within 45 minutes and was followed by strong convulsions after 85 minutes. The paroxysms were repeated 8 times during a period of 4 hours, and then the cat gradually recovered during the night.

(2) A cat weighing 1500 gm received 0.6 mg of sikimitoxin in 3 cc of water. The toxic symptoms produced were much more severe, death occurred within 45 minutes.

(3) A cat weighing 2100 gm received 0.4 mg of sikimitoxin in 2 cc of water. Death occurred within 3 hours after the usual toxic manifestations.

#### *Action of NaOH upon Sikimitoxin.*

A cat weighing 2000 gm received a mixture of 0.2 mg of sikimitoxin and 0.2 mg of NaOH in 4 cc of water. No toxic symptoms were observed except a little vomiting after one hour, while a few hours later, the cat showed an excellent appetite.

#### *Action of HCl upon Sikimitoxin.*

1 mg of Sikimitoxin was dissolved in 10 cc of water with the addition of 3 drops of conc. HCl. The whole was evaporated slowly to dryness over water bath with the addition of a fresh quantity of water from time to time. After 3 hours, the residue was taken up with 10 cc. of water, 3 cc of this solution, corresponding to 0.3 mg of sikimitoxin was given to a cat weighing 2200 gm. Salivation and slight vomiting occurred within 40 minutes, followed by a single paroxysm of characteristic convulsions. Finally the cat recovered after a few hours. The same dose, without previous treatment with HCl produces more severe toxic symptoms or is even fatal. The prolonged action of boiling water was found also to decrease the toxicity of sikimitoxin.

## DISCUSSION

From these experiments I conclude that sikimitoxin is the toxic principle present in the seeds of Mang-t'sao. Working with crude seeds on cats and dogs, Read, Kiang and Chen (1,3) found that 0.4 gm of seeds per kilogram of body weight was the minimum lethal dose; the M. L. D. in the case of sikimitoxin is 0.2. mg per kilogram. Calculating on the above basis, the amount of sikimitoxin present in the seeds is about 0.05 per cent.

The chief differences between Eijkman's sikimin and sikimitoxin can be seen from the following table.

SIKIMIN	SIKIMITOXIN
(1) Soluble with difficulty in cold water	Easily soluble in cold water.
(2) Faint turbidity with Mayer's reagent	No turbidity with Mayer's reagent
(3) 18.5 mg of substance killed a young dog in 3 hours (no body weight was given).	0.4 mg of substance killed a cat weighing 2100 gm in 3 hours.

## SUMMARY

- (1) There has been isolated from Bastard Anise the poisonous principle "*Sikimitoxin*". This principle differs from Eijkman's Sikimin in its physical and chemical properties and also in its greater toxicity.
- (2) *Sikimitoxin* is a white amorphous powder easily soluble in cold water, a feebly acid, non-nitrogenous body, non-precipitable with Mayer's reagent. It is present in bastard anise to about 0.05 per cent.
- (3) *Sikimitoxin* is exceedingly toxic. One tenth of a milligram per kilo produces in cats strong convulsions preceded by nausea and vomiting. Two tenths of a milligram are fatal.

The author wishes to express his indebtedness to Professor B. E. Read for suggesting the subject of this research.

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## 莽草中之毒素

趙承嘏

北京協和醫學校藥物學系

莽草出于中國廣西等省，性甚毒，食之可致命，形與八角茴香相似而實有異。(參觀本雜誌第一期伊博恩江鏡如二先生所著之莽草中毒及其解救法)。

一千八百八十年，德人愛克門氏(Eykman)在日本時，曾在莽草中取出一毒素，命名曰雪克明(Sikimin)，然此物是否係莽草中固有之物，或在提取時，因各種化學作用而產出，作者頗有懷疑之點。

作者今在莽草中提出一毒素，其性質與愛氏之雪克明不同，毒性亦過之。以少許(0.4 mg)注射于貓之體內，閱三小時即斃。

今定名此物曰莽草毒素(Sikimitoxin)。

莽草毒素之提取法，及其各種性質，并毒性之試驗等，請閱原文。