A CHEMICAL EXAMINATION
OF THE
SEED OF ILLICIUM RELIGIOSUM, SIEBOLD

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INTRODUCTION

The material in this investigation is the seeds of Illicium religiosum, Siebold Fam. Magnoliaceae known as Mang Tsao in Chinese, Shikimi no ki in Japanese, and commonly known as Japanese star anise or poison star anise. A brief description of the plant is as follows:

Scientific name

Illicium religiosum, Siebold Fam. Magnoliaceae

Synonyms

Chinese syn.[1]:	Mang Tsao	(mad herb)
	Shu Mang	(rat poison)
	Ao Woo Soo	
	Chun Tsao	(spring herb)
Japanese syn.[1]:	Shikimi	
	Ashikimi	(evil fruit)
	Seke	
	Kake	
	Yoka	
	Koyo	
	Tse	
	Tse Zden so	(herb before the temple)
	Yin Ba	
	Hari ma	
	Tsi ku sen	
	Yeun kiang	
	Hackijo	

[1] Am. Jour. of Pharm. vol. 101 p. 625

English syn.[1]: Poison star anise
Japanese star anise
False star anise

Habitat[2]

The plant was originally found in Szechoung, China and was introduced, in ancient times by Buddhist priests (Kaempfer). It now grows wild near Nagasaki and Tokyo and on the islands of Hackijo. The ecological habitat of the plant is: mountain slopes. It is sometimes cultivated in the gardens of south-eastern France.

Description of Plant

A complete description of Illicium religiosum made by Li Shih-Cheng[2] in Peng Tsao Kang Mu (1596) chapter 17 under the name of Mang Tsao; by Kaempfer in his Amoenitatum (1712) p.880 under the name of Somo Shikimi and other names; by Loureiro in the Flora Cochin Chinensis[2] (1790) p.353 under the name of Illicium anisatum and by Siebold in the Flora Japonica (1871) p.5 under the name of Illicium Japonicum.

The whole plant is about 6 to 20 feet high. The bark[3] is gray in color and has an aromatic odor. The leaves of this plant are shortly (about 1 cm.) petioled, thick, evergreen, oblong or oblong ovate, free from hairs with a wavy surface. The plant flowers in April. The flowers are about an inch and one half in diameter, greenish or very slightly yellowish-white with a waxy-like appearance. There are 12 to 20 petiols with 15 to 20 stamens.

1 Berichte, Schimmel & Co. (1893) p.46; (1909) p.51
2 Chen. S. Y. Am. Jour. of Pharm. 101, p.621-22
3 Luerssen, Christen., Hanb. d. system Botanik II p.583

The fruit is 25 mm. in diameter. It consists of eight carples arranged side by side in a circle having a depth of about 0.5 cm. There is a pistel on the upper side of each carple. The fruit is green and juicy in its unripe condition and reddish brown and shriveled when it is dried. The seeds are often shot out of an opening on the upper side of the carple as the fruit ripens.

The seed is usually about 0.7 cm. long and 0.5 cm. broad. It is covered with a thin hard waxy shell called the testa. It is brown in color, slightly lighter in color than true star anise. The wart-like hilum is light yellow or nearly white.[1] The hilum contains large crystalliods and few globiods. This is one of the chief structural differences between the seeds of true star anise and poison star anise.[2] The apex of the seed is not rounded but at the end of the keel terminales in a raised point. It has no odor and has an oily taste.

Uses

The Chinese use poison star anise as a toothache remedy, also in the treatment of certain forms of dermatitis, parasitism, etc. The Japanese plant the tree around their temples. The flowers are used to adorn the altar and in the decoration and display in religious feasts. Sometimes the plant is planted over graves because the Japanese people think that this poison plant keeps insects, animals, etc. away from the dead person. The seed alone is not used

1 Vogl, Mittheil des Wien Med. Dokt. Colliq.,7, pp. 167-173
2 Pfister, Rudolf Vierteljohrs Naturforschi. Gesellsch. Zuerich, 37 pp. 313-22

a great deal except that the expressed oil is used as a cheap lighting fluid oil. (At the present time the Lilly Laboratories are doing some work on the medical use of some of the Shikimic acid derivatives. This acid is found in both the seed and the fruit of Illicium religiosum.)[1]

Poison star anise is sometimes used as a substitute and an adulterant for true star anise (Illicium Anisium). Many cases of poisoning have been recorded because of such substitutions and adulterations.

[1] Lei, H. H., A. Ph. A. Journ. (1937) p. 900

The seed of poison star anise (Illicium religiosum) has been known as a poison seed for a long time. Since the fruits of poison star anise and the true star anise resemble each other so closely, many poison cases due to poison star anise can be found in literature due to the contamination or complete substitution of the seed of poison star anise for true star anise. One of the earliest cases of this kind was reported in 1880 in Leewarden in the Netherlands.[1] Since that time many such cases have been reported in literature from time to time.

Perhaps it was because of these substitutions in the commercial star anise that brought about the chemical investigations of poison star anise.[2] Eykman, in 1881, worked on the leaves and fruit of the plant. He also recorded the toxic principle to the present seed.

Bulir, J, 1912,[3] in his investigation of the seeds of both Illicium verum and Illicium religiosum found that the latter yeilded 1 per cent fatty oil. In this oil Oleic, linolic, stearic and palmitic acids were found and their percentages were computed.

In 1926 K. K. Chen[4] studied the fruit of Illicium religiosum making moisture determinations and ash determinations of both the seed and carple. The author found that the toxic principle was destroyed by alkalies but not by acids.

1 Pharm. Deekbl. 17 (1880), No. 4; Pharm. Journ. 40 p. 1067
2 Eykman, J. E. Pharm. Journ. 40 (1880-81) pp. 104 & 1066
3 Bulir, Jaromir Zeitschr. f. Untersuchung d. Nahrung 24 p.307
4 Chen, K. K. A. Ph. A. Journ. 15 p. 861

Chau, T. Q., 1927, isolated shihimitoxin from the fruit of Illicium religiosum as a white amorphous powder and found the M.L.D. for rats to be 0.2 gm. per Kgm. body weight.

S. Y. Chen made a study of the fruit (seed and carple) of Illicium religiosum, Siebold, making moisture and ash determinations and extractions with selective solvents such as petroleum ether, alcohol, chloroform, and acitone. He also studied the properties of shikimic acid and tried to compare it chemicaly with Benzoic acid. This work on shikimic acid and its derivatives is being continued by H. H. Lei at the University of Wisconsin.

EXPERIMENTAL WORK

The material used for this investigation is the seeds of the fruit Illicium religiosum Siebold collected in China and sent to H. H. Lei[1] in the air dried condition.

Moisture Determination by the xylene method (U.S.P.XI)

The drug was ground and weighed out in 10 gm. and 20 gm. portions. The samples were introduced into 250 c.c. round bottomed flask, about 100 c.c. of water-saturated xylene added, and the flask connected to a Dean-Stark apparatus. The mixture was boiled until no more water came over.

	I	II
Weight of sample	10.001 gm.	20.003
c.c. of water	0.475 c.c.	1.0 c.c.
Per cent of moisture	4.75	5.0

Other work on moisture determination of Illicium religiosum

K. K. Chen[2]	S. Y. Chen[3]	H. H. Lei[1]
3.44%	4.8% - 5.2%	4.8% - 5.0%

Ash Determination

A total ash determination was made according to the method given in the U.S.P.XI p. 473.

I. Total Ash

	I	II
Weight of sample	3.5326 gm.	3.3817 gm.
Weight of sample after ignition	0.0568 gm.	0.0523 gm.
Per cent of total ash	1.61	1.55

II. Acid-insoluble ash

After calculating the total ash, the above samples were used in determining the acid-insoluble ash.

1 H. H. Lei - Theses on Shikimic Acid & Deriv. at the University of Wisconsin
2 A. Ph. A. Journ. 1926
3 Am. Journ. of Journ. of Pharm. 1929

	I	II
Weight of total ash	0.0568 gm.	0.0523 gm.
Weight of ash after ignition and boiling with acid	0.0032 gm.	0.0034 gm.
Per cent of acid-insoluble ash	0.093	0.101

Other records of ash determinations

	K. K. Chen[1]	S. Y. Chen[1]
Total ash	1.472%	1.444% av.
Acid-insoluble ash	0.1921%[2']	I 0.068%[2'] II 0.098%

Extraction with selective solvents

I. Pentroleum Benzin

About 100 grams of the material were packed in an apparatus arranged for continous percolation, and completely extrated with petroleum benzin (Skelly solv. B,B. p. 65°). The petroleum benzin was allowed to evaporate spontaneously. About 20 grams of the extract resulted. It was a yellowish oily liquid with a slight bland odor. A thin skin-like layer formed when a small portion of the oil was left in a watch glass for a few weeks. This layer did not form when the oil was kept in a tightly stoppered erlenmeyer flask, even though it was kept for over 10 months.

Saponification value of the petroleum ether extract.

The saponification value of the petroleum ether extract was determined by heating an accurately weighed portion of the sample on a water bath with a known amount of standard alcoholic potassium hydroxide. The residual

1, Am. Journ. of Pharm. 1929 p. 343
2' These percentages are calculated considering the crude drug as 100%.

potassium hydroxide was titrated with standard acid and the saponification value was determined as directed in the U.S.P.XI p. 445. The results of this determination were:

Weight of sample	0.5175 gm.
cc. of N/2 alcoholic KOH added	24.84
cc. of N/2 HCl used	22.91
cc. of N/2 alcoholic KOH actually used	1.93
Saponification value	205.84

Acid value of the petroleum ether extract.

The acid value of the petroleum ether extract was determined by adding a known amount of standard KOH into a flask containing an accurately weighed portion of the sample. The residual KOH was titrated with standard HCl and the acid value determined as directed in the U.S.P.XI p. 444. The results were as follows:

Weight of sample	0.2365 gm.
cc. of N/2 alcoholic KOH added	12.18
cc. of N/2 HCl used	11.66
cc. of N/2 alcoholic KOH actually used	0.32
Acid number	18.88

Nonsaponifiable residue of the petroleum ether extract.

A sample of the petroleum ether extract weighing 2.3561 grams was saponified as stated above under saponification value. The volume of the resulting solution was approximately doubled by the addition of an equal volume of distilled water. The mixture was placed into a separatory funnel and extracted with three successive 50 cc. portions of diethyl ether. The ether solution was placed into a tared beaker and allowed to evaporate spontaneously. The percentage of nonsaponifiable material was determined.

Weight of the sample	4.6321 gm.
Weight of evaporating dish and nonsaponifiable residue	26.6011
Weight of evaporating dish	25.8634
Weight of nonsaponifiable residue	.7377 gm.
Per cent of nonsaponifiable residue	15.71

Test for sterols

A portion of the nonsaponifiable residue was dissolved in alcohol. A 1 per cent digitonin solution was added to the solution of non saponifiable matter. A white precipitation resulted indicating the presence of sterols.

A small amount of the non saponifiable matter was dissolved in chloroform. The chloroformic solution was then gently agitated with concentrated sulphuric acid. The chloroform layer became red where as the sulphuric acid assumed a green fluorescence when held against a black background.

Another sample of the non saponifiable matter was dissolved in a mixture of 2-3 drops of concentrated sulphuric acid drop by drop to the solution, a pink color appeared which upon addition of more acid changed to blue and then green.

These color tests indicate the presence of sterols.

Chloroform extraction

The marc from the petroleum ether extraction was further extracted with chloroform. The chloroform was evaporated spontaneously. The extract is a brown solid with no odor in particular.

The following yield was obtained from the remainder of the 100 grams of drug.

	Wt. of extract	% of Ext.
Sample No. 1	1.2 gm.	1.2
Sample No. 2	1.0 gm.	1.0

Because of the low yield of this extract, no further work was done on it.

Acetone extraction

The marc from the chloroform extraction was extracted

further with acetone in the same manner as described in the above extractions. The acetone was evaporated spontaneously. A very small amount of the extract was obtained by the use of this solvent. It was a brown semi-solid or a gummy solid.

The following yield was obtained from the remainder of the 100 grams of the drug:

	Wt. of Ext.	% of Ext.
Sample No. 1	1.2 gm.	1.2
Sample No. 2	1.1 gm.	1.1

Solubility of the acetone soluble portion

The solubility of the acetone soluble portion in various solvents was determined by dropping a very small portion of the extract in a test tube containing about 1 or 2 inches of solvent.

a. Acetone (cold). .partly soluble
b. Chloroform. . . .insoluble
c. Alcohol (cold). .soluble
d. Alcohol (warm). .very soluble
e. Petroleum ether .insoluble
f. Water (warm). . .soluble
g. Diethyl ether . .partly soluble

Saponification value of the acetone extract.

The saponification value of the acetone extract was determined by the method described under the saponification value determination of the petroleum ether extract. The results were as follows:

Weight of sample	1.0350 gm.
cc. of N/2 N alcoholic KOH added	24.84
cc. of N/2 N HCl used	20.97
cc. of N/2 N alcoholic KOH actually used	3.97
Saponification value	104.92

Because of the limited amount of the extract, no further tests could be made on it.

Alcohol extraction

The marc from acetone extraction was further extracted with alcohol (95% ethyl alcohol). After the alcohol was evaporated spontaneously, a light brown solid residue remained. There was no odor to this solid.

The following yield was obtained:

	Wt. of Ext.	% of Ext.[1]
Sample No. 1	5.9 gm.	5.9
Sample No. 2	5.1 gm.	5.1

The difference in the yields of these two samples may be due to incomplete extraction in the second sample.

Formic acid extraction

The marc from the alcohol extraction was removed as completely as possible from the continuous perculation apparatus and put into a 1000 cc. beaker. This marc was then covered with formic acid (76%) and allowed to macerate for about a week. During this time small amounts of acid were added from time to time in order to keep the level of the formic acid above that of the drug. At the end of the period the solvent was filtered off and put in a tared evaporating dish and set aside. After the formic acid was evaporated spontaneously, a dark brown odorless solid residue remained in the dish. The yield obtained was as follows:

	Wt. of Ext.	% of Ext.[1]
Sample No. 1	2.2 gm.	2.2
Sample No. 2	2.0 gm.	2.0

Tabulation of Extractions

	Av. %
Petroleum ether	21.6
Chloroform	1.1
Acetone	1.15
Alcohol	5.5
Formic acid	2.1
Residue	69.55

[1] Based on 100 grams of drug.

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Pharmacographia, p. 20

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A general description of the plant.

Eykman, J. E. 1881

Illicium religiosum, Sieb.; its poisonous constituents and essential and fatty oil.

Pharm. Journ., 40, p. 1046

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Mitth. des Wien. Med. Dokt. Colliq., 7, pp. 167-173

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Luessen, C. 1882

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Sur les principes aclefs de la Shimmia Japonica

Recueil d. Travaux chem., 3, p. 204 (Ber. Berichte, 17, p. 441)

SShimmini ($C_{15}H_{16}O_8$) was found in the wood of Illicium religiosum.

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Journ. de Pharm. et Chim., 18, p. 367 (Am. Journ. of Pharm., 101, p. 636)

Two cases of poisoning with Japanese star anise were recorded.

Barral, E. 1890

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Journ. de Pharm. et de Chim., 18, p. 367 (Am. Journ. of Pharm., 101, p. 336)

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Inoko, Y. 1890

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Chiugai Iji Shipo (Med. News of the World, Tokyo pp. 1245-48 and 1317-20) (Am. Journ. of Pharm., 101, p. 337)

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Fluekiger, F. A. 1891

Pharmakognosie de Pflanzenieiches Third ed. pp. 932-37

(A brief account of Illicium religiosum is given)

Oswald, -. 1891

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Analysis of true star anise and also a comparison of true star anise with poison star anise.

Pfister, -. -. 1892

(Zur Kenniuss d. echten und d. giftigen Sternanis.)

Vierleljahrs. Naturforschi. Gesellsch. Zuerich., 37, pp. 313-22

The author found that the seeds of Illicium religiosum and those of Illicium anisium, on close examination, different. He found that the seeds of Illicium religiosum contained large crystalliods and few globiods and those of Illicium anisium contained alurone grains having globiods with very few crystalliods.

Schimmel & Co. 1893

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Berichte S. & Co. - Oct. 1893 p. 46

Difference between poison star anise and true star anise in commerce. Phy. Const. of Oil of Poison Star Anise (vol. Oil)

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Poisoning with star anise

New York Med. Journ. 73, p. 642

Case of poisoning with star anise in the form of an infusion.

Schimmel & Co.. 1909

Oel von Illicium religiosum

Berichte S. & Co. Apr., 1909, p.51

Cineol, linalool and safrol were isolated from the oil of poison star anise.

Bulir, -. 1912

Zusammenselzung d. fetlen Oele d. Samen von Illicum vernm Hooke u. Illicum religiosum Sieb.

Zeilschr. f. Untersuchung. d. Nahrungs. u. Genussm., 24, p. 309

One per cent of fatty oil was found in Illicium religiosum which contained Oleic, linolic, stearic and palmitic acids.

Guirrero, L. E. D de Pag & Guirrero, A. L. 1916

Poisoning by Illicium religiosum

Philippine Journ. Sc., 11B., p. 203

Records of two poison cases due to poison star anise. This paper stated that poison star anise is 14 times more toxic than true star anise.

Wall, A. 1917

Handbook of Pharmacognosy p. 427

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Negrete, J. D. & Delarde, C. F. 1919

Contribucional Estudis de la Toxidad. del Illicium religiosum "Anis es rellodo del Japan. Part I & Part II Jour. A. Ph. A.

The effect of the toxic principle on different animals - dogs, rabbits, guinea pigs, pigeons, frogs, white rats and mestoes was studied. The author concluded that young animals are more susceptible to the drug than the older. The author goes on telling about the occurance of poisoning and the symptoms of the poisoning.
The second part of this paper explains the author's methods of preparing the toxid principle in the form of infusions and decoctions. He also found that the poisonous principle is absorbed by animal charcoal.

Chau, T. Q. 1927

Shikimitoxin, the toxic principle of Illicium religiosum

Chinese Journ. Physiol. Chem. I p. 213 (Am. Journ., Pharm. 101, p. 27

The author isolated shikimitoxin from the fruit of Illicium religiosum. (White amorph. pwd. liq. at 130°- M.L.D. for rat -.2 mg per Klo wt.)

Chen, K. K. 1926

A study of Illicium religiosum

A. Ph. A. Journ., 15, p. 861

The author records fatty oil from seed, also moisture and ash determinations. The toxic principle (which failed to crystalize) was destroyed by alkalies. The toxic principle was tried on rats.

Chen. S. Y. 1929

A Phytochemical Study of Illicium religiosum, Sieb. (Mongtsoa)

The Am. Journ. of Pharm., 101, p. 625 & 688

A study of the seeds and the carple of the plant trying to find the toxic principle in the seed and carple. Ash and moisture determinations were also made. The author stated that its toxic action is destroyed by alkalies.

Gildemeister, E. 1931

Die Ätherischen Öle III auflang., Bunt. II, p. 564 & 576.

The author gives a brief description of the fatty oil. also mentions the toxic principle in poison star anise.

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