A STUDY OF ILLICIUM RELIGIOSUM.

(MANG TSAO).*

BY K. K. CHEN.

I. INTRODUCTION.

The leaves of *Illicium religiosum* Sieb., "Japanese Staranise," or Mang Tsao, have long been known in Chinese medicine as a poisonous drug (1), and directed not to be taken internally or applied to the conjunctiva. The Chinese use it, by local application, in the treatment of toothache, certain forms of dermatitis, parasitism, etc. The fruits resemble those of the true staranise (*Illicium verum*) so closely that they are often mistaken for the latter, or sold as an adulterant. Poisoning cases from the use of such fruits have been recorded by Eijkman (2), Langgaard (3), Guerrero, Paz and Guerrero (4), and Read (5).

The present investigation deals with the dry fruit and consists of an attempt of isolating the toxic principle, a study of the volatile oil from the carpel, a study of the fatty oil from the seed, the determination of moisture, volatile substances and ash contents of the seed and of the carpel, respectively. The material used was that left over by Doctors Peter Tsiang and Carl F. Schmidt in their toxicological investigation at Peking Union Medical College, Peking, China.

II. THE NATURE OF THE TOXIC PRINCIPLE.

Eijkman (1881), while in Japan, succeeded in isolating a crystalline substance from the seeds, which produced toxic symptoms in dogs, and which he called shikimine. His method consisted of removal of the fatty oil with petroleum ether, extraction of the marc with alcohol acidified with acetic acid, and final separation with chloroform. He obtained 0.025 Gm. out of 2.5 kilograms of seeds.

In my investigation of the seed, the fatty oil was completely removed by ether. Ten grams of the marc were covered and shaken with a mixture of 1 cc. of 10 per cent hydrochloric acid, 74 cc. of alcohol, and 25 cc. of chloroform, for twenty-four hours. After filtration and evaporation, the residue from the filtrate, when dissolved in water with a trace of alcohol giving a cloudy solution, proves to be highly toxic to dogs, producing convulsions. While acidification does not destroy the toxic principle, it is not an essential step in its isolation from the seed, for a larger quantity of the marc (70 grams) when extracted continuously on a hot water-bath with alcohol-chloroform mixture (1:3), also yields the toxic substance in the residue of the extract on evaporation. In this residue, which was yellow, transparent and viscous, there were few cubic crystals. Two of these crystals separated mechanically, dissolved in water and injected into the lymph sac of a frog, caused convulsions. For purification, the whole residue was dissolved in water, shaken with petroleum ether, and subsequently treated with lead acetate, the excess of which was removed by the precipitation with hydrogen sulphide. The filtrate, upon evaporation to a small volume, contained the same potent substance, poisonous to dogs and frogs. It, however, refused to crystallize in spite of chilling in ice-salt mixture, rubbing of the inner side of the vessel, etc. It was thus not

possible to obtain the active principle in its pure state and submit it to elementary analysis. The quantity was small, and extensive investigation was not feasible. The solution did not show any visible reaction with any of the common alkaloidal reagents.

The toxic principle occurs not only in the seed but in carpels. An alcoholic extract, or an aqueous extract from the carpels after the removal of the volatile oil by steam distillation, proved to be poisonous to frogs, producing convulsions and death. The same difficulty was encountered here in the process of crystallization. When the solution containing the toxic principle is made alkaline with ammonium hydroxide or sodium hydroxide, it is not so toxic to animals as when the solution is acid. Alkalization appears to detoxify the principle.

Since this principle is difficultly obtainable in crystalline form, the biological test is the best way to ascertain its presence. The poisoning symptoms in dogs have been described by Eijkman (1881), and extensively studied by Langgaard (1881) in various species of animals.

In frogs, an injection into the anterior lymph sac results in weakness of limbs, hyperæmia of the anterior abdominal wall, indicating the local irritant action of the toxic principle, and finally tonic and clonic convulsions. During these convulsions the animal assumed the opisthotonos position, the respiration stopped and webs of the limbs spread out. Decerebration does not affect, but destruction of the medulla abolishes the convulsions. The latter are therefore due to the stimulation of medulla or upper cervical cord, similar to those after picrotoxin. When the convulsions cease, there is irregular twitching of the limbs. Death follows large doses.

In dogs, the symptom-complex after oral administration of the toxic solution consists of barking, scratching of ears, biting of the tail, shaking of the body, vomiting and defecation. Vomiting is possibly due to local irritation. Muscular twitching and weakness of hind legs precede paroxysms of violent convulsions. During the convulsions, there occur retraction of the head, gnashing of teeth, and continuous running movements, while the animal falls on its side. These paroxysms occupy about one minute each and occur four to fifteen minutes apart. A quantity of the solution equivalent to 0.4 Gm. of the seed per kilo of the dog's body weight is sufficient to produce these symptoms. With sublethal doses, the animal recovers after 7 to 8 paroxysms of convulsions; but with lethal doses, it dies during one of the paroxysms.

III. THE VOLATILE OIL OF THE CARPEL.

The oil can be obtained by steam distillation and cohobation. Out of 13.31 kilos of dry carpels, the following yield is recorded:

Original Oil	49.1696 Gm. = 0.3694 p.c.
Cohobated Oil (first and second together)	31.1453 Gm. = 0.2340 p.c.
Total	0.6034 p.c.

The freshly distilled oil is clear, transparent, light yellow, but turns reddish after drying over fused calcium chloride. On standing for 8 months, the original oil becomes red, and the cohobated oil orange. The odor of the original oil is agree-

able, and that of cohobated oil slightly empyreumatic. The taste is somewhat astringent and burning which persists for a few minutes. Both oils give a red color when shaken with a solution of ferric chloride, turning purplish red over night, and bluish purple on standing for several days. Neither oil congeals when chilled to —5° to —6° C. One cc. of the original oil is soluble in 9 cc. of 80 per cent alcohol. In the following table, the specific gravity was determined by a 10 cc. pycnometer, the specific rotation by a Lippich polariscope, the index of refraction by an Abbé refractometer, the solubility value by adding 1 cc. of absolute alcohol (d_{20} 0.7902) to 0.5 cc. of the oil and titrating the mixture with distilled water until permanent turbidity appears according to Dowzard (6), and the acid, ester and saponification values by the ordinary standard methods (7), using 2-cc. sample of the oil:

	Original oil.	Average.	Cohobated oil.	Average.
$d\,\frac{25}{25}$	0.9905		0.9790	
$(\alpha)\,^{25}_{D}$	−6.159°		−6.539°	
N_{20}	1.5007		1.4960	
Solubility Value	358 ⎫ 360 ⎭ 359		430 ⎫ 440 ⎭ 435	
Acid Value	4.247		4.272 ⎫ 4.286 ⎭ 4.278	
Ester Value	33.750		21.100 ⎫ 19.740 ⎭ 20.420	
Saponification Value	37.997		25.372 ⎫ 24.026 ⎭ 24.699	

The volatile oil proved to be quite toxic to frogs. When a small quantity was thoroughly suspended in a physiological saline solution, and a portion of it quickly measured out before the separation of the oil and injected into frogs, a volume equivalent to 0.0005 cc. of the oil per Gm. of the frog's body weight was found to be lethal to the frog, while a volume equivalent to 0.00025 cc. of the oil per Gm. of body weight produced depression for several hours but not death. The symptomatology is different from that seen after the administration of the toxic principle described above. It is a progressive depression, and death results without convulsions.

Schimmel and Company record (8) that the dry fruit yields 1 per cent of volatile oil, having d_{15} 0.9848, $(\alpha)_{D}$ —0°50′, congealing point —18° C., acid number 1.8, and ester number 12.9. Eijkman (9) demonstrated in the oil of fresh fruits and leaves, the presence of eugenol, schikimene, safrol, schikimic acid, protocate-chuic acid and schikimipicrin. Schimmel and Company report that the oil distilled from the dry fruit contains safrol, cineol and possibly linalool.

IV. THE FATTY OIL FROM THE SEED.

The oil can be extracted with ether from the ground seed by continuous extraction on a water-bath over ten hours until several drops of the distillate show no droplets of the oil on evaporation. The solvent is removed by spontaneous evaporation on a water-bath. The average yield of the oil from three extractions is 28.3 per cent as shown below:

Sample no.	Wt. of sample in grams.	Yield of oil in grams.	Yield of oil in p.c.	Average in p.c.
1	100	29.2	29.2 ⎫	
2	100	27.1	27.1 ⎬ 28.3	
3	100	28.7	28.7 ⎭	

The oil is yellow when fresh, brown on standing, turbid first but clear on standing with a heavy white sediment. It is tasteless, and has a slightly aromatic and pungent odor due to the admixture of a trace of volatile oil from the seed covering. It is soluble in ether, petroleum ether and warm alcohol. When a thin layer of the oil is exposed to the air, it solidifies in 2 to 3 months. The physical and chemical constants of the clear oil are tabulated below:

		Average.
$d\,\frac{25}{25}$	0.919	
N_{20}	1.4700	
Acid Value*	8.754 ⎫ 8.437	
	8.120 ⎭	
Saponification Value	197.3 ⎫	
	196.3 ⎬ 196.7	
	196.6 ⎭	
Iodine Value	99.6 ⎫	
	100.2 ⎬ 99.9	
	100.0 ⎭	

* Determined on the eighth day after extraction.

When 0.5 cc. of the oil (equivalent to 0.4 Gm. of the seed per kilo of dog's body weight) was made to an emulsion and given to a dog by a stomach tube, there was some evidence of restlessness but convulsions did not occur.

Eijkman (2) obtained 30.5 per cent of the fatty oil from dry seeds (one year old). Bulir (10) reported the yield of the oil to be 12.5 per cent with reference to the seed. His oil has d_{15} 0.92947, saponification number 193.4, iodine number 90.6, Hehner's number 95.0, and Reichert-Meissl's number 1.5. His oil contains 62.2 per cent of oleic acid, 9.8 per cent of linoleic acid, 22.5 per cent of palmitic acid, and 2.5 per cent of stearic acid.

V. DETERMINATION OF MOISTURE, VOLATILE SUBSTANCES AND ASH CONTENTS OF THE SEED AND OF THE CARPEL.

Samples of 4 Gm. of the powdered material were taken and studied in the same manner as in my investigation of Ma Huang published in This JOURNAL some time ago (11). The following results were obtained:

	From the seed.	Average.	From the carpel.	Average.
Moisture in p.c.	3.475 ⎫		3.420 ⎫	
	3.418 ⎬ 3.443		3.456 ⎬ 3.462	
	3.437 ⎭		3.510 ⎭	
Volatile substance in p.c.	0.787 ⎫		1.508 ⎫	
	0.800 ⎬ 0.805		1.510 ⎬ 1.506	
	0.827 ⎭		1.500 ⎭	
Ash in p.c.	1.437 ⎫		3.432 ⎫	
	1.495 ⎬ 1.472		3.445 ⎬ 3.432	
	1.485 ⎭		3.420 ⎭	

VI. ASH ANALYSIS OF THE SEED AND OF THE CARPEL.

The water-soluble, acid-soluble (in 10 per cent HCl), and insoluble portions of each ash were determined. The carpel, upon incineration, gives an ash, green in color. Upon qualitative tests, it was found to be due to the presence of manganese.[1] The latter was then quantitatively analyzed by conversion into permanganic acid and titrated against $N/50$ arsenious acid according to Bradley (12). The results are shown as follows:

Analysis for.	Ash from the seed.	Average.	Ash from the carpel.	Average.
H₂O-soluble in p.c.	18.78 17.40 15.32	.....17.17	71.45 70.54	........70.99
HCl-soluble in p.c.	69.74 69.39 70.20	.....69.78	23.96 24.05	........24.00
Insoluble in p.c.	11.48 13.21 14.48	.....13.06	4.59 5.41	.... 5.00
Manganese in p.c.			0.2239 0.2230 0.2327	.... 0.2265

VII. CONCLUSIONS.

(1) The dry fruit of *Illicium religiosum*, or Mang Tsao, contains a toxic principle, occurring in a small quantity both in seeds and in carpels. It is very difficultly crystallizable, soluble in chloroform, alcohol and water. It seems to be detoxified by alkalies, but not affected by acids. When administered to animals, the toxic principle has probably a local irritant action, and produces convulsions due to the stimulation of medulla or upper cervical cord.

(2) The carpel yields 0.60 p.c. of volatile oil which proves to be toxic to frogs. The symptomatology is one of progressive depression and not similar to that after the solid toxic principle. The physical and chemical constants of this oil have been recorded.

(3) The seed contains 28.3 p.c. of fatty oil which is almost non-poisonous. The common physical and chemical constants of this oil have been determined.

(4) The seed gives 1.472 p.c. of ash, while the carpel 3.432 p.c. The ash from the carpel contains 0.23 p.c. of manganese.

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EFFECT OF ACIDITY ON THE ACTIVITY OF PEPSIN IN THE SOLID STATE.*

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Literature references indicate that stability of pepsin in solution is affected by the p_H value of the solution. Examination of pepsin of the market revealed a considerable range in inherent acidity, hence it seemed worth while to set aside samples of dry pepsin of low and high acidity and test them from time to time to note if variation in its acidity had any effect on the stability of pepsin activity.

We set aside one sample with an acidity of 7.66% calculated as hydrochloric acid and another with an acidity of 2.43% calculated as hydrochloric acid. These were granular pepsins U. S. P. IX 1–3000. Both were kept in tightly stoppered bottles, kept at ordinary room temperature in the dark, but were opened from time to time to withdraw assay samples. In one year the pepsin with a high acidity had caked and deliquesced somewhat. A strong odor had likewise developed. The low acidity sample was still dry, free-flowing, granular and comparatively odorless.

These samples were tested for activity according to the U. S. P. IX method at intervals indicated, with the following results:

	Residue undigested albumen.	
	Low acid pepsin.	High acid pepsin.
After 3 months	.5 cc.	.75 cc.
After 6 months	.5 cc.	.75 cc.
After 9 months	.3 cc.	.7 cc.
After 12 months	.3 cc.	.7 cc.
After 18 months	.2 cc.	1.4 cc.
After 21 months	.65 cc.	1.7 cc.
After 24 months	2 cc.	4 cc.

These observations indicate that pepsin with a low acidity is more stable than with a high acidity.

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