Chinese Journal of Physiology, 1931, Vol. V, No. 4, pp. 345—352.

THE ALKALOIDS OF CHINESE GELSEMIUM, *KOU WEN*, GELSEMIUM ELEGANS, BTH.

T. Q. CHOU

With notes on their physiological properties by

C. PAK AND H. C. HOU

and Botanical identification by

J. C. LIU

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

Received for publication June 19, 1931

In previous communications on American gelsemium, the writer (1) reported the isolation of 4 alkaloids, gelsemine, gelsemicine, sempervine and an amorphous alkaloid as its active and toxic constituents, gelsemicine being the representative principle of the plant. The Chinese drug, Kou Wen, 鈎吻, identified as *Gelsemium Elegans*, Bth. by Ford (2) and also known under various local names as Tu-ken, 毒根, Tuan Ch'ang Ts'ao, 斷腸草, etc., is indigenous in Southern China and possesses very poisonous properties. Li Shih-chen (3) says that when people happen by mistake to eat the leaves mixed with vegetables, they die in the course of half a day. Medicinally the root is recommended for wounds, caked breast, perspiring feet and skin eruptions, in all cases externally. The present chemical investigation of this toxic drug has resulted in the isolation of 4 alkaloids to which the names Koumine, Kouminine, Kouminicine and Kouminidine are suggested by the writer. Koumine is the chief alkaloid, possessing rather similar physiological actions to gelsemine, but differing from the latter in its chemical constitution and properties. Kouminine is an amorphous base and forms well crystalline salts. Kouminicine has only been obtained in its amorphous and impure condition and found to be the most active principle present, producing the usual toxic effects of American gelsemium in mammals. Kouminidine is crystalline and

present only in small quantity. Mention should be made that no gelsemic acid could be found present. The raw material used in this piece of work, consisted of the stems, roots and leaves of the drug collected by the Superintendent of the Botanical Garden of Hongkong. A preliminary investigation of the physiological properties of these 4 alkaloids was made by Drs. C. Pak and H. C. Hou and a brief Botanical study by Prof. J. C. Liu to whom the writer is indebted for the notes appended to this paper.

EXPERIMENTAL

1,700 grammes of the Chinese drug Kou Wen in the form of stems, roots, and leaves were finely powdered and percolated with 95 per cent alcohol at room temperature for about a week. The alcoholic extract was separated and evaporated at low temperature to a syrup. The residue was taken up with a sufficient quantity of 2 per cent aqueous hydrochloric acid and filtered from the insoluble resinous matter. The acid solution, when allowed to stand at room temperature for about 2 weeks, deposited a further quantity of a non-basic resinous substance. The material, obtained on rendering the acid solution alkaline with sodium carbonate, was extracted several times with excess of ether and then with chloroform. The ethereal extract constitutes fraction 1, and the chloroform extract fraction 2. After a long and tedious study, Koumine, Kouminine and Kouminicine have been isolated from fraction 1 and Kouminidine from fraction 2.

1. *Koumine* $C_{20}H_{22}N_2O$.

The ethereal extract, as above described, was dried and distilled, when a quantity of a pale yellow basic product was obtained which crystallised readily from acetone in handsome well-formed prisms (see fig. 1). After recrystallisation from the same solvent, its melting point was constant at 170° C. It was readily soluble in alcohol, chloroform and benzene, less so in ether and insoluble in petroleum ether and water. It dissolved in concentrated sulphuric acid to a colourless solution which became slightly violet on the addition of a little Manganese dioxide, but not with potassium bichromate with which only a greenish yellow coloration was observed. A 1 per cent solution in alcohol in 1 dm tube gave a specific rotation $-2.65°$, whence $[\alpha]$ $23/D = -265°$. It had the molecular formula $C_{20}H_{22}N_2O$ according to the following analysis:—

(i) 0.1177 gm substance gave 0.3391 gm CO_2 and 0.0742 gm H_2O C=78.51; H=7.00

(ii) 0.1182 gm substance gave 0.3397 gm CO_2 and 0.0758 gm H_2O C=78.38; H=7.12

(iii) 0.1114 gm substance gave 9.4 cc moist nitrogen at 24°C and 758 mm pressure, N=9.39.

calculated for the formula $C_{20}H_{22}N_2O$, C=78.43; H=7.19; N=9.15.

It formed a monohydrochloride crystallising slowly from alcohol in prisms; melting point 255° C with decomposition. Its hydrobromide crystallised from alcohol in the presence of a little water in prisms melting at 269° C with decomposition.

Physiological tests. (By Dr. C. Pak)

An aqueous solution of its hydrochloride was used.

Frogs. The injection of 0.25 mg per gm body weight into the lymph sac produced depression of the C. N. S. which was followed by exagerated reflex and increased extensor tonus of the hind limbs but no convulsions. In dosage of 0.3 to 0.5 mg per gm body weight there was produced a complete paralysis of the C. N. S. The respiration ceased in the early stage while the heart was still beating. Electric stimulation of the sciatic nerve caused no response, whereas the direct stimulation of the muscles was effective. In one experiment in which one leg was ligated with the exception of the sciatic nerve, it showed a curare-like action on the motor nerve endings.

Mice. The subcutaneous injection of 0.3 to 0.7 mg per gm body weight caused restlessness within 10 minutes which was followed by violent tetanic convulsions of the whole body ending with death from failure of respiration, while the heart was still fibrillating.

Rabbit. 10 mg of substance was injected intravenously into a rabbit weighing 1.7 kg. The animal became quiet, the head falling down, and the hind legs stretched out. The respiration became a little slower and then gradually recovered. Three repeated injections of 10 mg each produced the same symptoms. Injections of 100 mg intravenously, immediately caused violent convulsions. After a minute, it died of failure of respiration. The pupil was not changed either by intravenous injection or by local instillation of 3.3 per cent solution.

2. *Kouminine.*

It can be easily separated from Koumine by taking advantage of the slight solubility of its hydrochloride in cold alcohol. The basic product from which Koumine had been removed as above described, was evaporated to dryness to get rid of acetone and the residue neutralised with an alcoholic solution of hydrochloric acid. Kouminine hydrochloride crystallised out readily on standing. After being re-

crystallised alternately from alcohol and water, it formed colourless prisms (see fig. 2) melting above 300° C. Its hydrobromide was prepared and purified in a similar way, melting also above 300° C. A 0.4 per cent solution of its hydrochloride in water was found to be optically inactive, whence $[\alpha]$ D=0°. Its hydrochloride dissolved in concentrated sulphuric acid to a colourless solution, which, on the addition of a crystal of potassium bichromate, became purple, brown and finally green. The free base, liberated from its purified salts by means of an alkali, has only been obtained as a colourless amorphous residue and refused to crystallise from any solvent tried. It is easily soluble in ether and most organic solvents and less so in water. The ethereal solution, when well dried and distilled, left behind a hard mass, melting indefinitely at about 115° C.

Fig. 1.
Koumine $C_{20}H_{22}N_2O$
m.p. 170°C
S.R.= −265°

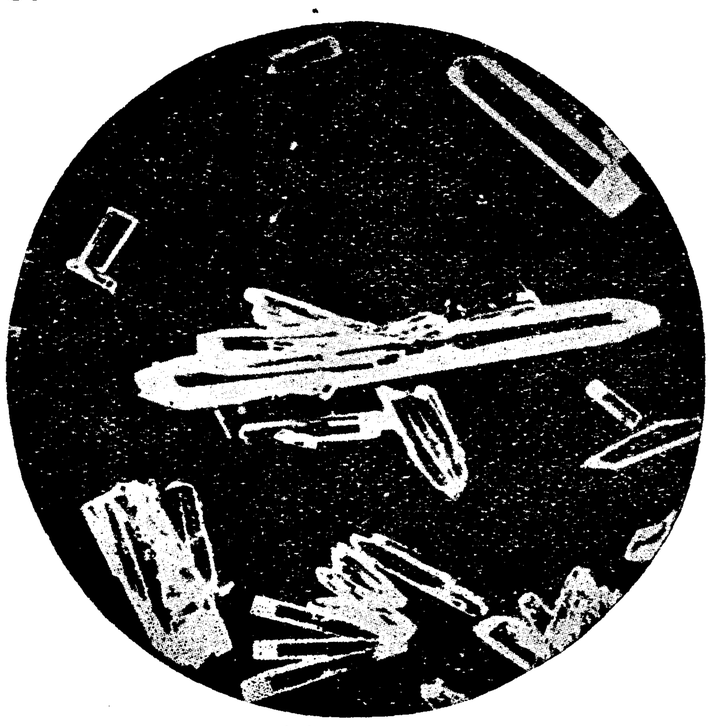

Fig. 2.
Kouminine hydrochloride
m.p. above 300°C
S.R.=0°

Physiological tests. (By Dr. C. Pak)

An aqueous solution of its hydrochloride was used.

Frogs. Dosage of 0.14 to 0.27 mg per gm body weight produced depression of the C. N. S. The reflexes were primarily increased and then depressed. It caused also a curare-like action on the motor nerve endings.

Mice. Dosage of 0.4 mg per gm body weight produced restlessness, slight excitement, tremor of muscles followed by violent convulsions. During the convulsions, the head was bent forward, the hind legs stretched out and death followed from failure of respiration.

Rabbit. Twice repeated intravenous injections of 20 mg to a rabbit weighing 1.5 kg produced no noticeable symptoms. The local instillation of 1 per cent solution caused no change in the pupil.

3. *Kouminicine.*

The basic residues, after the removal of Koumine and Kouminine as above described, were united, taken up with a 1 per cent solution of hydrochloric acid and the acid solution washed repeatedly with chloroform. The material, obtained on rendering the acid solution alkaline, was first extracted with ether to remove any Koumine and Kouminine still present and then with chloroform, when an amorphous basic product was obtained. It was evidently impure as neither the base nor any of its salts could be obtained in a crystalline condition. It was neutralised with hydrochloric acid and evaporated dryness over a water bath. The crude hydrochloride so obtained dissolved in water to a clear yellow neutral solution which was found to be optically leavo-rotatory.

Physiological tests. (By Dr. C. Pak)

An aqueous solution of its hydrochloride was used.

Frogs. The injection of 0.14 mg per gm body weight into the lymph sac produced gradual depression of the C. N. S. The respiration became very slow and within 20 minutes there was a characteristic trembling of the head and later the fore limbs. The reflex irritability was primarily increased and then depressed. Three hours later the frog was completely paralysed. It caused also a curare-like action on the motor nerve endings. Finally the heart was in diastolic standstill.

Rabbit. The intravenous injection of 0.7 mg per kg body weight produced within 10 minutes slow convulsive respiration and later trembling of the head. Violent convulsions of emprosthotonus type followed and sometimes started with a cry. The pupil was dilated, the respiration became gradually slower and labored, and the ear vessels showed cyanosis. Fifty minutes later it died of failure of respiration. A dosage of 0.26 mg per kg produced the same symptoms but the animal recovered in 40 minutes.

4. *Kouminidine.*

The chloroform extract (fraction 2) was dried and distilled, when a yellowish crystalline residue was obtained. It crystallised out more readily from acetone than Koumine. When crystallised pure from the same solvent, it formed colourless prisms (see fig. 3) melting at 200° C. It dissolved in concentrated sulphuric acid to a colourless solution, which on adding a crystal of potassium bichromate became greenish yellow, no purple coloration being observed. It was soluble in most organic solvents and in water to an alkaline solution. It formed a well crystallised hydrochloride and hydrobromide soluble in water.

On account of the small amount of substance in hand, no detailed physiological investigation was made. An injection of 4 mg of its hydrochloride produced no noticeable symptoms in the rabbit (test by Dr. H. C. Hou).

Fig. 3.

Kouminidine, m. p. 200°C.

Fig. 4.
Kou Wen
(Gelsemium elegans, Bth.*)*

On the botany of Kou Wen (鈎吻). (By Prof. J. C. Liu)

Two identifications have been given to the Chinese drug-plant "Kou Wen" fig. 4); namely *Gelsemium elegans*, Bth. and *Rhus Toxicodendron*, L., the poison,, ivy. As our sample of the drug bears simple, opposite and not ternate, alternate leaves, it can not be the latter. Without flowers and fruits, we are not in a position to give the final identification. But the sterile plant in our possession agrees well (with the description of *Gelsemium elegans*, Bth. as given by Ford (2).

Ford's description of Gelsemium elegans, Bth.	*Our sterile plant.*
A glabrous woody evergreen twiner.	A glabrous woody twiner.
Leaves—opposite, 2-4.5 in., long, ovate lanceolate, very acute at apex, entire and somewhat rounded at base, on a *petiole* 3-6 lines long *Stipules* minute, fugative, easily observed on young branches.	Leaves—opposite, 2.5-3.5-1-1.5 in. ovate—oblong, apex long—acute, base roundish—cuneate, entire *petioles*—3-4 lines; *Stipules* 0.

Mr. Ford further mentioned that the root is the part used in Chinese medicine though other parts of the plant are also toxic, judging from the few poisoning cases he reported. Regarding the localities where the plant is found, Mr. Ford says

that it is abundant on hills in the mainland, opposite Hongkong, such as near the base of Tai Mo Shan (3,000 ft.), etc. It climb rounds trees to a height of 12 ft., blooms from October to December and fruits April to May.

DISCUSSION

Chinese gelsemium, Kou Wen, is very rich in alkaloids, the total amount of which comes to about 0.3 per cent. Besides the four alkaloids as above isolated and described, there are still others left in the residue, for instance, one is found to have a melting point over 300° C and another soluble in caustic alkalies with phenolic properties. A more detailed investigation upon the alkaloids already or not yet isolated, can only be successively made by working on a large quantity of raw material. It is interesting to see that not a single alkaloid or gelsemic acid present in American gelsemium could be found in the Chinese species, although both plants possess rather similar physiological properties.

SUMMARY

From the Chinese drug, Kou Wen, *Gelsemium elegans*, Bth., 4 alkaloids have now been isolated and named Koumine, Kouminine, Kouminicine and Kouminidine respectively.

Koumine, the principal alkaloid, has the molecular formula $C_{20}H_{22}N_2O$, a specific rotation −265° and a melting point 170° C.

Kouminine is an amorphous alkaloid, but forms well crystalline salts.

Kouminicine has only been obtained in its amorphous and impure state. It is the most physiologically active substance present in the drug.

Kouminidine crystallises from acetone in prisms melting at 200° C and forms well crystallised salts.

A preliminary investigation of their physiological properties has been made.

The writer desires to express his thanks to the Superintendent of the Botanic Garden of Hongkong for supplying the raw material and Professors H. P. Chu and B. E. Read for suggesting this drug for research.

LITERATURE

1. Chou, T. Q. Chinese J. Physiol., 1931, **5**, 131-140; 295-300.
2. Ford, C. China Review, 1887, **15**, 214.
3. Li Shih-chen. Pen Ts'ao Kang Mu, Shanghai, 1597, cf, Stuart, G. A., Chinese Materia Medica, Shanghai, 1911.

中國鈎吻之有機鹼質

趙承嘏

附及關于生理性質及植物考之略記

朴柱秉　侯祥川　劉汝強

私立北平協和醫學院藥物學系,北平.

關于美國鈎吻之有機鹼質,作者已有詳細報告(見本雜誌第五卷第二期及第三期).今作者又從中國鈎吻中提及四種有機鹼質,定名爲鈎吻素子 (koumine) 鈎吻素丑 (kouminine) 鈎吻素寅 (kouminicine) 及鈎吻素卯 (kouminidine).此四者之化學及物理性質與在美國鈎吻中所提出者完全不同,惟生理性質則類似.

鈎吻素子之分子式爲 $C_{20}H_{22}N_2O$,旋轉度爲 $-265°$,熔點爲 170°C. 其性不甚毒,但爲中國鈎吻中之主要質素.鈎吻素丑爲一不結晶物,惟與酸類合成之有機鹽類則結晶極易.鈎吻素寅亦爲一不結晶物,其性甚毒,爲最重要之有效質素.其藥性足以代表中國鈎吻之全部.鈎吻素卯之結晶較易,熔點爲 200°C. 所得極少,不足供生理之研究.

上述四種有效質素之生理性質,已由朴柱秉及侯祥川二君作一簡單之試驗.詳細報告,當俟諸異日.鈎吻之植物考,由劉汝強君有所陳述.