The Alkaloids of the Chinese Drug Pai Pu*,†

By Henry M. Lee and K. K. Chen

It is well known that alkaloids occur in the species of *Stemona*, family *Stemonaceæ*. Table I is a résumé of the studies already recorded in literature. No information is as yet available concerning the chemical structure of these bases. Almost equally scanty is their pharmacological status. The only report dealing with the action of an alkaloid is that of Lobstein and Grumbach (5). These authors showed that their "stemonine" isolated from *S. tuberosa* caused paralysis of earthworms and frogs, reduced the chronaxie of frogs' muscle-nerve preparations and diminished the turtle's heart rate with increase in contraction soon followed by arrhythmia and depression.

Table I.—Alkaloids of *Stemona* Species

Species	Alkaloid	Empirical Formula	Melting Point, ° C.	Optical Rotation	Investigated by
S. japonica	Stemonine	$C_{17}H_{23}O_4N$	151	$[\alpha]_D^{16}$ −113.8°	Suzuki (1)
	Stemonidine	$C_{19}H_{29}O_5N$	116	$[\alpha]_D^{12}$ −7.6°	
S. ovata	Isostemonidine	$C_{19}H_{31}O_5N$	137	$[\alpha]_D$ −48.9°	Suzuki (2)
S. sessilifolia	Hodorine	$C_{19}H_{31}O_5N$	. . .	. . . . .	Furuya (3)
	Unnamed base	$C_{22}H_{33}O_4N$	86–87	$[\alpha]_D^{20}$ −47.1°	Schild (4)
S. tuberosa	Stemonine	$C_{22}H_{33}O_4N$	160	$[\alpha]$ +76.5°	Lobstein and Grumbach (5)
	Tuberostemonine	$C_{22}H_{33}O_4N$	86–88	$[\alpha]_D$ −25.4°	Suzuki (6, 7)

The Chinese drug Pai Pu is one of the *Stemona* species, although its exact botanical identity has not been established. Stuart (8) called it *S. tuberosa*, while the authors of "Botanical Nomenclature" (9) named it *S. sessilifolia*. The mere fact that Pai Pu gives rise to new alkaloids as shown in later sections is an indication that neither identification is correct. According to Pentsao Kang Mu (10), the root of this plant has been used in China as a cough remedy, an insecticide and in the treatment of pulmonary inflammation, scabies and insect bites. Recently Wang (11) reported that an alcoholic extract of Pai Pu was effective in killing lice.

EXPERIMENTAL

On account of the information furnished by Mr. Edward W. Miller that Pai Pu was attracting attention in modern medicine, we attempted to make a chemical investigation. Our material was obtained in the vicinity of Shanghai through the assistance of Mr. Miller. Briefly, two alkaloids were successfully isolated in crystalline form. They differed in both physical constants and chemical composition from the known alkaloids of *Stemona* species, as enumerated in Table I. One was water-soluble, melted at 138–138.5° C. and had the empirical formula $C_{21}H_{36}O_5N$; and the other was ether-soluble, melted at 105.5–106.5° C. and conformed to the empirical formula $C_{24}H_{37}O_4N$. The name sinostemonine is proposed for the higher melting compound, and paipunine for the other base. Certain pharmacological experiments were carried out with these new alkaloids.

I. CHEMICAL PART

The sample of Pai Pu, when assayed gravimetrically, was found to have a total alkaloidal content of 1.77 per cent. For isolation of the alkaloids, a quantity of 10 Kg. of the powdered root was percolated with ethyl alcohol until exhaustion. The alcohol of the extract was removed under diminished pressure and the residue was treated with dilute hydrochloric acid and filtered, to separate the resinous material. Crude alkaloids were then obtained by precipitation with ammonium hydroxide. Upon

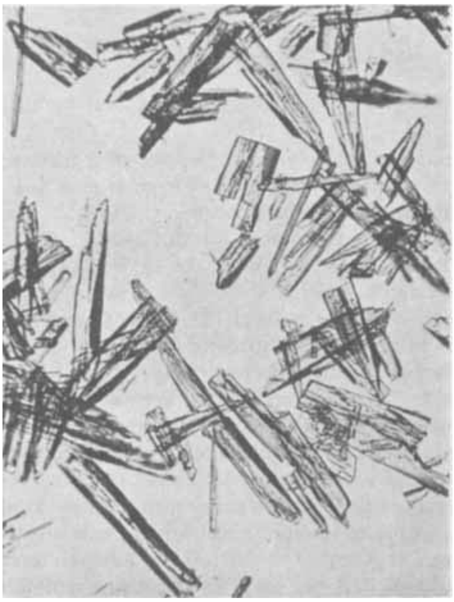

Fig. 1.—Photomicrograph of Paipunine.

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filtering and drying, the mass was repeatedly extracted with ether and the combined extracts were subjected to evaporation. This gave rise to paipunine which could finally be purified by recrystallization from a mixture of isopropyl alcohol and water. The remainder of the alkaloidal mass after ether extraction was dissolved in hot water. On cooling and standing, sinostemonine crystallized out in abundance.

Paipunine melts at 105.5–106.5° C. and is levorotatory, its specific rotation in acetone being $[\alpha]_D^{25}$ $-53.7°$. It forms elongated prisms as shown in Fig. 1 and exhibits a blue fluorescence in alcohol or acetone—very strongly under ultraviolet rays. It is easily soluble in alcohol, acetone, ether and dilute acids, but slightly soluble in water. It reacts to form a precipitate with Mayer's and Wagner's reagents and phosphomolybdic, phosphotungstic and picric acids. The results of combustion analyses conform to the empiric formula $C_{24}H_{37}O_4N$.

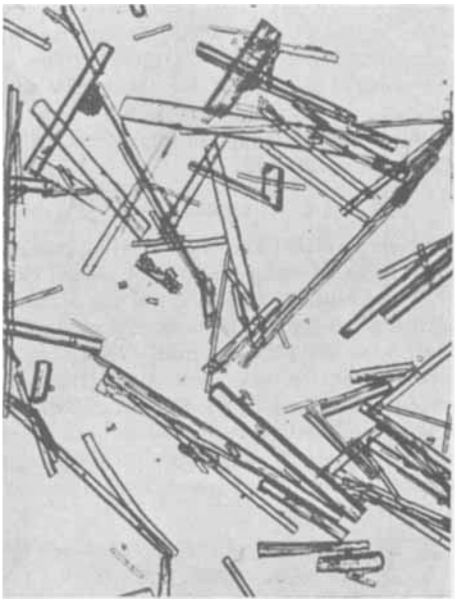

Fig. 2.—Photomicrograph of Sinostemonine.

Analysis. Calculated for $C_{24}H_{37}O_4N$: C, 71.41; H, 9.25; N, 3.47. Found: C, 71.45; H, 9.24; N, 3.66; C, 71.46; H, 9.39; N, 3.64.

Sinostemonine melts at 138–138.5° C. and crystallizes in needles as illustrated in Fig. 2. It has in water a specific rotation of $[\alpha]_D^{25}$ $-37°$, and is soluble in dilute acids and hot water, but less soluble in ether, alcohol and acetone. Like paipunine, it has a blue fluorescence in organic solvents, particularly under ultraviolet rays. It precipitates Mayer's and Wagner's reagents and phosphomolybdic, phosphotungstic and tannic acids. When heated in vacuum at 100° C., it loses an average weight of 3.38 per cent, less than one molecule of water of crystallization. Analytical data suggest the simple formula $C_{21}H_{36}O_5N$.

Analysis. Calculated for $C_{21}H_{36}O_5N$: C, 65.92; H, 9.49; N, 3.66. Found: C, 65.88; H, 9.37; N, 3.51. C, 65.67; H, 9.20; N, 3.63.

It becomes obvious that the composition and physical constants of paipunine and sinostemonine are not in agreement with those of any known alkaloids of *Stemona* species. Indeed, evidence favors the contention that they are new alkaloids.

II. PHARMACOLOGIC PART

1. Paipunine.—For pharmacological experiments the alkaloid was dissolved in dilute hydrochloric acid. Comparatively higher concentrations such as a 1 per cent solution required a low p_H, 2 to 3, otherwise the base would settle out. Frogs injected in the lymph sac in the dose of 0.1 mg. per Gm., mice injected intravenously in the dose of 22 mg. per Kg. and rabbits injected intravenously in the dose of 20 mg. per Kg., all developed clonic convulsions. Mice jumped high in the air during violent convulsions, while rabbits and frogs took opisthotonos position. The action is apparently on the medulla for in frogs decerebration did not alter the symptomatology but destruction of the medulla abolished the convulsions.

Table II.—Toxicity of Paipunine and Sinostemonine in Mice by Intravenous Injection

Alkaloid	Dose, mg. per Kg.	Number Died / Number Used	LD_{50} ± Standard Error, mg. per Kg.
Paipunine	25–28	0/18	38.95±2.09
	31	3/8	
	35	2/8	
	39	3/8	
	45	6/8	
	50	8/9	
'Sodium Amytal' immediately followed by Paipunine	56	1/8	81.62±8.60
	75	4/7	
	100	5/8	
	133	7/8	
Sinostemonine	600	1/5	757.0±53.5
	650	3/10	
	680	7/10	
	700	6/10	
	750	4/10	
	810	1/10	
	880	7/10	
	1000	5/5	

By intravenous injection of paipunine in mice, the median lethal dose (LD_{50} ± Standard Error) was determined to be 38.95 ± 2.09 mg. per Kg. as shown in Table II. In view of the fact that "Sodium Amytal" (Sodium Iso-amyl Ethyl Barbiturate, Lilly) is capable of detoxifying drugs which cause convulsions (12, 13, 14, 15, 16, 17, 18), such as cocaine, strychnine, picrotoxin, amidopyrine, dendrobine, coriamyrtin, tutin and harmine, it was tried here in mice in conjunction with paipunine. Indeed the results as contrasted in Table II strongly suggest the antagonism between paipunine and "Sodium Amytal." The latter appeared to detoxify slightly more than one LD_{50} of the former.

The action of paipunine on the frog's heart by perfusion into inferior *vena cava* is one of depression.

A concentration of 1:5000 caused a decrease in heart rate and amplitude of contraction, terminating in diastolic stoppage. After cardiac arrest, the ventricle responded by contraction to mechanical stimuli, and in most instances recovery took place when the medicated Ringer's solution was replaced by normal Ringer's solution. Vasoconstriction was observed in two out of three experiments in which the frog's legs were perfused with paipunine by the method of Laewen-Trendelenburg. There was no effect in the third animal.

In etherized cats intravenous injection of paipunine in the dose of 15–20 mg. (total) was followed sometimes by a rise, and other times by a fall, of carotid blood pressure. The results are difficult to interpret, but it is probable from the evidence in frog studies that the rise may be due to excessive vasoconstricting action as compared with cardiac depression, and *vice versa*. Respiration recorded with blood pressure showed depression followed by augmentation. It was not determined whether or not these changes were secondary to those of blood pressure.

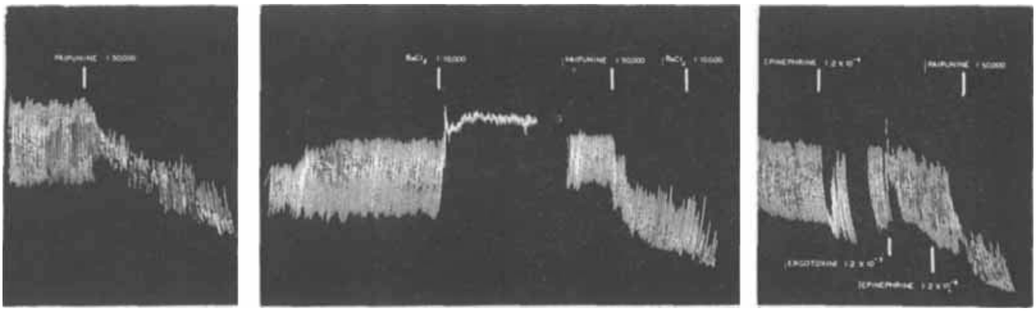

Fig. 3.—Illustration of the Effect of Paipunine on the Rabbit's Isolated Intestines.

Paipunine in the concentration of 1:50,000 inhibited the peristaltic movements of isolated rabbit's small intestines immersed in Locke's solution maintained at 38° C., as illustrated in Fig. 3. The action of barium chloride was abolished following paipunine. After ergotoxine had paralyzed the sympathetic endings, as evidenced by the epinephrine test, paipunine was still active. It is thus apparent that paipunine acts directly upon the smooth muscles of the intestine and not on the sympathetic nervous system.

The isolated guinea pig's uterus, on the other hand, responded to the application of paipunine by contractions in concentrations varying from 1:400,000 to 1:100,000. While the explanation for the deviation of action on the uterus from that on the intestine must be reserved for future exploration, it is possible that the smooth muscle of the guinea pig's uterus is not the same as that of the rabbit's intestine. Previous reports (19, 20, 21, 22, 23) published from this laboratory indicated that ephedrine, fritimine, retrorsine, dendrobine and seneciphylline also inhibited isolated intestines but stimulated the uterus.

2. *Sinostemonine.*—In all experiments, aqueous solutions of this alkaloid were used without acidification. The substance was much less toxic than paipunine. The median lethal dose (LD_{50} ± Standard Error) in mice by intravenous injection was found to be 757 ± 53.5 mg. per Kg. No systemic effects could be elicited even with relatively large doses. Its activity is therefore very low.

SUMMARY

1. The Chinese drug Pai Pu contains 1.77 per cent of total alkaloids.

2. Two new alkaloids have been isolated in crystalline form —paipunine, $C_{24}H_{37}O_4N$, and sinostemonine, $C_{21}H_{36}O_5N$.

3. Pharmacologically, paipunine is a convulsant, acting probably on the medulla. The median lethal dose in mice by intravenous injection is 38.95 ± 2.09 mg. per Kg. "Sodium Amytal" is capable of detoxifying more than one median lethal dose of paipunine in mice. Paipunine depresses the frog's heart in 1:5000 solution. It inhibits isolated rabbit's small intestines, but contracts the isolated guinea pig's uterus.

4. Sinostemonine has a median lethal dose of 757 ± 53.5 mg. per Kg. in mice when injected intravenously. It is practically inactive in other respects.

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Tannic Acid, U. S. P.

II. Relationship between Glucose and Tannic Acid

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It was claimed by Freda (1) in 1854 that tannic acid yielded on acid hydrolysis three molecules of gallic acid and one molecule of glucose, the glucose being a part of the tannic acid molecule. As a result of the study of the action of yeasts on tannic acid by Biddle (2) it was concluded that the glucose present in tannic acid is a free component and therefore an impurity.

EXPERIMENTAL

We endeavored to demonstrate the veracity of these seemingly conflicting theories by proving that glucose is not only a component part of tannic acid but an impurity as well. In order to do this, one per cent solutions of tannic acid were prepared and tested immediately by means of the Molisch reagent. A broad, distinct, violet band was formed. These one per cent solutions were then diluted to a strength of 1:1000. Using the Molisch reagent the violet band became narrower and fainter as the dilution increased. A solution freed of gallic acid also gave a positive test.

We therefore concluded that a sugar was present in the tannic acid samples as an impurity in varying amounts. As this reaction did not give any indication of the kind or amount of sugar, our attention was directed toward the possibility of preparing and isolating some derivative of the sugar in order to determine the kind and amount. If the amount of sugar was found to increase on hydrolysis it would prove possibly that the sugar existed in a combined form.

Osazones are usually crystalline in nature and can be easily purified by crystallization. The osazone prepared from the sugar in tannic acid after recrystallization from alcohol and pyridine was found to melt at 208° C. This led us to believe that the sugar was glucose and the fact that the sugar gave a negative Seliwanoff reaction was considered further proof of this.

The next step was the development of a method to determine the amount of osazone formed without the use of the tedious and unreliable gravimetric method. The unreliability of this method may be attributed to the solubility of the osazone in the large excess of phenylhydrazine acetate that is present in the mixture.

Knecht's method (3) of osazone estimation by means of titanium trichloride was modified by using methylthionine chloride instead of crystal scarlet. The solutions required were: standard titanium chloride solution prepared by mixing 50 cc. of commercial 20 per cent titanium chloride with 50 cc. of hydrochloric acid, specific gravity 1.175, and diluting to one liter with freshly distilled water; standard glucose solution containing 10 mg. per cc.; fifteenth molar methylthionine chloride solution; saturated sodium tartrate solution; and twenty-five hundredths per cent phenylhydrazine in glacial acetic acid.

The titanium chloride solution was standardized both in terms of milligrams of glucose per cc. of solution which is hereafter called the glucose factor and cc. of methylthionine chloride solution. The latter standardization was made at a temperature of 70° C. in the presence of hydrochloric acid. It is advisable to determine the glucose factor for each lot of commercial titanium chloride for accurate results. The end-point of the titration is the formation of a permanent blue color, which is advantageously seen by directing a beam of light from beneath the flask.

For the hydrolysis, a 20 per cent solution of each sample in approximately tenth-normal sulfuric acid was heated continuously on a steam bath. In order

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