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THE ALKALOIDS OF GELSEMIUM
I. GELSEMINE AND GELSEMICINE

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With notes on their Physiological Properties by

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Gelsemium, commonly known as yellow jasmine, consists of the rhizome and roots of *Gelsemium sempervirens*, Linné and *Gelsemium nitidum*, Michaux, indigenous to north America. It has long been used in medicine for its antispasmodic and analgesic properties, and is still recognised in several pharmacopoeias. Its active constituents were first thoroughly investigated by Wormley (10) who isolated an alkaloid, gelsemine and a peculiar acid, gelsemic acid. Some years later Sonnenschein (7) and Gerrard (3) made more detailed examinations on the alkaloid gelsemine and assigned to it the formulae $C_{22}H_{38}O_4N_2$ and $C_{24}H_{28}O_4N_2$ respectively. Gerrard's gelsemine was physiologically examined by Rouch (5) who mentioned its paralysing action on the excito-motor centres and by Tweedy (9) who noticed its mydriatic effect. Thompson (8) in 1887 ascribed to gelsemine the formula $C_{54}H_{69}O_{12}N_4$ and showed that it was accompanied in the plant by a second amorphous alkaloid which he named gelseminine. Both gelsemine and gelseminine were reinvestigated by Cushny (1) who proposed the formulae $C_{49}H_{63}O_{14}N_5$ and $C_{42}H_{47}O_{14}N_3$ respectively for the two bases and found that gelsemine was inactive to mammals but produced strychnine-like effects in frogs whilst gelseminine was highly potent 1 mg in the form of its hydrochloride being fatal to a rabbit weighing 2850 gm. Mention should be made of the work done by Moore (4), who isolated gelsemine in its pure state from the plant *Gelsemium sempervirens* Aiton having the formula $C_{20}H_{22}O_2N_2$,

specific rotation +15.9° in chloroform and m.p. 178°C and 2 amorphous alkaloids, one of which corresponded to Thompson's gelseminine. Dale (2), working with Moore's material, found that in doses of 0.1 gm, gelsemine produced no effect on rabbits; on the other hand 0.001 gm of the hydrochlorides of the mixed amorphous alkaloids injected into rabbits caused convulsions followed by death from respiratory failure. Sayre (6) and his colleagues made a series of investi gations of the active principles of gelsemium and showed that Thompson's gelseminine was not a single substance but a mixture of 3 alkaloids, to which the names sempervirine, gelsemidine and gelsemoidine were given, sempervirine being crystalline and the other two amorphous, but none of them being so potent as gelseminine itself.

With the object of getting some definite chemical knowledge of this important drug, the writer examined its alkaloidal constituents again and found that it contains more crystalline alkaloids than had hitherto been isolated. The raw material used consisted of the rhizome and roots of gelsemium of American origin. The present communication consists of the isolation and properties of gelsemine, its chief alkaloid and a second crystalline alkaloid to which the writer gives the name gelsemicine. Moore's formula for gelsemine $C_{20}H_{22}O_2N_2$ has now been confirmed, but its specific rotation was found to be inactive in alcohol and +10° in chloroform instead of +15.9° as given by Moore. Gelsemicine has been found to be as highly potent and toxic as gelsemium in mammals, 0.08 mg of its hydrochloride per kilo being fatal to rabbits.

A preliminary investigation of the physiological properties of gelsemine and gelsemicine is being made by Drs. K. K. Chen, C. Pak and H. C. Hou to whom the writer is indebted for the notes appended to this paper.

EXPERIMENTAL

Forty-eight kg of American gelsemium in the form of rhizome and roots were finely powdered and percolated with 95 per cent alcohol at room temperature for about a week. The alcohol extract was separated and evaporated to a syrup at a low temperature. The residue was taken up with a sufficient quantity of dilute hydrochloric acid and filtered from the resinous matter. The acid solution was then separated and allowed to stand at ordinary temperature for about 2 weeks. A crystalline deposit was obtained. This constitutes

fraction 1 and the acid solution fraction 2. The isolation of active constituents from these fractions proved a tedious matter. Gelsemine and gelsemicine, a new alkaloid, have, up to now been isolated in their pure state from fraction 2 as follows:—

1. Gelsemine $C_{20}H_{22}O_2N_2$.

The acid solution (fraction 2) was made alkaline with sodium carbonate and the liberated base extracted with chloroform. The chloroform solution was dried and distilled, and the residue crystallised out from acetone. The crude gelsemine so obtained was recrystallised several times from acetone until its m.p. became constant. As shown by Moore (4), gelsemine has the molecular formula $C_{20}H_{22}O_2N_2$ and crystallises out from acetone with one molecula of the solvent in prisms which lose their acetone at 120°C. This has now been confirmed by the following analysis:—

(i) 0.9770 gm substance (air dried), when heated to 115-120° until constant in weight, lost 0.1439 gm = 14.72 per cent
$C_{20}H_{22}O_2N_2$. CH_3COCH_3 requires acetone = 15.26 per cent

(ii) 0.1262 gm substance (dried at 120°C) gave 0.3453 gm CO_2 and 0.0773 gm H_2O
C = 74.62; H = 6.82. $C_{20}H_{22}O_2N_2$ requires C = 74.53 and H = 6 83

Fig. 1.
Gelsemine $C_{20}H_{22}O_2N_2$
M. p. 178°C
S. R. +10° in $CHCl_3$
M. L. D. 180 mg per kg (rats).

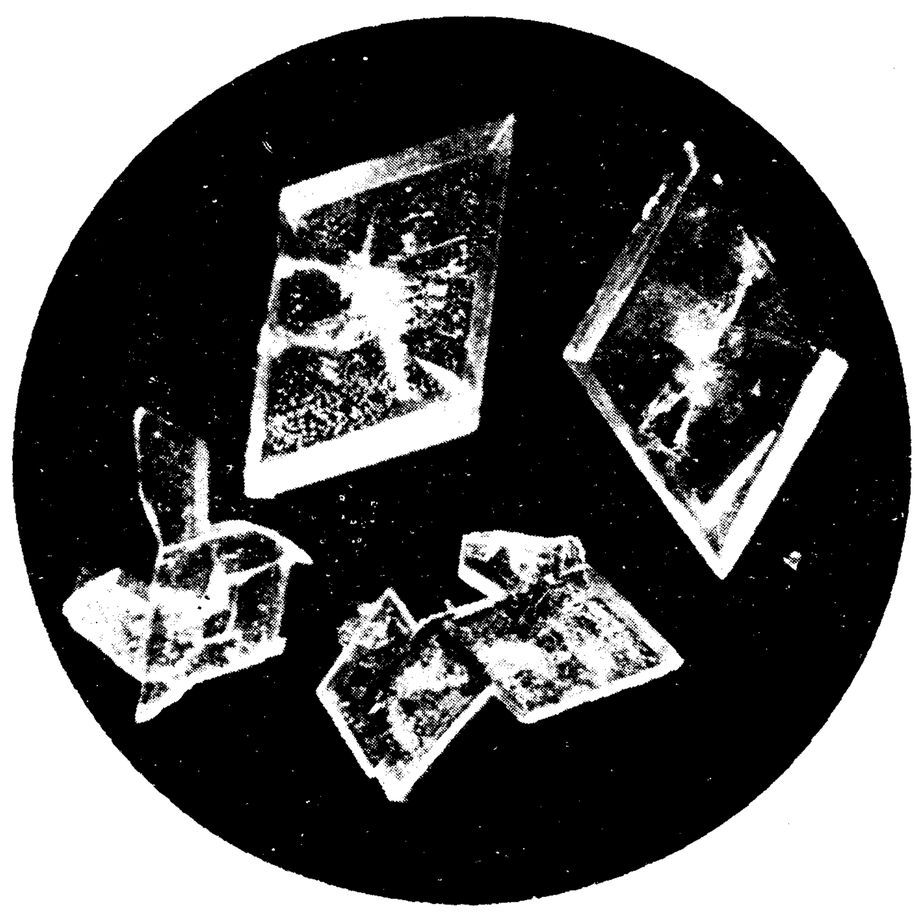

Fig. 2.
Gelsemicine $C_{20}H_{25}O_4N_3$
M. p. 171°C
S. R. −140° in alcohol
M. L. D. 0.08 mg per kg (rabbits).

When crystallised pure from acetone, gelsemine forms colourless long prisms (see fig. 1) melting at 178°C, and is easily soluble in ether,

benzene alcohol or chloroform. A 1 per cent solution in chloroform in 1 dm tube gives a specific rotation +0.1° whence (α) 24/D= +10° instead of +15.9° as given by Moore. Its solution in alcohol is optically inactive, the substance dried at 120°C being used in both cases. Gelsemine dissolves in conc. H_2SO_4 to a colourless solution which on the addition of a crystal of potassium dichromate or a little manganese dioxide becomes violet and then greenish yellow. It forms well crystallised hydrochloride and hydrobromide salts soluble in water.

2. Gelsemicine $C_{20}H_{25}O_4N_2$.

After the removal of gelsemine from fraction 2 as above, the basic residue was neutralised with hydrobromic acid in alcoholic solution. Gelsemine hydrobromide, being only slightly soluble in alcohol, crystallised out on long standing. The alcoholic mother liquor was then evaporated to dryness over a water bath and the residue taken up with water and filtered. The aqueous solution was then made alkaline with sodium carbonate and the liberated base extracted with chloroform. The chloroform solution was dried and distilled and the residue taken up with acetone. Gelsemicine crystallised out in the form of large orthorhombic crystals (see fig. 2). When crystallised pure, it melts at 171°C and is readily soluble in chloroform, acetone or alcohol, less so in ether or benzene and insoluble in water. A 1 per cent solution in alcohol in 1 dm tube, gives a specific rotation −1.40°, whence (α) 24/D= −140°. Differing from gelsemine, gelsemicine crystallised out from acetone without one molecule of the solvent and becomes easily coloured on exposure to the air and its solution in conc. H_2SO_4, colourless at first, gradually turns violet on standing. It has the molecular formula $C_{20}H_{25}O_4N_2$ according to the following analysis:—

(i) 0.1254 gm substance gave 0.3102 gm CO_2 and 0.0810 gm H_2O. C=67.46; H=7.17.

(ii) 0.1261 gm substance gave 0.3124 gm CO_2 and 0.0788 gm H_2O. C=67.56; H=6.94.

(iii) 0.2131 gm substance gave 14.7 cc moist nitrogen at 24°C and 771 mm pressure. N=7.81.

Calculated for the formula $C_{20}H_{25}O_4N_2$. C=67.22; H=7.00; N=7.84.

Its molecular weight was deduced from its platinum salt;

0.0989 gm Pt salt gave 0.0171 gm platinum on burning

Pt = 17.29

$(C_{20}H_{25}O_4N_2HCl)_2\ PtCl_4$ requires Pt = 17.36.

Gelsemicine forms a monohydrochloride which crystallises out from a mixture of alcohol and ether in the form of prismatic needles very soluble in water. The fraction 2 contains other alkaloids besides gelsemine and gelsemicine and its basic residue is kept for further investigation.

Notes on the physiological action of gelsemine and gelsemicine.

Gelsemine: (By Dr. K. K. Chen)

1. Toxicity. Minimal lethal dose (M. L. D.) of gelsemine was determined in white rats by intravenous injection. The results are given in the following table.

TABLE 1.

Toxicity of gelsemine HCl by intravenous injection in white rats.

2 per cent solution used

Dose *mg per kg*	No. of rats used	No. of rats survived	No. of rats died	M. L. D.
200 mg	1		1	
180 „	5	2	3	180 mg per kg
170 „	4	3	1	
160 „	1	1		

2. On blood pressure. Two pithed cats were used. An intravenous injection of 7 mg of gelsemine HCl into one resulted in a fall of blood pressure (see fig. 3) and that of 10 mg into another following a previous injection of 5 mg caused death of the cat

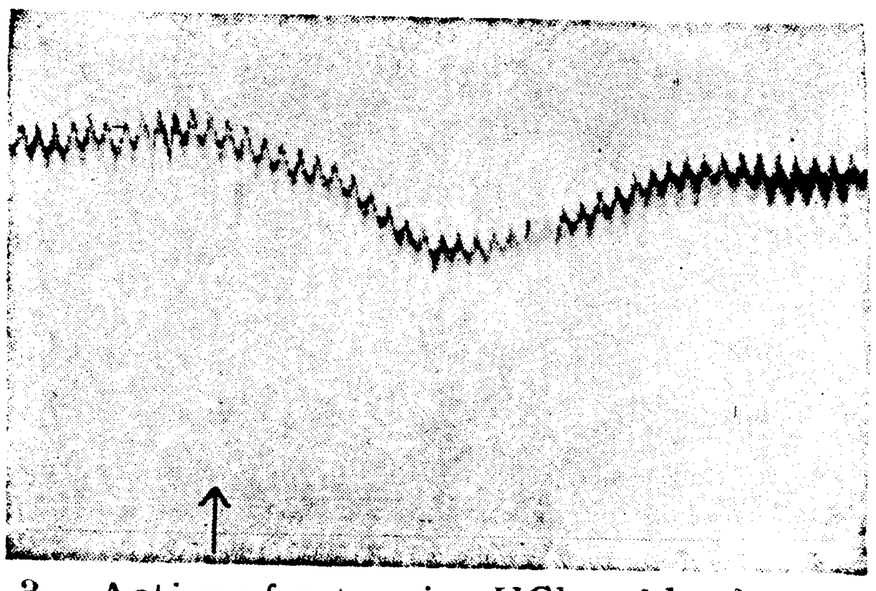

Fig 3 Action of gelsemine HCl on blood pressure.

Cat male 2 11 kg pithed At arrow 7 mg of gelsemine HCl were injected intravenously

3. On isolated intestines. In 13 experiments with isolated rabbit small intestine, gelsemine in the conc ntrations of 1:10,000 and 1:20,000 uniformly diminished the contractions (see fig. 4). Its action was not effected by previous treatment with nicotine or atropine. In one experiment; rgotamine seem d to antagonize its action, but the result from a single experiment should not be taken as conclusive evidence. The inhibition of the intestinal contractions by gelsemine may be then due to the stimulation of the sympathetic endings, or to th depre sion of th smooth muscl s. That barium chloride produces its usual effect (see fig. 4) i in favour of the former view.

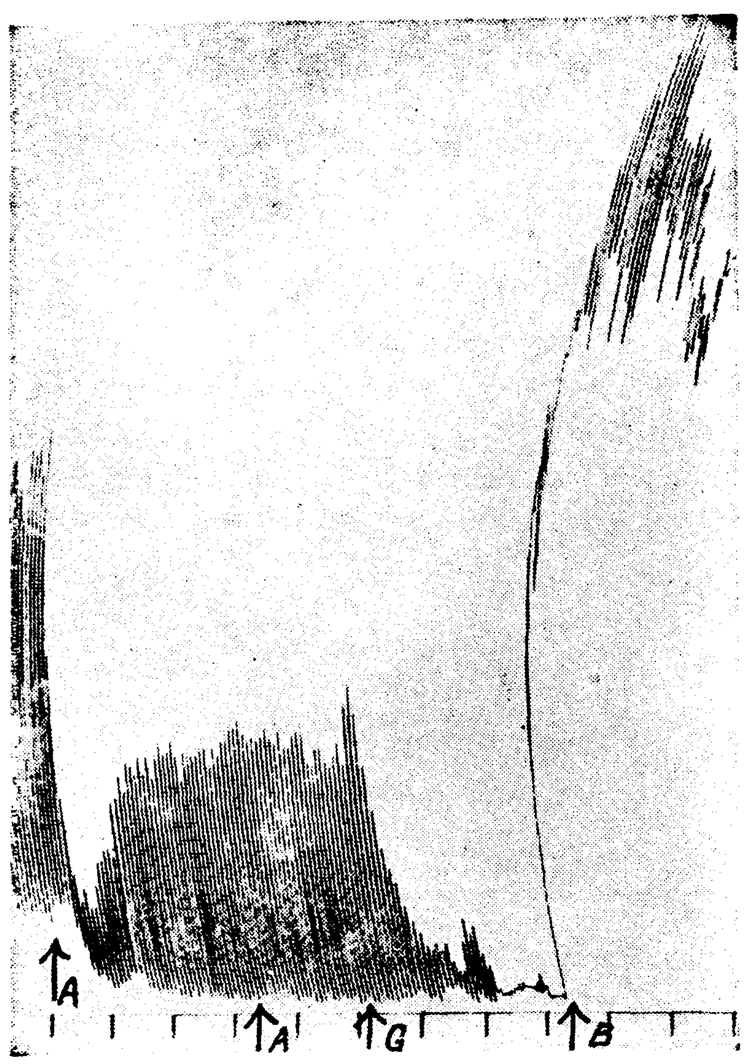

Fig. 4. Action of gelsemine HCl on isolated intestine.

A strip of rabbit's intestine, immersed in 50 cc of Locke's solution maintained at 38°C. At *A*, three drops of 1 per cent solution of atropine sulphate were added. At *G*, 5 mg of gelsemine HCl were added. At *B*, three drops of 10 per cent solution of $BaCl_2$ were added. Neither atropine nor barium altered the action of gelsemine.

4. Isolated uterus. In 5 experiments with isolated rabbit uteri and 3 with isolated guinea-pig uteri, gelsemine increased the contraction moderately (see fig. 5) The results also appear to exclude the possibility that gelsemine depresses smooth muscles.

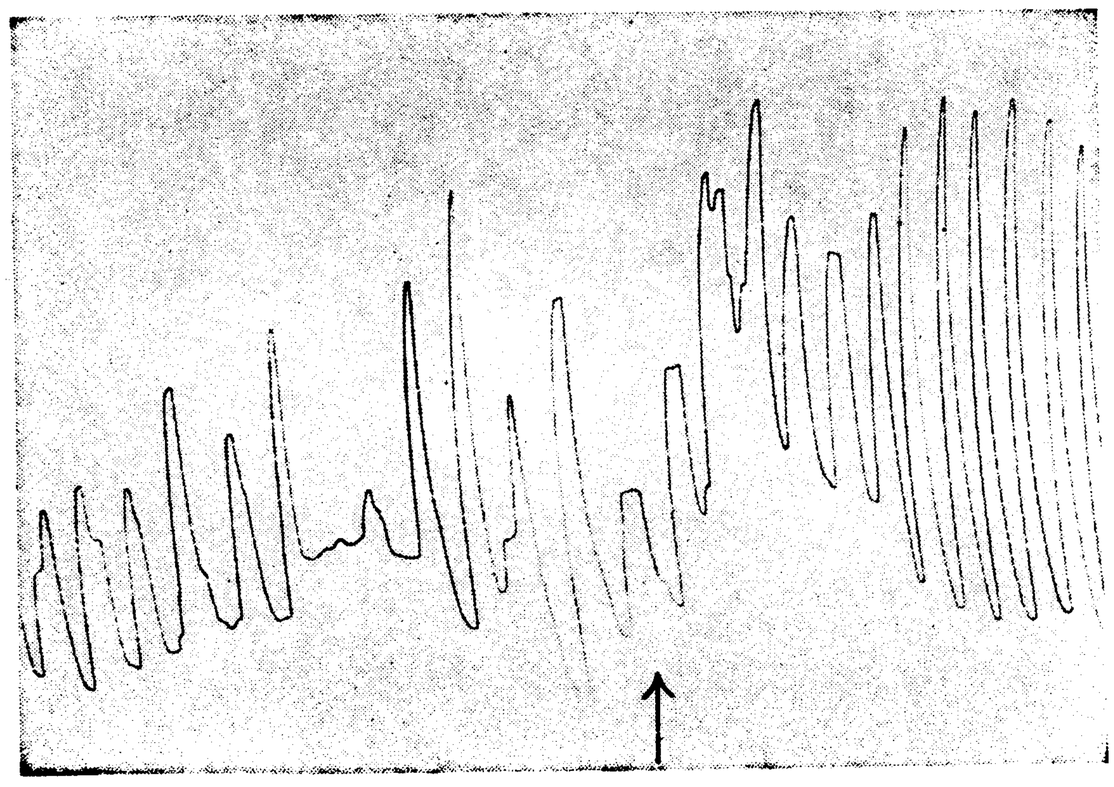

Fig. 5. Action of gelsemine HCl on uterus.

A strip of a virgin guinea-pig's uterus immersed in 50 cc of Locke' solution, maintained at 38°C. At arrow 10 mg of gelsemine HCl were added.

The action of gelsemine on the eye: (By Dr. C. Pak)

Mydriasis was observed in rabbits by local application of a 1 per cent solution of gelsemine HCl into the conjunctival sac. Its action was a purely peripheral one as indicated by the fact that the mydriasis was located only in the eye which received the drug. In one rabbit, the mydriatic effect was compared with that of homatropine by putting 1 drop of 1 per cent solution of gelsemine HCl into one eye and the same dose of homatropine into the other eye. The result was as follows:— With gelsemine HCl, maximal dilatation of the pupil to 3 mm occurred at the end of 3 hours and disappeared 24 hours later. On the other hand, the homatropine mydria is reached its maximum of 3.25 mm within 15 minutes, but lasted only for 8 hours. The mydriatic effect of gelsemine seems, therefore, to be about equal in strength to that of homatropine, but slower in action and longer in duration. The light reflex was incompletely lost. Local anesthesia of the cornea was not observed. Pilocarpin and physostigmine induced slight constriction of the pupil dilated by gels mine.

G l mici : (By Dr. H. C. Hou)

When administered intrav nously, gelsemicine produced the following symptoms in rabbits:— There w s a preliminary quietening down of the animal followed by excitement, weakness of extremities, shallow and slow respiration, convulsions, defecation, urination and salivation, and finally death from respiratory failure. After non-fatal dose, the excitem nt was followed by a period of apparent exhaustion. M. L. D. in the rabbit. was found to be 0.08 mg per kilo. When applied locally to the eye, it dilated the pupil with eventual loss of accommodation.

SUMMARY

From the rhizome and roots of American gelsemium, 2 alkaloids, gelsemine and gelsemicine have been isolated in their pure state. Gelsemicine is a new alkaloid.

Gelsemine has the molecular formula $C_{20}H_{22}O_2N_2$ m.p. 178°C when crystallised from acetone and a specific rotation +10° in chloroform. It is however found to be optically inactive in alcohol.

Gelsemicine has the molecular formula $C_{20}H_{25}O_4N_2$ a specific rotation −140° in alcohol, and crystallises from acetone in orthorhombic prisms melting at 171°C. It forms a monohydrochloride very soluble in water. Gelsemicine is highly potent producing the usual toxic effects of gelsemium in mammals, 0.08 mg of its HCl salt being fatal to rabbits.

A preliminary investigation of the physiological properties of both gelsemine and gelsemicine has been made.

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美國鈎吻之有機鹼質

其一. 鈎吻素甲及鈎吻素乙

趙承嘏

附及關於鈎吻素甲與乙之生理性質之略記

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美國鈎吻之研究已有六十餘年之歷史，而其最重要之有效質素,迄今尙未取出.今作者從美國鈎吻中提出二結晶物,命名爲鈎吻素甲 (gelsemine) 及鈎吻素乙 (gelsemicine). 鈎吻素甲已經前人所得,其藥性不足以代表鈎吻.鈎吻素乙則爲一新發現最重要之有機鹼質

鈎吻素乙之分子式爲$C_{20}H_{25}O_4N_2$,旋轉度爲−140° 熔點爲171°C.其性甚毒.兎之體重每公斤,如注射鈎吻素乙0.08公絲時,即能致死.滴於眼內,能使瞳孔放大.