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CONTRIBUTION TO THE CHEMISTRY OF STYLOPHORUM DIPHYLLUM.

BY J. O. SCHLOTTERBECK AND H. C. WATKINS.[1]

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STYLOPHORUM diphyllum, which is popularly known by the suggestive names yellow poppy and celandine poppy, belongs to the *Papaveraceae*. It grows in low woods from Ohio to Tennessee and westward to Wisconsin and Missouri. It is a perennial, herbaceous plant, with leaves pinnatifid in a manner similar to celandine. The flowers are deep yellow, poppy-like, and about an inch broad. The fruits are ovoid and tipped with the persistent style ; hence, the generic name *Stylophorum*. All parts of the plant exude a yellow juice, when bruised, which matches the color of the corolla. Under the name extra "large golden seal" the root is mentioned[2] as a possible accidental admixture of hydrastis.

Undoubtedly J. U. Lloyd was the first to undertake a chemical examination of this plant, but we have not been able to locate the account of his results. In a letter to us he writes that some twenty years ago his brother, C. G. Lloyd, called his attention to this plant and suggested that he make a chemical examination

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[1] Holder of the F. Stearns & Co. Fellowship in the School of Pharmacy, University of Michigan.

[2] Lloyd : "Drugs and Medicines of North America," 1, 95.

for alkaloids, since its relationship, botanically, indicated their presence. Acting upon this suggestion, Mr. Lloyd extracted a considerable quantity of the root and obtained a large alkaloidal product which he provisionally named stylophorine in order that it might have a place in literature. This product was sent to Professor Eykman, of Tokio, Japan, for critical study since he was at that time especially engaged in studying the constituents of the *Papaveraceae*. We have also failed to find in literature a report by this chemist upon *Stylophorum* or its alkaloids, but that he did concern himself with this subject is shown in an article by Schmidt[1] upon our plant in which he states that the results communicated by Ekyman agreed with his own. These two chemists believe that the principal alkaloid of *Stylophorum diphyllum*, and chelidonine of *Chelidonium majus* are identical.

A comparative examination of stylophorine[2] and chelidonine was made by Schmidt and Selle[3] in 1890. The identity was fully established and in addition they obtained evidence of the existence of two other alkaloids, but the isolation was not made because of scarcity of material. This is probably the extent of the work done on this plant at the time it was taken up by the authors.

Through the kindness of Professor Lloyd we were supplied with 50 pounds of the dried root which was collected by a professional root digger in the woods northeast of Cincinnati, Ohio. Quoting from his letter to us Mr. Lloyd says: "The *Stylophorum* has been collected and worked with the utmost care and no foreign substance is present in it. It was inspected piece by piece and you can use it with confidence."

METHOD OF EXTRACTION.

The drug in No. 20 powder was first thoroughly moistened with about 5 per cent. ammonia water for the purpose of liberating the bases from the acids with which they were combined in the plant, and then spread out in thin layers to dry at ordinary temperature. For the extraction a copper apparatus based upon the Soxhlet principle and of about 25 pounds' capacity was employed. Chloroform was the solvent used and extraction was continued until a portion of the liquid drawn from the bottom of the percolator did not, after the usual manipulation, respond to the

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[1] *Arch. d. Pharm.*, **226**, 622.

[2] While Lloyd applied the name stylophorine to the entire alkaloidal product, Schmidt, Selle, Eykman and others employed it specifically to the most abundant alkaloid.

[3] *Arch. d. Pharm.*, **228**, 96.

test for alkaloids. The chloroform was recovered from the percolate and the waxy, dark-colored residue repeatedly digested with acidulated (acetic acid) water upon the steam-bath until exhausted of its alkaloid. The combined reddish, transparent filtrates contained the total alkaloids as acetates.

The chloroform held by the marc was entirely recovered by passing high pressure steam in at the bottom of the percolator, condensing the mixed vapors of water and chloroform, and separating the two layers. The hot marc was at once percolated with distilled water for the purpose of extracting the soluble salts naturally existing in the drug as well as the ammonium salts of the acids originally combined with the bases. The aqueous percolate was concentrated to small volume, 10 per cent. of alcohol added for preservation, and set aside for later study.

The red solution of the acetates of the bases was made alkaline with ammonia water whereupon a very bulky, grayish precipitate was thrown down. This was collected on a large Buchner filter and thoroughly washed with water. The filtrate was now golden yellow in color instead of red, thus showing that the red color body is precipitated with ammonia. This indicates the presence of a base allied to the red salt-forming alkaloid of *Sanguinaria*, *Chelidonium*, and *Bocconia*. The yellow color of the filtrate also indicates the presence of a color body other than the yellow salt-forming alkaloid found in the allied plants. The yellow filtrate, as well as all filtrates from similar operations as long as they possessed considerable color, was reserved and worked later for the coloring-matter.

The alkaloids were redissolved in a small amount of glacial acetic acid to the usual bright red solution, diluted with water, reprecipitated with ammonia, and washed as before. This operation for preliminary purification was repeated a number of times until the precipitate was nearly white.

ISOLATION OF THE ALKALOIDS.

Successive convenient portions of the alkaloid were dissolved in acid, placed in a separator, about an equal volume of ether added and then ammonia to alkaline reaction. If not too much alkaloid be taken, a moment's vigorous shaking causes complete solution of the bases in the ether, which takes on a bluish fluorescence. Solution is only temporary, however, so that separation and filtration through a tuft of cotton must be made rapidly, to avoid premature crystallization. We have found wide-mouthed Erlenmeyer flasks of different sizes admirably suited for crystallizing since undue loss of ether is avoided and the alkaloid does not creep up the side of the dish. Generally the alkaloid begins to separate from the filtered solution in a very short time and sometimes even before it can be drawn from the separator. After no visible increase in crystallization takes place (generally after several hours). the supernatant ethereal solution, if it be practically colorless, is at once used again to shake out another portion of alkaloid, but if it be colored the ether is recovered by distillation, dried, and then used over again. If after several hours' standing no crystals separated from a colorless or nearly colorless solution, it was concentrated to small volume and again set aside to crystallize. The entire alkaloid was treated in this manner and all the filters and cotton plugs thrown into a waste jar for later recovery. The strictest economy was observed with the alkaloids, so we can say that practically none was lost.

It was noticed that crystals of two distinct forms would separate, sometimes both together, sometimes one form alone, and then again the other. The predominating alkaloid separated in the form of monoclinic prisms (Fig. 1), while the second crystallized in needles (Fig. 2). By close watching, these two forms could be completely separated by fractional crystallization. The different fractions of the former alkaloid melted from 130°–134° and of the latter from 197°–200°, both of course in the unpurified state.

By a continuous repetition of the above process of dissolving in acetic acid, precipitating with ammonia and shaking out with ether extending over a period of fully three months, the entire alkaloidal product was worked up. All fractions of the prisms were united as well as those of the needles.

A very interesting and peculiar property of the principal alkaloid, which we shall see later is identical with chelidonine, was accidentally discovered while scraping a large fraction of crystals from the sides of the crystallizing flask with a glass rod. As the rod rubbed against the crystals the faces seemed to reflect light to an unusual extent; consequently, the phenomenon of "triboluminescence"[1] suggested itself. The experiment was repeated in a perfectly dark room with greater success, an intense light being

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[1] *Ann. Phys.* (Wied.), **34**, 446; *Ber. d. chem. Ges.*, **34**, 1820.

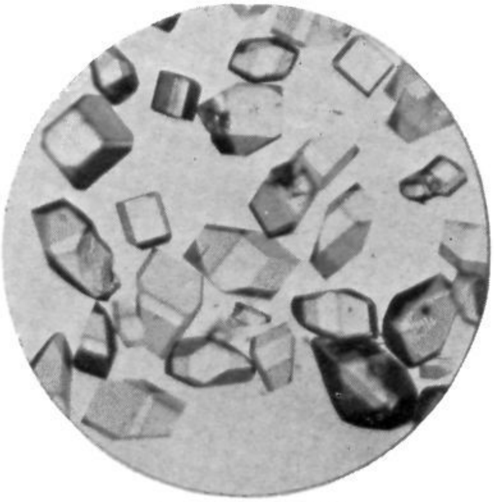

Fig. 1.—Chelidonine.
$C_{20}H_{19}NO_5.H_2O$.

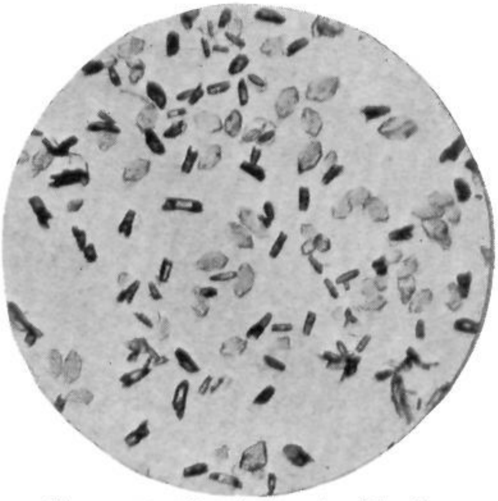

Fig. 2.—Chelidonine Hydrochloride.
$C_{20}H_{19}NO_5.HCl$.

Fig. 3.—Stylopine.
$C_{19}H_{19}NO_5$.

Fig 4.—Stylopine Hydrochloride.
$C_{19}H_{19}NO_5.HCl$.

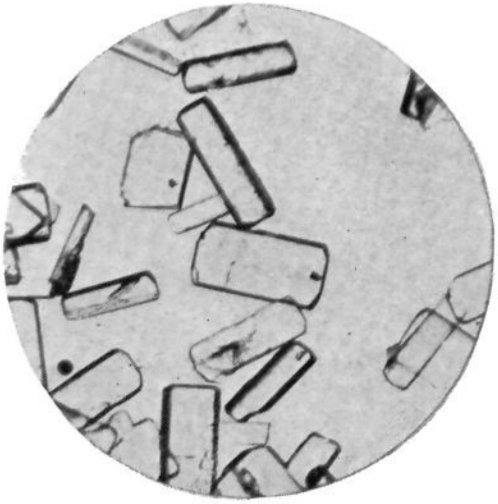

Fig. 5.—Protopine.
$C_{20}H_{19}NO_5$.

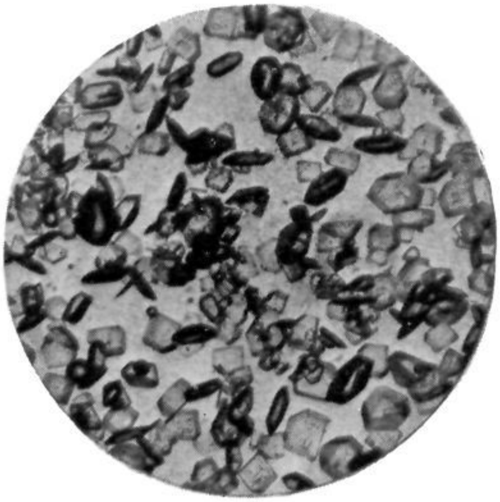

Fig. 6.—Diphylline.

emitted as the crystals were crushed. This is in some way connected with the crackling or snapping sound which we often heard coming from flasks in which very active crystallization was taking place. Whether an actual break in the crystals took place we cannot say. A solution in which an active growth of crystals was taking place was set in a dark room and watched for some time. Each crackling sound spoken of was accompanied by a feeble spark. When a flask containing large crystals was placed in the palm of the hand, the body heat was sufficient to produce the sound as well as the light. The same result was obtained when a flask of crystals was given a rotary motion. If a flask containing dry crystals be moderately shaken, the impact of the crystals against the side of the flask produced hundreds of intensely bluish white, scintillating sparks. These were best obtained with dry crystals from ether, not so well with those from chloroform or alcohol, or from mixtures of them. Covered with water, ether, or benzene the effect is produced equally well. By shaking a flask of dry crystals close to the face of a sensitive plate covered by a negative, a positive was obtained with a very short exposure. Other experiments are under way and will be reported upon later, but we are of the opinion that electricity of cleavage is the phenomenon manifested. Since of all the alkaloids of *Stylophorum* this one alone possessed this feature, it served as an excellent identifying characteristic in the isolation.

In the later mother ethereal liquids, three other alkaloids were separated by a very tedious and monotonous routine of fractional crystallization. These five alkaloids, all of which were obtained by the above method of isolation with ether as a solvent, we shall designate by the numerals I, II, III, IV, V. Alkaloid V was the last to be isolated because of its extreme solubility in ether, but its identity was practically established early in the process as was stated above.

ALKALOID I.

By far the greater portion of the total basic product consists of this alkaloid. Purification was effected as follows: The total product was dissolved in dilute sulphuric acid and treated with an excess of strong hydrochloric acid. The salt began to separate in a very short time in the form of a coarsely crystalline powder. After collecting on a filter it was dissolved in boiling water, rapidly filtered through a tuft of cotton, the solution cooled with ice-water and stirred to granulation. This was repeated at least ten times or until the hydrochloride became white. The combined filtrates were treated in a similar manner. A portion of the hydrochloride dissolved in water, converted into a free base and shaken out with ether, soon yielded crystals that melted at 136° (uncorr.). The remainder of the salt was so treated, and then recrystallized several times from hot alcohol and finally from a mixture of chloroform and alcohol. From the latter mixture large crystals fully $\frac{1}{4}$-inch long were easily obtained which melted at exactly the same point as before. From its melting-point, crystalline form, and behavior with solvents there is no question in our minds that this, the principal alkaloid of *Stylophorum*, is chelidonine.

Chelidonine has as yet been found in only one other plant, *viz.*, *Chelidonium majus*. It was first isolated by Godefroy[1] but in an impure state. Probst[2] was the first to obtain it pure and to study some of its properties. At about the same time Reuling[3] published a short article upon this base but he did not add much to the existing knowledge. A few years later Polex[4] concerned himself with the alkaloid and obtained it pure. Eykman[5] contributed an article upon chelidonine but it was separated from *Cheldonium majus* and not from *Stylophorum diphyllum*. He did not arrive at definite conclusions regarding the composition, however. Henschke[6] made a close study of chelidonine, likewise from *Chelidonium*. Schmidt and Selle[7] are the only ones who have published accounts of work done upon the alkaloids from *Stylophorum*.

There exists in literature considerable discrepancy regarding the composition of this alkaloid. Will[8] made combustions and calculated the formula $C_{40}H_{40}N_6O_6$ an expression that was variously interpreted as follows: Gerhardt[9] $C_{40}H_{19}N_3O_6$, Gmelin[10] $C_{42}H_{19}N_3O_6$, Löwig[11] $C_{40}H_{20}N_3O_6$, Limpricht[12] $C_{19}H_{17}N_3O_3$. Henschke, and Schmidt and Selle succeeded in obtaining concordant results and

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[1] *J. de Pharm.*, Dec., 1824.

[2] *Ann. Chem.* (Liebig), **29**, 113.

[3] *Ibid.*, **29**, 131.

[4] *Arch. d. Pharm.*, **16**, 77.

[5] *Rec. trav. chim. Pays-Bas*, **3**, 190.

[6] *Arch. d. Pharm.*, **226**, 624.

[7] *Ibid.*, **228**, 96.

[8] *Ann. Chem.* (Liebig), **35**, 113.

[9] *Traité* **4**, 210.

[10] Handbuch, IV, 1534.

[11] Lehrbuch, 1846.

[12] Lehrbuch, 1862.

the formula $C_{20}H_{19}NO_5.H_2O$. We believe that much of the difficulty encountered in the determination of the composition of this base rests in the fact that purification is effected with difficulty.

Having prepared a considerable quantity of chelidonine from *Stylophorum*, we took up its study mainly for the purpose of verifying the very latest results upon its composition.

For the determination of water of crystallization a small quantity of the freshly crystallized alkaloid was heated in an air-bath at a temperature of 100° to constant weight. Henschke used heat of 125° C., but at this temperature our product became gradually darker. Since neither Henschke nor Selle make mention of this in their report we were of the impression that our product must still be contaminated with impurities, therefore, the colorless alkaloid was recrystallized twice from alcohol and again heated but with the same result. Then a lower heat was tried and sufficiently prolonged to cause an appreciable loss of water, but again with the same change in color. Fearing that this coloration indicated a slight decomposition and therefore would vitiate results, an attempt was made to remove the water in a vacuum desiccator over sulphuric acid and phosphoric anhydride until the substance lost no weight.

A vacuum was maintained (with interruptions for weighing) for fully a month and it was noticed as increasing quantities of water were removed the alkaloid became more yellow. Unfortunately we could not complete the experiment because of an accident but it was plainly evident that with removal of water of crystallization even in the cold, discoloration takes place. Since light seems to hasten the change, there is in progress at this time an experiment to determine whether all the water is removed and also whether discoloration takes place in the dark.

A sample heated to constant weight at 100° for about fourteen hours lost 4.5 per cent. of its weight. According to the formula $C_{20}H_{19}NO_5.H_2O$, the theoretical amount of water is 4.8 per cent.

Several combustions were made with the air-dried material as well as with that dried to constant weight but the results were not as concordant as was desired and were therefore rejected. Excellent results were obtained with the nitrate which is easily made pure and which contains no water of crystallization. In the estimation of the acid in the hydrochloride by means of silver nitrate the filtrate from the precipitated silver chloride separated large needles of the nitrate of chelidonine. After recrystallizing several times from hot water and drying in a desiccator, the following results of the combustions were obtained :

I. 0.333 gram chelidonine nitrate gave 0.7044 gram carbon dioxide and 0.1524 gram water.

II. 0.350 gram chelidonine nitrate gave 0.1532 gram water.

III. 0.317 gram chelidonine nitrate gave 0.6738 gram carbon dioxide and 0.1354 gram water.

	Calculated for $C_{20}H_{19}NO_5.HNO_3$.	Found.		
		I.	II.	III.
Carbon	57.69	57.69	....	57.63
Hydrogen	4.84	5.08	4.86	4.74

Nitrogen was determined by the Dumas as well as by the Kjeldahl method. By the former method the following results were obtained :

I. 0.4926 gram of anhydrous chelidonine gave 0.0211 gram of nitrogen, or 4.27 per cent.

II. 0.551 gram of anhydrous chelidonine gave 0.022 gram of nitrogen, or 3.97 per cent.

Theory requires 3.96 per cent.

By the latter method the following results were obtained :

I. 0.412 gram of chelidonine gave 3.33 per cent. of nitrogen.

II. 0.486 gram of chelidonine gave 3.40 per cent. of nitrogen.

Theory requires 3.77 per cent.

The hydrochloride was prepared and purified as stated above. Air-dried material heated in an oven for several hours did not lose weight; therefore there is no water of crystallization. The acid was determined in the customary way with silver nitrate and weighing the washed and dried silver chloride.

I. 0.455 gram of chelidonine hydrochloride gave 0.1656 gram silver chloride.

II. 0.3406 gram of chelidonine hydrochloride gave 0.1264 gram silver chloride.

	Calculated for $C_{20}H_{19}NO_5.HCl$.	Found.	
		I.	II.
Hydrochloric acid	9.36	9.22	9.4

The analyses of the gold and platinum double salts verified the formula obtained from combustions of the nitrate. The gold salt was readily made by precipitating a hot acid solution of the hydrochloride of chelidonine with an excess of a 2 per cent. solution of gold chloride. The double salt separated as a voluminous, orange-red precipitate which was collected on a filter, thoroughly washed and dissolved in hot alcohol. Beautiful violet-red crystals bunched in rosettes separated in a short time and these were recrystallized and dried in a desiccator. Heating in an oven at 100° for several hours did not cause it to lose weight.

I. 0.0774 gram of the gold salt yielded, upon incineration, 0.022 gram gold.

	Calculated for $C_{20}H_{19}NO_5.HCl.AuCl_3$.	Found.
Gold	28.1	28.42

The platinum salt was made in the same manner, using a 5 per cent. solution of platinum chloride. The double salt separated as a yellowish precipitate which became denser and deeper colored when reprecipitated for purification. We did not succeed in obtaining crystals of this compound from ethyl or methyl alcohol. One small fraction that had been standing in methyl alcohol for several days had changed to fine silky needles but we were not able to repeat the operation. Heating to 100° to constant weight removes 3.4 per cent. water.

I. 0.136 gram of the water-free double salt left, upon incineration, 0.0236 gram platinum.

	Calculated for $(C_{20}H_{19}NO_5.HCl)_2PtCl_4.H_2O$.	Found.
Platinum	17.45	17.4
Water	3.1	3.4

Action of Ethyl Iodide.—Five grams of pure dried material were heated in a bomb tube with an excess of pure ethyl iodide at 130°–140° for four hours. When cold, the tube was opened and only a slight pressure noticed. In the bottom of the tube there was an insoluble portion yellowish in color and above it a transparent, light red liquid. The excess of ethyl iodide was driven off and the residue dissolved in boiling alcohol. It was filtered and set aside in a small Erlenmeyer flask. While crystals began to appear after a considerable length of time we found that layering with an equal volume of ether as employed by Henschke hastened crystallization materially. Tufts of fine, silky needles formed on the side of the flask and at the contact zone of the two liquids. Successive crops were obtained and all the combined fractions recrystallized from the same solvents several times but with loss of considerable material. Enough was left, however, to determine the iodine content with silver nitrate. The new compound was found to be free of water of crystallization.

I. 0.2038 gram of the compound gave 0.0948 gram silver iodide.

	Calculated for $C_{20}H_{19}NO_5.C_2H_5I$.	Found.
Iodine	24.94	24.8

Long contact of potassium hydroxide with a solution of this compound had no effect since the same compound with identical melting-point was again obtained in almost quantitative amount. This alkaloid is probably a tertiary base, as was shown by Henschke.

For the determination of methoxyl groups a modification of Zeissel's well-known method was employed, using ground-glass joints throughout the entire apparatus. After carrying on the operation for fully an hour not the least turbidity was noticed in the silver nitrate solution. Methoxyl groups are therefore absent.

By pouring a solution of the sulphate of chelidonine into a large excess of Wagner's reagent, an abundant chocolate-colored precipitate formed which, upon shaking vigorously, became denser and settled to the bottom. The collected precipitate was thoroughly washed and then dissolved in hot methyl alcohol and set aside in flat crystallization dishes. Two distinct forms separated, one in light red needles and the other in almost black prisms. These were separated mechanically and each recrystallized several times from hot methyl alcohol. When dried in a desiccator and the iodine estimated with thiosulphate solution, the light red crystals gave figures that agree with the formula for the triiodide $C_{20}H_{19}NO_5.HI.I_3$. The black prisms appear to have the formula $C_{20}H_{19}NO_5.HI.I_5$.

Finally a solution of the free alkaloid chelidonine, dissolved in absolute alcohol, gave an optical rotation of $[\alpha]_D = +115°24'$.

ALKALOID II.

This one is second in abundance and crystallizes in distinct needles as shown in Fig. 2. The needles which Selle obtained in such small quantity that only the melting-point (193°–195°)[1] could be made are probably the same substance. Since we have not found in literature anywhere a description of an alkaloid possessing the properties of this one we have decided to designate it by the name stylopine. All the fractions of the needles melting in the neighborhood of 200° were united and purified in the same manner employed with chelidonine. A pure product was obtained that possessed the constant melting-point of 202° (uncorr.).

This alkaloid is almost insoluble in hydrochloric acid, forming fine needles of the salt when strong hydrochloric acid is added to a

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[1] *Arch. d. Pharm.*, **228**, 108.

solution of the acetate. It is also insoluble in dilute sulphuric acid which serves excellently as a means of separation from chelidonine. The free alkaloid is very soluble in glacial acetic acid, much less so in dilute acid. The nitrate separates from aqueous solutions in very small clusters of needles which in mass appear almost jelly-like, or gelatinous.

Precipitates were obtained with well-known reagents as follows:

Tannic acid	slight white.
Potassium bismuth iodide	light yellow flocculent.
Potassium cadmium iodide	white.
Bromine water	yellow.
Phosphotungstic acid	white.
Potassium iodide	white needles.
Phosphomolybdic acid	dirty white.
Gold chloride	salmon to yellow ochre.
Platinum chloride	white to pale yellow.
Picric acid	deep lemon yellow.
Potassium dichromate	yellow.
Wagner's reagent	chocolate-brown.

The free alkaloid contains no water of crystallization. Combustions of the pure dry substance gave the following results:

I. 0.1926 gram stylopine gave 0.4712 gram carbon dioxide and 0.0958 gram water.

II. 0.1718 gram stylopine gave 0.0864 gram water.

III. 0.1704 gram stylopine gave 0.4206 gram carbon dioxide and 0.0854 gram water.

IV. 0.2714 gram stylopine gave 0.6680 gram carbon dioxide.

Four nitrogen determinations gave the following results:

I. 0.1768 gram stylopine gave 0.007478 gram nitrogen.

II. 0.177 gram stylopine gave 0.007292 gram nitrogen.

III. 0.1824 gram stylopine gave 0.00698 gram nitrogen.

IV. 0.1316 gram stylopine gave 0.005578 gram nitrogen.

	Calculated for $C_{19}H_{19}NO_5$.	Found.				
		I.	II.	III.	IV.	Average.
Carbon	66.83	66.72	....	66.78	67.1	66.86
Hydrogen	5.54	5.52	5.58	5.57	....	5.55
Nitrogen	4.10	4.23	4.12	3.83	4.24	4.10
Oxygen	23.46	....	....	....	....	23.49

The hydrochloride was made in the usual manner and purified by recrystallization from hot water. It contains no water of crystallization. For the determination of the formula of this salt the acid was estimated by precipitating with silver nitrate and weighing the washed and dried silver chloride. Only one estimation was made.

I. 0.0524 gram of the hydrochloride of stylopine gave 0.020 gram silver chloride or 9.7 per cent. hydrochloric acid.

	Calculated for $C_{19}H_{19}NO_5.HCl$.	Found.
Hydrochloric acid	9.65	9.7

The platinum salt was made by precipitating a hot acid solution of the hydrochloride with a 5 per cent. solution of platinum chloride. The precipitate was light yellow at first but became darker after a second precipitation for purification. It contains no water of crystallization.

I. 0.0406 gram of the platinum salt gave, upon incineration, 0.0074 gram platinum.

II. 0.015 gram of the platinum salt gave, upon incineration, 0.0028 gram platinum.

	Calculated for	Found.	
	$(C_{19}H_{19}NO_5.HCl)_2.PtCl_4$.	I.	II.
Platinum	17.7	18.2	17.9

Action of Ethyl Iodide.—Two grams of the pure alkaloid were heated in a hard-glass tube with an excess of freshly prepared ethyl iodide at 130° for about two hours. Even in the cold the change begins to take place, but heating is required to complete it. The new compound in the tube became slightly yellow in color and was decidedly more voluminous than the original alkaloid. After cooling, the tube was opened and the excess of ethyl iodide removed by distillation. The residue was then dissolved in boiling alcohol and when cold layered with ether and set aside as before. The successive small crops were all united and again dissolved in alcohol and set aside as before. The new compound, consisting of small clusters of fine needles, melts at 255°. The iodine content of this compound was determined in the usual manner.

I. 0.1062 gram of the double salt gave 0.0486 gram silver iodide.

II. 0.1086 gram of the double salt gave 0.050 gram silver iodide.

	Calculated for	Found.	
	$C_{19}H_{19}NO_5.C_2H_5I$.	I.	II.
Iodine	25.5	24.7	24.8

Strong potassium hydroxide is absolutely without action upon this compound. We have here probably a tertiary base, although the proof is not completed.

A modification of Zeissel's method was employed for the determination of methoxyl groups but not the least turbidity was noticed in the silver nitrate solution; hence, we must conclude that these groups do not exist in this base.

For the determination of hydroxyl groups a small quantity was heated in a flask with acetic anhydride and sodium acetate under a reflux condenser for several hours. A slight change in color of the solution took place. When the liquid was poured into cold water many small, oily drops separated which, after a time, collected on the sides of the dish in the form of a varnish which possessed a disagreeable odor. Repeated trials to secure crystals from this varnish were without results and as the material was so small in quantity, we abandoned this part of the work until a larger quantity is available. Crystalline periodides are easily obtained by the usual method but here, too, because of scarcity of material, no analyses could be made.

Potassium iodide throws down a white precipitate of fine needles from a solution of stylopine in acetic acid. Not enough material was at hand to make duplicate determinations of the iodine but the probabilities are that the compound has the formula $C_{19}H_{19}NO_5.HI$.

The pure alkaloid dissolved in absolute alcohol rotates polarized light $[\alpha]_D = -315°12'$.

ALKALOID III.

This alkaloid is probably third in abundance in the plant but only a small portion was obtained from the original total product for the reason, as we shall see later, that it is to some extent soluble in ammonia. In the separation of the two alkaloids already described, this one made its appearance in only one or two fractions in an almost uncontaminated condition. Small white warts, and colorless transparent prisms were about equally abundant in these fractions. Protopine was at once suspected, and from the melting-point of 203° in the unpurified condition and its characteristic color reaction with sulphuric acid and Erdmann's reagent our suspicions were practically confirmed.

It will be recalled that the alkaline aqueous liquids that had been washed with ether for the removal of alkaloids were reserved as long as they possessed a yellow color. After acidulating this liquid with acetic acid and concentrating to smaller volume it was found that a curdy precipitate was thrown down with potassium hydroxide. This was shaken with ether and the dissolved alkaloid filtered into a flask and set aside. In a very short time both warts and prisms separated which turned out to be protopine. All fractions of this alkaloid were united and purified in the usual manner and finally recrystallized from a mixture of alcohol and chloroform. The rather large prisms of protopine melted at 204°–205° (uncorr.).

To make doubly certain that this alkaloid was protopine one combustion was made.

0.4203 gram alkaloid gave 0.0467 gram carbon dioxide, and 0.196 gram water.

	Calculated for $C_{20}H_{19}NO_5$.	Found.
Carbon	67.98	67.92
Hydrogen	5.38	5.23

It contains no methoxyl groups as determined by Zeissel's method. A periodide was obtained which crystallized in beautiful rosettes of a wine-red color. The formula was not determined because of lack of sufficient material.

ALKALOID IV.

This base is present in very small quantity and was obtained with great difficulty during the long course of fractional crystallization. It generally separated out along with chelidonine but the relative quantity was so small that complete separation consumed nearly as much time as the isolation of all the other alkaloids. The mixed fractions of chelidonine and this alkaloid were dissolved in acetic acid, diluted with water, placed in a separator, made alkaline with ammonia and shaken out with ether. After filtering into a flask, close watch was kept of the progress of crystallization and as soon as a small quantity of crystals had separated the ether was decanted into another flask, and just as carefully watched for the appearance of further crystals. This was repeated over and over again until about twenty-five small fractions were obtained that melted in the neighborhood of 210°. These were combined and the process of purification fairly begun when we lost about two-thirds of the product by an accident. Enough was saved, however, to observe some of its properties. The perfectly pure alkaloid melts at 216° (uncorr.). It crystallizes at times in extremely thin plates, and from their position on the side of the flask reminded us of the wings of butterflies. The most common form is shown in Fig. 6.

The acetic acid solution behaved with the usual alkaloidal reagents practically in the same manner as stylopine with the exception that the hydriodide formed by the addition of potassium iodide solution is amorphous instead of crystalline.

Color tests were made as follows:

A few drops of a solution of the free alkaloid were placed into each of the concave depressions of a white porcelain testing plate, and evaporated on the water-bath to dryness. In this way a very thin film of the alkaloid was distributed over the surface of the depression. A drop of the reagent was let fall into the cavity and brought in contact with the alkaloid.

Nitric acid................	slowly dirty yellow, then passing through violet, wine-red, dull green, and finally reddish-brown.
Marquis' reagent..........	first violet-blue then wine-red.
Erdmann's reagent........	yellowish green to bright green.
Froehde's reagent.........	deep green, olive-green bordered with blue, finally all becoming blue.

Since this alkaloid seems not to have been found before, we have named it diphylline.

ALKALOID V.

In acid solutions this alkaloid is bright red in color, but is precipitated pure white with alkalies. The free alkaloid is extremely soluble in ether, more so than any of the others, which accounts for the fact that it is the last to crystallize out. The bluish fluorescent ethereal liquid, from which the four alkaloids mentioned had been eliminated because of their insolubility in ether, was treated with dry hydrochloric acid gas in a wash-bottle for an hour. The bright red salt collected on the exit tube, and on the bottom and side of the bottle. The ether was decanted and the red salt dissolved in hot water, cooled to just short of the crystallizing point, made alkaline with ammonia water and shaken out with ether. The filtered ethereal solution was set aside in a vacuum desiccator, over night. By morning almost white clusters of plates had separated that on exposure to air soon began to turn red. Not over 0.3 gram was obtained so that no analyses were attempted. This is without question the alkaloid which Schmidt and his pupils have named sanguinarine. From historical as well as etymological reasons it should be called chelerythrine, but we shall not add to the existing confusion by applying the names differently.

Organic Acids.—The organic acids which were in combination with the alkaloids in the original drug are now contained in the aqueous percolate of the marc in the form of ammonium salts. By the addition of strong alcohol most of the extraneous matter is removed in the form of a sticky mass. Convenient portions of the percolate were treated in a 5 liter flask with successive portions of 96 per cent. alcohol until the precipitate which formed balled together into a gummy mass, upon vigorous shaking, leaving the supernatant liquid light colored and almost transparent. The latter was filtered into a 20 liter ice-jar and set aside in a cool place. After a few hours very light, fluffy, voluminous hemispherical masses of fine needles began to separate on the side of the jar. By adding more alcohol from time to time these masses grew enormously and the solution finally became practically exhausted of this crystalline substance. The liquid was siphoned off and the crystals removed with a spatula, and what at first appeared to be a large yield was compressed to small volume. These crystals were found to consist of a potassium compound extremely soluble in water, practically insoluble in alcohol. No odor of ammonia could be detected when a portion was boiled with strong potassium hydroxide.

Several methods for the purification of this compound were tried but the following was found to be the most satisfactory. Since this compound forms an insoluble lead salt the aqueous solution of the entire yield was precipitated with a solution of lead acetate. The white granular precipitate was collected on a filter and washed until the filtrate scarcely gave a test for lead. The lead salt was then suspended in water and decomposed with hydrogen sulphide. After warming slightly, the lead sulphide was removed by filtration and the filtrate which contained the free acid evaporated slightly to remove excess of hydrogen sulphide. To this solution precipitated calcium carbonate was added until there was no more effervescence, the whole being kept hot. The nearly colorless filtrate was set aside and at the end of several hours a magma of fine needles of the calcium salt had separated. By recrystallizing several times from hot water the salt was obtained nearly pure white. The latter was brought into aqueous solution again and treated with a slight excess of the silver nitrate solution, which threw down pure white needles of silver salt. This was collected on a filter, slightly washed, dissolved in hot water and then treated with an excess of hydrochloric acid. The filtrate upon cooling separated a mass of silky needles of the free acid. Several recrystallizations furnished a pure product. It is very sour and forms salts with a number of bases.

Since *Stylophorum diphyllum* is so closely related to *Chelidonium majus*, botanically, we suspected that this acid is the same as the one found in the latter plant and named chelidonic acid by Probst. The characteristic yellow color of the potassium salt of chelihydronic acid, obtained when potassium hydroxide is added in excess to a solution of chelidonic acid, is also produced with our acid. A combustion was made of the desiccator-dried material, and the following results were obtained :

0.246 gram of the acid gave 0.3748 carbon dioxide and 0.0658 gram water.

	Calculated for $C_7H_4O_6.H_2O$.	Found.
Carbon	41.58	41.55
Hydrogen	2.97	2.97

Since chelidonic acid has been so thoroughly investigated by Lerch[1] and others, we have contented ourselves with mere identification.

The alcoholic liquid that had been deprived of potassium chelidonate was distilled *in vacuo* for the recovery of the alcohol and the aqueous residue remaining concentrated to small volume. The inert matter was removed with alcohol as before. The filtrate was set aside and after many days fine needles separated and projected from the side of the jar. These were removed, purified and found to be the ammonium salt of chelidonic acid.

Coloring-Matter.—As was stated above the filtered alkaline aqueous liquids, from which the alkaloids had been washed by means of ether, were reserved for the purpose of isolating the coloring-matter. The greater part of the protopine (alkaloid III) was recovered from this solution. The liquid still retained its full depth of color and was shaken out with several liberal portions of acetone. The deeply colored acetone solution was filtered and the acetone removed by distillation. The dark, bitter residue was taken up with a small quantity of water and treated with a solution of lead acetate. The precipitate was collected and then boiled with water which took out considerable of the coloring-matter. From the filtrate after concentration there separated, after several days, small crystals of a yellowish-red color which, when dissolved in water, imparted to it the color of the original solution. From analogy we believe that this body is the same as Probst's chelidoxanthin but the quantity was too small for further study. Some interesting questions have presented themselves in connec-

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[1] *Monatsh. Chem.*, **29**, 131.

tion with this substance which we hope to be able to answer in the near future.

SUMMARY.

Stylophorum diphyllum contains at least five alkaloids as follows:

Chelidonine	$C_{20}H_{19}NO_5.H_2O$,	melting-point	136°	(uncorr.).
Stylopine	$C_{19}H_{19}NO_5$	"	202°	"
Protopine	$C_{20}H_{19}NO_5$	"	204°-205°	"
Diphylline		"	216°	"
Sanguinarine				
Chelidonic acid	$C_7H_4O_6 + H_2O$			
Chelidoxanthin	(?)			

The study of this plant and its constituents will be prosecuted during the coming year, Professor Lloyd having again arranged for the collection of a large quantity of authentic material. We take pleasure in extending to Professor Lloyd our sincere thanks for this assistance without which the investigation would not have been possible.

We also take this opportunity of expressing our appreciation of the generosity of Messrs. F. Stearns & Co., for furnishing the funds necessary for the promotion of the work.

SCHOOL OF PHARMACY, UNIVERSITY OF
MICHIGAN, ANN ARBOR, MICH.

THE DISCOVERY OF NITROGLYCERINE IN AN EXHUMED BODY.

BY G. G. POND.

Received October 1, 1901.

THE writer has not been able to find any record of the detection of nitroglycerine in a dead body, in cases of suspected poisoning, though this substance is included in the accepted complete schemes for toxicological examination of human organs.

On the fourteenth of March, 1901, the stomach, liver, kidneys, and spleen of a woman of twenty-two years were brought to this laboratory for examination, direct from an autopsy which had been made on that date. This woman had died on the tenth of January previous, in convulsions supposed to have been brought about by the use of instruments, or by drugs or medicines administered with intent to produce abortion. The woman had been ill for only twenty-four hours, and during this time nothing had been administered by the attending physicians through the mouth ; small doses of morphia had been given hypodermically.