

loids with equal volumes of chloroform¹ until extraction is complete testing the final extracted residue with Mayer's reagent. Eight or more extractions are usually necessary. Each portion of chloroform extract is successively washed in another separatory funnel with 10 to 15 cc. of water and drawn off into a beaker or preferably into a 200-cc. Erlenmeyer flask and the combined extracts evaporated on the steam-bath using air blast. By evaporating the first four or five extracts, while the remaining extractions are being made, the process can be materially hastened.

Take up the alkaloidal residue in a few cc. of neutral alcohol warming on the steam-bath to insure complete solution, add 10 cc. *N*/10 sulphuric acid, dilute with about 20 cc. water and titrate back the excess acid with *N*/50 alkali using methyl red as indicator. One cc. *N*/10 acid = 36.4 mg. total alkaloid.

Tinctures.—Use 125 cc. sample, 2 cc. *N* acid and evaporate in a beaker and transfer into a volumetric flask as described in footnote 1.

Powdered Extracts.—These preparations require special treatment for their analysis. While it is possible to prepare fluid extracts from known weights of these solid extracts and then treat these solutions (for assay) in the manner described for the assay of fluid extracts and tinctures, this form of nux vomica preparation lends itself much more readily to analysis by means of an automatic extractor which has been developed in this laboratory. This device is equally serviceable for the other products of nux vomica and its description and application will follow in a later publication.

SUMMARY.

The steps involved in the extraction, purification, and determination of the total alkaloids in nux vomica preparations have been studied experimentally with the view to a simplification of the method of assay.

The following conclusions have been reached: (a) the extreme slowness with which the last portions of alkaloid are extracted by the solvent is probably due to the presence of some alkaloid, other than strychnine or brucine; (b) the extraction can be facilitated by careful control of the ammonia used; and (c) the essential purification can be effected by a simple preliminary precipitation. The latter step incidentally reduces the tendency for emulsion.

A shorter method is described and tables showing comparative results so obtained are given.

THE ISOLATION AND PROPERTIES OF THE ALKALOIDS AND OIL OF LARKSPUR SEED (*DELPHINIUM CONSOLIDIDA*).

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The present investigation was undertaken for the purpose of determining the insecticidal constituents of larkspur seed (*Delphinium consolida*) and of proprietary preparations made therefrom.

¹ Troublesome emulsions are rarely encountered after this preliminary purification but when they do occur the use of alcohol with the chloroform will be found advantageous and if not used in greater proportion than volume of CHCl_3 this will not vitiate the results as shown in Table III(b).

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Plants of the genus *Delphinium* have been investigated chemically by a number of workers,¹ but only two investigations have been made on the seed of the species *consolida*. In 1839, T. C. Hopkins (9)² described his methods and results of a chemical analysis of this seed. He found eight classes of substances, among them being a fixed oil and a bitter substance presumed to be identical with a vegetable alkaloid called "delphia" which had previously been found in the seed of *D. staphisagria*. The oil, colored green by chlorophyll, was obtained by extracting the seed with ether. To obtain the alkaloidal portion the seed was boiled with dilute sulfuric acid. The gums and other matter were removed with lead acetate solution, the excess of lead salt being removed with hydrogen sulfide, and magnesia was then added to the filtrate, thus precipitating the alkaloids. These were separated by filtration and dissolved in boiling alcohol from which they were deposited on evaporation. This crude product, from its bitterness, alkalinity, and physiological effects, was demonstrated to be alkaloidal in nature. Not much attempt was made to purify the products found and all that can be said in regard to the fixed oil and the alkaloidal substance is that their presence was demonstrated.

The second and only other investigation on the seed of *D. consolida* was made by O. Keller in 1910 (13). He treated the seed with 95% alcohol containing 0.5% hydrogen chloride. The alcohol was distilled off and water added to the residue so long as a turbidity resulted. The fatty impurities thus precipitated were removed by filtration and the acid filtrate containing the alkaloids was shaken with a chloroform-ether mixture to effect further removal of impurities. The solution was then made alkaline with sodium hydroxide and extracted exhaustively with a chloroform-ether mixture. The latter was extracted with 5% hydrochloric acid and the acid extract gently evaporated leaving the impure alkaloid-hydrochlorides. These were not crystallizable. In order to effect a separation of the different alkaloids in the mixture the salts were dissolved in water and the solution after being made alkaline with sodium hydroxide was extracted with ether. The ether solution on evaporation left a crystalline residue and an amorphous mass. Following the ether extraction a chloroform extraction was made. This yielded a second amorphous product. The crystalline product, called base A, was obtained in the form of 6-sided plates which, after repeated crystallization from alcohol, had the melting point 195–197° C. This product was soluble in most of the usual organic solvents, although not so readily in ether, and very little in water. The solutions showed an alkaline reaction. Attempts to obtain crystalline salts were unsuccessful. The average of 15 analyses, which, however, were not in good agreement (for which no reason could be found), gave

C—62.67%, H—8.69%, N—3.68%

Because the analyses failed to check satisfactorily no formula was given. Keller's findings may be summarized as follows: One crystalline alkaloid is present in the seed of *D. consolida* and the presence of other alkaloids is indicated.

METHOD OF INVESTIGATION.

The method adopted for this investigation was similar to that of Keller. Ten kg. of ground seed, in portions of 2 kg., were placed in an earthenware container;

¹ See bibliography appended.

² Numbers in parentheses refer to bibliography.

a quantity of 95% alcohol containing 0.5% hydrogen chloride was added, and the mixture mechanically agitated. When extraction was thought to be sufficiently complete the liquid was separated by filtration and distilled under reduced pressure on a water-bath at a temperature not over 60° C. until practically all the solvent was removed. The alcohol recovered in the distillate was used again in the extraction process. The concentrated extract separated on standing into 2 layers—an upper layer of oil, greenish in color, measuring about 3 liters, and a lower aqueous layer. These were separated. To the aqueous layer was added an equal volume of water and the mixture allowed to stand several days in order to deposit resinous matter. The addition of water also caused the separation of a small quantity of oil which was combined with the major portion previously obtained. The turbid aqueous solution was filtered under suction thus removing a blackish resinous substance. The filtrate, being the impure alkaloid solution, was clear and of a dark wine color.

PURIFICATION OF THE OIL.

The crude oil at first smelled strongly of alcohol but on standing in an open cylinder for several weeks lost most of this odor. A steam distillation on a small portion showed that no volatile oily constituent nor any volatile alkaloid was present, but a small amount of alcohol was detected. The main problem was to remove the alkaloids dissolved in the oil. To this end the oil was dissolved in twice its volume of ether and extracted repeatedly with 5% sulphuric acid. Katz (11) as a result of his work on *D. staphisagria*, had cautioned against the use of sulphuric acid on account of the formation of emulsions but it was found that the weaker organic acids, *e. g.*, acetic and tartaric, did not remove the alkaloids completely. In order to avoid troublesome emulsions the two liquids were not shaken together violently as is ordinarily done in extractions but were placed in a large bottle which was rotated slowly in a machine about a line parallel to its own axis. In this way, while the extractive process was slow, the desired result was accomplished and no trouble from emulsions was experienced. The last acid extract showed only a trace of alkaloids, using phosphotungstic acid as a test reagent, and it was considered that the oil was freed from alkaloidal matter. The oil was freed from ether by evaporation on the steam-bath. Alcohol was then found also to be absent.

The oil thus purified was of a greenish yellow color and had a slight characteristic odor resembling that of castor oil. The yield was 2540 g., equivalent to 25% of the weight of the seed.

The properties of the oil are as follows:

Specific gravity at 20° C.....	0.889
Refractive index at 20° C.....	1.4581
Saponification value.....	181.2
Acid value.....	189.5
Iodine value.....	87.8
Acetyl value.....	34.9
Unsaponifiable residue.....	1.28%
Elaidin test.....	Negative

Beside being soluble in such organic solvents as ether, petroleum ether, and chloroform, the oil is soluble in 95% alcohol.

The oil was set aside for future entomological tests.

TREATMENT OF THE ALKALOID SOLUTION.

Keller purified his alkaloids by washing the acidified alkaloid solution with a chloroform-ether mixture. While this undoubtedly removed some coloring matter and other impurities it must also have caused the loss of a considerable quantity of alkaloids as it was found that part of the alkaloids of larkspur are such weak bases as to be extracted from acid solution by chloroform. Therefore, instead of performing that operation the following method of purification was adopted.

A solution of lead subacetate was added to the solution containing the alkaloids. This produced a slimy precipitate which contained beside the organic matter a large amount of lead chloride (due to the hydrogen chloride used in the alcohol). The precipitate was filtered off and the excess of lead salt removed from the filtrate by precipitation with oxalic acid. The lead oxalate was filtered off and the filtrate saved for further treatment.

Preliminary work on the extraction of these alkaloids from aqueous solution brought out the fact that part of the alkaloids was extracted by organic solvents from acid solution while the balance was extractable only from alkaline solution. It was thought that because of this difference a means of separation of the various alkaloids was offered. Therefore, the alkaloid solution, in its acid condition, was extracted repeatedly with chloroform. Then the solution was made alkaline with sodium hydroxide and again extracted with chloroform until the latter failed to extract appreciable amounts of alkaloid. This gave 2 chloroform solutions designated A and B, respectively. On evaporation they left dark brown viscous residues which showed no tendency to crystallize and required further purification.

The residue from A (the chloroform extract of the acid solution) was dissolved in 5% sulphuric acid and the solution made alkaline with ammonia. A bulky white alkaloid precipitate was thus produced which was filtered off, redissolved in acid, and reprecipitated. It carried with it all the coloring matter present in the solution and it was found that this coloring matter could not be removed by the method of alternate extractions with ether and acid. A little more precipitate was obtained from the filtrate, the total amount being 23 grams. This product was difficultly soluble in ether, more readily in alcohol, and very readily in chloroform. The entire precipitate was dissolved in hot alcohol from which crystals were obtained on cooling. These crystals failed to give a sharp melting point. Repeated crystallizations also gave a product which melted indefinitely between 150° and 160° C.

As an aid in the identification of all the alkaloids met with in this work use was made of the petrographic microscope. The optical character was thus determined which afforded a means of identification not realized by chemical methods. These crystals, long irregular prisms, were biaxial negative, but in one fraction some biaxial positive crystals were detected, thus proving the presence of more than one alkaloid. It was thought that separation might be made by fractional precipitation with very dilute ammonia since other work showed that the positive alkaloid was not precipitated from weak ammoniacal solution whereas the negative alkaloid required but very slight excess of ammonia for precipitation. Accordingly, the material was dissolved in dilute sulphuric acid, precipitated with 1:10 ammonia, redissolved in acid, and reprecipitated, 10 times in all, and then recrystal-

lized from alcohol. The resulting crystals, however, showed the same irregular appearance as before and melted between 153° and 160° C. The work on this alkaloid (or mixture of alkaloids) was placed in abeyance after these and other attempts at purification and all that can be said about it is that it is precipitated by ammonia, is optically biaxial negative, melts between 153° and 160° C., is insoluble in water, slightly soluble in ether, more so in alcohol, and readily in chloroform.

The residue from A after precipitation with ammonia was extracted with ether and chloroform when it yielded biaxial positive crystals, which proved to be identical with the positive alkaloid isolated in a later fraction, and also a small amount of biaxial negative crystals, which had the same crystalline form as the negative alkaloid to be described later.

The residue, on evaporation of Solution B, was dissolved in 5% sulphuric acid, the solution made alkaline with sodium hydroxide and extracted first with ether until the ether failed to remove appreciable amounts of alkaloid, then with chloroform. These solutions are designated C and D, respectively.

Solution C, the ether extract, showed no tendency to crystallize on evaporation. It was taken to dryness and the residue dissolved in 5% sulphuric acid. When this solution was treated with ammonia in slight excess a white alkaloid precipitate was obtained which was probably identical with the previously mentioned alkaloid precipitated by ammonia and was added to it. When more ammonia was added to the solution further precipitation occurred but this precipitate immediately redissolved in the solution on stirring, thus showing it to be different from the other precipitate.

The ammoniacal solution on being concentrated by gentle evaporation deposited clear colorless crystals which were biaxial positive. The crystals were removed by filtration. In order to recover the material remaining in solution, the liquid was taken to dryness and the residue treated with alcohol thus dissolving out the alkaloid. This alcohol solution did not yield crystals on evaporation as was expected. Instead, a viscous mass was obtained and sulphates were found to be present. Therefore, it is evident that the alkaloid-sulphate had been formed in the evaporation of the ammoniacal solution. More crystals were obtained on extracting the alkaline solution with chloroform.

To this alkaloid, which is not previously mentioned in the literature, it is proposed to give the name "delsoline."

Delsoline crystallizes in brilliant crystals which resemble octahedra but are probably monoclinic. Crystal measurements of this alkaloid will be made and reported in the near future. The observed melting point is $207-209^{\circ}$ C. Delsoline is very soluble in alcohol and chloroform and fairly soluble in water. Solutions of it exhibit a strong alkaline reaction.

Analysis¹ of the compound gave the following results:

	C.	H.	N.
	62.07	8.49	2.88
Calculated for $C_{25}H_{41}NO_3$	62.07	8.55	2.90

The chloroform solution, D, on evaporation left a light brown amorphous mass. This was dissolved in 5% sulphuric acid and excess of ammonia added,

¹ Conducted by Mr. R. M. Hann of the Bureau of Chemistry.

but no precipitate was produced. The ammoniacal solution was extracted first with ether and then with chloroform. The ether solution was washed with water and the aqueous washings, on being warmed to drive off dissolved ether, deposited well-formed 6-sided crystals. From the ether solution on evaporation a further quantity of crystals was recovered and from the chloroform solution an additional crop was obtained.

These crystals were united and recrystallized from alcohol until the melting point was unchanged. The observed melting point was 198–199° C. This alkaloid crystallizes in the orthorhombic system and is deposited in 6-sided plates or prisms which are biaxial negative.¹ It is very soluble in chloroform and alcohol, appreciably soluble in water, and less so in ether. The chemical analysis² is as follows:

	C.	H.	N.
Observed	63.73	8.55	3.58
Calculated for $C_{21}H_{33}NO_6$	63.77	8.41	3.54

The crystalline alkaloid obtained by Keller is strikingly similar in its properties and composition to this product. Keller reports a melting point of 195–197° C. and states that the crystals are deposited as 6-sided columns. The various solubility relations are also, in general, in agreement. The analysis of Keller's compound based on the average of 15 analyses leads to the formula $C_{20}H_{33}NO_6$, which contains one carbon atom less than the formula obtained above. This close agreement alone indicates that Keller's alkaloid and the above described alkaloid are undoubtedly identical. He refrained from giving it a formula or a name as he regarded its composition to be insufficiently established. However, there appears to be no reason why this product should not now be regarded as a definite chemical compound and the name "delcosine" is proposed for it.

A method of purification devised for use with consolidine and which is of general application is as follows: The impure aqueous solution containing the alkaloids is made acid and a small amount of arsenious acid added. Hydrogen sulphide is passed through until all the arsenic is precipitated as arsenious sulphide. When this is filtered off the solution is found invariably to contain less coloring matter than before owing to the fact that arsenious sulphide has the property of adsorbing many accompanying substances. An impure delcosine solution treated in this way yielded crystals having the same properties as the product obtained from solutions which had not been thus treated, showing that neither the arsenic nor the hydrogen sulphide affected the alkaloid.

SUMMARY.

The pure oil of larkspur seed has been isolated and described. There have also been isolated two crystalline alkaloids, the compositions of which have been definitely established, and a third crystalline alkaloid, which has not yet been obtained in a pure state. Of the former two, delcosine, $C_{21}H_{33}NO_6$, melting point 198–199° C., is identical with a product previously obtained, while delsoline, $C_{25}H_{41}NO_8$, melting point 207–209° C., is an addition to the list of larkspur constituents.

¹ Crystal measurements of this alkaloid were made by Mr. R. M. Hann. The results will be reported separately.

² Conducted by Mr. R. M. Hann, of the Bureau of Chemistry.

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DIETHYLPHTHALATE II.

BY J. A. HANDY AND L. F. HOYT.

(Continued from p. 609, July number.)

III. TESTING FOR DIETHYLPHTHALATE IN BEVERAGES.

Preliminary tests on beverages, Table III, showed that the substances which react most similarly to diethylphthalate did not interfere with a correct interpretation of the diethylphthalate tests. Accordingly a series of alcoholic beverages containing various known amounts of diethylphthalate were subjected to the Handy-Hoyt and Calvert tests. Owing to lack of time the series of samples were not run