

STUDIES IN VARIOUS CONSTITUENTS OF THE SEED  
IN MEMBERS OF THE NATURAL ORDER  
RANUNCULACEAE

by

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## GENERAL INTRODUCTION.

The definition of the seed in chemical terms has not progressed far, largely because each plant species has characters individual to itself and over the flowering plants such is the degree of diversity that a very considerable amount of work is required. The major classes of compound such as the carbohydrates and proteins are at this stage of but little interest and this for two reasons. Where the differences between compounds are large each variation is common to many species. On the other hand, the extremely fine differences of say molecular structure in the proteins which are individual to a plant species and indeed probably condition the very species itself are so small as not to yield to present methods of chemical investigation. It is therefore to the smaller, apparently less significant groups of compounds that the investigator turns. The presence of a specific glucoside or an alkaloid very often characterises a plant species. Thus the chemical has a botanical interest. The potency of the compound often lends another interest.

The natural order Ranunculaceae is one extremely rich in alkaloids and although many of these bases have been the subject of chemical investigation, until comparatively recent years

little was known regarding them beyond recording their presence in particular species. The chemistry of the Aconite alkaloids and even more so that of the Delphinium alkaloids is still in the earliest stages of development, and until the recent work of Jacobs and Craig (1939 and 1940) on delphinine, little knowledge was available of the constitution of even a single individual.

The seeds of Delphinium Staphisagria have been used extensively in Europe as a parasiticide since the Middle Ages, and it is probably on this account that the first recorded investigations on the Delphinium species were made on these seeds. As far back as 1819, Brandes indicated the presence of an alkaline substance in these seeds which he named "Delphinin". This work was continued by Couerbe (1833), Erdmann (1864), Studer (1872) and Serck (1874), all of whom obtained more or less impure forms of delphinine. The presence of four different bases were described by Marquis (1877), who carried out an extensive investigation, and confirmed by Kara-Stojanow (1890), who disagreed however on the formula for delphinine. The properties of a fifth alkaloid - staphisagroine - were described by Ahrens (1899).

These investigations were carried a stage further by Katz (1900) who reported no formula or melting-point for delphinine but seems to have

obtained the pure material and performed the first experiments on chemical structure. Walz (1922), who carried out his investigations on Merck's crystallised delphinine, established the formula  $C_{34}H_{47}NO_9$ . He also prepared a crystalline salt - the acid oxalate - and did some preliminary work on the constitution. Walz' formula was confirmed by Markwood (1927) who extracted the delphinine from the seed and also examined the fixed oil. This was the position until the recent work of Jacobs and Craig (1939) who now give for delphinine the formula  $C_{33}H_{45}NO_9$  and who have carried out very extensive experiments on the structure of this molecule in continuance of the work of Walz (1922) and Keller (1925). In addition Jacobs and Craig (1941) have isolated a new alkaloid from the delphinine mother liquors by chromatographic adsorption which they have named staphisine and to which they assign the formula  $C_{22}H_{31}ON$  with reservation.

Meanwhile parallel investigations on numerous American species of Delphinium had been going on, promoted chiefly by the harmful physiological action of these plants when eaten by grazing cattle.

G. Heyl (1903) isolated a mixture of bases from the roots of various species each showing different quantities of the mixture, D. bicolor 27%,

D. Menziesii .35%, D. Nelsonii .72% and D. scopulorum 1.30% The seeds of the latter were also found to yield 1.18% of the mixed bases. This mixture was introduced into commerce under the name of Delphocurarine by Merck of Darmstadt as a substitute for curare. Its physiological properties were examined by Lohmann (1902) and from it Heyl was able to isolate a small quantity of a crystalline base to which he assigned the formula  $C_{23}H_{33}NO_7$ , but did not name.

Heyl, Hepner & Loy (1913) carried out assays of Delphinium Nelsonii, Delphinium glaucum and Delphinium Geyeri and determined the alkaloidal content of the different parts of each plant. More elaborate was the work of Beath (1919) who compared Delphinium Barbeyi, Delphinium Geyeri and Delphinium glaucescens, and not only reported the alkaloidal content of the different parts of each plant but examined the plants at different stages of growth. He was thus able to show that not only the quantity of alkaloid varied with the stage of growth, but that in the case of Delphinium Barbeyi changes in the nature of the alkaloids also took place. D. Barbeyi and D. Geyeri both yield toxic amorphous alkaloids very similar in composition. On hydrolysis these amorphous substances break down into less toxic crystalline products accompanied by an acid which is isomeric with aconitic acid and

which is also present uncombined in the juice of these two plants. The bases of D. glaucescens are crystalline and in no way resemble the crystalline products, either free or combined, of the other two species. Although numerous physical and chemical characters of the various active principles were determined, Beath does not appear to have established their percentage composition and no formulae are stated.

The presence of alkaloids in Delphinium Andersonii, Gray has also been recorded by Miller (1923). In 1936, Couch reported the extraction of 98% total alkaloids from Delphinium occidentale, the largest proportion of which was a new base  $C_{21}H_{33}NO_6$ , named deltaline by the author.

The most recent of the American species to be examined is Delphinium Brownii, Rydb. Manske (1938) investigated the nature of an alkaloid which could not be crystallised. On hydrolysis it yielded a crystalline base of indefinite composition, together with anthranilic and methyl-succinic acids. The author states this is the first occasion on which methyl-succinic acid has been extracted from natural sources and as this acid contains the isoprene carbon skeleton, makes the interesting suggestion that the Delphinium and Aconite alkaloids may be isoprene derivatives.

Besides Delphinium Staphisagria the seeds

of other European species have been the subject of investigation, notably Delphinium consolida and Delphinium Ajacis. Foremost in this work was Professor Oscar Keller and co-workers who in the course of investigations embracing the Hellebore group, recorded the presence of alkaloids in the seed of D. hybridum, D. formosum, D. rhinante, D. chinense and D. elatum, Keller (1925).

Delphinium consolida was one of the first species to be examined by Keller (1910) and from the seeds he isolated three bases - one crystalline, described as Base A, and two amorphous - none of which was sufficiently pure for analysis. This work was taken up by Markwood (1924) who obtained two crystalline alkaloids in a pure state and indicated the presence of a third. Of the pure bases one was obviously identical with Keller's Base A. Markwood named it delcosine and assigned to it the formula  $C_{21}H_{33}NO_6$ . The other,  $C_{25}H_{41}NO_8$  was named delsoline. Cionga and Iliescu (1941) continued this work and found delcosine and delsoline to be isomeric with one another and to both alkaloids they assigned the formula  $C_{25}H_{40}NO_7$ . The formation of salts and other derivatives was investigated.

Beyond a brief reference to the insecticidal value of the flowers by Benvenuti (1883), and a more elaborate examination of the seed from the same point of view by Williams (1914), there is only one



other recorded investigation of Delphinium Ajacis.

Keller and Völker (1913) carried out an extensive investigation of the seeds of this species and isolated two bases in a crystalline state. They also examined the physical and chemical properties of these substances which they named ajacine and ajaconine respectively. At first it was apparently their intention to continue the work of Keller (1910) on Delphinium Consolida, but after the first stages of extraction had been carried out they realised that the wrong material had been supplied and subsequent examination of plants grown from the seed led to its identification as the seed of Delphinium Ajacis.

The extraction of the alkaloids from the seed was carried out using a menstruum of 95% alcohol containing .5% hydrochloric acid. This alcoholic liquor was then concentrated by distillation in vacuo and fixed oil removed by shaking the acid residue with ether. The ether also took up a considerable quantity of the bases but these were recovered by shaking the ether solution with dilute sulphuric acid. This acid liquor was added to the principal extract which was then treated successively with (1) ammonia and ether, (2) ammonia and chloroform, (3) caustic potash and ether, and (4) caustic potash and chloroform. The total crude alkaloids, amounting to 1.4% of the seed,

were thus obtained in four principal fractions but only from two of these - the ethereal extracts - was it found possible to prepare crystalline products.

The first base isolated, which they named ajacine, was obtained from the ammoniacal liquor by shaking with ether. The other ethereal solution - the third fraction - yielded ajaconine. Ajacine was found to crystallise readily from 50 - 60% alcohol in the form of fine needles, melting-point  $142-143^{\circ}$ , but the yield of pure crystalline substance was relatively small owing to its tendency to resinify. It crystallised with one molecule of water of crystallisation and its formula was stated to be  $C_{15}H_{21}NO_4 \cdot H_2O$ , molecular weight 297.2. This however was not in very good agreement with the equivalent weight of 319 obtained by titration experiments. No mention is made of the indicator employed in this part of the work. It was observed solutions of ajacine in alcohol were characterised by a distinct blue fluorescence.

The salts of ajacine were found to be extremely soluble and difficult to crystallise and such as could be prepared were mostly basic, although there was also evidence of the formation of normal salts. The most readily crystallisable salt was the sulphate  $(C_{15}H_{21}NO_4)_4 H_2SO_4$ .

The chloride  $(C_{15}H_{21}NO_4)_2 \cdot HCl \cdot 2H_2O$  and aurichloride  $(C_{15}H_{21}NO_4)_2 \cdot HAuCl_4$  were obtained by precipitation methods, while both normal chloroplatinates  $(C_{15}H_{21}NO_4)_2 \cdot H_2PtCl_6$  and basic chloroplatinates  $(C_{15}H_{21}NO_4)_4 \cdot H_2PtCl_6$  were also prepared.

Ajaconine, melting-point  $162-163^\circ$ , was obtained in large colourless prisms by crystallisation from 95% alcohol. These, unlike ajacine, were anhydrous and did not lose their lustre on exposure. Analysis of this substance did not yield satisfactory results but it was found capable of combining with one molecule of methyl iodide to form a methiodide, written by Völker as  $C_{18}H_{31}NO_2 \cdot HI \cdot H_2O$ , from which the formula  $C_{17}H_{29}NO_2$  was assumed for ajaconine, although the author made this statement with reservation.

Like ajacine, the salts of ajaconine showed little inclination to crystallise and as the amount of the alkaloid was very limited, experiments were confined to the picrate and the gold and platinum salts. No formula for these salts was given, but the picrate was stated to have a melting-point lying between  $115-120^\circ$ . Völker's findings may therefore be summarised as follows:

Two crystalline alkaloids - ajacine and ajaconine - are present in the seeds of D.Ajacis and two other bases are indicated. The formula of ajacine is  $C_{15}H_{21}NO_4 \cdot H_2O$  while in the absence of further data

$C_{17}H_{29}NO_2$  is suggested as the formula of ajaconine. Ajacine is probably a tertiary base while ajaconine is a secondary base. The salts of these alkaloids are very soluble and difficult to crystallise.

As these results left a fairly wide field for further investigation and as a reasonable quantity of the seed of this particular species was available the first necessary conditions for an interesting problem were fulfilled. It was therefore decided to continue the work of Keller and Völker in the hope that by the use of more modern methods, further light would be thrown on the nature of the constituents, including those already known, of the seed of Delphinium Ajacis.

#### MATERIAL.

For the purpose of this work 5 lbs. of Larkspur seed were obtained from Messrs. David Bell, Limited, wholesale seedsmen, Leith. The seed was described as "Larkspur Branching Mixed", and had been imported by the seed merchant from Holland in which country these plants are cultivated for the supply of seed to the horticultural market. During the course of these investigations a further 5 lbs. of the same seed were obtained. This was described exactly as before and had been imported by the same firm from the same Dutch grower.

In order that there should be no dubiety

as to what botanical species the seeds belonged a small sample was drawn off from the main bulk and grown on. These were sown and grown in conformity with normal horticultural practice. The plants from both the consignments used were identical in every respect and the details concerning them are given below.

The plants of either lot could be divided into two well defined varieties.

- 1) Those with single corollas and long spurs.
- 2) Those with double corollas and shorter spurs.

All grew from 2 to 3 feet in height, and on the whole those of the "double" variety were taller and of stouter growth than the others. The flowers varied in colour - white, pink, mauve and blue being equally represented with all the intermediate shades.

Variety (1) A closer examination of individual flowers of this variety revealed that they all conformed very closely to the following description.

- (1.) Perianth of 5 petaloid sepals of which the posterior one is extended into a long spur.
- (2.) One single petal - or perhaps two joined - so shaped as to form a protective hood over the stamens

and pistil. This petal is attached to the posterior sepal in such a way that it helps to form the spur.

This whole arrangement of the perianth conveys the impression of a tubular effect which is entirely absent from the other variety.

(3.) Stamens, 15 - 16.

(4.) Carpels 1, forming a single ovary with many ovules on a parietal placenta.

(5.) Fruit, a follicle.

Variety (2) The flowers of this variety showed less uniformity, but all were distinguished by their flattish rosette appearance, as distinct from the tubular impression created by the other variety. They also showed the following characteristics:-

(1) Perianth of 5 petaloid sepals of which the posterior one is extended into a spur, shorter and much less significant than in the other variety.

In certain specimens the two lateral sepals also exhibited smaller rudimentary spurs.

(2) Corolla of 3 petals, distinct and none of them forming a hood. This characterises the simple "double" variety:

or

or

Corolla of 10 petals in two whorls with a further whorl of 4 rudimentary petals, which characterises the more elaborate type.

- (3) Stamens, 22.
- (4) Carpels 1 - 3, forming 1, 2 or 3 ovaries according to the number of carpels. Parietal placentation.
- (5) Fruit, a follicle or collection of same.

Specimens were submitted to the authorities at the Royal Botanic Gardens, Edinburgh, who identified them as showing the typical characters of Delphinium Ajacis although a few specimens showed other characters associated with the closely related species Delphinium orientale. It is well known that the garden variety of annual Delphinium to which this material belongs is composed of individuals, some tending to show the characters of Delphinium orientale while others show characters more towards D. Ajacis. The two species are not too well defined botanically, and indeed many competent authorities do not separate the species but include them both under Delphinium Ajacis. Specimens typical of all the forms occurring in the material were pressed and placed in safe custody in the Physiological section of the University Department of Botany.

ASSAY OF THE SEED.

Before proceeding with extraction and separation of the alkaloids from the main bulk of the seed, it was decided to carry out an alkaloidal assay on a small portion. Furthermore while Völker's methods of separation were in general quite commendable, it was clear that improvements could be introduced particularly into the initial stages. His process of extracting the seed with acidified alcohol, while completely successful in removing the alkaloids, dissolved out large quantities of fixed oil with equal facility. This led to considerable emulsification trouble at a later stage as the alcoholic extract contained, besides oil and alkaloids, resins and many other impurities. It was felt that initial extraction of the oil with petroleum ether of low boiling point would remove the principal factor promoting emulsification and in addition it was hoped by this means to separate the oil in such a state of purity that its physical and chemical characters could be studied. While carrying out the alkaloidal assay it was decided to put these theories to a practical test.

200 gm. of the seed were taken and bruised in a stone mortar until reduced to a rough powder. The powdered seeds were then transferred to a suitable vessel and macerated with  $1\frac{1}{2}$  litres of petroleum ether (boiling-point  $40/60^{\circ}$ ) for two days



with frequent agitation. The petroleum ether extract was then filtered off and the solvent recovered by distillation on a water-bath.

Meanwhile extraction of the seed was continued with two further quantities of petroleum ether which in turn were distilled. The oily residue was collected in a tared basin.

The defatted seed was now dried by exposure to air and sifted, the fine powder being separated from larger particles and whole seed which had escaped pulverisation. The unground portion was now bruised and macerated separately with a further litre of petroleum ether for one day. This extract was filtered and distilled as before, the small quantity of oily residue being added to the remainder already collected.

Extraction of fixed oil being virtually complete the seed marc was again allowed to dry in air. It was then returned to the vessel and extracted with four successive portions of 90% alcohol each of 1200 c.c. These were filtered off and concentrated in turn to small bulk by distillation under reduced pressure, temperature not exceeding 50°. The last liquor was colourless, but as it showed traces of alkaloid it was decided to dry off the seed marc again and powder the larger particles. After sifting through a fine sieve, the coarser portion, amounting to about 60% of the whole, was reduced to

a fine powder, but only with considerable effort as it consisted largely of seed-coats and was exceedingly resistant. The total seed powder was now returned to the vessel and again extracted with three further portions of 90% alcohol, each of 1 litre. The first of these liquors showed a considerable quantity of alkaloid indicating the necessity of having the seed in a fine state of comminution, if complete extraction was to be achieved. The final liquor showed only the merest traces.

These liquors were also concentrated by distillation in vacuo until the combined total alcoholic extract measured about 100 c.c. It was a clear reddish brown syrupy liquid and, to complete removal of the alcohol, was diluted with an equal volume of water and again distilled under reduced pressure. The liquor now presented a milky appearance due principally to the presence of fixed oil which quickly separated to the surface on standing. To facilitate removal of the oil, the extract was transferred to a separating funnel and acidified with 20 c.c. normal sulphuric acid which had first been used to rinse the distillation flask. The flask was further rinsed with 30 c.c. water, which washings were also added to the liquor in the separating funnel. 60 c.c. petroleum ether were now introduced and after thorough shaking the liquid

separated completely into two portions, of which the upper layer contained all the fixed oil in solution in the petroleum ether. This was carefully separated from the aqueous portion which was washed with a further portion of petroleum ether. Both petroleum ether liquors were added to the oily residues already collected. Most of the solvent was now removed from the oily residue by evaporation in vacuo, the oil being finally heated on a water-bath for 15 minutes until completely free from petrol odours. It was then cooled and weighed.

Total fixed oil in 200 gm. seed = 77.5 gm.  
= 38.75%.

Meanwhile it was shown by testing with Mayer's reagent that this oil contained a small quantity of alkaloid. It was accordingly dissolved in twice its volume of chloroform and shaken with three successive portions of dilute sulphuric acid. Each was tested with Mayer's reagent, the last showing only the merest traces of alkaloid. These acid liquors were added to the acid extract containing the main bulk of alkaloids forming a pale coloured solution which required no filtration.

This solution was now transferred to a separating funnel and made alkaline with ammonia, thereby liberating certain of the alkaloids in the form of a dense white precipitate which quickly curdled and floated in the liquor. This was

dissolved out by shaking with two successive volumes of ether, the ethereal solutions being set aside to evaporate in an open basin. No further extractions with ether were made at this stage, for although such extractions removed small quantities of alkaloid it had been found that such bases were not identical with the one precipitated by ammonia and it was desired as far as possible to make a separate estimate of each of the crude alkaloids.

On evaporation of the ether, the base remained as a yellowish-white, partly crystalline residue. This was dissolved in dilute sulphuric acid and re-extracted by making alkaline with ammonia and shaking out with several volumes of ether. The precipitated alkaloid was much whiter than on the previous occasion and the ether solutions virtually colourless. The ether was again removed by spontaneous evaporation in a tared basin, the residue being dissolved in 5 c.c. anhydrous alcohol and heated on a water-bath to constant weight.

Crude ether-soluble alkaloid in 200 gm. seed

(Base A : presumed ajacine) = .930 gm.

= .465%.

The original ammoniacal solution was now extracted with chloroform until it no longer yielded alkaloid to this treatment. Three extractions were

sufficient. The first was pale yellow in colour but the others quite colourless. They were set aside to evaporate in an open basin. The alkaloidal residue which was pale brown in colour and of a syrupy consistency contained darker particles of a resinous nature. To remove these the residue was dissolved in dilute sulphuric acid and filtered, the base being again recovered by making alkaline with ammonia and shaking out with chloroform. It was noted here that addition of ammonia to a solution of this base caused no precipitation. After spontaneous evaporation of the chloroform in a tared basin, the syrupy residue was dissolved in 5 c.c. anhydrous alcohol and dried to constant weight on a water-bath.

Crude chloroform-soluble alkaloid

in 200 gm.seed (Base B) = 1.234 gm.

= .617%

A large excess of strong caustic potash was now added to the original solution which was again extracted with ether until the ethereal extracts ceased to give a positive reaction with Mayer's reagent. As the alkaline liquor at this stage also gave no reaction for alkaloids it was rejected. The residue on evaporation of the ether liquors consisted of a white amorphous substance with which was associated some brown

viscous material. As this was obviously impure, it was redissolved in dilute acid, the solution filtered and the base reprecipitated with concentrated potassium hydroxide. On this occasion it formed a white curdy precipitate which was completely removed by shaking with ether. The residue on evaporation of the solvent was white and amorphous. It was taken up with anhydrous alcohol, 2 c.c., and dried on a water-bath to constant weight.

Crude ether-soluble alkaloid in 200 gm. seed

(Base C : presumed ajaconine) = .127 gm.

= .064%.

As a result of this preliminary investigation it was established that the material contained at least three alkaloids and a large quantity of fixed oil. It was also confirmed that no undue emulsification difficulties were encountered if this oil was removed by initial extraction with petroleum ether.

#### EXTRACTION OF THE SEED

Having decided on a suitable method of extraction, the main bulk of the seeds was treated in the manner outlined above. 2 kgm. were ground to a rough powder and after damping with petroleum

ether (b.p.  $40/60^{\circ}$ ) were packed in a stone percolator. More petroleum ether was then poured on until sufficient had been added to cover the powder. In all about  $2\frac{1}{2}$  litres were required. The powdered seed was thus macerated for three days before the liquid was allowed to percolate. The solvent was now recovered from this percolate by distillation and used for further extraction, while the oily residue was reserved. This process of macero-percolation was repeated a further three times each with  $2\frac{1}{2}$  litres of petroleum ether. The last percolate was colourless in contrast to the first two which were decidedly yellow, and yielded only 4 gm. of oily extract. The combined oily residues were then heated in vacuo at a temperature not exceeding  $60^{\circ}$  until free from the odour of the solvent. The yield at this stage was 534 gm. = 26.7%.

As this only represented about two thirds of the reported oil content, the powdered seed was allowed to dry in the air and after sifting, whole seed and coarser particles were reduced to a finer powder. It was then returned to the percolator and extracted with a further two quantities of petroleum ether, each of  $2\frac{1}{2}$  litres. These extracts were distilled as before and an additional 100 gm. of fixed oil = 5% were thus obtained. This was added to the previous quantity and reserved for further examination.

The seed marc was now again dried off until free from the odour of petroleum ether. It was then transferred to a suitable percolator and macerated with 5 litres of 90% alcohol for six days, after which the liquor was allowed to percolate. It was then concentrated by distillation under reduced pressure at a temperature not exceeding 60°. It was observed that the concentrated extract contained a considerable proportion of oil which separated readily. This showed that petroleum ether has less penetrating power than alcohol. The recovered alcohol was used for further extraction. In all ten percolations each with 5 litres of 90% alcohol were carried out, the last of these only showing the presence of mere traces of alkaloid when tested with Mayer's reagent. These liquors were all concentrated in turn, the combined extracts being set aside.

Having completed extraction of the seed with alcohol, a small portion of the marc was dried by exposure to air and examined to determine whether or not complete extraction of the alkaloids had been effected. Seven samples of this dried powder, each of 4 gm. were extracted with the following solvents:

- (1) 40 c.c. water.
- (2) 40 c.c. hydrochloric acid 1%.
- (3) 40 c.c. Prollius' Fluid  
 (ether 25: chloroform 8: )  
 (alcohol 2.5 : ammonia (10%) 1.)



The other four, before extraction, were each mixed with slaked lime, damped with water, allowed to stand for a day and dried off at 40°.

(4) 40 c.c. petroleum ether (40/60°).

(5) 40 c.c. ether.

(6) 40 c.c. chloroform.

(7) 40 c.c. alcohol.

After 24 hours these extracts were filtered off and examined with Mayer's reagent as follows.

	Extract	Treatment	Mayer's reagent.
(1) Water	Pale brown liquid. Somewhat frothy.	Acidified with dil. HCl.	Copious white precipitate
(2) HCl. 1%	As (1)	—	Copious white precipitate
(3) Prollius' Fluid	Clear colourless liquid	Evaporated to dryness & dissolved residue in dil. HCl.	Slight precipitate
(4) Petroleum Ether.	As (3)	As (3)	No precipitate
(5) Ether	As (3)	As (3)	Slight precipitate
(6) Chloroform	As (3)	As (3)	Slight dimness
(7) Alcohol	As (3)	As (3)	Slight precipitate

These results served to indicate that there were still small quantities of alkaloids present in the seed powder and that these could best be extracted by means of water or dilute hydrochloric acid.

As it was possible that the presence of dilute acid might induce hydrolysis of the alkaloids, especially during concentration, its use at this stage was undesirable. Further extraction of the seed marc was accordingly carried out with 10 litres water in which was dissolved 25% chloroform to prevent fermentation. After three days maceration, the liquid was poured off and filtered, and the filtrate concentrated by distillation under reduced pressure at a temperature not exceeding 55°. At first considerable trouble was experienced with frothing. The addition, from time to time, of a drop of capryl alcohol broke the froth immediately but the effect lasted only for a few minutes. It was found that this trouble could best be avoided by the addition to the filtrate of 25% of its own volume of alcohol. This mixture was then slowly sucked into the flask one drop at a time while distillation was proceeding and at approximately the same rate. The amount of liquid in the flask was thereby kept at a minimum and any tendency to froth was broken by the alcohol vapour introduced with each drop of the liquor.

Extraction with water, using 10 litres on each occasion, was carried out four times in all, the final liquor giving virtually no reaction for alkaloids when tested with Mayer's reagent. Each was concentrated in turn and the final concentrated liquor measured 2 litres. It was a thick, blackish-brown, muddy liquid, strongly acid to litmus, and on standing formed a considerable deposit which was removed by filtration. The liquor was set aside, and meanwhile fermentation was prevented by the addition of a few drops of chloroform.

The seed powder was now considered to be exhausted but before final rejection, 50 gm. of the dried powder was digested in the cold for four hours with 100 c.c. 2% hydrochloric acid. A few c.c. were filtered off and digestion continued on a water-bath for 30 minutes. The liquid was cooled and filtered. Both extracts were tested with Mayer's reagent but, as expected, with entirely negative results.

This completed the extraction of the principal constituents of the seed. These were now available in three extracts: (1) A petroleum ether extract; (2) An alcoholic extract; (3) An aqueous extract. Each of these was now the subject of a separate investigation.

PETROLEUM ETHER EXTRACT.

As it had previously been observed that the fixed oil as extracted by petroleum ether contained an appreciable quantity of alkaloidal matter the separation of this was the first essential. It was achieved by dissolving the total oily extract, amounting to 634 gm., in twice its volume of chloroform and shaking this with successive quantities of dilute sulphuric acid until tests showed complete removal of the alkaloids. The mixed acid solution which was virtually colourless, but cloudy owing to the presence of traces of oil, was clarified by filtration. The total alkaloids were then liberated by the addition of excess caustic potash and taken up by shaking with successive volumes of chloroform. After removal of most of the solvent by distillation under reduced pressure, evaporation to dryness was continued in a vacuum desiccator. 2.250 gm. crude mixed alkaloids were thus obtained as a pale brown toffee-like mass. This was dissolved in a small quantity of dilute sulphuric acid and set aside to be worked up with the crude alkaloids obtained from the alcoholic extract.

Meanwhile the chloroform-oil mixture was also distilled under reduced pressure until most of the chloroform had been recovered. After further evaporation in vacuo the oily residue was finally heated on a water-bath until free from the odour of

the solvent. The fixed oil as thus purified was of a deep golden-yellow colour and had a characteristic strong rank odour. In cold weather it partially solidified.

A careful analysis of the oil was carried out with the following results.

Specific gravity at 15.5°	0.905
Saponification value	192.9
Acid value	132.2
Iodine value (Wijs)	83.9
Unsaponifiable residue	1.04%

These values were determined by the methods described in the British Pharmacopoeia, 1932.

The oil was miscible with the usual organic solvents - ether, chloroform, acetone, petroleum ether and anhydrous alcohol - but not with 95% alcohol.

#### AQUEOUS EXTRACT

It was obvious from the consistency and colour of the aqueous extract that it contained much extraneous matter and that it would require considerable purification before any attempt could be made to remove the alkaloids. As a preliminary to clarification of the liquid by means of lead acetate, it was acidified with dilute hydrochloric acid. This caused the almost immediate formation

of a dark grey precipitate which remained suspended in the liquor and was only filtered off with difficulty. Having been formed in acid solution, this was most unlikely to be of the nature of an alkaloid. Nevertheless in conformity with the principle of rejecting no product without investigation this precipitated substance was examined. It was found to be of an organic nature, yielding little or no ash. It dissolved in ammonia and was reprecipitated by acids. It yielded ammonia on heating with soda lime and was probably a protein. It was definitely not an alkaloid and was in consequence rejected.

To the acidified mother liquor was now added Strong Solution of Lead Subacetate (British Pharmacopoeia 1932) until precipitation ceased. Excess of lead was removed as lead oxalate by the addition of oxalic acid. The copious deposit of lead salts and organic matter was removed by filtration and examined for alkaloids. Only mere traces were found indicating clearly that no loss of the bases had occurred, as oftens happens with this method of purification. That the process was exceedingly efficient as a means of removing extraneous matter was indicated by the filtered mother liquor which was of a pale sherry colour and in a condition suitable for extraction of the crude bases.

The addition of a large excess of strong caustic potash now precipitated the alkaloids which were entirely removed by extraction with several volumes of chloroform. The mother liquor, now being exhausted, was rejected. The crude bases were recovered by distillation of the mixed chloroform extracts and subsequent evaporation in vacuo of the alkaloidal residue. The yield was 0.655 gm. total crude alkaloids in the form of a pale brown toffee-like mass. This was dissolved in dilute sulphuric acid and set aside to be added to the principal extract in due course.

#### ALCOHOLIC EXTRACT

This extract, measuring 550 c.c., was a transparent reddish brown syrupy liquid, from which had separated a little fixed oil. It contained most of the alkaloids originally present in the seed. As a preliminary to removing the oil, it was diluted with an equal volume of water and distilled in vacuo at a temperature below 50° until free from alcohol. The residual liquor was now acidified with dilute sulphuric acid and transferred to a separator, the flask being rinsed with more acid. A considerable quantity of oil was now in evidence and was removed by shaking with three successive quantities of petroleum ether. The oil thus obtained was dark.

reddish brown in colour. It obviously contained many impurities and was rejected. To this defatted liquor were added the two alkaloidal solutions obtained from the "Petroleum Ether" and "Aqueous" extracts so that this combined solution now contained the total alkaloidal content of the seed.

Here a departure was made in the method of separation outlined earlier in the assay process. There were indications that certain of the alkaloids were weak bases and as such were readily extracted from acid solutions by organic solvents. Others were apparently only extractable from alkaline solution and it was thought this difference might be utilised to bring about their separation.

Accordingly the above acid solution was shaken repeatedly with chloroform but although ten extractions were carried out it was not found possible to remove all the weak bases by this method. The chloroform solution was highly coloured with resinous matter and was reserved for later investigation.

The acid solution was now rendered alkaline with excess of ammonium hydroxide, which immediately liberated a considerable quantity of alkaloid as a white flocculent precipitate. This was taken up by shaking with ether. Whilst standing overnight to allow time for the separation of the two liquids it was observed <sup>that</sup> the base crystallised out in the ether



to a slight extent in the form of small rosette-like clusters of needles. Further extractions with ether were made and the combined extracts reserved. The resulting ethereal solution was only slightly coloured and was characterised by an intense blue fluorescence. Although a considerable number of extractions were carried out on this occasion it was found later that for complete removal of the precipitated base not more than three or four are necessary. If extraction with ether is carried on beyond this limit other alkaloids, although only slightly soluble in this solvent, are taken up in appreciable amounts and lead to contamination of the principal alkaloid.

The ammoniacal mother liquor was now shaken with chloroform until it yielded no further alkaloidal material to this solvent. In all four extractions were required. The chloroform liquors were pale yellow in colour and were reserved.

As the mother liquor still gave a strong response to tests for alkaloids, caustic potash was added and extraction continued with ether. At first no precipitation occurred and it was only when a concentrated solution of the alkali was added in considerable quantity that the base was liberated. The base was very soluble in ether and was completely taken up in three extractions. Extraction was then continued with chloroform but no further alkaloids

were forthcoming. As tests on the mother liquor now showed removal of the alkaloids to be complete, this was rejected. The ether liquors were reserved for further investigation.

As a result of this work, the total alkaloids were now roughly separated in four extracts.

- (1) Weak bases - in chloroform.
- (2) Base A (presumed a jacine) in ether.
- (3) Base B - in chloroform.
- (4) Base C (presumed a jaconine) in ether.

Extracts (2) and (4) containing the bases already known were the first to be investigated.

#### BASE A.

The ethereal solution containing Base A was first distilled to remove the solvent, and it was observed at a point when most of the ether had been distilled that the alkaloid crystallised out in the residual liquor in masses of fine, white silky crystals. This liquor consisted largely of water which had been taken up by the large volumes of ether used during extraction. Sufficient dilute sulphuric acid was added to dissolve these crystals and the acid solution was transferred to a separator where the alkaloid was reprecipitated by the addition of excess ammonia and again taken up by ether. After three extractions, which were sufficient to

dissolve out the precipitated Base A, the mother liquor still retained a considerable quantity of alkaloid. This was completely removed by shaking with chloroform, the chloroform solution being added to Extract (3) since the bases were probably identical. This process of precipitation and extraction was repeated four times in all by which time most of the contaminating alkaloid had been removed and added to the appropriate extract. The final ether solution of Base A was transferred to an open basin and the solvent removed by spontaneous evaporation. The residue, a yellowish white amorphous substance, was dried in vacuo. Yield 8.50 gm.

The substance was now crystallised from 60% alcohol but difficulty was experienced with the first attempts. Like many Delphinium alkaloids Base 'A' resinifies fairly readily and the impure substance as obtained above contained a small quantity of resinified material. This not only introduced an objectionable impurity but during the process of crystallisation seemed to catalyse the formation of more resin with simultaneous degradation of the alkaloid. As a direct consequence the yield of the crystalline base was small. The crystalline material was eventually obtained by dissolving the freshly precipitated substance, still moist after collection and washing on a Buchner funnel, in a sufficient quantity of

warm alcohol (90%). The moist precipitate was used since the substance in this condition dissolves more rapidly and easily and the solution is less coloured than when the precipitate is dried beforehand. Drying, even in vacuo, promotes resinification. To the warm solution in alcohol was now added 50% of its volume of water at the same temperature, and on cooling the base crystallised in the form of fine silky needles suspended in clots throughout the liquid. It should be noted that the above amount of water is just sufficient to make the concentration of alcohol equal to 60%, and care must be taken not to exceed this quantity otherwise the alkaloid, instead of crystallising, will be precipitated as an amorphous mass.

The crystals were sucked off on a Buchner filter, dried and weighed. Yield 0.765 gm. They failed to give a sharp melting-point and were purified by repeated crystallisation in the manner described above until the product obtained melted sharply 134-135°. This melting-point and all others stated herein were determined by the use of the micro-melting point apparatus.

The pure air-dried crystals were analysed and the percentage composition of the substance determined. The methods employed were a micro-adaptation of the combustion process for carbon and hydrogen and Dumas' process for nitrogen. These

analyses and others which follow were carried out by Dr. G. Weiler, Oxford.

5.155 mg. substance yielded 11.680 mg.  $\text{CO}_2$  and 3.550 mg.  $\text{H}_2\text{O}$

= Carbon 61.79% : Hydrogen 7.65%.

6.513 mg. substance yielded 0.255 c.c. N at  $739/16^\circ$

= Nitrogen 4.50%.

The percentage of water of crystallisation was determined by drying over phosphorus pentoxide under reduced pressure at  $100^\circ$ .

4.929 mg. substance lost 0.296 mg. when dried for 3 hours at  $100^\circ$  under high vacuum

= 6.01%.

	C	H	N	$\text{H}_2\text{O}$
Found	61.79	7.65	4.50	6.01
Calculated for $\text{C}_{16}\text{H}_{21}\text{NO}_4 \cdot \text{H}_2\text{O}$	62.12	7.49	4.53	5.82

These analytical results are in good agreement with the theoretical. The alkaloid is undoubtedly identical with völker's ajacine, although

his formula,  $(C_{15}H_{21}NO_4 \cdot H_2O)$ , shows one carbon less than the above.

Ajacine,  $C_{16}H_{21}NO_4 \cdot H_2O$ , as obtained above occurs as fine white silky needles. It is readily soluble in alcohol, chloroform and acetone, and fairly soluble in ether. Both the ethereal and alcoholic solutions exhibit a powerful blue fluorescence which is characteristic of this base amongst the Delphinium alkaloids so far isolated. It is practically insoluble in water. Contrary to völker's statement the crystals do not lose their lustre on exposure, nor their water of crystallisation even when allowed to stand for several weeks over such dehydrating agents as fused calcium chloride.

The optical rotation was measured as follows.

0.0659 gm. ajacine in 10 c.c. chloroform = .659%.

Temperature  $16^\circ$   $\alpha_D + 35^\circ$  in 1 dm. tube.

$[\alpha]_D^{16^\circ} + 53^\circ$  in chloroform (C, 0.659).

A molecular weight determination was carried out by Rast's camphor method with the following results.

1.860 mg. ajacine dissolved in 20.175 mg. camphor

Depression =  $11^\circ$ .

Molecular Weight = 335.

Within the limits of experimental error this shows reasonable agreement with the calculated molecular weight of 309.4 and confirms that  $C_{16}H_{21}NO_4 \cdot H_2O$  is the true formula.

In the course of his investigations, Völker made several determinations of the equivalent weight of ajacine by volumetric methods. He dissolved the pure crystalline base in an excess of N/100 hydrochloric acid and back titrated with N/100 potassium hydroxide. By an unfortunate omission he does not state the indicator he used. His results for the equivalent weight varied from 319.0 to 341.5, but beyond stating that these are higher values than that required by his molecular weight of 297.2 the matter is not further discussed.

With a view to clearing up this point, and at the same time hoping to furnish further evidence in confirmation of the formula and molecular weight of 309.4, I carried out a similar titration experiment. The pure crystallised ajacine was dissolved in excess N/50 sulphuric acid and back titrated with N/50 sodium hydroxide. Methyl red was used as an indicator. The first titration gave a surprisingly high figure for the equivalent, 697.4, being more than double the molecular weight, but it was obvious that the colour change, using this indicator, did not take place till the formation of the basic salt.

The results being considerably above the theoretical, the titration was repeated, but precautions were taken to ensure that the substance was free from adhering moisture. Völker states that the crystals lose water of crystallisation on keeping and that the anhydrous substance gains weight by absorption. This had not been my experience but precautions were taken to remove any possible adhering moisture as this would give high results.

A small quantity of pure crystalline ajacine, 0.0525 gm., was carefully weighed and allowed to stand for 24 hours over phosphorus pentoxide in a vacuum desiccator. The substance then showed a slight tendency to acquire a yellowish tint, but far from losing weight was found to have made a very small gain and now weighed 0.0530 gm. It was returned to the vacuum desiccator for a further 48 hours, being weighed at the end of each 24 hour period, but no further change took place either in weight or appearance. No further darkening was observed and the crystals still retained their silky lustre. The weight remained at 0.0530 gm. This experiment thus demonstrates that pure crystalline ajacine is quite stable at normal temperatures and does not lose water of crystallisation even under high vacuum over phosphorus pentoxide. It showed the crystals



contained no adhering moisture. The increase in weight of 0.5 mg. was possibly due to slight resinification which would also account for the development of the slight yellow colour.

The titration was carried out in the same manner as before, using the same indicator, and it was observed that the change in colour of the titration solution from pink to yellow was gradual over 0.4 c.c. Readings were taken when the first colour change to orange took place. This was taken as the correct end-point since ajacine is a weak base and its salts with strong acids are in consequence slightly acid. The equivalent as obtained from this titration was 625. This figure is approximately double the molecular weight of 309.4 and, within the limits of experimental error, confirms the latter to a remarkable degree. It is also further evidence of the fact previously established by Völker that ajacine forms basic salts of the type  $(\text{Base})_2 \cdot \text{HCl}$ .

#### AJACINE OXALATE.

As a certain measure of success had been attained in preparing an oxalate of ajaconine (described later) inasmuch as good crystals of definite melting-point had been formed, it was considered that the same methods might be applied with equal success to the other bases which had been isolated. This was particularly desirable in the

case of ajacine as no satisfactory salts of this alkaloid had previously been obtained. Furthermore by using a dibasic acid such as oxalic acid, three types of salt were possible - acid, normal and basic - this last being one of the unusual characteristics of ajacine. It was therefore decided to try to crystallise the normal salt first and in the event of failure, to divide this solution into two parts, adding an equivalent of the base to one and an equivalent of the acid to the other. In this way solutions of the basic and acid salts would be formed.

Accordingly 0.62 gm. pure crystalline ajacine was dissolved in 45 c.c. anhydrous alcohol and an equivalent of oxalic acid, 0.126 gm., dissolved in an equal volume of the same solvent. 15 c.c. of each solution were reserved while the balance of the two solutions, containing exact equivalents for the normal salt, were mixed.

The mixture remained quite clear but the salt did not crystallise on standing, nor when the solvent was slowly removed in a vacuum desiccator. When the alcohol had entirely evaporated there was left a colourless transparent jelly which gradually hardened to a solid of glass-like consistency and appearance. There was no sign of crystallisation. Similar entirely negative results were obtained when alcohol 95%, 90% and 60% were each in turn used.

It was apparent alcohol was not a suitable medium for crystallising this salt so the solvent was removed by evaporation under reduced pressure and the transparent residue dissolved in 10 c.c. warm water. The substance was extremely soluble but the solution was distinctly cloudy and there was a suspicion that the salt was probably undergoing slight hydrolysis in the aqueous solution. The warm solution was cooled slowly and gradually concentrated in a vacuum desiccator but no crystallisation took place. It was evident that the normal oxalate was not readily crystallisable, so the alkaloidal residue was dissolved in 20 c.c. anhydrous alcohol and the solution divided into two equal parts. To one of these was added the reserved 15 c.c. ajacine solution and to the other the reserved 15 c.c. acid solution, thereby forming solutions of the basic and acid salts respectively.

It is convenient to discuss these separately. In the case of the acid salt the addition of a second equivalent of oxalic acid caused no apparent change in the solution, so after filtration, the solution was slowly concentrated in a vacuum desiccator. No crystallisation took place and eventually only a pale yellow syrup remained. The same disappointing results were experienced with diluted alcohols, 90%, 60% and 45% being each used in turn. Water was equally a failure as a crystallising medium. In each instance only a syrup

remained and it was observed after each operation that this syrup darkened in colour so that from a pale yellow to begin with it became reddish brown.

Having failed to crystallise the salt it was resolved to see if precipitation methods would be any more successful. The brown syrup was accordingly dissolved in 2 c.c. anhydrous alcohol and poured into 60 c.c. ether. A white cloudy precipitate formed immediately but instead of subsiding the substance slowly redissolved. As this was probably due to traces of water in the solution the experiment was repeated using anhydrous ether. The salt was recovered from the ether solution and dried in a vacuum desiccator until only a hard transparent varnish-like residue remained. This was redissolved in 5 c.c. anhydrous alcohol and poured quickly into 50 c.c. pure anhydrous ether. A copious white precipitate was immediately formed which rapidly clotted together on the bottom of the vessel. After a few minutes a brown patch was observed in the precipitate and this rapidly increased in size. The ether was sucked off on a Buchner funnel and the white precipitate collected on the filter but even before all the ether had filtered through, this was showing signs of discoloration. When the ether was entirely removed it darkened very rapidly, absorbing moisture from the atmosphere and forming a brown viscid mass. No

further experiments were possible but it is evident that this salt is very hygroscopic and shows a strong tendency to resinify.

The basic oxalate proved to be very unstable. It could not be crystallised from strong alcohol, and during attempts at crystallisation from dilute alcohol the base appeared to break down into simpler products. One of these crystallised out in the form of long needle-like crystals, but the quantity was too small to allow of any investigation beyond determining that it contained neither nitrogen nor oxalic acid.

#### BASE C.

The ethereal solution which contained this base was first distilled at a temperature not exceeding  $50^{\circ}$  until most of the ether had been removed. The concentrated liquor was highly coloured although the original solution had been virtually colourless. It was washed from the flask with dilute sulphuric acid and filtered into a separating funnel. The addition of a large volume of strong caustic potash liberated the alkaloid as a dense white precipitate. This was taken up by shaking with successive volumes of ether in the course of which much trouble was caused by the precipitation of considerable quantities of potassium sulphate. Later experiments confirmed that the use

of hydrochloric acid is preferable to sulphuric acid in extraction processes like the above involving caustic potash and ether, as potassium chloride is more soluble in strong alkaline solutions and is not precipitated on the addition of ether.

The alkaloid was now recovered from the mixed ether liquors by allowing these to evaporate slowly in an open basin. The alkaloidal residue was a pale brown viscid substance and obviously required further purification. It was redissolved in dilute hydrochloric acid and on addition of the acid considerable effervescence was observed showing the ether had taken up some of the alkali during extraction.

The resulting acid solution was still slightly coloured and after filtration the alkaloid was again liberated by the addition of an equal volume of strong caustic potash. The resultant precipitate was very dense and copious and quite obviously had absorbed a considerable proportion of alkali and other inorganic material. It was sucked off on a Buchner funnel but attempts to wash it free from inorganic impurities were only partially successful, and as part of the alkaloidal material was also taken up there was a strong suspicion that more than one base was present.

The alkaloidal precipitate was accordingly again dissolved in dilute hydrochloric acid to which solution were added the above washings. As it was

considered that the contaminating alkaloid might be ajacine, the solution was made alkaline with ammonia before precipitating in the usual way with caustic potash. As expected, the ammonia caused a substantial precipitate which was removed by shaking with ether. This ether solution was reserved for further treatment.

As a result of this preliminary extraction in the presence of ammonia, the aqueous liquor was practically colourless. The alkaloid was again recovered by precipitation with caustic potash and extraction with ether and after evaporation of the solvent remained as a soft pale brown jelly-like substance. With this was associated a small aqueous residue containing the usual inorganic impurities. As the presence of this aqueous liquor rendered the alkaloidal extract unsuitable for crystallisation, it was again dissolved in dilute hydrochloric acid and reprecipitated with potassium hydroxide. The precipitate was very dense and much whiter than hitherto. It was sucked off and dried in a vacuum desiccator over phosphorus pentoxide. Meanwhile the strongly alkaline mother liquor when tested for alkaloids with Mayer's reagent was found to give<sup>a</sup> definite positive response, and after standing for a few days clusters of needle shaped crystals were observed to grow in the solution. These were sucked off, dried and reserved for

examination later.

Meanwhile the precipitated base had dried to a light chalk-like consistency. Yield 2.20 gm. With a view to crystallisation, it was dissolved in a small quantity of 95% alcohol, forming a pale brownish coloured solution. A small quantity of potassium carbonate remained insoluble and was filtered off. A further quantity was precipitated on standing and it was apparent that the precipitated alkaloid had absorbed a considerable amount of caustic alkali which was being slowly deposited as potassium carbonate on standing. To facilitate the removal of this material, a stream of carbon dioxide was passed through the solution until precipitation of the carbonate ceased. This was removed by filtration and the solution set aside. After slow concentration the substance was eventually induced to crystallise in the form of compact masses of hard prismatic crystals. After recrystallisation from the same solvent these were obtained as large glistening well-defined prisms which melted sharply 165-166°.

For the estimation of water of crystallisation the crystals were dried over phosphorus pentoxide at 100° in vacuo.

17.583 mg. substance lost .022 mg. when dried for 5 hours at 100° under high vacuum

= 0.12%.



The base therefore contains only traces of adhering moisture and no water of crystallisation. This was confirmed by two further experiments on other samples of the alkaloid, both of which on being dried as above showed no loss in weight whatever.

With a view to determining the percentage composition of the alkaloid the following analyses were carried out.

(1) 3.769 mg. substance yielded 10.130 mg.  $\text{CO}_2$   
and 3.200 mg.  $\text{H}_2\text{O}$

= Carbon 73.30% : Hydrogen 9.43%.

4.165 mg. substance yielded 0.142 c.c. N  
at  $766/17^\circ$

= Nitrogen 4.04%.

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(2) 3.916 mg. substance yielded 10.510 mg.  $\text{CO}_2$   
and 3.150 mg.  $\text{H}_2\text{O}$

= Carbon 73.20% : Hydrogen 8.94%.

3.560 mg. substance yielded 0.130 c.c. N  
at  $748/16^\circ$

= Nitrogen 4.24%.

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Found	C	H	N
(1)	73.30	9.43	4.04
(2)	73.20	8.94	4.24
Calculated for $C_{21}H_{31}NO_3$	73.01	9.05	4.06

These results, which show good agreement with the theoretical, establish for this alkaloid the formula  $C_{21}H_{31}NO_3$  and this has been further confirmed by the analysis of certain salts of the base - notably the oxalate - and picrate. Although this formula does not agree with that suggested by Völker, a study of the properties of this compound leaves no doubt that it is identical with the base previously isolated and described as ajaconine.

The molecular weight was determined by Rast's camphor method with the following results.  
4.118 mg. ajaconine dissolved in 39.719 mg. camphor

Depression =  $13^{\circ}$ .

Molecular Weight = 319.

After allowing for experimental error this confirms with some certainty the molecular weight of 345.5 calculated from the above formula and therefore that this empirical formula corresponds

to the true formula of the base.

The equivalent weight of the base was now determined by volumetric methods. The pure crystallised ajaconine was dissolved in excess N/50 sulphuric acid and back titrated with N/50 sodium hydroxide. The end-point using phenolphthalein as an indicator was quite sharp and the equivalent thus obtained by this titration was 340.5. This shows close agreement with the calculated molecular weight of 345.5 and is therefore further confirmation of the true formula of the base as established by the above molecular weight determination.

As above obtained, ajaconine,  $C_{21}H_{31}NO_3$ , occurs in large well-defined glistening prisms which are anhydrous and do not lose their lustre on exposure. It is readily soluble in the usual organic solvents - alcohol, ether and chloroform, and can be crystallised from 95% alcohol. It is a strong base liberated from its salts by caustic potash but not by weaker alkalis such as ammonia.

The optical rotation was measured with the following results.

0.1612 gm. ajaconine in 10 c.c. chloroform = 1.612%.

temperature  $17^{\circ}$   $\alpha_D - 214^{\circ}$  in 1 dm. tube.

$\left[ \alpha \right]_D^{17^{\circ}} - 133^{\circ}$  in chloroform (C, 1.612).

AJACONINE OXALATE.

Völker reported that the salts of ajaconine showed little inclination to crystallise, and on account of the limited amount of material at his disposal he confined his experiments in this connection to the formation of the gold, platinum and picric acid compounds.

As no work seemed to have been done on the formation of salts with some of the simpler organic acids this appeared a promising field of investigation and a beginning was made with the oxalate. As there were at least two possible salts of this acid - the normal and acid salts - it was decided to combine one equivalent each of the acid and the base, thereby forming the normal salt and to add a second equivalent of the acid later.

Accordingly 366 gm. of the ajaconine were dissolved in 10 c.c. anhydrous alcohol and to this was added an exact equivalent of pure oxalic acid in the same solvent. The resulting mixed solution remained clear and colourless. It was slowly concentrated in vacuo but no crystallisation took place although there was distinct evidence of it on the sides of the vessel outwith the liquor. Eventually a syrup remained which on further drying to a glassy film showed a distinct radial structure. Apart from this there was further evidence of crystallisation at one point where numerous fine

needle crystals having grown out of the syrupy liquid were clustered together on the side of the vessel. Sufficient were collected to enable a melting-point to be taken and it was observed the substance softened and charred slightly at  $232^{\circ}$ , finally melting with decomposition at  $234^{\circ}$ .

Meanwhile the residue was redissolved in anhydrous alcohol but all attempts at crystallisation were unsuccessful. Alcohols 95% and 90% were also tried but each time with diminishing success and as the presence of water seemed to be a hindrance rather than a help, further experiments with diluted alcohols were abandoned. Acetone was also used but the salt was almost insoluble in this solvent.

Crystallisation having failed precipitation methods were now employed. After evaporation of the residue to dryness it was dissolved in a small quantity of anhydrous alcohol and poured into a relatively large volume of anhydrous ether. The salt was precipitated as a fine milky suspension which quickly adhered to the walls of the vessel and by forming a hygroscopic gummy mass which choked the filter defied all attempts to separate it from the solution.

Having thus failed to prepare the normal oxalate the entire salt was dried in vacuo and redissolved in anhydrous alcohol. A second equivalent of oxalic acid was now added and the clear solution concentrated in a vacuum desiccator. The

results to begin with were again disappointing, a transparent glassy residue remaining. This was redissolved in 95% alcohol and surprisingly it was not now so readily soluble as hitherto. Whereas 10 c.c. anhydrous alcohol had been ample to dissolve the residue of the normal oxalate without heat, the acid salt now required 30 c.c. and it was necessary to warm the solution. The results on this occasion were more successful and after concentration of the solution in vacuo, the salt crystallised in the form of bright glistening needles, many in the form of small rosette clusters, others singly or in pairs. These were removed and purified by recrystallisation from 95% alcohol.

The resulting crystals were small glistening needles which on drying in vacuo retained their lustre. They melted sharply with decomposition  $234 - 235^{\circ}$ , and are therefore identical with the crystals obtained during the attempted preparation of the normal salt. There was no loss in weight when they were dried over phosphorus pentoxide for several hours at  $100^{\circ}$  under high vacuum. They therefore contain no water of crystallisation.

In order to confirm that the acid salt had been prepared the oxalic acid in the molecule was estimated as calcium oxalate. Only a small quantity of the salt was available but the estimation was carried out with great accuracy and a control experiment demonstrated that results could be obtained to

within a very small percentage of error (1.4%).

The salt was dissolved in a small quantity of water and by the addition of excess calcium chloride in the presence of ammonia the oxalic acid was completely precipitated as calcium oxalate. This was carefully filtered off, washed with hot water and dissolved in a minimum of dilute hydrochloric acid. This solution was then acidified with dilute sulphuric acid and titrated with N/10 potassium permanganate.

Found  $\text{C}_2\text{H}_2\text{O}_4 = 20.55\%$ .

$\text{C}_{21}\text{H}_{31}\text{NO}_3 \cdot \text{C}_2\text{H}_2\text{O}_4$  requires  $\text{C}_2\text{H}_2\text{O}_4 = 20.67\%$ .

This result was in very good agreement with the theoretical and proved definitely that the substance was the acid salt.

The crystals were now analysed in order that the percentage composition of the substance might be determined.

4.314 mg. substance yielded 10.100 mg.  $\text{CO}_2$  and 3.040 mg.  $\text{H}_2\text{O}$ .

= Carbon 63.85% : Hydrogen 7.83%.

5.542 mg. substance yielded 0.158 c.c. N. at  $759/18^\circ$

= Nitrogen 3.33%

	C	H	N
Found	63.85	7.83	3.33
$C_{21}H_{31}NO_3 \cdot C_2H_2O_4$ requires	63.43	7.64	3.22

These results show good agreement with the theoretical and not only confirm the suggested formula  $C_{21}H_{31}NO_3 \cdot C_2H_2O_4$  for a jaconine acid oxalate but also the formula of the base itself.

A titration experiment was now carried out to determine the equivalent weight of the compound. Being the acid salt of a dibasic acid the equivalent weight of the substance should be equal to its calculated molecular weight. The salt was dissolved in an excess of N/50 sodium hydroxide and back titrated with N/50 sulphuric acid. Phenolphthalein was used as an indicator and quite a sharp end-point was obtained. The equivalent weight as thus determined was 438 which is in very close agreement with the calculated molecular weight of 435.5, corresponding to the formula  $C_{21}H_{31}NO_3 \cdot C_2H_2O_4$ .

#### AJACONINE ACETATE.

Having been successful in preparing an oxalate of a jaconine in pure crystalline form, attempts were now made to prepare salts of this base



with other simple organic acids, notably acetic acid. The methods employed in making the oxalate were again followed. Calculated equivalent quantities of the pure crystallised ajaconine and glacial acetic acid were separately dissolved in anhydrous alcohol and the solutions mixed. The resulting solution of ajaconine acetate was neutral to litmus and was concentrated very slowly in vacuo to a syrupy liquid which did not crystallise. In a vacuum desiccator this syrupy residue hardened to a transparent film which on exposure showed itself to be very hygroscopic. Further attempts were made to crystallise this salt from 90% alcohol but on each occasion the same syrupy hygroscopic residue remained and it was apparent the substance was not readily crystallisable.

The preparation of the formate was also attempted, but as this compound was even more hygroscopic and equally difficult to crystallise experiments in the formation of these salts were discontinued.

#### AJACONINE PICRATE.

Having experienced difficulties in preparing crystalline salts of ajaconine it was decided to try precipitation methods. Alkaloids as a rule form well defined compounds with picric acid and as these are usually insoluble in aqueous

or acid media they can very often be obtained by precipitation in an amorphous condition. Völker had already made some preliminary investigations on the picrate of this base and although his results were indifferent, it was decided in the first place to investigate the possibilities of his method.

To a solution of afaconine (1%) in dilute hydrochloric acid (N/5) was added an excess of picric acid in 1% solution. Hydrochloric acid of low concentration was deliberately used as Völker stated that strong acid prevents precipitation of the picrate. It was therefore surprising when no precipitation occurred and the experiment was repeated using a 5% solution of the base in just sufficient dilute hydrochloric acid to dissolve it. The first addition of picric acid caused the immediate formation of a yellow precipitate which quickly coagulated to a glutinous mass and adhered to the sides of the vessel. Further additions of picric acid were made till precipitation of the picrate ceased but it was found impossible to filter this substance on account of its viscid properties, and such as could be removed from the vessel immediately choked the filter. Furthermore it was now found that the precipitation of the picrate had by no means been complete and a large proportion still remained in solution. On exposure this substance formed a gelatinous skin on the surface

of the liquor which still further hindered filtration. From these experiments it is obvious a jaconine picrate is much more soluble than the usual alkaloidal picrates and for this reason alone preparation of this substance by the above methods is not to be recommended, especially when there is added to it the difficulties of filtration. Völker makes no mention of these difficulties and claims to have isolated a picrate of indefinite melting-point, 115-120°, but did not have sufficient to enable him to carry out an analysis of the product. In the light of my experience his claims in this respect are open to doubt and it is significant that the melting-point of his picrate is virtually the same as that of picric acid itself.

It was now decided to combine the picric acid and the base in anhydrous alcohol and in order to avoid, as far as possible, having excess of the acid the calculated molecular proportions were used. A transparent yellow solution resulted which was very slowly concentrated in a vacuum desiccator. No crystallisation took place but on the contrary, as the solution slowly evaporated the picrate separated as a soft mobile yellow substance after the manner of a heavy oil from water. On further evaporation this substance hardened to a transparent yellow film on the bottom of the vessel. Similar results were obtained using 60% alcohol.

As picrates are known to crystallise fairly readily from benzene the dried residue described above was digested in a water-bath with four successive portions of this solvent. Although the substance did not appear to be very soluble the solution was coloured an intense yellow. This hot benzene solution was set aside but no crystallisation took place either on cooling or after the solvent had been removed by slow evaporation in vacuo. Instead there remained the familiar transparent yellow residue which was surprisingly small in quantity considering the intense yellow colour of the solution and is an indication of the slight solubility of the picrate in benzene.

As it seemed most improbable that this substance would crystallise, precipitation methods were again resorted to. On account of the relative insolubility of the picrate in benzene this liquid was an obvious first choice. The substance was dissolved in a small quantity of anhydrous alcohol and poured quickly into the benzene. The results were disappointing for although a fine precipitate formed it could not be filtered off and settled out on standing as the familiar transparent varnish.

Much better results were obtained using pure anhydrous ether. The picrate was again dissolved in a small quantity of alcohol and this

solution poured into ten times its own volume of anhydrous ether. A copious yellow precipitate was immediately formed and within half an hour had completely subsided. It was quickly sucked off on a Buchner funnel, washed liberally with dry ether and dried over calcium chloride in a vacuum desiccator. It is necessary to continue washing with ether until all traces of alcohol have been removed otherwise the precipitate on drying hardens to a tough glue-like mass.

Meanwhile a control experiment was carried out and it was demonstrated that under the same experimental conditions picric acid itself is not precipitated when poured into ether. This showed that the picrate was unlikely to contain as an impurity any appreciable quantity of free picric acid.

As thus obtained ajaconine picrate is a pale yellow amorphous powder. It is very soluble in alcohol and fairly soluble in water, but almost insoluble in benzene, ether and chloroform. The melting-point is indefinite. Although the substance sinters as low as  $65-70^{\circ}$  it does not melt until  $95-98^{\circ}$ .

In order to establish the composition of this picrate an estimation was made of its nitrogen content.

2.876 mg. ajaconine picrate yielded  
0.233 c.c. N. at 755/15°

= Nitrogen 9.62%.

Found N = 9.62%.

$C_{21}H_{31}NO_3 \cdot C_6H_2OH(NO_2)_3$  requires N = 9.75%.

This result is in good agreement with the calculated quantity, and as other possible combinations involving two or more molecules of picric acid would contain larger percentages of nitrogen the formula of ajaconine picrate is shown to be  $C_{21}H_{31}NO_3 \cdot C_6H_2OH(NO_2)_3$ . Incidentally this is further indirect confirmation of the formula of the base itself.

Meanwhile the crystals unexpectedly recovered from the strongly alkaline ajaconine mother liquor were examined. These were obtained in clusters of stout needles, melting-point 95-98°, and gave the usual alkaloidal reactions. As ajaconine has a higher melting-point this substance was obviously a new alkaloid and as it had originally crystallised from an aqueous medium attempts were made to recrystallise it from water. These were only partially successful inasmuch as the crystals so obtained still showed an indefinite

melting-point.

0.1 gm. of the original crystals were dissolved in hot water and as expected were not very soluble, 100 c.c. water being required. Nevertheless crystallisation did not take place immediately on cooling and was only effected when the solution was concentrated on a water-bath to about one-fifth of its volume. Crystals then appeared in the hot liquor and were filtered off on cooling. As thus obtained the alkaloid was in the form of long fine colourless needles, the melting-point of which was still indefinite from  $95^{\circ}$  to  $100^{\circ}$ . The physical and chemical properties of this base await further study, but meanwhile it was demonstrated by experience that although this alkaloid is not precipitated from acid solution by ammonia it can be extracted completely from ammoniacal solutions by shaking with ether.

The ethereal solution mentioned on page 45 now came up for attention. This was obtained by extracting a solution of crude ajaconine with ether after making alkaline with ammonia, and in addition there were present other ether extracts believed then to contain ajaconine and obtained during the extraction of Base B. These mixed ethereal liquors were allowed to evaporate and the alkaloidal residue dissolved in dilute hydrochloric acid. After

filtration this solution was made alkaline with ammonia and the ajacine removed by one extraction with ether. The remaining alkaloids were precipitated by caustic potash and also taken up by ether. When this latter ether extract was evaporated to dryness, a considerable quantity of alkaloid remained in hard cake-like masses which being of a brownish colour were not yet pure enough for crystallisation. This residue was accordingly redissolved in dilute hydrochloric acid and to remove possible traces of ajacine was made alkaline with ammonia and shaken with ether. Very little ajacine was present as no precipitate was formed on addition of the ammonia. Nevertheless the alkaloids present were almost entirely removed from the ammoniacal solution by continued shaking with the ether, which was surprising, as this base was thought to be ajaconine.

On evaporation of this ethereal solution a colourless and partly crystalline residue remained which was considered sufficiently free from obvious impurities to be crystallisable.

It was accordingly dissolved in 95% alcohol forming a pale brown solution which was concentrated in vacuo. The base eventually crystallised in small needles. These were slightly coloured, but white transparent crystals, melting-point 108-110°, were



obtained by recrystallisation from the same solvent. Work is still proceeding on this base, but from information already available it is almost certainly identical with the base which crystallised from the strong potash liquor after precipitation of ajaconine.

#### WEAK BASES.

The solution containing these presumed weak bases had been obtained by a preliminary extraction of the original acid liquor with chloroform. These chloroform extracts which were much coloured were now distilled under reduced pressure and the chloroform recovered. The concentrated liquor was dried by further evaporation in vacuo until reduced to a dark brown semi-solid mass. The yield of total impure weak bases thus obtained was 5.12 gm. This gummy residue was now extracted with successive quantities of dilute sulphuric acid until only a hard brittle resin remained. This still retained alkaloid which was very difficult to remove by acid extraction and required special treatment. The difficulties arose principally from the insoluble nature of the resin which prevented the alkaloid coming in contact with the acid. To overcome this disadvantage the resinous substance was dissolved in a small quantity of alcohol. It was very soluble and dissolved readily. The concentrated alcoholic solution was

then poured into a large volume of dilute acid which precipitated the resin in a fine state of division and dissolved out most of the alkaloid. After filtration the acid solution was added to the principal acid extract. This process was repeated three times in all before the residue was finally exhausted.

The mixed acid liquors were made alkaline with ammonia and a dense white curdy precipitate was formed. This proved to be readily soluble on shaking with ether and as the ether solution showed an intense blue fluorescence the base was assumed to be ajacine. The ethereal extract was accordingly worked up in the manner already described under Base A and although the continual occurrence of resinous impurities caused considerable crystallization difficulties, sufficient of the pure anhydrous material was obtained to confirm its identity. When the anhydrous alkaloid was prepared by the spontaneous evaporation of an ether solution of the base it showed a melting-point  $70-73^{\circ}$ . This was in very good agreement with the melting-point ( $70-75^{\circ}$ ) of a sample of anhydrous ajacine prepared in a similar manner from the pure crystalline material.

As the alkaline mother liquor after removal of ajacine still contained considerable quantities

of alkaloid it was shaken with chloroform till exhausted. Investigation of a sample of this liquor revealed that the alkaloidal extract was very similar if not identical with the extract containing Base B. There seemed no point, therefore, in carrying on a separate investigation and the two chloroform solutions were accordingly mixed. The alkaline mother liquor now contained no further alkaloidal material and was rejected.

While this diversion did not reveal the presence of any new bases, nor any better methods of isolating those already known, it was an interesting practical confirmation that ajacine is decidedly a weak base forming compounds readily hydrolysed in aqueous solution. As expected, no ajaconine was present in this extract.

#### BASE B.

The chloroform solution containing Base B was pale reddish brown in colour and was obviously much contaminated with the resinous and other impurities which had given such trouble in previous extractions. The solvent was removed by distillation under reduced pressure and the residual liquor concentrated to a soft extract in the usual way. This alkaloidal residue was dissolved in dilute sulphuric acid and the solution filtered, thus removing much of the resinous impurity. The filtered

liquor was now made alkaline with ammonia and a small quantity of a jacin, which was thereby precipitated, was removed by shaking with ether. The principal alkaloid was now extracted with chloroform, the residue on removal of the solvent being now much paler in colour and obviously as pure as could be attained by these methods.

Crystallisation experiments carried out on small samples of this material revealed the fact that of various solvents used, acetone alone seemed likely to be productive of good results. The residue was accordingly dissolved in pure warm acetone in which it was very soluble, forming a reddish brown solution. This was filtered and allowed to stand for two days during which time considerable evaporation took place, the solution being now reduced to the consistency of a thick syrup. Although no crystallisation had taken place signs of this were not lacking round the sides of the vessel and it was not surprising when the addition of a little acetone caused the precipitation of a fine crystalline powder from the supersaturated solution. This was carefully filtered off, washed with acetone and dried in vacuo. The washings were added to the mother liquor which was again concentrated and yielded a further quantity of crystalline material. This process was repeated eight times in all with diminishing results, until the alkaloidal mass gave no further yield to this

treatment. It was then boiled with acetone under a reflux condenser but the results were again negative and so far as this particular base was concerned the residual mass was apparently exhausted. It may be remarked here that later work showed that this process could be carried out much more quickly by initial extraction with boiling acetone for one hour under a reflux condenser. Most of the alkaloid was thereby obtained in the first stage and the yield was complete by the third extraction. The residue was set aside for further treatment.

The crystalline substance thus isolated was a fine greyish white powder and tests showed that it was an alkaloid. By repeated crystallisation from acetone it was finally obtained pure in the form of large rhombic crystals which melted sharply  $210-211^{\circ}$ . They were found to be anhydrous. When dried for 6 hours over phosphorus pentoxide at  $100^{\circ}$  under high vacuum

6.422 mg. substance lost 0.012 mg.

= 0.19%.

This loss is obviously due to adhering moisture. The substance therefore contains no water of crystallisation.

An analysis of these crystals was carried out to determine the percentage composition of the base, and gave the following results.

4.030 mg. substance yielded 9.490 mg.  $\text{CO}_2$   
and 3.192 mg.  $\text{H}_2\text{O}$ .

= Carbon 64.22% : Hydrogen 8.80%.

3.674 mg. substance yielded 0.110 c.c. N  
at  $754/24^\circ$

= Nitrogen 3.41%

5.028 mg. substance yielded 11.835 mg.  $\text{CO}_2$   
and 3.975 mg.  $\text{H}_2\text{O}$ .

= Carbon 64.20% : Hydrogen 8.79%.

Found	C	H	N
(1)	64.22	8.80	3.41
(2)	64.20	8.79	
Calculated for $\text{C}_{22}\text{H}_{37}\text{NO}_6$	64.21	9.06	3.40

The close agreement of these values indicates that the base as thus crystallised is a pure and homogeneous substance. To it is assigned the formula  $\text{C}_{22}\text{H}_{37}\text{NO}_6$  and as it is a new addition to the list of Delphinium alkaloids, with no previous

mention in the literature, the name of "ajacinine" is here proposed for it.

Ajacinine, melting-point  $210-211^{\circ}$ , crystallises in large transparent orthorhombic crystals, which gradually lose their brilliance on keeping. It is very soluble in alcohol, chloroform and acetone and appreciably soluble in water and ether. Its aqueous solution is slightly alkaline to litmus. When a solution of ajacinine in chloroform is allowed to evaporate slowly in an open vessel, the base crystallises in an irregular ice-like formation and on standing this slowly loses its transparency, becoming opaque and white. This phenomena is closely associated with the tendency of the orthorhombic crystals to lose their brilliance and although not yet accounted for, is probably due to some change in crystalline structure. The melting-point remains unchanged by this development and the opaque substance can be reconverted into its crystalline counterpart by solution in chloroform and removal of the solvent.

A molecular weight determination was carried out by Rast's camphor method with the following results.

0.530 mg. ajacinine dissolved in 5.694 mg. camphor

Depression =  $9.3^{\circ}$ .

Molecular Weight = 400.

Within the limits of experimental error this shows quite good agreement with the calculated molecular weight of 411.5 and confirms that  $C_{22}H_{37}NO_6$  is the true formula.

A titration experiment to determine the equivalent of this base was now carried out in the same manner as with the two other alkaloids. 0.072 gm. of pure crystalline ajacinine was dissolved in excess N/50 sulphuric acid and back titrated with N/50 sodium hydroxide, methyl red being used as an indicator. Being a weak base the end-point was not sharp but a reading taken on the first colour change from pink to orange gave an equivalent of 418.5, which is confirmatory of the calculated molecular weight of 411.5.

Optical rotation measurements were made on the pure crystalline substance.

0.0389 mg. dissolved in 7 c.c. chloroform = 0.5557%.

Temperature  $17^{\circ}$   $\alpha_D + 29^{\circ}$  in 1 dm. tube

$\left[ \alpha \right]_D^{17^{\circ}} + 52^{\circ}$  in chloroform (C, 0.5557).

During all this work it was suspected that this alkaloid ajacinine might be an acetone derivative formed by condensation of one of the unknown bases with the acetone solvent during crystallisation. The iodoform reaction was carried out several times on the pure crystalline material



previously hydrolysed with caustic soda, but with inconclusive results. A slight aroma of iodoform could be detected on each occasion but as the quantity of afacinine used was very small, the sense of smell was not deemed a sufficiently accurate instrument to rely upon exclusively. It was therefore decided to determine the quantity of acetone, if any, present in the compound. For this purpose Messinger's method as modified by Goodwin (1920) was regarded as the most suitable. In this estimation, iodine is added to the solution of acetone in the presence of excess caustic soda. Iodoform is formed and iodine equivalent to the acetone present is thereby used up. Excess of iodine is taken up by the sodium hydroxide but is liberated when the solution is exactly neutralised and can be estimated by titration with sodium thiosulphate, using starch as an indicator. By difference the amount of iodine taking part in the iodoform reaction is known and from this factor the acetone present can be calculated.

For the purpose of this particular determination the compound had first to be hydrolysed to liberate any acetone which might be present. Accordingly 0.2760 gm. of the pure crystalline substance was introduced into a flask and dissolved in 10 c.c. N/1 sulphuric acid. 50 c.c. N/1 caustic

soda was then added and the solution heated in a water-bath for 10 minutes, the flask being closed meanwhile with a loosely fitting stopper to prevent loss of acetone. Having taken steps to hydrolyse the suspected acetone compound, the flask was then attached to a condenser and the solution distilled until reduced to 25% of its original volume. The distillate was carefully collected and estimated by Messinger's method as above described. For comparison a blank estimation using distilled water was carried out simultaneously. The result was entirely negative. No acetone whatever was present in the distillate and it is thereby confirmed that ajacinine is not a complex alkaloid-acetone compound, as was at once time suspected, but the alkaloid itself.

Experiments in preparing both the normal and acid oxalates of this base were unsuccessful owing to the difficulties of crystallisation. Precipitation methods were also tried but these salts, especially the acid salt, are very hygroscopic and syrupy residues were the only results achieved.

The alkaloidal residue from which ajacinine had been separated was now examined. After removal of the acetone, the dried powdered residue was a yellowish brown transparent substance which bore some resemblance to powdered colophony resin.

Its solutions with various solvents, including dilute sulphuric acid, were characterised by a powerful green fluorescence. The substance is apparently amorphous and so far all attempts at crystallisation have been unavailing. Different solvents were used - alcohol, acetone, ethyl acetate, benzene, petroleum ether and water - with varying results, but in no case was a crystalline product obtained. The substance was at first much contaminated with the other bases, especially ajacine and the unnamed base associated with ajaconine but these were eliminated by one or other of the above solvents. The substance is almost insoluble in ether and petroleum ether and precipitation methods taking advantage of this were tried but with disappointing results. The possibilities here have not yet been fully explored and experiments are still proceeding.

The most satisfactory results of all these attempts to obtain a pure product were achieved with petroleum ether 40/60°. After the failure of many of the crystallisation experiments, an effort was made to remove the impurities by extraction with those solvents, such as petroleum ether, in which the base itself seemed least soluble. Evaporation of the petroleum ether extract revealed a minute quantity of a white micro-crystalline powder which tests showed to be an alkaloid. The melting-point

of this substance was indefinite - about  $75^{\circ}$  - which was akin to that of the parent body. Therefore instead of dissolving out the impurity, it appeared as though the petroleum ether was extracting the base itself even though only in minute quantity.

Accordingly 5 gm. of the powdered impure substance was extracted for one hour with 500 c.c. boiling petroleum ether under a reflux condenser. The solution was filtered off while warm and extraction continued with 300 c.c. and then 200 c.c. of the same solvent. The mixed filtrates on cooling overnight were observed to deposit a small quantity of the base. The solution was distilled and when reduced to about 150 c.c. was poured into a beaker where the remaining solvent was removed by spontaneous evaporation. The residue, weighing about 1 gm., was a fine white crystalline powder.

This process of extraction was repeated several times until 4 gm. of the base had been recovered. During this time the fine crystalline powder as first extracted seemed to lose its crystalline character to some extent and had changed to a brittle white solid which adhered to the sides of the vessel like caked sugar. The observed melting-point was indefinite between  $75^{\circ}$  and  $85^{\circ}$ . For further purification a portion of this material

was subjected to a second series of petroleum ether extractions but the results were disappointing. Not only did the substance appear even less soluble than before but it showed a tendency to acquire a yellow tint and did not seem so pure. Further crystallisation experiments on this partially purified substance were no more successful than before.

Although obviously not yet sufficiently pure, an analysis of this substance was carried out. The results did not indicate a suitable formula but show an interesting approximation to the corresponding figures for the associated base ajacinine.

	C	H	N
Amorphous Base	62.97	8.45	3.47
Ajacinine	64.22	8.80	3.41

With a view to obtaining salts which might crystallise and lead to separation of the pure base, a few initial experiments in salt formation were carried out. Owing to lack of material these trial experiments were necessarily incomplete but sufficient information was forthcoming to indicate

that these derivatives have the disadvantage common to most of the salts of these alkaloids of being very soluble and difficult to crystallise. The hydrochloride and hydrobromide were both exceedingly hygroscopic, while the hydriodide decomposed during concentration forming with the base an insoluble brown iodine derivative. The picrate was exceedingly soluble for a salt of this type and could not be isolated although experiments are still proceeding. Lastly this base does not seem to form a methiodide, or if it does, this shows the same non-crystallisable tendencies.

To sum up, the extract containing Base B would appear to consist of two bases, one crystalline and the other amorphous. The latter base cannot be crystallised nor does it yield crystallisable salts. Its solubility relationships are similar to those of ajacine. Its aqueous solution shows a faint alkaline reaction and being a weak base can be extracted from acid solution by chloroform.

#### NOTE ON FIXED OIL.

In an initial survey of this work it is immediately obvious that in their general chemical composition the seeds of Delphinium Ajacis show a family resemblance to the seeds of other Delphinium species and especially to those of D. Staphisagria

and D.Consolida, whose seed constituents have been the most fully investigated. A study of the physical and chemical characters of the fixed oil would appear to place it in the group of non-drying vegetable oils, although further information regarding its actual chemical composition is necessary before a final decision can be made.

One interesting point is the unusually high acid value of 132.2 which expressed as oleic acid indicates the oil contains approximately 66% of free fatty acids. The amount of free fatty acids in commercial oils is often very considerable and is usually a variable factor depending on the method of production. For instance, in 89 samples of olive oil for lubricating use, Archbutt(1884) found from 2.2 to 25.1% of free oleic acid. Such excessive quantities are usually the result of preparation from fermented olive residues, but in superior grades of olive oil the proportion of free acid is much smaller. The same remarks apply to many of the other oils found in commerce. It would thus appear that in most fixed oils as present in the living seed, fruit or other plant organism, the fatty acids are combined as glyceryl esters. It is therefore a remarkable fact that the seeds of this Delphinium species should secrete an oil in which such a high proportion of the fatty acids are free

and not combined. Besides being an interesting biological fact raising much wider issues, it is perhaps one explanation of the remarkable insecticidal properties of the seeds of this and other *Delphinium* species. For instance, stavesacre (*Delphinium Staphisagria*) seeds have been used since the Middle Ages for the destruction of pediculi, and an ointment for this purpose was official in the British Pharmacopoeia from 1885 onwards until it was omitted from the 1932 edition. As it was prepared by extracting the stavesacre seed with hot melted fat, it obviously contained most of the fixed oil from the seed. Further, in an interesting paper, Williams (1914) described experiments on larkspur (*D. Ajacis*) seed and proved conclusively that the insecticidal value of extracts which he prepared was entirely due to the fixed oil present and not to their alkaloidal content. He gave no explanation of this fact but it seems to me not unlikely that it is connected with the high acid value of the oil.

The low specific gravity of 0.905 is another unusual feature of this oil and is probably the direct result of the high acid content. The specific gravity of oils belonging to the non-drying group range from 0.912 to 0.920 but all contain relatively small quantities of the free acids. On



the other hand, pure oleic acid of commerce has a specific gravity of 0.900 at 11.8°, (Allen's Commercial Organic Analysis, 5th Ed. Vol.2,543) so that a high proportion of free acid is bound to coincide with reduced specific gravity.

Finally, it is of special interest to note that Markwood(1924) in his work on the seed of D.Consolida and again in 1927 on the seed of D. Staphisagria isolated and described the fixed oils and in both cases high acid values and low specific gravities are recorded.

A question of some interest lies in the fact that few, if any, known vegetable oils show such high acid values. Should it eventually prove true that this is characteristic of the delphinium oils, and of them only, the biological significance of the matter will have to be further explored.

### SUMMARY

- (1) The seeds of Delphinium Ajacis contain approximately 39% of fixed oil and 1% total alkaloids. The fixed oil is of the non-drying variety and is characterised by a high acid value equivalent to 66% free fatty acids.
- (2) Five alkaloids are present, four of them crystalline and one amorphous. Of the four crystalline bases, ajacine and ajaconine had previously been described, while a third is a new addition to the list of Delphinium alkaloids and has been given the name of ajacine. The fourth base has not yet been obtained in a state of purity.
- (3) The formula  $C_{16}H_{21}NO_4 \cdot H_2O$  found for ajacine differs slightly from that suggested by Völker. Ajacine is a weak base and it has been confirmed that it forms basic salts of the type  $(Base)_2HCl$ . The oxalates of this alkaloid are very soluble, hygroscopic, and difficult to crystallise.
- (4) The formula of ajaconine, stated by Völker to be  $C_{17}H_{29}NO_2$  is now found to be  $C_{21}H_{31}NO_3$  and this is confirmed by an analysis of two of its compounds. It forms an acid oxalate

$C_{21}H_{31}NO_3 \cdot C_2H_2O_4$ , melting-point 234-235°  
with decomposition, which crystallises from  
alcohol in small glistening clusters of  
needles. By precipitation in anhydrous  
ether, a picrate  $C_{21}H_{31}NO_3 \cdot C_6H_2(O_2NO_2)_3$ ,  
melting-point 95-98°, is obtained as a pale  
yellow amorphous powder.

- (5) A new base, ajacinine, melting-point 210-211°,  
has been obtained in large orthorhombic  
crystals by crystallisation from acetone.  
It has the formula  $C_{22}H_{37}NO_6$  and is anhydrous.
- (6) The amorphous alkaloid has not yet been  
obtained in crystalline form. It is very  
soluble in most organic solvents except  
ether and petroleum ether. The purest  
form of this base yet obtained was prepared  
by repeated extraction with petroleum ether.

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