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Comparative study of Aconitum lutescens of the Bitterroot valley and the official Aconitum napellus

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A COMPARATIVE STUDY

of

ACONITUM LUTESCENS OF THE BITTER ROOT VALLEY

AND THE OFFICIAL ACONITUM NAPELLUS

by

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Preface

This thesis is the result of research involving Aconitum lutescens of the Bitter Root Valley. A thorough examination of all available literature revealed that very little work had been done on any of the North American species of Aconite and particularly had none been done on the western species, (with the exception of some on Aconitum Columbianum).

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Introduction

On account of the geographic differences in the several sections of this large state many kinds of plants are found within its borders. A great many resemble official drugs and grow in abundance within the Montana boundaries and especially in this district. Under such conditions the field for plant chemistry and pharmacological research is almost unlimited.

One of the plants native to this district and a member of the same genus as the official drug is Aconitum lutescens.

The official drug, Aconitum Napellus, is a native of southern and central Europe and imported from that country. A study of this native, yellow flowered Aconite was begun for the purpose of comparing it with the foreign medicinal species.

The name Aconitum is derived, according to Pliny, from the Black Sea port Aconie, or it may also come from the French Acone, a town in Bithynia where the plant grows plentifully. Others claim that the etymology of the drug's name is derived from the Greek "a", without and "konis", dirt, referring to the fact that the plant is able to grow on stony ground. Be that as it may, the first account of a plant designated Aconitum or Akoniton occurs in the writings of Theophrastus.

The genus Aconitum belongs to the Ranunculaceae commonly known as the Crow-foot or Buttercup family. The Ranunculaceae is now regarded as a primitive group of herbaceous dicotyledons closely related to the Magnoliaceae and other low woody dicotyledons. The family includes about thirty genera and twelve hundred species of herbaceous perennials including a few annuals and aquatic species, a few low shrubs and woody climbers. Many of the species contain an acrid poisonous juice. The Ranunculaceae has a wide distribution in the Arctic regions and at high altitudes in the mountains but members of the family are more or less rare in the tropics and in the southern hemisphere. Species of the genus under consideration (Aconitum), have been found growing at an altitude of 16,000 feet in the Himalayas. On the other hand, specimens of Aconitum lutescens sent to the College of Pharmacy, University of Florida at Gainesville, for the medicinal plant garden died out after one year.

The genus Aconitum consists of many perennial herbs. The leaves are palmately lobed or dissected and the flowers are showy in terminal racemes and panicles. The flower is zygomorphic with five colored petal-like sepals. The upper sepal is large and helmet shaped or prolonged saccate. The petals number two to five when present. The upper two

have long nectariferous claws and irregular spur-like blades concealed within the hood. The other three are lower and are either very minute or obsolete. The stamens are numerous, free and hypogynous; the pistils are three to four in number, sessile and free. The fruit consists of three or four beaked follicles containing numerous seeds. The genus is highly prized for its ornamental species and two species are used in medicine, namely Aconitum Napellus official in the U.S.P. and Aconitum ferox in the French Codex.

The genus Aconitum occupies a unique position in history, mythology and tradition. From the very earliest date its toxic properties have set it apart as something sinister and evil. It offered such a fascination of weirdness that it might well be imagined as growing in the "jardin funeste" of Rappaccini. It is not hard, therefore, to understand how this plant engaged the attention of earliest man as something evil in origin, and indeed this reputation of Aconite is encountered at the very threshold of history.

Like all history that of the "poison-lebre" began in the region of the myths. These plants have been mentioned in the mythology of many nations and have involved such characters as Thor, Thyr, Chiron, Medea and Perses.

To trace the history of Aconite one needs only to

chronicle the events of poisons and the important part they have played in world history.

Aconite is native to the Alps and Pyrenees as well as to the mountains and highlands of Germany, Austria, Denmark and Sweden. The whole of Siberia and the Himalaya mountains to the altitude of 16,000 feet are said to harbor the plant. Its distribution and the ease with which it could be obtained coupled with the early knowledge of its virulent properties account for its frequent appearance in the annals of crime.

Aconite as a drug or remedy received little attention from physicians of the Middle Ages although the literature of the period is full of references to it, for its poisonous nature was well-known to common people because it frequently caused death as the result of being gathered and eaten by mistake for radishes and carrots. In some parts of Europe, however, it appears to have been esteemed as a medicine. In a work published by the Welsh Mss. Society in 1861 entitled "The Physicians of Myddvai" (thirteenth century) it is designated as "a plant every physician should grow." The drug was introduced into medical practice by Storck of Vienna in 1762 who was very enthusiastic in recommending it, and it enjoyed some popularity as a medicine for some time but later passed into comparative

disuse. The discovery of the active principle, aconitine, was made in 1821 by the Genevese pharmacist, Perchier, and independently at about the same time by Geiger and Hesse and by Brandes.

A great deal of investigation has been conducted on the various species of aconite but the research work on Aconitum Napellus far exceeds that done on any other one species of the genus. On this account, therefore, this work will be a comparison of Aconitum Napellus official in the U.S.P. and Aconitum lutescens of the Bitter Root Valley, Montana.

Aconitum Napellus:

This species which is the most important member of the genus has been used for many centuries, and has attained much popularity as a medicine. It is commonly known as Aconite Root, Monkshood, Wolfsbane, Friar's Cap, Cuckoo's Cap, and Blue Rocket. The entire plant is acrid in taste and poisonous in action. At the present time, however, only the tuberous roots are official in the U.S.P. Several other countries still recognize the dried herbs and have formerly recognized the flowering tops. The literature is redundant with papers on Aconite and many erroneous conclusions have led to much confusion both chemically and pharmacologically.

The toxic properties of Aconite have been known since ancient times but the drug was not used medicinally until about the thirteenth century. It was introduced into regular practice in Vienna about 1762 by Störck. It was from this species that the active principle, aconitine, was obtained in 1821 by the Genevese pharmacist, Perchier. It was also isolated at about the same time by Geiger and Hesse, and also by Brandes. Morson has been credited with being the first person to obtain aconitine (1839) in crystalline form from the tubers of Aconitum Napellus. The pharmacology and therapeutics of Aconite was the subject of an

essay by Fleming (1844), for which he was awarded a gold medal by the University of Edinburgh.

Professor Berg (1862), subjected three species of Aconitum, namely A. Napellus L, A. Variegatum L., and A. Stoerkianum Reichenb., to a searching examination of their morphological and anatomical conditions. Professor Mittenheimer (1862) directed the attention to the importance of using wild grown aconite for pharmaceutical preparations instead of the cultivated.

Hirtz (1863) working on Aconitum Napellus found that the extract from the root possessed the peculiar properties of the plant more than twenty times as strong as that from the leaves. Also that Aconitine differed in its effects, somewhat, from the preparations of the plant and did not produce spasmodic symptoms as was the case with the latter.

C. Schroff (1863) published a thorough pharmacognostical and therapeutical history of the various species of Aconite which is given at some length in his handbook. He insisted on the presence, in Aconitum Napellus, of an acrid principle which was wanting in Aconitum Lycoctonum. Schroff also found that the alcoholic extract of Aconitum Napellus on keeping formed grape sugar which he thought might be a product of the decomposition of one of the active principles.

T. and H. Smith (1864) discovered a new alkaloid in

Aconitum Napellus which they named aconella. It did not appear to be poisonous and in many of its physical and chemical characters and in its atomic weight closely resembled narcotine. They considered the irregular effects of commercial aconitine to be due in part to contamination with aconella.

Professor Jellett (1864) confirmed the identity of narcotine and aconella by the discovery that their action was the same on polarized light.

Morson (1864) obtained from aconite root a body analogous to aconitine, though less powerful which he called napellina.

G. Krehbiel (1864) on examining a crystalline deposit present in tincture of aconite root found it to be cane sugar and lime, thus confirming a similar observation made by Professor Schroff in respect to this preparation and several of the narcotic extracts.

Professor W. Proctor Jr. (1866) made a comparative analysis of American and European Aconite and observed that the latter yielded about half as much aconitine as could be obtained from the former. He stated that the leaves of American Aconite (grown in Columbia) might be used when the plant was two years old but that the root should not be used before it was three or four years old.

In a paper (1871) on the alkaloids of the genus Aco-

nitum contained in the tubers of the different species, Professor Flückiger arrived at the conclusion that aconitine is contained in all blue-flowered European and Himalayan species of Aconitum.

The first aconitine to be prepared in crystalline and in apparently pure form was isolated from a mixture of the amorphous bases of tubers of Aconitum Napellus by Duquesnel in 1871. Duquesnel's aconitine was a faintly yellowish solid, crystallizing in transparent prisms sometimes a centimeter in length, and fusing at 188.5° C. Its solubility in water was one part in 4431 parts. It was readily soluble in chloroform and benzene, less readily in alcohol and ether and hardly at all in petroleum ether. It formed crystalline salts and alcoholic solutions of these salts were dextrogyrate; aqueous solutions of its salts were laevogyrate. It was easily hydrolyzed by acids, alkalis or water and high temperatures with the formation of aconine, benzoic acid and acetic acid. Duquesnel assigned the formula $C_{25}H_{39}(CH_3CO)(C_6H_5CO)NO_9$ to this alkaloid and called it acetylbenzoyl aconine.

C. R. Alder Wright has done a great deal of work on the alkaloids of the genus and particularly on those of Aconitum Napellus. Those investigated (1876) were some prepared by T. B. Groves and later described by him (1877).

The conclusion reached by Wright as a result of these experiments was that there was naturally present in Aconitum Napellus more than one alkaloid forming well-crystallized salts, or that changes produced in alkaloid by methods of extraction resulted in a mixture of at least two distinct salts, one of which, if not absolutely inert, was very much less active than the other. The results of these experiments were not entirely satisfactory for several reasons, chief of which was that methods of extraction adopted by Groves were likely to have brought about changes and decomposition of the alkaloids originally present and also because Duquesnel by a different method of extraction had obtained a considerable yield of a highly active well-crystallized body. Wright (1878) accordingly undertook to make a thorough revision of all the work that had been done. As a result of experiment on Aconitum Napellus treated by Duquesnel's process, he reached the following conclusions: (a) A crystallizable, alkaloid insoluble in a solution of potassium carbonate, difficulty crystallizable from ether and giving numbers agreeing with formula $C_{33}H_{43}NO_{12}$ was formed. (b) A second alkaloid or mixture of bases which did not crystallize well and did not yield crystalline salts was also formed. This alkaloid had a lower molecular weight than aconitine and contained more

carbon and hydrogen. (c) A third substance isolated consisted of a non-crystalline base or mixture of bases soluble in a dilute solution of potassium carbonate and possibly identical with (b). Duquesnel had assigned the formula $C_{27}H_{40}NO_{10}$ to aconitine. This differed from that of Wright which was, no doubt, due to imperfect purity of substances isolated and examined by him. Von Planta examined the amorphous substance and gave it the formula $C_{30}N_{47}NO_{7}$. At that time it was considered a mixture containing aconitine but whether it pre-existed in roots or whether it was formed in drying or during the process of extraction this worker was unable to say, but he did consider that "napelline" might be identical or closely allied to this amorphous substance. In a third report on the chemistry of the Aconite Alkaloids presented to the British Pharmaceutical Conference, (1879), C. R. Alder Wright working with A. P. Luff deduced from previously described experiments and results obtained by Groves, Duquesnel, Hübschmann, and others, the following facts: 1. Aconitum Napellus roots encountered in commerce contain a highly active crystallizable alkaloid which furnishes readily crystallizable salts. This they distinguished as <u>aconitine</u> and represented it by the formula $C_{33}H_{43}NO_{12}$. This alkaloid existed together with its

decomposition products in roots. 2. On saponification aconitine broke down into benzoic acid and aconine according to the following equation:

$$\underset{\text{Aconitine}}{C_{33}H_{43}NO_{12}} + H_2O = \underset{\text{Benzoic Acid}}{C_7H_6O_2} + \underset{\text{Aconine}}{C_{26}H_{39}NO_{11}}.$$

In addition to this, Wright and Luff also found another active alkaloid which was capable of being crystallized but did not readily yield crystallizable salts. This they named pseudoaconitine and assigned to it the formula $C_{36}H_{49}NO_{11}$. This likewise existed in the root with its decomposition products. Pseudoaconitine decomposed according to the following equation:

$$\underset{\text{Pseudo-aconitine}}{C_{36}H_{49}NO_{11}} + H_2O = \underset{\text{Di-Methyl Protocatechuic Acid}}{C_9H_{10}O_4} + \underset{\text{Pseudoaconine}}{C_{27}H_{41}NO_8}$$

A third alkaloid found by them was non-crystalline and did not give any crystalline salts and contained a higher carbon percentage than any of the other bases present. It was physiologically inert. A base was derived from Aconitum Napellus by Groves which though not crystalline itself gave well-crystallized salts. This base was comparatively inert and its salts were bitter and produced no lip-tingling. The name picraconitine with a formula

$C_{31}H_{45}NO_{10}$ was given this base. In an article published in 1879 C. R. Alder Wright and A. P. Luff told of obtaining certain well-crystallized salts by special manipulation, and found that the formula, $C_{36}H_{49}NO_{11}$ given to pseudoaconitine was incorrect. This first substance was a mixture of pseudoaconitine $C_{36}H_{49}NO_{12}$ and of a base derived therefrom by removal of water. Further that when pseudoaconitine was dissolved in a large excess of tartaric acid and hydrochloric acid and then heated from some hours at 100° C, apopseudaconitine, $C_{36}H_{47}NO_{11}$ was formed. They found, however, that when pseudaconitine was heated only with hydrochloric acid, it split up into veratric acid and pseudaconine, whereas, if only tartaric acid was used as a solvent, dehydration to apopseudaconitine took place. The substance heretofore regarded as pseudaconine was now considered to be a dehydrated derivative of a substance for which they proposed the name apopseudaconine. Acetyl apopseudaconitine with the composition $C_{26}H_{46}(C_2H_3O)NO_{11}$ was formed when pseudaconitine was heated at 100° C with a large excess of glacial acetic acid, losing the elements of water. This crystallized with water and thus resembled pseudaconine and apopseudaconitine. The derivatives of aconitine differed from those of pseudaconitine in this respect. Wright and Luff also made benzoylpseudaconitine by heating pseudaco-

nitine with benzoic anhydride. They found that aconitine formed a series of derivatives precisely similar to those of pseudaconitine and under almost identically the same conditions. These were: apo-aconitine, acetyl apoaconitine, benzoyl apoaconitine, aconine and apo aconine. As far as the authors were able to judge apopseudaconitine and apoaconitine were not inferior in activity to the parent base, so that there was no necessity for separating the "apo" derivatives should they be present. C. R. Alder Wright in a communication to the American Pharmaceutical Association reported (1881) certain characteristics of aconitine and allied alkaloids as observed during research done on this drug. He reported that aconitine to which he gave the formula of $C_{26}H_{35}NO_7\begin{cases}(OH)_3\\O.CO.C_6H_5\end{cases}$ was the chief if not the only active alkaloidal ingredient in the roots of Aconitum Napellus but that occurring with it were other amorphous alkaloids of lower molecular weight and containing a higher percentage of carbon. He found that if the amount of aconitine present, relatively to the amorphous bases was not considerable it was often impossible to get former to crystallize at all (on a small scale at least); a considerable amount of aconitine was retained in solution permanently by the agency of the amorphous alkaloids which thus caused considerable loss. Even after

repeated crystallization from ether or ether-petroleum spirit, aconitine retained mechanically minute quantities of the amorphous bases. This could, however, be wholly eliminated by conversion into a salt, crystallizing and then regenerating the alkaloid from the salt thoroughly freed from the mother-liquor which contained the amorphous base as a salt. When perfectly pure Wright found the melting point of aconitine in a capillary tube to be 183° - 184° C (corrected). Pure aconitine should contain close to the theoretical amount of carbon and hydrogen, viz. 61.39 and 6.67 per cent respectively. Wright found that these analytical figures were the only reliable means of distinguishing aconitine from the closely allied alkaloid previously described under the provisional name japaconitine which agreed very closely with aconitine in all the other above named points. Further work by C. R. Alder Wright collaborating with Rennie (1881) led to a report on alkaloids contained in the fresh aconite herb of the species Napellus grown at Foxton in Cambridgeshire. These men working together found that the quantity of active alkaloid contained in the herb was probably somewhat less than that contained in the roots, reckoned on the dry substance. It was concluded, however, that from these experiments it could hardly be said that it was a general

rule that aconite roots are richer in crystallizable aconitine than the dry herb. According to John Williams it had happened in his factory that no crystallizable aconitine but only non-crystallizable bases could be isolated from batches of roots worked up on the manufacturer's scale in exactly the same way as other batches which readily yielded crystals.

Mandelin (1885) in a contribution on the constitution and character of the alkaloids of aconite disagreed with conclusions arrived at by previous investigators, especially those of Wright. Mandelin accepted the existence of the two characteristic alkaloids but claimed that only aconitine and no pseudaconitine was found in tubers of Aconitum Napellus, while pseudaconitine and no aconitine occurred in Aconitum ferox. These two alkaloids, he claimed, were pharmacologically identical but that pseudaconitine having the larger molecule required the administration of a larger quantity to produce an equal effect. He also confirmed the splitting-up of these alkaloids but failed to recognize any difference between the resulting aconine and pseudaconine, and proposed that aconitine should be called <u>benzoyl-aconine</u> and pseudaconitine should be known as <u>veratroyl-aconine</u>. No indication was given that these opinions were based on analysis while Wright represented

them as differing in composition. Japaconitine obtained by Paul and Kingzett and later described by Wright and Luff as splitting up into benzoic acid and japaconine was claimed by Mandelin to be identical with aconitine or benzoylaconine. Hübschmann's napelline he considered to be unaltered aconitine with varying proportions of aconine. The greater or less activity and toxicity of tubers of Aconitum Napellus, Aconitum ferox and Aconitum Japonicum he held to be dependent on their richness in alkaloid and not upon any difference between the toxic power of the alkaloids contained in them.

The preparation of a crystalline alkaloid obtained from the roots of Aconitum Napellus by extraction with amyl alcohol was investigated by Wyndham R. Dunstan and W. H. Ince (1891). The aconitine as first obtained formed yellowish indistinct crystals which melted at 188.4° (corr.) and on combustion gave numbers agreeing fairly well with formula $C_{33}H_{43}NO_{12}$, proposed for aconitine by Wright and Luff. It was proved to be associated with a small quantity of a gummy amorphous base and when purified by repeated crystallization from a mixture of alcohol and ether afforded combustion numbers which agreed best with the formula, $C_{33}H_{45}NO_{12}$. The pure alkaloid they found crystallized in tabular prisms belonging to

the rhombic system. These crystals were very slightly soluble in water and light petroleum, more soluble in ether and alcohol, most soluble in benzene and chloroform. They melted at 188.5° (corr.). Contrary to statements of previous observers these workers found that aconitine in alcoholic solution was dextro-rotatory, (+ 10.78°) and that the aqueous solution of the hydrobromide was laevo-rotatory (- 30.47°). Two crystalline aurochlorides were obtained. One, $C_{33}H_{45}NO_{12} \cdot H\ AuCl$, melted at 135.5° (corr.); the other, a basic aurochloride, $C_{33}H_{45}NO_{12}AuCl_{31}$ melted at 129° (corr.). These compounds were obtained without difficulty and as these workers believed afforded trustworthy means of identifying aconitine. Aconitine they found was not appreciably affected by heating at a temperature below its melting point but above this point uncrystallized aconine was formed. Three aurochlorides of apoaconitine were obtained. One, $C_{33}H_{43}NO_{11}H\ AuCl$, melted at 141° (corr.). When this salt was crystallized from aqueous alcohol it became a hydrate, $C_{33}H_{43}NO_{11}H\ AuCl_4 \cdot H_2O$, melting at 129° (corr.) and isomeric with aconitine aurochloride into which it very readily changed. The third aurochloride was a direct combination product of the alkaloid with auric chloride, $C_{33}H_{43}NO_{11}AuCl_3$ and melted at 147.5° (corr.). These workers also obtained an

amorphous base from aconitine, together with benzoic acid, by prolonged heating with water in a closed tube. It appeared to be identical with the aconine of Wright and Luff. This same substance was formed together with a resinous substance when aconitine was heated with an alkali. Neither aconine nor its salts could be crystallized. After purification the amorphous base and its amorphous aurochloride afforded analytical data agreeing, respectively, with the formulas $C_{26}H_{41}NO_{11}$ and $C_{26}H_{41}NO_{11}H\ AuCl_4$. Aconitum Napellus plants grown by E. M. Holmes at instance of British Pharmaceutical Conference were examined for alkaloidal content by W. R. Dunstan and John C. Umney (1892). Their process of extraction of the alkaloids was such as to preclude the possibility of the occurrence of hydrolysis or other decomposition. The alkaloid soluble in ether was obtained as a gum-like mass incapable of crystallization. Conversion into a hydrobromide resulted in the separation of a crystallizable and an uncrystallizable salt. The crystalline hydrobromide was identified as the salt of aconitine, the crystalline and highly toxic alkaloid already described by one of the authors and W. H. Ince. The rotatory power of the pure hydrobromide in aqueous solution was ascertained to be -29.65°, a result which agreed with that recorded previously. Because some doubt

existed as to the solubility of aconitine in water, this constant was carefully redetermined with the pure specimen. The mean of two determinations showed a solubility of one gram in four thousand four hundred and thirty-one grams of water at 22°C. Jürgens (1886) had previously recorded a far greater solubility of one gram in seven hundred and forty-five grams of water at 22°. The non-crystallizable hydrobromide furnished an alkaloid resembling gum in appearance. It was soluble in ether and alcohol but only sparingly so in water. An aqueous solution was alkaline to litmus and very bitter. It did not, however, give rise to the tingling sensation so characteristic of aconitine. The base could not be crystallized; neither could the hydrochloride, sulphate or nitrate be prepared from it. The aurochloride was also amorphous. Owing to the fact that a crystalline compound could not be obtained it was difficult to gain conclusive evidence of its homogenity. The properties just recorded proved that it was not aconine or the base called by Wright and Luff, picraconitine which readily afforded crystalline salts. These workers proposed to assign to this substance the name of napelline which was first given to the alkaloid now known as pseudoaconitine and afterwards by Hubschmann to a substance proved by the work of Wright and Luff to be a mix-

ture chiefly composed of aconine. The napelline described above was considered to be associated with another amorphous base about which very little information could be secured at that time except that neither it nor its salts appeared to crystallize. An alkaloid extracted with chloroform proved to be identical with aconine, the amorphous base resulting from hydrolysis of aconitine. On combustion it afforded numbers corresponding with those of the formula $C_{26}H_{41}NO_{11}$. Its molecular weight determined by Raoult's method also corresponded with this formula. This work proved to Dunstan and Umney that the roots of the true Aconitum Napellus certainly contain three alkaloids, one of which is crystalline, viz. aconitine, and the other two are amorphous, viz: napelline and aconine. There were indications that a fourth alkaloid is present, an amorphous substance closely resembling napelline. Dunstan and Umney found that juice expressed from roots contained a large proportion of amorphous bases but very little aconitine the greater part of which remained in the root and could be extracted together with the remainder of amorphous alkaloids by exhaustion with amyl alcohol. The total quantity of amorphous bases amounted to more than twice that of aconitine. Very little of the physiological action of the three alkaloids was or had been investigated.

Work done up this date, however, pointed to the conclusion that crystalline aconitine was by far the most toxic of the alkaloids contained in Aconitum Napellus. Further investigation of the alkaloid found in roots of Aconitum Napellus, associated with aconitine for which Dunstan and Umney proposed the name napelline was carried out by Dunstan and E. F. Harrison (1893). They succeeded in crystallizing several of the salts notably those of the haloid compounds. Purification of these salts by repeated crystallizations resulted in a product of a constant melting point. The base regenerated from the pure hydrochloride was a colorless varnish-like substance slightly soluble in water though more so than aconitine, but readily soluble in alcohol, chloroform and less readily in ether. An alcoholic solution was feebly dextro-rotatory. By analysis napelline was proved to have the same composition as aconitine and is therefore represented by the same empirical formula, $C_{33}H_{45}NO_{12}$ and may be properly termed isoaconitine. With auric chloride napelline exhibited a remarkable reaction which sharply distinguished it from aconitine and most other alkaloids. A definite aurochloride, $C_{33}H_{45}NO_{12}HCl\ AuCl_3$ was not obtained. When solutions of napelline hydrochloride and auric chloride were mixed a yellow amorphous precipitate was thrown down as in the case of aconitine. When this was

crystallized from its solution in alcohol nearly colorless crystals of aurochlor-napelline, $C_{33}H_{44}(AuCl_2)NO_{12}$, separated. This is a derivative of napelline of which one atom of hydrogen of the molecular structure is replaced by the group, $AuCl_2$. The production of such a compound from napelline was unexpected. The first known alkaloidal derivative of this type was aurochlorocaffeine described by Dunstan and Shepheard. Aurochlor-napelline differed from aurochlorocaffeine in not being converted by action of hydrochloric acid into the aurochloride. When napelline was heated in a closed tube or under ordinary pressure with mineral acids it hydrolyzed readily. The hydrolysis was more rapidly effected by aqueous soda or potash which acted even in the cold. Further experimentation has shown that there are only two products of hydrolysis and these are both identical with the hydrolytic products of aconitine and, moreover, are formed in same proportion. The following equation represents the hydrolysis of napelline into aconine and benzoic acid as well as the hydrolysis of the isomeric aconitine:

$$C_{33}H_{45}NO_{12} + H_2O = C_{26}H_{41}NO_{11} + C_7H_6O_2$$

Both these substances were isolated in the pure state and aconine closely compared with that derived from aconitine and ascertained to be identical with it. From physiological experiments conducted with napelline it appeared doubtful

whether it would prove toxic to man except, perhaps, when given in large doses. Dunstan and Harrison examined the non-crystalline alkaloid obtained by Groves years before from Aconitum Napellus and named picraconitine. They came to the conclusion that picraconitine is nothing but impure isaconitine, an isomeride of aconitine.

Dunstan and Carr (1895) found that when aconitine is heated to its melting point, 186° - 190° C it loses about 10% of acetic acid which distills over leaving behind a new alkaloid which they proposed be called pyraconitine. The equation representing this decomposition is:

$$C_{33}H_{45}NO_{12} = C_2H_4O_2 + C_{31}H_{41}NO_{10}$$

Aconitine Acetic Acid Pyraconitine

This new alkaloid separates as an amorphous varnish-like substance sparingly soluble in water but readily soluble in alcohol, chloroform and acetone. It has no action on polarized light and is not poisonous in small doses. The alkaloid readily dissolves in acids forming salts which can be crystallized. Dunstan and Carr in their researches found that aconitine when heated to 190° C furnishes pyraconitine by losing acetic acid. Isaconitine and aconine do not undergo a similar decomposition. They also showed that while certain aconitine salts in slightly acid solution are very slowly converted into salts of isaconitine at 100° C their

conversion is rapidly effected by heating a neutral aqueous solution in a closed tube at 120° - 130° C during two or three hours.

Dunstan and Harrison (1895) working with pyraconitine were able to produce pyraconine and benzoic acid by hydrolysis. Pyraconine is amorphous and differs from aconine in several respects. It is soluble in water, ether and forms crystalline salts. The equation representing the hydrolysis of pyraconitine is:

$$C_{31}H_{41}NO_{10} + H_2O = C_7H_6O_2 + C_{24}H_{37}NO_9$$

Pyraconitine Benzoic Acid Pyraconine

Aconine has been shown to be produced by hydrolysis of aconitine and isaconitine. It is now conceded that in the hydrolysis of aconitine, isaconitine is first produced and from this aconine.

The tentative nature of reasonings on aconitine alkaloids is made more and more evident with each succeeding publication:

1. Aconitine, $C_{33}H_{45}NO_{12}$, is acetyl-benzoyl-aconine and on hydrolysis yields (1) benzoyl aconine (isaconitine) and acetic acid. (2) Aconine and benzoic acid (from the isaconitine); the production of acetic acid from aconitine Dunstan and Harrison considered might lead to a process of estimation of the alkaloid. 2. Pyraconitine is formed by

heating dry aconitine alone and from this pyraconine and benzoic acid may be produced by hydrolysis. Dunstan sums up the alkaloids thus:

1. Aconitine -- acetyl-benzoyl-aconine, $C_{33}H_{45}NO_{12}$.
2. Napelline -- Isaconitine, or picraconitine, benzoyl aconine, $C_{31}H_{43}NO_{11}$.
3. Pyraconitine, $C_{31}H_{41}NO_{10}$.
4. Aconine, $C_{24}H_{39}NO_{10}$.
5. Pyraconine, $C_{24}H_{37}NO_9$.

Edwin Richards and F. Ashley Roger in examining aconitum arrived at the following conclusions (1892):

1. Best material for preparation of aconitine is the root of Aconitum Napellus.
2. The alkaloid, aconitine, is found in the cambium, vascular bundles and sieve ducts.
3. The crystals of aconitine have the appearance of hexagonal thin plates with acute ends.
4. It is probable that two varieties, alpha and beta, aconitine exist. The melting point of alpha aconitine was found to be 182° - 184°. Beta aconitine melts at 178° - 180°. The beta form is also about six times as poisonous as the alpha.
5. These researchers submitted $C_{33}H_{43}N_2O_{12}$ as the empirical formula for aconitine.
6. Percentage of alkaloid obtained from fresh tubers was

0.71%; from dry tubers 0.14%.

M. Freund and P. Beck (1894) assigned $C_{34}H_{45}NO_{11}$ or $C_{34}H_{47}NO_{11}$ as the empirical formula for aconitine and stated that the body formed by long continued boiling of this alkaloid has the formula $C_{32}H_{45}NO_{10}$. They preferred to call it picraconitine rather than isaconitine, a name given by Wright to a similar body. The aconine of Wright they considered to be identical with a body they obtained by boiling alcoholic potash and picraconitine but according to their work the formula derived was $C_{25}H_{41}NO_9$ and not $C_{24}H_{39}NO_{10}$ as determined by Dunstan or $C_{26}H_{39}NO_{11}$ according to Wright. Freund and Beck were positive as to the correctness of these results.

According to J. T. Cash and W. R. Dunstan (1898) the extraordinary toxic power of aconitine is mainly dependent on the acetyl radicle in the molecule while the specific action of benzaconine depends on the existence in its molecule of the benzoyl radicle. Aconine which contains neither acetyl nor benzoyl group is very inert but both aconine and in a less degree benzaconine are said to act as antidotes to aconitine. These two workers regarded the composition and constitution of aconitine as being unsettled.

H. Hager (1894) stated that he had found in Aconite tubers alkaloidal strengths ranging from 0.6% to 1.25%

while Jürgens found only 0.2%. C. C. Keller reported percentages of five samples: 0.87%, 1.14%, 1.05%, 1.23% and 0.97%. The tubers which contained the least alkaloid contained considerable sugar from which fact it was concluded that they were not collected from flowering plants. Keller found 0.21% of alkaloid in aconite leaves.

F. Wentrup (1907) experimented to determine definitely whether the medicinal use of the "daughter" tubers along with the parent aconite tubers was justified. He found that the "daughter" tubers contained even a little more alkaloid than the parent tubers. Unfortunately, the tubers available were all deficient in alkaloid yielding only 0.34% to 0.52% whereas Keller had obtained from 0.87% - 1.23%.

Schulze and Berger (1925) in working on Aconitum Napellus found in the residue from aconitine extraction a new base which they called <u>neapelline</u>, $C_{32}H_{45}NO_8 \cdot 3H_2O$, and which they found to melt at 85° - 90° C. No crystalline salts could be prepared but analytical results showed a methylamino group and three methoxy groups. On hydrolysis this new base yielded benzoic acid, acetic acid and a base <u>neoline</u> which could not be crystallized. The hydrobromide obtained, $C_{23}H_{40}NO_6$ Br, melted at 210° C with decomposition. An uncrystallizable derivative of neoline

gave a gold salt which melted at 145°C and corresponded on analysis to only one acetyl group.

Pharmacology of Aconitum Napellus

Wilbur L. Scoville (1910) in commenting on the alkaloidal assay methods of the U.S.P. said that the chemical assay of aconite had been criticized much but that it had a real value. He considered that it could well be supplemented by the Squibb physiological test which would add much to its value.

Dr. A. B. Stevens (1911) contended that there was no basis at that time for claiming that the chemical assay of Aconite (U.S.P. VIII) was unreliable, except in the case of preparations of the drug that had been prepared by heat. The conclusions reached by Stevens for the Assay of Aconite were: 1. That aconite kept properly does not deteriorate, 2. That when deterioration does take place through improper care such deterioration may be detected by chemical assay, 3. That when deterioration is due to heat the weight of ether-soluble residue is increased, and the basic properties are decreased, hence it is easy to detect deterioration by volumetric assay, 4. That the Squibb test is valuable for comparison but that it is not suitable for pharmacopoeial standardization, 5. That chloroform should not be used in the assay of aconite, 6. That the chemical assay is not scientifically exact but that it is sufficiently accurate

for use in determining the quality of aconite or of its preparations.

Aconite preparations when tested physiologically show such great variability as to prove that the chemical method of the U.S.P. VIII for determining alkaloidal content of Aconitum Napellus was not a true measure of its activity. Preparations relatively rich in total alkaloids have a low toxicity whereas those comparatively low in total alkaloids have a high toxicity. Chemical methods of assay other than that of the U.S.P. VIII have been found to be equally unreliable.

The activity of aconite depends mainly upon its most active alkaloid, aconitine which is very readily hydrolyzed into benzaconine and later into aconine. Benzaconine, the product of the first stage of hydrolysis of aconitine, is the chief constituent of the substance named napelline and picraconitine by the older workers. Dunstan first named it isaconitine. Benzaconine and aconitine are sparingly soluble in water but readily soluble in alcohol. Aconine, an amorphous base is soluble in both water and alcohol. These alkaloids are present in the crude drug and may also be present in galenicals as decomposition products. Dunstan and Umney (1892) found aconite alkaloids remarkable in that they easily undergo hydrolysis.

Cash (1898) found that aconitine is the most active of the alkaloids. Either to the frog (Rana temporaria) or to the Guinea pig it is two hundred times more toxic than benzaconine and about twelve hundred times more toxic than aconine. In large doses aconitine produces marked effects upon the central nervous system. In warm blooded animals, the heart is first slowed due to a stimulation of the vagus center and this slowing is accompanied by a great fall in blood pressure. The heart rate then increases and later becomes irregular with the development of a marked arrhythmia between auricles and ventricles, the blood pressure fluctuating greatly. Applied to the tongue and mouth in dilute solutions aconitine produces the tingling sensation which is so characteristic of aconite.

Benzaconine in small doses causes a very slight rise in the blood pressure and an acceleration of the heart rate whereas with large doses a steady fall in blood pressure occurs which is accompanied by a decrease in the heart rate. There is also developed a change in rhythm between the auricles and the ventricles. No tingling is produced when benzaconine is applied to the tongue.

Aconine as compared with the former alkaloids is comparatively harmless. A slight rise in pressure usually occurs, due to a more complete systole of the heart. In

this respect aconine is antagonistic to aconitine and benzaconine. Like the latter it does not produce tingling.

Geo. B. Roth (1913) investigated seven methods of assay and found that of these the Squibb and the lethal guinea pig methods alone could be used with any degree of accuracy. His criticism against the Squibb method was the subjective factor which he regarded as detrimental. Roth concluded that the subjective factor is an objection since the results obtained by him for this method were so much lower than those of other observers. The guinea pig method was the most delicate toxic method investigated and showed little or no variability. The relative non-toxicity of the aconines in aconite and the parallelism noticed between the Squibb and guinea pig method indicated that the latter method is practically a measure of the aconitine content in aconite. A comparison of all results showed that the guinea pig method is the preferable method of assay.

Charles C. Haskell (1916) found the chemical standardization of aconite tincture useless. He considered the U.S.P. VIII method of chemical assay as not only useless but sufficiently misleading as to be more or less dangerous. He concluded that the lethal dose method for a guinea pig is the most convenient and satisfactory for arriving at a

standard. Later and more extensive tests by Haskell tended to prove that season influences in a decided way the resistance of guinea pigs to poisoning by tincture of aconite; that the cat method of Hatcher for assay of aconite is worthy of a more extended trial.

T. R. Fraser (1917) stated that of the one hundred and fifty known species of aconitum only two or three had been examined pharmacologically, although all those examined produced the same characteristic effects on the nervous system, secretions, circulation and respiration, yet they might be divided into two classes. One of these classes acts predominantly on the circulation, the other on the respiration. Those containing aconitine belong to the first class, those which yield pseudo-aconitine to the second category. Aconitum Napellus is the most efficient of the aconitine class. Aconitum heterophylloides and aconitum nagarum belong to the pseudoaconitine group.

Work done by E. E. Swanson and A. L. Walters (1923) on the standardization and stabilization of aconite preparations gave still further proof that the chemical method of assay is unreliable and suggested that this method be disregarded in the U.S.P. X. They found also that the lethal dose assay gives much better results in the hands of various technicians. Further work along this same

line carried out by E. E. Swanson (1924) showed that the decomposition or hydrolysis of the pure alkaloids of aconite does not take place in an acid alcoholic solution. Wright and Luff (1877, 1878, and 1879) had previously found that aconite alkaloids in an alkaline alcoholic solution hydrolyzed with remarkable ease. The U.S.P. tincture and fluidextract of aconite (U.S.P. IX) did not give an alkaline reaction toward litmus when freshly made or when aged. However, this was probably prevented by the alcohol. The alkaloids of aconite probably form combination salts with weak acids and these decompose readily into the free base. Additional experiments conducted by E. E. Swanson showed that the deterioration of the tinctures and fluid extracts is probably due to decomposition or hydrolysis of the alkaloids and may be a hydrogen ion concentration factor. Preparations containing an acid yielded to Swanson a higher pH value than the U.S.P. preparation. This difference is evidently due to the poor dissociating property of acetic acid as compared with that of hydrochloric acid. Continuing the work on the deterioration and stabilization of aconite preparations E. E. Swanson and Chester C. Hargreaves (1927) conducted a series of experiments on guinea pigs and mice with tinctures and fluid extracts of varying pH concentrations. They found

that the pH value did control the deterioration and stabilization of aconite preparations. From the results obtained they recommended that tinctures and fluidextracts of Aconite U.S.P. should have a pH value of 2.5 and not less than 3.00 in order to prevent deterioration. The amount of acid required to produce the desired pH depended upon the amount of alkaloids and inert material present in each lot of drug. These workers found that the guinea pig method agrees remarkably well with the white mouse method as far as standard aconite preparations are concerned but that this agreement vanishes when the preparation has undergone deterioration.

L. W. Rowe (1925) in comparing the degree of accuracy between the guinea pig and mouse methods showed the latter to be as accurate, if not more so, and just as dependable if an insusceptibility factor of 6.25 is used, the mouse being relatively more resistant. Also, from the standpoint of expense mice as test animals make this method more practical.

Manuel G. Jauregui (1927) in a study made on the methods for determining the minimum lethal dose of aconitine as a standard for biological assay of aconite preparations concluded: 1. That the U.S.P.X guinea pig method, carefully performed gives uniform results and offers no technical difficulties; 2. The lethal subcutaneous dose for cats and

dogs agrees fairly closely with that for guinea pigs; 3. Intravenous injections on anesthetized animals give unreliable results when the anesthetic is given in the usual manner; 4. If the anesthetic is given in fixed amounts proportional to the size of the animal or if respiratory failure is prevented by artificial respiration, the intravenous method to all appearances is reliable; 5. Lastly, vagus slowings of the heart can not be taken as a measure of aconitine toxicity.

James C. Munch and R. I. Grantham (1929) compared the toxicity of tinctures and fluidextracts of aconite to guinea pigs and rats. Their results showed a lesser toxicity to rats and to guinea pigs with increased age of the preparations. This fact was interpreted to mean that the hydrolytic cleavage products of aconitine are less toxic.

H. B. Haag and L. W. Hawkins (1930) examined four specimens of tincture of aconite manufactured under the names of well-known pharmaceutical houses. The assays were conducted according to the U. S.P. X method and proved that tinctures of aconite now offered for clinical use still show a great variation in physiological activity. Haag and Hawkins following up the work of Swanson and others examined the hydrogen ion concentration of

samples which they had previously tested biologically. Their findings also point to a close relationship between the stability and hydrogen ion concentration. They recommend that, if the preparation is to be retained in the pharmacopoeia it should be adjusted to a proper pH as proposed by Swanson. They further suggest that in the case of all drugs subject to deterioration, a statement should be made on the labels of these preparations giving date of manufacture and the time limit, if possible, beyond which the drug should not be used. Moreover, in view of the fact that digitalis leaf has been shown to be far more stable than any of its liquid preparations, it seems desirable that studies should be made to ascertain whether the same holds true for aconite.

Botany of Aconitum Napellus:

Arthur Meyer (1881) contributed a comprehensive study of the official aconite and of the more important species related to it. He classified the Aconites as follows:

I. Aconites having a rhizome.

Aconitum Lycoctonum L.

II. Aconites having poisonous tubers.

a. yellow flowered species
Aconitum Anthora L.

b. blue flowered species
(a) Indigenous (European)
Aconitum Napellus L.
Aconitum Paniculatum Lam.
Aconitum Variegatum L.
Aconitum Stoerckianum Rchb.

(b) Exogenous species
Aconitum ferox Wallich.
Aconitum uncinatum L.
Aconitum Fischeri Reich.

III. Aconites having non-poisonous tubers.
Aconitum heterophyllum Wallech.

This classification, while it does not include all the species of aconite known, gives, nevertheless, a skeleton for a classification of them all. Aconitum Lycoctonum, the only species having a several headed rhizome was selected by Linné as the type species of the genus. This species is a native of Europe and northern Asia (Siberia) and is very poisonous. It is a yellow-flowered form and commonly known as Yellow Wolfs-bone.

Aconitum Napellus attains a height of from 0.7 - 1.5 m. The underground portion consists of a fusiform parent tuberous root from which arise one or more lateral shoots, each forming a conical daughter tuberous root. The leaves are alternate, long stalked, with blades palmately cut into five or three segments, and each of these in turn divided into narrow segments. The inflorescence is a terminal raceme of violet blue flowers. The bracts are lanceolate, two in number beneath each pedicelled flower. The sepals are five in number, bluish-purple, the upper one helmet-shaped, laterally compressed, the two lateral ones blunt and ovate, the two lower oblong-lanceolate. The petals vary from two to eight and are hammer-like, the two posterior ones being covered by the hood of the posterior sepal. The stamens are numerous and hypogynous. There are three or four carpels with bilobed stigmas and the fruit consists of a like number of beaked follicles containing numerous, angular, wrinkled and acridly tasting seeds.

Experimental

Pharmacognosy of Aconitum Lutescens

Aconitum lutescens grows in Montana in the Bitter Root Valley. It is also found in Wyoming, Colorado, and New Mexico. Specimens found by Dean Mollett in the Big Hole Mountains near Sula were shoulder high. The plant in general has a stem 4-8 dm high. The stem of a specimen examined measured 6.5 dm and was smooth. The leaves are alternate with blades 5 - 8.5 cm broad, thin, glabrous, palmately divided into three deeply cut leaflets. These leaflets are not divided to base as in Aconitum Napellus, neither are they laciniately toothed as in the official aconite. Each leaflet of Aconitum Lutescens averages from 2 - 3.5 cm. in width and 4.5 - 7.2 cm. in length. In outline the leaves are ovate-lanceolate with apex acuminate and base cuneate. Each leaflet is also deeply and coarsely toothed three or four times above the middle. The petioles are from 3.5 cm - 6 cm long. The inflorescence consists of few racemes borne in axils of leaves, narrow, long for the plant and rather open. The pedicels and rachis are softly hirsute with straight viscid hairs standing at right angles. The rachis measures about 9 cm in length. Two bracts, lanceolate in outline

subtend each pedicelled flower. The sepals are five in number and cream colored. In plants growing in the shade without any access of light the flowers were observed to be pure white. The upper sepal is helmet-shaped, front almost straight with beak directed downwards. The lateral sepals are three-fourths as long as the hood, moderately oblique and concave. The lower sepals are one-fourth as long as the lateral. Two petals are present both hooded, clawed and possessing nectaries, and both being concealed in the hood or helmet. The fruit consists of either three or four beaked follicles which average 1 cm. in length and 2 mm. in width and which are sparingly pubescent.

Illustration number one shows the general habit of the plant in flower and in fruit.

Aconitum Napellus is usually collected in the fall after the overground portions have died down. Specimens of this species which grow in the medicinal plant garden at the University of Montana have been observed to be in flower as late as the first of October. Aconitum delphinifolium Rydb. considered closely related to the official species, flowers during July and August. It is indigenous to Alaska, Alberta and British Columbia and grows at an altitude of from 1800-3500 m.

The lengthened time of flowering of Aconitum Napel-

Illustration No. I Aconitum lutescens

lus may be influenced to some extent by the combined effect of differences in altitude, climate and cultivation. On the other hand, Aconitum lutescens grown in the medicinal plant garden had seeded and the overground parts had died down at the time the official species was still flowering. Aconitum lutescens A. Nelson, grows at an altitude of 1800 - 2500 m. along mountain streams in New Mexico, Montana and Idaho. It flowers during July only. Aconitum lutescens resembles Aconitum columbianum Nuttall in shape and size of leaves and Aconitum insigne Greene in the general appearance of the flowers excepting that those of Aconitum lutescens are smaller and ochroleucous while those of Aconitum insigne are generally blue. Aconitum columbianum Nuttal (Aconitum vestitum Greene) is native to British Columbia, Montana, New Mexico and California while Aconitum insigne Greene is indigenous to Colorado and Alberta, Canada. Coulter and Nelson (1909) reported Aconitum lutescens as growing in Wyoming, Colorado and New Mexico only.

Specimens of Aconitum lutescens were examined and found to be smaller and less sturdy than those of Aconitum Napellus grown in the medicinal plant garden or than those of Aconitum Columbianum. The leaves did not show as great a variation in form in the same plant as did those of Aconitum Columbianum the older leaves of which were dissected

almost to the midrib, in this respect resembling very closely those of Aconitum Napellus. The leaves of the species in question were also much thinner and venation was not so prominent as in Aconitum Columbianum and Aconitum Napellus. The dark blue flowers of Aconitum Napellus are disposed in terminal racemes while those of Aconitum lutescens occur in long, narrow, rather open racemes of pale cream-colored flowers. The flowers of Aconitum columbianum Nuttall are described in New Manual of Rocky Mountain Botany by Coulter and Nelson as being blue, sometimes pale or nearly white, few occuring in loose terminal, panicle-like racemes. A comparison of the flowers of Aconitum insigne Greene with those of Aconitum lutescens made from herbarium specimens showed a similarity in form and shape of flower but those of the latter were smaller and cream-colored whereas those of the former were violet-blue. Again, a mounted specimen in the herbarium of the Botany Department, University of Montana, identified as Aconitum columbianum and collected in Idaho was smaller, less sturdy in gross appearance than were others of the same species in this herbarium and in that of Department of Pharmacy. The leaves were smaller, more glabrous, thinner and did not show so great a differentiation of form. They were, moreover, not so

deeply dissected. The flowers were much smaller, and pale yellow with very narrow blue borders. This particular specimen of Aconitum columbianum was collected at an altitude of 6900 feet. The author has some doubt as to whether this specimen was hybridized by a crossing of the two species, columbianum and lutescens, or whether it was simply Aconitum columbianum altered by environment. If the latter is true then Aconitum lutescens may be a species established as a result of environmental conditions of altitude, soil and moisture.

Aconitum columbianum is the near relative of Aconitum Fischeri, one of the Japanese Aconites. This latter species is recognized as being morphologically and chemically distinct from Aconitum Napellus. If Aconitum lutescens is a descendant of, or an altered Aconitum columbianum, then we may reasonably expect to find that it differs from Aconitum Napellus in its outer and inner morphology and also in its chemistry.

Macromorphology of Aconitum lutescens tubers:

In shape the tubers of Aconitum lutescens are more or less fusiform or spindle-shaped, tapering at each end or in some instances slightly flattened at the crown. The daughter tubers are connected by a side branch. Externally they are dark brown in color and smooth. Rootlets are fairly numerous and the longest ones attain a length of 85 mm. The length of the tubers varies, ranging from 2.2 - 2.5 cm. Leaf buds are present at the crown and average 12 mm. in length and 4 mm. in width. The fracture of tuber is short and somewhat mealy. The taste is very bitter but quite free from acridity. Illustration number two represents a drawing of a typical tuber of Aconitum lutescens.

Compared with tubers of Aconitum Napellus those of Aconitum lutescens are considerably smaller. The dimensions for the U.S.P. species may be summarized as follows: 4-10 cm. in length and from 1-3.5 cm. in diameter at the crown, externally dark brown or grayish brown, smooth or longitudinally wrinkled, the upper end with a bud or remains of a bud scale and a stem scar; the other portions with numerous root scars or short rootlets; fracture short, horny and somewhat mealy; taste sweetish, soon becoming acrid and developing a tingling sensation followed by numbness.

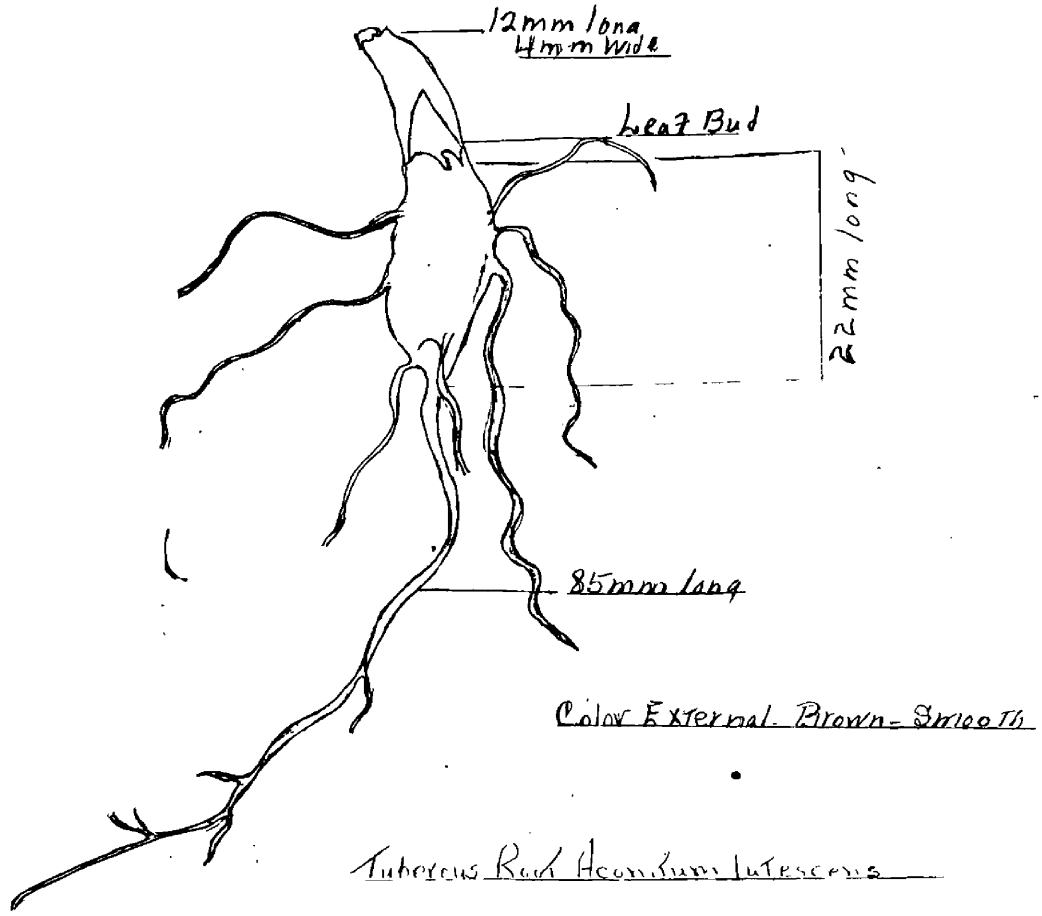
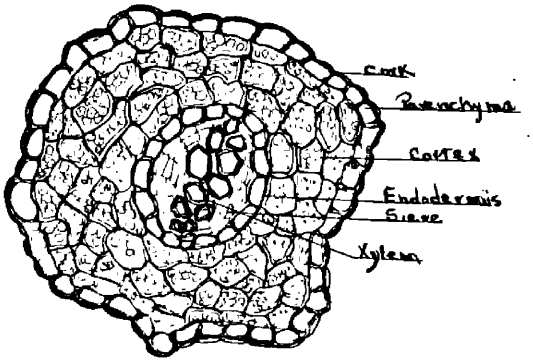


Illustration No. 2 - Tuberous Root
Aconitum lutescens

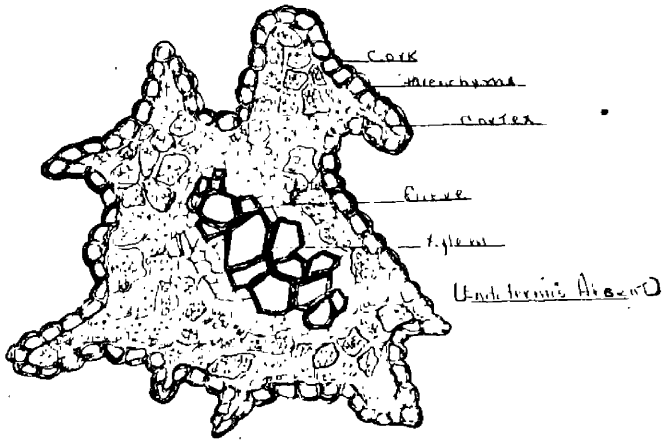
Histology of the Tuber and Rootlets of Aconitum lutescens:

Permanent slides of cross-sections of rootlets of Aconitum lutescens and Aconitum Napellus and cross and longitudinal sections of the tubers of both species were made according to the paraffin method and the following facts were noted: In cross-section the rootlets of Aconitum lutescens appear primitive in structure. An outer brown corky layer composed of one layer of very much flattened, compressed and suberized cells is present. Within this corky layer is found a single layer of tangentially elongated parenchyma cells totally devoid of starch grains. The next layer comprising the cortex consists of three to four layers of equal sized, parenchymatous, polyhedric cells in which are found numerous single and two to seven compound starch grains. Surrounding the vascular bundle is the endodermis or starch sheath composed of a single layer of tangentially elongated, slightly rounded cells. The vascular cylinder seems to be of the most primitive type, being solid, with xylem at center and surrounded by phloem. This apparently concentric type of bundle gives way to the radial arrangement in the tuber.

Cross-sections of the rootlets of Aconitum Napellus show the same characteristic structure of Aconitim lutescens with the exception that the endodermis is absent.



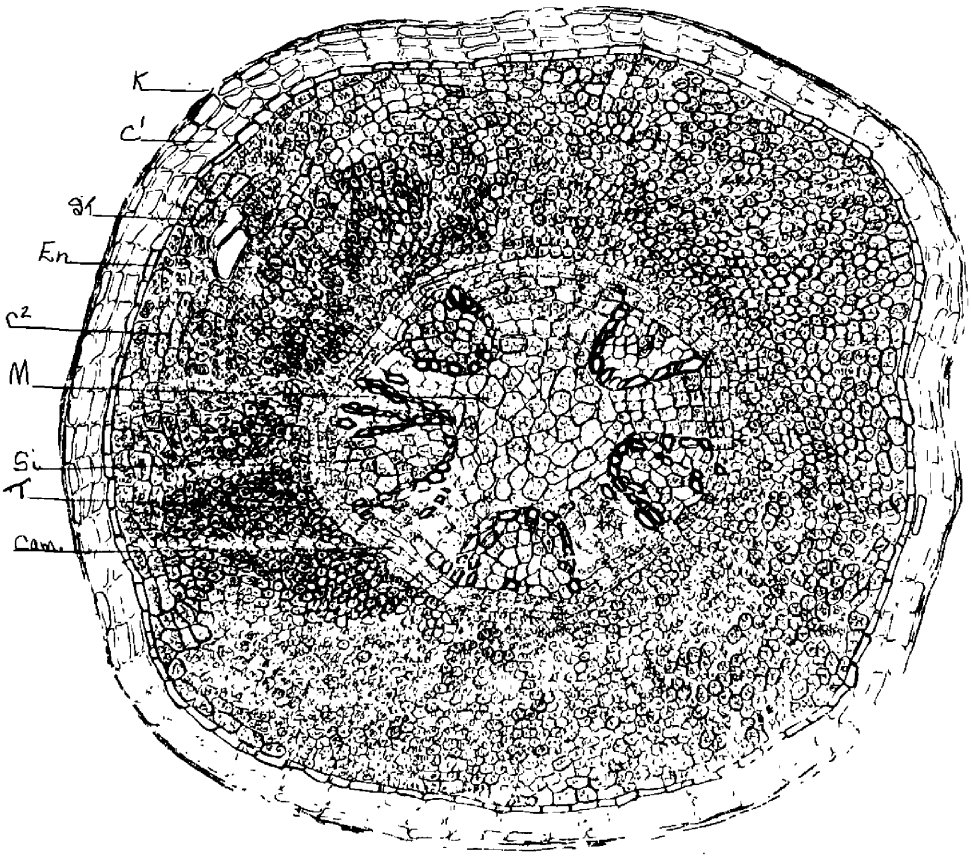
Cross Section Rootlet Aconitum lutescens



Cross Section Rootlet Aconitum Napellus

Illustration No. 3- Cross section Rootlets:
Aconitum lutescens
Aconitum Napellus

Cross-sections of tubers of Aconitum lutescens show an outer suberized layer of cork one cell thick. The cortex is composed of two distinct layers, an outer thin layer of primary cortex made up of three rows of elongated parenchyma cells totally devoid of starch grains and an endodermis of tangentially elongated cells separating the primary cortex from the inner. This inner zone or principal tissue is made up of from 30 - 35 rows of starch-containing parenchyma cells, more or less rounded and gradually becoming smaller as they approach the vascular bundles and pith. The cells of the principal tissue are packed with starch granules, both simple and compound. Stone cells are very few in number occurring in the inner zone of the cortex. The cambium is circular in outline and composed of three rows of flattened parenchymatous cells also filled with starch. The vascular cylinder is pentarch; the five collateral bundles are distributed or arranged to correspond with circular figure of cambium. Each bundle is separated by a medullary ray made up of rather tubular cells considerably larger than the cells of the innermost layer of principal tissue and penetrating them. The xylem of the bundles is bow-shaped, opening outwards. The phloem composed of sieve tubes and companion cells is enclosed by the arms of the xylem of each bundle. The



Cross Section Tuber Aconitum lutescens

K = Cork
C' = Outer zone of Cortex
Sl = Stone Cell
En = Endodermis
C2 = Inner zone of Cortex
M = Pith
Si = Vascular Tissue
T = Tissue of Aconitum lutescens
Cam. = Cambium

Illustration No.4 - Cross section Tuber:
Aconitum lutescens

cells of the phloem are larger than those found just outside the cambium, elongated and of rounded shapes. The pith or medulla surrounded by the bundles is made up of rounded polyhedric cells about seven such cells in diameter. There are very few if any intercellular spaces between these cells which exceed by about three times the size of the cells of principal tissue.

The longitudinal section shows that the several vessels of the tubers extend separately to the end of the root and do not anastomose, though they are often bent or curved. The tracheae show slit-like single pores or reticulate markings.

The histology of the tuber of Aconitum lutescens appears to simulate that of a Japanese Aconite described by D. V. Wasowiez (1881) and named Isaou. Two species of Japanese Aconite are substituted in medicine, namely Aconitum Fischeri and Aconitum uncinatum var. japonicum. It is impossible to know which, if either, of these is being described by D.V. Wasowiez.

The official Aconitum Napellus differs from Aconitum lutescens in the inner morphology of the root. Aconitum Napellus shows an outer layer of one or more rows of suberized cells with blackish-brown walls and a cortex made up of two zones. The outer zone is composed of 8-15 layers

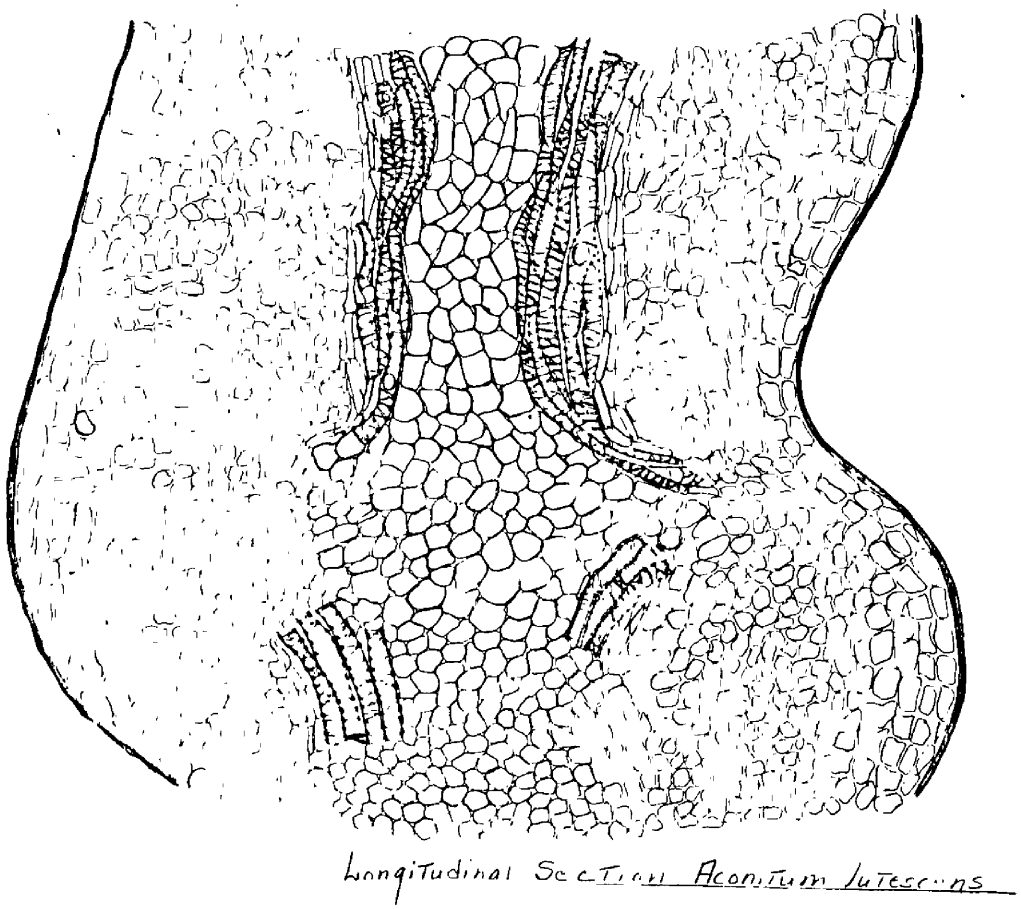


Illustration No. 5 - Longitudinal Section
Aconitum lutescens

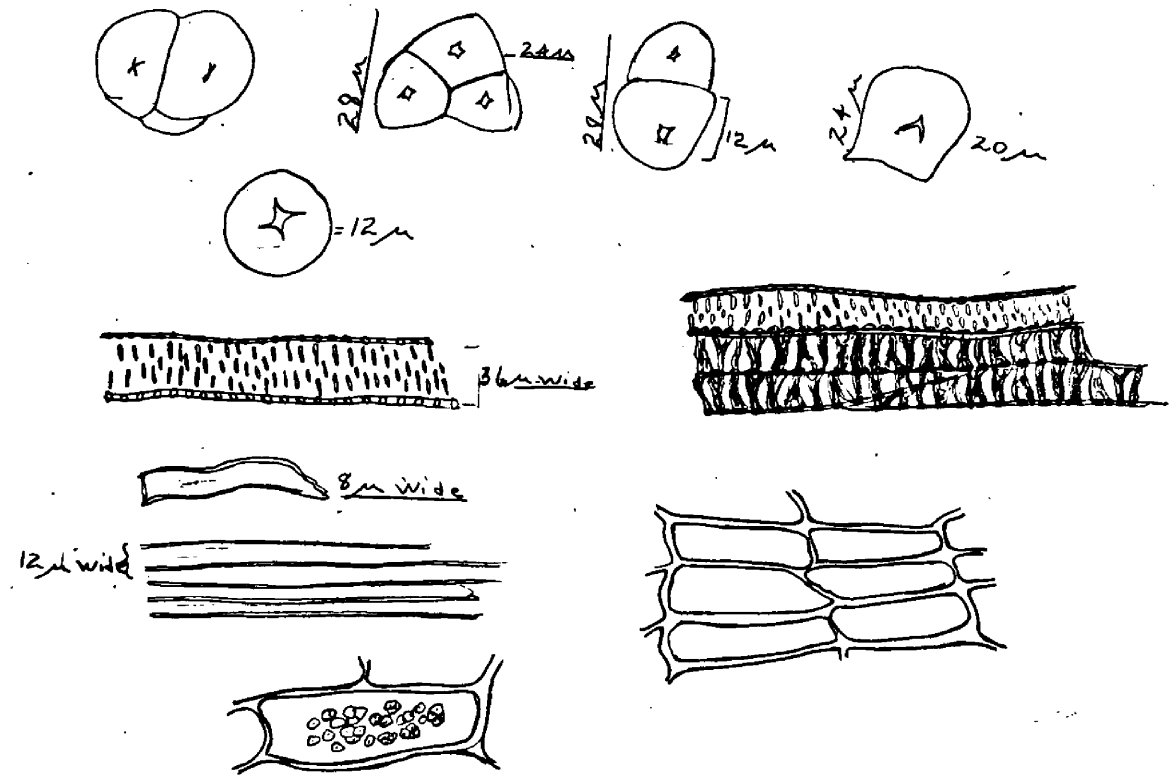
of parenchyma cells interspersed with stone cells. In Aconitum lutescens the stone cells are scattered and when present are distributed in inner zone. The outer zone of cortex in Aconitum lutescens is made up of but three or four layers of parenchyma cells. Twenty layers of starch containing parenchyma make up the inner broader zone of the official species whereas in Aconitum lutescens there are from thirty to thirty-five layers of starch containing parenchyma. The two zones in both species are separated one from the other by a modified endodermis. Aconitum Napellus possesses a five to eight angled cambium more or less star shaped within the angles of which and scattered along the entire cambial line occur collateral fibro-vascular bundles. As mentioned before, the cambium of Aconitum lutescens has a circular outline.

According to James C. Munch and H. H. Crosbie (1929) the U.S.P. X pharmacognostic description of Aconitum Napellus is inadequate in several particulars. They mention the "modified endodermis" of Aconitum Napellus which creates some speculation as to what the characteristics of such a structure should be. Also whether there really is any Aconitum Napellus on the market possessing a truly fusiform root. These workers report that a recent shipment of Aconite (1929) invoiced as Aconitum Napellus, while not dis-

agreeing greatly with any physical characteristic of the U.S.P. X, nevertheless, showed in cross-sections of daughter roots an almost perfect cambial zone. A cross-section made of a weak mother root showed the same circular cambial zone. Smaller auxillary bundles were noted between the main bundles which had the "Gestrechte V" form that seems to characterize all aconites. On the other hand, a cross-section of a tuber taken to be a sample of normal Aconitum Napellus showed the distinctly stellate cambial zone. It seems that Aconite is reaching the American market as a mixture of species and that even unskilled workmen can pick out these undesirable species by their outward appearances, but the official description is so indefinite that they cannot legally be rejected. The present pharmacopoeial description of the root makes it possible to exclude the Japanese and Indian Aconites but not some of the European species.

Preparatory to the analysis of the root and also to the making of the tincture for biological assay, the dried tubers of Aconitum lutescens were reduced to a No. 40 powder. This powder was greyish-brown in color and possessed only a slight odor. The starch grains were very numerous, spherical, plano-convex, single or two to seven compound with markings plain or clearly defined including a central cleft, forked or stellate hilum. The starch grains varied from

12 microns to 20 microns in diameter for single grains and from 12 to 24 microns in diameter and 28 microns in length for compound grains. The taste was very bitter and stimulated the flow of the saliva but it was free from any acridity. No tingling or benumbing sensation as is characteristic of Aconitum Napellus was produced in the mouth. Numerous tracheae averaged 20 to 36 microns in width. Bast fibers with oblique slits in the walls were found to be present in the powdered drug. Fragments of cork and parenchyma cells containing starch were also found. Stone cells were not in evidence in powdered Aconitum lutescens.



Microscopic No. 40 Powder - Aconitum lutescens

Illustration No. 6 - Powdered Aconitum lutescens

Chemical Analysis of Aconitum lutescens

Determination of Moisture in the Tubers

On October 21, 1930, tubers possessing good-sized buds and rootlets were collected from the medicinal plant garden of the University of Montana. On this date the official species, Napellus was still blooming while the overground parts of Aconitum lutescens had died down. Successive lots of the tubers were gathered on November 6, 1930, January 15, 1931, April 2, 1931. Each lot was treated in the following manner: The tubers were thoroughly washed and placed on blotting paper to take up excess moisture. The roots were then weighed, spread out on papers and allowed to dry to constant weight at room temperature. They were then transferred to an electric drying oven maintained at a constant temperature of 105° C and dried until there was no further loss in weight. The oven-dried material was then weighed . The per cent of moisture lost in air drying and oven drying was determined with the following results for the different lots of the roots:

Tubers	Oct. 21,1930	Nov. 6, 1930	Jan. 15,1931	Apr.2,1931
Air-dried	69¼%	50.75%	51.9%	62.6%
Oven-dried	76%	60.875%	54%	64.09%

These combined lots of tubers were comminuted into a number 40 powder.

Ash determinations were first made. These included: total ash, water soluble, acid insoluble ash, alkalinity of water soluble and alkalinity of water insoluble ash. In the titrations for alkalinity of water soluble ash 0.1N hydrochloric acid was used with methyl orange as the indicator. In titrating the alkalinity of water insoluble ash 15 cc. of 0.1N hydrochloric acid were added to the ash and the excess acid titrated back with 0.1N sodium hydroxide, methyl orange being used as the indicator.

An ash determination to determine total ash was likewise made on tubers of Aconitum lutescens collected by Dean C. E. Mollett on July 24, 1931 from the Big Hole Divide of the Bitter Root Valley. The results were widely different from those obtained from tubers of the previous year. It is assumed that the difference in year, season, locality and environment may account for the disparity. Duplicate determinations were made on powdered Aconitum lutescens of both seasons.

Total Ash	Determina. No. I	Determ. No. II,	Det. 1931 Drug I	Det. II
" " 1930 Drug				
" "	7.52%	7.45%	3.72%	3.697%

	1930 Drug Det. No. I	Det. No. II
Water Insol. Ash =	6.93%	7.06%
Acid Insoluble Ash =	3.609%	3.08%
Natural Ash =	3.911%	3.37%

The figures for alkalinity of water soluble ash and water insoluble ash made from powdered Aconitum lutescens were:

	Determination No. I	Determ. No. II
Alkalinity Water Soluble Ash = to neutralize alkalinity from one gram of sample.	1.052 cc $\frac{n}{10}$ Hcl	1.25cc $\frac{n}{10}$ Hcl.
Alkalinity Water Insoluble Ash	=4.39cc $\frac{n}{10}$ Hcl	5.46cc $\frac{n}{10}$ Hcl.

The U.S.P. requirement for acid insoluble ash of Aconitum Napellus is not more than 2%. Aconitum lutescens yields 1.345% more acid insoluble ash than the official species.

The organic analysis of Aconitum lutescens was carried out according to Dragendorff's method of plant analysis with modifications proposed by L. E. Sayre. Analyses were run on lots of drug collected from the medicinal plant garden, University of Montana and from the Bitter Root Valley, Big Hole Divide.

I. Approximately 5 gm. each of a no. 40 powder of Aconitum lutescens from the two lots were completely extracted with chloroform in a Saxhlet Extractor. After the chloroform was removed by evaporation there remained extractives orange brown in color, bitter in taste, and with a faint peculiar odor.

	Fall Drug 1930, Det. No. I.	Det. No. II	July Drug 1931 Det. No. I
Chloroform Ext.	3.73%	3.03%	1.94%

I. (b) Fixed Oil-- The chloroformic extractives were treated with small quantities of petroleum ether. The solutions were filtered and filtrate evaporated until free from petroleum ether. This petroleum ether extractive was calculated as a fixed oil. The per cent yields were:

1930 Drug, 1.629% and 1931 Drug (July) 1.36%.

These extractives were yellowish-orange in color, sticky and had an odor resembling that of asafoetida. They were then allowed to remain exposed to the air for several days and reweighed. There was no change in weight indicating the absence of volatile oil and also that the fixed oil is of a non-drying nature.

I. (c) Resin-- The filters and residues remaining after treatment with petroleum ether were macerated for 18 hours in 80% alcohol. The alcoholic solutions were filtered and evaporated to a small bulk on a steam bath and then poured into large volumes of water acidulated by addition of 5% sulphuric acid. The precipitates were collected in Gooch crucibles, weighed when dry, and calculated as resins. These resins were at first, flocculent, yellowish-white but became chocolate brown after filtration and drying. The yields of resin were respectively:

Fall 1930 Drug = 0.384%.

July 1931 " = 0.309%.

I. (d) Waxy Material-- The residues remaining after ex-

traction with 80% alcohol were weighed and calculated as waxy (?) material. The yields were:

Fall 1930 Drug = 0.189%

July 1931 " = 0.0166%

II. The dregs or mares remaining from the chloroform exhausted drug were next subjected to complete extraction with 80% alcohol.

II. (a) Resin-- The alcoholic extracts were evaporated on the steam bath and the concentrates poured respectively into large volumes of water. The precipitates thus obtained were colloidal in nature and represented resins not extracted by the previous treatment with chloroform. These resin precipitates collected also in Gooch crucibles were greenish-brown, bitter and had an odor resembling that of an old tobacco pipe. The yields for lots of the two years were:

Fall 1930 Drug = 0.0353%

July 1931 Drug = 0.958%

II. (b) Vegetable Acids-- A solution of neutral lead acetate was added to the two filtrates from IIa. Olive green precipitates were formed. These were removed from the mixtures by filtration and considered as vegetable acids (possibly aconitic acid). The precipitates were heated to constant weight. The differences between the residues (PbO) and original weights of precipitates were assumed as repre-

senting the number of grams of vegetable acid:

Fall 1930 Drug = 1.38%

July 1931 Drug = 0.893%

II. (c) Sugars Dissolved in Alcohol-- To 100 cc. each of the filtrates from IIb. solutions of lead subacetate were added. A slight cloudiness was produced in both filtrates. Lead was removed from solution by treatment with hydrochloric acid and hydrogen sulphide. The solutions were filtered, the filtrates neutralized with ammonia and made up to 200 cc. each with distilled water. Equal parts of Febling's Solution and filtrates (10cc) were boiled but no color changes were produced indicating absence of sugars dissolved in alcohol.

II. (d) Alkaloids-- To 50 cc. portions of filtrate remaining from II (a) qualitative tests for alkaloids were made on divided portions as follows:

Mayers Reagent yielded a yellow amorphous precipitate

Ammonia " brown gummy precipitate

Tannic Acid " a curdy tan-colored precipitate

Picric Acid " no precipitate

Gold Chloride T. S. yielded a brown precipitate which soon turned black.

Wagners Reagent yielded an orange brown precipitate

These precipitates were all heavy and without doubt consisted of other extractive, inert, or coloring matter.

The determination of the presence of alkaloids was later made on separate portions of these lots of drugs and is described more fully in the section on alkaloids.

III. Marcs remaining after previous treatments with chloroform and alcohol were macerated over night in water and then filtered. To small portions of the filtrate in test tubes the following reagents were added:

(1) Gelatin T. S.

(2) Quinine T. S.

(3) Ferric Chloride T. S.

No precipitates or color changes were obtained proving the absence of tannin or tannic acid. The vegetable acid present, therefore, is very likely aconitic acid which is found in other species of the genus. Aconitic acid is closely allied to citric acid which when heated to 175° C loses not only its water of crystallization but also a molecule of water from its own structural formula:

Citric Acid	Aconitic Acid
CH_2 COOH	CHCOOH
COH COOH	C-COOH
CH_2 COOH	CH_2 COOH.

Aconitic acid like citric acid is not poisonous.

III. (a) Gum-- To each of 25cc. portions of the filtrates from III contained in test tubes 50cc. of absolute alcohol were added. The test tubes were tightly closed and allow-

ed to stand for 48 hours at the end of which time muddy brown precipitates had formed which were considered as gum.

Fall 1930 Drug = 4.88% gum.

July 1931 Drug = 0.8104% gum.

III. (b) Albuminoid Matter-- The remainder of the filtrates from III were treated with tannic acid T. S. and any excess of tannin removed from the precipitates by treatment with hot alcohol. The precipitates were then collected in Gooch crucibles, dried to constant weight and calculated as albuminoid material:

Fall 1930 Dg. yielded 7.16% Albuminoid Mat.

July 1931 " " 7.88% " "

IV. (a) Inert Coloring Matter: Marcs remaining after chloroformic, alcoholic and aqueous extractions were next boiled for six hours with 500 cc. portions of water each aciduated with 5 cc. of sulphuric acid. Fresh portions of distilled water were added from time to time to keep the volumes at 500 cc. After filtration the acidity of the solutions was neutralized with 10% sodium hydroxide and the resulting liquids brought to the boiling point, a treatment yielding copious precipitates. These precipitates were removed by filtration and represented inert coloring matter. This was present in both lots but was calculated

only for the drug collected in the fall of 1930. The yield was .7044%.

IV. (b) Tests for Sugars-- 10cc. portions of filtrates from IV a, were tested with 10cc. each of Fehling's Solution (previously boiled to determine if solution itself was reduced). Heavy brick red precipitates of cuprous oxide, Cu_2O were formed indicating presence of reducing sugars.

Two 10cc. portions of filtrate from IVa were next heated with Barfoed's reagent. There was reduction confirming presence of monosaccharides. The starch which exists in abundance in the tubers undoubtedly became hydrolyzed during the procedure of analysis with formation of a monose (glucose). Mannitol which on oxidation yields mannose has been reported in Aconitum Napellus. Mannose is an isomer of glucose. The presence of Mannose in those plants in which it has been reported is, with the exception of manna, present in such small amounts that it is reasonable to suppose that the heavy precipitates of cuprous oxide formed were due to glucose resulting from the hydrolysis of starch present in the tubers of Aconitum lutescens.

V. Cutose-- The remaining dregs were treated with 500 cc. each of 2% NaOH and boiled for two hours, fresh portions of distilled water being added from time to time to keep volume of each determination at the 500 cc. mark. 50 cc.

portions of this sodium hydroxide extractive were evaporated and the residues weighed. To these residues was then added ammonio-cupric sulphate T. S. The sodium hydroxide extractives with the exception of a trace dissolved therein. The part insoluble in ammonio-cupric sulphate T. S. represents cutose or suberose, a substance associated with cellulose as protective matter in the cork of stems and roots.

VI. Cellulose-- The residues remaining from V after treatment with 2% sodium hydroxide were first washed with water, then with alcohol and lastly with ether. They were then thoroughly dried and the weight of each ascertained. The dried dregs were next incinerated to constant weight. The weights of the residues subtracted from the weights of the dried dregs gave the number grams of cellulose present in 5 grams of the tubers.

Fall 1930 Drug = 3.367% cellulose.

July 1931 Drug = 3.82% cellulose.

Summary - Organic Analysis

Aconitum lutescens

	Drug July 1931	Drug-Oct. 1930
Ash- total	3.7085% (ave)	7.485% (Ave.)
Natural Ash	Not determined	3.6405% (Ave.)
H_2O Soluble	" "	0.495% (Ave.)
Acid Insoluble	" "	3.344% (Ave.)
H_2O Insoluble	" "	6.99% (Ave.)
Volatile Oil	Absent	Absent
Fixed Oil	1.36%	1.629%
Resin Ic	0.509%	0.384%
Resin IIa	0.958%	0.0353%
Waxy (?) Matter	0.189%	0.187%
Organic Acids (Vegetable)	0.893%	1.38%
Glucose Dslvd. Alcohol	Absent	Absent
Extractive with Alkaloid	Present	Present
Gum	0.8104%	4.88%
Color Ext. & Albuminoid	7.16%	7.88%
Inert Coloring Matter	Present	.7044%
Starch & Allied Substances	" ++	Present ++
Cutose	Trace	Trace
Cellulose	3.82%	3.367%

From the above it can be seen that upon analysis Aconitum lutescens of fall and summer growth shows a wide variation in at least three respects, namely, total ash, resin and gum content.

The ash content of each organ changes during its growth or development. It has generally been understood that in leaves the ash content increases with age while in stems and roots it decreases. From the preceding summary we find such is not the case with tubers is Aconitum lutescens, those of fall or older growth yielding a much higher ash content than those gathered in July. The increase may be due to added silica encrusting cell walls or to an increased deposit of mineral salts in the tubers. Glancing down the summary a difference of almost one-half per cent in the amount of organic acids present in tubers of summer root and those of fall growth is noted. The organic acid present is assumed to be aconitic acid. This acid usually occurs in the root in the form of the calcium salt. Hence, it is altogether reasonable to suppose that there really is an increased amount of mineral matter present in the older roots which would be present in the form of oxides in the ash of the completely burned tubers. Moreover, the amount should vary considerably in the same plant in different situations indicating that in part these

materials are determined not by the "needs" of the plant but by the solutions which have a chance to wander into it.

The tabulated data show that the tubers of July growth contained only 0.8104% of gum whereas those of October growth 4.88% of gum. The reverse is true of the resin content, the tubers of July averaging 1.467% of resin while those of October only 0.4193%. Gums are in large part carbohydrates and arise from the transformation of cell wall and growing tissue in woody plants. It is, therefore, to be expected that the tubers of the fall season would show a greater gum content than those of the summer season. The conclusion may be drawn that the factors or conditions which favor the formation of gum or mucilage bring about a corresponding decrease in resin secretion.

It was also noticed that when a small amount of the powdered drug was agitated with water the solution frothed strongly and produced suds which did not disappear even after standing for an hour. This frothing was so typical of saponin that several tests were made for this principle. An aqueous extract of drug was utilized for this purpose and the following results were obtained:

1. Neutral Lead Acetate T. S. yielded a slight precipitate.
2. Basic Lead Acetate T. S. yielded a heavy yellowish white precipitate.

3. Gold Chloride T. S. was reduced.

4. Potassium Permanganate was decolorized.

5. Ammoniacal Silver Nitrate T. S. was reduced.

6. Alkaline Copper Solution gave first a greenish gelatinous precipitate, which in a few days settled and became yellowish-green in color. The supernatant liquid appeared blue-green and possessed a distinct fluorescence.

7. Barium Hydrozide T. S. yielded a precipitate.

Saponins form an interesting group of non-nitrogenous glucosides which are widely distributed among plants. Adonis, Cimicifuga, and Pulsatilla are the only genera of the Ranunculaceae which have, so far, been credited as containing one or more related saponins. Since qualitative tests for nitrogen were positive (confirming other positive tests for alkaloid) and also since glucosides and alkaloids are rarely found together in the same plant it may be concluded that the alkaloid or alkaloids of Aconitum lutescens give reactions identical with some of those ascribed to saponins. These same reactions are given by the alkaloids of other species of the genus, Aconitum.

Inorganic Qualitative Analysis of tubers of Aconitum Lutescens:

The plan of qualitative analysis followed was that of Bliss and Schaefer. It was made on two samples of ash from the tubers of Aconitum lutescens incinerated to constant weight. Summing up the results of these determinations the following cations and anions were found in the ash:

Cations	Anions
Group IV - Fe^{+++}	Group VI - SO_4^{--}
Group VI - Ca^{++}	Group IV - PO_4^{---}
Group VII- Mg^{++}, K^{+}	(1) Secondary phosphates (2) Neutral Ortho phosphates

The presence of silicates was indicated by acid insoluble ash.

The constituents of Aconitum Napellus are the alkaloids aconitine, benzaconine, aconine, isaconitine (napelline), aconitic acid chiefly combined with calcium, a little mannitol, a resin and starch. The inorganic constituents other than calcium are not listed by research workers.

The aconite with which Aconitum lutescens agrees most closely in respect to constituents is Aconitum heterophyllum (Wall.) an Indian aconite. Dr. M. Dunin V. Wasowiez

(1879) in undertaking a thorough examination of the root of this species found it to contain a soft fat, aconitic acid, an acid resembling ordinary tannic acid, cane sugar, vegetable mucilage, pectin bodies, the alkaloid atesin (non-toxic) and starch which was determined microscopically. The ash amounted to 2.331% of the perfectly dried roots and contained Al, Mg, Fe, K, and Ca united to Hcl, H_3PO_4, H_2SO_4 and H_2SiO_3.

No work has been done on Aconitum lutescens from an alkaloidal standpoint as far as the writer was able to discern from available literature. The work on Aconitum lutescens was undertaken in an effort to isolate, identify and to test the potency of the alkaloid or alkaloids.

Microchemical Tests Applied:

To several mounts made from a fresh tuber of Aconitum lutescens (Fall 1930 plant):

1. Concentrated sulphuric acid yielded a reddish coloration which became darker on standing (action on starch).
2. Gold and Sodium Chloride T. S. when applied to fresh sections of the tuber produced no crystals. Hence a conclusion was drawn that alkaloids, if present, produce only amorphous precipitates with this reagent.

Qualitative Tests for Alkaloid:

Two preliminary tests for alkaloids were made on tubers of Aconitum lutescens collected in October 1930 from the medicinal plant garden.

(1) 1.3285 grams of powdered drug were macerated in Modified Prollius fluid for twenty-four hours. The Modified Prollius Fluid was made according to the following formula:

Ether.....	250 cc.
Chloroform.....	80 cc.
Alcohol.....	25 cc.
Ammonia Water.....	10 cc.

This fluid containing the macerated drug was filtered and yielded a light brown colored filtrate. This was made alkaline with ammonia and shaken out in a separatory funnel with ether. This in turn was shaken out with acidulated water and separated from the ethereal layer. Mayers Reagent (1-20 normal) when added to acid-water solution gave no test for alkaloid.

Second Preliminary Test:

(2) 2.9384 Gm. of powdered Aconitum lutescens were macerated for 24 hours in Modified Prollius Fluid. The mixture was then filtered. The filtrate which was of light brown color was evaporated. Acidulated absolute alcohol was added to

the residue followed by a large volume of acidulated water. The solution was filtered through wet double filters and the filtrate divided into several portions each of which was tested with a different alkaloidal reagent:

(1) Mayer's Reagent yielded a light brown precipitate.

(2) Wagner's Reagent yielded a brown precipitate.

(3) Gold Chloride yielded no precipitate at first but on standing over night a light brown residue became evident.

(4) Picric Acid T. S. likewise yielded no precipitate at first but on standing over night it too developed a brown residue.

Qualitative Tests for Alkaloid in Tubers of Aconitum Lutescens Collected July, 1931.

1. One gram of drug in number 40 powder was digested with 50cc. of 1% sulphuric acid for 24 hours. It was then filtered and the filtrate made neutral with sodium hydroxide solution. This was then shaken out with chloroform and the aqueous layer drawn off. The chloroformic layer was again treated with acid and the aqueous layer drawn off as before and tested for alkaloid with Mayers Reagent and tannic acid T. S. No cloudiness or precipitate was evident indicating absence of alkaloid, or at least of one soluble in water.

From the result of this as well as from the results of the two foregoing tests it would seem that the alkaloid is more soluble in absolute alcohol than it is in chloroform, ether or acidulated water. The aconitines, however, are a class of alkaloids easily decomposed especially by strong reagents and the above treatment with solution of sodium hydroxide could easily have broken them down into benzoic acid and some inert base. The chloroformic layer remaining in separatory funnel was drawn off into a beaker and the chloroform allowed to evaporate spontaneously. A colorless varnish-like residue in which were detected small needle-like cystals was obtained. This residue had a rather agreeable odor. It was redissolved in chloroform, the chloroform evaporated, and to the resulting amorphous residue was added sulphuric acid containing sucrose. At first a brown coloration was produced followed by a red coloration which changed, gradually becoming violet and finally carmine. This color was quite lasting, changing after a day's standing to a dirty brown.

Second Qualitative Test on Tubers of Aconitum Lutescens of July 1931 Growth.

Following the Duquesnel Procedure 2 gm. of no. 40 powder

Aconitum lutescens were digested with 50 cc. of 95% alcohol containing 1% of tartaric acid and allowed to stand for 48 hours. The supernatant liquid was filtered off and distilled in vacuo. The residue obtained had the appearance of an orange brown varnish-like substance. It was soft, sticky, very bitter and contained white amorphous particles. A portion of this varnish-like residue plus the amorphous particles was dissolved in distilled water. The solution was affected quite readily with the formation, however, of a slightly cloudy mixture. This was filtered and divided into four portions each of which was tested for alkaloid.

(1) Mayers Reagent yielded a white amorphous precipitate.

(2) Wagners Reagent yielded a brown precipitate.

(3) Ammonia Water yielded no precipitate.

(4) Tannic Acid T. S. yielded a brown amorphous precipitate after 48 hours maceration. Small portions of the remaining residue were placed on watch crystals. To one portion concentrated sulphuric acid containing sucrose was added. This produced a yellow color changing to a dark reddish-brown. Neither nitric nor hydrochloric acid added to separate portions produced any color.

Third Qualitative Test on Tubers of July 1931:

Two gms. of powered Aconitum lutescens were macerated

for 24 hours in chloroform. The chloroformic solution was filtered and the solvent allowed to evaporate. The residue obtained was of an orange brown color admixed with a white amorphous substance containing minute needle-like crystals. The residue was redissolved in chloroform and, after subsequent evaporation of the solvent, was examined microscopically. The crystals obtained were small, colorless, needle-like in structure and occurred both singly and in rosettes. A part of the amorphous residue was again treated with concentrated sulphuric acid containing sucrose. A series of color changes, brown to reddish brown, violet and finally black, took place. From the foregoing tests it would seem that an alkaloid is present in Aconitum lutescens. This alkaloid is extracted to the greatest extent with alcohol acidulated with tartaric acid and chloroform. Moreover, it is practically amorphous and very difficult to obtain in crystalline form. It dissolves to quite an extent in water. The crystals obtained in the third qualitative test could possibly be aconitic acid which although not reported (1920) as being soluble in chloroform is soluble in water, alcohol and ether. The alkaloidal reagent tests as well as the color produced by concentrated sulphuric acid and sucrose agree with those given for "atisine" an alkaloid obtained from

Aconitum Heterophyllum Wall. But the reduction of gold chloride T. S., ammoniacal silver nitrate and Fehlings Solution is characteristic for aconine. Aconine, $C_{26}H_{41}NO_{11}$ is a powerful reducing agent and is a decomposition product of the hydrolysis of aconitine, $C_{33}H_{45}NO_{11}$. Professor Dunstan and Dr. F. W. Passmore (1892) studied the hydrolysis of aconitine by heating the pure aconitine with water in a closed tube at 150°C but were unable to obtain at any stage either picraconitine or methyl alcohol which Wright and Luff isolated from roots supposed to be Aconitum Napellus. Dragendorff and Jürgens asserted that the hydrolysis of aconitine proceeds in two stages; in the first stage benzoic acid and an alkaloid identical with picraconitine of Wright and Luff is formed; this is followed in the second stage by hydrolysis of picraconitine into benzoic acid, methyl alcohol and aconine which is the final product of the change. Dunstan and Passmore determined that alkaloid extracted from solution by ether is a mixture of aconine with unaltered aconitine. They found that the base when pure can not be crystallized but they did succeed in crystallizing several of its salts. They found aconine soluble in water, insoluble in ether and almost insoluble in chloroform. Dunstan and Passmore reported an aqueous solution of aconine as being slightly bitter and giving rise to a burning sensation in

the mouth. Aconine is also a product of the hydrolysis of jesaconitine, an alkaloid found in Japanese aconite of the "bush" sort. There are, therefore, three possibilities as to the character of the alkaloid of Aconitum lutescens. It either belongs to the non-toxic group atisines, found in Aconitum Heterophyllum and Aconitum Palmatum or it exists as aconine in the root of Aconitum lutescens or it is formed as the result of hydrolysis of aconitine, jesaconitine or japaconitine, the two latter being present in the two species of Japanese Aconite.

Qualitative Test for Nitrogen:

This was done to confirm the presence of alkaloid found by both qualitative tests and chemical assay.

The alcohol from 10 cc. of the tincture of Aconitum lutescens was evaporated leaving an orange brown residue. To this residue was added a large pellet of metallic sodium and the whole strongly fused. (Decomposition occurs with liberation of nitrogen which combines with metallic sodium to form sodium cyanide). The fused residue in test tube while still hot was plunged into cold water in which the fused mixture partially dissolved. This solution was filtered and divided into three portions. To each was added

a drop of 5% ferrous sulphate solution followed by two drops of a dilute aqueous solution of freshly prepared ferric chloride and one to two drops of dilute hydrochloric acid. A bluish-green color was produced in each of the three test tubes followed by a slight precipitation of Prussian Blue. A duplication of this experiment yielded similar results verifying the presence of alkaloid. The equations for the reactions are:

(a) $6\ NaCN + FeSO_4 = Na_4Fe\ C_6N_6 + Na_2SO_4$

(b) $3\ Na_4Fe\ C_6N_6 + 4\ Fe\ cl_3 = Fe_4(Fe\ C_6N_6)_3 + 12\ NaCl.$

Chemical Estimation of Alkaloid:

The present pharmacopoeia does not give a chemical assay for Aconitum Napellus since such an assay has been found to be comparatively useless in measuring the potency or toxicity of these tubers. The alkaloidal content of Aconitum lutescens was, however, determined by chemical means for purposes of comparison and to learn in what amount the alkaloid exists in the roots. The U.S.P. VIII method of assay was followed. A no. 40 powder consisting of ground tubers of Aconitum lutescens collected in July, 1931), was used. Ten grams of the powdered root were introduced into a 200 cc. Erlenmeyer flask and 75 cc. of a mixture of seven parts al-

cohol (by volume) and three parts distilled water were added; the flask was securely stoppered and agitated at intervals during four hours. A pledget of cotton wa placed in the bottom of a small cylindrical glass percolator and the contents of the flask transferred to the percolator. When the liquid had all passed through, more of the same menstruum was added until 150 cc. of percolate had been obtained. This was poured into a shallow porcelain evaporating dish and evaporated to dryness at a temperature not exceeding 60°C. 5 cc. of 0.1N sulphuric acid and 25 cc. of distilled water were added to the residue which, after solution had been effected, was filtered into a separatory funnel. The dish and filter were washed with 25 cc. of distilled water, and the washings added to the separator. 25 cc. of ether and 2 cc. of ammonia water were next introduced into the separator followed by 15 cc. of ether added after agitation. The lower layer was drawn off into a flask and the ether solution filtered into a beaker. This process was repeated two more times, shaking out with 10 cc. of ether each time. The combined ether solutions were evaporated to dryness and the residue dissolved in 3 cc. 0.1N sulphuric acid. (Factor 1.047). Five drops of cochineal T. S. were used as the indicator. This solution of residue in 0.1N H_2SO_4 was titrated with exactly 0.02N potassium hydroxide until the end point was reach-

ed as indicated by the formation of a pink color.

Final Reading Burette 0.02N KOH = 22.7 cc.

Initial " " = 0.0

No. cc. 0.02N KOH used= 22.7

Back titration with 0.1N H_2SO_4:

Final Reading Burette 0.1N H_2SO_4 = 11.2 cc.

Initial " " = 9.7 cc.

No. cc. 0.1N H_2SO_4 = 1.5

3cc. + 1.5cc. = 4.5cc $\frac{N}{10}$ H_2SO_4 x 1.047= 4.7115cc. exactly 0.1N H_2SO_4 used.

22.7 ÷ 5 = 4.5 cc. 0.1N KOH used.

4.7115 - 4.54cc = 0.1715 cc. 0.1N KOH required.

0.1715 cc. 0.1N KOH x 0.064 = 0.010976 Gm. alkaloid.

0.010976 x 10 = 0.10976% alkaloids present in Aconitum lutescens (July growth).

The U.S.P. VIII required that the dried tuberous root of Aconitum Napellus collected in autumn when assayed by this process yield not less than 0.5% aconitine. The difference in season, no doubt, has an important bearing on the per cent of alkaloid present. A larger amount should be present in the fall when decomposition of proteins occurs to a greater extent. (A lack of material from both lots of drug prevented further work along this line.)

The aconite alkaloids are now known to belong to three

well-defined groups:

1. The Aconitines which are highly poisonous.

2. Decomposition Products (aconines) of the aconitines which are scarcely toxic in the ordinary sense.

3. The atisines which are not toxic.

The aconitines may all be regarded as derived from a parent base, $C_{21}H_XN$[1] where X = 31, 33 or 35. Aconitines chemically are the diacetyl esters of polyhydroxyamino alcohols. The empirical formula of aconitine is $C_{34}H_{45}O_{11}N$. Aconitine melts at 197°-198°, is dextro-rotatory, + 14.61° and crystallises in prisms belonging to the rhombic system. It is soluble in chloroform or benzene, but less so in ether or dry alcohol and almost insoluble in water. Its salts crystallize well and are laevorotatory. When a salt of aconitine is heated to 120°-130° in aqueous solution it undergoes hydrolysis in two stages, yielding first acetic acid and a new base benzoyl aconine and eventually benzoic acid and aconine. Benzoyl aconine and aconine occur in such combination in the plant.

$$C_{33}H_{45}O_{11}N + H_2O = CH_3COOH + C_{32}H_{43}O_{10}N.$$

Aconitine — Acetic Acid — Benzoyl Aconine

$$C_{32}H_{43}O_{10}N + H_2O = C_6H_5COOH + C_{25}H_{39}O_9N.$$

Benzoic Acid — Aconine

Since the aconines are not toxic in the ordinary sense

and since the atisines show absolutely no toxicity, the non-poisonous species of aconite may, therefore, be considered as containing alkaloids belonging to the one class or the other. The amorphous residue obtained from Aconitum Lutescens resembles atisine, $C_{46}H_{74}N_2O_4$ (Broughton and M. Dunin V. Wasowiez) from Aconitum heterophyllum in physical properties and responds to some of the qualitative tests for atisine. If Aconitum lutescens is a variety of Aconitum Columbianum, a species related to Aconitum Fischeri, it is possible that the alkaloid is a decomposition product of japaconitine and occurs as aconine in the root. The presence of this base may account for the lessened toxicity of Aconitum Columbianum.

Fluckiger and Hanbury in their Pharmacographia contended that the poisonous qualities of Aconitum Napellus are not developed in certain localities. C. D. V. Schroff (1876) assumed a contradictory view claiming that the poisonous properties of aconite and other plants are not materially affected when growing wild in different localities. In other words, environment does not play any part in the production of the innocuous properties of certain species of aconite but the root and herb of some of the species are actually destitute of poisonous properties. If such is the case then Aconitum lutescens is not an altered Aconitum Columbianum or a de-

rived Aconitum Fischeri but a species belonging to the group of aconites destitute of poisonous principles. It has been proved, however, by organic analysis, that season, locality, and year play an important part in the kind and amount of constituents present in roots of Aconitum lutescens. Therefore, the writer favors the view that season, year and locality play an important rôle in the development of certain constituents of plants including such principles as alkaloids.

Bio-Assay of Aconitum lutescens:

The U.S.P. X requires that Aconitum Napellus be biologically assayed. Consequently, this procedure was applied to Aconitum lutescens in order that the pharmacodynamics of the two might be compared.

The U.S.P. X method of assay was followed and guinea pigs were used as test animals. This method requires that a tincture of the drug administered subcutaneously have a minimum lethal dose of not less than 0.00035 cc. nor more than 0.00045 cc. for each gram body weight of guinea pig. The assay directs the use of healthy animals weighing from 275 to 325 grams. The tincture is diluted with distilled water to make the dose about 1 cc. and injected under skin

of abdomen. The standard dose should kill at least two of every three guinea pigs within six hours.

Guinea pigs are especially well adapted for assay purposes because of their relatively slight variation in susceptibility due to age, sex, temperature, season, and so forth, as compared with a large variation found in frogs.

Apparatus-- A graduated syringe, a pipette graduated in $\frac{1}{100}$ cc, scissors, scales and a set of weights ranging from 1-500 grams.

Animals-- Male guinea pigs.

Preparation of Animal-- Hair was clipped from about one square inch of skin over the abdomen. The surface thus exposed was washed with alcohol and the animal weighed.

Preparation of Solution-- The alcohol was evaporated from 20 cc. of the tincture and then replaced with distilled water.

Method of Injection-- Subcutaneously in abdominal region.

Results of Experiments on Guinea Pigs

January 19, 1932

Aconitum lutescens

Subst.	Descr. of Animal	Wt.	Actual Dose of Dil.	Tm.	Remarks
Tinc.	Slate	400gm	0.160	11:25A.M.	No eff.
Tinc-ture	Tan	330gm	0.132	11:30A.M.	No eff.
"	Brown, Slate & Wh.	420gm.	0.168	11:35 "	"
	Date, Jan. 21, 1932. Five Time M.L.D. of Tr. Adm.				
Tinc-ture	White with Eyes Pink	360gm.	0.72	11:30A.M.	No eff.
"	White with Brown Spots ar. eyes	375gm.	0.75	11:30 "	"
"	Brown	410gm.	0.82	11:40 "	"

Five times the minimum lethal dose was administered because Aconitum luteseens in chemical assay showed the presence of only 0.1 of 1% of alkaloid. Chemically assayed, according to the U.S.P. VIII and IX, Aconitum Napellus should contain 0.5% aconitine. Therefore, in order to make a bio-assay comparison it was necessary to inject a dose equivalent to that of tincture of Aconitum Napellus.

A second bio-assay was also made using the lethal frog method. Four frogs of the ordinary grass variety were used in assaying the tincture. These frogs were kept in as nearly suitable conditions as possible, the temperature of storage tanks was kept as near to that of the room as possible. Frogs varying not more than 5 Gms. in weight were used. As in the official procedure, the alcohol from 20 cc. of the tincture was evaporated and replaced with distilled water. Ten times the minimum lethal dose was injected into the anterior lymph sac by first passing the needle of the syringe through the muscular tissue of a leg in order to prevent loss of part of injected fluid which is very possible on account of the relative inelasticity of the frog's skin.

Bio-Assay Tr. A. Lutescens on Frog

No.	Dose per Gm. Body Wt.	Description of Frog	Wt.	Time	Result
1	0.18 cc.	Grass Frog	45 Gm.	11:55a.m.	No effect
2	0.14 cc.	" "	35 Gm.	12:00 "	" "
3	0.16 cc.	" "	40 Gm.	12:02 "	" "
4	0.18 cc.	" "	45 Gm.	12:03 "	" "

10 x M. L. D. Injected

The results of these experiments show that the alkaloid present in tubers of Aconitum lutescens is non-toxic and, therefore, not aconitine.

The principal action of members of cardiac depressants to which Aconitum Napellus belongs is a lowering of the activity of the heart. The drug accomplishes this in two ways, namely:

1. By stimulation of the vagus mechanism.

2. By weakening of the cardiac muscle. The first effect is the only one utilized therapeutically since large doses of almost any drug may produce the second effect.

Aconitum heterophyllum and Aconitum palmatum belong

to the aconites containing the non-poisonous group of alkaloids known as the atisines.

On the other hand, aconine, a decomposition product of aconitine from Aconitum Napellus and of japaconitine and jesaconitine from the Japanese Aconites, behaves as a cardiac tonic. It exhibits a curare-like effect on the motor nerve endings of muscles. It is not considered poisonous in the usual sense, very large doses being required to produce death even in frogs.

Other Biological Tests for Alkaloids:

1. Squibb Taste Test for Aconitine.

A tincture of Aconitum lutescens made according to type process P. of U.S.P. X was diluted 1:70 with distilled water. Four cc. of this dilution were held in the anterior part of the mouth for a few seconds and then discharged. There was no tingling or benumbing sensation to the tongue or to any part of the mouth. There was, however, an increase in salivation caused by bitterness of the drug. The taste test was repeated within half an hour but the results were again negative.

2. Three drops of the 1:70 dilution were placed under the upper eyelid of the right eye. There was neither a

constriction nor a dilation of the pupil. (Solutions of aconitine constrict the pupil of the eye).

3. Fifteen minutes after the second taste test the writer experienced a choking sensation accompanied by a fall in the pulse rate to 64.

Cash investigated the physiological action of atisine and found it to be somewhat similar to aconine which is antagonistic to aconitine and exerts a curare-like effect on motor nerve endings of muscles.

Summary and Conclusions

1. Cross-sections of rootlets and tubers as well as longitudinal sections of tubers of Aconitum lutescens were made, examined, sketched and studied. The same procedure applied to the powdered drug. These studies revealed that, if certain ecological factors are taken into consideration Aconitum lutescens resembles Aconitum Columbianum in its outer morphology. There is also a marked histological similarity to tubers of Aconitum Fischeri, but since writers and workers disagree as to the true source of Japanese Aconite and also since there exists considerable confusion in regard to certain erratic structures and features of the daughter tubers and weak roots of Aconitum Napellus, it is not possible to determine with certainty the line of origin for Aconitum lutescens.

2. Quantitative results of organic analyses of tubers of Aconitum lutescens differed with season and regional sources of the plant. These analyses together with qualitative inorganic analyses revealed a general similarity to Aconitum heterophyllum, especially in the tendency of aqueous mixtures of the powdered root to froth on agitation. The acid insoluble ash also proved to be higher than the U.S.P. requirement for Aconitum Napellus.

3. Duquesnel's method yielded an amorphous alkaloidal substance. This material resembles atisine in physical properties and certain qualitative tests and color reactions. Its reducing properties simulate those of a saponin or of aconine.

4. A biological assay proved that Aconitum lutescens is non-toxic to guinea-pigs and frogs even in relatively large doses. In the assay the tincture was used and the hydrogen ion concentration may have had some influence in the results. Aconitum lutescens does not, however, bear any resemblance pharmacodynamically to Aconitum Napellus.

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