XXXII.—COMMUNICATIONS FROM THE RESEARCH
LABORATORY OF THE PHARMACEUTICAL SOCIETY
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Contributions to our Knowledge of the Aconite Alkaloids. Part II.
The Alkaloids of true Aconitum napellus.

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THE very discrepant observations of various workers as to the number, nature, and properties of the alkaloids contained in the different species of aconite have been attributed to several sources of error:—

i. To the confusion of different species. It is alleged that it is by no means easy to distinguish between the roots of the different species and varieties, and it is, therefore, highly probable that mixtures of roots have been examined. There is good reason to

believe that each species and possibly some varieties contain distinct alkaloids.

ii. The principal aconite alkaloids are remarkable for the ease with which they undergo decomposition, especially hydrolysis. Many of the processes hitherto used in extracting the alkaloids from the plant, as well as those adopted in "purifying" the products, must have led to decomposition of the natural alkaloids, and it is, therefore, probable that some of the alkaloids examined and described as constituents of the plant were in reality their decomposition products. The researches of Wright and his fellow workers afforded much valuable information on this point, and led them to the conclusion that "*A. napellus* roots contain as chief active constituent a crystallisable base aconitine, $C_{33}H_{43}NO_{12}$, together with less active amorphous bases probably formed therefrom during extraction." "On examination of the non-crystalline bases, they uniformly furnish numbers quite compatible with the notion that they are formed from the crystallisable bases (of *A. napellus* and *A. ferox*) by saponification, dehydration, polymerisation, &c., changes to which alkaloids as a class appear to be peculiarly prone" ("The Alkaloids of the Aconites." Part III. Trans., 1878).

iii. A third source of error resides in the circumstance that the determinations of the composition and properties of the alkaloids have been proceeded with before conclusive evidence of the purity of the material was obtained. The result is that mixtures have been examined and described as single alkaloids. This source of error is, perhaps, the most difficult to guard against, and much of the tediousness of this investigation has arisen from the length of time which it has been necessary to spend in discovering and carrying out, with very small quantities of material, processes of purification suitable to the exceptional character of these alkaloids.

In the present enquiry, every precaution has been taken to avoid the three important sources of error referred to above.

In a paper communicated to the Society last year by one of us in conjunction with Dr. W. H. Ince (Trans., 1891, 59), the principal properties of a crystalline alkaloid derived from carefully selected commercial roots of *Aconitum napellus* were fully described. The alkaloid had been extracted from the roots by means of a process which avoided all risk of decomposing the natural constituents. Special pains were taken to completely purify the base, and satisfactory evidence was furnished as to its homogeneity. This alkaloid was evidently the same substance as that first obtained by Wright from commercial roots of *A. napellus*, but in a purer condition. Two of the sources of error alluded to above as vitiating most of the previous work on the subject were thus removed. A third, however, still remained

to be dealt with. This alkaloid had been obtained from carefully selected commercial roots stated to be those of *A. napellus*, but it has been asserted by those botanists who have paid particular attention to this subject, notably by Mr. E. M. Holmes, that it is most difficult, if not impossible, to judge from the appearance of the roots alone whether they belong to the true *A. napellus*. It is necessary, for the purposes of identification, that the flowers and leaves should also be examined. On account of this doubt as to the authenticity of the roots from which this specimen of alkaloid had been obtained, we considered it desirable to ascertain whether this same alkaloid is present in the genuine *A. napellus*. We also wished to settle the question as to whether other alkaloids accompany it in the plant, for on this point there is at present no trustworthy information.

Fortunately we have had no difficulty in securing a small supply of the roots of the true *A. napellus*. The plants were selected and grown at Sevenoaks by Mr. E. M. Holmes at the instance of the British Pharmaceutical Conference. Their botanical characters have already been described by Mr. Holmes (*Trans. Brit. Pharm. Conf.*, 1888).

Alkaloidal Constituents of the Juice of the Roots.

As the fresh roots were moist with natural juice, an examination was made of the alkaloids contained in it, since it appeared interesting to determine to what extent the alkaloids are present in the juice as soluble salts, and how far they occur in an insoluble form in the cells of the root.

Ten kilos. of the fresh roots were submitted to hydraulic pressure, amounting to $1\frac{1}{2}$ tons to the square inch. By this means, about 4.5 kilos. of liquid were obtained. To complete the removal of the soluble alkaloidal salts, about 3.5 litres of water were allowed to soak into the pressed roots, which were then again submitted to hydraulic pressure. This operation furnished an additional 3.5 kilos. of liquid. A single drop of the mixed liquid, when placed on the end of the tongue, at once gave rise to the characteristic tingling sensation produced by crystalline aconitine. It was found to be almost impossible to filter the turbid liquid, and, owing to its viscosity, organic solvents (ether, chloroform, &c.), did not readily separate from it in distinct layers.

The greater part of the proteid was therefore precipitated by the addition of acetic acid, and the filtered solution shaken with chloroform, which removed a small quantity of resin and a little of the alkaloidal acetates. Excess of sodium hydrogen carbonate was then added to the acid liquid, and the alkaloid extracted by agitation with

chloroform; the chloroform solution, on evaporation, left a gummy, alkaloïdal residue. Since the amount of alkaloïd was small, the remainder of the roots (about 15 kilos.) was dealt with in an exactly similar manner, the juice being obtained by hydraulic pressure.

Examination of the Alkaloids from the Juice.—The brown resinous mass derived from the solution in chloroform showed no signs of crystallising, even after prolonged standing. Its weight was nearly 13·5 grams, and as about 25 kilos. of fresh roots had been used for its production it follows that the juice contains about 0·05 per cent. of the total alkaloïd present in the roots.

The mass was mixed with ether, and the mixture heated for some hours. About three-fourths of the total alkaloïd dissolved in the ether, nearly one-fourth remaining undissolved even after prolonged digestion. The total alkaloïd was thus divided into two portions, a greater part soluble in ether as well as in chloroform, and a smaller portion soluble in chloroform, but insoluble in ether.

Alkaloids Soluble in Ether.—The gummy residue left on evaporating the ether was dissolved in water acidified with acetic acid, and the liquid extracted with chloroform, which removed a little resin and also traces of alkaloïd, since acetates of the aconitine alkaloids are removed to a slight extent by chloroform from their aqueous solutions. Excess of sodium hydrogen carbonate was then added to the liquid, and the alkaloïd extracted by repeated shaking with ether. The ethereal solution was dried with calcium chloride, the greater part of the solvent distilled off, and the residual solution allowed to evaporate spontaneously, when it left a slightly coloured, gummy residue, which could not be crystallised. As an excessively dilute solution of this residue produced tingling of the tongue, it seemed likely that aconitine was present. The alkaloïd was converted into hydrobromide by exact neutralisation with dilute hydrobromic acid, and on slowly evaporating the neutral solution, a syrup was obtained which deposited a few crystals of *aconitine hydrobromide*, but the quantity was too small for analysis. An attempt was now made to separate the portion which did not further crystallise into two parts. Fractional precipitation of an alcoholic solution with ether, and fractional precipitation of the base itself, alike failed to effect any definite separation, and none of the fractions could be crystallised. Neither could the hydrochloride of the alkaloïd be crystallised. The aqueous solution of the hydrochloride gave an abundant precipitate with auric chloride, but the pale-yellow, amorphous aurochloride could not be crystallised either from its solution in chloroform or in alcohol.

These results proved that we were dealing for the most part with an amorphous alkaloïd containing a small quantity of aconitine.

Like aconitine, the amorphous alkaloid is soluble in ether and chloroform. It forms salts which do not appear to crystallise, and from which we were unable to separate a crystalline aconitine salt, although we had satisfactory evidence that this alkaloid was present in the substance.

The residual alkaline liquid which on extraction with ether had furnished the mixture of alkaloids above described was found still to contain alkaloid, which was removed by shaking with chloroform. The alkaloid thus extracted resembled that dissolved by ether. Neither the base nor its salts would crystallise. The aurochloride, however, deposited a few crystals from an alcoholic solution when this was slowly evaporated. These melted at 134.25° (corr.). Aconitine aurochloride melts at 135.5° .

Alkaloid Soluble in Chloroform but not in Ether.—The small quantity of alkaloid which was dissolved from the alkaline solution by chloroform and was not soluble in ether was converted into hydrobromide, and the solution of this salt concentrated by exposure in a vacuum desiccator; no crystallisation occurred, and attempts to produce crystalline salts were not successful. The base dissolved readily in water and alcohol, less readily in chloroform, and hardly at all in dry ether. Fractional precipitation of a solution in chloroform by ether did not lead to the isolation of a crystalline product. Although the base was nearly insoluble in dry ether, yet it may be partly removed from an alkaline solution by extraction with ether, especially in presence of the alkaloid above referred to as soluble in ether. This peculiarity accounts for the fact that some of this base was also found in the ether-soluble alkaloid obtained as above described.

This amorphous alkaloid resembled aconine in its properties, but the quantity obtained was insufficient for analysis.

The juice expressed from the roots, therefore, contains (1) a considerable quantity of amorphous alkaloid or alkaloids, soluble in ether; (2) a smaller quantity of an alkaloid, probably identical with aconitine; (3) a small amount of an amorphous alkaloid insoluble in ether, probably identical with aconine.

The greater part of the alkaloids had evidently remained in the roots, and has not been expressed with the juice.

Alkaloidal Constituents of the Pressed Roots.

The almost dry mass from which the juice had been expressed was now examined. It may be concluded that all the alkaloidal salts soluble in water had been removed from it by the previous operation.

The pressed roots were dried at about 50° , finely powdered, and the alkaloidal salts removed by percolation with steam-distilled fusel oil (b. p. $100-132^{\circ}$). The pale-yellow solution was shaken several

times with water containing 1 per cent. of sulphuric acid,* and the aqueous layer removed. Dissolved amyl alcohol was now extracted by shaking the acid liquid with chloroform, which also removed resin. Dilute ammonia was then added in very slight excess, and the solution extracted several times with ether. The ethereal solution of the alkaloid was dried with calcium chloride, the ether partly removed by distillation, and the remainder spontaneously evaporated.

The alkaline liquid from which the alkaloid soluble in ether had been removed was repeatedly extracted with chloroform, and the chloroform distilled off, when a resinous alkaloidal residue remained.

Alkaloid Soluble in Ether.—The residue from the ethereal solution was a slightly coloured gummy mass, which did not crystallise after prolonged standing. It was therefore converted into the hydrobromide by dissolution in very dilute hydrobromic acid, and the exactly neutral solution slowly evaporated† in a desiccator. By this means a crystalline hydrobromide was obtained, which, after recrystallisation and drying, melted at 163° (corr.). From this salt, which corresponded in its properties with aconitine hydrobromide, the alkaloid was regenerated by adding a slight excess of dilute ammonia to an aqueous solution, and extracting the base with ether. The dry ethereal solution, when spontaneously evaporated, deposited crystals, which melted at 188.6° (corr.), which has been shown in Part I of this investigation to be the melting point of pure aconitine. The aurochloride was prepared by precipitation in the usual manner, and subsequently crystallised from alcohol. When dry, it melted at 135.5° (corr.), the melting point of aconitine aurochloride.

In order to completely establish the identity of the alkaloid with aconitine, the aurochloride was burned. The following results were obtained :—

Weight of aurochloride, 0.155 gram; weight of carbon dioxide obtained, 0.2278 gram; weight of water obtained, 0.068 gram.

	Found.	Calculated for $C_{33}H_{45}NO_{11}, HAuCl_4$.
C	40.08 per cent.	40.12 per cent.
H	4.87 "	4.66 "
Au	19.7 "	19.99 "

* Very dilute sulphuric acid was employed instead of an organic acid, because sulphuric acid of this strength had been found not to hydrolyse aconitine under the conditions of the experiment, whilst some organic salts of aconitine occasion trouble by passing out of the acid liquid when it is shaken with chloroform.

† Aconitine is readily hydrolysed if evaporated in a solution acid with hydrobromic acid, but a neutral solution of the hydrobromide may be heated at 100° without decomposition.

These results prove the alkaloid to be aconitine, and incidentally furnish confirmatory evidence of the correctness of the formula $C_{33}H_{45}NO_{11}$, proposed for aconitine in Part I.

In the previous paper, it was shown that this alkaloid is remarkable in forming salts which are lævorotatory, although solutions of the alkaloid itself are dextrorotatory. The specific rotation of the hydrobromide now obtained was determined, with the following result:—

$$\alpha[20^\circ] = -1.1625^\circ \text{ (mean of four determinations); } l = 2 \text{ dm.; } \\ d \ 20/20^\circ = 1.0053; \ p = 1.95;$$

$$\text{whence, } [\alpha]_D = \frac{100 \times 1.1625}{2 \times 1.95 \times 1.0053} = -29.65.$$

This number agrees with that previously recorded in Part I, namely, $[\alpha]_D = -30.47^\circ$.

Having thus obtained a small supply of pure aconitine, the opportunity was taken of ascertaining the solubility of the alkaloid in water. As mentioned in the previous paper (Part I), Jürgens had recorded it as 1 gram in 726.4 grams of water at 22° , but we had observed that the pure alkaloid was far less soluble than this. A weighed quantity of aconitine was finely powdered, and digested with water at 22° during two days. A known quantity of the solution was evaporated to dryness, and the residue of aconitine weighed. The following results were obtained:—

I. 72.488 grams of solution yielded 0.0164 gram of aconitine.

Hence 1 gram of aconitine dissolves in 4420 grams of water.

II. 150.15 grams of solution yielded 0.0338 gram of aconitine.

Hence 1 gram of aconitine dissolves in 4442 grams of water.

The mean result of these two determinations is 1 in 4431, which may be taken as the solubility of aconitine in water at 22° .

Napelline.—The syrupy liquid from which all the aconitine hydrobromide had crystallised, after prolonged standing in a desiccator, was reconverted into alkaloid by the addition of a slight excess of ammonia to its aqueous solution. Ether extracted from this liquid an alkaloid, which, on evaporation of the ether, remained as a pale-yellow mass resembling a gum. It was almost entirely soluble in anhydrous ether. The ethereal solution, when evaporated, left a resinous mass, which could not be crystallised; when quite dry, it was easily powdered. The base dissolved only sparingly in water, but the aqueous solution was alkaline and extremely bitter; unlike aconitine, it produced no tingling sensation. The alkaloid is readily soluble in alcohol and ether, less readily in chloroform, and scarcely at all in light petroleum. Its solutions are lævorotatory.

The base dissolves in dilute acids forming salts which are left as varnishes on the evaporation of the solvent. Many unsuccessful attempts were made to crystallise the hydrobromide, hydrochloride, and nitrate both from alcoholic and from aqueous solutions. Alkalis precipitate the base from solutions of its salts in white flocks.

On the addition of auric chloride to an aqueous solution of the hydrochloride, a pale-yellow, amorphous aurochloride is precipitated. This, when dry, melted somewhat indefinitely near 110° . It could not be crystallised from its solution in alcohol or chloroform.

The properties of this alkaloid are being more fully investigated. It is evidently the substance which has been observed to cling obstinately to crystalline aconitine, and which appears as a "gummy ring" when solutions of impure aconitine are evaporated. The two alkaloids may, however, be almost completely separated by conversion into the hydrobromide, when the crystalline hydrobromide of aconitine may be removed from the syrupy residue of the uncrystalline hydrobromide of the new base. Since we were unable to obtain a crystalline compound or derivative of the substance, it was not possible to ascertain with certainty whether it was free from other impurity than aconitine; the last traces of the latter alkaloid are not easy to remove.

The facts above recorded prove that this amorphous base occurs naturally in *Aconitum napellus*, and that it is not a decomposition product of aconitine. Its properties do not correspond with those of the amorphous bases obtained by previous workers. It is distinguished from the piraconitine of Wright by not readily forming crystalline salts. It differs from aconine by its solubility in ether, and sparing solubility in water. We propose to revive for it the name *napelline*, which, although twice proposed for alkaloidal products derived from aconite roots, has never been widely used and is now no longer applicable to any single alkaloid. Its great advantages as a designation for a second alkaloid of *Aconitum napellus* are obvious. The name *napelline* was first applied by Wiggers to the alkaloid now known as pseudaconitine, and afterwards Hübschmann used it as a name for an alkaloid he extracted from *Aconitum napellus*, which the experiments of Wright showed to be a mixture chiefly composed of aconine, resulting from the decomposition of aconitine during its extraction from the root. The alkaloid, which we suggest should be called *napelline*, was observed to be present in the roots of *A. napellus*, to quite as large an extent as aconitine.

Considerable progress in the tedious work of purifying and characterising this alkaloid has since been made by one of us in conjunction with Mr. E. F. Harrison. The preparation of a crystalline compound of *napelline* has made it possible to obtain the alkaloid in an unques-

tionably pure state, but the operation is one of the most tedious and laborious that we have yet encountered in this troublesome inquiry. The pure alkaloid having been at last isolated, its physiological action is being examined. When separated from aconitine in the manner above described, napelline is still contaminated with another alkaloid, which does not crystallise or furnish crystalline salts. On account of its resemblance to napelline, we propose to call this substance *homo-napelline*.

Alkaloid Insoluble in Ether but Soluble in Chloroform.—The alkaline solution from which the alkaloid soluble in ether had been removed, was shaken with chloroform. This dissolved a resinous substance, which afforded alkaloïdal reactions. The mass was washed with water, which dissolved the greater part, leaving a little resin undissolved. By extracting the aqueous solution with chloroform, the alkaloid was removed, and remained after evaporation of the solvent, as an amorphous residue. This was found to be nearly but not quite insoluble in dry ether, a small quantity of napelline being dissolved. In order to completely remove napelline, the alkaloid was dissolved in chloroform, and the solution precipitated with anhydrous ether. By several repetitions of this process, a colourless, deliquescent resin was obtained, entirely soluble in cold water and in alcohol, but scarcely soluble in dry ether or light petroleum. Numerous attempts were made to crystallise the base, but without success. In all these respects this alkaloid corresponds with aconine, the basic hydrolytic product of aconitine, some of whose properties were described in Part I, and are now more fully elucidated in Part III, which follows this paper. The dry base was analysed with the following results:—

Weight of substance, 0·1078 gram; weight of carbon dioxide, 0·2258 gram; weight of water, 0·0776 gram.

	Found.	Calculated for $C_{26}H_{41}NO_{11}$.
C	57·12	57·44 per cent.
H	7·99	7·73 „

The base is thus proved to be *aconine*. There can be no doubt that this alkaloid actually exists in the roots of *A. napellus*, since the process used in its isolation is such that it could not have been formed by the hydrolysis of aconitine. The amount of aconine contained in the roots is very slightly if at all less than that of aconitine.

The precise relationship of aconine to aconitine has not hitherto been satisfactorily established. Its properties have suggested that it may not, perhaps, stand in the simple relation to aconitine which the equation representing its formation by hydrolysis would imply, $C_{33}H_{45}NO_{11} + H_2O = C_7H_6O_2 + C_{26}H_{41}NO_{11}$. Isomeric change or

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molecular condensation may have accompanied hydrolysis. In order to ascertain whether aconine is a polymeride of the formula $(C_{26}H_{41}NO_{11})_n$, the molecular weight of the base was determined by Raoult's method, acetic acid being used as the solvent. The following are the experimental data :—

Weight of substance.....	0.116 gram
Weight of solvent.....	6.1402 grams
Freezing point of solvent.....	14.973°
" solution	14.833°
Depression for 1 gram of substance to 100 grams of solvent	0.0741°
Mol. depression of acetic acid ..	39

Hence molecular weight of aconine = 526 ; molecular weight calculated from formula $C_{26}H_{41}NO_{11} = 543$.

This result is remarkably near that calculated for the formula $C_{26}H_{41}NO_{11}$, when it is remembered that a difference of 0.01° in the reading of the thermometer corresponds with a difference of about 40 in the molecular weight, and that no correction has been made for the formation of aconine acetate. The connection between aconine and aconitine has been placed beyond question by the experiments to be described in Part III.

This inquiry has proved that the roots of true *A. napellus* contain four alkaloids. Only one of these has so far been crystallised, namely, aconitine, whose chief physical and chemical properties, and those of its characteristic compounds, have been described in Part I (Trans., 1891). The quantity of the three uncrystalline alkaloids *aconine*, *napelline*, and *homo-napelline* occurring in the roots is more than twice as great as that of aconitine. The properties of *napelline* and *homo-napelline* are now being further investigated.

The physiological action of these four alkaloids derived from *A. napellus* is being examined. The results so far obtained prove that aconitine is by far the most poisonous of them, and, indeed, it appears to be the most toxic of the vegetable alkaloids.