

XCIX.—*Contributions to our Knowledge of the Aconite Alkaloids.* Part XIII. *On Atisine, the Alkaloid of Aconitum heterophyllum.*

By HOOPER ALBERT DICKINSON JOWETT, D.Sc. Lond., Research Fellow of the Pharmaceutical Society.

AMONGST the many species of *Aconitum* known to botanists, there is one—*Aconitum heterophyllum*—which especially attracts attention, because it is not poisonous. In this respect it differs from the other species of *Aconitum* which have been chemically examined. The roots of this plant are sold in the Indian bazaars as *atis*, or *atees*, and have long been used in India on account of their medicinal value, but very little is known concerning the chemical constituents of the root.

In 1873, Broughton (*Blue Book, East India Cinchona Cultivation*, 1877, 133) isolated an alkaloid from the roots of the plant, to which he gave the name *atisine*, and proposed for it the formula $C_{46}H_{74}N_2O_5$. The alkaloid was only obtained as an amorphous varnish, yielding, however, a crystalline sulphate and hydrochloride. The platini-chloride was also prepared, and the formula above given was deduced from the analyses of this salt, which, however, have not been published.

In 1879, Wasowicz (*Arch. Pharm.*, **214**, 193) isolated the same alkaloid from the roots of the plant, and also aconitic acid. He adopted Broughton's formula for atisine with very slight modification, and prepared the haloid salts in the crystalline condition. The hydriodide was found to be only sparingly soluble in cold water, and could thus be easily purified by recrystallisation; on analysis, results were obtained corresponding with the formula, $C_{46}H_{74}N_2O_4, HI, H_2O$.

In 1879, Dr. Alder Wright (*Year Book of Pharmacy*, 1879, 422) proposed for atisine the formula, $C_{22}H_{31}NO_2$, based on a single analysis of the amorphous aurichloride, the quantity at his disposal being too small for further examination.

In the course of the investigation described in this paper, the properties of the base and its salts have been carefully determined. A satisfactory method has been devised for the extraction of the roots and the preparation of pure material, and the presence of aconitic acid, mentioned by Wasowicz, has been confirmed.

Extraction of the Alkaloid from the Root, and its Purification.

The solvents, previously used to extract the alkaloidal salts from the aconite roots, have been chiefly alcohol acidified with 1 per cent. tartaric acid, or more recently amylic alcohol (steam-distilled fusel oil).

After a number of experiments with different solvents such as light petroleum, ether, alcohol, &c., had been made, it was found that the most satisfactory results were obtained by using a mixture of methylic and amylic alcohols, in the proportion of three volumes of methylic alcohol to one volume of amylic alcohol, the commercial wood spirit and fusel oil being employed for this purpose. The advantages of this solvent for the extraction of the roots are (i) the roots are completely extracted in a very short time, and the percolate does not contain very much colouring matter, resin, &c., although more than if amylic alcohol alone were used; (ii) the percolate can be reduced to a small bulk without very much heating, as the methylic alcohol can be distilled off at a low temperature under diminished pressure, thus preventing decomposition of the alkaloid by heat; (iii) the alcoholic solution thus obtained deposits a large quantity of fat and colouring matter, from which the alcohol containing the alkaloid in solution can be separated by decantation. The alkaloid can then be extracted by shaking the alcoholic solution with 1 per cent. aqueous sulphuric acid, and following the methods described when amylic alcohol alone is used. This solvent, which has since been successfully employed for the extraction of other species of aconite roots, possesses the advantage of both methylic and amylic alcohols with none of their attendant disadvantages.

About 60 lbs. of the root in fine powder were extracted by percolation with the above medium, and the percolate obtained, which was of a dark brown colour, was distilled over a water bath, when most of the methylic alcohol, together with a little amylic alcohol, was removed. On standing, the alcoholic solution deposited a large quantity of very dark coloured fat, which was separated by decantation; the solution in amylic alcohol was then repeatedly shaken with 1 per cent. aqueous sulphuric acid until no more alkaloid was extracted, the acid liquid shaken twice with chloroform to remove amylic alcohol, and then, after neutralisation, concentrated by evaporation on a water bath. The concentrated solution, which was

dark coloured, was rendered alkaline by aqueous sodium hydroxide, and then repeatedly shaken with ether or chloroform until no appreciable amount of alkaloid was any longer extracted. It is not possible, however, by this method to extract the whole of the alkaloid from the aqueous liquid.

The ethereal (or chloroform) solution, after being washed once or twice with water, is distilled, and the residue dissolved in dilute sulphuric acid, using as small an excess as possible. The solution thus obtained is dark brown, and still contains in addition to the alkaloidal salt, a large quantity of resinous and colouring matter, which prevents crystallisation when the solution is evaporated.

These attendant impurities are best removed by fractionally precipitating the alkaloid with aqueous sodium hydroxide, the first fractions containing nearly the whole of the impurities, whilst the later fractions consist of almost pure alkaloid; this fractionation is repeated several times, until the acid solution of the alkaloid is almost colourless. The freshly precipitated alkaloid, after being collected and washed, is suspended in water and dissolved in exactly the right quantity of dilute hydrochloric acid to form a neutral solution of atisine hydrochloride. This, when slowly evaporated on a water bath, yields crystals, which are collected and re-crystallised once or twice from water, and then from alcohol; on adding ether to the alcoholic solution until there is a faint permanent turbidity, and then setting it aside in a stoppered bottle, fine needle-shaped crystals of the pure hydrochloride are obtained. These are collected and dried in the usual manner.

For the extraction of the remaining traces of alkaloid in the different filtrates obtained during fractionation, and in the mother liquor after extraction with ether, a different method is employed. The solution is rendered faintly acid and excess of potassio-mercuric iodide added, the precipitate thus produced is first thoroughly washed several times by decantation with water, and then suspended in water, and decomposed by passing hydrogen sulphide through the mixture; the precipitate is then collected, extracted by boiling water until no more is dissolved, and the aqueous extracts mixed and allowed to cool; on standing, very faintly coloured crystals of atisine hydriodide separate, which may be purified by recrystallisation from hot water. A few crystals can be obtained by concentrating the original filtrate, but owing to the sparing solubility of the salt in cold water, scarcely anything is left in the mother liquors.

In this way the whole of the alkaloid is obtained as the sparingly soluble crystalline hydriodide, and the easily soluble hydrochloride, the amount of hydriodide contained in the mother liquor being so small that it may be neglected.

The total amount of alkaloid contained in the root is small, from 0·2 to 0·3 per cent., and no evidence was obtained of the existence of any alkaloid other than atisine.

Identification of Aconitic acid from the Root.

As the melting point of the acid obtained by Wasowicz did not agree with that recorded for aconitic acid by other observers, I prepared the acid from the root, and examined its properties. It was isolated by means of its lead salt, which was then decomposed by hydrogen sulphide, and purified by crystallisation from water until its melting point was constant. It is a white, crystalline substance, very soluble in ether or water, and, when dried at 100°, melted and decomposed with effervescence at 191·5° (corr.). Aconitic acid melts, and decomposes with effervescence, at 191°.

On analysis, the acid and its silver salt yielded the following data.

0·0382 acid required 12·5 c.c. N/20 soda.

0·0758 silver salt gave 0·0494 Ag. Ag = 65·17.

Calculated for $C_6H_6O_6$, 13·1 c.c. and 65·45 per cent. silver.

The acid is thus proved to be aconitic acid.

Composition and Properties of Atisine and its Salts.

The base, which has only been obtained as a colourless varnish, is prepared as follows. The aqueous solution of a salt (preferably the hydrochloride) is rendered alkaline by sodium hydroxide, and the alkaloid, which is precipitated in white flocks, is extracted by shaking with ether; the ethereal solution, after being shaken with a little water until free from alkali, is dried with calcium chloride, and the ether removed by distillation. The base is thus left as an amorphous, colourless varnish, which is slightly soluble in water, freely soluble in alcohol, ether, or chloroform, and insoluble in light petroleum. It readily undergoes decomposition when heated, becoming brown, and forming a resinous substance. All attempts to crystallise it from various solvents failed. Its alcoholic solution is lævorotatory, although the salts are dextrorotatory, a determination yielding the following results.

$$\alpha[19^\circ] = -2\cdot64^\circ; l = 2 \text{ dm}; c = 6\cdot728.$$

$$\text{Whence } \frac{100 \times 2\cdot64}{2 \times 6\cdot728} = [\alpha]_D = -19\cdot6^\circ.$$

Atisine hydrochloride, $C_{22}H_{31}NO_2 \cdot HCl$, is prepared by dissolving the base in exactly the requisite quantity of aqueous hydrochloric acid, and crystallising the salt from the aqueous solution thus obtained. It may also be crystallised from alcohol by the addition of ether. As

thus obtained, the pure hydrochloride forms long, transparent prisms, occurring either singly or in rosettes. The crystals are anhydrous, as there is no loss in weight when they are heated at 100—130° for five hours. The salt is very soluble in water and alcohol, less so in benzene and acetone, and sparingly in dry chloroform; it is insoluble in ether and in light petroleum. When dried at 100°, it melts and decomposes with effervescence at about 296° (corr.). The aqueous solution is dextrorotatory, two determinations yielding the following results :

$$(i) \alpha[19^\circ] = +0.61^\circ; l = 2dm; c = 1.691.$$

$$\text{Whence} \quad \frac{100 \times 0.61}{2 \times 1.691} = [\alpha]_D = +18.03^\circ.$$

$$(ii) \alpha[24^\circ] = +0.38^\circ; l = 2dm; c = 1.002.$$

$$\text{Whence} \quad \frac{100 \times 0.38}{2 \times 1.002} = [\alpha]_D = +18.9^\circ.$$

Mean of two determinations. $[\alpha]_D = +18.46^\circ$.

On analysis—

0.1776 of salt gave 0.0684 AgCl. Cl = 9.53.

$C_{22}H_{31}NO_2.HCl$ requires Cl = 9.38 per cent.

The molecular weight of the salt, determined by the depression of the freezing point of glacial acetic acid, gave the following result :

Weight of salt taken..... = 0.1676

Weight of solvent = 5.327

Depression of freezing point.. = 0.34°

$$\text{Whence} \quad 39 \times \frac{3.146}{0.34} = M = 360.8.$$

$C_{22}H_{31}NO_2.HCl$ requires 377.4.

Atisine hydrobromide, $C_{22}H_{31}NO_2.HBr$, prepared in a similar manner to the hydrochloride, crystallises from a mixture of alcohol and ether in long needles, which are anhydrous. It is freely soluble in water or alcohol, less so in chloroform, insoluble in ether, and melts and decomposes with effervescence at 273° (corr.). The aqueous solution of the salt is dextrorotatory, a determination giving the following results :

$$\alpha[15^\circ] = +0.386^\circ; l = 2dm; c = 0.7915.$$

$$\text{Whence} \quad \frac{100 \times 0.386}{2 \times 0.7915} = [\alpha]_D = +24.3^\circ.$$

Atisine hydriodide, $C_{22}H_{31}NO_2.HI$, cannot be obtained by the action of aqueous hydriodic acid on the base, since iodine is liberated, and a coloured substance—probably an iodo-derivative—formed. It is,

however, very easily prepared by adding Mayer's reagent to a solution of a salt of atisine, and treating the precipitate as before described. Atisine hydriodide is also precipitated on adding aqueous potassium iodide to a strong aqueous solution of an atisine salt. The hydriodide is best purified by dissolving it in boiling water, when, on cooling, an abundant deposit of crystals is obtained. It may also be crystallised by the spontaneous evaporation of an alcoholic solution, or by the addition of ether to the solution.

The crystals are anhydrous, transparent plates or tables, occurring singly or in rosettes; they are very sparingly soluble in cold, more so in hot water, sparingly soluble in alcohol or acetone, and almost insoluble in chloroform, ether, light petroleum, or benzene.

A determination of the solubility of this salt in water gave the following results:

I. 6.9924 solution at 90° gave 0.0926 salt.

Hence 1.3 grams of salt dissolves in 100 grams of water at 90°.

II. 8.7992 solution at 15° gave 0.0182 salt.

Hence 0.2 gram of salt dissolves in 100 grams of water at 15°.

The pure salt, when dried at 100°, melts and decomposes with effervescence at 279–280° (corr.). The aqueous solution of the salt is dextrorotatory, two determinations yielding the following results:

(i) $\alpha[19^\circ] = +0.19^\circ$; $l = 4dm$; $c = 0.1808$.

Whence
$$\frac{100 \times 0.19}{4 \times 0.1808} = [\alpha]_D = +26.2^\circ.$$

(ii) $\alpha[19^\circ] = +0.1^\circ$; $l = 2dm$; $c = 0.174$.

Whence
$$\frac{100 \times 0.1}{2 \times 0.174} = [\alpha]_D = +28.7^\circ.$$

Mean of two determinations $[\alpha]_D = +27.4^\circ$.

On analysis, the salt furnished the following results:

0.1652 gave 0.3468 CO₂ and 0.1086 H₂O. C = 57.02; H = 7.30.

0.0914 „ 0.1904 CO₂ „ 0.0598 H₂O. C = 56.77; H = 7.28.

0.1926 „ 0.097 AgI. I = 27.21.

0.0926 „ 0.0462 AgI. I = 27.02.

0.2636 „ 0.1312 AgI. I = 26.89.

0.3753 „ 11 c.c. moist nitrogen at 13° and 755.4 mm. N = 3.44

C₂₂H₃₁NO₂.HI requires C = 56.29; H = 6.82; N = 2.99;

I = 27.07 per cent.

Atisine nitrate, C₂₂H₃₁NO₂.HNO₃, is best obtained by adding a slight excess of aqueous silver nitrate to a hot aqueous solution of the pure hydriodide, filtering off the silver iodide, carefully removing the excess of silver nitrate by dilute aqueous hydrochloric acid, filtering

off the precipitated silver chloride, neutralising the filtrate with dilute ammonia, and concentrating the aqueous solution on the water bath. On cooling, the salt crystallises out in very well-defined hexagonal plates, which are best purified by recrystallisation from alcohol and ether. The crystals, which may occur singly or in rosettes, are, like the other salts of atisine, anhydrous; they are soluble in water, particularly in hot water, fairly so in benzene or acetone, more so in alcohol, sparingly in chloroform, and insoluble in ether or light petroleum. The dried salt melts sharply at 252° (corr.). The aqueous solution is dextrorotatory, a determination giving the following result:

$$\alpha[18^{\circ}] = +0.58^{\circ}; l = 2dm; c = 1.0255.$$

$$\text{Whence} \quad \frac{100 \times 0.58}{2 \times 1.0255} = [\alpha]_D = +28.3^{\circ}.$$

On analysis, the following data were obtained.

0.144 gave 0.3458 CO_2 and 0.1032 H_2O . C = 65.48; H = 7.96.

0.182 „ 0.4368 „ „ 0.1302 „ C = 65.43; H = 7.94.

0.1588 „ 9.5 c.c. moist nitrogen at 13° and 761.5 mm. N = 7.08.

$\text{C}_{22}\text{H}_{31}\text{NO}_2, \text{HNO}_3$ requires C = 65.34; H = 7.92; N = 6.93 p. c.

In the determination of the nitrogen contained in the hydriodide and nitrate, a difficulty was encountered similar to that described by Dunstan and Carr in the case of aconitine (Proc., 1896, 48), and concordant results were only obtained by mixing the salt with powdered copper oxide and lead chromate, and conducting the combustion in a vacuum.

Atisine aurichloride, $\text{C}_{22}\text{H}_{31}\text{NO}_2, \text{HAuCl}_4$, prepared by the usual methods, could only be obtained as an amorphous powder, soluble in alcohol, but insoluble in ether or light petroleum.

0.0682 gold salt, on ignition, gave 0.0202 Au. Au = 29.61.

$\text{C}_{22}\text{H}_{31}\text{NO}_2, \text{HAuCl}_4$ requires Au = 28.82 per cent.

Atisine platinumchloride, $(\text{C}_{22}\text{H}_{31}\text{NO}_2)_2, \text{H}_2\text{PtCl}_6$, is a yellow crystalline powder, sparingly soluble in cold, more so in hot, water, and melts sharply at 229° (corr.) with effervescence.

0.0609 gave 0.011 Pt. Pt = 18.06.

$(\text{C}_{22}\text{H}_{31}\text{NO}_2)_2, \text{H}_2\text{PtCl}_6$ requires Pt = 17.78 per cent.

The result of these analyses of pure material confirm conclusively the formula for atisine $(\text{C}_{22}\text{H}_{31}\text{NO}_2)$ proposed by Wright.

When atisine is treated with alkalis or acids under varying conditions, no acid is eliminated, but a new base is formed. To prepare the latter, atisine in alcoholic solution is heated with alkali for some hours, either in a sealed tube or in a reflux apparatus; the liquid is then neutralised with aqueous sulphuric acid, the alcohol removed

by distillation, the solution rendered alkaline with aqueous sodium hydroxide, and extracted with ether. On distilling off the ether, a non-crystalline, colourless varnish is left; none of the salts could be obtained crystalline, but the aurichloride and platinichloride were prepared and analysed, and the figures obtained on analysis agree best for a base represented by the formula $C_{22}H_{31}NO_2, H_2O$.

0.171 gold salt gave 0.239 CO_2 and 0.0784 H_2O . $C = 38.01$; $H = 5.08$. 0.1026 gave on ignition 0.0292 Au. Au = 28.4.

Two separate fractions (I and II) gave on analysis:

I. 0.0588 gave 0.0165 Au. Au = 28.06.

II. 0.0370 „ 0.0104 „ Au = 28.10.

$C_{22}H_{31}NO_2, H_2O, HAuCl_4$ requires $C = 37.82$; $H = 4.87$; Au = 28.08 per cent.

The platinichloride, which melts at 236° (corr.), was analysed:

0.0536 gave 0.0094 Pt. Pt = 17.53.

$(C_{22}H_{31}NO_2, H_2O)_2, H_2PtCl_6$ requires Pt = 17.21 per cent.

The same base was obtained when atisine was heated in aqueous solution with dilute sulphuric acid, the analysis of the platinichloride thus formed giving the following result:

0.0298 salt gave 0.0051 Pt. Pt = 17.11.

Calculated, Pt = 17.21 per cent.

When the hydriodide is heated with fuming hydriodic acid, no methylic iodide is formed, thus proving the absence of methoxyl groups in the molecule.

When atisine hydriodide is heated with soda lime, a white substance sublimes which has the odour of a cresol; this is insoluble in cold water, slightly soluble in hot water, but easily in alcohol or ether. Lack of material prevented the further examination of this decomposition product.

Professor Cash, of Aberdeen, who has examined the physiological action of atisine nitrate, reports that in small doses it is not toxic, and that its action in some respects resembles that of aconine.

The results of this investigation show that atisine does not present any close analogy to the alkaloïds of the other well known species of aconite (*A. napellus*, *ferox*, *japonicum*). The molecule is apparently less complex and much more stable, and since it yields a hydrate when heated with alkalis or acids, it might be inferred that it is the anhydride of atisine monhydrate ($C_{22}H_{33}NO_3$). There would thus be a relationship between these bases similar to that existing between aconine and pyraconine.

The material used in this investigation was collected in India, at

the instance of the Scientific Department of the Imperial Institute, and the inquiry has been conducted in the Research Laboratory of the Pharmaceutical Society.

In conclusion, I desire to express my warmest thanks to Professor Dunstan, both for having suggested the research, and for the very kind encouragement given to me during the progress of the work.
