

LXXXVIII.—*Taxine*.

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THAT, under certain circumstances, the leaves and fruit of the yew tree may be poisonous has long been known. Many instances, however, have been recorded in which no fatal result has followed from eating the leaves, and hence it has been alleged that the leaves vary in toxic character with the period of the year, or are different in different trees, and that certain animals are immune to the poison.

It has been alleged that the leaves from the male tree only are poisonous, leaves from the female tree being innocuous. It has been stated by some that the young, green shoots only contain the poison ; by others, that the fresh, green leaves have no fatal effect on cattle, but are poisonous when dry. It appears that the lower branches of yew trees in parks and grounds are constantly cropped by cattle without any ill effects. As regards the fruit, it is generally believed that the outer fleshy pulp is not poisonous, whilst the true seed contains a poison and is very dangerous.

There is little doubt that in the numerous recorded instances of fatal

effects resulting from eating yew leaves, there exists sufficient evidence to show that, under some circumstances, the yew tree contains an active poisonous principle. The most exhaustive experiments on the action of the yew poison of which we have any knowledge are those made known by Cornevin, in a work entitled "Des Plantes Vénéneuses et des Empoisonnements qu'elles déterminent." These are quoted by Mr. C. Whitehead in a communication to the *Journal of the Royal Agricultural Society* (1892, 3, 703). In the same journal (1893, 4, 141), a short account is given of some experiments carried out at the Royal Veterinary College, which are by no means conclusive as to the poisonous character of the yew. If a reference, however, is made to M. Cornevin's table as to the amounts necessary to kill different animals, it will be seen that the quantity taken by the animals in the Royal Veterinary College experiments was insufficient to cause death.

In view of the indefinite conclusions of the recorded observations of yew poisoning, it seemed desirable to learn more about the chemical character of the principle or principles that might be capable of being separated from the leaves. It is possible, of course, that the poisonous principle does not exist in the leaves, but is the product of the action of the juices of the stomach, or of fermentation during the drying of the leaves. If some knowledge could be gained of the constitution of the active principle, and of the action on it of different substances, an explanation might possibly be forthcoming of the apparently irreconcilable observations which have been made for so many years.

Chemical investigation of the poisonous principle in yew appears to have been first made by Bujardin, Schroff, and Lucas. Lucas (*Jahresber.*, 1856, 550) indicated the existence in yew of a specific alkaloid to which the name "taxin" was given.

In 1876, Marmé (*Medicinisches Centralblatt*, 14, 97) prepared from the yew an alkaloidal substance corresponding with the taxine described by Lucas. The leaves and seeds were repeatedly extracted with ether, and after distilling off the ether the residue was shaken with hot acidified water, filtered, and the taxine precipitated from the filtrate by ammonia. The substance is described by Marmé as a white, crystalline powder, scarcely soluble in water, readily soluble in acidified water, and in alcohol, ether, chloroform, benzene, or carbon disulphide, but insoluble in petroleum ether. It was free from smell, had a bitter taste, and gave precipitates with many of the alkaloidal reagents, but none with platinic chloride, gold chloride, mercuric chloride, and potassium platinicyanide. The taxine melted at 80°, was coloured red by the action of strong sulphuric acid, and gave no crystalline salts with ordinary acids. Marmé concluded that the alkaloid was present to a greater extent in the leaves than in the seeds.

Amato and Capparelli, in 1880 (*Ber.*, 13, 1999), made a further

attempt to isolate the alkaloid of yew and to examine its properties. The green needles of the yew were exhausted successively with ether, alcohol, water, and dilute sulphuric acid. From the ether solution, by Marmé's method, they obtained an alkaloid, described as crystalline and having a musty odour. The alcohol, water, and acid extracts of the leaves also yielded small quantities of the same alkaloidal substance. Besides this product, there was isolated from the ether-extract, a colourless, non-nitrogenous, crystalline substance, melting at 86—87°, readily soluble in boiling alcohol, but sparingly soluble in cold alcohol, to which they gave the name *milossin*.

A more complete examination of the active principle of yew was carried out by Hilger and Brande, who followed Marmé's method for separation of the alkaloid (*Ber.*, 1890, 23, 464). The ethereal extract of yew leaves was shaken with dilute sulphuric acid, and the acid solution precipitated with solution of ammonia. This was again extracted with ether, shaken out with dilute sulphuric acid, and precipitated with ammonia, and the process was repeated until a colourless precipitate was obtained. Hilger and Brande give the melting point as 82°, and assign to the alkaloid the formula $C_{37}H_{52}O_{10}N$. From the work of these chemists, it would appear that yew leaves contained a definite alkaloid, which they regarded as a tertiary base, producing salts resembling those produced from similar basic substances, and giving all the general reactions of an alkaloid.

In 1896, Vrèven (*Bull. Gén. Therap. Soc. Pharm.*, 1, 261) extracted taxine from yew according to the following process. Fresh yew leaves were cut up small and digested with water acidified with tartaric acid. The acid extract was evaporated to a small bulk and exhausted repeatedly with a mixture of equal parts of alcohol and water. This alcoholic extract was again evaporated, rendered alkaline with ammonia, and shaken with benzene, in which the alkaloid dissolved. The benzene solution was shaken with hydrochloric acid, the acid solution precipitated with ammonia, and extracted with ether. It was further purified in a similar manner, but Vrèven does not appear to have done any work on its chemical character.

Whilst in many respects the substance prepared by all the chemists mentioned is similar, there are notable differences in their descriptions of its properties. Hilger and Brande were unable to obtain the alkaloid in a crystalline form from any of its solvents, whilst the earlier observers describe it as crystalline. Marmé states that it gives no precipitate with gold chloride or platinic chloride, whereas Hilger and Brande prepared and analysed compounds of these salts with the alkaloid. Again, Marmé describes the alkaloid as having no smell, whilst Amato and Capparelli state that it has a "musty" odour. Hilger and Brande found that it is not soluble in benzene, whilst Marmé records

its solubility in that liquid. Vrêven's method of preparation, it will be observed, employs benzene for the purpose of extracting the alkaloid.

In all work so far carried out, no distinction had been made as to the sex of the tree from which the leaves for examination had been taken, but in 1892 Dr. Munro communicated to the *Journal of the Royal Agricultural Society* (3, 715) some results obtained by him on the examination of leaves from both male and female trees. He found an alkaloid which appeared to agree with taxine in both the male and female leaves, and although his results gave a greater quantity of crude alkaloid from the male than from the female leaves, Dr. Munro did not regard it as conclusively established that the amount of the alkaloid varied with the sex of the tree. He also pointed out the extreme difficulty of purifying taxine and the great loss attending the operation, and was of opinion that the loss was due to alteration in the composition of the substance.

Mr. R. A. Bray, of the Manor House, Shere, Guildford, has communicated to us the results of some work which he himself had carried out on the extraction of the alkaloid. He found, after trial of several processes, that water containing 1 per cent. of sulphuric acid yielded the best results.

In this general review of the chemical work carried out on yew leaves it will be seen that a substance giving the general tests for an alkaloid has been obtained by extracting yew leaves (1) with water acidified either with tartaric acid or sulphuric acid ; (2) with ether, and subsequently shaking the ethereal extract with an acid ; and (3) with hot alcohol, the method followed by Dr. Munro.

In the early stages of our work, considerable difficulties were met with in obtaining the alkaloid in quantity sufficient for investigating its properties by a method which would ensure that no change had occurred in it during the process of extraction, and which would at the same time enable the leaves or the extract to be used for the isolation of other substances.

The first experiments were directed to ascertain the best conditions under which a substance of the nature of an alkaloid could be extracted from yew, by the methods used by previous workers, or by other means which could be devised. The undried leaves from the female trees were cut up and extracted with water containing 1 per cent. of sulphuric acid, whereby 0.11 per cent. of an alkaloid were obtained. The leaves, dried in air at a low temperature, were powdered and treated in a similar manner, and taxine, equivalent to 0.12 per cent. on the green leaves, was obtained. It was found, however, that if, after the acidified extract had been made, the solution was evaporated to about one-sixth of its bulk, the quantity of alkaloid obtained

was much smaller, owing, as was subsequently proved, to changes experienced by the substance. Tartaric acid extracts of the green leaves and the air-dried leaves also yielded similar quantities of taxine, and, again, by evaporation of the solution there was a loss in the amount of alkaloid recovered. From an ether extract of the dry leaves, 0.08 per cent. was obtained, and from an extract with cold alcohol, 0.07 per cent. when calculated on the green leaves.

These preliminary experiments served to show that both from the green leaves and the air-dried leaves the alkaloid was obtained to about the same extent; that sulphuric acid or tartaric acid, in very weak solutions, easily extracted it; and that ether or alcohol might be used, though less completely, for obtaining the substance. In these experiments, the extraction was not prolonged until the leaves no longer yielded evidence of the presence of an alkaloid, and the above figures, from this cause and from evidence obtained later, are probably considerably below the true amount of alkaloid originally present in the leaves.

As is well known, acids are apt to produce change in products of the character of taxine. Accordingly, it was decided to operate upon larger quantities of material with alcohol. Two separate quantities of 8 lb., and 12 lb. of leaves, air-dried and powdered, were extracted with boiling alcohol until the leaves yielded practically no further substance. The residue obtained after evaporating the alcohol, and which would contain the alkaloid, amounted to 26 per cent. of the dry leaves. It was dark in colour (containing all the chlorophyll) and of wax-like consistency. It was treated several times with hot water containing 1 per cent. sulphuric acid and the aqueous extracts examined for alkaloid. The yield, however, was very considerably less than was expected; either the alkaloid had not been extracted from the dark fatty residue from the leaves by the hot sulphuric acid, or the action of this reagent, or the ammonia, had so altered the substance that it was not isolated by the subsequent treatment.

A further quantity of leaves was treated in the same manner, but with the use of hot water on the alcoholic extract, instead of dilute acid; the yield of alkaloid was considerably increased, but even now the amount was much smaller than was obtained in the preliminary work.

A re-examination of the residues furnished only slight additional amounts of alkaloid. It therefore became evident that the small quantity of alkaloid isolated was not due to accidental loss during manipulation, but to some change in the substance, brought about probably by the treatment with hot acid—the sticky, waxy, nature of the alcoholic residue requiring rather rigorous treatment to ensure thorough mixing with the acid.

A comparison was made between the taxine obtained by direct acid extract of the leaves and the taxine obtained from the alcoholic extract, and the substances appeared to be identical. Since, therefore, it was evident that the alcoholic treatment had not been successful in furnishing a satisfactory yield when employed on large quantities of leaves, and that the same substance was obtained by directly acting on the leaves with dilute sulphuric acid, the latter method was tried on a large scale. Ten and a half lb. of the air-dried, powdered leaves from the male tree were treated with 1 per cent. sulphuric acid for 5 or 6 days with occasional shaking. The liquid was strained, and the leaves pressed, and treated once more with dilute sulphuric acid; then again strained and pressed. Without evaporation, the acid extract was at once rendered alkaline with solution of ammonia and shaken with ether. The ether solution was removed and shaken with dilute acid. The acid solution was separated from the ethereal layer and rendered alkaline with ammonia, the alkaloid at this stage being thrown down as a brownish-coloured, bulky precipitate. The ammoniacal liquid, containing the partly precipitated alkaloid, was shaken with ether and the extraction with hydrochloric acid repeated as before. This was continued until no further colour was yielded to the ether in presence of hydrochloric acid. It was finally precipitated with ammonia solution, shaken thoroughly with ether, and the ether extract evaporated, dried in a vacuum over sulphuric acid, and weighed. The amount obtained was equivalent to 0.18 per cent. on the green leaves. It will be seen that this amount from the male leaves is greater than was obtained from the female leaves in the preliminary work, but the inference cannot be drawn that the actual quantity present in the leaves was greater. The experience gained during the conduct of the work showed the extreme ease with which the substance underwent change, and every effort was made in the later experiments to prevent as much as possible those changes taking place.

The alkaloidal substance, taxine, after drying in a vacuum, presented the appearance of very fine, glistening particles, extremely light, with no smell, and with a very bitter taste.

The substance is soluble in ether, acetone, alcohol, chloroform, or benzene, but insoluble in petroleum ether. It is almost insoluble in water, but dissolves in weak acid. The addition of dilute acid to the dry scales does not give complete solution; this is obtained by dissolving taxine in ether and shaking the ethereal solution with an acid. From the acid solution, the alkaloid is precipitated with solution of ammonia as a white, voluminous precipitate; as thus obtained, it has a characteristic odour. The alkaloid is, however, somewhat soluble in excess of ammonia solution, as after filtering from the precipitated alkaloid a further quantity is extracted from the filtrate with ether.

The alkaloid precipitated by ammonia and dried over sulphuric acid is a dull white, amorphous powder.

It was analysed, with the following results :

	I.	II.	III.	Calc. for $C_{37}H_{52}O_{10}N$.
Carbon	66.75	66.67	67.05	66.21
Hydrogen	8.07	8.17	8.19	7.83
Nitrogen	2.02	—	—	2.09

These numbers are in fair accord with those demanded by the formula $C_{37}H_{52}O_{10}N$, first given by Hilger and Brande.

At 82° , taxine practically melts, and at the same time undergoes some decomposition. At 100° , the loss amounts to 4.26 per cent., the vapours given off being slightly alkaline. There is no further loss on prolonging the heating at 100° beyond one hour.

Taxine does not crystallise. Attempts were made to crystallise it from its solvents, either used alone or mixed, but without success. In all cases, it dries as a hard, resinous-looking substance, similar to a varnish in appearance. It is not improbable that the alkaloid separated and described as taxine contains more than one substance of similar alkaloidal character.

The hydrochloric acid solution of the alkaloid gives the following reactions :

Solution of iodine in potassium iodide gives a dark chocolate-brown precipitate very readily ;

Mercuric iodide in potassium iodide, a white precipitate with a yellowish tinge ;

Phosphomolybdic acid, a yellow precipitate ;

Platinic chloride, a slight, pale yellow precipitate increasing in quantity on standing ;

Gold chloride, a voluminous, canary-yellow precipitate, very sparingly soluble in water ;

Potassium iodide and bismuth iodide, an abundant, orange-coloured precipitate ;

Picric acid, a yellow precipitate ;

Mercuric chloride solution, a white precipitate.

It will be seen that, in its behaviour with the reagents given, taxine resembles many alkaloidal substances.

As regards colour reactions, taxine behaves as follows :

Sulphuric acid (concentrated) gives a brown to purplish-blue coloration ;

Sulphuric acid with a little nitric acid gives a deep pink, almost red coloration ;

Sulphuric acid with molybdic acid gives a deep violet coloration ;

Sulphuric acid with potassium dichromate gives a fine purple-blue coloration ;

Nitric acid (concentrated) gives a slight brown tinge ;

Hydrochloric acid (concentrated) gives no coloration.

When the dry substance is heated in a tube, it is decomposed with formation of white fumes which condense as oily drops on the sides of the tube and have no alkaline reaction. When heated with soda-lime, ammonia is evolved.

That the substance readily undergoes change is proved by the following experiments. A weighed quantity of the alkaloid was heated for half-an-hour with water containing 2 per cent. of hydrochloric acid. On recovering the alkaloid again, only 70 per cent. was obtained. From a further quantity heated with a solution of 10 per cent. hydrochloric acid for 3 hours, only 5 per cent. of the alkaloid could be recovered. There are at least two substances formed in this decomposition ; one a white, non-nitrogenous and amorphous compound, with a definite melting point at 120° , soluble in alcohol or ether, but insoluble in acids or alkaline solutions. The other is a brown substance, which separates from the acid solution during the heating, is insoluble in ether, but soluble in alcohol. Owing to lack of material, these substances have not been further investigated.

Taxine combines with acids to form definite salts, but we have not been able to obtain any of these in a crystalline form. Several of these salts have been prepared, and some have been analysed. The method of preparation followed was to shake in a separating funnel an ethereal solution of the alkaloid with slightly less than the calculated amount of the acid required for molecular combination. The aqueous solution of the salt was then concentrated over sulphuric acid in a vacuum.

Of the simple salts, we have analysed the hydrochloride and the sulphate. The former gave, in two analyses, 4.98 and 5.35 per cent. of hydrogen chloride, the mean number, 5.16, agreeing exactly with that demanded by the formula $C_{37}H_{52}O_{10}N, HCl$. The sulphate was found to contain 6.94 per cent. of sulphuric acid, the formula $(C_{37}H_{52}O_{10}N)_2, H_2SO_4$ requiring 6.81 per cent.

If a solution of gold chloride in water is added to a hydrochloric acid solution of the alkaloid, a pale yellow precipitate is at once formed. This precipitate undergoes slight decomposition on drying in a vacuum, even in the dark. The gold chloride compound dissolves in alcohol, but does not crystallise from its solution on evaporation over sulphuric acid or by gradual addition of water. It is also readily soluble in acetone or chloroform. The melting point is 72.5° , but it is probable that the actual melting point is affected by the decompo-

sition which takes place at this temperature. Its composition would appear to be $C_{37}H_{52}O_{10}N, HAuCl_4$, although, on account of the ease with which it is decomposed, the analytical figures show a slight excess of gold above the theoretical amount. Analyses of several preparations gave the following numbers :

	(1).	(2).	(3).	(4). $C_{37}H_{52}O_{10}N, HAuCl_4$.	Calc. for. $C_{37}H_{52}O_{10}N, HAuCl_4$.
C	43.4	43.2	43.7	43.1	43.9
H	5.5	5.8	6.0	5.7	5.2
Au	20.8	21.7	20.4	19.3	19.1

The alcoholic solution of the alkaloid mixed with an alcoholic solution of gold chloride gives, on the addition of water, a pale yellow precipitate, a compound of the free alkaloid with gold chloride, which, after drying in a vacuum, melts with rapid decomposition at 132—134°. This compound is not absolutely stable on drying. Its composition would appear to be $C_{37}H_{52}O_{10}N, AuCl_3$. (Gold found, 21.0; calculated, 20.2 per cent.).

The alkaloid may be recovered from the gold chloride compounds without any change in its character.

Hilger and Brande describe the formation of a compound of taxine with ethyl iodide, obtained by heating the alkaloid with ethyl iodide, under pressure at 100°. We have prepared, but in a somewhat different manner, the corresponding methyl iodide compound. 0.302 gram of alkaloid was dissolved in benzene. To the solution was added, in slight excess, a solution of methyl iodide in benzene. At first, the mixture was clear, but in a few minutes a bulky, flocculent precipitate was formed. This was dried in a vacuum until no further loss in weight occurred. The white residue showed an increase in weight on the original substance of 0.0686 gram. The calculated increase, assuming that the compound $C_{37}H_{52}O_{10}N, CH_3I$ had been formed, is 0.064 gram. On analysis, it yielded :

C = 56.4 ; H = 7.4.

$C_{37}H_{52}O_{10}N, CH_3I$ requires C = 56.1 ; H = 6.8 per cent.

The substance is a white, amorphous powder which melts at about 121°.

Although the analytical numbers adduced, both for the alkaloid and its salts, are in substantial agreement with Hilger and Brande's formula, it cannot be said that this formula, to which there are obvious objections, is yet definitely established. Taxine has such feeble basic powers, and is itself so susceptible to change, that much further study is required in order to fix its true character and relations.

The physiological action of taxine was examined in 1876 by Borchero

(*Exper. Untersuch. u. Wirkung des Taxin, Göttingen, 1876*), who states that, when administered to frogs, rabbits, cats, and dogs, it depresses the action of the heart and interferes with the respiratory functions, and that death ensues from suffocation in a short time. It has been asserted, however, that taxine has no action on guinea-pigs. Further experiments are required to establish definitely whether the alkaloid is actually poisonous, and if so, how it acts; and whether, as alleged, certain animals are immune to it.

In conclusion, we desire to acknowledge our indebtedness to Mr. Bray of the Manor House, Shere, for his kindness in furnishing us with the material for our inquiry.

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