

XII.—*The Constituents of Piper ovatum.*

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THIS plant (*Piper ovatum* Vahl, or *Ottonia Vahlia*) grows in the West Indies, and is briefly described in Grisebach's "Flora of the West Indies," (p. 172).

It has been examined in this laboratory, at the suggestion of Mr. W. T. Thiselton Dyer, F.R.S., the Director of the Royal Gardens, Kew, who received it from Mr. John H. Hart, F.L.S., the Superintendent of the Royal Botanic Garden, at Trinidad, to whom we are indebted for a further supply of the material. The plant somewhat resembles Jaborandi in appearance, and has been employed in Trinidad as a remedy for snake bite, and as a cure for hydrophobia in animals. When chewed, it gives rise to a persistent tingling of the tongue and lips, which is followed by a sensation of numbness, accompanied by profuse salivation, in these respects recalling pellitory. Some irritation at the back of the throat is also observed. All parts of the plant—leaves, stem, and root—possess this property, but the leaves and root appear to act more powerfully than the stem; the leaves have an aromatic taste due to an essential oil which is absent from the root.

The following is a brief botanical description of *Piper ovatum*, taken partly from Grisebach and partly from our own observations.

Shrub, 2—4 feet high; root woody, spreading. Stems erect, slender, somewhat woody, branching in zig-zags, nodes tumid; leaves alternate, 3—6 inches long, 2—3 inches broad, ovate, pointed, broadly cuneate at base, papery opaque, glabrous; petiole channelled, naked, about $\frac{1}{8}$ — $\frac{1}{4}$ inch long. Flowers usually racemose, pedicles jointed with the flower. Bracts cucullate, usually at the base of a pedicel; stamens 4 (—3) inserted round the ovary; stigmas 4, sessile. Pericarp baccate, tetragonal. Berries pedicellate, somewhat longer than their pedicel, rounded at base, mucronate with a bluntish point. Habitat, Trinidad.

A number of the living plants have been brought to England, in Wardian cases, and are at present growing at Kew Gardens, but so far they have shown no signs of flowering.

Volatile Constituents.

As the aromatic taste of the leaves suggested the probability of the presence of a volatile oil, the leaves were distilled with steam in the usual manner and the distillate extracted with ether. By this means a small quantity of an almost colourless, volatile, aromatic oil was ob-

tained which was non-alkaloïdal and did not produce any tingling of the tongue. When cooled below 0° and stirred, the oil showed no tendency to solidify. Its density was $15^{\circ}/15^{\circ} = 0.9904$. It boiled at about 245° , nearly the whole passing over between 240° and 250° . It appears to be optically inactive, since a 6 per cent. solution in alcohol produced no rotation of the polarised ray. When dry hydrogen chloride was led into a dry ethereal solution of the oil, there was no separation of crystals, even after the liquid had been cooled to -13° . When a drop of the oil was dissolved in chloroform and strong sulphuric acid added, an intense crimson coloration was produced, passing into a reddish-violet. Aqueous alkalis did not act on the oil, and it formed no compound with sodium hydrogen sulphite. We have not had sufficient of the substance for analysis, but from the properties recorded above the volatile oil of *Piper ovatum* would appear to be a *sesquiterpene*. The leaves contain this oil to the largest extent, little or none being present in the root or stem.

Non-volatile Constituents.

The liquid remaining with the leaves after distilling off the oil with steam, though highly coloured, had none of the activity of the leaves themselves, and gave rise to little or no tingling when tasted; it was, therefore, clear that the active constituent was not appreciably soluble in water. This aqueous solution contained no alkaloïd, but a *sugar* having cupric reducing power was present, and also a considerable quantity of *potassium nitrate*. The leaves having been drained from the water were digested for some hours with boiling alcohol (50 per cent.), the process being repeated until they had lost nearly all their tingling property. The green alcoholic solution, which was highly active, was evaporated to dryness, the residue extracted with absolute alcohol, and the alcoholic solution fractionally precipitated by the addition of small quantities of water aided by the evaporation of some of the alcohol. The first precipitate contained nearly the whole of the chlorophyll, but scarcely any of the active constituent. The second precipitate also contained chlorophyll, but was active although not nearly so strongly as the filtrate; this second precipitate was, therefore, dissolved in alcohol and reprecipitated by water, the filtrate being added to the original filtrate, and the whole evaporated until the alcohol had been dissipated. The aqueous solution was then extracted by shaking with ether which removed the active constituent together with colouring matter. The residue left on evaporating the ether was dissolved in alcohol, and the solution fractionally precipitated with water, this process being repeated until the whole of the green colouring matter had been removed. Ether then extracted from the liquid, after the evaporation of the alcohol,

a light brown resin which was highly active in inducing tingling of the tongue and showed no signs of crystallisation even after prolonged standing.

The similarity in the physiological action of *Piper ovatum* to that of the medicinal pellitory has already been noticed, and in the latter case the action is usually attributed to a resin, about the properties of which, however, little is known.* The resin obtained from *P. ovatum* was fully examined in the hope of separating some crystalline active substance from it. It was soon found that the resin was not a single substance, in spite of the long process of purification to which it had already been submitted. It was further purified by dissolving it in ether, and fractionally precipitating the ethereal solution with light petroleum, in which the active constituent is nearly insoluble; by this means, it was obtained lighter in colour but still resinous. The resin was again dissolved in ether, and the solution shaken with weak aqueous ammonia, which removed a further quantity of inactive material. After the ethereal solution had been dried with calcium chloride, a resin, which was quite inactive, separated from the liquid.

The ethereal solution, when spontaneously evaporated, left a highly active substance which, however, was still resinous, and refused to crystallise. The resin was next dissolved in alcohol, and the solution fractionally precipitated by the addition of water; the first fraction was resinous and nearly inactive, the later fractions were lighter in colour than the first and highly active. On repeating the process on the later fraction, a bulky but very light mass of feathery crystals separated, which were nearly colourless and more active than any substance previously obtained. This material was, with difficulty, recrystallised several times from dilute alcohol, and lastly from hot, light petroleum. A further quantity was obtained from the purified resin by shaking the ethereal solution with ammonia, dissolving the residue in alcohol, and precipitating the solution with light petroleum, the precipitate being again dissolved in alcohol and reprecipitated with petroleum until it was capable of crystallising.

As will be inferred from the details which have been given, the separation of this crystalline active substance from the colouring matter, fat, wax, and resin which so obstinately adhere to it proved to be a very tedious operation, especially troublesome when the object was to obtain a sufficient quantity for analysis. Much time was spent in trying various methods of extracting the substance from the plant, and in purifying the product; the most successful plan was found to be the following, which, however, is still long and laborious.

* See the following paper.

The dried and finely-powdered material (leaves, root, and stem, all of which contain the active constituent) is repeatedly extracted with hot, light petroleum (boiling below 80°) until the active substance is almost entirely removed; the removal of the last traces is very troublesome. The active constituent is not readily soluble in light petroleum, but it is found to be advantageous to use this liquid because it removes a smaller quantity of other substances than is the case with better solvents of the active constituent, such as alcohol, ether, chloroform, or acetone, which dissolve so much colouring matter, resin, and fat that the subsequent purification of the active substance is very much lengthened.

The greater part of the light petroleum is distilled from the solution, and the residue is well shaken with alcohol (60 per cent.). By repeating this operation several times, nearly the whole of the active constituent passes into the alcohol, whilst most of the colouring matter, fat, essential oil, &c., remains in the layer of petroleum. On concentrating the dilute alcoholic solution, a considerable quantity of semi-crystalline active substance separates along with green colouring matter; this is partially purified by repeated crystallisation from dilute alcohol. By this means much of the resin, which so obstinately clings to it, is removed, and more is separated by shaking an ethereal solution with dilute aqueous ammonia. The dried ethereal solution may then be precipitated by the addition of small quantities of light petroleum, which throws out the resin in the first fraction, whilst the later fractions are crystalline and consist of the nearly pure active constituent. Finally, the substance is recrystallised by adding dry ether to its solution in absolute alcohol, and allowing the solution to evaporate spontaneously. The crystals which separate are dried on a tile in the air or in a desiccator; at 100° a little decomposition occurs, and the crystals become slightly yellow.

The preparation of sufficient material for analysis occupied a very long time, since the best process for isolating it is rather wasteful, as some of the active constituent is thrown down along with the resin during the fractional precipitation, and requires a succession of similar operations in order to recover it. Although we have used about 12 kilos. of the plant, we were not able to prepare more than about 2 grams of the pure active constituent. In all probability the plant does not contain as much as 0.1 per cent. of this substance, but it exerts so powerful a physiological action that from the effect produced by chewing the leaves or root one is led to expect a far larger proportion.

Properties and Composition of the Active Constituent, Piperovatine.

When pure, the active constituent forms colourless, light needles which often crystallise in rosettes. In appearance, these somewhat resemble the alkaloid caffeine, and melt at 123° (corr.), decomposing slightly. The substance is nearly insoluble in water, very sparingly soluble in light petroleum, and not much more so in dry ether. It dissolves readily in alcohol, chloroform, and acetone. The solution of the highly purified substance in strong alcohol has the curious property of apparently gelatinising when water is added to it in just sufficient quantity to cause precipitation; the dissolved substance being thrown out in a bulky mass of microscopic crystals resembling a jelly. On this account it is difficult to obtain it in well-defined crystals, but this may be managed by adding dry ether to the solution in absolute alcohol, and allowing the liquid to evaporate spontaneously. The substance appears to be optically inactive; an alcoholic solution containing 1.5 per cent. did not rotate the polarised ray. The compound contains carbon, hydrogen, nitrogen, and oxygen. Several combustions have been made with different specimens of the material, with the following results.

I.	0.1054	gave	0.2813	CO ₂	and	0.0858	H ₂ O.	C = 72.78;	H = 9.04.
II.	0.2023	„	0.5335	„	„	0.1484	„	C = 71.92;	H = 8.15.
III.	0.1847	„	0.4982	„	„	0.1391	„	C = 73.56;	H = 8.37.
IV.	0.1775	„	0.4810	„	„	0.1356	„	C = 73.90;	H = 8.49.

Of these four combustions, III and IV were made with the purest specimens, and give as a mean composition C = 73.73, H = 8.43 per cent., the percentage of nitrogen (determined by the absolute method) was 5.96. These data agree well with those calculated from the formula C₁₆H₂₁NO₂, which requires C = 74.13; H = 8.10; N = 5.40 per cent. The active constituent exhibits, therefore, the composition of a vegetable alkaloid, and shows some resemblance to piperine (C₁₇H₁₉NO₃), the alkaloid of pepper, a plant belonging to the same natural order.

Piperine has very feebly basic properties, and forms salts which are very unstable. The substance from *Piper ovatum*, to which we propose to assign the name *piperovatine*, is devoid of basic properties, being nearly insoluble in dilute acids, and therefore, from this point of view, does not come within the usual definition of an alkaloid. Nevertheless, further investigation will probably show that its constitution is precisely similar to that of such alkaloids as piperine, atropine, and aconitine, that is to say, it would seem from our observations to be composed of a basic pyridine nucleus (*e.g.*, piperidine), associated with an acid radicle, the acidity of the radicle exactly

neutralising the basic power of the other residue, so that the resulting derivative is neither basic nor acidic.

Piperovatine dissolves in glacial acetic acid, but may be precipitated unchanged by the addition of water. It is insoluble in dilute hydrochloric acid and in dilute sulphuric acid; on boiling it with these acids, hydrolysis appears to take place, and the solution now gives the reactions of an alkaloid, but we have not obtained sufficient of the product for identification. It appears, however, to be a pyridine derivative, and to present some analogies to piperidine; there would seem to be also an acid product of hydrolysis, but this we have never obtained except in traces.

Piperovatine is also devoid of acid properties. It does not dissolve in weak alkalis. Strong alkalis such as potash, especially when warm, appear to hydrolyse it, quickly forming the acid and the pyridine base referred to above.

Its decomposition products require a fuller examination than we have been able to subject them to with the small quantity of material at our disposal.

Physiological Action of Piperovatine.

The physiological action of piperovatine has been investigated for us by Professor Cash, F.R.S., of Aberdeen. He finds it to act as a temporary depressant of both motor and sensory nerve fibres, and also of sensory nerve terminations, producing some local anæsthesia. It acts as a heart poison, and also as a powerful stimulant to the spinal cord (in frogs), causing a tonic spasm somewhat resembling that produced by strychnine.

A 2.5 per cent. solution (in almond oil) produced no anæsthetic effect when rubbed on the skin, and it has been found generally to possess but little penetrative power, at all events in the form of this solution. When, however, the same solution is applied to the tongue, moderate local anæsthesia is produced, accompanied by a feeling of numbness and coldness, and much salivation.

In attempting to utilise the local anæsthetic effect of piperovatine, the want of penetrating power, the temporary character of its action, and, above all, its property of causing salivation, have interfered with its successful employment in the minor operations of dentistry. The solution of piperovatine in almond oil has, however, given promising results in affording temporary relief in painful superficial lesions.

It would therefore seem to be worth while to follow up in therapeutic trials the analogies, suggested by these preliminary experiments, of the physiological action of piperovatine to that of strychnine, cocaine, pilocarpine, and piperine. It is also clear that the action

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of piperovatine is very similar to that of the medicinal pellitory, the *Anacyclus Pyrethrum* of the Pharmacopœia, and it might be useful to determine how far this similarity can be traced, and also whether *Piper ovatum* or its alkaloid piperovatine could be used medicinally in the place of pellitory.

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