AURICULARINE—A NEW ALKALOID FROM THE ROOTS AND STEMS OF *HEDYOTIS AURICULARIA*.

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Chemical examination of the root and stem of *Hedyotis auricularia* has shown the presence of the following constituents: fatty matter yielding on saponification stearic and linoleic acids, a phytosterol, alizarin, oxalic acid, glucose, a crystalline alkaloid, auricularine ($C_{42}H_{55}ON_3$ m.p. 210°) and amorphous bases. Results of micro-analyses show that auricularine is different from the alkaloid hedyotine, isolated from the same plant. Unlike hedyotine, auricularine is a crystalline alkaloid with definite chemical characteristics.

The root of *Hedyotis auricularia* was examined by Dey and Lakshminarayanan (*Arch. Pharm.*, 1933 271, 485) and was found to contain, besides a fatty oil, glucosides, reducing sugars, colouring matter, tannins and albumins and an alkaloid named 'Hedyotine' ($C_{14}H_{22}O_2N_2$). The alkaloidal base could not, however, be obtained by them in a stable form, as they found it to change rapidly into a pasty mass on exposure to air. The hydrochloride and the nitrate of the alkaloid were, on the other hand, reported very stable, and could be obtained in crystalline condition.

A fresh chemical investigation of the plant was undertaken in this laboratory in order to isolate, if possible, hedyotine base in crystalline condition, and to find out if any other constituents were present to which the ascribed amoebicidal action of the drug could be attributed. As a result a stable, crystalline alkaloid has been isolated, though in very poor yield. But it appears to be different from hedyotine, because the results of micro-combustion show that its formula is $C_{42}H_{55}ON_3$, while hedyotine is stated to have a simpler formula, and further, it does not yield the coloured crystalline salts described by the previous workers (*loc. cit.*). The melting point of the picrate of the new crystalline alkaloid (217-18° decomp.) is also widely different from that of hedyotine picrate which is stated to melt at 265° with decomposition. It is, therefore, apparent that the crystalline alkaloid, now isolated, is different from hedyotine, and the name *Auricularine* is proposed for it. As the yield of the alkaloid is only about 0·001% of the dried plant, a sufficient quantity could not be isolated for a detailed examination.

The uncrystallisable residues left after the separation of auricularine still contained a large amount of alkaloidal matter from which no crystalline base, but only a microcrystalline hydriodide (m.p. 215-20° decomp.) could

be isolated. Further attempts to obtain crystalline material from this fraction are in progress.

The other constituents found to be present in the alcoholic extract of the drug are : fatty matter, yielding on saponification stearic and linoleic acids, a phytosterol, (m.p. 141-42°), alizarin, uncrystallisable substances which give colour reactions similar to those of oxymethylanthraquinone compounds, oxalic acid and glucose.

EXPERIMENTAL.

The material for the investigation consisting of the stem and root of the plant was supplied to us by the Forest Department of the Travancore State to whom we are deeply indebted.

Examination for alkaloid by extraction with Prollius's fluid gave marked reactions with the usual reagents.

The powdered plant was assayed for alkaloidal content by a gravimetric method based on the U.S.P. IX method for assay of Belladonna root, using as solvent a mixture of three volumes of ether and one volume of chloroform. The alkaloidal content of the plant was, on an average, 0·29% of the dried material.

Isolation of Auricularine.—25 Kg. of the powdered drug were extracted with 90% alcohol and the solvent was distilled off under reduced pressure. The residue (568 g.) was treated with benzene in order to remove fats and waxes, and the benzene extract (A) was reserved for further examination.

The residue that remained insoluble in benzene was repeatedly shaken with 2% sulphuric acid solution till the acid extract ceased to give alkaloidal reactions. The benzene extract (A) was also extracted with dilute sulphuric acid solution to recover any dissolved alkaloid, and the combined acid solution was shaken with ether to remove non-basic impurities. The aqueous acid solution was then nearly neutralised with 5% sodium carbonate solution, taking care that the solution remained distinctly acidic to litmus. The precipitated impurities were filtered off, excess of strong ammonia was added to the filtrate, and the solution was shaken with chloroform till no more alkaloid was extracted with the chloroform. The chloroform solution was washed with distilled water and dried with anhydrous sodium sulphate. On evaporating off the chloroform, a brownish residue was left (14·6 g.) which was dissolved in 10% acetic acid solution. To this solution was then added a strong solution of potassium nitrate till no further precipitation occurred. The precipitate was filtered off after standing overnight, and the filtrate was rendered alkaline by the addition of ammonia. The precipitated alkaloid was filtered under suction, washed thoroughly with

distilled water, and dried in a vacuum desiccator. The dried alkaloid was then extracted with benzene in a Soxhlet and the benzene solution was decolourised with animal charcoal and filtered. The residue from the benzene extract was dissolved in alcohol and the solution neutralised with 5% alcoholic solution of oxalic acid. On adding a little water and leaving it in the refrigerator for a day, thin glistening needles of auricularine oxalate separated out, which were rapidly filtered under suction and washed with 95% alcohol. The oxalate was recrystallised from 70% alcohol. On heating, auricularine oxalate turned brown at 185° and charred at 230° without melting.

The oxalate was dissolved in water, excess of ammonia was added to the solution, and the precipitated alkaloid was shaken with ether. The ethereal solution was shaken with water and dried with anhydrous sodium sulphate. On spontaneous evaporation of the ether, auricularine was obtained in the form of colourless rosettes of needles, yield 0·2 g. It was recrystallised from 90% alcohol. On heating, auricularine turned brown at 192° and melted with decomposition at 201°.

On drying in *vacuo* over phosphorus pentoxide at 100°, the crystals suffered a loss of weight of 2·65%. $C_{42}H_{52}ON_2$, H_2O requires a loss of weight of 2·71%. (Found in the dried sample: C, 78·24; H, 8·64; N, 10·62. $C_{42}H_{52}ON_2$ requires C, 78·07; H, 8·59; N, 10·85 per cent).

Auricularine picrate was obtained as a microcrystalline yellow powder by mixing an alcoholic solution of auricularine with an alcoholic solution of picric acid and recrystallising from alcohol. On heating, the picrate melted with decomposition and frothing at 217-18°.

The mother-liquors after the crystallisation of auricularine oxalate were diluted with water, made alkaline with strong ammonia, and shaken with ether. The ethereal solution was washed with water and dried with anhydrous sodium sulphate. The residue left after evaporating off the ether was dissolved in dilute acetic acid and mixed with a slight excess of a concentrated solution of potassium iodide. A cream coloured microcrystalline precipitate was obtained, which was washed with a dilute solution of potassium iodide and dried in a vacuum desiccator over sulphuric acid, yield 1·4 g. On heating, the hydriodide turned dark in colour at 195° and charred without melting at 215-20°. Attempts to obtain a crystalline base from the salt have not yet been successful.

Isolation of Alizarin.—The benzene extract (A) from above was treated with sodium hydroxide solution till the latter was no longer coloured violet. The addition of baryta water to the solution caused a purplish precipitate which was well washed with water to remove soluble barium compounds

The washed precipitate was then suspended in boiling water and mixed with hydrochloric acid when an orange coloured precipitate separated. This was filtered, washed and dried. On crystallising from benzene, reddish needles, m.p. 279°, were obtained which gave all the reactions characteristic of alizarin. The filtrate from the baryta precipitation did not yield any crystalline substance.

Fatty Constituents.—The residue from the benzene extract (A) after the alizarin had been separated was examined for fatty acids and non-saponifiable constituents by the usual methods.

Non-saponifiable Constituents.—A crystalline phytosterol, m.p. 141-42°, yielding a crystalline acetate, m.p. 128-29°, was isolated from this fraction.

Saturated Fatty Acids.—An acid, m.p. 67° and having a molecular weight of 342 was obtained which would appear to be stearic acid.

Unsaturated Fatty Acids.—The presence of linoleic acid was indicated in this fraction by the fact that on oxidation with alkaline potassium permanganate solution a tetrahydroxystearic acid, m.p. 154-55°, could be obtained.

Other Constituents.—The alkaline aqueous solution remaining after the separation of the alkaloids was neutralised with acetic acid and treated with a slight excess of neutral lead acetate solution. The precipitate obtained was washed and submitted to the method of separation of organic acids (Fleischer, *Arch. Pharm.*, 1874, 205, 97). The presence of oxalic acid was indicated by its typical reactions in the portion of the lead precipitate insoluble in ammonia.

The filtrate from the lead precipitate was treated with hydrogen sulphide, lead sulphide was filtered off, and the filtrate was concentrated under vacuum. The syrupy residue gave the usual reactions of a reducing sugar, and yielded an osazone, m.p. 105°, indicating the presence of glucose.

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