CHEMICAL ANALYSIS OF INDIAN MEDICINAL PLANTS. THE ACTIVE PRINCIPLE AND OTHER CONSTITUENTS OF *FUMARIA OFFICINALIS* BEDD.

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(Communicated by Dr. S. Dutt.)

(*Read March 25, 1937.*)

Fumaria officinalis Bedd., known in India by the vernacular names '*Shaheterah*' or '*Pitpapra*' (Hindustani), or '*Pipara*' (Bengali), is a common herb belonging to the natural order Fumariacæ. The plant has been in use in the Indian system of medicine for a very long time, being regarded as a diuretic and alterative. Besides, it is known to remove hepatic obstructions and is an aperient and expellent of humours but more especially of atrabiles. It has also been successfully used as a laxative, and is beneficial in dyspepsia depending upon the torpidity of the intestines, and in scrofulous skin affections. The taste of the plant is bitter, slightly acrid and astringent.

As regards its chemical composition, it has been a subject of numerous investigations. Peschier in 1829 (*J. Pharm.*, 1829, *17*, 280) found an alkaloidal principle in it, a fact which was later on corroborated by Hannon (*J. Chem. Med.*, 1852, (3), *8*, 705). The properties of this base which was termed fumarine were later on described in minor detail by Bettandier (*Pharm. Ztg.*, 1885, *24*, 542) and also by Reichwald (*ibid.*, 1889, *28*, 161). The drug was also supposed to contain fumaric acid and salts (Peschier, *loc. cit.*; Wincler, *Ann.* 1833, *4*, 230; Trommodorf, *J. Pharm.*, 1833, *25*, 152). Wehmer (*Die Pflazenstoffe*, 1931) mentions that Schlotterbeck (*Amer. Chem. Jour.*, 1900, *24*, 249) and also Schmidt (*Arch. Pharm.*, 1901, *239*, 401) showed that fumarine is probably identical with protopine. However, no reference to the latter work is found in abstracted chemical literature. Pictet (*Vegetable alkaloids*, 1913) retains the name fumarine and says that it crystallizes in prisms m.p. 199°C., has the formula $C_{21}H_{19}NO_4$ and is optically active.

The Indian variety of *Fumaria officinalis* was examined in this laboratory by Pendse and Dutt five years ago (*Ind. J. Med. Res.*, 1932, *22*, 663), but they could not isolate the alkaloid. Since the plant is successfully used in India in native medical practice, it was deemed proper by the present author to re-investigate the problem and to test the claims of the Western workers in view of the work of Pendse and Dutt with regard to the Indian variety. As a result of the present research, it has now been definitely shown that the drug contains 1% of inorganic salts, consisting of a mixture of potassium nitrate (70·9%) and potassium chloride (29·1%); a hydrocarbon 0·5% which has been identified

as penta-triacontane $C_{35}H_{72}$ and an alkaloidal principle (0·13%) identical with protopine ; besides tannins, phlobaphenes and sugars.

The presence of penta-triacontane in this drug is of sufficient interest in view of the hypothesis put forward by Channon and Chibnall (*Biochem. J.*, 1929, *23*, 168) regarding the metabolism and formation of paraffins in plants. According to their view the paraffins are formed from the fatty acids *via* the ketone. Stearone, the ketone derived from stearic acid, which is one of the most abundant fatty acids found in nature, on reduction will give pentatriacontane according to the scheme below :—

$$\underset{\text{(Stearic acid)}}{C_{17}H_{35}.COOH} \longrightarrow \underset{\text{(Stearone)}}{C_{17}H_{35}-CO-C_{17}H_{35}} \longrightarrow \underset{\text{(Penta-triacontane)}}{C_{35}H_{72}}$$

The diuretic properties of the drug are mainly due to the presence of such large quantities of potassium salts. Protopine, which was originally isolated from opium, has been found to exist widely in nature especially in plants belonging to the natural orders, Papaveracæ and Fumariacæ, e.g. *Macleya cordata*, *Stylophorum diphyllum*, *Sangunaria canadensis*, *Chelidonium majus* and *Corydalis veruyi*. It is a strong base and has powerful physiological properties. Protopine resembles cryptopine but solutions of the salts of the former have a bitter taste. In small doses protopine acts on frogs as a narcotic and in stronger doses paralyses the muscle-substance and the peripheral ends of the nerves. Upon mammals it has a poisonous action like that of camphor, but differs from it in paralysing the circulating organs. It has been isolated from *Fumaria officinalis* in the form of its acetate after the removal of tannins, inorganic and waxy materials, and is the active principle responsible for the physiological action of this drug.

Experimental.

The material used in these investigations was collected from the neighbourhood of Jhansi District (U.P.), dried in the shade and the entire plant, minus the roots, was crushed finely in an iron mortar. A weighed sample of this powdered material was exhaustively extracted in a Soxhlet's apparatus using different solvents when the following amounts of the extracts dried at 100° to constant weight were obtained :—

Benzene Extract.—A waxy-green mass was obtained which consisted mostly of chlorophyll and a white crystalline stuff of low melting point embedded in it. Yield 3·3%.

Chloroform Extract.—A yellowish green sticky mass smelling of sugars and giving reactions for alkaloids was obtained. Yield 4·5%.

Alcoholic Extract.—A yellow semi-solid mass containing some brown crystalline mass was obtained. It gave a yellow precipitate with lead acetate, a green colour with alcoholic ferric chloride and reduced Fehling's solution. Gave reactions for alkaloids. Yield 13·1%.

Aqueous Extract.—A brownish crystalline residue was obtained consisting of organic salts, giving tests for nitrates and chlorides. Reduced Fehling's solution and gave faint precipitates with alkaloidal reagents. Yield 5·1%.

2·5 kilograms of the well powdered material was then repeatedly extracted with boiling ethyl alcohol several times, in a big extraction flask of five litre capacity. The extracts were filtered hot and on cooling deposited a flocculent white crystalline precipitate. This was filtered and the dark green alcoholic extractive was concentrated whereby on cooling a further crop of the crystalline mass was obtained. The two crops were mixed together, and washed several times with warm alcohol, till a dirty white crystalline mass (A) was obtained containing a tinge of greenish colour. The washings were added to the alcoholic mother liquor.

The mother liquor from this mass, consisting of a thick syrupy liquid, was then further concentrated, the final concentrations being done in a vacuum dessicator under a very high vacuum. The semi-solid residue thus obtained was then extracted several times with hot benzene, under a reflux condenser, till the benzene extracts were perfectly colourless. Benzene removed all the chlorophyll and waxy material, and the mass then assumed a brownish-yellow appearance. The combined benzene extracts, on the complete removal of the solvent, gave a hygroscopic dark green mass, which on repeated washings with cold ethyl alcohol gave a dirty white amorphous residue which was added to the crystalline mass (A) obtained before.

The original alcoholic extract obtained after the removal of chlorophyll and wax through benzene was then dissolved in about one litre of alcohol. At this stage a little amount of white crystalline inorganic substance was left behind, which did not go into solution even on boiling. This was also added to the crystalline mass (A). The alcoholic solution was then treated with an alcoholic solution of lead acetate in order to free it from tannins and colouring matter. A flocculent yellow precipitate of the lead salt was formed, which was filtered over a filter pump, washed thoroughly with alcohol, suspended in ethyl alcohol and decomposed by passing a current of purified hydrogen sulphide. The filtrate obtained, after the separation of lead sulphide, was then concentrated under reduced pressure. A thick brownish-yellow residue was obtained, which failed to give any crystalline stuff and answered to all reactions of tannins. It was extracted with different solvents but no chemically pure substance could be isolated from it.

The clear alcoholic solution obtained, after removal of the lead lake, was then freed from excess of lead in the usual manner and concentrated to a small volume. A little acetic acid was added and the solution left for about a fortnight when fine silky needles separated out which were filtered and washed with small quantities of ethyl alcohol. The white crystalline needles (B) gave reactions for alkaloids and melted at 247–250°C. after previous shrinking at 237°C.

The mother liquor was again concentrated and gave a further yield of the acetate of the alkaloid which was filtered, washed and added to the first (B) The syrupy residue left behind was then dissolved in pure distilled water and on examination was found to contain a large amount of reducing sugars. A sample on treatment with phenyl-hydrazine gave an osazone (m.p. 206°C.), which was identified as glucasazone, showing the presence of glucose in the plant.

Examination of the crystalline mass (A).

Isolation of Potassium nitrate and potassium chloride.—The crystalline mass obtained as described above was dehydrated carefully in a vacuum dessicator over anhydrous calcium chloride, powdered finely and extracted repeatedly with hot boiling benzene. Benzene removed the organic matter leaving behind a dirty brownish-white amorphous inorganic material. This was dissolved in a small quantity of water, boiled with animal charcoal and filtered. On cooling the aqueous solution, a mixture of long needles and rhomblic plates was obtained. A further quantity was obtained on concentrating the mother liquor to a very small volume (total yield, 27·5 gms., i.e. 1·1% on the weight of dried drug). This was identified to be a mixture of potassium nitrate and potassium chloride. An estimation of the chloride content of this mixture gave 27·16 per cent of potassium chloride and 72·84 per cent of potassium nitrate. (2·0214 grams of the mixture gave 1·5561 grams of AgCl ; 27·16 per cent KCl requires 1·5562 grams of AgCl.)

Isolation of penta-triacontane.—The benzene extracts were concentrated to a very small volume and on being allowed to cool and stand, a large amount of crystalline matter began to separate. This was filtered and recrystallized several times from benzene and petroleum ether, till finally a white crystalline mass was obtained melting at 70–73°C. The final crystallizations were done through large volumes of ethyl alcohol several times, till colourless small needles were obtained (yield 13 grams) melting at 75-76°C. and the melting point did not rise further on subsequent crystallization. The compound which gave reactions for a hydrocarbon was identified to be penta-triacontane. It is a colourless, tasteless and odourless crystalline substance, which burns with a non-smoky luminous flame, giving a characteristic odour of burnt paraffin. It is soluble readily in benzene, less so in petroleum ether, alcohol, methyl alcohol and glacial acetic acid and insoluble in water. It distills unchanged at 329–331°C. at 15 m.m. pressure. It does not decolourize bromine in chloroform. (Found C, 85·19, 85·02 ; H, 15·01, 14·71 ; M.W. (Ebullioscopic in benzene) 479, 504, 489 ; $C_{35}H_{72}$ requires, C, 85·36 ; H, 14·64% M.W. 492.)

Examination of the alkaloidal content (B).

The crystalline acetate of the alkaloid obtained as described above was recrystallized twice from boiling ethyl alcohol till fine silky needles were obtained melting at 267°C. These were then dissolved in about 200 c.c. of boiling distilled water, cooled and treated with dilute solution of sodium hydroxide,

when the liberated base separated out in the form of gelatinous white precipitate. This was allowed to settle and then filtered. On crystallization from absolute methyl alcohol, small needles were obtained melting at 200–202°C. This was then recrystallized from a mixture of ethyl alcohol and chloroform when colourless needles were obtained melting at 206-207°C. (Yield 4·3 grams). It is extremely bitter and gives a beautiful violet colouration with a reddish tinge, which becomes blood red on standing with con. sulphuric acid, and a light reddish-violet colour with con. H_2SO_4 containing a little $K_2Cr_2O_7$. With con. H_2SO_4 and $KClO_3$ it gives a deep orange red colour and a very light yellow with concentrated HNO_3. With Frohde's reagent a very intense violet colour turning to olive green is developed. With Mendelins' reagent the crystals turn violet, and yield a green solution which becomes blue and finally violet. With phosphotungstic acid a white precipitate is formed and with phospho-molybdic acid a yellow precipitate. With K I and I_2 a flocculent brown precipitate and with tannic acid a flocculent white precipitate is obtained. With Mayer's reagent a flocculent white precipitate is obtained. It gives a yellow precipitate with gold chloride and platinic chloride. It is optically inactive. It gave no colour with ferric chloride and was insoluble in caustic alkalis. (Found C, 67·72, 67·59 ; H, 5·44, 5·52 ; N, 3·5, 4·00 $C_{20}H_{19}O_5N$ requires C, 67·99, H, 5·38 N, 3·97%.) From all its reactions and elementary analysis it has been identified to be protopine.

Protopine hydro-chloride.—Protopine (1 gram) was dissolved in the minimum quantity of absolute alcohol and treated with gaseous hydrochloric acid. On adding ether to the solution the hydrochloride separated out as fine colourless crystalline powder, m.p. 274°C. (Found Cl 9·00 ; $C_{20}H_{19}O_5N$ HCl requires Cl 9·18%.)

Protopine acetate.—Protopine (1 gram) was treated with the requisite amount of glacial acetic acid in alcohol. On standing the acetate crystallized out in fine needles, which were filtered, washed with alcohol and recrystallized from the same media when colourless silky needles were obtained, m.p. 268°C. (Found C, 63·72 ; H, 5·79 ; $C_{20}H_{19}O_5N$. CH_3CO-OH requires C, 63·92, H, 5·57%.)

Protopine picrate.—Protopine (0·5 gram) was dissolved in water and treated with an aqueous solution of picric acid whereby a flocculent yellow precipitate of the picrate was formed. It was filtered, washed and recrystallized from glacial acetic acid when micro-crystalline deep yellow needles were obtained, m.p. 249°C. (decomp.).

In conclusion the author wishes to express his sincere thanks to Dr. S. Dutt, D.Sc. (London), at whose suggestion this problem was undertaken, and who has generously given him at every stage most valuable advice and guidance.