Chemical Examination of the Roots and Leaves of *Saussurea Lappa*, Clarke. Part I.

By Sudhamoy Ghosh, Nihar Ranjan Chatterjee and Ashutosh Dutta.

In a recent paper on the treatment of asthma with *Saussurea appa*, Chopra (*Indian Med. Gazette*, 1928, **63**, April issue), gives the following short but very useful summary of the plant and its uses: "This plant belongs to the natural order *Compositae*, and is commonly called *Kuth* or *Patchak* in Hindi, *Kustha* or *Kashmirja* in Sanskrit and *Kust* in Arabic and Persian. It was for a long time confused with other species of costus and also with orris root but Guibourt first suggested the correct botanical source of this drug and his suggestion was confirmed by the researches of Falconer who visited Kashmir and found that *Aplotaxis lappa* growing there was undoubtedly *Auclandia costus*, now known as *Saussurea lappa*. It is a tall, very stout herb, with annual stem, heart-shaped leaves and thick perennial roots which are dug up in the months of September and October. It grows chiefly on the moist and open slopes of the Himalayas, especially those surrounding the valley of Kashmir and adjacent ranges, at a height of 2,000 to 13,000 feet above sea level. The best root is grown over an altitude of 9,000 to 13,000 feet. The root occur in crooked twisted pieces about 3 inches long, ½ to 1 inch in diameter and possesses a pungent aromatic odour like that of orris root; it is collected and exported to China in large quantities without any further preparation. It is also shipped to the Red Sea and Persian Gulf. The root fetches a very good price, as much as Rs. 385 (£28-10) being at present paid for a maund (80 lb.) of a good sample in Calcutta. In China it is chiefly used as an incense, as a spice and medicinally as a powerful aromatic stimulant, carminative, antispasmodic and in the form of an ointment for external application. It is a very old remedy of the Hindu medicine and has been described in the Nighantas as a remedy for cough, asthma, fever, dyspepsia and skin diseases. The Arabian medical writers describe it as a diuretic and anthelmintic and use it in the treatment of quartan fever and leprosy."

In a previous paper, Chopra and De (*Indian Med. Gazette*, 1924, 59, 540) gave an account of the antispasmodic action of the root in the treatment of bronchial asthma and, in the more recent paper referred to, Chopra has shown the remarkable effect of the drug in controlling the paroxysms of bronchial asthma.

In another paper recently communicated (*Indian J. Med. Res.*) Chopra and De have given a detailed description of the pharmacological action of the essential oil and the alkaloid (first isolated by us) which may be considered as the active principles chiefly responsible for the action of the drug in asthma. A summary of this is given later on.

Chemical Examination of the Root.

Of the active principles contained in the root, only the essential oil was thoroughly examined by Semmler and Feldstein (*Ber.*, 1914, 47, 2433). According to these workers, the essential oil has the following approximate composition. Camphene 0·04%; phellandrene 0·4%; terpene alcohol 0·2%; α-costene 6·0%; β-costene 6·0%; aplotaxene 20·0%; costol 7·0%; dihydrocostus lactone 15·0%; costus lactone 11·0%; costic acid 14·0%. The yield of the essential oil is stated to be about 1%.

No systematic study seems, however, to have been made for the other constituents. Hooper ("Pharmacographica Indica," 1890, Vol. II, p. 293) describes the presence of one solid and two liquid resins but he did not estimate their amount. He also mentions that he got evidence of the mere presence of an alkaloid, but he never isolated it. The isolation of the alkaloid was found by us to be a very difficult task and it took a long time to find out the proper technique for its purification.

The material employed in the present investigation was the genuine dry root supplied by the Kashmere State authorities, and we are grateful to them for this kind help.

For a systematic investigation, 500 gm. of the powdered root were extracted with petroleum ether (b. p. about 40°) for several days until exhausted. The extractive matter, freed from solvent, amounted to 6·4%. The extract, dissolved in petroleum ether, was shaken up repeatedly with water. The aqueous extracts, which had a bitter taste, were concentrated in *vacuo* and extracted in acid solution with ether. The small amount of bitter substance thus isolated was

amorphous and of non-alkaloidal nature. After extracting with water, the petroleum ether was removed and the residue was found to consist of an essential oil, some fixed oil and a little resin. The essential oil was separated by distillation in steam.

Ethereal Extract.—The roots, after extraction with petroleum ether, were extracted with ether until exhausted. The extract, which amounted to about 1·3%, gave tests for traces of tannins, a trace of the alkaloid and some of the bitter substances isolated above.

Absolute Alcoholic Extract.—After exhaustion with ether, the roots were exhausted with absolute alcohol, and the alcoholic extract amounted to about 11·8%. The residue, freed from alcohol, was taken up with water and the aqueous extract was shaken in slightly acid solution with ether. The residue from ethereal extract gave colour reactions for tannins. The aqueous solution was then made alkaline with ammonia and shaken repeatedly with ether and finally with chloroform. The ether extracted most of the alkaloid present.

The aqueous solution was next neutralised and precipitated with neutral lead acetate. The precipitate was washed, suspended in water and decomposed by sulphuretted hydrogen. The filtrate, after removal of lead sulphide, gave strong reactions for tannins, but nothing crystalline could be isolated. The filtrate from lead acetate precipitate was next precipitated by basic lead acetate. The precipitate was decomposed as before but nothing definite could be isolated. The filtrate from basic lead acetate was freed from lead and then evaporated in *vacuo*. Some of the bitter substance was isolated by chloroform, but nothing else of interest was obtained.

Aqueous Extract.—The roots, exhausted with alcohol, were then repeatedly extracted with hot water. On concentrating the aqueous extract in *vacuo*, a large amount of a white amorphous substance separated, which was purified and identified as inulin as described later. The yield of crude inulin amounted to nearly 18%. Some potassium nitrate and sugars were also detected in the aqueous extract.

Estimation of Essential Oil.—A weighed quantity of the powdered root was distilled in steam as long as the distillate was turbid. The distillate was then extracted with ether, the ethereal solution dried and ether removed. The average of two quantitative experiments gave 1·51% of essential oil for the sample examined. Previous experience showed that the yield of essential oil varied a good deal with different samples and was decreased by storage.

Resin.—A rough quantitative estimation of the amount of resin was carried out and the average of two estimations gave the value 6·0%.

Alkaloid (Assay).—The total alkaloidal content of a fresh sample as obtained by a general gravimetric method was found to be 0·052% as the average of 3 estimations. The amount of alkaloid varied in different samples and in one case it was extremely small.

Isolation of the Alkaloid.—About 1500 gm. of the powdered roots were extracted in the cold in a glass percolator with rectified spirit containing about 5% of ammonia until the alkaloid was all exhausted. The alcohol was recovered and the residue treated repeatedly with 1% sulphuric acid. The acid extract was made slightly alkaline with ammonia and shaken with chloroform. The chloroform was removed and the residue (still admixed with resinous matter) was dissolved in a little alcohol and neutralised with a little excess of tartaric acid. Water was added and the alcohol removed on the water-bath. The clear, pale yellow supernatant liquid was decanted off and concentrated on the water-bath until crystals were obtained. The highly coloured crystals were then dissolved in alcohol and precipitated by ether. The crystals were then washed several times with absolute alcohol to remove the excess of tartaric acid, resinous and colouring matters. They were next dissolved in a small quantity of hot water and decolourised with animal charcoal, filtered, and evaporated to crystallisation. The crystals were washed with absolute alcohol and then with ether and dried. They were perfectly colourless, needle-shaped crystals. Further chemical work with this new active alkaloid is in progress, and we have provisionally designated it as "Saussurine".

Inulin.—The crude inulin obtained before was dissolved in hot water, heated with animal charcoal and filtered hot. The precipitate obtained on cooling was dissolved in a small amount of hot water and precipitated by alcohol. On repeating the process, the substance was obtained as a perfectly white, amorphous powder without any taste. The air-dried sample melted at about 180° with decomposition and the melting point rose higher on prolonged drying in air oven. The substance was slightly soluble in cold water but dissolved easily in hot. It was insoluble in absolute alcohol, ether and benzene, but fairly soluble in dilute alcohol,

In aqueous solution the specific rotation, $[\alpha]_D^{25^\circ}$ was $-34{\cdot}0^\circ$. (The values for inulin given by different authors vary from -34° to -42°).

The estimations of C and H, the determination of the acetyl groups in the acetylated product, the osazone of the sugar obtained by its hydrolysis all confirmed the substance being a variety of inulin.

The large content of inulin in the roots can serve as an economic source of its manufacture. After the preparation of the liquid extract (see below), the residue (which is at present thrown away) can be utilised for its manufacture and will prove a useful by-product.

Examination of the Leaves.—The leaves were examined specially for essential oils and alkaloids. No essential oil could be obtained from the air-dry leaves supplied for examination. The alkaloidal content amounted to about 0·02% of the weight of the dry leaves. The nature of the alkaloid was the same as that found in the roots. It is, however, interesting to record that the alkaloid can be extracted much more easily from the leaves than from the roots owing to the absence of resinous and other matter present in the latter. And as the nature of the alkaloid is the same, the leaves, which are at present thrown away, may be utilised for the preparation of the pharmacologically active alkaloid.

" *Liquid Extract of Kut* ".—The liquid extract used for clinical trials is made by extracting the powdered roots repeatedly with 95% alcohol in the cold, and the solution is concentrated carefully until 1 c.c. of it corresponds to 1 g. of the root.

Pharmacological and Therapeutic Action.—The essential oil has antiseptic and disinfectant properties; it relaxes the involuntary muscle tissue; it is a cardiac stimulant, a carminative, an expectorant and a diuretic. The alkaloid, saussurine, has a depressant action on the vagus centre in the medulla as well as on the involuntary muscle fibres of the bronchioles and gastro-intestinal tract. It produces a slight but persistent rise of blood pressure and increases the force of contraction and amplitude of the ventricles.

The drug has a remarkable effect in controlling attacks of bronchial asthma especially those of the vagotonic type. The

paroxysms are cut short by the combined action of the essentia oil and the alkaloid present in the root. The essential oil during its excretion in the lungs not only relaxes the bronchial muscle but has a marked expectorant action which relieves turgescence of the mucosa.

The drug is also useful in persistent hiccough.

Summary.

I. Constituents isolated in roots : —

Essential oil ...	... 1·5%
Resin ...	about 6%
Alkaloid (saussurine)	... 0·05%
Inulin ...	... 18%

A very small amount of a bitter substance.

Tannins in small amount.

Fixed oil, KNO_3, sugars, etc.

II. Constituent isolated in leaves : —

Alkaloid	... 0·02%

In conclusion, we desire to express our indebtedness to Col. R. N. Chopra and Capt. P. De for the study of the pharmacological and therapeutic properties of the drug.

School of Tropical Medicine and Hygiene, Calcutta.

Received April 8, 1929