CHEMICAL COMPOSITION OF INDIAN SENNA LEAVES (*CASSIA ANGUSTIFOLIA*)

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The introduction of senna as an article of drug into the materia medica dates very far back. The Indian senna which is reputed for its high quality is extensively cultivated in the Southern districts of the Madras Presidency. Due to its commercial importance and the conflicting reports made by workers in the past regarding its composition it was considered desirable to re-examine the drug in detail.

The first systematic examination of the leaves was carried out by Tschirch and Hiepe.[1] From an aqueous percolate they claimed to have isolated an unstable yellowish crystalline compound of the composition, $C_{14}H_{10}O_5$, impure cathartic acid and senna-rhamnetin which was considered to be the same as iso-rhamnetin. From a dilute ammonia percolate they recorded that they obtained gluco-sennin, senna-emodin, senna chrysophanic acid and amorphous senna nigrin. Subsequently Tutin[2] made a more detailed analysis using an alcoholic extract. He divided it into two portions : (i) water-soluble portion, (ii) water insoluble resin. From the former he could isolate a glucoside of kæmpferol along with free kæmpferol, rhein, emodin and salicylic acid. From the resin he obtained fatty and waxy matter along with small quantities of kæmpferol, rhein and emodin. He was of opinion that cathartic acid and anthraglucosennin of Tschirch and Hiepe were impure mixtures. Further he could not detect the presence of any iso-rhamnetin or chrysophanic acid in the leaves. Though a number of papers have been published subsequently they are not of fundamental chemical importance and have not added to our knowledge of the chemical composition of the leaves.

There seems to exist considerable uncertainty regarding the nature of the inorganic salts present in senna. It is known that extractable inorganic salts can exert purgative action. During some of our experiments with water extracts, it was noticed that the addition of sulphuric acid precipitated a considerable amount of salts the main component of which was found to be calcium sulphate. Tutin was able to detect the presence of

magnesium salts. The ash of the entire leaves has therefore been analysed qualitatively and the important components estimated quantitatively. Most of the inorganic matter seems to be extractable with water. Calcium, magnesium and potassium exist as salts of organic acids and may account for a part of the purgative action of senna.

In our experiments on the isolation and characterisation of the organic compounds, it was found to be most convenient to remove the fatty and waxy matter at the beginning by extraction with light petroleum. By this means a good amount of such matter which would interfere with the isolation of other compounds at a later stage could be eliminated. From this extract was obtained after repeated purification using benzene, a colourless wax (senna wax) to the extent of more than 1 per cent. on the weight of the leaves. It could be divided into two fractions : (i) more sparingly soluble in benzene melting at 80–83° and found to be a wax-yielding myricyl alcohol as the main product of saponification ; (ii) more easily soluble in benzene melting at 87–88° consisting entirely of myricyl alcohol. Therefore it is clear that myricyl alcohol exists to a large extent in senna.

With a view to make a detailed examination of the flavonols, large quantities of the leaves extracted with light petroleum were employed. The alcoholic extracts of these leaves were divided into two fractions : (i) water-insoluble resin ; (ii) water-soluble material. Our preliminary experiments indicated that almost all of the flavonol portions went into the aqueous extract. This was therefore studied in detail by precipitating the pigments as the lead salts using neutral and basic lead acetates successively. It was convenient to use the two reagents since neutral lead acetate removed most of the resinous matter and subsequently when the bulk of the pigments was precipitated with the basic salt there was very little resin. The substances obtained by the decomposition of these lead salts were fractionally crystallised from alcohol. From the less soluble fraction a good yield of iso-rhamnetin could be obtained. The more soluble fraction yielded kæmpferol as the main component. It is therefore clear that iso-rhamnetin is an important component of the senna leaves and it is difficult to explain how Tutin failed to detect its presence in this material especially when it is present to the extent of nearly 50 per cent. of the flavonol mixture.

In the detailed study made by Tutin kæmpferol, emodin and rhein were obtained as mixtures at various stages requiring elaborate separation at each stage. The above procedure adopted by us yielded practically all the flavonols at one stage.

For isolating and examining the anthraquinone derivatives we have employed a different method. The whole of the alcoholic extract was subsequently extracted with boiling benzene, and then purified from resinous matter by repeated shaking with alkali, acidifying subsequently the alkaline solution and extracting again with benzene. By repeating this process, the anthraquinone derivatives were obtained in a pure condition (0·03%) and the mixture was found to consist mostly of rhein along with small quantities of emodin. It should be noted at this stage that of the three closely related anthraquinone derivatives rhein, emodin and chrysophanic acid, all the three were found by Tschrich and Hiepe, whereas the experiments of Tutin and ours indicate the presence of the first two only. It is probable that the occurrence of one or the other of these related compounds is dependent upon the conditions of soil and climate.

If in the process of isolating rhein outlined above a slight modification were introduced and the alcoholic extract boiled with hydrochloric acid prior to extraction with benzene the yield of rhein was almost doubled (0·07%). This could be explained as due to the existence of this anthraquinone compound in the leaf to a large extent as a glucoside insoluble in benzene.

Experimental

The leaves used for the present investigation were obtained from (The Agricultural Research Station) Koilpatti in the Tinnevelly District. The air-dried material was powdered in a grinder and preserved in glass stoppered jars.

The Ash.—The percentage of the ash in the leaves was determined by igniting the leaves in a platinum basin at a dull red-heat until a uniformly grey ash was obtained. It formed 9·6% by weight of the leaves and gave tests for the following radicals when analysed qualitatively: Iron, manganese, calcium, magnesium, potassium, sodium and phosphate, sulphate, carbonate, chloride and silicate. It was then analysed quantitatively for the important components. The following figures represent the amounts in terms of the oxides of the elements:

		%
SiO_2	(insoluble)	2·7
MgO	,,	7·2
CaO	,,	31·6
P_2O_5	,,	3·6

Isolation of Senna Wax.—4 kilo-grams of the leaf powder were extracted repeatedly with petroleum ether in a large continuous extractor,

until further extraction gave nearly a colourless solution. More than three-fourths of the solvent was distilled off and the concentrate allowed to stand. Since no crystalline product separated out of it even after several days, the remaining solvent was also removed as far as possible by distilling on a water-bath. The viscous green coloured residue could be purified best by employing benzene, other solvents and simple melting not being successful. It was taken in a wide-mouthed flask of 500 c.c. capacity and heated on a water-bath with sufficient benzene (250 c.c.) to give a clear solution. It was then quickly cooled with running water and kept undisturbed for 4 hours. A dark coloured solid gradually separated at the bottom. It was filtered and repeatedly washed with small amounts of benzene, whereby it was obtained in the form of a grey and non-sticky wax. It was then further purified by dissolving in a small quantity of hot benzene and allowing to crystallise. By repeating this process a number of times, fraction (I) was obtained in a perfectly colourless and crystalline form melting at 80–83° C.

The benzene mother-liquor and the subsequent washings were mixed together and concentrated to a small bulk. The solid that separated out was repeatedly recrystallised from benzene and thereby fraction (II) melting at 87–88 °C. was obtained.

Fraction I.—It was quite colourless, melted at 80–83° C. and formed nearly 1% of the weight of the dry leaves taken. It exhibited the properties of a wax. It was sparingly soluble in acetone, ether, chloroform, carbon tetrachloride and cold alcohol. It however readily dissolved in hot alcohol and when cooled separated in the form of a jelly-like mass. It had the following properties :

Saponification value	..	40·2
The acid value	..	5·2
Iodine value	..	2·1
Unsaponifiable matter	..	80%

The unsaponifiable matter was found to be identical in composition and properties with fraction (II) obtained from the benzene mother-liquor.

Fraction II : Myricyl Alcohol.—A preliminary examination of fraction (II) by heating it with seminormal alcoholic potash for 12 hours showed it to be completely unsaponifiable. It appeared to be a homogeneous compound and the analysis for carbon and hydrogen showed that it had the formula $C_{30}H_{62}O$ (Found : C, 82·0 ; H, 14·6 ; $C_{30}H_{62}O$ requires C, 82·2 ; H, 14·5%). From the melting point, it could be identified as myricyl alcohol. This was confirmed by preparing the acetyl derivative by heating it with sodium acetate and acetic anhydride for 5 hours, pouring the product

in a large volume of water and recrystallising the precipitate from ether. The acetyl derivative was obtained in the form of colourless plates melting at 70–71° C.

Isolation of Flavonols (Kæmpferol and Isorhamnetin)

The leaf powder left after extraction with petroleum ether was completely freed from the last traces of the solvent by blowing air through it. It was then repeatedly extracted with 95% alcohol till the process was complete. The dark coloured extract was concentrated to about 800 c.c. and filtered with suction. The filtrate was kept in a flask for several days but no crystalline product separated.

It was then poured into a large volume of water and the resinous mass that separated out was removed by filtration. The resin was again dissolved in alcohol and diluted with water when some more of it was brought into solution. This process was repeated thrice till all the water-soluble portion was extracted and the dark residue was finally rejected. The collected aqueous extract was concentrated to a small bulk under reduced pressure and allowed to stand for several days. Since it did not deposit any crystalline compound, it was treated in the following manner.

Neutral Lead Salt.—When treated with an excess of hot neutral lead acetate solution a dark brown precipitate was obtained. It was filtered and decomposed in the same way as the basic lead precipitate as described below. Besides large quantities of amorphous resin, it yielded the same flavonols and anthraquinone derivatives as the basic lead salt though in small amounts. The aqueous solution after hydrolysis responded to the tests for glucose and hence seemed to contain glucosides. This treatment with neutral lead acetate was found to be advantageous since it removed almost all resinous matter and rendered the subsequent study of the basic lead precipitate easy.

Basic Lead Salt.—The clear, brown filtrate obtained after the removal of the neutral lead precipitate, was boiled and treated with an excess of hot basic lead acetate solution. Even after the addition of a large amount of this reagent, it was found that all the colouring matter was not completely precipitated. By the cautious addition of a few c.c. of dilute ammonia, the precipitation was rendered complete. The canary yellow solid thus obtained was kept in fine suspension in water and decomposed by passing H_2S. The precipitated lead sulphide was removed by filtration and the resulting solution was concentrated under reduced pressure and finally allowed to evaporate in a desiccator. As no crystalline compound could be isolated from this after repeated attempts the whole of it was dissolved in water and hydrolysed with 7% sulphuric acid by heating the contents on

a water-bath. On cooling the flask a brown insoluble resin (A) separated. The clear solution obtained after the removal of the resin, was repeatedly ether-extracted and the mixed ether solution evaporated when an orange-yellow residue (B) was left behind. The remaining aqueous solution was found to contain glucose and hence the lead salt precipitate should consist of some glucosides.

Residue A.—The dark brown resin was not easily soluble in ether but readily dissolved in alcohol. To the alcoholic solution an excess of ether was added when a coloured resin separated. Since it yielded no crystalline solid on further treatment it was rejected. When the clear ether-alcohol solution was slowly concentrated a good amount of a clear orange yellow solid was obtained. It was found to be identical with residue (B) and the details of the experimental study are given below.

Residue B.—The material which was orange yellow in colour was found by a few preliminary tests to consist mostly of flavonol pigments along with small quantities of anthraquinone compounds. It was therefore dissolved in excess of alcohol and the solution was slowly concentrated as long as a clear yellow crystalline solid continued to separate. The mother-liquor (C) was turning darker red owing to the accumulation of the anthraquinone compounds. The crystalline solid melted between 280°–95° C., dissolved in aqueous alkali with a bright yellow colour and in concentrated sulphuric acid producing a deep blue fluorescence. It proved to be a mixture of flavonols (yield 0·1% of the leaves) and the separation was effected as below.

Isolation of Iso-rhamnetin.—The above mixture was subjected to fractional crystallisation using alcohol as solvent. The more sparingly soluble fraction was obtained in the form of greenish yellow crystals melting at 305–06° C. (Tutin gave the melting point as 302° and Heap and Robinson[3] 305°). The substance imparted a deep yellow colour to dilute alkali and its solution in concentrated sulphuric acid exhibited a green fluorescence. (Found : CH_3O, 9·4% ; required for $C_{16}H_{12}O_7$, 9·8%). By boiling it with acetic anhydride and sodium acetate, the acetyl derivative was obtained melting at 204°–05° C. thus confirming the identity of the compound with iso-rhamnetin.

Kæmpferol.—After the separation of iso-rhamnetin as above the alcohol mother-liquor was diluted with a few c.c. of water when a bright yellow crystalline substance was obtained. A little more of it was produced by slow concentration of the aqueous alcoholic solution. After further recrystallisation from dilute alcohol it appeared as tiny needle-shaped crystals and melted at 275°. With dilute alkali it gave a yellow colour and when

dissolved in concentrated sulphuric acid it gave a blue fluorescence which increased on standing. On acetylation with sodium acetate and acetic anhydride it formed an acetyl derivative which when recrystallised from alcohol gave colourless needle-shaped crystals melting at 120° C. On further heating it solidified and melted again at 182° C. This is a characteristic of kæmpferol acetate and hence the original flavonol was identified as Kæmpferol.

Separation of Anthraquinone Derivatives.—The red coloured mother-liquor (C) was evaporated so as to remove all alcohol and the residue was dissolved in a small quantity of ether. To the ether solution a large excess of petroleum ether was added when an orange yellow solid precipitated down. This was collected and dissolved in pyridine from which a small quantity of an orange yellow needle-shaped crystalline compound melting at 318°–20° C. was obtained. It gave red solutions with dilute alkali as well as with concentrated sulphuric acid. This was identified as rhein by comparison with an authentic sample. A mixture of the two did not show any depression in the melting point.

By evaporating the ether-petrol mother-liquor a small residue was obtained which when recrystallised from ethyl acetate yielded a very small amount of orange brown crystalline solid melting at 218° C. It exhibited the properties of emodin forming pink coloured solutions with alkali and sulphuric acid.

Isolation of Rhein.—Starting with a separate batch of senna-leaves, it was first extracted with petroleum ether and subsequently with methylated spirit as already described. The alcoholic solution was distilled in order to remove the solvent as completely as possible. The residue was then extracted with hot benzene repeatedly till all benzene-soluble portions were removed. The collected benzene extract was filtered and shaken in the cold with 1% potassium hydroxide solution. The alakli solution was separated and the extraction repeated till no more colouring matter could be removed by the alkali. The potash solution was then acidified with hydrochloric acid and the precipitate extracted with benzene. By repeated transference of colouring matter alternately into potassium hydroxide and benzene, it was rendered pure. Finally the benzene solution was shaken with aqueous sodium bicarbonate solution repeatedly till all rhein was extracted. On acidifying the bicarbonate solution an orange yellow solid was obtained which on recrystallisation from pyridine appeared as orange yellow needles, melting at 318°–20° C. and exhibited all the properties of rhein. Its acetyl derivative was obtained as yellow needles melting at 247° C. with decomposition.

By the above method the yield of rhein amounted to about 0·03% on the weight of the dry leaves. By adopting the following modification however a much higher yield (0·07%) of rhein could be obtained. This definitely indicates that a good portion of the rhein exists in the leaves in the form of a glucoside.

The alcoholic extract of the leaves was concentrated to a small bulk and treated with sufficient amount of concentrated hydrochloric acid so as to bring the acid concentration of the solution to 7%. It was then boiled for 2 hours and then distilled *in vacuo* to dryness. The residue was then extracted with benzene and the purification of rhein effected as already described.

The benzene solution from which rhein had been removed yielded a very small quantity of reddish brown solid on evaporation. It could be recrystallised from ethyl acetate when a pure substance melting at 218° C. and showing the properties of emodin was obtained. The quantity was however very small.

Summary

A detailed chemical examination of the Indian senna leaves has been made. The ash has been analysed. An aqueous extract of the leaves has been found to contain a good amount of calcium salts. A petroleum ether extract yields senna wax consisting of free myricyl alcohol admixed with regular wax. Methods have been worked out for obtaining the flavonol and anthraquinone group of compounds separately in the most convenient manner and in the best yield. The flavonol portion consists of iso-rhamnetin and kæmpferol in more or less equal quantities, whereas the anthraquinone portion contains mostly rhein along with small quantities of emodin.

REFERENCES

1. Tschirch and Hiepe .. *Arch. Pharm.*, 1900, **238**, 427.
2. Frank Tutin .. *J. Chem. Soc.*, 1913, 2006.
3. Tom Heap and Robert Robinson *Ibid.*, 1926, 2342.