

CHEMICAL EXAMINATION OF *ERYTHRINA INDICA*.

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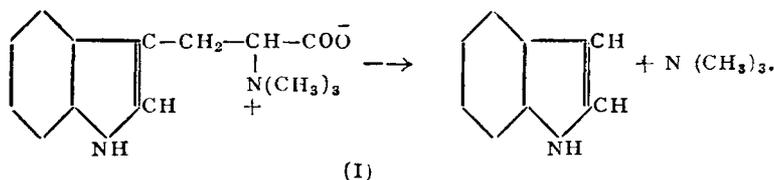
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Erythrina indica is known as the Indian Coral tree (English), *Parijata* (Sanskrit), *Pangra* (Hindi) and *Badita* (Telugu). The bark, the leaves and the seeds have long been used in the treatment of diseases and various valuable properties are attributed to them (Watt).¹ An attempt at a systematic investigation of the bark was made by Chopra, Ghosh and Sen in 1934.² From an alcoholic extract they isolated an impure alkaloid (0.05%) as a dark coloured varnish-like mass decomposing at 97° and two other water-soluble bases betaine and choline besides mineral matter chiefly potassium chloride and carbonate and a large quantity of resin. A preliminary chemical examination of the leaves was made by Chakravarti, Sitaraman and Venkatasubban³ who noticed that besides the usual components the leaves contained a substance of a complex nature melting at 83° C. and a mixture of alkaloids, one of which was isolated and found to melt at 117° C. No work on the composition or properties of these seems to have been done. Kafuku and Hata⁴ made a chemical study of the fixed oil present in the seeds collected in Formosa. No definite information is available about the chemistry of the basic components of the seeds except that they contain a poisonous alkaloid of unknown nature (Greshoff).⁵

From a study of the past literature it seemed to be possible that the seed, bark and leaf contain water-soluble alkaloids and hence attempts have now been made to isolate and identify them. A good sample of the seeds collected from the surroundings of Waltair was first extracted with light petroleum in order to isolate the fixed oil. In yield as well as in most of its properties the oil agreed closely with the sample examined by the Formosan workers. Ours however, had a lower iodine value and a higher solid acids content. The difference may be attributed to the influence of locality and climate.

The oil-free seeds yielded to alcohol a water-soluble alkaloid which formed a sparingly soluble nitrate melting at 220–22° C. It was found to reduce potassium permanganate and ferric chloride very readily, gave an intense violet colour with glyoxalic acid and sulphuric acid and underwent

decomposition with aqueous potash to yield trimethylamine and indole. It was therefore surmised to be hypaphorine, an alkaloid first isolated by Greshoff from *E. hypaphorus* whose constitution as (I) was established by Von Romburgh and Barger.⁶ The identity was established by comparison with an authentic sample of the nitrate kindly supplied by Prof. G. Barger, F.R.S., in regard to crystal structure, melting and mixed melting points and optical rotation.



The bark was examined in two ways. In one experiment the powdered bark was extracted directly with ethyl alcohol and in the other it was subjected to an additional preliminary extraction with cold ether. This modification did not produce any marked difference in the nature of the two extracts. On removing the alcohol the residue gave marked reactions for alkaloids. There was, however, plenty of resinous and mineral matter. The basic portion was found to be easily soluble in water and gave all the reactions for hypaphorine. But attempts to isolate the sparingly soluble nitrate ended in failure. It was felt that this was due to the large admixture with resin and mineral matter which made the separation impossible. The water-soluble portion of the alcoholic extract was therefore decomposed with boiling aqueous potash and the volatile products of decomposition were distilled and examined. Indole was found to be one of the products. A strongly basic water-soluble portion was converted into the chloroplatinate and the product examined. It was found to be a mixture of the chloroplatinates of trimethylamine and ammonia. The formation of indole and trimethylamine showed the presence in the bark of hypaphorine. The production of ammonia is obviously due to the decomposition of some accompanying plant substances. It is known that the amino-acid tryptophane gives the same colour reactions as hypaphorine. But it does not decompose into indole with aqueous alkali under the conditions employed.

The leaves also were found to contain hypaphorine as water-soluble substance. But it was not possible to isolate it owing to the same difficulties as in the case of bark. Further the amount of it present seemed to be much smaller.

With regard to the chemistry of other species of *Erythrina* reference should be made to the work of Maranon and Santos on *Erythrina variegata*⁷

and of Folkers and Major⁸ who very recently claim to have isolated a new alkaloid named erythroidine from *Erythrina americana*.

Experimental.

Seeds :

The fixed oil.—A good sample of the seeds was collected from the neighbourhood of Waltair, powdered and extracted with light petroleum (60–70°C.) in a Soxhlet extractor for 6 hours. When the operation was conducted vigorously this period was found to be sufficient for completely removing the fixed oil from the crushed seeds. The light petroleum solution was dried over fused calcium chloride, filtered and the solvent distilled off. The last traces of petroleum were removed by bubbling a current of nitrogen through the oil. Thus was obtained a pale yellow oil in a yield of about 11.3% of the air-dried seeds. On allowing it to stand for a week, a small quantity of a solid in the form of glittering flakes settled down. As much of the clear oil as possible was decanted off and the rest filtered off in order to separate this solid for examination. It was however too small to permit detailed study ; it seemed to be a fairly high melting plant wax.

The physical and chemical properties of the fatty oil were determined and the values are given below along with those obtained for the oil from the Formosan seeds for purposes of comparison.

	Waltair Seeds	Formosan Seeds
Density	0.8821 at 30° C.	0.8964 at 40° C.
Refractive index ..	1.4596 at 30° C.	1.4604 at 40° C.
Acid number	1.24	2.95
Saponification value ..	184.5	184.5
Iodine number	63.3	71.94
Unsaponifiable matter ..	0.81	0.497
Melting point	20–21° C.	..

The two samples agreed closely in most of their properties except in their iodine value. This may be the result of climatic differences which are known to have considerable effect on the degree of unsaturation of the fixed oils of seeds.

About 5 to 10 grams of the oil were saponified in the usual way using alcoholic potash (25 c.c.). After the saponification the soap was dissolved in sufficient quantity of water and boiled in a conical flask to remove as much alcohol as possible. It was then decomposed with dilute sulphuric acid, the liberated acids were made to separate on the top by continued boiling and the aqueous layer syphoned off. The fatty acids were repeatedly washed with water to remove all the mineral acids (tested with methyl orange). The whole was then filtered, the acid portion on the filter dried, dissolved in petroleum and after evaporating off the solvent weighed. The insoluble fatty acids formed 95.1% of the fatty oil.

As the oil seemed to contain very little resin no attempt was made to estimate resin acids. Separation of the saturated and the unsaturated fatty acids was effected by Twitchell's lead salt method.

	Waltair Seeds	Formosan Seeds
Saturated fatty acids ..	36.7	30.7
Unsaturated fatty acids ..	63.3	69.3

The higher percentage of saturated fatty acids in the Waltair sample accounts for its lower iodine value.

Since it has been stated by the Formosan workers that the unsaturated fatty acids are mainly linolic and oleic acids, the thiocyanogen value of our sample was determined with a view to calculate the proportions of the two acids. It was found to be 54.7 and the percentages of linolic and oleic acids as obtained from a calculation using this value are : oleic acid 84.4% and linolic acid 15.6% (Kafuku and Hata, oleic acid 80% and linolic acid 20%). The thiocyanogen value further leads to the calculated figure 36.7 for the saturated fatty acids which agrees with the value obtained by direct estimation. The lower linolic acid content and the higher saturated acid percentage of our samples thus accounts for its lower iodine value.

Isolation of an alkaloid, Hypaphorine.—The powdered seeds (500 gr.) that had been extracted with light petroleum were boiled under reflux with alcohol twice, six hours each time and the extract so obtained was distilled to remove almost all the alcohol. The residue was taken up with a small volume of hot water (30 c.c.) and filtered after cooling. The aqueous extract gave good tests for the presence of an alkaloid with Meyer's reagent and with phosphomolybdic acid. On acidifying the clear aqueous extract with

concentrated nitric acid (5 c.c.) a colourless crystalline precipitate was deposited rapidly. It was filtered. The mother-liquor gave no more solid on further addition of acid. The precipitated solid was dissolved in a small volume of hot water, filtered and acidified with nitric acid. The nitrate crystallised out as colourless flat needles and prisms melting at 220°–22° C. A further crystallisation as above rendered the salt quite pure, the melting point being raised to 222°–24° C. The yield of the pure salt was 1.2% of the weight of the whole seed. (Found: C, 54.0; H, 6.4; N, 13.9%; $C_{14}H_{19}N_3O_5$ required C, 54.4; H, 6.1; N, 13.6%.)

The substance was found to possess strong reducing properties. It decolorised potassium permanganate and reduced ferric chloride to the ferrous salt. It gave an intense violet colour with glyoxalic and sulphuric acids. When heated with aqueous potash (10%) it underwent decomposition into indole and trimethylamine. After decomposition the alkaline liquid was distilled to half its volume and in the distillate indole was identified by its reactions with sodium nitroprusside and para-dimethylamino-benzaldehyde. By collecting the distillate in aqueous hydrochloric acid, ether-extracting the solution to remove neutral products, concentrating it to a small bulk and treating with platinic chloride the chloroplatinate of trimethylamine was obtained. It melted at 236–38° C. and was found to be identical with a sample prepared from trimethylamine, the mixture melting also at the same temperature.

The alkaloid therefore seemed to be hypaphorine and its identity was established by comparison of the nitrate with an authentic sample of alkaloid nitrate kindly provided by Prof. G. Barger, F.R.S. The two samples were found to be identical in crystal structure (colourless flat needles and plates), to have the same melting point 222°–24° C. (Romburgh and Barger 215–20°) which was unaffected by admixture, and the same optical activity $[\alpha]_D^{25}$ 95.0° (Romburgh and Barger, 94.7°).

The residual cake.—The residue of the powdered seeds left after the removal of oil and hypaphorine was ashed in a platinum basin. It gave 4.2% of ash which was found to consist mainly of calcium, magnesium and potassium salts of phosphoric acid along with small quantities of chloride and some silica. The total nitrogen in the cake was 6.5% indicating that the proteins form about 40%.

Examination of the bark.—The bark offered difficulties in powdering since the inner portions consisted of tough fibre. It was therefore cut into small bits when fresh and then powdered after drying. About 7 kilograms of the bark were extracted with alcohol, each lot being boiled twice for

6 hours each time. When the extract was concentrated a large quantity of a colourless crystalline solid separated out. This consisted of mineral matter being mostly potassium chloride and carbonate. It was filtered off and alcohol evaporated as completely as possible. A voluminous dark brown semi-solid was left behind. This was extracted with about 50 c.c. of hot water and filtered. The brown aqueous extract gave good tests for alkaloids. A second extraction using about 20 c.c. of water removed a further small quantity of the basic substance, the residual resin retaining only insignificant amounts. This solution gave all the tests for the presence of hypaphorine.

Addition of concentrated nitric acid to the aqueous extract precipitated only resinous matter and no crystalline solid was produced. On the other hand, some decomposition with evolution of gas was noticed. With a view to isolate the base as the hydrochloride another portion of the aqueous extract was acidified with hydrochloric acid, the precipitated resin filtered off and the solution concentrated in a vacuum desiccator. The residue was found to be contaminated with a large amount of mineral matter and could not be purified.

The water-soluble basic portion was therefore treated with 10% aqueous potash and distilled till about half the volume of the liquid came over. The distillate which was strongly alkaline gave the requisite tests for indole whereas the original aqueous extract did not give the tests. When the distillate was rendered acid with hydrochloric acid, extracted with ether repeatedly to remove non-basic matter, concentrated to a small bulk and treated with a solution of platinic chloride, a yellow crystalline chloroplatinate was obtained. It melted almost completely between 230–35° though a small amount was left behind and it decomposed much higher. When boiled with a large volume of alcohol the insoluble residue melted at 236–38° and was found to be identical with the chloroplatinate of trimethylamine whereas the small portion that went into solution in alcohol was identified as the chloroplatinate of ammonia. The basic volatile matter therefore consisted mostly of trimethylamine along with a small amount of ammonia. It was therefore concluded that hypaphorine was present in the bark. This was supported by the colour reactions given by the aqueous extract mentioned above. The only other substance that can give these reactions is tryptophane. But this substance does not undergo decomposition under the conditions described above. The small amount of ammonia detected probably arose from an accompanying impurity. The bark gave about 15.5% of ash and the alcohol extracted a good portion of the mineral matter.

The leaves.—About 4 kilograms of the dry leaves were powdered, extracted with alcohol and the water-soluble portion of this extract examined as in the case of the bark. No sparingly soluble nitrate could be isolated though the extract gave prominent reactions for hypaphorine. It contained much more of impurities giving rise to ammonia on decomposition with alkali so that it was difficult to isolate trimethylamine chloroplatinate. Indole was, however, produced as one of the decomposition products thereby confirming the presence of hypaphorine.

Summary.

From the seeds of *Erythrina indica* a fixed oil and a water-soluble alkaloid have been isolated. The properties of the oil have been compared with those of the oil of the Formosan variety and found to differ in the degree of unsaturation. The alkaloid has been identified as hypaphorine. The bark contains, besides a large amount of mineral matter and resin, the same base which is also present in the leaves to a smaller extent.

REFERENCES.

1. *A dictionary of the economic products of India*, by G. Watt, 3, 269.
2. R. N. Chopra, S. Ghosh and B. N. Sen, *Ind. J. Med. Research*, 1934, 22, 265.
3. S. Chakravarti, M. L. Sitaraman and A. Venkatasubban, *J. Annamalai University*, 2, 238-42.
4. K. Kafuku and C. Hata, *C. Abs.*, 1934, 5266.
5. Greshoff, *Ber. Pharm. Ges.*, 1899, 9, 214.
6. P. Von Romburgh and G. Barger, *J.C.S.*, 1911, 2068.
7. J. Maranon and J. K. Santos, *Philippine J. Sci.*, 1932, 48, 563-80.
8. K. Folkers and R. T. Major, *J.A.C.S.*, 1937, 1580.