CHEMICAL EXAMINATION OF PLANT INSECTICIDES

Part I. Chemical Components of *Derris feruginea*

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There are about 25 species of Derris indigenous to India. Of these *D. ferruginea* belongs to the same sub-group as *D. elliptica* and bears a close resemblance to it[1]; careful examination is necessary to distinguish one from the other. *D. ferruginea* occurs in the tropical zone of the Eastern Himalayas, particularly in Assam.

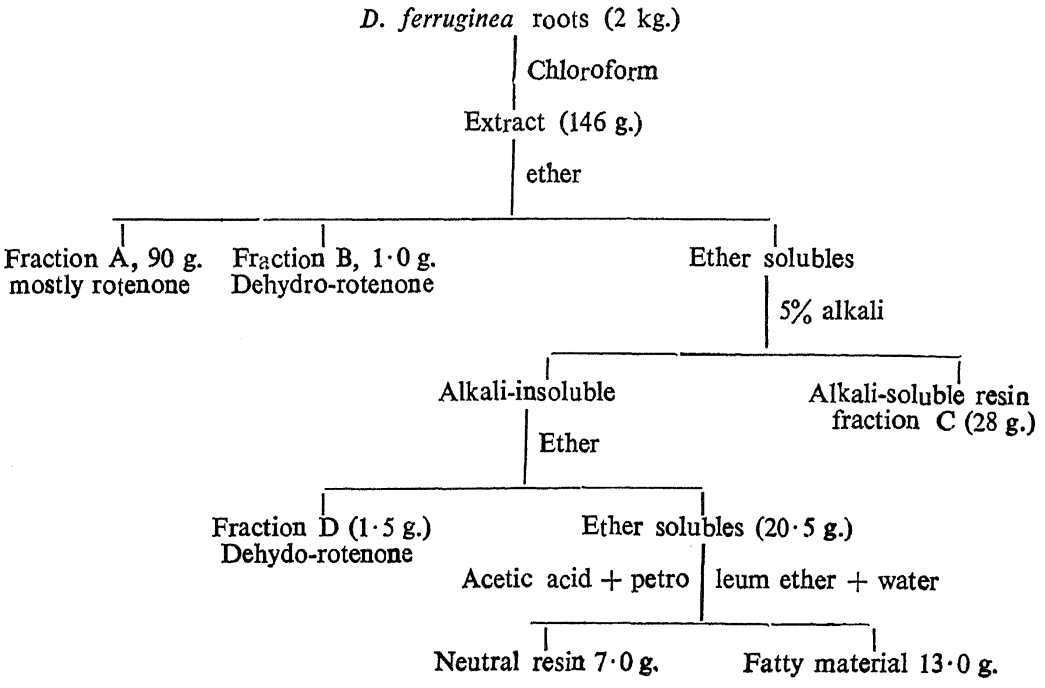
While making a general survey of the rotenone-bearing plants of India, Krishna and Ghose[2] noted that a species of derris from Assam contained 0·74% of rotenone. It was first mistaken to be *D. elliptica*[2] but later was correctly identified as *D. ferruginea*.[3] In a subsequent publication[4] these authors reported that the rotenone content of *D. ferruginea* varied from 0·74 to 2·4% while the ether extractives ranged from 2·5 to 4·5% and also mentioned that a sample of *D. ferruginea* grown by the Silviculturist, Assam, was found to contain as much as 6·1% of ether extractives but no rotenone. Thus there appeared to be two varieties of this plant, one of which was rotenone-bearing and the other rotenone-free. No detailed chemical investigation of these has been done so far.

Before undertaking a detailed examination of the roots of *D. ferruginea* and other plant materials, we have found it highly advantageous to carry out the Durham test on cut-sections of the samples. Durham himself first adopted this simple procedure to characterise plants as rotenone-bearing or rotenone-free. Later Worsley[5] applied this test for studying the distribution of rotenone in the plant cells. During the earlier days of work on derris, rotenone alone was considered to give this test, but subsequently a number of other compounds closely related to rotenone have been found to respond to it. A positive Durham test, therefore, should indicate the presence of rotenoids in the plant. It may here be pointed out that rotenoids like tephrosin, isotephrosin and dehydro-rotenone do not exhibit the series of colour changes in this reaction as given by the others. According to the generally accepted view, however, the tephrosins do not occur in the plant

as such, but are formed from deguelin by oxidation during the process of extraction.[6] Similar may be the case with the dehydro-compounds. Hence in testing the plant material directly, absence of the sequence of colours (red to blue or bluish green) in the Durham test may be taken as evidence of the plant being of the non-rotenoid type. This is supported by our experience of the detailed chemical investigation of a number of cases. The two varieties of *D. ferruginea* could be easily distinguished by this simple procedure.

D. ferruginea is of special interest in India, as it is the only indigenous species of derris so far known to contain a fairly good percentage of rotenone. Consequently samples obtained from Assam were subjected to detailed chemical examination. For extraction and separation of the components we have used in general the procedure adopted by Goodhue and Haller[7] for the study of the roots of *Tephrosia virginiana.* Chloroform is employed for the initial extraction of the root and ether and 5% sodium hydroxide for separation into fractions. The use of chloroform instead of ether renders the initial extraction quicker and minimises loss of solvent in the tropics. The rotenoid-free variety of *D. ferruginea* did not give any crystalline component though the extractives amounted to 6·5%. The extracted matter was liquid in consistency and most of it was soluble in petroleum ether.

The rotenoid-bearing roots gave 7·3% of extractives as a definite and clean solid of which more than 60% consisted of rotenone. The alkali-soluble fraction amounted to 20%, but no crystalline solid could be isolated



from it. From the alkali-insoluble portion only dehydro-rotenone could be obtained; attempts to get crystalline products from the other fractions by treatment with methyl alcoholic alkali or by dehydrogenation with iodine were not successful. The roots appear therefore to contain only rotenone and dehydro-rotenone. The scheme of extraction and separation is given above.

From the results presented in this paper it appears to be possible to distinguish between *D. elliptica* and *D. ferruginea* roots from their chemical composition. The former yields a considerably higher percentage of extractives generally and whatever may be this percentage, it is reported to contain a constant value of 40% of rotenone.[8] On the other hand, *D. ferruginea* yields a low percentage of extractives and rotenone amounts to more than 60% of it. Again as the alkali-soluble portion *D. elliptica* gives usually about 10% of the resin,[8,9] whereas nearly 20% is obtained from *D. ferruginea*. Further, the neutral resin, which forms an appreciable amount (30%) of the total resin in the former species, gives deguelin and elliptone. From the neutral resin of *D. ferruginea* no crystalline component could be isolated. Thus there seem to be marked differences between the resins of the two species, though the differences are not so marked as between *D. elliptica* and *D. malaccensis*.[8,9] This, no doubt, requires confirmation by examining some more samples of *D. ferruginea* before it could be conclusive.

Experimental

D. ferruginea (*rotenoid type*).

Medium-sized roots were obtained from Assam, coarsely powdered and the powder (2 kg.) was extracted five times in the cold with chloroform (5 litres each time). The combined extracts were distilled to remove the solvent. The solvent-free residue (146 g.), obtained as a solid, was treated with ether (350 c.c.) and the sparingly soluble fraction A (90 g.) was collected. By keeping the ether solution for a few days in an ice-chest, a very small amount of fraction B (1·0 g.) was obtained.

The ether solution was rapidly extracted four times with 50 c.c. portions of 5% aqueous potash. The combined alkali extracts were shaken once with ether, acidified and extracted again with ether. The alkali solubles, fraction C (28 g.), were separately examined. On keeping overnight in an ice-chest, the alkali-insoluble ether solution slowly deposited a small quantity of a crystalline material. This solid, D (1·5 g.) was filtered off.

The filtrate was dried over anhydrous sodium sulphate and after complete removal of ether, the residue was taken up in carbon tetrachloride

(50 c.c.) and kept for a week in the ice-chest with occasional stirring and shaking. As no crystalline material separated from this, the solvent was removed under reduced pressure and the residue (20·5 g.) taken in a small volume of ether and kept in an ice-chest. No crystalline material separated even from the ether solution. Therefore ether was removed and the residue was dissolved in glacial acetic acid (50 c.c.). Petroleum ether (50 c.c.) was added, followed by 5 c.c. of water to cause the separation of the layers. The acid layer was washed with petroleum ether and the petroleum ether layer with 90 acetic acid so as to effect better separation of the components. The petroleum ether layer was finally washed with alkali, evaporated and the fatty material (13·0 g.) recovered. The neutral resin (7·0 g.) was obtained from the acetic acid solution by precipitation with water and extraction with ether. This ether solution was rendered free of acetic acid by washing with 5% alkali and then washed with water free of alkali.

The neutral resin was treated with dilute methyl alcoholic alkali (50 c.c.) and boiled for 10 minutes. As no crystalline solid separated from this solution, it was acidified and the resin recovered by ether extraction. Then it was taken in absolute alcohol (50 c.c.) and to it was added fused sodium acetate (8 g.). The solution was kept gently boiling and to it was added, in small amounts, iodine (3 g.) in alcohol (30 c.c.). After the addition was over, the solution was set aside overnight; but no crystalline material separated.

Fraction A.—It formed the major bulk of the extract and was found to respond to the rotenone colour reactions. It melted at about 163° and was fractionated using alcohol. From 10 g. of the material the following two fractions were collected:

	Yield	Melting point	Durham test	Ferric chloride colour
Fraction (i) ..	7·5 g.	163–4°	Red–blue	Nil
Fraction (ii) ..	1·8 g.	160–1°	do.	do.

The two fractions were found to be identical with rotenone by mixed melting point determinations with an authentic sample.

Fraction B.—It had an indefinite melting point and was therefore purified by washing with petroleum ether to remove fatty impurities and then by crystallising from alcohol-chloroform mixture. An yellow crystalline solid (needles), melting at 217–8°, was obtained. In the Durham test, with nitric acid it gave red colour but no blue with ammonia. It did not give any ferric chloride colouration. It was identified as dehydro-rotenone by taking a mixed melting point with dehydro-rotenone obtained by the dehydrogenation of rotenone.

Fraction C.—By treatment with methyl alcohol, it gave only a waxy solid melting at about 85°. This solid did not give any colouration with nitric acid or ammonia in the Durham test. All attempts to get a crystalline component from this fraction by treatment with various solvents were unsuccessful.

Fraction D.—This fraction also had an indefinite melting point and therefore was first purified by treatment with petroleum ether. Then it was crystallised from alcohol. The crystalline product that was sparingly soluble in alcohol was filtered. It was yellow in colour (needles) and melted at 217–8°. From its properties it was identified as dehydro-rotenone. The alcoholic solution on cooling gave a gelatinous product, which could not be induced to crystallise by keeping in an ice-chest or by treatment with other solvents like acetone. Thus no crystalline product other than dehydro-rotenone could be obtained from this fraction.

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Summary

Detailed chemical examination of the roots of *D. ferruginea* has now been carried out. Of the two varieties, the rotenone-free one is further characterised by not giving any crystalline component. The rotenone-bearing variety yields besides rotenone (4·3%), only a small amount of dehydro-rotenone. The chemical chaarcteristics of rotenone-bearing *D. ferruginea* and *D. elliptica* are compared. It seems to be possible to use chemical composition to distinguish one from the other.

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