# CHEMICAL EXAMINATION OF PLANT INSECTICIDES

## Part VI. Root Bark of *Tephrosia lanceolata* Grah.

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*Tephrosia lanceolata* Grah. is a small herbaceous plant belonging to the family Leguminosæ and has the following special characters which distinguish it from other Tephrosia species. The plant is sub-erect, with the stems coloured distinctly red when fresh. Branches are green on the under-surface and reddish brown on the upper surface. The leaflets are lanceolate and their under-face is silvery. The flowers are small with the corolla thinly silky and red. The pods are more than an inch long and contain 5–6 seeds.

Most of the other Tephrosia species which have been examined in the past have been found to be toxic to fish and to yield crystalline compounds.[1] The root bark of *T. lanceolata* (for the identification we are indebted to the Systematic Botanist, Agricultural Research Institute, Coimbatore) was therefore examined by us for possible action as a fish poison. For our experiments we employed the fresh-water fish *Haplochilus panchax* available at Waltair. It was found that when an alcoholic extract of the powder was added to water in which the fish were kept, they showed toxic symptoms and died. For the quantitative study of toxicity we have employed the 'turning point', a term introduced by Krishnaswamy and Seshadri,[2] as the criterion. The concentration and time reported in this communication have reference to the turning point as defined by these authors. Tested in this manner the root bark powder exhibited definite toxicity in 19 minutes in a concentration of 66 p.p.m. or 6 minutes in a concentration of 166 p.p.m. It was therefore subjected to detailed investigation and the results are described below.

The plants required for the study were collected from sandy tracts round about Waltair where they grow wild and in fair profusion. The material was obtained in two seasons: (1) September 1950 and (2) May and June 1951. Part of the material collected in 1950 was subjected to extraction with ether and then with chloroform, while another part was extracted directly with chloroform. The results obtained in these experiments are,

or convenience, described after the results obtained with the 1951 collection which constituted the bulk of our material.

The powdered air-dried bark (1951 sample) was extracted with chloroform in the cold and the extract was concentrated to small bulk. Since no solid separated even after standing for a number of days at a low temperature, the solvent was completely removed under reduced pressure and the residue taken up in ether. The ether solution was extracted with aqueous potash thus effecting a separation into alkali-soluble and alkali-insoluble fractions. From the ether solution representing the alkali-insoluble fraction, a pale yellow crystalline solid, "Substance I", separated spontaneously. The mother-liquor from the crystals was concentrated to a syrupy consistency and treated with benzene. "Substance II" separated at this stage in a very small yield. The filtrate from Substance II, when further diluted with benzene and kept at a low temperature, yielded a crystalline solid, which could be separated into Substance I and another entity, "Substance III". The amorphous residue recovered from the mother-liquor was toxic to fish (5 p.p.m. toxic in 22 min.).

From the alkali-soluble portion no crystalline compound could be obtained. The amorphous substance was found to be toxic to fish (66 p.p.m. toxic in 11 min.).

The marc remaining after chloroform extraction was extracted with warm alcohol, and the extract concentrated to small bulk. Since no solid separated even after standing in the ice chest for a number of days, it was concentrated further and the residue poured with stirring into a large excess of water. The precipitated resinous material was divided into alkali-soluble and alkali-insoluble fractions by maceration with dilute aqueous alkali. The alkali-soluble portion was recovered by neutralising the filtered alkaline solution. It could be divided into two portions, chloroform-soluble and chloroform-insoluble. Thus the alcohol extract was obtained in three fractions, alkali-insoluble, alkali- and chloroform-soluble, and alkali-soluble but chloroform-insoluble. All of them were resinous and could not be crystallised from organic solvents. In fish tests with these fractions purging was observed in all the three cases, but only the first and the second were toxic (12 min. in a concentration of 10 p.p.m. or 6 min. in a concentration of 60 p.p.m. in the first case, and 140 min. in a concentration of 200 p.p.m. or 24 min. in a concentration of 320 p.p.m. in the second case). Attempts to prepare crystalline acetyl derivatives or to obtain crystalline degradation products by boiling with 10% aqueous potash yielded negative results in all the three cases.

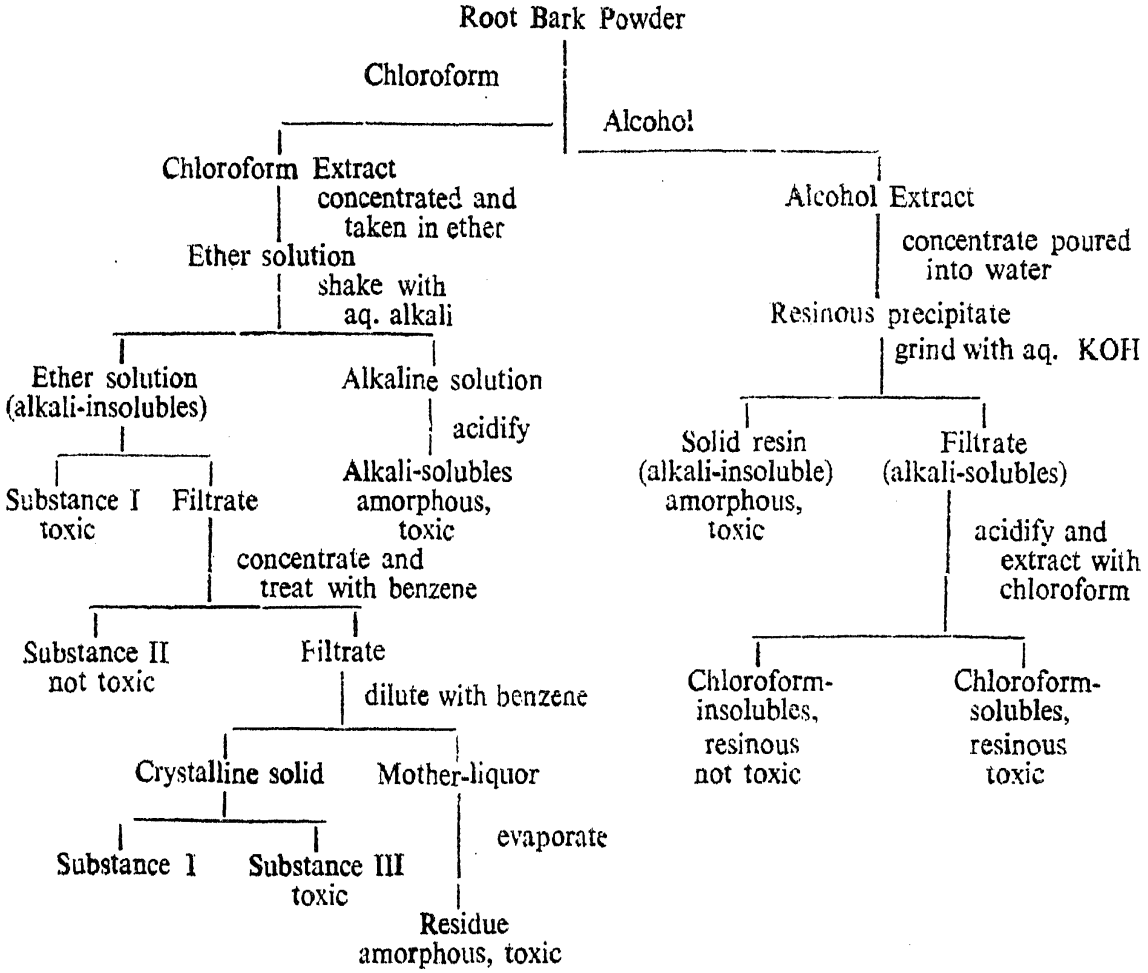
Thus from the 1951 sample three crystalline compounds, Substances I, II and III were isolated.

From the 1950 collection a 400 g. sample was extracted with chloroform and the chloroform extract examined in a manner analogous to that described under the 1951 sample. However only Substance I could be obtained as the crystalline component and it was isolated from the alkali-insoluble portion. Substances II and III could not be obtained, even though, as a result of the findings on the 1951 sample, they were specially looked for. Another 400 g. sample of the 1950 collection was extracted in stages, being first exhausted with ether before extraction with chloroform. The ether extract yielded a small amount of Substance I directly on concentration. The subsequent chloroform extract was worked up in the same manner as already described. Again only Substance I was obtained under the alkali-insoluble part and Substances II and III could not be got even after careful searching. The difference between the 1950 and 1951 samples with reference to the crystalline components is probably to be attributed to seasonal variations.

In view of the results obtained with the 1951 sample the marc of the 1950 sample remaining after the chloroform extraction was not subjected to extraction with alcohol.

Substance I (pale yellow needles from alcohol) melted at 187–88° and had the probable formula $C_{22}H_{22}O_4$. It was toxic to fish (17 min. in a concentration of 33 p.p.m.). Substance II (nodules), even after repeated recrystallisation, did not have a sharp melting point, 135–42°. It was not toxic to fish. In view of the very small amount available for study and the unsatisfactory nature of its purity it has not been analysed. Substance III (colourless woolly needles from alcohol) melted (with evolution of water vapour) at about 122°, then solidified and melted again at 147–49°, probable formula $C_{23}H_{14}O_4$, $2H_2O$. The anhydrous substance melted directly at 147–49° without previous sintering. It was much less toxic to fish than Substance I (12 hours in a concentration of 33 p.p.m.; the toxicity test could not be performed with higher concentrations because of the very low solubility of the substance in water). All the three substances seem to be new. The results of detailed examination of Substances I and III will be described in a future communication.

The course of the extraction and isolation of the different fractions is schematically represented below:




## Experimental

3·8 Kg. of the powdered air-dried root bark (1951 collection) was extracted with chloroform by maceration in the cold (5 times—each time 15 litres). The marc was then spread out on a tray to remove remnants of chloroform and extracted with methylated spirit in a metal Soxhlet extractor (only 1·5 kg. of the marc was employed for this purpose) until the extraction was complete. The extracted powder was discarded.

*Chloroform Extract.*—The combined chloroform extracts were concentrated to 500 c.c. and the concentrate left in the ice-chest for 15 days. No solid separated out. The solvent was removed completely under reduced pressure and the residue taken up in 1 litre of ether and left in the ice-chest. A small amount of resin sticking to the sides was removed after 2 days by decantation. The clear ether solution was rapidly extracted with 5% aqueous potassium hydroxide (4 × 100 c.c.) and then washed with water till neutral. Even during the washing with water a crystalline solid began to separate out. It was filtered after 24 hours and washed with ether (crude Substance I, yield 4·6 g.). The filtrate was dried over sodium sulphate, concentrated to 300 c.c. and left in the ice-chest. No solid separated even after 15 days. The solution was concentrated to a syrupy consistency and treated with

benzene (200 c.c.). A small quantity of solid that separated immediately was filtered off (impure Substance II, yield 0·1 g.). The filtrate was diluted with more benzene (200 c.c.) and left in the ice-chest for 10 days. The solid that had separated was filtered, washed with benzene and dried (impure mixture of Substances I and III, 4 g.). The semi-solid residue obtained by evaporation of the mother-liquor amounted to 200 g.

The alkaline solution was neutralised with 1 : 1 hydrochloric acid and the solution extracted with ether (5 × 100 c.c.). The ether solutions were washed neutral with water, dried over sodium sulphate, concentrated to 200 c.c. and left in the ice-chest for a month. No solid separated even then. It was next concentrated to a syrupy consistency and treated with benzene (100 c.c.) and left in the ice-chest. Even then no crystalline material could be obtained. Semi-solid residue obtained by evaporation of the solution weighed 45 g.

*Substance I.*—The impure substance after repeated crystallisation from rectified spirit yielded pure Substance I as pale yellow needles melting at 187–88° (3 g.). It was sparingly soluble in ether, moderately soluble in hot alcohol and readily soluble in chloroform and benzene. It did not give any colour with ferric chloride. With conc. sulphuric acid a yellow solution was obtained. In the Durham test it turned deep orange with nitric acid, but did not develop a blue colour with ammonia. It did not give any colour with acetic anhydride and sulphuric acid in chloroform solution (Liebermann-Burchard reaction) or on reduction with magnesium and hydrochloric acid (Found: C, 75·41; H, 6·40%; $C_{22}H_{22}O_4$ requires C, 75·43; H, 6·29%).

*Substance* II.—This was sparingly soluble in most of the organic solvents (ether, chloroform, acetone). It was moderately soluble in hot ethyl alcohol from which it was crystallised. Even after repeated crystallisations it could be obtained only as nodules and the melting point was not sharp, 135–42°. In the Durham test no colour was observed. Conc. sulphuric acid did not give any colour nor did reduction with magnesium and hydrochloric acid.

*Substance III.*—The impure mixture of I and III already referred to was first crystallised a few times from benzene-alcohol (1 : 1) whereby a sample melting indefinitely at 125–32° was obtained. This was then fractionally crystallised from rectified spirit. The less soluble fraction consisted of pale yellow needles melting at 187–88° (Substance I), while the more soluble consisted of colourless woolly needles melting (with evolution of water vapour) at about 122°, then solidifying and melting again at 147–49° (Substance III, yield 2 g.). Substance III was readily soluble in acetone, chloroform, warm benzene and hot alcohol. It did not give a colour with

ferric chloride or sulphuric acid or nitric acid. Reduction with magnesium and hydrochloric acid yielded only a pale yellow colour but not red. After drying in vacuum at 70° for one hour the substance melted at 147–49° without any previous sintering (Found: loss on drying at 110° *in vacuo*, 10·29%; $C_{23}H_{14}O_4$, $2H_2O$ requires loss on drying 9·23%. Found on the anhydrous sample: C, 77·91; H, 3·95%; $C_{23}H_{14}O_4$ requires C, 77·96, H, 3·96%.

*Alcohol Extract* (from 1·5 kg. of marc.).—The alcoholic extract in the Soxhlet extractor was concentrated to 500 c.c. and left in the ice-chest for 15 days. No solid separated. It was concentrated further to 100 c.c. and poured with stirring into 1 litre of water. The precipitate was allowed to settle overnight and the supernatant liquid was decanted off. The resinous solid was extracted with 4 × 50 c.c. of 2% aq. potassium hydroxide. The alkali-insoluble part was washed free from alkali and purified further by redissolving in alcohol and reprecipitation by pouring the solution into water (yield, 2 g. of reddish brown resin).

The alkaline solution was shaken with ether and the residue from the ether solution added to the alkali-insolubles. The aqueous layer was then neutralised with 1 : 1 hydrochloric acid, whereupon a certain amount of precipitate separated. The entire mixture was extracted with 5 × 100 c.c. of chloroform. A good amount of the material could not be extracted with chloroform and this remained as a layer floating in the aqueous phase. The chloroform solutions were collected, washed neutral, dried over sodium sulphate, concentrated to 50 c.c. and left in the ice-chest. No solid separated even after a number of days. The solution was poured into 500 c.c. of petroleum ether. The precipitate obtained was purified by re-solution in chloroform and reprecipitation by dilution with petroleum ether. The filtered and dried solid (resinous) amounted to 2·3 g.

The chloroform-insoluble portion which floated in the aqueous phase (see above para) was separated by filtration, washed free from acid and dried (4 g., resinous).

As already mentioned in the introduction, neither the alkali-insoluble part of the alcohol extract, nor the chloroform-soluble part of the corresponding alkali-solubles nor the chloroform-insoluble part of the alkali-solubles could be crystallised from organic solvents. For toxicity tests with these fractions (results described in the theoretical part), 10 c.c. of a 5% solution of gelatin per 500 c.c. of water was employed as stabiliser. The acetylation and hydrolysis with aq. potash were carried out on the following lines.

250 mg. of the resin was refluxed with 1 g. of fused sodium acetate and 5 c.c. of acetic anhydride for 4 hours on an oil-bath maintained at 140°. The mixture was cooled, poured into 200 c.c. of water with stirring and left overnight. The precipitated material was filtered and washed with water. It could not be crystallised from any solvents.

For alkaline hydrolysis 1 g. of the resin was boiled under reflux with 80 c.c. of 10% aq. potash for one hour. After cooling, the solution was neutralised with 1 : 1 hydrochloric acid and the mixture containing some precipitate was repeatedly extracted with ether. A small amount of material remained insoluble in ether in every case. It was filtered, washed and dried and examined separately. From the ether-soluble part of the fission products acidic and phenolic fractions were separated by shaking the ether solution with 5% aq. sodium carbonate and 5% aq. sodium hydroxide respectively. The remaining ether solution was washed with water, dried over sodium sulphate and evaporated to give the neutral products. The sodium carbonate and sodium hydroxide solutions were separately acidified and extracted with ether and the ether solutions evaporated to give the acidic and phenolic fractions. Crystalline compounds could not be obtained in any one of these experiments.

## SUMMARY

The isolation of three crystalline substances from the chloroform extract of the root bark of *Tephrosia lanceolata* Grah. is described. All of them seem to be new. These substances, as also certain amorphous fractions of the chloroform extract and the subsequent alcoholic extract have been studied for their toxicity towards fish and the results are recorded. Attempts to prepare crystalline acetyl derivatives or to obtain crystalline degradation products by alkaline hydrolysis of the different fractions of the alcoholic extract have yielded negative results.

## REFERENCES

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