CHEMICAL EXAMINATION OF PLANT INSECTICIDES

Part VII. Further Study of Some Amorphous Fractions from the Root-Bark of *Tephrosia lanceolata* Grah.

By S. Rangaswami, F.A.Sc. and B. V. Rama Sastry

(*From the Department of Pharmacy, Andhra University, Waltair*)

Received January 27, 1953

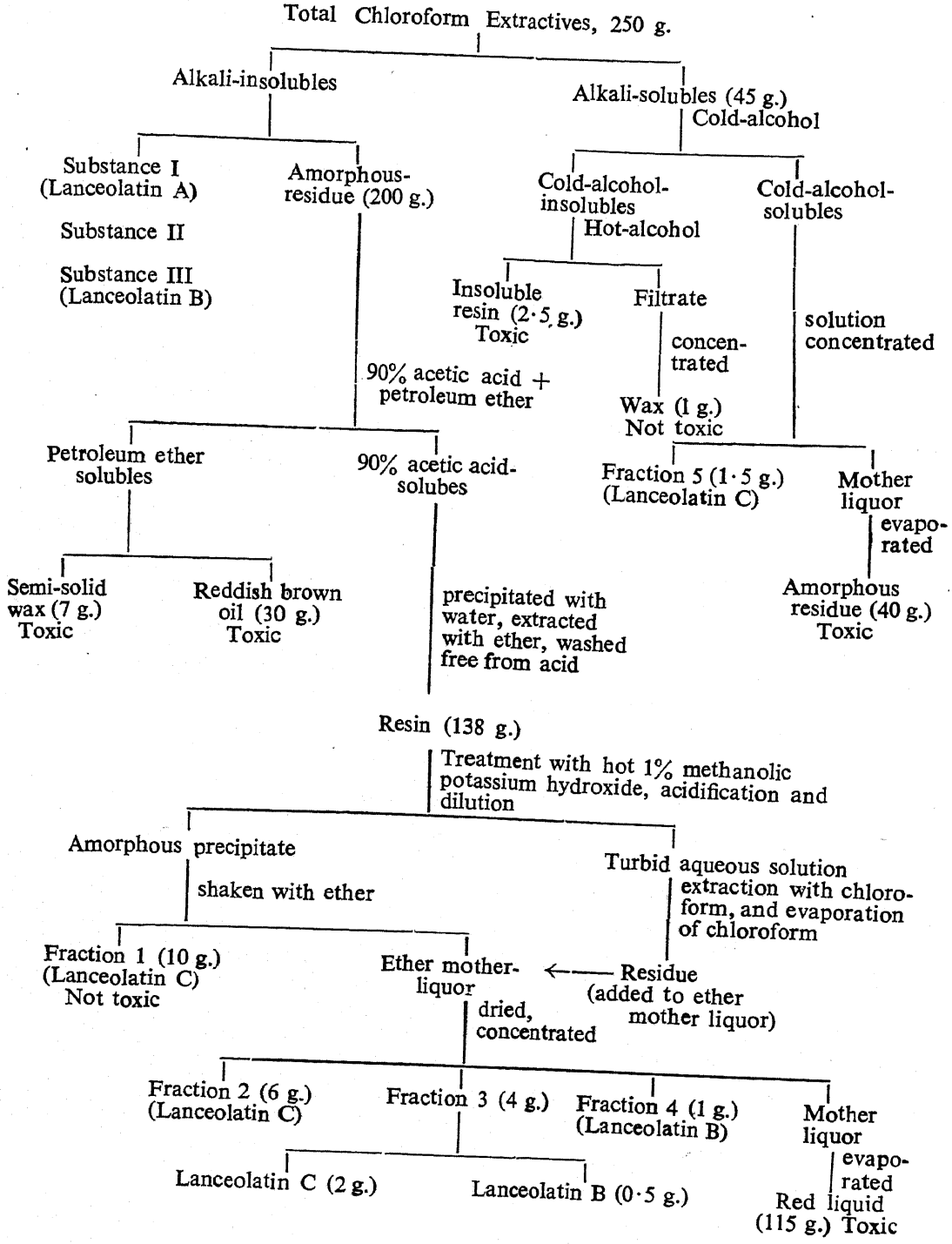
The powerful insecticidal property of rotenone led workers to believe at first that the insecticidal action of Derris root itself must be attributed to its rotenone content.[1] However, it was observed by later workers that extracts of Derris root poor in rotenone content or not having any rotenone at all could still be very active as insecticides.[2] It was further noticed that the amorphous fraction remaining after the removal of rotenone from various extracts exhibited high toxicity to fish and insects. A detailed study of these amorphous fractions resulted in the isolation of a number of crystalline substances closely related to rotenone like tephrosin, deguelin, toxicarol, malaccol and elliptone.[3] For the isolation of these, treatment of the extract with alkali was found to be essential, sometimes ordinary temperature being sufficient and sometimes higher temperatures being necessary. These substances were invariably obtained in an optically inactive form. It is now believed that they exist not as such in the resin but in the form of precursors or in some other unknown form of combination broken down by alkaline treatment yielding crystalline products.

In Part VI of this series[4] the isolation of three crystalline compounds (named then as Substances I, II and III) and some amorphous fractions from the chloroform and alcoholic extractives of the root-bark of *Tephrosia lanceolata* was described. As stated therein many of the amorphous fractions were toxic to fish. They have therefore been subjected to further examination now.

In view of the fact that Substances I and III have been met with in our laboratories not only in other parts of *T. lanceolata* but also in some other *Tephrosia* species it is now considered that it would be more convenient to give them more specific names. Consequently the names Lanceolatin A and Lanceolatin B are proposed for Substances I and III respectively of the previous communication; a name is not considered necessary for Substance II as it does not seem to occur widely.

The amorphous material remaining after the separation of Substances I (Lanceolatin A), II and III (Lanceolatin B) from the alkali-insolubles of the chloroform extractives was partitioned between 90% acetic acid and petroleum ether, according to the procedure described by Goodhue and

TABLE



Haller[5] in their study of the amorphous components of *Tephrosia virginiana* roots. The petroleum ether-soluble fraction could be separated into a waxy semi-solid and a thick reddish brown oil both of which exhibited toxicity towards fish. The 90% acetic acid-soluble fraction, on treatment with boiling methanolic potash, yielded two crystalline substances. One of them melted at 129°, had a composition corresponding to the formula $C_{24}H_{20}O_5$ and was non-toxic to fish. It is therefore considered to be a new substance and is designated as Lanceolatin C. The other crystalline substance proved to be identical with Lanceolatin B, *i.e.*, Substance III of the earlier communication. During this investigation it was observed that the substance could crystallise either in the hydrated form or in the anhydrous form depending upon the conditions employed, and that the hydrated form could lose its water slowly even on prolonged storage. The final mother liquor remaining after the removal of these substances yielded a reddish oil considerably toxic to fish.

The alkali-soluble part of the chloroform extractives of *Tephrosia lanceolata* was described in the earlier communication as amorphous. It has now been found that it could be divided into a cold alcohol-insoluble and a cold alcohol-soluble part. By treatment with boiling alcohol the former could be further divided into a more soluble waxy part which was non-toxic to fish and a less soluble resinous part which was toxic to fish. The cold alcohol-soluble part yielded on concentration a small crystalline fraction which proved to be identical with Lanceolatin C; the bulk of it, however, was amorphous and toxic to fish.

The course of the separations is shown in the above table.

Experimental

The separation of the total chloroform extractives of the root-bark of *Tephrosia lanceolata* into alkali-insolubles and alkali-solubles making use of 5% aqueous potassium hydroxide and the isolation of Substances I (renamed Lanceolatin A), II and III (renamed Lanceolatin B) from the former have been described in Part VI.

Alkali-insolubles.—The amorphous toxic residue recovered from the mother liquors after separation of Substance I (Lanceolatin A), Substance II and Substance III (Lanceolatin B) was taken up in 300 c.c. of glacial acetic acid and treated with 300 c.c. of petroleum ether, followed by 30 c.c. of water. The mixture was thoroughly shaken, and the layers were allowed to separate. The lower acetic acid layer was shaken with 3×50 c.c. of petroleum ether contained in a 2nd, 3rd and 4th separating funnel respectively. The petroleum ether solution in the first separator was washed with 3 further

quantities of 90% acetic acid, 50 c.c. each, and each of these washings passed in succession through the 2nd, 3rd and 4th separators containing petroleum ether as mentioned above. The four petroleum ether solutions and the four acetic acid solutions were separately united and treated further as described below.

The petroleum ether solution was washed with dilute alkali to remove acetic acid, then with very dilute hydrochloric acid to remove alkali and finally with water until free from acid, dried over anhydrous sodium sulphate, concentrated to 100 c.c. and left in the ice chest. A waxy semi-solid, reddish brown in colour, separated. The supernatent liquid was decanted off and the residue washed with petroleum ether by maceration and decantation of the supernatent liquid. The residual semi-solid could not be crystallised from any solvent. The solvent-free material (7 g., 0·18%) was toxic to fish (50 p.p.m. in 16 minutes; 100 p.p.m. in 10 minutes). Evaporation of the combined petroleum ether mother liquor and washings under vacuum left behind 30 g. (0·79%) of a thick reddish brown oil which was toxic to fish (20 p.p.m. in 42 minutes; 50 p.p.m. in 10 minutes; 200 p.p.m. in 5 minutes).

The 90% acetic acid-soluble fraction was recovered by dilution with water (1,000 c.c.) and extraction with ether (3 × 200 c.c.). The combined ether solution was extracted repeatedly with alkali until the acidity due to acetic acid was completely removed, then with 2% hydrochloric acid and finally with water till neutral. It was then dried over anhydrous sodium sulphate, filtered and concentrated to 200 c.c., the remaining ether being removed under suction.

The resulting neutral resin (138 g.; 2·8%) was dissolved in warm methyl alcohol (270 c.c.) and treated with 10% aqueous potassium hydroxide (30 c.c.). No solid separated. The solution was boiled under reflux for 15 minutes, cooled and left in the ice chest overnight. Still no solid separated. The alkali was neutralised with 1: 1 hydrochloric acid and the solution diluted with water (500 c.c.). An amorphous solid settled down. The turbid supernatent liquid was decanted into another flask, and the solid was washed with water by grinding and decantation of the aqueous liquid. The amorphous solid was then shaken with 500 c.c. of ether whereby the whole of the substance went into solution but immediately a yellow crystalline substance (Fraction 1) was deposited in the form of irregular plates and prisms. The substance was filtered off and thoroughly washed with ether. The ethereal filtrate was treated further as described below.

The turbid aqueous supernatent liquid and washings mentioned above were extracted with chloroform; the chloroform solution was washed free

from acid, dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure. The residue was added to the ethereal filtrate mentioned at the end of the last para.

The ether mother liquor from Fraction 1 was dried over anhydrous sodium sulphate, filtered and concentrated to 200 c.c. On keeping in the ice chest for fifteen days a yellow solid (Fraction 2) was deposited. The mother liquor from Fraction 2 deposited two more fractions (Fractions 3 and 4) at intervals of 10 days. No further crystalline fraction was obtained from the mother liquor even after keeping in the ice chest for a month. The residue obtained by evaporation of the solvent under vacuum was a red liquid miscible with alcohol, acetone and benzene. It was highly toxic to fish (50 p.p.m. in 11 minutes; 20 p.p.m. in 20 minutes).

Alkali-solubles.—The semi-solid (45 g.) representing the alkali-soluble fraction of the chloroform extract (*see* Part VI) was treated with 200 c.c. of methylated spirit. Most of it went into solution but a small quantity of a wax-like solid was found to remain undissolved. It was filtered and the filtrate was concentrated to 125 c.c. and left in the ice chest for 10 days, whereupon a yellow coloured crystalline fraction was deposited (Fraction 5). It was filtered. No more crystalline material could be obtained from the filtrate by further concentration and leaving in the ice chest for one month. The red semi-solid residue (40 g.) obtained by evaporating the solvent under vacuum was toxic to fish (34 p.p.m. in 17 minutes; 86 p.p.m. in 10 minutes; 171 p.p.m. in 6 minutes).

The wax-like solid mentioned above was boiled with methylated spirit (100 c.c.). A dark resinous solid remained insoluble. The alcohol solution was decanted off and the insoluble residue treated with further quantities of boiling methylated spirit (3 × 50 c.c.) and the solution was decanted off each time. The combined warm alcohol solution was filtered, concentrated to 100 c.c. and kept in the ice chest. The wax that was deposited was filtered and dried. The residue obtained by complete evaporation of the filtrate was insignificant in amount and was not examined further. The wax (dry weight 1 g.) melted at 75 to 84° C.; it did not answer Durham test and was not toxic to fish. (The fish lived for 24 hours in a concentration of 67 p.p.m. Tests with higher concentrations could not be carried out as the wax separated out even though gelatin was added as a stabiliser). No crystalline material could be obtained by taking the hot alcohol-insoluble reddish-brown resin in any other solvent. The solid amounting to 2·5 g. was toxic to fish, 50 p.p.m. in 43 minutes. (The initial solution was prepared with acetone; the acetone concentration in the final aqueous solution was below 0·1%).

Fraction 1. (Yield, 10 g.).—After crystallisation from methanol-acetone thick hexagonal plates, pale orange in colour and melting at 125–27°. On further crystallisation first from acetone and then from ethyl acetate, m.p 129°. This is referred to as Lanceolatin C.

Fraction 2. (Yield, 6 g.).—After two crystallisations from methanol-acetone and ethyl acetate it melted at 129° and was identical with Lanceolatin C.

Fraction 3. (Yield, 4 g.).—This was fractionally crystallised from methanol. Lanceolatin C crystallised out as the first crop. On concentration of the mother liquor and keeping in the ice chest another substance separated out in the form of pale yellow needles. After further crystallisation from benzene it was found to be identical with the substance comprising Fraction 4.

Fraction 4. (Yield, 1 g.).—On crystallisation from ethyl acetate and then from methylated spirit almost colourless stout needles were obtained. Colour reactions same as described under Substance III in Part VI. The m.p. of the present sample after drying in vacuum at 100° for 2 hours (*viz.*, 148–49°) was undepressed by admixture with a similarly dried sample of Substance III described in Part VI and now called Lanceolatin B (Found on sample dried in vacuum: C, 78·18; H, 4·59; $C_{23}H_{14}O_4$ requires C, 77·96; H, 3·96%).

Fraction 5. (Yield, 1·5 g.)—Crystallised from methanol-acetone it melted at 126–27°. On recrystallisation from methanol-chloroform it was obtained as yellow plates, m.p. 129°. The substance was identical with Lanceolatin C (mixed m.p. and colour reactions).

Lanceolatin C.—It crystallised from ethyl acetate as layers of pale orange thick hexagonal plates melting at 129°. It was sparingly soluble in ethyl and methyl alcohols, moderately soluble in ether, soluble in warm benzene and carbon tetrachloride and freely soluble in acetone and chloroform. In chloroform solution it was optically inactive. It was insoluble in aqueous potassium hydroxide but soluble in alcoholic potassium hydroxide. With alcoholic ferric chloride it gave a red colour. It dissolved in concentrated sulphuric acid forming a yellow solution. It gave a red colour in the Liebermann-Burchard reaction and a pale yellow colour on reduction with magnesium and hydrochloric acid. It did not answer Durham test and Gross-Smith-Goodhue test. The substance was not toxic to fish even after 24 hours in a concentration of 167 p.p.m. (Acetone was used to prepare the solution. The acetone concentration in the final test solution was slightly below 0·1%; in this concentration the solvent has no adverse effect on the fish). Owing to the sparing solubility of the substance the

toxicity test could not be conducted with higher concentrations (Found: C, 73·96; H, 5·24; $C_{24}H_{20}O_5$ requires C, 74·23; H, 5·15%).

Summary

The results obtained by a more detailed study of some of the amorphous fractions described in the previous communication on the root bark of *Tephrosia lanceolata* are herein recorded. Treatment of the amorphous residues after separation of Substance I (renamed Lanceolatin A), Substance II and Substance III (renamed Lanceolatin B) with methanolic potassium hydroxide has enabled the isolation of another crystalline substance, now named Lanceolatin C and further quantities of Lanceolatin B. Lanceolatin C is biologically inactive (fish test). The alkali-soluble portion of the chloroform extract of the root bark was also found to contain a small amount of Lanceolatin C. Several other active and inactive amorphous fractions are also described.

References

1. LaForge and Smith .. *J.A.C.S.*, 1929, **51**, 2574.
2. Nicolas Giovambattista .. *Rev. facultad Cienc. Quim.*, 1942, **17**, 83; *Chem. Abs.*, 1944, **38**, 3409.
   Arturo Mutinelli .. *Rev. Argentina agron.*, 1945, **12**, 291; *Chem. Abs.*, 1946, **40**, 4170.
3. Clark .. *Science*, 1930, **71**, 396; *J.A.C.S.*, 1930, **52**, 2461; *Ibid.*, 1931, **53**, 729.
   Cahn and Boam .. *J. Soc. Chem. Ind.*, 1935, **54**, 37 T.
   Buckley .. *Ibid.*, 1935, **54**, 285 T.
   Harper .. *J.C.S.*, 1940, 309.
4. Rangaswami and Sastry *Proc. Ind. Acad. Sci.*, 1952, **35A**, 166.
5. Goodhue and Haller .. *J.A.C.S.*, 1940, **62**, 2520.