

CHEMICAL EXAMINATION OF PLANT INSECTICIDES

Part II. Chemical Components of *Derris scandens*

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Derris scandens is a climbing shrub occurring in Eastern Himalayas, Bengal, the Central Provinces and Madras Presidency. It belongs to the sub-section Brachypterum of the genus *Derris*, to which *D. robusta* also belongs. It is distributed also in Ceylon, Siam, Australia and other warmer areas. The Ceylonese plant was reported to be of no insecticidal value, as it did not yield any ether extract.¹

Krishna and Ghose,² while making a survey of the rotenone-containing plant insecticides of India, examined the roots of *D. scandens* obtained from Chanda District (Central Provinces) and reported that they contained no rotenone. However, they mentioned that two crystalline substances, one melting at 185° and the other at 225°, were obtained from the ether extract. Our attention was drawn to this and as we had isolated from certain *Tephrosia* species compounds having similar melting points, we arranged to procure samples of *D. scandens* root from the Chanda District with a view to examine them. Due to war conditions and the shifting of the Andhra University from Waltair, the progress of work was very slow. Meanwhile the publication of Clark³ on 'Scandenin—a constituent of *D. scandens*' reached us towards the middle of the year 1944.

Clark examined *D. scandens* cultivated in a nursery at St. Petersburg, U.S.A., primarily to know whether it contained rotenone or not. By exhaustive extraction of the roots with ether, he obtained a mixture of crystalline substances none of which was identical with rotenone.³ The main component was a new compound melting at 231° and having the molecular formula, C₂₆H₂₆O₆. It was designated by him 'Scandenin'. A second substance which was also in appreciable amounts and gave little trouble in separation was lonchocarpic acid, previously found in *Lonchocarpus* species by Jones.⁴ Clark identified it by comparison with an authentic sample of lonchocarpic acid. The sample obtained from *D. scandens* sintered between 200° and 205° and melted at 223°. A third substance

melting at 190° was obtained only in small quantities and that too after elaborate purification. It was considered to be robustic acid⁵ mainly from its behaviour in the Durham test.

Though *D. scandens* grows in several parts of India, the root samples were obtained from the South Chanda Division of the Central Provinces for the reason already mentioned and a detailed examination was carried out. During the earlier part of this investigation, the roots were first exhausted with ether and then with chloroform. By concentrating the extracts in stages several fractions were obtained and studied in detail. It was easy to get a definite top fraction melting at $228-31^{\circ}$ and this seemed to be a definite chemical entity. This was designated D.S. I. Other fractions had indefinite melting points, and their behaviour indicated that they were composed of more than one entity. In order to obtain information useful for guiding the separation of the individual substances, chromatographic adsorption analysis was done using a small quantity of one of the fractions. Alumina was used as the adsorbent and benzene as the solvent. For elution chloroform-benzene mixture was employed and the various fractions were crystallised from benzene or alcohol. As a result of this study, it was clear that an individual substance melting at $217-18^{\circ}$ was present. This was marked D.S. II. Since yields suffered markedly in the adsorption analysis, methods of fractional crystallisation were explored for getting this substance in quantity and they were successful.

In later experiments the root powder was directly extracted with chloroform, ether being omitted. By this means all the extractives were obtained in one lot (7.0% yield) and the experimental work and loss of solvents minimised. The extract was separated into fractions using first ether and subsequently other solvents. Here again it was comparatively easy to separate D.S. I, melting at $228-31^{\circ}$ by virtue of its sparing solubility and its being the main crystalline component of the roots. D.S. II could also be obtained as the result of fractional crystallisation. Certain of the fractions consisted of mixtures which were difficult to separate by this procedure. They could, however, be dealt with by means of acetylation and fractional crystallisation of the acetyl derivatives. The sharp melting acetates were then hydrolysed. By this means a third compound melting at 196° was isolated and this was marked D.S. III. Thus the presence of three crystalline entities in the root could be established.

D.S. I exhibited a marked tendency to crystallise and came out as colourless stout rectangular prisms and rods. When crystallised from ordinary organic solvents its melting point was only $230-31^{\circ}$ but it could be

obtained purer with a higher melting point, 233–34° by conversion into its potassium salt and regeneration. The substance behaved as a weak acid and formed a sparingly soluble potassium salt which readily separated from the potash solution as silky crystals.

It was found to be non-rotenoid in character according to the Durham test and it gave no ferric chloride colour, though soluble in alkali. It did not reduce Fehling's solution nor did it respond to the magnesium-hydrochloric acid reduction test of flavones or the colour reactions of resinols and sterols. The characteristic blue colour given by the methylene-dioxy group was not obtained when the substance was warmed with gallic acid and concentrated sulphuric acid. Thus it could not be placed under any known group of compounds.

It had the molecular formula $C_{26}H_{26}O_6$ and formed a diacetate melting at 160–61°. On methylation it gave a dimethyl ether melting at 128–29°. By a comparison with the data reported by Clark,³ it could be identified as scandenin.

D.S. II had a crystal structure similar to that of scandenin and was soluble in alkali. Further, it did not give any colour with ferric chloride and did not respond to the Durham test. Like scandenin it contained one methoxyl; but it definitely differed from scandenin in being more soluble in alcohol, and in not yielding a sparingly soluble potassium salt. Its melting point was 217–18° and this was considerably depressed by admixture with scandenin. The composition too was different and agreed with the formula, $C_{26}H_{26}O_5$. On acetylation it gave a diacetate melting at 153–54° and on methylation a monomethyl ether melting at 116–17°. Its possible identity with lonchocarpic acid was examined in view of the findings of Clark relating to the American sample of *D. scandens*. For comparison an authentic sample of this acid was obtained from the Bureau of Entomology and Plant Quarantine, Washington, D.C. Besides the difference noted in Table I, the mixed melting point with lonchocarpic acid was depressed. Though the acetates had the same melting points, the mixture melted lower. Thus *D.S. II* seemed to be a new substance and was named 'Nallanin' derived from the local name of the plant 'Nalla thiga'.

D.S. III resembled *D.S. II* (nallanin) closely in several properties, but its melting point was lower (202°) and mixed melting points with scandenin and nallanin and also lonchocarpic acid were further lowered. Again in view of the suggestion of Clark that robustic acid is probably present in the American sample of *D. scandens*, *D.S. III* was compared closely with robustic acid obtained in a pure condition from *D. robusta*. The two were found to

be different. Consequently D.S. III is also considered to be a new entity and named 'Chandanin' derived from 'Chanda District' wherefrom the plant material was obtained. Chandanin melted at 201-2°, contained three methoxyls and had the molecular formula $C_{29}H_{30}O_7$. It formed a tetra-acetate and a tetramethyl ether, thereby indicating the presence of four phenolic hydroxyl groups. It seemed to be extraordinary in several respects. Table I brings out briefly the salient features of the compounds mentioned in the above paras:

TABLE I

| Substance | Melting point | Molecular formula | Acetate m.p. | Methyl ether m.p. | Remarks |
|--------------------|---------------|-------------------|--------------|-------------------|---------------|
| Scandenin (D.S. I) | 233-4° | $C_{26}H_{26}O_6$ | 160-1° | 128-9° | Present data |
| Do | 231° | " | 150° | 129° | Clark's data |
| D. S. II | 217-8° | $C_{26}H_{26}O_5$ | 153-4° | 116-7° | Present data |
| Lonchocarpic acid | 221° (202°) | $C_{26}H_{26}O_6$ | 153-4° | 154-5° | " |
| D. S. III | 201-2° | $C_{29}H_{30}O_7$ | 137-8° | 154-5° | " |
| Robustic acid | 205-6° | $C_{22}H_{20}O_6$ | 196-7° | 193-4° | " |
| Do | 190° | $C_{27}H_{24}O_8$ | — | 190° | Harper's data |

It is evident from the table that of the three components isolated from the *D. scandens* root, scandenin is the only one previously reported in the literature, whereas the other two appear to be new substances. Thus these roots do not contain any lonchocarpic acid or robustic acid.

As none of the compounds isolated responded to the rotenoid colour reaction (Durham test), it may safely be concluded that even the Indian sample of *D. scandens* does not possess rotenone or allied substances. The chief chemical component is scandenin, as in the case of the American sample, but the yields in the present case are far higher (1.5% compared to 0.5%). Nallanin and chandanin are present only in minor amounts and hence detailed work on these could not be carried out.

EXPERIMENTAL

The air-dried thick roots of *D. scandens* were made into thin shavings and extracted in two batches of 1,000 g. each.

1st batch.—It was extracted with ether (7.5 litres) by cold percolation and the extract was concentrated to small volume (300 c.c.); during the course of this concentration a crystalline solid began to separate. The mixture was set aside for a few days and the solid, fraction A (10.7 g.) was then filtered off. The mother-liquor on concentration to 150 c.c. yielded fraction B (3.5 g.). By further concentration fraction C (1.5 g.) was obtained.

The dried marc obtained after ether extraction was extracted in the cold with chloroform. The extract, after complete removal of the solvent and addition of ether (50 c.c.), gave fraction D (1.0 g.).

Fraction A.—It had a fairly sharp melting point, 225–8°, and was soluble in most of the organic solvents with the exception of ether and petroleum ether, in which it was sparingly soluble. It (9.0 g.) was crystallised from acetone and the following four fractions were collected: fraction (i) 2.5 g., m.p. 227–8°; (ii) 4.0 g., m.p. 228–31°; (iii) 1.0 g., m.p. 225–8°; and (iv) 0.75 g., m.p. 225–7°. All the fractions had the same crystal structure, rectangular prisms and rods, and they seemed to contain only one substance except for the presence of a small impurity in the last fractions. The substance melting at 228–31° is henceforward designated as D.S. I.

Fraction B.—This had an indefinite melting point, softening at 184° and melting clear at 208°. It was boiled with benzene (140 c.c.) and the sparingly soluble residue (B) collected on a filter. This had a melting point of 227° (sintering at 220°), which was undepressed by admixture with the pure product obtained from fraction A.

The benzene mother-liquor on cooling deposited a solid (B₂) melting at 185–206°. This was crystallised with the samples, having the same melting point range, obtained from the 2nd batch.

Fraction C.—This also had an indefinite melting point, 165–82°. On crystallisation from acetone and removal of the gelatinous impurities that separated in the beginning, a crystalline solid (C₁), m.p. 205–18°, was obtained. The filtrate on concentration yielded a product (C₂), m.p. 185–206°, identical with B₂. These were put together. By further concentration, fraction C₃, m.p. 165–80° was obtained; this seemed to have the same composition as the original fraction C, m.p. 165–82°. It was therefore considered better to apply the technique of chromatography to resolve this mixture. A pilot experiment was conducted starting with 170 mg. of the substance using alumina as adsorbent and benzene as solvent. The development and elution were carried out with chloroform-benzene mixture, the proportion of chloroform being increased in the later elutions. The final elution was carried out with alcohol. Several fractions were collected and the products were obtained by evaporation of the solvents and final crystallisation from benzene or alcohol. The results are tabulated below:

The above results indicated the presence in the mixture of a substance melting above 225°, another melting at 217–18° and possibly a third melting

TABLE II

| Fraction No. | Solvent | Melting point of the product | Remarks |
|--------------|---------------------------------------|------------------------------|---|
| I .. | Benzene mother-liquor | Nil | } Only development Sintering earlier Mixed m.p. with D.S. I depressed (195-220°); seemed to be a single component |
| II & III | Benzene-chloroform (6:1), 20 c.c. | ,, | |
| IV & V | ,, (3:1), ,, | ,, | |
| VI .. | ,, (2:1), ,, | 176° | |
| VII .. | ,, (1:3), ,, | 217-18° | |
| VIII .. | Chloroform 20 c.c. | 187-225° | } Appeared to be mixtures of D.S. I and fraction VIII |
| IX .. | ,, 35 ,, | 188-220° | |
| X .. | ,, 40 ,, | ,, | |
| XI .. | Chloroform-ethyl alcohol (1:3), 10 ,, | ,, | |
| XII .. | Ethyl alcohol 30 ,, | 195-215° | |

above 180°. This suggested that the fraction C₁ may be an impure form of the substance melting at 217-18° and not of D.S. I as it was originally considered to be. Therefore C₁ was recrystallised from alcohol, when a crystalline substance melting at 214-18° was obtained (mixed melting point with D.S. I from fraction A was depressed). This is henceforward designated as D.S. II.

Fraction D.—This has a melting point range of 210-18°. On recrystallisation from acetone, the following fractions were obtained: (i) melting at 228-9°, mixed melting point with D.S. I undepressed, (ii) melting at 223-9°, undepressed by D.S. I. No other substance was obtained from this fraction.

2nd batch.—The second batch was directly extracted with chloroform as this proved to be a better solvent than ether. The material was exhaustively extracted with this solvent (7.5 litres) which was recovered by distillation. The last traces of the solvent were removed by evaporation, at the laboratory temperature, in an open basin. The brown, viscous, semi-solid residue (70 g.) was taken in ether (300 c.c.) and set aside for a few days. Immediately after the addition of ether, a colourless crystalline solid began to separate. The solid (fraction A, 16.5 g.) was collected on a filter and washed with a small amount of ether. The ether solution on concentration (150 c.c.) yielded fraction B (2.4 g.). A further fraction C (1.3 g.) was obtained by concentrating the mother-liquor.

Fraction A.—Though it was uniformly crystalline, it melted at 218-25° and was therefore fractionated from acetone. The following fractions were collected:

Fraction C.—This also had an indefinite melting point, 170–210° and therefore was first purified by boiling with petroleum ether to remove any fatty matter present. Later it was washed with ether, crystallised from alcohol and the following fractions collected: Fraction (i) m.p. 195–210°, (ii) m.p. 212–15° and (iii) m.p. 175–200°. Of these only fraction (ii) had a fairly sharp melting point. Its mixed melting point with D.S. II (m.p. 214–18°) was not depressed.

Thus *D. scandens* obtained from the Central Provinces yielded three substances D.S. I, m.p. 228–31°, D.S. II, m.p. 214–18° and D.S. III, m.p. about 196°. The various fractions collected from the extracts may have the following probable composition:

- Fraction A: Mostly D.S. I
- „ B: D.S. I + D.S. III
- „ C: D.S. II + the other two substances.

D.S. I: (Scandenin).—It crystallised as colourless rectangular prisms and rods and exhibited a play of colours when viewed at different angles. Attempts to raise the melting point through crystallisation from solvents were not successful. It was readily soluble in chloroform, hot acetone and hot alcohol, less soluble in benzene, ether and petroleum ether. Though it was insoluble in water, it was found to be soluble in hot aqueous potash from which the sparingly soluble potassium salt readily separated on cooling as silky white crystals. On saturating the alkali solution with carbon dioxide the original substance was reprecipitated. After recrystallisation with alcohol it now melted at 233–34°.

The substance dissolved in concentrated sulphuric acid forming an intense orange-red solution without any visible fluorescence. In the Durham test, it did not give the rotenoid colour changes but was found to give a yellow solution with concentrated nitric acid which changed to orange-red on the addition of concentrated ammonia. The substance did not give any definite ferric chloride colouration, only an orange colour being observed. Fehling's solution was not reduced by it. It did not respond to either the magnesium-hydrochloric acid test of flavones or the colour reactions of resinols and sterols. When a speck of the substance was treated with gallic acid and concentrated sulphuric acid and gently heated, no blue colour was produced, thereby indicating the absence of methylene-dioxy grouping in the molecule. (Found: C, 71.7; H, 5.9; — OCH₃, 7.3; C₂₆H₂₆O₆ requires C, 71.8; H, 6.0; — OCH₃, 7.2%.)

Diacetate.—D.S. I (0.2 g.) was treated with acetic anhydride (3 c.c.) and sodium acetate (0.5 g.) and gently refluxed for 3 hours in an oil-bath

at 140°. It was then poured into cold water, when a colourless crystalline solid began to separate. It was filtered, washed with plenty of water and recrystallised from ethyl acetate-petroleum ether (rectangular plates). The acetate melted at 160–61°. (Found: C, 69·1; H, 6·0; $C_{30}H_{30}O_8$ requires C, 69·5; H, 5·8%.)

Dimethyl ether.—The substance (1·0 g.) in anhydrous acetone solution (75 c.c.) was treated with dimethyl sulphate (5 c.c.) and anhydrous potassium carbonate (12 g.) and refluxed on a water-bath for 36 hours. Then the solution was filtered from the carbonate, which was washed with sufficient quantity of dry acetone and the combined acetone solution was distilled to remove as much of the solvent as possible. Water was added to the residue and the solid product was filtered and air-dried (0·85 g.). The methyl ether crystallised from alcohol as long rectangular plates and needles and melted at 128–9°. (Found: C, 72·3; H, 6·6; — OCH_3 , 19·7; $C_{28}H_{30}O_6$ requires C, 72·7; H, 6·5; — OCH_3 , 20·1%.)

D.S. II (Nallanin).—On further crystallisation from alcohol, the melting point became sharper, 217–18°. Under the microscope its crystal structure was similar to that of scandenin (rectangular prisms and rods); but the mixed melting point with scandenin was depressed. It was compared with the sample of lonchocarpic acid obtained from Dr. Jones but was again found to be different as was evidenced by the mixed melting point and also the properties of its derivatives. It was more easily soluble in alcohol than scandenin. In its solubility in aqueous potash it resembled scandenin, though there was no quick separation of the potassium salt. In the Durham test, it also did not give the rotenoid colour changes, the change being from yellow to brick red. With concentrated sulphuric acid it formed an intense red solution without any visible fluorescence. There was no definite phenolic colour reaction with ferric chloride. (Found: C, 74·6; H, 6·5; — OCH_3 , 7·8; $C_{26}H_{26}O_5$ requires C, 74·6; H, 6·2; — OCH_3 , 7·4%.)

Diacetate. On acetylation with acetic anhydride and sodium acetate it gave a diacetate, m.p. 153–4°, which crystallised as rectangular rods from ethyl acetate; the mixed m.p. with the acetate of lonchocarpic acid was depressed. (Found: C, 71·4; H, 6·3; $C_{30}H_{30}O_7$ requires C, 71·7; H, 6·0%.)

Monomethyl ether.—The substance was methylated with methyl sulphate and potassium carbonate in anhydrous acetone medium by refluxing for 30 hours. The product crystallised from alcohol as long rectangular prisms and its melting point was 116–17°. (Found: C, 75·2; H, 6·6; — OCH_3 , 13·8; $C_{27}H_{28}O_5$ requires C, 75·0; H, 6·5; — OCH_3 , 14·3%.)

D.S. III (Chandanin).—On repeated crystallisation from alcohol its melting point rose to 201–2°. It resembled D.S. II very closely in all the

colour reactions but its mixed melting points with D.S. II and also with lonchocarpic acid were appreciably depressed. (Found: C, 71.2; H, 6.2; — OCH₃, 18.6; Molecular weight (Rast): 465. C₂₉H₃₀O₇ requires C, 71.0; H, 6.1; — OCH₃, 19.0%; Mol. weight, 490.)

In the Durham test, the colour change was from yellow to brick red. It was soluble in alkali, though no definite phenolic colouration was obtained with ferric chloride.

Tetra-acetate.—Chandanin was acetylated with acetic anhydride and sodium acetate and the crude product was crystallised from ethyl acetate-petroleum ether mixture. The acetate melted at 137–38° and appeared under the microscope as rectangular rods. (Found: C, 66.9; H, 5.5; C₃₇H₃₈O₁₁ requires C, 67.4; H, 5.8%.)

Tetra-methyl ether.—The methyl ether was prepared as in the case of D.S. I and D.S. II. It appeared under the microscope as rhombic prisms and melted at 154–55°; the mixed melting point with the methyl ether of lonchocarpic acid was depressed. (Found: C, 72.8; H, 6.7; C₃₃H₃₈O₇ requires C, 72.5; H, 6.9%.)

Our thanks are due to the Forest Officer, South Canada Division of the Central Provinces, for the supply of root samples and to Dr. Jones for the sample of lonchocarpic acid.

SUMMARY

The roots of *Derris scandens* obtained from the Central Provinces of India have now been investigated in detail. From the chloroform extractives (7.0%) three crystalline substances could be isolated and characterised. There is no rotenone. The major component is scandenin, as in the case of the American sample examined by Clark; but the yield is much higher. The other two substances appear to be new compounds and are therefore named 'Nallanin' and 'Chandanin'. Lonchocarpic and robustic acids are not found in the Indian roots.

Nallanin has the molecular formula C₂₆H₂₆O₅, contains one methoxyl and two hydroxyls, one of which seems to be phenolic and the other alcoholic. On the other hand, Chandanin has the molecular formula C₂₉H₃₀O₇ possesses three methoxyls and four phenolic hydroxyls; it seems to be rather extraordinary in its characteristics.

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