

CHEMICAL EXAMINATION OF PLANT INSECTICIDES

Part III. Chemical Components of *Derris Robusta*

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Received September 26, 1946

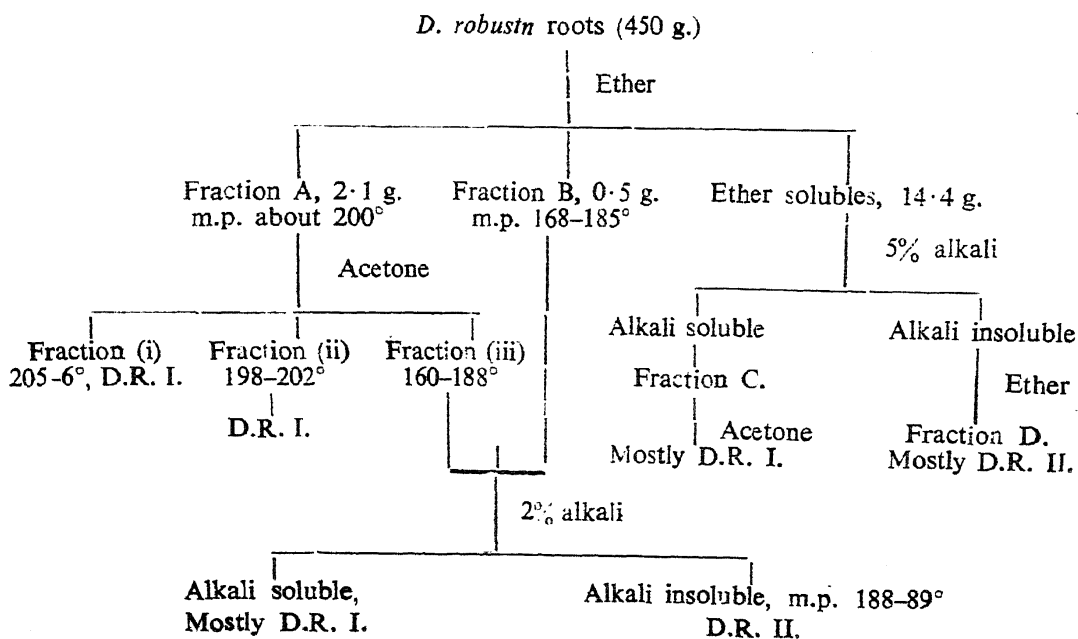
Derris Robusta is very closely related to *D. scandens* botanically as both belong to the same sub-section, Brachypterum of the genus *Derris*. The chief difference is that the former is an erect tree, 30-40 feet high, whereas the latter is a climber. The tree is widely distributed in the tropical areas of the world and occurs in India in the Eastern Himalayas and the Western Peninsula.

Krishna and Ghose¹ examined the roots obtained from Gauhati (Assam) and observed that they did not contain any rotenone. During the course of this examination they isolated from the ether extract a crystalline solid, melting at about 190° and sent it to Rothamsted for toxicity trials; it was found to be non-toxic to *Aphis rumicis* (the bean aphid). Chemical investigation of this sample was conducted by Harper² who concluded that it was not a rotenoid, as shown by the Durham test and other reactions, but was a carboxylic acid akin to lonchocarpic acid isolated by Jones.³ The crystalline substance was named by him 'Robustic acid' as it was found to possess acidic properties. He proposed the molecular formula $C_{27}H_{24}O_8$ for it from considerations of analytical values. It contained two methoxyl groups; it formed a mono-methyl derivative of the same melting point as the original substance and this was considered to be the methyl ester. Attempts to show the presence of a ketonic group by oximation were unsuccessful. Catalytic hydrogenation gave dihydro-robustic acid. He felt that the titration of the acid in hot alcohol was not a case of true neutralisation, as conflicting results were obtained and as the acid could not be recovered on acidification.

Since a detailed chemical examination of the roots of *D. robusta* had not been conducted by previous workers, it was considered desirable to undertake it. Further, two recent publications, one by Clark⁴ and the other by Jones and Haller⁵ made this study necessary. The first author reported the isolation of a substance, melting at 190°, from *D. scandens* which resembled

robustic acid. As we were examining the Indian *D. scandens*,⁶ we needed a sample of robustic acid for purposes of identification. In the second paper, the authors showed that lonchocarpic acid was not a carboxylic acid as it was originally thought to be. This led us to the expectation that robustic acid also may not be a carboxylic acid and that it may possibly be related to the two substances, scandenin and lonchocarpic acid. In this connection the close botanical relationship between *D. scandens* and *D. robusta* was present in our mind.

The roots of *D. robusta* obtained from Dr. Sri Krishna of the Forest Research Institute, Dehra Dun, were extracted with ether instead of chloroform, as robustic acid was originally isolated by the previous workers from an ether extract. The present sample of the roots gave 3.9% of ether extractives, which were studied on the lines outlined in an earlier publication.⁷ The fraction sparingly soluble in ether gave colour reactions similar to those of robustic acid² but was found to have a wide melting point range extending up to 200°. Therefore it was fractionated using acetone and as a result of this, a crystalline substance melting sharp at 205–6° was obtained. This was designated as D.R. I. A second crystalline substance (D.R. II) which melted at 188–9° and was insoluble in alkali, was isolated by the alkali treatment of the mixture present in the more soluble portions of the ether extract. This had a crystal structure similar to that of D.R. I and gave similar colour reactions but differed as regards solubility in alkali. Thus the roots of *D. robusta* gave two crystalline components, one melting at 205–6° (major component) and another melting at 188–9°.



Since D.R. I differed from robustic acid as described by Harper in certain of its properties, it was thought desirable to procure a sample of the acid from Dr. Krishna from whom the material used by Dr. Harper was also obtained and effect direct comparison. The crude sample obtained from Dr. Krishna had an indefinite melting point (170–90°) and was therefore purified by crystallisation from acetone. The top fraction melted at 205–6° and was identical with D.R. I. From the other fractions a sample of D.R. II could be obtained. Consequently it could safely be concluded that D.R. I is a purer sample of robustic acid than that employed by Harper for his investigation. It has a higher melting point. There are differences in analytical data as well; ours has given a methoxyl content of 16.2% whereas the methoxyl value recorded by Harper was 13.1%. In carbon percentage also there is a small difference.

Robustic acid can be obtained in a fairly well-developed crystalline form (elongated prisms). Like scandenin, it exhibits a play of colours when viewed at different angles. In solubility also, it closely resembles scandenin and forms a sparingly soluble potassium salt on treatment with aqueous alkali. The substance could be precipitated from the alkali solution either by treatment with mineral acid or by passing carbon dioxide. In the Durham test, it gives a green solution with nitric acid which changes to an unmistakable brilliant red colour on the addition of ammonia. It does not give any phenolic colouration with ferric chloride nor does it respond to the colour reactions of flavonols, resins or sterols.

Robustic acid contains two methoxyls; from considerations of analytical data and molecular weight determination, the molecular formula $C_{22}H_{26}O_6$ is now assigned to it. Just like scandenin, it could also be titrated with alkali in alcoholic solution and the original substance recovered without any difficulty on acidifying the alkaline solution. Hence the statement of Harper that the substance could not be recovered by acidification after titration with alkali does not seem to be correct. This view is further supported by the observation that robustic acid is quite stable to boiling aqueous alcoholic potash. The minimum molecular weight, 397, calculated from the titration data agrees closely with that obtained by Rast's method. It may be pointed out that Harper's titration values also lead to the same result.

Robustic acid gives a mono-acetate, melting at 196–7° and a mono-methyl ether, melting at 193–4° (*cf.* Harper's m.p. of 'methyl ester' 190°), thereby indicating the presence of one free hydroxyl group. The acetate is insoluble in alkali as is also the methyl ether. As robustic acid can be precipitated from an alkali solution by passing carbon dioxide, as it forms

an acetate which is insoluble in aqueous alkali, and as the methyl derivative does not regenerate robustic acid on treatment with alcoholic potash, it can be said with certainty that the acidic properties of robustic acid are not due to the presence of a carboxylic group as was originally thought but due to a strongly acidic hydroxyl group as in the case of scandenin and lonchocarpic acid. Hence the methyl derivative of robustic acid is a methyl ether and not a methyl ester. In all these properties there is close resemblance between robustic acid, scandenin and lonchocarpic acid. The name robustic acid should still be retained, although the substance is not a carboxylic acid, in order to avoid unnecessary confusion in the literature.

D. R. II has the same crystal structure as D.R. I (robustic acid) and also gives the same colour changes in the Durham test; but it is insoluble in alkali. As it has a lower methoxyl value (17.7%) than the methyl ether of robustic acid (23.2%), it could not be considered to be identical with the latter. From the analytical data, the molecular formula $C_{21}H_{20}O_6$ is assigned to it. Since it appears to be a new substance, it is designated 'Robustenin'.

EXPERIMENTAL

The air-dried roots of medium size (450 g.) obtained from the Forest Research Institute, Dehra Dun, were made into thin shavings and extracted three times with ether at the laboratory temperature soaking each time for 24 hours. The combined extracts (6 litres) were concentrated and the solvent-free residue (17 g.) was taken in a small volume of ether (75 c.c.). The undissolved crystalline material (A) was filtered (2.1 g.). After partial concentration of the filtrate and treatment with methyl alcohol, fraction (B) (0.5 g.) separated and was filtered off.

From the filtrate the solvents were removed under reduced pressure, the residue was taken up in ether and rapidly extracted with 5% aqueous potash. The alkali-soluble portion was liberated by acidification, filtered and washed with boiling petroleum ether to remove fatty matter and was then taken in ethyl alcohol (25 c.c.) and set aside. Next morning a crystalline solid (C) separated and was filtered. By concentration of the solution some more of the same solid was obtained.

The alkali-insoluble ether solution was dried over anhydrous sodium sulphate and the solvent completely removed. The solvent-free residue was taken in a small quantity of carbon tetrachloride (10 c.c.) and set aside. As no solid separated from this, the solvent was distilled under reduced pressure and the residue taken up in ether. The solid (D) that separated was collected.

From the rest of the neutral portion no other crystalline component could be obtained.

Fraction A.—It was colourless and crystalline in appearance. It melted at about 200° (with sintering earlier) and in the Durham test gave a green colour changing to evanescent red, as described by Harper for robustic acid. As its melting point was not quite definite and was higher than that recorded for robustic acid (190°) it was fractionated. Acetone was used for this purpose and the following three fractions were collected:

	Melting point	Crystal structure	Durham test	Ferric chloride colour
Fraction (i)	205-6°	Large rectangular prisms	Green to red	Negative
Do (ii)	198-202°	do	do	do
Do (iii)	160- 88°	do	do	do

Fraction (i) appeared to consist of a single entity and the melting point was not raised by further crystallisation. It was therefore marked D.R. I. Fraction (ii) consisted essentially of this and could be purified by further crystallisation whereas fraction (iii) behaved like a mixture.

Fraction B.—The solid had a wide melting point range, 168-85° but gave the colour changes in the Durham test similar to those given by D.R. I. Since fraction (iii) from (A) and this solid had similar melting point ranges, they were combined and treated with 2% aqueous alkali and the mixture heated and filtered. The clear solution was acidified and the product obtained was found to have a melting point of 192-96°. On crystallisation from alcohol the melting point rose and the product was found to be identical with D.R. I.

The alkali-insoluble solid was washed with hot water to remove any potassium salt that might have been present and then crystallised from alcohol. After two crystallisations it had a sharp melting point, 188-9° (mixed melting point with D.R. I. depressed). In the Durham test, the colour change was from dirty green to evanescent red. This substance was marked D.R. II.

Fraction C.—It melted fairly sharp at 189-91° and had the same properties as D.R. I. Recrystallisation raised the melting point to 193° and not further. However the mixture with D.R. I also melted at the same temperature and methylation of the substance yielded a methyl ether identical with that of D.R. I. It was therefore concluded that this fraction consisted

mainly of D.R. I. The cause of the lower melting point will receive further attention when more quantities of this fraction are again obtained.

Fraction D.—Its melting point was indefinite 170–80°, it was insoluble in alkali and gave in the Durham test the same colour changes as D.R. II. The quantity was too small to effect detailed purification.

Purification of the robustic acid sample obtained from Dehra Dun.—The crude sample (2.5 g., m.p. range 170–90°) was taken in boiling acetone (50 c.c.) and the insoluble portion, which was appreciable in amount, was filtered off. The first crop (0.7 g.) melted at 198–202° and appeared as colourless rectangular prisms. The melting point could be raised to 205–6° by another crystallisation and the mixed melting point with D.R. I was undepressed. The second crop (1.3 g.) had an indefinite melting point 170–88°. This on treatment with alkali gave an alkali-insoluble product identical with D.R. II.

D.R. I is hence forward designated robustic acid.

Robustic acid.—On slow crystallisation, it could be obtained in a fairly well-developed form and appeared as elongated prisms under the microscope. It was readily soluble in chloroform, boiling acetone and alcohol and less soluble in petroleum ether and benzene. It had greater solubility than scandenin in ether. Though it was insoluble in water, it was found to be soluble in warm aqueous potash from which the sparingly soluble potassium salt separated on cooling. On acidification of the alkali solution, the substance was reprecipitated.

Robustic acid dissolved in concentrated sulphuric acid forming an intense red solution without any visible fluorescence. In the Durham test it did not give the rotenoid colour changes but gave a green colour changing to brilliant red. This red colour was unmistakable and could not be missed. The compound did not respond to the sterol or resinol colour reactions. 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, 69.2; H, 5.2; OCH₃, 16.2%. Mol. weight (Rast) 404, C₂₂H₂₀O₆ requires C, 69.5; H, 5.3; 2-OCH₃, 16.4% Mol. weight 380. Cf. Harper's values C, 68.4; H, 5.1; OCH₃, 13.1%. Mol. weight (Rast) 496, 451].

Micro-titration.—The substance was dissolved in hot neutralised alcohol and titrated with standard alkali using phenolphthalein as the indicator. The end point was sharp (61.0 mg. of the substance required 4.65 c.c. of 0.033 N alkali. Mol. weight 397. Harper's values: 53.65 mg. required

7.05 c.c. of 0.02 N. NaOH; Mol. weight 380). The substance was recovered on acidification and dilution with water.

Mono-acetate.—The acetate was prepared by boiling the substance with acetic anhydride and sodium acetate. The product crystallised from ethyl acetate as rectangular rods and prisms melting at $196-7^{\circ}$; the mixed melting point with the original substance was depressed (Found: C, 68.1; H, 5.3; $C_{24}H_{22}O_6$ requires C, 68.2; H, 5.3%).

The acetate was found to be insoluble in 5% aqueous alkali even after warming the solution, thereby indicating the absence of a carboxylic group in the substance.

Mono-methyl ether.—The methylation was carried out in anhydrous acetone medium using dimethyl sulphate and potassium carbonate. On concentration of the acetone solution to a small volume, a crystalline solid, melting at $192-3^{\circ}$, was obtained; the mixed melting point with the original substance was considerably depressed. It was insoluble in aqueous alkali and did not yield robustic acid on treatment with alcoholic potash. The methyl ether was recrystallised from alcohol when it came out as prismatic needles melting at $193-4^{\circ}$ (Found: OCH_3 , 23.2%. $C_{23}H_{22}O_6$ requires 3- OCH_3 , 23.6%).

D.R. II (Robustenin).—It appeared as rectangular prisms under the microscope and gave in the Durham test a dirty green colour changing to evanescent red. With ferric chloride, no colouration was obtained [Found: C, 68.5; H, 5.7; OCH_3 , 17.7%. Molecular weight (Rast) 390; $C_{21}H_{20}O_6$ requires C, 68.5; H, 5.4; (2) OCH_3 , 16.9%; Molecular weight, 368]. As it appeared to be a new substance, it was designated 'robustenin'.

Our thanks are due to Dr. Sri Krishna of the Forest Research Institute, Dehra Dun for the supply of samples of the roots of *D. robusta* and also of a sample of the crystalline component of these roots.

SUMMARY

A detailed examination of *D. robusta* has now been made and two crystalline components isolated. The main component (D.R. I) melts at $205-6^{\circ}$ and agrees, in general, with the properties of robustic acid described by Harper except in melting point and analytical values. The same substance (D.R. I) was also obtained from the crude sample of robustic acid supplied by the Forest Research Institute, Dehra Dun, from where Dr. Harper also got his material. The name robustic acid has been retained for it. The molecular formula, $C_{22}H_{20}O_6$ agrees with the results more closely. The molecule contains two methoxyls and one hydroxyl group. The acid nature

of robustic acid has now been shown to be due to a phenolic hydroxyl just as in the case of scandenin and lonchocarpic acid. These three compounds seem to form a new type not having any simple relationship with rotenone.

The second component now named 'robustenin' melts at 188–89°, is neutral in nature and has the probable molecular formula $C_{21}H_{20}O_6$. It contains two methoxyls.

REFERENCES

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| 1. Krishna and Ghose | .. <i>Indian Forest Leaflet</i> , 1942, No. 2. |
| 2. Harper | .. <i>J. C. S.</i> , 1942, 181. |
| 3. Jones | .. <i>J. A. C. S.</i> , 1934, 56, 1247. |
| 4. Clark | .. <i>J. Org. Chem.</i> , 1943, 8, 489. |
| 5. Jones and Haller | .. —, 1943, 8, 493. |
| 6. Rao and Seshadri | .. <i>Proc. Ind. Acad. Sci.</i> , 1946, 24, 365. |
| 7. ——— | .. —, 1946, 24, 344. |