CHEMICAL EXAMINATION OF PLANT INSECTICIDES

Part V. A Comparative Study of Scandenin, Lonchocarpic Acid and Robustic Acid

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AMONG the crystalline non-rotenoid compounds occurring in leguminous plants scandenin, lonchocarpic acid and robustic acid seem to be the most important. They can be obtained in adequate quantities for study and seem to have an appreciable amount of toxicity though poor when compared with rotenone and related rotenoids. But the interesting point is that besides the negative Durham test they appear to have a number of other properties in common and to be closely related in their molecular structures thus constituting a type of chemical compounds. As a matter of fact Clark[1] claimed to have found all the three occurring together in the roots of American *Derris scandens* though this finding could not be supported from our study of the Indian material.[2] However between the individuals there are differences. These features have now been investigated more closely and are discussed in this paper.

Scandenin, the chief chemical component of the roots of *Derris scandens*, was first isolated by Clark[1] and with the small quantity of material available to him a brief chemical study was made. It was given the molecular formula $C_{26}H_{26}O_6$ and was found to have one methoxyl and two free hydroxyl groups capable of forming a diacetate and a dimethyl ether. It was acidic in character and produced sparingly soluble sodium and potassium salts. As the acetate was insoluble in aqueous alkali he concluded that the acid nature of the substance was not due to a carboxyl but due to the presence of an active hydroxyl group. The compound showed considerable unsaturation and absorbed three molecular proportions of hydrogen, though no definite hydrogenation products were isolated. The probability of the presence of *p*-hydroxy-benzoyl group in scandenin was indicated by the formation of *p*-hydroxy-benzoic acid on oxidation with alkaline hydrogen peroxide. No oxime or semicarbazone could be prepared. The substance did not give the Durham test. From these properties Clark concluded that scandenin bore no relationship to the rotenone group of fish poisons.

Since we could isolate a fairly good quantity of scandenin during the course of our examination[2] of *D. scandens* obtained from Central India, a more detailed study has now been carried out. The compound is definitely toxic to fish though very inferior to rotenone. It is optically inactive just like lonchocarpic and robustic acids.

The molecular formula, $C_{26}H_{26}O_6$ has now been confirmed. That scandenin has a minimum molecular size of 420 could be deduced from its methoxyl content, the titration with alkali and the estimation of potassium in the sparingly soluble potassium salt. Determination of the molecular weights of scandenin and of its methyl ether using Rast's method established that the molecular weight of scandenin is of the order 420 and not a multiple of it. The compound thus behaves like a monobasic acid and the salt is a monopotassium salt.

Scandenin is not only soluble in alkali but as mentioned above it could actually be titrated with alkali. However it is a weak acid and is thrown out of an alkaline solution by passing carbon dioxide. Besides its acetate being insoluble in alkali, its dimethyl ether does not undergo hydrolysis like an ester. Further scandenin is unaffected under conditions of esterification. These are in conformity with the suggestion of Clark that the acid property is due not to a carboxyl group but to an active hydroxyl group. We confirm that scandenin does not give ferric chloride colour and that it forms *p*-hydroxybenzoic acid readily on oxidation with alkaline hydrogen peroxide. We have also been unable to get derivatives indicative of the presence of a carbonyl group; no oxime or dinitrophenyl hydrazone could be prepared. But the study of the ultra-violet absorption spectrum mentioned later seems to indicate the presence of a carbonyl group. This is also supported by the toxic properties of the compound since most plant insecticides contain this group.

Clark[1] prepared the diacetate by boiling scandenin for 5 minutes with acetic anhydride and sodium acetate. As reported in Part II[2] by boiling for a longer period a purer product is obtained in better yield. Deacetylation with alcoholic potash regenerates scandenin. For purposes of methylation Clark used diazomethane and could obtain only poor yields of the dimethyl ether. By employing dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium very good yield of the pure dimethyl ether could now be readily produced.[2] Methylation with dimethyl sulphate and aqueous alkali forms the same product, but the yield is less. Attempts to isolate a monomethyl ether by employing one molecular proportion of dimethyl sulphate have been unsuccessful; only a mixture of the dimethyl ether and the potassium salt of scandenin are obtained.

Scandenin dissolves in concentrated sulphuric acid in the cold producing an intense orange red colour without any fluorescence. When the solution is poured on ice it gives a sulphonated product which does not melt below 270°; it is fairly soluble in alcohol, acetone and water, but insoluble in ether. In anqueous solution it gives a pure blue colour with ferric chloride. Attempts to crystallise it have not been successful. The experiment indicates that in cold sulphuric acid no simple isomerisation takes place and that the molecule of scandenin undergoes sulphonation readily.

Scandenin is found to be extraordinarily stable to alkali, boiling with alcoholic alkali, aqueous alcoholic alkali and even 50% aqueous potash producing no change. Almost all the scandenin could be recovered unchanged after this treatment. The potassium salt that is formed seems to be quite stable.

In order to get some idea of the nature of unsaturation in scandenin the reaction of bromine has been studied. Scandenin readily forms a well-defined bromo-derivative in acetic acid medium. The reaction seems to take place without elimination of hydrobromic acid and the bromo-derivative could therefore be taken to be an addition product. Its analysis indicates that it is a dibromide. In order to make sure that nuclear bromination is not involved owing to the influence of the phenolic hydroxyl groups, the dimethyl ether and the diacetate have also been subjected to treatment with bromine. They also behave similarly taking up two bromine atoms. It appears therefore that in all these reactions an ethylenic double bond is involved.

Lonchocarpic acid was first isolated by Jones[3] from an unknown species of Lonchocarpus from Venezula and was further studied by Jones and Haller.[4] It was found to have the formula $C_{26}H_{26}O_6$, to be soluble in sodium carbonate and stable to alcoholic alkali. Though at first it was considered to be a carboxylic acid the more recent work showed that the acid properties are due to a strongly acidic hydroxyl group only. Like scandenin, this also has one methoxyl and two hydroxyl groups. The diacetate is insoluble in aqueous alkali. Lonchocarpic acid formed both mono and dimethyl ethers which did not exhibit the properties of esters. Catalytic hydrogenation added 4 hydrogen atoms and a tetrahydro derivative could be prepared. Oxidation of lonchocarpic acid with alkaline hydrogen peroxide formed *p*-hydroxybenzoic acid.

In order to identify the crystalline substances isolated from *D. scandens*, a sample of lonchocarpic acid was obtained from Dr. H. A. Jones and a number of its derivatives prepared. We had also occasion to study its

properties and compare them with those of scandenin. They resemble in several respects. They have the same molecular formula, have one methoxyl and two hydroxyl groups in their molecules and thus form diacetates and dimethyl ethers. They are acidic in nature and are quite stable to alkali. Even in the hydrogen peroxide oxidation they behave alike forming *p*-hydroxybenzoic acid. They are also optically inactive.

Jones[3] reported that lonchocarpic acid had no insecticidal properties. In our tests using fresh-water fish it exhibited definite toxic properties. Though it is slower in its effect than even scandenin there is one noteworthy feature. The fish recovered when transferred to fresh water after the experiment in the case of scandenin as with most substances. But with lonchocarpic acid they all died. This showed that lonchocarpic acid is quite toxic though it may be slow acting.

The differences that we could so far note in their properties are as follows: Lonchocarpic acid is more easily soluble in alcohol than scandenin; it is also soluble in 5% sodium carbonate solution whereas scandenin forms a sparingly soluble salt. With concentrated sulphuric acid the former gives a deeper red solution. There is a small difference in the colour changes shown by the two substances under the Durham test.

Just as in the case of scandenin chemical tests for the presence of a carbonyl group are not given by lonchocarpic acid also. But the presence of such a group is indicated by the ultra-violet absorption spectrum and the agreement between the spectra of the two compounds is very close.

Even in regard to bromine addition there is resemblance between scandenin and lonchocarpic acid. The dimethyl ether and diacetate have been treated with excess of bromine in acetic acid solution. There is no elimination of hydrogen bromide and two atoms of bromine are found in the addition products.

In the course of their examination of the roots of *Derris robusta* Rao and Seshadri[6] made a preliminary study of robustic acid. They showed that its molecular formula is $C_{22}H_{20}O_6$ and thus it has 6 oxygen atoms just as scandenin and lonchocarpic acid. It is a dimethyl ether having a free hydroxyl group which is capable of acetylation and methylation. Thus the fully methylated ether has three methoxyl groups just as those of scandenin and lonchocarpic acid. Robustic acid is also definitely acidic in properties and can be titrated with alkali and it also forms a sparingly soluble potassium salt. Here too the acidic properties are due to a strongly acidic hydroxyl group which again does not give the ferric chloride colour. Like the other

two non-rotenoids it is also stable to treatment with aqueous alcoholic alkali. The potassium salt separates out and no reaction takes place. Robustic acid can be distinguished by the brilliant red colour which it finally gives in the Durham test.

The oxidation of robustic acid with alkaline hydrogen peroxide has now been carried out. *p*-Hydroxy benzoic acid could readily be separated from the products. By the action of bromine on robustic acid, its methyl ether and acetate a tetrabromo-compound is formed. This is a marked difference between this and the other two non-rotenoids.

Robustic acid again resembles scandenin and lonchocarpic acid in not giving chemical tests characteristic of a carbonyl group. Its ultra-violet absorption spectrum is almost identical with those of the other two non-rotenoids mentioned above. It also has some definite toxicity though it is also weak like the others.

It has already been mentioned that scandenin, lonchocarpic acid and robustic acid are extraordinarily stable to aqueous and alcoholic alkali. But their fully methylated ethers on the other hand, undergo decomposition easily with boiling aqueous alcoholic potash in the presence of zinc. The products consist mainly of neutral components isolated as viscous oils and the alkali-soluble portions are very small. There is correspondence in the behaviour of the three substances in this respect. The characterisation of the products is in progress.

Experimental

Scandenin

Molecular size.—As determined by the Micro-Zeisel method the methoxyl was 7·3%. From this the minimum molecular weight would be 425. Micro-titration using an alcoholic solution of scandenin and 0·1 N aqueous alkali also gave the value 425 for the equivalent weight (minimum molecular weight).

For obtaining the potassium salt, scandenin (1·0 g.) was dissolved in 2% potassium hydroxide (30 c.c.) by warming and the clear solution obtained after filtration was cooled. Large crystals of the potassium salt began to separate out and appeared as needles under the microscope. They were collected on a filter and washed with water to free them from adhering alkali. Finally they were washed with a small quantity of alcohol and dried in air.

The potassium salt (233·1 mg.) on ignition with two drops of concentrated sulphuric acid gave potassium sulphate (48·5 mg.). Therefore the minimum molecular weight of the potassium salt of scandenin is 418 or that

of scandenin 380. The higher yield of potassium sulphate and hence the lower value for the molecular weight of scandenin by this method may be due to some potassium hydroxide being retained by the potassium salt.

By Rast's method the molecular weight of scandenin was found to be 415, and that of its methyl ether 430. All the above results agree within limits of error with the molecular formula $C_{26}H_{26}O_6$ having a molecular weight of 434.

Sodium Salt.—Scandenin (40 mg.) was taken in chloroform (10 c.c.) and was extracted with 5% sodium carbonate solution (25 c.c.). In the first 5 minutes, turbidity developed with the separation of a solid at the interface. By shaking the mixture for some time and leaving overnight, a considerable amount of solid separated. It was filtered, washed successively with a small quantity of water, alcohol and ether and dried in air. The product left an ash on ignition, was soluble in warm water and the solution yielded scandenin on acidification. The sodium carbonate extract on acidification yielded some scandenin.

When the experiment was carried out with 5% sodium bicarbonate, neither was there any separation of the salt nor did the bicarbonate solution give any solid on acidification. Scandenin was therefore insoluble in bicarbonate.

Diacetate.—The preparation of the diacetate has already been described.[2] Its deacetylation could be effected as follows. The acetate (0·1 g.) was treated with 8% alcoholic potash (25 c.c.) when it readily went into solution giving a deep yellow colour. The solution was refluxed for ½ hour on a water-bath. Most of the alcohol was then distilled off after addition of sufficient amount of water (30 c.c.). No solid separated at this stage, thereby indicating complete hydrolysis of the acetate. The alkali solution was then acidified, the solid filtered and recrystallised from alcohol. It had a melting point 230–31° which was undepressed by admixture with scandenin.

Dimethyl ether.—The preparation of the dimethyl ether using dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium was already described. The following procedure could also be adopted.

Scandenin (1·0 g.) was dissolved in acetone (50 c.c.) and to it was added alternately in small quantities 5% aqueous alkali and dimethyl sulphate (10 c.c.) keeping the solution slightly alkaline throughout. The addition of the methylating agent was carried out in the course of 2 hours. At this stage a crystalline solid began to separate. The solution was kept overnight and then the excess of dimethyl sulphate decomposed by heating with alkali,

After the addition of a large volume of water, the crystalline material (0·85 g.) that separated was filtered. It was purified by treatment with ether, when some ether-insoluble material separated out. This was found to be the potassium salt of scandenin. The ether-soluble portion consisted of a crystalline solid (0·75 g.) which on recrystallisation from alcohol melted at 129°. It agreed with the sample of methyl ether obtained by methylation under anhydrous conditions.

Oxidation of Scandenin with Hydrogen Peroxide.—During the war strong solutions of hydrogen peroxide were not available for this experiment. The dilute solution used for pharmaceutical purposes was found to be unsuitable. However, a sample of 40 volume hydrogen peroxide gave good results and the procedure adopted is given below.

Scandenin (1·0 g.) was dissolved in 2% alkali (60 c.c.) and to the hot solution was added 8 c.c. of 40 vol. hydrogen peroxide in small portions (0·5 c.c. at a time). Towards the last stages some more alkali was added to keep the substance in solution. After complete addition of the hydrogen peroxide, the solution was boiled, cooled and filtered. Carbon dioxide was passed into the clear alkali solution and it was ether-extracted to remove the bicarbonate insoluble portion. This consisted mostly of resinous material and a small quantity of unoxidised scandenin.

The bicarbonate solution was acidified with hydrochloric acid and repeatedly extracted with ether. The combined ether extract was dried over anhydrous sodium sulphate and evaporated off. The oily liquid that remained behind gradually solidified (clusters of needles). It was purified by dissolving it again in 5% sodium bicarbonate solution and by extracting the acidified solution with ether. Finally it was recrystallised from water using a little animal charcoal. It melted at 210–11°, gave a reddish brown colouration with ferric chloride and the mixed melting point with an authentic sample of *p*-hydroxy benzoic acid was undepressed.

Lonchocarpic Acid.—The sample of lonchocarpic acid was supplied to us by the Insecticides Division of U.S. Department of Agriculture, Washington, under instructions from Dr. H. A. Jones. It was from lot No. 28–51. According to the information sent along with it, it was obtained from the original crude crystalline product of the extraction of the plant material by washing with hexane and crystallising twice from ethyl alcohol. It melted at 215–16° with sintering at 202°. It was further purified by recrystallisation from alcohol and then it melted at 220–21° without any sintering earlier. Mixed melting points with scandenin and also 'nallanin'[2] were considerably depressed. It was found to be more easily soluble in alcohol

than scandenin, was readily soluble in 5% sodium carbonate solution from which it was precipitated by the addition of acids. It did not form a sparingly soluble alkali salt like scandenin. In the Durham test, the change was from greenish yellow to brick red. With concentrated sulphuric acid a deep red solution was obtained.

The acetate was prepared by heating the substance with acetic anhydride and sodium acetate for 2 hours. It crystallised from ethyl acetate petroleum-ether mixture as rectangular rods and melted at 153–4°. The methylation of lonchocarpic acid was effected by boiling it with excess dimethyl sulphate and anhydrous potassium carbonate in anhydrous acetone medium for 12 hours. The dimethyl ether crystallised from alcohol as rectangular prisms melting at 154–55°. These agreed with the description of Jones and Haller.[4]

Robustic Acid.—Most of the properties of robustic acid have already been described.[6] Its oxidation with alkaline hydrogen peroxide was done as follows:

Robustic acid (1·0 g.) was dissolved in 2% sodium hydroxide (50 c.c.) and to the hot solution was added hydrogen peroxide (100 vols.; 10 c.c.) in small portions. Other details of the experiment were the same as in the oxidation of scandenin described earlier in this paper. No unoxidised robustic acid was found in this case and *p*-hydroxy benzoic acid was readily isolated from the acid fraction; its identity was established by comparison with an authentic specimen.

Bromo-derivatives.—The bromo derivatives were prepared by adding a solution of bromine in glacial acetic acid to a solution of the substances in the same solvent until the yellow colour persisted. The products were thrown out of the solution by the addition of a large volume of water and purified by recrystallisation from acetic acid. They are colourless solids with the exception of scandenin bromide which is yellow and are microcrystalline powders; they are easily soluble in ethyl acetate and benzene, moderately soluble in alcohol and acetic acid and very sparingly soluble in petroleum ether. They do not melt and run down the capillary tube at their melting points but either decompose or become transparent glassy masses sticking to the sides of the tube. The bromo-derivatives obtained from robustic acid, its methyl ether and acetate give characteristic deep blue solutions in sulphuric acid.

The following table gives a summary of the data relating to the bromo-derivatives.

Compound	Melting point	Bromine % Found	Bromine % Calculated for
1 Scandenin bromide ..	125° d.	27·4	$C_{26}H_{26}O_6Br_2$, 26·7
2 Scandenin acetate bromide ..	119–20° d.	22·9	$C_{30}H_{30}O_8Br_2$, 23·5
3 Scandenin methylether bromide ..	109° d.	26·6	$C_{28}H_{30}O_6Br_2$, 25·6
4 Lonchocarpic acid acetate bromide ..	125° d.	23·2	$C_{30}H_{30}O_8Br_2$, 23·5
5 Lonchocarpic acid methyl ether bromide ..	113° d.	25·9	$C_{28}H_{30}O_6Br_2$, 25·6
6 Robustic acid bromide ..	144°	46·3	$C_{22}H_{20}O_6Br_4$, 45·7
7 Robustic acid acetate bromide ..	148°	43·8	$C_{24}H_{22}O_7Br_4$, 43·1
8 Robustic acid methyl ether bromide ..	112°	44·0	$C_{23}H_{22}O_6Br_4$, 44·8

A preliminary study of the ultra-violet absorption spectra of scandenin, lonchocarpic acid and robustic acid in chloroform solution has now been made. The absorption regions at different concentrations agree closely and the maximum absorption for 0·002% solution is found between 3700 and 3100 A.U. Under the same conditions rotenone has the absorption band between 3300 and 2700 A.U. (see also Cahn, Phipers and Boam[5]). When compared with rotenone the absorption regions of the non-rotenoids are definitely displaced towards longer wave-lengths. The results would indicate the presence of a carbonyl group in conjugation with ethylenic bonds.

Substance	Absorption region
1 Rotenone ..	3300–2750 A.U.
2 Scandenin ..	3700–3100 ,,
3 Lonchocarpic acid ..	3700–3100 ,,
4 Robustic acid ..	3720–3000 ,,

Toxicity to fish.—Small fresh-water fish, *Haplochilus panchax*, were employed. The details of the method are the same as already described by Krishnaswamy and Seshadri.[7] The results are given in the following table:

ge = gelatin (1 gram) added per litre of water (see Murti and Seshadri).[8]

Compound	Concentration mg./litre*ge*	Turning time in minutes	Remarks
Scandenin ..	20	21·0	
	40	11·0	
Scandenin methyl ether ..	50	No effect in 4 hours	
Lonchocarpic acid ..	50	21·0	The fish did recover and died
Robustic acid ..	50	25·5	
Rotenone ..	1	6·5	

Our thanks are due to Dr. H. A. Jones for the sample of lonchocarpic acid used in this investigation.

SUMMARY

A more detailed study of scandenin is made and its important properties compared with those of lonchocarpic and robustic acids. The three non-rotenoids show marked resemblance. They do not give the Durham test, and are definitely acids owing this property not to carboxyl groups but to specially active hydroxyl groups. They are quite stable to aqueous and alcoholic alkali. The fully methylated ethers have three methoxy groups in all the three cases and these ethers undergo decomposition readily in alcoholic alkali in the presence of zinc, the main products being neutral in nature. The three compounds do not give chemical tests for the presence of carbonyl groups, exhibit definite toxicity to fish and have very similar ultra-violet absorption spectra. The toxicity and the spectra indicate the presence of carbonyl groups in conjugation with ethylenic bonds.

Oxidation with alkaline hydrogen peroxide yields *p*-hydroxy-benzoic acid with all the three compounds. In acetic acid solution scandenin and lonchocarpic acid add on two atoms of bromine; whereas robustic acid takes up 4 bromine atoms.

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ERRATUM

In Part II, Chemical Components of *Derris scandens*, *Proc. Ind. Acad. Sci.*, A, 1946, **24**, 374, *read* 'South Chanda Division' *for* 'South Canada Division'.