30. *The Active Principles of Leguminous Fish-poison Plants. Part VI. Robustic Acid.*

By Stanley H. Harper.

A substance isolated from the ethereal extract of *Derris robusta* has been shown to be a monocarboxylic acid, $C_{27}H_{24}O_8$ (less probably $C_{28}H_{26}O_8$), containing two methoxyl groups, to which the name *robustic acid* has been given. It is probably related to lonchocarpic acid, $C_{26}H_{26}O_6$, a monocarboxylic acid containing one methoxyl group isolated by Jones (*J. Amer. Chem. Soc.*, 1934, **56**, 1247) from a species of *Lonchocarpus*, as they both give the same sequence of colour changes in the Durham test.

In the course of examination of various plant materials for their insecticidal activity several species of *Derris* and *Derris* products have been tested. Among these was a colourless crystalline substance isolated from the ethereal extract of *Derris robusta* and forwarded by Dr. S. Krishna of the Forest Research Institute, Dehra Dun, India. Although this substance was non-toxic to *Aphis rumicis* (the bean aphis), a chemical examination has been made as far as the limited amount of material would allow.

The substance separated from the ethereal extract of *D. robusta* and crystallised from acetone or alcohol contained methoxyl groups but did not give a blue colour in the Durham test (see footnote, J., 1939, 1100). It was optically inactive in acetone or benzene solution. These properties are closely in accord with those of tephrosin, but, although the substance did not give a ferric chloride colour, it dissolved in aqueous potassium hydroxide to give a sparingly soluble potassium salt, from which the original material was recovered on acidification. Confirmation of its acidic properties was obtained by shaking an ethereal suspension with 5% sodium carbonate solution; the material slowly dissolved in the aqueous layer and could be recovered by acidification. This substance is therefore regarded as a carboxylic acid and the name *robustic acid* is proposed.

Analyses of robustic acid and of derivatives described subsequently for carbon and hydrogen do not distinguish between $C_{27}H_{24}O_8$ and $C_{28}H_{26}O_8$ for robustic acid and corresponding formulæ differing by CH_2 for the derivatives. The methoxyl contents, corresponding to two methoxyl groups in robustic acid, are more closely in accord with the formulæ with less carbon atoms, but the evidence is not sufficiently conclusive to enable a decision to be made. Analysis of the *potassium* salt shows the acid to be a monocarboxylic acid. Esterification in benzene with diazomethane gave the *methyl* ester. Attempts to show the presence of a keto-group by oximation were unsuccessful, but in view of the difficulty of oximating rotenone this cannot be regarded as proof of the absence of a keto-group. Catalytic hydrogenation to *dihydrorobustic acid* shows the presence of one double bond, and the slow rate of reduction suggests this to be cyclic (cf. the ease of reduction of rotenone with that of elliptone).

That the titration of robustic acid in hot alcohol, which leads to conflicting results, is not a true neutralisation, as it is in aqueous solution, is shown by the non-recovery of the acid on acidification; no definite products have so far been obtained.

Prior to this there has been only one instance of an acid being reported from *Derris* or *Lonchocarpus* species. This material, lonchocarpic acid, $C_{26}H_{26}O_6$, m. p. 201°, a monocarboxylic acid containing one methoxyl group, was isolated by Jones (*J. Amer. Chem. Soc.*, 1934, **56**, 1247) from an unknown species of *Lonchocarpus* from Venezuela. Through the kindness of Dr. H. A. Jones it has been possible to make a comparison of the two substances. Both behave similarly in the Durham test but in marked contrast to rotenone and toxicarol.

Because of their closely related origin and their similarity of behaviour in the Durham test it is thought that robustic and lonchocarpic acids are related both to each other and to rotenone, but from a consideration of their formulæ there does not appear to be any simple relationship between them. Robustic acid is not the methoxy-homologue of lonchocarpic acid, since this would necessitate a formula, $C_{27}H_{28}O_7$, which is ruled out by the analytical figures.

It is hoped to continue the investigation of robustic acid when more favourable conditions occur.

Experimental.

Microanalyses are by Drs. Weiler and Strauss, Oxford. Methoxyl determinations are by the author, using Clark's semimicro-method (*J. Assoc. Off. Agric. Chem.*, 1932, **15**, 136). Melting points are uncorrected.

Robustic acid crystallised from acetone or alcohol in colourless elongated prisms, m. p. 190°. Qualitative tests showed the absence of nitrogen, halogens, and sulphur (Found : C, 68·4, 68·5; H, 5·1, 5·1; OMe, 13·1, 13·2; *M*, Rast, 496, 451. $C_{27}H_{24}O_8$ requires C, 68·1; H, 5·1; 2OMe, 13·0%; *M*, 477. $C_{28}H_{26}O_8$ requires C, 68·6; H, 5·3; 2OMe, 12·7%; *M*, 491. $C_{27}H_{28}O_7$ requires C, 69·8; H, 6·1; 2OMe, 13·4%).

Titration in hot alcohol with sodium hydroxide gave a sharp end-point, but in excess of the theoretical value, and on acidification the acid was not recovered (53·65 mg. required 7·05 c.c. of 0·02N-sodium hydroxide. Calc., 5·62 c.c.).

By shaking the acid in ethereal suspension with 5% aqueous potassium hydroxide, immediate solution resulted, followed shortly by separation of the sparingly soluble *potassium* salt in colourless plates (50·0 mg. on ignition gave 7·4 mg. of potassium sulphate. Found : equiv. 550). The silver salt decomposed on drying.

Oxidation with iodine in alcohol in the presence of sodium acetate gave no recognisable product (cf. the formation of dehydrorotenone from rotenone). Robustic acid could not be methylated with methyl sulphate and alkali, but in one experiment, in which the reaction mixture was left overnight, a small quantity of substance, m. p. 167°, was obtained, insoluble in alkali. In the Durham test it gave the same sequence of colour changes as the acid, but the quantity was too small to permit further investigation (Found : OMe, 16·9%).

Esterification with diazomethane in benzene gave the *methyl* ester, which crystallised from chloroform–alcohol in plates, m. p. 190°, was insoluble in potassium hydroxide, and gave a marked depression of m. p. with robustic acid. In the Durham test the ester gave a brown colour with nitric acid, followed by a crimson colour on addition of ammonia (Found : C, 68·7; H, 5·3; OMe, 19·6. $C_{28}H_{26}O_8$ requires C, 68·6; H, 5·3; 3OMe, 19·0. $C_{29}H_{28}O_8$ requires C, 69·0; H, 5·6; 3OMe, 18·4%).

Robustic acid (250 mg.) was hydrogenated in ethyl acetate (20 c.c.) in the presence of platinum oxide (50 mg.). Absorption was slow, but a sharp break occurred corresponding to 1 mol. of hydrogen. After filtration and evaporation, the residue crystallised from alcohol to give oblique elongated parallelepipeds of *dihydrorobustic acid* (212 mg.), m. p. 180° (Found : C, 68·2, 68·0; H, 5·8, 5·8; OMe, 13·2. $C_{27}H_{26}O_8$ requires C, 67·8; H, 5·5; 2OMe, 13·0%. $C_{28}H_{28}O_8$ requires C, 68·3; H, 5·8; 2OMe, 12·6%). The crystals had a marked violet fluorescence. Esterification in benzene with ethereal diazomethane gave the *methyl* ester, which crystallised from chloroform–alcohol in prisms, m. p. 207—208° (Found : C, 68·85; H, 6·0; OMe, 20·0. $C_{28}H_{28}O_8$ requires C, 68·3; H, 5·5; 3OMe, 19·0%. $C_{29}H_{30}O_8$ requires C, 69·2; H, 6·0; 3OMe, 18·4%). Both this and the previous ester showed a tendency to give high values for methoxyl.

Durham test : The following sequence of colour changes was observed :

	HNO_3 ⟶		NH_3 ⟶
Robustic acid		green	red
Lonchocarpic acid		green	red
Toxicarol		red	green
Rotenone		red	blue

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