

# A NOTE ON GLABRIN, A NEW COMPONENT OF THE SEEDS OF *PONGAMIA GLABRA*

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IN a previous communication,<sup>1</sup> it was reported that by the extraction of the seed-cake of *Pongamia glabra* higher fatty acids along with some karanjin could be obtained. In our search for the presence of any further insecticidal principles, the cake was next subjected to extraction with alcohol and from this was obtained a small yield of a crystalline nitrogenous substance. Since its properties and reactions are not identical with any known compound, it is considered to be a new substance and it has been named Glabrin. It is best obtained from mature seeds since otherwise it has been noticed that the yield becomes practically negligible.

Glabrin melts at 290° with decomposition and has the empirical formula  $C_7H_{14}NO_4$ . It is very sparingly soluble in all the organic solvents. On the other hand it goes readily into solution in water. It is optically active and exhibits all the properties of  $\alpha$ -amino acids of the dicarboxylic type, the most important being the ninhydrin test and copper salt formation. Attempts to determine the molecular weight have not been quite successful. However, titration with alkali without formalin and with the addition of formalin (Sorensen's titration) fixes the minimum molecular weight at 580 and consequently the molecular formula may be tentatively given as  $C_{21}H_{42}N_3O_{12}$ . Attempts to hydrolyse it using hydrochloric acid were not successful.

Since the yield of the substance is very low (0.1%), its extraction extremely slow and since it has no marked physiological properties, no further detailed work has been undertaken.

## *Experimental*

The seed-cake of *Pongamia glabra* (3 kg.) left behind after extraction of the seeds with petroleum ether was freed from the solvent and was subsequently extracted with methylated spirits in a continuous extraction apparatus. The process was very slow and therefore it was continued for 60 hours. The receiver containing the extract was set aside for a few days to complete the deposition of the crystalline solid that began to separate. It was then

filtered and washed with light petroleum. On careful examination it was found to be a mixture and that the components could be separated easily because of their differences in solubility in alcohol. The one that went easily into solution in hot alcohol was identical with karanjin in all its properties. The second which was sparingly soluble in all the common organic solvents could not be recrystallised in the normal manner; but, however, advantage was taken of its solubility in water. The substance (3 g.) was dissolved in the minimum amount of water (10 c.c.) and then filtered so as to remove all the adhering impurities. The clear solution was then decolourised with vegetable carbon. Since attempts to obtain the substance in a pure condition, as for example by evaporating the aqueous solution, were unsuccessful, alcohol was added to the colourless aqueous solution in order to precipitate the substance. As the addition of alcohol did not bring about the separation of the substance, a large excess of ether was added to the aqueous alcoholic solution. Glabrin separated out as a shining colourless crystalline solid (rectangular plates). The mixture was allowed to remain overnight to facilitate the complete precipitation of the substance and was then filtered and air-dried.

Glabrin was found to melt with decomposition at  $290^{\circ}$  and contain nitrogen. [Found: C, 47.8; H, 8.0; N, 8.0%;  $C_7H_{14}NO_4$  requires C, 47.7; H, 8.0; N, 8.0%]. It went easily into solution in aqueous alkalis and acids. Further, its aqueous solution gave acidic reaction to phenolphthalein and litmus and produced effervescence with sodium carbonate and bicarbonate, indicating thereby the presence of one or more free carboxylic groups in the molecule. It exhibited optical activity and had a specific rotation,  $-56.1^{\circ}$ .

When an aqueous solution of glabrin was boiled with alkali, no ammonia was evolved. Further, it did not produce any precipitate with Nessler's reagent. This eliminates the possibility of glabrin being an ammonium salt. However, it produced a deep blue colour when a pinch of copper carbonate was added to its aqueous solution and the resulting copper salt was found to crystallise in leaflets. It did not give the tests for an amino group. But on reduction with sodium and alcohol, it responded to the carbylamine reaction. In this connection it has been found that aspartic acid also does not directly give the carbylamine reaction. Glabrin gave a blue colour when boiled with ninhydrin. This is a test which all acids having a free amino group in the  $\alpha$ -position to a free carboxylic group give. It is known that ninhydrin reaction is very sensitive in cases of amino acids and that ammonium salts also give a positive reaction provided the concentrations are sufficiently high. But the latter possibility has been ruled out as already described.

A solution of glabrin in water was titrated with standard alkali without and with the addition of formalin. The amount of alkali consumed in the latter titration was double that in the former. This clearly indicates the presence of one amino group and two carboxylic groups in the molecule. The calculated equivalent weight of the substance from the above data was 580.

Glabrin was subjected to acid hydrolysis using 20% hydrochloric acid with a view to obtain simpler products which could be more easily identified. But the resulting product was found to melt at the same temperature as glabrin and the mixed melting point of the substance was not depressed.

The toxic effect of glabrin on fish was studied by placing small fresh water fish in a solution of the substance in water (1 g. in 1000 c.c.). As there was no perceptible change in the fish even after 24 hours, it was concluded that glabrin is non-toxic fish.

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#### *Summary*

A new nitrogenous substance has been isolated from the seeds of *Pongamia glabra* and has been named Glabrin. Its composition and properties have been studied. It seems to belong to the group of complex amino acids and is non-toxic to fish.

#### REFERENCE

1. N. V. S. Rao and J. V. Rao .. *J.I.C.S.*, 1940, 27, 526.