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STRUCTURE OF GLABRIN

P.S. Sampath Kumar, V.V.S. Murti and T.R. Seshadri,
Department of Chemistry, University of Delhi, Delhi-7. India.

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Pongamia glabra is one of the commonest trees in India. For a long time, all parts of this tree have been known to possess curative action against various diseases.[1] From the seeds several furanoflavonoids have been isolated and their structures completely established. Glabrin was first isolated by Rao and Rao[2] in 1941 and later by Aneja et al.[3] in 1963, from the alcoholic extract of the ripe seeds. They fixed the mol. formula as $C_{21}H_{42}N_3O_4$ on the basis of Sorensen's titration. Due to its neutral behaviour towards many reagents and negative colour tests, even a tentative structure was not then possible. Hence, a detailed chemical and spectral study has now been undertaken to arrive at the correct structure of glabrin.

Chromatographically pure glabrin was obtained by crystallising the crude sample from alcohol-water-ether mixture. It melts with decomposition at 290-92°. It is chemically neutral and optically active, $[\alpha]_D^{20°}$ -54.82(5N HCl). It is insoluble in all common organic solvents except alcohol in which it is difficultly soluble, but is readily soluble in water. The compound isolated by us is in complete agreement with the older samples in regard to m.p., IR, rotation and TLC. But the analytical results are in better agreement with the formula $C_7H_{13}NO_4$ which is also supported by the mol. wt. 175 by mass spectrometry (mass calculated for $C_7H_{13}NO_4$ 175.0845, mass matched; 175.00, mass found; 175.0845).

Glabrin does not answer tests for amino acids, alkaloids or peptides. Further, it fails to answer the hypochlorite-iodate[4] and isatin tests[5] suggesting that it is neither a proline nor a pyrrolidine derivative.

It has no absorption in the UV region (220 to 360 nm) which shows that it is not unsaturated or aromatic in nature. The IR indicated -OH group (3509 cm^{-1}), C=O of carboxylate (1629 cm^{-1}) and a $=N^{\pm}$ group (1408 cm^{-1}). Though a band at 3330 cm^{-1} characteristic of diketopiperazines is present, this structure is not possible since, when glabrin was subjected to hydrolysis with 6N HCl, the hydrolysate failed to give the ninhydrin colour reaction. Its solubility, m.p., inertness towards most of the reagents and the presence of nitrogen were suggestive of pipecolic acid derivative. Further, pipecolic acid derivatives occur in fruits and seeds of leguminous plants, and P. glabra is a legume.

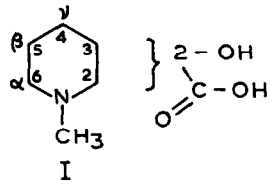
Glabrin formed a methyl ester with diazomethane; in its IR spectrum the band at 1629 cm^{-1} due to the carboxylate was absent and another peak due to the ester carbonyl appeared at 1727 cm^{-1}.

Glabrin formed a diacetate on acetylation with acetic anhydride and perchloric acid as catalyst. Its IR spectrum had strong peaks of the acetate at 1745 cm^{-1} and 1215 cm^{-1}. The NMR spectrum confirmed the presence of two acetoxyls by the signal at 2.9 δ integrating to six protons.

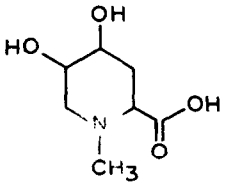
Glabrin formed an isopropylidine derivative when treated with acetone and a trace of con. HCl. It was purified by passing through a column of silica gel and eluting with ethyl acetate. The acetonide was a reddish brown viscous oil and showed broad intense peaks in the IR at 1370-1375 cm^{-1} corresponding to the gem-dimethyl group in the molecule. Further, in its NMR spectrum a signal at 1.22 δ integrating to six protons appeared.

Thus the presence of two hydroxyls in glabrin present at 1,3- or 1,2-glycol system was inferred. To settle between the alternatives, glabrin was treated with excess of sodium meta periodate and the amount of periodate consumed was determined by titrating with standard $Na_2S_2O_3$. One mole of glabrin consumed one mole of periodate thus fixing the hydroxyls as 1,2-glycol unit. $N\text{-}CH_3$ estimation indicated the presence of one such group.

Thus in the molecule of glabrin one N-CH_3 group, a 1,2-glycol unit and a -COOH group are present accounting for all the oxygen atoms and the nitrogen atom (partial formula I).

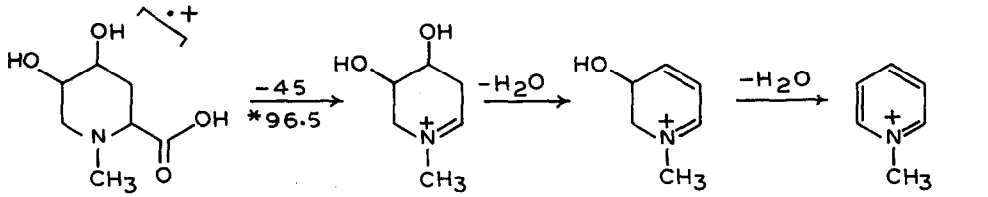


I



II

The α,β-dihydroxypiperidine carboxylic acid structure is ruled out since it embodies a carbinol -amine system which should make it unstable under acidic conditions[6,7] and hence the β,ν-structure is favoured. Then, there are three possibilities for the position of the -CO_2H group at 2,3 or 6. Glabrin has an intense band in IR at 1629 cm^{-1}, characteristic of the carboxyl α- to the N-atom, as found in the cases of L-pipecolic acid[8] and other α-amino acids. Of the two alternatives the mass spectrum of glabrin is best explained by structure (II). The intense peaks in the spectrum are at m/e 175, m/e 130, m/e 112 and m/e 94. The loss of 45 units is due to the loss of carboxylic group and is supported by the appearance of a meta stable peak at m/e 96.5. The other peaks at m/e 112 and m/e 94 are due to the loss of water in succession. These fragments can be rationalised as follows.



The structure (II) assigned for glabrin is also supported by its NMR spectrum: (CF_3COOD, 100 MHz); δ 2.36 and 2.76 (2H, multiplet, C_3-methylene),

3.34 (3H, s, N-CH_3), 3.76 (2H, d, C_6-methylene), 4.45-4.60 (3H, complex multiplet, C_2, C_4 and C_5-methines). The above data clearly fit the structure II and rule out alternatives. The other methylene signal viz. at C_3 is complex probably due to non-equivalence of the C_3-methylene protons and further coupling with the C_2 and C_4 protons. Hence glabrin has been assigned the structure of 4,5-dihydroxy-N-methyl pipecolic acid (II). In the case of glabrin most of the signals appeared relatively downfield and this downfield shift is ascribable to the influence of the solvent CF_3COOD employed. A similar observation has also been made both by Davis[9] and Booth *et al.*[10] To verify this solvent effect, a study of the NMR spectrum of the acetonide of glabrin was made in $CDCl_3$. As expected the signals now appeared in their expected positions and also in conformity with the structure assigned. NMR of acetonide of glabrin ($CDCl_3$, 60 MHz): δ 1.22 (6H, gem-dimethyl), 1.52 (1H, C_3-methylene), 1.85 (1H, C_3-methylene), 2.29 (3H, N-CH_3), 2.9 (2H, C_6-methylene and 3.25 (3H, C_2, C_4 and C_5-methines).

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