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CHEMICAL STUDIES IN THE GERMINATION METABOLITES OF PEGANUM HARMALA LINN

SALIMUZZAMAN SIDDIQUI, Z.H. ZAIDI and T. BURNEY

Postgraduate Institute of Chemistry, University of Karachi, Karachi 32

The changes in alkaloidal, amino acid and carbohydrate constituents at various stages of growth of *Peganum harmala* seeds were studied in detail. The seeds were first extracted with ether to remove fatty matter and then exhaustively extracted with alcohol. From the alcoholic extract bases were isolated and the base-free fraction was studied for amino acid and carbohydrate contents.

Peganum harmala Linn. is an important alkaloidal plant of economic importance which grows in abundance in Pakistan and widely occurs in other tropical countries. Goebel[1] was the first to report the presence of a base, harmaline, ($C_{13}H_{14}ON_2$), in the seeds of *Peganum harmala* in 1841. In 1847 Fritsche[2] isolated harmine ($C_{13}H_{12}ON_2$) while harmalol was obtained by Goebel and prepared by Fischer,[3] from harmaline. The fourth alkaloid peganine was isolated by Merk and later identified with vasicine[4], reported earlier by Hooper[5] from *Adhatoda vasica* Nees. 1,2,3-(Hydroxytrimethylene)-4-quinazoline and 2,3-trimethylene-4-quinozolone were isolated by Korestkaya[6] in 1958; and oxypeganine and oxopeganine were reported by Plekhanova[7] and Akanova in 1966. In 1962 Siddiqui[8] reported the isolation of an alkaloid harmidine, which melted at 258°C as against m.p. 239°-40°C recorded for harmaline, which was subsequently shown to be a mixture of harmine and harmidine. Siddiqui and Kemal[9] also reported the percentage yields of harmine and harmidine in the samples of *Peganum harmala* from different countries.

As against these exhaustive studies in the alkaloids of *Peganum harmala*, comparatively little attention has been given to its nonalkaloidal constituents. In 1960, Handa *et al.*[10] investigated the seed oil and reported its chemical composition. In 1964, Siddiqui and Kemal[9] communicated the isolation of an amino acid from the alcoholic extract of the seeds which melted at 295°C, analysed for $C_6H_{11}O_3N$, and was provisionally named as pegaline. From the preliminary structural studies it appeared to be a homologue of proline but it was subsequently identified by Ahmad and Khan[11] with 4-hydroxypipecolic acid in 1971. In 1969, Nahid and Zaidi[12] carried out a quantitative study of the amino acid and carbohydrate constituents of the alcoholic extract of the seeds after removal of the fatty and alkaloidal constituents.

In the present investigation the differences that occur in the alkaloidal, amino acid and carbohydrate constituents of *Peganum harmala* seeds, at various stages of germination, extending over a period of 6,10, and 20 days, were studied in detail. These results have been compared with the amino acid and carbohydrate constituents of the seeds reported by Nahid and Zaidi.[12]

Experimental and Results

The seeds were ground to a paste and exhaustively extracted with alcohol at room temperature (4 to 6 times) until a fresh extract contained only a negligible quantity of alkaloid. The bright red thick liquid obtained as residue after removal of the solvent under reduced pressure was digested with petroleum ether, to separate off the fatty matter. The ether-insoluble residue was treated with 10% ammonia and the total liberated bases were sucked and washed with water. The filtrate was repeatedly extracted out with ethyl acetate and then amyl alcohol to remove the residual bases. The aqueous phase was then repeatedly extracted out with ether to remove the major portion of dissolved amyl alcohol, and freed of the solvent *in vacuo*. The water-soluble residue B was subjected to quantitative studies in the amino acids.

(A) Bases

The isolation of the individual bases, harmidine, harmine and vasicine, from the total alkaloidal fraction A was carried out according to the general procedure recorded by Siddiqui.[8] Apart from these alkaloids a reddish yellow base which was previously isolated only in traces was obtained as a hydrochloride in crystalline form from seeds germinated over a period of 20 days. It was insoluble in most of the organic solvent, but crystallised out as a hydrochloride from alcoholic hydrochloric acid. It charred without melting at 200°C.

(B) Amino Acids and Carbohydrate

Amino acids and carbohydrates were separated on an Amberlite IR 120 packed column. First, the elution was carried out by deionised water and when the carbohydrates (B-1) were removed, the elution of amino acids (B-2) was achieved through 3% ammonia solution.

TABLE 1.—CARBOHYDRATE CONTENTS OF *Peganum harmala* SEEDS AT VARIOUS STAGES OF GERMINATION.

	Seeds	6 days	10 days	20 days
D-Glucose	+	++	++	++
D-Glactose	+	+	+	+
Sucrose	+	+	+	+
Arabinose	—	++	++	+
Lactose	—	+	+	+

(*B1*) *Carbohydrate.*—The carbohydrate fraction was evaporated *in vacuo* to a small volume and its composition was determined by paper chromatography. The solvent system, butanol–acetic acid–water (4:1:5) was used and the elution was carried out for 72 hr. The chromatogram was sprayed with aniline pthalate and dried at 80°C for 10 min. The sugars found to be present are shown in Table 1.

(*B2*)*Amino Acid.*—The quantitative estimation of amino acids was carried out by paper chromatography, using (a) n-butanol–acetic acid–water (4:1:5), n-butanol–pyridine–water (5:5:2) and (b) phenol–water (8:2) in the second dimension. After drying, the paper was sprayed with Cd–ninhydrin (0.5 g of ninhydrin in 50 ml of acetone, to this was added a solution of 0.05 g cadmium acetate dissolved in 1 ml glacial acetic acid and 5 ml of distilled water), dried in an oven for 10 min at 80°C and developed in a tank containing concd sulphuric acid for 3 hr. The spots were identified, extracted and the optical densities of their methanolic extracts (5 ml) were recorded on Unicam spectrophotometer S.P. 600, at 500 nm in 1-cm cell. From the optical densities μ moles of each amino acid was calculated with the help of calibration curves of the standard amino acids. Amino acid composition of 6,10 and 20 days is shown in Table 2.

TABLE 2.—AMINO ACID COMPOSITION OF *Peganum harmala* SEEDS AT DIFFERENT STAGES OF GERMINATION.

	μ moles/g			
Amino acid	Seeds10	6 days	10 days	20 days
Alanine	0 054	1 72	3 21	6 64
Threonine	T	0 382	1 70	3 51
Serine	T	0 1	0 14	0 186
Lysine	T	0 527	0 41	0 216
Crystine	—	T	0 10	0 369
Phenylalanine	0 011	0 500	0 45	3 095
Valine	0 062	0 302	0 41	0 754
Tyrosine	T	0 177	0 23	0 423
Leucine/isoleucine	0 032	2 146	2 27	4 44
Histidine	0 005	—	—	—
Glycine	0 007	—	—	—
Proline	0 054	+	+	+

Discussion

During the course of investigations it was observed that the alkaloidal contents decreased with increase in the period of germination. This finding is in agreement with the reports of earlier workers in regard to the decrease of the alkaloidal contents in the course of germination of other alkaloid-bearing seeds. In respect of the comparative percentage of harmine and harmidine in the germinated seeds, it has been noted in the present work, that the balance between harmine and harmidine shifts in favour of the former, as a result apparently of an oxidative process. It was striking to note that alkaloids were completely absent in the sprouts picked out of the seeds germinated over a period of 20 days. The qualitative estimation of carbohydrates indicate their utilization during germination and also the breakdown of polysaccharides. The amino acid composition shows marked quantitative variations at different stages of germination, their overall increase with the period of germination being probably due to the breakdown of seed protein.

The present work provides some interesting indications as to the fate of the alkaloidal constituents of the seeds in the process of germination. Further work under varying conditions of germination is projected to arrive at more definite conclusions regarding the role of the alkaloidal bases in the germination process.

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