

SOME STRUCTURAL FEATURES AND NEUROTOXIC ACTION OF A COMPOUND FROM *LATHYRUS SATIVUS* SEEDS

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'NEUROLATHYRISM' a syndrome characterized by such profound neurological disturbances as weakness, irritability, spasticity and rigidity of leg muscles, paralysis and at times death, has been described in humans subsisting for prolonged periods on the seed-meal of certain legumes belonging to the genus of *Lathyrus*.^{1,2} The existence of this crippling disease in an endemic form among the poor people in certain regions of Central India accustomed to consume *Lathyrus sativus* seeds (Kesari dal) as the major dietary constituent has been reported.³ However, the exact chemical and biochemical characteristics of the neurolathrogen(s) present in the pulse have not been elucidated so far. The lack of a convenient experimental organism capable of responding to the neurotoxic principles present in the seed has been the chief obstacle which impeded any progress. In a recent communication from this laboratory,⁴ we have detailed a procedure for the bulk isolation, from the aqueous ethanol-soluble fraction of the seed-meal of *L. sativus*, of a new ninhydrin-positive principle, present in the free form and toxic to several micro-organisms in minute amounts. Further, some of the physico-chemical properties of this compound and its degradation products have also been reported. This communication deals with our further findings on its structural aspects and profound neurotoxic action in experimental chicks.

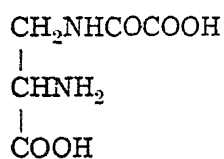
Earlier observations⁴ that the toxic principle yielded, on acid and alkaline hydrolysis, another ninhydrin-positive compound, distinctly different

from the parent compound in its behaviour on paper chromatograms, ion-exchange column chromatograms (Dowex-50) and in electrophoretic mobility, has now been followed up by the bulk isolation of the degradation product by ethanol precipitation from acid hydrolysates of the parent compound and crystallization from methanol. On the basis of its elementary analysis, melting point, paper chromatographic and electrophoretic characteristics, and the properties of its dipicrate and difluoroborate derivatives, it has been identified as α , β -diamino propionic acid, a C_3 -diamino monocarboxylic acid. It had a molar rotation $[M]_D^{22}$ of + 28.6 ($c = 2$, 6N HCl) establishing thereby that it is of L-configuration. When tested with ninhydrin according to Rosen⁵ the colour yield was 33% of that given by leucine on a molar basis, in agreement with the reported value in literature.⁶ A comparison of the solid-phase infra-red spectra (KBr disc) of its monohydrochloride with that of an authentic sample of L- α , β -diamino propionic acid monohydrochloride lends support to its identity with the proposed structure.

Absence of a positive reaction with Tollens reagent ruled out the possibility of an aldehyde type of group in the original toxic compound and its infra-red spectra did not show any characteristic peaks corresponding to either a —CN-function or a lactone ring. The elementary analysis indicated a difference of a C_2 -fragment between the parent compound and the diamino propionic acid moiety and this C_2 -fragment has since been isolated in a pure form from

the acid hydrolysates by repeated ether extraction and crystallization from ethanol. The latter fragment was identified as oxalic acid on the basis of its elementary analysis, melting point, the ability to decolorize acidic permanganate, behaviour on paper chromatograms and the colour reactions with indole⁷ and diphenylamine⁸ and the properties of its complex with ethanolamine.⁹ Quantitative estimation of both the diamino propionic acid and oxalic acid in the acid hydrolysate of the original compound showed their presence in 1 : 1 molar ratio.

The coupling of the parent compound with fluorodinitrobenzene (FDNB) of Sanger,¹⁰ yielded a DNP-derivative from which a free amino group could be liberated on hydrolysis with 6 N HCl. Further reaction with FDNB yielded a di-DNP amino-acid with characteristics identical with those of di-DNP diamino propionic acid. This, together with the loss of colour reaction with ninhydrin on treatment with $\text{Cu}(\text{NO}_3)_2$ reagent,¹¹ suggests the presence of only one free primary amino group in the original compound, the other being involved in the form of an acid-labile group possibly in the nature of $-\text{CONH}$ function. In support of this is its solid state infra-red spectra showing strong peaks characteristic of such a group. That, it is the α -amino group which is free, is indicated by ninhydrin colour reagent yield obtained with the original compound, which corresponds to 93% of that obtained with leucine on a molar basis, assuming the molecular weight of 175 for $\text{C}_5\text{N}_2\text{H}_7\text{O}_5$. On the basis of these observations, the following tentative structure for the toxic compound has been proposed.



β -N-oxalyl, α , β -diamino propionic acid. The total chemical synthesis to provide unequivocal proof for the proposed structure is currently in progress in this laboratory.

In view of the pronounced toxicity of this pure compound towards several micro-organisms,⁴ its influence on the development of the larva of *Corcyra cephalonica* St. has also been investigated, since in earlier experiments⁴ this organism has been shown to respond to the toxic principles of *L. sativus* seeds. It was found that the inclusion of the pure compound at 0.5% level in the basal wheat flour diet¹² inhibited the larval growth by over 60%.

In conformity with our earlier observations⁴ that the toxic principles reside in the aqueous ethanol-soluble fraction of *L. sativus* seeds, is the recent observation of Roy *et al.*¹³ who have demonstrated the development of neurological symptoms in chicks following injection of the concentrates of the aqueous ethanol-soluble fraction of *L. sativus* seeds. The pure toxic principle isolated from the pulse has, therefore, been examined for its neuro-lathrogenic action in chicks and was found to possess pronounced neurotoxic action.

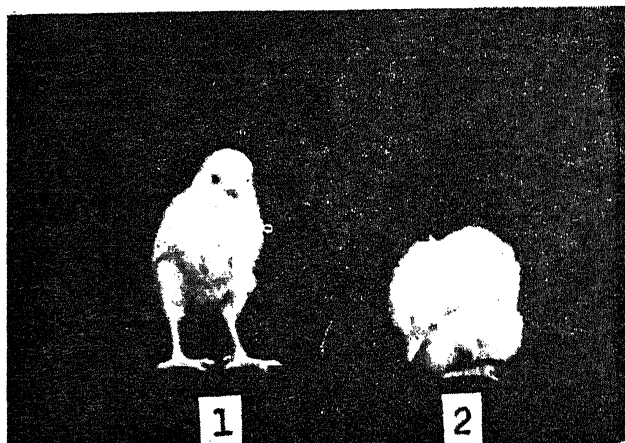


FIG. 1

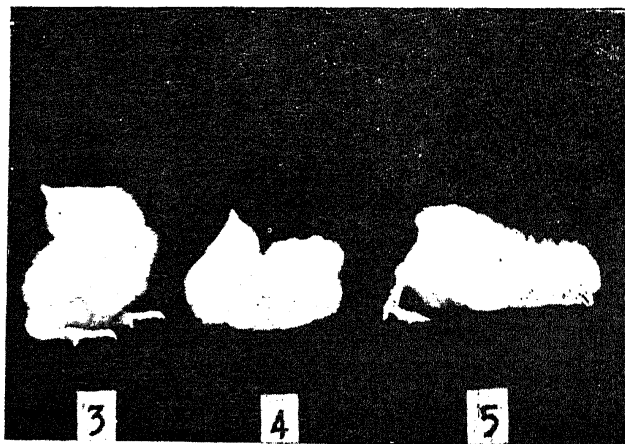


FIG. 2

FIGS. 1-2. No. 1. Control Chick. Nos. 2-5. Progressive symptoms in chicks administered toxic compound.

White Leghorn chicks, one day old and weighing 35-40 g./chick, obtained from the Central Poultry Farm, Hesserghatta, Bangalore, and maintained on the stock diet in electrically heated brooders were administered the toxic compound in aqueous solution (sodium salt, pH 6.8-7.0) at the level of 20 mg./chick. The chicks receiving the same amount of distilled water or the same molar concentration of L-glutamic acid in distilled water (pH 7.0) served as controls. Within 20-30 mins. after

a single intraperitoneal injection, all chicks (36 in number) developed typical and acute neurotoxic symptoms like inability to stand, head retraction, stiffening of the neck and extensor paralysis of the legs (Figs. 1, 2, Nos. 2-5). Administration through oral and subcutaneous routes required longer time (2-6 hours) to precipitate similar symptoms. At lower dosages (20-30 mg./chick) the birds recovered within 8-12 hours, while with larger dosages (30-60 mg./chick) or with continued daily dosage at lower levels the symptoms persisted even after 24 hours and became chronic in some and proved lethal to others. Injection of thiamine as hydrochloride (2 mg./chick) either before or after administration of the toxic principle neither prevented nor alleviated the toxic symptoms. It was also found that unlike the toxic substance intraperitoneal injection of either α, β -diaminopropionic acid or L-homo-arginine (recently isolated and characterized from *L. sativus*¹⁴) at comparable levels did not bring about any visible neural developments in experimental chicks. Detailed investigations are currently in progress in this laboratory to elucidate the biochemical and pharmacological basis of its neurotoxic action in this and other organisms.

The financial assistance of the Council of Scientific and Industrial Research, New Delhi, and The Rockefeller Foundation, New York, is gratefully acknowledged. Thanks are due to Dr. M. Sirsi for helpful discussions.

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