A NEW AMINO ACID, "PRECATORINE"

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The seeds of Abrus precatorius which grow wild in the southern part of Taiwan are claimed to be powerfully poisonous(1,2). Since 1932 the chemical components of Jequirity seeds have been studied by many Among the chemical comauthors(8-9) poner's, two indole positive substances, i. e. abrine (a-N-monomethyl-L-tryptophan) and hypaphorine (the betaine of L-tryptophan). have been identified. Both are the derivatives of an important essential amino acid, Ltryptophan. For the presence of these two L-tryptophan derivatives, we believe that there is an enzyme system which converts abrine to hypaphorine by methylation, or in the reverse by demethylation, and there might be an intermediate substance, i. e. a-N, N-dimethyl-L-tryptophan occurring in Jequirity seed. Since our laboratory is especially interested in the studies of indole derivatives, the purpose of the investigation was to isolate this new amino acid, and to study its physiological functions.

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In our laboratory, the new amino acid was crystallized and we named it "precatorine". In this paper, we shall describe the method of investigation of "precatorine" (a-N, N-dimethyl-L-tryptophan) from the alcohol soluble constituents of the seed. The method of identification and some properties of the new amino acid are also described.

EXPERIMENTAL AND RESULTS

After concentrating the alcohol extracts of the seed (ca. 2.5 kg.), crude abrine crystals were obtained and the syrupy mother liquor was diluted with a large amount of water.

Using adsorption-dialysis technique(10), a cellophane bag (No. 4465-A2 dialyzer tubing Arthus H. Thomas Company, cellulose. Philadelphia 5, U.S.A., 3 cm × 240 cm) containing an aqueous slurry of Amberlite IR 120 (in the acid form) was immersed into extract. Stirring was continued for 48 hours at room temperature. The cellophane bag was then taken out and washed with distilled water to remove any adhering contaminants. Then the resin in the bag was poured into a column (4 cm. in diameter) and was washed with distilled water (c a. 5 l.) until the eluants were clear. The washing was repeated with 0.5 N ammonia and indole positive yellow eluant was collected. The ammoniacal eluants were concentrated under reduced pressure to dryness and the residue was extracted with methyl alcohol. The methanol extracts were passed through alumina column (2 cm. × 25 cm. active alumina for absorption chromatography, Wako Pure Chemical Industries, L. T. D.) and eluted with methyl alcohol. After the first indole positive fraction had been collected it was concentrated under reduced pressure to one-third of its original amount. Then acetone was added. A large amount of needle-shaped crystals appeared, which decomposed at 142°C. The crystals gave no depression of m. p. with synthesized authentic trigonelline(9). tration and concentration of the filtrate gave rod-like crystals after standing 2 days in the Recrystallization from ethyl refrigerator. alcohol gave white-rod crystals of m.p. 261-262°C (decompose) which were identified to be &-N. N-dimethyl-L-tryptophan ("precato-

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rine"). The yield was 0.002%.

According to Van Romburgh and Barger⁽¹¹⁾, iodide of methyl-α-trimethylamino-β-indole-propionate, m. p. 197-199°C, can be synthesized when tryptophan is treated with methyl iodide and sodium hydroxide. Hypaphorine nitrate m.p. 215-219°C (decompose), can be isolated from the above iodide by hydrolysis with 1% aqueous sodium hydroxide and on acidification with concentrated nitric

acid. Instead of tryptophan, using precatorine (or abrine) as starting material, iodide of methyl-a-trimethylamino-s-indole propionate and hypaphorine nitrate can also be obtained, which give no depression of m.p. with the respective products obtained in the experiment of Van Romburgh and Barger.

This confirms the presence of precatorine.

The reactions were:

It was found that precatorine soluble in water, methyl alcohol, dil. acids and alkalies; slightly soluble in ethyl alcohol and insoluble in nonpolar solvents. It gave positive color reaction with Dragendorff's reagent, Ehrlich's reagent and was slightly positive to ninhydrin reaction. On polyamide thin layer chromatogram it gave a blue spot when sprayed with a mixture of equal volume of 1% aqueous ferric chloride solution and 1% aqueous potassium ferricyanide solution. It possessed the R_f value of 0.72 (Table 1) when developed in chloroform: ethylacetate: acetic acid=7:2:1(12).

The ultraviolet absorption (maximum absorption at 220 and 280 m μ) and infrared absorption of precatorine are shown in Fig. 1, 2 respectively. Specific rotation $[\alpha]_D^{29} = +113^\circ$. Three derivatives of precatorine such as

hydrochloride, picrate and chloroplatinate were also synthesized. The m.p. of these three derivatives were 244-245°C (decompose), 72-75°C and 195-196.5°C respectively.

DISCUSSION

Although many non-nitrogenous constituents, such as abralnin⁽⁴⁾, abranin chloride⁽⁵⁾, a compound having molecular formula C₈₀H₅₀O₂⁽⁶⁾, a flavon with m. p. 218-9°C⁽⁶⁾, gallic acid⁽⁶⁾ etc., have been reported in the seed of Abrus precatorius, precatorine is a nitrogen containing constituents of this seed other than abrine⁽⁶⁾, hypaphorine⁽⁶⁾ and trigonelline⁽⁹⁾. It is also interesting that all three kinds of N-methyl derivatives of L-tryptophan are present together in the seed. We must believe that there is an enzyme system that catalyze the methylation of

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Table 1. Some Physical and Chemical Properties of L-Tryptophan,
L-Abrine, L-Precatorine and L-Hypaphorine

Properties Substance	Tryptophan	Abrine	Precatorine	Hypaphorine
m. p.	289°C (decomp.)	295°C (decomp.)	261-2°C(decomp.)	237°C (decomp.)
R, value on polyamide TLC	0.24	0.52	0.72	adole prop <mark>ionale</mark>
Ninhydrin reaction	positive	slightly positive	slightly positive	slightly positive
Fecla+KaFe(CN) test	+ (blue) bad	istao + dipara	Brother + malbon	Box 851+ A Tella
Ehrlich's reagent	the restactive pr	ditw + decin	p. 213-74 C (Neoc	a see mutane one
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Citric acid-acetic anhydride reagent(18)*	his confirms the	any spinoib	a methog keeping	Midiw_Regions

^{*} This reagent is specific for tertiary amine.

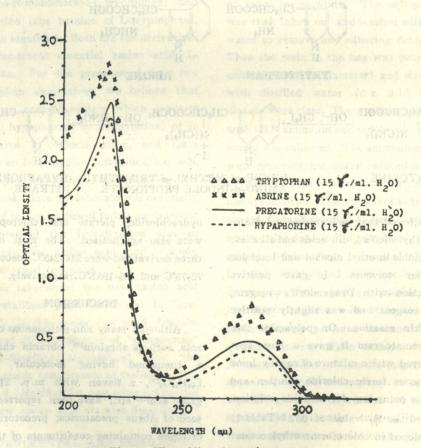


Fig. 1. Ultraviolet absorption spectra

abrine to hypaphorine or in the reverse by demethylation during the growth of the seed.

Abrine has been interested by many investigators for its nutritive value and

enzymatic studies^(14,15). Decarboxylation of abrine produced N-methyltryptamine which is an oxytocic active amine⁽¹⁶⁾ and also possesses many physiological functions^(17,18).

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Hypaphorine has a hyperexcitability on the frog⁽¹⁹⁾. The biochemical studies of precatorine are being undertaken in our laboratory now. The physiological function of the decarboxylation product of precatorine—N, N-dimethyltryptamine are also interested by us.

SUMMARY

From the alcohol extracts of the seeds of Abrus precatorius, using adsorption-dialysis technique and alumina chromatography, we can obtain a new amino $acid-\alpha$ -N, N-dimethyl-L-tryptophan. We named it "precatorine". The method of identification and some physicochemical properties of this new amino acid are described in this paper.

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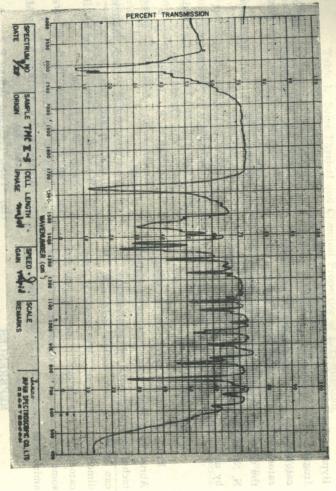
一種新氨基酸 "Precatorine"

董一致 徐千田 李宏圖 郭憲壽

(1971年8月10日受理)

由雞母珠豆之酒精抽出物,利用吸著——透析方 法和活性氧化鋁層色分析法,我們可分離出一種新氨 基酸—a-N, N-二甲基—L-色氨酸。 吾將它命名為 "Precatorine"。 Precatorine 為一白色柱狀結晶,融點為261~262°C(分解),它之一些物理化學性質及證明於本論文中均述及之。

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Fig. 2. Infrared absorption spectrum of Precatorine as Nujol mull

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