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SHORT COMMUNICATION

THE ABSENCE OF LOW-MOLECULAR-WEIGHT GUANIDO COMPOUNDS IN CASTOR BEAN SEEDS

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Abstract—In contrast to a previous report no free arginine, agmatine, or other low-molecular-weight guanido compound could be detected in seeds of *Ricinus communis* (Euphorbiaceae). A heat-stable, arginine-containing protein was isolated and identified with a previously known allergen.

INTRODUCTION

THE OCCURRENCE of agmatine in higher plants is cited in reviews, but original reports on its occurrence are few and leave much to be desired in their rigor.[1–4] Mourgue *et al.*[2] reported, in addition to agmatine and arginine, two other unknown guanido derivatives in castor bean seeds. We decided to confirm this report and, if possible, to identify the two unknown compounds. The paper of Mourgue *et al.*[2] does not give experimental details about the extraction procedure or the quantities of castor beans used. Letters requesting information on these points have not been answered. Therefore we have followed procedures that have been used successfully for extraction of such guanido compounds as arginine, canavanine, lathyrine and galegine.[5–8]

RESULTS AND DISCUSSION

Several variants of the extraction procedure were tried—for instance defatting with ether or chloroform before extraction, stirring the ground seeds with water for as long as 18 hr, grinding with a mortar and pestle, extraction with 75 per cent ethanol—0·1 N HCl. None of these variants offered any advantages over the standard method described in the Experimental section.

Paper chromatography (Table 1) showed a single Sakaguchi-positive spot present in castor bean extract that did not coincide with either arginine or agmatine. Unlike either of

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these knowns, the unknown also reacted with Ponceau S. By adding known agmatine to seeds and taking them through the entire procedure it was determined that 0·3 mg per 100 g of seed could have been detected. This is an amount several orders of magnitude smaller than quantities of unusual amino acids normally reported in seeds.[5–8] Electrolytic desalting made no difference; and since it can result in destruction of arginine and agmatine, it was not usually employed.

Paper strip electrophoresis and ion-exchange chromatography both indicated the presence of a single Sakaguchi-positive substance that was also reactive with Ponceau S. Electrophoretically it moved toward the cathode between arginine and agmatine. On the ion-exchange column it was eluted by distilled water while arginine and agmatine were retained until eluted with base. All of the Sakaguchi-positive material applied to the column (equivalent to 1·97 g arginine per 200 g seeds[9]) was recovered in the distilled water eluate.

The Sakaguchi-positive material was completely precipitated by saturated ammonium sulfate and, by gel filtration,[10] showed an approximate molecular weight of 10.[4] It therefore

TABLE 1. RESULTS OF PAPER CHROMATOGRAPHY

	R_fs in solvent				
	A	B	C	D	E
Arginine	0·04	0·08	0·20	0·72	0·89
Agmatine	0·10	0·34	0·26	0·91	0·91
Extract	0·00	0·00	0·00	0·74	0·81
Desalted extract	0·00	0·00	0·00	0·72	0·84

Spots detected with Sakaguchi reagent. Solvents were: (A) Paper treated with 0·066 N NaOH; *n*-BuOH–HOAc–H_2O (73:10:17). (B) Paper treated with 0·066 N Na_2CO_3; *n*-BuOH–HOAc–H_2O (73:10:17). (C) Paper treated with 0·066 N NaOH; *n*-BuOH–HOAc–H_2O (60:15:25). (D) Distilled water. (E) 50% acetic acid.

resembled the heat-stable allergenic protein fraction isolated by Spies and Coulson and designated CB-1A.[11] The CB-1A fraction has been found to be a mixture of eight different antigens that are chemically very similar.[12–15] Arginine contributes 26·6 per cent of the total nitrogen of the fraction.[11] We repeated isolation of the CB-1A fraction for comparison with our unknown material. The two were identical in chromatographic behavior, electrophoretic behavior, and color reactions. Amino acid analysis of our CB-1A preparation showed 26·2 per cent of the total nitrogen in arginine.

We conclude that the only heat-stable guanido compounds present in our sample of castor beans comprise a group of low-molecular-weight proteins contained in the CB-1A allergen preparation. Unless more evidence becomes available, citations of the presence of agmatine in castor beans should be regarded with doubt.

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EXPERIMENTAL

Preparation of Castor Bean Extract

200 g of seeds of *Ricinus communis* var. Cimarron were ground in a Waring Blendor for 2 min at room temp. using 2·5 ml distilled water per g of seed. The homogenate was strained through cheesecloth and centrifuged for 15 min at 6000 × *g*. The resulting creamy layer was skimmed from the top and discarded along with the precipitate. The aqueous layer was boiled for 10 min and filtered to remove coagulated protein. Extract was concentrated in a flash evaporator at 50–55° to about 5 ml and extracted with ether (3 × 20 ml). Final extract was brought to a volume of 10 ml. It was a clear, brownish liquid of pH 5·2. In a few experiments the extract was desalted electrolytically prior to chromatography.

Chromatography

Paper chromatography was done by the ascending technique on Whatman No. 1 paper. In repeating the procedure of Mourgue *et al.*[2] the paper was pretreated by dipping in 0·066 N NaOH and drying. Solvents are described in Table 1. TLC for molecular weight estimation followed the procedure of Roberts[10] using Superfine Sephadex G-50. Markers were hemoglobin and cytochrome c. For detection of guanido compounds the modified Sakaguchi reagent was used.[16] It was capable of detecting 0·03 mg of arginine. Protein was stained with Ponceau S (0·5% in 3% trichloracetic acid) and background color removed by successive washes in 5% acetic acid. Ponceau S did not stain either arginine or agmatine.

Electrophoresis

Since the usual cellulose acetate strips were destroyed by Sakaguchi reagent, electrophoresis was done on Whatman No. 1 paper strips at 400 V and 0·2 mA with acetate buffers of pH 3·5, 4·0, and 4·5. After 30 min of electrophoresis each strip was divided in half so that one half could be stained with Sakaguchi reagent and the other with Ponceau S.

Ion-Exchange Chromatography

Dowex $50H^+$ columns were washed with distilled water to pH 2·1. After adding the extract the column was washed with distilled water until no more Sakaguchi-positive materials came through. Basic compounds were then eluted with 0·5 N NH_4OH.

Preparation and Amino Acid Analysis of Castor Bean Allergen

Allergen was prepared by the method of Spies and Coulson[11] from 500 g of castor beans, hydrolyzed with 6 N HCl at 110° in a N_2 atmosphere for 30 hr, and analyzed with the Beckman Model 120c amino acid analyzer.

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