INDOLE ALKALOID BIOSYNTHESIS: PARTIAL PURIFICATION OF "AJMALICINE SYNTHETASE" FROM CATHARANTHUS ROSEUS

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SUMMARY

The "ajmalicine synthetase" system of Catharanthus roseus has been partially purified from callus, seedlings and mature plants. On gel filtration of the cell-free extract, four β-D-glucosidase isozymes were observed in seedlings and plants. Only two were present in the callus. A protein peak at 55,000 daltons in all three materials was capable of synthesizing ajmalicine from tryptamine and secologanin in the presence of NADPH. This "ajmalicine synthetase" rapidly lost its ability to synthsize ajmalicine, but retained the β-glucosidase activity.

INTRODUCTION

Ajmalicine, a heteroyohimbine type indole alkaloid from Catharanthus roseus, has hypotensive activity. The first cell-free system from ajmalicine biosynthesis in the callus was reported by our laboratory (1). Similar findings were confirmed by Stöckigt, et al. (2a) in a suspension culture of the same species.

Ajmalicine biosynthesis involves a coupling of tryptamine and secologanin to form vincoside which, in turn, is subject to hydrolysis, rearrangement, cyclization and reduction to give ajmalicine. Some of the steps are suspected to be non-enzymatic. A partial purification of the enzyme systems revealed some interesting properties of this process.

MATERIALS AND METHODS

C. roseus seeds were obtained from Burpee Co. Calluses were initiated and maintained in SH medium (3). The calluses retained vitality and capacity for ajmalicine formation after more than ten transfers. Cell-free preparations were carried out as described previously (1), except that 0.1M citrate-phosphate buffer at pH 6.4 was used throughout unless mentioned otherwise.

β-glucosidase was assayed with p-nitrophenyl-β-D-glucoside or by coupled enzyme assays (4). Ajmalicine was assayed by incubating the enzyme fraction with 0.5μCi [2-^{14}C] tryptamine bisuccinate, 5mM secologanin and 5mM NADPH in a total of 200μℓ. At the end of one hr incubation at 35°C, the alkaloids were extracted with $CHCl_3$ after the pH adjustment to 8.0. The products were analyzed by thin layer chromatography (1, 2a). Ajmalicine was identified by co-chromatography, radioscanning or autoradiography with authentic material.

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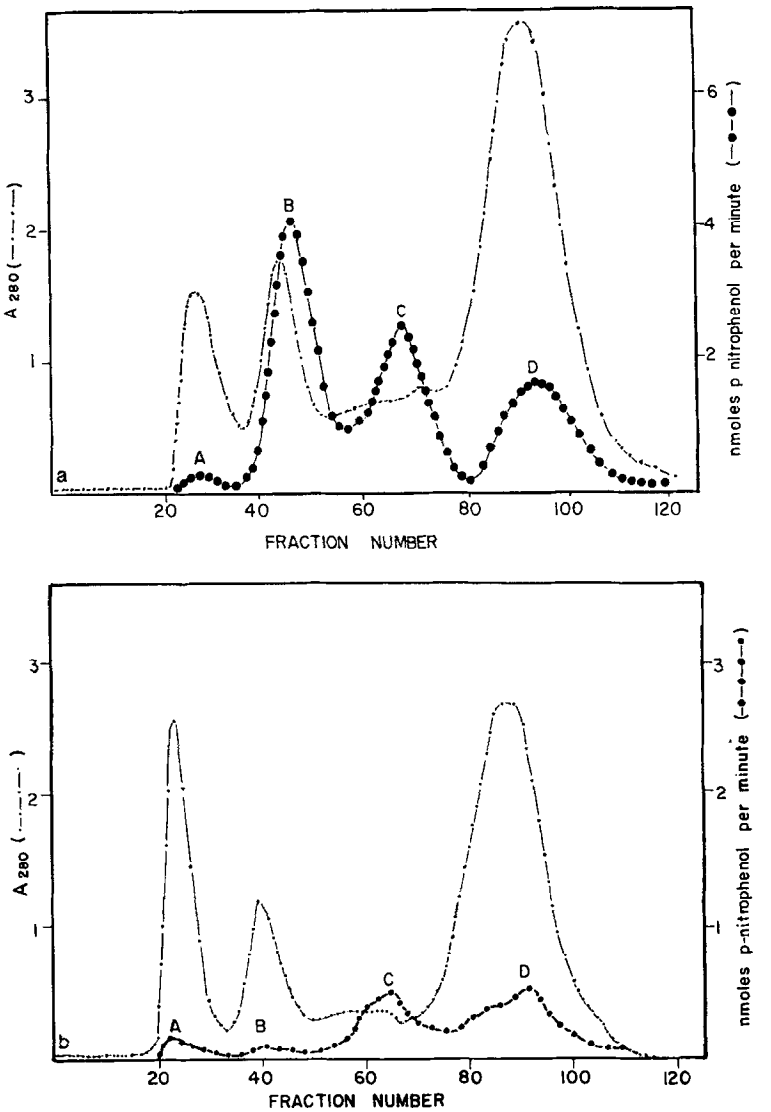

Fig. 1a. Ultrogel AcA34 Fractionation of 2-wk old C. roseus seedlings.
b. Ultrogel AcA34 Fractionation of 3-month old C. roseus plant.

RESULTS AND DISCUSSION

Based on the p-nitrophenyl-β-D-glucoside assay, four β-glucosidase isozymes (A, B, C, D) were observed on gel filtration of the 37,000 g supernatant of 2-week old seedlings and 3-month old plants (Fig. 1a, 1b). Their molecular weights were 182,000; 120,000; 55,000; and 8,000 respectively as estimated by comparing with protein standards. All of them were nonspecific

TABLE 1.

Glycosidases from seedlings and plants of C. roseus

Isozyme	Mol. wt.	Km value for pNP-β-Glc*	pNP-α-Glc*	pNP-β-Gal*	pNP-β-Fuc*
A	182,000	2.17mM	6.25mM	0.125mM	1.33mM
B	120,000	0.71mM	0.95mM	0.46 mM	2.63mM
C	55,000	0.51mM	0.98mM	0.63 mM	9.10mM
D	8,000	1.72mM	12.5 mM	2.17 mM	22.2 mM

*pNP-β-Glc = p-nitrophenyl-β-D-glucoside; pNP-α-Glc = p-nitrophenyl-α-D-glucoside
pNP-β-Gal = p-nitrophenyl-E-galactoside; pNP-β-Fuc = p-nitrophenyl-β-D-fucoside

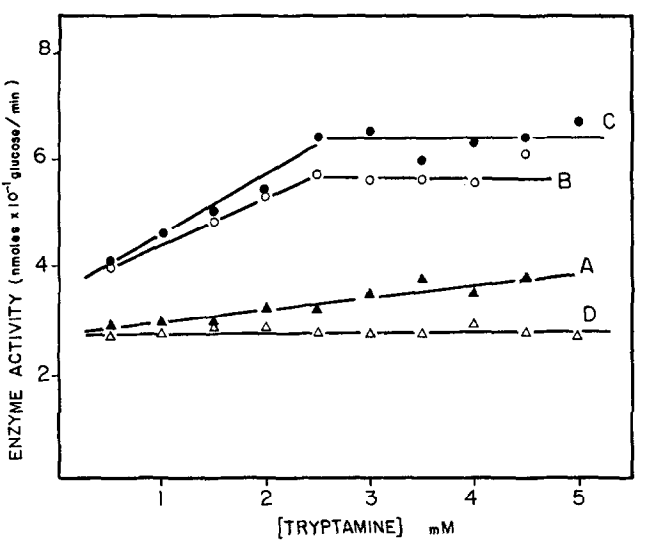

Fig. 2. Effect of Tryptamine on the glucosidase isozymes from seedlings.

glycosidases active towards the substrates shown in Table 1. Glycosidases B and C were activated by tryptamine, while A and D were not (Fig. 2).

Only two glucosidases were observed in the callus system; G_1 was

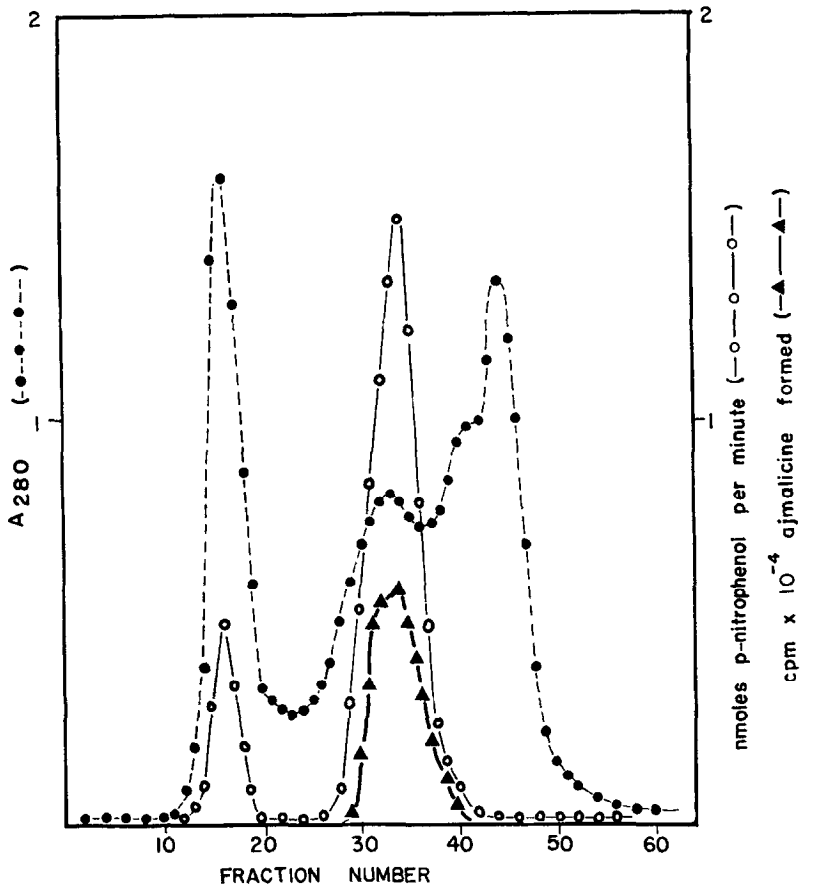

Fig. 3. Ultrogel AcA34 fractionation of "ajmalicine synthetase" from C. roseus callus.

eluted in the void volume (m.w. > 400,000), the other, G_{11} had a molecular weight of about 55,000 (Fig. 3).

Alkaloid assay revealed that only glucosidase C and G_{11} were capable of synthesizing ajmalicine from tryptamine and secologanin in the presence of NADPH. Incubation in the absence of NADPH led to accumulation of an intermediate similar to the one obtained by Stöckigt, et al. (2b). Structures 4 (2b), 8 (5) and 9 (6) represent the possibilities (Fig. 4), which are now being investigated by spectroscopic analysis.

The pH optimum for G_{11} was 5.0-5.5 but that for ajmalicine was about

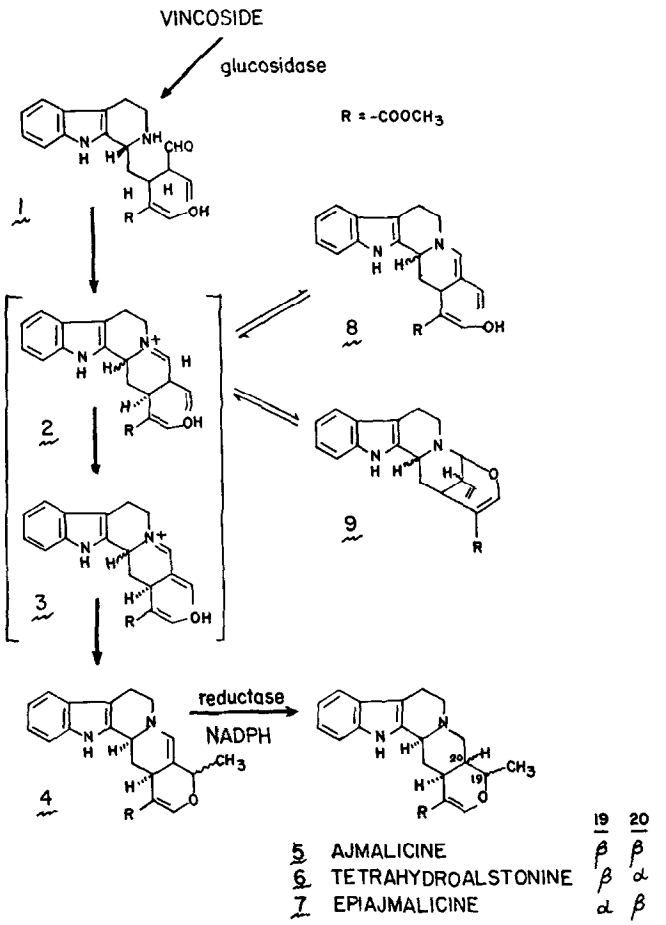

Fig. 4. Possible compounds obtained in the synthesis of ajmalicine.

6.4. On aging or further purification with CM Sephadex A50, the 55,000 dalton protein retained the glucosidase activity but lost the ajmalicine synthetase activity. These observations suggest that more than one protein is involved in ajmalicine biosynthesis. The questions remaining are whether C and G_{11} are identical and specific for the alkaloid pathway.

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