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

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Ajmalicine (5), 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.

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 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_I was eluted in the void volume (m.w. > 400,000), the other, G_{II} 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 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. The structures of several additional isolates from submerged tissue culture preparations are discussed together with the relationship of the above findings to the biosynthesis of Aspidosperma and Iboga alkaloids.

ACKNOWLEDGMENT

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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	0.46mM	2.63mM
C	55,000	0.51mM	0.98mM	0.63mM	9.10mM
D	8,000	1.72mM	12.5mM	2.17mM	22.2mM

*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

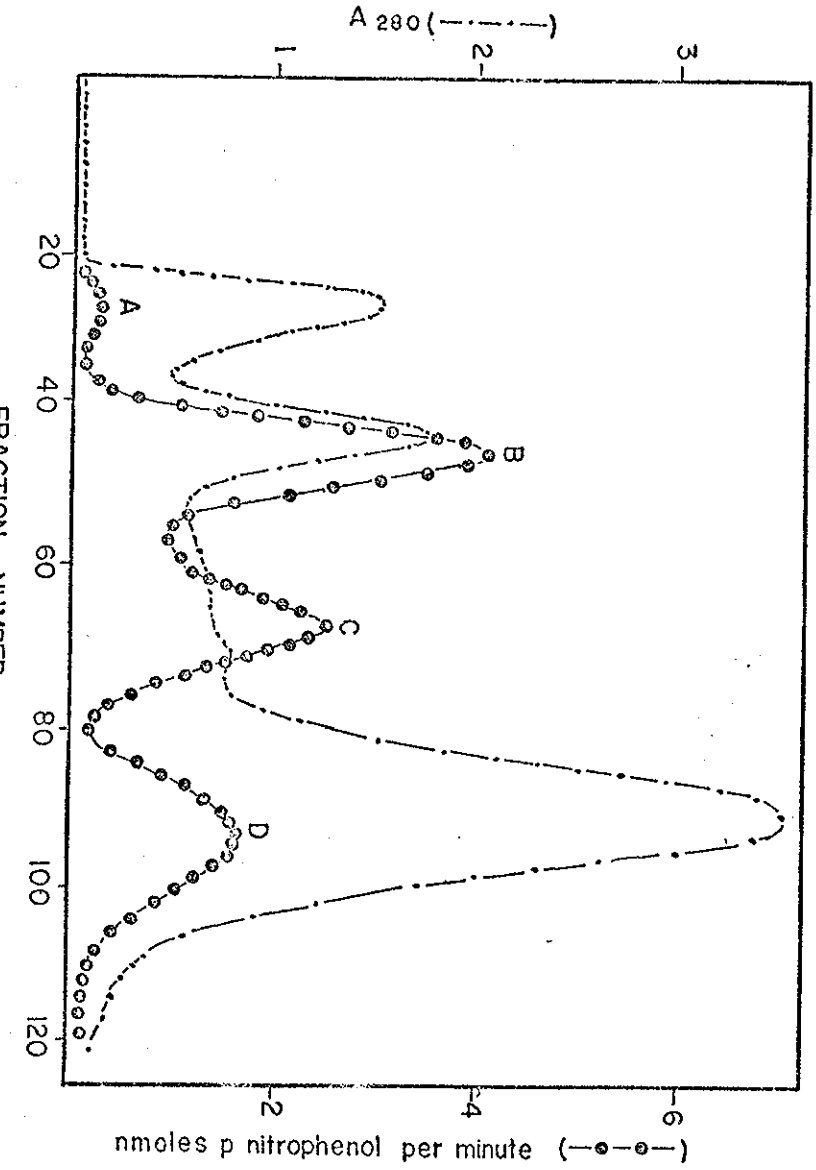


FIG. 1a Ultrogel AcA34 Fractionation of 2-wk old C. roseus seedlings.

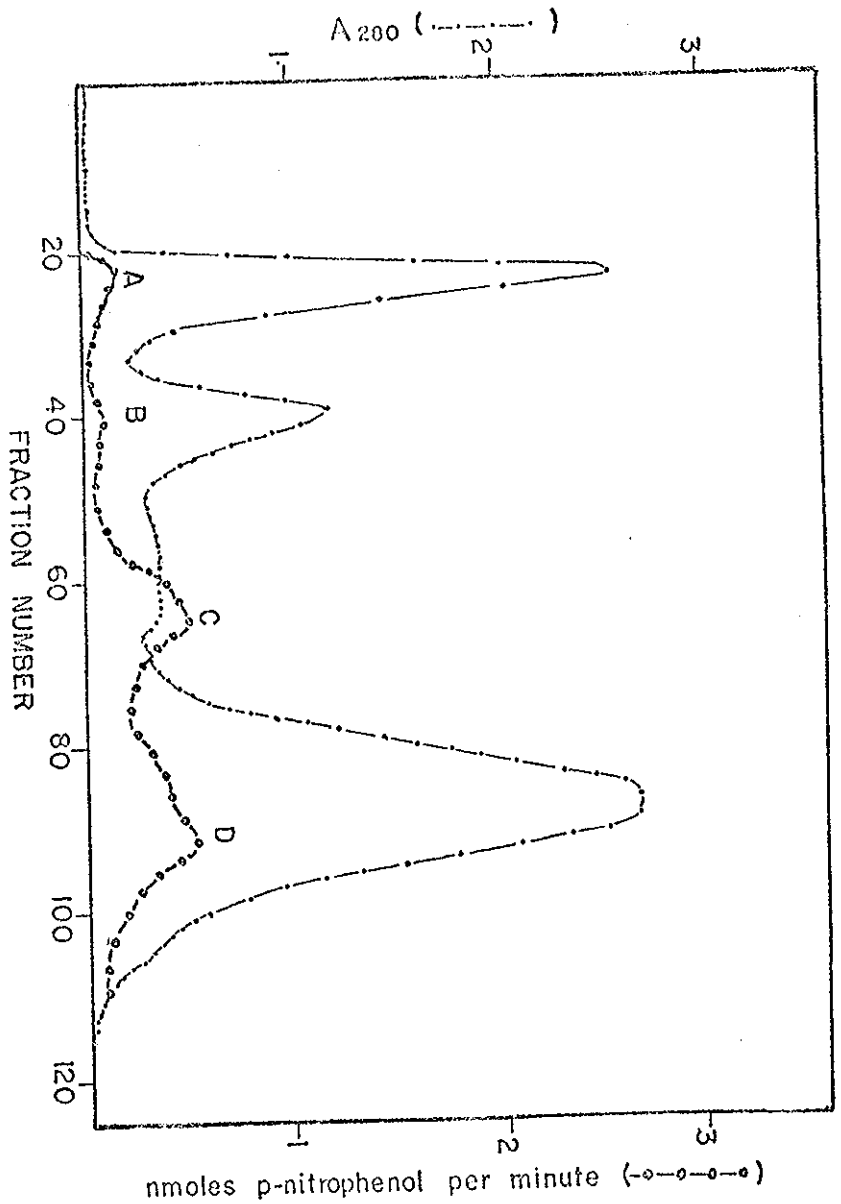


FIG. 1b Ultrogel AcA34 Fractionation of 3-month old C. roseus plant.

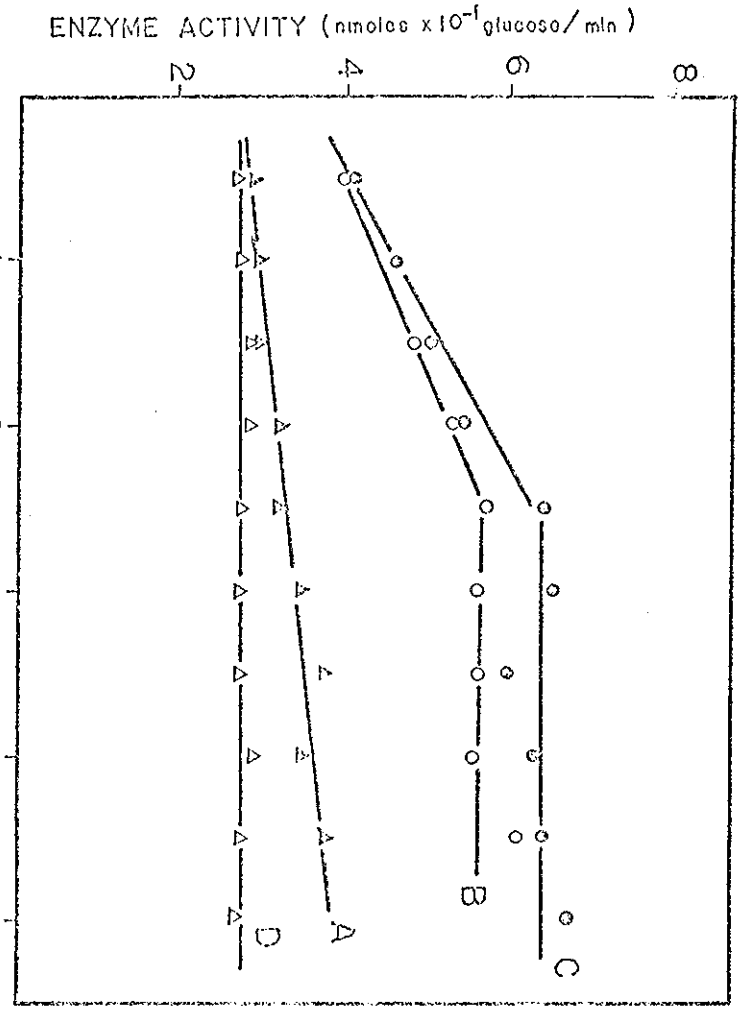


FIG. 2 Effect of Tryptamine on the glucosidase isozymes from seedlings

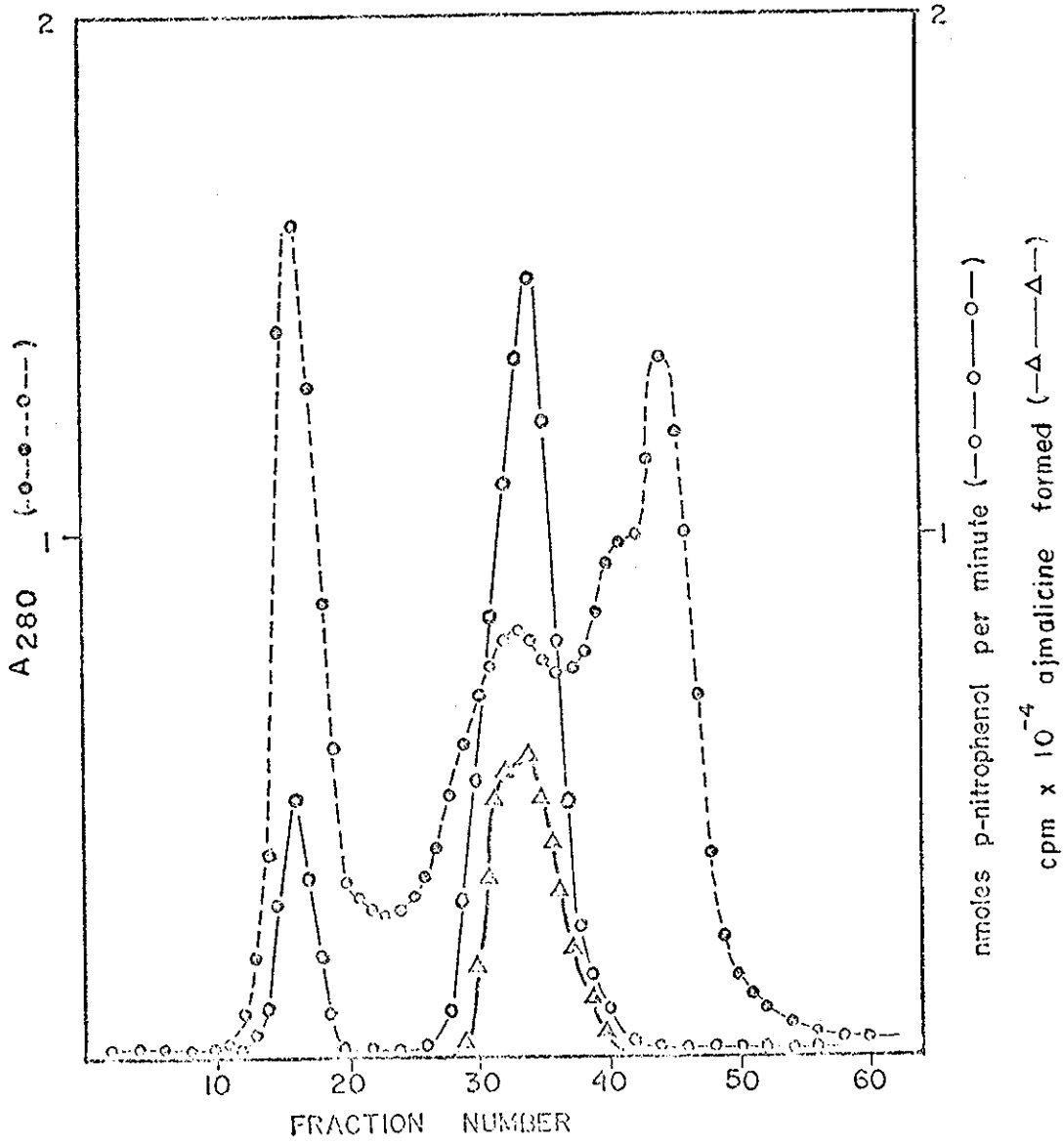
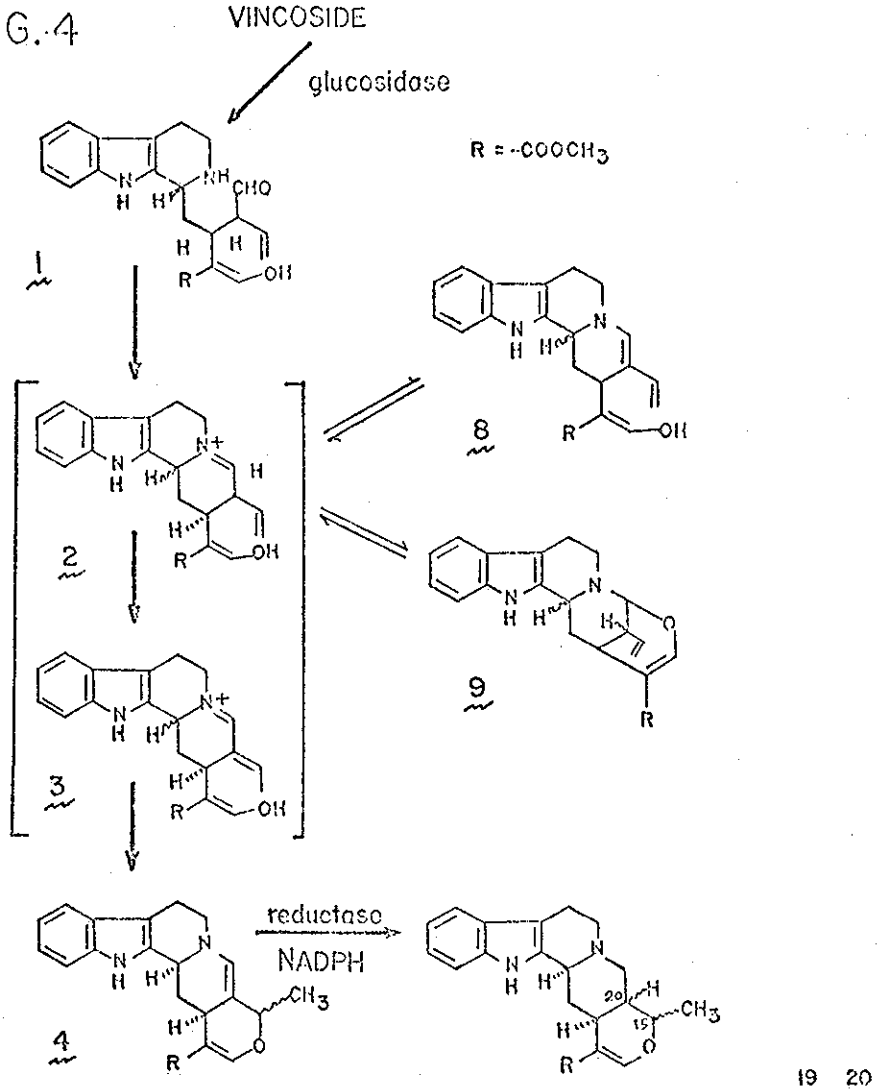


FIG. 3 Ultrogel AcA34 fractionation of "ajmalicine synthetase" from C. roseus callus

FIG. 4


VINCOSIDE
glucosidase
R = -COOCH3
reductase
NADPH
1
2
3
4
8
9
5 AJMALICINE β β
6 TETRAHYDROALSTONINE β α
7 EPIAJMALICINE α β

REFERENCES

1. Scott, A. I. and Lee, S.-L. (1975) J.Amer.Chem.Soc., 97, 6906.
2. (a) Stöckigt, J., Treimer, J. and Zenk, M. H. (1976) FEBS Lett., 70, 267.

   (b) Stöckigt, J., Husson, H. P., Kan-Fan, C. and Zenk, M. H. (1977) Chem. Comm., to be published.
3. Schenk, R. U. and Hildebrant, A. C. (1972) Can.J.Bot., 50, 199.
4. Sigma Chemical Co. (1972) Sigma Technical Bulletin No. 510.
5. Scott, A. I. and Yeh, C. L., unpublished results.
6. Brown, R. T., Chapple, C. Lyn, and Charalambides, A. A. (1974) J.C.S. Chem. Comm., 756.