Phytochemistry, 1972, Vol. 11, pp. 1405 to 1406. Pergamon Press. Printed in England.

SHORT COMMUNICATION

SYNTHESIS OF CARBOLINE ALKALOIDS BY PLANT CELL CULTURES

IVAN A. VELIKY

Division of Biology, National Research Council of Canada, Ottawa, Ontario, Canada

(*Received* 13 *July* 1971, *in revised form* 15 *September* 1971)

Abstract—A cell suspension culture of *Phaseolus vulgaris* possesses a biosynthetic potential for transforming tryptophan into alkaloids harman and norharman.

THE POSSIBILITY that plant cell cultures could be used for the production of useful metabolites has interested numerous researchers, but the tendency has been to assess formation of a metabolite known to be produced by plants of a particular species in cell cultures established from that species.[1–3] Such products are usually detectable in the cell culture, but the amount produced depends on a variety of factors such as the number of transfers of the culture and the environmental condition (nutrient supply, pH, temperature, etc.).

Plant cell cultures are also being used in more fundamental studies of cell physiology, and it is of interest to investigate the biosynthetic potential of such cells in the culture environment. The change from organized tissue in a whole plant to unorganized callus tissue or to the suspensions of cells and cell aggregates may affect the biochemistry of the cells quite markedly. An example of this is seen in the 5-fold increase in peroxidase activity in cell cultures established from *Phaseolus vulgaris* roots as compared to roots of the bean plant.

The harman alkaloids, β-carboline and its homologues, have been detected in species from nine plant families, including the Leguminoseae,[4,5] but have not been reported as occurring in *Phaseolus vulgaris*, the common kidney bean. These alkaloids contain an indole ring, and their biosynthesis has been linked to tryptophan, tryptamine, or their derivatives.[5]

In screening various cell lines for alkaloids, two Dragendorf positive spots were detected on a TLC of an extract of cells from a culture established from roots of *Phaseolus vulgaris*. The spots had a bright bluish–violet fluorescence in UV light, and reacted also positively with the iodoplatinate reagent. On silica gel plates (Eastman Chromatogram sheet No. 6061) developed with $CHCl_3$–$NHEt_2$ (9:1) the R_fs were 0·27 and 0·43. This system was one of ten used, most of which gave good separation of the two alkaloids.

The cells from which this extract was prepared had been cultured in 67-V medium,[6] which contains casein hydrolysate (NZ-Amine Type A, Sheffield Chemical, Norwich, New York) as the principal nitrogen source and thus contains 15–20 mg tryptophan/l. When grown in modified medium containing only inorganic nitrogen (ammonium and nitrate), this cell line grew normally but failed to produce these alkaloids, and when 18 amino acids

[1] E. J. STABA and P. LAURSEN, *J. Pharm. Sci.* **55**, 1099 (1966).
[2] WEN-NUEI CHAN and E. J. STABA, *Lloydia* **28**, 55 (1965).
[3] I. A. VELIKY and K. GENEST, *American Society of Pharmacognosy*, Annual Meeting, Vienna (1970).
[4] H. G. BOIT, *Ergebnisse der Alkaloid-Chemie bis* 1960, Akademie Verlag, Berlin (1961).
[5] K. MOTHES and H. R. SHUTTE (editors), *Biosynthese der Alkaloide*, p. 462, VEB Deutscher Verlag der Wissenschaften, Berlin (1969).
[6] I. A. VELIKY and S. M. MARTIN, *Can. J. Microbiol.* **16**, 223 (1970).

were tested individually as supplement to the inorganic medium only, tryptophan promoted alkaloid production. This suggested that these two alkaloids belonged to the indole ring-containing group.

The two alkaloids were purified, and their fluorescence used as a method of identification. The excitation and emission maxima, measured on a Farrand Spectrofluorometer MK-1 in N/10 H_2SO_4, were as follows:

R_f	Excitation max. nm	Fluorescence max. nm
0·27	373	445
0·43	368	430

These data indicate that the R_f 0·27 spot corresponds to β-carboline (norharman) and the R_f 0·43 spot to 1-methyl-β-carboline (harman) with excitation maxima 375 and 368 nm, and fluorescent maxima 445 and 432 nm, respectively. Authentic samples of these alkaloids (from commercial sources) were chromatographed with our purified preparations, and the results supported the fluorescence-based identifications. IR spectra also confirmed these identifications.

In an experiment in which 67-V medium was supplemented with additional tryptophan (100 mg/l.), the total quantity of alkaloid produced was not altered, but the proportion present as norharman increased. Thus, in the 67-V medium 46·9% of the alkaloid produced was harman, 53·1% norharman, while in the presence of added tryptophan the percentages were 28·9% and 71·1% respectively. There are indications, however, that some other factors (e.g. age of the culture, etc.) affect the proportion of the two alkaloids formed by *Phaseolus vulgaris* culture.

These findings indicate that a cell culture established from roots of a plant that is not known to produce alkaloid will produce harman and norharman when grown in the presence of tryptophan. These cells thus possess a biosynthetic potential for transforming tryptophan into two indole alkaloids.

Key Word Index—Phaseolus vulgaris; Leguminoseae; root culture; biosynthesis alkaloids; harman; norharman.