Plant Physiol. (1982) 69, 400–404
0032-0889/82/69/0400/05/$00.50/0

Accumulation of β-Carboline Alkaloids and Serotonin by Cell Cultures of *Peganum harmala* L.

I. CORRELATION BETWEEN PLANTS AND CELL CULTURES AND INFLUENCE OF MEDIUM CONSTITUENTS

Received for publication March 2, 1981 and in revised form August 12, 1981

FLORENZ SASSE, UTE HECKENBERG, AND JOCHEN BERLIN
Gesellschaft f. Biotechnologische Forschung m.b.H., D-3300 Braunschweig, West Germany

ABSTRACT

A number of cell cultures of *Peganum harmala* were initiated to check for a correlation between the harman alkaloid content of seedlings and cell lines derived therefrom. Despite a poor correlation between callus or suspension culture lines and parent plants, the mean alkaloid contents of strains derived from seedlings with higher alkaloid yields were nevertheless higher than the mean contents of strains derived from low yield plants. Generally, alkaloid accumulation decreased with the numbers of transfers. By permanent visual selection for fluorescent areas of the calluses, however, a mean content of 0.1% harman alkaloids and 0.1% serotonin could be maintained, which was 10 times higher than in unselected callus cultures.

The effects of medium constituents on harman alkaloid and serotonin accumulation were measured for a low yielding, faster growing suspension culture strain and a slowly growing, but high yielding cell line. This led to the development of a production medium without 2,4-dichlorophenoxyacetic acid and phosphate, and with Ca, Mg, and nitrate as sole macronutrients. When this production medium was used, the accumulation of harman alkaloids and serotonin was increased from 0.1 to 1% in the low yielding cell line and from 1 to 2% in the high yielding strain.

Plant cell cultures offer a convenient system for studying biochemical aspects of secondary metabolism of higher plants. This is best illustrated by the progress in the enzymology of flavonoids (8) and indole alkaloids (26). The formation of secondary compounds is rather low in most plant cell cultures, however, and conditions allowing higher synthesis of these compounds often have to be established first. This may be achieved by analytical and biochemical selection for highly productive strains from established cultures (3, 22, 27), or by altering the medium composition for higher production (10, 27, 28). Another strategy applied to obtain high producing strains is to start cultures from selected, high producing plants (12, 22, 27).

Heterotrophic callus cultures of *Peganum harmala* L. are known to produce low amounts of indole alkaloids of the β-carboline type. Harmine, harmaline, harmol, and harmalol have been identified and shown to constitute up to 0.2% of the dry mass of the cells (1, 15, 17). Traces of serotonin have also been found (1, 13). These rather simple compounds may provide a suitable system for studying the interaction of primary and secondary metabolic pathways. Using the above-mentioned techniques, we established cell lines of *P. harmala* with different levels of β-carboline alkaloids. Inasmuch as Nettleship and Slaytor (15) showed that the harman alkaloid content of callus cultures of *Peganum* was sensitive to changes in the medium composition, we tried to develop an induction system for alkaloid synthesis as has been demonstrated for *Catharanthus* cultures (10).

MATERIALS AND METHODS

Cell Cultures. Callus cultures of *P. harmala* L. were started from different organs of sterile grown seedlings. They were maintained on MS[1]-medium (14) with 2 μM 2,4-D and were subcultured every month. Stock suspension cultures were grown in the same medium in 200-ml Erlenmeyer flasks containing 70 ml medium. Cultures were incubated at 26°C in the dark on a gyratory shaker (120 rpm) and subcultured every 3 weeks. For media experiments, 0.5 g vacuum filtered 12-day-old cells of the stock suspension cultures were inoculated into 25 ml medium in 50-ml Erlenmeyer flasks.

Analytical Methods. Freeze-dried material was extracted with methanol for analysis. Harman alkaloids were determined fluorimetrically either directly in phosphate buffer (0.2 M, pH 5.0), allowing distinction between aromatic and 3,4-dihydro-β-carboline alkaloids, or after separation by HPLC (20). After separation on silica gel plates in $CHCl_3$/MeOH/25% NH_3 (10:4:1 and 5:4:1) alkaloids were quantified directly by their absorbance (harmine, harmol 320 nm; harmaline, harmalol 380 nm) and fluorescence (harmine, harmol: excitation 313 nm, emission 340 nm; harmaline, harmalol: excitation 365 nm, emission 415 nm) using a Shimadzu TLC-Scanner. Concentrations were expressed as harmine·HCl and harmaline·HCl equivalents.

Serotonin was also assayed fluorimetrically (excitation 394 nm, emission 505 nm) after incubation (75°C, 30 min) with ninhydrin (23). The analysis proved to be specific. Equimolar concentrations of 5-hydroxytryptophan, tryptophan, 6-hydroxytryptamine, tryptamine, harmine, harmol, harmalol, and IAA reached less than 5% of the serotonin fluorescence. Only high harmaline levels reaching 20% of the fluorescence of an equimolar solution of serotonin had to be considered. Calibration graphs were linear in a tested range from 0.01 to 2.0 μg serotonin·HCl/ml.

RESULTS

Callus Cultures. Four-week-old seedlings of *P. harmala*, grown from seeds which contained 4.4 to 9.0% harman alkaloids, showed an alkaloid content between 0.0 and 5.5%. Many callus cultures from different parts of the seedlings were initiated in order to find strains with highly different capacities for the synthesis of β-carboline alkaloids (Table I). After 4 weeks, the initiated calluses had an alkaloid content ranging from 0.01 to 2.3 mg/g dry mass (mean value 0.84 mg/g). The main alkaloids were harmine and

[1] Abbreviations: MS medium, Murashige and Skoog medium.

Table I. *Harman Alkaloid and Serotonin Concentrations of Seeds, Seedlings, and Calluses of P. harmula*

Data shown are means ± SD or maximum values related to dry mass. Number of seed samples (about 10 seeds each), seedlings, or callus strains analyzed in parentheses.

	Harman Alkaloids		Serotonin	
	Mean	Maximum	Mean	Maximum
		mg/g		
Seeds	62 ± 17 (7)	90	<1	
Seedlings (entire)	10.6 ± 11.5 (36)	55.4	<2	
Callus				
Growth cycles				
1	0.84 ± 0.94 (55)	2.34		
2	0.70 ± 0.65 (55)	3.13		
3	0.58 ± 0.78 (55)	4.18		
4	0.36 ± 0.26 (21)	1.16		
5	0.22 ± 0.16 (21)	0.66		
15	0.08 ± 0.04 (10)	0.16	0.08 ± 0.03 (10)	0.16
15[a]	0.97 ± 0.63 (10)	2.23	1.21 ± 0.48 (10)	2.32

[a] Selected for fluorescence under UV light for nine transfers.

harmalol. The molar ratio was 1.4 ± 0.3. No significant differences were found in calluses derived from root, shoot, or leaf. In general, there was a steady decrease of the alkaloid content from transfer to transfer. At the first passage, 8 of 55 callus strains showed an alkaloid concentration of less than 0.2 mg/g. After only two subcultures, 31 dropped under that level. For 36 callus lines, of which the parent seedlings were also analyzed, we found a very low correlation (after three growth cycles, correlation coefficient $r^2 = 0.22$) between the alkaloid content of the seedlings and the callus strains derived therefrom (Fig. 1). If the seedlings and the calluses were grouped in four classes according to their alkaloid content, calluses derived from seedlings of class 1 (lowest content) were found in class 4 of the calluses (highest contents) and vice versa. But if the mean alkaloid content of the calluses derived from class 1 of the seedlings (after three growth cycles 0.19 ± 0.16 mg/g) was compared with the mean content of calluses derived from class 4 (0.85 ± 1.39 mg/g), a 4 times higher value was found for the latter calluses. In addition, the callus with the highest content was derived from the seedling with the highest alkaloid content (Fig. 1).

Parallel to the decrease of alkaloid content from subculture to subculture, the calluses became whiter and smoother and showed less morphological differentiation. Macroscopic observation under UV light suggested a localization of the alkaloids in differentiated areas of the callus. The high fluorescence of harman alkaloids allowed a visual selection for cells with high yields of these compounds. When only fluorescent areas of the calluses were transferred, the initial high values were maintained. However, all of these analytically selected strains became low producers when the selection pressure was discontinued. Serotonin, which was only analyzed in the later subcultures, was found in equally high amounts as the harman alkaloids, and was also higher in selected strains.

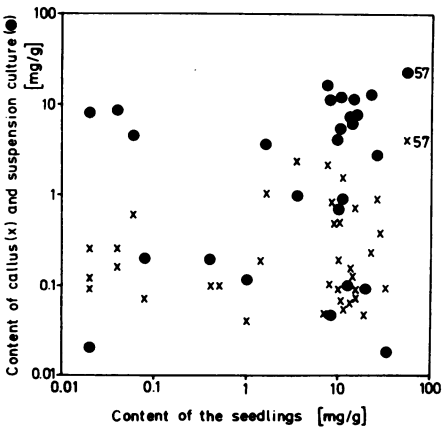

FIG. 1. Relation between harman alkaloid content of seedlings (4 weeks old), and the callus (X) and suspension cultures (●) derived therefrom (data of cultures after three growth cycles, one callus and one suspension culture from one seedling).

Suspension Cultures. From differently aged calluses (one or four growth cycles) we started 44 suspension cultures. At the end of the third growth cycle, they reached alkaloid values between 0 and 23 mg/g dry mass (mean value 4.7 ± 5.6 mg/g). Six strains had an alkaloid content > 10 mg/g dry mass, 19 a content <1 mg/g. The alkaloid concentrations of 27 strains together with the analyses of seedlings and calluses from which they were derived are shown in Figure 1. The correlation between alkaloid content of the parent seedlings and the suspension lines (after three growth cycles, $r^2 = 0.21$) was as poor as for the callus strains, but the mean value of the alkaloid contents of the suspension lines derived from class 4 (highest content) seedlings was twice as high (7.7 ± 8.2 mg/g) as that of strains derived from class 1 (3.6 ± 4.0 mg/g). There was also a low correlation between callus and suspension cultures ($r^2 = 0.29$).

Characterization of a High Yielding Cell Line. From the high yielding strains we chose one line (No. 57), which was initiated from leaf material, for further investigations. This line showed the highest alkaloid levels in the seedlings (55 mg/g), in callus culture (after three growth cycles, 4.2 mg/g), and the highest levels in suspension culture (after three growth cycles, 23 mg/g). During further suspension subcultures, the maximum alkaloid accumulation dropped to 1%, whereas the growth rate was nearly doubled. Characteristic data, which were relatively stable for more than 1 year without further selection, are shown in Figure 2. The culture reached stationary growth phase after 3 weeks (growth factor 5). The alkaloid content per dry mass decreased during the first 10 days by a factor of 2.5. This was due to diluting effects, since the dry matter increased by a factor of 3 during this period. Subsequently, harman alkaloids were accumulated up to 8 mg/g at day 24. The main alkaloids were the fully aromatic harmine and the 3,4-dihydro compound harmalol. Harmaline, harmol, and ruine were also detectable, but made up less than 10% of the total alkaloids. The ratio of aromatic alkaloids, *i.e.* mainly harmine, to the 3,4-dihydro compounds, *i.e.* mainly harmalol, ranged between 1.0 during the phase of the highest increase of the alkaloid content and 3.0 at the end of the growth cycle.

Serotonin, the other tryptophan-derived secondary metabolite,

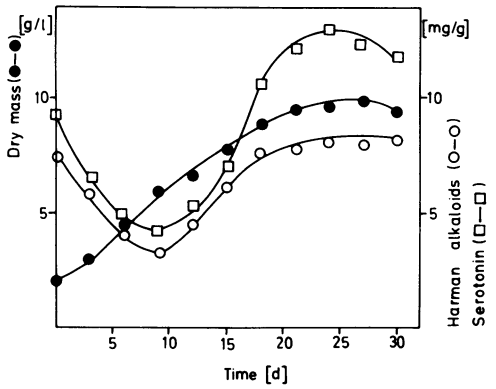

FIG. 2. Growth, harman alkaloid, and serotonin production (dry mass basis) of *P. harmala* suspension culture (strain 57) in MS-medium with 2 μM 2,4-D (average values of five growth cycles with two replicates).

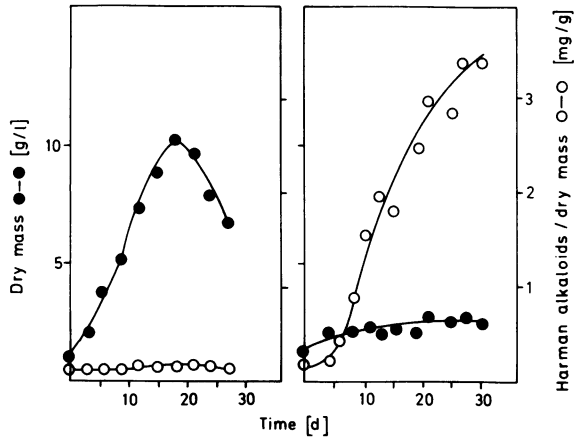

FIG. 3. Growth, harman alkaloid, and serotonin production (dry mass basis) of *P. harmala* suspension culture (strain 46) in MS-medium with 2 μM 2,4-D (a) and production medium (b) (average values of two cultures with two replicates.

accumulated in slightly higher amounts than the harman alkaloids, and within a growth cycle, the contents roughly paralleled the harman alkaloid accumulation (Fig. 2).

Microscopic observations of suspension cultures showed that highly fluorescing cells were bigger and had spindle-like shapes and lignificated cell walls (phloroglucinol-HCl test). This cell type seemed to increase in number at the beginning of the stationary growth phase. These cells also formed aggregates up to 3 mm in diameter.

Characterization of a Low Producing Cell Line. This strain (No. 46, Fig. 3) was derived from roots of a seedling with medium alkaloid content (1.6 mg/g), and also showed medium values in callus (after three growth cycles, 1.0 mg/g) and early suspension cultures (after three growth cycles, 3.8 mg/g). This strain formed smaller aggregates from the beginning and only fine material was subcultured, which resulted in a doubling of the growth rate. Line 46 reached stationary phase in 18 days, and the growth factor was 10. The harman alkaloid contents remained below 0.2 mg/g, and serotonin levels below 0.4 mg/g. For both compounds, we measured only small changes during the growth cycle.

Media Experiments. The effects of changes in the medium composition were analyzed for both cell lines described above. Increasing the 2,4-D concentration caused a decrease in alkaloid content; 10 μM 2,4-D reduced the alkaloid accumulation of strain 57 to 0.1 mg/g dry mass within one growth cycle, and the growth was not accelerated. When cells grown on normal medium with 2 μM 2,4-D were transferred to phytohormone-free medium (Fig. 4),

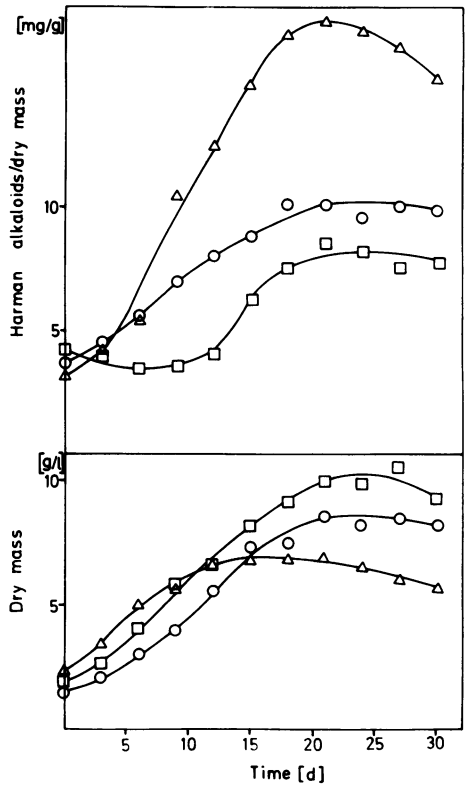

FIG. 4. Growth and alkaloid production (dry mass basis) of *P. harmala* suspension culture (strain 57) in different media (average values of three cultures with two replicates). (□——□), normal MS-medium with 2 μM 2,4-D; (○——○), MS-medium without 2,4-D; (△——△), production medium.

alkaloid accumulation started earlier and reached slightly higher final values than cells grown on normal 2,4-D concentration. The effect was more pronounced in low yielding cultures. In strain 46, the relative increase in alkaloid content after 3 weeks was 3-fold, in the high accumulating culture 57 it was only 1.2 (Fig. 4). In the absence of 2,4-D, growth of both strains was decreased, but the decrease was greater for the normally fast-growing strain 46 than for the slow-growing strain 57.

Investigations on the influence of macronutrients (Table II) on secondary metabolism showed that a lack of NO_3^-, K+, Ca^{2+}, and Mg^{2+} had negative effects on the harman alkaloid and the serotonin accumulation of strain 57. Omission of NH_4^+, K^+, or SO_4^{2-} led primarily to a reduction in serotonin concentration, whereas deficiency of total N completely abolished serotonin accumulation. In contrast, the harman alkaloid and serotonin production was greatly enhanced when phosphate was missing, and decreased with increasing P content of the medium. A combined K^+/PO_4^{3-} deficiency had the most pronounced stimulating effect on the accumulation of these secondary metabolites. In general, serotonin formation was more sensitive to negative treatments than harman alkaloid production.

Stepwise addition of nutrient salts to a sucrose solution showed that the harman alkaloid production of the low yielding strain 46 (Table III) and the high yielder 57 (not shown) was maximally raised when NH_4NO_3, $CaSO_4$, and $MgSO_4$ were added. Addition of phosphate clearly decreased the alkaloid content.

The data from the media experiments resulted in the formulation of a phytohormone-free production medium containing 10 mM $Ca(NO_3)_2$, 5 mM $Mg(NO_3)_2$, and normal concentrations of micronutrients, vitamins, and sugar. In this medium, the accumulation of the secondary metabolites started early. The maximum levels of harman alkaloids of the low yielding cell line

Table II. *Influence of Nutrient Elements on Growth and Harman Alkaloid and Serotonin Production of P. harmala Suspension Cultures (Strain 57)*

The values are means ± SD of four treatments. The culture time was 21 days.

Missing Media Component	Dry Mass		Harman Alkaloids[a]		Serotonin[a]	
	g/l	%	*mg/g*	%	*mg/g*	%
None	11.1 ± 1.1	100	7.5 ± 0.4	100	10.0 ± 2.2	100
2,4-D	9.6 ± 0.8	86	9.5 ± 0.6	127	12.0 ± 0.9	120
2,4-D, NH_4^+	9.7 ± 0.5	87	9.6 ± 0.8	128	5.8 ± 0.5	58
2,4-D, NO_3^-	7.4 ± 0.4	67	5.1 ± 0.3	68	2.4 ± 0.2	24
2,4-D, NH_4^+, NO_3^-	9.4 ± 0.3	85	0.9 ± 0.04	12	0.2 ± 0.02	2
2,4-D, K^+	8.5 ± 1.0	77	5.3 ± 0.4	71	3.3 ± 0.4	33
2,4-D, PO_4^{3-}	9.0 ± 0.5	81	13.2 ± 1.1	176	18.9 ± 2.2	189
2,4-D, K^+, PO_4^{3-}	9.2 ± 0.8	83	16.0 ± 1.2	213	20.6 ± 1.1	206
2,4-D, Ca^{2+}	8.5 ± 0.8	77	5.4 ± 0.2	72	3.3 ± 0.2	33
2,4-D, Mg^{2+}	6.5 ± 1.0	59	4.6 ± 0.2	61	2.7 ± 0.2	27
2,4-D, SO_4^{2-}	8.8 ± 1.0	79	10.3 ± 0.3	137	4.4 ± 0.4	44
2,4-D + PO_4^{3-} [b]	8.6 ± 0.6	77	5.9 ± 0.3	79	2.9 ± 0.2	29

[a] Related to dry mass.
[b] Ten mM KH_2PO_4 (normal MS 1.25 mM).

Table III. *Macronutrient Response of Harman Alkaloid Production (Dry Mass Basis) of P. harmala Suspension Cultures (Strain 46)*

The values are means ± SD of four treatments. The culture time was 21 days. The concentration of NH_4^+, Ca^{2+}, Mg^{2+}, K^+, NO_3^-, and PO_4^{3-} were the same as in the full MS medium (14).

Medium Components	Alkaloids
	mg/g
Sucrose + NH_4NO_3	0.38 ± 0.17
Sucrose + NH_4NO_3 + $CaSO_4$	3.07 ± 0.48
Sucrose + NH_4NO_3 + $CaSO_4$ + $MgSO_4$	3.97 ± 0.54
Sucrose + NH_4NO_3 + $CaSO_4$ + $MgSO_4$ + NaH_2PO_4	1.67 ± 0.34
Sucrose + NH_4NO_3 + $CaCl_2$ + $MgSO_4$ + KH_2PO_4 + KNO_3	1.44 ± 0.26
Full MS medium with 2 μM 2,4-D	0.18 ± 0.07

Table IV. *Examples of Harman Alkaloid and Serotonin Production of Different Suspension Cultures of P. harmala after Transfer to Production Medium (PM) in Comparison to Normal Grown Cultures (MS)*

(Culture time 3 weeks, relative standard deviation <20%).

Strain	Concentration per Dry Mass			
	Harman Alkaloids		Serotonin	
	MS	PM[a]	MS	PM[a]
	mg/g			
11	0.8	10.5		
46	0.3	1.5	0.2	4.9
51	0.1	2.0	0.5	3.6
57	7.4	17.0	8.0	16.7

[a] In the production medium, 2,4-D and macronutrients were missing, with the exception of 10 mM $Ca(NO_3)_2$ and 5 mM $Mg(NO_3)_2$.

increased 10-fold and of the high producing 2-fold (Figs. 3 and 4). The 10-fold increase by line 46 was not sufficient to reach the absolute values of strain 57. In testing the production medium with different cultures, we consistently found an increased accumulation of harman alkaloids and serotonin that was 2 to 20 times higher than in normally grown cultures (Table IV).

DISCUSSION

Investigations as to whether or not there is a correlation between the alkaloid content of a plant and a cell culture derived therefrom have been made with *Catharanthus roseus* (19, 27) and tobacco (9). Zenk *et al.* (27) found that suspension cultures from high yielding plants generally produce more alkaloids than cultures from low yielding parents, although they observed a large variation of the alkaloid content. Roller (19) showed that high and low yielding plants gave both high and low yielding callus cultures. She concluded that high yielding parents do not necessarily result in high yielding cell cultures. A strong correlation between nicotine content of callus cultures and the tobacco plants from which they were derived was reported by Kinnersley and Dougall (9), but those callus cultures were derived from plants which differed genetically at the two loci coding for production and accumulation of nicotine. For *P. harmala* we found a poor correlation between the alkaloid content of the seedling and the callus or suspension culture derived therefrom. However, we found that one has a better chance to get higher yielding strains from higher yielding parents. Thus, the proposed strategy of Zenk *et al.* (27) to select high yielding plants for the initiation of high yielding cultures seems to be a worthwhile approach despite the low correlation reported here. The low correlation might become understandable if one considers the remarkable variation found in ~100 protoplast-derived cell cultures of a single *Catharanthus* leaf (4). We also noted that various cell culture lines derived from one parent plant showed considerable differences in their capacity to form harman alkaloids.

The value of selecting directly for cells with increased capacity for the synthesis and accumulation of secondary compounds has been demonstrated (22, 27). This approach was also successfully applied to the *Peganum* cultures. As in many other cases, these analytically selected cell lines (22) were usually quite unstable without continuous selection. Evidently, it is not the high yield which causes the instability, since the high yielding cell line 57 proved to be quite stable, producing 2% alkaloids and serotonin over a long period without steady selection. An important question therefore seems to be how to stabilize analytically selected cell lines.

The alkaloid content of a cell culture is dependent upon the environmental conditions. From microorganisms (6) and also from cultured plant cells (5, 10, 25), it is known that growth and production of secondary compounds often behave antagonistically. A similar behavior was observed for *P. harmala* suspension cultures. During a growth cycle alkaloid accumulation was retarded in relation to cell mass production, and faster growing cell cultures produced less alkaloids. We were not able to follow the production of a single cell during a growth cycle, but microscopic

observations indicated that a cell starts production after having lost its meristematic character. As discussed for *Catharanthus* cells (10), it seems likely, that in a nonsynchronized cell culture, alkaloid content increases with increasing number of cells that reach stationary phase and concomitantly differentiate biochemically and morphologically. Faster growing cell cultures contained more meristematic cells and reached the stationary phase more quickly. They had less time to differentiate and to produce alkaloids because of the deficiency of needed media components. In microorganisms the relative levels of ATP, ADP, and AMP, expressed as energy charge (18), are discussed as important factors in regulating the relationship between primary and secondary metabolism. Many secondary metabolic processes are inhibited by Pi concentrations that are optimal or at least not inhibitory for growth (24).

In *P. harmala* callus cultures, Nettleship and Slaytor (15) observed a 2-fold increase in harmalol and harmine when phosphate was omitted from the medium. In our work with different suspension cultures, we could enhance harman alkaloid and serotonin production in all lines by transferring the cells into phosphate-free medium. The same effect was recently found for the formation of alkaloids and phenolics by *Catharanthus* cell cultures (10) and for cinnamoyl putrescine synthesis in *Nicotiana* cell cultures (11). Changing phosphate metabolism could be an important tool to switch over from primary to secondary metabolism. While the medium had to be deficient in phosphate, NO_3-N, Mg, and Ca were shown to be necessary in higher amounts for harman alkaloid production. K was not needed under these conditions.

The inhibiting effect of 2,4-D on secondary metabolism has been observed for different cultures (15, 16, 28) and also proved to be true for *P. harmala* suspension cultures. Cells transferred into the production medium accumulated up to 2% harman alkaloids related to dry mass, which corresponded to maximum values of the plant (7). Higher contents have only been found in seeds, where the main harman alkaloids are harmine and harmaline (7, 21). In plants and also in cell cultures, harmine and harmalol are dominant.

In the production medium, a serotonin accumulation >2% could be reached. This amount is remarkably high in relation to values hitherto reported from plant material. Serotonin is often found in fruits. Investigating *Juglans regia* L., Bergmann *et al.* (2) found serotonin only in the embryo, where 0.06% was reached, with most being formed after abscission. No serotonin was detected in leaves, stems, and roots of adult plants. Whether the high serotonin biosynthesis in suspension cultures of *Peganum* occurs only in cell cultures or in plants, too, has to be determined. Our results suggest that serotonin is a by-product of the biosynthetic pathway to the harman alkaloids. The formation of both products was influenced in the same way.

By having established cell strains with high and low yields of alkaloid and by the development of an induction system, we now have the prerequisites for studying regulatory aspects at the enzyme level.

Acknowledgments—We thank Prof. E. Leistner, Münster, for *P. harmala* seeds and Ms. Marita Buchholz for skillful technical assistance. We are also grateful to Dr. D. Monner for his linguistic help in the final version of this manuscript.

LITERATURE CITED

1. Barz W, H Herzbeck, W Hüsemann, G Schneiders, HK Mangold 1980 Alkaloids and lipids of heterotrophic, photomixotrophic and photoautotrophic cell suspension cultures of *Peganum harmala*. Planta Med 40: 137–148
2. Bergmann L, W Grosse, HG Ruppel 1970 Die Bildung von Serotonin in *Juglans regia* L. Planta 94: 47–59
3. Berlin J 1980 *p*-Fluorophenylalanine resistant cell lines of tobacco. Z Pflanzenphysiol 97: 317–324
4. Constabel F 1980 Advances in plant cell culture towards improved yields of secondary products. 6th International Fermentation Symposium, London, Ontario, Canada, p. 160
5. Constabel F, JP Shyluk, OL Gamborg 1971 The effect of hormones on anthocyanin accumulation in cell cultures of *Haplopappus gracilis*. Planta 96: 306–316
6. Drew SW, AL Demain 1977 Effect of primary metabolites on secondary metabolism. Annu Rev Microbiol 31: 343–356
7. Gröger D 1959 Zur Physiologie der Harman-Alkaloide. Planta Med 7: 461–470
8. Hahlbrock K, H Grisebach 1979 Enzymic controls in the biosynthesis of lignin and flavonoids. Annu Rev Plant Physiol 30: 105–130
9. Kinnersley AM, DK Dougall 1980 Correlation between the nicotine content of tobacco plants and callus cultures. Planta 149: 205–206.
10. Knobloch KH, J Berlin 1980 Influence of medium composition on the formation of secondary compounds in cell suspension cultures of *Catharanthus roseus* (L.) G. Don. Z Naturforsch 35c: 551–556
11. Knobloch KH, J Berlin 1981 Phosphate mediated regulation of cinnamoyl putrescine biosynthesis in cell suspension cultures of *Nicotiana tabacum*. Planta Med 42: 167–172
12. Kurz WGW, KB Chatson, F Constabel, JP Kutney, LSL Choi, P Kolodziejczyk, SK Sleigh, KL Stuart, BR Worth 1980 Alkaloid production in *Catharanthus roseus* cell cultures: Initial studies on cell lines and their alkaloid content. Phytochemistry 19: 2583–2587
13. McKenzie E, L Nettleship, M Slaytor 1975 New natural products from *Peganum harmala*. Phytochemistry 14: 273–275
14. Murashige T, F Skoog 1962 A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 473–497
15. Nettleship L, M Slaytor 1974 Adaptation of *Peganum harmala* callus to alkaloid production. J Exp Bot 25: 1114–1123
16. Phillips R, GG Henshaw 1977 The regulation of synthesis of phenolics in stationary phase cell cultures of *Acer pseudoplatanus*. J Exp Bot 28: 785–794
17. Reinhard E, G Corduan, OH Volk 1968 Nachweis von Harmin in Gewebekulturen von *Peganum harmala*. Phytochemistry 7: 503–504
18. Robbers JE, HG Floss 1970 Physiological studies on ergot: Influence of 5-methyltryptophan on alkaloid biosynthesis and incorporation of tryptophan analogs into protein. J Pharm Sci 59: 702–703
19. Roller U 1978 Selection of plants and plant tissue cultures of *Catharanthus roseus* with high content of serpentine and ajmalicine. *In* AW Alfermann, E Reinhard, eds, Production of Natural Compounds by Cell Culture Methods. Ges. f. Strahlen und Umweltforschung, Munich, pp. 95–104
20. Sasse F, J Hammer, J Berlin 1980 Fluorimetric and high-performance liquid chromatographic determination of harmane alkaloids in *Peganum harmala* cell cultures. J Chromatogr 194: 234–238
21. Schipper A 1960 Untersuchungen an *Peganum harmala*. Doctoral Thesis, University of Würzburg
22. Tabata M, T Ogino, K Yoshioka, N Yoshikawa, N Hiraoka 1978 Selection of cell lines with higher yield of secondary products. *In* TA Thorpe, ed, Frontiers of Plant Tissue Culture 1978. University of Calgary, pp. 213–222
23. Vanable JW, Jr 1963 A ninhydrin reaction giving a sensitive quantitative fluorescence assay for 5-hydroxytryptamine. Anal Biochem 6: 393–403
24. Weinberg ED 1974 Secondary metabolism: Control by temperature and inorganic phosphate. Dev Ind Microbiol 15: 70–81
25. Westcott RJ, GG Hensh.w 1976 Phenolic synthesis and phenylalanine ammonia-lyase activity in suspension cultures of *Acer pseudoplatanus* L. Planta 131: 67–73
26. Zenk MH 1980 Enzymatic synthesis of ajmalicine and related indole alkaloids. J Nat Prod (Lloydia) 43: 438–451
27. Zenk MH, H El-Shagi, H Arens, J Stöckigt, EW Weiler, B Deus 1977 Formation of the indole alkaloids serpentine and ajmalicine in cell suspension cultures of *Catharanthus roseus*. *In* W Barz, E Reinhard, MH Zenk, eds, Plant Tissue Culture and Its Biotechnological Application. Springer-Verlag, Berlin, pp. 27–43
28. Zenk MH, H El-Shagi, U Schulte 1975 Anthrachinone production by cell suspension cultures of *Morinda citrifolia*. Planta Med Suppl 79–101