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The lipid composition and conversion of tryptamine to N_b-acetyltryptamine in a *Catharanthus roseus* cell line without indole alkaloids*

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SUMMARY

A cell line, NA13-2, was selected as a rapidly growing colony of protoplasts from a UV(254 nm)-fluorescent cell line, NA13-1, which originated from a tryptamine-resistant strain of *Catharanthus roseus* NA13. Cell line NA13-2 lost the capability to produce indole alkaloids. Tryptophan fed to these cells was converted to N_b-acetyltryptamine as the major product. The free acetyl coenzyme A content of NA13-2 cells was 50% higher than in the mother cells. The total lipid content of the NA13-2 cells was 2.5-fold that in the NA13 cells. In spite of the similarity in the fatty acid content to that of the mother cell line NA13, the total lipid extract of NA13-2 cells appeared as a wax instead of an oil, resulting from the presence of sterol esters.

INTRODUCTION

Catharanthus roseus (periwinkle) produces a large number of indole alkaloids, many of which have pharmacological value as anticancer agents, hypoglycemic and hypotensive agents. Thus, there has been a considerable interest in the potential for producing these rare alkaloids by cell culture techniques. Since the isolation of the first cell-free system of *C. roseus* for biosynthesis of indole alkaloids [9], there has also been an interest in the enzymatic and metabolic regulation of alkaloid biosynthesis in *C. roseus* cell cultures.

This paper describes the isolation and characterization of a cell line of *C. roseus*, which lost the ability to produce indole alkaloids, but efficiently converted tryptamine to acetyltryptamine. This cell line also produced a high content of acetyl coenzyme A and sterol esters not normally found in the parent cell line.

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MATERIALS AND METHODS

The *C. roseus* cell line NA13 was a gift from Dr. Kenneth Wilson (Miami University, Oxford, OH). It was first isolated by Kinnersley [4] from a tryptamine-resistant cell line. The culture was maintained on Murashige-Skoog medium [8] for 5 years. A daughter cell line NA13-1 was isolated by repeated selection for fluorescence under UV light at 254 nm, and a fast-growing colony NA13-2 was selected from NA13-1 protoplasts. The NA13-2 cells were grown, as shaken suspension cultures in four 2.8 liter Fernbach flasks containing 1 liter of medium each, for 12 days under light (13.5×10^3 E $\cdot m^{-2} \cdot s^{-1}$) and at room temperature. The cells were then treated with 1.3 mM L-tryptophan and 0.014 mM riboflavin (as a photosensitizer) for 3–4 days. Finally, the cells were harvested by filtration through a Büchner funnel.

The cells were homogenized by blending in a Waring blender with cold ethanol in the ratio 1:3 (w/v). The homogenate was filtered and the filtrate evaporated to dryness. Two percent succinic acid was added to dissolve the alkaloids. Non-alkaloidal components were removed by extracting three times with petroleum ether (b.p. 35–60°C). The acidic aqueous alkaloidal fraction was brought to pH 8.0 by addition of solid sodium carbonate. This was then partitioned with 3 volumes of dichloromethane. The organic phase was analyzed for indole alkaloids by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Thin layer chromatographic analysis of alkaloids was performed with silica gel G plates. The plates developed in chloroform/methanol (9:1) were sprayed with 1% ceric ammonium sulfate (CAS) in orthophosphoric acid. Preparative HPLC was carried out with an ODS-3 column (Whatman Co.) eluted with the following solvent program: 40–65% solvent B in 20 min, then 65–90% B in 1 min, followed by 90% B for 15 min. (Solvent A = water with 0.1% ethanolamine; solvent B = acetonitrile with 0.1% ethanolamine.) Fourier transform infrared (FTIR) spectra of compounds (in dry film) were obtained with a DIGILAB FTS-14 instrument. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian CFT20 instrument. Mass spectra (MS) were obtained on a Finnigan 4500 mass spectrometer.

Thin layer chromatography was used for analyzing the lipids, using the following solvent systems on silica gel G plates: (1) for polar lipids, chloroform/methanol/acetic acid/water (50:35:4:2); (2) for non-polar lipids, hexane/ether/acetic acid (80:20:1); (3) for secologanin, chloroform/methanol (3:1). The lipids and secologanin were visualized by spraying the developed plate with concentrated sulfuric acid and heating the plate on a hotplate to char the organic spots. Quantitative analysis of the lipid contents on TLC plates was carried out by densitometric measurement of a photographic negative of the TLC plate.

For fatty acid analysis, the samples were saponified in 0.5 N KOH/methanol, 10–15% BF_3/-methanol. Gas chromatography of fatty acid methyl esters was performed on a Supelco column (6 ft × 0.25 in i.d. Glasspak, 10% SP2340 (75% cyanopropylsilicone) on 100–200 mesh chromosorb WAW). Nitrogen gas was used as a carrier at 13 ml/min. The temperature was set initially at 100°C (4 min), and then a gradient at 6°C/min was used up to 148°C, then 4°C/min to 200°C. The components were detected by a flame ionization detector.

Free acetyl coenzyme A in the cell extract was assayed by a coupled enzyme assay as described by Decker [2]. All organic solvents used were HPLC grade or spectral grade. All chemical reagents used were reagent grade.

RESULTS

TLC analysis of the dichloromethane extract of NA13-2 cells showed only two major components, A and B. Both gave yellow reactions with CAS spray (R_f of A = 0.05, R_f of B = 0.65), and intense yellow fluorescence under 254 nm UV light. These two components were purified by a small silica gel column (1.0 cm i.d. × 3 cm), eluted with 20–50% methanol in chloroform to obtain B, and then with 100% methanol containing 1% ammonium hydroxide to obtain A. The two components were fur-

ther purified by preparative HPLC on an ODS-3 Magnum column.

Compound A was identical to tryptamine by HPLC and TLC analyses. The infrared spectrum of a trifluoroacetic acid salt derivative of A was found to be identical to that of the same salt of tryptamine. Compound B was found to be N_b-acetyltryptamine (Fig. 1) based on the following spectroscopic data, compared to previous reports [1,3]: MS (CI, CH_4/NH_3): base *m/e* 203 (M + 1); MS (EI): 130.06 (100), 143.04 (94.67), 202.10 (12.10, M+). NMR ($CDCl_3$): 1.91 (3H, *s*, $-COCH_3$), 2.96 (2H, *t*, J = 6.4, $-CH_2-CH_2-$HAc), 3.48 (1H, *s*, –NH–CO), 3.64 (2H, *t*, J = 6.2, $-CH_2-CH_2-$NHAc), 7.02–7.25 (6H, *m*, indole ring). FTIR: 1660 (NH–CO).

From 4 liters of NA13-2 cell culture fed 1.3 mM L-tryptophan, a total of 21.73 g dry (200.5 g fresh) weight of cell mass was obtained. From this harvest, the cell contents of tryptamine and N_b-acetyl-

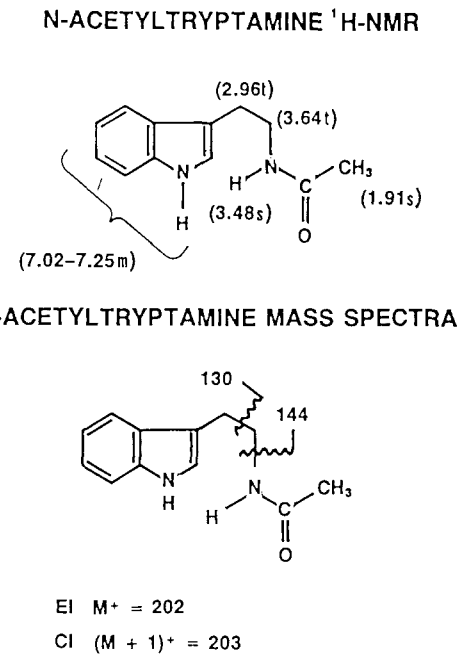

Fig. 1. Structural determination of N_b-acetyltryptamine.

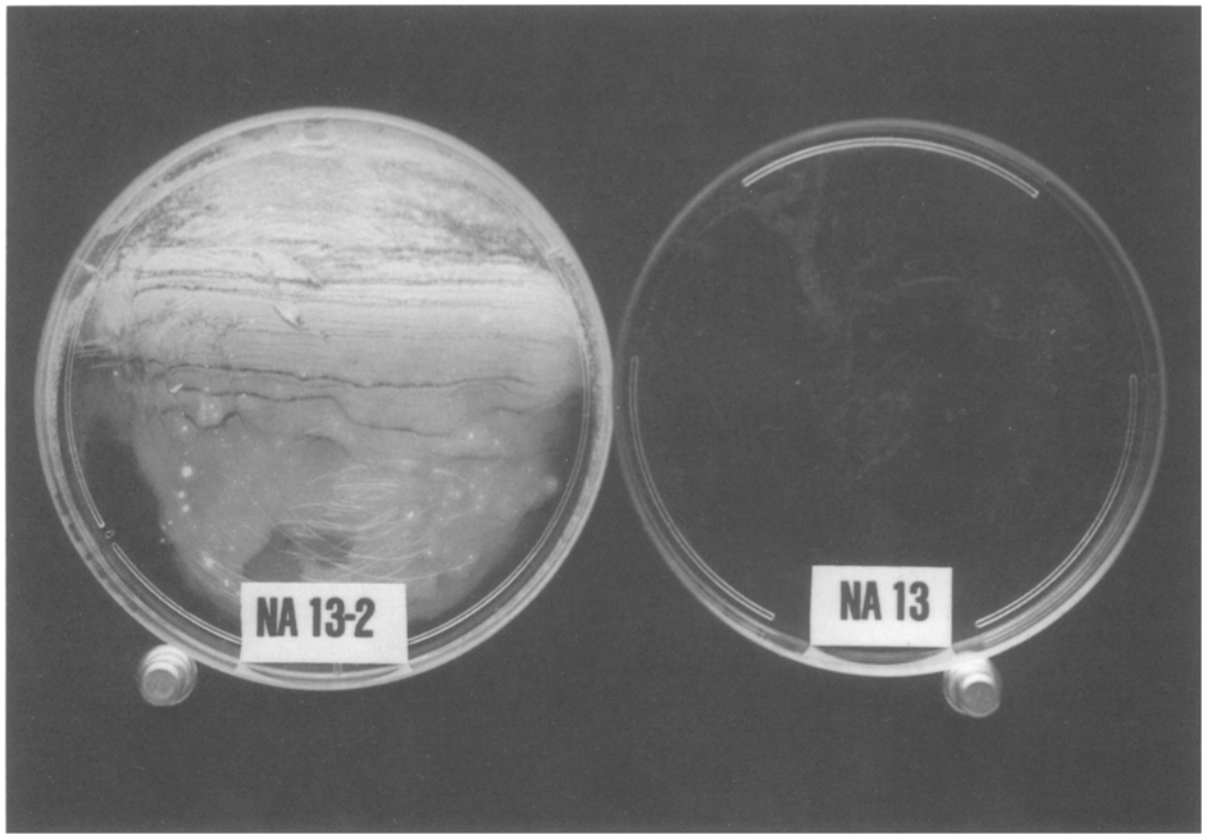

Fig. 2. Petroleum ether-extractable lipids from NA13 and NA13-2 cell lines of *C. roseus*.

tryptamine were 120 mg and 85 mg, respectively, corresponding to 0.55% and 0.39% based on cell dry weight. The yields of these components were 11.3% and 8.0%, respectively, based on the amount of tryptophan fed to the culture.

The petroleum ether extract of the NA13-2 cells was 3.1% (dry weight basis) compared to 1.2% in the NA13 cells. The lipid fraction in NA13-2 cells appeared as an opaque waxy solid, while the lipid in NA13 was a translucent oil (Fig. 2). Densitometric quantitation of the lipid zones showed that the sterol ester spot in NA13-2 extracts was almost 10-fold that in NA13 extracts (Fig. 3). Analysis of

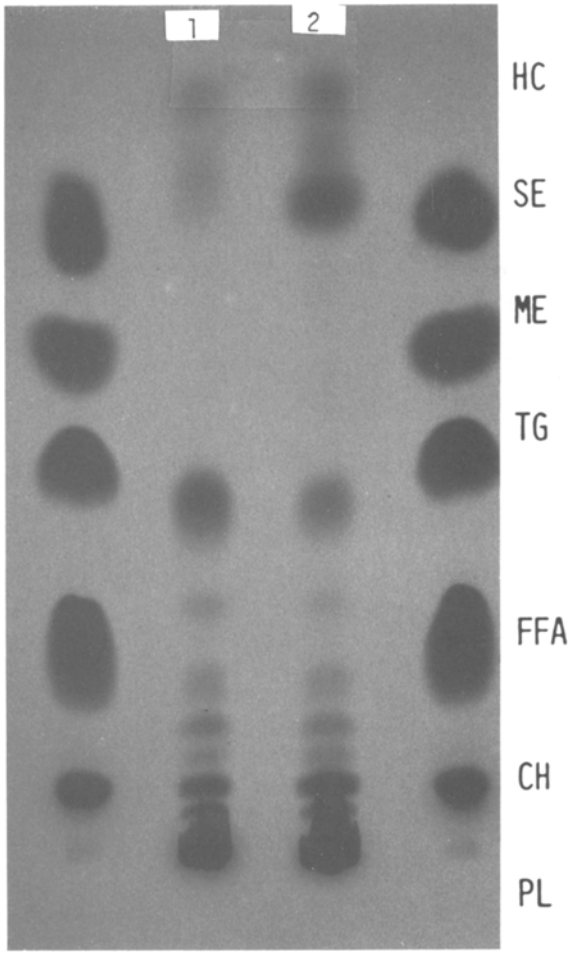

Fig. 3. Non-polar lipid analysis by TLC. Lane 1 = NA13; lane 2 = NA13-2; HC = hydrocarbons; SE = sterol esters; ME = monoesters; TG = triglycerides; FFA = free fatty acids; CH = cholesterol; PL = phospholipids.

Table 1

Fatty acid analysis of *C. roseus* cell lines by gas chromatography

Fatty acid	R.T.[a] (min)	Relative peak intensity	
		NA13	NA13-2
C-6	1.59	n.d.[b]	n.d.
C-8	3.49	n.d.	n.d.
C-10	6.89	n.d.	n.d.
C-12	9.68	n.d.	n.d.
C-14	12.27	0.35	0.40
C-16	15.22	23.52	18.07
C-17	16.72	0.23	0.34
C-18	18.25	1.66	1.01
C-18:1	18.97	32.30	32.38
C-18:2	20.19	31.23	38.44
C-18:3	21.58	7.36	5.46
C-20	21.19	0.48	0.58
A[c]	24.03	0.76	0.66
B[c]	24.65	n.d.	0.70
C[c]	27.27	0.54	0.63

[a] R.T. = retention time.
[b] n.d. = not detected.
[c] A, B, and C are unidentified components.

the fatty acid contents of the two cell lines showed no appreciable differences between the cell lines, both having palmitic acid, oleic acid and linoleic acid as the major components (Table 1).

The endogenous free acetyl coenzyme A in the cell extract as determined by a coupled enzyme assay revealed that the daughter cell line NA13-2 contained 77 nmol/g dry weight, a 50% higher acetyl coenzyme A content than in the NA13 cell line (51 nmol/g dry weight).

DISCUSSION

Indole alkaloids are derived from precursors in the acetate-mevalonate pathway and the tryptophan pathway as shown in Fig. 4. The *C. roseus* cell line NA13-2 lacked the ability to produce any indole alkaloid, although L-tryptophan fed to this cell culture was decarboxylated and N-acetylated on the side chain. Compared to the mother cell line NA13, the new cell line NA13-2 contained a 2.5-

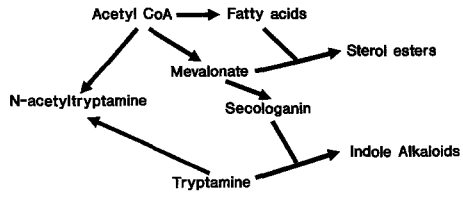

Fig. 4. Indole alkaloid biosynthesis and acetyl coenzyme A metabolism.

fold greater amount of petroleum ether-extractable lipids and a 10-fold greater amount of sterol esters.

Secologanin, the terpenoid precursor of the indole alkaloids, was not detected by TLC analysis of the NA13-2 cells. However, this cell line had a high level of endogenous acetyl coenzyme A, and was very active in the biosynthesis of sterol esters. Nevertheless, such activities did not appear to affect indole alkaloid biosynthesis in the cell line NA13-2.

Constabel (1986, private communication) also observed that some non-alkaloid-producing cell lines had large vacuoles with stored lipids within the cell. McFarlane et al. [7] reported that geraniol 10-hydroxylase (the enzyme leading to secologanin) was feedback-inhibited by catharanthine. Furthermore, Lee et al. [6] demonstrated (later confirmed by Kutney et al. [5]) that the biosynthesis of certain indole alkaloids can be increased more than 2-fold by the addition of a terpenoid-inducing, synthetic growth regulator to the culture medium. From these observations, it appears that regulation of indole alkaloid biosynthesis may be related to terpenoid biosynthesis, particularly in the post-mevalonate steps.

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