used to minimize quenching but even with larger amounts the pattern of elution of radioactivity was clearly very similar.

Table 1 gives the results from a typical experiment of three determinations made. In all cases nearly all the radioactive label was found in the benzene fraction which contains the aromatic residues. We conclude that, under the conditions described, methyl palmitate is eluted by benzene from an alumina chromatographic column. It is likely that other esters of long-chain monocarboxylic acids behave in the same manner.

This work was supported by National Science Foundation grant no. ENV 76-08310.

Received August 8, 1977

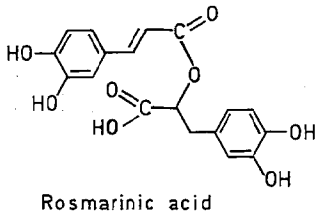
1. Walker, J.D., et al.: Bull Environ. Contam. Toxicol. *13*, 245 (1975)
2. Eglinton, G., Murphy, M.T.J. (eds.): Organic Geochemistry. Berlin: Springer 1969

Production of Rosmarinic Acid by Cell-Suspension Cultures of *Coleus blumei*

M.H. Zenk, H. El-Shagi, and B. Ulbrich

Lehrstuhl für Pflanzenphysiologie der Ruhr-Universität, D-4630 Bochum

It has been claimed that plant-cell cultures produce little, if any, of the particular "secondary" metabolites that are elaborated by the differentiated plant [1, 2]. However, we were able to demonstrate that *Morinda citrifolia* cell-suspension cultures could be induced to produce anthraquinones in concentrations exceeding those found in the differentiated plant by a factor of 9, and total yields of 2.5 g of secondary product per liter of medium were achieved [3]. In the course of studies on the biosynthesis of caffeoyl depsides [4] it became necessary to develop cell cultures capable of producing large quantities of rosmarinic acid (caffeoylester of 3,4-dihydroxyphenyllactic acid), the second



Rosmarinic acid

most common caffeoylester reported to occur in plants [5]. *Coleus blumei* (Labiatae) contains rosmarinic acid. Preliminary qualitative studies with *Coleus* tissue cultures, which had been maintained in this laboratory for a period of over 6 years, showed that rosmarinic acid is formed by these cultures [6]. A comparison of 15 commonly used tissue-culture media [7] showed that highest yields of the depside are obtained in B-5 medium [8]. Subsequently, it was demonstrated by testing 35 differently substituted phenoxyacetic acids at 10^{-5} *M* concentration in B-5 medium (without other hormones) that maximal production was supported by 2,4-dimethylphenoxyacetic acid (40% increase over controls grown in the absence of any hormone) while 2-chloro-4-fluoro-phenoxyacetic acid specifically inhibited ester formation (70%) without affecting cell mass production. Most reproducible yields were, however, obtained in the absence of any hormone, a condition which was finally adopted for all subsequent experiments. As shown in Figure 1 the concentration of sucrose is of great influence on the yield of rosmarinic acid production, 7% sucrose being optimal. At 2% sucrose, a concentration which most media routinely contain, only 13% of the maximal possible yield is obtained. Under these conditions of carbohydrate limitation rosmarinic acid synthesis can be stimulated over 100% by the addition of 500 mg L-phenylalanine/l, a biosynthetic precursor of this ester [5]. Under optimal conditions rosmarinic acid accumulated up to 13–15% of the dry weight of the cells. This value exceeds the concentration reached in the differentiated plant (shoot 2.8%; leaves 2.5%; root 2.1%) by a factor of 5. It is noteworthy that the maximal amount of secondary product is formed within the relatively short period of 13 days of growth; thereafter the cells rapidly autolyze. When the cells were grown in a 30-l airlift fermenter, yields of rosmarinic acid dropped by two-thirds and the maximal concentration was under these conditions reached only after about 20–25 days of growth. This shows that considerable scale-up problems occur. From fermenter-grown cells rosmarinic acid was isolated by standard methods [5] in cristalline form (2.2 g), m.p. 168–169 °C ([9]: 172 °C), MS: 360 (M^+), 198, 180, 163, 136, 123 m/e.

The *Coleus* system introduced here gives the highest yield (10 mmol/l medium) of any single secondary product formed in a plant-suspension culture thus far reported.

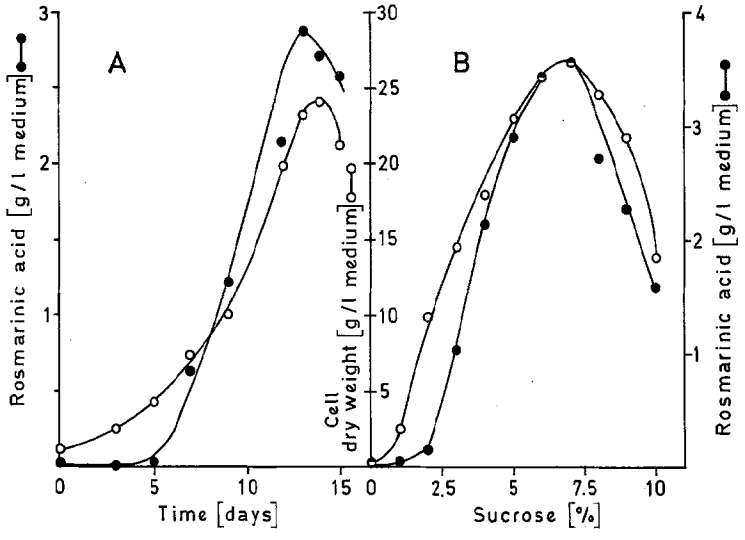


Fig. 1. A) Kinetics of dry-weight increase and rosmarinic acid synthesis in cell-suspension cultures of *C. blumei* (B-5 medium without hormones plus 5% sucrose). B) Effect of different sucrose concentrations on growth (as dry weight) and rosmarinic acid production (growth period 14 days). 100-ml Erlenmeyer flasks containing 25 ml of medium. Temperature 23 °C. 100 rpm gyratory shaking, continuous light (600 lux). Quantitative determinations were done by fluorometry on TLC plates (solvent system toluene/ethylformiate/HCOOH 50:40:10; rosmarinic acid: R_f 0.2) using a Zeiss KM 3 TLC-scanner

Supported by the Deutsche Forschungsgemeinschaft and the Bundesminister für Forschung und Technologie.

Received June 30, 1977

1. Krikorian, A.D., Steward, F.C., in: Plant Physiology, Vol. V b, p. 227 (F.C. Steward, ed.). New York-London: Academic Press 1969
2. for review: Teuscher, E.: Pharmazie *28*, 6 (1973)
3. Zenk, M.H., El-Shagi, H., Schulte, U.: Planta med. (Suppl.) 79 (1975)
4. Stöckigt, J., Zenk, M.H.: FEBS Letters *42*, 131 (1974)
5. Ellis, B.E., Towers, G.H.N.: Biochem. J. *118*, 291 (1970)
6. Ellis, B.E., Zenk, M.H.: unpublished
7. Koblitz, H.: Zell- und Gewebezüchtung bei Pflanzen. Stuttgart: G. Fischer 1972
8. Gamborg, O.L., Miller, R.A., Ojima, K.: Exptl. Cell Res. *50*, 151 (1968)
9. Hiller, K.: Pharmazie *20*, 574 (1965)

Secobarbital Suppression of Anaerobic Type of Rice Seedling Germination

H.A. Kordan

Department of Plant Biology, University of Birmingham, Birmingham B15 2TT (England)

Rice seedlings possess a highly efficient fermentation system which is capable of providing sufficient energy for coleoptilar extension growth but not for root or leaf growth when germinating under low environmental oxygen tensions [1, 2]. Opik [3] has pointed out that coleoptilar extension growth can occur to the extent of several centimeters in rice seedlings even when germinated under complete anaerobiosis. Thus, rice seedlings germinated in oxygen-poor environments manifest an anaerobic type of germination which has recently been designated as d^- seedlings [4, 5]. The similarity of the anaerobic type of rice seedling germination in nitrogen gas, in vacuo, and in the barbiturate Amytal in investigations concerning the influence of oxygen on chlorophyll formation indicated that Amytal did not interfere with the anaerobic type of germination [5]. Suppression of the anaerobic type of rice seedling germination by the barbiturate Secobarbital is reported here.

One hundred unhulled rice seeds (*Oryza sativa*, c.v. Calrose) surface-sterilized with sodium hypochlorite [6] were placed in a glass anaerobic jar and subjected to a continuous flow of nitrogen gas scrubbed with alkaline pyrogallol (anaerobic control) as described previously [4]. A 1-liter capacity tall form 'Pyrex' beaker without a spout was filled to a depth of 180 mm with glass-distilled water and another beaker was filled to a depth of 180 mm with a 3.05 m*M* solution of the acid form of Secobarbital (Sigma London, Chemical Co. Ltd.). One hundred surface-sterilized rice seeds were dropped into the liquid in each beaker, only those seeds being used which settled readily on the bottom of the beakers. Each beaker was loosely covered with a 'Pyrex' Petri dish top and both beakers and the anaerobic jar were placed under 3000–3500 lux illumination from Gro-Lux fluorescent lights set for an 18 h day (26–27 °C) and 6 h dark (24–25 °C) daily cycle.

Table 1 shows that coleoptilar extension growth was virtually completely suppressed in the Secobarbital-treated seedlings compared with coleoptilar extension growth in the seedlings in nitrogen gas and in glass-distilled water. None of the slightly emergent coleoptiles of the Secobarbital-treated seedlings showed any signs of greening, thus showing that this barbiturate also inhibited chlorophyll development as did Amytal [5]. All germinating seedlings in nitrogen gas (anaerobic control) were of the d^- type since anaerobiosis has been found to inhibit chlorophyll formation in light-germinated rice seedlings [4]. Coleoptile emergence in the germinating seedlings in glass-distilled water was followed by coleoptile greening and the subsequent development into normal green seedlings by day seven by virtue of their ability to supply themselves with sufficient oxygen for growth and development through photosynthetic activity.

On day seven, the Secobarbital-treated seedlings were washed as described previously [5] and resubmerged in glass-distilled water at 180 mm depth and placed under 3000–3500 lux illumination. The secobarbital-treated seedlings manifested the same pattern of coleoptilar extension growth and development into green seedlings as described above for the glass-distilled water environment after having been removed from the Secobarbital solution. This shows that the marked suppressive action of Secobarbital on coleoptilar extension growth was a reversible phenomenon.

Barbiturates have not been described as interfering with anaerobic metabolism in animal or plant organisms or cellular systems [7]. However, the marked suppressive action of Secobarbital on coleoptilar extension growth in submerged germinating rice seedlings shows that this barbiturate seems to interfere with the fermentative process(es) which brings about the anaerobic type of rice seedling germination in an oxygen-poor environment.

I thank The Dr. Hadwen Trust For Humane Research for funds received in support of this research, Dr. A.J. Oakes (United States Department of Agriculture, Beltsville, Maryland) for furnishing the rice seeds *Oryza sativa*, cv. Calrose, and Karin Brown for technical assistance.

Received July 12, 1977

1. Taylor, D.L.: Amer. J. Bot. *29*, 721 (1942)
2. Vlamis, J., Davis, A.R.: Plant Physiol. *18*, 685 (1943)
3. Opik, H.: J. Cell Sci. *12*, 725 (1973)
4. Kordan, H.A.: J. Exp. Bot. *27*, 105 (1976)
5. Kordan, H.A.: Ann. Bot. *41*, 257 (1977)
6. Kordan, H.A.: ibid. *41*, 673 (1977)

Table 1. Secobarbital suppression of germination

Treatment	Germination [%]	Time [days]	Mean coleoptile or shoot length [mm] (±S.E.)
Nitrogen gas	96	7	17.39 ± 0.81 (22.29)[a]
Distilled H_2O	94	7	53.71 ± 3.39[b] (68.86)[c]
Secobarbital	35 (91)[d]	7	0.78 ± 0.08

[a] Ratio of mean coleoptile lengths between nitrogen gas and Secobarbital
[b] Normal green seedlings
[c] Ratio of mean shoot and coleoptile lengths between distilled H_2O and Secobarbital
[d] Per cent germination and development into green seedlings after removal from Secobarbital given in brackets

