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PRODUCTION OF DIHYDROLEUCODIN FROM CALLUS LINES OF ARTEMISIA DOUGLASIANA BESSER

L. J. Pestchanker, M. Kurina, A. M. Giulietti and O. S. Giordano
Departamento de Química Orgánica.
Facultad de Química, Bioquímica y Farmacia.
Universidad Nacional de San Luis.
Chacabuco y Pedernera
5700 San Luis
ARGENTINA

SUMMARY:

Dihydroleucodin, the main secondary metabolite from *Artemisia douglasiana*, showed cytoprotective activity against chemically induced peptic ulcer in rats. In this work, cell lines of *A. douglasiana* were used to study the kinetic of growth and dihydroleucodin production.

INTRODUCTION:

A. douglasiana Bess., a plant that grows in the Cuyo region (Argentina), has often been used in folk medicine as an antiulcer agent. Our preliminary phytochemical analysis (unpublished) of specimens afforded beside the main product dihydroleucodin (DHL), other minor compounds. Recents studies on the pharmacological activity of DHL, showed it as a cytoprotective agent against chemically induced peptic ulcer in rats.

The objective of this work was the selection of DHL producer lines from *A. douglasiana* calli obtained from different origins and the kinetic study of cell growth and product formation.

MATERIALS AND METHODS:

Cell cultures: Plants and seeds of *A. douglasiana* were collected in El Durazno, San Luis, Argentina, between June and September 1988. The plant material was sterilized by treatment with 4% NaClO and 0.075% Triton X-100 for 15 min. After that the material was washed with sterilized bidistilled water x5. Explants were obtained from seedlings (cotyledons, hypocotyls, roots and leaves) and different adult plant parts (leaves and shoots).

The seeds were germinated on 0.8% agar medium containing Murashige and Skoog's basal medium (MS) (Murashige and Skoog, 1962) added 3% sucrose at 25∓1 °C and exposed to 16h. photoperiods for 30 days, using fluorescent lamps at approximately 1.8 $W/m^2.s$.

The explants were cultured in modified mineral nutrient medium (MS) with the addition of myoinositol (100 mg/l), sucrose (30 g/l), and RT vitamin complex (20 ml/l) (Khana and Staba, 1968). The pH was adjusted to 5.6-5.8 and the medium solidified with agar (9 g/l). This medium will be referred to as MSRT medium.

For callus initiation studies, explants from different tissue sources (seedling and explants) were cultured on 5 different media, which consisted of the MSRT medium with the addition of gibberellic acid (1 mg/l) supplemented with one of the following auxins: IAA, IBA, NAA, 2,4-D and 2,4,5-T at a concentration of 4 mg/l. The incubation conditions, were the same as mentioned above. Subcultures were carried out using the same medium in which the culture was initiated. In order to estimate the callus growth, calli were dried to constant weight at 45 °C.

DHL determination: the dry calli were crushed and extracted with methanol. The residue was percolated through a Silica Gel 60 column (0.5 g), concentrated and dissolved in a methanol:H_2O 4:1 mixture. This solution was analyzed by HPLC using a UV detector. A C-18 column eluted with methanol:H_2O 7:3 at a flow rate of 1.5 ml/min was used. The contents of DHL were determined by means of a standard calibration curve. Each value showed in the cultured tissue was the average of 6 measurements.

RESULTS AND DISCUSSION:

Calli from explants of different origins were initiated on 5 MSRT media containing gibberellic acid supplemented with IAA, IBA, NAA, 2,4-D, or 2,4,5-T respectively. Not all lines survived over the third subculture. After seven subcultures in the same medium 30 lines survived but only six of them maintained significant biosynthetic ability.

Table 1 shows the origin of each callus line and the plant growth regulator used in each case.

Clearly, DHL production depends on the explant origin and the plant growth regulator utilized. The best results were obtained when leaf explants from adult plants were grown on MSRT medium with IBA, and when

TABLE 1: DHL production in different origin calli of *A. douglasiana* Bess. after seven subcultures.

CALLUS	ORIGIN	PLANT GROWTH REGULATOR*	DHL CONTENT (mg/g)
Ho1	leaves**	IBA	0.46 ± 0.036
Ho2	leaves**	NAA	0.088 ± 0.007
Hi1	hypocotyls	IAA	0.42 ± 0.034
R1	roots	IBA	0.19 ± 0.015
Co2	cotyledons	2,4-D	0.074 ± 0.006
Co1	cotyledons	2,4,5-T	0.55 ± 0.044

(*) The plant cell regulator was added to MSRT medium supplemented with gibberellic acid. (**) From adult plants.

cotyledon explants were grown on MSRT medium with 2,4,5-T. These lines, and that obtained from roots of seedlings grown on MSRT medium supplemented with IBA and gibberellic acid, maintained their productive capacity during 12 subcultures.

In these studies the selection of cell lines of high productivity was made in the classic way in spite of recent reports demonstrating that the best way for the selection of cell lines with high productivity and a single chemotype is the isolation of protoplasts (Fujita, Takahashi and Yamada, 1985).

Figure 1 shows the growth and DHL production pattern of cell lines of *A. douglasiana* Bess. The dry weight was increased 4,5 and 10 times for Co1, Ho1 and R1 respectively at different periods of time.

The specific growth rates (μ) estimated were 0.1 and 0.11 d^{-1} for R1 and Ho1 calli respectively, while the value obtained for Co1 calli was about 0.061 d^{-1}. These values are within the range obtained for callus cultures of other species.

As regards, the kinetic of DHL production, the behavior of the three tested lines was similar: the maximum value obtained was followed by a quick fall, which could be attributed to the degradation of DHL, changes in the chemical structure or a switch phenomenon. The initial fall observed in the DHL contents in the tested lines was only due to the increase of biomass. DHL production began after 3 weeks of culture for the Co1 line and 4 weeks of culture for the R1 and Ho1 lines. These results were in accordance with stationary phase initiation. In all lines, DHL production was not associated to growth, which is a common phenomenon in secondary metabolite production (Dougall, 1985). In spite of the fact that both Ho1 and Co1 lines produced similar values of DHL, the latter showed a higher productivity (0.0196 mg/g.d for Co1 against 0.0109 mg/g.d for Ho1 lines).

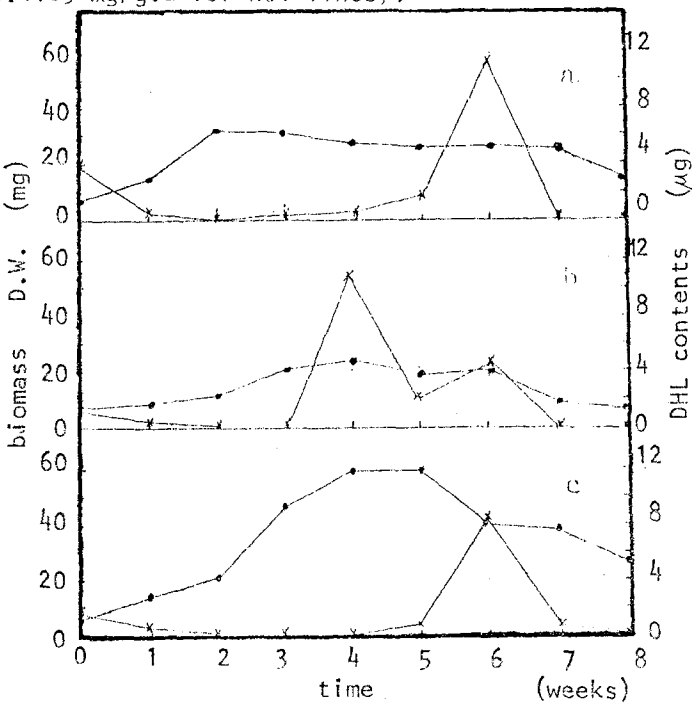

FIGURE 1: growth and DHL production pattern
a) Ho1 line.
b) Co1 line.
c) R1 line.
x DHL contents
. biomass

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