

DITERPENICS GLYCOSIDES ACUMULATIONS DINAMICS OF *STEVIA REBAUDIANA* (BERTONI) HEMSL. (*ASTERACEAE*) “IN VITRO” CULTIVATED

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Keywords: callous, hypocotyl, epicotyl, cotyledonal knot, primary leaflets, stevioside

Abstract: This paper analyses the content in diterpenics glycosides of *Stevia rebaudiana* Bert. (*Asteraceae*) „in vitro“ cultivated. There are presented comparative results about the stevioside’s concentration in the callous generated on different origins fragments, and leaves. The stevioside accumulates especially in callous that offer the possibility of its isolation in cellular crops.

Abbreviations: MS (1962), Murashige-Skoog medium, 1962; 2,4 D – 2, 4, dichlorfenoxiacetic acid, HPLC – high performance liquid chromatography.

INTRODUCTION

In green plant (Bridel, 1931), the stevioside is found in a concentration of 6%, at the beginning of vegetative period and it grows to 11,7%, at the end of vegetative period. Young stems have a sweet taste stronger than the old ones. However, the old leaves are sweeter than the young ones.

This paper is based on the analysis of diterpenic glycosides of *Stevia rebaudiana* Bert. species “in vitro” cultivated.

MATERIALS AND METHODS

In our experiments, we used, as an initial material, vitroplantes of *Stevia rebaudiana* Bert., that had been obtained out of meristheme crops at Biological Researches Institute from Cluj-Napoca.

As a source of explants for collousage, there were tested portions of 3 – 4 mm hypocotyl, epicotyl, cotyledonal knot and primary leaflets drow from the plantlets of about three weeks old.

The explants were inoculated upon hormonal variants of MS (1962), with an addition of 2,4 D, having concentrations of 2 mg/L. The axillary shooting multiplication was successfullly realised (Cachiță, 1987) upon MS (1962), without any improvements. At the same time, there was obtained a crop of *Stevia rebaudiana* Bert. using “in vivo” germination of seeds, as a control sample.

For analyzing the stevioside concentration, the callous and the leaves was fine broken up using mortaring. Extraction was sequentially realized, in a Soxhlet apparatus, with chlorophorm and, then, with methanol. Out of the obtained extracts, there were got corresponded dilutions for chromografcical analysis. The detection of the stevioside was realized using HPLC, a method that is usually used abroad (Kingham, 1984; Makapugay, 1984 and Nishiyama, 1991). For the chromatografcical analysis there was used a system consisting of a Supelcosil column, with 25 cm in length and 4.6 cm inner diametre, eluent acetonytril – water, with a flow of 0.5 mL/min., UV detector at 210 nm.

As a standard, there was used the stevioside of 95% purity, at a concentration of 1 mg/L, and the further observations was strictly made to this compound. The obtained results were processed on a computer, using a program dedicated to HPLC.

RESULTS AND THEIR INTERPRETATION

There was studied the capacity of biosynthesis of stevioside at callous of different "in vitro" origins level, comparatively with leaves of *Stevia rebaudiana* Bert., that was grown in normal conditions.

Out of the chromatograms that corresponded to the extract obtained from the leaves of *Stevia rebaudiana* grown in normal conditions, there was identified the stevioside, at a concentration of 0.7 mg/g leaves (fig. 1).

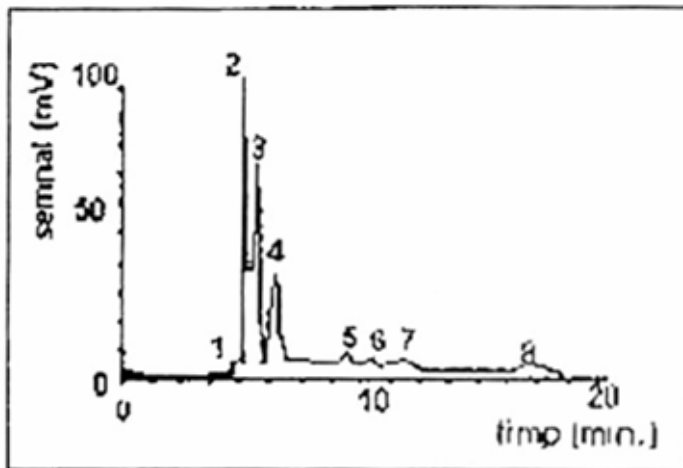


Fig.1- The stevioside's concentration in the extract of leaves "in vivo"(1-unknown;2-unknown;3unknown;4-steviozid; 5,6,7,8/unknown).

Out of the extracts from the "in vitro" explants, we obtained the following results:

- in the callous of hypocotyl, the stevioside was accumulated in a concentration of 6×10^{-4} mg/g material (fig. 2);

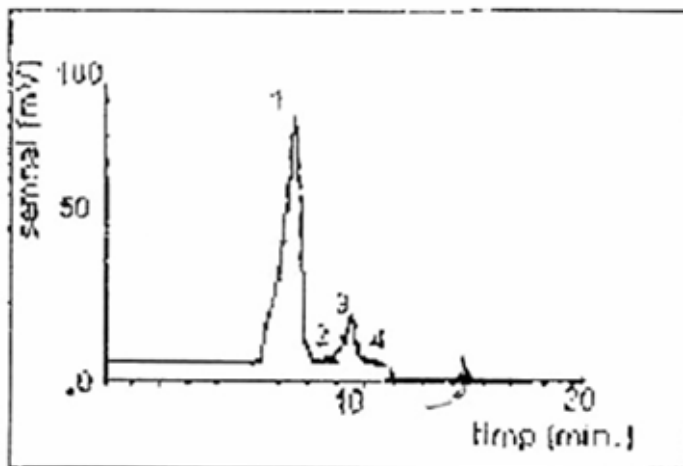


Fig.2. The stevioside's concentration in the callous generated of hipocotil (1-unknown;2-unknown;3-unknown; 4-steviozid).

- the callous generated on cotyledonal knot accumulated the stevioside in a concentration of 2×10^{-3} mg/g material (fig.3).

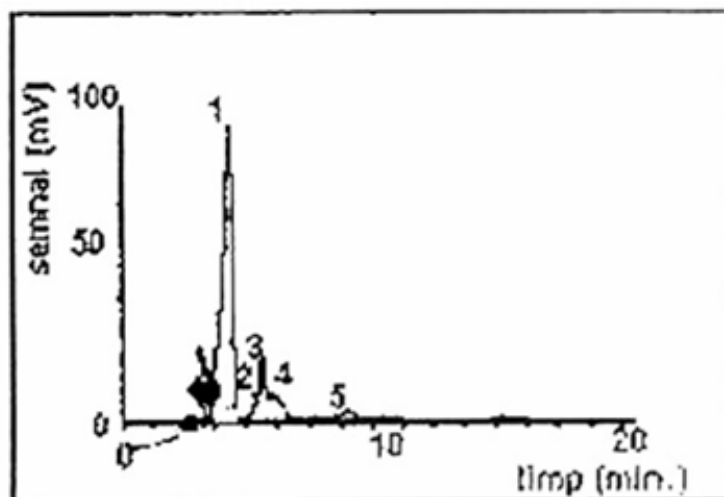


Fig.3. The stevioside's concentration in the callous generated of cotyledonal knot (1-unknown;2-unknown;3-unknown; 4-steviozid).

- the callous generated on the epicotyl accumulated the stevioside in a concentration of 10^{-2} mg/g material (fig. 4);

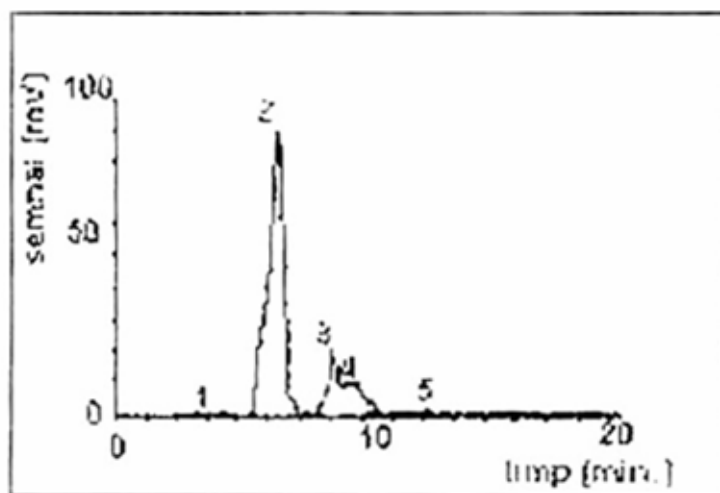


Fig.4. The stevioside's concentration in the callous generated of cotyledonal knot (1-unknown;2-unknown;3-unknown; 4-steviozid;5-unknown).

- in the callous generated on bits of primary leaflets, the stevioside was accumulated in a concentration of 2×10^{-4} mg/g material (fig. 5);

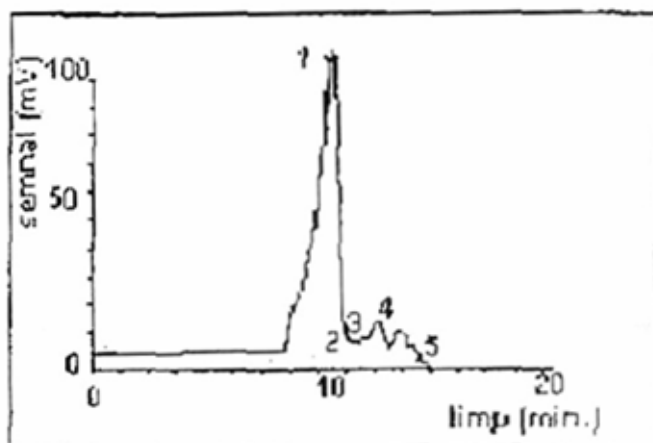


Fig.5. The stevioside's concentration in the callous generated of cotyledonal knot (1-unknown;2-unknown;3-unknown; 4-steviozid;5-unknown).

- The leaves "in vitro" of *Stevia rebaudiana* Bert. accumulated the stevioside in a concentration of 0.29 mg/g material (fig. 7).

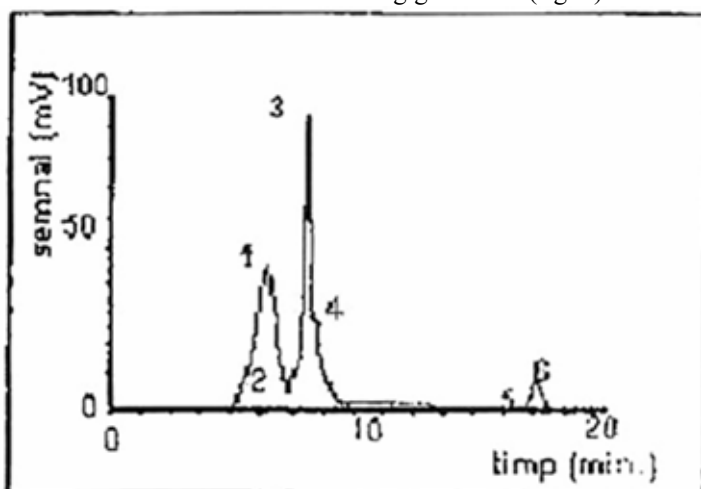


Fig.7- The stevioside's concentration in the extract of leaves "in vitro"(1-unknwn;2-unknown;3unknown;4-steviozid; 5,6-unknown).

- As a standard, there was used the stevioside of 95% purity, at a concentration of 1 mg/L, and the further observations was strictly made to this compound (fig.8).

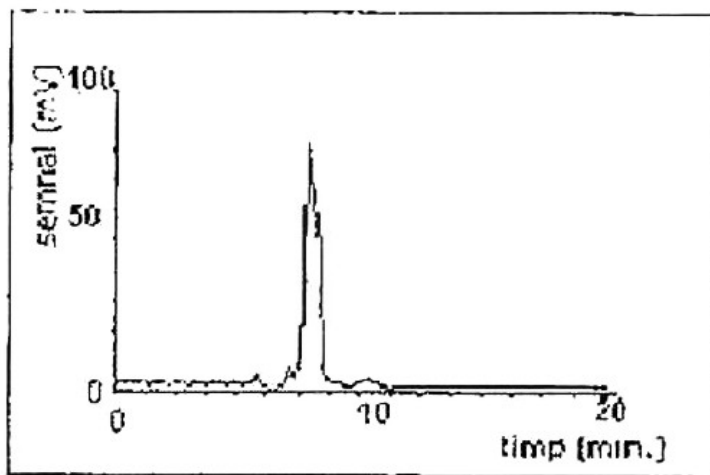


Fig.8 - The stevioside's concentration of 95% purity

CONCLUSIONS

Analyzing the chromatograms, it results that in the callous of different origins, the stevioside accumulated in concentrations between 2×10^{-4} mg/g and 10^{-2} mg/g vegetal material.

The great quantity of stevioside was accumulated in the callous generated by the fragments of epicotyl, in a concentration of 10^{-2} mg/g vegetal material. In raport with the control sample, all the extracts from callous had a smaller content in stevioside.

The presence of stevioside in the callous, give us the opportunity for its isolation in cellular cultures.

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