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OCCURRENCE OF BETULINIC ACID IN DIFFERENT CALLUS CULTURES OF *SOLANUM AVICULARE*

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Key Word Index—*Solanum aviculare*; Solanaceae; pora-pora; callus culture; triterpenoid production; betulinic acid; 3β-hydroxy-lup-20(29)-en-28-oic acid.

Abstract—Betulinic acid (3β-hydroxy-lup-20(29)-en-28-oic acid) was obtained from *in vitro* cultures of *Solanum aviculare* in yields up to 3% of the dry weight. This is a further example of overproduction by cells cultured *in vitro* of a product not found in the original parent plant.

INTRODUCTION

In recent years it has been shown that plant cells cultivated *in vitro* can serve as a source of natural products; for reviews see Berlin [1] or Curtin [2]. Much attention has been paid to the biosynthetic potential of plant cell cultures of several *Solanum* species due to the expected importance of the alkaloid solasodine as a raw material for the pharmaceutical industry [3]. Solasodine has been proved as a component in tissue cultures of *Solanum laciniatum* [4–9].

RESULTS AND DISCUSSION

We have established nearly 200 callus strains of a very closely related species, *Solanum aviculare* Forst [10]. These were initiated from leaves, stems and roots of sterile plantlets grown from aseptically germinated seeds we obtained from the botanical gardens in Gatersleben, Kew and Bordeaux. During our analysis of the chemical composition of extracts from callus strain KL 9 we found a relatively high amount (3% dry wt) of betulinic acid (identified by mp, MS, ^{1}H NMR), accompanied by sitosterol, stigmasterol, campesterol, solasonine, solamargine, γ-aminobutyric acid and other unidentified compounds. This fact led us to screen for the betulinic acid content in some of our other calli of *S. aviculare*.

The comparison of the betulinic acid content in nine other callus strains with their growth value under constant conditions showed no correlation between the two factors (Fig. 1). The large differences between various callus

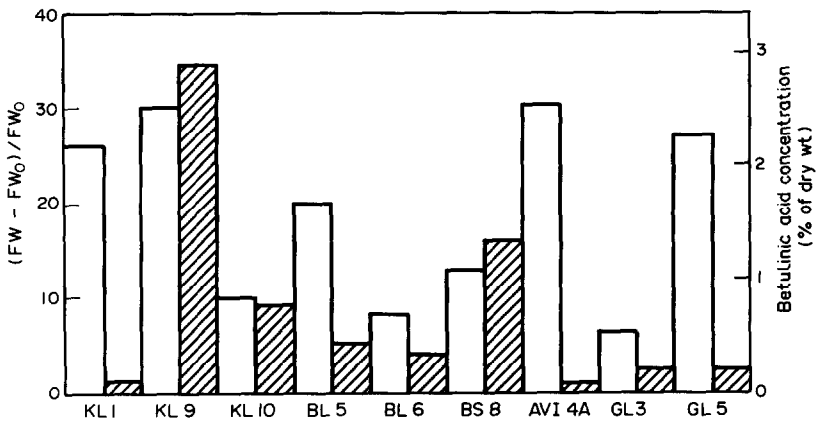

Fig. 1. Comparison of betulinic acid content and growth value in *Solanum aviculare* callus cultures. Open columns represent growth values while hatched columns indicate the betulinic acid content. FW = fr. wt at 25 days, FW_0 = initial fr. wt.

strains of the same species under standard conditions underlines the great variablity in gene expression to be expected when working with plant cells cultured *in vitro*.

Of great interest is not only the finding of betulinic acid in *Solanum* species itself, but also its relatively high production in the cell culture derived from a parent plant in which we could not detect any traces of betulinic acid. During the preparation of this manuscript Indrayanto *et al.* [11] reported the presence of lupane derivatives like betulinic acid in cell cultures of *Solanum* species, but no quantitative data are given.

EXPERIMENTAL

The callus cultures of *Solanum aviculare* were derived in 1979 and 1980 from plantlets aseptically grown on agar [10]. These were obtained from surface-sterilized seeds, kindly supplied by the Botanical Gardens in Gatersleben (G), Kew (K) and Bordeaux (B). Calli derived from leaves (L), stems (S) and roots (R) were cultivated for 3 years on agar media according to Murashige and Skoog, as modified by Linsmaier and Skoog [12] at 27° ± 1° in darkness. The media were supplied with 1 $\times 10^{-6}$ mol $l.^{-1}$ kinetine and 1 $\times 10^{-6}$ mol $l.^{-1}$ 2,4-dichlorophenoxyacetic acid. The regular subcultivation period was 25 days. Betulinic acid was isolated from the extract of 500 g fresh callus removed from agar. The callus of strain KL 9 was homogenized with 500 ml of MeOH, left standing overnight at room temp., filtered and concd on a vacuum rotary evaporator. Extract (2.0 g) was chromatographed on a column of 200 g silica gel Hermann 2, deactivated with 15% H_2O. For elution 2 l. $CHCl_3$ was used, followed by mixtures (2 l. each) of $CHCl_3$ with increasing contents of MeOH (1, 3, 5, 10, 30 and 50% of MeOH). Finally the column was eluted with 5 l. MeOH. The crude fractions were further purified by prep. TLC on silica gel Merck 60.

Betulinic acid was identified by MS and ^{1}H NMR, mp 286–291°, mmp 286–291°. Rapid quantitative analysis of the betulinic acid content in callus extracts was achieved by HPLC on a reverse phase column (SiC_{18} 10 μ, 150 × 3 mm) eluting with MeOH–10% 0.01 M H_3PO_4 (0.5 ml/min) with UV detection at 205 nm. For the analysis 10 g fresh callus were homogenized with 25 ml MeOH, the mixture left standing overnight, filtered and the filtrate evaporated *in vacuo* to dryness. To the dry extract 10 ml of the mobile phase were added, and aliquots of 10 μl were taken for HPLC analysis. Using these methods the detection limit for betulinic acid is about 0.1 μg. Each result is the average of three estimations on at least two different samples. This method is reliable for the betulinic acid concentrations of as low as 0.03% of dry wt.

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