Accumulation of Lignans in Suspension Cultures of *Linum mucronatum* ssp. *armenum* (Bordz.) Davis

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For the first time callus and suspension cultures of *Linum mucronatum* ssp. *armenum* were initiated, grown in darkness at 25 °C and analyzed for lignans. 6-Methoxypodophyllotoxin was the main lignan besides smaller amounts of podophyllotoxin isolated and identified by chromatographic methods as well as by ^{1}H NMR.

Introduction

Podophyllotoxin (PTOX) is needed as a precursor for chemical synthesis of the important anticancer drugs etoposide, teniposide and etopophos^R (Canel *et al.*, 2000). Podophyllotoxin is still extracted from rhizomes and roots of *Podophyllum hexandrum* and *P. peltatum* plants collected in the wild, because agricultural production of the plant material as well as chemical synthesis of PTOX are not economic. Therefore, there is a great interest in alternative sources of PTOX supply. Here we report about the initiation of suspension cultures from the Turkish plant species *Linum mucronatum* ssp. *armenum*, their growth characteristics and the accumulation of 6-methoxypodophyllotoxin (MPTOX) as the main lignan besides smaller amounts of podophyllotoxin (PTOX) (Fig. 1).

Results and Discussion

Sterile grown seedlings of *L. mucronatum* ssp. *armenum* were used for initiation of tissue cultures. In contrast to experience with other *Linum* species (Smollny *et al.,* 1998; Konuklugil *et al.,* 1999) an organogenic callus with leaflets developed, which did not loose its organogenic capacity completely, even not under longer subcultivation in suspension. Therefore, under the experimental conditions used these *in vitro* cultures may be referred to as shooty suspension cultures.

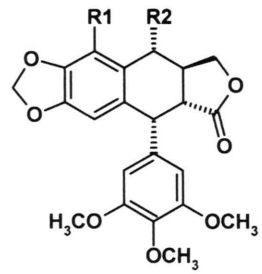

Fig. 1. Podophyllotoxin (R1 = H; R2 = OH), 6-methoxypodophyllotoxin (R1 = OCH_3; R2 = OH).

Two lignans were isolated from extracts of these suspension cultures using TLC and HPLC. They were identified by retention time and rf in HPLC and TLC, respectively (see Experimental), as well as by ^{1}H NMR (Table I) using reference compounds and in comparison with published data (e.g. Wichers *et al.*, 1991) as 6-methoxypodophyllotoxin (main product) and podophyllotoxin (minor compound). Nomenclature and numbering of both lignans is according the UPAC recommendations 2000 (Moss, 2000).

Table 1. ^{1}H-NMR data.

6-Methoxypodophyllotoxin: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.44 (2H, s, H-2′+H-6′), 6.30 (1H, s, H-3), 5.95 (2H, s, CH_2(pip)), 5.03 (1H, d, J = 8.8 Hz, H-7), 4.64 (1H, dd, J = 6.9, 8.8 Hz, H-9a), 4.54 (1H, d, J = 4.4 Hz, H-7′), 4.16 (3H, s, OCH_3 at C-6), 4.07 (1H, dd, J = 8.8, 10.1 Hz, H-9b), 3.81 (3H, s, OCH_3 at C-4′), 3.77 (6H, s, OCH_3 at C-3′ and C-5′), 2.89 (1H, m H-8), 2.75 (1H, dd, J = 4.4, 15.1 Hz, H-8′).

Podophyllotoxin: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.11 (1H, s, H-6), 6.52 (1H, s, H-3), 6.37 (2H, s, H-2′ + H-6′), 5.99 (1H, d, J = 1.3 Hz, H-a (pip)), 5.97 (1H, d, J = 1.3 Hz, H-b (pip)), 4.78 (1H, d, J = 8.8 Hz, H-7), 4.62 (1H, dd, J = 6.9, 8.8 Hz, H-9a), 4.60 (1H, d, J = 4.4 Hz, H-7′), 4.10 (1H, dd, J = 8.8, 10.1 Hz, H-9b) 3.81 (3H, s, OCH_3 at C-4′), 3.76 (6H, s, OCH_3 at C-3′ and C-5′), 2.84 (1H, dd, J = 4.4, 15.1, H-8′), 2.78 (1H, m, H-8).

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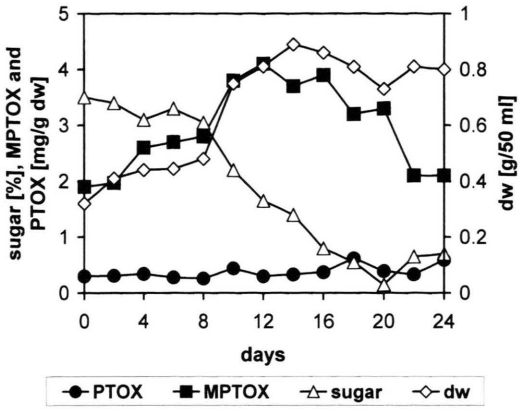

Fig. 2. Growth curve (dry weight) of dark grown suspension cultures of *Linum mucronatum* ssp. *armenum* and the amount of podophyllotoxin (PTOX) and 6-methoxy-podophyllotoxin (MPTOX) of the cells and the sugar content of the medium (%).

The suspension cultures reached about 0.9 g dry weight per 50 ml culture volume after 14 days of culture. The sugar of the medium was taken up by the cells until days 18/20. The cells contained about 4.1 mg/g dw MPTOX on day 12, whereas PTOX content was between about 0.3 and 0.6 mg/g dw throughout the culture period (Fig. 2). The total yield of lignans is comparable to those reported earlier for suspension cultures of *Linum album* and *L. nodiflorum*, respectively (Smollny *et al.*,1998; Konuklugil *et al.*, 1999).

Experimental

Callus and suspension cultures were established using standard methods (Seitz *et al.*, 1985) from seeds of *Linum mucronatum* ssp. *armenum* (Bordz.) Davis collected near Ankara and germinated under sterile conditions in 1997. Initiation of callus and suspension cultures as well as isolation and identification of lignans by ^{1}H NMR was as reported earlier (Konuklugil *et al.*, 1999). HPLC was performed as described by Smollny *et al.* (1992), the Rt for PTOX was about 8.2, for MPTOX about 10.3 min under the experimental conditions used. Additionally, for isolation of lignans TLC on silica gel plates (Merck TLC-plates no.5715) and the solvent systems chloroform:methanol (10:1, v:v; rf-value for MPTOX = 0.93 for PTOX = 0.70), chloroform:n-hexane (10:1, v:v; rf of MPTOX = 0.29; PTOX = 0.11), and diethylether:dichloromethane (6:1, v:v; rf of MPTOX = 0.87; PTOX = 0.69) were used.

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