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PRODUCTION OF SCOPADULCIOL BY CULTURED TISSUES OF *SCOPARIA DULCIS*

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Key Word Index—*Scoparia dulcis*; Scrophulariaceae; chemotype; SDX type; callus; multiple shoot; diterpenoid; scopadulciol.

Abstract—Callus and multiple shoots derived from an SDX-type plant of *Scoparia dulcis* were found to produce scopadulciol (SDY). The amounts of diterpenoid produced by the multiple shoots were the same as those of the callus and were five times higher than those of the multiple shoots from an SDB-type plant. Thus, the control of diterpenoid production is different between the SDX- and SDB-types.

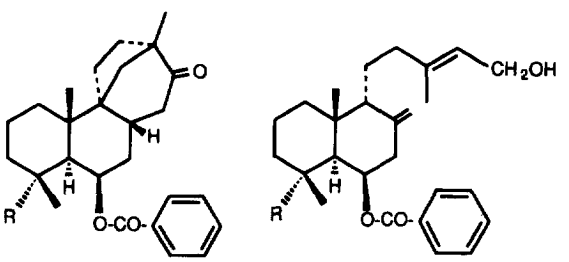
INTRODUCTION

Scoparia dulcis L., a perennial herb, is one of the medicinal plants traditionally used in the tropical and subtropical regions. From a Paraguayan collection of this plant, a tetracyclic diterpenoid named scopadulcic acid B (SDB, **1**) was isolated together with a labdane-type diterpenoid, scoparic acid A (SA, **2**) [1, 2]. SDB showed various biological activities such as inhibitory effects on gastric H^+, K^+-ATPase [3] and replication of herpes simplex virus type 1 (HSV-1) [4] as well as anti-tumour and anti-tumour promoting activities [5, 6]. HPLC analysis of diterpenoids in individual plants of this collection revealed the presence of two chemotypes, i.e. SA type and SDB type [7]. However, a different chemotype from that of the Paraguayan collection was observed in a Formosan collection which was characterized by the presence of scopadiol (SDX, **3**) and scopadulciol (SDY, **4**) [8]. SDY was found to be a novel inhibitor of gastric H^+, K^+-ATPase [9]. In addition, it showed a remarkable inhibitory effect on bone resorption stimulated by parathyroid hormone (PTH) [10].

In our previous studies on the production of diterpenoids by cultured tissues derived from an SDB-type plant, only SA was detected and only in a trace amount [11]. It was suggested that SDB production was suppressed in the undifferentiated cells [11]. In contrast, multiple shoots induced from an SDB-type plant were found to have capacity for SDB production although it was less than that of the parent plant [11]. As a result, SDB production appeared to be closely related to the differentiation of leaves. In this paper, we report on the results of our continuing study, where the production of SDY was examined by cultured tissues induced from the leaves of an SDX-type plant.

RESULTS AND DISCUSSION

Compact green callus tissues were induced when leaf segments of *S. dulcis* (SDX type) were placed on Murashige–Skoog's (MS) medium containing 5 μM 1-naphthaleneacetic acid (NAA) and 1 μM 6-benzyladenine (BA) in the light. The cell growth of the induced callus was remarkable when they were subcultured in Gamborg-B5 medium. HPLC analysis of the callus tissues confirmed that SDY was the main diterpenoid produced. The time-course of cell growth and production of SDY by callus tissues in suspension culture medium were examined. Growth of the cells in the light was much better than that in the dark (Fig. 1(A)), while the production of SDY in the light was less than that in the dark (Fig. 1(B)). Under



	R
1	COOH
2	COOH
3	CH_2OH
4	CH_2OH

*Author to whom correspondence should be addressed.

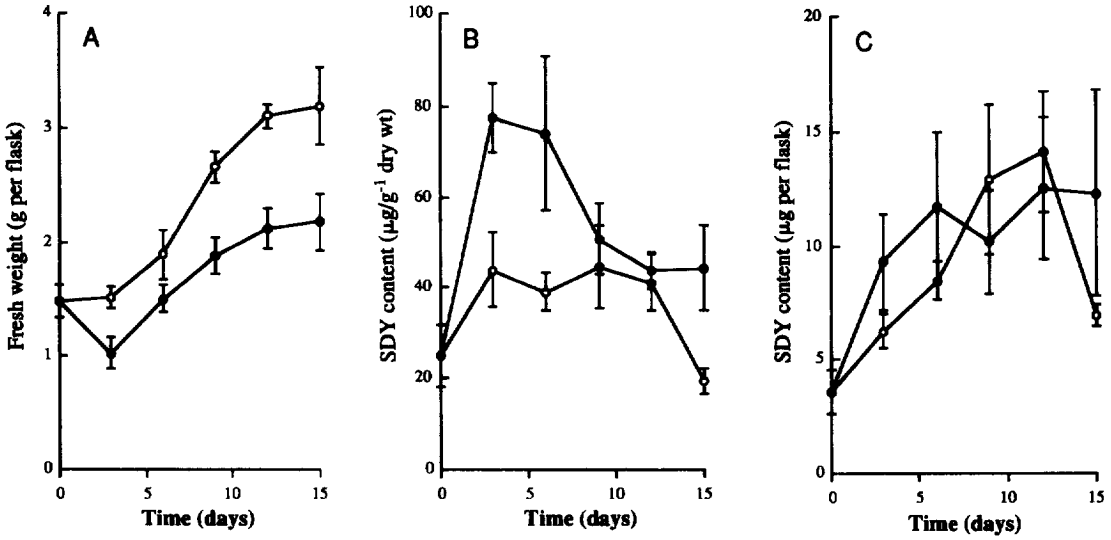


Fig. 1. Time courses of cell growth (A), SDY content in callus tissues (B) and total content of SDY (C) in suspension cultures of *S. dulcis* (SDX type). ○, In the light; ●, in the dark. Values are expressed as means ± SE of three replicates.

Table 1. Effect of photoperiod on cell growth and SDY content of callus tissues from SDX type plant of *S. dulcis**

Photoperiod (hr day^{-1})	Fr. wt (g per flask)	Dry wt (mg per flask)	SDY (μg g^{-1} dry wt)	SDY (μg per flask)
0 (n = 5)	0.6 ± 0.1	49 ± 10	21.8 ± 4.7	1.05 ± 0.21
12 (n = 4)	0.9 ± 0.3	78 ± 18	14.0 ± 2.7	1.09 ± 0.33
16 (n = 4)	1.3 ± 0.4	104 ± 29	10.7 ± 4.0	1.00 ± 0.43
24 (n = 4)	1.1 ± 0.3	87 ± 19	9.1 ± 1.0	0.80 ± 0.13

*B5 liquid medium containing 5 μM NAA and 1 μM BA was used. Values are expressed as means ± SE (n = 4 or 5).

both sets of culture conditions, the total content of SDY per flask increased with cell growth and reached a maximal level after 12 days culture (Fig. 1(C)). Over the 6–12 day period after inoculation, no significant difference was observed between the two cultural conditions.

The effects of photoperiod on SDY content and cell growth were then examined. As shown in Table 1, the SDY content decreased with extension of photoperiod and cell growth was best when cells were cultured with a 16 hr photoperiod per day. On the other hand, the total content of SDY per flask was highest with a 12 hr photoperiod per day. As previously reported [11], in the callus tissues from the SDB-type plant, SDB production was strongly suppressed and the accumulation of a trace amount of SA, a possible precursor of SDB, was observed. Consequently, it is noteworthy that the callus tissues from the SDX-type plant produced SDY, a reduced form of SDB, as a main diterpenoid, although their capability for SDY production was only 2–3% of that of the parent plant.

In our previous study, we found that diterpenoid production by multiple shoots of *S. dulcis* was much better than that by the callus tissues [11]. Therefore, we induced multiple shoots from leaf segments of SDX-type plants and subcultured them on MS medium containing 10 μM BA. The multiple shoots obtained under this cultural condition were found to produce both SDX and SDY. However, the content of SDY in the multiple shoots was the same as that of callus tissues. In order to examine the effects of photoperiod on SDY content and growth of the multiple shoots, inoculated multiple shoots were incubated in MS liquid medium containing 10 μM BA with 0, 12, 16 or 24 hr photoperiod per day. As shown in Table 2, the growth of the multiple shoots as well as the content of SDY and the total content of SDY per flask increased with extension of the photoperiod. The time-courses of SDY content and the growth of the multiple shoots with 12 and 24 hr photoperiod per day are illustrated in Fig. 2. The SDY content in the multiple shoots cultured under both illumination cycles reached the maximum level on the sixth day after inoculation, while the total content of SDY per flask increased with the period of culture up to the twelfth day and declined thereafter. The SDY content of the multiple shoots

Table 2. Effect of photoperiod on shoot growth and SDY content of multiple shoots from SDX type plant of *S. dulcis**

Photoperiod (hr day^{-1})	Fr. wt (g per flask)	Dry wt (mg per flask)	SDY (μg g^{-1} dry wt)	SDY (μg per flask)
0 (n = 5)	1.2 ± 0.2	97 ± 10	22.4 ± 2.9	2.18 ± 0.43
12 (n = 4)	2.5 ± 0.4	205 ± 21	26.1 ± 2.5	5.34 ± 0.72
16 (n = 4)	2.6 ± 0.4	209 ± 25	27.0 ± 2.6	5.68 ± 1.01
24 (n = 4)	2.8 ± 0.4	236 ± 33	28.0 ± 2.3	6.63 ± 1.19

*MS liquid medium containing 10 μM BA was used. Values are expressed as means ± SE (n = 4 or 5).

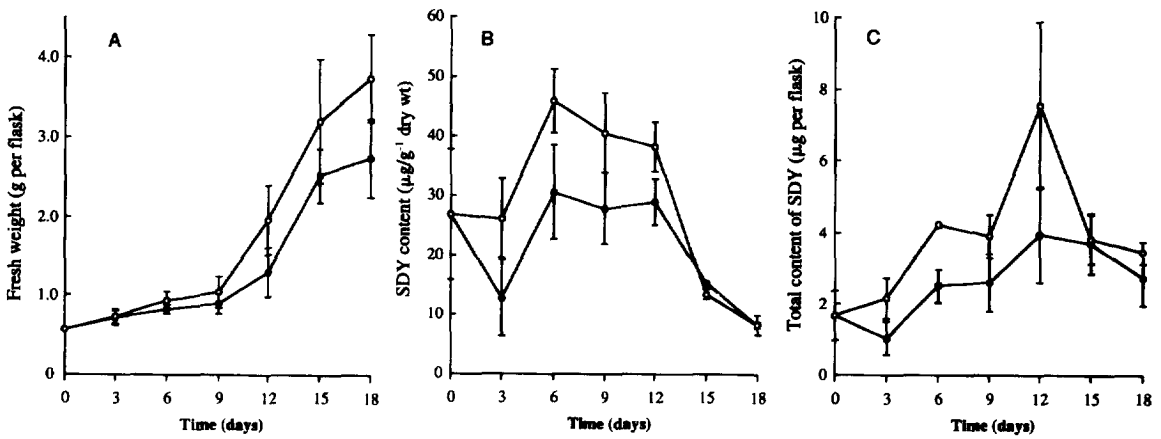


Fig. 2. Time courses of shoot growth (A), SDY content of shoots (B) and total content of SDY per flask (C) in suspension cultures of multiple shoots from *S. dulcis* (SDX type). ○, 24 hr light period day^{-1}; ●, 12 hr light period day^{-1}. Values are expressed as means ± SE of three replicates.

harvested on the twelfth day was 1.9% (12 hr photoperiod per day) or 2.6% (24 hr photoperiod per day) of that of parent plant. This level is about five times higher when compared with that of the multiple shoots from an SDB-type plant [11].

Thus, the expression of biosynthetic activities by cultured tissues for SDB type diterpenoids was suggested to be differently regulated between SDX- and SDB-type plants.

EXPERIMENTAL

Plant material and cell cultures. Scoparia dulcis was grown in the Herbal Garden of our University. After chemotype analysis of each plant, induction of callus and multiple shoots from SDX type plants and their subcultures were performed according to the method reported elsewhere [11]. Suspension cultures of the callus and the multiple shoots were initiated by transferring the primary subcultured tissues to B5 liquid medium containing 5 μM NAA plus 1 μM BA and MS liquid medium containing 10 μM BA, respectively. Each strain of callus tissues and multiple shoots was agitated on a reciprocal shaker at a speed of 140 strokes min^{-1} at 25° with a photoperiod of 0, 12, 16 or 24 hr light per day (8000 lux).

Extraction of diterpenoids and sample preparation. Harvested tissues were freeze-dried and pulverized. The powder was extracted with $CHCl_3$ and the samples for diterpene analysis were prepared as reported elsewhere [7].

Diterpene analysis. Content of SDY in cultured tissues was determined by HPLC as reported elsewhere [7].

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