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PRODUCTION OF (−)-EPICATECHIN BY *UNCARIA ELLIPTICA* CALLUS CULTURES

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Key Word Index—*Uncaria elliptica*, Rubiaceae, (−)-epicatechin production, flavonoid, plant tissue culture, callus

Abstract—The effects of sucrose concentration, light and darkness on the growth of callus culture from immature young leaves of *Uncaria elliptica* were studied The calli produced (−)-epicatechin (0 25–5 2 mg/g dry wt) and its production was affected by various conditions of growth. Low sucrose concentration (1–2%) was ideal for calli growth but did not significantly affect (−)-epicatechin production whilst light promoted its formation.

INTRODUCTION

The plant *Uncaria elliptica* R. Br exG. Don has been shown to contain high levels of rutin and moderate amounts of (−)-epicatechin in its various plant parts [1–3]. Rutin and its hydroxyethyl derivatives are marketed as drugs called 'Venoruton' and 'Paroven' for the treatment of blood capillary ailments in man [4]. (−)-Epicatechin is used in drugs called 'Catergen' and '(+)-Cianidanol-3 [4].

With the aim of establishing an experimental system for the controlled production of these two pharmaceutically useful flavonoids, tissue culture of *U elliptica* was studied In the present report, we describe the effects of sucrose concentration and continuous lighting on the growth of the callus cell line LKH 1/1 of the *Uncaria elliptica* plant. This is the first report on the *in vitro* formation of (−)-epicatechin, although we were unable to detect rutin.

RESULTS AND DISCUSSION

Effects of sucrose concentration

The effects of various concentrations of sucrose on the growth and (−)-epicatechin production in the *Uncaria elliptica* callus cultures are presented in Fig. 1 (a–e) The calli grew best with 1 to 2% sucrose medium and exhibited a dry weight increase of seven to twelve times that of their initial weight in five weeks The growth, however, decreased with increasing sucrose concentration. Calli which were grown in higher concentrations (3, 4 and 6%) of sucrose had larger percentage dry weight of tissue content, i e. less water content

In the dark the level of (−)-epicatechin showed no significant difference when different sucrose concentrations was used. This indicated that sucrose alone could not bring about increased level of the flavonoid. In the presence of light (Fig 1a–e), (−)-epicatechin content exhibited only a very slight elevation from 4 0 to 5.2 mg/g dry weight for sucrose concentrations in the range of 1 to 4% but it decreased to 4 0 mg/g dry weight again, at their respective peak value. Increasing sucrose concentration has been shown to stimulate secondary metabolite production in the callus culture of several plant species such as *Lithospermum erythrothizon* [5]. In contrast, the ubiquinone content in tobacco suspension culture was reported to increase with low sucrose concentration [6]

Effects of illuminance

In this study, we have found that (−)-epicatechin formation was stimulated by the exposure to continuous lighting. This is consistent with the reports for many other tissue culture studies that light generally stimulates the formation of flavonoids [7]. The (−)-epicatechin concentration in the calli peaked between 14 and 21 days after innoculation of calli and light exposure Its concentration ranged from 0 2 to 5 2 mg/g dry wt The flavonoid formation proceeded almost in parallel with the growing culture. The (−)-epicatechin content was at its maximum during the linear growth phase of the culture The observed decline in production as the culture entered the stationary phase (after *ca* four weeks of culture) could be due to a decline in the synthetic capacity of the cells and/or an increase in the turnover rate of this compound together with the onset of cell senescence. Such a growth-associated production pattern has also been reported in several other tissue cultures [e g. 8] and was different for many other growth-dissociated production pattern [9]

The pattern of (−)-epicatechin formation stimulated by light was independent of sucrose concentration The range of value of (−)-epicatechin content obtained (0 25–5.2 mg/g dry wt) was lower than those earlier reported for leaf and young stem (9.0–38 0 mg/g dry wt)

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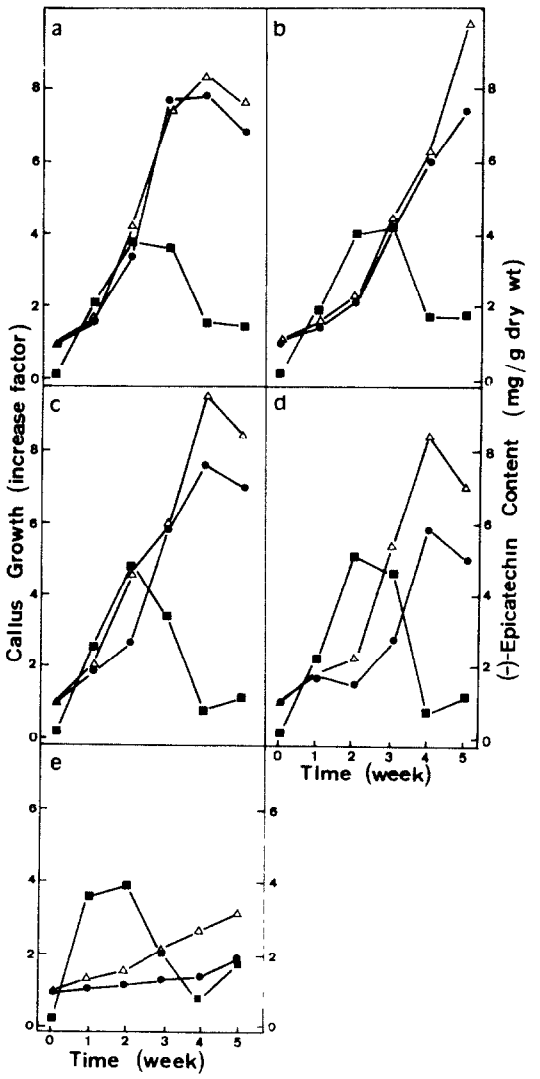

Fig 1 Time course of growth and (−)-epicatechin content (■--■) in LKH 1/1 callus of *Uncaria elliptica* grown in continuous light The tissues were cultured on ½ MS agar medium supplemented with 5 μM NAA, 0 1 μM 2-IP, and various sucrose concentrations (a) 1%, (b) 2%, (c) 3%, (d) 4% and (e) 6% Growth was expressed as an increase factor defined as the g wt of tissue at harvest per g wt of the inoculum (△- △) dry wt increase, (●– ●) fr wt increase

but comparable to those found in the 'woody' tissue and 'central' pith of the mature and old stems (3 0–5 0 mg/g dry wt) of the source plant [3]

EXPERIMENTAL

Tissue culture The young leaves of the *Uncaria* plant were excised and surface sterilized and used as explant to initiate callus formation Tissue culture was performed in 90 mm plastic petri dishes containing 25 ml of the nutrient medium solidified with 0 8% Phytagar Five times diluted Murashige and Skoog medium [10] supplemented with 1% sucrose, 5 μM α-naphthaleneacetic acid (NAA) and 0 1 M 6-γ,γ-dimethylallylaminopurine (2-IP) was used Calli were grown in the dark at 25±1° and subcultured every four weeks After nine subcultures, a callus line designated as LKH11 was selected and used for the present study Each petri dish was inoculated with four pieces of calli, each of 40 to 50 mg fr wt, in the above solid medium containing different concentrations (1, 2, 3, 4 and 6%) of sucrose They were grown separately in the dark or under continuous illumination from cool white fluorescent lamps at *ca* 4000 lux Growth was determined as fresh and dry weight each week for a period of 35 days For dry weight determination, the callus pieces were freeze-dried

Analytical procedure Flavonoids were extracted from lyophilized callus tissues using methanol [1] (−)-Epicatechin was identified by silica gel TLC [11], HPLC [3] and UV spectroscopy [12] In addition the following confirmatory tests were carried out for the identification of (−)-epicatechin (i) IR spectra 10 mg of flavonoids in KBr (200 mg) disks were examined, (ii) after recrystallization from aqueous ethanol twice, the compound showed m p 238–240 and $[\alpha]_D$-56 4 (MeOH, c 0 06), (iii) ^1H and ^{13}C NMR spectra dissolved in DMSO solvent were identical to those of an authentic sample

The flavonoid content was determined by HPLC [3] with a flow rate of 1 ml/min at a pressure of *ca* 3500 psi The elution programme used was solvent A acetonitrile (ACN)–H_2O (3 47), solvent B ACN–THF–HOAc–H_2O (18 1 1 20) The gradient elution profile was 0–10 min 100% A, 10–30 min 10% B in A (isocratic), 30–63 min 10–30% B in A (linear gradient), 63–78 min 30% B in A (isocratic) The stainless steel column used was a 30 cm × 4 0 mm i d MicroPak MCH-5-n-capp reversed-phase C_{18} column with 5 μm packing Detection was carried out at 280 nm

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