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Different kinetics of hydroquinone depletion in various medicinal plant tissue cultures producing arbutin

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Recently significant progress was made in production and accumulation of pharmaceutically important secondary metabolites by *in vitro* cultivated medicinal plant tissues. The biosynthetic capacity of such plant cell cultures can be utilised in biotransformation reactions with appropriate precursors added into the cultivation media. The glycoside arbutin is the main therapeutically active substance occurring in intact bearberry and other Ericaceae where it is biosynthesized from tyrosine. Arbutin is used as an antimicrobial agent [1, 2] in the treatment of urinary tract infections, it is also a powerful antitussive agent [3], it possesses an anti-inflammatory effect [4–6] and it is also known as effective inhibitor of the production of melanin [7]. This compound also reduces spore germination of decay fungi [8] and exhibits other biological effects. Its use as an indicator reagent in the assay of yeasts is well known. Isolation of arbutin from intact plant is rather time-consuming and economically inexpedient. Laboratory synthesis of arbutin is a multistep process. Hence it is obvious that the biotechnological production of arbutin and appropriate simple precursors in plant tissue cultures would be of practical interest to pharmaceutical chemists. We have found earlier [9] that bearberry plant tissues cultivated in conventional Murashige-Skoog (M-S) medium do not produce arbutin even when the M-S medium contained tyrosine or 4-hydroxybenzoic acid as precursors. However, the formation of arbutin in bearberry calluses cultivated in hydroquinone-treated M-S medium has been observed. Moreover, our previous studies [10] have also revealed that tissue cultures of other plants (that normally do not produce arbutin in intact state) are capable of glucosylating hydroquinone (HQ). This fact was also described for *Datura, Catharanthus* and *Rauwolfia* species [11–13]. The aim of this work was a comparative study of various plant tissue cultures *(Leuzea carthamoides, Bergenia crassifolia, Leonurus cardiaca, Rhodiola rosea, Datura meteloides)* with respect to the differences in the kinetics of HQ depletion leading to arbutin synthesis. For detailed long-term monitoring of the depletion of HQ during the biotransformation we employed an automated computer-aided FIA (flow-injection analysis) setup [14]. In the course of the experiment the following results were obtained.

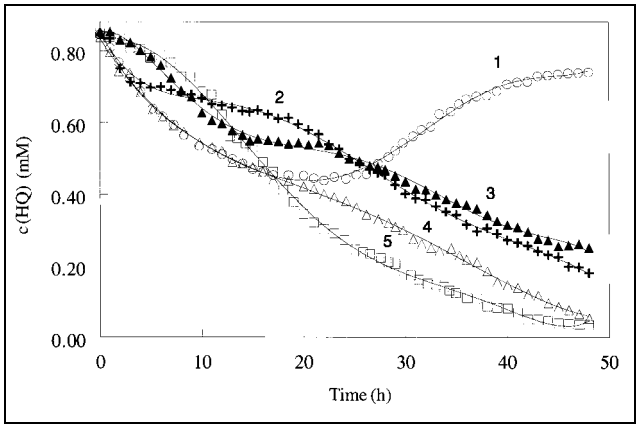

Fig. 1: Depletion of hydroquinone (HQ) from plant tissue cultivation media
Curves: 1: *Leuzea carthamoides*; 2: *Bergenia crassifolia*; 3: *Leonurus cardiaca*; 4: *Rhodiola rosea*; 5: *Datura meteloides*

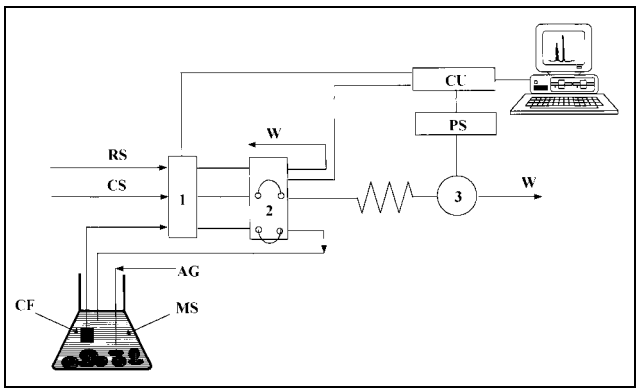

Fig. 2: Scheme of the automated flow-injection system for monitoring hydroquinone (HQ) concentration in plant tissue cultivation media
1: a 3-channel peristaltic pump; 2: dual sampling valve; 3: flow-through amperometric cell;
RS: reference sample (0.9 mM-HQ in pure plant cultivation medium); CS: carrier stream (0.1 M acetate buffer of pH 4.6); CU: control unit; PS: potentiostat; AG: inlet of agitation gas; MS: Murashige-Skoog cultivation medium (volume 300 ml); CF: polyamide cloth filter; W: waste

The course of time-dependent depletion of HQ is different in the various tissue cultures as depicted in Fig. 1. All the tissue cultures examined exhibit a relatively strong glycosylation activity during the initial 20 h of the experiment. After that time the *Leuzea* tissue culture shows the unique behaviour that the concentration of HQ in the cultivation medium gradually increases to approach its starting value in further 24 h, most probably due to the activation of hydrolytic enzymes. The tissue cultures of *Bergenia* and *Leonurus* biotransform HQ in two steps, namely during the initial 5 h (for *Bergenia*) or 10 h (for *Leonurus*) and then between the 20th and 48th hour; the period of "repose" corresponds to the night time. The highest depletion of HQ in the medium is attained with the tissue cultures of *Datura meteloides* and *Rhodiola rosea* where the conversion of HQ exceeds 90% after 48 h as confirmed by a HPLC determination of arbutin retained in the spent calluses [2]. The content of arbutin (calculated on dry mass of the culture) was 0.9%, 5.1%, 4.8%, 6.4% and 6.6% for *Leuzea*, *Bergenia*, *Leonurus*, *Rhodiola* and *Datura* which corresponds to 96.1 to 98.8% conversion of HQ to arbutin in the examined species.

It was further shown that the automated amperometric FIA system developed (Fig. 2) is suitable for a selective long-term monitoring of precursor changes in plant tissue cultivation media.

Experimental

The experiments were carried out with three-weeks grown callus cultures of five plant species, namely: *Leuzea carthamoides* DC. Asteraceae (1 mg 2,4 D + 1 mg K), *Bergenia crassifolia* (L.) Fritsch Saxifragaceae (10 mg IBA + 1 mg K), *Leonurus cardiaca* L. Lamiaceae (1 mg IAA + 1 mg K), *Rhodiola rosea* L. Crassulaceae (1 mg 2,4 D) and *Datura meteloides* DC. ex Dunal Solanaceae (1 mg 2,4 D); out of the species mentioned above only *Bergenia* produces arbutin in intact plant. A 20-g aliqout of a raw callus (approx. 1 g of dried mass; size of the swelled callus particles 1–5 mm) was suspended in 300 ml of air-purged M-S medium (+ corresponding stimulators) doped with HQ (starting concentration of HQ in the medium was 0.85 mmol · l^{-1}). The biotransformation changes in the medium were monitored at 23 ± 2 °C for 48 h, the measurements of HQ concentration being performed in 30 min intervals with use of the automated amperometric FIA setup (Fig. 2). Selective FIA assay of 2 μM to 20 mM HQ in M-S medium was carried out with the use of a three-electrode flow-through amperometric cell of the wall-jet type with 0.1 M acetate buffer of pH 4.6 as the carrier stream; working electrode spectrographic graphite rod (diam. 3 mm) impregnated with epoxide resin (working potential $E_w = +0.5$ V vs. reference SCE); auxiliary platinum wire electrode. The overal content of arbutin in the spent calluses was determined by HPLC [2]. The biotransformation experiments were performed in duplicate; the results of the parallel runs were practically identical.

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Coumarins from *Hypericum keniense* (Guttiferae)

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Hypericum keniense Sweinf. (Guttiferae), a shrub or small tree found growing in rain forests in the tropical East Africa, is a hitherto phytochemically uninvestigated species [1]. Guttiferae plant species are widely used in folk medicine and prior investigations into some of the species of this family led to the isolation of antiviral [2], antimicrobial [3, 4], antifungal [5] and cytotoxic [6–8] bioactive compounds including coumarins. In the present study, repeated chromatographic fractionation of the *n*-hexane and ethylacetate extracts of *H. keniense* stem bark afforded 5,7-dimethoxy-8-(3′-methylbut-2′-enyl)-coumarin (15 mg, 0.0010%), 8-(3′,3′-dimethoxyoxiranyl-methyl)-5,7-dimethoxy-chromen-2-one (71 mg, 0.0046%), toddanolactone (12 mg, 0.0008%), pimpinellin (62 mg, 0.0040%), the novel coumarin **1** and betulinic acid 756 mg (0.0484%). The chemical identities of these coumarins being reported for the first time from *H. keniense*, were established by comparing their physical and spectral data with those in the literature [9–12]. Compound **1** showed the elemental composition $C_{16}H_{18}O_5$ and was identified as (*E*)-8-(3′-Hydroxy-3′-methyl-1′-butenyl)-5,7-dimethoxycoumarin (5-methoxymurraol) from its ^{1}H NMR and ^{13}C NMR (Table 1) together with the MS, UV and IR spectroscopic data. To our knowledge, this is the first report on this position 8 substituted tertiary allylic alcohol of a 5,7-dimethoxy coumarin from a natural source. Compound **1** can be regarded as a biosynthetic intermediate of the naturally occurring 5,7-dimethoxycoumarins gleinene and gleinadiene [12].

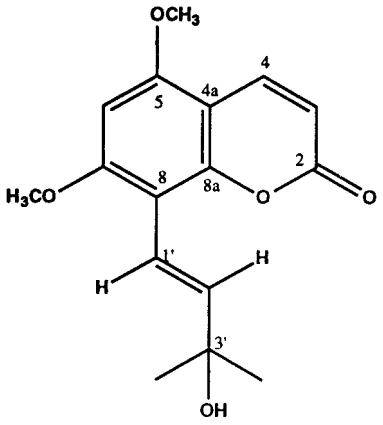

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Table: ^{1}H NMR and ^{13}C NMR data assignments and relevant HMBC correlations of (*E*)-8-(3′-hydroxy-3′-methyl-1′-butenyl)-5,7-dimethoxycoumarin (1)

Atom	^{13}C 75 MHz, $CDCl_3$	^{1}H 300 MHz, $CDCl_3$	Relevant HMBC's
2	161.1		
3	110.8	6.16, d, J = 9.6 Hz	C2, C4a
4	138.7	7.98, d, J = 9.6 Hz	C5
4a	103.6		
5	155.6		
6	90.2	6.32, s	
7	161.0		
8	106.4		
8a	153.4		
5-OCH_3	56.0	3.96, s	C5
7-OCH_3	55.9	3.94, s	C7
1′	114.2	6.86, d, J = 16.5 Hz	C8, C7, C8a, C3′
2′	141.8	6.90, d, J = 16.5 Hz	C4′, C5′
3′	77.6		
3′OH		2.02, s	
4′	30.0	1.46, s	
5′	30.0	1.46, s	