

alcohol [5], salicylic acid [6], and HQ [7] [8]. Our *Rauwolfia* culture, optimized for indole alkaloid production, has excellent glucosylating activity for alkaloids, because it synthesizes under optimum conditions 1.6 g/l medium of the glucoalkaloid raucaffricine from its aglycone vomilenine [9], and also glucosylates non-alkaloidal products.

From a range of phenolic compounds, incubated in the presence of [^{14}C]-UDPG¹) and a crude enzyme mixture of cultivated *Rauwolfia* cells, HQ was efficiently converted into its monoglucoside arbutin (9.5 pkat/mg protein) indicating the remarkable glucosylating enzymatic activity of the cells.

In vivo batch feeding of HQ (1 g, 5 g, 10 g/l nutrition medium) for 4 days to a *Rauwolfia* culture grown for 5 days in *Linsmaier* and *Skoog* (LS) medium [10] resulted in the formation of 2.5 g, 1.95 g, and 0.62 g of arbutin (= 4-hydroxyphenyl- β -D-glucopyranoside), respectively. Whereas the transformation of HQ at low concentration (1 g/l) was complete and cells survived, the higher HQ concentrations resulted in increasing cell death and decreasing biotransformation rates.

As already described for arbutin formation with cell suspensions of *Catharanthus roseus* (G.) DON, the successive addition of HQ or its continuous supply prevented such cell damage [8]. For the *Rauwolfia* cell culture, the following typical experimental conditions were found to be highly efficient. To obtain a high cell density, cells were allowed to grow for 7 days in 1 l of LS medium [10], resulting on average in the formation of 700 g of cells (22 g of cell d.wt.). After substitution of the nutrition medium by AP-II medium [9], cells were allowed to adjust to the new conditions for 4 days. Cells were then continuously fed for 6 days with HQ dissolved in AP-II medium containing sodium ascorbate (1.7 mM) to prevent HQ oxidation. The suspension volume increased finally to 1.5 l. Analysis of cell growth after 7 days indicated that the cells showed a slightly increased d.wt. up to a total of 26.6 g. Phytochemical analysis of the cells and the medium showed that the cells contained 6.3 g of arbutin (23.7% of d.wt.). The medium, however, contained 20.7 g of arbutin. At this point, it is not clear, whether the glucoside is excreted by the cells, or whether its content in the medium is completely due to cell lysis. We are now trying to find conditions where, at the end of the bioconversion process, complete cell lysis occurs, and the conversion product can more easily be isolated from the nutrition medium.

Besides arbutin, a HQ diglycoside (= 4-(hydroxyphenyl)- β -D-diglycoside, structure not shown) was also found to accumulate in the cells (2.3 g) and in the nutrition medium (6.5 g). In total, 35.8 g of HQ glycosides were synthesized by the cells in 1-l nutrition medium (1.5-l working volume), resulting in a final production rate of 18 g of arbutin and 5.87 g of the diglycoside per litre. Although the total bioconversion rate of HQ was only 83%, and 5% of free HQ could also be detected, 18 g arbutin/l is the highest value ever observed for a biotransformation or natural product formed in a plant-cell-culture system. This value exceeds the recently published production rate of arbutin by a factor of 2, which was observed under bioreactor conditions (20 l, 5 days, 9.2 g/l, 98% conversion rate) using a *Catharanthus roseus* cell culture [8].

Because arbutin preparations are widely used as urethral disinfectants and more recently have been employed in cosmetics for inhibition of melanin synthesis [8] [11], a new source of this glucoside could eventually be of biotechnological interest. It seems that *Shiseido Company* (Japan) is presently planning the commercial production of arbutin by *C. roseus* cultures [12], which has the advantage of not producing a HQ diglycoside as found in our *Rauwolfia* culture.

We believe, however, that the biotransformation rate described here could be enhanced by further optimization and by selecting high-producing cell lines. Moreover, our experiments clearly demonstrate that the capacity of plant cell cultures to produce natural products can be much higher than has usually been expected.

Experimental Part

Plant Cell Culture. Cell-suspension cultures of *R. serpentina* were cultivated under standard conditions in 1-l Erlenmeyer flasks at 25°, and 100 rpm (gyratory shaker) using LS medium [10]. Cell f.wt. was determined after filtration of the cells through nylon filter (mesh: 125 µm); d.wt. was measured after freeze drying of the cells.

Materials. UDP-[¹⁴C]glucose (11.7 GBq × mmol⁻¹) was obtained from Amersham (Braunschweig). Arbutin was purchased from Sigma (Deisenhofen).

Cell-Free Formation of Arbutin. Cells cultivated for 7 d in LS medium were harvested by suction filtration and frozen with liquid N₂. Protein extracts were obtained from 1 kg of cells stirred in 1.5 l of Tris-HCl (0.1M, pH 8.5) buffer, (NH₄)₂SO₄ precipitation (30–70%), and solubilized by 265 ml of the Tris buffer (5.7 mg of protein/ml). For an enzyme assay, 75 µl of this enzyme soln. were incubated in a total volume of 150 µl in presence of 0.5 mM UDP-[¹⁴C]glucose (62 MBq × mmol⁻¹) and 2 mM hydroquinone (120 min at 37°). After 15 min, the incubation mixture was chromatographed on silica-gel TLC plates G/UV₂₅₄ (0.2 mm, Merck, Darmstadt) with the solvent system AcOEt/MeOH/H₂O 7:2:1. Scanning for radioactive arbutin (instrument: Raytest 'Rita' 3200) showed a 10% formation (9.5 pkat/mg protein). HPLC analysis was carried out with a Merck-Hitachi system using a LiChroCart® 125-4 Super-spher 100 column (RP-18). With a flow rate of 1 ml/min the following solvent system was applied [8]: MeOH (5%) pH 2.5 adjusted with H₃PO₄ (detection at 230 nm) *t*_R (arbutin) 2.98, *t*_R (HQ) 3.88, *t*_R (diglycoside) 6.8 min.

In vivo Formation of Arbutin. Batch Feeding of HQ. *Rauwolfia* cells grown for 7 d in 1 l of LS medium were filtered off the nutrition soln. under sterile conditions and then cultivated for 4 d in 1 l of fresh LS medium containing 1 g, 5 g, and 10 g of HQ, respectively. HPLC of the medium and cell extracts (MeOH) showed arbutin formation of 100%, 15%, and 2.5%, respectively.

Continuous Feeding of HQ: Ca. 700 g of fresh cells obtained from 1 l of LS medium after cell growth of 7 d were filtered off and transferred to 400 ml of AP-II medium. After 4 d, 600 ml of AP-II medium containing 16 g of HQ (242 mM) and 200 mg of sodium ascorbate (1.7 mM) were continuously added over a period of 6 d. After an additional day, cells were harvested by filtration and extracted twice with MeOH under reflux for 20 min. The arbutin content of the extract and the nutrition medium was quantitated by HPLC.

Identification of Arbutin. After purification of the synthesized arbutin by TLC in the above mentioned solvent system, the mass spectral data, ¹H- and ¹³C-NMR, and the α_D value were in agreement with the data of commercially available arbutin.

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