Biotransformation of Progesterone by Cultured Cells of Marchantia polymorpha

Mamdouh S. A. Haridy, Mohamed-Elamir F. Hegazy, Abou El-Hamad H. Mohamed, Paul W. Paré, and Toshifumi Hirata

a Department of Botany, Faculty of Science, El-Minia University, El-Minia 61519, Egypt
b Chemistry of Medicinal Plants Department, National Research Center, Dokki, Cairo, Egypt. Fax: 20-23370931. E-mail: elamir77@yahoo.com
c Department of Chemistry, Aswan-Faculty of Science, South Valley University, Aswan, Egypt
d Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409-1061, USA
e Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan
f Pharmaceutical Laboratory, Center of Excellence for Advanced Sciences, National Research Center, Dokki 12622, Cairo, Egypt

* Author for correspondence and reprint requests

Z. Naturforsch. 65c, 599 – 602 (2010); received March 11/June 30, 2010

Cell suspensions of Marchantia polymorpha hydrogenate progesterone to 5α-pregnane-3,20-dione. Structure elucidation of the product was achieved by comprehensive NMR analyses.

Key words: Biotransformation, Marchantia polymorpha, Progesterone

Introduction

Plant suspension cultures exhibit a vast biochemical potential to generate secondary metabolites in vivo from plant-derived substrates (Charlwood and Rhodes, 1990; Lowe et al., 1996) as well as biotransformation of introduced substrates (Reinhard and Alfermann, 1980; Charlwood et al., 1986; Dia et al., 2001). Previously reported plant biocatalyst reactions include allylic hydroxylation, alcohol and ketone oxidation and reduction, respectively, and olefin reduction (Suga and Hirata, 1990; Ishihara et al., 2003). Plant culture-mediated biotransformations are now increasingly employed by synthetic chemists for structural modifications of various organic compounds.

In an earlier publication, cultured plant cells of Marchantia polymorpha and Nicotiana tabacum were reported to hydrogenate stereospecifically C,C double bonds of enones with enantiotopic discrimination to produce optically active ketone derivatives (Hegazy et al., 2006). Marchantia polymorpha cell suspension has proven to be particularly effective for a wide range of C,C double bonds of enone substrates with high conversion yield (Hirata et al., 2005).

Herein, we report the biotransformation of progesterone to 5α-pregnane-3,20-dione by Marchantia polymorpha cell suspensions with higher conversion yields than previously reported with other plant cell suspensions (2.67 – 13.3%) (Stohs, 1969; Furuya et al., 1971; Graves and Smith, 1967).

Results and Discussion

In screens for C,C double bond hydrogenation of naturally occurring plant terpenes by plant cell suspensions, several substrates were efficiently hydrogenated by Marchantia polymorpha (Hegazy et al., 2006). Progesterone (1) conversion to 5α-pregnane-3,20-dione (2) was observed in Marchantia polymorpha cell cultures as well as from the medium. The chemical structure of the product (Fig. 1) was determined by 1H NMR spectroscopy connectivity assignments based on 2D 1H-1H COSY and HMQC spectra. Progesterone dehydrogenation was confirmed by 13C NMR spectroscopy in which the spectrum exhibited the disappearance of the olefinic carbon signals at δC 124.3 and 171.3 ppm in 1, replaced by two carbon signals in 2 at δC 44.6 and 46.6 ppm for C-4 and C-5, respectively. Furthermore, the 1H NMR spectrum showed the disappearance of H-4 (δH 5.73 ppm) in 1 and the appearance of two multiplets in 2 at δH 2.2 – 2.40 and 1.50 ppm for H-4 and H-5, respectively. Protons and their connec-
tivity assignments were made based on 2D $^1$H-$^1$H COSY and HMQC spectra.

The closest progesterone synthetic analogue of \textbf{2} is $5\beta$-pregnane-3,20-dione (\textbf{2a}), which has been prepared by microbial transformation (Hu et al., 1995). The difference in the two structures lies in the presence of $5\alpha$ (H) in \textbf{2} instead of $5\beta$ (H) in \textbf{2a}.

The stereochemistry of H-5 in \textbf{2} was determined from the chemical shift of its carbon atom according to Blunt and Stothers (1977). These observations showed that the hydrogen attack at the conjugated C,C double bond takes place stereospecifically through a hydrogen atom addition on the re face at C-5 of \textbf{1} (Fig. 2) (Shimoda et al., 1996).

The product yield is based on the HPLC peak area under the curve and was expressed as relative percentage compared with the total amounts of the extracted reaction mixture; based on these
calculations 1 was observed to be reduced within a 10-day incubation period to give the dihydro derivative 2 in 37% yield.

Thus, it was found that the cultured suspension cells of *M. polymorpha* enantioselectively reduce the C,C double bond adjacent to the carbonyl group of 1 to yield 2.

### Experimental

#### General

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. $^1$H and $^{13}$C NMR spectra were obtained using a JEOL LA500 spectrometer using TMS as an internal standard reference. Mass spectra were performed using a JEOL SX-102A spectrometer with an ionizing energy of 70 eV. Analytical and preparative TLC were carried out on glass sheets (0.25 mm and 0.5 mm) coated with silica gel (Merck silica gel 60; GF254). HPLC was carried out on a Puresil C18 column (Waters) using CH$_3$CN/H$_2$O (2:3 v/v) as the eluent. Compounds were visualized by vanillin spraying and brief heating.

#### Substrate

Progesterone was commercially available (Aldrich Chemical Co.) and used without further purification for all biotransformation experiments.

#### Plant material

Cells of *M. polymorpha* (Ono et al., 1979) are routinely sub-cultured every 3 weeks using MSK-II medium (Katoh et al., 1980), containing 2% glucose, 0.1% inositol, 10 μM 2,4-dichlorophenoxyacetic acid (2,4-D), for ca. 10 years in the laboratory. Prior to use for biotransformation experiments, cells were transferred to MSK-II medium containing 2% glucose, 0.1% inositol, 10 μM 2,4-D and cultured on a rotary shaker (110 rpm) for 10 d at 25 °C under illumination (4000 lux). Cells were cultivated on a rotary shaker (75 rpm) at 25 °C for 3 weeks prior to use for actual biotransformation experiments.

Biotransformation of progesterone (1) with cultured cells of Marchantia polymorpha

*M. polymorpha* suspended cells (about 40 g fresh weight in 250 ml medium) were administered progesterone (1) (30 mg) in DMSO (0.3 ml) and incubated at 25 °C on a rotary shaker (110 rpm) under illumination. After incubation for 10 d, cells and medium were separated by suction filtration. Cells and medium were extracted successively with n-hexane, CHCl$_3$, and CHCl$_3$/MeOH (3:1 v/v). After comparison by TLC using n-hexane/EtOAc (5:1 v/v) as eluent, 5α-pregnane-3,20-dione (2) was separated as a product in n-hexane from medium and n-hexane and CHCl$_3$ fractions from cell extracts.

Transformation was tracked by TLC through taking aliquots from the culture daily. A negative control containing only plant cell suspension cultures and a positive control containing compound 1 in the medium were also prepared in order to check for the presence of plant metabolites in the cell culture and chemical changes as a result of a chemical reaction (if any) due to media and cell suspension components, respectively.

Crude fractions were combined and re-chromatographed on Sephadex LH-20 (2 x 60 cm) eluted with n-hexane/CH$_2$Cl$_2$/MeOH (7:4:0.25 v/v) followed by preparative TLC with n-hexane/EtOAc (5:1) to yield 5α-pregnane-3,20-dione (2). The conversion yield of compound 2 was determined by HPLC analysis and the structure identified by 1D and 2D NMR experiments.

5α-Pregnane-3,20-dione (2)

$[^{13}]$H NMR (CDCl$_3$): δ = 1.60 – 1.75 (2H, m, H-1), 2.2 – 2.4 (4H, m, H-2 and H-4), 1.50 (1H, m, H-5), 1.25 (1H, m, H-6a), 1.85 (1H, m, H-6b), 1.10 – 2.20 (1H, m, H-7), 1.50 (1H, m, H-8), 0.85 (1H, m, H-9), 1.10 – 1.40 (2H, m, H-11), 2.00 – 2.40 (2H, m, H-12), 0.61 (3H, s, H-18), 0.99 (3H, s, H-19), 2.10 (3H, s, H-21).

#### Acknowledgements

Financial assistance was provided in part by a grant from the Robert Welch Foundation (D1478).