Biotransformation of Progesterone by Cultured Cells of *Marchantia polymorpha*

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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). *M. 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 *M. 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 *M. polymorpha* (Hegazy *et al*., 2006). Progesterone (**1**) conversion to 5α-pregnane-3,20-dione (**2**) was observed in *M. polymorpha* cell cultures as well as from the medium. The chemical structure of the product (Fig. 1) was determined by ^{1}H NMR spectroscopy connectivity assignments based on 2D ^{1}H-^{1}H COSY and HMQC spectra. Progesterone dehydrogenation was confirmed by ^{13}C NMR spectroscopy in which the spectrum exhibited the disappearance of the olefinic carbon signals at δ_C 124.3 and 171.3 ppm in **1**, replaced by two carbon signals in **2** at δ_C 44.6 and 46.6 ppm for C-4 and C-5, respectively. Furthermore, the ^{1}H NMR spectrum showed the disappearance of H-4 (δ_H 5.73 ppm) in **1** and the appearance of two multiplets in **2** at δ_H 2.2–2.40 and 1.50 ppm for H-4 and H-5, respectively. Protons and their connec-

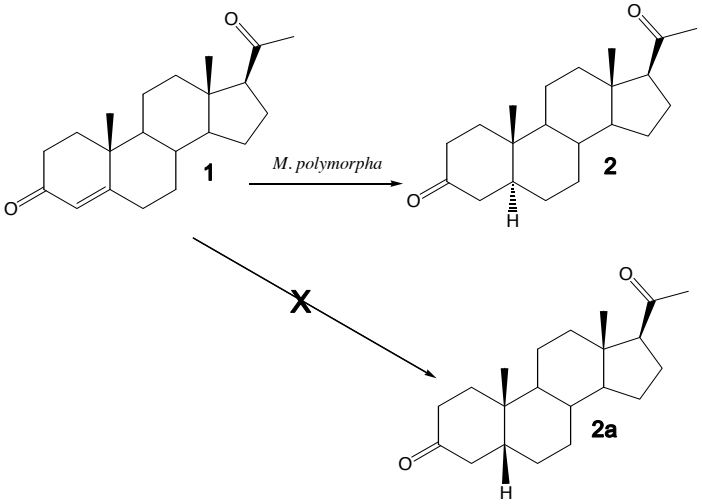

Fig. 1. Chemical structures of progesterone (**1**), 5*α*-pregnane-3,20-dione (**2**), and 5*β*-pregnane-3,20-dione (**2a**).

tivity assignments were made based on 2D ^{1}H-^{1}H COSY and HMQC spectra.

The closest progesterone synthetic analogue of **2** is 5*β*-pregnane-3,20-dione (**2a**), which has been prepared by microbial transformation (Hu *et al.*, 1995). The difference in the two structures lies in the presence of 5*α* (H) in **2** instead of 5*β* (H) in **2a**.

The stereochemistry of H-5 in **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 **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

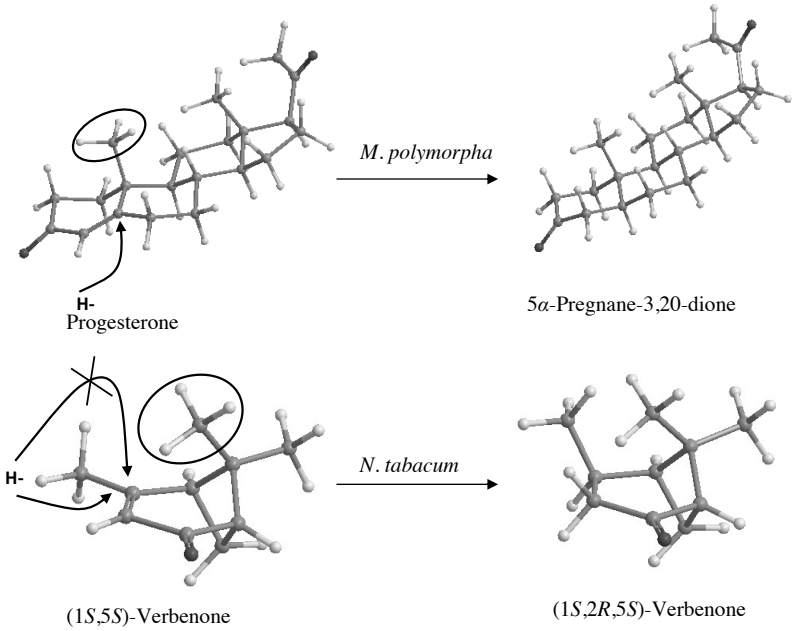

Fig. 2. The stereochemistry of progesterone reduction by *M. polymorpha* to 5*α*-pregnane-3,20-dione (top) proceeds in a way similar to that observed in the reduction of verbenone by cultured *Nicotiana tabacum* cells (bottom) as described by Shimoda *et al.* (1996).

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; GF_{254}). HPLC was carried out on a Puresil C18 column (Waters) using CH_3CN/H_2O (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_2Cl_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**)*

$[\alpha]_D^{25}$ +(39.0 ± 0.6)° (*c* 0.20, $CHCl_3$). – FABMS: *m/z* (rel. int.) = 316.2. – ^{1}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), 1.10–1.40 (2H, m, H-7), 1.50 (1H, m, H-8), 0.85 (1H, m, H-9), 1.10–1.40 (2H, m, H-11), 1.90–2.20 (1H, m, H-12a), 1.50 (1H, m, H-12b), 1.10–1.40 [m, H-14 (1H) and H-15 (2H)], 2.20–2.40 (2H, m, H-16), 2.51 (1H, t, J = 7.5 Hz, H-17), 0.61 (3H, s, H-18), 0.99 (3H, s, H-19), 2.10 (3H, s, H-21). – ^{13}C NMR ($CDCl_3$): δ = 38.9 (C-1), 38.1 (C-2), 211.9 (C-3), 44.6 (C-4), 46.6 (C-5), 29.7 (C-6), 31.6 (C-7), 35.4 (C-8), 53.7 (C-9), 34.9 (C-10), 21.4 (C-11), 33.6 (C-12), 44.2 (C-13), 56.5 (C-14), 24.4 (C-15), 22.8 (C-16), 63.7 (C-17), 44.6 (C-18), 13.4 (C-19), 209.5 (C-20), 31.5 (C-21) (Hu *et al.*, 1995).

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