CHEMISTRY LETTERS, pp. 2111-2112, 1993.

Stereoselective Reduction in the Biotransformation of Androstane Derivatives by Cell Suspension Cultures of *Marchantia Polymorpha*

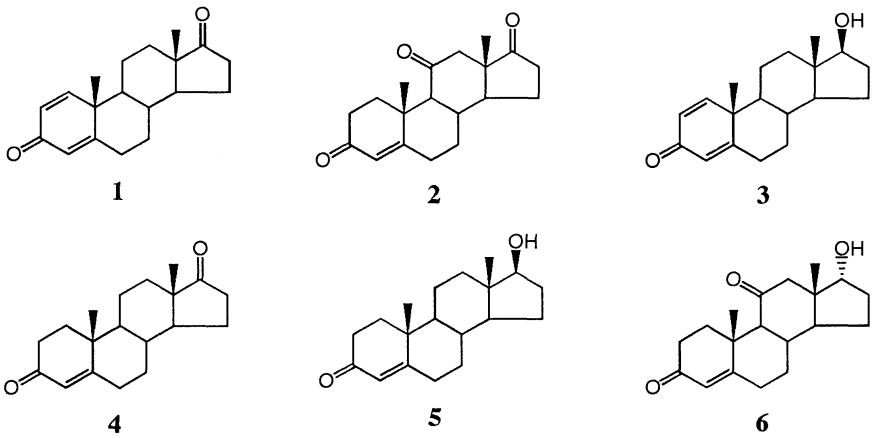
Hiroki HAMADA,* Shungo NAKA, and Halil KURBAN
Department of Applied Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700

Cell suspension cultures of *Marchantia polymorpha* mainly convert 1,4-androstadiene-3,17-dione to 17β-hydroxy-1,4-androstadiene-3-one and adrenosterone to 17α-hydroxy-4-androstene-3,11-dione.

Although transformation of steroids by microbes has been extensively studied,[1,2] and recently the metabolism of steroids in human organs, particularly in the liver, has been of considerable interest,[3] very few investigations on the biotransformation of steroids by plant tissue suspension cultures have been reported.[4]

Therefore we began studies of the biotransformation of steroids by plant tissue suspension cultures and we have reported the transformation of 4-androstene-3,17-dione and testosterone by cell suspension cultures of liverwort, *Marchantia polymorpha*.[5-7] We here report the biotransformation of androstane derivatives, 1,4-androstadiene-3,17-dione (**1**) and adorenosterone (**2**) by cell suspension cultures of *M. polymorpha* and discuss the stereoselective reduction of a carbonyl group at carbon-17.

A suspension of the cultured cells used in this study was prepared as described in our previous paper.[5] The callus tissues induced from gemma of liverwort *Marchantia polymorpha* were transplanted to a 300 ml conical flask containing 100 ml of MSK-2 medium (pH 5.8)[8] and were cultured with continuous shaking at 25 °C for 1 week in the light. The feeding and time-course experiments were carried out in a manner similar to that reported in Ref. 5; the substrate (10 mg) was administered to the above flask containing the suspension of the cultured cells under sterile conditions. The cultures were then incubated under continuous shaking at 25 °C for 3 days in the light. At regular time intervals, an aliquot (10 ml) of the incubated mixture was pipetted out under



sterile conditions and extracted with EtOAc. The relative percentages for peak integration of components in the reaction mixture was determined by HPLC. The products were identified by comparing their TLC, HPLC, GC-MS, and ^{1}H NMR characteristics with those of authentic samples.

1,4-Androstadiene-3,17-dione (**1**) was converted to 17β-hydroxy-1,4-androstadiene-3-one (**3**), 4-androstene-3,17-dione (**4**), and testosterone (**5**) as shown in Fig. 1. After 3 days incubation the formation of **3** was predominant. The yields of the two minor metabolites **4** and **5**, were each approximately 10% of metabolite **3**. The formation of **3** and **5** indicates that **1** is stereoselectively reduced to compounds with a β-hydroxyl group at C-17. The formation of **4** and **5** indicates that the C-C double bond connecting C-1 to C-2 of **1** is reduced to the compound **4**, and the carbonyl group at C-17 of **4** can still be stereoselectively reduced to the corresponding alcohol **5**. The fact that **4** was converted to **5** by cell suspension cultures of *M. polymorpha* already had been reported in our previous paper.[5] In spite of careful and repeated TLC and GC-MS analysis, no 17α-hydroxyl compound could be detected in the reaction mixture obtained.

4-Androstene-3,11,17-trione (**2**) was quantitatively converted to 17α-hydroxy-4-androsten-3,11-dione (**6**) as the sole product (**6**) after 3 days incubation (Fig. 1). This result indicates that the carbonyl group at C-17 of **2** is stereoselectively reduced to the corresponding alcohol **6**.

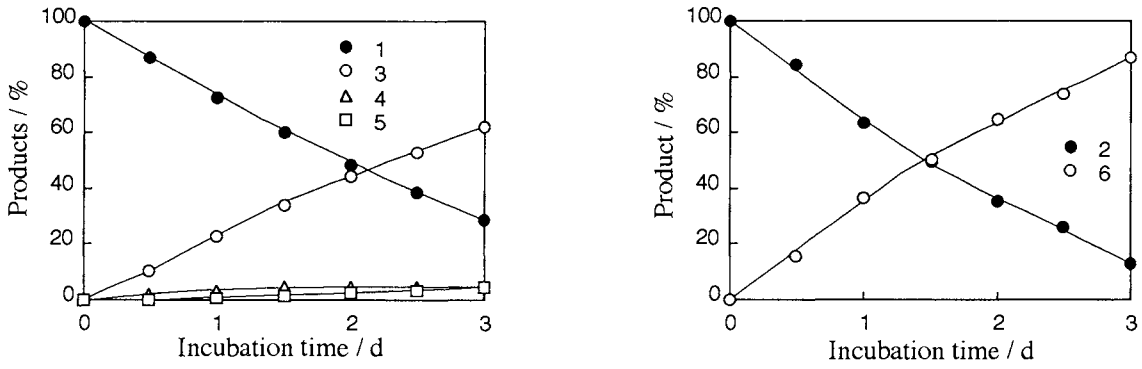


Fig. 1. The time course in the biotransformation of **1** and **2**.

Thus, it was found that the cell suspension cultures of *M. polymorpha* stereoselectively reduce the carbonyl group at position 17 of **1** from *re*-face and that of **2** from *si*-face and reduce the C-C double bond of C-1 and C-2 of **1**. It would be interesting to study the reason why the stereochemistry changes in the reduction of **1** and **2** and research on substrate specificity is now in progress.

References

1) J. S. Dahiya, *Planta Med.*, **58**, 31 (1992).
2) K. E. Smith, K. A. White, and D. N. Kirk, *J. Steroid. Biochem.*, **33**, 81 (1989).
3) D. R. Hawkins, "Biotransformations," Royal Society of Chemistry, London (1988), Vol 1, pp. 451-471.
4) T. Furuya, K. Kawaguchi, and M. Hirotani, *Phytochemistry*, **27**, 2129 (1988).
5) H. Hamada and S. Kawabe, *Life Sciences*, **48**, 613 (1991).
6) H. Hamada, S. Kawabe, F. Watanabe, M. Yanai, T. Kohishi, and H. Sumi, *Plant Tissue Culture Lett.*, **5**, 34 (1988).
7) H. Hamada, H. Konishi, H. J. Williams, and A. I. Scott, *Phytochemistry*, **30**, 2269 (1991).
8) Y. Ohta, K. Katoh, and K. Miyake, *Planta*, **136**, 229 (1977).

(Received August 13, 1993)