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HYDROXYLATION OF PROGESTERONE BY PLANT CELL SUSPENSION CULTURES OF *VINCA ROSEA*

GILAD E. GALLILI*, BORIS YAGEN† and RICHARD I. MATELES*

*Laboratory of Applied Microbiology and †Laboratory of Natural Products School of Pharmacy, Hebrew University–Hadassah Medical School, P.O. Box 1172, Jerusalem, Israel

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INTRODUCTION

Transformations of steroids by microorganisms are widely used in industry and have been extensively investigated [1, 2]. With the development of techniques permitting the growth of plant cells in suspension culture, the possibility of carrying out biotransformations with plant cells has arisen. Steroid biotransformations by plant cell suspension cultures have been reported [3–9] and the subject has been recently reviewed by Reinhard [10]. In most cases transformations involved reduction of double bonds, reduction of keto groups to hydroxyl groups, formation of glucosides, or esterification with fatty acids. Oxidation of an hydroxyl group to a keto group in testosterone has been reported [5], and hydroxylation of the glycoside digitoxin in the 12β- and 16β- positions have been noted [7 10]. In this work we show the *de novo* hydroxylation of a non-glycosylated steroid, progesterone, by suspension cultures of *Vinca rosea*.

RESULTS AND DISCUSSION

Progesterone, and its reduction product, 20β-hydroxypregn-4-en-3-one, were readily identified by their mps, IR, GLC, and TLC properties when compared to authentic samples. The third compound recovered after progesterone incubation with *V. rosea* was a crystalline material with the following properties: molecular ion at *m/e* 330; mp 201°, recrystallized from EtOAc and petrol; IR (KBr) cm^{-1}: 3470 (OH), 1692 (non-conjugated carbonyl), 1645 (conjugated carbonyl), 1625 (conjugated carbon–carbon double bond); NMR ($CDCl_3$): δ 0.75 (C-18), 1.13 (C-19), 2.1 (C-21), 3.16 (C-17), 5.66 (C-4), $[\alpha]_D^{25}$, +198.5° (*c*, 0.76 in $CHCl_3$); UV, λ_{max}: 246 nm (ε = 14 500). The IR and NMR spectra and the R_f on TLC were identical with those obtained from an authentic sample of 14α-hydroxyprogesterone (14α-hydroxypregn-4-ene-3,20-dione). Although the yield of 14α-hydroxyprogesterone was low (2–3%) it is therefore clear that plant cell suspension cultures are capable of carrying out *de novo* hydroxylation of non-glycosylated steroids.

EXPERIMENTAL

Cultures of *V. rosea*, well adapted to suspension culture, were inoculated into 250 ml flasks containing 100 ml Murashige–Skoog medium with 1 ppm of 2,4D. Incubation was carried out at 28° on a gyrotory shaker at 150 rpm. After 1 week of growth progesterone dissolved in EtOH (30 mg/ml) was added to a final concn of 300 mg/l. of broth, and incubation continued for a further 10–14 days. At this time the suspension was extracted 2× with 2 vol. of $CHCl_3$. The oil obtained after evapn of the $CHCl_3$ was treated with an Et_2O soln of CH_2N_2 to convert fatty acids to their methyl esters. The reaction mixture was left for 0.5 hr at room temp., and the Et_2O evapd yielding an oil. This oil was chromatographed on a column of Sephadex LH-20, using 40% $CHCl_3$ in petrol (40–60°) as eluent. Partly purified steroid fractions were rechromatographed on Florisil. Elution with EtOAc–petrol gave 3 fractions from which were crystallized the compounds described in the Results.

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