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Transformation of 3β-hydroxy-5-en-steroids by *Mucor racemosus*

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Abstract

The course of the transformation of three 3β-hydroxy-5-en-steroids with varying substituents at C-16 or/and C-17 by *Mucor racemosus* was first investigated. All the examined substrates were transformed, mainly 7α-hydroxylated. The fermentation of 3β-hydroxy-5-en-steroids with C16–C17 double bond or 16α, 17α-epoxy resulted in the formation of 16α-methyloxy-steriods. The characterization of the metabolites was performed by various spectroscopic methods such as IR, MS, ^{1}H NMR, ^{13}C NMR and 2D NMR. The relationships between the substrate structures and hydroxylated positions were also discussed.

Keywords: Microbial transformation; Hydroxylation; Steroids; *Mucor racemosus*

1. Introduction

The pharmaceutical industry has great interest in transformation of steroids for production of steroid hormones. Most important transformations of steroids were catalyzed by hydroxylases and dehydrogenases, but, regrettably, these catalysts are very unstable and expensive. Therefore, some intact microbial-cells have been employed in biotransformation of steroids [1]. *Colletotrichum lini* [2], *Bacillus* strains [3], *Fusarium culmorum* [4] and *Fusarium oxysporum* var. *cubense* [5] were successful in biotransformation of 3β-hydroxy-5-en-steroids.

The 7α-hydroxylation of steroids which is the most common reaction in the biotransformation of steroids has potential for industrial exploitation. A process for the 7α-hydroxylation of dehydroepiandrosterone (DHEA) and pregnenolone (PRG) has been presented [6]. It is claimed that 7α-hydroxylated derivative of DHEA may be useful in certain cancers and Alzheimer disease therapies, by increasing immune response, and as an anti-obesity agent, besides presenting anti-glucocorticoid action, and that most of these disease therapeutics are shared by the 7α-hydroxylated derivatives of PRG [6,7]. Some studies [8,9] indicated that 7α-hydroxylated metabolites of 3β-hydroxy-steroids increased immune response in mouse and might have anti-glucocorticoid potencies. These findings implied that 7α-hydroxysteroids might play a key role in regulation of glucocorticoid action and immune process.

The dimorphic fungus *Mucor racemosus* could be used to produce β-glucosidase [10], chitin deacetylases [11] and phytase [12]. It was involved in the commercial fermentation of Sufu, which is a fermented cheese-like soybean product in China and Vietnam [13]. The membrane-bound *M. racemosus* lipases could be entrapped in cryogel beads obtained from polyvinyl alcohol (PVA) by a freezing–thawing method in two-phase system, and the biocatalyst was applied for various hydrolysis and synthesis reactions [14]. It was also reported that some strains of *M. racemosus* could transform 4-ene-3-one steroids such as progesterone [15–20], 4-androstenedione [20,21] to its derivatives.

In our previous work, we reported the synthesis of 7α-hydroxy-dehydroepiandrosterone and 7β-hydroxy-dehydroepiandrosterone [22]. In this paper, we have focused on biotransformation of a series of 3β-hydroxy-5-en-steroid substrates by

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means of *M. racemosus* to study the correlation between the structures of substrates and the course of hydroxylation. We believe this is the first report of transformation of 3β-hydroxy-5-en-steroids with C16–C17 double bond or 16α, 17α-epoxy by *M. racemosus* to obtain 16α-methyloxy-steroids which may be a novel reaction in the field of microbial transformation of steroids.

2. Materials and methods

2.1. Instrumental methods

Melting points (mp) were determined on a TX5 melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded using KBr discs on a Bruker Vector-22 spectrometer. Mass spectra (MS) were obtained on an Esquire 3000 mass spectrometer by electrospray ionization (ESI). Optical rotations were measured in solution of methanol in 1-dm cells at 20 °C on a PerkinElmer 341 automatic spectropolarimeter. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Avance DPX-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane (TMS) as internal standard in DMSO-d_6. Chemical shifts (δ) were given in parts per million (ppm) relative to TMS. Couple constants (J) were given in hertz (Hz). The diffraction intensities of crystals were collected with graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) using a Rigaku R-AAXIS-IV diffractometer at 291 (2) K to a maximum 2θ value of 55°. The unit cell parameters were determined from the reflections collected on oscillation frames and were then refined. The data were corrected for Lorentz and polarization effects. The structures were solved by direct methods with SHELXS-97 [23] and subsequent Fourier-difference synthesis and refined by the full-matrix least-squares method on F^2 with SHELXS-97 [24]. Thin layer chromatography (TLC) was performed on 0.25 mm thick layer of silica gel G. Chromatography was performed with petroleum ether (bp 60–90 °C)/acetone (7:3) or chloroform/methanol (8:1) and visualized by spraying the plates with 50% sulfuric acid solution and heating in an oven at 100 °C for 3 min until the color developed.

2.2. Materials

The 3β-hydroxy-5-en-steroids (16-dehydro-pregnenolone, 16α, 17α-epoxy-3β-hydroxy-pregn-5-en-20-one and pregnenolone) were of chemical grade and obtained from Hunan Steroid Chemicals Co., Ltd., China. Silica gel G (100–200 mesh) was purchased from Qingdao Marine Chemical Factory, China. All chemicals and solvents were of analytical grade and obtained from Shenyang Chemical Company, China.

2.3. Maintenance and growth of microorganism

The strain of *M. racemosus* A.C.C.C.0401 was isolated from the samples collected from the forest in Mishan, Heilongjiang province, PRC, and identified by Agricultural Culture Collection of China. The strain was maintained on potato-2%-dextrose agar slope, grown at 27 °C, stored at 4 °C and freshly subcultured before using in the transformation experiment.

2.4. Incubation and biotransformation conditions

Ten 500 ml Erlenmeyer flasks, each containing 100 ml of sterilized potato-2%-dextrose broth (pH 7.0), were inoculated with freshly obtained spores from agar slope cultures and incubated for 2 days at 27 °C in rotary shaker (150 rpm). The 3β-hydroxy-5-en-steroid substrate (1.0 g) was dissolved in 20 ml acetone, respectively. To each 500 ml Erlenmeyer flasks, 2 ml of the acetone solution was added. Incubation was continued for 4 days at the same conditions.

2.5. Product isolation and analysis

All of the fermentation media were exhaustively extracted with ethyl acetate and filtered to separate the broth from the mycelium. After the extract was evaporated under reduced pressure, the residue was separated on silica gel column chromatography with petroleum ether/acetone (7:3) or $CHCl_3/CH_3OH$ (8:1) as eluent to obtain the metabolites, which were identified by melting points and spectra data.

3. Results

The following substrates were examined: 3β-hydroxy-pregn-5, 16 (17)-dien-20-one (16-dehydro-pregnenolone) (**1**), 16α, 17α-epoxy-3β-hydroxy-pregn-5-en-20-one (**2**), and 3β-hydroxy-pregn-5-en-20-one (PRG) (**3**). The course of transformation of the substrates was shown in Fig. 1.

3.1. Transformation of 3β-hydroxy-pregn-5, 16 (17)-dien-20-one (*1*)

Elution with petroleum ether/acetone (3:1) gave 3β-hydroxy-16α-methyloxy-pregn-5-en-7, 20-dione (**6**), mp: 158.8–160.1 °C; IR (KBr) ν_{max}: 3425, 2935, 2866, 1702, 1665, 1455, 1358, 1288, 1226, 1178, 1096, 1058 cm^{-1}; ^{1}H NMR: 3.27 (s, OCH_3), 3.72 (m, H-3), 5.72 (d, $J = 1.2$ Hz, H-6), 4.32 (m, H-16), 2.51 (d, $J = 6.4$ Hz, H-17), 0.66 (s, H-18), 1.19 (s, H-19), 2.19 (s, H-21) ppm; MS *m/z*: 383 $[M+Na]^+$, 399 $[M+K]^+$; Yield: 5.1%. Further elution with petroleum ether/acetone (7:3) gave 3β, 7α-dihydroxy-pregn-5, 16 (17)-dien-20-one (**4**), mp: 112.1–112.8 °C; $[\alpha]_D^{20} = -65.3°$ ($c = 0.101$, MeOH); IR (KBr) ν_{max}: 3480, 3415, 3366, 3309, 2934, 1660, 1588, 1460, 1432, 1372, 1233, 1055, 1019 cm^{-1}; ^{1}H NMR: 3.32 (m, H-3), 5.43 (d, $J = 4.8$ Hz, H-6), 3.69 (m, H-7), 6.90 (m, H-16), 0.82 (s, H-18), 0.93 (s, H-19), 2.21 (s, H-21) ppm; MS *m/z*: 353 $[M+Na]^+$, 369 $[M+K]^+$; Yield: 41.2%. Increasing the polarity of the solvent system with petroleum ether/acetone (3:2) gave 3β, 7α-dihydroxy-16α-methyloxy-pregn-5-en-20-one (**5**), mp: 192.2–198.0 °C; $[\alpha]_D^{20} = -89.6°$ ($C = 0.125$, MeOH); IR (KBr) ν_{max}: 3410, 2933, 1700, 1662, 1446, 1382, 1348, 1224, 1092, 948, 860 cm^{-1}; ^{1}H NMR: 3.60 (m, H-3), 3.22 (s, OCH_3), 5.61 (m, H-6), 3.38 (dd, $J = 3.2$, 6.8 Hz, H-7), 4.37 (dd, $J = 7.2$, 14.0 Hz, H-16), 2.60 (m, H-17), 0.64 (s, H-18), 0.98 (s, H-19),

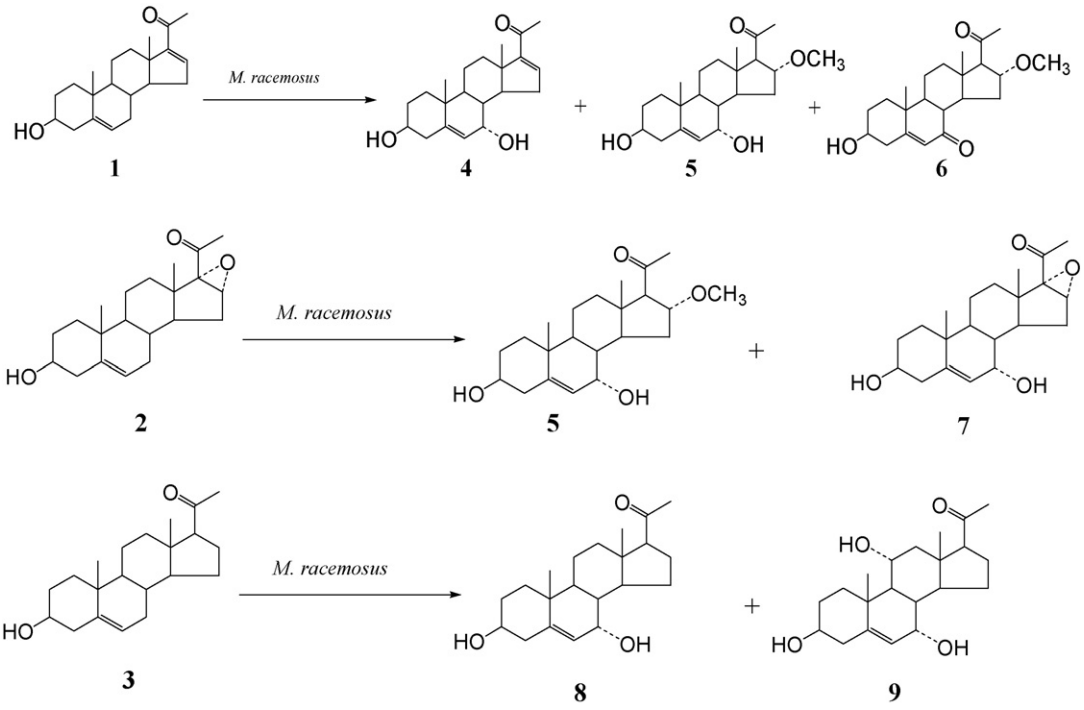

Fig. 1. Transformation of 3β-hydroxysteroids by *M. racemosus*.

2.21 (s, H-21) ppm; MS *m/z*: 385 $[M+Na]^+$, 401 $[M+K]^+$; Yield: 37.6%; X-ray diffraction for compound **5**: Colorless block crystals of **5** were grown from an ethyl acetate/petroleum ether solution. The crystal (0.20 mm × 0.18 mm × 0.16 mm) belongs to the orthorhombic system, space group $p2_12_12_1$ with $a = 12.231$ (2) Å, $b = 12.790$ (3) Å, $c = 12.936$ (3) Å, $V = 2023.6$ (7) Å^3, $Z = 4$, Dclcd = 1.190 g/cm^3. A total of 5620 reflections were collected. The refinement converged to the final $R = 0.0539$, Rw = 0.1049 for 3252 observed reflection [I > 2σ (I)] and 243 variable parameter.

3.2. Transformation of 16α, 17α-epoxy-3β-hydroxy-pregn-5-en-20-one (2)

Elution with petroleum ether/acetone (7:3) gave 3β, 7α-dihydroxy-16α, 17α-epoxy-pregn-5-en-20-one (**7**), mp: 185.3–186.5 °C; $[\alpha]_D^{20} = -65.3°$ ($c = 0.101$, MeOH); IR (KBr) ν_{max}: 3430, 3095, 2955, 2931, 2864, 1720, 1633, 1460, 1404, 1328, 1376, 1002, 817 cm^{-1}; ^{1}H NMR: 3.18 (m, H-3), 5.51 (d, $J = 4.8$ Hz, H-6), 3.68 (m, H-7), 4.02 (m, H-16), 1.05 (s, H-18), 1.01 (s, H-19), 2.07 (s, H-21) ppm; MS *m/z*: 369 $[M+Na]^+$; Yield: 64.1%; X-ray diffraction for compound **7**: Colorless block crystals of **7** were obtained by crystallization from MeOH. The crystal (0.20 mm × 0.18 mm × 0.18 mm) belongs to the monolinic system, space group $p2_1$ with $a = 7.5307$ (15) Å, $b = 10.358$ (2) Å, $c = 13.344$ (3) Å, $V = 1029.1$ (4) Å^3, $Z = 2$, Dclcd = 1.221 g/cm^3. A total of 3795 reflections were collected. The refinement converged to the final $R = 0.0478$, Rw = 0.1163 for 3656 observed reflection [I > 2σ (I)] and 255 variable parameter. Further elution gave 3β, 7α-dihydroxy-16α-methyloxy-pregn-5-en-20-one (**5**); Yield: 12.6%.

3.3. Transformation of 3β-hydroxy-pregn-5-en-20-one (3)

Elution with chloroform/methanol (8:1) gave 3β, 7α-dihydroxy-pregn-5-en-20-one (**8**), mp: 183.8–184.3 °C; IR (KBr) ν_{max}: 3420, 2935, 1698, 1661, 1458, 1433, 1358, 1229, 1186, 1054 cm^{-1}; ^{1}H NMR: 3.59 (m, H-3), 5.62 (dd, $J = 1.2$, 5.2 Hz, H-6), 3.87 (m, H-7), 2.61 (dd, $J = 9.2$, 18.4 Hz, H-17), 0.64 (s, H-18), 1.00 (s, H-19), 2.14 (s, H-21) ppm; MS *m/z*: 355 $[M+Na]^+$, 371 $[M+K]^+$; Yield: 60.3%. Further elution gave 3β, 7α, 11α-trihydroxy-pregn-5-en-20-one (**9**), mp: 268.1–269.1 °C; IR (KBr) ν_{max}: 3328, 2969, 2936, 1700, 1463, 1357, 1234, 1191, 1157, 1046, 1024, 955, 874 cm^{-1}; ^{1}H NMR: 3.33 (m, H-3), 5.44 (d, $J = 5.4$ Hz, H-6), 3.56 (d, $J = 8.48$ Hz, H-7), 3.81 (dd, $J = 5.6$, 10.3 Hz, H-11), 2.59 (dd, $J = 9.1$, 18.4 Hz, H-17), 0.50 (s, H-18), 1.00 (s, H-19), 2.07 (s, H-21) ppm; MS *m/z*: 371 $[M+Na]^+$; Yield: 19.6%.

The ^{13}C NMR signals of the compounds are presented in Table 1.

3.4. Identification of hydroxylated metabolites

The structure of the hydroxylation products were determined by a combination of IR, MS and two-dimensional NMR technique, of which the stereochemistry of two compounds was fully confirmed by X-ray diffraction study.

The mass spectrum of metabolite **4** showed the quasi-molecular ion in $[M+Na]^+$ at *m/z* 353, which suggested that one oxygen atom was incorporated into the substrate. The presence of signals at δ 5.43 (H-6) and δ 6.90 (H-16) in the ^{1}H NMR spectrum of compound **4** showed that the two double bonds C5–C6 and C16–C17 had been retained. The ^{1}H NMR spectrum showed a new downfield signal for the oxygen-bearing methine proton

Table 1
^{13}C NMR spectral of compounds **1**–**9**

No.	1	4	5	6	2	7	3	8	9
1	36.7	36.3	36.9	36.2	37.1	36.9	37.3	37.0	38.4
2	29.6	31.2	31.8	31.1	31.5	31.5	31.6	31.6	31.6
3	69.9	69.6	71.1	70.1	71.6	70.2	71.7	71.3	70.2
4	42.1	42.0	41.9	41.8	42.2	42.5	42.3	42.2	42.8
5	141.6	143.9	146.1	165.7	141.1	144.4	140.9	146.3	144.6
6	120.0	124.2	123.6	125.9	120.9	124.7	121.4	123.7	124.3
7	31.3	63.4	64.9	200.7	31.3	63.6	31.8	65.2	63.5
8	30.9	35.4	37.1	44.7	29.7	35.8	31.9	37.4	37.1
9	49.9	41.5	42.0	49.7	50.3	39.4	50.1	41.9	47.8
10	36.2	36.8	37.3	38.2	36.6	37.4	36.6	37.4	38.5
11	20.1	19.8	20.4	20.8	20.4	20.2	21.1	20.7	67.2
12	34.3	34.1	38.2	37.8	31.4	31.7	38.9	38.2	49.1
13	45.4	45.2	44.3	44.8	41.5	41.2	44.0	43.8	43.6
14	55.8	49.5	47.6	48.1	45.5	42.1	57.0	49.7	49.5
15	31.7	31.7	31.2	33.7	27.5	27.2	24.5	24.4	23.7
16	145.1	145.7	81.5	81.9	60.5	60.8	22.9	22.9	22.6
17	154.2	154.2	71.3	70.3	71.0	70.8	63.8	63.5	62.8
18	15.5	15.4	14.3	14.5	15.1	15.2	13.2	13.0	14.1
19	18.9	17.7	18.2	17.4	19.3	18.2	19.4	18.2	17.7
20	196.3	196.1	208.3	208.4	204.9	205.3	209.4	209.6	208.7
21	26.9	26.9	31.8	31.9	25.9	26.2	31.5	31.3	31.3
22			57.2	57.2					

at δ 3.69 ppm, which indicated introduction of a C-7 hydroxyl group in compound **4**. The hydroxyl group at C-7 was also confirmed by the connectivity between H-7 and the olefinic H-6 (δ 5.43 ppm) in the ^{1}H–^{1}H COSY spectrum of **4**. For hydroxylation at C-7, a new signal indicative of an oxygen-bearing methine carbon appeared at 63.4 ppm, and downfield shifts were observed for C-6 (δ 124.2 ppm) and C-8 (δ 35.4 ppm) and a γ-gauche upfield shift for C-9. In the NOESY spectrum of **4**, a cross-peak was observed between H-7 and H-6β, and the configuration of the hydroxyl group at C-6 was determined to be α.

The mass spectrum of metabolite **5** showed the quasi-molecular ion in $[M+Na]^+$ at *m/z* 385, suggesting the addition of 48 mass units to **1**. The IR spectrum of metabolite **5** showed characteristic absorption at 3410, 1700, 1662 cm^{-1} for hydroxyl and saturated ketone and double bond groups, respectively, which meant that the double bond C16–C17 of α, β-unsaturated ketone had been saturated. The ^{13}C DEPT experiments of **5** revealed a total of 22 carbon signals, of which four corresponded to methyl carbons, six to methylene carbons, eight to methine carbons and four to quaternary carbons, which suggested a methyl carbon into the substrate. Interpretation of HMBC spectrum revealed correlations between H_3-18 to C-12, C-13, C-14 and C-17; H_3-19 to C-1, C-9, C-10, and C-5; H_3-21 to C-20 and C-17, which suggested that the signal at δ 64.9 ppm was assigned to C-7. The signal appearing at δ 2.60 ppm was easily deduced to be resonating peak of H-17 from the HSQC spectrum. The ^{1}H–^{1}H COSY spectrum of **5** implied the connectivity for H-17 to H-16, the signal appearing at δ 4.37 ppm was assigned to H-16. The presence of a 16-methoxyl group was deduced from HMBC correlations between H-16 and the carbon at δ 57.2 ppm and δ 81.5 ppm, respectively. In the ^{1}H NMR spectrum of **5**, the signal appearing at δ 3.38 ppm was assigned to H-7, by the assistance of ^{1}H–^{1}H COSY and HMBC spectra. In order to confirm the configuration of C-16 methoxy group, a crystal of **5** was subjected to X-ray diffraction study. The result (Fig. 2) demonstrated it to be α-configuration and also confirmed the structure of **5** as deduced from spectral data.

The ^{13}C NMR data coupled with the DEPT and HSQC spectra showed the presence of two carbonyls and a methoxy group attached to C-16 in compound **6**. The disappearance of the methylene assigned to C-7 was noted. The large shielding effects experienced by the olefinic carbons suggested that the double bond C5–C6 was in conjugation with a carbonyl. The 7-carbonyl group was also supported by the absence of connectivity between the olefinic H-6 (δ 5.72 ppm) and H-7 in the ^{1}H–^{1}H COSY spectrum of **6**.

The mass spectrum of metabolite **7** showed the quasi-molecular ion in $[M+Na]^{\cdot+}$ at *m/z* 369, which suggested that one oxygen atom was incorporated into the substrate. The IR spectrum of **7** showed characteristic absorption at 3430, 1720, 1633 cm^{-1} for hydroxyl and saturated ketone and double bond groups, respectively. The chemical shift values of two oxygenated carbons at δ 60.8 and 70.8 ppm indicated the presence of an epidioxy group. The hydroxyl group at C-7 was deduced by

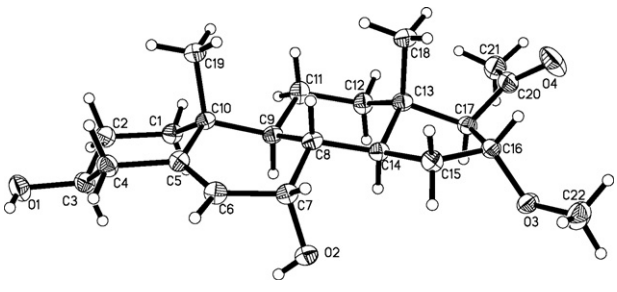

Fig. 2. X-ray diffraction analysis diagram of **5**.

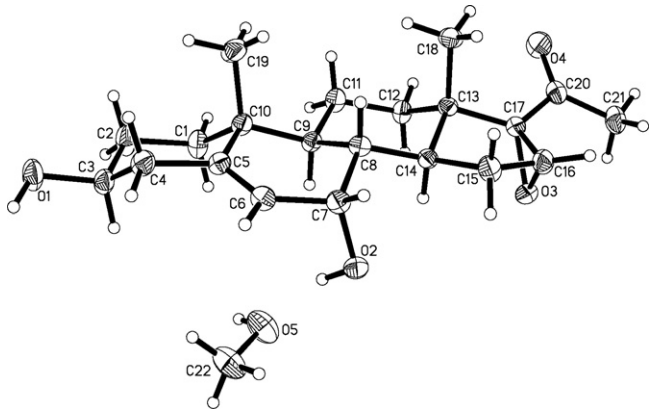

Fig. 3. X-ray diffraction analysis diagram of **7**.

a combination of two-dimensional NMR technique. The X-ray diffraction analysis of compound **7** (Fig. 3) showed 7-hydroxyl group as α-configuration. Therefore, the stereochemistry of compound **7** was fully confirmed.

The mass spectrum of metabolite **8** showed the quasi-molecular ion in $[M+Na]^+$ at *m/z* 355, which suggested that one oxygen atom was incorporated into the substrate. The hydroxyl group at C-7 was deduced by a combination of two-dimensional NMR technique. The presence of a 7-hydroxyl group was deduced from HMBC correlations between H-7 (*δ* 3.87 ppm) and the carbon at *δ* 146.3 ppm and *δ* 123.7 ppm, respectively. The hydroxyl group at C-7 was also confirmed by the connectivity between H-7 (*δ* 3.87 ppm) and the olefinic H-6 (*δ* 5.62 ppm) in the ^1H–^1H COSY spectrum of **8**. In the NOESY spectrum of **8**, a cross-peak was observed between H-7 and H-6β, and the configuration of the hydroxyl group at C-6 was determined to be α.

The mass spectrum of **9** showed the quasi-molecular ion in $[M+Na]^+$ at *m/z* 371, suggesting the addition of 32 mass units to **3**. The IR spectrum of metabolite **9** showed characteristic absorption at 3328, 1700, 1661 cm^{-1} for hydroxyl and saturated ketone and double bond groups, respectively. In the ^1H NMR spectrum, two new downfield signals were observed for oxygen-bearing methine protons at *δ* 3.56 ppm and *δ* 3.81 ppm, which indicated the hydroxyl groups introduced in compound **3** were at C-7α and C-11α. For hydroxylation at C-11, a new signal indicative of an oxygen-bearing methine carbon appeared at *δ* 67.2 ppm, and downfield shifts were observed for C-12 (*δ* 49.1 ppm) and a γ-gauche upfield shift for C-13. In the NOESY spectrum of **9**, a cross-peak was observed between H-7 and H-6β, and the configuration of the hydroxyl group at C-6 was determined to be α. Careful investigation on the NOESY spectrum of **9** revealed NOE correlations between H-11 and both of H_3-18 and H_3-19, and between H-11 and H-12β, which suggested that the hydroxyl group at C-11 had a α-configuration.

4. Discussion

The transformation of 3β-hydroxy-5-en-steroids with varying substituents at C-16 and/or C-17, i.e.: 16-dehydropregnenolone, 16α, 17α-epoxy-pregnenolone, and pregnenolone by *M. racemosus* was investigated. All the substrates were mainly hydroxylated at 7α position, depending on the structure of the substrate.

The possible pathways of biotransformation of the substrates by *M. racemosus* were shown in Fig. 4. The substrate **2** was changed into compound **7** first, and then transformed into compound **5** by methylation when the 16α, 17α-epoxy of compound **7** was opened. Transformation of both 16-dehydropregnenolone (**1**) and 16α, 17α-epoxy-pregnenolone (**2**), leads to the same compound **5**, therefore, compound **7** is suggested to be an intermediate in the transformation of 16-dehydropregnenolone by *M. racemosus*. We tried to detect the formation of 16α, 17α-epoxy steroid in the process of transformation of substrate **1**, but, unfortunately, no compound **2** or **7** was found. A possible bioconversion sequence from substrate **1** is of allylic hydroxylation at C-7 to form **4**, which was oxidized to the 16, 17-epoxide **7** followed by the methylation to a 7α-hydroxyl-16α-methoxyl steroid **5**. The formation of the 16α-methoxyl steroid seems to be catalyzed by the sterol methyltransferase (SMT). The latter would then be oxidized to the 7-ketone steroid **6**. Three compounds were produced by biotransformation of **1**, this infers

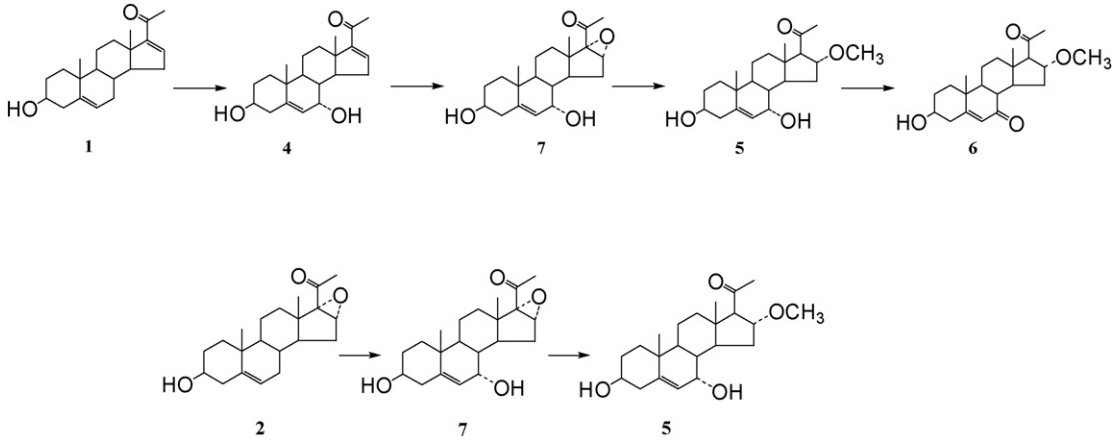

Fig. 4. The possible metabolic pathways of the biotransformation of the substrates by *M. racemosus*.

three distinct relative enzyme systems in the strain of *M. racemosus*. To our best knowledge, no information could be obtained of methylation or methoxylation at C-16 of steroids by the other fungi. However, soybean SMT in the presence of AdoMet catalyzes the transmethylation of the Δ^{24}-bond of the sterol side chain to produce phytosterols with a methyl (lene) or ethyl (idene) group at C-24 [25,26].

In the transformation by *M. racemosus*, the substrates of 3-ol-5-ene steroids with varying substituents were mainly hydroxylated at C-7α position. The fermentation of 3β-hydroxy-5-en-steroids with C16–C17 double bond or 16α, 17α-epoxy resulted in the formation of 16α-methyloxy-steriods. In the transformation of **3**, besides the mainly 7α-hydroxyl-steroids, there was a little 11β-hydroxyl-steroid to be found. The results obtained in transformation of three 3-ol-5-ene substrates by *M. racemosus* indicated that the presence of C5–C6 double bond had a significant impact on the position of hydroxylation.

In conclusion, this strain of *M. racemosus* may have a magnificent prospect in the pharmaceutical industry, because it can lead to the valuable 16α-methoxyl steroids and 11α-hydroxylated intermediate for steroidal drugs. Our research work will expend the applications of *M. racemosus*, and also provide a new method and compounds for the new steroid drugs screening.

Acknowledgement

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