Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 6, pp. 1563-1566, 1996

0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00181-4

Microbial Reduction of 2-(6-*m*-Methoxyphenyl-3-oxohexyl)-2,4,5-trimethylcyclopenta-1,3-dione with *Schizosaccharomyces pombe* (NRRL Y-164)

Shoei-Sheng Lee, Gwo-Sen Lin and K.C. Wang*

School of pharmacy, College of Medicine, National Taiwan University

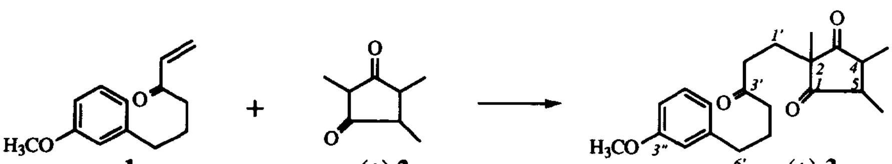
Taipei 100, Taiwan, Republic of China

Abstract: Racemic 2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4,5-trimethylcyclopenta-1,3-dione **3** was synthesized and resolved by reduction with *Schizosaccharomyces pombe* (NRRL Y-164) to give (-)-**4** and (+)-**5** in 42 and 36% yields, respectively.

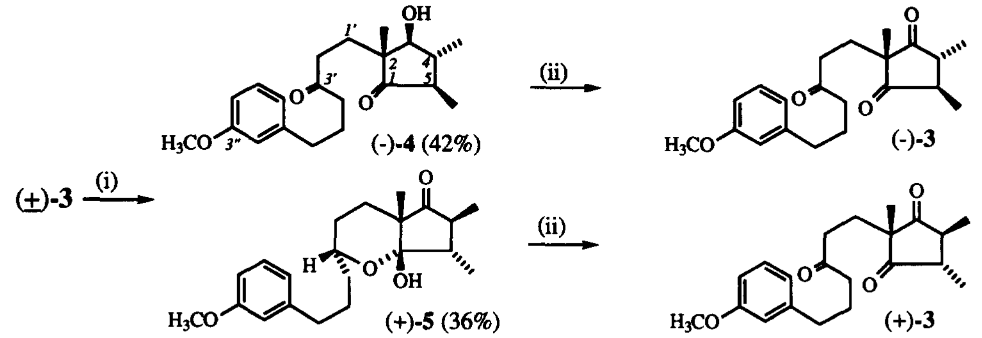
Previously we reported the preparation of **1** and microbial reduction of the prochiral 2-(6-*m*-methoxyphenyl-3-oxohexyl)-2-methylcyclopenta-1,3-dione with *Schizosaccharomyces pombe* (NRRL Y-164) to give (+)-2β-methyl-2α-(6-*m*-methoxyphenyl-3-oxohexyl)-3β-hydroxycyclopentanone in 65% yield[2].

Racemic 2,4,5-trimethylcyclopenta-1,3-dione **2** was prepared from condensation of meso-2,3-dimethyl succinic acid and propionyl chloride in the presence of $AlCl_3$[3]. Compound **2**[4] is present in the enol form as evidenced from its ^{1}H-NMR spectrum, a methyl singlet at δ 1.55 and two methyl doublets overlapping at δ 1.07 (J =7.2 Hz). Subsequent condensation of **1** and **2** gave racemic 2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4,5-trimethylcyclopenta-1,3-dione **3**[5]. When **3** was exposed to *S. pombe* (NRRL Y-164)[6], (-)-**4** and (+)-**5** were isolated in 42 and 36% yields, respectively.

Compound (-)-**4**[7], colorless oil with $[\alpha]_D^{24}$= -44.0(c=1.0, $CHCl_3$), has a molecular formula $C_{21}H_{30}O_4$ as deduced from its HREIMS *m/z* 346.2139 (calcd 346.2144). Its IR absorption at 3450 cm^{-1} and a carbinoyl proton signal at δ 3.45 (d, J =9.4 Hz) in the ^{1}H-NMR spectrum support the presence of a secondary hydroxyl group on the five-membered ring. That compound (-)-**4** is resistant to the Oppenauer oxidation but oxidizable by Jones reagent[8] to (-)-**3** supported this suggestion. In addition, the 2-Me carbon signal (δ 16.27) appearing more upfield than that of **3** (δ 19.57), both identified by HETCO and HMQC spectra, respectively, suggested further γ-gauche effect by 3β-OH present in (-)-**4**. The enhancement of 4-Me signal (δ 1.16, d) upon irradiation of H-3 (δ 3.45) built up their *cis* relationship. This irradiation also located H-1' (δ 1.76 and 1.72, each dt) and H-2' (δ 2.40, t). These analyses and later described correlation for stereochemistry determined (-)-**4** as (2*R*, 3*S*, 4*R*, 5*R*)- 2-(6-*m* -methoxyphenyl-3-oxohexyl)-3-hydroxy-2,4,5-trimethylcyclopentanone. This assignment agrees well with the fact that this microorganism reduced the C-3 carbonyl in **3**, corresponding to C-17 in steroid nucleus, to 17β-OH[2,9].



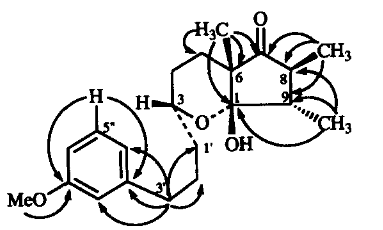
1 (±)-**2** (±)-**3**



(i) *Schizosaccharomyces pombe* (NRRL Y-164); (ii) CrO_3-H^+

Compound (+)-**5**[10], $[\alpha]_D^{25}$= +93.0 (c= 1.0, $CHCl_3$), mp. 65-66° C, has a molecular formula $C_{21}H_{30}O_4$ as deduced from its HREIMS *m/z* 346.2142 (calcd 346.4144). Its IR absorption at 3510 and 1730 cm^{-1} suggested hydroxyl and a ketone functions. The ^{13}C-NMR spectrum revealed a sole carbonyl carbon (δ 220.5), a dioxygenated carbon (δ 101.6, s) and an oxygenated methine (δ 69.2, d) which couples directly to a carbinoyl proton (δ 3.65), identified by a HMQC spectrum. The molecular formula of (+)-**5** provides seven ring and double bond equivalents while six of which were easily identified from the presence of an aryl ring, a carbonyl group and the five-membered ring in the molecule. The HMBC spectrum (Figure 1) showed that the dioxygenated carbon coupled to 6-Me singlet (δ 0.93) and 9-methyl doublet (δ 1.07), and the carbonyl carbon coupled to 6-Me and 8-Me. This afforded the exact substitution pattern for the five-membered ring and the dioxygenated carbon to be a hemiketal carbon ether-linked likely to C-3 to form a pyran ring accounting for the seventh equivalent. This suggestion was supported by the coupling pattern of the carbinoyl proton (H-3, δ 3.65, ddt, *J* =10.5, 1.7, 6.2 Hz) which indicated H-3 to be an axial proton coupling to the flexible C-1' protons (δ 1.38) (*J* =6.2 Hz), and the axial proton (δ 0.95) (*J* =10.5 Hz) and equatorial proton (δ 0.84) (*J* =1.7Hz) of C-4, clarified by a COSY-45 spectrum. Based on these analyses and supported by the X-ray diffraction analysis[11] (Figure 2) which also proved its essential optical purity, the structure of (+)-**5** was established unequivocally as (1*S*, 3*R*, 6*R*, 8*S*, 9*S*)- 1-hydroxy- 3-(3-*m* -methoxyphenylpropyl)-6,8,9-trimethyl-2-oxabicyclo[4,3,0]nonan-7-one.

Figure 1. Major HMBC correlations for (+)-5



The Jones oxidation of (-)-**4** gave (-)-**3** with $[\alpha]_D^{24}$= -61.5 (c= 1.0, $CHCl_3$), while oxidation of (+)-**5** gave (+)-**3** with $[\alpha]_D^{24}$= +61.0 (c= 1.0, $CHCl_3$). This chemical correlation and insignificant difference of specific rotation for (-)- and (+)-**3** provided solid support for the stereochemistry and optical purity of (-)-**4** as shown.

From above result, it is shown that successful resolution of (±)-**3** was achieved by reduction with *S. pombe* (NRRL Y-164) with the yield of 42% for (-)-**4** and 36% for (+)-**5**, respectively. This result also indicated that the microbial reduction of the carbonyl function corresponding to C-17 of steroid in (±)-**3** by this microorganism was not affected by the presence of a vicinal α-methyl group on the cyclopentane ring but was prohibited by the presence of a vicinal β-methyl substitution. Therefore, the side chain carbonyl function in (+)-**3** was reduced and leading to the formation of hemiketal product of (+)-**5**.

Currently, we are working on the asymmetric cyclization of the resolved (-)-**3** and (+)-**3** for the synthesis of 15,16-dimethylated steroid derivatives.

References and Notes

1. The physical data of the prepared and isolated compounds were obtained from the following instruments: JASCO IR report 100; Hitachi 150-20 Double Beam Spectrophotometer; JEOL JMX-HX110 Mass Spectrometer (70 eV); Bruker AMX-400 NMR Spectrometer in $CDCl_3$ or CD_3OD using solvent peak as reference standard. 2D NMR spectra were recorded by using Bruker's standard pulse program: in the HMQC and HMBC experiments, Δ= 1 s and *J* =140, 8 Hz, respectively, the correlation maps consisted of 512x 1K data points per spectrum, each composed of 16 to 64 transients.
2. Wang, K.C.; Liang, Chang-Hsing; Kan, Wai-Ming; Lee, Shoei-Sheng. *Bioorg & Med.Chem.* **1994**, *2*, 27.
3. Schick, H.; Lehman, G.; Hilgetag. *Chem. Ber.* **1969**, *102*, 3238.
4. **2.** IR (KBr) ν max 3200-2500, (br s, OH), 1650 (br s, [-C(OH)=C(Me)-C=O]), 1390 (br s), 1350 (br s), 1260, 1142, 1090 1002, 990, 968, 860 cm^{-1}; UV (MeOH) λ max (log ε) 248 (4.18) nm; ^1H NMR δ (CD_3OD) 2.71 (2H, m, H-4 and H-5), 1.55 (3H, s, 2-Me), 1.07 (6H, d, *J* = 7.2 Hz, 4,5-Me); ^{13}C-NMR δ (CD_3OD) 178.4 (s, C-1 and C-3), 111.4 (s, C-2), 41.3 (d, C-4 and C-5), 15.7 (q, 2-Me), 12.7 (q, 4- and 5-Me); HREIMS *m/z* $[M]^+$ 140.0836 (calcd for $C_8H_{12}O_2$ 140.0837); FABMS *m/z* $[M+H]^+$ 141 (100), 73 (91), 57 (84).
5. (±)-**3.** IR ($CHCl_3$) ν max 2965, 2935, 1766 (C=O), 1720 (C=O), 1600, 1585, 1452, 1370, 1260, 1140, 1042 cm^{-1}; UV (MeOH) λ max (log ε) 272 (3.20), 279 (3.17) nm; ^1H NMR δ ($CDCl_3$) 7.17 (1H, t, *J* = 7.8 Hz, H-5"), 6.72 (1H, br dd, *J* = 7.8, 1.5 Hz, H-6"), 6.71 (1H, br dd, *J* = 7.8, 1.5 Hz, H-4"), 6.68 (1H, br s, H-2"), 3.76 (3H, s, 3"-OMe), 2.54 (2H, t, *J* = 7.5 Hz, 6'), 2.45 (1H, dq, *J* = 6.7, 6.7 Hz) and 2.36 (1H, dq, *J* = 6.7 Hz) (H-4 and H-5), 2.33 (2H, m, H-4'), 2.32 (2H, m, H-2'), 1.82 (4H, m, H-1' and H-5'), 1.29 (3H, d, *J* = 6.7 Hz) and 1.26 (3H, d, *J* = 6.7 Hz) (4-Me and 5-Me), 1.07 (3H, s, 2-Me); ^{13}C- NMR δ ($CDCl_3$) 216.92 (s) and 216.87 (s) (C-1 and C-3), 209.2 (s, C-3'), 159.7 (C-3"), 143.1 (s, C-1'), 129.3 (d, C-5"), 120.9 (d, C-6"), 114.2 (d, C-2"), 111.3 (d, C-4"), 55.1 (q, 3"-OMe), 54.2 (s, C-2), 49.8 (d) and 49.1 (d) (C-4 and C-5), 41.8 (t, C-4'), 36.9 (t, C-2'), 35.0 (t, C-6'), 28.3 (t, C-1'), 24.9 (t, C-5'), 19.6 (q, 2-Me), 13.3 (q) and 13.1 (q) (4-Me and 5-Me); HREIMS *m/z* $[M]^+$ 344.1992 (calcd for $C_{21}H_{28}O_4$ 344.1988); EIMS *m/z* $[M]^+$ 344 (32), 261 (28), 177 (51), 169 (73), 154 (30), 141 (37), 134 (100), 121 (48).
6. The microorganism was maintained on a MP-#3 Agar (Maltose 4%, Proteose Peptone #3 1.5%, and Agar 3%) at 26° C for 11 days then transferred and grown in Nutrient broth-Dextrose medium (Nutrient broth 1.6% and Dextrose 4%) at 24-26° C on a rotary shaker (250 rpm, 1-in stroke). Transformation was carried out in 2-L Erlenmyer flasks containing 400 mL of medium. The substrate dissolved in DMF was added to the growing microorganism and the incubation was continued for 60 h.
7. (-)-**4.** $[\alpha]_D^{24}$= -44.0 (c= 1.0, $CHCl_3$); IR ($CHCl_3$) ν max 3450 (OH), 3125, 2850, 1720 (C=O), 1601, 1580, 1125, 1050 cm^{-1}; UV (MeOH) λ max (log ε) 272 (3.27), 279 (3.22) nm; ^1H NMR δ ($CDCl_3$) 7.16 (1H, t, *J*

= 7.8 Hz, H-5"), 6.72 (1H, br dd, J = 7.8, 1.5 Hz, H-6"), 6.71 (1H, br dd, J = 7.8, 1.5 Hz, H-4"), 6.68 (1H, br s, H-2"), 3.76 (3H, s, 3"-OMe), 3.45 (1H, d, J = 9.4 Hz, H-3), 2.55 (2H, t, J = 7.5 Hz, 6'), 2.40 (2H, t, J = 7.1 Hz, H-2'), 1.86 (1H, quintet, J = 7.5 Hz, H-5'), 1.76 (1H, dt, J = 11.7, 7.2 Hz) and 1.72 (1H, dt, J = 11.7, 7.2 Hz) (H-1'), 1.63 (2H, m, H-4 and H-5), 1.16 (3H, d, J =5.8 Hz, 4-Me), 1.08 (3H, d, J = 6.5 Hz, 5-Me), 0.88 (3H, s, 2-Me); ^{13}C-NMR δ ($CDCl_3$) 220.6 (s, C-1), 211.4 (s, C-3'), 159.6 (C-3"), 143.1 (s, C-1'), 129.3 (d, C-5"), 120.9 (d, C-6"), 114.3 (d, C-2"), 111.2 (d, C-4"), 80.4 (d, C-3), 55.1 (q, 3"-OMe), 51.6 (s, C-2), 49.7 (d, C-5) and 42.3 (d) (C-4), 41.9 (t, C-4'), 37.6 (t, C-2'), 35.0 (t, C-6'), 29.3 (t, C-1'), 25.1 (t, C-5'), 16.3 (q, 2-Me), 16.1 (q, 4-Me) and 12.7 (q, 5-Me); HREIMS *m/z* $[M]^+$ 346.2139 (calcd for $C_{21}H_{30}O_4$ 346.2144); EIMS *m/z* $[M]^+$ 346 (36), 328 (18), 212 (42), 197 (54), 177 (54), 142 (72), 121 (100).

8. Bowden, K.; Heilbron, I.M.; Jones, E.R.H.; Weedon, B.C.L. *J. Chem. Soc.* **1946**, 39.
9. Wang, K.C. (Syntex Corp): Mikrobiologische Reduktion, *Deutches Offenlengungsshift*, **1971**, 2,038,926.

10. (+)-**5**. colorless needle crystals, mp. 65-66° C (Me_2CO/hexane); $[\alpha]_D^{25}$= +93.0 (c= 1.0, $CHCl_3$); IR (KBr) ν max 3510 (OH), 2930, 1730 (C=O), 1610, 1585, 1285, 1250, 1160 cm^{-1}; UV (MeOH) λ max (log ε) 272 (3.25), 278 (3.22) nm; ^{1}H NMR δ ($CDCl_3$) 7.17 (1H, dt, J = 1.3, 7.6 Hz, H-5"), 6.74 (1H, br d, J = 7.6 Hz, H-6"), 6.72 (1H, br d, J = 7.6 Hz, H-4"), 6.70 (1H, br s, H-2"), 3.76 (3H, s, 3"-OMe), 3.65 (1H, ddt, J = 1.7, 10.5, 6.2 Hz, H-3), 2.55 (2H, t, J = 7.7 Hz, 3'), 2.00 (1H, m, H_{eq}-5), 1.99 (2H, m, H-8 and H-9), 1.68 (2H, m, H-2') and 1.46 (1H, dt, J = 4.5, 13.6 Hz, H_{ax}-5), 1.38 (2H, m, H-1'), 1.11 (3H, d, J = 6.7 Hz, 8-Me), 1.07 (3H, d, J = 6.2 Hz, 9-Me), 0.93 (3H, s, 6-Me), 0.95 (1H, m, H_{ax}-4), 0.84 (1H, m, H_{eq}-4); ^{13}C-NMR δ ($CDCl_3$) 220.5 (s, C-7), 159.6 (C-3"), 144.2 (s, C-1'), 129.2 (d, C-5"), 120.8 (d, C-6"), 114.3 (d, C-2"), 110.8 (d, C-4"), 101.6 (s, C-1), 69.2 (d, C-3), 55.1 (q, 3"-OMe), 51.0 (s, C-6), 49.0 (d, C-8) and 45.0 (d, C-9), 35.9 (t, C-3'), 35.4 (t, C-1'), 28.6 (t, C-4), 28.2 (t, C-5), 27.0 (t, C-2'), 21.1 (q, 6-Me), 14.0 (q, 8-Me), 9.1 (q, 9-Me); HREIMS *m/z* $[M]^+$ 346.2142 (calcd for $C_{21}H_{30}O_4$ 346.2144); EIMS *m/z* $[M+1]^+$ 347 (4), $[M]^+$ 346 (18), 331 (3), 212 (2), 188 (5) 162 (27), 134 (100), 121 (25).

11. Crystal data of (+)-**5**: orthorhombic p212121; a= 7.302 (3), b= 14.688 (6), c= 18.816 (6) Å, Z= 4. Intensity data were collected on a CAD-4 diffractometer with θ / 2θ scan mode, using monochromated MoKα radiation. Data were measured up to 2θ of 50°. A total of 2074 reflections were collected. Among them, 1000 were considered to be observed (> 2.05 σ (I)). Final agreement indices are R(F)= 0.041, WR(F)= 0.040, GoF= 1.57, based on anisotropic refinement of all non-hydrogen atoms.

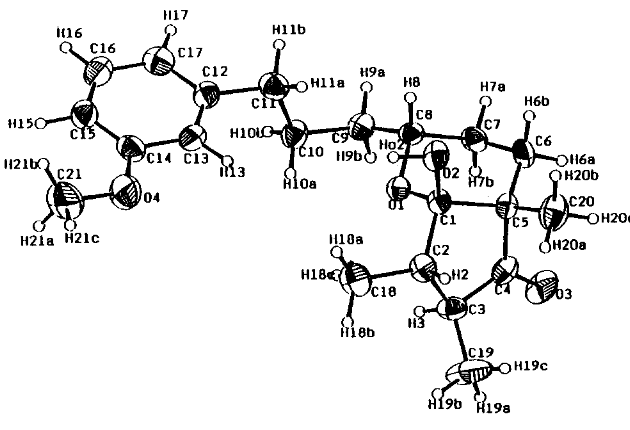


Figure 2. X-Ray Analysis diagram of (+)-**5**

12. We are grateful to NSC, R.O.C. for financial support of this research under Grant NSC 84-2331-B002-256 and NSC 85-2331-B-002-245.

(*Received in Japan* 15 *March* 1996; *accepted* 17 *April* 1996)