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Tetrahedron Letters 46 (2005) 8467–8470
Tetrahedron Letters

Regio- and stereoselective 12-*O*-demethylation of schizandrin into gomisin T, an important intermediate to gomisin A, by *Mortierella* sp. (TM-I1104)

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Received 5 September 2005; revised 5 October 2005; accepted 7 October 2005
Available online 21 October 2005

Abstract—A strain TM-I1104 identified as *Mortierella* sp. was discovered from soil as the most efficient fungus, which converted schizandrin into gomisin T in 91% regioselectivity by microbial 12-*O*-demethylation. Under optimum conditions, the yield of gomisin T reached around 80%. The faculty of 12-*O*-demethylation was specific on (+)-schizandrin (natural form) and the optical purity of gomisin T converted from (±)-schizandrin was 96% ee.

Gomisin A (TJN-101, **1**),[1] a dibenzocyclooctadiene lignan isolated from the fruits of *Schisandra chinensis* BAILL, has been reported to have important pharmacological effect, including inhibitory effect on chemical-induced liver injuries,[2] immunological hepatopathy,[3] and hepatocarcinogenesis,[4] and promoting effect on liver regeneration.[5] The total synthesis towards **1** in about 15% overall yield has been reported.[6] Targeting the synthesis of **1**, we have undertaken a straightforward access to **1** from schizandrin (**2**)[7] via gomisin T (**3**) by combination of a microbial transformation and subsequent two-step chemical transformations (Scheme 1).[8] The microbial 12-*O*-demethylation of **2** was a considerable key step in the strategy, which was achieved by using *Cunninghamella echinulata* var. *elegans* (ATCC 9245) in only 35% regioselectivity and only 25% yield in our previous investigation (Table 1, entry 1).[8]

In this letter, we report improvement of the biotransformation, which resulted in an *O*-demethylation from **2** to **3** in 80% yield and 91% regioselectivity by a *Mortierella* sp. fungus, TM-I1104. Moreover, we also describe high stereoselectivity for 12-*O*-demethylation of **2** by TM-I1104.

In the present study, seven commercial *Cunnninghamella* spp. were screened, and *C. echinulata* var. *elegans* (ATCC 8688a) was found as a fungus, which converted **2** into **3** in 73% regioselectivity and 21% yield (Table 1, entry 2). Further improvement of the regioselectivity and the yield by optimization of the culture condition was investigated by using the fungus ATCC 8688a. However, in spite of the improvement in the yield of **3**, the regioselectivity was not improved by the optimization at all (Table 1, entry 2, number in parentheses). Therefore, we carried out the next strategy in which the culture condition was optimized after finding the most regioselective 12-*O*-demethylation fungus by new screening.

Initially, we performed the screening with seven further commercial *Cunnninghamella* spp., and confirmed the regioselectivity. However, we could not find a high regioselective fungus (data not shown). Next, 384 fungal strains isolated from soil in Ibaraki, Japan, by the conventional streaking method on Potato Dextrose Agar plates containing 0.01% chloramphenicol, were screened for ability of microbial 12-*O*-demethylation of **2** in high regioselectivity. Each fungus was inoculated into 10 ml culture medium,[9] and then incubated at 25 °C with

Keywords: Biotransformation; *Mortierella*; TM-I1104; *Cunninghamella*; Schizandrin; Gomisin T; Gomisin A; *O*-Demethylation.
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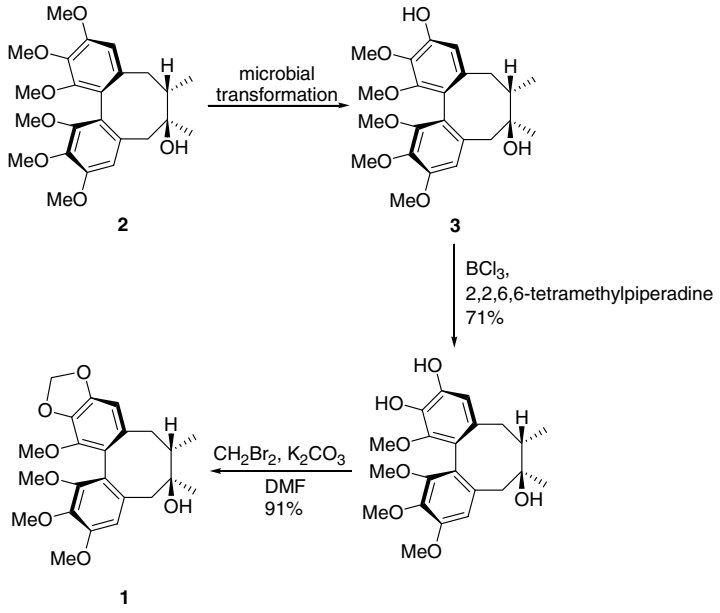
doi:10.1016/j.tetlet.2005.10.014



Scheme 1.

Table 1.

Entry	Fungi	Regioselectivity (%)[a]				Yield (%) of **3**
		3	**4**	**5**	**6**	
1[b]	*Cunninghamella echinulata* var. *elegans* (ATCC 9245)	35	15	23	27	25
2[b]	*Cunninghamella echinulata* var. *elegans* (ATCC 8688a)	73 (68)[c]	8 (11)[c]	7 (8)[c]	12 (13)[c]	21 (40)[c]
3[b]	*Cunninghamella echinulata* var. *elegans* (IFO 4441)	26	25	17	32	4
4[b]	*Cunninghamella echinulata* var. *elegans* (IFO 4443)	67	10	8	15	14
5[b]	*Cunninghamella echinulata* var. *elegans* (IFO 4446)	26	26	14	34	6
6[b]	*Cunninghamella echinulata* var. *elegans* (IFO 4447)	60	16	13	11	11
7[b]	*Cunninghamella echinulata* var. *elegans* (IFO 6156)	67	10	9	14	12
8[b]	*Cunninghamella echinulata* var. *elegans* (IFO 6334)	48	20	17	15	8

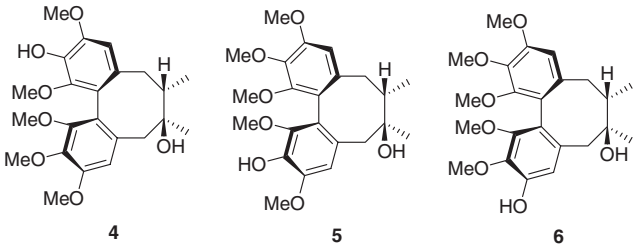
[a] % of ratio per four metabolites, **3**–**6**.

[b] Incubation was performed in the culture medium containing 0.5% potato dextrose broth (PDB), 2% glucose, 0.5% yeast extract, 0.5% NaCl, 0.5% K_2HPO_4, pH 7.0 at 25 °C for 2 weeks.

[c] Number in parentheses indicated the result of incubation in the culture medium containing 0.3% PDB, 3.5% corn starch, 3% yeast extract, 2% NaCl, 0.4% K_2HPO_4, 0.3% $ZnCl_2$, pH 7.0 at 30 °C for 2 weeks.

reciprocal shaking (120 strokes/min) for 2 weeks after addition of 5 mg **2** dissolved in 30 μl ethanol. Each culture broth was analyzed by HPLC.[10] Consequently, a fungus TM-I1104 identified as *Mortierella* sp. was discovered as the most competent fungus, which converted **2** into **3** in high regioselectivity.

Accordingly, optimization of the culture condition using TM-I1104 was carried out in terms of nutrient ingredients (carbon, nitrogen and mineral sources), pH, culture temperature and preincubation time, which resulted in the modified culture medium containing 4% glucose, 1% peptone, 0.07% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$, pH 5.5 at 25 °C and 2 days preincubation. Under the optimized culture conditions, the microbial 12-*O*-demethylation of **2** by TM-I1104 was investigated. On the basis of HPLC analysis of the culture broth, TM-I1104 gave the best result that **2** was converted into **3** in 80% yield and 91% regioselectivity. As shown in Figure 1, TM-I1104 did not produce unnecessary by-products, **4**, **5** and **6**, which most commercial *Cunninghamella* spp. produced as metabolites. Thus, TM-I1104 was the most competent fungus for 12-*O*-demethylation of **2**.



In a further study, the stereoselectivity of 12-*O*-demethylation by the fungus TM-I1104 was investigated using synthetic (±)-**2**[6a] as the substrate. After incubation for 2 weeks, the cultivation medium was collected by filtration. The filtrate was acidified by acetic acid and partitioned with diethyl ether. The diethyl ether extract was

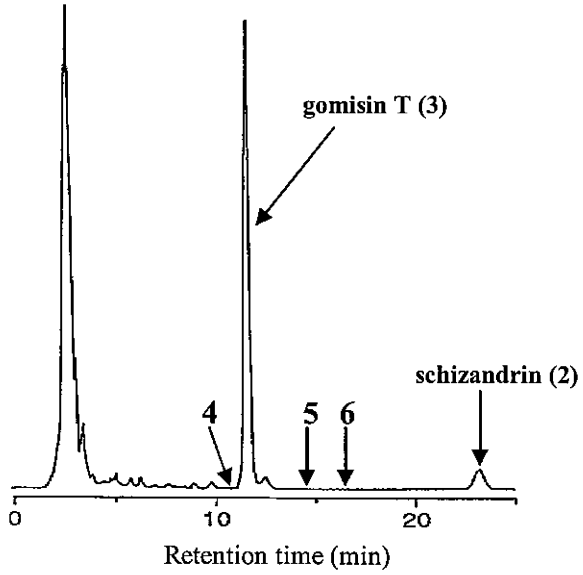


Figure 1. HPLC profile of culture broth[11] by *Mortierella* sp. (TM-I1104) analytical condition; column, YMC A-312 (C_{18}, 150 × 6 mm); flow rate, 1.0 ml/min; wavelength, 254 nm; mobile phase, H_2O–MeOH–CH_3CN–tetrahydrofuran (40:15:6:3, v/v). The retention times of **2** and **3** were 11.7 and 23.6 min, respectively. **4**, 13-norschizandrin; **5**, 2-norschizandrin; **6**, 3-norschizandrin.

separated by preparative HPLC to afford the produced **3** (amorphous powder, $[\alpha]_D^{25}$ +93.6), which was confirmed by comparison of the ^1H and ^{13}C NMR data[12] with those of natural **3**. The circular dichroism (CD) spectrum of produced **3** showed a positive Cotton effect at 249 nm ([θ] +80,442) and a negative Cotton effect at 215 nm ([θ] −93,821), indicating that the product possesses an *R*-biphenyl configuration.[7] On the other hand, the CD spectrum of the recovered substrate shows a positive Cotton effect at 215 nm ([θ] +103,109) and a negative Cotton effect at 249 nm ([θ] −94,055), indicating that the recovered substrate possesses an *S*-biphenyl configuration,[7] thus to be (−)-**2**. The optical purity of produced (+)-**3** was determined to be 96% ee by the following method. Produced (+)-**3** was first derived to (+)-**2**[13] by *O*-methylation with methyl iodide, then subjected to HPLC analysis using a chiral column.[14]

In conclusion, we have discovered a competent fungus, *Mortierella* sp. (TM-I1104), which converted **2** into **3** in 80% yield and 91% regioselectivity. This fact signifies that the microbial 12-*O*-demethylation of isolated natural **2** by TM-I1104 theoretically permits the total synthesis of **1** in 52% overall yield. Moreover, the microbial 12-*O*-demethylation of (±)-**2** by TM-I1104 produced **3** in 96% ee. This result indicates that synthetic (±)-**2** is also available as a starting material for the total synthesis of **1**.

Acknowledgements

We are grateful to Dr. Tatsuo Yokoyama (Institute for Fermentation, Osaka) for the identification of *Mortierella* sp. (TM-I1104). We also thank Dr. Masahide Tanaka for the offer of synthetic (±)-**2**.

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9. Culture medium for screening contains 0.5% potato dextrose broth, 2% glucose, 0.5% yeast extract, 0.5% sodium chloride, 0.5% potassium phosphate and pH 7.0.
10. The analysis of each culture broth by HPLC was done under the following conditions: column, YMC A-312 (C_{18}, 150 × 6 mm); mobile phase, H_2O–MeOH–CH_3CN–tetrahydrofuran (40:15:16:3); flow rate, 1 ml/min; detection, wavelength, 254 nm. The retention time of **2** and **3** were 11.7 and 23.6 min, respectively.
11. The incubation in the optimum condition was performed in the culture medium containing 4% glucose, 1% peptone, 0.07% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, pH 5.5 at 25 °C for 2 weeks.

12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, d, $J = 7.3$ Hz, H_3-8), 1.25 (3H, s, H_3-7), 1.82–1.89 (1H, m, H-8), 1.85 (1H, s, OH-7), 2.34 (1H, dd, $J = 14.2$, 7.8 Hz, H-9α), 2.37 (1H, d, $J = 13.2$ Hz, H-6β), 2.60 (1H, dd, $J = 14.2$, 1.5 Hz, H-9β), 2.67 (1H, d, $J = 13.2$ Hz, H-6α), 3.55 (6H, s, 1-OMe, 14-OMe), 3.90 (3H, s, 2-OMe), 3.91 (3H, s, 3-OMe), 3.92 (3H, s, 13-OMe), 5.72 (1H, br s, OH-12), 6.62 (2H, s, H-4 and H-11). ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.8 (C-17), 29.8 (C-18), 34.0 (C-9), 40.9 (C-6), 41.9 (C-8), 56.0 (3-OMe), 60.2 (1-OMe), 60.6 (14-OMe), 60.9 (2-OMe), 61.0 (13-OMe), 72.0 (C-7), 110.2 (C-4), 113.1 (C-11), 122.0 (C-15), 124.1 (C-16), 132.1 (C-5), 134.7 (C-10), 137.8 (C-13), 140.9 (C-2), 147.8 (C-12), 150.4 (C-14), 152.1 (C-1), 152.4 (C-3).
13. Derived (+)-**2**: Amorphous powder. $[\alpha]_D^{25}$ +82.0 (*c* 0.76, $CHCl_3$). MS *m*/*z*: 432 $[M]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, d, $J = 7$ Hz), 1.25 (3H, s), 1.80–1.94 (1H, m), 1.86 (1H, s), 2.32 (1H, d, $J = 14$ Hz), 2.33 (1H, dd, $J = 14$, 7 Hz), 2.68 (1H, dd, $J = 14$, 2 Hz), 2.70 (1H, d, $J = 14$ Hz), 3.59 (6H, s), 3.90 (6H, s), 3.92 (6H, s), 6.60 (1H, s), 6.53 (1H, s).
14. The analysis by HPLC was done under the following conditions: column, CHIRALCEL OJ (Daicel Chemical Industries, LTD); mobile phase, *n*-hexane–EtOH (95:5); flow rate, 1 ml/min; detection, UV (254 nm). The retention time of (+)-**2** and (−)-**2** were 9.55 and 7.12 min, respectively.