

## A Taxatetraene from Microbial Transformation of Sinenxan A

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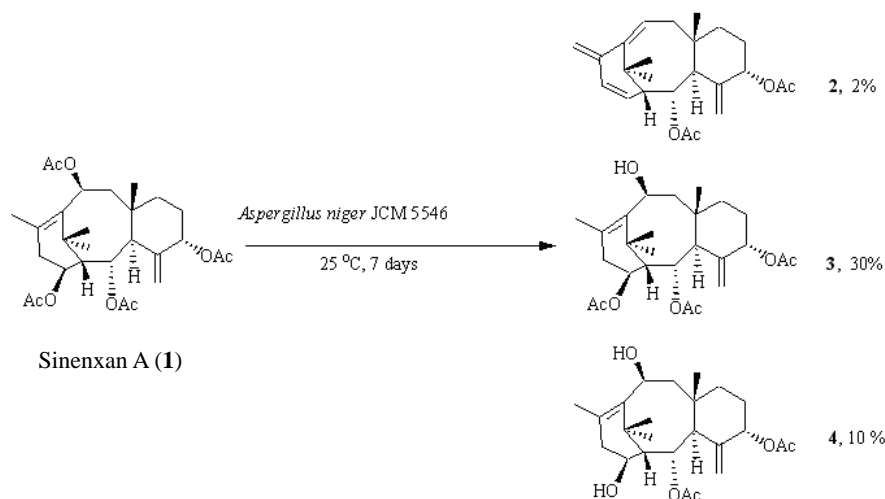
**Abstract:** Sinenxan A [ $2\alpha$ ,  $5\alpha$ ,  $10\beta$ ,  $14\beta$ -tetraacetoxytaxa-4(20), 11-diene, **1**] was biotransformed by a filamentous fungus, *Aspergillus niger* JCM 5546, and an unusual taxatetraene [ $2\alpha$ ,  $5\alpha$ -acetoxytaxa-4(20), 10(11), 12(18), 13(14)-tetraene, **2**], together with two known products,  $10\beta$ -deacetyl sinenxan A (**3**) and  $10\beta$ ,  $14\beta$ -dideacetyl sinenxan A (**4**) were produced.

**Keywords:** *Aspergillus niger*, sinenxan A, taxatetraene, biotransformation.

Sinenxan A,  $2\alpha$ ,  $5\alpha$ ,  $10\beta$ ,  $14\beta$ -tetraacetoxy-4(20), 11-taxadiene, is a taxoid isolated from the callus cultures of *Taxus* spp. in high yields (ca. 1~2% of dry weight)<sup>1</sup>. The rich resources and its taxane-skeleton endow its valuable potential for the semisynthesis of paclitaxel or other structurally related bioactive compounds. A number of studies on its structural modification by chemical and biocatalytic approaches were reported<sup>2-11</sup>. Lately, we also reported its highly regio- and stereoselective hydroxylation at C-9 and C-7 by *Ginkgo* cell suspension cultures and fungus *Abisidia coerulea* IFO 4011. As a part of our ongoing research on the biotransformation of this type taxanes and obtain other derivatives of interest, a number of species of microorganisms and suspended cell cultures of plants were investigated for their capacity to transform taxanes. Here, we report the biotransformation of sinenxan A by a fungus, *A. niger* and one unusual product derived from this bioprocess.

To 2-day-old cell cultures of *A. niger* JCM 5546 (purchased from Japan Collection of Microorganisms) 300 mg (in acetone) of **1** was added, and three products (**2**, **3** and **4**; **Scheme 1**) were obtained after 7 days of incubation by the flash column chromatography and pre-HPLC in the yields of 2%, 30% and 10%, respectively. Their structures were identified as  $2\alpha$ ,  $5\alpha$ -acetoxytaxa-4(20), 10(11), 12(18), 13(14)-tetraene (**2**),  $10\beta$ -deacetyl sinenxan A (**3**) and  $10\beta$ ,  $14\beta$ -dideacetyl sinenxan A (**4**) based upon the <sup>1</sup>H NMR, <sup>1</sup>H-<sup>1</sup>H COSY, <sup>13</sup>C NMR, DEPT, HMQC, HMBC, IR and HRMS spectral data. Compounds **3** and **4** are known compounds<sup>8</sup>, while **2** is a new compound of which NMR spectral data were showed in **Table 1**.

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**Scheme 1** Biotransformation of sinenxan A by *A. niger* JCM 5546**Table 1** NMR spectral data of compound 2<sup>a)</sup>

Position	<sup>13</sup> C <sup>b)</sup>	Connected <sup>1</sup> H <sup>c)</sup>	H-H correlation <sup>d)</sup>	HMBC <sup>e)</sup>
1	58.96 (d)	2.37 (dd, 2.7, 5.4)	H-2, H-14	H-3, H-13, H-14, H-16, H-17
2	70.91 (d)	5.53 (dd, 2.7, 7.6)	H-1, H-3	H-1, H-3
3	43.17 (d)	3.03 (d, 7.6)	H-2	H-1, H-5, H-2, H-9, H-19, H-20
4	144.20 (s)			H-3, H-20
5	77.36 (d)	5.11 (dd, 2.9, 3.2)	H-6	H-3, H-6, H-20
6	30.01 (t)	1.77 (m)	H-5, H-7	H-5, H-7
7	34.33 (t)	Ha: 2.05 (m); Hb: 1.12 (m)	H-6	H-6, H-9, H-19
8	46.12 (s)			H-2, H-6, H-7, H-9, H-19
9	39.76 (t)	Ha: 2.81 (dd, 12.9, 13.4); Hb: 1.72 (dd, 6.3, 13.9)	H-10	H-10, H-19
10	125.86 (d)	5.70 (dd, 6.1, 12.5)	H-9	H-9
11	147.82 (s)			H-1, H-9, H-10, H-13, H-16, H-17, H-18
12	150.70 (s)			H-10, H-13, H-14, H-18,
13	134.63 (d)	6.33 (d, 9.5)	H-14	H-14, H-18
14	127.04 (d)	5.58 (dd, 5.1, 9.5)	H-1, H-13	H-1, H-2, H-13
15	37.71 (s)			H-1, H-10, H-14, H-16, H-17
16	25.46 (q)	1.56 (s)	H-17	H-17
17	30.81 (q)	1.10 (s)	H-16	H-16
18	106.57 (t)	Ha: 4.92 (s); Hb: 4.72 (s)	H-13	H-13
19	20.98 (q)	0.98 (s)		H-3, H-7, H-9
20	113.82 (t)	Ha: 5.20 (s); Hb: 4.50 (s)	H-3	H-3, H-5
2- OCOCH <sub>3</sub>	170.11 (s)			H-2, 2- OCOCH <sub>3</sub>
5- OCOCH <sub>3</sub>	170.00 (s)			H-5, 5- OCOCH <sub>3</sub>
2- OCOCH <sub>3</sub>	21.52 (q)	2.01 (s)		
5- OCOCH <sub>3</sub>	21.25 (q)	2.03 (s)		

<sup>a)</sup> CD<sub>3</sub>Cl, 500 MHz for <sup>1</sup>H-NMR, 125 MHz for <sup>13</sup>C NMR, TMS, δ ppm; <sup>b)</sup> Multiplicities were determined by DEPT; <sup>c)</sup> Connections were determined by HMQC, multiplicities and coupling constants in Hz are in parentheses; <sup>d)</sup> Determined by <sup>1</sup>H-<sup>1</sup>H COSY; <sup>e)</sup> Correlations from C to the indicated protons.

The taxatetraene type structure of taxane has not been found in the natural *Taxus* plants yet, these results indicated that biotransformation technique could diversify the natural products. Moreover, in authors' opinion, this unusual structure might lead to a rather unique and interesting consideration on the biosynthesis of taxoid in nature.

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### References and Notes

1. Y. Q. Wu, W. H. Zhu, J. Lu, *et al.*, *Chin. Pharm. J.*, **1998**, *33*, 15.
2. G. Y. Huang, J. Y. Guo, X. T. Liang, *Acta Pharm. Sin.*, **1998**, *33*, 576.
3. S. H. Hu, X. F. Tian, W. H. Zhu, *et al.*, *J. Nat. Prod.*, **1996**, *59*, 1006.
4. S. H. Hu, X. F. Tian, W. H. Zhu, *et al.*, *Tetrahedron*, **1996**, *26*, 8739.
5. J. G. Dai, H. Z. Guo, D. Lu, *et al.*, *Tetrahedron Lett.*, **2001**, *42*, 4677.
6. J. G. Dai, M. Ye, H. Z. Guo, *et al.*, *Tetrahedron Lett.*, **2002**, *58*, 5659.
7. J. G. Dai, Y. J. Cui, W. H. Zhu, *et al.*, *Planta Medica*, **2002**, *68*, 1055.
8. J. G. Dai, M. Ye, H. Z. Guo, *et al.*, *Bioorganic Chemistry*, **2003**, *31*, 345.
9. J. G. Dai, S. J. Zhang, J. Sakai, *et al.*, *Tetrahedron Lett.*, **2003**, *44*, 1091.
10. J. G. Dai, M. Zhang, M. Ye, *et al.*, *Chin. Chem. Lett.*, **2003**, *14*, 804.
11. M. Zhang, D. L. Yin, J. Y. Guo, *et al.*, *Tetrahedron Lett.*, **2002**, *43*, 9425.
12. selected data of **2**: white powder, mp: 87-89 °C;  $[\alpha]_D^{20}$  -8.04 (*c* 0.58, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu$  3036, 1732, 1376, 1236, 1216, 1022 cm<sup>-1</sup>; HRESIMS *m/z* [M+H]<sup>+</sup> 385.2379 (calcd. for C<sub>24</sub>H<sub>33</sub>O<sub>4</sub> 385.2379), [M+Na]<sup>+</sup> 407.2199 (calcd. for C<sub>24</sub>H<sub>32</sub>O<sub>4</sub> Na, 407.2198).

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