Chinese Chemical Letters Vol. 14, No. 8, pp 804 – 806, 2003
http://www.imm.ac.cn/journal/ccl.html

Biotransformation of 14-Deacetoxy-13-oxo sinenxan A by *Ginkgo* Cell Cultures

Jun Gui DAI, Meng ZHANG, Min YE, Wei Hua ZHU, Ji Yu GUO, Xiao Tian LIANG*

Institute of Materia Medica, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100050

Abstract: 14-Deacetoxy-13-oxo sinenxan A (**1**) was converted to 9α-hydroxy-13-oxo-2α, 5α, 10β-triacetoxy-4(20),11-taxadiene (**2**) and 10β-hydroxy-13-oxo-2α,5α,9α-triacetoxy- 4(20), 11-taxadiene (**3**) by *Ginkgo* cell suspension cultures in 45% and 15% yields, respectively.

Keywords: Biotransformation, taxane, cell suspension cultures, *Ginkgo biloba* L.

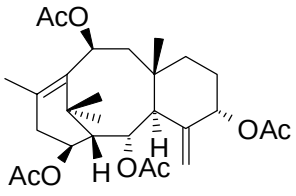
Sinenxan A, 2α, 5α, 10β,14β-tetraacetoxy-4(20),11-taxadiene, is a taxoid isolated from the callus cultures of *Taxus* spp. in high yield (*ca*. 1~2% of dry weight)[1,2]. The rich resources and its taxane-skeleton vest it valuable potential for the semisynthesis of paclitaxel or other structurally related bioactive compounds, such as "second -generation" taxoid anticancer agents and taxane-based multidrug resistant anticancer agents[3-5]. Many remarkable studies on its structural modification by chemical and biocatalytic approaches were reported[6-9]. Recently, we reported its highly regio- and stereo-selective hydroxylation at 9α position[10]. Here we report that the taxoid 14-deacetoxy-13-oxo sinenxan A[11], 14-deacetoxy-13-oxo-2α, 5α, 10β-triacetoxy-4(20), 11-taxadiene (**1**) obtained by chemical modification of sinenxan A, was also regio- and stereo-selectively hydroxylated at 9α position by *Ginkgo* cell suspension cultures.

Ginkgo cell suspension cultures were cultivated as described in reference[12]. **1** was efficiently bioconverted by *Ginkgo* cell suspension cultures. **1** was administered to the 15-day-old cell cultures, and two more polar products, **2** and **3**, were obtained by chromatographic methods after additional six days of incubation in the yields of 45% and 15%, respectively. Their structures were identified as 9α-hydroxy-13-oxo- 2α, 5α,10β-triacetoxy-4(20),11-taxadiene (**2**), 9α-hydroxylated derivative of **1**, and 10β-hydroxy-13-oxo-2α,5α,9α-triacetoxy-4 (20), 11-taxadiene (**3**) by ^{1}H, ^{13}C-NMR and FAB mass spectra, respectively (**Scheme 1**).

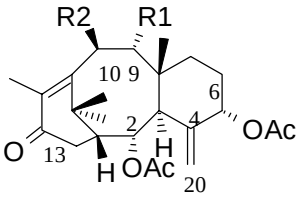
The results indicated that **1** could be regio- and stereoselectively hydroxylated at 9α position, too, and regioselectively deacetylated at C-10 position and acetylated at C-9 position by *Ginkgo* cell suspension cultures as well. The latter reaction(s) may be resulted from intra-molecular migration of acetoxyl group – from C-10 to C-9 by the

*E-mail: xtliang@public.bta.net.cn

Scheme 1



Sinenxan A



1 R_1=H, R_2=OAc
2 R_1=OH, R_2=OAc
3 R_1=OAc, R_2=OH

enzyme. These results suggested that the enzyme(s) responsible for the hydroxylation at 9α position display its high substrate-specialty, and, not restricted to sinenxan A only.Regarding this, the next step is to isolate and purify the responsible enzyme(s), then prepare 9α hydroxylated derivatives by using enzyme(s) instead of whole cells. The results also implied that biocatalysis might be a useful approach to prepare bioactive taxoids and intermediates for the semisyntheses of other bioactive agents. Furthermore, the fact of selective hydroxylation at 9α position of taxane may give some hints as to paclitaxel biosynthesis in *Taxus* plants.

Acknowledgment

This work is supported by the National Natural Science Foundation of China (to Jungui Dai, No. 30100230).

References and Notes

1. K. D. Cheng, W. M. Chen, W. H. Zhu, Q. C.Fang. PCT Int. Appl. WO9406, 740 (Cl. C07C35/37), 31 Mar **1994**, JP Appl. 92/249, 047, 18 Sep **1992**.
2. Y. Q. Wu, W. H. Zhu, J. Lu, Q. Hu, X. L. Li, *Chin. Pharm. J*., **1998**, *33*, 15.
3. J. M. Ford, W. N. Hait, *Pharnacol. Rev.*, **1990**, *42*, 155.
4. M. M. Gottesman, I. Pastan, *Annu. Rev. Biochem*., **1993**, *62*, 385.
5. I. Ojima, P. Y. Bounaud, R. J. Bernacki., *Chemtech June*, **1998**, 31.
6. G. Y. Huang, J. Y. Guo, X. T. Liang, *Acta Pharm. Sin.*, **1998**, *33*, 576.
7. S. H. Hu, X. F. Tian, W. H. Zhu, Q. C. Fang, *Chin. Chem. Lett.*, **1996**, *6*, 543.
8. S. H. Hu, X. F. Tian, W. H. Zhu, Q. C. Fang, *J. Nat. Prod*., **1996**, *59*, 1006.
9. S. H. Hu, X. F. Tian, W. H. Zhu, Q. C. Fang, *Tetrahedron*, **1996**, *26*, 8739.
10. J. G. Dai, H. Z. Guo, D. Lu, W. H. Zhu, D. Y. Zhang, J. H. Zheng, D. A. Guo, *Tetrehedron Lett*, **2001**, *42*, 4677.
11. M. Zhang, D. L. Yin, J. Y. Guo, X. T. Liang, *Chin. Chem. Lett*, **2002**, *13*, 135.
12. J. G. Dai, W. H. Zhu, Y. Q. Wu, Q. Hu, D. Y. Zhang, *Acta Pharm. Sin.*, **2000**, *2*, 151.
13. selected data of **2**: white powder; ^{1}H NMR (500 MHz, $CDCl_3$, δppm, JHz) 5.82 (d,1H, *J*=9.5, H-10), 5.47 (dd,1H, *J*=2.0, 6.0, H-2), 5.31 (s,1H, H-20a), 5.25 (brs, 1H, H-5), 4.89 (s, 1H, H-20b), 4.30 (d, 1H, *J*=9.5, H-9), 3.22 (d, 1H, *J*=6.0, H-3), 2.78 (dd, 1H, *J*=7.0, 19.5, H-14a), 2.32 (d, 1H, *J*=20, H-14b), 2.18 (dd, 1H, *J*=2.0, 5.5, H-1), 1.99 (s, 3H, H-18), 1.87 (m, 2H, H-6), 1.63 (s, 3H, H-16), 1.56 (m, 1H, H-7a), 1.25 (s, 3H, H-17), 1.24 (m, 1H, H-7b), 1.11 (s, 3H, H-19), 2.25, 2.17, 2.06 (s, 3H each, OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.16 (C-13), 170.27, 170.11, 169.67 [OAc(CO)], 150.93 (C-4), 142.22 (C-11), 137.62 (C-12), 117.08 (C-20), 78.25 (C-5), 76.0 (C-9), 75.58 (C-10), 69.89 (C-2), 48.60 (C-1), 44.87 (C-8), 42.97

(C-15), 37.76 (C-14), 37.13 (C-3), 36.03 (C-6), 29.68 (C-7), 28.57 (C-17), 25.54 (C-16), 21.39, 21.20, 21.14 [OAc (CH_3)], 17.59 (C-18), 13.91 (C-19); FABMS *m/z* $[M]^+$ 476 (for $C_{26}H_{36}O_8$).

14. selected data of **3**: white powder; ^1H NMR (500 MHz, $CDCl_3$, δppm, JHz) δ 5.74 (d, 1H, *J*=9.5, H-9), 5.52 (dd, 1H, *J*=2.0, 6.0, H-2), 5.33 (s, 1H, H-20a),5.25 (brs, 1H, H-5), 5.01 (d, 1H, *J*=9.5, H-10), 4.86 (s, 1H, H-20b), 3.22 (d, 1H, *J*=6.0, H-3), 2.81 (dd, 1H, *J*=7.0, 19.5, H-14a), 2.30 (d, 1H, *J*=19.5, H-14b), 2.19 (dd, 1H, *J*=2.0, 6.0, H-1), 1.99 (s, 3H, H-18), 1.89 (m, 2H, H-6), 1.81 (s, 3H, H-16), 1.58 (m, 1H, H-7a), 1.30 (m, 1H, H-7b), 1.26 (s, 3H, H-17), 0.91 (s, 3H, H-19), 2.17, 2.14, 2.06 (s, 3H each, OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.50 (C-13), 170.20, 169.21, 168.54 [OAc(CO)], 150.92 (C-4), 142.01 (C-11), 136.20 (C-12), 117.49 (C-20), 79.07 (C-9), 78.00 (C-5), 72.03 (C-10), 69.72 (C-2), 47.20 (C-1), 44.24 (C-8), 42.95 (C-15), 37.58 (C-14), 37.36 (C-3), 36.01 (C-6), 30.90 (C-7), 28.48 (C-17), 25.28 (C-16), 21.45, 21.32, 21.04 [OAc (CH_3)], 17.64 (C-18), 14.00 (C-19); FABMS *m/z* $[M]^+$ 476 (for $C_{26}H_{36}O_8$).

Received 5 September, 2002