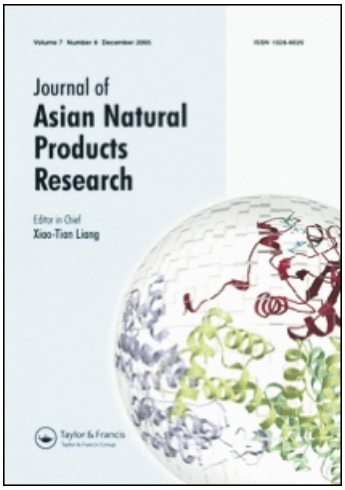

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Microbial transformation of dehydroandrographolide by *Cunninghamella elegans*

Xiu-Lan Xin[ab]; Xiao-Chi Ma[c]; Bao-Jing Zhang[c]; Dong-Hai Su[b]; Zhi-Ming Wu[b]; Xiao-Jie Wang[b]; Xiao-Yan Li[b]; Qi-Peng Yuan[a]
[a] Key Laboratory of Bioprocess of Beijing, Beijing University of Chemical Technology, Beijing, China [b] Biotechnology Application Center, Beijing Vocational College of Electronic Science and Technology, Beijing, China [c] School of Pharmaceutical Sciences, Dalian Medical University, Dalian, China

To cite this Article Xin, Xiu-Lan , Ma, Xiao-Chi , Zhang, Bao-Jing , Su, Dong-Hai , Wu, Zhi-Ming , Wang, Xiao-Jie , Li, Xiao-Yan and Yuan, Qi-Peng(2009) 'Microbial transformation of dehydroandrographolide by *Cunninghamella elegans*', Journal of Asian Natural Products Research, 11: 2, 187 — 191
To link to this Article: DOI: 10.1080/10286020802619280
URL: http://dx.doi.org/10.1080/10286020802619280

Journal of Asian Natural Products Research
Vol. 11, No. 2, February 2009, 187–191

Microbial transformation of dehydroandrographolide by *Cunninghamella elegans*

Xiu-Lan Xin[ac], Xiao-Chi Ma[b1], Bao-Jing Zhang[b], Dong-Hai Su[c], Zhi-Ming Wu[c], Xiao-Jie Wang[c], Xiao-Yan Li[c] and Qi-Peng Yuan[a]*

[a]*Key Laboratory of Bioprocess of Beijing, Beijing University of Chemical Technology, Beijing, China;* [b]*School of Pharmaceutical Sciences, Dalian Medical University, Dalian, China;* [c]*Biotechnology Application Center, Beijing Vocational College of Electronic Science and Technology, Beijing, China*

(*Received 8 September 2008; final version received 11 November 2008*)

The biotransformation of dehydroandrographolide (**1**) by *Cunninghamella elegans* was performed and four transformed products were obtained. Based on their extensive spectral data, the structures of these metabolites were identified as 3-oxo-dehydroandrographolide (**2**), 3-oxo-2β-hydroxy-dehydroandrographolide (**3**), 3-oxo-8β,17α-epoxydehydroandrographolide (**4**), 3,19-dihydroxy-7,11,13-*ent*-labdatrien-15,16-olide (**5**), respectively. Among them, products **3**–**5** are new compounds.

Keywords: biotransformation; dehydroandrographolide; *Andrographis paniculata*; *Cunninghamella elegans*; *ent*-labdane diterpenoids

1. Introduction

Andrographis paniculata is a common herb distributed in India, Thailand, and China [1]. Its stems and leaves are widely used as antivirus, antitumor, and hypotensive agents. The principal active constituents of *A. paniculata* are the *ent*-labdane diterpenoids such as andrographolide and dehydroandrographolide, which have characteristic A/B *cis* junctures with α-alkylidene γ-butyrolactone moiety and α-hydroxyl at C-3 position. They have anticancer [2], immunostimulant [3], and hypotensive [4] activities. In recent years, the structure–activity relationships of various andrographolide analogs in the biological activities of α-glucosidase inhibitory [5,6] and cytotoxic agents [7–9] were investigated, which indicate that they had potent bioactivities in antidiabetics and anticancer.

Microbial transformation is defined as an enzymatic reaction by microorganisms with metabolic activities to modify the structures of bioactive substrates. It has such advantages as high stereo- or regio-selectivity, as well as mild reaction conditions over chemical synthesis. Some reactions fulfilled with difficulty in the process of chemical synthesis are facile by microbial transformation. In addition, microbial transformation is a useful method to identify the metabolites as *in vitro* models [10]. In recent years, microbial biotransformation of diterpenoids has been frequently reported to modify their structures and obtain some new chemical entities. The unique and inexpensive source of diterpene provides the structural diversity for the preparation of hemisynthesis intermediate, chiral auxiliaries, and leading compounds.

In this paper, the biotransformation of dehydroandrographolide (**1**) by *Cunninghamella*

*Corresponding author. Email: yuanqp@mail.buct.edu.cn
[1]Email: maxc1978@sohu.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802619280
http://www.informaworld.com

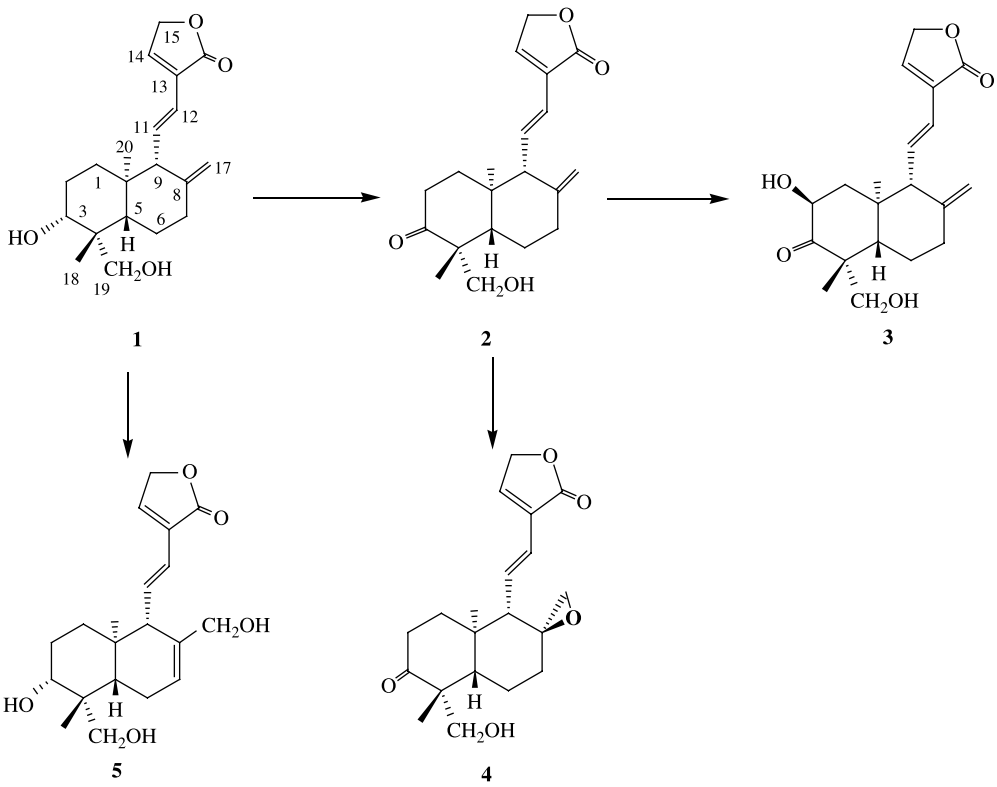

Figure 1. Possible biotransformation pathways of **1** by *C. elegans*.

elegans was performed to find out new chemical entities with the aim of improving its activities and solubility, due to its poor solubility in water limiting its clinical use. Four products were obtained, and their structures were elucidated on the basis of extensive spectral data, including 2D NMR. Among them, products **3**–**5** were new compounds.

2. Results and discussion

In the present study, compound **1** was added into the 36-h-old cultures of *C. elegans* AS 3.2028 and continued to incubate for an additional 6 days. And a blank culture control and substrate control were performed as described above. TLC and HPLC analyses showed that compound **1** was stable in the culture medium. In the preparative-scale biotransformation, a total amount of 500 mg of substrate was added to the culture liquid of *C. elegans*. After 6 days of incubation, four more polar products were isolated by chromatographic methods. Based on the extensive spectra of ^{1}H, ^{13}C, and 2D-NMR techniques, their structures were elucidated as 3-oxo-dehydroandrographolide (**2**), 3-oxo-2β-hydroxy-dehydroandrographolide (**3**), 3-oxo-8β,17α-epoxydehydroandrographolide (**4**), and 3,19-dihydroxy-7,11,13-*ent*-labdatrien-15,16-olide (**5**), respectively (Figure 1). Among them, compounds **3**–**5** are new products. And the spectral data of product **2** were in agreement with those reported in the literature [1].

Compound **3** was obtained as white powder. HR-MS of **3** showed an $[M+Na]^{+}$ ion peak at *m/z* 369.1677, indicating the molecular formula of $C_{20}H_{26}O_5$. In the ^{13}C NMR spectrum, two new carbon signals (δ 214.4 and 71.2) were observed. In HMBC spectrum, the carbon signal (δ 214.4) correlated

with H-2 (δ 5.03), H-19 (δ 3.77), H-1 (δ 2.41), and Me-18 (δ 1.55), suggesting that an oxo group was located at C-3 position. In addition, $^{1}H-^{1}H$ COSY spectrum showed that H-2 (δ 5.03) had correlations with H-1 (δ 1.65 and 2.41), indicating that a hydroxyl group to be introduced at C-2. In NOESY spectrum, H-2 (δ 5.03) had NOE enhancements with Me-20 (δ 1.27) and H-19 (δ 3.77 and 4.38), suggesting the 2-OH to be in β-configuration. On the basis of the above analysis, compound **3** was identified as 3-oxo-2β-hydroxydehydroandrographolide.

Compound **4** was assigned the molecular formula of $C_{20}H_{26}O_5$, on the basis of its HR-MS ion peak at *m/z* 369.1703 $[M+Na]^+$. By comparing with the spectrum of **2**, the ^{13}C NMR exhibited two new oxygen-bearing carbons (δ 58.3 and 50.7), together with the absence of two olefin carbons (δ 149.0 and 108.9) [1], suggesting it to be an epoxide derivative of compound **2**. The proton at δ 2.33 had the long-range correlations with C-8 (δ 58.3) and C-17 (δ 50.7) in HMBC spectrum. And H-17 (δ 2.93) correlated with C-7, C-8, and C-9. This evidence indicated that the substitution position of the epoxide should be at C-8 and C-17. The NOE enhancement between H-17 (δ 2.58 and 2.93) and H-11 (δ 6.83) was observed in NOESY spectrum. On the basis of the above analysis, compound **4** was identified as 3-oxo-8β, 17α-epoxy-dehydroandrographolide.

HR-MS of **5** gave an $[M+Na]^+$ ion peak at *m/z* 371.1861, indicating the molecular formula of $C_{20}H_{28}O_5$. By comparing with **1**, the ^{13}C NMR and DEPT spectra of **5** showed disappearance of a CH_2 signal (δ 110.1) and appearance of CH (δ 122.3) and CH_2 (δ 64.2), suggesting that compound **5** was a hydroxylated product of compound **1**, with the rearrangement of the double bond of C-8 and C-17. The alkene proton (δ 6.11) had HMBC correlations with C-5 (δ 50.7), C-6 (δ 23.7), C-9 (δ 58.4), and C-17 (δ 64.2). In $^{1}H-^{1}H$ COSY spectrum, the proton signal at δ 6.11 correlated with H-6 (δ 2.12 and 2.24). All evidence suggested that a double bond should be at C-7 and C-8 positions. In addition, the oxygen-bearing CH_2 signal (δ 64.2) had long-range correlations with C-8 (δ 138.1) and C-7 (δ 122.3), indicating a hydroxymethyl to be located at C-8. On the basis of the above analysis, compound **5** was identified as 3,19-dihydroxy-7,11,13-*ent*-labdatrien-15,16-olide.

The hydroxylation, dehydrogenation, and epoxidation reactions of the substrate by *C. elegans* AS 3.2028 were reported. A possible biotransformation pathway of **1** was proposed as shown in Figure 1.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT4A apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 243B polarimeter. The UV spectra were detected on a YV-1091 UV–vis spectrophotometer. The IR spectra were obtained on an Avatar 360 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) in pyridine-d_5 with TMS as internal standard. ESI-MS data were obtained by Bruker APEX IV FTMS. Agilent 1100 series HPLC equipped with diode array detector at 254 nm was used. Silica gel (200–300 mesh) was purchased from Qingdao Marine Chemical Group, Qingdao, China. All solvents including ethyl acetate, petroleum ether (60–90°C), and acetone are A.R. grade and were obtained from Beijing Chemical Reagents Company (Beijing, China). Dehydroandrographolide (**1**) was isolated from *A. paniculata* by the authors. The purity was above 98% determined by HPLC.

3.2 Microorganism

Mucor subtilissimus AS 3.2454, *M. spinosus* AS 3.3450, *M. spinosus* AS 3.2450, *M. spinosus* AS 3.3447, *M. subtilissimus* AS 3.2456, *M. polymorphosporus* AS 3.3443, *C. blakesleana lender* AS 3.970, *C. elegans* AS 3.1207, *C. elegans* AS 3.2028, *Alternaria*

Table 1. ^{1}H NMR spectral data of compounds **3**–**5** (pyridine-d_5, 500 MHz, δ in ppm, J in Hz).

H	3	4	5
1a, b	1.65 m, 2.41 m	1.46 m, 1.74 m	1.18 m, 1.70 m
2a, b	5.03 dd (6.0, 13.0)	2.37 m, 2.83 m	1.92 m, 2.02 m
3	–	–	3.64 m
5	1.67 m	1.60 m	1.41 m
6a, b	1.61 m, 1.72 m	1.79 m, 1.76 m	2.12 m, 2.24 m
7a, b	2.04 m, 2.45 m	1.49 m, 1.95 m	6.11 brs
9	2.50 brd (10.0)	2.33 brd (10.5)	2.75 brd (10.0)
11	7.23 dd (10.0, 16.0)	6.83 dd (10.0, 16.0)	7.00 dd (10.0, 16.0)
12	6.22 d (16.0)	6.30 d (16.0)	6.38 d (16.0)
14	7.25 brs	7.22 brs	7.26 s
15	4.71 brs	4.75 brs	4.72 brs
17a, b	4.82 d (2.0), 4.91 d (2.0)	2.58 d (4.5), 2.93 dd (4.5, 1.5)	4.26 brs
18	1.55 s	1.44 s	1.50 s
19	3.77 dd (3.0, 11.0), 4.38 dd (3.5, 11.0)	3.86 brd (11.0), 4.27 brd (11.0)	3.81 d (10.5), 4.56 d (10.5)
20	1.27 s	1.29 s	0.96 s

alternata AS 3.577, *A. alternata* AS 3.4578, *A. longipes* AS 3.2875, *Penicillium melinii* AS 3.4474, *P. janthinellum* AS 3.510, *Syncephalastrum racemosum* AS 3.264, *Trichoderma viride* AS 3.2942, *Rhizopus stolonifer* AS 3.3463, *R. stolonifer* AS 3.2050, *R. arrhizus* AS 3.2897, and *Curvularia lunata* AS 3.4381 were purchased from China General Microbiological Culture Collection Center in Beijing, China.

3.3 Culture medium

C. elegans AS 3.2028 was purchased from China General Microbiological Culture Collection Center in Beijing, China. All culture and biotransformation experiments using filamentous fungi were performed in potato medium, which was made with the following procedures: 200 g of peeled potatoes were boiled in water for 1 h, then the solution was filtered and the filtrate diluted with water to 1 l after addition of 20 g glucose. The culture medium was autoclaved at 121°C and 1.06 kg/cm^2 for 25 min [11].

3.4 Culture and biotransformation

Preparative-scale transformation of dehydroandrographolide by *C. elegans* AS 3.2028 was carried out in a 1000 ml Erlenmeyer flask. The flasks were placed on the rotary shakers, operating at 180 rpm at 28°C. After 36 h of preculture, the substrate (20 mg) in 1 ml acetone was added to 350 ml medium. In total, 500 mg of substrate were used. The incubation was continued under the above conditions for 6 additional days. The culture was filtered and the filtrate was extracted with the same volume of EtOAc for five times. The organic phase was collected and concentrated to dryness *in vacuo*.

The yellow extract (1.2 g) was applied to silica gel column and eluted with petroleum ether–acetone (in a gradient manner from 100:3 (v/v) to 1:1, at a flow rate of 1.5 ml/min). Fractions were monitored by HPLC and finally four transformed products including compounds **1** (51 mg, 10.2% yield), **2** (260 mg, 52.5% yield), **3** (6 mg, 1.2% yield), **4** (5 mg, 1% yield), and **5** (3.5 mg, 0.7% yield) were isolated.

3.4.1 3-oxo-2β-hydroxydehydroandrographolide (*3*)

Colorless solid (acetone); mp 153–154°C; $[\alpha]_D^{22}$ −15.6 ($c = 0.12$, MeOH); UV λ_{max} MeOH: 256 nm; IR (KBr) ν_{max} (cm^{-1}): 3380, 1741, 1637, 1100, and 889. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2.

Table 2. ^{13}C NMR spectral data of compounds **3**–**5** (pyridine-d_5, 125 MHz, δ in ppm).

C	3	4	5
1	49.9 t	39.3 t	39.0 t
2	71.2 d	36.0 t	28.6 t
3	214.4 s	213.6 s	80.4 d
4	55.4 s	54.9 s	42.7 s
5	56.9 d	55.9 d	50.7 d
6	24.2 t	22.6 t	23.7 t
7	36.5 t	35.9 t	122.3 d
8	148.3 s	58.3 s	138.1 s
9	61.3 d	58.9 d	58.4 d
10	39.4 s	39.4 s	36.3 s
11	134.8 d	131.0 d	136.5 d
12	122.7 d	125.0 d	122.5 d
13	128.6 s	128.6 s	128.9 s
14	145.6 d	145.7 d	144.7 d
15	70.2 t	70.2 t	70.2 t
16	172.7 s	172.6 s	172.8 s
17	110.1 t	50.7 t	64.2 t
18	20.8 q	21.5 q	23.4 q
19	65.5 t	65.4 t	64.9 t
20	16.7 q	15.5 q	16.1 q

HR-ESI-MS (*m*/*z*): 369.1677 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 369.1678).

*3.4.2 3-oxo-8β,17α-epoxydehydroandrographolide (**4**)*

Colorless solid (acetone); mp 152–153°C; $[\alpha]_D^{22}$ −23.6 ($c = 0.12$, MeOH); UV λ_{max} MeOH: 254 nm; IR (KBr) ν_{max} (cm^{-1}): 3400, 1710, 1665, and 908. ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; HR-ESI-MS (*m*/*z*): 369.1703 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 369.1678).

*3.4.3 3,19-dihydroxy-7,11,13-*ent*-labdatrien-15,16-olide (**5**)*

Colorless solid (acetone); mp 157–158°C, $[\alpha]_D^{22}$ −41.3 ($c = 0.12$, MeOH); UV λ_{max} MeOH: 259 nm; IR (KBr) ν_{max} (cm^{-1}): 3423, 1775, 1650, and 900. ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-ESI-MS (*m*/*z*): 371.1861 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_5Na$, 371.1834).

Acknowledgements

The authors thank Bureau of Science & Technology of Dalian City (2008J23JH042) and National Natural Science Foundation of China (No. 20576010) for financial support.

References

[1] L.X. Chen, F. Qiu, H.W. Liu, G.X. Qu, and X.Y. Yao, *Helv. Chim. Acta* **89**, 2654 (2006).
[2] R.J. Srinivasa, S.S. Genevieve, M. Charlie, S.H. Ahmad, H.L. Nordin, S.S. Mohammad, F.S. Malcolm, and S. Johnson, *Phytochemistry* **68**, 904 (2007).
[3] A. Puri, R. Saxena, R.P. Saxena, and K.C. Saxena, *J. Nat. Prod.* **56**, 995 (1993).
[4] X.F. Zhang and B.K. Tan, *Clin. Exp. Pharmacol. Physiol.* **27**, 358 (2000).
[5] G.F. Dai, H.W. Xu, J.F. Wang, F.W. Liu, and H.M. Liu, *Bioorg. Med. Chem. Lett.* **16**, 2710 (2006).
[6] H.F. Xu, G.F. Dai, G.Z. Liu, J.F. Wang, and H.M. Liu, *Bioorg. Med. Chem.* **15**, 4247 (2007).
[7] N. Srinivas, K.N. Vijay, S.R. Siva, K. Sridevi, and K.P. Mahesh, *Bioorg. Med. Chem. Lett.* **14**, 4711 (2004).
[8] R.J. Srinivasa, A.S. Hamzah, M.S. Sadd, N.H. Lajia, M.F.G. Stevens, and J. Stanslas, *J. Enzym. Inhib. Med. Chem.* **21**, 145 (2006).
[9] R.A. Kumar, K. Sridevi, N.V. Kumar, S. Nanduri, and S. Rajagopal, *J. Ethnopharmacol.* **92**, 291 (2004).
[10] X.C. Ma, J. Cui, J. Zheng, and D.A. Guo, *J. Mol. Catal. B: Enzymatic* **48**, 42 (2007).
[11] X.C. Ma, L.J. Wu, and D.A. Guo, *Enzyme Microb. Technol.* **38**, 367 (2006).