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Original Article

MICROBIAL METABOLISM OF AN ANTI-HIV AND ANTI-MALARIAL NATURAL PRODUCT ANDROGRAPHOLIDE

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ABSTRACT

Objective: Andrographolide (**1**), the main crystalline bitter principle of *Andrographis paniculata* nees. (also known as rice bitter in the West Indies) was first isolated by Gorter, and characterized as trihydroxy lactone. It was also isolated from *Holmskilodia sanguinea* in very good yield. It possesses a wide range of biological activities, which is also important in the therapeutic fields including anti-inflammatory, anti-malarial, anti-viral, immuno-stimulant, anti-HIV, and cardiovascular properties. In the present study, we first time studied the microbial metabolism of andrographolide (**1**) with *Cunninghamella elegans* (TSY 0865) and *Cephalosporium aphidicola* (IMI-68689).

Methods: Microbial cultures of the *C. elegans* and *C. aphidicola* were grown on Potato dextrose agar (PDA) at 25°C and stored at 4°C. Medium for *C. aphidicola* was prepared by mixing Glucose (50.0 g), KH_2PO_4 (1.0 g), $MgSO_4.7H_2O$ (2.0 g), Glycin (2.0 g), KCl (1.0 g) and *Gibberella trace* element solution (2.0 mL) into distilled water (1 L) and maintained pH at 5.6. While *C. elegans* medium was prepared by adding Glucose (10.0 g), peptone (5.0 g), KH_2PO_4 (5.0 g), yeast extract (5.0 g), NaCl (5.0 g) and glycerol (10 mL) into distilled water (1 L) and maintained pH at 5.6.

Results: Two compounds were obtained as transformed products. Based on physical and spectroscopic data, these have been identified as andropanolide (**2**) and 14-deoxy-11,12-didehydro andrographolide (**3**). Both compounds were previously obtained by the phytochemical investigation of *A. paniculata* and biotransformed product as well.

Conclusion: It could be concluded that *C. elegans* and *C. aphidicola* were able to produce oxidative derivatives of **1** in a regio- and stereoselective manner. Present investigation has been conducted for the first time with *C. elegans* and *C. aphidicola*. Incubation of **1** for 9 days with fungal strains yielded isomerized and oxidative products **2** and **3**. Structures of all metabolites were elucidated by using spectroscopic techniques.

Keywords: Microbial transformation, Andrographolide, Anti-HIV, *Andrographis paniculata*.

INTRODUCTION

Andrographolide (**1**) is an ideal substrate for biotransformation as it is readily isolated from plant extracts, having potential bioactivities. It is a key for the treatment value of plant *Andrographis paniculata*. The plant is commonly known as "Hempedu Bumi" (bile of the earth) and "pokok cerita" in Malaysia and it is also an established medicinal herb in China, India and Southeast Asia. Andrographolide (**1**) exhibits various pharmacological properties, such as anti-viral [1-2] anti-bacterial [3-5], cytotoxicity [6,7], hepatoprotective [8], immunostimulatory [9] and anti-inflammatory activities [10, 11].

According to a recent research study, microbial biotransformation is a unique and inexpensive resource of bioactive natural products [12-20]. The diversity of the possible reactions types in microbial transformation includes the process of oxidation, hydroxylation, esterification, isomerization, reduction, acetylation and hydrogenation [15-24]. Studies on the subject of microbial transformation of diverse compounds provide the foundation for roles of fungi in modifying the chemical structure [25-31]. Libraries of analogue compounds with unique structural modifications can be generated by microbial biotransformation stated [32-35]. Numerous microbial biotransformations of steroids and terpenes have been reported [13,14,25]. To the best of our knowledge, so far, only *Rhizopus stolonifer* and *Aspergillus ochraceus* have been confirmed to possess the ability to transform andrographolide [36, 37].

In the present study, we first time studied the microbial metabolism of **1** with *C. elegans* (TSY 0865) and *C. aphidicola* (IMI-68689). Two compounds (**2** and **3**) were obtained as transformed products. Based on physical and spectroscopic data, these have been identified as andropanolide (**2**) and 14-deoxy-11,12-didehydro andrographolide (**3**). Both compounds were previously obtained from the phytochemical investigation of *A. paniculata* [38,39] and from the biotransformation of andrographolide with *Rhizopus stolonifer* [37]. They also stated that the compound **2** was a result of hydration of the double bond at C-12(13), followed by dehydration which converts the configuration of the double bond to *Z*-form. Dehydration at C-14 in **1** resulted conjugated double bond producing compound **3**.

MATERIALS AND METHODS

General

Andrograholide (**1**) was purchased from sigma-aldrich (USA). Melting points were determined on a Yanaco MP-S3 apparatus. UV spectra were measured on a Shimadzu UV 240 spectrophotometer. JASCO DIP–360 Digital polarimeter was used to measure the optical rotations in chloroform by using 10 cm cell tube. FTIR-8900 Spectrophotometer was used to record IR spectra in $CHCl_3$.

The ^{1}H-NMR and 2D NMR spectra were recorded on a Bruker Avance III 400 spectrometer, while ^{13}C-NMR spectra were recorded on Bruker Avance III 400 spectrometer operating at 100 MHz using $CDCl_3$ as solvent. Chemical shifts were reported in δ (ppm), relative to $SiMe_4$ as internal standard, and coupling constants (J) were measured in Hz. The HREI MS were measured on Jeol HX 110 mass spectrometer. TLC was performed on Si gel precoated plates (PF_{254}, 20 × 20, 0.25 mm, Merck, Germany). Ceric sulphate in 10% H_2SO_4 spraying reagent was used for the staining of compounds on TLC. All reagents used were of analytical grades.

Fungi and culture conditions

Microbial cultures of the *C. elegans* (TSY 0865) was grown on Potato dextrose agar (PDA) at 25°C and stored at 4°C. *elegans* TSY 0865 medium was prepared by adding Glucose (10.0 g), peptone (5.0 g), KH_2PO_4 (5.0 g), yeast extract (5.0 g), NaCl (5.0 g) and glycerol (10 mL) into distilled water (1 L) and maintained pH at 5.6 [26].

Microbial cultures of the *C. aphidicola* (IMI-68689) were grown on Potato dextrose agar (PDA) at 25°C and stored at 4°C. Medium for *C. aphidicola* was prepared by mixing Glucose (50.0 g), KH_2PO_4 (1.0 g), $MgSO_4.7H_2O$ (2.0 g), Glycin (2.0 g), KCl (1.0 g) and *Gibberella trace* element solution (2.0 mL) into distilled water (1 L) and maintained pH at 5.6 [26].

General stage II fermentation and extraction procedure

Fermentation was carried out according to a standard stage II protocol [29]. Andrographolide (**1**) (600 mg) in acetone (15 mL) was evenly distributed among the 30 flasks (20 mg/0.5 mL in each flask), containing 24-h-old stage-II cultures. Fermentation was carried out for the further 9 days on a rotatory shaker (200 rpm) at 20° C. The culture media and mycelium were separated by filtration. The mycelium was washed with CH_2Cl_2 (2 L), and the filtrate was extracted with CH_2Cl_2 (12 L). The combined organic extracts were dried over anhydrous Na_2SO_4, evaporated under reduced pressure, and analyzed by TLC. The resulting brown gummy extracts (1.02, and 1.28 g for *C. elegans* and *C. .aphidicola*, respectively were purified by repeated column chromatorgaphy (petroleum ether/EtOAc gradients). From the *C. elegans* extracts, compound **2** (9.8 mg) was isolated. Similarly, from the *R. C. aphidicola* extracts, compound **3** (10.5 mg) was isolated.

Andropanolide (2): Amorphous solid (9.8 mg); $[\alpha][25]_{D:}$ -64° (*c* = 0.2, MeOH); **R_f:** 0.5 (Pet. Ether/EtOAc 80:20); EI-MS *m/z* (rel. int., %): *m/z* 350 [M^+] (6), 322 (17), 298 (56), 137 (100), 109 (78); HREI-MS (mol. formula, calcd value): *m/z* 350.1574 ($C_{20}H_{30}O_5$, 350.1583); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.51 (1H, t, $J_{(12,13)}$= 7.0 Hz, H-12), 4.82 (1H, brs, H_a-17), 4.72 (1H, d, $J_{(14,15)}$= 9.8 Hz, H-14), 4.52 (1H, brs, H_b-17), 4.41 (1H, dd $J_{(15(a,b))}$= 6.1 Hz, $J_{(15,14)}$= 9.9 Hz, H_a-15), 4.10 (1H, d, $J_{(19(a,b))}$= 10.5 Hz, H_a-19), 4.05 (1H, dd, $J_{(15(b,a))}$= 2.9 Hz, $J_{(15,14)}$= 9.9 Hz, H_b-15), 3.45 (1H, m, H-3), 3.42 (1H, d, $J_{(19(b,a))}$= 10.5 Hz, H_b-19), 1.20 (3H, s, Me-18), 0.73 (3H, s, Me-20);^{13}C-NMR ($CDCl_3$, 100 MHz) δ: see Table 1.

14-Deoxy-11, 12-didehydro andrographolide (3): Colorless crystalline solid (10.5 mg); $[\alpha][25]_{D:}$ +26° (*c* = 0.25, MeOH); **R_f:** 0.5 (Pet. Ether/EtOAc 85:15); M. .p.:- 203-204° C; EI-MS *m/z* (rel. int., %): *m/z* 332 [M^+] (8), 324 (10), 218 (5), 170 (49), 154 (80), 86 (73), 70 (100); HREI-MS (mol. formula, calcd value): *m/z* 332.1530 ($C_{15}H_{28}O_4$, 332.1553); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.02 (1H, d, $J_{(14,15)}$= 9.0 Hz, H-14), 6.85 (1H, dd, $J_{(11,12)}$= 16.2 Hz, $J_{(11,9)}$= 9.9 Hz, H-11), 6.15 (1H, d, $J_{(12,11)}$= 16.1 Hz, H-12), 4.80 (1H, d, $J_{(15,14)}$= 9.0 Hz, H-15a), 4.66 (1H, brs, H-17a), 4.49 (1H, brs, H-17b), 4.12 (1H, d $J_{(19(a-b))}$= 11.0 Hz, H-19a), 3.53 (1H, m, H-3), 3.37 (1H, d, $J_{(19(b-a))}$= 11.0 Hz, H-19b), 1.22 (3H, s, Me-18), 0.85 (3H, s, Me-20); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: see Table 1.

RESULTS AND DISCUSSION

Fermentation of andrographolide (**1**) ($C_{20}H_{30}O_5$) by *C. elegans* afforded metabolite **2**, which was identified as andropanolide. It was obtained as colorless needles, with an M^+ in HREI-MS at *m/z* 350.1574, corresponding to the formula $C_{20}H_{30}O_5$ (calcd350.1583). The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to the substrate **1**. Comparison between the two compounds revealed the downfield shifts of C-12 (δ 151.2) and C-14 (δ 69.9) in the ^{13}C-NMR spectrum, which can be rationalized on the basis of the absence of a γ-effect from H-11. The geometry of the double bond may be reversed in metabolite **2**, as compared to **1**. The β-configuration of C-14 was assigned on the basis of NOESY correlations between H-14 (δ 4.72) and H-12 (δ 6.51), and by comparison with the reported data [36,37].

Incubation of compound **1** with *C. aphidicola* for nine days afforded 14-deoxy-11,12-didehydroandrographolide (**3**) as a colorless solid. The HREI-MS of metabolite **3** exhibited an M^+ at *m/z* 332.1530 ($C_{20}H_{28}O_4$, calcd 332.1553). The ^{1}H-NMR spectrum of **3** was found to be substantially different from the substrate **1**, especially in two aspects. First the appearance of three downfield olefinic proton signals at δ 7.02 (brd), 6.85 (dd, *J* = 16.2 Hz, *J* = 9.9 Hz), and 6.15 (d, *J* = 16.1 Hz), and secondly the disappearance of C-14 hydroxy-bearing methine signal.

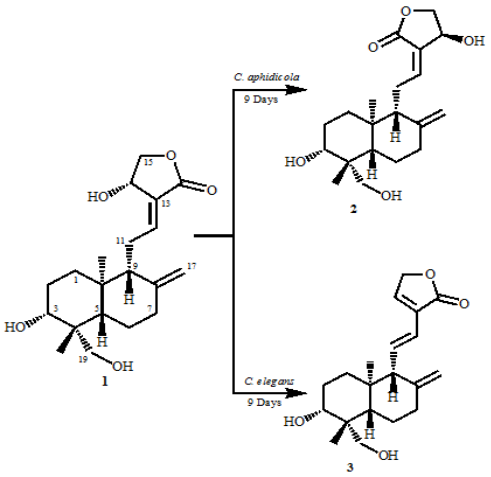
Table 1: The ^{13}C-NMR (100 MHz) chemical shift assignments of 2 and 3 in $CDCl_3$

Carbon No.	2		3	
	δ_C	Multiplicity	δ_C	Multiplicity
1	38.1	CH_2	39.5	CH_2
2	29.0	CH_2	33.1	CH_2
3	81.0	CH	81.2	CH
4	43.7	C	43.8	C
5	56.5	CH	55.8	CH
6	25.3	CH_2	23.8	CH_2
7	39.1	CH_2	37.8	CH_2
8	149.1	C	150.1	C
9	57.9	CH	62.9	CH
10	40.1	C	39.4	C
11	24.8	CH_2	136.5	CH
12	151.2	CH	122.5	CH
13	129.5	C	130.9	C
14	69.9	CH	146.8	CH
15	75.0	CH_2	71.7	CH_2
16	171.6	CH_2	174.8	C
17	108.6	CH_2	109.1	CH_2
18	65.0	CH_2	65.0	CH_2
19	23.4	CH_3	16.3	CH_3
20	15.7	CH_3	14.5	CH_3

The ^{13}C-NMR spectrum (Broad-band Decoupled and DEPT) (Table-1) of **3** showed downfield olefinic carbon signals resonated at δ 136.5, 146.8 and 122.5, were assigned to C-11, C-14 and C-12, respectively, while downfield methine signals at δ 71.7 and 109.1 were assigned OH at C-15 and C-17.

The migration of double bond from C-12/C-13 to C-11/C-12 was inferred from an additional olefinic proton signal at δ 6.85 and by comparison with the reported data [36,37].

The metabolite **3** was identified as 14-deoxy-11,12-didehydro andrographolide.



Scheme 1: Fermentation of andrographolide (1) by *C. elegans* and *C. aphidicola*.

CONCLUSION

In summary, present research on the biotransformation of **1** by *C. elegans* and *C. aphidicola* have been conducted for the first time. Fermenation of **1** for 9 days with fungal strains yielded isomerized and dehydrated products, **2** and **3**. Structures of all metabolites were elucidated by using spectroscopic techniques.

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Uttekar MM, Das T, Pawar RS, Bhandari B, Menon V, Nutan Bhat SV. Anti-HIV activity of semisynthetic derivatives of andrographolide and computational study of HIV-1 gp120 protein binding. Eur J Med Chem 2012;56:368–74.
2. Wang B, Li J, Huang WL, Zhang HB, Qian H, Zheng YT. Synthesis and biological evaluation of andrographolide derivatives as potent anti-HIV agents. Chinese Chem Lett 2011;22:781–4.
3. Arifullah M, Namsa ND, Mandal M, Chiruvella KK, Vikrama P, Gopal GR, *et al*. Evaluation of anti-bacterial and anti-oxidant potential of andrographolide and echiodinin isolated from callus culture of Andrographis paniculata Nees. Asian Pac J Trop Biomed 2013;3(8):604–10.
4. Aromdee C, Sriubolmas N, Wiyakrutta S, Suebsasna S, Khunkitti W. Effect of the derivatives of andrographolide on the morphology of Bacillus subtilis. Arch Pharmcal Res 2011;34(1):71–7.
5. Murugan K, Selvanayaki K, Al-Sohaibani S. Antibiofilm activity of Andrographis paniculata against cystic fibrosis clinical isolate Pseudomonas aeruginosa. World J Microb Biot 2010;27(7):1661–68.
6. Sharifuddin Y, Parry EM, Parry JM. The genotoxicity and cytotoxicity assessments of andrographolide *in vitro*. Food Chem Toxicol 2012;50(5):1393–8.
7. Sirion U, Kasemsook S, Suksen K, Piyachaturawat P, Suksamrarn A, Saeeng R. New substituted C-19-andrographolide analogues with potent cytotoxic activities. Bioorg Med Chem Lett 2012;22(1):49–52.
8. Singha PK, Roy S, Dey S. Protective activity of andrographolide and arabinogalactan proteins from Andrographis paniculata Nees. against ethanol-induced toxicity in mice. J Ethnopharmacol 2007;111(1):13–21.
9. Chang KT, Lii CK, Tsai CW, Yang AJ, Chen HW. Modulation of the expression of the pi class of glutathione S-transferase by Andrographis paniculata extracts and andrographolide. Food Chem Toxicol 2008;46(3):1079–88.
10. Hidalgo MA, Hancke JL, Bertoglio JC, Burgos RA. Andrographolide a new potential drug for the long term treatment of rheumatoid arthritis disease. Innovative Rheumatol 2013;1:246-70.
11. Deng S, Zhang BJ, Wang CY, Tian Y, Yao JH, An L, *et al*. Microbial transformation of deoxyandrographolide and their inhibitory activity on LPS-induced NO production in RAW 264.7 macrophages. Bioorg Med Chem Lett 2012;22(4):1615–8.
12. Sultan S, Zaimi M, Anouar EH, Shah SAA, Salim F, Rahim R, *et al*. Absolute configuration of 20β-hydroxyprednisolone, a biotransformed product of an Anti-Inflammatory drug prednisolone by marine endophytic fungus Penicilium lapidosum. Mol 2014;19(9):13775-87.
13. Choudhary MI, Atif M, Shah SAA, Sultan S, Erum S, Khan SN, *et al*. Biotransformation of dehydroabietic acid with microbial cell cultures and α-glucosidase inhibitory activity of resulting metabolites. Int J Pharm Pharm Sci 2014;6:375-8.
14. Sultan S, Choudhary MI, Khan SN, Fatima U, Atif M, *et al*. Fungal transformation of cedryl acetate and α-glucosidase inhibition assay, quantum mechanical calculations and molecular docking studies of its metabolites. Eur J Med Chem 2013;62:764–70.
15. Choudhary MI, Sultan S, Jalil S, Anjum S, Rahman AA, Fun HK. Microbial transformation of mesterolone. Chem Biodivers 2005;2:392-400.
16. Choudhary MI, Sultan S, Khan MTH, Atta-ur-Rahman. Microbial transformation of 17α-ethynyl-and 17α-ethylsteroids, and tyrosinase inhibitory activity of transformed products. Steroids 2005;70(12):798-802.
17. Choudhary MI, Sultan S, Yaqoob M, Musharraf SG, Yasin A, Shaheen F, *et al*. Microbial transformation of cortisol and prolyl endopeptidase inhibitory activity of its transformed products. Nat Prod Res 2003;17:389-95.
18. Choudhary MI, Sultan S, Hassan Khan MT, Yasin A, Shaheen F, Atta-ur-Rahman. Biotransformation of (+)-androst-4-ene-3, 17-dione. Nat Prod Res 2004;18(6):529-35.
19. Musharraf SG, Atta-ur-Rahman, Choudhary MI, Sultan S. Microbial transformation of (+)-adrenosterone. Nat Prod Lett 2002;16(5):345-9.
20. Sultan S, Ghani NA, Shah SAA, Ismail NH, Noor MZ, Naz H. Microbial transformation of anthraquinones-A Review. Biosci Biotechnol Res Asia 2013;10(2):577-82.
21. Shah SAA, Sultan S, Zaimi M. Biotransformation of tissue-specific hormone tibolone with fungal culture Trichothecium roseum. J Mol Struct 2013;1042:118-22.
22. Shah SAA, Sultan S, Adnan HS. A whole-cell biocatalysis application of steroidal drugs. Orient J Chem 2013;29(2):389-403.
23. Shah SAA, Sultan S, Adnan HS. Solid phase microbial transformation of cortexolone and prolyl endopeptidase inhibitory activity of the transformed products. Int J Pharm Pharm Sci 2011;3 Suppl 1: 1-6.
24. Casañola-Martín GM, Marrero-Ponce Y, Khan MTH, Ather A, Sultan S, Torrens F, *et al*. TOMOCOMD-CARDD descriptors-based virtual screening of tyrosinase inhibitors: evaluation of different classification model combinations using bond-based linear indices. Bioorg Med Chem 2007;15(3):1483-503.
25. Shah SAA, Tan HL, Sultan S, Faridz MABM, Shah MABM, Nurfazilah S, *et al*. Microbial-catalyzed biotransformation of multifunctional triterpenoids derived from phytonutrients. Int J Mol Sci 2014;15(7):12027-60.
26. Shah SAA, Sultan S, Hassan NB, Muhammad FKB, Faridz MABM, HussainFBM, *et al*. Biotransformation of 17α-ethynyl substituted steroidal drugs with microbial and plant cell cultures: a review. Steroids 2013;78(14):1312-24.
27. Azam SS, Reaz Uddin, Shah SAA, Zaheer-ul-Haq. Molecular docking studies of potent inhibitors of tyrosinase and α-glucosidase. Med Chem Res 2012;21(8):1677-83.
28. Choudhary MI, Shah SAA, Atta-ur-Rahman, Khan SN, Khan MTH. Alpha-glucosidase and tyrosinase inhibitors from fungal hydroxylation of tibolone. Steroids 2010;75(12):956-66.

29. Choudhary MI Shah SAA, Atta-ur-Rahman. Microbial transformation of anabolic steroids. Nat Prod Res 2008;22(15):1289–96.
30. Choudhary MI, Batool I, Shah SAA, Khan SN, Atta-ur-Rahman. Microbial transformation of oleanolic acid by Fusarium lini and □-glucosidase inhibitory activity of its transformed products. Nat Prod Res 2008;22(6):489-94.
31. Atta-ur-Rahman, Choudhary MI, Basha FZ, Abbas G, Khan SN, Shah SAA. Science at the interface of chemistry and biology: Discoveries of α-glucosidase inhibitors and antiglycation agents. Pure Appl Chem 2007;79(12):2263-8.
32. Choudhary MI, Yousuf S, Samreen, Shah SAA, Ahmed S, Atta-ur-Rahman. Biotransformation of physalin h and antileishmanial activity of transformed product. Chem Pharm Bull 2006;54(7):927-30.
33. Choudhary MI, Shah SAA, Sami A, Ajaz A, Shaheen F, Atta-ur-Rahman. Fungal metabolites of E-Guggulsterone and their antibacterial and antioxidant activities. Chem Biodivers 2005;2:516-24.
34. Choudhary MI, Batool I, Shah SAA, Nawaz SA, Atta-ur-Rahman. Microbial hydroxylation of pregnenolone derivative and cholinesterase inhibitory activity. Chem Pharma Bull 2005;53;1455-9.
35. Choudhary MI, Shah SAA, Musharraf SG, Shaheen F, Atta-ur-Rahman. Microbial trabsformation of dehydroepiandrosterone. Nat Prod Res 2003;17(3):215-20.
36. He X, Wang Y, Hu H, Wu Y, Zeng X. Novel bioconversion products of andrographolide by Aspergillus ochraceus and their cytotoxic activities against human tumor cell lines. J Mol Catal B Enzym 2011;68(1):89–93.
37. He X, Zeng X, Hu H, Wu Y. Cytotoxic biotransformed products from andrographolide by Rhizopus stolonifer ATCC 12939. J Mol Catal B Enzym 2010;62(3-4):242–7.
38. Matsuda T, Kuroyanagi M, Sugiyama S, Umehara K, Ueno A, Nishi K. Cell differentiation-inducing diterpenoids from Andrographis paniculata Nees. Chem Pharm Bull 1994;42(6):1216-25.
39. Pramanick S, Banerjee S, Achari B, Das B, Sibabrata AKS, Mukhopadhayay S, Neuman A, *et al*. Andropanolide and Isoandrographolide, Minor Diterpenoids from Andrographis paniculata: Structure and X-ray Crytallography Analysis. J Nat Prod 2006;69(3):403-5.