ISSN : 0974 - 7435 Volume 11 Issue 4

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(4), 2015 [133-137]

Biotransformation of asiaticoside and asiatic acid by *Nocardia sp* NRRL-5646

Xiao-Yun Zhang[1]* Ye-Wang Zhang[2]

[1]School of Food and Biological Engineering, Zhenjiang, 212013, People's Republic of China, (CHINA)

[2]School of Pharmacy, Jiangsu University, Zhenjiang, 212013, People's Republic of China, (CHINA)

E-mail : zhangxiaoyungu@ujs.edu.cn

ABSTRACT

Asiaticoside and asiatic acid are important active components of *Centella asiatica*. Transformation of asiaticoside and asiatic acid by *Nocardia sp* NRRL -5646 were investigated. The results indicated that asiaticoside could be removed glycoside and transformed to asatic acid or its isomer-arjulonic acid and asiatic acid sulfate by *Nocardia sp* NRRL -5646. The transformation results of asiatic acid by *Nocardia sp* NRRL -5646 showed that asiatic acid could be converted to asiatic acid sulfate.

KEYWORDS

Asiaticoside;
Asiatic acid;
Nocardia sp NRRL -5646;
Transformation;
Asiatic acid sulfate.

INTRODUCTION

Centella asiatica is an important medicinal herb. It has the pharmacological effects of wounds healing[1], mental disorders[2], atherosclerosis, fungicidal, antibacterial[3], antioxidant and anticancer[4,5]. Asiaticoside and asiatic acid are important active components of *Centella asiatica*[6]. The bio-availability in the digestive tract of asiaticosides after oral is lower because of large polarity, and the corresponding aglycone forms decomposed by intestinal bacteria are absorbed into the bloodstream to play its activity[7]. So asiaticosides is not suit for administration generally. Astic acid in *Centella asiatica* is much less than asiaticoside[8]. Preparation of asiatic acid and its derivatives from *Centella asiatica* is uneconomic and ineffective. While chemical method had some limitations for its complicated structure. Microbial transformation is environmentally friendly and significant in structural modification of natural products with stero-selectivities. Currently, microbial transformation of asaticoside and asiatic acid is very little reported at home and abroad[9-11]. In this work, asiaticoside and asiatic acid was transformed by *Nocardia sp* NRRL-5646 because of its broad array of useful enzymes and many of enzymes similar to those found in mammalians[12]. The transformation products of asiaticoside and Asiatic acid were investigated. The results were also of auxiliary significance for the metabolism of asiaticoside and asiatic acid in vivo.

MATERIALS AND METHODS

Chemicals

The substrates of asiaticoside (≥90%, assayed by HPLC) and asiatic acid (≥95%, assayed by HPLC) were purchased from guangxi changzhou natural

FULL PAPER

pharmaceutical co., ltd. (nanning, china). The substrates solution were prepared as 10 mg/ml (substrate/methanol). Methanol and acetonitrile (hplc-grade) and other chemicals (analytical grade) were purchased from sinopharm chemical reagent co.ltd. (shanghai, china).

Microorganism and transformation conditions

Nocardia sp. NRRL-5646 was obtained from Prof. J.P.N. Rosazza, University of Iowa. A 3% inoculum was inoculated to PDA medium, which were incubated for 48 h at 28ºC before addition 20 mg of asiaticoside or asiatic acid (prepared with ethanol as 10 mg/mL) per 30 ml cultures. Asiaticoside were transformed at 28ºC and asiatic acid were transformed at 28ºC or 25ºC for 72h, and then the cultures were collected to extract transformation products. Simultaneously, *Nocardia sp.* NRRL-5646 cultured at the same conditions without substrates was as the control.

Extraction of transformation products

The cultures were centrifugated at 8000 rpm for 15 min and the supernatant was extracted with butanol (asiaticoside as substrate) or ethyl acetate (asiatic acid as substrate) for three times. The organic layers from all extractions were pooled and evaporated to dryness in a vacuum. The resulting residue was reconstituted in 5 mL of methanol, and then filtrated to be subjected to LC-MS/MS separation and identification.

Separation and identification of the main transformation products by LC-MS/MS

The products were separated and identified by a Thermo Finnigan LXQ linear ion trap mass spectrometer, which was equipped with a Finnigan surveyor LC pump, a DAD detector, an electrospray ionization (ESI) source, triple quadrupole Quattro and a linear ion trap LXQ. Data acquisition was performed with Xcalibur 2.0 software (Thermo Finnigan).

The separation was performed on an ODS Hypersil C_{18} column (4. 6 mm×150mm, i.d., 5 μm; Thermo Corporation, USA). The mobile phase consisted of acetonitrile(A) and water(B). A gradient elution was used by linearly increasing A as follows: 0 min 5% A; 40 min 100% A; 50 min 5% A. The flow rate was 0.8 mL/min and the injection volume was 15 μL. The column temperature was maintained at 30 °C and wavelength detection was 205 nm.

Electrospray ionization (ESI) was performed in positive ion(+) or negative ion(-) detection mode. Typical ESI conditions were as follows: nitrogen drying gas and sheath gas flow were set as 5arb(+) and 35 rab(-) respectively; the voltage of electrosprary ionization were 4.5 kV(+) and 3.5 kV(-); capillary voltage were -30v(+) and 30v(-); the voltage of lens was -120v(+) and 120v(-); heated capillary temperature 300ºC; collision-induced decomposition voltage was 35v; m/z scaning range was from 350 to1200.

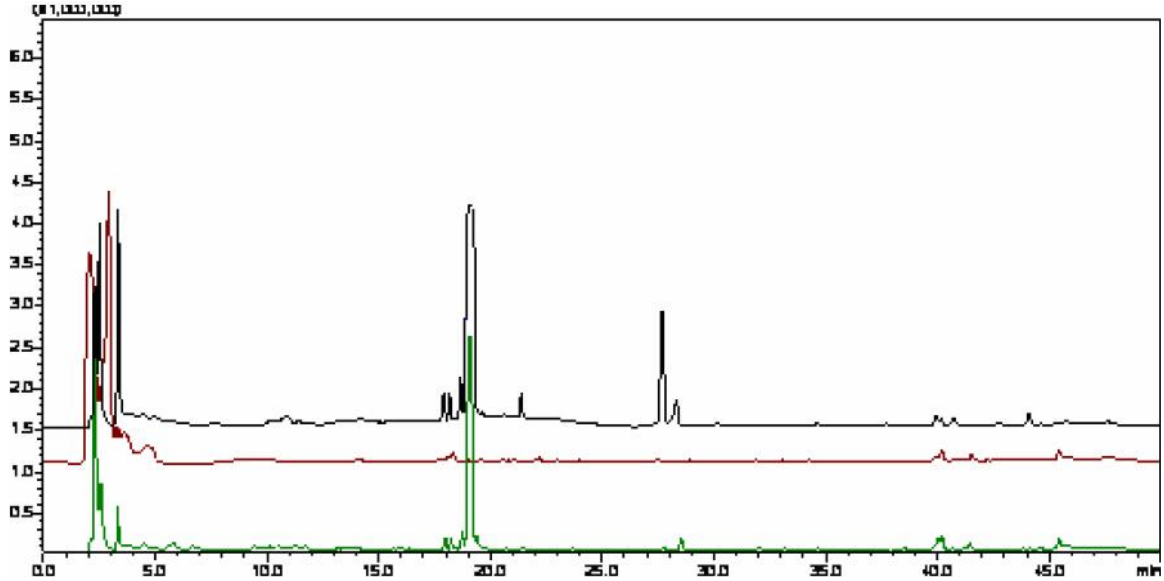

Figure 1 : LC spectra of the main products of asaticoside. From the bottom up as follows: asaticoside in 50% ethanol, the control, asaticoside transformed by *Nocardia sp* NRRL-5646 at 28°C

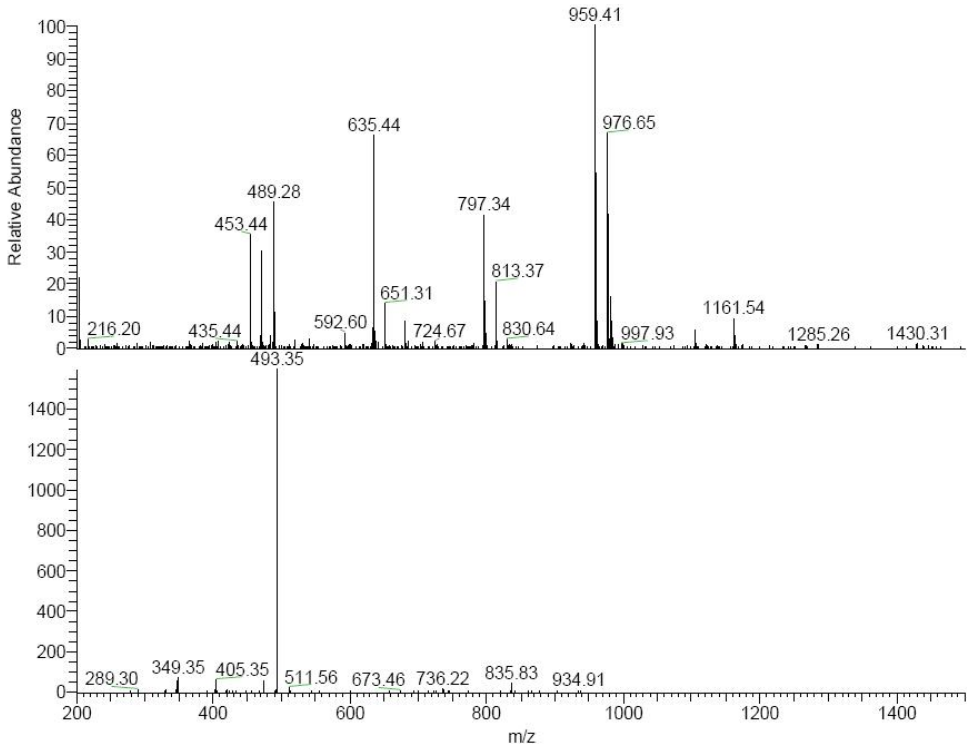

Figure 2 : Mass spectra of AS in the positive ion mode

Conversion rate of the substrate

The standard substrate solution were analyzed by HPLC and calibration curve were made as peak area and contration. Residual substrate were counted as the calibration curve above.

Conversion rate of asiaticoside or asiatic acid was caculated as the formula followed:

Conversion rate =

$$\frac{asiati\cos ide\,(asiatic\ acid)\ added - residual\ asiati\cos ide\,(asiatic\ acid)}{asiati\cos ide\,(asiatic\ acid)\ added} \times 100\%$$

RESULTS AND DISCUSSION

Sepration and identification of the main transformation products of asiaticoside

LC spectra of the extracts obtained from the biotransformation of asiaticoside presented well-resolved peaks, which indicated the formation of two main products(AS_1 and AS_2) in the presence of the substrate(AS) (Figure 1). Retention times for asiaticoside and its transformation products were 19.10 min(AS), 27.68 min(AS_2) and 28.54(AS_1) min, respectively. But the conversions rate of asiaticoside was only 40.10 %.

The mass spectra for peak AS showed signals for the molecular ions $[M+H]^+$ at m/z 959.41 and $[M+Na]^+$ at 981.87 respectively. It was proposed that the main fragment ion $[M+Na]^+$ at 493.34 (Figure 2) was glycoside fragmented from AS. The results also indicated that asiaticoside (AS) was residual.

The mass ions $[M-H]^-$ at 487.48 and mass ions $[2M-H]^-$ at 975.63 for peak AS_1 and its fragmentation pattern (Figure 3) indicated the presence of asiatic acid in transformation products. As Zhang J[13] and Cheng Z H[14], ursolic acid was converted to oleanolic acid which are formed by participation of retro-biosynthetic methyl migration by *Nocardia sp.* NRRL 5646. If the methyl migration happened to asiatic acid in this study, AS_1 was probably arjulonic acid—the isomer of asiatic acid. It was diffcult to identified asiatic acid and arjulonic acid by the results of LC-MS/MS. So AS_1 was asiatic acid or arjulonic acid.

As Figure 4, the mass ions $[M-H]^-$ at 567.15 for peak AS_2 and its fragmentation pattern conformed to asiatic acid sulfate. These results indicated that asiaticoside was transformed to asiatic acid and asiatic acid sulfate by *Nocardia sp.* NRRL-5646. It was consistent with the study of Weng J and[15] and Chasseaud L F[16]. They proposed that asiaticoside was metabolized to asiatic acid, and then asiatic acid was mainly

FULL PAPER

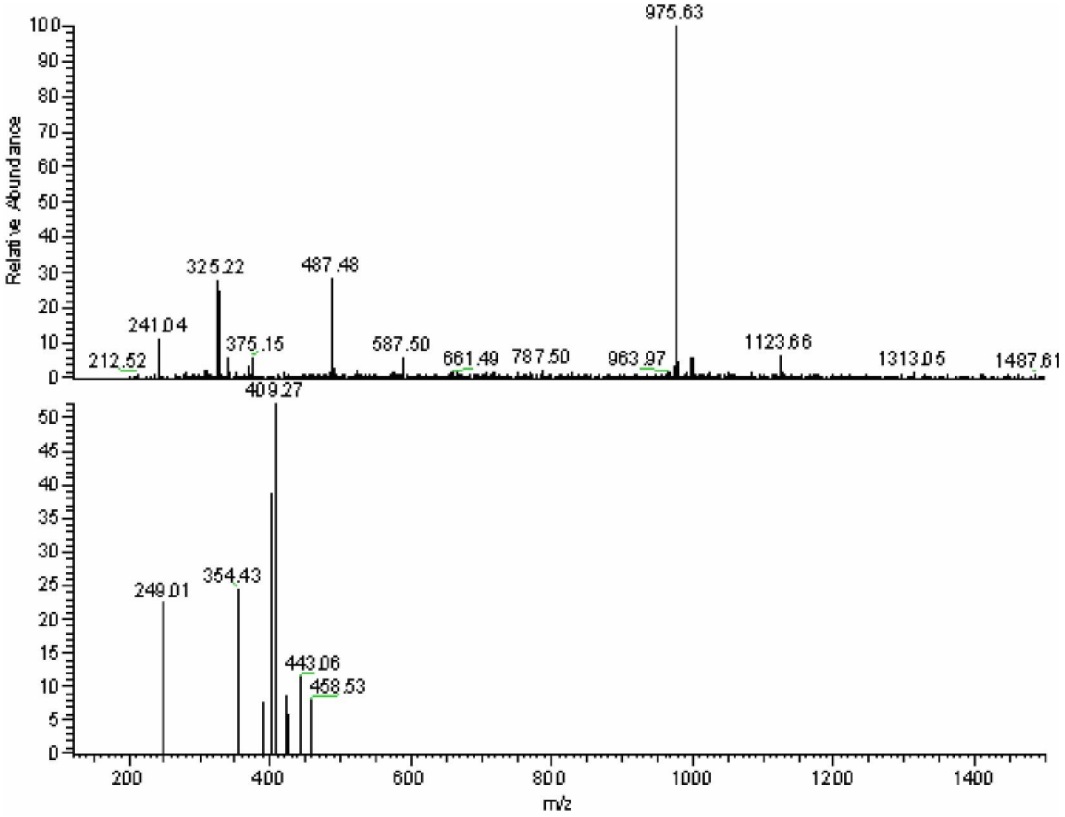

Figure 3 : Mass spectra of AS1 in the negative ion mode

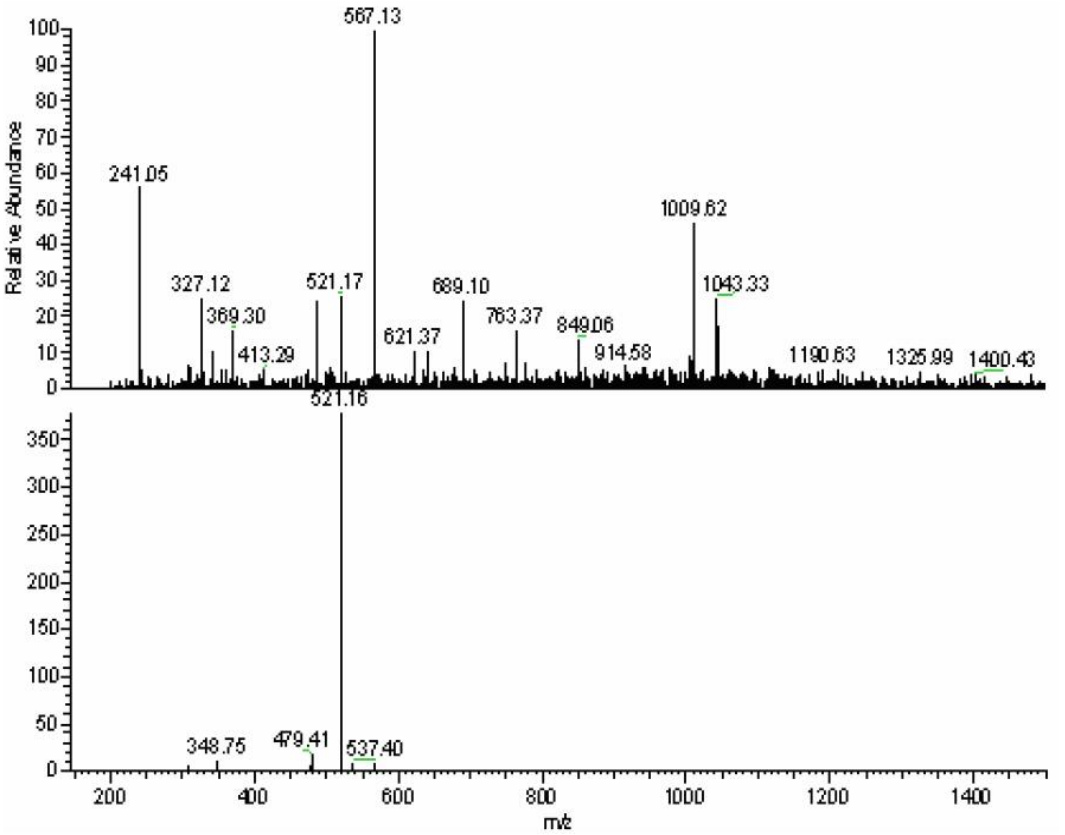

Figure 4 : Mass spectra of AS2 in the negative ion mode

converted to asiatic acid sulfate and glucuronic acid conjugates by intestinal flora of rat.

Sepration and identification of the main transformation products of asiatic acid

Fifure 5 showed that the main product of asiatic acid (AC) transformed by *Nocardia sp*. NRRL-5646 at 28°C was only AC_1, and the conversion rate was 100%. While asiatic acid was mainly transformed to AC_1 (27.90 min) and AC_2(22.70 min) at 25°C, and the conversion rate was 93.12%.

The molecular ion for AC and its fragmentation pattern was as same as that for AS_1 (Figure 3). This indicated that asiatic acid was not transformed completely or it was transformed to arjulonic acid[13,14] by *Nocardia sp* NRRL-5646 at 25°C.

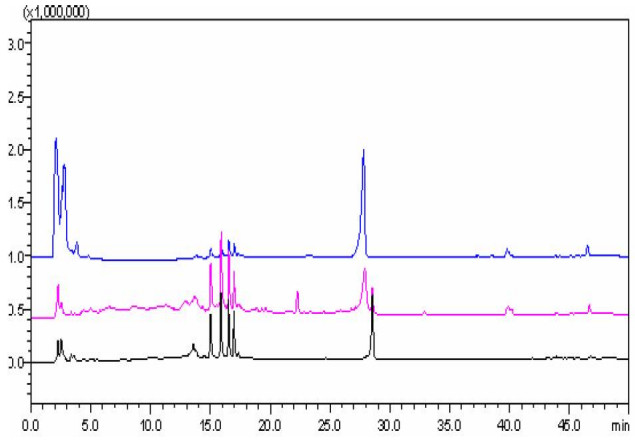

Figure 5 : LC spectra of the main products of asatic acid. From the bottom up as follows: asatic acid in ethanol, asatic acid transformed by *Nocardia sp* NRRL-5646 at 25°C and 28°C

The molecular ion for AC_1 and its fragmentation pattern was same as that for AS_2 (Figure 4). It also indicates that this main transformation product of asiatic acid was asiatic acid sulfate. This result is consistent with that asiatic acid from asiaticoside by removal of glycoside could be transformed to asiatic acid sulfate (AS_2).

CONCLUSIONS

The transformation results indicated that asiaticoside could be removed glycoside and mainly transformed to asiatic acid sulfate by *Nocardia sp* NRRL -5646. The products also include asatic acid or its isomer- arjulonic acid. And asiatic acid could be converted to asiatic acid sulfate. These results were consistent with the metabolism of asaticoside and asatic acid by intestinal flora of rat[15,16]. The results provide a method by which Asiatic acid and its derivatives were prepared. It was also of significance for auxiliary study of the metabolism of asiaticoside analogues in vivo.

ACKNOWLEDGEMENT

This research was financially supported by National Science Foundation of China (No. 21376110) and Jiangsu University (08JDG004).

REFERENCES

[1] L.Suguna, P.Sivakumar, G.Chandrakasan; Indian J.Exp.Biol., **34**, 1208 **(1996)**.
[2] M.V.R.Apparao Rao, K.Srinivasan, K.T.Rao et al; J.Res.Indian Med., **8**, 9 **(1973)**.
[3] O.A.Oyedeji, A.J.Afolayan; Pharm.Biol., **43**, 249 **(2005)**.
[4] G.Jayashree, M.G.Kurup, V.S.Sudarslal *et al*; Fitoterapia, **74**, 431 **(2003)**.
[5] T.D.Babu, G.Kuttan, J.Padikkala; J.Ethnopharmacol., **48**, 53 **(1995)**.
[6] M.F.He, Z.S.Meng, L.Q.Wo; J.China pharmaceutical university, **31**, 91 **(2000)**.
[7] J.Weng, Q.J.Lu, Y.Tian *et al*; Chinese traditional and herbal drugs, **37**, 1008 **(2006)**.
[8] J.G.Zeng, Y.F.Song; Primary Journal of Chinese material medica, **14**, 7 **(2000)**.
[9] W.N.He, J.G.Dai, M.Ye *et al*; J.Asian.Nat.Prod.Res., **12(9)**, 760-764 **(2010)**.
[10] F.F.Guo, X.Feng, Z.Y.Chu et al; J.Asian.Nat.Prod.Res., **15**, 15 **(2013)**.
[11] P.Du; The screening of asiaticosides microbial transformation strain and the purification, identification of transformation products, Master's Thesis, Shanghai normal university, Shanghai, **(2010)**.
[12] J.P.Rosazza; Novel derivatives of carnosic acid[P], US 20040014808.
[13] J.Zhang, Z.H.Cheng, B.Y.Yu et al.; Tetrahedron Lett., **46**, 2337 **(2005)**.
[14] Z.H.Cheng, B.Y.Yu, G.A.Cordell et al; Organic Lett., **6**, 3163 **(2004)**.
[15] T.Li, J.P.Rosazza; Appl.Environ.Microbiol, **66**, 684 **(2000)**.
[16] Y.J.Chen, J.P.Rosazza; Appl.Environ.Microbiol., **60**, 1292 **(1994)**.