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Biopreparation of an anti-inflammatory agent, diarctigenin, from arctiin isolated from *Arctium lappa* by *Rhizoctonia solani* AG-4

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ABSTRACT

In the preliminary screening for the plant-derived pesticides against *Rhizoctonia solani* Kühn AG-4 (RS AG-4), the indicator compounds arctiin (**1**) and arctigenin (**2**) in methanol extracts of *Arctium lappa* L. were consumed and transformed to other compounds. Thus, in the present study RS AG-4 was used as a biocatalyst and the biotransformation of arctiin (**1**) was investigated. Conversion of arctiin (**1**) to arctigenin (**2**) was achieved by the enzymatic hydrolysis of sugar moiety. In addition, an anti-inflammatory lignan dimer reported from the *Arctium* species, diarctigenin (**3**) was afforded in good yields. The HPLC monitoring of the biotransformation process indicated the possible mechanism. It would be an excellent method to produce a large scale of diarctigenin (**3**) for the successive medicinal examinations.

Small organic molecules purified from natural sources provide an incomparable source of inspiration for advances in organic chemistry and disease treatment due to their significant biological properties.[1–7] Although some compounds can be easily synthesized by chemical ways, some issues still remain, including complex operation, safety, pollution, and production cost. Biopreparation has been considered to be an economical technology by synthetic organic chemists in the development of new production routes for chemical and pharmaceutical compounds. As compared with chemical synthesis, biotransformation is a useful method to produce bioactive compounds with advantages such as high stereo- and regio-selectivity, as well as milder reaction conditions, simple operation procedures, and environmental safety.[8–10]

Arctium lappa L. is a perennial herb that has been cultivated as a vegetable in many countries for a long time. The seeds of *A. lappa* L. are extensively used in traditional medicines as diuretic, anti-inflammatory, and detoxifying agents. The *Arctium* genus is a plentiful source for the dibenzylbutyrolactone lignans, which are famous for their anti-proliferative and apoptotic effects. In the previous literature, a dimeric lignan, diarctigenin (**3**), was reported with significant inhibitory activity on nitric oxide (NO) production in LPS-activated mouse peritoneal macrophages.[11,12] Inflammation is related to morbidity and mortality of many diseases and is recognized as part of the complex biological response of vascular tissues to harmful stimuli. It is the host response to infection or injury, which involves the recruitment of leukocytes and the release of inflammatory mediators, including NO. Sustained NO release by inducible nitric oxide synthase (*i*NOS) and prostaglandin E2 (PGE2) production by cyclooxy-genase 2 (COX2) have been implicated as mediators of inflammation and are induced by bacterial lipopolysaccharide (LPS) or immunological stimuli. It has been reported that excess production of NO and PGE2 by macrophages and other cells exposed to endotoxins may contribute to septic shock, cerebral injury, myocardical ischemia, diabetes, arteriosclerosis, and other local or systemic inflammatory disorders.[13–16] Thus, inhibition of NO synthesis and PGE2 production stands as an important therapeutic goal. Due to the low cytotoxicity of diarctigenin,[12] it could be a good candidate for the treatment of various anti-inflammatory diseases. However, the natural abundance of this dimeric lignan is very limited for the successive bioactivity examinations.

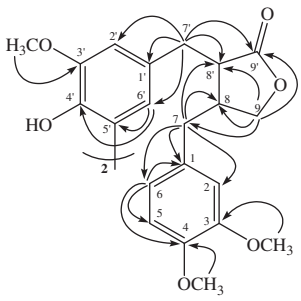

Figure 1. Chemical structure of diarctigenin (**3**) and significant HMBC (→) correlations.

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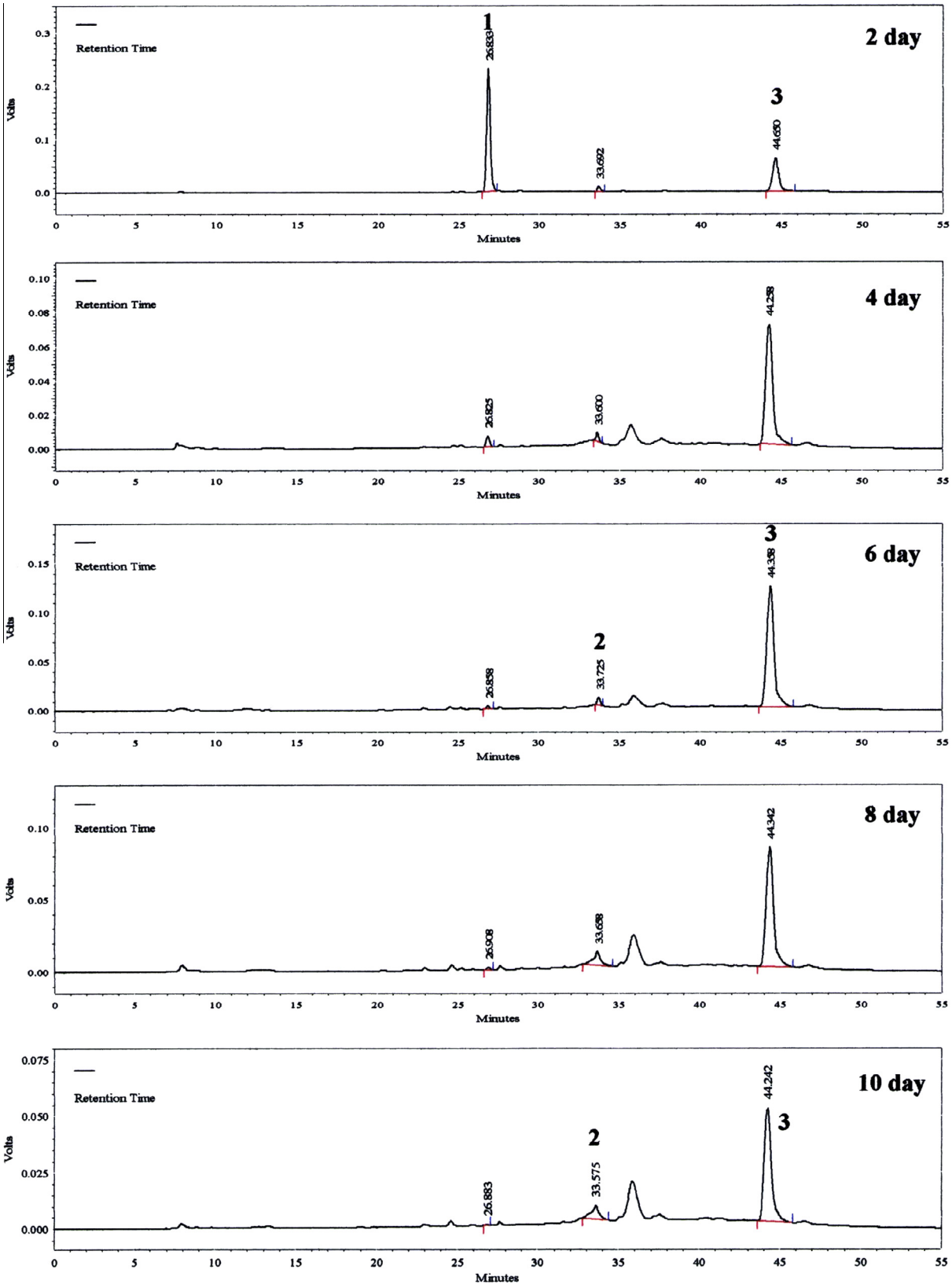

Figure 2. The HPLC chromatograms of the growth culture of RS AG-4 with arctiin (**1**) served as the starting substrate in the incubation period.

In addition, there was also no synthetic report regarding this compound until now.

Rhizoctonia solani Kühn caused serious damping-off diseases of numerous crops in Taiwan, especially it is a major problem for the commercial production of vegetable seedlings grown in cell-plug systems.[17,18] In our labs, we continued to focus on the discovery for the plant-derived pesticides against *R. solani* Kühn AG-4 (RS AG-4).[19] Among the tested extracts, the methanol extracts of seeds

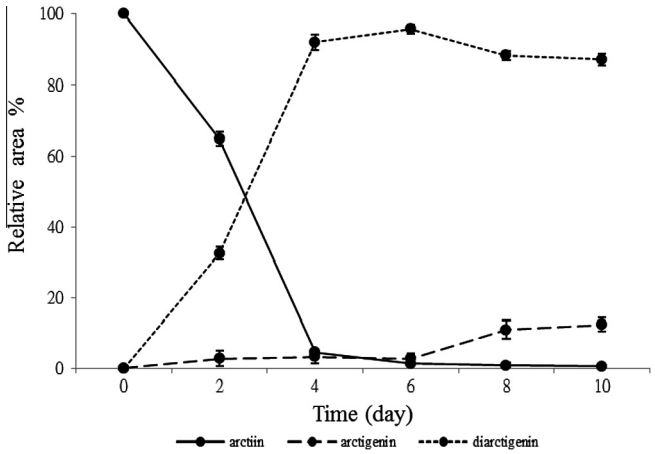

Figure 3. The relative abundances of arctiin (**1**), arctigenin (**2**), and diarctigenin (**3**) in the incubation period (n = 3–5).

of *A. lappa* L. (Arctii Fructus) did not show significant inhibition of the growth of plant pathogenic fungi. However, the indicator compound arctiin (**1**) in the methanol extracts of Arctii Fructus was transformed to other compounds according to our preliminary HPLC assays. Thus, in the present study the RS AG-4 was used as a biocatalyst and the biotransformation of arctiin (**1**) was investigated. In addition, the bioconversion was monitored by HPLC to determine the relative abundance of each component involved in the dynamic process.

Precultured RS AG-4 was transferred into a flask containing medium and cultivated at 27 °C for 3 days. After the growth of RS AG-4, arctiin (**1**) was added into the medium and cultivated for an additional 7 days, together with two controls, which contained either mycelia with medium or substrate dissolved in DMSO with medium. There were no metabolic products observed in two control experiments. After the fermentation, the culture medium and mycelia were filtered and fractionated by liquid–liquid partition to afford ethyl acetate and water soluble fractions, respectively. With the assistance of a combination of conventional chromatographic techniques, the ethyl acetate fractions afforded two compounds, arctigenin (**2**) (21%) and diarctigenin (**3**) (37%). The chemical structure of arctigenin (**2**) was characterized by comparison of its spectral and physical data with those reported in the literature.[11] Compound **3** was purified as optically active white amorphous powder with mp 89–92 °C and $[\alpha]_D^{25}$ −30 (c 0.1, $CHCl_3$). The positive-mode HR-ESI-MS of **3** showed a pseudomolecular ion peak at m/z 765.2870 corresponding to a molecular formula of $C_{42}H_{46}O_{12}$. The UV spectrum of compound **3** in methanol exhibited characteristic absorption maxima of a lignan skeleton at 284 and 225 nm.[20] The IR absorption bands at 3424, 1763, 1591 cm^{-1} displayed the presence of hydroxyl, carbonyl groups, and carbon–carbon double bond, respectively. In the ^1H NMR spectrum, a set of three mutually coupled ABX-type proton signals at δ 6.69 (1H, d, J = 8.4 Hz, H-5), 6.53 (1H, dd, J = 8.4, 2.0 Hz, H-6), and 6.46 (1H, d, J = 2.0 Hz, H-2), was indicative of a tri-substituted benzene ring. Two mutually *meta*-coupled doublets at δ 6.72 (1H, d, J = 2.0 Hz, H-6′) and 6.67 (1H, d, J = 2.0 Hz, H-2′) were characteristic of a tetra-substituted benzene ring. In addition, aliphatic methine and methylene signals at δ 4.14 (1H, m, H-9), 3.88 (1H, m, H-9), 2.96 (2H, d, J = 4.8 Hz, H-7′), 2.69 (1H, m, H-7), and 2.56 (3H, m, H-7, 8, 8′) were accounted for the butyrolactone-type lignan.[11] These spectral data were in accordance with the previously reported

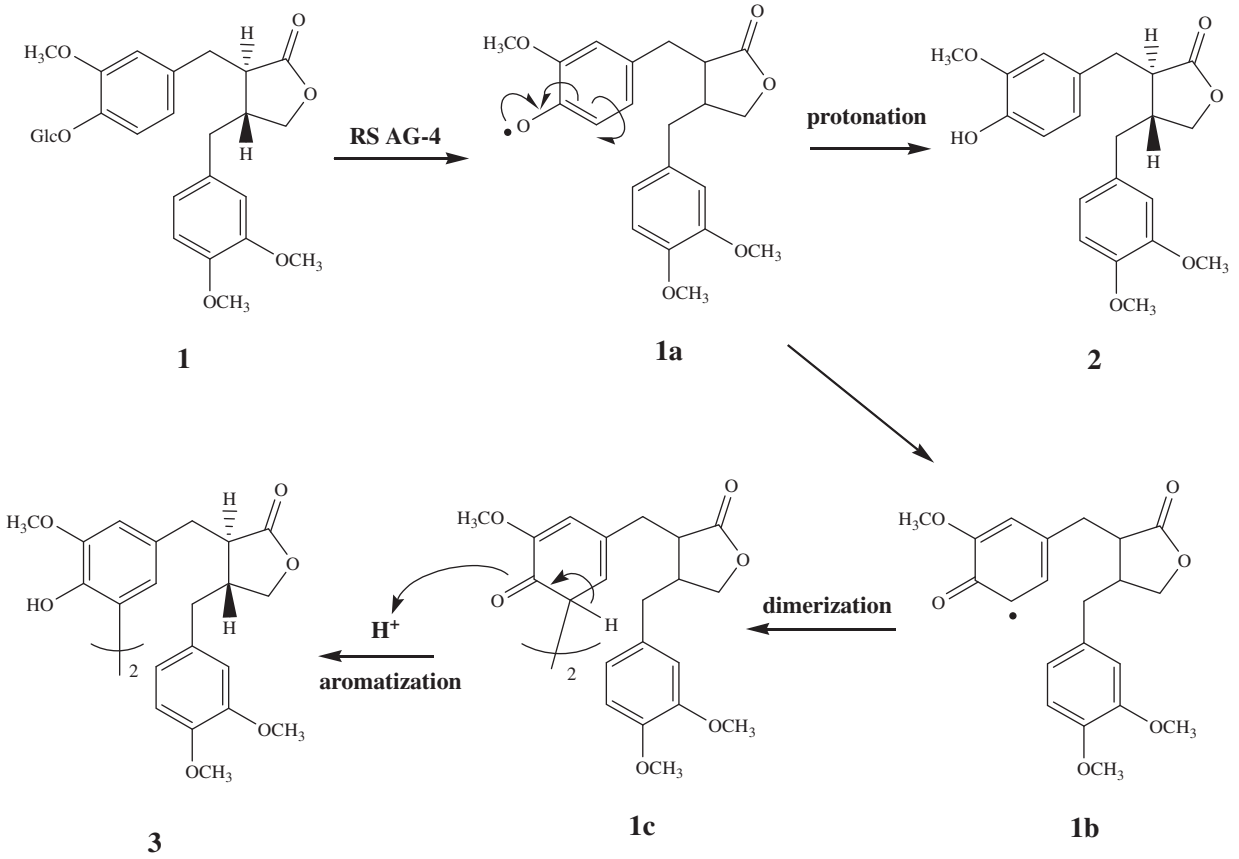

Figure 4. The plausible metabolic pathway for arctiin (**1**).

compound, diarctigenin (**3**).[11] The full assignments of ^{1}H and ^{13}C NMR signals were determined by the HMBC experiment (Fig. 1).

From the above spectral data, the chemical structure of compound **3** was concluded to be diarctigenin. This is the first biosynthetic Letter of diarctigenin and the present synthetic protocol provides an efficient synthetic pathway with satisfied yield (37%). In addition, the reagents used in this protocol were comparatively low cost, thus it would be suitable for the large-scale production of diarctigenin.

In order to investigate the biopreparation mechanism, the time course of biotransformation of arctiin (**1**) was analyzed by HPLC. The optimized HPLC analytical conditions for the crude products and the HPLC chromatograms of the indicator compounds arctiin (**1**), arctigenin (**2**), and diarctigenin (**3**) were displayed in the Supplementary data. The developed HPLC chromatographic analytical method was applied to assess the relative abundances of the compounds including arctiin (**1**), arctigenin (**2**), and diarctigenin (**3**) to evaluate the changes occurred during the incubation. The typical chromatograms of the crude products are displayed in Figure 2 and the relative abundances of arctiin (**1**), arctigenin (**2**), and diarctigenin (**3**) in the incubation period are shown in Figure 3. The content of starting material arctiin (**1**) was decreased significantly in the first 4 days; in contrast, arctigenin (**2**) and diarctigenin (**3**) were produced and their contents were increased. Until 6 days the contents of arctigenin (**2**) and diarctigenin (**3**) reached maxima and would not increase any more.

In another HPLC assay, only arctigenin (**2**) served as the starting substrate was incubated with the growth culture of RS AG-4, however, no metabolites of dimeric lignan diarctigenin (**3**) was found (see the Supplementary data). It suggested that the formation of diarctigenin should be related to the arctiin.

According to the results described above, the plausible metabolic pathway for arctiin (**1**) was proposed as shown in Figure 4. The glucose moiety connected with the lignan was hydrolyzed and resulted in a phenolic radical (**1a**). This radical could capture the hydrogen from the medium to afford the aglycone arctigenin (**2**). Part of the intermediate radical was converted into the phenylic radical (**1b**) and successive intermolecular dimerization yielded dimeric intermediate product (**1c**). Finally the aromatization of **1c** furnished the diarctigenin (**3**). Without the presence of arctiin (**1**), there is no accumulation of phenolic radical (**1a**) attributed to the formation of diarctigenin. Consequently, there is no production of dimeric lignan diarctigenin.

In the present study RS AG-4 was used as a biocatalyst and arctiin (**1**) was converted into arctigenin (**2**) and diarctigenin (**3**) both in satisfied yields. The current Letter would be an excellent method to produce a large scale of diarctigenin for the further medicinal and bioactivity examinations.

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Supplementary data

Supplementary data (experimental procedures, HPLC chromatograms, and spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.10.057.

References and notes

1. Frija, L. M. T.; Frade, R. F. M.; Afonso, C. A. M. *Chem. Rev.* **2011**, *111*, 4418–4452.
2. Wilson, R. M.; Danishefsky, S. *J. Org. Chem.* **2006**, *71*, 8329–8351.
3. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461–477.
4. Kingston, D. G. I. *J. Org. Chem.* **2008**, *73*, 3975–3984.
5. Butler, M. S. *Nat. Prod. Rep.* **2008**, *25*, 475–516.
6. Banwell, M. *Tetrahedron* **2008**, *64*, 4669–4670.
7. Baker, D. D.; Chu, M.; Oza, U.; Rajgarhia, V. *Nat. Prod. Rep.* **2007**, *24*, 1225–1244.
8. Zhang, H. F.; He, G. Q.; Liu, J.; Ruan, H.; Chen, Q. H.; Zhang, Q.; Wang, J. L.; Zhang, H. B. *Enzyme Microb. Technol.* **2008**, *43*, 25–30.
9. Chen, Q. H.; Liu, J.; Zhang, H. F.; He, G. Q.; Fu, M. L. *Enzyme Microb. Technol.* **2009**, *45*, 175–180.
10. Fu, M. L.; Wang, W.; Chen, F.; Dong, Y. C.; Liu, X. J.; Ni, H.; Chen, Q. H. *J. Agric. Food Chem.* **2011**, *59*, 7419–7426.
11. Han, B. H.; Kang, Y. H.; Yang, H. O.; Park, M. K. *Phytochemistry* **1994**, *37*, 1161–1163.
12. Park, S. Y.; Hong, S. S.; Han, X. H.; Hwang, J. S.; Lee, D.; Ro, J. S.; Hwang, B. Y. *Chem. Pharm. Bull.* **2007**, *55*, 150–152.
13. Hsieh, Y. H.; Chu, F. H.; Wang, Y. S.; Chien, S. C.; Chang, S. T.; Shaw, J. F.; Chen, C. Y.; Hsiao, W. W.; Kuo, Y. H.; Wang, S. Y. *J. Agric. Food Chem.* **2010**, *58*, 3153–3158.
14. Chen, J. J.; Lin, W. J.; Liao, C. H.; Shieh, P. C. *J. Nat. Prod.* **2007**, *70*, 989–992.
15. Pan, M. H.; Lai, C. S.; Dushenkov, S.; Ho, C. T. *J. Agric. Food Chem.* **2009**, *57*, 4467–4477.
16. Yun, K. J.; Koh, D. J.; Kim, S. H.; Park, S. J.; Ryu, J. H.; Kim, D. G.; Lee, J. Y.; Lee, K. T. *J. Agric. Food Chem.* **2008**, *56*, 10265–10272.
17. Huang, J. W.; Yang, S. H. *Plant Pathol. Bull.* **1992**, *1*, 26–30.
18. Shiau, F. L.; Chung, W. C.; Huang, J. W.; Huang, H. C. *Can. J. Plant Pathol.* **1999**, *21*, 368–374.
19. Kuo, P. C.; Yang, C. W.; Lin, T. C.; Lin, C. L.; Chen, G. F.; Huang, J. W. *J. Agric. Food Chem.* **2010**, *58*, 8618–8622.
20. Maiti, G.; Adhikari, S.; Roy, S. C. *Tetrahedron* **1995**, *51*, 8389–8396.