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Smith degradation, an efficient method for the preparation of cycloastragenol from astragaloside IV

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ABSTRACT

Cycloastragenol (CA) is the genuine sapogenin of astragaloside IV (ASI). This study focuses on the preparation of CA from ASI. Five hydrolysis methods were compared including H_2SO_4 hydrolysis, HCl hydrolysis, two-phase acid hydrolysis, mild acid hydrolysis, and Smith degradation. Seven hydrolysis products were purified, and five of them were identified as new compounds. The results indicated that Smith degradation was the most effective approach to prepare CA. In contrast, mild acid hydrolysis produced CA at a low yield, accompanied with the artificial sapogenin astragenol. The other three acid hydrolysis methods mainly produced astragenol. Furthermore, the reaction conditions for Smith degradation were optimized as follows: ASI was dissolved in 60% MeOH–H_2O solution, oxidized with 5 equiv. $NaIO_4$ for 12 h, followed by reduction with 3 equiv. $NaBH_4$ for 4 h, and finally acidified with 1 M H_2SO_4 at pH 2 for 24 h. Under the optimal conditions, CA could be prepared from ASI at a yield of 84.4%.

1. Introduction

Astragalus membranaceus, known as Huang-qi in Chinese, is a popular herbal medicine in China for the treatment of fatigue, and is also used as a food additive. It contains saponins, isoflavones, and polysaccharides [1–4]. Astragaloside IV (ASI) is a major saponin of Huang-qi, and has been reported to possess anti-aging, hepatoprotective, and cardiovascular-protective activities [5–8]. Cycloastragenol (CA) is the genuine sapogenin of astragaloside IV. Recently, it has been reported that CA could moderately increase telomerase activity and proliferative capacity of both CD4 and CD8 T cells, suggesting it could help delay the onset of cellular aging [9]. Further studies demonstrated CA as a telomerase activator to retard telomere shortening and enhance the antiviral function of human CD8 T lymphocytes in HIV-infected human donors [10]. CA has been introduced to the market as a promising new generation of anti-aging agent (nutritional supplement, TA-65®). Thus, it is necessary to establish an efficient method to prepare CA.

As a major constituent of *A. membranaceus*, astragaloside IV (ASI) could be easily prepared by microbial transformation of crude astragalosides with *Absidia corymbifera* [11]. ASI is a glycoside of CA, containing a xylose residue at C-3 and a glucose at C-6. A number of studies have tried to prepare CA from ASI. Kitagawa's group used enzymatic hydrolysis with crude hesperidinase and two-phase acid hydrolysis to obtain CA at a yield of 48.0% [12,13]. Mild acid hydrolysis could generate CA at a yield of 64% [14]. Smith degradation was also used to prepare CA from ASI, with a yield of 60.2% [15]. However, due to the labile 9,19-cyclopropane ring, the preparation of CA from ASI still suffers from the presence of by-products, particularly the artificial sapogenin, astragenol.

The present work has two objectives. The first one is to study the effects of different hydrolysis methods to prepare CA from ASI, including H_2SO_4 hydrolysis, HCl hydrolysis, two-phase acid hydrolysis, mild acid hydrolysis, and Smith degradation. The hydrolysis products from different methods were identified by spectroscopic techniques. The second objective is to establish an optimal reaction condition to prepare CA.

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2. Materials and methods

2.1. Materials

Astragaloside IV (purity > 98%) was purchased from Sanleng Biotechnology Co. Ltd., Guilin, China. Acetonitrile and formic acid were of HPLC grade (J.T. Baker, NJ, USA). De-ionized water was prepared by a Milli-Q system (Millipore, MA, USA). Solvents for sample extraction were of analytical grade.

2.2. High-performance liquid chromatography and mass spectrometry

HPLC analysis was performed on an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, a diode-array detector, an auto-sampler, and a column compartment. Samples were separated on an Agilent Zorbax Extend-C_{18} column (5 μm, ID 4.6 × 250 mm) (Agilent Technologies Inc., CA, USA). The mobile phase consisted of acetonitrile (A) and 0.1% formic acid aq. (B). A gradient elution program was used: 35−65% A for 0−10 min, 65−85% A for 10−15 min, and 85−100% A for 15−20 min. Flow rate, 1.0 mL/min; wavelength, 203 nm (scanned from 190 to 400 nm); column temperature, 35 °C. A GRACE Allsphere ODS-2 column (250 mm × 4.6 mm, 5 μm) was used to separate cycloastragenol (CA) from its isomer astragenol. The gradient program was as follows: 40−60% A for 0−13 min, 60−85% A for 13−25 min, and 85−100% A for 25−35 min. A Finnigan LCQ Advantage ion trap mass spectrometer (ThermoFisher, CA, USA) was connected to the HPLC system via an ESI interface (positive ion mode). Source-dependent parameters were as follows: sheath gas (N_2), 50 arb; auxiliary gas (N_2), 10 arb; spray voltage, 4.5 kV; capillary temperature, 330 °C; capillary voltage, 20 V; tube lens offset voltage, 54 V. Mass spectra full scan range, *m/z* 150–2000; collision energy, 35%; isolation width, 2.0 Th.

2.3. Structural identification of the hydrolysis products

Optical rotations were measured with a Perkin-Elmer 243B polarimeter at 20 °C. IR spectra were recorded in KBr with an Thermo Nicolet Nexus 470 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III-400 instrument (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) in pyridine-d_5. High-resolution mass spectra (HR-MS) were obtained on a Bruker APEX II Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.

2.4. Hydrolysis of astragaloside IV (ASI)

2.4.1. Smith degradation of astragaloside IV

An amount of 100 mg ASI was dissolved in 9 mL methanol, 6 mL water containing 500 mg $NaIO_4$ was then added, and the solution was stirred for 12 h at room temperature. The reaction was terminated with 0.21 g ethylene glycol in 10 mL water, and the reaction mixture was concentrated to remove methanol. Then, the residue was extracted with an equal volume of ethyl acetate for three times. The organic layer was washed with an equal volume of saturated NaCl water solution and then evaporated to dryness. The residue was re-constituted in 10 mL 60% methanol, and 15 mg $NaBH_4$ was added. The solution was stirred for 4 h at room temperature. About 10 mL water was added, and the pH was adjusted to 2.0 with 1 M H_2SO_4. The mixture was stirred for 24 h at room temperature. The reaction mixture was extracted with 20 mL ethyl acetate for three times, and the ethyl acetate layer was washed with saturated $NaHCO_3$ water solution and NaCl water solution, respectively. The organic layer was then evaporated to dryness. The crude products were separated by silica gel column chromatography and eluted with chloroform–methanol (50:1 and 30:1, *v/v*). From the 50:1 fraction, compound **8** was obtained. CA was obtained from the 30:1 fraction.

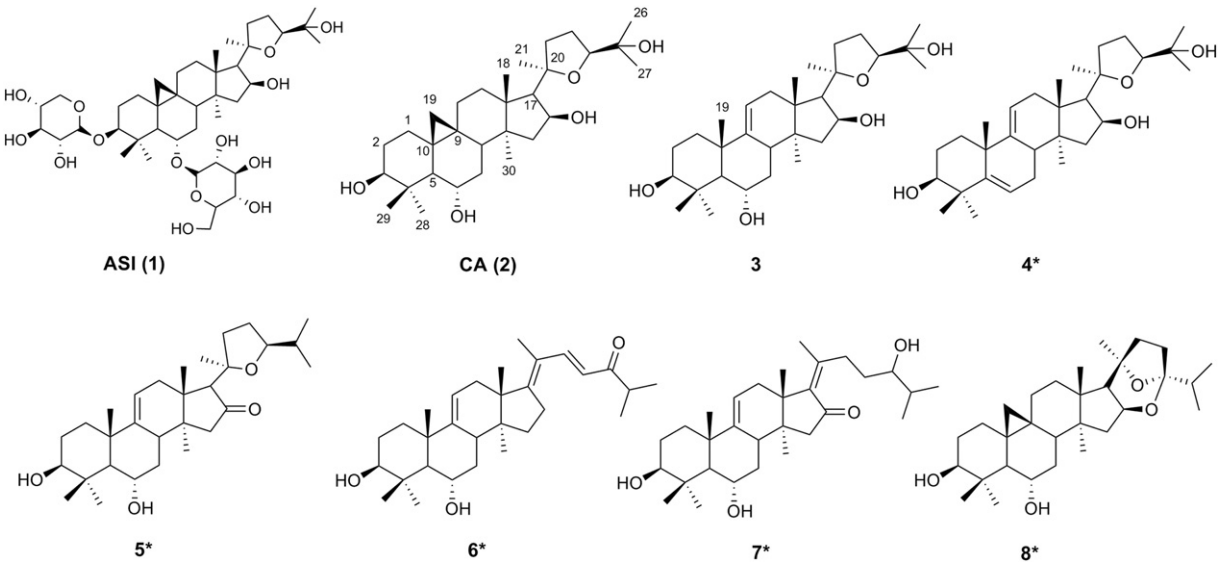

Fig. 1. Chemical structures of compounds **1**−**8** (* new compounds).