Iridoid glycosides from Gardeniae Fructus for treatment of ankle sprain

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ABSTRACT

The iridoid glycosides, genipin 1-O-beta-D-glucopyranoside (1) and genipin 1,10-di-O-beta-D-glucopyranoside (2), together with six known iridoid glycosides, genipin 1-O-beta-D-gentiobioside (3), geniposide (4), scandoside methyl ester (5), deacetylasperulosidic acid methyl ester (6), 6-O-methyldeacetylasperulosidic acid methyl ester (7), and gardenoside (8) were isolated from an EtOH extract of Gardeniae Fructus. The structures and relative stereochemistries of the metabolites were elucidated on the basis of 1D- and 2D-NMR spectroscopic techniques, high-resolution mass spectrometry, and chemical evidence. Geniposide (4), one of the main compounds of Gardeniae Fructus, was tested for treatment of ankle sprain using an ankle sprain model in rats. From the second to fifth day, the geniposide (4) (100 mg/ml) treated group exhibited significant differences (p < 0.01) with -21–34% reduction in swelling ratio compared with those of the vehicle treated control group. This indicated the potential effect of geniposide (4) for the treatment of disorders such as ankle sprain.

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1. Introduction

Ankle sprain is a partial or complete tear of ligaments that support the ankle (Bodien et al., 1995), with symptoms like pain, swelling, and bruising around the ankle in the acute stage. They may be caused by sudden twisting of the ankle, such as stepping on an uneven surface or in a hole, by taking an awkward step when running, jumping, or stepping up or down, or by inversion of the foot, which causes ankle to “roll over” when playing sports or exercising. Ankle sprain is one of the most common injuries in athletes, particularly in sports such as basketball, soccer, football, and volleyball (Kofotolis et al., 2007; Liu and Nguyen, 1999). Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, ibuprofen, naproxen, and piroxicam, have been used immediately post-injury and considered to be the best drug treatments due to their analgesic and anti-inflammatory effects (Buckwalter, 1995; Elder et al., 2001). However, taking such NSAIDs causes adverse effects, and is somewhat controversial regarding long-term healing (Stanley and Weaver, 1998; Stovitz and Johnson, 2003). For the treatment of soft tissue injuries, traditional drugs, such as Gardeniae Fructus (Guo et al., 1997a), Notoptis mergans (Guo et al., 1997b), and Cathami Flos (Guo et al., 1997c) etc., have also been used for hundreds of years in Korea, Japan, and China. However, their clinical efficacy and pharmacological effect need to be further studied.

Gardeniae Fructus, the dried ripe fruit of Gardenia jasminoides Ellis (Rubiacae), is widely used in traditional medicine for its cholagogic, sedative, diuretic, antiphlogistic, and antipyretic effects (Guo et al., 1997a). Additionally, it is an externally used drug with a long history and tradition in the treatment of sprain (Yao et al., 1991). Many iridoid glycosides were isolated from Gardeniae Fructus in previous investigations (Jin et al., 1991). Some of them, e.g. geniposide (4) (see Fig. 1), possess diverse biological activities such as anti-inflammatory, antithrombotic, neurotogenic, and cholagogic activities (Koo et al., 2006; Masaki et al., 1980; Suzuki et al., 2001; Yamazaki et al., 1996). However, there are few chemical and pharmacological studies related to Gardeniae Fructus for treating sprain (Yao et al., 1991). The specific aim of the present study was to investigate both the chemical constituents of Gardeniae Fructus and their potential for treatment of ankle sprain in a rat model.

2. Results and discussion

Two new iridoid glycosides 1 and 2 were isolated from the n-BUOH-soluble fraction of the EtOH extract of Gardeniae Fructus, together with six known iridoid glycosides (Fig. 1). The known
The compounds were identified as genipin 1-O-β-D-gentiobioside (3), geniposide (4) (Endo and Taguchi, 1973), scandoside methyl ester (5) (Guvencalp et al., 2006), deacetylasparulosidic acid methyl ester (6) (Damtoft et al., 1981; Kim et al., 2005), O-D-methyldeacetylasparulosidic acid methyl ester (7) (Machida et al., 2003), and gardenoside (8) (Farid et al., 2002) by comparing their physical and spectroscopic data with those of published literature.

Compound 1 was obtained as a white amorphous powder. The HR-FAB-MS spectrum of 1 showed a quasi-molecular ion at m/z 573.1757 (M+Na)\(^+\), corresponding to a molecular formula of C\(_{29}\)H\(_{36}\)O\(_{15}\). The structure of the compound was established from analyzing its \(^{1}H\) and \(^{13}C\) NMR spectroscopic data (Table 1) which were compared to those of 3, as well as the 2D-NMR spectra including HMOC, HMBC, COSY, and ROESY. Compound 2 showed similar UV, IR, and \(^{1}H\) and \(^{13}C\) NMR spectra to 3. Two glucopyranosyl signals were shown in the \(^{1}H\) and \(^{13}C\) NMR spectra of 2. A significant difference was observed in the \(^{1}H\) NMR spectrum, namely that the signals of H-10 [\(\delta = 4.60\) (1H, d, J = 12.6 Hz) and 4.27 (1H, d, J = 12.6 Hz)] of 2 were more downfield than those of 3 (\[\delta = 4.32\) (1H, d, J = 14.7 Hz) and 4.19 (1H, d, J = 14.7 Hz)]. This suggested that one glucopyranosyl moiety was connected to the 10-OH group of the aglycone. The HMBC experiment further supported this assignment as evidenced by the long-range correlations of H-10 to that of the anomeric carbon [\(\delta = 104.7\) (C-1')] of one glucose, and the anomeric proton H-1' [\(\delta = 4.34\) (1H, d, J = 7.8 Hz)] to \(\delta = 69.1\) (C-10). In addition, the proton signal at \(\delta = 5.26\) (1H, d, J = 7.8 Hz) of 1 showed a long-range correlation with the anomeric carbon of the other glucose at \(\delta = 103.3\) (C-1'). This indicated linkage between the glucose and the 1-OH group of the same pattern of 4 (Fig. 2). The configurations of the glycosidic linkage for two glucopyranosyl units were determined to be β on the basis of examination of the \(^{3}J\) values for the anomeric protons at \(7.5\) Hz (H-1') and \(7.8\) Hz (H-1'). The aglycone was also identified as genipin by analysis of the spectroscopic data of its hydrolysates produced as well as from a ROESY experiment (Fig. 2). Therefore, compound 2 was determined to be genipin 110-di-O-β-D-glucopyranoside.

Gardeniae Fructus, as a traditional medicine, is often externally used for the treatment of soft tissue injuries. Geniposide (4) is one of the main components for Gardeniae Fructus. Because it is a naturally occurring, biodegradable molecule with low cytotoxicity, it has recently been used in many biological investigations (Huang et al., 1998). In this study, the EtOH extract of Gardeniae Fructus and geniposide (4) isolated from the n-BuOH-soluble fraction of the EtOH extract were investigated for treatment of ankle sprain using a rat model. This model was first established in the research of acupuncture analgesia for ankle sprain (Ko et al., 2002), and lateral ankle sprain is also a common source of persistent pain in humans. To model this condition, the rat's right hind ankle was bent repeatedly, overextending lateral ligaments, for 4 min under ethyl ether anesthesia. The rat subsequently showed swelling of the ankle for the next several days. To estimate the degree of edema