Isolation of Flavonoids from Processed Aconiti Tuber

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A processed Aconiti tuber (Korean name, Kyeong-Po Buja) was extracted with 80% aqueous EtOH, and the concentrated extract was partitioned with EtOAc and water (H2O). From the EtOAc fraction, two flavonoids were isolated through repeated silica gel column chromatographies. From the result of physico-chemical data including NMR, mass spectrometry and IR, the chemical structures of the compounds were determined to be liquiritin (1) and liquiritigenin (2). This is the first study to isolate flavonoids (1) and (2) from the processed Aconiti tuber.

Key words: Aconitum carmichaeli, Kyeong-Po Buja, liquiritin, liquiritigenin, processed Aconiti tuber

The Aconiti tuber is a tuberous root of Aconitum carmichaeli Debeaux (Ranunculaceae), which has been an indispensable medicinal plant having cardiotonic, diuretic, and analgesic effects [Han et al., 1997]. Such preparations have been used for analgesic, anti-inflammatory, anti-rheumatic, and neurological indications for centuries [Ameri et al., 1998]. However, tuber of the species Aconitum has also been widely used as a source of poison for the execution of death penalty since the ancient times.

The Aconiti tuber contains toxic diterpene alkaloids such as aconitine, hyaconitine, and mesaconitine, which easily turn into less toxic alkaloids such as benzoylacetone, aconine, and pyroacanitine by heating or alkaline treatment through deacetylation, debenzoylation or oxidation reaction [Kitagawa et al., 1984b]. Therefore, the tuber of the species Aconitum has been used as herbal drug only after it was treated by immersion in salt solution or heating, commonly referred to as ‘Beop-Je’ or ‘Su-Chi’ in Korea, to reduce its toxicity [Park et al., 1990]. Currently, the processed Aconiti tuber is widely and safely used for the treatment of pain, neuronal disorders, and inflammation, with no problematic or annoying adverse effects [Murayama et al., 1991; Oyama et al., 1994; Ameri et al., 1998; Taki et al., 1998].

There are various processing method of Aconiti tuber such as ‘Yeom Buja’ (salted Aconiti tuber), ‘Po Buja’ (moist-heating Aconiti tuber) and so on, and the components of the processed Aconiti tuber vary significantly depending on the process method [Kitagawa et al., 1984a; Kim et al., 2002]. Kyeong-Po Buja, a type of ‘Po Buja’, is the Korean traditional process, which involves immersing ‘Yeom Buja’ in water for 24 h, peeling, cutting the tuber in two pieces, immersing once more in water to remove salt, heating for 3 h with black beans and licorice roots, again immersing in water for 24 h, and finally drying [Park et al., 1990].

Various alkaloids are reported as the principal components of the processed Aconiti tuber that manifest pharmacological activities [Hikino et al., 1979; Kitagawa et al., 1984b; Suzuki et al., 1993]. Except for the alkaloids, however, only few reports have been published on other pharmacologically active compounds of the processed Aconiti tuber. Therefore, this study was performed to identify other active components of the processed Aconiti tuber, resulting in the isolation of two flavonoids through repeated column chromatographies. This paper describes the isolation and the structural elucidation of the flavonoids.

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Abbreviations: cc, column chromatography; EI-MS, electron impact mass spectrometry; TLC, thin layer chromatography; TMS, tetramethylsilane

Materials and Methods

Plant materials. The processed Aconiti tuber was purchased from Kyungdong Market, Seoul, Korea, in June 2006, and was identified by Prof. Dae-Keun Kim, College of Pharmacy, Woosuk University, Jeonju, Korea. A voucher specimen (KHU061231) is reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Instruments and reagents. The melting points were determined on a Fisher-John apparatus and were not corrected. Optical rotation was measured on a JASCO P-1010 digital polarimeter (Tokyo, Japan). UV spectra were measured on a Shimadzu UV-1701 (Kyoto, Japan). IR spectra were obtained from a Perkin Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). EI-MS was recorded on a JEOL JMSXI-505-WA (Tokyo, Japan). \(^1\)H-NMR (400 MHz), and \(^13\)C-NMR (100 MHz) spectra were recorded on a Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA). Methanol-\(d_4\), with tetramethylsilane as an internal standard was purchased from Sigma (St. Louis, MO).

Extraction and isolation. The processed Aconiti tuber (10 kg) was extracted three times at room temperature with 80% aqueous EtOH (18 L\(\times\)3). The extracts were partitioned with water (3 L) and EtOAc (3 L). The concentrated EtOAc extract (45 g) was applied to a silica gel cc (10\(\times\)16 cm), eluted with a gradient of CHCl\(_3\):MeOH (20:1\(\rightarrow\)15:1\(\rightarrow\)10:1\(\rightarrow\)7:1\(\rightarrow\)5:1\(\rightarrow\)3:1\(\rightarrow\)1:1; each 1 L), and monitored by TLC to produce 27 fractions (PACE1 to PACE27). Fraction PACE10 [662 mg, \(R_v\), (elution volume/total volume) 0.35-0.40] was subjected to a silica gel cc (3.5\(\times\)17 cm) eluted with hexane:EtOAc (2:1, 4 L), yielding four fractions (PACE10-1 to PACE10-4) to ultimately produce compound 1 [25.4 mg, \(R_v\), 0.30-0.45, TLC (SiO\(_2\), F\(_{254}\)) \(R_f\), 0.30, hexane:EtOAc\(=\)1:1]. Fraction PACE21 [2.2 g, \(R_v\), 0.65-0.75] was applied to a silica gel cc (5\(\times\)15 cm) and eluted with CHCl\(_3\):MeOH (5:1, 7 L), yielding 15 fractions (PACE21-1 to PACE21-15) to ultimately produce compound 2 [113.4 mg, \(R_v\), 0.50-0.55, TLC (SiO\(_2\), F\(_{254}\)) \(R_f\), 0.35, CHCl\(_3\):MeOH\(=\)3:1].

Compound 1 (liquiritigenin): colorless crystals (MeOH); m.p. 203\(^\circ\)C; UV (MeOH) \(\lambda_{max}\) 313, 276 nm; \([\alpha]_D\) \(-22.5^\circ\) (c 0.21, MeOH); IR (KBr, v) 3200, 1640, 1600 cm\(^{-1}\); EI-MS m/z 256 [M]+; \(^1\)H-NMR (400 MHz, CD\(_3\)OD, \(\delta\)) 7.72 (1H, d, \(J\) = 8.8, H-5); 7.3 (2H, d, \(J\) = 8.4, H-2', 6'); 6.81 (2H, d, \(J\) = 8.4, H-3', 5'); 6.47 (1H, dd, \(J\) = 8.8, 2.0, H-6), 6.34 (1H, d, \(J\) = 2.0, H-8), 5.35 (1H, dd, \(J\) = 13.2, 2.8, H-2), 3.04 (1H, dd, \(J\) = 16.8, 13.2, H-3a), 2.67 (1H, dd, \(J\) = 16.8, 2.8, H-3b); \(^13\)C-NMR (100 MHz, CD\(_3\)OD, \(\delta\)) 193.4 (C-4), 166.6 (C-7), 165.4 (C-9), 158.8 (C-4'), 133.8 (C-1'), 129.7 (C-5), 128.9 (C-2', 6'), 116.2 (C-3', 5'), 114.8 (C-10), 111.6 (C-6), 103.7 (C-8), 81.0 (C-2), 44.9 (C-3).

Compound 2 (liquiritin): colorless crystals (MeOH); m.p. 209-211\(^\circ\)C; UV (MeOH) \(\lambda_{max}\) 313, 276 nm; \([\alpha]_D\) \(-56.0^\circ\) (c 0.21, MeOH); IR (KBr, v) 3300, 1640, 1600 cm\(^{-1}\); EI-MS m/z 418 [M]+, 256, 255; \(^1\)H-NMR (400 MHz, CD\(_3\)OD, \(\delta\)) 7.74 (1H, d, \(J\) = 8.8, H-5), 7.44 (2H, d, \(J\) = 8.4, H-2', 6'), 7.15 (2H, d, \(J\) = 8.4, H-3', 5'), 6.51 (1H, dd, \(J\) = 8.8, 2.0, H-6), 6.37 (1H, d, \(J\) = 2.0, H-8), 5.45 (1H, dd, \(J\) = 12.8, 2.8, H-2'), 4.95 (1H, d, \(J\) = 7.2, H-1'), 3.91 (1H, dd, \(J\) = 12.0, 1.6, H-6'a), 3.71 (1H, dd, \(J\) = 12.0, 5.6, H-6'b), 3.04 (1H, dd, \(J\) = 16.8, 12.8, H-3a), 2.73 (1H, dd, \(J\) = 16.8, 2.8, H-3b); \(^13\)C-NMR (100 MHz, CD\(_3\)OD, \(\delta\)) 193.0 (C-4), 166.6 (C-7), 165.2 (C-9), 159.0 (C-4'), 134.3 (C-1'), 129.7 (C-5), 128.7 (C-2', 6'), 117.7 (C-3', 5'), 114.9 (C-10), 111.7 (C-6), 103.7 (C-8), 102.1 (C-1'), 80.6 (C-2), 78.1 (C-5'), 77.9 (C-3'), 74.8 (C-2''), 71.3 (C-4''), 62.5 (C-6''), 45.0 (C-3).

Results and Discussion

When the EtOH extract of the processed Aconiti tuber was developed on the silica gel TLC, the spots showed a UV absorbance at 254 nm. In addition, a yellow colorization appeared when the TLC plate was sprayed with 10% H\(_2\)SO\(_4\) solution and then heated, which indicated the presence of flavonoids in the extracts. The EtOH extracts were partitioned with EtOAc and H\(_2\)O layers through solvent fractionation. The repeated silica gel cc of EtOAc fractions supplied two flavonoids, compounds 1 and 2. Structural identifications of these compounds were carried out by interpretation of extensive spectroscopic data and comparison with the data described in the literature.

Compound 1 showed absorbance bands due to hydroxyl (3300 cm\(^{-1}\)), conjugated carbonyl (1640 cm\(^{-1}\)),
and aromatic (1600 cm⁻¹) groups in the IR spectrum and a molecular ion peak at m/z 256 [M]+ in the EI-MS. In the 1H-NMR spectrum, four olefin methine signals at δH 7.31 (2H, d, J=8.4 Hz) and 6.81 (2H, d, J=8.4 Hz) due to a 1,4-disubstituted benzene ring, three olefin methine signals at δH 7.72 (1H, d, J=8.8 Hz), 6.47 (1H, dd, J=8.8, 2.0 Hz), and 6.34 (1H, d, J=2.0 Hz) of the typical ortho- and meta-coupled patterns indicating the presence of a 1,2,4-trisubstituted benzene ring were observed. An oxygenated methine signal at δH 5.35 (1H, dd) and a methylene signal at δH 3.04 (1H, dd) and 2.67 (1H, dd), with their respective coupling constants of 13.2 and 2.8 Hz, were also observed. In the 13C-NMR spectrum, fifteen carbon signals were observed. The multiplicity of each carbon was determined using a distortionless enhancement by polarization transfer experiment. One conjugated ketone signal at δC 193.4 (C-4), three oxygenated olefin quaternary signals at δC 166.6 (C-7), 165.4 (C-9), and 158.8 (C-4'), two olefin quaternary signals at δC 133.8 (C-1) and 114.8 (C-10), seven olefin methine signals at δC 129.7 (C-5), 128.9 (C-2'), 61.6 (C-3'), 51.1 (C-6), and 103.7 (C-8), one oxygenated methine signal at δC 81.0 (C-2), and one methylene signal at δC 44.9 (C-3) were observed. This led to the conclusion that compound 1 was a flavanone, with the 1,4-disubstituted and 1,2,4-trisubstituted benzene rings. Compound 1 was finally identified as liquiritigenin [(2S)-4',7-dihydroxyflavanone] by comparison of the physical and the spectral data with those in the literature [Fu et al., 2005].

Compound 2 was assumed to be a monoglycoside of compound 1 from the spectroscopic data of MS, 1H-NMR, and 13C-NMR. One each anomic proton signal at δH 4.95 (d, J=7.2 Hz) and anomic carbon signal at δC 102.1 (C-1') were observed. The chemical shifts of other glycosidic carbon signals at δC 78.1 (C-5'), 77.9 (C-3'), 74.8 (C-2'), 71.3 (C-4'), and 62.5 (C-6') suggested the presence of a β-glucopyranosyl moiety. The connection between the glucopyranosyl unit (C-1') and the C-4' of the aglycon was verified by the cross-peak observed between δH 4.95 (H-1') and δC 159.0 (C-4') in the heteronuclear multiple bonding connectivity spectrum. Thus, compound 2 was finally identified as liquiritigenin [(2S)-4',7-dihydroxyflavanone 4'-O-β-D-glucopyranoside] by comparison of the physical and the spectral data with those in the literature [Fu et al., 2005].

From the result of spectroscopic data including NMR, MS, and IR, the chemical structures of isolated two flavonoids were determined. This study marks the first isolation of liquiritigenin (1) and liquiritin (2) from the processed Aconiti tuber. These two flavonoids are the principal components of licorice [Hatano et al., 1991; Fu et al., 2005]. Liquiritigenin was reported to have antioxidant activity [Zou et al., 1996], inhibitory effect on xanthine oxidase and monoamine oxidase activity [Hatano et al., 1991; Pan et al., 2000], dose-dependant anti-allergic activity [Kakegawa et al., 1992], antibacterial activity [Hwang et al., 1989], inhibitory effect on angiogenesis [Kobayashi et al., 1995], Epstein-Barr virus inhibitory activity [Konoshima et al., 1989], and cytoprotective effect against cadmium-induced toxicity [Kim et al., 2004]. Liquiritin was reported to show anti-depressive effect [Zhao et al., 2006] and antibacterial activity [Tanaka et al., 2006].

The processed Aconiti tuber is well known in the traditional oriental medicine system as an effective natural resource for treating various diseases. Except for the alkaloids, however, there are only few reports on other pharmacologically active compounds of the processed Aconiti tuber. Further studies on these various components of the processed Aconiti tuber would be very useful for the development of medicinal materials. In summary, two flavonoids were isolated for the first time from the processed Aconiti tuber and identified as liquiritigenin and liquiritin by NMR, MS, and IR experiments.

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References


