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Separation and Identification of Cirsimarin from *Cirsium Pendulum* Fisch

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韓國産 엉겅퀴(*Cirsium Pendulum* Fisch)에서 Cirsimarin의 分離 및 確認

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Whole plant of *Cirsium pendulum* Fisch was extracted with methanol and a flavonoid glycoside was isolated from the methanol extract. The glycoflavonoid was identified as cirsimarin, 4′, 5-dihydroxy-6, 7-dimethoxyflavone-4′-β-D-glucopyranoside.

Introduction

Cirsium species have long been used for treatment of hemorrhage, scabies and various abdominal and intestinal disorders as folkloric remedies[1,2]. The authors have collected *Cirsium pendulum*, one of the common Cirsium species in Korea. The crude methanol extract showed some hepatotonic effects in our separate experiments[3]. It was decided to undertake a phytochemical investigations of this plant to seek a source of compounds with potential pharmacological activities since actually nothing has been known of the constituents of *Cirsium pendulum*.

Various flavonoids and flavonoid glycosides have been detected and isolated from various Cirsium species[4~10]. This paper describes the isolation of cirsimarin, 4′, 5-dihydroxy-6, 7-dimethoxyflavone-4′-β-D-glucopyranoside.

Experimental

Plant material The whole plants of *Cirsium pendulum* Fisch were collected from Daesungri, Kyungkido in June and air dried.

Separation of Cirsimarin (I) The dried and crushed whole plants (170g) were refluxed with 10 l of methanol for 8 hours and filtred. This procedure was repeated three times and the combined filtrate was concentrated under the vacuo yielding 37g of the crude extract. 25g of the crude extract was trituated with 200ml of the mixture of chloroform and methanol (1 : 1). The solid material which separated was filtred and washed with water. It was recrystallized from the mixture of ethanol and water (1 : 1) yielding 700mg of cirsimarin. mp. 244°~6° (melting once at 156°~8°). $C_{23}H_{24}O_{11}\cdot 1\frac{1}{2}H_2O$ Anal. Calcd.: C, 54.9; H, 5.4, Found: C, 54.6; H, 5.25, $[\alpha]_D^{23}$-57 (pyridine: EtOH=2 : 8) (reported: -70[6]), IR(KBr) ν_{max} (cm^{-1}) 3260 (-OH), 1650, 1605 (unsaturated ketone), UV λ_{max}^{EtOH} (nm) 279, 328, NMR (deuterated DMSO) δ 12.73 (1H, s, C_5-OH), 7.97 (2H, d, J=13Hz, $C_2', _6'$-H), 7.1 (2H, d, J=13Hz, $C_3', _5'$-H), 6.85 (2H, s, $C_{3,8}$-H), 5.17 (1H, m, glucosyl 1-H), 3.87 (3H, s, C_7-OCH_3), 3.7 (3H, s, C_6-OCH_3), MS M^+ at

m/e (%) 476 (1), 314 (100, aglycone) and other fragmentation peaks for cirsimaritin at 299, 285, 271, 181, 153, 118 etc.,

Cirsimarin pentaacetate (III) Cirsimarin (100mg) was refluxed with 5ml pyridine and 3ml of acetic anhydride for 3 hours. The reaction mixture was concentrated under the vacuo, dissolved in chloroform and washed with water. The chloroform layer was dried over anhydrous Na_2SO_4 and concentrated. It was applied to a silica gel column and eluted with the mixture of chloroform and methanol(9 : 1) to separate the acetate fraction which was recrystallized from isopropyl alcohol. mp. 152° IR(KBr) ν_{max} (cm^{-1}) 1760 (acetyl), 1635, 1608 (unsaturated ketone), NMR ($CDCl_3$) δ 7.74 (2H, d, J=13Hz, C_2',$_6'$-H), 7.04 (2H, d, J=13Hz, C_3',$_5'$-H), 6.87(1H, s, C_8-H), 6.47 (1H, s, C_3-H), 5.23 (4H, m, glucosyl), 4.20 (2H, m, glucosyl), 3.97 (3H, s, C_7-OCH_3), 3.7 (3H, s, C_6-OCH_3), 2.47 (3H, s, C_5-acetyl) 2.07 (12H, s, glucosylacetyl)

Hydrolysis of Cirsimarin (I) Cirsimarin (100mg) was refluxed with 20ml of the mixture of 2 M-HCl and ethanol (1 : 1) for 3 hours. The reaction mixture was extracted with chloroform. The chloroform layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The crude reaction product was applied to a silica gel column and eluted with the mixture of chloroform and methanol (9 : 1) to separate the aglycone, cirsimaritin (II) which was recrystallized from methanol mp. 256°~7°.

The aqueous layer of the reaction mixture after extraction with chloroform was neutralized with Ag_2CO_3, filtred off the solids and concentrated under the vacuo. The residue was found to be D-glucose by the direct comparison with the authentic sample by TLC (MeOH-$CHCl_3$-Acetone-NH_4OH=5-2-3-2 and BuOH-AcOH-H_2O=4-1-2).

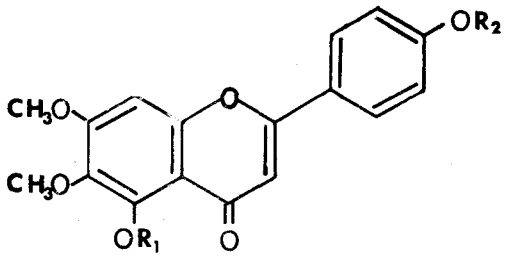
Results and Discussion

Cirsium pendulum Fisch (Compositae) is a perennial plant spreading by seeds and underground rootstocks. The stem is 30cm to 100cm high. Violet flowers with white head-hairs blossom from June to October. The whole plants were collected at Daesungri, Kyungkido in June. The dried plant material was extracted with methanol. The crude methanol extract was positive to flavonoid test with c-HCl and magnesium.

The solid precipitates (I), which did not dissolve in either chloroform or water, was separated from the crude methanol extract and recrystallized from EtOH-H_2O (1 : 1), mp. 244° ~6°. It was positive to flavonoid test (c-HCl-Mg) and glycoside test (anthrone - c-H_2SO_4). On hydrolysis, this flavonoid glycoside (I) produced an aglycone and D-glucose. Glucose was identified by the direct comparison with the authentic sample. The aglycone was identified as cirsimaritin (II) with the comparison of the reported mp., IR, UV, NMR and MS spectral data[6,11,12].

Acetylation of I with acetic anhydride and pyridine afforded a pentaacetate (III). The NMR spectra of III showed one aromatic acetyl group at δ 2.47 and four glycosidyl acetyl group at δ 2.07 suggesting one glucose unit is attached to the aglycone, cirsimaritin (II). The UV spectra of I showed absorption bands at 279 and 328nm. Aluminum chloride induced bathochromic shifts and the bands appeared at 301 and 349nm. Moreover, there was no differences in the UV spectrum with $AlCl_3$ alone and $AlCl_3$ with HCl suggesting that the C_5-OH is free. As long as C_5-OH is free, the glucose unit in I should be attached at C'_4-OH. The molecular rotation of I which was measured

$[\alpha]_D^{23}$-57 suggested the glycosidic linkage of β-orientation by Klyne's rule[13].



I : R_1=H, R_2=β-D-glucose
II : R_1, R_2=H
III : R_1=$COCH_3$, R_2=tetraacetyl glucose

Thus the flavonoid glycoside (I) was identified as cirsimarin, 4′,5-dihydroxy-6,7-dimethoxyflavone-4′-β-D-glucopyranoside. However cirsimarin was reported to be isolated from *Cirsium Martimum*[5] and *C. Tanakae sub. amorense*[6] by Morita et. al., no IR, UV, NMR or MS spectral data was available. This paper provides the details of the spectral data.

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