ISOLATION OF ESCULETIN FROM *Cichorium glandulosum* BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

H. K. Wu,[1,2] Z. Su,[1,2] A. Yili,[1] Z. P. Xiao,[1,2] B. Hang,[1] and H. A. Aisa[1]

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Cichorium glandulosum Boiss et Hout (Compositae, Asteraceae) is widely used in Uigur folk medicine as a cholagogic and diuretic agent, to improve the appetite, to increase digestion, and to cure liver diseases etc. [1]. This plant is widely distributed in Xinjiang but its chemical composition has not been reported with the exception of the analysis of its essential oil [2]. Esculetin is the active principle of *C. glandulosum*. Biological studies have established its hepatoprotective activity [3]. Herein we report a method for isolating and purifying esculetin by preparative high-speed countercurrent chromatography (HSCCC).

A crude sample of *C. glandulosum* was first analyzed by HPLC. The results showed that it contained esculetin and several unknown components. The sample was separated by HSCCC. Fractions containing esculetin were combined and dried. The yield of esculetin was 24 mg; the purity, 98%. Seeds (~1 kg) of *C. glandulosum* were extracted three times with ethanol (70%). The extracts were combined and evaporated to dryness under reduced pressure to afford a dry powder (150 g). The powder was dissolved in pure water (500 mL). The resulting solution was passed over a column of ion-exchange resin (1.1 kg, grade D 101) with elution by water (8 L) and ethanol (30%, 50, 70, and 95, 8 L each). The effluent from 50% ethanol was evaporated to dryness to afford a crude sample (223 mg).

The whole procedure was carried out as follows. An upper phase consisting of $CHCl_3$:CH_3OH:H_2O (4:3:2) was pumped into the multiple carbon column of the chromatograph (HSCCC, model GS-10A2) at flow rate 9 mL/min using a model NS-1007 pump to create a constant current. After the column was filled with the upper phase through the sample-injection system, a solution of dry extract (223 mg) dissolved in the upper phase was introduced to the column. Then the lower organic phase was pumped into the column at flow rate 2 mL/min with the column rotating at 800 rpm. The column effluent was monitored by a UV detector at 254 nm. Peaks were collected manually according to the chromatogram. The collected fractions were analyzed by HPLC using CH_3OH:CH_3CO_2H (0.3%) (31:69). The peak fraction obtained from the HSCCC chromatograph was identified using PMR and ^{13}C NMR spectral data.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.1 (1H, d, J = 9.6, H-3), 6.7 (1H, s, H-5), 6.9 (1H, s, H-8), 7.8 (1H, d, J = 9.6, H-4), 9.4 (1H, s, 6-OH), 10.2 (1H, s, 7-OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6): 160.6 (C-2), 150.0 (C-6), 148.3 (C-7), 144.3 (C-4), 142.5 (C-9), 112.1 (C-5), 111.4 (C-3), 110.7 (C-10), 102.5 (C-8). These data agreed well with those published [4] and identified the isolated compound as esculetin.

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1) Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumchi, 830011, China, South Beijing Road 40-1, fax (+86 991) 3835679, tel: (+86 991) 3835679, e-mail: haji@ms.xjb.ac.cn; 2) Graduate University of the Chinese Academy of Sciences, Beijing, 100039, China. Translated from Khimiya Prirodnykh Soedinenii, No. 1, p. 91, January-February, 2007. Original article submitted September 19, 2006.

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