Identification of Bioactive Compounds that Contribute to the α-Glucosidase Inhibitory Activity of Rosemary

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Extraction and Isolation

The dried leaves of rosemary (5.0 kg) were soaked with 95% methanol (3 × 7 L) at room temperature. The solvent was evaporated under reduced pressure to yield 272.3 g of extract. The crude extract was suspended in water (1.5 L) and then successively solvent partitioned with hexane, EtOAc, and *n*-butanol. Four layers with increasing polarity were obtained: hexane-soluble (88.5 g), EtOAc-soluble (38.0 g), BuOH-soluble (45.0 g) and H_2O-soluble (54.6 g) fractions. The hexane-soluble was subjected to D101 macroporous resin eluted with water-EtOH (1:0, 7:3, 1:1, 3:7, 1: 9, and 0:1, v/v) to afford six fractions(H1-H6). Fraction H1(0.81g) was further separated by CC using silica gel (4:1, petroleum ether-EtOAc) to give compound **18** (105 mg). Fraction H2 (4.2 g) was recrystallized with petroleum ether- EtOAc to afford compound **24** (103 mg) , and the mother liquid was chromatographed on a Sephadex LH-20 column ($CHCl_3$-MeOH, 2:1) to yield compound **12** (32 mg).and another impure yellow powder (0.97 g), which was purified by silica gel CC eluted with petroleum ether-EtOAc(4:1) to give compound **13** (10 mg). Compond 2**6** (17 mg) was obtained from fraction H3 (0.88 g) by Sephadex LH-20 ($CHCl_3$-CH_3OH, 1:1). Fraction H4 (0.75 g) was chromatographed by CC (petroleum ether-EtOAc, 4:1) to afford compound **17** (21mg). Fraction H5(0.8 g) was chromatographed on a Sephadex LH-20 column ($CHCl_3$-MeOH, 1:1) to yield compound **23** (12 mg). Fraction H6 (0. 2 g) was purified by RP-C 18 CC using MeOH-H_2O (1:1) to afford compound **20** (11 mg). The EtOAc-soluble was further fractionated by MPLC eluting with petroleum ether-EtOAc (from 15:1 to 0:1) to afford six fractions (E1-E4). Fraction E2 (1.3 g) was subjected to silica

gel CC eluted with petroleum ether-EtOAc (3:1) to afford yield compound **5** (17 mg). Fraction E3.1 (901 mg) was separated by preparative reversed-phase HPLC with MeOH/water (9:1) to give compound **30** (90 mg). Fraction E3.2 (1.1 g) was separated by preparative reversed-phase HPLC with MeOH/water (9:1) to give compound **31** (125 mg).