Isolation and Identification of Two New Flavanones and a Chalcone from *Citrus kinokuni*1)

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Two new flavanones and one chalcone were isolated from the peel of *Citrus kinokuni* Hort. ex TANAKA and identified as (2S)-5,6,7,8,4’-pentamethoxyflavanone (1), (2S)-5,6,7,3’,4’-pentamethoxyflavanone (2) and 2’-hydroxy-3,4,3’,4’,6’-pentamethoxychalcone (3). The structures of new compounds were elucidated by spectroscopic analysis.

Key words flavanone; chalcone; *Citrus kinokuni*; Rutaceae

Recently, we carried out primary screening of extracts of *Citrus* fruit to search for useful compounds for cancer chemoprevention.1,2) As a part of our studies on the constituents of these extracts, two new flavanones and one chalcone were isolated and identified as (2S)-5,6,7,8,4’-pentamethoxyflavanone (1), (2S)-5,6,7,3’,4’-pentamethoxyflavanone (2) and 2’-hydroxy-3,4,3’,4’,6’-pentamethoxychalcone (3) from the peel of *Citrus kinokuni* Hort. ex TANAKA, along with 21 known compounds.

(2S)-5,6,7,8,4’-Pentamethoxyflavanone (1) was obtained as a yellow oil. The molecular formula C20H22O7 was defined by a molecular ion peak at m/z 374.1366 in the high resolution (HR)-MS. The 1H-NMR spectrum showed three characteristic signals for H-2, H-3, and H-3eq at δ 5.38 (1H, dd, J=12.8, 3.1 Hz), 3.03 (1H, dd, J=16.5, 12.8 Hz) and 2.84 (1H, dd, J=16.5, 3.1 Hz), respectively, indicating that I had a flavanone skeleton. Five methoxy signals were observed at δ 4.05—3.83. In the aromatic proton region, A 2B2 signals at δ 6.94 were assigned to H-2 and H-3eq and the 13C-NMR spectrum showed five methoxy carbons were observed at lower magnetic field (δ 61.6—61.4), and the 1H-NMR signals (δ 3.90 (4H, d, J=8.5 Hz) and 3.92 (3H, d, J=15.6 Hz, H-8′) and 3.83 showed no enhancement of any aromatic proton signals. The 13C-NMR spectrum showed that the signals of two methoxy carbons were observed at lower magnetic field (δ 61.6, 61.3), and suggested that these two methoxy groups had substituents at both ortho positions, respectively.3) The H-5 and H-8 signals of flavonoids resonate at δ 7.5—7.6 and δ 6.5—6.9, respectively. Thus, the A-ring aromatic proton signal at δ 6.35 (1H, s) was assigned to H-8. Though 2 had no optical activity in a polarimeter at 589 nm,4) the CD spectrum showed a positive Cotton effect at 345 nm and a negative one at 313 nm, consistent with the S-configuration at C-2.4) The structure of 2 was deduced as (2S)-5,6,7,3’,4’-pentamethoxyflavanone.

2’-Hydroxy-3,4,3’,4’,6’-pentamethoxychalcone (3) was obtained as pale yellow needles, mp 134—136 °C. The molecular formula C20H22O7 was defined by a molecular ion peak at m/z 374.1368 in the HR-MS. The UV absorption (373 nm), the 1H-NMR signals (δ 7.74 (1H, d, J=15.6 Hz, H-β), 7.87 (1H, d, J=15.7 Hz, H-α)) and the 13C-NMR signals (δc 144.2 (C-β), 126.6 (C-α)) strongly suggested the presence of a chalcone skeleton. The 1H-NMR spectrum showed five methoxy signals (δ 4.03—3.70) and a hydrogen δ 4.04 (3H, s, H-8′).
bonded 2'-OH resonating at δ 13.88. The ABC type protons at δ 7.29 (1H, dd, J=8.5, 1.8 Hz), 7.32 (1H, d, J=1.8 Hz) and 7.02 (1H, d, J=8.3 Hz) could be assigned to H-6, H-2 and H-5 of a 3,4-methoxy-B-ring. In the NOE experiment, irradiation of the signal at δ 3.90 (3-OMe) caused 11% enhancement of the signal at δ 7.32 (H-2) and irradiation of the signal at δ 3.87 (4-OMe) caused 14% enhancement of the signal at δ 7.02 (H-5). These results indicated the presence of a 3,4-dimethoxy-B-ring. In the NOE experiment, irradiation of the methoxy signals at δ 4.03 and 3.96 caused 12% and 16% enhancement of the signal at δ 6.32, respectively. Two possibilities (3',4',6'- or 3',5',6'-methoxylated) remained for the A-ring substitution. The positions of the three methoxy groups on the A-ring were elucidated through the use of the heteronuclear multiple bond correlation (HMBC) experiment (Fig. 2). The key correlation for assignment of A-ring substitution was observed for H-5' (=1''), C-3', 2'-OH=C-1'. Thus, the A-ring aromatic proton signal at δ 6.32 (1H, s) was assigned to H-5', and 3 must therefore be 2'-hydroxy-3',4',3'6'-pentamethoxylchalcone.

The known compounds were fully characterized as scoparine (4), scopoletin (5), nobiletin (6),

Fig. 2. C–H Long-Range Correlation in the HMBC Spectrum of 2'-Hydroxy-3',4',3'6'-pentamethoxylchalcone (3)
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References and Notes