Natural Product Sciences
6(4) : 179~182 (2000)

Chemical Constituents of the Fruit of *Citrus junos*

Eun Jung Cho[1], Xianglan Piao[1], Longzhu Piao[1,2], Huishan Piao[2], Man Ki Park[1], Bak Kwang Kim[1] and Jeong Hill Park[1,*]

[1]Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea[2]College of Pharmacy, Yanbian University, Jilin 133000, China

Abstract – Nine compounds were isolated from the fruit of *Citrus junos*. Their structures were elucidated as 9-hydroxy-4-methoxypsoralen, auraptene, limonin, deacetylnomilin, cirsimaritin, narirutin, naringin, hesperidin and neohesperidin by physico-chemical evidences. 9-Hydroxy-4-methoxypsoralen and auraptene have not been reported from *C. junos* yet.

Key words – *Citrus junos*, Rutaceae, limonoid, flavonoid, coumarin, auraptene, 9-hydroxy-4-methoxypsoralen

Introduction

Citrus junos Sieb (*Rutaceae*), the hybrid of *C. inchangensis* and *C. reticulata* var. *austera* (Herman, *et al.*, 1989) has been cultivated mainly in Korea, China, and Japan (Kim, *et al.*, 1981). The fruit has been used as an aromatic bitter peptic, an expectorant, and a cough remedy in folk medicine (Kim, *et al.*, 1981). It is widely cultivated in southern seashore of Korea for the fruit. The fruit is mainly consumed as *junos* honey and *junos* juice in Korea. Limonoids (Bennett, 1971; Dreyer, *et al.*, 1976), coumarins (Wu, *et al.*, 1988; Wu, *et al.*, 1988) and flavonoids (Wu, 1989; Fukugawa, H. *et al.*, 1988) were reported from the fruit of *C. junos*. These compounds have various activities that include anticarcinogenic activity (Lam, *et al.*, 1994), antitumorigenic activity (Tanaka, *et al.*, 1997; Bracke, *et al.*, 1994), antihypertensive activity (Itoigawa, *et al.*, 1994), cholesterol-lowering activity (Kurowska, *et al.*, 1997) and antithrombogenic activity (Nogata, *et al.*, 1996).

This paper describes the isolation and identification of nine compounds from the fruit of *C. junos*. Among them, 9-hydroxy-4-methoxypsoralen and auraptene have not been reported yet from *C. junos*.

Experimental

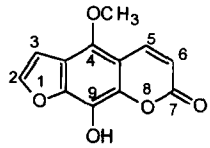
Fruits of *C. junos* were purchased from Garak agricultural and marine products wholesale market in Seoul.

Melting points were recorded on a Gallenkamp melting point apparatus (UK), UV spectra were measured on a Shimadzu UV-2100 UV/VIS spectrometer (Shimadzu, Japan). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Jeol JNM-GSX 300 spectrometer (Jeol, Japan). IR spectra were obtained on Perkin-Elmer 1710 spectrometer (USA) and mass spectra were obtained using VG TRIO-II GC/MS system (UK). Silica gel 60 and TLC plates were purchased from Merck (Germany).

Isolation of compounds – The fruit of *C. junos* (10 kg) was extracted with MeOH under reflux. Methanol was removed in reduced pressure and the extract (980 g) was partitioned between CH_2Cl_2 and water to yield CH_2Cl_2-soluble fraction (12.8 g). The aqueous layer was further extracted with *n*-BuOH to yield BuOH-soluble fraction (66.7 g).

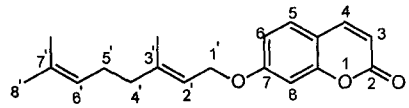
CH_2Cl_2-soluble fraction (10 g) was chromatographed over silica gel (50 g, 4×47 cm) using stepwise gradient elution with $CHCl_3$/MeOH mixture (100:1→5:1). Seven fractions (Fr. 1~Fr. 7) were obtained. Repeated column chromatography of each fraction yielded compound A (13.9 mg) and B (186.9 mg) from fraction 3, Compound C (152.9 mg) from fraction 4, Compound D (5.5 mg) and E (24.9 mg) from fraction 5. The BuOH-soluble fraction (20 g) was chromatographed over silica gel. Stepwise gradient elution with $CHCl_3$-MeOH-H_2O (90:20:1→MeOH) yielded 7 fractions (Fr. 1~Fr. 7). Repeated column chromatography of fraction 4 yielded compound F (22.5 mg), G (13.8 mg), H (26.4 mg) and I (13.5 mg).

Compound A (9-hydroxy-4-methoxypsoralen): Yellow crystal (MeOH), $C_{12}H_8O_5$, mp: 222-223°,



9-hydroxy-4-methoxypsoralen

$C_{12}H_8O_5$ (MW 232)



Auraptene

$C_{19}H_{22}O_3$ (MW 298)

Structure of compounds A and B

TLC R_f: 0.56 ($CHCl_3$/MeOH=20:1; Kieselgel $60F_{254}$), UV (MeOH) λ_{max}: 206, 323 nm, IR ν_{max} (KBr): 3356, 1708, 1147, 1592, 1481 cm^{-1}, Mass (EI+, *m/z*): 232 $[M]^+$, ^{1}H-NMR (300 MHz, DMSO-d_6, δppm): 10.07 (1H, s, 9-OH), 8.15 (1H, d, J=9.75 Hz, H-5), 8.03 (1H, d, J=2.19 Hz, H-2), 7.28 (1H, d, J=2.19 Hz, H-3), 6.30 (1H, d, J=9.75 Hz, H-6), 4.08 (3H, s, 4-OCH_3). ^{13}C-NMR (75 MHz, DMSO-d_6, δppm): 146.61 (C-2), 105.78 (C-3), 115.23 (C-3a), 145.90 (C-4), 107.51 (C-4a), 140.28 (C-5), 112.81 (C-6), 160.35 (C-7), 141.61 (C-8a), 125.87 (C-9), 147.31 (C-9a), 61.51 (4-OCH_3).

Compound B (auraptene): Amorphous solid (MeOH), $C_{19}H_{22}O_3$, mp: 68-69°, TLC R_f: 0.93 ($CHCl_3$/MeOH= 20:1; Kieselgel $60F_{254}$), UV (MeOH) λ_{max}: 206, 323 nm, IR λ_{max} (KBr): 1729, 1612, 1508, 1127 cm^{-1}, Mass (EI+, *m/z*): 298 $[M^+]$. ^{1}H-NMR (300MHz, $CDCl_3$, δppm): 7.61 (1H, d, J=9.51Hz, H-4), 6.22 (1H, d, J=9.51Hz, H-3), 7.33 (1H, d, J=8.55Hz, H-5), 6.82 (1H, dd, J=8.55, 2.43Hz, H-6), 6.80 (1H, d, J=2.43 Hz, H-8), 4.58 (2H, d, J=6.6Hz, H-1'), 5.44 (1H, t, J=6.6Hz, H-2'), 2.09 (4H, m, H-4', H-5'), 5.05 (1H, m, H-6'), 1.74 (3H, s, H-3'-CH_3), 1.64 (3H, s, H-8'), 1.58 (3H, s, 7'-CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$, δppm): 161.26 (C-2), 112.95 (C-3), 143.41 (C-4), 128.64 (C-5), 113.22 (C-6), 162.13 (C-7), 101.58 (C-8), 155.85 (C-9), 112.40 (C-10), 65.48 (C-1'), 118.39 (C-2'), 142.34 (C-3'), 39.49 (C-4'), 26.21 (C-5'), 123.59 (C-6'), 131.94 (C-7'), 25.63 (C-8'), 17.69 (7'-CH_3), 16.74 (3'-CH_3).

Compound C (limonin): Colorless needle ($CHCl_3$), $C_{26}H_{30}O_8$, mp: 298-300°, TLC R_f: 0.38 ($CHCl_3$/ MeOH=20:1), UV (MeOH) λ_{max}: 211 nm, IR ν_{max} (KBr) : 1757, 1262, 1029 cm^{-1}, Mass (FAB+, *m/z*): 471 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6, δppm): 7.71 (1H, s, H-21), 7.65 (1H, t, J=1.7Hz, H-23), 6.50 (1H, d, J=1.7 Hz, H-22), 5.46 (1H, s, H-17), 4.91 (1H, d, J= 13.0 Hz, H-19), 4.47 (1H, d, J=13.0 Hz, H-19), 4.10 (2H, s, H-15, H-1), 3.11 (1H, t, J=15.3 Hz, H-5), 2.76 (1H, d, J=16.3 Hz, H-2), 2.61 (1H, dd, J=16.3, 4.0 Hz, H-2), 2.55 (1H, dd, J=12.4, 3.1 Hz, H-9), 2.44 (1H, dd, J=15.0, 3.2 Hz, H-6), 2.26 (1H, dd, J=15.0, 3.2 Hz, H-6), 1.81 (1H, m, H-11β), 1.71 (1H, m, H-11α), 1.70 (1H, m, H-12β), 1.22 (1H, m, H-12α), 1.17 (3H, s, 4α-Me), 1.01 (3H, s, 4β-Me), 0.98 (3H, s, 8-Me), 1.09 (3H, s, 13-Me), ^{13}C-NMR (DMSO-d_6, δppm): 78.51 (C-1), 35.79 (C-2), 170.44 (C-3), 79.64 (C-4), 58.01 (C-5), 36.31 (C-6), 208.21 (C-7), 50.37 (C-8), 46.55 (C-9), 45.34 (C-10), 17.62 (C-11), 29.83 (C-12), 37.70 (C-13), 66.78 (C-14), 53.79 (C-15), 167.46 (C-16), 77.52 (C-17), 64.90 (C-19), 120.31 (C-20), 141.84 (C-21), 110.32 (C-22), 143.48 (C-23), 29.83 (4α-Me), 21.52 (4β-Me), 19.77 (17-Me), 17.10 (8-Me).

Compound D (deacetylnomilin): Amorphous solid, $C_{26}H_{32}O_8$, mp: 263-265°, TLC R_f: 0.30 ($CHCl_3$/MeOH= 20:1), UV (MeOH) λ_{max}: 205 nm, IR ν_{max}(KBr): 3419, 1717, 1119, 1027cm^{-1}, Mass (FAB+, *m/z*): 473 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6, δppm): 7.71 (1H, s, H-21), 7.65 (1H, t, J=1.7 Hz, H-23), 6.49 (1H, d, J=1.7 Hz, H-22), 5.42 (1H, s, H-17), 5.39 (1H, d, J=5.4 Hz, 1-OH), 3.81 (1H, s, H-15), 3.63 (1H, t, J=6.0 Hz, H-1), 3.01 (1H, t, J=14.4 Hz, H-5), 2.65 (2H, m, H-2), 2.59 (1H, d, J=11.0 Hz, H-9), 2.42 (1H, dd, J=14.2, 3.7 Hz, H-6), 2.28 (1H, dd, J=14.2, 3.7 Hz, H-6), 1.70 (2H, m, H-11), 1.37 (1H, m, H-12), 1.17 (1H, m, H-12α), 1.45 (3H, s, 4α-Me), 1.26 (3H, s, 4β-Me), 1.14 (3H, s, 10-Me), 1.09 (3H, s, 8-Me), 0.98 (3H, s, 13-Me). ^{13}C-NMR (DMSO-d_6, δppm): 68.39 (C-1), 39.09 (C-2), 170.85 (C-3), 83.84 (C-4), 49.44 (C-5), 38.88 (C-6), 208.77 (C-7), 52.04 (C-8), 43.75 (C-9), 44.25 (C-10), 16.74 (C-11), 31.32 (C-12), 36.85 (C-13), 65.79 (C-14), 52.69 (C-15), 167.24 (C-16), 77.56 (C-17), 120.20 (C-20), 141.62 (C-21), 110.26 (C-22), 143.38 (C-23), 23.10 (4α-Me), 33.03 (4β-Me), 20.22 (13-Me), 16.11 (8-Me), 15.95 (10-Me).

Compound E (cirsimaritin): Yellow crystal $C_{17}H_{14}O_6$, mp: 258-259°, TLC R_f: 0.29 ($CHCl_3$/MeOH=20:1), UV (MeOH) λ_{max}: 211, 273, 321 nm, IR ν_{max}($CHCl_3$): 3442, 1713, 1613, 1455 cm^{-1}, Mass (EI+, *m/z*): 314

[M+]. ^{1}H-NMR (C_5D_5N, δppm): 13.67 (1H, s, 5-OH), 12.74 (1H, s, 4'-OH), 7.96 (2H, d, J=8.8 Hz, H-2', H-6'), 7.27 (2H, d, J=8.8 Hz, H-3', H-5'), 6.96 (1H, s, H-3), 6.80 (1H, s, H-8), 3.99 (3H, s, 6-OCH_3), 3.87 (3H, s, 7-OCH_3). ^{13}C-NMR (C_5D_5N, δppm): 164.7 (C-2), 103.7 (C-3), 183.1 (C-4), 153.6 (C-5), 133.0 (C-6), 159.3 (C-7), 91.5 (C-8), 153.4 (C-9), 106.3 (C-10), 122.1 (C-1'), 128.9 (C-2', C-6'), 116.8 (C-3', C-5'), 162.8 (C-4'), 60.5 (6-OCH_3), 56.3 (7-OCH_3).

Compound F (narirutin): $C_{27}H_{32}O_{14}$, mp: 160-162°, Mass (FAB+, *m/z*): 581 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6, δppm): 12.03 (1H, s, 5-OH), 9.60 (1H, s, 4'-OH), 7.33 (2H, d, J=8.4 Hz, H-2', H-6'), 6.81 (1H, d, J=8.4 Hz, H-3', H-5'), 5.39 (1H, dd, J=10.4,2.4 Hz, H-2), 3.17 (1H, dd, H-3), 2.75 (1H, dd, H-3), 6.19 (1H, d, J=2.0 Hz, H-6), 6.16 (1H, d, J=2.0 Hz, H-8), 4.93 (1H, s, glc-1), 4.68 (1H, s, rha-1), 1.07 (3H, d, J=6.4 Hz, rha-6). ^{13}C-NMR (DMSO-d_6, δppm): 78.4 (C-2), 42.4 (C-3), 197.3 (C-4), 163.0 (C-5), 96.3 (C-6), 165.0 (C-7), 95.5 (C-8), 162.7 (C-9), 103.2 (C-10), 128.7 (C-1'), 128.4 (C-2', C-6'), 115.2 (C-3', C-5'), 157.6 (C-4'), 99.3 (glc-1), 72.9 (glc-2), 76.2 (glc-3), 69.6 (glc-4), 75.5 (glc-5), 66.0 (glc-6), 100.6 (rha-1), 70.2 (rha-2), 70.7 (rha-3), 72.0 (rha-4), 68.3 (rha-5), 17.8 (rha-6).

Compound G (naringin): $C_{27}H_{32}O_{14}$, mp: 171-173°, UV (MeOH) λ_{max}: 226, 281, 327 nm, IR ν_{max} ($CHCl_3$): 3421, 1647, 1520, 1457 cm^{-1}, Mass (FAB+, *m/z*): 581 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6, δppm): 12.04 (1H, s, 5-OH), 9.62 (1H, s, 4'-OH), 7.32 (2H, d, J=7.8 Hz, H-2', H-6'), 6.79 (2H, d, J=7.8 Hz, H-3', H-5'), 5.53 (1H, dd, J=11.6, 2.9 Hz, H-2), 3.20 (1H, m, H-3), 2.72 (1H, dd, H-3), 6.11 (1H, d, J=2.2 Hz, H-6), 6.08 (1H, d, J=2.2 Hz, H-8), 5.11 (2H, m, glc-1, rha-1), 1.15 (3H, d, J=6.1 Hz, rha-6). ^{13}C-NMR (DMSO-d_6, δppm): 79.1 (C-2), 42.4 (C-3), 197.7 (C-4), 163.4 (C-5), 96.7 (C-6), 165.2 (C-7), 95.5 (C-8), 163.2 (C-9), 103.7 (C-10), 129.0 (C-1'), 128.9 (C-2', C-6'), 115.6 (C-3', C-5'), 158.2 (C-4'), 100.8 (glc-1), 76.5 (glc-2), 77.5 (glc-3), 70.0 (glc-4), 77.3 (glc-5), 60.9 (glc-6), 97.8 (rha-1), 70.9 (rha-2), 70.8 (rha-3), 72.2 (rha-4), 68.7 (rha-5), 18.5 (rha-6).

Compound H (hesperidin): $C_{28}H_{34}O_{15}$, mp: 258-260°, UV (MeOH) λ_{max}: 203, 283, 326 nm, IR ν_{max} ($CHCl_3$): 3425, 1648, 1521 cm^{-1}, Mass (FAB+, *m/z*): 581 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6, δppm): 12.01 (1H, s, 5-OH), 3.72 (3H, s, 4'-OCH_3), 9.09 (1H, s, 3'-OH), 6.92 (3H, m, H-2', H-5', H-6'), 5.49 (1H, dd, J=12.2,3.2 Hz, H-2), 3.21 (1H, m, H-3), 2.76 (1H, dd, H-3), 6.14 (1H, d, J=1.95 Hz, H-6), 6.12 (1H, d, J=1.95 Hz, H-8), 4.96 (1H, s, glc-1), 4.52 (1H, s, rha-1), 1.08 (3H, d, J=6.1 Hz, rha-6). ^{13}C-NMR (DMSO-d_6, δppm): 78.8 (C-2), 42.2 (C-3), 197.4 (C-4), 163.5 (C-5), 96.8 (C-6), 165.6 (C-7), 96.0 (C-8), 162.9 (C-9), 103.7 (C-10), 131.3 (C-1'), 114.6 (C-2'), 146.9 (C-3'), 148.4 (C-4'), 112.4 (C-5'), 118.4 (C-6'), 101.0 (glc-1), 73.4 (glc-2), 76.7 (glc-3), 70.0 (glc-4), 75.9 (glc-5), 66.5 (glc-6), 99.9 (rha-1), 70.7 (rha-2), 71.1 (rha-3), 72.5 (rha-4), 68.7 (rha-5), 18.3 (rha-6), 56.1 (4'-OCH_3).

Compound I (neohesperidin): $C_{28}H_{34}O_{15}$, mp: 244-246°, Mass (FAB+, *m/z*): 581 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6, δppm): 12.07 (1H, s, 5-OH), 3.83 (3H, s, 4'-OCH_3), 9.18 (1H, s, 3'-OH), 7.00 (1H, m, H-2'), 6.99 (1H, m, H-5'), 6.94 (1H, m, H-6'), 5.54 (1H, dd, J=12.1,3.0 Hz, H-2), 3.28 (1H, m, H-3), 2.80 (1H, dd, H-3), 6.17 (1H, d, J=2.3 Hz, H-6), 6.15 (1H, d, J=2.3 Hz, H-8), 5.18 (1H, d, J=3.5 Hz, glc-1), 5.16 (1H, d, J=3.7 Hz, rha-1), 1.22 (3H, d, J=6.2 Hz, rha-6). ^{13}C-NMR (DMSO-d_6, δppm): 78.5 (C-2), 42.2 (C-3), 197.2 (C-4), 162.9 (C-5), 96.3 (C-6), 164.8 (C-7), 95.2 (C-8), 162.7 (C-9), 103.4 (C-10), 130.9 (C-1'), 114.2 (C-2'), 146.5 (C-3'), 148.1 (C-4'), 112.0 (C-5'), 118.0 (C-6'), 100.5 (glc-1), 76.2 (glc-2), 77.2 (glc-3), 69.6 (glc-4), 76.9 (glc-5), 60.5 (glc-6), 97.0 (rha-1), 70.4 (rha-2), 70.4 (rha-3), 71.9 (rha-4), 68.4 (rha-5), 18.1 (rha-6), 55.7 (4'-OCH_3).

Results and Discussion

Compound A, $C_{12}H_8O_5$, showed band for hydroxy group at 3356 cm^{-1}, carbonyl band at 1708 cm^{-1}, ester C-O at 1147 cm^{-1}, aromatic C=C at 1592 cm^{-1}, 1481 cm^{-1} in IR spectrum. The mass spectrum showed $[M]^+$ peak at *m/z* 232. The ^{1}H-NMR spectrum of compound A showed aromatic hydroxy signal at δ 10.08 (1H, s), aromatic methoxy signal at δ 4.08 (3H, s), a pair of doublets at δ 8.16 (1H, d, J = 9.75 Hz) and δ 6.30 (1H, d, J=9.75 Hz), α, β-furan protons at δ 8.03 (1H, d, J=2.19 Hz) and δ 7.28 (1H, d, J = 2.19 Hz) suggesting coumarin unsubstituted pyrone ring. The ^{13}C-NMR spectrum of compound A showed 12 carbon peaks. One methoxy carbon signal at δ 61.51, one carbonyl signal at δ 160.35, and ten conjugated carbons between δ 100 and 150 ppm. The NOE was observed at H-3 and H-5 on irradiation of methoxy methyl protons, and at H-2 and methoxy methyl protons on irradiation of H-3, which suggest methoxy group at C-4 position. Thus, compound A was identified as 9-hydroxy-4-methoxypsoralen which is isolated

from *Apium graveolens* (Garg, S. K., *et al.*, 1979) but not reported from *C. junos* yet.

Compound B, $C_{19}H_{22}O_3$, showed band for carbonyl at 1729 cm^{-1}, aromatic C=C at 1612, 1508 cm^{-1}, ester C-O at 1127 cm^{-1} in IR spectrum. The EI-MS spectrum showed $[M]^+$ peak at *m/z* 298. The ^{1}H-NMR spectrum of compound B displayed a pair of doublets at δ 7.61 (1H, d, J=9.5 Hz) and 6.22 (1H, d, J=9.5 Hz) suggesting coumarin unsubstituted pyrone ring. The ^{13}C-NMR spectrum of compound B showed 19 carbon peaks, one carbonyl signal at δ 161.26, twelve conjugated carbon signals between δ 101.58 and 162.13, one -OCH_2- at δ 65.48, and three methyl signals at δ 25.63, 17.69, 16.74 ppm. Based on these spectral data, compound B was identified as auraptene which is isolated from *Feronia elephantum* and *Pleiospermium alatum* (Talapatra, S. K., *et al.*, 1973; Bandara, B. M. R, *et al.*, 1988) but not from *C. junos* yet.

The structures of compounds C, D, E, F, G, H, and I were elucidated as limonin, acetylnomilin, cirsimaritin, narirutin, naringin, hesperidin and neohesperidin, respectively, by the comparison with reported data (Dreyer, D. L., *et al.*, 1976; Dreyer, D. L., 1965; Agrawal, P. K., 1989; Voirin, B., 1983; Markman, K. R., *et al.*, 1976).

References

Agrawal, P. K., Carbon-13 NMR of flavonoids, *Elsevier*, New York, 316-317 (1989).

Bandara, B. M. R. and Gunatilaka, A. A. L., Coumarins from *Pleiospermium alatum*. *Planta Med.* **54**, 91-92 (1988).

Bennett, R. D., Acidic limonoids of grapefruit seeds. *Phytochemistry* **10**, 3065-3038 (1971).

Bracke, M. E., Vennekens, K. M., Bruyneel, E. A., Vermeulen, S. J. and Mareel, M. M., The *Citrus* flavonoid tangeretin enhances cell-cell adhesion and inhibits invasion of human MCF-7/6 breast-carcinoma cell. *Abstracts of Papers of the American Chemical Society*, **208**, 81 (1994).

Dreyer, D. L., Bennett, R. D., Basa, S. C., Limonoids from *Atalantia*. Isolation and structure. *Tetrahedron* **32**, 2367-2373 (1976).

Dreyer, D. L., *Citrus* bitter principles-II. Application of NMR to structural and stereochemical problems. *Tetrahedron* **21**, 75-87 (1965).

Dreyer, D. L., *Citrus* bitter principles. III. Isolation of deacetylnomilin and deoxylimonin. *J. Org. Chem.* **30**, 749-751 (1965).

Fukugawa, H. *et al.*, A new flavonoid and other new compounds from *Citrus* plants. *Chem. Pharm. Bull.* **36**, 3292-3295 (1988).

Garg, S. K., Gupta, S. R. and Sharma, N. D., Minor phenolics of *Apium graveolens* seeds. *Phytochemistry* **18**, 352 (1979).

Han, D. Y., *et al.*, Modern Pharmacognosy, *Hak Chang Sa*, pp. 80-81 (1981).

Herman, Z., Hasegawa, S., Fong, Chi, H. and Ou, P., Limonoids in *Citrus ichangensis*. *J. Agric. Food Chem.* **37**, 850-851 (1989).

Itoigawa, M., Takeya, K. and Furukawa, Cardiotonic flavonoids from *Citrus* plants. *Biol. Pharm. Bull.* **17**, 1519-1521 (1994).

Kurowska, E. M., Borradaile, N., Meade, M., Spence, J. D. and Carroll, K. K., Cholesterol-lowering effects of dietary *Citrus* juices and their flavonoids. Studies in rats, mice and rabbits. *Atherosclerosis* **134**, 330 (1997).

Lam, L. K. T., Zheng, G. Q., Zhang, J. and Kenney, D. M., The importance of the B-ring of the limonoid nucleus to the cancer chemopreventive activity of *Citrus* limonoids. *Abstracts of Papers of the American Chemical Society* **208**, 79 (1994).

Markman, K. R., Ternai, B., ^{13}C NMR of flavonoids-II. Flavonoids other than flavone and flavonol aglycones. *Tetrahedron* **32**, 2607-2612 (1976).

Nogata, Y., Yoza, K., Kusumoto, K., Kohyama, N., Sekiya, K. and Ohta, H., Screening for inhibitory activity of *Citrus*-fruit extracts against platelet cyclooxygenase and lipoxygenase. *J. Agric. Food Chem.* **44**, 725-729 (1996).

Talapatra, S. K., Chaudhuri, M. K, Talapatra, B., Coumarins of the root bark of *Feronia elephantum*. *Phytochemistry* **12**, 236 (1973).

Tanaka, T., Kawabata, K., Kakumoto, M., Makita, H., Hara, A., Mori, H., Satoh, K., Murakami, A., Kuki, W., Takahashi, Y., Yonei, H., Koshimizu, K. and Ohigashi, H., *Citrus auraptene* inhibits chemically-Induced colonic aberrant crypt foci in male F344 rats. *Carcinogenesis* **18**, 2155-2161 (1997).

Tanizawa, H., Ohkawa, Y., Takino, Y., Miyase, T., Ueno, A., Kageyama, T. and Hara, S., Studies on natural antioxidants in *Citrus* species. I. Determination of antioxidative activities of Citrus fruits. *Chem. Pharm. Bull.* **40**(7), 1940-1942 (1992).

Voirin, B., UV spectral differentiation of 5-hydroxy- and 5-hydroxy-3-methoxyflavones with mono-(4'), di-(3`,4') or tri-(3',4',5')-substituted B rings. *Phytochemistry* **22**, 2107-2145 (1983).

Wu, T. S., Alkaloids and coumarins of *Citrus grandis*. *Phytochemistry* **27**, 3717-3718 (1988).

Wu, T. S., Flavonoids from root bark of *Citrus sinensis* and *C. nobilis*. *Phytochemistry* **28**, 3558-3560 (1989).

Wu, T. S., Huang, S. C., Jong, T. T., Lai, J. S. and Kuoh, C. S., Coumarins, Acridone alkaloids and a flavone from *Citrus*. *Phytochemistry* **27**, 585-587 (1988).

(Accepted November 16, 2000)