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A New Flavonoid and Other New Components from *Citrus* Plants[1)]

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A new flavonoid, citflavanone (**1**), and prenylated phenol derivatives, etrogol (**7**) and valencic acid (**8**), were isolated from roots and root barks of *Citrus natsudaidai* (natsudaidai), *C. medica* (etrog citron), *C. sinensis* (valencia orange), and several hybrid seedlings resulting from hyuga-natsu × hirakishu, and characterized. In order to determine the structure of citflavanone (**1**), prenylation of naringenin (**3**) was attempted to give 6- and 8-prenylnaringenin (**4** and **5**, respectively) as well as 6,8-diprenylnaringenin (**6**). The location of the prenyl moiety in **4** and **5** was established by means of the long-range selective proton decoupling technique in nuclear magnetic resonance spectrometry.

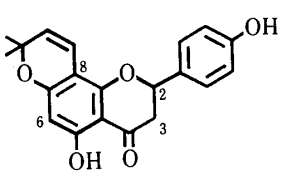
Keywords——*Citrus*; Rutaceae; flavonoid; flavanone; citflavanone; etrogol; valencic acid; prenyl; hybrid seedling

In continuing our phytochemical studies of *Citrus* plants,[2)] we have examined constituents of the roots and root barks of *Citrus natsudaidai* HAYATA (common name: natsudaidai), *C. medica* L. var. *ethrog* ENGL. (etrog citron), *C. sinensis* OSBECK (valencia orange), and several hybrid seedlings resulting from a cross of *C. tamurana* HORT. *ex.* TANAKA (hyuga-natsu) × *C. kinokuni* HORT. *ex.* TANAKA (hirakishu), and a new flavonoid, citflavanone (**1**), and two other new components, etrogol (**7**) and valencic acid (**8**), were isolated and characterized.

Results and Discussion

Structure of Citflavanone (1)

Citflavanone, a new flavonoid, was obtained as pale yellow prisms, mp 98—100 °C, $[\alpha]_D$ −3.7° (chloroform). The infrared (IR) spectrum gave absorption bands at ν_{max} 3300, 1640, 1620, and 1590 cm^{-1} indicating the presence of hydroxy, conjugated carbonyl, and phenyl



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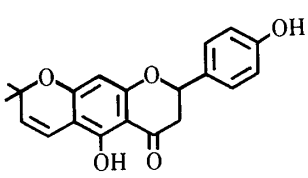

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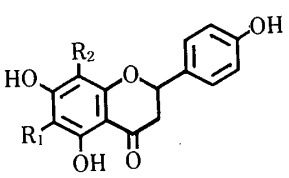
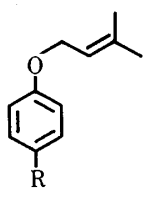


3: $R_1=R_2=H$
4: R_1=prenyl, $R_2=H$
5: $R_1=H$, R_2=prenyl
6: $R_1=R_2$=prenyl



7: $R=-CH_2CH_2OH$
8: $R=-COOH$

groups. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum revealed the presence of a dimethylpyran ring [δ 6.53 and 5.46 (each 1H, d, J=10 Hz), 1.44 and 1.42 (each 3H, s, 2×CH_3)], 1,4-disubstituted aromatic ring [δ 7.34 and 6.89 (each 2H, d, J=8.4 Hz)], and two hydroxy groups [δ 12.09 (1H, s, strongly hydrogen-bonded) and 4.90 (1H, br s)]. The ABX-type signals at δ 5.36 (1H, dd, J=3.0, 12.4 Hz), 3.06 (1H, dd, J=12.4, 17.1 Hz), and 2.79 (1H, dd, J=17.1, 3.0 Hz) are characteristic of protons attached to C-3 and C-2 of the flavanone ring. A 1H singlet at δ 5.99 was observed as an additional signal. The appearance of diagnostic fragment peaks at m/z 218 and 120, produced by a retro-Diels–Alder process at the B-ring in the flavanone nucleus,[3] suggested the location of a dimethylpyran ring on the A-ring. Based on these results, we assumed that citflavanone has the structure **1** or **2**. The yield of citflavanone from the plant was so small that we could not carry out further spectrometric analyses of citflavanone itself.

Thus, in order to confirm the direction of the dimethylpyran ring, syntheses of **1** and **2** were attempted. Treatment of naringenin (**3**)[4,5] with prenyl bromide on aluminium oxide surfaces[6] gave two kinds of mono-prenylnaringenin (A and B) as well as a diprenyl derivative. The presence of a prenyl substituent on the A-ring in both A and B was indicated by the follow results. a) Observation of a diagnostic fragment peak at m/z 220 produced by retro-Diels–Alder type fragmentation of the B-ring in the mass spectrum (MS).[3] b) Appearance of A_2B_2-type signals in the ^{1}H-NMR spectra (see Experimental). c) Appearance of a 1H singlet at δ 5.99 in the spectrum of A, and at δ 6.02 in that of B.

The location of the prenyl moiety at C-6 or C-8 in A and B was determined by a long-range decoupled carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrometric analysis. In the ^{13}C-NMR spectrum of A, the signal of the carbon carrying a proton with δ_H 5.99 was observed at δ_C 95.80 as a doublet (J=161.4 Hz), and this signal was found not to change after long-range selective proton decoupling (LSPD) of a strongly hydrogen-bonded hydroxy proton at δ_H 12.40. On the other hand, in the spectrum of B, the carbon signal at δ_C 96.88 due to the carbon bearing a proton with δ_H 6.02 was observed as a double doublet (J=161.4 and 7.3 Hz), and changed to a doublet (J=161.4 Hz) after LSPD of a hydrogen-bonded hydroxy proton at δ_H 12.00. These results established the location of the prenyl moiety as being at C-6 in A (formula **4**) and at C-8 in B (**5**); these compounds are known natural products.[7,8] The diprenylated compound was found to be identical with senegalensein (**6**)[5,9,10] by comparison of IR and ^{1}H-NMR spectral data with values in the literature.[11] No other prenylated product could be detected under these reaction conditions.

Next, treatment of **4** and **5** with phenylselenenyl chloride in dry ethyl acetate at −75 to −50 °C, followed by hydrogen peroxide and anhydrous magnesium sulfate afforded cyclization products[12,13] which showed spectral data in accord with structure **2** and **1**, respectively (see Experimental). The latter **1** was found to be identical (^{1}H-NMR, IR, ultraviolet (UV), MS, and co-thin layer chromatography (co-TLC) with the natural flavonoid, citflavanone. On the basis of these results, we proposed the structure **1** for citflavanone, except for the absolute stereochemistry.

Structure of Etrogol (7)

Etrogol was obtained as a colorless oil from roots and root barks of etrog citron. The molecular formula $C_{13}H_{18}O_2$ was proposed from the chemical ionization mass spectrum (CI-MS), and ^{1}H-NMR spectrum. The 4-substituted phenolic nucleus was suggested by UV, IR (see Experimental), and ^{1}H-NMR signals at δ 6.87 and 7.14 (each 2H, d, J=8.7 Hz). The ^{1}H-NMR signals at δ 5.49 (1H, t, J=6.7 Hz), 4.49 (2H, d, J=6.7 Hz), 1.79 (3H, s), and 1.74 (3H, s), together with the appearance of a MS fragment at m/z 138 [M−·CH_2CH=$C(CH_3)_2$+·H], and no shift of the UV bands in alkaline solution, revealed the presence of a prenyl moiety attached to a phenolic oxygen. The remaining two 2H triplets at δ 3.83 and

2.81, and IR bands at ν_{max} 3600 and 3450 (br) cm^{-1} indicated the structure [$-CH_2CH_2OH$] for the other substituent. On the basis of these spectral data, the structure **7** was proposed for etrogol.

Structure of Valencic Acid (8)

Valencic acid, colorless oil, was isolated from roots and root barks of valencia orange, natsudaidai, and several hybrid seedlings of hyuga-natsu × hirakishu. The molecular formula $C_{12}H_{14}O_3$ was deduced from the high-resolution MS. The ^{1}H-NMR signals at δ 8.05 and 6.95 (each 2H, d, J=8.7 Hz), together with the UV (λ_{max} 252 nm) and IR spectrum [ν_{max} 3400—2500 (br), 1690, and 1610 cm^{-1}] indicated that this compound is a *p*-hydroxybenzoic acid derivative. The presence of an O-substituted prenyl moiety deduced from the ^{1}H-NMR signals at δ 5.49 (1H, t, J=7.1 Hz), 4.59 (2H, d, J=7.1 Hz), 1.81 (3H, s), and 1.76 (3H, s) and MS fragments at m/z 138 and 121 corresponding to fragments of [$M-\cdot CH_2CH=C(CH_3)_2+\cdot H$] and [$M-\cdot OCH_2CH=C(CH_3)_2$]. Based on these results, we assigned the structure **8** to valencic acid.

Experimental

All melting points were measured on a micromelting point hot-stage apparatus (Yanagimoto). ^{1}H- and ^{13}C-NMR spectra were recorded on GX-270 (JEOL) and GX-400 (JEOL) spectrometers, respectively, in $CDCl_3$, unless otherwise stated. Chemical shifts are shown in δ-values (ppm) with tetramethylsilane (TMS) as an internal reference. Electron impact mass spectra (EI-MS) were taken with an M-52 (Hitachi) spectrometer having a direct inlet system, and CI and high-resolution MS spectra with an M-80 (Hitachi) spectrometer. UV spectra were determined in methanol and IR spectra were recorded in $CHCl_3$.

Extraction and Separation——The plant materials used were cultivated at Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture Forestry and Fisheries, Shimizu, Shizuoka.

The scientific names along with Japanese common names (in parentheses) of plant materials treated in this study as follow: *Citrus natsudaidai* HAYATA (natsudaidai), *C. medica* L. var. *ethrog* ENGL. (etrog citron), *C. sinensis* OSBECK (valencia orange), and several hybrid seedlings resulting from a cross of *C. tamurana* HORT. *ex.* TAKAHASHI (hyuga-natsu) × *C. kinokuni* HORT. *ex.* TANAKA (hirakishu).

The dried roots and root barks of each *Citrus* plant were extracted with acetone. The acetone extract was subjected to column and preparative thin layer silica gel chromatographies to obtain citflavanone (**1**), etrogol (**7**), and valencic acid (**8**) as new components as well as many kinds of coumarins.[14] The procedure for separation of these new components will be reported in detail elsewhere.

Citflavanone (1)——Pale yellow prisms from ether–hexane, mp 98—100 °C, $[\alpha]_D$ −3.7° (c=0.082, $CHCl_3$). 0.0023% in roots and root barks of natsudaidai and 0.0016% in those of several hybrid seedlings of hyuga-natsu × hirakishu. High-resolution MS: Calcd for $C_{20}H_{18}O_5$: 338.1154. Found: 338.1185. UV λ_{max} nm: 226, 264 (sh), 271, 295, 307 (sh), 336. IR ν_{max} cm^{-1}: 3300, 1640, 1620, 1590. ^{1}H-NMR δ: 12.09 (1H, s), 7.34 (2H, d, J=8.4Hz), 6.89 (2H, d, J=8.4 Hz), 6.53 (1H, d, J=10 Hz), 5.99 (1H, s), 5.46 (1H, d, J=10 Hz), 5.36 (1H, dd, J=3.0, 12.4 Hz), 4.90 (1H, br), 3.06 (1H, dd, J=12.4, 17.1 Hz), 2.79 (1H, dd, J=17.1, 3.0 Hz), 1.44 (3H, s), 1.42 (3H, s). MS m/z (%): 338 (M^+, 30), 324 (17), 323 (85), 218 (10), 204 (19), 203 (100), 149 (21), 121 (37), 120 (29).

Prenylation[6] of Naringenin (3)——Naringenin (**3**) (Tokyo Kasei Kogyo Co., Ltd.), 100 mg (0.37 mmol) in 2 ml of tetrahydrofuran (THF) was added to a slurry of 2.4 g of Al_2O_3 in ether. The solvent was evaporated off and 5 eq of prenyl bromide in ether–hexane (1 : 1) was added. The mixture was stirred for 2 h, then the Al_2O_3 was filtered off and washed with CH_2Cl_2, MeOH, and 1% HOAc–EtOAc. The solvent was evaporated off and the residue was purified by column and preparative TLC to afford 6-prenylnaringenin (**4**) (6.2 mg), 8-prenylnaringenin (**5**) (3.2 mg), and 6,8-diprenylnaringenin (**6**) (6.7 mg).

6-Prenylnaringenin (4)[7]——Colorless prisms from ether–hexane, mp 210—211 °C. UV λ_{max} nm: 221 (sh), 240 (sh), 294, 322. IR ν_{max} cm^{-1}: 3300 (br), 1640, 1620, 1600. ^{1}H-NMR δ: 12.40 (1H, s), 7.33 (2H, d, J=8.4 Hz), 6.88 (2H, d, J=8.4 Hz), 6.19 (1H, br), 5.99 (1H, s), 5.33 (1H, dd, J=12.8, 3.0 Hz), 5.25 (1H, t, J=7.4 Hz), 5.05 (1H, br), 3.36 (2H, d, J=7.4 Hz), 3.08 (1H, dd, J=12.8, 17.1 Hz), 2.78 (1H, dd, J=17.1, 3.0 Hz), 1.82 (3H, s), 1.76 (3H, s). ^{13}C-NMR (acetone-d_6) δ: 197.75 (s), 165.21 (s), 162.75 (s), 162.45 (s), 159.10 (s), 131.71 (s), 131.40 (s), 129.43 (2d), 124.06 (d), 116.65 (2d), 109.52 (s), 103.61 (s), 95.80 (d), 80.32 (d), 44.09 (t), 26.32 (q), 22.11 (q), 18.30 (q). LSPD: The doublet at δ_C 95.80 (J=161.4 Hz, C-8) did not change on irradiation of the signal at δ_H 12.40 (OH at C-5). MS m/z (%): 340 (M^+, 82), 325 (20), 297 (31), 285 (33), 220 (24), 205 (67), 192 (41), 177 (47), 165 (100).

8-Prenylnaringenin (5)[8]——Yellow oil. UV λ_{max} nm: 215 (sh), 225, 240 (sh), 294, 335. IR ν_{max} cm^{-1}: 3350 (br), 1640, 1620, 1600. ^{1}H-NMR δ: 12.00 (1H, s), 7.32 (2H, d, J=8.4 Hz), 6.88 (2H, d, J=8.4 Hz), 6.17 (1H, br), 6.02 (1H, s),

5.35 (1H, dd, $J=12.8$, 3.0 Hz), 5.19 (1H, t, $J=7.4$ Hz), 5.13 (1H, br), 3.31 (2H, d, $J=7.4$ Hz), 3.05 (1H, dd, $J=12.8$, 17.1 Hz), 2.80 (1H, dd, $J=17.1$, 3.0 Hz), 1.72 (6H, s). ^{13}C-NMR (acetone-d_6) δ: 198.00 (s), 165.47 (s), 163.44 (s), 161.54 (s), 159.06 (s), 131.64 (s), 131.53 (s), 129.27 (2d), 124.18 (d), 116.63 (2d), 108.78 (s), 103.76 (s), 96.88 (d), 80.19 (d), 43.90 (t), 26.35 (q), 22.73 (t), 18.33 (q). LSPD: The doublet ($J=161.1$ Hz) at δ_C 96.88 was found to show further fine split by 7.3 Hz. Irradiation of the proton at δ_H 12.00 (OH at C-5) changed this signal [δ_C 96.88 (C-6)] to a clear doublet. MS *m/z* (%): 340 (M^+, 100), 325 (32), 297 (32), 285 (32), 220 (27), 205 (89), 192 (50), 177 (72), 165 (79).

6,8-Diprenylnaringenin (6)[5,9,10]——Colorless oil. UV λ_{max} nm: 226, 296, 347. IR ν_{max} cm^{-1}: 3350 (br), 1630, 1620. ^{1}H-NMR δ: 12.32 (1H, s), 7.32 (2H, d, $J=8.7$ Hz), 6.87 (2H, d, $J=8.7$ Hz), 6.38 (1H, s), 5.32 (1H, dd, $J=3.0$, 12.8 Hz), 5.20 (3H, m), 3.34 (2H, d, $J=7.4$ Hz), 3.29 (2H, d, $J=7.1$ Hz), 3.04 (1H, dd, $J=12.8$, 17.1 Hz), 2.79 (1H, dd, $J=3.0$, 17.1 Hz), 1.81 (3H, s), 1.75 (3H, s), 1.71 (3H, s), 1.69 (3H, s). MS *m/z* (%): 408 (M^+, 100), 393 (17), 365 (14), 353 (34), 337 (45), 309 (24), 297 (27), 273 (31), 260 (23), 245 (28), 233 (46), 217 (12), 204 (20), 189 (90), 177 (46), 147 (12), 135 (16), 120 (23).

Cyclization[11] **of Prenylnaringenins (4) and (5)**——A dry ethyl acetate solution of phenylselenenyl chloride 22 mg (0.12 mmol) was added slowly to a stirred ethyl acetate solution of **5** (40 mg) (0.12 mmol) at -75——-50 °C. After 1 h, the solution was washed with water, dried over anhydrous $MgSO_4$, and evaporated. The residue was dissolved in THF, and 30% H_2O_2 (1 ml) and then anhydrous $MgSO_4$ (2 g) was added. The mixture was stirred for 1 h, washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was subjected to preparative TLC to give **1** (3.0 mg). **1** was found to be identical with citflavanone by UV, IR, and ^{1}H-NMR spectral comparisons and co-TLC. The same treatment of **4** (31 mg) gave **2** (11 mg) as a pale yellow oil. **2**: UV λ_{max} nm: 227, 265 (sh), 272, 294, 307, 356. IR ν_{max} cm^{-1}: 3300 (br), 1640, 1630, 1620, 1600. ^{1}H-NMR δ: 12.28 (1H, s), 7.31 (2H, d, $J=8.4$ Hz), 6.88 (2H, d, $J=8.4$ Hz), 6.61 (1H, d, $J=10$ Hz), 6.10 (1H, br), 5.95 (1H, s), 5.50 (1H, d, $J=10$ Hz), 5.33 (1H, dd, $J=12.8$, 3.0 Hz), 3.08 (1H, dd, $J=17.1$, 12.8 Hz), 2.77 (1H, dd, $J=17.1$, 3.0 Hz), 1.44 (3H, s), 1.43 (3H, s).

Etrogol (7)——Colorless oil. Content: 0.00064% in roots and root barks of Etrog citron. UV λ_{max} nm: 225, 275, 284 (sh), 326 (sh), UV λ_{max} (MeOH+KOH) nm: 225, 275, 284 (sh), 327 (sh). IR ν_{max} cm^{-1}: 3600, 3450 (br), 1610, 1510. ^{1}H-NMR δ: 7.14 (2H, d, $J=8.7$ Hz), 6.87 (2H, d, $J=8.7$ Hz), 5.49 (1H, t, $J=6.7$ Hz), 4.49 (2H, d, $J=6.7$ Hz), 3.83 (2H, t, $J=6.4$ Hz), 2.81 (2H, t, $J=6.4$ Hz), 1.79 (3H, s), 1.74 (3H, s). CI-MS (reactant gas: NH_3): *m/z* 224 ($M+NH_4$). EI-MS *m/z*: 138, 107.

Valencic Acid (8)——Colorless oil. Content: 0.001% from roots and root barks of valencia orange; 0.0021% from those of natsudaidai; 0.0009% from those of hybrid seedlings of hyuga-natsu × hirakishu. High-resolution MS: Calcd for $C_{12}H_{14}O_3$: 206.0941. Found: 206.0937. UV λ_{max} nm: 252. IR ν_{max} cm^{-1}: 3400—2500 (br), 1690, 1610. ^{1}H-NMR δ: 8.05 (2H, d, $J=8.7$ Hz), 6.95 (2H, d, $J=8.7$ Hz), 5.49 (1H, t, $J=7.1$ Hz), 4.59 (2H, d, $J=7.1$ Hz), 1.81 (3H, s), 1.76 (3H, s). MS *m/z* (%): 206 (M^+, 10), 139 (21), 138 (100), 121 (62).

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