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New Coumarins from *Citrus* Plant

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New coumarins, kiyomal (1), casegravol isovalerate (3), and *trans O*-methylgrandmarin (5) were isolated from several hybrid seedlings resulting from crosses of *Citrus unshiu* × *C. sinensis* × *C. iyo* and characterized by means of spectroscopic analysis and/or derivation from known coumarins.

Keywords *Citrus*; Rutaceae; coumarin; isovalerate; casegravol isovalerate; *trans O*-methylgrandmarin; kiyomal

During our phytochemical studies of *Citrus* plants,[1)] new coumarins, kiyomal (**1**), casegravol isovalerate (**3**), and *trans O*-methylgrandmarin (**5**), were isolated from roots of several hybrid seedlings resulting from crosses of [*Citrus unshiu* MARC. (miyagawa-wase) × *C. sinensis* OSBEC. (torobita orange)] (kiyomi) × *C. iyo* HORT. *ex* TANAKA (iyo).

Structure of Kiyomal (1) Kiyomal (**1**) was obtained as a colorless amorphous solid with the molecular formula $C_{12}H_{10}O_4$ as judged from the high-resolution mass spectrum (MS). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of kiyomal, a 3H singlet at δ 3.91 and two pairs of AB-type doublets at δ 7.66 and 6.27 (each 1H, J=9.4 Hz) and δ 7.43 and 6.89 (each 1H, J=8.7 Hz) together with a strong ultraviolet (UV) band at λ_{max} 321 nm and infrared (IR) absorptions at ν_{max} 1610 and 1730 cm^{-1} suggested the presence of a 7-methoxy-8-substituted coumarin nucleus in the molecule.[2)] Observations of a lower field 1H signal at δ 9.75 and a 2H signal at 3.98 coupled with each other (J=1.0 Hz), and a strong IR band at ν_{max} 1730 cm^{-1} revealed the structure of the side chain at C-8 as $-CH_2-CHO$. Next, 7-methoxy-8-allylcoumarin (**2**) from 7-hydroxycoumarin was treated with water saturated ozonized oxygen[3)] in ethyl acetate to give the aldehyde (**1**),[4)] which was found to be identical with natural kiyomal by comparisons of the ^{1}H-NMR and IR spectra. On the basis of these results, kiyomal can be represented by formula **1**, and has not previously been found as a natural product.

Structure of Casegravol Isovalerate (3) Casegravol isovalerate (**3**) was isolated as a colorless amorphous solid, $C_{20}H_{24}O_6$, $[\alpha]_D$ 0° (chloroform). The presence of the same 7-methoxy-8-substituted coumarin nucleus as in **1** was shown by ^{1}H-NMR signals at δ 7.62 (1H, d, J=9.4 Hz), 7.32 (1H, d, J=8.7 Hz), 6.87 (1H, d, J=8.7 Hz), 6.26 (1H, d, J=9.4 Hz), and 3.95 (3H, s), a characteristic UV band at λ_{max} 321 nm, and IR bands at ν_{max} 1730 and 1600 cm^{-1}. An AB-type doublet at δ 7.07 and 6.90 with a characteristic coupling constant (J=16.5 Hz) was assigned to a *trans*-disubstituted double bond, a 3H singlet at δ 1.45 to a methyl group attached to a carbon bearing an oxygen atom, and two doublets at δ 4.23 and 4.10 (each 1H) coupled with each other (J=11.1 Hz) to methylene protons located between an oxygen and a quaternary carbon atom. These ^{1}H-NMR spectral features of this coumarin were similar to those of casegravol (**4**),[5)] except for a downfield shift of the methylene protons. The appearance of signals at δ 0.95 (6H, d, J=6.1 Hz), 2.11 (1H, m), and 2.26 (2H, m), an IR band at ν_{max} 1730 cm^{-1} and a mass fragment base peak at m/z 245 [M^+-115 ($C_6H_{11}O_2$)] suggested the presence of an isovaleryl moiety as an ester. From these spectral data, we proposed the structure (**3**) for casegravol isovalerate.

Structure of *trans O*-Methylgrandmarin (5) *trans O*-Methylgrandmarin (**5**) was obtained as a colorless amorphous solid, $C_{16}H_{18}O_6$, $[\alpha]_D$ −13.4° (chloroform). A 5,7-oxygenated 8-substituted coumarin nucleus was indicated by the ^{1}H-NMR (acetone-d_6), IR, and UV spectral data as follows: δ 8.04 and 6.15 (coupled with each other, 1H, d, J=9.8 Hz) and 6.31 (1H, s), ν_{max} 1730, 1635, and 1610 cm^{-1}, and λ_{max} 259 and 328 nm.[2)] In the ^{1}H-NMR (acetone-d_6) spectrum, the appearance of methoxy signals at δ 3.96 and 3.64, geminal dimethyl signals at δ 1.43 and 1.48, and a 1H signal at δ 3.98 partially overlapped with a methoxy signal and coupled with protons at 4.43 (1H, d, J=3.0 Hz) and 4.47 (1H, d, J=5.4 Hz, disappeared with D_2O) suggested the presence of a 2′,2′-dimethyl-3′-hydroxy-4′-methoxydihydropyran ring system, which was also supported by the occurrence of a mass fragment base peak at m/z 235 (M^+-71) due to the loss of $(CH_3)_2C=CH(OH)$ radical from the molecular ion with a hydrogen transfer.[6)] The differential chemical shift value between two geminal methyls at C-2′ ($\Delta\delta$ 0.05 ppm) and the coupling constant ($J_{3',4'}$=3.0 Hz) suggested the *trans* orientation of substituents at C-3′ and 4′ in the dimethylpyran ring system.[6,7)] These findings led us to convert 5-methoxyseselin (**7**) into *trans O*-methylgrandmarin. Treatment of 5-methoxyseselin (**7**)[8)] isolated from *C. grandis* with *m*-chloroperbenzoic acid (*m*-CPBA) in methanol at room temperature for 1 h gave two diastereomeric isomers **5** and

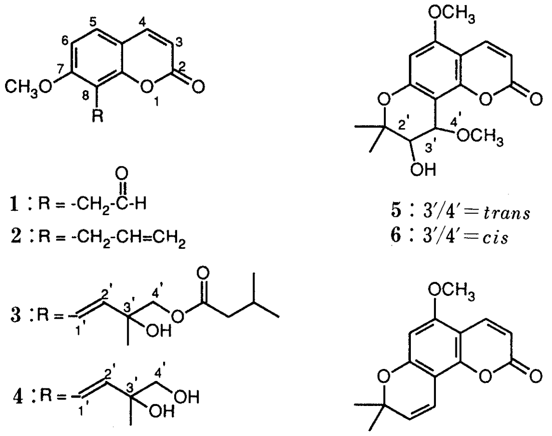

Fig. 1

6 after preparative silica gel thin-layer chromatography (TLC) in 50 and 15% yields, respectively. Easy cleavage of an oxiran ring of the intermediate followed by reaction with methanol at the benzylic C-4′ was considered to be a consequence of the effect of the methoxy group located *para* to the benzylic position. From analyses of the ^{1}H-NMR spectra, taking account of the $J_{3',4'}$ values and the differential chemical shift values of 2′-geminal dimethyls, structures **5** and **6** can be deduced for these products (see Experimental).[6,7] The synthetic *trans* isomer **5** was found to be identical with natural *trans O*-methylgrandmarin by comparisons of the ^{1}H-NMR and IR spectra. Consequently, the structure of *trans O*-methylgrandmarin was established as formula **5** except for the absolute stereochemistry.

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 high-resolution MS with an M-80 (Hitachi) spectrometer. UV spectra were determined in methanol and IR spectra were recorded in $CHCl_3$.

Extraction and Separation The roots of several hybrid seedlings resulting from crosses of [*Citrus unshiu* MARC. (miyagawa-wase) × *C. sinensis* OSBEC. (torobita orange)] (kiyomi) × *C. iyo* HORT. *ex* TANAKA (iyo) were used as materials. These *Citrus* plants were grown in the orchard of Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry, and Fisheries, Shimizu, Shizuoka.

The dried roots of *Citrus* plant (kiyomi × iyo) were extracted with acetone. The acetone extract was subjected to silica gel column chromatography. Successive elutions with benzene, benzene–isopropyl ether [(3 : 1)—(1 : 3)], and benzene–acetone (3 : 1) each gave 3—6 fractions. Each fraction was subjected repeatedly to preparative silica gel TLC developed with appropriate combinations of acetone, benzene, hexane, isopropyl ether, and chloroform to obtain kiyomal (**1**), casegravol isovalerate (**3**), and *trans O*-methylgrandmarin (**5**) in 0.0008, 0.0021, 0.001% yields, respectively, as well as known coumarins and other components. Details of the procedures of separation and characterization of known components will be reported elsewhere.

Kiyomal (1) Colorless amorphous solid. High-resolution MS: Calcd for $C_{12}H_{10}O_4$: 218.0578. Found: 218.0602. UV $\lambda_{\max}$ nm: 205, 219, 248, 257, 321. IR $\nu_{\max}$ cm^{-1}: 1730, 1610. ^{1}H-NMR δ: 9.75 (1H, t, J=1.0 Hz, –CHO), 7.66 (1H, d, J=9.4 Hz, H-4), 7.43 (1H, d, J=8.7 Hz, H-5), 6.89 (1H, d, J=8.7 Hz, H-6), 6.27 (1H, d, J=9.4 Hz, H-3), 3.98 (2H, d, J=1.0 Hz, benzylic-CH_2), 3.91 (3H, s, OCH_3). MS *m/z* (%): 218 (M^+, 40), 190 (22), 189 (100), 131 (64).

Ozonolysis[3] of 7-Methoxy-8-allylcoumarin (2) Ozonized oxygen was bubbled through H_2O and then through a solution of **2** (15 mg) in EtOAc (10 ml) at room temperature for 3 min. The solvent was evaporated off and the residue was subjected to preparative TLC (benzene : iso-Pr_2O = 1 : 2) to give **1** as a colorless oil (3.5 mg). This was found to be identical with kiyomal by IR and ^{1}H-NMR comparisons.

Casegravol Isovalerate (3) Colorless amorphous solid, $[\alpha]_D$ 0° ($CHCl_3$). High-resolution MS: Calcd for $C_{20}H_{24}O_6$: 360.1572. Found: 360.1591. UV $\lambda_{\max}$ nm: 212, 245, 254, 273, 283, 321. IR $\nu_{\max}$ cm^{-1}: 3500 (br), 1730, 1600. ^{1}H-NMR δ: 7.62 (1H, d, J=9.4 Hz, H-4), 7.32 (1H, d, J=8.7 Hz, H-5), 7.07 (1H, d, J=16.5 Hz, H-1′), 6.90 (1H, d, J=16.5 Hz, H-2′), 6.87 (1H, d, J=8.7 Hz, H-6), 6.26 (1H, d, J=9.4 Hz, H-3), 4.23 (1H, d, J=11.1 Hz, H-4′), 4.10 (1H, d, J=11.1 Hz, H-4′), 3.95 (3H, s, OCH_3), 2.38 (1H, br, OH), 1.45 (3H, s, 3-CH_3), [2.26 (2H, m), 2.11 (1H, m), 0.95 (6H, d, J=6.1 Hz); isovaleryl]. MS *m/z* (%): 360 (M^+), 258 (7), 246 (17), 245 (100), 203 (21).

***trans O*-Methylgrandmarin (5)** Colorless amorphous solid, $[\alpha]_D$ −13.4° (c=0.067, $CHCl_3$). High-resolution MS: Calcd for $C_{16}H_{18}O_6$: 306.1102. Found: 306.1186. UV $\lambda_{\max}$ nm: 209, 225, 250, 259, 328. IR $\nu_{\max}$ cm^{-1}: 3400 (br), 1730, 1635, 1610. ^{1}H-NMR δ: 7.97 (1H, d, J=9.8 Hz, H-4), 6.23 (1H, s, H-6), 6.17 (1H, d, J=9.8 Hz, H-3), 4.48 (1H, d, J=3.4 Hz, H-4′), 3.88 (1H, br, H-3′), 3.87 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 1.49 (3H, s, CH_3), 1.45 (3H, s, CH_3). MS *m/z* (%): 306 (M^+, 40), 235 (100), 234 (55), 219 (30), 205 (85).

Reaction of 5-Methoxyseselin (10)[7] with *m*-CPBA A methanolic solution (2 ml) of **7** (15 mg) and *m*-CPBA (30 mg) was stirred at room temperature for 1 h under dry conditions, then aqueous $NaHCO_3$ solution was added, and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried with anhydrous $MgSO_4$, and evaporated to dryness. The residue was subjected to preparative silica gel TLC (solvent, benzene : acetone = 10 : 1) to afford **5** (8.9 mg) and **6** (2.6 mg). **5**: Colorless needles from acetone, mp 174—177 °C. ^{1}H-NMR (acetone-d_6) δ: 8.04 (1H, d, J=9.7 Hz, H-4), 6.31 (1H, s, H-6), 6.15 (1H, d, J=9.7 Hz, H-3), 4.47 (1H, d, J=5.4 Hz, OH), 4.43 (1H, d, J=3.0 Hz, H-4′), 3.98 (1H, dd, J=5.4, 3.0 Hz, H-3′), 3.96 (3H, s, 5-OCH_3), 3.64 (3H, s, 4′-OCH_3), 1.48 and 1.43 (each 3H, s, 2′-CH_3). $\Delta\delta$ of 2′-$(CH_3)_2$ = 0.05 ppm. Synthetic **5** was found to be identical with natural *trans O*-methylgrandmarin by IR and ^{1}H-NMR comparisons. **6**: Colorless needles from acetone, mp 182—185 °C. UV $\lambda_{\max}$ nm: 210, 227, 251, 259, 330. IR $\nu_{\max}$ cm^{-1}: 3450 (br), 1730, 1630, 1605. ^{1}H-NMR δ: 7.98 (1H, d, J=9.4 Hz, H-4), 6.21 (1H, s, H-6), 6.17 (1H, d, J=9.4 Hz, H-3), 4.65 (1H, d, J=5.1 Hz, H-4′), 3.87 (3H, s, OCH_3), 3.82 (1H, dd, J=5.1, 7.4 Hz, H-3′), 3.81 (3H, s, OCH_3), 2.94 (1H, d, J=7.4 Hz, OH), 1.42 (6H, s, 2 × CH_3). ^{1}H-NMR (acetone-d_6) δ: 8.02 (1H, d, J=9.8 Hz, H-4), 6.28 (1H, s, H-6), 6.14 (1H, d, J=9.8 Hz, H-3), 4.58 (1H, d, J=4.7 Hz, H-4′), 4.16 (1H, d, J=7.7 Hz, OH), 3.94 (3H, s, OCH_3), 3.88 (1H, dd, J=4.7, 7.7 Hz, H-3′), 3.72 (3H, s, OCH_3), 1.43 (3H, s, CH_3), 1.41 (3H, s, CH_3). $\Delta\delta$ of 2′-$(CH_3)_2$ = 0.02 ppm.

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