Chem. Pharm. Bull. **38**(9) 2586—2588 (1990) Vol. 38, No. 9

Isolation of New Constituents from *Citrus* Plants

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Received March 5, 1990

A new coumarin and a new acridone–coumarin dimer named *trans*-grandmarin (1) and acrimarine-H (5), respectively, were isolated from roots of some *Citrus* plants (Rutaceae) and their structures are proposed on the basis of spectrometric analyses. Citaldoxime (7) was also isolated from roots of some *Citrus* plants and its structure was found to be identical with that of a γ-radiation-induced antifungal stress metabolite isolated from *Citrus* fruit.

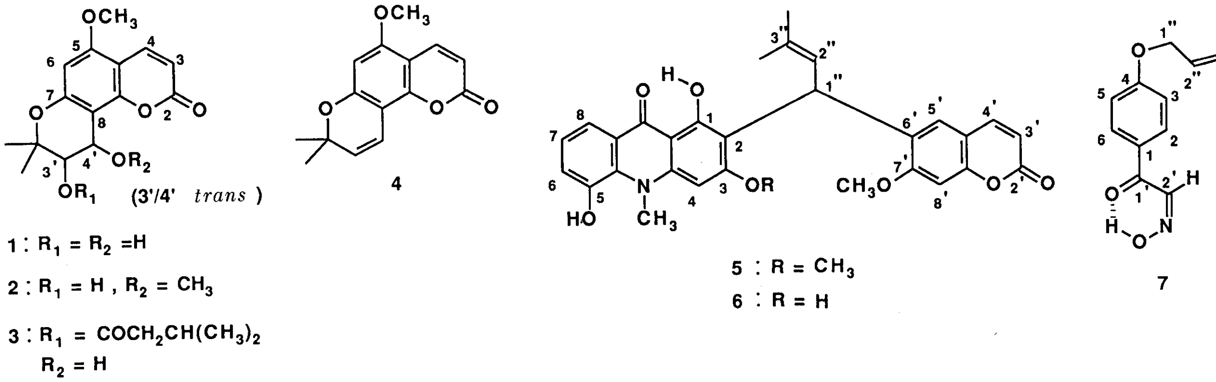
Keywords *Citrus*; Rutaceae; coumarin; acridone; acridone–coumarin dimer; *trans*-grandmarin; acrimarine-H; citaldoxime; oxime; prenyl

During our phytochemical studies of *Citrus* plants (Rutaceae), the isolation of many kinds of new coumarins and acridone alkaloids has been reported.[1)] Now, we wish to describe the isolation and structural elucidation of new constituents from *Citrus sinensis* OSBEC. *forma Valencia* (valencia orange) and several hybrid seedlings resulting from crosses of [*C. unshiu* MARC. (miyagawa-wase) × *C. sinensis* OSBEC. *forma Trovita* (trobita orange)] (kiyomi) × *C. iyo* HORT. *ex* TANAKA (iyo) and *C. Tamurana* HORT. *ex* TAKAHASHI (hyuga-natsu) × *C. kinokuni* HORT. *ex* TANAKA (hirakishu).

Results and Discussion

Structure of *trans*-Grandmarin (1) *trans*-Grandmarin (1) was isolated as a colorless oil, $[\alpha]_D$ +7.69° (chloroform), from roots of several hybrid seedlings resulting from a cross of hyuganatsu × hirakishu. The molecular formula was determined as $C_{15}H_{16}O_6$ by high-resolution mass spectrometry (HR-MS). The presence of a 5,7-oxygenated 8-substituted coumarin nucleus in the molecule was suggested by ultraviolet (UV) absorptions at λ_{max} 251, 260, and 330 nm, an infrared (IR) band at ν_{max} 1722 cm^{-1}, and proton nuclear magnetic resonance (^{1}H-NMR) signals at δ 8.00 (1H, d, J=9.75 Hz), 6.16 (1H, d, J=9.75 Hz), 6.23 (1H, s), and 3.87 (3H, s, OCH_3).[2)] The appearance of the remaining ^{1}H-NMR signals assignable to geminal methyls [δ 1.52 and 1.31 (each 3H, s)] attached to a carbon bearing an oxygen atom and vicinal protons [δ 4.92 and 3.81 (each 1H, d, J=6.72 Hz)] attached to carbinol carbons indicated the presence of a dihydroxygenated dimethylpyran ring system. The *trans* configuration of the glycol system was proposed on the basis of the large coupling constant (J=6.72 Hz) of the carbinol protons, the large chemical shift difference of the geminal methyls (Δ 0.21 ppm) on the pyran ring,[3)] and the lack of a nuclear Overhauser effect (NOE) between the two carbinol protons. The angular orientation of the dimethylpyran ring was suggested by the observation of NOE enhancement between the signals at δ 3.87 and 6.23, assignable to a methoxy proton and H-6 or -8, respectively. Finally, the structure **1** except for the absolute stereochemistry was established by direct comparison with the sample previously prepared from 5-*methoxyseselin* (**4**) with *m*-chloroperbenzoic acid (MCPBA).[4)] The *O*-methyl derivative (**2**), the isovalerate (**3**), and the *cis* isomer of **1** have already been found in *Citrus* plants.[4,5)]

Structure of Acrimarine-H (5) Acrimarine-H (**5**), a yellow oil, $[\alpha]_D$ 0° (acetone), was isolated from several hybrid seedlings resulting from a cross of kiyomi × iyo and was found to have the molecular formula $C_{30}H_{27}NO_7$ by HR-MS. The close similarity of the UV spectrum of **5** with that of acrimarine-G (**6**)[6)] suggested this compound to be an acridone–coumarin dimer such as **6**. In the ^{1}H-NMR spectrum, observation of an *N*-methyl signal at δ 4.04, a strongly hydrogen-bonded hydroxy proton at δ 14.71, two allyl methyls at δ 1.79 and 1.71, an olefinic proton at δ 5.97 (d, J=9.5 Hz) coupled with a methine proton at δ 5.74 (d, J=9.5 Hz), and two protons in an α,β-unsaturated carbonyl system at δ 7.68 and 6.21 (each d, J=9.5 Hz) also revealed the presence of an *N*-methyl-1-hydroxy-9-acridone and a prenylcoumarin nucleus in the molecule. Two predominant mass fragments at m/z 242 (94%) and 271 (100%) in the mass spectrum (MS) were assignable to fragments contain-



1 : $R_1 = R_2 = H$

2 : $R_1 = H$, $R_2 = CH_3$

3 : $R_1 = COCH_2CH(CH_3)_2$
$R_2 = H$

4

5 : $R = CH_3$

6 : $R = H$

7

Chart 1

ing a coumarin and an acridone nucleus, respectively. Observation of ABC-type proton signals [δ 8.05 (1H, d, $J=8.1$ Hz), 7.13 (1H, d, $J=8.1$ Hz), and 7.08 (1H, t, $J=8.1$ Hz)] including a more deshielded H-8 proton at δ 8.05 in the ^{1}H-NMR spectrum indicated a 5-substituted A-ring in the acridone nucleus. The remaining proton signals were two 3H singlets due to two methoxyls at δ 3.90 and 3.75, a D_2O-exchangeable broad singlet at δ 5.64, and three 1H singlets at δ 7.58, 6.68, and 6.24. In NOE experiments, irradiations of the *N*-methyl (δ 4.04) and the methoxy (δ 3.90) protons gave 9 and 11% enhancements of the signal at δ 6.23 (H-4), respectively. Irradiation of the methoxy protons at δ 3.75 caused an 18% increase of the signal at δ 6.60 (H-8′). On the basis of these spectral data, the structure of acrimarine-H was proposed to be represented by the formula **5**, corresponding to 3-*O*-methylacrimarine-G.[6)] Acrimarine-H (**5**) is the eighth example of the occurrence of acridone–coumarin dimers in *Citrus* plants.[6,7)]

Structure of Citaldoxime (7) Citaldoxime (**7**) was obtained as a colorless amorphous powder from roots of valencia orange and those of several hybrid seedlings resulting from a cross of kiyomi × iyo. The molecular formula $C_{13}H_{15}NO_3$ was proposed from the HR-MS. The chemical shifts and ^{1}H–^{13}C correlations in the H–C correlation spectrometry (COSY) spectrum are shown in Table I. The observation of ^{1}H-NMR signals at δ_H 8.04 and 6.97 (each 2H, d, $J=9.07$ Hz), UV bands at λ_{max} 203, 230, and 304 nm, IR bands at ν_{max} 1650, 1600, and 1570 cm^{-1}, and the base mass fragment at m/z 121, together with the signals of the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I), suggested the presence of a *p*-oxybenzoyl skeleton in the molecule. The presence of a prenyl (3-methyl-2-butenyl) moiety attached to an oxygen atom was also indicated by the ^{1}H- and ^{13}C-NMR signals (Table I) and the mass fragment at m/z 165 due to the loss of $\cdot CH_2CH=C(CH_3)_2$ from the molecular ion with transfer of one hydrogen atom. Further, a D_2O-exchangeable hydrogen-bonded hydroxy proton signal (δ_H 11.50) and 1H singlet at δ_H 7.78 due to a proton attached to sp^2 carbon at δ_C 149.75 in the NMR spectra were observed. Finally, the observation of a three-bond long-range correlation between C-1 (δ_C 130.23) and the proton at δ_H 7.87 in ^{1}H detected heteronuclear multiple bond connectivity (HMBC) experiments suggested the structure of citaldoxime to be represented by formula **7**. Other results of the HMBC experiments shown by arrows in Fig. 1 are also consistent with the structure **7**.

Recently, Dubery *et al.*[8)] reported the isolation of a γ-radiation-induced antifungal stress metabolite from peels of *C. sinensis* (valencia) and *C. lemon* (eureka) and the same formula as that of our citaldoxime (**7**) was assigned to their "compound 1" based on spectroscopic and single crystal X-ray structural analyses. From the results of a comparison of the spectral data of citaldoxime (**7**) with those of "compound 1" in the literature,[8)] citaldoxime (**7**) was concluded to be identical with "compound 1." Dubery *et al.* reported in their paper[8)] that **7** did not occur in non-γ-irradiated *Citrus* fruit. It is of interest to know that citaldoxime (**7**) does occur in normal (non-γ-irradiated) roots of some *Citrus* plants.

TABLE I. Chemical Shifts and H–C Correlations of NMR Spectra of Citaldoxime (7)

No. of Carbon	δ_H	δ_C
1	—	130.23
2, 6	8.04 (2H, d, $J=9.07$ Hz)	133.56
3, 5	6.97 (2H, d, $J=9.07$ Hz)	115.61
4	—	164.62
1′	—	188.02
2′	7.87 (1H, s)	149.75
N–OH	11.50 (1H, br)	—
1″	4.62 (2H, d, $J=6.72$ Hz)	66.29
2″	5.43 (1H, t, $J=6.72$ Hz)	120.89
3″	—	139.17
4″	1.73 (3H, s)	18.70
5″	1.71 (3H, s)	26.26

^{1}H- and ^{13}C-NMR spectra were measured in acetone-d_6 at 400 and 100 MHz, respectively.

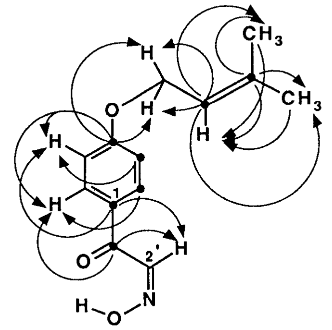


Fig. 1. H–C Long-Range Correlations in HMBC Spectrum ($J=8$ Hz) of Citaldoxime (7)

Experimental

IR spectra were recorded on a JASCO IR-810 IR spectrophotometer in $CHCl_3$, UV spectra on a JASCO UVIDEC-610C double beam spectrophotometer in MeOH, optical rotations on a DIP-181 polarimeter (JASCO) in $CHCl_3$ at 20 °C, and ^{1}H- and ^{13}C-NMR spectra on a JEOL GX-270 or GX-400 NMR spectrometer (with $SiMe_4$ as an internal standard) in $CDCl_3$, unless otherwise stated. Electron impact (EI)- and HR-MS spectra were measured on a Hitachi M-80 or JMS-HX-110 mass spectrometer. NOE enhancements were determined by differential NOE techniques, and ^{1}H–^{13}C COSY and HMBC ($J=8$ Hz) spectra were recorded on a JEOL GX-400 spectrometer. Preparative silica gel thin layer chromatography (TLC) was carried out with appropriate combinations of acetone, benzene, hexane, isopropyl ether, and chloroform.

***trans*-Grandmarin (1)** The acetone extract of dried roots (108 g) of several hybrid seedlings resulting from a cross of *C. Tamurana* HORT. *ex* TAKAHASHI (hyuga-natsu) × *C. kinokuni* HORT. *ex* TANAKA (hirakishu) was treated according to the procedure described previously.[4)] The benzene–acetone (3 : 1) eluate was subjected to preparative TLC to afford **1** as a mixture with *trans*-khellactone.[9)] The isolation of **1** from this mixture was accomplished by high performance liquid chromatography (HPLC) to afford **1** in 0.00093% yield. HPLC: column, Cosmosil $5C_{18}$ (4.6×150 mm); solvent, acetonitrile–H_2O (4 : 5); flow rate, 0.8 ml/min; detector, UV 254 nm. **1**: Colorless oil. $[\alpha]_D$ $+7.69°$ ($c=0.078$, $CHCl_3$). HR-MS: Calcd for $C_{15}H_{16}O_6$: 292.0947. Found: 292.0947. IR ν_{max} cm^{-1}: 3450, 1722, 1710, 1625, 1600. UV λ_{max} nm; 211, 229 (sh.), 251, 260, 330. ^{1}H-NMR (270 MHz) δ_H: 8.00 (1H, d, $J=9.75$ Hz, H-4), 6.23 (1H, s, H-6), 6.16 (1H, d, $J=9.75$ Hz, H-3), 4.92 (1H, d, $J=4.72$ Hz, H-4′), 3.87 (3H, s, 5-OCH_3), 3.81 (1H, d, $J=6.72$ Hz, H-3′), 2.65 (2H, br s), 1.52 (3H, s, 2′-CH_3), 1.31 (3H, s, 2′-CH_3). EI-MS m/z (%): 292 (M^+, 27), 222 (21), 221 (100), 220 (21), 192 (17), 191 (10), 164 (34), 149 (14). This compound was found to be identical with a synthetic sample obtained from **4** by treatment with MCPBA.[4)]

Acrimarine-H (5) The acetone extract of dried roots (161 g) of several hybrid seedlings resulting from a cross of [*C. unshiu* MARC. (miyagawa-wase) × *C. sinensis* OSBEC. (trobita orange)] (kiyomi)/*C. iyo* HORT. *ex* TANAKA (iyo) was subjected directly to preparative TLC to obtain **5** in 0.0006% yield. **5**: Yellow oil. HR-MS: Calcd for $C_{30}H_{27}NO_7$:

513.1786. Found: 513.1791. IR ν_{max} cm^{-1}: 3400 (br), 1720, 1620, 1600, 1570. UV λ_{max} nm: 206, 222, 258, 269, 288, 322. ^{1}H-NMR (400 MHz) δ_H: 14.71 (1H, s, OH), 8.05 (1H, d, $J=8.1$ Hz, H-8), 7.68 (1H, d, $J=9.5$ Hz, H-4′), 7.58 (1H, s, H-5′), 7.13 (1H, d, $J=8.1$ Hz, H-6), 7.08 (1H, t, $J=8.1$ Hz, H-7), 6.68 (1H, s, H-8′), 6.24 (1H, s, H-4), 6.21 (1H, d, $J=9.5$ Hz, H-3′), 5.97 (1H, d, $J=9.5$ Hz, H-2″), 5.74 (1H, d, $J=9.5$ Hz, H-1″), 5.64 (1H, br, OH), 4.04 (3H, s, N–CH$_3$), 3.90 (3H, s, 3-OCH$_3$), 3.75 (3H, s, 7′-OCH$_3$), 1.79 (3H, s, 3″-CH$_3$), 1.71 (3H, s, 3″-CH$_3$). EI-MS m/z (%): 513 (M$^+$, 40), 470 (77), 271 (100), 242 (94), 227 (71).

Citaldoxime (7) The acetone extracts of dried roots of *C. sinensis* OSBEC. *forma Valencia* (valencia orange) and several hybrid seedlings resulting from a cross of [*C. unshiu* MARC. (miyagawa-wase) × *C. sinensis* OSBEC. *forma Trobita* (trobita orange)] (kiyomi) × *C. iyo* HORT. *ex* TANAKA (iyo) were subjected to preparative TLC to afford **7** in 0.0014 and 0.0058% yields, respectively. **7**: Colorless amorphous powder. HR-MS: Calcd for $C_{13}H_{15}NO_3$: 233.1051. Found: 233.1079. IR ν_{max} cm^{-1}: 3550, 1650, 1600, 1570. UV λ_{max} nm: 203, 230, 304. ^{1}H-NMR (400 MHz) δ_H: 8.08 (2H, d, $J=9.08$ Hz, H-2, 6), 8.03 (1H, s, H-2′), 6.95 (2H, d, $J=9.08$ Hz, H-3, 5), 5.49 (1H, t, $J=6.7$ Hz, H-2″), 4.59 (2H, d, $J=6.7$ Hz, H-1″), 1.80 (3H, s, 4″-H), 1.71 (3H, s, 5″-H). ^{1}H- and ^{13}C-NMR (at 400 and 100 MHz, respectively in acetone-d_6): see Table I. EI-MS m/z (%): 233 (M$^+$, 4), 216 (22), 165 (50), 121 (100).

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