New Coumarins from *Citrus* Plants

Chihiro ITO,[a] Kazuko FUJIWARA,[a] Mayumi KAJITA,[a] Motoharu JU-ICHI,[b] Yuko TAKEMURA,[b] Yoshiko SUZUKI,[b] Kiyoko TANAKA,[b] Mitsuo OMURA,[c] and Hiroshi FURUKAWA*,[a]

Faculty of Pharmacy, Meijo University,[a] Tempaku, Nagoya, 468, Japan, Faculty of Pharmaceutical Sciences, Mukogawa Women's University,[b] Kōshien, Nishinomiya 663, Japan, and Okitsu Branch, Fruit Tree Research Station,[c] Ministry of Agriculture, Forestry and Fisheries, Shimizu 424-02, Japan.
Received March 20, 1991

Four new coumarins, named peroxytamarin (1), *cis*-casegravol (5), citrusarin-A (7), and citrusarin-B (8), were isolated from root of *Citrus* plants and their structures were elucidated by chemical and spectrometric methods. Citrusarin-B (8), a coumarin having both a dimethylpyran ring and a dihydrofuran ring in the molecule, was also synthesized.

Keywords coumarin; *Citrus*; Rutaceae; peroxytamarin; tamarin; casegravol; citrusarin; heteronuclear multiple bond connectivity

During our phytochemical studies of *Citrus* plants (Rutaceae), many kinds of new coumarins and acridone alkaloids have been isolated and characterized.[1] We report here the isolation of four new coumarins named peroxytamarin (**1**), *cis*-casegravol (**5**), and citrusarin-A (**7**), and -B (**8**), and their structural elucidation by chemical and/or spectral methods. Peroxytamarin (**1**) and *cis*-casegravol (**5**) were isolated from root of *Citrus sulcata* HORT. *ex* TAKAHASHI (Japanese name: sanbo-kan) together with many kinds of known coumarins and acridones. Citrusarin-A (**7**) and -B (**8**) are regioisomers, and both coumarins were also obtained from root of *C. Hassaku* HORT. *ex* Y. TANAKA (Japanese name: hassaku).

Results and Discussion

Structure of Peroxytamarin (1) Peroxytamarin (**1**) was isolated as a colorless oil, $[\alpha]_D$ $-5.3°$ ($CHCl_3$). The high-resolution mass spectrum (HR-MS) gave the molecular formula as $C_{15}H_{16}O_5$. The ultraviolet (UV) bands at λ_{max} 222, 252, 297 and 324 nm, infrared (IR) absorption at ν_{max} 1725 cm^{-1}, AB-type signals at δ_H 7.61 and 6.23 (each doublet, $J=9.4$ Hz), a methoxy signal at δ_H 3.91, and two 1H singlets at δ_H 7.25 and 6.79 in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum indicated the presence of 7-methoxy-6-substituted coumarin skeleton[2] in the molecule. Further, in the ^{1}H-NMR spectrum, ABX-type signals at δ_H 4.58 (1H, dd, $J=8.0$, 5.2 Hz), 2.92 (1H, dd, $J=14.1$, 5.2 Hz) and 2.87 (1H, dd, $J=14.1$, 8.0 Hz), two 1H singlets at δ_H 5.00 and 4.96 assignable to an *exo*-methylene protons, and a 3H singlet at δ_H 1.82 due to an allyl methyl group appeared. The 1H double-doublet at δ_H 4.58 in the ^{1}H-NMR spectrum and IR band at ν_{max} 3450 cm^{-1} together with the occurrence of typical mass fragments at *m/z* 260 and 258 corresponding to $[M^+ - \cdot O]$ and $[M^+ - H_2O]$, respectively, in the electron impact mass spectrum (EI-MS) suggested the presence of a hydroperoxy moiety in the molecule. These spectral data coupled with the appearance of a base fragment peak at *m/z* 189 arising from cleavage at the benzylic position indicated the structure of $[-CH_2-CH(OOH)-C(CH_3)=CH_2]$ for the side chain attached to C-6. For confirmation of the structure, the following two chemical reactions were carried out. 1) The hematoporphyrin-sensitized photo-oxygenation[3] of suberosin (**2**)[4] in oxygen gas gave two isomeric peroxygenated products. One of them was found to be identical with natural **1** by IR, UV, ^{1}H-NMR and mass spectrometric comparisons. The structure of the other reaction product was assigned as formula **3** on the basis of spectrometric analyses (see Experimental). 2) Treatment of peroxytamarin (**1**) with triphenylphosphine gave a colorless oil, $[\alpha]_D+8.9°$ ($CHCl_3$), which was found to be identical with tamarin (**4**), except for a difference of $[\alpha]_D$ value. Tamarin (**4**) was first isolated from *Ruta pinnata*,[5] and then from *Amyris balsamifera* by Burke and Parkins,[6] and *R*-stereochemistry was proposed for the specimen having $[\alpha]_D+27.3°$ ($CHCl_3$). From the results of the ^{1}H-NMR analysis using a chiral shift reagent, **4** derived from natural peroxytamarin (**1**) was to contain only about 5% excess of one of the enantiomers. According to the proposal by Bruke and Parkins,[6] the absolute stereochemistry of the negative $[\alpha]_D$ enantiomer of **1** was assigned as *R*, because **4** derived from **1** showed a positive $[\alpha]_D$ value. These results led us to conclude the structure of the major enantiomer of peroxytamarin to be as represented by formula **1**. The first isolation of a coumarin hydroperoxide from a plant was reported by Crombie *et al.*[7] in 1970. This is the fourth example of the isolation of a peroxygenated coumarin from a natural source.[8]

Structure of *cis*-Casegravol (5) *cis*-Casegravol (**5**) was obtained as a colorless oil, $[\alpha]_D$ $-12.1°$ ($CHCl_3$). The chemical ionization mass spectrum (CI-MS) using ammonia as a reactant gas showed the molecular ion $[M+NH_4]^+$ at *m/z* 294. The UV bands at λ_{max} 208, 255, 284 and 320 nm, IR band at ν_{max} 1720 cm^{-1}, and ^{1}H-NMR signals at δ_H 7.64 (1H, d, $J=9.4$ Hz, H-4), 6.26 (1H, d, $J=9.4$ Hz, H-3), and 3.94 (3H, OCH_3) suggested the 7-methoxycoumarin nucleus. The appearance of other AB-type doublets at δ_H 7.39 and 6.88 (each 1H, d, $J=8.7$ Hz) assignable to H-5 and H-6, respectively, indicated the presence of a side chain at C-8. The remaining ^{1}H-NMR signals showed the presence of a *Z*-disubstituted double bond [δ_H 6.30 and 5.96 (each 1H, d, $J=12.4$ Hz)], a methyl group attached to an oxygenated carbon [δ_H 1.25 (3H, s)], and a hydroxymethylene moiety [δ_H 3.57 and 3.36 (each 1H, d, $J=11.1$ Hz)]. These data together with the observation of mass fragments at *m/z* 259 and 245 corresponding to $[M^+ - \cdot OH]$ and $[M^+ - \cdot CH_2OH]$ ions, respectively, suggested the structure of the side chain as $[-CH=CH-C(OH)CH_3]-CH_2OH]$ having *Z*-configuration. On the basis of these results, we proposed the structure of *cis*-casegravol to be as shown by formula **5**, except for the absolute

stereochemistry. The racemic *E*-isomer named casegravol (**6**) was isolated from *Casearia graveolens* in 1980 and characterized.[9]

Structures of Citrusarin-A (7) and -B (8) Citrusarin-A (**7**) and -B (**8**) were isolated as pale yellow oils having $[\alpha]_D$ +5.2° and +3.0° ($CHCl_3$), respectively, and were found to have the same molecular formula $C_{19}H_{20}O_4$ by HR-MS analyses.

Citrusarin-A (**7**) showed UV bands at λ_{max} 210, 226, 284, and 340 nm and IR band at ν_{max} 1720 cm^{-1}. In the ^{1}H-NMR spectrum, two pairs of AB-type doublets at δ_H 7.94 and 6.07 (each 1H, d, J=9.5 Hz) and at δ_H 6.45 and 5.55 (each 1H, d, J=9.9 Hz), accompanied with signals of two methyl groups attached to oxygenated carbon at δ_H 1.46 (6H, s), were assignable to α- and β-protons on an α,β-unsaturated carbonyl system and two protons on the dimethylbenzopyran ring system, respectively. The lower chemical shift value of H-4 at δ_H 7.94 and the absence of other proton signals in the aromatic proton region in the ^{1}H-NMR spectrum, together with the results of the UV spectrum, suggested the presence of a 5,7-dioxygenated 6,8-disubstituted coumarin nucleus[2] having a dimethylpyran ring system in the molecule. The ramaining proton signals coupled with carbon signals in the ^{13}C-NMR spectrum were assigned to geminal methyls attached to a benzylic carbon [δ_H 1.52 and 1.26 (each 3H, s) and δ_C 43.99] and a secondary methyl [δ_H 1.39 (3H, d, J=6.6 Hz)] attached to an oxygenated methine carbon [δ_H 4.48 (1H, q, J=6.6 Hz) and δ_C 91.06]. The appearance of H–C long-range correlations in the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum between the oxygenated methine carbon at δ_C 91.06 and two methyl protons at δ_H 1.26 and 1.52, which further correlated to an aromatic carbon at δ_C 114.25 and a quaternary carbon at δ_C 43.99 indicated the presence of 2,3,3-trimethyldihydrobenzofuran system in the molecule. Based on these spectral data together with the HMBC data shown by arrows in Fig. 1, the structure of citrusarin-A should be depicted by either formula **7** or **8**.

On the other hand, citrusarin-B (**8**) showed UV bands at λ_{max} 204, 230, 293 and 328 nm and the IR band at ν_{max} 1720 cm^{-1}. The ^{1}H-NMR signal pattern of citrusarin-B resembled to that of citrusarin-A (**7**), except for some differences of chemical shifts of the signals due to H-4 and H-4′ (Table I). The mass fragmentation pattern of citrusarin-B showed a close similarity to that of **7**. The higher chemical shift value of H-4 [δ_H 7.75 (1H, d, J=9.5 Hz)] corresponds to that of coumarin bearing no oxygenated substituent at C-5.[2] However, in the HMBC spectrum (Fig. 2), observation of three-bond H–C correlations of H-4 (δ_H 7.75) to two oxygenated aromatic carbons at δ_C 149.89 and 156.17 suggested the presence of an *O*-substituent at C-5, as in the molecule of **7**. These data together with the HMBC data shown by arrows in Fig. 2 implied that citrusarin-B should be represented either by structure **8** or **7**. Therefore, citrusarin-A and -B were found to be regioisomers with regard to the location of the dimethylpyran and dihydrofuran rings attached to the 5,7-oxygenated coumarin nucleus.

The location of the pyran ring (or the dihydrofuran ring) either at C-5,6 or C-7,8 could not be confirmed by H–C long-range correlations in the HMBC spectra (Figs. 1 and

Table I. ^{1}H- and ^{13}C-NMR Data for Citrusarin-A (**7**) and -B (**8**)

Carbon No.	Citrusarin-A (**7**)		Citrusarin-B (**8**)	
	δ_H	δ_C	δ_H	δ_C
2		161.23		161.38
3	6.07 (1H, d, 9.5)	109.84	6.11 (1H, d, 9.5)	110.54
4	7.94 (1H, d, 9.5)	139.25	7.75 (1H, d, 9.5)	138.60
4a		103.64		99.06
5		150.27		156.17
6		101.84		117.91
7		158.09		153.17
8		114.25		102.88
8a		151.12		149.89
2′		77.86		77.44
3′	5.55 (1H, d, 9.9)	127.81	5.56 (1H, d, 9.9)	126.98
4′	6.45 (1H, d, 9.9)	115.90	6.80 (1H, d, 9.9)	115.63
2″	4.48 (1H, d, 6.6)	91.06	4.49 (1H, d, 6.6)	91.14
3″		43.99		44.11
2′-CH_3	1.46 (6H, s)	28.06	1.46 (3H, s)	28.09
		27.96	1.47 (3H, s)	28.05
2″-CH_3	1.39 (3H, d, 6.6)	14.23	1.40 (3H, d, 6.6)	14.27
3″-CH_3	1.52 (3H, s)	25.54	1.42 (3H, s)	25.59
	1.26 (3H, s)	21.17	1.18 (3H, s)	21.07

Spectra were measured at 400 (^{1}H) and 100 (^{13}C) MHz in $CDCl_3$. Values are in δ (ppm). Figures in parentheses are coupling constants (J) in Hz. Assignments were confirmed by H–H and H–C correlation spectroscopy (COSY) and HMBC (J=8 Hz) spectrometric analyses.

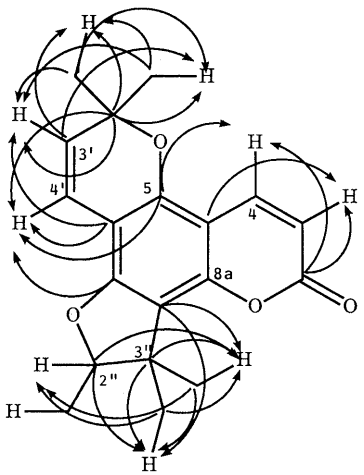

Fig. 1. CH Long-Range Correlations in the HMBC Spectrum (J=8 Hz) of Citrusarin-A (**7**)

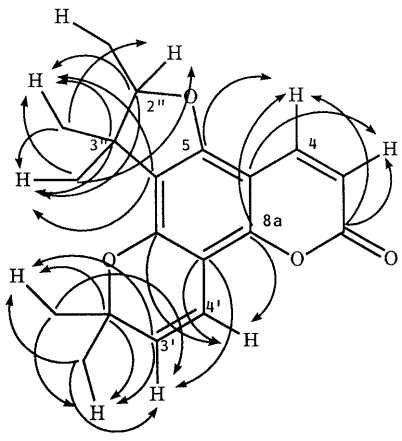

Fig. 2. CH Long-Range Correlations in the HMBC Spectrum (J=8 Hz) of Citrusarin-B (**8**)

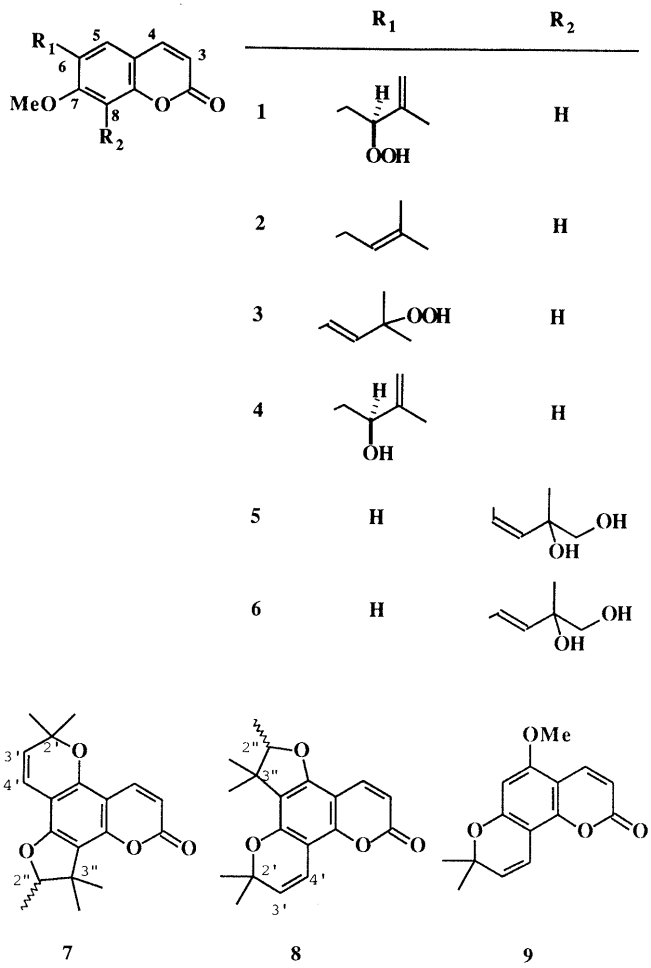

Chart 1

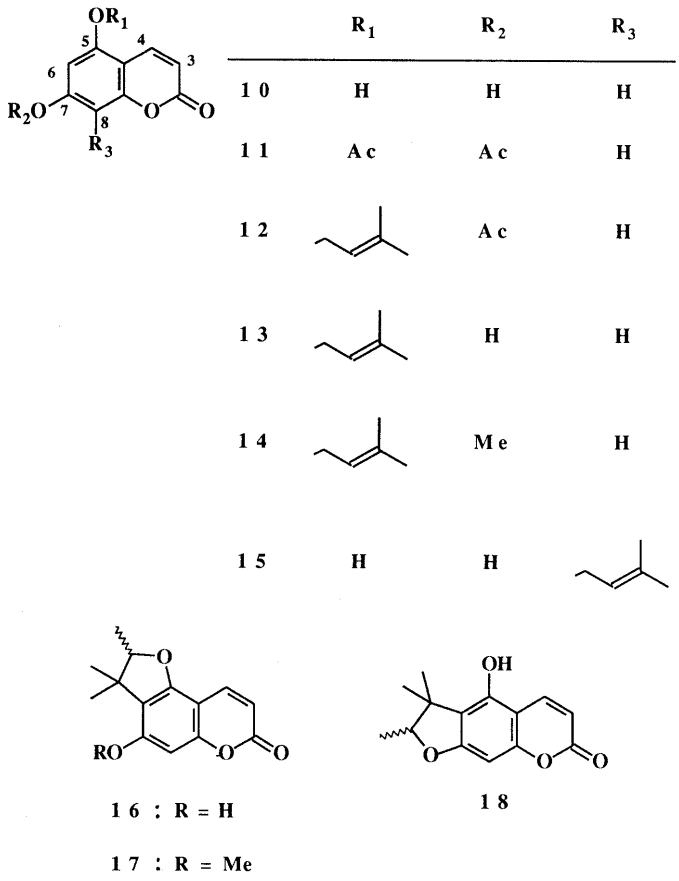

Chart 2

2) of citrusarin-A and -B, because the problem of the assignment of oxygenated aromatic carbon signals at δ_C 149.89 and 156.17 in the spectrum of citrusarin-B and at δ_C 150.27 and 151.12 in that of citrusarin-A, to either C-8a and C-5 or C-5 and C-8a, respectively, remained. The similarity of the chemical shift of the signal at δ_H 6.80 due to H-4′ on the pyran ring of citrusarin-B (**8**) to that of 5-methoxyseselin (**9**)[10] at δ_H 6.79 suggested the structure **8** for citrusarin-B.

To confirm this, we synthesized citrusarin-B (**8**) from 5,7-dihydroxycoumarin (**10**),[11] which was easily derived from phloroglucinol and ethyl propiolate. Treatment of the diacetate (**11**) obtained from **10** with 1-bromo-3-methylbut-2-ene in acetone in the presence of anhydrous potassium carbonate gave **12**,[12] which was hydrolyzed to afford the 5-prenylated coumarin (**13**).[12] The location of the prenyl ether at C-5 in **13** was confirmed by observation of nuclear Overhauser effect (NOE) between the *O*-methyl protons (δ_H 3.85) and two *meta*-coupled aromatic protons at δ_H 6.29 and 6.41 in the ^{1}H-NMR spectrum of the corresponding methyl ether (**14**).[12] A mixture of **13** and sodium acetate was heated at 190 °C for 20 min[13] gave three kinds of reaction products in 38 (A), 29 (B), and 27 (C) % yields. The spectroscopic data (UV, IR, and ^{1}H-NMR) of the major product (A) were in agreement with those of **15** reported by Murray and Jorge.[14] The ^{1}H-NMR spectra of the other products (B and C) showed the same signal pattern (see Experimental), which suggested the presence of a 2,3,3-trimethyldihydrofuran ring in the molecules, instead of a prenyl moiety and one of the *meta*-coupled aromatic protons on **13**. These data suggested the structures of B and C to be **16** and **18** (or *vice versa*), respectively. These products were considered to have been formed by Claisen rearrangement of the prenyl moiety on **13** followed by cyclization to give *ortho*-phenolic hydroxy groups. For confirmation of the structure, **14** was treated[13] with sodium acetate at 190 °C, as in the case of **13**, to give a cyclization product **17**, which was found to be identical with the *O*-methyl ether of B by spectrometric comparisons (UV, IR, MS, and ^{1}H-NMR). Therefore, we assigned the structures **16** for B and **18** for C.[15] Further treatment[16] of **16** with 3-chloro-3-methylbut-1-yne followed by cyclization at reflux temperature in diethylaniline afforded a colorless oil (**8**) in 37% yield, and this was found to be identical with natural citrusarin-B by spectrometric comparisons (UV, IR, MS, and ^{1}H-NMR). On the basis of the above results, the structures of citrusarin-A and -B were established as **7** and **8**, respectively, except for the absolute stereochemistry.

Experimental

Melting point was 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. HMBC spectra were measured at $J=8$ Hz on the GX-400. All mass spectra were measured under electron impact (EI) conditions, unless otherwise stated, using an M-80 (Hitachi) or a JMS-HX-110 (JEOL) spectrometer having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam

spectrophotometer (JASCO) in methanol, IR spectra on a IR-810 (JASCO) in $CHCl_3$, and optical rotations on a DIP-181 (JASCO) in $CHCl_3$. The preparative thin layer chromatographies (TLC) were done on Kieselgel 60 F_{254} (Merck).

Isolation and Separation of Peroxytamarin (1) and *cis*-Casegravol (5) The dried root (185 g) of *Citrus sulcata* HORT. *ex* TAKAHASHI (Japanese name: sanbo-kan) grown in the orchard of Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Shimizu, Sizuoka, was extracted with acetone at room temperature. The acetone extract (9.5 g) was chromatographed over silica gel with benzene–hexane (1 : 1 and then 10 : 1), benzene, benzene–acetone (20 : 1, 10 : 1, 4 : 1, and then 2 : 1), acetone, and methanol successively to give nine fractions. Each fraction was subjected to preparative TLC using appropriate mixtures of benzene, hexane, acetone, $CHCl_3$, isopropyl ether, ethyl acetate, CH_2Cl_2, and methanol as developing solvents to obtain 21 kinds of coumarins and 4 kind of acridones, as stated below. From fraction 4: tamarin[5,6] 15.2 mg, hopeyhopin[17] 2.6 mg, demethylauraptenol[18] 5.2 mg, seselin[19] 319 mg, xanthyletin[20] 2.2 mg, and a new coumarin, peroxytamarin (**1**) 17.7 mg. From fractions 4 and 5: demethylsuberosin[21] 2.9 mg, osthenol[22] 35 mg, osthenon[23] 14.7 mg, junosmarin[24] 4.0 mg, 5-hydroxyseselin[25] 3.9 mg, scoparone[26] 4.1 mg, nordentatin[27] 3.1 mg, natsucitrine-I[28] 5.0 mg, citprssine-I[26,29] 3.4 mg, 5-hydroxynoracronycine[29] 8.2 mg, and citracridone-I[29] 13.1 mg. From fraction 5: umbelliferone[30] 3.8 mg, *cis*-osthenon[31] 5.6 mg, and bisosthenon[32] 3.1 mg. From fraction 6: 7-methoxy-8-formylcoumarin[33] 6.1 mg, kiyomal[34] 1.1 mg, casegravol[9] 4.8 mg, (+)-decurusidinol[35] 18.3 mg, and a new coumarin. *cis*-casegravol (**5**) 1.9 mg. Known compounds were fully characterized by UV, IR, ^{1}H-NMR, and MS.

Peroxytamarin (1) Colorless oil. $[\alpha]_D$ −5.3° (c=0.12, $CHCl_3$). UV λ_{max} nm: 222, 244 (sh), 252, 297, 324. IR ν_{max} cm^{-1}: 3450 (br), 1725, 1620, 1560. ^{1}H-NMR δ: 8.23 (1H, br, OOH), 7.61 (1H, d, J=9.4 Hz, H-4), 7.25 (1H, s, H-5), 6.79 (1H, s, H-8), 6.23 (1H, d, J=9.4 Hz, H-3), 5.00 and 4.96 (each 1H, s, *exo*-CH_2), 4.58 (1H, dd, J=5.2, 7.9 Hz), 3.91 (3H, s, OCH_3), 2.92 (1H, dd, J=14.1, 5.2 Hz), 2.87 (1H, dd, J=14.1, 8.0 Hz), 1.82 (3H, s). MS m/z (%): 276 (M^+, 3), 260 (8), 258 (21), 206 (33), 205 (12), 191 (15), 190 (99), 189 (100), 177 (11), 175 (10), 162 (19), 161 (23), 160 (14), 159 (47), 147 (23). HR-MS Calcd for $C_{15}H_{16}O_5$: 276.0996. Found: 276.0982.

Photo-oxygenation[3] of Suberosin (2) Oxygen gas was bubbled through a solution of suberosin (**2**)[4] (50 mg) in pyridine (5 ml) containing hematoporphyrin (5 mg), and the solution was irradiated with a high-pressure Hg lamp using a Pyrex glass filter for 2 h. Then, the solvent was evaporated off. The residue was subjected to silica gel preparative TLC to afford 19.2 and 20.1 mg of **1** and **3**, respectively. Compound **1** was found to be identical with natural peroxytamarin by spectrometric comparisons (IR, UV, ^{1}H-NMR, and MS). **3**: Light yellow oil. IR ν_{max} cm^{-1}: 3450 (br), 1725, 1620, 1560. UV λ_{max} nm: 206, 223, 256, 296, 306, 339. ^{1}H-NMR δ: 7.63 (1H, d, J=9.4 Hz), 7.53 (1H, s), 6.89 (1H, d, J=16.5 Hz), 6.78 (1H, s), 6.34 (1H, d, J=16.5 Hz), 6.27 (1H, d, J=9.4 Hz), 3.92 (3H, s), 1.48 (6H, s). MS m/z (%): 276 (M^+, 11), 260 (34), 245 (55), 243 (20), 205 (34), 204 (28), 203 (64), 190 (22), 189 (100), 175 (21), 149 (30). HR-MS Calcd for $C_{15}H_{16}O_5$: 276.0996. Found: 276.0988.

Treatment of Natural Peroxytamarin (1) with Triphenylphosphine A methanolic solution (1 ml) of **1** (2.3 mg) and Ph_3P (1 mg) was stirred for 1 h at room temperature. The solvent was evaporated off *in vacuo*. The residue was subjected to preparative TLC to afford a colorless oil (**4**) (1.7 mg), $[\alpha]_D$ +8.9° (c=0.083, $CHCl_3$), IR ν_{max} cm^{-1}: 3050, 1720, 1620, 1560. UV λ_{max} nm: 206, 222, 253, 297, 328. ^{1}H-NMR δ: 7.63 (1H, d, J=9.4 Hz, H-4), 7.29 (1H, s, H-5), 6.81 (1H, s, H-8), 6.26 (1H, d, J=9.4 Hz, H-3), 4.94 (1H, s, H-4′), 4.85 (1H, s, H-4′), 4.32 (1H, dd, J=4.0, 8.7 Hz, H-2′), 3.91 (3H, s, 7-OCH_3), 3.01 (1H, dd, J=4.0, 14.1 Hz, H-1′), 2.76 (1H, dd, J=8.7, 14.1 Hz, H-1′), 1.83 (3H, s, 3′-CH_3). This was found to be identical with tamarin (**4**) by comparisons of the IR, UV, and ^{1}H-NMR data with those in the literature.[5] Tamarin (**4**), $[\alpha]_D$ +8.9° ($CHCl_3$), derived from natural **1** was found to contain only 5% excess of one of the enantiomers by ^{1}H-NMR analysis using a chiral shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium-(III). According to the literature,[6] the major enantiomer (**4**) having positive $[\alpha]_D$ can be assigned as having *R*-configuration in the chiral center on the side chain.

***cis*-Casegravol (5)** Colorless oil. $[\alpha]_D$ −12.1° (c=0.102, $CHCl_3$). UV λ_{max} nm: 208. 255, 284, 320. IR ν_{max} cm^{-1}: 3630, 3450 (br), 1720, 1600. ^{1}H-NMR δ: 7.63 (1H, d, J=9.4 Hz, H-4), 7.41 (1H, d, J=8.4 Hz, H-5), 6.86 (1H, d, J=8.4 Hz, H-6), 6.78 (1H, d, J=12.4 Hz, H-1′), 6.49 (1H, d, J=12.4 Hz, H-2′), 6.25 (1H, d, J=9.4 Hz, H-3), 3.90 (3H, s, 7-OCH_3), 2.25 (3H, s, 3′-CH_3). CI-MS m/z 294 [M+NH_4]$^+$. MS m/z (%): 260 (23), 259 (100), 257 (12), 245 (45), 231 (17), 219 (19), 213 (14), 203 (23).

Isolation of Citrusarin-A (7) and -B (8) The dried root (3200 g) of *Citrus Hassaku* HORT. *ex* Y. TANAKA (Japanese name: hassaku) was extracted with acetone at room temperature. The acetone extract (485 g) was subjected to silica gel column chromatography with benzene. The benzene eluate was further chromatographed on silica gel eluted successively with hexane and benzene–hexane (1 : 9 and then 1 : 4). The eluates was subjected repeatedly to preparative TLC with benzene–ethyl acetate (9 : 1), hexane–benzene (1 : 9), isopropyl ether, acetone–hexane (1 : 9), ethyl acetate–benzene (1 : 19), and/or acetone–hexane (1 : 19) to obtain citrusarin-A (**7**) (210 mg) and citrusarin-B (**8**) (5.1 mg) as well as other known compounds, which were characterized by spectral analysis (UV, IR, and ^{1}H-NMR).[36]

Citrusarin-A (7) Pale yellow oil. 0.00065% yield from the dry root. $[\alpha]_D$ +5.2° (c=0.172, $CHCl_3$). UV λ_{max} nm: 210, 226, 284, 340. IR ν_{max} cm^{-1}: 1720, 1640, 1625, 1610. HR-MS Calcd for $C_{19}H_{20}O_4$ 312.1359. Found: 312.1353. MS m/z (%): 312 (M^+, 58), 298 (35), 297 (100), 241 (11).

Citrusarin-B (8) Pale yellow oil. 0.000016% yield from the dry root. $[\alpha]_D$ +3.0° (c=0.148, $CHCl_3$). UV λ_{max} nm: 204, 230, 239 (sh), 247 (sh), 285 (fl), 293, 328. IR ν_{max} cm^{-1}: 1720, 1640, 1620. HR-MS Calcd for $C_{19}H_{20}O_4$: 312.1360. Found: 312.1358. MS m/z (%): 312 (M^+, 35), 298 (34), 297 (100), 295 (14), 241 (11), 149 (28), 141 (11).

5,7-Diacetoxycoumarin (11) A mixture of **10**[11] (7.4 g), acetyl anhydride (100 ml), and pyridine (7 ml) was refluxed for 2 h. The reaction mixture was poured into ice water, neutralized with $NaHCO_3$, and then extracted with ethyl acetate. The organic layer was washed with diluted aqueous $NaHCO_3$ and water, dried with anhydrous $MgSO_4$, and then concentrated to dryness to give **11** (6.3 g) as colorless prisms. **11**: mp 124—125 °C. UV λ_{max} nm: 206, 290, 313. IR ν_{max} cm^{-1}: 1780, 1740, 1630. ^{1}H-NMR δ_H: 7.73 (1H, d, J=9.7 Hz, H-4), 7.04 (1H, d, J=2.0 Hz, H-6 or -8), 6.98 (1H, d, J=2.0 Hz, H-8 or -6), 6.40 (1H, d, J=9.7 Hz, H-3), 2.40 (3H, s, OAc), 2.33 (3H, s, OAc). MS m/z (%): 262 (M^+, 7), 220 (22), 179 (12), 178 (100), 150 (48).

5-(2-Enyl-3-methylbut)oxy-7-acetoxycoumarin (12)[12] A mixture of **11** (30 mg) and 1-bromo-3-methylbut-2-ene (20.4 mg) in acetone (4.5 ml) was stirred in the presence of anhydrous K_2CO_3 (94 mg) for 14 h at room temperature. The reaction mixture was filtered and the filtrate was concentrated to dryness. The residue was dissolved in H_2O and extracted with ethyl acetate. The organic layer was washed with 5% K_2CO_3 and H_2O, and dried with anhydrous $MgSO_4$. The solvent was evaporated off. The residue was subjected to preparative TLC ($CHCl_3$: acetone = 40 : 1) to give **12** (9.0 mg) and **11** (5.0 mg) as colorless needles. **12**: mp 128—129 °C (lit.[12] mp 127—129 °C). UV λ_{max} nm: 210, 240, 304. IR ν_{max} cm^{-1}: 1770, 1738, 1618. ^{1}H-NMR δ_H: 8.05 (1H, d, J=9.8 Hz, H-4), 6.69 (1H, d, J=2.0 Hz, H-6 or -8), 6.51 (1H, d, J=2.0 Hz, H-8 or, -6), 6.28 (1H, d, J=9.8 Hz, H-3), 5.48 (1H, t, J=6.7 Hz, H-2′), 4.59 (2H, d, J=6.7 Hz, H-1′), 2.33 (3H, s, OAc), 1.82 (3H, s, CH_3), 1.75 (3H, s, CH_3). MS m/z (%): 288 (M^+, 2), 220 (57), 178 (83), 150 (21), 149 (21), 81 (12), 70 (16), 69 (100).

5-(2-Enyl-3-methylbut)oxy-7-hydroxycoumarin (13)[12] A methanolic solution (2 ml) of **12** (5.1 mg) and 1% aqueous $NaHCO_3$ (0.1 ml) was refluxed for 30 min, neutralized with diluted HCl, and then extracted with ethyl acetate. The organic layer was dried with anhydrous $MgSO_4$ and the solvent was evaporated off. The residue was subjected to preparative TLC (CH_2Cl_2: acetone = 20 : 1) to give **13** (4.9 mg) as colorless prisms. **13**: mp 134—136 °C. UV λ_{max} nm: 208, 249, 257, 330. IR ν_{max} cm^{-1}: 3300 (br), 1720, 1615. ^{1}H-NMR δ_H: 8.04 (1H, d, J=9.4 Hz, H-4), 6.63 (1H, d, J=2.0 Hz, H-6 or -8), 6.33 (1H, d, J=2.0 Hz, H-8 or -6), 6.11 (1H, d, J=9.4 Hz, H-3), 5.46 (1H, t, J=6.7 Hz, H-2′), 4.55 (2H, d, J=6.7 Hz, H-1′), 1.79 (3H, s, CH_3), 1.73 (3H, s, CH_3). MS m/z (%): 246 (M^+, 14), 179 (12), 178 (83), 150 (41), 70 (10), 69 (100).

***O*-Methylation of 13** A mixture of **13** (30 mg), anhydrous K_2CO_3 (18 mg) and methyl iodide (76.5 mg) in acetone (10 ml) was refluxed for 3 h. K_2CO_3 was filtered off and the filtrate was subjected to preparative TLC ($CHCl_3$: acetone = 20 : 1) to give colorless needles (**14**, 29.9 mg). **14**: mp 88—91 °C. UV λ_{max} nm: 208, 246, 255, 328. IR ν_{max} cm^{-1}: 1730, 1615. ^{1}H-NMR δ_H: 8.00 (1H, d, J=9.8 Hz, H-4), 6.41 (1H, d, J=2.0 Hz, H-6 or H-8), 6.29 (1H, d, J=2.0 Hz, H-8 or H-6), 6.14 (1H, d, J=9.8 Hz, H-3), 5.48 (1H, t, J=6.7 Hz, H-2′), 4.57 (2H, d, J=6.7 Hz, H-1′), 3.85 (3H, s, OCH_3), 1.82 (3H, s, CH_3), 1.76 (3H, s, CH_3). MS m/z (%): 260 (M^+, 19), 193 (22), 192 (99), 164 (60), 163 (14), 149 (17), 135 (15), 70 (11), 69 (100). Irradiation of the methoxy protons at δ_H 3.85 gave **4** and 12% NOE of the protons signals at δ_H 6.41 and 6.29, respectively.

Cyclization Reaction[13] of 13 A mixture of **13** (10 mg) and sodium

acetate (20 mg) was heated in an oil bath at 190 °C for 20 min. The residue was dissolved in a small amount of acetone and subjected to preparative TLC (iso-Pr_2O : acetone = 20 : 1) to give three kinds of pale yellow oils, A (*Rf*: 0.21) (**15**), B (*Rf*: 0.58) (**16**), and C (*Rf*: 0.46) (**18**) in 38, 29, and 27% yields, respectively. A (**15**):[14] UV λ_{max} nm: 208, 259, 331. IR ν_{max} cm^{-1}: 3400 (br), 1720, 1615. ^{1}H-NMR (acetone-d_6) δ_H: 8.03 (1H, d, J = 9.8 Hz, H-4), 6.66 (1H, s, OH), 6.48 (1H, s, H-6), 6.03 (1H, d, J = 9.8 Hz, H-3), 5.24 (1H, t, J = 7.0 Hz, H-2'), 3.40 (2H, d, J = 7.0 Hz, H-1'), 1.81 (3H, s, CH_3), 1.64 (3H, s, CH_3). MS *m/z* (%): 246 (M^+, 27), 191 (35), 97 (20), 83 (26), 73 (21), 71 (33), 70 (24), 69 (100). B (**16**): UV λ_{max} nm: 212, 258, 328. IR ν_{max} cm^{-1}: 3260 (br), 1710, 1630. ^{1}H-NMR δ_H: 7.82 (1H, d, J = 9.4 Hz, H-4), 6.57 (1H, s, H-8), 6.13 (1H, d, J = 9.4 Hz, H-3), 4.52 (1H, q, J = 6.4 Hz, H-2'), 1.78 (1H, br d, OH), 1.46 (3H, s, CH_3), 1.41 (3H, d, J = 6.4 Hz, CH_3), 1.21 (3H, s, CH_3). MS *m/z* (%): 246 (M^+, 41), 232 (21), 231 (100), 203 (19), 149 (46), 101 (12). C (**18**)[15]: UV λ_{max} nm: 210, 258, 331. IR ν_{max} cm^{-1}: 3400 (br), 1720, 1630. ^{1}H-NMR δ_H: 7.93 (1H, d, J = 9.8 Hz, H-4), 6.72 (1H, s, H-8), 6.39 (1H, s, OH), 6.16 (1H, d, J = 9.8 Hz, H-3), 4.45 (1H, q, J = 6.4 Hz, H-2'), 1.49 (3H, s, CH_3), 1.39 (3H, d, J = 6.4 Hz, CH_3), 1.24 (3H, s, CH_3). MS *m/z* (%): 246 (M^+, 41), 232 (17), 231 (100), 203 (16), 149 (31).

Cyclization Reaction[13] of 14 A mixture of **14** (20 mg) and sodium acetate (40 mg) was heated in an oil bath at 190 °C for 20 min. The reaction mixture was dissolved in acetone. The acetone solution was subjected to preparative TLC (hexane : acetone = 4 : 1) to give colorless prisms (**17**) (5.7 mg) together with the starting material (**14**) (5.3 mg). **17**: mp 107—110 °C. UV λ_{max} nm: 212, 228, 256 (sh), 322. IR ν_{max} cm^{-1}: 1730, 1630. ^{1}H-NMR δ_H: 7.77 (1H, d, J = 9.7 Hz, H-4), 6.35 (1H, s, H-8), 6.14 (1H, d, J = 9.7 Hz, H-3), 4.49 (1H, q, J = 6.4 Hz, H-2'), 3.86 (3H, s, OCH_3), 1.39 (3H, d, J = 6.4 Hz, CH_3), 1.40 (3H, s, CH_3), 1.16 (3H, s, CH_3). MS *m/z* (%): 260 (M^+, 54), 246 (20), 245 (100), 217 (17), 135 (16), 105 (73).

***O*-Methylation of 16** A mixture of **16** (10 mg) and methyl iodide (15 mg) in acetone (3 ml) was refluxed for 35 min in the presence of anhydrous K_2CO_3 (125 mg). The reaction mixture was filtered. The filtrate was subjected to preparative TLC ($CHCl_3$: hexane = 4 : 1) to give colorless prisms, mp 105—110 °C (10.9 mg). This product was found to be identical with **17** derived from **14** by UV, IR, ^{1}H-NMR, and MS comparisons.

Citrusarin-B (8) from 16 A mixture of **16** (40.7 mg), 3-chloro-3-methylbut-1-yne (169 mg), and anhydrous K_2CO_3 (338 mg) in acetone (8 ml) containing one drop of dimethylformamide was refluxed for 8 h. During the reaction, the butyne (169 mg) was added six times to the reaction mixture at intervals of 70 min. The reaction mixture was filtered, and the filtrate was concentrated to dryness. The residue was dissolved in diethylaniline (2 ml) and heated at 220 °C for 1 h in N_2 gas.[16] Diluted HCl was added to the mixture and the solution was extracted with diethyl ether. The extract was dried over anhydrous $MgSO_4$, and the solvent was evaporated off. The residue was subjected to preparative TLC (hexane : acetone = 4 : 1, and then 2 : 1) to give a pale yellow oil (19 mg), which was found to be identical with natural citrusarin-B (**8**) by spectrometric comparisons (UV, IR, ^{1}H-NMR, and MS).

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