The Structures of Claudimerines-A and -B, Novel Bicoumarins from *Citrus hassaku*[1)]

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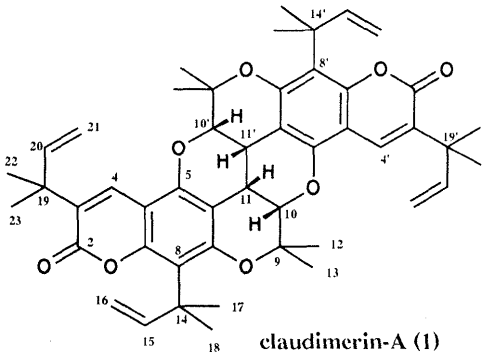
Two novel dimeric coumarins, claudimerin-A (1) and -B (3), were isolated from the roots of *Citrus hassaku* (Rutaceae). The chemical structures were elucidated by spectroscopic studies and/or single-crystal X-ray analysis. The main structural characteristic is a pyranopyran ring connecting two clausarin (2) units.

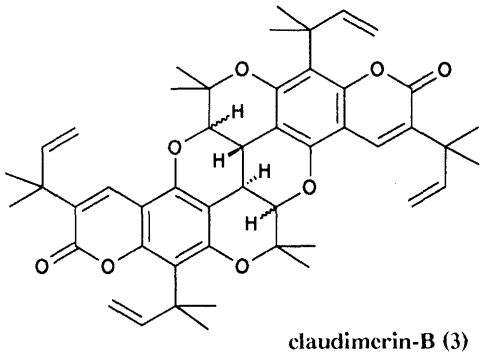
Key words bicoumarin; claudimerin-A; claudimerin-B; *Citrus hassaku*; Rutaceae

In our phytochemical investigations on the constituents of *Citrus* plants (Rutaceae), we have reported the isolation and structure elucidation of many kinds of novel coumarins and acridone alkaloids.[2)] Dimeric coumarins, khelmarins-A, -B,[3)] -C, bisnorponcitrin, bishassanidin,[4)] bisosthenon,[5)] bisclausarin,[6)] nordenletin,[7)] citrumarins-A—D,[8)] hassmarin,[9)] bisparasin,[10)] furobinordentatin and furobiclausarin[11)] are unique constituents of this genus. As a part of this program, we continued to investigate the constituents of *Citrus hassaku* HORT. *ex* TANAKA[4)] and isolated two novel dimeric coumarins containing a pyranopyran ring, named claudimerin-A (**1**) and claudimerin-B (**3**). Here we describe the isolation and structure elucidation of these novel coumarins.

Structure of Claudimerin-A (1) Claudimerin-A (**1**) was isolated as colorless cubes, mp 318—320 °C, $[\alpha]_D \pm 0°$ ($CHCl_3$). The molecular formula $C_{48}H_{54}O_8$ was obtained by high-resolution mass spectroscopy (HR-MS). The presence of a 5,7-dioxygenated coumarin nucleus was suggested by the UV [212, 244 (sh), 266, 292, 328 nm] and IR (1715, 1620, 1600 cm^{-1}) spectra.[12)] The ^{1}H-NMR spectrum showed a lone H-4 [δ 7.67 (s)] and two 1,1-dimethylallyl groups [δ 6.14 (1H, dd, J=10.7, 17.8 Hz), 4.74 (1H, dd, J=1.2, 17.8 Hz), 4.78 (1H, dd, J=1.2, 10.7 Hz), 1.54, 1.59 (each 3H, s), 6.09 (1H, dd, J=10.3, 17.8 Hz), 5.02 (1H, dd, J=1.2, 10.3 Hz), 5.03 (1H, dd, J=1.2, 17.8 Hz), 1.39, 1.40 (each 3H, s)]. The remaining signals at δ 3.55, 4.34 (each 1H, dd, J=0.7, 1.5 Hz), due to the hydrogens attached to the carbons at δ 25.96 (C-11) and 73.25 (C-10), were assigned to the protons on the connecting portions between the two coumarin moieties, based on the ^{1}H–^{13}C correlated spectroscopy (COSY) technique. In nuclear Overhauser effect (NOE) experiments, irradiation of the signal at δ 4.34 (H-10) induced a 10.5% increment of the signal at δ 3.55 (H-11). On irradiation of the signals at δ 3.55, 14.8 and 7.3% increments were observed in the signals at δ 4.34 and 1.50 (13-Me). The results, together with the J-values, suggested



claudimerin-A (1)



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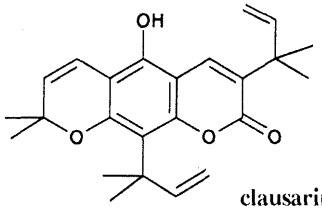

clausarin (2)

Chart 1

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the *cis* orientation of these protons on the 2,2-dimethyldihydropyran ring. As shown by the arrows in Fig. 1, the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum showed ^2J and ^3J correlations of methyl protons (δ 1.40 or 1.39) to the carbon at δ 128.70 (C-3) as well as the carbon at δ 145.70 (C-20) and from the methyl protons (δ 1.54, 1.59) to the carbon at δ 113.14 (C-8), suggesting the location of a 1,1-dimethylallyl moiety at C-3 and C-8 on the coumarin skeleton. These data suggested that **1** contained 10,11-dihydrogenated clausarin (**2**) units in the molecule. The observed number of signals was half of that expected and an intense fragment ion appeared at *m/z* 379, showing **1** to be a symmetrical coumarin dimer. The linkage structure of the two coumarin units could not be elucidated from the spectroscopic data and the presence of a novel skeleton was assumed. The complete structure and relative stereochemistry were obtained from a single-crystal X-ray analysis. The crystal data are as follows: monoclinic, space group $P2_1/c$, $a=15.960(2)$, $b=17.864(2)$, $c=17.961(2)$ Å, $\beta=116.08(1)°$, $V=4598(16)$ Å^3; $Z=4$, $D_c=1.180$ g/cm^3. The molecule of claudimerin-A found in an asymmetric unit is displayed in Fig. 2 with atomic labels. The asymmetric unit contains an acetone (solvent) molecule, but this is excluded from the figure for clarity. No abnormal bond parameter has been found in the molecule except for the double bonds of the 1,1-dimethylallyl group, where the unusually short atomic distances can probably be ascribed to the thermal motion of this group. The molecule has a dimeric feature of two units joined together like a hinge. The dihedral angle between the mean planes of two units is about 100°. Claudimerin-A (**1**) has a novel structure composed of two clausarin (**2**)[13] units linked symmetrically with the formation of the pyranopyran ring.

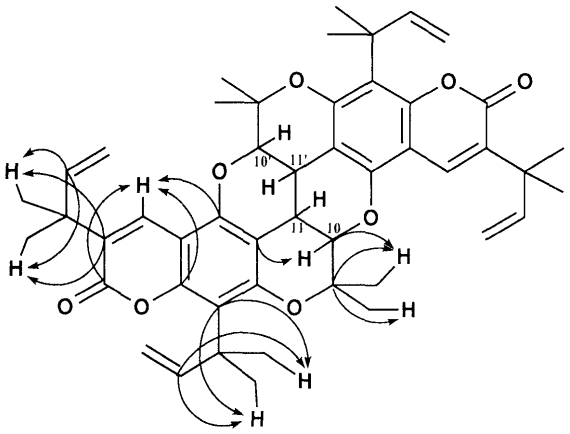

Fig. 1. C–H Long-Range Correlations in the HMBC Spectrum of Claudimerin-A (**1**)

Structure of Claudimerin-B (3) Claudimerin-B (**3**) was obtained as optically inactive colorless cubes, mp 356—358 °C (dec.). The molecular formula $C_{48}H_{54}O_8$, the same as that of claudimerin-A (**1**), was obtained from the molecular ion peak at *m/z* 758.3813 in HR-MS. The IR and UV spectra (see Experimental) indicated the presence of a 5,7-dioxygenated coumarin nucleus.[12] The ^{1}H-NMR spectrum showed signals due to H-4 of the coumarin skeleton, two 1,1-dimethylallyl groups and a 3,4-disubstituted 2,2-dimethylpyran ring. Because the observed signals were half of what was expected, a symmetrically dimerized structure was suggested. Comparisons of the ^{1}H- and ^{13}C-NMR data of claudimerin-B (**3**) with those of claudimerin-A (**1**) and an HMBC experiment (Fig. 3) revealed the structural similarity of both compounds. The prominent features distinguishing **3** from **1** were the difference of the coupling constants of H-10 and H-11. The coupling constants of H-10 and H-11 of claudimerin-A (**1**) were 1.5 and 0.7 Hz, indicating a *cis* orientation. Relatively large *J*-values (2.9, 6.4 Hz) of H-10 and H-11 compared with **1** were observed in claudimerin-B (**3**),

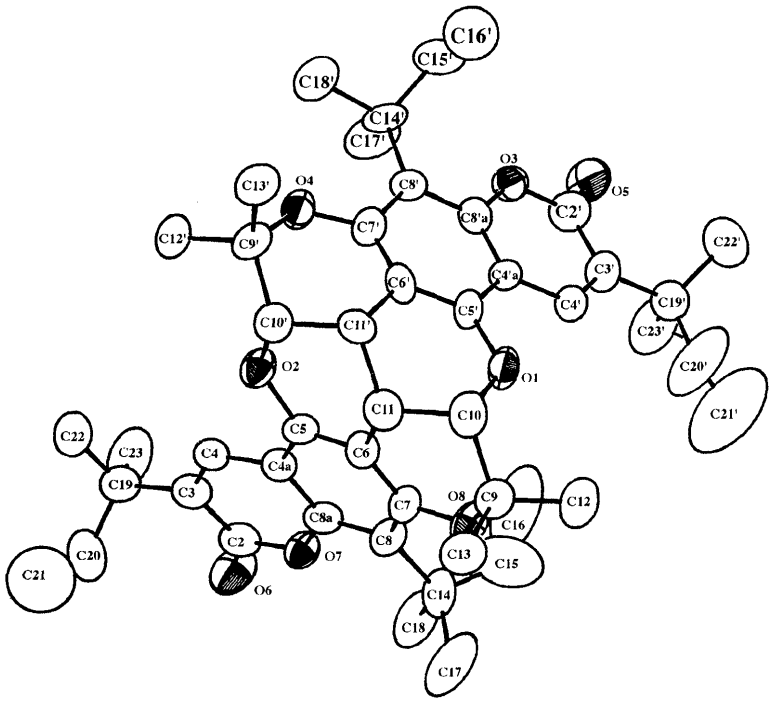

Fig. 2. Perspective View of Claudimerin-A (**1**) with Atomic Numbering

suggesting the presence of *cis* and *trans* orientations among the four protons at H-10, H-11, H-10′ and H-11′. The *trans–trans–trans* and *cis–trans–cis* linkage of two coumarin units were assumed. In the NOE experiment, irradiation of the signal at δ 4.55 (H-10 or H-10′) induced 24.9% and 6.5% increments of the signals at δ 2.81 (H-11′ or H-11) and 1.75 (13-CH_3 or 13′-CH_3), respectively. When the signal at δ 2.81 was irradiated, 24.9% and 11.3% increments were observed in the signals at δ 4.55 and 1.24 (12-CH_3 or 12′-CH_3). Examination by using nuclear Overhauser enhancement spectroscopy (NOESY) showed cross peaks between the signals at δ 4.55 and 1.75, δ 4.55 and 2.81, and δ 1.24 and 2.81, respectively. Since the chemical shifts for H-10/H-10′; H-11/H-11′; 13-CH_3/13′-CH_3 and 12-CH_3/12′-CH_3 were the same, the relative configurations of four protons on the pyranopyran ring, whether *trans–trans–trans* form or *cis–trans–cis* form, could not be determined from these results. From the above-mentioned results, the structure of claudimerin-B was assigned as **3** except for the relative configuration of the linkage. Because of the lack of optical activity of claudimerin-A and -B, it can be assumed either that they are artifacts or that they are formed in the plant cells without the participation of enzymes. The presence of a pyranopyran ring is rare and claudimerin-A and -B are the first coumarins containing this moiety from natural sources.

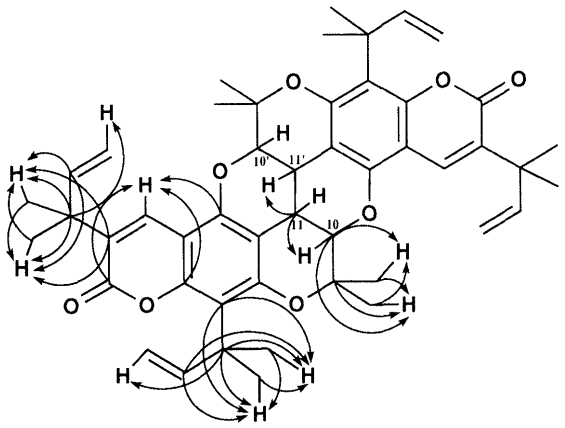

Fig. 3. C–H Long-Range Correlations in the HMBC Spectrum of Claudimerin-B (**3**)

Experimental

Melting points were measured on a Yanagimoto micromelting point hot-stage apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL GX-270, GX-400 and GSX-500 spectrometers. Chemical shifts are shown on the δ (ppm) scale with tetramethylsilane (TMS) as an internal reference. HMBC spectra were measured at J= 8 Hz on the GX-400. Electron impact (EI)- and HR-MS were taken with a JMS-HX-110 spectrometer having a direct inlet system. UV spectra were recorded on a Shimadzu UV-160A spectrometer in EtOH, and IR spectra on a Shimadzu IR-450 spectrometer in $CHCl_3$. The preparative thin-layer chromatography (PTLC) was done on Kieselgel 60 F_{254} (Merck).

Isolation of Claudimerin-A (1) and Claudimerin-B (3) The acetone extract (485 g)[4] of dried roots (3.2 kg) of *C. hassaku* HORT. *ex* TANAKA (Rutaceae) collected at Innoshima, Hiroshima, was subjected to silica gel column chromatography eluted successively with hexane, benzene, CH_2Cl_2, acetone and MeOH. The hexane eluate (18.7 g) was subjected to repeated column chromatography to isolate claudimerin-B (**3**) (24.1 mg). The benzene eluate (4.6 g) was subjected repeatedly to PTLC with isopropyl ether and benzene–acetone (8:2) to give claudimerin-A (**1**) (23.0 mg).

Claudimerin-A (1) Colorless cubes, mp 318—320 °C (from $CHCl_3$),

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** and **3**

	1		**3**	
	δ_H	δ_C	δ_H	δ_C
2		159.67		159.45
3		128.70		130.73
4	7.67 (2H, s)	132.27	7.85 (2H, s)	131.72
4a		102.78		109.59
5		148.08		148.52
6		95.83		106.02
7		154.17		152.49[b]
8		113.14		117.47
8a		152.82		152.21[b]
9		77.76		78.33
12-CH_3	1.70 (6H, s)	24.62	1.24 (6H, s)	19.47
13-CH_3	1.50 (6H, s)	29.13	1.75 (6H, s)	27.68
10	4.34 (2H, dd, J=0.7, 1.5 Hz)	73.25	4.55 (2H, dd, J=2.9, 6.4 Hz)	78.05
11	3.55 (2H, dd, J=0.7, 1.5 Hz)	25.96[a]	2.81 (2H, dd, J=2.9, 6.4 Hz)	30.81
14		41.02		41.13
17-CH_3	1.54 (6H, s)	29.64	1.67 (6H, s)	29.27
18-CH_3	1.59 (6H, s)	25.87	1.70 (6H, s)	29.71
15	6.14 (2H, dd, J=10.7, 17.8 Hz)	150.08	6.34 (2H, dd, J=17.3, 10.8 Hz)	149.26
16	4.74 (2H, dd, J=1.2, 17.8 Hz)	107.69	4.93 (2H, dd, J=1.0, 10.8 Hz)	108.76
	4.78 (2H, dd, J=1.2, 10.7 Hz)		4.97 (2H, dd, J=1.0, 17.3 Hz)	
19		40.06		40.31
22-CH_3	1.39 (6H, s)	25.82[a]	1.51 (6H, s)	25.98
23-CH_3	1.40 (6H, s)	26.05[a]	1.51 (6H, s)	25.98
20	6.09 (2H, dd, J=10.3, 17.8 Hz)	145.70	6.22 (2H, dd, J=10.5, 17.8 Hz)	145.45
21	5.02 (2H, dd, J=1.2, 10.3 Hz)	111.75	5.11 (2H, dd, J=1.0, 10.5 Hz)	112.04
	5.03 (2H, dd, J=1.2, 17.8 Hz)		5.12 (2H, dd, J=1.0, 17.8 Hz)	

a, *b*) Assignments may be interchanged.

$[\alpha]_D \pm 0°$ ($c=0.226$, $CHCl_3$). UV λ_{max}^{EtOH} nm: 212, 244 (sh), 266, 292, 328. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1620, 1600. HR-MS Calcd for $C_{48}H_{54}O_8$ 758.3819. Found: 758.3821. EI-MS *m/z* (%): 758 (base peak, 100), 744 (11), 743 (50), 675 (9), 380 (8), 379 (21), 378 (4), 365 (11), 364 (7), 363 (8), 351 (10), 349 (5), 311 (7). ^{1}H- and ^{13}C-NMR ($CDCl_3$) δ: see Table 1. NOE: irradiation at δ 4.34—10.5% enhancement at δ 3.55; irradiation at δ 3.55—14.8% and 7.3% enhancement at δ 4.34 and 1.50.

Claudimerin-B (3) Colorless cubes, mp 356—358 °C (dec.) (from $CHCl_3$), $[\alpha]_D \pm 0°$ ($c=0.140$, $CHCl_3$). UV λ_{max}^{EtOH} nm: 215, 242 (sh), 264 (sh), 324. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1620, 1605. HR-MS Calcd for $C_{48}H_{54}O_8$ 758.3819. Found: 758.3813. EI-MS *m/z* (%): 760 (M^+, 20), 758 (base peak, 100), 744 (26), 743 (44), 675 (12), 380 (20), 379 (46), 378 (15), 365 (21), 364 (13), 363 (17), 351 (11), 349 (13), 311 (11). ^{1}H- and ^{13}C-NMR ($CDCl_3$) δ: see Table 1. NOE: irradiation at δ 4.55—24.9% and 6.5% enhancement at δ 2.81 and 1.75; irradiation at δ 2.81—24.9% and 11.3% enhancement at δ 4.55 and 1.24.

Crystal Structure Determination and Refinement of Claudimerin-A (1) Plate crystals suitable for X-ray analysis were obtained by slow evaporation of an acetone solution of claudimerin-A (**1**). Preliminary examination of a crystal on an Enraf–Nonius CAD4 diffractometer indicated a monoclinic unit cell and the space group $P2_1/c$ was suggested from the systematic absence of reflections. A least-squares refinement of the setting angles of 25 reflections, collected in the range of $14.6° < 2\theta < 20.5°$, led to the crystal data. Diffracted intensities were measured with graphite-monochromated MoK_α radiation ($\lambda=0.71073$ Å). The tables of data collection parameters (positional parameters, individual bond lengths and bond angles) are available on request. Net intensities were reduced to a set of relative structure factors by the application of standard Lorenz and polarization factors. No absorption correction was made. The structure was solved by the direct method and refined by least-squares techniques.[14] Most non-hydrogen atoms of a molecule in an asymmetric unit were found in the initial E-map. Subsequent difference Fourier (DF) syntheses revealed all non-hydrogen atomic positions. The non-hydrogen atoms were refined with anisotropic thermal parameters, and hydrogen atoms bound to carbon were included in calculated positions as fixed parameters. Final cycles of full-matrix, least-squares refinement were carried to convergence at $R=0.087$ and $R_w=0.091$.[15] The final difference Fourier was judged to be essentially featureless. Tables of the atomic coordinates for non-hydrogen atoms with the isotropic equivalent thermal factors are available from one of the authors (K. H.).

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$R=\Sigma||F_o|-|F_c||/\Sigma|F_o|$, $R_w=[\Sigma_w(|F_o|-|F_c|)^2/\Sigma_w F_o^2]^{1/2}$ with unit weight.