Structure of Pummeloquinone, a New Coumarin–Naphthoquinone Dimer Isolated from *Citrus* Plants

Chihiro Ito,[a] Tomohisa Ono,[a] Emiko Tanaka,[a] Yuko Takemura,[b] Toshimi Nakata,[b] Hiromi Uchida,[b] Motoharu Ju-ichi,*,[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] Nishinomiya, Hyogo 663, Japan, and Okitsu Branch Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries,[c] Okitsu, Shimizu, Shizuoka 424-02, Japan. Received July 7, 1992

Pummeloquinone (1), the third example of a coumarin–naphthoquinone dimer from a natural source, was isolated from the roots of several hybrid seedlings resulting from a cross of May pummelo × Marsh grapefruit (Rutaceae) grown in an orchard at Okitsu, Shizuoka, and its structure was elucidated by chemical and spectrometric methods. Synthesis of 1 was also achieved by Diels–Alder reaction between *trans*-dehydroosthol (3) and 2-methoxy-1,4-benzoquinone followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation.

Keywords pummeloquinone; coumarin; *Citrus*; naphthoquinone; dehydroosthol; 2-methoxy-1,4-benzoquinone

Many kinds of coumarins are present in *Citrus* plants (Rutaceae).[1] As part of our continuing studies of constituents of *Citrus* plants,[2] we have obtained a new coumarin–naphthoquinone dimer named pummeloquinone (**1**) from the roots of several hybrid seedlings resulting from a cross of pummelo (*Citrus grandis*) cv. May pummelo × Marsh grapefruit (*C. paradisi*) grown at the orchard of the Fruit Tree Research Station, Okitsu, Shizuoka, and its structure was established by chemical and spectrometric methods, including H–C correlation spectroscopy (COSY) and H–C long-range COSY. Pummeloquinone (**1**) is the third example of a coumarin–naphthoquinone dimer to have been isolated from a natural source.[3,4]

Results and Discussion

Pummeloquinone (**1**) was obtained as yellow prisms, mp >250 °C. The molecular formula was established as $C_{22}H_{16}O_6$ by high resolution mass spectrometry (HR-MS). The UV spectrum showed bands at λ_{max} 206, 220 (sh), 248, 256, 283, and 322 nm, and IR bands due to three carbonyl moieties appeared at ν_{max} 1720, 1685, and 1650 cm^{-1}. Characteristic ^{1}H- and ^{13}C-NMR signals due to a 7-oxygenated 8-substituted coumarin nucleus included *ortho*-located aromatic protons at δ_H 6.94 and 7.49 (each, d, J=8.8 Hz), AB-type signals at δ_H 6.21 and 7.68 (each d, J=9.8 Hz) due to α- and β-protons on an α,β-unsaturated carbonyl moiety and a lower field carbonyl carbon signal at δ_C 160.94. In a nuclear Overhauser effect (NOE) experiment, irradiation of one of the two methoxy proton signals at δ_H 3.77 gave a 14% enhancement of the doublet at δ_H 6.94 (H-6), one of the *ortho*-coupled aromatic protons, indicating the location of this methoxy group to be at C-7 on the 8-substituted coumarin nucleus. The remaining signals of a pair of *meta*-coupled protons at δ_H 7.29 and 8.02, a lone olefinic proton (δ_H 6.12, s), an aryl methyl group (δ_H 2.51), and a methoxy proton (δ_H 3.81) were assignable to an 8-substituted 2-methoxy-6-methylnaphthoquinone moiety, taking account of the appearance of two carbonyl carbon signals at δ_C 179.60 and 185.03 in the ^{13}C-NMR spectrum, two C=O stretching absorptions at ν_{max} 1685 and 1650 cm^{-1} in the IR spectrum, and the following results of NOE experiments. Irradiation of an aryl methyl signal at δ_H 2.51 gave 12% and 9% enhancements of the *meta*-coupled proton signals at δ_H 7.29 (H-7′) and 8.02 (H-5′), respectively. Irradiation of the methoxy signal at δ_H 3.81 gave an 18% enhancement of the singlet at δ_H 6.12 (H-3′). Locations of the methoxy and methyl groups at C-2′ and C-6′, respectively, on the naphthoquinone skeleton were established by the following results of H–C long-range COSY experiments. One of the angular carbons (δ_C 126.96) on the naphthoquinone skeleton showed three-bond H–C correlations with both *meta*-coupled aromatic protons [δ_H 8.02 (H-5′) and 7.29 (H-7′)], and another angular carbon at δ_C 133.45 on the naphthoquinone nucleus, only with the singlet proton at δ_H 6.12 (H-3′). On the basis of the spectral results described above coupled with other results of H–C long-range COSY experiments shown in Fig. 1 by arrows, we assigned the structure **1** to pummeloquinone.[5]

From the biogenetic viewpoint, pummeloquinone (**1**) is considered to be derived from the previously known coumarin, *trans*-dehydroosthol (**3**)[6] and 2-methoxy-1,4-benzoquinone through a [4+2] cycloaddition reaction.

Next, we tried to synthesize pummeloquinone (**1**) from the corresponding coumarin, *trans*-dehydroosthol (**3**)[6] and 2-methoxy-1,4-benzoquinone. *trans*-Dehydroosthol (**3**) prepared from commercially available umbelliferone (**4**)[7] according to Reisch and Bathe[5] was treated with

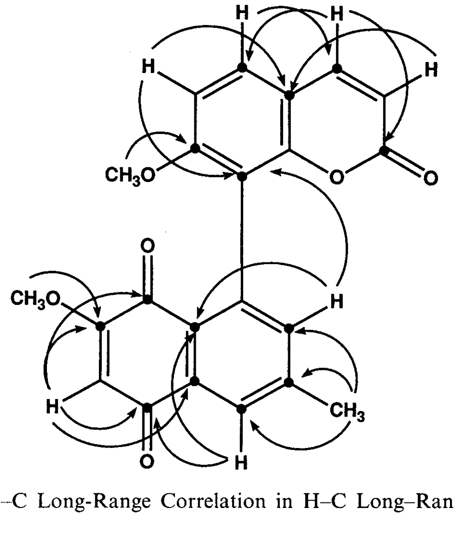

Fig. 1. H–C Long-Range Correlation in H–C Long-Range COSY of **1**

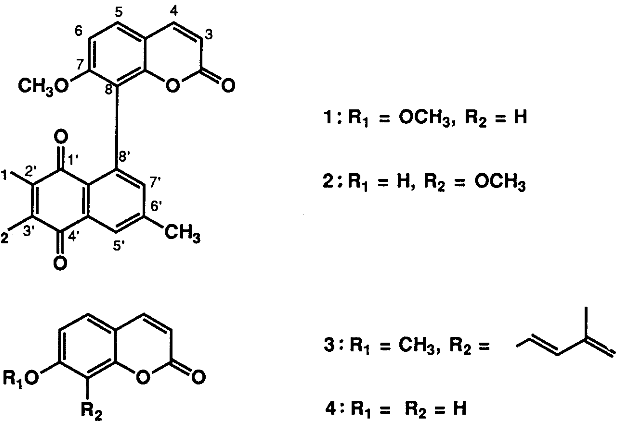

Chart 1

Table I. ^{1}H- and ^{13}C-NMR Data for Pummeloquinone (**1**) and **2**

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2	—	160.94	—	160.98
3	6.21 (d, 9.8)	113.17	6.21 (d, 9.8)	113.20
4	7.68 (d, 9.8)	143.60	7.69 (d, 9.8)	143.64
4a	—	113.09	—	113.05
5	7.49 (d, 8.8)	127.98	7.50 (d, 8.8)	127.88
6	6.94 (d, 8.8)	107.56	6.96 (d, 8.8)	107.59
7	—	159.48	—	159.32[b]
7-OMe	3.77 (s)	56.19[a]	3.78 (s)	56.17[a]
8	—	117.95	—	118.41
8a	—	151.98	—	151.93
1′	—	179.60	—	184.87
2′	—	160.56	5.91 (s)	110.96
2′-OMe	3.81 (s)	56.26[a]	—	—
3′	6.12 (s)	108.70	—	159.38[b]
3′-OMe	—	—	3.85 (s)	56.23[a]
4′	—	185.03	—	180.58
4′a	—	133.45	—	132.40
5′	8.02 (d, 2.0)	127.50	8.06 (d, 1.5)	128.01[c]
6′	—	144.85	—	143.48
6′-Me	2.51 (s)	21.80	2.50 (s)	21.55
7′	7.29 (d, 2.0)	137.88	7.31 (d, 1.5)	138.97
8′	—	132.99	—	132.19
8′a	—	126.96	—	128.01[c]

Spectra were measured at 400 (^{1}H) and 100 (^{13}C) MHz in $CDCl_3$. Values are in δ (ppm). All proton signals except for methyls correspond to 1H. Figures in parentheses are coupling constants (J) in Hz. Assignments were confirmed by H–H, H–C, and H–C long-range (J = 5 Hz) COSY analyses. *a, b*) Assignments may be interchanged. *c*) Overlapped signal.

2-methoxy-1,4-benzoquinone under three different reaction conditions. First, according to the method reported by Reisch and Bathe,[5] a toluene solution of the coumarin and the benzoquinone was left at room temperature for 3 d under nitrogen gas, followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), giving two adducts[8] in 8 and 1% yields. The major adduct was found to be identical with a natural specimen of pummeloquinone (**1**). Under the analogous reaction conditions reported by Wu *et al.*[9] (benzene solution in a sealed tube at 125 °C for 19.5 h, followed by DDQ treatment), the desired adduct (**1**) was obtained in only 6% yield. Next, we tried to heat a mixture of the two starting materials neat at 150 °C for 1 min, followed by DDQ treatment, to give **1** in 20% yield along with a minor adduct (**2**) in 2% yield. The minor adduct (**2**) showed the same molecular formula $C_{22}H_{16}O_6$ as that of **1** in HR-MS, and the signal pattern in the ^{1}H-NMR spectrum of **2** also showed close similarity to that of **1** (Table I), except for a 0.21 ppm high-field shift of the lone quinoid proton signal at δ_H 6.12 (H-3′) in the spectrum of **1**. In an H–C long-range COSY experiment on **2** after the assignment of carbon signals by H–C COSY, a pair of *meta*-coupled aromatic protons (H-5′ and 7′) and the lone quinoid proton (H-2′) showed three-bond correlations with a carbon at δ_C 128.01 (C-8a), indicating the location of a methoxy group and a methyl group at C-3′ and -6′, respectively, on the 1,4-naphthoquinone nucleus. On the basis of these spectrometric results, we assigned structure **2**, corresponding to a regio-isomer of **1**, to the minor adduct.

Experimental

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 $CDCl_3$. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal reference. The H–C long-range COSY spectrum was measured at J = 5 Hz on the GX-400. All mass spectra were measured under electron impact (EI) conditions, using an M-80 (Hitachi) spectrometer having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in methanol, CD on a J-600 (JASCO) in methanol, and IR on an IR-810 (JASCO) in $CHCl_3$. The preparative TLC were done on Kieselgel 60 F_{254} (Merck).

Isolation of Pummeloquinone (1) The plant material used in this study was identified as several hybrid seedlings resulting from a cross of pummelo (*Citrus grandis* (L.) Osbeck) cv. May pummelo × Marsh grapefruit (*C. paradisi* Macf.). May pummelo is a hybrid variety resulting from a cross of hassaku (*C. hassaku* hort. ex Tanaka) × Hirado pummelo (*C. grandis* (L.) Osbeck). The genetic background of the sampled seedlings is considered to be predominantly pummelo. The plant was grown in the orchard of the Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry, and Fisheries, Shimizu, Shizuoka.

The dried root (500 g) of the plant was extracted with acetone at 40 °C. The acetone extract (56.2 g) was chromatographed over silica gel and eluted successively with hexane, benzene, CH_2Cl_2, acetone, and MeOH. The CH_2Cl_2 eluent was further subjected to silica gel chromatography eluted with acetone–CH_2Cl_2. The eluent was rechromatographed under medium pressure with ethyl acetate–benzene (1 : 9). Further separation and purification by preparative TLC using MeOH–$CHCl_3$ (1 : 9) and acetone–hexane (1 : 1) as developing solvents gave pummeloquinone (**1**) (8.0 mg) as yellow prisms.

Pummeloquinone (**1**): Yellow prisms, mp >250 °C. UV λ_{max} nm: 206, 220 (sh), 248, 256, 283, 322. IR ν_{max} cm^{-1}: 1720, 1685, 1650, 1625, 1610. EI-MS *m/z* (%): 376 (M^+, 50), 345 (100), 302 (11), 274 (11), 189 (12). HR-MS Calcd for $C_{22}H_{16}O_6$: 376.0946. Found: 376.0960. ^{1}H- and ^{13}C-NMR: see Table I. Difference NOE: irradiation of 2′-OCH_3 (δ_H 3.81), 18% enhancement of H-3′ (δ_H 6.12); irradiation of 7-OCH_3 (δ_H 3.77), 14% enhancement of H-6 (δ_H 6.94); irradiation of 6′-CH_3 (δ_H 2.51), 12 and 9% enhancements of H-7′ (δ_H 7.29) and H-5′ (δ_H 8.02), respectively.

Synthesis of Pummeloquinone (1) a)[5] A solution of **3**[5,6] (13.8 mg, 0.057 mmol) in toluene (1.5 ml) and a solution of 2-methoxy-1,4-benzoquinone (23.6 mg, 0.171 mmol) in $CHCl_3$ (1.0 ml) were mixed and left under N_2 gas at room temperature for 3 d. The solvent was evaporated *in vacuo* and the residue was dissolved in $CHCl_3$ (3 ml). This solution was refluxed with DDQ (13.5 mg, 0.057 mmol) for 4 h. The solvent was evaporated and the residue was subjected to silica gel preparative TLC ($CHCl_3$: hexane : acetone = 10 : 3 : 1) to afford **1** (1.6 mg) and **2** (0.2 mg), which showed *Rf* values of 0.42 and 0.35, respectively. Compound **1** was found to be identical with the natural specimen of pummeloquinone (**1**) by IR and ^{1}H-NMR comparisons.

b) A mixture of **3** (14.1 mg, 0.058 mmol) in benzene (6 ml) and 2-methoxy-1,4-benzoquinone (24.0 mg, 0.174 mmol) in ethanol (1.0 ml) was heated in a sealed tube at 125 °C for 19.5 h. The reaction mixture was treated with DDQ (13.7 mg, 0.058 mmol) in the same manner as in a) to give **1** (1.4 mg) and **2** (0.7 mg).

c) A mixture of **3** (15.2 mg, 0.063 mmol) and 2-methoxy-1,4-

benzoquinone (26.1 mg, 0.189 mmol) was heated neat at 150 °C for 1 min. The reaction mixture was dissolved in $CHCl_3$ (3 ml) and refluxed with DDQ (14.9 mg, 0.063 mmol) under N_2 gas for 4 h. The solvent was evaporated *in vacuo* and the residue was subjected to silica gel preparative TLC to obtain **1** (4.8 mg) and **2** (0.4 mg) in 20 and 2% yeilds, respectively. **2**: yellow prisms, mp >250 °C. UV λ_{max} nm: 206, 220 (sh), 248, 254, 282, 323. IR ν_{max} cm^{-1}: 1720, 1690, 1655, 1625, 1610. EI-MS *m/z* (%): 376 (M^+, 62), 345 (100), 317 (74), 302 (63), 274 (38), 189 (26). HR-MS Calcd for $C_{22}H_{16}O_6$: 376.0946. Found: 376.0957. ^1H- and ^{13}C-NMR: see Table I. Difference NOE: irradiation of 3'-OCH_3 (δ_H 3.85), 16% enhancement of H-2' (δ_H 5.91); irradiation of 7-OCH_3 (δ_H 3.78), 15% enhancement of H-6 (δ_H 6.96); irradiation of 6'-CH_3 (δ_H 2.50), 8 and 7% enhancements of H-7' (δ_H 7.31) and H-5' (δ_H 8.06), respectively.

References and Notes

1) R. D. H. Murray, J. Mendez, and S. A. Brown, "The Natural Coumarins. Occurrence, Chemistry and Biochemistry," John Wiley & Sons Ltd., New York, 1982, pp. 27—45.
2) C. Ito, K. Fujiwara, M. Kajita, M. Ju-ichi, Y. Takemura, Y. Suzuki, K. Tanaka, M. Omura, and H. Furukawa, *Chem. Pharm. Bull.*, **39**, 2509 (1991) and references cited therein.
3) Zs. Roza, I. Mester, J. Reisch, and K. Szendrei, *Planta Medica*, **55**, 68 (1989).
4) H. Ishii, J.-I. Kobayashi, H. Seki, and T. Ishikawa, *Chem. Pharm. Bull.*, **40**, 1358 (1992).
5) J. Reisch and A. Bathe, *Justus Liebigs Ann. Chem.*, **1988**, 543.
6) C. Ito and H. Furukawa, *Chem. Pharm. Bull.*, **35**, 4277 (1987); F. Bohlmann, H. Franke, and C. Zdero, *An. Quim.*, **68**, 765 (1972).
7) A. Hashimoto and T. Kawana, *Yakugaku Zasshi*, **55**, 183 (1935); W. Steck and M. Mazurek, *Lloydia*, **35**, 418 (1972).
8) Reisch and Bathe[5] obtained only one adduct, which was characterized as **1**.
9) T.-S. Wu, M.-J. Liou, C.-J. Lee, T.-T. Jong, A.-T. McPhail, D. R. McPhail, and K.-H. Lee, *Tetrahedron Lett.*, **30**, 6649 (1989).