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A NOVEL DIMERIC COUMARIN FROM *CITRUS LUMIA*

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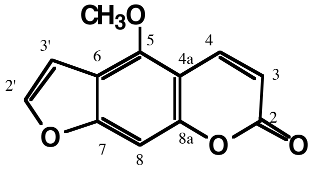
Abstract - A new dimeric coumarin, bergapten dimer (**2**), was isolated from the peel of *Citrus lumia* Risso in addition to the known nine coumarins, one triterpene, one steroid, and two unidentified flavones. The structure of the dimer was elucidated by X-Ray crystallographic analysis.

Many kinds of *Citrus* plants have been studied in our laboratories and various compounds including acridone alkaloids and coumarins were isolated and identified.[1] Recently we carried out a primary screening of extracts of *Citrus* fruit to search useful compounds for cancer chemoprevention and found good results in some of the extracts.[2] In the process of identifying active principles from these extracts, a new dimeric coumarin was isolated from the peel of *Citrus lumia* Risso in addition to the known compounds, which were friedelin, β-sitosterol, limettin, psolaren, bergapten (**1**), umbelliferone, scoparone, hydrangetin, heraclenol, byabangelicin, 7,8-dimethoxycoumarin and two unidentified flavones.



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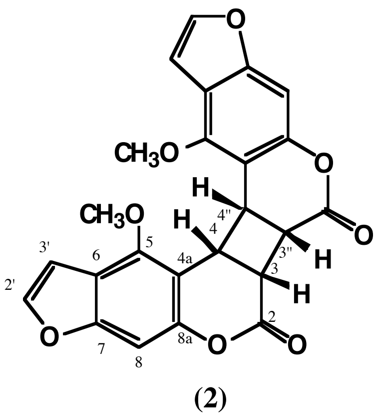

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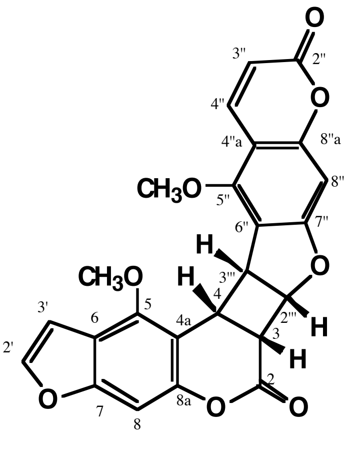

(3)

Recently, Lima *et al.* reported the isolation and the structure elucidation of furocoumarin dimer, psoralen dimer, from the roots of *Dorstenia lindeniana*.[3] It prompted us to report the isolation and structural characterization of a new dimeric coumarin, bergapten dimer (**2**).

Bergapten dimer (**2**) was obtained as optically inactive colorless prisms, mp 253-256 °C. The molecular formula $C_{24}H_{16}O_8$ was defined by molecular ion peak at m/z 433.0926 in the HR-FABMS. The ^{1}H-NMR spectrum showed the presence of three aromatic protons [δ 7.43 (2H, d, J = 2.2 Hz), 6.75 (2H, d, J = 2.2 Hz), 6.71 (2H, s)], one methoxy group (δ 3.89) and two methine protons [δ 4.44 (2H, m), 3.99 (2H, m)]. The two doublets at δ 7.43 and 6.75 were assumed to the signals of H-2' and H-3' of furan ring. In the NOE experiment, irradiation of the methoxy signal at δ 3.89 caused 2%and 15% enhancement of the signals at δ 4.44 and 6.75. These results indicated that the two signals at δ 6.71 and 3.89 were due to H-8 and methoxy group attached to C-5. It means that the linear orientation of furan ring (bergapten unit) existed. The dimeric structure of **2** was proposed by the absence of the characteristic signals of H-3 and H-4 of coumarin nucleus and the observed number of signals which were half of that expected in the ^{1}H NMR spectrum. This was supported by the ^{13}C NMR spectrum. It showed two doublets at δ 39.5 and 37.4 which required saturated carbons with only one hydrogen for each. The ^{1}H NMR spectrum also gave multiplet signals similar to an AA'BB' system for these protons at δ 3.99 (H-3) and 4.44 (H-4). In the NOE experiment, irradiation of the signal at δ 3.99 (H-3) enhanced the signal at δ 4.44 (H-4), and the other way around. These data strongly suggested the presence of the cyclobutane moiety and symmetrical structure in the dimer.[4] Based on the spectral analysis of high field NMR spectroscopy such as COSY, HMBC and HMQC, the individual proton resonances were assigned to the corresponded carbon and the plane symmetry structure of the dimer was proposed as **2**. However, the question of the regiochemistry of a head-to-head or a head-to-tail dimer, and the stereochemistry of the four protons on the cyclobutane ring, were still remained. The HMBC spectrum (Figure 1) could not clear the regiochemistry of the

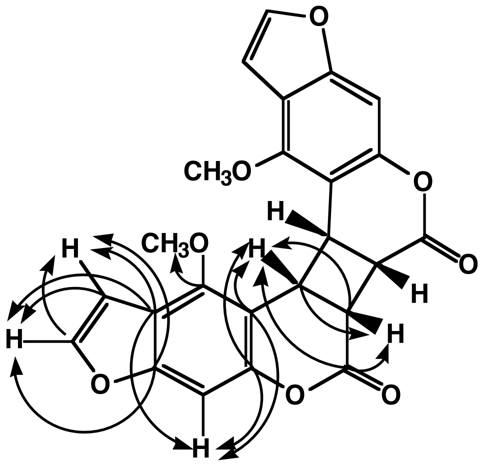

Figure 1. C-H Long-Range Correlations in the HMBC Spectrum of Bergapten dimer (**2**)

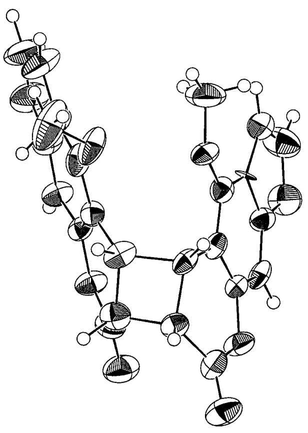

Figure 2. ORTEP Drawing of **2**

cyclobutane linkage part of the dimer.

Because of a small quantity obtained as natural isolate, photodimerization of bergapten (**1**) was carried out to prove the structure of the dimer in the following manner.[5 - 7]

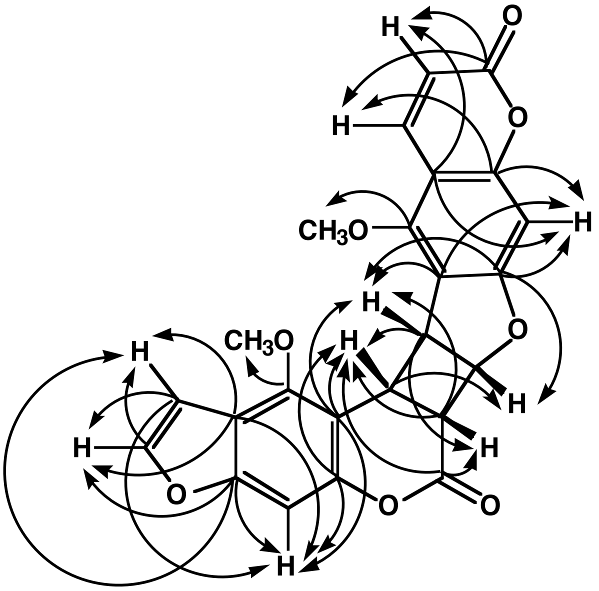

Figure 3. C-H Long Range Correlations in the HMBC Spectrum of Compound B (**3**)

Irradiation of bergapten (**1**) in solid-state using a high-pressure Hg lamp through a Pyrex glass filter for 168h at room temperature gave two dimeric products, compound A (**2**) and B (**3**) in 14.2% and 1.0% yields, respectively, accompanied with a 18.5% recovery of bergapten. Compound A obtained as a major product was found to be identical with natural isolate by spectral comparisons (UV, IR and ^{1}H NMR). To confirm the stereochemistry, compound A was subjected to a single crystal X-Ray analysis. The derived molecular structure (Figure 2) established the head-to-head *syn* stereochemistry for the cyclobutane moiety of the dimer.

In general, UV irradiation of coumarin gave a mixture of isomers, head-to-head (*syn, anti*) and head-to-tail (*syn*, *anti*) dimer, and usually a head-to-head *syn* dimer as a major product.[8] However, the spectrum of compound B (**3**) suggested unsymmetrical structure though the HR-FABMS gave the molecular formula $C_{24}H_{16}O_{8}$ same with bergapten dimer (**2**). The existence of a strong absorption band at 330 nm in the UV spectrum indicated conjugation in bergapten moiety which was lost in bergapten dimer (**2**). The ^{1}H NMR spectrum showed distinctive signals of H-4’’ and H-3’’ [δ 7.66, 6.00 (each 1H, d, J = 9.5 Hz)] of coumarin skeleton, H-2’ and H-3’ [δ 7.44 (1H, d, J = 2.6 Hz), 6.87 (1H, dd, J = 2.6, 0.7 Hz)] of furan ring, H-8 and H-8’’ [δ 6.64 (1H, d, J = 0.7 Hz), 6.40 (1H,s)]. The signals due to four methine protons (δ 5.64, 4.86, 4.46, 4.03) and two methoxy groups (δ 4.20, 3.91) also appeared. In the NOE experiment, irradiation of the methoxy signal at δ 4.20 caused 14% enhancement of the signal at δ 6.87 (H-3’), indicating the methoxy group attached to C-5. On the other hand, irradiation of the another methoxy signal at δ 3.91 showed 2% and 11% enhancement of the signals at δ 7.66 (H-4’’) and 4.86 (H-3’’’). These results suggested the existence of H-4’’, H-3’’ and 5’’-methoxy group. The correlation of four methine protons in the H-H COSY spectrum and the NOE between the methine signal at δ 4.86 (1H, t, J = 7.0Hz) and the methoxy group at δ 3.91 indicated that the dimerization occurred between the furan ring and the pyran-2-one ring. The structure of **3** was further elucidated through the use of HMBC experiment (Figure 3). The key correlations for the assignments around the cyclobutane ring were observed for H-3’’’ / C-6’’, C-7’’, C-4a, C-3 ; H-2’’’ / C-7’’, C-4 ; H-4 / C-3’’’, C-2, C-3, C-8a, C-4a ; H-3 / C-2, C-3’’’. The stereochemistry around the cyclobutane ring was solved by NOE experiment. Each of the enhancement among four methine protons in the NOE experiment (see EXPERIMENTAL) revealed the stereochemistry as *cis-syn-cis*. Based on the above findings, the structure of compound B was established as **3**. This is the first example to be shown this kind of dimer during a process of the photodimerization of coumarins.

The known compounds were identified by direct comparison with authentic samples and spectral data.[9 - 15]

EXPERIMENTAL

Extraction and Isolation *Citrus lumia* Risso was cultivated and collected in Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Okitsu, Shizuoka. The dried peels (1.5 kg) of *Citrus lumia* Risso was extracted with acetone (3250 mL) at room temperature (3 days x 2) and under reflux for 7.5 h. The acetone extract (94.7 g) dissolved in 600 mL of water was first extracted with 600 mL of AcOEt three times and then extracted with 450 mL of n-butanol three times. The AcOEt extract (11.37 g) was chromatographed over silica gel with toluene, CH_2Cl_2, CH_2Cl_2 - acetone (9:1), acetone, and MeOH, successively. Each eluate was further subjected to preparative TLC repeatedly and furnished the known compounds, friedelin (5.4 mg), β-sitosterol (24.3 mg), limettin (5.1 mg), psolaren (4.7 mg), bergapten **(1)** (0.8 mg), umbelliferone (1.9 mg), scoparone (4.4 mg), hydrangetin (1.3 mg), heraclenol (14.2 mg), byabangelicin (9.5 mg), 7,8-dimethoxycoumarin (1.2 mg) and two unidentified flavones (6.8 mg and 18.6 mg, respectively) as well as a bergapten dimer **(2)** (3.0 mg). The dimer was obtained from the CH_2Cl_2 eluate [solvent system : AcOEt - benzene (3:7), acetone - benzene (1:9)].

Bergapten dimer (2) Colorless prisms (acetone), mp 253-256 °C, $[\alpha]_D$ 0° (c=0.0415, $CHCl_3$); HR-FABMS m/z: 433.0926 ($[M+H]^+$, found), 433.0924 (calcd for $C_{24}H_{17}O_8$); EI-MS m/z: 432 $[M]^+$, 217, 216 (base peak), 201, 188, 173, 145; UV λ_{max} (MeOH, nm) : 221, 252 (sh), 263 (sh), 288, 301; IR ν_{max} ($CHCl_3$, cm^{-1}) : 1764, 1600, 1469; ^1H-NMR ($CDCl_3$,δ) : 7.43 (2H, d, J = 2.2Hz, H-2’), 6.75 (2H, d, J = 2.2Hz, H-3’), 6.71 (2H, s, H-8), 4.44 (2H, m, H-4), 3.99 (2H, m, H-3), 3.89 (6H, s, 5-OMe); Differential NOE : irradiation of 5-OMe (δ 3.89) gave 2% and 15% NOE at H-4 (δ 4.44) and H-3’ (δ 6.75); irradiation of H-3 (δ 3.99) gave 8% NOE at H-4; irradiation of H-4 gave 9% NOE at H-3; ^{13}C-NMR ($CDCl_3$, δ) : 165.1 (s, C-2), 156.3 (s, C-7), 150.7 (s, C-5), 150.1 (s, C-8a), 144.0 (d, C-2’), 112.4 (s, C-6), 104.6 (d, C-3’), 102.6 (s, C-4a), 93.9 (d, C-8), 58.8 (q, 5-OMe), 39.5 (d, C-3), 37.4 (d, C-4).

Photodimerization of Bergapten Crystalline bergapten (**1**) (245.7 mg, Fluka, Japan) was irradiated with a high pressure Hg lamp through a Pyrex glass filter for 168 h (8 h x 21days) at rt. The solid was dissolved in acetone and subjected to flush column chromatography [solvent system : AcOEt - hexane (4:6), acetone - hexane (4:6)] and preparative TLC [solvent system : acetone - $CHCl_3$ (1:9), AcOEt - hexane (7:3)] to give a compound A **(2)** (34.8 mg) and compound B **(3)** (2.4 mg). Compound A was found to be identical with the natural bergapten dimer on the basis of spectral data comparison (UV, IR and ^1H NMR).[16]

Compound B (3) Pale yellow oil; HR-FABMS m/z: 433.0930 ($[M+H]^+$, found), 433.0924 (calcd for

$C_{24}H_{17}O_8$); EI-MS m/z: 216 [M/2]$^+$, 201, 188, 173, 145; FAB-MS m/z: 433 [M+H]$^+$; UV λ_{max} (MeOH, nm) : 228 (sh), 254 (sh), 262 (sh), 301 (sh), 330, 350 (sh); IR ν_{max} (CHCl$_3$, cm^{-1}) : 1725, 1625, 1469; ^{1}H-NMR (CDCl$_3$, δ) : 7.66 (1H, d, J = 9.5 Hz, H-4’’), 7.44 (1H, d, J = 2.6 Hz, H-2’), 6.87 (1H, dd, J = 2.6, 0.7 Hz, H-3’), 6.64 (1H, d, J = 0.7 Hz, H-8), 6.40 (1H, s, H-8’’), 6.00 (1H, d, J = 9.5 Hz, H-3’’), 5.64 (1H, t, J = 7.0 Hz, H-2’’’), 4.86 (1H, t, J = 7.0 Hz, H-3’’’), 4.46 (1H, dt, J = 1.5, 7.0 Hz, H-4), 4.20 (3H, s, 5-OMe), 4.03 (1H, dd, J = 1.5, 7.0 Hz, H-3), 3.91 (3H, s, 5’’-OMe); Differential NOE : irradiation of H-3 (δ 4.03) gave 6% and 7% NOE at H-4 (δ 4.46) and H-2’’’ (δ 5.64); irradiation of H-4 gave 6% and 9% NOE at H-3’’’ (δ 4.86) and H-3; irradiation of H-3’’’ gave 9%, 5% and 11% NOE at 5’’-OMe (δ 3.91), H-2’’’ and H-4; irradiation of H-2’’’ gave 8% and 4% NOE at H-3 and H-3’’’; irradiation of 5’’-OMe gave 2% and 11% NOE at H-4’’ (δ 7.66) and H-3’’’; irradiation of 5-OMe (δ 4.20) gave 14% NOE at H-3’ (δ 6.87); ^{13}C-NMR (CDCl$_3$, δ) : 166.3 (s, C-7’’), 163.1 (s, C-2), 161.1 (s, C-2’’), 156.6 (s, C-8’’a), 156.3 (s, C-7), 153.7 (s, C-5’’), 150.0 (s, C-5 or C-8a), 149.7 (s, C-8a or C-5), 144.0 (d, C-2’), 138.7 (d, C-4’’), 111.8 (s, C-6), 111.0 (d, C-3’’), 109.9 (s, C-6’’), 106.4 (s, C-4’’a), 104.8 (d, C-3’), 101.7 (s, C-4a), 93.8 (d, C-8’’), 93.7(d, C-8), 82.8 (d, C-2’’’), 59.1 (q, 5’’-OMe), 58.6 (q, 5-OMe), 48.2(d, C-3’’’), 41.1 (d, C-3), 34.7 (d, C-4).

X-Ray Structure Determination of the Bergapten Dimer For X-Ray diffraction analysis, colorless transparent prisms of the dimer were grown from acetone. A crystal measuring 0.35 x 0.20 x 0.15 mm was selected form among them and used for all data collection. Unit cell constants and intensity data were obtained with a Mac Science MXC18 diffractometer controlled by the DIP2000 (Mac Science) program package, using graphite-monochromated Mo *K*α radiation (λ= 0.71073 Å), in the 2θ-ω scan mode. Crystal data of the bergapten dimer : $C_{24}H_{16}O_8$; M=432.00 ; monoclinic; space group $P2_1/n$; *a*=11.364000 (0) Å ; *b*=27.743999 (0) Å ; *c*=12.731000 (0) Å ; β=104.620003 (0)° ; *V*=38840100098 (0) Å^3; *Z*=8 ; *Dx*=1.530 Mgm^{-3} ; D*m*=1.500 Mgm^{-3}. Reflections (1897) with intensity above the $3\sigma(I)$ level were used for the structure determination. The structure was solved by a direct method using *CRYSTAN SIR*92 (Mac Science) and refined by the full-matrix least-squares method with anisotropic temperature factors for non-hydrogen atoms. All hydrogen atoms were clearly located on the difference Fourier maps and refined with isotropic temperature factors. The final *R* value was 0.054 (R_w=0.069).

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16 Isolation of other products are in progress.