TWO NEW BICOUMARINS FROM A *CITRUS* PLANT[1]

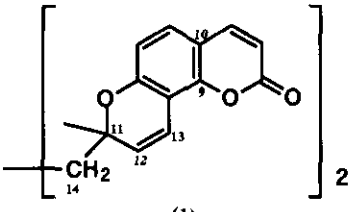
Shinobu Ikeda,[a] Yuko Takemura,[a] Motoharu Ju-ichi,*[a] Chihiro Ito,[b] and Hiroshi Furukawa[b]

Faculty of Pharmaceutical Sciences, Mukogawa Women's University,[a] Nishinomiya, Hyogo 663-8179, Japan, Faculty of Pharmacy, Meijo University,[b] Tempaku, Nagoya 468-8503, Japan

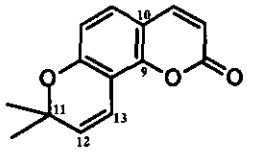
Abstracts - Two new bicoumarins, named biseselin (**1**) and yukomarin (**3**), were isolated from the roots of Yuko (*Citrus yuko* Hort. ex Tanaka) and their structures were elucidated by spectroscopic analyses.

In the course of our research on the phytochemical aspects of *Citrus* plants, we have isolated many new coumarins and acridone alkaloids.[2] On the constituents of the roots of Yuko (*Citrus yuko* Hort. ex Tanaka), we already reported the isolation and structure elucidation of new acridone alkaloids (yukomine, citracridone-III, yukocitrine and yukodine),[3,4] an acridone-lignan (acrignine-A)[5] and a bicoumarin (furobinordentatin).[6] Further studies resulted in the isolation of two new bicoumarins, named biseselin (**1**) and yukomarin (**3**). In this paper we deal with the isolation and structural elucidation of these two new bicoumarins.

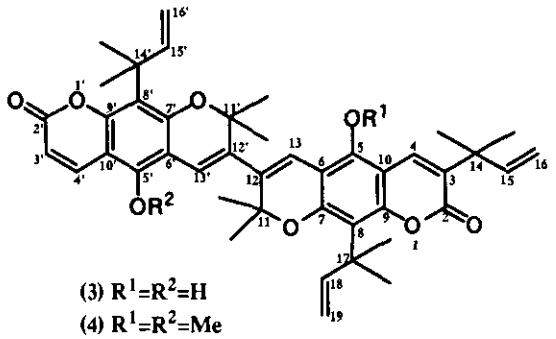
Biseselin (**1**) was isolated as colorless powder, $[\alpha]_D$ 0°, and its molecular formula $C_{28}H_{22}O_6$ has been deduced from the HR-MS showing the exact mass at m/z 454.1418. The IR (1630, 1590 cm^{-1}) and UV (218, 265, 295, 305, 332 nm) spectra showed characteristic absorptions of 7-oxygenated 8-substituted coumarin.[7] The ^{1}H-NMR spectrum indicated the characteristic signals due to H-4, H-3, H-5 and H-6 of coumarin skeleton [δ 7.60, 6.23 (each 1H, d, J=9.5 Hz), 7.20, 6.69 (each 1H, d, J=8.4 Hz)]. The remaining signals at δ 6.93, 5.65 (each 1H, d, J=9.9 Hz), 1.41 (3H, s) and 1.86 (2H, m) suggested the



(1)



(2)



(3) R^1=R^2=H
(4) R^1=R^2=Me

presence of 2,2-disubstituted pyran ring of which one substituent is methyl and the other is methylene group. These spectral features were similar to those of seselin (**2**)[8] except for the signals on the pyran ring. The observed numbers of proton and carbon signals were half of that expected, this coumarin was assumed to be symmetrical dimeric compound of seselin. The presence of methylene group was confirmed by negative signal at δ_C 35.9 in DEPT experiment. From the above result, the linkage of two seselin moieties was established to locate at C-14 of both moieties, and the structure of biseselin was concluded as **1**.

Yukomarin (**3**) was isolated as colorless powder, $[\alpha]_D$ 0°. The HR-MS showed molecular ion peak at m/z 690.3167, corresponding to the molecular formula $C_{43}H_{46}O_8$. The IR (1712, 1606, 1540 cm^{-1}) and UV [223 (sh), 301 (sh), 331 nm] spectra indicated the presence of coumarin skeleton.[7] The ^{1}H-NMR spectrum showed signals assignable to H-4', H-3' [δ 8.16, 6.08 (each 1H, d, J=9.9 Hz)] and H-4 [δ 8.01 (1H, s)] of coumarin nucleus, three 1,1-dimethylallyl groups [δ 6.32 (1H, dd, J=10.6, 17.6 Hz), 6.31 (1H, dd, J=10.6, 17.2 Hz), 6.20 (1H, dd, J=10.6, 17.6 Hz), 5.07 (1H, d, J=17.6 Hz), 5.02 (1H, d, J=10.6 Hz), 4.93 (1H, d, J=17.6 Hz), 4.92 (1H, d, J=17.2 Hz), 4.84 (1H, br d, J=10.6 Hz), 4.83 (1H, br d, J=10.6 Hz), 1.65, 1.64, 1.44 (each 6H, s)], and two 3,5,6-trisubstituted 2,2-dimethylpyran ring [δ 6.77, 6.75 (each 1H, s), 1.58, 1.57 (each 6H, s)]. The connectivity of these segments was examined by HMBC experiments (Figure 1). The cross peaks of H-13 (δ 6.75) to C-12' (δ 136.5), C-11 (δ 80.3), C-7 (δ 155.1), C-6 (δ 108.43) and C-5 (δ 149.9), and of H-13' (δ 6.77) to C-12 (δ 136.4), C-11' (δ 80.1), C-7' (δ 156.0), C-6' (δ 108.39) and C-5' (δ 149.4) indicated the linkage of two pyranocoumarin units between C-12 and C-12'. Though the cross peaks of C-5 (δ 149.9) to H-4 (δ 8.01) and H-13 (δ 6.75), and C-5' (δ 149.4) to H-4' (δ 8.16) and H-13' (δ 6.77) were observed, the orientation of both pyran rings could not be determined. To establish the orientation of both pyranocoumarin moieties, yukomarin was transformed to *O,O'*-dimethyl ether (**4**) by treatment with diazomethane. In the NOE experiments of **4**, irradiation of the methoxy signal at δ 3.916 showed 4% and 8% increment of the signal at δ 8.03 (H-4') and 6.627 (H-13'). When the another methoxy signal at δ 3.922 was irradiated, 5% and 12% enhancement were observed on the signals at δ 7.82 (H-4) and 6.626 (H-13). The above results revealed the linear orientation of both pyranocoumarins. Based on these results, we assigned the structure (**3**), composed of clausarin[9] and nordentatin,[10] to yukomarin.

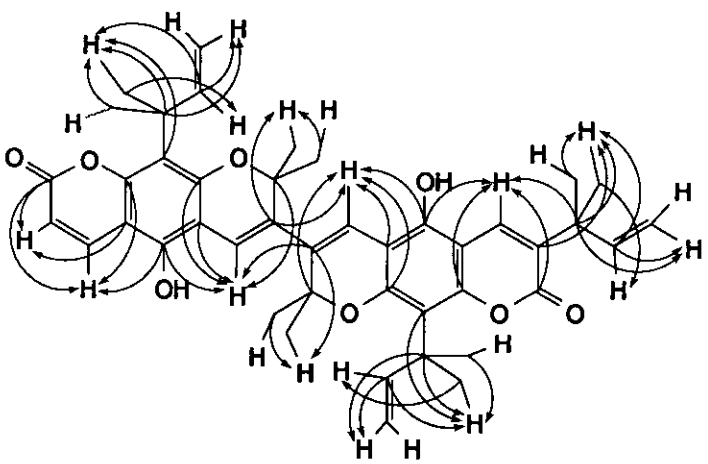


Figure 1 C-H Long-Range Correlations in the HMBC spectrum of yukomarin (2)

EXPERIMENTAL

Extraction and Isolation: The CH_2Cl_2 eluate (127.6 g) and acetone - MeOH eluate (177.29 g) obtained by silica gel column chromatography[6] were further submitted to repeated silica gel column chromatography and PTLC, respectively. Biseselin (**1**)(3.2 mg) was obtained from the acetone and MeOH eluate by purification with PTLC [silica gel, solvent systems: AcOEt-benzene (2:8), isopropyl ether, acetone-benzene (1:9)]. Yukomarin (**3**)(4.2 mg) was isolated from the CH_2Cl_2 eluate by purification with PTLC [silica gel, solvent systems: acetone-$CHCl_3$ (2:8), isopropyl ether].

Biseselin (**1**): Colorless powder, $[\alpha]_D$ 0° (*c*=0.004, $CHCl_3$); HR-MS m/z: 454.1418 (M^+, found), 454.1416 (calcd for $C_{28}H_{22}O_6$); EI-MS m/z: 454 (M^+), 214, 213 (base peak), 185, 128; UV λmax (EtOH, nm): 218, 265, 295, 305, 332; IR νmax ($CHCl_3$, cm^{-1}): 1630, 1590; ^{1}H-NMR ($CDCl_3$, δ): 7.60 (1H, d, J=9.5 Hz, H-4), 7.20 (1H, d, J=8.4 Hz, H-5), 6.93 (1H, d, J=9.9 Hz, H-13), 6.69 (1H, d, J=8.4 Hz, H-6), 6.23 (1H, d, J= 9.5 Hz, H-3), 5.65 (1H, d, J=9.9 Hz, H-12), 1.86 (2H, m, H-14), 1.41 (3H, s, 11-Me); ^{13}C-NMR ($CDCl_3$, δ): 160.9 (s, C-2), 156.5 (s, C-7), 150.2 (s, C-9), 143.8 (d, C-4), 129.3 (s, C-10), 129.3 (d, C-12), 127.9 (d, C-5), 116.0 (d, C-13), 113.3 (d, C-6), 112.7 (d, C-3), 109.0 (s, C-8), 79.9 (s, C-11), 35.9 (t, C-14), 27.1 (q, 11-Me).

Yukomarin (**3**): Colorless powder, $[\alpha]_D$ 0° (*c*=0.034, $CHCl_3$); HRMS m/z: 690.3167 (M^+, found), 690.3193 (calcd for $C_{43}H_{46}O_8$); EIMS m/z: 690 (M^+), 279, 278, 149; UV λmax (EtOH, nm): 203, 223 (sh), 301 (sh), 331. IR νmax ($CHCl_3$, cm^{-1}): 1712, 1606, 1540, 1508; ^{1}H-NMR (acetone-d_6, δ): 8.16 (1H, d, J=9.9 Hz, H-4'), 8.01 (1H, s, H-4), 6.77 (1H, s, H-13'), 6.75 (1H, s, H-13), 6.32 (1H, dd, J=10.6, 17.6 Hz, H-18 or H-15'), 6.31 (1H, dd, J=10.6, 17.2 Hz, H-15' or H-18), 6.20 (1H, dd, J=10.6, 17.6 Hz, H-15), 6.08 (1H, d, J=9.9 Hz, H-3'), 5.07 (1H, d, J=17.6 Hz, H-16), 5.02 (1H, d, J= 10.6 Hz, H-16), 4.93 (1H, d, J=17.6 Hz, H-19 or H-16'), 4.92 (1H, d, J=17.2 Hz, H-16' or H-19), 4.84 (1H, br d, J=10.6 Hz, H-16' or H-19), 4.83 (1H, br d, J=10.6 Hz, H-19 or H-16'), 1.65 (6H, s, 14'-Me or 17-Me), 1.64 (6H, s, 17-Me or 14'-Me), 1.58 (6H, s, 11'-Me or 11-Me), 1.57 (6H, s, 11-Me or 11'-Me), 1.44 (each 6H, s, 14-Me). ^{13}C-NMR (acetone-d_6, δ): 160.7 (s, C-2'), 159.6 (s, C-2), 156.0 (s, C-7'), 155.4 (s, C-9'), 155.1 (s, C-7), 154.5 (s, C-9), 151.0 (d, C-18), 150.9 (d, C-15'), 149.9 (s, C-5), 149.4 (s, C-5'), 146.7 (d, C-15), 140.4 (d, C-4'), 136.5 (s, C-12'), 136.4 (s, C-12), 134.2 (d, C-4), 129.5 (s, C-3), 118.02 (d, C-13), 117.96 (d, C-13'), 115.4 (s, C-8'), 114.9 (s, C-8), 112.1 (t, C-16), 110.7 (d, C-3'), 108.8 (t, C-16'), 108.6 (t, C-19), 108.43 (s, C-6), 108.39 (s, C-6'), 105.5 (s, C-10), 105.4 (s, C-10'), 80.3 (s, C-11), 80.1 (s, C-11'), 41.61 (s, C-14'), 41.55 (s, C-17), 40.9 (s, C-14), 30.3 (q x2, 14'-Me or 17-Me), 30.0 (q x2, 17-Me or 14'-Me), 26.8 (q x2, 11'-Me or 11-Me), 26.8 (q x2, 11-Me or 11'-Me), 26.4 (q x2, 14-Me).

O, O'-Dimethylyukomarin (**4**) Ethereal diazomethane solution prepared as usual manner was added to yukomarin (1.5 mg) in MeOH (5 mL) and the mixture was allowed to stand overnight at rt. After evaporation of the solvent, the residue was purified by PTLC (silica gel) using AcOEt - benzene (1 : 9) to give *O, O'*-dimethylyukomarin (**4**)(0.8 mg) as colorless oil. HR-MS m/z: 718.3517 (M^+, found), 718.3513 (calcd for $C_{45}H_{50}O_8$); EI-MS m/z: 718 (M^+, base peak), 704, 703, 359, 219; UV λmax (MeOH, nm): 224 (sh), 301, 351, 367 (sh); IR νmax ($CHCl_3$, cm^{-1}): 1716, 1608, 1579; ^{1}H-NMR (acetone-d_6, δ): 8.03 (1H, d, J=9.5 Hz), 7.82, 6.627, 6.626 (each 1H, s), 6.34 (1H, dd, J=10.6, 17.6 Hz), 6.33 (1H,

dd, J=10.6, 17.6 Hz), 6.22 (1H, dd, J=10.6, 17.6 Hz), 6.20 (1H, d, J= 9.5 Hz), 5.10 (1H, dd, J=1.1, 17.6 Hz), 5.06 (1H, dd, J=1.1, 10.6 Hz), 4.96 (1H, dd, J=1.1, 17.6 Hz), 4.95 (1H, dd, J=1.1, 17.6 Hz), 4.89 (1H, dd, J=1.1, 10.6 Hz), 4.88 (1H, dd, J=1.1, 10.6 Hz), 3.922, 3.916 (each 3H, s), 1.68 (12H, s), 1.63, 1.62, 1.46 (each 3H, s).

ACKNOWLEDGEMENT

The authors express their deep gratitude to Misses K. Suwa and S. Takeyama, Mukogawa Women's University, for their measurements of MS and NMR spectra. A part of this study was supported by a Grant-in-Aid (No. 02041054 to H. F.) for Scientific Research Program from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES AND NOTES

1. Part XXXI of "Constituents of Domestic Citrus Plants". Part XXX: Y. Takemura, M. Wada, M. Ju-ichi, C. Ito, and H. Furukawa, Chem. Pharm. Bull., accepted.
2. Y. Takemura, J. Kuwahara, M. Ju-ichi, M. Omura, C. Ito, and H. Furukawa, Heterocycles, 1997, **45**, 1411, and references cited therein.
3. M. Ju-ichi, Y. Takemura, C. Ito, and H. Furukawa, Heterocycles, 1991, **32**, 1781.
4. Y. Takemura, H. Uchida, M. Ju-ichi, M. Omura, C. Ito, K. Nakagawa, T. Ono, and H. Furukawa, Heterocycles, 1992, **34**, 2123.
5. Y. Takemura, M. Abe, M. Ju-ichi, C. Ito, K. Hatano, M. Omura, and H. Furukawa, Chem.Pharm.Bull., 1993, **41**, 406.
6. Y. Takemura, M. Ju-ichi, K. Hatano, C. Ito, and H. Furukawa, Chem.Pharm.Bull., 1994, **42**, 2436.
7. R. D. H. Murray, J. Mendez, and S. A. Brown, "The Natural Coumarins", p. 27, John Wiley & Sons Ltd., New York, 1982.
8. K. Kato, Acta Crystallogr., Sect. B, 1970, **26**, 2022.
9. F. Anwer, A. Shoeb, R. S. Kapil, and S. P. Popli, Experientia, 1977, **33**, 412.
10. H. Fuhrer, T. R. Govindachari, B. S. Joshi, and B. R. Pai, Ind. J. Chem., 1970, **8**, 198.

Received, 16th January, 1998