STRUCTURE OF MARSHDIMERIN AND BISOSTHENON-B, TWO NEW BICOUMARINS FROM THE ROOTS OF MARSH GRAPEFRUIT[1]

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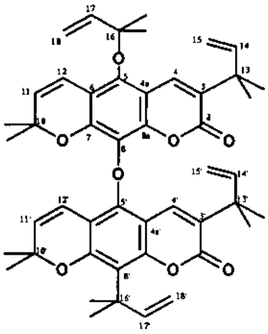
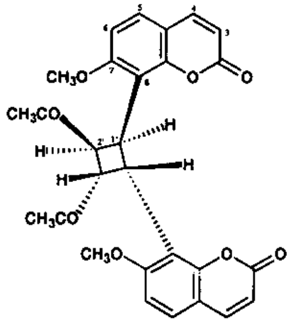
Abstracts ——The structures of two dimeric coumarins named marshdimerin (1) and bisosthenon-B (2) from the roots of Marsh grapefruit (*Citrus paradisi* Macf.) were elucidated on the basis of their spectral data.

In continuation of our study on the constituents of *Citrus* plants, we have isolated and characterized many new acridone alkaloids and coumarins. In our preceding papers, we reported the structure elucidation of twelve new acridone alkaloids (azacridone-A,[2] citbismine-A, -B, -C,[3] -D, -E,[4] furoparadine, trans-dihydrocitracridone-I,[5] marshdine, marshmine,[6] margrapine-A, -B[7]) from the roots of Marsh grapefruit (*C. paradisi* Macf.). Further examination on the constituents of this plant has resulted in isolation of two new dimeric coumarins, marshdimerin (1) and bisosthenon-B (2). In this paper, we describe the structure elucidation of **1** and **2**.

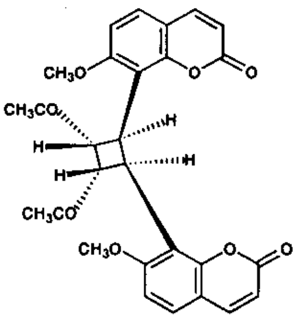
Marshdimerin (1) was obtained as a colorless oil and the molecular formula $C_{48}H_{54}O_8$ was established by HRMS (*m/z* 758.3837). The IR (1718, 1612 cm^{-1}) and UV [230, 269(sh), 276, 328, 348 (sh) nm] spectra indicated the presence of coumarin skeleton.[8] The ^1H-NMR spectrum showed two singlets at δ 7.84 and 7.65, characteristic signals of H-4 of coumarin skeleton,[9] therefore, the presence of two coumarin nuclei



(1) marshdimerin



(2) bisosthenon-B



(3) bisosthenon

Table 1. ^{1}H- and ^{13}C-NMR spectral data of marshdimerin (1)

	δC	δH		δC	δH
2	158.7		2'	159.3	
3	130.5		3'	130.9	
4	135.2	7.84	4'	133.5	7.65
4a	110.4**		4a'	106.7**	
5	143.8		5'	149.1	
6	115.4		6'	111.4	
7	145.9		7'	154.6	
8	129.3		8'	118.3	
8a	144.9		8a'	152.8	
10	76.4		10'	76.9	
10-Me	29.8*	1.40	10'-Me	27.3*	1.40
	29.9*	1.40		27.9*	1.40
11	129.2	5.49 (d, 9.8)	11'	129.4	5.56 (d, 9.8)
12	118.6	6.55 (d, 9.8)	12'	117.2	6.62 (d, 9.8)
13	40.6		13'	40.2	
13-Me	26.1	1.49	13'-Me	25.9	1.26
	26.1	1.49		25.9	1.26
14	145.5	6.19 (dd, 10.7, 17.5)	14'	145.3	5.96 (dd, 10.1, 17.1)
15	112.3	5.10 (br d, 17.5)	15'	111.7	4.89 (dd, 10.3, 1.3)
		5.09 (br d, 10.7)			4.88 (dd, 17.5, 1.3)
16	84.2		16'	41.1	
16-Me	26.2	0.80	16'-Me	29.4	1.66
	27.1	1.05		29.5	1.66
17	142.4	5.99 (dd, 10.7, 17.1)	17'	150.1	6.32 (dd, 10.7, 17.5)
18	114.4	5.18 (br d, 17.1)	18'	108.1	4.94 (dd, 17.5, 0.9)
		5.12 (br d, 10.7)			4.87 (dd, 10.7, 0.9)

Spectra were taken in $CDCl_3$. Values are in ppm (δH and δC).

The coupling constants (*J* values) in parentheses are in Hz.

*,**: may be interchanged.

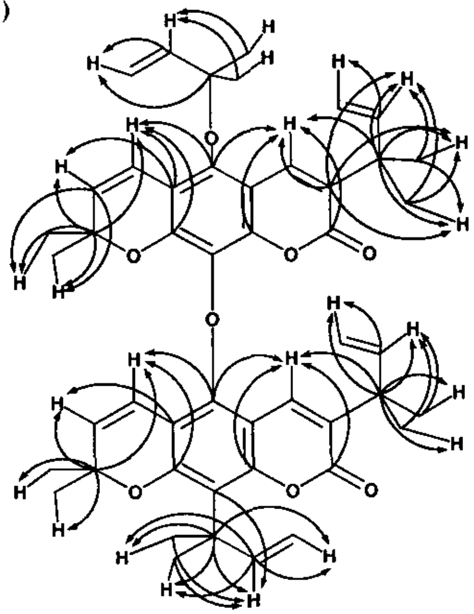


Figure 1. HMBC correlations found for marshdimerin (1)

and substituent at C-3 of both skeletons was suggested. The signals at δ 6.62, 5.56 (each 1H, d, J=9.8 Hz), δ 6.55, 5.49 (each 1H, d, J=9.8 Hz) and δ 1.40 (12H, s) suggested the presence of two 2, 2-dimethylpyran rings and four set of signals [δ 6.32 (1H, dd, J=10.7, 17.5 Hz), 4.94 (1H, dd, J=17.5, 0.9 Hz), 4.87 (1H, dd, J=10.7, 0.9 Hz), 1.66 (6H, s); 6.19 (1H, dd, J=10.7, 17.5 Hz), 5.10 (1H, br d, J=17.5 Hz), 5.09 (1H, br d, J=10.7 Hz), 1.49 (6H, s); 5.99 (1H, dd, J=10.7, 17.1 Hz), 5.12 (1H, br d, J=10.7 Hz), 5.18 (1H, br d, J=17.1 Hz), 0.80, 1.05 (each 3H, s); 5.96 (1H, dd, J=10.1, 17.1 Hz), 4.89 (1H, dd, J=10.3, 1.3 Hz), 4.88 (1H, dd, J=17.5, 1.3 Hz), 1.26 (6H, s)] indicated the existence of four 1,1-dimethylallyl groups. Further information was obtained by 2D-NMR analyses. In the HMBC spectrum (Figure 1), C-5 (δc 143.8) showed cross peaks to H-4 (δH 7.84) and H-12 (δH 6.55), on the other hand C-5' (δc 149.1) revealed cross peaks to H-4' (δH 7.65) and H-12' (δH 6.62). The ^3J-connectivity was also observed between H-4 and C-13 (δc 40.6), and between H-4' and C-13' (δc 40.2). These connectivity revealed the linear orientation of both pyran rings and the presence of 1,1-dimethylallyl groups at C-3 of both coumarin skeletons. In the ^{13}C-NMR spectrum, one of the sp^3 carbon signal among the four 1,1-dimethylallyl groups resonated at lower field (δc 84.2) compared with other three carbon signals (δc 40.6, 40.2, 41.1), indicating that the one 1,1-dimethylallyl group was *O*-substituted and other three were *C*-substituted. Determination of the linkage of two coumarin moieties and the location of one *O*-substituted 1,1-dimethylallyl group was achieved by NOE experiment. Irradiation of the H-4 (δ 7.84) signal showed 4 % and 2 % increments of the signal at δ 6.19 (H-14) and 5.99 (H-17), respectively. When the H-4' (δ 7.65) signal was irradiated, 4 % increment was observed on the signal at δ 5.96 (H-14'). From these results, the ether linkage of two coumarin nuclei was assigned between C-8 and C-5', and the location of one 1,1-dimethylallyl group exists as ether at C-5.

Bisosthenon-B (2) was isolated as a colorless oil, $[\alpha]_D$ +24.53° ($CHCl_3$), the molecular formula $C_{28}H_{24}O_8$ was established by HRMS (*m/z* 488.1467). The IR (1725, 1600 cm^{-1}) and UV [265(sh), 326 nm] spectra indicated the presence of coumarin skeleton.[8] The ^1H-NMR spectrum showed characteristic signals of H-4, H-5, H-6 and H-3 of coumarin skeleton at δ 7.55 (1H, d, J=9.4 Hz), 7.32 (1H, d, J= 8.6 Hz), 6.89 (1H, d, J=8.6 Hz), 6.15 (1H, d, J=9.4 Hz), two methine signals at δ 4.95 and 4.07 (each 1H, dd, J=3.4, 6.0 Hz) one methoxy group at δ 3.96 (3H, s) and one acetyl group at δ 2.12 (3H, s). The number of proton signals was half of that expected, the coumarin was considered to have symmetrical dimeric structure. Comparison of ^1H-NMR spectral data of **2** with that of known bisosthenon (3)[10] showed that both compounds were quite similar. The prominent feature of distinguishing **2** from **3** was the difference of the chemical shift of one of methine proton. The difference suggested the stereoisomeric structure about the cyclobutane ring. NOE experiments allowed assignment of the regiochemical placement of two methine protons. In the case of bisosthenon (3), irradiation of acetyl signal at δ 2.27 showed 3 % and 4 % increments of the signals at δ 4.94 (H-1') and 4.81 (H-2'); no increments were observed between two methine protons. In the experiment of bisosthenon-B (2), irradiation of the acetyl group at δ 2.12 gave 5 % and 3 % increments of the signals at δ 4.07 and 4.95; irradiation the methine proton signal at δ 4.95 showed 7 % increments on the signal at δ 4.07; irradiation the signal at δ 4.07 caused 7 % increments of the signal at δ 4.95. From the above results, the relative stereochemistry of four methine protons on the cyclobutane ring were assigned as *trans, cis, trans*. Previously, we reported the photolysis of osthenon to afford bisosthenon (16%), compound B (11%), compound C (2%), and *cis*-osthenon (9%).[10] The spectral data of compound C were identical with those of bisosthenon-B and identified with direct comparisons of TLC. From the above results, the structure of bisosthenon-B was determined as **2**.

EXPERIMENTAL

Isolation The toluene and CH_2Cl_2 eluate obtained through the SiO_2 column chromatography of the acetone extract (138g) of Marsh grapefruit (*C. paradisi* Macf.)[6] was subjected to repeated preparative thin layer chromatography [solvent: n-hexane:AcOEt (8:2), benzene:AcOEt (29:1), benzene:acetone (8:2), $CHCl_3$:acetone (9:1), benzene:MeOH (19:1)]. Marshdimerin (1)(9.0 mg) and bisosthenon-B (2)(2.0 mg) were obtained from toluene and CH_2Cl_2 eluate, respectively.

Marshdimerin(1) Colorless oil, UV λ_{max} (MeOH) 230, 269 (sh), 276, 328, 348 (sh) nm; IR ν_{max} ($CHCl_3$) 1718, 1612 cm^{-1}; HRMS m/z 758.3837 (M^+, Calcd for $C_{48}H_{54}O_8$ 758.3819); EIMS m/z 758 (M^+), 743, 380, 379, 365, 363; ^1H-NMR and ^{13}C-NMR ($CDCl_3$): see Table 1.; NOE: irradiation at δ 7.65 - 4 % enhancement at δ 5.95; irradiation at δ 7.84 - 4 % and 2 % enhancement at δ 6.19 and 5.99.

Bisosthenon-B(2) Colorless oil, $[\alpha]_D$ +24.53° (c=0.1467, $CHCl_3$), UV λ_{max} (EtOH) 206, 265 (sh), 326 nm; IR ν_{max} ($CHCl_3$) 1725, 1710, 1600 cm^{-1}; HRMS m/z 488.1467 (M^+, Calcd for $C_{28}H_{24}O_8$ 488.1471); EIMS m/z: 488 (M^+), 446, 445, 403, 244, 229, 215, 213 (base peak), 203, 201, 189, 131; ^1H-NMR ($CDCl_3$) δ 7.55 (2H, d, J= 9.4 Hz), 7.32 (2H, d, J= 8.6 Hz), 6.89 (2H, d, J= 8.6 Hz), 6.15 (2H, d, J= 9.4 Hz), 4.95 (2H, dd, J= 3.4, 6.0 Hz), 4.07 (2H, dd, J= 3.4, 6.0 Hz), 3.96 (6H, s), 2.12 (6H, s); NOE: irradiation at δ 3.96 - 14 % enhancement at δ 6.89; irradiation at δ 2.12 - 5 % and 3 % enhancement at δ 4.07 and 4.95; irradiation at δ 4.95 - 7 % and 3 % enhancement at δ 4.07 and 2.12; irradiation at δ 4.07 -

7 % and 4 % enhancement at δ 4.95 and 2.12; ^{13}C-NMR ($CDCl_3$) δ 207.6 (s, C-3'), 161.9 (s, C-2), 160.5 (s, C-8a), 143.6 (d, C-4), 127.6 (d, C-5), 116.7 (s, C-8), 113.2 (d, C-3), 113.0 (s, C-4a), 108.2(d, C-6), 56.1 (q, 7-OCH_3), 49.4 (d, C-2'), 33.8 (d, C-1'), 27.5 (q, 3'-CH_3).

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