STRUCTURE OF BISCLAUSARIN, A NEW BICOUMARIN FROM CITRUS PLANT[1]

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Abstract —— A new type bicoumarin, named bisclausarin, was isolated from the roots of Citrus hassaku (Rutaceae) and the structure was elucidated as 1 on the basis of spectroscopic data.

On continuing our investigations on the constituents of Citrus plants, we reported the isolation of many kinds of acridone alkaloids and coumarins.[2] We studied the constituents of the roots of Citrus hassaku Hort. ex Y. Tanaka and isolated a new dimeric coumarin, named bisclausarin (1). The acetone extracts of the dried roots of the plant were subjected to silica gel column, preparative centrifugal thin-layer, and preparative thin-layer chromatographies to yield bisclausarin along with many other coumarins and acridone alkaloids.[3] This paper deals with the structure elucidation of bisclausarin (1) particularly by the use of 2D nmr method.

Bisclausarin was isolated as an unstable oily compound. The uv (λ_{max}: 214, 330 nm) and ir (ν_{max}: 1715, 1610 cm^{-1}) spectra showed typical absorptions of 5,7-dioxygenated coumarin skeleton[4] and the ^{1}H-nmr spectrum indicated the presence of characteristic H-4 (δ 8.02, s), two 1,1-dimethylallyl groups [δ 6.34 (1H, dd, J= 17.48, 10.42 Hz), 6.22 (1H, dd, J= 17.48, 10.42 Hz), 5.08 (1H, dd, J= 17.48, 1.35 Hz), 5.07 (1H, dd, J= 10.42, 1.35 Hz), 4.95 (1H, dd, J= 17.48, 1.35 Hz), 4.86 (1H, dd, J= 10.42, 1.35 Hz), 1.66, 1.46 (each 6H, s)]. The remaining signals at δ 6.77 (1H, s) and 1.59 (6H, s) suggested the presence of one olefinic proton and geminal methyl groups. Because of its unstability, this compound was transformed to the methyl ether (2) by treatment of diazomethane to obtain further information of its structure. O-Methylbisclausarin (2), colorless needles, mp >300^{0}, showed the molecular ion peak at m/z 786, thus the molecular formula $C_{50}H_{58}O_{8}$ was established. Though the number of observed signals in the ^{1}H- and ^{13}C-nmr spectra were just half of expected, bisclausarin was assumed to have a symmetrical dimeric structure. The linear type pyranocoumarin structure was revealed by NOE experiments of 2. Irradiation of the signal at δ 6.53 afforded the 4% NOE on the each signal at δ 3.86 and 1.57. On the other hands, when irradiated the signal at δ 7.75, signals at δ 6.19 and 3.86 were affected. These results indicated that two singlets at δ 7.75 and 6.53 were assignable to H-4 and H-11, respectively and this coumarin was also proved to be the linear type pyranocoumarin. The ^{1}H- and ^{13}C-nmr spectra of 2 showed a

Table 1 ^{1}H-Nmr Data of 2 Compared with Those of Clausarin (3)

	2	3
H-4	7.75 (s)	7.85(s)
9-Me	1.57 (12H, s)	1.47*(6H,s)
H-10		5.67 (d,J=9.77 Hz)
H-11	6.53 (s)	6.50 (d,J=9.77 Hz)
1'-Me	1.68 (12H,s)	1.63 (6H, s)
H-2'	6.34 (dd,J=17.58,10.50 Hz)	6.29 (dd,J=10.50,17.34 Hz)
H-3'	4.99 (dd,J=17.58,0.98 Hz)	4.93 (dd,J=1.22,17.58 Hz)
	4.92 (dd,J=10.50,0.98 Hz)	4.85 (dd,J=1.22,10.50 Hz)
1"-Me	1.49 (12H,s)	1.43*(6H,s)
H-2"	6.19 (dd,J=17.58,10.50 Hz)	6.18 (dd,J=10.50,17.58 Hz)
H-3"	5.11 (dd,J=17.58,0.98 Hz)	5.11 (dd,J=0.97,10.50 Hz)
	5.10 (dd,J=10.50,0.98 Hz)	5.07 (dd,J=0.97,10.50 Hz)
O-Me	3.86 (6H,s)	

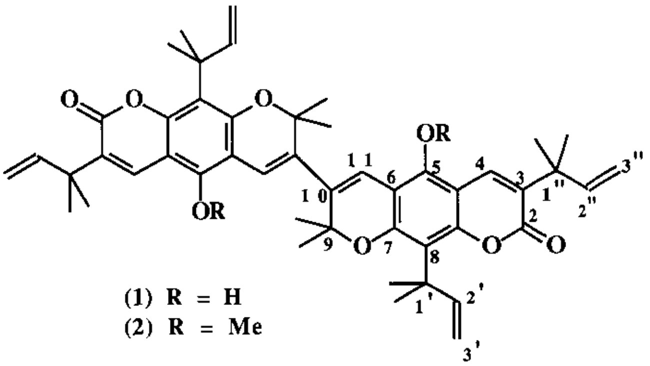
* Assignments may be interchanged.

similar signal pattern to those of clausarin (3) (Tables 1 and 2), except for the following signals: H-11 and H-10 at δ 6.50 and 5.67 (each 1H, d, J= 9.77 Hz) in 3 appeared as a singlet at δ 6.53 (2H, s) in 2, C-10 at δ 129.28 (d) in 3 resonated at δ 137.18 (s) in 2. These differences indicated that the binding position is C-10 of both coumarin nuclei.

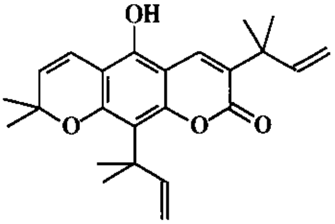
Table 2. ^{13}C-Nmr Spectral Data of 2 and 3

	2	3
C-2	159.22	160.80
C-3	130.92	128.53
C-4	132.59	134.18
C-4a	108.18	104.44
C-5	151.22	147.04
C-6	112.39	106.38
C-7	154.12	155.11
C-8	118.83	115.20
C-8a	153.39	153.20
C-9	79.48	79.00
9-Me	26.72	27.27
C-10	137.18	129.28
C-11	117.52	115.84
C-1'	41.18*	40.88
1'-Me	29.30	29.50
C-2'	149.58	150.12
C-3'	108.37	107.94
C-1"	40.38*	40.18
1"-Me	26.08	26.19
C-2"	145.47	145.63
C-3"	112.10	111.90

*Assignments may be interchanged.



(1) R = H
(2) R = Me



(3)

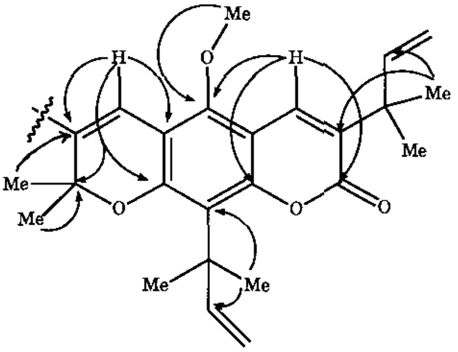


Figure 1 HC long range correlations in HC long range COSY spectrum(J = 5 Hz) of metylbisclausarin

The linking position of two coumarin nuclei and the location of the 1,1-dimethylallyl group was further confirmed by ^{13}C-^{1}H COSY and long-range ^{13}C-^{1}H COSY experiments. As shown in Figure 1, the H-4 at δ 7.75 showed long-range correlations with the carbons at δ 159.22 (C=O), 153.39 (C-8a) and 151.22 (C-5). On the other hand, the proton at δ 6.53 showed long-range correlations with the carbons at δ 112.39 (C-6), 154.12 (C-7), 137.18 (C-10) and 79.48 (C-9). One of the 1,1-dimethylallyl proton at δ 1.49 showed long-range correlations with the carbon at δ 130.92 (C-3) and 145.47 (C-2"), the other methyl proton singlet at δ 1.68 showed correlations with the carbons at δ 118.83 (C-8) and 149.58 (C-2') indicating the location of these 1,1-dimethyallyl groups at C-3 and C-8, respectively. The methyl signal of dimethylpyran ring at δ 1.57 showed long-range correlations with the carbons at δ 79.48 (C-9) and 137.18 (C-10). Thus, the linking position of two coumarin nuclei was concluded to C-10. From the above results, the structure of bisclausarin was proved to be 1.

Bicoumarins isolated from plant sources have been known in nature about 40 so far,[5] most of them have the structure that two coumarin units are linked by a methylene, an ether bridge, a carbon-carbon bond or a C_{10} alkyl residue (monoterpene unit) between aromatic rings. Bisclausarin is the first example of dimeric coumarin having direct carbon-carbon linkage at the pyran ring.

EXPERIMENTAL

Extraction and Isolation: The dried roots (3.2 Kg) of *Citrus hassaku* collected at Innoshima was extracted with acetone at room temperature for 30 days. The acetone extract (485 g) was chromatographed over silica gel with successive elution with hexane, benzene, CH_2Cl_2, acetone and MeOH (each 3 l). The benzene eluate was subjected to preparative centrifugal chromatography using CH_2Cl_2. Further separation with preparative thin layer chromatography (solvent: isopropyl ether, hexane-acetone, acetone-$CHCl_3$) gave bisclausarin (1) (39 mg).

Bisclausarin(1): Yellow oil, ir ν_{max} ($CHCl_3$) cm^{-1}: 1715, 1610; uv λ_{max} (MeOH) nm: 214, 330; ^{1}H-nmr (acetone-d_6,δ): 8.02 (2H, s), 6.77 (2H, s), 6.34 (2H, dd, J= 17.48, 10.42 Hz), 6.22 (2H, dd, J= 17.48, 10.42 Hz), 5.08 (2H, dd, J= 17.48, 1.35 Hz), 5.07 (2H, dd, J= 10.42, 1.35 Hz), 4.95 (2H, dd, J= 17.48, 1.35 Hz), 4.86 (2H, dd, J= 10.42, 1.35 Hz), 1.66, 1.59, 1.46 (each 12H, s).

O-Methylbisclausarin (2): Ethereal diazomethane was added to a methanolic solution (5 ml) of bisclausarin (1) (23 mg) and the mixture was left to stand overnight. Then, the solvent was distilled off, and the residue was purified by ptlc to afford (2) (13.4 mg). colorless needles (from acetone); mp >300^0 ; high ms: m/z 786.4135 (M^+, found), 786.4131 (calcd for $C_{50}H_{58}O_8$); ir ν_{max} ($CHCl_3$) cm^{-1}: 1715, 1612, 1580; uv λ_{max} (MeOH) nm: 207, 228 (sh), 303, 352; eims m/z: 786 (M^+, base peak), 772, 369, 368, 313, 237, 236; ^{1}H-nmr ($CDCl_3$, δ): see Table 1. ^{13}C-nmr ($CDCl_3$, δ): see Table 2.

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REFERENCES AND NOTES

1 Part XI on "Studies on the Constituents of Domestic Citrus Plants" ; Part X: M. Ju-ichi, Y. Takemura, M. Azuma, K. Tanaka, M. Okano, N. Fukamiya, C. Ito, and H. Furukawa, submitted to Chem. Pharm. Bull.

2 C. Ito, S. Tanahashi, Y. Tani, M. Ju-ichi, M. Omura, and H. Furukawa,

Chem. Pharm. Bull., 1990, 38, 2586 ; M. Ju-ichi, M. Inoue, K. Sakiyama, M. Yoneda, and H. Furukawa, Heterocycles, 1987, 26, 2077, and references cited therein.

3 Isolation and characterization of known compounds isolated from this plant will be reported elsewhere.

4 R. D. H. Murray, J. Mendez, and S. A. Brown, "The Natural Coumarins" , John Wiley & Sons Ltd., New York, p.27, 1982.

5 S. C. Basa, Phytochemistry, 1988, 27, 1933; H. Fischer, A. Roemer, B. Ulbrich, and H. Arens, Planta Med., 1988, 54, 398 ; K. Baba, Y. Tabata, M. Taniguti, and M. Kozawa, Phytochemistry, 1989, 28, 221; P. C. Joshi, S. Mandel, and P. C. Das, Phytochemistry, 1989, 28, 1281; L. H. Gu, X. Li, S. Q. Yan, and T. R. Zhu, Acta Pharm. Sinica, 1989, 24, 744; C. Zdero, F. Bohlmann, and H. M. Niemeyer, Phytochemistry, 1990, 29, 326; K. Baba, M. Taniguti, Y. Yoneda, and M. Kozawa, Phytochemistry, 1990, 29, 247; C. Ito, T. Mizuno, S. Tanahashi, H. Furukawa, M. Ju-ichi, M. Inoue, M. Muraguchi, M. Omura, D. R. McPhail, and A. T. McPhail, Chem. Pharm. Bull., 1990, 38, 2102; C. Ito, M. Matsuoka, T. Oka, M. Ju-ichi, M. Niwa, M. Omura, and H. Furukawa, Chem. Pharm. Bull., 1990, 38, 1230.

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