A New Biscoumarin from *Citrus* Plants

Chihiro Ito,[a] Mayumi Nakagawa,[a] Mami Inoue,[b] Yuko Takemura,[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] Koshien, Nishinomiya 663. Japan and Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries,[c] Shimizu, Shizuoka 424–02, Japan. Received February 19, 1993

A new dimeric coumarin (biscoumarin) named bisparasin (1) was isolated from some hybrid plants of *Citrus* species. On the basis of spectrometric analyses, the structure was characterized as a [2+4] cycloaddition adduct between *trans*-dehydroosthol (2) and citrubuntin (3).

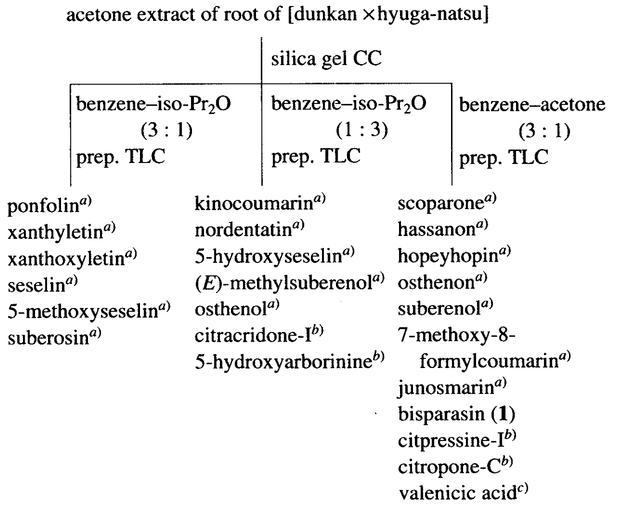
Keywords bisparasin; coumarin; *Citrus*; Rutaceae; biscoumarin; Diels–Alder

From the roots of several hybrid seedlings resulting from crosses of *Citrus paradisi* Macf. (duncan) × *C. sinensis* Osbeck (hamlin) and Okitsu No. 17[1] × *C. tamurana* Tan. (hyuga-natsu) (Rutaceae), a new homoacridone alkaloid, citropone-C,[2] has been isolated and its structure determined. In a further investigation of this plant, a new biscoumarin named bisparasin (**1**) was isolated along with a number of known coumarins and acridones (Chart 1).[3] We describe here the structural elucidation of a new biscoumarin, bisparasin (**1**), by spectrometric methods.

The acetone extract of the plant was subjected successively to silica gel column chromatography and preparative silica gel TLC to obtain a new biscoumarin, bisparasin (**1**), and the structure was characterized by NMR, MS, UV, and IR spectra.

Results and Discussion

Bisparasin (**1**) was isolated as a racemic colorless oil, and the molecular formula $C_{30}H_{28}O_6$ was determined by high-resolution (HR) MS. The UV spectrum showed absorptions characteristic of the 7-oxygenated coumarin chromophore[4] and the IR spectrum had a band (ν_{max} 1720 cm^{-1}) due to lactone carbonyl groups. In the ^1H- and ^{13}C-NMR spectra (Table I), signals assignable to *ortho*- and *para*-located aromatic protons along with two methoxy and two unsaturated lactone moieties suggested the presence of two 7-methoxycoumarin nuclei having a substituent at C-6 and C-8, respectively. Further, analysis of the ^1H- and ^{13}C-NMR spectra using ^1H–^1H and ^1H–^{13}C correlation spectroscopies (COSY) indicated the presence of a cyclic monoterpene (C_{10}) unit containing a *trans*-disubstituted double bond [δ_H 6.33 and 6.96 (each d, $J=16.8$ Hz)], a tertiary methyl [δ_H 1.23 (s)] attached to an allyl carbon [δ_C 40.13 (C-11)], a trisubstituted double bond [δ_H 5.31] having a methyl [δ_H 1.81], and *vicinal* located methylenes [δ_H 1.70—1.81 (2H, m), 2.10 (1H, m), and 2.25 (1H, m); δ_C 33.65 and 27.84 (each t)]. The linked arrangement of these



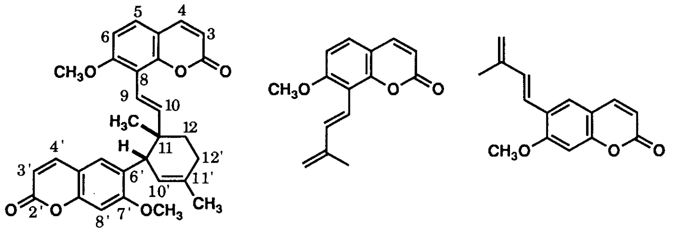
a) Coumarins. *b*) Acridones. *c*) Others. CC: column chromatography.

Chart 1. Isolation Procedure of Bisparasin (**1**)

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

Carbon No.	δ_H	δ_C
2	—	160.94
3	6.25 (d, 9.4)	113.02
4	7.60 (d, 9.4)	143.91
4a	—	113.20
5	7.23 (d, 8.7)	126.29
6	6.77 (d, 8.7)	107.46
7	—	159.71
7-OCH_3	3.76 (3H, s)	55.63
8	—	114.79
8a	—	152.28
9	6.33 (d, 16.8)	115.50
10	6.96 (d, 16.8)	143.39
11	—	40.13
12	1.70—1.81 (2H, m)	33.65
11-CH_3	1.23 (3H, s)	25.03
2′	—	161.67
3′	6.15 (d, 9.4)	112.32
4′	7.65 (d, 9.4)	144.21
4′a	—	111.78
5′	7.42 (s)	129.67
6′	—	129.56
7′	—	160.88
7′-OCH_3	3.77 (3H, s)	55.98
8′	6.66 (s)	97.94
8′a	—	154.37
9′	3.95 (br s)	43.24
10′	5.31 (br s)	123.76
11′	—	134.56
12′	2.10 (m) 2.25 (m)	27.84
11′-CH_3	1.81 (3H, br s)	23.38

Values are in δ ppm. Each signal corresponds to 1H, unless otherwise stated. Figures in parentheses are coupling constants (*J*) in Hz. Assignments were confirmed by ^1H–^1H and ^1H–^{13}C COSY and HMBC spectrometric analyses.



1 2 3

Chart 2

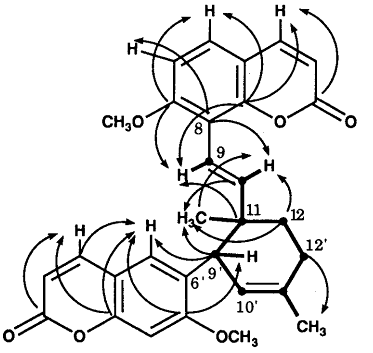


Chart 3. Significant ^{13}C–^{1}H Three-bond Correlations in the HMBC Spectrum of **1**

moieties was elucidated from the 1H detected heteronuclear multiple bond connectivity (HMBC) spectrum, as indicated by arrows in Chart 3. This partial structure was supported by the appearance of a base mass fragment peak at m/z 242, presumably formed by a retro-Diels–Alder type cleavage of the cyclohexene ring in the monoterpene portion.

Moreover, the C(8)–C(9) linkage between one of the coumarin units and the monoterpene unit was suggested by the presence of three-bond (^{3}J) correlations between the lower proton [δ_H 6.33 (H-9)] on the *trans*-olefin, and an angular carbon [C-8a (δ_C 152.28)], which further correlated to H-4 (δ_H 7.60) and H-5 (δ_H 7.23) in the HMBC spectrum. On the other hand, the methine carbon at δ_C 43.24 (C-9′) showed a ^{3}J correlation to H-5′ (δ_H 7.42), which further correlated to C-4′ and C-7′ on another coumarin unit and this together with the observation of a correlation between H-9′ and C(7′), indicated the presence of a C(6′)–C(9′) linkage in the molecule.

Next, the stereochemistry of **1** was elucidated by an nuclear Overhauser effect (NOE) experiment. Irradiation of the 11-methyl signal (δ_H 1.23) gave an 8% enhancement of the signal at δ_H 3.95 (H-9′), which also showed a 5% increment on irradiation of H-10′ (δ_H 5.31), suggesting a *cis* relationship of the 11-methyl group and H-9′, *i.e.*, *cis* arrangement of two coumarin units on the cyclohexene ring. The presence of a 4% NOE enhancement between one of the *trans*-olefinic protons (H-10) and H-5′ on the coumarin nucleus also supported the relative stereochemistry at C-11 and C-9′.

On the basis of these results, the structure **1** was assigned to bisparasin, which is thus a regio-isomer of isothamnosin A reported by Gonzalez *et al.* in 1977.$^{5)}$ Biogenetically, bisparasin (**1**) was considered to be formed from *trans*-dehydroosthol (**2**)$^{6)}$ and citrubuntin (**3**)$^{7)}$ through a [2+4] cycloaddition reaction.$^{8-10)}$

Experimental

^{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 (TMS) as an internal reference. HMBC spectra were measured at $J=8$ Hz on the GX-400. MS were taken 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, IR spectra on an IR-810 (JASCI) in $CHCl_3$, and optical rotations on a DIP-181 (JASCO) in $CHCl_3$ at 25 °C. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

Extraction and Isolation The plant materials used in this study were grown in the orchard of Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Shimizu, Shizuoka.

a) The dried roots (132 g) of several hybrid seedlings resulting from a cross of *C. paradisi* MACF. (dunkan) × *C. sinensis* OSBECK (hamlin) were extracted with acetone at room temperature. The acetone extract was subjected to silica gel column chromatography and eluted successively with benzene, benzene–isopropyl ether (3 : 1 and 1 : 3), benzene–acetone (3 : 1), acetone, and MeOH. The benzene–acetone (3 : 1) fraction was further subjected to preparative TLC to obtain **1** (3.9 mg) as well as known monomeric coumarins, acridones and other compounds (Chart 1).$^{2)}$

b) Roots (500 g) of several hybrid seedlings resulting from a cross of Okitsu No. 17$^{1)}$ × *C. tamurana* TAN. (hyuga-natsu) were extracted with acetone under reflux. The acetone extract (54 g) was subjected to silica gel column chromatography successively eluted with benzene, 50% benzene–CH_2Cl_2, CH_2Cl_2, EtOAc, acetone, and MeOH. The MeOH eluate was further chromatographed on silica gel using benzene, EtOAc–benzene, and MeOH as solvents. The EtOAc–benzene fraction was purified by preparative TLC iso-Pr_2O, acetone–$CHCl_3$ (1 : 9) to obtain **1** (3.4 mg).

Bisparasin (1) Colorless oil, $[\alpha]_D$ ±0° ($c=0.039$). UV λ_{max} nm: 206, 228, 247 (sh), 256, 285, 324. IR ν_{max} cm^{-1}: 1720, 1620, 1600. ^{1}H- and ^{13}C-NMR: Table I. EI-MS m/z (%): 484 (M^+, 10), 243 (15), 242 (100), 227 (23), 211 (18), 199 (3), 183 (5). HR-MS Calcd for $C_{15}H_{14}O_3$: 242.0941. Found: 242.0918. Calcd for $C_{30}H_{28}O_6$: 484.1885. Found: 484.1900.

References and Notes

1) Okitsu No. 17 is a hybrid unnamed selection resulting from a cross of hyuga-natsu (*C. tamurana* TAN.) × sweet orange (*C. sinensis* (L.) OSBECK) cv. Fukuhara.
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