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BIYOUXANTHONES A – D, PRENYLATED XANTHONES FROM ROOTS OF *HYPERICUM CHINENSE*

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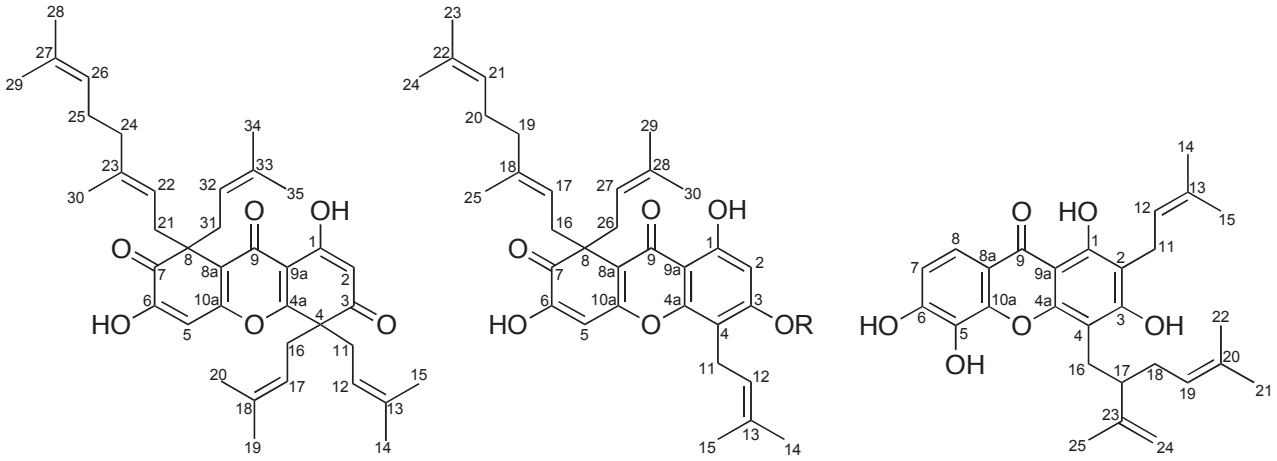
Abstract - Four new prenylated xanthones, biyouxanthones A – D (**1** – **4**), were isolated from roots of *Hypericum chinense*. The gross structures of **1** – **4** were elucidated from spectroscopic data, and **2** was assigned as a racemate by chiral HPLC analysis. Biyouxanthones A (**1**) and B (**2**) exhibited anti-viral activity against hepatitis C virus (HCV).

The plants, belonging to the genus *Hypericum* (family Clusiaceae), are known to be a traditional medicine for the treatment of burns, bruises, swelling, inflammation, and anxiety as well as bacterial and viral infections.[1-4] In our continuing search for bioactive compounds from *Hypericum* spp,[5] three new prenylated xanthones possessing 2-hydroxycyclohexa-2,4-dienone moiety, biyouxanthones A – C (**1 – 3**), and one new prenylated xanthone having a 5-methyl-2-(prop-1-en-2-yl)hex-4-enyl group, biyouxanthone D (**4**), were isolated from roots of *H. chinense*. In this paper, we describe the isolation, structure elucidation of **1** – **4** and anti-HCV activity of **1** and **2**.



1 **2** : R = Me **4**

3 : R = H

The dried roots of *H. chinense* were extracted with MeOH, and the extracts were partitioned successively with *n*-hexane, EtOAc, and H_2O. *n*-Hexane-soluble portions were separated by a silica gel column (*n*-hexane/EtOAc) and then a Sephadex LH-20 column (MeOH) to afford a mixture of xanthone derivatives, which was purified by C_{18} reversed-phase HPLC (MeOH/H_2O) to yield biyouxanthones A (**1**, 0.00017%), B (**2**, 0.00016%), and C (**3**, 0.00087%). EtOAc-soluble portions were purified by a silica gel column ($CHCl_3$/MeOH) and a Sephadex LH-20 (MeOH) column chromatographies, and C_{18} reversed-phase HPLC (MeOH/H_2O, 0.1% TFA) to give biyouxanthone D (**4**, 0.00012%).

The molecular formula of biyouxanthone A (**1**), $C_{38}H_{48}O_6$, was revealed by HRESIMS (*m/z* 599.3366 $[M-H]^-$, Δ –0.6 mmu). IR absorptions at 1671 and 1636 cm^{-1} implied the presence of carbonyl

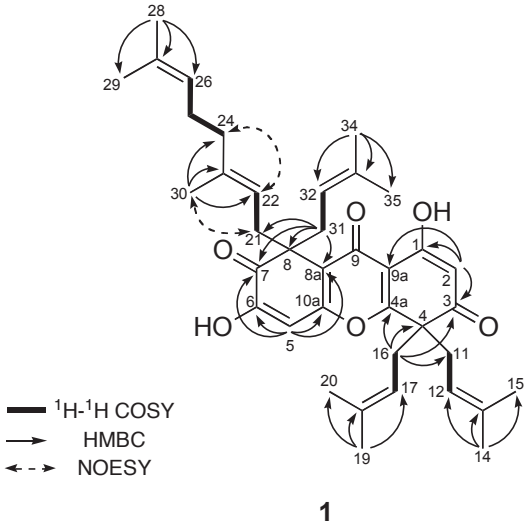


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Figure 1. Selected 2D NMR correlations for biyouxanthone A (**1**)

functionalities. ^{1}H and ^{13}C NMR data of **1** (Table 1) revealed the presence of three carbonyl groups, seven trisubstituted olefins, two tetrasubstituted olefins, two sp^3 quaternary carbons, six sp^3 methylenes, nine tertiary methyls, and one hydrogen-bonded hydroxy group. ^{1}H–^{1}H COSY correlations for H_2-11 and H-12, H_2-16 and H-17, and H_2-31 and H-32, and HMBC cross-peaks of H_3-14 to C-12, C-13, and C-15, H_3-19 to C-17, C-18, and C-20, H_3-34 to C-32, C-33, and C-35 suggested the presence of three prenyl groups. The

Table 1. ^{1}H and ^{13}C NMR data for biyouxanthone A (**1**) in $CDCl_3$

position	δ_C	δ_H (*J* in Hz)
1	170.0	-
2	103.6	5.62 (1H, s)
3	195.9	-
4	57.6	-
4a	173.5	-
5	106.5	6.47 (1H, s)
6	160.1	-
7	200.6	-
8	56.3	-
8a	121.3	-
9	177.0	-
9a	112.2	-
10a	152.5	-
11	38.4	2.81 (2H, m)
12	117.0	4.82 (1H, t, *J* = 6.9)
13	135.5	-
14	25.7	1.55 (3H, s)
15	17.9	1.47 (3H, s)
16	38.6	2.70 (2H, m)
17	117.1	4.80 (1H, t, *J* = 6.9)
18	135.6	-
19	25.7	1.55 (3H, s)
20	17.7	1.48 (3H, s)
21	37.5	3.40 (1H, dd, *J* = 14.5, 7.5)
		2.79 (1H, m)
22	116.9	4.60 (1H, t, *J* = 7.5)
23	139.8	-
24	39.8	1.79 (2H, m)
25	26.5	1.91 (2H, m)
26	123.5	4.90 (1H, t, *J* = 7.2)
27	131.7	-
28	25.6	1.63 (3H, s)
29	17.6	1.51 (3H, s)
30	16.1	1.48 (3H, s)
31	37.5	3.41 (1H, dd, *J* = 14.5, 7.5)
		2.79 (1H, m)
32	117.1	4.55 (1H, t, *J* = 7.5)
33	136.1	-
34	25.7	1.51 (3H, s)
35	17.7	1.48 (3H, s)
1-OH	-	13.53 (1H, s)

presence of a geranyl group was implied by ^{1}H–^{1}H COSY correlations for H_2-21 and H-22, H_2-24 and H_2-25, H_2-25 and H-26, and HMBC cross-peaks of H_3-30 to C-22, C-23, and C-24, and H_3-28 to C-26, C-27, and C-29. NOESY correlations for H_2-21 to H_3-30 and H-22 to H_2-24 suggested the 22*E* configuration for the geranyl group. From these data, **1** was presumed to be a xanthone derivative possessing one geranyl and three prenyl groups. The chemical shift of OH-1 (δ_H 13.53) revealed the presence of hydrogen-bonded hydroxy group at C-1. The substitution pattern of A-ring (C-1 to C-4, C-4a, and C-9a) was deduced from HMBC cross-peaks of H-2 to C-1, C-3, and C-9a, and H_2-16 to C-3, C-4, C-4a, and C-11. HMBC correlations for H_2-31 to C-7, C-8, C-8a, and C-21 indicated that C-7, C-8a, a prenyl group, and a geranyl group were attached to an sp^3 quaternary carbon (C-8). Connectivities of C-8a to C-10a, C-10a to C-5, and C-5 to C-7 were deduced from HMBC correlations for H-5 to C-6, C-7, C-8a, and C-10a. Thus, the gross structure of biyouxanthone A was elucidated to be **1** (Figure 1).

The molecular formula of biyouxanthone B (**2**), $C_{34}H_{42}O_6$, was established by HRESIMS (*m/z* 545.2892 $[M-H]^-$, Δ −1.1 mmu). IR absorptions at 1668 and 1645 cm^{-1} implied the presence of carbonyl functionalities. ^{1}H and ^{13}C NMR data of **2** (Table 2) suggested that **2** was a xanthone derivative possessing one geranyl, two prenyl, one methoxy, and two hydroxy groups. Carbon chemical shifts of ring B (C-5 to C-8, C-8a, and C-10a) and C-16 to C-30 were similar to corresponding carbon chemical shifts of **1**. The substitution pattern of A-ring (C-1 to C-4, C-4a, and C-9a) as shown in Figure 2 were deduced by HMBC cross-peaks of a hydrogen-bonded hydroxy proton (OH-1) to C-1, C-2, and C-9a, H_2-11 to C-3, C-4, and C-4a, H-2 and 3-OMe to C-3, and the NOESY correlation between H-2 and 3-OMe. Thus, the gross structure of biyouxanthone B was elucidated to be **2**. Biyouxanthone B (**2**) showed no specific rotation. Chiral HPLC analysis of biyouxanthone B (**2**) gave two peaks with almost same peak areas due to (−)-biyouxanthone B (t_R 19.0 min) and (+)-biyouxanthone B (t_R 24.0 min). In

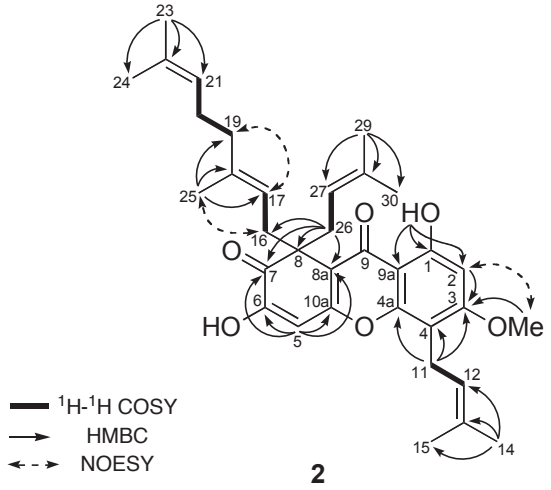

Figure 2. Selected 2D NMR correlations for biyouxanthone B (**2**)

addition, patterns of Cotton effects for respective enantiomers in the CD spectra were symmetrical each other. From these data, biyouxanthone B (**2**) was assigned as a racemate.

Biyouxanthone C (**3**) showed the pseudomolecular ion peak at *m/z* 531 $[M-H]^-$ in the ESIMS, and the HRESIMS analysis revealed that the molecular formula of **3** was $C_{33}H_{40}O_6$, whose molecular weight is

Table 2. ^1H and ^{13}C NMR data for biyouxanthones B (**2**) and C (**3**) in $CDCl_3$

	2		**3**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	161.0	-	160.4	-
2	95.2	6.41 (1H, s)	99.6	6.33 (1H, s)
3	162.6	-	160.6	-
4	107.2	-	104.6	-
4a	153.2	-	153.7	-
5	108.8	6.52 (1H, s)	108.7	6.52 (1H, s)
6	159.2	-	159.0	-
7	201.4	-	201.4	-
8	55.9	-	55.9	-
8a	116.2	-	116.4	-
9	179.7	-	179.6	-
9a	104.6	-	105.1	-
10a	151.7	-	151.8	-
11	21.5	3.44 (2H, m)	21.7	3.51 (2H, m)
12	121.8	5.21 (1H, t, *J* = 7.2)	121.1	5.28 (1H, t, *J* = 6.8)
13	131.9	-	135.1	-
14	25.7	1.70 (3H, s)	25.8	1.78 (3H, s)
15	17.9	1.83 (3H, s)	17.9	1.87 (3H, s)
16	37.7	3.48 (1H, m)	37.7	3.44 (1H, m)
		2.81 (1H, dd, *J* =13.2, 7.8)		2.80 (1H, dd, *J* =14.8, 7.8)
17	117.8	4.69 (1H, t, *J* =7.8)	117.7	4.68 (1H, t, *J* =7.8)
18	139.0	-	139.0	-
19	39.6	1.80 (2H, m)	39.6	1.83 (2H, m)
20	26.6	1.86 (2H, m)	26.5	1.80 (2H, m)
21	123.9	4.89 (1H, t, *J* = 6.7)	123.9	4.88 (1H, t, *J* = 6.8)
22	131.3	-	131.4	-
23	25.5	1.60 (3H, s)	25.5	1.60 (3H, s)
24	17.8	1.49 (3H, s)	17.9	1.50 (3H, s)
25	16.2	1.48 (3H, s)	16.2	1.48 (3H, s)
26	38.0	3.48 (1H, m)	37.9	3.44 (1H, m)
		2.83 (1H, dd, *J* =12.5, 7.5)		2.83 (1H, dd, *J* =13.9, 7.1)
27	117.7	4.66 (1H, t, *J* =7.5)	117.6	4.65 (1H, t, *J* =7.1)
28	135.2	-	135.3	-
29	25.7	1.50 (3H, s)	25.7	1.50 (3H, s)
30	17.5	1.50 (3H, s)	17.5	1.50 (3H, s)
1-OH	-	13.25 (1H, s)	-	13.23 (1H, s)
3-OH	-	-	-	-
6-OH	-	6.98 (1H, brs)	-	-
3-OMe	56.0	3.91 (3H, s)	-	-

smaller by 14 as compared with that of biyouxanthone B (**2**). ^{1}H and ^{13}C NMR data of biyouxanthone C (**3**) (Table 2) were similar to those of **2**, except for the absence of the signal for a methoxy group at C-3. These data suggested that **3** had a hydroxy group at C-3 in place of a methoxy group of **2**. It was supported by detailed analyses of 2D NMR spectra of **3** (Figure 3). Thus, the gross structure of biyouxanthone C was elucidated to be **3**.

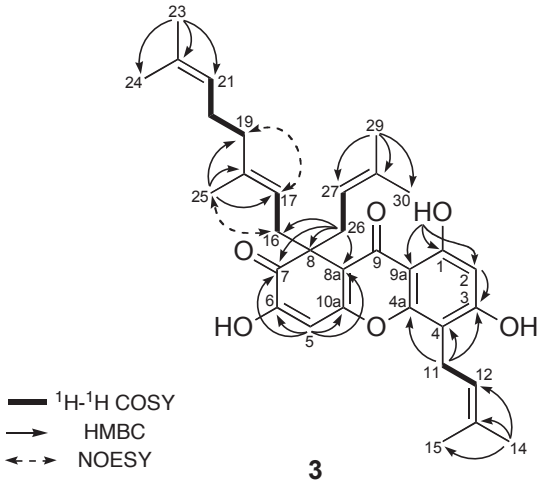

Figure 3. Selected 2D NMR correlations for biyouxanthone C (**3**)

Biyouxanthone D (**4**) had a molecular formula of $C_{28}H_{32}O_6$ based on HRESIMS. ^{1}H and ^{13}C NMR data of **4** (Table 3) revealed that **4** was a xanthone derivative possessing one prenyl group, four hydroxy groups, and one monoterpene substituent. The substituent was elucidated to be a 5-methyl-2-(prop-1-en-2-yl)hex-4-enyl group by ^{1}H–^{1}H COSY correlations for H_2-16 and H-17, H-17 and H_2-18, and H_2-18 and H-19, and HMBC cross-peaks of H_3-21 to C-19, C-20, and C-22, and H_3-25 to C-17, C-23, and C-24. The locations of these substituents were deduced from the HMBC correlations as shown in Figure 4. Thus, the gross structure of biyouxanthone D was elucidated to be **4**. Biyouxanthone D (**4**) showed optical rotation, $[\alpha]^{23}_{D}$ +37.9, while the stereochemistry of C-17 was not assigned.

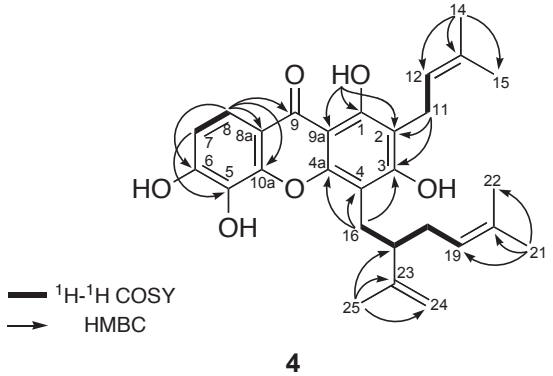

Figure 4. Selected 2D NMR correlations for biyouxanthone D (**4**)

Biyouxanthones A (**1**) and B (**2**) inhibited the HCV core protein level in the culture of HCV-infected human hepatoma Huh7 cells (89% and 61%, respectively) at 10 μM.

Table 3. ^{1}H and ^{13}C NMR data for biyouxanthone D (**4**) in $CDCl_3$

position	δ_C	δ_H (*J* in Hz)
1	158.4	-
2	108.6	-
3	160.5	-
4	105.8	-
4a	152.7	-
5	130.2	-
6	149.0	-
7	112.3	6.98 (1H, d, *J* = 8.8)
8	118.1	7.77 (1H, d, *J* = 8.8)
8a	114.0	-
9	180.5	-
9a	102.6	-
10a	144.7	-
11	21.5	3.49 (2H, d, *J* = 7.2)
12	121.0	5.27 (1H, t, *J* = 7.2)
13	136.4	-
14	25.8	1.80 (3H, s)
15	17.9	1.87 (3H, s)
16	27.2	2.93 (1H, dd, *J* = 14.0, 6.4)
		2.84 (1H, dd, *J* = 14.0, 7.3)
17	47.7	2.39 (1H, quint, *J* = 7.0)
18	31.8	2.23, 2.15 (each 1H, dt, *J* = 15.2, 6.7)
19	122.6	5.20 (1H, t, *J* = 6.7)
20	133.8	-
21	25.8	1.75 (3H, s)
22	17.9	1.62 (3H, s)
23	150.0	-
24	110.6	4.76, 4.69 (each 1H, brs)
25	19.6	1.75 (3H, s)
1-OH	-	13.32 (1H, s)
3-OH	-	6.34 (1H, s)

EXPERIMENTAL

General Experimental Procedures

Optical rotations were recorded on a JASCO P-1030 digital polarimeter. IR, UV, and CD spectra were recorded on JASCO FT/IR-230, Shimadzu UV-1600PC, and JASCO J-720 spectrophotometers, respectively. NMR spectra were measured by a JEOL ECA 500 spectrometer. The 7.26 and 77.0 ppm resonances of residual $CHCl_3$ were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESIMS spectra were recorded on a JEOL JMS 700-TZ spectrometer.

Plant Material

Hypericum chinense was cultivated at the botanical garden of the University of Tokushima and collected in January 2006. Herbarium specimens were deposited in Experimental Station for Medicinal Plants Studies, Hokkaido University (specimen number: UTP98014).

Extraction and Isolation

Roots of *H. chinense* (2.52 kg, dry) were extracted with MeOH (10 L x 3), and the extracts were partitioned between *n*-hexane (1 L x 3) and H_2O (1 L). The *n*-hexane-soluble portions were subjected to a silica gel column (*n*-hexane / EtOAc), a Sephadex LH-20 column (MeOH), and then C_{18} reversed-phase HPLC [LUNA 5μ C18(2), Phenomenex, 10 x 250 mm; flow rate 3.0 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 95:5] to afford biyouxanthones A (**1**, 4.2 mg, 0.00017%), B (**2**, 3.9 mg, 0.00016%), and C (**3**, 21.8 mg, 0.00087%). EtOAc-soluble portions were purified by a silica gel column ($CHCl_3$/MeOH) and a Sephadex LH-20 (MeOH) column chromatographies, and C_{18} reversed-phase HPLC (Mighty sil RP-18, Kanto Chemical Co. Ltd, 10 x 250 mm; flow rate 2.0 mL/min; UV detection at 254 nm; eluent MeOH/H_2O, 9:1, 0.1% TFA) to give biyouxanthone D (**4**, 2.9 mg, 0.00012%).

Biyouxanthone A (1): yellow amorphous solid; $[\alpha]^{23}_D$ ±0 (*c* 0.1 $CHCl_3$); UV (MeOH) λ_{max} 306 (ε 3100) and 418 (4400) nm; IR (film) ν_{max} 3347, 1671, and 1636 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 599 $[M-H]^-$; HRESIMS: *m/z* 599.3366 $[M-H]^-$ (calcd for $C_{38}H_{47}O_6$, 599.3372).

Biyouxanthone B (2): yellow amorphous solid; $[\alpha]^{23}_D$ ±0 (*c* 0.3 $CHCl_3$); UV (MeOH) λ_{max} 260 (ε 12900), 280 (10100), 314 (13400), and 421 (9200) nm; IR (film) ν_{max} 3367, 1668, and 1645 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESIMS *m/z* 545 $[M-H]^-$; HRESIMS: *m/z* 545.2892 $[M-H]^-$ (calcd for $C_{34}H_{41}O_6$, 545.2903).

Biyouxanthone C (3): yellow amorphous solid; $[\alpha]^{23}_D$ ±0 (*c* 0.3 $CHCl_3$); UV (MeOH) λ_{max} 250 (ε 10800), 277 (10900), and 419 (5600) nm; IR (film) ν_{max} 3347, 1670, and 1645 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESIMS *m/z* 531 $[M-H]^-$; HRESIMS: *m/z* 531.2747 $[M-H]^-$ (calcd for $C_{33}H_{39}O_6$, 531.2750).

Biyouxanthone D (4): yellow amorphous solid; $[\alpha]^{23}_D$ +37.9 (*c* 0.5 $CHCl_3$); UV (MeOH) λ_{max} 254 (ε 33600), 286 (11300), and 331 (16000) nm; IR (KBr) ν_{max} 3430, 2923, 1617, and 1591 cm^{-1}; ^1H and ^{13}C NMR data (Table 3); ESIMS *m/z* 487 $[M+Na]^+$; HRESIMS: *m/z* 487.2088 $[M+Na]^+$ (calcd for $C_{28}H_{32}O_6Na$, 487.2097).

Chiral HPLC analysis of biyouxanthone B (2)

Biyouxanthone B (**2**) was subjected to a chiral HPLC [Chiralpak AD; Daicel Chemical Industry, Ltd., 4.6 x 250 mm; *n*-hexane / *i*-PrOH (99:1); flow rate 0.5 mL/min; UV detection at 254 nm] to afford (–)-biyouxanthone B (t_R 19.0) and (+)-biyouxanthone B (t_R 24.0 min). (–)-Biyouxanthone B: $[\alpha]^{21}_D$ –9.6 (*c* 0.1 $CHCl_3$); CD (MeOH) λ_{ext} 280 (Δε –0.96), 257 (+0.28), 237 (–0.88), 226 (–0.23), 220 (–0.49), and 211 (+4.52) nm. (+)-Biyouxanthone B: $[\alpha]^{21}_D$ +13.7 (*c* 0.1 $CHCl_3$); CD (MeOH) λ_{ext} 280 (Δε +0.79), 257 (–0.55), 237 (+0.63), 226 (+0.14), 220 (+0.60), and 211 (–3.67) nm.

Measurement of anti-HCV activity

Huh7 cells infected with HCV JFH-1 isolate[6] were treated with biyouxanthones A – D (**1** – **4**) for 3 days. HCV core protein in cell culture supernatants was quantified using an enzyme immunoassay (Ortho HCV antigen ELISA Kit; Ortho Clinical Diagnostics, Tokyo, Japan), following the manufacturer's instructions.

ACKNOWLEDGMENTS

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REFERENCES

1. A. Nahrstedt and V. Butterweck, *Pharmacopsychiatry*, 1997, **30**, 129.
2. M. Dostalek, J. Pistovcakova, J. Jurica, J. Tomandl, I. Linhart, A. Sulcavá, and E. Hadasova, *Life Sciences*, 2005, **78**, 239.
3. M. A. Medina, B. Mrtínez-Proveda, M. I. Amores-Sánchez, and A. R. Quesada, *Life Sciences*, 2006, **79**, 105.
4. L. Beerhues, *Phytochemistry*, 2006, **67**, 2201.
5. N. Tanaka, T. Kubota, H. Ishiyama, Y. Kashiwada, Y. Takaishi, J. Ito, Y. Mikami, M. Shiro, and J. Kobayashi, *Heterocycles*, 2009, **79**, 917.
6. T. Wakita, T. Pietschmann, T. Kato, T. Date, M. Miyamoto, Z. Zhao, K. Murthy, A. Habermann, H. G. Krausslich, M. Mizokami, R. Bartenschlager, and T. J. Liang, *Nat. Med.*, 2005, **11**, 791.