THE ^{1}H AND ^{13}C NMR CHEMICAL SHIFT ASSIGNMENTS FOR THIRTEEN BUFADIENOLIDES ISOLATED FROM THE TRADITIONAL CHINESE DRUG CH'AN SU

Yoshiaki KAMANO[a1], Toshihiko NOGAWA[a2], Ayano YAMASHITA[a3] and George R. PETTIT[b,*]

[a] *Faculty of Science, Kanagawa University, 2946, Tsuchiya, Hiratsuka, Kanagawa 259-1293, Japan; e-mail: [1] kamano@info.kanagawa-u.ac.jp, [2] toshinogawa@mac.com, [3] kotake@info.kanagawa-u.ac.jp*

[b] *Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287-2404, U.S.A.*

Received October 1, 2001
Accepted December 1, 2001

Dedicated to the memory of an outstanding steroid chemist Dr Václav Černý.

The ^{1}H and ^{13}C NMR spectra of thirteen natural bufadienolides, which were isolated from the Chinese drug Ch'an Su, were assigned using a combination of 2D NMR spectra that included ^{1}H-^{1}H COSY, NOESY, HMQC, and HMBC techniques.

Keywords: Ch'an Su; Bufadienolides; Steroids; NMR spectroscopy, ^{1}H and ^{13}C; Assignment of signals; Cardiotonic; Antitumor activity.

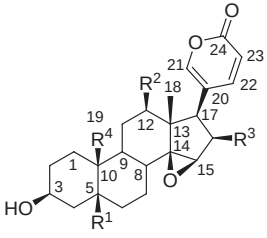
The naturally occurring bufadienolides comprise a class of steroids bearing a 5-substituted 2*H*-pyran-2-one moiety at position C-17β (ref.[1]) and are now well-known constituents of certain animals and plants[2]. Complex mixtures of bufadienolides from toads of the Bufonidae[3] have been used in Chinese (Ch'an Su) and Japanese (Sen So) traditional medicine for at least several millenia. Ch'an Su is a product of the skin secretion of local toads such as *Bufo gargarizans* Cantor or *Bufo melanostictus* Schneider. The principal biologically active components of Ch'an Su are bufadienolides, which show a broad spectrum of potent biological activities that includes cardiotonic, hypertensive, antineoplastic[4] and antiviral[4d] activities.

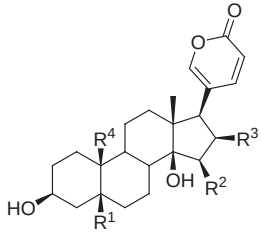
In 1958 we began to investigate the bufadienolides as potential antineoplastic agents and directed considerable attention and resources to making some of these very interesting steroids more readily available by total[5a–5f] and semisyntheses[6a–6i] for anticancer evaluations. Initial focus was on the classic bufadienolide, bufalin[7], where evidence is now accumulating that

doi:10.1135/cccc20011841

the cancer cell growth inhibiting and blood pressure regulating substance may be a normal trace constituent of human serum[4c]. As part of our early evaluations of the bufadienolides we were able to find a number of these steroids with cancer cell line inhibitory[4a,6] and/or *in vivo*[6b] antineoplastic activity and that validated our early assumptions[3]. Recently, we reported cancer cell growth[8] structure–reactivity relationships for a selection of bufadienolides, new separation methods using[9] Sephadex LH-20, and conformational preference of the 2*H*-pyran-2-one ring in 17β position as analyzed by spectroscopic and computational methods[10].

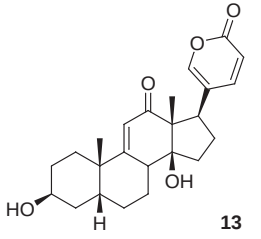
In our continuing investigation of Ch'an Su components[4a], we isolated thirteen previously known bufadienolides, cinobufaginol (**1**), desacetylcinobufaginol (**2**), resibufaginol (**3**), 12β-hydroxyresibufogenin (**4**), 12β-hydroxycinobufagin (**5**), cinobufotalin (**6**), desacetylcinobufagin (**7**), resibufagin (**8**), hellebrigenin (**9**), 15β-hydroxybufalin (**10**), 5β-hydroxybufotalin (**11**), desacetylbufotalin (**12**), and argentinogenin (**13**). Although these compounds had been isolated from natural sources or synthesized from related bufadienolides, the NMR data were incomplete. Since the majority of our earlier chemical investigations of the bufadienolides were conducted with NMR





1, $R^1 = R^2 = H$, $R^3 = OCOCH_3$, $R^4 = CH_2OH$
2, $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = CH_2OH$
3, $R^1 = R^2 = R^3 = H$, $R^4 = CH_2OH$
4, $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_3$
5, $R^1 = H$, $R^2 = OH$, $R^3 = OCOCH_3$, $R^4 = CH_3$
6, $R^1 = OH$, $R^2 = H$, $R^3 = OCOCH_3$, $R^4 = CH_3$
7, $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = CH_3$
8, $R^1 = R^2 = R^3 = H$, $R^4 = CHO$

9, $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = CHO$
10, $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_3$
11, $R^1 = OH$, $R^2 = H$, $R^3 = OCOCH_3$, $R^4 = CH_3$
12, $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = CH_3$



13

instruments contemporary at the time, the renewal supply of the thirteen bufadienolides allowed an opportunity to study these steroids by current 2D NMR techniques. In turn this was expected to update the 1987 study of Robien[11] and others.

EXPERIMENTAL

The Chinese traditional drug Ch'an Su was obtained in a Hong Kong folk medicinal market. The Ch'an Su was ground into rough powder and extracted successively (3×) with methanol and ethanol–water (1 : 1) at room temperature for five days. The extract was concentrated at reduced pressure to provide a residue, which was subjected to column chromatography on HP-20 (DIAION). Elution with methanol–water followed by successive column chromatography on silica gel, Sephadex LH-20 and C_{18}-HPLC afforded the bufadienolides.

NMR spectra were recorded at room temperature using a JEOL ECP-500 spectrometer at 500 MHz for ^{1}H, and 125 MHz for ^{13}C. Bufadienolides **4**, **6**, **7**, and **13** were dissolved in $CDCl_3$, **1**–**3**, **5**, and **9**–**12** in CD_3OD and **8** in $CDCl_3$–CD_3OD (7 : 1) owing to solubility requirements. These samples (10–20 mg) were dissolved in about 0.5 ml of solvent using a 5-mm sample tube. Chemical shifts, referenced to TMS as internal standard, are reported in ppm (δ-scale). Coupling constants (*J*) are expressed in Hz. Processing of data was performed using the program JEOL DELTA, running on a Silicon Graphics work station. All 2D NMR experiments were conducted using the field gradient mode. The ^{1}H NMR spectra were recorded with a spectral width of 7.5 kHz (with 16 384 data points). For ^{13}C NMR spectra, the spectral width was 31.4 kHz with 32 768 data points. HMQC spectra were obtained with an f2 spectral width of 4.4 kHz (512 data points) and f1 spectral width of 23.3 kHz (128 data points zero filled to 512). Relaxation delay was 1.5 s. HMBC spectra were collected with the same spectral width and relaxation delay as with the HMQC spectra using a long-range coupling constant of 8 Hz. The ^{1}H-^{1}H COSY and NOESY spectra were obtained using a spectral width of 4.4 kHz (512 data points for f2 and 128 zero filled to 512 for f1). Relaxation delay was 1.0 s for the ^{1}H-^{1}H COSY. NOESY results were obtained using a 1.5 s relaxation delay with 1.0 s mixing time and processed in the phase-sensitive mode.

RESULTS AND DISCUSSION

The ^{1}H and ^{13}C NMR chemical shift assignments are presented in Tables I–III. The complete NMR assignments of these compounds were made using the data provided by two-dimensional NMR techniques that included ^{1}H-^{1}H COSY, NOESY, HMQC, and HMBC spectra. As a typical example of the assignments made for each compound, the analysis of cinobufaginol (**1**) follows. The ^{1}H NMR spectrum showed H-21, H-22, and H-23 signals at 7.36, 8.02, and 6.24 ppm, respectively, for the 2*H*-pyran-2-one ring and a characteristic bufadienolide pattern. The spectrum of **1** was similar to that of cinobufagin (one of the major components of Ch'an Su) except for the presence of a pair of doublets coupled to each other at 3.47 and 3.79 ppm, and absence of a 19-methyl signal. This suggested that **1** was a 19-hydroxy

TABLE I

^{1}H NMR chemical shifts (ppm, δ-scale) and coupling constants (*J*, Hz) for bufadienolides **1**–**6**

Position	**1**	**2**	**3**	**4**	**5**	**6**
1α	1.41	1.87	1.82	1.52 (2 H)	1.50	1.88 td (14.7, 5.5)
1β	1.87	1.40	1.42		1.29	1.40
2α	1.57	1.58 (2 H)	1.58 (2 H)	1.54 (2 H)	1.48	1.63 (2 H)
2β	1.65				1.63	
3α	4.03 brt (2.8)	4.04 br s	4.03 br s	4.414 br s	4.05 br s	4.21 br s
4α	1.96 td (14.2, 2.8)	1.95 td (13.8, 2.8)	1.95	1.85	1.96	2.10 dd (15.1, 3.2)
4β	1.34	1.37	1.35	1.36	1.32	1.58
5β	2.19	2.19	2.19	1.83	1.80	–
6α	1.18	1.18	1.17	1.23	1.21	1.37
6β	1.84	1.82	1.82	1.87	1.91	1.69
7α	1.07 qd (13.3, 3.7)	1.06 qd (13.8, 4.1)	1.07 qd (12.8, 3.7)	0.93 qd (13.3, 4.1)	1.04	0.86 qd (12.8, 4.1)
7β	1.49	1.49	1.49	1.49	1.46	1.66
8β	2.14 td (11.9, 3.7)	2.09 td (12.4, 4.1)	2.05 td (11.9, 3.7)	1.96 td (11.9, 3.7)	2.02	2.09 td (12.8, 3.7)
9α	1.82	1.79	1.79 td (11.9, 2.8)	1.63 td (11.9, 3.2)	1.77	1.52 td (11.5, 3.2)
11α	1.60	1.59	1.63	1.78 dt (12.8, 4.1)	1.74	1.61
11β	1.39	1.37	1.38	1.37	1.39	1.41
12α	1.49	1.46	1.46	3.39 dd (11.5, 4.1)	3.48 dd (11.5, 4.1)	1.43
12β	1.80	1.75	1.70	–	–	1.78
15α	3.75 d(0.9)	3.60 d (0.9)	3.61 s	3.52 s	3.72 s	3.64 s
16α	5.48 dd (9.6, 1.4)	4.72 dd (9.2, 1.4)	2.42 dd (15.1, 10.1)	2.35 ddd (15.6, 10.5, 1.4)	5.46 d (8.7)	5.45 dd (9.6, 0.9)
16β	–	–	1.92	2.00 d (15.6)	–	–
17α	2.92 d (9.6)	2.68 d (9.2)	2.58 d (10.1)	3.11 d (9.2)	3.43 d (9.6)	2.81d (9.6)
18β	0.81 s	0.78 s	0.77 s	0.73 s	0.74 s	0.82 s
19β	3.47 d (11.5) 3.79 (11.5)	3.46 d (11.5) 3.79 d (11.0)	3.46 d (11.0) 3.81 d (11.5)	1.00 s	1.00 s	0.98 s
21	7.36 s	7.39 d (1.8)	7.44 d (1.8)	7.34 d (2.3)	7.37	7.17 br s
22	8.02 brd (7.3)	8.09 d (8.3)	7.90 dd (9.6, 2.3)	7.73 dd (9.6, 2.3)	8.01	7.91 br s
23	6.24 dd (10.1, 0.9)	6.21 d (10.1)	6.26 d (9.6)	6.25 dd (9.6, 0.9)	6.24 dd (9.6, 0.9)	6.22 d (10.1)
CH_3CO	1.85 s	–	–	–	1.86 s	1.90 s

TABLE II

^{1}H NMR chemical shifts (ppm, δ-scale) and coupling constants (*J*, Hz) for bufadienolides **7–13**

Position	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1α	1.51 (2 H)	1.55	2.13	1.47 (2 H)	1.80	1.84 td (13.8, 5.5)	2.47
1β		1.86	1.67		1.39	1.40	1.76
2α	1.55	1.52	1.67 (2 H)	1.65	1.56	1.61 (2 H)	1.61
2β		1.69		1.46	1.72		1.76
3α	4.14 br s	4.14	4.14 brs	4.05 br t (2.3)	4.14 br s	4.16 br s	4.11 br s
4α	1.90 td (13.8, 2.5)	1.84	2.18	2.09	2.22 dd (14.7, 2.8)	2.10 dd (9.6, 2.8)	1.64
4β	1.35	1.50	1.49	1.33	1.48	1.51	1.53
5β	1.78	2.33	–	1.75	–	–	1.91
6α	1.22	1.39	1.66	1.20	1.37	1.35	1.51
6β	1.85	1.57	2.10	2.02	1.71	1.69	2.06
7α	0.94 qd (12.8, 3.7)	0.97 qd (14.2, 3.7)	1.37 qd (13.3, 3.7)	1.54 (2 H)	1.19 qd (12.4, 4.1)	0.88 qd (13.3, 5.5)	1.57
7β	1.49	1.55	2.16		1.98	1.64	2.02
8β	2.03 td (1.9, 3.7)	2.30 td (12.4, 3.2)	1.94 td (12.4, 3.2)	2.11	1.64	2.06 td (11.9, 3.7)	2.75 dd (12.8, 5.5)
9α	1.60 td (11.9, 3.7)	1.71	1.71	2.11	1.59	1.50	–
11α	1.55	1.55	1.55	1.41	1.47	1.57	–
11β	1.32	1.93	1.18 qd (13.3, 3.7)	1.28	1.29	1.37	–
12α	1.37	1.41	1.41 td (13.3, 3.7)	1.19	1.42	1.40	–
12β	1.72	1.73	1.50	1.78	1.57	1.75 dd (9.6, 2.8)	–
15α	3.58 s	3.55 s	2.06	4.00 dd (7.3, 1.8)	2.65 dd (15.6, 9.6)	3.55 s	1.85
15β	–	–	1.65	–	1.82	–	1.71
16α	4.75 d (9.2)	2.40 ddd (14.2, 10.5, 1.4)	2.21	2.46	5.50 td (8.7, 1.4)	4.70 d (8.7)	2.04
16β	–	1.97	1.73	1.75	–	–	1.73
17α	2.61 d (9.2)	2.51 d (10.1)	2.54 dd (9.6, 6.9)	3.05 t (9.6)	2.97 d (8.7)	2.62 d (9.2)	3.77 dd (9.6, 6.9)
18β	0.80 s	0.87 s	0.67 s	0.85 s	0.78 s	0.79 s	0.81 s
19β	0.98 s	9.49 d (1.8)	10.06 s	1.01 s	0.93 s	0.97 s	1.29 s
21	7.23 d (1.8)	7.30 d (1.8)	7.42 d (2.3)	7.47	7.43 d (1.4)	7.25 d (1.8)	7.50 d (2.3)
22	7.98 dd (9.6, 2.3)	7.83 dd (9.6, 2.3)	7.98 dd (9.6, 2.3)	7.51 dd (9.6, 2.3)	8.24 dd (9.6, 1.8)	8.04 dd (9.6, 2.3)	7.78 dd (9.6, 2.3)
23	6.21 d (9.6)	6.27 d (9.6)	6.28 d (10.1	6.31 dd (9.6, 0.9)	6.21 dd (9.2, 0.9)	6.23 d (9.6)	6.28 d (9.6)
CH_3CO	–	–	–	–	1.84 s	–	

TABLE III
^{13}C NMR chemical shifts (ppm, δ-scale) for bufadienolides **1–13**

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	24.5	24.2	24.2	29.5	30.7	24.8	29.5	20.8	18.5	31.3	26.2	24.9	31.7
2	28.5	28.2	28.2	27.8	28.5	27.9	27.9	26.1	27.6	28.5	28.5	27.6	31.7
3	67.7	67.4	67.4	66.7	67.6	68.0	66.8	65.2	68.0	67.9	69.1	67.7	66.6
4	34.3	34.1	34.1	33.3	34.1	36.4	33.3	32.1	38.6	34.4	37.8	36.8	35.8
5	30.4	30.0	30.0	36.0	37.5	74.4	35.9	28.8	75.8	38.0	76.2	74.7	37.2
6	26.9	26.7	26.7	25.7	26.9	34.4	25.7	27.6	37.5	27.7	35.9	34.0	25.3
7	21.6	21.4	21.4	20.9	21.8	23.1	20.7	20.8	25.2	21.6	24.6	23.1	21.5
8	34.8	34.6	35.0	32.8	33.8	32.1	33.1	33.8	43.0	36.6	42.1	32.3	40.1
9	40.3	40.1	40.2	34.8	35.7	42.7	39.3	38.1	40.6	33.3	40.1	42.8	133.4
10	40.9	40.6	40.6	35.3	36.3	40.9	35.5	51.3	56.3	36.8	41.9	41.0	41.4
11	22.5	22.2	22.4	30.2	30.7	21.3	20.9	20.9	23.6	20.9	22.8	21.4	140.9
12	41.4	41.2	40.5	75.0	76.2	40.0	40.1	39.5	41.6	32.6	41.4	40.1	200.0
13	46.7	46.4	46.4	51.1	52.6	45.0	45.4	45.3	49.5	48.2	50.7	45.2	58.7
14	73.9	73.7	76.0	73.9	72.4	72.4	72.8	74.6	85.7	86.3	85.0	72.6	83.1
15	61.1	63.3	61.2	59.7	60.7	59.4	61.9	59.9	32.3	76.6	41.2	62.1	33.6
16	76.9	73.1	33.2	32.3	76.6	74.6	72.6	32.5	29.7	37.8	75.7	71.9	28.0
17	51.7	53.2	48.6	42.8	47.1	50.2	52.4	47.7	52.0	46.7	58.1	52.1	44.1
18	17.8	17.7	17.2	11.1	12.3	17.2	17.3	16.8	17.1	17.1	17.3	17.3	16.2
19	66.6	66.3	66.2	23.7	24.1	16.8	23.7	206.3	210.2	24.1	17.1	16.8	27.5
20	118.7	119.9	124.5	122.1	118.4	116.1	117.1	122.8	124.9	120.7	119.4	118.0	120.9
21	153.8	152.7	151.8	150.4	153.7	151.3	150.9	149.9	150.6	150.7	152.9	150.8	150.4
22	151.2	152.0	149.6	147.1	151.0	148.3	148.8	147.8	149.3	148.4	152.1	149.9	146.7
23	114.4	113.6	115.4	115.2	114.2	113.9	114.1	115.2	115.5	115.7	113.2	113.5	115.4
24	164.3	164.8	164.4	162.2	164.0	161.7	162.2	163.0	164.8	164.7	164.5	163.2	162.3
$CH_3\mathbf{C}O$	171.9	–	–	–	171.6	170.2	–	–	–	–	172.0	–	–
$\mathbf{C}H_3CO$	20.7	–	–	–	20.4	20.5	–	–	–	–	21.0	–	–

derivative of cinobufagin. In the HMQC spectrum, relationships between carbons and protons were determined. The ^{1}H-^{1}H COSY spectrum revealed the connectivities C-2 to C-11, C-16 to C-17, and C-22 to C-23. In the HMBC spectrum, HMBC correlations of H-1 to C-5 and C-9, H-3 to C-1 and C-5, H-11 to C-10, H-12 to C-9 and C-14, H-15 to C-13, H-17 to C-14 and C-15, and H-18 to C-12, -14, and -17 allowed assignment of the steroid skeleton. The HMBC correlations H-19 to C-1 and C-5 suggested that the CH_2OH moiety was located on C-10. The H-2′ assignable to an acetyl signal showed an HMBC correlation to H-1′, which was connected to C-16 by an HMBC correlation of H-16 to C-1′. Therefore, these were assigned to the acetoxy group at C-16. The connectivity between C-17 and C-20 was supported by the HMBC correlations H-17 to C-21 and C-22 and H-16 to C-20. The connectivity through C-20 of the pyran ring was thereby confirmed.

The α and β protons of the molecule were assigned employing the phase sensitive NOESY spectrum. NOESY correlations of H-18 and H-19 identified the axial protons, H-1β, H-8, and H-11β. The H-18 proton was also correlated to the one of the protons at C-12, and that proton was assigned H-12β. The H-3 proton showed NOESY correlations with both α and β protons at C-2 and C-4. These correlations suggested that H-3 was equatorial and hence α-oriented. The α orientation of H-17 was assigned by NOESY correlations with both protons at C-12. The signal of H-15 on the carbon bearing the 14,15-epoxy group was assigned as α-oriented by a NOESY correlation with H-7α, and the 14,15-epoxy group was confirmed to have the β orientation. A NOESY correlation of H-12α/H-16 showed that the 16-acetoxy group was β-oriented. The NOESY correlations H-2α/H-9, H-4α/H-7α, H-5/H-1β, H-5/H-4β, H-6β/H-8, and H-7β/H-8 revealed the assignments for all the skeleton protons. Thus, the ^{1}H and ^{13}C NMR spectra of **1** were satisfactorily assigned.

In an analogous manner, the ^{1}H and ^{13}C NMR spectra of bufadienolides **2**–**13** were assigned and the results are shown in Tables I–III. The NMR data will be useful for future identification and structure elucidation of other bufadienolides and cardenolides.

We thank for financial assistance Outstanding Investigator Award (to G. R. P.) CA-44344-01A1-12, 1RO1-CA-90441-01, from the Division of Cancer Treatment and Diagnosis, National Cancer Institute, Department of Health and Human Services; and the Arizona Disease Control Research Commission; the R. B. Dalton Endowment; and Dr F. Hogan for other assistance.

REFERENCES

1. a) Linde H., Hofer P., Meyer K.: *Helv. Chim. Acta* **1966**, *49*, 1234; b) Hofer P., Linde H., Meyer K.: *Helv. Chim. Acta* **1960**, *43*, 1955; c) Rees R., Schindler O., Deulofeu V., Reichstein T.: *Helv. Chim. Acta* **1959**, *42*, 2400; d) Horinger N., Zivanov D., Linde H. H. A., Meyer K.: *Helv. Chim. Acta* **1972**, *55*, 2549.
2. Krenn L., Kopp B.: *Phytochemistry* **1998**, *48*, 1.
3. Pettit G. R., Green B., Dunn G. L.: *J. Org. Chem.* **1970**, *35*, 1367.
4. a) Nogawa T., Kamano Y., Yamashita A., Pettit G. R.: *J. Nat. Prod.* **2001**, submitted; b) Numazawa S., Honma Y., Yamamoto T., Yoshida T., Kuroiwa Y.: *Leukemia Res.* **1995**, *19*, 945; c) Jing Y., Ohizumi H., Kawazoe N., Hashimoto S., Masuda Y., Nakajo S., Yoshida T., Kuroiwa Y., Nakaya K.: *Jpn. J. Cancer Res.* **1994**, *85*, 645; d) Kamano Y., Satoh N., Nakayoshi H., Pettit G. R., Smith C. R.: *Chem. Pharm. Bull.* **1988**, *36*, 326.
5. a) Pettit G. R., Green B., Dunn G. L.: *J. Org. Chem.* **1970**, *35*, 1377; b) Pettit G. R., Herald C. L., Yardley J. P.: *J. Org. Chem.* **1970**, *35*, 1389; c) Pettit G. R. , Fessler D. C., Paull K. D., Hofer P., Knight J. C.: *J. Org. Chem.* **1970**, *35*, 1398; d) Knight J. C., Pettit G. R., Brown P.: *J. Org. Chem.* **1970**, *35*, 1415; e) Pettit G. R., Kasturi T. R., Knight J. C., Jaeggi K. A.: *J. Org. Chem.* **1970**, *35*, 1410; f) Pettit G. R., Dias J. R.: *J. Org. Chem.* **1971**, *36*, 3207.
6. a) Pettit G. R., Kamano Y., Bruschweiler F., Brown P.: *J. Org. Chem.* **1971**, *36*, 3736; b) Kamano Y., Pettit G. R.: *Experientia* **1972**, *28*, 768; c) Pettit G. R., Kamano Y.: *J. Org. Chem.* **1972**, *37*, 4040; d) Pettit G. R., Kamano Y.: *J. Chem. Soc., Perkin Trans. 1* **1973**, 725; e) Kamano Y., Pettit G. R.: *J. Org. Chem.* **1973**, *38*, 2202; f) Pettit G. R., Kamano Y.: *J. Org. Chem.* **1974**, *39*, 3003; g) Kamano Y., Pettit G. R., Inoue M.: *J. Org. Chem.* **1974**, *39*, 3007; h) Kamano Y., Pettit G. R., Tozawa M., Komeichi Y., Inoue M.: *J. Org. Chem.* **1975**, *40*, 2136; i) Kamano Y., Pettit G. R., Brown P., Inoue M.: *Tetrahedron* **1975**, *31*, 2359; j) Kamano Y., Pettit G. R., Inoue M., Tozawa M., Smith C. R., Weisleder D.: *J. Chem. Soc., Perkin Trans. 1* **1988**, 2037; k) Kamano Y., Drašar P., Pettit G. R., Tozawa M.: *Collect. Czech. Chem. Commun.* **1987**, *52*, 1325; l) Pettit G. R., Kamano Y., Drašar P., Inoue M., Knight J. C.: *J. Org. Chem.* **1987**, *52*, 3573.
7. a) Pettit G. R., Houghton L. E., Knight J. C., Bruschweiler F.: *J. Org. Chem.* **1970**, *35*, 2895; b) Pettit G. R., Houghton L. E., Knight J. C., Bruschweiler F.: *Chem. Commun.* **1970**, 93.
8. a) Kamano Y., Kotake A., Hashima H., Inoue M., Morita H., Takeya K., Itokawa H., Nadachi N., Segawa T., Yukita A., Saitou K., Katsuyama M., Pettit G. R.: *Bioorg. Med. Chem.* **1998**, *6*, 1103; b) Jing Y., Ohizumi H., Kawazoe N.: *Jpn. J. Cancer Res.* **1994**, *85*, 645.
9. Kamano Y., Nogawa T., Kotake A., Tozawa M., Pettit G. R.: *J. Liq. Chromatogr. Rel. Technol.* **1999**, *22*, 2455.
10. a) Kamano Y., Kotake A., Nogawa T., Hashima H., Tozawa M., Morita H., Takeya K., Itokawa H., Matsuo I., Ichihara Y., Drašar P., Pettit G. R.: *Collect. Czech. Chem. Commun.* **1998**, *63*, 1663; b) Kamano Y., Kotake A., Hashima H., Morita H., Takeya K., Itokawa H.: *Heterocycles* **1998**, *49*, 275.
11. Robien W., Kopp B., Schabl D., Schwarz H.: *Prog. Nucl. Magn. Reson. Spectrosc.* **1987**, *19*, 131.