Sarcophytonolides A – D, Four New Cembranolides from the Hainan Soft Coral *Sarcophyton* sp.

by **Rui Jia**[a]), **Yue-Wei Guo***[a]), **Ernesto Mollo**[b]), and **Guido Cimino**[b])

[a]) State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Zu Chong Zi Rd. 555, Zhangjiang Hi-Tech Park, Shanghai 201203, P. R. China (phone: +86-21-50805813; e-mail: ywguo@mail.shcnc.ac.cn)

[b]) Istituto di Chimica Biomolecolare-CNR, I-80078 Napoli

Four new cembranolide diterpenes, sarcophytonolides A – D (**1** – **4**), were isolated from a Hainan soft coral *Sarcophyton* sp. Their structures were elucidated on the basis of detailed spectroscopic analysis and by comparison with related model compounds.

Introduction. – Soft corals of the genus *Sarcophyton* (family Alcyoniidae) have been reported to contain a variety of diterpenes, of which cembranoids represent the most commonly encountered structural type. Most of these diterpenes have been regarded as defensive, competitive, reproductive or pheromonal substances, playing a functional role in the survival of these corals [1]. In particular, some of them exhibited very interesting biological activities [2]. The genus *Sarcophyton* is prolific in the South China Sea. In the course of our ongoing studies on biologically active substances from Hainan marine organisms [3], we made a collection of the soft coral *Sarcophyton* sp. off Ximao island, Hainan Province, China. Chemical investigation of the Et_2O-soluble fraction from the Me_2CO extract of the animal resulted in the isolation of four new cembranolides, named sarcophytonolides A – D (**1** – **4**; *Fig. 1*). This paper describes the isolation and structure elucidation of these new compounds.

Results and Discussion. – Freshly collected animals from Sanya, Hainan Province, China, in the South China Sea, were immediately put at $-20°$ and kept frozen until used. Specimens of *Sarcophyton* sp. were extracted exhaustively with acetone. The acetone extract was then partitioned between Et_2O and H_2O. The Et_2O-soluble fraction was repeatedly subjected to column chromatography (silica gel and *Sephadex LH-20*) followed by reversed-phase HPLC to afford pure compounds **1** – **4**.

Sarcophytonolide A (**1**) was isolated as colorless oil. Its molecular formula, $C_{21}H_{32}O_3$, was deduced from its HR-EI-MS (*m/z* 332.2356 (M^+)), indicating six degrees of unsaturation. Further spectral data (see also *Tables 1* and *2*) and their comparison with those of the model compounds **5** [4] and **6** [5] allowed us to determine the structure of **1** as depicted in *Fig. 1*.

The ^{13}C-NMR data of **1** (*Table 1*) revealed the presence of 1 C=O, 3 trisubstituted C=C, 1 trisubstituted epoxide, 6 CH_2, 1 CH, 4 Me groups, and 1 MeO group. The total of 20 C-atoms, besides the MeO, pointed to a diterpene. The C=O, 3 C=C, and epoxide moieties left one site of unsaturation, which was attributed to a

© 2005 Verlag Helvetica Chimica Acta AG, Zürich

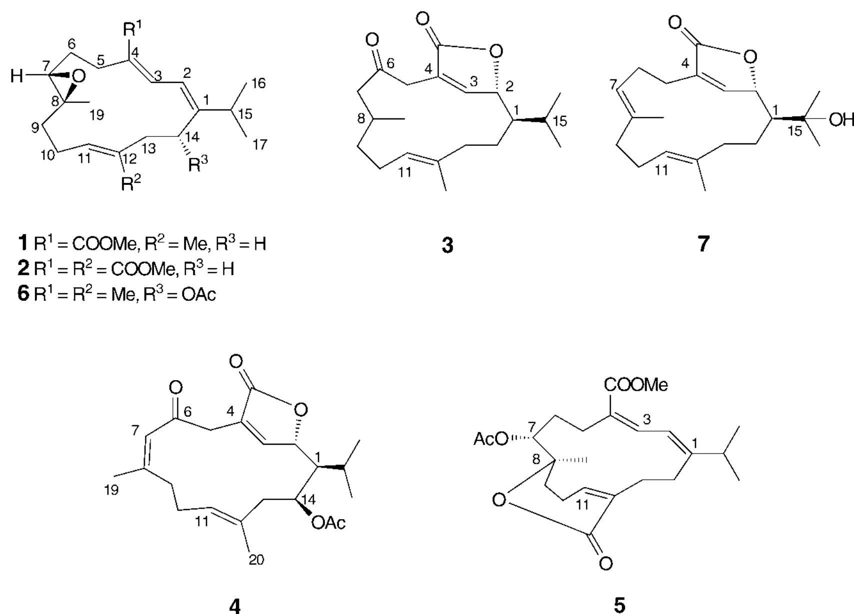

Fig. 1. *Structures of compounds* **1**–**7**. Trivial numbering.

monocyclic skeleton. In the ^{1}H-NMR spectrum, 2*d* at δ 6.67 and 7.01 (each $J = 11.7$ Hz, 1 H) were assigned to a β- and γ-positioned proton of a dienoate moiety, the latter being in agreement with the UV maximum at 283 nm (log ε 3.15) and the IR band at 1710 cm^{-1}. The third olefinic proton (δ 5.03 (br. *t*)) had thus to be at a separate trisubstituted C=C. The OCH signal, a *dd* at δ 2.79 ($J = 7.4$, 4.9 Hz), was attributed to a proton at the epoxide moiety. A *s* at δ 3.75 (3 H) was assigned to a MeO group. The ^{1}H,^{1}H-COSY plot revealed the presence of an ^{i}Pr group due to resonances at δ 1.08 (*d*, $J = 6.8$ Hz, 3 H), 1.09 (*d*, $J = 6.8$ Hz, 3 H), and 2.39 (*m*, 1 H). The partial structure of a dienoate moiety with an ^{i}Pr substituent at the δ position was further confirmed by the comparison of ^{1}H- and ^{13}C-NMR data of **1** with that of model compound **5** [4], which also allowed to assign the cembrane C-skeleton to **1**. In addition, three isolated spin systems (H−C(5) to H−C(7), CH$_2$(9) to H−C(11), and CH$_2$(13) to CH$_2$(14)) were established by the ^{1}H,^{1}H-COSY data (*Fig. 2*). The epoxide proton (H−C(7)) was coupled to CH$_2$ protons at δ 1.86 (*m*, H$_a$−C(6)) and 1.75 (*m*, H$_b$−C(6)), which, in turn, were coupled to CH$_2$ protons at 2.50 (*m*, CH$_2$(5)). The downfield shifts of the latter protons suggested that the CH$_2$ group was attached to a C=C bond. An olefinic proton at 5.03 (br. *t*, $J = 6.7$ Hz, H−C(11)) was coupled allylically to Me protons (δ 1.62 (*s*, Me−C(20))) and to CH$_2$ protons at δ 1.96 (*m*, CH$_2$(10)), the latter being further coupled to CH$_2$ protons at δ *ca.* 1.76 (CH$_2$(9)). At this stage, the epoxide moiety and the isolated C=C bond were concluded to be located between C(7) and C(8) and between C(11) and C(12), respectively.

We attempted to determine the configuration of **1** by NOESY experiments (*Fig. 3*). A NOESY correlation between H−C(2) at δ 7.01 and H−C(15) of the ^{i}Pr group indicated (*E*) configuration for the C(1)=C(2) bond. A NOESY correlation between H−C(2) and the MeO group of COOMe at δ 3.75, together with the *trans* relation of H−C(2) and H−C(3) ($J = 11.7$ Hz), implied a (*Z*)-configuration for the C(3)=C(4) bond. The (*E*)-configuration of the C(11)=C(12) bond was suggested by the chemical shift of Me(20) (δ(C) 17.3) [5]. Finally, analogously to model compound **6** [5], the relative configuration of the stereogenic centers (C(7) and C(8)) was established as (7*R**,8*R**), the same as that of **6**, by NOE experiments with **1** (*Fig. 3*).

Sarcophytonolide B (**2**) was isolated as a colorless oil. Its HR-EI-MS suggested a molecular formula $C_{22}H_{32}O_5$. Analysis of its spectral data revealed a close structural relationship with compound **1**. In fact, the main difference appeared in the ^{13}C-NMR spectrum (*Table 1*) with the absence of 1 Me group and the presence of an additional

Table 1. *¹H- and ¹³C-NMR Data* ($CDCl_3$) *for Compounds* **1** *and* **2**[a]). Trivial numbering.

	1		**2**	
	δ(H)	δ(C)[b])	δ(H)	δ(C)[b])
C(1)	–	157.7 (*s*)	–	157.1 (*s*)
H−C(2)	7.01 (*d*, *J* = 11.7)	120.0 (*d*)	6.89 (*d*, *J* = 11.5)	120.2 (*d*)
H−C(3)	6.67 (*d*, *J* = 11.7)	138.0 (*d*)	6.83 (*d*, *J* = 11.5)	138.2 (*d*)
C(4)	–	125.3 (*s*)	–	125.9 (*s*)
CH_2(5)	2.50 (*m*)	31.4 (*t*)	2.88, 2.15 (2*m*)	32.8 (*t*)
CH_2(6)	1.86, 1.75 (2*m*)	26.6 (*t*)	2.16, 1.38 (2*m*)	26.4 (*t*)
H−C(7)	2.79 (*dd*, *J* = 7.4, 4.9)	60.4 (*d*)	2.78 (*dd*, *J* = 10.5, 1.8)	62.2 (*d*)
C(8)	–	60.6 (*s*)	–	61.3 (*s*)
CH_2(9)	1.82, 1.70 (2*m*)	36.4 (*t*)	2.18, 0.96 (2*m*)	39.0 (*t*)
CH_2(10)	1.96 (*m*)	22.0 (*t*)	2.27 (*m*)	26.6 (*t*)
H−C(11)	5.03 (*t*, *J* = 6.7)	127.3 (*d*)	6.93 (*dd*, *J* = 9.4, 7.3)	141.3 (*d*)
C(12)	–	134.0 (*s*)	–	133.1 (*s*)
CH_2(13)	2.12 (2*m*)	38.3 (*t*)	2.46, 2.09 (2*m*)	28.8 (*t*)
CH_2(14)	2.42, 2.30 (2*m*)	28.4 (*t*)	2.32, 2.22 (2*m*)	29.3 (*t*)
CH_2(15)	2.39 (*m*)	34.8 (*d*)	2.43 (*m*)	37.1 (*d*)
Me(16)	1.08 (*d*, *J* = 6.7)	22.2 (*q*)	1.09 (*d*, *J* = 6.8)	21.5 (*q*)
Me(17)	1.09 (*d*, *J* = 6.7)	22.0 (*q*)	1.11 (*d*, *J* = 6.8)	21.4 (*q*)
C(18)	–	168.0 (*s*)	–	167.5 (*s*)
Me(19)	1.21 (*s*)	18.4 (*q*)	1.29 (*s*)	15.3 (*q*)
Me(20) or C(20)	1.62 (*s*)	17.3 (*q*)	–	167.8 (*s*)
COOMe(21)	3.75 (*s*)	51.2 (*q*)	3.79 (*s*)	51.4 (*q*)
COOMe(22)	–	–	3.75 (*s*)	51.7 (*q*)

[a]) *Bruker DRX-400-MHz* spectrometer, chemical shifts in ppm referred to $CDCl_3$ (δ(H) 7.26), *J* in Hz. [b]) By DEPT sequence.

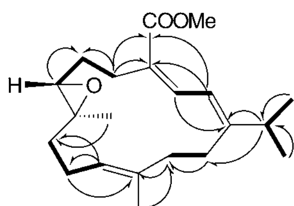

Fig. 2. *¹H,¹H-COSY Correlations* (bold lines) *and selected key HMBC correlations* (arrows) *in* **1**

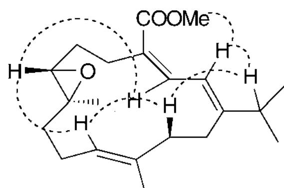

Fig. 3. Selected NOESY correlations in **1**

α,β-unsaturated methyl ester moiety (δ 167.5 and 51.7), in agreement with a molecular-mass increase of 44 for **2**. ¹H,¹H-COSY, HMQC, and HMBC experiments allowed the unambiguous assignment of the structure of **2**. Especially, HMBC correlations of the olefinic proton at δ 6.93 with C(12) (δ 133.1) and C(20) (δ 167.8) established the

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *for Compounds* **3** *and* **4**[a]). Trivial numbering.

	3		4	
	δ(H)	δ(C)[b])	δ(H)	δ(C)[b])
H−C(1)	1.46 (*m*)	45.8 (*d*)	1.52 (*d J* = 9.6)	50.9 (*d*)
H−C(2)	4.81 (*dd*, *J* = 9.6, 1.0)	83.7 (*d*)	5.03 (*d*, *J* = 9.6)	81.2 (*d*)
H−C(3)	7.26 (*s*)	151.3 (*d*)	7.58 (*s*)	152.0 (*d*)
C(4)	–	127.4 (*s*)	–	128.8 (*s*)
CH_2(5)	3.50, 3.16 (each *d*, *J* = 15.7)	40.3 (*t*)	3.58, 3.09 (each *d*, *J* = 13.5)	40.2 (*t*)
C(6)	–	205.9 (*s*)	–	196.1 (*s*)
CH_2(7) or H−C(7)	2.37 (*m*)	2.37 (*m*)	50.0 (*t*)	6.01 (*s*)
125.2 (*d*)				
H−C(8)	1.74 (*m*)	28.6 (*d*)	–	158.8 (*s*)
CH_2(9)	1.38, 1.36 (2*m*)	35.7 (*t*)	3.35, 2.16 (2*m*)	31.2 (*t*)
CH_2(10)	2.12, 1.95 (2*m*)	24.3 (*t*)	2.29, 2.18 (1*m*)	24.8 (*t*)
H−C(11)	4.97 (*t*, *J* = 7.1)	127.1 (*d*)	4.79 (*t*, *J* = 5.5)	124.5 (*d*)
C(12)	–	133.9 (*s*)	–	132.2 (*s*)
CH_2(13)	2.10, 1.93 (2*m*)	38.7 (*t*)	2.26, 2.11 (2*m*)	41.2 (*t*)
CH_2(14) or H−C(14)	1.58, 1.14 (2*m*)	23.2 (*t*)	5.07 (*m*)	71.8 (*d*)
H−C(15)	2.08 (*m*)	29.5 (*d*)	2.14 (*m*)	25.9 (*d*)
Me(16)	0.99 (*d*, *J* = 6.8)	18.4 (*q*)	1.10 (*d*, *J* = 7.0)	18.9 (*q*)
Me(17)	1.00 (*d*, *J* = 6.8)	19.9 (*q*)	1.08 (*d*, *J* = 7.0)	24.8 (*q*)
C(18)	–	172.8 (*s*)	–	172.7 (*s*)
Me(19)	0.93 (*d*)	20.7 (*q*)	1.76 (*s*)	23.1 (*q*)
Me(20)	1.60 (*s*)	16.1 (*q*)	1.57 (*s*)	18.0 (*q*)
AcO	–	–	2.06 (*s*)	170.8 (*s*), 21.1 (*q*)

[a]) *Bruker-DRX-400-MHz* spectrometer; chemical shifts in ppm referred to $CDCl_3$ (δ(H) 7.26), *J* in Hz. [b]) By DEPT sequence.

position of this second COOMe moiety at C(12). Thus, sarcophytonolide B (**2**) is a 12-(methoxycarbonyl) analog of **1**.

Sarcophytonolide C (**3**) was assigned the molecular composition $C_{20}H_{30}O_3$ by HR-EI-MS (M^+ at *m/z* 318.2194) and its ^{13}C-NMR spectrum. The spectral data (see also *Table 2*) and their comparison with those of the known cembranoid **7**, of which the structure was determined by X-ray-diffraction analysis [6], established the structure of **3**. The configuration at C(8) could not be determined.

The presence of two trisubstituted C=C bonds (δ(C) 152.0 (*d*), 128.8 (*s*), 124.5 (*d*), and 132.2 (*s*)) and two C=O groups (δ(C) 196.1 and 172.7), accounting for four of the six degrees of unsaturation, suggested **3** to be bicyclic. The 1D-NMR data and their comparison with those of the known cembranoid **7**, suggested the presence of an α,β-unsaturated γ-lactone (as in **7**), a keto group, a Me-bearing trisubstituted C=C bond, and an ^{i}Pr group. Analysis of the ^{1}H,^{1}H-COSY plot readily allowed to recognize five spin systems (H−C(1) to H−C(3), H−C(1) to H−C(13), H−C(1) to H−C(15), C(16), C(17), CH_2(5) (*AB*-type), and H−C(7) to H−C(11)). In the HMBC experiment of **3**, the position of ^{i}Pr at C(1) was confirmed by the long-range correlations H−C(1)/C(2), C(3), C(4), C(13), C(14), C(15), C(16), and C(17), Me−C(16)/C(1), and Me−C(17)/C(1). The position of the α,β-unsaturated γ-lactone at C(4) (α), C(3) (β), C(2) (γ), and C(18) (C=O) was deduced from the HMBC correlations H−C(2)/C(1), C(3), C(4), and C(15), and H−C(3)/C(2), C(4), C(5), and C(18). The keto group at C(6) was confirmed by HMBC correlations CH_2(5)/C(6), C(4), C(3), and C(7). The Me group at C(12) was revealed by the HMBC correlations Me−C(20)/C(11), C(12), and C(13), and H−C(11)/C(10), C(13), and C(20). The remaining Me group was placed at C(8), mainly based on the biogenetic consideration and supported by the HMBC correlations H−C(8)/C(7) and C(6). Finally the configuration of the two C=C bonds was suggested to be the same as in **1** on the basis of the NOESY experiment. The relative configuration at C(1)

and C(2) of **3** was elucidated by NOE difference experiments. Irradiation of H–C(2) resulted in enhancement of H–C(15) implying that H–C(1) and H–C(2) are positioned on opposite sides of the ring.

Sarcophytonolide D (**4**), isolated as a UV-absorbing oil (λ_{max} 227 nm), showed IR and NMR data similar to those of **3**. Careful comparison of their ^{1}H- and ^{13}C-NMR data (*Table 2*) revealed that **4** differs from **3** by the presence of an additional trisubstituted C=C and an AcO group. NOE Data confirmed the proposed structure.

In the ^{1}H-NMR spectrum of **4**, a low-field *s* at δ 6.01 suggested that the additional C=C bond was located at C(7), in conjugation with the keto group at C(6). This assignment was confirmed by the diagnostic HMBC correlations H–C(7)/C(6) (δ 196.1), C(8) (δ 158.8), Me(19) (δ 23.1), and C(5) (δ 40.2). The AcO group was placed at C(14), as suggested by the δ(C) of both C(1) and C(13) which were shifted downfield with respect to those of **3** (*Table 2*); this was confirmed by the diagnostic long-range correlations H–C(14) (5.07)/C(12), C(1), C(2), and C(13). Analogously to **3**, the relative configuration of H–C(1), [H–C(2)], and H–C(14) was elucidated by the NOE correlations. The NOEs H–C(1) suggested that both H–C(1) and H–C(14) were α-oriented. Once again, the configuration at C(7)=C(8) and C(11)=C(12) was inferred to be (*Z*) and (*E*), respectively, by either the ^{13}C-NMR chemical shifts of Me(19) and Me(20) [5] or by the NOE H–C(7)/Me(19).

It should be pointed out that the conformational mobility/flexibility of the 14-membered macrocycle of cembranoids renders the configurational assignments of the stereogenic centers by NOESY or NOE difference experiments somewhat risky. Unambiguous assignments can only be obtained by more advanced techniques, *e.g.*, by X-ray-diffraction analysis.

Compounds **1**–**4** were tested for cytotoxicity against A-549 and HL-60 tumor cell lines. But they are inactive at a concentration of 20 μg/ml. Other bioassays such as antibacterial, and anti-inflammatory tests are currently ongoing.

This research work was financially supported by the *National Marine 863 Project* (No. 2003AA624030), *National Natural Science Foundation for Outstanding Chinese Youths* (No. 30125044), and partly founded by *State Key Program of Basic Research of China* (No. 2004CB518905), and *Program of Basic Research of Shanghai Science and Technology Committee* (No. 04ZR14156).

Experimental Part

General. Column chromatography (CC): commercial silica gel (*Qing Dao Hai Yang Chemical Group Co.*; 200–300 and 400–600 mesh). TLC: precoated silica gel plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60 F-254*). Reversed-phase HPLC: *Agilent 1100* instrument for liquid chromatography, with a *VWD-G1314A* detector at 210 nm; semi-prep. *ODS-HG-5* column (5 μm, 10 mm (i.d.) × 25 cm) for purification. Optical rotations: *Perkin-Elmer 241MC* polarimeter. UV Spectra: *Varian Cary-300-Bio* spectrophotometer. IR Spectra: *Nicolet Magna-FT-IR-750* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-400* spectrometer; δ in ppm with residual $CDCl_3$ (δ(H) 7.26; δ(C) 77.0) as internal standard, *J* in Hz. MS: *Finnigan MAT-95* mass instrument; in *m*/*z*.

Biological Material. The specimens of the *Sarcophyton* sp., identified by Prof. *R.-L. Zhou* of the South China Sea Institute of Oceanology, Chinese Academy of Sciences, were collected along the cost of Ximao island, Hainan Province, China, in December 2002, at a depth of −20 m and were frozen immediately after collection. A voucher specimen is available for inspection at the Institute of Materia Medica, SIBS-CAS (No. LS163).

Extraction and Isolation. The frozen animals (257 g, dry weight) were cut into pieces and extracted exhaustively with acetone at r.t. (3 × 1.5 l). The org. extract was evaporated and the residue partitioned between Et_2O and H_2O. The Et_2O soln. was evaporated to give a dark brown residue (5.3 g), which was fractionated by CC (silica gel, 0 → 100% acetone/light petroleum ether): 3 fractions with R_f 0.5, 0.45, and 0.15 on TLC (petroleum ether/AcOEt 2:1; blue spots after spraying with H_2SO_4). The 3 fractions were further purified CC (*Sephadex LH-20*, petroleum ether/$CHCl_3$/MeOH 2:1:1) followed by CC (silica gel): pure **1** (10.2 mg), **2**

(9.1 mg), and **3**/**4**. The latter was separated by reversed-phase HPLC (semi-prep. *ODS-HG-5*, MeCN/H_2O 75 : 25, 2.0 ml/min): pure **3** (5.9 mg) and **4** (4.3 mg).

Sarcophytonolide A (*(1*R**,4*Z*,6*E*,10*E*,14*R**)-10,14-Dimethyl-7-(1-methylethyl)-15-oxabicydo[12.1.0]pentadeca-4,6,10-triene-4-carboxylic Acid Methyl Ester*; **1**): Colorless oil. $[\alpha]_D^{20} = 1.8$ ($c = 0.57$, $CHCl_3$). UV (MeOH): 283 (3.15). IR (KBr): 2958, 1710, 1627. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-EI-MS: 332.2356 ($C_{21}H_{32}O_3^+$; calc. 332.2352).

Sarcophytonolide B (*(1*R**,4*Z*,6*E*,10*Z*,14*R**)-14-Methyl-7-(1-methylethyl)-15-oxabicyclo[12.1.0]pentadeca-4,6,10-triene-4,10-dicarboxylic Acid Dimethyl Ester*; **2**): Colorless oil. $[\alpha]_D^{20} = 118.0$ ($c = 1.65$, $CHCl_3$). UV (MeOH): 287 (3.35), 217 (3.48). IR (KBr): 2956, 1713, 1622. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-EI-MS: 376.2278 ($C_{22}H_{32}O_5^+$; calc. 376.2250).

Sarcophytonolide C (*(8*E*,12*R**,13*R**)-5,9-Dimethyl-12-(1-methylethyl)-14-oxabicyclo[11.2.1]hexadeca-1(16),8-diene-3,15-dione*; **3**): Colorless oil. $[\alpha]_D^{20} = 31.0$ ($c = 0.20$, $CHCl_3$). UV (MeOH): 227 (2.13). IR (KBr): 2920, 1738, 1280. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-EI-MS: 318.2194 ($C_{20}H_{30}O_3^+$; calc. 318.2195).

Sarcophytonolide D (*(4*Z*,8*E*,11*R**,12*R**,13*R**)-11-(Acetyloxy)-5,9-dimethyl-12-(1-methylethyl)-14-oxabicyclo[11.2.1]hexadeca-1(16),4,8-triene-3,15-dione*; **4**): Pale viscous oil. $[\alpha]_D^{20} = -17.0$ ($c = 0.17$, $CHCl_3$). UV (MeOH): 231 (2.25). IR (KBr): 2922, 1761, 1689, 1622. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-EI-MS: 374.2091 ($C_{22}H_{30}O_5^+$; calc. 374.2094).

REFERENCES

[1] J. C. C. Coll, *Chem. Rev.* **1992**, *92*, 613.
[2] A. S. R. Anjaneyulu, R. G. Venkateswara, *J. Indian Chem. Soc.* **1997**, *74*, 272.
[3] W. Zhang, Y.-W. Guo, E. Mollo, G. Cimino, *Helv. Chim. Acta* **2004**, *87*, 2341; W. Zhang, Y.-W. Guo, E. Mollo, G. Cimino, *Helv. Chim. Acta* **2004**, *87*, 2919; Z.-Y. Shao, D.-Y. Zhu, Y.-W. Guo, *J. Nat. Prod.* **2002**, *65*, 1675.
[4] J. A. Toth, B. J. Burreson, P. J. Scheuer, *Tetrahedron* **1980**, *36*, 1307.
[5] T. Iwagawa, R. Nakashima, K. Takayama, H. Okamura, M. Nakatani, M. Doe, K. Shibata, *J. Nat. Prod.* **1999**, *62*, 1046.
[6] C.-Y. Duh, S.-K. Weng, M.-Y. Chiang, C.-F. Dai, *J. Nat. Prod.* **1999**, *62*, 1518.

Received January 31, 2005