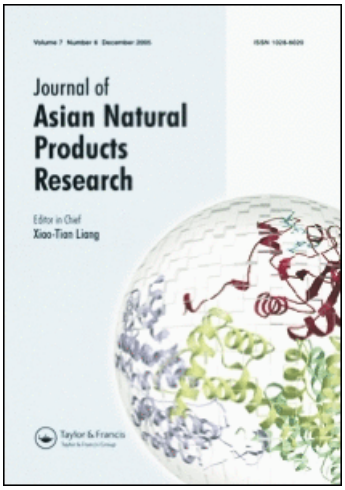


**Journal of Asian Natural Products Research**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

# Manoalide derivatives from a sponge, *Luffariella* sp.

Guang-Xiong Zhou[a]; Tadeusz F. Molinski[b]
[a] College of Pharmacy, Jinan University, Guangzhou, China [b] Department of Chemistry, University of California, Davis, CA, USA

**To cite this Article** Zhou, Guang-Xiong and Molinski, Tadeusz F.(2006) 'Manoalide derivatives from a sponge, *Luffariella* sp.', Journal of Asian Natural Products Research, 8: 1, 15 — 20
**To link to this Article: DOI:** 10.1080/10286020500246022
**URL:** http://dx.doi.org/10.1080/10286020500246022

*Journal of Asian Natural Products Research*,
Vol. 8, No. 1–2, January–March 2006, 15–20

Taylor & Francis
Taylor & Francis Group

# Manoalide derivatives from a sponge, *Luffariella* sp.

GUANG-XIONG ZHOU†* and TADEUSZ F. MOLINSKI‡

†College of Pharmacy, Jinan University, Guangzhou 510632, China
‡Department of Chemistry, University of California, Davis, CA 95616, USA

*(Received 28 September 2004; revised 14 December 2004; in final form 28 May 2005)*



A new derivative of manoalide, 24-n-propyl-O-manoalide (**1**) together with manoalide (**4**) and four known derivatives (**2**, **3**, **5**, **6**) were isolated from the methanolic extract of a sponge. Their structures were elucidated on the basis of spectroscopic method. All the compounds showed significant cytotoxicity against HCT-116 cell line by MTS assay. Secomanoalide indicated antifungal activity on fungal lines *Candida glabrata, Candida krusei* and *Candida albicans* by *in vitro* antibiotic assay.

*Keywords*: Sponge; 24-β-n-Propyl-*O*-manoalide; Manoalide; Cytotoxicity; Antifungal

## 1. Introduction

Manoalide, first reported in 1980 from the marine sponge *Luffariella variabilis* in Palau, West Caroline [1], showed significant activity *in vitro* against *Streptomyces pyogenes* and *Staphylococcus aureus*, cytotoxicity, anti-inflammatory activity and irreversible inhibition activity to the enzyme phospholipase $A_2$ [1,2]. A series of isomers or derivatives of manoalide have been isolated from the same sponge species or the sponges of the same genus. Manoalide and its isomer secomanoalide possess the same trimethylcyclohexenyl and γ-hydroxybutenolide moieties and antibiotic activity [3]. Two other isomers, luffariellins A and B, have the same γ-hydroxybutenolide group as manoalide but a different structure in the other parts, with same anti-inflammatory activity [4]. Luffariolide J and its 24-methyl-*O*-derivative luffariolide H, having the same γ-hydroxybutenolide moiety as manoalide and with a double bond-moved trimethylcyclohexenyl group, showed similar antibiotic activity [5]. *E*- and *Z*-neo-manoalides, possessing the same trimethylcyclohexenyl and γ-hydroxybutenolide groups with a changed attachment position of the long-chain part and an additional hydroxymethyl group, also showed antibiotic activity and cytotoxicity [3]. The dehydroxyl or 24-methyl-*O*-derivatives of manoalide have been found from sponge or by chemical conversion [3,6]. In our search of marine natural products from sponges with antifungal and cytotoxic activity, the methanolic extract of a dark brown sponge (*Luffariella*

*Corresponding author. E-mail: guangxzh@sina.com

*Journal of Asian Natural Products Research*
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500246022

sp.) from the West Atlantic region showed positive results for antifungal and cytotoxic activities. The investigation of the active fractions from solvent/solvent partition and subsequent chromatography on silica gel column and reversed-phase HPLC resulted in the isolation of 24-β-n-propyl-*O*-manoalide, a new cytotoxic derivative of manoalide (**1**), together with manoalide (**4**) and four other known derivatives: 4*E*,6*E*-dehydromanoalide (**2**), secomanoalide (**3**), 24-β-methyl-*O*-manoalide (**5**) and 24-α-methyl-*O*-manoalide (**6**). This paper describes the isolation and characterization of these compounds as well as their cytotoxic and antifungal activities.

## 2. Results and discussion

Compound **1** was colourless gum. HRFAB-MS exhibited a quasi-molecular ion peak at *m/z* 481.2975 ($[M + Na]^+$) corresponding to the molecular formula $C_{28}H_{40}O_4$. The molecular formula was further supported by the peaks at *m/z* 481.3 ($[M + Na]^+$) and 939.3 ($[2M + Na]^+$) in the ESI-MS spectrum. $^{13}C$ NMR and DEPT spectra gave four $CH_3$, ten $CH_2$, five CH and seven quaternary carbon signals. HSQC spectra showed the signal at $\delta$ 28.6 representing two methyl carbons. The carbon signal at $\delta$ 97.3 was correlated with two methine protons at $\delta$ 4.91 (s) and 6.22 (s), suggesting the presences of two acetal carbons. $^1H$ NMR and $^{13}C$ NMR spectra of **1** revealed the presence of three trisubstituted double bonds [$\delta_C$ ($\delta_H$) 118.8 (6.10)/167.0, 120.5 (5.68)/137.1, 122.9 (5.13)/137.0] and one tetrasubstituted double bond (136.8/127.0). Extensive analyses of $^1H-^1H$ COSY and HSQC spectra of **1** showed the existence of $-OCH_2CH_2CH_3$, $-CH_2CH_2CH_2-$, $-CH_2CH_2-$, $=CHCH_2CH_2-$ and $=CHCH_2CH=$ fragments in the molecule. The γ-hydroxybutenolide moiety was secured by UV absorption at 235 nm, IR band at 1764.5 $cm^{-1}$ and NMR data at $\delta_C/\delta_H$ 170.5/–, 118.8/6.10, 167.0/–, 62.8/4.83. The attachment of an n-propyl group at C-24 was elucidated on the basis of the comparison of the chemical shift of C-24 ($\delta$ 97.3) in **1** with that of C-24 ($\delta$ 91.7) in manoalide (**4**). Some important HMBC correlations (figure 1) resulted in the connection of the fragments and the final structural determination of **1**. The absolute configuration of C-4 in **1** was considered as *R* because C-4 in natural manoalide had been determined to be *R* by two research groups with different methods [7,8]. The relative configuration between H-4 and H-24 should be *trans* on the basis of the similarity of chemical shifts of H-4, H-5, H-6 and H-24 in **1** with those of 24*R*-methy-*O*-manoalide but not 24*S*-methyl-*O*-manoalide [9]. Thus, C-24 in **1** should be an *R* configuration. The fact that 24*S*-methyl-*O*-manoalide can be converted into the 24*R* isomer in MeOH with DMP and

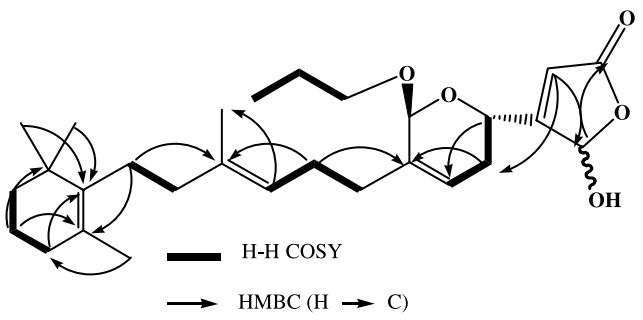


Figure 1. Key $^1H-^1H$ COSY, HMBC correlations of **1**.

*p*-TsOH suggests a 24*R* isomer with a 24-methoxy group on the same side of H-4 on the pyran ring as a more stabile isomer in thermodynamics [9]. However, the orientation of the OH group at C-25 in **1** was not determined as those in manoalide and other derivatives.

Compounds **5** and **6** were identified as a pair of diastereomers at C-24. Their $^1$H NMR spectra showed one more methoxyl signal at $\delta_H$ 3.46 and 3.49, respectively compared with those of manoalide [7]. Compounds **2**–**6** were identified by the comparison of the NMR and MS data with those previously reported in the literatures [1,3,6].

With the consideration of chemical correlation, could **4** be the precursor of **1**, **3**, **5** and **6**, even **2**, as shown in figure 2? In fact, **4** had been converted into **3**, **5** and **6** under some conditions. However, we were not sure whether we had created similar conditions in the process of extraction, partition and separation for the same conversion of **4** into **1**, **3**, **5** and **6**.

All the compounds (**1**–**6**) showed significant cytotoxicity ($IC_{50}$ from 0.32 to 4.26 μg/ml) against HCT-116 cell lines by the MTS method (see table 1). Although the structural changes do not affect activity too much, compound **1** showed the strongest activity ($IC_{50}$ 0.32 μg/ml). Secomanoalide (**3**) showed antifungal activity against *C. glabrata* (MIC 100 μg/ml), *C. krusei* (MIC 30 μg/ml), *C. albicans* ATCC (MIC 30 μg/ml) and *C. albica*ns 96–786 (MIC 30 μg/ml). However, other compounds were inactive at test amount (150 μg/disc).



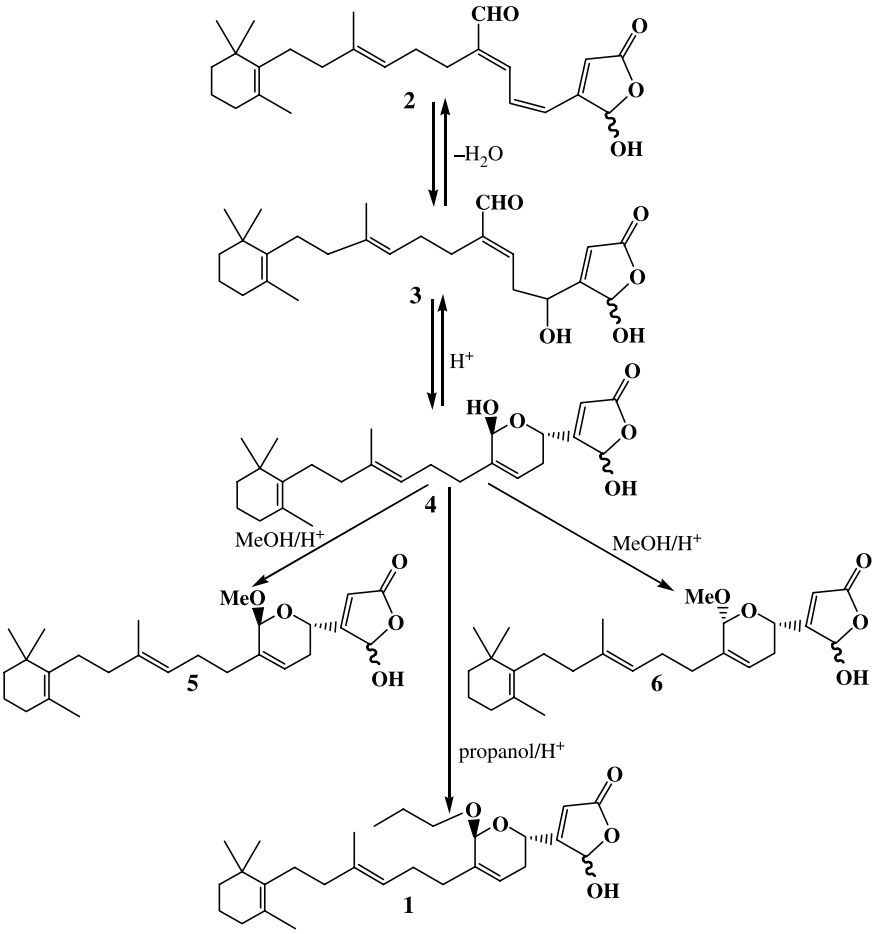


Figure 2. Possible conversion for compounds **1**–**6**.

Table 1. The $IC_{50}$ (μg/ml) for compounds **1**–**6** against HCT-116 cell lines.

| | *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|---|
| $IC_{50}$ | 0.32 | 1.26 | 4.26 | 1.97 | 1.09 | 1.21 |

## 3. Experimental

### *3.1 General experimental procedures*

NMR and $^{13}C$ NMR spectra were obtained using a Varian Inova 400 MHz spectrometer at 400 and 100 MHz, respectively. 2D NMR spectra were also run in the same instrument. Solvents used in extraction or chromatography were HPLC-grade or distilled from glassware system. HRFAB-MS was run on a VG 1FHF double-focusing high-resolution mass spectrometer. ESI-MS data were carried out on a ThermoFinnigan Surveyor LC and LC Deca ion-trap with infusion in MeOH. Antifungal assays were carried out using a modification of a standard microtitre broth dilution assay. Cytotoxicity assays against HCT-116 cell lines were performed using cultured HCT-116 cell lines incubated with MTS.



### *3.2 Animal material*

The sponge *Luffariella* sp. (01-09-049) was collected by hand using scuba gear in Pohnpei (Federated States of Micronesia) in September 2001, and kept frozen until needed. A voucher specimen was preserved at the Department of Chemistry, University of California, Davis, CA, USA.

### *3.3 Assay of biological activities*

**3.3.1 Cytotoxicity assay**. Assay for cytotoxicity against human colon tumour cell line HCT-116 was performed following the general process. Compounds in DMSO were assayed and run against etoposide as positive control. HCT-116 were incubated in 96-well plates for 72 h before addition of MTS. Well absorbances ($\lambda_{490nm}$) were corrected for background and expressed as percentage of the negative control (DMSO only).

**3.3.2 Antifungal assay**. MIC measure of compounds for antifungal activities was performed with a series of diluted solutions of compounds in DMSO. The fungal lines in liquid media were incubated in 16-well plates together with compounds for about 20 h, with DMSO as negative control. The clear wells with minimum compounds were counted for the MIC concentration.

### *3.4 Extraction and isolation*

The sponge was collected and kept frozen until work-up. The lyophilised tissue (108.0 g) was exhaustively extracted with MeOH and the methanol extract was partitioned successively with hexane, $CHCl_3$ and n-butanol after adjustment of the $H_2O$ content at each step. The $CHCl_3$-soluble fraction (6.78 g), which exhibited antifungal activity and cytotoxicity, was

further separated by gradient silica gel chromatography (40–63 μm, EtOAc in hexane, then MeOH in $CH_2Cl_2$). The active fractions (500 mg) were purified by HPLC (Si column EtOAc/hexane and C-18 reversed phase $CH_3CN/H_2O$) to obtain compounds **1** (3.0 mg), **2** (7.0 mg), **3** (35.0 mg)**, 4** (15.5 mg)**, 5** (2.5 mg) and **6** (3.0 mg).

**3.4.1 Compound 1**. Colourless oil**,** IR (film, $CHCl_3$) $\nu_{max}$ 3357.5, 2962.1, 2927.4, 2865.7, 1764.5, 1261.2, 1097.3, 1027.9, 800.3 $cm^{-1}$; UV $\lambda_{max}$ ($CH_3CN$) 235 nm; $^{13}C$ NMR (100 MHz, Acetone-$d_6$) $\delta$ 170.9 (C-1), 161.4 (C-3), 145.5 (C-6), 146.0 (C-7), 138.0 (C-11), 137.2 (C-14), 29.8 (C-5), 127.8 (C-15), 124.5 (C-10), 122.1 (C-2), 98.3 (C-24, 25), 70.3 (C-4), 60.2 (C-26), 41.5 (C-12), 40.9 (C-18), 36.0 (C-19), 33.8 (C-16), 33.7 (C-27), 29.3 (C-13), 29.1 (C-9), 27.3 (C-20, 21), 24.1(C-8), 20.6 (C-17), 20.4 (C-20), 16.5 (C-23), 11.5 (C-28); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 170.5 (C-1), 167.0 (C-3), 137.1 (C-7), 137.0 (C-11), 136.8 (C-14), 127.0 (C-15), 122.9 (C-10), 120.5 (C-6), 118.8 (C-2), 97.3 (C-24), 97.3 (C-25), 70.4 (C-26), 62.7 (C-4), 29.0 (C-5), 40.3 (C-8), 39.8 (C-18), 35.0 (C-19), 32.7 (C-9), 32.6 (C-16), 27.9 (C-13), 26.1 (C-12), 28.6 (C-20, 21), 23.0 (C-27), 19.8 (C-22), 19.5 (C-17), 16.1 (C-23), 10.7 (C-28); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.22 (1H, s, H-25), 6.10 (1H, s, H-2), 4.91 (1H, s, H-24), 4.84 (3H, t, $J = 7.2$ Hz, H-4), 2.27 (2H, brs, H-5), 5.68 (1H, brs, H-6), 2.03 (2H, dt, $J = 13.6$, 4.0 Hz, H-8), 5.13 (1H, t, $J = 6.8$ Hz, H-10), 2.12 (2H, t, $J = 5.6$ Hz, H-12), 3.76 (1H, dt, $J = 9.6$, 6.4 Hz, H-26a), 3.46 (1H, dt, $J = 9.6$, 6.8 Hz, H-26b), 2.15 (2H, t, $J = 6.4$ Hz, H-9), 2.02 (2H, m, H-13), 1.90 (2H, t, $J = 6.0$ Hz, H-16), 1.64 (s, H-22), 1.60 (3H, s, H-23), 1.60 (2H, m, H-27), 1.55 (2H, m, H-17), 1.42 (2H, m, H-18), 0.99 (6H, s, H-20, 21), 0.95 (3H, t, $J = 7.6$ Hz, H-28); HRFAB-MS: *m/z* 481.2975 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_4Na$, 481.2930); ESI-MS: *m/z* 481.3 ($[M + Na]^+$), 939.3 ($[2M + Na]^+$).

**3.4.2 Compound 5**. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.20 (1H, s, H-25), 6.10 (1H, s, H-1), 5.69 (1H, t, $J = 3.6$ Hz, H-6), 5.14 (1H, t, $J = 5.6$ Hz, H-10), 4.81 (1H, s, H-4), 4.79 (1H, s, H-24), 3.46 (3H, s, 24-OMe), 2.28 (1H, s, H-5), 2.11 (4H, m, H-9, 13), 2.03 (4H, d, $J = 4.0$ Hz, H-10, 12), 1.91 (2H, t, $J = 6.0$ Hz, H-16), 1.64 (3H, s, H-22), 1.60 (3H, s, H-23), 1.56 (2H, m, H-17), 1.41 (2H, m, H-18), 0.99 (6H, s, H-20, 21); ESI-MS *m/z* 453.3 ($[M + Na]^+$). The data were consistent with 24*R*-methyl-*O*-manoalide [9].

**3.4.3 Compound 6**. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.18 (1H, s, H-25), 6.10 (1H, s, H-1), 5.74 (1H, t, $J = 3.6$ Hz, H-6), 5.12 (1H, t, $J = 5.6$ Hz, H-10), 5.07 (1H, s, H-24), 4.68 (1H, s, H-4), 3.49 (3H, s, 24-OMe), 2.37 (1H, s, H-5), 2.12 (4H, m, H-9, 13), 2.03 (4H, d, $J = 4.0$ Hz, H-10, 12), 1.91 (2H, t, $J = 6.0$ Hz, H-16), 1.65 (3H, s, H-22), 1.60 (3H, s, H-23), 1.55 (2H, m, H-17), 1.41 (2H, m, H-18), 0.99 (6H, s, H-20, 21); ESI-MS *m/z* 453.3 ($[M + Na]^+$). The data were consistent with 24*S*-methyl-*O*-manoalide [9].

## References

[1] E.D. Silva, P.J. Sheuer. *Tetrahedron Lett*., **21**, 1611 (1980).
[2] R.S. Jacobs, P. Culver, R. Langdon, T. O'Brien. *Tetrahedron*, **41**, 981 (1985).
[3] E.D. Silva, P.J. Sheuer. *Tetrahedron Lett*., **22**, 3147 (1981).
[4] M.R. Kernan, D.J. Faulkner. *J. Org. Chem*., **52**, 3081 (1987).
[5] M. Tsuda, T. Endo, Y. Mikami, J. Fromont, J. Kobayashi. *J. Nat. Prod*., **65**, 1507 (2002).

[6] B.C.M. Potts, R.J. Capon, D.J. Faulkner. *J. Org. Chem*., **57**, 2965 (1992).
[7] V.E. Amoo, S. De Bermardo, M. Weigele. *Tetrahedron Lett*., **29**, 2401 (1988).
[8] M. Kobayashi, T. Okamoto, K. Hayashi, N. Yokoyama, T. Sasaki, I. Kitagawa. *Chem. Pharm. Bull*., **42**, 265 (1994).
[9] M. Namikoshi, S. Suzuki, S. Meguro, H. Nagai, Y. Koike, A. Kitazawa, H. Kobayashi, T. Oda, T. Yamada. *Fisheries Sci*., **70**, 152 (2004).