24-O-ethylmanoalide, a manoalide-related sesterterpene from the marine sponge Luffariella cf. variabilis

Anne Gauvin-Bialecki, Maurice Aknin, Jacqueline Smadja

Molecules **2008**, *13*, 3184-3191; DOI: 10.3390/molecules13123184

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

24-*O*-Ethylmanoalide, a Manoalide-related Sesterterpene from the Marine sponge *Luffariella* cf. *variabilis*

Anne Gauvin-Bialecki *, Maurice Aknin and Jacqueline Smadja

Université de la Réunion, Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, 97 715, Saint-Denis, La Réunion, France

* Author to whom correspondence should be addressed; E-mail: anne.bialecki@univ-reunion.fr; Tel: +262 262 93 81 97; Fax: +262 262 93 81 83.

Received: 4 November 2008; in revised form: 5 December 2008 / Accepted: 11 December 2008 / Published: 15 December 2008

Abstract: A new manoalide-related sesterterpene, 24-*O*-ethylmanoalide (**3**), was isolated from the Indian Ocean sponge *Luffariella* cf. *variabilis*, together with the known compounds manoalide (**1**), seco-manoalide, manoalide monoacetate and 24-*O*-methyl-manoalide (**2**). The structure of compound **3** was elucidated by interpretation of its spectroscopic data.

Keywords: *Luffariella* cf. *variabilis*; Demospongiae, Manoalide-related sesterterpene, 24-*O*-ethylmanoalide.

Introduction

Marine sponges of the family Thorectidae (e.g. *Luffariella* [1-15], *Hyrtios* [16, 17], *Thorectandra* [18], *Fasciospongia* [19-23], and *Aplynopsis* [24]) are known to be a rich source of novel bioactive sesterterpenoids. Some of them containing a γ-hydroxybutenolide moiety showed a strong anti-inflammatory activity. Manoalide (**1**), for example, the first sesterterpene to be reported from the Palauan sponge *Luffariella variabilis* by De Silva and Scheuer [1], has been extensively investigated as a potent inhibitor of phospholipase A_2 (PLA_2) [25-33]. Subsequently, many related metabolites with PLA_2 inhibitory activity were reported [4, 25, 34-39]. In the course of our search for biologically

active compounds from Indian Ocean marine organisms, our chemical investigation of a sponge from Mayotte Island belonging to the genus *Luffariella*, yielded manoalide (**1**) together with the known seco-manoalide [2], manoalide monoacetate [18], and 24-*O*-methylmanoalide (**2**) [13], as well as a new constituent which we have named 24-*O*-ethylmanoalide (**3**). In this paper, we describe the isolation and structure determination of compound **3**.

Results and Discussion

The MeOH-$CHCl_3$ extract of *Luffariella* cf. *variabilis* was subjected to solvent partitioning, as outlined in the Experimental section. The hexane fraction was repeatedly fractionated by silica gel column chromatography, followed by normal phase HPLC to afford manoalide monoacetate, 24-*O*-methylmanoalide (**2**) and 24-*O*-ethylmanoalide (**3**). The CCl_4 and $CHCl_3$ fractions were combined and chromatographed on a silica gel column to furnish manoalide (**1**) and seco-manoalide. The latter was further purified by normal phase HPLC. The known compounds manoalide (**1**), seco-manoalide, manoalide monoacetate and 24-*O*-methylmanoalide (**2**) were identified through comparison of their physical data (NMR and EIMS) with published information [1-3, 13, 16, 18].

Compound **3** was obtained as a colorless glass. The IR spectrum contained three bands at 3410, 1790 and 1762 cm^{-1}, typical of a γ-hydroxybutenolide moiety, and a band at 1098 cm^{-1} supporting the presence of an ether group. The EIMS showed a molecular peak at *m/z* 444. This datum together with its ^{1}H- and ^{13}C-NMR spectra (Table 1) suggested the molecular formula $C_{27}H_{40}O_5$. The mass spectrum showed an intense peak at *m/z* 137 and fragments ions at *m/z* 121, 107 and 95 derived from the *m/z* 137, implying the presence of the alkylated cyclohexenyl end group $C_{10}H_{17}$ commonly generated by manoalide-related sesterterpenes [18]. The ^{1}H- and ^{13}C-NMR of **3** were almost identical with those of manoalide (**1**). However, they showed the characteristic signals of an additional ethoxy group [δ_H 3.55, 3.83 (2H, m, H-26), δ_H 1.23, 1.24 (3H, t, J = 7.0 Hz, H-27), δ_C 64.0, 64.3 (C-26), and δ_C 15.3, 15.4 (C-27)]. The ether linkage between C-24 and C-26 was suggested by the ^{13}C-NMR chemical shift of C-24 which resonated at a lower field (δ_C 97.1, 97.2) than the C-24 of (**1**) bearing an hydroxyl group (δ_C 91.2, 91.5). These data suggested structure **3** for 24-*O*-ethylmanoalide (Figure 1). Besides, pairs of two signals due to the same carbons or protons were detected in the ^{1}H- and ^{13}C-NMR spectra of **3** as similar to the signals of manoalide [16], which are ascribable to a mixture of stereoisomers. Compound **3** includes three asymmetric carbon atoms; C-4, C-24 and C-25. The axial nature of C-4 i.e. its *R*-configuration, was deduced from its coupling constants to the C-5 protons (10.5, 3.4 Hz) [1]. C-24 in **3** was also presumed to be an *R*-configuration. Indeed, the relative configuration between H-4 and H-24 was established to be *trans* on the basis of the similarity of chemical shifts of H-4, H-5, H-6 and H-24 in **3** with those of 24*R*-*O*-methylmanoalide and not 24*S*-*O*-methylmanoalide [13]. Therefore it was deduced that **3** is a mixture of C-25 epimers with *R*-configuration at C-4 and C-24.

It is interesting to note that compounds **2** and **3** may be suspected to be artifacts due to experimental procedure. Manoalide is indeed a hemiacetal and its extraction under some particular conditions - as shown in Figure 1 - would be expected to produce compounds **2** and **3**. If the conversion of **1** into **2** may be explained by the use of MeOH in the process of extraction [13], however the conversion of **1** into **3** requiring the use of EtOH/H^+ remains unexplained. In the same way, in a previous report by

Zhou and Molinski [14], manoalide (**1**) was presumed to be precursor of 24-*O*-propylmanoalide (**4**) (Figure 1), a manoalide derivative isolated from the Palauan sponge *Luffariella variabilis*.

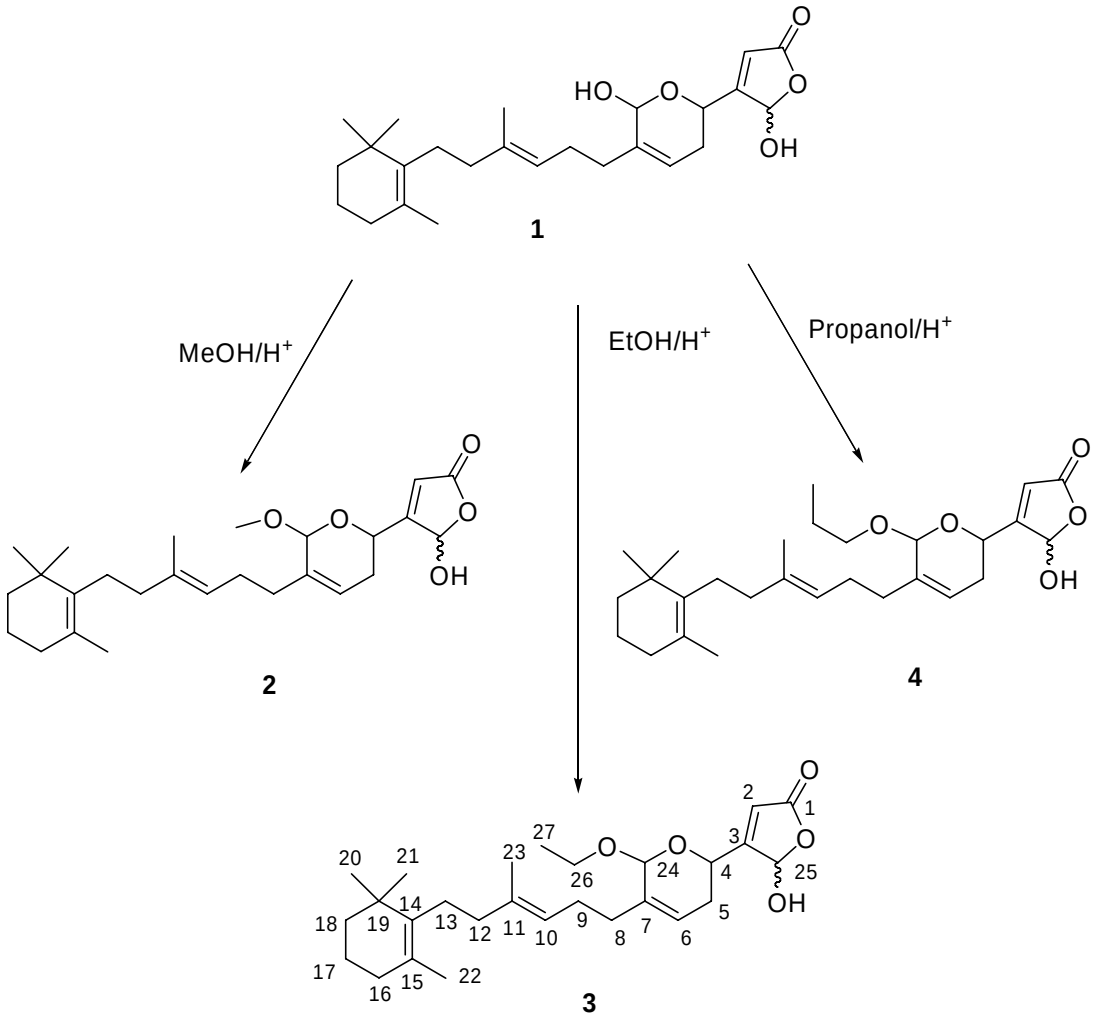
Table 1. NMR Spectroscopic Data ($CDCl_3$) for 24-*O*-ethylmanoalide (**3**)[a].

position	δ_C	δ_H (*J*, Hz)
1	170.3, 170.4	
2	117.5, 118.4	6.02, 6.19 s
3	167.4, 167.7	
4	62.3, 63.2	4.78, 4.86 dd (3.4, 10.5)
5	28.8, 29.1	2.20 m
6	120.6, 120.8	5.66 m
7	136.8, 137.1	
8	32.7	2.10 m
9	26.1	2.10 m
10	122.9	5.12 t (6.1)
11	137.1	
12	40.3	2.00 m
13	27.9	2.00 m
14	136.9	
15	127.1	
16	32.8	1.88 t (6.2)
17	19.6	1.53 m
18	39.9	1.39 m
19	35.0	
20	28.7	0.97 s
21	28.7	0.97 s
22	19.9	1.58 s
23	16.1	1.62 s
24	97.1, 97.2	4.89, 4.92 s
25	97.1, 97.7	6.09, 6.23 s
26	64.0, 64.3	3.55, 3.83 m
27	15.3, 15.4	1.23, 1.24 t (7.0)

[a] Measured at 400 MHz (^1H) and 100 MHz (^{13}C).

However, according to the authors, the conditions of the process of extraction, partition and separation applied could not justify the conversion of **1** into **4**. Thus, on the basis of the above results, we suggest that 24-*O*-ethylmanoalide (**3**) and 24-*O*-propylmanoalide (**4**) be considered as "true" metabolites produced by a biosynthetic pathway, rather than artifacts arising from the isolation procedure.

Figure 1. Possible chemical conversion of **1** into **2**, **3** and **4**.



Experimental

General

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were determined on a Perkin-Elmer 1600 FT-IR spectrometer. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded on a Brucker AMX-400, in $CDCl_3$, with TMS as internal standard. Chemical shifts were reported in ppm and coupling constants (*J*) were reported in Hz. EI mass spectra were obtained on a Jeol AX-500 mass spectrometer. HPLC was performed on a Spectraseries P100 equipped with a differential refractometer (Thermoseparation products – Refractomonitor). A Merck Lichrospher Si-60 column (25 cm × 10 mm i.d.) was used.

Animal material

The sponge *Luffariella* cf. *variabilis* (order Dictyoceratida, family Thorectidae) collected off Mayotte Island (Indian Ocean), in November 1995, was kept frozen until used. The material was

identified by Dr N. Boury-Esnault (Station Marine d'Endoume – Marseille – France) and Pr P. Bergquist (School of Biological Sciences – Auckland – New Zealand). A voucher sample AGL-2-97M, has been deposited at the Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (University of Reunion Island – France).

Extraction and Isolation

Frozen sponge tissue (1,343 g dry weight after extraction) was cut up and homogenized in a Waring-blender in MeOH/$CHCl_3$ (1:2). After filtration, the solvent was removed under reduced pressure to give the crude material (33.4 g), which was successively partitioned between equal volumes of aqueous MeOH, percentage adjusted to produced a biphasic solution, and a solvent series of *n*-hexane (yield 5.71 g), CCl_4 (yield 11.95 g) and $CHCl_3$ (yield 7.44 g). The remaining H_2O soluble were extracted but did not contain any compounds of interest. A portion of the *n*-hexane fraction (2.98 g) was repeatedly subjected to silica gel columns using eluents of increasing polarity from 5% EtOAc in *n*-hexane to 10% EtOAc in *n*-hexane, to afford a mixture of manoalide monoacetate, 24-*O*-methyl-manaolide (**2**) and 24-*O*-ethylmanaolide (**3**). The resulting material was purified by semi-preparative HPLC over normal phase silica with hexane/EtOAc (7.5:2.5) to yield pure manoalide monoacetate (18 mg, 0.0026%, dry wt), **2** (13 mg, 0.0019%) and **3** (19 mg, 0.0027%). CCl_4 and $CHCl_3$ solubles were combined on the basis of TLC, and a 4.38 g portion was fractionated by silica gel column chromatography eluted with *n*-hexane/EtOAc using a step gradient of increasing EtOAc (9:1 to 7:3) to afford pure manaolide (**1**) (99 mg, 0.033%) and impure seco-manoalide. Final purification *via* HPLC using Si gel column with *n*-hexane/EtOAc (2:3) gave pure seco-manoalide (76 mg, 0.025%).

24-O-ethylmanoalide (**3**): colourless glass; $[\alpha]^{25}_D$ + 63° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3410, 2925, 1790, 1762, 1098, 1040 cm^{-1}; ^1H- and ^{13}C-NMR, see Table 1; EI mass spectrum *m/z* 444 $[M]^+$ (22), 426 (3), 398 (9), 380 (2), 261 (5), 203 (4), 177 (6), 137 (100), 123 (12), 121 (12), 107 (9), 95 (26), 81 (22).

Acknowledgements

This research was supported by the Regional Council of Reunion Island.

References

1. De Silva, E.D.; Scheuer, P.J. Manoalide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis* (Polejaeff). *Tetrahedron Lett.* **1980**, *21*, 1611-1614.
2. De Silva, E.D.; Scheuer, P.J. Three new sesterterpenoid antibiotics from the marine sponge *Luffariella variabilis* (Polejaeff). *Tetrahedron Lett.* **1981**, *22*, 3147-3150.
3. Kernan, M.R.; Faulkner, D.J.; Jacobs, R.S. The luffariellins, novel anti-inflammatory sesterterpenes of chemotaxonomic importance from the marine sponge *Luffariella variabils*. *J. Org. Chem.* **1987**, *52*, 3081-3083.

4. Albizati, K.F.; Holman, T.; Faulkner, D.J.; Glaser, K.B.; Jacobs, R.S. Luffariellolide, an anti-inflammatory sesterterpene from the marine sponge *Luffariella* sp. *Experientia* **1987**, *43*, 949-950.
5. Kernan, M.R.; Faulkner, D.J.; Parkanyi, L.; Clardy, J.; De Carvalho, M.S.; Jacobs, R.S. Luffolide, a novel anti-inflammatory terpene from the sponge *Luffariella* sp. *Experientia* **1989**, *45*, 388-390.
6. König, G.M.; Wright, A.D.; Sticher, O. Four new antibacterial sesterterpenes from a marine sponge of the genus *Luffariella*. *J. Nat. Prod.* **1992**, *55*, 174-178.
7. Potts, B.C.M.; Capon, R.J.; Faulkner, D.J. Luffalactone and (4E,6E)-dehydromanoalide from the sponge *Luffariella variabilis*. *J. Org. Chem.* **1992**, *57*, 2965-2967.
8. Butler, M.S.; Capon, R.J. The luffarins (A-Z), novel terpenes from an australian marine sponge, *Luffariella geomatrica*. *Aust. J. Chem*. **1992**, *45*, 1705-1743.
9. Tsuda, M.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. Luffariolides A-E, new cytotoxic sesterterpenes from the okinawan marine sponge *Luffariella* sp. *J. Org. Chem.* **1992**, *57*, 3503-3507.
10. Kobayashi, J.; Zeng, C.M.; Ishibashi, M.; Sasaki, T. Luffariolides F and G, new manoalide derivatives from the okinawan marine sponge *Luffariella* sp. *J. Nat. Prod.* **1993**, *56*, 436-439.
11. Reddy, M.V.R.; Harper, M.K.; Faulkner, D.J. Luffasterols A-C, 9,11-secosterols from the Palauan sponge *Luffariella* sp. *J. Nat. Prod.* **1997**, *60*, 41-43.
12. Tsuda, M.; Endo, T., Mikami, Y., Fromont, J.; Kobayashi, J. Luffariolides H and J, new sesterterpenes from a marine sponge *Luffariella*. *J. Nat. Prod.* **2002**, *65*, 1507-1508.
13. Namikoshi, M.; Suzuki, S.; Meguro, S.; Nagai, H.; Koike, Y.; Kitazawa, A.; Kobayashi, H.; Oda, T.; Yamada, J. Manoalide derivatives from a marine sponge *Luffariella* sp. collected in Palau. *Fish. Sci.* **2004**, *70*, 152-158.
14. Zhou, G.X.; Molinski, T.F. Manoalide derivatives from a sponge, *Luffariella* sp. *J. Asian Nat. Prod. Res.* **2006**, *8*, 15-20.
15. Ettinger-Epstein, P.; Motti, C.A.; De Nys, R.; Wright, A.D.; Battershill, C.N.; Tapiolas, D. M. Acetylated sesterterpenes from the Great Barrier reef sponge *Luffariella variabilis*. *J. Nat. Prod.* **2007**, *70*, 648-651.
16. Kobayashi, M.; Okamoto, T.; Hayashi, K.; Yokoyama, N.; Sasaki, T.; Kitagawa, I. Marine natural products. XXXII. Absolute configurations of C-4 of the manoalide family, biologically active sesterterpenes from the marine sponge *Hyrtios erecta*. *Chem. Pharm. Bull.* **1994**, *42*, 265-270.
17. Bourguet-Kondracki, M.L.; Debitus, C.; Guyot, M. Biologically active sesterterpenes from a new caledonian marine sponge *Hyrtios* sp. *J. Chem. Res.* **1996**, 192-193.
18. Cambie, R.C.; Craw, P.A.; Bergquist, P.R.; Karuso, P. Chemistry of sponges, III. Manoalide monoacetate and thorectolide monoacetate, two new seterterpenoids from *Thorectandra excavatus*. *J. Nat. Prod.* **1988**, *51*, 331-334.
19. De Rosa, S.; De Stefano, S.; Zavodnik, N. Cacospongionolide: a new antitumoral sesterterpene, from the marine sponge *Cacospongia mollior*. *J. Org. Chem.* **1988**, *53*, 5020-5023.
20. Montagnac, A.; Païs, M.; Debitus, C. Fasciospongides A, B, and C, new manoalide derivatives from the sponge *Fasciospongia* sp. *J. Nat. Prod*. **1994**, *57*, 186-190.

21. De Rosa, S.; Crispino, A.; De Giulio, A.; Iodice, C.; Pronzato, R.; Zavodnik, N. Cacospongionolide B, a new sesterterpene from the sponge *Fasciospongia cavernosa*. *J. Nat. Prod.* **1995**, *58*, 1776-1780.
22. De Rosa, S.; Crispino, A.; De Giulio, A.; Iodice, C.; Tommonaro, G. Cavernosolide, a new sesterterpene from a Tyrrhenian sponge. *J. Nat. Prod.* **1997**, *60*, 844-846.
23. De Rosa, S.; Carbonelli, S. Two new luffarin derivatives from the Adriatic sea sponge *Fasciospongia cavernosa*. *Tetrahedron* **2006**, *61*, 2845-2849.
24. Ueoka, R.; Nakao, Y.; Fujii, S.; Van Soest, R.W.M.; Matsunaga, S. Aplysinoplides A-C, cytotoxic sesterterpnes from the marine sponge *Aplysinopsis digitata*. *J. Nat. Prod.* **2008**, *71*, 1089-1091.
25. Soriente, A.; De Rosa, M.; Scettri, A.; Sodano, G.; Terencio, M.C.; Paya, M.; Alcaraz, M.J. Manoalide. *Curr. Med. Chem.* **1999**, *6*, 415-431.
26. De Freitas, J.C.; Blankmeier, L.A.; Jacobs, R.S. In vitro inactivation of the neurotoxic action of β-bungarotoxin by the marine natural product, manoalide. *Experientia* **1984**, *40*, 864-865.
27. Lombardo D.; Dennis, E.A. Cobra venom phospholipase A2 inhibition by manoalide. *J. Biol. Chem.* **1985**, *260*, 7234-7240.
28. Glaser K.B.; Jacobs, R.S. Molecular pharmacology of manoalide. Inactivation of bee venom phospholipase A2. *Biochem. Pharm.* **1986**, *35*, 449-453.
29. Glaser K.B.; Jacobs, R.S. Inactivation of bee venom phospholipase A2 by manoalide. A model based on the reactivity of manoalide with amino acids and peptide sequences. *Biochem. Pharmac.* **1987**, *36*, 2079-2086.
30. Bennett, C.F.; Mong, S.; Clarke, M.A.; Kruse, L.I., Crooke, S.T. Differential effects of manoalide on secreted and intracellular phospholipases. *Biochem. Pharm.* **1987**, *36*, 733-740.
31. Glaser, K.B.; De Carvalho, M.S.; Jacobs, R.S.; Kernan, M.R.; Faulkner, D.J. Manoalide: structure-activity studies and definition of the pharmacophore for phospholipase A_2 inactivation. *Mol. Pharmacol*. **1989**, *36*, 782-788.
32. Jacobson, P.B.; Marshall, L.A.; Sung, A.; Jacobs, R.S. Inactivation of human synovial fluid phospholipase A_2 by the marine natural product, manoalide. *Biochem. Pharm.***1990**, *39*, 1557-1564.
33. Ortiz, A. R.; Pisabarro, M. T.; Gago, F. Molecular model of the interaction of bee venom phospholipase A_2 with manoalide. *J. Med. Chem*. **1993**, *36*, 1866-1879.
34. Deems, R.A.; Lombardo, D.; Morgan, B.P.; Mihelich, E.D.; Dennis, E.A. The inhibition of phospholipase A_2 by manoalide and manoalide analogues. *Biochim. Biophys. Acta* **1987**, *917*, 258-268.
35. Reynolds, L.J.; Morgan, B. P.; Hite, G.A.; Mihelich, E.D.; Dennis, E.A. Phospholipase A_2 inhibition and modification by manoalogue. *J. Am. Chem. Soc.* **1988**, *110*, 5172-5177.
36. Potts, B.C.M.; Faukner, D.J. Phospholipase A_2 inhibitors from marine organisms. *J. Nat. Prod.* **1992**, *55*, 1701-1717.
37. Reynolds, L.J.; Mihelich, E.D.; Dennis, E.A. Inhibition of venom phospholipase A_2 by manoalide and manoalogue. *J. Biol. Chem.* **1991**, *266*, 16512-16517.

38. Potts, B.C.M.; Faulkner, D.J.; De Carvalho, M.S.; Jacobs, R.S. Chemical mechanism of inactivation of bee venom phospholipase A_2 by the marine natural products manoalide, luffariellolide, and scalaradial. *J. Am. Chem. Soc.* **1992**, *114*, 5093-5100.
39. De Rosa, M.; Giordano, S., Scettri, A., Sodano, G., Soriente, A., Pastor, P.G.; Alcaraz, M.J.; Paya, M. Synthesis and comparison of the antiinflammatory activity of manoalide and cacospongionolide B analogues. *J. Med. Chem.* **1998**, *41*, 3232-3238.

Sample Availability: Not avaiable.