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An Antimalarial Cembranolide from Tanzania Soft Corals, *Lobophytum Crassum* (von Marenzeller (1886) and *L. Rotundum* (Tixier-Durivault (1957)

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Abstract—Bioscreening guided fractionation of the extracts of soft corals *Lobophytum crassum* and *L. rotundum* using brine shrimp larvae cytotoxicity assay led to the isolation of a cembranolide diterpene (E,E,E)-6,10,14-trimethyl-3-methylene-3a,4,7,8,11,12,15,15a-octahydrocylotetradeca[b]furan-2(3H)-one (1). This diterpene, identified as cembranolide compound 1, was found to be active against the multidrug-resistant and chloroquine-sensitive strains of *Plasmodium falciparum* malaria parasite in vitro. It also possessed cytotoxic properties.

INTRODUCTION

Recent investigations of marine organisms as sources of new drugs has shown that marine environment is an exceptional reservoir of pharmacological active compounds (Anderson and Williams, 2000). A number of useful pharmaceutical metabolites bearing unique structural features have been isolated from marine biota in the last three decades (Faulkner, 2002; Blunt *et al.*, 2003; Blunt *et al.*, 2004) in studies conducted in USA, Europe, Japan and Australia. However, most of these studies were not focused on isolation of compounds for the treatment of neglected diseases. This prompted the present investigation, aimed at isolation of potent bioactive compounds for treatment of tropical diseases.

Preliminary screening for cytotoxicity (Brine Shrimp Test = BST) and for antimicrobial activity of thirty soft corals which were collected from the coasts of Mbudya island off Dar es Salaam, and Pange reef in Zanzibar indicated three *Lobophytum* species *L. crassum, L. rutundum* and *L. venustum* and one *Sinularia* species, to be most active (Nyanda 1991). Bioassay (BST) guided isolation of the active constituent(s) has led to the isolation of the same cembranolide 1 as the bioactive constituent of the dichloromethane extracts of *L. crassum* and *L. rotundum.*

MATERIALS AND METHODS

General method

All solvents were redistilled and Merck Si gel 60 (230-400 mesh) was used for vacuum flash chromatography. Infra Red (IR) spectra were taken on Shimadzu IR-435 Spectrophotometer and Nuclear Magnetic Resonance (NMR) experiments were conducted in $CDCl_3$ with a Bruker AM 400 instrument. Signals are reported in parts per million (δ), referenced to the solvent used. High Resolution Mass Spectra (HRMS) were measured on a Varian VG 7070E instrument at 70 eV. Ultra Violet (UV) spectra were run on a Shimadzu UV-240 spectrophotometer. Antimalarial tests were conducted at the Swiss Tropical Institute, Basle

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Switzerland, using multidrug-resistant and chloroquine-sensitive strains K1 and NF54 of *Plasmodium falciparum.*

BST using brine shrimp larvae (*Artemia salina*) were used as the indicator organisms, through lethal test conducted as described by Meyer *et al.* (1982). LC_{50} values were determined using percent mortality of the shrimp nauplii.

Biological Materials, collection and identification

Lobophytum crassum von Marenzeller (1886) and *L. rotundum* Tixier-Durivault (1957) were collected from Pange reef in Zanzibar (06° 11.659' S; 039° 07.819' E) on 15 April 1992. The corals were identified by Dr. L.P. van Ofwegen of the National Natuurhistorisch Museum (RMNH), Leiden, Netherlands and Prof. Y. Beneyahu of the Department of Zoology, Tel Aviv University, Israel (Beneyahu and van Ofwegen 1992). Voucher specimens are preserved at RMNH and at the Zoological Museum of Tel Aviv University (ZMTAU) with voucher numbers *L. crassum* (RMNH Coel. 181917, ZMTAU Co 26276), *L. rotundum* (RMNH Coel. 18922).

Extraction and Isolation

Dried soft corals *L. crassum* (730 g) and *L. rotundum* (900 g) were soaked separately and consecutively in petroleum ether, followed by dichloromethane and methanol for 48 hours in each solvent. The extracts were concentrated in vacuo to yield petroleum ether, dichloromethane and methanol extracts. The CH_2Cl_2 solubles from both soft coral species exhibited cytotoxicity against brine shrimp larvae and were thus subjected to vacuum liquid chromatography (VLC), eluting with hexane containing increasing amounts of ethyl acetate. Elution of the VLC column with 10% EtOAc in C_6H_{14} gave a bioactive fraction (BST) that contained 1 as the only major compound (TLC). Repeated column chromatography ultimately gave crude 1 which was purified by gel filtration on sephadex LH-20 eluting with a mixture of MeOH-CH_2Cl_2 (1:1). The cembranolide was obtained as a colourless oil with yields for *L. crassum* of 149 mg (0.21%) and for *L. rotundum* of 360 mg (0.4%), based on the weight of the dried material. UV λ_{max} (MeOH), 210 nm (ε 10057). IR ν ($CHCl_3$), 2909, 1768, 1661, 1437, 1281, 1120, 947, 942 and 833 cm^{-1}. HRFABMS m/z 300.2088 $[M]^+$ (calculated for $C_{20}H_{28}O_2$, 300.2090). ^1H- and ^{13}C-NMR (Table 1).

RESULTS AND DISCUSSION

A compound identified as cembranolide 1 with a molecular formula $C_{20}H_{28}O_2$, was established by High Resolution Fast Atom Bombardment Mass Spectrum (HRFABMS). The ^1H-NMR ($CDCl_3$) (spectral data are shown in Table 1) absorption at δ 6.26 and 5.65 indicates the presence of an α-exomethylene group conjugated to a γ-lactone (Yamaguchi, 1970). This was supported by the ^{13}C-NMR spectrum (see Table 1), which consisted of a carbonyl and a methine resonance at δ 170.4 and 81.8 respectively (Francenus *et al.*, 1987).

The ^{13}C-NMR spectrum revealed signals for nine sp^2 carbons. The molecular formula requires seven degrees of unsaturation, three were accounted for by the lactone ring and its conjugated exomethylene group. The other three were assigned to the remaining six sp^2 carbons. Thus, the seventh degree of unsaturation suggested the molecule to be bicyclic, by analogy with other diterpenes isolated from coelenterates (e.g. Scheuer, 1973), it appeared that the compound possessed a cembranoid skeleton.

The ^1H-NMR spectrum showed a peak at δ 2.7 which was attributed to the allylic proton H3a. A peak that appeared at δ 4.30 was assigned to the lactonic methine proton (H15a), this chemical shift value suggests a homoallylic link (Bowden *et al.*, 1977) presented in compound 1 rather than an allylic connection shown in compound 2 (Figure 1) which requires a δ value of *ca* 5.35 (Coll *et al.*, 1977). The suggested setup is corroborated by a Correlated Spectroscopy (COSY) spectrum which showed a vicinal neighborhood of protons resonating at δ 2.44 and 2.04, and H15a. Thus, C15 must be allylic and its two protons resonate at δ 2.44 and 2.04. More evidence is obtained from the spin coupling of methine proton (ddd, J = 8.4, 3.5, 2.9 Hz), which indicated an allylic CH_2 group at C15 thus a C13-C14 double bond. Absorptions at

Table 1. ^{1}H- and ^{13}C-NMR spectral data for the isolated cembranolide compound 1

Position*	δ_H (J in Hz)		δ_C		HMBC
	Observed	Ref. (Ahond 1979)	Observed	Ref. (Ahond 1979)	
3a	2.71 dddd (6.7, 3.6, 2.2, 1.9)	2.62 (m)	44.9	45.0	C2,C3,C16,C15,C15a
15a	4.30 ddd (8.4, 3.6, 2.9)	4.17ddd (8.5, 4, 3)	81.8	81.9	C2,C3,C3a,C4,C14,
15	2.06 (obsc.), 2.44 dd (4.3, 3.4)	n.r	44.9	45.0	C3a,C13,C15a,C19
13	4.90 (m)	4.90 (m)	120.5	120.8	C11,C14,C15,C19
12	2.25 (m), 2.15 (m)	n.r	38.1	38.3	C10,C14
11	2.06 (m), 2.15 (m)	n.r	38.6	38.7	C9,C10,C13,C18
9	5.05 (m)	4.90 (m)	124.2	124.5	C7,C10,C11,C18
8	2.25 (m), 2.15 (m)	n.r	24.4	24.5	C6,C10
7	2.06 (m)	n.r	33.7	33.7	C5,C9,C17
5	5.05 (m)	4.90 (m)	128.1	128.4	C3a,C17
4	2.25 (obsc.)	n.r	24.5	24.5	C3,C3a,C6C15a
16α,β	5.65 d (1.8), 6.26 d (2.1)	5.50 d (1.0), 6.10 d (1.5)	122.2	122.3	C2,C3,C3a
17	1.70 (s)	1.62 (s)	17.4	17.4	C5,C6,C7
18	1.70 (s)	1.58 (s)	16.5	15.9	C9,C10
19	1.60 (s)	1.58 (s)	15.9	16.5	C13,C14,C15
2			170.3	170.6	
3			139.6	140.1	
6			133. 5	137.4	
10			129.5	129.8	
14			137.1	133.7	

n.r = not reported

*Assignment was assisted by NOESY, 1H-1H and 1H-13C COSY spectra

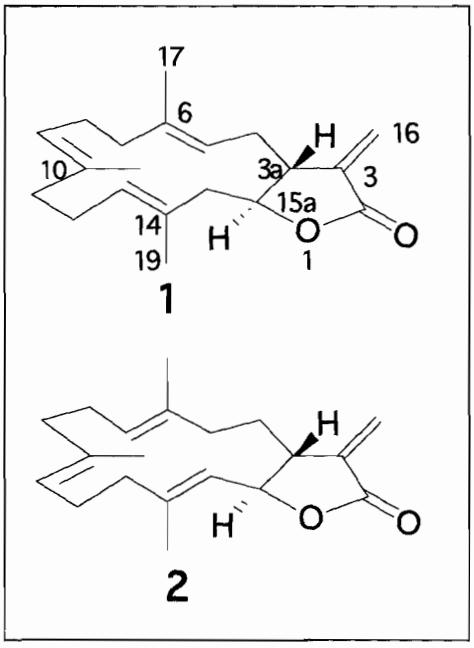

Fig. 1. The structures of cembranolide compound 1 and its analog compound 2

δ 4.90 and 5.05 which are due to three vinylic protons (H13, H5 and H9), and two singlets at δ 1.70 (6H) and 1.66 (3H) which were attributed to the three vinyl methyl groups confirm the presence of three tri-substitued double bonds in the molecule, as indicated by ^{13}C-NMR spectral data. The COSY spectrum revealed a vicinal relationship between H3a (δ 2.71) and two protons at δ 2.25, and this confirmed that both H4's resonate at δ 2.25. Hence C4 is allylic, furthermore, homoallylic H4's would appear at *ca* δ 1.4 (c.f. spectrum of compound 2, Coll *et al.*, 1977). The absence of a signal between δ 3.0 and 3.2 in the ^{1}H-NMR spectrum implied the absence of doubly allylic protons in compound 1 (Jackman and Sternhell, 1969).

^{13}C-NMR chemical shifts of the three methyl carbons (δ 15.9, 16.5 and 17.4) indicate an *E* stereochemistry of the double bonds, since in cyclic systems the chemical shift of a methyl carbon on an *E*-trisubstituted double bond is about 15-16 ppm, while that of methyl carbon on a *Z*-trisubstituted double bond is 23-24 ppm (Johnson

and Jankowiski, 1972). The *E*-stereochemistry for the three endocyclic double bonds in compound 1 was corroborated by NOE measurements, since the Nuclear Overhauser Enhancement Spectroscopy (NOESY) spectrum showed no interactions between vinylic protons (H5, H9 and H13) and the methyl protons (H17, H18 and H19) respectively. The NOESY spectrum also established the stereochemistry at the lactone ring junction. The fact that it does not exhibit any NOE between H3a and H15a is clear proof that these protons are spatially very well separated. This is only possible if H3a and H15a exist in an anti-relationship, and hence a trans-stereochemsitry for the lactone ring junction in compound 1. Apparently, this stereochemistry is the most common among cembranolides which have been found in alcyonarian soft corals. Gorgonian soft corals tend to yield cembranolides which have opposite stereochemical configurations at C3a and C15a (Bowden *et al.*, 1984). Compound 1 has also been isolated from *Lobophytum crasscopiculatum* (Ahond *et al.*, 1979)

Cembranolide compound 1 was found to be cytotoxic against brine shrimp Artemia salina larvae with LC_{50} = 1.15 mg/ml. The compound also demonstrated antimalarial activity in vitro against both multidrug-resistant and chloroquine-sensitive strains K1 and NF54 of *Plasmodium falciparum* with IC_{50} values of 2.5 and 1.8 µg/ml respectively.

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REFERENCE:

Ahond, A., Bowden, B.F., Coll, J.C., Fourneron, J.D. and Mitchell, S.J. (1979) Studies of Australian Soft Corals XII- Further cembranolides diterpenes from *Lobophytum crassospiculatum* and a correction of a previous stereochemical assignment *Aust. J. Chem.* **32:** 1237-1280.

Anderson, R.J. and Williams, D.E. (2000) Pharmaceuticals from the sea. *Issues in Environmental Sciences and Technology* **13:** 55-79.

Benayahu, Y. and Ofwegen van, L.P. (1992) Notes on Alcyonacea (Octocorallia) from Tanzania Zool. Meded. Leiden, **66:** 139-154

Blunt, J.W., Copp, B.R., Munro, M.H.G., Northcote, P.T. and Prinsep, M.R. (2003) Marine natural products. *Nat. Prod. Rep.*, **20:** 1 - 48.

Blunt, J.W., Copp, B.R., Munro, M.H.G., Northcote, P.T. and Prinsep, M.R. (2004) Marine natural products. *Nat. Prod. Rep.*, 21, 1-49.

Bowden, B.F., Brittle, J.H., Coll, J.C., Liyanage, N., Mitchell, S.J. and Stokie, G.J. (1977) Studies of Australian Soft Corals VI- A new cembranolide diterepene from the soft coral *Lobophytum crassum* (Coelenterata, Anthozoa, Octocorallia, Alcyonacea) *Tetrahedron Lett.* **41:** 3661-3662.

Bowden, B.F., Coll, J.C., Decosta, M.S.L., Mackay, F.M., Mahendra, M., De Silva, E.D. and Wills, R.H. (1984) The structure determination of a new cembranolide diterpene from the soft coral *Lobophytum cristigalli* (Coelenterata, Octocorallia, Alcyonacea) *Aust. J. Chem.* **37:** 545-552

Coll, J.C., Mitchell, S.J. and Stokie, G.J. (1977) Studies of Australian Soft Corals II- A novel cembrenoid diterpene from *Lobophytum michaelae Aust. J. Chem.* **30:** 1859-1863

Faulkner, D.J. (2002) Marine natural products. *Nat. Prod. Rep.*, **19:** 1-48.

Francenus, W., Huber, J. F., Pungor, E., Rechnitz, G. A., Simon, W. and West, S. T. (Eds). (1987) Tables of spectral data for structural determination of organic compounds. Springler Verlag, Berlin.

Jackman, L.M. and Sternhell, S. (1969) Application of NMR spectroscopy in organic chemistry, Pergamon Press, Oxford.

Johnson, L.F. and Jankowiski, W.C. (1972) Carbon-13 NMR spectra. John Wiley, New York.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E. and McLaughlin, J.E. (1982) A convenient general bioassay for active plant constituents. *Planta Med.* **45:** 31-34

Nyanda, J. N. (1991) Screening for antimicrobial compounds from some Tanzanian soft corals MSc. Thesis, University of Dar es Salaam, Dar es Salaam, Tanzania

Scheuer, P.J. (1973) Chemistry of Marine Natural Products, Academic press, New York.

Yamaguchi, K. (1970) Spectral data of natural products Vol 1. Elsevier, Amsterdam.