Bioactive Terpenes from the Soft Coral *Heteroxenia* sp. from Mindoro, Philippines

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Z. Naturforsch. **55c,** 82–86 (2000); received August 1999

Corals, *Heteroxenia,* Cadinene Sesquiterpenes, Structure Elucidation, Bioactivity

A marine soft coral species of the genus *Heteroxenia* collected from Mindoro Island, Philippines yielded two cadinene sesquiterpenes, (+)-α-muurolene (**1**) and a novel derivative (+)-6-hydroxy-α-muurolene (**2**), as well as the biologically active polyhydroxysterol, sarcoaldosterol A (**3**). The structure of the novel compound was unambiguously established on the basis of NMR spectroscopic (^{1}H, ^{13}C, COSY, ^{1}H-detected direct and long range ^{13}C-^{1}H correlations) and mass spectrometric (EIMS) data. All compounds were active against the phytopathogenic fungus *Cladosporium cucumerinum.* The isolated terpenes were also active in the brine shrimp lethality test.

Muurolane represents the basic structure of the muurolene sesquiterpenes. Muurolane, a (1*R*, 6*R*, 7*S*)-7-(2-propyl)-4,10-dimethylbicyclodecane, is a cadinene sesquiterpene which was first isolated from an industrial extract of resinous stumps of *Pinus silvestris* produced in Muurola, Finland (Aschan, 1929). The first report of the isolation of cadinene dates back to the first half of the last century. Cadinenes are widely distributed in terrestrial plants and animals. In a review article in 1989, approx 165 naturally occurring cadinene derivatives were reported (Bordoloi *et al.,* 1989). In the marine environment, muurolene type sesquiterpenes, namely, a-muurolene and 7-hydroxymuurolene, were first reported from the soft coral *Heteroxenia fuscescens* (Kashman *et al.,* 1978). Heteroxenia spp. are also known to produce the sesquiterpene (+) cubebol, trisnorsesquiterpene clavukerin A, and bicyclogermacrene (Bowden *et al.,* 1983; Kobayashi *et al.,* 1983; Kobayashi *et al.,* 1984). Heteroxenia spp. are extremely soft and fleshy organisms with interesting growth and survival strategies that utilize a novel detachment strategy in the face of competitors by floating in the water column and reattaching to substrates remote from competition (Dai *et al.,* 1991). A species of *Heteroxenia* collected in the Philippines yielded the sesquiterpene **1** and the novel derivative **2**. Two-dimensional homonuclear correlation spectroscopy (^{1}H COSY) and heteronuclear ^{1}H-detected ^{13}C multiple quantum coherence (HMBC, HMQC) spectra afforded independent, unambiguous confirmation of the signal assignments, substituent position, and total structure of the novel compound **2** (Table I).

Materials and Methods

Specimens of the soft coral species of the genus *Heteroxenia* sp. were collected by snorkelling off the shores of Mindoro Island, Philippines, in April 1994. The samples were encountered on a very shallow reef, located in a well-protected cove unexposed to the prevailing wind, where the water is quiet. They are attached to pieces of coral just above the lowest tide level and are also seen as irregular limp masses partially covering the stones to which they are attached. In life, they are vari-

0939–5075/2000/0100–0082 $ 06.00

ously colored, exhibiting light hues of yellow, brown, blue, and lilac. The stalk, the polyp bodies, and the tentacles are differently tinged. The polyps are united on the base through a mass of coenenchyme which is traversed by an extensive endodermal canal system. The united portion of the polyps form a fleshy, soft columnar, stalk which is sometimes branched or spreadout, from which the non-retractile free portions of the polyps extend. The polyps, 1.5 mm in length, are numerous and are well spaced or closely set on the upper portion of the stalk. The apical portion of the stalk forms an expended disk or capitulum. The siphonozoids are absent, while the autozoids are solid-looking, more or less rounded, about 1 mm in length and 0.44 mm in diameter. The apical end flattens and divides into eight knobs, 0.6–0.7 mm long with two or three small wart-like pinnules on their sides.

The samples were frozen immediately and then freeze-dried prior to transport to the University of Würzburg, Germany. A voucher fragment is kept in 70% ethanol under the voucher no. RE-36.04.94 in the Nationaal Natuurhistorisch Museum, Leiden.

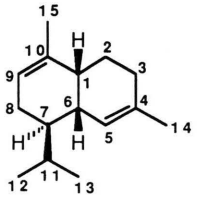
The freeze-dried samples of *Heteroxenia* (104 g) were extracted with acetone and MeOH (300 ml x 2 for each) successively. The concentrated total acetone-methanol extract was partitioned between EtOAc (50 ml x 5) and H_2O (50 ml). The organic fraction was taken to dryness (11.16 g) and chromatographed over a Si gel column (mobile phase hexane:EtOAc, 80:20), and ten fractions were obtained. The non-polar fractions 2 and 3 afforded **1** (24.2 mg, 0.023%) and the major compound, **2** (214.7 mg, 0.093%), respectively. The polar fraction 7 contained the biologically active polyhydroxy sterol **3** (28.2 mg, 0.012%). The pure compounds **1** and **2** were obtained by rechromatography on Si 60 Lobar columns using different ratios of hexane:EtOAc as eluent, 90:10 and 70:30, respectively. The polyhydroxysterol **3** was obtained by further purification of fraction 7 on a reversed phase RP18 column with MeOH:H_2O (70:30) as mobile phase.

^1H NMR and ^{13}C NMR spectra were recorded on Bruker ARX 400 NMR and AVANCE DMX 600 NMR spectrometers. Mass spectra (EIMS) were measured on a Finnigan MAT 8430 mass spectrometer. Optical rotations were determined on a Perkin Elmer-241 MC Polarimeter. UV spectra were recorded in hexane. Solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with Si gel 60 F254 (Merck, Darmstadt, Germany). The compounds were detected by spraying the TLC plates with anisaldehyde reagent.

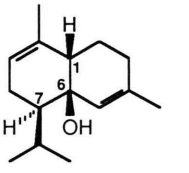
For antibiotic activity, the agar plate diffusion assay was used (Edrada *et al.*, 1998) and for the detection of fungicidal activity against the plant pathogenic fungus *C. cucumerinum*, the bioautographic method has been utilized (Edrada *et al.*, 1998; Gottstein *et al.*, 1984). The brine shrimp lethality test was also performed as previously described (Edrada *et al.*, 1998, Meyer *et al.*, 1982).

Results and Discussion

The organic fraction of *Heteroxenia* sp. (family Xeniidae) was chromatographed over a Si gel column utilising hexane and EtOAc as the mobile phase. The non-polar fractions afforded **1** and the major compound, **2** (Fig. 1). The polar fraction contained the biologically active polyhydroxysterol **3** (Fig. 1), which contains the 3b,5a,6b-trihy-



1



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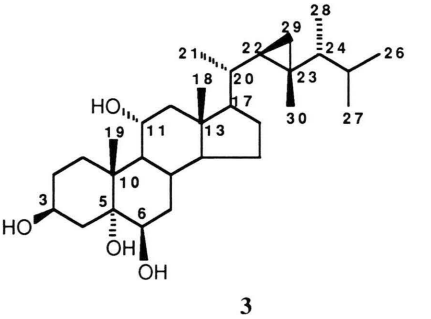

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Fig. 1. Isolated compounds from the soft coral species of the genus *Heteroxenia*.

droxysterol nucleus common to all sterols previously isolated from the family *Xeniidae* (Kitagawa *et al.,* 1986, Sjöstrand *et al.,* 1981). Sarcoaldosterol A (**3**) was obtained as a white amorphous powder; $[\alpha]_D$ −53.35 ° (*c* 0.41, $CHCl_3$); ($C_{30}H_{52}O_4$); EIMS (70 eV) *m/z* $[M]^+$ 476 (24); $[M\text{-}H_2O]^+$ 458(24), $[M\text{-}2H_2O]^+$ 440 (34), $[M\text{-iPr}]^+$ 433 (34), $[M\text{-}3H_2O]^+$ 422(20), 414(36), 396(38), 369(38), 332(40), 315(40), 305(48), 297(54), 287(100), 269(68), 109(58), 95(70), 83(82), 69(80), 55(84), 43(92). The ^{13}C and ^1H NMR spectra of the isolated sterol are identical to those of sarcoaldosterol A which was recently isolated from a *Sarcophyton* species collected from Okinawa (Umeyama *et al.,* 1996).

(+)-α-Muurolene (**1**) was obtained as a colorless viscous oil; $[\alpha]_D$ +51.16 ° (*c* 0.23, $CHCl_3$), (lit. $[\alpha]_D$ +54 ° [Kashman *et al.*, 1978], +53 ° [Beechan *et al.*, 1978], −85 ° [Westfelt, 1966]); ($C_{15}H_{24}$); EIMS (70 eV) *m/z* $[M]^+$ 204(18), $[M\text{-iPr}]^+$161(45), 133(52), 119(62), 105(66), 91(58), 69(40), 55(46), 43(100). The isolated sesquiterpene (+)-α-muurolene **1** showed the molecular ion peak $[M]^+$ at *m/z* 204 in the EIMS which is compatible with the molecular composition of $C_{15}H_{24}$. The mass spectral fragmentation pattern with intense ion peaks at *m/z* 161 ($C_{12}H_{17}$), *m/z* 133 ($C_{10}H_{13}$), *m/z* 119 (C_9H_{11}), and *m/z* 105 (C_8H_9), suggests a characteristic cadinene type of sesquiterpene (Beechan *et al.,* 1978). Its NMR spectral data are identical to those already published in the literature (Beechan *et al.,* 1978; Hill *et al.,* 1968; Kashman *et al.,* 1978; Westfelt, 1966). (+)-α-Muurolene (**1**) is an optical antipode of a compound known from *Pinus silvestris* and other terrestrial plants. It has been previously noted that sesquiterpenes from coelenterates often tend to have structures enantiomeric or antipodal to those from terrestrial sources (Beechan *et al.,* 1978).

(+)-6-Hydroxy-α-muurolene (**2**) was obtained as a yellow viscous oil; UV λmax (hexane) 203; $[\alpha]_D$ +23.03 ° (*c* 1.41, $CHCl_3$); ($C_{15}H_{24}O$); EIMS (70 eV) *m/z* $[M]^+$ 220 (44); $[M\text{-}H_2O]^+$ 202(26), $[M\text{-iPr}]^+$ 177 (26), 187 (8), $[M\text{-iPr-}H_2O]^+$159(100), 135(25), 131(18), 121(18), 110(22), 109(23), 91(24), 82(26), 69(24), 55(22), 43(22). Compound **2** showed the molecular ion peak $[M]^+$ at *m/z* 220 in the EIMS which is compatible with the molecular composition of $C_{15}H_{24}O$. From the mass spectral fragmentation, intense ion peaks at *m/z* 109 (C_7H_9O) and *m/z* 110 ($C_7H_{10}O$) indicate the presence of a hydroxyl group either at C-1 or C-6. In order to clarify this, a full NMR analysis was undertaken. The ^{13}C and ^1H NMR spectra are similar but not identical to those of **1** (Table I). The major difference of derivative **2** to that of **1** is the presence of a tertiary hydroxyl group which was indicated in the ^{13}C NMR spectrum by a resonance at δ 73.1. The hydroxyl substitution is positioned at C-6 and thereby deshielding of C-1, C-5 and C-7 by 8.8, 3.3, and 4.8 ppm, respectively. 1D and 2D COSY ^1H NMR spectra of **2** identified fragments belonging to the isopropyl and substituted olefinic systems, although the signal overlap made this difficult. The types of groupings present were established from the ^{13}C, DEPT-135 and direct ^{13}C-^1H correlation (HMQC). Finally the unambiguous connectivity of these were elucidated from the long-range ^{13}C-^1H correlation (HMBC) as shown

Table I. ^{13}C- and ^1H- NMR data of **2** in $CDCl_3$.

No.	δC, multiplicity[a]	δH, multiplicity	Coupling constant in Hz
1	49.8 d	1.83 bd	$J_{(1ax\text{-}2ax)}$= 13.0 $J_{(1ax\text{-}2aq)}$= small
2A	26.7 t	1.99 m	
2B		1.37 ddd	$J_{(2ax\text{-}1ax)}$= 13.0 $J_{(2ax\text{-}3ax)}$= 8.0 $J_{(2ax\text{-}3eq)}$= 5.5
3A	30.4 t	2.05 bm	
3B		1.92 bm	
4	135.7 s		
5	127.1 d	5.51 bs	
6	73.1 s		
7	43.8 d	1.61 ddd	$J_{(7ax\text{-}8ax)}$= 11.0 $J_{(7ax\text{-}8eq)}$= 3.5 $J_{(7ax\text{-}11)}$ = 6.0
8A	21.6 t	2.02 bm	
8B		1.92 bm	
9	121.6 d	5.50 bs	
10	134.5 s		
11	26.1 d	2.12 ddd	$J_{(11\text{-}12)}$= 7.0 $J_{(11\text{-}13)}$= 7.0 $J_{(11\text{-}7ax)}$= 6.0
12	17.9 q	0.90 d	$J_{(12\text{-}11)}$= 7.0
13	23.1 q	0.99 d	$J_{(13\text{-}11)}$= 7.0
14	23.5 q	1.70 bs[b]	
15	21.9 q	1.69 bs[b]	

ax = axial; eq = equatorial

[a] ^{13}C chemical shifts for **1**: 41.0d (C-1), 24.5t (C-2), 30.4t (C-3), 135.8s (C-4), 123.8d (C-5), 36.6d (C-6), 39.0d (C-7), 24.6t (C-8), 121.1d (C-9), 134.0s (C-10), 26.5 (C-11), 15.9q (C-12), 21.3q (C-13), 23.7 (C-14), 21.5q (C-14). From Kashman *et al.,* 1978, assignments for C-1, C-6, and C-11 need interchanging.

[b] Long range allylic couplings were observed in the COSY between H-5 and H-14, H-9 and H-15, H-9 and H-1.

Table II. Unambiguous long-range correlations in the HMBC spectra for compound **2**.

H No.	CH correlation
1	C-2
2A	C-3, C-10
2B	C-1, C-3, C-4, C-6, C-10
3A	C-1, C-4
3B	C-4
5	C-1,C-3, C-14
7	C-6, C-8, C-11, C-12, C-13
8A	C-6
8B C-6	
9	C-8
11	C-6, C-7, C-12
12	C-7, C-11, C-13
13	C-7, C-11, C-12
14	C-3, C-4, C-5
15	C-1, C-9, C-10

in Table II. The two 2D heteronuclear spectra allowed the assignment and differentiation of the overlapping ^{1}H signals through correlation with the well resolved ^{13}C signals. A ROESY experiment was recorded to establish the relative stereochemistry at the asymmetric centres, C-7 and C-1. NOEs were observed from H-7_{ax} to Me-12 and H-5 to Me-13 and H-11. The NOE from H-2ß to H-7_{ax} confirms that the side chain at C-7 is equatorial while the ring junction has the *cis* conformation. Compound **2** is thus, a *cis*-(1*R*, 7*S*)-7-(2-propyl)-(6-hydroxyl)-4,10-dimethylbicyclodecane,4(5), 9(10)-diene.

Both sesquiterpenes were found to be active against the plant pathogenic fungus *C. cucumerinum* causing a zone of inhibition of 17 mm and 30 mm at a concentration of 0.80 µmol for **1** and its hydroxyl derivative **2**, respectively. Both derivatives were also found to be active against the gram positive bacterium *B. subtilis* causing a zone of inhibition of 7 and 12 mm for **1** and **2**, respectively at a concentration of 100 µg per disc. Compound **2** was found to be more active than its parent compound in both assays. However, only **2** was found to be lethal to brine shrimps with a LC_{50} of 6.49 µg/ml while **1** was inactive.

Compound **3** was also found to be anti-fungal against the *C. cucumerinum* causing a zone of inhibition of 28 mm at a concentration of 0.80 µmol. However, **3** was found to be inactive on the bacteria *B. subtilis, S. aureus,* and *E. coli.* It was found to be active in the brine shrimp lethality assay with a LC_{50} of 4.5 µg/ml.

Acknowledgments

Financial support by grants of the DFG (SFB 251) and by the „Fonds der Chemischen Industrie" to P. P. is gratefully acknowledged. Furthermore, we would like to thank Dr. Dieter Gross and Monika Kummer (IPB, Halle, Germany) for giving us the opportunity to perform bioassays with *C. cucumerinum*, and C. Kakoschke and B. Jaschok-Kentner for recording NMR data (GBF, Braunschweig). We thank Pedro C. Gonzales of the Zoological Division, Philippine National Museum and Frederick Agcaoili, Gerlie C. de los Reyes, and Imelda G. Peña of the College of Pharmacy, University of the Philippines Manila, and the rest of the members of the expedition crew, Allan Edrada, Gladys Edrada, and Arlene Si for their help with the collection of the soft coral specimens. R. A. E. wishes to thank the DAAD (Deutscher Akademischer Austauschdienst) for a scholarship.

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