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CHELODANE, BAREKOXIDE, AND ZAATIRIN—THREE NEW DITERPENOIDS FROM THE MARINE SPONGE *CHELONAPLYSILLA ERECTA*

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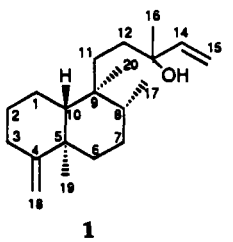
ABSTRACT.—Three new diterpenoids, chelodane [**1**], barekoxide [**2**], and zaatirin [**3**], were isolated from a marine sponge, *Chelonaplysilla erecta*, collected in the Red Sea. The structure determination of the three compounds, which belong to three different types of diterpenoids, was achieved by 1D and mainly 2D nmr measurements.

In the course of studying pharmacologically active metabolites from sponges (1) we have investigated the components of a *Chelonaplysilla erecta* (Keller) (Aplysillidae, Dendroceratida), the CH_2Cl_2-MeOH (95:5) extract of which showed antitumor activity. We report herein the isolation and structure elucidation of three new diterpenes designated chelodane [**1**], barekoxide [**2**], and zaatirin [**3**]. Other diterpenes from *Chelonaplysilla* sp. were reported earlier (2–4). Diterpenoids from marine origin are well known from algae, soft and horny corals, and sponges (5).

RESULTS AND DISCUSSION

Si gel chromatography of the crude extract of the sponge with increasing percentages of EtOAc in petroleum ether resulted in compounds **1–3** in 0.4, 0.04, and 0.04% (dry wt) yields, respectively.

Compound **1**, chelodane, $C_{20}H_{34}O$ (obtained from the cims, *m/z* 291 $[MH]^+$; ^{13}C data see Table 1), ν max 3600, 3480, 2900, and 900 cm^{-1}, is an unsaturated alcohol. Of the four degrees of unsaturation of the molecule, two belong to double bonds: i.e., an exomethylene group (δ_C 160.4 s, 103.1 t, and δ_H 4.47 bs, 4.65 bs, 1H each) and a vinyl group (δ_C 145.8 d, 111.5 t, and δ_H 5.71 (1H, dd, J = 17.4, 10.5), 5.14 (1H, dd, J = 17.4, 1.5), and 4.92, (1H, dd, J = 10.5, 1.5 Hz). On the basis of the latter data and the 4 Me's (Table 1), compound **1** was suggested to be a bicyclic diterpene. Furthermore, an intense fragment at *m/z* 191 ($C_{14}H_{23}$, 50%) in the mass spectrum suggested a methylated decalin portion (6). Comparison of the nmr data of **1** with the decalin moiety of the sigmoscepterellins (7) and with the chemical shifts of the side



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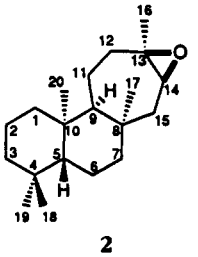

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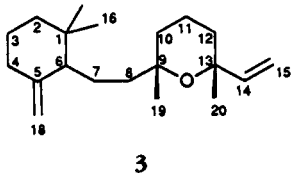

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TABLE 1. ^{1}H- and ^{13}C-nmr Chemical Shifts (ppm)[a] of Chelodane [1] and Protons to Which Long-Range Correlations Were Observed in the HMBC Experiment.

Position	δ_C	δ_H	*CH* Correlations	
1	22.1 t	1.52, 1.25		21.9[b]
2	28.9 t	1.75, 1.27	3′	27.6[b]
3	33.5 t	2.25, 2.18	18′	33.1[b]
4	160.4 s		Me-19	160.2[b]
5	40.4 s		6, 18′, Me-19	40.2[b]
6	37.8 t	1.60, 1.58	Me-19	37.5[b]
7	27.9 t	1.43, 1.35	Me-17	28.7[b]
8	37.0 d	1.35	Me-17, Me-20	36.7[b]
9	39.3 s		Me-17, Me-20	39.1[b]
10	49.1 d	1.12	11′, Me-19, Me-20	48.8[b]
11	32.2 t	1.30, 1.25	12, Me-20	31.2[b]
12	35.6 t	1.32, 1.18	11′, Me-16	
13	72.5 s		15, 15′, Me-16	73.3[c]
14	145.8 d	5.71	12′, 15′, Me-16	145.1[c]
15	111.5 t	5.14, 4.92		111.4[c]
16	28.2 q	1.09		27.9[c]
17	16.1 q	0.79	Me-20	18.4[b]
18	103.1 t	4.65, 4.47		102.6[b]
19	21.2 q	1.02		20.9[b]
20	18.5 q	0.70		15.9[b]

[a]At 360 MHz, C_6D_6; apostrophe on a proton indicates the lower field proton in a geminal pair.

[b]^{13}C values of the corresponding carbons in sigmosepterellin (7).

[c]^{13}C values of the corresponding carbons in manool (8).

chain of manool (8) (Table 1), suggested a structure for **1** composed from the latter two moieties. Intensive nmr measurements and particularly one- and several-bond CH correlations derived from HMQC (9) and HMBC (10) experiments (Table 1) fully confirmed the proposed structure. Long range correlations established the connectivities between all carbons except for C-1. However, the vicinity of the latter methylene to H_2-2 on the one hand and to H-10 on the other hand was confirmed by a decoupling experiment. Thus, while irradiating H-1 at δ 1.52 ppm, changes in the pattern of H-2 at δ 1.75 ppm and of H-10 at δ 1.12 were observed.

The latter changes in the signals of H-2 and H-10 confirmed the continuity of C-1 to C-3 and the bond of C-1 to C-10 (forming a second six-membered ring). As the six-membered structure of ring B as well as the six-carbon side chain were unequivocally confirmed by long range CH correlations, chelodane has to be a substitued decalin (excluding a priori a possible perhydroazulene structure). The good agreement of the carbon chemical shifts of the decalin moieties in **1** and sigmoscepterellin suggested for **1** the same relative configuration at C-5, -8, -9, and -10 as in sigmoscepterellin (7). This stereochemistry is in good agreement with the measured nOe's between methyls 17, 19, and 20, all being on the same side of the molecule. The relative stereochemistry of C-13 remains unassigned. Chelodane [**1**] is a new clerodane diterpene (11).

The second compound, barekoxide [**2**], had the same $C_{20}H_{34}O$ formula (*m/z* 291 $[MH]^+$) as **1**. However, in the absence of unsaturated bonds, compound **2**, possessing four degrees of unsaturation, had to be tetracyclic. Carbon chemical shifts of δ 59.8 s and 60.0 d ppm, together with a triplet at 2.58 (1H, $J = 7.1$) assigned to the proton seated on the latter carbon atom, suggested an epoxide as the only functionality of the molecule. Compound **2** with its five methyl groups (Table 2) was proposed to be a

TABLE 2. ^{1}H- and ^{13}C-nmr Chemical Shifts (ppm)[a] of Barekoxide [2] and Protons to which Long-Range Correlations Were Observed in the HMBC Experiment.

Position	δ_C	δ_H	CH Correlations	
1	40.2 t	1.58, 1.55	2′, 3	39.0[b]
2	19.1 t	1.38, 1.34	1′, 3	19.3
3	42.1 t	1.33, 1.08	1, Me-18, Me-19	42.1
4	33.5 s		3, Me-18, Me-19	33.2
5	56.2 d	0.58	6, 7, Me-18, Me-19, Me-20	56.4
6	18.8 t	1.36, 1.25	5, 7, Me-18	19.9
7	44.2 t	1.28, 1.02	5, 15′, Me-17	43.2
8	37.7 s		7, 7′, 9, 11, 11′, 15, 15′, Me-17	
9	64.4 d	0.60	6, 11, 11′, 12, 12′, 15′, Me-17, Me-20	55.7
10	38.9 s		1, 1′, 2′, 5, 9, 15′, Me-20	36.9
11	20.3 t	1.51, 1.12	9, 12, 12′	15.4
12	36.6 t	1.88, 1.42	9, 11, 11′, Me-16	35.8
13	59.8 s			
14	60.0 d	2.59	11, 12, 12′, 15, 15′, Me-16	
15	47.9 t	1.70, 1.22	7, 9, 14, Me-17	
16	22.7 q	1.20	12, 12′	
17	19.8 q	0.84	12, 12′	
18	33.5 q	0.86	3′, 5, Me-19	33.4
19	21.7 q	0.80	3, 5	21.7
20	16.2 q	0.72	9, Me-18	15.4

[a]At 500 MHz, C_6D_6; apostrophe on a proton indicates the lower field proton in a geminal pair.
[b]The chemical shifts of the corresponding carbons in manoyl oxide (13).

carbo-tricyclic diterpene. One- and multi-bond CH-correlations (HMQC and HMBC experiments), measured on a 360 MHz instrument, established several parts of the molecule including a seven-membered ring bearing two of the methyls and the trisubstituted epoxide. However, only after repeating the CH-correlation experiments on a 500 MHz instrument (Table 2), whereupon all protons and carbons were separated, could the complete epoxycarbotricyclic structure of **2** be established. The most important correlations, establishing the size and link between the three rings, were the connectivities of the angular carbons C-5, -8, -9, and -10 with their neighbor protons (^{2}J to ^{4}J couplings).

The suggested structure was also in full agreement with the partial structures which were deduced from a homo-COSY experiment (H to H correlations, apostrophe indicates the lower-field proton within a geminal pair): 1/3′, 3/5, 3/Me-19, 5/6, 5/6′, 5/9, 5/Me-20, 7/7′, 7/Me-17, 9/11, 9/11′, 9/12, 9/12′, 9/Me-20, 11/11′, 11/12, 11/12′, 12/12′, 12/Me-16, 12′/14, 12′/Me-16, 14/15, 14/15′, 14/Me-17, 15′/15, 15′/Me-17.

Barekoxide [**2**] has the rare structure of a carbo-tricyclic diterpene with two six-membered (AB) and one seven-membered (C) rings. Strobic acid (12) and the related strobal are among the only known diterpenes with the same six-, six-, and seven-membered rings. However, as the location of the methyls of **2** differs, compound **2** represents a new diterpenoid skeleton. Comparison of the ^{13}C chemical shifts of rings AB of **2** with corresponding known δ_C values of similarly substituted rings AB of other terpenoids (e.g., the AB-trans rings of manoyl oxide) (7) (Table 2) determined the AB-trans stereochemistry as shown in **2**. Furthermore, the suggested stereochemistry of **2**, with methyls 17, 19 and 20 in the 1,3-diaxial positions, was unequivocally confirmed by nOe's between the latter three methyls, measured from 1D (*d*-nOe) and 2D (phase sensitive NOESY) experiments. Additional nOe's that further supported the structure

were the following: 2/3′, 2/Me-18, 5/Me-18, 7′/15′, 7′/Me-20, 9/Me-17, 9/Me-20, 11′/Me-20, 12/12′, 14/15, 14/Me-17, 15/15′, 15′/Me-17, Me-17/Me-16.

The configuration of the epoxide group in **2** was determined to be β (opposite to methyls 16, 17, 19, 20) on the grounds of an nOe between Me-17 and Me-16, an nOe between Me-17 and H-14, and the multiplicity of the latter oxirane proton (δ 2.58 brt, $J = 7.1$ Hz). The latter nOe's and J values of H-14 define the conformation of ring C, that is, a conformation in which carbons 11 to 15 are approximately in the same plane. The nOe between Me-17 and Me-20, regardless of the stereochemistry of C-9, is (according to a Dreiding model) only possible when Me-16 is in the α orientation (the same direction as Me's 17, 19, and 20).

The configuration of C-9 was also determined by measured nOe's. Thus, the H-9α configuration was deduced from nOe's between Me-20 and H-9α as well as between the Me-17 and H-9α and H-14α. The H-9α signal appears as a double doublet with coupling constants of 11.6 and 1.8 Hz, J values that agree well with a twisted boat conformation of ring B in which Me-17 and Me-16 are in spatial proximity as required for the explanation of the measured nOe between the two (see above). The expected energy increase in case of a twisted boat is expected to be diminished in part due to cancellation of the 1,3-diaxial interaction between Me-17 and Me-20. Furthermore, the nOe between Me-20 and H-7′ (vide supra) is also in good agreement with the suggested twisted boat conformation of ring B.

Outstanding in the ^{13}C-nmr spectrum of **2** was the 64.4 ppm δ_C value of C-9 [instead of an expected value of 58–61 ppm (7)]. This downfield shift may originate from the twisted boat conformation of ring B and the particular conformation of the seven-membered ring.

The third isolated compound, zaatirin [**3**], also had the formula $C_{20}H_{34}O$ (*m/z* 291 $[MH]^+$, and ^{13}C nmr data, Table 3) with four degrees of unsaturation. Two double bonds, an exo-methylene [δ_c 148.4 s, 109.3 t, and δ_H 4.71 (1H, brs) and 4.52 (1H, d, $J = 2.2$)] and a vinyl group [δ_c 147.9 d, 109.9 t and δ_H 5.91 (1H, dd, $J = 17.6$, 10.9), 5.03 (1H, d, $J = 17.8$) and 4.90 (1H, d, $J = 10.9$)], indicate a bicyclic molecule.

Because of the absence of OH and C=O absorptions in the ir spectrum and the existence of two oxygen-bearing carbon atoms (δ_c 72.8 s and 73.9 s), the single oxygen of the molecule had to be part of an ethereal bridge. Furthermore, the relative low-field of two of the four methyls (δ_H 1.27 s and 1.30 s) suggested they were in the α,α′ positions to the ethereal oxygen. As with compounds **1** and **2**, the structure assignment was mainly achieved from the HMQC and HMBC experiments (Table 3).

The CH correlations determined unequivocally two portions of the molecule, **a** and **b**, leaving out of the structure only two methylenes. Of the two halves of the molecule, moiety **a** was unequivocally confirmed by comparison of its ^{13}C-nmr data with the relevant data of dehydroambliol A (13).

Assuming compound **3** to be a diterpene with an undivided carbon skeleton, the two β,β′ methylenes of the ether moiety (C-10 and C-12, **b**), must be linked among themselves in one of the following ways: directly, thus leaving three methylenes to form the bridge between the two rings of **3**; through one methylene; or through two methylenes, to form in the three cases either a THF, THP, or an oxepane ring. The THF ring was immediately excluded on the grounds of ^{13}C-nmr data; comparison of the carbon chemical shifts of the heterocycle of **3** with those of the THF ring of davanone (14) showed a difference of ca. 10 ppm for the carbons in the α,α′ to the oxygen positions. The THP ring was preferred over the oxepane mainly on biogenetic considerations; since transformation of geranyllinalool (GeLi), to **3** can occur through intermediate **4** as shown in Scheme 1. Thus, zaatirin [**3**] most probably has the known diterpene skeleton of the algal metabolite caulerpol (15). The relative stereochemistry

TABLE 3. ^{1}H- and ^{13}C-nmr Chemical Shifts (ppm)[a] of Zaatirin [3] and Protons to Which Long-Range Correlations Were Observed in the HMBC Experiment.

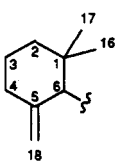
Position	δ_C	δ_H	*CH* Correlations
1	35.1 s		2, 2′, 3, Me-16, Me-17
2	36.3 t	1.45, 1.18	3, 4, Me-16, Me-17
3	24.0 t	1.55 (2H)	2′, 4, 4′
4	32.6 t	2.15, 2.05	2′
5	148.4 s		
6	55.2 d	1.70	2, 18, 18′, Me-16, Me-17
7	16.8 t	1.55, 1.50	
8	35.3 t	1.52, 1.28	11, Me-19
9	73.9 s		10′, Me-19
10	41.2 t	1.78, 1.38	Me-19
11	21.2 t	1.65, 1.62	
12	34.2 t	1.65, 1.40	Me-20
123	72.8 s		14, 15, 15′, Me-20
14	147.9 d	5.91	
15	109.9 t	5.03, 4.90	
16	26.8 q	0.91	Me-17
17	28.5 q	1.02	2, 2′, Me-16
18	109.3 t	4.71, 4.52	
19	28.3 q	1.27	
20	29.9 q	1.30	12

[a]At 500 MHz, C_6D_6; apostrophe on a proton indicates the lower field proton in a geminal pair.

of two out of the three chiral chenters of **3**, C-9 and C-13, was determined, from the nmr data, to be cis. An nOe between H-14 and Me-12 and the absence of such an effect between this proton and Me-19 suggested the vinyl group to be equatorial. Conformational considerations suggest the C-9 cyclohexylethyl substituent also to be equatorial; thus both Me-19 and Me-20 should be axial. Comparison of the ^{1}H chemical shifts of these two methyl groups and the vinyl protons (H-14, H-15, and H-16) with the corresponding ones in the caparrapi oxides (16) fully supports the 1,3-diaxial configuration of Me-19 and Me-20. The chirality of C-6, however, remains unassigned.

The biogenesis of each one of the three new compounds **1–3** is readily understandable in light of the knowledge of the biogenesis of other known terpenoids, starting from geranylgeranyol pyrophosphate (GeGePP) or geranyllinalol pyrophosphate (GeLiPP) (17). While compounds **2** and **3** keep the original carbon skeleton of GeGePP (or GeLiPP) the biogenesis of compound **1** has to involve several well known 1,2-shifts of protons and methyl groups—the same route, for example, as the one leading to the bicyclic portion of sigmoscepterellin (7).

None of the three new compounds was responsible for the cytotoxicity of the extract of the sponge. Work on the more polar metabolites from the active fraction is ongoing.



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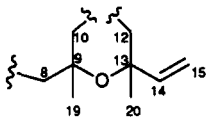

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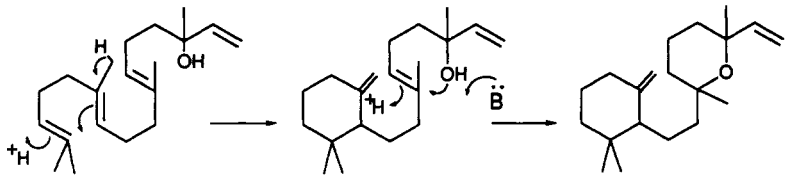

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SCHEME 1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Low resolution mass spectra were recorded on a Finnigan 4021 mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM 360 spectrometer and a 500 MHz GE OMEGA instrument.

COLLECTION, EXTRACTION AND ISOLATION.—The sponge was collected near Ras-Zaatir in the entrance to Marsa Bareka in the southern part of the Gulf of Eilat. A voucher specimen was kept in our collection in Tel Aviv (# TA-YK 2905). Compounds **2** and **3** were named after the place of collection and the name of compound **1** was derived from chelonaplysilla and clerodane, the diterpene skeleton of **1**. The sample was deep frozen immediately after collection, freeze-dried (20 g dry wt), and then extracted with $CHCl_3$ and 5% MeOH. The crude extract (200 mg) was separated by flash chromatography on Si gel H by using solvents of gradually increasing polarity from petroleum ether through EtOAc to give, in elution order, zaatirin [**3**] (8 mg), barekoxide [**2**] (8 mg), and chelodane [**1**] (80 mg). For nmr data see Tables 1–3. The proton coupling constants of each clear multiplet are given below for each compound.

Chelodane [**1**].—A foaming oil: ir ($CHCl_3$) 3600, 3480, 2900, 1620, 1450, 1330, 900 cm^{-1}; cims *m/z* (rel. int.) $[MH]^+$ 291 (3), $[MH-H_2O]^+$ 273 (100), 257 (10), 217 (25), 204 (15), 191 ($C_{14}H_{23}^+$) (50), 177 (20), 163 (25), 149 (25), 95 (30); ^{1}H nmr δ 5.71 (1H, dd, J = 17.4, 10.5, H-14), 5.14 (1H, dd, J = 17.4, 1.5, H-15′), 4.92 (1H, dd, J = 10.5, 1.5, H-15), 4.65 (brs, H-18′), 4.47 (1H, brs, H-18), 2.25 (1H, dt, J = 13.7, 5.1, H-3′), 2.18 (1H, bd, J = 13.7, H-3). *Anal.* found C 82.87, H 11.65; $C_{20}H_{34}O$ requires C 82.69, H 11.80%.

Barekoxide [**2**].—A foaming oil: ir ($CHCl_3$) 2920, 2800, 720 cm^{-1}; cims *m/z* (rel. int.) $[MH]^+$ 291 (42), $[MH-H_2O]^+$ 273 (100), 257 (5), 217 (10), 191 (15), 177 (20), 163 (15), 149 (10), 137 (10); ^{1}H nmr δ 2.58 (1H, t, J = 7.1, H-14), 1.88 (1H, dd, J = 14.0, 7.3 H-12′), 1.70 (1H, dd, J = 14.0, 6.7, H-15′), 1.58 (1H, dd, J = 7.9, 3.1, H-1′), 1.42 (1H, dd, J = 14.0, 7.0, H-12), 1.02 (1H, dd, J = 12.8, 3.9, H-7), 0.60 (1H, dd, J = 11.6, 2.2, H-9), 0.58 (1H, dd, J = 11.6, 2.4, H-5). *Anal.* found C 82.52, H 11.90; $C_{20}H_{34}O$ requires C 82.69, H 11.80%.

Zaatirin [**3**].—An oil: ir ($CHCl_3$) 2900, 2850, 1440, 880 cm^{-1}; cims *m/z* (rel. int.) $[MH]^+$ 291 (10), $[MH-H_2O]^+$ 273 (100), 217 (4), 206 (10), 179 (20), 177 (20), 149 (25), 139 (20), 137 (20), 123 (10), 109 (10); ^{1}H nmr δ 5.91 (1H, dd, J = 17.6, 10.9, H-14), 5.03 (1H, d, J = 8, H-15′), 4.90 (1H, d, J = 10.9, H-15), 4.71 (1H, brs, H-18′), 4.52 (1H, d, J = 2.2, H-18).

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