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ScienceDirect
Tetrahedron
Tetrahedron 62 (2006) 12092–12097

Novaxenicins A–D and xeniolides I–K, seven new diterpenes from the soft coral *Xenia novaebrittanniae*

Ashgan Bishara,[a] Amira Rudi,[a] Israel Goldberg,[a] Yehuda Benayahu[b] and Yoel Kashman[a,*]

[a]*School of Chemistry, Tel Aviv University, Ramat Aviv 69978, Israel*
[b]*Department of Zoology, Tel Aviv University, Ramat Aviv 69978, Israel*

Received 19 July 2006; revised 28 August 2006; accepted 15 September 2006
Available online 31 October 2006

Abstract—Seven new diterpenes, novaxenicins A–D (**1**–**4**) and xeniolides I–K (**5**–**7**), have been isolated from the Kenyan soft coral *Xenia novaebrittanniae*. The structures and relative stereochemistry of the compounds were elucidated by interpretation of MS, COSY, HSQC, HMBC, and NOESY experiments. The structure of novaxenicin A (**1**) was secured by X-ray diffraction analysis. Compound **5** possesses anti-bacterial activity at a concentration of 1.25 μg/ml and compound **2** induces apoptosis in transformed mammalian cells at a concentration of 1.25 μg/ml.

1. Introduction

Octocorals of the genera *Xenia* (order Alyconaceae, family Xeniidae) are a rich source of diterpenoids containing nine-membered carbocylic rings.[1–3] Xenicin isolated from *Xenia elongata* was the first reported compound.[4] Soon after additional members of the group were reported from the Red Sea *Xenia macrospiculata*.[5,6] The structures of the *Xenia* diterpenoids have been divided by us into three groups: xenicins or xenicane type (containing a dihydropyran-cyclononane skeleton), xeniolides (possessing a δ-lactone-cyclononane skeleton), and xeniaphyllanes (possessing a prenylated caryophyllene skeleton).[7]

Dozens of *Xenia*-isoprenoides have since been reported (53 reports)[3] with all kinds of modifications in the ring system as well as in the prenyl side chain. More recently two additional types of compound have been added, i.e., the xeniaethers[8] and the azamilides.[9] Antheliolide A isolated from *Anthelia glauca*, is an example of a more complex penta cyclic secondary metabolite of mixed-biogenesis (C_{20}+C_4) incorporating the xeniaphyllane ring system.[10]

The present work describes the isolation and structural elucidation of seven new *Xenia* diterpenes, designated as novaxenicins A–D (**1**–**4**) and xeniolides I–K (**5**–**7**) according to their structures (Figs. 1 and 2). The compounds were isolated from the Kenyan soft coral *X. novaebrittanniae* (Ashworth, 1900) collected in Kitagamwa, southern Kenya (04° 48′ 49″ S, 39° 21′ 60″ E, February, 2004). The collection was done on a reef, at a depth of 8 m, characterized by highly diverse soft coral fauna. The colonies there form large patches, growing mainly on dead colonies of stony corals. The type locally of species is New Britain and Loyalty Islands; later it was recorded in the northern Red Sea.[11] Further records, not yet verified, are from Philippines, the Malay Archipelago, the Great Barrier Reef Australia, and New Caledonia.[12]

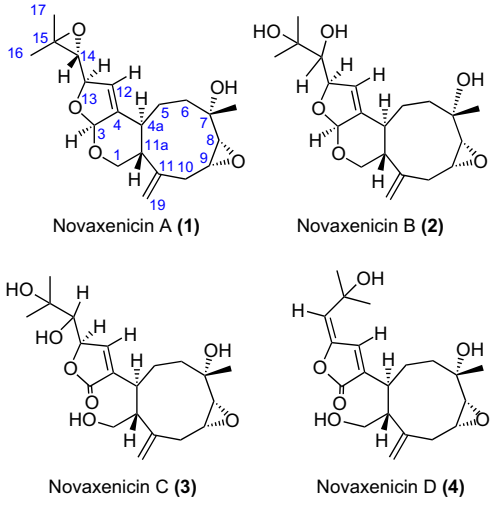

Figure 1. Novaxenicins A–D (**1**–**4**).

Keywords: Soft coral; Diterpenes; Novaxenicins; Xeniolides.
* Corresponding author. Tel.: +972 3 6408419; fax: +972 3 6409293; e-mail: kashman@post.tau.ac.il

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doi:10.1016/j.tet.2006.09.050

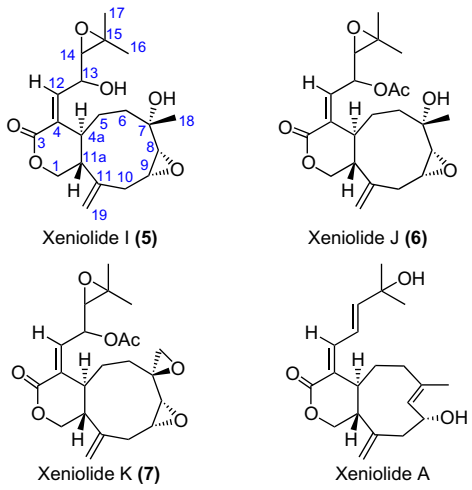

Figure 2. Xeniolides A and I–K (**5**–**7**).

2. Results and discussion

The ethyl acetate extract of the *Xenia* (35 g, dry weight) was repeatedly chromatographed over Sephadex LH-20 columns, followed by vacuum–liquid chromatography (VLC) over silica gel to yield the seven new compounds.

The CIMS spectrum of **1** exhibited a pseudo molecular ion $[M+H]^+$ at *m/z* 349. The molecular formula $C_{20}H_{28}O_5$ was suggested by the ^{13}C NMR data and later confirmed by X-ray diffraction analysis (see below). Both the ^1H NMR and the ^{13}C NMR spectra of **1** and most other herewith described compounds, were well resolved in C_6D_6 (Tables 1–3). The ^{13}C and ^1H NMR experiments revealed the presence of an exocyclic double bond (δ_C 142.1 s, 118.7 t and δ_H 4.90 t, 4.97 br s), one tri-substituted double bond (δ_C 142.1 s, 118.7 d and δ_H 5.75 br s), two epoxides (δ_C 61.7 d, 60.9 d and 64.9 d, 58.5 s and δ_H 2.36 d, 2.84 ddd and 3.06 d),

Table 1. ^{13}C NMR spectral data of compounds **1**–**7**[c]

C	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[a]
1	69.4 t	69.7 t	64.6 t	63.9 t	69.9 t	70.5 t	69.4 t
3	108.0 d	107.4 d	173.1 s	169.5 s	169.1 s	169.9 s	168.7 s
4	143.3 s	143.8 s	136.8 s	136.2 s	135.8 s	137.8 s	136.3 s
4a	35.2 d	36.1 d	33.0 d	32.1 d	36.2 d	35.4 d	33.9 d
5	25.7 t	28.9 t	29.1 t	28.2 t	32.5 t	31.6 t	32.9 t
6	33.9 t	34.8 t	35.7 t	33.8 t	32.7 t	31.9 t	27.8 t
7	72.2 s	72.3 s	72.1 s	71.8 s	70.9 s	70.6 s	54.7 s
8	61.7 d	62.4 d	61.8 d	61.3 d	62.5 d	62.5 d	55.7 d
9	60.9 d	61.7 d	60.5 d	60.3 d	59.1 d	59.4 d	58.0 d
10	29.1 t	29.1 t	28.5 t	27.3 t	34.8 t	33.9 t	33.1 t
11	142.1 s	143.4 s	147.1 s	144.2 s	144.3 s	143.0 s	142.4 s
11a	53.9 d	55.0 d	54.1 d	53.5 d	44.4 d	44.6 d	43.7 d
12	119.9 d	118.6 d	151.1 d	137.6 d	141.0 d	134.9 d	135.8 d
13	85.0 d	89.3 d	82.6 d	147.3 s	66.3 d	69.4 d	69.6 d
14	64.9 d	78.1 d	78.3 d	122.0 d	65.0 d	62.8 d	62.6 d
15	58.5 s	71.1 s	72.0 s	70.9 s	59.5 s	59.7 s	59.0 s
16	18.5 q	25.3 q	28.4 q	30.9 q	19.1 q	18.4 q	18.0 q
17	24.2 q	26.2 q	27.2 q	31.0 q	24.1 q	24.6 q	24.5 q
18	31.8 q	32.1 q	32.6 q	31.5 q	32.7 q	32.5 q	48.4 t
19	118.7 t	119.7 t	118.9 t	119.8 t	119.8 t	116.8 t	116.4 t
Ac						169.6 s, 20.8 q	169.0 s, 20.7 q

[a] Recorded in $CDCl_3$ solution measured at 100 MHz.
[b] Recorded in d_6-DMSO solution measured at 100 MHz.
[c] Multiplicities were determined by DEPT and HSQC experiments.

one methylenoxy group (δ_C 69.4 t, and δ_H 3.39 t, 3.80 dd), one methineoxy group (δ_C 85.0 d and δ_H 5.75 s), one methinedioxy group (δ_C 108.0 d, δ_H 5.58 s), and one *tert*-alcohol (δ_C 72.2 s).

The above functionalities account for four of the seven degrees of unsaturation of **1**, suggesting three additional rings. The COSY spectrum revealed the presence of three spin systems (I–III) depicted in Figure 3. HMBC correlations connected the latter three spin systems enabling the construction of the planar structure of **1** as shown in Figure 3.

The relative stereochemistry of most of **1** was determined by the analysis of coupling constants and from NOESY cross-peaks (Fig. 4). A cis-configuration for the 8(9) epoxy moiety, a trans-configuration for H-4a and H-11a and a ca. 90° dihedral angle between H-12 and H-13, were determined from the measured 4.4,[13,14] 11.0,[14] and 0 Hz coupling constants, respectively.

Further support for the suggested stereochemistry came from NOE cross-peaks depicted in Figure 4. NOEs between CH_3-18 and H-6β (the same side as H-11a) suggested that this methyl group was also β, an NOE between H-3 and H-13 determined them to be cis-oriented and an NOE between CH_3-16 and H-14 distinguished between the geminal methyl pair. The suggested stereochemistry of the nine-membered ring is the same as in known *Xenia* diterpenes as, e.g., in isoxeniatin C[9] and in the blue coral secondary metabolites, helioxenicins A–C.[14] Helioxenicin C, possessing the same ring system as compound **1**, is closest in structure to **1**, as seen from the NMR data. Novaxenicin A differs from helioxenicin C in C-1 (a CH_2O group against a lactol) and in the configuration of C-3.

The full relative stereochemistry of **1** was secured by X-ray diffraction analysis (Fig. 5 and Section 4). The absolute configuration of this light atom structure cannot be reliably determined from diffraction data. Yet, it is more likely (based on the Flack parameter)[15] to be the one shown in Figure 5 and known for the other *Xenia* diterpenoids in the series.

The sensitivity of the conformation of the nine-membered ring to small structural changes is revealed by changes in the proton chemical shifts and coupling constants, e.g., (δ_H 2.63 d, 2.43 d; H-8), (δ_H 3.03 m, 3.12 ddd; H-9), (δ_H 3.52 br t, δ_H 3.85 dd; H-4a) and (δ_H 2.64 br t, δ_H 3.21 dd; H-11a) (Table 2), for compounds **3** and **4**, respectively.

The CIMS spectrum of novaxenicin B (**2**) exhibited a pseudo molecular ion at *m/z* 367 $[M+H]^+$. The molecular formula $C_{20}H_{30}O_6$ was determined by HRCIMS. ^{13}C and ^1H NMR revealed high similarity to **1**, namely, **2** possessing the same tricyclic ring system, but differing in the side chain. The 14(15) epoxide of **1** is exchanged by a 14,15-diol in **2** (δ_C, 78.1 d and 71.1 s for C-14 and C-15, respectively) (Fig. 1). The latter change of functionality brought about a change in the vicinal coupling constant between H-13 and H-14, from 8.7 Hz for **1** to 3.3 Hz for **2**. The expected conformational mobility of the side chain prevented the determination of the chirality of C-14. Moreover, as it is unknown if the 14,15-epoxide of **1**, that is, expected to be obtained from the 14(15) double bond, is the precursor

Table 2. ^{1}H NMR spectral data of novaxenicins A–D (**1**–**4**)[c]

H	**1**[a]	**2**[a]	**3**[b]	**4**[a]
1	3.80 dd (11.4, 4.6)	3.65 dd (11.8, 4.6)	3.21 ddd (11.2, 4.3, 0.9)	3.63 dt (11.4, 4.1)
	3.39 t (11.4)	3.32 t (11.8)	3.05 m	3.48 dd (11.4, 4.1)
3	5.58 s	5.43 s		
4a	3.51 br t (11.2)	3.45 br t (11.2)	3.52 br t (11.8)	3.85 dd (11.6, 8.6)
5	1.65 m	1.81 m	1.85 t (14.1)	1.92 dt (1.6, 14.9)
	1.50 dt (2.6, 14.9)	1.49 dt (1.9, 14.1)	1.43 m	1.67 m
6	1.83 dt (2.8, 14.9)	1.73 m	1.64 t (14.1)	1.23 m
	1.30 dq (14.9, 2.6)	1.25 m	1.35 m	1.21 m
8	2.36 d (4.0)	2.29 d (4.2)	2.63 d (4.4)	2.43 d (4.0)
9	2.84 ddd (12.0, 5.0, 4.0)	2.78 ddd (10.0, 5.1, 4.2)	3.03 m	3.12 ddd (11.3, 5.1, 4.0)
10	2.61 dt (1.5, 12.0)	2.45 ddd (12.4, 10.0, 0.9)	2.75 t (12.1)	2.90 dt (5.1, 11.3)
	2.54 dd (12.0, 5.0)	2.48 dd (12.4, 5.1)	2.52 br t (12.1)	2.78 br dt (1.7, 12.9)
11a	2.40 dt (4.6, 11.2)	2.35 dt (4.6, 11.2)	2.64 br t (11.8)	3.21 dd (11.6, 4.1)
12	5.75 br s	6.04 s	7.28 s	8.31 s
13	4.78 dddd (8.7, 3.9, 2.6, 0.9)	5.20 br t (3.3)	5.19 br s	
14	3.06 d (8.7)	3.74 d (3.3)	3.48 d (4.6)	5.70 s
16	1.37 s, 3H	1.20 s, 3H	1.19 s, 3H	1.34 s, 3H
17	1.22 s, 3H	1.42 s, 3H	1.12 s, 3H	1.38 s, 3H
18	1.23 s, 3H	1.21 s, 3H	1.24 s, 3H	1.23 s, 3H
19	4.97 br s	4.89 s	5.14 s	5.28 s
	4.90 d (1.3)	4.83 s	5.08 s	5.18 s

[a] Recorded in C_6D_6 solution measured at 400 MHz.
[b] Recorded in d_6-DMSO solution measured at 400 MHz.
[c] The *J*-values in hertz are indicated in parentheses.

Table 3. ^{1}H NMR spectral data of xeniolides I–K (**5**–**7**)[c]

H	**5**[a]	**6**[b]	**7**[b]
1	3.96 dd (10.6, 4.2)	4.18 dd (10.7, 4.0)	4.32 dd (11.1, 4.5)
	3.75 t (10.6)	3.94 t (10.7)	4.05 dd (11.1, 9.2)
4a	3.56 ddt (11.0, 1.5, 4.0)	3.89 m	3.82 dd (11.6, 5.6)
5	1.89 m	1.82 m	1.79 m
	1.49 m	1.64 m	1.75 m
6	2.08 ddd (15.0, 11.1, 3.6)	2.18 ddd (14.6, 10.6, 2.6)	2.10 ddd (15.2, 7.2, 4.6)
	1.23 m	1.60 m	1.93 ddd (15.2, 9.3, 4.8)
8	2.20 d (3.8)	2.82 d (3.9)	3.23 d (3.8)
9	2.58 dt (10.9, 3.8)	2.99 dt (11.3, 3.9)	3.07 dt (11.1, 3.8)
10	2.85 dd (13.4, 10.9)	3.08 dd (13.2, 11.3)	2.97 dd (14.0, 11.1)
	2.51 dd (13.4, 3.8)	2.85 dd (13.2, 3.9)	2.80 dd (14.0, 3.8)
11a	2.41 m	2.64 br dt (4.0, 11.9)	2.77 m
12	6.84 dd (9.4, 1.5)	6.29 dd (1.5, 10.1)	6.43 dd (10.9, 0.8)
13	4.56 dd (9.4, 8.1)	5.40 dd (10.1, 8.4)	5.32 dd (10.9, 8.4)
14	2.70 d (8.1)	2.96 d (8.4)	2.98 d (8.4)
16	1.39 s, 3H	1.39 s, 3H	1.37 s, 3H
17	1.18 s, 3H	1.35 s, 3H	1.36 s, 3H
18	1.13 s, 3H	1.36 s, 3H	2.83 d (5.6)
			2.57 d (5.6)
19	4.81 s	5.19 br s	5.20 s
	4.59 s	5.01 d (1.2)	5.06 d (1.5)
Ac		2.01 s, 3H	2.01 s, 3H

[a] Recorded in C_6D_6 solution measured at 400 MHz.
[b] Because of the better resolution, the NMR of **6** and **7** were taken in $CDCl_3$ at 400 MHz.
[c] The *J*-values in hertz are indicated in parentheses.

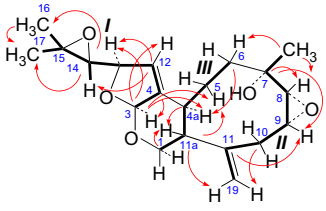

Figure 3. ^{1}H–^{1}H COSY (I–III) and selected HMBC correlations for novaxenicin A (**1**).

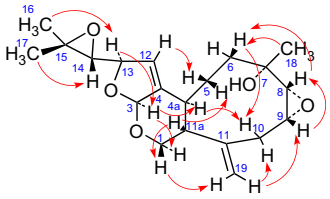

Figure 4. Selected NOESY for novaxenicin A (**1**).

of **2**, no conclusion about the configuration of C-14 could be reached.

Novaxenicin C (**3**) was found to possess a molecular formula $C_{20}H_{30}O_7$ as established from its HRESIMS (*m*/*z* 405.1907, $[M+Na]^+$), implying six degrees of unsaturation. The NMR data of compound **3**, the most polar compound among the seven (several OH groups, ν_{max} 3560 cm^{-1}) had to be determined in d_6-DMSO. The NMR data revealed the presence of the following functionalities: an exocyclic double bond (δ_C 118.9 t, 147.0 s), an α,β-unsaturated butenolide (δ_C 173.1 s, 136.8 s, 151.1 d, 82.6 d), an epoxide (δ_C 61.8 d, 60.5 d), a methyleneoxy group (δ_C 64.6 t), one methineoxy group

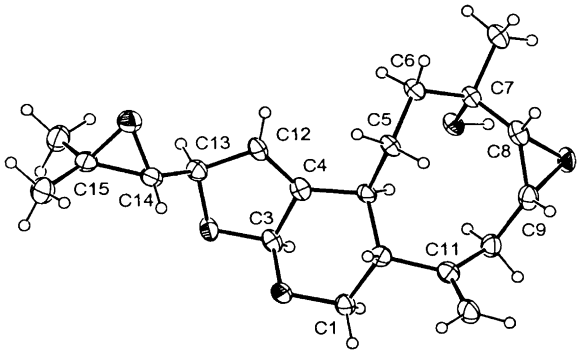

Figure 5. ORTEP representation of novaxenicin A (**1**) as determined by signal-crystal X-ray analysis.

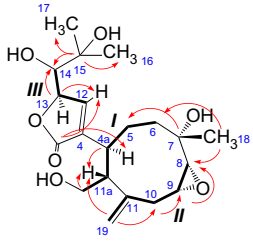

Figure 6. ^{1}H–^{1}H COSY (I–III) and HMBC correlations for novaxenicin C (**3**).

(δ_C 78.3 d), and two tertiary alcohols (δ_C 72.1 s, 72.0 s) carrying, together, three methyl groups (δ_H 1.19 s, 1.12 s, and 1.24 s).

The above functionalities account for five of the six degrees of unsaturation of **3** suggesting one additional ring. The COSY spectrum revealed the presence of three spin systems (I–III, Fig. 6), which could be linked together by CH-correlations (HMBC) to establish the planar structure of **3** (Fig. 1). Novaxenicin C possesses two of the three rings constructing the ring system of novaxenicins A and B (**1** and **2**). The relative stereochemistry of **3** was established, as for **1** and **2**, from the coupling constants and NOEs (Fig. 7), i.e., the chirality of the five asymmetric centers of the nine-membered ring is the same as in **1** and **2**. Additionally, a 13*R** configuration is assumed on the basis of common biogenesis of **1**–**4**. The configuration of C-14 on the other hand, as in the case of **2**, remains unsolved.

The CIMS spectrum of **4** exhibited a pseudo molecular ion $[M+H]^+$ at *m*/*z* 365. The molecular formula was determined by HRMS to be $C_{20}H_{28}O_6$, which agrees with the loss of a molecule of water from **3**. The ^{13}C and ^{1}H NMR spectra of **4** are almost identical to those of **3**, the only major difference being an additional $\Delta^{13(14)}$ double bond (δ_C 147.3 s, 122.0 d) instead of the 14-hydroxy group. NOEs between CH_3-16 and CH_3-17 to H-12 and H-14, respectively, established the $\Delta^{13(14)}$ *E* configuration and point to a preferred conformation around the 14(15) double bond. Notable in the ^{1}H NMR spectrum of **4** is the low field chemical shift of H-12 (δ_H 8.31 s) due to its β-position to the lactone carbonyl group and its allylic position to the Δ^{13} bond.

The three additional isolated compounds (**5**–**7**) possessing the ring system of xeniolide A were designated xeniolides I–K (Fig. 2).

The CIMS spectrum of xeniolide I (**5**) exhibited a molecular ion $[M+H]^+$ at *m*/*z* 365. The molecular formula was determined by HRCIMS to be $C_{20}H_{28}O_6$. The NMR data of **5** revealed, in addition to the same substituted nine-membered rings of **1**–**4**, a δ-lactone conjugated to an exocyclic

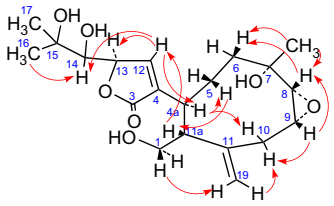

Figure 7. Observed NOESY for novaxenicin C (**3**).

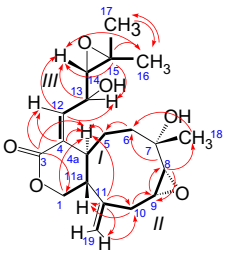

Figure 8. COSY (I–III) and selected HMBC correlations for xeniolide I (**5**).

tri-substituted double bond (δ_C 69.9 t, 169.1 s, 135.8 s, 141.0 d) condensed to the nine-membered ring, a methineoxy group (C-13, δ_C 66.3 d), and a tri-substituted epoxide (C-14,15, δ_C 65.0 d, 59.5 s) with two substituting CH_3 groups (Me-16,17, δ_C 19.1 q, 24.1 q). The COSY spectrum (Fig. 8) revealed for **5** the presence of three spin systems (I–III) that were joined together by long-range CH-correlations (HMBC) (Fig. 8), establishing its planar structure (Fig. 2). The *E* configuration of the 4(12)-double bond as in xeniolide A was established from the low field chemical shift of H-12 (δ_H 6.84, against 6.40 in case of the *iso*-isomer)[16] and NOEs between H-13 and H-5. Proton H-13 in all three new xeniolides (**5**–**7**) possesses two large coupling constants (ca. 8 and 10 Hz) with its neighbors, H-12 and H-14, pointing to two anti-conformations but, because of conformational mobility of the side chain, it was impossible to suggest unambiguously the stereochemistry of C-13 and C-14.

Xeniolide J (**6**), $C_{22}H_{30}O_7$ (HRCIMS), is the 13-acetoxy derivative of **5** (Fig. 2). Comparison of the ^{1}H NMR spectrum of **6** with that of **5**, Table 3, showed a downfield shift of H-13 from 4.56 dd (*J*=9.4, 8.1) to 5.40 dd (*J*=10.1, 8.4).

Xeniolide K (**7**), $C_{22}H_{28}O_7$ (HRCIMS), possesses, according to the NMR data, the same side chain (C-4 to CH_3-16, CH_3-17) and butanolide (C-1, C-4a, and C-11a) as **6**, but differs in the substitution of the nine-membered ring. Namely, the 18-methyl group disappeared and instead showed up a new 1,1-disubstituted epoxide (δ_C 54.7 s, 48.4 t), as in the havannahines,[17,18] as confirmed by HMBC correlations from C-6 and C-7 to H-18,18′.

Comprehensive comparison of the NMR data of **7** with that of the havannahine isomers (xenicins possessing the 7(18), 8(9) diepoxy moieties),[18] exhibited good agreement with havannahine itself, over the other isomers. The suggested structure is also in good agreement with the NOESY cross-peaks (Fig. 9), though, the epimeric 7(18) epoxide cannot

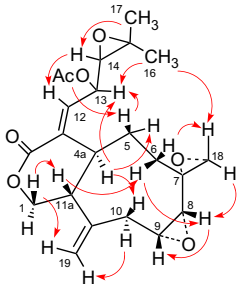

Figure 9. Selected NOESY correlations for xeniolide K (**7**).

unambiguously be excluded. Change of the epoxide stereochemistry is not expected to significantly influence the observed NOEs.

All new compounds and especially xeniolide K are very acid sensitive because of the epoxy groups.

Compound **5** possesses anti-bacterial activity at a concentration of 1.25 μg/ml (*Escherichia coli* and *Bacillus subtilis*) and compound **2** induces apoptosis in transformed mammalian cells at a concentration of 1.25 μg/ml.[19]

Noteworthy is an earlier report on *X. novaebrittanniae* collected at Laing Island, Papua-New Guinea, affording three different compounds, a xenicin derivative and two *iso*-xeniolide A derivatives. Isolation of divergent secondary metabolites from the same soft coral collected from different localities is well known—the reason for this still being a source for debate.[20]

3. Conclusion

The four novaxenicins (**1**–**4**) together with helioxenicin C[14] build an interesting group of compounds related to the *Xenia* diterpenoids. Biogenetically, the novaxenicins and the xeniolides (Figs. 1 and 2) may be obtained from a similar precursor, regarding the common two xeniolide rings,[7] namely, a metabolite possessing a 1-methyleneoxy and a 3-carboxaldehyde group. The latter can then undergo different routes that will lead to either xeniolides (oxidation of C-3, before or after C-1 to C-3 cyclization), or form a dihydrofurane or a butenolide ring, from C-3 to C-13, by the attack of the C-3 lactol OH or carboxylic group on C-13, to create the novaxenicins. It is unlikely that during the isolation process the two stable ring systems will convert one to the other.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained with a Jasco P-1010 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-500 and Avance-400 spectrometers. ^{1}H, ^{13}C, COSY, HSQC, NOESY, and HMBC were recorded using standard Bruker pulse sequences. EIMS, CIMS, and HRMS measurements were recorded on a Fisons, Autospec Q instrument. Electrospray MS measurements were performed on an AppliedBiosystems Q-STAR Pulsar instrument (ESI-QqTOF).

4.2. Biological material

The soft coral *X. novaebrittanniae* (Ashworth, 1900) was collected at Kitagamwa in February, 2004. A voucher specimen is deposited at the Zoological Museum, Tel Aviv University, Israel (ZMTAU CO 32253).

4.3. Extraction and isolation

Freeze-dried soft coral (KB-2177, 35 g) was homogenized and extracted with ethyl acetate (0.2 l×3) to give after evaporation a brown gum (3.5 g). The gum was chromatographed on a Sephadex LH-20 column, eluting with hexane–$CHCl_3$–MeOH (2:1:1), 12 fractions of 20 ml were collected.

Interesting fractions from the Sephadex column were further separated on the methanol-washed silica gel (VLC) as follows: fraction 5 (46 mg) afforded with hexane–ethyl acetate (7:3) compound **1** (18.5 mg 0.052% dry weight) and with 2:3 compounds **6** (3.9 mg 0.011% dry weight) and **7** (4.5 mg 0.012% dry weight). Fractions 7 and 8 (98 mg) afforded with hexane–ethyl acetate (2:3) compounds **2** (19 mg 0.0547% dry weight) and **5** (6.2 mg 0.017% dry weight). Fractions 9 and 10 (49 mg) afforded with hexane–ethyl acetate ratio 3:7 compound **4** and with 2:8 compound **3**. Compound **4** (9.4 mg 0.026% dry weight) was further purified by HPLC (RP-18) eluted with CH_3CN–H_2O (85:15), and compound **3** (3.2 mg 0.009% dry weight) with CH_3CN–H_2O (80:20).

4.3.1. Novaxenicin A (1). Colorless crystals (crystallized from acetone); mp 168–172 °C; $[\alpha]_D^{22}$ −34 (*c* 0.98, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3436, 3023, 2838, 1729, 1426, 1331, 1219, 1016, 929 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; CIMS *m/z* 349 $[M+H]^+$ (86), 331 (35) ($[M+H]^+-H_2O$), 259 (55) ($[M+H]^+-C_4H_{10}O_2$), 71 (75) (C_4H_7O), 277 (100) ($[M+H]^+-C_4H_8O$).

4.3.2. Novaxenicin B (2). Pale yellow oil; $[\alpha]_D^{22}$ −37 (*c* 1.81, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3684, 3500, 3019, 1731, 1425, 1215, 1031, 928 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 1 and 2; CIMS *m/z* 367 $[M+H]^+$ (8), 365 (10) ($[M+H]^+-H_2$), 347 (16) ($[M+H]^+-H_2-H_2O$), 278 (85) ($[M+H]^+-C_4H_9O_2$), 151 (100) ($[M+H]^+-C_{13}H_{12}O_3$); HRCIMS *m/z* 367.2042 (Calcd for $C_{20}H_{30}O_6$, 367.2046).

4.3.3. Novaxenicin C (3). Pale yellow oil; $[\alpha]_D^{22}$ −12 (*c* 0.3, MeOH); IR (MeOH) ν_{max} 3684, 3480, 3018, 1754, 1424, 1211, 1030, 922 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 1 and 2; ESIMS *m/z* 405.17 $[M+Na]^+$; HRESIMS *m/z* 405.1907 (Calcd for $C_{20}H_{30}O_7Na$, 405.1883).

4.3.4. Novaxenicin D (4). Pale yellow oil; $[\alpha]_D^{22}$ −77 (*c* 0.94, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3680, 3019, 1754, 1426, 1216, 1031, 927 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 1 and 2; CIMS *m/z* 365 $[M+H]^+$ (20), 347 (85) ($[M+H]^+-H_2O$), 83 (100) (C_5H_7O); HRCIMS *m/z* 365.1881 (Calcd for $C_{20}H_{28}O_6$, 365.1885).

4.3.5. Xeniolide I (5). Pale yellow oil; $[\alpha]_D^{22}$ +41 (*c* 0.17, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3480, 3020, 1750, 1616, 1216, 1032, 926 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 1 and 3; CIMS *m/z* 365 $[M+H]^+$ (20), 329 (100) ($[M+H]^+-2H_2O$), 347 (40) ($[M+H]^+-H_2O$), 293 (40) ($M+H]^+-C_4H_8O$); HRCIMS *m/z* 365.1963 (Calcd for $C_{20}H_{28}O_6$, 365.1964).

4.3.6. Xeniolide J (6). Pale yellow oil; $[\alpha]_D^{22}$ +10 (*c* 0.13, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3627, 3020, 1751, 1630, 1204, 1015, 922 cm^{-1}; ^{13}C and ^{1}H NMR see Tables 1 and 3; CIMS *m/z* 407 $[M+H]^+$ (15), 347 (82) ($[M+H]^+-C_2H_4O_2$), 329 (100) ($[M+H]^+-C_3H_{10}O_2$); HRCIMS *m/z* 407.2067 (Calcd for $C_{22}H_{30}O_7$, 407.2069).

4.3.7. Xeniolide K (7). Pale yellow oil; $[\alpha]_D^{22}$ −27 (*c* 0.28, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3475, 3011, 1745, 1640, 1204, 1160, 970 cm^{-1}; ^{13}C and ^1H NMR see Tables 1 and 3; EIMS *m/z* 404 $[M]^+$ (12), 345 (100) ($[M]^+-C_2H_3O_2$), 287 (55) ($[M]^+-C_5H_9O_3$); HREIMS *m/z* 404.1831 (Calcd for $C_{22}H_{28}O_7$, 404.1835).

4.4. X-ray crystallographic analysis

The measurements were carried out on a Nonius KappaCCD diffractometer at low temperature (ca. 110 K) in order to optimize the precision of the crystallographic determination, with Mo Kα radiation.

Crystal data: $C_{20}H_{28}O_5$, *M*=348.42, monoclinic, space group $P2_1$, *a*=10.9750(11), *b*=7.7323(4), *c*=11.9516(12) Å, β=114.545(3)°, *V*=922.59(14) $Å^3$, *Z*=2, *T*=110(2) K, D_c=1.254 g cm^{-3}, μ(Mo Kα)=0.09 mm^{-1}, 2245 unique reflections to $2\theta_{max}$=51.4°, 229 refined parameters, R_1=0.058 for 3191 observations with $I>2\sigma(I)$, R_1=0.095 (wR_2=0.119).

The molecule geometry, including that of epoxide fragments, revealed common bond lengths and bond angle characteristics, and the polar space group of the crystal is consistent with the chiral nature of this compound. The central six-membered ring adopts a chair conformation, the unsaturated five-membered ring is planar, and the eight-membered ring has a strain-optimized conformation. In the crystal, neighboring molecules displaced along **b** in both directions are O–H···O hydrogen bonded to one another, thus forming supramolecular chains along the polar direction. These bonds involve the ethereal *O*-atom of the six-membered ring of one species as proton acceptor and the hydroxyl group of another unit as proton donor [O···O 2.940(3) Å, O–H···O 151°]. Side-packing of these chains perpendicular to **b** is stabilized by van der Waals forces.

Crystal data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CDCC deposition number 613498. The supplementary crystallographic data for this paper can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1233 336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk/).

Acknowledgements

We thank Dr. Ayelet Sacher (of the Maiman Institute for Proteome Research, Tel Aviv University) for performing the electrospray mass measurements. Special thanks are due to Mr. Charles O. Oduol, Assistant Director of Fisheries, Mombasa, Ministry of Agriculture and Rural Development, Fisheries Department, Kenya, for issuing the collection permit for the research expedition. Micha Ilan and Ruth Gottlieb (National Center for High Throughput Screening (HTS) of Novel Bioactive Compounds, Tel Aviv University) are acknowledged for their biological evaluation of the compounds.

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