Kitungolides A, B, and C, New Diterpenes from a Soft Coral of a New Genus
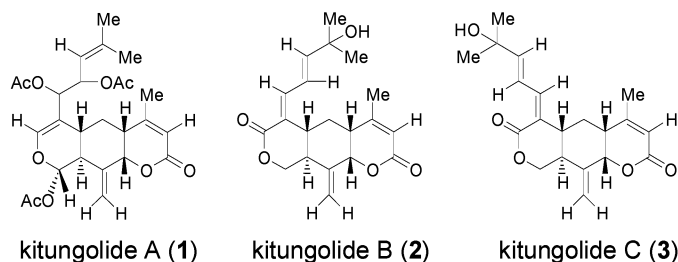
Liat Chill,[†] Amira Rudi,[†] Yehuda Benayahu,[‡] Michael Schleyer,[§] and Yoel Kashman*,[†]

School of Chemistry and Department of Zoology, Tel-Aviv University, Ramat Aviv 69978, Israel, and Oceanographic Institute, Durban, Republic of South Africa

kashman@post.tau.ac.il

ABSTRACT

Three novel compounds, designated kitungolides A (1), B (2), and C (3), were isolated from a soft coral of a new genus collected at Kitungamwe, Kenya. The three new compounds are of a unique heterotricyclic skeleton. The structures and relative stereochemistry of the compounds were elucidated by interpretation of MS, COSY, HMQC, HMBC, and NOESY experiments.

In the search for bioactive substances from marine invertebrates,[1,2] three novel diterpenoids designated kitungolides A (1),[3] B (2),[4] and C (3)[5] were isolated from a Kenyan soft coral of a new genus.[6] All three compounds are of a unique heterotricyclic skeleton consisting of an unprecedented hexahydrocoumarine system. In this paper we present the isolation and structure determination of these novel marine metabolites.

* To whom correspondence should be addressed. Phone: +972-3-640-8419. Fax: +972-3-640-9293.
† School of Chemistry, Tel Aviv University.
‡ Department of Zoology, Tel Aviv University.
§ Oceanographic Institute.

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(3) Kitungolide A (1): an oil; [α]$^{21}_D$ +36.5° (*c* 0.71, CHCl$_3$); for ^{1}H and ^{13}C NMR data, see Table 1; CIMS *m/z* 489 [M + H]+, 429 [M + H − Ac]+ (15), 369 [M + H − 2Ac]+ (90), 327 (95), 309 [M + H − 3Ac]+ (100), 277 (45), 259 (30); HRCIMS *m/z* 489.2124 (calcd for C$_{26}$H$_{33}$O$_9$, 489.2126, Δ = 0.1 ppm).
(4) Kitungolide B (2): an oil; [α]$^{21}_D$ +70.6° (*c* 0.11, CHCl$_3$); for ^{1}H and ^{13}C NMR data, see Table 1; CIMS *m/z* 345 [M + H]+ (60), 327 [M + H − H$_2$O]+ (100), 301 (90), HRCIMS *m/z* 345.1702 (calcd for C$_{26}$H$_{32}$O$_9$, 345.1702, Δ = −0.1 ppm).
(5) Kitungolide C (3): an oil; [α]$^{21}_D$ +54.5° (*c* 0.07, CHCl$_3$); for ^{1}H and ^{13}C NMR data, see Table 1; CIMS *m/z* 345 [M + H]+ (60), 327 [M + H −H$_2$O]+ (100), 301 (90), HRCIMS *m/z* 345.1699 (calcd for C$_{26}$H$_{32}$O$_9$, 345.1702, Δ = 0.8 ppm).

kitungolide A (1)

kitungolide B (2) [4(11)*E*]
kitungolide C (3) [4(11)*Z*]
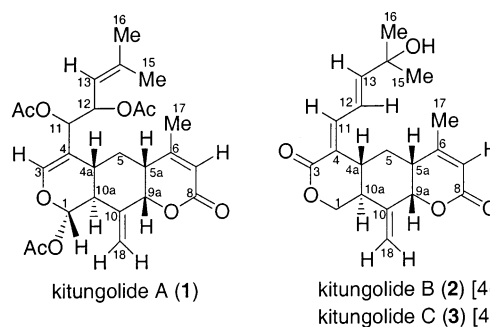
The CIMS of **1** exhibited a pseudomolecular ion [M + H]+ at *m/z* 489. The molecular formula C$_{26}$H$_{32}$O$_9$ was

(6) For description of the coral, see Supporting Information.
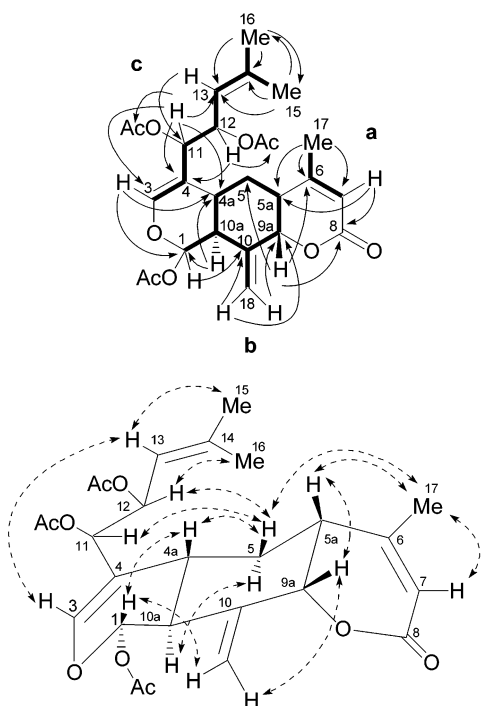
Figure 1. COSY (—), selected HMBC (→ (solid)), and NOESY (↔ (dashed)) correlations of kitungolide A (**1**).

determined by HRMS. The IR spectrum (CHCl₃) suggested the presence of an ester group (1724 cm^{-1}) as well as a terminal methylene (890 cm^{-1}). The ^{13}C NMR and ^{1}H NMR experiments revealed the presence of three acetate groups (δ_C 169.7 s and 20.8 q; 169.8 s and 21.4 q; 170.0 s and 21.2 q; δ_H 2.00, 2.01, and 2.11 s, 3H each, respectively), one acetal [δ_C 90.7 d, δ_H 6.20 ($J = 9.5$ Hz)], one enol ether [δ_C 143.5 and 110.2 s, δ_H 6.40d ($J = 2.0$ Hz)], one β-methyl-$\alpha\beta$-unsaturated lactone [δ_C 164.1 s, 116.2 d, 161.2 s 21.2 q, δ_H 5.87 s, 2.05 s (3H)], one exocyclic double bond (δ_C 141.2 s, 112.7 t, δ_H 4.75 s, 5.15 s) and a $-CH=C(CH_3)_2$ group [δ_C 118.5 d, 140.3 s, 25.8 q, 18.8 q, δ_H 5.13 d ($J = 5.2$ Hz), 1.74 s (3H), 1.69 s (3H)]. The above functionalities account for 8 of the 11 degrees of unsaturation of **1**, suggesting a tricyclic structure for kitungolide A.

The COSY spectrum revealed the presence of three spin systems (**a**−**c**) as shown in Figure 1. HMBC correlations observed from both H-3 and H-11 to C-4a indicated the connection between C-4 (of fragment **c**) and C-4a (of frament **b**). Another HMBC correlation from H-3 to C-1 established the connection between O-2 (of fragment **b**) and C-3 (δ_C 143.5, of fragment **a**) to create the enol ether, closing a 1-acetoxy-3,4-dihydropyrane ring. HMBC correlations from both H-7 and H-9a to C-8 determined the lactone functionality to be connected to C-7 and to C-9a. The connectivity between C-5a and C-6 was implied by the correlation from H-17 to C-5a. Thus, the planar structure of **1** was completed (Figure 1), presenting a unique ring system, part of which is an unprecedented hexahydrocoumarine.

The relative stereochemistry of **1** was determined by analysis of the coupling constants of protons 1, 10a, 4a, 5B,

5A (A and B denote downfield and upfield resonances, respectively), and 5a. H-10a is a triplet presenting a coupling constant of 12.1 Hz due to couplings to both H-1 and H-4a. Therefore, H-10a, H-1, and H-4a should all be in the quasi-axial position of the 3,4-dihydropyrane ring that adapts a half chair conformation.[7] H-5B is a quartet presenting a coupling constant of 12.5 Hz due to coupling with H-4a, H-5A, and H-5a, thus implying that H-5B and H-5a are also quasi-axial. H-5a is a broad doublet presenting a coupling constant of 12.1 Hz due to coupling to H-5B only. Therefore, it was deduced that H-9a occupies the quasi-equatorial position and the qausi-axial position of C-9a is occupied by the oxygen atom of the lactone moiety. A set of correlations observed in the NOESY experiment (Figure 1) further corroborated the suggested structure.[8]

Noteworthy is the fact that in **1**, the 3-substituted 1-acetoxy-3,4-dihydropyrane ring of the new tricyclic skeleton is the same as in the xenicins as part of the 2-oxabicyclo[7.4.0]-tridecane system.[9−11] The side chain of the dihydropyrane ring of **1**, namely, C-11 to C-16, is regiochemically identical to the side chain (C-12 to C-17) in the xenicins. The NMR data of the dihydropyrane ring and the side chain of **1** fully agree with those of the xenicins. The latter compounds appear in two diastereomeric configurations of C-12 and C-13 in different cases. In xenicin,[9] the configuration is 12R*,13R*, whereas in 13-epi-9-desacetylxenicin,[12] the configuration is 12R*,13S*. The differences in the ^{1}H and ^{13}C chemical shifts of the diastreomeric centers C-12 and C-13 between xenicin and 13-epi-9-desacetylxenicin are small, and therefore the relative stereochemistry of the stereogenic carbons C-11 and C-12 in **1** could not be determined.

The CIMS spectra of **2** exhibited a pseudomolecular ion [M + H]$^+$ at m/z 345. The molecular formula $C_{20}H_{24}O_5$ was determined by HRMS. ^{13}C NMR and ^{1}H NMR experiments revealed that a β-methyl $\alpha\beta$-unsaturated lactone [δ_C 164.1 s, 116.3 d, 160.6 s 21.9 q, δ_H 5.77 s, 1.99 s (3H)] and an exocyclic double bond (δ_C 142.4 s, 112.8 t, δ_H 4.71 s, 5.16 s) were present in **2**, as well as another ester/lactone group (δ_C 168.0) and a conjugated diene system. Since six of the nine unsaturations were accounted for by the above analysis, compound **2** was determined to be of a tricyclic nature. Analysis of one- and two-dimensional NMR spectra (Table 1 and Figure 2) of **2** led to the construction of two spin systems (**d**, **e**). HMBC correlations observed from both H-1A (δ_H 4.46,δ_C 68.0) and H-11 (δ_H 7.24) to C-3 (δ_C 165.0 s) indicated the connection between C-4 and C-3 and the connection of C-1 to C-3 (via O-2) to create the $\alpha\beta$-$\gamma\delta$-

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(8) The NOE between H-18A and H-9a, which almost overlap in CDCl₃, was measured in DMSO-d_6, where these protons are well separated [δ_H 4.86 (H-9a) and 5.26 (H-18A, which in DMSO-d_6 resonates at a lower field than H-18B].

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**Table 1.** NMR Data of Kitungolides A (**1**), B (**2**) and C (**3**) in CDCl$_3$

	1[a]		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	90.7	6.20 d (9.5)	68.0	4.46 dd (10.4, 2.6) 4.10 t (10.4)	69.4	4.57 dd (10.8, 3.7) 4.25 t (10.8)
3	143.5	6.40 d (2.0)	168.0		165.0	
4	110.2		not observed		not observed	
4a	38.3	2.36 t (12.1)	40.9	2.55 bt (12.2)	42.3	2.37 bt (12.5)
5	30.4	2.52 bd (12.1) 1.30 q (12.1)	32.4	2.30 bd (13.5) 1.42 q (12.8)	30.2	2.22 m 1.49 q (11.9)
5a	42.1	2.25 bd (12.1)	42.1	2.36 dt (12.5, 4.3)	41.5	2.27 m
6	161.2		160.6		160.0	
7	116.2	5.87 s	116.3	5.77 t (1.3)	116.6	5.83 s
8	164.1		164.1		165.0	
9a	81.2	4.77 d (2.0)	80.3	4.76 d (3.2)	80.3	4.75 d (2.8)
10	141.2		142.4		141.5	
10a	41.6	2.75 t (12.1)	39.9	2.71 bt (12.3)	37.5	2.70 bt (12.9)
11	72.7	5.26 d (5.2)	142.2	7.24 d (11.7)	142.6	6.52 dd (10.7, 2.0)
12	70.8	5.57 dd (9.3, 5.2)	120.8	6.45 dd (15.0, 11.7)	123.7	7.50 dd (15.5, 10.8)
13	118.5	5.13 d (9.3)	152.4	6.21 d (15.0)	151.8	6.17 d (15.7)
14	140.3		70.0		71.0	
15	25.8	1.74 s	29.6	1.30 s	29.3	1.35 s
16	18.8	1.69 s	29.6	1.30 s	29.4	1.35 s
17	21.2	2.05 s	21.9	1.99 s	21.5	2.05 s
18	112.7	5.15 s, 4.75 s	112.8	5.16 s, 4.71 s	114.0	5.21 s, 4.78 s

[a] Acetate groups of **1**: 1-OAc (δ_C 170.0 s and 21.2 q, δ_H 2.11 s), 11-OAc (δ_C 169.8 s and 21.4 q, δ_H 2.01 s), and 12-OAc (δ_C 169.7 s and 20.8 q, δ_H 2.00 s).

unsaturated lactone system. Other HMBC correlations from both H-7 and H-17 to C-5a established the connection between C-5a and C-6. The connectivity between C-8 and C-7 was deduced on the basis of HMBC correlations from

Figure 2. COSY (—), selected HMBC (→ (solid)), and NOESY (↔ (dashed)) correlations of kitungolide B (**2**).

both H-17 and H-7 to C-8. The E geometry was assigned to the $\Delta^{4(11)}$ double bond on the basis of NOESY correlations between both H-4a, H-5A, and H-12 as well as between H-11 and H-13. In this configuration of the double bond, H-11 (δ_H 7.24) is closest to the carbonyl group and therefore should be the most deshielded vinyl proton by the carbonyl group, as is the case here. Analysis of the coupling constants of protons 10a, 4a, 5B, 5A, and 5a established the relative stereochemistry of the various asymmetric centers. H-10a is a triplet presenting a coupling constant of 12.3 Hz due to couplings to both H-1B and H-4a. Therefore, H-10a, H-1, and H-4a should all be in the quasi-axial position. H-5B is a quartet presenting a coupling constant of 12.8 Hz due to coupling with H-4a, H-5A, and H-5a, thus implying that H-5B and H-5a are also quasi-axial. H-5a is a double triplet of 12.5 and 4.3 Hz due to coupling to H-5B, H-5A, and H-9a, respectively. Therefore, it was deduced that H-9a occupies the quasi-equatorial position. The quasi-axial position of C-9a is occupied by the oxygen atom of the ester moiety. Correlations observed in the NOESY experiment of **2** (Figure 2) further corroborated the suggested relative stereochemistry. As in the case of **1**, the unprecedented hexahydrocoumarine system of the heterocycle is present also in compound **2**, and the relative stereochemistries of the stereogenic centers of this system in both compounds are identical.

The CIMS spectra of **3** exhibited a pseudomolecular ion [M + H]$^+$ at m/z 345. The molecular formula was determined by HRMS to be identical to that of **2**, namely, C$_{20}$H$_{24}$O$_5$. The ^{13}C NMR and ^{1}H NMR spectra of **3** were almost identical to those of **2**, the only major differences were observed in the vinyl proton and carbon regions. Therefore,
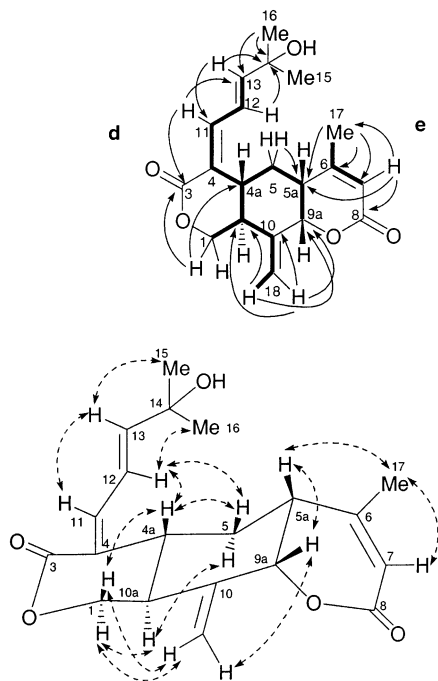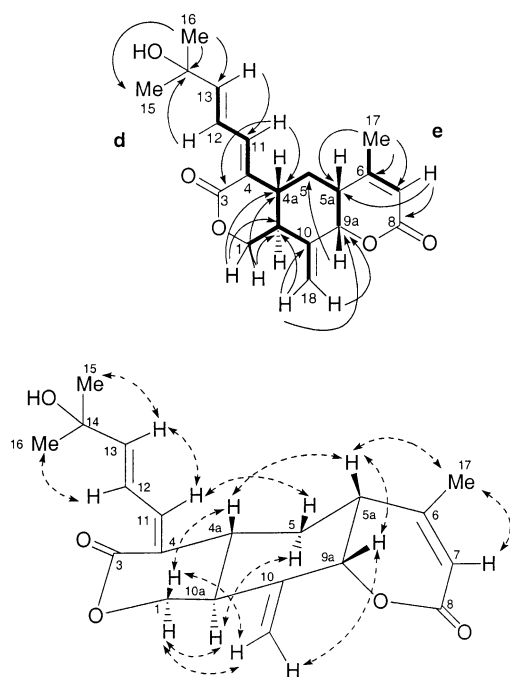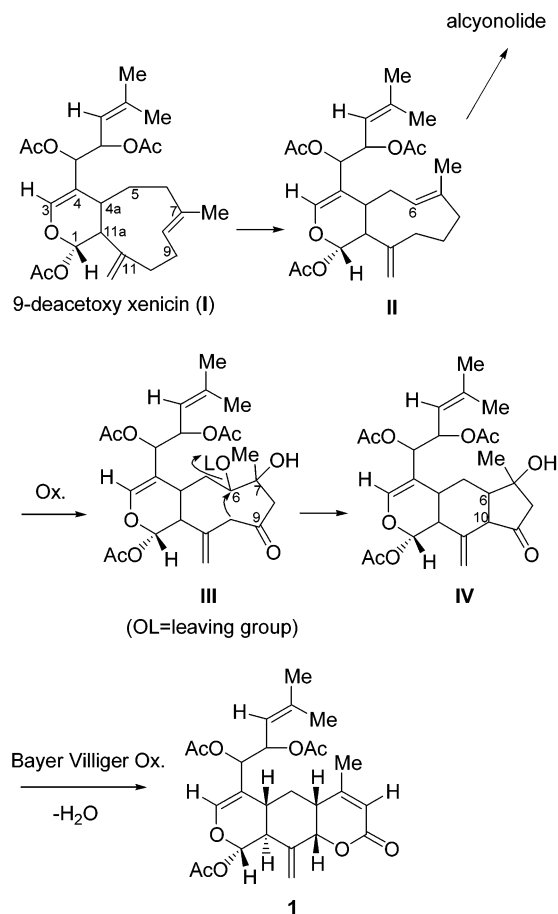
Figure 3. COSY (—), selected HMBC (→ (solid)), and NOESY (↔ (dashed)) correlations of kitungolide C (**3**).

Figure 4. Suggested biogenesis of kitungolide A.

it was concluded that **2** and **3** differ in the configuration of the diene system. In the case of **3**, H-12 (δ_H 7.50) was the most deshielded proton, which implies its spatial proximity to the carbonyl group and thus establishes the *Z* geometry for the $\Delta^{4(11)}$ double bond. NOESY correlations between H-11 and H-5 further corroborated this conclusion.

As mentioned above, in **1**, the unprecedented hexahydro-coumarine system is fused with a 3-substituted 1-acetoxy-3,4-dihydropyrane ring that is a characteristic of the xenicins as part of the 2-oxabicyclo[7.4.0]tridecane system.[9−11] In **2**, the hexahydrocoumarine system is condensed with a sub-stituted δ-lactone ring, a moiety that characterizes the [7.4.0]-tridecane system of the xeniolids[13,14] and the isoxeniolids.[12,15] Moreover, in the xenicins[9] as well as in the xeniolides,[16] the dihydropyrane and the δ-lactone rings, respectively, are trans-fused to the nine-membered ring. Thus, the relative stereo-chemistry of C-4a and C-10a of the kitungolides is identical to that of C-4a and C-11a of the xenicins and the xeniolides. This implies that kitungolides A−C are biogenetically related to the xenia diterpenoids. It is suggested that kitungolide A is obtained from 9-deacetoxy xenicin (**I**)[9] via the Δ^6-isomer (**II**) as shown in Figure 4. The latter Δ^6-isomer was assumed to be the precursor of the seco-xenicin alcyonolide.[17] It is

suggested that the Δ^6-isomer (**II**) oxidizes to the 6,7-dihydroxy-9-oxo derivative (**III**) which then, by an internal nucleophilic reaction from C-10 (allylic and α to the carbonyl group) to C-6 (**IV**), followed by Bayer−Villiger oxidation[18] of the β-hydroxyketone (**IV**) and water elimination, affords compound **1**. Kitungolides B and C are obtained in a similar manner from xeniolide A[13] and isoxeniolide A,[12] respectively.

Supporting Information Available: General experimen-tal procedures, NMR data (¹H NMR, ¹³C NMR, COSY, and HMBC), and proton NMR spectra of Kitungolides A−C. This material is available free of charge via the Internet at http://pubs.acs.org.

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