Nardosinanols A−I and Lemnafricanol, Sesquiterpenes from Several Soft Corals, *Lemnalia* sp., *Paralemnalia clavata*, *Lemnalia africana*, and *Rhytisma fulvum fulvum*†

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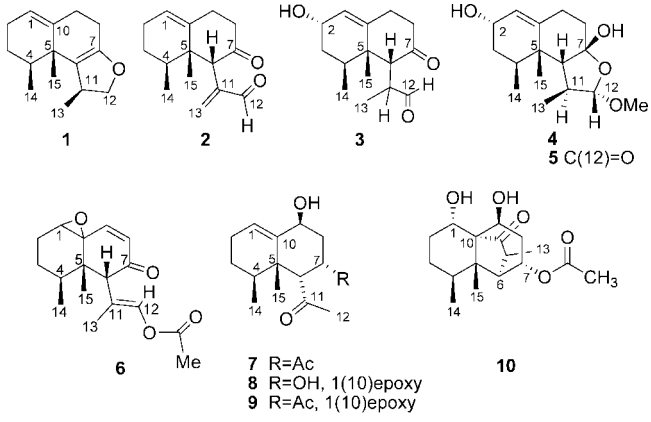
Ten new sesquiterpenes, nardosinanols A−I (**1**−**9**) and lemnafricanol (**10**), have been isolated from several Kenyan soft corals, i.e., from *Lemnalia* sp., *Paralemnalia clavata*, *Lemnalia africana*, and *Rhytisma fulvum fulvum*. The structures and relative stereochemistry of these compounds were elucidated by interpretation of MS, COSY (^{1}H−^{1}H correlations), HSQC, HMBC, and NOESY NMR spectroscopic experiments and in the case of **5** also by chemical transformation to compounds **11** and **12**. Nine compounds (**1**−**9**) are based on the nardosinane skeleton (**1**–**6** are nardosinanes and **7**–**9** nornardosinanes). Lemnafricanol (**10**) possesses a novel tricyclic skeleton. Compounds **3**, **7**, and **10** were found to be toxic to brine shrimp with LC_{50} values of 4.0, 0.35, and 0.32 μM, respectively.

Soft corals of the genera *Lemnalia*, *Paralemnalia*, and *Rhytisma* are a rich source of sesquiterpenoids and norsesquiterpenoids, containing a variety of skeleta, including the nardosinane skeleton.[1–10] Dozens of compounds have thus far been reported with several ring systems.[11]

The present work describes the isolation of 10 sesquiterpenes, nardosinanols A−I (**1**–**9**) and lenmafricanol (**10**), from *L. africana*, *P. clavata*, *R. fulvum fulvum*, and *Lemnalia* sp. (Due to the lack of any revision on the genus *Lemnalia*, identification of most species is problematic and uncertain; therefore, no species name can be given at this stage.) Six compounds (**1**–**6**) were assigned as nardosinanes, three as nornardosinanes (**7**–**9**), and one, lemnafricanol (**10**), as a sesquiterpene possessing an unprecedented tricyclic carbon skeleton.



Results and Discussion

Lemnalia sp. collected in south Kenya afforded nardosinanol A (**1**). The CIMS of **1** exhibited a pseudomolecular ion $[M + H]^+$ at *m/z* 219, suggesting, together with the ^{13}C NMR data, a molecular formula of $C_{15}H_{22}O$. The ^{13}C NMR and ^{1}H NMR experiments (Tables 1 and 2) revealed the presence of two double bonds, one trisubstituted (δ_C 139.9 C, 122.4 CH; δ_H 5.30 m) and the other tetrasubstituted (δ_C 148.1 C, 115.5 C), and one methyleneoxy group (δ_C 71.2 CH_2; δ_H 4.17 t, 3.82 dd). The latter functionalities account for two of the five degrees of unsaturation of **1**, suggesting three rings. The COSY spectrum revealed the presence of three spin systems (*I*–*III*) depicted in Figure 1. HMBC correlations connected the latter three spin systems, enabling the construction of the planar structure of **1**, as shown in Figure 1. The relative configuration of **1** was determined by analysis of NOESY cross-peaks (Figure 1). NOEs between all three methyl groups, CH_3-13, CH_3-14 and CH_3-15, suggested that they are all on one side, the β-side, in agreement with NOEs between H-4 and H-11 on the opposite α-side. The suggested configuration is the same as in the corresponding centers of the *Lemnalia africana* nardosinane sesquiterpene 2-deoxylemnacarnol (**a**)[1] (Figure 2). The relative configuration was well established from the NMR data, especially the ^{13}C resonances. Nardosinanol A (**1**) differs from **a** by a 7-hydroxy group instead of a 6(7) double bond, with all other carbon shifts in good agreement with compound **a**. Throughout this report, on the basis of biogenetic considerations, albeit not confirmed, we have adopted for the drawings the absolute configuration of 2-deoxy-12-oxolemnacarnol.[1]

The CIMS of nardosinanol B (**2**), isolated from *P. clavata*, exhibited a pseudomolecular ion at *m/z* 233 $[M + H]^+$. The molecular formula, $C_{15}H_{20}O_2$, was determined by HRCIMS and from the ^{13}C NMR spectrum. NMR data revealed considerable similarity to **1**; namely, **2** possesses the same nardosinane ring system, but differs in a carbonyl at C-7 and in the C-(11–13) segment (δ_C 135.3 CH_2, 145.6 C, 191.2 CH; δ_H 6.18 s, 5.43 s 9.09 s) (Table 1). Nardosinanol B (**2**) is close in structure to (*1E*)-2-[(1*S*,8*S*,8a*S*)-8,8a-dimethyl-2-oxo-1,2,3,4,6,7,8,8a-octahydronaphthalen-1-yl]prop-1-en-1-yl acetate (**b**), a *Paralemnalia* sp. sesquiterpene[2] (Figure 2) but differs in the three-carbon appendix, i.e., an α,β-unsaturated aldehyde in place of an enol-acetate. The structure of **2** was determined by HMBC and COSY correlations (Figure 3). Nardosinanols B and A (**2** and **1**) have the same relative configuration of the C-4 and C-5 chiral centers, as determined for **2** by analysis of NOESY cross-peaks (Figure 3).

R. fulvum fulvum, previously *Parerythropodium fulvum fulvum*, was studied by our group, and the two, yellow and gray, morphs were found to be rich in sesquiterpenes.[4] The present Kenyan soft coral afforded in addition to several earlier reported sesquiterpenes, 4,10-diacetoxy-5-oxo-1,12-neolemna-2,8-diene,[14] 6α-acetyl-4β,5β-dimethyl-1(10)-α-

† Dedicated to Dr. G. Robert Pettit of Arizona State University for his pioneering work on bioactive natural products.

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Table 1. ^{13}C NMR Spectroscopic Data of Compounds **1**–**12** (measured at 100 MHz)

C	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[c]	**6**[a]	**7**[b]	**8**[b]	**9**[b]	**10**[b]	**11**[b]	**12**[b]
1	122.4	124.0	126.0	124.5	126.5	62.5	128.9	56.9	56.7	67.9	124.9	132.8
2	25.1	25.3	64.1	63.8	64.3	25.7	27.8	20.9	20.8	31.9	64.0	128.2
3	26.4	26.1	36.0	35.3	36.1	23.2	26.2	24.0	23.9	27.9	35.6	31.1
4	33.3	33.1	28.2	28.7	29.7	33.2	34.7	30.2	30.9	36.6	26.4	28.2
5	35.8	42.2	44.3	40.8	42.1	39.2	41.2	39.1	39.4	47.3	43.4	40.0
6	115.5	55.1	58.3	59.5	58.1	61.9	57.5	59.9	56.8	46.6	61.2	56.6
7	148.1	206.1	211.3	106.2	107.1	196.2	69.2	65.5	68.8	69.7	211.5	210.0
8	27.2	34.7	39.1	35.6	35.9	149.8	33.8	35.3	31.9	33.2	38.1	34.5
9	33.1	30.4	31.0	28.6	28.3	127.9	74.8	75.6	75.4	66.5	30.0	32.4
10	139.9	137.1	143.4	145.2	143.9	60.7	141.2	62.1	61.7	62.5	140.9	83.8
11	34.7	145.6	45.3	42.5	40.8	117.4	210.5	211.8	208.3	41.4	38.5	33.3
12	71.2	191.2	202.1	109.8	182.2	131.3	35.2	35.1	34.3	228.4	175.2	172.0
13	18.8	135.3	13.0	19.9	18.6	13.4				14.3	17.2	15.0
14	15.2	14.6	15.3	16.1	16.6	14.5	15.6	15.4	15.3	12.0	14.7	14.3
15	16.3	19.8	19.3	19.0	20.1	18.2	21.2	17.3	17.2	18.4	19.6	13.1
Ac						166.6	169.8		170.1	169.5		
						19.6	21.7		21.2	21.3		
OMe				56.0							51.9	

[a] Recorded in C_6D_6 solution. [b] Recorded in $CDCl_3$ solution. [c] Recorded in $CDCl_3$ + MeOD (4:1).

Table 2. ^1H NMR Spectroscopic Data of Compounds **1**, **2**, **4**, **6**, **7**, and **10**[e]

H	**1**[a]	**2**[b]	**4**[c]	**6**[a]	**7**[d]	**10**[d]
1	5.30 brs	5.36 brs	5.68 brd (5.0)	2.98 brd (2.0)	5.80 brs	3.86 td (11.2, 4.8)
2	1.97 (2H)	1.79 (2H)	4.05 brs	1.85	2.10	2.03
				1.50	2.05	1.85
3	1.45 (2H)	1.17 (2H)	1.57 (2H)	1.39	1.42 (2H)	1.46 (2H)
				0.82		
4	1.63	1.50	2.08	1.45	1.57	1.42
6		4.26 s	1.81 dd (10.6, 1.6)	3.14 s	3.48 d (5.3)	2.22 brs
7					5.71 dt (12.2, 5.3)	5.09 ddd (13.0, 7.3, 3.9)
8	2.49 (2H)	2.49	2.04 td (13.5, 4.4)	5.67 d (10.0)	2.23 dt (12.2, 4.1)	2.36 dd (13.0, 7.3)
		2.16	1.89 brd (13.5)		2.01	1.84
9	2.07 (2H)	2.48	2.49 tdt (13.5, 5.0, 1.6)	6.14 d (10.0)	4.50 brs	4.33 brd (6.5)
		2.05	2.13 dt (13.5, 5.0)			
11	2.97		1.90			2.45 d (7.5)
12	4.17 t (8.6)	9.09 s	4.55 d (4.4)	7.45 s	2.19 s	
	3.82 dd (8.6, 3.0)					
13	1.21 d (6.7)	6.18 s	1.24 d (6.7)	1.71 s		1.31 d (7.5)
		5.43 s				
14	1.06 d (6.6)	0.68 d (6.9)	0.87 d (6.7)	0.52 d (6.7)	0.93 d (6.5)	0.84 d (6.5)
15	1.11 s	0.90 s	1.08 s	1.08 s	1.39 s	1.21 s
Ac				1.67 s	2.09 s	2.06 s
OH-1						3.37 d (11.2)
OH-7			2.79 brs			
OH-9						2.56 brs
OMe-12			3.35 s			

[a] Recorded in C_6D_6 solution measured at 400 MHz. [b] Recorded in C_6D_6 solution measured at 500 MHz. [c] Recorded in $CDCl_3$ solution measured at 400 MHz. [d] Recorded in $CDCl_3$ solution measured at 500 MHz. [e] The *J* values in Hz are indicated in parentheses.

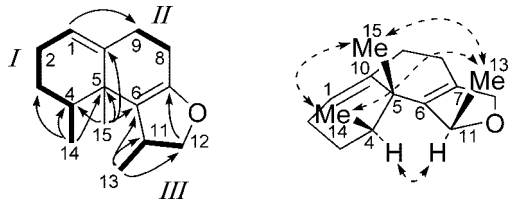


Figure 1. $^1H-^1H$ COSY (—) (*I*–*III*), selected HMBC (↷), and NOE (dotted arrow) correlations for nardosinanol A (**1**).

epoxy-2α-hydroxy-7-oxodecalin,[4] 2-oxolemnacarnol,[4,15] and a guaiane-type sesquiterpene,[16,17] and also nardosinanols C, E, and F (**3**, **5**, and **6**).

The CIMS of nardosinanol C (**3**), isolated from *R. fulvum fulvum*, exhibited a pseudomolecular ion at *m/z* 251 $[M + H]^+$, suggesting, together with the ^{13}C NMR resonances and HREIMS, the molecular formula $C_{15}H_{22}O_3$. On comparing the NMR data of compounds **3** and **2** (Table 1), it was revealed that both possess the same nardosinane ring system, but differ at C-2 and C-13, with **3** possessing a hydroxy instead of 2-methylene and a methyl instead of the 11-methylene of **2**. Comparison of the ^{13}C NMR spectrum of **3** with that of **11** enabled the resonance assignments (Table 2). The α-configuration of the C-2 hydroxy group was determined by comparing the $J_{1,2}$ value of **3** with those of elongatol A (**f**), isolated from *Nephthea elongata*[10] (Figure 2) and its 2-epimer analogue (0 Hz).[6,18]

The second isolated compound from *R. fulvum fulvum*, nardosinanol D (**4**), was assigned with the elemental formula $C_{16}H_{26}O_4$, as shown by its CIMS and ^{13}C NMR spectra. That the *m/z* 265 peak agrees with $[M + H - H_2O]^+$ was clear from the required four oxygen atoms (Table 1). The ^{13}C NMR and HSQC spectra showed signals for four methyls, three sp^3 methylenes, five sp^3 methines, one sp^2 methine, two sp^3 quaternary carbons, and one sp^2 quaternary carbon (Table 1). The ^{13}C NMR and ^1H NMR experiments revealed the presence of (a) a double bond (δ_C 145.2 C, 124.5 CH; δ_H 5.68 brd) and (b) an α,α′-hydroxymethoxytetrahydrofuran ring (δ_C 106.2 C, 109.8 CH; δ_H 4.55 d; $^1J_{CH}$ = 170 Hz and δ_C 56.0 CH_3; δ_H 3.35 s; $^1J_{CH}$ = 141 Hz). The above functionalities accounted for two of the four degrees of unsaturation of **4**, requiring two additional rings. The structure of nardosinanol D (**4**), possessing the α,α′-hydroxymethoxytetrahydrofuran ring, was determined by COSY and HMBC correlations (Figure 4). The

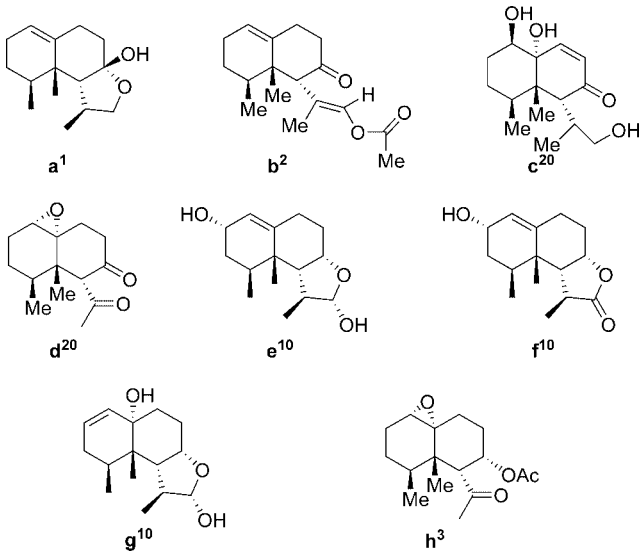


Figure 2. Known nardosinane and nornardosinane sesquiterpenes.

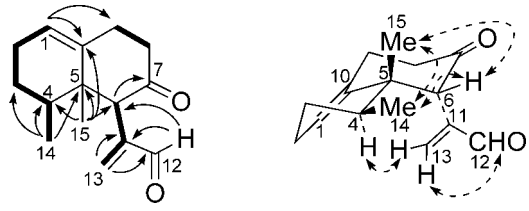


Figure 3. Selected ^{1}H−^{1}H COSY (—), HMBC (⌒), and NOE (dotted arrow) correlations for nardosinanol B (**2**).

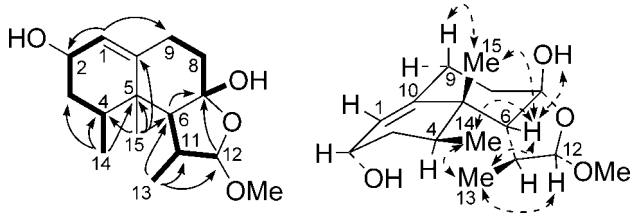
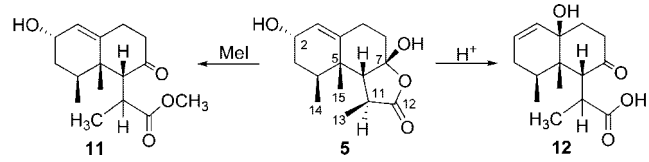


Figure 4. Selected ^{1}H−^{1}H COSY (—), HMBC (⌒), and NOE (dotted arrow) correlations for nardosinanol D (**4**).

α-configuration of the hydroxyl at C-2 was determined by comparison of the $J_{1,2}$ value of **4** with those of **e**[10] (Figure 2) and its 2-epimer analogue (0 Hz).[6,18] The relative stereochemistry of **4** was determined by analyzing the NOESY cross-peaks (Figure 4). NOEs between CH_3-13, H-6, H-12, and CH_3-14; CH_3-14 and H-6; CH_3-15, H-6, and H-9ax; and OH-7, H-6, and H-8eq suggested all three methyl groups, H-6, OH-7, and H-12, to be on the same β-side, in agreement with a NOE correlation between OCH_3-12 and H-8ax assigned on the α-side (Figure 4). The spectroscopic data and the relative configuration of **4** showed similarities to those of **e**[10] (Figure 2) except for a 7β-hydroxy-12α-methoxy group in **4**, as opposed to a 7β-methine-12α-hydroxy group at **e**.[10]

The CIMS of a third compound from *R. fulvum fulvum*, nardosinanol E (**5**), exhibited a pseudomolecular ion $[M + H - H_2O]^+$ at *m/z* 249, and, together with the HRCIMS and ^{13}C NMR carbon resonances, a molecular formula of $C_{15}H_{22}O_4$ was determined (loss of water in the MS was clear from the demand for four oxygen atoms from the ^{13}C NMR spectrum). The ^{13}C NMR δ values of **5** closely resembled those of **4** except for replacement of the acetal moiety of **4** by a γ-lactone in **5** (Table 1). Compound **5** is closest in structure to elongatol B (**f**) (Figure 2), isolated from *Nephthea elongata*.[10] The α-configuration of the 2-hydroxy group

Scheme 1. Chemical Transformations of Nardosinanol E (**5**) to Compounds **11** and **12**



was determined, as for **4**, by comparing the $J_{1,2}$ value of **5** with those of **f**[10] and its 2-epimer analogue (0 Hz).[6,18] The relative configuration of **5** was determined by analysis of NOESY cross-peaks that were similar to the one observed for **4** (Figure 4). NOEs between CH_3-13, H-4, and H-6; CH_3-14 and H-6; and CH_3-15, H-6, and H-9β suggested all methyl groups and H-6 to be on the β-side. As OH-7 was not observed in the ^{1}H NMR spectrum, the stereochemistry of OH-7 is suggested to be β, based on the *J*-values and chemical shifts of **5**, in comparison to **4**. Nardosinanol E (**5**) has the same relative configuration as **f**[10] (Figure 2).

Opening of the lactol moiety of **5** was achieved by treating the compound with CH_3I/K_2CO_3 in acetone to yield derivative **11** (Scheme 1). Compound **11** possesses a 7-ketone (δ_C 211.5 C) and an 11-carbomethoxy ester (δ_C 51.9 OCH_3, 175.2 C; δ_H 3.35 s). NOESY cross-peaks of **11** confirmed the stereochemistry of **5**; *inter alia*, a peak between CH_3-15, H-3β, H-6β, and H-9β determined the 6β configuration, as in **5**. As H-6 is in the α-position to the ketone and **11** was obtained under basic conditions, the 6β (the natural) configuration is the thermodynamically more stable one. The lactol of **5** also opens up under acidic conditions, e.g., a trace of acid in $CDCl_3$, to afford the 7-keto (δ_C 210.0 C), 12-carboxylic acid (δ_C 172.0 C). However, along with the lactol opening, under the acidic conditions, an allylic rearrangement of the 2-hydroxy-1(10)-ene moiety to the 10-hydroxy-1-ene took place, to afford compound **12** (Scheme 1). Comparison of ^{13}C NMR chemical shifts of **12** with those of **g**[10] suggested that the configuration of the hydroxy at C-10 is β [δ_C 83.8 C(OH-β), for **12**, against δ_C 78.4 C(OH-α) for **g**]. All other chiral centers of **12** possess almost the same ^{13}C NMR values as **11**, as expected, pointing to the same stereochemistry.

The CIMS of nardosinanol F (**6**), isolated from *P. clavata*, exhibited a pseudomolecular ion at *m/z* 291 $[M + H]^+$. The molecular formula, $C_{17}H_{22}O_4$, was determined by HRCIMS and from the ^{13}C NMR spectrum. COSY and HMBC correlations, depicted in Figure 5, established the gross structure of **6**. Nardosinanol F (**6**) resembles compound **b**[2] (Figure 2) in its 7-oxo and 11(12)-enol-acetate moieties. In addition, **6** possesses a 8(9)-disubstituted double bond (δ_C 149.8 CH, 127.9 CH; δ_H 5.67 d, 6.14 d) and an epoxide at C-1(10) (δ_C 62.5 CH, 60.7 C; δ_H 2.98 brd). The above functionalities accounted for five of the seven degrees of unsaturation, requiring **6** to be bicyclic. The relative stereochemistry of **6** was determined by analysis of NOESY cross-peaks (Figure 5). NOEs between H-6, CH_3-14, and CH_3-15 suggested all to be on the β-side. Hence, the enol-acetate has to be α, the more stable configuration, as evidenced by attempting basic epimerization. After one week compound **6** did not undergo basic epimerization at C-6, as shown by the ^{1}H NMR spectrum of **6** in d_5-pyridine. The configuration of the epoxide in **6** as well as in compounds **8** and **9** remains unknown. For both the α and β stereochemistry H-1, C-2, C-5, and C-9 are all in the same plane and do not define the configuration of the epoxide. Essentially, **6** is composed of a similar ring system to that of parathylone isolated from *Paralemnalia thyrsoides* (Figure 2, **c**),[19] and nardosinanol F (**6**) carries an epoxide instead of a 1,10-diol in **c**.[19] Close in structure also is compound **d**, isolated from *Paralemnalia thyrsoide*[19] (Figure 2), carrying a 1(10)-epoxide.

The soft coral *L. africana* afforded nardosinanols G−I (**7**–**9**) and lemnafricanol (**10**). The HRCIMS and ^{13}C NMR data of compound **7** revealed a pseudomolecular ion of $C_{16}H_{23}O_3$ $[M + H - H_2O]^+$.

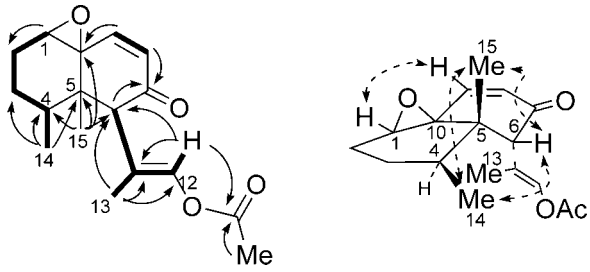
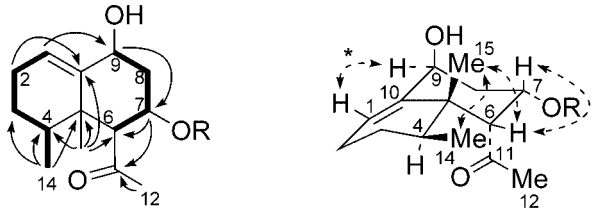


Figure 5. Selected ^{1}H−^{1}H COSY (—), HMBC (⟳), and NOE (dotted arrow) correlations for nardosinanol F (**6**).



* for all three compounds

7 R=Ac
8 R=H , 1(10)epoxy
9 R=Ac , 1(10)epoxy

Figure 6. Selected ^{1}H−^{1}H COSY (—), HMBC (⟳). and NOE (dotted arrow) correlations for nardosinanol G (**7**).

The DEPT spectrum evidenced four methyls, three methylenes, four methines, and four quaternary carbons. In turn, the ^{1}H and ^{13}C NMR spectra showed the presence of (a) a secondary methyl (δ_C 15.4 CH_3; δ_H 0.93 d, J = 6.5 Hz), (b) a tertiary methyl (δ_C 21.2 CH_3; δ_H 1.39 s), (c) a secondary hydroxyl on C-9 (δ_C 75.6 CH; δ_H 4.50 brs), (d) a trisubstituted olefin (δ_C 128.9 CH, 141.2 C; δ_H 5.80 brs), (e) a $COCH_3$ group at C-6 (δ_C 210.5 C, 35.2 CH_3; δ_H 2.19 s), and (f) an acetate at C-7 (δ_C 169.8 C, 21.7 CH_3; δ_H 2.09 s). The IR absorption at 3565 cm^{-1} and two absorptions at 1733 and 1712 cm^{-1} and the NMR signals indicated the presence of a hydroxyl, an acetate, and a methyl ketone (Tables 1 and 2). The structure of **7** was determined by COSY and HMBC correlations (Figure 6), and the latter correlations determined the acetate to be at C-7 (δ_C 69.2 CH). The relative configuration of **7** was established from NOESY cross-peaks (Figure 6), namely, between Me-14 and Me-15; Me-15 and H-6; and H-6 and H-7, suggesting all to be on the β-side. NOE correlations between H-7 and H-8β and between H-8α and H-9 led to the conclusion that H-7 and OH-9 are on the same β-side. Hence, both the acetyl and the methyl ketone groups are at the α-side. Nardosinanol G (**7**) possesses parts of the known *Paralemnalia thyrsoides* sesquiterpene **h** (Figure 2)[3] [a 1(10)-epoxide at **h** instead of a 1(10) double bond and 9β-hydroxyl group at **7**].

The CIMS of nardosinanol H (**8**), the second compound isolated from *L. africana*, exhibited a pseudomolecular ion at *m/z* 255 [M + H]$^+$. The molecular formula, $C_{14}H_{23}O_4$, was determined by HRCIMS. The 1D and 2D NMR data revealed **8** to be the 7-desacetyl-1(10)-epoxy analogue of **7**. HMBC and COSY correlations confirmed the structure of nardosinanol H (**8**) (Figure 6). The relative configuration of **8** was deduced from NOE cross-peaks (Figure 6) exhibiting similar NOEs to those observed for **7**. Furthermore, a NOE correlation between H-1 axial (δ_H 2.98 d, J = 2.7 Hz) and H-9 equatorial (δ_H 3.46 brt, J = 2.7 Hz) suggested the epoxide to be on the α-side. Compound **8** differs from compound **h** in C-7 and C-9: two secondary hydroxyl groups, for **8**, against an acetate at C-7 for **h**. The compounds have the same configuration of the C-(4–6) chiral centers.

The third nardosinanol (I, **9**), isolated from *L. africana*, was assigned a molecular formula of $C_{16}H_{24}O_5$ and was determined to be the 7-acetoxy derivative of **8**. Comparison of the ^{1}H NMR spectrum of **9** with that of **8** showed a downfield shift of H-7 from 4.63 ddd (J = 12.4, 5.3, 4.9 Hz) to 5.71 dt (J = 12.1, 5.2 Hz) in **9**.

The fourth compound isolated from *L. africana*, lemnafricanol (**10**), was assigned by the HRCIMS and ^{13}C NMR data to have a pseudomolecular ion of $C_{17}H_{27}O_5$. The DEPT spectrum showed resonances for three methyls, three methylenes, five methines, and four quaternary carbons. The IR absorptions at 3577 and 1717 cm^{-1} indicated the occurrence of hydroxyl and carbonyl groups. The ^{1}H and ^{13}C NMR spectra showed the presence of the following groups: (a) two secondary methyls (δ_C 14.3 CH_3; δ_H 1.31 d, J = 7.5 Hz and δ_C 12.0 CH_3; δ_H 0.84 d, J = 6.5 Hz), (b) a tertiary methyl (δ_C 18.4 CH_3; δ_H 1.21 s), (c) two methinoxy groups (δ_C 67.9 CH, δ_H 3.86 dt and δ_C 66.5 CH, δ_H 4.33 brd) and two hydroxyl protons (δ_H 3.37 d, 2.56 brs), (d) an acetate (δ_C 169.5 C, 21.3 CH_3; δ_H 2.06 s), and (e) a, most likely, cyclopentanone (δ_C 228.4 C). The COSY spectrum revealed the presence of two spin systems (*I, II*) (Figure 7). HMBC correlations of (a) C*H*$_3$-13 to C-6, C-11, and C-12, (b) H-1, H-6, and H-11 to the carbonyl C-12 atom, and (c) C*H*$_3$-14 and *C*H$_3$-15, and vice versa, connected the latter two spin systems, enabling the construction of **10**, as shown in Figure 7. The above functionalities revealed that lemnafricanol (**10**) is a tricyclic compound. Lemnafricanol possesses a novel sesquiterpene tricyclic skeleton. The relative stereochemistry of **10** was deduced from a NOESY experiment. NOE correlations between H-6, CH_3-13, and CH_3-15; H-7, H-8β, and CH_3-15; and CH_3-14 and CH_3-15 suggested all to be on the β-side, in agreement with NOE correlations between H-8α, H-9, and H-11; OH-1 and H-9 were assigned on the same α-side (Figure 7).

Possible relationships between the 10 reported compounds (**1**–**10**) are shown in Scheme 2. Compounds **2** and **3** are the aldehyde-bearing nardosinanes and are likely to be the precursors of nardosinanes containing enol-acetates, like **6**, and di- or tetrahydrofuran rings like **1**, **4**, and **5**. It is suggested that compounds **7**–**9** are derived from a 7,9-dioxygenated nardosinane. It is also suggested that lemnafricanol (**10**) is obtained by the attack of the double bond of an isopropylene group on a carbocation at C-10.

The toxicity of the various compounds on brine shrimp larvae has been checked, and it was found that nardosinanols C (**3**) and G (**7**) and lemnafricanol (**10**) exhibited LC_{50} values of 4.0, 0.35, and 0.32 μM, respectively.

Classical taxonomy of the Octocorallia has assigned the soft coral genera *Lemnalia* and *Paralemnalia* to the family Nephtheidae, and *Rhytisma* to the family Alcyoniidae. However, it is interesting to note that mitochondrial protein-coding sequences analysis reveals that these three genera form a clade that is quite distinct and distant from the rest of the Nephtheidae.[12] A more recent study, examining DNA of *Paralemnalia* and *Rhytisma* samples from Eilat, Israel (Red Sea), has confirmed the earlier result: they form a clade together, not with the Nephtheidae, but actually closer to the soft coral family Xeniidae.[13] Hence, the present findings on the similarity of natural products of the examined soft coral species might be related to the close phylogenetic relationships among them, contradicting classical taxonomy. It is worth noting that often during freeze-drying, volatile sesquiterpenes are lost.[4] This has to be taken into account for comparative purposes. Additionally, because of symbiotic zooxanthella, the source of the sesquiterpenes is unknown.

Experimental Section

General Experimental Procedures. Optical rotations were obtained with a JACSO 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 spectra were mesured using standard Bruker pulse sequences. EIMS, CIMS (using isobutane), and HRMS measurements were recorded on a Fisons Autospec Q instrument. Electrospray MS measurements were performed on an Applied Biosystems Q-STAR Pulsar instrument (ESI-QqTOF).

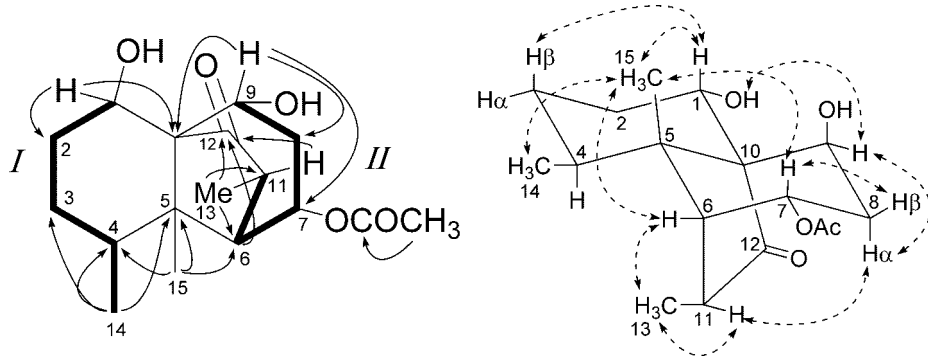
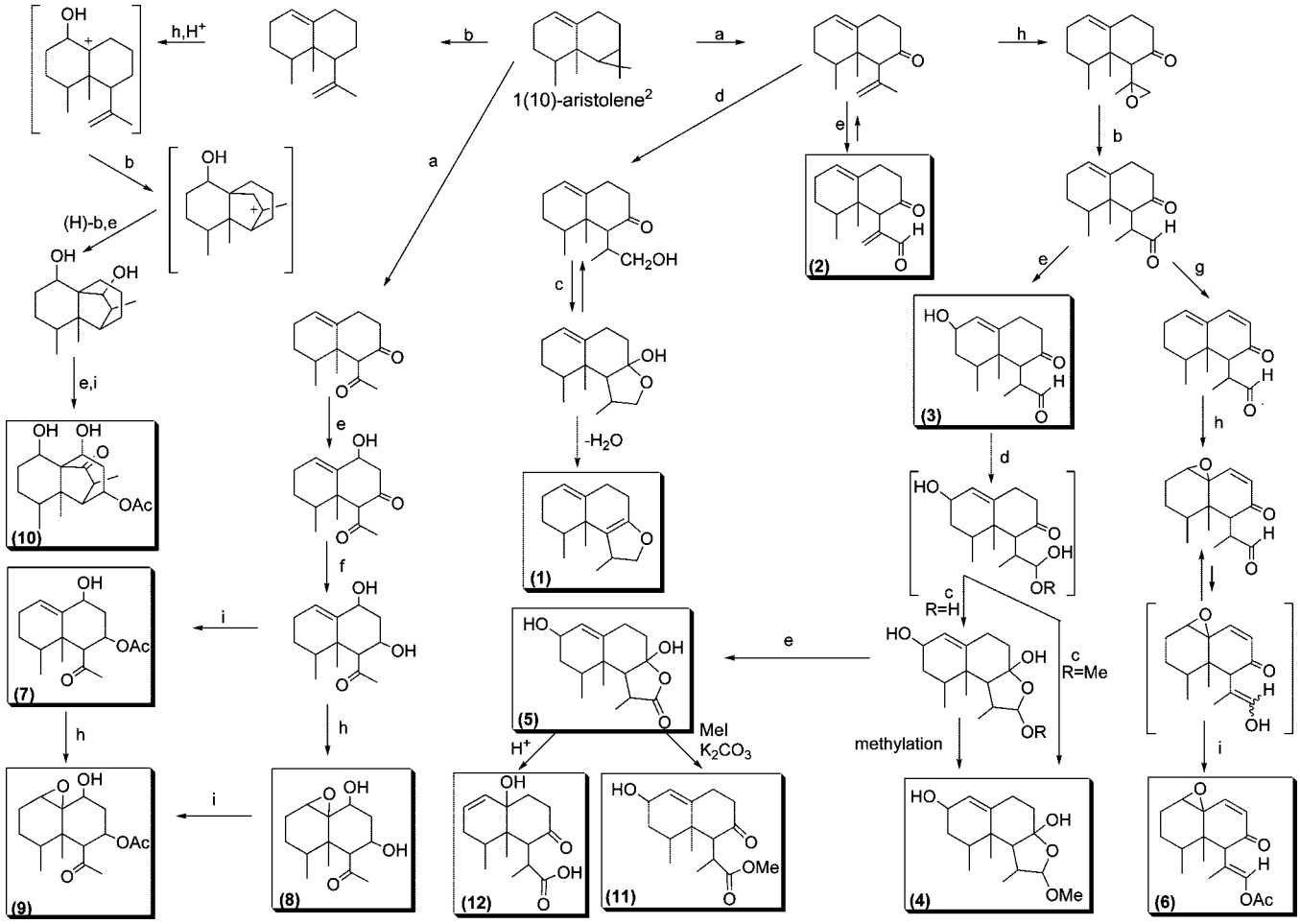


Figure 7. Selected $^1H-^1H$ COSY (—), HMBC (↷), and NOE (dotted arrow) correlations for lemnafricanol (**10**): (a) oxidative cleavage, (b) rearrangement, (c) lactolization, (d) hydration or methanolation, (e) oxidation, (f) reduction, (g) double-bond formation, (h) epoxidation, (i) acetylation. *The order of the different steps might be different.

Scheme 2. Suggested Biogenesis for Compounds **1**–**10**, Starting from 1(10)-Aristolene[2] or a Diastereomer



Biological Material. The soft coral *L. africana* (May, 1898) was collected at Fundu (deep gap south), Pemba Island, Tanzania (15–25 m, 1 December 2004). This species has been previously recorded from various reef sites in the Indian Ocean.[20] Voucher specimens are deposited at the Zoological Museum of Tel Aviv University (collection numbers ZMTAU CO 32690 and 3268), as are all the other species reported here. *Rhytisma fulvum fulvum* (Forskal, 1775) was obtained at Nuambe Wambe, Pemba Island, Tanzania (20–25 m, 9 December 2004, ZMTAU CO 32727) and has been previously obtained from various Indo-Pacific localities.[21] *Paralemnalia clavata*[20] was originally described from Madagascar and in the present study was collected at Nuambe Wambe (details above, ZMTAU CO 32727). *Lemnalia* sp. was collected from Kitagamwa, southern Kenya (6–8 m, 13 December, 2004, ZMTAU CO 32291). Due to lack of any revision on the genus *Lemnalia*, identification of most species is quite difficult and uncertain. Therefore, no species name can be given at this stage to ZMTAU CO 32291. All the collection sites are exposed to very strong tidal currents and thus are appropriate for flourishing of rich fauna.

Extraction and Isolation. Extraction of *Lemnalia* sp. and Isolation of Nardosinanol A (1). A freeze-dried sample of *Lemnalia* sp. (17 g) was homogenized and extracted with *n*-hexane to give, after evaporation, 1.0 g of extract. The extract was chromatographed on a Sephadex LH-20 column, eluting with *n*-hexane−MeOH−CH_2Cl_2 (2:1:1) + 0.01% TFA and afterward subjected to vacuum-liquid chromatography (VLC) over silica gel, using *n*-hexane with increasing proportions of EtOAc as eluent, to give compound **1** (3.0 mg, 0.02% dry weight).

Nardosinanol A (1): unstable, colorless oil; ^1H and ^{13}C NMR (Tables 1 and 2); CIMS *m/z* 219 (100) $[M + H]^+$; 203 (34) $[M + H - CH_3]^+$; HREIMS *m/z* 218.1680 $[M + H]^+$ (calcd for $C_{15}H_{22}O$, 218.1671).

Extraction of *P. clavata* and Isolation of Nardosinanols B (2) and F (6). A freeze-dried sample of *P. clavata* (10 g) was homogenized and extracted with EtOAc−MeOH−CH_2Cl_2 (5:5:1) to give, after evaporation, a brown gum (1.4 g). The gum was partitioned between aqueous MeOH, *n*-hexane, and CH_2Cl_2 under a Kupchan procedure.[22] The CH_2Cl_2 extract (290 mg) and *n*-hexane extract (160–170 mg) were each subjected to VLC over silica gel, using *n*-hexane with increasing proportions of EtOAc as eluent. Similar fractions from both columns were united to give 42 mg of a mixture. This mixture was chromatographed on a Sephadex LH-20 column, eluting with *n*-hexane−MeOH−CH_2Cl_2 (2:1:1), and on a silica gel column, using *n*-hexane with increasing proportions of EtOAc as eluent to give compounds **2** and **6**, with *n*-hexane−EtOAc (9.3:0.7) for **2** and with (9.5:0.5) for **6** (3.6 mg, 0.04% and 3.5 mg, 0.04% dry weight, respectively).

Nardosinanol B (2): white powder; $[\alpha]^{23}_D$ –228 (*c* 0.2, $CHCl_3$); ^1H and ^{13}C NMR (Tables 1 and 2); CIMS *m/z* 233.2 (100) $[M + H]^+$; 215.2 (28) $[M + H - H_2O]^+$; 173.1 (5) $[M + H - COCH_2]^+$; HRCIMS *m/z* 233.1537 $[M + H]^+$ (calcd for $C_{15}H_{21}O_2$, 233.1542).

Nardosinanol F (6): colorless oil; $[\alpha]^{23}_D$ +151 (*c* 0.14, $CHCl_3$); ^1H and ^{13}C NMR (Tables 1 and 2); CIMS *m/z* 291.2 (100) $[M + H]^+$; 231.1 (55) $[M + H - CO_2CH_3]^+$; 191.1 (18) $[M + H - C_5H_7O_2]^+$; HRCIMS *m/z* 291.1601 $[M + H]^+$ (calcd for $C_{17}H_{23}O_4$, 291.1596).

Extraction of *R. fulvum fulvum* and Isolation of Nardosinanols C (3), D (4), and E (5). The EtOAc−MeOH−H_2O (5:5:1) extracts of freeze-dried *R. fulvum fulvum* (10 g) were subjected to partition by the method of Kupchan.[22] The CH_2Cl_2 phase was chromatographed over a Sephadex LH-20 column eluted with *n*-hexane−MeOH−CH_2Cl_2 (2:1:1) followed by VLC over silica gel, using *n*-hexane with increasing proportions of EtOAc as eluents to give compounds **4** (12 mg 0.08% dry weight) and **5** (18 mg 0.12% dry weight). Compound **3** (11 mg 0.03% dry weight) was received applying the same methodology from a different LH-20 fraction.

Nardosinanol C (3): colorless oil; $[\alpha]^{23}_D$ –160 (*c* 0.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.84 (1H, brd, $J = 4.7$ Hz, H-1), 4.14 (1H, brs, H-2), 1.57 (2H, m, H-3, H-3′), 1.92 (1H, m, H-4), 2.85 (1H, d, $J = 8.8$ Hz, H-6), 2.42 (2H, m, H-8, H-8′), 2.68, 2.53 (each 1H, m, H-9, H-9′), 2.69 (1H, m, H-11), 9.62 (1H, d, $J = 3.1$ Hz, H-12), 0.94 (3H, d, $J = 7.2$ Hz, CH_3-13), 0.78 (3H, d, $J = 6.7$ Hz, CH_3-14), 0.91 (3H, s, CH_3-15); ^{13}C NMR (Table 1); CIMS *m/z* 251.1 (25) $[M + H]^+$; 249.1 (35) $[M + H - H_2]^+$; 233.1 (100) $[M + H - H_2O]^+$; HREIMS *m/z* 250.1565 $[M + H]^+$ (calcd for $C_{15}H_{22}O_3$, 250.1569).

Nardosinanol D (4): yellow oil; $[\alpha]^{23}_D$ –50 (*c* 0.41, $CHCl_3$); ^1H and ^{13}C NMR (Tables 1 and 2); CIMS *m/z* 265.0 (5) $[M + H - H_2O]^+$; 233.0 (100) $[M + H - H_2O - CH_3OH]^+$ (because of unstability of **4** it was impossible to get a HRMS).

Nardosinanol E (5): yellow oil; $[\alpha]^{23}_D$ −168 (*c* 0.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.57 (1H, d, $J = 5.0$ Hz, H-1), 3.93 (1H, brs, H-2), 1.52 (1H, td, $J = 13.0$, 4.0 Hz, H-3), 1.44 (1H, m, H-3′), 1.90 (1H, m, H-4), 2.10 (1H, d, $J = 10.5$ Hz, H-6), 2.05, 1.62 (each 1H, m, H-8, H-8′), 2.39, 1.96 (each 1H, m, H-9, H-9′), 2.40 (1H, m, H-11), 1.36 (3H, d, $J = 7.0$ Hz, CH_3-13), 0.76 (3H, d, $J = 6.7$ Hz, CH_3-14), 0.98 (3H, s, CH_3-15); ^{13}C NMR (Table 1); CIMS *m/z* 249 (100) $[M + H - H_2O]^+$; HRCIMS *m/z* 249.1483 $[M + H]^+$ (calcd for $C_{15}H_{21}O_3$, 249.1491).

Methylation of 5 to 11. Compound **5** (2 mg) was treated with MeI (1 mL) and K_2CO_3 (1 mg) in acetone (2 mL) at room temperature for 12 h, to afford after evaporation and filtration through filter paper compound **11** (1 mg), a yellow oil: $[\alpha]^{23}_D$ –176 (*c* 0.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.73 (1H, d, $J = 5.3$ Hz, H-1), 4.02 (1H, brs, H-2), 1.65, 1.58 (each 1H, m, H-3, H-3′), 2.60 (1H, m, H-4), 2.45 (1H, d, $J = 6.7$ Hz, H-6), 2.76, 2.40 (each 1H, m, H-8, H-8′), 2.65, 2.41 (each 1H, m, H-9, H-9′), 2.97 (1H, quin, $J = 6.7$ Hz, H-11), 1.15 (3H, d, $J = 6.7$ Hz, CH_3-13), 0.88 (3H, d, $J = 6.9$ Hz, CH_3-14), 0.89 (3H, s, CH_3-15), 3.65 (3H, s, OCH_3); ^{13}C NMR (Table 1); CIMS *m/z* 280 $[M + H]^+$.

Rearrangment of 5 to 12. Compound **5** (7 mg) in acidic $CDCl_3$ (0.5 mL) afforded after 3 days compound **12**, a yellow oil: $[\alpha]^{23}_D$ −50 (*c* 0.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.10 (1H, d, $J = 10.0$ Hz, H-1), 5.98 (1H, ddd, $J = 10.0$, 4.4, 2.0 Hz, H-2), 2.22 (1H, m, H-3), 1.86 (1H, ddt, $J = 18.4$, 10.6, 2.0 Hz, H-3′), 2.27 (1H, m, H-4), 2.59 (1H, brd, $J = 6.8$ Hz, H-6), 2.50 (1H, ddd, $J = 16.0$, 5.7, 1.2 Hz, H-8), 2.30 (1H, m, H-8′), 2.23 (1H, m, H-9), 2.09 (1H, ddd, $J = 12.1$, 5.7, 2.0 Hz, H-9′), 2.80 (1H, quin, $J = 6.8$ Hz, H-11), 1.19 (3H, d, $J = 6.8$ Hz, CH_3-13), 0.88 (3H, d, $J = 6.5$ Hz, CH_3-14), 0.85 (3H, s, CH_3-15); ^{13}C NMR (Table 1); CIMS *m/z* 266 $[M + H]^+$.

Extraction of *L. africana* and Isolation of Nardosinanols G−I (7–9) and Lemnafricanol (10). A freeze-dried sample of *L. africana* (15 g) was homogenized and extracted with EtOAc−MeOH−H_2O (5:5:1) to give, after evaporation, a brown gum (2.5 g). The gum was partitioned between aqueous MeOH, *n*-hexane, and CH_2Cl_2 under a Kupchan procedure.[22] The CH_2Cl_2 phase (0.4 g) was chromatographed on a Sephadex LH-20 column, eluting with *n*-hexane−MeOH−CH_2Cl_2 (2:1:1) to give 12 fractions, which were subjected subsequently to VLC over silica gel, using *n*-hexane with increasing proportions of EtOAc as eluent to afford with *n*-hexane−EtOAc (7.5:2.5) compound **8** (3 mg 0.02% dry weight), with *n*-hexane−EtOAc (7:3) **7** (3 mg 0.02% dry weight) and **9** (7 mg 0.05% dry weight), and with *n*-hexane−EtOAc (8:2) **10** (15 mg, 0.1% dry weight).

Nardosinanol G (7): $[\alpha]^{23}_D$ −97 (*c* 0.14, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3026, 2993, 1733, 1558 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); CIMS *m/z* 263 (10) $[M + H - H_2O]^+$; HRCIMS *m/z* 263.1639 (calcd for $C_{16}H_{23}O_3$, 263.1647).

Nardosinanol H (8): $[\alpha]^{23}_D$ −45 (*c* 0.69, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3409, 2935, 1711, 1558 cm^{-1}; ^{13}C NMR (Table 1); CIMS *m/z* 255 (10) $[M + H]^+$; HRCIMS *m/z* 255.1602 (calcd for $C_{14}H_{23}O_4$, 255.1596).

Nardosinanol I (9): $[\alpha]^{23}_D$ –95 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3022, 1716, 1708, 1521 cm^{-1}; ^{13}C NMR (Table 1); CIMS *m/z* 279 (70) $[M + H - H_2O]^+$; HRCIMS *m/z* 279.1592 (calcd for $C_{16}H_{23}O_4$, 279.1596).

Lemnafricanol (10): $[\alpha]^{23}_D$ −54 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3577, 3013, 1717, 1653, 1459 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); CIMS *m/z* 311 (10) $[M + H]^+$; HRCIMS *m/z* 311.1867 (calcd for $C_{17}H_{27}O_5$, 311.1854).

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