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Negombins A–I, New Chlorinated Polyfunctional Diterpenoids from the Marine Sponge *Negombata* Species

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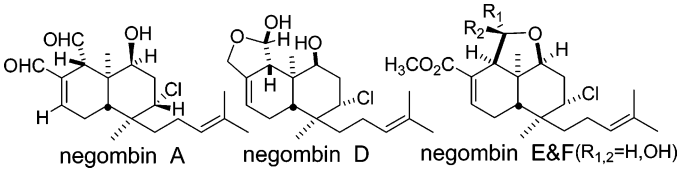
ABSTRACT



Nine novel compounds designated negombins A–I (1–9) were isolated, together with latrunculin, from the Tanzanian sponge *Negombata* sp. The nine are sacculatane type diterpenes, previously only known from liverworts. The structures of the compounds were elucidated by interpretation of MS and 1D and 2D NMR spectra. A possible biogenesis initiated by the naturally rare chloronium ion is suggested, possibly hinting to a guest microorganism as the source of the compounds. Compound 4 is toxic to brine shrimp larvae.

In the framework of searching for bioactive compounds from marine invertebrates[1,2] and our long-standing interest in the metabolites of the sponge *Latrunculia magnifica*[3](presently *Negombata* sp.),[4] we investigated three specimens of this sponge collected at Pemba Island, Tanzania[5]

The ethyl acetate extract of the freeze-dried sponge (5 g dry weight) was separated by sequential chromatographies on Sephadex LH-20 (eluting with hexane/CHCl3/MeOH 2:1:1) and silica gel (eluting with hexane/ethyl acetate) to afford negombins A−I (**1**−**9**) in quantities of 2−12 mg each.

The EIMS of **1**[6] exhibited a molecular ion $[M]^+$ at *m*/*z* 352 for which a formula of $C_{20}H_{29}O_3Cl$, with six degrees of unsaturation, was determined by HRMS. The IR (1725, 1708, 1678 cm^{-1}) together with the ^1H NMR spectra (δ_H 9.56s, 9.70d) suggested the presence of two aldehyde groups. The ^1H NMR and ^{13}C NMR experiments (Table 1) revealed in addition to the two CHO groups (δc 193.0d, 203.8d), the presence of two trisubstituted double bonds (δc 138.2s, 152.8d, most likely conjugated to a CO, and 123.7d, 132.1s; δ_H 7.05q and 5.07t) a hydroxymethine (δc 72.0d, δ_H 3.68q) and, in agreement with the MS peak-cluster, a chloromethine group (δc 61.0d, δ_H 4.55dd). The above functionalities account for four of the six degrees of unsaturation of **1**, suggesting a bicyclic structure for negombin A. The COSY spectrum revealed the presence of three spin systems (**a**−**c**) as shown in Figure 1. HMBC correlation, (Table 1 and Figure 1) established the complete planar structure of **1**. Key starting points for interpretation of the CH correlations were the ones

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[†] School of Chemistry.
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(5) The red orange sponge is growing on steep reef walls exposed to strong water currents and was collected at a depth of 22−25 m. Voucher specimens (ZMTAU PO 25464-25466) are deposited at the Zoological Museum, Tel Aviv University, Israel. A new collection and comprehensive work is required for identification of the sponge which is close to *Sigmosceptrella* another genus, besides the *Negombata*, of the Podospongiidae family.

(6) Negombin A (**1**), an oil. $[\alpha]^{20}_D$ −10.8 (*c* 0.9 $CHCl_3$) (for ^1H and ^{13}C NMR see Table 1). IR ($CHCl_3$) ν_{max} 3054, 2986, 2036, 1708, 1678, 1272 cm^{-1}. EIMS *m*/*z* 352 $[M]^+$(20), 334 $[M - H_2O]^+$ (35), 309 (20), 281 (20), 221 (43), 157 (50), 69 (100). HREIMS *m*/*z* $[M - H_2O]^+$ 334.1687 (calcd for $C_{20}H_{27}O_2Cl$, 334.1693).

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Table 1. NMR Data of Negombin A (**1**)[a,b]

position	δ_C	δ_H (J in Hz)	HMBC (C to H)[c]
1	72.0 CH	3.68q (4.9)	OH, 3, 2a, 2b, 13
2	35.3 CH_2	2.25m 2.24m	
3	61.0 CH	4.55dd (10.0, 6.6)	9, 5, 2a, 2b, 14, 15b
4	41.6 C		14
5	38.5 CH	1.95dd (9.7, 9.5)	13, 14
6	25.8 CH_2	2.52m 2.50m	
7	152.8 CH	7.05q (3.6)	12
8	138.2 C		6a, 6b, 11, 12
9	52.6 CH	3.50q (2.9)	7, 11, 12, 13
10	41.7 C		13
11	193.0 CH	9.70d (4.8)	7
12	203.8 CH	9.56s	9
13	15.5 CH3	1.00s	1
14	17.0 CH_3	1.05s	3
15	38.2 CH_2	1.62m 1.40ddd (15.6,11.9,5.3)	14
16	21.3 CH_2	2.02m 1.80m	
17	123.7 CH	5.07t (6.8)	19, 20
18	132.1 C		17, 19, 20
19	25.9 CH_3	1.69s	17, 20
20	17.5 CH_3	1.61s	17, 19

[a] Data recorded in $CDCl_3$ on Bruker Avance 400 and 500 MHz instruments (100 MHz for ^{13}C and 500 MHz for all other spectra). [b] The CH correlations were assigned by an HSQC experiment. [c] The letters a and b for a methylene pair denote the upper (a) and lower (b) field protons.

from the four methyl groups, two aldehydes, and the hydroxyl- and chloromethine functionalities.

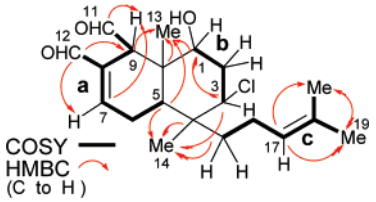


Figure 1. COSY and key HMBC correlations of **1**.

The relative stereochemistry of **1** was determined by analysis of the coupling constants of the protons of the functional groups (Table 1) and NOE cross-peaks (Figure 2). NOEs

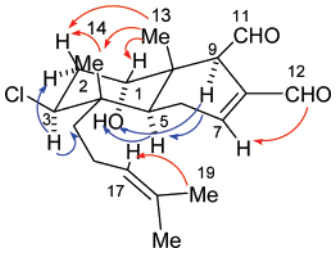
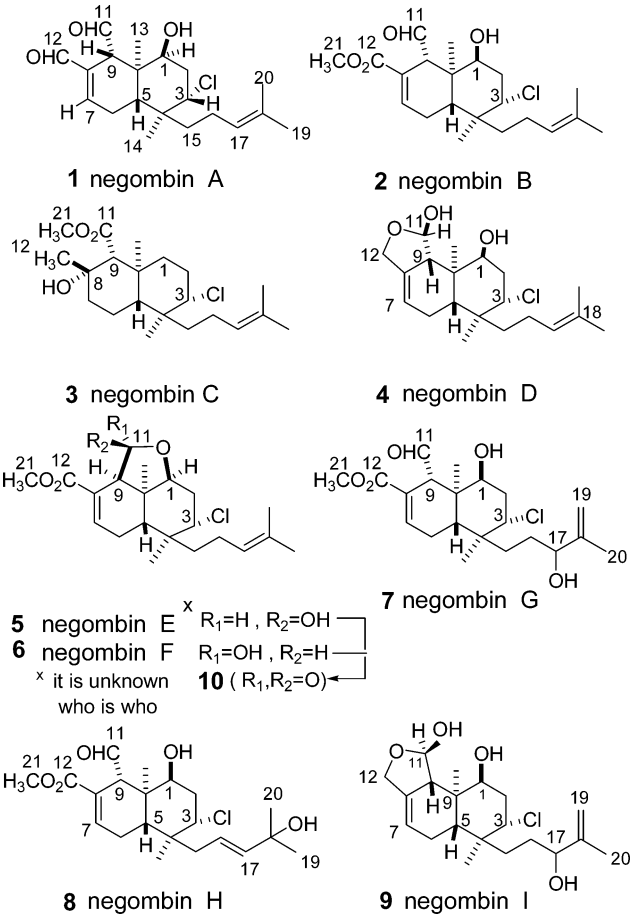


Figure 2. Key NOEs of negombin A.

between H-9α (β assigned for the methyl side), H-5α and OH(1); between H-1β and CH_3-13; between Me-14 and -13 and H-2β; and between H-5α and H-3α established the configuration of the OH and Cl groups and the trans ring fusion. A 9.7 Hz coupling constant of H-5α, 10.0 Hz of H-3α, and 4.9 Hz of H-1β confirmed their axial, axial, and equatorial configurations, respectively, completing the relative stereochemistry of **1**. Tentatively, based on the known absolute stereochemistry of the drimane-class terpenoids, for example, that of sacculatnes and polygodial, the same stereochemistry was suggested for **1**−**9**.

Close in structure to negombin A was negombin B (**2**).[7] The only difference between the two being replacement of the CHO(12) group by a carbomethoxy group (δc 168.0s, 52.0q, δ_H 3.80s, 3H). A change which, as expected, influenced the resonances of the Δ^7-bond (δc 139.9 and 142.1, δ_{H-7} 7.22). Similar NOEs to those observed for **1** pointed to the same stereochemistry.



Negombin C (**3**), the second, after **1**, most abundant compound,[8] exhibited the highest peak at m/z 370, agreeing

(7) Negombin B (**2**), an oil. $[\alpha]^{20}_D$ −38.7 (c 0.16 $CHCl_3$) (for ^1H and ^{13}C NMR data see Supporting Information). CIMS m/z 383 $[MH]^+$ (100), 367 (30), 351 (65), 299 (55). HRCIMS m/z 383.1975 (MH^+) (calcd for $C_{21}H_{32}O_4Cl$, 383.1981).

(8) Negombin C (**3**), an oil. $[\alpha]^{20}_D$ +36.3 (c 0.9 $CHCl_3$) (for ^1H and ^{13}C NMR data see Supporting Information). CIMS m/z 371 $[MH]^+$ (30), 353 $[MH - H_2O]^+$ (100), 335 (35), 317 (75). HRCIMS m/z $[MH - H_2O]^+$ 353.2245 (calcd for $C_{21}H_{34}O_2Cl$, 353.2239).

with $C_{21}H_{35}O_3Cl$ $[M]^+$. NMR experiments revealed the presence of a methyl ester (δc 51.3q, δ_H 3.70s, 3H), replacing the 11-CHO group of **1** and **2**, and a tertiary alcohol group (δc 70.2s) instead of the 12-aldehyde of **1**. The C-12 position of the additional methyl group (δ_H 1.12s) was determined from its HMBC correlations from C-9, C-8 (δc 70.2s), and C-7 (Table 1). Further $^2J_{CH}$ and $^3J_{CH}$ HMBC correlations, to H-9, -13, and -21 (C-11 to H-9, -21; C-12, -13 to H-9; and C-1, -5, -9, -10 to H-13), were in full agreement with the suggested structure. In addition to the above changes the 1-hydroxyl group of **1** and **2**, in the second ("right") ring, was absent in **3** ($\delta_{C(1)H2}$ 40.4t; δ_H 1.35, 1.40).

The stereochemistry of the two, C-8 and -9, chiral centers were determined from NOE cross-peaks between the CO_2CH_3 protons and methyls-13 (on the β-side) and methyl-12; between H-9α, H-7α, H-5α, and CH_3-12 on the α-side (Figure 2), hence, both CH_3-12 and the CO_2CH_3 group on the trans decalin ring system (confirmed by a NOE between CH_3-13 and -14), are equatorial.The EIMS of negombin D

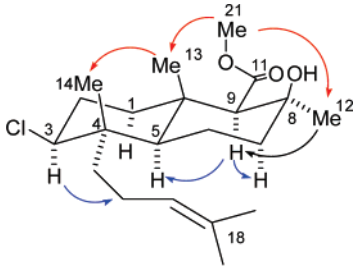


Figure 3. Key NOEs of negombin C.

(**4**)[9] exhibited a $[M - H_2O]^+$ ion at *m/z* 336 ($C_{20}H_{29}O_2Cl$), HRMS. Loss of a molecule of water became evident from the requirement of 20 carbon atoms (^{13}C NMR) and three oxygen atoms for a lactol and a hydroxyl group[10] (see below). Half of the molecule of **4**, the "right" portion, was identical with the corresponding half (C_{1-4}−C_{20}) in **1** and **2**. The other "left" cyclohexene ring is fused to a five-membered lactol (δ_{C-11} 98.7d, δ_{H-11} 5.30d (J = 5.6 Hz), and δ_{C-12} 69.2t, δ_{H-12} 4.25d and 4.50d, J = 11.5 Hz). Vicinal coupling between H-9 and the lactol proton H-11, and allylic-coupling between the AB system of CH_2(12) and H-7 confirmed the position of the lactol ring. NOEs between H-11β and CH_3-13 and between OH(11α) and H-9α established the suggested C-11 stereochemistry.

An additional pair of compounds were the epimeric negombins E and F [**5** and **6**][11] which could not be completely separated from each other (each was obtained in ca. 80% purity). Both **5** and **6** exhibited the same pseudo-molecular $[M - H_2O]^+$ peak. Loss of water was deduced from the 21 carbon resonances in the ^{13}C NMR spectrum and the need of four oxygen atoms (a lactol and a CO_2CH_3 group). The difference between **5** and **6** and negombin B (**2**) was the replacement of the 1-hydroxy-9-carboxaldehyde functionality of **2** by a lactol group (δ_{C-11} 101.7 and 97.0 and δ_{H-11} 5.22d (J = 4.5 Hz), 5.61dd (J = 5.3, 2.2 Hz) for **5** and **6**, respectively). As far as could be judged from the NMR spectra of **2**, **5**, and **6**, they are not in equilibrium in $CDCl_3$. Jones oxidation of both **5** and **6** afforded the corresponding lactone **10**.[12]

Three other compounds, negombins G−I (**7**−**9**) were obtained in minute quantities only. Negombins G and H possess the same substituted decalin system as negombin B (**2**), and negombin I possesses the same bicyclic system as negombin D (**4**); the three differ from **2** and **4** in the side chains.

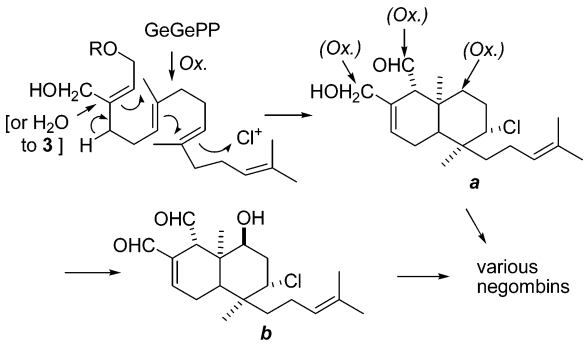
The EIMS spectrum of **7**[13] exhibited a $[M - H_2O]^+$ ion at *m/z* 380. The molecular formula was determined by HRMS of the $[M - H_2O]$ peak and ^{13}C-resonances to be $C_{21}H_{31}O_5Cl$. The NMR data of the bicyclic system was almost identical to those of **2**; differences were only observed in the side chain, namely, replacement of the $-CH{=}C(CH_3)_2$ terminus of **1**−**6** by a $-CH(OH)C(CH_3){=}CH_2$ functionality (δ_{C-17} 76.3d, δ_{C-18} 147.3s, and δ_{C-19} 111.3t; δ_{H-17} 4.00t, δ_{H-19} 4.95s and 4.88s, and δ_{H-20} 1.70s).

Negombin H (**8**)[14] possesses the same formula as **7**, and the NMR data of the ring system were found to be almost identical to those of **2** and **7**. Differences were observed in the NMR of the side chain suggesting a $-CH_2CH{=}CH{-}C(CH_3)_2OH$ terminus (δ_C 142.9d, 120.3d, 70.6s, δ_H 5.56dt (15.5, 7.3), 5.75d(15.5), 1.31s(3H), 1.30s(3H)).

Negombin I (**9**),[15] *m/z* 352 $[M - H_2O]^+$, $C_{20}H_{29}O_3Cl$, comprises the ring system of **4** and the side chain of **7** (δ_C 79.0s, 145.0s, 115.0t, 17.6q; δ_H 3.86t, 5.10s and 5.05s 1.70s).

Outstanding in the structure of the negombins is the chlorine atom. A suggested biogenesis, shown in Scheme 1,

Scheme 1. Suggested Biogenesis for the Negombins



starts with a chloronium ion. While isoprenoid cyclizations initiated by bromonium ion are well-known in the marine environment, electrophilic attack of a double bond by Cl^+

(9) Negombin D (**4**), an oil. $[\alpha]^{20}_D$ −1.7 (*c* 0.5 $CHCl_3$) (for ^1H and ^{13}C NMR data see Supporting Information). EIMS *m/z* 336 $[M - H_2O]^+$ (75), 321 (100). HREIMS *m/z* 336.1849 $[M - H_2O]^+$ (calcd for $C_{20}H_{29}O_2Cl$, 336.1853).

(10) Acetylation of **4** with Ac_2O/pyridine at room temperature overnight afforded the expected 9,11-diacetate [δ_H 2.04, 2.16, 3H each 6.05d (H-11), 4.90bs (H-9)].

(11) Negombin E and F (**5**, **6**), an oil (for ^1H and ^{13}C NMR data see Supporting Information). CIMS *m/z* 365 $[MH - H_2O]^+$ (100). HRCIMS *m/z* $[MH - H_2O]^+$ 365.1883 (calcd for $C_{21}H_{29}O_3Cl$, 365.1876).

is rare and is only reported for cyanobacteria.[16,17] Hence, isolating the negombins only from the Tanzanian *Negombata* sponge[18] suggests their origin may be a guest microorganism within the sponge. The latter notion receives further support from the isolation of the pungent tasting bioactive polygodial, with a drimane-skeleton,[19] and sacculatol, with the same skeleton as the negombins, from liverworts.[20] Negombin D (**4**) exhibited toxicity in concentration of 0.1 mg/mL to brine shrimp larvae.[21] The small available amounts of material prevented further tests.

Supporting Information Available: NMR data (^{1}H NMR, and ^{13}C NMR) for negombins A−I including COSY, HSQC, and HMBC for negombin A. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Jones oxidation ($Na_2Cr_2O_7$ in acetone) of compounds **5** and **6** afforded the corresponding lactone **10** (replacement of the anomeric C-11 signal by lactone resonances: δ_C 174.0 (C-11), 53.6 (C-1), 83.1 (C-9); δ_H 3.40s (H-1) and 4.33bs (H-9).

(13) Negombin G (**7**), an oil (for ^{1}H and ^{13}C data see Supporting Information). EIMS *m/z* 380 $[M - H_2O]^+$ (10), 348 (10), 319 (25), 251(55), 215 (100). HREIMS *m/z* 380.1752 $[M^+ - H_2O]^+$ (calcd for $C_{21}H_{29}O_4Cl$, 380.1747).

(14) Negombin H (**8**), an oil (for ^{1}H and ^{13}C data see Supporting Information). EIMS *m/z* 380 $[M - H_2O]^+$ (15), 348 (10), 319 (25). HREIMS *m/z* 380.1741 $[M - H_2O]^+$ (calcd for $C_{21}H_{29}O_4Cl$, 380.1747).

(15) Negombin I (**9**), an oil (for ^{1}H and ^{13}C NMR see Supporting Information). EIMS *m/z* 352 $[M - H_2O]^+$ (30), 334 (30), 319 (40), 253 (40), 235 (45). HREIMS *m/z* 352.6767 $[M - H_2O]^+$ (calcd for $C_{20}H_{29}O_3Cl$, 352.6771).

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(17) Radical chlorination, on the other hand, is well known, for example, hydrogen substitution of methyl protons to produce mono-, di- and trichloromethyl groups.

(18) Dozens of studied Red Sea *Negombata* sp. were never found to contain negombins.

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