ORGANIC LETTERS
2004
Vol. 6, No. 22
4013−4016

Likonides A and B: New Ansa Farnesyl Quinols from the Marine Sponge *Hyatella* sp.

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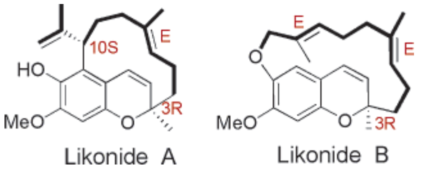
Received August 18, 2004

ABSTRACT



Two novel ansa farnesyl quinols, designated likonides A and B, were isolated together with avarone from the Kenyan sponge *Hyatella* sp. The compounds are of a unique ansa structure. The structures and stereochemistry of the compounds were elucidated by interpretation of MS, two-dimensional NMR, and CD experiments.

Marine secondary metabolites of mixed C_{15} + C_6 biogenesis are well-known from algae and especially from sponges.[1−4] More than 100 sesquiterpene quinones or quinols from marine sources have been reported.[4] Many of them exhibit a variety of promising biological activities such as cytotoxic, antiviral, antimicrobial, and immunomodulatory effects.[4,5] Well-known examples are the avarone and avarol[6,7] and ilimaquinone.[8]

Tunicates, contrary to sponges and algae, are poor in isoprenoids, and only a few examples of farnesylated quinones and quinols, C_{15} + C_6 metabolites are known.[1,3] Among the latter are the longithorones,[9] longithorols,[10] and floresolides,[11] unique para- and metacyclophane ansa compounds, exclusively reported from *Aplidium* spp. ascidians.

In our continuing focus on the chemistry of Kenyan invertebrates,[12,13] we report here the isolation and structure elucidation of avarone (**1**, Figure 6) and two novel ansa compounds, designated likonides A and B (**2** and **3**), of unprecedented structure from the Kenyan sponge *Hyatella* sp. The sponge was collected off Likoni in a site known as “Wall”, a steep reef, almost vertical, at a depth of 16−28 m. Previous reports on *Hyatella* sponges by us[5] and others[3,14,15] already described the isolation of sesquiterpene quinones such as compound **1**. Hence, it was of interest to

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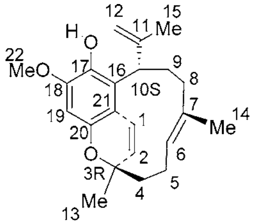
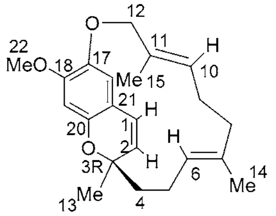
10.1021/ol048358d CCC: $27.50

find other metabolites embodying the farnesyl and quinol building blocks in a completely different way.

The ethyl acetate extract of the freeze-dried sponge (23 g) was separated by sequential chromatographies on Sephadex LH-20 (eluting with hexane/$CHCl_3$/MeOH, 2:1:1) and silica gel (eluted with hexane and ethyl acetate) to afford avarone **1** (15 mg, 0.06% dry weight), **2** (15 mg, 0.06% dry weight), and **3** (1 mg, 0.004% dry weight). Compound **1** of MW 358 was determined according to its one- and two-dimensional NMR data to be avarone previously isolated from sponges.[6,7]



likonide A (**2**)



likonide B (**3**)

The EIMS of **2** exhibited a molecular ion at *m*/*z* 340. The molecular formula $C_{22}H_{28}O_3$ (9° of unsaturation) was determined by ^{13}C NMR and HRMS data.[16] The NMR experiments revealed the presence of a cis-disubstituted double bond (δ_H 6.45 and 5.43 d, $J = 9.9$ Hz), a trisubstituted double bond ($-CH{=}C(CH_3)-$, δ_H 5.25 bd and 0.93 s,[17] 3H), an isopropenyl group (δ_H 4.79, 4.85, and 1.63 s, 3H), a quaternary methyl group, most likely next to an oxygen atom (δ_H 1.36 s), and a pentasubstituted benzene ring (Table 1). Two of the benzene substituents are a methoxyl (δ_H 3.73 s) and a hydroxyl (δ_H 6.85 s). The single benzene proton (δ_H 6.31 s) has, according to its high-field resonance, to be seated ortho to the latter two oxy substituents.

Table 1. NMR Data for Compounds **2** and **3** in DMSO-d_6[a]

position	b	δ_C	**2** δ_H (*J* in Hz)[c]	δ_C	**3** δ_H (*J* in Hz)[c]
1	CH	122.3	6.45 d (9.9)	123.8	6.37 d (9.8)
2	CH	126.6	5.43 d (9.9)	125.0	5.40 d (9.8)
3	C	76.2		78.6	
4	CH_2	42.5	1.80 1.70	41.2	1.66 1.56
5	CH_2	25.2	2.05 1.88	22.8	2.08 2.00
6	CH	121.9	5.25 bd (7.1)	126.8	4.86t (7.1)
7	C	131.7		129.9	
8	CH_2	34.9	2.30 bd (13.9) 1.45 t (13.9)	38.8	1.98 1.62
9	CH_2	29.0	2.08 1.92	22.4	2.05 1.90
10	CH	42.8	3.94 d (4.0)	131.1	4.76 t (7.2)
11	C	148.4		131.0	
12	CH_2	109.1	4.85 s 4.79 s	79.5	4.38 d (11.4) 4.07 (11.4)
13	CH_3	31.0	1.36 s	30.0	1.41 s
14	CH_3	19.7	0.93 s	14.1	1.32 s
15	CH_3	22.7	1.63 s	14.4	1.60 s
16	C	123.6		119.3[f]	6.60 s
17	C	139.1		140.0	
18	C	147.5[d]		153.0	
19	CH	98.4	6.31 s	100.5	6.33 s
20	C	147.7[d]		151.0	
21	C	116.9		113.0	
22	CH_3	55.9	3.73 s	55.0	3.66 s

[a] Measured at 500 MHz (^1H) and 100 MHz (^{13}C). [b] Multiplicity based on DEPT experiment. [c] Proton correlation to C-atoms based on HMQC experiment. [d] Assignments can be switched. [e] Notations a and b denote high-field and low-field protons of the geminal pair. [f] CH for **3**.

The COSY spectrum revealed three spin systems as shown by the bold lines in Figure 1. Most instructive for the structure elucidation were the CH correlations (Figure 1). First, the strong HMBC correlations of the four methyl groups with their neighboring C-atoms were analyzed, and the resulting unities were then extended by the COSY correlations. Additional CH correlations from the clear resonances of H-1,-2,-6,12a,b and H-19, through 2−5 bonds to neighboring C-atoms, corroborated the planar structure of **2**. Key correlations (Figure 1) were from H-1 to C-3,-4,-13,-16,-17, 20, and −21 (^2J to ^4J); H-2 to C-19 (^5J); H-19 to C-17 and -21; O**H** to C-16,-17, and C-18; and from the OC$\mathbf{H}_3$ to C-18 and C-19. the *E* configuration of the C-6(7) double bond was suggested on the basis of the relative high-field chemical shift of C-5 (δ_C 25.2) due to a γ-effect with Me-14 and a NOE between the latter and H-5. The relative stereochemistry of **2** was determined mainly by analysis of the measured NOEs (Figure 2). To diminish the transannular strain, between the chromene and the bridging chain (C-4 to -10) in the ansa molecule, C-4 has to be pseudoaxial, thus

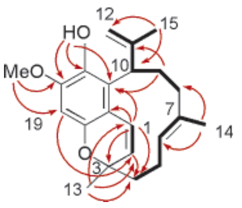


Figure 1. COSY (−) and selected HMBC (→) correlations of **2**.

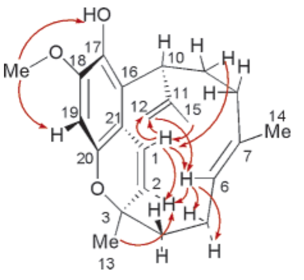


Figure 2. Key NOE correlations of **2**.

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(16) Likonide A (**2**): an oil; $[\alpha]_D$ +85 (*c* 0.56 MeOH); CD λ_{275} + 0.4, λ_{330} + 1.5 (CH_3OH); ν_{max} 3540, 2930, 1633, 1609, 1480 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 340 [M^+]; HREIMS *m*/*z* 340.2034 (calcd for $C_{22}H_{28}O_3$ 340.2031).

(17) The high field of the vinyl methyl group is caused by the benzene ring.

fixing the dihydropyrane conformation, i.e., the angle of the 1(2) double bond relative to the benzene ring. The direction of the isopropenyl group, on C-10, was determined to be on the same side as the chromene 1(2)-double bond on the basis of NOEs between CH_3-15, H-12a, H-12b, and H-1 (Figure 2). Key NOEs from the OCH_3 to the O**H** and H-19 and between H-6 and H-2 confirmed the suggested structure. The absolute stereochemistry of **2** could be determined according to the Cotton effect of the styrene chromophore of the chromene system.[19,20] The absolute configuration of the C-3 position of **2** was deduced on the basis of Kikuchi's method.[19,20] The CD spectrum of likonide A exhibited a positive Cotton effect around the 275 nm region (MeOH, $\Delta\xi + 0.4$) due to the styrene chromophore of the chromene moiety, indicating that the latter ring has a negative chirality (a left-handed helix) (Figure 5). As (vide supra) $CH_2(4)$ adopts the pseudoaxial conformation, the absolute configuration of C-3 is *R*, and hence the chirality of C-10 is *S*.

The EIMS of likonide B (**3**) also exhibited a molecular peak at *m*/*z* 340. The molecular formula was determined by ^{13}C NMR and HRMS data[21] to be identical to that of **2**, $C_{22}H_{28}O_3$. The NMR data of **3** pointed to a structure similar to that of **2**, i.e., to the existence of a chromene system, two double bonds (besides the chromene one), and, most likely, also a bridging chain. The above functionalities account for the 9° of unsaturation of the molecule. However, significant differences were also observed, namely, no isopropenyl group was seen but rather a second trisubstituted double bond (δ_H 4.76 t, 1H and 1.60 s, 3H), the benzene ring was found to carry two para-positioned protons (δ_H 6.33 and 6.60 s), a methylenoxy group showed up (δ 4.07 and 4.38 d $J = 11.4$ Hz), and the phenol O**H** disappeared.

The structure determination of **3** was achieved in the same manner as described for **2**. Again, three spin systems were observed by the COSY experiment and the complete planar structure was concluded from the HMBC correlations (Figure 3). Important were the additional correlations from the H_2-12 AB-system to C-10 and C-17 and the correlations of the second aromatic proton, H-16, to C-1,-18, and -20.

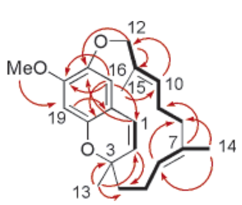

Figure 3. COSY (−) and key HMBC (→) correlations of **3**.

Compounds **2** and **3** differ in the cyclization mode of the bridging chain, namely, whereas in **2** C-10 binds to C-16, leaving a free ortho OH group, in **3** C-12 forms an ethereal bond with the phenol group leaving C-16 nonalkylated. The structure of **3** was corroborated by the measured NOEs as shown in Figure 4. Compound **3** possesses a single chiral center, C-3, the absolute configuration of which was determined, as in the case of **2**, by the positive Cotton effect of the styrene chromophore of the chromene system to be 3*R*.[18,19]

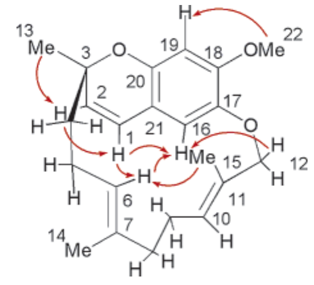

Figure 4. Key NOE correlations of **3**.

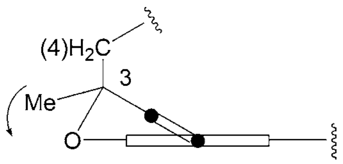

Figure 5. Perspective drawing of the chromene moiety of likonides A and B (a left-handed helix).

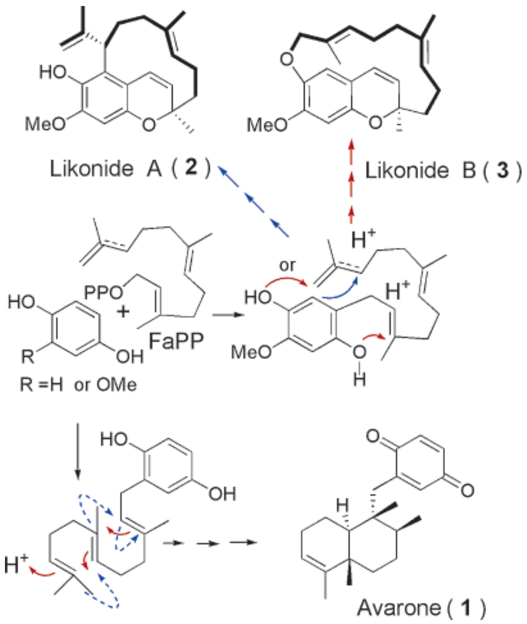

Figure 6. Suggested biogenesis for compounds **1**−**3** (general outline).

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(18) A different, less likely, substitution pattern was excluded by NOE correlations.

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Likonides A and B are new ansa compounds. Likonide A can be considered a 1-oxa-[9]metacyclophane, [10]orthocyclophane, or 2,5-(4-methyl-7-isopropenyl-hept-3-enylene)-chromene, and likonide B can be looked upon as a 1-oxa-[13]metacyclophane, 1,12-dioxa-[12]paracyclophane, or 2,6-(2,6-dimethylnona-2,6-dienylene)-chromene.

From the biogenetic point of view, it was interesting to find sesquiterpene quinones (like **1** and other analogues that appear in minute amounts) and the new ansa compounds (**2** and **3**) together in the same sponge. The latter coexistence demonstrates different ways by which farnesyl and activated (to alkylation) hydroxylated benzenes can interact (Figure 6). Compounds **2** and **3**, although resembling the *Aplidium* tunicate longithorones and others alike,[9−11] are of different ring sizes and assembly and they are the first examples of these ansa compounds from sponges.

Acknowledgment. We thank PharmaMar Madrid for financial support. Special thanks are due to Mr. Charles O. Oduol, Assistant Director of Fisheries, Mombasa, Fisheries Department, Ministry of Agriculture and Rural Development, Kenya, for issuing the collection permit for the research expedition. Shimrit Perkol and Tali Yacobovitch are acknowledged for assistance in the fieldwork.

Supporting Information Available: NMR data of **2** and **3** (^{1}H spectra and full HMBC and NOE correlations). This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Likonide B (**3**): an oil; $[\alpha]_D$ +27 (*c* 0.08, MeOH); CD λ_{275} + 0.4, λ_{330} + 1.5 (CH_3OH); ν_{max} 3687, 3050, 1604, 1503, 1465 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 340 [M$^+$]; HREIMS *m*/*z* 340.2027 (calcd for $C_{22}H_{28}O_3$ 340.2031).