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Three New Sesquiterpene Hydroquinones From Marine Origin

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Abstract: Three new sesquiterpene hydroquinones peyssonol A and B (**5** and **6**) and hyatellaquinone (**7**) have been isolated from the alga *Peyssonnelia sp.* and the sponge *Hyatella intestinalis*. The structure of the three compounds was determined mainly by NMR measurements. Peyssonol A (**5**) is the first reported bromo sesquiterpene hydroquinone. Several of the new compounds were found in a preliminary test to inhibit the reverse transcriptase of human immunodeficiency virus (HIV).

Marine algae and sponges have yielded a variety of compounds of mixed biogenesis that are based on the farnesyl hydroquinone skeleton, e.g. zonarol, avarol and ilimaquinone, compounds **1-3** respectively[1-4]. Reports that avarol (**2**)[5], derivatives of avarone (**4**)[6,7] and ilimaquinone (**3**)[8] show activity against HIV has resulted in renewed interest in these compounds[9].

During a survey of marine organisms for anti HIV RTs activities (reverse transcriptases of human immunodeficiency virus) we have isolated from the active anti HIV RTs extracts of the alga *Peyssonnelia sp.* (family Peyssonneliaceae) two new compounds of the farnesyl hydroquinone group. The structures of the two compounds, **5** and **6**, designated peyssonol A and peyssonol B, respectively, together with another closely related sponge metabolite, named hyatellaquinone (**7**) isolated from *Hyatella intestinalis* (Lamarck)(family: Spongiidae) are the subject of this report.

The Red Sea *Peyssonnelia sp.* alga was frozen shortly after collection and kept frozen until the work up. The methanol-ethyl acetate (1:1) extract of the freeze-dried specimen (390 mg) was partitioned between aq. MeOH and hexane, CCl_4 and $CHCl_3$. The hexane and CCl_4 solubles were chromatographed in hexane-MeOH-$CHCl_3$ (2:1:1) over Sephadex LH-20 and selected fractions were purified further by vacuum liquid chromatography over silica gel to afford peyssonol A (**5**) and peyssonol B (**6**), as non-crystalline gums (ca. 0.01% yield, each, dry wt).

Compound **5**, $C_{22}H_{29}BrO_3$ (*m/z* 420/422;1:1) showed hydroxyl (3450 cm^{-1}) and carbonyl (1655 cm^{-1}) absorptions in the IR spectrum. The ^1H NMR spectrum of **5** (500 MHz, C_6D_6) showed three quaternary methyl signals (δ 0.80, 0.90, 1.05), the AB part of an ABX spin system (δ 2.30t, J=12 and 2.70dd, J=12,4), a methine-bromide resonance (δ 3.70dd, J=10,3), an exocyclic methylene absorption (δ 4.20d,

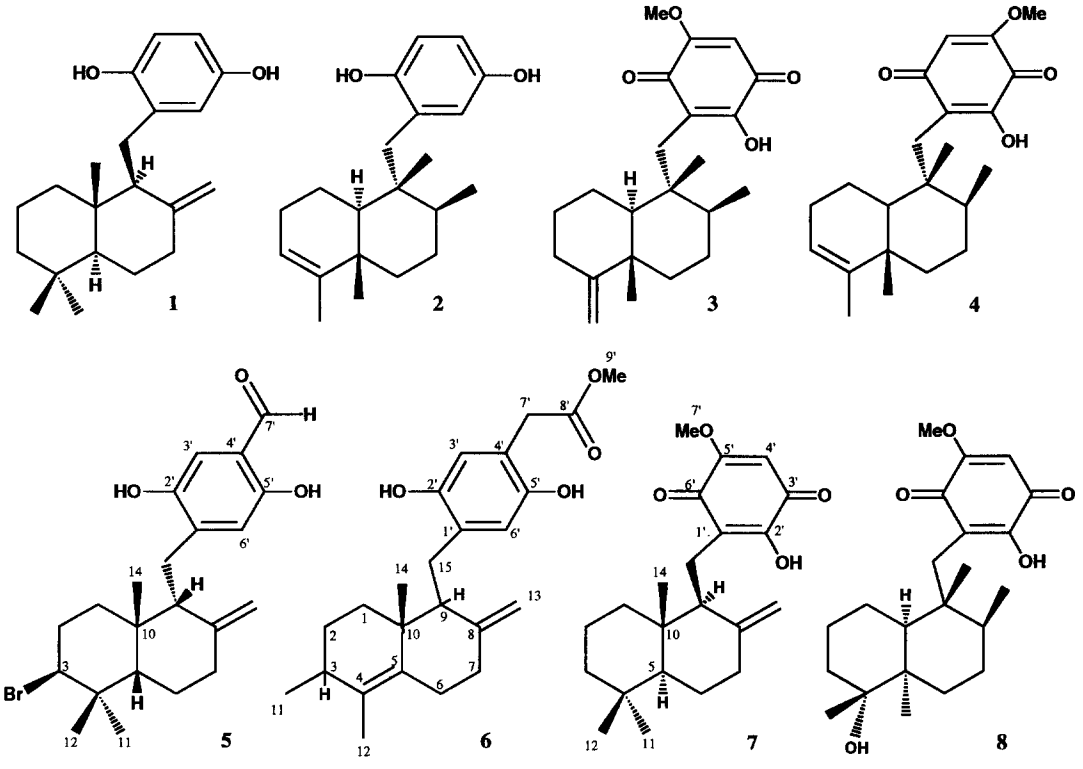
4.50d, J=2, 2H), two singlet aromatic proton signals (δ 5.80, 6.70 in C_6D_6 and 6.62, 6.85 in $CDCl_3$), an aldehyde singlet (δ 9.40) and a sharp phenol singlet (δ 11.20, suggesting it to be β to the CO group). The ^{13}C NMR spectrum (Table 1) exhibited all 22 carbon atoms and confirmed the above functionalities. The molecular formula (8 degrees of unsaturation) and the above spectral features indicated that peyssonol A (**5**) contained, in addition to the phenyl group two other rings. 2D NMR experiments, namely, COSY, HMQC and HMBC measurements (Table 1) established unequivocally a drimane skeleton for **5**, similar to the one of the brown seaweed metabolite zonarol (**1**)[1]. To the best of our knowledge peyssonol A (**5**) is the first bromo-farsenyl hydroquinone marine metabolite.

On the basis of the ^{13}C NMR data a cis-decalin configuration was suggested for **5**. Most characteristic for the distinction between cis and trans 4,4,10-trimethyl decalins are the chemical shifts of C5 and the angular 10-methyl (C14 of **5**). Namely, chemical shifts of ca. 46(d) and 22.5 (q) ppm for C5 and the 10-methyl, respectively, are expected for the cis isomer against ca.55(d) and 14(q) ppm for the trans decalin[10,11]. No ^{13}C NMR data was published in 1973 for zonarol (**1**), however, comparison of the ^{13}C data of compounds **5** and **7**, *vide infra* (Tables 1 and 3), suggested clearly the cis configuration for **5** and the trans one for **7**. Furthermore, a non-steroidal conformation, in which Me-14 is axial to ring A, was determined on the basis of the following nOe's: Me-14/H-2ax, (δ 2.14)dq, J=3, 13)) (3%); Me-14/Me-12ax(5%); Me-14/H-9 (4%); Me-11eq/H-3ax, (δ 3.70 (dd, J=3,10)) Me-11/H-6b(δ 1.39)(3%); Me-11/Me-12 (4%); Me-12/Me-14 (5%); Me-12/H-2ax. (3%); Furthermore, the benzyl group is suggested to be α-equatorial, if it would have been in the β-orientation (as Me-14 and H-5) it could have been expected that the large benzyl group would change the non-steroidal conformation of the decalin to a steroidal one, a conformation which does not agree with the measured nOe's. The 1′,2′,4′,5′-tetrasubstituted pattern suggested for the aromatic portion of **5** is based on the carbon chemical shifts[12] (which are almost the same in C_6D_6 and $CDCl_3$) and mainly on the long-range two and three bond CH-correlations (Table 1).

The molecular formula for peyssonol B (**6**), $C_{24}H_{32}O_4$, was established by high resolution mass spectrometry (m/z 384. 2298, Δmmu -3), in full agreement with the ^{13}C NMR data (Table 2). The IR spectrum of **6** showed a hydroxyl (3400 cm^{-1}) and a carbonyl (1710 cm^{-1}) absorption, and its ^1H NMR, exhibited one quaternary methyl signal (δ 0.94), a methyl doublet (δ 1.04), a vinyl-methyl singlet (δ 1.64), the AB part of an ABX system (δ 2.75d, J=15 and 2.80dd, J=11, 15) a sharp two proton signal (δ 3.55), a methoxy signal (δ 3.70), an exocyclic methylene absorption (δ 4.70, 4.85, each 1H) and two aromatic proton signals (δ 6.50 and 6.70). The ^{13}C NMR data of **6** (Table 2) fully supported a 1′,2′,4′,5′-tetrasubstituted p-hydroquinone, two double bonds and a methyl ester. The latter functionalities (seven degrees of unsaturation) required for **6** two additional rings. Comprehensive 2D NMR measurements, summarized in Table 2, established the two parts of peyssonol B (**6**); i.e. a tetrasubstituted p-hydroquinone and a rearranged drimane system. The substitution pattern of the aromatic ring, first suggested by the chemical shifts, was unequivocally confirmed by the two and three bond CH-correlations (a HMBC experiment, Table 2). The same HMBC experiment also established, definitely, the substitution pattern of the decalin, however, the relative configuration of the chiral C3 and C9 centers could not be determined unequivocally because of the unpredictable stereochemistry of the twisted cyclohexane rings. Insufficient material prevented us from preparing derivatives of **6** which may have been more suitable for stereochemical studies. Worth mentioning is the $C_{14}H_{21}$

fragment (*m/z* 189) in the mass spectrum of **6**, in good agreement with the suggested structure.

Obtaining both **5** and **6** from the same alga suggests the 3-bromodrimane **5** (or may be a compound with another good C3 leaving group) to be the natural precursor of **6**. That is, leaving of the C3-substituent followed by a 1,2-shift of one of the C4 methyl groups, to C3, and sequentially abstraction of H-5 will lead to the 4(5) double bond.



While isolating compounds **5** and **6** from the alga we have isolated another closely related compound designated hyatellaquinone (**7**) from the Indo-Pacific sponge *Hyatella intestinalis* (Lamarck)(order: Dictyoceratida). From the ethyl acetate extract (140 mg) of this sponge (20 g) we have isolated after solvent partitioning followed by Sephadex LH-20 chromatography (hexane-$CHCl_3$-MeOH, 2:1:1) three compounds (a few mgs, of each). Two of the compounds were found to be the known sponge metabolites ilimaquinone (**3**)[4] and the recently reported smenochromene A[11]. The third compound hyatellaquinone (**7**) $C_{22}H_{30}O_4$ (*m/z* 358. 2147, Δmmu +2) showed in the IR spectrum hydroxyl (3400 br cm^{-1}) and quinone (1656, 1649, 1642 cm^{-1}) absorptions. The ^1H NMR spectrum of **7** showed three quaternary methyl signals (δ 0.75, 0.80, 0.85), a characteristic benzyl AB part of an ABX system (δ 2.52,(dd, J=14, 3) and 2.63,(dd, J= 14, 11)), a methoxyl singlet (δ 3.80), an exocyclic methylene absorption (δ 4.65s, 4.68s, 2H) and a sp^2 singlet (δ 5.80). From the NMR data it was evident that compound **7** is isomeric with ilimaquinone (**3**). Both embody the same substituted quinone moiety, but differ in the decalin system. 2D NMR experiments summarized in Table 3 established, unequivocally, a drimane skeleton for **7**.

Table 1 NMR Data of Peyssonol A (5)

Proton	$^{13}C^{d}$	$^{1}H^{d}$	COSY^{d} to H	HMBC^{d} to C	Proton	$^{13}C^{d}$	$^{1}H^{d}$	COSY^{d} to H	HMBC^{d} to C
1a	37.2t	0.92m	2a,2b,1b		11	31.3q	1.05s	12	12,5,4,3
1b		1.42m	14,2a,2b,1a	14,10,3	12	19.2q	0.90s	11	11,5,4,3
2a	32.1t	1.96m	2b,1a,1b	10,4,3,1	13a	111.4t	4.20t (2)	13b	9,7
2b		2.14qd (13,3)	2a,1a,1b	10,4.3,1	13b		4.50t (2)	13a	9,7
3	69.5d	3.70dd (10,3)	2a,2b	12,5	14	22.5q	0.80s	1b	9,5,1
4	40.1s								
5	46.0d	1.15m	6b	14,12,10,6,4,3	15a	29.0t	2.30t (12)	15b,9	9,8,6′,2′,1′
6a	25.6t	1.15m	7a,7b,6b	10,4	15b		2.70dd (12,4)	15a,9	9,8,6′,2′,1′
6b		1.39m	7a,7b,6a	10,8					
7a	32.2t	1.89m	7b,6a,6b	13,9,8,5	1′	140.0s			
7b		2.05m	7a,6a,6b	13,9,8,5	2′	146.0s			
8	146.1s				3′	117.6d	5.80s		7′,5′,2′,1′
9	57.3d	1.85m	15a,15b	15,14,13,10,8,7	4′	118.2s			
				5	5′	156.0s			
10	39.0s				6′	120.2d	6.70s		15,5′,4′,2′
					7′	195.8d	9.40s		5′,3′
					OH		11.20s		6′,5′

a: the high field and b: the low field proton in a geminal pair. d: 500 MHz in C_6D_6

Table 2 NMR Data of Peyssonol B (6)

Proton	$^{13}C^{c}$	$^{1}H^{c}$	COSY^{d} to H	HMBC^{c} to C	Proton	$^{13}C^{c}$	$^{1}H^{c}$	COSY^{d} to H	HMBC^{c} to C
1a	26.8t	1.40m	2a,2b,1b	14,10,9,3	11	19.1q	1.04d (7)	3	4,3,2
1b		1.70m	14,2a,2b,1b	14,10.9,3					
2a	33.2t	1.66m	2b,1a,1b	11,10,9,4	12	18.7q	1.64s	6a	5,3
2b		1.68m	3,2a,1a,1b		13a	107.1t	4.70d (1.5)	13b,7a, 9	9,7a,7b
3	35.4d	2.00m	11,2b	12,5,4,2,1					
4	129.3s				13b		4.85d (1.5)	13a,7a, 9	9,7a,7b
5	135.2s								
6a	27.8t	1.94m	me-12,7a,7b,6b	13,10,8,7,5,4	14	19.6q	0.94s	1b	10,9,5,1
6b		2.60m	7a,7b,6a	13,10,8,7,5,4	15a	24.2t	2.75d (15)	15b,9, 6′	10,9,8,6′,2′
7a	37.7t	1.94m	13a,13b,7b,6a 6b	13,9,8,6,5	15b		2.80dd (15,11)	15a,9, 6′	10,9,8,6′,2′
7b		2.30m	7a,6a,6b	13,9,8,6,5					
8	148.0s								
9	54.0d	2.25m	15a,15b,13a, 13b	15,14,13,10,8,5	1′	129.1s			
10	41.0s				2′	147.4s			
					3′	117.2d	6.50s	7′	16,7′,5′
					4′	118.1s			
					5′	148.1s			
					6′	118.8d	6.70s	15a,15b	15,4′,2′
					7′	37.2t	3.55s	3′	8′,5′,4′,3′
					8′	173.1s			
					9′	52.1q	3.70s		8′,7′
					OH		6.80s		
					OH		4.50s		

a: the high field and b: the low field proton in a geminal pair. c: 500 MHz in $CDCl_3$

d: 500 MHz in C_6D_6

Comparison of the NMR data of the drimane moiety of **7** and **5** (Tables 1 and 3) pointed clearly to a different stereochemistry in the two, (different chemical shifts of C5 and C14 due to γ-effects)[16], *vide supra*. Furthermore, nOe's between Me-14 and Me-12 (5%) and CH_2-15 (2%) were in agreement with a trans decalin system and also determined the β-equatorial orientation of the benzyl group. Thus, **7** possesses the same drimane system as in zonarol (**1**) (the proton NMR data of the decalin system in **7** was in full agreement with the limited 60 MHz NMR data reported for **1** in 1973)[1].

Three publications in the literature[13-15] report secondary metabolites from *Hyatella sp.*, two reports are on metabolites from *H. intestinalis*[13,14] describing the isolation of spongian diterpenoids and scalarane sesterterpenes from two different collections of the same sponge.

The third report[15] from an unidentified *Hyatella sp.* describes a new quinone, chiatoquinone (**8**) which has much in common with compound **7**.

The bio-activity of compounds **5-7** will be reported elsewhere.

Table 3 NMR Data of Hyatellaquinone (7)

Proton	$^{13}C^c$	$^1H^c$	COSY^c to C	HMBC^c	Proton	$^{13}C^c$	$^1H^c$	COSY^c to H	HMBC^c to C
1a	38.7t	1.40m	2a,2b,1b	14,5	11	33.6q	0.85s		12,5,4,3
1b		1.76brd (12)	2a,2b,1a	10,5,3,2	12	21.7q	0.80s	3a	11,5,4,3
					13a	106.2t	4.65s	7a	9,7
2a	19.4t	1.52m	3a,3b,2b,1a,1b	10,4,1	13b		4.68s	9,7b	9,7
2b		1.60m	3a,3b,2a,1a,1b	10,4,1	14	14.4q	0.75s	9	10,9,5,1
3a	42.1t	1.22m	12,3b,2a,2b	12,4					
					15a	18.8t	2.52dd (14,3)	15b,9	10,9,8,6′,2′,1′
3b		1.40m	3a,2a,2b	4,1	15b		2.63dd (14,11)	15a,9	10,9,8,6′,2′,1′
4	33.6s								
5	55.4d	1.16dd (13,2)	6a,6b	10,9,6,4,1	1′	120.2s			
					2′	156.3s			
6a	24.4t	1.30qd (13,4.5)	7a,7b,6b,5	10,8,7,5	3′	181.2s			
					4′	102.0d	5.80s		6′,5′,2′
6b		1.70m	7a,7b,6a,5	10,8,5	5′	162.2s			
7a	38.3t	1.91td (13,4.5)	13a,7b,6a,6b	13,8,6,5	6′	181.0s			
					7′	56.7q	3.80s		5′
7b		2.29dq (13,2)	13b,7a,6a,6b	13,9,6,5	OH		7.34s		
8	148.3s								
9	54.1d	2.36brd (11)	15a,15b,13b 14	15,14,13,10,8					
10	40.2s								

a: the high field and b: the low field proton in a geminal pair c: 500 MHz in $CDCl_3$

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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.- IR spectra were recorded neat on a Nicolet 205 FT-IR spectrometer, UV spectra for solutions in MeOH were taken with a Uvikon-931 spectrophotometer, and NMR spectra, for solutions in $CDCl_3$ or C_6D_6, with a Bruker ARX-500 spectrometer operating at 500 and 125 MHz for ^1H NMR and ^{13}C NMR, respectively. Mass spectra were measured with a Finnigan TSQ-70 spectrometer. Optical rotations were measured for solutions in MeOH with a Perkin-Elmer 241 polarimeter with a 10 cm microcell.

COLLECTION AND EXTRACTION - *Pessonnelia sp* was collected at depths of 10-15 m near Zaabargad island, the Red Sea. The alga was frozen immediately after collection. A voucher specimen is kept in Tel-Aviv collection (#TA-YK-3645). The freeze-dried organism (50 g) was extracted in MeOH-EtOAc (1:1) to give after evaporation a gum (390 mg). The extract was partitioned between aq. MeOH and hexane, CCl_4 and $CHCl_3$. The hexane (160 mg) and CCl_4 (150 mg) solubles were chromatographed in hexane - MeOH-$CHCl_3$ (2:1:1) over Sephadex LH-20 and then by vacuum liquid chromatography over a silica gel column eluted with hexane and increasing percentage of EtOAc to afford two new compounds **5** (4.6 mg) and **6** (8 mg). *H. intestinalis* was collected at Sodwana Bay, South Africa by divers using SCUBA, during the summer of 1992.

The sponge *Hyatella-intestinalis* was frozen immediately after collection. A voucher specimen was kept in Tel-Aviv collection, (# TASA-125). The freeze-dried organism (20 gr) was extracted with EtOAc to give after evaporation a gum (140 mg). The extract was partitioned between aq. MeOH and hexane, CCl_4 and $CHCl_3$. The CCl_4 soluble (30 mg) was chromatographed in hexane-MeOH-$CHCl_3$ (2:1:1) over Sephadex LH-20 to give compound **7** (6 mg).

COMPOUND 5.- An oil:$[\alpha]_D^{25}$+ 2.0^0(c=0.1, $CHCl_3$);Cims *m/z* $[M+H]^+$ 421 (100%),$[M-Br]^+$ 341 (81%); ν_{max} 3600-3300 br, 2927, 1655, 1650 cm^{-1}; λ_{max}(MeOH) 364 (970), 270 (2400), 238 (3510); δ_H (C_6D_6) and δ_C(C_6D_6)see Table 1.

COMPOUND 6.- An oil $[\alpha]_D^{25}$ - 57^0 (c=0.1, $CHCl_3$); Cims *m/z* $[M]^+$ 384 (100%), $[M-OH]^+$ 367 (66%), $[C_{24}H_{32}O_2]^+$357 (42%) $[C_{23}H_{29}O_2]^+$ 337 (36%),$[C_{14}H_{21}]^+$189 (82%) δ_H($CDCl_3$) and δ_C($CDCl_3$) see Table 2.

COMPOUND 7.- An oil $[\alpha]_D^{25}$+15.6^0 (c=0.5, $CHCl_3$); Cims *m/z* $[M]^+$ 358 (43%),$[C_{14}H_{21}]^+$191 (82%) $[M-C_{14}H_{21}]^+$ 168(100%); ν_{max}3400 br,2937, 1656,1642, 1606, 1218; λ_{max} (MeOH) 287(1180), 205(1730); δ_H ($CDCl_3$) and δ_C($CDCl_3$) see Table 3.

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