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POPOLOHUANONE A AND B. TWO NEW SESQUITERPENOID AMINOQUINONES FROM A PACIFIC SPONGE, DYSIDEA SP.

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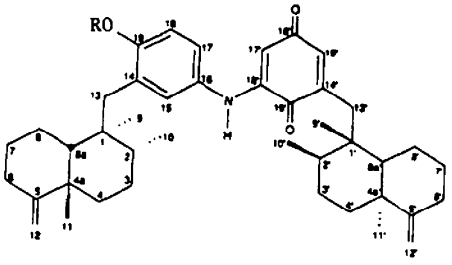
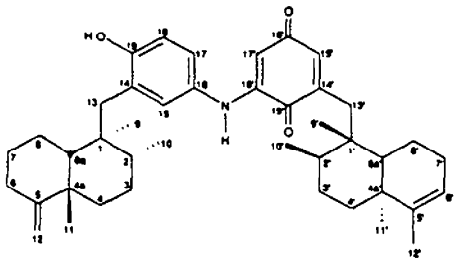
Abstract: Popolohuanone-A (1) and B (2) are two new sponge metabolites of composition $C_{42}H_{57}NO_3$. Their structures, which were determined by spectral and chemical correlations, comprise two molecules of a benzenoid moiety each linked to a rearranged drimane sesquiterpene and connected by an amino function.

Some C_{21} compounds, which are biogenetically derived from sesquiterpene and benzenoid moieties, have been isolated from marine sponges and brown algae.[1] Interest in these compounds has been heightened by reports of biological properties, which include *in vivo* antileukemia activity,[2] *in vitro* inhibition of replication of the AIDS virus,[3] and inhibition of tubulin polymerization.[4] A few nitrogenous derivatives have also been reported, in which the benzenoid moiety bears an amine function.[5,6] We wish to describe two compounds, popolohuanone[7] A (1) and B (2), from a Dysidea sp. sponge, in which two C_{21} entities, one a quinone, the other a hydroquinone, derived from the known arenarone (3) and arenarol (4)[8]. Both of these compounds are also present in the sponge.

A large specimen of purple-blue Dysidea sp. (0.80 kg) frozen shortly after collection at Port Moresby, Papua New Guinea, and kept frozen until work-up, was extracted three times with methanol. After concentration the crude residue was partitioned between hexane and water. The hexane fraction was chromatographed with hexane-ethyl acetate over silica gel yielding the known arenarone (3, 0.18%), followed first by a complex mixture of pigments which eluted as a broad dark blue band, and then arenarol (4, 0.31%). Compounds 3 and 4 were identified by their IR, ^{1}H NMR, ^{13}C NMR, UV and mass spectra.[8] Appropriate fractions containing the strongly colored mixture of pigments were combined and purified successively first on Sephadex LH20 in chloroform/methanol then on silica gel in hexane/ethyl acetate, and finally by sequential high-pressure liquid chromatography (HPLC) on a silica bonded-phase column. The clean complex mixture of purple-blue pigments (15 mg, 0.01%) was resolved by reverse-phase HPLC on a C-18 bonded-phase column with methanol/water (98:2) to yield popolohuanone-A (1, 8 mg) and popolohuanone-B (2, 3 mg). A third strongly colored fraction containing at least three additional purple-blue pigments (< 3 mg) was also isolated but because of lack of material was not investigated further.

The predominant isomer 1, a non-crystalline solid with molecular formula $C_{42}H_{57}NO_3$ as determined by HRMS, showed hydroxyl (3600-3510 cm^{-1}), amino (3440-3300 cm^{-1}) and carbonyl (1655 and 1638 cm^{-1}) absorptions in the infrared and UV maxima (hexane) at 222, 266 and 512 nm, compatible with a benzoquinone chromophore. Upon acetylation, pigment 1 formed a monoacetate 1a, which lacked hydroxyl absorption but exhibited NH bands at 3400-3350 cm^{-1} in the infrared. The N-H group in popolohuanone-A may be regarded as a vinylogous amide





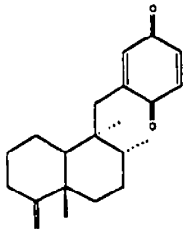
1 R = H

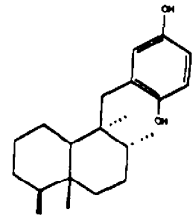
1a R = AC

2

and therefore would be expected to resist acetylation. Acetate 1a was no longer purple-blue (in solution) but rather pink [UV absorptions (hexane) at 220, 266 and 488 nm]. Compound 1 also reacted slowly with ammonia at 25°C to yield a light brown solution irreversibly, and with 5% HCl (25°C, slow) to produce a colorless solution. In its ^{1}H NMR spectrum ($CDCl_3$) compound 1 showed two exchangeable protons at δ 6.00 (1H, br s) and 7.22 (1H, br s), but the signals corresponding to the terpene protons of popolohuanone-A (1) were similar to those in the ^{1}H NMR spectra of arenarone (3) and arenarol (4).[8] The ^{13}C NMR spectrum of 1 displayed 42 resonances. A ^{1}H-detected heteronuclear multiple bond ^{1}H-^{13}C correlation experiment (HMBC) facilitated the complete assignment of both ^{13}C and ^{1}H (Table I) resonances for popolohuanone-A.[6]

The molecular formula ($C_{42}H_{57}NO_3$) and the spectral data indicated that popolohuanone-A constituted a combination of an amine bearing two benzenoid sesquiterpenes related to arenarone (3) and arenarol (4). This conclusion was also supported by the mass spectrum of 1 which showed successive loss of two $C_{14}H_{23}$ (m/z 191) fragments corresponding to two norsesquiterpene fragments leaving a $C_{14}H_{11}NO_3$ (m/z 241) fragment which corresponds to the bis-benzylamino moiety of popolohuanone-A.





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A more detailed ^{1}H NMR analysis (by COSY and nOe measurements) of the high field region confirmed that 1 has the same cis-fused rearranged drimane unit as do 3 and 4.[8] Normal and long range COSY experiments confirmed all carbon-carbon bond connectivities of 1 in the high field region of the ^{1}HNMR spectrum. Furthermore, irradiation of the quaternary methyl signals at δ 0.93 and δ 0.90 caused the methylene signals at δ 2.47 - 2.78 (C-13 and C-13') to experience an nOe. This represents evidence that Me-9 and Me-9' are vicinal to the benzylic methylenes. During the same experiments no nOe effect was

observed near δ 1.37 or δ 1.24, where the C-8a and C-8a' methine protons absorb, suggesting that the C-9 and C-9' quaternary methyls are trans to the C-8a and C-8a' protons. Also, since no enhancement was observed near δ 1.42 or δ 1.34 (signals of the C-2 and C-2' protons) a trans relationship is assumed between the Me-9 and Me-9' and the C-2 and C-2' protons. Irradiation at δ 1.06 and δ 1.10 (Me-11 and Me-11') caused one of the C-8 and C-8' protons near δ 1.70 as well as the C-8a and C-8a' protons near δ 1.37 and δ 1.24, to experience an nOe. These results suggest a cis- decalin ring junction. Moreover, comparison (Table I) of the ^{13}C chemical shifts of the terpenoid moieties of popolohuanone-A (1) with those of arenarone (3) and arenarol (4) shows close similarity, which suggests identical relative stereochemistry.

The structure of minor component 2 was determined by direct comparison of the ^{1}H and ^{13}C NMR data of 1 and 2. Popolohuanone-B (2), is a non-crystalline solid, composition $C_{42}H_{57}NO_3$, isomeric with 1. Its ^{1}H and ^{13}C NMR spectra differ from those of 1 by lacking one sp^2 exocyclic methylene present in 1. Instead, the ^{1}H NMR spectrum of 2 showed a new trisubstituted olefinic proton at δ 5.14 and a new olefinic methyl at δ 1.53. These features suggest that one of the exocyclic double bonds in 1 must have shifted into an endocyclic position in 2. Comparison of the ^{13}C chemical shifts of the terpenoid moieties of popolohuanone-B (2) with those of 3 and 4 allowed placement of the new endocyclic double bond in the position shown in 2. In general, the terpenoid ^{13}C NMR shifts for arenarone (3), where the terpenoid skeleton is linked to a quinone residue, are slightly upfield from those for arenarol (4), where the terpenoid skeleton is linked to a hydroquinone moiety. It must be noted that in popolohuanone-A (1), where both terpenoid double bonds are exocyclic, the ^{13}C NMR shifts for both drimanes appear as closely spaced pairs that correlate nicely with the chemical shifts for both arenarone (3) and arenarol (4). Hence, it is a reasonable assumption that for most if not all of these pairs, the downfield peaks arise from the sesquiterpene linked to the reduced benzenoid moiety, while the upfield signals correspond to the quinoid-linked drimane. In the ^{13}C NMR spectrum of 2 only the upfield members of the drimane doublets are shifted, which places the new endocyclic olefin on the oxidized side of the molecule.

Popolohuanone-A (1) was not cytotoxic against KB cells at 10, 5 and 1 μg/mL. Antifungal activity tests (up to a maximum concentration of 64 μg/disk) all gave negative responses against Candida albicans.

EXPERIMENTAL SECTION

General Procedures. Infrared spectra were recorded on a Perkin-Elmer Model 1420 spectrometer and Ultraviolet spectra on a Hewlett-Packard Model 8452A diode array spectrophotometer. Mass spectra were measured on a VG-70SE instrument and NMR spectra on a General Electric GN OMEGA 500 instrument at 500-MHz (^{1}H) and 125-MHz (^{13}C) respectively; signals are reported in parts per million (δ) downfield from internal tetramethylsilane. Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Merck silica gel 60 (230-240 mesh) was used for column chromatography. TLC

TABLE I. ^{13}C (125-MHz)[a] AND ^{1}H(500-MHz)[b] NMR SPECTRAL DATA OF POPOLOHUANONE-A(1) IN CD_2Cl_2 AND PROTONS TO WHICH LONG-RANGE CORRELATIONS WERE OBSERVED IN THE HMBC EXPERIMENT

atom	^{13}C(mult)b	^{1}H(mult,J(Hz), intgrtn)	HlMBC(^{1}H)	atom	^{13}C(mult)b	^{1}H(mult,J(Hz), intgrtn)	HMBC(^{1}H)
1	44.0(s)		3,4,8a,9,10,13	1'	44.4(s)		3',8',8a',9',10,13'
2	38.0(d)	1.42(m,1H)	3,4,9,10,13	2'	38.6(d)	1.34(m,1H)	3',4',9',10',13'
3	27.9(t)	1.26(m,1H) 1.58(m,1H)	2,4,10	3'	28.0(t)	1.28(m,1H) 1.60(m,1H)	4',10'
4	38.1(t)	1.02(m,1H) 2.01(m,1H)	3,11	4'	38.1(t)	1.17(m,1H) 2.09(m,1H)	3',11'
4a	39.6(s)		3,4,6,8a,11,12	4a'	39.7(s)		3',4',8a',11',12'
5	154.0(s)		4,6,7,8a,11	5'	153.7(s)		4',6',7',8a',11',12'
6	32.2(t)	2.15(m,2H)	7,8,12	6'	32.1(t)	2.49(m,2H)	8',12'
7	22.8(t)	2.17(m,2H)	6,8a	7'	22.2(t)	2.06(m,2H)	8',8a'
8	25.3(t)	1.70(m,2H)	6,7,8a	8'	25.0(t)	1.87(m,2H)	6',8a'
8a	47.0(d)	1.37(m,1H)	4,8,9,11,13	8a'	47.5(d)	1.24(m,1H)	4',7',8',9',11',13'
9	19.2(q)	0.94(s,3H)	8a,13	9'	19.3(q)	0.95(s,3H)	2',8a',13'
10	18.1(q)	1.00(d,6.5,3H)	3	10'	17.5(q)	0.95(d,6.6,3H)	3'
11	33.2(q)	1.06(s,3H)	8a	11'	33.0(q)	1.10(s,3H)	8a'
12	106.2(t)	4.73(d,6.5,2H)	6	12'	105.9(t)	4.74(d,2.0,2H)	6'
13	37.7(t)	2.60(d,14.0,1H) 2.78(d,14.0,1H)	8a,9,15,18	13'	35.4(t)	2.47(d,14.0,1H) 2.68(d,14.0,1H)	8a',9',15'
14	127.3(s)		13,18	14'	142.6(s)		13'
15	128.2(d)	6.96(d,2.6,1H)	13,17,18	15'	139.6(d)	6.43(d,2.3,1H)	13',17'
16	129.8(s)		15,17,18	16'	186.5(s)		
17	122.4(d)	6.92(dd,2.6,8.5,1H)	15,18	17'	99.3(d)	5.90(d,2.3,1H)	15'
18	116.5(d)	6.78(d,8.5,1H)	15,17	18'	145.0(s)		17'
19	153.5(s)		13,15,17,18	19'	184.5(s)		13',15',17'
					NH	7.26(br s,1H)	15,16,17,17',19'
					OH	5.80(br s,1H)	

[a]The chemical shifts are in δ units. Assignments were aided by conventional COSY, RCT-COSY, HMQC and homonuclear spin-decoupling experiments.

[b]Multiplicities of the carbon atoms were determined by a DEPT Experiment in $CDCl_3$.

analyses were carried out using Analtech precoated silica gel glass plates. Solvents were freshly distilled prior to use. Partisil 5μm silica gel semipreparative (10mm X 50cm) and 5μm C-18 semipreparative (10mm X 50cm) columns were used for HPLC separations with an ultraviolet detector.

Collection and Extraction. Dysidea sp. was collected in Papua New Guinea and frozen shortly after collection. The sponge (0.80 kg) was cut into small pieces and blended with methanol three times at room temperature with 1L portions. The combined methanolic extracts were concentrated on a rotary evaporator, and the residue was partitioned between hexane and water. After concentration of the hexane solubles (9.2 g) a portion of the crude extract (1.5g) was chromatographed over silica gel (70g) with hexane-ethyl acetate (9:1). A fraction (0.24g) was shown by ^{1}HNMR, ^{13}CNMR, IR, UV and mass spectra to contain predominantly arenarone (3). A second fraction, which eluted as a dark blue band, consisted of a complex mixture of pigments which was sequentially chromatographed over Sephadex LH-20 with chloroform-methanol (1:1) and silica gel with hexane-ethyl acetate (9:1). Partial purification by HPLC over a silica bonded-phase column with hexane-ethyl acetate (8.2) gave a clean mixture (15mg) of popolohuanones A (1) and B(2). A third fraction (0.41g) which eluted with hexane-ethyl acetate (7:3) was shown by ^{1}H and ^{13}CNMR, IR, UV and mass spectra to be pure arenarol (4).

Popolohuanone-A (1). Separation and purification of 1 and 2 was achieved by sequential HPLC chromatography of the clean mixture of pigments (15mg), first over a silica bonded-phase column with 100% chloroform followed by a C-18 bonded-phase column with 2% aqueous methanol: 1 (8 mg; 0.006%) was obtained as an intense purple-blue non-crystalline dust; IR($CHCl_3$) 3600-3510 (br,-OH), 3440-3300 (br,-NH), 2953 (s), 1655 (w), 1638 (s), 1584 (s), 1503 (w), 1263 (s), 1099 (s), 826 (s) cm^{-1}; UV (hexane) λ_{max} 222, 266, 512 nm (log ε 4.28, 4.21, 3.58); HRFABMS (DTE/DTT Matrix): 626.4587 ($C_{42}H_{60}NO_3$ requires 626.4575), 435.2774 ($C_{28}H_{37}NO_3$ requires 435.2774), 244.0963 ($C_{14}H_{14}NO_3$ requires 244.0973); LRFABMS (3-NBA Matrix: m/z 624 $[M + H]^+$; fits $C_{42}H_{58}NO_3$; HREIMS: m/z 623.4113 ($C_{42}H_{57}NO_3$ requires 623.4338); EIMS: m/z 623 (18), 433(33), 243(15), 191(18), 190(23), 175(51), 95(57), 81(53), 69(56); ^{1}H and ^{13}CNMR (see Table 1). All attempts to measure the specific rotation of popolohuanone-A failed even after repeated dilutions in $CHCl_3$. Apparently the strongly colored solutions do not allow the passage of polarized light through the cell.

Popolohuanone-B(2). Fine, non-crystalline purple-blue dust (3 mg; 0.002%); IR ($CHCl_3$): 3600-3510 (br,-OH), 3440-3300 (br,-NH), 2940 (s), 1675 (s), 1635 (s), 1585 (s), 1515 (s), 1345 (s), 770 (s) cm^{-1}; UV (hexane) λ_{max} 222, 266, 512 nm (log ε 4.08, 3.98, 3.36); HREIMS: m/z 623.3986 ($C_{42}H_{57}NO_3$ requires 623.4338); EIMS: m/z 623(15), 433(33), 243(13), 191(13), 190(6), 175(15), 121(23), 95(93); LRFABMS (DTE/DTT Matrix): 626 (fits $C_{42}H_{60}NO_3$), 435, 244, 191, 135; ^{1}HNMR (CD_3OD); δ 6.97 (1H, d, J=2.5 Hz), 6.92 (1H, dd, J=2.5, 8.5 Hz), 6.78 (1H, d, J=8.5 Hz), 6.37 (1H, d, J=2.5 Hz), 5.78 (1H, d, J=2.5 Hz), 5.14 (1H, m), 4.71 (1H, br s), 4.67 (1H, br s), 2.80-2.40 (4H, two overlapping AB quartets, J=14.0 Hz), 1.40-1.60 (complex m), 1.53 (3H, s), 1.50-1.10 (complex m), 1.04 (3H, s), 1.01 (3H, s),

0.97 (3H, d, J=6.2 Hz), 0.95 (3H, d, J=6.2 Hz), 0.92 (3H, s), 0.85 (3H, s); in CD_2Cl_2 two exchangeable protons are observed at δ 7.20 (1H, br s) and 5.47 (1H, br s); ^{13}CNMR ($CDCl_3$): δ 186.41, 184.01, 153.34, 153.34, 144.39, 144.02, 142.21, 139.55, 129.42, 127.75, 126.78, 122.21, 120.66, 116.32, 105.86, 99.27, 46.83, 46.60, 43.64, 42.22, 39.33, 38.50, 37.74, 37.67, 37.57, 36.74, 36.06, 35.27, 33.04, 31.87, 27.54, 27.47, 26.56, 24.86, 22.52, 20.06, 19.45, 19.08, 18.07, 17.99, 17.81, 16.77.

Popolohuanone-A acetate (1a): A solution of 1 (1mg), acetic anhydride (2 drops) and pyridine (1 drop) in hexane (5 mL) was stirred at 25°C for about 2 h. The acetylated derivative was pink in solution. The following spectroscopic data were recorded after purification by HPLC over a silica bonded-phase column with hexane–chloroform (3:7): IR ($CHCl_3$) 3400-3350 (br,-NH), 2980 (s), 2950 (s), 1757 (s, -O-C=O), 1665 (s), 1640 (s), 1590 (s), 1520 (s) cm^{-1}; UV (hexane) λ_{max} 220, 266, 488 nm; ^{1}HNMR ($CDCl_3$): δ 7.1-7.0 (4H, complex m), 6.4 (1H, d, J=2.4 Hz), 6.1 (1H, d, J=2.4 Hz), 4.5 (4H, m), 2.7-2.4 (4H, complex m), 2.3 (3H, s), 2.2-1.1 (complex m), 1.07 (3H, s), 1.05 (3H, s), 0.98 (3H, d, J=6.2 Hz), 0.94 (3H, d, J=6.2 Hz), 0.92 (3H, s), 0.89 (3H, s); LRMS: m/z 665 (M^+); fits $C_{44}H_{59}NO_4$. Prominent fragments are observed at m/z 665 (11), 475(30), 433(9), 285(21), 243(16), 191(50), 190(52), 175(100).

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