Table 1. NMR Data for 3 (C_6D_6)

C #	δ C	δ H[a] (m, Hz)	^{1}H-^{1}H COSY	(^{1}H Correlation)
1	28.2	1.18 (s)	-	3
2	98.9	OH, 1.68 (s)	-	1, 3, 20
3	47.2	1.25 (dq, 10.8, 6.3)	4, 21	1, 4, 5, 20
4	69.7	3.58 (dddd, 4.4, 5.4, 10.8, 12.2)	3, 5a, 5b	5b, 20
		OH, 0.78 (d, 5.4)		
5	41.3	1.14 (q, 12.2)	4, 5b, 6	-
		1.71 (ddd, 2.5, 4.4, 12.2)	4, 5a, 6	
6	68.3	3.84 (dtd, 2.5, 6.4, 12.2)	5a, 5b, 7a, 7b	7b
7	39.7	2.18 (br ddd, 6.4, 7.3, 13.7)	6, 7b, 8	6, 8, 10
		2.36 (br ddd, 6.4, 7.3, 13.7)	6, 7a, 8	
8	128.4	5.69 (dt, 14.7, 7.3)	7a, 7b, 9, 11	6, 7a, 7b
9	133.2	6.12 (dddd, 1.0, 1.5, 10.2, 14.7)	7a, 7b, 8, 10	7b, 8
10	128.9	6.10 (ddd, 1.0, 10.2, 14.7)	9, 11, 12	11, 12
11	138.8	5.54 (dd, 7.8, 14.7)	8, 10, 12	12, 13a, 13b, 21
12	35.0	2.32 (m)	11, 13a, 13b, 22	10, 11, 13a, 21
13	47.9	1.92 (dd, 7.9, 13.2)	12, 13b	11, 12, 15, 21, 22
		2.07 (m)	12, 13a	
14	133.8	-	-	12, 13a, 13b, 16,
15	126.2	5.16 (br t, 6.8)	16, 23	13a, 13b, 16, 17, 22
16	27.8	2.07 (m)	15	15, 17, 18
17	34.4	2.07 (m)	18, 19a, 19b	15, 16, 18, 19a, 19b
18	138.8	5.79 (ddt, 17.1, 10.2, 6.8)	17, 19a, 19b	16, 17, 19b
19	114.8	4.98 (ddd, 1.4, 2.5, 10.2)	17, 18, 19b	17
		5.04 (ddd, 1.5, 2.5, 17.1)	17, 18, 19a	
20	12.3	1.12 (d, 6.3)	3	4
21	20.1	0.97 (d, 6.9)	12	11, 12, 13a
22	16.0	1.50 (s)	-	13a, 15

[a] With geminal protons, the smaller δ-value is given the *a* designation, the larger δ-value is given the *b* designation.

H-5a and H-6 (12.2 Hz). In addition, H-5b displayed typical equatorial-axial couplings of 4.4 ($J_{4\text{-}5b}$) and 2.5 Hz ($J_{5b\text{-}6}$). The equatorial placement of the tertiary methyl group at C-2 was based upon its ^{13}C chemical shift value of δ 28.2, compared to its axial epimer (see below) with a δ value of 21.9.

The stereochemistry of the two disubstituted double bonds of **3** was assigned as *E* on the basis of their vicinal coupling constants of 14.7 Hz ($J_{8,9}$ and $J_{10,11}$). The absence of any significant nOe enhancement of the olefinic methyl (C-22, δ 1.50) upon irradiation of the adjacent olefinic hydrogen (H-15, δ 5.16), and *vice versa*, suggested that the trisubstituted double bond was also in the *E*-configuration. In confirmation of this assignment, the olefinic methyl displayed a chemical shift of δ 16.0; this upfield δ value results from the γ-shielding effect of the C-16 methylene, which is *cis* to the olefinic methyl in the *E*-isomer. In the *Z*-isomer, the olefinic methyl would be expected to absorb in the vicinity of δ 24.[12] The relative configuration at C-12 remains undetermined.

Hemiketal **3** was partially converted to its C-2 epimer, hemiketal **4**, when it was allowed to stand in methanol. When subjected to reverse-phase TLC (C_{18}), **3** provided small amounts of its C-2 epimeric methyl ketal, **5**, as well. The HRFABMS of methyl ketal **5** established its molecular formula as $C_{23}H_{38}O_3$; its structure was deduced from ^1H, ^{13}C, COSY, and HMQC NMR spectral data. Two major differences between **5** and **3** in the ^1H and ^{13}C spectra were observed: **5** displayed a new methyl singlet (δ 3.01, 47.37) and the tertiary methyl group at C-2 had shifted from δ 28.2 in **3** to δ 21.9 in **5**. Epimerization of **3** at C-2 followed by methyl ketal formation would account for these spectral differences. HRFABMS established a molecular formula of $C_{22}H_{36}O_3$ for hemiacetal **4**. Its spectral data were virtually identical to those of **5** except for the absence of signals for the ketal methyl group.

The Faulkner group has just reported a homologous pair of hemiketals **(6/7)** from a *Raspailia* sp. collected in Palau.[13] In that work, the absolute configurations at C-2, C-3 and C-4 were determined by the Mosher ester method. Given the identical chemical shift data and comparable optical rotations of **3** and **6** and **4** and **7**, it is likely that our isolates have the same absolute stereochemistry around the pyran ring. The paucity of compound precluded oxidative cleavage of **3** to 2-methyl levulinic acid to determine the configuration at C-12.[14]

EXPERIMENTAL

General Procedures

NMR spectra were obtained on a Varian VXR 500 MHz spectrometer in C_6D_6, with the solvent as the internal reference. A 5 mm indirect detection probe was employed for all NMR experiments. Low- and high-resolution mass spectra were collected on a Finnigan MAT95 spectrometer in the positive ion mode. IR spectra were obtained on a Perkin-Elmer model 1600 FTIR. UV spectra were determined on a Beckman DU 64 spectrophotometer and optical rotations were measured on a Perkin-Elmer model 241 polarimeter.

Animal Material

Haliclona sp. was collected from the underside of overhanging rock substrates at depths of 12-15 meters off Rottnest Island in Southwestern Australia in March, 1989. The sponge was green in color with amorphous lobes and a high mucous content. Identification was made by P. Jane Fromont and voucher specimens were deposited in the Smithsonian Sorting Center.

Extraction and Isolation

The frozen sponge (450 g) was ground with dry ice, thawed, stirred in distilled H_2O at 4°C for 4 h and then filtered. The marc was freeze-dried and extracted successively with CH_2Cl_2-MeOH (1:1 v/v) and MeOH. The combined organic filtrates were concentrated *in vacuo* to give 4.19 g (0.93%) of extract.

The crude extract was subjected to VLC in several batches on Diol-60 columns, eluting successively with hexane, CH_2Cl_2, EtOAc, acetone, and MeOH. The hexane fraction from 552 mg of crude extract was subjected to VLC on silica gel with 5% CH_2Cl_2-hexane followed by reverse phase TLC on C-18 with 5% H_2O-MeOH (2x) affording 11.0 mg of bromoether **1** (2% of crude extract) and impure bromoether **2**. The latter was purified by silica gel TLC with 15% $CHCl_3$-hexane (3x) to give 14.5 mg of **2** (2.6 %).

A 198 mg sample of the CH_2Cl_2 fraction from the Diol-60 column was subjected to silica gel TLC with 20% i-PrOH-hexane to give 9.6 mg (1.7%) of hemiketal **3**. When this sample was recovered from a methanol solution, a mixture of hemiketals **3** and **4** was obtained. Separation was effected by silica gel TLC with 15% i-PrOH-hexane to give 5.5 mg of **3** and 2.6 mg of **4**. A small amount (1.2 mg) of methyl ketal **5** was obtained from the CH_2Cl_2 fraction of the diol column after reverse-phase TLC on C-18 with 10% H_2O-MeOH followed by silica gel TLC with 20% i-PrOH-hexane.

3 [(2*S**,3*R**,4*S**)-2,4-Dihydroxy-2,3-dimethyl-6-(6,8-dimethyltrideca-2*E*,4*E*,8*E*,12-tetraenyl)tetrahydropyran)]: oil; $[\alpha]_D$ +67.4° (*c* = 0.43, CH_2Cl_2); UV λ_{max} (i-PrOH) 232 nm, (log ϵ = 4.52), 227 nm (sh, log ϵ = 4.50); IR ν_{max} (film) 3386, 2820, 1641, 1454, 1381, 1172, 1078, 1014, 989, 918 cm^{-1}; ^1H and ^{13}C NMR (C_6D_6) see Table 1; LREIMS *m/z* 348 (M^+, 2), 331 (4), 313 (7), 307 (2), 272 (2), 239 (4), 222 (7), 204 (27), 203 (33), 149 (29), 145 (32), 128 (23), 127 (100), 109 (54), 101 (20), 95 (63), 94 (23), 93 (22), 81 (39), 79 (31), 73 (31), 67 (37), 55 (45), 43 (81), 41 (32), 18 (10); HREIMS *m/z* 348.2662 (calcd for $C_{22}H_{36}O_3$, 348.2666); ^1H and ^{13}C NMR data are presented in Table 1.

4 [(2*R**,3*R**,4*S**)-2,4-Dihydroxy-2,3-dimethyl-6-(6,8-dimethyltrideca-2*E*,4*E*,8*E*,12-tetraenyl)tetrahydropyran)]: oil; $[\alpha]_D$ +110° (*c* = 0.20, CH_2Cl_2); ^1H NMR (C_6D_6) identical to that of **5** except for the additional singlet at δ 3.01 in **5**; ^{13}C NMR (C_6D_6) δ 12.1 (C-20), 16.1 (C-22), 20.1 (C-21), 21.9 (C-1), 27.8 (C-16), 34.4 (C-17), 35.0 (C-12), 39.6 (C-7), 41.2 (C-5), 47.9 (C-13), 48.6 (C-3), 68.5 (C-6), 69.6 (C-4), 101.6 (C-2), 114.8 (C-19), 126.2 (C-15), 128.3 (C-8), 128.8 (C-9), 133.4 (C-10), 133.8 (C-14), 138.8 (C-11), 138.8 (C-18); HRFABMS *m/z* [MH^+] 349.2746 (calcd for $C_{22}H_{37}O_3$, 349.2744).

5 [(2*R**,3*R**,4*S**)-4-Hydroxy-2-methoxy-2,3-dimethyl-6-(6,8-dimethyltrideca-2*E*,4*E*,8*E*,12-tetraenyl)tetrahydropyran)]: oil; ^1H NMR (C_6D_6) δ 0.71 (1H, d, J=5.4 Hz, OH), 0.96 (3H, d, 6.8, H-21),

1.14 (3H, d, 6.9, H-20), 1.15 (1H, q, 12.2, H-5a), 1.22 (3H, s, H-1), 1.31 (1H, dq 10.2, 6.8, H-3), 1.50 (3H, br s, H-22), 1.70 (1H, ddd, 2.0, 4.9, 12.2, H-5b), 1.92 (1H, dd, 7.8, 13.2, H-13a), 2.05 (5H, m, H-13b, 16, 17), 2.16 (1H, ddd, 5.9, 6.8, 13.7, H-7a), 2.31 (1H, m, H-12), 2.33 (1H, m, H-7b), 3.01 (3H, s, H-23), 3.52 (1H, dddd, 2.0, 5.9, 6.4, 12.2, H-6), 3.62 (1H, dddd, 4.9, 5.4, 10.2, 12.2, H-4), 4.99 (1H, dd, 2.0, 10.3, H-19a), 5.04 (1H, dd 2.0, 17.1, H-19b), 5.16 (1H, br t, 6.8, H-15), 5.53 dd, 7.3, 14.7, H-11), 5.69 (1H, dt, 14.7, 6.8, H-8), 5.79 (1H, ddt, 17.1, 10.3, 6.4, H-18), 6.10 (1H, ddd, 1.0, 10.2, 14.7, H-10), 6.12 (1H, dddd, 1.0, 1.5, 10.2, 14.7, H-9); ^{13}C NMR (C_6D_6) δ 12.0 (C-21), 16.1 (C-23), 20.1 (C-22), 21.9 (C-20), 27.8 (C-16), 34.4 (C-17), 35.0 (C-12), 39.5 (C-7), 41.1 (C-5), 47.4 (C-24), 47.9 (C-13), 48.6 (C-3), 68.5 (C-6), 69.5 (C-4), 101.6 (C-2), 114.7 (C-19), 126.2 (C-15), 127.5 (C-8), 128.8 (C-9), 133.3 (C-10), 133.8 (C-14), 138.8 (C-11), 138.8 (C-18); LREIMS 331 (M-31) (2), 313 (5), 298 (1), 272 (6), 254 (1), 236 (1), 221 (6), 203 (10), 185 (5), 159 (57), 145 (5), 141 (51), 127 (27), 109 (100), 87 (42), 81 (40), 79 (32), 67 (23), 55 (44), 43 (42), 41 (26), 31 (13), 18 (20); HRFABMS *m/z* [MH$^+$] 363.2900 (calcd for $C_{23}H_{39}O_3$, 363.2901).

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QUASSIOLS B-D, NEW SQUALENE TRITERPENES FROM *QUASSIA MULTIFLORA*

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Abstract: Three new squalene triterpenes, quassiols B (**2**), C (**3**) and D (**4**), were isolated from the roots of *Quassia multiflora*. Their structures were established by a combination of 2D NMR experiments, which included DQF-COSY, HMQC, HMBC and NOESY sequences.

Although squalene is of importance in the biosynthesis of triterpenoids and steroids, there are relatively few reports on the isolation of squalene derivatives from nature.[3-11] In a preliminary account we recently reported the isolation of a new squalene triterpene, quassiol A (**1**), from the roots of *Quassia multiflora* (Simaroubaceae).[3] Quassiol A belongs to a small group of squalene triterpenes possessing one or more tetrahydrofuran moieties that were recently isolated from plants belonging to the Simaroubaceae family, some of which display antineoplastic activity.[4,5] We have further investigated the extracts of *Q. multiflora* and report here the details of the isolation of quassiol A (**1**) in addition to the isolation and characterization of three further squalene triterpenes, quassiols B (**2**), C (**3**) and D (**4**). Previous investigations of *Q. multiflora* has resulted in the isolation of a number of biologically active quassinoids[12-17] and alkaloids,[12,18,19] in addition to tirucallane triterpenes[17,20] and phenolic compounds.[17,21]

The ^1H and ^{13}C NMR spectra of quassiol A (**1**) were previously recorded in CD_3OD in which the proton and carbon resonances were observed as well resolved signals.[3] However, when the spectra were recorded in $CDCl_3$, some of both the proton and carbon resonances were doubled. In

Table 1. ^{1}H NMR Assignments of Quassiols A (**1**), A Diacetate (**1a**), B (**2**), C Diacetate (**3a**), and D Diacetate (**4a**).[a]

Position	1	1a	2	3a	4a
1	1.24	1.19	1.25	1.67	1.68
3	3.82, 3.83	3.75	3.82	5.10	5.11
4	1.89	1.80	1.90	2.12	2.10
	1.89	1.80	1.90	2.12	2.02
5	2.11	1.97	2.11	1.45	1.55
	1.48	1.58	1.50	1.32	1.43
7	3.58, 3.56	4.94	3.56	3.75	4.84
8	1.56	1.74	1.59	1.79	1.73
	1.41	1.60	1.37	1.79	1.65
9	2.20, 2.22	1.96	2.24	1.93	1.95
	2.09, 2.06	1.96	2.06	1.56	1.95
11	5.19	5.14	5.22	5.02	5.13
12	2.04	2.00	2.02	1.71	1.99
	2.04	2.00	2.02	1.55	1.99
13	2.04	2.00	2.02	1.98	1.99
	2.04	2.00	2.02	1.78	1.99
14	5.19	5.14	5.14	5.10	5.13
16	2.22, 2.20	1.95	1.95	1.98	1.95
	2.06, 2.10	1.95	1.95	1.89	1.95
17	1.58	1.75	1.73	1.80	1.73
	1.35	1.64	1.65	1.58	1.65
18	3.36, 3.37	4.85	4.84	4.87	4.84
20	1.60	1.55	1.56	1.52	1.55
	1.37	1.42	1.43	1.42	1.43
21	2.12	2.12	2.08	1.99	2.10
	2.00	2.02	2.08	1.99	2.02
22	5.13	5.11	5.11	5.10	5.11
24	1.67	1.68	1.68	1.67	1.61
25	1.11	1.11	1.12	1.62	1.61
26	1.14	1.19	1.15	1.19	1.16
27	1.60	1.58	1.61	1.18	1.58
28	1.60	1.58	1.59	1.54	1.58
29	1.14	1.15	1.16	1.14	1.16
30	1.61	1.62	1.62	1.62	1.68
7-Ac	- -	2.06	- -	- -	2.10
11-Ac	- -	- -	- -	2.06	- -
18-Ac	- -	2.10	2.10	2.09	2.10

[a]The ^{1}H NMR spectra were recorded at 500 MHz in $CDCl_3$. Assignments based on DQF-COSY, HMQC and HMBC experiments.