Clathrins A−C: Metabolites from a Southern Australian Marine Sponge, *Clathria* Species

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A *Clathria* sp. collected in the Great Australian Bight has yielded the novel metabolites clathrins A (**6**), B (**7**), and C (**8**). Structures were assigned to clathrins A−C on the basis of spectroscopic analysis. Clathrin A (**6**) represents a plausible biosynthetic intermediate that provides an inferred link between marine sesquiterpene/benzenoids and mixed terpene/shikimate biosynthesis.

Many novel metabolites of mixed biosynthesis have been isolated from marine sources. Featured among these are a large array of sesquiterpene/benzenoids, some of the earliest examples of which include avarol (**1**),[1] spongiaquinone (**2**),[2,3] and ilimaquinone (**3**) (Chart 1).[4,5] This class of compound is thought to arise from mixed biogenesis, with separate pathways accounting for both terpenoid and benzenoid subunits. Although the various terpenoid subunits featured in this structure class can be derived from traditional mevalonate terpenoid biosynthesis, the origins of the benzenoid subunit are less clear. With well over 100 examples described, it is possible to draw some general observations. The benzenoid subunit is typically at the hydroquinone or quinone level of oxidation and can feature hydroxy, acetoxy, methoxy, amino, bromo, chloro, sulfate, carboxy, or amino acid substituents. On occasion, the benzenoid subunit is cyclized to the terpene chain via either carbocyclic or ether linkages. Another structural subset consists of dimers through the benzenoid subunit. Although the absence of experimental evidence makes it impossible to unambiguously establish the biosynthetic source(s) of the benzenoid subunit, shikimic acid has been speculated[6,7] as a possible precursor (albeit without elaboration). Supportive of this shikimate proposition is the observation that on those occasions when the benzenoid subunit is substituted by carbon, in the form of an aldehyde, methyl ester, or carboxylic acid functionality, the regiochemistry is *exclusively* meta to the attached terpene residue—suggesting that the carbon substituent is acquired from the precursor "shikimate" and not introduced during subsequent biosynthetic transformations. Dehydration and aromatization of the "shikimate" residue could lead to the required benzenoid moiety. Two examples of suitably substituted aromatic metabolites are zonaric acid (**4**)[8] from the brown alga *Dictyopteris undulata* collected in the Pacific Ocean near southern California and the acyclic isomer (**5**)[9] from a southern Australian brown alga *Perithalia caudata*. This shikimate pathway would have more currency if suitably substituted nonaromatic metabolites of this structure class could be found.

In this report we describe an investigation into the chemistry of a southern Australian marine sponge, *Clathria* sp. (family Microcionidae). This sponge yielded clathrin A (**6**), which represents the first example of a marine sesquiterpene/benzenoid metabolite in which the "benzenoid" residue retains a nonaromatic "shikimate" character. The *Clathria* sp. also yielded the novel metabolite clathrin B (**7**) and its oxidized artifact clathrin C (**8**).

Results and Discussion

The crude EtOH extract of the *Clathria* sp. was decanted and concentrated in vacuo, after which the residue was triturated with CH_2Cl_2. The CH_2Cl_2-soluble material was subsequently subjected to rapid silica filtration followed by normal-phase HPLC to yield three pure compounds (**6**−**8**).

The molecular formula determined for clathrin A (**6**) ($C_{22}H_{34}O_2$ 0.5 Δmmu) required six double-bond equivalents. Analysis of the ^{1}H and ^{13}C NMR ($CDCl_3$, 400 MHz) data for **6** revealed resonances consistent with a terminal isopropyl (^{1}H δ 0.87, 2d; ^{13}C δ 22.3, 2q), a secondary methyl (^{1}H δ 1.09, d; ^{13}C δ 20.6, q), two 1,2-disubstituted double bonds (^{1}H δ 6.25, dd, 5.58, dd, and δ 5.37, dd, 5.37, dt; ^{13}C δ 124.8, d, 137.8, d and δ 135.4, d, 127.9, d), as well as a trisubstituted double bond bearing an olefinic methyl (^{1}H δ 5.85, d, 1.74, s; ^{13}C δ 124.0, d, 139.2, s, 15.0, q) and a trisubstituted double bond bearing a carboxylic acid functionality (^{1}H δ 7.12, d; ^{13}C δ 137.4, s, 142.0, d, 172.8, s). The presence of the conjugated carboxylic acid was further confirmed by characteristic absorptions in the IR (3010 and 1690 cm^{-1}) and UV (275 nm) spectra. The UV spectrum also supported an acyclic conjugated diene functionality (236 nm).

A 2D NMR analysis (^{1}H−^{1}H COSY and ^{1}H−^{13}C gHMBC; see Table 1) of **6** revealed a connectivity sequence supportive of the structure fragments C2′ to C6′and C3′ to C12. Together with the observations made above, the gross structure for clathrin A (**6**) was determined to be as shown.

The magnitude of $J_{5,6}$ (15.1 Hz) was characteristic of an E $\Delta^{5,6}$ stereochemistry, while the upfield ^{13}C NMR chemical shift of the 3-Me (15.0 ppm) was consistent with an E $\Delta^{3,4}$ stereochemistry. Unfortunately, overlapping ^{1}H NMR resonances for H-8 and H-9 prevented an experimental measure of $J_{8,9}$, and NOE measurements did not shed light on the $\Delta^{8,9}$ stereochemistry. Assignment of this stereochemistry was achieved through ^{13}C NMR comparisons with the model compounds (*E*)-2,6-dimethyl-3-heptene and (*Z*)-2,6-dimethyl-3-heptene.[10] From the published data[10] it is apparent that the stereochemistry about the double bond in these model compounds influences the ^{13}C NMR chemical shifts for the olefinic carbons (E δ 137.7 and 138.8; Z δ 126.1 and 138.1). The corresponding ^{13}C NMR shifts for **6** (δ 127.9 and 135.4) supported a Z $\Delta^{8,9}$ stereochemistry. Clathrin A (**6**) is optically active and incorporates two chiral centers, although at this time the absolute stereochemistry about C-7 and C-3′ in **6** remains unknown.

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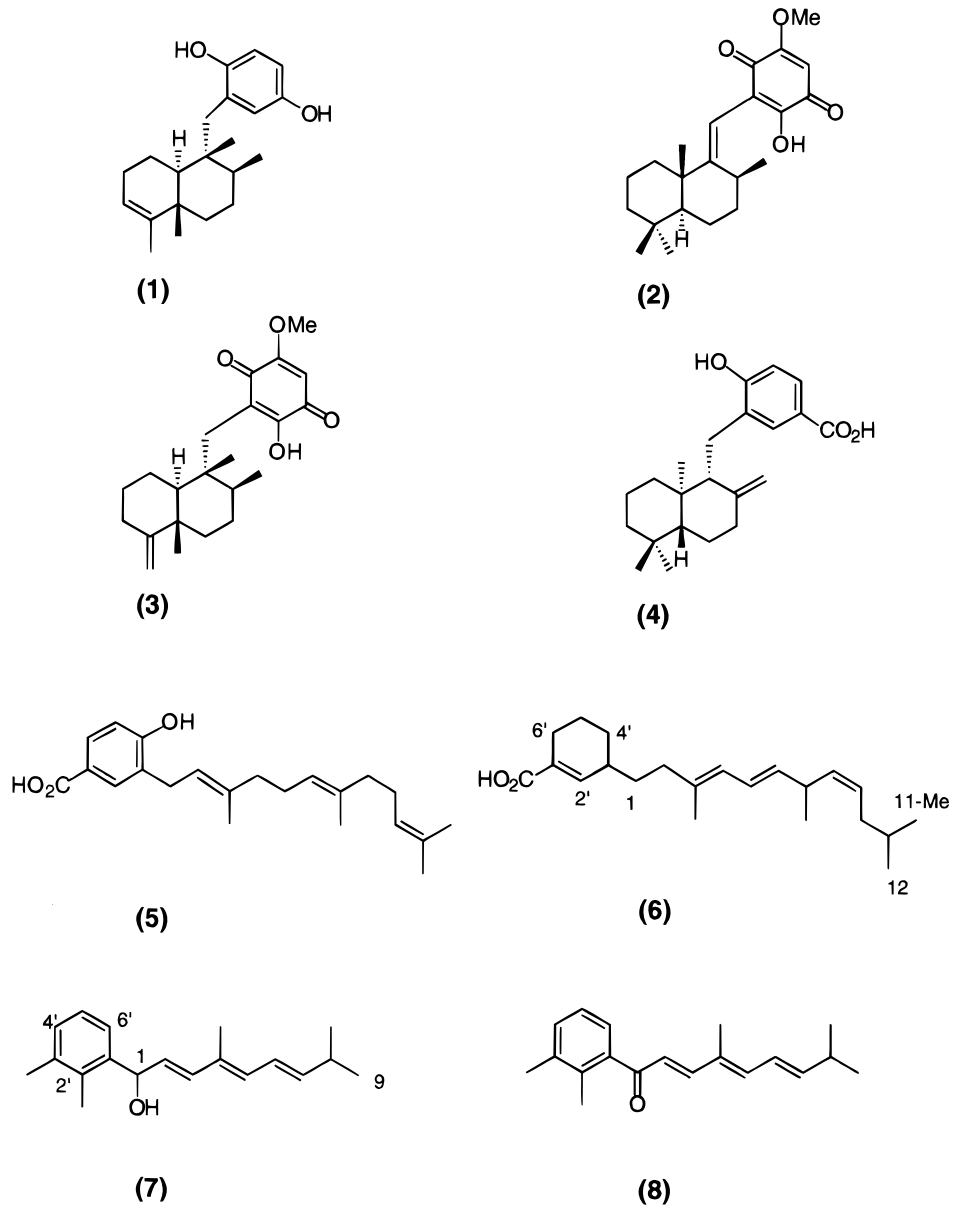
Chart 1



The molecular formula attributed to clathrin B (**7**) ($C_{19}H_{26}O$) on the basis of NMR and low-resolution mass spectral analysis [clathrin B underwent air oxidation to clathrin C (**8**) prior to acquisition of HREIMS data] required seven double-bond equivalents. IR analysis identified the presence of a hydroxy functionality (3440 cm^{-1}). The NMR data for (**7**) revealed resonances for three mutually coupled aromatic protons of a 1,2,3-trisubstituted aromatic ring (^1H δ 7.18, d, 7.08, d, and 6.87, dd), two aromatic methyls (^1H δ 1.61, s and 2.26, s; ^{13}C δ 12.4, q and 15.0, q), an olefinic methyl (^1H δ 1.93, s; ^{13}C δ 8.3, q), an isopropyl unit (^1H δ 1.04, d; ^{13}C δ 22.4, q), and a series of deshielded methines. Two-dimensional $^1H-^1H$ COSY analysis revealed a sequence of correlations from H-4′ to H-6′, from H-1 to H-3, and from H-5 to the isopropyl terminus. Extension of this analysis to include gHMBC data (see Table 2) revealed a full sequence of correlations assembling the gross structure for clathrin B (**7**) as shown.

The magnitude of $J_{2,3}$ (15.6 Hz) and $J_{6,7}$ (14.9 Hz) defined a common *E* stereochemistry, while the stereochemistry about $\Delta^{4,5}$ was established as *E* based on the upfield ^{13}C NMR shift for the 4-Me (δ 18.3, q). This latter argument was confirmed by the observation of NOEs to H-2 (6.3%) and H-6 (5.4%) on irradiation of the ^1H NMR resonance for 4-Me. Although clathrin B (**7**) was optically active, attempts to determine the absolute stereochemistry were thwarted by oxidative degradation to the achiral artifact clathrin C (**8**). The NMR data for clathrin C (**8**) revealed the absence of an H-1 resonance and corresponding simplification of the H-2 multiplet (dd to d) consistent with oxidation of the C-1 2°-OH to a ketone. Supportive of this was the absence of a hydroxy and the appearance of a carbonyl absorbance in the IR spectrum (1680 cm^{-1}) and the observation of a characteristic conjugated ketone resonance in the ^{13}C NMR spectrum (182.5 ppm).

Although clathrin C (**8**) was independently isolated during the course of these investigations, given the ease with which it was formed by air oxidation of clathrin B (**7**) we are inclined to believe that **8** is an artifact of the isolation process. Clathrin A (**6**) would appear to be derived from mixed terpene/shikimate biosynthesis, and its discovery lends support to speculation of similar biosynthetic origins for other marine sesquiterpene/benzenoids. Clathrin B might also be derived from mixed biosynthesis or, alternatively, could be viewed as a rearranged norditerpene.

Table 1. NMR ($CDCl_3$, 400 MHz) Data for Clathrin A (**6**)

no.	$^{13}C^a$ ppm	^1H (δ, m, J Hz)	COSY	gHMBC ^1H to ^{13}C
1′	137.4			
2′	142.0	7.12, d, 4.9	H-3′, H_2-1	C-4′
3′	41.9	2.18, m	H-1, H_2-4′, H-2′	C-2′, C-4′
4′	31.1	1.87, m	H-3′, H_2-5′	C-3′, C-5′
5′	30.5	2.28, tt, 3.4, 4.7	H-4′, H_2-6′	C-4′, C-6′
6′	24.3	2.47, t, 4.7	H_2-5′	C-1′, C-2′
1	28.4	1.26, m	H-1′, H_2-2	C-2
2	28.8	2.05, t, 5.1	H_2-1	C-3
3	139.2			
4	124.0	5.85, d, 10.8	H-5	C-3, C-5, 3-Me
5	124.8	6.25, dd, 10.8, 15.1	H-4, H-6	C-4, C-6, C-7
6	137.8	5.58, dd, 7.1, 15.1	H-5, H-7	7-Me
7	39.7	2.88, m	H-6, H-8/9, 7-Me	C-6, C-8, C-9, 7-Me
8	135.4	5.37, m	H-7, H_2-10	C-7, C-10, 7-Me
9	127.9	5.37, m	H-7, H_2-10	C-7, C-10, 7-Me
10	42.0	1.88, dd, 4.8, 7.0	H-8/9, H-11	11-Me, C-8, C-9, C-12
11	26.9	1.60, m	H_2-10, 11−Me, H_3-12	C-10, 11-Me, C-12
11-Me	22.3	0.87, d, 6.7	H-11	C-10, C-11
12	22.3	0.87, d, 6.7	H-11	C-10, C-11
7-Me	20.6	1.09, d, 6.8	H-7	C-6, C-8
3-Me	15.0	1.74, s		C-3, C-4
12	172.8			

[a] ^{13}C NMR assignments supported by gHMQC, DEPT 90° and 135° NMR experiments.

Table 2. NMR ($CDCl_3$, 400 MHz) Data for Clathrin B (**7**)

no.	$^{13}C^a$ ppm	^1H (δ, m, J Hz)	COSY	gHMBC ^1H-^{13}C
1′	152.9			
2′	130.2			
3′	120.7			
4′	116.4	7.18, d, 7.4	H-5′	C-6′, 2′-Me, 3′-Me
5′	122.3	6.87, dd, 7.4, 7.4	H-4′, H-6′	C-4′, C-6′, 3′-Me
6′	125.4	7.08, d, 7.4	H-5′	C-4′, C-5′, C-1′
1	92.5	4.68, d, 7.8	H-2	C-1′, C-2
2	133.7	5.90, dd, 7.8, 15.6	H-1, H-3	C-1, C-3
3	112.8	6.54, d, 15.6	H-2	C-2, C-4
4	133.0			
5	133.3	6.13, d, 11.0	H-6	4-Me, C-6
6	124.0	6.36, dd, 11.0, 14.9	H-5, H-7	C-5, C-7, 4-Me
7	143.9	5.78, dd, 6.9, 14.9	H-6, H-8	C-5, C-6, C-8
8	31.7	2.41, m	H-7, H_3-9, 8-Me	C-7, 8-Me, C-9
8-Me	22.4	1.04, d 6.8	H-8	C-7, C-8
9	22.4	1.04, d, 6.8	H-8	C-7, C-8
4-Me	8.3	1.93, s		C-4
2′-Me	12.4	1.61, s		C-2′
3′-Me	15.0	2.26, s	H-4′	C-3′, C-4′, C-5′
1-OH		5.38, brs	H-1	

[a] ^{13}C NMR assignments supported by gHMQC, DEPT 90° and 135° NMR experiments.

Experimental Section

General Methods. For general experimental procedures see Urban et al.[11]

Animal Material. A *Clathria* sp. [899 g dry wt] was collected via epibenthic sled off the coast of Cape Arid, Western Australia. The sponge was diced, steeped in EtOH, and stored at −18 °C until required. A voucher specimen was deposited with the Museum of Victoria, registry number F77049.

Growth form, macrobenthic, stalked, branching flabelliform (15 mm); color in life, bright orange red, beige in ethanol; texture, compressible, spongy, fibrous; surface, opaque, glossy with circular tubercules; oscules, conspicuous, lateral, slightly sunken with a membranous lip; spicules, megascleres oxeas, telescoped, mucronate (350−400 × 5 μm), styles, with occasional spined bases (250−300 × 3−8 μm), acanthostyles, with short spines 1 (50 × 5 μm); microscleres, arcuate isochelae (20 μm); ectosome, a distinct layer of plumose brushes of oxeas forming a continuous palisade in parts, covered by ectosomal collagen in which chelate microscleres are scattered; choanosome, an anastomosing reticulation of thick spongin fibers becoming plumose in the subectosome, cored by multispicualr tracts of styles and acanthostyles, with acanths occasionally echinating and connecting fibers, unispicular bispicular; styles and acanthothostyles, scattered in abundant collagen; lacking differentiation of axial and extra-axial regions.

Extraction and Isolation. The sponge was extracted with EtOH and the concentrated extract partitioned into CH_2Cl_2-, MeOH-, and H_2O-soluble fractions. The CH_2Cl_2-soluble fraction was then fractionated by rapid silica filtration using a 10% stepwise gradient from hexane to EtOAc, followed by normal-phase HPLC (2 mL/min 15% EtOAc/hexane through a Phenomenex 5 μ silica 250 × 10 mm column) to yield clathrin B (**7**) (152 mg, 0.53% dry wt), clathrin C (**8**) (32 mg, 0.11%), and clathrin A (**6**) (120 mg, 0.42%) in order of elution.

Clathrin A (6): yellow oil; $[\alpha]_D$ +34.3° (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 3010 and 1690 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 275 (4.6), 236 (4.1); ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 330 $[M]^+$ (5), 314 (8), 271 (6), 254 (12), 205 (15), 159 (55), 135 (60), 95 (66), 55 (100); HREIMS *m*/*z* 330.2560 (calcd for $C_{22}H_{34}O_2$, 330.2555).

Clathrin B (7): yellow oil; $[\alpha]_D$ −25.4° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3440 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 327 (3.6), 285 (4.8), 267 (4.0); ^1H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 270 $[M]^+$ (4), 225 (2), 205 (1), 200 (2), 194 (5), 190 (10), 198 (15), 178 (56), 174 (100); HREIMS, sample decomposed prior to measurement.

Clathrin C (8): yellow oil; IR (film) ν_{max} 1698 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 307 (4.1), 295 (4.0), 285 (4.0); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.08, d, J = 7.1 Hz, 8-Me, $H_3$9; 2.01, s, 4-Me; 2.28, s, 2′-Me; 2.45, m, H-8; 2.54, s, 3′-Me; 5.86, dd, J =

6.0, 12.0 Hz, H-7; 6.30, d, $J = 12$ Hz, H-5; 6.44, dd, $J = 12.0$, 12.0 Hz, H-6; 6.52, d, $J = 15.1$ Hz, H-3; 7.01, d, $J = 15.1$ Hz, H-2; 7.07, d, $J = 7.8$ Hz, H-6′; 7.11, dd, $J = 7.8$, 7.1 Hz, H-5′; 7.28, d, $J = 7.1$ Hz, H-4′; ^{13}C NMR ($CDCl_3$, 400 MHz) δ 8.3, 4-Me; 12.4, 2′-Me; 15.0, 3′-Me; 22.4, 8-Me, C-9; 31.7, C-2; 112.8, C-3; 116.4, C-4′; 120.7, C-3′; 122.3, C-5′; 124.0, C-6; 125.4, C-6′; 130.2, C-2′; 133.0, C-4; 133.3, C-5; 133.7, C-2; 143.9, C-7; 182.5, C-1; 150.9, C-1′; EIMS *m*/*z* 268 $[M]^+$ (69), 237 (13), 199 (91), 159 (100), 144 (60), 115 (80), 71 (80); ESIMS *m*/*z* 269 [M + H]; HREIMS *m*/*z* 268.1824 (calcd for $C_{19}H_{24}O$, 268.1827).

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