Clathriol, a Novel Polyoxygenated 14*β* Steroid Isolated from the New Zealand Marine Sponge *Clathria lissosclera*

Robert A. Keyzers,[†] Peter T. Northcote,*[,†] and Vicky Webb[‡]

School of Chemical and Physical Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand, and National Institute of Water and Atmospheric Research (NIWA), Greta Point, Wellington, New Zealand

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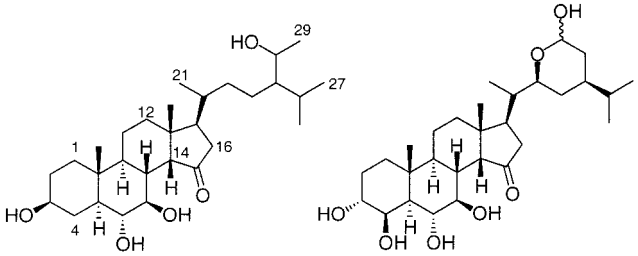
Clathriol (**1**), a highly oxygenated steroid with the unusual 14*β* configuration, was isolated from the New Zealand marine sponge *Clathria lissosclera*. The structure was elucidated from a combination of NMR and mass spectroscopy. Clathriol showed *in vitro* antiinflammatory activity against human neutrophyl and rat mast cells.

Marine sponges have proven to be a rich source of novel steroids. Recently, a number of polyoxygenated steroids have been reported with potent biological activities.[1−3] We wish to report the isolation of a new antiinflammatory polyhydroxy steroid with the unusual *cis* C/D ring fusion. In the course of screening crude extracts of marine sponges for the presence of secondary metabolites, it became apparent by ^{1}H NMR spectroscopy that the organism *Clathria lissosclera* Berquist & Fromont, collected at a depth of 100 m near Three Kings Islands, New Zealand, contained a novel polyoxygenated compound. A methanolic extract obtained from 610 g of the same sponge was partitioned on polymeric reversed-phase support to yield 450 mg of a partially purified extract that appeared to contain a new polyoxygenated steroid. Subsequent separation by MPLC afforded 26.2 mg of clathriol (**1**).



1 **2**

A molecular formula of $C_{29}H_{50}O_5$ for **1** was established by electrospray HRMS from the observation of a molecular ion in both positive and negative ion modes, (479.37542 $[M + H]^+$ Δ 3.7 ppm; 477.35816 $[M - H]^-$ Δ 1.5 ppm). All 29 carbons and 46 protons attached to carbon were observed in the ^{13}C and ^{1}H NMR spectra. One-bond proton carbon connectivity was determined by DEPT and HSQC experiments as listed in Table 1. Evidence of four exchangeable protons was obtained from an electrospray HRMS spectrum of **1** run in negative ion mode with D_2O and CD_3OD used as the injection solvent where a molecular ion of 481.39026 was observed, corresponding to the exchange of four deuteriums. The presence of hydroxyls was supported by a strong OH stretch (3410 cm^{-1}) observed in the IR spectrum. The presence of a saturated ketone was indicated by a ^{13}C NMR resonance at 221.9 ppm and a C=O IR stretch at 1734 cm^{-1}. With no further evidence of unsaturation in the NMR spectra, the remaining degrees of unsaturation required a tetracyclic structure.

The most prominent features of the ^{1}H NMR spectrum, four resolved oxymethine resonances between 4.5 and 3.0 ppm, were found to belong to two separate spin systems from an analysis of COSY and HSQC-TOCSY experiments. Three methylenes (C-1, C-2, C-4), three methines (C-5, C-8, C-14), and three oxymethines (C-3, C-6, C-7) were assigned to a linear sequence of carbons (C-1 to C-8, C-14) on the basis of COSY and HSQC-TOCSY correlations. Methine C-9 and methylenes C-11 and C-12 were connected in a linear fashion to C-8 from HSQC-TOCSY and HMBC correlations, establishing the first spin system. Similarly, four methyls (C-21, C-26, C-27, C-29), two methylenes (C-22, C-23), three methines (C-20, C-24, C-25), and the final oxymethine (C-28) were assembled into a 24-(1-hydroxyethyl)cholestane side chain. Attachment of a methine (C-17) and a methylene (C-16) to C-20 on the basis of HMBC and COSY correlations completed the second spin system.

The three six-membered rings of a steroidal skeleton were established from analysis of the strong HMBC correlations of the protons of the angular methyls C-18 and C-19. Correlations from the protons of CH_3-19 (δ_H 0.83) to a quaternary carbon C-10 (δ_C 36.9), C-1, C-5, and C-9 revealed the connectivity of the A and B rings. Similarly, correlations from the protons of CH_3-18 (δ_H 1.19) to a quaternary carbon C-13 (δ_C 42.6), C-12, C-14, and C-17 established the presence of ring C and the attachment of the side chain. HMBC correlations from H-8, H-14, and H-16*β* to the ketone carbon C-15 provided evidence of the last two carbon bonds C-14 to C-15 and C-15 to C-16 establishing the five membered ring D. Further evidence of the placement of the ketone is provided by the deshielded chemical shift of the proton resonances of H-14, H-16α, and H-16*β* and a further HMBC correlation from H-16*β* to C-14.

The relative stereochemistry of 10 of the 13 stereogenic centers of clathriol (**1**) was assigned from a combination of ^{1}H−^{1}H coupling constants and NOE correlations. The magnitude of ^{1}H−^{1}H coupling constants was determined from the ^{1}H NMR, ^{1}H−^{1}H homonuclear decoupling experiments, and a phase-sensitive double quantum filtered COSY experiment. NOE correlations were detected in a ROESY experiment and a series of 1D GOESY experiments. The normal steroid conformation and configurations of rings A, B, and C were confirmed by NOE correlations from the angular methyls over the *β* face of the molecule.

* To whom correspondence should be addressed. Tel: 644 463 5960. Fax: 644 463 5237. E-mail: peter.northcote@vuw.ac.nz.
[†] Victoria University of Wellington.
[‡] National Institute of Water and Atmospheric Research.

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Table 1. ^{13}C (75 MHz) and ^{1}H (300 MHz) NMR Data (CD_3OD) for Clathriol (**1**)

	$^{13}C^a$		$^{1}H^b$			HMBC
position	δ (ppm)	mult	δ (ppm)	mult, J (Hz)	COSY	(H to C)
1α	38.2	CH_2	0.93	dd(13.5,10)	1*β*, 2α	2, 3, 10, 19
1*β*			1.71	d(11)	1α, 2α	2, 3, 5
2α	31.5	CH_2	1.71	mult	1α, 1*β*, 3	1, 3, 4, 10
2*β*			1.34	mult	1α, 1*β*, 3	3
3	71.8	CH	3.47	dddd(13.5, 10, 4.5, 3)	2α, 2*β*, 4α, 4*β*	
4α	33.0	CH_2	2.15	br d(11.5)	3, 4*β*, 6	2, 3, 6, 10
4*β*			1.16	mult	3, 4α	6, 7, 10
5	48.5	CH	1.10	mult	5, 6	
6	75.1	CH	3.06	dd(10.5, 9)	4α, 5, 7	4, 5, 7
7	75.6	CH	4.30	dd(10.5, 9)	6, 8	6, 8, 14
8	40.4	CH	1.62	ddd(12, 10.5, 3)	7, 14	6, 7, 9, 10, 11, 14, 15
9	47.2	CH	0.93	mult	11*β*	
10	36.9	C				
11α	22.8	CH_2	1.52	mult	11*β*	8, 9, 12, 13
11*β*			1.25	mult	9, 11α, 12α, 12*β*	
12α	39.0	CH_2	1.17	mult	11*β*	
12*β*			1.37	mult	11*β*, 14	18
13	42.6	C				
14	53.4	CH	2.84	dd(3, 2)	8, 12*β*, 16α	8, 9, 12, 13, 15, 18
15	221.9	C				
16α	38.1	CH_2	2.18	mult	14, 16*β*, 17	13, 15, 17, 20
16*β*			2.36	dd(20, 10)	16α, 17	13, 14, 15, 17, 20
17	49.8	CH	1.72	mult	16α, 16*β*, 21	13, 15, 20, 21
18	19.4	CH_3	1.19	s		12, 13, 14, 17
19	13.7	CH_3	0.83	s		1, 5, 9, 10
20	35.4	CH	1.91	mult	21	
21	19.8	CH_3	0.94	d(6.5)	17, 20	17, 20, 22
22α	33.5	CH_2	1.60	mult		16, 20
22*β*			0.90	mult		
23α	25.8	CH_2	1.36	mult		24, 25, 28
23*β*			1.14	mult		
24	52.6	CH	1.08	mult	28	22, 23, 25, 26, 27, 29
25	29.4	CH	1.86	mult		23, 24, 26, 27, 28
26	19.4	CH_3	0.88	d(6.5)		24, 25, 27
27	21.5	CH_3	0.91	d(6.5)		24, 25, 26
28	69.6	CH	3.77	quin(6)	24, 29	23, 24, 25, 29
29	20.7	CH_3	1.14	d(6.5)	28	24, 28

[a] Multiplicity determined from DEPT and HSQC experiments. [b] Carbon connectivity determined from an HSQC experiment, multiplicity determined from ^{1}H, 1D-TOCSY, and homonuclear 2DJ experiments

In particular, 1,3 diaxial NOE correlations between CH_3-19 and H-2*β*, H-4*β*, H-6, and H-8 established the *trans* ring fusion and chair conformations of the A and B rings, as well as the axial position of CH_3-19. Similarly NOE correlations from CH_3-18 to H-8 and H-20 confirmed the chair conformation of ring C and the *β* configurations of CH_3-18 and the 24-(1-hydroxyethyl)cholestane side chain (C-20 to C-29).

The hydroxyl of C-3 was assigned as *β* (equatorial) on the basis of the multiplicity of H-3. This proton, in an axial position, shows coupling constants of greater than 10 Hz to both H-2*β* and H-4*β*. H-3 shows small couplings of less than 5 Hz to both of the equatorial protons at C-2 and C-4 (H-2α, H-4α). A strong NOE correlation from H-3 to H-5 confirms this assignment. H-6 showed NOE correlations to both H-8 and CH_3-19, which together with its large 1,2 *trans* diaxial coupling to H-5, established the equatorial placement of the C-6 hydroxyl on the α face of ring B. The hydroxyl at C-7 was assigned as *β* (equatorial) on the basis of large scalar couplings of H-7 in an axial position to both H-6 and H-8 and NOEs observed from H-7 to the α axial protons on C-5 and C-9. Interestingly, NOEs from H-7 were also observed in both 1D GOESY and ROESY experiments to H-6, H-8, and H-14 assigned to the *β* side of the molecule, perhaps indicating flexibility in this region.

Almost all steroids have a *trans* C/D ring fusion with H-14 in an α (axial) configuration. In both the 1D GOESY and ROESY experiments of clathriol (**1**), however, the H-14 proton resonance showed strong correlations to both H-8 and CH_3-18 on the *β* face of the molecule. The unusual *β* configuration of H-14 was confirmed from the small coupling of H-14 to H-8 (3 Hz) and weak *W* coupling to protons on C-12 and C-16 consistent with an equatorial placement on ring C. Assuming normal steroid configuration, the above observations allow the stereochemistry of the cyclic portion of clathiol (**1**) to be assigned as 3*S*, 5*S*, 6*R*, 7*R*, 8*R*, 9*S*, 10*R*, 13*R*, 14*R*, 17*R*.

The first reported naturally occurring 14*β* steroid, contignasterol (**2**) isolated from a marine sponge of the genus *Petrosia*, has many features in common with **1**, including identical substitution and relative stereochemistry of the B, C, and D rings. Contignasterol (**2**) was reported to inhibit histamine release by rat peritoneal mast cells in a dose-dependent manner with an IC_{50} of 0.8 ± 0.32 *μ*M.[5] Clathriol (**1**) was found to inhibit histamine release in peritoneal mast cells by 72% at a concentration of 30 *μ*M. Clathriol (**1**) was also found to inhibit the activation of human periferal blood neutrophyls by 76% at a concentration of 30 *μ*M.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Unity-INOVA 300 spectrometer. All chemical shifts (δ) were referenced to the residual solvent peak (CD_3OD: ^{1}H δ 3.31 ppm; ^{13}C δ 49.0 ppm). Short- and long-range $^{1}H-^{13}C$ correlations were determined with gradient-enhanced inverse-detected HSQC and HMBC experiments. 1D TOCSY and 2D HSQC-TOCSY experiments were performed

with spin lock mixing times of 15−80 ms. NOE enhancements were detected with GOESY and ROESY experiments with a 0.5 s mixing time. Electrospray HR mass spectrometry was carried out on a PE Biosystem Mariner 5158 TOF mass spectrometer. Infrared spectra were measured on a Biorad FTS-7 spectrometer. Thin layer chromatographic (TLC) analyses were performed using Merck Kieselgel (Aufoilen) 60 F_{254} plates. TLC plates were visualized by spraying with 50% $MeOH/H_2SO_4$ (conc). All solvents used were glass distilled prior to use. HP20 (Diaion) and Amberchrom CG-161M (TosoHaas) poly(styrene divinylbenzene) resins were used for reversed-phase chromatography.

Animal Material. *Clathria lissosclera* was collected by dredging off the Three Kings Islands, North Island, New Zealand, at 100 m depth (voucher specimen #MNP0090 stored at NIWA).

Extraction and Isolation. A single frozen specimen (610 g wet wt) was cut into 2 cm pieces and extracted with methanol (2 × 1.5 L) for 24 h. The second and first methanolic extracts were passed through a glass column packed with 100 mL of HP20 beads preequilibrated with methanol. The eluents were combined with distilled water (3 L) and were passed through the same column. Finally the resulting eluent was diluted with water (6 L) and passed through the column. The column was then washed with water (300 mL) and eluted with 300 mL fractions of (1) 40% acetone/water (2) 80% acetone/water, and (3) 100% acetone. Fraction 2 was concentrated to dryness to yield 451.8 mg of a brown amorphous solid. The brown solid was chromatogrammed on a 20 × 1.5 cm glass column packed with TosoHaas Amberchrom and eluted with increasing concentrations of acetone in water. The 49%−61% acetone in water fractions were combined and concentrated to dryness to give 116.8 mg of a pale yellow solid, which was chromatogrammed in a similar manner. The 51%−53% acetone in water fractions were combined and concentrated to dryness to yield **1** as a pale yellow solid (26.2 mg).

Clathriol (1): orange char R_f 0.4 (CH_2Cl_2−MeOH, 19:1); $[\alpha]^{20}_D$ +22.6° (*c* 1.4, MeOH); IR (KBr) ν_{max} 3410, 2958, 2872, 1734, 1584, 1055 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESHRMS pos. *m*/*z* 479.37542 (calcd for $C_{29}H_{51}O_5$ 479.37365), neg. *m*/*z* 477.35816 (calcd for $C_{29}H_{49}O_5$ 477.35745).

Biological Assays. Human peripheral blood neutrophyl activation was measured as superoxide production after stimulation with either PMA or fMLP as previously described.[6] Rat mast cell histamine release inhibition was measured as previously described.[5]

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Supporting Information Available: NMR spectra of **1** are available including ^1H, ^{13}C, DEPT, COSY, HSQC, HMBC, ROESY, and HSQC-TOCSY. This material is available free of charge via the Internet at http://pubs.acs.org.

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