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Aust. J. Chem. **2003**, *56*, 279–282

Clathriol B, a New 14β Marine Sterol from the New Zealand Sponge *Clathria lissosclera*

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A new anti-inflammatory sterol, clathriol B (1.3 mg), was isolated from the New Zealand marine sponge *Clathria lissosclera* collected at the Three Kings Islands. Clathriol B possesses the unusual 14β stereochemistry, a structural feature that occurs naturally only in marine sponges.

Manuscript received: 21 August 2002.
Final version: 17 December 2002.

Marine sponges have proven to be a continuing rich source of sterols exhibiting novelty in both structure and biological activity.[1–8] As part of our ongoing screening of marine sponges for interesting secondary metabolites, work with *Clathria lissosclera* (Berquist and Fromont) indicated the presence of several polyoxygenated compounds. Extraction of a bulk sample of the same sponge led to the recently reported isolation of the novel sterol, clathriol A (1) (see Diagram 1),[9] which possessed the unusual 14β C/D *cis*-ring fusion stereochemistry, a feature that occurs naturally only in marine sponge steroids. Further work on an extract of this sponge has yielded a second 14β sterol, clathriol B (2).

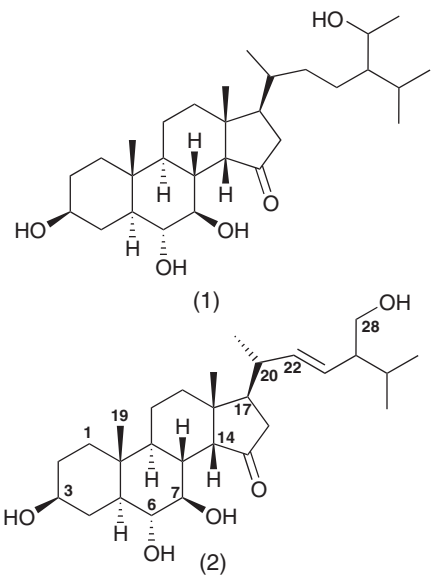

Diagram 1

A 610 g sample of sponge material, collected from a depth of 100 m by dredging near the Three Kings Islands, New Zealand, was extracted with MeOH. Crude partitioning on a polymeric solid support yielded 450 mg of a complex mixture of compounds. Further isolation using reverse-phase medium-pressure liquid chromatography (MPLC) resulted in the isolation of 26.3 mg of (1).[9] Side fractions possessed smaller amounts of several other similar compounds. Subsequent repeated chromatography on silica gel afforded the novel compound clathriol B (2) (1.3 mg).

A molecular formula of $C_{28}H_{46}O_5$ was determined for (2) by observation of pseudo-molecular ions in both positive and negative modes [pos. m/z 463.3423 (calc. for $C_{28}H_{47}O_5$ 463.3418); neg. m/z 461.3294 (calc. for $C_{28}H_{45}O_5$ 461.3273)] of electrospray high-resolution mass spectroscopy (HRMS) spectra. All 28 carbons and 42 protons attached to carbon were observed in ^{13}C and ^{1}H NMR spectra. One bond carbon–proton connectivity was determined in a heteronuclear single-quantum correlation (HSQC)–distortionless enhancement by polarization transfer (DEPT) experiment as listed in Table 1. The presence of a saturated ketone was indicated by a ^{13}C NMR resonance at δ 222.3 and an IR C=O stretch at 1728 cm^{-1}. The presence of a disubstituted double bond was indicated by deshielded chemical shifts in both the ^{13}C NMR (δ 134.6, 130.3) and ^{1}H NMR (δ 5.63, 5.26) spectra. With no other evidence of multiple bonds, the remaining degrees of unsaturation required compound (2) to have a tetracyclic structure.

Detailed correlation spectroscopy (COSY) and 1D total correlation spectroscopy (TOCSY) analysis indicated the presence of two separate ^{1}H spin systems. These systems were established by means of correlations observed in the most prominent features of the ^{1}H NMR spectrum, namely, two olefinic methines, three oxygenated methines, and two

 10.1071/CH02167 0004-9425/03/04279

Table 1. ^{13}C and ^{1}H NMR data for clathriol B (2)
^{13}C data recorded at 75 MHz; ^{1}H data recorded at 300 MHz. Solvent $CDCl_3$

Position	^{13}C [A]		^{1}H [B]		COSY	HMBC (H to C)
	δ (ppm)	Multiplicity	δ (ppm)	Multiplicity, J (Hz)		
1α	36.8	CH_2	0.94	Multiplet	1β, 2α, W19	–
1β	–	–	1.69	Multiplet	1α, 2α, 2β	–
2β	30.4	CH_2	1.36	Multiplet	1β, 2α, 3	–
2α	–	–	1.77	Multiplet	1α, 1β, 2β, 3	–
3	71.1	CH	3.54	dddd (14.6, 10.3, 4.3, 3.8)	2α, 2β, 4α, 4β	–
4β	32.1	CH_2	1.24	Multiplet	3, 4α	2, 6
4α	–	–	2.24	Multiplet	3, 4β, 5	–
5	47.0	CH	1.16	Multiplet	4β, 6	–
6	73.5	CH	3.16	dd (10.3, 9.1)	5, 7	–
7	74.5	CH	4.26	dd (10.6, 8.8)	6, 8	6
8	38.9	CH	1.58	dt (11.8, 4.2)	7, 14	7, 9, 15
9	46.0	CH	0.89	Multiplet	11α, 11β	–
10	36.0	C	–	–	–	–
11α	21.3	CH_2	1.20[C]	Multiplet	9, 11β, 12β	–
11β	–	–	1.50	Multiplet	9, 11α	–
12α	36.5	CH_2	1.19[C]	Multiplet	–	–
12β	–	–	1.36	Multiplet	11α, W14, W17	9, 18
13	41.7	C	–	–	–	–
14	51.7	CH	2.74[C]	Multiplet	8, W12β, W16α	8, 9, 12, 13, 15
15	222.3	C	–	–	–	–
16β	37.9	CH_2	2.17	br d (19.9)	16α, 17, W14	13, 15, 20
16α	–	–	2.40	dd (19.9, 9.3)	16β, 17	15, 20
17	48.2	CH	1.76	dd (10.8, 2.7)	16α, 16β, 20, W12β	15, 22
18	18.9	CH_3	1.21[C]	s	–	12, 13, 14, 17
19	13.5	CH_3	0.80	s	W1α	1, 5, 9, 10
20	34.9	CH	2.75[C]	Multiplet	17, 21, 22	17, 22, 23
21	19.6	CH_3	0.99	d (7.0)	20	17, 20, 22
22	134.6	CH	5.63	dd (15.8, 7.5)	20, 23	20, 21, 24
23	130.3	CH	5.26	dd (15.3, 8.0)	22, 24	20, 24, 28
24	52.3	CH	1.96	Multilpet	23, 25, 28a, 28b	–
25	29.3	CH	1.58	Multiplet	24, 26, 27	23, 24, 26, 27, 28
26	19.6	CH_3	0.81	d (6.7)	25	24, 25, 27
27	20.8	CH_3	0.86	d (6.7)	25	24, 25, 26
28a	64.7	CH_2	3.45	t (10.4)	24, 28b	23, 24
28b	–	–	3.64	dd (10.6, 4.1)	24, 28a	23

[A] Multiplicity determined from a HSQC-DEPT experiment.
[B] Carbon connectivity determined from a HSQC-DEPT experiment. Multiplicity determined from ^{1}H, 1D TOCSY, and ^{1}H homonuclear decoupled experiments.
[C] Can be interchanged.

signals attributed to an oxygenated methylene. The first spin system connected a linear sequence of carbons from C1 to C14, as determined by COSY correlations. The attachment of a side branch (C9, C11, and C12) was difficult to ascertain in the COSY spectrum due to spectral overlap and was established from a series of 1D TOCSY spectra. Selective irradiation of H7 sequentially revealed, as the mixing time was increased, the resonances of H8 and H9, establishing the C8 to C9 bond. Selective irradiation of H12β revealed the resonance of H11β, and thereby indicated the adjacent nature of the C11 and C12 methylenes. Finally, a resolved cross peak in the COSY spectrum between H9 and H11 established the C9–C11 connectivity and completed this proton spin system. The second spin system connected another chain of carbons from C16 (δ 37.9) to C28 (δ 64.7) as determined by COSY cross peaks.

Identification of the three six-membered rings of (2) was established on the basis of strong heteronuclear multiple-bond correlations (HMBCs) from both 18-CH_3 (δ_C 18.9, δ_H 1.21) and 19-CH_3 (δ_C 13.5, δ_H 0.80). Strong correlations from the protons of 19-CH_3 to C1, C5 (δ 47.0), C9 (δ 46.0), and quaternary carbon C10 (δ 36.0) connected rings A and B. Similar correlations from 18-CH_3 to C12 (δ 36.5), C14, C17 (δ 48.2), and quaternary carbon C13 (δ 41.7) gave evidence for the presence of ring C. Final determination of ring D was established by strong HMB correlations from H8, H14, H16α, and H16β to C15, thereby completing the tetracyclic structure required for (2). Further evidence for the placement of ketone C15 is given by the deshielded chemical shifts of H14 (δ 2.74), H16α (δ 2.40), and H16β (δ 2.17), as well as by the weak W-coupling observed in the COSY spectrum between H14 and H16α. All NMR spectral correlations for the tetracyclic portion of (2) were in complete agreement with those of (1), as substitution of these rings is identical in both compounds. The chemical shifts of carbons C21 to C28 and all their attached protons were also in agreement

with those of previously published sterols with the same side chain.[10,11]

The relative stereochemistry of 11 of the 12 possible stereogenic centres in (2) and the geometry of the olefin were established from a combination of ^{1}H–^{1}H homonuclear couplings and nuclear Overhauser effect (NOE) correlations. The magnitudes of the ^{1}H–^{1}H homonuclear couplings were determined from ^{1}H NMR and ^{1}H homonuclear decoupling experiments. NOE correlations were observed in both rotating-frame Overhauser enhancement spectroscopy (ROESY) and a series of 1D gradient nuclear Overhauser spectroscopy (GOESY) experiments. The normal sterol conformation of rings A, B, and C was confirmed by NOE correlations from angular methyls 18-CH_3 and 19-CH_3 over the β face of the molecule. In particular, 1,3-diaxial NOE correlations from 19-CH_3 to H2β, H4β, and H6 established the existence of the normal *trans* ring fusion between, and chair conformations of, rings A and B. Strong NOE correlations from 18-CH_3 to both H8 and H20 confirmed the chair conformation of ring C and identified the orientation of the side chain as being on the β face of the molecule.

The hydroxyl attached to C3 was established as being equatorial (β) on the basis of the multiplicity of H3. This proton resonance shows two large couplings of greater than 10 Hz to both H2α and H4α, and shows two small couplings of less than 5 Hz to both H2β and H4β, which is consistent with the placement of H3 in an axial orientation. A strong NOE from H3 to H5 helped confirm this assignment. Strong NOE correlations from H6 to both H8 and 19-CH_3, along with the two large 1,2-*trans* diaxial coupling constants measured between H6 to both H5 and H7 allowed placement of the hydroxyl of C6 as equatorial. The hydroxyl attached to C7 was assigned as equatorial on the basis of the two large (>10 Hz) couplings from H7 to H6 and H8, and strong NOEs from H7 to H5 and H9.

The β placement of H14 was indicated by a strong NOE from H14 to H8 and from weak W-couplings observed between H14 and both H12β and H16β. The assignment of H14 as β in (1) was strengthened by observation of a strong NOE from 18-CH_3 to H14. However, in (2) H14 spectrally overlaps with H20, both of which are in close spatial proximity to 18-CH_3, and prevents any valid inference being made from observed NOEs.

Restricted rotation of the side chain between C17 and C20 was indicated by the weak coupling between their attached protons (as indicated by the lack of a measurable coupling constant between H17 and H20), which is indicative of a bond angle of approximately 90° between H17 and H20. This observation is consistent with a single conformation, and several NOE correlations support this assignment. NOE correlations from H23 to 18-CH_3 and H16β, along with another correlation from H22 also to 18-CH_3, clearly places the olefin above ring D (on the β face of the molecule) in an antiperiplanar relationship to H17. The *R* configuration of C20 is confirmed by the observation of an NOE correlation from H16β to 21-CH_3 as illustrated in Figure 1.

The geometry of the olefin was assigned as *E* due to the large couplings measured between H22 and H23 (15.8 Hz).

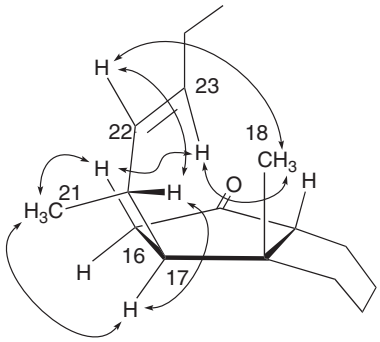

Fig. 1. Selected NOE correlations establishing the stereochemistry of C20.

Strong NOE correlations from H22 to H20, and from H23 to H16β, H21, and H24 helps to confirm this assignment. NOEs from both H22 and H23 to 18-CH_3 also confirm the β orientation of the side chain. Assuming normal steroid stereochemistry, these observations allow (2) to be assigned as 3*S*, 5*S*, 6*R*, 7*R*, 8*R*, 9*S*, 10*R*, 13*R*, 14*R*, 17*R*, 20*R*, and 22(*E*).

Clathriol A (1) was found to be an anti-inflammatory agent that inhibits the production of superoxide from human peripheral blood neutrophils stimulated with *N*-formyl-methionine-leucine-phenylalanine (fMLP) or phorbol myristate acetate (PMA) (IC_{50} 33 and 140 μM, respectively), whilst clathriol B (2) returned values of 27 and 130 μM, respectively, in the same assay.[12]

In conclusion, a novel sterol with moderate anti-inflammatory activity has been isolated from the New Zealand sponge *Clathria lissosclera* using a variety of normal and reverse-phase chromatography. Clathriol B (2) possesses the rare 14β stereochemistry, a structural feature that is only found to occur naturally in marine sponges.

Experimental

General Experimental Procedures

^{13}C NMR were recorded on a Bruker Avance 300 spectrometer. All other NMR spectra were recorded on a Varian Unity-INOVA 300 spectrometer. All chemical shifts were referenced internally to the residual solvent peak ($CDCl_3$: δ_H 7.26, δ_C 77.0). Short and long range ^{1}H–^{13}C correlations were determined with gradient-enhanced inverse-detected HSQC and HMBC experiments, respectively. NOE correlations were detected with GOESY and ROESY experiments with a 0.5 s mixing time. 1D TOCSY experiments were performed with spin-lock-mixing times of 10–80 ms. Electrospray HRMS was carried out on a PE Biosystem Mariner 5158 TOF mass spectrometer. Thin-layer chromatography analyses were performed using Merck Kieselgel (Alufoilen) 60 F_{254} plates. TLC plates were visualized by spraying with 1 : 1 MeOH/H_2SO_4 followed by dipping in a vanillin solution (1% vanillin in ethanol). All solvents were glass distilled prior to use. HP20 (Diaion) and Amberchrom CG-161M (TosoHaas) poly(styrene divinylbenzene) resins were used for reverse-phase chromatography. Merck Keiselgel 60 (0.0623–0.200 mm) was used for normal-phase separation.

Animal Material

Clathria lissosclera was collected by dredging off the Three Kings Islands, North Island, New Zealand at a depth of 100 m (voucher specimen no. MNP0090 stored at the National Institute of Water and Atmospheric Research (NIWA), Greta Point, Wellington, New Zealand).

Extraction and Isolation

A single frozen specimen (610 g wet weight) was cut into 2 cm pieces and extracted in MeOH (2 × 1.5 L) for 24 h. The second and first MeOH extracts were passed through a glass column packed with 100 mL HP20 beads pre-equilibrated with MeOH. The eluents were combined with distilled H_2O (3 L) and were passed through the same column. Finally, the resulting eluent was diluted with H_2O (6 L) and passed through the column. The column was washed with H_2O (300 mL) and eluted with 300 mL fractions of 40% acetone/H_2O, 80% acetone/H_2O, and 100% acetone. Fraction two was concentrated to dryness to yield 451.8 mg of a brown amorphous solid. The brown solid was chromatogrammed on a 20 cm × 1.1 cm glass-packed column with Amberchrom and eluted with increasing concentrations of acetone in H_2O. The 49–61% acetone in H_2O fractions were combined and concentrated to dryness to give 116.8 mg of a pale yellow solid which was rechromatogrammed in a similar manner. The 51–53% acetone in H_2O fractions were combined and concentrated to dryness to give clathriol A (1) (26.3 mg). The 48–50% acetone in H_2O fractions were combined and concentrated to dryness to give 17.1 mg of a mixture of sterols. Repeated normal-phase chromatography on silica gel using mixtures of MeOH in CH_2Cl_2 (between 0 and 10% MeOH) gave 1.3 mg of (2). A mixture of several other co-eluting sterols (5.5 mg) was obtained which could not be resolved.

Clathriol B (2). H_2SO_4 charring, blue, R_F 0.38 (MeOH/CH_2Cl_2, 1 : 19). $[\alpha]_D$ −29.4° (*c* 0.71 in CH_2Cl_2). ν_{max} (cm^{-1}) 3376, 2928, 1728, 1602, 1461, 1206, 1153, 1056. δ_H and δ_C see Table 1. Electrospray ionization HRMS pos. m/z 463.3422, neg. m/z 461.3294.

Biological Assays

Human peripheral blood neutrophil activation was measured as superoxide production after stimulation with either PMA or fMLP in a micro-plate assay as previously described.[12]

Acknowledgments

This research was supported in part by a grant (CO1X0001) from the PGSF (New Zealand). Sponge specimens were collected by Michael Page, National Institute of Water and Atmospheric Research (NIWA), Nelson. The sponge was identified by Belinda Glasby. Anti-inflammatory assays were performed by An Tan.

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