Clathsterol, a Novel Anti-HIV-1 RT Sulfated Sterol from the Sponge *Clathria* Species

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Received March 2, 2001

As part of a search for novel inhibitors of humandeficiency virus type 1 (HIV-1) reverse transcriptase (RT), the MeOH−EtOAc extract of a Red Sea sponge, *Clathria* sp., was shown to be active. Bioassay-guided fractionation of the extract yielded a novel sterol sulfate, clathsterol (**1**), which is responsible for the activity and is active at a concentration of 10 μM. The structure of **1** was established mainly by interpretation of spectral data and a chemical transformation.

Sulfated sterols have been described from a wide variety of marine organisms, particularly sponges and echinoderms, and several of these steroidal sulfates have exhibited a broad range of activities.[1−8] For example, halistanol sulfate, the most common sulfated sterol, first isolated from the sponge *Halichondria moorei*, is antimicrobial, hemolytic, ichthyotoxic, and active against HIV-1.[1,3] Weinbersterol disulfate, isolated from the sponge *Petrosia weinbergi*, exhibited in vitro activity against both feline leukemia virus and HIV-1.[4] Ibisterol sulfate, isolated from *Topsentia* sp., was active against HIV-1.[5,6] Halistanol sulfates A−E were found to be active in thrombin receptor assay.[7] Halistanol disulfate B was found to be an inhibitor of endothelin converting enzyme.[8] The latter sulfated sterols have in common the 2β,3α sulfoxy groups, some of them a third 6α sulfate, and they differ in the steroidal side chain.

As part of continuing research for biologically active natural products with activity against human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT),[9] the organic extract of the Red Sea sponge *Clathria* sp. (family Microcionidae, order Poecilosclerida, class Demospongiae) was found to be active.

The freeze-dried sponge, collected in the Dahlak archipelago (Eritrea), was extracted several times with a 1:1 mixture of EtOAc−MeOH. Bioassay-directed fractionation of the $CHCl_3$-soluble portion of the crude extract, which exhibited anti-HIV-RT activity, yielded clathsterol (**1**) after repeated column chromatorgaphy on Sephadex LH-20 and RP-18.

Compound **1**, $[\alpha]_D$ +28° (*c* 0.35, CH_3OH), which was obtained as a white powder, displayed a molecular ion at *m*/*z* 905 $[M + Na]^+$ by positive ion FABMS corresponding to $C_{39}H_{64}O_{15}S_2Na_2$. The IR spectrum exhibited strong absorptions at 1247 and 1730 cm^{-1}, consistent with the presence of sulfate and ester functionalities, respectively. As the ^{13}C NMR spectrum showed three carbonyl groups (δ_C 170.4, 172.3, and 172.4 ppm) and lacked double bonds, to satisfy the seven degrees of unsaturation, clathsterol has to be tetracyclic. The ^{13}C and ^1H NMR spectra (Table 1) supported the existence of nine methyl groups (three methyl singlets, including one $OCOCH_3$ group, three methyl doublets, and three triplets). The two methyl singlets at δ_H 0.85 and 0.88, three methyl doublets at δ_H 0.92, 0.86, and 0.79, and three methines resonating at δ_C 54.5, 56.8, and 53.9 suggested a steroidal skeleton for the four rings of **1**.

Comparing the NMR data of **1** (Table 1) and the data of halistanol disulfate B suggested the same diaxial 2β,3α-disulfate structure[8] for clathsterol. The latter suggestion was further supported by the H_2-1 to H_2-4 COSY correlations as well as the HMBC CH-correlations from methyl-19 to carbons 1, 5, 9, and 10.

The ^1H NMR spectrum (Table 1) supported the existence of an additional four oxygenated methines (to C-2 and -3) (δ_H 4.80, 3.94, 5.38, and 5.25 ppm). In the COSY spectrum, the latter four protons were found to belong to two spin systems which could be linked together by a TOCSY experiment. One system comprises H-15 and -16 and the other H-22 and -23. The location of the first system was suggested from the proton correlations with H-17, H-20, and Me-21, and that of the second was suggested from its correlations with H-20 and Me-21, thereby joining them both. Most important for the assignment of the methinoxy groups was the identification of H-14, -17, and -20 by means of their CH-correlations with Me-18 and -21 (Table 1). The chemical shift of H-16 (δ 3.94), which is about 1 ppm or more upfield when comapred to the resonances of H-15, -22, and -23, suggested that it carries a free hydroxy group, while the three other oxygenated methines are esterified. Interestingly, the 16-OH group did not acetylate under the regular Ac_2O−pyridine conditions, at room temperature, most likely due to severe spatial hindrance of the latter hydroxy group by the neighboring 15-acetate and the highly substituted side chain. Comparison of the NMR data of ring D of **1** with ring D of the starfish *S. caroli* carolisterols A−C, which carry the 15α,16β-dihydroxy groups (and especially the coupling constants),[10] pointed clearly to the same 15α,16β stereochemistry in both.

The COSY and TOCSY data of **1** also established the presence of two butyrate esters (C-1′ to -4′ and C-1″ to -4″, Table 1) in addition to the acetate group (δ_H 1.92, δ_C 21.3q and 170.4s).

Determination of the location of the three esters was achieved from an HMBC experiment (Table 1), namely, C-22 and C-23 carry the butyrate esters while C-15 is acetylated. Most indicative for the assignment were the correlations of the three carbonyl carbons with the alkyl residue of the acid, from one side, and the oxygenated methine from the other side (Table 1).

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10.1021/np010121s CCC: $20.00
Published on Web 10/16/2001

Table 1. ^{1}H and ^{13}C NMR Data for Clathsterol[a]

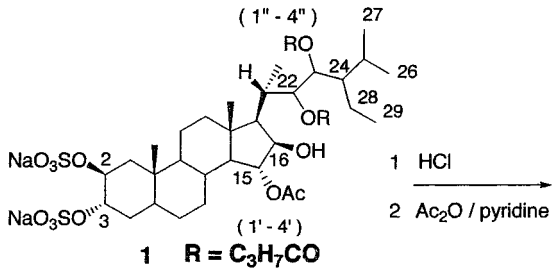
no.	δ_C (mult)[b]	δ_H (mult, *J* in Hz)	HMBC[d]	no.	δ_C (mult)	δ_H (mult, *J* in Hz)	HMBC
1	37.2 t	1.92, 1.03	19	20	30.4 d	2.18	21
2	73.0 d	4.25 brs	3	21	11.9 q	0.92 d (6.5, Me)	
3	73.1 d	4.28 brs	2	22	74.5 d	5.38 d (9.1)	21, 23
4	28.8 t	1.50, 1.42		23	73.8 d	5.25 dd (9.1, 1.4)	22, 25
5	38.2 d	1.42	19	24	44.9 d	1.29	22, 26, 27, 28, 29
6	30.4 t	1.46, 0.92		25	25.4 d	2.25	26, 27
7	27.5 t	1.15, 1.05		26	23.4 q	0.86 d (6.5, Me)	27
8	33.7 d	1.55		27	18.1 q	0.79 d (6.5, Me)	26
9	54.5 d	0.56	19	28	17.8 t	1.55, 1.45	29
10	34.9 s		19	29	12.4 q	0.88 t (6.5, Me)	
11	20.2 t	1.42, 1.10		1′[c]	172.4 s		22, 2′, 3′
12	40.1 t	1.83, 1.08	18	2′	35.6 t	2.12, 2.14	4′
13	42.7 s		18	3′	17.2 t	1.32, 0.95	4′
14	56.8 d	1.08	18	4′	13.5 q	0.86 t (6.5, Me)	
15[f]	84.9 d	4.80 dd (10.6, 2.3)	14	1″[c]	172.3 s		23, 2″, 3″
16	77.3 d	3.94 ddd (10.6, 4.8, 2.3)		2″	35.7 t	2.25, 2.18	4″
17[e]	53.9 d	1.21 dd (10.6, 6.5)	18, 21	3″	18.1 t	1.08, 0.85	4″
18	13.8 q	0.85 s		4″	13.5 q	0.88 t (6.5, Me)	
19	14.0 q	0.88 s		15 Ac	170.4 s		1.92
					21.3 q	1.92 s	

[a] Data recorded in DMSO at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b] CH assignments are based on the HMQC spectrum. [c] 1′−4′ and 1″−4″ are the C atom numbers of 22-OBu and 23-OBu, respectively. [d] C to H. [e] *J*-values measured from a selective TOCSY experiment. [f] $J_{14\alpha,15\beta} = 10.6$; $J_{15,16} = 2.3$; $J_{16,OH} = 4.8$; $J_{16,17} = 10.6$ Hz.

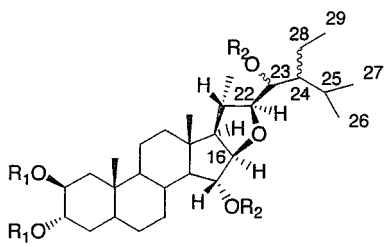
The last structural feature clarified was the substitution at C-24 of the side chain. The NMR data (Table 1) pointed clearly to an additional ethyl group in **1**. HMBC correlations of both the extra ethyl group and the side chain-end isopropyl group to the same carbon atom established the place of the ethyl group.[11]

While the relative, and most likely also the absolute, stereochemistry of C-2, -3, -15, and -16 of **1** was established from the NMR data, the configuration of the four chiral centers of the side chain (C-20, -22, -23, and -24) could not be similarly achieved due to conformational lability.

Experiments conducted to remove the sulfate groups by heating **1** in a mixture of dioxane−pyridine as well as basic hydrolysis to take off the ester groups, with 1% K_2CO_3 in CH_3OH, failed to give single pure compounds. On the other hand, acid hydrolysis, 6 N HCl, changed compound **1** into **2**. For further purification of the latter, which was not characterized, it was acetylated with Ac_2O in pyridine, at room temperature, to give triacetate **3** after chromatography.



1 $R = C_3H_7CO$



2 R_1 = H ; $R_2 = C_3H_7CO$

3 R_1 = Ac ; $R_2 = C_3H_7CO$

Compound **3** was obtained as a solid gum, $[\alpha]_D$ +33°, which no longer showed anti-HIV-1 RT activity. The mass spectrum of **3** established a molecular formula of $C_{39}H_{62}O_9$ [*m/z* 675, $(MH)^+$], and the NMR data (Table 2) showed the hydrolysis of the 2,3-disulfoxy groups and their replacement by two acetates.[1] A third acetoxy group on C-15 was also evident from the NMR data (Table 2). According to H-15's coupling constants, the acetate kept its original stereochemistry, as in **1**, through the acid hydrolysis and re-acetylation. Suprisingly, one of the two butyrate esters remained intact in **3**. The location of the latter ester on C-23 was determined by CH-correlations (HMBC results, Table 2; namely, C-1′ to H-23, C-28 to H-23, and C-23 to H-22). The above-discussed four ester groups (three acetates and one butyrate) together with the four steroidal rings required, for **3**, another ring to fulfill the nine degrees of unsaturation. The 13 ppm downfield shift of C-16 and C-22 pointed clearly to an ethereal bridge between the two, forming a THF ring. The remaining of $J_{14\alpha,15\beta} = 10.6$ Hz and $J_{15\beta,16\alpha} = 2.1$, 2.3 Hz (Tables 1 and 2), essentially unchanged, also suggested that the chirality of C-15 and -16 did not change. Indeed, NOEs measured for **3** (Figure 1) confirmed, unambiguously, the relative stereochemistry of the THF and ring D chiral centers. According to the results, C-20 has the regular (20S) steroidal chirality and hydrogen atoms 16, 17, and 22 are α-oriented. As with compound **1** (and for the same reasons), here too the chirality of C-23 and 24 could not be established. Also, from the chirality of C-22 in **3**, it is impossible to deduce the stereochemistry of this center in **1**, as the mechanism of closure of the THF ring at C-22 is not unambiguous; there is a possibility of neighbor group participation during the hydrolysis of the 23-butyrate group. Clathsterol is, therefore, 2*β*,3α-disulfoxy-15α-acetoxy-16*β*-hydroxy-22*ξ*,23*ξ*-dibutyroxy-24*ξ*-ethylcholestane. The biological activity of **1** will be reported elsewhere.

Experimental Section

General Experimental Procedures. IR spectra were obtained with a Bruker Vector 22 spectrophotometer. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker ARX-500 spectrometer. All chemical shifts are reported with respect to TMS ($\delta_H = 0$) and $CDCl_3$ ($\delta_C = 77.0$). MS data were recorded on a Fisons Autospec-Q spectrometer, and $[\alpha]_D$ values were recorded on a Jasco P-1010 polarimeter.

Animal Material. The sponge was collected near Dahlak Island in Dahlak archipelago, Eritrea, using scuba at a depth

Table 2. ^{1}H and ^{13}C NMR Data for Compound **3**[a]

no.	δ_C (mult)[b]	δ_H (mult, *J* in Hz)	HMBC[d]	no.	δ_C (mult)	δ_H (mult, *J* in Hz)	HMBC
1	37.7 t	1.90, 1.38	19	20	35.8 d	2.03	
2	70.1 d	4.90 bs		21	18.6 q	0.86 d (6.5, Me)	17
3	69.8 d	4.87 bs		22	87.2 d	3.52 dd (8.7, 6.9)	21
4	29.7 t	1.75, 1.42		23	75.4 d	5.27 dd (8.7, 2.7)	22
5	39.2 d	1.45	19	24	46.3 d	1.55	26, 27, 29
6	30.9 t	1.60 (2H)		25	26.5 d	2.01	26, 27
7	27.6 t	1.22 (2H)		26	22.9 q	0.95 d (6.5, Me)	
8	34.5 d	1.78		27	18.0 q	0.86 d (6.5, Me)	
9	54.6 d	0.70	19	28	18.6 t	1.65 (2H)	23, 29
10	35.4 s		19	29	13.8 q	0.92 t (6.5, Me)	
11	20.2 t	1.50, 1.25		1′[c]	170.1 s		23, 2′, 3′
12	39.3 t	1.68, 1.10	18	2′	36.4 t	2.28 dt (2.5, 6.5, 2H)	4′
13	42.1 s		16, 18	3′	18.3 t	1.66 (2H)	4′
14	59.7 d	1.42	18, 19	4′	13.9 q	0.96 t (6.5, Me)	
15[e]	80.1 d	5.11 dd (10.6, 2.1)	14	2 Ac	168.4 s		2.01
16	90.2 d	4.14 dd (7.7, 2.1)	15		21.5 q	2.01 s (Me)	
17	63.7 d	1.75		3 Ac	168.4 s		
18	17.0 q	0.87 s (Me)			21.3 q	2.02 s (Me)	2.02
19	12.8 q	0.89 s (Me)		15 Ac	168.3 s		2.02
					21.3 q	2.02 s (Me)	

[a] Data recorded in $CDCl_3$ at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b] CH assignments are based on the HMQC spectrum. [c] 1′−4′ are the C atom numbers of the 23-OBu. [d] C to H correlations. [e] $J_{14\alpha,15\beta} = 10.6$; $J_{15,16} = 2.1$; $J_{16,17} = 7.7$ Hz.

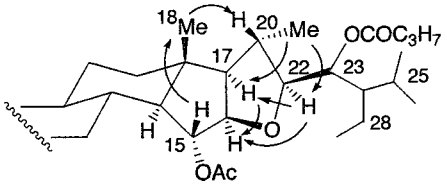


Figure 1. Key NOEs of **3**.

of 10 m during February 1998. A voucher sample is deposited at the Zoological Museum, Tel-Aviv University (no. ET406, sp 25205). The sponge has digits radiating from a single holdfast like gorgonian, tough, finely fibrous. It is a *Clathria* sp.; it resembles *C. reinwardti*, common in Indonesia, but certainly differs from it.

Extraction and Isolation. After collection, the sponge was immediately frozen and kept at −20 °C. The frozen sponge was freeze-dried and then extracted (22 g, dry wt) with a mixture of MeOH−EtOAc (1:1) × 3 to give a dark brown gum (1.2 g). Solvent partitioning between $CHCl_3$ and H_2O gave crude **1** (120 mg) in the organic phase.

The latter material was chromatographed three times sequentially, on Sephadex LH-20 eluted with MeOH and once on RP-18, to give **1** (13 mg) as a gum, $[\alpha]_D$ +28° (*c* 0.15, CH_3-OH); IR (neat) 3300, 2900, 1730, 1247 cm^{-1}; FABMS *m*/*z* (%) 905 $[M + Na]^+$ (20), 819 (18), 803 (75), 781 (48), 699 (25), 683 (100), 665 (48); HR-FABMS (*m*/*z*) 905.3373 $[(M + Na)^+$, calcd for $C_{39}H_{64}O_{15}S_2Na_3$ 905.3380]; NMR data, see Table 1.

Hydrolysis of 1 to 2 and Acetylation to 3. After overnight reflux of **1** (10 mg) in 6 N HCl (5 mL), the cooled solution was extracted with EtOAc (3 × 10 mL). The dried organic solution (anhydrous Na_2SO_4) was evaporated and chromotographed on Si gel. The fraction that came out with EtOAc−hexane, 8:2 (compound **2**) (5 mg), was acetylated overnight at room temperature with Ac_2O−pyridine, 1:1 (1 mL). The solvent was then evaporated and the residue chromatographed on Si gel to yield with EtOAc−hexane, 1:4, compound **3** (2.5 mg) as an oil: $[\alpha]_D$ +33° (*c* 0.15, MeOH); IR (neat) 2900, 1730 cm^{-1}; CIMS *m*/*z* (%) 675 $[MH]^+$ (100), 647 (20), 615 $[M - HOAc]^+$ (55), 587 $[M - C_4H_7O_2]$ (98); FABMS *m*/*z* (%) 697 $[M + Na]^+$ (100), 587 (60); NMR data see Table 2.

Acknowledgment. The authors acknowledge Dr. R. van Soest, Zoological Museum, Amsterdam, for the identification of the sponge.

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NP010121S