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WEINBERSTEROL DISULFATES A AND B, ANTIVIRAL STEROID SULFATES FROM THE SPONGE *PETROSIA WEINBERGI*

Hao H Sun[1]*, Sue S. Cross, Malika Gunasekera and Frank E Koehn
Division of Biomedical Marine Research
Harbor Branch Oceanographic Institution, Inc
5600 Old Dixie Highway, Fort Pierce, Florida 34946, USA

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Abstract - Weinbersterol disulfates A (**1**) and B (**2**), two new sulfated tetrahydroxy steroids with an unprecedented cyclopropane-containing side chain, were isolated from the sponge *Petrosia weinbergi*. Both compounds are active in vitro against feline leukemia virus, and **1** is also active in vitro against HIV The structures of these two compounds were assigned mainly on the basis of spectral data.

Sulfate esters of polyhydroxylated sterol are uncommon among marine sponges [2-5] In the course of our screening program for new bioactive agents from marine organisms, we have isolated two novel antiviral tetrahydroxylated steroid disulfates, designated as weinbersterol disulfates A (**1**) and B (**2**), from the sponge *Petrosia weinbergi* Van Soest (order Haplosclerida, family Petrosidae) Both compounds possess an unprecedented cyclopropane-containing side chain, showing one more example for the diversity in the side chain structures of sponge sterols [6-8] It was found that weinbersterol disulfates A and B exhibited in vitro activity (EC_{50} = 4 0 and 5 2 μg/ml, respectively) against the feline leukemia virus (FeLV)[9], and **1** also showed activity against the human immunodeficiency virus (EC_{50} = 1 0 μg/ml)[10]

The extract of the sponge *Petrosia weinbergi* collected off Acklin Island, Bahamas was found to be active in antiviral screening against a panel of viruses Solvent partitioning of the extract and centrifugal countercurrent chromatography of the active partition, followed by reversed-phase HPLC yielded weinbersterol disulfates A (**1**) and B (**2**)[11] The molecular formula of **1** was determined as $C_{30}H_{50}O_{10}S_2Na_2$ on the basis of high resolution FAB mass measurement of the molecular ion species at m/z 703 2578 (major) and 681 2733 (minor) corresponding to $[M+Na]^+$ and $[M+H]^+$, respectively An intense fragmentation ion at m/z 601 3141 was interpreted as loss of $NaSO_3(+H)$ from $[M+Na]^+$ EIMS failed to show the molecular ion but exhibited the ions at m/z 422 and 404 corresponding to $[M-2NaHSO_4-H_2O]^+$and $[M-2NaHSO_4-2H_2O]^+$, respectively, suggesting the presence of two sulfoxyl and two hydroxyl groups The IR spectrum exhibited strong absorptions at 1235 and 1215 cm^{-1}, characteristic of sulfates The ^{13}C NMR spectrum and DEPT measurement of **1** (Table I) indicated the presence of 30 carbons including six methyls, ten methylenes, ten methines and four quaternary carbons, among which three methines (δ 74 4, 75 9, 76 3) and one quaternary carbon (δ 78 1) are oxygen-bearing Its ^1H NMR spectrum (Table I) supported the existence of three oxygenated methines (δ 4 52, 4 71, 4 74) The presence of a methylcyclopropane feature was evident from the couplings of a methine proton at δ 0 68 (m) to two geminal-coupled methylene protons at δ -0 20 (dd, J=5 5, 4 2 Hz) and 0 48 (dd, J=8 6, 4 2 Hz), and to a methyl at δ 1.10 (d, J=6 7 Hz) Two other methyl doublets at

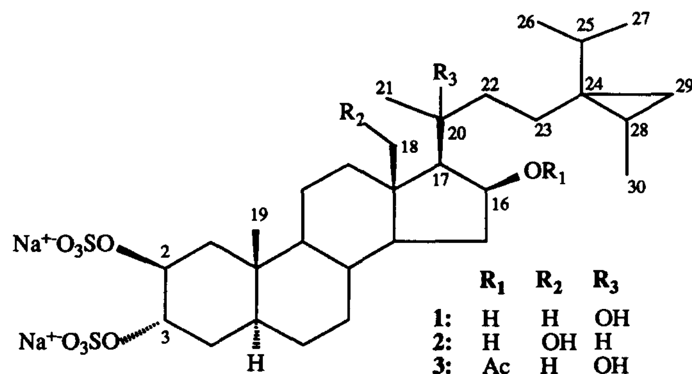


Table I ^{13}C and ^{1}H NMR Assignments of Weinbersterol Disulfates A (1) and B (2)[a]

	1		2	
C#	^{13}C δ	^{1}H δ (m, J Hz)	^{13}C δ	^{1}H δ (m, J Hz)
1	39 1	1 40, 2 02	39 0	1 37, 2 05
2	76 3	4 74 (br m, $W^1/2$=8 Hz)	76 4	4 71 (br m, $W^1/2$=8 Hz)
3	75 9	4 71 (br m, $W^1/2$=8 Hz)	76 1	4.69 (br m, $W^1/2$=8 Hz)
4	30 4	1 57, 1 77	30 5	1 56, 1 77
5	40 1	1 53	40 3	1 52
6	29 0	1 23	29 1	1 25
7	32 9	0 91, 1 67	33 2	0 95, 1 71
8	35 3	1 50	35 6	1 65
9	56 5	0 84	56 9	0 76
10	36 3	-	36 5	-
11	21 6	1 42	22 4	1 46
12	41 8	1 13, 2 07	38 8	1 06, 1 96
13	44 2	-	47 5	-
14	55 6	0 69	55 0	1 10
15	38 0	1 27, 2 18 (ddd, 12 9, 7 8 7 8)	38 5	1 29, 2 15 (ddd, 12 9, 7 8, 7 8)
16	74 4	4 52 (br ddd, 7 8, 7 8, 4 5)	72 8	4 22 (br ddd, 7 8, 7 8, 4 5)
17	60 9	1 15	62 1	1 14
18	15 4	1 09 (s)	62 7	3 55 (d, 11 5), 3 94 (d, 11 5)
19	14 2	0 99 (s)	14 1	0 96 (s)
20	78 1	-	32 3	1 77
21	26 3	1 18 (s)	19 1	0 95 (d, 6 5)
22	42 5	1 52, 1 72	33 9	1 03, 1 51
23	28 5	1 26, 1 37	30 1	1 15, 1 45
24	28 7	-	28 8	-
25	33 0	1 30	33 1	1 26
26	20 9	1 00 (d, 7 0)	20 8	0 95 (d, 7 0)
27	20 8	1 03 (d, 7 0)	20 7	1 02 (d, 7 0)
28	19 1	0 68 (m)	18 7	0 69 (m)
29	20 6	-0 20 (dd, 5 2, 4 2), 0 48 (dd, 8 6, 4 2)	20 3	-0 24 (dd, 5 5, 4 2), 0 46 (dd, 8 6, 4 2)
30	14 1	1 10 (d, 6 7)	14 3	1 08 (d, 6 7)

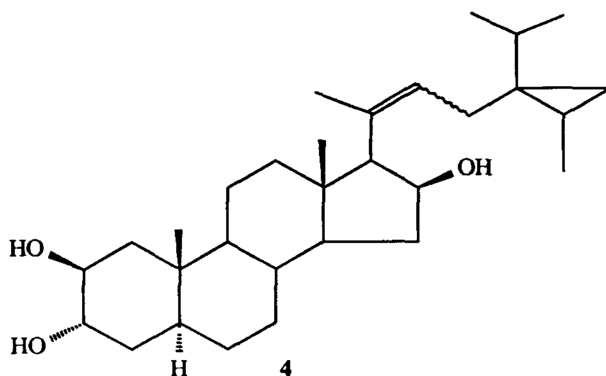
[a]Recorded in CD_3OD, where ^{1}H multiplicity not specified, ^{1}H δ deduced from crosspeaks in HETCOR spectrum

δ 1.00, 1 03 and three methyl singlets at δ 0.99, 1 09 and 1 18 were also observed in the ^{1}H NMR spectrum A 2D INADEQUATE NMR experiment performed on a 150 mg sample of **1** and in conjunction with its HMBC, HETCOR, COLOC and COSY data (Table II) established the complete connectivity for the molecule This led us to propose for **1**, a 2,3,16,20-tetraoxygenated steroidal structure with an unusual methylisopropyl cyclopropane group at the terminus of the side chain.

Treatment of compound **1** with acetic anhydride/pyridine afforded the monoacetate **3**, and analysis of the ^{1}H NMR chemical shifts for the C-16 methine suggested esterification at C-16. Solvolysis of **1** in pyridine/dioxane at 140°C for 18 hours[12] yielded the desulfated derivative **4** The ^{1}H and ^{13}C NMR spectra of **4** revealed the desulfurization occurring at the C-2 and C-3 oxygens, dehydroxylation at C-20 and C-22 occurred as well Therefore, the positions of sulfoxyl groups at C-2 and C-3, and hydroxyl groups at C-16 and C-20 were established.

Table II 2D INADEQUATE, HMBC, and COLOC NMR Data of Weinbersterol Disulfate A (1)

C#	INADEQUATE Coupled C#	HMBC Coupled H#	COLOC Coupled H#	C#	INADEQUATE Coupled C#	HMBC Coupled H#	COLOC Coupled H#
1	10	3,19	19	16	17		15
2		1,3	1,4	17	13,16,20	15,18,21	18,21
3	4	1,2	1,4	18	13		
4	3,5			19	10		
5	4,6,10	1,3,7,19	1,7,19	20	17	21,22	21,22
6	5,7	5		21			
7	6,8			22	23		21
8	7,9,14			23	22	22,28,29	
9	8,10,11	19	7,12,19	24	29	22,28,29	
10	1,5,9,19	1,2	1,4,19	25	26,27	29	26
11	9			26	25		27
12	13	11,18	18	27	25		26
13	12,14,17,18	15,18	18	28		29	29,30
14	8,13,15	12,18	12,18	29	24		30
15	14			30		29	29



Comparison of the ^{13}C NMR shifts of 1 with those published for halistanol sulfate[2] and 5α-cholestane-2β,3α,26-triylsulfate[13] suggested the existence of trans diaxial disulfate (2β,3α) and trans AB ring juncture (5α-H) Furthermore, the ^{13}C shifts for each carbon on ring A in the desulfated derivative 4 was also in agreement with that published for 5α-spirostane-2β,3α-diol[14], and differed from those reported for the corresponding 2α,3α- and 2α,3β-diols[14-16]. ^{1}H NMR data for 1 revealed a typical α-H_{16} at δ 4 52 (br ddd)[15-18], which was coupled to $H_{17\alpha}$, $H_{15\alpha}$, and $H_{15\beta}$ with couplings of 7.8, 7 8 and 4.5 Hz, respectively The stereochemistry of the remaining 3 chiral centers, C-20, C-24 and C-28, was not assigned We have not attempted to establish the stereochemistry of C-20 and were unable to establish the relative stereochemistry of C-24 and C-28 of the cyclopropyl ring by performing a series of difference NOE experiments. Irradiation of CH_3-26, CH_3-27, CH_3-30, CH-28, CH_2-29 gave no detectable NOEs on the adjacent protons

High resolution FABMS measurement and ^{13}C NMR data established the formula of $C_{30}H_{50}O_{10}S_2Na_2$ for weinbersterol disulfate B (2) By ^{1}H and ^{13}C NMR analyses (Table I), including COSY, HETCOR, HMBC data, 2 appeared to have the 2β,3α,16β-triol, 2β,3α-disulfate structure in a 5α-H steroid, and to possess the terminal methylisopropyl cyclopropane group in the side chain, analogous to 1. However, the position of the fourth hydroxyl group is different from that in 1 In the ^{1}H NMR spectrum of 2, the C-18 and C-21 methyl singlets which were present in 1, had disappeared, while a methyl doublet at δ 0 95 (J=6 5 Hz) and two geminal-coupled hydroxymethylene doublets at δ 3 55 (J=11 5 Hz) and 3 94 (J=11 5 Hz) were observed instead Each of these protons were found to have cross peaks with C-17 at δ 62 1 in the HMBC spectrum Correlations were also observed from the two hydroxymethylene protons to C-13 (δ 47 5) and C-14 (δ 55 0) We thus placed the fourth hydroxy at C-18 and established the structure of weinbersterol disulfate as 2

EXPERIMENTAL

ISOLATION OF WEINBERSTEROL DISULFATES A (1) AND B (2) - The sponge *Petrosia weinbergi* Van Soest[19] (943 g) was collected on June 17, 1985 at a depth of 100 feet off the Southwestern tip of Acklin Island, Bahamas and stored frozen A taxonomic voucher specimen was deposited at Harbor Branch Oceanographic Institution, Inc , Indian River Coastal Zone Museum, catalog number 003 00044 (DBMR No 17-VI-85-1-14) A small portion (1g) of the sponge was homogenized with methanol, and the resulting extract was found to be active against FeLV Isolation of the active components was performed by following FeLV activity The sponge was (870 g) extracted with 1 1 methanol-chloroform After evaporation of the organic solvents under reduced pressure, the aqueous suspension was sequentially extracted with ethyl acetate, 1 1 ethyl acetate-n-butanol, and n-butanol The latter two active extracts (2.5 and 6 3 g, respectively) were pooled and a portion (2 0 g) of the combined extract was fractionated on an Ito high speed countercurrent chromatograph ($CHCl_3$-i-PrOH-MeOH-H_2O, 9 2 12 10, lower phase stationary) to yield 60 fractions Of these, three successive active fractions were rechromatographed on a reversed-phase C-18 HPLC column (MeOH-H_2O-$CHCl_3$, 60 40 5) to yield two active components, weinbersterol disulfates A (1) and B (2) After repeating the chromatographic procedures described above three more times, 170 mg of 1 and 50 mg of 2 were obtained Both compounds were recrystallized as white opaque needles (unsuitable for X-ray analysis) from MeOH-H_2O-$CHCl_3$ (60 40 5)

1 $[\alpha]_D$=+20° (c=1 75, MeOH), m.p. 182°C; HRFABMS: $[M+Na]^+$ 703.2578 (calcd for $C_{20}H_{50}O_{10}S_2Na_2$+Na, Δ - 3.9 mmu), $[MH]^+$ 681.2733 (calcd for $C_{20}H_{50}O_{10}S_2Na_2$+H, Δ - 1 4 mmu), $[M+Na-NaSO_3+H]^+$ 601.3141 (calcd for $C_{30}H_{51}O_7SNa$+Na, Δ 1.0 mmu); LREIMS (rel %) $[M-2NaHSO_4-H_2O]^+$ 422(4), 407(3), 404(2), 379(5), 360(3), 295(6), 271(20), 253(16), 211 (6), 193(6), 161(13), 157(15), 147(17), 133(16), 119(20), 105(28), 91(30), 81(23), and 64(100); IR (KBr): 3425, 2930, 1620, 1440, 1375, 1235, 1215, 1060, 970, 940, 880, and 785 cm^{-1}; ^1H & ^{13}C NMR. Table I

2 $[\alpha]_D$=+32° (c=0.10, MeOH); m.p. 191°C; HRFABMS: $[M+Na]^+$ 703 2570 (calcd for $C_{30}H_{50}O_{10}S_2Na_2$+Na, Δ 4.0 mmu); LREIMS (rel. %)· 441(4), 422(55), 410(62), 404(36), 392(21), 379(16), 310(48), 297(100), 285(71), 269(47), 257(29), 187(28), 173(28), 157(42), 145(42), 145(51), 133(56), 119(58), 105(90), and 91 (82), IR (KBr): 3450, 2930, 2860, 1630, 1450, 1380, 1240, 1220, 1065, 975, 935, 880, 790, and 745 cm^{-1}, ^1H & ^{13}C NMR· Table I.

ACETYLATION OF 1 A solution of 1 (7 mg) in pyridine (1 ml) and acetic anhydride (0.5 ml) was stirred at room temperature overnight. After addition of H_2O, the solution was evaporated to dryness under reduced pressure The residue was chromatographed by HPLC with an ODS column (60 40 5 MeOH H_2O $CHCl_3$) to yield the acetylated product 3 (5 mg). HRFABMS. $[M+Na]^+$ 745 2665 (calcd for $C_{32}H_{52}O_{11}S_2Na_2$+Na, Δ - 2 1 mmu), $[M+H]^+$ 723 2850 (calcd for $C_{32}H_{52}O_{11}S_2Na_2$+H, Δ - 2 5 mmu); LREIMS (rel %). 482(5), 464(3), 439(2), 422(16), 404(15), 379(21), 361(12), 337(3), 323(6), 297(13), 271(20), 253(22), 191(18), 147(50), 133(39), 119(52), 105(67), 91(63), 81(47), 64(100), 55(53); ^{13}C NMR (CD_3OD) δ 14.0 (C-30), 14 3 (C-19), 15 7 (C-18), 19 4 (C-28), 20 8 (C-27), 20.9 (C-29), 21 0 (C-26), 21.6 (OAc), 21 7 (C-11), 26 4 (C-21), 28 3 (C-23 and C-24), 29 0 (C-6), 30.4 (C-4), 32 8 (C-7), 33.1 (C-25), 35.3 (C-8), 35.7 (C-15), 36 5 (C-10), 39 0 (C-1), 40.1 (C-5), 41 6 (C-12), 42 6 (C-22), 44 5 (C-13), 55.7 (C-14), 56 4 (C-9), 62 9 (C-17), 76 1 (C-16), 76 4 (C-3), 76 7 (C-2), 78.4 (C-20) and 171 7 (OAc), ^1H NMR (CD_3OD): δ -0 20 (1H, dd, J=5 2, 4 2 Hz, H-29), 0 45 (1H, dd, J=8 6, 4.2 Hz, H-29), 0.64 (1H, m, H-28), 0.74 (1H, m), 0 97 (3H, d, J=7 0 Hz, CH_3-26), 1 00 (3H, s, CH_3-19), 1 01 (3H, d, J=7.0 Hz, CH_3-27), 1 08 (3H, s, CH_3-18), 1 09 (3H, d, J=6 4 Hz, CH_3-30), 1 23 (3H, s, CH_3-21), 1 2-1 8 (m), 2.05 (3H, s, 16-OAc), 2 08 (m), 2 35 (1H, m, Hα-15), 4 72 (1H, m, H-3), 4 76 (1H, m, H-2), and 5 34 (1H, ddd, J=7 8, 7.8, 5 4 Hz, H-16).

SOLVOLYSIS OF 1 A solution of 1 (10 mg) in pyridine (0 5 ml) and dioxane (0 5 ml) was heated at 140°C for 18 h in a stoppered reaction vial After the solution cooled, H_2O (1 ml) was added, and the solution was extracted with n-BuOH (2x2 ml). The extract was evaporated to dryness under reduced pressure, and then chromatographed by HPLC with an ODS column (90 10 1 MeOH.H_2O $CHCl_3$) to yield the desulfated product 4 (0.5 mg). HRFABMS: $[M+Na]^+$ 481.3692 (calcd for $C_{30}H_{50}O_3$+Na, Δ 3.3 mmu), LRFABMS (rel %) $[M+Na]^+$ 481 (13), $[M+H]^+$ 459 (15), 441 (51), 391 (18), 342 (20), 317 (21), 307 (100), 289 (78), 273 (18), 220 (18), 186 (22) and 176 (44), ^{13}C NMR ($CDCl_3$). δ 14.4 (C-19 and C-30), 15 3 (C-18), 19.2 (C-28), 19 8 (C-29), 20 0 (C-27), 20.1 (C-26), 21 5 (C-11), 27 9, 29 3 (C-6), 30 1 (C-4), 31 4, 31 5, 31 7, 34 1, 35 4 (C-8), 35 7 (C-10), 38 7, 38 9 (C-1), 40 4(C-5), 42 7 (C-12), 44 5 (C-13), 54 6 (C-14), 55 4 (C-9), 64 8 (C-12), 70 6 (C-3), 71 8 (C-2), 72 2 (C-16), 127 7 (C-22), and 132 4 (C-20), ^1H NMR ($CDCl_3$) δ -0 25 (1H, dd, J=5 3, 4 2 Hz, H-29), 0 42 (1H, dd, J=8 7, 4 2 Hz, H-29), 0 63 (1H, m, H-28), 0 7-0 9 (m), 0 91 (3H, s, CH_3-19), 0 94 (3H, d, J=6 8 Hz, CH_3-26), 0 97 (3H, s, CH_3-18), 0 97 (3H, d, J=6 7 Hz, CH_3-27), 1 05 (3H, d, J=6 3 Hz, CH_3-30), 1 1-1 9 (m), 1 63 (3H, s, CH_3-21), 2 10 (1H, d, J=7 0 Hz, H-17), 2 19 (1H, ddd, J=12 8, 8 0, 7.2 Hz, Hα-15), 3 84 (1H, m, H-3), 3 86 (1H, m, H-2), 4 25 (1H, br ddd, J=8 0, 7 0, 5 3 Hz, H-16), and 5 31 (1H, t, J=5 5 Hz, H-22)

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