Sarcochromenol Sulfates A-C and Sarcohydroquinone Sulfates A-C, New Natural Products from the Sponge Sarcotragus spinulosus

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SARCOCHROMENOL SULFATES A–C AND SARCOHYDROQUINONE SULFATES A–C, NEW NATURAL PRODUCTS FROM THE SPONGE *SARCOTRAGUS SPINULOSUS*

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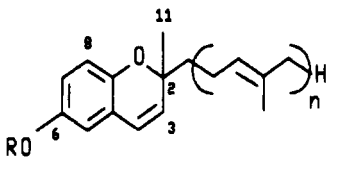
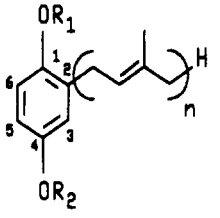
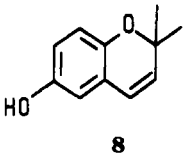
ABSTRACT.—New Na^+,K^+-ATPase inhibitors, sarcochromenol sulfates A [**1**], B [**2**], and C [**3**] and sarcohydroquinone sulfates A [**4**], B [**5**], and C [**6**], have been isolated from the sponge *Sarcotragus spinulosus*. Four of them (**1**, **4–6**) and all six corresponding acetates **1a–6a** have been isolated, and their structures have been established by spectral methods and chemical transformations.

Prenylated hydroquinones and related secondary metabolites have previously been isolated from sponges (1–3) and urochordates (4,5). Some of them show protection against tumor development in test animals (4,5). Recently the first sulfated derivative **7** of this series was reported from the sponge *Dysidea* sp. (6). In the course of our search for bioactive metabolites from marine invertebrates (7), we found that extracts of the sponge *Sarcotragus spinulosus* (Schmidt) (family Thorectidae, order Dictyoceratida, class Demospongiae), collected in the Pacific Ocean between Norfolk Island and New Zealand, inhibited Na^+,K^+-ATPase (8). Bioassay-guided fractionation of the crude extract led to the isolation of a fraction containing new sulfated metabolites **1–6** as active components. Compound **1** proved to be an isomer of **7**.

An EtOH extract of the lyophilized sponge was partitioned between 90% EtOH and hexane and then with $CHCl_3$. The $CHCl_3$-soluble material (4.2 g) was chromatographed on Silpearl and Sephadex LH-20 columns to give an active fraction (0.321 g). It was established that this fraction consisted of sulfated derivatives by ir spectroscopy (band at 1220 cm^{-1}) and transformation to less polar compounds after solvolysis with pyridine/dioxane (90°, 1 h) (9). The fraction was separated into two parts. One part (0.17 g) was desulfated by solvolysis, then acetylated and subjected to cc on Si gel. The rest of the material was used for the direct isolation of active principles. Two subfractions (SF-1 and SF-2) obtained from the first part were separated by hplc (Zorbax-ODS, 95% aqueous Me_2CO) to give six derivatives: chromenol acetates **1a–3a** from SF-1 and hydroquinone acetates **4a–6a** from SF-2. Compounds **1a–3a** have similar uv and nmr







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1	R=SO_3Na, n=5	**4**	R_1=H, R_2=SO_3Na, n=6
1a	R=Ac, n=5	**4a**	R_1=R_2=Ac, n=6
2	R=SO_3Na, n=6	**5**	R_1=R_2=SO_3Na, n=7
2a	R=Ac, n=6	**5a**	R_1=R_2=Ac, n=7
3	R=SO_3Na, n=7	**6**	R_1=H, R_2=SO_3Na, n=8
3a	R=Ac, n=7	**6a**	R_1=R_2=Ac, n=8
		7	R_1=SO_3Na, R_2=H, n=6
		9	R_1=R_2=H, n=3-7
		10	R_1=Me, R_2=SO_3Na, n=7

spectra, and the presence of 2-substituted 2-methylchromenol moieties and all-trans-polyprenylic side chains was recognized from these data (5,10,11). The acetates differ from each other in their physical constants, chromatographic behavior and eims: **1a** *m/z* 558 $[M]^+$, **2a** *m/z* 626 $[M]^+$, **3a** *m/z* 694 $[M]^+$. These mass spectra are consistent with structures for **1a–3a**, having five, six, and seven isoprene units in their side chains, respectively. Uv as well as nmr spectra of compounds **4a–6a** are also very similar to each other. These data were indicative of all-trans-polyprenylic side chains, connected with a hydroquinone moiety (1,6). The acetates show the following eims data: *m/z* **4a** 602 $[M]^+$; **5a** 670 $[M]^+$; **6a** 738 $[M]^+$. Thus it was established that **4a–6a** have six, seven, and eight isoprene units in their side chains, respectively.

Sarcochromenol sulfate A [**1**] and sarcohydroquinone sulfates **4–6**, corresponding to **1a** and **4a–6a**, respectively, were obtained from the other part of the active fraction by repeated hplc on a Zorbax-ODS column (65% aqueous Me_2CO).

Based on an $[M - Na]^-$ peak at *m/z* 595 in the fabms of **1**, it was concluded that the sodium *O*-sulfate fragment was present in **1** at C-6, and this was confirmed by conversion of **1** into **1a** by solvolysis and acetylation. Moreover, comparison of ^{1}H-nmr spectra of **1** and 2,2-dimethylchromenol [**8**] (5) showed the main difference between these spectra to be the chemical shifts of H-5 and H-7 due to the location of a functional group at C-6 in **1** (Table 1).

Compounds **2** and **3** were isolated only in admixture with compounds **5** and **6**, respectively. In their fabms $[M - Na]^-$ ions were observed at *m/z* 663 and 731.

Metabolites **4–6** were then studied by fabms and nmr as well as transformed into **4a–6a** as described above. $[M - Na]^-$ peaks at *m/z* 597, 665, and 733 for fabms of **4–6**, respectively, indicated these metabolites to be hexa-, hepta- and octaprenylated hydroquinone sodium monosulfates.

Hexaprenylhydroquinone sulfate [**7**] has recently been isolated from the sponge

TABLE 1. Nmr Spectra of Aromatic Moieties of Sarcochromenol Sulfate A [**1**], Sarcohydroquinone Sulfates A [**4**], B[**5**], and C [**6**], Sulfate Derivative **7** (6), and 2,2-Dimethylchromenol [**8**] (5), 2-Polyprenylhydroquinones (general formula **9**) as Model Compounds.

Position	Compound				
	1	**8**	**4–6**	**7**	**9**
1			146.2	142.8	148.9
2			130.9	120.0	131.2 6.46 m
3	5.65 d, J = 9.5 Hz	5.47 d, J = 10.0 Hz	119.6, 7.00 d, J = 3.0 Hz	116.8, 6.65, J = 3.0 Hz	113.8, 6.46 m
4	6.37 d, J = 9.5 Hz	6.12 d, J = 10.0 Hz	148.2	153.7	150.9
5	6.94 d, J = 3.0 Hz	6.57 d, J = 2.0 Hz	120.5, 6.94 dd, J = 8.5, 3.0 Hz	113.6, 6.55 dd, J = 8.0, 3.0 Hz	116.5, 6.46 m
6			122.7, 6.67 d, J = 8.5 Hz	131.2, 7.19 d, J = 8.0 Hz	117.3, 6.46 m
7	7.02 dd, J = 3.0, 8.5 Hz	6.50 bs			
8	6.66 d, J = 8.5 Hz	6.50 bs			
11	1.35 s	1.33 s			

Dysidea sp. (6). The nmr spectra of this material differ from the corresponding spectra of compounds **4–6**, although their uv spectra were almost identical (λ max at 283 nm for **4–6** and λ max 281 nm for **7**).

It was proposed on the basis of chemical shift analysis of ^{1}H and ^{13}C signals of the aromatic moiety that the *O*-sulfate group in sarcohydroquinones is located at C-4. In fact, comparison of nmr spectra for compounds **4–6**, 1-*O*-sulfated metabolite **7** (6), and nonsulfated 2-polyprenyhydroquinones (1,11,12) showed that in the ^{13}C-nmr spectra of 2-polyprenylhydroquinone derivatives the signal of C-2 is more sensitive to sulfation at C-1 than other signals. It was shifted from δ 131.2 to 120.0 in the spectrum of **7**, while in the spectra of **4–6** this signal was observed at almost the same position when compared with nonsulfated hydroquinone derivatives. Signals for H-3, H-5, and H-6 are easily assayed in these spectra. Therefore shifts of H-3 and H-5 (+0.54 and +0.48, respectively) signals in the ^{1}H-nmr spectra of **4–6** as well as the shift of the H-6 signal (+0.73) in the spectrum of **7** when these spectra were compared with the spectra of nonsulfated derivatives **9** indicate a 4-*O*-sulfate moiety in **4–6** and a 1-*O*-sulfate fragment in **7** (Table 1).

Compound **5** was treated with MeI/K_2CO_3 in Me_2CO to confirm the C-4 position of sulfate groups in sarcohydroquinones. An nOe experiment with the resulting methyl ether **10** enhanced the ortho proton at C-6 (6.84 ppm) after irradiation of OMe signal at 3.8 ppm, indicating that the sulfate must be placed at C-4.

Thus, structures of sarcochromenol sulfates A–C have been established as 6-sulfates of 2-(pentaprenylmethyl)-, 2-(hexaprenylmethyl)-, and 2-(heptaprenylmethyl)-2-methylchromenes. Sarcohydroquinone sulfates A–C have been shown to be 4-sulfates of 2-hexa-, 2-hepta-, and 2-octaprenylhydroquinones.

Sulfated metabolites **1** and **4–6** inhibit the activity of Na^+,K^+-ATPase from the rat brain with IC_{50} 1.6×10^{-6}, 1.6×10^{-6}, 1.4×10^{-6}, and 1.3×10^{-6} M, respectively.

It is interesting that sulfatases with different substrate specificity can be present in sponges. Thus one enzyme in *Dysidea* sp. (6) sulfates the hydroxyl at C-1 of hydroquinone derivatives and another enzyme in *S. spinulosus* sulfates the hydroxyl at C-4.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) and optical rotations ($[\alpha]_{578}$) were determined on a Boethius and a Perkin-Elmer 141 apparatus, respectively. Combustion analyses were carried out on a Perkin-Elmer 240 instrument. The ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WM-250 spectrometer in CD_3OD (for **1–6**) and $CDCl_3$ (for **1a–6a**) with TMS as internal standard. Mass spectra under electron impact (70 eV) and fast atom bombardment conditions (registration of negative ions, +7.5 kV) were measured on an LKB 2091 spectrometer. Ir spectra were recorded on a Specord 75 IR spectrometer in $CHCl_3$ or KBr. Uv spectra were recorded for EtOH or hexane solutions on a Specord M-40 spectrophotometer. Preparative hplc was conducted utilizing a DuPont 8800 instrument equipped with a differential refractometer RIDK-102, 1.5 ml/min. Semi-preparative columns with Zorbax ODS (25 cm × 4.6 mm) were used. Tlc and low pressure liquid chromatography were performed on Si gel L (Chemapol, Czechoslovakia) 5/40 μ and 40/100 μ, respectively. Visualization of spots on tlc was with a solution of EtOH (50 ml) in concentrated H_2SO_4 (70 ml), 100°, 5 min.

ISOLATION OF SARCOCHROMENOL SULFATES, SARCOHYDROQUINONE SULFATES, AND THEIR ACETATES.—*S. spinulosus* was collected in September 1988 at a depth of 400 m in the Tasmanian Sea (32°27′S, 167°36′E) by dredging. A voucher specimen (no. 07-293) was lodged at the Zoological Museum, Zoological Institute of the USSR Academy of Sciences, St. Petersburg. The sponge (500 g dry wt) was lyophilized, cut into small pieces, and extracted with EtOH (2 × 2.5 liters). The combined EtOH extracts were filtered, concentrated under vacuum, and partitioned between hexane (3 × 400 ml) and 90% aqueous EtOH (400 ml). The EtOH layer was separated, diluted with H_2O (100 ml), and partitioned with $CHCl_3$ (3 × 400 ml). The $CHCl_3$ extracts were concentrated to a dark brown oil (4.2 g). The $CHCl_3$-soluble materials (4.2 g) were dissolved in a minimum volume of $CHCl_3$-EtOH (3:1) and chromatographed on a column of Si gel and then of Sephadex LH-20 in the same system. Active material (0.321 g) was obtained,

part of which (0.17 g) was desulfated by solvolysis in dioxane-pyridine (1:1) (2 ml), 90°, 1 h (9) and acetylated by Ac_2O-pyridine mixture (1:1) (2 ml), 25°, 3 h. Subfractions SF-1 (44 mg) and SF-2 (99 mg) were obtained after concentration under vacuum and flash chromatography on a column of Si gel with hexane-EtOAc (20:1). Further hplc (Zorbax ODS, 95% aqueous Me_2CO) gave **1a–3a** from SF-1 and **4a–6a** from SF-2.

The other portion of active material (0.151 g) was used for direct separation of sarcochromenol sulfate A [**1**] and sarcohydroquinone sulfates A [**4**], B [**5**], and C [**6**] by hplc (Zorbax ODS, 65% aqueous Me_2CO).

Sarcochromenol A acetate [**1a**].—Compound **1a** (15.0 mg): mp 25–26° (EtOH); relative retention time (rrt) = 1.0; $[\alpha]_{578}$ −39.2° (*c* = 1.3, $CHCl_3$), uv λ max (hexane) 253 (ε 3370), 266 (ε 3670), 285 (ε 1120), 319 (ε 3160); eims *m/z* (rel. int.) 558 (3.8), 543 (2.3), 489 (1.5), 421 (1), 353 (1), 285 (2), 203 (51); ^{1}H nmr ($CDCl_3$) 5.60 (d, 9.5, H-3), 6.30 (d, 9.5, H-4), 6.75 (m, H-5, -7, -8), 1.40 (s, Me-11), 5.12 (m, 5 × CH=), 1.58 (s, Me), 1.60 (s, Me), 1.69 (s, 4 × Me), 2.28 (s, OAc).

Sarcochromenol B acetate [**2a**].—Compound **2a** (14.5 mg): colorless oil; rrt = 1.26; $[\alpha]_{578}$ −21.7° (*c* = 0.23, $CHCl_3$); uv λ max (hexane) 252 (ε 2000), 266 (ε 2300), 286 (ε 730), 319 (ε 2000); eims *m/z* (rel. int.) 626 (5%), 611 (1.3), 557 (0.3), 489 (1), 421 (1), 353 (5), 285 (6), 203 (100); ^{1}H nmr ($CDCl_3$) 5.60 (d, 9.5, H-3), 6.30 (d, 9.56, H-4), 6.75 (m, H-5, -7, -8), 1.40 (s, Me-11), 5.12 (m, 6 × CH=), 1.58 (s, Me), 1.60 (s, Me), 1.69 (s, 5 × Me), 2.28 (s, OAc).

Sarcochromenol C acetate [**3a**].—Compound **3a** (3.4 mg): colorless oil; rrt = 1.68; $[\alpha]_{578}$ −5° (*c* = 0.1, $CHCl_3$); uv λ max (hexane) 253 (ε 2800), 266 (ε 3000), 285 (ε 850), 319 (ε 2700); eims *m/z* (rel. int.) 694 (14%), 679 (7), 625 (4), 557 (6), 489 (7), 421 (1.5), 353 (25), 285 (30), 203 (100); ^{1}H nmr ($CDCl_3$) 5.60 (d, 9.5, H-3), 6.30 (d, 9.5, H-4), 6.75 (m, H-5, -7, -8), 1.40 (s, Me-11), 5.12 (m, 7 × CH=), 1.58 (s, Me), 1.60 (s, Me), 1.69 (s, 6 × Me), 2.28 (s, OAc).

Sarcohydroquinone A acetate [**4a**].—Compound **4a** (16.6 mg): colorless oil; rrt = 1.00; uv λ max (hexane) 259 (ε 870), 269 (ε 940); eims *m/z* (rel. int.) 602 (6), 687 (2.5), 533 (9), 465 (18), 423 (1), 397 (25), 329 (7), 261 (5), 203 (100), 191 (70), 189 (80); ^{1}H nmr ($CHCl_3$) 7.00 (m, H-3, -5, -6), 5.23 (t, 7, *HC*=CH_2-Ar), 5.12 (t, 7, 5 × CH=), 3.23 (d, 7, CH_2-Ar), 2.04 (m, 10 × CH_2), 1.60 (s, 5 × Me), 1.67 (s, 2 × Me), 2.28 (s, OAc), 2.31 (s, OAc).

Sarcohydroquinone B acetate [**5a**].—Compound **5a** (20.2 mg): mp 37–38° (EtOH); rrt = 1.26; uv λ max (hexane) 257 (ε 660), 268 (ε 820); eims *m/z* (rel. int.) 670 (16), 655 (1), 601 (9), 533 (17), 465 (48), 397 (52), 329 (11), 261 (14), 203 (100), 191 (56), 189 (60); ^{1}H nmr ($CHCl_3$) 7.00 (m, H-3, -5, -6), 5.23 (t, 7, *HC* = CH_2-Ar), 5.12 (t, 7, 6 × CH=), 3.23 (d, 7, CH_2-Ar), 2.04 (m, 12 × CH_2), 1.60 (s, 6 × Me), 1.67 (s, 2 × Me), 2.28 (s, OAc), 2.31 (s, OAc).

Sarcohydroquinone C acetate [**6a**].—Compound **6a** (31.4 mg): mp 45–46° (EtOH); rrt = 1.68; uv λ max (hexane) 255 (ε 800), 267 (ε 1000); eims *m/z* (rel. int.) 738 (11%), 723 (1), 669 (6), 601 (11), 533 (15), 465 (25), 397 (26), 329 (10), 261 (5), 203 (100), 191 (100), 189 (50); ^{1}H nmr ($CHCl_3$) 7.00 (m, H-3, -5, -6), 5.23 (t, 7, *HC* = CH_2-Ar), 5.12 (t, 7, 7 × CH=), 3.23 (d, 7, CH_2-Ar), 2.04 (m, 14 × CH_2), 1.60 (s, 7 × CH_3), 1.67 (s, 2 × CH_3), 2.28 (s, OAc), 2.31 (s, OAc); ^{13}C nmr ($CDCl_3$) 148.2 s (C-4), 146.2 s (C-1), 130.9 s (C-2), 122.5 d (C-6), 120.7 d (C-5), 119.7 d (C-3), 137.5 s, 135.1 s, 134.8 s (6 × =C<), 124.4 d, 124.3 d, 123.9 d, 122.8 d (6 × C=), 39.8 t, 26.8 t, 25.6 t (10 × CH_2), 28.7 t (CH_2-Ar), 16.0 q (5 × Me), 16.2 q, 17.6 q (2 × Me).

Sarcochromenol sulfate A [**1**].—Compound **1** (2.8 mg): amorphous solid; rrt = 1.30; uv λ max 251 (ε 2300), 266 (ε 2700), 285 (ε 1100), 318 (ε 2000); fabms *m/z* $[M - Na]^-$ 595; ^{1}H nmr (CD_3OD) 5.65 (d, 9.5, H-3), 6.37 (d, 9.5, H-4), 6.94 (d, 3.0, H-5), 7.02 (dd, 3.0, 8.5, H-7), 6.66 (d, 8.5, H-8), 1.35 (s, Me-11), 5.10 (m, *HC*=), 1.67 (s), 1.57 (s, 2 × Me), 1.69 (s, 4 × Me).

Sarcohydroquinone sulfate A [**4**].—Compound **4** (3.0 mg): amorphous solid; rrt = 1.00; uv λ max (EtOH) 283 (ε 2700); fabms *m/z* $[M - Na]^-$ 597; ^{1}H nmr (CD_3OD) 7.00 (d, 3.0), 6.94 (dd, 8.5, 3.0), 6.67 (d, 8.5), 5.34 (t, 7, Ar-CH_2-*HC*=C), 5.11 (t, 7, 5 × *HC*=), 3.28 (t, 7, CH_2-Ar), 2.04 (m, 10 × CH_2), 1.60 (s, 5 × Me), 1.67 (s), 1.71 (s, 2 × Me).

Sarcohydroquinone sulfate B [**5**].—Compound **5** (5.0 mg): amorphous solid; rrt = 2.00; uv λ max (EtOH) 283 (ε 2700); fabms *m/z* $[M - Na]^-$ 665; ^{1}H nmr (CD_3OD) 7.00 (d, 3.0), 6.94 (dd, 8.5, 3.0), 6.67 (d, 8.5), 5.34 (t, 7, Ar-CH_2-*HC*=), 5.11 (t, 7, 6 × *HC*=), 3.28 (t, 7, CH_2-Ar), 2.04 (m, 12 × CH_2), 1.60 (s, 6 × Me), 1.67 (s), 1.71 (s, 2 × Me).

Sarcohydroquinone sulfate C [**6**].—Compound **6** (1.4 mg): amorphous solid; rrt = 3.00; uv λ max (EtOH) 283 (ε 2600); fabms *m/z* $[M - Na]^-$ 733; ^{1}H nmr (CD_3OD) 7.00 (d, 3.0), 6.94 (dd, 8.5, 3.0), 6.67 (d, 8.5), 5.34 (t, 7, Ar-CH_2-*HC*=), 5.11 (t, 7, 7 × HC=), 3.28 (t, 7, CH_2-Ar), 2.04 (m, 14 × CH_2), 1.60 (s, 7 × Me), 1.67 (s), 1.71 (s, 2 × Me).

TREATMENT OF COMPOUND **5** WITH K_2CO_3/Me I TO GIVE COMPOUND **10**.—Compound **10** (2.5 mg), colorless oil, was obtained from compound **5** (2.5 mg) by refluxing in dry Me_2CO (2 ml) with 21 mg K_2CO_3 and 200 μl Me I during 8 h. ^1H nmr (7.08 (m, H-3, -5), 6.84 (d, 8.5), 5.29 (m, Ar-CH_2-*H*C=), 5.11 (t, 7), 3.28 (t, 7), 2.04 (m, 12 × CH_2), 1.60 (s, 6 × Me), 1.67 (s), 1.71 (s, 2 × Me).

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