# Unstable Enol Sulfates from a Two-Sponge Association

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# UNSTABLE ENOL SULFATES FROM A TWO-SPONGE ASSOCIATION

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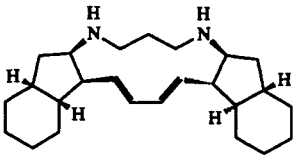
ABSTRACT.—An inseparable two-sponge association, consisting of a *Haliclona* sp. and a choristid sponge, yielded two unstable enol sulfates, (1*E*)-2-(3′,4′-dihydroxyphenyl)ethylene sulfate [**3**] and (1*Z*)-2-(3′,4′-dihydroxyphenyl)ethylene sulfate [**4**]. Methanolysis converted the enol sulfates into 2-(3′,4′-dihydroxyphenyl)-1,1,2-trimethoxyethane [**5**] by an oxidative mechanism.

On many occasions we have observed a reddish orange sponge of the genus *Haliclona* overgrowing a bright yellow aerophobic sponge of the family Stellettidae (order Choristida). The choristid sponge is completely obscured by the overlying sponge and appears to communicate with the environment via the *Haliclona* sp., suggesting a shared aquiferous system. Spicules of the choristid sponge, which is 3–5 cm thick, protrude through its collagenous cortex into the *Haliclona* crust, that is 2–10 mm thick, depending on the thickness of the underlying choristid sponge. Although the overgrowth of one sponge by another could well be an accidental event, this example appears to be a consistent association. Detailed examination of two specimens of the two-sponge association, one from Pohnpei and the other from the Philippines, revealed that, despite differences in the physical form of the underlying choristid sponge, the taxonomy and chemistry of the associated sponges were identical. Because it was impossible to separate the two sponges cleanly, we investigated the chemistry of the two-sponge association. Both specimens contained the same mixture of haliclonadiamine [**1**] (1) and papuamine [**2**] (2) that had previously been isolated from a free-living specimen of *Haliclona* sp., which cannot be distinguished from the *Haliclona* sp. involved in this association. In addition to the alkaloids **1** and **2**, the two-sponge association has also yielded two unstable enol sulfates, (1*E*)-2-(3′,4′-dihydroxyphenyl)ethylene sulfate [**3**] and (1*Z*)-2-(3′,4′-dihydroxyphenyl)ethylene sulfate [**4**], that have few precedents among marine natural products (3). Coincident with the completion of this manuscript, we became aware that Ohta and co-workers had described the isolation of (1*Z*)-2-(3′,4′-dihydroxyphenyl) ethylene sulfate [**4**] under the name isojaspisin from a Japanese *Jaspis* sp. (4).

In our initial studies of the specimen from the Philippines we observed that if the crude aqueous extract was evaporated to dryness, the organic material could not be redissolved in any solvent. The formation of an insoluble polymer was accompanied by the release of sodium hydrogen sulfate, which was identified by its ir spectrum. The enol sulfates were isolated from the specimen from Pohnpei by extraction of the sponges with $Me_2CO$ followed by careful evaporation of the $Me_2CO$ to obtain a concentrated aqueous solution that was immediately subjected to chromatography on $C_{18}$ reversed-phase packings. Enol sulfates **3** and **4** were readily separated by hplc on a $C_{18}$ reversed-phase column using 3% $CH_3CN$ in $H_2O$ as eluant and were obtained as white powders after lyophilization.

The molecular formulas for the anions of (1*E*)-2-(3′,4′-dihydroxyphenyl)-ethylene sulfate [**3**] and (1*Z*)-2-(3′,4′-dihydroxyphenyl)ethylene sulfate [**4**] were determined by negative-ion hrfabms as $C_8H_7O_6S^-$, and X-ray fluorescence spectroscopy indicated that the major cations were sodium and potassium in approximately the same proportions as in sea water. The ir spectra of both **3** and **4**



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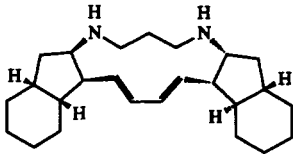

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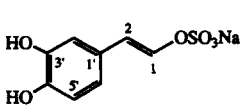

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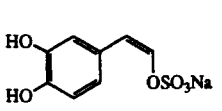

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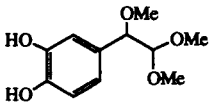

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contained strong sulfate bands at ca. 1260 and 1230 $cm^{-1}$ in addition to the bands at ca. 3370 (phenol), 1655–1660 (enol), and 1600–1605 (aromatic) $cm^{-1}$. The uv absorptions at 300 nm in **3** and 296 nm in **4** indicated that the enol double bond was conjugated to the aromatic ring and the bathochromic shifts to 350 nm on addition of base were typical of phenols. The $^1$H- and $^{13}$C-nmr spectra were assigned by interpretation of the COSY, HMQC, and HMBC experiments (Table 1). The major differences between **3** and **4** were observed in the $^1$H-nmr spectra, in which the coupling constants for the enol protons (13 Hz in **3** and 7 Hz in **4**) clearly indicated that the two compounds were geometrical isomers. The sulfate group must be at C-1 since a hydroxyl group at that position would be unstable with respect to the corresponding aldehyde.

Initial attempts to isolate the enol sulfates **3** and **4** from the Philippines specimen were unsuccessful because they reacted with the MeOH used as the extraction solvent to obtain 2-(3′,4′-dihydroxyphenyl)-1,1,2-trimethoxyethane [**5**]. The structural elucidation of **5** was accomplished in a straightforward manner by interpretation of spectral data. Because the sulfates **3** and **4** are derivatives of the enol form of 3′,4′-dihydroxyphenylacetaldehyde, the formation of **5** requires a formal oxidation at the carbon adjacent to the aromatic ring. The conversions of the pure enol sulfates **3** and **4** into the trimethoxy derivative **5** were followed by $^1$H-nmr spectroscopy. Samples of **3** (ca. 12 mg/ml) and **4** (ca. 3 mg/ml) were stored in nmr tubes in MeOH-$d_4$ at 4° and the spectra were measured at daily, then weekly, intervals.

TABLE 1. $^1$H- (200 MHz, MeOH-$d_4$) and $^{13}$C-Nmr (50 MHz, MeOH-$d_4$) Data for the Enol Sulfates **3** and **4**.

| Position | Compound | | | |
|---|---|---|---|---|
| | **3** | | **4** | |
| | $\delta_C$ | $\delta_H$ (mult., *J*) | $\delta_C$ | $\delta_H$ (mult., *J*) |
| 1 | 138.7 | 7.10 (d, 13) | 136.7 | 6.63 (d, 7) |
| 2 | 116.0 | 6.09 (d, 13) | 111.3 | 5.41 (d, 7) |
| 1′ | 128.1 | | 128.5 | |
| 2′ | 113.4 | 6.75 (d, 2) | 117.0 | 7.22 (d, 2) |
| 3′ | 146.3 | | 145.9 | |
| 4′ | 145.6 | | 145.5 | |
| 5′ | 116.5 | 6.67 (d, 8) | 115.9 | 6.69 (d, 8) |
| 6′ | 119.2 | 6.60 (dd, 8, 2) | 122.3 | 6.90 (dd, 8, 2) |

The transformation of **3** and **4** to the trimethoxy derivative **5** occurred cleanly but slowly and after 2 months at 4° approximately 50% conversion of **3** and 15% conversion of **4** were observed. No signals for intermediates were observed in the nmr spectra. The difference in the observed reaction rates implies that the oxidation reaction proceeds through a planar methylene quinone intermediate, which is destabilized by steric interactions in the (*E*) isomer. Since oxygen was not excluded from the nmr tubes, we propose that the most likely transient intermediate is the methylene quinone, 4-(ethylene-2′-carboxaldehyde)-2-hydroxycyclohexa-2,5-diene or possibly the corresponding 2′-methoxy-2′-sulfate derivative.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The solvents used for extractions were freshly distilled and the solvents used for reversed-phase chromatography or spectroscopy were purchased as hplc or mass spectral grades, respectively. Uv spectra were recorded on a Perkin-Elmer Lambda 3B spectrophotometer and ir spectra were obtained on a Perkin-Elmer 1600 series Ft spectrophotometer. $^1$H- and $^{13}$C-nmr spectra were obtained on Bruker WP-SY 200 and Varian Unity 500 (COSY, HMQC, and HMBC experiments) spectrometers using $CD_3OD$ or $CDCl_3$ as solvents. Low-resolution mass spectra were obtained in a Hewlett Packard 5988A spectrometer and high-resolution mass spectra were obtained from the Mass Spectrometry Facility, University of California, Riverside.

DESCRIPTION OF THE TWO-SPONGE ASSOCIATION.—The two-sponge association (sample #POH93-098) was collected by hand using scuba (−30 m) on the southwest side of Pohnpei, Micronesia. The association consists of a bright yellow aerophobic choristid sponge, the surface of which is completely covered by a 2–10 mm thick layer of a *Haliclona* sp., referred to as "*Haliclona* sp. red" by van Soest (5). While the *Haliclona* sp. is found throughout the western Pacific in the free-living state, we have never encountered the choristid sponge without its associated *Haliclona*. The choristid sponge forms a globular mound, averaging 8 cm long and 3–5 cm thick, possessing a well-defined collagenous cortex and a decidedly radiate skeleton of oxea (ca. 1500 μm) with oxyasters (12–16 μm). No triaenes or other microsclere types are present.

Reduced spiculation among choristid sponges–the condition in which a sponge is comparable to an established genus excepting the absence of a spicule type–has long been recognized, but until a better understanding of the phylogenetic relationships of such sponges has been achieved there is no adequate classification of this choristid sponge. We therefore regard this choristid as an undescribed member of the family Stellettidae. It is most similar to either a "reduced" *Rhabdastrella* (without sterrasters) or a "lipotriaenose" *Stelletta*. A voucher specimen of this two-sponge association (registry #P1149) is on deposit in the Scripps Institution of Oceanography Benthic Invertebrate Collection.

The specimen from the Philippines (NCI-1249) was collected in Palawan (−20 m) and was identical in all respects except overall morphology to the specimen from Pohnpei. Although $^1$H-nmr signals assigned to the enol sulfates **3** and **4** were observed in a crude aqueous extract of this specimen, the compounds decomposed to obtain a polymer or reacted with MeOH during attempted purification.

EXTRACTION AND ISOLATION.—The Philippine specimen (540 g wet weight) was extracted with MeOH (1 liter) and the solvent evaporated to obtain a residue that was partitioned between EtOAc (3×200 ml) and $H_2O$ (200 ml). The aqueous extract was evaporated to dryness and the residue was triturated with MeOH. The MeOH solubles were partitioned between $NaHCO_3$ solution and EtOAc. The material that was insoluble in MeOH consisted of the polymeric material that was insoluble in all solvents together with some $H_2O$-soluble material that did not contain signals in the aromatic region of the $^1$H-nmr spectrum. Evaporation of the EtOAc extract gave a mixture of haliclonadiamine [**1**] and papuamine [**2**], as judged by nmr spectroscopy, and were not purified further. The original EtOAc fraction was evaporated to dryness and the residue was partitioned between MeOH (200 ml) and hexane (200 ml). The MeOH-soluble material was subjected to flash chromatography on Si gel using eluents from hexane to EtOAc. Fractions eluting with hexane-EtOAc (1:1) contained **5** (8 mg, 0.006% dry wt) as the only uv-active compound.

A portion of the two-sponge association (120 g dry wt) from Pohnpei was cut into small pieces and was twice extracted overnight with $Me_2CO$ (400 ml). The alkaloids **1** and **2** were detected by tlc but were not pursued. The extracts were combined and concentrated under reduced pressure at 30° until most of the $Me_2CO$ was removed (the solution should not be evaporated to dryness). One tenth of the resulting aqueous phase was applied to a $C_{18}$ Sep-Pak cartridge (10 g) that had previously been equilibrated with $H_2O$. Fractions were eluted with $H_2O$ (fractions 1–13), $H_2O$-$CH_3CN$ (9:1)

(fractions 14–23), $H_2O$-$CH_3CN$ (8:1) (fractions 24–34), and $CH_3CN$ (fractions 35–50). A mixture of **3** and **4**, which was eluted in fractions 4–12, could be detected by tlc on Si gel (BuOH-$H_2O$-AcOH, 12:5:3), as a strongly uv-active spot ($R_f$ 0.7). Fractions 4–12 were combined and 20% of the solution was concentrated to 5 ml and chromatographed (10 injections) on a Dynamax $C_{18}$ column eluting with 3% $CH_3CN$ in $H_2O$ (3 ml/min, detection at 250 nm) to obtain (1*E*)-2-(3′,4′-dihydroxyphenyl)ethylene sulfate [**3**] (12 mg, 2.24% dry wt, $R_t$, 12.5 min) and (1*Z*)-2-(3′,4′-dihydroxyphenyl)ethylene sulfate [**4**] (3 mg, 0.56% dry wt; $R_t$, 14.5 min).

*(1E)-2-(3′,4′-Dihydroxyphenyl)ethylene sulfate* [**3**].—Obtained as a white solid: mp >300° (dec); uv (MeOH) λ max (log ε) 204 (4.78), 260 (4.41), 300 nm (3.99), (MeOH+KOH) 208 (4.63), 243 (4.28), 298 (4.16), 350 nm (4.11); ir (KBr) ν max 3373 (OH), 1656 (C=C), 1601, 1530, 1305, 1261, 1234, 1113, 1037, 861, 746, 620 $cm^{-1}$; $^1H$ nmr (MeOH-$d_4$, 200 MHz), see Table 1; $^{13}C$ nmr (MeOH-$d_4$, 50 MHz), see Table 1; eims (70 eV) *m/z* 152, 123, 77; hrfabms, *m/z* 230.9953 $[M-Na]^-$ ($C_8H_7O_6S$ requires 230.9963); X-ray fluorescence spectroscopy showed the presence of sulfur, sodium, and potassium.

*(1Z)-2-(3′,4′-Dihydroxyphenyl)ethylene sulfate* [**4**].—Obtained as a white solid: mp >300° (dec); uv (MeOH) λ max (log ε) 216 (4.57), 257 (4.47), 296 nm (3.90), (MeOH+KOH) 217 (4.49), 263 (4.26), 300 (4.02), and 350 nm (3.93); ir (KBr) ν max 3369 (OH), 1659 (C=C), 1604, 1528, 1344, 1263, 1230, 1105, 1007, 818, 736, 623 $cm^{-1}$; $^1H$ nmr (MeOH-$d_4$, 200 MHz), see Table 1; $^{13}C$ nmr (MeOH-$d_4$, 50 MHz), see Table 1; eims (70 eV) *m/z* 152, 123, 77; hrfabms, *m/z* 230.9964 $[M-Na]^-$ ($C_8H_7O_6S$ requires 230.9963).

*2-(3′,4′-Dihydroxyphenyl)-1,1,2-trimethoxyethane* [**5**].—Obtained as a colorless oil; uv (MeOH) λ max (log ε) 220 nm (3.92), 280 (3.58); (MeOH+KOH) 244 nm (3.94), 298 (3.74); ir ($CHCl_3$) ν max 3376 (OH), 2933, 1284, 1191 $cm^{-1}$; $^1H$ nmr ($CDCl_3$, 200 MHz) δ 6.91 (1H, d, *J*=2 Hz, H-2′), 6.83 (1H, d, *J*=8 Hz, H-5′), 6.75 (1H, dd, *J*=8 and 2 Hz, H-6′), 5.84 (1H, br s, OH), 5.42 (1H, br s, OH), 4.34 (1H, d, *J*=6 Hz, H-2), 4.07 (1H, d, *J*=6 Hz, H-1), 3.42 (3H, s, OMe), 3.23 (3H, s, OMe), 3.22 (3H, s, OMe); $^{13}C$ nmr ($CDCl_3$, 50 MHz) δ 144.1, 143.7, 129.8, 121.0, 114.9, 114.5, 106.4 (C-2), 83.3 (C-1), 56.6 (OMe), 55.7 (OMe), 54.6 (OMe); eims (70 eV) *m/z*, 228 $[M]^+$, 197, 166, 153, 137, 109, and 75.

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