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Petromyzonin, a Hexahydrophenanthrene Sulfate Isolated from the Larval Sea Lamprey (*Petromyzon marinus* L.)

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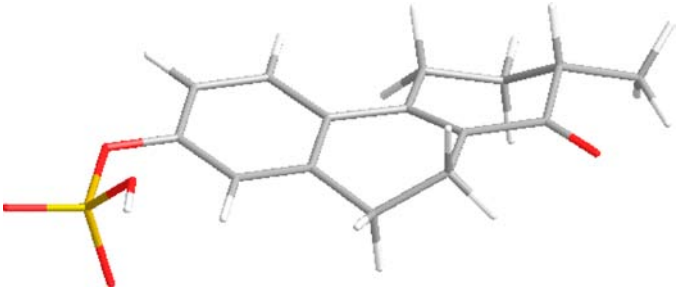
ABSTRACT



A new hexahydrophenanthrene sulfate was identified from water conditioned with sea lamprey larvae (*Petromyzon marinus*) and named petromyzonin. Its structure was unequivocally elucidated on the basis of spectroscopic analyses including comparison with spectra of known compounds. The absolute configuration was determined by electronic circular dichroism. Petromyzonin may function as a chemical signal, as it elicited responses in electro-olfactogram recording with a dynamic concentration−response relationship and a detection threshold of 10^{-11} M.

The sea lamprey (*Petromyzon marinus*) is a jawless vertebrate with an anadromous life history. Sea lamprey are born in freshwater, migrate to the ocean to feed, and then return to freshwater to spawn. This species relies heavily upon a number of chemical cues to mediate key aspects of its life cycle, including migration and reproduction.[1−3] Sea lamprey have been shown to be a prolific source of structurally interesting secondary metabolites, such as bile acids, bile salts, and steroids, and some of them exhibit intriguing biological activities.[2−9] In our further investigation of metabolites from larval sea lamprey, a naturally occurring hexahydrophenanthrene with a sulfate group was obtained and named petromyzonin. Herein we report the isolation, structure elucidation, and bioactivity of petromyzonin.

Water conditioned with sea lamprey larvae was extracted by XAD 7HP resin. The extract (42 L) was lyophilized, suspended in methanol, and then separated by repeated column chromatography on silica gel and Sephadex LH-20 to yield petromyzonin (Supporting Information).

Petromyzonin was obtained as a colorless oil with $[\alpha]_D^{25}$ +31.0 (*c* 0.10, MeOH). The molecular weight was determined by using negative ion electrospray ionization coupled with a quadruple time-of-flight mass analyzer (ESI-QTOF-MS) that gave rise to a pseudomolecular ion of *m*/*z* 307.06 (ESI-QTOF, $[M - H]^-$). The presence

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of sulfur was suggested by the low density mass offset of +2 amu (^{34}S) for the parent ions and confirmed by the characteristic loss of 80 amu at m/z 227.11 $[M - SO_3]^-$.[10] The high-resolution negative ion mass spectrum [(HR-ESI-QTOF-MS) at m/z 307.0646 $(M - H)^-$ and 227.1082 $(M - SO_3)^-$] matched well with the expected molecular formula of $C_{15}H_{16}O_5S$ (calcd m/z 307.0640, Δ 0.6 amu) and fragment $C_{15}H_{15}O_2$ (calcd m/z 227.1072, Δ 1.0 amu), respectively. These data indicated eight degrees of unsaturation of petromyzonin.

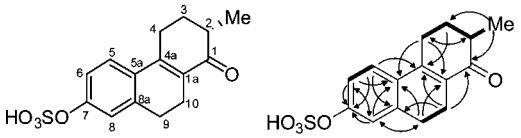

Figure 1. Structure, key $^1H-^1H$ COSY (bold lines), and HMBC (arrows) correlations of petromyzonin.

The ^1H NMR spectrum (methanol-d_4, Table 1) contained signals for three methines attributed to an ABX spin system at δ_H 7.49 (1H, d, J = 8.5 Hz, H-5), 7.20 (1H, dd, J = 8.5, 2.4 Hz, H-6), and 7.18 (1H, d, J = 2.3 Hz, H-8), an additional methine at δ_H 2.46 (1H, ddq, J = 11.7, 6.8, 2.0 Hz, H-2), four methylenes at δ_H 2.22 (1H, m, H-3a), 1.82 (1H, m, H-3b), 2.76 (1H, m, H-4a), 2.87 (1H, m, H-4b), 2.71 (1H, ddd, J = 18.1, 11.7, 6.6 Hz, H-9a), 2.81 (1H, m, H-9b), 2.62 (1H, m, H-10a), and 2.31 (1H, m, H-10b), and a methyl at δ_H 1.17 (3H, d, J = 6.8 Hz, H-11). The ^{13}C NMR spectrum (Table 1) exhibited 15 carbons including one methyl, four methylenes, four methines, and six quaternary carbons, of which the signals at δ_C 203.6 (qC) were attributable to a ketone, whereas those at δ_C 132.0 (qC), 151.4 (qC), 127.0 (CH), 131.9 (qC), 120.3 (CH), 155.1 (qC), 121.3 (CH), and 141.1 (qC) were attributable to a trisubstituted aromatic ring and an olefinic moiety. Furthermore, the remaining elemental composition resulted in the formation of two aliphatic rings to account for the remaining degrees of unsaturation. The carbon signals were assigned to the relevant protons through an HSQC two-dimensional NMR experiment. In an ABX coupling system, the proton signals at δ_H 7.49 (H-5) showed a long-range correlation to the carbon signal at δ_C 155.1 (qC) in the HMBC spectrum (Figure 1); both proton signals at δ_H 7.20 (H-6) and 7.18 (H-8) displayed long-range correlations to the carbon signals of C-5a and C-7. Based on the relevant correlation signals, the aromatic ring in this compound can be assigned as Figure 1 and Table 1. The gCOSY spectrum showed correlations between CH_2-9 and CH_2-10. The remaining deprotonated olefinic moiety was assigned after elucidating the trisubstituted aromatic ring. The key long-range correlations from H-9 to C-8, C-5a, and C-1a from H-5 to C-4a and from H-10 to C-8a and C-4a in the gHMBC spectrum indicated that the deprotonated vinyl and 1,2-disubstituted ethanyl correlated to produce 1,4-disubstituted but-1-enyl moiety and connected to C-5a and C-8a on the aromatic ring to form the 3,4-disubstituted 1,2-dihydronaphthalenyl moiety. The gCOSY spectrum displayed signals between H_2-4/H_2-3, H_2-3/H-2, and H-2/H_3-11, respectively, indicating the presence of a 2-substituted butanyl fragment. The long-range correlations from methyl to C-1, from H-10 to C-1, from H-3 to C-4a, and from H-4 to C-5a and C-1a further supported the connection between C-4 and C-4a as well as between C-1 and C-1a. The degree of unsaturation agreed with the above-mentioned data. In addition to elucidating the carbon skeleton, the sulfate moiety was assigned to C-7 on the basis of its downfield shift at δ_C 155.1 (qC), and the remaining positions were left without substituents.[6] Thus, the planar structure of petromyzonin was assigned.

Table 1. ^1H (900 MHz, J in Hz) and ^{13}C NMR (225 MHz) Spectroscopic Data for Petromyzonin in Methanol-d_4

no.	δ_H	δ_C
1		203.6 (qC)
1a		132.0[a] (qC)
2	2.46 (ddq, J = 11.7, 6.8, 2.0 Hz, 1H)	42.0 (CH)
3	2.22 (m, 1H), 1.82 (m, 1H)	31.5 (CH_2)
4	2.76 (m, 1H), 2.87 (m, 1H)	26.4 (CH_2)
4a		151.4 (qC)
5	7.49 (d, J = 8.5 Hz, 1H)	127.0 (CH)
5a		131.9[a] (qC)
6	7.20 (dd, J = 8.5, 2.4 Hz, 1H)	120.3 (CH)
7		155.1 (qC)
8	7.18 (d, J = 2.3 Hz, 1H)	121.3 (CH)
8a		141.1 (qC)
9	2.81 (m, 1H), 2.71 (ddd, J = 18.1, 11.7, 6.6 Hz, 1H)	28.9 (CH_2)
10	2.62 (m, 1H), 2.31(m, 1H)	21.0 (CH_2)
11	1.17 (d, J = 6.8 Hz, 3H)	16.0 (CH_3)

[a] Data are interchangeable.

To establish the absolute configuration of petromyzonin, its electronic circular dichroism (ECD) spectrum was experimentally recorded, which showed a positive Cotton effect at 230, 305, and 345 nm (Figure 2). The theoretical ECD was then calculated with a time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d) level.[11−13] The calculated ECD spectrum was produced by SpecDis software[14] and is shown in Figure 2. The absolute configuration at C-2 of petromyzonin was determined as *S*.

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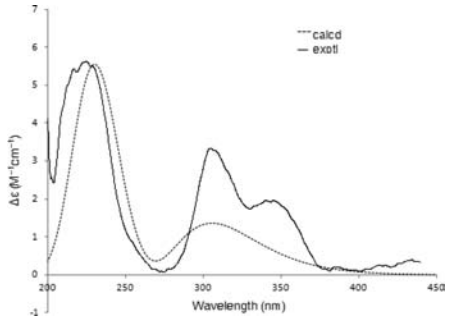

Figure 2. Experimental and calculated ECD spectra and stereostructure of petromyzonin (in MeOH).

To the best of our knowledge, petromyzonin possesses a novel carbon skeleton that has not previously been discovered in nature. Petromyzonin also represents the first example of a hexahydrophenanthrene conjugated with a sulfate moiety. A carbon skeleton identical to that of petromyzonin was reported as an intermediate in synthetic studies.[15] Two synthesized compounds based on this skeleton, 2,6-dimethyl-1,2,3,4,9,10-hexahydrophenanthrenone, have been reported.[16,17] One compound, with a methyl at C-6, was a product via hydrolysis and rearrangement of spiranes under anhydride and Pd−C catalysis.[16] The other compound, with a methoxyl group at C-7, was an intermediate in the synthetic pathway for estrone.[17] This intermediate was used to construct the cyclopentanone structure (ring D) of a steroid with an aromatic ring (ring A), which proved to be highly successful in the synthesis of equilenin. Despite the similarities, the carbon skeleton of petromyzonin is unlikely an artifact because the critical chemical reaction conditions for synthesizing 2-methyl-1,2,3,4,9,10-hexahydrophenanthrenone are unavailable in nature.

Petromyzonin is the first example of hexahydrophenanthrene isolated from sea lamprey and from nature. So far, only a few compounds, including 3-ketopetromyzonol sulfate (3KPZS),[2] petromyzonamine disulfate (PADS),[3,5] petromyzosterol disulfate (PSDS),[3,5] petromyzestrosterol,[6] 3-ketoallocholic acid (3KACA),[7] 3,12-diketo-4,6-petromyzonene-24-sulfate (DKPZS),[8] and allocholic acid (ACA),[9] were isolated from the different life stages of sea lamprey. All of these compounds belong to classes of bile acids, bile salts, or steroids, consisting of a steroidal carbon skeleton, and were found to be highly stimulatory for sea lamprey olfactory organs.[2,3,5−8] Petromyzonin may provide a new template for the research on sea lamprey metabolites.

The olfactory sensitivity to petromyzonin was assessed by EOG recording. The olfactory epithelia of adult male sea lamprey was stimulated with increasing concentrations of petromyzonin, and the recorded responses were normalized (Supporting Information Figure S1). The detection threshold for petromyzonin was 10^{-11} M (n = 6). This threshold was only 1 order of magnitude higher than the threshold (10^{-12} M) for 3KPZS, a known lamprey pheromone. Nonetheless, the threshold for petromyzonin was almost 3 orders of magnitude lower than that of the most potent amino acid, L-arginine (threshold at 10^{-8} M on the same test animal). The concentration−response curve for petromyzonin showed a steep increase in responses as the concentration was augmented, which is typical of the involvement of a specific receptor in the detection of an odorant (Figure S1). These data showed that petromyzonin, with a hexahydrophenanthrene carbon skeleton, is also a new class of compound that acts as a potent odorant in vertebrates. Its biological function warrants further examination.

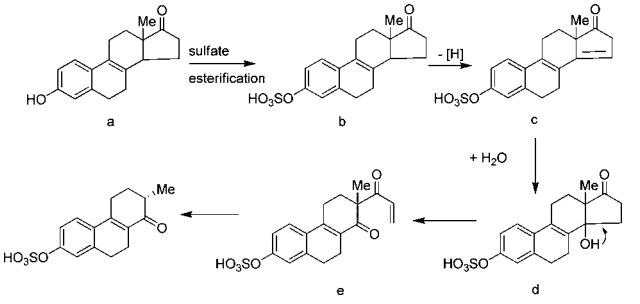

Figure 3. Plausible biosynthetic pathway from estrone to petromyzonin.

It is notable that the petromyzonin is also similar to estrone by possessing an aromatic ring (ring A). Estrone is the first estrogenic hormone to be isolated with a classic A ring aromatic steroid and the starting material for a number of fertility-regulating hormones in vertebrate animals.[18] In the sea lamprey, estradiol has been measured.[19] It is possible that petromyzonin is a product from estrone since both compounds have an identical aromatic ring (ring A). Here we postualte a possible biosynthetic pathway from estrone to petromyzonin, as shown in Figure 3. We hypothesize that estrone first conjugates with a sulfate group to add the atoms necessary for the construction of the substituted A ring, which is followed by dehydrogenation to afford a tetracyclic intermediate (c). Subsequently, a H_2O is added to generate a saturated D ring. Finally, a migration and elimination reaction of ring D results in petromyzonin.

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Supporting Information Available. Experimental details; NMR, MS, and HR-MS spectra of petromyzonin. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.