A New Iodinated Metabolite and a New Alkyl Sulfate from the Senegalese Sponge *Ptilocaulis spiculifer*

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The hydrophilic extract of the Senegalese sponge *Ptilocaulis spiculifer* has been analyzed. It has been shown to contain dakaramine (**1**), a new tyrosine derivative containing iodine, an unusual feature for sponge metabolites. In addition, the new alkyl sulfate **2** (as a counterion of **1**) and the known ecdysonic sterol **3** were isolated from the same extract.

Tyrosine-derived halometabolites frequently occur in marine organisms and are known to play basic functions related to the survival of the living creatures producing them.[2] Bromine is by far the halogen most frequently found in these metabolites; for example, bromotyrosine derivatives are constantly detected among the secondary metabolites of the Demospongiae (order Verongida); thus, they have been used for taxonomic purposes.[3−5] Iodinated halometabolites, biosynthetically related to tyrosine, are much less widespread in the marine environment, since they have been isolated just in a few algae and invertebrates.[6,7]

This paper describes the isolation and the structure determination of **1**, a new example of this structural class from a specimen of *Ptilocaulis spiculifer* (Lamarck, 1814; family Axinellidae, order Axinellida) recovered on a *Palythoa* sp. (Zoanthid) as a host organism. The hydrophilic extract of this organism also contained the new alkyl sulfate **2**, which was isolated as the counterion of **1**, and the known ecdysonic sterol **3**.

Specimens of *P. spiculifer* were collected along the coast of Senegal in October 1994 and extracted with CH_2Cl_2−MeOH. The crude extract was partitioned between diisopropyl ether and water, and the water-soluble material was purified by chromatography on an RP-18 column and successively by HPLC to give pure compound **3** and a fraction consisting of **1** and **2** in a 1:1 ratio. This fraction was partitioned between $CHCl_3$ and aqueous 0.1 M Na_2CO_3. The $CHCl_3$ layer yielded pure compound **1**, while the aqueous layer was neutralized with solid NH_4Cl, and passed through an RP-18 column using as eluent first H_2O and then MeOH. The MeOH fraction contained pure compound **2**.

Compound **3** was identified as meristerone A, an ecdysone first isolated from kaladana (*Ipomea calonyction*) seeds in 1977, by comparison of its spectral properties with those reported in the literature.[8]

Combined analysis of HRFABMS and ^{13}C-NMR spectra of **1** indicated the molecular formula $C_{15}H_{24}I_2N_2O$. Inspection of ^{1}H-NMR and COSY spectra of the diprotonated form **1p** allowed the identification of oxypropylidene [$-OCH_2CH_2CH_2-$, δ 4.13, (H_2-7), 2.37 (H_2-8), and 3.53 (H_2-9)] and ethylidene [$-CH_2CH_2-$, δ 3.23, (H_2-1) and 2.95 (H_2-2)] chains.

The ^{1}H-NMR spectrum also contained two 6H singlets at δ 2.96 and δ 2.85, attributable to two dimethylammonium functions. These were located as end-groups of both the oxypropylidene and the ethylidene chains on the basis of a ROESY experiment, showing intense correlation peaks of the singlet at δ 2.96 with the multiplet at δ 3.53 (H_2-9) and of the singlet at δ 2.85 and the multiplet at δ 3.23 (H_2-1). The above substructures contained all the sp^3 carbon atoms present in **1p** as evidenced by the ^{13}C-NMR spectrum; the five CH_2 resonances in the high-field region at δ 71.7, 59.5, 57.2, 30.1, 26.6 were assigned to the relevant carbon atoms through an HMQC experiment, whereas the resonances of the two pairs of methyl groups were coincident (δ 43.8).

The remaining four sp^2 carbon signals [δ 157.7 (C), 141.6 (CH), 138.6 (C), and 91.7 (C)] were indicative of a symmetrically tetrasubstituted benzene ring. This was confirmed by the ^{1}H-NMR spectrum, which showed in the low-field region only one 2H singlet at δ 7.85. The unusually high field of the signal at δ 91.7 clearly distinguished the sp^2 carbon atoms linked to the iodine atoms, while the deshielded carbon atom resonating at δ 157.7 must be linked to the sole oxygen atom in the molecule, thus establishing the C6-O-C7 connection.

The presence of the C2−C3 bond is obvious at this stage and was confirmed by the long-range ^{1}H−^{13}C coupling of the aromatic carbon atom resonating at δ 138.6 (C-3) with the methylene protons H_2-2, evidenced through an HMBC experiment. Finally, the aromatic protons were located at the position 4 and 4′ on the basis of their ROESY correlation with H_2-2, and the iodine atoms were therefore located at the biogenetically expected positions 5 and 5′.

The ^{1}H-NMR spectrum of the free amine, which was easily assigned through a COSY experiment, was similar to that of **1p**. As expected, the chemical shifts of the methyl and methylene groups linked to the nitrogen atoms were all upfield shifted (see the Experimental Section).

The molecular formula $C_8H_{17}O_4S$ for the optically active ($[\alpha]^{25}_D$ +0.5°) compound **2** was suggested by the negative ion HRFABMS spectrum and was confirmed by the ^{13}C-NMR spectrum, which excluded the alternative $C_8H_{18}O_4P$ on the basis of the absence of any ^{13}C/^{31}P coupling for ^{13}C -NMR signals.

Structure **2** devoid of the stereochemistry was deduced through a detailed interpretation of its ^{1}H-NMR spectrum aided by a COSY experiment, which evidenced a single spin system comprising all the protons of the molecule, and by the HMQC spectrum, which simplified the interpretation of the COSY spectrum through the identification of three pairs of diastereotopic methylene protons. The NMR assignments of the carbon and the proton resonances are reported in the Experimental Section. The proposed structure was then confirmed by treatment of **2** with 1,4-dioxane/pyridine, which gave (*R*)-3-ethyl-4-methylpentan-1-ol (**4**) identified by comparison of its spectral and optical properties with those

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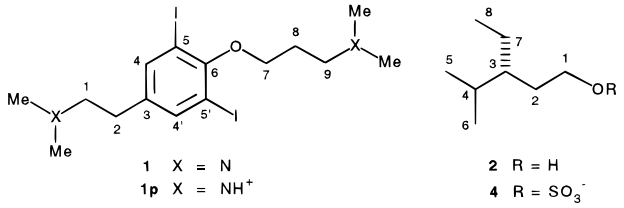
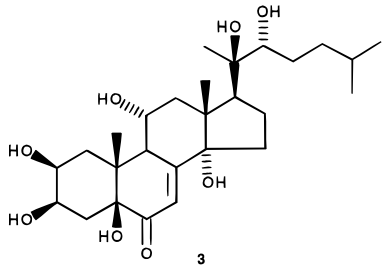
previously reported.[9] This experiment also allowed us to assign the stereochemistry of C-3 in compound **2** as *R*.

To the best of our knowledge, sulfate **2** is a novel compound; its parent alcohol has not been reported as a naturally occurring compound but has been synthesized as a starting material for the preparation of bioactive products.[10] The alcohol recollects the side chain of 24-ethyl steroids, from which it could be derived by oxidative fission between C-20 and C-22 bond, a reaction involved in basic pathways of the sterol metabolism in living organisms.





Experimental Section

General Experimental Methods. FABMS were obtained on a VG Prospec-Autospec (55 eV) using a glycerol matrix. ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker AMX-500 spectrometer in CD_3OD solution. ^{1}H-NMR chemical shifts were referenced to the residual solvent signal (δ 3.34). ^{13}C-NMR spectra were referenced to the center peak of the solvent at δ 49.0. The multiplicities of ^{13}C resonances was determined by DEPT experiments. Optical rotation were performed on a Perkin-Elmer 192 polarimeter with a sodium lamp operating at λ = 589 nm and a 10-cm microcell. HPLC was performed on a Varian 2050 apparatus equipped with an RI-3 refractive index detector, using Hibar columns.

Collection, Extraction, and Isolation of Compounds 1−3. Specimens of *P. spiculifer* were collected (depth 30 m) by SCUBA in October 1994 at Dakar (Senegal). A voucher specimen is deposited at the Istituto di Zoologia, Genova, Italy. The sponge (100 g dry wt) was air dried and then extracted twice with CH_2Cl_2−MeOH 1:1 (250 × 2) at room temperature. After evaporation of the solvent, the oily residue (6.7 g) was partitioned between water and diisopropyl ether. The water-soluble material (4.8 g), after evaporation to dryness, was redissolved in MeOH and filtered. The MeOH-soluble layer was purified by passing it through an RP-18 column, and a fraction (72 mg) eluted with H_2O−MeOH (4:6) was further fractioned by HPLC on a Hibar Superspher RP-18 column with MeOH−H_2O (7:3) as eluent, leading to pure compound **3** (3.2 mg, 0.003%) and a fraction (45 mg) consisting of **1** and **2** in a ratio about 1:1. This fraction was partitioned between $CHCl_3$ and aqueous 0.1 M Na_2CO_3. The $CHCl_3$ fraction was dried over Na_2SO_4 and evaporated, yielding pure compound **1** (30.3 mg, 0.03%). The aqueous layer was neutralized with solid NH_4Cl and chromatographed on an RP-18 column, using as eluent first H_2O and then MeOH. The MeOH fraction, taken to dryness, contained pure compound **2** (10.5 mg, 0.01%).

Compound 1: HRFABMS (positive ion mode) *m/z* 503.0097 ([M + H]$^+$, $C_{15}H_{25}I_2N_2O$ gives 503.0056); UV (MeOH) λ max (log ϵ) 223 (4.12), 237 (sh, 3.82), 277 (3.06), 286 (3.01); IR (dry film) ν max 2955, 1578, 1458, 1385, 1250, 1042, 984; ^{1}H-NMR (CD_3OD) δ 7.73 (H_2-4,4′, s), 4.02 (H_2-7, t, J = 6.1 Hz), 2.73 (H_2-1 and H_2-9), 2.56 (H_2-2, m), 2.36 (NMe_2-1, s), 2.32 (NMe_2-9, s), 2.13 (H_2-8, m).

Compound 1p. 1 (2 mg) was dissolved in 100 μL of trifluoroacetic acid and then taken to dryness in vacuo, leading to its diprotonated form **1p:** ^{1}H-NMR (CD_3OD) δ 7.85 (H_2-4,4′, s), δ 4.13 (H_2-7, t, J = 6.1 Hz), 3.53 (H_2-9, m), 3.23 (H_2-1, m), 2.96 (NMe_2-9, s), 2.95 (H_2-2, m), 2.85 (NMe_2-1, s), 2.37 (H_2-8, m); ^{13}C-NMR (CD_3OD) δ 157.7 (s, C-7), 141.6 (d, C-4,4′), 138.6 (s, C-3), 91.7 (s, C-5,5′), 71.7 (t, C-7), 59.5 (t, C-1), 57.2 (t, C-9), 43.8 (q, NMe_2-1 and NMe_2-9), 30.1 (t, C-2), 26.6 (t, C-8).

Compound 2: $[\alpha]^{25}_D$ +0.5° (MeOH, c = 0.01); HR-FABMS (negative ion mode) *m/z* 209.0873 ([M]$^-$, $C_8H_{17}O_4S$ gives 209.0848); IR (dry film) ν max 2959, 1466, 1214, 1042, 756; ^{1}H-NMR (CD_3OD) δ 4.07 (H_2-1, m), 1.78 (H-4, m), 1.72 (H-2a, m), 1.55 (H-2b, m), 1.41 (H-7a, m), 1.30 (H-7b, overlapped), 1.22 (H-3, overlapped), 0.93 (H_3-8, t, J = 7.5 Hz), 0.90 (H_3-5 or H_3-6, d, J = 7.1), 0.88 (H_3-6 or H_3-5, d, J = 6.8); ^{13}C-NMR (CD_3OD): δ 68.2 (t, C-1), 43.3 (d, C-3), 30.9 (t, C-2), 30.3 (d, C-4), 24.1 (t, C-7), 19.8 and 19.2 (2q, C-5 and C-6), 12.3 (q, C-8).

Compound 3. Meristerone A (**3**) was identified by comparison of its spectral properties with those reported in the literature.[8]

Solvolysis of 2. Compound **2** (5 mg) in dioxane (100 μL) and pyridine (100 μL) was heated at 120 °C for 2 h in a sealed tube. The solution was evaporated to dryness, giving (*R*)-3-ethyl-4-methylpentan-1-ol (**4**) identified by comparison of its spectral and optical properties with those reported in the literature ($[\alpha]^{25}_D$ +6.0, $CHCl_3$, c = 0.004 (lit.[9] $[\alpha]^{25}_D$ +6.9, $CHCl_3$, c = 5.2)).

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