Ophirapstanol Trisulfate, a New Biologically Active Steroid Sulfate from the Deep Water Marine Sponge Topsentia ophiraphidites

Sarath P. Gunasekera, Susan H. Sennett, Michelle Kelly-Borges, and Robert W. Bryant

J. Nat. Prod., **1994**, 57 (12), 1751-1754• DOI: 10.1021/np50114a024 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 12, pp. 1751-1754, December 1994

OPHIRAPSTANOL TRISULFATE, A NEW BIOLOGICALLY ACTIVE STEROID SULFATE FROM THE DEEP WATER MARINE SPONGE *TOPSENTIA OPHIRAPHIDITES*

SARATH P. GUNASEKERA,* SUSAN H. SENNETT, MICHELLE KELLY-BORGES,[1]

Harbor Branch Oceanographic Institution, Inc., 5600 U.S. 1, North, Fort Pierce, Florida 34946

and ROBERT W. BRYANT

Schering Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033

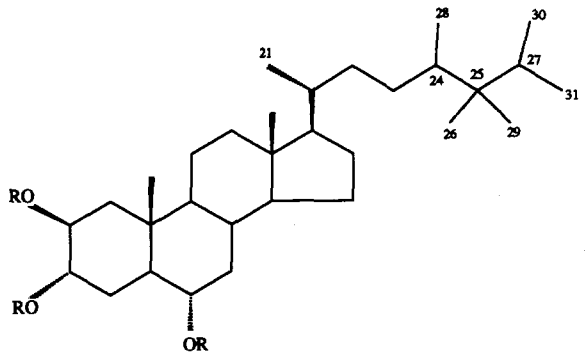
ABSTRACT.—Ophirapstanol trisulfate [**1**], a new steroid trisulfate related to sokotrasterol trisulfate was isolated from a deep water marine sponge *Topsentia ophiraphidites*. Compound **1** exhibited significant inhibition in the guanosine diphosphate/G-protein RAS exchange assay. The structure elucidation of **1** and ophirapstanol [**2**] by nmr spectroscopy is described.

The occurrence of sulfate esters of polyoxygenated sterols in sponges has been well documented (1). Most of the sulfated sterols have novel alkylation patterns in their side-chains, but a few sterols containing a C_{12} side-chain are known (2–7). During our ongoing program to isolate biologically active marine natural products, we found that an EtOH extract of the marine sponge *Topsentia ophiraphidites* (de Laubenfels, 1934) showed in vitro activity in the guanosine diphosphate/G-protein RAS exchange assay (GDPX). This assay determines the ability of inhibitors to affect the exchange of guanine nucleotides bound to $p21^{RAS}$ that is active in cell cycling and signal transduction (8). The mitogenic activity of normal RAS is modulated by a GTP-GDP cycle. RAS is able to transform cells when in the GTP-bound conformation but not in GDP-bound state. Mutant forms of RAS found in human cancer have impaired GTPase activity and therefore remain constitutively complexed to GTP which leads to unregulated cell proliferation. The mutant form of RAS protein (p21) has been examined extensively because its activated forms are found in 20% of most types of human cancer and over 50% of colon and pancreatic carcinomas. In this paper, we report the isolation and structure determination of a novel stanol trisulfate designated ophirapstanol trisulfate [**1**], which is active in the GDPX assay. This compound is related to the reported sokotrasterol sulfate (7) which possesses an unsaturated C_{12} side-chain. We believe this stanol trisulfate **1** to be the first reported example of this particular combination of functionalities.

The sponge *T. ophiraphidites* was collected by manned submersible in the Gulf of Mexico at a depth of 168 m. An EtOH extract of the frozen sponge yielded a brownish extract that was partitioned between EtOAc and H_2O. The H_2O-soluble fraction was further partitioned between BuOH and H_2O. Reversed-phase C_{18} hplc of the latter H_2O-soluble partition with 25% H_2O/MeOH containing 0.1% TFA yielded ophirapstanol trisulfate [**1**]. Compound **1** exhibited an activity of $IC_{50}=2$ μg/ml in the GDPX assay. The trihydroxystanol **2** obtained by hydrolysis of **1** was not tested for activity since this assay was discontinued.

The molecular formula of **1** was determined as $C_{31}H_{53}O_{12}S_3Na_3$ on the basis of high-resolution negative-ion fab ms measurement of the molecular ion species at *m/z* 759.2544 corresponding to $(M-Na)^-$. The twelve oxygen atoms in the molecular formula, taken together

[1]Current address: Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK.



1 R=$SO_3^-Na^+$
2 R=H

with three sulfur and three sodium atoms, suggested the presence of three sulfate groups in the molecule. This was confirmed by the presence of the characteristic ir bands at 1243 and 1063 cm^{-1}. From a combination of APT, DEPT, and HMQC experiments, the 31 resonance lines in the ^{13}C-nmr spectrum were assigned to three quaternary carbons, eight methines, three oxygen-bearing methines, nine methylenes, and eight methyls. Comparison of the ^{13}C-nmr chemical shift values of **1** with those reported in the literature for halistanol sulfate (9) strongly suggested the presence of a cholestane nucleus having an A/B trans ring junction with oxygen substitution at C-2, C-3, and C-6. The ^1H-nmr spectrum confirmed the presence of three esterified hydroxy methines (δ 4.18, 4.73, and 4.76), and the presence of eight methyl groups. The splitting pattern observed for these methyls confirmed the presence of four tertiary methyls and four secondary methyls. The ^1H-nmr chemical shift values for methyl groups at δ 0.68 (s), 1.04 (s), and 0.93 (d) were in agreement with the reported values for C-18, C-19, and C-21, respectively (9). The chemical shift value of δ 0.93 (d, J=6.5 Hz) for C-21 methyl indicated the 20*S* configuration (10).

In the COSY nmr spectrum, the narrow multiplet signal for H-2 at δ 4.76 showed couplings to H-3 at δ 4.73 and to both H-1 methylene protons at δ 2.08 and 1.45. The narrow multiplet for H-3 showed couplings to H-2, and to signals for H-4 protons at δ 1.77 and 2.25, respectively, thus establishing the proton connectivity of the steroid A ring. The results from the HMBC nmr experiment indicated long-range C-H correlations from H-2 to C-10 (δ 37.6) and C-4 (δ 25.0), and from H-3 to C-1 (δ 39.2) and C-5 (δ 45.3), thus unambiguously establishing the oxygenation pattern of the A ring. Furthermore, these results eliminated possible methylation at C-4. Also, in the COSY nmr spectrum, the C-6 proton observed at δ 4.18 (ddd, J=11.0, 11.0, and 4.3 Hz) indicated couplings to the C-5 methine proton (δ 1.63), and to the C-7 methylene protons (δ 1.05 and 2.34). The long-range C-H correlations observed from H-6 to C-4 and C-5 confirmed the position of this last oxymethine proton. The large coupling constants observed established axial (βH) configuration. Assignment of the stereochemistry of the C-2 and C-3 sulfoxy groups was based on the coupling information obtained from COSY nmr and difference decoupling experiments. The narrow multiplets for H-2 and H-3 with width at half height of 8 Hz, indicated lack of a large vicinal coupling (>5 Hz) constant and thus precluded an axial-axial coupling of H-2 or H-3 with any neighboring protons. These data established a 2β,3α,6α-trisulfoxy configuration con-

sistent with that reported for halistanol sulfate (9) and sokotrasterol sulfate (7).

A combination of APT and DEPT nmr spectra revealed the presence of one quaternary, three methine, two methylene, and six methyl carbons in addition to the signals accounted for by the stanol nucleus, and thus confirmed the presence of an uncommon C_{12} side-chain in the molecule. The structure of the side-chain was established by analyzing the results from COSY, TOCSY, HMQC, and HMBC nmr experiments. In the COSY spectrum of **1**, the methyl protons observed at δ 0.93 (H-21) revealed coupling to the methine proton at δ 1.37 (H-20), which in turn was coupled to a methine proton at δ 1.16 (H-17) and to one of the methylene protons at δ 0.90 (H-22). The latter methylene proton indicated coupling to one of the methylene protons at δ 1.08 (H-23). In the TOCSY experiment of **1**, the methylene proton observed at δ 0.90 (H-22) revealed couplings to the geminal proton at δ 1.55 (H-22), vicinal protons at δ 1.37 (H-20) and 1.60 (H-23), and distant protons at 1.16 (H-17), 1.25 (H-24), and 0.80 (Me-28). The irradiation of the heptet at δ 1.72 (H-27) collapsed the two methyl doublets at δ 0.82 (Me-30) and 0.80 (Me-31) into two singlets and thus established the presence of a terminal isopropyl group in the side-chain. Both COSY and TOCSY experiments indicated that the heptet was coupled only to the two methyl groups and thus indicated that this isopropyl group was attached to the remaining quaternary carbon. The remaining two methyl groups appeared at δ 0.69 (Me-26) and 0.71 (Me-29) were attached to the quaternary carbon and thus completed the structure of the side-chain. In the long-range C-H correlated (HMBC) spectrum there were cross-peaks appearing from H-28 or H-31 (δ 0.80) and H-30 to C-25 (δ 38.7), from H-26 and H-29 to both C-24 (δ 41.3) and C-27 (δ 34.5), and from H-21 to C-17 (δ 57.3) and C-22 (δ 36.7). These connectivities confirmed the arrangement of the methyl groups in the side-chain. The structure of ophirapstanol trisulfate [**1**] was thus established based on the above data.

Acid hydrolysis of **1** gave ophirapstanol [**2**] as colorless needles. The high-resolution mass spectrum established a molecular formula of $C_{31}H_{56}O_3$ which was supported by nmr data.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were obtained on a Midac Ft-ir M 1200 spectrophotometer. ^{13}C-Nmr spectra were measured on a Bruker AM-360. ^{1}H- and all 2D nmr spectra were measured on a Bruker AMX-500. Chemical shifts were referenced to CD_3OD solvent signal at 3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C. All 2D spectra were run non-spinning. The high-resolution mass spectra were obtained on a Finnigan MAT95Q mass spectrometer at the Department of Chemistry, University of Florida. Optical rotations were measured with a Jasco DIP 360 digital polarimeter.

ANIMAL MATERIAL.—The sponge *Topsentia ophiraphidites* (phylum Porifera, class Demospongiae, order Halichondrida, family Halichondriidae) (11) was collected by the Johnson-Sea-Link I manned submersible from the Gulf of Mexico at a depth of 168 m. A taxonomic voucher specimen is deposited in the Harbor Branch Oceanographic Museum (DBMR no. 4-II-88-1-026).

EXTRACTION AND ISOLATION.—The freshly thawed sponge (90 g, wet wt) was extracted three times with EtOH. The concentrated extract was then partitioned between EtOAc and H_2O. The H_2O-soluble fraction (1.9 g) which showed activity in the GDPX assay (100% inhibition) was repartitioned between BuOH and H_2O. Both the BuOH and H_2O fractions gave a similar activity profile of 100% inhibition at the concentration of 200 μg/ml. The H_2O-soluble fraction was then subjected to reversed-phase hplc (C_{18}, 5 μm, 250×10 mm i.d.) with 25% H_2O/MeOH acidified with 0.1% TFA to give pure ophirapstanol trisulfate [**1**]. The yield was not calculated as no effort was made to quantitatively isolate **1** in the BuOH-soluble fraction.

Ophirapstanol trisulfate [**1**].—Obtained as a white powder (42 mg): $[\alpha]^{24}$D 17.3° (c=0.12, MeOH); ir (dry film) ν max 3479 (br), 3105, 2961, 2305, 1398, 1243, 1213, 1063, 931 cm^{-1}; ^{1}H nmr (CD_3OD, 500 MHz) δ 4.76 (1H, br s, H-2), 4.73 (1H, br s, H-3), 4.18 (1H, ddd, J=11.0, 11.0, and 4.3 Hz, H-6), 2.34 (1H, ddd, J=12.2, 4.3, and 4.1 Hz, H-7), 2.25 (1H, br d, J=14.5 Hz, H-4), 2.08 (1H, br d, J=14.0 Hz, H-1), 1.99 (1H, br d,

J=12.5 Hz, H-12), 1.85 (1H, m, H-16), 1.77 (1H, ddd, J=14.5, 12.7, and 3.0 Hz, H-4), 1.72 (1H, hept, J=6.6 Hz, H-27), 1.63 (1H, ddd, J=12.7, 11.0, and 3.0 Hz, H-5), 1.60 (1H, m, H-23), 1.60 (1H, m, H-15), 1.55 (1H, m, H-8), 1.55 (1H, m, H-11), 1.55 (1H, m, H-22), 1.45 (1H, dd, J=14.0 and 3.6 Hz, H-1), 1.37 (1H, m, H-20), 1.30 (1H, m, H-11), 1.30 (1H, m, H-16), 1.25 (1H, m, H-24), 1.16 (1H, m, H-17), 1.15 (1H, m, H-12), 1.10 (1H, m, H-14), 1.08 (1H, m, H-23), 1.05 (1H, br dd, J=12.2 and 11.0 Hz, H-7), 1.04 (3H, s, Me-19), 0.93 (3H, d, J=6.5 Hz, Me-21), 0.90 (1H, m, H-22), 0.82 (3H, d, J=6.6 Hz, Me-30), 0.80 (3H, d, J=6.6 Hz, Me-31), 0.80 (3H, d, J=6.5 Hz, Me-28), 0.75 (1H, ddd, J=11.4, 11.0, and 3.2 Hz, H-9), 0.71 (3H, s, Me-29), 0.69 (3H, s, Me-26), 0.68 (1H, m, H-15), 0.68 (3H, s, Me-18); ^{13}C nmr (CD_3OD, 90.5 MHz) δ 78.7 (d, C-6), 75.6 (d, C-2), 75.5 (d, C-3), 57.6 (d, C-14), 57.3 (d, C-17), 55.8 (d, C-9), 45.3 (d, C-5), 43.8 (s, C-13), 41.3 (d, C-24), 41.1 (t, C-12), 40.1 (t, C-7), 39.2 (t, C-1), 38.7 (s, C-25), 37.7 (d, C-20), 37.6 (s, C-10), 36.7 (t, C-22), 35.2 (d, C-8), 34.5 (d, C-27), 29.1 (t, C-16), 28.6 (t, C-15), 25.1 (t, C-23), 25.0 (t, C-4), 21.8 (t, C-11), 19.9 (q, C-26), 19.8 (q, C-29), 19.6 (q, C-21), 17.8 (q, C-30), 17.8 (q, C-31), 15.3 (q, C-19), 14.6 (q, C-28), 12.5 (q, C-18); hrfabms (triethanolamine) m/z 759.254, Δ 5.0 mmu for $C_{31}H_{53}O_{12}S_3Na_2(M-Na^+)^-$, m/z 737 (12), 669 (3), 657 (100), 635 (16), 555 (38), 537 (18), 469 (3).

HYDROLYSIS OF **1**.—Compound **1** (10 mg) was hydrolyzed in 3 N HCl (70°, 0.5 h), and the hydrolysate was diluted with H_2O and extracted with EtOAc. The solvent was evaporated and crystallized in $CHCl_3$ to give ophirapstanol [**2**] as colorless crystals (5 mg). Mp 258–260°; $[\alpha]^{24}D$ 23.0° (c=0.05, MeOH); ir (dry film) ν max 3359, 2965, 2937, 2848, 1448, 1379, 1035 cm^{-1}; ^{1}H nmr ($CDCl_3$/10% CD_3OD) δ 3.74 (1H, br s, H-3), 3.72 (1H, br s, H-2), 3.28 (1H, ddd, J=11.0, 11.0, and 4.3 Hz, H-6), 1.90 (1H, m, H-7), 1.90 (1H, m, H-12), 1.82 (1H, br d, J=14.5 Hz, H-4), 1.76 (1H, m, H-16), 1.67 (1H, ddd, J=14.5, 12.7, and 3.0 Hz, H-4), 1.63 (1H, m, H-27), 1.60 (1H, br d, J=14.0 Hz, H-1), 1.52 (1H, m, H-23), 1.47 (1H, m, H-15), 1.43 (1H, m, H-22), 1.42 (1H, m, H-11), 1.38 (1H, m, H-8), 1.35 (1H, dd, J=14.0 and 3.6 Hz, H-1), 1.35 (1H, m, H-5), 1.29 (1H, m, H-20), 1.20 (1H, m, H-16), 1.18 (1H, m, H-11), 1.15 (1H, m, H-24), 1.07 (1H, m, H-12), 1.07 (1H, m, H-17), 0.98 (1H, m, H-14), 0.95 (1H, m, H-23), 0.89 (3H, s, H-19), 0.83 (3H, d, J=6.5 Hz, H-21) 0.81 (1H, m, H-22), 0.80 (1H, m, H-7), 0.72 (3H, d, J=6.5 Hz, H-30), 0.70 (3H, d, J=6.5 Hz, H-31), 0.68 (3H, d, J=6.5 Hz, H-28), 0.65 (1H, ddd, J=11.4, 11.0, and 3.2 Hz, H-9), 0.61 (3H, s, H-29), 0.60 (1H, m, H-15), 0.58 (3H, s, H-26), 0.56 (3H, s, H-18); ^{13}C nmr ($CDCl_3$/10% CD_3OD, 90.5 MHz) δ 70.6 (d, C-2), 70.0 (d, C-3), 69.4 (d, C-6), 56.6 (d, C-14), 56.3 (d, C-17), 54.9 (d, C-9), 46.0 (d, C-5), 42.9 (s, C-13), 41.8 (t, C-7), 40.5 (t, C-12), 40.3 (d, C-24), 40.2 (t, C-1), 37.9 (s, C-25), 36.8 (s, C-10), 36.7 (d, C-20), 35.8 (t, C-22), 34.1 (d, C-8), 33.5 (d, C-27), 28.4 (t, C-16), 27.8 (t, C-15), 25.5 (t, C-4), 24.4 (t, C-23), 21.0 (t, C-11), 19.6 (q, C-26), 19.5 (q, C-29), 19.2 (q, C-21), 17.5 (q, C-30), 17.5 (q, C-31), 15.3 (q, C-19), 14.3 (q, C-28), 12.2 (q, C-18); hrfabms (dithiothreitol and dithioerythritol, 3:1) m/z 499.418, Δ 5.0 mmu for $C_{31}H_{56}O_3Na$ $(M+Na)^+$, m/z 423 (38), 391 (8), 309 (64), 258 (40).

GUANOSINE DIPHOSPHATE/G-PROTEIN RAS EXCHANGE ASSAY (GDPX).—This assay was performed according to the procedure described by Hall and Self (12). Human recombinant RAS was supplied by Schering-Plough Research Institute, and ^{3}H-GDP was supplied by New England Nuclear.

ACKNOWLEDGMENTS

We are grateful to Dr. Peter McCarthy for helpful discussions and Dr. Shirley Pomponi for her assistance in the identification of the sponge sample. We thank Dr. Paul Kirschmeier of SPRI for supplying recombinant RAS protein and for help in development of the GDPX assay and David Powell of the University of Florida for mass spectral measurements. This is Harbor Branch Oceanographic Institution Contribution No. 1055.

LITERATURE CITED

1. M.V. D'Auria, L. Minale, and R. Riccio, *Chem. Rev.*, **93**, 1839 (1993).
2. R.G. Kerr, S.L. Kerr, G.R. Pettit, D.L. Harold, T.L. Groy, and C. Djerassi, *J. Org. Chem.*, **56**, 58 (1991).
3. L.N. Li and C. Djerassi, *Tetrahedron Lett.*, **22**, 4639 (1981).
4. L.N. Li and C. Djerassi, *J. Am. Chem. Soc.*, **103**, 3606 (1981).
5. L.N. Li, U. Sjorstrand, and C. Djerassi, *J. Org. Chem.*, **46**, 3867 (1981).
6. L.K. Shubina, T.N. Makarieva, A.I. Kalinovsky, and V.A. Stonik, *Khim. Prir. Soedin*, 232 (1985).
7. T.N. Makarieva, L.K. Shubina, A.I. Kalinovsky, V.A. Stonik, and G.B. Elyakov, *Steroids*, **42**, 267 (1983).
8. D.R. Lowy and B.M. Willumsen, *Ann. Rev. Biochem.*, **62**, 851 (1993).
9. N. Fusetani, S. Matsunaga, and S. Konosu, *Tetrahedron Lett.*, **22**, 1985 (1981).
10. M. Koreeda and N. Koizumi, *Tetrahedron Lett.*, 1641 (1978).
11. M.C. Diaz, S.A. Pomponi, and R.W.M. van Soest, *Sci. Mar.*, **57**, 283 (1993).
12. A. Hall and A.J. Self, *J. Biol. Chem.*, **261**, 10963 (1986).

Received 27 June 1994