Novel HIV-Inhibitory Halistanol Sulfates F-H from a Marine Sponge, Pseudoaxinissa digitata

G. Bifulco, I. Bruno, L. Minale, and R. Riccio

J. Nat. Prod., **1994**, 57 (1), 164-167• DOI:
10.1021/np50103a026 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 1, pp. 164-167, January 1994

NOVEL HIV-INHIBITORY HALISTANOL SULFATES F–H FROM A MARINE SPONGE, *PSEUDOAXINISSA DIGITATA*

G. BIFULCO, I. BRUNO, L. MINALE,* and R. RICCIO

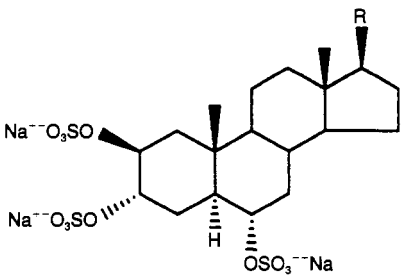
Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II," via D. Montesano, 49, 80131 Napoli, Italy

ABSTRACT.—Three new steroid sulfates **2–4** related to halistanol sulfate [**1**] were isolated along with the known halistanol sulfate C [**5**] from the marine sponge *Pseudoaxinissa digitata*. Halistanol sulfates F [**2**] and G [**3**] proved to be cytoprotective against HIV.

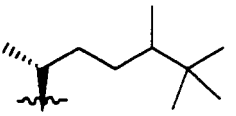
Halistanol sulfates are a group of sulfated polyhydroxysteroids from sponges, which are very attractive because of their biological activity. They are characterized by the same 2β,3α,6α-trisulfoxy functionalities (e.g., halistanol sulfate [**1**]), differing only in their side chains, and have been isolated from sponges of the family Halichondriidae (1–3) and more recently from a species of *Epipolasis* (4). McKee *et al.* (5) have recently reported the discovery of a further member of this series, ibisterol sulfate, from a *Topsentia* sp., which combines a $\Delta^{9(11)}$ olefin and a methyl group at C-14 with the 2β,3α,6α-trisulfoxy functionalities and a cyclopropane-containing side chain. Halistanol sulfate and ibisterol sulfate were cytoprotective against HIV.

In the course of our continuing studies of bioactive metabolites from marine invertebrates we have collected a sponge, *Pseudoaxinissa digitata* Cabioch (order Axinellida, family Axinellidae) in the Mediterranean sea off Tunisia. Extraction and isolation afforded three new halistanol sulfates named halistanol sulfates F [**2**], G [**3**], and H [**4**] along with the known halistanol sulfate C [**5**] (4). This paper deals with the isolation and structure elucidation of these compounds.

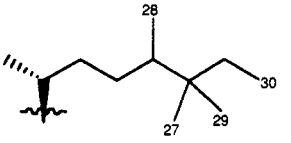
The *n*-BuOH-soluble portion of the aqueous Me_2CO extract of the frozen sponge (300 g) was fractionated on Sephadex LH-20, followed by dccc and reversed-phase hplc to yield **2–5**. The ^{1}H- and ^{13}C-nmr spectra readily implied that all compounds **2–5** had a steroid sulfate nucleus identical with that of the known halistanol sulfate [**1**] (1) (Tables 1,2). This was supported by negative ion fab mass spectra: **2**, *m/z* 745 [St $(SO_3Na)_2SO_3]^-$, 723 [St $(SO_3Na)(SO_3H)SO_3]^-$, 643 [723 $-SO_3]^-$; **3**, *m/z* 717 [St $(SO_3Na)_2SO_3]^-$, 695 [St $(SO_3Na)(SO_3H)SO_3]^-$; **4**, *m/z* 715 [St $(SO_3Na)_2SO_3]^-$, 693 [St $(SO_3Na)(SO_3H)SO_3]^-$, 613 [693 $-SO_3$]; **5**, *m/z* 703 [St $(SO_3Na)_2SO_3]^-$, 681 [St $(SO_3Na)(SO_3H)SO_3]^-$, 601 [681 $-SO_3]^-$. The structure of the side chain of halistanol sulfate F [**2**] was elucidated by analysis of 2D ^{1}H- and ^{13}C-nmr data. The fabms and nmr spectra indicated the presence of a saturated C_{11} side chain containing two secondary methyls (δ_H 0.97 and 0.85 ppm; δ_C 19.6 and 14.6 ppm), two tertiary methyls (δ_H 0.82 and 0.83 ppm; δ_C 24.6 and 24.4 ppm), and an ethyl group (δ_H 0.84 t, 1.28 q; δ_C 8.5 and 33.8 ppm). The COSY spectrum allowed the connectivities C-20 to C-28 to be determined and established the location of the secondary methyls on the steroid side chain at the biogenetically common positions 20 and 24. Thus, the two tertiary methyl groups and the ethyl group must be placed at the end of the side chain. A comparison of the ^{13}C-nmr data of halistanol sulfate F [**2**] with those of halistanol sulfate [**1**] (1) strongly supported the unusual side chain in **2**. The signals from C-1 to C-23 match closely in the two spectra; the presence of an ethyl group at C-25 in **2**, instead of the methyl group in **1**, causes upfield shifts at the signals of C-24, C-27, and C-29 (γ effects; C-24: 42.3 vs. 45.4, **2** vs. **1**; C-27, C-29: 24.6, 24.4 vs. 27.8 ppm; **2** vs. **1**)



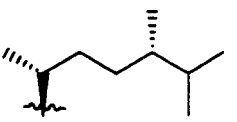
1 R=



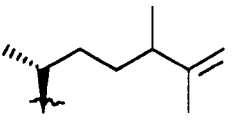
2 R=



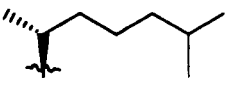
3 R=



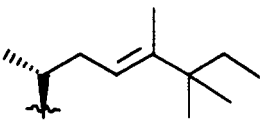
4 R=



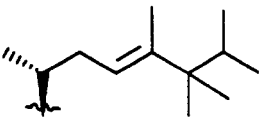
5 R=



6 R=



7 R=



and a downfield shift of C-25 (β effect; C-25: 37.5 vs. 34.1, **2** vs. **1**). The 20*R* stereochemistry is implied by the ^{1}H-nmr chemical shift of Me-21 (6). Halistanol sulfate F is the fourth example of a C-25 methylated naturally occurring sterol. However, in the polar steroids fraction of the sponge *Trachyopsis halichondroides*, a halistanol-sulfate-like component, 26-norsokotrasterol sulfate [**6**], has been found with a similar side chain but with a double bond at the C-23 position (3). It was supposed that the formation of 26-norsokotrasterol as well as sokotrasterol [**7**] (2) originated from a 24-methyl-25(26)-ene side chain through two and three consecutive methylations by SAM (S-adenosylmethionine), respectively. Recently Djerassi and his group (7) have elucidated the biosynthesis of mutasterol, a Δ^5-3β-OH sterol with a 24-methylene-25,26-dimethyl cholestene side chain from a Caribbean sponge, by feeding selective radioactive precursors. They showed that codisterol (24*S*-methylcholesta-5,25-dien-3-β-ol) is efficiently transformed into mutasterol. Interestingly, *P. digitata* contains halistanol sulfate H [**4**], the potential precursor of 26-norsokotrasterol sulfate

TABLE 1. ^{13}C-nmr (125 MHz, CD_3OD) Data of Compounds **2–4**.

Carbon	Compound		
	2	**3**	**4**
C-1	40.0	40.2	40.1
C-2	75.3	75.7	75.5
C-3	75.3	75.7	75.5
C-4	25.1	25.2	25.1
C-5	45.2	45.5	45.3
C-6	78.5	78.9	78.7
C-7	39.2	39.4	39.2
C-8	35.2	35.3	35.2
C-9	55.8	56.0	55.9
C-10	36.5	37.4	37.7
C-11	21.9	21.9	21.9
C-12	41.1	41.3	41.2
C-13	43.8	43.9	43.8
C-14	57.2	57.6	57.5
C-15	25.2	25.2	25.1
C-16	29.2	29.2	29.1
C-17	57.6	57.8	57.7
C-18	12.6	12.5	12.5
C-19	15.3	15.3	15.3
C-20	37.5	37.5	36.9
C-21	19.6	19.4	19.2
C-22	36.6	34.2	32.2
C-23	28.6	31.8	34.8
C-24	42.3	40.6	42.8
C-25	37.5	32.9	151.0
C-26	33.8	18.2	110.2
C-27	24.6	20.7	20.7
C-28	14.6	16.0	18.7
C-29	24.4	—	—
C-30	8.5	—	—

[**6**], sokotrasterol sulfate [**7**], and halistanol sulfate F [**2**].

Halistanol sulfate G [**3**] had a side chain composed of four methyls, two methylenes, and three methines, reminiscent of a standard 24-methyl cholestane side chain, which was confirmed by the ^{13}C-nmr data (8). The 24*S* configuration is suggested from the ^{13}C-nmr chemical shifts of 26- and 27-methyls (9).

Halistanol sulfate H [**4**] had a mol wt 2 mass units smaller than **3**. The ^{1}H-nmr spectrum included one two-proton olefinic signal at δ_H 4.72 (brs) and the signal for 27-methyl shifted to δ_H 1.67 (s), whereas the ^{13}C-nmr spectrum included two sp^2 carbon signals at δ_C 110.2 (CH_2) and 151.0 ppm (C). The ^{1}H- and ^{13}C-nmr spectra also revealed the presence of two secondary methyl groups, two methylenes, and two methynes, in agreement with a 24-methyl-25(26)ene side chain, which was evident from the comparison of the ^{13}C-nmr data with those of **3**. The cholestane side chain of **5** was evident from the ^{13}C-nmr data (8) and comparison with those reported for halistanol C (4).

Halistanol sulfates F [**2**] and G [**3**] were tested in the NCI primary anti-HIV screen and proved to be cytoprotective against HIV-1 (EC_{50} 3 and 6 μg/ml, respectively) and HIV-2.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker AMX-500 (^{1}H at 500 MHz, ^{13}C at 125 MHz), δ (ppm), *J* in Hz, spectra referred to CHD_2OD signal at 3.34 ppm and to central carbon CD_3OD signal at 49.0 ppm. Mass spectra were measured on a ZAB VG mass spectrometer equipped with fab source [in glycerol or glycerol-thioglycerol (3:1) matrix, Xe atoms of 2–6 kV]. Reversed-phase hplc was performed on a C18 ODS-2 column (30 cm×8 mm i.d.; flow rate 5 ml/min), Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401.

EXTRACTION.—The animals, *P. digitata*, were collected in Tunisia in June 1991 and identified by Professor M. Sarà, Istituto di Zoologia, Università di Genova; a voucher specimen is preserved there. The organisms (900 g) were chopped and soaked in Me_2CO (1 liter, 24 h, twice). The Me_2CO extract was evaporated in vacuo, the residue was partitioned between H_2O and Et_2O, and then the aqueous residue was re-extracted with *n*-BuOH. Evaporation of the *n*-BuOH extract afforded 7.8 g of glassy material, which was chromatographed in two runs on a column of Sephadex LH-20 (4×100 cm) with MeOH as eluent. Fractions (6 ml) were collected and analyzed by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5). The first-eluting fractions 20–90 (1.6 g in total) contained mainly a mixture of nucleosides and other polar compounds; the last-eluting fractions 150–180 (1.3 g) contained lipidic ethers, while the fractions 90–150 (850 mg) contained the sulfated steroids. These latter fractions were subjected to dccc using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the descending mode (the upper phase was the stationary phase, flow rate 15 ml/h; 5-ml fractions were collected and monitored by tlc). Fractions 40–50 (180 mg) were evaporated, and the residue was submitted to

TABLE 2. ^{1}H-nmr (500 MHz, CD_3OD) Data of Compounds **2–4** [coupling constants (Hz) in parentheses].

Proton	Compound		
	2[a]	**3**	**4**
H-1	2.12 brd (14.5) 1.50 dd (14.5; 4)	2.12 brd (14.5) 1.50 dd (14.5; 4)	2.12 brd (14.5) 1.50 dd (14.5; 4)
H-2	4.84 brs	4.84 brs	4.84 brs
H-3	4.78 brs	4.78 brs	4.78 brs
H-4	2.32 brd (15) 1.83 dt (15; 2.7)	2.32 brd (15) 1.83 dt (15; 2.7)	2.32 brd (15) 1.83 dt (15; 2.7)
H-5	1.68 dt (2.7; 11.2)	1.68 dt (2.7; 11.2)	1.68 dt (2.7; 11.2)
H-6	4.22 dt (4.4; 11.2)	4.22 dt (4.4; 11.2)	4.22 dt (4.4; 11.2)
H-7	2.40 dt (4.4; 12.2)	2.40 (4.4; 12.2)	2.40 dt (4.4; 12.2)
H-18	0.72 s	0.72 s	0.72 s
H-19	1.09 s	1.09 s	1.09 s
H-20	1.40 m		
H-21	0.97 d (6.6)	0.98 d (7)	0.98 d (7)
H-22	0.91 m		
H-23	1.65 m		
H-24	1.14 m		
H-26	1.28 q (7)	0.83 d (7)	4.72 brs
H-27	0.82 s	0.83 d (7)	1.67 s
H-28	0.85 d (6.5)	0.91 d (7)	1.03 d (7)
H-29	0.83 s	—	—
H-30	0.84 t (7)	—	—

[a]Assignments based on ^{1}H-^{1}H COSY NMR experiment.

reversed-phase hplc, MeOH–H_2O (4:6) to collect compound **4** (5 mg), compound **5** (12 mg), compound **3** (9 mg), and compound **2** (30 mg) eluted in this order. ^{1}H- and ^{13}C-nmr spectra are in Tables 1 and 2; fabms data are in the text.

ACKNOWLEDGMENTS

Halistanol sulfates F [**2**] and G [**3**] were tested in the NCI's primary anti-HIV screen, and we thank Drs. J.H. Cardellina II, T.C. McKee, J. McMahon, and M. Boyd of the National Cancer Institute, Frederick, Maryland. The chemical work was supported by CNR (P.F. Chimica fine II), Rome and Ministero dell-Università e della Ricerca Scientifica e Tecnologica "Murst," Rome. Mass spectra were provided by "Servizio di spettrometria di massa del CNR e dell'Università di Napoli;" the assistance of the staff is gratefully acknowledged. We also thank Professor M. Sarà, Istituto di Zoologia, Università di Genova for the identification of the sponge.

LITERATURE CITED

1. N. Fusetani, S. Matsunaya, and S. Konosu, *Tetrahedron Lett.*, **22**, 1985 (1981).
2. T.N. Makariewa, L.K. Shubina, A.I. Kalinowsky, V.A. Stonik, and G.B. Elyakov, *Steroids*, **42**, 267 (1983).
3. T.M. Makav'era, P.S. Dmitrenok, L.K. Shubim, and V.A. Stonik, *Chem. Nat. Compd.*, 312 (1988).
4. S. Kanazawa, N. Fusetani, and S. Matsunaga, *Tetrahedron*, **48**, 5467 (1992).
5. T. McKee, J.H. Cardellina II, M. Tischler, K.M. Serader, and M.R. Boyd, *Tetrahedron Lett.*, **34**, 389 (1993).
6. W.R. Nes, T.E. Vorkey, and K. Kravitz, *J. Am. Chem. Soc.*, **99**, 260 (1977).
7. G.R. Kerr, S.L. Kerr, and C. Djerassi, *J. Org. Chem.*, **56**, 63 (1991).
8. T.W. Blunt and T.B. Stothers, *Org. Magn. Reson.*, **9**, 439 (1977).
9. J.L.C. Wright, A.G. McInnes, S. Shimizu, D.G. Smith, J.A. Walker, D. Idler, and W. Khalil, *Can. J. Chem.*, **56**, 1978).

Received 1 June 1993