Notes

Absolute Configuration of 2,6-Dimethylheptyl Sulfate and Its Distribution in Ascidiacea

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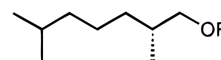
2,6-Dimethylheptyl sulfate (**1**) was isolated for the first time from a marine ascidian from *Policitor adriaticus* Drasche (class Ascidiacea, order Aplousobranchiata, family Polycitoridae). In this work, the absolute stereochemistry at C-2 of **1** was determined by ^{1}H-NMR analysis of its (+)- and (−)-methoxy(trifluoromethyl)phenylacetic acid (MTPA) esters (Mosher's method). The distribution of **1** was investigated among the three orders (Aplousobranchiata, Phlebobranchiata, and Stolidobranchiata) of the class Ascidiacea. Compound **1** showed cytotoxicity (LC_{50} = 17.8 μg/mL) in the *Artemia salina* bioassay and was not toxic (not toxicity at 100 μg/mL) in a fish (*Gambusia affinis*) lethality assay.

In the course of our search for polar metabolites from marine organisms, we recently isolated the sulfated normonoterpenoid **1** from the ascidian *Policitor adriaticus* Drasche (class Ascidiacea, order Aplousobranchiata, family Polycitoridae), collected in the northern Adriatic.[1] Compound **1**, which was characterized without assignment of its absolute stereochemistry, is the first sulfated normonoterpenoid to be isolated from a marine ascidian. At the same time, Tsukamoto[2] reported the isolation of some sulfated alkanes and alkenes, including **1**, as a racemate, from different Japanese ascidians.

The knowledge of absolute stereochemistry for the bioactive metabolites is of fundamental importance because, generally, only one of the stereoisomers shows biological activity. In this study, we describe the absolute stereochemistry at C-2 of **1** using Mosher's (^{1}H) method,[3] its distribution in different ascidians, and its toxicity to *Artemia salina* and *Gambusia affinis*.

The absolute stereochemistry at C-2 of **1** was achieved by ^{1}H-NMR analysis of (+) and (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters [Mosher's (^{1}H) method]. Mosher's (^{1}H) method is generally used to elucidate the absolute configuration of secondary alcohols.[4] Recently, Minale and co-workers[5] have used Mosher's method on primary alcohols in the determination of the absolute stereochemistry at C-25 of steroids hydroxylated at C-26. The signals of the H-26 protons in the ^{1}H-NMR spectra of the 26-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters of 25*S* isomers were much closer together for the (+)-MTPA derivative than for the (−) derivative; the reverse was true for the 25*R* isomers.

The alcohol **2**, obtained by solvolysis of **1** with pyridine−dioxane,[1] was treated with an excess of (+)- and



1 R = $SO_3Na(K)$

2 R = H

3 R = (+)MTPA

4 R = (-)MTPA

(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA−Cl) to yield (+)-MTPA (**3**) and (−)-MTPA (**4**) esters, respectively. The methylene proton signals of the (+)-MTPA ester appeared as well separated double doublets at δ 4.07 (J = 10.7, 6.7 Hz) and 4.23 (J = 10.7, 5.8 Hz), while in the spectrum of the (−)-MTPA ester they appear as a doublet at δ 4.15 (J = 6.1 Hz). These observations are in agreement with an *R* configuration for **2**.

The distribution of **1** was investigated among the three orders (Aplousobranchiata, Phlebobranchiata, and Stolidobranchiata) of the class Ascidiacea. Similar amounts (about 0.04% dry wt after extraction) of **1** were found in both members of the order Aplousobranchiata analyzed, *P. adriaticus* and *Aplidium conicum* Olivi (family Polyclinidae). Smaller amounts (about 0.01% dry wt) were found in *Phallusia mammilata* Cuvier (order Phlebobranchiata, family Ascidiiae), *Botryllus schlosseri*, Pallas (order Stolidobranchiata, suborder Styelidae family Botryllinae), *Phallusia fumigata* Grube (order Phlebobranchiata, family Ascidiiae), and *Ascidia virginea* Müller (order Phlebobranchiata, family Ascidiiae). The sulfate **1** was not detected in the extract of *Ciona intestinalis* Linne (order Phlebobranchiata, family Cionidae), *Styela plicata* Leseur (order Stolidobranchiata, suborder Styelidae, family Styelinae), *Microcosmus sulcatus* Coquebert (order Stolidobranchiata, family Pyuridae), or *Halocynthia papillosa* Linne (order Stolidobranchiata, family Pyuridae). The compounds isolated from the six ascidians were identified as 2,6-dimethylheptyl sulfate by comparison of spectral data

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with those of the authentic sample, and the compounds also had the same optical rotation as **1**.

Compound **1** showed a good activity (LC_{50} = 17.8 μg/mL) in the *Artemia salina* bioassay[6,7] and was not toxic (no toxicity at 100 μg/mL) in the fish (*Gambusia affinis*) lethality assay.[8]

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. IR cards 3M (KC-0061) were purchased from Carlo Erba Reagenti, Italy. Optical rotations were measured on a JASCO DIP 370 polarimeter, using a 10-cm microcell. EIMS were recorded on an Fisons TRIO 2000 spectrometer, coupled with an INTEL computer; FABMS were recorded on a VG analytical ZAB2SE double-focusing mass spectrometer, equipped with a cesium gun operating at 25 keV (2 μA) using glycerol (G) as matrix. ^{1}H-NMR spectra were recorded at 500 MHz with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X32 control. Chromatographies were performed using precoated Merck F_{254} plates, Kieselgel 60 powder, and Lichroprep RP-18 (40−63 μm).

Animals. *P. adriaticus* (voucher no. TPA/R94), *A. conicum* (TAC/R94), *B. schlosseri* (TBS/R94), and *M. sulcatus* (TMS/R94) were collected by dredging (−15 m) at Rovinj (Figarola, Bagnole Islands), Croatia, in September 1994. *H. papillosa* (THP/R95) and *A. virginea* (TAV/R94) were collected by dredging (−15 m) at Rovinj (Figarola, Bagnole Islands), Croatia, in October 1995. *P. mammilata* (TPM/V93), *C. intestinalis* (TCI/V93), and *S. plicata* (TSP/V93) were collected by scuba diving in the Lagoon of Venice, Italy, in May 1993. *P. fumigata* (TPF/N95) was collected by scuba diving in the Bay of Naples (Punta Epitaffio), Italy, in September 1995. The animals were frozen at −20 °C until extraction. A voucher specimen of each animal is maintained in the collection of ICMIB−CNR, Naples.

Extraction and Isolation of 2,6-Dimethylheptyl Sulfate. The frozen animals, *P. adriaticus* (50 g dry wt after extraction), *A. conicum* (45 g), *P. mammilata* (38 g), *B. schlosseri* (25 g), *C. intestinalis* (30 g), *S. plicata* (36 g), *M. sulcatus* (48 g), *H. papillosa* (43 g), *A. virginea* (52 g), and *P. fumigata* (47 g) were extracted with 4 × 500 mL of H_2O-Me_2CO (1:1), and after elimination of the solvent *in vacuo*, the aqueous residues were introduced onto a Lichroprep RP-18 (30 g) column, desalted with 1000 mL of H_2O, and eluted with 500 mL of MeOH. The MeOH fractions gave, after evaporation, a mixture partially soluble in MeOH. The MeOH-soluble portion was chromatographed on a Si gel column and eluted with $CHCl_3$−MeOH (4:1) to give 2,6-dimethylheptyl sulfate, 20 mg [0.04%; $[\alpha]_D$ +4.7° (*c* 0.18, $CHCl_3$)], 16 mg [0.035%; $[\alpha]_D$ +4.6° (*c* 0.15, $CHCl_3$)], 3.8 mg [0.01%; $[\alpha]_D$ +4.9° (*c* 0.03, $CHCl_3$)], 3.2 mg [0.013%; $[\alpha]_D$ +4.4° (*c* 0.03, $CHCl_3$)], 6.1 mg [0.012%; $[\alpha]_D$ +4.8° (*c* 0.06, $CHCl_3$)], and 4.5 mg [0.01 %; $[\alpha]_D$ +4.5° (*c* 0.04, $CHCl_3$)] for *P. adriaticus*, *A. conicum*, *P. mammilata*, *B. schlosseri*, *A. virginea*, and *P. fumigata*, respectively. The compounds isolated from the six ascidians showed the same R_f on TLC developted with $CHCl_3$−MeOH (4:1). The spectral data (^{1}H-NMR, IR, and MS) were in excellent agreement with those previously reported.[1]

Solvolysis of 2,6-Dimethylheptyl Sulfate. Compound **1** (15 mg) was heated at 110 °C overnight in dioxane (3 mL) and pyridine (3 mL). The cooled solution was neutralized with HCl 2 N and extracted with $CHCl_3$ (3 × 5 mL). The combined extract was evaporated *in vacuo* to give the alcohol **2** (10 mg).

Preparation of the Mosher Esters 3 and 4. Compound **2** (4 mg) was esterified with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (Aldrich) (20 μL) in dry pyridine (0.5 mL) for 2 h at room temperature to give, after the removal of the solvent, (+) methoxy-(trifluoromethyl)phenylacetic acid (MTPA) ester **3** (2 mg): $[\alpha]_D$ +7.9 (*c* 0.002, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (6H, d, J = 7.1 Hz), 0.90 (3H, d, J = 6.7 Hz), 1.12 (1H, m), 1.28 (3H, m), 1.53 (3H, m), 1.84 (1H, m), 3.55 (3H, s), 4.07 (1H, dd, J = 10.7, 6.7 Hz), 4.23 (1H, dd, J = 10.7, 5.8 Hz), 7.41 (3H, m) and 7.52 (2H, m).

(−)-Methoxy(trifluoromethyl)phenylacetic acid (MTPA) ester **4** (2 mg) was prepared using (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (Aldrich): $[\alpha]_D$ −17.2 (*c* 0.002, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.85 (6H, d, J = 6.6 Hz), 0.91 (3H, d, J = 6.6 Hz), 1.11 (1H, m), 1.26 (3H, m), 1.53 (3H, m), 1.83 (1H, m), 3.55 (3H, s), 4.15 (2H, d, J = 6.1 Hz), 7.41 (3H, m) and 7.51 (2H, m).

Biological Assays. A brine shrimp (*Artemia salina*) lethality assay performed as previously described[6,7] gave LC_{50} 17.8 μg/mL for **1**. A fish lethality assay using *Gambusia affinis* performed as already described,[8] showed that **1** was not toxic at doses up to 100 μg/mL.

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