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Structure and Synthesis of a Unique Isonitrile Lipid Isolated from the Marine Mollusk *Actinocyclus papillatus*

Emiliano Manzo,[†] Marianna Carbone,[†] Ernesto Mollo,[†] Carlo Irace,[‡] Antonio Di Pascale,[‡] Yan Li,[§] Maria Letizia Ciavatta,[†] Guido Cimino,[†] Yue-Wei Guo,*[,§] and Margherita Gavagnin*[,†]

Consiglio Nazionale delle Ricerche (CNR), Istituto di Chimica Biomolecolare (ICB), Via Campi Flegrei, 34, 80078 Pozzuoli (Na), Italy, Dipartimento di Farmacologia Sperimentale, Facoltà di Farmacia, Università 'Federico II' di Napoli, Italy, and State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China

ywguo@mail.shcnc.ac.cn; margherita.gavagnin@icb.cnr.it

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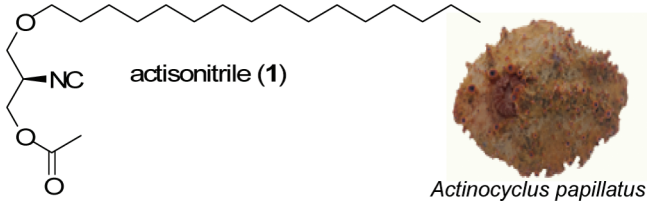
ABSTRACT

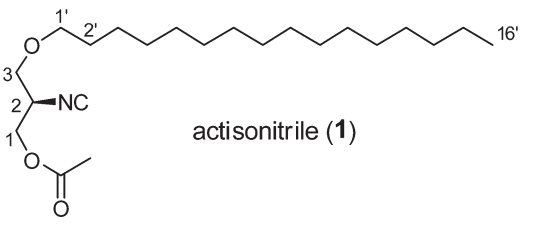


The first chemical study of an Actinocyclidae nudibranch, *Actinocyclus papillatus*, resulted in the isolation of (−)-actisonitrile (1), a lipid based on a 1,3-propanediol ether skeleton. The structure was established by spectroscopic methods, whereas the absolute configuration of the chiral center was determined by comparing the optical properties of natural actisonitrile with those of (+)- and (−)-synthetic enantiomers, opportunely prepared. Both (−)- and (+)-actisonitrile were tested in preliminary in vitro cytotoxicity bioassays on tumor and nontumor mammalian cells.

Lipids are undoubtedly representatives of chemical biodiversity. Fatty acids, fatty alcohols, and lipids containing them are ubiquitous in nature, and a number of structures occur in both terrestrial and marine organisms, even though the latter organisms have proven to be a major source of unique structures with promising bioactivity.[1] In particular, ether lipids usually occurring as minor components[2,3] have attracted the attention of medicinal chemists due to their antineoplastic[4] and other pharmacological activities.[3,5] Typical ether lipids are monoalkyl ethers of glycerin, also called alkyl/alkenyl glyceryl ethers.

In the course of our screening program for cytotoxic compounds from marine mollusks, we have isolated a unique ether lipid from a single specimen of the nudibranch *Actinocyclus papillatus*, belonging to the never studied Actinocyclidae family. The structure of this novel compound, which we named actisonitrile (**1**), is characterized by the presence of a 1,3-propanediol moiety bearing an isonitrile group at the C-2 position.

The isolation, structure elucidation, and synthesis of actisonitrile are herein reported together with the results of a preliminary bioactivity evaluation.



[†] Istituto di Chimica Biomolecolare (ICB).
[‡] Università `Federico II' di Napoli.
[§] Chinese Academy of Sciences.

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The mantle and the inner organs from one individual of *A. papillatus*, collected by scuba along the coast of Wei Zhou Island (South China Sea) during May 2007, were separately extracted with acetone. The TLC chromatographic analysis of the diethyl ether soluble portion of both extracts showed the presence in the mantle of a main metabolite (R_f 0.70, light petroleum ether/diethyl ether 1:1) which was not detected in the inner organs. The subsequent fractionation of the mantle extract (172.1 mg) by Sephadex LH20 chromatography, followed by further silica gel purification, afforded pure actisonitrile (**1**, 8.8 mg, 5.0%) as the major component (for details, see the Supporting Information).

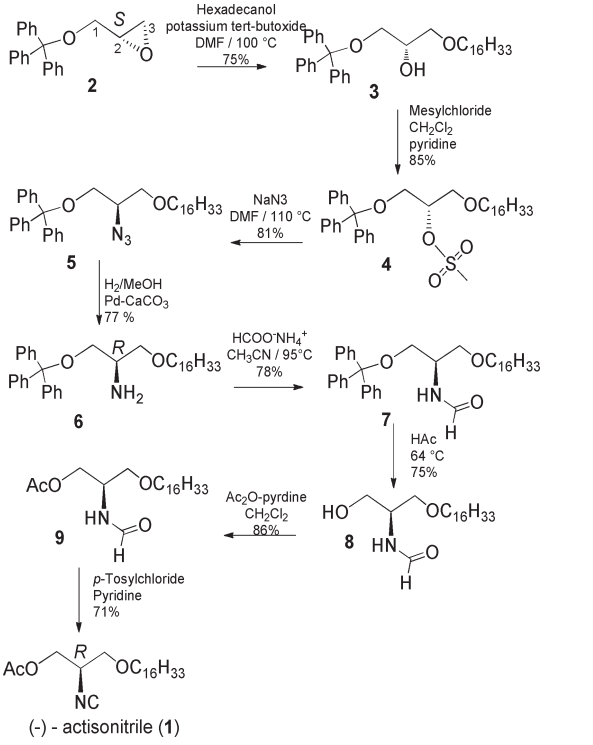
Actisonitrile (**1**)[6] showed in the HRESIMS spectrum a sodiated molecular peak at m/z 390.2973 according to the molecular formula $C_{22}H_{41}NO_3$. The co-occurrence in the ESIMS spectrum of a base peak at m/z 363, corresponding to the loss of HCN, suggested the presence of an isonitrile group. This hypothesis was further confirmed by the characteristic IR absorption at 2140 cm^{-1} as well as by the diagnostic signal at δ 158.7 in the ^{13}C NMR spectrum.

The ^1H NMR spectrum of **1** showed signals at δ 0.88 (3H, t, J = 7 Hz, H_3-16′), 1.20−1.38 (26H, overlapped multiplets, H_2-3′/ H_2-15′), 1.57 (2H, m, H_2-2′), 3.48 (2H, app t, J = 7 Hz, H_2-1′) that were attributed to a saturated C_{16} fatty alcohol. A typical set of signals at δ 3.63 (2H, m, H_2-3), 3.96 (1H, m, H-2), 4.20 (1H, dd, J = 11, 7 Hz, H-1a), 4.30 (1H, dd, J = 11, 4 Hz Hz, H-1b)] that could be attributed to a glyceryl-like fragment was also observed. The proton spectrum was completed by a 3H singlet at δ 2.12 assigned to an acetyl group. These data were consistent with a structure containing an acetylated 2-isonitrile-1,3-propanediol moiety connected to a fatty alcohol residue through an ether linkage. Analysis of the $^1H-^1H$ COSY experiment confirmed the defined spin systems. The ^{13}C NMR spectrum of **1** was very indicative. Along with the expected resonances due to the alkyl chain carbons of the fatty alcohol, the spectrum contained two oxygenated methylene signals at δ 69.1 (C-3) and 62.7 (C-1) and a methine carbon at δ 53.1 (C-2) indicating that the secondary carbon in the C_3 fragment had to be linked to the isonitrile group.

According to this structural hypothesis, the HMBC spectrum of **1** showed diagnostic correlations between C-3 and H_2-1′ as well as between the carbonyl of the acetyl residue and H_2-1.

Therefore, the structure of actisonitrile (**1**) was determined to be 3-(hexadecyloxy)-2-isonitrile-propyl acetate. This compound exhibits structural features without any precedent as it incorporates an isonitrile function in the 1,3-propanediol fragment. Actisonitrile (**1**) could be considered either a glyceryl-like ether in which the 2-OH group is replaced by an isonitrile function or a serinol (2-aminoglycerol) derivative. In this case, the isonitrile group should originate by dehydration of a corresponding *N*-formylserinol precursor. Even though glycerolipids are very common in marine organisms,[1] the occurrence of a certain number of serinolipids from tunicates,[7−10] sponges,[11] and cyanobacteria[12] implies that both biosynthetic pathways could be plausible. However, further studies are necessary to elucidate the true origin of this molecule.

Scheme 1. Synthesis of **1**



In order to assign the absolute configuration of C-2, which is the only chiral center of actisonitrile (**1**), the stereospecific total synthesis of both (−)- and (+)-enantiomers was carried out. The synthesis of each compound was accomplished in eight steps, as outlined in Scheme 1. The levorotatory enantiomer (−)-**1** was prepared starting from the commercially available *S*-(−)-glycidyl trityl ether (**2**). The opening of the epoxide **2** under basic conditions allowed the introduction of the fatty alcohol hexadecanol to the less substituted position. The subsequent mesylation of the intermediate **3**, followed by reaction with sodium azide, led to the formation of C−N bound at the C-2 position of the 1,3-propanediol moiety affording the

(6) Actisonitrile (**1**): $[\alpha]_D$ −7.0 (c = 0.27, $CHCl_3$); CD (*n*-hexane) $[\theta]_{204}$ 850; IR (liquid film) 2925, 2854, 2140, 1748, 1228 cm^{-1}; ^1H NMR values ($CDCl_3$) δ 0.88 (3H, t, J = 7 Hz, H_3-16′), 1.20−1.38 (26H, overlapped multiplets, H_2-3′/ H_2-15′), 1.57 (2H, m, H_2-2′), 2.12 (3H, s, $OCOCH_3$), δ 3.48 (2H, app t, J = 7 Hz, H_2-1′), 3.63 (2H, m, H_2-3), 3.96 (1H, m, H-2), 4.20 (1H, dd, J = 11, 7 Hz, H-1a), 4.30 (1H, dd, J = 11 and 4 Hz Hz, H-1b); selected ^{13}C NMR ($CDCl_3$) δ 14.1 (C-16′), 20.6 ($COCH_3$), 29.6 (C-2′), 53.1 (C-2), 62.7 (C-1), 69.1 (C-3), 72.0 (C-1′); ESIMS m/z 390 (M + Na), 363 (M − HCN + Na)$^+$; HR ESIMS calcd for $C_{22}H_{41}NO_3Na$ 390.2984, found 390.2973.

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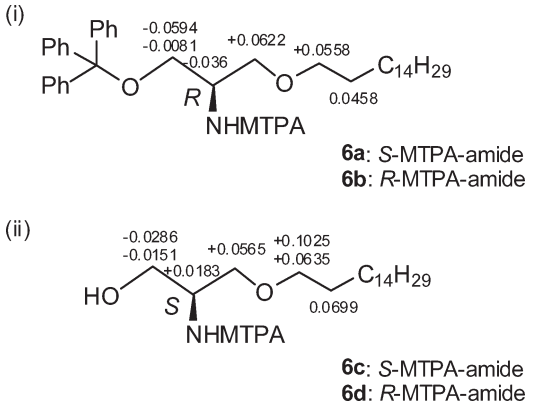

Figure 1. $\Delta\delta$ (ppm) for (i) Mosher derivatives **6a** and **6b** and (ii) the corresponding detritylated MTPA amides **6c** and **6d**.

corresponding azide **5**. Because introduction of the azide group is operated predominantly through a S_N2 mechanism, we decided to verify the inversion of configuration at C-2 by applying the modified Mosher method[13,14] to the amino derivative **6**, which was obtained by reduction with Lindlar's catalyst of azide **5**. Treatment of compound **6** with (*R*)- and (*S*)-MTPA chlorides in dry CH_2Cl_2 and DMAP afforded the corresponding (*S*)-MTPA (**6a**) and (*R*)-MTPA (**6b**) amides, respectively. The two Mosher derivatives were characterized by 2D-NMR experiments (see the Supporting Information). The $\Delta\delta$ ($\delta S - \delta R$) values observed for the signals of protons close to the amino group at C-2 indicated the *R* configuration (Figure 1i). With the aim of excluding a possible interaction of the trityl residue on the observed $\Delta\delta$ values that could invalidate the applied Mosher method, the evaluation of the proton chemical shift differences was also made on the detritylated MTPA amide derivatives (**6c** and **6d**) obtained by acetolysis of **6a** and **6b** (see the Supporting Information). According to the different priority order in **6c** and **6d** with respect to **6a** and **6b**, the *S* configuration was observed at C-2 (Figure 1ii).

Having confirmed the inversion of configuration at C-2, amine **6** was submitted to a formylation reaction. According to Reddy et al.,[15] the less expensive ammonium formate was used as *N*-formylating agent. The resulted formyl derivative **7** was then submitted to acetolysis to provide by removal of the trityl group the corresponding alcohol **8**. Compound **8** was acetylated affording the intermediate **9**. The subsequent dehydration of formamide **9** with *p*-tosyl chloride in pyridine led to (−)-3-(hexadecyloxy)-2-(*R*)-isonitrile-propyl acetate, (−)-actisonitrile **1**.

The dextrorotatory enantiomer (+)-3-(hexadecyloxy)-2-(*S*)-isonitrile-propyl acetate, (+)-actisonitrile, was prepared starting from (*R*)-(+)-glycidyl trityl ether and

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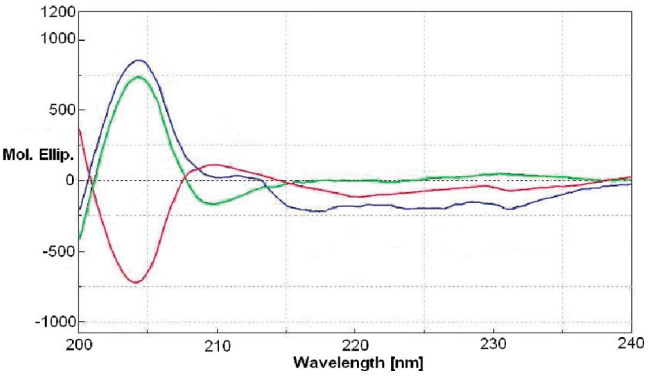

Figure 2. CD curves (θ) of natural actisonitrile (blue), synthetic (−)-actisonitrile (green), and synthetic (+)-actisonitrile (red).

following the same synthetic strategy (for details, see the Supporting Information).

The optical properties including the CD absorption of both synthetic products were compared with those of natural **1** (Figure 2). The negative optical rotation value and the positive CD profile of natural **1**[6] showed results similar to those of synthetic (−)-enantiomer,[16] thus assigning the *R* absolute stereochemistry.

By considering interest in the biological properties of ether lipids,[3−5] the bioactivity of both (−)- and (+)-actisonitrile enantiomers were tested in a fluorimetric culture cytotoxicity assay on tumor and nontumor mammalian cells (see the Supporting Information). Without showing appreciable bioselectivity, both enantiomers exhibited a parallel concentration-dependent toxic profile, displaying IC_{50} values within the micromolar range. In particular, (−)-**1** and (+)-**1** show moderate cytotoxicity against nontumor H9c2 rat cardiac myoblast cells (IC_{50} 23 ± 6 and 23 ± 6 μM, respectively).

The selective distribution of actisonitrile (**1**) in the external tissues of *A. papillatus* as well as its cytotoxicity could suggest a possible involvement of this molecule in the defensive mechanisms of the nudibranch. Specific ecological assays will be conducted in order to confirm this hypothesis.

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Supporting Information Available. Full experimental procedures, 1D and 2D NMR spectra of compound **1**, and spectral data of all synthetic intermediates including Mosher derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

(16) Synthetic (−)-(*R*)-actisonitrile: $[\alpha]_D$ −7.9 (c = 0.20, $CHCl_3$); CD (*n*-hexane) $[\theta]^{204}$ 748.