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Sinulasulfoxide and sinulasulfone, sulfur-containing alkaloids from the Indonesian soft coral *Sinularia* sp.

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ABSTRACT

Chemical analysis of the Indonesian soft coral *Sinularia* sp. (order Alcyonacea, family Alcyoniidae) afforded two new alkaloids, named sinulasulfoxide (**1**) and sinulasulfone (**2**), characterized by an amide linkage between a phytanic acid moiety and an uncommon sulfur-containing unit. Their complete stereostructures were elucidated by interpretation of MS and NMR data along with CD analysis and chemical modifications. Sinulasulfoxide (**1**) proved to moderately inhibit LPS-induced NO release.

Soft corals belonging to the family Alcyoniidae are by far the dominant reef dwelling octocorals in the Indo-West Pacific area. These organisms, characterized by a great variety in colors, shapes, and sizes, have been recognized as prolific producers of a wide array of secondary metabolites, particularly cembrane diterpenoids and steroids, often characterized by uncommon structural features and potent bioactivities.[1–3] Since some of these soft corals host large populations of zooxanthellae symbionts, a biosynthetic role for these microorganisms has been postulated.[4]

We have recently started a research project aimed at the evaluation of the chemical composition and of the biomedical potential of marine octocorals from the Indonesian coasts,[5–7] one of the richest biodiversity hot spots of the oceans. As part of this investigation, we have been analyzing a specimen of *Sinularia* sp. (order Alcyonacea, family Alcyoniidae), collected along the island of Siladen, in the Bunaken Marine Park of Manado (North Sulawesi, Indonesia). From the organic extract of this organism, we have recently reported the isolation of chloroscabrolides,[8] a class of chlorinated norcembranoids, and of polyhydroxylated steroids endowed with antagonistic activity against the farnesoid X-activated receptor.[9]

Inspection of more polar fractions obtained from the organic extract of *Sinularia* sp. has now resulted in the isolation of two new sulfur-containing alkaloids, named sinulasulfoxide (**1**) and sinulasulfone (**2**), along with the known furanosesquiterpenoid **3**[10] (Fig. 1). In this Letter we will provide details about the stereostructural elucidation of these metabolites and the results of the evaluation of their inducible NO-synthase (iNOS) protein inhibitory activity.

Colonies of *Sinularia* sp. (580 g wet weight) were homogenized and repeatedly extracted with MeOH and $CHCl_3$ at room temperature. The obtained organic extract (6.8 g) was chromatographed by MPLC over silica gel eluting with a gradient system of increasing polarity from *n*-hexane to EtOAc to MeOH. Fractions eluted with EtOAc were further purified by reversed-phase HPLC (eluent MeOH/H_2O 96:4) to afford sinulasulfone (**2**, 1.0 mg), fractions eluted with EtOAc/MeOH 9:1, purified by HPLC (eluent MeOH/H_2O 9:1), gave sinulasulfoxide (**1**, 3.9 mg), while fractions eluted with EtOAc/*n*-hexane 9:1 were purified by HPLC (eluent EtOAc/*n*-hexane 85:15) to give the known furanosesquiterpene (*E*)-5-(2,6-dimethylocta-5,7-dienyl)furan-3-carboxylic acid (**3**, 3.2 mg) identified by comparison of its spectral data with those reported in the literature.[10]

Sinulasulfoxide (**1**)[11] was obtained as an optically active amorphous solid and showed pseudomolecular ion peak at *m*/*z* 424 $[M+Na]^+$ in the ESI-MS (positive ions). The molecular formula $C_{23}H_{47}NO_2S$ was assigned to sinulasulfoxide on the basis of HR-ESIMS measurements.[11] The ^1H NMR spectrum of **1** (CD_3OD)

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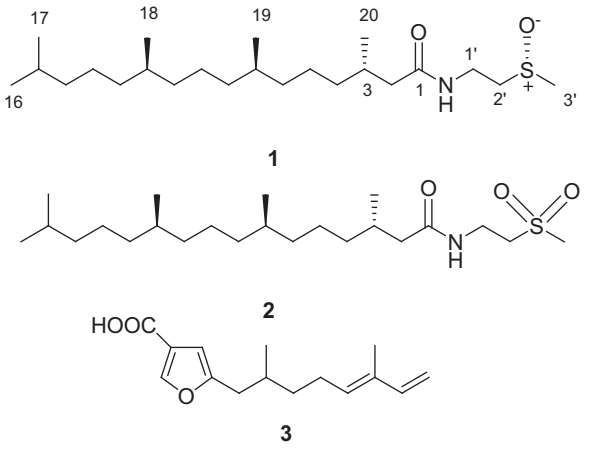

Figure 1. Chemical structures of sinulasulfoxide (**1**), sinulasulfone (**2**) and of the known furanosesquiterpenoid **3**.

showed six methyl resonances (five doublets at δ 0.94, 0.90, 0.89, 0.88, and 0.88, in addition to a deshielded methyl singlet at δ_H 2.70), a broad cluster of signals between δ_H 1.10–1.50, three methine multiplets at δ_H 1.54, 1.99, and 2.20, and four relatively deshielded methine signals at 2.94, 3.07, 3.58, and 3.64. All these proton signals were associated to those of the directly attached carbon atoms by means of the 2D NMR HSQC experiment, and, in particular, the multiplets resonating in the midfield region could be sorted out as two methylene groups (δ_C 43.5 and 53.9, respectively). The ^{13}C NMR spectrum of **1** (CD_3OD) showed the signal of a single unprotonated carbon, a carbonyl resonating at δ_C 174.9.

The COSY spectrum of **1** evidenced the mutual coupling of the two deshielded methylenes, which constituted an isolated spin system (Fig. 2), while the remaining multiplets could be organized in a single saturated polyisoprenoid spin system starting from the relatively deshielded methylene H_2-2 (δ_H 2.20 and 1.99) and terminating with the two methyl groups H_3-16 and H_3-17 to build up a phytanic acid moiety. The presence of an amide linkage between this moiety and 2-methylsulfinyl ethanamine fully accounted for the molecular formula and the correlations coming from the g-HMBC experiment (Fig. 1). In particular, the key g-HMBC cross-peaks of both H_2-2 and H_2-1′ with C-1 and of H_3-3′ with C-2′ confirmed the planar structure of sinulasulfoxide (**1**).

Sinulasulfoxide includes four stereogenic centers, three carbon atoms (C-3, C-7, C-11), and the sulfur atom. The assignment of the absolute configuration at this latter center was achieved through analysis of the CD spectrum of **1**. Indeed, Mislow et al.[12] have demonstrated the existence of a direct correlation between the absolute configuration of methyl alkyl sulfoxides and their optical activity in terms of the signs and rotational strengths of selected Cotton effects. In the absence of strongly perturbing groups, as in the case of monoallyl(aryl) or diallyl(aryl) sulfoxides, a negative Cotton effect, centered at the absorption band in the region 220–240 nm in acetonitrile, was found to be indicative of the *R* configuration. This correlation proved to be not significantly influenced by the presence of stereogenic centers on the alkyl group itself; since in sinulasulfoxide (**1**) the first asymmetric carbon is six-atoms far from the sulfur center, this rule could be confidently applied. The CD spectrum of **1** showed an absorption band at λ = 236 nm, whose

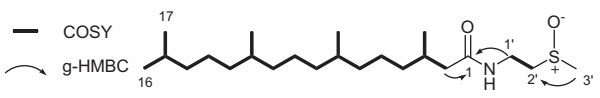

Figure 2. COSY (bold) and key g-HMBC correlations evidenced for sinulasulfoxide (**1**).

negative sign ($\Delta\varepsilon$ = −0.8), according to Mislow's rule, allowed the assignment of the *R* configuration at the sulfur atom in **1**.

Phytanic acid is a branched polyisoprenoid of considerable biological interest which, in the ocean, should be produced by sediment bacteria through oxidation and hydrogenation of the phytol unit present in the chlorophyll side chain.[13] Since two diastereomers of phytanic acid have been found in nature (3*S*,7*R*,11*R* and 3*R*,7*R*,11*R*), an evaluation of the configurational features of the phytanic acid unit present in sinulasulfoxide was needed. To this aim, sinulasulfoxide (1.2 mg) was treated with 70% acetic acid at 80 °C for 2 days, and the obtained mixture was partitioned between EtOAc and water. The organic phase was finally purified by HPLC (*n*-hexane/EtOAc 95:5) to obtain pure phytanic acid, whose ^{1}H NMR spectrum and $[\alpha]_D$ (−3.9, *c* 0.1) value were compared with those reported in the literature for the two diastereoisomers.[14] This comparison suggested its assignment as the 3*S*,7*R*,11*R* isomer.

Sinulasulfoxide is a unique secondary metabolite constituted by a phytanic acid linked to a sulfoxide-containing unit through an amide bond. The 2-methylsulfinyl ethanamine unit has been very rarely found in the structures of natural compounds and some *Piper* alkaloids[15] or psammaplin N from the sponge *Aplysinella rhax*[16] are among the few examples. On the other hand, its homolog 3-methylsulfinyl propanamine, the decarboxylated methionine sulfoxide, has been much more frequently reported.

Sinulasulfone (**2**)[17] is a secondary metabolite strictly related to sinulasulfoxide (**1**), from which it differs only for oxidation at the sulfur carbon. Compound **2** showed pseudomolecular ion peak at *m*/*z* 440 $[M+Na]^+$ in the ESI-MS (positive ions) and its molecular formula $C_{23}H_{47}NO_3S$, assigned on the basis of HR-ESIMS measurements, presented an additional oxygen atom compared to that of sinulasulfoxide. The ^{1}H and ^{13}C NMR spectra of **2**[17] closely paralleled those of **1** and strongly suggested the attachment of the additional oxygen atom at the sulfur atom. In particular, the ^{1}H NMR spectrum showed that, while the signals of the phytanic moiety were practically superimposable to those detected for **1**, consistent differences could be evidenced in the signals of the sulfur containing moiety. In particular, the methyl singlet was downfield shifted to δ_H 2.95, while the two methylenes of this moiety resonated as mutually coupled triplets at δ_H 3.22 (the S-linking) and 3.77 (the N-linking). The multiplicity of these protons was in perfect agreement with the lack of chirality of the sulfone group. All the proton and the carbon resonances of **2** were assigned on the basis of detailed 2D NMR (COSY, HSQC, and g-HMBC) analysis. Oxidation of sinulasulfoxide (**1**, 1.5 mg) with *meta*-chloroperoxybenzoic acid (rt, 3 h) afforded **2** in nearly quantitative yields, thus finally supporting the stereostructure assignment of sinulasulfone as reported in **2**. To our knowledge, sinulasulfone (**2**) is only the second sulfone-containing derivative from a soft coral, the first being austrasulfone, recently isolated as a neuroprotective agent from *Cladiella australis*.[18] Sinulasulfoxide (**1**) was left in MeOH/H_2O, EtOAc, and $CHCl_3$ solutions for one week at room temperature but no detectable changes were observed in any of the three solvents. Although this plays in favor of a genuine origin, we cannot exclude that sinulasulfone (**2**) could be an artifact obtained by oxidation of sinulasulfoxide during the isolation procedure.

It is interesting to notice that one of the taxonomic markers of *Sinularia* soft corals is the presence of spermidine amides of long chain fatty acids, such as the strongly cytotoxic compound **4**[19] or the geranylneroic derivative sinulamide (**5**)[20] (Fig. 3), showing H,K ATPase inhibitory activity. Sinulasulfoxide and sinulasulfone innovate this class of amphipathic compounds by showing, for the first time, a sulfur-containing polar moiety. To our knowledge, the role of this class of compounds in the producing organism has never been investigated.

Sinulasulfoxide (**1**) and furanosesquiterpene **3** were evaluated for inducible NO-synthase (iNOS) protein inhibitory activity. iNOS

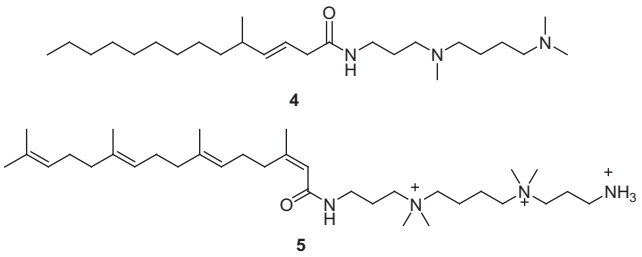

Figure 3. The structures of some spermidine derivatives obtained from *Sinularia* species.

is regulated by inflammatory mediators (LPS, cytokines), and the excessive production of NO by iNOS has been implicated in the pathogenesis of the inflammatory response.[21] In this assay, the production of NO_2^- (stable metabolite of NO) was evaluated as a parameter of macrophage activation and iNOS induction. Unstimulated J774 cells generated undetectable (<5 nmol/mL) amounts of NO_2^-, while stimulation of the cells with LPS (1 μg/mL) for 24 h produced a dose-dependent release of NO_2^- (15.6 ± 0.1 nmol/mL). After incubation of the cells with a concentration 30 μM of sinulasulfoxide (**1**) or of compound **3**, a moderate inhibition of NO_2^- production (22.6% and 25.7%, respectively), was observed.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.05.095. These data include MOL files and InChiKeys of the most important compounds described in this article.

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11. *Sinulasulfoxide (**1**).* Amorphous colorless solid; $[\alpha]_D^{25}$ +11.7 (*c* 0.2, MeOH); (+)ESI-MS (positive ions) *m*/*z* 424 [M+Na]$^+$. IR (KBr) ν_{max} 3288, 1662, 1630, 1609, 1255, 1050, 1014, 990 cm^{-1}; CD (CH_3CN): λ = 236 nm ($\Delta\varepsilon$ = −0.8); HR-ESIMS: *m*/*z* 424.3229 (calcd for $C_{23}H_{47}NaNO_2S$ 424.3225); ^{1}H NMR (CD_3OD, 500 MHz, *J* in Hz): 3.64 (1H, m, H-1′a), 3.59 (1H, m, H-1′b), 3.07 (1H, m, H-2′a), 2.94 (1H, m, H-2′b), 2.70 (3H, s, H_3-3′), 2.20 (1H, dd, *J* = 11.5, 6.5, H-2a), 1.99 (1H, t, *J* = 11.5, H-2b), 1.95 (1H, m, H-3), 1.54 (1H, non, *J* = 6.5, H-15), 1.41 (2H, m, H-7 and H-11), 1.25–1.30 (14H, m, H_2-5, H_2-6, H_2-8, H_2-9, H_2-10, H_2-13, H_2-14), 1.20 (2H, m, H-4a, H-13a), 1.10 (2H, m, H-4b, H-13b), 0.94 (3H, d, *J* = 6.5 Hz, H_3-20), 0.90 (3H, d, *J* = 6.5 Hz, H_3-16), 0.89 (3H, d, *J* = 6.5 Hz, H_3-17), 0.88 (6H, d, *J* = 6.5 Hz, H_3-18 and H_3-19); ^{13}C NMR (CD_3OD, 125 MHz): 174.9 (C-1), 53.9 (C-2′), 43.7 (C-2), 39.3 (C-14), 37.5 (C-3′), 37.4 (C-6, C-8, C-10, C-12), 37.0 (C-4), 35.5 (C-1′), 32.7 (C-7, C-11), 30.9 (C-3), 28.1 (C-15), 24.8 (C-5), 24.6 (C-9), 24.3 (C-13), 21.9 (C-16, C-17), 21.0 (C-18, C-19), 20.2 (C-20).
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