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The Aignopsanes, a New Class of Sesquiterpenes from Selected Chemotypes of the Sponge *Cacospongia mycofijiensis*

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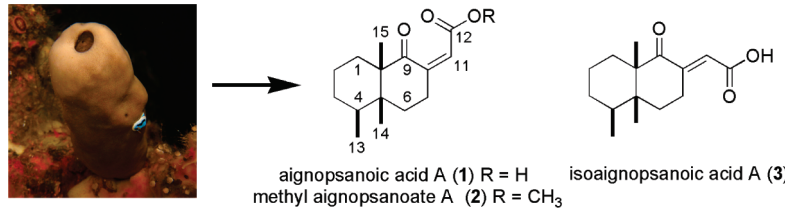
ABSTRACT



aignopsanoic acid A (**1**) R = H
methyl aignopsanoate A (**2**) R = CH_3

isoaignopsanoic acid A (**3**)

A survey of individual specimens of northern Papua New Guinea derived *Cacospongia mycofijiensis* has yielded novel sesquiterpenes, aignopsanoic acid A (1), methyl aignopsanoate A (2), and isoaignopsanoic acid A (3). The structures and absolute configurations of 1−3 were established using NMR data, X-ray crystallography results, and an analysis of CD properties. Two of these metabolites, 1 and 2, were moderately active against *Trypanosoma brucei*, the parasite responsible for sleeping sickness.

We now believe that chemocentric investigations of the Indo Pacific marine sponge *Cacospongia mycofijiensis* can be almost as rewarding as those reported in the past for *Theonella swinhoei*.[1] For example, our recent exhaustive exploration of the breadth of metabolites that could be obtained from a Fijian collection (coll. no. 89126) of *C. mycofijiensis* provided exciting results. There were five distinct chemotypes that could be isolated from this sponge consisting of the sesterterpene dendrolasin,[2] the fijianolide polyketides,[3] three distinct mixed PKS−NRPS structures including mycothiazole,[4] latrunculin A,[5] and the unprecedented thiopyrone CTP-431.[6] Another dimension to this

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Table 1. ^{1}H and ^{13}C NMR[a] Data of **1**−**3**

	1[d]			**2**[d]			**3**[d]		
position	δ_C	type[b]	δ_H (J in Hz)	δ_C	type[b]	δ_H (J in Hz)	δ_C	type[b]	δ_H (J in Hz)
1ax	30.2	CH_2	1.40 (td, 13.8, 4.2)	30.2	CH_2	1.37 (td, 13.8, 4.8)	30.4	CH_2	1.40 (m)[c]
1eq			2.05 (bddd, 13.2, 4.8, 2.4)			1.98 (ddd, 13.8, 3.6, 1.2)			2.05 (dt, 13.2, 3.0)
2ax	22.3	CH_2	1.60 (m)[c]	22.2	CH_2	1.72 (m)	22.5	CH_2	1.50 (m)[c]
2eq			1.60 (m)[c]			1.50 (m)			1.40 (m)[c]
3ax	30.0	CH_2	1.40 (m)[c] 1.30 (m)	30.2	CH_2	1.40 (m)	30.1	CH_2	1.40 (m)[c]
3eq			1.30 (m)			1.30 (m)			1.30 (m)
4	35.2	CH	1.49 (m)	34.2	CH	1.55 (m)	35.1	CH	1.50 (m)[c]
5	42.0	C		42.5	C		40.1	C	
6ax	30.2	CH_2	1.85 (td, 14.4, 4.8)	31.1	CH_2	1.84 (td, 14.4., 4.8)	28.4	CH_2	1.81 (td, 14.4, 5.4)
6eq			1.78 (ddd, 14.4, 6.0, 2.4)			1.78 (ddd, 14.4, 5.4, 2.4)			1.74 (ddd, 14.4, 6.6, 1.2)
7ax	31.0	CH_2	2.70 (dddd, 15.6, 14.4, 6.0, 3.0)	30.5	CH_2	2.63 (dddd, 15.0, 14.4, 6.0, 3.0)	23.7	CH_2	2.55 (dddd, 18.0, 14.4, 6.6, 3.0)
7eq			2.44 (dddd, 15.6, 4.8, 2.4, 0.6)			2.40 (dddd, 15.0, 4.8, 2.4, 0.6)			3.61 (ddt, 18.0, 5.4, 1.2)
8	149.3	C		152.0	C		153.6	C	
9	211.5	C		207.0	C		206.2	C	
10	55.4	C		54.3	C		53.6	C	
11	126.1	CH	5.95 (dd, 3.0, 0.6)	121.0	CH	5.75 (dd, 3.0, 0.6)	121.1	CH	6.50 (dd, 3.0, 1.2)
12	165.6	C		166.5	C		168.9	C	
13	15.8	CH_3	0.82 (d, 7.2)	15.7	CH_3	0.83 (d, 7.2)	15.6	CH_3	0.87 (d, 6.6)
14	15.5	CH_3	0.89 (s)	15.3	CH_3	0.91 (s)	15.5	CH_3	0.91 (s)
15	22.2	CH_3	1.06 (s)	21.2	CH_3	1.12 (s)	22.3	CH_3	1.01 (s)
OMe				51.7	CH_3	3.70 (s)			

[a] Measured at 600 MHz (^{1}H) and 125 MHz (^{13}C). [b] Carbon type determined by DEPT and HMQC experiments. [c] Partially overlapped by other signals. [d] Measured in $CDCl_3$. For more complete NMR data of compounds **1**−**3**, see Tables S1−S3 and Figures S1−S23 in the Supporting Information.

pattern resides in the biogeographical variations in *C. mycofijiensis* metabolites that we have recently summarized,[3,5] culminating in the isolation of 18-*epi*-latrunculol A. This important compound is being further pursued as a preclinical lead for cancer therapeutics because of its demonstrated solid tumor selectivity unaccompanied by significant microfilament disruption activity.

The initial stimulus justifying yet one more detailed study of *C. mycofijiensis* came about by our fortuitous discovery in 2007 of a relatively large community of this sponge (coll. no. 07327, whose partial collection yielded 1.7 kg) on reefs in the Kimbe Bay region of Papua New Guinea. Historically, Milne Bay Papua New Guinea specimens, as well as those from Fiji and the Solomon Islands, have been a repeated source of just two major metabolites—latrunculin A and dendrolasin—and were always devoid of the fijianolides and mycothiazole.[3,7] A preliminary LC-MS survey of this extract obtained from a pooled collection (0.8 kg) indicated these past observations did not hold, which was surprising. The fijianolides (515, $M + H^+$) and mycothiazole (405, $M + H^+$) were present as major metabolites accompanied by latrunculin A (404, $M - H_2O + H^+$) (see Chart S1 in Supporting Information). A number of previously unreported masses from this species were also observed in this extract which prompted us to more carefully explore the chemistry of individual specimens culled out from the bulk collection. The next step that was the basis of our further chemical investigation consisted of a parallel LC-MS screening of extracts from the individual specimens. Eventually, members of an entirely new sesquiterpene class, possessing a unique bicyclic framework, were isolated and characterized, and to celebrate this discovery we have coined the name aignopsane[8] for this family. Described at this time are the structures of three such compounds along with their biological activity properties.

The separate workup of 15 distinct sponge colonies (coded as 07327A-O) using the high-throughput approach of accelerated solvent extraction (ASE) was the initial step in this project. This yielded three different polarity fractions coded XFH (hexane processing), XFD (dichloromethane processing), and XFM (methanol processing). The most remarkable outcome came from the further investigation of the XFD fractions. A subset of these, 07327A and G, were observed to contain three unrecognizable metabolites with relatively low molecular masses at *m/z* 251 as a major metabolite and *m/z* 265/233 as minor constituents (see Chart S2 in the Supporting Information). These were also observed by LCMS-ELSD in the crude extracts of the remaining sponge material 07327A (138 g) separately processed by traditional extraction using a modified Kupchan partition scheme[6] (see Chart S3, Supporting Information). Further investigation of the dichloromethane solvent partition fraction (coded FD) of coll. no. 07327A afforded, after reverse-phase HPLC purification, aignopsanoic acid A (**1**, 6.3 mg), methyl aignopsanoate A (**2**, 1.4 mg), and isoaignopsanoic acid A (**3**, 1.6 mg), accompanied by latrunculol A (1.1 mg),[5] fijianolide B (2.3 mg), and latrunculin A (1.9 mg). The major metabolite, **1**, was pursued in the initial total structure elucidation.

(7) We previously reported on the chemistry of *C. mycofijiensis* from Papua New Guinea collections in the Milne Bay region from depths above 25 meters. Our recent 2007 collections were off the coast of New Britain (Kimbe bay) at depths between 30 and 40 m, which may explain this observed regional biogeographical variation for the presence of the fijianolides and mycothiazole chemotypes.

(8) There are many compounds whose names are based on the cacospongia prefix, such as the cacospongins, cacofurans, and the cacospongionolides. To avoid confusion, the new name is based on an anagram of the organism genus.

A concise process was used to characterize the overall structure of aignopsanoic acid A (**1**) $[\alpha]^{23}_{D}$ 42.0 (*c* 0.14, CH_3OH), whose molecular formula of $C_{15}H_{22}O_3$ was set from HRESITOFMS, based on the $[M + Na]^+$ ion *m/z* 273.15606 (calcd for $C_{15}H_{22}O_3Na$, 273.15102). Each of the oxygens, but not all of the five degrees of unsaturation, could be located in the ^{13}C NMR data outlined in Table 1. These consisted of a trisubstituted double bond (δ_C 149.3, 126.1), a very downfield peak (δ_C 211.5) of a ketone, and one upfield carboxylic acid signal (δ_C 165.6). Together such observations demanded the presence of two carbocyclic rings as scaffolds to attach the three nongeminal methyl groups. The ^1H NMR data (Table 1) revealed two methyl singlets (δ_H 1.06, 0.89) and one methyl doublet [δ_H 0.82 (d, $J = 7.2$ Hz)]. The most obvious next conclusion was that **1** was a sesquiterpene containing a fused bicyclic ring system. Further insights came once the 2D NMR data sets had been collected and analyzed revealing the key correlations shown in Figure 1. Finally, these data enabled the drafting of substructures **A**−**D**, and the gHMBC spectrum possessed correlations providing unequivocal support to combine these fragments in just one way resulting in gross structure **E**.

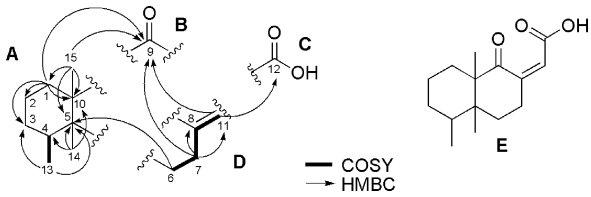

Figure 1. Substructures and 2D NMR correlations of **1**.

The total structure elucidation of aignopsanoic acid A (**1**) was as follows. The initial geometry of its bicyclic ring was based on the ^{13}C chemical shifts for three CH_3 (δ_C 15.5, 15.8, and 22.2) groups of **1** that were similar to those of three such groups (δ_C 16.3, 16.4, and 18.3) in (+) nakamurol (**4**), a rare sponge-derived thelepogane diterpene, possessing a *cis*-fused decalin core.[9] This conclusion was quickly reaffirmed from the NOE results summarized in Figure 2, including enhancement observed from H_3-14 to H_3-13 and H_3-15 indicating that all three methyls were on the same side of a plane. Similar NOE correlations were also observed for the A/B ring constituents of **4**.[10] The large 1,2 trans diaxial coupling ($J = 14.4$ Hz) observed from H-6_{ax} to H-7_{ax} along with a key 1D NOE correlation from H-4 to H-7_{ax} further supported the chair−chair orientation shown for the bicyclic ring system. The trisubstituted double bond regiochemistry was established by the NOE correlations from H-7 to H-11. The ultimate proof of the relative stereochemical features proposed for **1** in Figure 2 came from X-ray crystallographic analysis whose outcome is shown in Figure 3.

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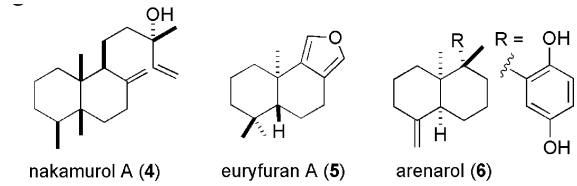

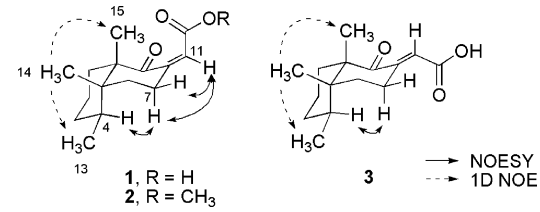

Figure 2. Significant 1D NOE correlations for compounds **1**−**3**.

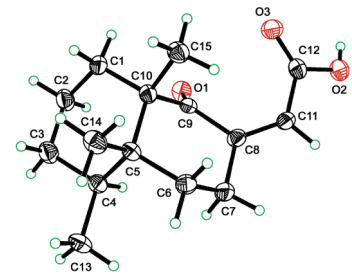

Figure 3. X-ray crystal structure of aignopsanoic acid A (**1**).

The absolute configuration of **1** was determined from its CD spectrum (Figure S24, Supporting Information). On the basis of the octant rule for cyclohexanones,[11] the positive Cotton effect (CE) at 262 nm for $n\rightarrow\pi^*$ and the negative CE at 228 nm for $\pi\rightarrow\pi^*$ indicated that the configuration of **1** is as depicted. Accordingly, the final assigned structure can be summarized as (4*S*, 5*R*, 10*S*) aignopsanoic acid A (**1**). Interestingly, (+) **4** has the same absolute configuration as **1** at the decalin ring chiral centers.

The second compound isolated was **2**, $[\alpha]^{23}_{D}$ −60.4 (*c* 0.09, CH_3OH), of molecular formula of $C_{16}H_{24}O_3$ based on the $[M + Na]^+$ ion *m/z* 287.16271 (calcd for $C_{16}H_{24}O_3Na$ 287.16177) which differed from **1** by $+CH_2$. It was quickly evident that **2** was simply the methyl ester (δ_H 3.70, A = 3, δ_C 51.7) of **1**. The ^1H and ^{13}C NMR (Table 1) data were almost identical between this pair. That they shared the same absolute stereochemical features was also clear from: (a) the gCOSY and $^{2-3}J_{H,C}$ HMBC correlations observed for **2**(see Table S2 in Supporting Information), (b) the NOESY results summarized in Figure 2, and (c) the CD spectrum (Figure S24 in Supporting Information) which showed a positive CE at 256 nm and negative CE at 225 nm. Thus, this compound can be concluded to be 4*S*,5*R*,10*S*-methyl aignopsanoate A (**2**).

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Compound **3**, $[\alpha]^{23}_{D}$ −45.0 (*c* 0.08, CH_3OH), proved to be a diastereomer of **1** as it possessed the same molecular formula. Similar ^{1}H and ^{13}C NMR data for the pair were evident from a side-by-side comparison. The exception was that for **3** versus **1** there was a noticeable downfield shift for the methylenes H-7_{ax} (δ_H 2.55) and H-7_{eq} (δ_H 3.61) and the vinylic proton H-11 (δ_H 6.50). The same additional gCOSY and $^{2-3}J_{H,C}$ HMBC connectivities observed for **1** were also seen for **3** (see Table S3 in Supporting Information). Likewise, the NOE enhancement of H_3-14 to H_3-13 and H_3-15, shown in Figure 2, established that **1** and **3** had the same *cis*-decalin ring junction and that all three methyls were on the same facial plane. Consistent with the proposed double bond geometry of **3** as *E* and **1** as *Z* was that H-11 was at the lowest field for **3** due to the anisotropic deshielding of the proximal oxygen. Analogous to that observed above, the CD results (see Figure S24 in Supporting Information) of **3** with positive CE at 278 nm and negative CE at 237 nm confirmed that the absolute configuration was conserved for **1**−**3**.

The biological activity properties of sesquiterpenes **1**−**3** were assessed against the parasite *Trypanosoma brucei*, the protozoan species responsible for the infectious disease African trypanosomiasis (sleeping sickness).[12] Compounds **1** and **2** showed moderate inhibition in the assay with IC_{50} values of 6 and 16 μg/mL, respectively, whereas **3** was inactive at 25 μg/mL. Interestingly, the configuration of the *E*/*Z* double bond appears to modulate the activity. These values are on par with that observed for natural products investigated by others[13] and are less potent than the clinically used agent melarsoprol.[14]

The unusual molecular framework present in the aignopsanes discovered here has no counterpart in natural products chemistry. Sesquiterpenes have been a focus of investigations for many decades, and the knowledge based on the fused bicyclic ring containing natural sesquiterpenes is substantial. For example, the 1972 seminal review by Devon and Scott summarized 52 such frameworks.[15] Our survey of the more recent literature dealing with this class, based on *Natural Products Reports* reviews by Fraga, indicates that during the past five years, on average, there are only four new bicyclic sesquiterpene frameworks reported annually.[16] This circumstance heightens the impact of our discovery, and some additional relevant observations to further calibrate the relationship of our findings to those of others are as follows. A drimane class sesquiterpene, euryfuran (**5**), has also been shown to co-occur with fijianolide A and latrunculin A from an unidentifiable Indo Pacific marine sponge[17] that we believe to be *C. mycofijiensis*. Euryfuran has also been shown to be biosynthesized in nudibranchs by the traditional mevalonate biosynthetic pathway.[18] It would appear that the next step in the biosynthetic creation of the aignopsane skeleton would occur via a rearrangement of a drimane to a 4,9-friedo drimane intermediate.[19] This latter framework is richly represented in mero-sesquiterpenes, commonly isolated from sponges, such as in arenarol (**6**).[20]

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Supporting Information Available: 10 tables and 24 figures are provided which include the experimental procedures, 1D and 2D NMR, and X-ray crystallography data of compounds (**1**−**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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