Employing Dereplication and Gradient 1D NMR Methods to Rapidly Characterize Sponge-Derived Sesterterpenes

Chad C. Stessman, Rainer Ebel, Angelo J. Corvino,[§] and Phillip Crews*

Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California, Santa Cruz, California 95064

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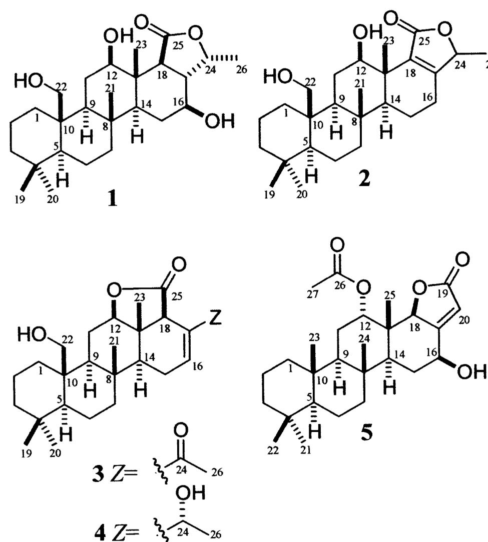
The sesterterpene constituents of two Indo-Pacific sponges were investigated and rapidly characterized using aggressive dereplication methods along with gradient 1D NMR techniques. *Lendenfeldia frondosa* yielded three sesterterpenes: 12*β*,16*β*,22-trihydroxy-24α-methylscalar-25*β*,24α-olide (**1**), the 24 epimer of a known compound; 12*β*,22-dihydroxy-24-methylscalar-17-en-24,25-olide (**2**), a known compound; and 22-hydroxy-24-methylsedn-16-en-24-one-12*β*,25*β*-olide (**3**), a new compound. A *Hyrtios* sp. sponge yielded known 12α-acetoxy-16*β*-hydroxyscalarolbutenolide (**5**).

Sponge-derived tetra- or pentacyclic sesterterpenes appear to be well known as more than 60 such compounds have been described in the literature.[1] Dereplication of such compounds is often not straightforward despite recent publications[2] outlining alternatives beyond those traditionally used in the antibiotic field. We have found that focusing on the pattern of low-field ^{13}C NMR signals along with substructure searching of databases is an effective combination for dereplication or de novo structure elucidation of polycyclic sesterterpenes.[3] Such an approach helps to quickly overcome the problem lack of diagnostic MS fragment peaks and the myriad of overlapping signals occurring in the aliphatic region of their ^{1}H and ^{13}C NMR spectra.[4] In fact, many publications reporting polycyclic sesterterpenes contain incompletely assigned ^{1}H NMR spectra, which further illustrates the challenges in translating spectroscopic data for this class into unique final structures with all stereochemical elements defined.

From the viewpoint of *de novo* structure elucidation, some of the more recently introduced NMR techniques offer a concise approach to this process. Examples of these methods are inverse detected two-dimensional experiments,[5] the use of pulsed field gradients to obtain 2D data,[6] or measuring spectra at increasingly higher field. Alternatively, the more direct approach of using classic 1D methods, such as difference NOE, is not usually practical for unraveling the structures of polycyclic sesterterpenes. Outlined below is the useful alternative of employing gradient shaped pulse experiments in a 1D mode. We illustrate that gradient 1D TOCSY pulse sequences can be used in the irradiation of a set of nonoverlapping peaks using an array of mixing times.[7] This causes excitation of the entire spin system, resulting in data sets from which coupling constant data can be extracted, even when some of the chemical shifts are closely spaced. It is the nuance of employing the pulsed field gradient method to obtain the 1D NOE data that adds to the power of this approach.[8]

The gradient 1D NMR techniques plus aggressive dereplication efforts were initially applied to study the sesterterpene constituents of *Lendenfeldia frondosa* (coll. no. 98115) obtained near Wewak, Papua New Guinea. The concentrated methanol crude extract of this sponge was fractionated according to the UCSC standard extraction scheme.[9] A solvent partition fraction (CH_2Cl_2, labeled as "FD") was further debulked by filtration through Sephadex LH-20. The major fraction was then subjected to reversed-phase HPLC to yield 12*β*,16*β*,22-trihydroxy-24α-methylscalar-25*β*,24α-olide (**1**),[10,11] the 24-epimer of a known compound; 12*β*,22-dihydroxy-24-methylscalar-17-en-24,25-olide (**2**), a known compound;[12] and 22-hydroxy-24-methylsedn-16-en-24-one-12*β*,25*β*-olide (**3**), a new major component of this sponge extract.



The structural characterization of these compounds began by obtaining high-resolution MS data. Once their molecular formulas were in hand, these data plus unsaturation deduced from low-field ^{13}C NMR peaks were used as seeds for dereplication via MarinLit database[1] searches. Thus, electrospray ionization time-of-flight mass spectrometric (ESI-TOF-MS) analysis of the first compound gave an $m/z = 457.2938$ $[M + Na]^+$ for a molecular formula of $C_{26}H_{42}O_5$. The six unsaturation equivalents required by this formula plus a single ester moiety identified from the ^{13}C NMR δ 177.7, together with the requisite pentacyclic frame, were used to filter the large number of literature hits. Initial side-by-side comparison of the ^{13}C NMR data of **1**, shown in Table 1, with that of 12*β*,16*β*,22-trihydroxy-24*β*-

§ Participant in the UCSC NIH Bridges Summer Research Institute.
* To whom correspondence should be addressed. Tel: 831 459 2603. Fax: 831 459 2935. E-mail: phil@chemistry.ucsc.edu.

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Table 1. NMR Data for **1**, **3**, and **5** in $CDCl_3$ at 500/125 MHz

	1		3			5	
no.	^{13}C (δ)	^{1}H (δ, mult., *J*, Hz)	^{13}C (δ)	^{1}H (δ, mult., *J*, Hz)	HMBC	^{13}C (δ)	^{1}H (δ, mult., *J*, Hz)
1	34.6t	β 2.24bd, 13.6	35.1t	β 2.25bd, 11.5	H3,H22,H22	39.9t	α 0.58ddd, 13.0, 13.0, 3.5
		α 0.74m		α 0.80dd, 11.5, 11.5			β 1.62bd, 13.0
2	18.6t[h]	1.42m, 1.58m	17.6t	1.51m	H1,H3	18.5t	1.42m
3	41.9t[g]	α 1.10bt	41.7t[a]	1.20m, 1.45m	H1,H2,H20,H19	42.0t[i]	α 1.12dd, 12.5, 12.5
		β 1.42m					β 1.40m
4	33.1s		33.3s		H3,H19, H20	33.4s	
5	56.8d[e]	0.85d, 12.7	57.7d	1.01d, 12.7	H1,H3,H7,H19,H20	56.6d	0.88d, 12.7
6	18.2t[h]	1.41m, 1.52m	18.5t	β 1.42m	H7	18.3t	α 1.40bd
				α 1.55m			β 1.60bq
7	42.4t[f]	α 0.93m	41.7t[a]	α 1.10m	H21	42.1t[i]	α 1.09dd, 12.5, 12.5
		β 1.80bd, 13.6		β 1.74bd, 12.2			β 1.83bd, 12.5
8	37.5s		38.3s		H7, H14,H15,H21	38.1s	
9	57.1d[e]	0.92d, 11.2	61.4d	1.09d, 11.0	H7,H11,H21,H22	52.3d	1.26d, 14.5
10	42.4s[f]		43.5s[b]		H22	37.0s	
11	31.2t	1.44m, 1.98m	24.4t[c]	2.28m	H9	21.7t	β 1.64dd, 14.5, 14.5
							α 1.96d, 14.5
12	79.7d	3.38dd, 11.2, 3.9	89.9d	3.79dd, 11.2, 4.4	H9,H11,H23	74.4d	4.88dd, 3.0, 2.5
13	41.9s[g]		43.3s[b]		H14,H18,H23	43.9s	
14	58.8d	0.91m	50.9d	1.25dd, 7.0, 7.0	H15,H21, H23	47.2d	1.46dd, 12.7, 2.0
15	28.9t	1.99m, 2.02m	24.6t[c]	2.35dd, 7.0, 3.0	H14	31.2t	β 1.52q, 12.7
							α 2.21ddd, 12.7, 7.5
16	72.8d	3.61bs, $w_{1/2}$ = 25	140.8d	6.68dd, 3.0, 3.0	H15	68.4d	4.52ddd, 10.7, 7.3, 1.9
17	51.6d	2.02m	136.7s		H15,H18	172.4s	
18	58.5d	2.03m	55.5d	3.10bs	H23	82.2d	4.91d, 1.5
19	33.9q	0.78s	21.9q	0.79s	H3,H5,H20	173.3s	
20	21.9q	0.86s	34.0q	0.90s	H3,H5,H19	112.3d	5.95t, 1.9
21	16.3q	1.02s	16.4q	1.17s	H14	33.3q	0.86s
22	62.8t	4.01d, 11.7 3.84d, 11.7	62.8t	3.91d, 11.8 4.08d, 11.8	H5	21.4q	0.81s
23	12.4q	1.05s	13.7q	0.90s	H14,H18	16.2q	0.81s
24	81.9d	4.36dp, 6.4. 2.4	197.9s		H26	17.2q	0.90s
25	177.7s		173.4s		H18	12.1q	0.77s
26	20.2d	1.65d, 6.3	27.5q	2.40s		169.5s	
27						21.4q	2.12s

[a−i] Can be interchanged.

methylscalar-25β,24α-olide, previously isolated by our group from *Lendenfeldia frondosa*[10] and Rao from *Phyllospongia dendyi*,[11] indicated a close match.

Similarities between our observed and literature ^{13}C and ^{1}H shifts for the residues of the A, B, and C rings of **1** confirmed that these were *trans* fused. The C/D ring junction was also determined to be *trans* fused based upon similarities in the shifts of observed and literature shifts of Me-23 (δ 12.4, 1.05, $CDCl_3$, lit. δ 11.9, 0.92, DMSO-d_6) and CH-14 (δ 58.8, 0.91, $CDCl_3$, lit. δ 58.4, DMSO-d_6, ^{1}H unreported). The assignment of the three contiguous stereocenters in ring D [C-18 (δ 58.5), C-17 (δ 51.6), and C-16 (δ 72.8)] was verified next. The orientation of H-18α was deduced through arguments based upon the shift of Me-23 as previously outlined.[11] The remaining two stereocenters were assigned on the basis of the couplings measured to H-16 (δ 3.61, bs, $w_{1/2}$ = 25 Hz, see S5a; and bdt J = 4.8, 13.2, 13.2 Hz see S5b), uniquely consistent with an H-17β/H-16α orientation.

Assignment of the E ring stereochemistry of **1** was based on the ^{1}H pattern of H-24, δ 4.37. This peak was previously described[10,11] as being a doublet of quartets, J = 10 or 8 and 6 Hz for the parent compound and 16-acetyl derivative, respectively. A β-24 methyl stereochemistry was assigned for both compounds.[10,11] The appearance of H-24 (δ 4.38) in **1** was assigned as a doubled pentet, J = 2.3 and 6.4 Hz. Previously,[10] we addressed the two possible H-17/H-24 orientations and estimated diagnostic data as $^{3}J_{17-24}$ = 10.7 Hz *trans* (168°) and 6.9 Hz *cis* (34°). Thus, the observed $^{3}J_{17-24}$ = 6.4 Hz for **1** is consistent with *cis* geometry, indicating a α-methyl. No stereochemical significance could be implied from the $^{4}J_{24-18}$ = 2.3 Hz. Interestingly the 26β-Me of epi-**1** also exhibits $^{4}J_{24-18}$ = 3.0 Hz.[10,11] Thus, our compound **1** is the 24- epimer of the compound previously reported in the literature.

Similarly, the evaluation of **2**, the second compound isolated, began with LRESI-MS data (m/z = 417, $[M + H]^+$) and a ^{13}C APT count proposed as $C_{26}H_{38}$. The LRMS and NMR data were consistent with a final molecular formula of $C_{26}H_{40}O_4$ (seven unsaturation equivalents). These data were used as input for dereplication, along with moieties consisting of a carbonyl (^{13}C NMR δ 175.2), a tetrasubstituted double bond (^{13}C NMR δ 166.2s, 135.4s), and five rings. Three structures were obtained as hits (Table S1). Comparison of the observed ^{13}C shifts with literature values for each compound indicated a unique match to the structure and relative stereochemistry, assigned at seven of the eight chiral sites, in **2** originally characterized from the dorid nudibranch *Chromodoris sedna* by Faulkner.[12]

Applying an analogous dereplication approach to characterize compound **3** was initially unsuccessful. The ESI-TOF-MS $[M + H]^+$ m/z 437.2672 was used to define the molecular formula as $C_{26}H_{38}O_4$, consistent with the APT tally of $C_{26}H_{37}$. The ^{13}C/^{1}H NMR resonances at δ 197.9s, 173.4s, 140.8d/δ 6.68, 136.7s were used to identify three of the eight unsaturation equivalents as two carbonyls and a trisubstituted double bond. These residues could be imbedded into substructure **A** using two different data sets. First, g-HMBC correlations were observed from δ 3.10 (H-18) to 173.4 (C-25), 136.7 (C-17), 43.3 (C-13), and 13.7 (C-23); from δ 2.40 (H_3-26) to 197.9 (C-24); from δ 1.25 (H-14) to 13.7 (C-23); and from δ 0.90 (H_3-23) to 50.9 (C-14). Second, a gradient shaped pulse ^{1}H NMR 1D TOCSY experiment involving irradiation at δ 2.35 (H_2-15) resulted in excitement of resonances at δ 6.68 (H-16), 3.10 (H-18), and 1.25 (H-14). Third, a gradient shaped pulse 1D NOE

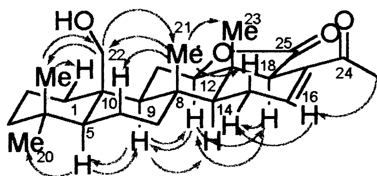
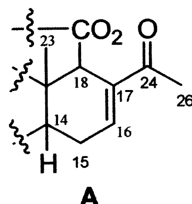


Figure 1. Important 1D NOE correlations for **3**.

correlation was observed through irradiation at δ 2.40 (H_3-26), resulting in excitement of the resonance at δ 6.68 (H-16).



Using $C_{26}H_{38}O_4$ and substructure **A** as seeds for dereplication was not initially productive. Only one rather distant match, 22-hydroxy-24-methyl-12,24-dioxoscalar-16-en-25-al (shown in Table S2), was obtained.[13] Interestingly, the ^{13}C NMR data indicated **3** possessed the AB ring system of this compound, also present in **1** and **2**. Using that substructure as a MarinLit search parameter yielded 10 additional hits (Table S2), but no structures provided an exact match to our data. The most relevant member of this set was sednolide (**4**),[14] whose formula, $C_{26}H_{40}O_4$, differs from that of **3** by a count of H_2. The structure of sednolide, established via X-ray crystallography, was accompanied with only partial ^{1}H and no ^{13}C NMR data. While only a limited side-by-side comparison of the NMR data between **3** and **4** was possible, it appeared this pair differed simply in the oxidation state at C-24. The supporting diagnostic shifts were as follows: (a) δ 1.35 d (H_3-26 **4**) vs δ 2.40 s (H_3-26 **3**), (b) δ 5.52 m (H_3-16 **4**) vs δ 6.69 dd (H_3-16 **3**), (c) δ 2.91 bs (H-18 **4**) vs δ 3.10 bs (H-18 **3**), and (d) δ 4.46 bq (H-24 **4**) vs no signal in **3**. Additionally, the −OH in **3** was set at C-22 and not C-21 or C-23 on the basis of the abnormally downfield shift of H-1β (δ 2.25 in **3** vs δ 1.62 in **5**) (see below) and HMBC correlations δ 3.91/4.08 (H_2-22) to δ 35.1 (C-1) and 61.4 (C-9).

At this point, substantiation of the final structure proposed for **3** could now be rapidly accomplished. A series of gradient shaped pulse ^{1}H NMR g-1D-TOCSY data (see Experimental Section) provided invaluable data. These results unraveled aliphatic proton spin systems, with badly overlapping resonances in the normal ^{1}H NMR spectrum, consisting of H_2-1−H_2-2−H_2-3; H-5−H_2-6−H_2-7; and H-9−H_2-11−H-12. Connectivity between these spin systems was made from the gHMBC data shown in Table 1. Determination of the relative stereochemistry was addressed using ^{13}C chemical shift considerations and results from a series of shaped pulse gradient 1D NOE experiments. The characteristic ^{13}C shifts of methyls 21 and 23 indicated they were axial and that the BCD rings were *trans* fused. The axial orientation of C-22 and the *trans*-fused AB rings were established from the NOE data summarized in Figure 1.

A combination of spectroscopic data and dereplication steps were used to characterize the sesterterpene isolated from a *Hyrtos* sp. (96600FD) collected in Indonesia. The molecular formula, $C_{27}H_{40}O_5$, was set from high-resolution ESI-TOF-MS $[M + H]^+$ m/z = 445.2944. The eight unsaturations could be divided into five rings along with an α,β-unsaturated carboxylate [δ 172.4 (C-17), 173.3 (C-19), 112.3 (C-20)] and an acetate [δ 169.5 (C-26) and 21.4 (C-27)]. Using these constraints as MarinLit search parameters[1] revealed seven reasonable matches (see Table S4). A three-step process was used to filter these structures. The ^{1}H NMR low-field resonances at δ 5.95 (bs, H-20), 4.91 (bs, H-18), 4.88 (dd, J = 3.0, 2.5 Hz, H-20), and 4.52 (bs, H-12) were consistent with four of the seven structures based on a 12,16-dihydroxyscalarolbutenolide framework with one of the hydroxyl groups being acetylated. The relative downfield ^{1}H NMR shift of H-12 (δ 4.88) as compared to H-16 (δ 4.52) indicated the OAc site as C-12. Next, NMR data were obtained consisting of a gHMBC spectrum and a series of ^{1}H NMR g-1D-TOCSY spectra. These allowed the badly overlapping non-Me upfield resonances to be confidently disentangled and divided into four distinct spin systems. For example, a g-1D-TOCSY spectrum (60 ms mixing time) via irradiation at δ 1.96 (H-11α) (see Figures S4 and compare to Figure S11) revealed the spin system consisting of H-9$_{ax}$ (J = 14.5 Hz), H-11β_{ax} (J = 14.5, 14.5 Hz), and H-12$_{eq}$. Further, the preceding ^{1}H NMR Js indicated an α-C-12 OAc leaving structure **5** as the only fit. Finally, side-by-side comparison of our experimental ^{13}C shifts with the literature confirmed **5** to be 12α-acetoxy-16β-hydroxyscalarolbutenolide, previously isolated from the marine nudibranch *Chromodoris inornata*[15] and the sponge *Spongia matamata*.[16]

In summary, we have illustrated above a concise way to meet the challenge of rapid dereplication or structure elucidation for polycyclic terpenoids. An expedient approach to deal with such compounds consists of obtaining ESI-TOF MS data to establish the molecular formula that, along-with substructures identified from ^{13}C NMR data, is used as the seed for dereplication. In cases where a large number of hits are obtained, a useful next step is to filter these possibilities by unraveling and assign the overlapping aliphatic ^{1}H NMR resonances using gradient 1D NMR techniques such as g-1D-TOCSY and g-1D-NOE.

Experimental Section

General Experimental Procedures. NMR spectra were collected at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Multiplicities of ^{13}C NMR peaks were determined using DEPT and gHMQC data. Spin systems were determined using g-1D-TOCSY using the approach that included a q3 shape pulse (Gaussian cascade 180° inversion), with mixing times arrayed between 0 and 150 ms at 30 ms increments. Additional g-1D-TOCSY parameters and pulse sequence are shown in Figure S13. Connectivity between these spin systems was determined using gHMBC data. Relative stereochemistry was established through a series of g-1D-NOESY again using a q3 shape pulse (Gaussian cascade 180° inversion)) experiments, whose g-1D-NOESY parameters and pulse sequences appear in Figure S14). High-resolution mass measurements were obtained on a benchtop ESI-TOF apparatus. Other procedures were as previously published.[17]

Biological Material, Collection, and Identification. Sponges of the species *Lendenfeldia frondosa* (Lendenfeld, 1889) (Spongiidae, Dictyoceratida) (coll. No. 98115, 2.8 kg) were collected using scuba from coral heads off the coast of Wewak, Papua New Guinea, (S 03°24.983′, E 143°37.899′) at depths of 15−20 ft. This sponge was identified by Dr. M. C. Diaz (UCSC, IMS) in reference to properties described in the literature.[18] Samples of *Hyrtios erectus* (Keller 1889) (Thorectidae, Dictyoceratida) (coll. No. 96600, 1.3 kg) were collected using scuba from Sulawesi, Indonesia, (N 01°25.719′, E 125°108.21′). The sponge was also identified by Dr. M. C. Diaz (UCSC, IMS) in reference to properties described in the literature.[18] Voucher specimens and underwater photos are available (from P.C.).

Extraction and Isolation. Both sponges were preserved in the field according to our standard procedures and transported back to the laboratory at ambient temperature.[17] Each collection was extracted with MeOH (3×), after which the solvent was removed and the resulting oil was partitioned between hexanes and 10% aqueous MeOH. The MeOH layer was adjusted to 50% aqueous MeOH and extracted with CH_2Cl_2. The CH_2Cl_2 layer (98115FD) was evaporated and yielded a red-brown oil (9.09 g). A portion of this extract was subjected to chromatography on Sephadex LH-20 (1:1 MeOH−CH_2Cl_2), yielding a fraction (313 mg) that after reversed-phase (C_{18}) HPLC (gradient 10% aqueous MeOH to 100% MeOH) gave **3** (4.3 mg), **1** (3.8 mg), and **2** (2.6 mg) (see Figures S8 and S9). The CH_2Cl_2-soluble extract of the second sponge (96600FD) was evaporated and yielded a brown oil (2.02 g). This was passed through silica gel (100−200 mesh) with 1:4 EtOAc−hexanes to yield 55.9 mg of a fraction that after reverse-phase (C_{18}) HPLC (gradient 10% aqueous MeOH to 100% MeOH) yielded **5** (5.0 mg) (see Figures S11 and S12).

12β,16β,22-Trihydroxy-24α-methylscalar-25β,24α-olide (1): colorless solid; $[\alpha]_D$ +42.1° (*c* 0.076, CH_2Cl_2); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), in Table 1 (also see Figures S5 and S6); HRESIMS $[M + Na]^+$ obsd 457.2938, calcd for $C_{26}H_{42}O_5Na$, 457.2925.

22-Hydroxy-24-methylsedn-16-en-24-one-12β,25β-olide (3): colorless solid; $[\alpha]_D$ +18° (*c* 0.022, CH_2Cl_2) epi-$\mathbf{1}_{lit.}$ $[\alpha]_D$ +15.8° (*c* 0.91, pyridine); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), in Table 1 (also see Figures S9 and S10); g-1D ^1H TOCSY spin systems H-1α(δ 0.80)−1β(2.25)−H_2-2(1.51)−H_2-3(1.20,1.45); H-5(1.01)−H-6α(1.42)−H-6β(1.55)−H_2-7(1.74,1.10); H-9(1.09)−H_2-11(2.28)−H-12(3.79); H-14(1.25)−H_2-15(2.35)−H-16(6.68)−H-18(3.10); HRESIMS $[M + H]^+$ obsd 437.2672, calcd 437.2686 for $C_{28}H_{37}O$.

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Supporting Information Available: Results of database searches and NMR spectra of **1**, **2**, **3** (^1H, ^{13}C NMR) and **5** (^1H, ^{13}C NMR, and a partial g-1D-TOCSY) are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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