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USING SPECTROSCOPIC AND DATABASE STRATEGIES TO UNRAVEL STRUCTURES OF POLYCYCLIC BIOACTIVE MARINE SPONGE SESTERTERPENES

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ABSTRACT: Three new tricyclic sesterterpenes, aplysolides A (**1a**) and B (**1b**) and aplyolide A (**2**), have been characterized from an *Aplysinopsis* Indo-Pacific marine sponge. The structure elucidation of these compounds was accomplished using data from a new generation of 2D NMR strategies including multiple quantum coherence (HMQC and HMBC) and rotating frame NOE (ROESY). An additional approach during the early phases of structure elucidation made use of our personal database with searchable fields including substructures, APT formulae, and molecular formulae.

INTRODUCTION

Our laboratory has a continuing interest in the sesterterpene chemistry of marine sponges[1,2]. Sesterterpenes are often active in a variety of bioassays; most notable are the anti-inflammatory properties of the manoalides[3] and the scalarins[4]. The process of characterizing the first sesterterpene natural products, isolated in the early 1950's, uniformly took many years.[2] Now, the interval between isolation and complete characterization is remarkably short. Our contribution to this symposium in print will illustrate structure elucidation methodologies which we commonly employ in efficient characterizations of unknown, biologically active, non-crystalline marine natural products.

Recent advancements in NMR technology have made this the dominant tool for the study of complex natural products. The most frequently measured NMR data, including coupling constants (J's), chemical shifts (δ's) and relaxation times (T_1's or NOE's), are of obvious value in organic structure analysis. These data are frequently applied to assign relative stereochemistry, or to analyze the conformations of natural products. A snag in this seemingly straightforward process often occurs when complex natural products are in hand whose ^1H resonances are substantially overlapping or when ambiguities exist in the assignments of their completely resolved ^{13}C resonances. These obstacles can be largely overcome by two-dimensional (2D) NMR methods such as homonuclear correlation or heteronuclear shift-correlation spectroscopy, commonly termed ^1H-^1H COSY and ^1H-^{13}C COSY respectively. Unfortunately, tens to hundreds of milligrams of sample[5] are often required to obtain high quality 2D ^1H-^{13}C COSY NMR spectra in reasonable time periods via the usual technique of ^{13}C nucleus detection. Inverse correlation techniques for obtaining such COSY spectra by ^1H nucleus observation provide a solution[6] to this problem because a gain of 30:1 in sensitivity (as compared to ^{13}C observation) is achieved. These methods are based on the indirect detection of ^{13}C nuclei by establishing a multiple quantum coherence to a proton signal. For nuclei with gyromagnetic ratio (G), the enhancement of sensitivity possible in the inverse detection strategy is given by: $(G_H/G_C)^{3/2} \cdot (G_H/G_C)$, where $S/N \approx (G_H/G_C)^{3/2}$ and enhancement at the outset of an experiment by ^1H excitation $\approx (G_H/G_C)$.

Both NMR and non-NMR strategies were used to establish the structures of new sesterterpenes **1a**, **1b**, and **2**. Key

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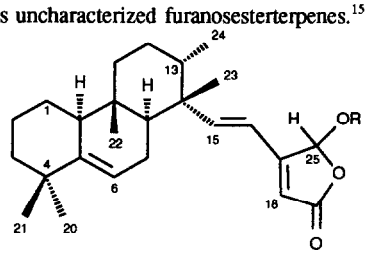
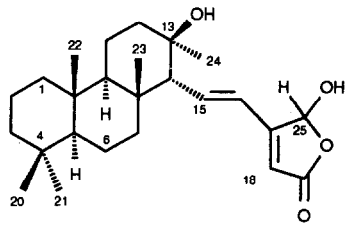
inverse NMR data are $^{1}J_{CH}$ interactions from HMQC (heteronuclear multiple quantum correlation)[7,8] and $^{2\text{-}3}J_{CH}$ interactions from HMBC (heteronuclear multiple bond correlation)[9]. An important complementary strategy is the so-called SUPER-COSY method,[10] which emphasizes $^{4}J_{H\text{-}H}$ and $^{5}J_{H\text{-}H}$ couplings along with revealing weakly coupled geminal and vicinal proton coupled pairs. Another valuable tool we will illustrate is the use of a personalized structure database[11a]. We call on our database to rapidly gather benchmark data for verifying proposed substructures or to highlight biogenetic patterns which can assist in interconnecting sets of substructures. Database queries, which can be used singularly or in combination, consist of a drawing of a partial structure, specifying a molecular formula based on high resolution MS, or inputting a partial APT (attached proton test) molecular formula derived from 1D or 2D ^{13}C NMR data[11b]. An eclectic approach is implemented during the final phases of the structure analysis which is usually aimed at solving questions of stereochemistry and conformation. Approaches we wish to illustrate are the use of ^{13}C NMR shift increment effects[12], NOE data derived from ROESY (rotating frame Overhauser enhancement spectroscopy) measurements[13], and qualitative estimates of $^{3}J_{H\text{-}C}$ couplings from HMBC correlations.

RESULTS AND DISCUSSIONS

Sponges belonging to the Dictyoceratida family Thorectidae are a source of either sesterterpenoids or modified indoles or in rare instances, both.[14] This work commenced when a member of an uncommon Thorectidae genus, *Aplysinopsis*, was collected (#88102, Fiji Is.) and identified as *A.* cf. *elegans*. A review by Bergquist and Wells noted that *A. elegans* contains uncharacterized furanosesterterpenes.[15]



1 a,b R = H (α or β)
1 c,d R = Ac (α or β)



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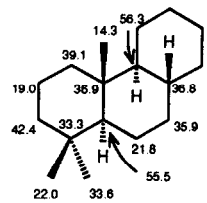

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A mixture of aplysolide A (**1a**) and B (**1b**) could not be separated; subsequent acetylation followed by normal phase HPLC yielded pure **1c** and **1d**. An APT formula of $C_{25}H_{35}$ was calculated for **1a** by analyzing the ^{13}C HMQC spectrum of **1c** (Figure 1) and subtracting the count of an acetate. This formula, plus the presence of five methyls, indicated a sesterterpene. Furthermore, a tetracyclic skeleton was evident for **1** based on low-field NMR peaks for eight unsaturated carbons, including two carbonyls (see Table 1 and discussions below) for **1c**, in comparison to its molecular formula of $C_{27}H_{38}O_4$ established by HREIMS (426.2784, Δ 1.4 mmu of calcd). Resonances expected for a furan ring were not observed.

Extensive study of the spectroscopic properties of aplysolide acetate **1c** constituted our strategy to establish its structure as well as those of the related compounds **1a,b,d**. A heterocyclic ring, substructure **A**, was proposed based on ^{13}C NMR ($CDCl_3$) peaks diagnostic of a γ-acetoxy butenolide ß-substituted with a *trans* double bond. Characteristic resonances were observed for two carbonyl groups (δ 169.1, -OAc; and 170.3, C-19), one hemiacetal carbon (δ 92.2, C-25), and four sp^2 carbons (δ 115.4, C-18; 117.1, C-16; 156.6, C-15; and 160.2, C-17). An isolated AB vinyl proton pair, H-15/16 at δ 6.04

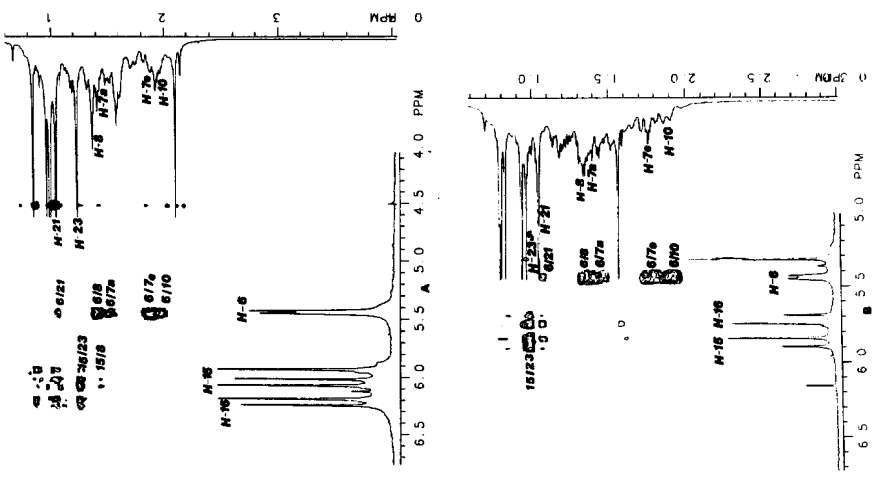


Figure 2. Expanded portion of the long range ^{1}H-^{1}H COSY at 300 MHz of **1c**. A) In $CDCl_3$. B) In C_6D_6.

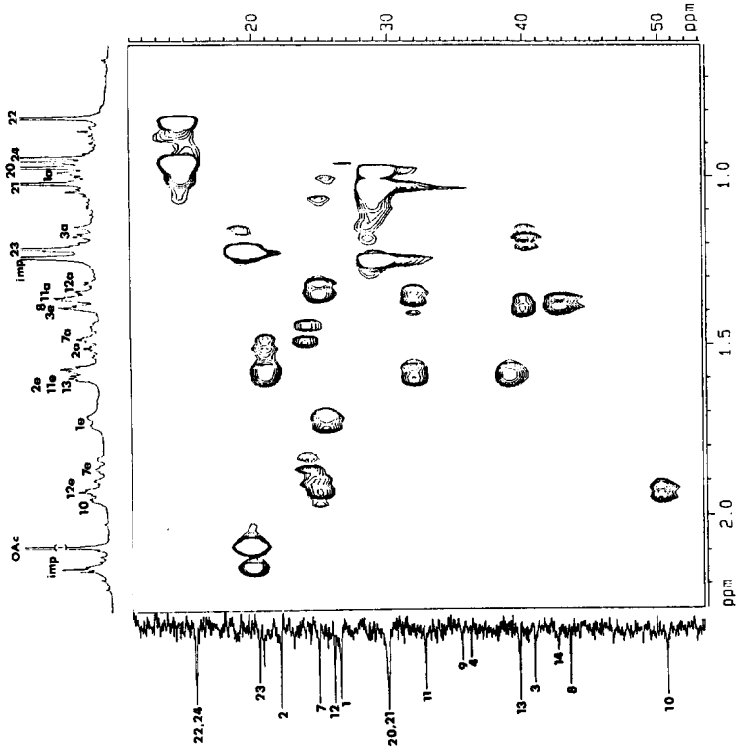


Figure 1. High-field region of the ^{1}H-^{13}C HMQC ($CDCl_3$; 500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrum of **1c**. For protons: a= axial; e= equatorial.

and 6.22 (d, J= 16 Hz) showed that **A** was terminated by a quaternary C-14. A methyl was also attached at C-14, as shown in **A**, because of $^{4}J_{H\text{-}H}$ and $^{5}J_{H\text{-}H}$ correlations observed in a ^{1}H-^{1}H SUPER-COSY NMR spectrum (Figure 2) between this singlet methyl (δ 1.22) and both olefinic signals. Substructure **A** is somewhat different from the butenolides present in other sponge higher terpenoids such as the mokupalides[16], luffariellins[17], manoalide[18], and thorectolide[19].

The characterization of the remaining monounsaturated tricarbocyclic system with its attached methyls (^{1}H NMR three singlets and one doublet) was next. The presence of a methyl at C-14 in substructure **A** intimated that the remaining tricyclic ring system was a methyl-rearranged terpenoid skeleton. This was substantiated during the analysis of COSY data which used the vinyl C/H-6 as an anchor point. Proton H-6 (δ 5.44) appeared as a simple doublet (J = 6 Hz, Figure 2) and must be attached to a trisubstituted double bond. Fortunately, a potential pitfall of constraining H-6 as adjacent to a methine site was averted. Assignment of a =C(H)-CH_2- was justified by the $^{3}J_{H\text{-}H}$ coupling observed from the vinyl proton to a dt at δ 1.85 (Figure 1) which was clearly geminal to a dd at δ 1.45 as this pair showed mutual correlation to a δ 24.7 carbon in the HMQC spectrum (Figure 1). This double bond was proposed at C-5/6 to explain the very small chemical shift differences in the C-4 geminal Me's (δ 29.7 and 29.8) versus the large spread in shifts of the corresponding Me's of podocarpane (**3**)[26]. Moreover, both C-4 Me's exhibited a clear $^{3}J_{C\text{-}H}$ correlation to C-4 in the HMBC spectrum (Figure 3 and Table 2). Partial structure **B** could now be proposed based on the $^{3\text{-}5}J_{H\text{-}H}$ correlations observed between H-6 and five other H's in the SUPER-COSY spectrum of Figure 2. Parallel evidence for **B** was also derived from the $^{3}J_{CH}$ HMBC correlations observed from H-6 to three

Table 1. ^{13}C and ^{1}H NMR Data

	1c[a]		Verrucosin-A **4** [a,b]
Atom	^{13}C(mult)	^{1}H (mult,J in Hz)	^{13}C (mult)
1	26.2 (t)	1.72 (m)[c] 1.12 (m)[d]	28.4 (t)
2	21.8 (t)[c]	1.58 (m)[c] 1.48 (m)[d]	28.8 (t)
3	40.6 (t)	1.37 (m)[c] 1.18 (m)[d]	40.8 (t)
4	35.8 (s)		38.0 (s)[c]
5	146.1 (s)		144.9 (d)
6	116.3 (t)	5.44 (br d,6)	113.9 (d)
7	24.7 (t)	1.85 (dt,12,6,6) 1.45 (br dd,12,8)[d]	35.0 (t)
8	43.2 (d)	1.37 (m)	37.3 (s)[c]
9	35.2 (s)		36.3 (s)[c]
10	50.5 (d)	1.95 (m)	51.7 (d)[c,e]
11	32.5 (t)	1.57 (m)[c] 1.37 (m)[d]	29.7 (t)
12	25.8 (t)	1.90 (m)[c] 1.32 (m)[d]	29.7 (t)
13	39.5 (d)	1.57 (m)	29.0 (d)
14	42.4 (s)		36.7 (d)
15	156.6 (d)	6.04 (d,16)	
16	117.1 (d)	6.22 (d,16)	
17	160.2 (s)	5.93 (s)	
18	115.4 (d)		
19	170.3 (s)		
20	29.8 (q)	0.98 (s)	29.6 (q)
21	29.7 (q)	1.03 (s)	28.3 (q)
22	15.6 (q)	0.83 (s)	18.7 (q)
23	20.6 (q)	1.22 (s)	18.1 (q)
24	15.6 (q)	0.96 (d,7)	21.4 (q)
25	92.2 (d)	7.13 (s)	
$\underline{COCH_3}$	169.1 (s)		
$\underline{COCH_3}$	20.3 (s)	2.09 (s)	

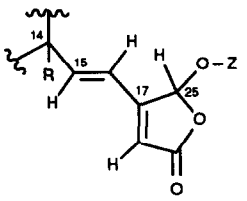
[a]In $CDCl_3$ [b]ref. 20 [c]Interchangeable
[d]Equatorial [e]Axial [f]our reassignment

Table 2. Selected 2D NMR Correlations of 1c[a]

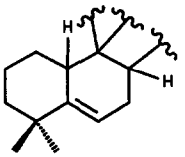
	^{1}H-^{13}C HMBC ($CDCl_3$)		ROESY (C_6D_6)
	Long range correlations to C[f]		NOE Correlations
	$^{2}J_{C\text{-}H}$	$^{3}J_{C\text{-}H}$	to H[f]
H-1e		C-3	
H-2e		C-4,C-10	
H-3e		C-1	
H-3a		C-20	
H-6		C-8,C-10,C-4	
H-8		C-23	H-10
H-11e	C-9	C-13	
H-11a			H-10
H-15	C-16,C-14	C-23,C-25	
H-16	C-15	C-14,C-25	
H-18		C-25,C-16,C-15	H-15
H-20	C-4	C-3,C-5,C-21	H-10
H-21	C-4	C-3,C-5,C-20	H-6
H-22	C-9	C-8,C-10,C-11	
H-23	C-14	C-8,C-13,C-15	H-16,H-22
H-24	C-13	C-12,C-14	H-8,H-11a,H-15

[a] 500 MHz for ^{1}H.

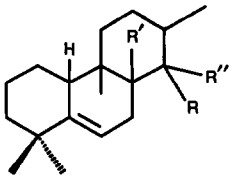
other C's as summarized in Table 2. Furthermore, the ^{13}C NMR resonance at δ 26.2 assigned to C-1 was considerably shielded as compared to the C-1 shift (δ 39.1) of **3** due to the absence of a C-10 Me ß-effect. The shift of C-1 provided the foundation for other assignments shown in the HMBC spectrum (Figure 3) including contours at the intersection of C-3 ··· H-1e, C-1 ··· H-3e, C-20 ··· H-3a and C-3 ··· H-20/21.



A R=Me, Z=Ac
A' R=Z=H

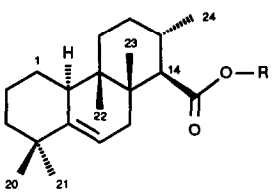


B



C

A search of our sponge-nudibranch natural products database files using substructure **B** did not give any matches. However, a repeat search with **B** having C-8 constrained as quaternary revealed two related compounds of general structure **C** (R' = Me, R" = H): verrucosin-A (**4**) and B isolated from the nudibranch *Doris verrucosa*[20]. The ^{13}C shifts of verrucosin-A, whose framework is based on an X-ray crystallography study, proved helpful. Good agreement was observed between the ^{13}C shifts of aplysolide A acetate (**1c**) and verrucosin-A (**4**) for C-1 to C-6, C-9 to C-12, C-20, and C-21 (Table 1). Substructure **C** (R' = H, R" = Me) could now be justified for aplysolide A acetate. The additional correlations noted in Figures 1-3 indicated the union of **A** and **C** (R' = H, R" = Me) for the final gross structure **1c**.



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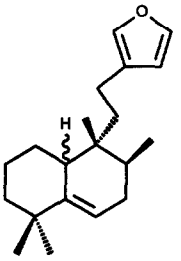
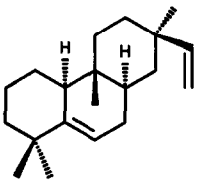


5a H-10 = β
5b H-10 = α



6

The stereochemical features of aplysolide A acetate were studied next. The ^{13}C methyl chemical shifts at δ 20.6 (C-23), and 15.6 (C-24) were indicative of two axial methyl groups, and the methyl chemical shift of δ 15.6 (C-22) was consistent with an axial methyl at a *trans*-BC ring junction. Also the ^{1}H NMR shift of Me-22 (δ 0.83) matches the literature data for C-9 methyl groups in general structure **C** which have been assigned as axial when shielding is observed from the Δ5 double bond. Axial methyl shift ranges are δ 0.62 - 0.69 for diterpenes[21] and δ 0.81 - 0.84 for triterpenes[23]. The H-10 stereochemistry was most likely axial, but resolving a similar point of stereochemistry has, in the past, caused difficulties for others. For example, the H-10 stereochemistry of an ambliol-B dehydration product was first proposed as **5a**[21], but later revised to **5b** based on X-ray crystallographic results[22]. The H-10 stereochemistry of a series of triterpene adianenoic acid derivatives of general structure **C** has never been determined.[23] Our belief that the C-10 chemical shift

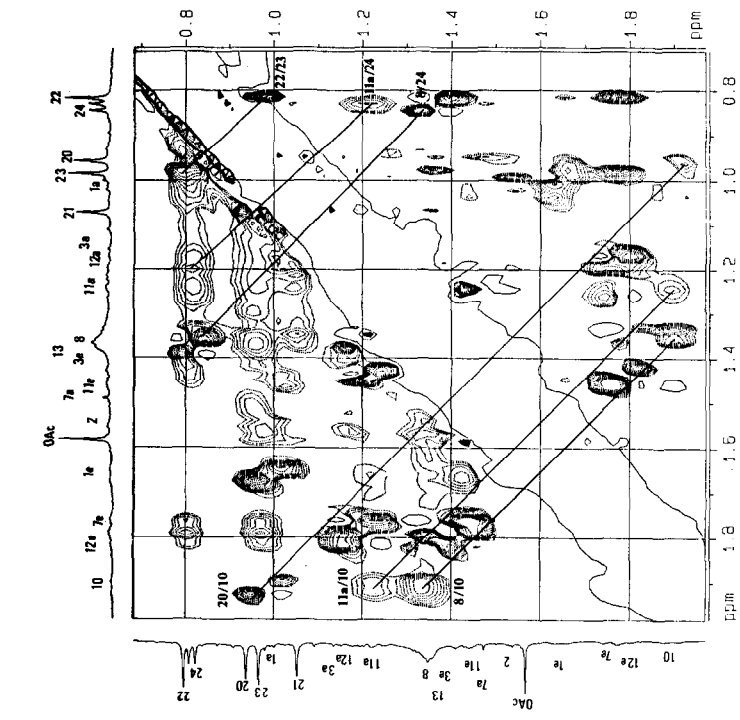


Figure 4. High-field region of the ROESY (C_6D_6, 500 MHz) spectrum of **1c**. Note: a = axial; e = equatorial.

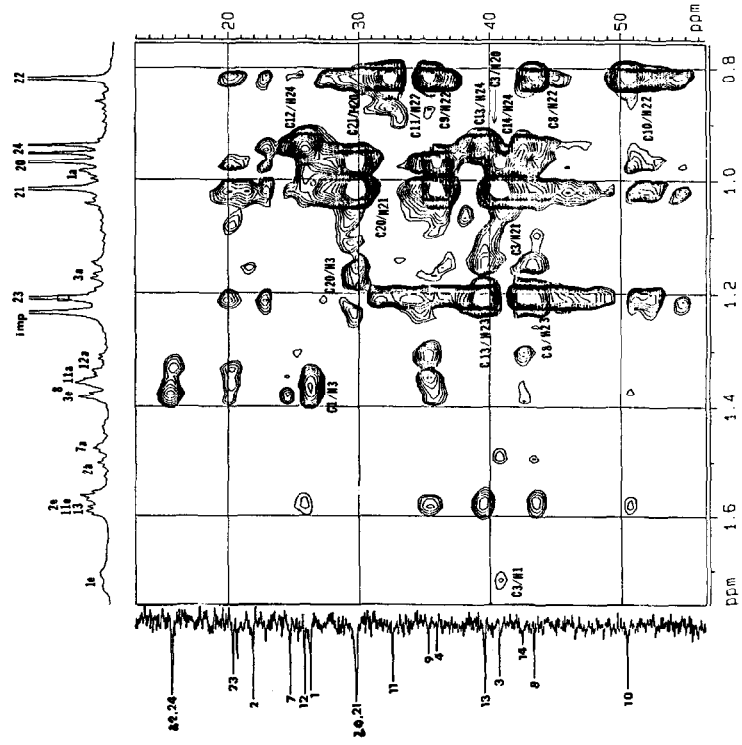
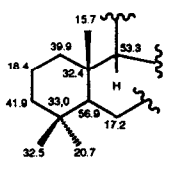


Figure 3. High-field region of the ^1H-^{13}C HMBC ($CDCl_3$ 500 MHz for ^1H, 125 MHz for ^{13}C) spectrum of **1c**.

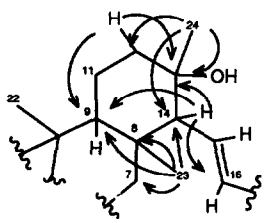
of tricyclics of general structure **C** might be sensitive to the geometry of the ring junction stereochemistry was not justified because this assignment was uncertain in key models including **4** (C-10 = 36.1 or 51.7)[20], **5b** (C-10 = 33.7 or 40.3)[22], and **6** (C-10 = 36.1 or 47.3)[24]. A ROESY spectrum of **1c** in (C_6D_6, Figure 4 and Table 2) provided definitive results as an Overhauser correlation was seen from H-10 to axial protons H-8, H-11, and Me-20. All of the other stereochemical assignments proposed above were vindicated by Overhauser correlations from Me-23 to Me-22 and H-16, and from Me-24 to H-8 and H-11a (Table 2). The additional correlations summarized in Table 2 indicated that the side chain attached to C-14 was rigid and was in the plane defined by Me-23···C-14···C-11. Also, only the conformer with H-15 *syn* to H-18 appeared to be present.

The related compound aplysolide B acetate (**1d**) showed spectroscopic data, especially ^{13}C shifts, which were nearly identical to those of **1c** with the exception of shifts in the vicinity of the butenolide substructure. Thus, aplysolides A and B were concluded to be C-25 epimers.

Aplyolide A (**2**), a yellow oil, was clearly a sesterterpene as evidenced by the $C_{25}H_{36}$ APT formula and the five ^1H NMR Me singlets (Figure 5). A minuscule mass spectral peak molecular ion (LRFAB) at *m/z* 403 ($C_{25}H_{39}O_4$, M^++H) was accompanied by an intense M^+-H_2O+H peak at *m/z* 385 of formula $C_{25}H_{37}O_3$ (HRFAB *m/z* 385.2738, Δ 0.5 of calcd). The seven unsaturations of **2** were comprised of three multiple bonds [^{13}C NMR peaks (Figure 6) of a C=O (δ171.6 (s), and two C=C's δ 162.6 (s), 145.4 (d), 122.6 (d), 114.4 (d)]; consequently, there must be four rings. A butenolide moiety was identified first by analogy to the ^{13}C and ^1H NMR resonances of **A** in **1**, including such resonances in **2** as δ 98.4 (C-25), 171.6 (C-19), 114.4 (C-18) and 162.6 (C-17). Further evidence came from the IR OH and C=O bands [3310, 1757 and 1734 cm^{-1}]. The entire substructure **A'** with a *trans*-propenyl terminated by a methine at C-14 was based on the data above and on the ^1H NMR ABX pattern [δ 6.26 (1H, d, J = 16 Hz), 6.51 (1H, dd, J = 16 & 11 Hz), and 1.95 (1H, d, J = 11 Hz] and the $^4J_{HH}$ correlation peak observed between the ^1H signals at δ 5.89 (H-18) and 6.28 (H-16) in a ^1H-^1H NMR TOCSY[25] spectrum. The remaining three rings were assumed to be carbocyclic and it seemed that the structures of aplyolide and the aplysolides were closely related. However, since the remaining upfield 17 proton signals were complex and substantially overlapping making the ^1H-^1H COSY NMR difficult to decipher, no additional substructural features could be proposed from the ^1H NMR data. Additional subunits **D** and **E** were assembled once ^1H-^{13}C COSY NMR data became available. Initially, sample size was a limitation as only 15 mg of material was available, but high quality 2D NMR data were obtained by the inverse techniques of HMQC (Figure 5) and HMBC (Figure 6). Array **D** was proposed when a majority of the AB-ring and the three Me ^{13}C NMR shifts of podocarpane **3**[26] were located in the spectrum of **2** (see data accompanying structure **D**). The long-range correlations seen in Figure 6 from the protons of Me-22 along with additional ones to C-1 and C-10 plus the correlations from the protons of Me-21/20 to C-3 and C-5 (Table 3) confirmed substructure **D**. A search of our sponge-nudibranch database using the constraints of the APT formula range $C_{25}H_{35}$ - $C_{25}H_{37}$ and substructure **D** plus two additional carbocyclic rings provided just two matches, suvanine anion (**7**)[1b] and deactyl luffolide (**8**)[27]. The lead provided by these compounds enabled a composite structure **2** (without stereochemistry) to be proposed. It was consistent with the ^1H-^{13}C COSY NMR correlations shown for substructure **E**. Moreover, the



D



E

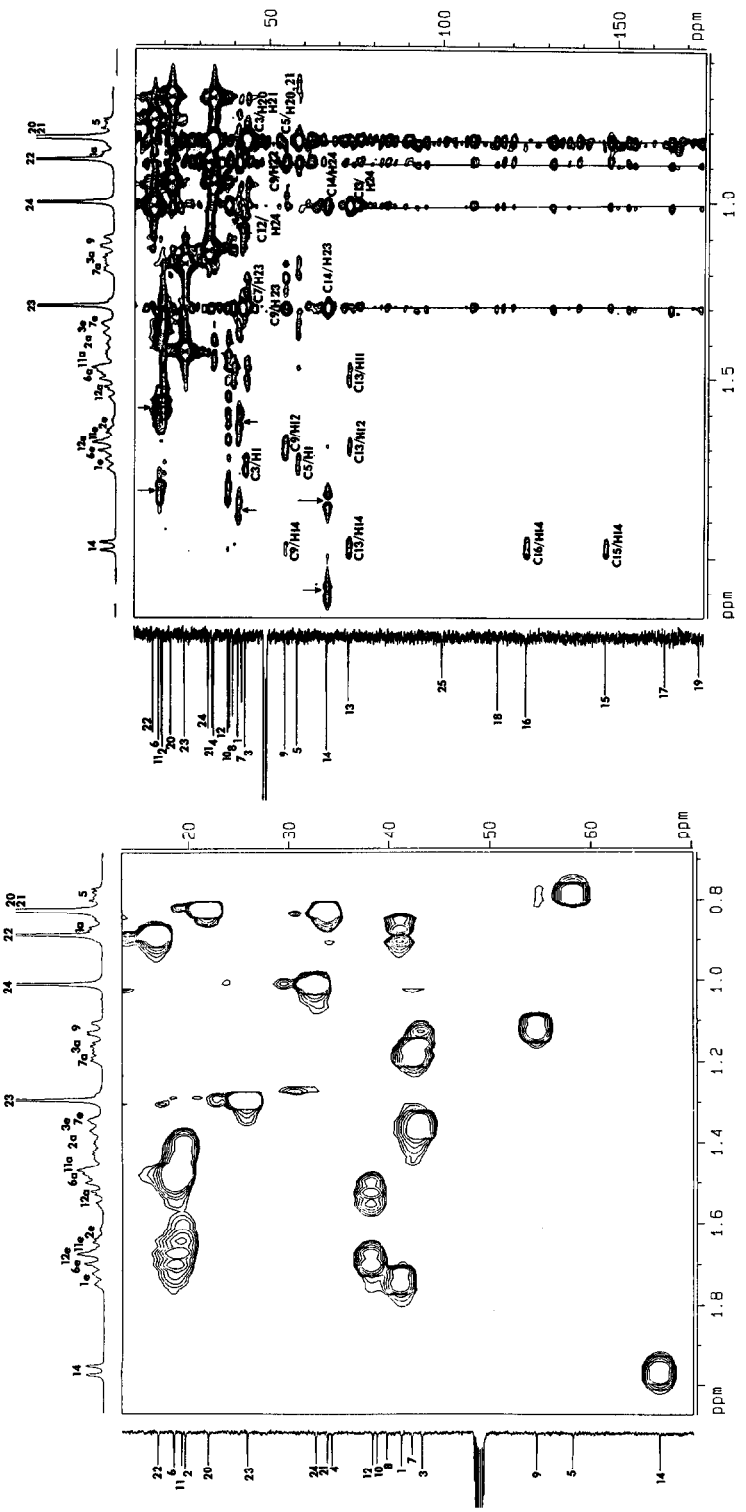


Figure 5. High-field region of the ^{1}H-^{13}C HMQC spectrum of **2**. (CD_3OD; 500 MHz for ^{1}H; 125 MHz for ^{13}C). For protons; a = axial; e = equatorial.

Figure 6. High-field region for ^{1}H and all ^{13}C resonances of the ^{1}H-^{13}C HMBC spectrum of **2**. The conditions are those of Figure **5**. Incompletely suppressed $^{1}J_{CH}$'s are indicated by x or ->. The t_1 noise peaks commonly observed for intense methyls are indicated by the vertical bar.

^{13}C NMR shifts of its ABC ring carbons and associated methyls, excepting those at C-7/12/13, were close to those of the corresponding carbons in suvanine anion (**7**) as shown in Table 4.

Table 3. Selected $^{2\text{-}3}J_{C\text{-}H}$ Correlations from ^{1}H-^{13}C HMBC (CD_3OD) Data of **2**

H#	Long range correlations to C# $^{2}J_{C\text{-}H}$	$^{3}J_{C\text{-}H}$
H-1e	C-3,C-5	
H-12e	C-13	C-9
H-14	C-13,C-15	C-9,C-16
H-15	C-16	C-17
H-16	C-17	C-14,C-18,C-25
H-18	C-17,C-19	C-16,C-25
H-20/21	C-4	C-3,C-5,C-21/20
H-22	C-10	C-1,C-5,C-9
H-23	C-7,C-8,C-9,C-14	
H-24	C-13	C-12,C-14
H-25	C-17	C-18,C-19

* 500 MHz for ^{1}H.

It was clear that each of the three carbocyclic rings of **2** adopts regular chair conformations, and that AB-*trans* fused rings were present. These conclusions were based on the characteristic chemical shifts at axial Me's-22 & 20, at equatorial Me-21 and equatorial Me-24 (see **3** and data in Ref. 12), and on characteristic vicinal J's to axial H-5 (13 and 3 Hz), to equatorial H-7 (5 and 5 Hz), and to axial H-9 (13 and 3 Hz). Since $^{3}J_{C\text{-}H}$'s are dihedral angle dependent they can also be used to estimate conformation[28] via qualitative comparison of HMBC cross-peak intensities to geminal protons[29]. Such HMBC correlations are observed from H-12e to C-9 or H-1e to C-3 and none are observed for the respective axial H's (Figure 6). These data also indicate that rings A and C are regular chairs.

The interpretation of the ^{13}C NMR shift of Me-23 (δ 24.6) was problematic. This value was close to the shift of Me-23 (δ 26) located at the BC-*cis* ring junction of suvanine (**7**), but in between the shift expected for a methyl at the ring junction of a tricyclic skeleton with a methyl at a *trans* (δ 18.1) or *cis* (δ 30-33) ring junction, based respectively on ^{13}C NMR data of cheilanthatriol (**9**)[30] and suvanine degradation products **7a** and **7b**[1b]. Other determining influences on this shift in these latter compounds are the number and geometry of substituents at positions C-13/14. Thus, δ and γ ^{13}C NMR chemical shift increments[12,31] were used to fine tune calculated shifts of two plausible stereochemical possibilities as follows. A *trans*-BC ring junction model, represented by structure **2y** with an axial R group at C-14 (this geometry was subsequently verified from ROESY data), has a calculated Me-23 shift of 24 - 27 ppm[32], whereas a *cis*-BC ring junction model, represented by structure **2n** with an axial R group at C-14 along with an axial OH group C-13, has a calculated Me-23 shift of 33 - 34 ppm.[33] The experimental Me-23 shifts were in better agreement with those of **2y**.

The ROESY spectrum shown in Figure 7 provided the final definitive data to assign the stereochemistry shown in **2**. Important ROESY correlations were from

Table 4. ^{13}C and ^{1}H NMR Data

	2[a]		(**7**)[b]
Atom	^{13}C(mult)	^{1}H (mult,J in H_z)	^{13}C(mult)
1	39.9 (t)	1.74 (dt,13,5,5)[e]	41.5(t)
		0.87 (m)[g]	
2	18.4*(t)	1.65 (m)*	18.3 (t)
		1.42 (m)[g]	
3	41.9 (t)	1.36*	41.6 (t)
		1.14[g]	
4	33.0 (s)		32.9 (s)
5	56.9 (d)	0.79 (d,13,3)	56.5 (d)
6	17.2 (t)	1.71 (m)*	17.9 (t)
		1.48 (m)[g]	
7	40.9 (t)	1.36 (13,5,5)*	34.8 (t)
		1.19 (m)[g]	
8	38.4 (s)		38.0 (s)
9	53.3 (d)	1.11 (dd,13,3)	52.5 (d)
10	37.4 (s)		38.4 (t)
11	18.0*(t)	1.67 (m)*	19.7 (t)
12	37.0 (t)	1.69 (dt,13,3,3)*	23.9 (t)
13	71.6 (s)		118.4 (s)
14	65.4 (d)	1.95 (d,11)	41.7 (d)
15	145.4 (d)	6.51 (dd,11,16)	24.9 (t)
16	122.6 (d)	6.26 (d,16)	23.0 (t)
17	162.6 (s)		124.9 (s)
18	114.4 (d)	5.89 (s)	111.0 (d)
19	171.6 (s)		142.9 (d)
20	20.7 (q)	0.81 (s)	21.6 (q)
21	32.5 (q)	0.81 (s)	33.2 (q)
22	15.7 (q)	0.87 (s)	17.7 (q)
23	24.6 (q)	1.28 (s)	26.0 (q)
24	31.4 (q)	0.99 (s)	133.3 (d)
25	98.4 (d)	6.28 (s)	138.8 (d)

[a]In CD_3OD [b]In DMSO-d_6 (ref. 1b)
*Interchangeable [e]Equatorial [g]Axial

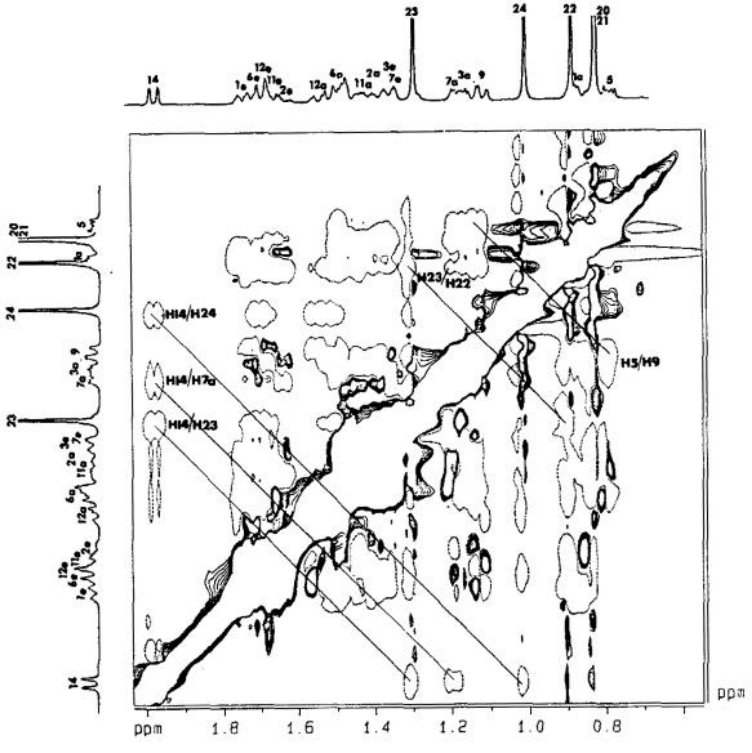


Figure 7. High-field region of the ROESY spectrum of **2**. (CD_3OD; 500 MHz for ^1H). Note: a = axial; e = equatorial.

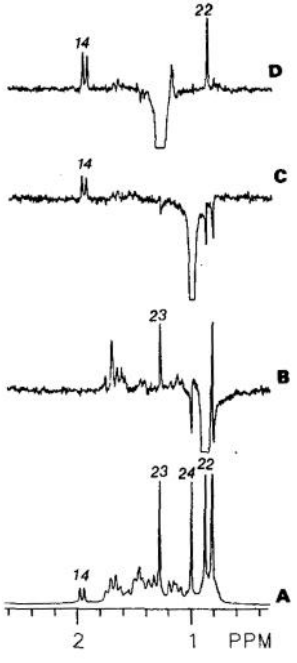
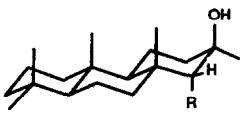
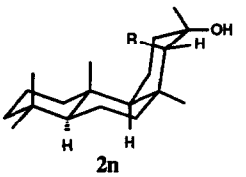


Figure 8. The 300 MHz for ^1H NMR spectrum (A) in CD_3OD of **1** from 0.4 to 2.6 ppm. The difference NOE spectra (B, C, D) resulting from the irradiation of the signals corresponding to H-22 (B), H-24 (C), and H-23 (D) are shown as positive NOE effects.

H-14 to Me-23 and Me-24. Additional meaningful ROESY correlations were between respectively Me-22 and Me-23, H-9 and H-15, or H-12a and H-15. Unquestionably, structure **2y** was in agreement with these data while alternative **2n** was not. Quantitative NOE data obtained from a one-dimensional difference experiment (Figure 8) gave enhancements: Me-22 to Me-23 = 4%, Me-23 to H-14 = 3%.

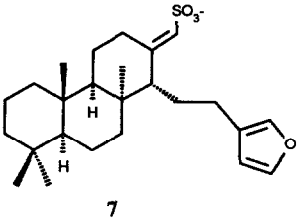


2y



2n

The aplysolides and aplyolide A were evaluated at Syntex Research by Dr. M. J. Ernest, in anti-inflammatory assays by testing for inhibition to human PMN PLA2 enzyme. An endogenous arachidonic acid release assay was employed with manoalide[3] (IC_{50}≈2μM vs PLA2) as a positive control. Aplyolide A showed 100% inhibition at 30μM and an IC_{50} = 10.5 μM. The acetate of aplyolide A was slightly more active and exhibited 60% inhibition at 10μM, but no IC_{50} was determined. These represent mild positive activity results. The remaining compounds, inactive when tested at 30 μM, included a mixture of aplysolides A and B and aplysolide A acetate.



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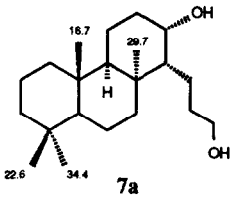
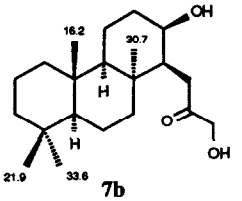
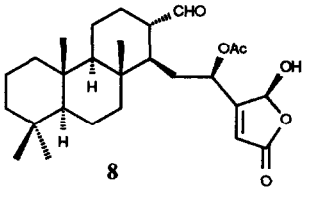


7a



7b



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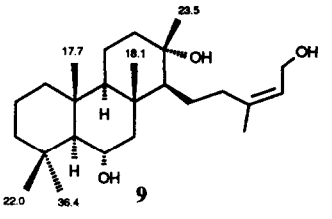

9

CONCLUDING REMARKS

It is rare to find rearranged isoprenoid carbon skeletons among the sesterterpenes that have been isolated to date from marine organisms. The only exceptions appear to be a ring contracted manoalide derivative, luffariellin A[34], and methyl rearranged compounds such as palauolide[2] and furoscalarol[2]. The aplysolides can be added to this small inventory. The carbon skeleton of the aplysolides (**1**) is new, and a most probable biogenesis involves a concerted rearrangement of the methyl groups at C-10 and C-8 of the **2** framework followed by expulsion of a proton to give the $\Delta^{5(6)}$ double bond. The AB rings of the aplysolides are similar to those of the rimuene type diterpenes (**6**) which have a rearranged rosane skeleton[24].

A concise approach to the structure elucidation of complex, non-crystalline terpenoids, available in only milligram amounts, has been illustrated above. We have shown how compounds of known structural families, such as **2**, can be proficiently characterized. First 1D ^{13}C NMR data, then 2D ^{1}H-^{1}H COSY NMR data are used to deduce both a complete

CH formula (the so-called APT MF) and a few key substructures. Next, both the APT formula and the short list of substructures are used as input in database searches which provide biogenetic insights to construct complete structures. A parallel tactic can also be employed to characterize new compounds of unknown structural families such as **1a** and **1b**. In this case there is an important difference in that a comprehensive list of substructures must be generated. This demands extensive 2D NMR data, especially from long-range COSY NMR data emphasizing the detection of $^{3\text{-}5}J_{H\text{-}H}$ and $^{2\text{-}3}J_{C\text{-}H}$ correlations. The inverse detection tactics of HMQC and HMBC are essential for obtaining such data on limited amounts of sample. For this latter case, database searches of substructures are less useful in providing biogenetic precedents for insights as to the proper way to interconnect substructures. Rather, long-range COSY NMR data, often gathered from parallel runs in different solvents, must be exhaustively obtained and analyzed. Overhauser effect data should also be sought. The possibility of incorrect interpretations due to the ever present situation of overlapping proton resonances must be carefully considered to insure that NOE data is accurately interpreted and that the substructures are correctly joined. Ideally, stereochemical and conformational assignments are finalized by considering two or more types of data (e.g., $^{3}J_{HH}$, $^{3}J_{CH}$, NOE) which lead to the same conclusion. Although results from computer molecular modeling were not employed in this study, we have in the past combined this approach with results of NOE and ^{1}H NMR vicinal J measurements to derive relative stereochemical[35] or conformational analysis relationships[36].

EXPERIMENTAL SECTION

The NMR spectra were recorded at 99.5 MHz for ^{1}H, 25.0 MHz for ^{13}C, or at 300 MHz for ^{1}H, 75 MHz for ^{13}C, or at 500.14 MHz for ^{1}H, 125.77 for ^{13}C. Multiplicities of ^{13}C NMR resonances were determined from APT or DEPT data, and COSY experiments were done at ^{1}H frequencies of 300 or 500 MHz. Low resolution electron impact mass spectrometry data were obtained at U.C.S.C., while high resolution mass spectral data were obtained from the U.C. Berkeley MS laboratory. High performance liquid chromatography (HPLC) was done using columns that included 10 μ ODS or 10 μ silica. All solvents were distilled and dried for HPLC use and were spectral grade for spectroscopy.

Two-Dimensional NMR Procedures. Literature pulse sequences were used for the APT[5], ^{1}H-^{1}H COSY[5], ^{1}H-^{13}C COSY[5], ROSY[13], HMQC[6], and HMBC[6] experiments. The parameters used in this work are as follows. HMQC: 2 X 128 X 1024 data matrix size (two separate sets of data, with 1024 data points in t_2 and 128 data points in t_1); 32 scans (preceded by one dummy scan) per t_1 value; recycle delay = 2.5 s, 800 ms "weft" delay period; broad band (16 W) ^{13}C-decoupling during the acquisition period; 6-Hz Gaussian and 90°-shifted sine bell filtering in t_2 and t_1, respectively. HMBC: 128 X 512 data matrix size; 128 scans (preceded by 2 dummy scans) per t_1 value; recycle delay = 2.5 s; 36 μs 90° ^{13}C pulse width; Δ_1 and Δ_2 durations of 3.5 and 55 ms, respectively; sine bell filter and 39-Hz Gaussian filtering in t_2 and t_1, respectively. COSY: 1 X 256 X 1024 data matrix size; 200-ms repetition delay; 32 scans per t_1 increment; sine bell filtering in t_2 and t_1, followed by application of a magnitude calculation. ROESY experiments with a $(90\text{-}t_1\text{-}90\text{-}\tau_m\text{-}90\text{-acquire})_n$ pulse-sequence and phase-cycling scheme were designed to separate the real and imaginary parts of the t_1 dimension. Three ROESY data sets (256 X 1K) were collected with mixing times (τ_m) of 100, 250, and 600 ms using a delay of 3.0 s between scans. The sample conditions were: 15 mg of **1** in 0.4 ml of CD_3OD and 10 mg of **2b** in 0.4 ml $CDCl_3$.

Database Searches. We have created a marine natural products database using the ChemBase software package which operates on an IBM compatible PC.[37] This database is organized along phyletic lines and the subset of ≈ 1100 records

of sponge/nudibranch natural products was searched at several points during this research. An important searchable field that we have created is the Attached Proton Test (APT) Molecular Formula which is a total of CH_n's by type. Symmetry has also be taken into account so that the stored formula will exactly match that derived from an experimental APT spectrum, which are usually acquired in such a way that peak intensities cannot be integrated.

Identification. The sponge (collection no. 88102) *Aplysinopsis* cf. *elegans* (Family Thorectidae, Order Dyctioceratida, voucher specimens & underwater photo available from PC) was identified by Ms. M. C. Diaz (U.C.S.C., Institute of Marine Sciences). This organism can be regularly collected from Fiji. Our voucher specimen no. 88102 was carefully examined and exhibits the following characteristics: color - a brown exterior and tan interior which becomes grey-black when dried; shape - three small coalescent tubes; consistency - hard to tear; surface - conulose, very marked, high conules with blunt ends; ectosome - only an organic skin; choanosome - fibroreticle, primaries cored with sand, secondaries not cored; spicules - none; fibers - laminated, with pith. Its properties do not appear to match any species described to date; the closest match is with *A. elegans*.

Isolation Procedures. The preserved sponge (120 g wet weight) was soaked three times with MeOH for 24 hr. The solvent was decanted and the oil concentrated to yield 5.7 g of a crude viscous oil. As detected by the ^1H and ^{13}C NMR spectra, sesterterpenes were the major constituents of the extract. The crude oil (5.7 g) was then successively partitioned between equal volumes (500 ml of aqueous MeOH, percent adjusted to produce a biphase solution) and a solvent series of hexanes (600 mg), CCl_4 (760 mg), and CH_2Cl_2 (260 mg). The CH_2Cl_2 fraction was chromatographed on silica gel (CH_2Cl_2/MeOH) followed by normal phase HPLC (EtOAc/hexanes 1:1) yielding pure **2** (17 mg). The hexanes and CCl_4 partition fractions were separately chromatographed on silica gel (CH_2Cl_2/ MeOH) followed by normal phase HPLC (AcOEt/hexanes, 22:78) to yield an inseparable mixture of **1a** and **1b**. This mixture was acetylated with Ac_2O and pyridine (1:1) overnight at room temperature, concentrated under reduced pressure, and then subjected to normal phase HPLC (AcOEt/hexanes, 6:94) which afforded the monoacetylated compounds **1c** (12 mg) and **1d** (4 mg).

Aplysolide A acetate (1c): colorless oil; $[\alpha]_D$ = -20° (c = 0.7 g/100 mL, $CHCl_3$); IR (neat) 2941, 1777, 1758, 1640, 1383, 1364, 1214, 1160, 1076 cm^{-1}; UV (MeOH) λ_{max} 269 (E = 29,000); NMR (C_6D_6, [atom number] δ in ppm from Me_4Si ^{13}C at 75 MHz, ^1H at 300 MHz, multiplicities, *J* (Hz) [1] 26.2 (t), 1.65 & 1.00 (m, 2H); [2] 21.9 (t), 1.55 (m, 2H); [3] 40.6 (t), 1.35 & 1.10 (m, 2H); [4] 35.7 (s); [5] 145.8 (s); [6] 116.6 (d), 5.44 (br d, J = 6.0 Hz); [7] 24.5 (t), 1.76 & 1.45 (m, 2H); [8] 42.9 (d), 1.32 (m); [9] 35.1 (s); [10] 50.4 (d), 1.88 (br d, J = 12.6 Hz); [11] 32.4 (t), 1.45 & 1.25 (m); [12] 25.7 (t), 1.70 & 1.35 (m); [13] 39.2 (d), 1.35 (m); [14] 41.8 (s); [15] 154.8 (d), 5.87 (d, J = 16.8 Hz, 1H); [16] 117.1 (d), 5.72 (d, J = 16.8 Hz, 1H); [17] 159.4 (s); [18] 115.4 (d), 5.33 (s, 1H); [19] 169.4 (s); [20] 29.6 (q), 1.04 (s, 3H); [21] 29.5, 0.92 (s, 3H); [22] 15.4 (q), 0.78 (s, 3H); [23] 19.9 (q), 0.96 (s, 3H); [24] 15.38 (q), 0.81 (d, J = 7.5 Hz, 3H); [25] 91.9 (d), 7.03 (s, 1H); [$\underline{CO}CH_3$] 168.5 (s); [$CO\underline{CH_3}$] 19.8 (q), 1.57 (s, 3H); NMR data in other solvents are in Table 2; HREIMS 426.2784 (M^+, $C_{27}H_{38}O_4$, Δ 1.4 mmu of calcd); LRCIMS (isobutane) (%) 427 [M^++H (38)], 368 [M^+-OAc (73)], 340 (12), 257 (19), 61 (100); LREIMS (isobutane) (%): 426 [M^+ (3)], 384 (2), 367 (22), 352 (17), 257 (12), 229 (45), 175 (100).

Aplysolide B acetate (1d): colorless oil; $[\alpha]_D$ = -9° (c = 0.4 g/100 mL, $CHCl_3$); IR (neat) 2933, 1733, 1759, 1640, 1350, 1335, 1213, 1160, 1024 cm^{-1}; UV (MeOH) λ_{max} 265 (ε = 28,500); ^1H 300 MHz, NMR ($CDCl_3$, [atom number] δ in ppm from Me_4Si, multiplicities, *J* (Hz)) [6] 5.44 (br d, J = 5.7 Hz), [15] 6.08 (d, J = 16.8 Hz), [20/21] 1.05 & 1.04 (s), [22] 0.84 (s), [23] 1.22 (s), [24] 0.94 (d, J = 7.2 Hz), [25] 7.13 (s, 1H), [$CO\underline{CH_3}$] 2.21 (s); ^{13}C 75 MHz NMR ($CDCl_3$,

[atom number assignments based on analogy with **1c**] δ in ppm from Me_4Si, multiplicities), [1] 26.2 (t); [2] 21.8 (t); [3] 40.8 (t); [4] 35.9 (s); [5] 146.0 (s); [6] 116.6 (d); [7] 24.8 (t); [8] 42.8 (d); [9] 35.3 (s); [10] 50.3 (d); [11] 32.5 (t); [12] 25.9 (t); [13] 39.9 (d); [14] 42.6 (s); [15] 156.3 (d); [16] 117.2 (d); [17] 159.9 (s); [18] 115.8 (d); [19] 170.3 (s); [20] 29.7 (q); [21] 29.5; [22] 15.5 (q); [23] 20.2 (q); [24] 15.7 (q); [25] 92.3 (d); [$\underline{CO}CH_3$] 169.3 (s); [$CO\underline{CH_3}$] 20.8 (q); HREIMS 426.2783 (M^+, $C_{27}H_{38}O_4$, Δ 1.3 mmu of calcd.); LRCIMS (isobutane) (%) 427 [M^+ +H (38)], 368 [M^+ -OAc (73)], 340 (12), 257 (19), 61 (100); LREIMS (%) 426 [M^+, 3], 384 (2), 367 (22), 352 (17), 257 (12), 229 (45), 175 (100).

Aplyolide A (2): colorless oil; $[\alpha]_D$ = -20° (c = 0.3 g/100 mL, MeOH); IR (neat) 3310, 2946, 1757, 1734, 1639, 1456, 1388, 1311, 1131, 978 cm^{-1}; UV (MeOH) λ_{max} 269 (ε = 51067); NMR (CD_3CN, [atom number] δ in ppm from Me_4Si ^{13}C at 75 MHz, ^1H at 300 MHz, multiplicities, *J* (Hz)) [1] 39.9 (t), 1.70 & 0.85 (m, 2H); [2] 19.3 (t), 1.40 (m, 2H); [3] 41.9 (t), 1.30 & 1.10 (m, 2H); [4] 33.1 (s); [5] 56.6 (d), 0.90 (m, 1H); [6] 18.2 (t), 1.40 (m, 2H); [7] 40.8 (t), 1.30 & 1.10 (m, 2H); [8] 38.5 (s); [9] 53.0 (d), 1.10 (m, 1H); [10] 37.4 (s); [11] 19.0 (t), 1.4 (m, 2H); [12] 37.0 (t), 1.65 (m, 2H); [13] 71.7 (s); [14] 65.5 (d), 1.92 (d, under CD_3CN, 1H); [15] 145.7 (d), 6.42 (dd, J = 11.1 & 15.9 Hz, 1H); [16] 122.4 (d), 6.24 (d, J = 15.9 Hz, 1H); [17] 162.0 (s); [18] 114.4 (d), 5.83 (s, 1H); [19] 171.2 (s); [20] 21.8 (q), 0.79 (s, 3H); [21] 32.7 (d), 0.80 (s, 3H); [22] 16.8 (q), 0.85 (s, 3H); [23] 25.7 (q), 1.24 (s, 3H); [24] 31.8 (q), 0.94 (s, 3H); [25] 97.6 (d), 6.18 (s, 1H); NMR data in other solvents are in Table 1; HRFABMS (positive ion) 403 (M^+ +H), 385.2738 (M^+ - H_2O +H, $C_{25}H_{37}O_3$, Δ 0.5 of calcd); LRCIMS (isobutane) (%) 385 [M^+ - H_2O +H, (85)], 368 (29), 289 (40), 261 (20), 231 (52), 193 (100).

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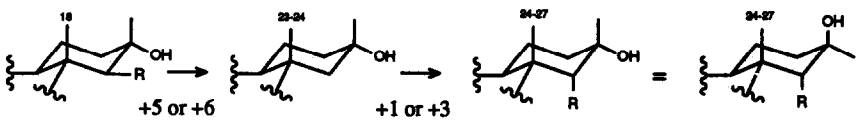

9 **2y**

33. In this case we are considering the difference in shift effects to a methyl which is *syn*-diaxial to either an oxygen or a methyl. This point has been previously considered by Stothers (see Ref. 31) who showed that Me or OH substituents gamma to a methyl and in a rigid *syn*-axial arrangement result in a similar deshielding effect at the latter. Others including ourselves have also commented on the usefulness of this effect in making stereochemical assignments. Analysis of data for polycyclic models assumed to have chair six membered rings indicates the following ranges for this substituent effect: 1,4 Me-Me +2.9 to +3.4, 1,4 Me-OH + 1.3 to 3.3. This is based on the Me shifts in the following sources: (a) Ref 1b and refs. within. (b) Ref. 12 and refs. within. (c) Ref. 31 and refs. within. (d) Englehardt, G.; Jancke, H.; Zeigan, D. *Org. Magn. Reson.* **1976**, *8*, 655.

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