construction of 1,3-diols. We are currently testing the use of other carbon nucleophiles such as propargylsilanes, crotylstannanes, aryltitanium trichlorides, and alkylaluminum reagents[21] with **2a** and **b** and related aldehydes having an additional chiral center at the α-position.

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[21] We have discovered that certain alkylaluminum reagents add to aldehydes in the presence of $TiCl_4$: Reetz, M. T.; Kesseler, K., unpublished results.

Amphimedine, New Aromatic Alkaloid from a Pacific Sponge, *Amphimedon* sp. Carbon Connectivity Determination from Natural Abundance ^{13}C–^{13}C Coupling Constants

Francis J. Schmitz,* Santosh K. Agarwal, and
Sarath P. Gunasekera

Department of Chemistry, University of Oklahoma
Norman, Oklahoma 73019

Paul G. Schmidt

Oklahoma Medical Research Foundation
Oklahoma City, Oklahoma 73104

James N. Shoolery

Varian Associates, Palo Alto, California 94303

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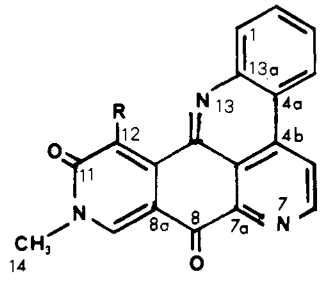
Although a wide variety of novel natural products have been isolated from marine organisms, relatively few alkaloids have been reported.[1] We report here a new, cytotoxic fused pentacyclic aromatic alkaloid obtained from a Pacific sponge. Owing to the paucity of protons in the molecule, extensive use was made in the structure elucidation of long-range proton–^{13}C couplings and also natural-abundance ^{13}C–^{13}C couplings obtained from a two-dimensional double-quantum coherence experiment.[2-4] The latter technique has been used rarely for structure elucidations to date,[5] but it is extremely useful for highly fused systems such as the one described herein.



1 **R = H**
2 **R = Br**

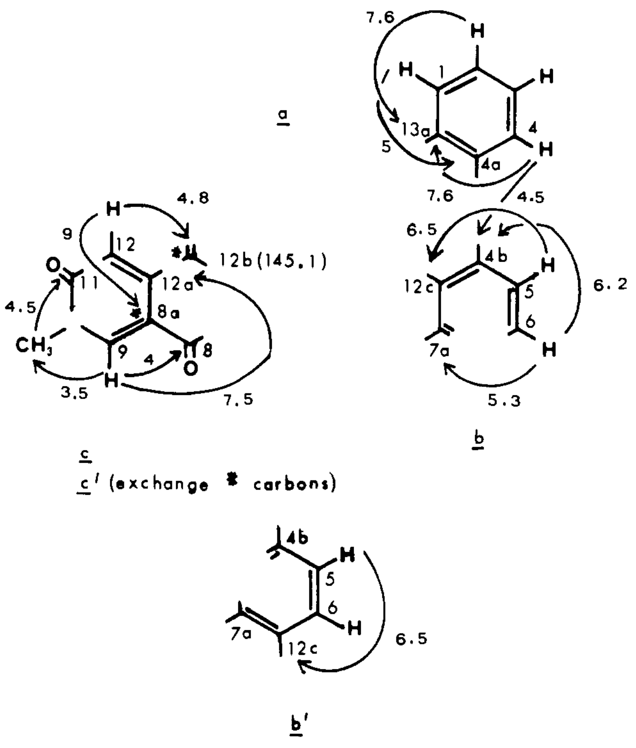


Figure 1. Partial structures from proton–proton and H–C–C–C coupling.

The new alkaloid, amphimedine (**1**), a sparingly soluble yellow solid, mp >360 °C, was isolated from an *Amphimedon* sp. of sponge collected at Guam Island at −3 m. Room-temperature (CH_2Cl_2; $CHCl_3$–MeOH; MeOH) and hot Soxhlet extracts ($CHCl_3$) of freeze-dried specimens were chromatographed sequentially over silica gel and alumina ($CHCl_3$–MeOH) to give pure amphimedine. High-resolution mass spectral analysis established the formula $C_{19}H_{11}N_3O_2$ (*m/e* 313.08547, +0.35 mass error) and indicated a very stable structure, since only a few fragment ions were observed, corresponding to losses of CH_3, CO, CHO, and HCN. The UV absorption of **1** [absolute ethanol, λ_{max} 210 nm (19690), 233 (39393), 281 (9099), 341 (6060)] changed significantly upon addition of $NaBH_4$ [λ_{max} nm 235 (12879), 280 (9090)], indicating the presence of conjugated ketone. Two intense carbonyl absorptions, 1690 and 1640 cm^{-1}, were thus attributed to α,β-unsaturated ketone and amide functionalities, respectively; no OH or NH absorptions were observed in the IR.

Owing to the very limited solubility of **1** in common organic solvents, most NMR spectral data were obtained in trifluoroacetic acid-*d* (TFA-*d*) and TFA-*d*–$CDCl_3$ (2:1); these data are summarized in Table I. Proton homonuclear decoupling confirmed that protons on carbons 1–4 were on contiguous atoms of an aromatic ring, that protons at C-5 and -6 constituted an isolated vicinal pair, and that protons at C-8 and -12 were each isolated. NOE enhancements were observed between H-4 and -5 and also between H-9 and the *N*-methyl protons (H-14). The ^{13}C data confirmed the presence of an amide carbonyl (C-11, δ 165.9) and a cross-conjugated ketone (C-8, δ 175.0).[6]

Since over half of the carbons in amphimedine were nonprotonated, further information regarding the skeletal network was sought from three-bond proton–carbon couplings, which were identified by a series of single-frequency decoupling experiments; see Table I and Figure 1. Analysis of these data confirmed the presence of partial structures a, b (or b′), and c (or c′), Figure 1, but did not provide evidence for joining the fragments. A link between carbons 4a and 4b was indicated by a three-bond coupling between H-4 and C-4b, but an H-5 to C-4a coupling was not observed.

When maximum use of proton and proton–carbon coupling did not unambiguously resolve the structure, attention was turned to determination of natural-abundance ^{13}C–^{13}C one-bond couplings

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[1] Fenical, W. *Science* (*Washington, D.C.*) **1982**, *215*, 923.

[2] Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* **1980**, *102*, 4849.

[3] Bax, A.; Freeman, R.; Frenkiel, T. A.; Levitt, M. H. *J. Magn. Reson.* **1981**, *43*, 478.

[4] Mareci, T. H.; Freeman, R. *J. Magn. Reson.* **1982**, *48*, 158.

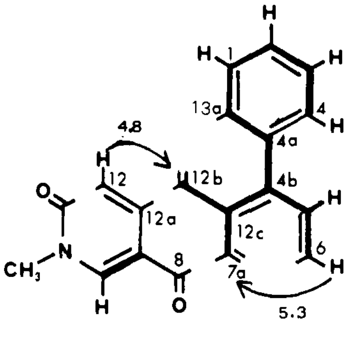
[5] (a) Cf.: Pinto, A. C.; Gonçalves, M. L. A.; Filho, R. B.; Neszmelyi, A.; Lukacs, G. *J. Chem. Soc., Chem. Commun.* **1982**, 293. (b) Freeman, R., Frenkiel, T. A. *J. Am. Chem. Soc.* **1982**, *104*, 5545.

[6] Levy, G. C.; Lichter, R. L.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance Spectroscopy"; Wiley: New York, 1980; Chapter 5.

Table I. Proton and Carbon-13 Data for Amphimedine (1)

C	δ H^a at C	H, m, J (Hz)	δ C^b	$J_{\text{H-C}}{}^b$		$J_{\text{H-C-C-C}}{}^b$	^{13}C-^{13}C^c coupled pairs obsd
1	8.68	d, 8.5	133.1	169.1	d	7.2 (H-3)	1-2, 1-13a
2	8.39	t, 8.5	137.4	164.5	dt	8.75 (H-4), 3 (H-1, -3)	2-1, 2-3
3	8.22	t, 8.5	132.5	166	d	8.4 (H-1)	3-2, 3-4
4	8.97	d, 8.5	125.8	162	d	8.0 (H-2)	
4a			120.5		m	5.0 (H-1), 9.5 (H-3)	4a-4b, 4a-13a
4b			146.2		dd	4.5 (H-4), 6.2 (H-6)	4b-4a, 4b-5, 4b-12c
5	9.53	d, 7	125.2	173	d	4.0 (H-6)	5-4b, 5-6
6	9.29	d, 7	139.0	196	d	2.5 (H-5)	6-5
7a			139.8		d	5.3 (H-6)	7a-12c
8			175		d	3.5 (H-9)	
8a			113.5		d	9.0 (H-12)	8a-9
9	9.20	s	147	185.7	q	4.0 (H-14)	9-8a
11			165.9		m	5.5 (H-9), 4.5 (H-14)	
12	8.52	s	115	175.9			
12a			143.9		dd	3 (H-12), 7.5 (H-9)	
12b			145.1		d	4.75 (H-12)	12b-12c
12c			119.0		d	6.5 (H-5)	12c-12b, 12c-7a, 12c-4b
13a			147.9		dd	7.6 (H-2), 7.6 (H-4)	13a-1, 13a-4a
14	4.10	s	40	144.8		3.5 (H-9)	

^{a} 300 MHz, TFA-d/CDCl$_3$ (2:1). ^{b} 75 MHz, TFA-d/CDCl$_3$ (2:1); assignments are based on single-frequency decoupling experiments. ^{c} 75 MHz, TFA-d.



d

Figure 2. Partial structure showing carbon connectivities (heavy lines) from ^{13}C–^{13}C couplings.

by using the two-dimensional INADEQUATE experiment described recently by the Freeman group,[2-4] from which it is possible to determine the connectivity of the entire carbon network. Due to the limited amount of sample available in our case, ~300 mg, and long T_1 values associated with the nonprotonated carbons, not all of the ^{13}C–^{13}C doublet pairs[3] in **1** were observable after a 40-h acquisition.[7] However, those that were observed provided the crucial information (see Table I) needed to confirm that partial structures b and c were valid, that C-12b was joined to C-12c and C-4a to C-4b, and hence that partial structure d, Figure 2, could be assumed. Insertion of a nitrogen atom at position 7 in d was dictated by the large H–C coupling (196 Hz) of C-6,[8] the low-field positions of H-6 (δ 9.27) and C-6 (δ 139.0),[9] and the three-bond coupling between C-7a and H-6. Insertion of another nitrogen atom between C-12b/C-13a was indicated by the downfield position of these carbons (δ 145.1 and 147.9), the relatively high-field position of C-4a and C-12c (δ 120.5 and 119.0),[9] and the lack of any three-bond coupling between H-1 and a carbon that might have been located at position 13.[10] Bonds between C-11/C-12 and C-8/C-7a were postulated to give the final structure **1**, which has two stable six-membered rings containing carbonyl groups that are consistent with the infrared data.[11]

Structure **1** is also consistent with ^{13}C T_1 data (20 MHz) with C-12c, C-12b, and C-8 having the longest T_1's.[12,13]

Bromination of **1** (Br$_2$/AcOH) in an effort to obtain a crystalline derivative suitable for X-ray analysis yielded primarily a monobromo product formulated as **2**.[14] This selective bromination is consistent with the stabilization afforded by the amide nitrogen to the intermediate involved in attack by bromine at C-12.

We propose that protonation of **1** occurs at the nitrogen at position 7 on the basis of the downfield shifts of H-6 and -5 in TFA-d/CDCl$_3$ relative to CDCl$_3$ (+0.64 and 0.16 ppm, respectively),[9a,15] the similarity of the H–C coupling of C-6 with that of the α-carbon in pyridinium ion (190.7 Hz),[8] and the shorter T_1 for C-7a (1.66 s) compared to C-12b and -13a (4.15 and 3.13 s, respectively).[13] Protonation at one of the carbonyl oxygens is also presumed to occur, thereby altering the enamide–ketone system and producing the change from bright yellow crystals to a dark red solution in acid media (TFA or H$_2$SO$_4$–H$_2$O).

Although the double-quantum coherence experiment for establishing carbon–carbon connectivities from natural-abundance ^{13}C–^{13}C couplings is clearly a powerful tool for determining structures, application to biologically active natural products remains limited due, in part, to the substantial quantities of sample, several hundred milligrams, required for a complete analysis in a reasonable amount of time. The current example illustrates the fact that when sample availability is limited, even a partial set of carbon connectivities obtained from the double-quantum experiment can be used to great advantage with data from the more sensitive proton–carbon coupling experiments to solve challenging structures such as **1**, which represents a new alkaloid skeleton.

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Registry No. 1, 86047-14-5; **2**, 86047-15-6.

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(7) The 2-D spectrum was obtained with a Varian XL-300 superconducting spectrometer using the standard software available in the instrument.

(8) (a) Seel, H.; Günther, H. *J. Am. Chem. Soc.* **1980**, *102*, 7051. (b) Wehrli, F. W.; Wirthlin, T. "Interpretation of Carbon-13 NMR Spectra"; Heyden: New York, 1976; p 55.

(9) (a) Pugmire, R. J.; Grant, D. M. *J. Am. Chem. Soc.* **1968**, *90*, 697. (b) Reference 5, p 122.

(10) Cf. the couplings of H-4 to C-2, -4b, and -13a.

(11) Four- and eight-membered rings containing carbonyl groups would result from alternate bonding of the carbonyl groups to C-7a and C-12 in d, and these would be highly incompatible with the IR data.

(12) T_1's (20 MHz TFA-d/CDCl$_3$ (2:1)): 1–4, 5, 6, 9, 12 (0.14–0.16 s); 7a (1.7 s); 11 (1.4 s); 4b, 8a, 12a, 13a (3.0–3.3 s), 4a (3.8 s); 8, 12b (4.1 s); 12c (6.4 s).

(13) Cf. ref 5, chapter 8. Norton, R. S.; Croft, K. D.; Wells, R. J. *Tetrahedron* **1981**, *37*, 2341. Norton, R. S.; Wells, R. J. *Tetrahedron Lett.* **1980**, *21*, 3801.

(14) ^{1}H NMR of **2** differs from **1** only in absence of the δ 8.52 signal (H-12).

(15) Proton shifts in CDCl$_3$ (300 MHz): δ 8.38 (H-1), 7.99 (H-2), 7.87 (H-3), 8.65 (H-4), 9.37 (H-5), 8.65 (H-6), 8.81 (H-9), 8.15 (H-12).