and fast atom bombardment mass spectroscopy, which showed an M + 1 molecular peak at m/z 1311.8 (calculated = 1312.1). These studies were also extended to the longer peptides, YIIKGVFWDPACVIA (**4**) and DESGPGSMSSKCVLS[15] (**5**) (Scheme I), both of which were farnesylated under similar reaction conditions to afford the desired products $\mathbf{4}_p$ and $\mathbf{5}_p$ (Scheme I) in 84% and 85% yield,[17] respectively.

This method has provided the first practical method for regioselective isoprenylation of cysteine thiols in unprotected peptides and should permit systematic studies of regioselective isoprenylation of cysteine thiols in peptides and proteins. Several farnesylated peptides prepared by this method have been used to assay the activity of protein prenyltransferase in yeast.[4] A variety of different isoprenylating reagents are now under investigation in our laboratory.

Acknowledgment. We thank Professors Paul Bartlett at the University of California at Berkeley, Daniel Santi at the University of California at San Francisco, and Tomikazu Sasaki at the University of Washington for helpful discussions.

(17) The structures of peptides $\mathbf{4}_p$ and $\mathbf{5}_p$ were identified by amino acid analysis and fast atom bombardment mass spectroscopy, which gave an M + 1 peak at m/z 1899.2 (calculated = 1898.4) for peptide $\mathbf{4}_p$ and m/z 1687.2 (calculated = 1687.0) for peptide $\mathbf{5}_p$.

Clavepictines A and B: Cytotoxic Quinolizidines from the Tunicate *Clavelina picta*[1]

Michael F. Raub and John H. Cardellina, II*,[2]

Natural Products Laboratory, Department of Chemistry Montana State University, Bozeman, Montana 59717

M. Iqbal Choudhary, Chao-Zhou Ni, and Jon Clardy

Department of Chemistry, Baker Laboratory Cornell University, Ithaca, New York 14853

Michael C. Alley

National Cancer Institute, Building 560, Room 32-60 Frederick, Maryland 21702-1201

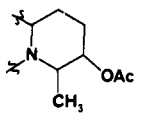
Received July 23, 1990

In our investigations of the chemistry and biological significance of the secondary metabolites of Bermudian tunicates,[3] we found cytotoxicity (KB: IC_{50} = 12 μg/mL) and antimicrobial activity in the organic soluble extracts of *Clavelina picta*. From substantial collections (1–2 kg) made in the summer of 1984 and the spring of 1987, we have found the first quinolizidines from a tunicate and have evaluated the cytotoxicity of these novel compounds.

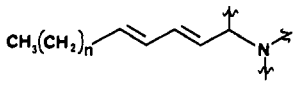
Clavepictine A was obtained from the extracts by a sequence of solvent partitioning ($CHCl_3$ vs 40% aqueous MeOH), gel permeation chromatography (Bio-Beads S-X4 and Sephadex LH-20), reversed-phase low-pressure chromatography (C_{18}, H_2O–MeOH gradient), and centrifugal countercurrent chromatography (CCC; hexane–CH_2Cl_2–CH_3CN, 10:3:7) as a colorless oil.[4] It could also be obtained naturally as the hydrochloride salt (IR 2500 cm^{-1}; positive Beilstein test). High-resolution MS provided the molecular formula $C_{22}H_{37}NO_2$, requiring five sites of unsaturation. One was readily attributed to an acetate ester (IR 1730 cm^{-1}; ^1H NMR δ 2.05, 3 H, s; MS m/z 305 (M^+ – 42). Two more comprised a conjugated heteroannular diene (λ_{max} 232 nm), leaving two rings to be placed in the molecule.

The ^{13}C NMR spectrum revealed the expected acetate carbonyl and four protonated olefinic carbons, but also contained four signals for methines bearing hetero atoms. One (δ 73.4) obviously carried the acetate; therefore, the remaining three (δ 58.0, 53.0, 49.1) all bore nitrogen. All the remaining carbons were methylenes or methyls, indicating that all branch or juncture points were attached to hetero atoms. Noteworthy was the presence of two methyl groups besides the acetate methyl. (See Table I.)

Two part structures could be constructed without difficulty. A nitrogen-bearing methine (δ 3.5) was found to be coupled to a methyl group (δ 1.09) and to the acetate-bearing methine (δ 4.8). A sequence of two methylenes was found to connect the acetate-bearing methine to a nitrogen-bearing methine at δ 3.08, completing part structure **1a**. The second part structure began with the third nitrogen-bearing methine (δ 3.8), which was coupled to a terminus of the diene system (δ 5.65). As expected from the UV and ^{13}C NMR data, the olefinic protons were sequentially connected on adjacent carbons and the opposite terminus was coupled to an allylic methylene at δ 2.0, which was coupled into a methylene envelope at δ 1.2. Irradiation at δ 1.2 revealed a terminal methyl group at δ 0.9, giving part structure **1b**.

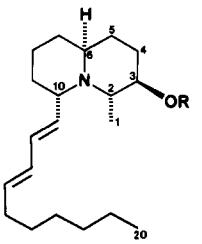


1a



1b

All that remained was to define the size of the second ring or the length of the alkadienyl side chain. Mass spectral cleavage of the alkadienyl chain would provide the necessary evidence, but fragmentation α to the nitrogen would require rupture of an sp^2–sp^3 carbon–carbon bond. This ion (m/z 210) was weak in the mass spectrum of clavepictine A, but catalytic reduction (Pd/C) gave a tetrahydro derivative whose mass spectrum featured a base peak at m/z 210, corresponding to loss of an alkyl chain from a quinolizidine nucleus bearing methyl and acetoxyl substituents. Thus, the gross structure of clavepectine A had to be **1**.



1 R = Ac
2 R = H

The relative stereochemistry of **1** was gleaned from NOE experiments. Irradiation of the methyl group (C-1) attached to the α-methine (C-2) elicited responses from its vicinal neighbor (H-2) and the ring-juncture α-methine (H-6), indicating a 1,3-diaxial relationship of the methyl group and H-6. The small coupling constant between H-2 and H-3, the acetate-bearing methine, and the observation of an NOE between the two suggested that they were trans-diequatorial, putting the acetoxyl substituent in an axial position. No NOE was observed between H-6 and H-10, the third α-methine, but one was observed between H-10 and H-12, suggesting that the alkadienyl side chain was positioned so as to bisect the "plane" of the attached ring. Moreover, an NOE was revealed between H-2 and H-11. These data could best be accommodated by a cis ring juncture in the quinolizidine, an equatorial alkadienyl substituent, and as noted above, axially disposed methyl and acetoxyl groups.

(1) Contribution No. 1280 from the Bermuda Biological Station.
(2) Address correspondence to this author at the National Cancer Institute, Building 1052, Room 121, Frederick, MD 21702-1201.
(3) (a) Kinzer, K. F.; Cardellina, J. H., II. *Tetrahedron Lett.* **1987**, *28*, 925–926. (b) VanWagenen, B. C.; Cardellina, J. H., II. *Tetrahedron Lett.* **1989**, *30*, 3065–3068.
(4) $[\alpha]_D$ –75.6° (*c* 0.7, CH_2Cl_2); IR ν_{max} (neat) 3015, 2932, 2861, 1735, 1453, 1375, 1252, 1163, 1130, 1105, 1039, 992 cm^{-1}; UV λ_{max} (EtOH) 230 nm (ε = 19 000); EIHRMS m/z 347.2831 ($C_{22}H_{37}NO_2$ requires 347.2824); EIMS m/z (relative intensity) 347 (M^+, 6), 332 (9), 288 (9), 260 (100), 210 (4), 150 (7).

0002-7863/91/1513-3178$02.50/0

Table I. NMR Data for Clavepictines A (**1**) and B (**2**)[a]

carbon no.	**1** ^{13}C[b]	**1** ^{1}H[c]	**2** ^{13}C[c]	**2** ^{1}H[c]
1	14.0	1.09, d, 3 H (7)	16.7	1.29, d, 3 H (6)
2	53.0	3.50, dq, 1 H (7, 3)	58.8	3.31, br m, 1 H (6)
3	73.4	4.70, m, 1 H (3)	70.6	3.61, br s, 1 H
4	29.9	1.73, m, 1 H (14.4)	29.3	1.81, m, 1 H
		1.82, m, 1 H (14.4, 11.1)		1.91, m, 1 H
5	22.6	0.97, m, 1 H (10.2)	26.2	1.80, m, 1 H
		1.93, m, 1 H (11.1, 10.2)		1.46, m, 1 H
6	49.1	3.12, br d, 1 H	47.2	3.13, br m, 1 H
7	32.6[d]	1.47, m, 2 H	28.3	1.60–1.65, m, 2 H
8	21.5[e]	1.47, m, 2 H	20.6	1.40–1.45, m, 2 H
9	29.8[e]	1.58, m, 1 H (7.3)	28.1	1.65–1.73, m, 2 H
		1.20, m, 1 H		
10	58.0	3.84, ddd, 1 H (8.5, 7.3, 3)	60.8	4.03, br q, 1 H (6.2)
11	136.2	5.67, dd, 1 H (15.3, 3)	135.1	5.87, dd, 1 H (15.3, 6.2)
12	130.9	6.31, dd, 1 H (15.3, 10.5)	130.9[f]	6.38, dd, 1 H (15.3, 10.5)
13	133.4	6.15, dd, 1 H (15.2, 10.5)	131.2[f]	6.21, dd, 1 H (15, 10.5)
14	130.1	5.73, m, 1 H (15.2)	136.9	5.63, m, 1 H (15)
15	32.6[d]	1.31, m, 2 H	32.9	2.05, q, 2 H
16, 17, 18	29.3	1.24–1.12, m, 6 H	26.7 (16)	1.44, m, 2 H
	28.9		32.9 (17, 18, 19)	1.27–1.38, m, 6 H
	22.6		29.1	
			22.8	
19	31.7	1.24–1.12, m		
20	17.1	0.85, t, 3 H (6)	14.2	0.81, t, 3 H (6.8)
21	170.2			
22	25.7	2.13, s, 3 H		

[a] Chemical shifts are in δ. Multiplicities are those observed. Coupling constants (J) given are those that could be measured. [b] Recorded in $CDCl_3$. [c] Recorded in C_5D_5N. [d–f] Interchangeable.

Improbable as this first seemed, molecular modeling studies revealed that the proposed configuration was energetically quite stable compared to the other possibilities.[5] Efforts to resolve this issue unequivocably by hydrolysis and preparation of a heavy atom derivative for X-ray diffraction analysis failed, but the hydrolysis product [$Ba(OH)_2$] proved to be identical with a major compound isolated by CCC from the aqueous methanol phase of the solvent partitioning scheme, **2**,[6] clavepictine B. The amino alcohol **2** slowly crystallized from CH_3CN–CH_2Cl_2, and its structure was confirmed by X-ray diffraction analysis.[7] Figure 1 is a computer-generated perspective drawing of the final X-ray model. The diffraction experiment did not define the absolute configuration, so the enantiomer shown is an arbitrary choice. The conformation of the quinolizidine appears to be governed by having the extended alkadienyl chain in an equatorial orientation. The quinolizidine has a cisoid ring fusion, presumably because the alternative trans conformation would have a diaxial interaction between the alkenyl chain at C-10 and the methyl at C-2. Both piperidine rings are in the chair conformation; the alternate arrangement of chairs would have the alkenyl chain in an axial orientation. The end of the alkenyl side chain is disordered.

Clavepictines A and B inhibit growth of murine leukemia and human solid tumor cell lines (P-388, A-549, U-251, and SN12K1) at concentrations less than 9 μg/mL (IC_{50} = 1.8–8.5 μg/mL) and effectively kill each cell line at less than 25 μg/mL (LC_{50} = 10.1–24.7 μg/mL) under conventional culture conditions.[8]

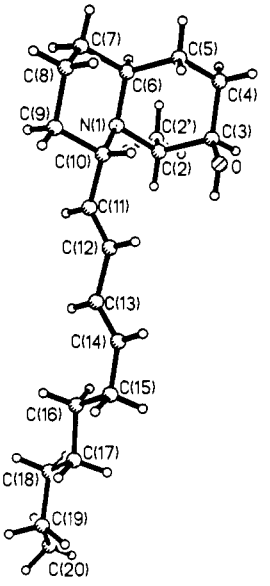


Figure 1. Computer-generated perspective drawing of the final X-ray model of clavepictine B (**2**).

No quinolizidines have been reported previously from tunicates, although a single piperidine has been reported from a tunicate[9] and may be derived from a similar polyketide pathway.

(5) Raub, M. F. Ph.D. Dissertation, Montana State University, 1990.

(6) Mp 70–72 °C; $[\alpha]_D$ +27.1° (*c* 0.03, CH_2Cl_2); IR ν_{max} (neat) 3408, 3015, 2928, 2855, 1715, 1454, 1374, 1257, 1156, 1112, 1047, 989 cm^{-1}; UV λ_{max} (EtOH) 230 nm (ϵ = 18 000); EIHRMS *m/z* 305.2720 ($C_{20}H_{30}NO$ requires 305.2719); EIMS *m/z* (relative intensity) 305 (M^+, 24), 290 (51), 260 (68), 234 (34), 168 (34), 150 (38), 41 (100).

(7) Clavepictine B crystallized in the monoclinic space group $P2_1$ upon slow evaporation of a CH_3CN–CH_2Cl_2 solution at 4 °C. Accurate lattice constants of a = 12.095 (4) Å, b = 5.895 (2) Å, c = 14.086 (3) Å and β = 99.34 (2)° were determined from a least-squares fit of 20 diffractometer-measured 2θ values. The asymmetric unit consisted of one molecule of composition $C_{20}H_{35}NO$. A total of 1364 diffraction maxima were collected by using θ–2θ scans with graphite-monochromated Cu Kα radiation, and of these, 1282 (94%) were judged observed ($|F_o|$) $\geq 6\sigma(|F_o|)$. The structure was solved with direct methods and refined by using full-matrix least-squares techniques with anisotropic heavy atoms and fixed isotropic riding hydrogens to a conventional discrepancy index of 0.065 for the observed reflections. See the paragraph entitled Supplementary Material Available for additional crystallographic details.

(8) IC_{50} refers to the concentration that inhibits tumor cell growth to 50% of that of untreated tumor cells, while LC_{50} refers to the concentration that reduces tumor cell counts to 50% of the cell count at the drug addition, T_0. See: Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

(9) Ishibashi, M.; Ohizumi, Y.; Sasaki, T.; Nakamura, H.; Hirata, Y.; Kobayashi, J. *J. Org. Chem.* **1987**, *52*, 450–453.

Acknowledgment. We thank Dr. L. J. Sears for the mass spectral measurements and Prof. Ivan Goodbody for identifying the tunicate. This work was supported by grants from the National Cancer Institute, in part, and the Office of Sea Grant, Department of Commerce.

Registry No. 1, 132621-83-1; **2**, 132621-84-2.

Supplementary Material Available: Fractional coordinates, temperature factors, interatomic distances, and interatomic angles for clavepictine B (**2**) (4 pages). Ordering information is given on any current masthead page.

Do Rotational Barriers Dictate the Regioselectivity in the Ene Reactions of Singlet Oxygen and Triazolinedione with Alkenes?

Michael Orfanopoulos,* Manolis Stratakis, and Yiannis Elemes

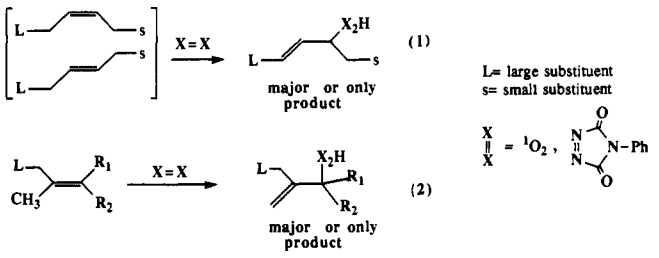
Department of Chemistry, University of Crete 71110 Iraklion, Crete, Greece

Frank Jensen

Department of Chemistry, University of Odense DK-5230 Odense M, Denmark

Received December 3, 1990
Revised Manuscript Received February 4, 1991

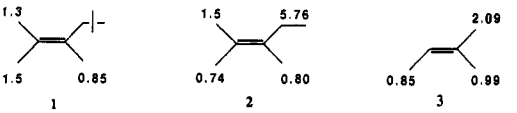
Recently the regioselective ene reactions of singlet oxygen and triazolinedione (TAD) with alkyl-substituted ethylenes have attracted considerable attention. For example, it has been shown that singlet oxygen and TAD react with unsymmetrical *cis*-alkenes with regioselective double-bond formation at the larger group (eq 1).[1] Furthermore, the reaction with tetrasubstituted alkenes was found to favor hydrogen abstraction from the alkyl group that is geminal to the larger substituent of the double bond (eq 2).[2] This remarkable geminal selectivity was recently rationalized[2a] in terms of rotational-barrier differences within the alkyl groups of the double bond.



These arguments, which previously have been utilized by Houk and co-workers[3] in order to explain the singlet oxygen side-selectivity in trisubstituted olefins, have been shown to correctly predict ene regiochemistry in a number of tetrasubstituted olefins. The lower the calculated rotational barrier, the higher the reactivity of the alkyl group.[2a,3] For example, molecular mechanics calculations showed[2a] that the methyl group geminal to the neopentyl group in 2,3,5,5-tetramethyl-2-hexene (**1**) has the lowest rotational barrier and is the most reactive. Furthermore, the ethyl group in 2,3-dimethyl-2-pentene (**2**) has a much higher rotational barrier (5.76 kcal/mol) than the methyl groups and is totally inactive. Similar trends hold with 2-methyl-2-butene (**3**). The numbers shown with structures **1**-**3** are rotational barriers calculated by MM2.

Table I. Relative Yields of Ene Products and Rotational Barriers of Methyl Groups

	% Ene Product		Rotational Barriers (Kcal/mol)	
	With $^1O_2$	With PTAD	HF/STO-3G	HF/3-21G
CD_3 ← 76 CH_3 ← 24 (E)-4	76 24	53 47	1.63 1.11	2.27 1.64
CH_3 ← 74 CD_3 ← 26 (Z)-4	74 26	58 42	1.63 1.11	2.27 1.64
CD_3 ← 14 CH_3 ← 86 5	14 86	18 82	1.64 0.40	2.26 1.03
← 73 ← 27 cis-6	73 27	100 0	0.56	
← 0 ← 100 gem-6	0 100	0 100	0.91	
← 69 ← 31 trans-6	69 31	100 0	1.51	
← 36 Ph ← 64 7	36 64		1.22 1.45	



These interesting results prompt us to report here our own findings, which demonstrate that barriers to rotation do not always predict the regioselectivity in the ene reaction of $^1O_2$ or TAD with alkenes. We will show, as we have already pointed out,[2b] that it is the nonbonded interactions in the isomeric transition states that control product formation and that barriers to rotation are irrelevant.

The results are summarized in Table I. In alkenes **4** the methyl groups occupy different stereochemical environments and are expected to show different rotational barriers and reactivities. It is therefore ideally suited for this purpose. Deuterium labeling allows us to distinguish the ene product distribution, and isomers (*E*)- and (*Z*)-**4** can be prepared in high stereochemical purity.[4]

(1) (a) Orfanopoulos, M.; Stratakis, M.; Elemes, Y. *Tetrahedron Lett.* **1989**, 4875-8. (b) Elemes, Y.; Stratakis, M.; Orfanopoulos, M. *Tetrahedron Lett.* **1989**, 6903-6.

(2) (a) Clennan, E. L.; Chen, X.; Koola, J. J. *J. Am. Chem. Soc.* **1990**, *112*, 5193-9. (b) Orfanopoulos, M.; Stratakis, M.; Elemes, Y. *J. Am. Chem. Soc.* **1990**, *112*, 6417-9. (c) Ensley, H. E.; Carr, R. V. C.; Martin, R. S.; Pierce, T. E. *J. Am. Chem. Soc.* **1980**, *102*, 2836-8. (d) Adam, W.; Griesbeck, A. *Synthesis* **1986**, 1050-1. (e) Orfanopoulos, M.; Foote, C. S. *Tetrahedron Lett.* **1985**, 5991-4. (f) Akasaka, T.; Misawa, Y.; Goto, M.; Ando, W. *Tetrahedron* **1989**, *45*, 6657-66. (g) Clennan, E. L.; Chen, X. *J. Org. Chem.* **1988**, *53*, 3124-5.

(3) Houk, K. N.; Williams, J. C., Jr.; Mitchell, P. M.; Yamaguchi, K. *J. Am. Chem. Soc.* **1981**, *103*, 949-51.

(4) Alkenes (*E*)-**4** and **5** were prepared by the following method: Wittig coupling of the stabilized ylide methyl(triphenylphosphoranylidene)propionate with 2,2-dimethylpropanal and 2-methylpropanal respectively gave the *E* configuration of the corresponding esters as the only isomer (GC analysis on an SE-30 10 ft × 1/8 in. column). Complete reduction of these esters with a $LiAlD_4/AlCl_3$ mixture gave in two steps compound (*E*)-**4**: ^1H NMR ($CDCl_3$) δ 5.17 (m, 1 H), 1.71 (d, J = 1.6 Hz, 3 H), 1.08 (s, 9 H). Reaction of methyl(triphenylphosphoranylidene)propionate-*3,3,3-d*$_3$ with 2,2-dimethylpropanal gave the *E* d_3 ester as the only isomer. Reduction of this ester with a $LiAlH_4/AlCl_3$ mixture gave in two steps compound (*Z*)-**4**: ^1H NMR (acetone-d_6) δ 5.16 (m, 1 H), 1.63 (d, J = 1.6 Hz, 3 H), 1.07 (s, 9 H). **5**: ^1H NMR ($CDCl_3$) δ 4.93 (br d, J = 8.8 Hz, 1 H), 2.5 (m, 1 H), 1.60 (br s, 3 H), 0.91 (d, J = 6.4 Hz, 6 H).

0002-7863/91/1513-3180$02.50/0