produce triplets[15] (which could presumably sensitize singlet oxygen) and also singlet oxygen directly.[16] All attempts to observe, via flash spectroscopy, triplets or singlet oxygen production by trapping with β-carotene in ^{1}DCA*-sensitized photooxidations have thus far proved unsuccessful.[17]

(15) H. Schomberg, H. Staerk, and A. Weller, *Chem. Phys. Lett.*, **21**, 433 (1973).
(16) A. Mayeda and A. J. Bard, *J. Am. Chem. Soc.*, **95**, 6223 (1973).
(17) L. T. Spada, D. S. Steichen, and C. S. Foote, unpublished results. Unfortunately, β-carotene and other singlet oxygen quenchers are too rapidly destroyed in this system to be useful in product studies.

Structures of the Didemnins, Antiviral and Cytotoxic Depsipeptides from a Caribbean Tunicate[1]

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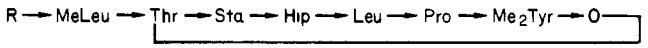
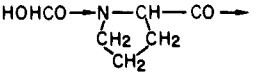
We have recently discovered a class of depsipeptides in a Caribbean tunicate of the family Didemnidae (a species of the genus *Tridemnum*)[2] and have reported their isolation, separation, and biological properties elsewhere.[3] In brief, the didemnins inhibit the growth of both RNA and DNA viruses, are highly cytotoxic to L1210 leukemic cells, and protect mice against P388 leukemia and B16 melanoma. For example, didemnin A shows 50% inhibition of Coxsackie virus and equine rhinovirus (both RNA) and *Herpes simplex*, type 2 (DNA), at 1.5 μg/mL and *Herpes simplex*, type 1, at 3 μg/mL, while didemnin B has ID_{50} = 0.0011 μg/mL vs. L1210 leukemic cells, T/C up to 199 vs. P388 leukemia, and T/C 160 vs. B16 melanoma. We report here the structures of these very promising new compounds **1–3**, where Hip is hydroxyisovalerylpropionyl, O-CH[CH(CH$_3$)$_2$]COCH(CH$_3$)-CO.



1 (didemnin A), R = H

2 (didemnin B), R = $CH_3CHOHCO-$



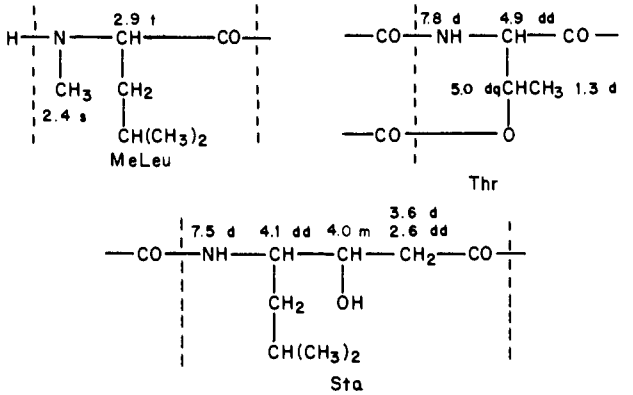
3 (didemnin C), R = $CH_3CHOHCO-$

The didemnins were separated over silica gel and assigned molecular weights of 942 (didemnin A, major component), 1111 (B, minor component), and 1014 (C, trace component) from their field desorption (FD) mass spectra. Initial structural efforts centered on didemnin A, as the simplest and most abundant component. Didemnin A was concluded to be a peptide from its ^{1}H NMR spectrum, with broad doublets (NH) at 8.3, 7.8, and 7.5 ppm. Hydrolysis with 6 N hydrochloric acid gave 1 mol each of *N*-methylleucine (MeLeu), threonine (Thr), leucine (Leu), proline (Pro), *N,O*-dimethyltyrosine (Me$_2$Tyr),[4] and statine (Sta).[5]

(1) Presented in part at the 3rd International Symposium on Marine Natural Products (International Union of Pure and Applied Chemistry and Société Chimique de Belgique), Brussels, Sept 16, 1980.
(2) Identification as family Didemnidae was carried out by Dr. Charles C. Lambert, California State College, Fullerton, and identification as a *Tridemnum* species by Dr. Françoise Lafargue, Laboratoire Arago, Banyuls-sur-mer, France.
(3) K. L. Rinehart, Jr., J. B. Gloer, R. G. Hughes, Jr., H. E. Renis, J. P. McGovren, E. B. Swynenberg, D. A. Stringfellow, S. L. Kuentzel, and L. H. Li, *Science (Washington, D.C.)*, in press.
(4) F.-J. Marner, R. E. Moore, K. Hirotsu, and J. Clardy, *J. Org. Chem.*, **42**, 2815–2818 (1977).

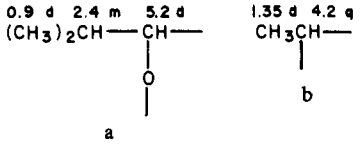
The amino acids were identified by FD mass spectrometry (FDMS) of the mixture, with M + H peaks at *m/z* 146 (MeLeu), 132 (Leu), 116 (Pro), and 210 (Me$_2$Tyr), an M – H_2O peak at *m/z* 101 for Thr, and M at *m/z* 175 for Sta. They were also identified by gas chromatography/mass spectrometry (GC/MS) of the amino acids' trifluoroacetyl *n*-butyl ester derivatives. In addition, they were quantitated by GC, and their identities confirmed by coinjection with derivatives of authentic samples. Statine was assigned as the allo (erythro) isomer by its coelution with the synthetic *R,S* isomer,[5] while gas chromatography on an optically active column[6] indicated Leu, MeLeu, Pro, Thr, and Me$_2$Tyr to have the L configuration.

The individual amino acids were characterized in the ^{1}H NMR spectrum of didemnin A by extensive spin decoupling, which established MeLeu as N-terminal, Thr as O- as well as N-acylated, and the hydroxyl group of Sta to be free, as shown. Didemnin



A reacts with acetic anhydride on the methylamino group of MeLeu and the hydroxyl group of Sta to give a diacetyl derivative, as judged by the product's molecular weight of 1026, two new $COCH_3$ groups at 1.9 and 2.1 ppm, and the shift of the appropriate protons to 2.8 (–N(Ac)–CH_3, MeLeu), 5.1 (AcNCH<, MeLeu) and 5.3 ppm (AcO–CH<, Sta).

Addition of the formulas for the amino acids' aminoacyl units (MeLeu, $C_7H_{14}NO$; Thr, $C_4H_6NO_2$; Sta, $C_8H_{15}NO_2$; Me$_2$Leu, $C_{11}H_{13}NO_2$; Pro, C_5H_7NO; Leu, $C_6H_{11}NO$) gave $C_{41}H_{66}N_6O_9$, 786.4891. The high resolution electron impact (HREI) mass spectrum of didemnin A gave a molecular ion peak at 942.5678. Subtraction of the two numbers yielded 156.0787 ($C_8H_{12}O_3$) as the unaccounted-for residue of didemnin A. The $C_8H_{12}O_3$ unit can be identified in the ^{1}H NMR spectrum as containing the units a and b and in the ^{13}C NMR spectrum as containing one ketone



carbon (205.1 ppm) and one carboxyl-type carbon (one of seven near 175 ppm, in addition to the six of the amino acids). The only reasonable combination of a and b with a keto and a carboxyl carbon in a $C_8H_{12}O_3$ unit is as a hydroxyisovalerylpropionyl (Hip) group. All the carboxyl carbons occur as amides or esters since didemnin A does not react with diazomethane.

The order of linkage of these seven units was assigned by partial hydrolysis of didemnin A in base and acid, followed by HRFDMS

(5) D. H. Rich, E. T. Sun, and A. S. Boparai, *J. Org. Chem.*, **43**, 3624–3626 (1978).
(6) R. C. Pandey, J. C. Cook, Jr., and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **99**, 8469–8483 (1977).

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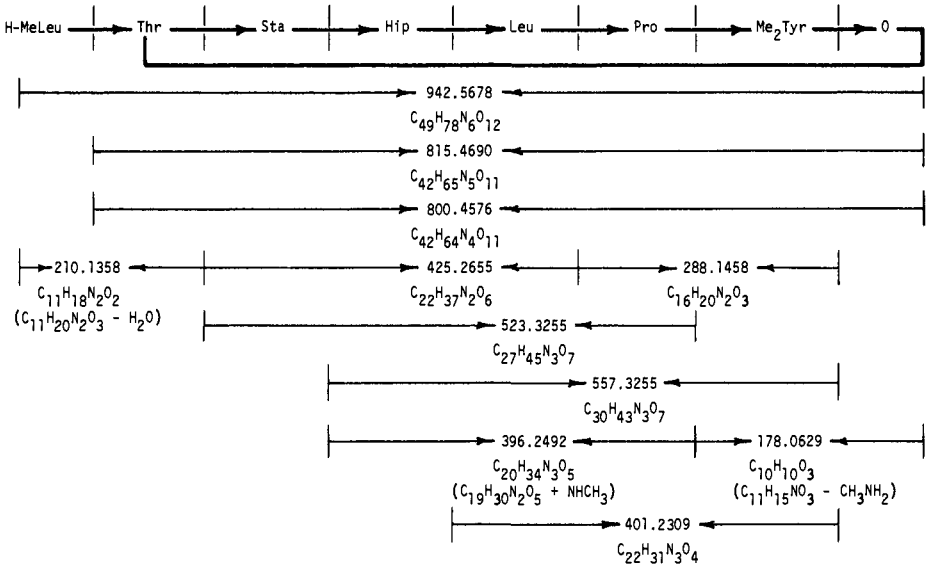
Table I. HRFD Mass Spectral Peaks in Hydrolyzates of Didemnin A

ion	composition	assignment
576.3279[a]	$C_{30}H_{46}N_3O_8$	H-Hip→Leu→Pro→Me_2Tyr-OH + H
557.3094[a-c]	$C_{30}H_{43}N_3O_7$	H-Hip→Leu→Pro→Me_2Tyr-OH − H_2O
539.2987[a]	$C_{30}H_{41}N_3O_6$	H-Hip→Leu→Pro→Me_2Tyr-OH − $2H_2O$
420.2492[a,b]	$C_{22}H_{34}N_3O_5$	H-Leu→Pro→Me_2Tyr-OH + H
386.2646[b,d]	$C_{19}H_{36}N_3O_5$	H-MeLeu→Thr→Sta-OH − H_2O + H
383.2214[a,b]	$C_{22}H_{29}N_3O_3$	H-Leu→Pro→Me_2Tyr-OH − $2H_2O$
288.1467[a-c]	$C_{16}H_{20}N_2O_3$	Pro→Me_2Tyr
178.0631[a]	$C_{10}H_{10}O_3$	$CH_3OC_6H_4CH$=CHCOOH
156.0794[a,b]	$C_8H_{12}O_3$	Hip

[a] 0.1 N NaOH, room temperature, 2 h. [b] 6 N HCl, 110 °C, 10 min. [c] Trifluoroacetic acid, room temperature, 48 h. [d] Accompanied by peaks at m/z 404[b] and 368.[b]

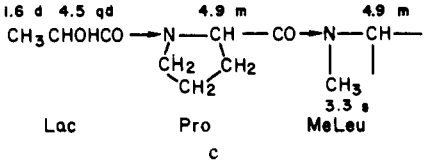
Scheme I. Fragment Ions Observed in the High Resolution Electron Impact Mass Spectrum of Didemnin A



on the molecular ions of the resultant mixtures (Table I). The major peptide peaks observed due to the two lactone cleavages were $C_{30}H_{45}N_3O_8$ (which must contain Me_2Tyr, Hip, Pro, and Leu from its degree of unsaturation and oxygen content) and $C_{19}H_{36}N_3O_5$ (which must then contain MeLeu, Thr, and Sta), which together account for all the component acids of didemnin A. Smaller peptides (e.g., $C_{22}H_{34}N_3O_5$, Me_2Tyr, Pro, Leu; $C_{16}H_{20}N_2O_3$, Me_2Tyr, Pro) were also observed. On the basis of these data, Hip, the hydroxy acid, must be N-terminal in the C_{30} peptide, while Leu could be C-terminal or between Hip and the C_{16} dipeptide.

These peptide linkages are confirmed in the HREI mass spectrum of didemnin A (Scheme I), which contains fragment ions corresponding to the C_{30} (Hip, Leu, Pro, Me_2Tyr), C_{22} (Leu, Pro, Me_2Tyr), and C_{16} (Pro, Me_2Tyr) peptides. Sequencing of the C_{30} peptide is completed by the assignment of the *p*-methoxycinnamic acid ion ($C_{10}H_{10}O_3$) at m/z 178.0629, which shows the Me_2Tyr group to be linked as an ester, hence C-terminal in the C_{30} peptide, and confirmed by the Hip→Leu→Pro–$NHCH_3$ ion at m/z 396.2492. Similarly, the ion at 425.2655 ($C_{22}H_{37}N_2O_6$), containing Sta in addition to Hip and Leu, shows Sta to be adjacent to Hip, hence C-terminal in the C_{19} peptide. The ion at m/z 800.4576 (M − $C_7H_{14}N_2O$) shows MeLeu is linked as an amide. Thus, the structure of didemnin A is assigned as **1**.

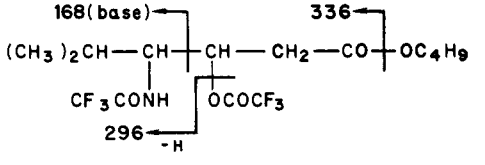
The molecular formula of didemnin B was established as $C_{57}H_{89}N_7O_{15}$, differing from didemnin A by only $C_8H_{11}NO_3$, by HRFDMS on the M + H ion at 1112.6442, while a major peak at m/z 942 in the mass spectra of B and the occurrence of nearly all of the EIMS peaks of didemnin A indicated a close similarity to the major component. Acidic hydrolysis of didemnin B to the component amino acids followed by GC of the *N*-trifluoroacetyl *n*-butyl ester derivatives indicated an additional mole of Pro, while decoupling studies of the ^{1}H NMR spectrum showed the unit c as a modification of the N terminus of didemnin A. Thus, the structure of didemnin B is assigned as **2**.



c

Similarly, the HRFD mass spectrum of the trace component, didemnin C, contains a molecular ion at m/z 1014.5873 ($C_{52}H_{82}N_6O_{14}$); its ^{1}H NMR spectrum contains a shifted MeLeu *N*-methyl group at 2.8 ppm; the GC/MS trace of the derivatized amino acids from its hydrolyzate is the same as that from didemnin A; and large peaks appear in the EI mass spectrum at m/z 172.1552 and 200.1283 [$CH_3CHOHCON(CH_3)CH(CH_2$-*i*-$C_3H_7)R^+$, R = absent and CO]. Although lack of adequate sample has prevented more extensive studies, its structure is tentatively assigned as **3**.

Each of the didemnins (A–C) is accompanied by a minor but varying amount of a lower homologue. The nature of the homology was clarified by the observation that GC traces of the derivatized amino acids always contain minor amounts of an amino acid identified by GC/MS as a lower homologue of statine, for which we propose the name norstatine [$(CH_3)_2CHCHNH_2CHOHCH_2COOH$] and whose derivative (**4**) fragments in the



4

manner shown. The lower homologous peptides can then properly be designated as nordidemnins A–C, in which norstatine replaces the statine unit of **1–3**.

Acknowledgment. This work was supported in part by research grants from the National Institute of Allergy and Infectious Diseases (AI 4769), the National Institute of General Medical Sciences (GM 27029), and the National Science Foundation (PCM 77-12584). We thank Dr. D. H. Rich, University of Wisconsin, and Dr. R. E. Moore, University of Hawaii, for samples of statine and *N,O*-dimethyltyrosine, respectively, Dr. M. L. Moore, University of Illinois, for assistance with chiral GC, and Dr. Françoise Lafargue, Université Pierre et Marie Curie, and Dr. Charles C. Lambert, California State College, Fullerton, for tunicate identifications.

Regioselectivity in the Intramolecular Ene Reaction of Cyclopropene Derivatives

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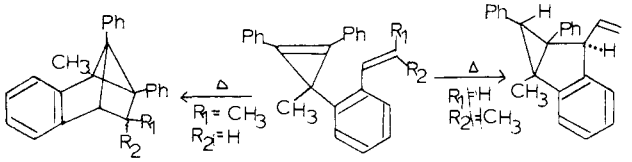
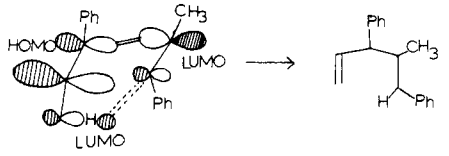
Received December 1, 1980

The thermal addition of an alkene to another olefin possessing an allylic hydrogen, the so-called "ene" reaction, is one of the most simple and versatile reactions of organic chemistry.[1] Though radical[2] and other mechanisms[3] have been advanced, the addition is usually considered to proceed in a symmetry-allowed concerted process[4-8] involving a six-membered cyclic transition state, unless prohibited by steric factors.[9] Olefins with strained double bonds seem particularly prone to enter into ene reactions. For example, cyclopropene derivatives are known to undergo ready dimerization via the ene process.[10,11] Considerable interest has recently been focused on intramolecular examples of this reaction.[12] In this communication, we wish to describe a novel regiochemical effect associated with the intramolecular ene reaction of tetrasubstituted cyclopropene derivatives.

As an extension of our studies dealing with intramolecular cycloaddition reactions of cyclopropene derivatives,[13] we have examined the thermal and triplet sensitized behavior of a series of 3-(*o*-alkenylphenyl)-substituted cyclopropenes. Thermolysis of the trans-substituted cyclopropene **1** at 175 °C resulted in a [2 + 2] cycloaddition reaction to produce a 4.6:1 mixture of exo and endobenzotricycloheptene **2**.[14] In marked contrast, heating

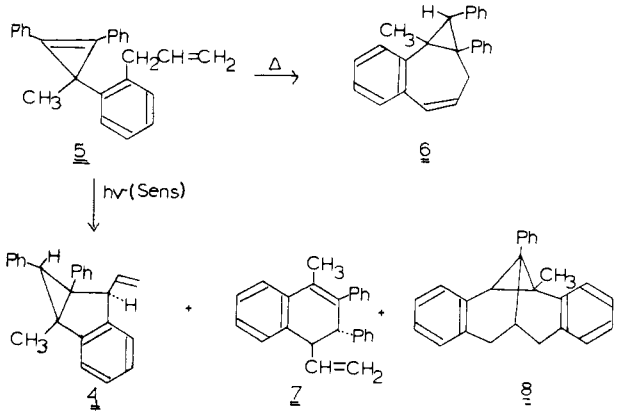


Scheme I



a sample of the isomeric cis-isomer **3** gave rise to benzobicyclohexane **4** in quantitative yield. We suggest that **4** most reasonably arises from **3** by a concerted ene reaction. The geometry necessary for this type of reaction is easily achieved with the *Z*-substituted cyclopropene. Although bimolecular ene reactions of cyclopropenes are known,[10,11] the above case constitutes the first example of an intramolecular version of this reaction.

Additional examples which would establish the generality and scope of the intramolecular ene reaction were sought. With this in mind we investigated the thermolysis of the closely related 3-(*o*-allylphenyl)-substituted cyclopropene **5**. Heating a sample of **5** gave rise to the ene product **6** in quantitative yield.[15] The



NMR spectrum (C_6D_6, 90 MHz) of **6** consists of signals at δ 1.19 (s, 3 H), 2.14 (ddd, 1 H, J = 13.3, 6.1, and 2.0 Hz), 2.54 (dd, 1 H, J = 13.3 and 7.4 Hz), 2.62 (s, 1 H), 6.01 (ddd, 1 H, J = 10.7, 7.4, and 6.1 Hz), 6.62 (d, 1 H, J = 10.7 Hz), and 6.72–7.58 (m, 14 H). The sensitized irradiation of **5** was also studied and was found to produce a mixture of three products. Chromatography of the mixture on silica gel gave benzobicyclohexene **4**[15] (60%) together with dibenzotricyclodecane **8** (30%) and dihydronaphthalene **7** (10%). Assignment of the minor component as dihydronaphthalene **7** was made on the basis of its NMR spectrum and by its oxidation to the corresponding aromatic hydrocarbon: NMR **7** ($CDCl_3$, 90 MHz) δ 2.07 (s, 3 H), 3.55 (d, 1 H, J = 7.5 Hz), 3.79 (s, 1 H), 4.89–5.15 (m, 2 H), 6.21 (ddd, 1 H, J = 17.5, 10.0, and 7.5 Hz), 6.88–7.54 (m, 14 H). The structure of **8** was easily assigned on the basis of its straightforward spectral data:[16] NMR ($CDCl_3$, 90 MHz) δ 1.37 (s, 3 H), 2.22 (d, 1 H, J = 15.5 Hz), 2.37 (d, 1 H, J = 15.5 Hz), 2.60 (s, 1 H), 2.92 (t, 1 H, J = 6.6 Hz), 3.20 (dd, 1 H, J = 15.5) and 6.6 Hz), 3.42 (dd, 1 H, J = 15.5 and 6.6 Hz), and 6.73–7.49 (m, 13 H).

The triplet state of cyclopropene **5** can readily abstract a hydrogen from the neighboring benzylic carbon to produce a biradical intermediate which either collapses to give **4** or undergoes cyclopropyl ring opening in competition with coupling.[17] The ring-opened species **10** would be expected to cyclize in a disrotatory fashion to give dihydronaphthalene **7**. The formation of dibenzotricyclodecane **8** in the sensitized irradiation represents an

(1) For a review, see: Hoffmann, H. M. R. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 566.
(2) Chia, H. A.; Kirk, B. E.; Taylor, D. R. *Chem. Commun.* **1971**, 1144.
(3) Agami, C.; Andrac-Taussig, M.; Prevost, C. *Bull. Soc. Chim. Fr.* **1966**, 173; **1966**, 1195.
(4) Arnold, R. T.; Dowdall, J. F. *J. Am. Chem. Soc.* **1948**, *70*, 2590.
(5) Dai, S.; Dolbier, W. R., Jr. *J. Am. Chem. Soc.* **1972**, *94*, 3953.
(6) Stephenson, L. M.; Mattern, D. L. *J. Org. Chem.* **1976**, *41*, 3614.
(7) Benn, F. R.; Dwyer, J.; Chappell, I. *J. Chem. Soc., Perkin Trans. 2* **1977**, 533.
(8) Inagaki, S.; Fujimoto, H.; Fukui, K. *J. Am. Chem. Soc.* **1976**, *98*, 4693.
(9) Lambert, J.; Napoli, J. J. *J. Am. Chem. Soc.* **1973**, *95*, 294.
(10) Breslow, R.; Dowd, P. *J. Am. Chem. Soc.* **1963**, *85*, 2729.
(11) Dowd, P.; Gold, A. *Tetrahedron Lett.* **1969**, 85.
(12) Oppolzer, W.; Snieckus, V. *Angew. Chem., Int. ed. Engl.* **1978**, *17*, 476.
(13) Padwa, A. *Acc. Chem. Res.* **1979**, *12*, 310.
(14) Padwa, A.; Rieker, W. F. *J. Org. Chem.* **1979**, *44*, 3273.
(15) The NMR spectrum of structures **4** and **6** suggest that the phenyl group is located in the exo position of the cyclopropyl ring. Satisfactory spectral and analytical data were obtained for each new compound.
(16) Attack of the ortho position of the triplet state of **5** on the adjacent double bond can occur in two ways. The NMR data obtained fits structure **8** better than the alternative regioisomer.
(17) Padwa, A.; Blacklock, T. J.; Chou, C. S.; Hatanaka, N. *J. Am. Chem. Soc.* **1979**, *101*, 5743.

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