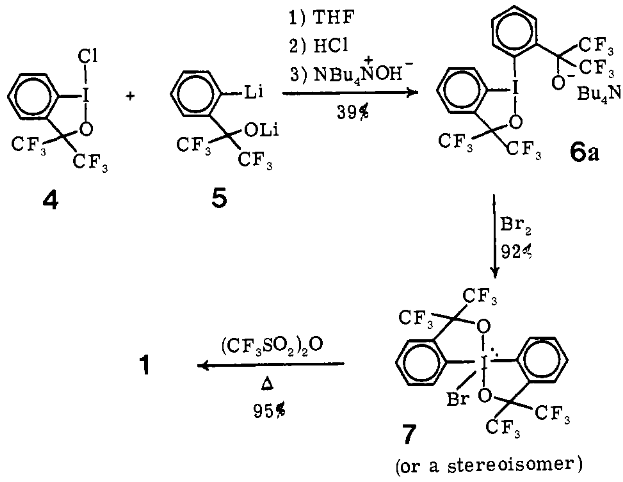
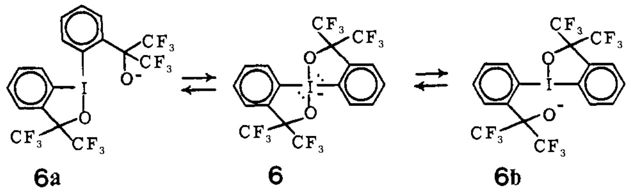
Scheme I



with the ^{19}F NMR spectrum. Even at temperatures as low as −60 °C, the spectrum shows evidence for only two types of CF_3 peaks as expected for **1**. An X-ray crystallographic structure determination, to be published later,[12] confirms the proposed distorted TBP structure to be correct. The O–I–O angle is 162.8°.[12]

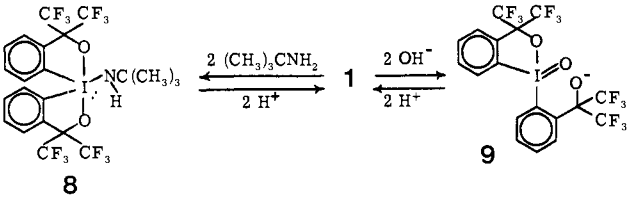
The synthesis of **1** is outlined in Scheme I. Preparations of **4**[5,13] and **5**[6] have been described in earlier papers. Alkoxydiaryliodinane **6a**[14] was at first believed to exist as bicyclic 12-I-4 species **6** (or its trans isomer), species analogous to XeF_4, a 12-Xe-4 species. The ^{19}F NMR spectrum at room temperature shows only a single sharp singlet.[14] Low-temperature ^{19}F NMR spectroscopy, however, shows the ground states to be unsymmetrical (**6a** and **6b**) with a ΔG^* of ca. 12 kcal/mol[15] at the coalescence temperature, −80 °C, for the two CF_3 singlets at 60 MHz.



Bromoperiodinane **7**[16] provides the first example of a bond joining bromine to iodine(V). It is stable for an indefinite period at room temperature and does not react with atmospheric moisture. It reacts with tetrahydrofuran or acetonitrile to give reduced product **6a** with bromination of solvent. The periodonium triflate is, under the same conditions, inert toward these solvents. A molecular weight determination by osmometry showed the triflate to be almost completely dissociated in acetonitrile.[3] These observations indicate that the I–Br bond in **7** is covalent. Its reactivity toward certain solvents and its insolubility in other solvents have prevented us from obtaining NMR data to support the proposed structure of **7**.

The low electrophilicity observed for sulfuranes **2a,b** finds a parallel in the relatively low electrophilicity of the periodonium ion of **1**, despite the positive charge on iodine. The periodonium ion does not react with water in aqueous tetrahydrofuran, acetonitrile, or acetone nor with methanol or pyridine in acetonitrile. It does react with stronger nucleophiles such as *tert*-butylamine and hydroxide ion.

The reaction of *tert*-butylamine with **1** in acetonitrile provides a compound whose ^{19}F NMR spectrum is consistent with a structure such as **8**.[17] Reaction of **1** with tetraethylammonium



hydroxide in acetonitrile forms a compound whose ^{19}F NMR spectrum is consistent with a structure such as **9**.[18] Both **8** and **9** react with acid to regenerate **1**. It has not yet been possible to isolate **8** or **9** as pure crystalline solids. Further work on these and related reactions with nucleophiles is in progress.

Acknowledgment. This research was supported in part by a grant from the National Science Foundation (CHE 79-07-7905692). NMR facilities of the University of Illinois NSF Regional Instruments Facility (CHE 79-16100) and mass spectrometer facilities supported through the National Institute of General Medical Sciences (GM 27029) were used to obtain the spectral data.

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(12) Dess, D. B.; Martin, J. C., to be published.

(13) Amey, R. L.; Martin, J. C. *J. Org. Chem.* **1979**, *44*, 1779.

(14) Mp 200–202 °C; ^{1}H NMR (CD_3CN) δ 7.58 (1 H, t), 7.76 (1 H, t), 7.94 (1 H, d, H ortho hexafluorocumyl group), 8.01 (1 H, d, H ortho I); ^{19}F NMR (25 °C) (THF) δ −76.23 (12 F, s); ^{19}F NMR (−80 °C) −72.93 (12 F, br s); ^{19}F NMR (−100 °C) δ −71.33 (6 F, s), −73.89 (6 F, s); mass spectrum (field desorption) *m/e* 853 ($M^{+}\cdot$). Anal. ($C_{13}H_{42}F_{12}IO_2N$) C, H, F, I, N.

(15) Gutowsky, H. S.; Holm, C. H. *J. Chem. Phys.* **1956**, *25*, 1228.

(16) Mp 190–196 °C; mass spectrum (10 eV), *m/e* (rel intensity) 690, 692 (7, $M^{+}\cdot$), 621, 623 (30, $M^{+}\cdot - CF_3$), 611 (49, $M^{+}\cdot$ − Br). Anal. ($C_{18}H_8F_{12}BrIO_2$) C, H, F.

(17) ^{19}F NMR (CH_3CN) δ −72.97 (2.9 F, q), −73.48 (3.5 F, q), −74.35 (2.9 F, q), −75.42 (2.8 F, q).

(18) ^{19}F NMR (CH_3CN) δ −74.12 (5.9 F, m), −74.88 (2.3 F, q), −76.93 (2.8, q).

Involvement of the Azide Radical in the Quenching of Singlet Oxygen by Azide Anion in Water

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Received September 3, 1981

Reactive oxygen intermediates generated in aqueous environment are responsible for many of the reactions observed in biological and chemical systems. It is a difficult task to determine which intermediate(s) is involved in a particular system. The utilization of nitrone spin traps[1] has proved useful for the identification of the hydroxyl (·OH) and superoxide (O_2^-) radical intermediates.[2] In 1975, Ching and Foote[3] demonstrated that cyclic nitrones quench $^1O_2$ in $CDCl_3$. We extended these results to aqueous medium and demonstrated that nitrone spin traps react with singlet oxygen ($^1O_2$) in water[4] with rate constants of $\sim 10^8$ M^{-1} s^{-1}. This reaction is readily followed by oxygen uptake measurements and demonstrates that nitrones can effectively be utilized in the detection of these three major oxygen intermediates.

A major diagnostic for $^1O_2$ in aqueous medium has been the quenching of $^1O_2$ by the azide anion, N_3^-.[5] A kinetic study[4] using oxygen uptake revealed that N_3^- quenches the reaction between nitrones and $^1O_2$ with a rate constant $k = 1.5 \times 10^9$ M^{-1} s^{-1} (compared to literature value of 2×10^9 M^{-1} s^{-1}).[6] The mechanism of this quenching is considered to proceed through a charge-transfer complex[5] ($O_2^{-}\cdots N_3$). This communication gives

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(1) Janzen, E. G.; Liu, J. I. *J. Magn. Reson.* **1973**, *9*, 510–512.

(2) Harbour, J. R.; Chow, V.; Bolton, J. R. *Can. J. Chem.* **1974**, *52*, 3549–3552.

(3) Ching, T. -Y.; Foote, C. S. *Tetrahedron Lett.* **1975**, *44*, 3771–3774.

(4) Harbour, J. R.; Issler, S. L.; Hair, M. L. *J. Am. Chem. Soc.* **1980**, *102*, 7778–7779.

(5) Foote, C. S. *Org. Chem.* (*N.Y.*) **1979**, *40*, 139–171.

(6) Gupta, A. K.; Rohatgi-Mukherjee, K. K. *Photochem. Photobiol.* **1978**, *27*, 539–543.

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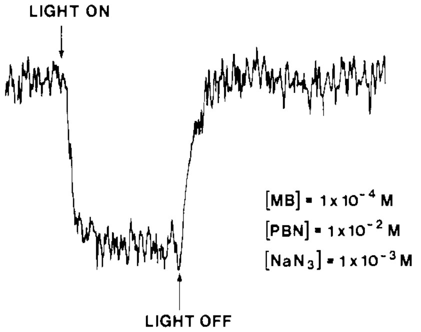

Figure 1. Time dependence of the ESR intensity as a function of illumination. The magnetic field is fixed at the minimum of one of the lines of the $N_3\cdot$ adduct of PBN. [MB] = 1 × 10^{-4}, [PBN] = 1 × 10^{-2}, and [NaN_3] = 1 × 10^{-3} M.

evidence for the identification and participation of $N_3\cdot$ in the quenching of $^1O_2$ by N_3^- in aqueous medium as detected indirectly with the spin-trapping technique.[7]

Methylene blue (MB) was selected as a sensitizer for the generation of $^1O_2$ in aqueous medium. In situ illumination of a MB solution containing both phenyl-*tert*-butylnitrone (PBN) and N_3^- resulted in the generation of a spin adduct of PBN arising from the trapping of a nitrogen-centered radical (A^{N_1} = 15.2 G; A^{N_2} = A^H = 2.1 G). These splittings are essentially equivalent to those reported by Rehorek et al.[8] for the $N_3\cdot$ spin adduct of PBN and by Kremers and Singh[9] and Janzen et al.[10] Several additional experiments were carried out which confirm the results of these workers.[9,10] Photolysis of peroxydisulfate ($S_2O_8^{2-}$) generates a strong oxidizing agent (SO_4^-)[11] which should oxidize N_3^- to $N_3\cdot$. In the presence of PBN, the SO_4^- spin adduct is observed when N_3^- is absent.[12] However, upon addition of N_3^-, the SO_4^- is no longer observable, and a nitrogen-centered adduct appears with splittings equivalent to those found above in the MB system. Replacing $S_2O_8^{2-}$ by H_2O_2 gave the same results upon photolysis. This is consistent with the light-induced homolysis of H_2O_2 to yield $\cdot OH$ radicals which can also oxidize N_3^- to $N_3\cdot$. In addition, direct photolysis of N_3^- with only PBN present also gave this adduct but to a lesser degree. When 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO)[1] or 4-pyridyl *N*-oxide *N*-*tert*-butylnitrone (4-PyOBN)[13] were used instead of PBN in the above type of experiments, equivalent results were obtained with adducts having A^{N_1} = 14.5, A^{N_2} = 3.1, and A^H = 14.5 G for DMPO and A^{N_1} = 14.8, A^{N_2} = 2.0, and A^H = 2.0 G for 4-PyOBN. Therefore, the spin adducts generated during the quenching of $^1O_2$ by N_3^- correspond to the $N_3\cdot$ adducts.[8-10] Having established this fact, it is necessary to now ascertain the origin of $N_3\cdot$ radical to determine whether it results from the quenching reaction in a significant way.

The $N_3\cdot$ adduct is unstable and decays to half-intensity in approximately 2 s after the light is turned off (Figure 1). Hence, a steady state is reached where the rate of formation of the adduct equals the rate of decay of the adduct. However, as the oxygen is consumed in this system, the magnitude of the signal steadily decreases. This is illustrated in Figure 2 for the partially nitrogen-purged sample. The maximum in signal intensity is observed at 0.3 min for this system. This can be compared to maxima of 1.5 and 2.2 min for the air- and oxygen-saturated cases, which is consistent with an involvement of oxygen in the creation of the $N_3\cdot$ adduct. O_2 uptake experiments under equivalent conditions reveal that more than 50% of the dissolved oxygen is consumed by the time the maximum is reached in the air-saturated case.

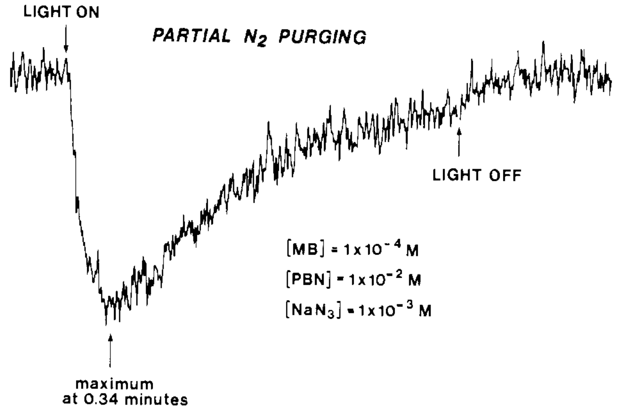

Figure 2. Magnitude of one of the lines of the $N_3\cdot$PBN adduct as a function of time of illumination. This sample was partially N_2 purged. [MB] = 1 × 10^{-4}, [PBN] = 1 × 10^{-2}, and [NaN_3] = 1 × 10^{-3} M.

The instability of the radical adduct makes quantification of the number of spins more difficult. Nevertheless, using the fact that a steady state has been achieved, it is possible to determine roughly the number of azide radicals created during the illumination period in this closed system. This calculation, using 2,2,6,6-tetramethylpiperidinyloxy as a free radical standard, shows that ~3 × 10^{-5} M $N_3\cdot$ adduct was generated which can be compared to the [O_2] of 2 × 10^{-4} M in air-saturated water. As indicated above, this illumination period is sufficient to consume most of the dissolved oxygen. Hence, at least 15% of the quenching of $^1O_2$ by N_3^- results in trapped $N_3\cdot$ radicals. This result demonstrates that the generation of $N_3\cdot$ adduct is a significant event in this system. It must be remembered that oxygen consumption in this system occurs mainly through the reaction of PBN and $^1O_2$ to form a diamagnetic product.

It was previously demonstrated that, in the absence of azide anion, small quantities of the hydroxyl adduct are formed during the photolysis of methylene blue solutions.[4] However, these adducts are produced in amounts three orders of magnitude less then the oxygen consumed. In addition, once formed, they are stable and reach a limiting concentration quickly. With 4-MePyBN,[13] the $\cdot OH$ adduct is generated in equivalent amounts both with and without azide. These results rule out the generation of $N_3\cdot$ from a hydroxyl radical generated in these systems.

A possible origin of $N_3\cdot$ radical would be the direct oxidation of N_3^- by the excited triplet state of MB. The backreaction for this process would be very fast, but could be slowed down by a concomitant reduction of O_2 to O_2^-. However, previous work[4] on this system has shown that a common intercept is observed in a plot of $[PBNO_2]^{-1}$ vs. $[PBN]^{-1}$, confirming that N_3^- is not quenching the excited states of MB.[5] This immediately suggests that a direct oxidation of N_3^- is not occurring. This is supported by the D_2O experiments presented below which revealed a 3-fold increase in $N_3\cdot$ adduct upon replacing H_2O by D_2O. A direct oxidation should be relatively unaffected by deuteration of the solvent and one would therefore predict no significant change in $N_3\cdot$ adduct production. Finally, no preceptible increase in O_2 uptake was observed when a 1 × 10^{-4} M MB solution containing 1 × 10^{-3} M N_3^- was illuminated. These results taken together suggest that direct oxidation of N_3^- is not the path by which $N_3\cdot$ adduct arises.

In order to further substantiate the direct connection between $N_3\cdot$ adduct formation and $^1O_2$ quenching by N_3^-, three experiments were conducted in which one experimental parameter was varied. For this discussion, the following reactions are defined with the assumption that the $N_3\cdot$ radical arises through eq 1 (k_1 = 1.5 × 10^9 $M^{-1}s^{-1}$ and k_2 = 1.4 × 10^8 M^{-1} s^{-1}).

$$N_3^- + {}^1O_2 \xrightarrow{k_1} N_3^- + {}^3O_2 \quad r_1 = k_1[N_3^-][{}^1O_2] \qquad (1)$$

$$\mathrm{PBN} + {}^1O_2 \xrightarrow{k_2} \text{unknown product} \quad r_2 = k_2[\mathrm{PBN}][{}^1O_2] \qquad (2)$$

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[7] Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31–40.

[8] Rehorek, D.; Thomas, P.; Henning, H. *Inorg. Chim. Acta* **1979**, *32*, L1–L2.

[9] Kremers, W., Singh, A *Can. J. Chem.* **1980**, *58*, 1592–1595.

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[11] Chawla, O. P.; Fessenden, R. W. *J. Phys. Chem.* **1975**, *79*, 2693–2700.

[12] Harbour, J. R.; Hair, M. L. *Can. J. Chem.* **1979**, *57*, 1150–1152.

[13] These nitrone spin traps were obtained from Spin Trap Producers, Guelph, Ontario N1H 6L8, Canada.

$$\mathrm{PBN} + \mathrm{N_3}\cdot \xrightarrow{k_3} \text{adduct} \quad r_3 = k_3[\mathrm{PBN}][\mathrm{N_3}\cdot] \qquad (3)$$

$$\mathrm{N_3}\cdot + \mathrm{O_2}^- \xrightarrow{k_4} \mathrm{N_3}^- + {}^3\mathrm{O_2} \quad r_4 = k_4[\mathrm{N_3}\cdot][\mathrm{O_2}^-] \qquad (4)$$

Variation of N_3^- Concentration. In this experiment, the concentration of PBN was fixed at 1×10^{-3} M with the sensitizer MB at 1×10^{-4} M. The ESR intensity of the azide adduct was then determined for N_3^- concentrations of 1×10^{-4} and 4×10^{-3} M. Since $k_1 \equiv 10k_2$, $r_1 = r_2$ when the concentration of N_3^- equals 1×10^{-4}. In this case, 50% of the ${}^1O_2$ molecules generated will be quenched by N_3^-. However, when the $[N_3^-]$ is increased by 40 times, essentially all of the ${}^1O_2$ molecules will be quenched and the amount of radicals observed should double. In fact, an increase of 2.5 was observed.

Variation of PBN Concentration. In this case, the $[N_3^-]$ was fixed at 1×10^{-3} M with $[MB] = 1 \times 10^{-4}$ M and $[PBN]$ was 1×10^{-3} vs 1×10^{-2} M. For 1×10^{-2} M, $r_1 = r_2$, and 50% of the ${}^1O_2$ molecules would react with N_3^-. However, at 1×10^{-3} M PBN, $r_1 = 10r_2$ and ~90% of the ${}^1O_2$ molecules would be quenched by N_3^-. The ESR predicted outcome depends on the lifetime of the $N_3\cdot$ radical and the rate constants k_3 and k_4. If essentially all the $N_3\cdot$ radicals are trapped, then one would expect that the ESR intensity would be halved by the order of magnitude increase in [PBN]. If, however, $N_3\cdot$ is relatively short lived, r_3 will be increased due to the increase in [PBN], and an increase of ~5 in ESR intensity would be predicted. The experimentally observed change was an increase in signal intensity of 2. This suggests that the $N_3\cdot$ radical is relatively short lived as well as demonstrating that the observed change is within the predicted limits.

Variation of ${}^1O_2$ Lifetime. In this experiment, $[PBN] = 1 \times 10^{-3}$, $[N_3^-] = 1 \times 10^{-3}$, and $[MB] = 1 \times 10^{-4}$ M. The lifetime of the ${}^1O_2$ was varied by using D_2O vs. H_2O. This increases the lifetime of ${}^1O_2$ from 2 to 20 μs,[14] provided of course there is no other species present which quenches ${}^1O_2$. However, this is not the case since both N_3^- and PBN are present and reactive to ${}^1O_2$, thereby preventing ${}^1O_2$ from achieving its natural lifetime. With H_2O as the solvent, $r_1 = 10r_2$ and ~90% of the ${}^1O_2$ produced will react according to eq 1. Replacing H_2O by D_2O will not change this ratio; yet, the number of available ${}^1O_2$ molecules will increase because of the longer inherent lifetime. Previous work has shown[4] that at a quencher concentration of 10^{-3} M, a 2.3 increase in rate is observed (this value approaches 10 as the [quencher] is reduced). Therefore, a 2.3 increase in radical concentration is predicted while experimentally we observed a 3-fold increase.

These three experiments are all consistent with the predicted change in ESR intensity of the $N_3\cdot$ radical adduct if in fact it arises during the quenching of ${}^1O_2$ by N_3^-. Additionally, these results are quantitively consistent within reasonable limits of errors and are in agreement with a charge-transfer mechanism of physical quenching in which the charge-transfer complex dissociates into free $N_3\cdot$ and O_2^- radicals.

$${}^1\mathrm{O_2} + \mathrm{N_3}^- \rightarrow \{\mathrm{O_2}^- \cdots \mathrm{N_3}\cdot\} \rightarrow \mathrm{O_2}^- + \mathrm{N_3}\cdot \qquad (5)$$

The PBN then traps the $N_3\cdot$ radical to produce the ESR spectrum observable. It is feasible that the spin trap interacts with the complex in such a way as to cause dissociation. The O_2^- adduct is not observed in this system which is not unexpected due to difficulties of trapping O_2^- in aqueous media.[15] In addition, O_2^- disproportionates at these pH's to O_2 and H_2O_2 with a significant rate.[16] In the case where PBN is not present, this N_3^- quenching mechanism with dissociation may still occur but then reacts by eq 4 to be consistent with physical quenching. Finally, these results suggest that at least 15% of the charge-transfer complexes formed dissociate into free ions prior to recombination. The fact that PBN may not react with all the $N_3\cdot$ radicals formed (eq 3) makes this a minimum value. Spin trapping thus gives the first spectroscopic evidence for participation of N_3 radicals in the quenching of ${}^1O_2$ by N_3^- in H_2O.[17]

Registry No. $N_3\cdot$, 12596-60-0; O_2, 7782-44-7; N_3^-, 14343-69-2; PBN spin adduct, 58200-47-8; DMPO spin adduct, 80387-88-8; 4-PyOBN spin adduct, 80387-89-9; PBN, 3376-24-7.

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(17) It is interesting to note that azido radicals are produced during the formation of azidohydroperoxides in the sensitized photooxygenation of olefins quenched by N_3^-. Foote, C. S.; Fujimoto, T. T.; Chang, Y. C. *Tetrahedron Lett.* **1975**, 45–47.

Structure of the Cyclic Peptide Dolastatin 3 from *Dolabella auricularia*[1]

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Devens Gust, Masuo Inoue, and Cherry L. Herald

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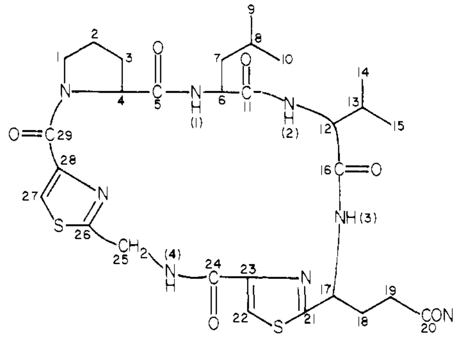
Received August 10, 1981

The Indian Ocean sea hare *Dolabella auricularia*[3] has been found to be an exceptionally productive source of new anticancer biosynthetic products.[4,5] Recently, we recorded preliminary observations concerning our discovery of nine antineoplastic and/or cytotoxic substances in *D. auricularia*.[4a] Dolastatins 1–9 were obtained in about 1-mg amounts each from 100 kg of the wet sea hare. Because of the 1-mg quantities, and lack of crystallinity, structural elucidation of the dolastatins has presented an ample challenge.

We are now pleased to report assignment of unique cyclic peptide[6] structure **1** to the powerful cell growth inhibitory (murine P388 lymphocytic leukemia cell line $ED_{50} < 1 \times 10^{-4} - 1 \times 10^{-7}$ μg/mL)[7] dolastatin 3: colorless amorphous solid from methylene



1, *cyclo*[Pro-Leu-Val-(gln)Thz-(gly)Thz], dolastatin 3

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(1) Part 83 of "Antineoplastic Agents". For part 82, refer to: Pettit, G. R.; Cragg, G. M.; Gust, D.; Brown, P.; Schmidt, J. M. *Can. J. Chem.*, submitted for publication.

(2) Dedicated to the memory of our friend and colleague Professor Peter Brown who expired on March 25, 1981.

(3) This species (Mollusca phylum, Aplysiomorpha order, Aplysiidae family) designation also includes, e.g., *D. andersoni*, *D. californica*, *D. ecaudata*, and *D. scapula*. See ref 4a, footnote 6.

(4) (a) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482. (b) Pettit, G. R.; Ode, R. H.; Herald, C. L.; Von Dreele, R. B.; Michel, C. *J. Am. Chem. Soc.* **1976**, *98*, 4677–4678.

(5) Some of the *D. auricularia* constituents such as dolatriol[4b] and related dolastanes may originate from dietary utilization of certain brown algae (particularly from the semitropical *Dictyota* genus, Dictyotaceae family). Evidence for such a chain of biological events has been obtained by Fenical and colleagues: Sun, H. H.; McConnell, O. J.; Fenical, W.; Hirotsu, K.; Clardy, J. *Tetrahedron* **1981**, *37*, 1237–1242. See also: Amico, V.; Oriente, G.; Piattelli, M.; Tringali, C.; Fattorusso, E.; Magno, S.; Mayol, L. *Ibid.*, **1980**, *36*, 1409–1414.

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