by C–S bond fission and H^+ migration, and at 697.2592 ($C_{32}H_{41}N_8O_6S_2$ requires 697.2590) to a loss of S_2H,[14] which is compatible only with **2**.

The biosynthetic origin of ulicyclamide (**1**) and of ulithiacyclamide (**2**) is unknown. Didemnid ascidians are a unique group of invertebrate chordates which harbor symbiotic unicellular algae[15] that possess photosynthetic viability.[16] However, little is known about chemical transport between host and symbiont or about the nitrogen-fixing ability of the algae.

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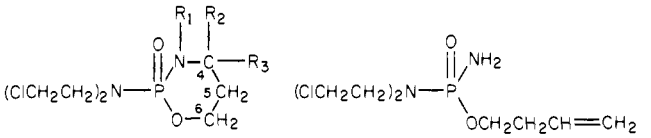
A New Oxidized Derivative of Cyclophosphamide Obtained from Ozonolysis of O-3-Butenyl *N,N*-Bis(2-chloroethyl)phosphorodiamidate

Sir:

The antitumor agent cyclophosphamide (**1**) exerts its cytostatic action after oxidation in vivo by liver microsomes.[1,2] Chemical synthesis of the primary oxidation product in vivo, 4-hydroxycyclophosphamide (**2**), has been described by Takamizawa et al.[3] By this procedure, **2** can be isolated after reduction of 4-hydroperoxycyclophosphamide (**3**), a compound that was synthesized by ozonolysis of O-3-butenyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (**4**). Fenton oxidation of **1** led also to the formation of peroxy compounds[4–6] that were identified as **3** and 4-peroxycyclophosphamide (**5**). In aqueous solution, **3** and **5** are spontaneously converted to **2**.[7] As a consequence, these three compounds exhibit the same cytotoxic properties in in vitro systems. Since **3** and **5** are much more stable than **2** on storage at −20 °C, they are of practical value for in vitro studies on cytostatic effects of **1**.

Routinely, we synthesized **3** by a modified procedure of Takamizawa et al.[3] The modifications led, however, to an unexpected observation, i.e., the occurrence of an as yet unknown oxidized derivative of **1** that could be isolated as a precursor of **3**. Our procedure implied the following:



1, $R_1 = R_2 = R_3 = H$
2, $R_1 = R_2 = H$, $R_3 = OH$
3, $R_1 = R_2 = H$, $R_3 = OOH$
5, ($R_1 = R_2 = H$, $R_3 = O$-)$_2$
6, R_1, $R_2 = O$; $R_3 = H$
7, $R_1 = H$; R_2, $R_3 = O$

4

A solution of 2 g of **4** in 120 mL of acetone/water (2:1), slightly alkalinized with NH_4OH, was ozonized[8] in the presence of 5 mL of 30% H_2O_2 at 0 °C. The reaction was followed by TLC on silica gel, with CH_2Cl_2/*n*-BuOH (9:1) as eluant. After ca. 30 min, the alkylating spot[9] of the starting material ($R_f = 0.4$) had completely disappeared, and almost all alkylating activity was found at $R_f = 0.2$. Acetone was extracted from the reaction mixture with 3 volumes of CH_2Cl_2. The aqueous phase was freeze-dried and the remaining oil dissolved in a small amount of acetone. After the insoluble material was filtered off, ether was added to the filtrate until saturation was reached. From this, a product with rather polar characteristics crystallized at −20 °C (soluble in water, acetone; insoluble in CH_2Cl_2, ether). Isolation of the product had to be carried out as quickly as possible because of its tendency to decompose. The overall yield after crystallization from acetone/ether was ~20%. Crystals of the compound were hygroscopic; when stored dry at −20 °C, its stability is comparable to **3**.

We tentatively identified the compound as 2-bis(2-chloroethyl)aminotetrahydro-2*H*-3,4-epoxy[1,3,2]oxazaphosphorine 2-oxide (**6**). ^1H NMR, ^{13}C NMR, IR, and field desorption (FD) mass spectral analyses[10] were consistent with this structure: ^1H NMR (acetone-*d*, Me_4Si; Varian HA-100) δ 1.75–2.15 (2 H, m, C_5H_2), 3.25–3.85 [8 H, m, $(CH_2CH_2Cl)_2$], 3.90–4.30 (2 H, q, C_6H_2), 5.25 (1 H, t, $J = 6$ Hz, C_4H); ^{13}C NMR (Varian XL-100, solvent and reference acetone-*d*, −40 °C) 33.6 (C_5), 41.8 ($C_{\beta,\beta'}$), 48.5 ($C_{\alpha,\alpha'}$), 61.1 (C_6), 97.5 ppm (C_4).[11] In the ^{13}C NMR "off-resonance" spectrum, the C_4 signal appears as a doublet (while all other signals appear as a triplet), indicating that only one proton is bound at the C_4 atom. IR(KBr): 3410, 3320, 2965, 2945, 2900, 1570, 1455, 1365, 1255, 1205, 1175, 1125, 1090, 1040, 985, 940, 870, 760, and 740 cm^{-1}. FD mass spectrum: *m/z* 274 (2Cl, M^+, relative abundance 75), 141 (2Cl, $[HN(CH_2CH_2Cl)_2]^+$, relative abundance 100), 92 (1Cl, $[CH_2{=}NHCH_2CH_2Cl]^+$, relative abundance 30). Accurate mass measurements of the molecular ion in the FD mode using high resolution and peak matching gave a value of *m/z* 274.0026 (theoretical 274.0041 for $C_7H_{13}O_3N_2Cl_2P$). An isomeric structure of **6**, i.e., the *N*-oxide of the corresponding 3,4-unsaturated compound (Schiff base), is possible from the IR data (absorption at 1570 cm^{-1} is in the >C=N– stretching vibration region) but is unlikely since no peak was detected in the FD mass spectrum at *m/z* 258 $(M - 16)^+$. It has been shown that the loss of oxygen from substances which carry a nitroso group is a characteristic feature in FD mass spectrometry.[12] Also, a possible conversion from **6** to 4-oxocyclophosphamide (**7**) on the probe of the mass spectrometer is unlikely because under the experimental conditions employed **7** is known to give abundant $(M + H)^+$ ions at *m/z* 275 whereas

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6 gives the base peak at m/z 274 and only a minor protonation contributes to m/z 275. A conversion of 6 to 7 on the emitter should also be recognizable from the fragmentation pattern and the best anode temperature. However, no other signals than those stated could be detected between m/z 141 and m/z 400, and the best anode temperature for 6 is 10 mA below that for 7.

Elemental analysis was in agreement with the proposed chemical formula with two molecules of crystal water. Analysis by Röntgen diffraction was unsuccessful because of the instability of the crystalline product.

The reaction mechanism that leads to the formation of 6 is not fully clear. Takamizawa et al.[13] have proposed a mechanism for the formation of 3 in which cleavage of a primary ozonide was followed by cyclization of the resulting zwitterion fragment (cf. ref 13). We suggest that under our conditions, especially the slightly alkaline solution, cyclization occurs, preferably to yield 6.

Compound 6 can be converted to 3, possibly by reaction with its crystal water, by dissolving it in acetone/CH_2Cl_2 and refluxing the solution for 30 min. The yield of this reaction was ~70%. Refluxing for a longer period of time primarily resulted in the formation of 5. Conversion of 6 into the stable isomer 7 was also noticed under these conditions. Direct preparation of 3 from the ozonolysis reaction mixture, by refluxing the CH_2Cl_2 extract together with the oil remaining from the freeze-dried aqueous phase, gave an overall yield of 3 of ~30% after crystallization from ether.

Since 6 can be converted to 3, it is likely that the oxazirane group is cis to the phosphoryl oxygen, because it is known that the peroxide group of 3 also has the cis configuration.[14,15] A small amount of the trans isomer of 6 (less than 10%) may contaminate the isolated crystals since the ^1H NMR spectrum shows a small triplet at δ 5.42 (J = 6 Hz) next to the triplet at δ 5.25. Peter et al.[16] have observed that ozonation of 1 leads to the formation of 3, 7, and an unstable compound that was suggested to be the trans diastereomer of 3. The described chromatographical and chemical behavior leads us to the suggestion that Peter et al. might also have observed 6.

Compound 6 also exhibits cytostatic activity in vitro. On BHK cells, it has the same cytostatic action as 3 (ED_{50} = 7–10 μM when drugs are left in the medium for the full period of growth). On $3T3_f$ cells, the cytotoxic capacity of 6 is even a little stronger in comparison with 3 (6, ED_{90} = 9 μM; 3, ED_{90} = 17 μM; drug treatment, 1 h).

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Flow Pyrolysis and Direct and SiF_4-Sensitized Laser-Induced Decomposition of Tetralin. Identification of Retro-[2 + 4] Cleavage as the Primary Homogeneous Thermal Decomposition Channel

Sir:

The thermal chemistry of tetralin (1) has been under intense investigation recently,[1] due to interest in the fate of hydrogen donors which are used as recycle liquids in the solvent refining of coal. The reactivity of tetralin appears to be dependent on the presence of hydrogen, hydrogen-acceptor molecules, different types of reactor surfaces, and surface history. This has resulted in conflicting data[2] in the literature and confusion as to what the thermal reactivity is in the absence of any catalytic effects. In an effort to resolve this situation, we report the reactivity of tetralin resulting from several different methods of activation.

Three methods for energization of 1 were investigated: conventional flow pyrolysis, infrared multiphoton excitation, and sensitized infrared laser thermal activation. All three give rise to six major products: benzocyclobutene (2), styrene (3), *o*-allyltoluene (4), indene (5), 1,2-dihydronaphthalene (6), and naphthalene (7) (see Scheme I), and several minor products.[3] The primary concern of this work is to delineate the energetics of the ethylene-loss channel (giving rise to 2 and 3) vs. the hydrogen-loss channel (giving rise to 6 and 7) without interference from catalytic effects.

A number of parameters affected the product distribution for the decomposition in a flow reactor,[4] including the composition of the surface, the history of the surface, and the pressure of the system (see Table I). However, except at the lowest pressure, where the two reaction channels became comparable, dehydrogenation was always the predominant decomposition mode. Unconditioned surfaces, higher pressures, and longer contact times in the reactor led to more dehydrogenation of 1.

Multiphoton excitation of 1 in the gas phase was accomplished[5] with a pulsed CO_2 TEA laser[6,7] tuned to 945.99 cm^{-1}. All six major products[8] found from the pyrolysis also resulted from photolysis of tetralin, including one additional product, phenylacetylene.[8] The distribution of these products, however, was at variance with the distribution from the pyrolysis (see Table I); the major reaction channel for the multiphoton dissociation of tetralin involved ethylene loss.

In the third method of activation, 1 and varying pressures of SiF_4, an inert sensitizer[10] which absorbs strongly in the infrared,

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(2) For example, compare ref 1a and 1e for the static pyrolysis results.
(3) The minor products have been tentatively identified as toluene, ethylbenzene, 1,4-dihydronaphthalene, and *o*-ethylstyrene. Traces of other unidentified aromatics were also observed. In nearly all experiments, none of the above products amounted to more than 1% of the total reaction mixture.
(4) For a description of the flow reactor, see: M. D'Amore, R. G. Bergman, M. Kent, and E. Hedaya, *J. Chem. Soc., Chem. Commun.*, 49 (1972).
(5) A sample cell consisted of a 2-cm i.d. by 20-cm long Pyrex cell with KCl windows mounted at the Brewster angle. The cell was pumped to $<10^{-5}$ torr on a grease-free vacuum line and was pressured to 0.325 torr of 1 for all photolysis experiments. Pressures of all gases were measured with a capacitance manometer.
(6) The laser pulse consisted of a 100-ns pulse followed by a 1-μs tail with an approximately 50:50 energy distribution between the pulse and tail. The energy per pulse was 1.3 J.
(7) A 15-cm focal length NaCl lens was used to focus the beam. During a photolysis, the focal point was located at approximately the midpoint of the cell. The laser beam was attenuated by placing a gas cell containing SF_6 in front of the photolysis cell. The intensity was varied by changing the pressure of SF_6. The energy density at the focal point was 46 J/cm^2 at 0.8 J/pulse and 23 J/cm^2 at 0.4 J/pulse.
(8) At low fluencies, 7 was not observed, presumably due to lack of secondary decomposition of 6.
(9) Phenylacetylene was identified by GC (MS)–mass spectroscopy of the photolysis mixture. It was not a product in the flow pyrolysis or the sensitized photolysis. In the direct irradiation experiments, this product could result from dehydrogenation of vibrationally excited styrene.

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