One possible explanation for the nonappearance of the pyrene radical cation in the sensitization experiment lies in the bimolecular nature of the proposed photoionization mechanism; the efficiency of the production of ions from the triplet–triplet annihilation will be given by

$$\varphi = \frac{\gamma}{1 + (1/[^3Py^*])(k_1/k_2)}$$

where γ is the probability of a triplet–triplet annihilation leading to ion formation, k_1 and k_2 are rate constants for the disappearance of the pyrene triplets by all first-order and by all second-order processes, and $[^3Py^*]$ is the initial concentration of triplets produced by the flash. Obviously, as $[^3Py^*]$ becomes smaller, φ will decrease, perhaps to the point where ion production falls below the detection limit of our instrument.

To settle this question we repeated the experiment with a sensitizer with a more favorable absorption spectrum, fluoranthene (the triplet of which also lies about 0.2 eV above the pyrene triplet). This enabled us to produce approximately the same concentration of triplets by sensitization as was obtained by direct excitation in Figure 1. Since the buildup and decay kinetics of the triplets were approximately the same in both cases, we could assume that the maximum triplet concentration (not measurable because of scattered flash light) was the same in both cases. The result is shown in Figure 1B. As before, the pyrene radical cation is produced only when direct excitation is used (solid curve in Figure 1B) and not when the pyrene triplet is produced *via* excitation of the added sensitizer fluoranthene (dashed curve), in spite of the fact that the concentration of sensitized triplets has increased by a factor of 2. The possibility that initially formed radical cations of pyrene disappear by an electron-transfer reaction with the sensitizer can be ruled out, since 1,2-benzopyrene and fluoranthene have larger oxidation potentials in acetonitrile (1.25 and 1.45 V *vs.* sce, respectively) than pyrene (1.20 V).

The nonoccurrence of the photoionization when the triplets are produced indirectly is thus not a result of the dependence of φ on the triplet concentration, but rather indicates that the contribution of the triplet–triplet annihilation mechanism, or indeed of any mechanism involving the triplet state, is negligible. The same conclusion has been reached by Richards, *et al.*,[5] in a laser study of the pyrene photoionization in ethanol. We are planning further experiments with the aim of obtaining more information about this photoionization reaction.

Acknowledgments. Thanks are due to Mrs. S. Reiche and Miss U. Heine for valuable assistance with the flash experiments.

(5) J. T. Richards, G. West, and J. K. Thomas, *J. Phys. Chem.*, **74**, 4137 (1970).

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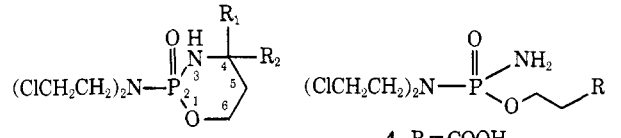
Received November 22, 1972

Studies on Cyclophosphamide Metabolites and Their Related Compounds. II.[1] Preparation of an Active Species of Cyclophosphamide and Some Related Compounds

Sir:

The mechanism of the *in vivo* activation of cyclophosphamide (**1**) to the cytostatically effective species has been a matter of considerable interest in recent years.[2] In 1963, Brock and Hohorst[3] found that liver microsomal oxidation is responsible for the activation process. Hill and coworkers,[4,5] and also the authors,[1] isolated 4-ketocyclophosphamide (**2**) and its ring-opened carboxylic acid **4** as the urinary metabolites of animals treated with cyclophosphamide, which indicates that the metabolic reaction of cyclophosphamide involves mainly oxidation at the C-4 position on the oxazaphosphorine ring. However, these oxidized metabolites were found to be cytostatically less active than cyclophosphamide in *in vivo* experiments[1,6,7] indicating that the activation of cyclophosphamide occurs in an earlier phase of the oxidation. Thus, 4-hydroxycyclophosphamide (**3**) or the ring-opened aldehyde **5** have recently been proposed as the active species.[6]

We now report the preparation of 4-hydroxycyclophosphamide (**3**) and some related compounds which exhibit pronounced cytostatic activities in both *in vivo*



1, $R_1 = R_2 = H$
2, $R_1, R_2 = O$
3, $R_1 = H$; $R_2 = OH$
8, $R_1 = H$; $R_2 = OOH$

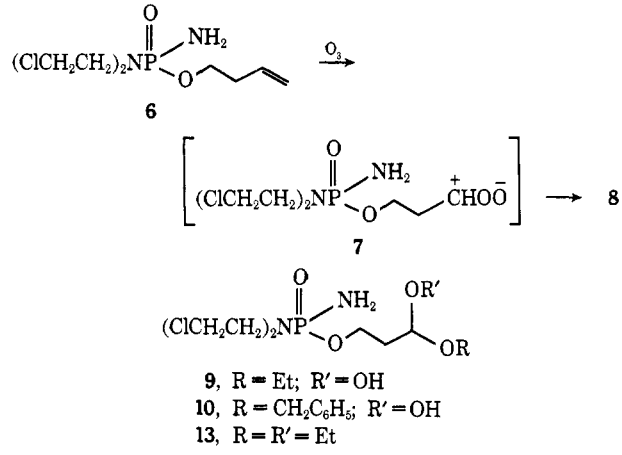
4, R = COOH
5, R = CHO
11, R = COOEt
12, R = $COOCH_2C_6H_5$

and *in vitro* experiments, confirming that C-4 hydroxylation is indeed responsible for the activation of cyclophosphamide.

Reaction of $POCl_3$ with 3-buten-1-ol in CH_2Cl_2 at $-10°$ for 4 hr, followed by treatment with *N,N*-bis(2-chloroethyl)amine hydrochloride and NEt_3 at -5 to $-10°$ for 3 hr, and finally with NH_3 at 0° for 2 hr, afforded *O*-3-butenyl *N,N*-bis(2-chloroethyl) phosphorodiamidate (**6**),[8] mp 20°, in 73% overall yield. Attempts to prepare the aldehyde **5** or its ring-closed equivalent **3** by ozonolysis of the olefin **6** under various conditions were unsuccessful, the corresponding peroxygenated product being obtained instead as follows.

(1) Part I: A. Takamizawa, Y. Tochino, Y. Hamashima, and T. Iwata, *Chem. Pharm. Bull.*, **20**, 1612 (1972).
(2) For example, see (a) H. Arnold, F. Bourseaux, and N. Brock, *Naturwissenschaften*, **45**, 64 (1958); (b) H. M. Rauen and K. Norpoth, *ibid.*, **52**, 477 (1965); (c) G. E. Foley, O. M. Friedman, and B. P. Drolet, *Cancer Res.*, **21**, 57 (1961); (d) H. Arnold and F. Bourseaux, *Arzneim. Forsch.*, **13**, 927 (1963); (e) H. M. Rauen and K. Norpoth, *ibid.*, **17**, 599 (1967); (f) J. L. Cohen and J. Y. Jao, *Proc. Amer. Ass. Cancer Res.*, **10**, 14 (1969); (g) N. Brock and H.-J. Hohorst, *Cancer (Philadelphia)*, **20**, 900 (1967).
(3) N. Brock and H.-J. Hohorst, *Arzneim. Forsch.*, **13**, 1021 (1963).
(4) D. L. Hill, M. C. Kirk, and R. Struck, *J. Amer. Chem. Soc.*, **92**, 3207 (1970).
(5) R. F. Struck, M. C. Kirk, L. B. Mellett, S. El Dareer, and D. L. Hill, *Mol. Pharmacol.*, **7**, 519 (1971).
(6) H.-J. Hohorst, A. Ziemann, and N. Brock, *Arzneim. Forsch.*, **21**, 1254 (1971).
(7) K. Norpoth, E. Golovinsky, and H. M. Rauen, *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 377 (1970).
(8) Elemental analysis and molecular weight determination are consistent with the assigned structure.

Ozonization of **6** in a mixture of acetone and H_2O (2:1) at 0° with a slight excess of O_3 gave 2-[bis(2-chloroethyl)amino]-4-hydroperoxytetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide (**8**)[8] (4-hydroperoxycyclophosphamide) in *ca.* 10% yield, mp 107–108°; ir_{max}^{Nujol} (cm^{-1}): 3310, 3080, 1237, 1210, 1035, 926, 840; $nmr_{TMS}^{DMSO\text{-}d_6}$: δ 1.92 (2 H, m, C_5–H_2), 3.01–4.02 (8 H, m), 4.21 (2 H, m, C_6–H_2), 4.71–5.11 (1 H, d of m, $J_{H,P}$ = 24.5, $J_{H,NH}$ = 5.0, $J_{H,CH}$ = 3.0 Hz, C_4–H), 5.81[9] (1 H, d of d, $J_{H,P}$ = 7.0 Hz, NH), 11.51[9] (1 H, s, OOH). The structure assignment for **8** was based on the spectral properties cited and on the following chemical behavior: **8** readily oxidizes KI to I_2, and it gives 4-ketocyclophosphamide (**2**) in good yield on treatment with $SOCl_2$–pyridine. It can be rationalized that **8** is produced by intramolecular cyclization of Criegee's zwitterion[10] intermediate **7** which was intercepted by adduct formation with alcohols (*vide infra*). The yield of **8** was markedly increased (50–60%) when excess of hydrogen peroxide or *tert*-butyl hydroperoxide was added to the ozonization reaction mixture.[11] Deoxygenation of **8** by triphenylphosphine in CH_2Cl_2 at 0° afforded 4-hydroxycyclophosphamide (**3**)[8] as labile crystals in 40% yield, mp 47.5–48.5°; ir_{max}^{Nujol} (cm^{-1}): 3240, 3180, 1240, 1215, 1195, 1053, 980; $nmr_{TMS}^{DMSO\text{-}d_6}$: δ 1.80 (2 H, m, C_5–H_2), 3.00–3.87 (8 H, m), 4.20 (2 H, m, C_6–H_2), 4.90 (1 H, d of m, $J_{H,P}$ = 21 Hz, C_4–H), 5.15[9] (2 H, m, NH, OH). Action of hydrogen peroxide upon **3** regenerated **8** in good yield.



9, R = Et; R′ = OH
10, R = $CH_2C_6H_5$; R′ = OH
13, R = R′ = Et

When the ozonolysis of **6** was carried out in the presence of alcohol, the zwitterion **7** could be captured as the open-chain hemiacetal hydroperoxide. Thus, ozonization of **6** in CH_2Cl_2 containing an excess amount of EtOH gave *O*-(3-ethoxy-3-hydroperoxy) *N*,*N*-bis(2-chloroethyl)propylphosphorodiamidate (**9**)[8,12] in 50% yield. Similarly, in the presence of benzyl alcohol, **6** gave **10**.[8,12] On treatment with $SOCl_2$–pyridine, **9** and **10** afforded the corresponding esters **11**[1] and **12**[1] in good yield. Deoxygenation of **9** by triphenylphosphine yielded an unstable mixture[13] from which no characterizable product could be isolated in a pure state. An alternative attempt to obtain **5** by acid hydrolysis of **13**, which was prepared by the reaction of *N*,*N*-bis(2-chloroethyl)aminophosphorodichloridate[14] with γ-hydroxypropionaldehyde diethylacetal followed by treatment with NH_3, was unsuccessful because of facile elimination of the C_3 unit of **5** to give acrolein.

As expected, 4-hydroxycyclophosphamide (**3**) exhibited high cytostatic activity in the preliminary bioassay. For example, it inhibited the growth of Yoshida sarcoma in rats (inoculated at 10^7 cells/rat) by over 95% (determined by tumor weight after 7 days) at 5 mg/kg (iv) administration. For L1210 leukemia in BDF_1 mice (inoculated at 4×10^4 cells/mouse), 50 mg/kg (iv) gave a 100% ILS (per cent increase in life-span over control).[15] The most striking activity shown by **3** was its *in vitro* activity against B-HeLa cells (ED_{50} 0.6 μg/ml).[16] 4-Hydroperoxycyclophosphamide (**8**) also exhibited high cytostatic activity in both *in vivo* and *in vitro* experiments with almost equal potency, which indicates that **8** can be readily converted into **3** by biological reduction. Both **3** and **8** were shown to be metabolized into **2** and **4** by isolating these metabolites from the urine of rabbits.[17]

The results presented so far give confirmatory support to the previous proposal that the first step of the activation of cyclophosphamide involves C_4-hydroxylation of its oxazaphosphorine ring.

Acknowledgment. The authors are indebted to Mr. Shoji Sakai and Mr. Itsuo Makino for their technical assistance.

(9) Signal assignment was made on the basis of deuterium exchange.
(10) R. Criegee and G. Wenner, *Justus Liebigs Ann. Chem.*, **564**, 9 (1946).
(11) The role of the added hydroperoxides is to prevent dimerization or decomposition of **8**.
(12) Obtained as a rather unstable oil, but purifiable by column chromatography (silica gel).
(13) The mixture gives typical aldehyde reactions and the following spectral properties: ir_{max}^{film} 1720 cm^{-1}; $nmr_{TMS}^{CDCl_3}$ δ 9.8 (t, *J* = 1.2 Hz), which differed slightly from the reported data for **5** (R. F. Struck and D. L. Hill, *Proc. Amer. Ass. Cancer Res.*, **13**, 50 (1972)).
(14) O. M. Friedman and A. M. Seligman, *J. Amer. Chem. Soc.*, **76**, 655 (1954).
(15) The LD_{50} of **3** was 240 mg/kg in DS mice, which was slightly increased as compared to **1** (LD_{50} 380 mg/kg).
(16) Recently, Hill and coworkers reported on the possible enzymatic conversion of cyclophosphamide to **3** or **5** which showed also the remarkable *in vitro* activity (D. L. Hill, W. R. Laster, Jr., and R. F. Struck, *Cancer Res.*, **32**, 658 (1972)).
(17) The isolated amount of **2** and **4** was approximately the same as produced from **1**.

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Received July 24, 1972

Flash Photolysis of Michler's Ketone in Solution. Rate Constants for Decay and Triplet Excimer Formation[1]

Sir:

The relationship between the electronic configuration of the lowest lying triplet state (n,π*, π,π*, or charge transfer) and the photochemical reactivity of ketones has been a question of great interest in photochemistry.[2–4] In a classic study, Porter and his coworkers[2] proposed, based on absorption and emission spectroscopy and solvent shifts, that for benzophenones with substituents

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(2) G. Porter and P. Suppan, *Trans. Faraday Soc.*, **61**, 1664 (1965); **62**, 3375 (1966); *Pure Appl. Chem.*, **9**, 499 (1964).
(3) P. Suppan, *Ber. Bunsenges. Phys. Chem.*, **72**, 321 (1968).
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