($C_7H_7^+ - CH_5$) and ($C_7H_7^+ - CH_3$), are shifted to higher masses in the spectrum of $C_7H_4D_3^+$ from *p*-$CD_3C_6H_4NO_2$ (Figure 1G), strongly supporting the tolyl structure **3** as the major form of these ions.[19] Although appearance potentials indicate that $C_7H_7^+$ ions from *m*- and *p*-$CH_3C_6H_4Br$ and *o*- and *m*-$CH_3C_6H_4I$ have structure **3** on formation,[5] their CA spectra show that only the ions formed from the iodo compounds have retained this structure for 10^{-5} sec. Similarly, in contrast to previous conclusions,[6] our data indicate that ions of structure **3**, not **1**, are formed from *p*-$CH_3C_6H_4COCH_3$.

(19) However, we find for *p*-$CD_3C_6H_4NO_2$ and *p*-$CH_3C_6D_4NO_2$ that the metastable decompositions used in the previous study[6] involve complete H/D scrambling, indicating that the differences noted by these authors are due to differences in ion internal energy, not to differences in ion structure.[9,16]

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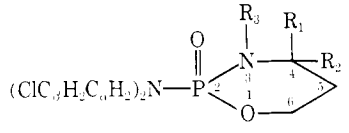
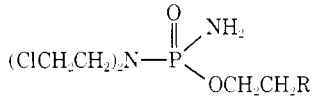
4-Hydroperoxidation in the Fenton Oxidation of the Antitumor Agent Cyclophosphamide

Sir:

It is becoming increasingly clear that monooxidation at C-4 of cyclophosphamide (I) is essentially responsible for the activation of this antitumor agent by the drug-metabolizing enzymes of the liver.[1-3] The occurrence of aldophosphamide (VI) after activation of I by microsomal preparations[1,2] and the production of acrolein under such conditions[4] are understandable on the basis of prior C-4 hydroxylation. 4-Ketocyclophosphamide (V) and a ring-opened carboxylic acid (VII), which have



I, $R_1 = R_2 = R_3 = H$
II, $R_1 = OOH$; $R_2 = R_3 = H$
III, $R_1 = R_2 = H$; $R_3 = OH$
IV, $R_1 = OH$; $R_2 = R_3 = H$
V, $R_1, R_2 = O$; $R_3 = H$



VI, R = CHO
VII, R = COOH

been identified as end products of metabolism, are ineffective as cytostatic agents.[5-10]

(1) D. L. Hill, *Proc. Amer. Ass. Cancer Res.*, **12**, 67 (1971).
(2) D. L. Hill, W. R. Laster, Jr., and R. F. Struck, *Cancer Res.*, **32**, 658 (1972).
(3) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino, and K. Yamaguchi, *J. Amer. Chem. Soc.*, **95**, 985 (1973).
(4) R. A. Alarcon and J. Meienhofer, *Nature (London)*, *New Biol.*, **233**, 250 (1971).
(5) D. L. Hill, M. C. Kirk, and R. F. Struck, *J. Amer. Chem. Soc.*, **92**, 3207 (1970).
(6) H.-J. Hohorst, A. Ziemann, and N. Brock, *Arzneim. Forsch.*, **21**, 1254 (1971).

As mentioned earlier,[11] studies of the Fenton oxidation of I led to the isolation of a crystalline compound, to which the structure N_3-hydroxycyclophosphamide (III) was tentatively assigned. The compound was obtained *via* a precursor that has now also been isolated. We wish to discuss here the identification of 4-hydroperoxycyclophosphamide (II) among the Fenton oxidation products of I and to report on the spontaneous conversion of II to 4-hydroxycyclophosphamide (IV) instead of III. The *in vivo* occurrence of IV in mice treated with I is also reported.

After room temperature exposure for some hours, or on reflux, a CH_2Cl_2 extract of a mixture of Fenton oxidation products of I, obtained by the addition of 1 mol of $FeSO_4$ to 1 mol of H_2O_2 and 0.5 mol of I in an unbuffered solution, was shown by tlc on silica gel in CH_2Cl_2–*n*-BuOH (9:1) to contain a product that appeared at first hand difficult to explain on the basis of C-4 hydroxylation. It was present in a higher amount when a modified Fenton system with a H_2O_2–$FeSO_4$ ratio of 1.5 was used, which would be expected to favor the generation of OOH· radicals (*cf.* ref 12). The compound was shown to oxidize triphenyltetrazolium chloride to the red formazan. Its isolation with the aid of preparative tlc on silica gel in CH_2Cl_2–EtOH (95:5) yielded a crystalline compound: mp 106° dec; yield, 4% relative to the amount of I submitted to oxidation; ir_{max}^{KBr} (cm^{-1}) 3220, 2970, 2905, 1460, 1370, 1325, 1250, 1230, 1210, 1135, 1055, 980, 960, 890, 820, and 730, the broad peak at 3220 cm^{-1} being displaced to 2335 cm^{-1} on deuterium exchange; nmr (TMS, $CDCl_3$) (HA-100 spectrometer) δ 1.70–2.40 (2 H, m), 3.05–3.70 (9 H, m), 3.90–4.80 (2 H, m), 5.23 (1 H, d of q, $J_{P,H}$ = 30 Hz), 6.58 (1 H, br s). On deuterium exchange the signal at δ 6.58 disappeared and the quartets at δ 5.23 degenerated to triplets. Quantitatively, the observed spectra suggested exchange of only one proton; assuming this, an unforced interpretation of the nmr spectral data was possible. Treatment of the compound with H_2O_2 did not give rise to the formation of II (*cf.* ref 3). This was in clear contrast to the behavior (under identical conditions) of an as yet unidentified product of reduction of II by triphenylphosphine that was obtained in a nearly pure state after column chromatography and that probably is one of the two diastereomeres of 4-hydroxycyclophosphamide (IV).

Because of the elemental analytic, ir, and nmr data, and the findings on treatment with H_2O_2, the compound was initially identified as N_3-hydroxycyclophosphamide (III). Exact similarity of the ^{13}C nmr data for the compound with those obtained by Struck, *et al.*,[13]

(7) J. E. Bakke, V. J. Feil, and R. G. Zaylkie, *J. Agr. Food Chem.*, **19**, 788 (1971).
(8) K. Norpoth, E. Golovinsky, and H. M. Rauen, *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 377 (1970).
(9) R. F. Struck, M. C. Kirk, L. B. Mellett, S. El Dareer, and D. L. Hill, *Mol. Pharmacol.*, **7**, 519 (1971).
(10) A. Takamizawa, Y. Tochino, Y. Hamashima, and T. Iwata, *Chem. Pharm. Bull.*, **20**, 1612 (1972).
(11) C. Benckhuysen, J. van der Steen, and J. G. Westra, VIIth International Congress of Chemotherapy, Prague, Aug 23–28, 1971, Abstract No. B-3/9.
(12) R. O. C. Norman and J. R. Lindsay Smith, "Oxidases and Related Redox Systems," T. E. King, H. S. Mason, and M. Morrison, Ed., Vol. I, Wiley, New York, N. Y., 1965, p 131.
(13) R. F. Struck, M. C. Thorpe, W. C. Coburn, Jr., and W. R. Laster, Jr., *J. Amer. Chem. Soc.*, submitted for publication.

for 4-hydroxycyclophosphamide[14] and investigation with the ^{13}C nmr "off-resonance" technique, however, necessitated reconsideration of this conclusion. In the "off-resonance" spectrum the signals at 62.64, 49.08, 41.89, and 28.30 ppm (C_6, $C_{\alpha\alpha}$, $C_{\beta\beta}$, and C_5, respectively; *cf.* ref 13) are found as triplets, whereas the signal at 86.73 ppm appears as a doublet, giving conclusive evidence for the structure of 4-hydroxycyclophosphamide (IV).

The gradual appearance of IV in the extracts indicated the existence of a precursor of the compound. Chromatography on 0.2-mm layers of silica gel, solvent system CH_2Cl_2–*n*-BuOH (9:1), proved useful for the isolation of this precursor, which was in other procedures easily converted to IV: mp of the crystalline compound 102.5–103.5° (uncorrected); ir_{max}^{KBr} (cm^{-1}) 3320, 3110, 2960, 2800, 1465, 1435, 1330, 1245, 1215, 1165, 1090, 1045, 985, 960, 930, 840, and 750; on deuterium exchange the absorptions above 2000 cm^{-1} at 3320, 3110, and 2800 cm^{-1} disappeared, while absorptions at 2470, 2440, 2300, and 2060 cm^{-1} appeared; nmr (TMS, $CDCl_3$) (HA-100 spectrometer) δ 1.80–2.20 (2 H, m, C_5H_2), 3.15–4.40 (9 H, m, C_6H + exocyclic H), 4.45–4.85 (1 H, m, C_6 H), 4.90–5.40 (2 H, m, N–H + C_4 H), 11.61 (1 H, OOH). On deuterium exchange δ 11.61 disappeared and 2 H δ 4.90–5.40 became 1 H, indicating the exchangeability of two protons. Elemental analysis pointed to a structure containing four O atoms. The similarity of the ir spectrum in Nujol with that described by Takamizawa, *et al.*,[3] for synthetically prepared 4-hydroperoxycyclophosphamide (II) provided additional support for the assignment of this structure to the compound.

The *in vivo* occurrence of IV has been observed. One and one-half hours after ip administration to BC_3H_f mice of [^{14}C]cyclophosphamide (600 mg/kg; sp act. 1.0 μCi/mg) dissolved in 6% dextran solution in isotonic saline, the fluid withdrawn from the peritoneal cavity was found to contain a small amount of ^{14}C-labeled IV.

In an aqueous solution of neutral pH, IV is apparently readily converted to VI. This is among others clearly indicated by the recovery of the theoretical amount of acrolein from IV on incubation for 4–5 hr at pH 7.2 and 37°, as determined by gas chromatography. A lag phase of 30 min was noticed for the release of acrolein. Since the same findings hold true for a similarly treated solution of II, this hydroperoxy compound must also spontaneously be converted to VI, from which the elimination of acrolein apparently occurs. The latter is inferred from the finding that the hydrolysis of the alkylating groups was markedly retarded in comparison with the release of acrolein.

In vitro both II and IV are cytostatically active against BHK cells. When the drug-supplemented medium was left on the cells during the full period of growth, an ED_{50} of 2–3 μg/ml (7–10 μM) was found for both compounds. For the cytotoxic acrolein[15] an ED_{50} of 7–13 μM was measured under these conditions. However, when the compounds acted upon the cells during only the first 30 min of a 48-hr period of growth, the ED_{50} value for acrolein varied from 50 to over 120 μM in three separate experiments, while for II and IV ED_{50} values between 20 and 28 μM (6–8 μg/ml) were measured (two experiments).

More elaborate studies of the relative efficacies of the various *in vitro* active metabolites of I are expected to reveal more about the role that is played by their conversion to aldophosphamide and by their spontaneous breakdown, both intra- and extracellularly, to the two cytotoxic substances acrolein and *N*,*N*-bis-(2-chloroethyl)phosphorodiamidic acid.[16]

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(14) The authors are most grateful to Dr. R. F. Struck, *et al.*, Southern Research Institute, Birmingham, Ala., for their willingness to make their paper[13] available before publication for comparing the spectral data.

(15) R. A. Alarcon, *Arch. Biochem. Biophys.*, **106**, 240 (1964).

(16) O. M. Friedman, E. Boger, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963).

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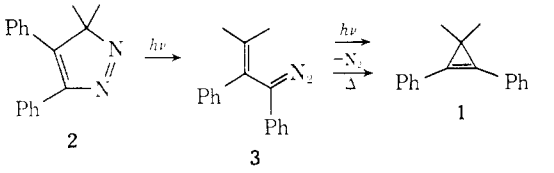
Electronic Excited States of Small Ring Compounds. Cyclopropene and the Vinylcarbene[1]

Sir:

As part of a continuing study of the thermal and photochemical interconversions of three-membered rings, 1,3 dipoles, and 1,3 biradicals,[2] we wish to report preliminary results for a cyclopropene system.

3,3-Dimethyl-1,2-diphenylcyclopropene (**1**)[3] was prepared from 5,5-dimethyl-3,4-diphenyl-5*H*-pyrazole (**2**)[4,5] in 92% yield by photolysis in 9:1 benzene–pyridine[6] above 350 nm;[7a] less than 1% of *cis*- and *trans*-1,2-diphenyl-3-methylbutadiene[8] (**4** and **5**, respectively) was formed. During the photolysis the solution turned deep red suggesting the diazo compound **3** was an intermediate (see eq 1). This was confirmed by generating



(1)

3[9] in high yield by photolyzing **2** between 330 and 410

(1) Contribution No. 86 from the Photochemistry Unit.

(2) D. R. Arnold, A. B. Evnin, and L. A. Karnischky, *Pure Appl. Chem.*, **24**, 523 (1970).

(3) New compounds gave satisfactory spectral and elemental analyses.

(4) Addition of 2-diazopropane to diphenylacetylene gave **2** in 3% yield.

(5) W. H. Williams and W. R. Dolbier, *J. Amer. Chem. Soc.*, **94**, 3955 (1972).

(6) This reaction is sensitive to acid catalysis.

(7) Filter solutions: (a) 1 cm, 4×10^{-3} *M* $BiCl_3$ in 10% HCl; (b) 2 cm, 1.0 *M* $CoSO_4 \cdot 7H_2O$ and 0.1 *M* $NiSO_4 \cdot 6H_2O$ in 5% H_2SO_4, and 1 cm, 0.4 *M* $SnCl_2 \cdot 2H_2O$ in 10% HCl.

(8) The structure of these compounds has been established by independent synthesis.

(9) The instability of **3** precludes isolation but the assigned structure is consistent with the spectral properties: nmr (CCl_4) δ 1.87 (3 H, s, CH_3), 1.81 (3 H, s, CH_3); ir (CCl_4) 2070 cm^{-1} (diazo band); uv (benzene) 510 nm (ϵ *ca.* 50).