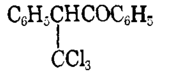
The rearrangements could also be carried out in other solvents, both polar and nonpolar, like methanol, 2-propanol, benzene, or carbon tetrachloride. However in bromotrichloromethane the enol tosylates gave trichloromethyl ketones[6] or their decomposition products, the dichloromethylene ketones. Thus, **1a** and **3** were converted in this solvent to **4**[8] [oil; uv max



$C_6H_5\underset{CCl_3}{CH}COC_6H_5$

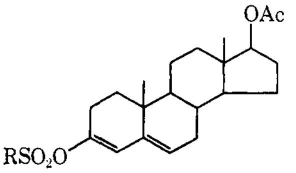
4

$MeCOCH{=}C(Cl)_2$

5

(EtOH) 252 nm (14,000); nmr ($CDCl_3$) δ 5.74 (s, 1, COC*H*)] (30%) and **5**[6b] (24%), respectively, after heating for 3 hr at 100° with benzoyl peroxide.

When an acetonitrile solution of the dienol tosylate **6a**[7] [mp 138–139°; uv max (EtOH) 226 nm (ϵ 24,000); nmr ($CDCl_3$) δ 5.67 (s, 1, H at C-4), 5.38 (m, 1, H at C-6), 0.86 (s, 3, H at C-19)] was heated in an evacuated tube at 100° for 25 hr, it rearranged in 90% yield to the 6β-tosyl derivative **7a** [mp 185–186°; uv max 227 nm (ϵ 27,000); nmr ($CDCl_3$) δ 5.56 (s, 1, H at C-4), 3.84 (d, 1, J = 7.5 Hz, H at C-6), 1.49 (s, 3, H at C-19)].



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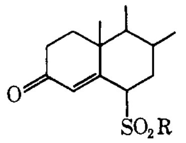


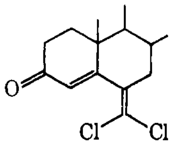
7

a, R = $CH_3C_6H_4$
b, R = CH_3

This 1,5-rearrangement also appears to proceed by a free-radical mechanism, since its rate was enhanced by the addition of benzoyl peroxide (90% yield after 2.5 hr) and inhibited by traces of hydroquinone.

Heating **6a** in ether solution containing ether peroxides also results in **7a**. The same rearrangement also took place on irradiation of **6a** with a filament lamp (70% after 21 hr) or stronger light sources, such as Westinghouse sun lamps (60% after 1 hr) and Philips germicidal lamps (40% after 2.5 hr), the two latter emitting at 300–400 nm and 254 nm, respectively.

In this case too, other solvents did not interfere with the rearrangement, except trichlorobromomethane, which reacted with the dienol tosylate **6a** when heated at 100° yielding *ca.* 85% of the dichloromethylene derivative **8**,[6c,8] and only 10% of the 6β-sulfone **7a**.



8

The dienol mesylate **6b**[9] [mp 138–139°; uv max (C_6H_{12}) 241 nm (ϵ 23,000); nmr ($CDCl_3$) δ 5.97 (s, 1, H at C-4), 5.53 (m, 1, H at C-6), 0.98 (s, 3, H at C-19)] similarly but somewhat less readily gave the 6β-mesyl derivative **7b** [mp 241–242°; uv max (EtOH) 243 nm (ϵ 12,000); nmr ($CDCl_3$) δ 6.00 (s, 1, H at C-4), 3.95 (d, 1, J = 7.5 Hz, H at C-6), 1.39 (s, 3, H at C-19)].

Heating in the presence of benzoyl peroxide at 100° for 5.5 hr gave 90% of **7b**, but in the absence of peroxide or upon irradiation with a filament lamp the starting mesylate was recovered unchanged.

The two main factors influencing the free-radical rearrangement of enol sulfonates are, thus, the relative stabilities of the free radicals obtained by homolysis of the O–S bonds and the stereochemical accessibility of the β-carbon atom of the enol sulfonates to an attack by the sulfonyl radical.

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(6) Analogous free-radical addition reactions of trichlorobromomethane to enol acetates and enol ethers are well known; *cf.* (a) C. Walling, "Free Radicals in Solutions," John Wiley & Sons, Inc., New York, N. Y., 1957, pp 247–272; (b) S. Searles, R. A. Sanchez, R. L. Soulen, and D. G. Kundiger, *J. Org. Chem.*, **32**, 2655 (1967); (c) S. Liisberg, W. O. Godtfredsen, and S. Vangedal, *Tetrahedron*, **9**, 149 (1960).

(7) Prepared in 80% yield from testosterone acetate and tosyl anhydride in dimethylformamide; *cf.* ref 1a.

(8) J. Libman, M. Sprecher, and Y. Mazur, *J. Amer. Chem. Soc.*, **91**, 2062 (1969).

(9) Prepared in 60% yield from testosterone acetate and mesyl anhydride in dimethylformamide.

(10) Taken in part from the Ph.D. Thesis of Norbert Frydman to be submitted to the Feinberg Graduate School of the Weizmann Institute of Science.

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Singlet Oxygen Sources in Ozone Chemistry. Decomposition of Oxygen-Rich Intermediates

Sir:

We have recently shown that decomposition of the triphenyl phosphite–ozone adduct[1] provides a convenient method for accomplishing chemical oxygenations involving singlet oxygen.[2–5] This result suggested that a number of known reactions of ozone with such substrates as sulfides, sulfoxides, amines, and phosphines are also potential sources of singlet oxygen.

We now wish to report that ozonization of even relatively unreactive substrates including hydrocarbons, ethers, and alcohols, where oxygen-rich intermediates, possibly hydrotrioxides, are formed, can also be expected to lead to sources of singlet oxygen. Evidence is presented that this prediction is realized in the cases of isopropyl alcohol and isopropyl ether.

Hydrotrioxides have been postulated as intermediates or unstable products in the ozonization of such diverse substrates as hydrocarbons,[6–8] silanes,[9] ethers,[10,11] alcohols,[7] amines,[12,13] aldehydes,[14] and diazo compounds.[15] Evidence has also been presented confirming

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(1) Q. E. Thompson, *J. Amer. Chem. Soc.*, 83, 846 (1961).

(2) R. W. Murray and M. L. Kaplan, *ibid.*, 90, 537 (1968).

(3) R. W. Murray and M. L. Kaplan, *ibid.*, 90, 4161 (1968).

(4) E. Wasserman, R. W. Murray, M. L. Kaplan, and W. A. Yager, *ibid.*, **90**, 4160 (1968).

(5) R. W. Murray and M. L. Kaplan, *ibid.*, 91, 5358 (1969).

(6) G. A. Hamilton, B. S. Ribner, and T. M. Hellman, "Oxidation of Organic Compounds," Vol. III, Advances in Chemistry Series, No. 77, American Chemical Society, Washington, D.C., p 15.

(7) M. C. Whiting, A. J. N. Bolt, and J. H. Parish, "Oxidation of Organic Compounds," Vol. III, Advances in Chemistry Series, No. 77, American Chemical Society, Washington, D.C., p 4.

(8) J. E. Batterbee and P. S. Bailey, *J. Org. Chem.*, 32, 3899 (1967).

(9) J. D. Austin and L. Spialter, "Oxidation of Organic Compounds," Vol. III, Advances in Chemistry Series, No. 77, American Chemical Society, Washington, D.C., p 26.

(10) R. E. Erickson, R. T. Hansen, and J. Harkins, *J. Amer. Chem. Soc.*, 90, 6777 (1968).

(11) C. C. Price and A. L. Tumolo, *ibid.*, 86, 4691 (1964).

(12) P. S. Bailey, D. A. Mitchard, and A.-I. Y. Khashab, *J. Org. Chem.*, 33, 2675 (1968).

(13) P. S. Bailey and J. E. Keller, *ibid.*, 33, 2680 (1968).

(14) H. M. White and P. S. Bailey, *ibid.*, 30, 3037 (1965).

(15) P. S. Bailey, A. M. Reader, P. Kolsaker, H. M. White, and J. C. Barborak, *ibid.*, **30**, 3042 (1965).

the existence of trioxides.[16,17] In addition, bis(perfluoroalkyl)trioxides have been shown to have surprising thermal stability.[18,19] In many of these cases the oxidized substrate is postulated as arising from decomposition of the hydrotrioxide with a concomitant release of molecular oxygen. We have now shown that in at least two of these cases decomposition of the unstable product produced upon ozonization leads to typical singlet oxygen reactions with suitable acceptors.

The cases chosen for study are those of isopropyl alcohol and isopropyl ether in which Whiting[7] and Erickson,[10] respectively, have reported unusually stable peroxidic products of ozonization which were assigned the hydrotrioxide structure, and which decomposed to give off oxygen in each case.

In a typical experiment 5 ml of isopropyl ether was ozonized neat at −70 to −80° using an ozone stream containing *ca.* 0.20 mmol of O_3/min. The total O_3 absorbed was determined by subtracting the titer of a gas trap containing sodium iodide which was placed in series with the reaction vessel. The ozonized solution was allowed to stand for 15 min after ozone treatment was completed and then was purged for 15 min with a nitrogen stream. The cold solution was then treated with various singlet oxygen acceptors or other reagents. The solution was then permitted to warm first to equilibrium with a carbon tetrachloride–CO_2 bath (−25°), then in an ice bath, and finally to room temperature.

Qualitative experiments with rubrene and tetraphenylcyclopentadienone (tetracylone) showed that 0.2 mmol (in CH_2Cl_2) of each of these highly colored reagents was completely bleached by samples of isopropyl ether which had absorbed *ca* 2.7 mmol of ozone and then warmed to −10 to +5°. This temperature range corresponds to the reported temperature (−10°) of maximum gas evolution from ozonized isopropyl ether. Rubrene *endo*-peroxide[20] was isolated in 47% yield[22] by crystallization of the crude bleaching product from ether–pentane solutions. The expected product from the reaction of singlet oxygen with tetracyclone, *cis*-dibenzoylstilbene, was also isolated in 38% yield[23] by column chromatography on neutral alumina, mp 219–220° (lit.[24] mp 216–217°).

Quantitative assays of bleaching of tetracyclone were made spectrophotometrically by measuring decreases in the absorbance maximum at 510 mμ.[25] Bleaching of excess tetracyclone was carried out at *ca.* 10^{-1}–10^{-2} *M* tetracyclone.

Using this technique it was possible to show that in the case of isopropyl ether 21.4% of the absorbed ozone was available to bleach tetracyclone, presumably as singlet oxygen. In the case of isopropyl alcohol, 3.13% of the absorbed ozone was available for bleaching. Since tetracyclone is not a particularly reactive singlet oxygen acceptor, these figures should be regarded as lower limits only. At any rate, the tetracyclone bleaching technique can be useful in determining whether substrates will be useful as singlet oxygen precursors at least on a relative basis.

The procedure described for isopropyl ether was also used to give the expected singlet oxygen product, 2,3-dimethyl-3-hydroperoxy-1-butene, in 61% yield (crude product) from 2,3-dimethyl-2-butene. When 2-methyl-2-butene was used as the substrate the hydroperoxides obtained could be reduced to 3-methyl-1-buten-3-ol and 2-methyl-1-buten-3-ol in a ratio of 57:43 which compares favorably with the value (52:48) previously cited[26] as being characteristic of singlet oxygen reactivity. We have also recently reported[27] that singlet oxygen from the triphenyl phosphite–ozone adduct gives these two alcohols in the ratios of 51:49 and 56:44 in methylene chloride and methanol solvent, respectively.

Control experiments demonstrated that no visible bleaching of tetracyclone occurs at −60 to −80° during 24 hr storage with the ozonized isopropyl ether, but that the tetracyclone was completely bleached when the solution was warmed to 0°. A fivefold excess of *t*-butyl hydroperoxide did not decrease the concentration (*ca.* 10^{-2} *M*) of tetracyclone in CH_2Cl_2 during 15 min at room temperature as measured spectrophotometrically. Solutions of tetracyclone were also stable to air and excess 30% hydrogen peroxide. These results indicate that the oxygen-rich intermediates present do not bleach tetracyclone below the temperature at which they decompose and that possible by-product hydroperoxides or hydrogen peroxide are not efficient bleaching agents.

Reasonable possibilities for the structure of the oxygen-rich species produced upon ozonization of isopropyl ether include the hydrotrioxide suggested by Erickson[10] or an ether–ozone complex. We are inclined to the hydrotrioxide structure on the basis of the observations given above and those which follow. The oxygen-rich intermediate fails to give the normal ozonolysis reaction with tetramethylethylene or diisopropylethylene. Such a reaction might have been expected for an ether–ozone complex. Erickson's work indicates that the intermediate ultimately gives acetone, isopropyl alcohol, and isopropyl acetate.[10] At least two of these products, the alcohol and acetone, along with singlet oxygen, are very conveniently rationalized in terms of the concerted decomposition of the hydrotrioxide *via* a six-membered ring transition state. This transition state would be analogous to that proposed by Russell[28] for the self-reaction of secondary peroxy

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[16] P. D. Bartlett and P. Gunther, *J. Amer. Chem. Soc.*, 88, 3288 (1966).

[17] P. D. Bartlett and G. Guaraldi, *ibid.*, 89, 4799 (1967).

[18] L. R. Anderson and W. B. Fox, *ibid.*, 89, 4313 (1967).

[19] P. G. Thompson, *ibid.*, 89, 4316 (1967).

[20] The *endo*-peroxide thus obtained was identical with an authentic sample prepared by self-sensitized photooxidation in CH_2Cl_2. In our hands no sharp melting point was observed for any sample of the *endo*-peroxide. Gradual color change from a yellow powder to a red solid was noted from 160 to 185° (lit.[21] mp 197°).

[21] C. Moureau, C. Dufraisse and L. Girard, *C. R. Acad. Sci.*, 186, 1166 (1928).

[22] In all cases yields are based on acceptor concentration.

[23] In the tetracyclone example, a trace of sodium methoxide was added prior to warm-up. While the exact function of the added base is still being investigated, it seems possible that it might lead to a smoother decomposition of the hydrotrioxide *via* its anion.

[24] D. R. Berger and R. K. Summerbell, *J. Org. Chem.*, **24**, 1881 (1959).

[25] Absorbance measurements were made in CH_2Cl_2 solution on a Beckman DU-2 uv-visible spectrophotometer at a slit width of 0.020 mm with a reproducibility of ±3%. A Beer's law plot of 510-mμ absorbance *vs.* concentration was perfectly linear in the concentration range 10^{-3}–10^{-4} *M*.

[26] C. S. Foote, S. Wexler, and W. Ando, *Tetrahedron Lett.*, 4111 (1965).

[27] R. W. Murray, J. W.-P Lin, and M. L. Kaplan, *Ann. N. Y. Acad. Sci.*, in press. Presented at the International Conference on Singlet Molecular Oxygen and its Role in Environmental Sciences, N. Y., N. Y., Oct 1969.

[28] G. A. Russell, *J. Amer. Chem. Soc.*, 79, 3871 (1957).

radicals. Such a reaction has recently been shown to give singlet oxygen by Howard and Ingold.[29]

The low-temperature nmr spectrum of ozonized isopropyl ether contains small peaks which can be assigned to isopropyl acetate and acetone as well as two new peaks at 8.53 and −2.98[30] with an area ratio of approximately 6:1. These latter absorptions decay and disappear at *ca.* −10° which is the same temperature at which gas evolution is observed. The absorption at 8.57 can be assigned to the methyl groups attached to the carbon bearing the hydrotrioxy group in the hydrotrioxide. The compounds 2-methoxyisopropyl-*t*-butyl peroxide and 2,2-bis(*t*-butylperoxy)propane have absorptions at 8.64 and 8.59, respectively, for the comparable methyl groups.[31] The absorption at −2.98 would then tentatively be assigned to the proton of the hydrotrioxy group. Taken together these observations would seem to favor the hydrotrioxide structure.

These results also suggest that our earlier proposal[3] that singlet oxygen may be produced in the atmosphere from a variety of reactions of ozone with organic substrates should now be extended to include substrates of even relatively low reactivity such as saturated hydrocarbons. The increasing concentrations of ozone in polluted atmospheres as well as the suggestion[32] that atmospheric singlet oxygen may be involved in the important NO to NO_2 conversion in such atmospheres makes these results of particular importance to the air pollution problem.

Acknowledgment. We thank the National Air Pollution Control Administration, Consumer Protection and Environmental Health Service, Public Health Service, for support of this work through Grant No. AP 00925-01.

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[29] J. A. Howard and K. U. Ingold, *J. Amer. Chem. Soc.*, **90**, 1056 (1968).

[30] The nmr spectra were taken on a Varian Associates HA-100 nmr spectrometer beginning at −41°. The chemical-shift values are τ values relative to internal TMS.

[31] R. D. Youssefyeh and R. W. Murray, *Chem. Ind.*, 1531 (1966).

[32] A. U. Khan, J. N. Pitts, Jr., and E. P. Smith, *Environ. Sci. Technol.*, 1, 656 (1967).

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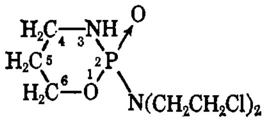
Isolation and Identification of 4-Ketocyclophosphamide, a Possible Active Form of the Antitumor Agent Cyclophosphamide

Sir:

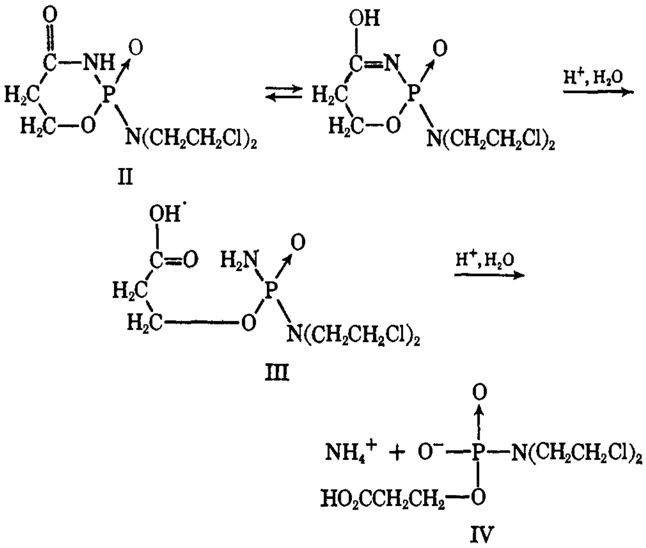
The mechanism of activation of cyclophosphamide (I), an agent widely used in the treatment of many types of cancer,[1] has been a matter of significant interest for several years.[2] We wish to report our results on the isolation and identification of a cyclophosphamide metabolite from dog urine in which the tetrahydro-2*H*-1,3,2-oxazaphosphorine ring has not been opened.



I

Urine from a dog injected intravenously with 20 mg/kg of side chain labeled ^{14}C-cyclophosphamide (0.02 μCi/mg) was collected for 6 hr. The sample contained 14% of the total dose. Several volumes of ethanol were added and the insoluble materials removed by filtration. Ethanol was removed at 30° under reduced pressure, and the remaining solution was passed through a DEAE-Sephadex A-25 column, previously equilibrated with 0.02 *M* NH_4HCO_3. The column was washed with this buffer, and a linear gradient of 0.02–0.2 *M* NH_4HCO_3 was applied. Three major radioactive materials were obtained. The first was cyclophosphamide, which was eluted in washing the column. The second was further purified by paper chromatography and Sephadex G-10 column chromatography.

This metabolite upon mass spectral analysis gave a molecular ion at *m/e* 274, and infrared analysis showed a band at 1695 cm⁻¹, which is indicative of an amide carbonyl group. The metabolite (*ca.* 100 μg) was crystallized from ethanol, and several crystals were isolated and dried. The crystals, which melted sharply at 148–149°, were analyzed again by mass spectral and infrared methods. Mass spectral analysis gave a molecular ion at *m/e* 274 and a base peak at *m/e* 225, corresponding to loss of $-CH_2Cl$. A strong band appeared at 1695 cm⁻¹ in the infrared spectrum of the crystalline metabolite along with the two strongest bands which appeared at 1630 and 1615 cm⁻¹ and suggested the presence of −C=C− and/or −C=N− group(s). These differences in the infrared spectra of the total metabolite fraction and the crystalline metabolite suggested possible keto–enol tautomerism. The crystalline sample was dissolved in distilled water (pH *ca.* 6), allowed to stand at room temperature for 1 hr, frozen for 3 days, and lyophilized. Two strong bands appeared at 3130 (broad) and 1395 cm⁻¹ (sharp) in the infrared spectrum of the residue and clearly indicated the presence of NH_4^+. Thus, the compound is subject to hydrolytic decomposition in weak acid solution.



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[1] See, for example: R. Nissen-Meyer and H. Höst, *Cancer Chemother. Rep.*, **9**, 51 (1960); M. P. Sullivan, *ibid.*, **51**, 393 (1967); M. E. Haggard, *ibid.*, **51**, 403 (1967); W. W. Sutow, *ibid.*, **51**, 407 (1967).

[2] See, for example: N. Brock and H.-J. Hohorst, *Arzneim. Forsch.*, **13**, 1021 (1963); O. M. Friedman, *Cancer Chemother. Rep.*, **51**, 327 (1967); J. L. Cohen and J. Y. Jao, *Proc. Amer. Ass. Cancer Res.*, **10**, 14 (1969); H. M. Rauen and K. Norpoth, *Arzneim. Forsch.*, **17**, 599 (1967); H. M. Rauen and K. Norpoth, *Naturwissenschaften*, **52**, 477 (1965).