Tetrahedron Letters,Vol.24,No.28,pp 2819-2820,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

[E]-AZOXYBIS(METHYLENE) BIS(3-CHLOROBENZOATE): POTENT MUTAGEN FROM REACTION OF HEXAMETHYLPHOSPHORAMIDE, N-METHYLHYDROXYLAMINE AND [E]-NITROSOMETHANE DIMER WITH 3-CHLOROPEROXYBENZOIC ACID

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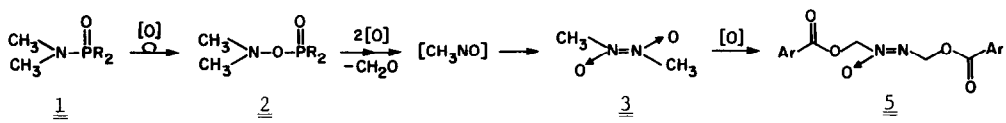
Summary. [E]-Azoxybis(methylene) bis(3-chlorobenzoate), an exceptionally potent mutagen (3050 revertants/nmol) in the Salmonella typhimurium (strain TA100) assay, is formed in trace (< 1%) quantity via [E]-nitrosomethane dimer on treatment of hexamethylphosphoramide or N-methylhydroxylamine with 3-chloroperoxybenzoic acid.

We recently reported[1] that hexamethylphosphoramide (HMPA) (1, R = NMe_2) and some analogs upon treatment with 3-chloroperoxybenzoic acid (CPBA) (3-5 equivalents) in acetone at 25°C undergo N-oxidation and rearrangement to the corresponding P-dimethylaminooxyphosphonous derivatives (2). Further oxidation of 2 yields formaldehyde and [E]-nitrosomethane dimer (3) as the major nonphosphorus-containing products. Similar oxidation of N-methylhydroxylamine (4) also yields 3. The reaction mixtures of 1-4 and some related compounds with CPBA in acetone[2] possess high mutagenic activity in the Salmonella typhimurium mutagenesis assay[3] which could not be attributed to any compounds on the major reaction pathway.[1] The nature of the mutagen in these peracid reactions was of interest since HMPA is a carcinogen and oxidatively-activated promutagen.[4] We have now identified the mutagen (5) in these CPBA reaction mixtures (Scheme).



1 2 3 5

Scheme. Oxidation of dimethylphosphoramides (1) via P-dimethylaminooxyphosphonous derivatives (2) and [E]-nitrosomethane dimer (3) to [E]-azoxybis(methylene) bis(3-chlorobenzoate) (5). [O] = 3-chloroperoxybenzoic acid. Ar = 3-chlorophenyl.

Careful chromatography[5] with fraction monitoring by mutagenesis assays[3,6] revealed a single mutagen and led to near quantitative isolation of diester 5[7] from reaction mixtures of each of 1, 3 and 4 with CPBA in acetone as colorless plates (benzene/pentane), m.p. 124-126°C. Tentative identification of 5 was made by normal spectroscopic methods.[8] In view of its exceptional biological activity, the structure of 5 was confirmed by X-ray crystallography.[9]

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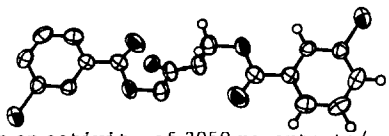
X-Ray Structure of 5



Pure 5 is a direct-acting mutagen with an activity of 3050 revertants/nmol, a potency rarely encountered and generally associated with highly active carcinogens.[10] It is deactivated by the rat liver S9 fraction and on hydrolysis by butyrylcholinesterase, indicating that an intact ester linkage is required for activity. Clearly 5 is not the mutagen formed on HMPA bioactivation since it requires abiotic substituents for its formation. Speculation on the mutagenic mode of action of 5 might include hydrolysis/fragmentation to an alkylating species, cf cycasin analogs.[11] Clarification of these points requires synthetic routes to 1,4-disubstituted azoxymethanes.

Acknowledgments. Dr. F. J. Hollander, CHEXRAY facility, Department of Chemistry, University of California, Berkeley, performed the crystal structure analysis. This study was supported by National Institutes of Health Grant No. PO1 ES00049.

References and Notes

1. I. Holden, Y. Segall, E. C. Kimmel, and J. E. Casida, Tetrahedron Lett., 23, 5107 (1982).
2. Other compounds showing high mutagenic activity on CPBA oxidation for 2-48 hr in acetone at 25°C were: $(Me_2N)_2P(O)OP(O)(NMe_2)_2$; $(Me_2N)_2P(O)Cl$; $Me_2NP(O)(OEt)_2$; $Me_2NP(O)(OPh)_2$; $Me_2NOP(O)(NMe_2)_2$; $Me_2NOP(O)(OEt)_2$; $MeNHP(O)(OEt)_2$; $Me_2NC(O)Cl$; $Me_2NSO_2NMe_2$. Potent mutagens are formed with CPBA from the nonphosphorus-compounds 3 and 4 in acetone or methanol but from HMPA and $Me_2NP(O)(OEt)_2$ only in acetone. No mutagenic activity resulted on treatment of 3 with 3-chlorobenzoic acid or of MeN=N(O)Me or $MeCO_2CH_2N$=N(O)Me with CPBA in acetone.
3. B. N. Ames, J. McCann, and E. Yamasaki, Mutation Res. 31, 347 (1975).
4. J. A. Zapp, Jr., Am. Ind. Hyg. Ass. J. 36, 916 (1975); J. Ashby, J. A. Styles, and D. Paton, Br. J. Cancer 38, 418 (1978).
5. Florisil column with hexane-dichloromethane then μPorasil HPLC with chloroform-acetonitrile.
6. Reaction mixtures were quenched with dimethyl sulfoxide to destroy excess CPBA (bactericidal) prior to incubation with S. typhimurium (strain TA100).
7. Isolated yields of crystalline 5 were 0.3% from 1, 0.7% from 3, and 0.1% from 4.
8. $[M^+]$ 382.0123; calculated for $C_{16}H_{12}N_2O_5Cl_2$, 383.0122: ^{1}H NMR (250 MHz, $CDCl_3$), δ7.3-8.2 (8H, m); 6.01 (2H, t, J 1.4 Hz); 5.77 (2H, t, J 1.4 Hz): ν_{max} 1750 cm^{-1}.
9. The correct space group for this molecule is $P2_1$. Refinement within this space group, however, led to poor residuals, bad convergence, and inconsistent bond distances. Refinement in space group $P2_1/c$, with the unique oxygen atom disordered about the inversion center, led to a clean and rapid convergence (R 5.6% for 919 observed data, $(F_2 > 3\sigma(F^2))$). This latter space group, though technically wrong, gives a more consistent representation of the structure than does refinement in the (correct) space group $P2_1$.
10. J. McCann, E. Choi, E. Yamasaki, and B. N. Ames, Proc. Nat. Acad. Sci. USA 72, 5135 (1975). Examples for the TA100 strain are (revertants/nmol): N-methyl-N'-nitro-N-nitrosoguanidine (1375); 4-nitroquinoline-1-oxide (2906); aflatoxin B_1 (7057) (+S9).
11. M. H. Benn, P. Kazmaier, J. Chem. Soc. Chem. Comm., 887 (1972).

(Received in USA 14 April 1983)