Tetrahedron Letters,Vol.23,No.49,pp 5107-5110,1982 0040-4039/82/495107-04$03.00/0
Printed in Great Britain

PERACID-MEDIATED N-OXIDATION AND REARRANGEMENT OF DIMETHYLPHOSPHORAMIDES PLAUSIBLE MODEL FOR OXIDATIVE BIOACTIVATION OF THE CARCINOGEN HEXAMETHYLPHOSPHORAMIDE (HMPA)

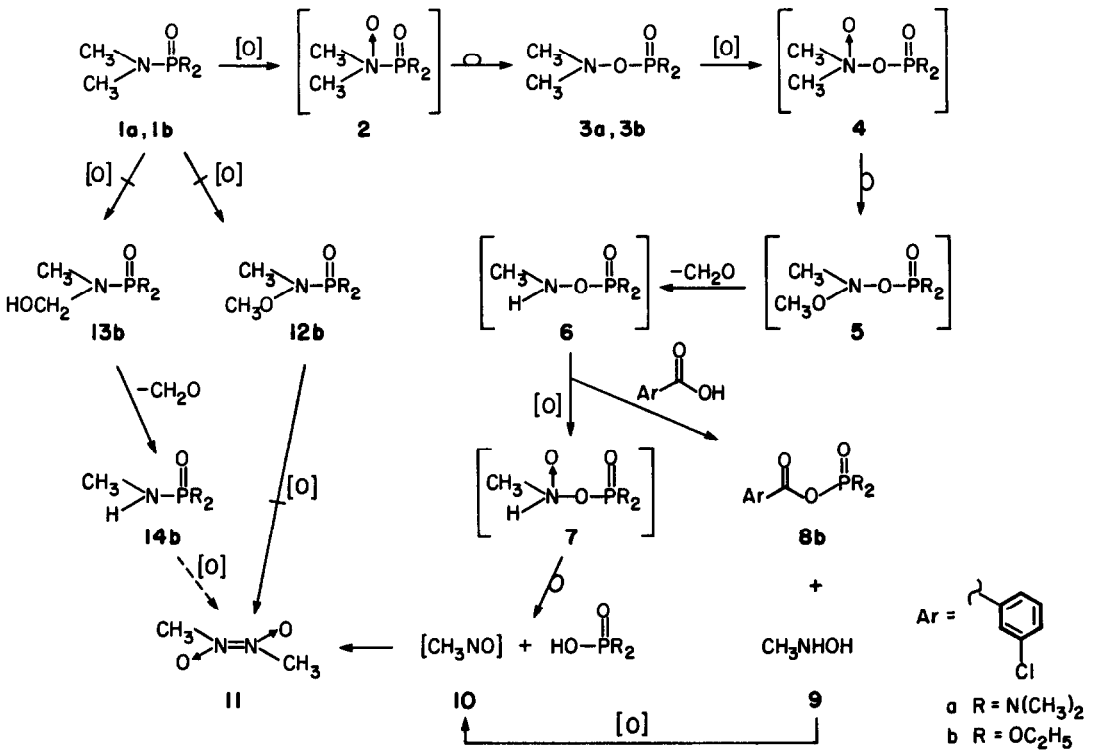
Ian Holden*, Yoffi Segall[a], Ella C Kimmel and John E Casida
Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences
University of California, Berkeley, California 94720

Summary Dimethylphosphoramides react with m-chloroperoxybenzoic acid (MCPBA) in anhydrous acetone to yield the previously unknown P-dimethylamino-oxyphosphonous derivatives via N-oxidation and rearrangement Further MCPBA oxidation yields formaldehyde and nitrosomethane, isolated as its trans-dimer These reactions provide a possible biomimetic model for the metabolic activation of hexamethylphosphoramide as a mutagen and carcinogen

Hexamethylphosphoramide (HMPA) (1a), an extensively-used solvent in organic synthesis, is a powerful rodent carcinogen [1] It is detected as a mutagen in a cell transformation assay but only when activated by a liver enzyme preparation [2] Metabolism of HMPA in rats and liver preparations involves N-demethylation with liberation of formaldehyde, possibly via the N-hydroxymethyl, N-methyl derivative from rearrangement of an N-oxide [3,4] Analogous reactions occur on metabolic conversion of the insecticide octamethyldiphosphoramide (schradan) $[(Me_2N)_2P(O)OP(O)(NMe_2)_2]$ to a potent cholinesterase inhibitor [3,4] These metabolic reactions and activation of the insecticide are reproduced in part on oxidation with permanganate or a peracid [3,4] The existence of dimethylphosphoramide N-oxides is not established despite their potential importance as bioactivated metabolites or intermediates in the biological activity of dimethylphosphoramides We therefore examined the reactions of HMPA and related compounds with m-chloroperoxybenzoic acid (MCPBA) as a possible biomimetic model (Scheme)

The reaction of HMPA with MCPBA (> 99%) in anhydrous d_6-acetone at 25°C was monitored directly by ^1H and ^{13}C NMR (internal tetramethylsilane) and ^{31}P NMR [referenced from external $(MeO)_3P(O)$ in $CDCl_3$] with parallel studies on 1b [5] (which has the advantage of a single dimethylamino group) and on their possible oxidation products. Treatment of 1b for 24 hr with up to two equivalents of MCPBA gave strong ^{31}P NMR (δ +3.48) and ^1H NMR signals (δ 2 81, singlet) These chemical shifts and particularly the lack of ^{31}P-^1H coupling in the ^1H NMR suggested the formation of dimethylaminooxyphosphonous derivative 3b, instead of dimethylphosphoramide N-oxide 2b, a speculation confirmed by synthesis and spectral comparison.[6] The analogous product 3a from HMPA was similarly identified [6] Thus, the dimethylphosphoramide N-oxide (2) is strongly implicated as an intermediate undergoing a rearrangement reaction to the dimethylaminooxyphosphonous derivative (3) [7]

[a]Current address Israel Institute for Biological Research, Ness-Ziona, P O B 19, 70450, Israel



Scheme Oxidation of hexamethylphosphoramide (1a) and O,O-diethyl N,N-dimethylphosphoramide (1b) to the corresponding P-dimethylaminooxyphosphonous derivatives (3a, 3b) and nitrosomethane dimer (11)

One dimethylamino group of HMPA and this moiety of 1b form two terminal products in near quantitative yield (^{1}H NMR) within 24 hr on treatment of 1a and 1b with a five-fold excess of MCPBA. Disappearance of the N,N-dimethyl doublet (^{1}H δ ~2 7) is accompanied by appearance of singlets corresponding to formaldehyde (^{1}H δ 8.15) and trans-nitrosomethane dimer (11) (^{1}H δ 3.85). Formaldehyde (steam distillation) was further characterized as its methylene bis-dimedone derivative (m p 189-190°C) The identity of dimer 11 was confirmed by ^{13}C NMR (δ 47.2) and chemical ionization (methane)-mass spectrometry (CI-MS) (M+1$^+$ 91) on the crude reaction mixture and by preparative TLC isolation and comparison with synthetic cis- and trans-dimers [8] Nitromethane (^{1}H δ 4 35) is not present in the reaction mixture and would be detected if formed since it is resistant to MCPBA oxidation Dimer 11 and formaldehyde were the principal terminal products on MCPBA oxidation not only of 1a and 1b but also of schradan and tetra-

methylphosphorodiamidic chloride [$(Me_2N)_2P(O)Cl$] This dimer was not formed from O,O-diphenyl N,N-dimethylphosphoramide Phosphorus-containing products from 1b evident by CI-MS were 8b and O,O,O,O-tetraethyl pyrophosphate

Potential intermediates in the conversion of 1a and 1b to 11 and formaldehyde were examined by subjecting them to MCPBA oxidation under the same conditions Dimethylaminooxyphosphonous derivatives 3a and 3b quantitatively yielded 11 within 2 hr Other potential organophosphorus intermediates[9] (12b, 13b and 14b) were treated with excess MCPBA but gave no dimer (11) N-Methylhydroxylamine (9) (free base in acetone) reacted instantaneously with MCPBA, giving an intense, transient blue flash due to nitrosomethane (10) and quantitative formation of dimer 11 These findings establish two portions of the pathway in the Scheme, i e 1 → 2 → 3 and 9 → 10 → 11 Formaldehyde liberation might involve formation and degradation of the N-hydroxymethyl, N-methylphosphoramide (13) but this would generate the corresponding monomethylphosphoramide (14) which was not detected, 14b reacts slowly with MCPBA forming small amounts of 11 but only after several days indicating it is not an intermediate in this oxidation The proposed rearrangement reaction for conversion of 4 to 5 accommodates the subsequent formaldehyde liberation on forming 6 and provides for an oxidative pathway in addition to that from initial methyl hydroxylation The reaction sequence for conversion of 3 to 11 is uncertain but clearly requires two further oxidation steps Alternative pathways are shown in the Scheme, one via N-methylhydroxylamine (10) and the other via N-oxide 7, in each case with concommitant formation of the appropriate phosphoric acid or anhydride (8b or tetraethyl pyrophosphate)

HMPA readily undergoes biological N-demethylation to 14a and formaldehyde [4] Unidentified metabolites may include some of the compounds shown in the Scheme The carcinogenicity of formaldehyde[10] may contribute to that of HMPA[11] but seems insufficient to account for the high potency of this phosphoramide N-Methylhydroxylamine is a mutagen[12] and might react directly[13] or require further oxidation, e g to nitrosomethane (10) Thus, 9 gives 11 with MCPBA (this study) or cis-11 with periodate[14] via 10 Although not an alkylating agent itself, nitrosomethane might be expected to react with nucleophiles at nitrogen Nitrosomethane tautomerizes to formaldehyde oxime,[15] which might also contribute to the overall biological activity

We have used the Ames mutagenesis assay[16] with Salmonella typhimurium strain TA-100 to evaluate the mutagenic activity of all compounds (except 8) indicated without brackets in the Scheme and also formaldehyde oxime, formaldoxime HCl and nitromethane None of these compounds was detected as a mutagen (< 0 05 revertants/μg) even on addition of the microsomal activation system The Ames assay may not be an appropriate indicator of carcinogenicity in the present series[2] or the ultimate mutagen and carcinogen may be a compound other than those tested, e g. proposed intermediate 5 or 10 In addition, the reaction mixture of HMPA and MCPBA is highly mutagenic due to a single trace component (~ 7000 revertants/μg) as we will detail elsewhere Care should therefore be taken in using HMPA with peracids and possibly other oxidants

Acknowledgment Supported in part by National Institutes of Health Grant PO1 ES00049. Helpful comments were provided by L O Ruzo and W M Draper of this laboratory

References and Notes

1 Nasal turbinate tumors are induced in rats exposed to a vapor level of 50 ppb HMPA for one year J A Zapp, Jr , Am Ind Hyg Ass J 36, 916 (1975), K P Lee, H J Trochimowicz, and C F Reinhardt, The Toxicologist 1, 128 (1981)

2 J Ashby, J A. Styles, and D Anderson, Br. J Cancer 36, 564 (1977), J Ashby, J. A Styles, and D Paton, Br J Cancer 38, 418 (1978)

3 B W Arthur and J E Casida, J Econ Entomol 51, 49 (1958)

4 A R Jones and H Jackson, Biochem Pharmacol 17, 2247 (1968)

5 Compound 1b [$(EtO)_2P(O)Cl$/excess gaseous dimethylamine/Et_2O at 0°C], b.p 38°C/0 2 mm Hg, ^1H δ 4 04 (4H, dq, J 7 Hz each), 2 70 (6H, d, J 10 Hz), 1 34 (6H, t, J 7 Hz), ^{31}P δ +8 22

6 Compounds 3a and 3b were synthesized by reacting the relevant chloridate in tetrahydrofuran for 5 hr at 25°C with N,N-dimethylhydroxylamine (free base from refluxing HCl salt with equiv NaH in tetrahydrofuran) in the presence of Et_3N 3a, b p 85-87°C/0 3 mm Hg, ^1H δ 2 75 (6H, s), 2 68 (12H, d, J 10 Hz) 3b, b p 68°C/0 05 mm Hg, ^1H δ 4 19 (4H, dq, J 7 Hz each), 2 81 (6H, s), 1 36 (6H, dt, J 1 Hz, 7 Hz), ^{31}P δ +3 48.

7 Analogous rearrangements are proposed for phosphorothiolate S-oxides to phosphinyloxysulfenates, i e =P(O)-S(O)-alkyl → =P(O)-O-S-alkyl [Y Segall and J E Casida, Tetrahedron Lett 23, 139 (1982)] On a similar basis, oxidation of $Me_2N-SO_2-NMe_2$, m p 69-70°C (^1H δ 2 83), with 3 equiv MCPBA gives a single product (δ 2 83, 3 10, 3H each) tentatively suggested to be $Me_2N-O-SO_2-NMe_2$

8 cis-Nitrosomethane dimer (^1H δ $CDCl_3$ 4 2) was obtained by vapor phase photolysis of t-butyl nitrite (254 nm/quartz) and trans-dimer (^1H δ $CDCl_3$ 3 9) by thermolysis of cis-dimer [(C S Coe and T F Doumani, J Am Chem Soc 70, 1516 (1948)] or peracid oxidation of N-(benzylidene)methylamine [K G Taylor, M -S Chi, and M S Clark, Jr , J Org Chem 41, 1131 (1976)]

9 Compound 12b (made as for 3a and 3b with N,O-dimethylhydroxylamine), b p 63-64°C/0 3 mm Hg, ^1H δ 4 20 (4H, dq, J 7 Hz each), 3 50 (3H, s), 2 92 (3H, d, J 12 Hz), 1 28 (6H, t, J 7 Hz) 13b (from 14b/1 equiv 40% aqueous formaldehyde/trace Na_2CO_3 for 18 hr at 25°C), decomposition on attempted distillation, ^1H δ 4 62 (2H, d, J 15 Hz), 4 06 (4H, dq, J 7 Hz each), 2 75 (3H, d, J 9 Hz), 1 30 (6H, t, J 7 Hz) 14b (made as for 1b with excess gaseous methylamine), b p 84°C/0 2 mm Hg, ^1H δ 4 10 (4H, dq, J 7 Hz each), 2 70 (6H, d, J 10 Hz), 1 30 (6H, t, J 7 Hz)

10 F Perera and C Petito, Science 216, 1285 (1982)

11. A R Dahl, W M Hadley, F F Hahn, J M Benson, and R O McClellan, Science 216, 57 (1982)

12 P Marfey and E Robinson, Mutation Res 86, 155 (1981)

13 C Janion and D Shugar, In Molecular Mechanisms of Genetic Processes, N B Dubinin and D M Gol'dfarb, Ed , pp 106, Halsted Press, Wiley, New York, 1975

14 T Emery and J B Neilands, J Am Chem Soc 82, 4903 (1960)

15 K A Jensen and A Holm, Mat -Fys Medd -K Dan Vidensk Selsk 40, 1 (1978), Chem Abstr 89, 110480b (1978)

16. B N Ames, J McCann, and E Yamasaki, Mutation Res 31, 347 (1975)

(Received in USA 30 August 1982)