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CHEMICAL ACTIVATION OF NITROSAMINES INTO MUTAGENIC AGENTS

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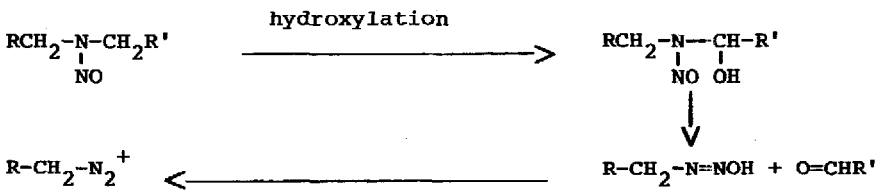
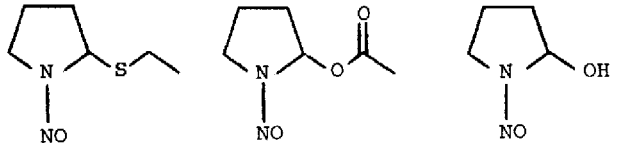
The role of nitrosamines as environmental carcinogens has been extensively investigated.[1] In this group of compounds nitrosopyrrolidine (NP) is of particular interest because of its proven occurrence in, for example, fried bacon[2] and tobacco smoke[3] and its structural similarity to the ubiquitous amino acid proline.[4] NP has been shown to produce liver and testicular tumors when administered to rats.[5] It has been suggested that nitrosamines are activated by enzymatic α-hydroxylation to an α-hydroxy species which, on cleavage, provides a diazohydroxide having the ability to alkylate nucleic acids, possibly by way of a diazonium ion.[1,6] Intrinsic to this scheme and so far unrecognized is the



possibility that the α-hydroxynitrosamine behaves as a transportable form of a potential alkylating agent. Support for the above mechanism is found in the isolation of specifically alkylated nucleic acid bases from rats treated with

nitrosamines,[7] and also the recent demonstration that rat liver metabolises nitrosopyrrolidine to an, as yet unknown, mutagen, active on Salmonella typhimurium.[8] In this report we describe a synthesis of α-acyloxynitrosamines[9] and the demonstration that α-acetoxynitrosopyrrolidine (α-NP)[11] is a powerful mutagen, in its own right, requiring no liver microsomal activation. This finding complements a recent and independent observation that α-acetoxydimethylnitrosamine is mutagenic.[13] Thus nitrosopyrrolidine was metallated[14] (-78°, lithium diisopropylamide, THF) and then treated with ethyl methoxycarbonyl disulfide[15] (-78°) to provide the α-thioethyl derivative (1)[16], purified by column chromatography (silica gel; E.M. Reagents, 70-230 mesh) in benzene-ether gradient (isolated yield 20-40%), nmr ($CDCl_3$): δ 1.32 (t, 3H, J 7 Hz), 1.85-3.1 (m, 6H), 3.45-3.9 (m, 2H), 5.7-5.9 (m, 1H). This substance (1) was chlorinated (Cl_2 (1 equiv.), CH_2Cl_2, -78°) and treated at this temperature with triethylammonium acetate (1 equiv.) to yield, after chromatography (silica gel) α-NP (2)[17] (yield 24%), nmr ($CDCl_3$): δ 1.8-2.4 (m, 4H), 2.09 (s, 3H), 3.2-3.8 (m, 2H), 7.0-7.15 (m, 1H); IR (film): 1740 cm^{-1}.



1 2 3

The mutagenicity of compound (2) was determined by employing S. typhimurium strains TA 1535 and TA 1975 obtained from Bruce N. Ames. Both strains carry a missense mutation in the histidine operon. TA 1535 also has a deletion through the gal-bio-uvr B region and both TA 1535 and TA 1975 carry a deep rough (rfa) mutation.[18] Qualitative mutagenic activity with and without microsomal activation was performed by the method described by Ames.[19] The substances NP, α-NP, and N-methyl-N'-nitro-nitrosoguanidine (NG) were tested in this system. Ethanol (20 µl) was used as a negative control and NG as a positive control. The plates were incubated at 37°C for 48 hours and then examined.

As can be seen from the Table α-NP is mutagenic against TA 1535 and TA 1975 without microsomal activation. This is in contrast to the results obtained with NP which is only mutagenic when incubated with the microsomal preparation. These results clearly indicate that α-NP is mutagenic per se or that S. typhimurium has the ability to convert it to a mutagen.

These results are in accord with the hypothesis that α-NP is hydrolyzed on the plate to α-hydroxy-N-nitrosopyrrolidine (3) which is a mutagen per se, a finding which adds support to the idea that activation of nitrosamines to carcinogens occurs by way of microsomal α-hydroxylation.

Table
Mutagenic Activity[20]

Compound	No Activation		Activation	
	TA 1535	TA 1975	TA 1535	TA 1975
EtOH	—	—	—	—
NG	+	+	+	+
NP	—	—	+	+
α-NP	+	+	+	+

Notes: + = presence of revertants (his^+).
— = presence of spontaneous revertants only.

WARNING. Nitrosamines, which are mutagenic without liver activation, such as the α-acetoxy compounds are potentially extremely hazardous chemicals, being "contact carcinogens" perhaps at concentrations much lower than the parent nitrosamines. All manipulations of such compounds must be done with this possibility in mind.

References and Notes

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20. Qualitatively, it should be noted that incubation of α-NP with rat liver microsomes resulted in a decreased number of revertants.

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