Methylation with (*Z*)-Methyl-*ONN*-azoxymethanol: the Nature of the Reactive Species

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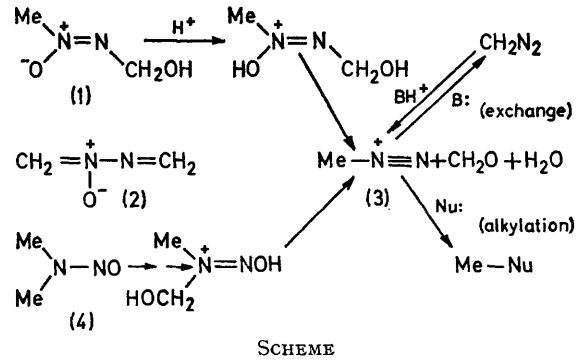
Summary Reaction of [$^2H_5$]-(*Z*)-Methyl-*ONN*-azoxymethyl acetate with *p*-cresol yielded [$^2H_3$]methyl *p*-tolyl ether; a result consistent with fragmentation to yield methanediazonium cation as the methylating species.

INTEREST in the poisonous properties of *Macrozamia* and *Cycas* seeds led to the initial identification of the toxins as glycosidic derivatives of (*Z*)-methyl-*ONN*-azoxymethanol (MAM) (**1**),[1] and subsequently to the recognition that the toxicity was associated with the enzymatic release of the very labile aglycone (**2**).[2] The revelation that MAM was mutagenic and a potent carcinogen[3] stimulated studies of its mode of action which revealed that this extraordinary natural product functioned as a methylating agent *in vitro*,[4] and *in vivo*.[5] To account for this it has been proposed that MAM is a precursor for the *in situ* generation of: diazomethane, formed *via* formaldazine monoxide (**2**),[4a] or directly;[6] or the methanediazonium cation and thence the methyl cation.[7]

In an attempt to clarify the nature of the reactive methylating species we examined the behaviour of [$^2H_5$]-MAM.



SCHEME

Azoxymethane dissolved in D_2O underwent base catalysed (NaOD) exchange of protons in both methyl groups: at room temperature the exchange proceeds at a rate convenient for monitoring by n.m.r.; the protons in the methyl group attached to *unoxidised* nitrogen exchange fastest. The [$^2H_6$]azoxymethane so obtained was then converted[8] into [$^2H_5$]MAM acetate.

When this product was treated with a large excess of *p*-cresol in the presence of catalytic amounts of sulphuric acid[4a] at 75°, the methyl *p*-tolyl ether formed was predominately [$^2H_3$]methyl labelled (n.m.r. and m.s.): repeated experiments with MAM acetate containing *ca.* 6% residual protons in the methyl group of the alcohol yielded a methyl ether containing not more than 15% of protons in its methyl group (values relative to 3H = 100%) by n.m.r.; approximately 70%[$^2H_3$] and 30%[$^2H_2$] by mass spectroscopy.

Under similar conditions, azoxymethane did not yield any methyl *p*-tolyl ether, so direct transfer of methyl groups from MAM or its acetate seems unlikely. We therefore interpret our results as consistent with the fragmentation of MAM, generated by transacetylation, to yield the methanediazonium cation (**3**) which is then efficiently trapped by nucleophile before extensive exchange reactions occur (Scheme).[9]

A similar result has been obtained in experiments with [$^2H_6$]-*N*-nitrosodimethylamine (**4**)[10] and the similarity in biological action of this compound and MAM very probably reflects generation of a common reactive species (**3**).[7] Our conclusions also correspond to those reached by Nagasawa *et al.*[11] on the basis of the non-incorporation of deuterium into the methyl group of methanol formed during the decomposition of MAM in deuterium oxide.

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