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SHORT COMMUNICATIONS

Further metabolites of hexamethylphosphoramide

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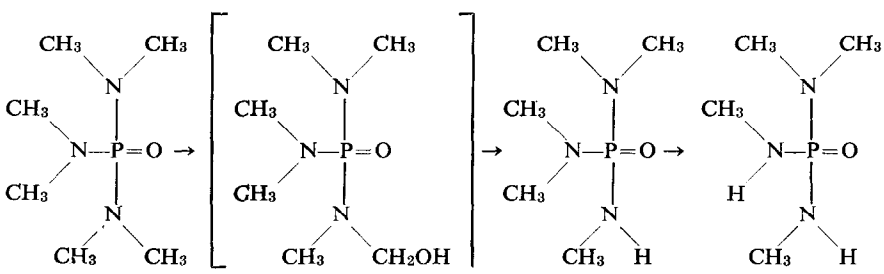
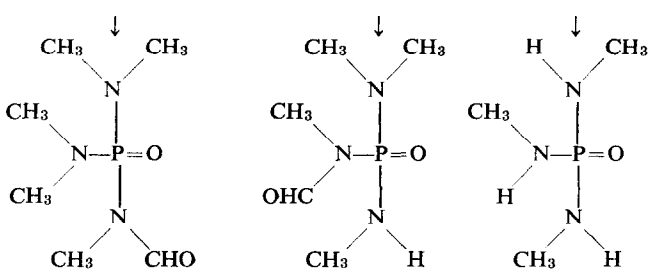
HEXAMETHYLPHOSPHORAMIDE (HMPA, I) possesses antifertility activity in rodents[1] and is an insect chemosterilant.[2] In rats and mice it is degraded by a stepwise loss of methyl groups[3] to pentamethylphosphoramide (PMPA, III), *N*, *N*, *N'*, *N''*-tetramethylphosphoramide (IV) and *N*, *N'*, *N''*-trimethylphosphoramide (V) whereas in the housefly PMPA is the only metabolite.[4] Both liver slices and permanganate oxidation have shown that the demethylation reaction involves the loss of the carbon as formaldehyde and the methylol (II) has been proposed[3] as the intermediate. Borkovec has recently demonstrated[5] PMPA, *N*-formyl-pentamethylphosphoramide (VI) and formaldehyde to be oxidation products of HMPA by reaction with molar permanganate confirming that the methylol (II) is the most logical *in vitro* intermediate.

Further investigations into the metabolism of HMPA in the rat has led to the isolation of VI as a minor metabolite so that both *in vitro* and *in vivo* oxidations appear to occur by similar mechanisms. Long term administration of PMPA to rats gave *N'*-formyl-*N*, *N*, *N'*, *N''*-tetramethylphosphoramide (VII) as a further metabolite so that analogous methylol intermediates are probably involved at each demethylation stage.



I II III IV



VI VII V

Together with the previously known metabolites of HMPA,[3] compound VI possessed no antifertility activity in rats. ^{14}C-labelled VI (prepared from uniformly labelled ^{14}C-HMPA by oxidation with molar permanganate[5]) was metabolised by rats to PMPA and similarly VII gave *N*, *N*, *N'*, *N''*-tetramethylphosphoramide (IV) and *N*, *N'*, *N''*-trimethylphosphoramide (V).

Both VI and VII are ineffective as housefly chemosterilants though in this species VI is reported to be metabolised to PMPA.[5] Assuming that the transformation of VI to PMPA proceeds reductively *via* the methylol (II) in both rats and houseflies, the biological activity of HMPA in these species must be associated with the molecule itself and not be due to the methylol (II).[3]

METHODS

HMPA and PMPA were administered separately in the drinking water (0·05%) to groups of six female Wistar rats for 5 weeks, the filtered urine collected and frozen in solid carbon dioxide. The urine (approximately 1 l. in each case) was extracted and chromatographed on Whatmans SG-31 Chromedia as previously described[3] to remove HMPA, PMPA and *N, N, N′, N″*-tetramethylphosphoramide. The columns were then eluted with methanol and the extracts chromatographed on freshly activated[3] preparative TLC plates (1·5 mm) of silica gel G in solvent A (chloroform–ethanol 3–1). From HMPA-administered urine, the area R_f 0·44–0·55 (developed yellow with molybdate reagent[6]) was eluted with methanol, evaporated *in vacuo*, dissolved in chloroform and purified on a small cellite column. This gave a pale yellow oil (23 mg) characterised as *N*-formyl-pentamethylphosphoramide (VI) by reference to an authentic sample.[5] M$^+$ 193·098782; $C_6H_{16}N_3PO_2$ requires M$^+$ 193·098014 (error 3 p.p.m.). IR (chloroform) 1695 cm^{-1} (CHO). R_f 0·52 on 250 μ silica gel G in solvent A.

From PMPA-administered urine the area R_f 0·30–0·40 was similarly extracted from preparative TLC plates as a yellow gum (18 mg) and characterised as *N′*-formyl-*N, N, N′, N″*-tetramethylphosphoramide (VII) by reference to an authentic sample.[5] M$^+$ 179·081778; $C_5H_{14}N_3PO_2$ requires M$^+$ 179·082364 (error 3 p.p.m.). IR (chloroform) 1690 cm^{-1} (CHO). R_f 0·41 on 250 μ silica gel G in solvent A.

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Alteration in tyramine metabolism by ethanol

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PRETREATMENT of animals with ethanol alters the pattern of metabolism of the biogenic amines serotonin and norepinephrine.[1–3] These amines are deaminated by monoamine oxidase with the formation of an aldehyde intermediate which is either oxidized by aldehyde dehydrogenase to the corresponding acid or reduced by alcohol dehydrogenase to the corresponding alcohol. Ethanol causes an increase in the production of the alcohol and a decrease in the production of the acid from an administered dose of serotonin or norepinephrine. In the course of an investigation of the metabolism of tyramine-1-^{14}C in the rat we have studied the effect of ethanol on the metabolism of this