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PARACETAMOL OXIDATION: SYNTHESIS AND REACTIVITY OF N-ACETYL-p-BENZOQUINONEIMINE

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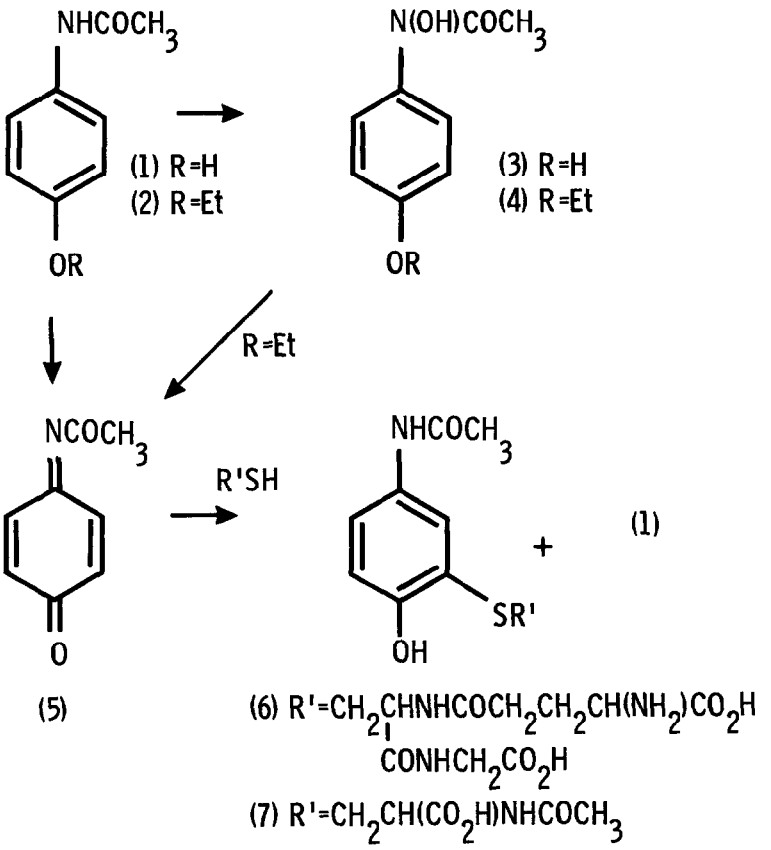
Summmary: A convenient synthesis of N-acetyl-p-benzoquinoneimine is described, its reaction with glutathione and N-acetyl cysteine proceeds not only by adduct formation but also by a reductive pathway.

The widely used analgesic paracetamol (1) has been shown to cause liver necrosis in man and experimental animals when administered in high doses.[1,2] Its toxicity has been attributed to an electrophilic metabolite which is formed by a microsomal cytochrome P-450 dependent mixed function oxidase.[3] With therapeutic doses of paracetamol it has been proposed[4] that the potent electrophile is detoxified by addition of glutathione. At higher doses the glutathione is depleted and this allows covalent binding to cellular macromolecules. The severity of liver necrosis caused by paracetamol correlates with the amount of covalent binding.[5]

The glutathione adduct formed from the reactive intermediate, subsequently undergoes modification of the peptide moiety and is excreted as the mercapturic acid derivate (7). The site of sulphur attachment has been established as C-3 by comparison of a sample obtained from a paracetamol overdose patient[6] with an authentic sample prepared by synthesis.[7] It has been proposed[8] that N-acetyl-p-benzoquinoneimine (5) is the reactive intermediate which causes glutathione depletion. It is presumed to arise[9] by dehydration of N-hydroxy-paracetamol (3), formed through a route analagous to that which converts[10] phenacetin (2) to N-hydroxy-phenacetin (4). It has recently been demonstrated, however, that N-hydroxy-paracetamol (3) is not a metabolite of paracetamol (1) in microsomal incubations.[11]

We suggest that the oxidation of paracetamol may occur without the intermediary of an N-hydroxy compound. There are at least two plausible mechanisms for this reaction. Firstly by formation of N-hydroperoxide[12] which then loses the elements of hydrogen peroxide and secondly, direct oxidation by superoxide derived from the Fe (II) oxygen complex of the cytochrome P-450.[13]



(5)

(6) R'=$CH_2CH(CONHCH_2CO_2H)NHCOCH_2CH_2CH(NH_2)CO_2H$

(7) R'=$CH_2CH(CO_2H)NHCOCH_3$

It has already been demonstrated[14] that paracetamol (1) can be oxidised chemically, the product was trapped in low yield as a Diels Alder adduct. The structure of this adduct is consistent with the formation of quinoneimine (5) during the oxidation. We have found that the quinoneimine (5) is not as unstable as previously suggested. It can be synthesised by lead tetraacetate oxidation of paracetamol in benzene in 50% yield, and stored in benzene solution for up to 24 h at 10°. More conveniently, paracetamol can be oxidised to benzoquiononeimine (5) with freshly prepared silver oxide. The reaction proceeds almost quantitatively (room temperature, 2h) with either benzene or chloroform as solvent and little decomposition (benzene solution) was observed even after one week.

The 200 MHz ^{1}H-NMR spectrum in deuterochloroform exhibited a 4H-AB quartet at δ 7.01, 6.66 (J = 9Hz, olefinic H) and a 3H singlet at δ 2.33. The expected solvent dependence[15] was observed. Thus the downfield olefinic resonances in deuterobenzene had Δ = + 0.77 ppm

[Δ = $\delta(CDCl_3)-\delta(C_6D_6)$] the upfield olefinic resonances Δ = + 0.66 ppm and the methyl singlet Δ = + 0.60 ppm. The 50 MHz ^{13}C-NMR spectrum in deuterochloroform confirmed the structure : δ 186.0, 185.1, 153.2 (3xs, quinone C, imine C, carbonyl C), 135.0, 134.9 (2xd, olefinic C), 25.2 (q, methyl C). Finally, the electronic spectrum confirmed that the imine had an extended chromophore $\lambda_{max}^{CHCl_3}$ 258 nm.

The availability of stable solutions of benzoquinoneimine (5) has enabled a study of its reaction with glutathione and N-acetyl cysteine at physiological pH to be undertaken. Previous studies have relied upon generation in situ either by base catalysed decomposition[9] of N-hydroxy paracetamol (3) or electrochemical generation[16] in the presence of a large excess of paracetamol (1).

Concentrations of benzoquinoneimine (5) in solution were determined by reduction with ascorbic acid to paracetamol (1). This was then quantified by a standard reverse phase hplc method using phenacetin as internal standard. Although it is extremely base sensitive reaction of benzoquinoneimine (5) with glutathione proceeded smoothly in a two phase benzene/150 mM phosphate buffer system at pH 7.4 and 37^{o}C. Utilising 1 mM benzoquinoneimine reactions could be carried out with glutathione concentrations in the physiological range (4 mM). Two separate reactions were observed: firstly glutathione adduct formation (6) and secondly reduction to paracetamol (1). The glutathione adduct (6) was found to have identical chromatographic[17,18] properties with an authentic sample prepared by a microsomal incubation of glutathione and paracetamol[18] (1). The identity of the paracetamol (1) was confirmed by isolation and comparison with an authentic sample.

An almost identical mode of reactivity was observed with N-acetyl cysteine. Thus, an N-acetyl cysteine adduct (7) was formed and reduction to paracetamol (1) took place. The structure of the adduct was confirmed by comparison with an authentic sample kindly supplied by Dr R S Andrews of Sterling Winthrop.

These observations imply that benzoquinonemine can function as an oxidising agent as well as an electrophile. We are currently extending our studies to determine whether oxidation of intracellular proteins and peptides can take place and what the consequences are for the viability of the cell. We are also exploring the chemistry of benzoquinoneimine both as an oxidising agent and as an electrophile.

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