A Novel Iron(III) Catalysed Degradation of Aliphatic Aldehydes to their Lower Homologues with Implications for Lipid Peroxidation Chemistry

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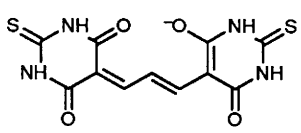
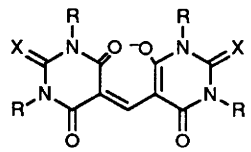
In the presence of various barbituric acids and oxygen, aliphatic aldehydes are degraded to their lower homologues and bis(barbituroyl)methine chromogens in an iron catalysed process which can account for the generation and structure of the transient 452 nm chromogenic species in the thiobarbituric acid test and has implications for aldehyde toxicity and mutagenicity.

The thiobarbituric acid (TBA) test is widely used in the quality control of foodstuffs and in pharmacological studies to determine levels of malonaldehyde (MDA) generated by *in vivo* lipid peroxidative processes associated with cell deterioration and necrosis. It entails the formation in aqueous acid of the stable anionic chromogen (**1**) ($\lambda_{max.}$ 532 nm; $\varepsilon_{max.}$ 156 000 mol^{-1} dm^3 cm^{-1})[1] from TBA and MDA or compounds which readily degrade to MDA. Less obviously, hydroperoxydienes, alka-2,4-dienals, and alk-2-enals, which are typical products of lipid peroxidation, also produce chromogen (**1**) (pseudo-MDA activity) in addition to a second chromogen ($\lambda_{max.}$ 452 nm) which is unstable and which has not been characterised.[2—4] Saturated aldehydes give no chromogen (**1**) but produce the unstable chromogen under the conditions of the TBA test.[5] In this communication we report first on the nature and genesis of this labile species.

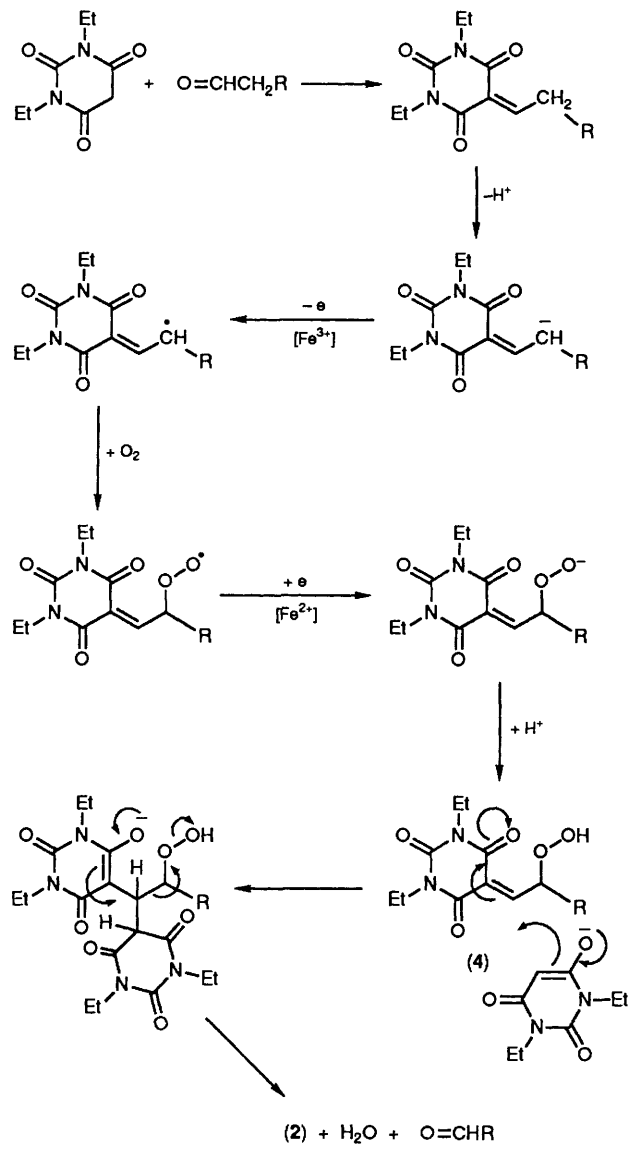


(1)

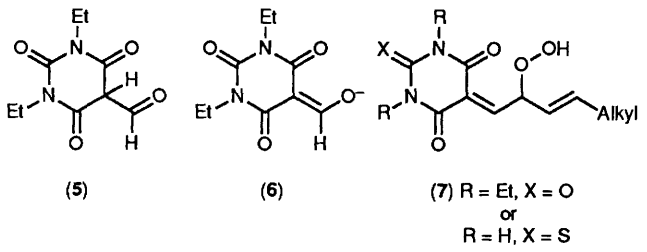


(2) R = Et, X = O
(3) R = H, X = S

Our studies show that an analogous chromogen ($\lambda_{max.}$ 414



Scheme 1. Proposed mechanism for the iron catalysed oxidative formation of chromogen (**2**) and the lower aldehydic homologue.



nm) of limited stability is formed from hexanal and barbituric acid, both under the conditions of the TBA test and in anhydrous alcoholic solutions containing a little pyridine. In the anhydrous solvent, hexanal and *N*,*N*′-diethylbarbituric acid (NDBA) produce a corresponding chromogen ($\lambda_{max.}$ 419 nm), which is stable at temperatures up to 70 °C, and has UV-VIS absorption and chromatographic properties (HPLC and TLC) which are indistinguishable from those of the anion (**2**) ($\lambda_{max.}$ 419 nm, $\varepsilon_{max.}$ 30 700 mol^{-1} dm^3 cm^{-1}), obtained as

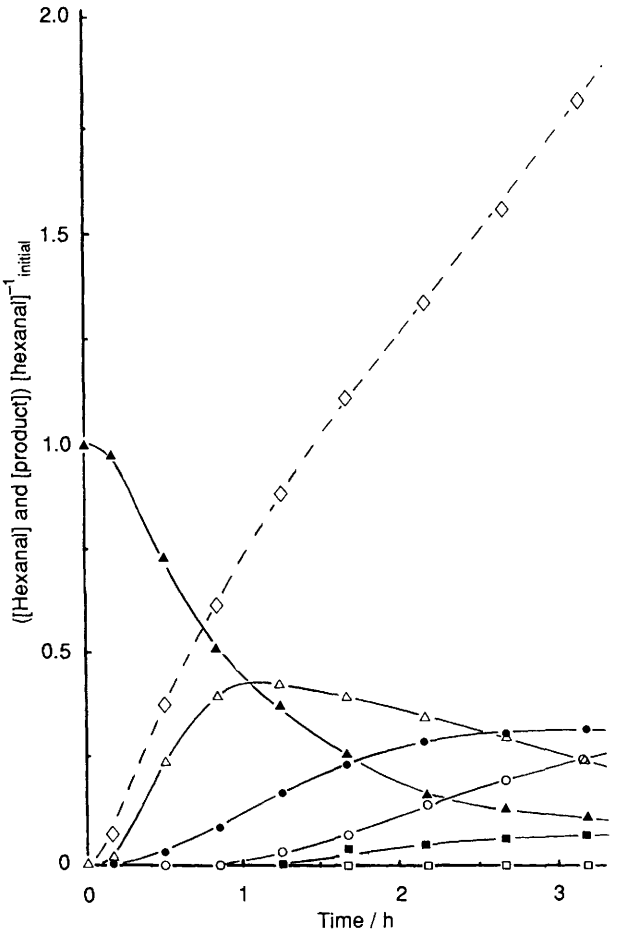


Figure 1. Product formation from hexanal (1.6×10^{-4} M) in anhydrous ethanol containing pyridine (4.75×10^{-2} M), NDBA (1.01×10^{-2} M), and $FeCl_3$ (8.6×10^{-5} M) at 50 °C. Key: chromogen (**2**) (◊), hexanal (▲), pentanal (△), butanal (●), propanal (○), ethanal (■), and methanal (□).

the pyridinium salt from NDBA and ethyl orthoformate in ethanol–pyridine (95 : 5). This finding clearly points to (**2**) as the product formed from hexanal and NDBA and it follows that the labile 452 nm chromogen from aldehydes and thiobarbituric acid in the TBA test is the analogous anion (**3**). In 1959 H. Schmidt suggested that this chromogen was the protonated form of the anion (**3**) on the sole basis of the position of the absorption maximum.[6] This proposal has been either overlooked or disregarded on the grounds that the contribution of just one carbon of the aldehyde to the chromogen was improbable. Reports suggesting that the 452 nm chromogen was an intermediate in the formation of (**1**) have done little to help clarify the structure.[7] Our findings strongly support Schmidt's structural proposal with the minor amendment that the chromogen is anionic.

The rate of formation of the 419 nm chromogen from hexanal and NDBA can be increased by addition of iron(III). However, no chromogen is formed in solutions with high iron loadings when EDTA is added. Degassed solutions with high iron loadings produce relatively little chromogen. Formation of the corresponding chromogens from thiobarbituric and barbituric acids under aqueous acid conditions is similarly inhibited. The genesis of these compounds is therefore associated with an iron mediated oxygenative process and in this respect parallels the genesis of (**1**) from alk-2-enals and related pseudo-MDA active species.[4] We suggest that the

mechanisms are closely related. In Scheme 1 the key features of our recently proposed mechanism for pseudo-MDA activity[4] are adapted to account for the formation of (**2**). It is envisaged that all but the first two steps in this process occur within the co-ordination sphere of the metal. When only adventitious iron is present the process can be regarded as catalytic in the metal.† Formation of the aldehydic intermediate (**5**) from the hydroperoxide (**4**) seems unlikely under the anhydrous conditions and even if this aldehyde was formed, the enolate (**6**) would predominate to ensure a very sluggish further condensation with NDBA. In the case of alka-2-enals, the hydroperoxidic intermediate is considered to be (**7**).

On the basis of the above mechanism the lower aldehydic homologue is a product of the reaction. This will be free to generate further chromogen (**2**) when excess NDBA and oxygen are present, and in principle straight chain aldehydes could be degraded to methanal, one carbon at a time. With hexanal as substrate, we have monitored aldehyde levels in a reaction solution over a period of three hours (see Figure 1 for conditions). Our findings show a decrease in [hexanal], increase and subsequent decrease in [pentanal], and progressive increases in [butanal], [propanal], and [ethanal]. Over the same period the yield of chromogen (**2**) increases at a rate which closely parallels the rate of carbon–carbon bond fission, entirely in accord with the proposed stepwise degradation.

We believe that these findings do more than simply clarify the nature and formation of the transient 452 nm chromogen often observed in the TBA test. Aliphatic monoaldehydes from lipid peroxidation, both saturated and unsaturated, display pathogenic activities associated with the cross-linking of proteins, the cross-linking of protein to DNA, and the cross-linking of DNA, leading to the 'ageing' of tissues, cell mutations, impairment of cell function, and necrosis.[8,9] In the case of three carbon cross-linkages, assumptions that such mono-aldehydes are converted to MDA, which then undergoes double condensations with amino or equivalent centres on the macromolecules, are common.[9] These and our earlier findings demonstrate (i) that an alternative cross-linking mechanism for two appropriate nucleophilic centres is possible without the generation of the dialdehydic species, and (ii) that one straight chain aldehyde molecule has the potential to effect numerous single carbon cross-linkages when presented with an array of appropriate nucleophilic centres, iron, and oxygen. It is possible that free MDA, detected in biological media by techniques which do not subject the components to the hot aqueous acidic conditions of the TBA test, is largely a product of the relatively slow hydrolytic breakdown of three carbon cross-linked materials rather than their precursor.

It is intended to explore further the role of iron catalysed oxygenative coupling processes in the wide and largely unexplored chemistry of cell breakdown. We gratefully acknowledge financial support from the Northcott Devon Medical Foundation (for T. B.) and the SERC (for R. D.).

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† Kikugawa's observation[3] that the yield of (**1**) from alka-2,4-dienals in the TBA test is approximately doubled by the addition of t-butyl hydroperoxide (1 mM) suggests that oxy-radicals are also capable of acting as one-electron acceptors in this process.

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