Novel antioxidant compounds

Thesis

UNRESTRICTED

NOVEL ANTIOXIDANT COMPOUNDS

A Thesis submitted by

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in partial fulfilment of the requirements for the Ph.D. of The Open University

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NOVEL ANTIOXIDANT COMPOUNDS

Adrian Parry

Abstract

The autoxidation process, mechanism of action of radical-trapping antioxidants and the problem of textile yellowing are described and reviewed. Structures implicit in the function of an antioxidant by promotion of radical stabilisation are identified and textile yellowing is attributed largely to reactions of antioxidants with NO_2.

The reaction of ethanolic solutions of 2,6-di-*t*-butyl-4-methylphenol (BHT) with several concentrations of gaseous NO_2 are investigated. The main products are 2,6-di-*t*-butyl-4-methyl-4-hydroxy-2,5-cyclohexadienone (MQOL) and 2,6-di-*t*-butyl-4-methyl-4-nitro-2,5-cyclohexadienone (MQN). Additional products are observed when lower concentrations of BHT and NO_2 are employed. The major product, MQN, is shown to be unstable in aqueous ethanolic solutions and the yellowing component of the reaction solutions is identified as 2,6-di-*t*-butyl-4-nitrophenol (NP).

Two methods for screening potential compounds for antioxidant activity involving the inhibition of BHT product formation and the NO_2^-/NO_3^- ratio are described. They indicate that kojic acid, maltol and 2,5-dimethyl-4-hydroxyfuranone possess similar degrees of antioxidant activity to BHT. This is shown to be consistent with antioxidant function being promoted by the structural features identified.

A quantitative NO_2-yellowing test is described which allows rapid determination of the yellowing indices (OU_{YI}) of test compounds. The results support the use of kojic acid and maltol as alternatives to BHT.

An investigation of kojic acid indicates that it is both nitrosated by gaseous NO_2, and undergoes a complicated series of reactions. Investigations with 3-methylcyclopentane-1,2-dione confirm that cyclic 1,2-diones both reduce and trap gaseous NO_2, a possible strategy for reducing yellowing.

The foregoing studies indicate possible structural requirements for a non-yellowing antioxidant and the synthesis of several *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl) cyclic imides has been accomplished. This has provided a group of new compounds which show similar antioxidant activity to BHT, but are less prone to NO_2-yellowing than 4-substituted hindered phenols possessing labile hydrogen atoms. Studies on the solvolysis of these cyclic imide compounds suggest that sufficient stability to obtain a commercially useful antioxidant may be imparted by the bulky alkyl substituents in the imide ring, as in the case of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-3,3-dimethylglutarimide and *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-2,3-diethyl-2,3-dimethylsuccinimide which have been successfully prepared.

Acknowledgements

I would like to thank my supervisors Professor B. C. Challis and Dr. R. R. Hill for their enthusiasm and guidance during this project. I would also like to thank The British Textile Technology Group for their financial backing of this project, and in particular Dr. T. Sagar, Dr. V. Wilson and Mr O. Bangee for their comments on the work that I have undertaken.

I acknowledge the friendly advice of my colleagues in the Chemistry Department at The Open University, in particular, Dr. J. A. Challis for her help with mass spectrometry, Mr G. Jeffs for his help with chromatographic problems great and small and for his efforts in purifying the hindered imide, to Mr. G. Howell for running NMR samples and to Mr. B. Cook for performing mass spectral analysis.

Finally , I would like to give special thanks to my parents and my girlfriend Sarah, for their help and encouragement during the preparation of this thesis.

Contents

CHAPTER 1

Introduction

1.1. The problem

The discolouration (yellowing) of textiles in use and in storage has become more widespread and has been related to the environmental pollutant nitrogen dioxide (NO_2) interacting with small amounts of chemicals (antioxidants) used to retard the degradation of packaging materials (Wagner, 1982, Oughton, 1985). The increased occurrence of yellowing therefore results from higher levels of NO_2 associated with urbanisation, industrialisation and the use of motor vehicles and extensive use of the hindered phenolic antioxidant 2,6-di-*t*-butyl-4-methylphenol (BHT), which has been identified as the principal source of yellowing compounds (Wagner, 1982, Schmid and Krucker, 1986). Yellowing is often not observed until textiles are taken from storage and it can result in large financial losses. The present project was conceived to provide an alternative to BHT which, possessed a similar degree of antioxidant activity but did not produce significant NO_2-yellowing. This introduction first outlines both the process by which antioxidants function and the autoxidation products formed from BHT. Then, the structural features promoting antioxidant activity, the measurement of antioxidant activity, the chemistry of gaseous NO_2 and possible antioxidant-NO_2 reactions are discussed. Finally, the problem of textile yellowing and the objectives of the project are described.

1.2. The autoxidation process

Autoxidation or the oxidative deterioration of organic substances, proceeds in three stages; initiation, propagation and termination. Initiation occurs when an adventitious radical ($X\cdot$) abstracts a hydrogen atom from the organic substrate, which can then combine with oxygen to form the reactive peroxy radical (Scheme 1.1). There are many sources of these adventitious radicals, such as metal ions, halogens and even NO_2.

Scheme 1.1. Initiation.

$$X^{\bullet} + RH \longrightarrow XH + R^{\bullet}$$

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

Propagation of the peroxy radicals may proceed either by abstraction of a hydrogen atom from the organic substrate (to produce further alkyl radicals which can in turn react to produce more peroxy radicals) or by reaction with unsaturated groups (Scheme 1.2).

Scheme 1.2. Propagation.

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

$$ROO^{\bullet} + C{=}C \longrightarrow ROO\text{-}C\text{-}C^{\bullet}$$

The termination stage involves both homogeneous and heterogeneous combination of radicals to non-radical products (Scheme 1.3), which may result in the oxidative degradation of the substrate.

Scheme 1.3. Termination.

$$\left.\begin{array}{l} 2R^{\bullet} \\ R^{\bullet} + ROO^{\bullet} \\ 2ROO^{\bullet} \end{array}\right] \longrightarrow \text{Non-radical Products}$$

Antioxidants are added to the organic substrates to retard the process of autoxidation.

1.3. Antioxidant compounds

Antioxidants are classified in two ways according to their mechanism of action. Phosphorus and organic sulphur compounds such as (*1*) and (2), are used as preventative or peroxide decomposing antioxidants. Their mode of action involves the decomposition of a hydroperoxide to an alcohol with the oxidation of the sulphur or phosphorus atom

(Scheme 1.4). The ability of phosphorus and sulphur to exist in several oxidation states facilitates the efficiency of peroxide decomposing antioxidants. The major limitation in their use is that other antioxidants must be added to ensure effective stabilisation of substrates.

$(C_{18}H_{37}O)_3P$ $(C_{18}H_{37}OCOCH_2CH_2)_2S$

(*1*) (*2*)

Scheme 1.4. Typical mode of action of preventative or peroxide-decomposing antioxidants.

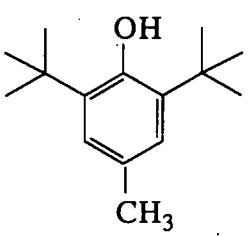
$$R'OOH + (RO)_3P \longrightarrow (RO)_3P{=}O + R'OH$$

Radical-trapping or chain-breaking antioxidants act as a source of hydrogen atoms for the reactive peroxy radicals resulting in the formation of the less reactive hydroperoxide (ROOH) and stable antioxidant radical (A·) (Scheme 1.5). All subsequent discussion will concentrate on compounds that act in this manner.

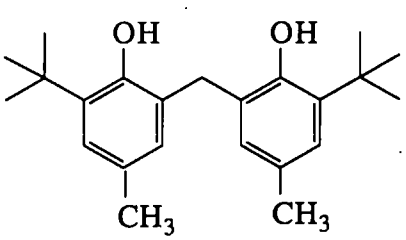
Scheme 1.5. Mode of action of radical-trapping or chain-breaking antioxidants.

$$ROO^{\bullet} + HA \longrightarrow ROOH + A^{\bullet}$$

The most commonly used radical-trapping antioxidants are hindered phenolic compounds such as 2,6-di-t-butyl-4-methylphenol (BHT) (*3*) and Cyanox 2246 (*4*) with alkyl substituents at the 2- and 6-positions.



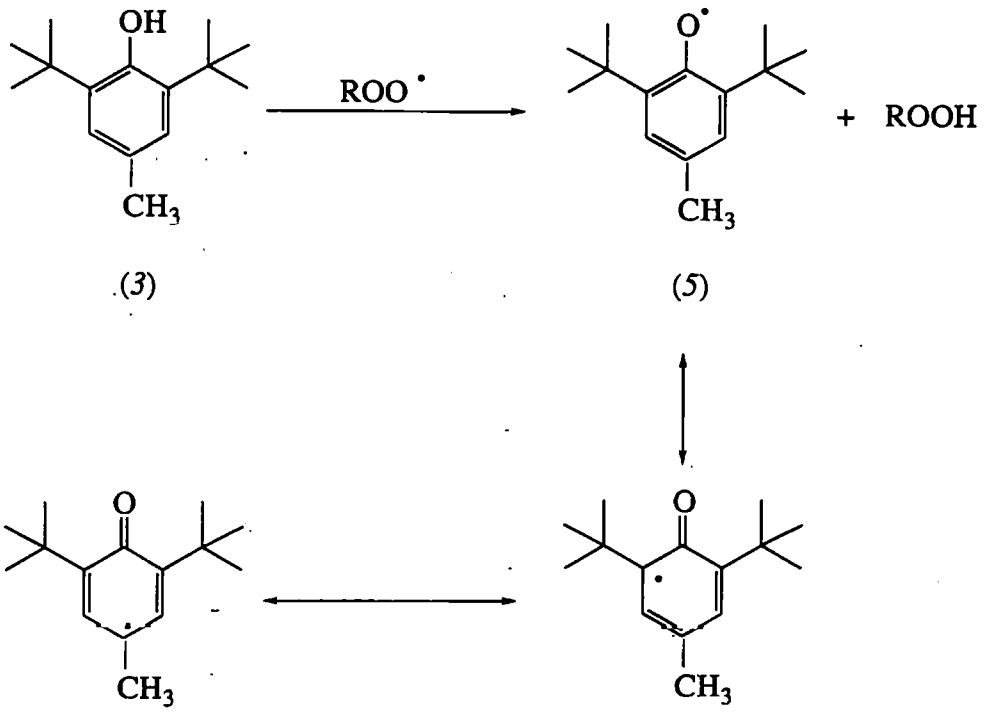
(*3*)



(*4*)

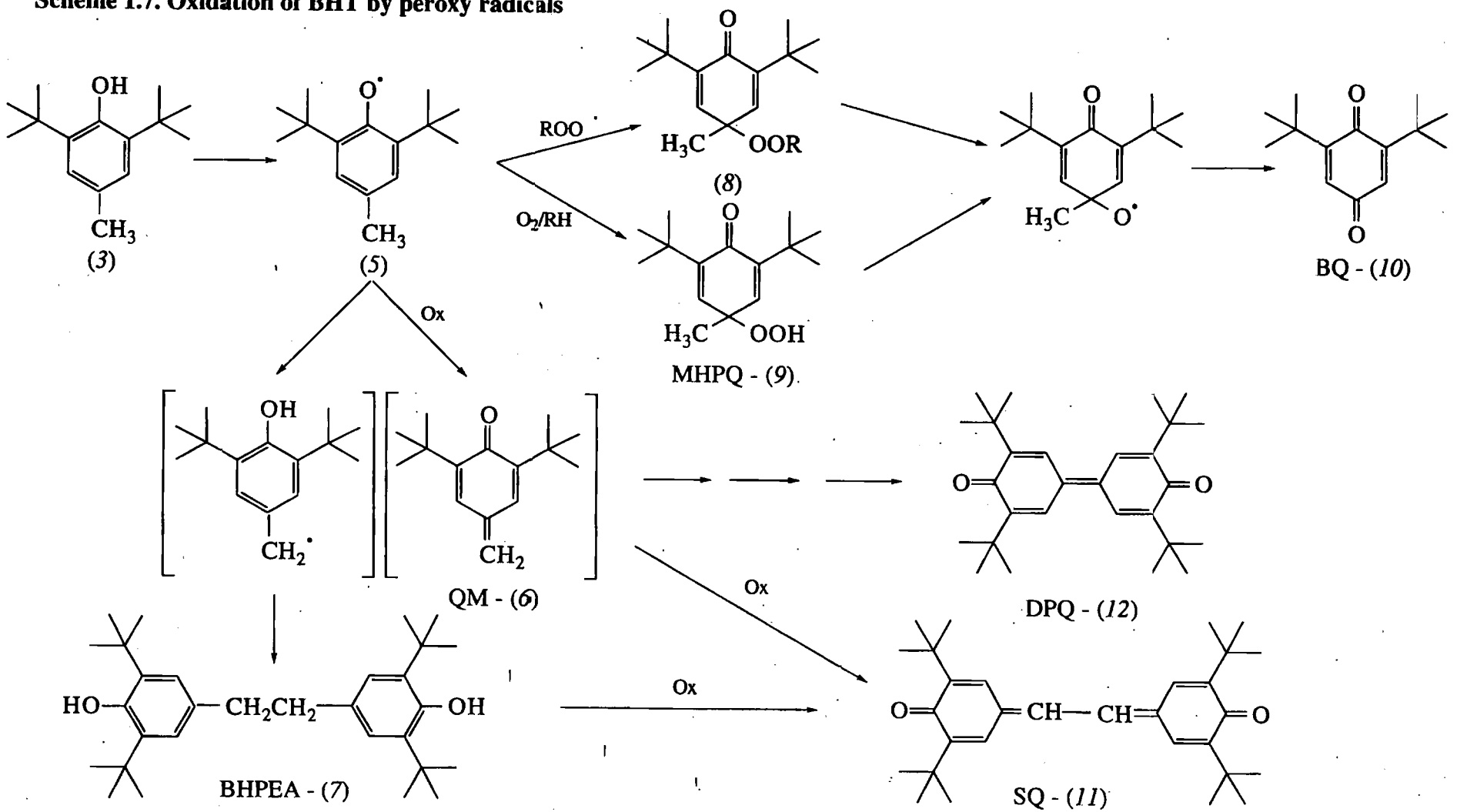
The antioxidant radical (A·) which forms must be stable so that further reaction with the substrate does not occur. This stabilisation is improved when the unpaired electron is delocalised, as in the radical formed from BHT (*5*) (Scheme 1.6). Variation of the 4-substituent is used to facilitate incorporation of the antioxidant into various materials.

Scheme 1.6. Formation and delocalisation of BHT radical.



The autoxidation of BHT has been extensively studied by Pospisil (1988, 1991) who demonstrated formation of quinonoid products (Scheme 1.7.). The chemical transformations have been deduced from experiments performed under model conditions. The initial step is removal of the phenolic hydrogen to form the phenoxy radical (*5*). Disproportionation of (*5*) leads to the formation of 2,6-di-*t*-butylbenzoquinone methide (QM) (*6*). Coupling of benzyl radicals formed by formal rearrangement of (*5*) results in the formation of 1,2-bis-(3,5-di-*t*-butyl-4-hydroxyphenyl)ethane (BHPEA) (*7*). Recombination of (*5*) with an alkylperoxy radical results in the formation of an alkylperoxycyclohexadienone (*8*). Combination of (5) with oxygen gives 2,6-di-*t*-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadiene-1-one (MHPQ) (*9*). Both hexadienones (*8*) and (*9*) react to form 2,6-di-*t*-butyl-1,4-benzoquinone (BQ) (*10*). Further coupling reactions of QM result in the formation of 3,3′,5,5′-tetra-*t*-butyl-stilbene-4,4′-quinone (SQ)(*11*) and 3,3′,5,5′-tetra-*t*-butyl-dipheno-4,4′-quinone (DPQ) (*12*). The oxidation of BHT proceeds with the removal of more than one hydrogen, and the number of peroxy radicals deactivated is normally two or less.

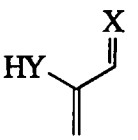
Scheme 1.7. Oxidation of BHT by peroxy radicals



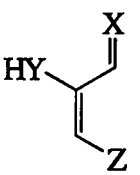
1.4. Structural features promoting antioxidant activity

Consideration of the radicals formed by a variety of radical-trapping antioxidants indicates that the two structural features in Scheme 1.8 are implicit in the function of an antioxidant by promotion of radical stabilisation.

Scheme 1.8. Structural features implicit in the function of an antioxidant by promotion of radical stabilisation.



(*13*)

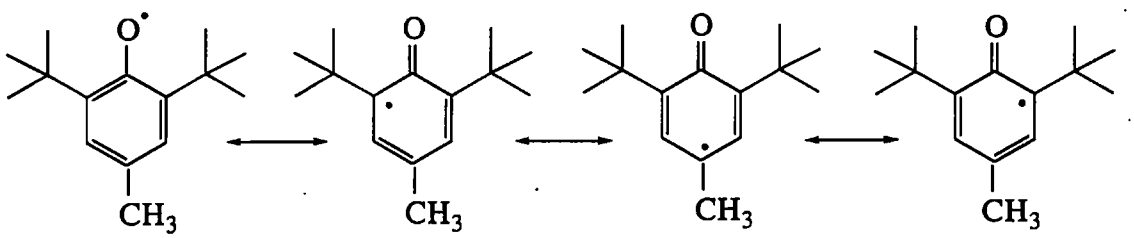


(*14*)

where: X - heteroatom or carbon
Z,Y - heteroatom

The stabilisation of a radical derived from structure (*13*), a feature of the hindered phenolic antioxidants where X = carbon and Y = oxygen, is illustrated for BHT (Scheme 1.9). The radical (*5*) is stabilised by conjugation around the aromatic ring.

Scheme 1.9. Promotion of radical stabilisation by conjugation.

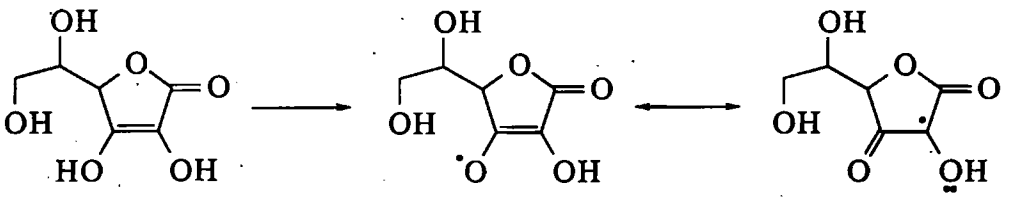


(*5*)

The stabilisation of a radical generated from structure (*14*), a feature of many biological antioxidants, is fully consistent with the captodative effect (Viehe *et al.*, 1985), where electron-donating (X) and withdrawing (Z) groups are adjacent to the radical centre.

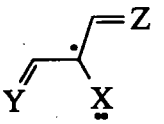
Vitamin C (*15*) and vitamin E (*16*) are well known biological antioxidants, the latter preventing lipid autoxidation by rapid donation of hydrogen atoms to lipid radicals. The radicals formed as a result of hydrogen donation (Schemes 1.10. and 1.11.) possess the structural features previously described.

Scheme 1.10. Formation of Vitamin C radical with structural features identified.

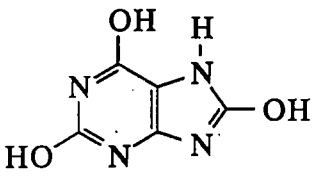


(*15*)

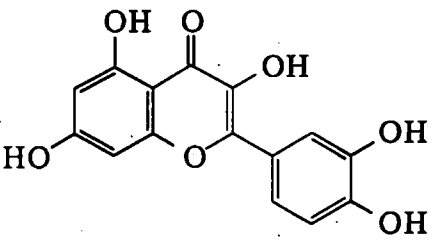
Structural feature contributing to radical stabilisation by the captodative effect.



Many other naturally occurring compounds identified as antioxidants, such as uric acid (*17*) (Halliwell *et al.*, 1992) and quercetin (*18*) (Hudson, 1990), possess the structural feature (*14*).

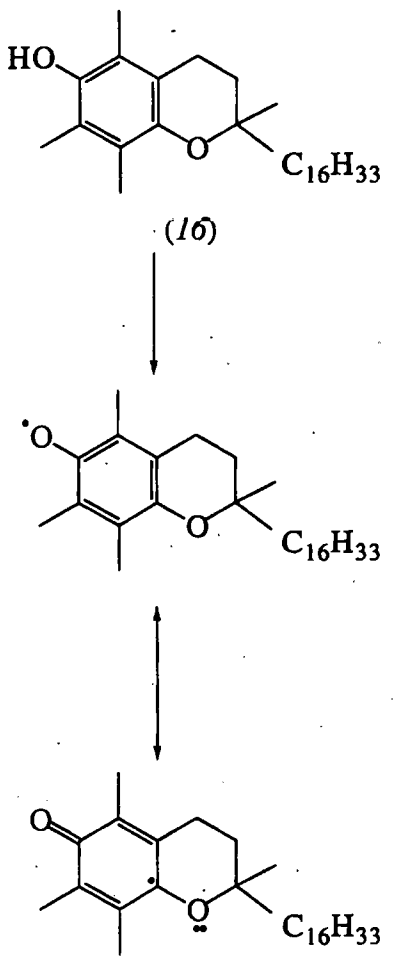


(*17*)

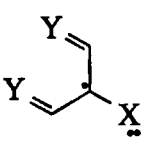


(*18*)

Scheme 1.11. Formation of Vitamin E radical with structural features identified.



Structural feature contributing to radical stabilisation by the captodative effect.



1.5. Measurement of antioxidant activity

The development of a compound as an antioxidant requires demonstration that it either prevents or retards oxidative deterioration. Three main types of testing procedure are used to measure antioxidative efficiency. The first involves the prediction of the performance of materials under working conditions, the second and third measure physical and chemical changes in model compounds under idealised conditions. All the methods are described in detail by Scott (1993), and some of the more widely used will be summarised here.

The evaluation of antioxidant efficiency of compounds under working conditions has been carried out by incorporating the antioxidant into a matrix of lubricating oils (Pederson, 1956, Wasson and Smith, 1953), polymers (Henman, 1979, Humphris and Scott, 1974, 1982) and foodstuffs (Kurechi and Kunugi, 1983, Ragnarsson *et al.*, 1977, Tanizawa *et al.*, 1983). Under normal atmospheric conditions, the rate of oxidative deterioration of such a system is too slow to accurately measure the extent of oxidation. It is therefore necessary to accelerate oxidation by using abnormal conditions, such as increased temperature and pressure.

For example, polymers such as polypropylene and polyethylene for antioxidant studies are subjected to both mechanical and physical stress. The Melt Flow Index (MFI) is measured by the number of grams of polymer extruded through a standard orifice in ten minutes at an elevated temperature under a standard load. Polymer samples are often tested in this manner (Henman, 1979, Humphris and Scott, 1974, 1982) and the more efficient antioxidants are those which give lower melt flow indices. Physical stress measurement using the thermal stability of polymer sheets has been determined by placing them in an oven at 100°C and 150°C in an air or oxygen stream and measuring the onset of colour (Patel *et al.*, 1972) or embrittlement (Chakraborty and Scott, 1977).

A variety of physical methods to determine antioxidant efficiency have been applied to both technological matrices and model compounds. Oxygen absorption has been used to predict induction times for antioxidant efficiency in model compounds (Scott, 1972,

1981, 1983,) and polymers (Chan *et al.*, 1978, Hartless and Trozzolo, 1974). The oxygen uptake is measured by changes in volume or pressure at constant temperature either manometrically (Hawkins *et al.*, 1959), by differential pressure transducers (Ingham *et al.*, 1975) or by gas burette (Hammond *et al.*, 1955). IR spectroscopy has also been used to monitor the thermal oxidation of polymer films, by measuring absorbance at the carbonyl stretching frequency (Chakraborty and Scott, 1977), which increases as a result of oxidation. The analysis involves the use of a stable absorption peak elsewhere in the spectra to correct for any variations in thickness during oxidation, the results being expressed as a carbonyl index.

Differential thermal analysis (DTA) and differential scanning calorimetry (DSC) provide information about heat effects associated with physical changes within a polymer matrix as it is oxidised, and have been used to assess the efficiency of antioxidants (Howard, 1973, Van Sickle and Pond, 1978).

Oxidation potentials, half-wave potentials, and rate constants have all been employed to measure chemical changes within a matrix to determine antioxidant efficiency. The oxidation and half-wave potentials are measures of the reducing power of a compound, the half-wave potential being equal to the oxidation potential minus a constant. Both have been used to measure antioxidant efficiency (Penketh, 1957, Nash *et al.*, 1958), and have allowed a critical value to be determined. Any compound possessing a potential below this value could be assumed to exhibit useful antioxidant properties. Other work utilises rate constants for the bimolecular reaction of the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) with phenolic and amine antioxidants (Blois, 1958, McGowan *et al.*, 1959).

The procedures described above measure different types of antioxidant activity. As the present study is primarily concerned with radical-trapping antioxidants only methods specific to determining this type of activity are relevant.

1.6. Chemistry of NO_2

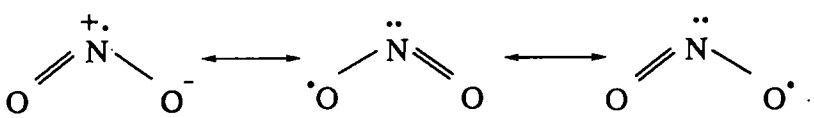
Having described the mechanism by which antioxidants function and the methods for testing their efficiency, it is beneficial to outline the oxidant properties of NO_2, the other factor involved in textile yellowing.

Nitrogen dioxide exists in equilibrium with its dimer, dinitrogen tetraoxide (Equation 1.1). At ambient temperature and pressure, N_2O_4 is approximately 50% dissociated (Redmond and Wayland, 1968). The dissociation increases with increasing temperature and of course, with dilution. As an environmental pollutant, NO_2 exists entirely as a monomer, but dimers may form transiently via heterogeneous absorption.

$$N_2O_4 \rightleftharpoons 2NO_2 \qquad (1.1)$$

NO_2 is a radical that can act as an oxidising and nitrating agent, and a nitrosating agent via the NO_2 dimer. Because the unpaired electron can reside on any of the three atoms (Scheme 1.12), NO_2 reacts at both the *N*- and *O*-atoms.

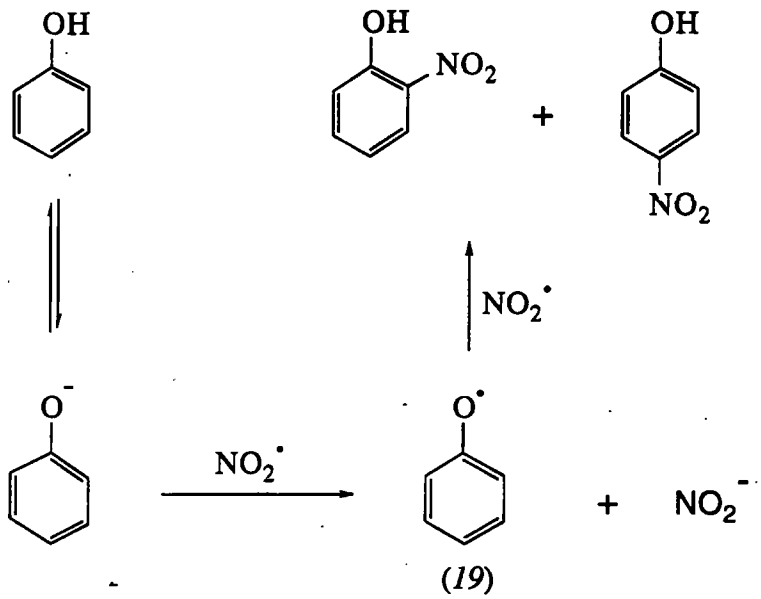
Scheme 1.12. Resonance forms of nitrogen dioxide.



Depending on the type of substrate, reaction may lead to either addition or substitution products. Both reactions can generate radical intermediates which may react further with NO_2. Atmospheric levels of NO_2 are typically below 10ppm and therefore this discussion will concentrate on the reactions of substrates with dilute NO_2.

The reaction of the phenolate anion with NO_2 produces nitration at the 2 and 4 positions (Prutz *et al.*, 1985) (Scheme 1.13). The reaction proceeds via a phenoxy radical (*19*) generated in an initial electron transfer to NO_2, which then reacts with a second molecule of NO_2 to give the nitro substitution products, 2-nitrophenol and 4-nitrophenol. Only the phenolate ion and not the neutral phenol appear to electron transfer to NO_2.

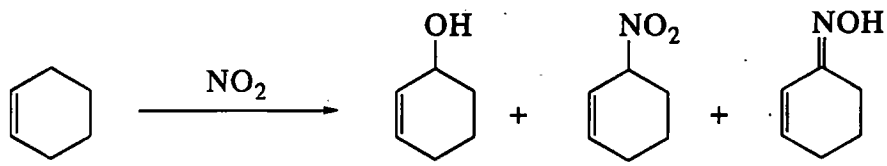
Scheme 1.13. Reaction of phenol with NO_2.



(*19*)

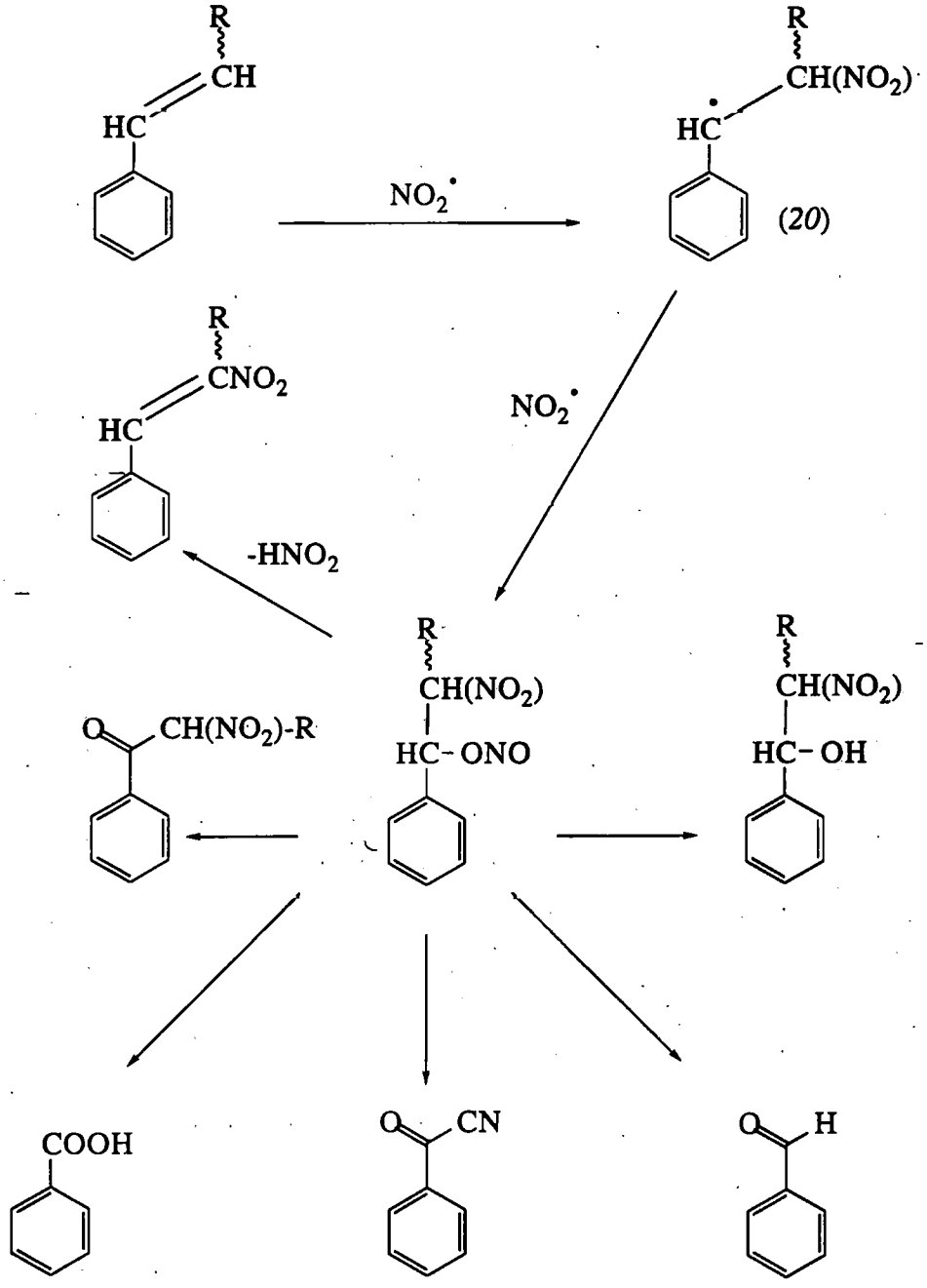
Substrates containing allylic hydrogen atoms undergo hydrogen abstraction reactions with dilute NO_2 (up to 10,000ppm) to give an allylic radical, which reacts further to give substitution products as shown by the reaction of NO_2 (78ppm) in nitrogen, air or oxygen with cyclohexene (Pryor *et al.*, 1982) (Scheme 1.14).

Scheme 1.14. Products formed from the reaction of cyclohexene with dilute NO_2 (Pryor *et al.*, 1982).



The reaction of styrene and related alkenes with 10,000ppm NO_2 results in a large number of products from the initial substitution of NO_2 into the double bond, and further reactions of the resultant radical intermediate (*20*) with a second mole of NO_2 (Bryant, 1989) (Scheme 1.15). Increased conjugation of the double bond reduces the reactivity, as found for stilbenes (R = Ph).

Scheme 1.15. Products from the reaction of styrene and related alkenes with NO_2 (Bryant, 1989).



1.7. NO_2-antioxidant reactions

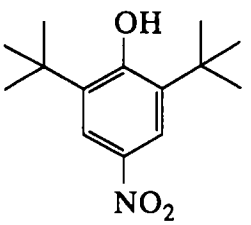
As an oxidant NO_2 may be expected to react with antioxidants to give NO_2^- or HNO_2 and A·. Thus, NO_2 will accelerate the loss of antioxidant and the formation of its

decomposition products. As a relatively stable radical NO_2 will also combine with A· to form products different from those of autoxidation.

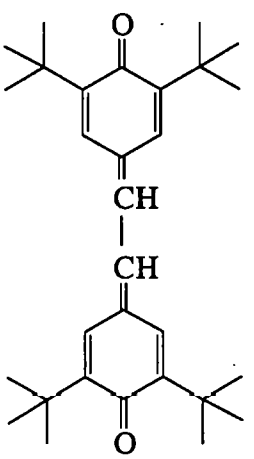
Thus, the reactions of NO_2 with organic compounds have features that can be identified with, and distinguished from, those of antioxidant chemistry. Both need careful consideration in the evaluation of the yellowing potential of new antioxidants by NO_2.

1.8. Textile yellowing

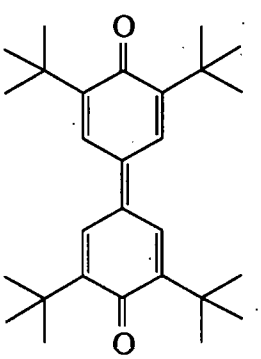
The first documented evidence for the components of textile yellowing was produced by Wagner (1982), following mass spectral analysis of yellow extracts from fabric samples. He found small amounts of 2,6-di-*t*-butyl-4-nitrophenol (NP) (*21*), 3,3′,5,5′-tetra-*t*-butyl-stilbene-4,4′-quinone (SQ) (*11*) and 3,3′,5,5′-tetra-*t*-butyl-dipheno-4,4′-quinone (DPQ) (*12*) and proposed that these compounds were responsible for the yellow colouration. Their incidence was linked to hindered phenol antioxidants, such as BHT and 2,6-di-*t*-butylphenol, used in the plastic packaging of garments which were sufficiently volatile to migrate from the plastic packaging to the textile surface.



NP - (*21*)

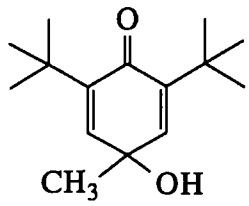


SQ - (*11*)

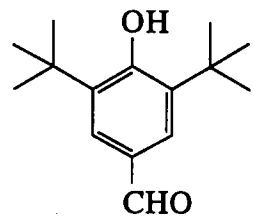


DPQ - (*12*)

Subsequently, Schmid and Krucker (1985) identified 2,6-di-*t*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadiene-1-one (MQOL) (*22*), QM (*6*), NP (*21*) and 3,5-di-*t*-butyl-4-hydroxy-benzaldehyde (BALD) (*23*) by GC-MS in a simulated yellowing test using cotton fibre soaked with BHT following exposure to 200ppm NO_2 in air for four days.

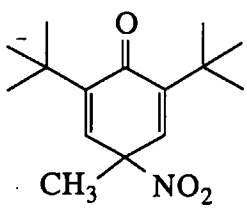


MQOL - (*22*)

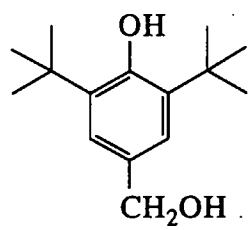


BALD - (*23*)

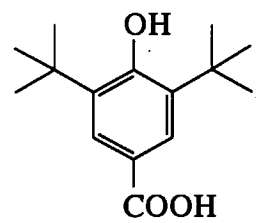
Wilson (1989) carried out more extensive work using BHT spotted onto TLC plates. The reaction site on the plate was adjusted to either pH4 or 6.5 then exposed to gaseous NO_2. 2,6-Di-*t*-butyl-4-methyl-4-nitro-2,5-cyclohexadiene-1-one (MQN) (*24*) and MQOL were identified by Rf and UV spectra after reaction with 5ppm NO_2 for 6 h at pH4; further reaction gave 3,5-di-*t*-butyl-4-hydroxybenzylalcohol (BALC) (*25*) and NP after 18 h, and SQ and BALD after 42 h. In addition, 3,5-di-*t*-butyl-4-hydroxybenzoic acid (BAC) (*26*), BQ (*10*) and three other unidentified compounds were detected in reactions carried out at pH 6.5.



MQN - (*24*)



BALC - (*25*)



BAC - (*26*)

Thus, the various studies have identified MQN, MQOL, BALD, BALC, BAC, NP, QM, BQ, SQ, and DPQ, as products from the reaction of adsorbed BHT with gaseous NO_2. As most of the experiments did not exclude oxygen, autoxidation may play a role in the formation of some of these products.

Of the compounds identified; NP, SQ and DPQ seem to be the most likely contributors to textile yellowing. The formation of NP involves the reaction of NO_2 with BHT, while the coupled products, SQ and DPQ result from the oxidation of BHT which may be enhanced by NO_2.

1.9. Objectives of the present project

The autoxidation process, mechanism of action of radical trapping antioxidants and the problem of textile yellowing have been reviewed. There is a need for alternative compounds to BHT, which possess similar antioxidant activity but do not produce significant NO_2 yellowing. The overall aim of the project is to identify, synthesise and evaluate suitable alternative compounds.

This aim is best achieved by first clarifying the processes by which BHT forms yellow products. For example, by determining the product distribution for reaction of BHT with NO_2 under various conditions, the components of textile yellowing may be identified with more certainty and allow structural and other criteria for non-yellowing antioxidant compounds to be recognised. In practise this requires suitable procedures to critically assess antioxidant activity and NO_2 yellowing capabilities. New antioxidants can then be synthesised and tested as suitable replacements for BHT and related compounds.

The objectives may therefore be summarised:

1. Determine how BHT forms yellow compounds by obtaining quantitative data on the product distribution from reactions of BHT solutions with gaseous NO_2 and use these data to determine the identity of the yellowing components.

2. Identify structural criteria for non-yellowing antioxidant compounds.

3. Establish procedures for critically assessing a potential compound's antioxidant activity and NO_2 yellowing capabilities.

4. Select available compounds and synthesise new compounds for further study, with antioxidant activity comparable with or better than BHT, but without propensity to NO_2-yellowing.

CHAPTER 2

Reaction of BIIT with nitrogen dioxide

2.1. Introduction

Previous studies of the reactions of BHT with NO_2 were reviewed in Chapter 1. Most involved the identification and characterisation of products, from reactions carried out in heterogeneous phases. In this chapter, the influence of reagent concentrations, time of reaction and pH on the product distribution for reactions of BHT in solution with gaseous NO_2 are reported, and the mechanistic implications discussed.

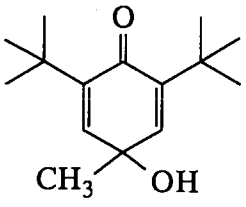
The main objective of this work was to develop regimes for the exposure to gaseous NO_2 and product analysis for subsequent application to the evaluation of new antioxidants. With GC, GC-MS and HPLC as the principal analytical tools, BHT in both neutral and basic solutions of aqueous ethanol was reacted with an NO_2 supply diluted with air.

2.2.1. Product analysis by GC and GC-MS

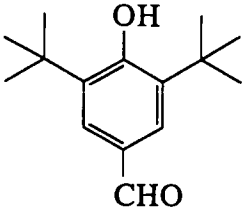
The extract from reaction of neutral 10mM BHT in aqueous ethanol (70%v/v) sparged with NO_2 (900vpm*) in air for 2 h (Reaction 1) (Table 2.1), was shown to contain 2,6-di-*t*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone (MQOL)(*22*), BHT and 3,5-di-*t*-butyl-4-hydroxybenzaldehyde (BALD)(*23*) by GC comparison with authentic compounds. The main component of the reaction mixture was unreacted BHT. The GC analysis of a similar reaction carried out under alkaline conditions (Reaction 2), showed the presence of MQOL, BHT, BALD and five unidentified peaks of significant magnitude. GC-MS analysis (Table 2.2) confirmed MQOL, BHT and BALD, and the MS data suggested 2,6-di-*t*-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadienone (MHPQ)(*9*) and an ethoxy-derivative of MQOL, 2,6-di-*t*-butyl-4-ethoxy-4-methyl-2,5-cyclohexadienone (EQOL)(*27*) for two of the unidentified components corresponding to m/z 252 and 264, with GC retention times 10.7 and 12.2 min.

* - volume parts per million

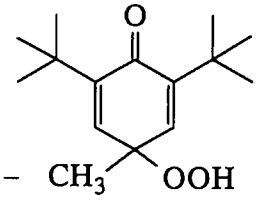
The highly retained peaks at 20.4, 21.2 and 22.2 min showing M^+ ions at m/z 414, m/z 368 and m/z 398, respectively, remain unidentified, but must be BHT-coupled products because of the large molecular ions.



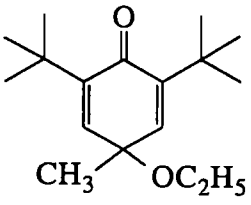
MQOL - (*22*)



BALD - (*23*)



MHPQ - (*9*)



EQOL - (*27*)

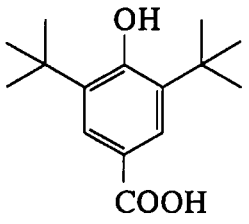
GC-MS analysis of the reaction extract from NO_2 exposure over 4 h (Reaction 3) showed the presence of MQOL, BHT, EQOL and BALD and the unidentified compound with M^+ at m/z 368 (GC retention time 24.8 min).

The GC-MS analysis of the 30mM BHT reaction extract (Reaction 4) (Table 2.3), indicated the presence of MQOL, BHT, MHPQ, EQOL, BALD and the three unknowns with M^+ ions at m/z 414, m/z 368 and m/z 398, respectively. 3,5-Di-*t*-butyl-4-hydroxybenzoic acid (BAC)(*26*), and a series of unidentified compounds with M^+ ions at m/z 276, m/z 262, m/z 278 and m/z 318 corresponding to GC peaks at 10.1, 13.1, 14.7 and 16.4 min, respectively, were also present. The undesignated compounds are BHT-related because their mass spectra show the loss of 15 m/z fragments from the molecular ion, corresponding to the loss of methyl from the *t*-butyl group.

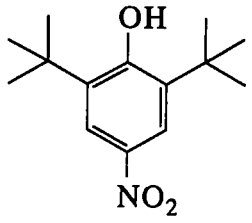
The reaction of 1mM BHT with NO_2 for 4 h (Reaction 6) gave an extract showing the presence of MQOL, MHPQ, BAC, EQOL, BALD, 2,6-di-*t*-butyl-4-nitrophenol (NP)(*21*) and an unidentified compound with M^+ ion at m/z 262.

In summary the reactions (1-6) show an alkaline solution of BHT generates more products than a similar reaction carried out under neutral conditions. Furthermore,

increasing the concentration of BHT produces a greater variety of products including ones of high mass, probably formed from two molecules of BHT.



BAC - (*26*)



NP - (*21*)

Table 2.1. Results for GC analyses of BHT-NO_2 reaction extracts.

	GC Component - Retention Time/min (Area x 10^3)				
Reaction No.	MQOL	- BHT	BALD	NP	Unknowns
1	6.3 (3.6)	8.2 (23.3)	13.1 (1.5)	-	-
2	6.3 (8.0)	8.2 (135.0)	13.1 (11.1)	-	10.7 (3.7) 12.2 (1.8) 23.1 (0.3) 24.8 (1.4) 26.4 (3.1)
3	6.7 (39.1)	8.3 (48.8)	13.2 (10.7)	-	10.3 (3.3) 11.6 (4.2) 24.8 (1.3)
4	6.3 (11.0)	8.2 (79.7)	13.1 (4.1)	-	9.0 (2.9) 10.8 (3.7) 11.2 (9.2) 12.4 (2.4) 14.9 (5.9) 15.6 (6.3) 18.7 (2.2) 23.1 (4.6) 24.8 (4.8) 26.4 (3.1)
6	6.2 (10.4)	-	13.3 (1.4)	14.8 (1.4)	12.2 (6.0) 13.0 (1.2) 14.8 (4.5)
STD	6.1	8.4	13.3	14.8	

Table 2.2. Products formed from the reaction of 10mM BHT with 200vpm NO_2 for 2 h in alkaline solution (Reaction 2) determined by GC-MS.

Retention Time (Scan No.)	m/z	Product
4.0 min (98)	236 ($M^{+\cdot}$, 5%), 221 (16), 193 (10), 180 (56)	MQOL (*22*)
4.7 min (132)	220 ($M^{+\cdot}$, 33%), 205 (100).	BHT (*5*)
5.3 min (160)	252 ($M^{+\cdot}$, 5%), 237 (8), 196 (18), 181 (20), 153 (100)	MHPQ (*9*)
11.3 min (455)	264 ($M^{+\cdot}$, 35%), 249 (100), 219 (28)	EQOL (*27*)
12.2 min (498)	234 ($M^{+\cdot}$, 14%), 219 (45), 203 (4), 191 (15), 28 (100)	BALD (*23*)
20.4 min (901)	414 ($M^{+\cdot}$, 8%), 399 (2), 205 (100)	Unknown
21.2 min (939)	368 ($M^{+\cdot}$, 50%), 353 (14), 219 (32), 57 (100)	Unknown
22.2 min (989)	398 ($M^{+\cdot}$, 30%), 341 (15), 285 (5), 28 (100)	Unknown

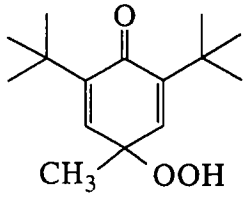
Table 2.3. Products formed from the reaction of 30mM BHT with 200vpm NO_2 for 4 h in alkaline solution (Reaction 4) determined by GC-MS.

Retention Time (Scan No.)	m/z	Product
4.0 min (114)	236 ($M^{+\cdot}$, 5%), 221 (16), 193 (10), 180 (56)	MQOL (*22*)
4.7 min (144)	220 ($M^{+\cdot}$, 33%), 205 (100).	BHT (*3*)
5.5 min (216)	252 ($M^{+\cdot}$, 5%), 237 (5), 196 (22), 181 (20), 153 (100)	MHPQ (*9*)
8.2 min (344)	250 ($M^{+\cdot}$, 3%), 235 (3), 220 (3), 205 (8), 193 (35)	BAC (*26*)
10.1 min (396)	276 ($M^{+\cdot}$,12%), 261 (8), 219 (100), 203 (10)	Unknown
11.3 min (509)	264 ($M^{+\cdot}$, 35%), 249 (100), 219 (28)	EQOL (*27*)
12.2 min (560)	234 ($M^{+\cdot}$, 25%), 219 (100), 203 (5), 191 (28)	BALD (*23*)
13.1 min (605)	262 ($M^{+\cdot}$, 15)%, 247 (9), 219 (100), 203 (13)	Unknown
14.7 min (700)	278 ($M^{+\cdot}$,18%), 263 (100), 247 (4), 235 (18), 219 (7), 203 (18)	Unknown
16.4 min (799)	318 ($M^{+\cdot}$,12%), 303 (7), 261 (10), 219 (100)	Unknown
19.8 min (929)	414 ($M^{+\cdot}$, 11%), 399 (4), 219 (25), 205 (100)	Unknown
21.0 min (983)	368 ($M^{+\cdot}$, 50%), 353 (14), 219 (32) 57 (100)	Unknown
22.9 min (1023)	398 ($M^{+\cdot}$, 30%), 341 (15), 285 (5) 28 (100)	Unknown

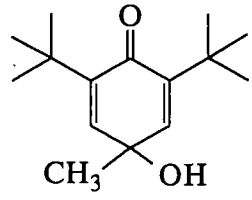
The reactions (1-6) confirm some of the products previously reported (Wilson, 1989 and Oughton, 1986) and reveal several new unidentified products some of which may be BHT-coupled. The majority of the unidentified compounds may arise from coupling of the 3,5-di-*t*-butyl-4-hydroxy benzyl moiety with reactive species in the extract, because of the fragment ion at m/z 219 in their spectra. The major products from the reaction of BHT with gaseous NO_2 appear to be MQOL and BALD. The exact distribution of products is uncertain, however, as experiments on a standard solution of BHT and its products taken through the extraction procedure gave poor recovery. This was attributed to the volatility of BHT and possibly, some of its reaction products. It was therefore decided to employ HPLC for quantitative analysis.

2.2.2. Product Analysis by HPLC

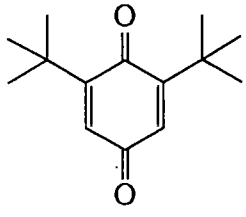
The reagent used in these experiments was a mixture of NO_2 with air, so all experiments presented an opportunity for reaction with O_2 as well as NO_2. The total products therefore consist of those arising from reaction with NO_2 plus those formed by autoxidation. An oxidation experiment using an alkaline solution of 1mM BHT exposed to air for 12 h showed MHPQ (*9*) (80.1%), MQOL (*22*) (7.6%), BQ (*10*) (2.5%) and BALD (*23*) (1.3%) as the major autoxidation products.



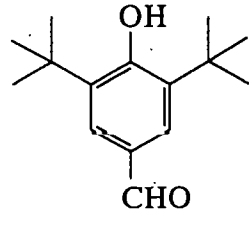
MHPQ - (*9*)



MQOL - (*22*)



BQ - (*10*)



BALD - (*23*)

Most reaction products were identified and quantitated by comparison of retention time, UV spectrum, peak purity parameter and peak area with those of authentic compounds. Authentic standards were not available, however, for MHPQ, 3,5-di-*t*-butyl-4-hydroxybenzylalcohol (BALC)(*25*) and EQOL, but plausible identification and quantitation was made on the basis of the following considerations:

(i) MHPQ and EQOL were shown to be reaction products by molecular ions in GC-MS spectra.

(ii) MHPQ and EQOL are structurally similar to MQOL as BALC is to BHT. The similarity in both peak purity parameters and UV spectra for each set of compounds is consistent with their assumed assignments (Table 2.4).

(iii) MHPQ and MQOL should have similar retention times whereas EQOL should be more retained in reverse phase liquid chromatography.

(iv) As detector response factors correspond with extinction coefficients at the detection wavelength, the assumption of closely similar response factors is reasonable for compounds with closely similar UV spectra. This assumption was used to quantitate MHPQ, EQOL, and an unknown at retention time 17.1 min.

In control experiments, BHT was found to aspirate by gas purging at a rate of 0.03 mMh^{-1} from the alkali solution and 0.05 mMh^{-1} from the neutral solution. Taking this into account where appropriate, the percentage yield (Y_i) for each product P_i was determined using Equation 2.1.

$$Y_i = \frac{P_i \times 100}{C\text{-}N\text{-}U} \qquad (2.1)$$

where P_i – measured concentration of product i (mM)

C – starting concentration of BHT (mM) from control solution

N – unreacted BHT (mM)

U – BHT lost by evaporation (mM)

Table 2.4. Peak Purity Parameter and Uv max. data for BHT and reaction products.

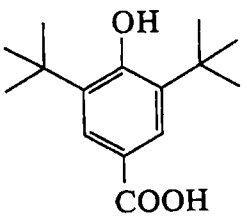
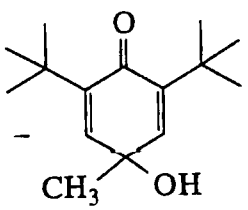
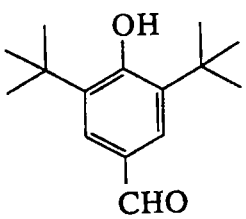
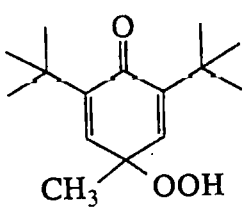
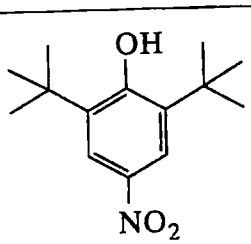
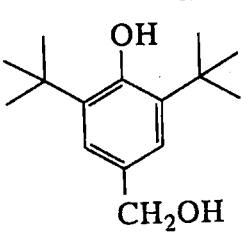
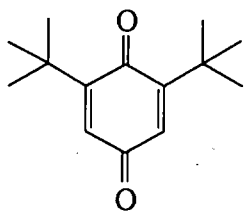
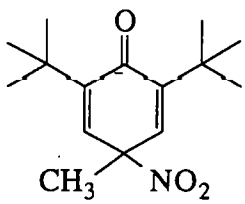
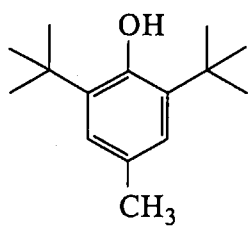
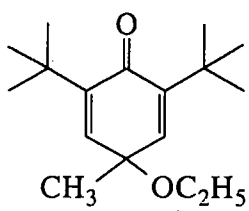
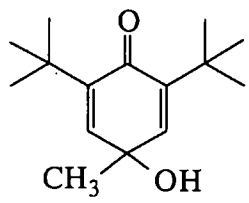
Compound	Structure	Uv max. (nm)	Peak Purity (λ215-367)	Retention Time (min)
BAC		192, 253	240.7	5.3
MQOL		232	230.8	9.5
BALD		198,219, 275	258.6	9.9
MHPQ		232	230.5	10.3
NP		317	290.4	12.6
BALC		274	223.0	13.4

Table 2.4. Peak Purity Parameter and Uv max. data for BHT and reaction products (cont.).

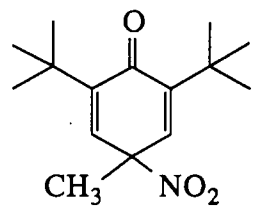
Compound	Structure	Uv max. (nm)	Peak Purity (λ215-367)	Retention Time (min)
BQ		251	250.0	14.1
MQN		230	229.0	14.9
BHT		273	224	15.9
Unknown	-	230	231.5	17.1
EQOL		234	230.8	18.8

The quantitative data for the majority of reactions does not account for all of the BHT reacted. Some products may have been lost due to aspiration while others may not have been identified by HPLC. The presence of a large unresolved solvent front in many chromatograms suggests some products were not retained and therefore not identified (see Figure 6.3.).

The results (Table 2.5) show that in all but two cases the main products of the reaction of BHT with NO_2 are MQOL (*22*) and MQN (*24*). More products are observed when lower concentrations of BHT and NO_2 are employed, where BALC, BALD, BAC, NP and EQOL have been identified. The converse was observed with samples analysed by GC and GC-MS. This conflict may however be due to further reactions promoted by the extraction procedure prior to GC analysis.



MQOL - (*22*)



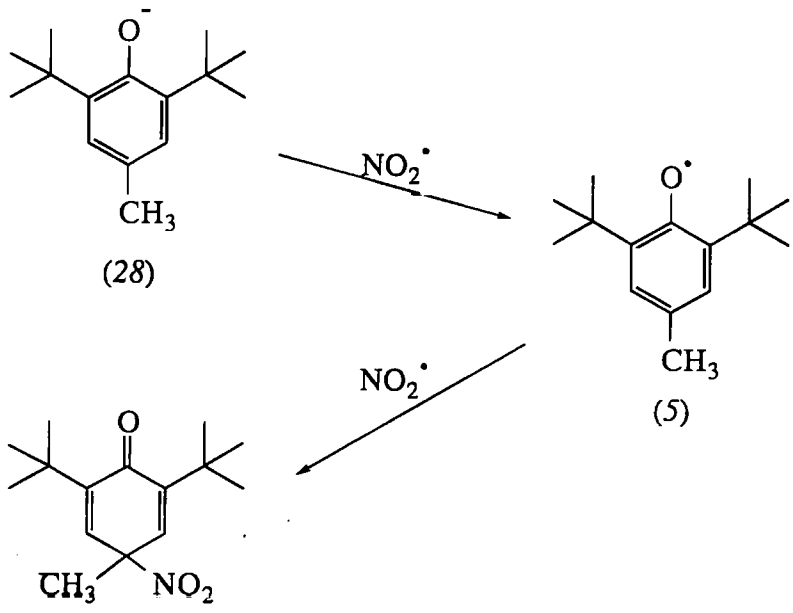
MQN - (*24*)

The reaction of 1.2mM BHT in an alkaline aqueous ethanol solution with 200vpm NO_2 for 4 h produces BAC (0.3%), MQOL (17.6%), BALD (0.6%), MHPQ (5.0%), NP (0.4%), BALC (2.8%), BQ (2.8%), MQN (18.3%), EQOL (1.2%) and an unknown component (3.7%) (Reaction 6). The main products of this reaction are MQOL and MQN. The formation of MQN probably arises by the reaction of NO_2 with the phenoxy radical (*5*), formed from the phenoxy anion (*28*) (Scheme 2.1) as suggested by Smeltz (1982).

Table 2.5. Results for the HPLC analysis of BHT-NO_2 reaction solutions.

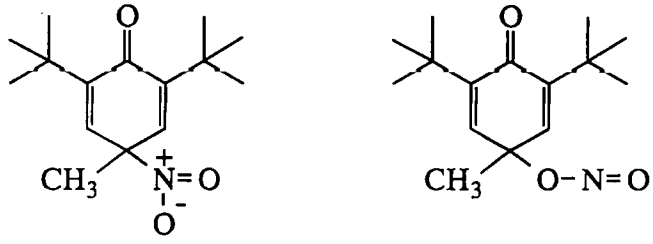
React. No.	Reaction Conditions	BHT Data		HPLC Retention time (min) - Component yield (%)										
		N (mM)	U (mM)	5.3 BAC	9.5 MQOL	9.9 BALD	10.3 MHPQ	12.7 NP	13.5 BALC	14.1 BQ	15.0 MQN	17.1 UK	18.9 EQOL	Total
4	30mM BHT 200vpm NO_2 4 hr alkaline	10.3	0.12	–	25.1	–	–	–	–	–	30.5	–	–	
5	1.2mM BHT 200vpm NO_2 0.4 hr alkaline	0.850	0.012	–	8.3	–	3.3	–	27.5	–	18.3	–	–	57.4
6	1.2mM BHT 200vpm NO_2 4 hr neutral	-	0.12	0.3	17.6	0.6	5.0	0.4	2.8	2.8	13.9	3.7	1.2	48.3
7	1.2mM BHT 200vpm NO_2 0.4 hr neutral	0.593	0.22	–	17.1	–	–	–	1.8	–	44.7	–	–	63.6
8	1.2mM BHT 10vpm NO_2 8 hr alkaline	0.174	0.24	–	16.2	1.1	49.7	–	4.7	2.2	8.6	0.8	2.9	86.2
9	12mM BHT 200vpm NO_2 0.4 hr alkaline	11.33	0.012	–	–	-	–	–	-	–	51.6	–	–	51.6
10	12mM BHT 200vpm NO_2 4 hr alkaline	3.389	0.12	–	20.1	–	40.3	–	20.5	–	20.9	–	5.9	107.2

Scheme 2.1. Formation of MQN.



(*28*) (*5*) (*24*)

As discussed in Chapter 1, an NO_2 molecule may react as an oxygen or a nitrogen centered radical. Physical evidence suggests that the MQN formed contains a C-N linkage (*24a*). This is further supported by diode array data.

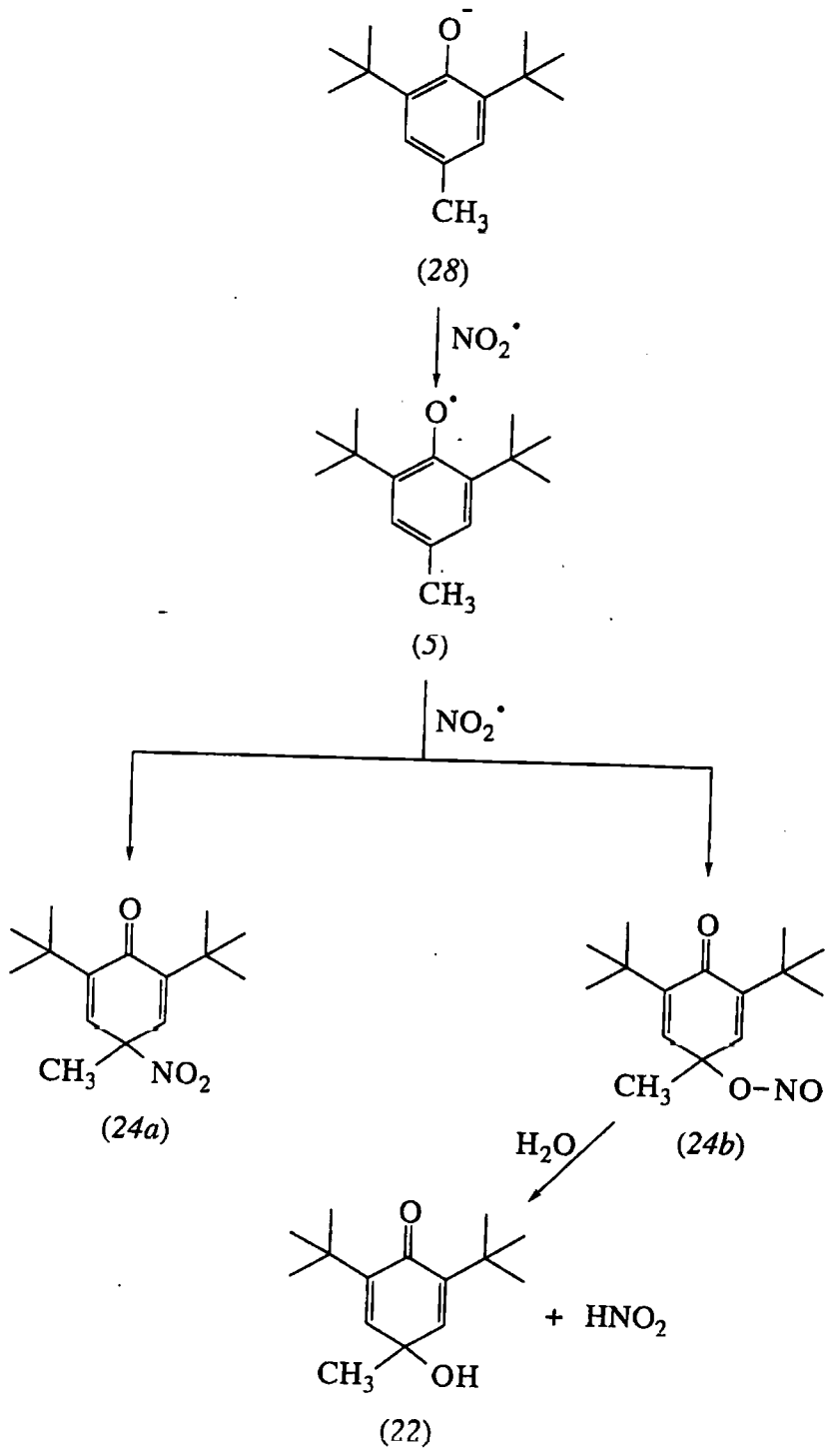


(*24a*) (*24b*)

The purity parameters ($PuP_{215-367}$) for MQOL and MHPQ which possess a C-O linkage at the 4-position in the quinonoid structure are 230.8 nm and 230.5 nm respectively. If the C-O linkage was to be present within MQN then a similar purity parameter would be observed. However this is not the case, the purity parameter for MQN is 229.0 nm and therefore suggests the C-N linkage is present in MQN.

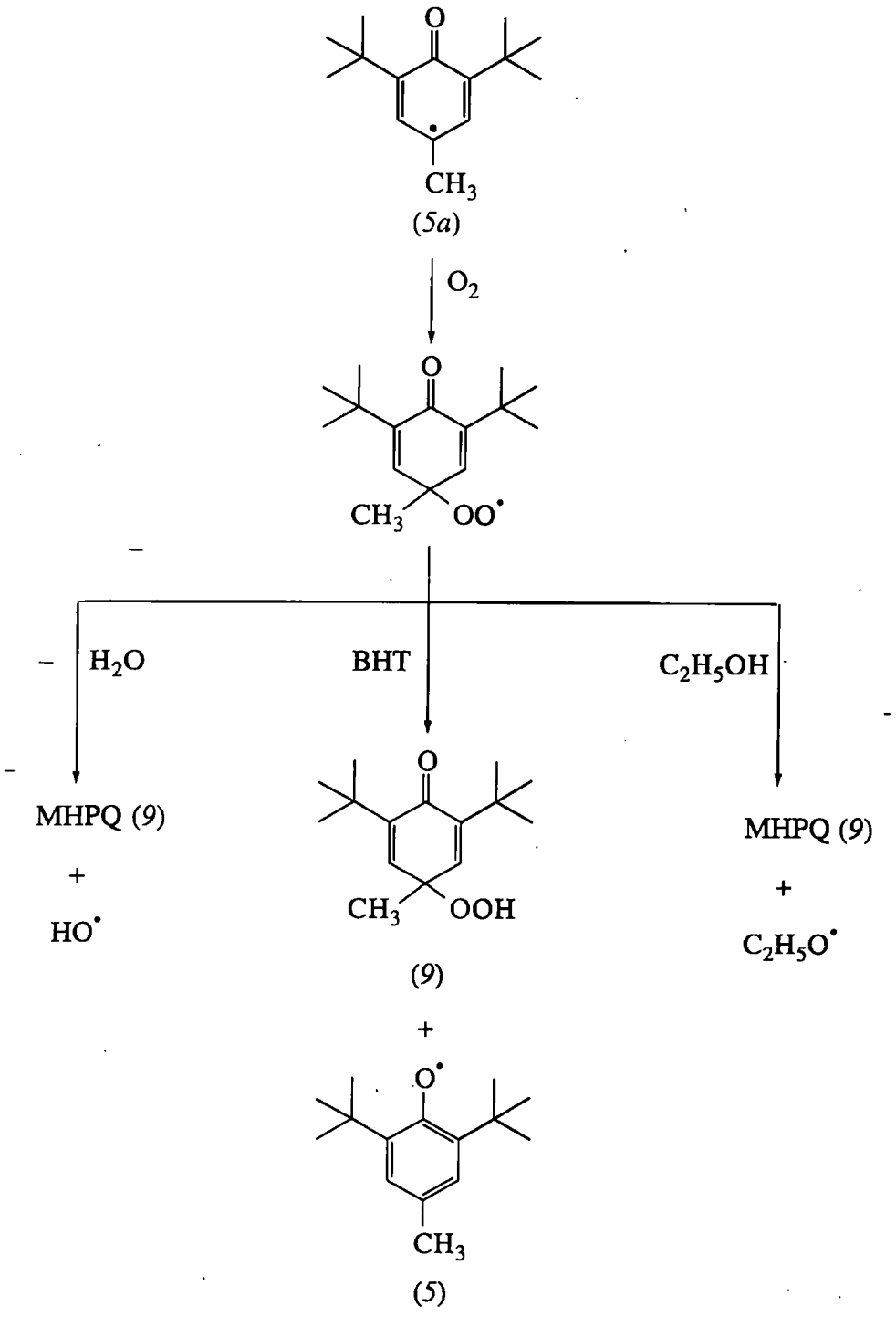
The existence of the unstable nitrite ester form of MQN (*24b*) would provide a satisfactory explanation for the formation of MQOL as the other main product (Scheme 2.2).

Scheme 2.2. Formation of MQN and MQOL.



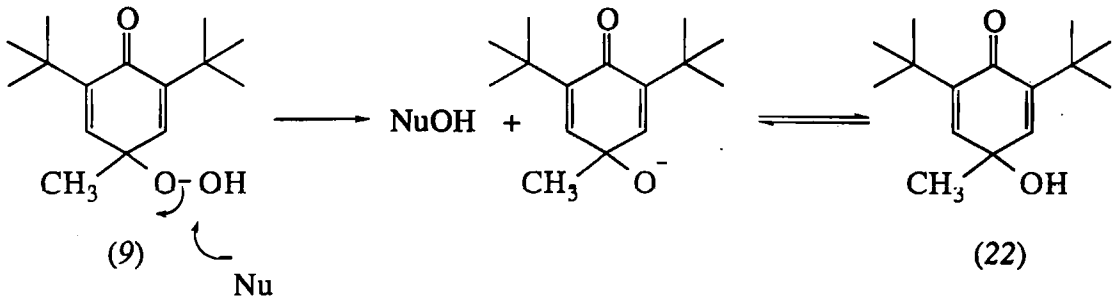
The formation of MHPQ (*9*) proceeds by reaction of the BHT radical (*5a*) with oxygen, followed by hydrogen abstraction from either the solvent or another molecule of BHT (*3*) (Scheme 2.3). When BHT is the hydrogen donor, the resulting BHT radical (*5*) formed may react with further oxygen to generate more MHPQ in a chain reaction. If the solvent was the hydrogen donor, both HO· and C_2H_5O· could be generated and would possibly provide an plausible pathway to EQOL.

Scheme 2.3. Formation of MHPQ.



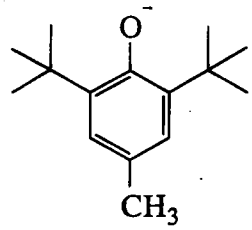
MQOL may be formed both by hydrolysis of the nitrite ester (*24b*, Scheme 2.2) and by decomposition of MHPQ as shown by the oxidation experiment using 1mM alkaline BHT exposed to air for 12 h. The decomposition of MHPQ may proceed *via* direct attack of a nucleophilic species (solvent etc.) on the peroxide bond (Scheme 2.4). Further reaction of MQOL leads to the formation of BQ possibly *via* nucleophilic displacement (Scheme 2.5).

Scheme 2.4. Decomposition of MHPQ.



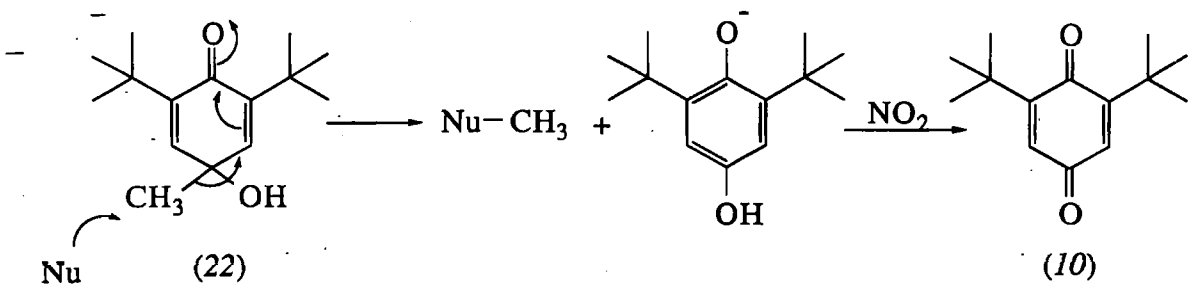
(9) (22)

where Nu = H_2O, EtOH, NO_3^-, NO_2^-, etc.



(28)

Scheme 2.5. Formation of BQ.

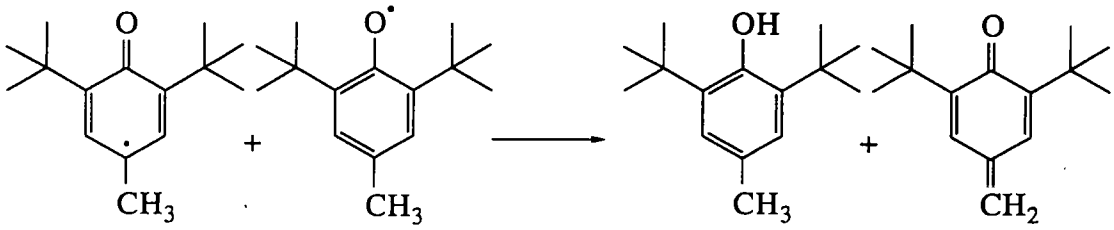


(22) (10)

EQOL is not seen in the absence of NO_2 and its formation is best explained by the combination of $C_2H_5O\cdot$ generated from the solvent acting as a hydrogen donor and a BHT radical.

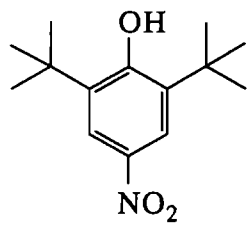
The formation of the minor products BALC, BALD, BAC and NP can be rationalised by the initial formation of 2,6-di-*t*-butylbenzoquinone methide (QM, *6*) by disproportionation (Scheme 2.6).

Scheme 2.6. Formation of quinone methide.



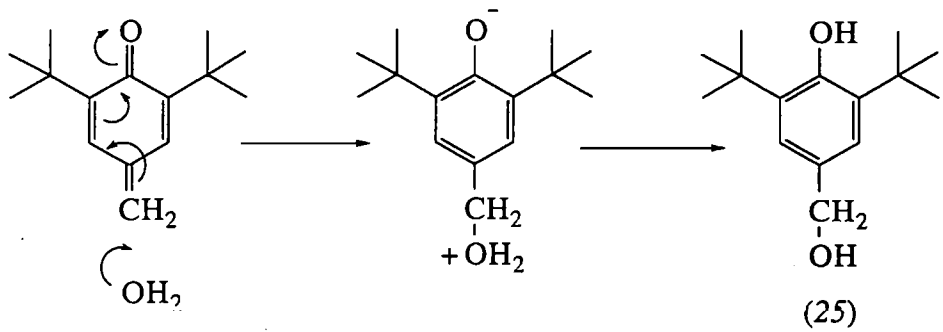
(*6*)

QM reacts with water to give BALC (*25*) (Scheme 2.7), and successive hydrogen abstractions by BHT or NO_2 would lead to BALD (*23*) (Scheme 2.8) and BAC (*26*) (Scheme 2.9). The nitrophenol, 2,6-di-*t*-butyl-4-nitrophenol (NP, *21*) may then be generated from BAC by reaction with NO_2 and loss of CO_2. A similar stepwise oxidation to NP has been suggested by Wilson (1989).



(*21*)

Scheme 2.7. Formation of BALC



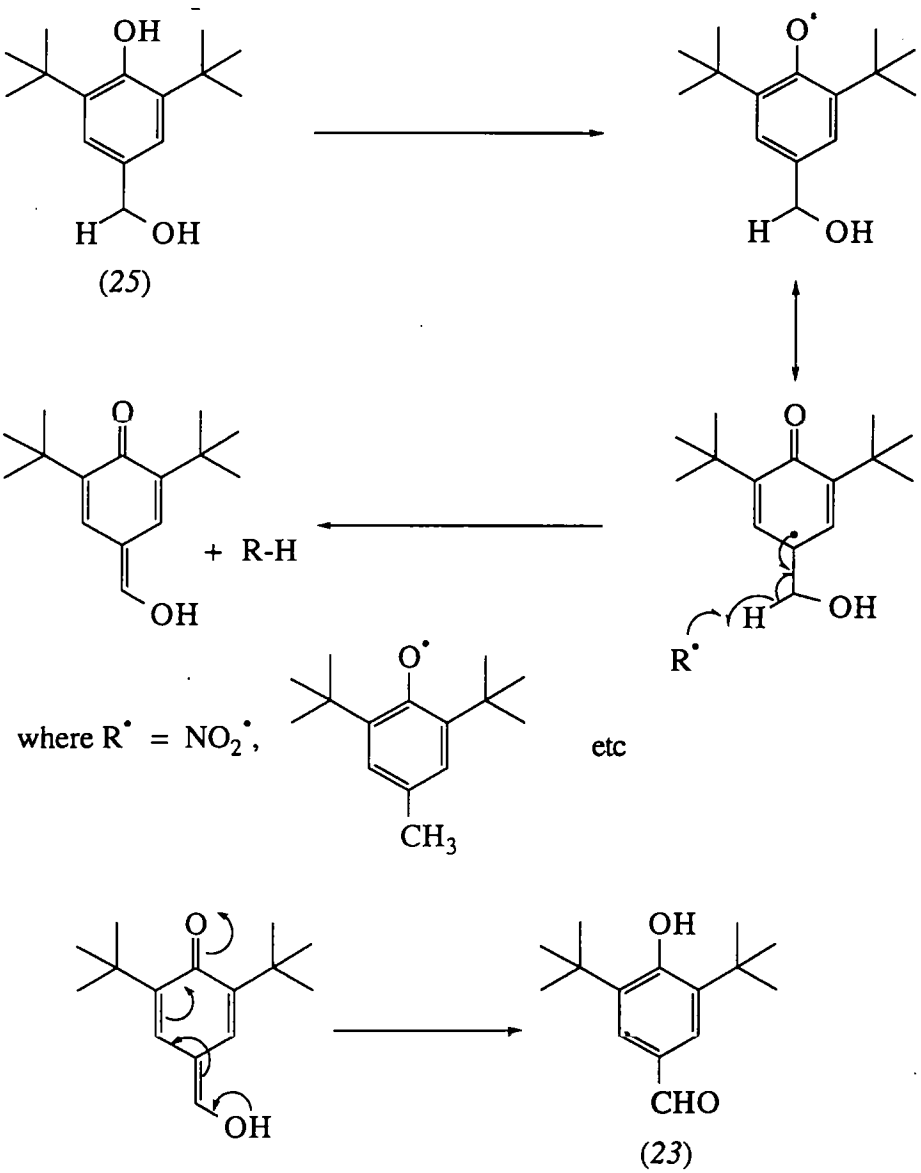
(*25*)

The peak at 17.06 min in the HPLC appears to be due to a quinonoid-type product, as indicated by both purity parameter and UV spectra, but no further information on its structure is available.

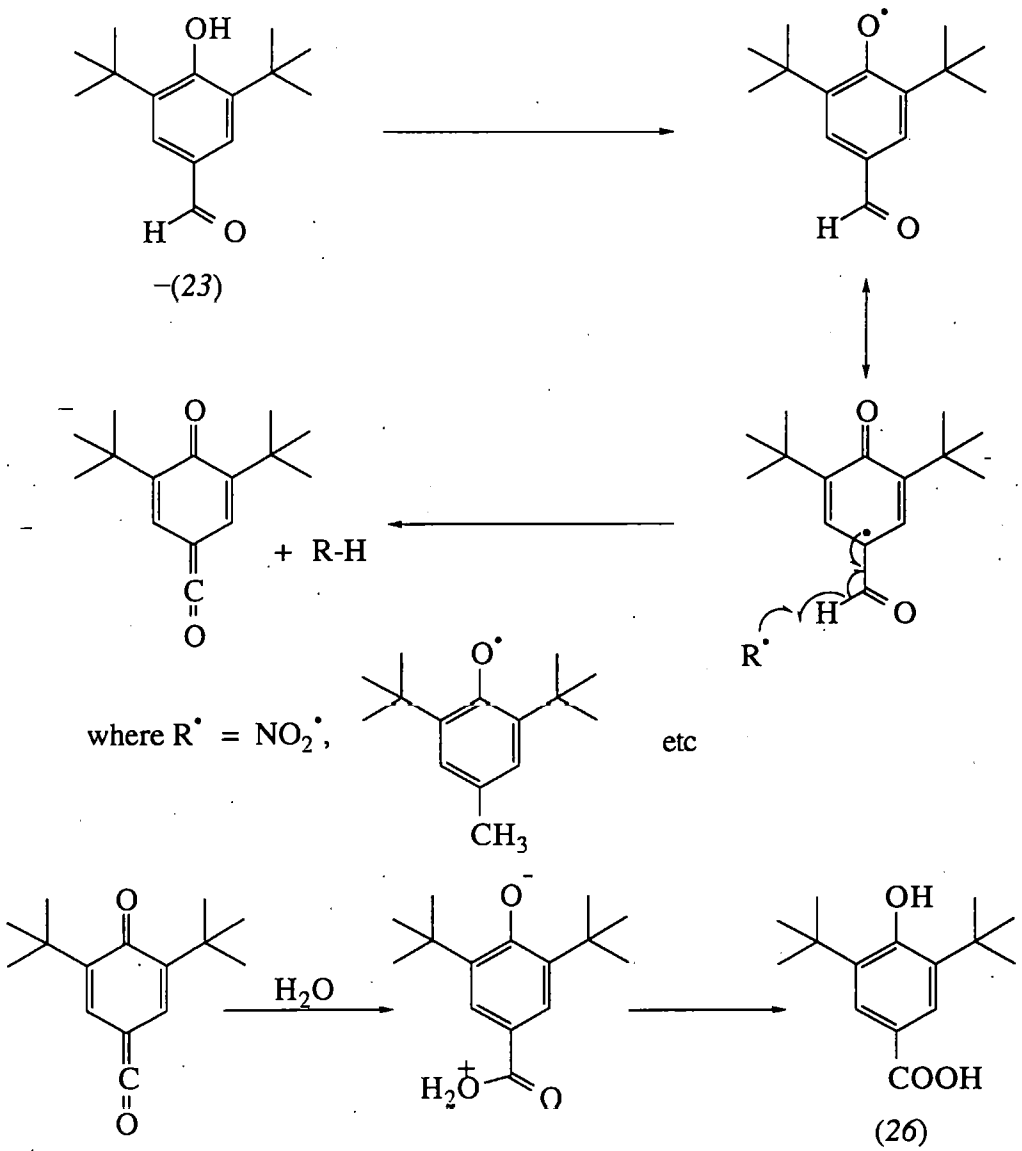
Increasing the concentration of BHT ten-fold (Reaction 10), but otherwise maintaining the conditions of the reaction, leaves some substrate unreacted and gives a

smaller range of products. The primary products MQOL, MQN and MHPQ dominate but the secondary products BALC and EQOL are also observed. The excess BHT present favours the formation of primary products, with the secondary products (such as BAC, BALD and NP) only being formed when the bulk of the BHT has been consumed. The reaction involving the same amount of NO_2 with a lower concentration of BHT over a longer period of time (Reaction 8) leaves less BHT unreacted. The main portion of the products are those formed by autoxidation, this being more extensive than the reaction with NO_2. Nonetheless, the primary products MQOL and MQN are observed along with secondary products BALC, BALD and EQOL.

Scheme 2.8. Formation of BALD.



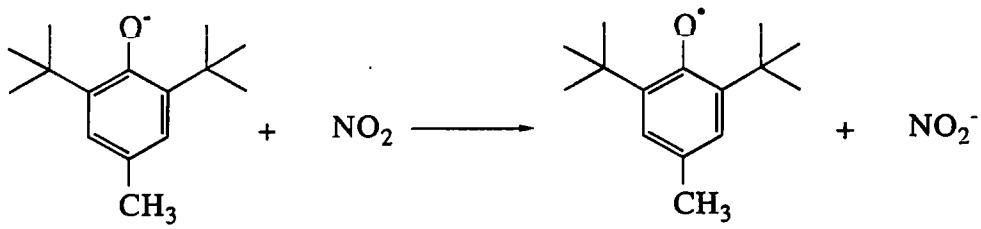
Scheme 2.9. Formation of BAC.



The effect of time on autoxidation and the reaction with NO_2 is seen when 12mM BHT is exposed to 200vpm NO_2 for 0.4 h, (Reaction 9) and for 4 h (Reaction 10). The shorter time results in only one product MQN and no autoxidation products. It appears that decreasing the concentration of either NO_2 or BHT produces a wider range of products. The decrease in concentration of NO_2 allows more chance of products involving reaction with oxygen to be formed, whereas decreasing the BHT concentration allows more chance for the formation of secondary products.

So far we have been concerned with BHT in alkaline solution, and have been considering the reactions of the phenolate anion. The effect of using neutral reaction conditions reduces the range of products. Thus, reaction of 1mM BHT under neutral conditions for 0.4 h (Reaction 7) gives only MQN, MQOL and BALC (cf. Reaction 5 in alkali discussed earlier). This reflects the higher concentration of phenoxy anions (*28*) in alkaline solution leading to more electron transfer and therefore a higher concentration of phenoxy radicals (*5*) (Scheme 2.10).

Scheme 2.10. Reaction of phenoxy anion with nitrogen dioxide



(*28*) (*5*)

All of the products detected, except the nitrite ester and EQOL, have previously been observed by Wilson (1989). However, the coupled products DPQ and SQ reported by Wilson were not found in the present study (standard RT 33.87 min, $PuP_{215-367}$ 327.1 nm and 36.1 min, $PuP_{215-367}$ 268.7 nm, respectively) and the only coupled products observed were seen in GC-MS (m/z 414 and m/z 398). The reason for this may be the relatively concentrated BHT solutions, used for GC assays, coupled with an elevated temperature leading to the formation of dimers within the GC injector. Wilson's detection of dimeric products may also relate to locally high concentrations of BHT as spots on TLC plates. In

the absence of dimeric compounds, the yellow colouration of reaction solutions has to be attributed to the formation of NP.

2.2.3. Maturation of solutions

During the HPLC analysis, it was noted that the reaction solutions were unstable unless acidified. Samples were therefore quenched in 0.1M HCl on sampling prior to analysis by HPLC. In order to investigate further the relationship between products, changes in the unquenched reaction solutions were monitored over several hours.

The maturation of the 0.4 h reaction solution over 73 h resulted in a decrease in the concentration of MQN (0.013mM to 0.002mM) and increase in an unassigned component at 19.9 min (0.014mM to 0.023mM), equivalent to the peak observed previously at 17.1 min. The concentrations of MQOL, MHPQ, BALC and BHT remained unchanged (Figure 2.1).

After 22 h, the 4 h reaction solution also showed a decrease in the concentration of MQN (0.088mM to 0.001mM) and increases in MQOL (0.033mM to 0.068mM), BALD (0.025mM to 0.064mM) and the peak at RT 19.9 min (0.052mM to 0.209mM) (Figure 2.2). The concentrations of MHPQ, BALC, BHT and EQOL remain unchanged.

The peak at 19.9 min had the same retention time as BQ; however, the purity parameters for this peak are variable both immediately on sampling {upslope $PuP_{215-367}$ 247.2 nm, apex $PuP_{215-367}$ 237.2 nm and downslope $PuP_{215-367}$ 228.6 nm} and after 22h maturation {upslope $PuP_{215-367}$ 245.6 nm, apex $PuP_{215-367}$ 237.2 nm and downslope $PuP_{215-367}$ 222.6 nm) which is indicative of two, coeluting components. One of these components may be BQ, but the identity of the other is unknown.

It appears that the decrease in MQN concentration in both reaction solutions relates to an increase in the concentration of the unassigned peak at 19.9 min. Maturation studies of authentic 0.6mM MQN in 70% (v/v) ethanol/water show a decrease in the

concentration of MQN from 0.557mM to 0.042mM over 146 hr and an increase in unretained components, and therefore discount this explanation.

After 430 h the only product peak evident was an unretained peak at 3.7 min ($PuP_{215-367}$ 227.1 nm). Smeltz (1982) showed that MQN degrades to QM and that this compound was stable at concentrations less than 0.2%. The purity parameter for this peak is indicative of a quinonoid type compound. On this basis the peak observed at 3.7 min, resulting from the degradation of MQN could be assigned to QM.

2.2.4. Investigation of yellowing component

The HPLC analysis of BHT-NO_2 reaction solutions show no evidence of the BHT-coupled products observed in previous studies (Wilson, 1989). The intense yellow colouration produced from the reaction of 1.2mM BHT must therefore be attributed to the formation of NP. HPLC investigations of the solutions using a detector wavelength of 450nm confirm the presence of NP in the reaction solution. Furthermore, the UV-vis spectra of authentic 1mM NP in 70% (v/v) ethanol/0.1M NaOH and of the reaction solution show a common absorbance band at 450nm (Figure 2.3), although there is little correlation at shorter wavelengths due to the presence of other compounds in the reaction mixture.

Figure 2.1. Variation in concentration of products with time for 0.4h BHT reaction.

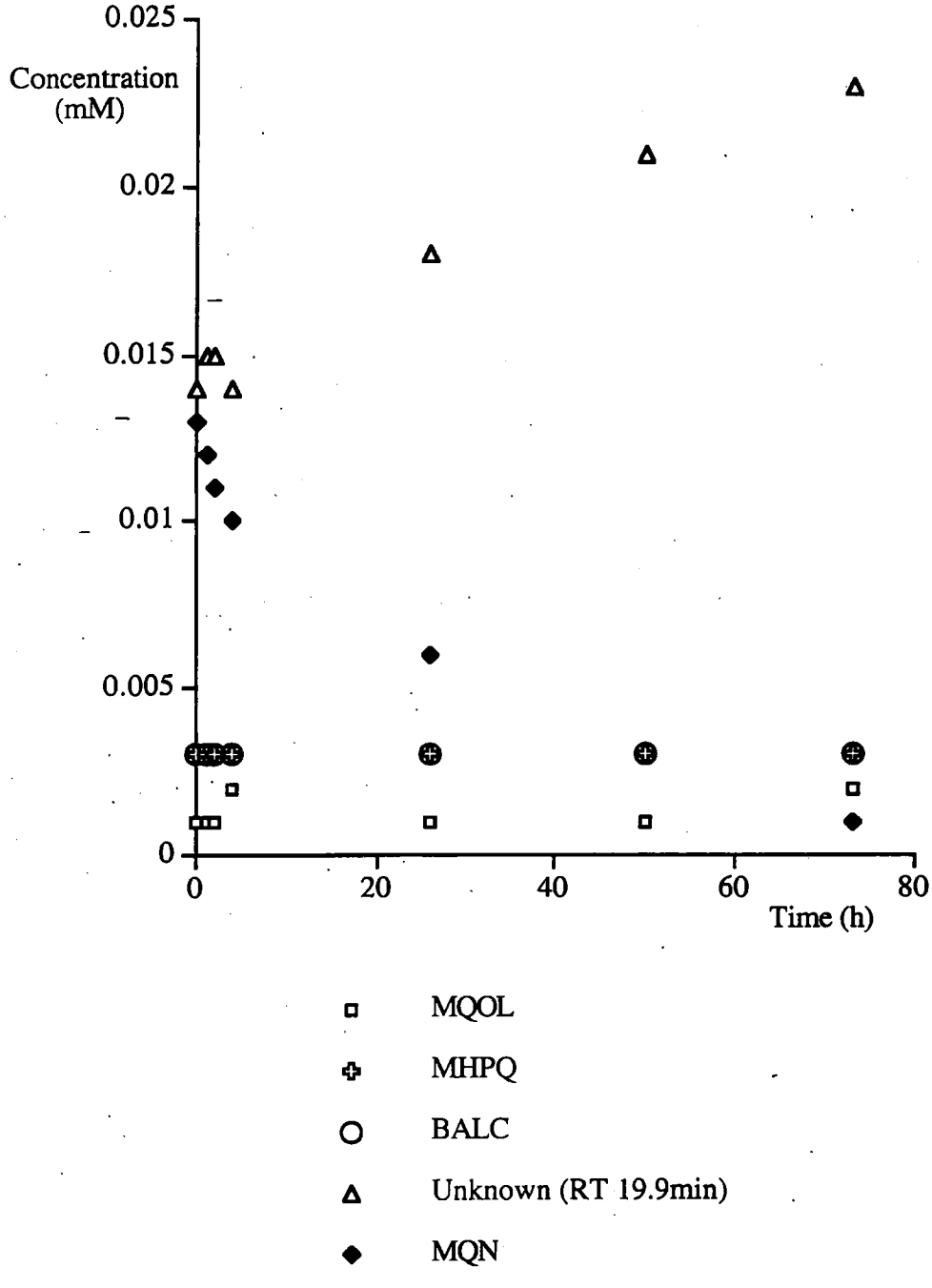


Figure 2.2. Variation in concentration of products with time for 4h BHT reaction.

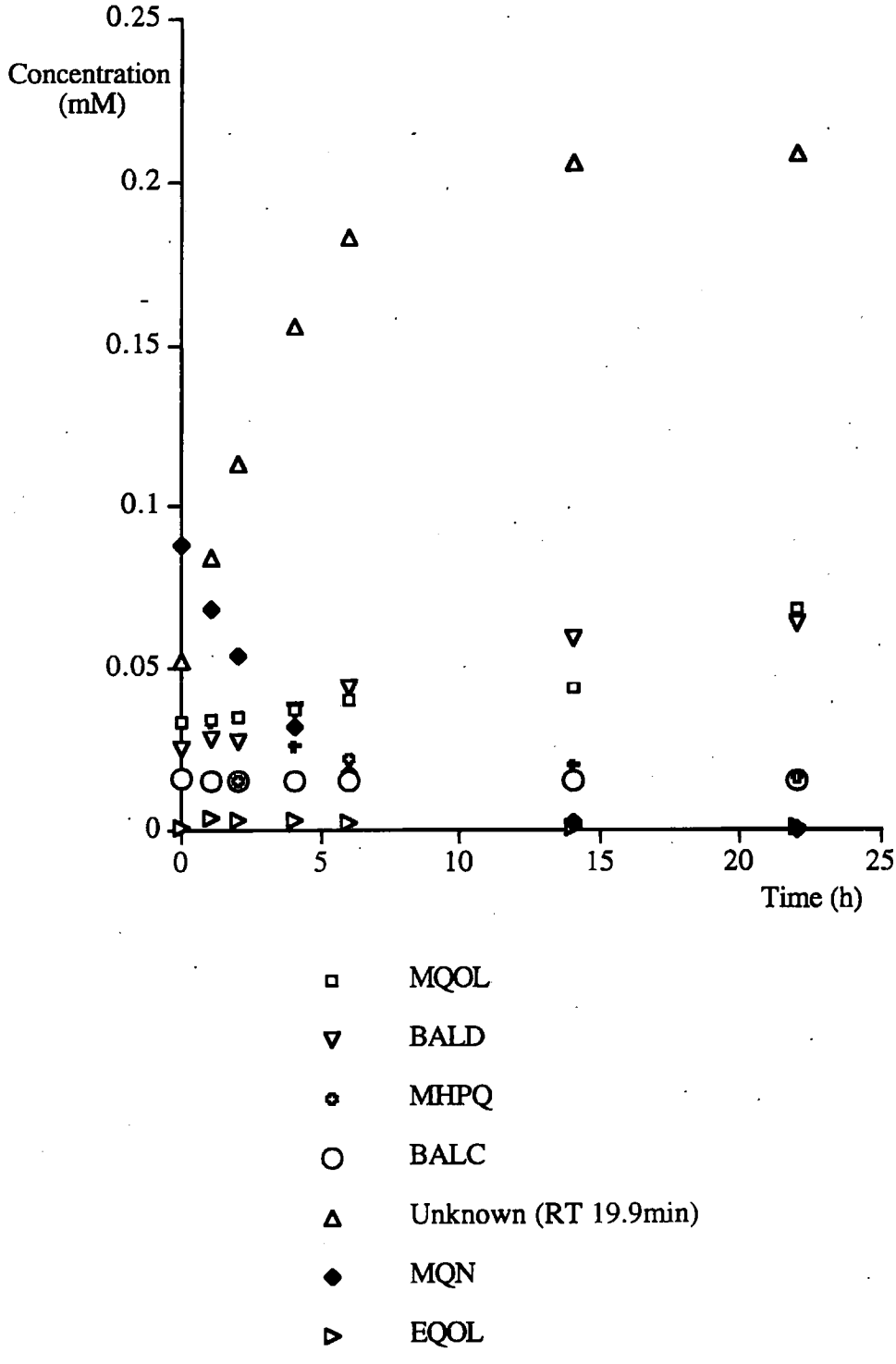
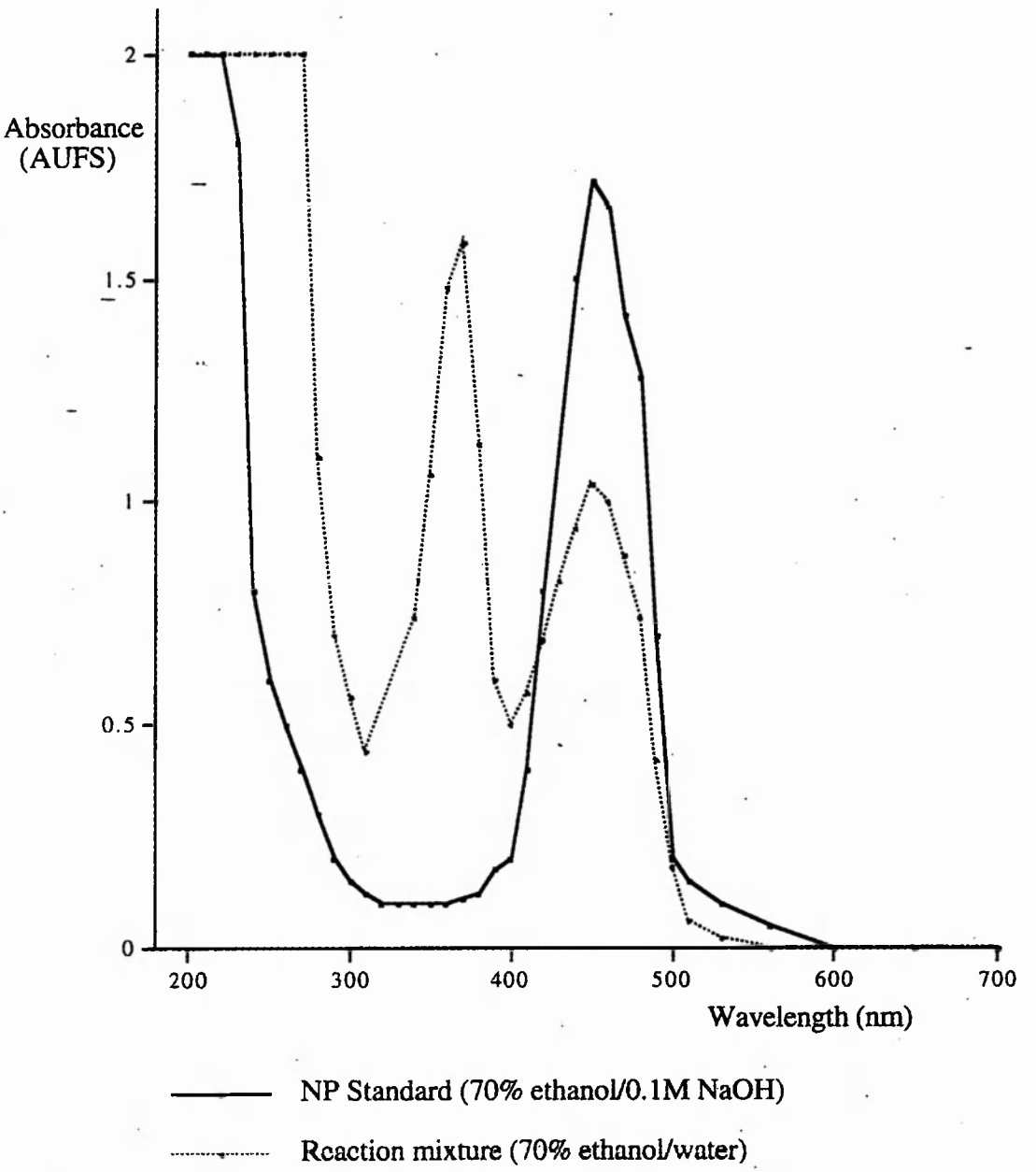


Figure 2.3. UV-vis spectra of a NP standard and reaction solution.



2.2.5. Summary

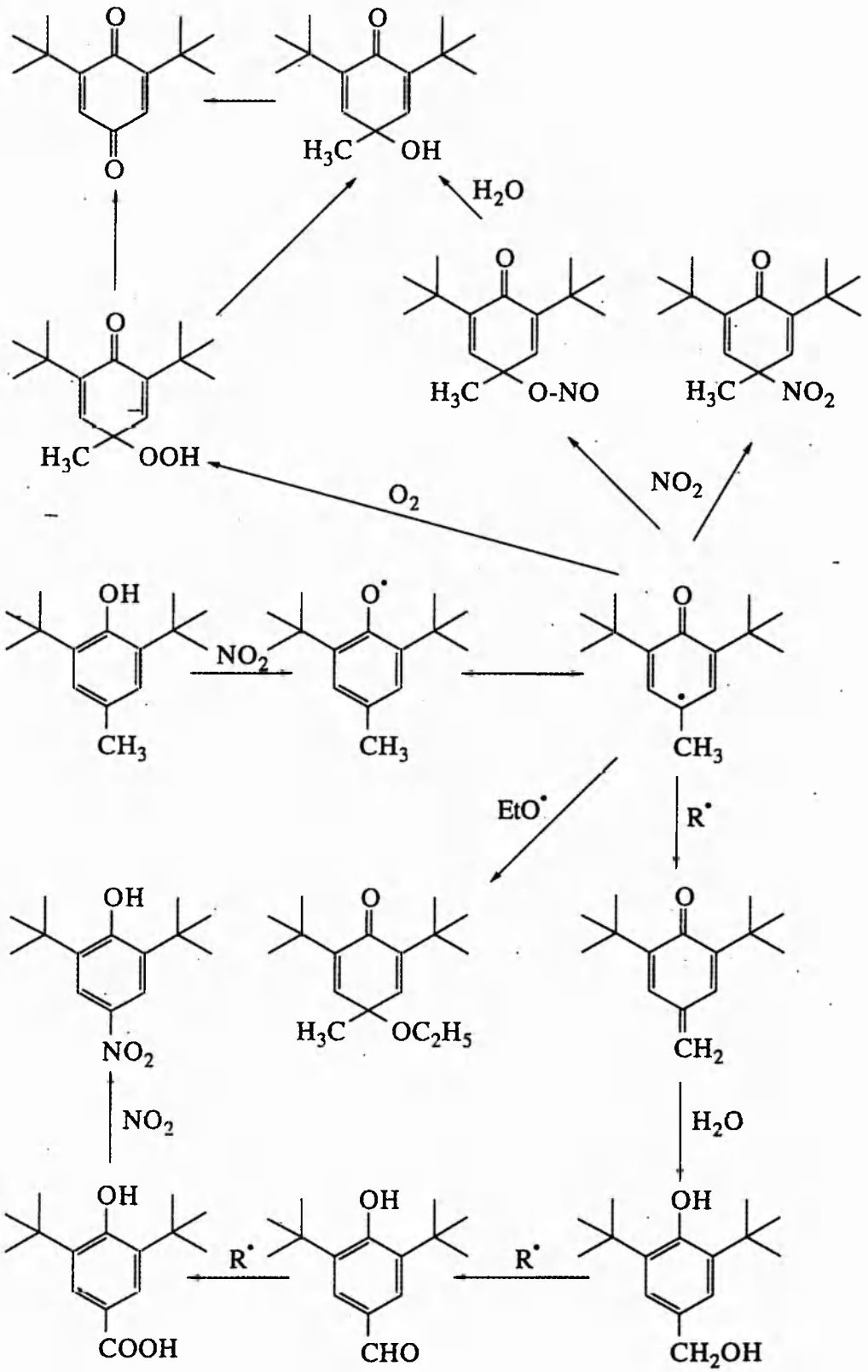
The GC data qualitatively confirm the formation of MQOL, BALD and NP by retention times. The GC-MS data allow further identification of MHPQ, BAC and EQOL. There remain several unidentified products. The HPLC data show that the main products of the reaction between NO_2 and BHT are MQOL and MQN. Further products are observed when lower concentrations of BHT and NO_2 are employed, where BALC, BAC, BALD, NP and EQOL have been identified. The proposed formation of products is shown in Scheme 2.11. The major product, MQN has been shown to be unstable in aqueous ethanolic solutions, probably degrading to QM. The yellowing component of the reaction solutions has been identified as NP and not the coupled compounds observed previously (Wilson, 1989).

2.3. Conclusions

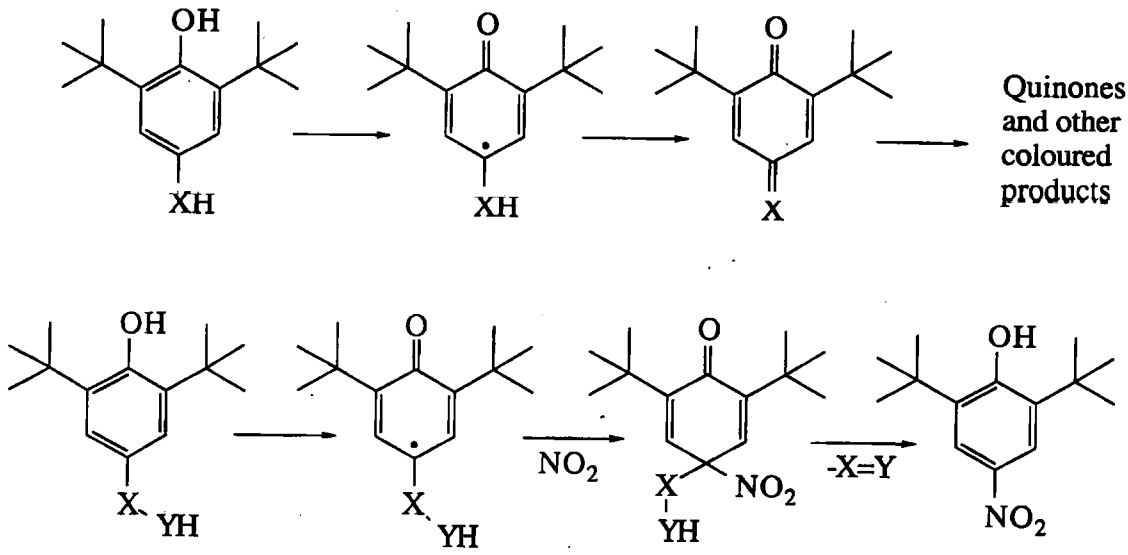
BHT reacts in solution to produce several products, *via* the action of NO_2, oxygen and secondary reactions. The principle yellow component of the reaction is the secondary product 2,6-di-*t*-butyl-4-nitrophenol, although a minor contribution could arise from quinonoid compounds. Therefore NO_2-yellowing of antioxidants may be avoided either by rejecting electron rich aromatic compounds altogether or inhibiting their nitration by suitable structural modification. The results of the investigation of various non-aromatic compounds incorporating a furanone or pyranone ring are reported in the following chapter.

Further consideration of the nitration process suggests that yellow products are formed from the reaction of NO_2 with 4-substituted hindered phenols in a manner outlined by Scheme 2.12.

Scheme 2.11. Proposed formation of BHT products.



Scheme 2.12. Origins of NO_2-yellowing of phenolic antioxidants



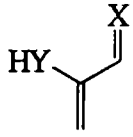
This suggests that two important criteria for avoiding a yellowing reaction with NO_2 are the absence of hydrogen atoms on either X or Y in 4-substituted phenols. New compounds meeting these criteria are the subject of Chapter 4.

CHAPTER 3

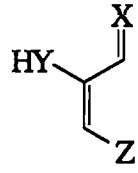
Evaluation of selected non-phenolic compounds

3.1. Introduction

In Chapter 1, potential antioxidant properties were associated with compounds having one of two general structures (*13*) and (*14*).



(*13*)

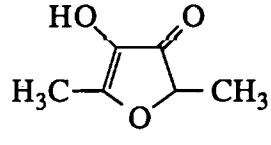


(*14*)

where: X - heteroatom or carbon
Z,Y - heteroatom

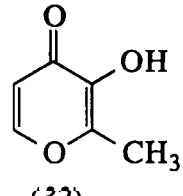
A search of the literature showed that these structural features are present in commercially available compounds (*31*) to (*34*), known to possess antioxidant activity.

2,5-Dimethyl-4-hydroxyfuranone (*31*) has been identified as an antioxidant formed in the L-cysteine/Maillard reaction (Eiserich *et al.*, 1992).



(*31*)

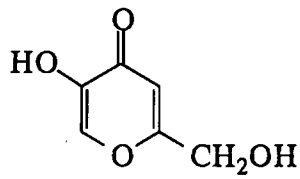
3-Hydroxy-2-methyl-4-pyranone or maltol (*32*) is effective in retarding lipid peroxidation (Kim and Yi, 1982), and has been identified as an antioxidant component of ginseng (Shin *et al.*, 1990, Han *et al.*, 1985).



(*32*)

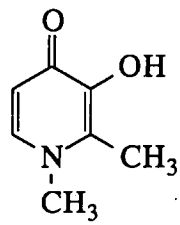
5-Hydroxy-2-hydroxymethyl-4-pyranone or kojic acid (*33*) retards oxidation by metal chelation (Morel *et al.*, 1992), and several Japanese patents claim kojic acid as an

antioxidant in food (Sansho, 1981 and Sansei, 1982) and in cosmetics (Egawa *et al.*, 1990).



(*33*)

1,2-Dimethyl-3-hydroxypyridone (*34*) has also been shown to retard oxidation by metal chelation (Van der Kraaij *et al.*, 1989).



(*34*)

The aim of work reported in this Chapter was to develop methods for determining both antioxidant activity and NO_2 yellowing, and to apply these methods to selected non-phenolic compounds. For the most promising candidates, products formed by reaction with gaseous NO_2 were briefly examined by GC-MS, with a view to identifying structural modifications that might improve their performance and acceptability.

3.2. Evaluation of antioxidant activity

Many procedures to measure antioxidant activity have been described in Section 1.5. and are reviewed by Scott (1993). As the present work was primarily concerned with radical trapping antioxidants, three methods of measuring this behaviour were examined for their suitability to quantitate the antioxidant activity of selected test compounds.

3.2.1. Oxidation of linoleic acid

The oxidation of linoleic acid has been widely used to measure antioxidant activity (Tanizawa *et al.*, 1983, Ragnarsson *et al.*, 1977 and Tamura and Shibamoto, 1991). The method of Tanizawa *et al.*, (1983) appears to be a convenient and efficient screen for antioxidant compounds. It utilises the oxidation of linoleic acid by air bubbling, and determination of the resultant peroxide content (POV) by titration and thiobarbituric acid (TBAV) by UV. These parameters are then used to calculate an Inhibitory Ratio (I.R.) (Equation 3.1.), which is a measure of antioxidant activity. The method was briefly examined to determine the antioxidant activity of the selected test compounds.

$$\text{I.R. (\%)} = \frac{(A - B) \times 100}{(A - C)} \qquad (3.1)$$

where, A - POV (TBAV) of linoleic acid after incubation,

B - POV (TBAV) of linoleic acid after incubation with an antioxidant,

C - POV (TBAV) of linoleic acid before incubation.

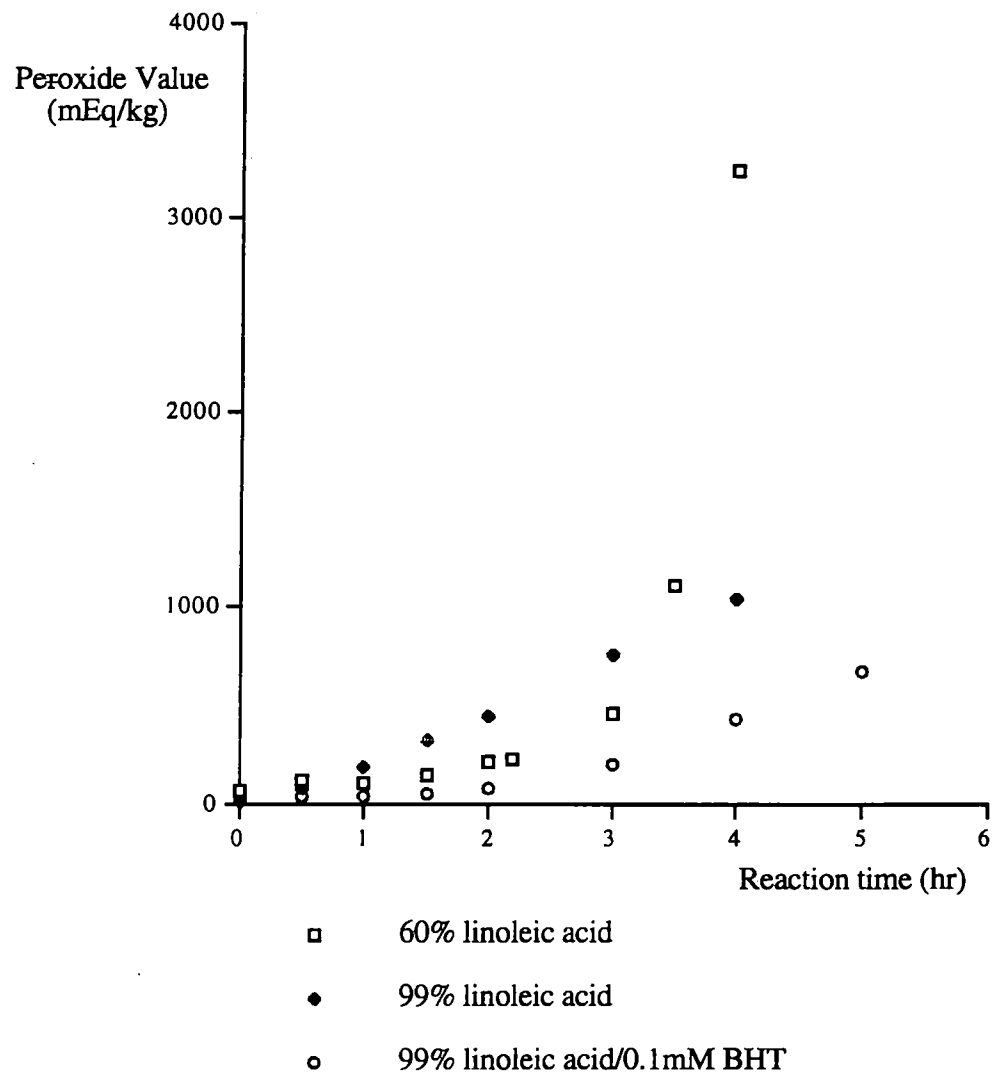
Initial experiments carried out over a period of two weeks, using 60% linoleic acid (the remainder being oleic acid, 23% and linolenic acid 6%) in the absence of antioxidant, gave irreproducible peroxide (POV) and thiobarbituric acid (TBAV) values (Table 3.1.). The variation in POV was attributed to the slow aerial oxidation of linoleic acid, which produced increasing peroxide values that differed significantly from those obtained by Tanizawa *et al.* (1983). However, the variation in TBAV could not be explained and further work with 60% linoleic acid was therefore discontinued.

Table 3.1. Peroxide and thiobarbituric acid values for the oxidation of linoleic acid (60%).

Run Number	POV (mEq/kg)	TBAV (abs/g)
1	6.2	338
2	11.5	-
3	18	1.6
4	36	12.4

The variation of POV with the duration of air bubbling using 60% linoleic acid, 99% linoleic acid and 99% linoleic acid containing 100µl 0.1mM BHT was determined by the removal and assay of aliquots of the linoleic acid at timed intervals. The results (Figure 3.1.) suggest that some form of inhibition occurs for the 60% linoleic acid as the plot shows a general similarity with that for 99% linoleic acid containing the antioxidant BHT. This unexpected inhibition may account for the discrepancies between the initial experiments using 60% linoleic acid and those of Tanizawa *et al.* The 99% linoleic acid shows the linear variation of POV with time reported by Tanizawa *et al.*, and it was therefore used in all subsequent experiments.

Figure 3.1. Variation in POV with time



The I.R. values of BHT and two of the selected compounds using 99% linoleic acid are summarised in Table 3.2. These experiments were carried out by adding 100µl aliquots of 0.1mM antioxidant in decahydronaphthalene to $5cm^3$ of linoleic acid in the reaction vessel to give an antioxidant concentration of 2mM. The very low values for both maltol and 2,5-dimethyl-4-hydroxyfuranone may be misleading insofar as the high temperature and air flows necessary for the procedure, result in the loss of compounds more volatile than BHT. Further, compared to the I.R. of 50% for a similar concentration of BHT reported by Tanizawa *et al.* (1983), the large I.R. value obtained for BHT here suggests that concentrations of 2mM antioxidant are too high to allow significant oxidation of the linoleic acid to critically test relative antioxidant activities.

Table 3.2. Inhibitory ratios for selected test compounds.

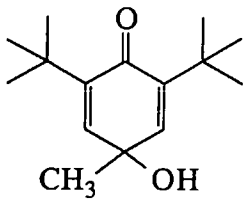
Test Compound	Inhibitory Ratio (%)
BHT	92.4
Maltol	22.8
2,5-Dimethyl-4-hydroxyfuranone	5.0

It appears that linoleic acid oxidation is intrinsically unsuitable for assessing the antioxidant activities of the volatile compounds under investigation and alternative procedures were therefore examined.

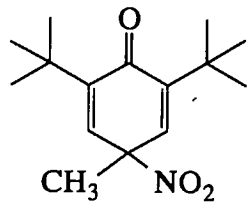
3.2.2. Competitive inhibition of BHT-NO_2 products

Competitive inhibition of the formation of products from the oxidant and substrate in the presence of an antioxidant is a potential method for the measurement of antioxidant efficiency. The reaction of BHT with gaseous NO_2 could be used in this way.

The reaction of BHT with NO_2 leads to the formation of the products MQOL (*22*) and MQN (*24*) via an initial electron transfer. Thus, the effect of added antioxidants on the formation of these products should assess antioxidant activity relative to BHT.



(*22*)



(*24*)

The exposure of 10mM BHT in 70% ethanol/water to 900vpm NO_2 for 1h was used as a control, and compared with reactions under similar conditions using 10mM BHT and 10mM of the test antioxidant. The reaction solutions were then assayed for BHT-NO_2 products by HPLC. The results are summarised in Table 3.3.

Table 3.3. Concentration of BHT products for competitive inhibition experiments.

Test Compound	[MQOL] (mM)	[MQN] (mM)	[Total] (mM)
BHT	0.017	0.140	0.157
Vitamin C	0.001	0.005	0.006
Kojic acid	0.008	0.007	0.015
Maltol	0.008	0.062	0.070
1,2-Dimethyl-3-hydroxypyridone	0.008	0.127	0.135
2,5-Dimethyl-4-hydroxyfuranone	0.020	0.157	0.177

The results for Vitamin C illustrate the high degree of inhibition attained by a compound known to possess antioxidant activity (Hudson, 1990). The other results show that kojic acid and maltol inhibit the formation of BHT-NO_2 products, but less effectively than Vitamin C. The lack of inhibition by 2,5-dimethyl-4-hydroxyfuranone may reflect its

volatility (Eiserich *et al.*, 1992), and that by 1,2-dimethyl-3-hydroxypyridone is not understood.

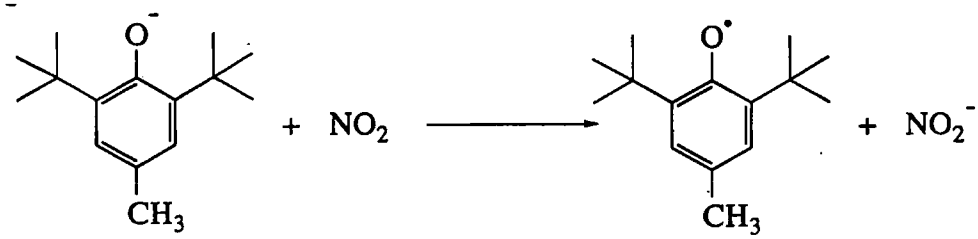
3.2.3. NO_2^-/NO_3^- ratio

The hydrolysis of NO_2 via the N_2O_4 dimer gives one mole of NO_2^- and one mole of NO_3^-, resulting in a NO_2^-/NO_3^- ratio of one (Equation 3.2).

$$N_2O_4 + 2HO^- \longrightarrow NO_2^- + NO_3^- + H_2O \qquad (3.2)$$

When electron transfer occurs, as in the presence of an antioxidant, this ratio is increased by the formation of additional NO_2^- (Scheme 3.1.). The NO_2^-/NO_3^- ratio may therefore provide a measure of antioxidant efficiency.

Scheme 3.1. Formation of NO_2^- by electron transfer with BHT.



The effect of test compounds on the NO_2^-/NO_3^- ratio was determined by exposure of a 10mM solution of test compound in 70% (v/v) ethanol/0.1M sodium hydroxide to NO_2 (900vpm) at 25°C. Aliquots of solution were analysed for NO_2^- and NO_3^- by ion chromatography.

The results summarised in Table 3.4 show that 2,5-dimethyl-4-hydroxyfuranone participates most readily in electron transfer to NO_2, and maltol the least. The similar NO_2^-/NO_3^- ratios for BHT and Vitamin C illustrate the effectiveness of BHT as an antioxidant.

Table 3.4. Normalised* NO_2^-/NO_3^- ratios.

Compound	NO_2^-/NO_3^- Ratio
2,5-Dimethyl-4-hydroxyfuranone	12.6
BHT	10
Vitamin C	9.8
Kojic acid	7.8
Maltol	2.9

* - Values normalised to BHT.

The NO_2^-/NO_3^- ratio is, of course, a measure of electron transfer capability only. The contribution of radical trapping by hydrogen donation to antioxidative efficiency is determined by other factors, such as radical stability and ease of hydrogen atom abstraction.

3.2.4. Summary

The assessment of antioxidant efficiency by the inhibition of linoleic acid oxidation is unsuitable for volatile compounds such as 2,5-dimethyl-4-hydroxyfuranone. The BHT-NO_2 product and NO_2^-/NO_3^- ratio experiments confirm that kojic acid, maltol and 2,5-dimethyl-4-hydroxyfuranone are antioxidants. These methods, however only assess the compound's ability to act as an electron transfer agent to $NO_2^{\bullet}$. As well as being relevant to the NO_2-yellowing problem, both methods provide an initial screening for antioxidant activity rather than a quantitative evaluation.

3.3. NO_2-yellowing

Two BSI methods exist for the measurement of colour changes in fabrics and polymers. Thus, the discolouration of fabrics is determined by visual comparison of fabric strips to a scale of grey intensity (BSI, 1990), whilst that of plastics involves the measurement of transmittance at 420nm (BSI, 1976). A method specific to NO_2-yellowing developed by McEwan and Murdoch (1981) involves the saturation of a methanolic solution of the test compound with gaseous NO_2 followed by determination of a yellowing index by UV measurements.

A method based on that of McEwan and Murdoch (1981) was used here. Thus, the NO_2 yellowing of the test compounds was determined by exposure of a 10mM antioxidant in 70% (v/v) methanol/0.05M phosphate buffer at pH 7.0 and 25°C to 900vpm NO_2 for 7 h, followed by development of colour for 15 h at 25°C and measurement of the UV-vis spectrum (200-700nm). The results for five antioxidants expressed as the OU yellowing index (OU_{YI}, defined in Section 6.3.1.) are reported in Table 3.5.

Table 3.5. OU yellowing indices (OU_{YI}) for the test compounds.

Antioxidant Compound	OU Yellowing Index (OU_{YI})
BHT	8.06
2,5-Dimethyl-4-hydroxyfuranone	0
Maltol	0.64
Kojic acid	3.86
1,2-Dimethyl-3-hydroxypyridone	36.7

These data show that kojic acid, maltol and 2,5-dimethyl-4-hydroxyfuranone are less prone to NO_2-yellowing than BHT. This is not surprising as none of these compounds can react to generate the aromatic nitration products associated with the

yellowing of BHT. The large OU_{YI} value for 1,2-dimethyl-3-hydroxypyridone is unexpected and discounts this compound as an alternative to BHT.

3.4. NO_2-reactions

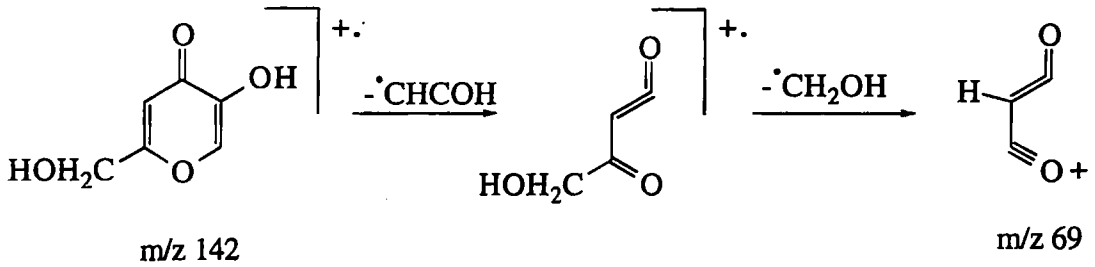
The antioxidant efficiency and NO_2-yellowing data suggests that kojic acid, maltol and 2,5-dimethyl-4-hydroxyfuranone are potential replacement antioxidant compounds for BHT. All of these compounds have other properties (e.g. odour, high volatility), however, that makes them impractical in this role. The question then arises whether these compounds can be structurally modified to minimise their undesirable properties. Unfortunately, insufficient time was available to explore this prospect, but as a preliminary to further deliberations an attempt was made to characterise the products formed by the reaction of gaseous NO_2 with the alternative antioxidants. This work, too, was not completed as planned due to insufficient time. However, the reactions of kojic acid in organic solvents with relatively high concentrations of gaseous NO_2 were carried out, and the reaction mixtures examined by GC and GC-MS. Results and conclusions from these preliminary experiments are briefly reported below.

3.4.1. Kojic acid

Kojic acid (0.02M) dissolved in methyl formate or dioxane, was allowed to react with gaseous NO_2 (4mM) at 25°C. After work up with methanol, the extracts were analysed by GC and GC-MS. Blank reactions with solvent and gaseous NO_2 were carried out to distinguish peaks arising from the reaction of NO_2 with the kojic acid rather than solvent. The GC output for reactions in dioxane (Figure 3.2.) and methyl formate (Figure 3.3.) suggest the formation of a common, major volatile product (Scan 765 and Scan 712 respectively) along with 5-7 other minor products. Attempts to characterise these products via GC-MS in both EI and CI modes proved unsuccessful.

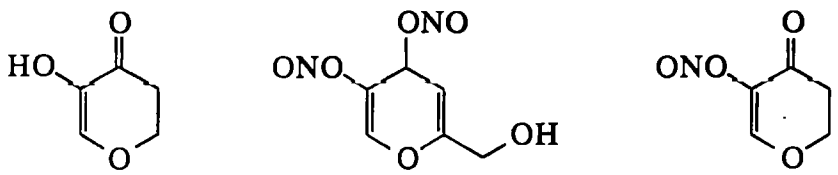
Studies on the MS fragmentation of 4-pyranones (Katritzky, 1984), such as kojic acid and maltol, show that an ion peak at m/z 69 is formed as a result of retro-Diels-Alder cleavage of the molecular ion and subsequent loss of the side chain. The process is illustrated for kojic acid in Scheme 3.2.

Scheme 3.2. Retro-Diels-Alder cleavage of kojic acid.



m/z 142 m/z 69

These characteristic and other fragment ions suggest the following structures for products (*35*) {m/z 114 (M^+, 64%), 85 (57), 69 (100)} and (*36*) {m/z 202 (M^+, 2%), 172 (2), 143 (92), 101 (72), 69 (46), 44 (100)} from reactions in dioxane, and (*37*) {m/z 143 (M^+, 100%), 115 (58), 69 (78), 59 (53), 44 (50)} and (*36*) {m/z 202 (M^+, 2%), 172 (2), 143 (92), 101 (72), 69 (46), 44 (100)} from reactions in methyl formate.



(*35*) (*36*) (*37*)

These structural assignments are tentative, but if confirmed, the products show that under the present reaction conditions, gaseous NO_2 acts as a nitrosating agent towards kojic acid.

Figure 3.2. GC-MS chromatogram of the products from the reaction of 0.02M kojic acid in dioxane with gaseous NO_2 (4mM) at 25°C.

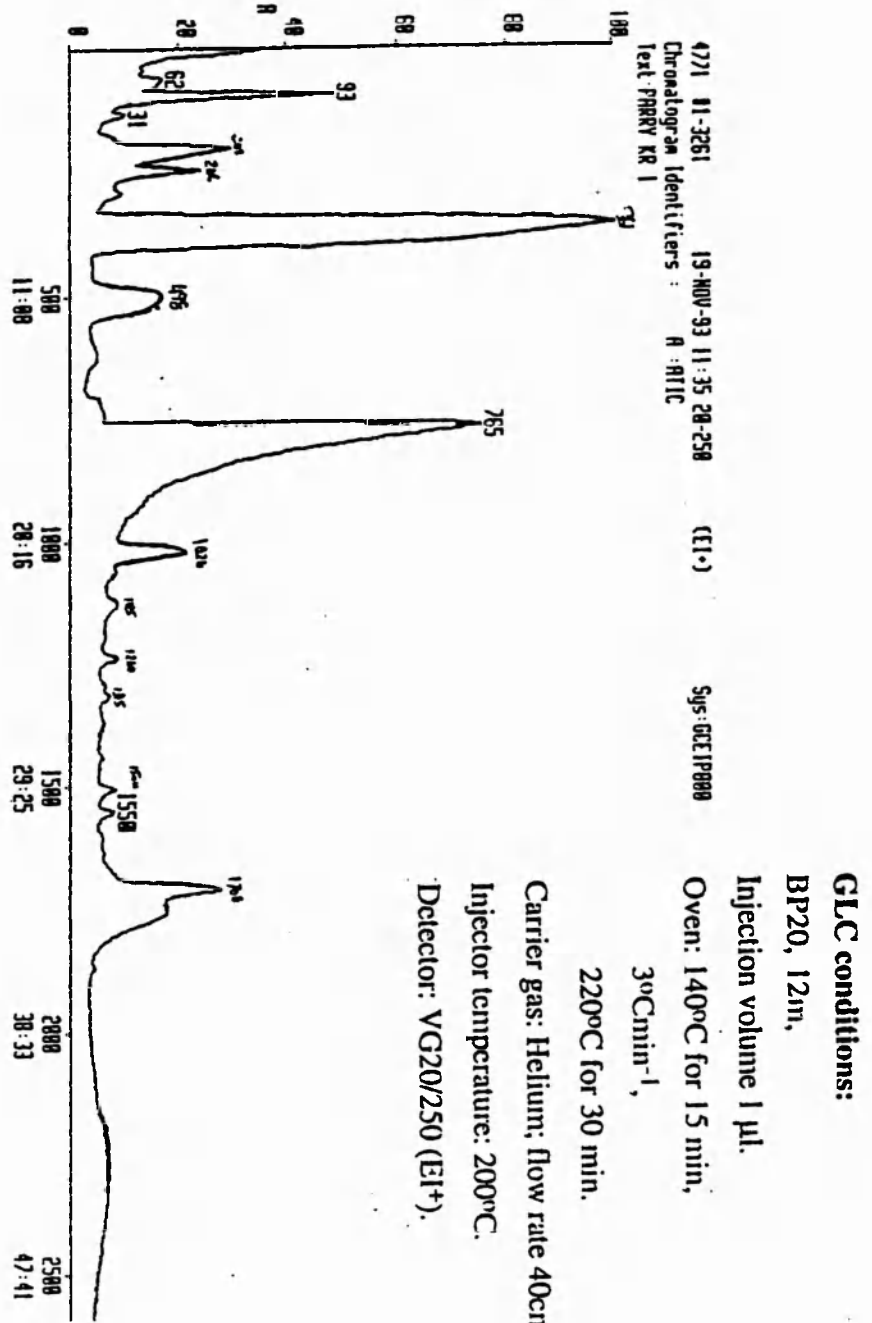
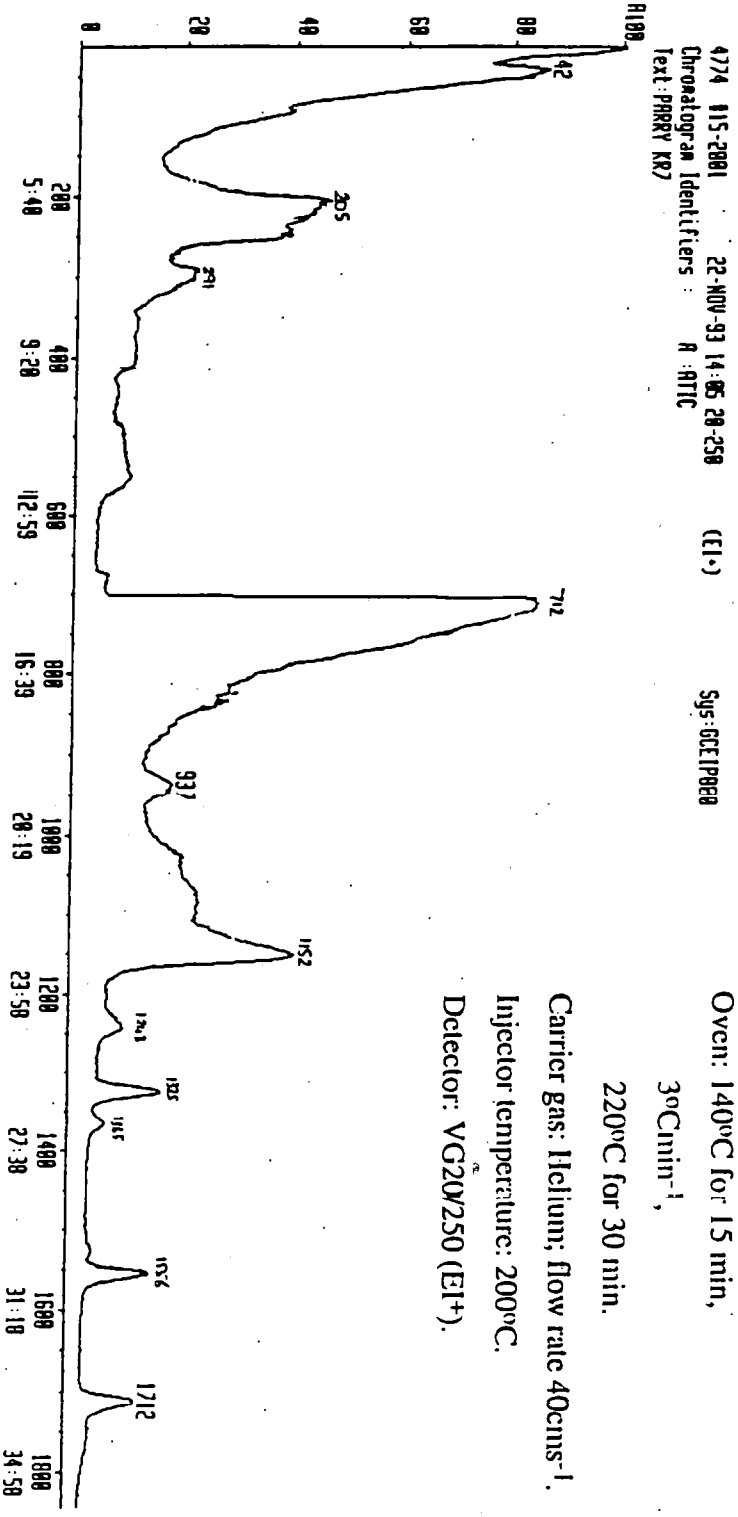


Figure 3.3. GC-MS chromatogram of the products from the reaction of 0.02M kojic acid in methyl formate with gaseous NO_2 (4mM) at 25°C.



GLC conditions:

BP20, 12m,

Injection volume 1 µl.

Oven: 140°C for 15 min,

3°Cmin^{-1},

220°C for 30 min.

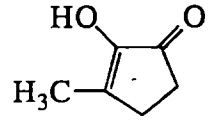
Carrier gas: Helium; flow rate 40cms^{-1}.

Injector temperature: 200°C.

Detector: VG20/250 (EI+).

3.4.2. 3-Methyl-1,2-cyclopentanedione

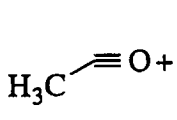
In an attempt to understand the formation of products from the reaction of kojic acid with gaseous NO_2, the simpler diketone compound, 3-methylcyclopentane-1,2-dione (*38*) was investigated.



(*38*)

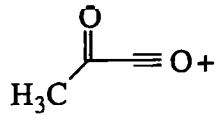
Although not proven here to have antioxidant activity, 3-methylcyclopentane-1,2-dione is chemically similar to 2,5-dimethyl-4-hydroxyfuranone and possesses the structural characteristics necessary for radical stabilisation.

3-Methylcyclopentane-1,2-dione dissolved in methyl formate was allowed to react with gaseous NO_2 (4mM) at 25°C. After work up with methanol, the extracts were also analysed by GC and GC-MS. Blank reactions with solvent and gaseous NO_2 were carried out to distinguish peaks arising from the reaction of NO_2 with the 3-methylcyclopentane-1,2-dione rather than the methyl formate. Molecular ions have been assigned to products so that ions of lower mass are derived by sensible fragmentation. In particular the loss of 15 and 18 mass units from a molecular ion being indicative of the loss of methyl and hydroxy substituents, respectively. Further, the presence of m/z 43, m/z 71 and m/z 99 within spectra lends support for the presence of the ions (*39*), (*40*), and (*41*).



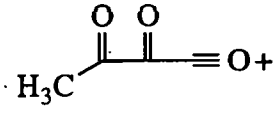
m/z 43

(*39*)



m/z 71

(*40*)



m/z 99

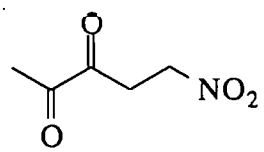
(*41*)

GC-MS analysis of the extract from the reaction of 0.025M 3-methylcyclopentane-1,2-dione in methyl formate with gaseous NO_2 at 25°C (Figure 3.4.) suggests the product distribution shown in Table 3.6. The formation of most of these

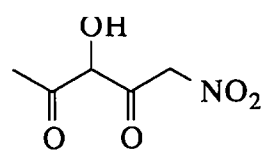
tentatively assigned products can be rationalised by a pathway where NO_2 reacts as an oxygen centered radical at the 3-position of the radical generated by hydrogen abstraction from 3-methylcyclopentane-1,2-dione (Scheme 3.3.).

This reaction appears to give rise to several products resulting from the cleavage of the weak nitrogen oxygen bond, which are further complicated by methylation from the methyl formate solvent. The formation of the diketone (*43*) (M^+ 128, Scan 2444); {identified by comparison with the library spectrum of the isomeric 3-hydroxy-5-methylcyclopentane-1,2-dione} could arise from the nitrite ester (*42*), itself produced by hydrogen abstraction from 3-methyl-1,2-cyclopentanedione by NO_2 followed by the addition of NO_2. The ring opening of diketone (*43*) could result in the formation of levulinic acid (*44*) (M^+ 116, Scan 2497) and 4-acetoxy-2-butanone (*45*) (M^+ 130, Scan 1247) *via* subsequent reaction with methyl formate: both (*43*) and (*44*) have been confirmed by spectral comparison with authentic materials and GC retention times. The diketone (*43*) may also react further to form the two products (isomers *46a* or *46b*) and (*47*) (M^+ 142, Scan 2246 and Scan 2173). Products (*46*) and (*47*) may react with the methyl formate solvent in various ways to give (*48*) (M^+ 156, Scan 1710), (*49*) (M^+ 156, Scan 1976) and (*50*) (M^+ 174, Scan 1910).

Formation of the two N-containing products (*51*) and (*52*) {tentatively assigned in Table 3.6.} is difficult to rationalise. They may arise from extensive decomposition of the substrate or from an impurity. Insufficient time was available to pursue these matters further by either time-dependent product studies and/or the independent synthesis of authentic compounds.



(*51*)



(*52*)

Scheme 3.3. Proposed pathway for the reaction of 3-methylcyclopentane-1,2-dione and NO_2 as an oxygen centered radical.

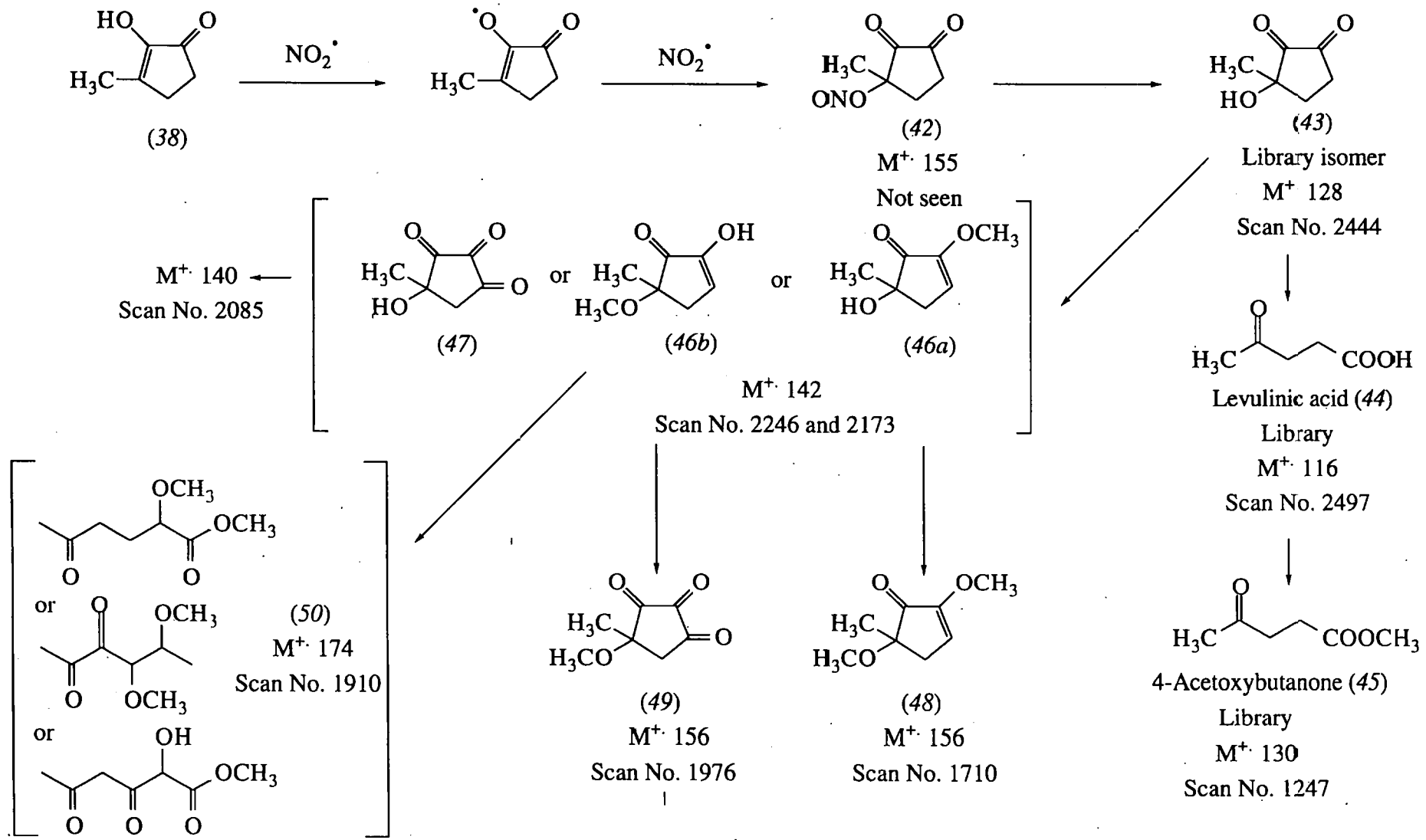
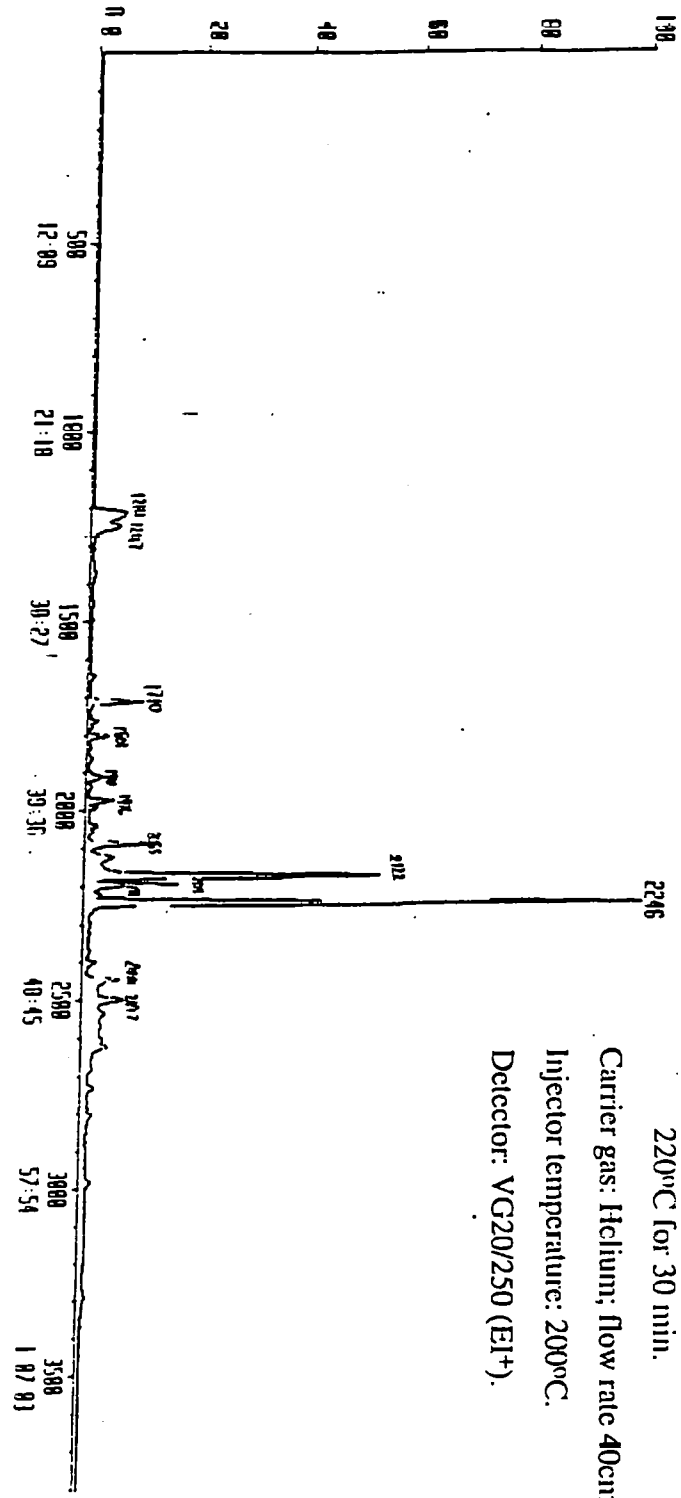


Figure 3.4. GC-MS chromatogram of the products from the reaction of 0.025M 3-methyl-1,2-cyclopentanedione in methyl formate with gaseous NO_2 (4mM) at 25°C.



GLC conditions:

BP20, 12m,

Injection volume 1 µl.

Oven: 90°C for 10 min,

2°Cmin^{-1},

220°C for 30 min.

Carrier gas: Helium; flow rate 40cms^{-1}.

Injector temperature: 200°C.

Detector: VG20/250 (EI+).

Table 3.6. Relative intensities and fragmentation of major products from the reaction of 0.025M 3-methyl-1,2-cyclopentanedione in methyl formate with gaseous NO_2 (4mM) at 25°C.

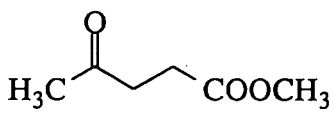
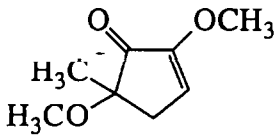
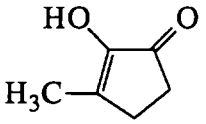
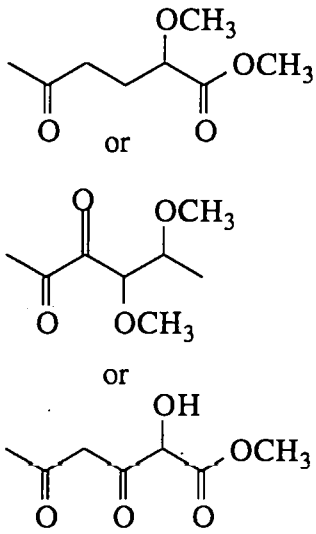
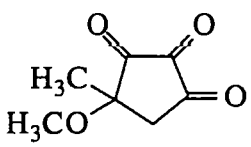
Scan No.	Product	Relative GC Peak Area [a]	Fragmentation (m/z)
1247	 4-Acetoxybutanone (*45*)	0.21	130 (3), 115 (21), 99 (23), 88 (11), 43 (100)
1710	 (*48*)	0.08	156 (17), 141 (4), 126 (100), 111 (13), 94 (70), 81 (30), 55 (72)
1801		0.26	112 (100), 97 (9), 83 (45), 69 (52), 55 (76), 41 (59)
1910	 (*50*)	0.27	174 (4), 159 (3), 118 (16), 99 (12), 43 (100)
1976	 (*49*)	0.27	156 (32), 141 (15), 126 (72), 113 (75), 81 (69), 72 (89), 53 (83), 43 (100)

Table 3.6. Relative intensities and fragmentation of major products from the reaction of 0.025M 3-methyl-1,2-cyclopentanedione in methyl formate with gaseous NO_2 (4mM) at 25°C (cont.).

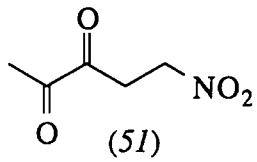
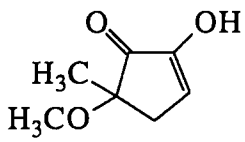
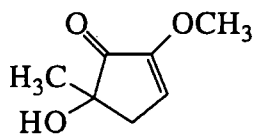
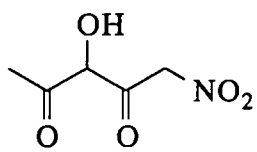
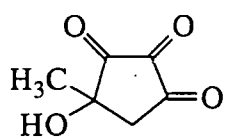
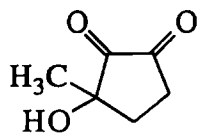
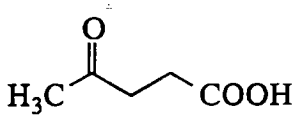
Scan No.	Product	Relative GC Peak Area [a]	Fragmentation (m/z)
2085	Unknown	0.23	140 (2), 116 (2), 109 (4), 99 (63), 71 (18), 43 (100)
2122	 (*51*)	0.95	145 (4), 127 (6), 110 (32), 99 (35), 43 (100)
2173	 (*46b*) or  (*46a*)	0.22	142 (6), 127 (3), 112 (50), 99 (79), 43 (100)
2194	 (*52*)	0.03	175 (5), 158 (9), 126 (25), 88 (100), 69 (43), 58 (48), 43 (74)
2246	 (*47*)	1.00	142 (28), 125 (9), 114 (12), 99 (28), 71 (47), 43 (100)

Table 3.6. Relative intensities and fragmentation of major products from the reaction of 0.025M 3-methyl-1,2-cyclopentanedione in methyl formate with gaseous NO_2 at 25°C (cont.).

Scan No.	Product	Relative GC Peak Area [a]	Fragmentation (m/z)
2444	 (*43*) Library isomer	0.40	128 (25), 110 (13), 100 (9), 57 (42), 43 (100)
2497	 Levulinic acid (*44*)	0.13	116 (3), 101 (3), 99 (4), 73 (10), 56 (27), 43 (100)

[a] - relative to largest GC peak centred at Scan 2246.

3.4.3. Summary

GC-MS analysis of extracts from the reaction of kojic acid with gaseous NO_2 has identified some nitrosation products and several unidentified compounds. The chemistry of these processes is beyond the bounds of the present investigation, but similar studies with 3-methylcyclopentane-1,2-dione and gaseous NO_2 suggest reduction of NO_2 occurs with subsequent formation of addition and ring opened products. Due to extensive methylation of the products by methyl formate, further work should use a simpler solvent and work-up procedure.

3.5. Conclusions

The antioxidant testing methods reported above suggest that kojic acid, maltol and 2,5-dimethyl-4-hydroxyfuranone possess similar degrees of antioxidant activity to BHT and support the structural hypothesis for antioxidant properties by promotion of radical

stabilisation. The methods, however, only provide a screen for antioxidant activity rather than a quantitative assessment.

Results from the NO_2-yellowing tests suggest that kojic acid and maltol might be useful non-yellowing alternatives to BHT.

The investigation of the NO_2-chemistry of kojic acid indicates that it is nitrosated by gaseous NO_2, and probably undergoes a complicated series of reactions. Investigations with 3-methylcyclopentane-1,2-dione suggest that cyclic 1,2-diones both reduce and trap gaseous NO_2.

CHAPTER 4

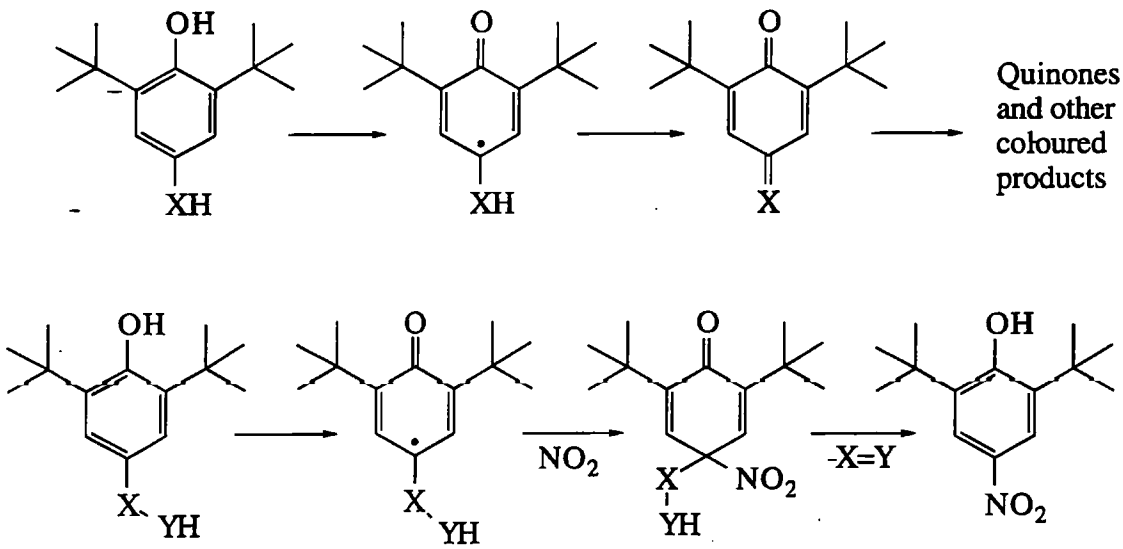
Synthesis and evaluation of BHT-related compounds

4.1. Introduction

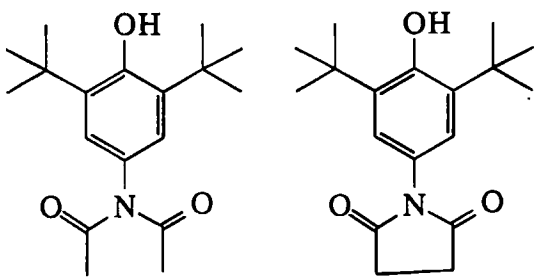
The investigations with kojic acid and 3-methyl-1,2-cyclopentanedione indicate that non-phenolic compounds would trap gaseous NO_2 and prevent its action as an oxidant. Before embarking on major syntheses involving new and more practicable examples of non-phenolic antioxidants, 4-substituted hindered phenols related to BHT were re-evaluated as suitable non-yellowing antioxidant compounds.

The reaction of 4-substituted hindered phenols with NO_2 gives rise to yellow products principally by the processes of Scheme 4.1.

Scheme 4.1. Origins of NO_2-yellowing of phenolic antioxidants.



Thus, yellowing could be largely avoided if the 4-substituent contained no α- or β-hydrogen atoms, as in the case of *N*-acetylated aminophenols like 2,6-di-*t*-butyl-*N,N*-diacetyl-4-aminophenol (*53*) and *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide (*54*).



(*53*) (*54*)

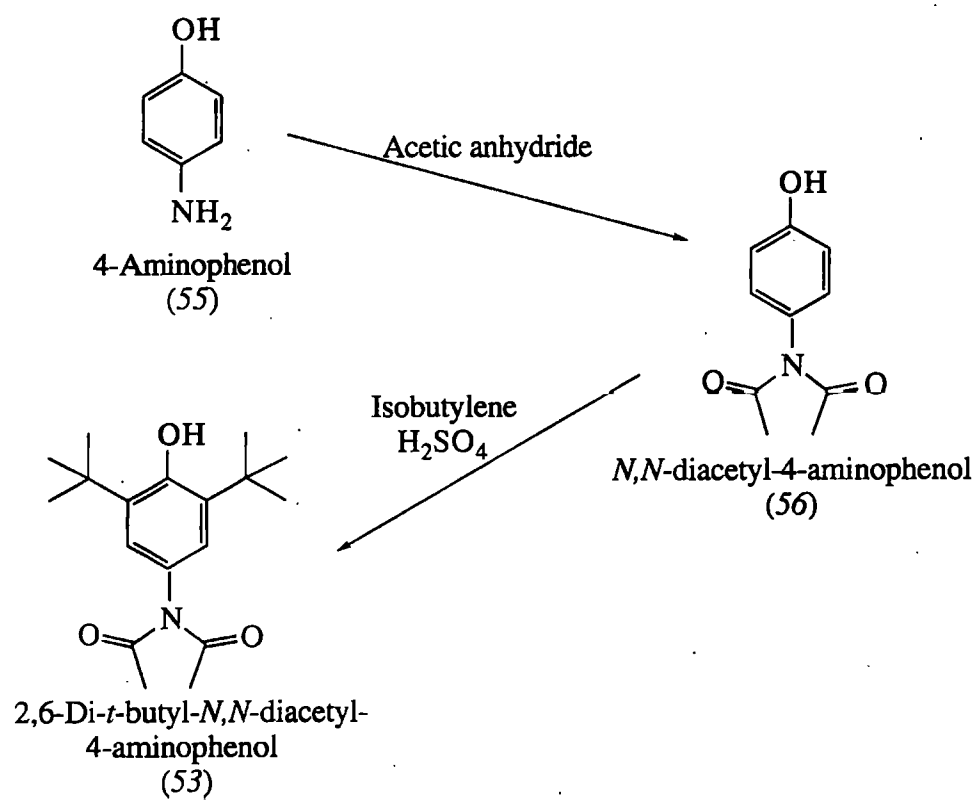
Interestingly, Vitamin E also fulfils this criterion for a non-yellowing antioxidant, but it is expensive and not particularly stable.

4.2. Syntheses

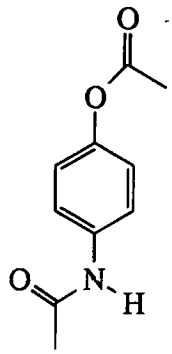
4.2.1. Preparation of 2,6-di-*t*-butyl-*N*,*N*-diacetyl-4-aminophenol (*53*)

A synthesis of the 2,6-di-*t*-butyl-*N*,*N*-diacetyl-4-aminophenol (*53*) involving acetylation of 4-aminophenol (*55*) with acetic anhydride followed by alkylation of the aromatic ring using isobutylene is outlined in Scheme 4.2.

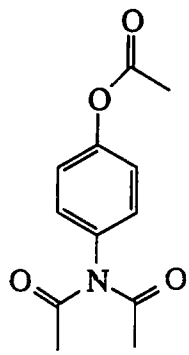
Scheme 4.2. Potential synthesis of 2,6-di-*t*-butyl-*N*,*N*-diacetyl-4-aminophenol (*53*).



Acetylation of 4-aminophenol, however, gives only *N*,*O*-diacetyl-4-aminophenol (*57*). The formation of an *N*,*O*-diacetyl derivative relates to deactivation of the amine group following the addition of one *N*-acetyl substituent. Addition of a second *N*-acetyl function becomes more difficult and reaction proceeds preferentially at the *O*-atom.



(*57*)

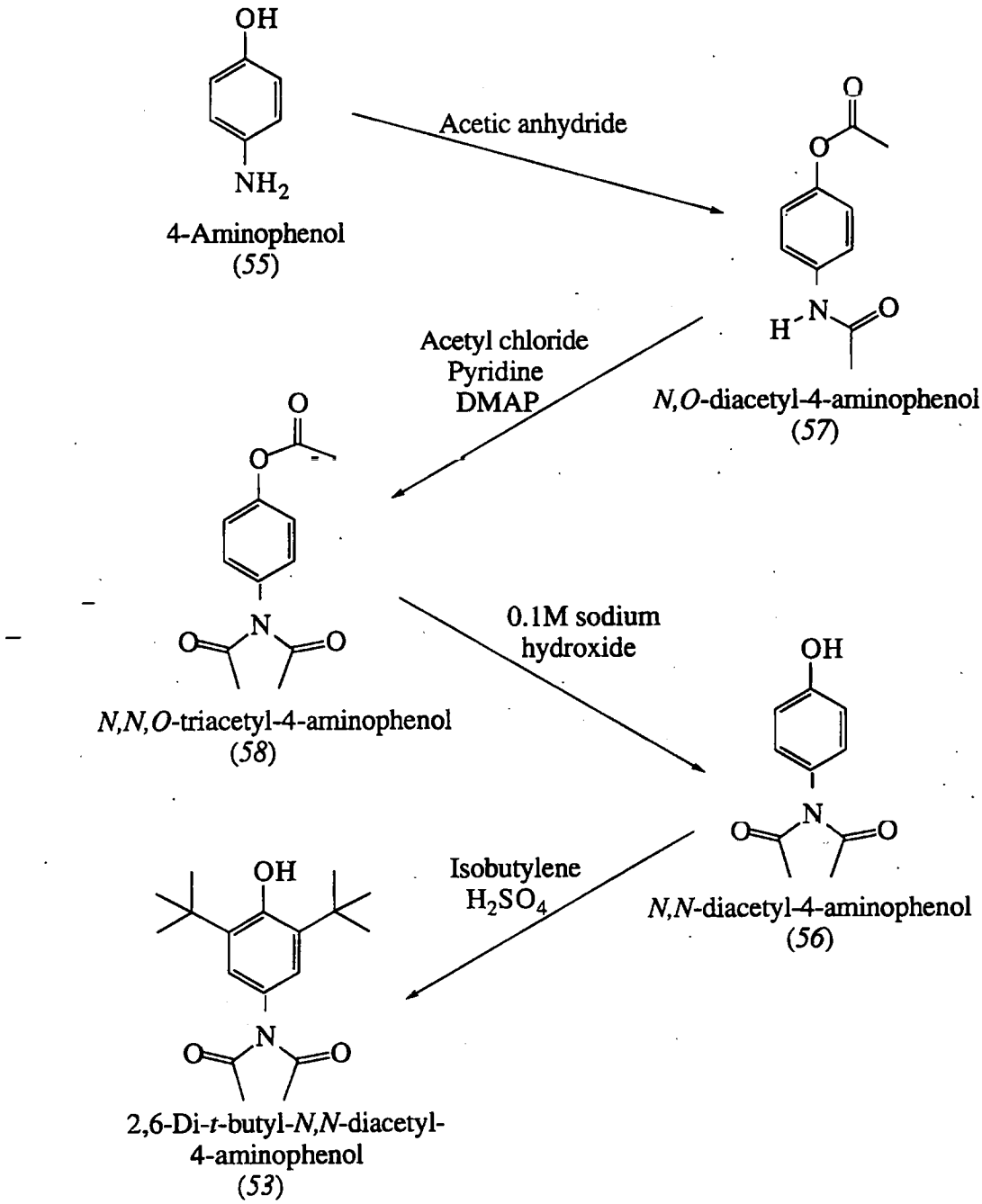


(*58*)

Further acetylation of *N,O*-diacetyl-4-aminophenol using 4-dimethylaminopyridine (DMAP) (Aldrich, 1986) as catalyst and more vigorous conditions produced a mixture of unreacted starting material (up to 10% by ^{1}H NMR) and *N,N,O*-triacetyl-4-aminophenol (*58*). Attempts to separate the two compounds by chromatography were unsuccessful. By increasing the reaction time from 12 to 100 h the amount of unreacted *N,O*-diacetyl-4-aminophenol was reduced to only 2.5%. The synthesis of 2,6-di-*t*-butyl-*N,N*-diacetyl-4-aminophenol was therefore amended to include the extra acetylation step followed by the subsequent hydrolysis of the *O*-acetyl function (Scheme 4.3). The small *N,O*-diacetyl-4-aminophenol contaminant was considered acceptable for the subsequent steps.

Initial investigation of the hydrolysis of *N,N,O*-triacetyl-4-aminophenol using aqueous sodium hydroxide (0.1M) at 25°C gave *N*-acetyl-4-aminophenol (*59*). Further reactions using more dilute alkali resulted in the formation of mixtures of *N,O*-diacetyl- and *N*-acetyl-4-aminophenol. To determine whether any of the required *N,N*-diacetyl-4-aminophenol (*56*) was formed during the hydrolysis, the reaction of *N,N,O*-triacetyl-4-aminophenol in 50% (v/v) methanolic borate buffer (pH 9.0) was monitored by HPLC.

Scheme 4.3. Revised synthesis of 2,6-di-*t*-butyl-*N*,*N*-diacetyl-4-aminophenol



The results (Table 4.1) show a time dependent increase in percentage peak area for *N*-acetyl-4-aminophenol, *N,O*-diacetyl-4-aminophenol and an unidentified peak (RT 20.53 min), with a corresponding decrease in percentage peak area for *N,N,O*-triacetyl-4-aminophenol over the first thirty minutes of reaction.

Table 4.1. Percentage peak areas for the hydrolysis of *N,N,O*-triacetyl-4-aminophenol in 50% methanol-borate buffer (pH 9.0)

Reaction Time (min)	Percentage Peak Area (%)			
	N-acetyl-4-aminophenol RT 8.87 min	*N,N*-diacetyl-4-aminophenol RT 20.53 min	*N,O*-diacetyl-4-aminophenol RT 21.63 min	*N,N,O*-triacetyl-4-aminophenol RT 25.13 min
0	-	-	63.41	36.59
5	-	3.95	61.77	34.28
10	-	5.68	67.17	27.15
20	-	7.90	73.25	18.85
30	3.15	8.44	79.96	8.45
60	7.36	9.50	76.70	6.43
120	22.93	8.26	67.25	1.56
1140	75.27	3.12	21.61	-

The unidentified peak is assigned to the required *N,N*-diacetyl-4-aminophenol for the following reasons:

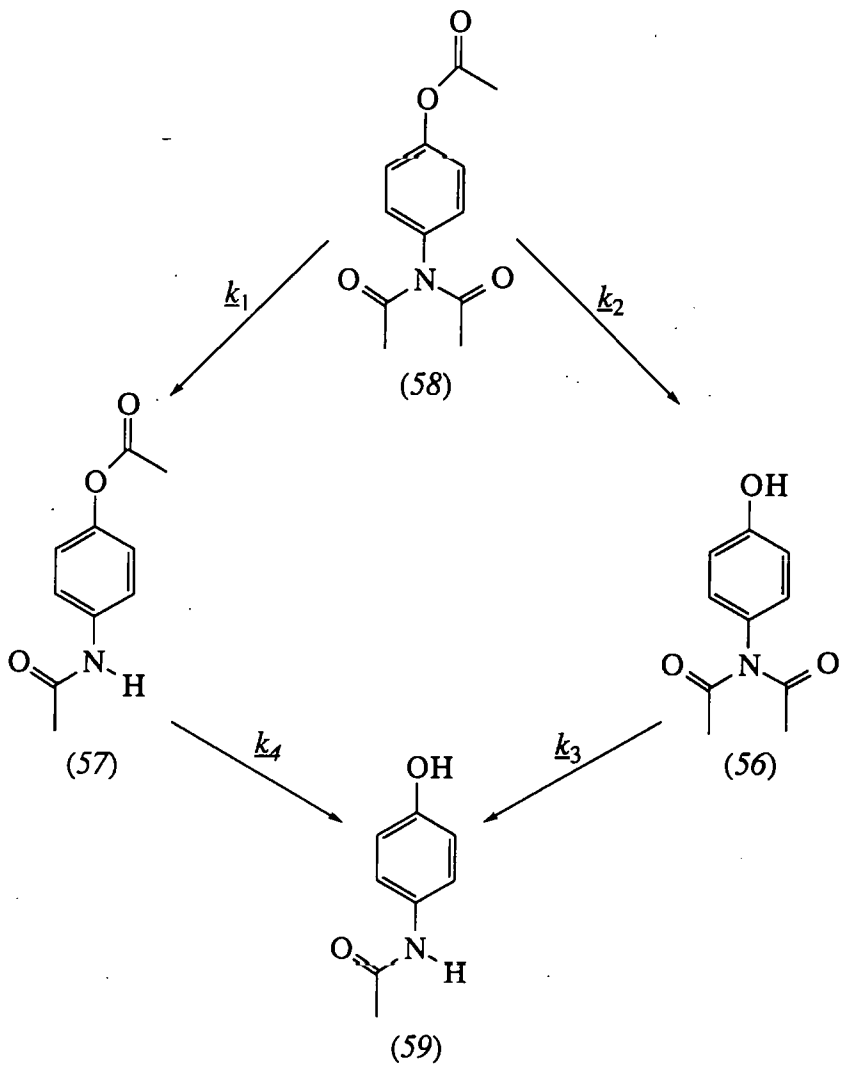
i) Variation in purity parameter - The change in purity parameter from *N,O*-diacetyl-4-aminophenol ($PuP_{210-367}$ 237.50 nm) to *N*-acetyl-4-aminophenol ($PuP_{210-367}$ 239.17 nm) is an increase of 2 nm. A similar change is observed from *N,N,O*-triacetyl-4-aminophenol ($PuP_{210-367}$ 222.30 nm) to the unidentified peak ($PuP_{210-367}$ 224.50 nm) indicating the loss of the *O*-acetyl function from *N,N,O*-triacetyl-4-aminophenol.

ii) Retention time - *N*-acetyl-4-aminophenol has a retention time (RT) of 8.87 min and *N,O*-diacetyl-4-aminophenol 21.63 min. It is expected that *N,N*-diacetyl-4-aminophenol has a retention time between these two, as for the unidentified peak at 20.53 min.

The rate of *O*-acetyl-hydrolysis of *N,N,O*-triacetyl-4-aminophenol in Scheme 4.4, (step k_2), must be faster than the rate of *N*-acetyl-hydrolysis of *N,N*-diacetyl-4-

aminophenol (step k_3) as the latter is seen by chromatography. It is not possible to determine whether the *N*-acetyl-4-aminophenol is formed from the hydrolysis of *N,N*-diacetyl-4-aminophenol (step k_3) or *N,O*-diacetyl-4-aminophenol (step k_4). However, the presence of *N*-acetyl-4-aminophenol in this reaction mixture shows that *N,N*-diacetyl-4-aminophenol hydrolyses readily, and suggests that 2,6-di-*t*-butyl-*N,N*-diacetyl-4-aminophenol is too labile to be a useful antioxidant. Thus, it was decided to synthesise cyclic imide derivatives such as *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide (*54*).

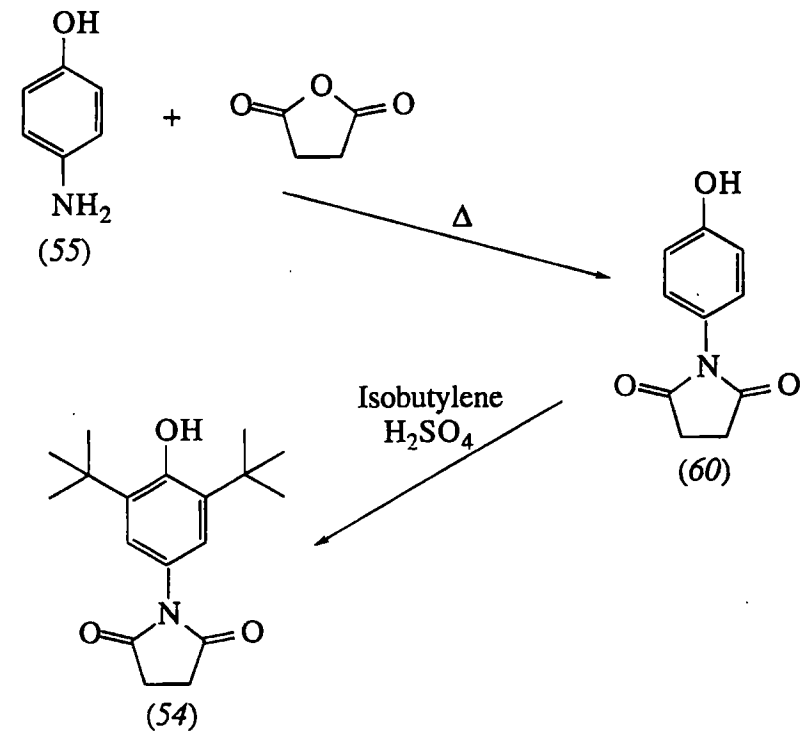
Scheme 4.4. Hydrolysis of *N,N,O*-triacetyl-4-aminophenol



4.2.2. Preparation of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide (*54*)

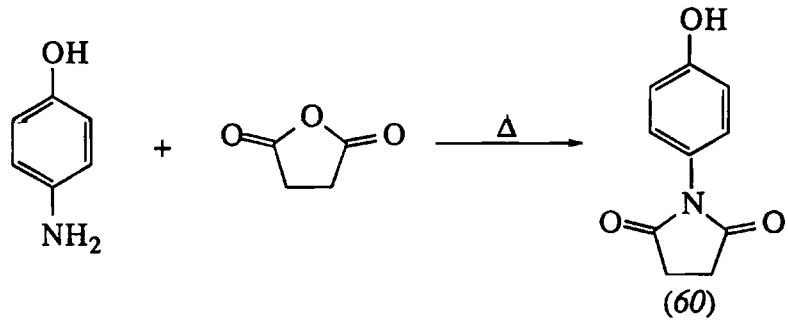
The formation of phthalimide derivatives of amines are well-known from the Gabriel synthesis of amino acids. Vogel, for example, describes procedures for the ready fusion of substituted anilines with 3-nitrophthalic anhydride. The synthesis of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide was therefore envisaged via two steps: the first, the fusion of 4-aminophenol (*55*) with succinic anhydride to form the cyclic *N*-oxyphenyl succinimide (*60*); and the second, alkylation of this compound with isobutylene and sulphuric acid (Scheme 4.5).

Scheme 4.5. The envisaged synthesis of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide (*54*).



No difficulty was encountered with the fusion of succinic anhydride and 4-aminophenol to give *N*-oxyphenylsuccinimide (Scheme 4.6)

Scheme 4.6. Preparation of *N*-oxyphenyl-succinimide (*60*) from 4-aminophenol.

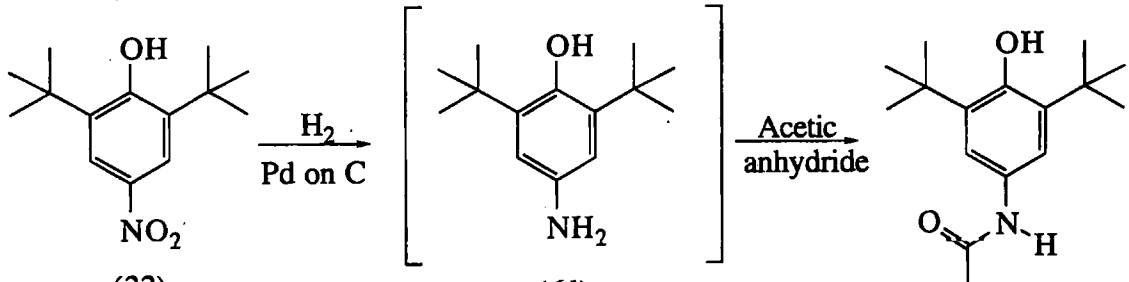


(*60*)

To find suitable conditions for the alkylation reaction, an experiment was first carried out using 4-methylphenol, isobutylene and sulphuric acid to synthesise BHT. Using a method based on that carried out by Stillson *et al.* (1945), BHT was prepared in acceptable yield (28%). Application of the method to the alkylation of *N*-oxyphenylsuccinimide resulted in the recovery of unreacted starting material. Further examination of the literature procedures for the synthesis of BHT showed the use of pressurised reaction vessels was required to obtain high yields. As this technology was unavailable at The Open University, an alternative method for the preparation of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide was investigated.

Barnes and Hickenbottom (1961) prepared 4-acetamido-2,6-di-*t*-butylphenol (BHPN) (*62*) by the catalytic reduction of 2,6-di-*t*-butyl-4-nitrophenol (*22*) followed by an *in situ* reaction with acetic anhydride (Scheme 4.7).

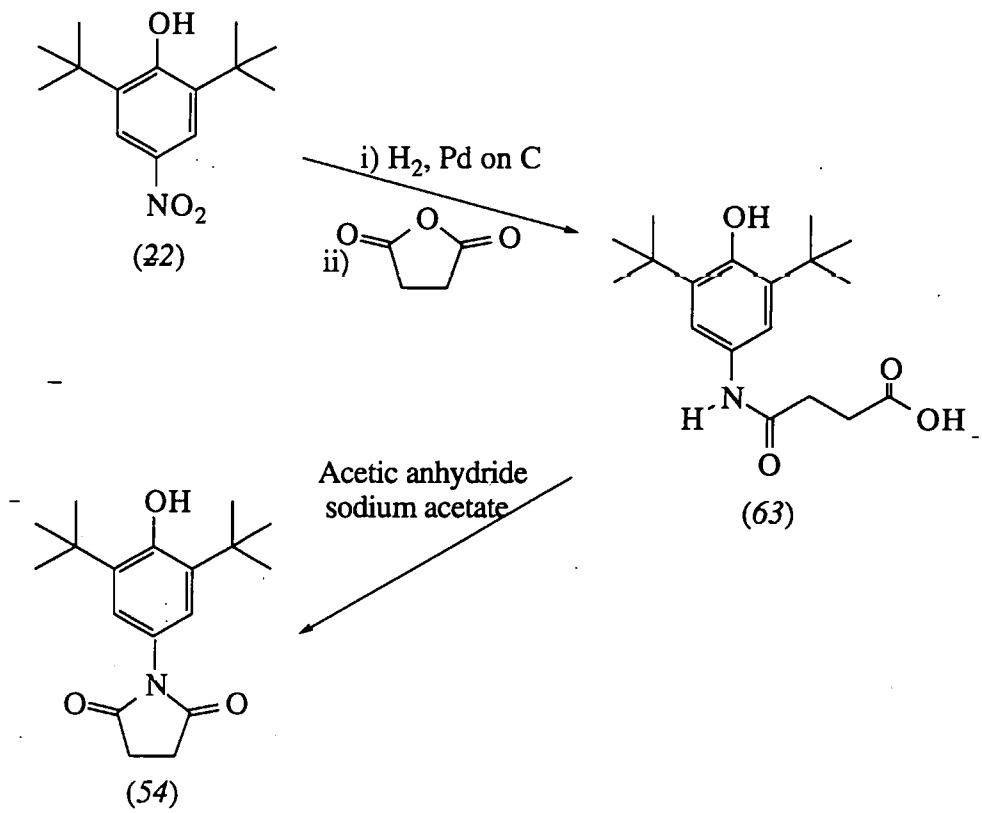
Scheme 4.7. Preparation of 4-acetamido-2,6-di-*t*-butylphenol (*62*).



(*22*) (*61*) (*62*)

This procedure using succinic anhydride in place of acetic anhydride should produce *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinamic acid (*63*). Subsequent dehydration of this product then leads to the formation of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide (Scheme 4.8).

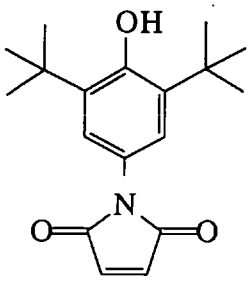
Scheme 4.8. Preparation of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide (*54*) from 2,6-di-*t*-butyl-4-nitrophenol (*22*).



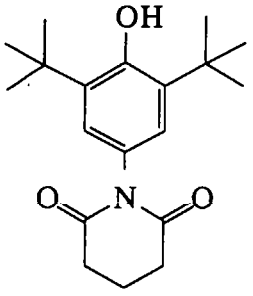
4.2.3. Preparation of other *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-imides

The procedure was used successfully to prepare *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide (BHPS) and the related imides (*64*)-(*68*) whose structures are given in Table 4.2. in yields of 40 to 65%.

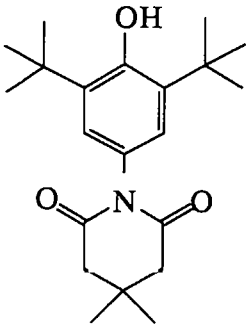
Table 4.2. Cyclic imides obtained by condensation of cyclic anhydrides with 2,6-di-*t*-butyl-4-aminophenol.



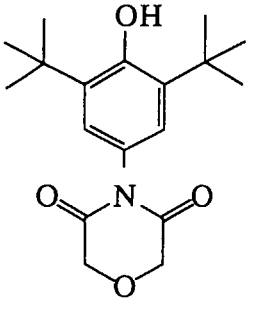
N-(3,5-di-*t*-butyl-4-hydroxyphenyl)-maleimide
BHPM
(*64*)



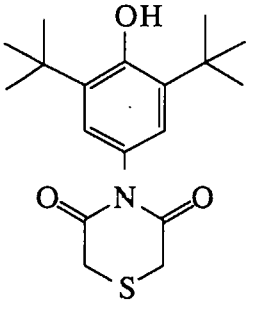
N-(3,5-di-*t*-butyl-4-hydroxyphenyl)-glutarimide
BHPG
(*65*)



N-(3,5-di-*t*-butyl-4-hydroxyphenyl)-3,3-dimethyl-glutarimide
BHPMG
(*66*)



N-(3,5-di-*t*-butyl-4-hydroxyphenyl)-diglycolarimide
BHPDG
(*67*)



N-(3,5-di-*t*-butyl-4-hydroxyphenyl)-thiodiglycolarimide
BHPTG
(*68*)

4.3. Yellowing and antioxidant properties

To assess their potential utility as antioxidants, the cyclic imides were examined for the development of colour on exposure to gaseous NO_2 and for their antioxidant activity. Also, a variety of polymer incorporation tests were applied at BTTG to one compound, *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide to provide an additional assessment of antioxidant activity.

4.3.1. Yellowing indices, OU_{YI}

As described in Chapter 3., the OU_{YI} is a quantitative estimate of the absorbance of a methanolic solution of the antioxidant above $\lambda = 400$nm following exposure to gaseous NO_2. The results in Table 4.3 show that all the cyclic imides except BHPM produce less NO_2 yellowing than BHT. The adverse result for BHPM relates to the compound's yellow colouration. It was noted that the yellow colouration of the BHT solution increased on standing, whereas that for BHPS remained unchanged.

The mono-acetyl derivative BHPN was included in these tests as it possesses structural characteristics that should promote NO_2 yellowing.

Table 4.3. Yellowing index results for the phenolic cyclic imides.

Cyclic Imide	OU_{YI}
BHPS (*54*)	1.60
BHPM (*64*)	22.0
BHPG (*65*)	3.16
BHPDG (*67*)	2.43
BHPTG (*68*)	4.66
BHPN (*62*)	6.16
BHT (*3*)	5.72

The OU_{YI} = 6.16 for BHPN (*62*) (0.34 units higher than BHT) supports the hypothesis that the absence of α- or β-hydrogen atoms on a 4-substituent within a hindered phenol minimises the yellowing.

4.3.2. NO_2^-/NO_3^- ratios

As explained in Chapter 3., the NO_2^-/NO_3^- ratio is a direct measure of the antioxidant compound's ability to reduce NO_2 to NO_2^-. The ratios reported in Table 4.4 (normalised to BHT) show BHPS is the only cyclic imide to perform better than BHT as an electron transfer agent to NO_2. In the light of this finding, it was decided to apply the polymer testing methods available at BTTG to BHPS.

Table 4.4. NO_2^-/NO_3^- ratio results for the phenolic cyclic imides.

Cyclic Imide	NO_2^-/NO_3^- ratio
BHPS (*54*)	11.3
BHPM (*64*)	6.6
BHPG (*65*)	4.7
BHPDG (*67*)	6.2
BHPTG (*68*)	5.6
BHPN (*62*)	6.6
BHT (*3*)	10.0

4.3.3. Polymer Tests

Polymer incorporation tests are widely used to evaluate antioxidants, as explained in Chapter 1. The compound under investigation is formulated with a suitable polymer by dissolution in a volatile organic solvent, evaporation of the solvent and processing in a variety of ways.

A study carried out at BTTG (Bangee, 1994), to evaluate commercially available phenolic antioxidants as processing and long term stabilisers of polypropylene provided the opportunity to test BHPS. The polymer samples were assessed for process stability by the melt flow index, and for long term stability by thermal analysis.

4.3.3.1. Process stability

The stability of the antioxidant doped propylene to the thermal and mechanical stresses of processing was determined by repeated extrusion followed by measurement of changes in melt viscosity. The results for several phenolic-antioxidants (Table 4.5) show BHPS is the least effective against changes in melt viscosity. Thus, BHPS is a poorer polymer processing antioxidant than BHT.

Table 4.5. Melt flow indices as a function of the number of extrusions for polypropylene formulations containing phenolic antioxidants (Bangee, 1994).

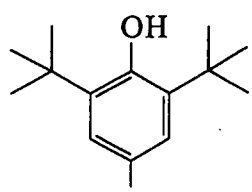
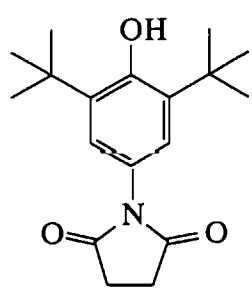
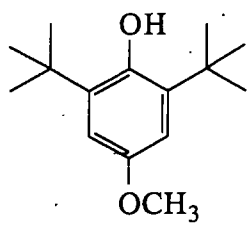
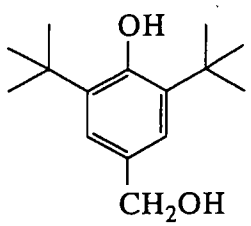
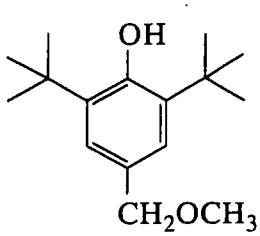
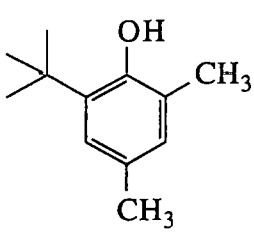
	Melt Flow Index		
Antioxidant	1st pass	3rd pass	5th pass
Antioxidant free polypropylene	9.07	40.28	64.00
 BHT (*3*)	4.83	7.36	9.20
 BHPS (*54*)	5.40	9.08	-

Table 4.5. Melt flow indices as a function of the number of extrusions for polypropylene formulations containing phenolic antioxidants (Bangee, 1994).

Antioxidant	Melt Flow Index		
	1st pass	3rd pass	5th pass
Antioxidant free polypropylene	9.07	40.28	64.00
 BHA (*69*)	3.46	5.36	8.24
 AO 754 (BALC, *26*)	3.70	4.80	6.52
 AN 62 (*70*)	4.89	7.84	-
 Ionol K (*71*)	4.47	6.72	9.28

4.3.3.2. Long term stability

The long term stability of antioxidants in polymers was measured by Differential Thermal Analysis (DTA). DTA determines the oxidative induction time of the polypropylene samples by measuring temperature differentials between the doped sample and a reference in a supply of oxygen at 190°C. The results (Table 4.6) show that BHPS retards the onset of oxidative degradation better than all the other antioxidants except Ionol K. Thus, BHPS performs better than BHT as an antioxidant in this test.

Table 4.6. Oxidative induction times for phenolic antioxidants (Bangee, 1994).

Antioxidant	Oxidative induction time (min.sec)
Antioxidant free polypropylene	< 1
BHT (*3*)	2.00
BHPS (*54*)	3.40
BHA (*69*)	3.20
AO 754 (*26*)	2.50
AN 62 (*70*)	2.10
Ionol K (*71*)	4.45

4.3.4. Electrochemical measurements

Electrochemical measurements have been widely used to assess antioxidant activity (Nash *et al.*, 1958 and Penketh, 1957). For example, Nash *et al.*, (1958) determined the half-wave potentials of a series of phenolic compounds and compared them with antioxidant activities derived from mineral oil oxidation. This revealed that the greatest antioxidant activity is found for compounds with a half-wave potential between 30 and 300mV. Also, Penketh (1957) measured the oxidation potentials of several phenolic and

amino compounds and compared them to an oxidative induction time. The outcome was that any compound possessing a potential below 0.70v exhibited useful antioxidant properties.

The results of electrochemical measurements (Table 4.7) show that BHT and the mono-acetylated BHPN undergo two step oxidations, while the imides undergo three step oxidations. Studies of the anodic oxidation of BHT (Ronlan, 1971 and Vermillion and Pearl, 1964) have shown this oxidation to proceed via initial formation of the phenoxy radical (*5*) followed by loss of a second electron to give the quinone methide derivative (*6*) (Scheme 4.9).

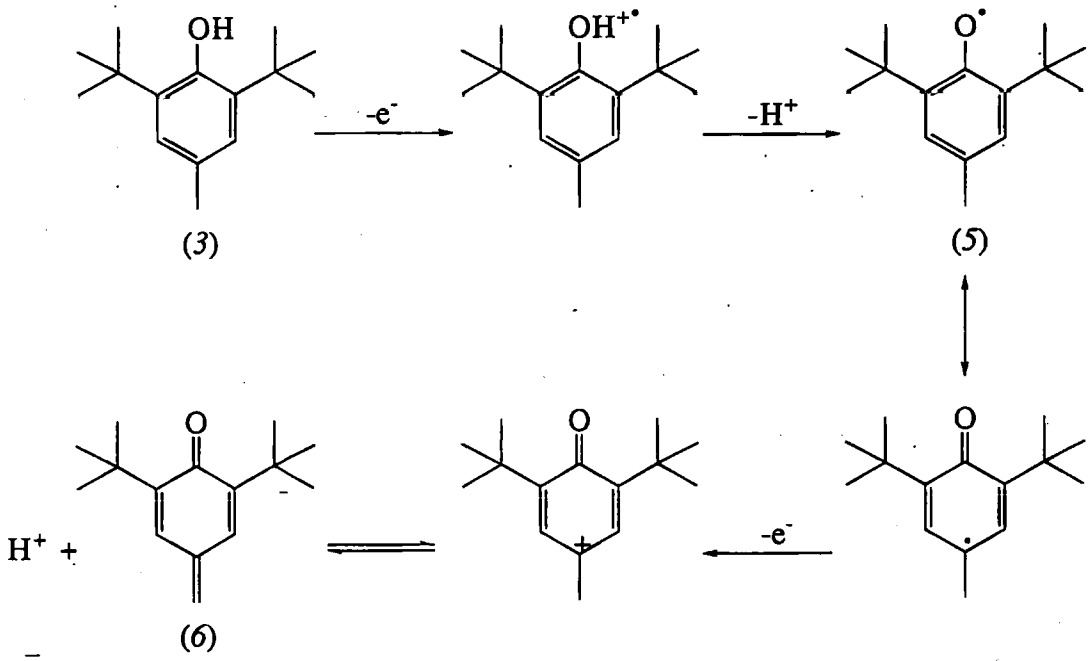
BHPN can undergo a similar oxidation (Scheme 4.10) as it possesses an α-hydrogen atom. In contrast, cyclic imide compounds lack α-hydrogen atoms but the origin of the additional step is not obvious even taking the high potential into account.

The results (Table 4.7) of cyclic voltammetry measurements for the phenolic cyclic imides and BHT, corrected to a saturated calomel electrode (SCE), show that the initial electron loss occurs at a very similar potential for all compounds. Thus, these data suggest the phenolic cyclic imides are as good as BHT as antioxidants.

4.3.5. Summary

The results of the above tests indicate that BHPS is the most efficient non-yellowing antioxidant of the present series of phenolic cyclic imides. The polymer tests, however, show that BHPS lacks stability under thermal and mechanical stress. It was therefore decided to investigate the stability of the phenolic cyclic imides to direct the synthesis of more stable analogues.

Scheme 4.9. Mechanism for the electrochemical oxidation of BHT.



Scheme 4.10. Proposed mechanism for the electrochemical oxidation of BHPN.

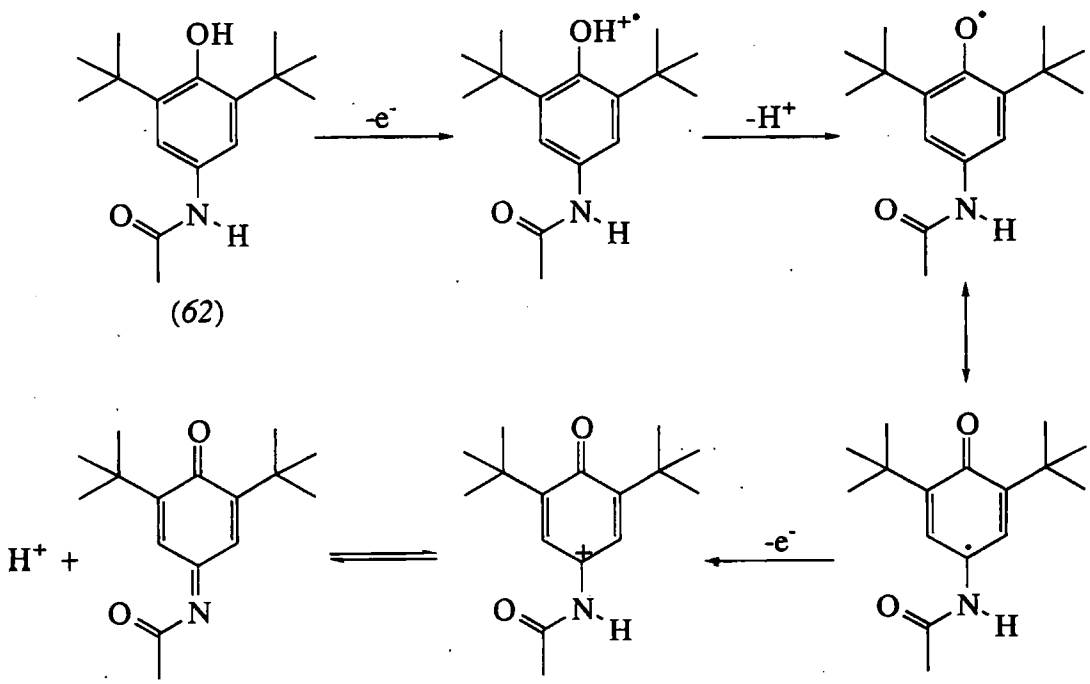


Table 4.7. Oxidation potentials for the phenolic cyclic imides and BHT

Compound	Potential, E_{SCE} (v)
BHT	0.593
	1.083
BHPS	0.577
	0.879
	1.295
BHPM	0.585
	0.877
	1.225
BHPG	0.581
	0.879
	1.397
BHPMG	0.597
	0.895
	1.421
BHPDG	0.589
	0.883
	1.497
BHPTG	0.598
	0.885
	1.418
BHPN	0.609
	0.977

4.4. Stabilities of cyclic imides

Assuming that the instability of BHPS relates to opening of the imide ring, attention was focused on structural factors influencing this process.

The stability of cyclic anhydrides (Eberson, 1964, Eberson and Welinder, 1971 and Eberson and Landstrom, 1972) and N-phenyl succinimides (Herd *et al.*, 1966) have been ascertained *via* their rates of alkaline hydrolyses in aqueous media. From these data, Eberson and Welinder (1971) determined equilibrium constants for the interconversion between several succinic acids and their corresponding anhydrides (Table 4.8),

where Rate = k_{-1}[Diacid] and k_1[Anhydride], respectively.

$$\text{Diacid} \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} \text{Anhydride} + H_2O$$

Table 4.8. Equilibrium data for diacids in aqueous solution at 60°C (Eberson and Welinder, 1971).

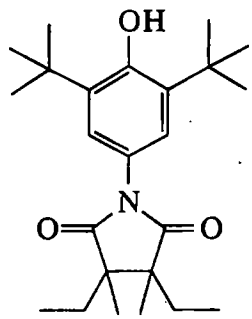
Diacid	k_1 (min^{-1})	k_{-1} (min^{-1})
Succinic	1.22	9.0×10^{-6}
Methylsuccinic	1.41	5.9×10^{-5}
2,2-Dimethylsuccinic	1.07	1.1×10^{-4}
dl-2,3-Dimethylsuccinic	1.30	3.2×10^{-4}
Trimethylsuccinic	0.93	-
Tetramethylsuccinic	0.105	0.018
dl-2,3-Diethyl-2,3-dimethylsuccinic	0.0045	0.0155
meso-2,3-diethyl-2,3-dimethylsuccinic	0.0098	0.010

The results of Table 4.8 indicate that alkyl substituents increase anhydride stability, the most stable being 2,3-diethyl-2,3-dimethyl succinic anhydride. The alkaline hydrolyses of *N*-phenylsuccinimides by Herd et al. (1966) show similarly that alkyl substitution decreases the rate of reaction, which implies increased stability of the imide.

With these data in mind, the sterically hindered *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl) imide based on 2,3-diethyl-2,3-dimethyl succinimide was prepared, and the stabilities of all of the phenolic cyclic imides with respect to alkaline hydrolysis determined.

4.4.1. Synthesis of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-2,3-diethyl-2,3-dimethylsuccinimide (*72*)

Initial attempts to prepare *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-*meso*-2,3-diethyl-2,3-dimethylsuccinimide (*72*) (BHPDS) followed the method devised for the other imides.



BHPDS
(*72*)

Thus, a catalytically reduced solution of 2,6-di-*t*-butyl-4-nitrophenol was reacted *insitu* with *meso*-2,3-diethyl-2,3-dimethylsuccinic anhydride. This gave a dark red solution subsequently identified as the coupled product of 2,6-di-*t*-butyl-4-aminophenol. The presence of adventitious air in the reaction mixture had led to oxidation and coupling of the 2,6-di-*t*-butyl-4-aminophenol (*61*) as it formed. It was evident that more vigorous, anaerobic conditions were necessary to synthesise BHPDS, probably due to the stability and therefore low reactivity of the highly substituted succinic anhydride.

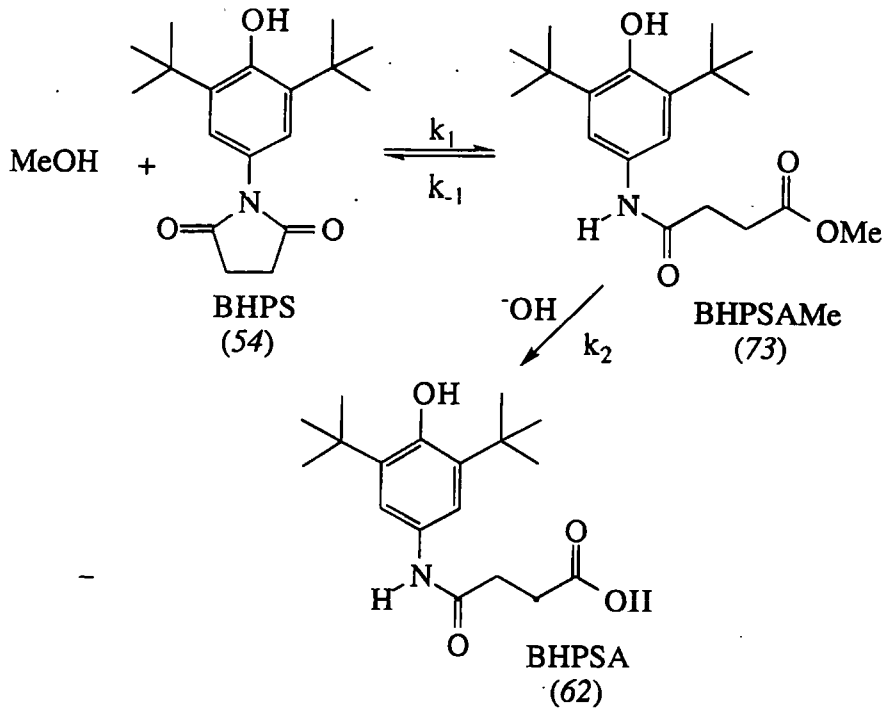
Thus, a solution of the catalytically reduced 2,6-di-*t*-butyl-4-nitrophenol was filtered and evaporated to dryness under nitrogen, and the residue in toluene was reacted in a sealed tube with excess *meso*-2,3-diethyl-2,3-dimethyl anhydride at 220°C. MS analysis of the resulting red residue showed the presence of the required BHPDS ($M^{+\cdot}$ 387), but ^{1}H-NMR analysis showed the anhydride starting material was the major component. Using a solid phase extraction procedure, a very small amount of BHPDS was successfully isolated and characterised.

These results indicate that BHPDS is likely to be a very stable compound because of the inherent difficulty in its formation and the high stability of the anhydride reagent.

4.4.2. Stabilities of the phenolic cyclic imides

The lability of BHPS in polymer incorporation tests may relate to the ease of nucleophilic reaction at the imide group. Preliminary experiments showed that BHPS degraded to the half-acid methyl ester, *N*-(3,5-di-t-butyl-4-hydroxyphenyl)-succinamic acid methyl ester (BHPSAMe) (*73*) in methanolic solutions. Further HPLC monitoring of both BHPS and BHPSAMe in 50% (v/v) aqueous methanol at pH9 showed that an equilibrium existed between the two compounds, with a slower concurrent conversion to *N*-(3,5-di-t-butyl-4-hydroxyphenyl)-succinamic acid (BHPSA) (*62*) (Scheme 4.11) arising from the difference in nucleophilicity of alkoxide and hydroxide ions. Measurement of the equilibrium constant for the imide half-acid interconversion is an accessible means of evaluating the stability of the cyclic imides, which could direct refinements to the imide structure to increase its stability.

Scheme 4.11. Solvolysis of BHPS in 50% (v/v) aqueous methanol at pH9.



The stabilities of the phenolic cyclic imides were therefore determined *via* the initial *pseudo* first order rate constants for the ring-opening (Equation 4.1) and ring-closure (Equation 4.2) reactions in 50% (v/v) aqueous methanol at 25°C.

$$\text{Rate} = k_1[\text{Imide}] \qquad (4.1)$$

$$\text{Rate} = k_{-1}[\text{Half-acid}] \qquad (4.2)$$

The pH values of the reaction solutions are operational rather than absolute as they were prepared from alcohol and aqueous buffers at pH 7 and 9. The pH of these solutions was then determined using electrodes calibrated in entirely aqueous standard buffers. The 10-fold difference for BHPS in both rates of solvolysis and cyclisation in 'pH 9' and 'pH 7' buffers correlate well with the experimental operational pH difference of the reaction solutions.

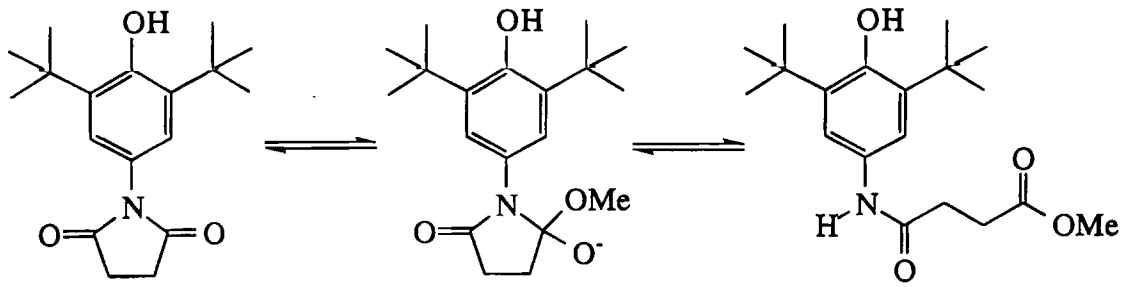
Table 4.9. Kinetic data for the solvolysis of cyclic imides at 25°C.

Cyclic Imide	Reaction conditions	'pH'	*Pseudo* first order rate constant k_1 ($mMmin^{-1}$ x 10^{-3})	*Pseudo* first order rate constant k_{-1} ($mMmin^{-1}$ x 10^{-3})	Equilibrium constant K_{eq}
BHPS	50% (v/v) Methanol/ pH 9 buffer	8.82	7.1	12.0	0.592
BHPS	50% (v/v) Methanol/ pH 7 buffer	8.00	0.63	0.9	0.700
BHPS	50% (v/v) Ethanol/ pH 9 buffer	10.32	13.0	15.0	0.867
BHPM	50% (v/v) Methanol/ pH 9 buffer	8.82	1200	310	3.871
BHPG	50% (v/v) Methanol/ pH 9 buffer	8.82	5.7	$< 10^{-3}$	-
BHPMG	50% (v/v) Methanol/ pH 9 buffer	8.82	0.081	0.023	3.522
BHPDG	50% (v/v) Methanol/ pH 9 buffer	8.82	210	$< 10^{-3}$	-
BHPTG	50% (v/v) Methanol/ pH 9 buffer	8.82	4.3	$< 10^{-3}$	-

The results, summarised in Table 4.9, show that the 3,3-dimethylglutarimide derivative (BHPMG) is the most kinetically stable compound. The effect of structure on the rates of solvolysis of the imides can be rationalised by steric effects on the reaction mechanism. Solvolysis is most likely to proceed by initial attack of alkoxide ion at the carbonyl C-atom to form a tetrahedral intermediate (*74*) with subsequent ring opening to

form the methyl ester; i.e. the reverse of that observed for the hydrolysis of imides by Hargreaves *et al.*, (1970) (Scheme 4.12).

Scheme 4.12. Solvolysis of BHPS by methanol.



(*54*) (*74*) (*73*)

For this mechanism, three factors could affect the rate of solvolysis depending on the rate-limiting step:

1) polar contributions of any groups attached to the carbon atom adjacent to the carbonyl group of the imide, which would affect the electron deficiency of the carbonyl carbon atom;

2) steric inhibition by substituents to the attacking alkoxide ion;

3) steric acceleration or retardation by substituents on ring opening.

The introduction of a double bond into the 5-membered ring of the imide produces a 200 fold increase in solvolysis rate. Similar increases have been attributed previously (Eberson and Landstrom, 1972) to ring strain. The maleimide BHPM however, possesses a planar structure compared to the puckered form of the succinimide BHPS, which makes BHPM more susceptible to nucleophilic attack than BHPS.

The glutarimide BHPG exists in either half-chair or chair conformations and is more flexible than BHPS. The increased flexibility does not produce a significant difference in the rate of solvolysis. The presence of a sulphur atom within the six-membered ring, as in BHPTG has no effect on the rate of solvolysis. However, the introduction of an oxygen atom into the six-membered ring of the glutarimide, as in BHPDG causes an increase in the

solvolysis rate because of the increased positive charge on the carbonyl carbon-atom arising from the β-oxygen.

The introduction of two γ-methyl groups in the glutarimide BHPMG produces a 70-fold decrease in the rate of solvolysis. This reduction probably arises from the steric effects of the methyl groups, which inhibit attack of an approaching nucleophile. A similar effect is seen with di-alkyl substituted anhydrides (Eberson, 1964), where the slowest rate of solvolysis is observed for 3,3-diethylglutaric anhydride.

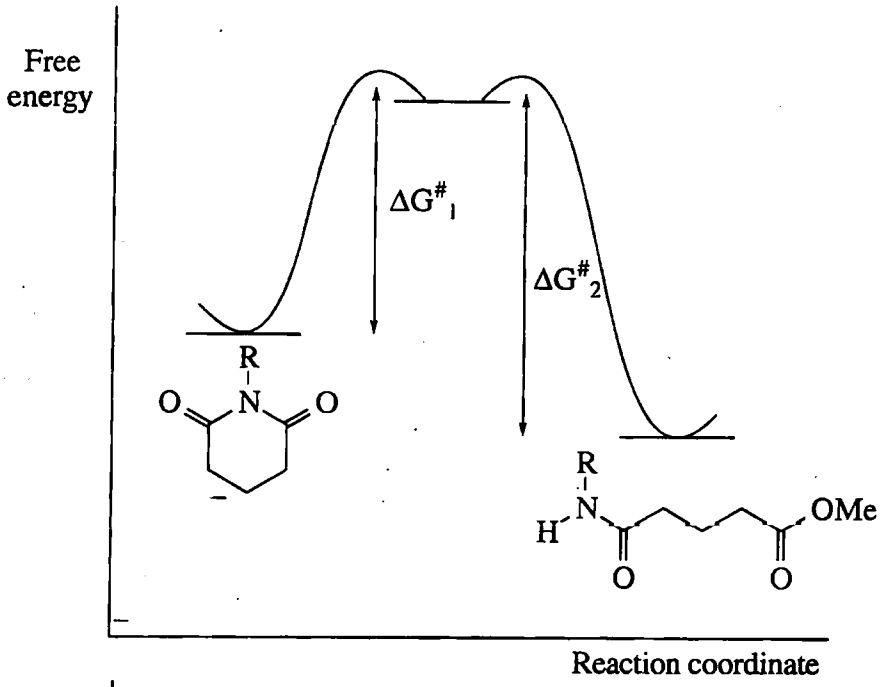
Ring closure proceeds readily only for the 5-membered cyclic imides and the most hindered glutarimide, BHPMG (Table 4.9). This result may be rationalised by considering the free energy changes (Scheme 4.13).

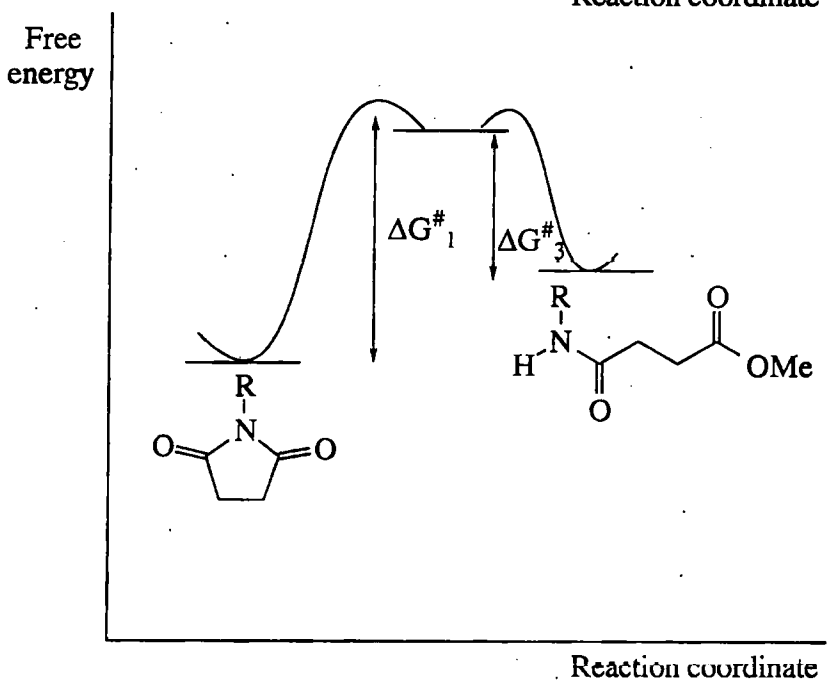
The free energies of activation ($\Delta G^{\#}_1$) for solvolysis (ring opening) of the five-membered rings of BHPS and BHPM, and the six-membered rings of BHPG, BHPDG and BHPTG must be similar because all reactions proceed at similar rates. The corresponding free energies of activation for ring closure of these five- and six-membered rings ($\Delta G^{\#}_3$ and $\Delta G^{\#}_2$, respectively) must differ such that $\Delta G^{\#}_3 < \Delta G^{\#}_2$. Thus, ground state free energy differences produce the barrier to ring closure reactions for BHPG, BHPDG and BHPTG. The two methyl groups in BHPMG must raise the ground state energy of the open chain substrate as ring closure can be observed.

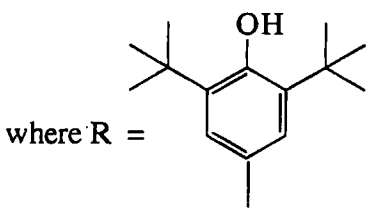
4.5. Conclusions

The synthesis of several *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl) cyclic imides has provided a group of new compounds showing similar antioxidant activity to BHT. Furthermore, they are less prone to NO_2-yellowing than BHT. Studies on the solvolysis of these cyclic imide compounds suggest that sufficient stability to obtain a commercially useful antioxidant may be imparted by bulky alkyl substituents in the imide ring, as in the case of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-2,3-diethyl-2,3-dimethylsuccinimide.

Scheme 4.13. Thermodynamic control of ring closure







CHAPTER 5

Conclusions

The most common incidence of textile yellowing is due to the interaction of NO_2 and the hindered phenolic antioxidant, 2,6-di-*t*-butyl-4-methylphenol. There is a need for an alternative antioxidant to BHT, which possesses a similar degree of antioxidant activity, but does not produce significant NO_2-yellowing. This was set up as the overall aim of the project.

The origins of textile yellowing have required consideration of the process by which antioxidants function, the autoxidation products formed from BHT, the chemistry of gaseous NO_2, possible antioxidant-NO_2 reactions and the problem of textile yellowing. Antioxidant chemistry covers a very diverse range of topics and further information may be found that lead to a greater understanding of the complicated processes involved.

In order to achieve the overall objective of the project, it was necessary to identify with more certainty the coloured components of textile yellowing. This was accomplished by obtaining quantitative data on the product distribution of reactions of ethanolic solutions of BHT with a variety of concentrations of gaseous NO_2. These investigations not only identified the yellowing component as 2,6-di-*t*-butyl-4-nitrophenol, but also made it possible to identify structural criteria for non-yellowing antioxidant compounds by consideration of the mechanisms involved. Thus textile yellowing may be avoided by the use of either, non-aromatic antioxidant compounds, or hindered phenolic antioxidants with 4-substituents that do not possess α or β hydrogen atoms. These criteria can be used to synthesise and assess the suitability of compounds as non-yellowing antioxidants.

The quantitative data for the product distribution of reactions of BHT with NO_2 have identified the 4-substituted cyclohexadienone, EQOL, and a variety of unassigned components. To fully confirm the assignment of EQOL within HPLC chromatograms authentic material should be prepared and checked against reaction extracts for purity parameter and retention time. Further investigations of reaction

solutions may provide more information concerning the identity of unassigned components.

In developing alternative antioxidant compounds to BHT, it was necessary to design a means of assessing a potential compound's antioxidant activity and NO_2-yellowing capabilities. The development of two antioxidant testing methods, the inhibition of BHT product formation and the NO_2^-/NO_3^- ratio only provided a means of screening potential compounds for antioxidant activity, and a more accurate method would have to be used for the appraisal of antioxidant activity if any further antioxidant development studies were to be carried out. The yellowing test developed, determines the yellowing indices (OU_{YI}) of test compounds by exposure of a solution to gaseous NO_2. These tests were then used to assess the suitability of any potential antioxidant compounds.

In the literature study of antioxidant compounds, two structural features were identified as being necessary for efficient radical trapping antioxidant activity. The first feature found in hindered phenolic antioxidants involved the stabilisation of a radical by conjugation. The second, shown to be present within a variety of naturally occurring compounds is centred on the captodative stabilisation of a radical. This latter feature was found to be present in some hydroxyfuranone and hydroxypyranone compounds.

Antioxidant activity measurements for the non-aromatic hydroxyfuranone and hydroxypyranone compounds showed that kojic acid, maltol and 2,5-dimethyl-4-hydroxyfuranone possessed a similar degree of antioxidant activity as BHT. Furthermore, the yellowing test supports the use of suitably modified hydroxyfuranone and hydroxypyranone compounds as alternatives to the NO_2-yellowing antioxidant BHT.

The reactions between kojic acid and NO_2 carried out indicate that kojic acid is nitrosated by gaseous NO_2 and undergoes a complicated series of reactions. Many of the products of these reactions remain unidentified and in order to fully

understand the reactions involved the synthesis of authentic compounds would have to be performed.

The results for the reaction of the simpler diketone, 3-methylcyclopentane-1,2-dione and gaseous NO_2 suggest that reduction of NO_2 occurs with the subsequent formation of addition and ring opened products. Due to the extensive methylation of the products by methyl formate, further work should use a simpler solvent and work up procedure. Again the synthesis of authentic compounds would have to be performed to fully elucidate the pathways involved.

The NO_2 investigations confirmed that non-phenolic compounds possessing a 1,2-dione group within a ring system provide a useful means for trapping NO_2, a possible strategy for reducing yellowing. Derivatives of these compounds suitably substituted to reduce volatility and further enhanced for antioxidant behaviour could provide an alternative to the hindered phenolic antioxidant BHT and should be investigated.

Investigation of a non-nitrating hindered phenol system resulted in the synthesis of several *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl) cyclic imides, which have provided a group of new compounds showing similar antioxidant activity to BHT. Furthermore, they are less prone to NO_2-yellowing than 4-substituted hindered phenols possessing labile hydrogen atoms. Kinetic studies on the solvolysis of these cyclic imide compounds suggest that sufficient stability to obtain a commercially useful antioxidant may be imparted by bulky alkyl substituents in the imide ring, as in the case of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-3,3-dimethylglutarimide and *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-2,3-diethyl-2,3-dimethylsuccinimide which have been successfully prepared. In order to fully substantiate the use of such alkyl-substituted compounds as alternatives to BHT, polymer incorporation studies should be carried out.

The potential of compounds structurally similar to BHT could also be considered, where the imido group is replaced by other heteroatoms which may further enhance antioxidant potential.

There remains considerable scope for non-yellowing antioxidant compounds based on the imide-substituted hindered phenols. The presented work has indicated some potentially useful directions for future work in this area..

CHAPTER 6

Experimental

6. Experimental

6.1 Instrumentation

The physical properties of compounds were determined on the following instruments. ^{1}H and ^{13}C NMR spectra taken on a Jeol EX400FT spectrometer, using tetramethylsilane as the internal reference were supplied by Mr G. Howell of the Chemistry Department. Infra-red spectra were measured on a Nicolet 205 FT-IR spectrometer. UV-vis spectra were measured on a Kontron UV spectrophotometer. Mass spectra recorded on a VG20-250 quadrupole spectrometer in either the electron impact (EI+) or fast atom bombardment (FAB+) mode were supplied by Mr B. Cook of the Chemistry Department. Melting points were measured on an Electrothermal Digital Melting Point apparatus and are uncorrected. Elemental microanalyses were provided by Medac Ltd.

6.2. Substrates, products and reagents

Reagents and solvents were obtained from commercial sources unless otherwise stated. 2,6-Di-t-butyl-4-nitrophenol (NP), 2,6-di-*t*-butyl-4-methyl-4-nitro-2,5-cyclohexadiene-1-one (MQN), 2,6-di-*t*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadiene-1-one (MQOL), 1,2-bis(2,5-di-*t*-butyl-4-hydroxyphenyl)ethane (BHPEA), 3,3′,5,5′-tetra-*t*-butyl-dipheno-4,4′-quinone (DPQ) and 3,3′,5,5′-tetra-*t*-butyl-stilbene-4,4′-quinone (SQ) were kindly supplied by V. Wilson (BTTG, Manchester). 3,5-Di-*t*-butyl-4-hydroxybenzylalcohol (BALC) was supplied by Lancaster. 2,6-Di-t-butyl-1,4-benzoquinone (BQ), 3,5-di-*t*-butyl-4-hydroxy-benzaldehyde (BALD), 3,5-di-*t*-butyl-4-hydroxybenzoic acid (BAC) and 2,6-di-*t*-butyl-4-methylphenol (BHT)(m.p. 70.5°C, lit. m.p. 71°C (CRC Handbook)), linoleic acid (99% and 60%), decahydronaphthalene, 3-methyl-1,2-cyclopentanedione, 3-hydroxy-2-methyl-4-pyrone, 3-hydroxy-1,2-dimethyl-4(1H)-pyridone, kojic acid, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, ascorbic acid (vitamin C), cyclohexane, sodium dodecyl sulphate, thiobarbituric acid, *t*-butanol, potassium dihydrogen orthophosphate, methyl formate (99%), dioxane (99%), sodium acetate, 4-acetoxy-2-butanone, levulinic acid, dimethyl oxalate, 4-aminophenol,

dimethylaminopyridine, N-acetyl-4-aminophenol, 4-methylphenol, 2,6-di-*t*-butylphenol (99%), palladium on activated charcoal (10%), acetic anhydride (99%), thiodiacetic acid (98%), phosphorus trichloride (98%), succinic anhydride (99%), maleic anhydride (99%), glutaric anhydride (97%), 3,3-dimethylglutaric anhydride (99%), diglycolic anhydride (90%), hydrazine sulphate, sodium cyanide, bromine, anthracene and 2-butanone were supplied by Aldrich Chemical Company Limited. Potassium iodide (AR), starch soluble, acetic acid (AR), chloroform (AR), 1-butanol, pyridine, hydrochloric acid, sulphuric acid, 0.1M sodium hydroxide (SVS) and 0.1M sodium thiosulphate (CVS) were supplied by BDH Limited. Dichloromethane, 2,2,4-tetramethylpentane, tetrahydrofuran (AR), methanol (AR), acetonitrile (HPLC), toluene, diethyl ether, and ethyl acetate were supplied by Rhone Poulenc. Tetrabutylammonium tetrafluoroborate was supplied by Fluka. Ethanol (99%) was supplied by Hayman Limited. Nitrogen dioxide (900vpm and 50vpm in air), dinitrogen tetraoxide, isobutylene, hydrogen, nitrogen and air were supplied by British Oxygen Special Gases. All chemicals were used as supplied without purification.

6.3. Analytical methods

6.3.1. GC analyses

GC analyses were performed on a Pye Unicam 4500 instrument, using a SGE BP5 or BP20 capillary column (12M x 0.53mm I.D.), with on column injection and a flame ionisation detector (FID) at 250°C. Specific conditions and retention times for each antioxidant are summarised in Table 6.1. Injection volumes were usually 1µl and data collection was carried out using a Shimadzu G-RIB integrator.

Quatitation was performed using gravimetrically prepared solutions of authentic material (when available) in the same solvent as that used for assay.

Table 6.1. GC conditions for compounds.

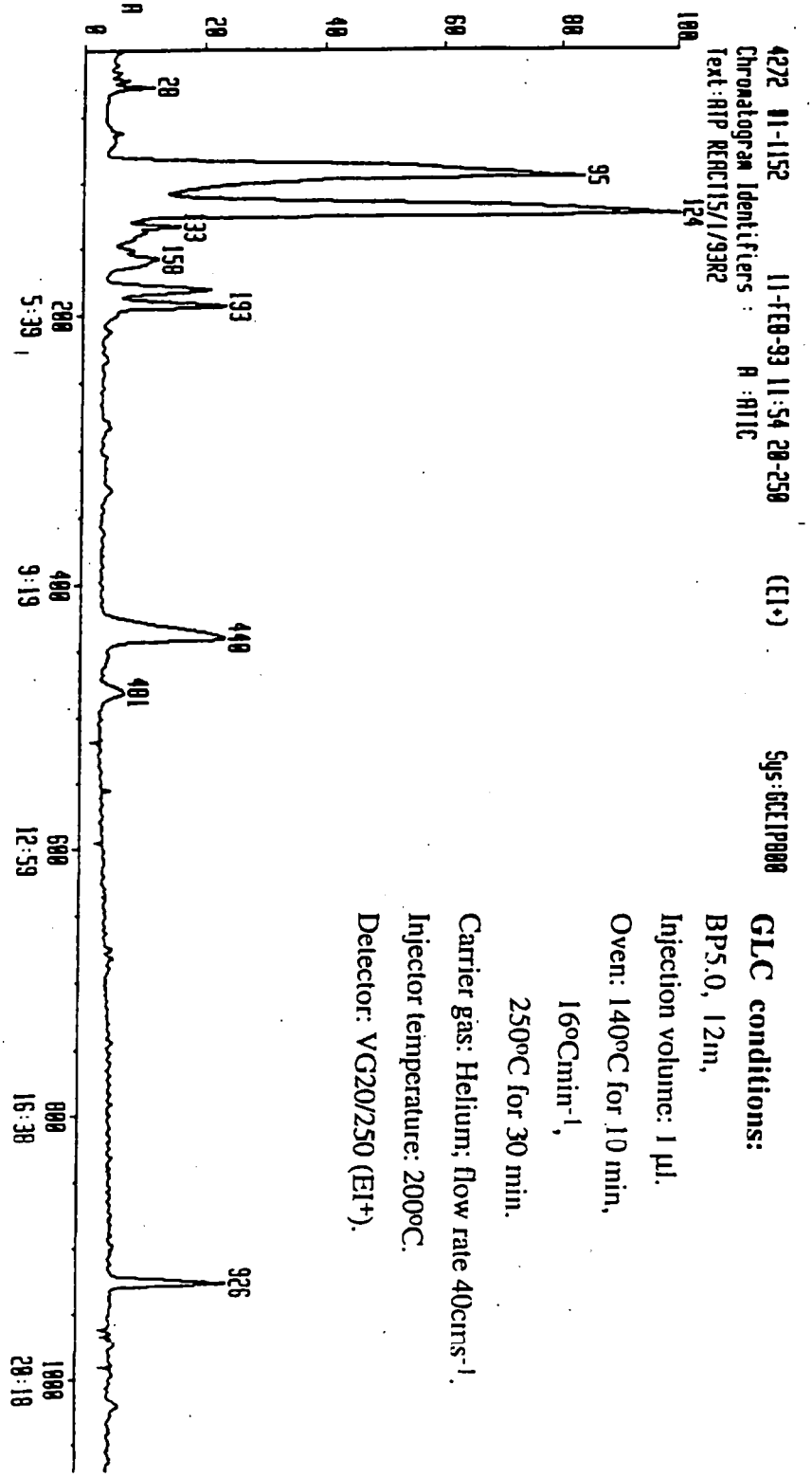
Compound	GC Column	Temperature Program	Retention Time (min)
BHT	BP 5.0	140°C (for 10 min), ramp: 16°C min^{-1}, final temperature 250°C (for 30 min)	8.0
Kojic acid	BP 5.0	60°C (for 2 min), ramp: 16°C min^{-1}, final temperature 250°C (for 10 min)	10.4
Maltol	BP 5.0	100°C	10.7
3-methyl-1,2-cyclopentane-dione	BP 20	90°C (for 10 min), ramp 2°C min^{-1}, final temperature 220°C	25.3

6.3.2. GC-MS analyses

Samples were analysed using a Hewlett Packard 5890 GC coupled to a VG20-250 quadrupole mass spectrometer calibrated with perfluorokerosene (Fluka) and tuned with heptacosafluorotributylamine (Fluka). Spectra were run at an electron energy of 70 eV and a source temperature of 200°C. Scans were taken every 1.1 seconds. Injections were made onto a SGE BP5 or BP20 capillary column (12M x 0.32mm I.D.) with a helium flow at 5 psi. and using an oven temperature program.

Products were identified by GC-MS analyses of authentic samples on the same instrument or by comparison with library spectra. Figure 6.1 shows a typical example of a GC-MS chromatogram for the products from the reaction of 10mM BHT with NO_2 (200vpm) in 70% (v/v) ethanol/water at 25°C (Reaction No. 3).

Figure 6.1. Typical GC-MS chromatogram for the products from the reaction of 10mM BHT with NO_2 (200vpm) in 70% (v/v) ethanol/water at 25°C (Reaction No. 3).



6.3.3. HPLC Analyses

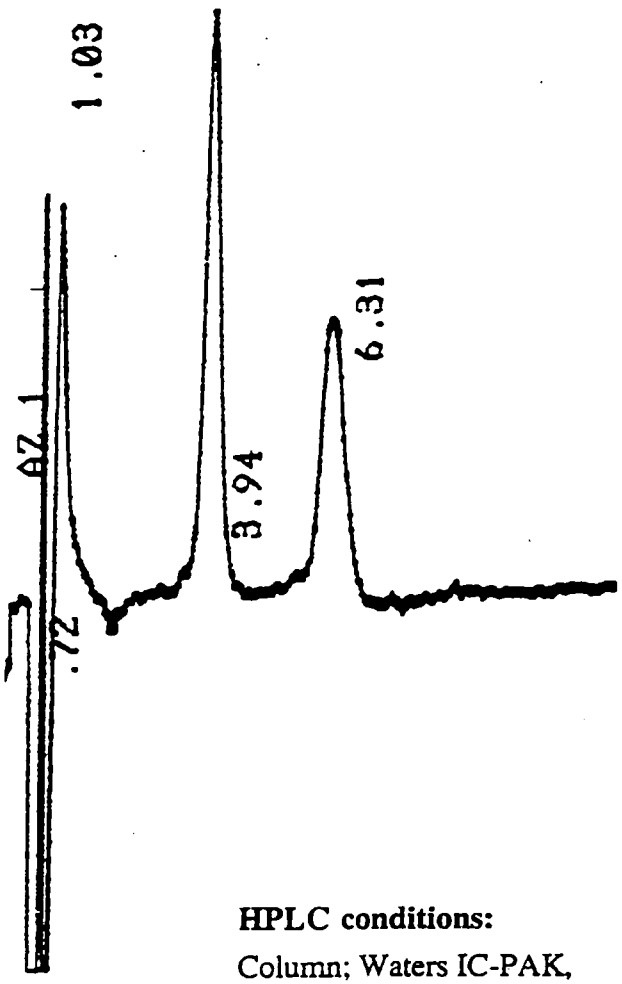
All eluents were prepared where possible from HPLC grade reagents, solvents and deionised water (Elgastat B.102 deioniser, input distilled water). The eluents were degassed by bubbling helium (BOC) through the solutions for fifteen minutes prior to use. Data collection was carried out using either a Spectraphysics chromjet integrator or a Varian Chromstar data system.

Concentrations were calculated from peak areas calibrated against gravimetrically prepared solutions of authentic material in the same solvent as that used for the assay, unless otherwise stated. Reproducibility of injections was better than ± 5% and reported concentrations are the average of at least two injections.

The HPLC assay of nitrite and nitrate ions was performed by ion chromatography using the following apparatus: Milton Roy Constametric 3000 HPLC pump, Waters IC PAK column, Milton Roy Conductomonitor III detector and Waters 712 WISP autosampler; eluent, borate/gluconate buffer (pH 8.4) (Waters). Sample injection volume was 100 µl. Figure 6.2 shows a typical chromatogram for nitrite and nitrate ions. The retention times for nitrite and nitrate were 4.2 and 6.5min, respectively, while the limit of detection was 10 µM.

BHT product analyses were performed using a Varian 5000 LC pump, Waters Novapak C18 column (4mm x 30cm), Varian 9060 diode array spectrometer and an acetonitrile-water gradient (Eluent A: acetonitrile, Eluent B: 5% acetonitrile in distilled water. T_0 50% A, T_{25} 95% A). Sample injection volume was 20 µl. Figure 6.3 shows a typical chromatogram for the products from the reaction of 1mM BHT with NO_2 (200vpm) in 70% (v/v) ethanol/water at 25°C (Reaction No. 6).

Figure 6.2. Typical HPLC chromatogram for a 100 mM standard solution of nitrite and nitrate ions in water.



HPLC conditions:
Column; Waters IC-PAK,
Injection volume; 100 µl,
Mobile Phase; borate/gluconate buffer (pH 8.4),
Flow rate; 1.0cm^3min^{-1},
Detector; Conductivity.

Figure 6.3. Typical HPLC chromatogram for the products from the reaction of 1mM BHT with NO_2 (200vpm) in 70% (v/v) ethanol/water at 25°C (Reaction No. 6).

HPLC conditions:

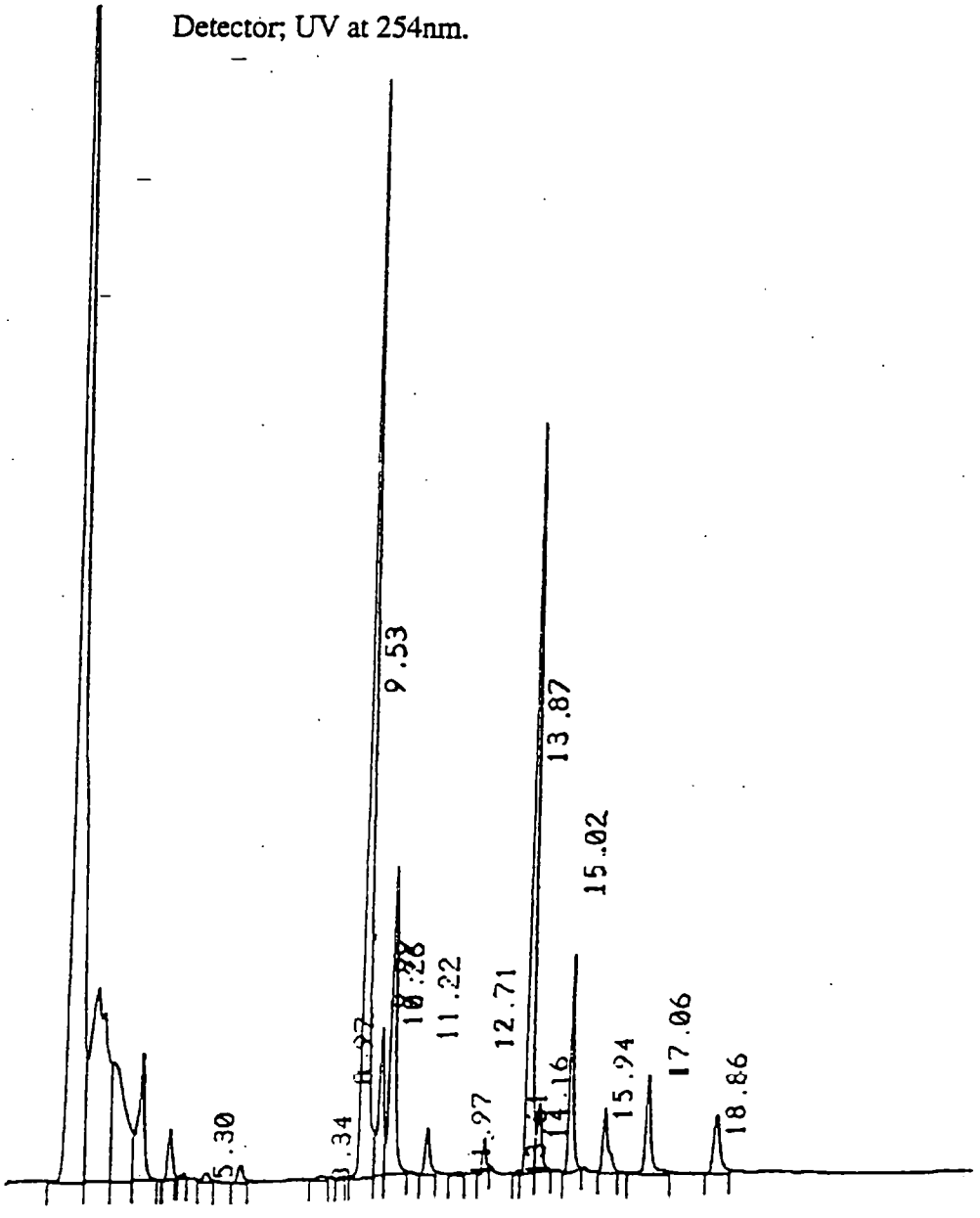
Column; Waters Novapak C18 (4mm x 30cm),

Injection volume; 20 µl,

Mobile Phase; acetonitrile-water gradient (Eluent A: acetonitrile, Eluent B: 5% acetonitrile in distilled water. T_0 50% A, T_{25} 95% A),

Flow rate; 1.0cm^3min^{-1},

Detector; UV at 254nm.



The assay of products from the hydrolysis of *N,N,O*-triacetyl-4-aminophenol was carried out using a Varian 5000 LC pump, Waters Novapak C18 column (4mm x 30cm), Varian 9060 diode array spectrometer and an acetonitrile-phosphate buffer gradient (Eluent A: acetonitrile, Eluent B: 5% acetonitrile in 0.05M KH_2PO_4 buffer pH 6.25. T_0 100% B, T_{10} 100% B, T_{20} 80% B). Sample injection volume was 20 µl. Figure 6.4 shows a typical chromatogram for the products from the hydrolysis of 1mM *N,N,O*-triacetyl-4-aminophenol in 50% (v/v) methanol/0.025M sodium tetraborate buffer pH 9.0 at 25°C after 5 min.

Assays of *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-imide solvolysis solutions were carried out using a Milton Roy Constametric 3000 IIPLC pump, Jones Apex ODS II column (4.6 mm x 30 cm), Milton Roy UV detector and the eluents described in Table 6.2. Sample injections of 20 µl were carried out using a Waters 712 WISP autosampler. Figure 6.5 shows a typical chromatogram for the solvolysis of 10mM *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide in 50% methanol/ 0.1M KH_2PO_4 buffer pH 7.0 at 25°C after 20 min.

The peak purity parameter (PuP) was used to confirm peak identity. The PuP is determined as an average wavelength weighted by the absorbance squared between two wavelength values, I_s and I_e (Equation 6.1, Webster, 1991). It is weighted towards wavelengths of higher absorbance values.

$$PuP = \frac{\Sigma (E^2_n \times I_n)}{\Sigma E^2_n} \qquad (6.1)$$

where E_n - absorption at I_n,

I_n - wavelength in spectral range,

I_s - starting wavelength of the spectral range,

I_e - end wavelength of the spectral range.

Figure 6.4. Typical HPLC chromatogram for the products from the hydrolysis of 1mM *N,N,O*-triacetyl-4-aminophenol in 50% (v/v) methanol/0.025M sodium tetraborate buffer pH 9.0 at 25°C after 5 min.

HPLC conditions:

Column; Waters Novapak C18 (4mm x 30cm),

Injection volume; 20 µl.

Mobile Phase; acetonitrile-phosphate buffer gradient (Eluent A: acetonitrile, Eluent B: 5% acetonitrile in 0.05M KH_2PO_4 buffer pH 6.25. T_0 100% B, T_{10} 100% B, T_{20} 80% B),

Flow rate; 1.0cm^3min^{-1},

Detector; UV at 254nm.

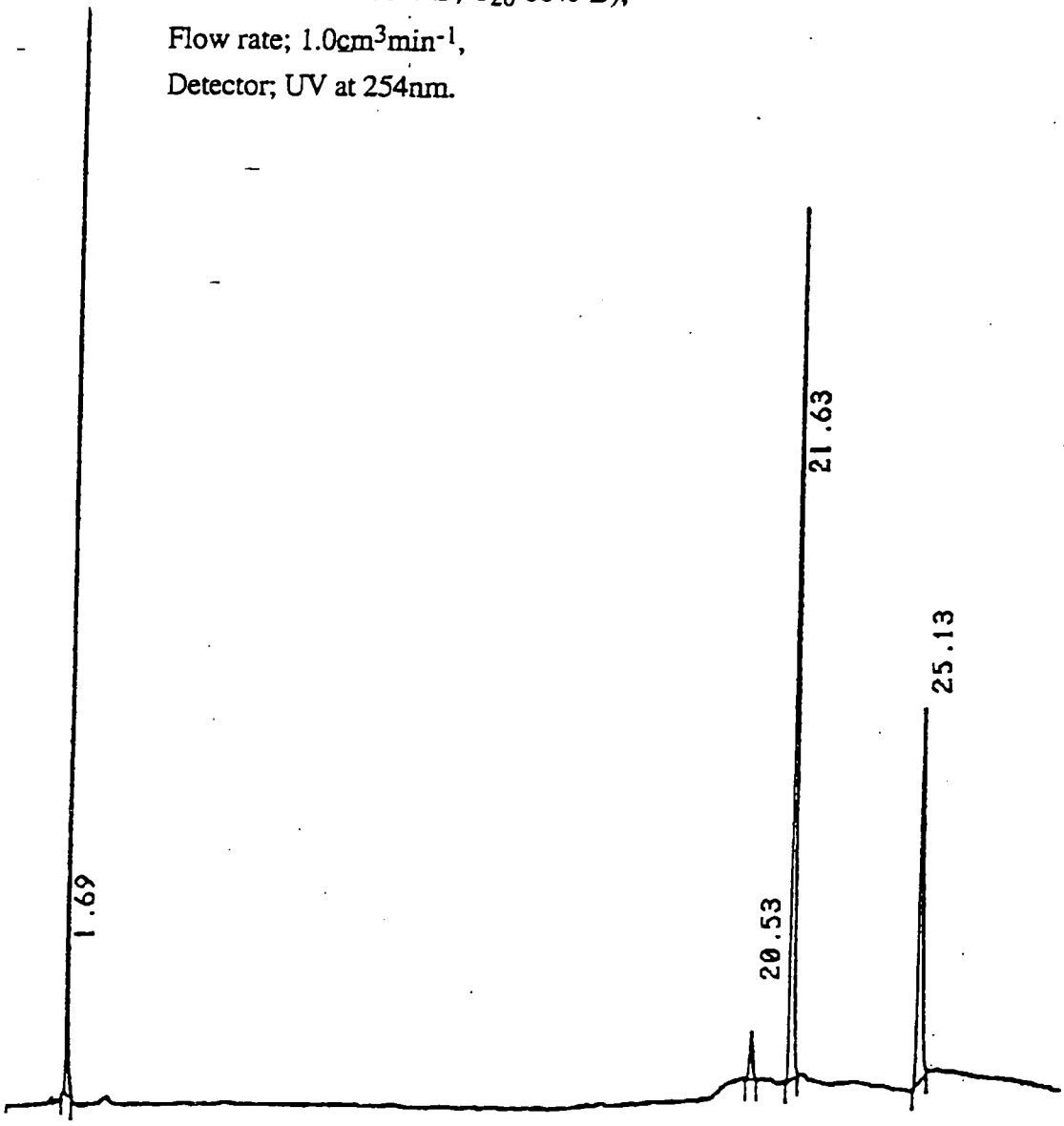
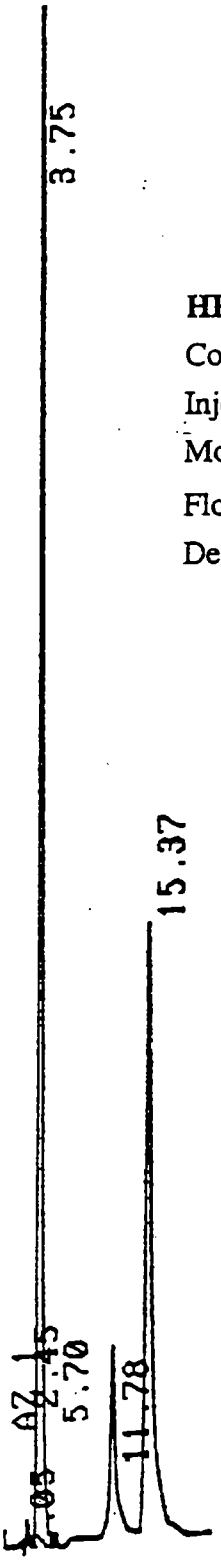


Figure 6.5. Typical HPLC chromatogram for the solvolysis of 10mM *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-succinimide in 50% (v/v) methanol/ 0.1M KH_2PO_4 buffer pH 7.0 at 25°C after 20 min.



HPLC conditions:
Column; Jones Apex ODS II (4.6mm x 30cm),
Injection volume; 20 µl,
Mobile Phase; 50% acetonitrile/ 0.01M KH_2PO_4 pH 5.0,
Flow rate; 1.0cm^3min^{-1},
Detector; UV at 254nm.

Table 6.2. HPLC eluents and retention times for *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-imides and esters.

Compounds	Eluent	Retention Time (min)
BHPS, BHPSAMe	50% acetonitrile/ 0.01M KH_2PO_4 pH 5.0	10.8, 14.2
BHPS, BHPSAEt	50% acetonitrile/ 0.01M KH_2PO_4 pH 5.0	10.8, 19.2
BHPM, BHPMAMe	50% acetonitrile/ 0.01M KH_2PO_4 pH 5.0	18.4, 12.3
BHPG, BHPGAMe	50% acetonitrile/ 0.01M KH_2PO_4 pH 5.0	10.9, 14.9
BHPMG, BHPMGAMe	57% acetonitrile/ 0.01M KH_2PO_4 pH 5.0	13.8, 19.8
BHPDG, BHPDGAMe	60% methanol/ 0.01M KH_2PO_4 pH 5.0	10.2, 21.5
BHPTG, BHPTGAMe	60% methanol/ 0.01M KH_2PO_4 pH 5.0	14.4, 23.4

6.3.4. Calibration of NO_2 gas concentrations

Different NO_2 gas concentrations were generated from 900vpm or 50vpm NO_2 by dilution with air or nitrogen, using a Signal Series 850 Gas Blender. Settings of the gas blender were calibrated by bubbling the diluted NO_2 gas streams for a timed period into 0.1M sodium hydroxide. The NO_2 produces equal amounts of nitrate and nitrite (6.2), which can be related to the NO_2 gas concentration.

$$2NO_2 + 2OH^- \longrightarrow NO_2^- + NO_3^- + H_2O \qquad (6.2)$$

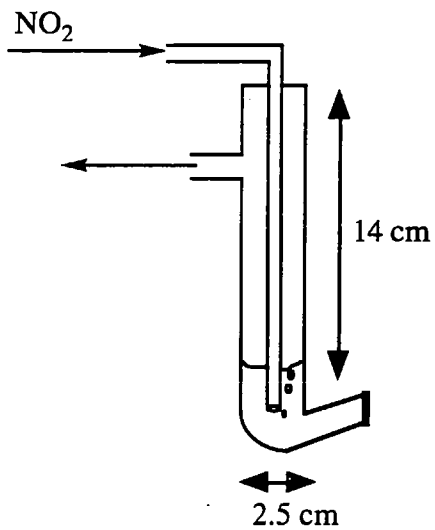
Assay of nitrite and nitrate by ion chromatography (see Section 2.3.) gave the NO_2 concentrations delivered by various settings of the blender within ± 5%.

6.4. Reaction methods with gaseous NO_2

6.4.1. Determination of yellowing indices

A 10mM test solution of antioxidant was prepared in 70% (v/v) ethanol/aqueous 0.05M KH_2PO_4. An aliquot (20 cm^3) of the test solution was placed in the reaction vessel (Figure 6.6.) and gaseous NO_2 (900vpm) passed through the solution at 100 cm^3min^{-1} for seven hours through a gas distribution tube (P100, BDH) at ambient temperature. The solution was then transferred quantitatively to a 25 cm^3 volumetric flask, made up to volume with reaction solvent and developed for 15 h at 25°C.

Figure 6.6. Reaction vessel design.



Construction: - Glass

The UV-vis spectrum (200-700nm) of the solution was recorded in a 1cm cuvette against a 70% (v/v) ethanol/aqueous 0.05M KH_2PO_4 blank. As a control, the UV-vis spectrum (200-700nm) of the unreacted antioxidant test solution was also recorded in a 1cm cuvette against a 70% (v/v) ethanol/aqueous 0.05M KH_2PO_4 blank. The yellowing index (OU_{YI}) was determined from the absorbance data by means of equation 6.3.

$$OU_{YI} = \frac{W_1}{W_2} \times 10 \qquad (6.3)$$

where, W_1 - difference in absorbance area (weight) above 400nm (g) between exposed antioxidant test and control solution,

W_2 - area (weight) of absorbance corresponding to 0.25 AU x 100nm[a] (g).

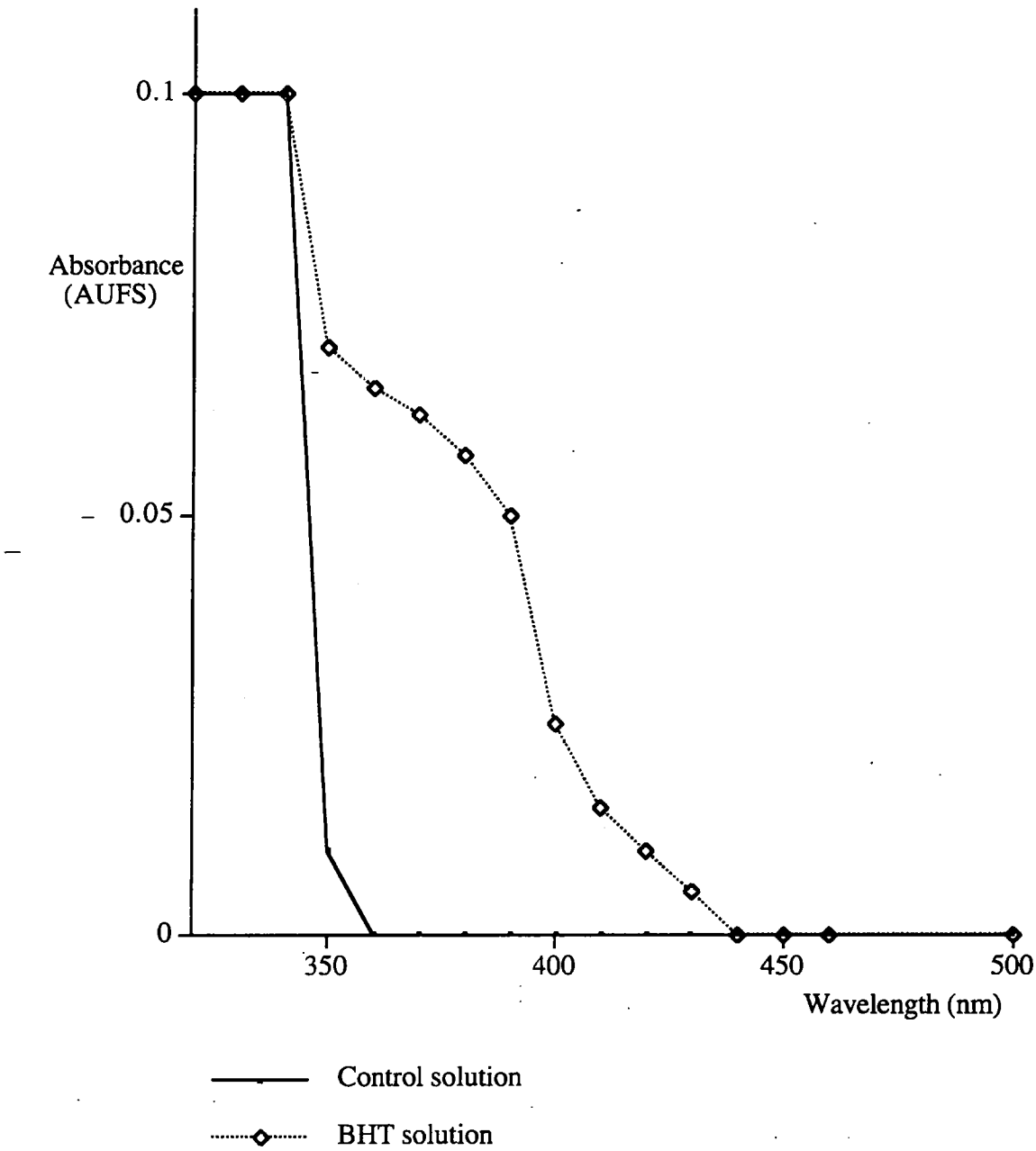
[a] - this figure allows for the standardisation of each determination as a result of the use of different types of chart paper.

Figure 6.7. shows a typical UV spectrum for the NO_2 yellowing reaction solution for 10mM BHT. This gives a OU_{YI} value of 6.69. Typically OU_{YI} determinations were the average of at least two experiments and agreed to within ± 5%.

6.4.2. Reactions of BHT with gaseous NO_2

The BHT solution (20 cm^3, 1 to 30mM) in 70% (v/v) ethanol/water in the reaction vessel (described in section 3.1) at 25°C was sparged with nitrogen for 10 min. Gaseous NO_2 from the gas blender (10 to 900 vpm) was passed through the solution at 40 cm^3min^{-1} for timed periods. Finally, the solution was sparged with nitrogen for 10 min. The reaction solution was then either extracted and analysed by GC and GC-MS or an aliquot (5 cm^3) quenched in 50% (v/v) 0.1M hydrochloric acid/ethanol (5 cm^3) and analysed by HPLC. For the GC assays, the reaction solution was transferred quantitatively to a 25 cm^3 round-bottomed flask and the ethanol evaporated *in vacuo*. The residue was extracted into dichloromethane (2 x 5 cm^3), separated, the dichloromethane evaporated off and then reconstituted in 2,2,4-trimethylpentane (0.5 cm^3).

Figure 6.7. Typical UV-vis spectrum for a NO_2-yellowing reaction solution for 10mM BHT.



The reactions of BHT with NO_2 carried out are summarised in Table 6.3.

Table 6.3. Reactions of BHT with NO_2

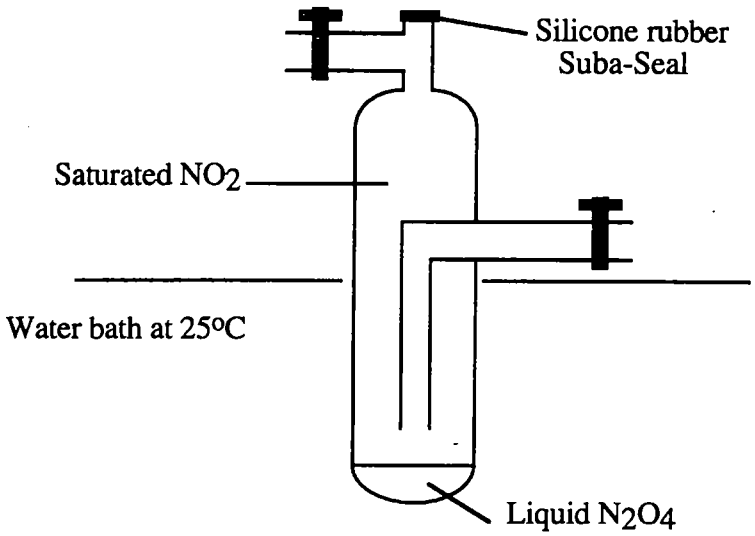
Reaction No.	[BHT] mM	[NO_2] vpm	Time (h)	pH (*)	Analysis
1	10	900	2	neutral	GC
2	10	200	2	alkaline	GC,GCMS
3	10	200	4	alkaline	GC,GCMS,HPLC
4	30	200	4	alkaline	GC,GCMS,HPLC
5	1	200	0.4	alkaline	HPLC
6	1	200	4	alkaline	GC,GCMS,HPLC
7	1	200	4	neutral	HPLC
8	1	10	8	alkaline	HPLC
9	12	200	0.4	alkaline	HPLC
10	12	200	4	alkaline	HPLC

*Alkaline – solution made alkaline with 1M NaOH (0.5 cm^3) and neutralised after reaction with 1M HCl. Neutral - reaction as typical procedure.

6.4.3. Reactions of non-phenolic compounds with gaseous NO_2

Ca. 200mg of compound was dissolved in methyl formate or dioxane (50 cm^3) contained in a Suba-seal stoppered flask at 25°C. Gaseous NO_2 (180 cm^3) was added via a gas syringe (Pressure-Lok) from a glass resevoir as shown in Figure 6.8., which supplied a constant level of saturated NO_2 vapour at the temperature required. The mixture was left stirring at ambient temperature until the compound had reacted fully (as shown by TLC). This usually required ca. 1 h. Further reaction was stopped by sparging the solution with nitrogen, the solvent was evaporated and the residue reconstituted in methanol. The mixture was then analysed by GC and GC-MS.

Figure 6.8. NO_2 reservoir.



Construction: - Glass
Teflon taps

6.5. Kinetics of imide formation and solvolysis

Both reactions were followed by monitoring the loss of substrate and formation of product similtaneously by reversed-phase HPLC.

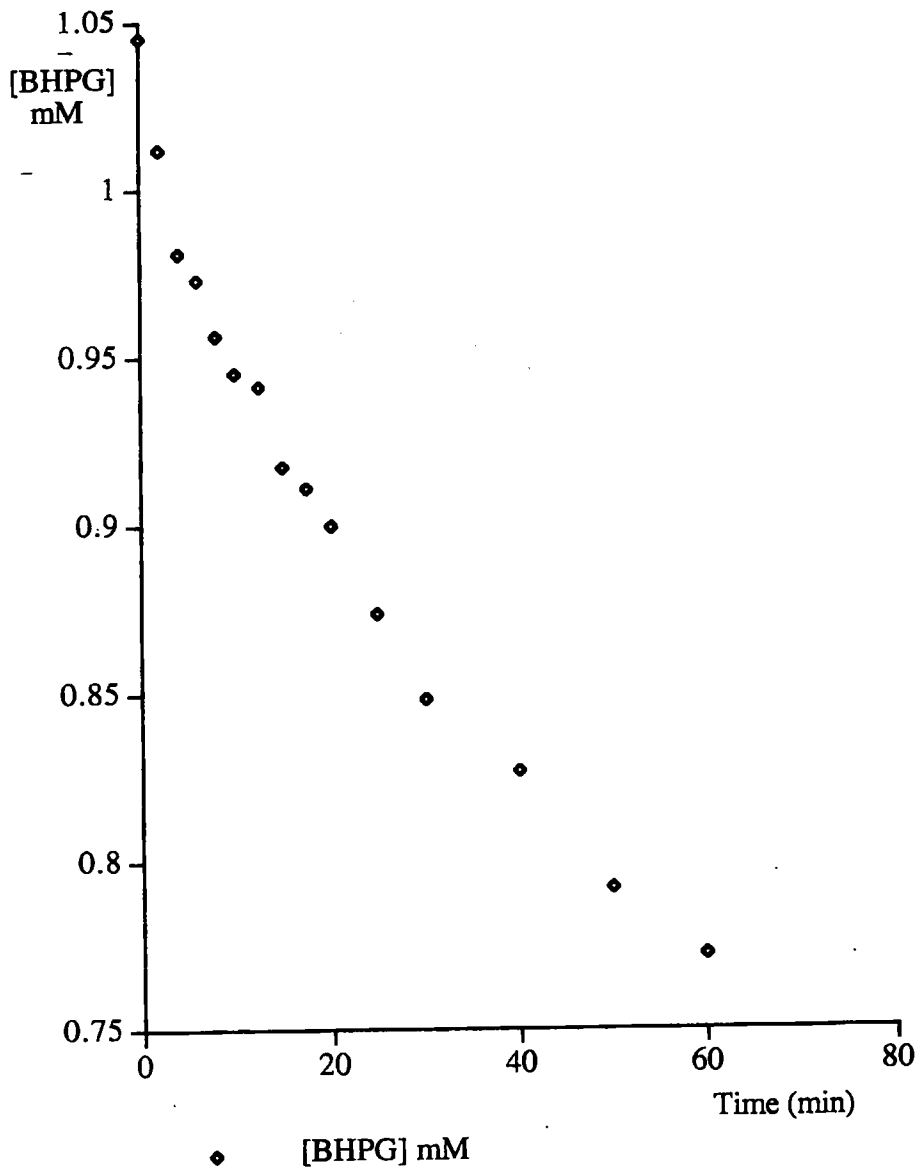
Usually, a stock solution of the substrate (10mM) was prepared in acetonitrile (except BHPTD - where tetrahydrofuran was used). An aliquot of the stock solution (1 cm^3) was diluted with 50% (v/v) methanol/aqueous buffer (pH 9.0 - 0.025M sodium tetraborate or pH 7.0 - 0.1M KH_2PO_4) in a volumetric flask (100 cm^3) to give the reaction solution. After thorough mixing the volumetric flask was immersed in a thermostatted water bath at 25°C and the reaction timing commenced. At timed intervals aliquots (2 cm^3) were removed, quenched in 50% (v/v) 0.1M HCl/acetonitrile (3 cm^3) and then assayed by HPLC against a set of gravimetrically prepared standards of authentic substrates and products by comparison of peak areas.

Initial rates were determined by inspection from plots of the concentration of imide/ester (mM) against time. The rates were converted into pseudo first-order rate coefficients assuming equation 6.4 applies.

$$\frac{-d[\text{Substrate}]}{dt} = \frac{d[\text{Product}]}{dt} = \underline{k}_0[\text{substrate}] \qquad (6.4)$$

Usually, values of $\underline{k}_0$ from the loss of substrate and formation of product agreed to ± 5%, and a mean value was taken. Figure 6.9. shows a typical plot for the reaction of BHPG in 50% (v/v) methanol/0.025M sodium tetraborate pH 9.0 at 25°C. This gives a value of $\underline{k}_0$ = 5.7 x 10^{-3} mMmin^{-1}.

Figure 6.9. Typical graph of concentration of imide against time for the solvolysis of BHPG in 50% (v/v) methanol/0.025M sodium tetraborate pH 9.0 at 25°C.

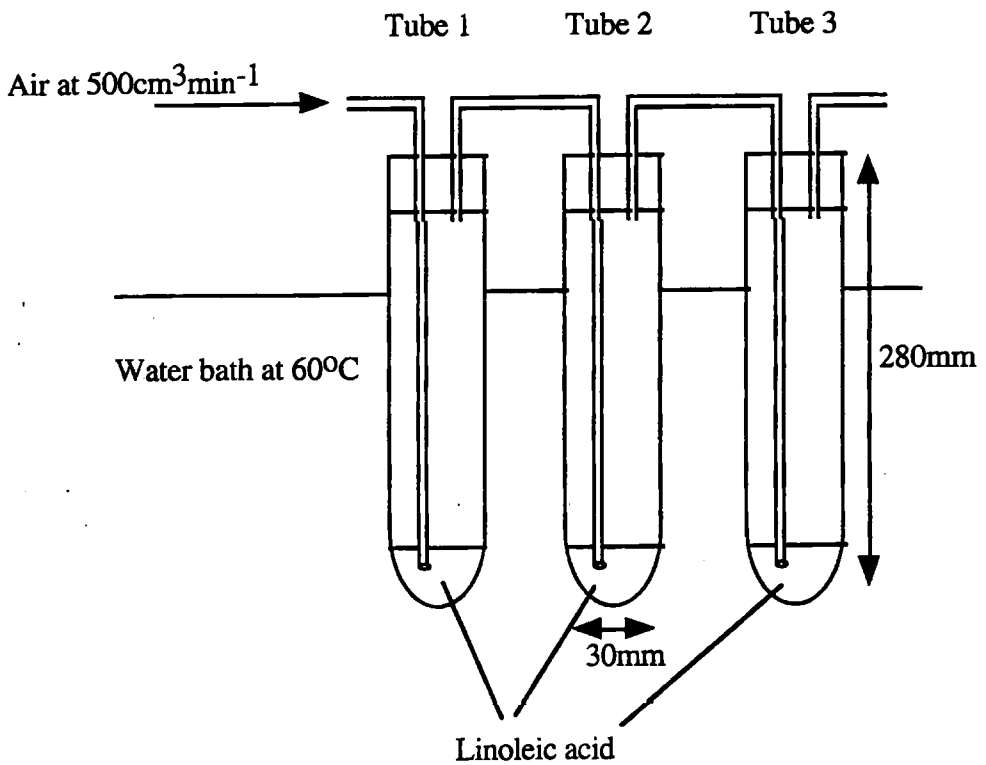


6.6. Measurement of antioxidant efficiencies

6.6.1. Inhibition of linoleic acid oxidation

Linoleic acid (5 cm^3) was added to each of two or more boiling tubes (30 mm diam. x 280mm) connected in series by a glass gas manifold (Figure 6.10). The first tube typically contained linoleic acid without added antioxidant and was used to determine uninhibited oxidation. The remaining tubes contained linoleic acid with 100µl aliquots of 0.1mM antioxidant in decahydronaphthalene. The tubes were incubated at 60°C in a water bath for 2 hours with air flowing through a 2mm internal diameter tube at 500 cm^3min^{-1}. After oxidation the peroxide value (POV) and thiobarbituric acid value (TBAV) of each tube were determined.

Figure 6.10. Linoleic acid oxidation apparatus.



Construction: - Glass

6.6.1.1. POV determination of oxidised linoleic acid

An aliquot (1g) from one of the boiling tubes was accurately weighed into a 100 cm^3 conical flask and dissolved in acetic acid-chloroform (3:2 v/v, 20 cm^3). Saturated potassium iodide solution (1 cm^3) was added and the mixture shaken vigorously for 1 min. The mixture was allowed to stand in the dark for a further minute, and distilled water (30 cm^3) added. The solution was titrated with 0.1M sodium thiosulphate solution using starch solution (0.5% w/v, 1 cm^3) as an indicator and the POV derived from equation 6.5.

$$\text{POV (mEq/kg)} = \frac{(T_0 - T_1) \times M \times 10^3}{G} \qquad (6.5)$$

where, T_0 - titre without added antioxidant (blank, cm^3),

T_1 - titre with added antioxidant (sample, cm^3),

G - weight of aliquot taken for POV analysis (g),

M - molarity of thiosulphate solution (mol dm^{-3}).

6.6.1.2. TBAV determination of oxidised linoleic acid

An aliquot (20mg) from one of the boiling tubes was weighed accurately into a test tube (15 cm^3). Sodium dodecyl sulphate solution (3% w/v, 0.5 cm^3) and 2.0M acetic acid buffer solution (pH 3.6, 1.5 cm^3) were added with stirring, followed by thiobarbituric acid solution (0.8% w/v, 1.5 cm^3) and distilled water (0.5 cm^3). The mixture was then heated for 75 min in a boiling water bath, followed by chilling for 5 min at ambient temperature. After adding 0.2M hydrochloric acid (1.0 cm^3) and 1-butanol-pyridine (15:1 v/v, 5.0 cm^3) the mixture was shaken vigorously and then allowed to separate (10 min). The absorbance of the 1-butanol layer measured in a 1cm cuvette against a 1-butanol reference at 532nm gave TBAV by dividing the absorbance by the sample weight (Equation 6.6).

$$TBAV = \frac{Abs_{532}}{W} \qquad (6.6)$$

where, Abs_{532} - sample absorbance at 532nm,

W - weight of sample taken for TBAV analysis (g).

6.6.1.3. Calculation of efficacy of antioxidants

The inhibitory ratio (I.R.) was calculated from both POV and TBAV indeces using equation 6.7.

$$I.R.\ (\%) = \frac{(A - B) \times 100}{(A - C)} \qquad (6.7)$$

where, A - POV (TBAV) of linoleic acid after incubation,

B - POV (TBAV) of linoleic acid after incubation with 2mM antioxidant,

C - POV (TBAV) of linoleic acid before incubation.

6.6.2. NO_2^-/NO_3^- ratio

A 10mM solution of the test compound was prepared in 70% (v/v) ethanol/0.1M sodium hydroxide. Immediately, an aliquot (30 cm^3) was transferred to the reaction vessel (described in section 3.1.) at 25°C. NO_2 (900vpm) was bubbled through the solution at 100 cm^3min^{-1} for 30 min. At timed intervals aliquots (2 cm^3) were removed and analysed for NO_2^- and NO_3^- by ion chromatography with peak area comparison against authentic standards. The NO_2^-/NO_3^- ratio was calculated from equation 6.8.

$$NO_2^-/NO_3^- \text{ ratio} = \frac{[NO_2^-]}{[NO_3^-]} \qquad (6.8)$$

where, $[NO_2^-]$ - concentration of NO_2^- (mM),

$[NO_3^-]$ - concentration of NO_3^- (mM).

NO_2^-/NO_3^- ratios determined were typically the average of at least two experiments and gave values which agreed to better than ± 5%.

6.6.3. Competitive inhibition of BHT/NO_2 products

In this procedure, the antioxidant efficiency was determined from the substrate's ability to competitively inhibit the reaction of BHT with gaseous NO_2.

Solutions of test compound (20 mM) and BHT (20 mM) were prepared in 70% (v/v) ethanol/water. 5 cm^3 of each solution was placed in the reaction vessel (described in section 3.1) and NO_2 (900vpm) passed through the solution at 100 cm^3min^{-1} for one hour at 25°C. The solution was analysed by HPLC and the BHT reaction products MQOL and MQN were quantitated. A reaction carried out similarily using 5 cm^3 of 20mM BHT plus 5 cm^3 70% (v/v) ethanol/water was used as a control. The antioxidant efficiency was determined from equation 6.9.

$$\text{Antioxidant efficiency} = \frac{[BHT]_R}{[BHT]_C} \qquad (6.9)$$

where, $[BHT]_R$ - sum of MQN and MQOL products from reaction of NO_2 with BHT in the presence of test compound,

$[BHT]_C$ - sum of MQN and MQOL products from reaction of NO_2 with BHT only.

Antioxidant efficiency determinations were typically the average of at least two injections and gave values which agreed to better than ± 2%.

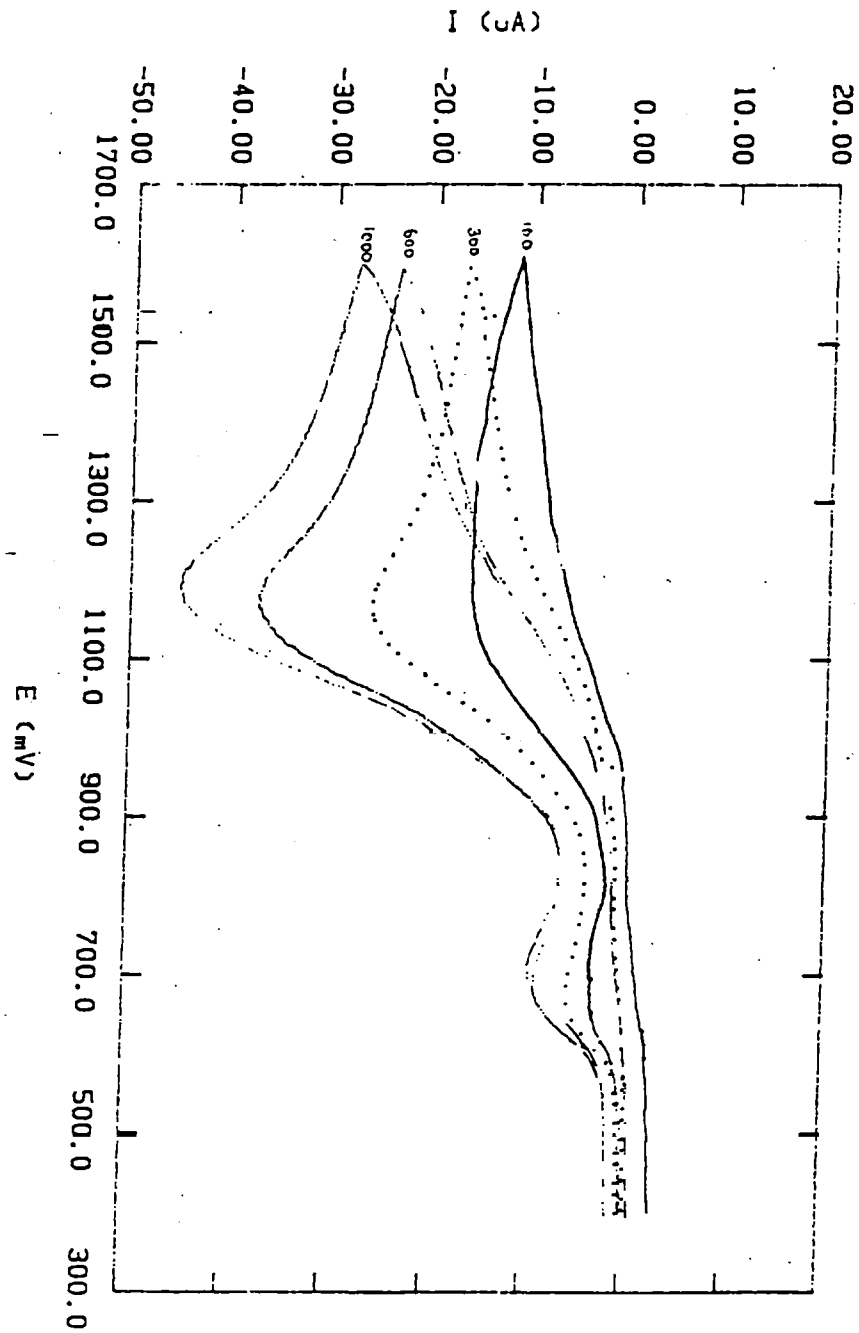
6.6.4. Electrochemical measurements

Electrochemical measurements of the antioxidant compounds were measured by cyclic voltammetry against an anthracene standard.

Solutions of test compound (2.0 mM) plus 0.1M tetrabutylammonium tetrafluoroborate were prepared in acetonitrile. An aliquot (10 cm^3) of the solution was placed in a reaction vessel and purged with nitrogen. Using platinum disc working- and platinum wire counter-electrodes with a silver reference electrode, the current was determined as the potential cycled from zero to 2.00v using a Princeton Applied Research PAR Model 273A Potentiostat/Galvanostat controlled by Research Electrochemistry software 275/250 and an IBM computer. In independent experiments the contribution of the solvent and electrolyte to the measured current was determined and then subtracted from the current measurements above to provide background-corrected data.

The background-corrected voltammograms were plotted on a Could Colorwriter 6120. Figure 6.11. shows a typical voltammogram for 2.0mM BHT. The potentials were referenced to an aqueous saturated calomel electrode by repeating the measurements using anthracene in place of antioxidant.

Figure 6.11. Typical voltammograms for the electrochemical oxidation of a 2.0mM BHT in 0.1M tetrabutylammonium tetrafluoroborate in acetonitrile at 100, 300, 600, 1000 mvsec^{-1} scan speeds.



6.7. Syntheses

N,O-Diacetyl-4-aminophenol (*57*)

4-Aminophenol (1.5g, 0.014mol) and acetic anhydride (3.0 cm^3) were heated under reflux for one hour. The residue was dissolved in dichloromethane (50 cm^3), washed with 5% sodium hydrogen carbonate (3 x 50 cm^3), and after drying over magnesium sulphate, the solvent was removed on a rotary evaporator. Recrystallisation from hexane gave *N,O*-diacetyl-4-aminophenol (0.7g, 26%), m.p. 153-153.5°C (lit.150-151°C, Tingle and Williams (1915)); ν_{max} (KBr) 3369 (NH), 1747 (C=O), 1691 (C=O); δ_H (400mHz, $CDCl_3$) 7.77 (1H, s, NH), 7.47 (2H, d, J 8.79 Hz, aromatic CH), 7.01 (2H, d, aromatic CH), 2.29 (3H, s, ester-CH_3), 2.14 (3H, s, amide-CH_3); δ_C (100mHz, $CDCl_3$) 169.7 (ester C=O), 168.3 (amide C=O), 146.7 (phenol C), 135.6 (aromatic CN), 121.9 (aromatic CH), 120.8 (aromatic CH), 24.4 (ester CH_3), 21.1 (amide CH_3); m/z (EI +ve) 193 (M^+, 14%), 151 (M^+-$COCH_3$, 94), 109 ($C_6H_4OHNH_2$, 100).

N,N,O-Triacetyl-4-aminophenol (*58*)

N,O-Diacetyl-4-aminophenol (1.0g, 0.005mol) and dimethylaminopyridine (0.2g) were dissolved in pyridine (20 cm^3). Acetyl chloride (2 cm^3) was added dropwise with constant stirring. The mixture was heated under reflux for 40 h. The residue was dissolved in dichloromethane (50 cm^3), washed with 5% sodium hydrogen carbonate (3 x 50 cm^3), and after drying over magnesium sulphate, the solvent was removed on a rotary evaporator.

Recrystallisation from cyclohexane gave a mixture of unreacted *N,O*-diacetyl-4-aminophenol and *N,N,O*-triacetyl-4-aminophenol (75% by ^1H NMR, 0.7g), m.p. 77-78.5°C; ν_{max} (KBr) 1749 (C=O), 1713 (C=O), 1666 (C=O); δ_H (400mHz, $CDCl_3$) for N,N,O-triacetyl-4-aminophenol, 7.19 (2H, d, J 7.32 Hz, aromatic CH), 7.14 (2H, d, aromatic CH), 2.31 (3H, s, ester-CH_3), 2.27 (6H, s, amide-CH_3); δ_C (100mHz, $CDCl_3$) 172.9 (ester C=O), 168.9 (amide C=O), 150.7 (phenol C), 136.7 (aromatic CN), 129.7

(aromatic CH), 123.0 (aromatic CH), 27.0 (ester CH_3), 21.1 (amide CH_3); m/z (EI +ve) 235 (M^+, 2%, for *N,N,O*-triacetyl-4-aminophenol), 193 (M^+, 30%, for *N,O*-diacetyl-4-aminophenol), 151 (M^+-$COCH_3$, 56), 109 ($C_6H_4OHNH_2$, 100).

N-Acetyl-4-aminophenol (*59*)

N,N,O-Triacetyl-4-aminophenol (0.2g, 0.001mol) was dissolved in 0.1M sodium hydroxide (10 cm^3). After 15 minutes stirring the mixture was neutralised with solid carbon dioxide, extracted into ethyl acetate (50 cm3) and washed with 0.1M hydrochloric acid (2 x 30 cm^3). The ethyl acetate was dried over magnesium sulphate and the solvent was removed on a rotary evaporator to yield *N*-acetyl-4-aminophenol, m.p.168-169°C (lit. 167-168°C, Tingle and Williams (1915)); ν_{max} (KBr) 3323 (N-H), 1654 (C=O); δ_H (400mHz, CD_3OD) 7.30 (2H, d, J 8.79 Hz, aromatic CH), 6.72 (2H, d, aromatic CH), 2.07 (3H, s, amide-CH_3); δ_C (100mHz, CD_3OD) 171.3 (amide C=O), 155.4 (phenol C), 131.7 (aromatic CN), 123.3 (aromatic CH), 116.5 (aromatic CH), 23.5 (amide CH_3); m/z (EI +ve) 151 (M^+, 28%), 109 ($C_6H_4OHNH_2$, 100), 80 (26).

N-Oxyphenylsuccinimide (*60*)

Succinic anhydride (4.0g, 0.04mol) and 4-aminophenol (1.5g, 0.014mol) were placed in a Pyrex beaker (100 cm^3) and heated in a muffle furnace at 180 °C for 12 hours. The residue was recrystalised from ethanol/acetic acid (50/50 v/v). m.p. 278-280°C (lit 275-276°C, Wirths (1895); ν_{max} (KBr) 3265 (phenol OH), 1695 (C=O); δ_H (400mHz, CD_3SOCD_3) 9.70 (1H, phenol OH), 7.01 (2H, d, J 8.79Hz, aromatic CH), 6.82 (2H, d, aromatic CH), 2.73 (4H, s, CH_2); δ_C (100mHz, CD_3SOCD_3) 177.1 (C=O), 157.0 (phenol C-OH), 128.2 (aromatic C-N), 123.6 (aromatic C-H), 115.9 (aromatic C-H), 28.2 (CH_2); m/z (EI +ve) 191 (M^+, 100%), 163 (14), 135 (C_6H_4OHNCO, 39), 109 ($C_6H_4OHNH_2$, 59).

2,6-Di-*t*-butyl-4-methylphenol (*3*)

The method of Stillson *et al.* (1945) was used to prepare compound (*3*). Thus, 4-methylphenol (5.0g, 0.05mol) was dissolved in toluene (20 cm^3) and sulphuric acid

added (0.25g). The solution was placed in a boiling tube (2 cm^3 x 30cm^3) which was immersed in a water bath at 60°C. Isobutylene was bubbled through the solution for 6 h. The mixture was then washed with 0.1M sodium hydroxide (2 x 20 cm^3) and water (2 x 20 cm^3). The toluene solution was concentrated *in vacuo* to yield 2,6-di-*t*-butyl-4-methylphenol (3.1g, 28%), m.p. 70.5°C (lit. m.p. 71°C (CRC Handbook)); ν_{max} (KBr) 3627 (phenol OH), 2955, 2914, 2817 (C-H); δ_H (400mHz, $CDCl_3$) 6.98 (2H, s, aromatic CH), 5.12 (1H, s, phenolic OH), 2.27 (3H, s, CH_3), 1.43 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 151.5 (phenol C), 135.7 (aromatic CN), 128.2 (aromatic C-*t*-butyl), 125.5 (aromatic CH), 34.2 (C-CH_3), 30.3 (*t*-butyl CH_3), 21.2 (CH_3); m/z (EI +ve) 220 (M^+, 35%), 205 (M^+-CH_3, 100).

Alkylation of *N*-oxyphenylsuccinimide

N-Oxyphenylsuccinimide (0.5g, 0.003mol) was dissolved in *t*-butanol (20 cm^3) and sulphuric acid added (0.25g). The solution was placed in a boiling tube (2 cm^3 x 30cm^3) which was immersed in a water bath at 60°C. Isobutylene was bubbled through the solution for 7 h. The solution was then washed with 5% sodium hydrogen sulphate (2 x 20 cm^3) and water (2 x 20 cm^3). The *t*-butanol solution was concentrated in vacuo. ^{1}H NMR analysis showed the residue to be unreacted starting material.

2,6-Di-*t*-butyl-4-nitrophenol (*22*)

The method of Meek *et al.* (1968) was used to prepare compound (*22*). Thus, to a stirred solution of 2,6-di-*t*-butylphenol (5g, 0.024mol) in ice cold cyclohexane (30 cm^3), was added dropwise acetic acid/nitric acid (50% v/v, 3 cm^3). The solid 2,6-di-*t*-butyl-4-nitrophenol was filtered, washed with water and dried. Recrystallisation from hexane gave pale yellow needles of 2,6-di-*t*-butyl-4-nitrophenol (2.2g, 36%), m.p. 151.5-152.5°C (lit. 153-154°C); (Found: C, 67.04; H, 8.34; N, 5.55. $C_{14}H_{21}NO_3$ requires C, 66.91; H, 8.42; N, 5.57); ν_{max} (KBr) 3556 (phenol OII), 1513, 1581 (NO_2), 887cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 8.12 (2H, s, aromatic CH), 5.96 (1H, s, phenolic OH), 1.48 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 159.5 (phenol COH), 140.7 (aromatic CNO_2), 136.6 (aromatic C-*t*-butyl), 121.3 (aromatic CH), 34.5 (C-CH_3), 29.9 (CH_3); m/z (EI +ve) 251

(M^+, 20%), 236 (M^+-CH_3, 100), 220 (M^+-C_2H_7,10), 208 (M^+-C_3H_7, 46), 192 (M^+-C_4H_9, 14), 91 ($C_6H_5N^+$, 11), 57 ($C_4H_9^+$, 17).

4-Acetamido-2,6-di-*t*-butylphenol (*62*)

The method of Barnes and Hickinbottom (1961) was used to prepare compound (*62*). Thus, 2,6-di-*t*-butyl-4-nitrophenol (3g, 0.012mol) and palladium on activated charcoal (10%, 0.4g) were suspended in ethyl acetate (70 cm^3). The mixture was stirred under a positive pressure of hydrogen until a colourless solution remained. Acetic anhydride (1.5g, 0.015mol) was added and the solution stirred for an hour. The filtered solution was washed with 5% sodium hydrogen carbonate (3 x 50 cm^3), and after drying over magnesium sulphate, the solvent was removed on a rotary evaporator. Recrystallisation from hexane gave 4-acetamido-2,6-di-*t*-butylphenol (2.4g, 75%), m.p. 170.5-171.0°C (lit. 170-170.5°C); (Found for C, 73.20; H, 9.53; N, 5.24. $C_{16}H_{25}NO_2$ requires C, 72.97; H, 9.57; N, 5.32); ν_{max} (KBr) 3625 (phenol-OH), 3314 (NH), 3023 (aromatic CH), 1667 (C=O), 1606 (C=C), 1435cm^{-1}(CN); δ_H (400mHz, $CDCl_3$) 7.57 (1H, s, NH), 7.29 (2H, s, aromatic CH), 5.07 (1H, s, phenolic OH), 2.10 (3H, s, CH_3), 1.36 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 168.5 (C=O), 150.7 (phenol COH), 136.4 (aromatic CN), 129.9 (aromatic C-*t*-butyl), 118.2 (aromatic CH), 30.1 (C-CH_3), 26.9 (*t*-butyl CH_3); m/z (EI +ve) 263 (M^+, 85%), 248 (M^+-CH_3, 100), 221 (M^+-CH_2C=O, 22), 206 (M^+-C_4H_9, 30), 192 (M^+-C_5H_{11}, 14), 164 (M^+-C_7H_{15}, 10), 91 ($C_6H_5N^+$, 10), 57 ($C_4H_9^+$, 25).

Thiodiacetic anhydride

Thiodiacetic anhydride was prepared using the method of Morrill *et al.* (1961). A mixture of thiodiacetic acid (21.6g, 0.13 mol), phosphorus trichloride (6.1g, 0.04 mol) and chloroform (50 cm^3) was warmed at 60°C with stirring for one hour. The mixture was heated under reflux for one hour, after which a further portion of phosphorus trichloride (3g, 0.02 mol) was added. The mixture was heated under reflux for another hour. The hot solution was decanted through a filter, the residue on the filter washed with a small amount of chloroform and the filtrate cooled on ice. Thiodiacetic anhydride was obtained as colourless needles (12g, 63%) m.p. 88-90°C (lit. 89-90°C); ν_{max} (KBr) 1752 (C=O),

1173cm^{-1} (C-O-C); δ_H (400mHz, CD_3COCD_3) 3.88 (4H, s, CH_2); δ_C (100mHz, CD_3COCD_3) 163.8 (C=O), 33.8 (CH_2); m/z (EI +ve) 132 (M^+, 60%), 60 (100).

2,3-Diethyl-2,3-dimethylbisazonitrile

2,3-Diethyl-2,3-dimethylbisazonitrile was prepared using the method of Eberson (1960). Hydrazine sulphate (48g) and sodium cyanide (38g) were dissolved in distilled water (750 cm^3). 2-Butanone (48g) was added and the mixture stirred vigoursly for 48 hours. The mixture was washed with diethyl ether (2 x 200cm^3). The combined organic extracts were dried over anhydrous magnessium sulphate and concentrated *in vacuo*. The remaining oil was dissolved in acetic acid/hydrochloric acid (150 cm^3, 2:1 v/v), and cooled to 5°C. Bromine (20cm^3) dissolved in 70% aqueous acetic acid (200 cm^3) was added over two minutes with vigorous stirring. The mixture was stirred for a further 30 minutes and then poured onto ice water (1500 cm^3). The crude bisazonitrile was collected, dried and recrystallised from petroleum ether (30-60°C) to yield white needles (26.6g, 20.1%), m.p. 50-51°C (lit. 50-51°C); δ_H (400mHz, $CDCl_3$) 2.18 (2H, m, ethyl-CH_2), 2.08 (2H, m, ethyl-CH_2), 1.70 (3H, s, methyl-CH_3), 1.67 (3H, s, methyl-CH_3), 1.04 (2H, t, J 7.3Hz, ethyl-CH_3), 1.02 (3H, t, ethyl-CH_3); δ_C (100mHz, $CDCl_3$) 118.2 (CN), 73.3 (C-CN), 31.6 (ethyl-CH_2), 31.5 (ethyl-CH_2), 23.8 (methyl-CH_3), 23.5 (methyl-CH_3), 8.7 (ethyl-CH_3), 8.6 (ethyl-CH_3); m/z (FAB +ve glycerol) 193 (MH^+, 45%), 83 (MH^+-$C_2H_5CCH_3CN$, 100).

2,3-Diethyl-2,3-dimethylsuccinonitrile

2,3-Diethyl-2,3-dimethylbisazonitrile (20g, 0.1mol) was dissolved in carbon tetrachloride (125 cm^3) and heated under reflux for 20 hours. After cooling the carbon tetrachloride was evaporated off and the residue recrystallised from toluene to yield *meso*-2,3-diethyl-2,3-dimethylsuccinonitrile (4.2g, 26%), m.p. 98-99°C (lit. 99-100°C); ν_{max} (KBr) 2236 (CN); δ_H (400mHz, $CDCl_3$) 2.01 (2H, m, J=7.32Hz, ethyl-CH_2), 1.64 (2H, m, ethyl-CH_2), 1.47 (6H, s, methyl-CH_3), 1.18 (6H, t, ethyl-CH_3); δ_C (100mHz, $CDCl_3$) 120.7 (CN), 44.9 (C-CN), 28.2 (ethyl-CH_2), 19.1 (methyl-CH_3), 9.8 (ethyl-CH_3); m/z (FAB+ve glycerol) 165 (MH^+, 100%).

dl-2,3-Diethyl-2,3-dimethylsuccinonitrile was recrstallised from the mother liquor (3.1 , 19%), m.p. 43-44.5°C; ν_{max} (KBr) 2236 (CN); δ_H (400mHz, $CDCl_3$) 2.01 (2H, m, J=7.32Hz, ethyl-CH_2), 1.64 (2H, m, ethyl-CH_2), 1.47 (6H, s, methyl-CH_3), 1.18 (6H, t, ethyl-CH_3); δ_C (100mHz, $CDCl_3$) 120.7 (CN), 44.9 (C-CN), 28.2 (ethyl-CH_2), 19.1 (methyl-CH_3), 9.8 (ethyl-CH_3); m/z (FAB+ve glycerol) 165 (MH^+, 100%)

meso-2,3-Diethyl-2,3-dimethylsuccinic anhydride

meso-2,3-Diethyl-2,3-dimethylsuccinonitrile (5g, 0.03mol) was dissolved in sulphuric acid (25 cm^3), water (10 cm^3) and glacial acetic acid (30 cm^3), and heated under reflux for 20 hours. The reaction mixture was poured onto water (500 cm^3) and the organic layer taken up in ether (200 cm^3). The ether solution was washed with 5% sodium hydrogen carbonate (200 cm^3) and water (200 cm^3). The ether solution was concentrated in vacuo to yield crude *meso*-2,3-diethyl-2,3-dimethylsuccinic anhydride (3.5, 64%), m.p. 31-33°C; ν_{max} (KBr) 1849, 1785 (C=O); δ_H (400mHz, $CDCl_3$) 1.74 (4H, m, J=7.32Hz, ethyl-CH_2), 1.27 (6H, s, methyl-CH_3), 1.01 (6H, t, ethyl-CH_3); δ_C (100mHz, $CDCl_3$) 175.5 (C=O), 52.1 (C-C=O), 26.6 (ethyl-CH_2), 17.9 (methyl-CH_3), 8.8 (ethyl-CH_3); m/z (FAB+ve, glycerol) 184 (MH^+, 16%), 112 ($C_2H_5CH_3C{=}CCH_3C_2H_5$, 70), 85 ($MH^+$-$C_2H_5CCH_3CN$, 100).

_N_-(3,5-Di-_t_-butyl-4-hydroxyphenyl) acid amides

The following general procedure was used for the preparation of acid amides.

2,6-Di-*t*-butyl-4-nitrophenol (3g, 0.012mol) and palladium on charcoal (10%, 0.4g) were suspended in ethyl acetate (70 cm^3). The mixture was stirred under a positive pressure of hydrogen until a colourless solution remained. The appropriate anhydride (0.015 mol) dissolved in ethyl acetate (30 cm^3) was added and the solution stirred for an hour. The filtered solution was washed with 0.1M sodium hydroxide (3 x 50 cm^3). The combined aqueous extracts were acidified with 50% hydrochloric acid and extracted with ethyl acetate (3 x 50 cm^3). After drying over magnesium sulphate, the solvent was removed on a rotary evaporator.

After brief storage the compounds showed discolouration which prevented reliable CHN analysis despite several attempts.

_N_-(3,5-Di-_t_-butyl-4-hydroxyphenyl)-succinamic acid (_62_)

Recrystallisation from chloroform yielded a white powder (1.96, 51%); m.p. 164.5-165.0 °C (dec.); ν_{max} (KBr) 3632 (phenol OH), 3300-2300 (COOH), 3332 (NH), 1716 (acid C=O), 1655cm^{-1} (amide C=O), 1437 (C-N), 1260 (C-O); δ_H (400mHz, CD_3COCD_3) 8.96 (1H, s, NH), 7.50 (2H, s, aromatic CH), 5.83 (1H, s, phenolic OH), 2.64 (2H, d, J 6.24, CH_2-COOH), 2.60 (2H, d, CH_2-NH), 1.40 (18H, s, _t_-butyl CH_3); δ_C (100mHz, CD_3COCD_3) 174.1 (acidic C=O), 170.2 (amide C=O), 150.5 (phenol C-OH), 138.8 (aromatic CN), 132.9 (aromatic C-_t_-butyl), 117.2 (aromatic CH), 35.3 (C-CH_3), 32.1 (CH_2-COOH), 30.3 (_t_-butyl CH_3), 29.5 (CH_2-NH); m/z (EI +ve) 321 (M^+, 100%), 306 (M^+-CH_3, 25), 288 (M^+-(CH_3+$H_2$0), 39), 221 ($C_{14}H_{21}NO^+$, 74), 57 ($C_4H_9^+$, 35), 55 ($CH_2CHC{=}O^+$, 28).

_N_-(3,5-Di-_t_-butyl-4-hydroxyphenyl)-maleamic acid

Recrystallisation from chloroform yielded a yellow powder (2.63g, 68.9%); m.p. 201.2-202.0 °C (dec.); ν_{max} (KBr) 3632 (phenol OH), 3300-2300 (COOH), 3295 (NH), 1700 (acid C=O), 1626 (amide C=O), 1438 (C-N), 1260cm^{-1} (C-O); δ_H (400mHz, CD_3COCD_3) 10.2 (1H, s, COOH), 7.60 (2H, s, aromatic CH), 6.70 (1H, d, J 12.7, CH-COOH), 6.42 (1H, s, phenolic OH), 6.34 (1H, d, CH-NH), 1.43 (18H, s, _t_-butyl CH_3); δ_C (100mHz, CD_3COCD_3) 164.9 (acidic C=O), 164.8 (amide C=O), 152.6 (phenol C-OH), 139.0 (aromatic CN), 135.9 (CH-COOH), 133.3 (CH-NH), 130.3 (aromatic C-_t_-butyl), 118.6 (aromatic CH), 35.3 (C-CH_3), 30.4 (_t_-butyl CH_3); m/z (EI +ve) 319 (M^+, 44%), 304 (M^+-CH_3, 16), 286 (M^+-(CH_3+$H_2$0), 22), 221 ($C_{14}H_{21}NO^+$, 100), 57 ($C_4H_9^+$, 39), 55 ($CH_2CHC{=}O^+$,19).

_N_-(3,5-Di-_t_-butyl-4-hydroxyphenyl)-glutaramic acid

Recrystallisation from chloroform yielded a white powder (3.20g, 80.1%); m.p. 176.5-177.2°C (dec.); ν_{max} (KBr) 3627 (phenol OH), 3300-2300 (COOH), 3319

(NH), 1698 (acid C=O), 1628cm^{-1} (amide C=O); δ_H (400mHz, CD_3COCD_3) 10.61 (1H, s, COOH), 8.93 (1H, s, NH), 7.53 (2H, s, aromatic CH), 5,83 (1H, s, phenolic OH), 2.42 (2H, t, CH_2-COOH), 2.40 (2H, t, CH_2-NH), 1.96 (2H, q, J 7.32, CH_2), 1.40 (18H, s, *t*-butyl CH_3); δ_C (100mHz, CD_3COCD_3) 174.5 (acidic C=O), 170.9 (amide C=O), 150.6 (phenol C-OH), 138.8 (aromatic CN), 132.9 (aromatic C-*t*-butyl), 117.3 (aromatic CH), 36.5 (CH_2-acid), 35.3 (C-CH_3), 33.5 (CH_2-NH), 30.6 (*t*-butyl CH_3), 21.6 (CH_2); m/z (EI +ve) 335 (M^+, 72%), 320 (M^+-CH_3, 14), 221 ($C_{14}H_{21}NO^+$, 100).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-3,3-dimethylglutaramic acid

Recrystallisation from chloroform yielded a white powder (1.71g, 57%); m.p. 214-215°C (dec.); ν_{max} (KBr) 3639 (phenol OH), 3300-2300 (COOH), 3345 (NH), 1709 (acid C=O), 1636 cm^{-1} (amide C=O); δ_H (400mHz, CD_3COCD_3) 11.58 (1H, s, COOH), 9.23 (1H, s, NH), 7.52 (2H, s, aromatic CH), 5,93 (1H, s, phenolic OH), 2.46 (2H, s, CH_2-COOH), 2.45 (2H, s, CH_2-NH), 1.42 (18H, s, *t*-butyl CH_3), 1.15 (6H, s, CH_3); δ_C (100mHz, CD_3COCD_3) 175.9 (acidic C=O), 172.4 (amide C=O), 152.1 (phenol C-OH), 139.8 (aromatic CN), 131.5 (aromatic C-*t*-butyl), 118.8 (aromatic CH), 48.9 (CH_2-acid), 46.7 (CH_2-NH), 35.6 (*t*-butyl C-CH_3), 34.2 (glutaramic C-CH_3), 30.7 (t-butyl CH_3), 28.4 (glutaramic CH_3); m/z (EI +ve) 363 (M^+, 25%), 330 (M^+-(CH_3+H_2O), 5), 221 ($C_{14}H_{21}NO^+$, 100).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-diglycolamic acid

Recrystallisation from chloroform yielded a white powder (3.95g, 98.3%); m.p. 169.9-170.5°C (dec.); ν_{max} (KBr) 3630 (phenol OH), 3300-2200 (COOH), 3292 (NH), 1730 (acid C=O), 1629 (amide C=O), 1438 (C-N), 1270 (C-O), 1143cm^{-1} (C-O-C); δ_H (400mHz, CD_3COCD_3) 9.25 (1H, s, NH), 7.57 (2H, s, aromatic CH), 5.93 (1H, s, phenolic OH), 4.33 (2H, s, CH_2-COOH), 4.22 (2h, s, CH_2-NH), 1.44 (18H, s, *t*-butyl CH_3); δ_C (100mHz, CD_3COCD_3) 172.2 (acidic C=O), 168.2 (amide C=O), 151.1 (phenol C-OH), 138.9 (aromatic CN), 131.8 (aromatic C-*t*-butyl), 117.6 (aromatic CH), 72.4 (CH_2-COOH), 69.7 (CH_2-NH), 35.3 (C-CH_3), 30.6 (*t*-butyl CH_3); m/z (EI +ve) 337 (M^+, 100%), 322 (M^+-CH_3, 43), 91 ($C_6H_5N^+$, 12), 57 ($C_4H_9^+$, 60).

N-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-thiodiglycolamic acid

Recrystallisation from chloroform yielded a white powder (2.62g, 62.2%); m.p. 167.5-168.0°C (dec.); ν_{max} (KBr) 3628 (phenol OH), 3300-2500 (COOH), 3307 (NH), 1715 (acid C=O), 1628cm^{-1} (amide C=O); δ_H (400mHz, CD_3COCD_3) 11.40 (IH, s, COH), 9.22 (1H, s, NH), 7.52 (2H, s, aromatic CH), 5.89 (1H, s, phenolic OH), 3.51 (2H, s, CH_2-COOH), 3.50 (2H, s, CH_2-NH), 1.41 (18H, s, *t*-butyl CH_3); δ_C (100mHz, CD_3COCD_3) 171.4 (acidic C=O), 167.8 (amide C=O), 151.0 (phenol C-OH), 138.9 (aromatic CN), 132.3 (aromatic C-*t*-butyl), 117.6 (aromatic CH), 37.2 (CH_2-COOH), 35.2 (C-CH_3), 34.7 (CH_2-NH), 30.4 (*t*-butyl CH_3); m/z (EI +ve) 353 (M^+, 100%), 338 (M^+-CH_3, 9),57 ($C_4H_9^+$, 57).

N-(3,5-Di-*t*-butyl-4-hydroxyphenyl) acid methyl esters

N-(3,5-di-*t*-butyl-4-hydroxyphenyl) acid amide (2.0g) was dissolved in methanol (50 cm^3). Sulphuric acid (0.5 cm^3) was added and the mixture stirred for up to 48 hours. The mixture was evaporated to dryness. The residue was reconstituted in ethyl acetate (50 cm^3), washed with 5% sodium hydrogen carbonate (2 x 50 cm^3) and water (50 cm^3). After drying over magnesium sulphate, the solvent was removed on a rotary evaporator. Recrystallisation from chloroform/hexane gave the corresponding acid methyl ester.

After brief storage the compounds showed discolouration which prevented reliable CHN analysis despite several attempts.

N-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-succinamic acid methyl ester (*73*)

Recrystallisation from chloroform yielded a white powder (1.96, 51%); m.p. 164.5-165.0 °C (dec.); ν_{max} (KBr) 3632 (phenol OH), 3332 (NH), 1716 (ester C=O), 1655 (amide C=O), 1436 cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 7.93 (1H, s, NH), 7.31 (2H, s, aromatic CH), 5.06 (1H, s, phenolic OH), 3.67 (3H, s, OCH_3), 2.73 (2H, t, J 6.34, CH_2-COOMe), 2.63 (2H, t, CH_2-NH), 1.38 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 173.7 (ester C=O), 169.6 (amide C=O), 150.5 (phenol C-OH), 136.4 (aromatic CN), 130.0 (aromatic C-*t*-butyl), 117.6 (aromatic CH), 51.8 (OCH_3), 34.4 (C-CH_3), 31.7

(CH_2-COOMe), 30.1 (*t*-butyl CH_3), 29.3 (CH_2-NH); m/z (EI +ve) 335 (M^+, 60%), 320 (M^+-CH_3, 6), 304 (M^+-OCH_3, 12), 221 ($C_{14}H_{21}NO^+$, 100).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-maleamic acid methyl ester

Recrystallisation from chloroform yielded a white powder (1.4g, 67%); m.p. 145-146°C (dec.); ν_{max} (KBr) 3567 (phenol OH), 3283 (NH), 1731 (ester C=O), 1661 (amide C=O), 1436 cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 10.44 (1H, s, NH), 7.45 (2H, s, aromatic CH), 6.42 (1H, d, J 13.19, CH_2-COOMe), 6.18 (1H, s, CH_2-NH), 5.12 (1H, s, phenolic OH), 3.84 (3H, s, OCH_3), 1.44 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 167.2 (ester C=O), 161.4 (amide C=O), 151.0 (phenol C-OH), 140.4 (CH-COOMe), 136.5 (aromatic CN), 129.7 (aromatic C-*t*-butyl), 124.5 (CH-NH), 117.9 (aromatic CH), 52.7 (OCH_3), 34.4 (C-CH_3), 30.2 (*t*-butyl CH_3); m/z (EI +ve) 333 (M^+, 27%), 318 (M^+-CH_3, 8), 301 (M^+-OCH_3, 10), 69 (100).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-glutaramic acid methyl ester

Recrystallisation from chloroform yielded a white powder (1.40g, 67%); m.p. 130.5-132.0 °C (dec.); ν_{max} (KBr) 3542 (phenol OH), 3366 (NH), 1720 (ester C=O), 1675 (amide C=O), 1436 cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 7.57 (1H, s, NH), 7.32 (2H, s, aromatic CH), 5.07 (1H, s, phenolic OH), 3.67 (3H, s, OCH_3), 2.43 (2H, t, J 7.33, CH_2-COOMe), 2.40 (2H, t, CH_2-NH), 2.03 (2H, quin., CH_2), 1.40 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 173.7 (ester C=O), 170.3 (amide C=O), 150.5 (phenol C-OH), 136.3 (aromatic CN), 129.8 (aromatic C-*t*-butyl), 117.6 (aromatic CH), 51.5 (OCH_3), 36.1 (CH_2-COOMe), 34.3 (C-CH_3), 32.9 (CH_2-NH), 30.1 (*t*-butyl CH_3), 20.8 (CH_2); m/z (EI +ve) 349 (M^+, 56%), 318 (M^+-OCH_3, 10), 221 ($C_{14}H_{21}NO^+$, 100).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-3,3-dimethylglutaramic acid methyl ester

Recrystallisation from chloroform yielded a white powder (0.51g, 25%); m.p. 151.5-152.0 °C (dec.); ν_{max} (KBr) 3520 (phenol OH), 3301 (NH), 1718 (ester C=O), 1646 (amide C=O), 1436 cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 8.44 (1H, s, NH), 7.35 (2H,

s, aromatic CH), 5.05 (1H, s, phenolic OH), 3.74 (3H, s, OCH_3), 2.43 (2H, t, J 7.33, CH_2-COOMe), 2.40 (2H, t, CH_2-NH), 2.03 (2H, quin., CH_2), 1.40 (18H, s, *t*-butyl CH_3), 1.14 (6H, s, CH_3); δ_C (100mHz, $CDCl_3$) 174.7 (ester C=O), 169.7 (amide C=O), 150.9 (phenol C-OH), 136.9 (aromatic CN), 130.9 (aromatic C-*t*-butyl), 117.8 (aromatic CH), 52.3 (OCH_3), 48.8 (CH_2-COOMe), 45.2 (CH_2-NH), 34.4 (*t*-butyl-C-CH_3), 34.0 (C-CH_3), 30.1 (t-butyl CH_3), 28.9 (CH_3); m/z (EI +ve) 377 (M^+, 18%), 346 (M^+-OCH_3, 10), 314 (M^+-OCH_3), 221 ($C_{14}H_{21}NO^+$, 100).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-diglycolamic acid methyl ester

Recrystallisation from chloroform yielded a white powder (0.8g, 38%); m.p. 110-112ºC (dec.); ν_{max} (KBr) 3638 (phenol OH), 3354 (NH), 1748 (ester C=O), 1661 (amide C=O); δ_H (400mHz, $CDCl_3$) 8.59 (1H, s, NH), 7.40 (2H, s, aromatic CH), 5.12 (1H, s, phenolic OH), 4.25 (2H, s, CH_2-COOMe), 4.19 (2H, s, CH_2-NH), 3.80 (3H, s, OCH_3), 1.44 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 170.4 (ester C=O), 166.8 (amide C=O), 150.9 (phenol C-OH), 136.5 (aromatic CN), 129.1 (aromatic C-*t*-butyl), 117.8 (aromatic CH), 71.7 (CH_2-COOMe, 68.9 (CH_2-NH), 52.2 (OCH_3), 34.4 (C-CH_3), 30.1 (*t*-butyl CH_3); m/z (EI +ve) 351 (M^+, 100%), 336 (M^+-CH_3, 16), 320 (M^+-OCH_3, 2).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-thiodiglycolamic acid methyl ester

Recrystallisation from chloroform yielded a white powder (0.76g, 37%); m.p. 103-104 ºC (dec.); ν_{max} (KBr) 3577 (phenol OH), 3330 (NH), 1735 (ester C=O), 1639 (amide C=O); δ_H (400mHz, $CDCl_3$) 8.50 (1H, s, NH), 7.34 (2H, s, aromatic CH), 5.07 (1H, s, phenolic OH), 3.71 (3H, s, OCH_3), 3.42 (2H, s, CH_2-COOMe), 3.35 (2H, s, CH_2-NH), 1.44 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 170.5 (ester C=O), 165.8 (amide C=O), 150.9 (phenol C-OH), 136.4 (aromatic CN), 129.4 (aromatic C-*t*-butyl), 117.6 (aromatic CH), 52.7 (OCH_3), 37.4 (CH_2-COOMe, 34.8 (CH_2-NH), 34.4 (C-CH_3), 30.1 (*t*-butyl CH_3); m/z (EI +ve) 367 (M^+, 30%), 352 (M^+-CH_3, 3), 43 (100).

N-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-succinamic acid ethyl ester

N-(3,5-di-*t*-butyl-4-hydroxyphenyl) acid amide (2.0g) was dissolved in ethanol (50 cm^3). Sulphuric acid (0.5 cm^3) was added and the mixture stirred for 48 hr. The mixture was evaporated to dryness. The residue was reconstituted in ethyl acetate (50 cm^3), washed with 5% sodium hydrogen carbonate (2 x 50 cm^3) and water (50 cm^3). After drying over magnesium sulphate, the solvent was removed on a rotary evaporator. Recrystallisation from chloroform yielded a white powder (1.2g, 57%); m.p. 144.3-145 °C (dec.); ν_{max} (KBr) 3583 (phenol OH), 3290 (NH), 1729 (ester C=O), 1652 (amide C=O), 1436 cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 7.47 (1H, s, NH), 7.30 (2H, s, aromatic CH), 5.05 (1H, s, phenolic OH), 4.14 (2H, q, J 7.33, OCH_2), 2.73 (2H, t, J 6.83, CH_2-ester), 2.63 (2H, t, CH_2-amide), 1.41 (18H, s, *t*-butyl CH_3), 1.26 (3H,t, ethyl-CH_3); δ_C (100mHz, $CDCl_3$) 173.2 (ester C=O), 169.6 (amide C=O), 150.6 (phenol C-OH), 136.4 (aromatic CN), 129.8 (aromatic C-*t*-butyl), 117.7 (aromatic CH), 60.8 (OCH_2), 34.4 (C-CH_3), 32.0 (CH_2-acid), 30.1 (*t*-butyl CH_3), 29.6 (CH_2-amide), 14.1 (ethyl-CH_3); m/z (EI +ve) 349 (M^+, 65%), 304 (M^+-OC_2H_5, 18), 221 ($C_{14}H_{21}NO^+$, 100).

N-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-imides

The following general procedure was used for the preparation of imides, except *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-2,3-diethyl-2,3-dimethylsuccinimide (*72*).

The appropriate acid amide (2g) was added to a stirred solution of acetic anhydride (20 cm^3) and sodium acetate (0.8g). The mixture was heated under reflux for two hours with stirring, the cooled solution poured onto 5% sodium hydrogen carbonate (100 cm^3) and extracted with ethyl acetate (2 x 50 cm^3). The combined organic extracts were washed with 5% sodium hydrogen carbonate (3 x 100 cm^3) and water (50 cm^3). After drying over magnesium sulphate, the solvent was removed on a rotary evaporator.

N-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-succinimide (*54*)

Recrystallisation from toluene gave white needles (1.09, 58%); m.p. 187.9-188.2°C; (Found: C, 71.21; H, 8.30; N, 4.61. $C_{18}H_{25}NO_3$ requires C, 71.26; H, 8.31; N,

4.62%); ν_{max} (KBr) 3552 (phenol OH), 1721 (C=O), 1439cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 6.97 (2H, s, aromatic CH), 5.38 (1H, s, phenolic OH), 2.81 (4H, s, CH_2), 1.42 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 176.9.(C=O), 154.0 (phenol C-OH), 136.6 (aromatic CN), 123.6 (aromatic C-*t*-butyl), 123.5 (aromatic CH), 34.9 (C-CH_3), 30.0 (*t*-butyl CH_3), 28.3 (CH_2-CH_2); m/z (EI +ve) 303 (M^+, 37%), 288 (M^+-CH_3, 100), 232 (M^+-C_5H_{11}, 10), 57 ($C_4H_9^+$, 25), 55 ($CH_2CHC{=}O^+$, 33).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-maleimide (*64*)

Recrystallisation from toluene gave yellow crystals (0.75, 40%); m.p. 206.8-207.3°C; (Found: C, 72.21; H, 7.75; N, 4.50. $C_{18}H_{23}NO_3$ requires C, 71.73; H, 7.69; N, 4.65%); ν_{max} (KBr) 3555 (phenol OH), 1769 (C=O), 1434cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 7.02 (2H, s, aromatic CH), 6.81 (2H, s, CH), 5.34 (1H, s, phenolic OH), 1.44 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 170.3.(C=O), 153.8 (phenol C-OH), 136.6 (aromatic CN), 134.4 (CH=CH), 123.7 (aromatic C-*t*-butyl), 122.5 (aromatic CH), 34.4 (C-CH_3), 30.8 (*t*-butyl CH_3); m/z (EI +ve) 301 (M^+, 40%), 286 (M^+-CH_3, 100), 258 (M^+-C_3H_7, 10), 202 (M^+-C_7H_{15}, 7), 121 (17), 91 ($C_6H_5N^+$, 23), 57 ($C_4H_9^+$, 24), 55 ($CH_2CHC{=}O^+$, 11).

_N_-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-glutarimide (*65*)

Recrystallisation from toluene gave white needles (1.04g, 54.4%); m.p. 245.0-246.2°C; (Found: C, 71.96; H, 8.62; N, 4.32. $C_{19}H_{27}NO_3$ requires C, 71.89; H, 8.57; N, 4.47%); ν_{max} (KBr) 3565 (phenol OH), 1762 (C=O), 1435cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 6.81 (2H, s, aromatic CH), 5.29 (1H, s, phenolic OH), 2.79 (4H, t, J 6.35Hz, CH_2), 2.07 (2H, q, CH_2) 1.42 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 172.9.(C=O), 153.7 (phenol C-OH), 136.4 (aromatic CN), 126.5 (aromatic C-*t*-butyl), 124.7 (aromatic CH), 34.3 (C-CH_3), 33.2 (C=O-CH_2), 30.1 (*t*-butyl CH_3), 17.3 (CH_2); m/z (EI +ve) 317 (M^+, 37%), 302 (M^+-CH_3, 100).

***N*-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-3,3-dimethylglutarimide (*66*)**

Recrystallisation from toluene gave white needles (1.2g, 63%); m.p. 150.5-151°C; (Found: C, 73.01; H, 9.03; N, 4.03. $C_{21}H_{31}NO_3$ requires C, 73.01; H, 9.04; N, 4..05%); ν_{max} (KBr) 3370 (phenol OH), 1729, 1685 (C=O), 1435cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 6.79 (2H, s, aromatic CH), 5.30 (1H, s, phenolic OH), 2.66 (4H, s, CH_2), 1.42 (18H, s, *t*-butyl CH_3), 1.20 (6H, s, glutarimide CH_3); δ_C (100mHz, $CDCl_3$) 172.3.(C=O), 153.7 (phenol C-OH), 136.4 (aromatic CN), 126.3 (aromatic C-*t*-butyl), 124.7 (aromatic CH), 46.7 (CH_2), 34.3 (C-CH_3), 30.1 (*t*-butyl CH_3), 29.2 (glutarimide C-CH_3), 27.8 (glutarimide CH_2); m/z (EI +ve) 345 (M^+, 30%), 330 (M^+-CH_3, 100).

***N*-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-diglycolarimide (*67*)**

Recrystallisation from toluene gave white needles (1.24g, 65.3%); m.p. 219.2-220.0°C; (Found: C, 67.91; H, 7.92; N, 4.35. $C_{18}H_{25}NO_4$ requires C, 67.69; H, 7.89; N, 4.39%); ν_{max} (KBr) 3588 (phenol OH), 1749 (C=O), 1433 (C-N), 1143cm^{-1} (C-O-C); δ_H (400mHz, $CDCl_3$) 6.90 (2H, s, aromatic CH), 5.37 (1H, s, phenolic OH), 4.45 (4H, s, CH_2), 1.42 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 169.9.(C=O), 154.2 (phenol C-OH), 136.7 (aromatic CN), 124.7 (aromatic C-*t*-butyl), 123.7 (aromatic CH), 68.1 (CH_2), 34.8 (C-CH_3), 30.1 (*t*-butyl CH_3); m/z (EI +ve) 319 (M^+, 33%), 304 (M^+-CH_3, 100), 276 (M^+-C_3H_7, 8), 87 (15), 57 ($C_4H_9^+$, 23).

***N*-(3,5-Di-*t*-butyl-4-hydroxyphenyl)-thiodiglycolarimide (*68*)**

Recrystallisation from toluene gave cream needles (1.00g, 52.7%); m.p. 251.0-252.5°C; (Found: C, 64.59; H, 7.56; N, 4.13. $C_{18}H_{25}NSO_3$ requires C, 64.45; H, 7.51; N, 4.18%); ν_{max} (KBr) 3581 (phenol OH), 1725 (C=O), 1431cm^{-1} (C-N); δ_H (400mHz, $CDCl_3$) 6.83 (2H, s, aromatic CH), 5.31 (1H, s, phenolic OH), 3.68 (4H, s, CH_2), 1.42 (18H, s, *t*-butyl CH_3); δ_C (100mHz, $CDCl_3$) 169.6.(C=O), 153.4 (phenol C-OH), 139.8 (aromatic CN), 127.8 (aromatic C-*t*-butyl), 124.2 (aromatic CH), 34.4 (C-CH_3), 31.5 (CH_2), 30.4 (*t*-butyl CH_3); m/z (EI +ve) 335 (M^+, 43%), 320 (M^+-CH_3,100), 162 (9), 91 (15), 57 ($C_4H_9^+$, 30).

_N_-(3,5-Di-_t_-butyl-4-hydroxyphenyl)-2,3-diethyl-2,3-dimethylsuccinimide (_72_)

2,6-Di-_t_-butyl-4-nitrophenol (1g, 0.004mol) and palladium on charcoal (10%, 0.4g) were suspended in ethyl acetate (50 cm^3). The mixture was stirred under a positive pressure of hydrogen until a colourless solution remained. The solution was filtered under nitrogen and the ethyl acetate removed in vacuo. The residue remaining was resuspended in toluene (30 cm^3) and placed in a Carius tube (50 cm^3) with *meso*-2,3-diethyl-2,3-dimethylsuccinic anhydride (2g, 0.01mol). The tube was sealed and placed in an muffle furnace at 170°C for 48 h. The solvent was removed on a rotary evaporator to yield a red oil. A portion of the oil (0.5g) was dissolved in aqueous acetonitrile (4 cm^3, 50% v/v) and placed into a Jones C_{18} EC cartridge column (20 cm^3, 5g) which had been preconditioned with acetonitrile (50 cm^3). The column was washed with aqueous acetonitrile (50 cm^3, 60%v/v), and the fractions containing the product eluted with aqueous acetonitrile (4x50 cm^3, 40%v/v). The acetonitrile was removed on a rotary evaporator and the water removed by freeze-drying to yield *N*-(3,5-di-*t*-butyl-4-hydroxyphenyl)-2,3-diethyl-2,3-dimethylsuccinimide (40mg, 2.5%); m.p. 125-124°C; (M^+ = 387.2773, $C_{24}H_{37}NO_3$ ± < 0.01 mmu); ν_{max} (KBr) 3584 (phenol-OH), 1705 (C=O); δ_H (400mHz, $CDCl_3$) 6.97 (2H, s, aromatic CH), 5.30 (1H, s, phenolic OH), 1.80 (4H, m, J 7.32Hz, ethyl-CH_2), 1.42 (18H, s, *t*-butyl CH_3), 1.29 (6H, s, methyl-CH_3), 1.03 (6H, t, ethyl-CH_3); δ_C (100mHz, $CDCl_3$) 181.9 (C=O), 153.6 (phenol C-OH), 136.5 (aromatic CN), 123.7 (aromatic C-*t*-butyl), 123.0 (aromatic CH), 50.3 (C-C=O), 34.4 (C-CH_3), 30.6 (*t*-butyl CH_3), 27.2 (ethyl-CH_2), 19.0 (methyl-CH_3), 9.3 (ethyl-CH_3); m/z (EI+ve) 387 (M^+, 53%), 372 (M^+-CH_3, 100), 344 (M^+-C_3H_7, 2), 330 (M^+-C_4H_9, 3).

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