THE SYNTHESIS OF DEUTERIUM LABELED BUTYLATED HYDROXYTOLUENE (BHT)

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SUMMARY

Attempts were made to synthesize 3,5-di-t-butyl-4-hydroxytoluene containing a deuterium label. 1. p-cresol was alkylated with (D_8)-isobutylene. 2. 2,6-di-t-butyl-4-hydroxytoluene was reacted with deuterated trifluoroacetic acid giving direct exchange. 3. 2,6-dideutero-4-hydroxytoluene was synthesized and alkylated with isobutylene and with 2-methyl-2-chloropropane and $AlCl_3$. 4. BHT was treated with D_2O and Pt_2O in a catalytic exchange process. All methods gave labeled BHT but in all cases a mixture of isomers was found.

Key Words: Butylated hydroxytoluene, BHT, deuterium exchange, alkylation.

INTRODUCTION

The widespread use of 3,5-di-t-butyl-4-hydroxytoluene (butylated hydroxytoluene; BHT) as an antioxidant in the food, cosmetic and petroleum industries has prompted many investigations of the toxicology, metabolism and excretion of this substance. The biotransformation studies have been undertaken using ^{3}H, ^{13}C and ^{14}C labelled BHT as well as non-labeled material, and the metabolic fate of BHT in laboratory animals has now been largely resolved. Knowledge of the metabolism and excretion of BHT and its metabolites in humans, however, remains inadequate (1,2).

In studies of biotransformation and excretion of xenobiotics, the non-labeled substrate may give rise to qualitative and quantitative uncertainties; radio-labeled materials on the other hand may not be desirable from ethical aspects, particularly when long lived β-emitters are used to label compounds with slow or unknown elimination kinetics. The use of stable nuclides for such investigations has therefore become of interest in all laboratories with

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access to a mass spectrometer. BHT-^{13}C for example has been synthesized and its metabolism in man has been studied (2). The cost of ^{13}C labeling remains a significant disadvantage to the use of this nuclide, and this has been the motivating force in this study of the synthesis of deuterium labeled BHT.

EXPERIMENTAL

General Methods: All solvents and chemicals were reagent grade, used without further purification except for p-hydroxytoluene which was freshly distilled before use. Samples of t-butyl (D_9) alcohol (99 atom % D), D_2O (99.7 atom % D) and D_2SO_4 (99 atom % D) were purchased from Merck, Sharpe and Dohme Co. Ltd., Montreal. NMR spectra were obtained using a Varian Model A60-D spectrometer. GC analyses were performed with a Hewlett-Packard Model 5700 or 5710 gas chromatograph, and GCMS measurements were made with a Hewlett-Packard Model 5980A GCMS.

Reaction products were identified by their NMR and mass spectra when isolated, or by retention times observed upon GC analysis of reaction mixtures. Melting points are reported uncorrected.

3,5-di-(D_8)-t-butyl-4-hydroxytoluene ((D_{16})-BHT): The treatment of (D_9)-t-butyl alcohol (5 g; 60 mmoles) with H_2SO_4 (0.25 ml) at reflux temperature lead to the generation of (D_8)-isobutylene. This gas was slowly bubbled through a mixture of 4-hydroxytoluene (2.6 g, 24 mmoles) and H_2SO_4 (30 µl). The apparatus was constructed to allow collection of unreacted (D_8)-isobutylene in a cold trap. Upon depletion of the isobutylene generator (ca 4.5 hr) the cold trap was warmed slowly to room temperature allowing the unreacted (D_8)-isobutylene to pass through the reaction mixture a second time. The reaction mixture was then diluted with water (50 ml) and boiled for 2½ hr. An oily layer separated from the mixture and crystallized upon cooling. The crystals were isolated by filtration, washed repeatedly with water and with 50% aqueous ethanol and air dried. Analysis of this material by GC indicated 99% BHT

(m.p. 65-70°C), with 3-t-butyl-4-hydroxytoluene as the only impurity. The reaction yield (4.16 g) based on (D_9)-t-butyl alcohol and (D_{16})-BHT was 75% of theory.

2,6-dideutero-3,5-di-t-butyl-4-hydroxytoluene ((D_2)-BHT):

1. BHT (1.1 g, 5 mmoles) was dissolved in a mixture of $(CF_3CO)_2O$ (0.1 mole) and D_2O (0.1 mole), and stirred continuously in a sealed vessel for 50 hours at room temperature. The solution was then diluted with D_2O (10 ml), and extracted three times with ethyl ether. The ether was washed twice with 5 ml portions of water, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to yield a yellow oil (0.71 g). The oil was subjected to GC analysis, which indicated the presence of at least 5 components, including BHT, 3-t-butyl-4-hydroxytoluene and 4-hydroxytoluene.

2. To a mixture of TFA : $(CF_3CO)_2O$ = 5:2 (3 ml) was added (D_2)-4-hydroxytoluene (1.6 g, 14.5 mmole) (see below). Isobutylene gas, generated by refluxing 5% H_2SO_4 in t-butyl alcohol, was passed through this solution for 72 hours at 40°C. The mixture was diluted with water, neutralized with Na_2CO_3 and extracted with ethyl ether. The ether extract was found on GC analysis to contain only BHT and 3-t-butyl-4-hydroxytoluene in the ratio of 17:3.

3. (D_4)-4-hydroxytoluene (0.6 g, 5.35 mmoles) (see below) was mixed with 2-methyl-2-chloropropane (5 ml) and cooled to 0°C in ice. After addition of $AlCl_3$ (0.5 g) the mixture was gradually heated to 50°C, then stirred at this temperature for 18 hours. The reaction mixture was diluted with water, filtered to remove insoluble salts and then extracted with ethyl ether. The ether extract was dried over $MgSO_4$, then reduced *in vacuo* to 1 ml for GC analysis. Three compounds, 4-hydroxytoluene, BHT, and 3-t-butyl-4-hydroxytoluene were found to be present in the ratio 9:29:62.

2,3,5,6-tetradeutero-4-hydroxytoluene ((D_4)-4-hydroxytoluene): A solution of 4-hydroxytoluene (1.08 g; 10 mmoles) in a mixture of D_2SO_4 (0.1 mole) and D_2O (0.4 mole) was heated at 50°C and shaken occasionally over a period of 24 hours.

The mixture was poured into aqueous sodium carbonate to neutralize the D_2SO_4, then extracted 3 times with ethyl ether. The ether extract was dried over anhydrous $MgSO_4$, and the ether removed in vacuo to yield a viscous liquid (0.64 g) which crystallized on prolonged standing (m.p. 30-32°C). GC analysis of an ether solution of this material indicated that only 4-hydroxytoluene was present.

2,6-dideutero-4-hydroxytoluene ((D$_2$)-4-hydroxytoluene): (D_4)-4-hydroxytoluene (2.6 g; 23 mmoles) was dissolved in TFA (0.79 moles) and water (1.33 moles). This solution was stirred for 16 hours at 65°C, diluted with water and neutralized with Na_2CO_3 and then extracted with ethyl ether. The ether solution was dried over $MgSO_4$ and then the ether was removed in vacuo to give the desired product (0.81 g; m.p. 30-32°C).

RESULTS AND DISCUSSION

The initial approach to the synthesis of deuterated BHT was to alkylate 4-hydroxytoluene with labeled isobutylene which has been generated from (D_9)-t-butyl alcohol. The product obtained displayed ions in the mass spectrometer at m/e 228 through m/e 238, indicating a mixture of compounds incorporating from 8 to 18 deuterons (figure 1). The most prominent isomers had molecular ions of m/e 236 corresponding to the incorporation of 16 deuterium atoms, as expected for (D_{16})-BHT, and m/e 235 for (D_{15})-BHT. These two isomers together accounted for ca 44% of the total mass in the molecular ion region (m/e 228-238) of the mass spectrum. This very significant loss of deuterium from the t-butyl substituents was not anticipated even though strongly acidic conditions were used for generation of (D_8)-isobutylene and for catalysis of the butylation step. However, a simple mechanism for exchange during generation of isobutylene could be postulated.

NMR spectral data for (D_{16})-BHT (Table 1) indicated little or no exchange of aromatic protons during the alkylation process since the ratio of aromatic protons to phenolic protons to methyl protons remained 2 : 1 : 3, as in BHT.

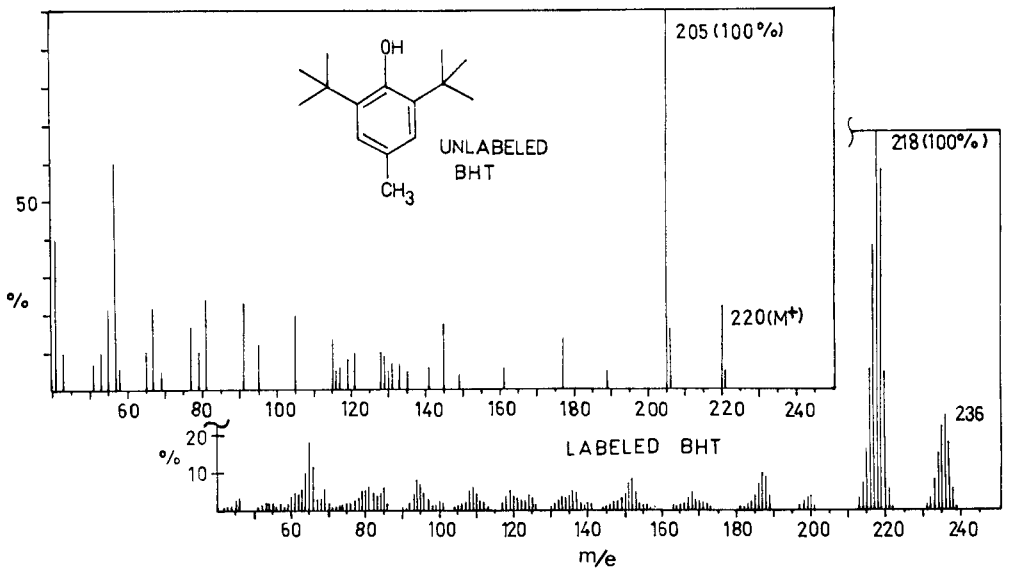


Figure 1: Mass spectra of BHT and deuterium labeled BHT.

Fully substituted (D_{16})-BHT molecules would have integrated for only 2 protons in the t-butyl proton region. The extra 1.5 protons per molecule in the synthesized compound represents an enrichment of 90% of the expected value.

The IR spectrum of this mixture of isotopes referred to as (D_{16})-BHT was characterized by prominent absorption bands at 3650, 2930 and 2220 cm^{-1}, the latter being the region in which the C-D stretch of aliphatic methyl groups would be found (3). Other peaks observed were at 3070, 2860 and 2130 cm^{-1}.

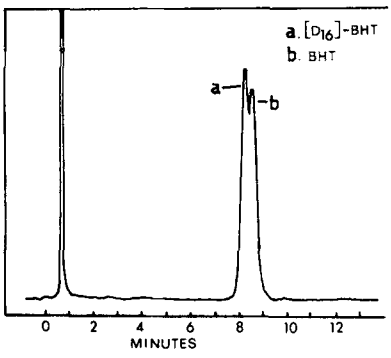
Table 1: NMR spectral data for BHT and (D_{16})-BHT

Compound	δ	Multiplicity	Integration	Assignment
BHT	6.92	s	2	aromatic H
	4.86	s	1	phenolic H
	2.23	s	3	methyl H
	1.40	s	18	t-butyl H
(D_{16})-BHT	6.94	s	2	aromatic H
	4.92	s	1	phenolic H
	2.27	s	3	methyl H
	1.39	s	3.5	t-butyl H

The (D_{16})-BHT displayed a change in physical properties from BHT. The observed wide melting point range (65° to 70°C) was attributed to the mixture of isotopes present in the sample. A difference between the retention times of BHT and (D_{16})-BHT on GC analysis (Figure 2) is presumably due to a decrease in polarity of the deuterated material. The GC peak corresponding to (D_{16})-BHT was also examined by continuous MS scanning over the range m/e 195 to m/e 250 at 3 second intervals. Each scan displayed a range of M^+ ions from m/e 228 (D_8) to m/e 238 (D_{18}). This indicated that the decreased retention time of the deuterated sample was virtually independent of the number of deuterons incorporated although the major ion varied from m/e 236 at the start of the GC peak to m/e 234 at the end.

It appeared from the information gathered that (D_{16})-BHT of higher overall deuterium enrichment, and consequently with a lesser number of isomers, could be synthesized in this reaction by substitution of D_2SO_4 for H_2SO_4 during isobutylene generation and in the alkylation step. It was also apparent, however, that because of the significant change in polarity resulting from the incorporation of up to 18 atoms of deuterium per molecule, such an extensively deuterated material would not be desirable for absorption, metabolism and excretion studies in laboratory animals. Metabolic studies have shown extensive oxidation of the t-butyl groups (2) and the substitution of deuterium for hydrogen in these positions would undoubtedly lead to quantitative if not qualitative differences in the fate of this compound. The synthesis of (D_{16})-BHT was not pursued further for these reasons.

The reaction of BHT in deuterated TFA solution (prepared from D_2O and trifluoroacetic anhydride) gave dealkylation as well as incorporation of deuterium into t-butyl groups and aromatic positions. The GC analysis of the reaction mixture showed 4-hydroxytoluene and 3-t-butyl-4-hydroxytoluene as well as BHT. The GCMS of the BHT peak showed m/e values from 228 to 235 for the M^+ ion. The incorporation of deuterium suggested an alkylation-dealkylation process.



3% OV-225; 6 ft; 60 ml/min;
130°C flame ionization

Figure 2: Gas Chromatograph trace of BHT and (D_{16})-BHT.

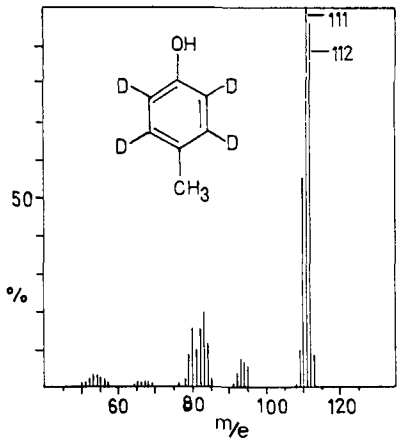


Figure 3: Mass spectrum of (D_4)-4-hydroxytoluene.

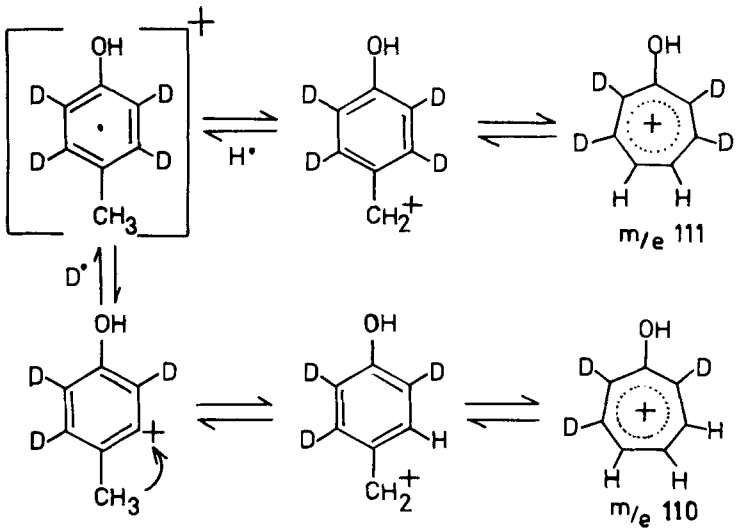
The NMR spectrum could not be interpreted due to the mixture of compounds present and the various isotopes of each compound. The GCMS also indicated that from 2 to 4 deuterons had been incorporated into 4-hydroxytoluene (M^+ m/e 112; base peak m/e 110), and that up to 11 deuterons were present in the 3-t-butyl-4-hydroxytoluene molecules (M^+ m/e 175, base peak m/e 170).

The 2,6-protons of BHT appeared to have undergone deuterium exchange in the presence of D-TFA. However, 2,6-exchange could not be effected with 4-hydroxytoluene in D-TFA; only the 3,5-protons underwent exchange. In the presence of D_2SO_4, on the other hand, 95% exchange of the aromatic protons was achieved, as determined by the NMR spectrum. The mass spectrum of this (D_4)-p-hydroxytoluene (figure 3) was of interest in that the parent ion was at m/e 112, and the base peak was at m/e 111, with a strong ion at m/e 110 (55% of base). In view of the virtually quantitative incorporation of deuterium into the aromatic centers, this suggested that two major fragmentation routes were operational. Fragmentation by the normally accepted route (3) involving formation of a benzylic ion by loss of $H^{\cdot}$ with subsequent rearrangement to a (D_4)-tropylium ion and a second

process, by the formation of a phenylic ion through loss of D· with subsequent rearrangement to a benzylic ion and finally to a (D_3)-tropylium ion (Scheme 1).

In an attempt to avoid what appeared to be acid catalyzed exchange of aliphatic protons during alkylation, (D_4)-4-hydroxytoluene was alkylated with t-butyl chloride in the presence of $AlCl_3$. Label scrambling was still observed and the yield of BHT was low. The addition of base (Na_2CO_3 or triethylamine) to prevent exchange by neutralizing acid generated during the reaction only served to further reduce the yield of BHT, and consequently this reaction was deemed unsuitable for our purposes.

Treatment of (D_4)-4-hydroxytoluene with TFA resulted in the removal of two deuterons. Comparison of the NMR spectrum to that of a published NMR spectrum for BHT (4) showed that 3,5-positions now both contained protons. The mass spectrum displayed a molecular ion at m/e 110 and a base peak at m/e 109. The M^+-2 peak was 32% of the base peak. The NMR spectrum showed only minor peaks for protons in the 2,6-positions; integrating for ∿10% of the value of the peak for the 3,5-protons. Treatment of the (D_2)-4-hydroxytoluene with isobutylene



Scheme 1

in TFA gave a high yield of BHT, with some 3-t-butyl-4-hydroxytoluene in an otherwise clean reaction. It was apparent from the mass spectrum that the integrity of the label had been lost and a series of isomers were now present in the BHT.

The exchange labeling of 4-hydroxytoluene with D_2O in the presence of PtO_2 resulted in extensive deuteration of both aliphatic (94%) and aromatic (82%) centers, as determined by integration of the NMR spectrum. The molecular ion occurred at m/e 115 and the base peak at m/e 113 (M^+-2). The incorporation of deuterium in the methyl group was undesirable because this center is known to be oxidized in sequential steps to the corresponding carboxylic acid (5) in mammalian systems thus leading to loss of the label on this methyl group.

CONCLUSIONS

Deuterium labeled BHT has been prepared by alkylation of 4-hydroxytoluene with deuterium labeled isobutylene, by exchange of BHT protons in D-TFA and by exchange of the aromatic protons of 4-hydroxytoluene in D_2SO_4 with subsequent alkylation using TFA / isobutylene or t-butyl chloride with $AlCl_3$. In all cases random exchange occurred. The total ion population in the mass spectra during analysis of these compounds was distributed among a series of isomers of the same compound. Consequently each individual isomer gave a weak spectrum and the GCMS technique was rendered less sensitive and virtually useless for mixtures having low concentrations of compounds. The NMR spectra were not interpretable in some instances because of the number of molecular isomers present.

The incorporation of 8 or more deuterons per molecule in the t-butyl substituents also altered the GC retention time for the deuterated BHT. Although it was conceivable that a deuterated BHT consisting almost entirely of (D_{18})-BHT could be prepared from (D_9)-t-butyl alcohol and 4-hydroxytoluene, this material was not synthesized because of its unsuitability for metabolic studies.

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