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PII: S0308-8146(97)00122-2

Food Chemistry, Vol. 61, No. 1/2, pp. 101–105, 1998

0308-8146/98 $19.00 + 0.00

Antioxidant properties of butylatedhydroxytoluene refluxed in ferric chloride solution

S. Duan, X. C. Weng,* X. W. Dong, Y. P. Liu, H. P. Li & J. R. Jin

Lipid Research Laboratory, Yantai University, Shandong, People's Republic of China, 264005

(Received 21 February 1997; revised version received 21 May 1997; accepted 21 May 1997)

1,2-Bis (3,5-*di*-*tert*-butyl-4-hydroxyphenyl)-ethane (BBH PE), 3,3′,5,5′-*tetra*-*tert*-butyl-stilbene-4,4′-quinone (TBSQ), 3,3′,5,5′- *tetra*-*tert*-butylhydroxy-stilbene-4,4′-quinone (TBHSQ) and 4-hydroxy-3,5-*tert*-butylbenzaldehyde (HBBA) were isolated from a mixture of butylatedhydroxytoluene (BHT) refluxed in ferric chloride solution by using TLC and identified by mass, NMR, IR and UV spectra. Their antioxidant properties were investigated individually and in comparison with BHT and butylatedhydroxyanisole (BHA) on the Oxidative Stability Instrument (OSI) at 95, 100 and 105°C, separately. This demonstrated that BBHPE was the strongest antioxidant of the compounds isolated, much stronger even than BHT and as strong as BHA. The phenolic compound, HBBA, structurally similar to BHT, had much weaker antioxidant effect than BHT and even weaker than the quinones, TBSQ and TBHSQ. The strength of the antioxidant activity of the compounds decreased in the order: BHA, BBHPE > > BHT > TBSQ, TBHSQ > HBBA. As temperature increased, the protection factors (Pf) of BHA and BHT increased, but those of BBHPE, TBSQ, TBHSQ and HBBA decreased slightly. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Autoxidation occurs widely in nature. It is involved in rancidity of fats and oils, deterioration of fatty foods and aging of rubber and other petrochemical products.

BHT and BHA are widely used to protect these fats and oils, fatty foods and their raw materials from autoxidation since they are cheap and have high antioxidant activity. The antioxidant activity and degradation of BHT and BHA have been comprehensively studied (Campbell and Coppinger, 1952; Moore and Walters, 1954; Anderson and Huntly, 1963; Horswill *et al*., 1966; Harano *et al*., 1967; Leventhal *et al*., 1976). It is well known that iron is traditionally used for cooking vessels and equipment and is still widely used in some developing countries, e.g. China. When food products or their raw materials are stored, transported and processed, iron contaminates them readily. Ferrous and ferric irons are well known lipid autoxidation catalysts and can effectively initialize and accelerate autoxidation of lipids (Pokorny, 1987; Gordon and Weng, 1992; Weng, 1993*b*).

In this experiment, the products of BHT refluxed in ferric chloride solution and their antioxidant properties were investigated and a novel compound, 3,3′,5,5′-*tetra*-*tert*-butylhydroxystilbene-4,4′-quinone (TBHSQ) and its antioxidant effects, are reported here. The results may explain the antioxidant mechanism of BHT and autoxidation catalytic mechanism of ferric iron. Also, the results indicate what compounds are produced after or during BHT acts as an antioxidant.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

BHA and BHT, food grade antioxidants, were purchased from Chemical Material Company, Guanzhou, China. $FeCl_3$. $6H_2O$, C.P., brown crystalline, was purchased from Jinshan Chemical Factory, China. Silica gel F254, was obtained from Qingdao Ocean Chemical Factory, China. Lard was rendered in the laboratory from fresh pig fat tissue, purchased from Yantai Slaughter House, China.

Reaction of BHT in ferric chloride solution

Five g BHT, 20 g $FeCl_3$. $6H_2O$ and 50 ml water were mixed in a 250 ml conical flask with an one meter long air condenser. The mixture was heated (on a heating plate) stirred with a magnetic stirrer and refluxed for

4 h. The organic layer was colourless liquid, floating on top of the aqueous layer which was red at the beginning, but at the end, the organic layer became orange-red and the aqueous layer straw green. After cooling to room temperature, the mixture was extracted with diethyl ether and chloroform (4:1, v/v) three times. The organic solvents were removed with a rotary evaporator.

Isolation and purification of compounds

Thin-layer chromatography (TLC) plates were made by coating 1 mm silica gel F254 onto 20×20 cm glass plates.

Six bands appeared on TLC plates when the mixture was developed with the solvent system, petroleum ether-chloroform-ethyl acetate (60:40:0.5). The six compounds were separately: unreacted butylatedhydroxytoluene (BHT, band 1); 1,2-bis(3,5-*di-tert*-butyl-4-hydroxyphenyl)-ethane (BBHPE, white crystalline, band 2); The compound 3 was too small in quantity to be identified; 3,3′,5,5′-*tetra-tert*-butylstilbene-4,4-quinone (TBSQ, red crystalline, band 4); 3,3′,5,5′-*tetra-tert*-butylhydroxystilbene-4,4′-quinone (TBHSQ, red crystalline, band 5) and 4-hydroxy-3,5-*di-tert*-butylbenz-aldehyde (HBBA, white crystalline, band 6). When they were developed with the solvent petroleum ether-chloroform-ethyl acetate (80:20:0.5) on TLC plates their Rf values were 0.58, 0.54, 0.52, 0.34, 0.28 and 0.21, respectively. TBSP, the compound of band 3, TBSQ, TBHSQ and HBBA were purified by TLC with the solvent system petroleum ether-chloroform-ethyl acetate, but with different ratios: 70:30:1, 50:50:2, 50:50:2, 50:48:2. But BBHPE was purified by TLC with petroleum ether-chloroform-ethyl acetate-pyridine (80:20: 0.5: 0.5).

Recording spectra

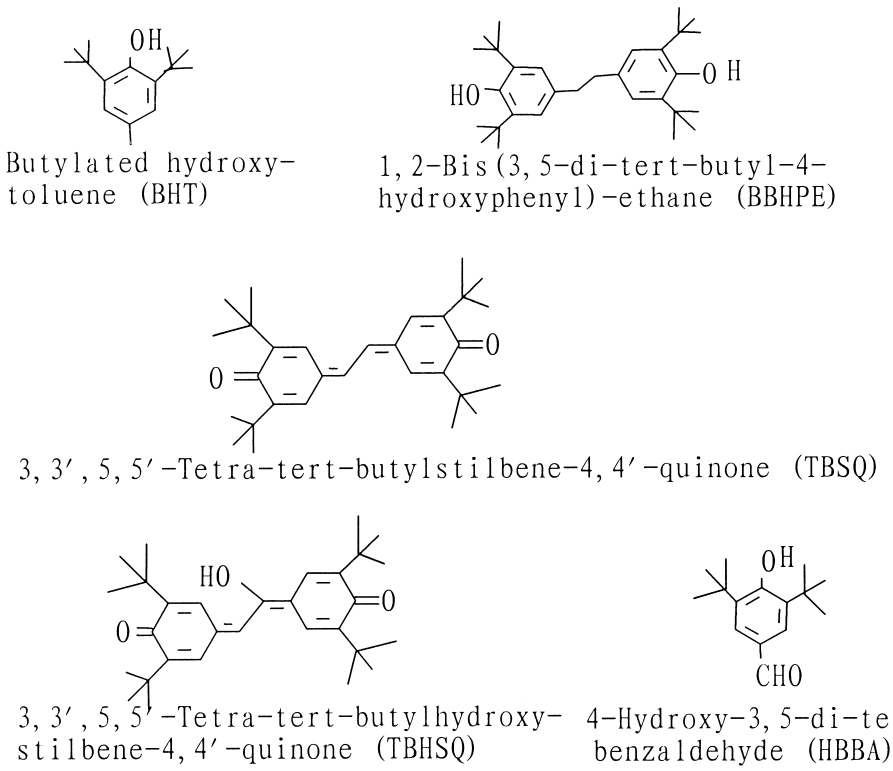
The ultraviolet (UV) spectra were recorded with a Hitachi UV-3400 and the ethanol was purified according to the Paquot method (1979) and used freshly as a solvent. The infrared (IR) spectra were recorded with a JASCO-IR810 and the discs were made with KBr. The mass spectra were recorded with a QP-1000 mass spectroscopic instrument. The PMR and ^{13}CNMR were recorded with a NMR 90 MHz spectroscopic instrument. $CDCl_3$ was used as solvent and TMS was used as internal standard.

Antioxidant activity

Antioxidant activity was studied in lard with an Omnion OSI, Massachusetts USA, at 95, 100 and 105°C. The air flow rate was fixed at 20 liters h^{-1}. BHA and BHT were used as comparison samples.

RESULTS AND DISCUSSION

The spectral data of BBHPE, TBSQ, TBHSQ and HBBA are listed in Table 1. BBHPE was white and crystalline and identified by mass, PMR, IR and UV spectra. It had a molecular ion peak at 438 in its mass spectrum. There were peaks at 437 (M–H), 219 (C–C



Scheme 1. BHT, BHA and the compounds isolated from mixture of BHT refluxed in ferric chloride solution.

Table 1. Spectral data and melting points of BHT, BBPHE, TBSQ, TBHSQ, HBBA

MS, m/z (relative abundance)	NMR, chemical shift ppm (no. of peaks and H)	IR wavenumber (cm^{-1})	UV, nm (abs A)
	Butylated hydroxytoluene (BHT)		
		3680, 2980, 2890, 1428	283 (0.36),
		1395, 1360, 1300, 1225	274 (0.36)
		1206, 1147, 860, 765	215 (1.15)
	1,2-Bis (3,5-*di-tert*-butyl-4-hydroxyphenyl) ethane (BBHPE)		
438 (M, 21.2)	1.49 (S, 36 H), 2.85 (S, 4H)	3700, 2990, 2950	283 (0.28)
439 (M + 1,6.4)	5.08 (S, 2H), 7.04 (S, 4H)	2900, 1430, 1360	277 (0.28)
437 (25.5), 221 (6.4),		1310, 1227, 1160	230 (0.75)
220 (66.0), 219 (100),		1120, 873, 780, 763	
203 (21.2), 204 (10.6)			
189 (6.4), 161 (14.9), 57 (100)			
	3,3′,5,5′-*tetra-tert*-butylstilbene-4,4′-quinone (TBSQ)		
434 (M,100),	1.32 (S, 18H), 1.36 (S, 18H)	3035, 2995, 2990	451.2 (1.62),
435 (M + 1,32)	7.03 (D, 2H), 7.25 (S, 2H)	1900, 1605, 1565	425.0 (1.06)
436 (M + 2,8)	7.52 (D, 2H)	1460, 1260, 1080	296.2 (0.43)
420 (36), 219 (60)			281.3 (0.08)
57 (63)			233.3 (0.31)
	3,3′, 5,5′-*tetra-tert*-butylhydroxystilbene-4,4′-quinone (TBHSQ)		
450 (M, 36.2)	1.25 (S, 36H), 6.48 (S, 2H)	3700, 3050, 3000	442 (1.37)
449 (M-H, 34.0)	7.02 (S, 1H), 7.24 (S, 2H)	2950, 2900, 1652	420 (1.21)
435 (5.3)	10.58 (S, 1H)	1613, 1600, 1480	315 (0.66)
434 (6.4)		1460, 1432, 1390	255 (2.33)
394 (10.6)		1360, 1310, 1240	
393 (10.6)			
337 (21.3), 336 (17.4)			
263 (47.7), 262 (46.8), 220 (8.5)			
219 (12.8), 163 (16.0), 161 (1.8), 57 (100).			
	4-Hydroxy-3,5-*di-tert*-butylbenzaldehyde (HBBA)		
234 (M, 23.3)	1.48 (S, 18H), 5.84 (S, 1H)	3480, 2995, 1668	280 (2.10)
235 (M + 1,4.3)	7.72 (S, 2H), 9.85 (S, 1H)	1596, 1578, 1430	270.4 (2.57)
220 (17.0)		1298, 1260, 1200	225.6 (2..20)
219 (100)			
203 (4.3), 191 (21.3), 175 (8.5)			
159 (6.4), 115 (10.6), 91 (16), 57 (57.4)			

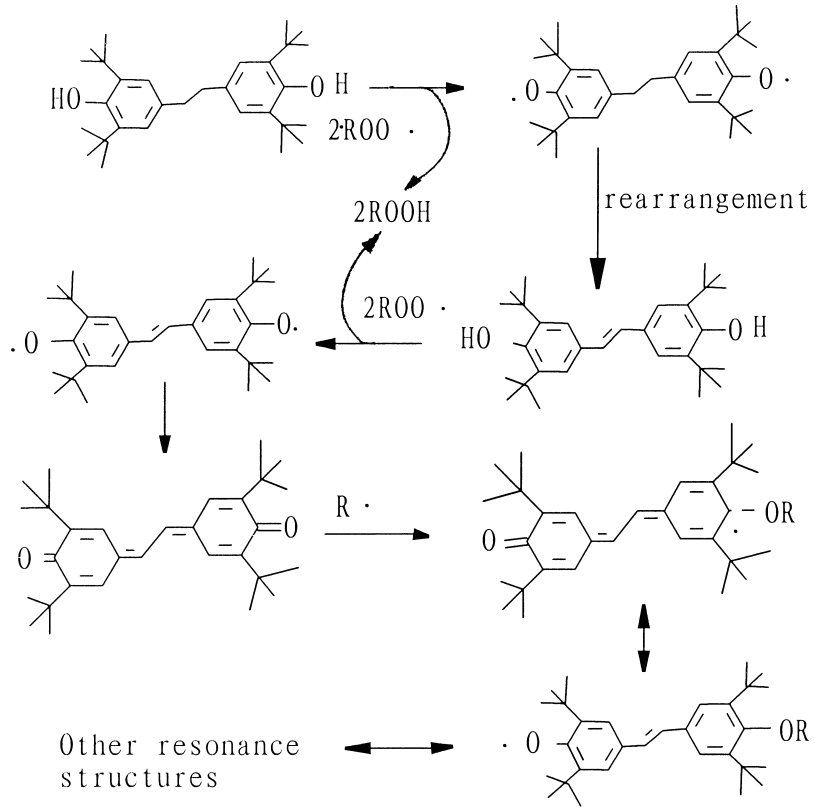
bond broken of ethane) in mass spectra. Its UV and IR spectra were very similar to those of BHT. PMR showed at 1.50 ppm of singlet peak (36 H of 4-*tert*-butyl groups), 2.85 ppm, a singlet (4-H of ethane group), 5.08 ppm, a singlet (2-H of two hydroxy groups), 7.05 ppm, singlet (4-H of four aromatic rings C–H).

TBSQ was a red crystalline structure, identified by mass, PMR, ^{13}CNMR, IR and UV spectra. It had a base peak at 434 for the molecular ion, 419 (M–CH3). Its PMR spectrum showed that there were resonances at 1.32, 1.36, 7.03, 7.25 and 7.52 ppm. Because of the whole molecule being conjugated, the two rings were locked and could not rotate freely; this structure made hydrogen on the 2 and 6 positions, and hydrogen on the tetra-butyl groups of 3 and 5 positions not equivalent. There were ^{13}CNMR peaks at 29.71, 35.46, 35.85, 124.29, 133.17, 133.95, 136.29, 150.04, 150.43, and 186.47 ppm. However, all carbons of methyl groups are equivalent, but 3,5-carbons, 2,6-carbons and *tert*-butyl carbons linked directly to 3,5 positions are not equal in ^{13}CNMR spectra. The resonance at 186.41 ppm of its ^{13}CNMR spectrum confirmed that a carbonyl group existed. There was no strong absorption in the range 3400–3700 cm^{-1}, but very strong absorption at 1605 cm^{-1} in the IR spectra. So this meant there were carbonyl groups, but no hydroxyl group in the molecule. It had strong absorption at 451.2 nm in the UV spectrum.

TBHSQ was red and crystalline, identified by mass, NMR, IR and UV spectra. It had characteristic peaks at 450 (Molecular ion), 449 (M–H), 435 (M–CH_3), 393 (M–C(CH_3)) in the mass spectrum. Its PMR showed it had resonances at 1.25, 6.48, 7.02, 7.24 and 10.58 ppm. It had strong absorption at 3700, 1690 and 1650 cm^{-1} in the IR spectrum. This demonstrated the compound had both hydroxyl and carbonyl groups.

HBBA was a white crystalline compound, identified by mass, NMR, IR and UV. It had characteristic peaks at 234 (Molecular ion), 235(M + 1), 219(M–CH_3), 191(M–CH_3–CO). It resonated at 1.48, 5.84, 7.72 and 9.85 ppm in its PMR spectrum. It had strong absorption at 3480 and 1668 cm^{-1} which demonstrated it had both hydroxyl and carbonyl groups. The structures of the four compounds are shown in Scheme 1.

The induction periods (IP) and protection factors (Pf) are listed in Table 2. The results demonstrate that BBHPE had very strong antioxidant activity, almost as strong as BHA, but much stronger than that of BHT. BBHPE was a strong antioxidant because its oxidation compounds, 3,3′,5,5-*tetra-tert*-butylstilbene-4,4′-phenol (TBSP) and 3,3′,5,5′-*tetra-tert*-butylstil-bene-4,4′-quinone



Scheme 2. The antioxidant mechanism of BBHPE and its oxidation products.

(TBSQ) could still act as quite strong antioxidants after providing the hydrogens to interrupt free radical chain reactions of autoxidation as shown in Scheme 2. Actually, some quinones are very strong antioxidants and their antioxidant activity has been studied by Weng and Gordon (1992), and Weng (1993*a*).

HBBA had a similar phenolic structure to BHT, but a formo group substitutes a methyl group. However, its antioxidant activity is much weaker than that of BHT, and obviously weaker than that of the quinones, TBSQ, TBHSQ. This is mainly because, first, HBBA cannot form a new strong antioxidant after antioxidation like BHA and second, the aldehyde group is a strong electron-withdrawing group and weakens the antioxidant ability markedly. Weng (1993*a*) suggested that electron withdrawing groups, such as carbonyl and carboxyl groups, weaken antioxidant activity of phenolic antioxidants, but electron donating groups, such as methoxyl and alkyl groups, strengthen antioxidant activity of phenolic antioxidants.

When temperature increased 10°C, all the IPs of the lard containing 0.04% compounds shortened by 2–3 times. Both Pfs of BHA and BHT increased quite obviously, but the Pfs of BBHPE, TBSQ, TBHSQ and HBBA even decreased slightly when the temperature rose (Table 2). This is probably because it is favorable

Table 2. The induction periods (IP, hours)[a] and the protection factors (Pf)[b] of the lard containing 0.04% different compounds at 95, 100, 105°C by OSI instrument

Compounds	Control	BHA	BHT	BBHPE	TBSQ	TBHSQ	HBBA
95°C							
IP(h)	5.4 ± 0.1	77.0*	39.6 ± 0.2	76.0 ± 0.7	27.9 ± 0.2	21.9 ± 0.3	14.0 ± 0.0
Pf	1.0 ± 0.02	14.3*	7.3 ± 0.04	14.1 ± 0.15	5.2 ± 0.04	4.1 ± 0.0	52.6 ± 0.00
100°C							
IP(h)	3.2 ± 0.0	46.9 ± 1.0	24.7 ± 0.1	45.0 ± 0.0	15.0 ± 0.5	12.3 ± 0.5	6.8 ± 0.1
Pf	1.0 ± 0.0	14.4 ± 0.31	7.7 ± 0.03	14.1 ± 0.0	4.7 ± 0.16	3.8 ± 0.1	62.1 ± 0.03
105°C							
IP(h)	1.8 ± 0.0	31.3 ± 0.4	16.6*	24.3 ± 0.0	9.7 ± 0.2	7.9 ± 0.0	4.3 ± 0.2
Pf	1.0 ± 0.00	17.4 ± 0.22	9.2*	13.5 ± 0.00	5.4 ± 0.1	4.4 ± 0.00	2.3 ± 0.10

[a]IP results are duplicates.
[b]Pf = The IP of lard with antioxidant/the IP of control lard.
*The results are single.

for BHA and BHT to produce stronger antioxidants, such as the compounds isolated, after acting as antioxidants, at higher temperatures, but not for BBHPE, TBSQ, TBHSQ and HBBA.

ACKNOWLEDGEMENTS

The authors thank the National Science Foundation Committee of China and the Education Commission of Shandong Province for grants; Dr Jiang Wu, Yantai University, for precious suggestions; Professor Hanshui Zhao and Professor Cuiping Li, Shandong University, for recording NMR spectra.

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