Antioxidant Activity of Quinones Extracted from Tanshen (*Salvia miltiorrhiza* Bunge)

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Six compounds have been solvent extracted from tanshen and separated by column and thin-layer chromatography. The structures have been identified by spectroscopic methods as rosmariquinone, dehydrorosmariquinone, miltirone I, tanshinone II_A, cryptotanshinone, and dihydrotanshinone. The antioxidant activity of these quinones in lard at 100 °C was determined with a Rancimat. The following structural features are associated with increased activity: (1) additional conjugated double bonds in the A ring; (2) a dihydrofuran ring rather than a furan ring; (3) an isopropyl substituent ortho to a quinone carbonyl rather than a dihydrofuran ring. An addition product has been isolated from the reaction between 9,10-phenanthrenedione and methyl oleate, and it is proposed that the quinones act as primary antioxidants by addition of the lipid radical to the quinone to form a stabilized radical which interrupts the autoxidation chain reaction.

INTRODUCTION

Tanshen (*Salvia miltiorrhiza* Bunge) is a wild herbal plant which has been used in traditional Chinese medicine because of its tranquilizing, sedative, circulation-promoting, and bactericidal effects (Fang et al., 1976).

A wide range of quinones have been shown to be present in tanshen (Tateishi et al., 1971, Chien et al., 1978; Okumura et al., 1961; Luo et al., 1988; Fang et al., 1976; Kakisawa et al., 1969; Baillie and Thomson, 1968) including rosmariquinone (also known as miltirone), which has been identified as one of the antioxidant components of rosemary. A recent paper describes the isolation of seven quinones from tanshen and an investigation of their antioxidant activity. It was found that dihydrotanshinone (I), tanshinone I (II), methylenetanshinquinone (III), cryptotanshinone (IV), tanshinone II_B (V), and danshenxinkun B (VI) act as antioxidants in heated lard, whereas tanshinone II_A (VII) has no antioxidant properties (Zhang et al., 1990).

This paper extends the investigation of the antioxidant properties of quinones from tanshen in heated fat by including dehydrorosmariquinone (VIII), miltirone I (IX), and rosmariquinone (X) and also includes a study of the product formed when a model quinone is heated in methyl oleate to identify the mechanism by which the quinones act as antioxidants.

MATERIALS AND METHODS

Materials and Reagents. Tanshen was purchased from a Chinese Medicine Co. in Zhengzhou, People's Republic of China.

Pig fat was purchased at Reading Slaughter House and rendered in the laboratory. 9,10-Phenanthrenedione was purchased from Aldrich Chemical Co. Ltd., Gillingham, U.K., and purified by recrystallization from ethyl acetate. Methyl oleate (99%) was purchased from Sigma Chemical Co., Poole, U.K.

Isolation and Purification of Quinones from Tanshen. The procedure for isolating the quinones from tanshen was as follows. Dry, clean tanshen rhizomes were powdered with a mill, and the powder (1.3 kg) was extracted with hexane (3 L) for 3 days at room temperature. The hexane solution was kept overnight and then filtered. The solvent was removed with a rotary evaporator to leave a residue (11.4 g). The residue was separated into seven colored fractions by column chromatography with silica gel 60 (400 g for 3-g sample). The fractions were eluted with hexane–ethyl acetate (200 mL, 3:1), hexane–acetone (375 mL, 4:1), hexane–acetone (100 mL, 4:1), hexane–ethyl acetate (100 mL, 3:1), hexane–chloroform (400 mL, 7:3), hexane–ethyl acetate (100 mL, 3:1), hexane–ethyl acetate (120 mL, 1:1), hexane–acetone (100 mL, 4:1), hexane–acetone (100 mL, 3:2), hexane–acetone (100 mL, 2:3), hexane–acetone (100 mL, 1:4), acetone (200 mL), and methanol (200 mL).

Rosmariquinone was isolated by preparative TLC from fraction 1 (light red) using hexane–ethyl acetate (4:1) (R_f = 0.65), followed by benzene–acetone (20:1). It was recrystallized from ethyl acetate.

Dehydrorosmariquinone was isolated by preparative TLC from fraction 1 using hexane–ethyl acetate (4:1) (R_f = 0.55), followed by benzene–acetone (20:1) twice. Miltirone 1 and tanshinone II_A were isolated by preparative TLC from fraction 2 (bright red) with hexane–ethyl acetate (4:1) twice and benzene–acetone (20:1). The brown band was separated and recrystallized from ethyl acetate as dark brown-red crystals, identified as miltirone 1. The red band was recrystallized from ethyl acetate and identified as tanshinone II_A.

Cryptotanshinone and dihydrotanshinone were isolated by preparative TLC from fraction 7 (black). Hexane–chloroform–ethyl acetate–formic acid (50:39:11:3) was used to elute the blank TLC plates, and then the sample was separated with chloroform–ethyl acetate (20:1) and chloroform. The yellow band was scraped off, and cryptotanshinone was isolated as yellow needles and purified by two recrystallizations from ethyl acetate.

Dihydrotanshinone was isolated from the yellow band by further preparative TLC of the supernatant isolated by filtration of crude cryptotanshinone. Preparative TLC with benzene–pyridine (9:1) yielded a brown band, from which dihydrotanshinone was isolated and purified by recrystallization as dark brown crystals from ethyl acetate.

A sample of 9,10-phenanthrenedione (0.48%) was added to methyl oleate (6 g) and stored in an oven at 50 °C for 65 days. The sample retained the yellow color of phenanthrenedione. The product mixture was separated by successive preparative TLC with silica gel. Elution with hexane–ethyl acetate (4:1) gave five bands with R_f = 0.72 (methyl oleate), 0.59 (intense blue), 0.41 (thin blue), 0.24 (phenanthrenedione), and 0. The blue bands were colored only under long-wavelength UV radiation. The product in the intense blue band was extracted and purified by TLC with the same solvent system. Then the product was successively purified by TLC using hexane–acetone (10:1), R_f = 0.39, and hexane–ethyl acetate–acetone (55:5:3), R_f = 0.42. The main band (blue under UV radiation) was purified by TLC with hexane–ethyl acetate (7:3), R_f = 0.70, and toluene–acetone (10:1), R_f = 0.71. The final product was extracted with hexane, and evaporation of the solvent left a pale yellow liquid. The spectral data for the liquid are given in Table III.

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Table I. Spectral and Melting Point Data of the Compounds Isolated from Tanshen

MS; *m/z* (relative abundance)	NMR chemical shift, ppm (no. of peaks and H)	IR wavenumber, cm^{-1}	UV, nm (abs *A*)	mp, °C
		Rosmariquinone, Experimental Data		
282 (M, 5), 267 (2), 254 (38), 239 (100), 224 (18), 209 (12), 197 (7), 170 (10), 165 (13), 152 (18), 115 (9), 40 (53)	1.15 (d, 6 H), 1.26 (s, 6 H), 1.64 (m, 2 H), 1.78 (m, 2 H), 3.01 (m, 1 H), 3.16 (t, 2 H), 7.06 (s, 1 H), 7.10 (d, 1 H), 7.58 (d, 1 H)	3080, 2965, 2940, 2866, 1670, 1657, 1634, 1581, 1565, 1454, 1390, 1259, 1210, 925, 584	436 (0.368), 360 (0.266), 258 (2.537), 213.5 (2.0)	100–101
		Literature Data from Ho (1985)		
282 (7), 267 (5), 254 (52), 239 (17), 224 (17), 219 (6), 209 (9), 197 (8), 165 (10)	1.17 (d, 6 H), 1.30 (s, 6 H), 1.64 (m, 2 H), 1.79 (m, 2 H), 3.02 (m, 1 H), 3.17 (t, 2 H), 7.09 (s, 1 H), 7.12 (d, 2 H), 7.60 (d, 1 H)	3090, 2960, 2940, 1649, 1570, 1560, 1450, 1410, 1380, 1300, 1250, 1140, 920		94–95
		Data from Hayashi (1970)		
282.161	1.30 (6 H), 1.50 (6 H), 1.71 (4 H), 3.02 (1 H), 3.20 (2 H), 7.10 (1 H), 7.34 (2 H)	1680, 1660, 1635	436 (log 3.52), 362 (3.36), 260 (4.55)	100
		Dehydrorosmariquinone, Experimental Data		
280 (M, 2.5), 267 (5), 252 (50), 238 (23), 237 (100), 223 (74), 209 (20), 194 (15), 179 (28), 165 (32), 152 (18)	1.15 (d, 6 H), 1.25 (s, 6 H), 2.25 (d, 2 H), 3.0 (m, 1 H), 6.3 (m, 1 H), 7.09 (d, 1 H), 7.12 (t, 1 H), 7.5 (d, 1 H), 7.85 (d, 1 H), 7.89 (d, 1 H)		461 (0.125), 363 (weak), 270 (0.75), 208 (0.64)	
		Literature Data from Luo (1988)		
	1.15 (d, 6 H), 1.25 (s, 6 H), 2.25 (d, 2 H), 3.0 (m, 1 H), 6.3 (m, 1 H), 6.93 (d, 1 H), 7.06 (s, 1 H), 7.85 (d, 1 H), 7.89 (d, 1 H)	2958, 1683, 1662, 1647, 1635, 1575, 1560, 1382		
		Miltirone I Experimental Data		
264 (M, 2.3), 237 (19), 236 (100), 221 (30), 208 (13), 193 (34), 178 (36), 165 (17), 152 (12), 89 (7)	1.25 (d, 6 H), 2.70 (s, 3 H), 3.10 (m, 1 H), 7.20 (s, 1 H), 7.40 (two d, 2 H), 7.53 (t, 1 H), 8.32 (d, 1 H), 9.27 (d, 1 H)	417 (0.117), 360 (weak), 289 (0.391), 232 (0.82), 210 (1.013)		219
		Literature Data from Luo (1988)		
266 (M + 2, 7.8), 264 (M, 1.4), 250 (8.2), 236 (100), 221 (33.2)	1.20 (d, 6 H), 2.68 (s, 3 H), 3.10 (m, 1 H), 7.20–7.60 (m, 3 H), 8.25 (d, 2 H), 9.20 (d, 2 H)	1640, 1620, 1580, 1360, 1218, 940, 840, 780	236, 289, 425	221–223
		Tanshinone II_A, Experimental Data		
295 (M + 1, 21), 294 (M, 100), 279 (40), 261 (70), 251 (53), 233 (9), 222 (9.5), 189 (6), 178 (15), 165 (19), 115 (5), 89 (5)			454 (0.139), 351 (0.137), 268 (0.946), 250 (0.75), 223 (0.881)	202–204
		Literature Data from Kakisawa (1968)		
	1.30 (s, 6 H), 1.7 (m, 4 H), 2.25 (d, 3 H), 3.15 (t, 2 H), 7.10 (q, 1 H), 7.48 (q, 2 H)	3150, 1690, 1670, 1583, 1535	460, 352, 269, 252, 224	
		Data from Zhang (1990)		
294 (100), 279 (52), 261 (87), 251 (73), 233 (19), 222 (14), 165 (33), 178 (20), 152 (16)		2950, 2817, 1686, 1667, 1580, 1534, 1453, 1429, 1403, 1389, 1287, 1193, 1170, 1152, 961, 917, 838, 707		202–204
		Cryptotanshinone, Experimental Data		
297 (20, M + 1), 296 (M, 61), 281 (10), 268 (35), 254 (21), 253 (100), 235 (10), 227 (4), 209 (4), 171 (13), 152 (10), 128 (9), 115 (8)	1.3 (s, 6 H), 1.34 (d, 3 H), 1.65 (m, 2 H), 1.78 (m, 2 H), 3.21 (t, 2 H), 3.59 (m, 1 H), 4.38 (q, 1 H), 4.88 (t, 1 H), 7.48 (d, 1 H), 7.62 (d, 1 H)	2955, 2925, 2872, 1685, 1654, 1625, 1558, 1462, 1403, 1170, 942, 842, 700	445 (0.253), 356 (0.236), 292 (0.52), 272 (2.14), 263 (2.48), 218 (1.59)	187–188
		Literature Data from Zhang (1990)		
296 (64), 281 (10), 268 (32), 253 (100), 235 (14), 178 (5), 171 (18), 152 (10)		2857, 1695, 1647, 1626, 1575, 1481, 1441, 1342, 1212, 1176, 1156, 948, 919, 704		174–175
		Data from Baillie (1968)		
		2950, 1680, 1648, 1620, 1553, 1460, 1400, 1333, 1193, 1140, 1160, 941, 840, 700	447 (3.48), 355 (3.41), 290 (3.96), 272 (4.41), 263 (4.47), 221 (4.26)	184–185 or 174–175 (*dl* mixture)

Table I (Continued)

MS; m/z (relative abundance)	NMR chemical shift, ppm (no. of peaks and H)	IR wavenumber, cm^{-1}	UV, nm (abs A)	mp, °C
		Dihydrotanshinone I, Experimental Data		
279 (M + 1, 5), 278 (M, 20), 250 (43), 236 (20), 235 (100), 221 (4), 207 (7), 189 (7), 179 (10), 169 (7), 152 (8.7), 139 (16), 89 (8), 40 (43)		3129, 2968, 2873, 1685, 1656, 1632, 1592, 1572, 1472, 1423, 1380, 1355, 1298, 1241, 1177, 1022, 930, 827, 790,760, 660	410 (0.17), 333 (0.123), 290 (0.63), 241 (0.96), 214 (0.89)	222–223
		Literature Data from Zhang (1990)		
278 (17), 250 (55), 235 (100), 222 (4), 207 (10), 189 (8), 179 (26), 169 (9), 165 (9)		2857, 1684, 1653, 1626, 1563, 1481, 1429, 1337, 1304, 1181, 961, 939, 843, 799		223–225
		Data from Inouye (1969)		
	1.42 (d, 3 H), 2.64 (s, 3 H), 3.6 (m, 1 H), 4.37 (d, 1 H), 4.94 (t, 1 H), 7.3–7.8 (m, 3 H), 8.18 (d, 1 H), 9.20 (d, 1 H)	1680, 1650, 1622	414 (3.61), 335 (3.48), 292 (4.20), 242 (4.40), 216 (4.30)	202 (*dl* mixture)

The quinones were identified by spectroscopic methods; 400-MHz ^1H NMR spectra were recorded by the SERC NMR service at the University of Warwick, and a 500-MHz ^1H NMR spectrum of miltirone 1 was recorded at the University of Oxford. Ultraviolet spectra were recorded on a Perkin-Elmer λ5 spectrometer, infrared spectra of KBr disks were recorded on a Perkin-Elmer 577 spectrometer, and mass spectra were recorded on a Kratos MS 80 RFA mass spectrometer with electron impact ionization, electron energy 70 eV. The spectroscopic data for each pure compound isolated from tanshen are given in Table I.

Antioxidant studies were performed on 0.02% solutions of antioxidants in lard with a Metrohm Rancimat Model 617. Six determinations on a standard sample indicated good reproducibility of induction periods with a coefficient of variation of 2.9%.

DISCUSSION

The quinonoid antioxidants from tanshen (*S. miltiorrhiza* Bunge) were extracted with hexane and separated by column chromatography and preparative TLC. Hexane was chosen for the extraction since this has been shown to be effective in extracting the lipids with antioxidant properties from tanshen (Gordon and Weng, 1992). The elution sequence by column chromatography showed that rosmariquinone and dehydrorosmariquinone eluted in fraction 1, miltirone 1 and tanshinone II_A in fraction 2, and cryptotanshinone and dehydrotanshinone in fraction 7. A mixture of tanshinone 1 and methylenetanshinone eluted in fractions 3–5, but the pure compounds were not isolated, and therefore studies on this mixture are not reported in this paper. Initial elution with hexane–ethyl acetate (3:1) appears to have advantages over the use of diethyl ether with increasing amounts of acetone used by Zhang et al. (1990) since these workers did not isolate rosmariquinone, dehydrorosmariquinone, and miltirone 1.

All samples were stored at −20 °C after isolation. The reactive nature of the quinones was revealed during evaporation of the solvent in the isolation of cryptotanshinone, when TLC indicated that partial decomposition had occurred but recrystallization was an effective method of purifying the quinones.

The structure of dehydrotanshinone 1 (I) was confirmed by the mass, IR, and UV spectra and by the melting point, which agrees with that of Zhang et al. (1990). The mass spectrum shows peaks at 278 (M^+), 250 (M − CO), 235 (M − C_3H_7), and 221 (M − CH_3CH_2CHO). The UV absorption spectrum agrees well with that of Inouye and Kakisawa (1969), who reported the isolation of the *dl* mixture, but the higher melting point of the sample isolated in the present work suggests the presence of a single enantiomer.

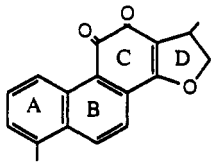
The mass spectrum of cryptotanshinone (IV) agrees well with that reported by Zhang et al. (1990). The relative intensities of the base peak (253, M − C_3H_7) and the high mass ions, namely 296 (M^+), 281 (M − CH_3), 268 (M − CO), and 235, are very similar to those reported by Zhang et al. (1990). The NMR spectrum of cryptotanshinone has not been found in the literature. The resonances of H-5, H-6, and H-7 are similar to those of rosmariquinone, but the presence of the perhydrofuran ring is revealed by the higher chemical shifts of H-9 and H-10 in cryptotanshinone. The quartet at 4.38 ppm is due to the ^1H on C-2 trans to the ^1H on C-1, whereas the triplet at 4.88 ppm is due to the cis ^1H on C-2, which has strong coupling both to the geminal proton and to the cis ^1H on C-1.

The identification of tanshinone II_A (VII) relies on the similarity of the mass spectrum and melting point to those reported by Zhang et al. (1990) and on the similarity of the UV spectrum to that reported by Kakisawa et al. (1969). The molecular ion (294) is the base peak in the mass spectrum and peaks at 279 (M − CH_3), 261 (M − CH_3, H_2O), 251 (M − CO, CH_3), 233 (M − CO, CH_3, H_2O), 222 (M − CH_3CH_2CO) are characteristic.

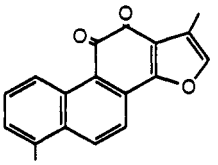
The mass and UV spectra of dehydrorosmariquinone (VIII) are reported, but no literature values have been found for comparison. Peaks at 280 (M^+), 252 (M − CO), and 237 (M − CO, CH_3) are consistent with the proposed structure. The NMR spectrum agrees well with that reported by Luo et al. (1988), and the resonances are assigned to the protons in the structure shown in Figure 1. The similarity of the resonances at H-1, H-2, H-9, H-10, and H-11 to those of rosmariquinone is in agreement with the common structural features.

Miltirone 1 (IX) has been identified by mass, NMR, and UV spectra and the melting point, which can be compared with those reported by Luo et al. (1988). The base peak of miltirone 1 is at 236 (M − CO), but the molecular ion (264) is of low intensity. The presence of a peak more intense than the molecular ion at M + 2 (266) in Luo's spectrum casts doubt on the purity of his sample. The peaks at 221 (M − C_3H_7) and 193 (M − CO, CH_3) can readily be assigned. The high field applied in the NMR spectrum of the present sample has separated the resonances at 7.20, 7.39, 7.40, and 7.53 ppm more clearly than in Luo's spectrum. The probable assignments are indicated in Figure 1.

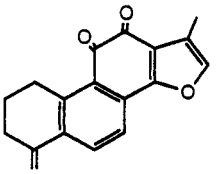
The structure of rosmariquinone (X) was confirmed by the mass spectrum and NMR, IR and UV spectra, which agree quite closely with those of Ho et al. (1985) and Hayashi et al. (1970). Characteristic ions in the mass spectra include 282, the molecular ion, 267 (M − CH_3), 254 (M − CO), 239 (M − C_3H_7), 224 (M − C_3H_7, CH_3), and 209 (M − C_3H_7, $2CH_3$). The M − CO peak is characteristic of 1,2-quinones. Probable identification of the protons in the NMR spectrum is indicated in Figure 1. The deshielding



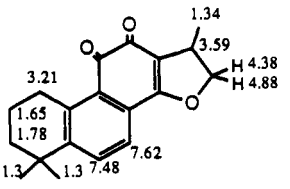
(I) Dihydrotanshinone I, or 1,2-Dihydro-1,6-dimethyl phenanthro[1,2-b] furan-10, 11-dione



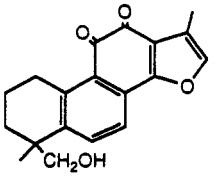
(II) Tanshinone I, or 1,6-Dimethyl phenanthro-[1,2-b] furan--10,11-dione



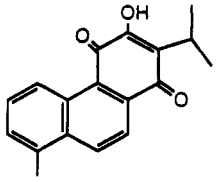
(III) Methylenetanshinone, or 7,8,9-Trihydro-1-methyl-6-methylene phenanthro[1,2-b] furan-10,11-dione



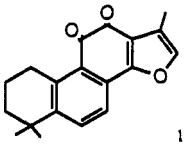
(IV) Cryptotanshinone, or 1,2,7,8, 9-Pentahydro-1,6,6-trimethyl phenanthro[1,2-b] furan-10,11-dione



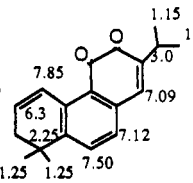
(V) Tanshinone II $_B$, or 7,8,9-Trihydro-1,6-dimethyl-6-hydroxymethyl phenanthro-[1,2-b] furan-10,11-dione



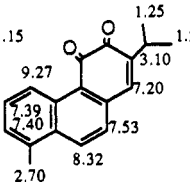
(VI) Danshenxinkun B, or 3-Hydroxy-8-methyl-2-(1-methyl-ethyl)-1,4-phenanthrendione



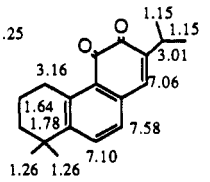
(VII) Tanshinone II$_A$ or 7,8,9-Trihydro-1, 6,6-trimethyl phenanthro[1,2-b] furan-10, 11-dione



(VIII) Dehydrorosmariquinone, or 8,8-Dimethyl-7-hydro-2-(1-methyl-ethyl)-3,4-phenanthrendione



(IX) Miltirone I, or 8-Methyl-2-(1-methyl-ethyl)-3,4-phenanthrendione



(X) Rosmariquinone, or 5,6,7-Trihydro-8,8-dimethyl-2-(1-methylethyl)-3,4-phenanthrendione

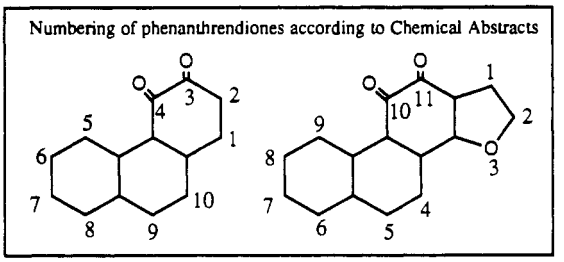


Figure 1. Structures of quinones from tanshen (numbers for compounds IV, VIII, IX, and X refer to chemical shift of protons in the NMR spectrum).

effect of the δ carbonyl group on the protons of C-5 is clearly evident, increasing the chemical shift to 3.16 ppm. The assignment of frequencies to H-6 or H-7 and H-9 or H-10 is tentative, since the frequencies are very similar. The melting point was 100–101 °C compared with 100 °C reported by Hayashi et al. (1970) and 94–95 °C reported by Ho et al. (1985), which suggests that Ho's sample may have been impure. The quinonoid structure of the isolated compounds is confirmed by the IR peaks at 1630–1685 cm^{-1}, which compare with those of 9,10-phenanthrenedione at 1637–1677 cm^{-1}.

The order of antioxidant activity of the quinones tested on the Rancimat at 100 °C was rosmariquinone, dihydrotanshinone, miltirone 1 > dehydrorosmariquinone > cryptotanshinone > tanshinone II$_A$. This sequence compares with that found by Zhang et al. (1990), who found that the antioxidant activity fell in the order dihydrotanshinone I > tanshinone I > methylenetanshinquinone, cryptotanshinone > tanshinone II$_B$, danshenxinkun B > tanshinone II$_A$. For the effect of structure on activity to be interpreted, the protection factor, P_F, has been calculated according to

$$P_F = \frac{\text{induction period for (lard + antioxidant)}}{\text{induction period for lard}}$$

and P_F values are quoted in Table II. Although the absolute protection factors for tanshinone II$_A$, cryptotanshinone, and dihydrotanshinone I differ in the present study from those calculated from the data of Zhang et al. (1990), the order of activity of the three quinones is the same in the two studies. The order of activity of BHA, BHT, and cryptotanshinone is also identical in the two studies. The stability of the lard used is likely to be one of the factors affecting the value of the protection factor. Consequently, only P_F values determined with the same lard can be compared. Thus, the high P_F value for rosmariquinone is partly due to the short induction period of the lard control sample.

Table II. Antioxidant Properties of Quinones and Standards at 100 °C

compound	induction period, h	P_F	P_F[a]
lard control 1	4.3	1	1
dihydrotanshinone 1	23.5	5.5	4.1
miltirone 1	22.9	5.3	
dehydrorosmariquinone	17.9	4.2	
tanshinone II$_A$	6.8	1.6	1.0
tanshinone I			2.8
methylenetanshinquinone			3.0
tanshinone II$_B$			2.2
dashenxinkun B			1.8
9,10-phenanthrenedione	12.7	2.95	
lard control 2	2.1	1	1
rosmariquinone[b]	13.4	6.4	
cryptotanshinone[b]	7.4	3.5	2.6
BHA[b]	12.2	5.8	3.7
BHT[b]	6.3	3.0	2.2

[a] P_F calculated from data of Zhang et al. (1990). [b] Induction periods determined using lard 2.

The antioxidant character of the isolated quinones is clearly associated with aromatic *o*-quinones. Thus, 9,10-phenanthrenedione has a similar antioxidant character with a P_F value of 2.95 when tested as an antioxidant in lard at 0.02% concentration (Table II).

Table III. Spectral Data for Product from Reaction of Methyl Oleate and 9,10-Phenanthrenedione

mass (m/z)[a]	UV (A) in hexane	IR in CCl_4, cm^{-1}	NMR in $[^2H]CCl_3$, ppm
506 (7.2), 505 (35.9), 504 (100), 473 (2.1), 211 (2.6), 210 (15.8), 209 (7.8), 207 (2.2), 181 (7.1), 180 (16.5), 165 (4.0), 152 (4.3), 55 (5.1)	372 (0.070), 353 (0.062), 314 (0.391), 300 (0.325), 275 (0.485), 257 (1.581), 250 (1.3), 226 (0.7)	3182 (w), 2960 (s), 2932 (s), 2860 (s), 1742 (s), 1632 (m), 1500 (m), 1486 (m)	8.6 (d, 2 H), 8.15 (d, 2 H), 7.69 (m, 1 H), 7.59 (t, 2 H), 7.52 (m, 3 H), 4.37 (d, 2 H), 4.21 (t, 1 H), 3.65 (d, 3 H), 2.27 (d, 2 H), 1.78 (m, 3 H), 1.6 (m, 7 H), 1.25 (m, 22 H), 0.9 (m, 8 H)

[a] peaks with m/z below 100 are given where the intensity is greater than 5% of the base peak.

The following effects of variations in quinone structure on antioxidant activity can be deduced:

(1) Compounds with an aromatic A ring have strong antioxidant properties, whereas compounds lacking an aromatic A ring are less effective as antioxidants. Thus, dihydrotanshinone I is more effective than cryptotanshinone and miltione 1 is more effective than dehydrorosmariquinone.

(2) If the A ring is nonaromatic, an additional conjugated double bond in the A ring increases the antioxidant properties. Hence, dehydrorosmariquinone is more effective than tanshinone II_A.

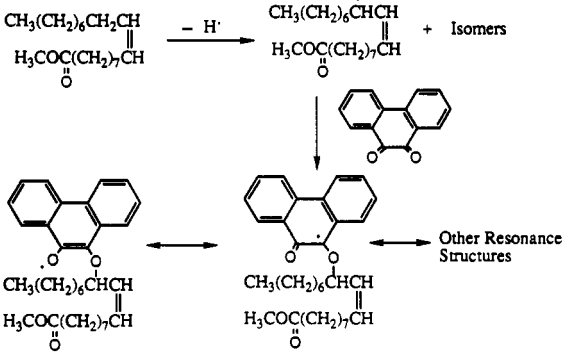
(3) A dihydrofuran ring increases stability compared with a furan ring. Hence, dihydrotanshinone I is a more effective antioxidant than tanshinone I. However, a dihydrofuran ring reduces activity compared with an isopropyl group since rosmariquinone is more active than cryptotanshinone.

Iron (2.2 ppm) is very effective in reducing the antioxidant effect of rosmariquinone with a reduction in induction period from 13.4 to 3.9 h, just as it does for α-tocopherol, where it reduces the induction period from 10.0 to 5.6 h. Hence, it is clear that the action of the quinone is not due to metal chelation. The sequence of activity of the 3,4-phenanthrenedione derivatives isolated from tanshen is consistent with the sequence of stability of a radical derived from the quinones. It therefore appears that the antioxidant activity of o-phenanthrenedione derivatives is a primary antioxidant effect, with a stabilized radical being formed from the quinone.

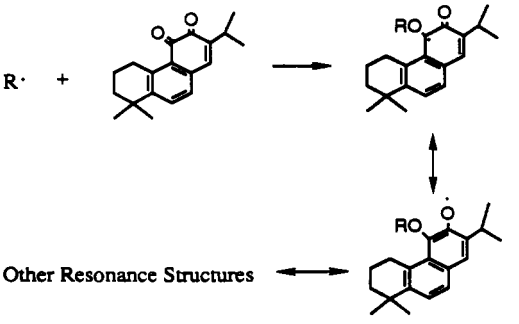
To confirm this hypothesis and gain information about the identity of the radical, a sample of 9,10-phenanthrenedione was heated with methyl oleate at 50 °C. The mass spectrum of a product isolated from the reaction indicated a peak at m/z 504, which corresponds to the addition of the mass of methyl oleate (296) to 9,10-phenanthrenedione (mass 208). The relative intensities of the molecular ion, M + 1, and M + 2 peaks were 100:35.9:7.2, which are similar to the theoretical ratios of 100:37.0:7.4 for a compound with the molecular formula $C_{33}H_{44}O_4$. The product was clearly not a single pure compound as shown by the complex NMR spectrum (Table III). The IR spectrum indicates that the quinone structure has been lost since the characteristic IR absorbances of 9,10-phenanthrenedione are absent, and the remaining C=O band at 1742 cm^{-1} is at a similar wavenumber to that of methyl oleate (1745 cm^{-1}). The product does not appear to have a free phenolic hydroxyl group since treatment with sodium hydroxide in methanol does not give the shift in the UV spectrum exhibited by phenols.

It is known that radicals add to quinones to form stabilized radicals (Roberts and Caserio, 1965), allowing some quinones to be inhibitors in free radical chain reactions. Consideration of the likely reaction path indicates that a radical derived from methyl oleate adds to a quinonoid oxygen atom to yield a radical which is stabilized by extensive conjugation (Scheme I). Since methyl oleate can yield radicals at C-8, C-9, C-10, and C-11 as shown by the formation of four hydroperoxides in other oxidation studies (Frankel et al., 1977), it would be expected that the product isolated is a mixture of four products. Compounds in which the two carbonyl oxygen atoms are not equivalent would be expected to give a mixture of eight radicals by addition of methyl oleate. The contribution of the resonance canonical in which the initial carbon radical becomes an aromatic oxygen radical, as shown in Scheme I, is clearly an important factor in the stability of the radical. In the case of rosmariquinone and similar quinones, the oxygen radical is stabilized not only by its aromatic character but also by the presence of two bulky groups in the ortho positions (Scheme II).

Scheme I. Formation of Addition Products from Methyl Oleate and 9,10-Phenanthrenedione



Scheme II. Antioxidant Activity of Rosmariquinone



It is likely that other products were formed in the reaction including products formed by the addition of alkoxy or peroxy radicals to the quinone, but these were not isolated. An aromatic A ring or any additional conjugated double bonds would increase the stability of the radical as found in the present study.

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