Synthesis and Antioxidant Activity of Rosmariquinone and Several Analogues

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Rosmariquinone (**1**) and six analogues were chemically synthesized using an ultrasound-promoted Diels−Alder cycloaddition in yields of 35−90%. The analogues included substitution of the isopropyl at carbon 13 (C-13) with a hydrogen (**5**), methyl (**6**), or *tert*-butyl (**4**) substituent. The hydrogen-substituted analogue had the lowest yield at 35%, due in part to the instability of the compound to air, while the highest yields were achieved for the methyl (85%) and *tert*-butyl (90%) analogues. The 60% yield obtained for the C-14 methyl analogue (**7**; no C-13 isopropyl) may have been caused by the *meta*-substituted catechol inhibiting the cycloaddition. The final two analogues were ring A modifications and included the removal of one C-4 methyl (**3**; 80% yield) or both C-4 methyl (**2**; 85% yield) groups. The analogues were tested against rosmariquinone in light-sensitized oxidation of stripped soybean oil. Analogues **5** and **6** were significantly ($P < 0.05$) better antioxidants than rosmariquinone and all other analogues. The antioxidant properties of compounds **2**−**7** were not significantly different ($P < 0.05$) from each other while compounds **2** and **4** had significantly ($P < 0.05$) lower antioxidant activity than rosmariquinone. This study demonstrated the importance of structural characteristics of antioxidants and that natural antioxidants, such as rosmariquinone, can be improved through chemical modification.

Keywords: *Antioxidant activity; Diels−Alder cycloaddition; rosmariquinone; ultrasound*

INTRODUCTION

Many natural product syntheses have been directed toward the production of medicinal compounds, and little attention has been given to the production of compounds for fields other than medicine. The production of natural food antioxidants through synthetic methods increases yields and reduces their costs of supply to the food industry. For example, the synthetic antioxidant butylated hydroxytoluene (BHT) costs $1.80/lb while the natural counterpart, tocopherols, cost $14.64/lb (Haumann, 1991), thus demonstrating the substantial cost savings to the users of synthetic antioxidants. A second advantage of synthetic organic chemistry is in the production of analogues of a naturally occurring compound. Miller and Quackenbush (1957a,b) and Dugan et al. (1951) identified structural characteristics of phenolic antioxidants and found that simple variation in side groups (e.g., *tert*-butyl versus methyl) improved the activity of an antioxidant. The same idea can be applied to a naturally occurring compound where the substitution of functional groups at different regions of the molecule may bring about increased activity of the compound. This type of improvement would be difficult if not impossible to control in cell cultures and/or in the plant system. Antioxidants isolated from rosemary (*Rosmarinus officinalis* L.) have been given a great deal of attention because of their strong antioxidant activity, but only carnosol, carnosic acid, and rosmariquinone (RQ) have been produced synthetically.

Several synthetic methods were developed for the total synthesis of carnosol, carnosic acid, and their dimethyl ethers (Meyer and Schindler, 1966; Meyer et al., 1968; Shew and Meyer, 1968). The synthetic routes to carnosol and carnosic acid have been given little attention over the last 25−30 years due to low synthetic yields and the large quantities (2.7−26%) of these antioxidants that can be found in rosemary and sage extracts (Cuvelier et al., 1994). Rosmariquinone (Houlihan et al., 1985) or miltirone (Hayashi et al., 1970) isolated from rosemary and Chinese sage, respectively, was found to be a minor component of the plant materials. Hall et al. (1994) isolated between 16 and 230 ppm (0.0016−0.023%) RQ from a commercial rosemary oleoresin which represents a concentration that is 100 times lower than the amount of carnosol or carnosic acid in the commercial rosemary oleoresin. For this reason, little attention has been given to the separation of RQ from the commercial rosemary oleoresin or as an antioxidant. Unlike carnosol or carnosic acid, RQ can be synthetically produced in yields as high as 60% (total synthesis yield) and large quantities can be easily produced. Several synthetic methods have been developed over the last 20 years following various synthetic routes.

There are three general approaches for the synthesis of RQ. The first synthetic method (Nasipuri and Mitra, 1973; Chang et al., 1990, 1991; He et al., 1990) follows the approach in which ring C was constructed first

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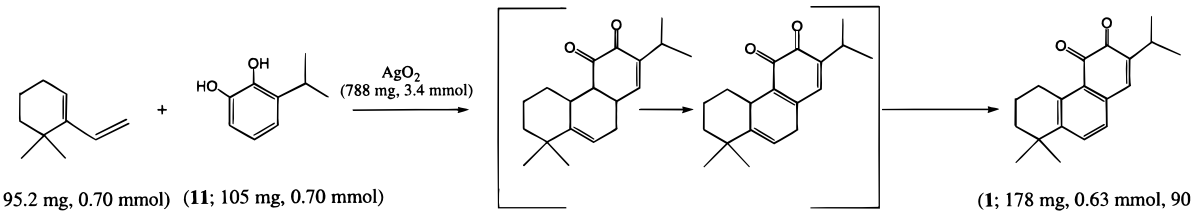


Figure 1. Reaction sequence of the ultrasound-promoted Diels−Alder cycloaddition as postulated by Lee et al. (1990) for the synthesis of miltirone (i.e., rosmariquinone; **1**).

followed by ring B and finally ring A. The resulting overall yields of 3−3.5% limits the cost-effectiveness of this synthetic approach. The second method follows a semisynthetic route using carnosol as the starting material (Luis et al., 1996). This method resulted in a yield of 42% and, provided carnosol is readily available, represents a more efficient method to obtain RQ compared to the earlier synthetic methods. The third and most productive method involves the Diels−Alder cycloaddition reaction between 3-isopropyl-*O*-benzoquinone and 6,6-dimethyl-1-vinylcyclohexene (Knapp and Sharma, 1985; Lee et al., 1990). The Diels−Alder cycloaddition method of Lee et al. (1990) resulted in significant improvement in the overall yield of RQ (i.e., miltirone) when compared to the method of Knapp and Sharma (1985). Lee et al. (1990) reported a cycloaddition yield of 93% (60% overall yield), while Knapp and Sharma (1985) were only able to achieve a 30% (20% overall yield). The significant improvement in the Diels−Alder approach between Lee et al. (1990) and Knapp and Sharma (1985) was the elimination of an external oxidation step prior to the cycloaddition step. A second advantage to the ultrasound-promoted cycloaddition was that less catechol (i.e., *o*-benzoquinone), the more expensive reactant, was needed to produce higher yields. A variety of abietanoid *o*-quinones have been produced using ultrasound-promoted cycloadditions (Lee and Snyder, 1990). Because these authors have demonstrated the application of ultrasound for the production of a wide variety of *o*-quinones, the use of ultrasound represents a potential synthetic method for the production of RQ analogues.

The antioxidant activity of RQ in various lipid systems has been demonstrated (Houlihan et al., 1985; Weng and Gordon, 1992; Hall et al. 1994) while analogues of RQ (excluding tanshinones) have not been tested. Due to the limited research on the structural characterization of RQ, the objectives of this study were to synthesize several RQ analogues, including the appropriate starting materials, and to compare the antioxidant activity of the RQ analogues to that of RQ. The general approach will be through the use of ultrasound-promoted Diels−Alder reaction. To determine the importance of the isopropyl group (*ortho* position), substitution will be made using *tert*-butyl, methyl, or hydrogen in place of isopropyl (Figure 1, Table 1). To determine the importance an *ortho* substituent, an analogue bearing a *meta* methyl in place of the *ortho* isopropyl will be synthesized. Last, the significance of ring A dimethyl substitution will be investigated with analogues bearing no methyl or single methyl on carbon 4 (Table 1).

EXPERIMENTAL PROCEDURES

General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 MHz General Electric NMR instrument (300 MHz for ^{1}H, 75 MHz for ^{13}C). Chemical shift data are reported as parts per million (δ) and referenced to the internal residual peak of deuteriochloroform (7.25 ppm) for ^{1}H NMR and to the center peak of the triplet (77.0 ppm) for ^{13}C NMR. ^{1}H shifts and couplings were assigned using ^{1}H−^{1}H chemical shift correlated spectroscopy (i.e., COSY) while ^{13}C−^{1}H heteronuclear chemical shift correlated spectroscopy (i.e., HETCOR) was used to define the ^{13}C resonances. Infrared (IR) absorption spectra were recorded on an Analect model RFX-30 FT-IR spectrophotometer using a potassium bromide cell. Ultraviolet data were collected using a Milton Roy Genesys 5 UV−vis spectrophotometer. Low-resolution mass spectra were obtained using a Hewlett-Packard 5790 GC-MS system and a Chiral-val column (Alltech, State College, PA) using both chemical (methane) and electron impact ionization. Uncorrected melting point determinations were completed using open Pyrex capillary tubes and a Mel-Temp apparatus. All flash column chromatography was completed on E. Merck silica gel 60 and thin-layer chromatography on commercial silica gel plates (Analtech Silica HLF 250 m; Fisher Scientific, St. Louis, MO). Solvents for chromatography and for reactions were purchased from Fisher, and other chemical reactants were purchased from Aldrich Chemical Co. (Milwaukee, WI). When required, the solvents were dried over an appropriate drying agent under nitrogen atmosphere and used immediately. Reactions involving moisture-sensitive reagents were performed under dry nitrogen in flame-dried flasks. Evaporation of solvents was performed first on a Büchi rotary evaporator and then at 0.10 mmHg.

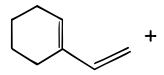
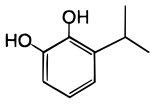
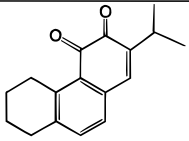
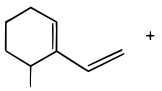
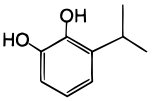
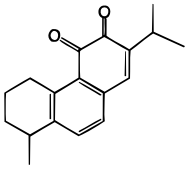
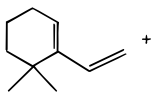
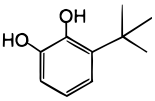
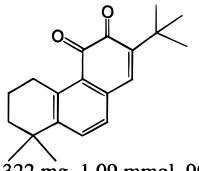
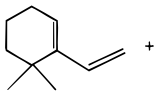
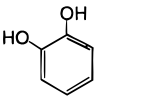
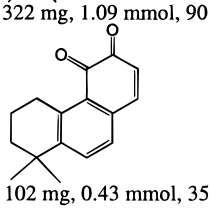
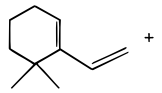
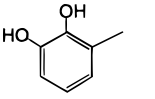
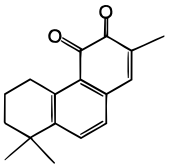
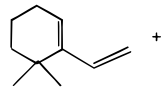
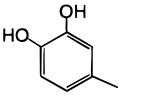
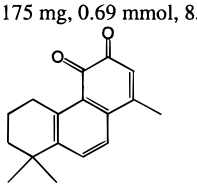
Synthetic Procedures. The production of the vinylcyclohexene compounds (**8**−**10**; Table 1) was completed using the method of Tanis and Abdallah (1986). The 1-vinylcyclohexene (**8**) and 6-methyl-1-vinylcyclohex-1,2-ene (**9**) were synthesized from the available cyclohexanone and 1-methylcyclohexanone, respectively. However, the reactant 6,6-dimethyl-1-vinylcyclohexene (**10**) synthesis required that 2,2-dimethylcyclohexanone be synthesized using the method of Boatman et al. (1965) prior to Grignard addition and subsequent dehydration.

3-Isopropyl catechol (**11**) was synthesized following the methods of Edwards and Cashaw (1955) and Tsuruta and Mukai (1968) while 3-*tert*-butyl catechol (**12**) was produced using the method of Casiraghi et al. (1980). All other catechols (**13**−**15**; Table 1) were purchased from Aldrich.

The Diels−Alder cycloaddition was completed using the ultrasound method of Lee et al. (1990) using the appropriate catechol and vinylcyclohexene (Table 1). A general synthetic method for RQ is listed below, and all other analogues were prepared and purified in the same manner following the conditions in Table 1. Physical data are listed in Table 2.

1,2,3,4-Tetrahydro-4,4-dimethyl-13-(1-methylethyl)-11,-12-phenanthrenedione (RQ, 1; Figure 1). A mixture of 3-isopropylcatechol (**11**; 105 mg, 0.69 mmol), 6,6-dimethyl-1-vinylcyclohexene (**10**, 95.2 mg, 0.69 mmol), and silver oxide (788 mg, 3.40 mmol) in ethanol (1.5 mL) was subjected to ultrasonication for 3 h at 24 °C. The mixture was filtered, and the solvent was removed in vacuo. The *o*-quinone was separated from the crude material using flash chromatography on silica gel (hexane, 100%; hexane:ethyl acetate 90:10 v/v) to give a mixture of regioisomers. The final purification was done using flash chromatography on silica gel eluting with hexane: ethyl acetate (100:0; 98:2; 95:5; 90:10). The solvent was removed in vacuo to afford a red oil. The oil was recrystallized from hexane to give RQ as red crystals (178 mg, 0.63 mmol, 90%): mp 100−101 °C; IR (KBr) 2959, 2932, 2870, 1660, 1630,

Table 1. Diels–Alder Cycloaddition Conditions for the Production of RQ Derivatives

vinylcyclohexene	catechol[a]	reaction conditions[b]	product
 (**8**; 150 mg, 1.39 mmol)	 (**11**; 211 mg, 1.39 mmol)	Ag_2O (1,284 mg, 5.56 mmol)	 (**2**; 300 mg, 1.18 mmol, 85 %)
 (**9**; 200 mg, 1.64 mmol)	 (**11**; 249 mg, 1.64 mmol)	Ag_2O (1,515 mg, 6.56 mmol)	 (**3**; 351 mg, 1.31 mmol, 80 %)
 (**10**; 164 mg, 1.21 mmol)	 (**12**; 218 mg, 1.21 mmol)	Ag_2O (1,118 mg, 4.84 mmol)	 (**4**; 322 mg, 1.09 mmol, 90 %)
 (**10**; 164 mg, 1.21 mmol)	 (**13**; 133 mg, 1.21 mmol)[c]	Ag_2O (1,118 mg, 4.84 mmol)	 (**5**; 102 mg, 0.43 mmol, 35 %)
 (**10**; 110 mg, 0.81 mmol)	 (**14**; 100 mg, 0.81mmol)[c]	Ag_2O (843 mg, 3.65 mmol)	 (**6**; 175 mg, 0.69 mmol, 85 %)
 (**10**; 110 mg, 0.81 mmol)	 (**15**; 100 mg, 0.81mmol)[c]	Ag_2O (843 mg, 3.65 mmol)	 (**7**; 123 mg, 0.49 mmol, 60 %)

[a] Catechol is converted to *o*-quinone prior to cycloaddition. [b] All reactions run under ultrasound and anhydrous ethanol. [c] Catechol purchased from Aldrich.

1581, 1563, 1460, 1387, 1294, 1258, 1227, 1141, 934 cm^{-1}; ^1H NMR δ 7.56 (d, J = 7.97 Hz, 1H), 7.09 (d, J = 7.97 Hz, 1 H), 7.06 (s, 1 H), 3.14 (t, J = 6.38 Hz, 2 H), 2.99 (sept, J = 6.82, 1 H), 1.76 (m, 2 H), 1.62 (m, 2 H), 1.28 (s, 6 H), 1.14 (d, J = 6.89, 6 H); ^{13}C NMR δ 182.3, 181.4, 149.6, 145.0, 144.8, 144.3, 139.8, 134.2, 133.7, 128.0, 127.8, 37.6, 34.3, 31.5 (2 C), 29.8, 26.7, 21.3 (2 C), 18.9; LRMS (EI, 70 eV) m/z 254 ($M^+ - 28$); (CI, methane) m/z 283 (M + 1); UV–vis (methanol) λ_{max} (ϵ) 209 (14 222), 257 (17 730), 359 (1803), 437 (2184).

1,2,3,4-Tetrahydro-13-(1-methylethyl)-11,12-phenanthrenedione or Normiltirone (2; Table 1). Isolated as red crystals (300 mg, 1.181 mmol, 85%): mp 101–102 °C; IR (KBr) 2931, 2869, 1652, 1563, 1458, 1418, 1382, 1255, 917 cm^{-1}; ^1H NMR δ 7.30 (d, J = 7.5 Hz, 1H), 7.09 (s, 1 H), 7.06 (d, J = 7.5 Hz, 1 H), 3.20 (t, J = 6.4 Hz, 2 H), 3.04 (sept, J = 6.8 Hz, 1 H), 2.80 (t, J = 6.4 Hz, 2 H), 1.80 (m, 4 H), 1.18 (d, J = 6.9 Hz, 6 H); ^{13}C NMR δ 182.2, 181.3, 145.0, 144.7, 140.7, 139.9, 135.9, 134.6, 128.1, 127.5, 30.5, 28.7, 26.9, 22.8, 21.9, 21.4 (2 C); LRMS (EI, 70 eV) m/z 253 (M − 1); (CI, methane) m/z 255 (M + 1); UV–vis (methanol) λ_{max} (ϵ) 209 (10 630), 257 (13 487), 359 (1194), 437 (1695).

1,2,3,4-Tetrahydro-4-methyl-13-(1-methylethyl)-11,12-phenanthrenedione (3; Table 1). Isolated as red crystals (351 mg, 1.31 mmol, 80%): mp 93–94 °C; IR (KBr) 2960, 2934, 2872, 1661, 1574, 1464, 1388, 1254, 1110, 1110, 940 cm^{-1}; ^1H NMR δ 7.41 (d, J = 7.5 Hz, 1H), 7.07 (d, J = 7.5 Hz, 1 H), 7.06 (s, 1 H), 3.21 (t, J = 5.7 Hz, 1 H), 3.01–3.10 (m, 2 H), 2.95 (sept, J = 5.7 Hz, 1 H), 1.79–1.84 (m, 2 H), 1.73 (m, 1 H), 1.53 (m, 1 H), 1.27 (d, J = 7.5 Hz, 3 H), 1.15 (d, J = 7.5 Hz, 6 H); ^{13}C NMR δ 181.9, 181.0, 145.9, 144.9, 144.8, 139.9, 135.2, 134.5, 128.0, 127.7, 32.9, 29.5, 28.8, 26.6, 22.6, 21.2 (2 C), 19.2; LRMS (EI, 70 eV) m/z 267 (M-1); (CI, methane) m/z 269 (M + 1); UV–vis (methanol) λ_{max} (ϵ) 209 (13 475), 260 (13 741), 359 (1492), 437 (2134).

1,2,3,4-Tetrahydro-4,4-dimethyl-13-(1,1-dimethylethyl)-11,12-phenanthrenedione (4; Table 1). Isolated as red-orange crystals (322 mg, 1.09 mmol, 90%): mp 124–126 °C; IR (KBr) 2958, 2869, 1661, 1582, 1562, 1460, 1366, 1221, 1142, 911 cm^{-1}; ^1H NMR δ 7.58 (d, J = 7.5 Hz, 1H), 7.14 (s, 1 H), 7.10 (d, J = 7.5 Hz, 1 H), 3.15 (t, J = 6.6 Hz, 2 H), 1.76 (m, 2 H), 1.63 (m, 2 H), 1.28 (s, 15 H); ^{13}C NMR δ 182.7, 181.8, 149.4, 146.5, 144.0, 140.4, 134.4, 133.6, 128.2, 37.8, 34.6, 34.3, 31.6

Table 2. Physical Data for Rosmariquinone Analogues

analogue	mp (°C)	^{1}H NMR (δ)	^{13}C NMR (δ)	IR (KBr) (cm^{-1})	UV−vis (MeOH) (λ_{max} (ϵ))	LRMS(m/z) CI (CH_4)	LRMS(m/z) EI (70 eV)
2	101−102	7.30 (d, J = 7.5 Hz, 1H), 7.09 (s, 1 H), 7.06 (d, J = 7.5 Hz, 1 H), 3.20 (t, J = 6.4 Hz, 2 H), 3.04 (sept, J = 6.8 Hz, 1 H), 2.80 (t, J = 6.4 Hz, 2 H), 1.80 (m, 4 H), 1.18 (d, J = 6.9 Hz, 6 H)	182.2, 181.3, 145.0, 144.7, 140.7, 139.9, 135.9, 134.6, 128.1, 127.5, 30.5, 28.7, 26.9, 22.8, 21.9, 21.4 (2 C)	2931, 2869, 1652, 1563, 1458, 1418, 1382, 1255, 917	257 (13 487), 359 (1194), 437 (1695)	255	253
3	93−94	7.41 (d, J = 7.5 Hz, 1H), 7.07 (d, J = 7.5 Hz, 1 H), 7.06 (s, 1 H), 3.21 (tt, J = 5.7 Hz, 1 H), 3.01−3.10 (m, 2 H), 2.95 (sept, J = 5.7 Hz, 1 H), 1.79−1.84 (m, 2 H), 1.73 (m, 1 H), 1.53 (m, 1 H), 1.27 (d, J = 7.5 Hz, 3 H), 1.15 (d, J = 7.5 Hz, 6 H)	181.9, 181.0, 145.9, 144.9, 144.8, 139.9, 135.2, 134.5, 128.0, 127.7, 32.9, 29.5, 28.8, 26.6, 22.6, 21.2 (2 C), 19.2	2960, 2934, 2872, 1661, 1574, 1464, 1388, 1254, 1110, 1110, 940	260 (13 741), 359 (1492), 437 (2134)	269	267
4	124−126	7.58 (d, J = 7.5 Hz, 1H), 7.14 (s, 1 H), 7.10 (d, J = 7.5 Hz, 1 H), 3.15 (t, J = 6.6 Hz, 2 H), 1.76 (m, 2 H), 1.63 (m, 2 H), 1.28 (s, 15 H)	182.7, 181.8, 149.4, 146.5, 144.0, 140.4, 134.4, 133.6, 128.2, 37.8, 34.6, 34.3, 31.6 (2 C), 29.6, 29.0 (3 C), 22.5, 18.9	2958, 2869, 1661, 1582, 1562, 1460, 1366, 1221, 1142, 911	251 (11 646), 356 (1616), 428 (2346)	297	294
5	90−92	7.61 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 8.7 Hz, 1 H), 7.15 (d, J = 8.7 Hz, 1 H), 6.35 (d, J = 8.7 Hz, 1 H), 3.17 (t, J = 6.0 Hz, 2 H), 1.78 (m, 2 H), 1.63 (m, 2 H), 1.28 (s, 6 H)	181.5, 181.4, 151.0, 146.9, 145.0, 133.9, 133.7, 129.3, 128.4, 126.4, 37.6, 31.6 (2 C), 31.3, 29.9, 18.9	2957, 2933, 2865, 1661, 1558, 1457, 1246, 1138, 852	251 (13 172), 356 (1201), 419 (1717)	241	240
6	106−107	7.57 (d, J = 7.5 Hz, 1H), 7.12 (s, 1 H), 7.05 (d, J = 7.5 Hz, 1 H), 3.16 (t, J = 6.6 Hz, 2 H), 2.0 (s, 3 H), 1.76−1.80 (m, 2 H), 1.61−1.65 (m, 2 H), 1.28 (s, 6 H)	181.9, 181.6, 149.5, 144.3, 142.9, 134.8, 134.2, 133.7, 128.2, 127.5, 37.5, 34.3, 31.5 (2 C), 29.7, 18.8, 14.9	2961, 2929, 2861, 1692, 1570, 1462, 1264, 1143	257 (12 649), 359 (1444), 434 (1927)	255	253
7	119−120	7.60 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1 H), 6.23 (s, 1 H), 3.09 (t, J = 6.6 Hz, 2 H), 2.29 (s, 3 H), 1.69 (m, 2 H), 1.57 (m, 2 H), 1.24 (s, 6 H)	182.8, 181.3, 154.7, 150.8, 144.3, 134.4, 133.4, 129.2, 126.3, 124.6, 37.6, 34.4, 31.7 (2 C), 30.1, 21.0, 19.1	2946, 2866, 1654, 1619, 1566, 1446, 1377, 1285, 1248, 859	254 (12 453), 356 (1201), 413 (1422)	255	253

(2 C), 29.6, 29.0 (3 C), 22.5, 18.9; LRMS (EI, 70 eV) *m/z* 294 (M − 2); (CI, methane) *m/z* 297 (M + 1); UV−vis (methanol) λ_{max} (ϵ) 218 (11 058), 251 (11 646), 356 (1616), 428 (2346).

1,2,3,4-Tetrahydro-4,4-dimethyl-11,12-phenanthrenedione (5; Table 1). Isolated as red-brown crystals (102 mg, 0.42 mmol, 35%): mp 90−92 °C; IR (KBr) 2957, 2933, 2865, 1661, 1558, 1457, 1246, 1138, 852 cm^{-1}; ^1H NMR δ 7.61 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 8.7 Hz, 1 H), 7.15 (d, J = 8.7 Hz, 1 H), 6.35 (d, J = 8.7 Hz, 1 H), 3.17 (t, J = 6.0 Hz, 2 H), 1.78 (m, 2 H), 1.63 (m, 2 H), 1.28 (s, 6 H); ^{13}C NMR δ 181.5, 181.4, 151.0, 146.9, 145.0, 133.9, 133.7, 129.3, 128.4, 126.4, 37.6, 31.6 (2 C), 31.3, 29.9, 18.9; LRMS (EI, 70 eV) *m/z* 240 (M^+); (CI, methane) *m/z* 241 (M + 1); UV−vis (methanol) λ_{max} (ϵ) 209 (10 703),251 (13 172), 356 (1201), 419 (1717).

1,2,3,4-Tetrahydro-4,4-dimethyl-13-methyl-11,12-phenanthrenedione (6; Table 1). Isolated as red-orange crystals (175 mg, 0.69 mmol, 85%): mp 106−107 °C; IR (KBr) 2961, 2929, 2861, 1692, 1570, 1462, 1264, 1143 cm^{-1}; ^1H NMR δ 7.57 (d, J = 7.5 Hz, 1H), 7.12 (s, 1 H), 7.05 (d, J = 7.5 Hz, 1 H), 3.16 (t, J = 6.6 Hz, 2 H), 2.0 (s, 3 H), 1.76−1.80 (m, 2 H), 1.61−1.65 (m, 2 H), 1.28 (s, 6 H); ^{13}C NMR δ 181.9, 181.6, 149.5, 144.3, 142.9, 134.8, 134.2, 133.7, 128.2, 127.5, 37.5, 34.3, 31.5 (2 C), 29.7, 18.8, 14.9; LRMS (EI, 70 eV) *m/z* 253 (M^+ − 1); (CI, methane) *m/z* 255 (M + 1); UV−vis (methanol) λ_{max} (ϵ) 209 (11 299), 257 (12 649), 359 (1444), 434 (1927).

1,2,3,4-Tetrahydro-4,4-dimethyl-14-methyl-11,12-phenanthrenedione (7; Table 1). Isolated as red-orange crystals (123 mg, 0.49 mmol, 60%): mp 119−120 °C; IR (KBr) 2946, 2866, 1654, 1619, 1566, 1446, 1377, 1285, 1248, 859 cm^{-1}; ^1H NMR δ 7.60 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1 H), 6.23 (s, 1 H), 3.09 (t, J = 6.6 Hz, 2 H), 2.29 (s, 3 H), 1.69 (m, 2 H), 1.57 (m, 2 H), 1.24 (s, 6 H); ^{13}C NMR δ 182.8, 181.3, 154.7, 150.8, 144.3, 134.4, 133.4, 129.2, 126.3, 124.6, 37.6, 34.4, 31.7 (2 C), 30.1, 21.0, 19.1; LRMS (EI, 70 eV) *m/z* 253 (M^+ − 1); (CI, methane) *m/z* 255 (M + 1); UV−vis (methanol) λ_{max} (ϵ) 209 (10 987), 254 (12 453), 356 (1201), 413 (1422).

Oxidation Evaluation. *Analysis of Soybean Oil Components.* Tocopherols (α, δ), β-carotene, and chlorophyll standards were obtained from Sigma Chemical Co. (St. Louis, MO). γ-Tocopherol was obtained from Eastman Chemical Products, Inc. (Kingsport, TN). The tocopherols were determined by the high-performance liquid chromatography method of Carpenter (1979). Chlorophyll and carotenoids were spectrophotometrically analyzed by modified Association of Official Analytical Chemists (AOAC) methods (1984) (Hall and Cuppett, 1993).

Antioxidants. *tert*-Butylhydroquinone (TBHQ) was obtained from Eastman. RQ and analogues were synthesized as discussed above (see the synthetic procedures section).

Stripping of Soybean Oil. Commercial soybean oil (SBO) was purchased from a local supermarket and stored in the dark at −18 °C until needed. The oil was stripped using the modified method of Kiritisakis and Dugan (1985) which included a batch process rather than a column stripping (Hall, 1996). Stripping was conducted under a nitrogen atmosphere until tocopherol was no longer detected by HPLC (2−4 h). The bleaching material was filtered under a stream of nitrogen, and the solvent was then removed in vacuo from the stripped SBO at 30 °C.

Final purification was completed by re-suspending the SBO into solvent (2.0 times the SBO content) and passing it through a column containing the purification material [silicic acid (36.4%); absorptive magnesia (27.2%; magnesium oxide); Hyflo SuperCel (18.2%) and Florisil (18.2%) packed over Actisil (0.1 parts to the amount of PM)]. The SBO/solvent mixture was passed through the column under nitrogen and vacuum. Purification was monitored by spectrophotometry at 436 nm (β-carotene) and 452 nm (Lutein) and was re-purified if pigments were observed. Solvent was evaporated under vacuum at 30 °C and the stripped SBO was stored at −18 °C for no longer than 4 weeks.

Oxidation of Soybean Oils. Stripped soybean oil (100 g) was weighed into 110 mL glass jars. RQ, RQ analogues, and TBHQ were added to separate containers of stripped soybean oil at a concentration of 200 ppm (0.02%). Each sample was thoroughly mixed to ensure complete dispersion of the antioxidants. TBHQ served as the positive control while an untreated sample served as the negative control.

The jars were randomly placed under two, 15 W cool fluorescent lamps at a level sufficient to illuminate 4200 lux of fluorescent radiation at 25 ± 1 °C. Aluminum foil was placed in the open areas between the side of the jars and the bottom of the lamps to create uniform lighting. Duplicate peroxide values (PV) were determined every 24 h during the light exposure using the American Oil Chemists Society (AOCS; 1989 official method Cd-8-53) until a PV of 20 meq/kg was reached for each oil sample. When necessary, PV were taken after 12 h rather than 24 h.

Statistical Analysis. The entire oxidation evaluation was completed three times and the data were analyzed by analysis of variance (ANOVA) using Statistical Analysis System (SAS) software (1985). The least significant differences (Steel and Torrie, 1980) were used to determine a 95% confidence level ($P < 0.05$) between the mean number of hours required to reach a PV of 20 meq/kg for the treatments. The synthesis was completed three times, and the analytical data were reported as an average.

RESULTS AND DISCUSSION

Synthesis. To elucidate the structure of the RQ analogues, spectral data of the analogues were compared to RQ spectral data. The infrared spectrum of RQ exhibited an aromatic C−H stretching at 3059 cm^{-1} and, more importantly, a conjugated ketone system at 1659 cm^{-1}. The UV−vis scan indicated four primary absorption signals at 209, 257, 359, and 437 nm. The absorbance maximum at 257 nm indicated the presence of an aromatic ring system while 359 and 437 nm indicated further substitution and conjugation within the molecular structure. All infrared and UV−vis data were in agreement with those reported by Houlihan et al. (1985), Knapp and Sharma (1985), and Lee et al. (1990).

The ^1HNMR spectrum of RQ indicates several regions which correspond to functional groups. RQ has a doublet at δ 1.14 ppm which corresponds to the protons of the methyl groups of the isopropyl region (Figure 2A; protons at carbon 16 [C-16] and 17 [C-17]). The singlet at δ 1.28 ppm indicated the six protons of the two methyl groups of the aliphatic ring (Figure 2A; C-18 and C-19). The methine protons (δ 2.99 ppm) correspond to the protons at C-15, located in the isopropyl region, while the triplet at δ 3.15 ppm indicated aliphatic protons at C-1. Other aliphatic protons were found between δ 1.6 and 1.8 ppm. The final region of concern is that of the aromatic protons. RQ has three aromatic signals at δ 7.06, 7.09, and 7.56 ppm (Figure 2A). As a general rule, the proton at C-14 will give a singlet signal and those at C-6 and C-7 doublet signals. The proton at C-6 is influenced by the methyl protons (C-18 and C-19) of the aliphatic ring and shifts the signal upfield (δ 7.09 ppm) from the aromatic protons at C-7.

The ^{13}CNMR spectrum of RQ confirmed the presence of the aromatic, aliphatic, and alkyl carbons and supported the presence of the carbonyl functional groups at δ 181 and 182 ppm (Figure 2B; C-11 and C-12). The aromatic region (δ 125−160 ppm) represents all carbons containing double bonds. The most intense signals (C-6, C-7, and C-14) are attached to protons while the weak signals indicate quaternary carbons (Figure 2B; C-5, C-8, C-9, C-10, and C-13). C-9 and C-13 are influenced by the magnetic field of the oxygen atoms and, as a result, shift furthest downfield in comparison to the other aromatic carbons. Carbons C-5 and C-10 are influenced by the methyl protons and aliphatic protons, respectively, and shift upfield when compared to C-8

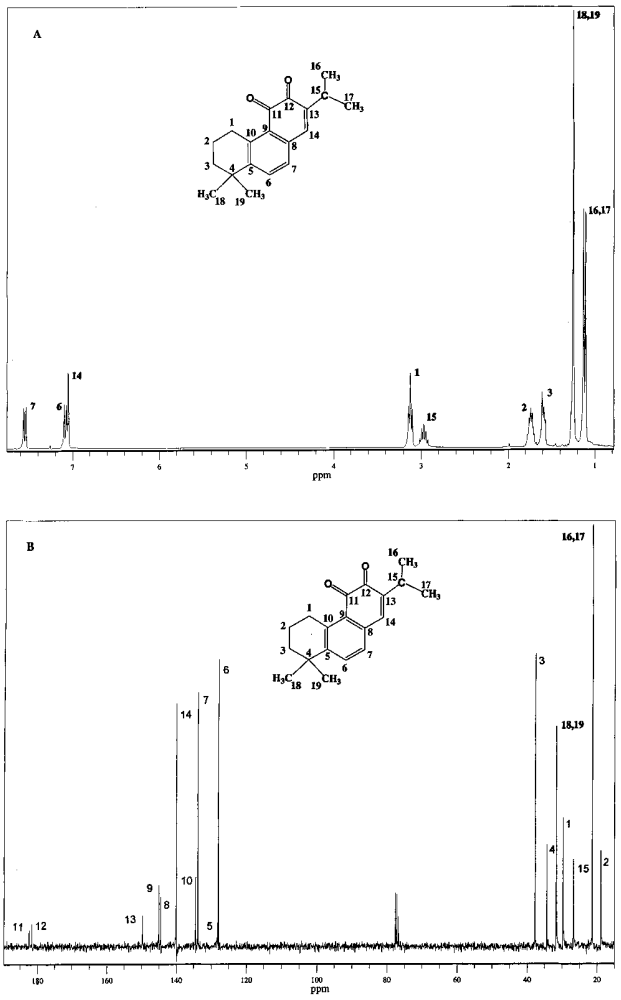


Figure 2. ^{1}HNMR (A) and ^{13}CNMR (B) spectra of rosmariquinone.

and C-9. The signal for C-5 is the weakest due to the strong shielding of the methyl protons. The alkyl and aliphatic region (δ 14−45 ppm) can be easily identified and correlated to the appropriate protons using the HETCOR technique. The methyl carbons can be found at δ 30 ppm (aliphatic ring) and δ 21 ppm (isopropyl group) and each corresponds to two carbons. The HETCOR also established the location of the aliphatic carbons (C-1, C-2, and C-3) at δ 29, 19, and 38 ppm, respectively. C-4 and C-15 give the weakest signals due to the neighboring methyl groups. The final analytical data includes mass spectrometry and is used primarily to support the proposed structure. In the case of RQ, electron impact ionization mass spectrometry resulted in a molecular ion of 254 *m/z* (M − 28) while the chemical ionization resulted in molecular ion peak of 283 *m/z* (M + 1).

In all analogues, the infrared and UV−vis data were similar to that of RQ, as would be expected. Ring A alterations included the removal of one C-4 methyl (**3**; Table 1) and both C-4 methyl groups (**2**; Table 1). The removal of one methyl group resulted in a change in the the δ 2.9−3.3 ppm region of the spectrum. The biggest change was the presence of the triplet signal at δ 3.2 and a quartet at 3.0 ppm and indicates the proton at C-4 position. The removal of both C-4 methyl groups resulted in the formation of a triplet signal at δ 2.9 ppm and indicated the presence of the C-4 protons. In the absence of C-4 methyl groups, the characteristic set of multiplets for C-2 and C-3 becomes one multiplet signal. The ^{13}CNMR spectrum of compounds **2** and **3** are similar to RQ and all spectral data for compounds **2** and **3** are in agreement with Lee et al. (1990).

The replacement of the C-13 isopropyl group with a tertiary butyl group (**4**, Table 1) results in the loss of the methine ^{1}H signal (δ 2.99 ppm) and doublet signal (δ 1.14 ppm). Because the methyl groups give the equivalent chemical shift, the singlet represents the methyl protons at C-16, C-17, C-18, C-19, and C-20. The carbon spectrum is similar to that of RQ with the exception that C-16, C-17, and C-18 are represented by a single peak at δ 29 ppm while the methyl carbons (C-16 and C-17) are found near δ 22 ppm.

The removal of ring C substituents (**5**; Table 1) results in a change in the aromatic region of the ^{1}HNMR spectrum. The protons at C-6 are strongly influenced by the C-16 protons and, as a result, shift upfield from δ 7.09 ppm in RQ to δ 6.35 ppm in compound **5**. The proton at C-13 and C-7 are shifted downfield due to the deshielding properties of the aromatic protons. The *o*-benzoquinones have similar downfield signals as with C-13 and C-14 and give support to the labeling of these protons. The intensity of the C-13 and C-14 signal in the ^{13}CNMR spectrum indicated the C−H bonding and can be supported by the HETCOR techniques.

When the isopropyl group at C-13 is simply replaced with a methyl group (**6**; Table 1), several noticeable changes in the ^{1}HNMR spectrum can be observed. The loss of the methine signal (septet, δ 2.99 ppm), normally found in the RQ spectrum, can be explained by the loss of the two methyl groups at C-15. Because of the loss of C-15 methyl groups, no hydrogens are present on neighboring carbons (C-16 and C-17 as in RQ for example; Figure 2a) to give a septet signal, and for this same reason, the C-15 methyl gives rise to a singlet signal. The chemical shift of the C-15 protons also moves upfield. The loss of the C-15 methyl groups also explains the disappearance of the doublet signal, found in RQ, at δ 1.28 ppm. The final difference in the proton spectrum is the downfield shift of the C-14 proton beyond that of the C-6 proton signal. The reason for this is that the C-15 methyl group has less of an influence on the magnetic field of the C-14 proton, in comparison to the isopropyl influence, thus allowing the C-14 proton to shift downfield. The ^{13}CNMR spectrum remains similar to RQ except that the signal for C-15 can be found at δ 15 ppm instead of δ 26.7 ppm as in RQ.

1,2,3,4-Tetrahydro-4,4-dimethyl-14-methyl-11,12-phenanthrenedione (**7**, Table 1) has a ^{1}HNMR spectrum similar to compound **6** (Table 1). The ^{1}HNMR spectrum for the C-14 methyl substituted compound has singlets at δ 2.29 and 6.23 ppm. The singlet at δ 2.29 ppm and the loss the methine signal (δ 2.99 ppm) support the presence of the methyl substitution based on the discussion above for the C-13 methyl substituted compound. The reduced shielding of the C-15 methyl protons by the C-12 oxygen atom results in a downfield shift of the C-15 proton's signal. The opposite holds true for the C-13 proton, where the shielding effects of C-15 and C-12 result in an upfield shift (δ 134 ppm) compared with the δ 144 ppm shift observed for C-14 of analogue **6**. All other carbons remain at similar chemical shifts.

The ultrasound-promoted Diels−Alder reaction resulted in yields between 35 and 90% for the RQ analogues. The poorest yield (35%) occurred in the nonsubstituted ring C compound 1,2,3,4-tetrahydro-4,4-

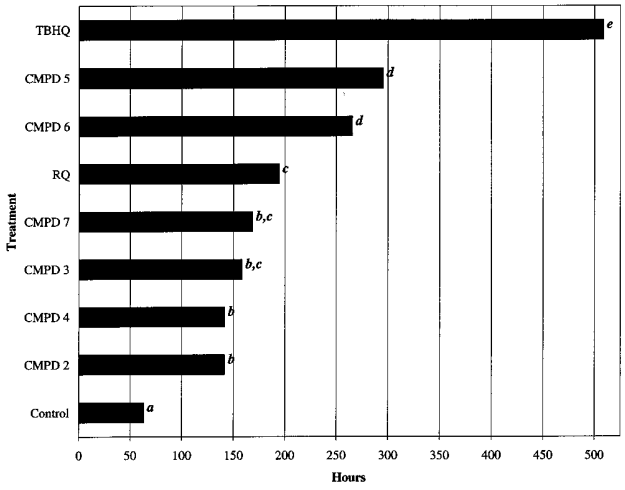


Figure 3. Number of hours RQ (**1**), RQ analogues (**2**−**7**), and TBHQ delayed the oxidation of stripped SBO to a level of 20 meq/kg. Different letters represent significant differences ($P < 0.05$) between treatments.

dimethyl-11,12-phenanthrenedione (**5**). There are two primary reasons for the low yield of this cycloaddition: (1) Several regioisomers may be produced during the cycloaddition due in part to the catechol which lacks *ortho* substitution and (2) Chang et al. (1990) reported the synthesis of compound **5** but found that the compound was unstable upon storage. The second lowest yield (60%) was found in the 14-methyl-substituted compound 1,2,3,4-tetrahydro-4,4-dimethyl-14-methyl-11,12-phenanthrenedione (**7**). The presence of the methyl at the *meta* position may have inhibited the cycloaddition. The C-13 methyl (**6**), C-13 *tert*-butyl (**4**), and C-13 isopropyl (**2** and **3**) analogues were formed in yields between 80 and 90%, indicating the importance of the C-13 (*ortho*) substitution in the orthoquinone. The starting diene appears to have little effect on the reduced yields of the cycloaddition products. From this synthetic study, the ultrasound-promoted Diels−Alder was a valuable method for the synthesis of structurally similar phenanthrenediones.

Antioxidant Comparison. The light-sensitized oxidation study involving RQ and analogues in stripped soybean oil provided some unexpected results (Figure 3). As expected, TBHQ had the highest ($P < 0.05$) antioxidant activity (AOA) and delayed oxidation to the 20 meq/Kg level for 500 h, while the nearest RQ derivative (**5**) was significantly ($P < 0.05$) lower at 294 h. Compounds **5** and **6** had the best AOAs of the analogues and were significantly ($P < 0.05$) better than the remaining analogues, including RQ. Although compounds **5** and **6** had comparable AOAs, the methyl substitution (**6**) was slightly greater (294 h versus 268 h) but not statistically different. Hall and Cuppett (1997) proposed that the antioxidant mechanism of RQ relies on the ability of RQ to convert to the active 11,-12-dihydroxy compound arucadiol which then participates in hydrogen donation to a free radical. The formation of this 11,12-dihydroxy structure would then be expected to occur in all RQ analogues and thus act as a phenolic antioxidant. The AOA of the hydrogen (**5**) or methyl (**6**) C-13-substituted analogues was surprisingly better ($P < 0.05$) than that of RQ. The *ortho* substitution of phenolic antioxidants with large or bulky side groups usually improved the AOA of a phenolic compound (Miller and Quackenbush, 1957a,b). Large *ortho* alkyl (e.g., isopropyl) substitutions enhance the electron density of the hydroxyl (OH) group, which in turn decreases the bond dissociation energy of the AO−H, resulting in an antioxidant that will readily donate a hydrogen to the lipid radical. Also, phenoxy radical can couple through the *para* position enhancing the stability of the radical; preventing the antioxidant radical from participating in free radical propagation reactions (Miller and Quackenbush, 1957a,b; Gordon, 1990). Since the hydrogen (**5**) and methyl (**6**) are less bulky than the C-13 isopropyl of RQ, the AOA was expected to be worse than that of RQ.

For analogue **4**, *tert*-butyl substitution at C-13 had an AOA that was significantly ($P < 0.05$) worse than that of RQ. This was an unexpected result since the bulkier C-13 *tert*-butyl was expected to give a better AOA for reasons mentioned above. The addition of a methyl at the C-14 position (**7**) resulted in an AOA that was not significantly different from RQ or analogue **3**. Both compounds **3** and **7** had slightly lower protective times of 157 and 168 h, respectively, when compared to RQ. These same analogues had nonsignificant AOA differences when compared to analogues **2** and **4**. All analogues were found to control the oxidation relative to the SBO control thus demonstrating the AO potential for the analogues.

SUMMARY AND CONCLUSIONS

The AOA of the RQ analogues was found to vary depending on the substitution pattern. The unexpected improvement in the AOA by C-13 methyl (**6**) and C-13 hydrogen (**5**) and the unexpected decrease in AOA by the C-13 *tert*-butyl substitution (**4**) suggest that the AOA is not completely dependent on the hydrogen donating activity observed in phenolic antioxidants. The observed AOA of the RQ analogues probably is due to the ability of the compounds to undergo a rearrangement to the 11,12-dihydroxy analogues (Hall and Cuppett, 1997) following a photooxidation mechanism similar to that of tanshinone IIa proposed by Kusumi et al. (1976). The bulky *tert*-butyl C-13 substitution may interfere with the peroxide polymer formation thus eliminating or reducing the concentration of the important 11,12-dihydroxy intermediates that form during the breakdown of the peroxide polymer. A more likely explanation may be that the C-13 *tert*-butyl substitution undergoes a radical coupling reaction with the C-12 oxygen radical intermediate (i.e., C-11,12 semiquinone) similar to the mechanism proposed by Kurechi and Kunugi (1983) for intermediates formed from the photooxidation of TBHQ. Under this mechanism, the C-12 hydroxyl group would not form resulting in a compound that had fewer hydrogen, which could be donated to the lipid radical, than the 11,12-dihydroxy intermediates, thus explaining the reduced AOA observed in analogue **4**. The opposite may be true for the C-13 methyl and hydrogen substitution in that the important 11,12-dihydroxy intermediate form quickly, thus creating the active analogues.

The difference between AOA of the C-13 methyl (**6**) and C-14 methyl (**7**) compounds may be due to the observations that *meta*-substituted phenols tend to be less active due to the inability to stabilize the phenoxy radicals (Miller and Quackenbush, 1957a,b). The significant reduction of AOA observed in analogue **2** is not completely understood at this time but may be due to the polymerization processes which reduces the concen-

tration of the active intermediates which form during the photoenolization as proposed by Kusumi et al. (1976).

On the basis of the results of this study, the RQ analogues exhibited various AOA in a photooxidation environment and thus demonstrate the importance of chemically modifying natural antioxidants to improve antioxidant capabilities.

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