8-Methylethoxyquin: A Possible Replacement for Ethoxyquin

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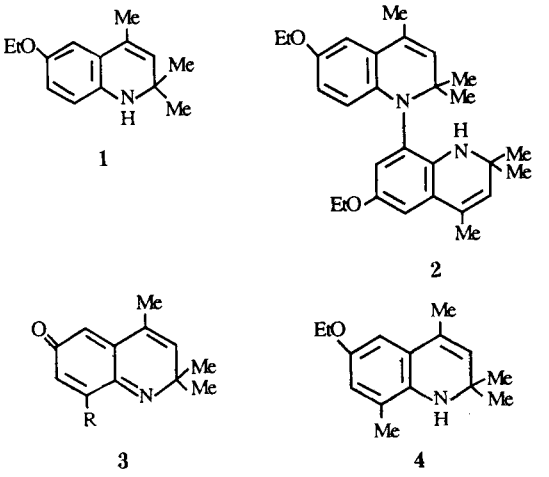
When acting as an antioxidant, ethoxyquin is converted mainly to a 1,8′-dimer and to a quinone-imine, with the latter itself acting as a powerful antioxidant. The 8-methyl derivative of ethoxyquin was prepared in the expectation that this would not form a dimer but more quinone-imine and, therefore, be a more powerful antioxidant. On oxidation of 8-methylethoxyquin, the dimer was absent and the quinone-imine was formed in high yield. Despite this, 8-methylethoxyquin was a less efficient antioxidant than ethoxyquin in fish oil and meal.

KEY WORDS: Ethoxyquin, ethoxyquin oxidation products, 1,2-dihydro-6-ethoxy-2,2,4,8-tetramethylethoxyquin, fish oil oxidation, fish meal oxidation, 8-methylethoxyquin, Warburg manometric equipment.

Ethoxyquin (1) is an effective antioxidant used primarily in fish meal and various feeds. Oxidation of lipids results in decreased nutritional and energy value. It can also cause heat production in fish meal, leading, in exceptional cases, to spontaneous combustion of stored material (1-3). Antioxidants also help to preserve carotene and vitamins A and E (4-6). Ethoxyquin has been used as a post-harvest dip for apples, to inhibit the development of brown spots (scald) and to protect pigments in spices (7-11).

We have already shown that when it acts as an antioxidant, ethoxyquin is converted to two major products—the 1,8′-dimer (2) and the quinone-imine (3, R=H) (12). The latter shows marked antioxidant activity, but the dimer has little or no antioxidant activity (13). Taimr *et al.* (14) found the same oxidation products of ethoxyquin when oxidized with silver oxide or lead dioxide. Other oxidation media also led to formation of the dimer, but not the quinone-imine. de Koning and van der Merwe (15) confirmed our findings of the two oxidation products of ethoxyquin in fish meal, although their results indicate some antioxidant properties of the dimer in fish oil, but at somewhat different conditions.

We now describe the preparation of the new compound 1,2-dihydro-6-ethoxy-2,2,4,8-tetramethylquinoline (8-methylethoxyquin) (4). We expected that the 8-methyl group would block the formation of the 1-8′-dimer and increase the yield of quinone-imine (3, R=CH_3), making 8-methylethoxyquin a more efficient antioxidant. Structures 1-4 can be seen in Scheme 1.



SCHEME 1

MATERIALS AND METHODS

Synthesis of 8-methylethoxyquin. 4-Ethoxy-2-methylaniline (8) was prepared according to the method of Büchi *et al.* (16).

A solution of the nitrophenol (5) (35 g) in ethanolic potassium hydroxide (2.5 M, 100 mL) was refluxed for 30 min. The solvent was then removed on a rotary evaporator, and the residual solid was washed with ether (3 × 250 mL) to leave potassium 3-methyl-4-nitrophenoxide (6) (42.0 g, 96%; m.p. *ca.* 300°C, decomp).

The phenate (6) (35 g) was heated in a closed pressure vessel (150°C, 24 h) with ethanol (44 mL) and ethyl bromide (25 g). The product was transferred to a flask, and the solvent was removed (rotary evaporator). The residue was mixed with aqueous sodium hydroxide (0.1 M, 500 mL) and extracted with ether (2 × 500 mL and 2 × 250 mL). The combined ether solutions were washed with water until the washings were neutral, and then filtered. After removal of solvent and of water (by co-distillation with acetone), 4-ethoxy-2-methylnitrobenzene (7) (27.5 g, 83%, m.p. 50-51°C) was obtained as a brown solid. A portion of this was purified by sublimation for spectroscopic examination.

^{1}H (300 MHz, $CDCl_3$, 0.5 M, chemical shifts, ppm): 1.44 (3H, *t*, J = 7.9 Hz, CH_3CH_2), 2.62 (3H, *s*, CH_3Ar), 4.10 (2H, *q*, J = 7.9 Hz, CH_3CH_2), 6.80 (2H) and 8.98 (1H) aromatic C*H*.

^{13}C (75 MHz, $CDCl_3$, 0.5 M, chemical shifts, ppm): 14.59 (CH_3CH_2), 21.62 (CH_3Ar), 64.20 (CH_3CH_2), 112.24, 117.93, 137.00, 142.12, 162.54 (aromatic carbon atoms).

Mass spectrum: *m/e* 181 (M^+, 92, mass 181.07372, calc. 181.07393), 164 (M − OH, mass 164.07031, calc. 164.0712), 151 (M − NO, 6), 136 (M − OEt or 164 − C_2H_4, 95), 123 (151 − C_2H_4, 61) and 108 (123 − CH_3, 71).

Without further purification, the nitro compound (7) (25 g) was hydrogenated in ethanol solution (200 mL) with Raney nickel (10 g as supplied by Aldrich Chemical Co., Milwaukee, WI) as catalyst. When hydrogen absorption ceased, the solution was separated from the nickel, and the solvent was evaporated in a rotary evaporator. The residue, dissolved in ether (200 mL), was extracted with aqueous hydrochloric acid (1 M, 3 × 100 mL), and the combined acidic extracts were made alkaline (5M sodium hydroxide solution). 4-Ethoxy-2-methylaniline (8) was extracted from the alkaline soluton with ether (3 × 200 mL)

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and, after removal of this solvent, the amine (19.7 g, 95%, containing some unreduced nitro compound and some *N*-ethyl-4-ethoxy-2-methylquinoline) was eluted from a column of silica (M60, Crosfield, Warrington, England) with petroleum ether (b.p. 40–60°C), containing increasing proportions of ether by using a simple gravity system. A portion of the purified product was submitted to spectroscopic examination.

Compound 8 appeared as a blue spot (R_f 0.16) after spraying the thin-layer chromatography plate (SiO_2, petroleum ether, b.p. 40–60°C, and diethyl ether, 80:20) with phosphomolybdic acid. The *N*-ethyl derivative appeared as a second blue spot (R_f 0.57). Compound 8 gave the following spectroscopic data:

^{1}H (300 MHz, $CDCl_3$, 0.5M, chemical shifts, ppm): 1.32 (3H, *t*, J = 8.5 Hz, CH_3CH_2), 2.12 (3H, *s*, CH_3Ar), 3.30 (2H, *s*, NH_2), 3.90 (2H, *q*, J = 8.5 Hz, CH_3CH_2), 6.60 (3H, aromatic C*H*).

^{13}C (75 MHz, $CDCl_3$, 0.5 M, chemical shifts, ppm): 15.01 (CH_3CH_2), 17.66 (CH_3Ar), 63.95 (CH_3CH_2), 112.95, 115.97, 117.21, 123.91, 138.21 and 151.91 (aromatic carbon atoms).

Mass spectrum: *m/e* 151 (M^+, 90), 122 (M − Et, 100), 106 (M − OEt, 23).

The *N*-ethyl derivative gave the following spectroscopic data: ^{1}H (300 MHz, $CDCl_3$, 0.5M, chemical shifts, ppm): 1.30 (3H, *t*, J = 7.3 Hz, CH_3CH_2NH), 1.39 (3H, *t*, J = 7.3 Hz, CH_3CH_2), 2.13 (3H, *s*, CH_3Ar), 2.80 (1H, *s*, N*H*), 3.17 (2H, *q*, J = 7.3 Hz, CH_3CH_2NH), 3.95 (2H, *q*, J = 7.3 Hz, CH_3CH_2O) 6.58 (1H) and 6.70 (2H) (aromatic C*H*).

^{13}C (75 MHz, $CDCl_3$, 0.5 M, chemical shifts, ppm): 15.07 (CH_3CH_2NH), 15.12 (CH_3CH_2O), 17.67 (CH_3Ar), 39.24 (CH_3CH_2NH), 64.15 (CH_3CH_2O), 110.95, 112.69, 117.88, 123.62, 140.86 and 150.87 (aromatic carbon atoms).

Mass spectrum: *m/e* 179 (M^+, 100), 164 (M − CH_3, 57), 150 (M − C_2H_5, 100), 136 (164 − C_2H_4, 57) and 122 (150-CO).

The amine (8) (2.6 g) was placed in a pressure vessel along with acetone (4.0 g) and iodine (50 mg), flushed with nitrogen and then heated to 170°C for 48 h. The reaction mixture was dissolved in ether (100 mL) and extracted with aqueous hydrochloric acid (0.1M, 2 × 100 mL to remove unreacted amine and then 1.0M, 2 × 100 mL). The two final extracts were combined, washed with diethyl ether (50 mL), made alkaline (aq. sodium hydroxide, 5M) and extracted with ether (2 × 100 mL). The extracts, after washing with water and removal of solvent, gave a dark oil (0.99 g) which was purified by chromatography through a column of silica (M60, Crosfield, 20 × 2 cm) by elution with petroleum ether (b.p. 40–60°C) containing increasing proportions of ether. The dihydroquinoline (4) was a yellow oil (0.56 g, 14%) which solidified (m.p. 42–43°C) and seemed pure by thin-layer chromatography. It had the following spectroscopic properties.

^{1}H (300 MHz, $CDCl_3$, 0.5M, chemical shifts, ppm): 1.28 (6H, *s*, $(CH_3)_2C$), 1.37 (3H, *t*, J = 7.7 Hz, CH_3CH_2), 1.98 (3H, *d*, J = *1.5 Hz*, CH_3C-4), 2.10 (3H, *s*, CH_3C-8), 3.35 (1H, *s*, N*H*) 3.97 (2H, *q*, J = 7.7 Hz, CH_3CH_2), 5.37 (1H, *s*, C-3*H*), 6.57 (1H, *d*, J = 2.3 Hz, C-7 or C-5*H*), and 6.61 (1H, *d*, J = 2.3 Hz, C-5 or C-7*H*).

^{13}C (75 MHz, $CDCl_3$, 0.5 M, chemical shifts, ppm): 15.09 (CH_3CH_2), 17.14 (CH_3C-4), 18.84 (CH_3C-8), 30.73 [$(CH_3)_2$], 51.58 (*C*-2), 64.20 (CH_3CH_2) 135.45 (*C*-10), 150.45 (*C*-6), 108.82, 116.49 and 129.07 (*C*-3,5,7), and 120.83, 122.56 and 128.83 (*C*-4,8,9).

Mass spectrum: *m/e* 231 (M^+, 5), 216 (M − CH_3, 100), 202 (M − C_2H_5, 5) 188 (216 − C_2H_4, 50), 173 (188 − CH_3, 5), 158 (173 − CH_3, 45).

Ultraviolet spectrum (petroleum ether, nm): maxima at 251 (log ε 3.43) and 358 (log ε 3.20) [ethoxyquin (ethanol): 229 (4.35) and 358 (3.41)].

The following spectroscopic equipment was used: Bruker (Karlsruhe, Germany) AM 300 at 300 (H) and 75 (C) MHz, AEI MS 902 double-focusing mass spectrometer at 70 eV, and a Pye-Unicam SP8-100 ultraviolet spectrometer (Cambridge, United Kingdom).

Oxidation of 8-methylethoxyquin with tert-butyl hydroperoxide. 8-Methylethoxyquin (50 mg) in ethanol (5 mL), an equimolar amount of *tert*-butyl hydroperoxide (30 mg, 70%) and an aqueous solution of ferrous ammonium sulfate (43 mg in 1 mL) were mixed and stirred in the dark for 24 h. Water was then added (25 mL), and the product was extracted with ether (25 mL). A sample of ethoxyquin was treated in the same way for comparison. The reaction product was separated into its components by preparative thin-layer chromatography (SiO_2) with a mixture of petroleum ether (b.p. 40–60°C) and diethyl ether (80:20) as developing solvent.

The major product was a yellow amorphous solid (R_f 0.37, 60% yield), which was identified as the quinone imine (**3**, R = CH_3). No dimer was identified.

^{1}H (300 MHz, $CDCl_3$, 0.5M, chemical shifts, ppm): 1.38 (6H, *s*, $(CH_3)_2C$), 1.97 (3H, *d*, J = 1 Hz, CH_3C-4), 2.22 (3H, *d*, J = 1 Hz, CH_3C-8), 6.27 (1H, *d*, J = 1 Hz, C*H*-3), 6.44 (1H, *s*, C*H*-7 or 5) and 6.40 (1H, *d*, J = 1 Hz, C*H*-5 or 7).

^{13}C (75 MHz, $CDCl_3$, 0.5 M, chemical shifts, ppm): 17.95 [CH_3-C(4)], 28.77 [$(CH_3)_2C$], 61.37 [CH_3-C(8)], 120.17 (2), 126.97, 131.02 (2), 131.75, 145.42, 148.58 and 168.51, (aromatic carbon atoms).

Mass spectrum: *m/e* 201 (M^+, 60), 186 (M − CH_3, 14), 172 (M − CHO) and 158 (186 − CO, 100).

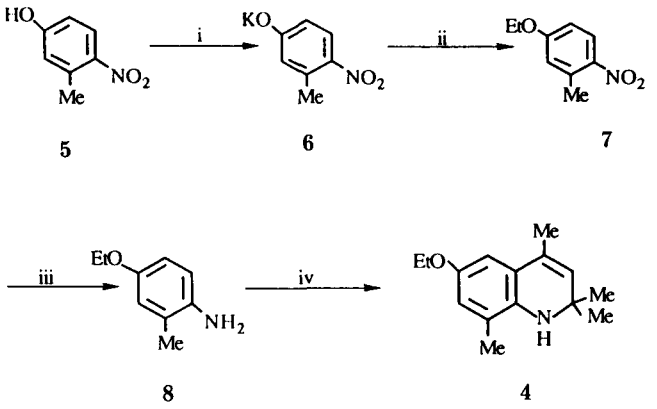
Antioxidant properties. In studies of the antioxidant properties of 8-methylethoxyquin, oxygen uptake was measured by the Warburg manometric technique, as detailed previously (13). All oxygen uptake measurements were run in duplicate and parallel studies were carried out with ethoxyquin.

RESULTS AND DISCUSSION

1,2-Dihydro-6-ethoxy-2,2,4,8-tetramethylquinoline (8-methylethoxyquin, 4) was prepared by the reaction sequence set out in Scheme 2. The reagents are: (i) KOH, EtOH (96% yield); (ii) EtBr (83%); (iii) H_2,Ni (95%); and (iv) $COMe_2$,I_2 (14% yield).

The phenol (**5**), as its potassium salt (**6**), reacted with ethyl bromide to give the ethoxy compound (**7**). This nitro compound was reduced, and the amine (**8**) was treated with acetone and iodine to furnish the desired dihydroquinoline derivative (**4**). The first three steps proceeded in excellent yield (76% overall). The final reaction between amine and acetone occurred only in poor yield (14%), and no attempt has been made to improve this.

When ethoxyquin is oxidized, it furnishes the dimer (**2**) and the quinone imine (**3**, R = H), and we have already shown that the latter has strong antioxidant properties



5 6 7 8 4

SCHEME 2

(13). We prepared the 8-methyl derivative of ethoxyquin in the expectation of blocking the formation of dimer and increasing the yield of quinone-imine (3, R = Me). When 8-methylethoxyquin was oxidized with *tert*-butyl hydroperoxide in the presence of ferrous iron, we obtained the quinone-imine in about 60% yield, and there was no evidence of any dimer.

The antioxidant properties of ethoxyquin and its 8-methyl derivative were compared in fish oil and fish meal. The results (Table 1) show ethoxyquin to be a more effective antioxidant than its 8-methyl derivative in fish oil at each of the three concentrations examined (0.01, 0.05 and 0.10%). On the basis of our experiment, showing that the 8-methyl group blocked dimer formation and increased the quantity of quinone-imine, this was an unexpected result. However, a similar result was obtained with fish meal, although the difference between the two antioxidants was less marked than in the oil (Table 2).

In explanation we suggest that despite the higher yield of quinone-imine from 8-methylethoxyquin, this is a less effective antioxidant than the quinone-imine from ethoxyquin itself. de Koning and Milkovitch (17) found that 1,2-dihydro-8-ethoxy-2,2,4-trimethylquinoline had virtually no antioxidant properties. This might suggest differences in the antioxidant properties of the 6- and 8-quinone-imine. They also found that 1,2-dihydro-6,8-dimethoxy-2,2,4-trimethylquinoline had less antioxidant properties than ethoxyquin, which could support our conclusion that substituting hydrogen by another group in the 8-position reduces the antioxidant properties of the 6-quinone-imine. Of course, the comparison has been made on a weight basis, which represents a 6% lower molar concentration of the 8-methyl derivative.

TABLE 1

Oxygen Uptake in Fish Oil in the Presence of Ethoxyquin and of 8-Methylethoxyquin

	Oxygen uptake (μmol/g)						
Time (h)	20	50	80	140	180	260	300
No antioxidant	67	—[a]	—	—	—	—	—
Ethoxyquin (%)							
0.01	4	10	18	99	—	—	—
0.05	4	10	13	25	33	56	145
0.1	2	5	8	13	17	26	33
8-Methylethoxyquin (%)							
0.01	1	15	107	—	—	—	—
0.05	4	9	16	32	55	168	—
0.1	4	9	16	28	36	52	—

[a]—, Indicates these measurements were not made.

TABLE 2

Oxygen Uptake in Fish Meal in the Presence of Ethoxyquin and of 8-Methylethoxyquin

	Oxygen uptake (μmol/g)					
Time (h)	20	50	100	200	300	400
No antioxidant	213	417	630	883	1081	1232
Ethoxyquin (%)						
0.1	243	398	543	728	834	946
0.5	107	150	184	204	213	223
8-Methylethoxyquin (%)						
0.1	301	446	582	747	892	1009
0.5	121	175	204	223	247	262

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