The Antioxidant Properties of Ethoxyquin and of Some of Its Oxidation Products in Fish Oil and Meal

Snorri Thorisson[a,1], **Frank Gunstone**[a,*] and **Roy Hardy**[b]
[a]Chemistry Department, The University of St. Andrews, Fife, KY16 9ST, United Kingdom and [b]The Torry Research Station, Aberdeen, AB9 8DG, United Kingdom

Ethoxyquin is an effective antioxidant for both fish oil and fish meal but its antioxidant activity often follows an initial burst of pro-oxidant activity. Some oxidation products of ethoxyquin are shown to be powerful antioxidants as determined from measurements by the Warburg manometric technique. Of the compounds examined, 2,6-dihydro-2,2,4-trimethyl-6-quinone imine and ethoxyquin nitroxide showed marked antioxidant activity.

KEY WORDS: Ethoxyquin, ethoxyquin dimer, ethoxyquin nitroxide, ethoxyquin oxidation products, fish meal, fish oil, 2,6-dihydro-2,2,4-trimethyl-6-quinone imine, Warburg apparatus.

Changes occurring during lipid oxidation can be followed in several ways. These techniques fall into three main categories: quantitative determination of primary (hydroperoxides) and secondary oxidation products (carbonyl compounds, malondialdehyde, *etc.*), measurement of the falling levels of unsaturated compounds in the lipid, and measurement of oxygen uptake.

We have employed the third method in this study by using the relatively simple Warburg constant-volume technique (1), which measures changes in oxygen (air) pressure in a closed flask of known volume during oxidation. Oxygen uptake measurements have been widely used with fish meal and have also been found to be suitable for products such as freeze-dried meats (2). The validity of the method depends on two assumptions: (i) that pressure decrease arises only from the reaction between oxygen and lipids and (ii) that no gases, such as carbon dioxide, are evolved during the reaction.

There is independent evidence that protein oxidation is insignificant compared to lipid oxidation (3,4), and bacterial growth, which might be expected to produce carbon dioxide, is not important in fish meal. However, some carbon dioxide is evolved from fish meal, but the amount is not significant below 60°C (5). The main advantage of the manometric technique is its accuracy, sensitivity, and simplicity. A disadvantage, which applies to the experiments on fish meal, is that the rate of lipid oxidation may be influenced by the rate of diffusion of oxygen through the meal (6).

EXPERIMENTAL PROCEDURES

Source of experimental material. Samples of fish meal from South Africa and Chile were provided for us by the International Association of Fish Meal Manufacturers (IAFMM). They were sealed under nitrogen while still fresh, but it was discovered later that the Chilean meal already contained ethoxyquin. The fat content of the meal was determined by a modification of Bligh and Dyer's procedure (7).

Samples of oil sealed under nitrogen were supplied along with the meals, and a further sample for one experiment was obtained from a fish meal factory in Aberdeen, Scotland. In addition, oil was obtained from frozen fresh mackerel and herring by mixing the minced fish (heads and viscera removed) with anhydrous sodium sulfate (10:3) followed by centrifugation (2000 rpm, 20 min). Cod liver oil was supplied by the Torry Research Station, Aberdeen, Scotland.

Oxidation studies. All the oxidation studies were carried out at 30°C. Oil samples (5 g) were placed in conical flasks (25 mL) and fish meal samples (40–50 g) were contained in larger conical flasks (100 mL). After insertion of the flasks in a water bath, the manometers were kept open for one hour to permit thermal equilibrium. After each reading, air exchange in the flasks was ensured by sucking out air and letting fresh air in a few times. The pressure drop was never allowed to exceed 50–60 mm mercury between readings.

Antioxidants were mixed with oil by placing an appropriate volume of antioxidant solution into the flask and removing the solvent before addition of the oil. Addition of antioxidant to fish meal was carried out in one of two ways. One involved dissolving the antioxidant in a small amount of oil obtained from a sample of the same meal and mixing this with the meal by means of a Kenwood stirrer. In the alternative procedure, antioxidant solution was added to the meal, then shaken well in a large volume of hexane (200 mL), which was finally removed on a rotary evaporator. Controls were treated in the same way.

Samples were oxidized in duplicate, and average values were reported in terms of oxygen uptake (μmol/g oil). It was assumed that the meal contained 10% residual oil.

RESULTS AND DISCUSSION

The following discussion refers to oxidation carried out at 30°C.

Influence of ethoxyquin on oxidation of fish oil. When added to fresh herring oil, ethoxyquin appeared to act as a pro-oxidant in the early stages of reaction at concentration levels of 0.01, 0.05 and 0.1%. It showed marked antioxidant activity at the 0.5% level but it was not as efficient as butylated hydroxytoluene (BHT) at the 0.05 and 0.5% concentrations (Table 1).

In a second experiment, with a crude oil obtained from a fish meal plant in Aberdeen, ethoxyquin was as effective an antioxidant from the beginning at all levels (0.01, 0.05, 0.1 and 0.5%) as BHT (0.05 and 0.5%) (Table 2).

Ethoxyquin also acted as an antioxidant for oils from Chilean and South African fish meal plants. This effect was apparent at the 0.1% level and more marked at 0.5% (Tables 3 and 4).

At this point we suspected that ethoxyquin reacted with oxidized fish oil to give one or more oxidation products, some or all of which were antioxidants. We confirmed this

[1]Present address: Icelandic Fisheries Laboratory, Reykjavik, Iceland.

*To whom correspondence should be addressed.

TABLE 1

Herring Oil—Uptake of Oxygen with Time

	Oxygen uptake (μmole/g)		
	50 h	100 h	200 h
Ethoxyquin (%)			
0	5	18	39
0.01	9	25	36
0.05	16	35	52
0.1	16	30	42
0.5	8	15	23
	50 h	100 h	145 h
BHT (%)			
0.05	1	2	3
0.5	1	2	3

TABLE 2

Fish Oil from a Fish Meal Plant—Uptake of Oxygen with Time

	Oxygen uptake (μmole/g)			
	50 h	100 h	200 h	300 h
Ethoxyquin (%)				
0	11	19	39	100
0.01	4	6	8	10
0.05	3	4	8	12
0.1	3	4	5	7
0.5	1	2	2	3
BHT (%)				
0.05	5	7	10	13
0.5	3	4	7	9

TABLE 3

Oil from a Chilean Fish Meal Plant—Uptake of Oxygen with Time

	Oxygen uptake (μmole/g)			
	100 h	200 h	400 h	700 h
Ethoxyquin (%)				
0	30	100	200	258
0.1	5	11	29	61
0.5	1	4	12	27

TABLE 4

Oil from a South African Fish Meal Plant—Uptake of Oxygen with Time

	Oxygen uptake (μmole/g)			
	100 h	200 h	300 h	500 h
Ethoxyquin (%)				
0	78	118	178	242
0.1	15	29	40	58
0.5	8	19	32	56

in an experiment with mackerel oil in which the oil was oxidized slightly before addition of the ethoxyquin (0.1%) (Table 5). After a short period of slow oxygen uptake (approx. 150 h), oxygen was taken up in an approximately linear fashion up to 600 h in the absence of ethoxyquin. If ethoxyquin was added before reaction with oxygen, there was an initial reaction (up to an intake of about 20 μmole O_2/g oil), which then stopped. If the oil was oxidized to 20, 50 and 90 μmole O_2/g oil before addition of ethoxyquin, the antioxidant effect was immediate and efficient. We have shown in an earlier paper (8) that ethoxyquin (1) is oxidized mainly to a quinone imine (2) and a 1,8′-dimer (3) (Scheme 1).

Influence of ethoxyquin on oxidation of fish meal. We had supplies of fish meal from Chile and from South Africa but our initial experiments showed that the meal from Chile already contained ethoxyquin. We therefore confined our experiments to the South African meal.

In one experiment, oxidation was followed over 1600 h without ethoxyquin and with ethoxyquin at 0.1 and 0.5% levels (based on oil in the fish meal). The ethoxyquin behaved as a weak antioxidant at the lower level of incorporation and was more effective at the higher level. In a subsequent experiment, attention was focussed on the first 150 h. As with the oils, ethoxyquin behaved first as a pro-oxidant before it exercised its antioxidant activity. The beneficial effect of the ethoxyquin became apparent only after a reaction time of 70–80 h. BHT (0.1 and 0.5%) was a poorer antioxidant in this meal.

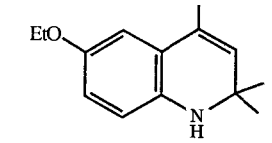
Influence of ethoxyquin oxidation products on oxidation of fish oil. The oxidation of herring oil at 30°C was examined without antioxidant and with ethoxyquin (0.01 and 0.1%) or its nitroxide (0.01 and 0.1%) (4). The two compounds behaved in a similar manner. Antioxidant activity was low at the 0.01% level but significant at the higher level (0.1%) (Table 6).

When the nitroxide was added to fresh oil, it acted initially as a pro-oxidant and then as an antioxidant. It acted quickly when added to pre-oxidized oil, suggesting that it also was oxidized to a product with antioxidant activity (Table 7). We have not pursued the nature of this product. Our present results differ from earlier claims that the nitroxide is a superior antioxidant to ethoxyquin (9,10). To check whether this reported activity arises from the pure nitroxide or some impurities, we examined the crude reaction product from which the nitroxide had been

TABLE 5

Mackerel Oil—Ethoxyquin (0.1%) Added at Different Levels of Oxygen Uptake—Uptake of Oxygen with Time

	Oxygen uptake (μmole/g)				
	200 h	200 h	300 h	400 h	500 h
No ethoxyquin	5	40	100	142	179
Oxygen uptake prior to addition of ethoxyquin (μmole/g)					
0	9	19	24	25	25
20	5	20	24	19	17
50	5	50	56	55	54
90	5	50	97	97	97



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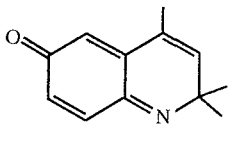

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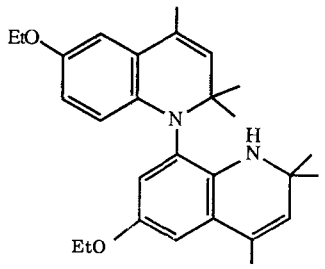

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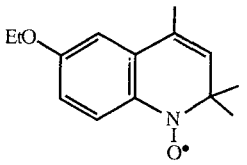

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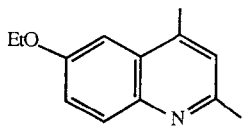

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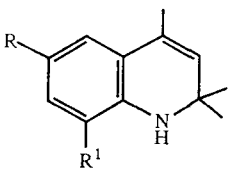

6

a R = OMe R^1 = H
b R = OPr R^1 = H
c R = OPr^i R^1 = H
d R = H R^1 = OMe
e R = H R^1 = OEt
f R = OMe R^1 = OMe

SCHEME 1

TABLE 6

Herring Oil—Addition of Ethoxyquin or Its Nitroxide—Uptake of Oyxgen with Time

Antioxidant		Oxygen uptake (μmole/g)				
		100 h	200 h	400 h	600 h	800 h
None		23	51	165	277	358
Ethoxyquin	0.01%	27	61	178	277	356
Ethoxyquin	0.10%	33	43	52	58	66
Nitroxide	0.01%	28	53	163	251	324
Nitroxide	0.10%	28	41	53	61	70

isolated but found little difference from the pure nitroxide. We concluded that ethoxyquin nitroxide (4) was not the oxidation product from which ethoxyquin derived its antioxidant properties.

The next series of experiments involved the addition of quinone imine (2) to mackerel oil and to fish meal. The quinone imine was prepared from ethoxyquin by oxidation with *t*-butylhydroperoxide and ferrous sulfate or with

TABLE 7

Mackerel Oil—Ethoxyquin Nitroxide (0.1%) Added at Different Level of Oyxgen Uptake—Uptake of Oxygen with Time

	Oxygen uptake (μmole/g)				
	100 h	200 h	300 h	400 h	500 h
No antioxidant	6	43	98	140	179
Oxygen uptake prior to addition of nitroxide (μmole/g)					
0	26	32	33	35	35
20	6	26	35	35	33
50	6	43	66	67	67
80	6	43	92	97	103

oxidized linoleate as described in our previous paper (8). There is evidence that quinones and quinone imines have antioxidant properties by virtue of their ability to act as radical acceptors (11–15).

With mackerel oil, ethoxyquin (0.1%) behaved as expected. Some oxidation occurred before antioxidant behavior was observed. With the quinone imine (0.1%), however, there was slow oxygen uptake throughout the experiment (Table 8). In South African fish meal, the quinone imine behaved similarly to ethoxyquin at 0.1 and 0.5% concentrations but it showed no superior antioxidant behavior.

The ethoxyquin dimer (3) at 0.1% level showed little or no antioxidant behavior in the oxidation of mackerel oil.

2,4-Dimethyl-6-ethoxyquinoline (5) was examined in cod liver oil and compared with ethoxyquin (0.01 and 0.1%). Ethoxyquin had only a small effect at 0.01% concentration but the quinoline had none. At 0.1%, ethoxyquin showed the expected antioxidant effect after a short period of a slightly faster oxygen uptake than observed with the control. The quinoline had a marked pro-oxidant effect at 0.1% concentration so that the oxygen uptake was approximately doubled compared to the control.

In a recent paper, de Koning and Milkovitch (16) compared the antioxidant effect of ethoxyquin (1) with some related compounds. The ethers **6a–6c** behaved like ethoxyquin. Compounds **6d** and **6e** with ether groups at position 8 rather than position 6, showed little or no antioxidant activity but the *bis* ether **6f** was midway in its behavior. The difference between the 6- and 8-ethers is not easy to explain.

In summary, when ethoxyquin acts as an antioxidant, it is converted to the dimer (3) and to other oxidation

TABLE 8

Mackerel Oil—Addition of Ethoxyquin (0.1%) or Quinone Imine (0.1%)

Antioxidant	Oxygen uptake (μmole/g)			
	100 h	300 h	500 h	1000 h
None	10	158	211	555
Ethoxyquin	10	43	51	66
Quinone imine	0	5	8	13

products. The dimer shows neither pro-oxidant or antioxidant properties but the quinone imine (2) and the nitroxide (4) are powerful antioxidants.

ACKNOWLEDGMENTS

We thank the International Association of Fish Meal Manufacturers for financial assistance and for the supply of some materials. Dr. Stuart Barlow of the Association took part in regular helpful discussions.

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[Received January 3, 1992; accepted April 9, 1992]