in in vitro systems by Maillard-type reactions and has genotoxic properties. Availability of a good synthetic method will facilitate generating additional data on this particular chemical as a potential carcinogen arising during cooking.

ACKNOWLEDGMENT

We thank R. Conrad Jones for reviewing the data, William Biles, In Vitro CORE Facility, for technical assistance in generating the data on genotoxicity in the Ames and the Williams tests, and Clara Horn for editorial support.

LITERATURE CITED

Adolfsson, L.; Olsson, K. *Acta Chem. Scand., Ser. B* **1983**, *B37*, 157.

Ashby, J.; de Serres, F. J. *Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program*; Elsevier/North-Holland Biomedical: Amsterdam, 1981.

Barnes, W. S.; Lovelette, C. A.; Tong, C.; Williams, G. M.; Weisburger, J. H. *Carcinogenesis* **1985**, *6*, 441.

Grivas, S. *Acta Chem. Scand., Ser. B* **1986**, *B40*, 404.

Grivas, S.; Olsson, K. *Acta Chem. Scand., Ser. B.* **1985**, *B39*, 31.

Hatch, F. T. *EHP, Environ. Health Perspect.* **1986**, *67*, 93.

Hatch, F. T., Nishimura, S., Powrie, W. D., Kolonel, L. N., Eds. *EHP, Environ. Health Perspect.* **1986**, *67*, 3–157.

Howes, A. J.; Beamand, J. A.; Rowland, I. R. *Food Chem. Toxicol.* **1986**, *24*, 383.

Jägerstad, M.; Grivas, S. *Mutat. Res.* **1985**, *144*, 131.

Jägerstad, M.; Grivas, S.; Olsson, K.; Reuterswärd, A. L.; Negishi, C.; Sato, S. In *Genetic Toxicology of the Diet*; Knudsen, I., Ed.; Alan R. Liss: New York, 1986; pp 155–168.

Kadin, S. B. *J. Org. Chem.* **1973**, *38*, 1348.

Kaiser, G.; Harnasch, D.; King, M. T.; Wild, D. *Chem.-Biol. Interact.* **1986**, *57*, 97.

Kasai, H.; Nishimura, S.; Wakabayashi, K.; Nagao, M.; Sugimura, T. *Proc. Jpn. Acad., Ser. B* **1980a**, *56B*, 382.

Kasai, H.; Yamaizura, Z.; Wakabayashi, K.; Nagao, M.; Sugimura, T.; Yokoyama, S.; Miyazawa, T.; Nighimura, S. *Chem. Lett.* **1980b**, 1391.

Kasai, H.; Shioni, T.; Sugimura, T.; Nishimura, S. *Chem. Lett.* **1981**, 657.

Kier, L. E.; Brusick, D. J.; Auletta, A. E.; Von Halle, E. S.; Brown, M. M.; Simmon, V. F.; Dunkel, V.; McCann, J.; Mortelmans, K.; Prival, M.; Rao, T. K.; Ray, V. *Mutat. Res.* **1986**, *168*, 69–240.

Knudsen, I., Ed. *Genetic Toxicology of the Diet*; Alan R. Liss: New York, 1986.

Le Bris, M.-T. *Bull. Soc. Chim. Fr.* **1976**, 921.

Matsushima, T. In *Molecular Interrelations of Nutrition and Cancer*; Arnott, M. S., van Eys, J., Wang, Y.-M., Eds.; Raven: New York, 1982; pp 35–42.

Negishi, C.; Wakabayashi, K.; Tsuda, M.; Sato, S.; Sugimura, T.; Saito, H.; Maeda, M.; Jägerstad, M. *Mutat. Res.* **1984**, *140*, 55–59.

Ohgaki, H.; Hasegawa, H.; Kato, T.; Suenaga, M.; Ubukata, M.; Sato, S.; Takayama, S.; Sugimura, T. *Environ. Health Perspect.* **1986**, *67*, 129.

Olsson, K.; Grivas, S. *Acta. Chem. Scand., Ser. B* **1986**, *B40*, 486.

Sugimura, T.; Sato, S.; Ohgaki, H.; Takayama, S.; Nagao, M.; Wakabayashi, K. In *Genetic Toxicology of the Diet*; Knudsen, I., Ed.; Alan R. Liss: New York, 1986; pp 85–108.

Tanaka, T.; Barnes, W. S.; Weisburger, J. H.; Williams, G. M. *Jpn. J. Cancer Res. (Gann)* **1985**, *76*, 570–576.

Taylor, R. T.; Fultz, E.; Knize, M. *Environ. Health Perspect.* **1986**, *67*, 59–93.

Weisburger, J. H. In *Diet. Nutrition and Cancer*; Hayashi, Y., Nagao, M., Sugimura, T., Takayama, S., Tomatis, L., Wattenberg, L. W., Wogan, G. N., Eds.; Japan Science Society: Tokyo, 1986; pp 11–26.

Weisburger, J. H.; Williams, G. M. In *Chemical Carciongens*, 2nd ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984; Vol. 2, pp 1323–1373.

Wynder, E. L., Leveille, G. A., Weisburger, J. H., Livingston, G. E., Eds. *Environmental Aspects of Cancer: The Role of Macro and Micro Components of Foods*; Food and Nutrition: Westport, CT, 1983.

Received for review October 21, 1986. Revised May 18, 1987. Accepted July 7, 1987. Research was supported by USPHS Grants CA-24217 and CA-42381 from the National Cancer Institute.

Nitroxides Derived from Ethoxyquin and Dihydroethoxyquin as Potent Anti-Nitrosamine Agents for Bacon

Keki R. Bharucha,* Charles K. Cross, and Leon J. Rubin

Attempted preparation of ethoxyquin nitroxide from ethoxyquin according to the procedure of Lin and Olcott resulted in dealkylation to give the corresponding hydroxy nitroxide as a dimer. The same behavior was also observed with dihydroethoxyquin. These nitroxides derived from ethoxyquin and dihydroethoxyquin were shown to be the most potent anti-nitrosamine agents for bacon, yet discovered with complete inhibition of nitrosamines at 20 ppm and in some instances as low as 10 ppm. Significant reduction was also observed at 1 ppm.

Earlier work from these laboratories (Bharucha et al., 1985) has shown that ethoxyquin, dihydroethoxyquin, and their analogues are excellent inhibitors of nitrosamine formation in bacon. As exemplified by ethoxyquin, it was postulated that they function in the case of nitrosopyrrolidine, by competing with proline for the available nitrosating species, the initially formed *N*-nitrosoethoxyquin undergoing rearrangement to 8-nitrosoethoxyquin prior to (air) oxidation to 8-nitroethoxyquin. The possibility was entertained that ethoxyquin may also function by first undergoing oxidation to ethoxyquin nitroxide (I), which would then trap nitric oxide (the nitrosating species) to give ethoxyquin nitrite (II). The latter could then undergo thermal rearrangement directly to give nitroethoxyquin (III) or alternatively undergo thermal dissociation to give N_2O_4 and ethoxyquin radical, which could then be converted to the nitroxide and thus carry on the chain. These transformations are depicted schematically in Figure

Canada Packers Inc., Toronto, Ontario, Canada M6N 1K4.

0021-8561/87/1435-0915$01.50/0

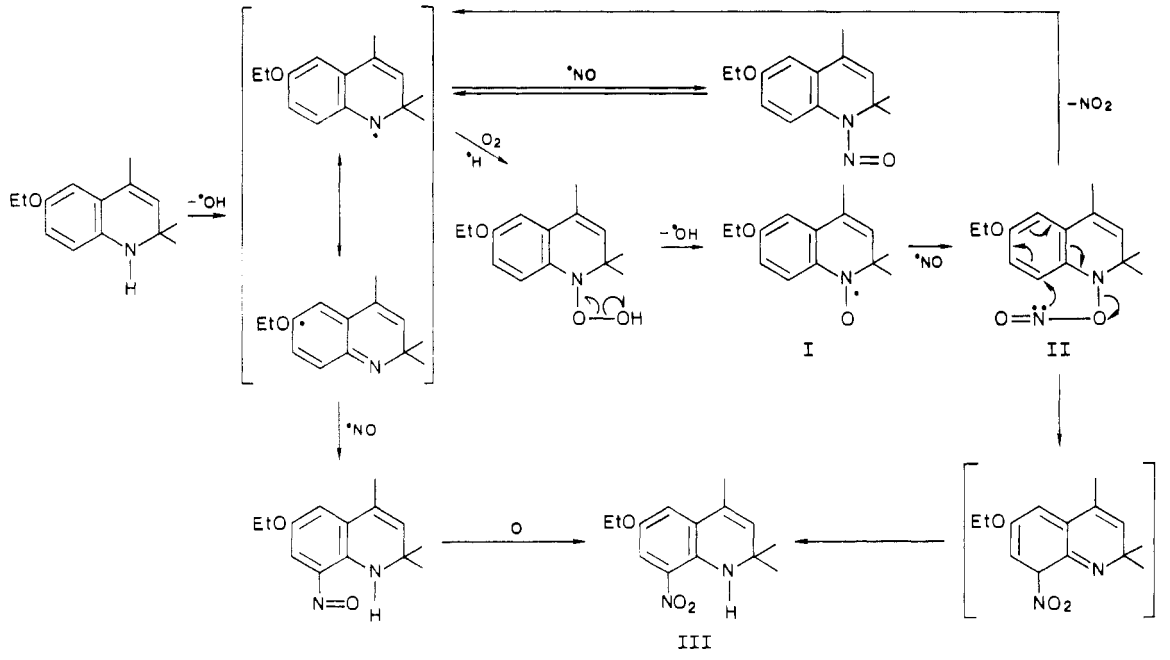


Figure 1. Mode of action of ethoxyquin.

1, which outlines the various possible modes of action of ethoxyquin. The intermediacy of ethoxyquin nitroxide is rendered all the more attractive by the observation of Lin and Olcott (1975) that ethoxyquin nitroxide is a more effective antioxidant than ethoxyquin itself.

Nitroxides from ethoxyquin and dihydroethoxyquin were therefore synthesized and tested in bacon. The results form the subject matter of the present report.

EXPERIMENTAL SECTION

Safety note: Many nitrosamines have been shown to be highly carcinogenic compounds in test animals. All experiments should be done in a well-ventilated area. Safety gloves should be worn whenever nitrosamines are being handled.

Preparation of Nitroxide from Ethoxyquin (Lin and Olcott, 1975). A mixture of freshly distilled ethoxyquin (4.62 g), sodium tungstate dihydrate (0.47 g), ethylenediaminetetraacetic acid disodium salt (0.82 g), absolute ethanol (190 mL), and water (50 mL) was stirred magnetically. Over a period of 10–15 min 30–35% hydrogen peroxide (40 mL) was added at room temperature. A deep red-brown solution formed. After 4 h, water (200 mL) and solid $NaHCO_3$ to saturation were added. The mixture was extracted with benzene. The benzene extract was washed four times with water, dried ($NaHCO_3$), and evaporated to dryness in vacuo on a 30–35 °C water bath; weight of dark brown oil, 4.27 g.

A column of silicic acid (100 g, 65–200 mesh) was prepared in chloroform. The brown oil was added and eluted with chloroform. Fractions: I, 150 mL (yellow band) → brown oil, 1.80 g (A); II, 200 mL (yellow band) → brown oil, 0.96 g (B); III, 200 mL (yellow band) → brown oil, 1.90 g (C); IV, 200 mL (yellow band) → brown oil, 0.64 g (D); V, 400 mL (yellow band) → brown oil, 0.14 g (E). Fraction C was chromatographed on silicic acid (200 g of Mallinckrodt CC7 special, 200–325 mesh) in ethanol-free chloroform. The elution was performed with ethanol-free chloroform. Fractions: I, 2800 mL → brown gum, 0.03 g (F); II, 450 mL → brown gum, 0.24 g (G); III, 450 mL → brown gum, 0.97 g (H); IV, 300 mL → brown gum, 0.57 g (I); V, 2700 mL → brown solid, 0.27 g (J). Fraction H, which turned semisolid on standing, was used for the first bacon test. Crystallization of a portion of H (0.5 g) from light petroleum ether with a minimum of added dichloromethane under refrigeration for 28 h produced an orange-red solid: 0.36 g; mp 116–119 °C.

Elemental analysis. Anal. Calcd for desethylethoxyquin nitroxide (MW 204) ($C_{12}H_{14}NO_2$): C, 70.56; H, 6.91; N, 6.86; O, 15.67. Found: C, 70.32; H, 6.59; N, 7.55; O, 15.79. NMR ($CDCl_3$): δ 1.59 (s, 6 H, 2-CH_3), 2.08 (s, 4-CH_3), 6.12 (1 H, br s, 3-H), 6.28 (1 H, d, 5-H, $J_{5,7}$ = 2 Hz), 6.6 and 8.0 (AB pattern, Ar H, J = 6 Hz). ESR: Electron spin resonance spectroscopy showed the absence of an unpaired electron in the molecule. The solvent used was chloroform. Mass spectral analysis: The parent ion has a mass of 204. Mass 203 is roughly twice as abundant as 204. The base peak occurs at 186, probably indicating the loss of OH from 203.

Desethyldihydroethoxyquin Nitroxide. The method described above for nitroxide formation from ethoxyquin was applied to dihydroethoxyquin (4.60 g). Column chromatography of the crude reaction mixture (4.01 g) gave a brown solid (1.84 g) that was crystallized from dichloromethane–light petroleum ether. A brown solid (0.95 g), mp 130–132 °C, was isolated. This material was used for the bacon experiments.

Elemental analysis. Anal. Calcd for desethyldihydroethoxyquin nitroxide ($C_{12}H_{16}NO_2$): C, 69.87; H, 7.83; N, 6.79; O, 15.51. Found: C, 69.97; H, 7.57; N, 7.03; O, 15.43. NMR ($CDCl_3$): δ 1.34 (3 H, d, 4-CH_3), 1.55 (7 H, br, 2-CH_3 and one of 3-H or 4-H), 1.7, 1.95, and 2.12 (3 H, OH, both 3-H or one 3-H and 4-H), 6.25 (1 H, d, 5-H, $J_{5,7}$ = 2 Hz), 6.49 and 7.87 (AB pattern, Ar H, J = 9 Hz). The doublet at δ 6.49 due to 7-H was further split by 5-H. ESR: A very weak triplet apparently due to the nitrogen coupling to the unpaired electron is present. The triplet is further split into three triplets. The spectrum is very weak, and we therefore doubt that the free radical is the major portion of the sample. Mass spectral analysis: The parent ion has a mass of 206. Mass 205 is twice as abundant as 206. The base peak occurs at 158.

The amount of nitroxide required to produce the desired level of additive was incorporated as a slurry in 2 mL of winterized soybean oil to 2-lb lots of sequentially sampled side bacon. The method of sampling to obtain equivalent

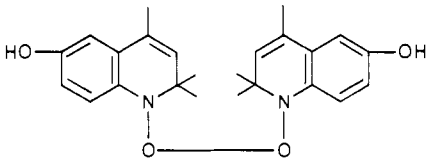
samples has been described in an earlier publication (Bharucha et al., 1980).

The bacon samples were fried immediately for 12 min under the standard conditions described in Bharucha et al. (1979), which produces maximum amounts of nitrosamines. Volatile nitrosamines were analyzed in the cook-out fat by the colorimetric method described earlier (Cross et al., 1978).

RESULTS AND DISCUSSION

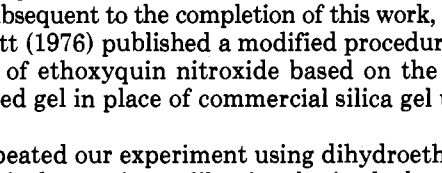
The oxidation of ethoxyquin to ethoxyquin nitroxide was carried out by Lin and Olcott (1975) at room temperature for 4 h using 30% hydrogen peroxide. They obtained the crude product as a viscous oil that was then fractionated on a silicic acid column with chloroform. A red oil, obtained in 42% yield, was purported to be ethoxyquin nitroxide. When we attempted to duplicate Lin and Olcott's procedure, we obtained a dark brown oil that upon chromatography on a silicic acid column as recommended by the authors gave only brown oils.

When the middle fraction was rechromatographed and the chloroform eluates collected in various lots, one of the middle fractions, which was a brown gum, slowly turned semisolid and deposited red-orange crystals, mp 116–119 °C, on crystallization from light petroleum ether and a little dichloromethane. Elemental analysis and molecular weight determination by mass spectrometry indicated that the orange-red material was desethylethoxyquin nitroxide, dealkylation having taken place during nitroxide formation. The absence of the ethoxy group was also substantiated by a proton NMR spectrum showing no peaks characteristic of the ethoxy group. Lack of any signal in the ESR spectrum showed that there were no unpaired electrons present, strongly suggesting that the compound is a dimer of the following structure:



Since a number of attempts to duplicate Lin and Olcott's results by varying various parameters in the oxidation failed, we communicated our findings to Prof. Olcott. In a private communication (1976) he informed us that he too had experienced difficulties in duplicating his earlier work. Subsequent to the completion of this work, Wu, Lin, and Olcott (1976) published a modified procedure for the isolation of ethoxyquin nitroxide based on the use of a neutralized gel in place of commercial silica gel used originally.

We repeated our experiment using dihydroethoxyquin in place of ethoxyquin; we likewise obtained a brown solid, mp 130–132 °C, which on the basis of elemental analysis, NMR, MS, and ESR spectroscopy was assigned the dimeric structure



corresponding to the nitroxide obtained from ethoxyquin. Once again the loss of the ethoxy group is noteworthy.

The nitroxides derived from ethoxyquin and dihydroethoxyquin were applied to bacon slices as a slurry in winterized soybean oil. As results in Tables I and II show, these compounds are excellent anti-nitrosamine agents for bacon, lowering the nitrosamine content in the cook-out fat of bacon to below our detection limit of 3 ppb at the 20 ppm level and in some instances even at 10 ppm. The fact that at levels as low as 1 ppm these compounds still show pronounced effect makes them the most potent nitrosamine-inhibiting agents for bacon, yet discovered.

Table I. Effect of the Desethylethoxyquin Nitroxide on Nitrosamine Formation in Fried Bacon Cook-Out Fat

sample	amt added nitroxide, ppm	nitrosamines[a] in cook-out fat, μmol × 10^{-2} kg	
		test	control
1	100	nd	18
2	50	nd	19
3	10	3	19
4	1	13	19
5	20	nd	8
6	10	nd	8
7	1	nd	8
8	20	nd	38
9	10	5	38
10	1	16	38

[a] Detection limit about 3 μmol × 10^{-2}/kg. nd = not detected.

Table II. Effect of the Desethyldihydroethoxyquin Nitroxide on Nitrosamine Formation in Fried Bacon Cook-Out Fat

sample	amt added nitroxide, ppm	nitrosamines[a] in cook-out fat, μmol × 10^{-2}/kg	
		test	control
1	20	3	35
2	10	13	35
3	1	20	35
4	20	nd	36
5	10	6	36
6	1	22	36

[a] Detection limit about 3 μmol × 10^{-2}/kg. nd = not detected.

The superior anti-nitrosamine properties of these nitroxides compared to those of the parent compounds ethoxyquin and dihydroethoxyquin strongly suggest that at least in part the action of the latter is mediated through the former.

Although nitroxides derived from ethoxyquin and dihydroethoxyquin are excellent inhibitors of nitrosamine formation, it is not recommended that they be used in bacon without prior toxicological testing.

Registry No. Desethylethoxyquin nitroxide, 75074-95-2; desethyldihydroethoxyquin nitroxide, 75074-93-0.

LITERATURE CITED

Bharucha, K. R.; Cross, C. K.; Rubin, L. J. *J. Agric. Food Chem.* **1979**, *27*, 63–69.

Bharucha, K. R.; Cross, C. K.; Rubin, L. J. *J. Agric. Food Chem.* **1980**, *28*, 1274–1281.

Bharucha, K. R.; Cross, C. K.; Rubin, L. J. *J. Agric. Food Chem.* **1985**, *33*, 834–839.

Cross, C. K.; Bharucha, K. R.; Telling, G. M. *J. Agric. Food Chem.* **1978**, *26*, 657–660.

Lin, J. S.; Olcott, H. S. *J. Agric. Food Chem.* **1975**, *23*, 798–800.

Olcott, H. S., University of California, Davis, Santa Barbara, CA, private communication, March 23, 1976.

Wu, T.; Lin, J. S.; Olcott, H. S. *J. Agric. Food Chem.* **1976**, *24*, 1255.

Received for review July 15, 1986. Accepted May 20, 1987.