JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

AUGUST 2000
VOLUME 48, NUMBER 8

Purification of Ethoxyquin and Its Two Oxidation Products

Ping He and Robert G. Ackman*

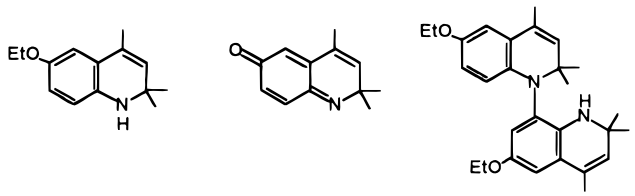
Canadian Institute of Fisheries Technology, DalTech, Dalhousie University, Halifax, Nova Scotia, B3J 2X4, Canada

2,6-Dihydro-2,2,4-trimethyl-6-quinolone (QI) and 1,8′-bis(1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) (DM) are two oxidation products of 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyquin, EQ). This paper describes several methods for the purification of technical grade EQ and for the production of pure QI and DM as standards with the purity required (>99%) for calibration of quantitative determination methods. EQ of high purity was obtained through vacuum distillation followed by a quick column chromatographic purification on silica gel. Preparative scale purity DM could be obtained through recrystallization from methanol, but QI could be purified only by a high-pressure liquid chromatographic method.

Keywords: *Ethoxyquin; oxidation; purification*

INTRODUCTION

Ethoxyquin (EQ, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is a synthetic antioxidant, which may also be on labels as Santoquin. To fulfill its function, EQ can be oxidized to a series of compounds under different conditions. Among these oxidation products, a quinone imine compound (QI, 2,6-dihydro-2,2,4-trimethyl-6-quinolone) and a dimer (DM, 1,8′-bis(1,2,-dihydro-6-ethoxy-2,2,4-trimethylquinoline)) were identified as the major oxidation products of EQ in fish meal (Thorisson et al., 1992). Their structures are shown in Figure 1. Obtaining pure EQ, QI, and DM as standards is critical to the establishment of calibration lines for quantitative determination. However, these standards are not commercially available. This paper describes our experience in detail on the purification of EQ, QI, and DM.



I II III

Figure 1. Chemical structures of EQ (I), QI (II), and DM (III).

EXPERIMENTAL PROCEDURES

Column Chromatography (CC). CC was carried out on a silica gel column (30 × 2 cm). Silica gel (60−200 mesh, grade 62, Aldrich Chemical Co., Milwaukee, WI) was heated in an oven at 170 °C overnight before using. A slurry was made by mixing 50 g of silica gel with 150 mL of hexane and was added in portions to the column. The column was gently tapped between additions of each portion, and extra pressure was applied by N_2 at the top to pack the silica gel slurry tightly. Finally, a plug of glass wool was added to the top of the column. The sample was loaded on the glass wool and then eluted by mixtures of diethyl ether in hexane at a flow rate of 7 mL min^{-1} under a slight extra pressure of N_2.

High-Pressure Liquid Chromatography (HPLC). Analytical HPLC was executed with a Waters 6000A solvent delivery system, a Waters Model U6K injector, and a Waters 450 variable-wavelength UV detector (Waters, Milford, MA). The mobile phase was acetonitrile and 0.01 M CH_3COONH_4 (80:20 v:v) at a flow rate of 1 mL min^{-1}. The column was a CSC-S ODS-2 column (30 × 0.39 cm, 10 μm, CSC Inc.,

* To whom correspondence should be addressed. Tel: (902) 494-6030. Fax: (902) 420-0219. E-mail: odorjr@is.dal.ca.

10.1021/jf991193g CCC: $19.00
Published on Web 07/27/2000

Montreal, PQ, Canada) with a guard column (3 cm, Waters, Milford, MA).

Gas Chromatography−Mass Spectrometry (GC-MS). GC-MS was carried out with a PE 990 gas chromatograph (Perkin-Elmer, Norwalk, CT) fitted with a DB-1 fused silica capillary column (59 m × 0.25 mm i.d., Chromatographic Specialties Inc., Brockville, ON) passed directly into a Finnigan 700 ion trap detector (Finnigan Corp., San Jose, CA) form of mass spectrometer. The column operating temperature was 150 °C for EQ and QI determinations and 260 °C for DM determination. Data acquisition was executed by a computer program provided by Finnigan Corp. (San Jose, CA).

Purification of EQ. EQ (100 g, from the former Canada Packers Fine Chemicals Division, Mississauga, ON, Canada; an ethoxyquin, No. E8260, of 75% purity is now available from Sigma-Aldrich, Oakville, ON, Canada) was distilled with stirring in an all-glass apparatus under high vacuum (≤1 mmHg). The condensate was collected in three fractions, A (35 g), B (20 g), and C (15 g), boiling in the range 126−139 °C at 0.20−0.95 mmHg. The residue (30 g) was discarded. An aliquot of B (about 2 g) was further purified by CC as described above. The initial impurities were eluted by 2% ethyl ether in hexane (200 mL), and pure EQ (1.2−1.4 g) was then eluted by 5% ethyl ether in hexane (200 mL).

Purification of QI and DM. After 26 h of EQ oxidation (Thorisson et al., 1992), the mixture was made basic (NaOH, pH >10) and extracted with hexane (50 mL × 4). The combined hexane solutions were then washed with 1 M HCl (50 mL × 3), and QI was extracted into the aqueous acid solution while DM remained in the hexane phase. Most of the hexane containing DM was removed on a vacuum rotary evaporator, and the concentrated solution was transferred into a test tube. Crude DM was obtained by removing the rest of the hexane with a stream of N_2. Pure DM was obtained by repeated recrystallization of the crude DM from MeOH. The aqueous portion was again made basic (NaOH, pH >10) and was then extracted with hexane (50 mL × 3). The combined hexane solutions were washed with H_2O and dried over Na_2SO_4. After removal of the solvent, a red oil containing EQ, QI, and other oxidation products was obtained. The red oil was then streaked on silica gel TLC plates and developed with a mixture of hexane, ether, and triethylamine (40:60:5 v:v:v). The yellow QI band (no fluorescence under UV) was immediately scraped into a test tube and recovered with freshly distilled diethyl ether to obtain crude QI. The crude QI was further purified on the same HPLC system used for analyses (He and Ackman, 2000), operated in semipreparative mode.

RESULTS AND DISCUSSION

Silica gel CC (Winell, 1976; Wu et al., 1976; Thorisson et al., 1992; He and Ackman, 2000) and a vacuum distillation method (de Koning, 1987) have been commonly used for EQ purification, and a preparative HPLC method was introduced to purify QI and DM (Thorisson et al., 1992). The previously published methods either were incomplete in details or required equipment not available in this laboratory. Our comprehensive study began with a technical grade of EQ that was a very viscous black liquid, containing several components. The impurities probably consisted of the EQ oxidation products and some other compounds such as phenetidine (Taimr and Prusìkov, 1991). The major peak, EQ, had an area percentage of 86% of peaks on the HPLC chromatogram. This percentage did not represent the actual purity, since the response factor of the UV detector was not the same for different compounds. However, it was used later to roughly compare the purity between different EQ samples by assuming that they had similar compositions.

A number of trials were conducted for purification by CC. When activated silica gel (heated at 170 °C for 15 h) was used, and a "flash chromatography" principle (Leonard et al., 1995) was applied for column preparation and sample elution, 5% ethyl ether in hexane was found to be a good choice for the isolation of EQ from other impurities. However, the highest purity of EQ obtained by this means was less than 93% (peak area percentage) when analyzed by HPLC, still far from an analytical standard.

The original EQ sample was then purified by vacuum distillation. Among the three fractions collected, fraction A contained several distinct compounds with the EQ peak area percentage being about 90%. Fractions B and C, having similar compositions, contained only a few small peaks besides EQ (area percentage >96%). After distillation, B was further purified by CC, and the purity of EQ recovered was greater than 99% on HPLC (no other peak found). To get good resolution on the CC column used, the maximum load applied had to be 2 g or less of EQ. The recovery of pure EQ was 60−70%.

The purified EQ standard was examined by GC-MS, and its MS spectrum perfectly matched the literature (Thorisson et al., 1992). The molecular ion peak appeared at *m/e* 217 (M^+). The principal fragments were at *m/e* 202, 188, 174, and 145. The fragment of *m/e* 202 (base peak) was produced by losing a methyl radical from M^+. The fragment *m/e* 188 represented loss of an ethyl radical at the 6-O position. The fragment *m/e* 174 had the structure of a quinone. It is probably produced by losing a neutral $CH_2{=}CH_2$ molecule from the molecular ion due to the McLafferty rearrangement (McLafferty, 1959) and then by further loss of a methyl group. The composition of the fragment *m/e* 145 was not clear.

Modifications of the purification procedure for QI and DM were studied after they were prepared according to a published method (Thorisson et al., 1992). HPLC analysis of the black reaction mixture showed that the EQ was almost completely oxidized. The mixture contained three other major compounds besides QI and DM. After an acid wash, crude QI was recovered in the aqueous layer, but crude DM stayed in the organic solvent. The crude DM was recovered as a black solid. Using methanol as solvent (Kato and Kanohta, 1985), DM could be purified quite simply in a test tube through repeated recrystallization. This modification was especially suitable for preparative scale purification of the DM standard. On the other hand, there was no obvious simple and rapid method for QI purification. QI has to be purified by a combination of TLC separation and analytical scale HPLC purification if preparative scale HPLC is not available.

The purified QI was a yellow-green solid; the purified DM was a white solid. Their purity (peak areas for both compounds on HPLC >99%) was confirmed with the appearance of only one peak for each on GC-MS. Their structures were also confirmed by GC-MS, the MS spectra of both compounds being in agreement with the literature (Thorisson et al., 1992). For QI, major peaks appeared at *m/e* 187, 172, 159, 144, and 117. The molecular ion peak appeared at *m/e* 187 (M^+). Like the EQ molecular ion, a methyl group was lost to form the fragment *m/e* 172. The QI molecular ion could follow another pathway to form the fragment *m/e* 159 by losing a neutral CO molecule. This new fragment (*m/e* 159) could again release a methyl radical to form the *m/e* 144 fragment, which became the base peak of the spectrum. The structure of the fragment *m/e* 117 is not clear. The MS spectrum of DM contained one major peak (*m/e* 417)

and several small peaks (*m*/*e* 432, 402, 387, 373). Only two of these peaks can be easily explained. The molecular ion peak is at *m*/*e* 432 (M^+). The base peak of the spectrum is accounted for by the loss of one methyl radical, yielding a corresponding *m*/*e* value of 417.

EQ and its most frequently reported oxidation products are all unstable. Any standard or reference material may have to be repurified after even a short storage period. Fortunately, the procedures described for this purpose are relatively simple.

ACKNOWLEDGMENT

Funding for the study, provided by the Natural Sciences and Engineering Research Council of Canada through a grant awarded to Dr. R. G. Ackman, is gratefully acknowledged. Special thanks are due to Dr. J. A. Pincock of the Chemistry Department of this university for allowing us to do the vacuum distillation in his laboratory.

LITERATURE CITED

de Koning, A. J. Ethoxyquin—A note on its refractive index and some of its spectral data. *Fat Sci. Technol*. **1987**, *89*, 103−106.

He, P.; Ackman, R. G. HPLC determination of ethoxyquin and its major oxidation products in fresh and stored fish meals and fish feeds. *J. Sci. Food Agric*. **2000**, *80*, 10−16.

Leonard, J.; Lygo, B.; Procter, G. Flash chromatography. In *Advanced Practical Organic Chemistry*, 2nd ed.; Blackie Academic and Professional: London, 1995; pp 184−227.

Kato, S.; Kanohta, K. Chromatographic studies of the autoxidation products of ethoxyquin and its photochemical conversion. *J. Chromatogr*. **1985**, *324*, 462−468.

McLafferty, F. W. Mass spectrometric analysis: rearrangements in vinyl derivatives. *Anal. Chem*. **1959**, *31*, 2072−2075.

Taimr, L.; Prusìkov, M. Chromatographic behaviour of the antioxidant ethoxyquin and its transformation products. *J. Chromatogr*. **1991**, *587*, 355−358.

Thorisson, S.; Gunstone, F. D.; Hardy, R. Some oxidation products of ethoxyquin including those found in autoxidising systems. *Chem. Phys. Lipids* **1992**, *60*, 263−271.

Winell, B. Quantitative determination of ethoxyquin in apples by gas chromatography. *Analyst* **1976**, *101*, 883−886.

Wu, T.; Lin, J. S. Olcott, H. S. Modified procedure for purification of ethoxyquin and ethoxyquin nitroxide. *J. Agric. Food Chem*. **1976**, *24*, 1255.

Received for review November 3, 1999. Accepted June 2, 2000.

JF991193G