benefits of the higher lysine and tryptophan contents.

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Howard L. Mitchell*
Donald B. Parrish
Margo Cormey
Clyde E. Wassom

Department of Biochemistry (H.L.M., D.B.P., M.C.)
Department of Agronomy (C.E.W.)
Kansas State University
Manhattan, Kansas 66506

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Modified Procedure for Purification of Ethoxyquin and Ethoxyquin Nitroxide

The commercial silica gel used originally for the column separation of ethoxyquin nitroxide (Lin, J. S., Olcott, H. S., *J. Agric. Food Chem.* **23**, 798 (1975)) is no longer available. The present procedure depends upon use of a neutralized gel which may have applicability in the chromatography of other stable free radicals.

The procedure used by Lin and Olcott (1975) to separate the free radical, ethoxyquin nitroxide, from other products of the oxidation of ethoxyquin has had to be modified because the silica gel originally used for the column chromatography (Silic AR CC7, 200–325 mesh, Mallinckrodt) is no longer available. The following procedure for preparing a suitable gel yielded satisfactory columns. To 500 g of silica gel (Bio-Sil A, 200–400 mesh, Bio-Rad) in a 2000-ml round-bottomed flask was added, in portions and with stirring, 1500 ml of methanol in which had been dissolved 5 g of sodium bicarbonate and 0.5 g of EDTA disodium salt (Matheson). The methanol was removed by filtration (coarse fritted disk) and the product dried at 100 °C in a draft oven.

Purified ethoxyquin for the synthesis was obtained from technical grade ethoxyquin (Santoquin, Monsanto) by chromatography on a column of the silica described above with chloroform–hexane (1:1). Ethoxyquin eluted as a yellow fraction; yield about 80%. The red material remaining on the column has not been characterized. Ethoxyquin was oxidized as described previously (Lin and Olcott, 1975) for 1.5 h and fractionated on a column of the above silica with chloroform–hexane (2:1) (column size, 450 mm × 25 mm, 65 g dry silica, eluent rate, 2.5 ml/min). A first fraction was colorless, the second was unchanged ethoxyquin, and the third was deep red ethoxyquin nitroxide; yield about 35%. By thin-layer chromatography (13179 silica gel, Eastman) with chloroform–hexane (2:1), ethoxyquin had R_f 0.66 and the nitroxide had R_f 0.33. The column effluent nitroxide fraction gave a single spot but after the solvent was removed by vacuum evaporation, the residue showed two spots only one of which was unchanged nitroxide.

Ethoxyquin nitroxide is unstable in acid media; hence it was necessary to use a silica gel which had been neutralized. The treated gel was in the pH range 6.8–7.2. The same procedure may be useful in the isolation of other free radicals that are unstable in acid media (Forrester et al., 1968).

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Thea Wu
James S. Lin
Harold S. Olcott*

Institute of Marine Resources
Department of Food Science and Technology
University of California
Davis, California 95616

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