A Research Note

# Identification of 2–Methyl–3–Furanthiol in the Steam Distillate from Canned Tuna Fish

DONALD A. WITHYCOMBE and CYNTHIA J. MUSSINAN

## ABSTRACT

The volatile components of canned tuna fish were isolated by steam distillation followed by solvent extraction. Analysis of the extract using a sulfur-specific detector and coupled gas chromatography - mass spectrometry permitted the identification of 2–methyl–3–furanthiol, a compound with an intense beef extract aroma. Mass spectral data are presented.

## INTRODUCTION

MANY FURANTHIOL DERIVATIVES have been identified in foods (e.g., Tressl and Silwar, 1981; Macleod and Ames, 1985). These compounds are normally present at very low concentrations. However, since they have extremely low taste and odor thresholds, they make a significant contribution to food flavor. The flavor properties of a number of furanthiol derivatives were discussed by Evers et al. (1976). Although their importance to food flavor is well recognized, the identification of these compounds from natural food matrices has been extremely difficult. It has only been since the advent of inert, glass capillary, chromatography columns used in conjunction with inert mass spectrometer interfaces and computer data handling systems that the identification of these unstable, trace components could be achieved.

This paper reports the identification of 2–methyl–3–furanthiol from tuna fish steam distillate.

## MATERIALS & METHODS

### Sample preparation

Ninety-four hundred grams of Code Brand fancy, solid, water-pack, Albacore tuna fish were blended with 6.5 kg of distilled water and placed in a 22L distillation flask equipped with a cold water condenser and a 12L steam generator charged with 9L of distilled water. Ten liters of water were distilled from the tuna fish. The distillate, possessing a characteristic canned tuna fish aroma, was then extracted with diethyl ether for 48 hr in a 12L liquid-liquid extractor. The ether extract was dried over anhydrous sodium sulfate and concentrated by careful distillation in a Kuderna-Danish concentrator (Kontes Glass Co., Vineland, NJ). Final concentration was accomplished under a slow stream of nitrogen.

### Analysis

As an aid in the detection of sulfur compounds, the concentrate was analyzed using a Tracor MT-220 gas chromatograph, modified to simultaneously detect sulfur and/or nitrogen containing compounds (Withycombe et al., 1976).

The sample was analyzed by coupled gas chromatography-mass spectrometry using a Varian MAT-111 mass spectrometer equipped with a Varian SS 100 data system using a Varian 620L computer and operated in the continuous scanning mode. The column used was a 400 ft × 0.032 in i.d. glass capillary column coated with SE-30. The oven temperature was programmed from 60°C to 190°C at 1°C/min.

*Author Mussinan is with International Flavors & Fragrances, Inc., 1515 Highway 36, Union Beach, NJ 07735. Author Withycombe is deceased.*

## RESULTS & DISCUSSION

USING AN EFFLUENT SPLITTER at the end of the GC column, an aroma profile was obtained on the tuna fish extract. A small peak was observed which exhibited an intense, pleasant, "beef extract" aroma. Since this component eluted on the tailing edge of a major component, furfuryl alcohol, it was necessary to use subtraction techniques to obtain a clean mass spectrum. After subtracting the mass spectrum of furfuryl alcohol and the chromatographic background, we were able to identify the component of interest as 2–methyl–3–furanthiol. The identification was confirmed by comparing the mass spectrum to that of authentic material synthesized in our laboratory (Evers et al., 1976). Additional confirmation was obtained by matching $I_E$ values, that is, the retention indices relative to a series of ethyl esters of normal alkanoic acids (van den Dool and Kratz, 1963). Figure 1 compares the mass spectrum of the unknown compound to that of the reference standard.

## REFERENCES

Evers, W.J., Heinsohn, H.H., Mayers, B.J., and Sanderson, A. 1976. Furans substituted at the three position with sulfur. In "Phenolic, Sulfur,

—Continued on page 660

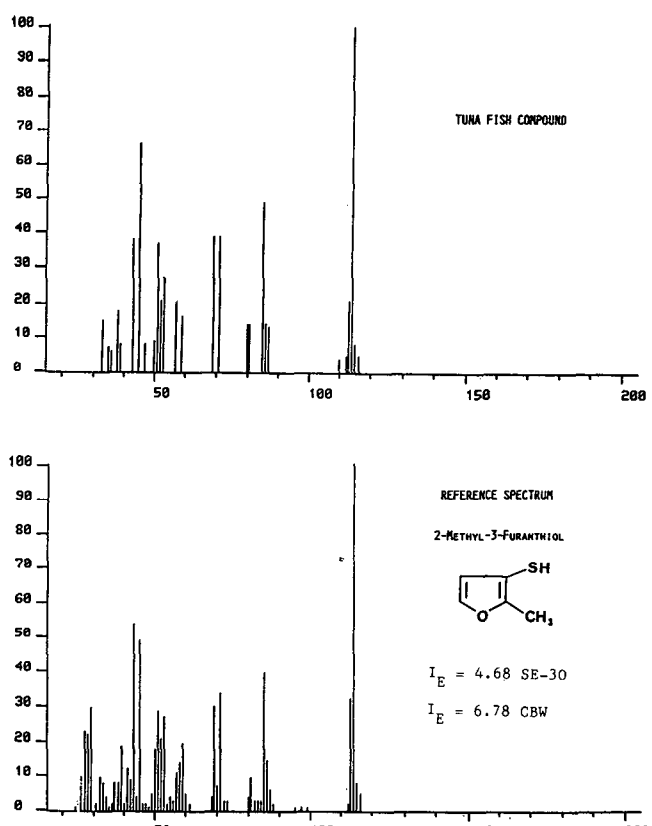


*Fig. 1—Mass spectrum of tuna fish compound and 2-methyl-3-furanthiol reference spectrum.*

Case No.: IPR2023-00208
U.S. Patent No. 11,013,250



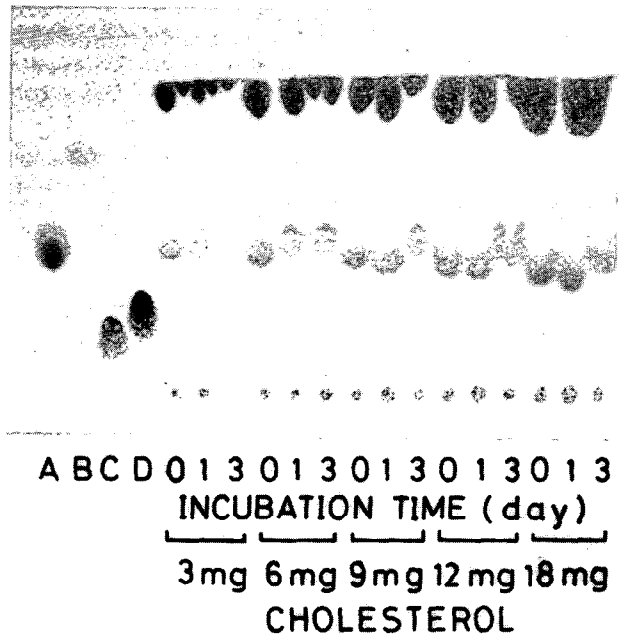


*Fig. 2—Degradation of egg yolk-cholesterol as detected by thin-layer chromatography: A: cholesterol, B: 4-cholesten-3- one, C: 1,4-androstadiene-3,17-dione, D; 4-androstene-3,17-dione.*

free cholesterol by *R. equi* No. 23, as reported previously (Aihara et al., 1986; Watanabe et al., 1987).

Most cholesterol in egg yolk has been reported to be present in the low density lipoprotein (LDL) particle (Gornall and Kuksis, 1971). Egg yolk LDL particles appear to be spheres of lipid surrounded by a layer of glycoprotein, phospholipid and cholesterol with hydrophobic groups on the inside and hydrophylic groups oriented to the outside. A large portion of the cholesterol in LDL (about 92% of total cholesterol) is extractable by ether (Evans et al., 1973). This means that cholesterol in egg yolk LDL is not involved in strong interactions with macromolecules. Thus, cholesterol could probably be degraded more easily by the degradative enzymes of *R. equi* No. 23 than cholesterol present in other classes of lipoproteins which could not be extracted by ether.

We did not investigate the degradation of cholesterol esters in this study, because cholesterol esters are present in egg yolk in only small amounts (5-8%), as reported by Bitman and Wood (1980). At the present, it is unknown whether *R. equi* No. 23 produces cholesterol-ester hydrolase.

It is not clear from the results of this study whether cholesterol degradation in egg yolk resulted from the action of cholesterol- degrading enzymes alone or from the combined action with other enzymes such as lipase, phospholipase and protease. Further studies are needed to elucidate the mechanism of cholesterol degradation by *R. equi* No. 23 in egg yolk and its LDL component.

## REFERENCES

Aihara, H., Watanabe, K. and Nakamura, R. 1986. Characterization of production of cholesterol oxidase in three *Rhodococcus* strains. J. Appl. Bacteriol. 61: 269.
Bair, C.W. and Marion, W.W. 1978. Yolk cholesterol in eggs from various avian species. Poultry Sci. 57: 1260.
Bitman, J. and Wood, D.L. 1980. Cholesterol and cholesterol ester of eggs from various avian species. Poultry Sci. 59:2014.
Evans, R.J., Bauer, D.H., Bandeman, S.L., Vaghefi, S.B., and Flegal, C.J. 1973. Structure of egg yolk very low density lipoprotein. Polydispersity of the very low density lipoprotein and the role of lipovitellenin in the structure. Arch. Biochem. Biophys. 154: 493.
Flynn, M.A., Nolph, G.B., Flynn, T.C., Kahrs, R., and Krause, G. 1979. Effect of dietary egg on human serum cholesterol and triglycerides. Am. J. Clin. Nutr. 32: 1051.
Gornall, D.A. and Kuksis, A. 1971. Molecular species of glycerophosphatides and triglycerides of egg yolk lipoprotein. Can. J. Biochem. 49: 51.
Kummerow, F.A., Kim, Y., Hull, J., Pollard, J., Ilnov, P., Dorossiev, D.L., and Valek, J. 1977. The influence of egg consumption on the serum cholesterol level in human subjects. Am. J. Clin. Nutr. 30: 664.
Porter, M.W., Yamanaka, W., Carlson, S.D., and Flynn, M.A. 1977. Effect of dietary egg on serum cholesterol and triglyceride of human males. Am. J. Clin. Nutr. 30: 490.
Roberts, S.L., McMurry, M.P., and Connor, W.E. 1981. Does egg feeding (i.e., dietary cholesterol) affect plasma cholesterol levels in humans? The results of a double-blind study. Am. J. Clin. Nutr. 34: 2092.
Turfitt, G.E. 1944. Microbiological agencies in the degradation of steroids. I. The cholesterol-decomposing organisms of soils. J. Bacteriol. 47: 487.
Watanabe, K., Aihara, H., Tachi, N. and Nakamura, R. 1987. Degradation of 4-cholesten-3-one and 1,4-androstadiene-3,17-dione by cholesterol-degrading bacteria. J. Appl. Bacteriol. 62: 151.
Watanabe, K., Shimizu, H., Aihara, H. Nakamura, R., Suzuki, K. and Komagata, K. 1986. Isolation and identification of cholesterol-degrading *Rhodococcus* strains from food of animal origin and their cholesterol oxidase activities. J. Gen. Appl. Microbiol. 32: 137.
Ms received 12/3/87; accepted 12/9/87.

 From page 658

and Nitrogen Compounds in Food Flavors," (Ed) Charalambous, G. and Katz, I. ACS Symposium Series No. 26. American Chemical Society, Washington, DC.
MacLeod, G. and Ames, J. 1985. 2–Methyl–3–(methylthio)furan: a meaty character impact aroma compound identified from cooked beef. Chem. Ind. 5: 175.
Tressl, R. and Silwar, R. 1981. Investigation of sulfur-containing components in roasted coffee. J. Agric. Food Chem. 29: 1078.
van den Dool, H. and Kratz, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11: 463.
Watson, J.T. and Biemann, K. 1965. Direct recording of high resolution of mass spectra. Anal. Chem. 37: 844.
Withycombe, D.A., Walradt, J.P., and Hruza, A. 1976. Simultaneous detection of nitrogen and sulfur containing flavor volatiles. In "Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors," (Ed.) Charalambous, G. and Katz, I. ACS Symposium Series No. 26. American Chemical Society, Washington, DC.
Ms received 11/8/87; accepted 11/12/87.



Motif Exhibit 1095, Page 2 of 2