

A NEW FUROXAN DERIVATIVE AND ITS PRECURSORS FORMED BY THE REACTION
OF SORBIC ACID WITH SODIUM NITRITE

Toshihiko Osawa, Yukio Kito and Mitsuo Namiki

Department of Food Science and Technology, Nagoya University

Chikusa-ku, Nagoya, 464, Japan

Keiichi Tsuji

The Institute of Physical and Chemical Research

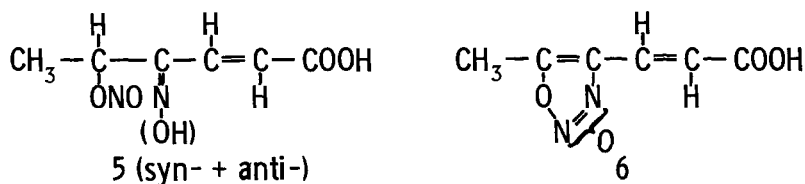
Wako, Saitama, 351, Japan

Abstract; A new furoxan derivative (3) and its precursors (4a and 4b) were isolated from the reaction mixture of sorbic acid with sodium nitrite, and their structures were determined by spectroscopic and X-ray crystallographic analysis.

Sorbic acid (trans, trans-2, 4-hexadienoic acid), which has been widely used as a food preservative of low toxicity, and sodium nitrite, that is also used in meat processing, react together to produce a mixture showing the strong mutagenic activity towards bacteria¹). We have been concerned with the study of the products and mechanism of this reaction and isolated two products, 1,4-dinitro-2-methyl pyrrole (1) and ethylnitrolic acid (2), as the main mutagenic principles in the reaction mixture²⁾³). Recently we have isolated from the reaction mixture a new product named substance F that seemed to carry a furoxan ring system, as well as two isomeric products that were presumably the precursors to substance F in the course of the reaction (pre-F 1 and 2). We have assumed the tentative structure for substance F but this remained inconclusive⁴). Here we wish to report the determination of their unambiguous structures, mainly by spectroscopic and X-ray crystallographic analysis.

Sorbic acid (1.12g) and sodium nitrite (5.52g) dissolved in 1 liter distilled water was warmed at 60°C for 30 min, keeping pH of the solution at 3.5 by automatic addition of 1 N sulfuric acid⁵). After pH adjustment to 4.2, the mixture was extracted with ethyl acetate, and subsequent purification on silica gel column (Wakogel C-100) and by preparative tlc (Wakogel B5-FM) yielded 70mg of substance F (m.p. 160-162°C) and respective amounts of 1 and 2. Elemental analysis of substance F showed C;42.39%, H;3.65% and N;16.47% (calc. for C₆H₆N₂O₄: C;42.36%, H;3.56% and N;16.47%). Molecular ion peak of substance F was observed at m/e 170.0325 (C₆H₆N₂O₄) and other peaks were observed at m/e 140(M⁺-NO), 126(M⁺-CO₂), 110(M⁺-N₂O₂) and 93(M⁺-N₂O₂-OH). IR spectrum shows ν_{\max}^{KBr} 3100-2500cm⁻¹ and 1695cm⁻¹ (carboxyl), 1658cm⁻¹ (double bond) and 1600cm⁻¹ (C=N-). ¹H-NMR spectrum (d₆-acetone, TMS) exhibited a 3H singlet at δ 2.34 (C-CH₃), two 1H doublets at δ 6.76 and δ 7.47 (J=16.7Hz) attributable to olefinic protons, and a broad singlet at δ 10.49 (disappeared by addition of D₂O) due to carboxylic proton and it

Literature search revealed that our products 4a and 4b have very much in common with the products previously obtained by Tanaka et al. by the reaction of sorbic acid with N_2O_3 in chloroform⁸⁾, in regard to the physical and spectroscopic characteristics. They have given the structure 5 to this compound. Moreover, it is to be noted that it was converted to another product by acid treatment, to which they have attributed the structure 6⁸⁾. The latter product also show significant similarity to our substance F in its nature. We therefore performed X-ray crystallographic analysis of the substance F in order to confirm the structure 3.



A single crystal of substance F, obtained from ethyl acetate-methanol, was shown to belong to monoclinic space group $P2_1/C$ with $a=7.101$, $b=11.110$, $c=9.955\text{\AA}$ and $\beta=106.41^\circ$. The unit cell contains four molecules. Intensity data were measured on a Syntex P3/F fourcircle diffractometer using $\text{MoK}\alpha$ radiation. Corrections were made in the usual way and the structure has been solved by Syntex XTL System using program 'MULTAN'⁹⁾ and refined by a full matrix least squares method. From bond lengths and temperature factors, all carbons, nitrogens and oxygens were identified. After refinement using anisotropic temperature factors for carbon, nitrogen and oxygen, all hydrogen atoms were located on a difference map (Fig. 1). R factor for observed reflections' is 0.073 at the present stage.

We believe that the crystallographic results provided a firm basis for the structure 3 given to the substance F. It looks certain now that at least under conditions employed the reaction of sorbic acid with sodium nitrite can proceed as shown in the scheme. The biological activities of the products 3, 4a and 4b are under investigation. Whether pre-F 1 and 2 can be the precursors to the mutagenic products 1 and 2 or not is not yet known at this stage.

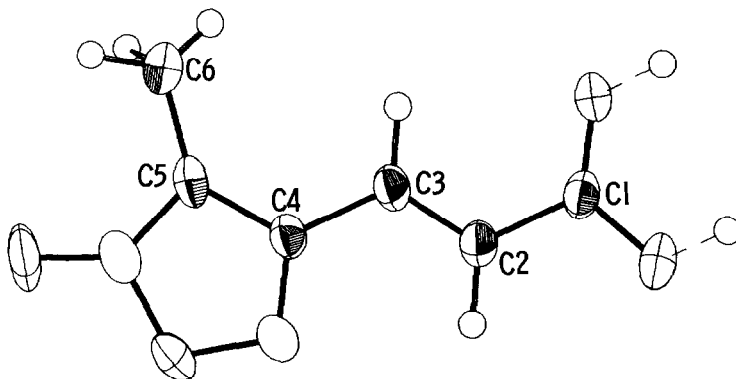


Fig. 1. Molecular structure of substance F.

Acknowledgement. This work was supported by a grant from Nissan Science Foundation. The authors wish to thank to Mr. T. Uraji and Miss H. Ishibashi for their excellent technical assistance and also to Prof. T. Goto of our Department, Prof. K. Namiki of Sugiyama women's University and Dr. T. Kada of National Institute of Genetics for valuable discussions and suggestions. They are grateful to Mr. A. Wada and staffs of JASCO Instruments for X-ray crystallographic analysis.

Footnotes and References.

- 1) T. Kada, Ann. Rep. Nat. Inst. Genet. (Japan), 24 43 (1974).
- 2) Y. Kito, M. Namiki and K. Tsuji, Tetrahedron, 34, 505 (1978).
- 3) M. Namiki and T. Kada, Agr. Biol. Chem., 39, 1335 (1975).
- 4) Chemical structure of 3 was presented by M. Namiki, Y. Kito, T. Uraji and H. Ishibashi at the meeting of Agricultural Chemical Society of Japan in Yokohama (1978, April).
- 5) M. Namiki, T. Osawa and K. Namiki, presented at ACS/CSJ Chemical Congress (Hawaii), 1979, April.
- 6) 3, 4-diacetyl furoxan was prepared according to the Ungnade and Kissinger's method (J. Org. Chem., 24, 666, 1959), and ^{13}C -NMR spectrum exhibits two carbons at 111.43 and 153.30ppm (s) due to furoxan ring (d_6 -acetone, TMS).
- 7) H. Siegerman in "Techniques of Chemistry". Vol. V, Pt. I, John Wiley and Sons, 1974, p. 667.
- 8) K. Tanaka, K.C. Chung, H. Hayatsu and T. Kada, J. Fd. Cosmet. Toxicol., 16, 209 (1979).
- 9) Modified "MULTAN, A Computer Program for the Automatic Solution of Crystal Structures" by P. Main, M. M. Woolfson and G. Germain, Dept. of Physics, University of York, York, England and Laboratoire de Chemie, Universite de Lourain, Lourain, Belgium (May, 1971) for Syntex XTL System.

(Received in Japan 6 August 1979)