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

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Abstract; A new furoxan derivative $(\underline{3})$ and its precursors $(\underline{4a} \text{ and } \underline{4b})$ 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 (<u>trans</u>, <u>trans</u>-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 (<u>1</u>) and ethylnitrolic acid (<u>2</u>), 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. $160-162^{\circ}C$) 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 $C_6H_6N_2O_4$: C;42.36%, H;3.56% and N;16.47%). Molecular ion peak of substance F was observed at m/e 170.0325 ($C_6H_6N_2O_4$) and other peaks were observed at m/e 140(M^+ -NO), 126(M^+ -CO₂), 110(M^+ -N₂O₂) IR spectrum shows v^{KBr} 3100-2500 cm⁻¹ and 1695 cm⁻¹ (carboxy1), 1658 cm⁻¹ and $93(M^+ - N_2O_2 - OH)$. (double bond) and 1600 cm^{-1} (>C=N-). 1 H-NMR spectrum (d₆-acetone, TMS) exhibited a 3H singlet at δ2.34(C-CH₃), two lH doublets at δ6.76 and δ7.47 (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

has the UV absorption at $\lambda_{\text{max}}^{\text{EtOH}}$ 233nm (ϵ =24,000). ¹³C-NMR spectrum (d₆-acetone, TMS) showed a methyl carbon at $\delta 8.6(q)$, two olefinic carbons at $\delta 127.8$ and $\delta 129.6$ (d, respectively), two carbons in a furoxan ring ($\delta 112.5$ and $\delta 154.5$, s) and carboxyl carbon at $\delta 166.0$ (s). These data, especially the comparion of the ¹³C-NMR spectrum of substance F with that of 3, 4-diacetylfuroxan⁶), established the presence of the furoxan ring system in the molecule of substance F, and the structure 3 was given to substance F.

As the reaction of sorbic acid with sodium nitrite yielded only limited amount of pre-F products, the reaction was repeated at 4°C in an ice-bath with other conditions unchanged. By densitometry on tlc it was found that the amount of the pre-F species reached maximum after 12 hrs and then decreased, while, after 48 hrs, the products <u>1</u>, <u>2</u> and <u>3</u> appeared in the reaction mixture as the main reaction products. The mixture reacted for 12 hrs at 4°C was worked up in the similar manner, and yielded the crude pre-F and it was separated into two components (1:1) by means of preparative HPLC (column; Bondapack C-18 porasil B and solvent system; H₂0: MeOH:acetic acid=90:10:1). Both of these products were quantitatively converted into <u>3</u> by reaction with sodium nitrite at pH 3.5, 60°C for 30min, and are very likely the precursors to <u>3</u> in the course of the reaction and named pre-F 1 and 2, respectively.

Pre-F1 and 2 (m.p. 128-9 and 165-6°C, respectively) have the same molecular ion peak at m/e 188.0450 ($C_6H_8N_2O_5$) and also other peaks at m/e 142 (M⁺-NO₂) and 124 (M⁺-NO₂-H₂O) as well. IR spectra of both also showed the same absorption at v_{max}^{KBr} 2800-3600cm⁻¹ (carboxyl), 1670cm⁻¹ (double bond) and 1560 and 1380cm⁻¹ (nitro). They were both polarographically reducible, and their least negative half wave potentials (-0.43 and -0.46V vs SCE, respectively in pH 2,60 tris-buffer) fell in the range of those for unconjugated C-nitro compounds⁷). They also absorb similarly in uv range λ_{max}^{EtOH} 253nm (ε =18,000). The difference in the spectroscopic data between pre-F1 and 2 were observed only in ¹H-NMR spectra (d₆-acetone, TMS).

Pre-F 1 exhibits a 3H doublet at $\delta 1.78$ (d, J=6.9Hz) due to C-methyl protons, 1H of $-C\underline{H}-NO_2$ at $\delta 5.97$ (q, J=6.9Hz) and two olefinic protons at $\delta 6.42$ and $\delta 7.80$ (d, J=16.7Hz). Pre-F 2 shows a 3H doublet at $\delta 1.78$ (d, J=6.9Hz) and a 1H proton at $\delta 5.90$ (q, J=6.9Hz) and two olefinic protons were observed at $\delta 6.31$ and $\delta 6.95$ (d, J=16.7Hz). From these spectroscopic data and the fact that pre-F 1 and 2 can be easily transformed to 3, we assumed the structures of pre-F 1 and 2 as $\underline{4a}$ and $\underline{4b}$ (syn- and anti- conformers).

$${}^{6}CH_{3} \xrightarrow{-5}{C} = {}^{4}C \xrightarrow{-3}{C} = {}^{2}C \xrightarrow{-1}COOH + NaNO_{2} \xrightarrow{-pH 3.5}{4^{\circ}C} \left(CH_{3} \xrightarrow{-}CH \xrightarrow{-}$$

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Literature search revealed that our products $\underline{4a}$ and $\underline{4b}$ have very much in common with the products previously obtained by Tanaka et al. by the reaction of sorbic acid with N₂O₃ 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 $\underline{6^8}$. 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 P2₁/C with a=7.101, b=11.110, c=9.955Å and β =106.41°. The unit cell contains four molecules. Intensity data were measured on a Syntex P3/F fourcircle diffractometer using MoKa 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 $\underline{3}$ 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 $\underline{3}$, $\underline{4a}$ and $\underline{4b}$ are under investigation. Whether pre-F l and 2 can be the precursors to the mutagenic products $\underline{1}$ and $\underline{2}$ or not is not yet known at this stage.



Fig. 1. Molecular structure of substance F.

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Footnotes and References.

- 1) T. Kada, <u>Ann. Rep. Nat. Inst. Genet.</u> (Japan), <u>24</u> 43 (1974).
- 2) Y. Kito, M. Namiki and K. Tsuji, <u>Tetrahedron</u>, <u>34</u>, 505 (1978).
- 3) M. Namiki and T. Kada, Agr. Biol. Chem., 39, 1335 (1975).
- 4) Chemical structure of <u>3</u> 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).
- 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 (<u>J. Org.</u> <u>Chem.</u>, <u>24</u>, 666, 1959), and ¹³C-NMR spectrum exhibits two carbons at 111.43 and 153.30ppm (s) due to furoxan ring (d₆-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.

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