[Contribution from the Research Department of Eaton Laboratories, Inc.]

# Furans. III. Crystalline Modifications of 5-Nitro-2-furaldehyde Semicarbazone

By Robert F. Raffauf[1] and F. L. Austin[2]

During the course of study of a number of nitrofuran derivatives prepared for bacteriostatic examination,[3] it was observed that one of them, 5-nitro-2-furaldehyde semicarbazone, existed in two different crystalline modifications obtained as a result of different preparations of the compound.

## Experimental

**Preparation.**—5-Nitro-2-furaldehyde semicarbazone has been prepared in these laboratories by two general methods: (A) by the addition of an alcoholic solution of the aldehyde to a buffered solution of semicarbazide hydrochloride at room temperature, and (B) by warming the aldehyde diacetate and semicarbazide hydrochloride with dilute mineral acid. In the first instance, the product was obtained as a voluminous precipitate of lemon-yellow needles (I), while that obtained by method (B) was orange-yellow in color and prismatic in character (II). Both forms were washed with alcohol and dried to constant weight prior to analysis. The needles (I) melted with gross decomposition at 230–232° (β-form), whereas the prisms (II) melted with decomposition at 238–240° (α-form).

*Anal.* Calcd. for $C_6H_6N_4O_4$: C, 36.4; H, 3.03. Found: (α) C, 36.5; H, 3.19; (β) C, 36.4; H, 2.98.

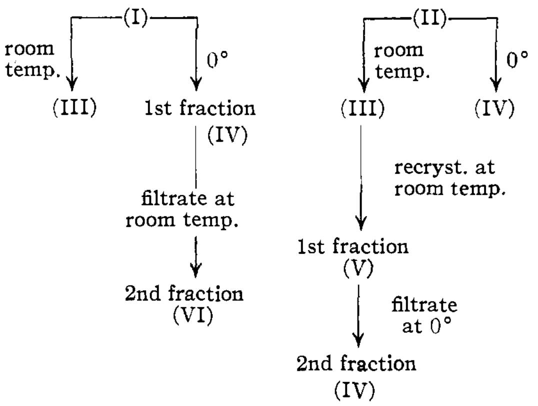
**Crystallization Studies.**—Using freshly distilled furfuryl alcohol as the solvent, the behavior of the compound under varied conditions of crystallization demonstrated that the two forms could be interconverted merely by modifying these conditions. One gram of either (I) or (II) was dissolved in 80 cc. of warm (85°) furfuryl alcohol. The solution was filtered and, when cooled to room temperature, was diluted with 20 cc. of a 1:1 mixture of ethanol and ether. On standing at room temperature, the solution slowly deposited a homogeneous crop of small light yellow-orange prisms (III), whereas at 0° light yellow rectangular plates (IV) were obtained.

In a second experiment, a sample of the prisms (III) was crystallized at room temperature from furfuryl alcohol alone. A small first crop of crystals (V) was removed, washed with ethanol–ether (1:1), and the mixture of filtrate and washings was cooled to 0°. A second crop of crystals was obtained similar in appearance to the plates (IV).

In a third experiment, a sample of (I) was crystallized at 0° from furfuryl alcohol alone. A small crop of (IV) was removed, washed with ethanol–ether (1:1), and the combined filtrate and washings was allowed to stand at room temperature. A second fraction (VI) was obtained similar in gross appearance to (III).

In all cases, the solutions were protected from the light due to the photosensitive nature of the compound. A summary of these crystallization studies is given in the chart.

**X-Ray Data.**—The relationships between the several crystal fractions were established by means of their X-ray powder diffraction patterns. The accompanying photographs (Fig. 1) indicate clearly the identity of fractions (I) and (IV) (β-form, m. p. 230–232° dec.), and of (II), (III), (V) and (VI) (α-form, m. p. 238–240 dec.), and thus establish the fact that the interconversion of the two forms was effected by varying the conditions of crystallization.



"The diffraction patterns were taken with CuK$_\alpha$ radiation using Buerger precision powder cameras of 57.3-mm. radius. The samples of 200-mesh powder were packed into 0.64-mm. ethyl cellulose tubes and sealed with a drop of Duco cement. Eastman No-Screen X-ray film was used, and loaded into the cameras in asymmetric position. The exposures were identical; two hours at approximately 30 kv. peak and 20 ma."[4]

**Ultraviolet Absorption Studies.**—An examination of the ultraviolet absorption characteristics of aqueous solutions of crystal fractions (I)–(IV) in the region 220–400 mμ showed them to be identical, with maxima at 260 and 375 mμ and $\epsilon_{\text{max.}}$ = 13200 and 15800, respectively (Fig. 2). The method used for obtaining the spectra was that given in a previous paper.[5]

**Freezing Point Depression Studies.**—The method of Sidgwick[6] for distinguishing between isomers and polymorphs was applied to the two forms of 5-nitro-2-furaldehyde semicarbazone using phenol ($K_f$ = 7.27). The addition of a small amount of the less soluble α-form to a saturated solution of the β-form caused no depression, but rather a final slight elevation of the freezing point of the mixture. Depression of the f. p. by β-form = 2.52°; solubility = 6.87 g./100 g.; β + α = 2.54°. After a time, the depression caused by β + α was diminished 0.2°. This behavior satisfies Sidgwick's criterion for cases of polymorphism.

**Miscellaneous Observations.**—It was hoped to gain some information by a study of the optical properties of the crystals. These were

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[3] Dodd and Stillman, *J. Pharmacol.*. **82** 11 (1944).

[4] From a report by Dr. H. P. Klug, formerly of the School of Chemistry of the University of Minnesota, now of the Mellon Institute, Pittsburgh, Pa., whose aid in compiling and interpreting the X-ray data is gratefully acknowledged.

[5] R. F. Raffauf, Paper I, This Journal, **68**, 1765 (1946).

[6] N. V. Sidgwick, *J. Chem. Soc.*, **107**, 672 (1915).

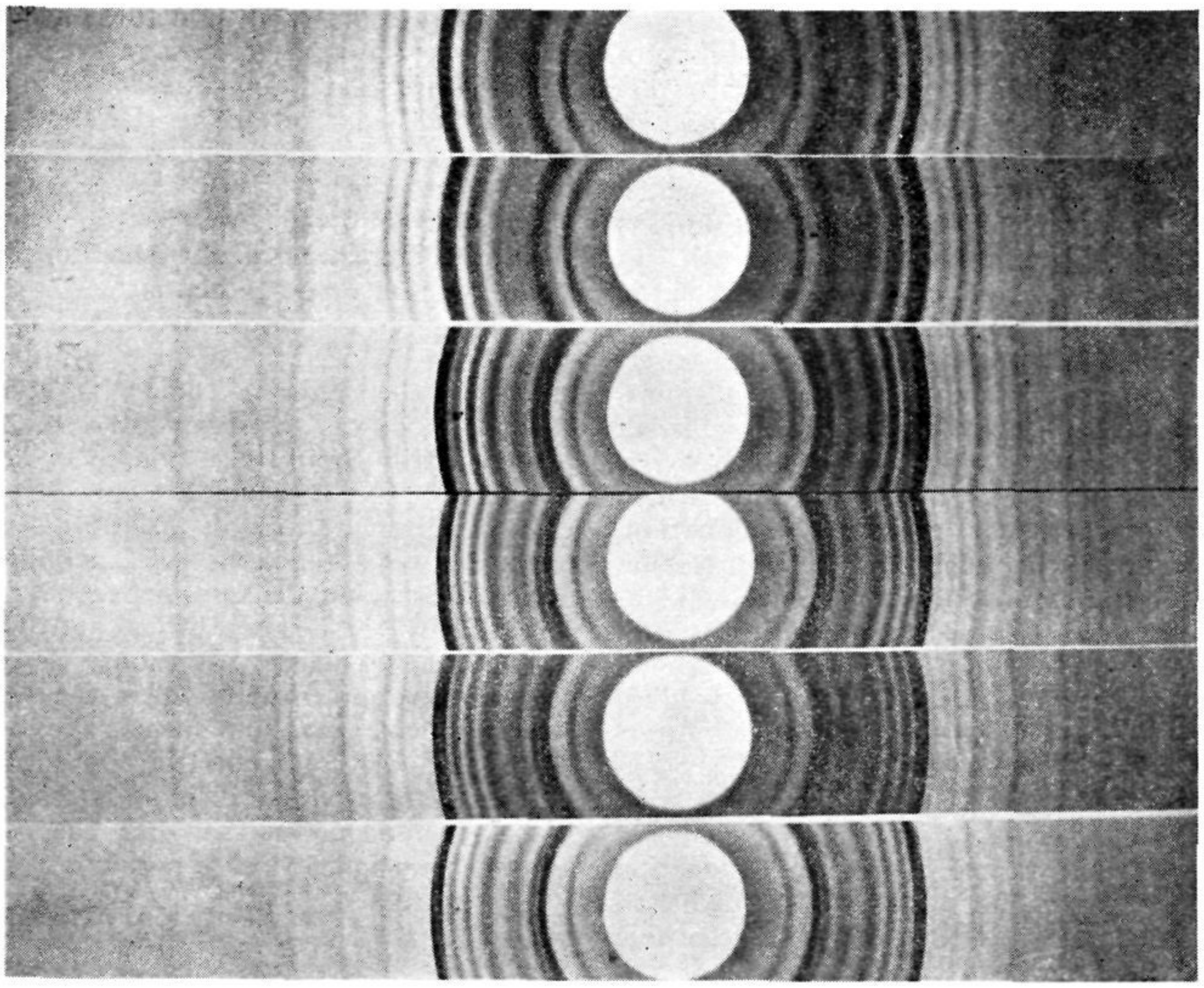

Fig. 1.—X-Ray diffraction patterns of crystal fractions of 5-nitro-2-furaldehyde semicarbazone: from top to bottom: fractions I, IV, II, III, V, VI.

not well adapted to observation under crossed nicols. The prisms ($\alpha$-form) showed both parallel and oblique (38°) extinction. A few interference figures were obtained which indicated that both forms are biaxial with a large axial angle.

Using a variety of solvents, there was no detectable difference in the rate or degree of absorption of the two forms on a chromatograph column of "Neutrol."

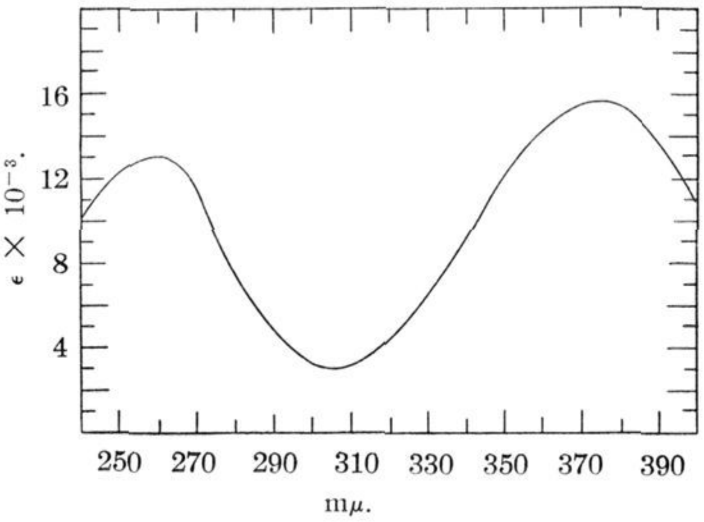

Fig. 2.—Ultraviolet absorption spectrum of 5-nitro-2-furaldehyde semicarbazone; $c$ = 11.4 mg./l. in water; $l$ = 1.0 cm.

## Discussion

In the case of 5-nitro-2-furaldehyde semicarbazone, structural theory permits a *syn–anti* relationship at the carbon-nitrogen linkage. The results of the X-ray diffraction study clearly indicate that a fundamental difference between the two forms does in fact exist in the solid state, *i. e.*, the obvious differences cannot be ascribed merely to a single molecular species capable of existence in different crystal habit. By analogy with the oximes, and by reason of a few recorded cases of carbon–nitrogen isomerism in the semicarbazones,[7] a *syn–anti* relationship between the two forms was considered. It has been shown for the isomeric furaldoximes and nitrofuraldoximes that the position of the absorption maxima of the *syn*-isomers in the near ultraviolet is located at higher wave lengths than is true of the *anti*-forms.[5] Unlike the oximes, however, aqueous solutions of the two forms were indistinguishable spectrophotometrically. This failure of the modifications to preserve their identity in solution was also true for other solvents. This was evident from the cryoscopic studies in phenol, and from solubility studies in furfuryl alcohol where temperature of crystallization appeared to be the

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[7] Henderson and Heilbron, *J. Chem. Soc.*, **107**, 1740 (1915); Read and Smith, *ibid.*, **121**, 1863 (1922); Wilson and McCaulay, *ibid.*, **125**, 841 (1924); Hopper, *ibid.*, **127**, 1282 (1925); Bruzau, *Ann. Chim.*, [11] **1**, 257 (1934).

governing criterion for the formation of a given modification. Unfortunately, the insolubility of the compound in non-polar media prevented extension of the work to solvents of this character.

Obviously, observations on solutions are not necessarily characteristic of the situation which may exist in the solid state. Thus, solutions of tautomeric substances exhibit physical and chemical properties which may be expected on the basis of the relative proportions of the two forms present at a given time. Yet, in certain cases, the individual compounds comprising a tautomeric pair have been isolated and crystallized. When one deals with a substance which exists in more than one crystalline form for which classical structural theory provides no explanation, polymorphism is the customary interpretation regardless of the stability or persistence of identity of those forms. For example, Straus and Demus[8] found two forms of dibiphenylylcarbinol which were very stable, uninfluenced by seeding with each other and which were only slowly interconverted under various conditions. They exhibited different absorption characteristics in benzene. Similar cases of polymorphism have been cited by Naves and Korpi[9] in connection with their work on the oxalates of *dl*-menthol. Since various recorded cases of polymorphic forms show such marked differences in the degree to which their identity is retained, v. Auwers and Schaum[10] made a somewhat artificial classification of them into five groups. It is apparent that the physical and chemical behavior of many of these is such that they would be designated geometrical isomers if structural theory had so permitted. Obviously, where classical structural formulas are inadequate, it is necessary to find an explanation at the level of atomic arrangements within the molecule. This has been accomplished for the polymorphic forms of anhydrous oxalic acid[11] for which Raman spectra and infrared absorption indicated a *cis-trans* relationship at the carbon–carbon bond. This has been confirmed by Dunitz and Robertson[12] who deduced from an electron diffraction study of the hydrated acid that the carbon–carbon bond was slightly contracted and would possess attributes of the ethylenic linkage.

Infrared studies, which are expected to furnish more precise information on the discrete differences between the two solid modifications of 5-nitro-2-furaldehyde semicarbazone, have been initiated by these laboratories, and will be published at a later date. Without reference to the structural organization of the molecule which can produce two crystalline forms, the behavior of these modifications, particularly with reference to their interconvertibility and loss of identity in solution, is consistent with the simpler and more conventional cases of polymorphism.

## Summary

Two crystalline forms of 5-nitro-2-furaldehyde semicarbazone have been observed and their physical properties investigated. The gross behavior of the two forms is typical of that conventionally assigned to the more common examples of polymorphism.

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[8] Straus and Demus, *Ber.*, **59**, 2427 (1926).

[9] Naves and Korpi, *Helv. Chim. Acta*, **30**, 1219 (1947).

[10] v. Auwers and Schaum, *Ber.*, **62**, 1675 (1929).

[11] Duval and Lecomte, *Compt. rend.*, **212**, 389 (1941).

[12] Dunitz and Robertson, *J. Chem. Soc.*, 142 (1947).

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# Paper Chromatography in Synthetic Organic Chemistry. Microgram Scale Syntheses of Labeled Monoiodotyrosine, Diiodotyrosine and Thyroxine[1]

BY R. M. LEMMON, WINIFRED TARPEY AND KENNETH G. SCOTT

The availability of labeled organic iodide of high specific activity is very desirable for tracer studies of thyroid metabolism. Investigations into some of the differences in the thyroid activity of normal and tumor-bearing animals are now under way at the University of California Medical School and, in connection with these studies, we have carried out *in vitro* syntheses of $I^{131}$-labeled monoiodotyrosine, diiodotyrosine and thyroxine on a microgram scale. In order to isolate and identify the products of these syntheses we have made use of the technique of paper chromatography.[2] This technique, which has already proved so valuable in amino acid work, provided us with a method by which our products could be isolated from their respective reaction mixtures without the addition of any inactive carrier. The positions of the amino acids on the chromatograms were located by preparing radioautographs of the chromatograms. The identity of the material in a given spot was established by preparing mixed chromatograms of the eluted material with a known sample of monoiodotyrosine, diiodotyrosine or thyroxine. Although a number of procedures for the quantitative estimation of

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[2] R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem J.*, **38**, 224 (1944).