200 ml. of methanol was reduced in a low pressure hydrogenator over 24 hr. The insoluble solid and catalyst were filtered and recrystallized from 550 ml. of nitromethane to give 13.4 g. (63.5%). An analytical sample decomposed at 224–225°, $\lambda_{max.}$ 5% EtOH 348 m$\mu$ ($\epsilon$ 20,750). The infrared spectrum (mull) had bands at 3.0, 3.05, 5.8 and 6.3 $\mu$.

*Anal.* Calcd. for $C_8H_9N_3O_2S$: C, 45.50; H, 4.30; S, 15.15. Found: C, 45.55; H, 4.48; S, 15.15.

The acetyl derivative was prepared in 98% yield by heating the amine in acetic anhydride at steam bath temperature. After several recrystallizations from a 2:1 mixture of nitromethane and dimethylformamide, the compound decomposed at 269–271°, $\lambda_{max.}$ 2% DMF 338 m$\mu$ ($\epsilon$ 21,000). The infrared spectrum (mull) had bands at 3.1, 5.78, 5.95, 6.0 and 6.42 $\mu$.

*Anal.* Calcd. for $C_{11}H_{10}N_3O_3S$: C, 47.41; H, 4.38; S, 12.67. Found: C, 47.05; H, 4.77; S, 12.69.

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# The Metabolism of 5-Nitro-2-furaldehyde Acetylhydrazone

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The identification of 5-acetamido-2-furaldehyde acetylhydrazone in the urine of rabbits fed 5-nitro-2-furaldehyde acetylhydrazone is reported. Identification of 1,2-diacetylhydrazine and evidence for 5-amino-2-furaldehyde acetylhydrazone, 5-diacetylamino-2-furaldehyde acetylhydrazone and 5-nitro-2-furoic acid also are given. A yellow material, found in the urine of animals fed 5-nitro-2-furaldehyde acetylhydrazone, can be synthesized from a photochemical product of 5-nitro-2-furaldehyde diacetate.

A reductive metabolic pathway for nitrofuraldehyde hydrazone derivatives analogous to that demonstrated for certain aromatic

nitro compounds[1] has been postulated frequently.[2-4] Evidence for such a transformation has been limited to spectral alterations observed following exposure of various nitrofurans to either mammalian[2] or bacterial[3,4] systems. Postulated products have not been isolated and characterized.

Attempts to isolate and characterize products of the chemical reduction of nitrofuraldehyde derivatives have been relatively unsuccessful.[3b,3c,5] The recent work of Ebetino, *et al.*[6] however, has shown that aminofurans and their acetylated derivatives can be isolated after chemical reduction of the corresponding nitrofuran.

The present study is concerned with the metabolism of 5-nitro-2-furaldehyde acetylhydrazone (I). Products isolated from the urine of rabbits to which I was administered have been sufficiently characterized to prove a reductive pathway for the metabolism of I. Evidence for other pathways also is presented.

The 2,4-dinitrophenylhydrazone of 5-acetamido-2-furaldehyde (II) was isolated from the urine of rabbits dosed with I after the addition of 2,4-dinitrophenylhydrazine in hydrochloric acid to the urine, extraction with ethyl acetate and chromatography of the extract on alumina. This isolated derivative was identical with an authentic sample by melting point, ultraviolet spectra in neutral and in alkaline solution, infrared spectrum and $R_f$ value on paper chromatographs. Analytical results supported the empirical formula.

The isolation of this derivative from rabbit urine indicated the presence of 5-acetamido-2-furaldehyde acetylhydrazone (III). The acidic reagent will react with I under comparable conditions to give the 2,4-dinitrophenylhydrazone of 5-nitro-2-furaldehyde; a comparable reaction was assumed here. Additional evidence for the existence of III in urine is given by the demonstration of $C^{14}$ in the $R_f$ area corresponding to III after the administration of I labelled with $C^{14}$ in the acetylhydrazone side chain (see below).

For the isolation of 5-acetamido-2-furaldehyde acetylhydrazone (III), urine from rabbits dosed with I was extracted repeatedly with

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(4) R. E. Asnis, *Arch. Biochem. Biophys.*, **66**, 208 (1957).

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ethyl acetate. The extracts were concentrated, dried, and chromatographed on alumina. Elution with ethyl acetate and with ethanol yielded III and a small amount of a compound believed to be 5-diacetylamino-2-furaldehyde acetylhydrazone (IV). Acetylation of synthetic 5-amino-2-furaldehyde acetylhydrazone (V) yielded a crude product which, upon chromatography, gave results identical with those of the urine extract. $R_f$ values on paper chromatographs and ultraviolet spectra at pH 7 and at pH 12 were identical (Table I). The alkaline spectral shift indicated in Table I was reversible. Neither III nor IV, synthetic or isolated, could be crystallized to satisfactory purity for elemental analysis.

For infrared spectra, the column eluates were dried directly on KBr. Spectra of isolated and synthetic materials were identical and consistent with the postulated structures. In IV, absorption bands at 5.75 (with shoulder) and 6.05 $\mu$ were present, indicating a triacetyl compound. The location of the third acetyl is not known with certainty. Because of the regular progression of ultraviolet absorption maxima and $R_f$ values (Table I), the compound is written as a diacetylamino derivative rather than a 1,1-diacetylhydrazone.

TABLE 1

PROPERTIES OF COLUMN ELUATES

| Compound | $R_f$[a] | λ max pH 7 (mμ) | λ max pH 12 (mμ) |
|---|---|---|---|
| $(CH_3CO)_2N$-furan-$CH{=}NNHCOCH_3$[b] IV | 0.94 | 320 | 370 |
| $CH_3CONH$-furan-$CH{=}NNHCOCH_3$ III | 0.81 | 328 | 358 |
| $H_2N$-furan-$CH{=}NNHCOCH_3$[c] V | 0.67 | 350 | [d] |

[a] Chromatographed on Whatman 1 paper in 1-butanol:95% ethanol:0.5 $N$ $NH_4OH$ (4:1:1). [b] Probable structure, synthetic and isolated. [c] Synthetic only. [d] Unstable.

A portion of the isolated acetamidofuran (III) was converted to a 2,4-dinitrophenylhydrazone. A product with the same ultraviolet maxima and melting point as those of the same derivative of 5-acetamido-2-furaldehyde (II, described above) was obtained. Acid hydrolysis of the diacetylaminofuran (IV) was followed spectrally. The initial 320 mμ absorption maximum shifted to longer wave lengths in agreement with the expected IV (320 mμ) → III (328 mμ) → V (350 mμ) and/or 5-amino-2-furaldehyde (350 mμ).[6]

Trace amounts of the aminofuran (V) were eluted from alumina columns when urine extracts or synthetic III and IV were chromatographed. This probably represented degradation on the column. Paper chromatography of fresh rabbit urine indicated only traces of the nitro- (I) and aminofuran (V). In contrast, urines of I-dosed chickens showed similar quantities of the nitro- (I), acetamido- (III) and aminofuran (V).

In rabbit urine, 5-nitro-2-furoic acid (VI) was also present following the oral administration of I. Identification was based on $R_f$ value and spectral data after elution from the paper. An orange zone with the $R_f$ of 1,2-diacetylhydrazine was found after spraying the chromatograph with Ehrlich reagent (*p*-dimethylaminobenzaldehyde in $H_2SO_4$). 1,2-Diacetylhydrazine, the major excretion product of acetylhydrazine in rabbits,[7] was isolated by fractionation on Dowex 50 ($H+$) and IR-45 ($OH^-$) ion exchange resins according to the procedure of McKennis, *et al.*[7] A white crystalline product was obtained. Infrared spectrum, $R_f$ value and melting point corresponded to that of authentic 1,2-diacetylhydrazine. A mixture melting point was not depressed. These results, indicating hydrolysis of the —CH=N— linkage, are not surprising in view of the lability of I under conditions of acidity comparable to those found in the mammalian stomach.[2c]

In a chicken dosed orally with I, where acid hydrolysis of I in the more weakly acidic gastrointestinal tract[8] is less likely, 1,2-diacetylhydrazine, acetylhydrazine, or 5-nitro-2-furoic acid was not detectable by chemical spot-tests on paper chromatographs of the urine. In the rabbit, the same results were obtained if intraperitoneal injection of I were used to avoid the acidic gastrointestinal tract. The reduced nitrofurans and traces of unchanged I were found, regardless of the route of administration of I. These results indicated metabolism of the nitrofuran I via enzymatic reduction and, in appropriate circumstances, metabolism of the products of acid hydrolysis (Fig. 1).

Paul, *et al.*[2c] reported a yellow crystallizable material which absorbed at 412.5 m$\mu$ in the urine of rabbits fed 5-nitro-2-furaldehyde semicarbazone. We have found a similar metabolite in the urine of rabbits, rats, dogs, and monkeys after feeding I; ultraviolet absorption maxima occurred at 415 and 317 m$\mu$. This metabolite (VII) does not appear to be derived by the pathway outlined in Fig. 1. In rat experiments, administration of VII did not yield the reduced

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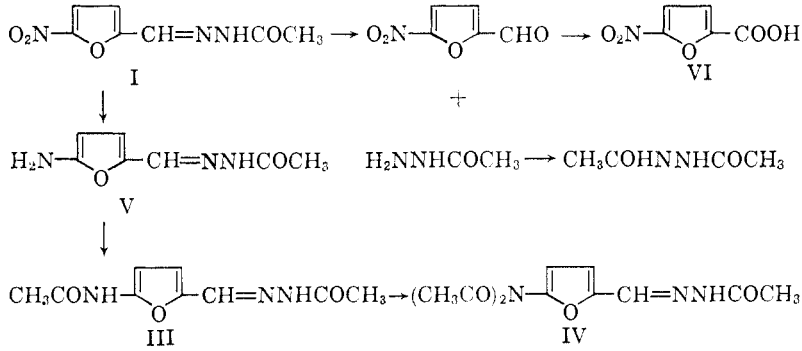


Fig. 1.—Metabolism of 5-nitro-2-furaldehyde acetylhydrazone.

nitrofurans nor did dosing the reduced nitrofurans yield VII. Both a nitro group and an azomethine linkage were necessary for VII formation.

Mild acid hydrolysis of VII yielded a product absorbing at 400 m$\mu$ (VIII). This product could also be produced photochemically from 5-nitro-2-furaldehyde diacetate (IX). Irradiation of dry crystalline IX in sunlight followed by solution in warm ethanol and extraction with water-ethyl acetate yielded a labile compound in the aqueous layer absorbing at 400 m$\mu$. This product was coupled immediately with 1-isopropylidene-2-acetylhydrazine in dilute hydrochloric acid to yield a derivative absorbing at 415 m$\mu$. Ultraviolet spectrum and $R_f$ value corresponded to that of the urine metabolite VII. Both yielded a red dinitrophenylhydrazone derivative with the same $R_f$ and ultraviolet absorption maximum. Concentration of the solution containing VII precipitated a solid, but attempts to obtain a pure crystalline product were unsuccessful; "polymers" seemed to form with each recrystallization. Experiments to identify VII and the metabolic pathway of its formation continue.

In order to assess the quantitative significance of the various metabolites, two $C^{14}$-labeled nitrofurans, $\overline{O(NO_2)C{=}CHCH{=}CC^{14}H{=}NNHCOCH_3}$ (X) and $\overline{O(NO_2)C{=}CHCH{=}CCH{=}NNHC^{14}OCH_3}$ (XI) were fed to rats and the urines chromatographed and examined for labeled metabolites. VII accounted for 20% of the radioactivity in both urines while about 6% of the urine radioactivity was 5-acetamido-2-furaldehyde acetylhydrazone (III). Total radioactivity in a 4 hr. urine collection represented 5% recovery of the administered $C^{14}$.

The data on the metabolites of 5-nitro-2-furaldehyde acetylhydrazone (I) presented here prove a reductive pathway of metabolism in the rat, rabbit and chicken. Acid hydrolysis of I in the gastrointestinal tract accounts for the presence of 5-nitro-2-furoic acid and 1,2-diacetylhydrazine in the urine of some species. These are the metabolites of the hydrolytic products, 5-nitro-2-furaldehyde and acetylhydrazine.[7,11] An additional independent pathway leading to the formation of a labile metabolite absorbing at 415 mμ also has been established, in confirmation of previous reports.[2c]

## Experimental[11]

Compounds were fed by stomach tube to 3 kg. New Zealand white rabbits, male and female, usually in carboxymethylcellulose suspension at a 100 mg./kg./day level for a maximum of 5 days. Urine was collected 0–6 days, filtered through glass wool, and frozen until used. For intraperitoneal dosing, two male 2 kg. rabbits received 150 mg. and 250 mg. of I, respectively. Urine was collected for 24 hr. and chromatographed. Male and female Wistar rats weighing 200 g. were dosed perorally at 100 mg./kg. and urine was collected for 4 hr. A 3 kg. Rhode Island Red-Barred Rock cross hen was dosed orally with 100 mg./kg. and urine was collected for 5 hr. according to the method of Sperber.[9]

Samples were chromatographed on Whatman #1 filter paper in 1-butanol: 95% ethanol:0.5 *N* $NH_4OH$ (4:1:1). Ehrlich's reagent[7] was used to detect hydrazine derivatives and 0.5 *N* NaOH to detect nitro groups (a brown color was obtained). Merck alumina, Amberlite IR-45 and Dowex 50-X4 were used in column separations. The syntheses of I and IX have been described elsewhere.[12,13]

For the $C^{14}$ experiments, two labeled nitrofurans X, 1.1 μc./mg., and XI, 1.7 μc/mg., were synthesized.[14] These compounds were each administered perorally to one female rat at 100 mg./kg. Urine was collected for 4 hr. Total radioactivity of urine samples was determined using a Packard Tri-Carb Liquid Scintillation Spectrometer with the suspending medium of Gordon and Wolfe.[15] The $C^{14}$ activity of labeled areas on paper chromatographs were measured with a Nuclear Chicago Actigraph II, Model C-100 B coupled with Analytical Count Ratemeter, Model 1620B and Model R1000 Rectilinear Recording Milliammeter.

**5-Acetamido-2-furaldehyde 2,4-dinitrophenylhydrazone: Isolation from Urine.**

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(11) The authors are indebted to several members of the staff of Eaton Laboratories: Mr. Frank Ebetino provided the synthetic nitrofuraldehyde and aminofuraldehyde derivatives, Dr. Claude Spencer, the 1,2-diacetylhydrazine, and, together with Dr. John Howard, the infrared spectra. Mr. George Klein synthesized the $C^{14}$-labeled compounds; assistance in the hen studies was provided by Dr. R. C. Bender. G. Gustin and associates of the Analytical Section performed all analyses.

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—Urine of rabbits dosed with 5-nitro-2-furaldehyde acetylhydrazone (I) was acidified with HCl to pH 3.5 and the solids removed by centrifugation. The urine was extracted once with an equal volume of ethyl acetate to remove fat-like materials and 0.4 volume of 0.2% 2,4-dinitrophenylhydrazine in 2 *N* HCl was added. After standing for 1 hr. at room temperature, the urine was extracted twice with about 0.2 volume of ethyl acetate, the extracts were concentrated *in vacuo*, and the light colored solids were removed by centrifugation. The ethyl acetate solution was dried with sodium sulfate and chromatographed on alumina in ethyl acetate. The acetamido derivative was eluted as a brownish-orange band with ethyl acetate. The eluate was concentrated *in vacuo* to a syrup. The derivative could be crystallized from nitromethane as a dull orange solid, m.p. 251–255° dec.; $\lambda_{max.}$ 413 m$\mu$ ($\epsilon$ 30,000) in ethanol, $\lambda_{max.}$ 505 m$\mu$ in dilute NaOH; and $R_f$ 0.81. The infrared spectrum (KBr) showed bands at 3.05, 6.0, 6.2, 6.45, 7.6 and 9.9 $\mu$.

**Independent Synthesis.**—3-(5-Acetamido-2-furfurylideneamino)-2-oxazolidinone[6] was treated with the 2,4-dinitrophenylhydrazine reagent and the product recrystallized from nitromethane. Physical data were identical with those of the isolated derivative.

*Anal.* Calcd. for $C_{13}H_{11}N_5O_6$: C, 46.85; H, 3.33; N, 21.02. Found: Synthetic: C, 46.66; H, 3.48; N, 21.16. Isolated (a): C, 46.02; H, 3.41; N, 20.47. (b): C, 47.16, H, 4.00; N, 20.93.

**5-Acetamido-2-furaldehyde Acetylhydrazone (III) and 5-Diacetylamino-2-furaldehyde Acetylhydrazone (IV): Isolation from Urine.**—Urine from I-dosed rabbits was extracted 4 to 6 times with equal volumes of ethyl acetate. The extracts were concentrated *in vacuo*, the solids removed by centrifugation, and the extract dried with sodium sulfate. The solution was chromatographed on alumina in ethyl acetate. A small amount of the diacetylamino metabolite (IV) was eluted with ethyl acetate, and the acetamido metabolite (III) with 95% ethanol. Neither compound could be crystallized. Characterizing data are given in Table I. For infrared spectra, the column eluates were dried directly on KBr, *in vacuo* at room temperature until the solvent evaporated and, subsequently, *in vacuo* at 53° for 1.5 hr. The infrared spectrum for IV showed bands at 2.95, 5.75 (with shoulder), 6.05, and 9.85 $\mu$. The acetamidofuran III had bands at 3.1, 5.95, 6.1 (shoulder), and 9.85 $\mu$.

A solution of metabolite III was treated with dinitrophenylhydrazine in HCl and the product recrystallized from nitromethane. A derivative with ultraviolet maximum at 413 m$\mu$, maximum in alkali at 505 m$\mu$ and m.p. 251–253° dec. was obtained. These values are also those of the same derivative of independently synthesized 5-acetamido-2-furaldehyde (II).

**Independent Synthesis.**—The crude product of the reaction of acetic anhydride and 5-amino-2-furaldehyde acetylhydrazone (V)[6] was dissolved in ethyl acetate and a small amount of residue was removed by filtration. The ethyl acetate solution was chromatographed on alumina as described above for the urine extract, with identical results (Table I). Infrared spectra also were identical. An ethyl acetate solution of IV from this column was evaporated to dryness at room temperature in a stream of air and hydrolyzed in 0.8 *N* HCl at 70°. After 6 min. the ultraviolet maximum had shifted from 320 m$\mu$ to 330 m$\mu$. After 12 min. the peak was at 340 m$\mu$ and at 30 min. at 350 m$\mu$.

**1,2-Diacetylhydrazine.**—The procedure of McKennis, *et al.*[7] was followed to yield a white crystalline product, m.p. 130–131°. A synthetic product melted

at 134–135° and a mixture m.p. was 131–132° (lit.,[7] 138–139°). The infrared spectra (Nujol mull) for synthetic and isolated products were identical.

**Isolation of VII.**—This compound was isolated from rabbit urine in low yield by the procedure of Paul, *et al.*[2c] Comparison with the photochemically produced product is given below.

**Photochemical Product of 5-Nitro-2-furaldehyde Diacetate.**—Dry 5-nitro-2-furaldehyde diacetate (IX) (100 g.) was irradiated with frequent mixing in flat-sided Roux bottles which had been flushed with nitrogen and stoppered. About one hour of midday sunlight caused the white solid to turn green. Irradiation in air resulted in yellowing and decreased the yield. The green crystals were dissolved in 500–600 ml. of warm (about 50°) ethanol, then 600 ml. of water and 1500–2000 ml. of ethyl acetate were added. The yellow water–alcohol layer was separated and the brown-red ethyl acetate layer washed once with about 100 ml. of water. The combined aqueous layers were extracted twice with equal volumes of ethyl acetate to remove residual IX yielding an aqueous phase with absorption maxima at 400 and 300 mμ. The ratio of absorbances at 400:300 mμ is 2.1:1. 1-Isopropylidene-2-acetylhydrazine (1 g.) and 1 ml. of concd. HCl/500 ml. of solution were added. After standing overnight in the refrigerator, the solution was concentrated *in vacuo* to yield a brownish-yellow solid with absorption maxima at 415 and 320 mμ; recrystallization from water did not give a pure product. VII isolated from urine[2c] yielded upon hydrolysis in dilute HCl at 70° a product which absorbed at 400 mμ, and which corresponded in $R_f$ in the butanol:ethanol:$NH_4OH$ solvent with that of the photochemically produced VIII. Both of these compounds had $R_f$ 0.5 and both VII compounds had $R_f$ 0.20. Solutions of either, on standing for 0.5 hr. at room temperature with an equal volume of 0.2% 2,4-dinitrophenylhydrazine in 2 *N* HCl, yielded red derivatives with $R_f$ 0.27 and $\lambda_{max.}$ 462 mμ. An infrared spectrum (KBr pellet) of isolated VII showed no nitro group absorption and bands at 5.75, 5.95 and 6.9 μ.

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# Synthesis and Biological Activity of Pyridoxine Analogs

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The syntheses of three new analogs of pyridoxine have been described. On biological evaluation in rats, 3-chloro-4,5-bis(hydroxymethyl)-2-methylpyridine hydrochloride (I) was a weak vitamin $B_6$ antagonist compared with deoxypyridoxine.