can probably also be applied to species other than the rabbit. However, extensive transformation to metabolites of unknown structures and pharmacological activity appears to occur, which will be discussed in a separate report.

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Synthesis and Biological Activity of a Novel Analog of Nitrofurantoin

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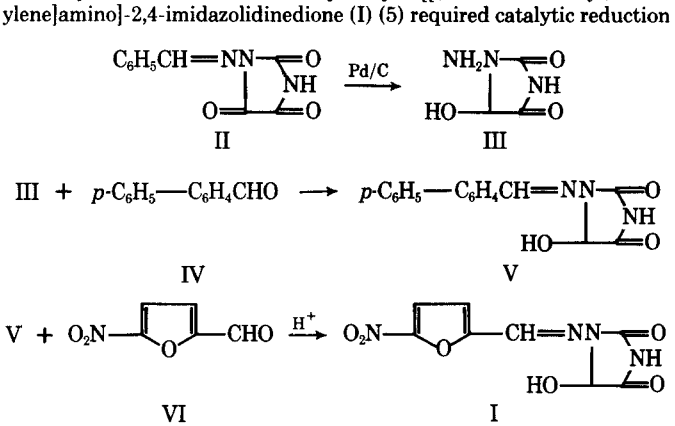
Abstract □ The synthesis of 5-hydroxy-1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione is described, and its antibacterial activity is reported.

Keyphrases □ Nitrofurantoin analog—synthesized, screened for antibacterial activity □ 2,4-Imidazolidinedione, substituted—synthesized, screened for antibacterial activity □ Antibacterial activity—substituted 2,4-imidazolidinedione evaluated

Because of the continuing interest (1–3) in the chemotherapeutic properties of 1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinediones, the 5-hydroxy analog of nitrofurantoin[1] was prepared by utilizing the general preparative route to 5-hydroxy-1-(substituted amino)-2,4-imidazolidinediones (4, 5).

DISCUSSION

The synthesis of the desired 5-hydroxy-1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione (I) (5) required catalytic reduction of 1-[(phenylmethylene)amino]imidazolidinetrione (II) to yield 1-amino-5-hydroxy-2,4-imidazolidinedione (III). Compound III was not isolated but was trapped with 1,1′-biphenyl-4-carboxaldehyde (IV) to yield 1-[[[(1,1′-biphenyl)-4-yl]methylene]amino]-5-hydroxy-2,4-imidazolidinedione (V). Exchange on the 1-amino-5-hydroxyimidazolidinedione moiety of V was accomplished by stirring V and 5-nitro-2-furancarboxaldehyde (VI) in the presence of an acid catalyst, which yielded the desired product I (Scheme I).



Scheme I

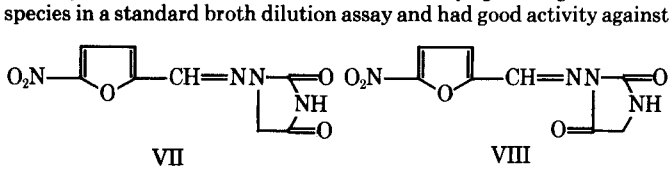
Evidence supporting reduction of II at the 5-oxo group to give a 5-hydroxy-1-substituted 2,4-imidazolidinedione was found by comparing the NMR spectrum of I with spectra of nitrofurantoin (VII) and its isomer 3-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione (VIII) (6). The NH signal of I is at 11.5 ppm, the N_3H signal of VII occurs at 11.2 ppm, and the N_1H signal of VIII is at 8.57 ppm, indicative of a N_3H proton for I rather than an N_1H proton. These NMR data confirm that I is a 5-hydroxy-1-(substituted amino)-2,4-imidazolidinedione and not a 5-hydroxy-3-(substituted amino)-2,4-imidazolidinedione. The structure of I is thus a 5-hydroxy-substituted nitrofurantoin.

Compound I was tested for antibacterial activity against eight bacterial species in a standard broth dilution assay and had good activity against

Table I—Serial Dilution Method for I and Nitrofurantoin[a]

Organism	MIC, μg/ml I	MIC, μg/ml Nitrofurantoin
Staphylococcus aureus (Mi-12)	50	12.5
Escherichia coli (Es-90)	12.5	6.2
Salmonella typhosa (SaD-13)	25	6.2
Aerobacter aerogenes (Ae-6)	>100	100
Pseudomonas aeruginosa (Ps-44)	>100	>200
Shigella flexneri (Sh-378)	25	12.5
Proteus mirabilis (Pr-91)	>100	200
Hemophilus vaginalis (He-127)	3.1	1.5

[a] Activities are given in MIC values (minimum inhibitory concentrations).



VII VIII

[1] Furadantin, Eaton Laboratories, Division of Morton-Norwich Products, Inc.

Table II—Serial Dilution Method for the Urine of Rats Dosed with I[a]

Collection Interval, Hours after Dosing	*Escherichia coli* (Es-90)	*Proteus mirabilis* (Pr-91)	*Aerobacter aerogenes* (Ae-6)	*Pseudomonas aeruginosa* (Ps-44)
0—4	48	48	<6	<6
4—8	<6	<6	<6	<6

[a] Activities are given in RID values (reciprocal of the highest inhibitory dilution).

all organisms tested except *Aerobacter aerogenes, Pseudomonas aeruginosa,* and *Proteus mirabilis* in a standard minimum inhibitory concentration (MIC) serial dilution test (Table I). Antibacterial activity of nitrofurantoin was measured by the serial dilution method to have a direct comparison of MIC values in the same species. Nitrofurantoin exhibited better general antibacterial activity than I for the bacteria tested. MIC values for nitrofurantoin against various bacterial species also may be found in the literature (7, 8).

Urine collected in 0.1 *N* HCl from rats fasted overnight prior to receiving a single oral dose (30 mg/kg) of I was evaluated for antibacterial activity in a broth dilution assay (Table II) (9). Rats dosed with I excreted urine having excellent antibacterial activity against *Escherichia coli* and *P. mirabilis* (Table II). The *in vivo* antibacterial activity was measured in a standardized rat unilateral pyelonephritis model (Table III) (10); efficacy against *E. coli* and *P. mirabilis* was apparent. The estimated oral 72-hr LD_{50} for I in mice was 668 mg/kg. Urine from rats or dogs fed nitrofurantoin (VII) contained no detectable I, and urine from rats fed I contained no detectable VII, as indicated by TLC with three different solvent systems [(*a*) 1-butanol–ethanol–ammonium hydroxide, (*b*) ethyl acetate–hydrochloric acid, and (*c*) 1-butanol–ethanol–acetic acid] on nitromethane extracts (pH 2.0) of appropriate urines and standards, internal standards, control urines, and spent, extracted urines.

EXPERIMENTAL[2]

1-[[[(1,1′-Biphenyl)-4-yl]methylene]amino] -5-hydroxy-2,4-imidazolidinedione (V)—In each of two 2-liter reduction vessels were placed 1-(benzylideneamino)imidazolidinetrione (87 g, 0.40 mole) (1), palladium-on-charcoal (5% with 50% water, 20 g), and methanol (500 ml). The mixtures were reduced on a Parr apparatus until about 95% of the theoretical three equivalents of hydrogen was taken up (24 hr). The combined reduction mixtures were filtered, and the insoluble residue was rinsed with methanol (600 ml). The combined methanolic solution was made acidic with concentrated hydrochloric acid, and then 1,1′-biphenyl-4-carboxaldehyde (73 g, 0.40 mole) was added. The mixture was stirred for 2 hr, and the resulting solid was collected (21 g, 28% yield). A sample was recrystallized from absolute ethanol, mp 224–225°.

Anal.—Calc. for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.19; H, 4.52; N, 14.10.

5-Hydroxy-1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione (I)—In tetrahydrofuran (500 ml) were placed V (20 g, 0.070 mole), VI (11 g, 0.080 mole), and concentrated hydrochloric acid (5 ml). The solution was refluxed for 1 hr and concentrated under reduced pressure to a yellow solid. Benzene (100 ml) was added, and the mixture was again concentrated to a solid. The solid was stirred for 30 min with anhydrous ether (400 ml), and the mixture was filtered to yield the desired product I (15 g, 88% yield). Recrystallization of a sample from acetonitrile gave the white product, mp 213–214°; NMR: δ 5.68 (d, 1H, CH, J = 10 Hz, s after exchange), 7.18 (d, 1H, furan CH, J = 4 Hz), 7.47 (d, 1H, OH, J = 10 Hz, exchange), 7.75 (d, 1H, furan CH, J = 4 Hz), 8.18 (s, 1H, azomethine CH), and 11.5 (broad s, 1H, NH, exchange).

Anal.—Calc. for $C_8H_6N_4O_6$: C, 37.80; H, 2.38; N, 22.05. Found: C, 37.77; H, 2.35; N, 22.00.

NMR Spectrum of Nitrofurantoin (VII)—Signals were observed at δ 4.40 (s, 2H, CH), 7.15 (d, 1H, furan CH, J = 4 Hz), 7.78 (d, 1H, furan CH, J = 4 Hz), 7.82 (s, 1H, azomethine CH), and 11.2 (broad s, 1H, NH, exchange).

NMR Spectrum of 3-[[(5-Nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione (VIII)—The synthesis of this compound was reported previously (6); NMR: δ 4.10 (s, 2H, CH), 7.33 (d, 1H, furan CH, J = 4 Hz), 7.66 (d, 1H, furan CH, J = 4 Hz), 8.57 (s, 1H, NH, exchange), and 9.51 (s, 1H, azomethine CH).

[2] A Varian Associates A-60A instrument was used for NMR spectra, which were run in dimethyl sulfoxide-d_6 with tetramethylsilane as the internal standard. Melting points were determined in open capillary tubes with a Mel-Temp melting-point apparatus and are uncorrected.

Table III—Standard Rat Bacterial Pyelonephritis Efficacy[a]

Organism	Treated	Untreated
Escherichia coli (Es-90)	2.6	4.9
Proteus mirabilis (Pr-91)	3.8	4.8
Aerobacter aerogenes (Ae-6)	4.5	5.2
Klebsiella pneumoniae (Kl-10)	4.9	5.5

[a] Activities are expressed as the $\log_{10}$ of the geometric mean of the viable bacterial titers of the infected kidneys from 10 rats (per group) dosed 10 mg/kg twice daily for 14 days with I.

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