Metabolic and Photochemical Hydroxylation of 5-Nitro-2-furancarboxaldehyde Derivatives

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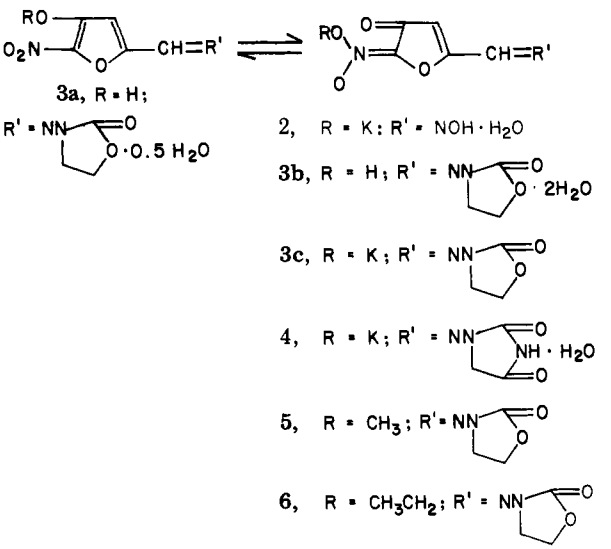
The potassium salt of 1-[[(5-*aci*-nitro-4,5-dihydro-4-oxo-2-furanyl)methylene]amino]-2,4-imidazolidinedione (4) was isolated from the urine of rats fed nitrofurantoin. An aldehyde absorbing at 400 nm was synthesized photochemically, in less than 1% yield, from 5-nitro-2-furancarboxaldehyde diacetate (1), and the hydroxylamine (2), 3-amino-2-oxazolidinone (3a–c), and 1-amino-2,4-imidazolidinedione (4) derivatives were prepared. On the basis of ir and NMR data 2, 3b,c, and 4 are considered derivatives of 4-hydroxy-5-nitro-2-furancarboxaldehyde which are mainly in the *aci*-nitro form. Methyl and ethyl nitronic esters of 3b were synthesized. The photochemical hydroxylation of 1 also yields 3,4-dihydroxy-5-nitro-2-furancarboxaldehyde, isolated as 3-[[(3,4-dimethoxy-5-nitro-2-furanyl)-methylene]amino]-2-oxazolidinone (7).

A pathway of nitrofuran metabolism which leads to the formation of bright yellow, polar, labile metabolites absorbing near 415 nm has been reported.[2] The metabolites were found in the urine of rats, rabbits, dogs, chickens, and man after dosing with nitrofurans. Solid materials were isolated, but not identified, from the urine of rabbits[3] and rats[4] fed nitrofurazone,[5] from swine fed furazolidone,[6,7] and from chickens fed nihydrazone.[2,8] The latter, although not identified, could be synthesized by coupling 1-isopropylidine-2-acetylhydrazine with the aldehyde product of the photochemical reaction of 5-nitro-2-furancarboxaldehyde diacetate (1). Spectral and chromatographic evidence for the identity of photochemical and metabolic products was reported.[2] On the basis of chemical and spectral properties described in this paper, as well as the fact that hydroxylation is a known biotransformation mechanism, these compounds are considered tautomeric mixtures of 4-hydroxy-5-nitro-2-furancarboxaldehyde derivatives and the corresponding *aci*-nitro forms.

Scheme I[a]



[a] Each structural formula represents the major tautomer as determined from its ir spectrum.

Results

A yellow, 417-nm absorbing metabolite of nitrofurantoin[1] was isolated from rat urine on Sephadex columns and cellulose plates and crystallized from aqueous potassium acetate. Comparison of uv, ir, and NMR spectra proved the identity of the metabolite and a photosynthetically hydroxylated product (4). Recovery of the drug as the 405–417-nm metabolite varies with species and compound administered. In the chicken about 15% and in the rat up to 40% of the dose recovered in urine is the furan-ring hydroxylated metabolite; in man, this pathway appears minor.

Reaction of the 400-nm absorbing photoproduct of 1 with hydroxylamine hydrochloride in potassium acetate buffer yielded a crystalline product $C_5H_5N_2O_6K$. The ir spectrum showed strong absorption at 1430 cm^{-1} and a relatively weak nitro stretching band at 1380 cm^{-1}. In the NMR spectrum there were only two hydrogens, singlets at 7.6 and 6.7 ppm. These data suggested structure 2.

The 3-amino-2-oxazolidinone derivative, after recrystallization from water and drying in vacuo for 24 h over P_2O_5, gave an elemental analysis consistent with structure 3a. The infrared spectrum of 3a showed strong nitro group stretching bands at 1470 and 1330 cm^{-1} and a relatively weak band at 1400 cm^{-1}. When the recrystallized product was dried in vacuo for 24 h over NaOH, the dihydrate 3b resulted. The ir spectrum of 3b showed a strong band at 1400 cm^{-1} and relatively weak NO_2 bands. On the basis of their infrared spectra, 3a and 3b are considered mixtures of tautomers with the *aci*-nitro form predominating in the dihydrate 3b. The intense 1430-cm^{-1} absorption shown by the potassium salts 2, 3c, and 4 indicates that these compounds are predominately in the *aci*-nitro form. The strong acidity of 3b is indicated by a pK_a of 2.7. Since both 3b and the potassium salt 3c have the same ultraviolet maximum, 3b in dilute aqueous solution must approach complete ionization. Compound 3b is relatively stable as a dilute aqueous solution ($t_{1/2}$ of a 0.1% solution is 2 days). The half-life of 3a (neat) is ca. 5 days under atmospheric pressure and >26 days in vacuo. The potassium salts are stable.

When crude 3a was treated with diazomethane, as an ethereal suspension, the major product was the methyl nitronic ester 5, which showed a strong band at 1400 cm^{-1} and a weak NO_2 band at 1330 cm^{-1}. The minor product of the methylation was 3-[[(3,4-dimethoxy-5-nitro-2-furanyl)methylene]amino]-2-oxazolidinone (7). The elemental analysis, ir, uv, melting point, and R_f on thin-layer chromatography agreed with the corresponding values of an authentic sample of 7 synthesized from 3,4-dimethoxy-2,5-furandicarboxylic acid. The isolation of both mono- and dimethoxy compounds from the methylation of 3a indicates that the photochemical hydroxylation of 1 produced both 4-hydroxy- and 3,4-dihydroxy-2-furancarboxaldehyde.

A synthesis of nitronic esters by treating salts of nitroparaffins with trialkyloxonium fluoborates has been reported by Kornblum and Brown.[9] This procedure gave the ethyl nitronic ester 6 in 6% yield from 3c and in 39% yield from the corresponding sodium salt. The ir spectrum

showed an intense band at 1400 cm^{-1} and only slight NO_2 absorption. The NMR spectrum showed the C-3 proton as two bands of equal intensity at δ 7.02 and 6.84 ppm, which may be attributed to "syn" and "anti" forms. The nitronic esters, which may be compared with un-ionized *aci*-nitro compounds, show a uv maximum at 385 nm; thus it appears to be the ionization of the *aci*-nitro compounds which accounts for the bathochromic shift to 415 nm. Southwick et al.[10] report the nitro enol, 3-hydroxy-4-nitro-5-phenyl-3-pyrrolin-2-one. This is an acidic, water-soluble compound which shows an intense maximum at 358 nm for both the acid and sodium salt. The NMR spectrum of the methylated product established the 3-methoxy-4-nitro structure, which was confirmed by $NaBH_4$ reduction. Since the methylated derivative had its principal uv maximum at 257 nm, the bathochromic shift to 358 nm for the hydroxynitro compound was attributed to its ionization.

Two pathways of nitrofuran metabolism in animals were described earlier.[2] Acid hydrolysis of the azomethine linkage in the gastrointestinal tract yields 5-nitro-2-furancarboxaldehyde which is excreted as 5-nitro-2-furoic acid and a hydrazine derivative which may be acetylated. A second route involves reduction of the nitrofuran to the 5-aminofuran which is acetylated to form the 5-acetamidofuran or 5-diacetylaminofuran. A third pathway is oxidative metabolism of the side chain of the nitrofuran.[11] Isolation of 4 from urine of rats fed nitrofurantoin[1] establishes a fourth metabolic pathway, hydroxylation of the furan ring.

Experimental Section

Ultraviolet spectra were determined on a Beckman DU spectrophotometer and infrared spectra were obtained from potassium bromide pellets on a Perkin-Elmer Infracord. NMR spectra were determined on a Varian A-60A spectrometer using Me_4Si as an internal standard. Melting points were taken on a Fisher-Johns block checked with known compounds. Elemental analyses are represented by the symbols of the elements analyzed for; if any element falls more than 0.4% from the theoretical value, the obtained value is reported.

Potassium 1-[[(5-*aci*-Nitro-4,5-dihydro-4-oxo-2-furanyl)methylene]amino]-2,4-imidazolidinedione Monohydrate (4). Rats were dosed orally with nitrofurantoin[1] at 100 mg/kg. Urine was collected for 6 h. The pH of the urine was adjusted to 4.8 and the urine was extracted with 2 vol of nitromethane. The aqueous phase was chromatographed on a Sephadex G-10 (Pharmacia) column in water. The yellow band was collected, concentrated in vacuo, and chromatographed on 0.5-mm silica gel H (Brinkmann) thin-layer plates in acetone–water (95:5). The yellow band at R_f 0.8 was eluted with 80% methanol and the methanol was evaporated in a stream of nitrogen. Potassium acetate was added; after standing overnight at 4°, yellow crystals were obtained. The crystals were washed with cold water and dried in vacuo at room temperature.

Synthesis of the photochemical product of 5-nitro-2-furancarboxaldehyde diacetate (1) was described earlier.[2] Irradiation of the dry solid in sunlight, solution in ethanol at 60°, dilution with water, and extraction of the unreacted 1 with ethyl acetate yielded an aqueous solution of an aldehyde absorbing at 400 nm in ca. 0.4% yield. The aldehyde was coupled with 1-amino-2,4-imidazolidinedione in dilute HCl. After concentration in vacuo, addition of potassium acetate, and cooling, yellow crystals were obtained. Spectrometric data for metabolite and photoproduct were identical. Because the sample of isolated metabolite was small, only the photoproduct was analyzed for C, H, and N: uv max (H_2O) 417 nm; ir 1430 (NO–OK), 1620 (CH=N–), 1780 cm^{-1} (COO–); NMR (Me_2SO-d_6) δ 7.30 (s, 1, CH=N), 6.82 (s, 1, 3 CH) and 4.29 (s, 2, CH_2). Anal. ($C_8H_7N_4O_7K$) C, H; N: calcd, 18.06; found, 17.56.

Potassium 5-*aci*-Nitro-4,5-dihydro-4-oxo-2-furancarboxaldehyde Oxime Monohydrate (2). The aldehyde photoproduct was coupled with NH_2OH·HCl in potassium acetate buffer. Concentration in vacuo and cooling yielded a crystalline product which was recrystallized from water at 50° and dried in vacuo at room temperature: uv max (H_2O) 405 nm (ϵ 18 000); ir 1430 (NO–OK), 1670 cm^{-1} (CH=NOH); NMR (Me_2SO-d_6) δ 7.6 (s, 1, CH=N), 6.7 (s, 1, 3 CH). Anal. ($C_5H_5N_2O_6K$) C, H, N, K.

3-[[(4-Hydroxy-5-nitro-2-furanyl)methylene]amino]-2-oxazolidinone Hemihydrate (3a). The photochemically synthesized aldehyde was coupled with 3-amino-2-oxazolidinone in 1.5 M HCl. The crystalline product (0.25% yield) was twice recrystallized from water at 60° and dried in vacuo for 24 h over P_2O_5: uv max (H_2O) 415 nm (ϵ 23 800); ir 1330 and 1470 (NO_2), 1630 (CH=N–), 1750 cm^{-1} (COO–); pK_a = 2.7 (by potentiometric titration). Anal. ($C_8H_8N_3O_{6.5}$) C, H, N.

Recrystallization from water followed by drying for 24 h over sodium hydroxide yielded the dihydrate **3b**: uv max (H_2O) 415 nm (ϵ 22 200); ir 1400 (NO–OH), 1650 (CH=N), 1740 cm^{-1} (COO–).

Potassium 3-[[(5-*aci*-Nitro-4,5-dihydro-4-oxo-2-furanyl)methylene]amino]oxazolidinone (3c). The photochemical product was coupled with 3-amino-2-oxazolidinone and crystallized from potassium acetate buffer. The product was recrystallized from water (50°): uv max (H_2O) 415 nm (ϵ 21 300); ir 1440 (NO–OH), 1750 cm^{-1} (COO–). Anal. ($C_8H_6N_3O_6K$) C, H; N: calcd, 14.61; found, 15.05.

Methyl Nitronic Ester of 3-[[(5-*aci*-Nitro-4,5-dihydro-4-oxo-2-furanyl)methylene]amino]-2-oxazolidinone (5). To a suspension of 0.91 g (3.3 mmol) of crude **3a** in 300 ml of anhydrous ether was added 20 ml (ca. 10 mmol) of diazomethane solution in ether with stirring and ice bath cooling. After stirring for 1 h the product was collected and dried in vacuo. The product was triturated with 75 ml of water and filtered, and the cake was washed with 20 ml of water. The filtrate contained 0.14 g (15%) of starting material based on absorbance at 415 nm. The precipitate, which after drying in vacuo weighed 0.56 g (60%), was twice recrystallized from acetonitrile: uv max (SDA 30) 385 nm (ϵ 18 700); ir 1390 (NO–OCH_3), 1630 (CH=N–), 1780 cm^{-1} (COO–). Anal. ($C_9H_9N_3O_6$) C, H, N.

After concentration of the acetonitrile mother liquors, 0.09 g (9.7%) of product was obtained. Two recrystallizations from nitromethane–SDA 30 (1:5) yielded 0.05 g of 3-[[(3,4-dimethoxy-5-nitro-2-furanyl)methylene]amino]-2-oxazolidinone (**7**). Melting point, R_f, ir, and uv max and ϵ value corresponded to that of authentic **7**. Anal. ($C_{10}H_{11}N_3O_7$) C, H, N.

3,4-Dimethoxyfuran. 3,4-Dimethoxy-2,5-furandicarboxylic acid was synthesized from ethyl diglycolate and diethyl oxalate.[12] A mixture of 44 g (0.20 mol) of the dicarboxylic acid, 5 g of copper chromite, and 200 ml of quinoline was heated rapidly to 160° (N_2 atmosphere) and maintained at 160–175° for 30 min. After cooling, 300 ml of ether was added and the mixture was filtered. An additional 500 ml of ether was added and the ether was extracted with 150 ml of concentrated HCl in 300 ml of ice water. The ether phase was extracted three times with a solution of 100 ml of concentrated HCl in 200 ml of ice water and two times with 200 ml of 1 M NaOH, 10% HCl, and H_2O saturated with NaCl and was dried (Na_2SO_4). After evaporation of the solvent, the residue was distilled to yield 14.5 g (57%) of 3,4-dimethoxyfuran: bp 34–36° (0.3 mm). Anal. ($C_6H_8O_3$) C, H.

3,4-Dimethoxy-2-furancarboxaldehyde. In a flask equipped with drying tube was placed 14.5 g (0.11 mol) of 3,4-dimethoxyfuran and 70 ml of DMF. $POCl_3$ (15 ml) was added during 1 h at −12 to −3° and the solution was stirred for an additional hour. After 24 h the reaction mixture was poured into 250 ml of ice water and a solution of 70 g of sodium acetate in 200 ml of water was added. After stirring for 0.5 h the solution was cooled and the product collected. The filtrate was adjusted to pH 5.9 with NaOH (5 M) and cooled, and the product was collected. The combined products (17.8 g) were recrystallized from SDA 30: uv max (SDA 30) 284 nm (ϵ 17 300). Anal. ($C_7H_8O_4$) C, H.

3,4-Dimethoxy-2-furancarboxaldehyde Oxime. A solution of 5.0 g (0.03 mol) of 3,4-dimethoxy-2-furancarboxaldehyde and 4 g (0.06 mol) of NH_2OH·HCl in 15 ml of pyridine was refluxed for 2.5 h. After concentration in vacuo the residue was diluted with water and cooled, and the product was collected (4.46 g, 81%); uv max (SDA 30) 280 nm (ϵ 15 600).

3,4-Dimethoxy-5-nitro-2-furancarboxaldehyde Oxime. 3,4-Dimethoxy-2-furancarboxaldehyde oxime (3.5 g, 0.02 mol) was

added with stirring to 35 ml of concentrated H_2SO_4 in 10 min at -5°. Fuming HNO_3 (0.9 ml) was added with stirring during 45 min at -3 to -8°. After stirring for 0.5 h at -8°, the reaction mixture was poured into a stirred mixture of 350 ml of ice water and 200 ml of ethyl acetate. The aqueous phase was washed with ethyl acetate. The combined ethyl acetate solution was washed with Na_2CO_3 solution and with water and dried (Na_2SO_4). After evaporation of the solvent, the residue was crystallized from SDA 30 (0.67 g, 15%): uv max (SDA 30) 353 nm (ε 12 700). Anal. ($C_6H_7N_2O_6$) C, H, N.

3-[[(3,4-Dimethoxy-5-nitro-2-furanyl)methylene]amino]-2-oxazolidinone (7). To a solution of 0.3 g of 5-nitro-3,4-dimethoxy-2-furancarboxaldehyde oxime in 15 ml of SDA 32 at 55° were added 5 ml of 24% H_2SO_4 and a solution of 0.3 g of 3-amino-2-oxazolidinone in 2 ml of water. The solution was heated to 75° during 0.5 h and cooled. The crude product (0.23 g) was recrystallized from SDA 30 containing a small amount of nitromethane: mp 198°; uv max (SDA 30) 370 nm (ε 18 900); ir 1350 (NO_2), 1590 (CH=N-), 1770 cm^{-1} (COO-); TLC on silica gel chromagram sheet (Eastman) in $CHCl_3$-CH_3NO_2-CH_3OH (7:2:1), R_f 0.92; in $CHCl_3$-CH_3NO_2 (8:2), R_f 0.44. Anal. ($C_{10}H_{11}N_3O_7$) C, H, N.

Ethyl Nitronic Ester of 3-[(5-*aci*-Nitro-4,5-dihydro-4-oxo-2-furanyl)methylene]amino]-2-oxazolidinone (6). The photosynthetically prepared aldehyde was coupled with 3-amino-2-oxazolidinone in acid and crystallized from sodium acetate buffer. A stirred slurry of 0.69 g (2.6 mmol) of finely powdered sodium salt in 40 ml of methylene chloride was cooled in an ice bath and a solution of 0.8 g (4.2 mmol) of triethyloxonium fluoborate in 10 ml of methylene chloride was added rapidly.[9] After stirring for 5 h, 25 ml of nitromethane and ca. 0.5 g of filter aid was added. The solid was washed with nitromethane; the combined filtrate and wash was extracted three times with water, dried over Na_2SO_4, and concentrated to low volume in vacuo. The solid was recrystallized from 35 ml of acetonitrile to yield 0.27 g (39%) of orange crystals; uv max (SDA 30) 385 nm (ε 20 800); ir 1400 (NO-OC_2H_5), 1630 (CH=N); NMR (Me_2SO-d_6) δ 7.54 (s, 1, CH=N), 7.02 (s, 0.5, 3 CH), 6.84 (s, 0.5, 3 CH), 4.4 (m, 4, $-CH_2CH_2-$), 3.9 (q, 2, $-CH_2O$), 1.29 (t, 3, CH_3-). Anal. ($C_{10}H_{11}N_3O_6$) C, H, N.

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References and Notes

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Antidepressant and Anticonvulsant Activity of 1-(5-Phenyl-4-oxo-2-oxazolin-2-yl)-4-substituted Piperazines

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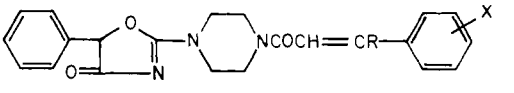
1-(5-Phenyl-4-oxo-2-oxazolin-2-yl)-4-substituted cinnamoylpiperazines and 1-(5-phenyl-4-oxo-2-oxazolin-2-yl)-4-carbamoylpiperazine and derivatives were synthesized and evaluated for antidepressant activity in the mouse Dopa potentiation test. 1-(5-Phenyl-4-oxo-2-oxazolin-2-yl)-4-carbamoylpiperazine and derivatives were further evaluated for anticonvulsant activity in the audiogenic seizure test in mice.

As a continuing study in our laboratories for the modification of 2-amino-5-phenyl-2-oxazolin-4-one (pemoline) in search of potential CNS drugs,[1,2] it is of interest to replace the amino group by substituted piperazines. It was reported that some substituted cinnamoylpiperazines showed a strong sedative activity with a weak antihistamine activity.[3] Also, 1-heterocyclo-4-substituted carbamoylpiperazines were found to have anticonvulsant activity when tested against audiogenic seizure in rats.[4,5] Accordingly, several 1-(5-phenyl-4-oxo-2-oxazolin-2-yl)-4-(substituted cinnamoyl)piperazines and 1-(5-phenyl-4-oxo-2-oxazolin-2-yl)-4-carbamoylpiperazine and derivatives were synthesized.

Chemistry. 2-Acetamido-5-phenyl-2-oxazolin-4-one (1) was obtained by heating 2-amino-5-phenyl-2-oxazolin-4-one with acetic anhydride.[6] Reaction of 1 with 2 equiv of piperazine in dioxane at room temperature yielded 1-(5-phenyl-4-oxo-2-oxazolin-2-yl)piperazine (2) (Scheme I). This method is preferred to the procedure of heating 2-amino-5-phenyl-2-oxazolin-4-one with piperazine in xylene.[7] 1-(5-Phenyl-4-oxo-2-oxazolin-2-yl)-4-substituted cinnamoylpiperazines (**3a–e**) (Table I) were prepared by reaction of **2** with substituted cinnamoyl chlorides. 1-(5-Phenyl-4-oxo-2-oxazolin-2-yl)-4-carbamoylpiperazine

Table I. 1-(5-Phenyl-4-oxo-2-oxazolin-2-yl)-4-(substituted cinnamoyl)piperazines



Compd	X	R	Mp, °C	Yield, %[a]	Formula[b]
3a	4-F	H	241-243	64	$C_{22}H_{20}FN_3O_3$
3b	4-OCH_3	H	211-212	64	$C_{23}H_{23}N_3O_4$
3c	3,4,5-(OCH_3)$_3$	H	222-223	61	$C_{25}H_{27}N_3O_6$
3d	3-Br-4,5-(OCH_3)$_2$	H	227-229	69	$C_{24}H_{24}BrN_3O_5$
3e	3,4,5-(OCH_3)$_3$	CH_3	164-165	54	$C_{26}H_{29}N_3O_6$

[a] After recrystallization from EtOH. [b] All compounds were analyzed for C, H, and N.