thione (**1c**), 4,5-dimethyl-2-imidazolidinethione (**1d**),[7] and tetrahydro-2-pyrimidinethione (**1e**),[6] respectively, with **2a**. The crude bromide salts were converted to the free bases and then to the chloride salts for characterization. Thin layer chromatography of **3c–e** in several solvent systems indicated that the compounds were homogeneous.

2-Bromo-1-(5-nitro-2-furyl)-1-propanone (2c).—Ethyl 5-nitro-2-furyl ketone[8] (25.0 g, 0.15 mole) was dissolved in warm CCl_4 (200 ml). The solution was kept at 60–70° while bromine (24.0 g, 0.15 mole), dissolved in CCl_4 (50 ml), was added rapidly. Initially there was no reaction. The mixture was refluxed with stirring for 0.5 hr. After this time, the reaction became very exothermic; stirring and heating were stopped until the reaction abated. Then stirring and heating were continued until HBr evolution ceased. The solution was boiled with charcoal for 5 min and filtered. The filtrate was concentrated to *ca.* 50 ml; the product precipitated after the addition of 400 ml of petroleum ether (bp 30–60°). The yield of crude product melting at 56–58° was 24.0 g (65.0%). Recrystallization of a sample from petroleum ether (bp 30–60°) raised the melting point to 59.5–60°.

Anal. Calcd for $C_7H_6BrNO_4$: C, 33.89; H, 2.44; Br, 32.22. Found: C, 34.20; H, 2.48; Br, 32.58.

6,7-Dihydro-2-methyl-3-(5-nitro-2-furyl)-5H-imidazo[2,1-*b*]-thiazolium Chloride (3g).—A mixture of **2c** (124.0 g, 0.5 mole) and **1a** (51.0 g, 0.5 mole) in dimethylformamide (500 ml) was heated at 130–140° for 10 min. The mixture was allowed to cool to room temperature, diluted with ether (400 ml), and filtered to yield 120 g (70.0%) of crude bromide salt. The salt was dissolved in water and neutralized with aqueous Na_2CO_3 solution. The red free base was collected, washed with water, and dried at 100° to yield 86.0 g (95%). A sample of the free base (36.0 g, 0.14 mole) was stirred into concentrated HCl (25 ml) to give a yellow lumpy paste. The paste was stirred with 2-propanol (350 ml), diluted with ether, and filtered. The product was dried at 110° to yield 38.0 g (95%) of crude chloride salt. A portion of the salt (36.0 g) was recrystallized from 2-propanol (10 ml/g) (charcoal) to give 31.0 g of **3a** decomposing at 255° when placed on a preheated melting point block.

3-Chloro-4-(5-nitro-2-furyl)-3-buten-2-one (8).—To a solution of **6** (145.0 g, 1.57 moles) and **7** (141.0 g, 1.0 mole) in acetic acid (500 ml) was added concentrated H_2SO_4 (100 ml) during 10 min at 20–25°. The dark red solution was allowed to stand in the refrigerator for 6 days. The precipitate was collected, washed with 2-propanol, and air dried. The crude material was recrystallized from ethyl acetate (charcoal) to yield 96.0 g (44.5%) of **8** melting at 118–120°. Further recrystallization of the material from 2-propanol raised the melting point to 120–121°.

Anal. Calcd for $C_8H_6ClNO_4$: Cl, 16.45; N, 6.50. Found: Cl, 16.48; N, 6.48.

The nmr spectrum ($CDCl_3$) displayed three singlets at τ 7.5 (3H), 2.7 (2H), and 2.45 (1H).

1-Chloro-4-(5-nitro-2-furyl)-3-buten-2-one (5).—A mixture of **7** (107.0 g, 0.76 mole) and **9**[10] (182.0 g, 0.517 mole) in benzene (1200 ml) was refluxed for 1 hr. The benzene was removed under reduced pressure, and the residue was recrystallized from methanol to yield 92.0 g (82.9%) of crude **5** melting at 134–136°. A portion of the product (70.0 g) was recrystallized from 2-propanol to give 53.0 g of **5** melting at 137–138°.

Anal. Calcd for $C_8H_6ClNO_4$: C, 44.56; H, 2.81; Cl, 16.45. Found: C, 44.64; H, 2.89; Cl, 16.47.

The nmr spectrum (DMSO) showed singlets at τ 5.25 (2H), 3.2, 2.91, 2.72, 2.68, 2.58, 2.30 (overlap), and 2.24 with J = 16 cps.

6,7-Dihydro-3-[2-(5-nitro-2-furyl)vinyl]-5H-imidazo[2,1-*b*]-thiazolium Chloride (10).—A mixture of **1a** (31.0 g, 0.3 mole) and **5** (65.0 g, 0.3 mole) in absolute ethanol (*ca.* 3000 ml) was refluxed for 4 hr. The reaction mixture was cooled and filtered to give 66.0 g of crude **10** decomposing at 245°. Concentration of the filtrate followed by cooling resulted in a second crop of 14.8 g decomposing at 245°; total yield 80.8 g (89.7%). Recrystallization of crude **10** from methanol (charcoal) did not affect the decomposition point.

Anal. Calcd for $C_{11}H_{10}ClN_3O_3S$: C, 44.08; H, 3.36; N, 14.02. Found: C, 44.22; H, 3.58; N, 14.24.

Acknowledgments.—The authors gratefully acknowledge the aid of Mr. George Klein who prepared compound **8**, of Mr. Nicholas Harris who prepared **3b** by method B, and of Mrs. Patricia Curtis, Mr. Frederick Abbott, and Mr. Benjamin Stevenson for the preparation of chemical intermediates. Mr. Grant Gustin and Mr. Marvin Tefft performed the microanalyses. The microbiological data were obtained by Dr. Warren Carey, Mr. Eric Russell, and Mr. Richard Dobson. The nmr spectra were obtained from and interpreted by Professor Jerrold Meinwald of the Chemistry Department, Cornell University.

Nitrofuryl Heterocycles. III.[1] 3-Alkyl-5-(5-nitro-2-furyl)-1,2,4-triazoles and Intermediates

HOMER A. BURCH AND WARREN O. SMITH

Chemical Research Division, The Norwich Pharmacal Company, Norwich, New York

Received January 5, 1966

The synthesis of several N-acylamido-5-nitro-2-furamidines and 3-alkyl-5-(5-nitro-2-furyl)-1,2,4-triazoles is described. The antibacterial testing data for these and other derivatives are discussed.

A continuing search for new chemotherapeutic nitrofurans led to an investigation of 1,2,4-triazole derivatives and intermediates.

Chemistry.—The compounds herein reported were prepared by a modification of the method of Browne and Polya[2] for the synthesis of 3-aryl-5-alkyl-4H-1,2,4-triazoles. Thus, ethyl 5-nitro-2-furimidate hydrochloride[3] was treated with various alkyl hydrazides in the presence of 1 equiv of base to yield N-acylamidoamidines (I). The physical and analytical properties of I are summarized in Table I. Cyclization of the N-acylamidoamidines I was effected readily in refluxing phosphorus oxychloride solution or refluxing glacial acetic acid solution to give the 3-alkyl-5-(5-nitro-2-furyl)-4H-1,2,4-triazoles (II) listed in Table II. These reactions are summarized as shown in Scheme I. In addition, triazole IIa (R = H) was acetylated with acetic anhydride and carbamoylated with methyl isocyanate in dimethylformamide solution to give III and IV, respectively. No satisfactory method was found for establishing the position of ring-nitrogen substitution in III and IV. Alkylation of triazole IIb

(1) For paper II in this series see H. A. Burch and L. E. Benjamin, *J. Med. Chem.*, **9**, 425 (1966).

(2) E. J. Browne and J. B. Polya, *J. Chem. Soc.*, 5149 (1962).

(3) W. R. Sherman and A. Von Esch, *J. Med. Chem.*, **8**, 25 (1965).

SCHEME I

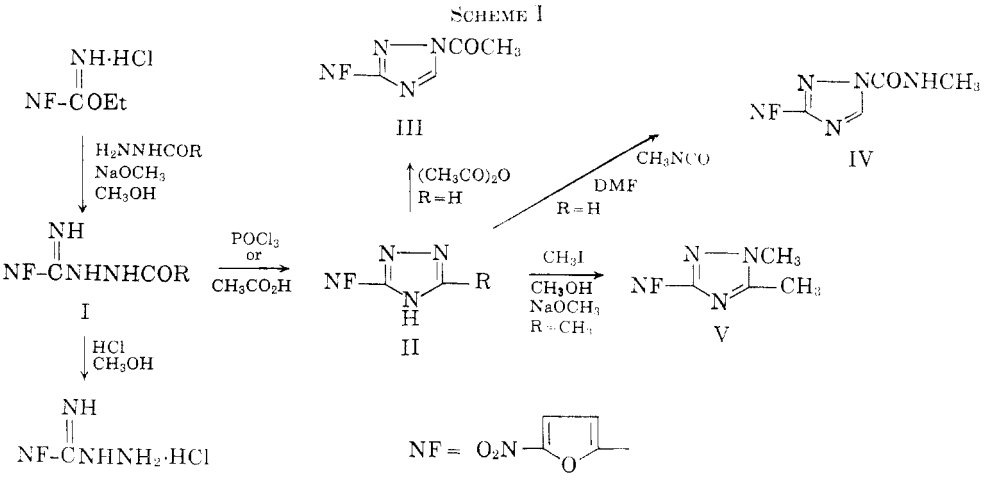
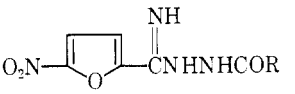


TABLE I

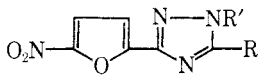
N-ACYLAMIDOAMIDINES



I	R	Mp, °C	Yield, %	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
a	H	259–260	30.6	$C_6H_6N_4O_4$	36.37	36.59	3.05	3.22	28.28	28.27
b	CH_3	224–225	83.5	$C_7H_8N_4O_4$	39.64	39.76	3.80	4.06	26.41	26.35
c	C_2H_5	204–205	49	$C_8H_{10}N_4O_4$	42.48	42.44	4.46	4.49	24.77	24.48
d	$(CH_2)_2CH_3$	199–200	71.5	$C_9H_{12}N_4O_4$	45.00	44.80	5.04	5.08	23.33	23.42
e	$CH(CH_3)_2$	221–222	83.5	$C_9H_{12}N_4O_4$	45.00	44.97	5.04	5.18	23.33	23.19

TABLE II

5-(5-NITRO-2-FURYL)-1,2,4-TRIAZOLES



No.	R	R'	Mp, °C	Yield, %	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
IIa	H	H	259–260	27.4	$C_6H_4N_4O_3$	40.01	40.00	2.24	2.26	31.31	31.47
b	CH_3	H	254.5–256	76.5	$C_7H_6N_4O_3$	43.30	43.36	3.12	3.28	28.86	28.78
c	C_2H_5	H	181.5–183.5	51.5	$C_8H_8N_4O_3$	46.15	46.08	3.87	3.94	26.92	26.84
d	$(CH_2)_2CH_3$	H	150–151.5	73	$C_9H_{10}N_4O_3$	48.65	48.58	4.54	4.50	25.22	25.25
e	$CH(CH_3)_2$	H	173–175	44	$C_9H_{10}N_4O_3$	48.65	48.52	4.54	4.71	25.22	25.27
f	CH_2CN	H	250–251	50	$C_5H_3N_5O_3$	43.84	43.93	2.30	2.33	31.96	31.99
III	H	$COCH_3$	165–165.5	69.5	$C_8H_6N_4O_4$	43.25	43.28	2.72	2.72	25.22	25.28
IV	H	$CONHCH_3$	257–259	47.7	$C_8H_7N_5O_4$	40.51	40.72	2.97	3.18	29.53	29.45
V	CH_3	CH_3	184.5–185	34.4	$C_8H_8N_4O_3$	46.15	46.14	3.87	4.01	26.92	26.76

($R = CH_3$) with methyl iodide–sodium methoxide in methanol solution gave a dimethyl derivative assigned structure V based on the work of Atkinson and Polya.[4] Attempts to establish conclusively the structure of V by treating ethyl 5-nitro-2-furimidate with $H_2NN(CH_3)COCH_3$ followed by cyclization failed. Alcoholysis of N-acylamidoamidine (Ia) with cold methanolic HCl solution gave the N-aminoamidine VI in 73% yield.

Screening Results.[5]—It can be seen from Table III that all of the N-acylamidoamidines (I) and triazoles (II–V) possess broad antibacterial activity *in vitro* against both gram-positive and gram-negative bacteria with but one exception. All of the compounds are inactive against one strain of *Pseudomonas aeruginosa* at the drug levels tested. In general, lengthening of the carbon chain at position 3 in triazoles II causes a decrease in activity, whereas the reverse effect is suggested by an inrease in the carbon chain length of the acyl group in N-acylamidoamidines (I). Of more interest, however, is the *in vivo* activity in mice that was demonstrated for most of the compounds herein reported. These data are summarized in Table IV. The most active compounds (Ia and IIa) contain no alkyl substituents at position 3. Increasing the carbon chain length of R decreases the activity. Acetylation, carbamoylation, and alkylation of the triazole ring giving III, IV, and V, respectively, does not enhance

(4) M. R. Atkinson and J. B. Polya, *J. Chem. Soc.*, 3319 (1954).

(5) The *in vitro* and *in vivo* biological data were obtained using the methods described by F. F. Ebetino, W. F. Carey, and B. F. Stevenson *J. Med. Chem.*, **6**, 633 (1963).

TABLE III

In Vitro ANTIBACTERIAL ACTIVITY OF I–VI

	Minimal inhibitory concentration, μg/ml[a]									
No.	*Staphylococcus aureus* Mi-6[b]	*Erysipelothrix insidiosa* Er-4	*Streptococcus pyogenes* StA-1	*Streptococcus agalactiae* StB-12	*Escherichia coli* Es-2	*Escherichia coli* Es-L	*Salmonella typhosa* SaD-13	*Aerobacter aerogenes* Ae-6	*Proteus vulgaris* Pr-12	*Pseudomonas aeruginosa* Ps-44
Ia	12	12	12	100	3	6	6	25	100	>200
b	25	25	12	200	6	12	6	100	200	>200
c	12	3	50	50	3	25	3	100	100	>100
d	12	3	25	50	3	25	3	100	200	>200
e	25	25	50	100	6	25	3	100	200	>200
VI	25	3	25	100	3	6	3	200	>200	>200
IIa	6	6	6	100	0.75	3	0.75	50	100	>200
b	12	1	1	200	1	12	1	100	200	>200
c	6	25	12	200	1	12	3	200	>200	>200
d	6	50	25	100	3	50	12	200	>200	>200
e	12	50	25	200	3	100	12	>200	>200	>200
f	50	6	3	50	6	12	6	>50	>50	>50
III	12.5	6	3	100	1.5	3	1.5	25	100	>200
IV	6	12.5	3	50	0.75	3	1.5	25	50	>50
V	12	100	50	>200	0.75	3	1.5	100	200	>200
Nitrofurazone[c]	12.5	12.5	6	12.5	3	12.5	3	100	100	>100

[a] Minimum inhibitory concentration is the lowest concentration of compound that prevents visible growth after 24 hr of incubation. [b] Eaton Laboratories strain number. [c] Furacin®, for comparison.

TABLE IV

In Vivo ACTIVITY OF I–VI

	ED_{50} (mice), mg/kg	
No.	*S. aureus*	*S. typhosa*
Ia	100	30
b	160	32
c	>200	50
d	>200	46
e	112	50
VI	>100	>100
IIa	40	19
b	126	43
c	122	126
d	>100[a]	>25
e	>25[a]	50
f	>200	163
III	70	35
IV	100	50
V	151	76
Nitrofurazone[b]	50	100

[a] Toxic at higher levels. [b] Furacin®, for comparison.

the activity compared to the unsubstituted derivatives IIa and IIb. In general, the compounds herein reported gave better protection in mice against gram-negative bacteria than against gram-positive bacteria. It is interesting to note that removal of the acyl group from Ia to give VI causes loss of *in vivo* activity but does not affect appreciably the *in vitro* activity. Toxicological studies on several of the compounds in Table IV are in progress.

Experimental Section[6]

N-Formamido-5-nitro-2-furamidine (Ia).—To a stirred solution of 30.0 g (0.55 mole) of sodium methoxide in 1000 ml of methanol was added 121 g (0.55 mole) of ethyl 5-nitro-2-furimidate hydrochloride[3] and 33.0 g (0.55 mole) of formhydrazide. The solution was refluxed for 1 hr and concentrated to dryness *in vacuo* on a steam bath, and the residue was shaken with 100 ml of ice–water. The crude product was filtered, washed with water, and recrystallized from 95% ethanol (charcoal) from which Ia separated as yellow needles. Other derivatives of I in Table I were prepared from the appropriate alkyl hydrazide.

3-(5-Nitro-2-furyl)-4H-1,2,4-triazole (IIa).—A solution of 34.0 g (0.17 mole) of Ia in 110 ml of glacial acetic acid was refluxed for 4 hr and chilled thoroughly, and the crude product was collected by filtration. Two recrystallizations from aqueous acetic acid (charcoal) gave the product as tan micro crystals. Other derivatives of II in Table II were prepared by this method from the appropriate I.

3-Methyl-5-(5-nitro-2-furyl)-4H-1,2,4-triazole (IIb).—A solution of 51.0 g (0.24 mole) of Ib in 200 ml of $POCl_3$ was refluxed for 1 hr. The cooled mixture was poured cautiously into ice–water and stirred for 1 hr. The crude product was collected by filtration, washed with cold water, and air dried on the funnel. Recrystallization from aqueous ethanol (charcoal) gave the product as pale yellow needles.

1-Acetyl-3-(5-nitro-2-furyl)-1H-1,2,4-triazole (III).—A solution of 45.0 g (0.25 mole) of IIb in 300 ml of acetic anhydride was heated on a steam bath for 2 hr and concentrated to dryness *in vacuo* on a steam bath, and the residue was recrystallized from acetic anhydride (charcoal). The product separated as pale yellow needles.

1-Methylcarbamoyl-3-(5-nitro-2-furyl)-1H-1,2,4-triazole (IV).—A solution of 50.0 g (0.28 mole) of IIa in 300 ml of dimethylformamide containing 50 ml of methyl isocyanate was heated on a steam bath with stirring for 45 min. The hot solution was treated with charcoal and filtered. The filtrate was diluted with 300 ml of water and the mixture was chilled thoroughly. The crude product was collected by filtration and washed with water. Recrystallization was effected rapidly by taking up portions of the crude product in boiling 95% ethanol (charcoal), filtering the mixture by suction, and cooling the filtrate as quickly as possible. The product separated as long yellow needles melting at 188.5–190° followed by resolidification and decomposition at 257–259°. Prolonged heating in alcohol converted IV to IIa.

2,3-Dimethyl-5-(5-nitro-2-furyl)-1H-1,2,4-triazole (V).—To a stirred suspension of 40.0 g (0.21 mole) of IIb in 1000 ml of methanol was added 11.3 g (0.21 mole) of sodium methoxide. After refluxing the mixture for 20 min, 40 ml of methyl iodide was added dropwise within 10 min. The mixture was refluxed for an additional 1.5 hr and concentrated to dryness *in vacuo* on a steam bath, and the residue was slurried in cold water. The crude product was filtered, washed with water, and recrystallized from methanol (charcoal) to give V as yellow needles.

N-Amino-5-nitro-2-furamidine Hydrochloride (VI).—To 500 ml of methanol containing 50 g of dry HCl was added in portions

(6) All melting points were determined on a hot stage (Mel-Temp) melting apparatus and are corrected.

with stirring below 10° 196 g (0.99 mole) of Ia. Stirring was continued in the cold for 3 hr, and then at room temperature overnight. Dilution with 2 vol of anhydrous ether precipitated the product. The crude product was filtered, taken up in 100 ml of boiling methanol, and treated with charcoal, and the mixture was filtered. Dilution of the cooled filtrate with ether gave VI as pale yellow crystals melting at 192–193° in a yield of 149 g (72.7%).

Anal. Calcd for $C_5H_6N_4O_3 \cdot HCl$: C, 29.06; H, 3.42; Cl, 17.16; N, 27.12. Found: C, 28.72; H, 3.48; Cl, 17.12; N, 27.01.

Acknowledgments.—We wish to thank Mr. Grant Gustin and Mr. Marvin Tefft for elemental analysis, and Mr. R. A. Dobson for the testing data.

Nitrofuryl Heterocycles. IV[1]. 4-Amino-2-(5-nitro-2-furyl)quinazoline Derivatives

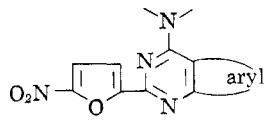
HOMER A. BURCH

Chemistry Division, The Norwich Pharmacal Company, Norwich, New York

Received January 5, 1966

Thirty-five 4-(substituted amino)-2-(5-nitro-2-furyl)quinazolines were prepared and found to possess broad *in vitro* antibacterial activity against a variety of organisms. Several compounds were also active *in vivo* against *Staphylococcus aureus* infections. The most active compound contained the 4-bis(2-hydroxyethyl)amino group. A new molecular grouping responsible for enhancing the antibacterial activity of nitrofurans is postulated.

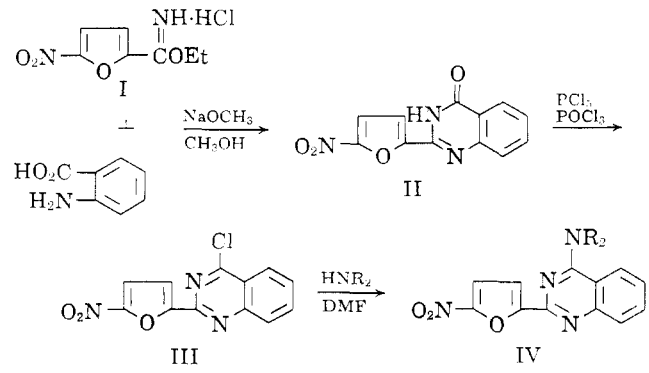
In the previous papers of this series[1] it was shown that the attachment of a heterocyclic ring system to the 2-position of the 5-nitrofuran nucleus frequently gave antimicrobial agents. It is the intent of this and a succeeding paper to demonstrate that the attachment of a condensed pyrimidine ring system to the 2-position of the nitrofuran ring will also give derivatives possessing exceptional antibacterial activity. This ring system is represented by the following general formula. This paper is concerned with the synthesis



and biological evaluation of 4-amino-2-(5-nitro-2-furyl)quinazoline derivatives.

Chemistry.—The general procedure of Dymek and Berezowski[2] was followed to prepare quinazolinone (II) from ethyl 5-nitro-2-furimidate (I).[3] Chlorination of II with phosphorus pentachloride solution gave the 4-chloro derivative III in an over-all yield of about 72% from I. Displacement of the chloro group in III with a variety of amines proceeded smoothly in dimethylformamide (DMF) solution to give the amino derivatives IV listed in Table I. These reactions are summarized in Scheme I.

SCHEME I



Screening Results.—The 4-amino derivatives IV were screened for *in vitro* and *in vivo* antibacterial activity according to the procedures described previously.[4] It can be seen in Table II that the 35 derivatives of IV herein reported possess broad *in vitro* activity against both gram-positive and gram-negative organisms. The activity of several of the derivatives (**13–16**, **18**, **19**, **22–26**, **28–31**, and **33**) against both *Pseudomonas aeruginosa* and *Proteus vulgaris* is particularly noteworthy. The most active compounds contain the 2-hydroxyethylamino group, $NRCH_2CH_2OH$, in which R is H (**7**), alkyl (**13–16**), or hydroxyalkyl (**18** and **19**). These compounds also demonstrated good activity *in vivo* against *Staphylococcus aureus* infections in mice. The *in vivo* data are summarized in Table III. The next most active group of compounds contain the dialkylaminoalkylamino grouping (**22–33**). Except for compound **33** (γ-morpholinopropylamino) this group demonstrated good *in vitro* activity but failed to show *in vivo* activity at the levels tested. The toxicity of several compounds described in Table II is being investigated.

Experimental Section

All melting points were taken on a hot stage (Mel-Temp) melting apparatus and are corrected.

2-(5-Nitro-2-furyl)-4(3H)-quinazolinone (II).—To a stirred solution of 27.0 g (0.5 mole) of sodium methoxide in 500 ml of methanol was added 110 g (0.5 mole) of ethyl 5-nitro-2-furimidate hydrochloride[3] and then 68.5 g (0.5 mole) of anthranilic acid. The mixture was refluxed for 4 hr and concentrated to dryness *in vacuo* on a steam bath, and the residue was shaken with 500 ml of ice–water. The mixture was acidified with acetic acid. The crude product was filtered, washed with water, and dried to give 114 g (89%) of II. Recrystallization of a sample from DMF (charcoal) gave the product as yellow micro needles decomposing above 300°.

(1) For the previous paper in this series see H. A. Burch and W. O. Smith, *J. Med. Chem.*, **9**, 405 (1966).

(2) W. Dymek and L. Berezowski, *Dissertationes Pharm.* **15**, 23 (1963); *Chem. Abstr.*, **59**, 11491b (1963).

(3) W. R. Sherman and A. Von Esch, *J. Med. Chem.*, **8**, 25 (1965).

(4) F. F. Ebetino, W. F. Carey, and B. F. Stevenson, *ibid.*, **6**, 633 (1963).