under reduced pressure, and the residue extracted into $Et_2O$. The aqueous solution was neutralized with HOAc and the solid which formed was filtered, washed with $H_2O$, and dried, affording 0.7 g of product. *Anal.* ($C_{23}H_{29}F_3N_4O_2$) C, H, F, N.

3,3-Diphenyl-1,5-dimethylpyrrolidin-2-one (5-Nitro-2-pyridyl)-hydrazone (13). A mixture of 4a (9.9 g, 0.03 mol) and 2-chloro-5-nitropyridine (5.1 g, 0.032 mol) in pyridine (100 ml) was refluxed until evolution of gas had ceased. The pyridine was evaporated under reduced pressure; the residue was taken up in $H_2O$, acidified with dilute HCl, extracted into $CH_2Cl_2$, and dried (anhydrous $Na_2SO_4$). Evaporation of the solvent left a dark resinous oil (16.6 g) which was eluted through a column of silica gel with PhH–EtOAc (19:1), affording 6.0 g of a red crystalline product. Recrystallization of the crude material from PhH–Skellysolve B gave 2.0 g of product as red prisms. *Anal.* ($C_{23}H_{23}N_5O_2$) C, H, N.

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# Nitrofuryltriazole Derivatives as Potential Urinary Tract Antibacterial Agents

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A series of 5-(5-nitro-2-furyl)-1,2,4-triazoles was synthesized and their activity as potential urinary tract antibacterial agents was tested. Many of the compounds showed a higher antibacterial activity than nitrofurantoin (XII) especially against gram-negative bacteria. After po administration in dogs only a few of the compounds were excreted in the urine in an antibacterially active form. All the compounds were less toxic than XII in mice. Five compounds were tested for emetic effect in cats and found less active than XII. The *in vitro* enzymatic degradation of different nitrofuryltriazoles showed no clear correlation to the excretion. Five compounds were tested for their excretion in man and 3-amino-5-(5-nitro-2-turyl)-1,-2,4-triazole (IVa) and 3-hydroxymethylamino-5-(5-nitro-2-furyl)-1,2,4-triazole (IVb) showed the highest excretion with 15% of given dose compared with 30% for XII. IVa was selected for further study. It was found active in experimental pyelonephritis in rats and showed a higher activity than XII in a test against 423 bacterial strains isolated from patients with urinary tract infection. A preclinical tolerance study in man showed that higher doses of IVa than of XII could be given without causing nausea and vomiting. However, four cases of suspected allergic reactions of drug fever type were reported for IVa. Two clinical studies showed that IVa had a therapeutic effect against urinary infections.

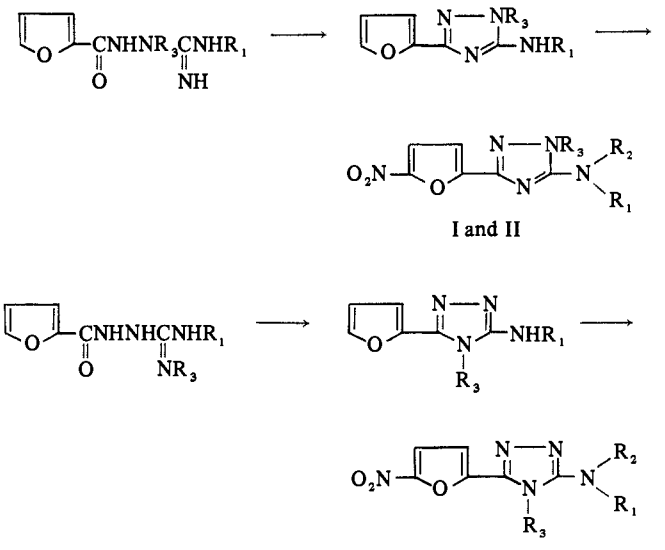
A new urinary tract antibacterial agent should fulfil the following basic criteria. It should have high antibacterial activity against microorganisms causing urinary tract infections, should be well absorbed perorally, and be excreted in the urine in amounts sufficient to give effective concentrations of the active substance.

Many nitrofuran derivatives have been synthesized and found to possess a good antibacterial activity *in vitro*, but only a few of these have been reported to be excreted in the urine in an antibacterially active form.[1,2] Nitrofurans are known to be inactivated rapidly in the body.[1]

Nitrofurantoin is a nitrofuran compound which largely fulfils the basic criteria for an effective urinary tract antibacterial agent and is indeed one of the clinically more important drugs. We have been searching for other nitrofuran derivatives with at least the same level of activity and broad antibacterial spectrum as nitrofurantoin and with less side effects. A series of 5-(5-nitro-2-furyl)-1,2,4-triazoles (Table I) was synthesized and subjected to the following screening tests: *in vitro* antibacterial activity against bacteria causing infections in the genitourinary tract, *in vivo* urinary excretion, acute toxicity, acute emetic effect, and metabolic susceptibility.

**Chemistry.** A number of the nitrofuran compounds in series I, II, and IV (Table I) were obtained by ring closure of furoylaminoguanidines to 3-amino-5-(2-furyl)-1,2,4-triazoles,[3] which were then acetylated and nitrated in a mixture of concentrated $HNO_3$ and $H_2SO_4$ (Scheme I). In later

Scheme I



experiments, protection of the amino group before nitration was obviated by employing an equivalent amount of $HNO_3$. The furan ring was nitrated first and thereafter the amino group. Derivatives of the nitrofuran compounds were prepared by alkylation, acylation, nitrosation, or nitration. The hydroxymethyl-substituted nitrofurans IIb and IVb were obtained by reacting IIa and IVa, respectively, with formaldehyde in DMF. Alkylation of Ia,b with MeI in the presence of NaOEt yielded a mixture of 1- and 2-methylated compounds, IIIa,d and IIa,g, respectively.

Acetylation of the nitrofurylaminotriazoles with excess $Ac_2O$ gave diacetylated compounds. The corresponding monoacetyl compounds were obtained by selective hydrolysis of the former in water.

Treatment of the nitrofurylaminotriazoles with mono- and dichloroacetyl chloride in benzene gave the monoacylamino derivatives, while benzoylation of Ia with benzoyl chloride in pyridine at 40° occurred at a ring nitrogen atom, possibly in position 2. On heating the latter compound, it rearranged to the more stable benzoylamino compound Il.

The nitroso compounds If and Ig were obtained by treating Ib and Ic, respectively, with $NaNO_2$ in dilute HCl. Nitration of Ic and IVa with concentrated $HNO_3$ in concentrated $H_2SO_4$ gave the nitroamino compounds Ih and IVf, respectively. Va was obtained by diazotizing Ia in HCl.

Vb–e and VIb were obtained according to the synthetic route in Scheme II. Attempts to hydrolyze the triazoles VII–X were unsuccessful. The methylsulfinyl and methylsulfonyl groups proved to be better leaving groups than the methylthio group[4] and reacted easily with NaOH and NaOMe to give compounds X and XI, respectively. When VII was nitrated with an excess of $HNO_3$, the methylthio group was oxidized and compound Vc was obtained.

**Biological Results and Discussion. I. Antibacterial Activity** *in Vitro*. The nitrofuryltriazoles I–VI possessed broad spectrum *in vitro* antibacterial activity against representative bacteria except *Pseudomonas aeruginosa* as shown in Table I when the twofold serial dilution technique[5] was employed. The validity of these values was studied separately and it was found that a MIC value never differs more than ± one step from the true value. The highest activity was shown against the gram-negative bacteria *Escherichia coli* and *Klebsiella aerogenes* with MIC values as low as 0.4 μg/ml. Against *Proteus vulgaris* the activity was low and only a few compounds showed high activity against *Alkaligenes faecalis*. Many of the nitrofuryltriazoles tested were more active than nitrofurantoin against gram-negative bacteria. Against gram-positive bacteria, our compounds were fairly active against *Staphylococcus aureus* and *Streptococcus facecalis* while the activity against *β-haemolytic streptococci* was low.

It is difficult to find any structure-activity relationship which is valid for all bacteria tested. Against the gram-negative bacteria some correlations can, however, be perceived. Thus, methylation of the triazole ring especially in position 1 and 2 increases the antibacterial activity against *E. coli*. Acetylation of the free amino group in the different nitrofuryltriazoles decreases the activity against *K. aerogenes* and *A. faecalis*.

Compound IVf, with a nitroamino substituent in position 3, showed especially low activity. This compound is an acid with $pK_a$ around 3. It is known that acidic nitrofurans show low activity[6] probably depending on difficulties in penetrating the bacteria. The same is probably true for compound VIa which is base protonated at physiological pH.

The *in vitro* antibacterial activity of IVa was compared with nitrofurantoin in a MIC test (repeated 15 times). IVa was found to be 1.9, 3.2, and 1.6 times more active than nitrofurantoin against *E. coli*, *K. aerogenes*, and *A. faecalis*, respectively. The differences were significant ($p < 0.05$).

Scheme II

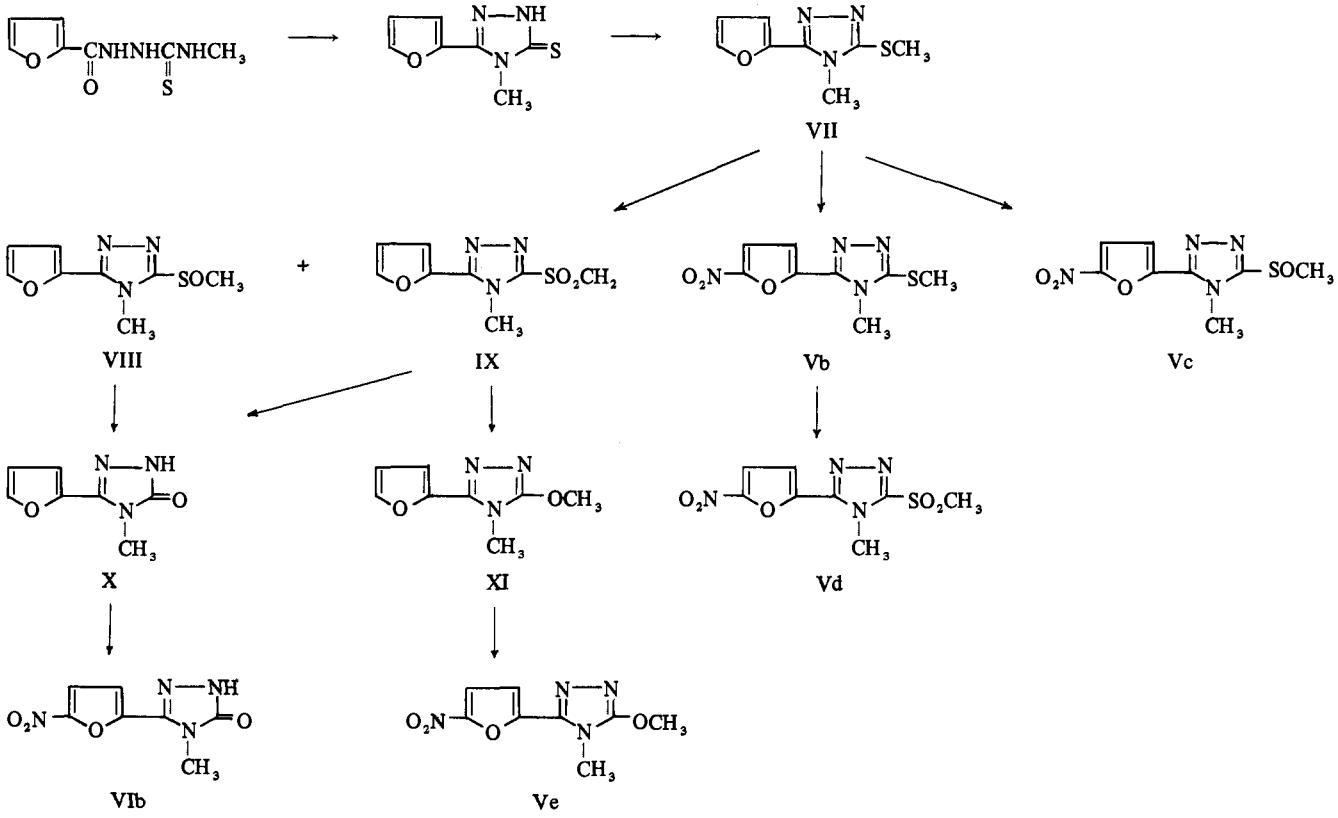
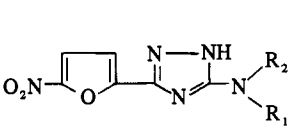


Table I



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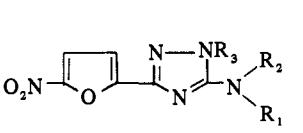
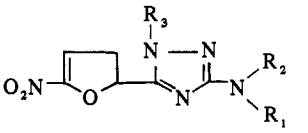


II



III

| Compd | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Method | Yield, % | Mp, °C | St-a[e] |
|---|---|---|---|---|---|---|---|---|---|
| Ia | H | H | | | | A, B | 47, 61 | 286–288.5 dec | 12.5 |
| b | $CH_3$ | H | | | | A, B | 55, 59 | 279–280 dec | 12.5 |
| c | $C_2H_5$ | H | | | | A | 48 | 255–258 dec | 12.5 |
| d | $C_3H_7$ | H | | | | A | 29 | 180–182.5 dec | 12.5 |
| e | $C_3H_5$ | H | | | | | 2.5 | 209–211.5 | 25 |
| f | $CH_3$ | NO | | | | D | 46 | 181–185 dec | 12.5 |
| g | $C_2H_5$ | NO | | | | D | 84 | 141–146 dec | 12.5 |
| h | $C_2H_5$ | $NO_2$ | | | | C | 77 | 136.5–137 dec | 12.5 |
| i | CHO | H | | | | E | 80 | 325–328 dec | 25 |
| j | $COCH_3$ | H | | | | A | 53 | 350–351 dec | 12.5 |
| k | $COCHCl_2$ | H | | | | E | 59 | 196–199 | 25 |
| l | COPh | H | | | | E | 50 | 321–324 dec | 0.8 |
| IIa | H | H | $CH_3$ | | | A, F | 27 | 299–301 dec | 6.3 |
| b | $CH_2OH$ | H | $CH_3$ | | | G | 56 | 151–154 dec | 6.3 |
| c | $COCH_3$ | H | $CH_3$ | | | A, E | 40, 74 | 191–192 | 12.5 |
| d | $COCH_3$ | $COCH_3$ | $CH_3$ | | | E | 90 | 178–181.5 dec | 12.5 |
| e | H | H | COPh | | | E | 70 | 260–261 dec | 12.5 |
| f | H | H | $CONHCH_3$ | | | | 77 | 231–235 dec | 6.3 |
| g | $CH_3$ | H | $CH_3$ | | | F | 8 | 194–201 | 6.3 |
| IIIa | H | H | $CH_3$ | | | F | 12 | 227–229 | 6.3 |
| b | $COCH_3$ | H | $CH_3$ | | | E | 51 | 214–215.5 | 12.5 |
| c | $COCH_3$ | $COCH_3$ | $CH_3$ | | | E | 92 | 120–122 | 12.5 |
| d | $CH_3$ | H | $CH_3$ | | | F | 12 | 191–193 dec | 12.5 |
| IVa | H | H | $CH_3$ | | | A, B | 49, 69 | 282–283 dec | 12.5 |
| b | $CH_2OH$ | H | $CH_3$ | | | G | 92 | 196–199 dec | 12.5 |
| c | $COCH_3$ | H | $CH_3$ | | | A, E | 42, 75 | 284–286 dec | 25 |
| d | $COCH_2Cl$ | H | $CH_3$ | | | E | | 149.5–152 dec | 1.6 |
| e | $COCH_3$ | $COCH_3$ | $CH_3$ | | | E | 77 | 165–167 dec | 6.3 |
| f | $NO_2$ | H | $CH_3$ | | | C | 59 | 159.5–160 dec | 50 |
| g | H | H | $C_2H_5$ | | | A | 36 | 242–245 dec | 25 |
| h | $COCH_3$ | H | $C_2H_5$ | | | A | 56 | 208–212.5 | 25 |
| Va | | | H | Cl | | | 51 | 165.5–167 | 6.3 |
| b | | | $CH_3$ | $SCH_3$ | | | 71 | 174–176 | 25 |
| c | | | $CH_3$ | $SOCH_3$ | | | 48 | 183–185 | 50 |
| d | | | $CH_3$ | $SO_2CH_3$ | | | 49 | 198.5–202.5 | 6.3 |
| e | | | $CH_3$ | $OCH_3$ | | | 32 | 176.5–179 dec | 12.5 |
| VIa | | | $CH_3$ | $NH_2^+I^-$ | $CH_3$ | F | 56 | 236.5–237.5 dec | 50 |
| b | | | H | O | $CH_3$ | | 43 | 270–274 dec | 6.3 |
| XII | Nitrofurantoin | | | | | | | | 12.5 |

[a] All compounds were inactive to *Pseudomonas aeroginosa* at the highest test level, 200 μg/ml. [b] Individual values or mean value ± S.D. [c] Only some of the urinary samples were analyzed with the polarographical method. [d] A cup-plate assay with a sensitive *E. coli* as test organ-

For *Staph. aureus* no difference in activity was obtained.

II. **Urinary Excretion.** (a) In **Dogs.** The po absorption and the urinary excretion of the nitrofuryltriazoles were tested in dogs. A single dose of 5 mg/kg was given orally to 2–18 dogs and the total excretion was measured for 0–6 hr using both polarographic and microbiological (*E. coli*) assay techniques.

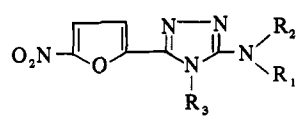
The polarographic method[7] measures the total amount of nitrofuran derivatives excreted in the urine as a sum of the parent nitrofuran compound and all possible metabolites containing an intact nitrofuran group.

The microbiological method[8] measures the amount of the antibacterially active compounds that has been excreted, expressed in terms of the parent compound.

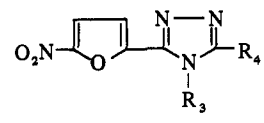
With the exception of IVf, none of the nitrofuryltriazoles were excreted as well as nitrofurantoin in the urine of dogs. For nitrofurantoin and some of the compounds in Table I, both assay techniques gave similar results. This indicates that the compounds excreted are the parent compounds and not active metabolites. Most of our compounds were excreted to some extent in the urine according to the polarographic assay but the amounts of active substances as measured by the microbiological assay were always lower, indicating that these compounds were metabolized to antibacterially less active compounds.

The compounds in series V and VI (see Table I) do not appear from the microbiological assay to be excreted in the urine. These compounds lack an amino substituent in position 3. An amino substituent in this position seems thus to be a prerequisite for good excretion.

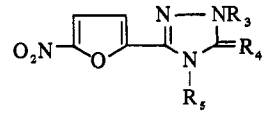
In the series where a 3-amino substituent is present, I–IV, further N-substitution in the triazole ring modifies the urinary excretion. Some structure–activity rules may be inferred. Thus, an *N*-alkyl substitution in position 4 (series IV) renders the compounds excretable. Compounds with *N*-alkyl substitution in position 1 and 2 (series III and II) are generally not excreted. Exceptions are the compounds IIb,c and IIId. The same is also found for the compounds in series I with a hydrogen on the ring nitrogen where only compounds Ib and Ic are excreted.



IV



V



VI

| Antibacterial activity *in vitro*, MIC, μg/ml[a] | | | | | | Total urinary excretion in dogs,[b] % of given dose | | | Acute toxicity in mice, approx $LD_{50}$ in mg/kg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| β-Str | Str-f | E-c | Kl-a | Al-f | Pr-v | No. of dogs | Polarographic method | Microbiological method[d] | ip | po | po/ip |
| 200 | 12.5 | 6.3 | 6.3 | 12.5 | >200 | 2 | 2.5 | 0, 3 | 1460 | 4800 | 3.3 |
| 200 | 25 | 3.2 | 12.5 | 50 | >200 | 4 | 9 ± 4 | 7 ± 4 | 600 | 2400 | 8.0 |
| 100 | 25 | 6.3 | 50 | 50 | >200 | 2 | 3, 7 | 2, 2 | 440 | 1760 | 4.0 |
| >200 | 12.5 | 12.5 | 50 | 50 | >200 | 2 | 2, 7 | 0, 0 | 150 | >800 | >5.3 |
| 200 | 25 | 6.3 | 25 | 100 | >200 | | | | | | |
| 200 | 25 | 6.3 | 100 | 3.2 | 200 | 2 | 5, 12 | 0, 1 | 300 | 400 | 1.5 |
| 200 | 25 | 12.5 | 100 | 25 | 200 | 2 | 5, 5 | 0, 0 | 150 | 440 | 1.5 |
| >200 | 12.5 | 12.5 | 200 | 50 | >200 | 2 | 7, 8 | 0.5, 1 | 150 | 600 | 4.0 |
| >200 | 25 | 6.3 | >200 | 12.5 | >200 | 2 | 0, 0.2 | 0, 0 | >1600 | >6400 | |
| >200 | 12.5 | 12.5 | 100 | >200 | >200 | 2 | 0, 0.2 | 0, 0.3 | >1600 | 6400 | |
| >200 | 6.3 | 12.5 | 100 | 50 | 200 | 2 | 7, 9 | 0, 0.3 | 880 | 1460 | 1.7 |
| >200 | 200 | 3.2 | >200 | >200 | >200 | 2 | 0, 0 | 0, 0 | >3200 | >6400 | |
| >200 | 6.3 | 0.8 | 0.8 | 50 | >200 | 2 | 2, 3 | 0.1, 0.2 | >1600 | >6400 | |
| >200 | 6.3 | 1.6 | 1.6 | 50 | 100 | 8 | 6 ± 1 | 2 ± 1 | 600 | 3520 | 5.9 |
| >200 | 6.3 | 0.8 | 12.5 | >200 | >200 | 4 | 5, 5[c] | 8 ± 1 | 300 | 600 | 2.0 |
| >200 | 12.5 | 0.8 | 12.5 | >200 | >200 | 2 | 1, 1 | 0.4, 2 | 300 | 1460 | 4.9 |
| >200 | >200 | 3.2 | >200 | >200 | >200 | 2 | | 0, 0 | >1600 | >6400 | |
| >200 | 12.5 | 0.8 | 3.2 | 25 | >200 | 2 | 0, 0.1 | 0, 0 | >1600 | | |
| 12.5 | | 0.8 | | | | 2 | 5, 5 | 1, 2 | 300 | 730 | 2.4 |
| >200 | 25 | 0.4 | 3.2 | 25 | 100 | 2 | 1, 2 | 0, 0 | 160 | 880 | 5.6 |
| >200 | 100 | 3.2 | 100 | 200 | >200 | 2 | 1, 1 | 0, 0 | 300 | 600 | 2.0 |
| >200 | 100 | 6.3 | 100 | 200 | >200 | 2 | 1, 1 | 0, 0 | 300 | 600 | 2.0 |
| 50 | | 1.6 | | | | 2 | 7, 8 | 9, 10 | 300 | 880 | 2.9 |
| 100 | 6.3 | 3.2 | 12.5 | 25 | 50 | 15 | 6 ± 4 | 5 ± 4 | 730 | 1460 | 2.0 |
| 25 | 12.5 | 3.2 | 50 | 25 | 100 | 18 | 11 ± 5 | 9 ± 4 | 600 | 2910 | 4.9 |
| 200 | 12.5 | 6.3 | 100 | 50 | 100 | 3 | 3, 4, 5 | 4, 5, 5 | 1200 | >6400 | >5.3 |
| 50 | 12.5 | 1.6 | 50 | 50 | 100 | 8 | 4, 15, 16[c] | 5 ± 4 | 300 | 1200 | 4.0 |
| 200 | 50 | 6.3 | 100 | 50 | 100 | 11 | 13 ± 5 | 13 ± 5 | 600 | 1760 | 2.9 |
| 100 | 100 | 50 | >200 | 200 | >200 | 5 | 29 ± 11 | 63 ± 37 | 300 | 730 | 2.4 |
| >200 | 6.3 | 3.2 | 25 | 6.3 | 200 | 4 | 10, 12[c] | 2 ± 1 | 440 | | |
| >200 | 12.5 | 3.2 | 100 | 50 | >200 | 4 | 14 ± 7 | 9 ± 3 | 300 | | |
| 200 | 6.3 | 3.2 | 12.5 | 3.2 | 100 | 4 | 4 ± 2 | 0.1 ± 0.1 | 180 | 440 | 2.4 |
| 200 | 50 | 3.2 | 12.5 | 25 | 200 | 3 | | 0, 0, 0 | 360 | 440 | 1.2 |
| >200 | | 6.3 | | | | 2 | 2, 2 | 0, 0 | 300 | 600 | 2.0 |
| 200 | >200 | 6.3 | 100 | 200 | 200 | 4 | | 0 ± 0 | >1600 | 5820 | <3.6 |
| >200 | 50 | 6.3 | 12.5 | 50 | 200 | 2 | 0, 0.1 | 0, 0 | 360 | 600 | 1.7 |
| >200 | >200 | 50 | 100 | 200 | 200 | 4 | 3 ± 2 | 0 ± 0 | 440 | 1460 | 3.3 |
| 200 | 100 | 6.3 | 12.5 | 50 | 100 | 2 | 0, 0 | 0, 0 | 880 | 2910 | 3.3 |
| 50 | 6.3 | 6.3 | 50 | 50 | 100 | 26 | 19 ± 8 | 17 ± 6 | 150 | 360 | 2.4 |

ism. [e]St-a = *Staph. aureus*, β-Str = *haem. strept.*, Str-f = *Strept. faecalis*, E-c = *E. coli*, Kl-a = *K. aerogenes*, Al-f = *A. faecalis*, Pr-v = *P. vulgaris*.

Compound IVf shows an oustandingly high excretion, 63% of the dose measured microbiologically. Measured polarographically the excretion was 29%. This discrepancy may be ascribed to the presence of metabolites which are antibacterially more active than the parent compound. The excretion of IVf was blocked by Probenicid. This shows that IVf is excreted actively by the acid transport system in the renal tubular cells.[7] The compound IVf has a high excretion, but owing to its low antibacterial activity, further development as a urinary tract antibacterial agent was not justified.

**(b) In Man.** The urinary excretion of some of our compounds was tested in man. A single dose of 3 mg/kg was given orally and the total urinary excretion was followed for 0–6 hr. The assay techniques were the same as in the experiment with dogs. The results are found in Table II. Generally, the excretion was higher in man than in dog; however, the correlation is sufficiently good to justify the use of the dog as a test animal in studies on the excretion of different nitrofuran compounds. About 15% of a given dose of IVa and IVb was excreted, which is approximately half that of nitrofurantoin. As, however, IVa showed a higher antibacterial activity than nitrofurantoin, it was regarded as a promising candidate for further investigation as a urinary tract antibacterial agent.

**III. Metabolic Studies** *in Vitro*. It was of interest to investigate whether the degree of urinary excretion of the nitrofuryltriazole derivatives could be correlated to the rate of metabolic degradation.

It is known that nitrofurans are inactivated in the body by reduction of the nitro group.[1] The rate of the reduction of some nitrofuryltriazoles in the presence of reductive enzymes from the dog liver was therefore studied. The enzyme preparation and the incubation conditions used are described in the Experimental Section and the results presented in Table II. The inactivation of the nitrofurans is expressed as the precentage of the original compound reduced in 1 hr (measured polarographically). A plot of the logarithm of the concentration of nitrofuran against time afforded a straight line showing first-order kinetics (Figure 1).

No correlation was obtained between the excretion of

Table II

| Compd | Metabolic inactivation (1 hr) by crude liver enzyme prepn[a] No. of expt | % | Urinary excretion (%) of the po dose in 0–6 hr[b] Dogs, 5 mg/kg No. | Polarographic | Microbiological | Homo, 3 mg/kg No. | Polarographic | Microbiological | Emetic effect in cats No. of cats showing vomiting/no. of cats tested 200 | 100 | 50 | 25 | 12.5 | 6 | 3[d] | Approx $ED_{50}$, mg/kg,[c] po |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ia | 3 | 31 ± 6 | 2 | 2, 5 | 0, 3 | 6 | 7 ± 1 | 5 ± 5 | 2/5 | 0/10 | 0/5 | | | | | 250 |
| Ib | 2 | 14 ± 7 | 4 | 9 ± 4 | 7 ± 4 | 3 | 5, 7, 10 | 3, 4, 4 | 2/5 | 2/4 | 0/5 | | | | | 100 |
| Ik | 2 | 0 | 2 | 2, 9 | 0, 0.3 | 5 | 3 | <1 | | 5/5 | 2/5 | 3/5 | 0/5 | | | 20 |
| IIa | 2 | 9 ± 7 | 2 | 2, 3 | 0.1, 0.2 | | | | | | | | | | | |
| IVa | 4 | 21 ± 5 | 15 | 6 ± 4 | 5 ± 4 | 6 | 14 ± 5 | 13 ± 6 | 3/5 | 0/5 | 1/10 | 1/5 | 1/5 | 0/5 | | 100 |
| IVb | 1 | 21 ± 10 | 18 | 11 ± 5 | 9 ± 4 | 7 | 13 ± 4 | 17 ± 7 | | 2/3 | 1/5 | 0/5 | 0/5 | | | 75 |
| Va | 2 | 2 ± 7 | 4 | 4 ± 2 | 0.1± 0.1 | | | | | | | | | | | |
| VIa | 2 | 44 ± 7 | 4 | 3 ± 2 | 0 | | | | | | | | | | | |
| VIb | 2 | 38 ± 7 | 2 | 0, 0 | 0, 0 | | | | | | | | | | | |
| Nitrofurantoin | 6 | 40 ± 4 | 26 | 19 ± 8 | 17 ± 6 | 4 | 36 ± 4 | 30 ± 5 | | 2/2 | 5/6 | 5/5 | 5/5 | 4/5 | 0/5 | 5 |

[a]Mean value with 95% confidence limits. [b]Individual values or mean value ± S.D. [c]Estimated dose given vomiting in 50% of cats. [d]Doses in mg/kg.

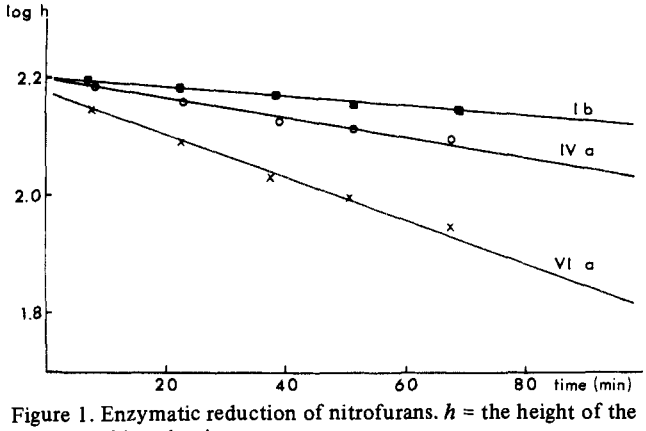



Figure 1. Enzymatic reduction of nitrofurans. $h$ = the height of the polarographic reductive wave.

nitrofuryltriazoles in the urine and their metabolic degradation in the *in vitro* test. Most of the nitrofuryltriazoles were metabolized slower than nitrofurantoin. The compounds Ik, IIa, and Va were metabolized very slowly, yet their urinary excretion was low. Obviously, slow degradation is not the sole requirement for a high rate of excretion.

IV. **Toxicity Test in Mice.** In order to gain a general idea of the pharmacological activity and peroral absorption of the the nitrofurans, a simple toxicity test[10] was performed in mice. The toxicity of the various compounds was recorded after ip or po administration according to a standard dose scale of 12,800, 6400, 3200, 1600, . . ., 3 mg/kg to groups of three mice for each dose.

The toxicity results are shown in Table I. The ip $LD_{50}$ of the nitrofuryltriazoles tested here ranged from >3200 to 150 mg/kg, the latter figure being the same as that for nitrofurantoin. Perorally, all the new nitrofuryltriazoles show lower acute toxicity than nitrofurantoin. A comparison of the po/ip toxicity ratio revealed no marked tendencies. The compounds Ia-d show that an extension of the alkyl chain confers increased ip and po toxicity parallel with increased hydrophilic character. The three isomers IIa, IIIa, and IVa with a methyl group in position 2, 1, or 4 show markedly different toxicities. Some substituents, for example, NO, $NO_2$, and Cl, seem to increase the toxicity significantly compared with the parent, unsubstituted compound.

V. **Emetic Activity in Cats.** As vomiting and nausea are known side effects of the nitrofurans, it was of interest to compare the ability of the various nitrofurans to elicit vomiting in cats. The various nitrofurans were given perorally in doses of 200, 100, 50, 25, 12, 6, and 3 mg/kg. The cats were watched for vomiting for a period of 0–6 hr and an approximate $ED_{50}$ for vomiting was obtained (Table II). All five compounds tested produced a lower incidence of vomiting than nitrofurantoin. Ia,b and IVa were at least 20 times less active than nitrofurantoin.

VI. **Further Biological Trials with Compounds IVa and IVb.** Compounds IVa and IVb were selected for further investigation as potential urinary tract antibacterial agents. Prat, *et al.*,[11] studied the antibacterial effect of these two compounds and nitrofurantoin on experimental acute ascending *E. coli* pyelonephritis in rats which were given 20 mg/kg of nitrofuran compound in a stomach tube three times daily for 7 days. The kidneys of the animals treated with IVa and IVb were significantly less infected than the untreated controls ($p < 0.05$). Animals treated with nitrofurantoin showed no significant differences with either the controls of the animals treated with IVa and IVb.

The antibacterial activity of compound IVa, compared with that of nitrofurantoin, was further documented in an *in vitro* study against 423 bacterial strains isolated from the urine of patients with urinary tract infections.† The bacteria were classified as *Coliforms* 226, *Enterococci* 77, *Proteus spheroides* 71, *Klebsiella* 40, *Pseudomonas aeruginosa* 9.

The sensitivity test was performed according to the method of Ericsson, *et al.*[12] IVa was more active than nitrofurantoin against the coliform bacteria and the bacteria belonging to the group of *Klebsiella*. There was no difference in activity against bacteria belonging to the *Proteus* group and all the strains of *Pseudomonas* were resistant to both compounds at the highest test level (100 μg/ml). Against *Enterococci* nitrofurantoin showed higher activity than IVa.

VII. **Preclinical and Clinical Studies with Compound IVa.** The promising results obtained with compound IVa encouraged us to test the compound in man.

A human preclinical tolerance study was conducted and the $ED_{50}$ with regard to adverse effects for compound IVa, nitrofurantoin, and placebo was defined by means of a single blind trial in 20 healthy young men.‡ Different po doses

†This examination was performed by B. Elgefors and J. Kjellander, Clinical Bacteriological Laboratory, Reglonsjukhuset, Örebro, Sweden.

‡This examination was performed by J. Ekstedt and A. Broman, Pharmacological Department, University of Uppsala.

were given according to latin square design at an interval of 3–4 days. Every subject noted his symptoms. Nausea and vomiting were the chief complaints and the corresponding $ED_{50}$ for IVa was found to be approximately 1300 mg and for nitrofurantoin approximately 350 mg.

However, four cases of suspected drug allergy to compound IVa with high fever and pain in joints and muscles 9–11 hr after the second dose of compound IVa were reported. One of the four subjects developed urticaria. As the first dose of IVa was given 1 week earlier, immunization might have been developed during this period. Similar reactions were not found with nitrofurantoin. These symptoms are similar to the allergic reactions of drug fever type reported for nitrofurantoin.[13]

Intra- and epicutaneous skin tests were performed on the suspected allergic subjects but no reactions were obtained against IVa. One of the subjects was tested with a third dose and developed the same symptoms, even more pronounced.

The first clinical trial was performed with compound IVa on 14 patients with chronic or recurrent urinary infections.§ The patients were treated with a dose of 4 × 150 mg daily for 2 weeks. In nine (64%) patients the outcome was successful and the urine was free from bacteria 2 weeks after completed treatment. Two (14%) patients were free of bacteria after the treatment but 2 weeks later the infection recurred. The remaining three patients' infection failed to respond to the therapy. However, a high percentage of the patients (60%) complained of adverse effects from the compound, mainly in the form of gastrointestinal disturbances.

In a second clinical trial, compound IVa was given to 12 patients (average age 79 years) with chronic or recurrent urinary tract infections.# The dose was 3 × 200 mg daily and the treatment was extended to 3 weeks. In four (33%) patients the urine was still sterile 2 weeks after completed treatment. In four (33%) patients with bacteria-free urine after treatment, reinfection appeared 2 weeks later. The remaining four (33%) patients still showed bacteria in the urine after treatment. No side effects were observed during the treatment.

The clinical studies, which included patients with urinary tract infections which had earlier been resistant to therapy, show that compound IVa has a clinical effect. However, in view of the side effects observed during the preclinical and clinical studies, further investigations are required in order to establish the clinical value of compound IVa.

## Experimental Section

The structures of all compounds were assigned on the basis of compatible ir, nmr, and mass spectra and satisfactory analyses. Ir spectra were measured in KBr disks in a Unicam Sp200 spectrophotometer; nmr spectra were obtained at 60 Mc on a Perkin-Elmer T60 instrument; mass spectra were measured at 70 eV in a LKB9000 instrument. The melting points are uncorrected. Tlc was recorded on Merck silica gel $F_{254}$. All compounds were analyzed for C, H, N, S, and halogen and analytical results were within ±0.4% of the calculated values.

**Chemistry. Method A. Nitration of 3-Acetylamino-5-(2-furyl)-1,2,4-triazoles Ia–d, Ij, IIa, IIc, IVa, and IVc.** An appropriate 3-amino-5-(2-furyl)-1,2,4-triazole derivative was acetylated by refluxing in an excess of $Ac_2O$ for 1 hr. The acetylated compound either precipitated on cooling or was recovered by evaporation of the excess of $Ac_2O$. The acetylated compound (0.5 mol) was added in portions to a mixture of 50 ml of concentrated $HNO_3$ (0.72 mol) and 450 ml of concentrated $H_2SO_4$ at 0° over a period of 45 min. The mixture was stirred for another 30 min and then poured into ice. The precipitate was filtered and the acidic solution was neutralized to give a further crop of the acetylamino compound.

The corresponding amino compounds were obtained by hydrolyzing the acetylamino compounds in 500 ml of 2 *M* HCl at 100° for 1–3 hr. The acidic solution was neutralized and the amino compound precipitated. The product was filtered and reprecipitated from 2 *M* HCl.

**Method B. Nitration of 3-Amino-5-(2-furyl)-1,2,4-triazoles Ia,b and IVa.** A solution of 0.1 mol of a 3-amino-5-(2-furyl)-1,2,4-triazole derivative in 70 ml of concentrated $H_2SO_4$ was prepared at 0°. To this was added a solution of 7.1 ml of 65% $HNO_3$ (0.1 mol) in 30 ml of concentrated $H_2SO_4$ over a period of 30 min at −3 to 0°. The solution was stirred for another 45 min and then poured into ice. The pH of the acidic solution or mixture was adjusted to 5 with concentrated $NH_4OH$. The precipitate was filtered and reprecipitated from a 2 *M* HCl solution. In the synthesis of IVa a maximum yield of 69% was obtained using 10% excess of $HNO_3$.

**Method C. Nitration of 3-Amino-5-(5-nitro-2-furyl)-1,2,4-triazoles Ih and IVf.** Ic and IVa, respectively, were added in small portions to a mixture of 7.2 ml of concentrated $HNO_3$ and 75 ml of concentrated $H_2SO_4$. The addition was complete in 10 min and the reaction mixture was stirred for another 5 min and poured into ice. The product was collected by filtration and recrystallized from EtOH.

**Method D. Nitrosation Reactions. If and Ig.** An aqueous solution of 0.7 g (0.01 mol) of $NaNO_2$ was added dropwise to a mixture of 0.009 mol of 3-alkylamino-5-(5-nitro-2-furyl)-1,2,4-triazole and 20 ml of 2 *M* HCl. The reaction mixture was stirred for 2 hr and the precipitated product collected by filtration.

**Method E. Acylation Reactions. Ii–l, IIc–e, IIIb,c, and IVc–e.** Ii was obtained by refluxing Ia with an excess of formic acid for 1 hr. Ii precipitated on cooling and was recrystallized from DMF. IId, IIIc, and IVe were obtained by boiling 0.03 mol of the corresponding amino compounds IIa, IIIa, and IVa, respectively, in 100 ml of $Ac_2O$ for 3 hr. The reaction solution was cooled and a smaller part of monoacetylated product was removed by filtration. The filtrate was evaporated to dryness and the residue recrystallized: IId in $Ac_2O$; IIIc and IVe from EtOH. IId, IIIc, and IVe were hydrolyzed to the corresponding monoacetylated compounds IIc, IIIb, and IVc, respectively, by boiling in $H_2O$ for 3 hr.

Ik was obtained by refluxing 1 mol of Ia with 2 mol of dichloroacetyl chloride in $C_6H_6$ for 6 hr. The product was filtered and recrystallized from a mixture of $H_2O$–EtOH (1:1).

IVd was obtained in the same way by boiling 1 mol of IVa with 2 mol of monochloracetyl chloride in $C_6H_6$ for 66 hr. The HCl salt of IVd was collected by filtration. The free base was obtained by treating the salt with an equivalent amount of NaOH in $H_2O$. The product was recrystallized from MeOH.

IIe was obtained by reacting 1 mol of Ia with 2 mol of benzoyl chloride in pyridine at 40° for 1.5 hr. The product was collected by filtration and recrystallized from nitromethane. On heating, IIe rearranged to II.

When the above reaction was performed at 115° the main product was II. The structures of II and IIe were confirmed by their ir and nmr spectra. The ir spectrum of II showed a peak for C=O at 1695 $cm^{-1}$, and for $NH_2$ at 3500 and 1695 $cm^{-1}$, whereas the ir spectrum of IIe showed a peak for C=O at 1660 $cm^{-1}$.

**Method F. Methylation Reactions. IIa,g, IIIa,d and VIa. Methylation of Ia.** To 2100 ml of MeOH 175.5 g (0.9 mol) of Ia and 56.2 ml (0.9 mol) of MeI were added. The mixture was refluxed and a solution of NaOMe, prepared from 21.7 g (0.9 mol) of Na in 300 ml of MeOH, was added dropwise over a period of 30 min. The reaction mixture was refluxed for 1 hr and then cooled and filtered to give 155.7 g of a mixture of IIa and IIIa. The product was recrystallized from 1700 ml of glacial AcOH to give 67.5 g of IIa. The AcOH solution was evaporated. The residue was treated with 400 ml of glacial AcOH for 6 hr. 31.6 g, mainly compound IIa, did not dissolve and was collected by filtration. The filtrate was evaporated and the residue was recrystallized three times from 650, 600, and 550 ml of nitromethane to give 23 g of compound IIIa. The purity was determined with tlc [MeOH–$C_6H_6$ (1:3)].

Methylation of Ib. To 80 ml of MeOH 10 g (0.048 mol) of Ib and 8 g (0.057 mol) of MeI were added. The reaction mixture was refluxed and a solution of NaOMe, prepared from 1.1 g (0.048 mol) of Na and 25 ml of MeOH, was added dropwise over a period of 40 min to the refluxing reaction mixture. The solution was refluxed for 2 hr and then cooled and filtered. Crude IIId (1.8 g) was obtained and recrystallized from MeOH to yield 1.3 g (12%) of IIId.

The mother liquor was evaporated, and the residue was treated

§ This clinical trial was performed by P. Å. Örsten, Renal Clinic, St. Erik's Hospital, Stockholm, Sweden.

# This clinical trial was performed by A. Dymling, Geriatric Clinic, Lasarettet, Kungälv, Sweden.

with hot AcOEt. On cooling, 1.7 g of a product precipitated which was mainly composed of NaI and unidentified product. AcOEt was evaporated and the residue treated with water and recrystallized twice from AcOEt to yield 0.9 g (8%) of IIg.

Tlc ($C_6H_6$) was used to identify the products in the separation work. IIg was identical with the product obtained by nitration of 5-(2-furyl)-2-methyl-3-methylamino-1,2,4-triazole.[3] The poor yield is due to difficulties in the separation of the products.

**Methylation of IVa.** To a solution of 5 g (0.025 mol) of IVa in 50 ml of DMSO in a closed vessel was added 1.6 ml (0.025 mol) of MeI. The vessel was placed in an oven at 80° for 10 hr. The DMSO was evaporated *in vacuo* and the residue was treated with 30 ml of acetone. VIa (4.9 g, 56%) was collected by filtration and recrystallized from EtOH to give 3.2 g (36%) of pure product.

**Method G. Hydroxymethylation Reactions. IIb and IVb. Hydroxymethylation of IIa.** A mixture of 5 g (0.024 mol) of IIa, 12.5 ml of 40% formalin, and 10 ml of DMF was heated at 90° for 6 hr and then cooled. An unidentified product (0.85 g) was filtered off. $H_2O$ was added to the filtrate and the mixture was stored in a refrigerator for 4 days and then filtered. The product was washed with acetone and 3.2 g (56%) of IIb remained: ir 3380 $cm^{-1}$ (OH); nmr (DMSO-*d*) δ 4.77 m ($CH_2$), 5.67 t (OH), 7.60 t (NH).

Hydroxymethylation of **IVa.** A mixture of 430 g (2.05 mol) of IVa, 980 ml of 40% formalin, and 280 ml of DMF was heated at 90° for 4 hr. The reaction mixture was chilled and filtered. The product was washed with acetone and dried at 60° *in vacuo* for 2 days: yield 450 g (92%) of IVb; ir 3330 $cm^{-1}$ (OH); nmr (DMSO-*d*) δ 4.77 m ($CH_2$), 5.69 t (OH), 7.30 t (NH).

**Preparation of Vb–e, VIb, VII–XI (Scheme II). Compound VII.** A solution of 44 g (0.6 mol) of MeNCS in 100 ml of EtOH was added dropwise to a solution of 76 g (0.6 mol) of 2-furoylhydrazine in 150 ml of EtOH. The reaction was exothermic and a product precipitated. The reaction mixture was refluxed for 1.5 hr and then cooled and filtered. 1-Furoyl-4-methylthiosemicarbazide (110 g, 92%) was obtained, mp 196–200° dec. This product was cyclized by boiling in 400 ml of $H_2O$ in the presence of 70 g (0.7 mol) of $NaHCO_3$. The $H_2O$ solution was acidified and 100 g (99%) of 5-(2-furyl)-4-methyl-1,2,4-triazoline-3-thione was collected by filtration, mp 195–198°. This product was methylated in 1 l. of EtOH by dropwise addition of 94 g of MeI. The reaction was strongly exothermic. After complete addition, the reaction mixture was boiled for 0.5 hr and cooled. VII HI salt (370 g, 82%) was obtained by filtration, mp 178–183° dec. The free base was obtained by neutralizing the HI salt in $H_2O$ with NaOH and extracting the $H_2O$ solution with $CHCl_3$, mp 80–83°.

Nitration of Compound VII to Compounds Vb and Vc. A. A solution of 6.95 ml (0.1 mol) of concentrated $HNO_3$ in 20 ml of concentrated $H_2SO_4$ was prepared at 0°. This was added dropwise at 0° to 19.5 g (0.1 mol) of VII partly dissolved in 60 ml of concentrated $H_2SO_4$. The addition took 30 min and the dark reaction mixture was stirred for another 15 min and then poured into ice. The pH of the acidic mixture was adjusted to pH 1 with 33% NaOH with cooling. A product precipitated which was collected by filtration and thoroughly washed with $H_2O$ to remove inorganic salts. A yield of 17.2 g (71%) of yellow crystals of Vb was obtained. Recrystallization from EtOH did not raise the melting point.

B. A solution of 19.1 ml (0.275 mol) of concentrated $HNO_3$ in 200 ml of concentrated $H_2SO_4$ was prepared at 0°. To this solution 45 g (0.231 mol) of finely ground VII was added in portions over a period of 30 min at 0°. The reaction solution was stirred for another 45 min and then poured into ice. The acidic solution was neutralized with 33% NaOH with cooling. The precipitate obtained was collected by filtration and thoroughly washed with $H_2O$ to remove inorganic salts, giving 26.8 g (48%) of crude Vc. After two recrystallizations from a mixture of MeOH–DMF (7:1), 15.8 g (27%) of orange needles of Vc remained.

**Compound Vd.** To a mixture of 8.4 g (0.035 mol) of Vb in 50 ml of glacial AcOH, 90 ml of 30% $H_2O_2$ was added dropwise and the mixture was stirred for 8 hr at room temperature. All the substance dissolved and after a while the product began to precipitate. The precipitate was collected by filtration and recrystallized twice from a mixture of EtOH–DMF (1:1) to give 4.7 g (49%) of Vd.

**Compound X.** To a solution of 195.2 g (1 mol) of VII in 400 ml of glacial HOAc, 100 ml of 33% $H_2O_2$ was added dropwise at 15–20°. The reaction solution was stirred for 4 hr and another 130 ml of $H_2O_2$ was added. After stirring another 4 hr, the reaction solution was made alkaline and 102.9 g of crude IX precipitated. The $H_2O$ phase was extracted with $CHCl_3$. On evaporation 100.6 g of a mixture of VIII and IX was obtained. The two fractions were mixed and hydrolyzed to X without any further separation and purification. Pure VIII melted at 133–135° and pure IX melted at 72–76°. Both compounds VII and VIII formed a complex with $H_2O_2$ which could be decomposed in alkaline solution.

The crude reaction products VIII and IX were hydrolyzed in 1 l. of 2 *M* NaOH at 80° for 4 hr. The cold solution was acidified with 5 *M* HCl and 113 g (68%) of X precipitated which was recrystallized from EtOH: mp 168.5–171°; ir 1690 $cm^{-1}$ (C=O).

**Compound VIb.** A solution of 7.5 ml of concentrated $HNO_3$ in 75 ml of concentrated $H_2SO_4$ was prepared at 0°. To this solution 12.4 g (0.075 mol) of finely ground X was added in portions over a period of 30 min. The dark reaction solution was stirred for another 45 min and then poured into ice. A product precipitated and was collected by filtration. A crude VIb (6.8 g, 43%) was obtained and was recrystallized from nitromethane to yield 4.1 g (26%) of pure VIb.

**Compound XI.** A solution of 10 g (0.044 mol) of IX in 100 ml of MeOH containing 0.048 mol of NaOMe was refluxed for 4 hr. The MeOH was evaporated and the residue treated with 3 l. of $Et_2O$. Crude sodium salt of X (6 g) was separated by filtration. The ether solution was evaporated to a volume of 250 ml. By filtration 4.8 g of 5-(2-furyl)-3-methoxy-4-methyl-1,2,4-triazole (XI) was collected: mp 128–130°; nmr ($CDCl_3$) δ 4.19 ($OCH_3$).

**Compound Ve.** To a solution of 2.3 ml of concentrated $HNO_3$ in 20 ml of concentrated $H_2SO_4$ 4.5 g (0.0251 mol) of XI was added in portions at 0°. The addition took 15 min and the reaction mixture was stirred for another 30 min at 0° and then poured into ice. The acid solution was neutralized with concentrated $NH_4OH$ to give 5.2 g of crude product. Recrystallization from $H_2O$ gave 1.8 g (32%) of Ve as yellow crystals.

**3-Allylamino-5-(5-nitro-2-furyl)-1,2,4-triazole (Ie).** A mixture of of 26 g (0.137 mol) of 3-allylamino-5-(2-furyl)-1,2,4-triazole and 400 ml of $Ac_2O$ were refluxed for 2.5 hr. The $Ac_2O$ was evaporated to a volume of 50 ml. On cooling, 25 g of 3-acetylallylamino-5-(2-furyl)-1,2,4-triazole precipitated, mp 90–93.5°. Another crop of 3.6 g was obtained by addition of $H_2O$ to the mother liquor, total yield 90%.

To a solution of 5.5 ml of fuming $HNO_3$ in 31 ml of $Ac_2O$ 10 g (0.043 mol) of 3-acetylallylamino-5-(2-furyl)-1,2,4-triazole and added in portions at −2 to −6°. The reaction mixture was stirred for 30 min after complete addition and then poured into ice. Product (2.1 g) was collected by filtration. This was refluxed with 2 *M* HCl for 1 hr. The acid solution was neutralized and 0.9 g of product precipitated. Recrystallization from $H_2O$ gave 0.25 g (2.5%) of Ie. Attempts to synthesize Ie according to method A failed.

**3-Amino-2-methylcarbamoyl-5-(5-nitro-2-furyl)-1,2,4-triazole (IIf).** To a mixture of 8.8 g (0.045 mol) of Ia and 60 ml of DMF, 5.1 g (0.09 mol) of MeNCO was added. The reaction mixture was heated for 1 hr on a water bath and 8.7 g (77%) of IIf was collected by filtration and recrystallized from DMF: ir 1725 (C=O), 3500, 1650 $cm^{-1}$ ($NH_2$).

**3-Chloro-5-(5-nitro-2-furyl)-1,2,4-triazole (Va).** A solution of 76 g (1.1 mol) of $NaNO_2$ in 500 ml of $H_2O$ was added dropwise to a mixture of 195 g (1 mol) of Ia and 1950 ml of concentrated HCl at 0° over a period of 2 hr. The reaction mixture was stirred at room temperature for 14 hr and then heated at 80° for 3 hr. The acidic solution was neutralized with 5 *M* NaOH to give 166 g of crude product which was collected by filtration. This product was recrystallized from $H_2O$ giving 110 g (51%) of Va.

**Metabolic Studies *in Vitro*. Enzyme Preparation.** The liver enzymes used in the metabolic studies were isolated from dog liver in the following way. The animal was sacrificed and the liver removed immediately, cooled on ice, and cut in small pieces. The liver tissue (5 g) was homogenized in 50 ml of a 0.066 *M* Tris buffer, pH 7.2, containing KCl (final concentration 0.1 *M*) during cooling with ice. KCl is necessary as supporting electrolyte in the polarographic analysis of the nitrofurans. The homogenate was centrifuged for 15 min at 40,000 rpm. The clear supernatant was collected and used in the metabolic experiments. It was analyzed for total N according to Kjeldahl and found to contain between 25 and 27 mg of N/ml. The solution was stored at −30°.

**Kinetic Measurements.** The rate of the enzymatic reduction of different nitrofurans was determined in the following way. The enzyme solution described above was diluted with two parts of the same Tris buffer as used earlier. This solution (4.5 ml) was placed in a polarographic vessel which was thermostated at 37°. The solution was then deaerated by passing nitrogen through it. A polarogram of the enzyme solution was run to obtain a blank value. Thereafter, 0.5 ml of a $10^{-3}$ *M* solution of the nitrofuran compound in DMSO was added to the enzyme solution. The reaction solution was deaerated for 2 min and then the reduction was followed by running polarograms after about 10, 20, 35, 45, and 65 min. As the nitrofurans are sensitive to light, daylight was excluded from the polarographic vessel.

The height of the reduction wave is proportional to the concentration of the nitrofuran compound. The logarithm of the heights was plotted against the time in a diagram of the type presented in Figure 1, from which per cent reduced compound after 1 hr reaction could be calculated. The values in Table II are the mean values with calculated 95% confidence limits assuming the same variation in measurements with all compounds.

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# Antiradiation Agents. Substituted 2-Pyridyloxy and 2-Quinolyloxy Derivatives of *S*-2-(Alkylamino)ethyl Hydrogen Thiosulfates and 3-Alkylthiazolidines and Substituted 2-Pyridyloxy Derivatives of 2-(Alkylamino)ethanethiols and Corresponding Disulfides†

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Substituted 2-pyridyloxy and 2-quinolyloxy derivatives of *S*-2-(alkylamino)ethyl hydrogen thiosulfates (Table II), 3-alkylthiazolidines (Table V), and substituted 2-pyridyloxy derivatives of 2-(alkylamino)-ethanethiols (Table III) and corresponding disulfides (Table IV) were synthesized as antiradiation agents by the appropriate aziridine ring-opening reactions of substituted 2-{[(1-aziridinyl)alkyl]oxy}pyridines and -quinolines. 5-Substituted 2-chloropyridines and substituted 2-chloroquinolines were prepared for heterocyclic ether-forming reactions by treatment with the Na salts of 1-aziridinealkanols to give the aziridinylalkyloxy derivatives. 5-Halo-2-pyridyl ethers resulted in the highest antiradiation activity regardless of route of administration or type of sulfur-covering group. *S*-2-({5-[(5-Chloro-2-pyridyl)oxy]-pentyl}amino)ethyl hydrogen thiosulfate (**15**) and *S*-2-({5-[(3,5-dichloro-2-pyridyl)oxy]pentyl}amino)-ethyl hydrogen thiosulfate (**11**) afforded 87% survival of mice in the 30-day test at 19 ($^1/_{12}$ of $LD_{50}$ dose) and 12.5 mg/kg ip ($^1/_{14}$ of $LD_{50}$ dose), respectively. In view of the dearth of agents effective perorally, remarkable good radioprotection was found on oral administration of thiazolidines substituted in the 3 position with 5-halo-2-pyridyloxypentyl or -hexyl groups; 5-chloro- (**102**) and 5-iodo-2-{[6-(3-thiazolidinyl)hexyl]oxy}pyridine (**103**) hydrochlorides resulted in survival rates of 73% at 150 mg/kg po (0.25 of $LD_{50}$ dose) and 93% at 300 mg/kg po (0.5 of $LD_{50}$ dose), respectively.

Expansion of several series of antiradiation agents[1-3] of the substituted 2-aminoethanethiol type led to derivatives of 2-pyridyl and 2-quinolyl ethers as highly effective antiradiation compounds. Thiols, disulfides, thiazolidines, and Bunte salts were compared to determine which sulfur-covering group would result in optimum activity. An objective of the antiradiation program has been to produce a drug which is effective when administered orally. Extensive development of the present series was undertaken because several compounds, thiazolidines in particular, showed remarkably good activity in the peroral test.

N-Substituted aziridines (Table I) were key intermediates leading to Bunte salts **1–54** (Table II) by reaction[3,4] with $(NH_4)_2S_2O_3$ and to thiols **55–69** (Table III) by reaction[3,5] with $H_2S$. Thiols were oxidized[3] to disulfides **70–80** (Table IV) and treated[3,6] with sodium formaldehyde bisulfite to give thiazolidines **81–134** (Table V). The required 1-aziridinealkanols [$HO(CH_2)_nN(CH_2)_2$] were conveniently prepared from polymethylene chlorohydrins using the displacement reaction previously described[3] for simpler 1-alkylaziridines. Substituted 2-chloro- or 2-bromopyridines and -quinolines were treated with the Na salt of 1-aziridinealkanols in refluxing THF to synthesize the heterocyclic ethers Het-O-$(CH_2)_nN(CH_2)_2$. No attempt was made to rigorously purify the new 1-substituted aziridines given in Table I.

Several novel 2-chloropyridines were prepared, although not all were used successfully in the displacement reaction. Reductive methylation of 3,3′-(methylenediimino)bis(6-chloropyridine) gave 2-chloro-5-(dimethylamino)pyridine;

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