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The Reactions of Nitrofurans with Bacteria—II.* Reduction of a Series of Antibacterial Nitrofurans by *Aerobacter aerogenes*

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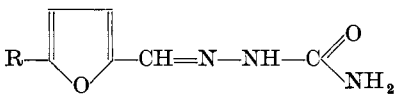
Introduction

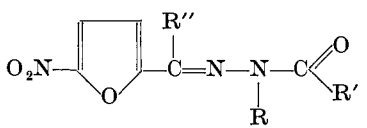
In a previous paper,[1] the reduction of nitrofurazone (5-nitro-2-furaldehyde semicarbazone I; R = —NO_2) by *Aerobacter aero-*



I

genes was reported; the initial metabolic product was thought to contain either a hydroxylamino (I; R = —NHOH) or an amino (I; R = —NH_2) group. We now advance evidence for the formulation of biologically reduced nitrofurazone as the corresponding amino compound, and interpret the effects of pH changes and storage on the spectrophotometric characteristics of solutions of the latter compound.

The reactions of *A. aerogenes* with nitrofurans having the structural formula II, in which R = —H while R″ = —CH_3, —CH_2OH



II

and R′ = —$NHCH_3$, —CH_3, —$CONH_2$ and —$CONHCH_2CH_2OH$, are also reported. Some of the results have been summarized elsewhere.[2, 3]

* Reference 1 is to be considered as Part I of this series.

Experimental Methods

Materials

Nitrofuran compounds. Nitrofurazone (NF 7) and NF 106 were supplied by Smith, Kline and French Laboratories Ltd. Furamazone (NF 84), NF 57, NF 62, NF 64 and NF 89 were supplied by Eaton Laboratories, New York. In Table I the compounds used, together with their melting points (uncorrected) and ultraviolet absorption characteristics, are recorded. The manufacturers' code numbers are included to facilitate reference to the compounds which are not known by recognized names.

5-Nitro-2-furaldehyde anil was prepared by the addition of a slight excess of aniline to 5-nitro-2-furaldehyde in absolute ethanol, to yield yellow plates (from ethanol) m.p. 129° (Drisko and McKennis[6] gave m.p. 127·5°, Fenech, Tommasini and La Rosa[8] gave m.p. 127–8°). Methyl 5-nitro-2-furoate was prepared by esterification of 5-nitro-2-furoic acid with diazomethane to yield pale yellow crystals (from water), m.p. 82° (Freure and Johnson[7] gave 81·6°, corrected).

Organism and culture medium. These were as described in Part I.[1]

Preparation of bacterial suspensions. A sufficient volume from 16 h, 250 ml, cultures of *A. aerogenes*, grown at 40° with positive pressure aeration was centrifuged at 18,000 ***g*** for 4 min, and the cells washed twice, and re-suspended in distilled water. The final volume of suspension was adjusted as described previously.[1]

Preparation of test suspensions. The reactions between *A. aerogenes* and the various compounds were carried out in single strength culture medium (see reference 1) contained in glass-stoppered flasks. The culture medium and drug solutions were placed in the flasks and allowed to reach 40° before the addition of the bacterial suspension. A distilled water control was substituted for the bacterial suspension and incubated simultaneously. Samples were withdrawn for analysis after timed intervals.

Spectrophotometric measurements. The test suspensions were centrifuged before spectrophotometric examination of the solution between 220–450 mμ (1 cm cuvettes; Uvispek, H 700). At each sampling interval, the test suspension was also examined between 500–800 mμ, with culture medium blanks, to test for bacterial

growth; previously,[1] no gross change in the surface properties of the bacteria occurred during reduction of nitrofurazone, so that any change in optical density may be attributed to growth of the organism.

The effects of pH change on the ultraviolet absorption curves of the reaction products of NF 57 and NF 62 were observed in the presence of McIlvaine buffer (pH range 3·0–8·0).

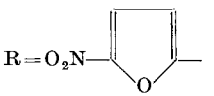
Polarographic measurements. Compounds NF57, NF62 and nitrofurazone only were examined, using the apparatus and technique described previously.[1] Solutions of chemically reduced nitrofurazone were examined after adding buffer (pH 7·0) and Analar potassium chloride to a final concentration of 0·1 M. The effects of *A. aerogenes* in single strength glucose-free culture medium containing Analar potassium chloride (0·1M) on the polarographic behaviour of NF57 and NF62 were observed without removing the bacteria, since identical polarograms were obtained in the presence and absence of bacteria provided the suspensions were cooled rapidly to room temperature and measured within 15 min. Half-wave potentials were measured against a mercury pool anode.

Calibration curves were prepared for solutions of NF57 (1–10 μg/ml) and NF62 (1–10μg/ml); those for nitrofurazone were described in Part I.[1]

Chemical reduction of nitrofurazone. 0·5 g nitrofurazone, 1·0 g of 5 per cent palladium on charcoal and 25 ml absolute ethanol were shaken with hydrogen at room temperature and atmospheric pressure. When hydrogen uptake was complete the suspension was filtered through dry filter paper into a dry flask in an atmosphere of dry nitrogen. A further 25 ml of absolute ethanol was used for rinsing purposes. A 1 in 1,000 dilution of the alcoholic solution in distilled water was prepared and spectrophotometric measurements made immediately. In some experiments the alcoholic solution was allowed to age, fresh dilutions being made at intervals for examination and comparison with the stored initial dilution.

When the alcoholic solution was evaporated to dryness with a minimum of heat, under reduced pressure and in an atmosphere of dry nitrogen, dark red scales were obtained; an infrared analysis was made within 2 h of preparation.

Table I The formulae, melting points (uncorrected) and ultraviolet absorption characteristics of the nitrofurans used in the investigation



Code No.	Structure and systematic chemical name	Recognized name	m.p. °C*	λ_{max} in mμ	log ε†	λ_{max} in mμ	log ε†
			SECTION A				
NF 7	R—CH=N—NHCONH$_2$ 5-nitro-2-furaldehyde semicarbazone	nitrofurazone	238	260 [ε 13,200 at 260 mμ]‡	4·12	375 [ε 15,800 at 375 mμ]‡	4·2
NF 57	R—C(CH$_3$)=N—NHCONH$_2$ 5-nitro-2-furyl methyl ketone semicarbazone		248–50	261 [ε 13,250 at 260 mμ]‡	4·14	377 [ε 14,000 at 375 mμ]‡	4·15
NF 62	R—CH=N—NHCONHCH$_3$ 5-nitro-2-furaldehyde 4′-methyl semicarbazone		201–2	265 [ε 12,600 at 265 mμ]‡	4·10	381 [ε 15,700 at 380 mμ]‡	4·20
NF 64	R—CH=N—NHCOCH$_3$ N-acetyl-5-nitro-2-furaldehyde hydrazide		230–5	253	4·11	364	4·23

NF 84	R—CH=N—NHCOCONH$_2$ 5-nitro-2-furaldehyde semioxamazone	Furamazone	275	270	4·01	363 [$E^{1\%}_{1\,cm}$ 752 at 362 mμ]§	4·28
NF 89	R—CH=N—NHCOCONHCH$_2$CH$_2$OH 5-nitro-2-furaldehyde 5′-(2-hydroxyethyl) semioxamazone		242–4	253	4·07‖	362 [$E^{1\%}_{1\,cm}$ 704 at 362 mμ]§	4·28‖
NF 106	R—C(CH$_2$OH)=NNHCONH$_2$ 5-nitro-2-furyl hydroxymethyl ketone semicarbazone		204–7	260	4·10	374 [$E^{1\%}_{1\,cm}$ 540 at 375 mμ]§	4·14
			SECTION B				
	R—CH=NC$_6$H$_5$ 5-nitro-2-furaldehyde anil		129	228	4·21¶	310	4·12¶
NF 5	R—COOCH$_3$ methyl 5-nitro-2-furoate		82	305 [$E^{1\%}_{1\,cm}$ 602 at 304 mμ]§	4·03		
NF 33	R—NO$_2$ 2,5-dinitro-furan		101·5–102	233 [ε 7,500 at 230 mμ]‡	3·91	310 [ε 11,500 at 310 mμ]‡	4·15

* All compounds except NF 5 and NF 33 melt with decomposition.
† In distilled water unless otherwise stated.
‡ Raffauf.[4]
§ Paul, Austin, Paul and Ells.[5]
‖ In 20 per cent v/v ethanol.
¶ Substance dissolved in dimethylformamide, since virtually insoluble in water, and then diluted with water (final concentration of dimethylformamide was 1 per cent). This procedure was also applied to NF 153 and NF 180 (see Part 3 of this series), when the values were identical with those obtained in the absence of dimethylformamide.

Also, alcoholic solutions of 5-amino-2-furaldehyde anil and methyl 5-amino-2-furoate were obtained from the corresponding nitro compounds by reduction with palladized charcoal and hydrogen as described for the reduction of nitrofurazone. The compounds were unstable and isolation of pure material was not achieved.

Bacteriostatic tests. The bacteriostatic concentrations against *A. aerogenes* of the nitrofurans described in Table I were assessed using the glucose–inorganic salts medium. Sterile glucose solution and the nitrofuran solutions were added aseptically to the medium after sterilization. The inoculum consisted of one drop of a 16 h culture of *A. aerogenes* (optical density at 500 mμ = 0·660*) added from a standard dropping pipette. The tubes were incubated at 40° and examined for the presence or absence of visible growth after 18 and 24 h.

Results

Spectrophotometric Observations

Fig. 1 shows the absorption curves obtained upon spectrophotometric examination of solutions of NF 62 after contact with *A. aerogenes* for various times: the results for nitrofurazone were

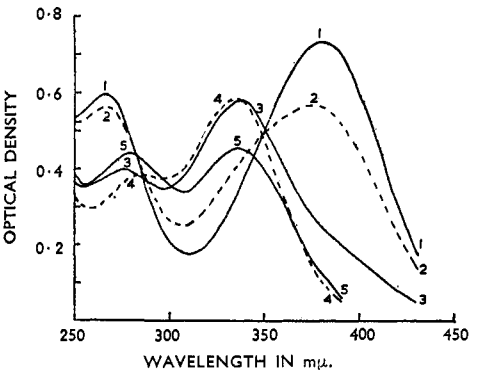


Fig. 1. Ultraviolet absorption curves of supernatant solutions obtained from contact of *A. aerogenes* with 9·9 μg/ml NF 62, in single-strength culture medium at 40·0°. Curve 1, 9·9 μg/ml NF 62 (reference curve), curves 2, 3, 4 and 5 represent the solutions obtained after $\frac{1}{4}$, $\frac{3}{4}$, $1\frac{1}{2}$ and 3 h contact respectively

* 5 mm cuvettes.

presented previously.[1] The general pattern observed with the other nitrofurans listed in Table I, section A, is similar: gradual loss of the longer wavelength absorption peak (360–380 mμ) with the appearance of a new absorption peak (see reference 1) having a maximum at wavelengths either longer or shorter than that of the parent compound, depending upon the structure of the latter. Table II shows the location of the absorption peaks of the nitrofuran solutions before and after contact with *A. aerogenes* in culture medium at pH 7·0. The new absorption peak slowly

Table II. Summary of the ultraviolet absorption spectra of some nitrofurans before and after reduction by *A. aerogenes**

Compounds	Ultraviolet absorption maxima			
	Before reduction†		After reduction‡	
	λ in mμ	λ in mμ	λ in mμ	λ in mμ
NF 7 (nitrofurazone)	260	375	272	333
NF 57	265	375	270	325
NF 62	265	380	280	335
NF 64	255	365	265	347
NF 84 (Furamazone)	270	363		375
NF 89	250	360		375
NF 106	260	374	255–75§	335

* Growth of the inoculum was indicated during these experiments.
† In culture medium pH 7.
‡ In culture medium, pH not less than 5·5–6, i.e. not sufficiently acid to cause a bathochromic shift due to proton addition.
§ Shoulder on the curve, peak masked by rising end absorption.

disappeared upon storage of the solutions and this was accompanied by increases in absorption in the 260 mμ region.

There were increases in the optical densities of the bacterial suspensions between 500 and 800 mμ caused by growth; acidity also developed but was not sufficient to give spectral shifts.

The amount of decomposition of five aminofurans after 90 min

at 40°, calculated from the shifts in the absorption peaks, is shown in Table V.

Fig. 2 shows the ultraviolet absorption curves of the solutions after contact of *A. aerogenes* with NF106; here, the 374 mμ absorption peak shows gradual hypochromic and hypsochromic shifts with increasing time of contact and only a transient peak between 320–350 mμ in contrast with the results for NF62 shown in Fig. 1.

In Table IV are shown the absorption maxima for 2-nitro-,

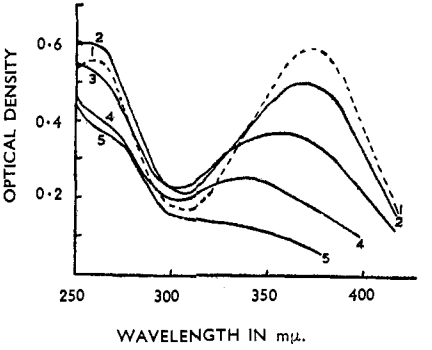


Fig. 2. Ultraviolet absorption curves of supernatant solutions obtained from contact of *A. aerogenes* with 10·0 μg/ml NF106 in single-strength culture medium at 40·0°. Curve 1, 10·0 μg/ml NF106 (reference curve), curves 2, 3, 4 and 5 represent the solutions obtained after ¼, ¾, 1½ and 3 h contact respectively

2,5-dinitro- and 5-amino-2-nitro-furan; the latter being obtained by bacterial reduction of the dinitro compound.

The ultraviolet absorption peaks in the region 325–335 mμ of bacterially reduced NF57 and NF62 underwent bathochromic shifts (325 to 385 mμ and 335 to 370 mμ respectively) upon changing the pH of the solution from 7 to 3·5. Solutions of chemically reduced 5-nitro-2-furaldehyde anil exhibited a shift from 385 to 410 mμ upon similar pH changes, whereas the ultraviolet absorption peak of solutions of chemically reduced methyl 5-nitro-2-furoate remained unchanged at 311–312 mμ over the pH range 2 to 8.

Polarographic Results

NF 57 and NF 62 exhibited two reduction steps, $E_{\frac{1}{2}}$ $-0\cdot34$ and $-1\cdot42$ V and $E_{\frac{1}{2}}$ $-0\cdot34$ and $-1\cdot30$ V respectively, similar to those already reported for nitrofurazone.[1]

Straight-line calibrations were obtained on plotting the diffusion current of the first reduction steps against concentration between 1–10 μg/ml for both NF 57 and NF 62.

Upon contact with *A. aerogenes*, solutions of NF 57 and NF 62 showed similar polarographic behaviour: decrease in the diffusion current of the first reduction step but no immediate decrease in the diffusion current of the second reduction step. These results are in agreement with those previously reported for the nitrofurazone–*A. aerogenes* system.[1]

Chemical Reduction of Nitrofurazone

Using palladized charcoal, hydrogen uptake was virtually complete after the absorption of three molecules of hydrogen per molecule of nitrofurazone. The ultraviolet absorption spectrum of an aqueous dilution of the alcoholic reaction solution initially showed a single absorption peak at 333 mμ. On storage of this aqueous dilution, the 333 mμ peak showed a hypochromic shift which was accompanied by a hyperchromic shift in the region 250–290 mμ ultimately resulting in a peak at 272 mμ; the former peak eventually disappeared. The transformation was slower in alcoholic solution.

The effects of pH changes on the 333 mμ absorption peak were identical with those reported previously[1] for solutions of the product of bacterial reduction of nitrofurazone (λ_{max}, also 333 mμ), i.e. a bathochromic shift to 370 mμ caused by proton addition.

Polarographic measurements on an aqueous solution (stored for 24 h) having absorption maxima at 333 mμ and 272 mμ showed two reduction steps: $E_{\frac{1}{2}}$ $-1\cdot35$ and $-0\cdot85$ V; these were small compared with the residual current of the supporting medium.

Bacteriostatic Results

The minimum inhibitory concentrations are recorded in Table III, each figure being the mean of five results.

Table III. Minimum inhibitory concentrations of some nitrofurans against *A. aerogenes*, expressed as μg/ml

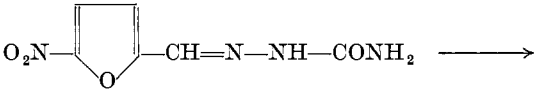
Compounds	18 h incubation at 40°	24 h incubation at 40°
NF 7 (nitrofurazone)	12·5	20·0
NF 57	20·0	30·0
NF 62	20·0	25·0
NF 64	5·0	10·0
NF 84 (Furamazone)	10·0	15·0
NF 89	>30·0	
NF 106	60·0	>60·0

Discussion

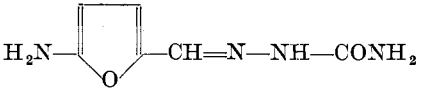
Bacterial Reduction of Nitrofurazone (NF 7)

Structure of Reduced Nitrofurazone

We previously showed[1] by spectrophotometric and polarographic measurements that the unstable product (λ_{max} 333 mμ) of the reduction of nitrofurazone by *A. aerogenes* contained either a hydroxylamino or an amino group (I; R = —NHOH or —NH_2). Hydrogenation, in which three molecules of hydrogen per molecule of nitrofurazone (III) are taken up, yields a product with an absorption peak at 333 mμ, indicating that the amino compound IV is the chief component of the product of hydrogenation.



III



IV

The infrared absorption spectrum of the freshly prepared reduced material established that the main product possessed an intact furan ring and semicarbazide side-chain, while the absorption of the nitro group of the starting material had disappeared. The development of the 272 mμ absorption peak at the expense of

the 333 mμ peak upon storage of an aqueous solution of the reduced material is attributed to the cleavage of the furan ring; polarographic data supported this conclusion since solutions exhibiting both these absorption peaks had two reduction steps, $E_{\frac{1}{2}}$ $-1 \cdot 35$ V against a mercury pool anode due to the reduction of the azomethine group[1, 9] and $E_{\frac{1}{2}}$ $-0 \cdot 85$ V which may be attributed to reduction of the product of furan ring cleavage (the diffusion current of the latter step increases at the expense of the former upon storage of the solutions).

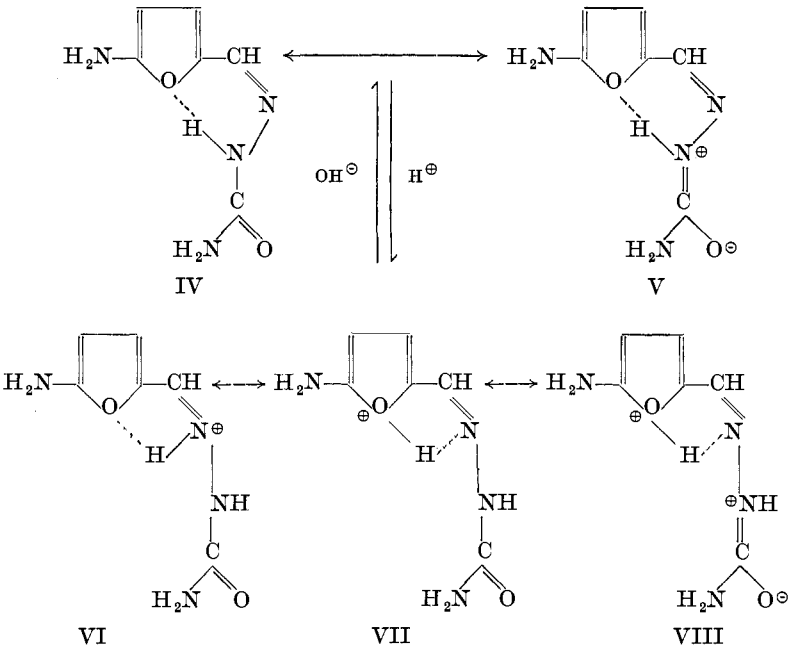
The identical character of the products of biological and chemical reduction of nitrofurazone exhibiting an absorption peak at 333 mμ is established by the similarity of shape of the ultraviolet absorption curves, the comparable bathochromic shifts upon adding acid to the aqueous solution, the similarity of the changes of ultraviolet absorption characteristics upon storage of the aqueous solutions, and the similarities of the polarographic characteristics.

The primary product of reduction of nitrofurazone by *A. aerogenes* is therefore 5-amino-2-furaldehyde semicarbazone (IV); the alternative formulation as the hydroxylamino compound (I; R = —NHOH) is excluded by the above evidence, although the latter has been reported by various workers[10–18] as the product of reduction of nitrofurazone by several biological systems. The evidence quoted by these workers for the identity of the reduction product was not unequivocal since it rests mainly upon a consideration of Raney nickel reduction under aqueous conditions (Austin[3]), in which reduction is accompanied by ring cleavage. (Ring cleavage also accompanied reduction when water was substituted for ethanol in the hydrogenation reported in the experimental section, since, in the reaction solution, the single absorption peak of compound IV (λ_{max} 333 mμ) is replaced by two peaks (λ_{max} 333 and 272 mμ) similar to those observed during bacterial reduction of nitrofurazone and reductions involving Raney nickel.[3])

The decomposition of nitrofurazone by various bacteria has been described by Ohyama;[19] the initial product was apparently 5-amino-2-furaldehyde semicarbazone, although the spectrophotometric and polarographic data presented did not fully support this conclusion.

Effect of pH Changes and Storage on 5-Amino-2-furaldehyde semicarbazone

The bathochromic and hypochromic absorption shifts previously reported to occur on addition of acid to a solution containing reduced nitrofurazone may be explained in terms of the following resonance hybrids:

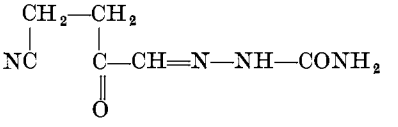


IV and V represent the main structures contributing to the resonance hybrid having the 333 mμ peak. VI, VII and VIII represent the main structures contributing to the resonance hybrid having the 370 mμ peak.

Addition of a proton to the 5-amino group would be expected to cause a hypsochromic shift, by analogy with aminobenzene derivatives. Support for the suggestion that the nitrogen atom in the β-position to the furan ring is involved was provided by reduction of 5-nitro-2-furaldehyde anil, when the amino compound showed a bathochromic shift from 385 to 410 mμ on addition of acid. Further support for the conclusion that the 5-amino

group is not involved was provided by the observation that the absorption peak of a solution containing methyl 5-amino-2-furoate remained unchanged at 311–312 mμ upon pH changes over the range pH 2–8. Analogous bathochromic shifts on proton addition have been observed in azo compounds (see, for example, references 20, 21).

Austin[3] was able to isolate small quantities of glyoxyl propionitrile monosemicarbazone (IX) from aged solutions of chemically



IX

reduced nitrofurazone and has suggested that the former is the product of an isomeric transformation of the reduced compound (IV).

Bacterial Reduction of Other Nitrofurans

The spectrophotometric measurements using solutions of the other nitrofurans in Table I (except NF 106) in contact for various times with *A. aerogenes* reveal a common pattern similar to that observed for nitrofurazone (see, for example, Fig. 1). The new absorption maxima in the region 320–375 mμ which appeared in the solutions are attributed to the primary metabolic products; the gradual hypochromic shifts of these peaks upon storage of the solutions, with the concomitant development and then increase of an absorption peak in the region 260–280 mμ (Table II), indicates the lack of stability of these primary products. The fact that the maxima of these primary products are at shorter wavelengths in certain cases (NF 7, NF 57, NF 62 and NF 64) than that of the parent structure, but at longer wavelengths in others (NF 84 and NF 89), does not indicate anomalies in the reduction pattern (see below).

It seems reasonable to assume that a common metabolic pathway obtains for these drugs, which differ only in the nature of the side-chains. Further evidence was sought in the case of the two compounds NF 57 and NF 62, by polarographic determinations

and a study of the spectrophotometric changes of solutions of the metabolic products upon changes of pH. The half-wave potentials of NF57 (−0·34 and −1·42 V) and of NF62 (−0·34 and −1·30 V) are similar to those observed for nitrofurazone (−0·35 and −1·35 V)[1, 9, 18]; the first reduction steps are attributed to the nitro groups and the second reduction steps to the azomethine bonds.[1, 9, 22] A gradual decrease in the diffusion currents of the first reduction steps of solutions of NF57 and NF62 exposed to *A. aerogenes* indicated the metabolic change of the nitro groups; the lack of an initial decrease in the diffusion current for such solutions for the second reduction steps indicated that the azomethine bond was unchanged. The actual positions of the absorption maxima of the metabolic products further indicate the integrity of the furan ring and the semicarbazone chain in these substances. The bathochromic shifts observed on changing the pH of solutions of the primary reduction products from NF57 and NF62 prove that proton addition occurs upon a group associated with the conjugation of the system.

It is therefore concluded that the reaction of *A. aerogenes* with the nitrofurans recorded in Table I, section A, yields the corresponding aminofurans which exhibit absorption maxima at the wavelengths recorded in Table II; these compounds are relatively unstable, undergoing furan ring fission to yield open-chain analogues of IX exhibiting absorption maxima in the region 260–280 mμ.

The ultraviolet absorption curves (Fig. 2) obtained using solutions derived from the interaction of *A. aerogenes* with NF106 exhibited characteristics different from those described above, in that the decrease of the nitrofuran peak at 374 mμ with increasing time of drug–bacteria contact was accompanied only by the development of a transient new peak in the region 330–380 mμ. Since the compound differs from NF57 only by the replacement of a $—CH_3$ by a $—CH_2OH$ group, it seems reasonable to assume that the metabolic pathway is similar; the metabolically derived aminofuran corresponding to NF106, therefore, is considered to be so unstable that furan ring opening to a compound analogous to IX occurs so readily that the aminofuran can scarcely be detected spectrophotometrically in the presence of the unchanged nitro compound.

The increase in the optical densities in the region 500–800 mμ

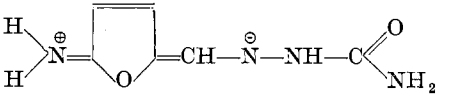
of bacterial suspensions in culture medium containing the nitrofurans of Table I, section A, in the concentrations used in the present experiments indicated that limited growth is occurring during metabolism of the drugs. These nitrofurans exert a bacteriostatic effect upon *A. aerogenes* (see Table III). In agreement with other workers,[22, 23] no relation is observed between the ease of reduction of the nitro group (as approximately indicated by the rate of decrease in the longer wavelength nitrofuran absorption peaks) and bacteriostatic activity.

Stability of the Aminofurans Derived from Antibacterial Nitrofurans

The relative stability of the various amino compounds derived from the above nitrofurans is explicable in terms of the different electronic and steric effects obtaining in the various compounds. It is possible to prepare 5-amino-2-furoic esters,[24] whereas all attempts to prepare α-aminofuran have proved abortive—electron withdrawal from the amino group, as indicated in X and XI,

X XI

reduces the tendency to furan ring opening in the former compounds. Similarly, 5-amino-2-furaldehyde semicarbazone is stabilized by electron withdrawal from the amino group towards the semicarbazide chain (see XII) and the hydrogen bonding of the

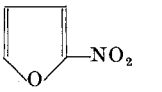
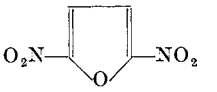
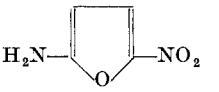


XII

H atom of the —NH group to the oxygen atom of the furan ring (see IV) which will reduce the tendency towards furan ring cleavage. It is significant that substitution of the —NH group[25] yields aminofurans which are too unstable to be detected spectrophotometrically.

The presence of a 2-nitro group also leads to partial stabilization of an aminofuran, e.g. bacterial reduction of 2,5-dinitrofuran yields a solution containing 5-amino-2-nitrofuran (Table IV).

Table IV. Comparison of ultraviolet absorption maxima of three nitrofurans in aqueous solution*

					
λ in mμ	ε†	λ in mμ	ε‡	λ in mμ	ε
225	3,400	233	8,200	masked	
315	8,100	310	13,000	355	14,000§

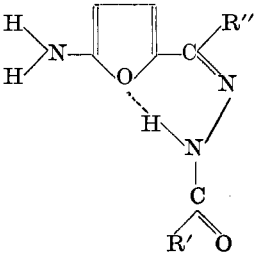
* Mono- and di-nitrofuran figures quoted for distilled water; 5-amino-2-nitrofuran was in culture medium pH 7·0.

† Values given by Raffauf.[4]

‡ Raffauf[4] gave ε 7,500 and 11,500 at 230 and 307 mμ respectively.

§ Value obtained by plotting the change in optical density at the absorption peak against time and extrapolating to obtain the density at zero time. A straight-line relationship was observed.

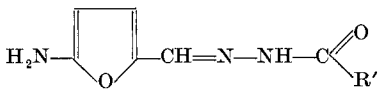
In compounds of type XIII, the strength of the O—H—N hydrogen bonding will be influenced by the acidic character of the hydrogen atom. This will itself be influenced by the elec-



XIII

tronic character of group R′—increase in the electron repelling properties of the group will reduce the acidity of this H atom and thus the strength of the hydrogen bonding; the stabilizing electron drift from the 5-amino group will also be adversely affected. In Table V, the relative stabilities of the amino deriva-

Table V. Approximate percentage decomposition of five aminofurans after 90 minutes at 40·0°*

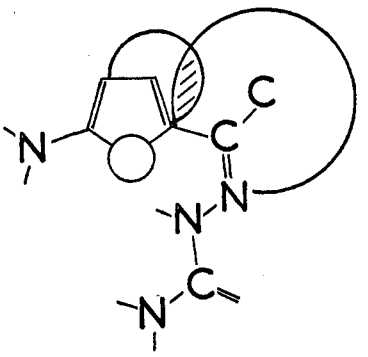


R′	Percentage decomposition†
—$CONH_2$	6·0
—$CONHCH_2CH_2OH$	9·0
—CH_3	10·0
—NH_2	14·0
—$NHCH_3$	22·0

* pH 5·5–6 in each case.
† Calculated from the hypochromic shift in the absorption peak.

tives are recorded. The electron-repelling properties of R′ (XIII) will be in the following order —$NHCH_3$ > —NH_2 > —CH_3 > —$CONHCH_2CH_2OH$ > —$CONH_2$, the last two groups in fact being electron-attracting. Although the values quoted in Table V are only approximate, it is of interest that the stability of these aminofurans is increased as R′ is altered in the direction of decreased electron-repelling properties.

Compounds of the general formula XIII, in which R″ = —CH_3 or —CH_2OH, are even less stable than those recorded in Table V, e.g. in the compound in which R″ = —CH_3 and R′ = —NH_2, a 39 per cent loss occurs during 90 min at 40°, and the amino compound (XIII; R″ = —CH_2OH, R′ = —NH_2) from NF106 undergoes ring cleavage much more readily than the other amino compounds. Models indicate that upon rotation of the group R″, the steric requirement of this group would tend to prevent the coplanarity of the furan ring (see XIV) and the chelate ring; resonance stabilization and electron withdrawal from the amino group is therefore reduced, as will be the strength of the O—H—N bonding with consequent facilitation of furan ring cleavage. The greater steric requirement of the —CH_2OH than the —CH_3 group accounts for the fact that the amino compound (XIII; R″ = —CH_2OH, R′ = —NH_2) derived from NF106 is much less stable than that (XIII; R″ = —CH_3, R′ = —NH_2) derived from NF57.

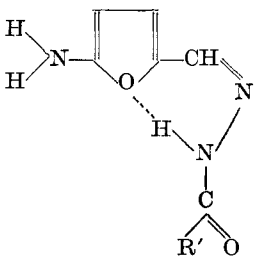


XIV

Generalized plane diagram representing the steric interaction in XIV between the R″ group (—CH_2OH) and the furan ring. The partial circles represent the van der Waals' zones of the respective atoms or groups. Maximum dimensions are used so that the steric interaction shown is maximal, and rotation of the —CH_2OH group together with other uncertainties associated with assigning dimensions to such a diagram may reduce the steric non-bonded interactions below those apparent in the above diagram. The diagram is drawn to scale, using the values for bond lengths and bond angles quoted by Wheland[26] for furan, acetoxime and urea; the data for the latter compounds are used in the apparent absence of information concerning semicarbazide.

Ultraviolet Absorption Spectra of Aminofurans

The locations of the absorption maxima of various aminofurans of general formula XV (Table II, NF 7, NF 62, NF 64, NF 84 and



XV

NF89) may be expected to be dependent upon the electronic character of group R′; a decrease in the electron-repelling properties of this group would enhance the electronic drift from the 5-amino group and so lead to a bathochromic shift in the absorption peak.

The differences in the peak locations between the compounds possessing strong electron-repelling groups (where tautomeric effects are possible)— —NH_2 (NF7; λ_{max} 333 mμ), —$NHCH_3$ (NF62; λ_{max} 335 mμ) and a weak electron-repelling group —CH_3 (NF64; λ_{max} 347 mμ), and the large difference between these and the ones observed when R′ is electron-attracting— —$CONH_2$ (NF84; λ_{max} 375 mμ), —$CONHCH_2CH_2OH$ (NF89; λ_{max} 375 mμ), indicate the validity of the above argument and provide further support for the foregoing formulations of the aminofurans.

Since the electron drift in the molecules of the parent nitrofurans (—NO_2 group exhibits −I and −T effects) is away from the side-chain, the order of the above peak locations should be reversed in the parent nitro compounds, but the differences should be less marked. This hypothesis is supported by the results shown in Table II; when R′ = —NH_2 (NF7) or —$NHCH_3$ (NF62), λ_{max} is 375 mμ, but when R′ = —$CONH_2$ (NF84) and —$CONHCH_2CH_2OH$ (NF89), λ_{max} is 363 and 360 mμ respectively.

Summary. *A. aerogenes* reduces nitrofurazone to 5-amino-2-furaldehyde semicarbazone, which is also produced by palladized charcoal reduction of the nitrofuran. The effects of pH changes on the ultraviolet absorption spectrum of the amino compound are explained. Storage of 5-amino-2-furaldehyde semicarbazone in aqueous solution results in furan ring cleavage; the changes in absorption spectra are interpreted.

The initial products of bacterial reduction of nitrofurans of general formula II, in which R = —H and R′ = —NH_2, —$NHCH_3$, —CH_3, —$CONHCH_2CH_2OH$, etc., are shown to be the corresponding aminofurans by a combination of spectrophotometric and polarographic methods. The stability of various aminofurans obtained by bacterial reduction of the corresponding nitro compounds is discussed. The ultraviolet absorption spectra of the aminofurans and their parent antibacterial nitrofurans are interpreted.

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