Journal of Medicinal and Pharmaceutical Chemistry

VOL. 1, No. 2 (1959)

The Reaction of Nitrofurans with Bacteria—III. Reduction of a Series of Antibacterial Nitrofurans (Type B Compounds*) by *Aerobacter aerogenes*

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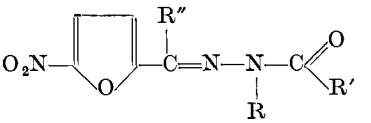
The preceding paper in this series[1] dealt with the reduction of nitrofurans of structural type (I), in which R = —H in every compound and R′ and R″ were varied.



I

We now report on compounds in which R = alkyl or hydroxyalkyl or is part of a ring involving R′. The differences in the bacterial reduction products and an explanation of the ultraviolet absorption and polarographic properties of the reduced compounds are presented.

Experimental Methods

Materials

Nitrofuran compounds. Nitrofurantoin (NF153), furazolidone (NF180) and NF148 were supplied by Smith, Kline and French Laboratories Ltd. Furadroxyl (NF67) and NF61 were supplied by Eaton Laboratories, New York. Table I shows the compounds, their melting points (uncorrected) and ultraviolet absorption characteristics. The manufacturers' code numbers are also given.

Organism and culture medium. These were described in Part I.[4]

Preparation of the bacterial and test suspensions. The methods were described in Part II[1] of this series. The compounds listed

* Type B compounds are those nitrofurans (I) in which R = alkyl or is part of a ring involving R′; type A are those in which R = —H.

in Table I were examined; the initial concentrations used were approximately 10 μg/ml in each case.

Spectrophotometric measurements. The apparatus and techniques used for measurements during and after drug–bacteria contact were as described in Part II[1] (all compounds in Table I were examined).

The ultraviolet absorption curves for nitrofurantoin were determined in solutions of a boric acid–potassium chloride–sodium hydroxide buffer at pH 7·8–10 (and at pH 7·0 in culture medium).

Polarographic measurements. The apparatus and techniques were as described in Part II.[1] Only nitrofurantoin and NF61 were examined; calibration curves were obtained over the range 1–20 μg/ml and 1–10 μg/ml respectively.

Bacteriostatic tests. The technique described in Part II[1] was employed. The optical density of the *Aerobacter aerogenes* culture used was 0·660 at 500 mμ in 5 mm cuvettes.

Results

Spectrophotometric Results

The ultraviolet absorption curves obtained upon examination of solutions of nitrofurantoin after contact with *A. aerogenes* for various times are shown in Fig. 1. The results using the other

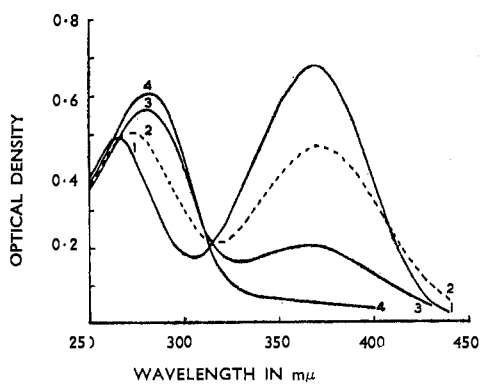
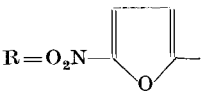
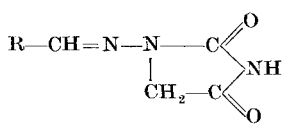
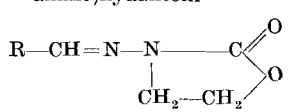


Fig. 1. Ultraviolet absorption curves of supernatant solutions obtained from contact of *A. aerogenes* with 10·0 μg/ml nitrofurantoin in single-strength culture medium (glucose omitted) at 40·0°. Curve 1, 10·0 μg/ml nitrofurantoin (reference curve), curves 2, 3 and 4 represent the solutions obtained after $\frac{1}{2}$, 3 and 23 h contact respectively

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



Code No.	Structure and systematic name	Recognized name	m.p. in °C*	λ_{max} in mμ	log ε†	λ_{max} in mμ	log ε†
NF 61	R—CH=N—N(CH_3)$CONH_2$ 5-nitro-2-furaldehyde 2′-methyl semicarbazone		213·5	268 [ε 13,600 at 265 mμ]‡	4·14	385 [ε 16,100 at 385 mμ]‡	4·22
NF 67	R—CH=N—N(CH_2CH_2OH)$CONH_2$ 5-nitro-2-furaldehyde 2′-(2-hydroxyethyl) semicarbazone	Furadroxyl	216·5	270	4·10	387 [$E^{1\%}_{1\,cm}$ 670 at 387 mμ]§	4·21
NF 148	R—CH=N—N(CH_3)$CONHCH_3$ 5-nitro-2-furaldehyde 2′:4′-dimethyl semicarbazone		168–9	272	4·13	390	4·20
NF 153	 1-(5-nitro-2-furfurylidene amino)hydantoin	nitrofurantoin	270–1	270	4·11‖	375	4·25‖
NF 180	 N-5-nitro-2-furfurylidene-3′-amino-2′-oxazolidinone	furazolidone	268–9	260	4·10	370	4·22

* All compounds melt with decomposition. † In distilled water unless otherwise stated.
‡ Raffauf.[8] §Paul, Austin, Paul and Ells.[3] ‖ In culture medium at pH 7·0.

nitrofurans listed in Table I were similar, i.e. progressive hypochromic shift of the absorption peak in the region 370–390 mμ and hyperchromic and bathochromic of the other absorption peaks (260–272 mμ). The locations of the absorption peaks obtained after exposing the nitrofurans to the bacteria are shown in Table II.

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
NF 61	268	385	280
NF 67 (Furadroxyl)	270	387	283
NF 148	272	390	290
NF 153 (nitrofurantoin)	270	375	282
NF 180 (furazolidone)	260	370	268

* Growth of the inoculum was indicated during these experiments.
† In culture medium at pH 7.
‡ In culture medium, pH not less than 5·5–6, uncorrected for background of cell exudate.

Growth of the bacteria in the medium during the course of the experiments was indicated by an increase in the optical density of the suspensions in the region 500–800 mμ.

Polarographic Results

Nitrofurantoin (NF 153) exhibited two reduction steps at pH 7·0 ($E_{\frac{1}{2}}$ −0·3 and −1·25 V); NF 61 behaved similarly ($E_{\frac{1}{2}}$ −0·35 and −1·27 V). All half-wave potentials were measured against a mercury pool anode. Calibration curves of nitrofurantoin and NF 61 were linear over the range 1–20 and 1–10 μg/ml respectively, employing the first reduction steps in each case. The polarographic behaviour of solutions of nitrofurantoin and NF 61 in the presence and absence of bacteria was identical providing the precautions described in the previous paper were observed.

During the interaction of *A. aerogenes* and nitrofurantoin, there was a parallel decrease in the diffusion current for each

reduction step and the concomitant appearance and then increase in diffusion current of a third reduction step having $E_{\frac{1}{2}}$ −0·85 V (see Fig. 2).

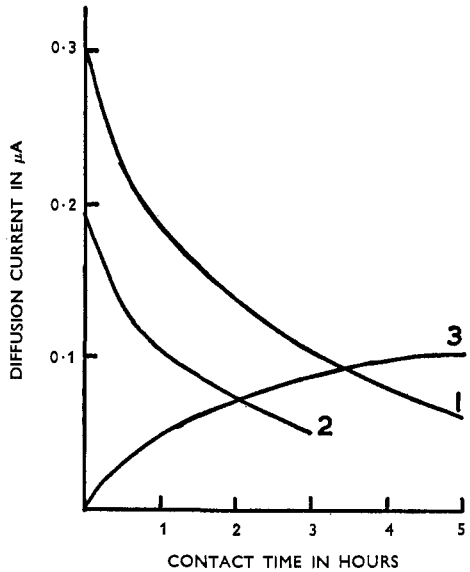


Fig. 2. Summary of the polarographic changes observed during contact of *A. aerogenes* with nitrofurantoin in single-strength culture medium (glucose omitted) at 40·0° and pH 7·0. Curve 1 represents the reduction step having $E_{\frac{1}{2}}$ −0·3V, curve 2 represents the reduction step having $E_{\frac{1}{2}}$ −1·25V, curve 3 represents the reduction step having $E_{\frac{1}{2}}$ −0·85V. All half-wave potentials measured against mercury pool anode

A similar pattern was observed upon polarographic investigation of NF 61 in contact with *A. aerogenes* for various times. The new reduction step which appeared, as those of the parent compound decreased, had $E_{\frac{1}{2}}$ −0·98 V.

Bacteriostatic Results

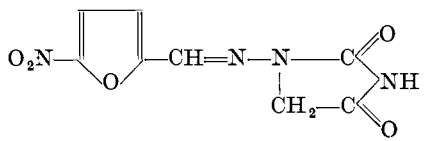
The minimum inhibitory concentrations, defined as that concentration of drug preventing visible growth after 18 and 24 h incubation at 40°, are recorded in Table III, each figure being the mean of five results.

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

Compounds	18 h incubation at 40°	24 h incubation at 40°
NF 61	20·0	30·0
NF 67 (Furadroxyl)	>50·0	
NF 148	>50·0	
NF 153 (nitrofurantoin)	20·0	40·0

Discussion

All the nitrofurans of Table I exhibited similar changes in ultraviolet absorption curves upon contact with *A. aerogenes* for various times, and consequently only two compounds of the series, nitrofurantoin (II) and NF 61, were chosen for more



II

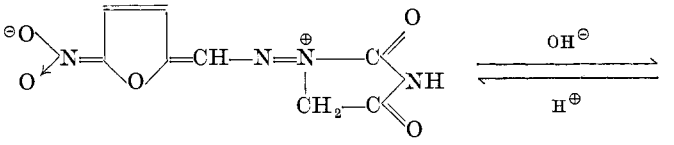
detailed examination. The spectrophotometric pattern developing with time of drug–bacteria contact was different from that reported for type A nitrofurans.[1]

Ultraviolet Absorption Spectra

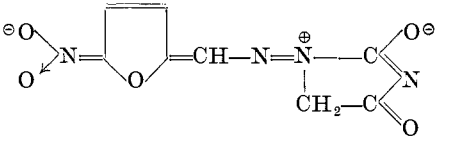
Stuckey[5] has shown that the bathochromic shift observed in hydantoins upon changing from neutral to alkaline conditions may be attributed to the acidity of the hydrogen atom of the 3-NH group and the contribution of intermediate structures to the resonance hybrid under alkaline conditions.

In nitrofurantoin it is therefore reasonable to assume that, because of the electron withdrawing power of the nitro group, structure III contributes significantly to the resonance hybrid exhibiting an absorption peak at 375 mμ at pH 7·0, while struc-

ture IV will be the main structure of the resonance hybrid with an absorption peak at 390 mμ under alkaline conditions.



III



IV

The absorption spectrum of nitrofurazone solutions (and of other type A nitrofurans) remained constant over the pH range 2·0–8·0, whereas the above bathochromic shift occurred upon changing the pH of solutions of nitrofurantoin from 7·0 to 8·5; this shift was reversed on reversing the pH changes. The expected more acidic character of nitrofurantoin than that of nitrofurazone is therefore demonstrated.

The ultraviolet absorption spectra of other type B nitrofurans in aqueous solution may be considered in terms of structure I and structures analogous to III contributing to the resonance hybrid exhibiting an absorption maximum at about 380 mμ. In compounds of general formula I in which R = —CH_3 and R′ = —NH_2 or —$NHCH_3$ (NF61 and NF148), and R = —CH_2CH_2OH and R′ = —NH_2 (NF67), the contribution of structures analogous to III would be expected to be greater than those in which R and R′ were combined to form part of the five-membered hydantoin (NF153) or oxazolidinone (NF180) ring system because of both the planarity and electronic characteristics of these rings. The absorption peaks of the latter type should, therefore, be located at a shorter wavelength than those of the former type. The results recorded in Table I (NF153, 375 mμ, and NF180, 370 mμ, whereas NF61, 385 mμ, NF67, 387 mμ, and NF148, 390 mμ) indicate the validity of the above conjecture.

Reaction of Nitrofurantoin with A. aerogenes

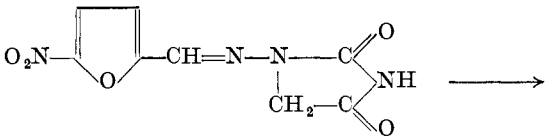
The ultraviolet absorption curves shown in Fig. 1 demonstrate the gradual loss of the longer wavelength absorption peak of nitrofurantoin upon contact with the bacteria, but the emergence of a new peak at 282 mμ, masking the shorter wavelength absorption peak of the drug, indicated that the loss cannot be attributed to a simple uptake of the drug. Reduction of the nitro to an amino group would be expected to yield a compound with an absorption peak between 320–400 mμ (*cf.* Part II[1]); the absence of such a peak indicates that if the aminofuran is formed, it is not sufficiently stable to be detected in the spectrophotometric measurements.

It seems reasonable to assume by comparison with the reactions reported in Part II,[1] that reduction of the nitro group might be accompanied by facile furan ring fission, thus accounting for the lack of an absorption peak of the aminofuran and the development of the peak at 282 mμ. Polarographic measurements provided the evidence.

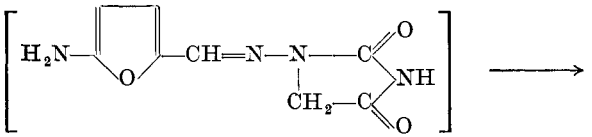
The polarographic reduction steps exhibited by nitrofurantoin at pH 7·0 are assigned to the nitro group ($E_{\frac{1}{2}}$ −0·3 V) and to the azomethine bond ($E_{\frac{1}{2}}$ −1·25 V) by analogy with those observed for nitrofurazone and other nitrofurans (see Sasaki[6, 7] and Beckett and Robinson[1, 4]). The simultaneous decrease in *both* reduction steps (see Fig. 2) upon contact with bacteria, in contrast with the loss of only the first reduction step of nitrofurazone and type A nitrofurans (Part II[1]), indicates not only the gradual loss of the nitro group but also the simultaneous loss of the azomethine bond conjugated with the furan nucleus.

The reaction of nitrofurantoin with *A. aerogenes* may therefore be represented as shown in the formulae at the top of p. 163, compound V being too unstable to be detected spectrophotometrically. The development and gradual increase (Fig. 2) of the reduction step having $E_{\frac{1}{2}}$ −0·85 as the reduction steps of the parent compound decrease (contrast with the measurements with nitrofurazone, NF 57 and NF 62, in Parts I and II[1, 4]) are consistent with the above. Polarographic measurements using NF 61 gave results similar to those for nitrofurantoin.

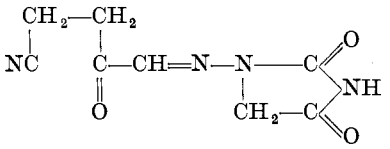
It is concluded that the type B nitrofurans listed in Table I, all of which give similar changes in the ultraviolet absorption



II



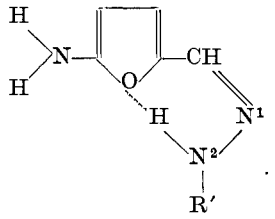
V



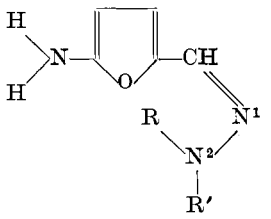
VI

pattern upon interaction with *A. aerogenes*, are reduced to the corresponding aminofurans which are so unstable that furan ring cleavage immediately occurs to yield VI and its analogues. Using differential spectrophotometry, the absorption peak of VI was located at 287 mμ.

In the amino compounds derived from type A nitrofurans, the 2-NH— group is presumed to hydrogen bond to the oxygen of the furan ring (VII); this was considered as contributing to the



VII



VIII

stability of the furan ring (see Part II[1]). In those from type B nitrofurans (VIII) such hydrogen bonding is impossible, and

consequently these compounds are very unstable and furan ring cleavage virtually accompanies the reduction of the nitro group.

No relation between the ease of reduction of the nitrofurans investigated and their bacteriostatic activity against *A. aerogenes* was observed (*cf.* Part II[1]).

Summary. Information is presented concerning the reactions of *A. aerogenes* with nitrofurans of general formula I, in which R = alkyl or is part of a ring involving R′. The ultraviolet absorption curves (and polarographic characteristics of certain examples) obtained during the bacterial reduction of these compounds are interpreted. The lack of stability of the corresponding aminofurans is discussed.

Acknowledgements. The authors thank Smith, Kline and French Laboratories Ltd. and Eaton Laboratories Incorporated for supplying the compounds used in this and the previous paper; they also thank Mr. A. H. J. Cross for valuable suggestions and discussions during the course of this work.

(*Received 15 September, 1958.*)

References

[1] Beckett, A. H. and Robinson, A. E. *J. med. pharm. Chem.*, **1**, 135 (1959)
[2] Raffauf, R. F. *J. Amer. chem. Soc.*, **72**, 753 (1950)
[3] Paul, H. E., Austin, F. L., Paul, M. F. and Ells, V. R. *J. biol. Chem.*, **180**, 345 (1949)
[4] Beckett, A. H. and Robinson, A. E. *J. Pharm., Lond.*, **8**, 1072 (1956)
[5] Stuckey, R. E. *J. chem. Soc.*, 331 (1947)
[6] Sasaki, T. *Pharm. Bull. (Japan)*, **2**, 99 (1954)
[7] Sasaki, T. *Pharm. Bull. (Japan)*, **2**, 104 (1954)