J. Pharm. Pharmacol., 1964, **16**, 663–669 Received March 6, 1964

Antibacterial properties of 5-nitro-2-furylglyoxylidene derivatives

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5-Nitro-2-furylglyoxal has been prepared and nine new derivatives synthesised. These showed similar *in vitro* antibacterial activity to the corresponding 5-nitrofurfurylidene analogues. The antibacterial activity of the 5-nitro-2-furylglyoxylidenes could not be demonstrated in the serum or urine of rats after oral administration.

MANY diverse structures have been based upon the 5-nitrofuran molecule following the original work of Dodd & Stillman (1944), which led to the development and use of nitrofurazone (5-nitrofurfuraldehyde semicarbazone) (Dodd, 1946) as an effective antibacterial agent. In view of the success of the nitrofurans in a variety of clinical infective conditions, the 5-nitro-2-furylglyoxylidene derivatives were considered to be worth examining as potential antibacterial drugs.

Soldabols & Hillers (1960) obtained the dihydrate of 5-nitro-2-furyl glyoxal, and Gualtieri, Riccieri & Stein (1962) demonstrated its reactivity by preparing several unstable aromatic glyoxylidene derivatives. Later Caradonna, Gualtieri & Riccieri (1962) demonstrated the bacteriostatic activity of 5-nitro-2-furylglyoxal monosemicarbazone *in vitro*. We obtained 5-nitro-2-furylglyoxal as an oil by oxidation of 2-acetyl-5-nitrofuran (Hayes & O'Keefe, 1954) with selenium dioxide in aqueous acetic acid. From this were prepared derivatives of 1-aminohydantoin and 3-amino-2-oxazolidone, the latter being found to possess high antibacterial activity *in vitro*. In view of this high activity, the series of derivatives of 5-nitro-2-furylglyoxal was extended and compared with analogous derivatives of 5-nitrofurfuraldehyde for antibacterial activity *in vitro* and acute toxicity in mice.

Experimental

CHEMICAL

All melting-points are uncorrected. Yields are based on 5-nitro-2-furylglyoxal dihydrate.

5-*Nitro*-2-*furylglyoxal* (I). 2-Acetyl-5-nitrofuran (Hayes & O'Keefe, 1954) (40 g) in acetic acid (110 ml) was heated under reflux with selenium dioxide (28·4 g in 10 ml water) for 3 hr. The hot reaction mixture was then filtered (kieselguhr) to remove metallic selenium, and concentrated *in vacuo*. The *glyoxal* was obtained as a dark red oil (26·1 g, 45%).

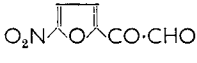

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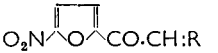
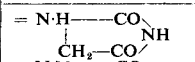
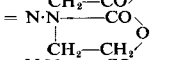
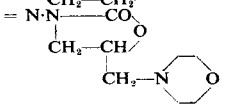
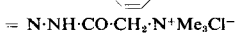
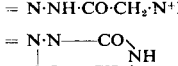
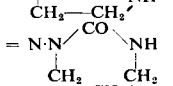
TABLE 1. 5-NITRO-2-FURYLGLYOXYLIDENE DERIVATIVES



Derivative No.	Parent compound	R	Recrystallisation solvent	% yield	m.p. °	Formula	Found % C	Found % H	Found % Cl	Found % I	Found % N	Required % C	Required % H	Required % Cl	Required % I	Required % N
II	1-Aminohydantoin		Ethanol	40	241	$C_5H_6N_4O_4$	40·4	2·5			20·9	40·6	2·3			21·0
III	3-Amino-2-oxazolidone ..		Acetic acid	82	230	$C_5H_7N_3O_4$	43·0	2·9			16·5	42·7	2·8			16·6
IV	3-Amino-5-morpholinomethyl-2-oxazolidone ..		Nitroethane	57	213	$C_{14}H_{16}N_4O_7$	47·9	4·8			15·8	47·7	4·6			15·9
V	IV as hydrochloride		—	—	235–240	$C_{14}H_{17}N_4O_7Cl$			9·1					9·1		
VI	IV as methiodide		Aqueous ethanol	—	232–235	$C_{15}H_{19}IN_4O_7$	37·5	4·1		25·9	11·3	36·4	3·9		25·7	11·3
VII	*p*-Hydroxybenzhydrazide ..		Nitroethane	70	248	$C_{13}H_{11}N_3O_4$	50·7	3·0			13·6	51·5	3·0			13·9
VIII	(Hydrazinoformylmethyl)-trimethylammonium chloride		Water/ethanol	66	242	$C_{11}H_{15}N_4O_5Cl$	41·2	4·5	11·2		17·8	41·2	4·7	11·1		17·6
IX	1-Amino-2-imidazolidone ..		Dimethylformamide	86	251	$C_6H_8N_4O_6$	43·0	3·2			22·0	42·9	3·2			22·2
X	1-Aminotetrahydro-2-pyrimidone		Nitroethane	60	261	$C_{10}H_{10}N_4O_6$	45·3	4·0			21·1	45·1	3·8			21·1

Derivatives of 5-Nitro-2-furylglyoxal (II–X; Table 1). The general preparative method consisted of reacting the glyoxal with the amine or amine salt in ethanol or aqueous ethanol, and is illustrated by the following preparation of two of the products.

(*a*) 5-Nitro-2-furylglyoxal (1·0 g) in ethanol (10 ml) was heated with 1-aminohydantoin hydrochloride (Jack, 1959) (0·6 g) in boiling water (2·0 ml) for 15 min. The *product* (II) was filtered, dried and twice crystallised (ethanol) to yield fine pale yellow needles (0·65 g, 40%) m.p. 238–241° (decomp.) ν_{max} 3400, 3300 (NH), 1740 (C : O), 1645 (CO·NH), 1580 (C : N), 1515 and 1345 cm^{-1} (NO_2).

(*b*) To a warm solution of 5-nitro-2-furylglyoxal (12·7 g) in ethanol (60 ml) was added, with mixing, a warm solution of 3-amino-5-morpholinomethyl-2-oxazolidone (13·2 g) (Gever, 1957) in ethanol (70 ml). After heating under reflux for 1 hr the reaction mixture was cooled, and the *product* (IV) collected. Recrystallisation from nitroethane gave yellow blades (12·5 g, 57·3%), m.p. 214–215°, ν_{max} 3200 (NH), 1780 (C:O), 1650 (CO·NH), 1580 (C:N), 1510 and 1340 cm^{-1} (NO_2).

Derivatives of 5-nitrofurfuraldehyde (XI–XIX, Table 2). These were prepared according to literature methods as follows: the derivatives of 1-aminohydantoin (XI) (Jack, 1959), of 3-amino-2-oxazolidone (XII) (Gever, O'Keefe, Drake, Ebetino, Michels & Hayes, 1955), of 3-amino-5-morpholinomethyl-2-oxazolidone (XIII) and of its hydrochloride (XIV) and methiodide (XV) (Gever, 1957), of *p*-hydroxybenzhydrazide (XVI) (Carron, Jullien, Julia & Garczynska, 1963), of hydrazinoformylmethyl)-trimethylammonium chloride (XVII) (Ward, 1953), of 1-amino-2-imidazolidone (XVIII) (Michels & Gever, 1956) and of 1-aminotetrahydro-2-pyrimidone (XIX) (Michels, 1960).

BIOLOGICAL

Bacteriostatic activity. Compounds were dissolved in distilled water or acetone purified by distillation over potassium permanganate. The solutions were serially diluted by 2-fold steps in nutrient broth (Oxoid No. 2–CM 67) and each tube was inoculated with 0·1 ml of an 18 hr culture of one of the following organisms:

Staphylococcus aureus (benzylpenicillin-resistant), *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris* (all isolated and identified at St. Luke's Hospital, Bradford) and *Bacillus subtilis* (NCTC 6276). Determinations using *Salmonella typhimurium* (Strain 305, Allen & Hanburys Ltd., Veterinary Department), *Salmonella dublin* (Strain 98, Allen & Hanburys Ltd., Veterinary Department) and *Streptococcus faecalis* (E186, PHLS (Strep. R.L.)) were made in glucose-peptone water. Each tube was then incubated at 37° for 24 hr, and the minimal inhibitory concentrations (MIC) were determined as the lowest concentrations of compounds which prevented growth visible to the naked eye.

TABLE 2. A COMPARISON OF THE MINIMUM INHIBITORY CONCENTRATIONS AND ACUTE TOXICITY OF DERIVATIVES OF GLYOXAL AND FURFURALDEHYDE

Parent compound	Derivative No.	MIC μg/ml (24 hr) *Staph. aureus*	*B. subtilis*	*Pr. vulgaris*	*Ps. aeruginosa*	*E. coli*	*Kl. aerogenes*	*Salm. typhimur.*	*Salm. dublin*	*Strep. faecalis*	LD50 mg/kg in mice (24 hr) *Oral*	*Intra-peritoneal*
5-Nitro-2-furylglyoxylidene derivatives												
1-Aminohydantoin	II	128	64	256	—	512	512				>1000	110 (90–120)
3-Amino-2-oxazolidone	III	1	4	8	—	8	8	2	3	1	>1000	88 (86–91)
3-Amino-5-morpholinomethyl-2-oxazolidone	IV	8	4	64	—	16	64				300–500	64 (50–81)
IV hydrochloride	V	16	16	132	—	64	64				300–400	65 (60–71)
V methiodide	VI	>256 <1000	64	>256 <1000	—	>256 <1000	>256 <1000				>1000	390 (350–430)
p-Hydroxybenzhydrazide	VII	256	>256 <1000	>256 <1000	>256 <1000	>256 <1000	>256 <1000				>1000	>1000
(Hydrazinoformylmethyl-)trimethyl-ammonium chloride	VIII	64	32	64	—	128	64				>1000	470 (400–560)
1-Amino-2-imidazolidone	IX	2	8	32	—	16	8	3	2	4	>1000	410 (340–470)
1-Aminotetrahydro-2-pyrimidone	X	32	128	32	—	64	128				>1000	1100 (780–1400)
5-Nitrofurfurylidene derivatives												
1-Aminohydantoin	XI†	64	32	128	—	16	16				>1000	110 (100–130)
3-Amino-2-oxazolidone	XII*	4	4	16	—	2	1	2	1	2	>1000	1600 (1400–1800)
3-Amino-5-morpholinomethyl-2-oxazolidone	XIII	8	4	32	—	4	16				>1000	1000
IV hydrochloride	XIV	8	4	128	—	16	16				≧1000	190 (150–240)
V methiodide	XV	>256 <1000	64	>256 <1000	—	>256 <1000	>256 <1000				>1000	430 (380–490)
p-Hydroxybenzhydrazide	XVI	>256 <1000	8	>256 <1000	>256 <1000	>256 <1000	256				>1000	1000
(Hydrazinoformylmethyl-)trimethyl-ammonium chloride	XVII	128	128	256	—	128	256				>1000	440 (400–480)
1-Amino-2-imidazolidone	XVIII	4	4	64	—	8	4				>1000	1000
1-Aminotetrahydro-2-pyrimidone	XIX	8	8	4	—	16	64				600	720 (500–1000)

* Furazolidone. † Nitrofurantoin.

The effect of serum on the antibacterial activity of compounds was determined by estimating the MIC using nutrient broth containing 25 or 50% horse serum.

Bactericidal activity. Bactericidal tests were carried out by subculturing from tubes not showing growth in the bacteriostatic tests, onto nutrient agar (Oxoid Blood Agar No. 2–CM271). After overnight incubation the presence or absence of growth was noted.

Bacteriostatic activity of serum and urine in rats. Groups of four albino rats (60–80 g) were dosed orally with 1 g/kg of the compound suspended in 1% methylcellulose. Control groups received 1% methylcellulose alone. Blood and urine samples were taken 1 and 2 hr later. The blood was allowed to clot, centrifuged and serum removed. 0·1 ml samples of serum and urine were then placed in 5 mm diameter wells in agar plates seeded with *Staph. aureus*, *E. coli* or *Kl. aerogenes*. The plates were refrigerated at 4° for 90 min, then incubated overnight at 37° and examined for growth inhibitory zones around the wells.

Acute toxicity in mice. Albino male mice (20–25 g) were used and randomised into groups of five. Compounds were standardised for particle size between 100- and 200-mesh sieves and the resultant powder suspended in distilled water for oral administration or in normal saline for intraperitoneal injection using 1% methylcellulose as suspending agent. After administering the compounds in a dose volume of 0·2 ml/20g mouse, the percentage mortality was recorded after 24 hr and 5 days. The LD50 was computed by the method of Litchfield & Wilcoxon (1949).

Results

Antibacterial activity in vitro. The minimum inhibitory concentrations of the compounds tested against a number of organisms are shown in Table 2.

Subcultures taken at 24 hr from the bacteriostatic test samples showed that only the 1-aminotetrahydro-2-pyrimidone derivatives were bactericidal at concentrations within one or two tubes of the MIC. The other derivatives only demonstrated bactericidal activity at higher concentrations.

TABLE 3. THE EFFECT OF SERUM ON THE BACTERIOSTATIC ACTIVITY OF THREE NITROFURAN DERIVATIVES

No.	Derivative	Concentration of horse serum %	MIC μg/ml (24 hr)				
			Staph. aureus	*B. subtilis*	*Pr. vulgaris*	*E. coli*	*Kl. aerogenes*
XII	3-(5-nitrofurfurylidene-amino)-2-oxazolidone (furazolidone)	0	4	4	16	2	1
		25	32	32	64	16	32
		50	64	64	128	32	32
III	3-(5-nitro-2-furylglyoxyl-ideneamino)-2-oxazolidone	0	1	4	8	8	8
		25	16	16	32	32	32
		50	32	32	64	64	64
IX	1-(5-nitro-2-furyl-glyoxylideneamino)-2-imidazolidone	0	2	8	32	16	8
		25	32	32	128	128	64
		50	64	128	128	128	128

With the three derivatives (III, IX and XII) tested in the presence of horse serum, it was found that in a 50% serum concentration, bacteriostatic activity was markedly reduced (Table 3). In several instances, a 25% serum concentration had the same effect.

TABLE 4. THE BACTERIOSTATIC EFFECT OF URINE AND SERUM FROM RATS TESTED ORALLY WITH NITROFURAN COMPOUNDS

No.	Derivative	Bacteriostatic activity 1 hr after oral administration					
		Serum			Urine		
		Staph. aureus	*E. coli*	*Kl. aerogenes*	*Staph. aureus*	*E. coli*	*Kl. aerogenes*
III	3-(5-Nitro-2-furylglyoxylidene-amino)-2-oxazolidone	O	O	+	O	O	O
XII*	3-(5-nitrofurfurylideneamino-)2-oxazolidone	O	+	+	O	+	+
IX	1-(5-Nitro-2-furylglyoxylidene-amino)-2-imidazolidone	O	O	O	O	O	O
XVIII	1-(5-Nitrofurfurylideneamino)-2-imidazolidone	O	+	+	+	+	+
X	Tetrahydro-1-(5-nitro-2-furylgly-oxylideneamino)-2-pyrimidone	O	O	O	O	O	O
XIX	Tetrahydro-1-(5-nitrofurfurylidene-amino)-2-pyrimidone	O	O	O	+	+	+
	Control 1% methylcellulose	O	O	O	O	O	O

O = Inactive. + = Bacteriostatic activity. * Furazolidone.

Bacteriostatic effect in rat serum and urine. Table 4 shows the bacteriostatic effect of three 2-furylglyoxylidene and the three corresponding furfurylidene derivatives in the serum and urine of rats 1 hr after oral administration of 1 g/kg of compound suspended in 1% methylcellulose in distilled water.

Acute toxicity in mice. The 24-hr LD50 values in mg/kg are shown in Table 2. After 5 days the mortality did not alter in either the orally or intraperitoneally dosed groups. Intraperitoneally, the 2-furylglyoxylidene derivatives were more toxic than the furfurylidene derivatives in most instances. The 1-aminohydantoin and 1-aminotetrahydro-2-pyrimidone derivatives were similarly toxic, as were the quaternary derivatives. The derivatives of *p*-hydroxybenzhydrazide possessed very low toxicity. Orally, both the 2-furylglyoxylidene and furfurylidene series exhibited low acute toxicity in mice, but the 2-furylglyoxylidene derivative of 3-amino-5-morpholinomethyl-2-oxazolidone and its hydrochloride, and the furfurylidene derivative of 1-aminotetrahydro-2-pyrimidone were more toxic. In all instances the urine was a yellow to red colour, and sedation and respiratory depression preceded death.

Discussion

In the 2-furylglyoxylidene and furfurylidene series the minimal inhibitory concentrations *in vitro* were about the same. The resistance of *E. coli* and *Kleb. aerogenes* to 1-(5-nitro-2-furylglyoxylideneamino)-hydantoin was anomalous in this respect. Only moderate activity

against some organisms was found when three pairs of compounds were tested *in vivo* in the rat (Table 4). These findings partially agree with the report (Interscience Conference, 1963) claiming good results with 1-(5-nitrofurfurylideneamino)-2-imidazolidone in animals and man. Even the large oral dosage of 1 g/kg in the rat did not give blood levels sufficiently high to inhibit the growth of *Staph. aureus*, and activity against *Ps. aeruginosa*, even *in vitro*, was not found. No activity was found in the serum of rats treated with tetrahydro-1-(5-nitrofurfurylideneamino)-2-pyrimidone. The 2-furylglyoxylidene analogues showed no activity in either the urine or the serum. This may be due to their breakdown in the animal body to give inactive metabolites in contrast to the furfurylidenes, which may be active themselves *in vivo* or may be broken down to give metabolites possessing antibacterial activity.

Acknowledgements. Acknowledgement is made to Dr. W. A. Cox for work with *Salm. typhimurium*, *Salm. dublin* and *Strept. faecalis*, and to Mrs. T. P. S. Tweedie and Mr. A. Harrison for technical assistance.

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