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Antibacterial Action of Nitro-furan Derivatives

Since 1944 attempts to synthesize nitro-furan derivatives have been undertaken, quite independently from Stillman and Dodd, by Dr. H. Saikachi and his collaborators in the Pharmacy Department of Kyoto University. In one of the whole 130 derivatives, i.e. 2-(5-nitro)-furfural semicarbazone, we found in 1946 a predominant antibacterial action against grampositive as well as gramnegative bacteria. The works of Stillman and Dodd were wholly unknown to us at that time.

Since then the antibacterial power in vitro, diffusibility and toxicity of about 60 among 130 synthesized nitro-furan derivatives have been examined in our Institute.

The following is a brief statement of our results:

I. Antibacterial Action in vitro

Serial dilution of each derivative was made with bouillon (pH 6.5) and the minimal concentration of growth inhibition of aerobic bacteria was estimated.

1) 5-nitro-furfural derivatives (See Table 1).

The antibacterial power of 5-nitro-furfural semicarbazone (No. 1, which is called "furacin" in the U.S.) is highest of 12 derivatives. The power of No. 2 and No. 3 is inferior to that of No. 1. But Nos. 2 and 3 are different from No. 1, and easily soluble in the water. 5-nitro-furfural phenylsemicarbazone (No. 4) shows a high titer of 1:320,000 against tubercle bacilli, though it is not very effective, against other bacteria.

2) 5-nitro-furylacrylyl derivatives (See Table 2).

The antibacterial power of 5-nitro-furylacrylyl amide (No. 13) is two times as much as that of furacin. Its solubility is so small as the latter.

3) 5-nitro-furoyl derivatives (See Table 3).

5-nitro-furoylamide (No. 18) is easily soluble in water, and has almost the same antibacterial power as furacin. It is also interesting that thiazole or pyrimidine including derivatives (No. 21, 25 and 31) have strong affinity with staphylococci.

4) 5-nitro-furylacrolein derivatives (See Table 4).

5-nitro-furylacrolein semicarbazone (No. 35) and 5-nitro-furylacrolein amino-guanidine (No. 36) are the most noteworthy derivatives. The former is scarcely soluble in water, but has two times or more antibacterial power as compared with furacin. The antibacterial power of the latter (No. 36) against grampositive bacteria is as strong as the former (No. 35), and it is soluble in water.

5) 5-nitro-furylsulfonamide (See Table 5).

5-nitro-furylsulfonamide (No. 46) has almost the same antibacterial potency as furacin, and is soluble.

6) 5-nitro-furylsulfide (See Table 6).

5, 5'-dinitro-2, 2'-difurylsulfide (No. 52) has strong antibacterial power (1: 800,000) against staphylococci.

7) Other derivatives (See Table 7).

There are scarcely found any noteworthy derivatives as compared with furacin.

II. Antibacterial Action against Anaerobic Bacteria (See Table 8)

The antibacterial power against *Cl. tetani*, *Cl. oedematis maligni* (*V. septique*) and *Cl. novyi* of 8 kinds (No. 1, 2, 13, 14, 18, 19, 52 and 53) was examined with the liver-bouillon dilution of the derivatives.

The derivatives have almost the same antibacterial power as homosulfamin.

III. Diffusibility

Each 5 ml of the following composition was poured in a small reagents glass:

1.5% agar	100 ml
1.0% NaNO ₃	0.5 „
0.1% methylene blue	3.5 „
Suspension of staphylococci	0.1 „

0.5 ml of diluted derivatives was put in the glass, and reagents glasses were kept 18-20 hours in an ice-box and 24 hours in an incubator. The inhibitory length of bacterial growth was estimated.

The result is shown in the Table 9. Nitro-furan derivatives have generally strong diffusibility like penicillin.

IV. Toxicity

Toxicity of 10 derivatives, expressed in 50% lethal dose per 10 g mouse (Reed and Münch), is given in the Table 10.

5-nitro-furylacrolein semicarbazone (No. 35) is least toxic

V. Fastness Gaining of Bacteria against Nitro-furan Derivatives

The serial cultivation of susceptible strains of *Staphylococcus aureus*, *Escherichia coli communis* and *Bacillus subtilis* was conducted in the 5-nitro-furfural semicarbazone or 5-nitro-furfurylidene aminoguanidine containing media. Each bacterium gains fastness quickly from the 1st to the 10th generation. From the 10th generation on the resistance of the bacteria increases step by step till it reaches the climax in the 25-30th generations. The resistance of *Staphylococcus* becomes, then, 30 times larger, and that of *Escherichia coli* and *Bacillus subtilis* ca. 20 times larger as compared with the original strain. The value of fastness is remarkably inferior to that by penicillin or streptomycin. It is noteworthy that the gained fastness of bacteria against nitro-furan derivatives can not be revived, even if the bacteria are cultivated in drugfree media.

- 1) Dodd, M. C. & Stillman, W. B.: J. Pharmacol. **82**, 10 (1944).
- 2) Dodd, M. C.: J. Pharmacol. **86**, 311 (1946).
- 3) Gramer, D. L. & Dodd, M. C.: J. Bact. **51**, 293 (1946).
- 4) Eaton Laboratory.: J. A. M. A. **134** (1947).
- 5) Goi, J.: Kokin Busshitsu Kenkyu, **2**, 40 (1947).
- 6) Kimura, R., Higashi, N., Goi, J. & Saikachi, H.: J. Penicillin, **2**, 62 (1948).
- 7) William, C. W. & Dodd, M. C.: J. Bact. **56**, 649 (1948).
- 8) Takahashi, T., Saikachi, H. *et al.*: Yakugaku Zasshi, **69**, 284 (1949).
- 9) Takahashi, T., Saikachi, H. *et al.*: *Ibid.* **69**, 286 (1949).
- 10) Ikegaki, K.: Kokin Busshitsu Kenkyu, **2**, 442 (1949).
- 11) Ikegaki, K.: *Ibid.* **3**, 15 (1950).
- 12) Ikegaki, K.: Yakugaku Kenkyu, **22**, 148 (1950).
- 13) Ikegaki, K.: Kokin Busshitsu Kenkyu, **3**, 179 (1951).
- 14) Ikegaki, K.: *Ibid.* **3**, 199 (1951).
- 15) Ikegaki, K.: *Ibid.* **3**, 209 (1951).

Table 1. 5-nitro-furfural derivatives. (unit = 10,000, $R = \text{NO}_2 - \text{C}_6\text{H}_4 - \text{O}$)

Bacteria	Derivatives		Hours		Staphylococcus aureus	Streptococcus haemolyticus	Diplococcus pneumoniae Type I	Escherichia coli communis	Eberthella typhosa	Shigella dysenteriae	Pseudomonas aeruginosa	
			24	96	24	96	24	96	24	96	24	96
No. 1.	R-CH=N-NH-CONH_2		20	16	2	1	2	1	20	10	<0.5	<0.5
No. 2.	$\text{R-CH=N-NH}\cdot\text{C}\begin{smallmatrix} \text{NH}_2 \\ \diagup \\ \text{NH} \end{smallmatrix}\cdot\text{HCl}$		10	8	8	4	2	1	5	16	<0.5	<0.5
No. 3.	$\text{R-CH=N-NH}\cdot\text{C}\begin{smallmatrix} \text{NH}_2 \\ \diagup \\ \text{NH} \end{smallmatrix}\cdot\frac{1}{2}\text{H}_2\text{SO}_4$		10	8	8	4	2	1	5	16	<0.5	<0.5
No. 4.	$\text{R-CH=N-NH-CONH-}\langle\text{---}\rangle$		5	2.5				<0.5	<0.5	1		
No. 5.	R-CH=N-NH-COOH		10	10				2.5	2.5	2.5	<0.5	<0.5
No. 6.	$\text{R-CH=N-}\langle\text{---}\rangle\text{OH}$		2.5	1	1	<0.5	1	<0.5	0.5	<0.5	0.5	<0.5
No. 7.	$\text{R-CH=N-}\langle\text{---}\rangle\text{COOH}$		2.5	1	1	<0.5	1	<0.5	<0.5	<0.5	<0.5	<0.5
No. 8.	$\text{R-CH=N-}\langle\text{---}\rangle\text{SO}_2\text{NH}_2$			1					<1	<5		<0.5
No. 9.	$\text{R-CH=N-}\langle\text{---}\rangle\text{SO}_2\text{NHCOCCH}_3$			<1		<1				<1		
No. 10.	$\text{R-CH=N-NH}\cdot\text{C}\begin{smallmatrix} \text{NH}_2 \\ \diagup \\ \text{S} \end{smallmatrix}$			5		<0.5		<0.5	1	1	1	<1
No. 11.	$\text{R-CH=N-}\langle\text{---}\rangle\text{SC}_2\text{H}_5$		2.5	2.5				<0.5	<0.5	1	1	<0.5
No. 12.	$(\text{R-CH=N-NH-CO})_2$		2.5	1				<0.5	<0.5	1	<0.5	<0.5

Table 2. 5-nitro-furylacrylyl derivatives. (unit=10,000, R=NO₂-)

Derivatives	Staphylo- coccus aureus		Strepto- coccus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
Hours	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 13. R-CH=CH-CONH ₂	40	20	4	2	2	1	40	20	40	20	20	10	1	<0.5
No. 14. R-CH=CH-COOH	20	10	2	1	2	1	10	5	10	5	10	5	<0.5	<0.5
No. 15. R-CH=CH-CONH- 	16	10					2.5	1	10	5	8	5	<0.5	<0.5
No. 16. R-CH=CH-CONH-  Cl	10	8		<0.5		<0.5		<1		<1		<1		
No. 17. R-CH=CH-CONH-  -O-CH ₃	10	8					1	0.5	2.5	1				

Table 4. 5-nitro-furylacrolein derivatives. (unit=10,000, $R=NO_2-\text{C}_6\text{H}_4-\text{O}-$)

Derivatives	Bacteria	Staphylococcus aureus		Streptococcus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
		24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 34. $R-CH=CH-CHO$		20	10	<1	<1	<1	<1	10	5	10	5	10	5	<1	<1
No. 35. $R-CH=CH-CH=N-NH-CONH_2$		40	20	10	8	4	2	20	20	20	16	32	16	<1	<1
No. 36. $R-CH=CH-CH=N-NH-C(=NH_2)NH_2$ HCl		32	20	16	8	4	2	25	2.5	5	5	10	5	<1	<1
No. 37. $R-CH=CH-CH=N-NH-C(=NH_2)NH_2$ N-C ₂ H ₅		2.5	1					2.5	1	5	2.5	2.5	1	<1	<1
No. 38. $R-CH=CH-CH=N-NH-CONH-C(=O)-C_2H_5$		<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
No. 39. $R-CH=CH-CH=N-NH-C(=O)-Cl$		<1	<1			<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
No. 40. $R-CH=CH-CH=N-NH-C(=O)-C_6H_4Cl(m)$		1	<1			<1	<1	<1	<1	<1	<1	<1	<1		
No. 41. $R-CH=CH-CH=N-NH-C(=O)-O-CH_3$		<1	<1					<1	<1	<1	<1	<1	<1	<1	<1
No. 42. $R-CH=CH-CH=N-NH-C(=O)-CO_2C_2H_5$		2.5	1					1	<1	1	<1	1	<1	<1	<1
No. 43. $R-CH=CH-CH=N-NH-C(=O)-OH$		10	5	<1	<1	<1	<1	2.5	1	2.5	1	2.5	1		
No. 44. $R-CH=CH-CH=N-NH-CONH-C(=O)-N$		2.5	1					<1	<1	<1	<1				
No. 45. $(R-CH=CH-CH=N-N)_2$		<1	<1					<1	<1	<1	<1				

Table 5. 5-nitro-furylsulfonamide derivatives. (unit=10,000, R=NO₂-)

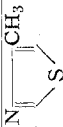
Bacteria	Staphylo- coccus aureus		Strepto- coccus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
Derivatives	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 46. R-SO ₂ NH ₂	20	10	<1	<1	<1	<1	10	10	20	10	10	5	<1	<1
No. 47. R-SO ₂ NH- 	2.5	1			<1	<1	1	<1	1	<1	1	<1	<1	<1
No. 48. R-SO ₂ NH- 	5	2.5			<1	<1	1	<1	1	<1	<1	1	<1	<1
No. 49. R-SO ₂ NH- 	1	<1			1	<1	2.5	1	2.5	1	<1	<1		
No. 50. R-SO ₃ K	<1	<1			<1	<1	<1	<1	<1	<1	2.5	1	<1	<1

Table 6. 5-nitro-furylsulfide derivatives. (unit=10,000, R=NO₂-)

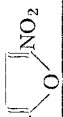
Bacteria	Staphylo- coccus aureus		Strepto- coccus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
Derivatives	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 51. R-S-  -NO ₂	10	5	2	1			5	2.5	5	2.5	5	2.5	<1	<1
No. 52. R-S- 	80	60	<1	<1			5	2.5	5	2.5	10	5		

Table 7. Other derivatives. (unit =10,000, R=NO₂-)

Bacteria	Staphylo- coccus aureus		Strepto- coccus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
Derivatives	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 53. R-C=C-COOC ₂ H ₅ COCH ₃	5	2.5			<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
No. 54. R-C=C-COOC ₂ H ₅ COOC ₂ H ₅	1	<1			<1	<1	<1	<1	<1	<1	<1	<1		
No. 55. R-C≡N	5	2.5	<1	<1	2	1	10	5	10	5	10	5	<1	<1
No. 56. N-CH=NOH	10	5	4	2	2	1	10	5	10	5	10	5	<1	<1

Table 8. Antibacterial action of nitro-furan derivatives against anaerobic bacteria

Bacteria	Cl. tetani		V. septique		Cl. novyi	
Derivatives	24	96	24	96	24	96
No. 1. R-CH=N-NH-CONH ₂	16000	8000	16000	8000	8000	8000
No. 2. R-CH=N-NH-C()-NH ₂ HCl	16000	16000	16000	8000	8000	4000
No. 13. R-CH=CH-CONH ₂	16000	8000	16000	16000	8000	<4000
No. 14. R-CH=CH-COOH	8000	8000	16000	8000	8000	4000
No. 18. R-CONH ₂	8000	4000	6000	6000	4000	<4000
No. 19. R-CONH- 	6000	4000	8000	4000	<4000	<4000
No. 52. R-S-  NO ₂	8000	6000	6000		<6000	
No. 53. R-CH=C-COOC ₂ H ₅ COCH ₃	4000	4000	4000	<4000	<4000	<4000

Table 9. Diffusibility of nitro-furan derivatives (mm)

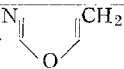
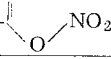
Derivatives	Concentration				
	1: 10,000	1: 20,000	1: 40,000	1: 80,000	1: 160,000
No. 1. $R-CH=N-NH-CONH_2$	17.0	14.5	12.0	9.0	5.5
No. 2. $R-CH=N-NH \cdot C \begin{smallmatrix} NH_2 \\ \diagup \\ NH \end{smallmatrix} HCl$	17.0	14.5	12.5	9.5	5.5
No. 13. $R-CH=CH-CONH_2$	18.0	15.5	13.0	7.5	4.0
No. 14. $R-CH=CH-COOH$	14.5	9.0	6.5	3.5	0
No. 18. $R-CONH_2$	9.5	6.0	2.5	0.5	0
No. 30. $R-CONH-$ 	14.0	11.0	8.0	5.0	1.0
No. 35. $R-CH=CH-CH=N-NH-CONH_2$		17.5	15.0	11.0	6.5
No. 36. $R-CH=CH-CH=N-NH \cdot C \begin{smallmatrix} NH_2 \\ \diagup \\ NH \end{smallmatrix} HCl$	18.5	16.5	14.5	11.5	7.0
No. 46. $R-SO_2NH_2$		14.5		9.8	
No. 52. $R-S-$  NO_2	23.0	21.5	19.5	17.0	15.0
No. 56. $R-CH=NOH$	11.0	7.5	3.5	1.5	0

Table 10. 50% lethal dose per 10 gr mouse (mg)

	subcutan.	per os
No. 1. $R-CH=N-NH-CONH_2$	6.25	2.08
No. 2. $R-CH=N-NH \cdot C \begin{smallmatrix} NH_2 \\ \diagup \\ NH \end{smallmatrix} HCl$	1.2	1.43
No. 13. $R-CH=CH-CONH_2$	3.13	1.88
No. 14. $R-CH=CH-COOH$	4.35	2.12
No. 18. $R-CONH_2$	1.46	1.88
No. 35. $R-CH=CH-CH=N-NH-CONH_2$	11.11	9.26
No. 36. $R-CH=CH-CH=N-NH \cdot C \begin{smallmatrix} NH_2 \\ \diagup \\ NH \end{smallmatrix} HCl$	1.0	1.5
No. 46. $R-SO_2NH_2$	2.18	2.5
No. 53. $R-CH=C-COOC_2H_5$ $COCH_3$	1.25	
No. 56. $R-CH=NOH$	0.31	