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ANTISEPTIC

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The present invention relates primarily to antiseptics, i. e. compounds which restrain or inhibit the growth of putrefactive micro-organisms, and is concerned more particularly with antiseptics of the furan series—specifically nitrofurans.

Antiseptics are, of course, known. However, they are frequently bound up with undesirable characteristics, such as toxicity, tendency toward undue irritation of or injury to tissue to which they are applied, lack of stability, insolubility, etc. These undesirable characteristics are, in general, ascribable to the presence conventionally in most antiseptics, of toxic heavy metals (e. g. mercurials), phenolic hydroxyl groups (e. g. antiseptics of the phenol type), halogens (iodine or the like), etc.

A desideratum relative to antiseptics is that they shall be free of defects of known substances of the character above outlined, i. e. that the antiseptics shall be non-toxic, relatively non-irritant, non-corrosive, stable and soluble, without however, sacrificing relative antiseptic activity.

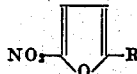
A primary object of this invention therefore is to provide a new series of organic antiseptics which shall fulfill the above characterized desideratum.

The invention is based upon the surprising and unexpected discovery that the nitrofurans comprise compounds which possess marked antiseptic activity without entailing the prejudicial characteristics of toxicity, undue irritativeness, etc., to which reference has above been made.

More specifically stated, it has been found that outstanding antiseptic activity of useful character can be realized from a wide variety of furan compounds provided that these compounds contain a nitro group in an α -position, i. e. in the 2- or 5-position in the furan ring. It is within the scope of the present invention that these nitro-furan compounds may be substituted in at least one of the remaining alpha (2 or 5) and beta positions 3 and 4 by hydrogen, or an alkyl, hydroxyalkyl, acyloxyalkyl, oximidoalkyl, semicarbozonoalkyl, hydrazonoalkyl, diacyloxyalkyl, carboxyalkenyl, carbalkoxyalkenyl, acyl, carbalkoxy, halo-

geno-carbalkoxy, carbamyl, or dialkylcarbamyl radical or the like.

The preferred derivatives of nitrofuran within the scope of the present invention may conveniently be represented by the following chemical formula:



wherein R represents the aforementioned wide variety of substituents of which the following specific ones may be cited by way of example:

H, CH₃, C₂H₅, CH₂OH, CH₂OOCCH₃, CH=NOH,
CH=N—NHCOHN₂, CH=N—NH₂,
CH(OOCCH₃)₂, CH=CHCOOH,
CH=CHCOOC₂H₅, COCH₃, COC₂H₅, COC₃H₇,
COOCH₃, COOC₂H₅, COOCH₂CH₂Cl,
COHN₂, CON(C₄H₉)₂

The general formula above given is intended to include the various position isomers due to the location of the R group.

For the sake of clearness, it may be indicated that the positions in the furan ring may be designated as follows:



The organic antiseptics according to the present invention may be used, severally or in admixture, for example as constituents of aqueous antiseptics, antiseptic tinctures, antiseptic creams and ointments, deodorants and other pharmaceutical preparations. In some cases, the compounds of the present invention may impart preservative and/or bactericidal properties to the preparations into which they are incorporated.

An advantageous characteristic of the nitrofurans which come within the purview of this invention is their solubility in simple solvents frequently used in pharmaceutical preparations, e. g. water, oil, etc. The solubilities are, in general, of a magnitude to make it possible to use aqueous solutions as well as ointments of essentially oily

base in actual practice and in making tests of the character hereinafter set forth. By way of illustration, aqueous antiseptic preparations and antiseptic ointments may be produced as follows, according to the present invention:

Preparation of 2-formyl-5-nitrofuran di-acetate and aqueous antiseptic solution

A nitrating mixture of 60 cc. acetic anhydride and 27 cc. fuming nitric acid (sp. gr. 1.5) is stirred at -5° C. while a solution of 16 grams of furfural diacetate in 15 cc. of acetic anhydride is added dropwise. Stirring is continued 1 hour after addition is complete, and the mixture poured onto 250 grams of crushed ice. The oil is separated, more is obtained by ether extraction of the aqueous layer, and the final ether solution containing all the oil is washed thoroughly with sodium bicarbonate and then twice with water. The ether is removed and an equal volume of pyridine is added. Vigorous reaction occurs. The solution is then poured onto ice, diluted to one liter, and chilled. The solid product is filtered and recrystallized twice from petroleum ether. White crystals M. P. $91-92^{\circ}$ C. Yield=4 grams or 20%.

An aqueous antiseptic solution is readily prepared by dissolving 1 gram in one liter of hot water and filtering. This solution will give a cup plate zone of 8 mm. (F. D. A. method—*Staph. aureus*).

Preparation of antiseptic ointments containing nitrofuran

The nitrofuran, 2-formyl-5-nitrofuran diacetate, just described or various other nitrofuran antiseptics can be incorporated readily into ointments. With a petrolatum base, the nitrofuran can be dissolved by warming in a small portion of the base, and adding this to the main portion in a Lenart mixer. Various ingredients can then be sifted in.

When a combined base, for example petrolatum+white wax+lanolin, is used, the whole amount of base can be mixed and the nitrofuran can be stirred into the melted base.

In vanishing cream containing glyceryl monostearate, the nitrofuran can be incorporated into the glyceryl monostearate.

ANTISEPTIC ACTIVITY OF NITROFURANS

The nitrofuran derivatives above indicated as coming within the purview of this invention, and many others, have been prepared and subjected, among other things, to a test for inhibitory antiseptic activity according to the standard cup plate method (described in Circular No. 198, U. S. Department of Agriculture, Food and Drug Administration, December 1931), and have been found to be excellent antiseptics.

The following Table I sets forth the results obtained according to the said standard cup plate method (F. D. A. Circular 198) with aqueous solutions of representative compounds according to this invention in connection with *Staphylococcus aureus*. The cup plate results shown are expressed in terms of mm. of clear zone (i. e. zone in which the growth of bacteria is inhibited), and show the penetration as well as the inhibitory action on *Staphylococcus aureus*. The Kolmer test on *Staph. aureus*, also shown in Table I, represents the maximum dilution at which the antiseptic still inhibits the growth of the bacteria.

TABLE I

Cup plate antiseptic tests and Kolmer test for inhibitory action

Compound	Conc. (aqueous)	<i>Staph. aureus</i> cup plate zone (F. D. A. test for antiseptic action)	<i>Staph. aureus</i> Kolmer dilution
2-carbethoxy-5-nitrofuran.....	1:500	Mm. 4.0	$\frac{1}{50,000}$
2-acetyl-5-nitrofuran.....	1:500 1:1000 1:5000	16.5 14.5 8.5	$\frac{1}{80,000}$
2-butyl-5-nitrofuran.....	1:1500 1:2500 1:5000	12.0 9.0 7.0	$\frac{1}{30,000}$
2-formyl-5-nitrofuran diacetate.....	1:1000	8.0	-----
2-ethyl-5-nitrofuran.....	1:500	11.0	-----
2-propyl-5-nitrofuran.....	1:1750	3.0	-----

Note.—See Circular No. 198, U. S. Department of Agriculture, December 1931, "Methods of Testing Antiseptics."

These results compare very favorably with the activity against *Staphylococcus aureus* in many of the best known prior art antiseptics.

THE ANTISEPTIC ACTIVITY IN DEODORANT CREAMS

An important characteristic of the antiseptics of the present invention is their property of remaining effective in a deodorant cream with petrolatum or other ointment or cream base.

The following table sets forth results as to antiseptic activity obtained by the standard cup plate method (F. D. A. Circular 198) on *Staphylococcus aureus* with the following illustrative nitrofuran antiseptics in a deodorant cream containing a petrolatum base.

TABLE II

Cup plate antiseptic tests on deodorant creams

Compound	Concentration	<i>Staph. aureus</i> cup plate zone
5-nitro-furfuryl acetate.....	1:500	Mm. 9.0
2-formyl-5-nitrofuran diacetate.....	1:500	8.0
2-carbomethoxy-5-nitrofuran.....	1:500	8.5
2-carbethoxy-5-nitrofuran.....	1:500	8.0
2-acetyl-5-nitrofuran.....	1:500	16.0
2-propionyl-5-nitrofuran.....	1:500	15.0
Ethyl 5-nitro-furacrylate.....	1:500	8.0
2-ethyl-5-nitrofuran.....	1:500	8.0
2-propyl-5-nitrofuran.....	1:500	5.0
Deodorant cream without antiseptic.....	-----	0.0

PERSISTENCE OF ACTIVITY IN DEODORANT CREAMS

In the deodorant use of an antiseptic cream the ability of the antiseptic to remain active is of considerable importance. When deodorant creams with a petrolatum base containing nitrofuran antiseptics are spread on parchment and immersed in a broth inoculated with a mixed culture (*Staph. aureus* plus organisms from the human axilla), the persistence of antiseptic ac-

tion is remarkable. This is shown in Table III.

In this test for persistence of antiseptic action, 0.25 g. of the deodorant cream are spread evenly over a 25 sq. cm. strip of parchment paper. The parchment paper is then placed in a culture tube containing 10 cc. of artificial perspiration (culture broth) which has been inoculated with a mixed culture (*Staph. aureus* plus organisms from the human axilla), using approximately 500,000 organisms. After 24 hours incubation at 37° C., the tube is observed for bacterial growth, the paper is transferred to a fresh tube, incubated, and the transfer process repeated until bacterial growth occurs. The tubes are incubated at 37° C. for 48 hours after the transfer and observed for delayed growth. The persistence of antiseptic efficiency of the deodorant cream is measured by the number of transfers possible before growth is observed. A + sign signifies growth.

TABLE III

Transfer test—Persistence of antiseptic activity in deodorant creams

Compound	Conc. in cream	First transfer			Second transfer			Third transfer		
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
2-carbethoxy-5-nitrofur.	Per cent									
2-acetyl-5-nitrofur.	0.2	—	—	—	—	—	—	—	—	—
2-acetyl-5-nitrofur.	0.1	—	—	—	—	—	—	—	—	—
Ethyl 5-nitrofuracrylate.	0.2	—	—	—	—	—	—	—	—	—
5-nitro-furacrylic acid.	0.1	—	—	—	—	—	—	—	—	—

FUNGISTATIC ACTION

A further characteristic of antiseptics according to this invention is the fungistatic action which is exhibited thereby. Results obtained with some of the more active nitrofurant antiseptics, when tested at a concentration of 1:500 in a cream with a petrolatum base, are shown in Table IV. The cup plates were incubated 48 hours at room temperature.

TABLE IV

Fungistatic action of deodorant creams (petrolatum base)

Compound	Concentration in cream	<i>Trichophyton rosaceum</i> cup plate zone
2-carbethoxy-5-nitrofur.	1:500	Mm. 10
2-acetyl-5-nitrofur.	1:500	18
Dibutyl 5-nitrofuramide.	1:500	6

NON-IRRITANT AND NON-TOXIC CHARACTER OF NITROFURAN ANTISEPTICS

The relatively non-irritant and non-toxic character of the nitrofurant antiseptics is shown in Table V. The irritation was tested by subcutaneous injection of the rabbit ear followed by intravenous Trypan blue. The irritation work was controlled by the use of solutions producing standard irritation. Toxicity was obtained by intraperitoneal administration of the solid compound in a gelatin capsule. The toxicity is given as the level at which 50% of the test animals die.

The intravenous dye test for vascular permeability due to irritants is a well known laboratory method of testing for irritation. See, for example, "A method for the quantitative estimation of chemical irritation," J. H. Weatherby, *Journal of Laboratory and Clinical Medicine*, 25, 1199-1204 (1940).

TABLE V

Trypan blue irritation studies (rabbit) toxicity (intraperitoneal)

Compound	Solub. in water	Safe concentration (no irritation)		Toxicity (Intraperitoneal) mg./kg.	
		Saline	Liq. petrolatum	Rat	Rabbit
2-carbethoxy-5-nitrofur.	1:5000	1:5000	1:250	800	700
Ethyl 5-nitrofuracrylate.	1:3000	1:3000	1:250	850	500
2-acetyl-5-nitrofur.	1:300	1:300	1:1000	200	50
2-propionyl-5-nitrofur.	1:600	1:1000	1:250	250	100
2-butyryl-5-nitrofur.	1:1000	1:1000	-----	300	150

¹ Maximum solubility.

NITROFURANS IN OINTMENTS

Still another important characteristic of nitrofurans within the scope of this invention is the property which they exert, when incorporated into ointments, of imparting high antiseptic power (as shown in tests according to the aforementioned standard cup plate method) while involving an extremely and unusually low toxicity (as determined in tests on laboratory animals).

Thus, nitrofurans such, for example, as 2-acetyl-5-nitrofurant and 2-propionyl-5-nitrofurant in a petrolatum base ointment at an antiseptic concentration of 1:1000 produce, in the cup plate test, clear zones of 10 mm. or more. The corresponding toxicity in the rat (level at which more than 50% die) is of the extremely low order of 200-250 mg. per kg. of body weight. Good results are also obtainable, for example, with 2-ethyl-5-nitrofurant and 2-propyl-5-nitrofurant.

Among the new compounds developed in this work the most active, useful antiseptics are 2-formyl-5-nitrofurant semicarbazone, 2-propionyl-5-nitrofurant, 2-butyryl-5-nitrofurant and 2-ethyl-5-nitrofurant. In the preparation of one of these new compounds, namely, 2-propionyl-5-nitrofurant, the nitration step is typical of the procedure used on all of the nitrofurant antiseptics.

One mole of aluminum chloride is mixed with 1250 cc. carbon disulfide and one mole of propionyl chloride in a 3-neck, 3-liter flask. A solution of 74 cc. of furan in 250 cc. carbon disulfide is added slowly. Temperature is held at 10° C. At the end of the addition, the mixture is stirred 1 hour at room temperature and then poured onto cracked ice. The carbon disulfide is distilled off and the residue steam distilled. The distillate is made alkaline, extracted with ether and the ether extract washed and dried. Propionyl furan, B. P. 81.2° C. Yield=50 grams or 40%.

To 148 cc. of acetic anhydride is added 67 cc. of fuming nitric acid (sp. gr. 1.5) slowly with stirring and below -5° C. A solution of 31 grams

of propionyl furan in 50 cc. acetic anhydride is run in slowly at -5°C ., the mixture stirred for $\frac{1}{2}$ hour and poured onto cracked ice. The product is extracted twice with ether (150 cc.) and 50 cc. pyridine added to the ether extract. The solution is warmed to start the reaction and diluted with water when reaction is complete. The product is filtered off, recrystallized from petroleum ether and then sublimed. 2-propionyl-5-nitrofuran. White crystals M. P. $69-70^{\circ}\text{C}$. Yield 8.5 grams (20% based on propionyl furan).

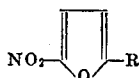
An aqueous antiseptic solution can be prepared from 2-propionyl-5-nitrofuran by dissolving 1 gram in 600 cc. of hot water and filtering. This solution will give a cup plate zone of 16.5 mm. (*Staph. aureus*) and 16.0 mm. (*Bact. typhosum*).

It is apparent from the foregoing that the present invention makes available a new series of organic antiseptics which are non-corrosive and practically non-irritant. These new antiseptics do not contain a heavy metal, such as mercury, nor do they contain the phenolic hydroxyl group. The new antiseptics possess lower toxicity than commonly used antiseptics. They are sufficiently soluble and stable to be of practical use in aqueous antiseptics, in antiseptic tinctures, in antiseptic creams and ointments, in deodorants, etc.

It will of course be understood that the antiseptic action of the furan derivatives according to the present invention is not limited to the bacteria above employed by way of example but that such antiseptic action extends also to various other bacteria such as *Bact. typhosum*, etc.

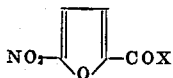
Having described the invention, what is claimed is:

1. A pharmaceutical preparation having bacteria-growth inhibiting properties and containing, as the essential active ingredient, a nitrofuran of the formula



wherein R represents a member of the group consisting of hydrogen and the alkyl, hydroxyalkyl, acyloxyalkyl, oximidoalkyl, semicarbazonoalkyl, hydrazonoalkyl, diacyloxyalkyl, carboxyalkenyl, carbalkoxyalkenyl, acyl, carbalkoxy, halogeno-carbalkoxy, carbamyl and dialkylcarbamyl radicals.

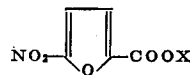
2. A pharmaceutical preparation having bacteria-growth inhibiting properties and containing as the essential active ingredient, a nitrofuran of the formula



wherein X represents alkyl.

3. A pharmaceutical preparation having bacteria-growth inhibiting properties and contain-

ing as the essential active ingredient, a nitrofuran of the formula



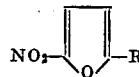
wherein X represents alkyl.

4. A pharmaceutical preparation having bacteria-growth inhibiting properties and containing as the essential active ingredient, a nitrofuran of the formula



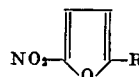
wherein Y represents acyloxy.

5. A pharmaceutical preparation having bacteria-growth inhibiting properties and comprising an aqueous solution containing, as the essential active ingredient, a nitrofuran of the formula



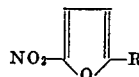
wherein R represents a member of the group consisting of hydrogen and the alkyl-hydroxyalkyl, acyloxyalkyl, oximidoalkyl, semicarbazonoalkyl, hydrazonoalkyl, diacyloxyalkyl, carboxyalkenyl, carbalkoxyalkenyl, acyl, carbalkoxy, halogeno-carbalkoxy, carbamyl and dialkylcarbamyl radicals.

6. A deodorant composition containing, as the essential active ingredient, a nitrofuran of the formula



wherein R represents a member of the group consisting of hydrogen and the alkyl, hydroxyalkyl, acyloxyalkyl, oximidoalkyl, semicarbazonoalkyl, hydrazonoalkyl, diacyloxyalkyl, carboxyalkenyl, carbalkoxyalkenyl, acyl, carbalkoxy, halogeno-carbalkoxy, carbamyl and dialkylcarbamyl radicals.

7. A pharmaceutical preparation having bacteria-growth inhibiting properties and comprising the ointment base having homogeneously dispersed therethrough, as the essential active ingredient, a nitrofuran of the formula



wherein R represents a member of the group consisting of hydrogen and the alkyl, hydroxyalkyl, acyloxyalkyl, oximidoalkyl, semicarbazonoalkyl, hydrazonoalkyl, diacyloxyalkyl, carboxyalkenyl, carbalkoxyalkenyl, acyl, carbalkoxy, halogeno-carbalkoxy, carbamyl and dialkylcarbamyl radicals.

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