SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SUBSTITUTED 2,3-DIHYDRO-4H-PYRANS

L. G. Stepanova, N. A. Keiko,
T. N. Malkova, A. G. Leont'eva,
T. Ya. Pushechkina, and M. G. Voronkov

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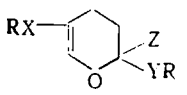
There has of late been an increase in the numbers of hospital pyoinflammatory diseases in which the primary etiological role is played by *Staphylococcus aureus*, which is increasingly accompanied by Gram-negative microflora (*Pseudomonas aeruginosa* and *Proteus*). For this reason, the search for compounds which effectively suppress these microorganisms is of some urgency.

We have synthesized a number of 2-formyl-2,5-dialkylthio-2,3-dihydro-4H-pyrans (Ia-f) and examined their antimicrobial activity. Compounds (Ib) and (Id) are dimers of 2-alkylthioacroleins, which we have previously obtained by the Mannich condensation of the appropriate alkylthioacetaldehydes with formaldehyde in the presence of diethylamine hydrochloride at room temperature [2].



Ia-f, II, III, IVa-d V

R = Me (Ia, IVa), Et (Ib, IVb, d, V), Pr (Ic), Bu (Id, II, III, IVc), *i*-Bu (Ie), *t*-Am (If); X = O (IVa-c), S (Ia-f, II, III, IVd, V). Y = O (IVa-d V), S (Ia-f, II, III); Z = CHO (Ia-f, IVa-d V), COOH (II), COOK (III).

It has been shown for the first time that 2-formyl-2,5-dibutylthio-2,3-dihydro-4H-pyran (Id) can be obtained by the Knoevenagel condensation (see [1]) of butylthioacetaldehyde with formaldehyde in the presence of potassium fluoride.

Oxidation of (Id) with silver oxide in alkaline medium at 20°C gives the acid (II). Reaction of this with potassium hydroxide gives the salt (III).

Dimerization of 2-ethoxyacrolein (Diels-Alder reaction) affords 2-formyl-2,5-diethoxy-2,3-dihydro-4H-pyran (IV) [6], which on treatment with ethanethiol gives the product of replacement of the 5-ethoxy group by ethylthio (V). The purity and structures of the products were confirmed by ^{1}H NMR and IR spectroscopy, and by elemental analysis (Table 1).

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained on a UR-20 instrument (East Germany) in microlayers, and ^{1}H NMR spectra on a Tesla-BS-487B spectrometer (Czech SSR) (80 MHz) in CCl_4, internal standard hexamethyldisiloxane.

TABLE 1. Constants of Dihydropyrans Obtained

Compound	Yield, %	bp, °C (mm Hg)	n_D^{20}	d_4^{20}	Found, %			Empirical formula	Calculated, %		
					C	H	S		C	H	S
Ia	31	126—7 (3)	1.5553	1.2242	47.04	5.76	30.98	$C_9H_{12}O_2S_2$	47.05	5.87	31.4
Ic	45	127—8 (3)	1.5176	1.1094	55.19	7.52	24.45	$C_{14}H_{24}O_2S_2$	55.34	7.68	24.63
Ie	50	101 (5)	1.5115	1.0528	58.27	8.46	21.96	$C_{12}H_{20}O_2S_2$	58.3	8.32	22.2
If	45	152—3 (1)	1.5150	1.0436	60.88	8.90	19.88	$C_{14}H_{28}O_2S_2$	60.76	8.89	20.27
II	80		1.5300	1.1358	55.10	8.00	21.00	$C_{14}H_{24}O_3S_2$	55.30	7.90	21.10
III	90	185 (dec.)	—	—	49.00	6.64	18.63	$C_{14}H_{23}O_3S_2K$	49.11	6.71	18.72
V	60	111—5 (2)	1.4930	1.1062	55.90	7.70	14.30	$C_{10}H_{18}O_3S$	55.5	7.40	14.80

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TABLE 2. Antimicrobial Activity of (Id) against Staphylococci from Various Sources

Source of isolate	Number of strains	Number of strains showing activity (A and B) at the given concentration of (Id), expressed in μg/ml									
		bacteriostatic (A)					bactericidal (B)				
		1.5	3.1	6.2	12.5	25.0	3.1	6.2	12.5	25.0	50.0
Suppurative foci	47	17	26	4	—	—	13	24	6	4	—
Feces	28	—	14	7	6	1	—	12	9	4	3
Blood	12	—	—	2	3	7	—	—	2	2	8
Sweat	26	—	16	8	2	—	—	15	7	4	—
Oral and nasal secretions	13	—	2	7	2	2	—	2	1	—	10
Total . . .	126	17	58	28	13	19	13	53	25	14	21

TABLE 3. Antimicrobial Activity of (Id) against Staphylococci of Various Phage Groups

Phage group of staphylococcus	Number of strains	Number of strains showing activity (A and B) at the given concentration of (Id), expressed in μg/ml									
		bacteriostatic (A)					bactericidal (B)				
		1.5	3.1	6.2	12.5	25.0	3.1	6.2	12.5	25.0	50.0
1	54	17	37	—	—	—	13	34	7	—	—
2	16	—	12	3	1	—	—	12	2	2	—
3	18	—	3	12	2	1	—	1	10	4	3
Mixed 1, 2, and 3	8	—	6	2	—	—	—	4	3	1	—
Nontypical	30	—	—	11	10	9	—	4	9	7	10
Total . . .	126	17	58	28	13	10	13	55	31	14	13

2-Formyl-2,5-diisobutylthio-2,3-dihydro-4H-pyran (Ie). A mixture of 5.8 ml of 33% formaldehyde and 7.6 g (0.07 mole) of diethylamine hydrochloride was neutralized with 10% sodium carbonate solution to pH 7.0. Isobutylthioacetaldehyde (9.3 g, 0.07 mole), 0.01 g of hydroquinone, and 40 ml of alcohol were then added, and the mixture heated at 60°C for 1 h. The organic layer was separated, the aqueous layer extracted with ether, and the extract and organic layer dried over magnesium sulfate. Vacuum distillation afforded 5 g (50%) of (Ie). Compounds (Ia), (Ic), and (Id) were obtained similarly.

2-Formyl-2,5-dibutylthio-2,3-dihydro-4H-pyran (Id). A mixture of 3.1 g (0.02 mole) of butylthioacetaldehyde, 1.8 g (0.02 mole) of paraformaldehyde, and 1.2 g (0.02 mole) of potassium fluoride in 15 ml of pyridine was heated at 50°C for 3 h. According to GC, the reaction mixture contained 60% of (Id).

2,5-Dibutylthio-2,3-dihydro-4H-pyran-2-carboxylic Acid (II). To a solution of 7.4 g (0.025 mole) of (Id) in 100 ml of dioxane was added dropwise with tap-water cooling a solution of 8.75 g (0.05 mole) of silver nitrate in 7 ml of water, and 3.1 g (0.075 mole) of sodium hydroxide in 7 ml of water. The temperature of the mixture was then brought slowly to ambient, and kept at this temperature for 1 h with vigorous stirring. The precipitated silver oxide was filtered off, washed 5-7 times with water, and the filtrate and washings extracted with ether. To the aqueous portion remaining after ether extraction was added dropwise at 10°C the calculated amount of conc. HCl, and the organic layer separated and the aqueous layer extracted with ether. The extract and organic layer were dried over magnesium sulfate. Removal of the ether gave 6 g (80%) of (II). For purification, a solution of the salt was again prepared, extracted with ether to remove impurities, acidified with the calculated amount of HCl, and the ether extracts dried over magnesium sulfate. Thorough removal of the ether under oil pump vacuum gave (II).

Potassium 2,5-Dibutylthio-2,3-dihydro-4H-pyran-2-carboxylate (III). A mixture of 3.4 g (0.01 mole) of the acid (II) in 20 ml of alcohol and 0.77 g (0.0055 mole) of potassium carbonate in 20 ml of water was heated for 1 h at 60°C. On cooling, 1.9 g (90%) of (III) separated.

TABLE 4. Antimicrobial Activity of 2-Formyl-2,5-dibutylthio-2,3-dihydro-4H-pyran (Id) on a Solid Nutrient Medium

Concentration of (Id), %	Staph. aureus		Staph. epidermidis		Ps. aeruginosa		Pr. mirabilis		Bac. cereus	
	1	2	1	2	1	2	1	2	1	2
1	—	—	—	—	—	—	—	—	—	—
0.5	—	—	—	—	—	—	—	—	—	—
0.25	—	—	—	—	i.c.	i.c.	—	—	—	—

Note. 1 and 2 represent microbial loadings of 10,000 and 100 microbial cells per 0.1 ml respectively. The signs + and - correspond to the presence or absence of microbial growth. i.c. are individual colonies.

2-Formyl-2-ethoxy-5-ethylthio-2,3-dihydro-4H-pyran (V). To a mixture of 30.1 g (0.15 mole) of (IVb) and 0.03 g (0.017 mole) of toluene-p-sulfonic acid, cooled to 0°C, was added 9.6 g (0.15 mole) of ethanethiol. The mixture was stirred for 1.5 h at ambient temperature, then for 0.25 h at 40°C and neutralized with the calculated amount of methanolic sodium methoxide. Vacuum distillation afforded 9.6 g (60%) of (V) (calculated on (IV) reacted).

The physicochemical constants of the products are given in Table 1.

EXPERIMENTAL (BIOLOGY)

The bacteriostatic activity of (I-V) against various strains of microorganisms was determined by serial dilution [4] in a liquid nutrient medium, with an initial concentration of the compounds of 200 μg/ml. Antistaphylococcal activity in (I-V) was dependent on the ring substituent. Activity was greater when the dihydropyran (I) contained an alkylthio (other than CH_3S) group and an aldehyde group (Table 2), while the 2-formyl-2,3-dihydro-4H-pyrans (Ia), (IV), and (V), which contain CH_3S or alkoxy groups, were inactive.

Compounds (IVa-c) were obtained as described in [3, 6, 7]. For (Ia, f), (II), (III), and (IVa-d), the minimum bacteriostatic concentration was not found, for (Ib) it was 100 μg/ml, (Ic) 50 μg/ml, (Id) 3.1 μg/ml, and (Ie) 25 μg/ml. The LD_{50} values were (mg/kg): (Ia) 2440, (Ib) 136, (Ic) 2667, (Id) 640, and (IVa) 476. Oxidation of the aldehyde group in (Id) to carboxyl (II) also resulted in loss of activity. The antimicrobial activity of the dihydropyrans (Ia-e) decreased in the following order of changes in the alkyl radical attached to sulfur: $t\text{-}C_5H_{11} << C_4H_9 > i\text{-}C_4H_9 > C_3H_7 > C_2H_5 >> CH_3$. The greatest antistaphylococcal activity was shown by (Id), which was not highly toxic (LD_{50} 640 mg/kg). The antistaphylococcal activity of (Id) was examined in 126 strains of staphylococci, most of which (80) displayed polyresistance to the antibiotics used. In view of the different epidemiological significance of staphylococci of different phagotypes, the dependence of the antistaphylococcal activity of (Id) on the source and phagotypic attribution was studied (Tables 2, 3, and 4).

The data presented in Table 2 show that the minimum bacteriostatic (1.5-3.1 μg/ml) and bactericidal (3.1-6.2 μg/ml) concentrations of (Id) are seen for strains of staphylococci isolated from suppurative foci in surgical patients, and from the sweat in staphylococcal pneumonia and enterocolitis. However, the staphylococci obtained from the blood and from healthy bacterial carriers were less sensitive, the minimum bacteriostatic and bactericidal concentrations in this case being 6.25-25.0 and 12.5-50.0 μg/ml respectively.

The bacteriostatic and bactericidal activity of (Id) is also dependent on the phagotypic attribution of the staphylococci (Table 3). The growth of staphylococci of phage group 1 was retarded by (Id) in concentrations of 1.5-3.1 μg/ml. For nonclassified staphylococcal strains, however, this value was 6.2-25.0 μg/ml. Accordingly, the bactericidal concentrations of (Id) against these staphylococci were twice as great.

Hence, the strains of staphylococci isolated from patients with clinical symptoms of staphylococcal infection, belonging to phage group 1 ("hosphital" phagotypes) were more sensitive to (Id).

The antimicrobial activity of (Id) was also examined on a solid nutrient medium at various microbial loadings of *Staph. aureus, Staph. epidermidis, Ps. aeruginosa, Pr. mirabilis, Bac. cereus*. At concentrations of (Id) of 0.5 and 1%, none of the organisms showed any growth (Table 4).

The antimicrobial activity observed in (Id) led to its being examined as a disinfectant. The bacteriostatic activity of (Id) was examined by the method for the determination of bactericidal activity in novel disinfectants described in [5], using cambric fabric contaminated with test cultures. To determine the bactericidal activity of (Id), *Staph. aureus* strain 209-P was used. The compound was used as the 1 and 0.5% solutions in a mixture of water and dimethyl sulfoxide (12:1) (0.5 ml of solution in each test). Disinfectant activity was measured daily for 6-7 days.

Staphylococcal growth was seen only at concentrations of (Id) of 0.25% following 5 minute exposure of the test material to the disinfectant solution, and only on day 3. When 0.5 or 1% solutions of (Id) were used, growth of the microorganisms was not seen even by the seventh day.

The acute toxicity of (Id) was measured in mongrel white mice of both sexes weighing 20-24 g, by the intraperitoneal route in doses of 3000, 600, 120, and 24.5 μg/kg. The lethal dose LD_{50}, calculated by Kerber's method, was 640 mg/kg.

It follows that (Id) shows high antimicrobial activity and low toxicity, so that it may be used as a disinfectant agent.

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