

Antimicrobial Properties of Some Mixed Diesters of Aliphatic Diols

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ABSTRACT

Twenty compounds, diesters of ethylene glycol, diethylene glycol, or *cis*-2-butene-1,4-diol, were screened for antimicrobial activity against a gram-positive bacterium, *Staphylococcus aureus*; a gram-negative bacterium, *Escherichia coli*; a mold, either *Aspergillus flavus* or *A. species*; and a yeast, either *Candida albicans* or *Torula species*. All of the compounds were inhibitory against two or more of the test organisms, and eight mixed diesters were inhibitory against all four types of organisms. Each of these eight compounds had a benzoate group at one ester function and an acyl group other than benzoyl, mostly fatty acyl groups of 12-22 carbons, at the other.

INTRODUCTION

Many long chain fatty acid derivatives, particularly *N*-substituted amides, possess antimicrobial activity (1-5), as do some of the free acids and simple fatty esters (6,7). The availability of a group of diesters of aliphatic diols prepared for use in other research (8) gave us a unique opportunity to investigate the antimicrobial activity of these compounds. Diesters of three diols were tested: ethylene glycol, diethylene glycol, and *cis*-2-butene-1,4-diol. All contained a benzoate group at one ester function, and an aliphatic or aromatic grouping other than benzoyl at the other ester function, with one exception. The dibenzoate of diethylene

glycol was tested along with the mixed diesters for comparative purposes.

EXPERIMENTAL PROCEDURES

Preparation of the diesters used in this study is reported elsewhere (8). Difco Dehydrated Mycological Agar at pH 7.0 was used to test inhibition of the organisms selected by the compounds being screened. The microorganisms used were from stock cultures: *Staphylococcus aureus*, ATCC 12692; *Escherichia coli*, ATCC 25922; *Aspergillus sp.*; *Aspergillus flavus*, ATCC 11495; *Candida albicans*, ATCC 753; and *Torula sp.* The *Aspergillus sp.* and *Torula sp.* are organisms which are stock cultures of the LSU Food Science Department and were isolated from contaminated foods. After incubating the cultures for 48 hr at room temperature, suspensions of the microorganisms were prepared. One loop (1/8 in.) of spores of sporeformers was removed from the cultures and placed in 5 ml sterile 0.5% saline. With nonsporeformers, one loop of vegetative cells was suspended in 5 ml sterile 0.5% saline; the suspension served as the inoculum for the estimation of activity against microbial growth.

Agar plates were inoculated by placing three drops of the suspension on the agar. Microorganisms were spread over the surface of the plates with sterile glass rods. Paper discs (6.5 mm diameter) made from Whatman No. 1 filter paper were used in the evaluation of the liquid compounds, and stainless steel cylinders (5 mm ID) were used for the

TABLE I
Antimicrobial Activity of Some Diesters of Aliphatic Diols

Sample number	C ₆ H ₅ COOR ₁ OR ₂		Antimicrobial activity ^a					
	R ₁	R ₂	Microorganism ^b					
			A	B	C	D	E	F
1	-CH ₂ CH ₂ -	Lauroyl	++	+	++	-	++	-
2		Palmitoyl	oo	+	oo	-	+	-
3		Oleoyl	++	+	++	-	++	-
4		Erucoyl	o	oo	oo	-	oo	-
5		12-Acetoxyleoyl	o	oo	oo	-	oo	-
6	-CH ₂ CH ₂ OCH ₂ CH ₂ -	Lauroyl	oo	oo	oo	-	o	-
7		Palmitoyl	oo	oo	o	-	-	o
8		Oleoyl	o	oo	oo	-	oo	-
9		Erucoyl	+	oo	+	-	+	-
10		12-Acetoxyleoyl	oo	oo	oo	-	+	-
11		Trimethylacetyl	+	oo	-	+	-	oo
12		Furoyl	oo	oo	+	-	oo	-
13		p-Toluoyl	+	o	oo	-	oo	-
14		Benzoyl	o	oo	o	-	-	oo
15		-CH ₂ CH=CHCH ₂ -	Lauroyl	++	++	-	o	-
16	Palmitoyl		+	o	o	-	-	+
17	Oleoyl		++	o	+	-	-	++
18	Erucoyl		++	o	o	-	-	++
19	Trimethylacetyl		++	+	-	+	-	++
20	Hydrocinnamoyl		++	oo	-	o	-	+

^a ++ = Zone of inhibition was at least 0.5 cm beyond disc or cylinder area at 120 hr.

+ = Zone of inhibition was < 0.5 cm beyond disc or cylinder area at 120 hr.

oo = Organism failed to grow on disc or cylinder area at 120 hr.

o = Slight growth on the saturated disc or cylinder area at 120 hr.

- = Not tested.

^b A = *Staphylococcus aureus*, B = *Escherichia coli*, C = *Aspergillus species*, D = *Aspergillus flavus*, E = *Candida albicans*, F = *Torula species*.

solid compounds (samples 2, 7, and 16 of Table I). The paper discs, completely saturated with the liquid test compound, were placed on the surface of agar plates inoculated with test organisms. Solid compounds were placed in stainless steel cylinders in direct contact with the inoculated plates. No carrier solvent was employed. At least three experiments were made at different times, with duplicate plates for each compound tested. All plates were incubated at the optimal temperature for each organism, 37 C for *S. aureus* and *E. coli* and 30 C for the other organisms, and readings were taken after 24, 48, 72, and 120 hr.

RESULTS AND DISCUSSION

Nineteen mixed diesters and 2-benzoyloxyethoxyethyl benzoate were screened for activity against a gram-positive bacterium, *Staphylococcus aureus*; a gram-negative bacterium, *Escherichia coli*; a mold, either *Aspergillus flavus* or *A. species*; and a yeast, either *Candida albicans* or *Torula species*. The data reveal that all 20 compounds appreciably inhibited activity of two or more of the test organisms, and that eight of them were effective against all four types of organisms. In examining the data in Table I, it should be borne in mind that compounds rated oo (organism failed to grow on saturated disc or solid) are not necessarily inferior to those rated + (zone of inhibition was < 0.5 cm) or ++ (zone of inhibition was > 0.5 cm), as failure to inhibit growth of an organism beyond the point of actual application to the plate may result from inability to diffuse through the culture medium rather than from low antimicrobial activity.

In general, the mixed diesters of all three diols showed a broad spectrum of antimicrobial activity, most of them completely inhibiting growth of three or more of the test organisms, at least in the area of application of the compound. The effectiveness of the dibenzoate ester, Sample 14, which was somewhat lower than most of the mixed diesters, indicates that the benzoyl grouping which is common to all of the other compounds is not the primary moiety responsible for antimicrobial activity of these com-

pounds. Among the mixed diesters of ethylene glycol, those with the laurate, palmitate, and oleate functions were particularly effective against all of the organisms tested. Among the mixed diesters of diethylene glycol, those containing the erucate, 12-acetoxyoleate, trimethylacetate, and furoate moieties were effective inhibitors against the four organisms tested. Among the diesters of *cis*-2-butene-1,4-diol, only the one containing the trimethylacetate moiety was strongly inhibitory against all four of the test organisms, but the remaining five esters (laurate, palmitate, oleate, erucate, and hydrocinnamate) showed a high degree of inhibition against the gram-positive bacteria (*S. aureus*) and the yeast (*Torula species*).

In this study, the compounds were tested against a limited number of organisms. However, the effective inhibition of all of the organisms by several of the compounds, and of some of the organisms by all of the compounds, indicates the desirability of a more thorough investigation and suggests that some of these compounds may have potential utility as biostatic additives in commercial products. Some of the compounds have good plasticizer properties (8) and might serve the dual role of plasticizer and antimicrobial agent in some specialty applications.

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