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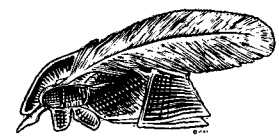

Antimicrobial Properties of Some Fatty Ester-Amides

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ABSTRACT

Twenty-seven fatty acid derivatives having both the amide and ester functions 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 effectively inhibited at least one of the organisms, and 19 of them were inhibitory against 3 or more. These 27 compounds were derivatives of diethanolamine or of N-methylethanolamine. The amide acyl moieties included the lauroyl, palmitoyl, oleoyl, erucoyl, benzoyl, and *p*-toluoyl groupings. The ester moieties included the lauroyl, palmitoyl, oleoyl, erucoyl, trimethylacetyl, benzoyl, *p*-toluoyl, and furoyl groupings. The activity exhibited by many of these materials suggests that they may have potential utility as biostatic additives in commercial products.

INTRODUCTION

Many N-substituted amides of long chain fatty acids have been shown to possess antimicrobial activity (1-5). Various fatty esters have also been shown to exhibit antimicrobial activity (6-8). The availability from other research (9) of a series of compounds possessing both ester and amide functions in the same molecule gave rise to investigations of the antimicrobial activity of a variety of fatty compounds that have these two functional groups.

EXPERIMENTAL PROCEDURES

Preparation of the ester-amides used in this study is reported elsewhere (9). The purpose of the simple screening technique described here was to obtain some general information on the likelihood of the compounds tested having antimicrobial properties if added to commercial products. A manufacturer interested in using any of these compounds should perform comprehensive investigations to ascertain the relative degree of inhibition that could be attained with

TABLE I

Antimicrobial Activity of Some Fatty Ester-Amides

Sample No.[a]	Amide Acyl	Ester Acyl, R_1	Antimicrobial activity[b] microorganisms[c] A	B	C	D	E	F
1	Benzoyl	Lauroyl	oo	oo	---	oo	---	oo
2	Benzoyl	Oleoyl	++	+	+	---	---	oo
3	Benzoyl	Erucoyl	oo	oo	o	---	---	oo
4	Lauroyl	Benzoyl	oo	+	o	---	---	oo
5	Lauroyl	Furoyl	+	o	o	---	---	oo
6	Lauroyl	Trimethylacetyl	o	+	---	o	---	oo
7	Oleoyl	Benzoyl	+	o	o	---	---	+
8	Oleoyl	Furoyl	o	oo	---	oo	---	o
9	Oleoyl	Trimethylacetyl	o	oo	---	oo	---	o
10	Benzoyl	Lauroyl	oo	oo	oo	---	o	---
11	Benzoyl	Palmitoyl	oo	oo	oo	---	oo	---
12	Benzoyl	Oleoyl	oo	oo	oo	---	oo	---
13	*p*-Toluoyl	Lauroyl	o	o	o	---	---	oo
14	*p*-Toluoyl	Oleoyl	o	+	oo	---	---	+
15	Lauroyl	Benzoyl	o	oo	o	---	---	o
16	Lauroyl	Furoyl	++	oo	++	---	---	++
17	Lauroyl	*p*-Toluoyl	+	oo	o	---	---	++
18	Lauroyl	Trimethylacetyl	++	o	---	+	---	++
19	Palmitoyl	Benzoyl	+	oo	oo	---	oo	---
20	Palmitoyl	Furoyl	o	oo	oo	---	oo	---
21	Oleoyl	Benzoyl	+	o	o	---	---	+
22	Oleoyl	Furoyl	o	o	oo	---	oo	---
23	Oleoyl	*p*-Toluoyl	+	oo	oo	---	o	---
24	Oleoyl	Trimethylacetyl	o	oo	---	oo	---	+
25	Erucoyl	Benzoyl	oo	oo	oo	---	o	---
26	Erucoyl	Furoyl	++	++	---	o	---	++
27	Erucoyl	*p*-Toluoyl	+	oo	---	oo	---	+

[a]Structure for Nos. 1-9, $RCON(CH_2CH_2OR_1)_2$; structure for No. 10-27, $RCON(CH_3)$-$CH_2CH_2OR_1$.

[b]++ = Zone of inhibition at least 0.5 cm beyond disc or cylinder area at 120 hr. + = Zone of inhibition less than 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 disc or cylinder area at 120 hr.

[c]A = *Staphylococcus aureus*; B = *Escherichia coli*; C = *Aspergillus* species; D = *Aspergillus flavus*; E = *Candida albicans*; F = *Torula* species.

any specific microorganisms under normal conditions of product use and in accordance with the chemical and physical properties of the product. Results reported in this paper are merely an indication that these compounds do possess antimicrobial properties and might be effective for many commercial applications.

Difco Bacto dehydrated nutrient agar at pH 6.8, Difco Bacto dehydrated yeast mycological agar at pH 4.5 and Difco dehydrated mycological agar at pH 7.0 were used to test inhibition of the bacteria, yeast and mold cultures, respectively. The microorganisms were from stock cultures: *Staphylococcus aureus,* ATCC 12692; *Escherichia coli,* ATCC 25922; *Aspergillus flavus,* ATCC 11495; *Aspergillus* sp.; *Candida albicans,* ATCC 753; and *Torula* sp. The *Aspergillus* sp. and the *Torula* sp. are organisms that are stock cultures of the Louisiana State University Food Science Department and were isolated from contaminated foods. After the cultures were incubated 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 solution. With nonspore formers, one loop of vegetative cells was suspended in 5 ml steril 0.5% saline solution; the suspension served as the inoculum for the estimation of activity against microbial growth.

Agar plates were inoculated by placing 3 drops of the suspension on the agar. Microorganisms were spread over the surface of the plates with steril glass rods. Paper discs (6.5 mm diam) made from Whatman No. 1 filter paper were used in the evaluation of the liquid compounds, and stainless steel cylinders (5 mmID) were used for the solid compounds (samples 11, 19-21, and 25 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. Readings were taken after 24, 48, 72, and 120 hr.

RESULTS AND DISCUSSION

The 27 ester-amides listed in Table I 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 27 compounds effectively inhibited at least one of the test organisms, and that 19 of them were effective against 3 or more. In Table I, compounds rated oo are not necessarily inferior to those rated + or ++ because failure to inhibit the growth of an organisms beyond the point of actual application to the plate may result from inability of the compound to diffuse through the culture medium rather than from low antimicrobial activity.

The ester-amides are derivatives of diethanolamine or N-methylethanolamine, and each contains one or more fatty acyl moieties in the molecules. Though there are individual differences in the antimicrobial activities of analogous compounds in the two series, neither type of compound appears to be superior to the other on an overall basis under the conditions of these tests. Both series have examples of compounds which are strongly inhibitory toward the four types of organisms against which they were screened. The benzamides, examples 1-3 and 10-12, were particularly effective against both types of bacteria. With only two exceptions, they were also effective against the molds and yeasts. There appeared to be some loss in effectiveness when the benzoyl moiety was at the ester function rather than the amide.

Other acyl amide moieties included the *p*-toluoyl, lauroyl, palmitoyl, oleoyl, and erucoyl groupings. All had examples with activity against at least three types of organisms. The three erucamides tested, samples 25-27, all displayed this wide range of antimicrobial activity. The three compounds were derived from N-methylethanolamine and had the benzoyl, furoyl, and *p*-toluoyl moieties in the ester grouping.

The tests carried out in this study were screening tests against a limited number of microorganisms; however, the activity displayed by many of the compounds indicates that a more thorough investigation is warranted. Some of these compounds may have potential utility as biostatic additives in commercial products. Some of them have good plasticizing properties (9) and could probably serve a dual role as plasticizer and antimicrobial agent in some specialty applications.

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