Antimicrobial Activity of Some Fatty Acid Derivatives

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ABSTRACT

Twelve fatty acid amide or ester derivatives 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. These compounds were adducts of unsaturated fatty derivatives in which the addends were hexachlorocyclopentadiene, thiolacetic acid, bromotrichloromethane, or O,O-diethylphosphorodithioic acid. All of the new compounds appreciably inhibited the activity of at least one of the test organisms, and most of them showed activity against all four types of organisms. The hexachlorocyclopentadiene adduct of 2-(2-ethoxyethoxy)ethyl oleate was especially potent in this regard.

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 this kind of activity (6-8). In the present work, a number of modified fatty amides or esters available from other research were screened for antimicrobial activity. A high level of activity was exhibited by several of the new compounds. These compounds were adducts of various addends to unsaturated amides or esters. The addends were hexachlorocyclopentadiene (HCCPD), thiolacetic acid, bromotrichloromethane, and O,O-diethylphosphorodithioic acid.

EXPERIMENTAL PROCEDURES

The materials were reagent grade and were purchased from commercial sources. The unsaturated esters and amides were prepared by conventional methods. Some of the adducts have been previously reported (9). Preparation of the others is described below. NMR spectra verified the structure of the addition products and confirmed the elimination of unsaturation by the absence of olefinic protons in the 5.5 ppm region.

Preparations

2,2'-Thiobis[ethyl 8-(1,4,5,6,7,7-hexa chloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octanoate]: The HCCPD adduct of oleic acid was prepared by heating a mixture of oleic acid (150 g, 0.53 mole) and HCCPD (295 g, 1.08 moles) under nitrogen in a flask equipped with a condenser for 30 hr at 136 C, and working up the reaction mixture as previously described (10).

The adduct (137 g, 0.25 mole), 2,2'-thiodiethanol (13.8 g, 0.11 mole), 50 ml of benzene, and 0.5 g of 2-naphthalenesulfonic acid were placed in a flask equipped with a reflux condenser and Dean-Stark trap. The temperature was raised to reflux and maintained there until water ceased to azeotrope. The reaction mixture was passed through an activated alumina column to remove unesterified adduct and stripped, giving an essentially quantitative yield of the product, which analyzed: S, 2.35 (theory, 2.67); Cl, 36.25 (theory, 35.60).

8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octyl 8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octanoate: This adduct of HCCPD and oleoyl oleate was prepared by the procedure described (10), using 30 g (0.056 mole) of the ester and 61.2 g (0.22 mole) of HCCPD. The product analyzed: Cl, 39.80 (theory, 39.45).

2,3-Bis[8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octanoyloxymethyl]-1,4,5,6,7,7-hexachlorobicyclo-[2.2.1]-5-heptene: This adduct of HCCPD and 1,4-bis(oleoyloxy)butene was prepared by the procedure described (10), using 24 g (0.05 mole) of the diester and 82 g (0.30 mole) of HCCPD. The product analyzed: Cl, 44.10 (theory, 44.46).

Ethyl 10(11)-bromo-11(10)-trichloromethylundecanoate: Samples of 5 g (0.02 mole) of ethyl undecenoate and 14 g (0.07 mole) of bromotrichloromethane were placed in a flask, mixed well, and exposed to a cobalt-60 (γ-radiation) source to initiate a free radical chain reaction. After irradiation for 19 hr, the mixture was removed and the excess bromotrichloromethane was eliminated by stripping at reduced pressure. The residue was dissolved in benzene, passed through a column of activated alumina, eluted with a mixture of 1:1 benzene-ethanol, and the solvent was removed by stripping at reduced pressure. The product, obtained in quantitative yield, had d_4^{30} 1.3130 and analyzed: Br, 19.75 (theory, 19.48).

Ethyl 9(10)-bromo-10(9)-trichloromethylstearate: This compound was prepared by the procedure described for the analogous undecanoate, using 5 g (0.02 mole) of ethyl oleate and 9.6 g (0.05 mole) of bromotrichloromethane. The product had d_4^{30} 1.1617 and analyzed: Br, 15.20 (theory, 15.71).

N-9(10)-Acetylthiostearoyl-N'-methylpiperazine: This compound was prepared by the procedure described in the literature (11), using 5 g (0.014 mole) of N-oleoyl-N'-methylpiperazine and 2.1 g (0.028 mole) of thiolacetic acid. The product analyzed: N, 6.45 (theory 6.36); S, 7.10 (theory, 7.27).

N-(11-Acetylthioundecanoyl)hexamethyleneimine: This compound was prepared by the procedure described in the literature (11), using 9 g (0.034 mole) of N-undecenoylhexamethyleneimine and 5.2 g (0.068 mole) of thiolacetic acid. The product had d_4^{30} 1.0001, n_D^{30} 1.4542, and analyzed: N, 4.25 (theory, 4.10); S, 9.10 (theory, 9.39).

N,N-Bis(3-acetylthiopropyl)-9(10)-acetylthiostearamide: This compound was prepared by the procedure described in the literature (11), using 10 g (0.028 mole) of N,N-diallyloleamide and 12.6 (0.17 mole) of thiolacetic acid. The product had d_4^{30} 1.0204, n_D^{30} 1.5018, and analyzed: N, 2.34 (theory, 2.37); S, 15.80 (theory, 16.30).

1,3-Bis[9(10)-O,O-diethylphosphorodithiostearoyl]imidazolidine: This compound was prepared by placing 15 g (0.025 mole) of 1,3-bis(oleoyl)imidazolidine, 18.6 g (0.10 mole) of 0,0-diethylphosphorodithioic acid, and 5 ml of benzene (to produce a homogenous solution) in a flask and irradiating it in a cobalt-60 source for 24 hr. The flask was then removed and the contents dissolved in benzene and neutralized with 5% KOH. The mixture was washed with water, dried over Na_2SO_4, filtered, and stripped of solvent at reduced pressure. The product was obtained in essentially quantitative yield and analyzed: N, 2.95 (theory, 2.88); P, 6.10 (theory, 6.36); S, 12.85 (theory, 13.17).

Antimicrobial Testing

The purpose of the simple screening technique described

TABLE I

Antimicrobial Activity of Some Fatty Acid Derivatives

Sample no.	Compound	Antimicrobial activity[a] Microorganisms[b] A	B	C	D	E	F
1	2,2'-Thiobis[ethyl 8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octanoate]	++	++	++	--	--	+
2	2-(2-Ethoxyethoxy)ethyl [8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octanoate]	+++	+++	+++	--	--	+++
3	8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)-8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octanoate	o	oo	o	--	--	o
4	2,3-Bis[8-(1,4,5,6,7,7-hexachloro-3-octylbicyclo-[2.2.1]-5-hepten-2-yl)octanoyloxymethyl]-1,4,5,6,7,7-hexachlorobicyclo-[2.2.1]-5-heptene	+	o	+	--	--	o
5	2,2'-Oxybis[ethyl 9(10)-bromo-10(9)-trichloromethylstearate]	+	oo	+	--	--	oo
6	Ethyl 9(10)-bromo-10(9)-trichloromethylstearate	++	oo	--	+	+	--
7	Ethyl 10(11)-bromo-11(10)-trichloromethylundecanoate	+	oo	--	oo	+	--
8	N-9(10)-Acetylthiostearoyl-N'-methylpiperazine	o	o	o	--	--	+
9	N-(11-Acetylthioundecanoyl)hexamethyleneimine	+	+	++	--	--	++
10	N,N-Bis(3-acetylthiopropyl)-9(10)-acetylthiostearamide	+	oo	--	++	--	++
11	1,3-Bis[9(10)-0,0-diethylphosphorodithiostearoyl] imidazolidine	oo	oo	oo	--	--	oo
12	3-(0,0-Diethylphosphorodithio)propyl 11-(0,0-diethylphosphorodithio)-undecanoate	+	oo	--	o	--	oo

[a]+++ = Completely inhibited growth of organisms on agar in petri dish. ++ = The zone of inhibition was at least 0.5 cm beyond disc area at 120 hr. + = The zone of inhibition was less than 0.5 cm beyond disc area at 120 hr. oo = Organism failed to grow on disc area at 120 hr. o = Slight growth on the disc area at 120 hr. -- = Not tested.

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

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 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 used 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 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 spore formers 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 sterile 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 sterile glass rods. Paper discs (6.5 mm diameter) made from Whatman No. 1 filter paper were used in the evaluation of the compounds. The paper discs, completely saturated with the liquid test compounds, were placed on the surface of agar plates inoculated with test organisms. 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

Twelve addition compounds of unsaturated fatty esters or amides 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 of the compounds appreciably inhibited activity of at least one of the test organisms, and that eight of them were effective against all four types of organisms. These eight compounds included all four types of addition compounds used in this study. In examining the data in Table I, it should be borne in mind that compounds rated oo (organism failed to grow on disc area) are not necessarily inferior to those rated + (zone of inhibition was less than 0.5 cm) or ++ (zone of inhibition was at least 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.

The potent activity of sample 2, the HCCPD adduct of 2-(2-ethoxyethoxy)ethyl oleate, is especially noteworthy. It completely inhibited the growth of all organisms against which it was tested. This compound is currently being further tested for possible medical, pharmacological, and industrial uses. Sample 1, the HCCPD adduct of 2,2'-thiobis-(ethyl oleate), also showed potent activity, while the remaining HCCPD adducts, samples 3 and 4, had only fair antimicrobial activity.

The three bromotrichloromethane addition products, samples 5-7, also showed activity against all four types of organisms, indicating that compounds of this type are worthy of further investigation for antimicrobial uses. Two of the thiolacetic acid addition products, samples 9 and 10, also had good activity, as has been shown for other similar fatty acid derivatives (11). The diethylphosphorodithioic acid addition products, samples 11 and 12, appear to have somewhat poorer activity than the other types of compounds tested, but did inhibit growth of most of the organisms in the actual area of contact of the saturated disc

with the inoculated agar.

These results indicate that compounds of the types studied may have potential utility as biostatic additives in commercial products.

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