Sulfur Derivatives of N,N-Disubstituted Amides of Long Chain Fatty Acids and their Antimicrobial Activities

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ABSTRACT

Mercaptoacetic acid was added to the internal double bond of N,N-disubstituted oleamides and the terminal double bond of 11-undecenoylmorpholine. Unreacted starting materials were removed by distillation and as urea complexes. The internal carboxyl group of N,N-disubstituted-9(10)-(carboxymethylthio)stearamides and the terminal carboxyl group of 11-(carboxymethylthio)undecanamide were esterified. Screening for broad range antimicrobial activity against *Candida albicans, Staphylococcus aureus, Escherichia coli,* and *Aspergillus species* indicated that all compounds tested were active. However, 9(10)-(carbomethoxymethylthio)stearoylmorpholine and 9(10)-carbethoxymethylthio)stearoyl-4-methylpiperidine were the most effective and strongly inhibited the growth of all organisms tested.

INTRODUCTION

The free radical addition of mercaptoacetic acid to esters of oleic and undecenoic acid have been reported (1). The addition of a mercaptan to an olefinic bond is a well known reaction and generally takes place by a free radical mechanism (2-6). Many N,N-disubstituted amides of long chain fatty acids have been shown to be antimycotic (7-11), as have been phosphonated fatty amides (12). We have continued our investigation of the antimycotic behavior of N,N-disubstituted amides, and now present the preparation and evidence of antimicrobial activity of some sulfur containing N,N-disubstituted amides.

EXPERIMENTAL PROCEDURES

All of the materials were reagent grade and purchased from commercial sources. The unsaturated amides were prepared as described previously (13-16).

9(10)-(Carboxymethylthio)stearoylmorpholine

N-oleoylmorpholine, 158 g (0.44 mole), and 124.5 g (1.35 mole) mercaptoacetic acid, were placed in a flask and stirred for 2 hours at 90 C. Excess mercaptoacetic acid then was removed by distillation at reduced pressure. Nuclear magnetic resonance (NMR) spectra of the residue showed unsaturation, so unreacted N-oleoylmorpholine was removed by the urea complex procedure of Swern (17). NMR spectra then showed no unsaturation.

9(10)-(Carbopropoxymethylthio)stearoylmorpholine

Ten grams (0.02 mole) of 9(10)-(carboxymethylthio)-stearoylmorpholine, 4.2 g (0.07 mole) propyl alcohol, 0.1 g 2-naphthalenesulfonic acid and benzene were placed in a flask equipped with reflux condenser and Dean-Stark trap. The mixture refluxed for 8 hours or until water ceased to be azeotroped. The mixture was cooled, dissolved in benzene, washed with water, dried over anhydrous sodium sulfate, filtered, and the filtrate passed through a column of activated alumina as described previously (13). The benzene fraction was discarded and the alcohol fraction retained. Solvent was removed by stripping at reduced pressure.

11-(Carboxymethylthio)undecanoylmorpholine

Five grams (0.02 mole) of 10-undecenoylmorpholine and 2.3 g (0.025 mole) mercaptoacetic acid were placed in a flask and stirred with a stirring bar for 2 hours at 60 C. Excess mercaptoacetic acid then was removed by distillation at reduced pressure.

11-(Carbethoxymethylthio)undecanoylmorpholine

Six grams (0.017 mole) of 11-(carboxymethylthio)-undecanoylmorpholine, 4.8 g (0.11 mole) anhydrous ethyl alcohol, and 0.1 g 2-naphthalene-sulfonic acid were placed in a flask, equipped with reflux condenser, and refluxed for 16 hours. The mixture was dissolved in benzene, washed with water, dried over anhydrous sodium sulfate, filtered, and the filtrate passed through a column of activated alumina as described previously (13). The product was recovered as described for 9(10)-(carbopropoxymethylthio)stearoylmorpholine. Remaining amides were prepared as described above.

Densities were determined pycnometrically in a thermostated bath at 30 ± 0.1 C. The refractive indices were determined at 30 C by using the D sodium line. The melting point was determined on a Fisher-Johns apparatus and was uncorrected.

Suspensions of the test organisms were prepared by transferring a loop of spores from stock cultures into sterile saline. Each hardened agar plate, made of Difco dehydrated mycological agar (Difco, Detroit, MI) at pH 7.0, was inoculated with 3 drops of the suspension spread over the surface with a sterile glass rod. These plates were used to estimate the antimicrobial activity of the compounds. Filter paper discs, 6.5 mm in diameter, made from Whatman No. 1 filter paper, were used to evaluate the liquid compounds, and a stainless steel cylinder, 5 mm internal diameter (ID), was used for the solid compound, Sample 14. The paper discs, wetted until completely saturated with test compound, and the stainless steel cylinder containing the test solid, were placed on the surface of the agar plates inoculated with the test organisms. To eliminate any errors that could result from an insufficient number of tests, a minimum of 3 experiments, at different times, employing duplicate plates were run for each compound. All plates were incubated at the optimum growing temperature for each organism, and the readings were taken after 24, 48, 72, and 120 hours.

RESULTS AND DISCUSSION

The densities and refractive indices of the various amides are reported in Table I, as is the melting point of the solid undecanoyl derivative. Elemental analyses in all cases agreed with calculated values within limits of experimental error. Yield of the products from the preparative steps were 80-90%.

The antimicrobial activity of these sulfur derivatives was screened against the following organisms: *Candida albicans, Staphylococcus aureus, Escherichia coli,* and *Aspergillus species.* The data showed that all of the sulfur containing ester-amides significantly inhibited at least 2 of the organisms. The various C_{18} morpholine derivatives, samples 1-6, showed a progressive decrease in antimicrobial activity as the chain length increased in the alkyl substituent of the ester group. Maximum activity was shown by the derivative containing the methyl substituent, 9(10)-carbomethoxymethylthio)stearoylmorpholine, with + or ++ for all 4 orga-

TABLE I

Physical Properties and Antimicrobial Activity of Sulfur-Containing Fatty Amides

Compound	Density 30 C	N^{30} D	Antimicrobial Activity[b] Microorganisms[c] A	B	C	D
9(10)-(Carbomethoxymethylthio)stearoylmorpholine	0.9979	1.4865	++	+	+	++
9(10)-(Carbethoxymethylthio)stearoylmorpholine	0.9996	1.4849	++	0	+	++
9(10)-(Carbopropoxymethylthio)stearoylmorpholine	0.9988	1.4838	00	00	+	++
9(10)-(Carbobutoxymethylthio)stearoylmorpholine	0.9901	1.4840	0	00	+	00
9(10)-(Carbopentoxymethylthio)stearoylmorpholine	0.9889	1.4831	00	0	00	00
9(10)-(Carbohexoxymethylthio)stearoylmorpholine	0.9725	1.4822	0	0	00	++
9(10)-(Carbo-3-methyl-1-butoxymethylthio)stearoylmorpholine	0.9866	1.4813	+	0	00	+
9(10)-(Carballyloxymethylthio)stearoylmorpholine	0.9941	1.4877	+	+	00	+
N,N-Bis(2-ethoxyethyl)-9(10)-(carbethoxymethylthio)stearamide	0.9780	1.4717	++	0	00	0
N,N-Bis(2-ethoxyethyl)-9(10)-carbo-3-methyl-1-butoxymethylthio)stearamide	0.9575	1.4698	0	0	00	0
N,N-Dibutyl-9(10)-(carbo-3-methyl-1-butoxymethylthio)stearamide	0.9439	1.4687	00	00	0	+
N-methyl-N-butyl-9(10)-(carbo-3-methyl-1-butoxymethylthio)stearamide	0.9512	1.4736	00	00	00	0
9(10)-(Carbethoxymethylthio)stearoyl-4-methylpiperdine	0.9496	1.4807	++	+	+	++
11-(Carbethoxymethylthio)undecanoylmorpholine[a]			0	00	00	00

[a]mp = 42.5-43.5 c.

[b]++ = The zone of inhibition was at least 0.5 cm beyond disc at 120 hr; + = The zone of inhibition was less than 0.5 cm beyond disc at 120 hr; 00 = Organism failed to grow on disc at 120 hr; 0 = Slight growth on the saturated disc at 120 hr.

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

nisms tested. Branching or unsaturation in the alkyl substituent of the ester group, samples 7 and 8, did not significantly enhance the antimicrobial activity of these morpholine derivatives.

Comparison of Sample 2 with Sample 14 indicated that products obtained by derivatization of the acid group of terminal C_{11} fatty acid amides were not more active than those obtained by the derivatization of the internal unsaturated bond of C_{18} fatty acid amides.

Samples 9-12 showed that the change in amine moiety from morpholine to N,N-bis(2-ethoxyethyl)amine, N,N-dibutylamine, or N-methyl-N-butylamine, adversely affected antimicrobial behavior. However, a 4-methylpiperidine derivative (sample 13) showed antimicrobial activity comparable with or better than the most effective analogous morpholine derivative. This 4-methylpiperidine derivative was highly inhibitory, + or ++, to all 4 organisms tested.

The broad range of antimicrobial activity of 9(10)-(carbomethoxymethylthio)stearoyl morpholine and 9(10)-(carbethoxymethylthio)stearoyl-4-methylpiperidine suggest their potential use in biostatic products.

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