Free Radical Addition of Thiolacetic Acid to Esters and Amides of Unsaturated Acids and Screening of the Products for Antimicrobial Activity

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ABSTRACT

Free radical addition of thiolacetic acid to terminal and internal double bonds of mono-and di-unsaturated amides, as well as to unsaturated esters, was achieved by irradiation with Cobalt-60. Screening for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Aspergillus* species, *Torula* species, and *Candida albicans* indicated that terminal addition products may be more active than internal addition products.

INTRODUCTION

The addition of thiolacetic acid to a variety of unsaturated compounds, aldehydes, ketones, and alcohols in the presence of peroxides has been previously reported (1). Thiolacetic acid adds to the terminal rather than to the 10-position of 10-undecenoic acid (2). Free radical additions of dialkyl phosphites to unsaturated amides initiated by irradiation with Cobalt-60 has been reported from this laboratory (3). As a continuation of this work, thiolacetic acid was added to the terminal and nonterminal double bonds of N,N-disubstituted fatty amides and esters. Since many N,N-disubstituted fatty amides have been shown to be antimycotic (4-6), these new sulfur compounds were screened for antimicrobial activity.

EXPERIMENTAL PROCEDURES

Procedures for the preparation of the unsaturated esters and amides are described elsewhere (7-10).

Materials

All of the materials were of reagent grade, purchased from commercial sources.

Irradiations

Irradiations were carried out using the SRRC Cobalt-60 source (11). The samples were irradiated at dose levels of 1.03×10^6 R/hr. The calibrations were based on ferrous-ferric dosimetry in 0.5N H_2SO_4 (12).

Methods

The disubstituted 11-acetylthioundecanamides, and N-monosubstituted or N,N-disubstituted-9(10)-acetylthio-octadecanamides, were prepared from 1 mol of the amide and 3 mol of thiolacetic acid. The materials were mixed well in a flask and exposed in the SRRC Cobalt-60 (γ-radiation) source to initate free radical chain reaction. After irradiating for 18-24 hr, the mixtures were removed from the irradiating source, dissolved in benzene, neutralized with 5% sodium carbonate, washed with water, dried over anhydrous sodium sulfate, filtered, and stripped. Isolated yields were 90% or better.

NMR spectra verified the addition products, revealing the absence of olefinic protons in the 5.5 ppm region and the appearance of a sharp singlet at 2.3 ppm (three protons from the acetyl group), overlapping the triplet at 2.0-2.4 ppm (two hydrogens from the methylene alpha to the carbonyl), total integration, 5 protons. A signal at 3.6 ppm was also observed for the protons adjacent to the nitrogen atom.

Densities were determined pycnometrically in a thermostated bath at 30 ± 0.1 C. Refractive indices were determined at 30 C with a precision Bausch and Lomb refractometer, using D sodium line. Melting points were determined on a Fisher-Johns apparatus.

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. Difco Dehydrated Mycological Agar at pH 7.0 was used to test the inhibition of the organisms selected by the compounds being screened. Suspensions of the organisms were prepared by transferring a loop (1/8 in.) of spores into 5 ml sterile 0.5% saline. Hardened agar plates were inoculated by placing three drops of the suspension on the agar. Sterile glass rods were used to spread the microorganisms over the surface of the plates. These plates were employed in estimation of activity against microbial growth. Filter paper discs, 6.5 mm diameter, made from Whatman No. 1 filter paper, were used to evaluate the liquid compounds, and 5 mm ID stainless steel cylinders were used for the solid compounds (samples 8, 9, and 16). The paper discs, completely saturated with the liquid compound being tested, were placed on the surfaces of the agar inoculated with the test organisms. To eliminate any errors which could result from an insufficient number of tests, at least three experiments were made at different times with duplicate plates for each compound tested. All plates were incubated at the optimum growing temperature for each organism (*S. aureus* and *E. coli* were incubated at 37 C, the other organisms were incubated at 30 C), and readings were taken after 24, 48, 72, and 120 hr periods.

RESULTS AND DISCUSSION

Thiolacetic acid has been known to add to a variety of unsaturated compounds in the presence of peroxides via a free radical mechanism. Because free radical addition of dibutyl phosphites to unsaturated amides by the use of a Cobalt-60 source was successful, we used Cobalt-60 to initiate free radical addition of thiolacetic acid to unsaturated esters and amides. The densities, refractive indices, and elemental analyses of the various sulfur-containing esters and amides are reported in Table I along with the melting point ranges of the solid derivatives.

Antimicrobial activity of these sulfur derivatives was screened against the following organisms: *Staphylococcus aureus, Escherichia coli, Aspergillus* species, *Torula* species, and *Candida albicans.* With the exception of samples 1 and 3, all of the sulfur derivatives substantially inhibited at least one of these organisms. In Table II, compounds rated xx (organisms that failed to grow on saturated disc) are not necessarily inferior to those rated + (zone of inhibition

TABLE I

Elemental Analyses and Properties of S-Containing Amides and Esters

	Density 30 C	N_D^{30}	mp(C)[a]	% C		% H		% N		% S	
				Exp.	Theory	Exp.	Theory	Exp.	Theory	Exp.	Theory
N,N-Dibutyl-9(10)-acetylthiostearamide	0.9314	1.4769		71.16	71.58	11.85	11.80	3.10	2.98	7.07	6.81
N-Methyl-N-butyl-9(10)-acetylthiostearamide	0.9331	1.4776		70.25	70.22	11.60	11.55	3.26	3.28	7.67	7.48
N,N-Bis(2-ethoxyethyl)-9(10)-acetylthiostearamide	0.9476	1.4690		67.58	67.04	11.36	11.05	2.76	2.79	6.46	6.38
N-9(10)-Acetylthiostearoylmorpholine	0.9893	1.4898		67.34	67.39	10.44	10.36	3.23	3.27	7.68	7.48
N-9(10),12(13)-Diacetylthiostearoylmorpholine	1.0435	1.5043		62.45	62.22	9.39	9.24	2.81	2.79	12.81	12.75
N-(3-Acetylthiopropyl)-9(10)-acetylthiostearamide	0.9957	1.4944		63.37	63.37	10.03	9.79	2.84	2.95	13.62	13.51
N,N-Dibutyl-11-acetylthioundecanamide	0.9476	1.4790		67.84	67.91	11.12	11.13	3.83	3.77	8.66	8.62
N-11-Acetylthioundecanoylmorpholine			37-38	61.84	61.96	9.38	9.48	4.14	4.25	9.77	9.71
N-11-Acetylthioundecanoylpiperidine			27-29	66.02	66.03	10.10	10.16	4.27	4.28	9.84	9.77
N-11-Acetylthioundecanoyl-2,6-dimethylmorpholine	1.0237	1.4925		63.83	63.86	9.74	9.87	3.89	3.92	9.17	8.96
(2-Ethoxyethoxy)ethyl 9(10)-acetylthiostearate	0.9698	1.4662		66.08	65.77	10.66	10.62			6.91	6.74
1,4-Bis[9(10)-Acetylthiostearoyloxy]-2-acetylthiobutane	0.9976	1.4849		65.75	65.35	10.16	9.78			11.02	11.35
3-(Acetylthiopropyl)9(10)-acetylthiostearate	0.9998	1.4849		63.28	63.05	9.61	9.77			13.59	13.72
2,2′-Oxybis [ethyl 9(10)-acetylthiostearate]	0.9830	1.4784		66.95	67.12	10.28	10.24			8.31	8.13
2,2′-Thiobis[ethyl 9(10)-acetylthiostearate]	0.9899	1.4865		65.33	64.97	9.97	9.91			11.55	11.80
2,3-(Diacetylthio)propyl 11-acetylthiostearate			38.5-39.5	53.79	53.30	7.61	7.60			21.11	21.30

[a]Uncorrected

TABLE II

Antimicrobial Activity of Some S-Containing Amides and Esters

Sample number	Compound	Antimicrobial activity[a] Microorganisms[b] A	B	C	D	E
1	N,N-Dibutyl-9(10)-acetylthiostearamide	x	x	x	x	
2	N-Methyl-N-butyl-9(10)-acetylthiostearamide	+	x	+	x	
3	N,N-Bis(2-ethoxyethyl)-9(10)-acetylthiostearamide	x	x	x	x	
4	N-9(10)-Acetylthiostearoylmorpholine	+	xx	+	++	
5	N-9(10),12(13)-Diacetylthiostearoylmorpholine	+	xx	++	++	
6	N-(3-Acetylthiopropyl)-9(10)-acetylthiostearamide	+	+	++	+	
7	N,N-Dibutyl-11-acetylthioundecanamide	+	x	++	+	
8	N-11-Acetylthioundecanoylmorpholine	++	+	+	++	
9	N-11-Acetylthioundecanoylpiperidine	++	++	+	++	
10	N-11-Acetylthioundecanoyl-2,6-dimethylmorpholine	+	+	+	++	
11	(2-Ethoxyethoxy)ethyl 9(10)-acetylthiostearate	+	x	x	++	
13	1,4-Bis[9(10)-Acetylthiostearoyloxy]-2-acetylthiobutane	x	x	x	++	
13	3-(Acetylthiopropyl) 9(10)-acetylthiostearate	x	+	+		+
14	2,2'-Oxybis[ethyl 9(10)-acetylthiostearate]	+	xx	++	++	
15	2,2'-Thiobis[ethyl 9(10)-acetylthiostearate]	+	xx	++	++	
16	2,3-(Diacetylthio)propyl 11-acetylthiostearate	+	xx	++	++	

[a] ++ = The zone of inhibition was at least 0.5 cm beyond disc or cylinder area at 120 hr.
+ = The zone of inhibition was less than 0.5 cm beyond disc or cylinder area at 120 hr.
xx = Organism failed to grow on disc or cylinder area at 120 hr.
x = Slight growth on the saturated disc or cylinder area at 120 hr.

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

<0.5 cm) or ++ (zone of inhibition at least 0.5 cm); failure to inhibit growth of an organism beyond the area of the treated filter paper disc may be due to inability to diffuse through the culture medium, rather than low antimicrobial activity.

Maximum activity for the C_{18} fatty acid derivatives obtained where thiolacetic acid was added to the internal double bond of unsaturated amides (samples 1-5) was shown for N-9(10)-acetylthiostearoylmorpholine (sample 4) and N-9(10),12(13)-diacetylthiostearoylmorphine (sample 5), both of which strongly inhibited the growth of three of the four organisms tested. Sample 5 also indicates that activity was not appreciably enhanced by the addition of 2 mol of thiolacetic acid to the internal double bonds of N-linoleoylmorpholine. However, N-(3-acetylthiopropyl)-9(10)-acetylthiostearamide (sample 6) indicates that activity was increased by the addition of 1 mol of thiolacetic acid to the internal double bond and 1 mol to the external double bond of N-allyloleamide. This compound inhibited the growth of all four test organisms, suggesting that it may be a broad spectrum antimicrobicide.

The addition of thiolacetic acid to the external double bond of N,N-disubstituted-10-undecenamides (samples 7-10) shows that all had broad antimicrobial spectra and inhibited the growth of all four test organisms, except N,N-dibutyl-11-acetylthioundecanamide (sample 7), which inhibited the growth of three of test organisms. It appears, therefore, that terminal addition products may be more active than internal addition products.

Most of the six sulfur-containing esters (samples 11-16) that were screened for antimicrobial activity showed considerable activity against three of the test organisms. The esters, however, unlike the amides, do not appear to have a broad antimicrobial spectrum.

Several of the N,N-disubstituted acetylthioacylamides have broad antimicrobial spectra, suggesting that they might have potential utility in biostatic products.

REFERENCES

1. Brown, R., W.E. Jones, and A.R. Pinder, J. Chem. Soc. 2123 (1951).
2. Koenig, N.H., and D. Swern, J. Am. Chem. Soc. 79:4235 (1957).
3. Mod, R.R., J.A. Harris, J.C. Arthur, Jr., F.C. Magne, G. Sumrell, and A.F. Novak, JAOCS 49:634 (1972).
4. Novak, A.F., J.M. Solar, R.R. Mod, F.C. Magne, and E.L. Skau, Ibid. 46:249 (1969).
5. Novak, A.F., J.M. Solar, R.R. Mod, F.C. Magne, and E.L. Skau, Appl. Microbiol. 18:1050 (1969).
6. Mod, R.R., F.C. Magne, G. Sumrell, and A.F. Novak, JAOCS 52:455 (1975).
7. Magne, F.C., R.R. Mod, and E.L. Skau, Ind. Eng. Chem. 50:617 (1958).
8. Mod, R.R., F.C. Magne, and E.L. Skau, JAOCS 41:237 (1964).
9. Mod, R.R., F.C. Magne, and E.L. Skau, Ibid. 42:941 (1965).
10. Magne, F.C., R.R. Mod, G. Sumrell, R.E. Koos, and W.E. Parker, Ibid. 52:494 (1975).
11. Arthur, J.C., Jr., F.A. Blouin, and R.J. Demint, "The SRRL Cobalt-60 Facility," USDA, ARS 72-21, August 1960.
12. Schuler, R.H., and A.O. Allen, J. Chem. Phys. 24:56 (1956).

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