Free Radical Addition of Dialkyl Phosphites to N,N-Disubstituted Amides of Unsaturated Fatty Acids and Screening of the Products for Antimicrobial Activity[1]

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ABSTRACT

Free radical addition of dibutyl phosphites to terminal and internal double bonds of monounsaturated amides was attained in high yield. The reaction was initiated by irradiation using Cobalt-60. Attempts to add diphenyl phosphite to the unsaturated amides failed with the amides being recovered unchanged. Similar attempts to add dialkyl phosphites to N-linoleoylmorpholine resulted in products that were deficient in phosphorus. Screening for antimicrobial activity against *Escherichia coli, Trichosporon capitatum, Trichoderma viride* and *Candida lipolytica* indicated that terminal addition products may be more active than the internal addition products, with the former strongly inhibiting the growth of all four test organisms.

INTRODUCTION

The free radical addition of dialkyl phosphites to terminal and nonterminal olefins in the presence of peroxides or UV radiation has been previously reported (1,2). It has been shown that the free radical-catalyzed addition of dialkyl phosphites to unsaturated fatty acid esters is a general reaction and that fair to good yields are readily obtained in the presence of peroxides or UV radiation (3). Dialkyl 11-phosphonoundecanoates and P,P-dialkyl 9(10)-phosphonostearates have been shown to be good primary plasticizers for poly(vinyl chloride) resins (4). Many N,N-disubstituted amides of long chain fatty acids have been shown to possess antimycotic activity (5,6). This publication deals with the free radical addition of dialkyl phosphites to terminal and nonterminal double bonds of N,N-disubstituted amides, and subsequent evaluation of these derivatives for antimicrobial activity. The free radical additions of the dialkyl phosphites to the unsaturated amides were initiated by irradiation using Cobalt-60.

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EXPERIMENTAL PROCEDURES

Materials

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

The procedures for the preparation of the unsaturated amides are described elsewhere (7-9).

Irradiations

Irradiations were carried out using the SRRL Cobalt-60 source (10). 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 (11).

Methods

The 11-dibutylphosphonoundecanoylmorpholine or N,N-disubstituted-9(10)-dialkylphosphonooctadecanamides were prepared from 1 mole 10-undecenoylmorpholine or N,N-disubstituted oleamide and 3 moles dibutyl phosphite. These materials were placed in a flask, mixed well, and exposed in the SRRL Cobalt-60 (γ-radiation) source to initiate free radical chain reaction. After irradiating for 18-24 hr, the mixtures were removed from the irradiation source, dissolved in Skelly B, passed through a column of activated alumina and stripped. IR spectra confirmed the preparation of the phosphonoamides and the absence of dibutyl phosphite. The IR spectrum of N,N-dibutyl-9(10)-dibutylphosphonoctadecanamide prepared by irradiation was identical with that prepared from the same two reactants using peroxide to initiate the reaction.

Densities were determined pycnometrically in a thermostated bath at 30 ± 0.1 C. The refractive indices were determined at 30 C with a precision Bausch and Lomb

TABLE I

Physical Properties and Antimicrobial Activity of Phosphonated Fatty Amides

Compound	Density, 30 C	N^{30} D	Antimicrobial activity[a] microorganism[b] A	B	C	D
N,N-Dibutyl-9(10)-dibutylphosphonooctadecanamide	0.9223	1.4534	xx	—	—	—
N,N-Bis (2-ethoxyethyl)-9(10)-dibutylphosphonooctadecanamide	0.9501	1.4538	xx	x	xx	x
N-(9[10]-Dibutylphosphonooctadecanoyl)-2,6-dimethylmorpholine	0.9661	1.4618	+	x	x	x
N-Ethyl-N-(3-ethoxypropyl)-9(10)-dibutylphosphonooctadecanamide	0.9411	1.4541	x	x	x	x
N-(9[10]-Dibutylphosphonooctadecanoyl)piperidine	0.9385	1.4667	+	x	x	xx
N-(9[10]-Dibutylphosphonooctadecanoyl)N'-methylpiperazine	0.9514	1.4605	xx	xx	x	xx
N,N-Dimethyl-9(10)-dibutylphosphonooctadecanamide	0.9495	1.4559	x	xx	xx	x
N-(9[10]-Dimethylphosphonooctadecanoyl)-4-methylpiperidine	0.9482	1.4643	xx	xx	x	x
N-Methyl-N-butyl-9(10)-dibutylphosphonooctadecanamide	0.9150	1.4557	xx	xx	xx	x
N-(9[10]-Dibutylphosphonooctadecanoyl)morpholine	0.9740	1.4611	x	x	x	x
N-(11-Dibutylphosphonoundecanoyl)morpholine	1.0233	1.4728	++	++	++	++

[a]++ = 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. xx = Organism failed to grow on disc at 120 hr. x = Slight growth on the saturated disc at 120 hr. - = No inhibition detectable.

[b]A = *Escherichia coli*; B = *Trichosporon capitatum*; C = *Trichederma viride*; D = *Candida lipolytica*.

refractometer using the D sodium line. NMR spectra were determined in deuterochloroform solution with a Varian-A-60-A spectrometer, using tetramethylsilane as an internal reference. The microorganisms used were obtained from stock cultures. Difco Dehydrated Mycological Agar at pH 7.0 was used to test the inhibition of the test organisms by the compounds being screened. Suspensions of the test organisms were prepared by transferring a loop of spores into sterile saline. Hardened agar plates were inoculated by placing 3 drops of the suspension onto the agar. The microorganisms were spread over the surface of the plates with sterile glass rods. These plates were employed in the activity estimation against microbial growth. Filter paper discs 6.5 mm in diameter, made from Whatman no. 1 filter paper were used to evaluate the compounds. The paper discs, wetted until they were completely saturated with the test compound, 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 three experiments, at different times, employing duplicates plates were made for each compound under test. All plates were incubated at the optimum growing temperature for each organism and the readings were taken after 24, 48, 72 and 120 hr periods.

RESULTS AND DISCUSSION

The availability of Cobalt-60 as a source of radiation energy in our laboratory presented a unique opportunity to investigate the feasibility of employing this energy as an initiator in free radical reactions. Although costs are high, the radiation energy furnishes a high potential energy and therefore must be used for new applications in order to produce new chemicals that cannot be realized in any other way.

Since dialkyl phosphites have been known to add to terminal and nonterminal olefins in the presence of decomposing peroxides or UV radiation, they were chosen for this study. Dibutyl phosphite added readily to the terminal double bond of 10-undecenoylmorpholine and to the internal double bonds of substituted amides of oleic acid (3:1 ratio of reactants) using Cobalt-60 as the initiator at room temperature to give 90% yield of the 1:1 adduct after 18-20 hr of radiation at 1.03×10^6 R/hr. IR spectra established the presence of the phosphonates and the absence of the phosphites by showing absorption bands at 8.05 microns, P→O stretching 9.4 microns, (P)-O-C (aliphatic) out-of-phase stretching vibration; 9.8 microns, (P)-O-C (aliphatic) in-phase stretching vibrations; 10.2 microns, P-O (pentavalent phosphorus) vibration; and absence of an absorption band at 4.1 microns, P-H vibration for the phosphite group. NMR spectra showed no significant impurities, revealing the absence of olefinic protons in the 5.5 ppm region, and the presence of a quartet at 3.8-4.2 ppm (four hydrogens from two methylene groups adjacent to the two oxygen atoms); a triplet at 3.0-3.5 ppm (four hydrogens from two methylene groups adjacent to the nitrogen atom); and a triplet at 2.0-2.4 ppm (two hydrogens from the methylene group alpha to the amide carbonyl). The physical properties of the various phosphorus derivatives are shown in Table I. Elemental analysis in all cases agreed with calculated values within limits of experimental error.

Attempts to add diphenyl phosphite to the internal double bond of N,N-dibutyloleamide and the external double bond of 10-undecenoylmorpholine failed, and both unsaturated amides were recovered unchanged. This may possibly be due to steric effects.

Likewise, attempts to add 2 moles dibutyl phosphite or dimethyl phosphite to the internal double bonds of N-linoleoylmorpholine resulted in products which were deficient in phosphorus. The phosphorus analysis revealed that not even one mole of dialkyl phosphite added to either double bond of the unsaturated amide. A NMR spectrum revealed that the olefinic protons were still present in the product.

The antimicrobial activity of these phosphorus derivatives was screened against the following organisms: *Escherichia coli, Trichosporon capitatum, Trichoderma viride* and *Candida lipolytica*. The data reveal that, with the exception of the N-ethyl-N-(3-ethoxypropyl) octadecanamide and N-octadecanoylmorpholine derivatives all of the phosphonated amides displayed significant inhibition against at least one of these organisms. In examining the data in Table I it should be borne in mind that compounds rated xx (organisms that failed to grow on saturated disc) are not necessarily inferior to those rated + (zone of inhibition less than 0.5 cm) or ++ (zone of inhibition greater than 0.5 cm), as failure to inhibit the growth of an organism beyond the area of the treated filter paper disc may result from inability to diffuse through the culture medium rather than from low antimicrobial activity.

Although the data are scant, it appears that the derivatives obtained by the addition of the dibutyl phosphite to the terminal bond of unsaturated amides may be more active than derivatives involving an internal double bond. This is illustrated by the fact that 9,10-dibutylphosphonooctadecanoylmorpholine shows only slight activity against the organisms tested, whereas 11-dibutylphosphonoundecanoylmorpholine strongly inhibited the growth of all four tests organisms. The broad antimicrobial activity displayed by the latter compound indicates that a more thorough investigation is warranted and suggests that compounds of this type may have potential utility as biostatic additives in commercial products.

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G.J. Boudreaux did NMR spectra and interpretations.

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