Preparation of Pyrazolines from Methyl 4-oxooctadec-2(*E*)-enoate and Their Antimicrobial Activity

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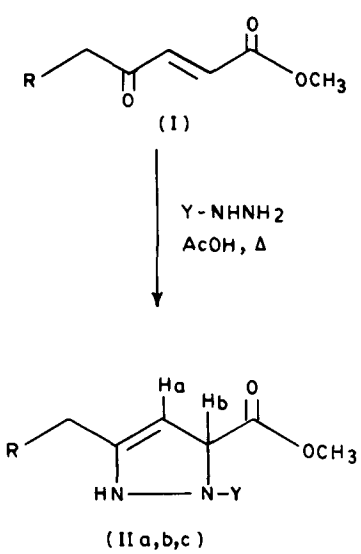
Reactions of methyl 4-oxooctadec-2(*E*)-enoate (I) with hydrazine, phenyl hydrazine and tolylhydrazine provided pyrazoline derivatives (IIa-c) in excellent yields. The structures of pyrazolines (IIa-c) were established by IR, NMR and mass spectral analysis. These pyrazoline derivatives showed antifungal and antibacterial activities.

The chemistry of pyrazolines has attracted attention because of their antibacterial, insecticidal and acaricidal properties (1,2). Pyrazolines can be conveniently prepared by reaction of α,β-unsaturated carbonyl compounds with hydrazine or substituted hydrazines (3). The objectives of this investigation were to synthesize fatty pyrazolines from methyl 4-oxooctadec-2(*E*)-enoate. Furthermore, the pyrazolines (IIa-c) were screened for their antibacterial and antifungal potential.

EXPERIMENTAL PROCEDURES

Infrared spectra were recorded as thin films or as nujol mulls on a Pye Unicam SP-3-100 spectrophotometer and calibrated against polystyrene. NMR spectra were recorded at 60 MHz using a Varian A-60 spectrometer. Chemical shifts were observed in ppm with tetramethylsilane as the internal standard. The abbreviations, "s, t, m and br," denote "singlet, triplet, multiplet and broad," respectively. Mass spectra were recorded using a JEOL-JMS D300 instrument. In the MS study, only the structures justifying fragments have been included. Thin layer chromatography (TLC) was carried out by standard procedures using petroleum ether (40-60°C) and diethyl ether as the developing solvent.



R = $CH_3(CH_2)_{12}$; Y = H (IIa), C_6H_5 (IIb), $CH_3C_6H_4$ (IIc).

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Methyl 4-oxooctadec-2(*E*)-enoate (I) was prepared by allylic oxidation of methyl octadec-2(*E*)-enoate as reported earlier (4).

General procedure for preparation of fatty pyrazolines. A solution of I (1 g, 3.2 m mol), hydrazine and acetic acid (2 ml) in benzene (20 ml) was refluxed at 70°C under nitrogen atmosphere. Benzene was removed under reduced pressure. The reaction mixture was diluted with diethyl ether and washed successively with 5% aq. soln. of sodium bicarbonate and water. It was dried over anhydrous sodium sulfate. Evaporation of the solvent followed by preparative TLC on silica gel (petroleum ether/diethyl ether/acetic acid, 50/50/1, v/v/v) of the residue gave II. Compounds prepared by this method follow.

3-Tetradecyl-5-(methoxycarbonyl)-3-pyrazoline (IIa). Reaction of I (1 g, 3.2 mmol) with hydrazine (0.6 g, 18.7 mmol) in benzene (20 ml) and acetic acid (2 ml) at 70°C for 4 hr furnished IIa as a light brown viscous oil (1 g) in 95.7% yield. Found: C, 70.30; H, 11.20; N, 8.58. $C_{19}H_{36}N_2O_2$ requires: C, 70.32; H, 11.18; N, 8.63%. Spectral data are given in the discussion section.

3-Tetradecyl-1-phenyl-5-(methoxycarbonyl)-3-pyrazoline (IIb). Reaction of I (1 g, 3.2 mmol) with phenyl hydrazine (2 g, 18.5 mmol) in benzene (20 ml) and acetic acid (2 ml) at 70°C for 6 hr afforded IIb as a viscous oil (1.1 g) in 85.3% yield. Found: C, 74.91; H, 10.10; N, 6.93. $C_{25}H_{40}N_2O_2$ requires: C, 74.95; H, 10.06; N, 6.99%.

3-Tetradecyl-1-tolyl-5-(methoxycarbonyl)-3-pyrazoline (IIc). Reaction of I (1 g, 3.2 mmol) with tolylhydrazine (2.25 g, 18.4 mmol) in benzene (20 ml) and acetic acid (2 ml) at 70°C for 5 hr gave a viscous oil (1.2 g) in 90.2% yield. Found: C, 75.38; H, 10.25; N, 6.68. $C_{26}H_{42}N_2O_2$ requires: C, 75.31; H, 10.21; N, 6.76%.

Microbial activity. One percent stock solutions of the compounds (IIa-c, each) were prepared in acetone. From the stock solutions, concentrations of 800, 600, 400 and 200 ppm were prepared in distilled water for testing antimicrobial activity *in vitro* against test fungi and bacteria. Pure cultures of the microbes were maintained in culture tubes on potato dextrose sugar (for fungi) and tryptone broth (for bacteria). Ten ml of nutrient medium were poured into petri dishes. In one set of petri dishes 2 ml solution of each compound was incorporated separately. In another set, 2 ml solution of acetone in distilled water (800, 600, 400, 200 ppm) was added. Then each set was inoculated with test microorganisms by aseptic transfer from the culture tubes to the surface of the nutrient medium. The samples were incubated at 28-30°C for one week in case of fungi and at 37°C for 48 hr for bacteria. Per cent control was calculated. Ten replicates were taken for each microorganism and each compound.

RESULTS AND DISCUSSION

Reaction of methyl 4-oxooctadec-2(*E*)-enoate (I) with hydrazine in the presence of acetic acid led to the formation

of 3-tetradecyl-5-(methoxycarbonyl)-3-pyrazoline (IIa). Its IR spectrum showed bands at 3270 (NH), 1725 (ester C=O) and 1620 cm^{-1} (C=C). NMR spectrum showed signals at δ 6.52 d (Ha), 3.74 s ($CO_2C\underline{H}_3$), 3.67 m (Hb, partially merged with the signal of ester protons), 2.3 m ($C\underline{H}_2$ α to the ring), 2.08 m and 1.88 m (2 × N<u>H</u>, D_2O exchangeable), 1.25 brs (12 × $C\underline{H}_2$) and 0.88 t (terminal $C\underline{H}_3$). The two clearly separated signals at δ 2.08 and 1.88 of the hydrazo moiety (-HN-NH-) of the structure IIa were due to the nonequivalent nature of these protons. The mass spectrum of compound (IIa) did not show the molecular ion peak at m/z 324 ($C_{19}H_{36}N_2O_2$). Other ions at m/z 323 (M-H, 7.9) and 322 (M-2H, 17) were observed. Structure-justifying peaks with their intensities are given in Figure 1.

Compound (I) was reacted with phenyl hydrazine in the presence of acetic acid. Final work up of the reaction mixture and preparative TLC purification afforded (IIb). Its IR spectrum displayed bands at 3300 (NH), 1725 (ester C=O), 1620 (C=C), 1540 (C-N) and 1600, 1505, 750, 690

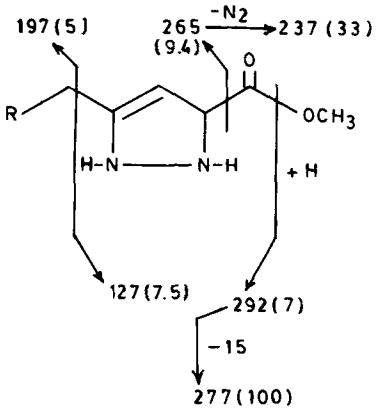

FIG. 1. MS fragmentation of compound IIa.

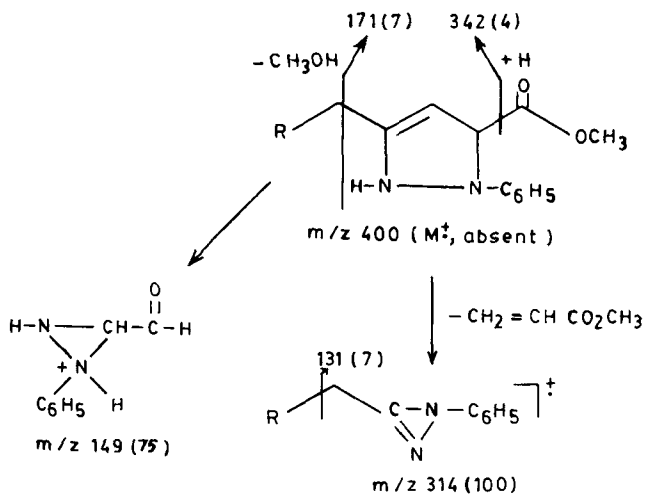

FIG. 2. MS fragmentation of compound IIb.

cm^{-1} (monosubstituted benzene). In addition to the signals normally present in the spectrum of long chain esters, the NMR spectrum of IIb showed absorptions at δ 7.2 br m ($C_6\underline{H}_5$), 5.91 d (Ha), 3.68 d (Hb, merged in part to the ester proton signals at δ 3.72), 2.34 m ($C\underline{H}_2$ α to the ring) and 1.91 br (N<u>H</u>, D_2O exchangeable). The mass spectrum furnished corroborative evidence in support of structure IIb (Fig. 2) by exhibiting mass ions at m/z 342, 314, 223 and 171. These data agreed with the formulation of IIb as 3-tetradecyl-1-phenyl-5-(methoxycarbonyl)-3-pyrazoline.

Similarly, compound (I) reacted with tolylhydrazine and gave 3-tetradecyl-1-tolyl-5-(methoxycarbonyl)-3-pyrazoline (IIc). Its IR spectrum displayed significant bands at 3320 (NH), 1730 (ester C=O), 1615 (C=C), 1600, 1510, 820 (aromatic, disubstituted) and 1545 cm^{-1} (C-N). The NMR spectrum showed important signals at δ 7.5, 7.3 m (aromatic protons), 5.78 d (Ha), 3.65 d (Hb, merged in part to the ester proton signal at δ 3.7), 2.32 m ($C\underline{H}_2$) α to ring) and 1.65 br (N<u>H</u>, D_2O exchangeable). MS of compound IIc gave a fragmentation pattern similar to that of IIb.

The antimicrobial activity of various seed oils, fatty acids and their derivatives has been studied (5-7). The compounds (IIa-c) were screened for antifungal activity against *Aspergillus flavus, A. fumigatus, A. niger, A. sydowii, Acremonium* sp., *Alternaria alternata, Curvularia clavata, Fusarium moniliforme, Cladosporium citrinum* and *Penicillium citrinum* at 800, 600, 400 and 200 ppm. All three compounds (IIa-c) were active and showed 100% colonial growth inhibition on test fungi. The compounds were also examined for antibacterial test against *Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus* sp. and *Escherichia coli* at the same concentration and showed 100% colonial growth inhibition.

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