Synthesis and Antimicrobial Activity of Fatty 2-Morpholinones Prepared from Epoxy Fatty Acid Methyl Esters[1]

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Reactions of methyl 10,11-epoxyundecanoate (I), methyl 9,10-epoxyoctadecanoate (II) methyl 12,13-epoxy-*cis*-9-octadecenoate (III) and methyl *trans*-2,3-epoxyhexadecenoate (IV) with glycine in dimethylformamide (DMF) in the presence of anhydrous $AlCl_3$ as catalyst have yielded 5(6)-[8′-carbomethoxyoctyl]-2-morpholinone (V); 5(6)-[7′-carbomethoxyheptyl]-6(5)-octyl-2-morpholinone (VI); 5(6)-[10′-carbomethoxydec-*cis*-2-enyl)-6(5)-pentyl-2-morpholinone (VII), and 5-tridecyl-6-carbomethoxy 2-morpholinone (VIII), respectively in excellent yield. The products have been characterized with the help of spectral data and microanalysis. Antifungal and antibacterial screening of (V-VIII) showed pronounced activity against four bacteria and seven fungal species.

Compounds containing morpholinone nuclei are known to possess biological as well as useful industrial properties. They are used biologically as analgesics (1), germicides (2) and antiallergics (3), and industrially as hair grooming aids (4), dispersants for lubricating oils (4), moisture-resistant adhesives and dye receptors for resins such as polyethylene and polypropylene (4). Upon reaction with amino acids or their esters, oxiranes gave 2-morpholinone derivatives (3, 5, 6). These reports focussed our attention on the synthesis of fatty 2-morpholinones from epoxy fatty acids using amino acids such as glycine as condensing agents, and on their exploitation as antimicrobes. In continuation of our studies on the synthesis of fatty heterocycles such as 2-oxazolidones (7—9) and 2-oxazolines (10, 11) from epoxy fatty acids, we report herein the synthesis of some fatty 2-morpholinone derivatives from the reactions of methyl 10,11-epoxyundecanoate (I), methyl 9,10-epoxyoctadecanoate (II), methyl 12,13-epoxy-*cis*-9-octadecenoate (III) and methyl *trans*-2,3-epoxyhexadecenoate (IV) with glycine in dimethylformamide (DMF) as solvent and anhydrous $AlCl_3$ as catalyst. Further, these morpholinones were screened for their antimicrobial potential.

EXPERIMENTAL PROCEDURE

The spectroscopic and chromatographic methods used have been described in our earlier communications (9, 10).

Methyl esters (I) and (II) were prepared by m-chloroperbenzoic acid oxidation of methyl 10-undecenoate and methyl-*cis*-9-octadecenoate, respectively. III was isolated from *Vernonia anthelmintica* seed oil. IV [m.p. 35–36°C] was prepared as given in our earlier communication (9).

Reaction of epoxy fatty acid methyl esters (I–IV) with glycine. Equimolar amounts of epoxide (I–IV) and glycine were heated under reflux in DMF (20–25 ml) in the presence of a catalytic amount of anhydrous $AlCl_3$ (0.05 g) for 1–2 hr. The reaction mixtures were then allowed to cool to room temperature, poured into water and extracted with ether. The ether extracts were washed successively with water to remove the final traces of glycine and DMF and dried over anhydrous Na_2SO_4. Viscous oils were obtained in each case after evaporation of the solvent in vacuo. Products were purified by crystallization from ether/petroleum ether or by chromatography on silica.

Antimicrobial activity. Stock solutions (1%) of the test compounds were prepared in acetone. From the stock solutions, concentrations of 800, 600, 400 and 200 ppm were prepared in distilled water. Potato dextrose agar and trypotone broth (TB) agar were used for the cultures of fungi and bacteria, respectively. Ten ml of nutrient medium (agar + nutrient medium) was poured into petri dishes aseptically. Then 0.3 ml of an overnight culture of liquid medium was poured into test tubes containing three ml of soft medium (13 g TB + 7 g agar/1000 ml). This inoculum was seeded on the agar plates. Then, 0.2 ml of solution of the compounds was soaked on filter discs (Schleicher + Schuell no. 740-E, .5″ diameter) and the discs were kept on the seeded plates. Acetone (800–200 ppm) was used as a control. The petri dishes were incubated at 28 ± 2°C for one week for fungi and at 37 ± 2°C for 40 hr for bacteria. After the incubation the petri dishes were observed for growth inhibition zones and the radial growth was measured. Percent growth inhibition was calculated with respect to the control. Ten replicates were run simultaneously for each treatment.

RESULTS AND DISCUSSION

Methyl epoxy esters (I–IV) on refluxing with glycine in DMF in the presence of anhydrous $AlCl_3$ as catalyst afforded their corresponding 2-morpholinones (V–VIII) (Scheme 1). These products have been characterized with the help of spectral data and microanalysis.

Product V (98% yield) gave white crystals from petroleum ether, m.p. 60–62°C. Microanalysis gave C, 61.9; H, 9.2; N, 5.1. $C_{14}H_{25}O_4N$ requires C, 62.0; H, 9.3; N, 5.2. It revealed characteristic IR bands at 1720 and 1090 cm^{-1} for the 2-morpholinone ring along with bands at 3400 (NH), 1740 ($\underline{C}OOCH_3$), 1450, 1370, 1180 and 740 cm^{-1}. The NMR spectrum of this compound gave characteristic signals at δ 4.1 s (2H, 3-CH_2 in ring), 3.52 m (2H, 5-CH_2 in ring), 3.40 m(1H, 6-CH in ring) along with other signals at δ 3.65 s (3H, -$\underline{C}OOCH_3$), 2.5 s (NH), 2.25 m(2H, -$C\underline{H}_2$ $COOCH_3$) and 1.35 br, s (chain -CH_2). Appearance of weak multiplets at δ 3.0 (5-CH_2-NH) and δ 3.78 (6-C$\underline{H}$-O) suggested the formation of another isomer 6-(8′-carbomethoxyoctyl)-2-morpholinone

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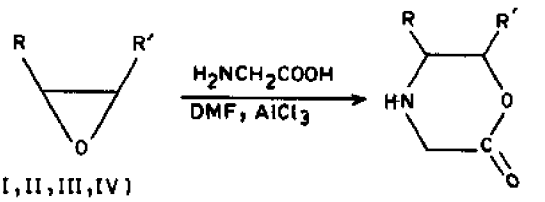


(I, II, III, IV) (V, VI, VII, VIII)

I: R = H; R′ = $(CH_2)_8$ $COOCH_3$

V: R = H; R′ = $(CH_2)_8$ $COOCH_3$ and R = $(CH_2)_8$ $COOCH_3$; R′ = H

II: R = $CH_3(CH_2)_7$; R′ = $(CH_2)_7$ $COOCH_3$

VI: R = $CH_3(CH_2)_7$; $(CH_2)_7$ $COOCH_3$ and R = $(CH_2)_7$ $COOCH_3$; R′ = $CH_3(CH_2)_7$

III: R = $CH_3(CH_2)_4$; R′ = $CH_2CH{=}CH{-}(CH_2)_7$ $COOCH_3$

VII: R = $CH_3(CH_2)_4$; R′ = $CH_2(CH{=}CH)(CH_2)_7$ $COOCH_3$ and R = $-CH_2-CH{=}CH-(CH_2)_7$ $COOCH_3$; R′ = $CH_3(CH_2)_4$

IV, VIII: R = $CH_3(CH_2)_{12}$; R′ = $COOCH_3$

SCHEME 1

in a small amount. These data confirmed the structure of V as 5(6)-[8′-carbomethoxyoctyl]-2-morpholinone.

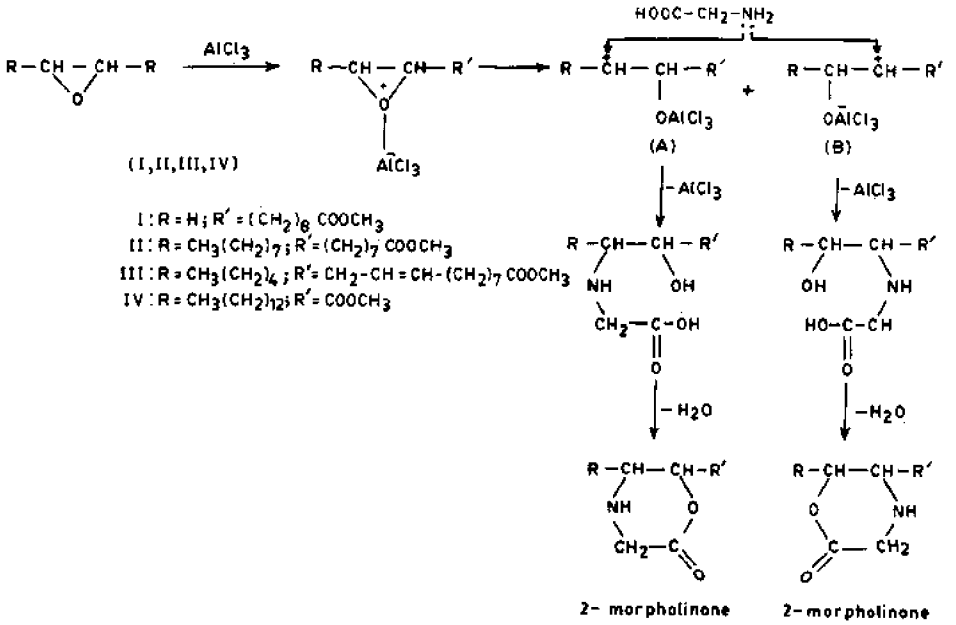
Product VI gave a clear liquid in 85% yield. Found C, 68.20; H, 10.6; N, 3.7. $C_{21}H_{39}O_4N$ requires C, 68.2; H, 10.6; N, 3.8. Its IR spectrum also gave the 2-morpholinone ring vibrations. Its NMR spectrum gave signals at δ 4.0 s (2H, 3-CH_2 in ring), 3.85 m(1H, CH-O), 3.5 m(CH-NH) along with bands at δ 2.4 s (NH), 3.65 s (3H, $COOCH_3$), 1.37 br, s (chain CH_2) and 0.9 t (3H, terminal CH_3). The product (VI) was assumed to be an isomeric mixture and characterized as 5(6)-[7′-carbomethoxyheptyl]-6(5)-octyl-2-morpholinones.

Product VII gave a clear liquid in 88% yield. Found C, 68.6; H, 10.1; N, 3.7. $C_{19}H_{35}O_4N$ requires C, 69.0; H, 10.1; N, 3.8. It gave IR and NMR spectral values almost identical to those of product (VI) except that there were additional NMR signals at δ 5.5 m(2H, -CH=CH) and signals at δ 2.20–2.25 br, m (integrating for six protons for α-CH_2 to $COOCH_3$ and two α-CH_2 to double bonds. This product was also assumed to be an isomeric mixture and characterized as 5(6)-[10′-carbomethoxydec-*cis*-2-enyl]-6(5)-pentyl-2-morpholinones.

Product VIII was a liquid in 88% yield. Found C, 66.8; H, 10.3; N, 4.1. $C_{19}H_{35}O_4N$ requires C, 66.8; H, 10.3; N, 4.0. Its IR spectrum also gave the 2-morpholinone ring vibrations and its NMR spectrum gave characteristic signals at δ 4.2 d (J = 4 Hz, 1H, 6-CH adjacent to $COOCH_3$), 4.0 s (3-CH_2 in ring), 3.6 m (5-CH adjacent to NH), 3.8 s (3H, $COOCH_3$), 2.14 br, s (NH), 1.27 br, s (chain CH_2) and 0.91 t (terminal CH_3). These data confirmed the structure of product VIII as 5-tridecyl-6-carbomethoxy-2-morpholinone.

The reaction of epoxides with glycine in the presence of anhydrous $AlCl_3$ should give rise to isomeric 2-morpholinones. In the case of the terminal epoxide (I), one isomer [5-(8′-carbomethoxyoctyl)-2-morpholinone] predominates over the other [6-(8′-carbomethoxyoctyl)-2-morpholinone]. In the case of the α,β-epoxide (IV), the isomer [5-tridecyl-6-carbomethoxy-2-morpholinone] was formed almost exclusively, while in internal epoxides (II) and (III), a 1:1 mixture of both isomers was found. This behavior can be explained on the basis of the probable mechanism formulated in Scheme 2.

In the case of the terminal epoxide (I), two structural carbonium ions are possible (secondary and primary). The attack of the glycine moiety to the more stable carbonium ion (secondary) results in the formation of 2-morpholinone, 5-(8′-carbomethoxyoctyl)-2-morpholinone (V) in the major amount. In the case of the α-β-epoxy ester (IV), the more stable carbonium ion (A) results in the formation of isomer (VIII), whereas



I: R = H; R′ = $(CH_2)_8$ $COOCH_3$

II: R = $CH_3(CH_2)_7$; R′ = $(CH_2)_7$ $COOCH_3$

III: R = $CH_3(CH_2)_4$; R′ = $CH_2-CH{=}CH-(CH_2)_7$ $COOCH_3$

IV: R = $CH_3(CH_2)_{12}$; R′ = $COOCH_3$

SCHEME 2

TABLE 1

Effect of 2-Morpholinones on Colonial Growth (% inhibition)[a]

Test compounds	ppm	Fungi							
		Cladosporium harbarum	*Aspergillus flavus*	*A. niger*	*A. nidulans*	*A. sydowii*	*Fusarium oxysporium*	*Curvularia clavata*	*Penicillium citrinum*
V($C_{14}H_{25}O_4N$)	800	++	++	++	++	++	++	++	++
	600	++	++	++	++	++	++	++	++
	400	52	70.	++	80	61	++	++	++
	200	25	56.25	++	20	11	++	++	++
VI($C_{21}H_{39}O_4N$)	800	++	++	++	++	++	++	++	++
	600	++	++	++	++	++	++	++	++
	400	++	++	++	++	++	++	++	++
	200	++	70.	++	60	++	++	50	++
VII($C_{21}H_{37}O_4N$)	800	++	++	++	++	++	++	++	++
	600	++	85.	90	69	++	90	89	++
	400	++	60.	60	32	++	55	45	79
	200	++	20.	30	07	25	25	12	43
VIII($C_{19}H_{35}O_4N$)	800	++	++	++	++	++	++	++	++
	600	++	++	++	++	++	++	++	++
	400	++	++	++	++	++	++	++	++
	200	++	29.	++	++	++	++	++	++

[a]++, 100% inhibition.

the less stable carbonium ion (B) due to the adjacent electron withdrawing carbonyl group results in the formation of the isomeric product of morpholinone in a small amount. In the case of the internal epoxy esters II and III, the formation of two structural carbonium ions of almost equal stability results in an approximately 1:1 ratio of isomers VI and VII.

The 2-morpholinones (V–VIII) were screened for antibacterial activity against *Staphylococcus aureus*, *Micrococcus* sp., *Pseudomonas aerugenosa* and *Escherichia coli* at concentrations of 800, 600, 400 and 200 ppm and showed 100% colonial growth inhibition. These compounds (V–VIII) were also screened for antifungal activity at the same concentrations; the results are shown in Table 1. It was found that compounds VI and VIII were the most effective and strongly inhibited the growth of all organisms except *A. flavus* (VI, VIII, 200 ppm), *A. nidulus* (VI, 200 ppm) and *C. clavata* VI, 200 ppm). Compounds V and VIII were found to be less effective.

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