

# Topical Mosquito Repellents XIII: Cyclic Analogs of Lactic Acid

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**Abstract** □ Numerous cyclic analogs of lactic acid were synthesized to ascertain whether they might act as agonists or antagonists of lactic acid, a known attractant for mosquitoes. These compounds were evaluated with an *in vitro* blood-feeding test system and an *in vivo* cloth test. Two of the compounds in the blood-feeding test system showed biphasic results, acting as attractants at low concentrations and as repellents at higher concentrations. Several compounds (III, V, VII, and X) repelled *Aedes aegypti* mosquitoes in the blood-feeding test system. However, in the *in vivo* cloth test system, only III repelled the mosquitoes significantly.

**Keyphrases** □ Lactic acid cyclic analogs—topical mosquito repellents, *in vivo* testing, humans □ Mosquitoes—topical repellents, lactic acid cyclic analogs, *in vivo* testing, humans □ Repellents, mosquito—topical use in humans, lactic acid cyclic analogs

Numerous cyclic analogs of lactic acid have been synthesized and evaluated as mosquito repellents using an *in vitro* blood-feeding test system (1). Some of these compounds also were evaluated by an *in vivo* cloth test<sup>1</sup>.

In addition to L-lactic acid, several compounds related to lactic acid attract *Aedes aegypti* mosquitoes (2). Certain receptors on the antennae of the female *A. aegypti* mosquito respond to lactic acid, but *N,N*-diethyl-*m*-toluamide blocks this response (3). Thus, an antagonist to lactic acid may exhibit repellent activity and an agonist may attract the mosquitoes.

A survey of chemical classes with repellent behavior (4, 5) indicated that  $\alpha$ -keto and  $\alpha$ -hydroxy carbonyl compounds were among those showing increased frequency of repellent activity.

The structure-activity relationships of these compounds were investigated in structures that varied in the separation of the oxygen functions (hydroxyl or RO) from the carbonyl functions. The alkyl substituents were varied to give a boiling-point range since volatility is an important variable affecting the duration of topical repellency. Table I indicates the structures, boiling points or melting points, and repellency data for compounds used against *A. aegypti* (yellow fever) mosquitoes.

## EXPERIMENTAL<sup>2</sup>

**Chemistry**—Ethyl 2-Butyl-1-hydroxycyclopentanecarboxylate (6) (IV)—2-*n*-Butyl-1-hydroxycyclopentanecarbonitrile (7) (5.0 g, 29.9 mmoles), anhydrous ethanol (1.38 g, 29.9 mmoles), *p*-toluenesulfonic acid

monohydrate (5.69 g, 29.9 mmoles), and 5 ml of anhydrous benzene were stirred overnight at 90°. Water and benzene were added, the organic layer was washed with 20% Na<sub>2</sub>CO<sub>3</sub> and water and then dried over sodium sulfate, and the solvent was evaporated. Distillation of the residue afforded IV (bp 71–72°/0.5 mm Hg) as a colorless liquid, 1.0 g (16% yield); IR (film): 3460, 2900, 2830, 1720, 1430, 1370, 1230, 1050, 880, and 760 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>):  $\delta$  4.27 (q, 2H), 3.1 (s, 1H), 1.6–2.2 (m, 7H), 1.27 (t, 3H), 1.05–1.4 (m, 6H), and 0.87 (t, 3H) ppm.

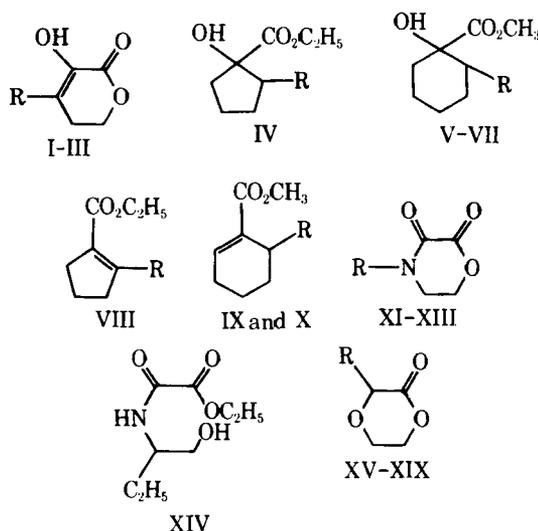
*Anal.*—Calc. for C<sub>12</sub>H<sub>22</sub>O<sub>3</sub>: C, 67.25; H, 10.35. Found: C, 67.70; H, 10.60.

*Methyl 2-Butyl-1-hydroxycyclohexanecarboxylate* (V)—2-*n*-Butyl-1-hydroxycyclohexanecarbonitrile (7) (7.2 g, 39.7 mmoles) and 20 volume % H<sub>2</sub>SO<sub>4</sub> (26 ml) in acetic acid (35 ml) were stirred at 110° for 27 hr. After cooling, the reaction mixture was poured into cold water and extracted with ether. The carboxylic acid was extracted with dilute sodium hydroxide, the aqueous alkaline solution was acidified with concentrated hydrochloric acid, and the solution was extracted with ether. The combined ether layers were dried over sodium sulfate–charcoal and were concentrated to yield 0.91 g of a yellow oil.

A solution of diazomethane in ether was added slowly at 0° to a stirred solution of 2-*n*-butyl-1-hydroxycyclohexanecarboxylic acid (0.91 g, 4.54 mmoles) in 10 ml of ether until nitrogen evolution ceased. The reaction mixture was stirred for 40 min at 0° and for 30 min at room temperature. After the addition of 1 ml of 3 *N* acetic acid, the solution was extracted with sodium bicarbonate and water, and the extract was dried over magnesium sulfate and concentrated. Distillation afforded V (bp 85–86°/0.5 mm Hg) as a colorless liquid, 0.90 g (93% yield); IR (film): 3480, 2920, 2850, 1730, 1460, 1390, 1240, 1160, 1100, and 1020 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>):  $\delta$  3.76 (s, 3H), 3.64 (s, 3H), 3.36 (s, 1H), 2.2–2.5 (m, 3H), 1.0–1.9 (m, 12H), and 0.86 (t, 3H) ppm.

*Anal.*—Calc. for C<sub>12</sub>H<sub>22</sub>O<sub>3</sub>: C, 67.26; H, 10.35. Found: C, 67.20; H, 10.22.

(1-Acetoxy-2-heptylcyclohexyl)carbonitrile—Five grams (0.022 mole) of (2-heptyl-1-hydroxycyclohexyl)carbonitrile was dissolved in 50 ml of acetic anhydride. After addition of 0.1 g of 4-dimethylaminopyridine as a catalyst, the mixture was allowed to stand at room temperature for 2 days. The excess acetic anhydride was removed *in vacuo*. The residue was taken up with saturated sodium bicarbonate and extracted three times with ether. After drying with anhydrous magnesium sulfate and



<sup>1</sup> The *in vivo* cloth test system was developed by the Agricultural Research Laboratories, U.S. Department of Agriculture, Gainesville, Fla.

<sup>2</sup> Melting points were determined on a capillary melting-point apparatus and are uncorrected. Boiling points were determined using a short-path distillation apparatus and are uncorrected. IR (Perkin-Elmer 735B) and NMR (Varian Associates T-60 or EM-390) spectra were taken of all compounds and were completely consistent with the assigned structures. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Stanford University, Stanford, Calif.

Table I—Physical and Biological Properties of Cyclic Analogs of Lactic Acid

Compound	R	Boiling Point or Melting Point	Refer- ence	Repellent Activity (Membrane Feeder Test)			Repellent Activity (Cloth Test), days	Minimum Effective Dose, mg/cm <sup>2</sup>	
				ED <sub>50</sub>	ED <sub>90</sub>	r		15 min	24 hr
I	CH <sub>3</sub>	72–75°	8	—	No effect	—	—	—	
II	CH <sub>2</sub> CH <sub>3</sub>	—	— <sup>a</sup>	0.10	0.64	–0.77	0	0.25 >1.0	
III	(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	—	— <sup>b</sup>	0.035	0.094	–0.93	6	0.063 0.25	
IV	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	71–72°/0.5 mm Hg	— <sup>c</sup>	—	No effect	—	—	—	
V	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	85–86°/0.5 mm Hg	— <sup>c</sup>	0.0060	0.31	–0.87	>1	0.125 >1.0	
VI	(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	92–93°/0.5 mm Hg	— <sup>d</sup>	0.096	0.27	–0.95	>1	0.5 >1.0	
VII	(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	130°/0.5 mm Hg	— <sup>c</sup>	0.0073	0.064	–0.93	0	>1.0 —	
VIII	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	74–75°/0.5 mm Hg	— <sup>c</sup>	—	No effect	—	—	—	
IX	(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	88°/0.5 mm Hg	— <sup>e</sup>	—	Bimodal effect	—	0	>1.0 —	
X	(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	124°/0.5 mm Hg	— <sup>c</sup>	0.058	13	–0.33	0	>1.0 —	
XI	CH <sub>2</sub> CH <sub>3</sub>	56–66°	— <sup>f</sup>	—	—	—	—	—	
XII	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	63–64°	— <sup>c</sup>	—	Bimodal effect	—	—	—	
XIII	(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	62.5°	— <sup>g</sup>	—	—	—	—	—	
XIV	—	—	— <sup>c</sup>	—	No effect	—	0	>1.0 —	
XV	CH <sub>2</sub> CH <sub>3</sub>	52–55°/0.5 mm Hg	9	0.31	12	–0.80	—	—	
XVI	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	85–88°/0.5 mm Hg	9	—	No effect	—	0	0.25 >1.0	
XVII	(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>	90–92°/0.5 mm Hg, 48–49°	— <sup>h</sup>	—	—	—	—	—	
XVIII	(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	100–102°/0.5 mm Hg, 47–49°	— <sup>i</sup>	—	—	—	—	—	
XIX	(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>	111–113°/0.5 mm Hg, 60–62°	— <sup>j</sup>	—	—	—	—	—	
Diethyl toluamide	—	100°/0.5 mm Hg	—	0.031	0.10	–0.81	6	0.016 0.25	

<sup>a</sup> Compound II was prepared as described previously for I (8). IR (CDCl<sub>3</sub>): 3400, 2950, 2880, 1705, 1475, 1430, 1390, 1280, and 1190 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 5.6–6.1 (m, 1H), 4.4 (t, 2H), 2.05–2.65 (m, 4H), and 1.07 (t, 3H) ppm. *Anal.*—Calc. for C<sub>7</sub>H<sub>10</sub>O<sub>3</sub>: C, 59.14; H, 7.09. Found: C, 57.42; H, 7.21 (hygroscopic because a part of the lactone always is hydrolyzed). <sup>b</sup> Compound III was prepared as described previously for I (8). IR (CDCl<sub>3</sub>): 3450, 2940, 2860, 1710, 1480, 1430, 1390, 1295, 1200, and 1100 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 5.6–6.1 (m, 1H), 4.37 (t, 2H), 2.05–2.65 (m, 4H), 1.03–1.7 (m, 6H), and 0.9 (t, 3H) ppm. *Anal.*—Calc. for C<sub>10</sub>H<sub>16</sub>O<sub>3</sub>: C, 65.19; H, 8.75. Found: C, 63.98; H, 8.53 (hygroscopic because a part of the lactone always is hydrolyzed). <sup>c</sup> See *Experimental*. <sup>d</sup> Compound VI was prepared as described under *Experimental* for V. IR (CDCl<sub>3</sub>): 3500, 2920, 2850, 1720, 1460, 1390, 1245, 1160, 1095, and 1010 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 3.76 (s, 3H), 3.38 (s, 1H), 1.05–2.5 (m, 17H), and 0.86 (t, 3H) ppm. *Anal.*—Calc. for C<sub>13</sub>H<sub>24</sub>O<sub>3</sub>: C, 68.38; H, 10.59. Found: C, 68.60; H, 10.74. <sup>e</sup> Compound IX was prepared as described under *Experimental* for X. IR (film): 2900, 2840, 1710, 1645, 1460, 1440, 1390, 1360, 1250, 1090, and 770 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 6.86 (t, 1H), 3.7 (s, 3H), 2.4–2.7 (m, 1H), 2.0–2.3 (m, 2H), 1.5–1.7 (m, 4H), 1.1–1.5 (m, 8H), and 0.87 (t, 3H) ppm. *Anal.*—Calc. for C<sub>13</sub>H<sub>22</sub>O<sub>2</sub>: C, 74.24; H, 10.54. Found: C, 74.13; H, 10.47. <sup>f</sup> Compound XI was prepared as described under *Experimental* for XII. IR (film): 2960, 2930, 2870, 1760, 1680, 1490, 1450, 1375, 1325, 1200, 1080, 1030, 960, 890, and 820 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 4.58 (t, 2H), 3.2–3.9 (m, 4H), and 1.2 (t, 3H) ppm. <sup>g</sup> Compound XIII was prepared as described under *Experimental* for XII. IR (film): 2900, 2830, 1750, 1675, 1460, 1370, 1325, 1190, and 1075 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 4.53 (t, 2H), 3.70 (t, 2H), 3.50 (t, 2H), 1.1–1.8 (m, 8H), and 0.87 (t, 3H) ppm. *Anal.*—Calc. for C<sub>10</sub>H<sub>17</sub>NO<sub>3</sub>: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.00; H, 8.51; N, 6.93. <sup>h</sup> Compound XVII was prepared as described previously (9). IR (film): 2920, 2840, 1745, 1470, 1415, 1385, 1350, 1290, 1225, 1205, 1140, 1125, 1090, and 940 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 4.17–4.60 (m, 3H), 3.74–4.10 (m, 2H), 1.7–2.2 (m, 2H), 1.1–1.7 (m, 6H), and 0.9 (t, 3H) ppm. *Anal.*—Calc. for C<sub>9</sub>H<sub>16</sub>O<sub>3</sub>: C, 62.77; H, 9.36. Found: C, 62.89; H, 9.32. <sup>i</sup> Compound XVIII was prepared as described previously (9). IR (film): 2900, 2820, 1720, 1465, 1410, 1380, 1340, 1300, 1280, 1210, 1180, 1120, 1070, 1005, 940, 880, and 740 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 4.2–4.6 (m, 3H), 3.7–4.1 (m, 2H), 1.7–2.2 (m, 2H), 1.1–1.7 (m, 8H), and 0.9 (t, 3H) ppm. *Anal.*—Calc. for C<sub>10</sub>H<sub>18</sub>O<sub>3</sub>: C, 64.49; H, 9.74. Found: C, 64.40; H, 9.80. <sup>j</sup> Compound XIX was prepared as described previously (9). IR (mineral oil): 2900, 2820, 1740, 1460, 1420, 1380, 1310, 1230, 1150, 1080, 960, 905, 860, and 740 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 4.2–4.65 (m, 3H), 3.75–4.1 (m, 2H), 1.7–2.2 (m, 2H), 1.1–1.7 (m, 10H), and 0.9 (t, 3H) ppm. *Anal.*—Calc. for C<sub>11</sub>H<sub>20</sub>O<sub>3</sub>: C, 65.97; H, 10.07. Found: C, 66.22; H, 9.94.

evaporating, 5.3 g of a yellow oil was obtained and was sufficiently pure for the following reaction.

(2-Heptyl-1-hydroxycyclohexyl)carbonitrile—A mixture of 15 g (0.076 mole) of 2-heptylcyclohexanone, 19 g (0.22 mole) of acetone cyanohydrin, and 0.3 g of triethylamine was allowed to stand at room temperature for 2 hr. After acidification with sulfuric acid in acetone, the excess acetone cyanohydrin was distilled *in vacuo* (60°/1.0 mm Hg). The residue was taken up in ether and washed with water. After drying over sodium sulfate and evaporating, 15 g of a reddish oil was obtained and was sufficiently pure for further reactions.

Methyl (2-Heptyl-1-hydroxycyclohexyl)carboxylate (VII)—Five grams (0.0187 mole) of (1-acetoxy-2-heptylcyclohexyl)carbonitrile was dissolved in a mixture of 19 ml of acetic acid, 1 ml of acetic anhydride, and 4 ml of sulfuric acid. After standing at room temperature for 4 hr, 3 ml of water was added. Three grams (0.0435 mole) of granular sodium nitrite was added slowly to the cooled and stirred mixture. The suspension was stirred under cooling for 30 min and at room temperature for 1 hr. The mixture was poured into ice water and extracted three times with ether.

The combined extracts were evaporated *in vacuo*, and the residue was taken up with 20 ml of methanol. After addition of 10 ml of 40% KOH, the mixture was refluxed for 1 hr, poured into water, and extracted three times with ether. The aqueous phase was acidified with 10% H<sub>2</sub>SO<sub>4</sub> and extracted three times with dichloromethane. After drying with anhydrous magnesium sulfate, an ethereal solution of diazomethane was added slowly to the stirred and cooled acid solution. After decolorization with activated charcoal and evaporation, the crude product was distilled *in vacuo* to give 2.5 g of a colorless oil (130° air bath temperature/0.35 mm Hg). A second distillation gave 2.4 g (50% yield) of pure product, bp 118°/0.3 mm Hg.

*Anal.*—Calc. for C<sub>15</sub>H<sub>28</sub>O<sub>3</sub>: C, 70.27; H, 11.01. Found: C, 69.42; H, 10.70.

Ethyl 2-Butyl-1-cyclopentene-1-carboxylate (VIII)—Compound IV (2.3 g, 10.73 mmoles) in 2.5 ml of pyridine was treated dropwise at 0° with 1.4 ml of thionyl chloride, heated for 1 hr at 140° (oil bath temperature), and poured after cooling into ice water. The ester was extracted with

ether, and the extract was dried over sodium sulfate–charcoal and concentrated *in vacuo*. Distillation (bp 74–75°/0.5 mm Hg) afforded VIII as a colorless liquid, 1.8 g (86% yield); IR (film): 2940, 2850, 1700, 1640, 1470, 1380, 1270, 1210, 1105, and 1045 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 4.18 (q, 2H), 1.6–2.8 (m, 8H), 1.26 (t, 3H), 1.1–1.16 (m, 4H), and 0.9 (t, 3H) ppm.

*Anal.*—Calc. for C<sub>12</sub>H<sub>20</sub>O<sub>2</sub>: C, 73.43; H, 10.27. Found: C, 73.03; H, 10.03.

Methyl 6-Heptyl-1-cyclohexene-1-carboxylate (X)—2-*n*-Heptyl-1-hydroxycyclohexanecarbonitrile (7) (2.0 g, 8.95 mmoles) in 2.2 ml of pyridine was treated dropwise at 0° with 1.2 ml of thionyl chloride, and the mixture was heated for 1 hr at 140° (oil bath temperature) and poured after cooling into ice water. The desired product was extracted with ether, and the extract was dried over sodium sulfate–charcoal and concentrated *in vacuo* to yield 1.6 g of a light-yellow oil.

A solution of 6-*n*-heptylcyclohex-1-enecarbonitrile (1.3 g, 6.33 mmoles) in 2.6 ml of 50 volume % H<sub>2</sub>SO<sub>4</sub> and 6.5 ml of acetic acid was stirred at 110° for 18 hr. To this ice-cooled and well-stirred reaction mixture, a solution of sodium nitrite (0.52 g, 7.6 mmoles) in 2 ml of water was added slowly. After stirring for 20 min at room temperature and 1.5 hr at 80°, the reaction mixture was poured into water and extracted with ether. The ether solution was extracted several times with dilute sodium hydroxide, and the combined alkaline layers were acidified with concentrated hydrochloric acid and extracted with ether. The ether layers were dried over sodium sulfate–charcoal and concentrated to yield 0.93 g of a yellow oil.

Esterification of 6-*n*-heptylcyclohex-1-enecarboxylic acid (0.93 g, 4.14 mmoles) with diazomethane (as described for V) afforded X after distillation (bp 124°/0.5 mm Hg) as a light-yellow oil, 0.84 g (95% yield); IR (film): 2900, 2840, 1715, 1645, 1440, 1390, 1360, 1250, 1090, and 770 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>): δ 6.84 (t, 1H), 3.7 (s, 3H), 2.4–2.65 (m, 1H), 2.0–2.3 (m, 2H), 1.1–1.7 (m, 16H), and 0.87 (t, 3H) ppm.

*Anal.*—Calc. for C<sub>13</sub>H<sub>26</sub>O<sub>2</sub>: C, 75.58; H, 10.99. Found: C, 75.34; H, 10.82.

4-Butyl-2,3-morpholinedione (XII)—A mixture of diethyl oxalate (6.24 g, 42.7 mmoles), 2-(butylamino)ethanol (5.0 g, 42.7 mmoles), and potassium hydroxide (0.1 g) was heated at 110° for 2 hr, and the ethanol

that formed was distilled off. To remove all of the ethanol, the reaction mixture was heated for another 2 hr at 100° under reduced pressure (40 mm). After cooling to room temperature, the partially crystallized residue was acidified by the addition of 0.2 ml of concentrated sulfuric acid and dissolved in methylene chloride. The solution was washed several times with water, dried over sodium sulfate, and concentrated *in vacuo*. Recrystallization from ether afforded XII as colorless flakes, 5.5 g (75% yield); IR (KBr): 2920, 2840, 1745, 1670, 1480, 1440, 1360, 1180, 1070, 1030, and 940  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ):  $\delta$  4.53 (t, 2H), 3.70 (t, 2H), 3.50 (t, 2H), 1.14–1.8 (m, 4H), and 0.93 (t, 3H) ppm.

*Anal.*—Calc. for  $\text{C}_8\text{H}_{13}\text{NO}_3$ : C, 56.13; H, 7.65. Found: C, 56.39; H, 7.77.

*Ethyl* [[1-(Hydroxymethyl)propyl]amino]oxoacetate (XIV)—2-Amino-1-butanol (5.0 g, 56.1 mmoles) in 70 ml of anhydrous ethanol was added over 2.5 hr to a stirred solution of diethyl oxalate (8.2 g, 56.1 mmoles) in 50 ml of anhydrous ethanol at 50°. After cooling, some crystalline by-product was filtered off, and the solution was concentrated *in vacuo*. The residue was purified by low-pressure liquid chromatography (8% ethanol in dichloromethane) to give XIV as a light-yellow oil, 5.2 g (49% yield); IR ( $\text{CDCl}_3$ ): 3370, 2960, 2880, 1740, 1690, 1540, 1470, 1385, 1320, 1280, 1220, and 1040  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ ):  $\delta$  7.7 (d, 1H), 4.4 (q, 2H), 3.5–4.2 (m, 4H), 1.35 (t, 3H), 1.2–1.8 (m, 2H), and 0.93 (t, 3H) ppm.

*Anal.*—Calc. for  $\text{C}_8\text{H}_{15}\text{NO}_4$ : C, 50.78; H, 7.99; N, 7.40. Found: C, 50.65; H, 8.01; N, 7.30.

**In Vivo Testing Cloth Test**—The cloth test screens limited quantities of chemicals as mosquito repellents. The test compound (50 mg) was placed in a 2-dram vial to which 0.75 ml of acetone or other solvent was added. When the chemical was thoroughly dissolved, a 50- $\text{cm}^2$  (5 × 10-cm) piece of muslin bandage was rolled, placed in the vial, and mixed with the solution, and the vial was sealed until the chemicals were tested. The rate of cloth treatment was 1.0  $\text{mg}/\text{cm}^2$ . After treatment, the cloths were kept refrigerated in the vials for at least 24 hr.

At the start of a test, vials were removed from the refrigerator and allowed to warm to room temperature. The cloth then was removed from the vial, stapled over a 4 × 9-cm opening cut into a 12.7 × 20.3-cm file card, and allowed to dry for 15 min before testing.

The subject testing the compounds covered his or her arm with a nylon stocking and wore a rubber glove over the hand and wrist to protect against bites. The card and attached cloth patch were then taped over the nylon-covered forearm so that only the treated cloth allowed access to the skin by the mosquitoes. The arm was then exposed to a stock cage of *A. aegypti* mosquitoes (~1500) for 1 min. More than three bites through the treated cloth in 1 min denoted failure of the chemical. If the chemical did not fail, it was stored at room temperature and retested in 24 hr and daily thereafter until it failed. Two standard repellents, diethyltoluamide and dimethyl phthalate, were tested concurrently.

When a sufficient quantity of the candidate material was available, the minimum effective dose (MED) was determined. The testing method was the same, but the treatment rate was reduced by one-half until more

than three bites per minute were received at the lowest dose. The minimum effective doses were determined only on the fresh (air dried for 15 min) and 1-day-old treatments.

## RESULTS AND DISCUSSION

Several of the synthesized compounds were solids; and although two compounds (I and XII) were evaluated in the membrane feeder test (1), the others (XI, XIII, and XVII–XIX) were not evaluated since solids with melting points greater than 40° do not repel significantly.

Two compounds (IX and XII) showed weak repellent activity at high concentrations and attracted the mosquitoes at lower concentrations in the membrane feeder test. These compounds are under study in these laboratories for their effects on the lactic acid receptor of *A. aegypti* mosquitoes.

There is not always good agreement between the *in vitro* membrane feeder test and the *in vivo* cloth test. However, the membrane feeder test is useful as a rapid screening method when many compounds are studied.

The most interesting compounds from this series were III and VII, which will be studied further on mice and Mexican hairless dogs prior to any human skin tests. From the cloth test results, III seems to be the most promising.

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