

## In Vitro and In Vivo Cytotoxicities and Antileishmanial Activities of Thymol and Hemisynthetic Derivatives

Sara Robledo,<sup>1\*</sup> Edison Osorio,<sup>1,2</sup> Diana Muñoz,<sup>1</sup> Luz Marina Jaramillo,<sup>1,2</sup> Adriana Restrepo,<sup>1</sup> Gabriel Arango,<sup>2</sup> and Iván Vélez<sup>1</sup>

Programa de Estudio y Control de Enfermedades Tropicales PECET<sup>1</sup> and Grupo de Investigación en Sustancias Bioactivas GISB,<sup>2</sup> Universidad de Antioquia, Medellín, Colombia

Received 25 November 2004/Returned for modification 27 November 2004/Accepted 29 November 2004

**The in vitro and in vivo antileishmanial and cytotoxic activities of thymol and structural derivatives in comparison to those of Glucantime were studied. The results showed here suggest that thymol and hemisynthetic derivatives have promising antileishmanial potential and could be considered as new lead structures in the search for novel antileishmanial drugs.**

Thymol, a *p*-cymene-derived compound, is widely used in medicine for its antimicrobial, antiseptic, disinfectant, and wound-healing properties (1, 3, 6, 10, 12, 16). Because derivatives of *p*-cymene have leishmanicidal activity (2, 8, 11) and are considered important basic structures for development of novel antiparasitic drugs (9), in this study the thymol structure was chemically modified and its derivatives were evaluated for cytotoxic and antileishmanial activities.

The capacity to kill *Leishmania (Viannia) panamensis* (MHOM/CO/87/UA/UA140) promastigotes and U-937 was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] method, following a previously reported methodology (19). After 96 h of incubation in the presence of the compounds, the viability of promastigotes and cells was determined according to the formazan produced after addition of MTT. Parasites and cells cultivated in the absence of the compounds but maintained under the same conditions were used as controls. A control of dimethylsulfoxide was also included.

The effect against intracellular amastigotes was evaluated by microscope examination of infected U-937 cells incubated in the presence of the compounds, following a previously reported methodology (15, 19). Cells grown in RPMI medium were adjusted to 10<sup>6</sup> cells/ml. In each well of a 24-well cell culture plate containing a 12-mm-diameter coverslip glass, 10<sup>5</sup> cells were dispensed and incubated in the presence of phorbol myristate acetate for 48 h. Adherent cells were exposed to promastigotes (25:1, parasite/cell) during 2 h (34°C, 5% CO<sub>2</sub>). Nonattached cells and free parasites were washed, and fresh medium was added; 24 h later, the medium was replaced with medium containing the compound. The range of concentration for every compound was selected according to the toxicity previously observed in the U-937 cells. Infected cells were exposed to the compounds for 96 h, changing the medium after 48 h. Cells were washed, fixed with methanol, and stained with Giemsa. Infected cells cultivated in the absence of compounds served as a control of infection. Two hundred cells were evaluated in every well. Reduction of the parasite load was deter-

mined by dividing the number of amastigotes obtained in the presence of each compound by the number of amastigotes obtained in the absence of them (15, 19). All the compounds were evaluated in three independent experiments, each in triplicate. Their cytotoxic and antileishmanial activities were compared with those of Glucantime. Results are expressed as 50% effective concentrations for parasites and 50% lethal concentrations for cells, calculated by the probit method (7).

The in vivo effectivity was evaluated in Golden hamsters (*Mesocricetus auratus*) experimentally infected with *L. (V.) panamensis*. Male and female outbred adult hamsters (*n* = 5 per group), maintained according to institutional guidelines, were inoculated intradermally in the snout (13) with 10<sup>6</sup> infective promastigotes (14). Treatment schedules began when lesions were conspicuous (17). Compounds were solubilized in carboxymethylcellulose and administered orally at a dosage of 40 mg/kg of body weight/day for 10 days. A control group received carboxymethylcellulose, and a second control group received Glucantime by intramuscular injections at the same dose. Animals were evaluated for lesion size prior to treatment and at days 15 and 30 after treatment was finished by measuring the diameter of the nose. Their parasitological stage was determined by culture of material obtained from the lesions in NNN medium (13). Animals were also bled to evaluate serum level of glucose, uric acid, and bilirubin, and animals were monitored for changes in behavior or weight. Finally, hamsters were sacrificed as recommended, and the parasite burden was estimated by a semiquantitative limiting dilution culture assay of tissue samples adjusted to 0.1 mg/ml of medium, serially fourfold diluted, and incubated for 4 weeks at 26°C (13, 18). Parasite burden was expressed as the maximum dilution in which parasites were detected by examination under an inverted microscope.

The hemisynthesis of the compounds was analyzed by thin-layer chromatography, silica gel column chromatography, the combined gas chromatography–mass spectrometry (MS) technique, and UV spectral analysis. All of the products were identified by the <sup>1</sup>H nuclear magnetic resonance (NMR), <sup>13</sup>C NMR, and MS techniques. The pathways for synthesis, the structures of the compounds, and their chemical properties are summarized in Fig. 1.

\* Corresponding author. Mailing address: Calle 62 No. 52-59, Lab 632, Medellín, Colombia. Phone: (574) 210 6503. Fax: (574) 210 6511. E-mail: srobledo@guajiro.udea.edu.co.

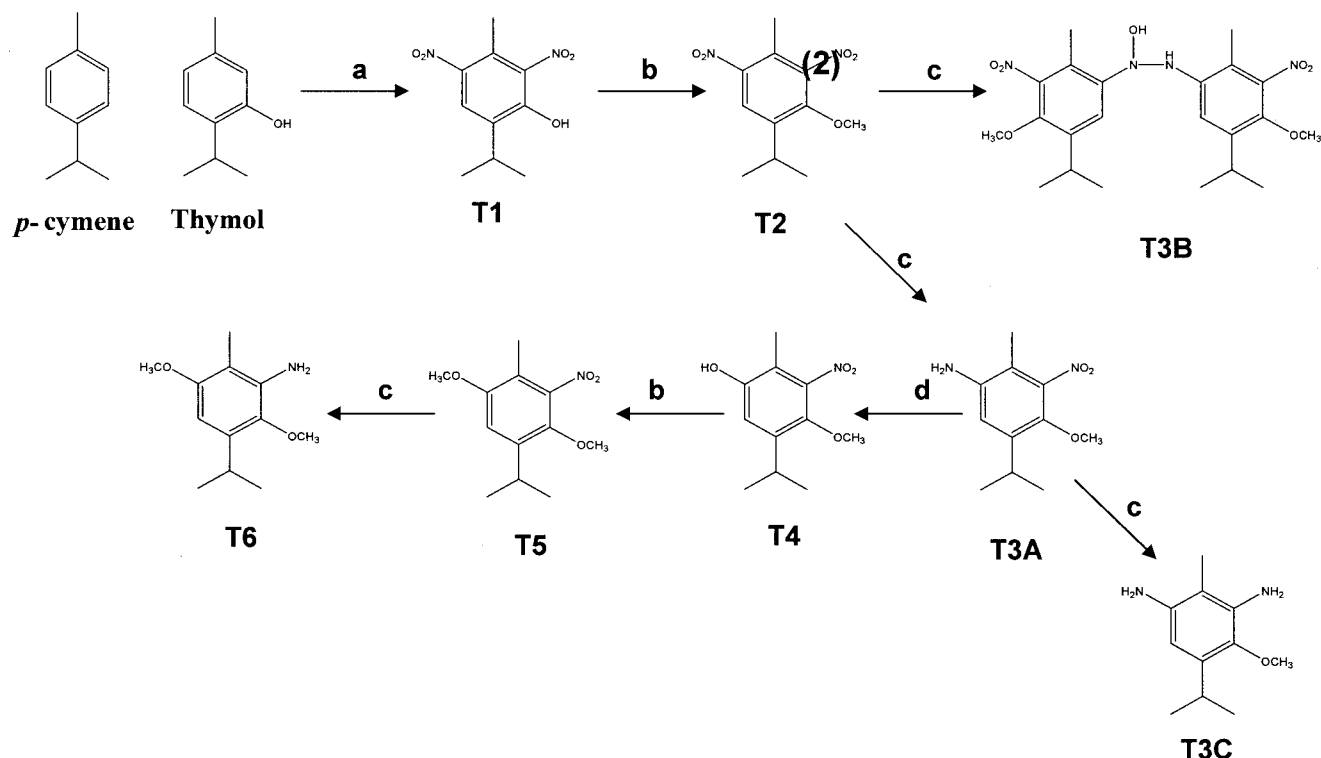
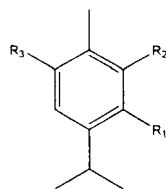


FIG. 1. The pathway for synthesis, the structure, and the chemical properties of aromatic monoterpenes derived from thymol. T1, 6-isopropyl-3-methyl-2,4-dinitro-phenol [ $C_{10}H_{12}N_2O_5$ , pf 51 to 52°C; UV (ethyl alcohol [EtOH])  $\lambda$  max (log  $\epsilon$ ) 211.0 (4.12), 267.0 (3.68), 399.0 (3.87) nm; (EtOH + NaOH, 0.1 M)  $\lambda$  max (log  $\epsilon$ ) 209.0 (4.11), 262.0 (3.72), 383.0 (4.12) nm; IR (KBr)  $\nu_{max}$  3,401 (OH), 3,037 (Ar-H), 2,969 (CH), 2,875 (CH<sub>3</sub>), 1,547 (NO<sub>2</sub>), 1,333 (NO<sub>2</sub>), 1,235 (CO, phenol); MS  $m/z$  241 [ $M + 1$ ]<sup>+</sup> (52), 240 [ $M$ ]<sup>+</sup> (15), 223 [ $M-OH$ ]<sup>+</sup> (100), 177 [ $M-OH-NO_2$ ]<sup>+</sup> (19), 149 (12), 119 (13), 104 (7), 91 [ $C_7H_7$ ]<sup>+</sup> (35), 39 (13); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.90 (1H, s, H-5), 3.40 (1H, sep,  $J = 7.0$  Hz, H-7), 1.30 (6H, d,  $J = 7.0$  Hz, H-8,9), 2.60 (3H, s, H-10), 9.80 (1H, br s, OH); <sup>13</sup>C NMR JMOD (CDCl<sub>3</sub>)  $\delta$  154.12 (C-1), 138.18 (C-2), 126.40 (C-3), 138.18 (C-4), 127.44 (C-5), 129.00 (C-6), 27.73 (C-7), 22.24 (C-8), 22.24 (C-9), 16.86 (C-10)]; T2, 1-isopropyl-2-methoxy-4-methyl-3,5-dinitro-benzene [ $C_{11}H_{14}N_2O_5$ , pf 80 to 82°C; UV (EtOH)  $\lambda$  max (log  $\epsilon$ ) 214.0 (4.11), 238.0 (4.04), 291.0 (3.41) nm; IR (KBr)  $\nu_{max}$  3,473 (NH<sub>2</sub>), 3,386 (NH<sub>2</sub>), 3,035 (Ar-H), 2,964 (CH), 2,870 (CH<sub>3</sub>), 1,630 (NH<sub>2</sub>), 1,526 (NO<sub>2</sub>), 1,377 (NO<sub>2</sub>), 1,240 (CO, ether), 1,109 (COC, ether) cm<sup>-1</sup>; MS  $m/z$  224 [ $M$ ]<sup>+</sup> (100), 209 (15), 207 (17), 175 (3), 164 (10), 147 (11), 91 (3), 39 (3); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.00 (1H, s, H-6), 3.35 (1H, sep,  $J = 7.0$  Hz, H-7), 1.30 (6H, d,  $J = 7.0$  Hz, H-8,9), 2.45 (3H, s, H-10), 3.90 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR JMOD (CDCl<sub>3</sub>)  $\delta$  131.00 (C-1), 152.50 (C-2), 143.52 (C-3), 124.96 (C-4), 143.52 (C-5), 125.24 (C-6), 27.31 (C-7), 23.59 (C-8), 23.59 (C-9), 14.58 (C-10), 64.25 (OCH<sub>3</sub>); T3A, *N,N'*-bis-(5-isopropyl-4-methoxy-2-methyl-3-nitro-phenyl)-*N*-hydroxy-hydrazine [ $C_{22}H_{30}N_4O_7$ , pf 140 to 142°C; UV (EtOH)  $\lambda$  max (log  $\epsilon$ ) 211.0 (4.41), 238.0 sh (4.10), 320.0 (4.08) nm; IR (KBr)  $\nu_{max}$  3,036 (Ar-H), 2,968 (CH), 2,872 (CH<sub>3</sub>), 1,532 (NO<sub>2</sub>), 1,455 (NOH), 1,340 (NO<sub>2</sub>), 1,266 (CO, ether), 1,102 (COC, ether), 1,054 (NOH) cm<sup>-1</sup>; MS  $m/z$  462 [ $M$ ]<sup>+</sup> (12), 429 (86), 355 (88), 73 (100); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.10 (1H, s, H-6), 7.20 (1H, s, H-6'), 2.90 (2H, sep, H-7,7'), 0.80 (12H, d, H-8,9,8',9'), 1.95 (3H, s, H-10), 1.87 (3H, s, H-10'), 3.53 (3H, s, OCH<sub>3</sub>), 3.48 (3H, s, OCH<sub>3</sub>), 3.02 (1H, br s, NH); <sup>13</sup>C NMR JMOD (CDCl<sub>3</sub>)  $\delta$  143.28 (C-1), 123.62 (C-2), 155.03 (C-3), 148.62 (C-4), 126.90 (C-5), 124.23 (C-6), 27.27 (C-7), 23.88 (C-8), 23.88 (C-9), 13.52 (C-10), 64.24 (OCH<sub>3</sub>), 139.00 (C-1'), 123.50 (C-2'), 157.16 (C-3'), 141.78 (C-4'), 126.00 (C-5'), 122.62 (C-6'), 27.27 (C-7'), 23.72 (C-8'), 23.72 (C-9'), 13.14 (C-10'), 64.10 (OCH<sub>3</sub>); T3C, 5-isopropyl-4-methoxy-2-methyl-benzene-1,3-diamine [ $C_{11}H_{18}N_2O$ , pf 94 to 95°C; UV (EtOH)  $\lambda$  max (log  $\epsilon$ ) 212.0 (4.08), 290.0 (3.52) nm; IR (KBr)  $\nu_{max}$  3,467 (NH<sub>2</sub>), 3,376 (NH<sub>2</sub>), 3,246 (NH<sub>2</sub>), 3,035 (Ar-H), 2,961 (CH), 2,870 (CH<sub>3</sub>), 1,610 (NH<sub>2</sub>), 1,225 (CO, ether), 1,151 (COC, ether) cm<sup>-1</sup>; MS  $m/z$  195 [ $M + 1$ ]<sup>+</sup> (60), 194 [ $M$ ]<sup>+</sup> (100), 179 (95), 162 (21), 136 (10), 91 (7), 65 (7), 39 (5); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.80 (1H, s, H-6), 3.40 (1H, sep,  $J = 6.9$  Hz, H-7), 1.20 (6H, d,  $J = 6.9$  Hz, H-8,9), 2.20 (3H, s, H-10), 3.90 (3H, s, OCH<sub>3</sub>), 3.09 (4H, br s, NH<sub>2</sub>); <sup>13</sup>C NMR JMOD (CDCl<sub>3</sub>)  $\delta$  142.08 (C-1), 112.35 (C-2), 140.79 (C-3), 142.21 (C-4), 131.20 (C-5), 114.55 (C-6), 26.73 (C-7), 24.00 (C-8), 24.00 (C-9), 12.12 (C-10), 64.13 (OCH<sub>3</sub>); T4, 5-isopropyl-4-methoxy-2-methyl-3-nitro-phenol [ $C_{11}H_{15}NO_4$ , pf 149 to 151°C; UV (EtOH)  $\lambda$  max (log  $\epsilon$ ) 214.0 (3.98), 278.0 (3.40) nm; (EtOH + NaOH 0.1 M)  $\lambda$  max (log  $\epsilon$ ) 213.0 (4.10), 241.0 (3.93), 297.0 (3.50) nm; IR (KBr)  $\nu_{max}$  3,403 (OH), 3,036 (Ar-H), 2,967 (CH), 2,872 (CH<sub>3</sub>), 1,530 (NO<sub>2</sub>), 1,374 (NO<sub>2</sub>), 1,197 (CO, phenol) cm<sup>-1</sup>; MS  $m/z$  226 [ $M + 1$ ]<sup>+</sup> (58), 225 [ $M$ ]<sup>+</sup> (100), 208 (58), 193 (2), 176 (4), 164 (8), 150 (10), 121 (18), 105 (8), 91 (18); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  6.75 (1H, s, H-6), 3.25 (1H, sep,  $J = 6.8$  Hz, H-7), 1.21 (6H, d,  $J = 6.8$  Hz, H-8,9), 2.12 (3H, s, H-10), 3.79 (3H, s, OCH<sub>3</sub>), 5.40 (1H, br s, OH); <sup>13</sup>C NMR JMOD (CDCl<sub>3</sub>)  $\delta$  151.02 (C-1), 115.21 (C-2), 142.70 (C-3), 142.70 (C-4), 129.15 (C-5), 115.50 (C-6), 27.11 (C-7), 24.26 (C-8), 24.26 (C-9), 14.60 (C-10), 64.49 (OCH<sub>3</sub>); T5, 1-isopropyl-2,5-dimethoxy-4-methyl-3-nitro-benzene [ $C_{12}H_{17}NO_4$ , pf 54 to 56°C; UV (EtOH)  $\lambda$  max (log  $\epsilon$ ) 209.0 (4.01), 276.0 (3.33) nm; IR (KBr)  $\nu_{max}$  3,036 (Ar-H), 2,966 (CH), 2,871 (CH<sub>3</sub>), 1,534 (NO<sub>2</sub>), 1,373 (NO<sub>2</sub>), 1,264 (CO, ether), 1,091 (COC, ether) cm<sup>-1</sup>; MS  $m/z$  240 [ $M + 1$ ]<sup>+</sup> (58), 239 [ $M$ ]<sup>+</sup> (100), 224 (12), 190 (3), 178 (12), 162 (17), 147 (4), 105 (7), 91 (6), 79 (2), 39 (3); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  6.80 (1H, s, H-6), 3.35 (1H, sep,  $J = 6.9$  Hz, H-7), 1.20 (6H, d,  $J = 6.9$  Hz, H-8,9), 2.10 (3H, s, H-10), 3.80 (3H, s, OCH<sub>3</sub>), 3.86 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C JMOD (CDCl<sub>3</sub>)  $\delta$  130.01 (C-1), 142.00 (C-2), 142.00 (C-3), 118.15 (C-4), 154.52 (C-5), 110.15 (C-6), 27.28 (C-7), 24.30 (C-8), 24.30 (C-9), 15.01 (C-10), 64.41 (OCH<sub>3</sub>), 56.81 (OCH<sub>3</sub>); and T6, 3-isopropyl-2,5-dimethoxy-6-methyl-phenylamine [ $C_{12}H_{19}NO_2$ , pf 67 to 69°C; UV (EtOH)  $\lambda$  max (log  $\epsilon$ ) 208.0 (4.02), 275.0 (3.30) nm. IR (KBr) 3,469 (NH<sub>2</sub>), 3,379 (NH<sub>2</sub>), 3,036 (Ar-H), 2,966 (CH), 2,870 (CH<sub>3</sub>), 1,534 (NH<sub>2</sub>), 1,132 (CO, ether), 1,091 (COC, ether) cm<sup>-1</sup>. MS  $m/z$  209 [ $M$ ]<sup>+</sup> (100), 194 (49), 177 (7), 162 (4), 134 (8), 119 (5), 91 (1), 65 (1); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  6.20 (1H, s, H-6), 3.30 (1H, m, H-7), 1.20 (6H, d, H-8,9), 2.00 (3H, s, H-10), 3.80 (3H, s, OCH<sub>3</sub>), 3.76 (3H, s, OCH<sub>3</sub>), 3.34 (2H, br s, NH<sub>2</sub>); <sup>13</sup>C NMR JMOD (CDCl<sub>3</sub>)  $\delta$  140.01 (C-1), 156.10 (C-2), 139.86 (C-3), 98.38 (C-4), 156.00 (C-5), 109.70 (C-6), 27.15 (C-7), 24.58 (C-8), 24.58 (C-9), 15.01 (C-10), 61.13 (OCH<sub>3</sub>), 56.43 (OCH<sub>3</sub>). Reaction conditions: (a) HNO<sub>3</sub>/CH<sub>3</sub>COOH; (b) (CH<sub>3</sub>)<sub>2</sub>SO<sub>4</sub>/Na<sub>2</sub>CO<sub>3</sub>-acetone; (c) Fe/HCl-CH<sub>3</sub>OH; (d) NaNO<sub>2</sub>/H<sub>2</sub>SO<sub>4</sub>.

TABLE 1. In vitro cytotoxicities and anti-leishmanial activities of aromatic monoterpene isoprenoids<sup>a</sup>

Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	LC <sub>50</sub> <sup>b</sup>	EC <sub>50</sub> <sup>c</sup>		IS <sup>d</sup>	
					Prom	Am	Pro	Am
Thymol	OH	H	H	>400.0 ± 0	194.3 ± 3.9	>400.0 ± 0	>2.0	>1.0
T1	OH	NO <sub>2</sub>	NO <sub>2</sub>	101.7 ± 0.7	0.4 ± 0	<b>58.8 ± 7.7</b>	148.1	1.7
T2	OCH <sub>3</sub>	NO <sub>2</sub>	NO <sub>2</sub>	176.3 ± 18.7	0.31 ± 0.03	176.3 ± 0	568.7	1.0
T3A	OCH <sub>3</sub>	NO <sub>2</sub>	NH <sub>2</sub>	410.4 ± 2.4	17.1 ± 1.0	318.0 ± 34.3	24.0	1.3
T3B	OCH <sub>3</sub>	NO <sub>2</sub>	C <sub>11</sub> N <sub>3</sub> H <sub>16</sub> O <sub>4</sub>	220.2 ± 25.7	10.0 ± 1.0	210.0 ± 0	22.0	1.0
T3C	OCH <sub>3</sub>	NH <sub>2</sub>	NH <sub>2</sub>	87.1 ± 6.3	8.2 ± 1.2	<b>26.7 ± 1.0</b>	10.6	3.3
T4	OCH <sub>3</sub>	NO <sub>2</sub>	OH	376.9 ± 74.4	>100.0 ± 0.0	<b>43.4 ± 1.5</b>	NC <sup>e</sup>	<b>8.7</b>
T5	OCH <sub>3</sub>	NO <sub>2</sub>	OCH <sub>3</sub>	368.3 ± 85.0	>100.0 ± 0	131.5 ± 0.9	NC	2.8
T6	OCH <sub>3</sub>	NH <sub>2</sub>	OCH <sub>3</sub>	23.8 ± 0.4	>100.0 ± 0	<b>13.6 ± 4.0</b>	NC	1.8
Glucantime				416.4 ± 0	400.0 ± 1.2	6.7 ± 0.1	1.0	62.1

<sup>a</sup> All the data reported are the average of three independent experiments conducted in triplicate and are expressed as the mean plus or minus the standard deviation. R, chemical substitution.

<sup>b</sup> Cytotoxic activity (50% lethal concentration) in U-937 human promonocytic cells (μg/ml) ± SD.

<sup>c</sup> Leishmanicidal activity (μg/ml) ± SD; Prom, promastigotes; Am, amastigotes. Boldface indicates the most active compounds.

<sup>d</sup> IS, index of selectivity (LC<sub>50</sub> in U-937/50% effective concentration [EC<sub>50</sub>] in Prom or Am).

<sup>e</sup> NC, not calculated.

The spectra of <sup>1</sup>H NMR showed the protons of the isopropyl chain in the form of an A<sub>6</sub>X system. The protons of the aromatic methyl in position *para* resonate as a singlet of about 2.2 to 2.4 ppm. MS studies showed peaks for the oxygenated derivatives of *p*-cymene at [M-15]<sup>+</sup>, due to loss of the methyl group and the formation of a substituted hydroxytropylium ion. The presence of the tropylium ion [C<sub>7</sub>H<sub>7</sub>]<sup>+</sup> at *m/z* = 91 is also characteristic. Peaks were also observed at [M-43]<sup>+</sup>, these being due to the elimination of the isopropyl chain.

The cytotoxic and antileishmanial activities for each of the compounds are summarized in Table 1. Only the T6 compound showed leishmanicidal activity against amastigotes at concentrations lower than 20 μg/ml, and the T1, T3C, and T4 compounds were active at concentrations less than 60 μg/ml. Similar to previous observations (4, 5, 14, 15), the activity against

promastigotes was substantially different from that detected against amastigotes (Fig. 2), but unlike Glucantime, most of the compounds presented stronger activity against promastigotes than amastigotes. Given that the amastigote is the parasitic form responsible for the disease, it should constitute the chemotherapeutic target in studies of new antileishmanial agents. Most of the compounds were shown to be toxic only at concentrations of >100 μg/μl (Table 1). Indeed, the toxicity of the T3A compound was comparable to that of Glucantime. Although none of the compounds proved to be better than Glucantime, a similar reduction in lesion size was obtained after treatment with T3A; however, as can be seen in Table 2, while Glucantime essentially eradicated the parasite from the lesion, only thymol and T1 decreased the parasite burden, by 46 and 23%, respectively.

None of the compounds seem to be toxic to the animals

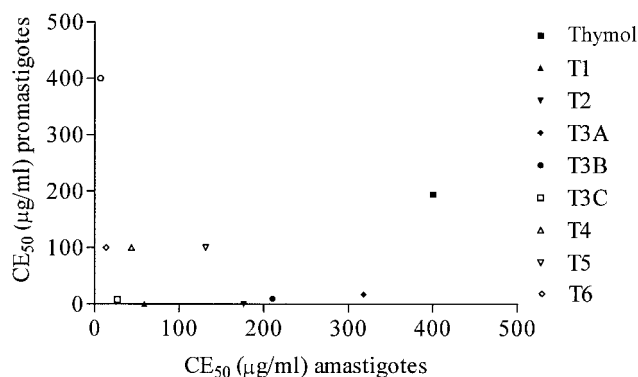


FIG. 2. Activities of monoterpene isoprenoids: correlation between intracellular amastigotes and promastigotes of *L. (V.) panamensis*. The mean of the results obtained in triplicate with both promastigotes and intracellular amastigotes is expressed as 50% of the effective concentration (CE<sub>50</sub>), calculated by the probit method. Spearman *r* = 0.4068.

TABLE 2. In vivo cytotoxicities and antileishmanial activities of aromatic monoterpene isoprenoids<sup>a</sup>

Compound	Corporal weight (mg) on day <sup>b</sup> :			% Reduction of lesion size on day <sup>b</sup> :		% Reduction of parasite burden 30 days posttreatment	
	0	15	30	15	30	Giemsa stain smears	Limiting dilution assay
Thymol	112.3	118.5	127.5	0	0	67.8	46.0
T1	88.7	90.2	91.5	5.7	5.7	33.5	23.5
T2	119.1	124.6	133.1	0	0	0	0
T3A	112.4	123.8	120.2	15.1	26.7	0	0
T3B	104.7	112.5	113.5	0	0	0	0
T4	102.0	116.9	121.2	0	0	0	0
Glucantime	96.8	100.1	101.8	16.7	18.8	100	100
Vehicle	110.4	109.0	111.7	0	0	0	0

<sup>a</sup> Mean of five animals per group.

<sup>b</sup> Days posttreatment.

based on corporal weight, behavior, and serum levels of bilirubin, uric acid, and glucose; however, more complete studies to test their toxicity potential need to be carried out. While complete cure did not occur, the absence of toxicity of these products will facilitate daily oral treatment for longer periods. In conclusion, these results suggest that thymol and its derivatives have promising antileishmanial potential as oral therapy and could be considered new lead structures in the search for novel antileishmanial drugs.

Our thanks to Ferney Ospina for synthesis of the compounds and to Wiston Quiñones for  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra. We gratefully acknowledge J. Santiago Mejía for valuable suggestions and comments.

This study was funded by Colciencias (contract 1115-05-353-96), the ECOS-Nord/ICFES/COLCIENCIAS/ICETEX programme (code no. 99SO3), and Universidad de Antioquia (code SCPP33).

#### REFERENCES

1. Aeschbach, R., J. Loliger, B. C. Scott, A. Murcia, J. Butler, B. Halliwell, and O. I. Aruoma. 1994. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chem. Toxicol.* **32**:31–36.
2. Castro, C., M. Jiménez, and M. González. 1992. Inhibitory effect of piquerol A on the growth of epimastigotes of *Trypanosoma cruzi*. *Planta Med.* **58**: 281–282.
3. Cenas, N., A. Nemeikaite, E. Sergedienė, H. Nivinskas, Z. Anusevicius, and J. Sarlauskas. 2001. Quantitative structure activity relationships in enzymatic single-electron reduction of nitroaromatic explosives: implications for the cytotoxicity. *Biochim. Biophys. Acta* **1528**:31–38.
4. Chulay, J., L. Fleckenstein, and D. Smith. 1988. Pharmacokinetics of antimony during treatment of visceral leishmaniasis with sodium stibogluconate or meglumine antimoniate. *Trans. R. Soc. Trop. Med. Hyg.* **82**:69–72.
5. Delmas, F., C. Di Giorgio, M. Robin, N. Azas, M. Gasquet, C. Detang, M. Costa, P. Timon-David, and J. P. Galy. 2002. In vitro activities of position 2 substitution-bearing 6-nitro- and 6-amino-benzothiazoles and their corresponding anthranilic acid derivatives against *Leishmania infantum* and *Trichomonas vaginalis*. *Antimicrob. Agents Chemother.* **46**:2588–2594.
6. Didry, N., L. Dubreuil, and M. Pinkas. 1994. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharm. Acta Helv.* **69**:25–28.
7. Finney, J. D. 1971. Probit analysis: statistical treatment of the sigmoid response curve, 3rd ed. Cambridge University Press, Cambridge, United Kingdom.
8. Hocquemiller, R., D. Cortes, G. Arango, S. Myint, A. Cave, A. Angelo, V. Munoz, and A. Fournet. 1991. Isolation et synthèse de l'espintanol, nouveau monoterpène antiparasitaire. *J. Nat. Prod.* **54**:445–452.
9. Kayser, O., and A. Kiderlen. 2003. Delivery strategies for antiparasitics. *Exp. Opin. Investig. Drugs* **12**:197–207.
10. Kulevanova, S., A. Kaftandzieva, A. Dimitrovska, G. Stefkov, T. Grdanoska, and N. Panovski. 2000. Investigation of antimicrobial activity of essential oils of several Macedonian Thymus L. species (Lamiaceae). *Boll. Chim. Farm.* **139**:276–280.
11. Mikus, J., M. Harkenthal, D. Steverding, and J. Reichling. 2000. In vitro effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. *Planta Med.* **66**:366–368.
12. Ogaard, B., E. Larsson, R. Glans, T. Henriksson, and D. Birkhed. 1997. Antimicrobial effect of a chlorhexidine-thymol varnish (Cervitec) in orthodontic patients. A prospective, randomized clinical trial. *J. Orofac. Orthop.* **58**:206–213.
13. Osorio, Y., P. C. Melby, C. Primes, B. Chandrasekar, N. Guarín, and B. Travi. 2003. The site of cutaneous infection influences the immunological response and clinical outcome of hamsters infected with *Leishmania panamensis*. *Parasite Immunol.* **25**:139–148.
14. Rhayour, K., T. Bouchikhi, A. Tantaoui Elaraki, K. Sendide, and A. Remmal. 2003. The mechanism of bactericidal action of oregano and clove essential oils and of their phenolic major components on *Escherichia coli* and *Bacillus subtilis*. *J. Essent. Oil Res.* **15**:286–292.
15. Robledo, S., A. Valencia, and N. Saravia. 1999. Sensitivity to Glucantime of *Leishmania Viannia* isolated from patients prior to treatment. *J. Parasitol.* **85**:360–366.
16. Shapiro, S., and B. Guggenheim. 1995. The action of thymol on oral bacteria. *Oral Microbiol. Immunol.* **10**:241–246.
17. Travi, B., J. E. Martínez, and A. Zea. 1993. Antimonial treatment of hamsters infected with *Leishmania (V.) panamensis*: assessment of parasitological cure with different therapeutic schedules. *Trans. R. Soc. Trop. Med. Hyg.* **87**:567–569.
18. Travi, B., Y. Osorio, P. C. Melby, B. Chandrasekar, L. Arteaga, and N. Saravia. 2002. Gender is a major determinant of the clinical evolution and immune response in hamsters infected with *Leishmania* spp. *Infect. Immun.* **70**:2288–2296.
19. Weniger, B., S. Robledo, G. Arango, E. Deharo, R. Aragon, V. Munoz, J. Callapa, A. Lobstein, and R. Anton. 2001. Antiprotozoal activities from Colombian plants. *J. Ethnopharmacol.* **78**:193–200.