J. Braz. Chem. Soc., Vol. 13, No. 2, 151-157, 2002.
Printed in Brazil - ©2002 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Novel Derivatives of Kaurenoic Acid: Preparation and Evaluation of their Trypanocidal Activity

Henriete S. Vieira[a], Jacqueline A. Takahashi[a], Alaíde B. de Oliveira[b], Egler Chiari[c] and Maria Amélia D. Boaventura*[*a]

[a]*Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte - MG, Brazil*

[b]*Departamento de Produtos Farmacêuticos, Faculdade de Farmácia , Universidade Federal de Minas Gerais, Av. Olegário Maciel, 2360, 30180-112, Belo Horizonte - MG, Brazil*

[c]*Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte - MG, Brazil*

O ácido caurenóico, um diterpeno caurânico, mostrou-se ativo *in vitro* contra formas tripomastigotas do *Trypanosoma cruzi*. Uma ação lítica sobre os eritrócitos foi uma das limitações encontradas para esta atividade. A síntese de doze derivados deste ácido: quatro amidas, quatro aminas (e três cloridratos) e quatro oximas foi realizada, com o objetivo de se tentar diminuir ou eliminar esse efeito secundário e, se possível, aumentar a atividade em relação ao material de partida. Dentre esses compostos, um mostrou-se mais ativo que o ácido caurenóico, mas também apresentou lise discreta de eritrócitos; outro não apresentou este efeito, mas a atividade não foi aumentada em relação àquela apresentada pelo ácido caurenóico.

Kaurenoic acid, a kauranic diterpenoid, presents *in vitro* activity against trypomastigote forms of *Trypanosoma cruzi*, showing, however, lytic activity on blood erythrocytes, as a side effect. The syntheses of twelve new derivatives of kaurenoic acid, four amides, four amines (and three hydroclorides) and four oximes, was carried out aiming at the improvement of the therapeutic activity and without the side effect. Among the derivatives prepared, one compound showed enhanced trypanocidal activity *in vitro* towards *Trypanosoma cruzi* trypomastigote erythrocytic forms, when compared to kaurenoic acid, but continued to show discrete lytic activity on erythrocytes; another compound showed a level of activity similar to that of kaurenoic acid, but without lysis.

Keywords: kaurenoic acid, kaurenic hydrochloride salts, kauranic oximes, trypanocidal activity

Introduction

Chagas' disease affects approximately 24 million people from Southern California to Argentina and Chile.[1] The agent of this disease, *Trypanosoma cruzi*, is transmitted by the faeces of triatomine bugs (like *Triatoma infestans*), which are hematophagous insects. Transmission by blood transfusion is an important source of infection in urban areas, through migration of infected persons from endemic rural areas. Gentian violet is used to treat blood for transfusion but it is far from satisfactory for this purpose.[2] In the search for a suitable replacement for blood clearance many compounds have been tested.[3-6]

Kaurenoic acid (**1**) is known to exhibit interesting biological activities, including antimicrobial, cytotoxic, antiinflammatory and antiprotozoal properties.[7] Alves *et al*.[8] have described its activity against trypomastigote forms of *T. cruzi*. However, the acid showed a blood lytic activity on erythrocytes and also low solubility in the biological medium used for the test.

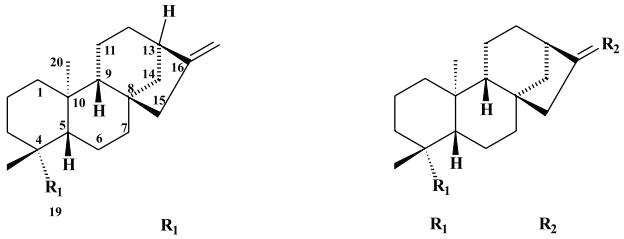
We describe in this paper the preparation of some derivatives of kaurenoic acid (**1**) aiming at the improvement of the trypanocidal activity, without the previously mentioned inconvenients.

Experimental

General experimental procedures

Melting points were determined with a Kofler hot plate apparatus and are uncorrected. The optical rotations were measured on a Perkin Elmer 341 polarimeter. IR spectra

*e-mail: dianadb@dedalus.lcc.ufmg.br



	R_1
1	CO_2H
2	$CON(CH_2CH_2CH_3)_2$
3	$CON(CH_2CH_3)_2$
4	CON
5	CON
6	$CH_2N(CH_2CH_2CH_3)_2$
7	$CH_2N(CH_2CH_3)_2$

	R_1
8	CH_2N
9	CH_2N
10	$CH_2N^+H(CH_2CH_2CH_3)_2\ .Cl^-$
11	$CH_2N^+H\ Cl^-$
12	$CH_2N^+H\ Cl^-$

	R_1	R_2
13	CO_2CH_3	CH_2
14	CH_2OH	CH_2
15	CO_2CH_3	O
16	CH_2OH	O
17	CO_2CH_3	NOH (Z)
18	CO_2CH_3	NOH (E)
19	CH_2OH	NOH (Z)
20	CH_2OH	NOH (E)

were recorded on a Shimadzu/IR-408 spectrophotometer. IR absorption bands are expressed in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker Avance DPX 200 operating at 200 and 50 MHz. The chemical shifts are reported in δ values (ppm) relative to the solvent $CDCl_3$ (δ = 7.26 for ^1H NMR and δ 77.01 for ^{13}C NMR). Mass spectrometry spectra (GC-EI-MS) were obtained from a GCQ Finnigan-ION TRAP instrument and they were performed with an ionizing energy of 70 eV. Silica Gel used for flash chromatography was Merck WC4790-005 (230-400 mesh).

Kaurenoic acid (**1**) was isolated from green fruits of *Xylopia frutescens*,[9] *Xylopia sericea*[10] and aerial parts of *Wedelia paludosa*,[11] using the same procedures.

Syntheses

Amides: A mixture of triphenylphosphine (30.0 mmol) and carbon tetrachloride (50.0 mL) was heated under reflux for 5h under nitrogen. The solution was then cooled, kaurenoic acid (**1**, 3.0 mmol), $[\alpha]_D^{25}$: -114.81° ($CHCl_3$, c 1.9), in carbon tetrachloride, was added and the reaction mixture heated under reflux for 0.5h.[12] The solvent was removed, the amine (60.0 mmol) was added to the residue containing the unstable acid chloride and the mixture kept for 24h, at room temperature, under nitrogen. After removal of excess of amine, the residue was submitted successively to chromatography on a silica gel column with ascendant polarities of hexane/ethyl acetate and to flash chromatography on silica gel (hexane/ethyl acetate 9:1).

ent-kaur-16-en-19-N,N-di-n-propylamide (**2**, 623 mg, 49%): oil ; $[\alpha]_D^{25}$: - 22.7° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 2900 (C-H), 1650 (C=C), 1625 (C=O) (KBr); ^1H NMR (200 MHz, $CDCl_3$) δ 0.88 (6H, t, *J* 7.4 Hz, 3'-CH_3), 0.97 (3H, s, 20-CH_3), 1.25 (3H, s, 18-CH_3), 1.40-1.80 (4H, m, 2'-CH_2), 2.61 (1H, br s, 13-CH), 3.22 (4H, br t, *J* 8.0 Hz, 1'-CH_2), 4.72 (1H, br s, 17a-CH_2) and 4.78 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1; EI-MS: *m/z* (rel. int.) 385 (53) ($M^{\bullet+}$), 370 (18) (M^+-CH_3), 342 (15) (M^+-C_2H_4), 257 (100) (M^+-$CONC_6H_{14}$).

ent-kaur-16-en-19-N,N-diethylamide (**3**, 540 mg, 46%): white crystals, mp 98 °C; $[\alpha]_D^{25}$: - 33.0° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 2900 (C-H), 1650 (C=C), 1625 (C=O) (KBr); ^1H NMR (200 MHz, $CDCl_3$) δ 0.98 (3H, s, 20-CH_3), 1.24 (3H, s, 18-CH_3), 1.3 (6H, t, *J* 7.4 Hz, 2'-CH_3), 2.62 (1H, br s, 13-CH), 3.10-3.40 (4H, m, 1'-CH_2), 4.72 (1H, br s, 17a-CH_2) and 4.77 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1.

ent-kaur-16-en-19-pyrrolidinamide (**4**, 725 mg, 62%): white crystals, mp 69 °C; $[\alpha]_D^{25}$: - 43.9° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 2900 (C-H), 1650 (C=C), 1625 (C=O) (KBr); ^1H NMR (200 MHz, $CDCl_3$) δ 0.95 (3H, s, 20-CH_3), 1.19 (3H, s, 18-CH_3), 1.6-2.0 (4H, m, 2'-CH_2), 2.61 (1H, br s, 13-CH), 3.20-3.60 (4H, m, 1'-CH_2), 4.72 (1H, br s, 17a-CH_2) and 4.77 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1; EI-MS: *m/z* (rel. int.) 355 (60) ($M^{\bullet+}$), 340 (35) (M^+-CH_3), 327 (10) (M^+-C_2H_4), 312 (25) (M^+-C_3H_7), 257 (43) (M^+-$CONC_4H_8$), 113 (100) (M^+-242).

ent-kaur-16-en-19-piperidinamide (**5**, 835 mg, 68%): white crystals, mp 95 °C; $[\alpha]_D^{25}$: - 13.65° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 2900 (C-H), 1650 (C=C), 1625 (C=O) (KBr); ^1H NMR (200 MHz, $CDCl_3$) δ 0.97 (3H, s, 20-CH_3), 1.26 (3H, s, 18-CH_3), 1.30-1.70 (6H, m, 2'- CH_2 and 3'-CH_2), 2.63 (1H, br s, 13-CH), 3.49 (4H, br t, *J* 5,0 Hz, 1'-CH_2), 4.72 (1H, br s 17a-CH_2) and 4.78 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1. EI-MS: *m/z* (rel. int.) 369

(56) ($M^{\bullet+}$), 354 (28) (M^+-CH_3), 326 (22) (M^+-C_3H_7), 257 (47) (M^+-$CONC_6H_{10}$-), 127 (100) (M^+-242).

Amines: The amide (1.4 mmol), in dry THF (10.0 mL), was cooled to 0 °C, under nitrogen, and treated with DIBAL-H (7.0 mL, 25% in toluene, 12.0 mmol). After stirring at room temperature for 1h, the excess of the reagent was destroyed by addition of MeOH (10 mL) and H_2O (0.5 mL).[13] The solid aluminium salts were filtered, the organic solvent was removed and the aqueous phase was extracted several times with CH_2Cl_2. The solvent was evaporated under reduced pressure to give the pure amine.

ent-kaur-16-en-19-N,N-di-n-propylamine (**6**, 385 mg, 80%): oil ; $[\alpha]_D^{25}$: - 108.1° ($CHCl_3$, c 0.77); IR ν/cm^{-1}:2900 (C-H), 1650 (C=C) (KBr) ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.85 (6H, t, *J* 7.2 Hz, 3'-CH_3), 0.91 (3H, s, 18-CH_3), 1.01 (3H, s, 20-CH_3), 1.30-1.50 (4H, m, 2'-CH_2), 2.00-2.10 (1H, m, 19a-CH_2), 2.29-2.39 (4H, m, 1'-CH_2), 2.59 (1H, d, *J* 14.2 Hz, 19b-CH_2), 2.63 (1H, br s, 13-CH), 4.73 (1H, br s, 17a-CH_2) and 4.78 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1.

ent-kaur-16-en-19-N,N-diethylamine (**7**, 360 mg, 75%): white solid, mp 48 °C; $[\alpha]_D^{25}$: - 32.2° ($CHCl_3$, c 0.77); IR ν/cm^{-1}: 2900 (C-H), 1650 (C=C) (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (3H, s, 18-CH_3), 0.96 (6H, t, *J* 7.0 Hz, 2'-CH_3), 1.02 (3H, s, 20-CH_3), 1.80-2.10 (1H, m, 19a-CH_2), 2.48 (4H, q, *J* 7.0 Hz, 1'-CH_2), 2.58 (1H, d, *J* 14.2.Hz, 19b-CH_2), 2.62 (1H, br s, 13-CH), 4.72 (1H, br s, 17a-CH_2) and 4.77 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1.

ent-kaur-16-en-19-pyrrolidinamine (**8**, 417 mg, 87%): white solid, mp 91 °C; $[\alpha]_D^{25}$: - 70.8° ($CHCl_3$, c 0.77); IR ν/cm^{-1}: 2900 (C-H), 1650 (C=C) (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.95 (3H, s, 18-CH_3), 1.02 (3H, s, 20-CH_3), 1.90-2.30 (4H, m, 2'-CH_2), 2.26 (1H, d, *J* 13.4 Hz, 19b-CH_2), 2.45-2.65 (5H, m, 1'-CH_2 and 13-CH), 2.66 (1H, d, *J* 13.4 Hz, 19b-CH_2), 4.72 (1H, br s, 17a-CH_2) and 4.78 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1.

ent-kaur-16-en-19-piperidinamine (**9**, 400 mg, 83%): white solid, mp 99 °C; $[\alpha]_D^{25}$: - 41.7° ($CHCl_3$, c 0.77); IR ν/cm^{-1}: 2900 (C-H), 1650 (C=C) (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89 (3H, s, 18-CH_3), 1.02 (3H, s, 20-CH_3), 1.20-1.50 (2H, m, 3'-CH_2), 1.50-1.70 (4H, m, 2'-CH_2), 1.80-2.00 (1H, m, 19a-CH_2), 2.20-2.50 (4H, m, 1'-CH_2), 2.40-2.60 (1H, m, 19b-CH_2), 2.63 (1H, br s, 13-CH), 4.73 (1H, br s, 17a-CH_2) and 4.78 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1.

Hydrochloride salts: The amine (0.2 mmol), in dry CH_2Cl_2 (5.0 mL), was treated with gaseous hydrochloric acid, generated *in situ* from sulfuric acid and sodium chloride, for 10 min at room temperature, to give the hydrochloride.

ent-kaur-16-en-19-N,N-di-n-propylamonium chloride (**10**, 42 mg, 50%): oil; $[\alpha]_D^{25}$: - 51.5° (CH_3OH, c 0.77); IR ν/cm^{-1}: 2900 (C-H), 2700 (N^+-H), 1650 (C=C) (KBr); ^{1}H

Table 1. ^{13}C NMR chemical shift values for diterpenes **1** to **12**

C	1	2	3	4	5	6	7	8	9	10	11	12
1	40.7	41.9	41.9	41.7	41.9	40.6	40.6	40.7	40.6	39.9	39.7	41.0
2	19.1	20.4	20.4	19.9	20.2	20.1	18.6	18.5	18.7	17.9	17.8	19.1
3	37.8	39.4	39.5	38.6	39.5	36.8	36.6	37.5	36.4	36.1	36.7	37.4
4	43.7	46.5	46.3	45.9	46.1	44.0	38.9	38.6	39.2	38.2	37.3	38.3
5	57.1	62.4	62.4	61.6	61.6	57.5	57.3	57.6	56.9	57.4	57.7	59.4
6	21.8	21.4	23.1	23.0	22.9	18.1	20.1	20.2	20.1	18.1	19.8	20.9
7	41.3	42.4	42.4	42.3	42.3	41.6	41.6	41.6	41.7	41.1	40.7	42.2
8	44.2	44.5	44.5	44.5	44.5	44.2	44.2	43.9	44.2	43.9	43.8	45.2
9	55.1	56.4	56.4	56.1	56.4	56.4	56.4	56.4	56.4	56.0	55.8	57.5
10	39.7	40.1	40.1	40.0	40.1	39.3	39.2	39.3	39.3	38.9	38.9	40.3
11	18.4	18.5	18.5	18.5	18.5	18.5	18.2	18.2	18.2	17.9	17.7	18.9
12	33.1	33.1	33.2	33.2	33.2	33.3	33.2	33.3	33.3	32.9	32.9	34.2
13	43.8	43.8	43.9	43.9	43.9	44.0	43.9	44.0	44.0	43.7	43.6	45.3
14	39.7	39.6	39.6	39.5	40.1	39.8	39.8	39.8	39.8	39.5	39.5	40.7
15	48.9	48.9	48.9	49.0	49.0	49.1	49.0	49.1	49.0	48.7	48.6	50.0
16	155.9	156.3	156.4	156.3	156.3	156.1	155.8	155.9	156.0	155.3	155.0	156.5
17	102.9	102.6	102.6	102.7	102.7	102.8	102.9	102.9	102.8	102.9	103.2	103.8
18	28.9	28.3	28.0	27.3	27.9	28.7	28.6	29.2	28.4	28.2	28.3	26.9
19	184.3	175.9	175.8	176.0	176.4	57.2	55.7	59.4	60.5	56.7	61.5	63.0
20	15.6	18.7	18.7	18.1	18.3	18.6	18.6	18.6	18.6	18.4	18.1	18.7
1'	-	50.7	42.5	48.3	47.2	58.7	49.2	57.4	57.7	58.2	58.2/ 57.7	58.0
2'	-	23.1	13.5	24.9	26.2	20.1	11.9	24.1	26.7	19.9	23.7/ 23.3	23.4
3'	-	11.3	-	-	24.8	11.9	-	-	24.2	11.4	-	22.2

NMR (200 MHz, CD_3OD) δ 0.96 (6H, t, *J* 6.2 Hz, 3'-CH_3), 1.01 (3H, s, 20-CH_3), 1.35 (3H, s, 18-CH_3), 1.55-1.65 (4H, m, 2'-CH_2), 2.55-2.70 (2H, m, 19a-CH_2 and 13-CH), 2.80-3.10 (4H, m, 1'-CH_2), 3.26 (1H, d, *J* 12.4 Hz, 19b-CH_2), 4.75 (1H, br s, 17a-CH_2), 4.80 (1H, br s, 17b-CH_2) and 10.9-11.0 (1H, N^+-H); ^{13}C NMR (50 MHz, CD_3OD): see Table 1.

ent-kaur-16-en-19-pyrrolidinamonium chloride (**11**, 53 mg, 78%): mp 256 °C; $[\alpha]_D^{25}$: - 64.8° (CH_3OH, c 0.54); IR ν/cm^{-1}: 2900 (C-H), 2700 (N^+-H), 1650 (C=C) (KBr); ^1H NMR (200 MHz, CD_3OD) δ 0.97 (3H, s, 20-CH_3), 1.35 (3H, s, 18-CH_3), 2.00-2.15 (2H, m, 2'a-CH_2), 2.30-2.50 (2H, m, 2'b-CH_2), 2.55-2.75 (2H, m, 1'a-CH_2), 2.65 (1H, br s, 13-CH), 2.86 (1H, br d, *J* 13.2 Hz, 19a-CH_2), 3.35 (1H, d, *J* 13.2 Hz, 19b-CH_2), 3.80-4.30 (2H, m, 1'b-CH_2), 4.75 (1H, br s, 17a-CH_2), 4.81 (1H, br s, 17b-CH_2) and 11.0-11.3 (1H, N^+-H); ^{13}C NMR (50 MHz, CD_3OD): see Table 1.

ent-kaur-16-en-19-piperidinamonium chloride (**12**, 55 mg, 70%): mp 203 °C; $[\alpha]_D^{25}$: - 48.5° (CH_3OH, *c* 0.62); IR ν/cm^{-1}: 2900 (C-H), 2700 (N^+-H), 1650 (C=C) (KBr); ^1H NMR (200 MHz, CD_3OD) δ 1.01 (3H, s, 20-CH_3), 1.13 (3H, s, 18-CH_3), 1.20-1.30 (2H, m, 3'-CH_2), 1.70-1.90 (4H, m, 2'-CH_2), 2.90-3.20 (1H, N^+-H), 2.55 (1H, br s, 13-CH), 2.85 (1H, d, *J* 13.8 Hz, 19a-CH_2), 3.30-3.41 (4H, m, 1'-CH_2), 3.37 (1H, d, *J* 13.8 Hz, 19b-CH_2), 4.65 (1H, br s, 17a-CH_2) and 4.71 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, CD_3OD): see Table 1.

Route for Oximes: Oximes were prepared following the sequence:
carboxylic acid → methyl ester → norketone → oximes **17** and **18**;
carboxylic acid → methyl ester → alcohol → norketone → oximes **19** and **20**.

Methyl ent-kaur-16-en-19-oate (**13**): Kaurenoic acid (**1**, 1.0 g, 3.3 mmol) in ethyl ether was treated with diazomethane in ethyl ether (obtained from Diazald treated with potassium hydroxide) to yield the methyl *ent*-kaur-16-en-19-oate (**13**, 1.0 g, 100%): mp 82 °C (lit.[14] 84-86 °C); $[\alpha]_D^{25}$: - 107.5° ($CHCl_3$, c 1.2); IR ν/cm^{-1}: 2900 (C-H), 1725 (C=O), 1650 (C=C) (KBr); ^1H NMR (200 MHz, $CDCl_3$) δ 0.83 (3H, s, 20-CH_3), 1.17 (3H, s, 18-CH_3), 2.64 (1H, br s, 13-CH), 3.64 (3H, s, 21-CH_3), 4.74 (1H, br s, 17a-CH_2) and 4.77 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2.

ent-kaur-16-en-19-ol (**14**): Methyl ester **13** (500 mg, 1.6 mmol), in dry THF (50 mL), was added dropwise to a suspension of $LiAlH_4$ (50.0 mg, 1.3 mmol), in dry THF (10.0 mL) under nitrogen. After 3h reflux, ethyl acetate was added, the mixture was washed with dil. NaOH and the product was recovered from ethyl acetate. It was submitted to flash chromatography on silica gel (hexane/ethyl acetate 7:3) to yield *ent*-kaur-16-en-19-ol (**14**, 382 mg, 84%), crystallized from $CHCl_3$: mp 140 °C (lit.[14]141-143 °C); $[\alpha]_D^{25}$: - 68.4° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 3400, (O-H), 2900 (C-H), 1650 (C=C) (KBr); ^1H NMR (200 MHz, $CDCl_3$) δ 0.96 (3H, s, 18-CH_3), 1.01 (3H, s, 20-CH_3), 2.63 (1H, m, 13-CH), 3.44 (1H, d, *J* 10.9 Hz, 19a-CH_2), 3.75 (1H, d, *J* 10.9 Hz, 19b-CH_2), 4.73 (1H, br s, 17a-CH_2) and 4.79 (1H, br s, 17b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2.

Table 2. ^{13}C NMR Chemical Shift Values for Diterpenes **13** to **20**

C	13	14	15	16	17	18	19	20
1	40.8	40.8	40.8	40.4	40.7	40.6	40.4	40.4
2	19.2	18.3	18.9	18.5	19.3	19.1	19.1	18.2
3	38.2	35.8	37.2	35.6	37.9	37.9	35.6	35.6
4	43.9	38.7	42.8	38.7	43.0	42.7	38.7	38.7
5	57.1	56.9	56.8	56.7	56.8	56.8	56.6	56.7
6	21.9	21.6	20.9	19.5	21.6	21.5	20.2	20.1
7	41.3	41.4	41.1	41.4	40.8	40.9	41.2	41.2
8	44.2	44.2	43.7	42.4	43.7	43.7	43.0	42.7
9	55.1	56.3	54.4	55.1	54.6	54.4	55.7	55.5
10	39.4	39.3	39.4	39.4	39.5	39.4	39.3	39.2
11	18.4	18.2	18.9	18.2	19.0	18.4	18.2	18.2
12	33.1	33.3	29.5	29.5	27.9	31.2	28.0	31.3
13	43.8	44.0	48.2	47.8	37.8	40.6	38.0	40.8
14	39.7	39.7	37.7	37.3	38.5	38.6	38.5	38.6
15	48.9	49.3	55.4	55.0	46.4	43.7	46.6	43.8
16	155.9	155.6	222.5	222.4	167.8	168.3	168.3	168.6
17	102.9	102.9	-	-	-	-	-	-
18	28.9	27.1	28.6	27.1	28.7	28.7	27.1	27.1
19	178.1	65.6	178	65.5	177.9	177.9	65.5	65.5
20	15.4	18.1	15.8	18.5	15.5	15.5	18.2	18.2
21	51.1	-	51.6	-	51.1	51.1	-	-

Ketones: The alkenes 19-ester (**13**) and alcohol (**14**) (1.0 mmol), in 50.0 mL THF/H_2O (1:1) were treated with sodium periodate (5.0 mmol), plus one crystal of osmium tetroxide and left overnight at room temperature.[15] The reaction mixtures were treated with a solution of sodium bisulfite 10% and washed with solution of sodium thiosulfate 10%. The residues were submitted to flash chromatography on silica gel to afford:

Methyl ent-16-oxo-17-norkauran-19-oate (**15**, 256 mg, 81%), elution with hexane/ethyl acetate (95:5): mp 130 °C (lit.[14]142-145° C); $[\alpha]_D^{25}$: - 55.2° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 2900 (C-H), 1745 (C=O), 1725 (C=O) (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89 (3H, s, 20-CH_3), 1.19 (3H, s, 18-CH_3), 2.40 (1H, br s, 13-CH) and 3.66 (3H, s, 21-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2.

ent-16-oxo-17-norkauran-19-ol (**16**, 209 mg, 72%) elution with hexane/ethyl acetate (9:1): mp 154 °C (lit.[16] 154-157 °C); $[\alpha]_D^{25}$: - 36.9° ($CHCl_3$, *c* 0.38); IR ν/cm^{-1}: 3300, (O-H), 2900 (C-H), 1745 (C=O) (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (3H, s, 18-CH_3), 1.08 (3H, s, 20-CH_3), 2.40 (1H, br s, 13-CH), 3.48 (1H, d, *J* 10.9 Hz, 19a-CH_2) and 3.75 (1H, d, *J* 10.9 Hz, 19b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2.

Oximes: 2.0 mL of a sodium hydroxyde solution (1.5 mol L^{-1}) was added to a solution of ketone (0.6 mmol), hydroxylamine hydrochloride (1.2 mmol), in 12.0 mL of EtOH/ H_2O (8:2), and this solution was kept for 15 min at room temperature.[17] After removal of EtOH, the organic phase was extracted with ethyl ether, the ethereal phase was dried over anhydrous sodium sulfate and concentrated. The residue was submitted to flash chromatography on silica gel to yield the *Z* and *E* oximes:

Methyl ent-16Z-oxime-17-norkauran-19-oate (**17**, 90 mg, 42%), elution with hexane/ethyl acetate (9:1), fractions 11-15: white solid, mp 172 °C; $[\alpha]_D^{25}$: - 74.32° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 3300 (O-H), 2900 (C-H), 1725 (C=O), 1540 (C=N) (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.85 (3H, s, 20-CH_3), 1.18 (3H, s, 18-CH_3), 3.32 (1H, br s, 13-CH) and 3.65 (3H, s, 21-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2. EI-MS: *m/z* (rel. int.) 333 (33) ($M^{\bullet+}$), 316 (100) (M^+-OH), 302 (10) (M^+-OCH_3), 274 (30) (M^+-CO_2CH_3).

Methyl ent-16E- oxime-17-norkauran-19-oate (**18**, 95 mg, 45%), elution with hexane/ethyl acetate (9:1), fractions 41-46: white solid, mp 184 °C; $[\alpha]_D^{25}$: - 112.7° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 3300 (O-H), 2900 (C-H), 1725 (C=O), 1540 (C=N) (KBr); ^{1}*H N*MR (200 MHz, $CDCl_3$) δ 0.84 (3H, s, 20-CH_3), 1.18 (3H, s, 18-CH_3), 2.76 (1H, br s, 13-CH) and 3.65 (3H, s, 21-CH_3); ^{13}*C N*MR (50 MHz, $CDCl_3$): see Table 2.

ent-16Z-oxime-17-norkauran-19-ol (**19**, 55 mg, 28%), elution with hexane/ethyl acetate (7:3), fractions 23-32: oil; $[\alpha]_D^{25}$: - 105.2° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 3300, (O-H), 2900 (C-H), 1540 (C=N) (KBr): ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.97 (3H, s, 20-CH_3), 1.03 (3H, s, 18-CH_3), 3.34 (1H, br s, 13-CH), 3.45 (1H, d, *J* 10.9 Hz, 19a-CH_2) and 3.75 (1H, d, *J* 10.9 Hz, 19b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2.

ent-16E-oxime-17-norkauran-19-ol (**20**, 63 mg, 33%), elution with hexane/ethyl acetate (7:3), fractions 38-52: white solid, mp 191 °C; $[\alpha]_D^{25}$: - 37.7° ($CHCl_3$, *c* 0.77); IR ν/cm^{-1}: 3300, (O-H), 2900 (C-H), 1540 (C=N) (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.97 (3H, s, 20-CH_3), 1.02 (3H, s, 18-CH_3), 2.76 (1H, br s, 13-CH), 3.45 (1H, d, *J* 10.9 Hz, 19a-CH_2) and 3.75 (1H, d, *J* 10.9 Hz, 19b-CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): see Table 2. EI-MS: *m/z* (rel. int.) 305 (31) ($M^{\bullet+}$), 288 (73) (M^+-OH), 274 (100) (M^+-CH_2OH).

Bioassay

Albino mice acutely infected with the Y strain of *Trypanosoma cruzi* were used to obtain trypomastigotes. The parasite at a density of $2x10^6$ cells/mL was introduced into each flat-bottomed test tube (4.0 mL, 56x13 mm). Each compound (0.02 moles) was dissolved (or suspended) in 0.2 mL of dimethyl sulfoxide (DMSO) plus TC 199 (2.0 mL) and aliquots (0.1, 0.05 and 0.025 mL) of this solution (or suspension) was mixed with infected blood (0.2 mL) plus TCM 199 medium to complete 0.4 mL. Control tubes with DMSO and gentian violet (125 μg mL^{-1}) were run in parallel. All tubes were incubated for 24 h at 4 °C. Thereafter, 5 μL of the suspension were examined microscopically for the presence of motile organisms, and only those samples in which 100% of the parasites were killed, were considered active. Samples showing 50% mortality of *T. cruzi* trypomastigotes were considered partially active. Below this value the samples were considered inactive.

Results and Discussion

Syntheses

The use of carbon tetrachoride and triphenylphosphine[12] instead of thionyl chloride or phosphrus pentachloride to prepare the acid chlorides, in amide syntheses, led to the maintenance of the exocyclic double bond in the kaurane skeleton of all products. The presence of the amide group was comproved by a band at 1625 cm^{-1} of amide I, in the IR spectra and by a signal of δ 175.0-177.0, in the ^{13}C NMR spectra. The mass spectra (EI) showed, besides M^+, significant M^+-CONR peaks.

The best procedure for amide reduction to amines was obtained with DIBAL-H. ^{13}C NMR spectra showed the lack

of signal related to the amide group and the presence of a new methylene carbon; deshielding of C-1' was also observed, when compared to the spectrum of the starting amide. The ^{1}H NMR spectra showed shielding of hydrogens H-18 , H-1', H-2'and H-3', possibly due to the nitrogen lone eletron pair.

The amines were treated with gaseous HCl, in dry CH_2Cl_2, to give the corresponding hydrochlorides. The ^{1}H NMR spectra showed deshielding of hydrogens H-18, H-19, H-1'and H-2', possibly due to the positively charged nitrogen and C-H bond polarization. The ^{13}C NMR spectra showed a shielding of C-2' e C-3'and deshielding of C-19.

Oximes were obtained from methyl *ent*-kaur-16-en-19-oate (**13**) and *ent*-kaur-16-en-19-ol (**14**), both prepared from kaurenoic acid (**1**). Compounds **13** and **14** were transformed into their corresponding norketones **15** and **16**.

The reaction of these ketones with hydroxylamine furnished, in both cases, the two possible isomeric oximes: *Z* and *E* (**17-20**). Oxime formation was confirmed by the signal of C-16, in ^{13}C NMR spectra, displaced from δ 222.0 to δ 176.0, characteristic of the C=NOH bond. The chemical shift values of H-13, C-13 and C-15, in the ^{1}H and ^{13}C NMR spectra were taken as the basis for the isomer assignments:[18] in *Z*-oximes H-13 is deshielded, due to steric hindrance and C-13 is shielded, when compared to *E*-oximes. Similarly, H-15 in *E*-oximes is deshielded and C-15 shielded, compared to *Z*-oximes.

The mass spectra (EI) of these oximes showed M^+ peaks consistent with molecular formulas $C_{20}H_{34}NO_3$ (for C-19 ester) and $C_{20}H_{31}NO_2$ (C-19 alcohol). $[M\text{-}OH]^+$ and $[M\text{-}CH_2OH]^+$ fragments were also observed.

Trypanocidal activity

The most significant results of trypanocidal activity evaluation of the kaurenoic acid (**1**) derivatives synthetised in this work are presented in Table 3. The solubility improvement of the hydrochlorides in the biological medium used for the test, by means of the positive charge on nitrogen, was poor, the hydrophobic part of the molecule still preponderanting.

However, some compounds exhibited good results. Compound **19** manifested the same level of activity as kaurenoic acid (**1**), but showed discrete erythrocyte lysis at the lowest concentration tested and high lysis at the higher concentration (Table 3), while compound **11** was as active as **1**, and without lysis.

Drugs used clinically as trypanocidal agents such as pentamidine and nifurtimox possess C=N bonds in their molecules. Therefore, the oximes obtained were tested and the Z-isomer **17** turned out to be more active than **1**, but this activity was accompanied by discrete lysis of erytrocytes.

Table 3. *In vitro* activity of diterpenes **1**, **11**, **17** to **20** against *Trypanosoma cruzi* trypomastigotes

Compound	Concentration (10^{-3}mol L^{-1})		
	2.27	1.14	0.57
Kaurenoic acid (**1**)	A TL	A TL	PA DL
ent-kaur-16-en-19-pyrrolidinamonium chloride **(11)**	A	A	PA
methyl *ent*-16Z-oxime-17-norkauran-19-oate **(17)**	A HL	A HL	A DL
methyl *ent*-16E-oxime-17-norkauran-19-oate (**18**)	A HL	A HL	PA DL
ent-16Z-oxime-17-norkauran-19-ol (**19**)	A HL	A HL	PA DL
ent-16E-oxime-17-norkauran-19-ol (**20**)	A HL	PA DL	PA DL
Controls: DMSO and Gentian Violet	I A	I A	I A

A: Absence of *T. cruzi* (active).
PA: About 50% of trypomastigotes killed (partially active).
I: Below 50% of trypomastigotes killed (inactive).
TL: Total lysis of erythrocytes.
HL: High lysis of erythrocytes.
L: Discrete lysis of erythrocytes.

Conclusion

This paper reports the synthesis of twelve new kaurane diterpenes aiming at an improvement of trypanocidal activity in relation of kaurenoic acid (**1**). This was achieved by one of the tested compounds, methyl *ent*-16Z-oxime-17-norkauran-19-oate (**17**). *Ent*-kaur-16-en-19-pyrrolidinamonium chloride (**11**) and two oximes, methyl *ent*-16E-oxime-17-norkauran-19-oate (**18**) and *ent*-16Z-oxime-17-norkauran-19-ol (**19**) did not improve the trypanocidal activity, but they did show reduced lysis of erythrocytes. The remaining compounds were completely inactive against trypomastigote forms of *T. cruzi* at the concentrations used.

Acknowledgments

To FINEP, FAPEMIG, CAPES and CNPq for grants and financial support.

References

1. Schofield, C. J.; *Br. Med. Bull*. **1985**, *41*, 187.
2. Rezende, J. M.; Zupelli, W.; Bafutto, M. G.; *Rev. Goiana Med.* **1965**, *11*, 35.
3. Hammond, D. J.; Cover, B.; Gutteridge, W. E.; *Trans. Roy. Soc. Trop. Med. Hyg.* **1984**, *78*, 91.
4. Boza, S. S.; Cassels, B. K.; *Planta Med.* **1996**, *62*, 98.
5. Chiari, E.; Oliveira, A. B.; Prado, M. A. F.; Alves, R. J.; Galvão, L. M. C.; Araujo, F. G.; *Antimicrob. Agents Chemoter.* **1996**, *40*, 613.
6. Hammond, D. J.; Hogg, J.; Gutteridge, W. E.; *Exper. Parasitol.* **1985**, *60*, 32.
7. Ghisalberti, E. L.; *Fitoterapia* **1997**, *LXVIII*, 303.
8. Alves, T. M. A.; Chaves, P. P. G.; Santos, L. M. S. T.; Nagem, T. J.; Murta, S. M. F.; Geravolo, I. P.; Romanha, A. J.; Zani, C. L.; *Planta Med*. **1995**, *61*, 85.
9. Takahashi, J. A.; Boaventura, M. A. D.; Bayma, J. C.; Oliveira, A. B.; *Phytochemistry* **1995**, *40*, 607.
10. Takahashi, J. A.; Vieira, H. S.; Boaventura, M. A. D.; Hanson, J. R.; Hitchcock, P. B.; Oliveira, A. B.; *Quim. Nova* **2001**, *24*, 616.
11. Vieira, H. S.; Takahashi, J. A.; Boaventura, M. A. D.; *Fitoterapia* **2001**, *72*, 854.
12. Croft, K. D.; Ghisalberti, E. L.; Jefferies, P. R.; Knox, J. R.; Mahoney, T. J.; Sheppard, P. N.; *Tetrahedron* **1974**, *30*, 3663.
13. Winterfeldt, E.; *Synthesis* **1975**, 617.
14. Henrick, C. A.; Jefferies, P. R.; *Aust. J. Chem*. **1964**, *17*, 915.
15. Castellaro, S. J.; Dolan, S. C.; MacMillan, J.; Willis, C. L.; *Phytochemistry* **1990**, *29*, 1823.
16. Jefferies, P. R.; Payne, T. G.; *Aust. J. Chem.* **1965**, *18*, 1441.
17. La Mattina, J. L.; Suleske, R. T.; In *Organic Synthesis*; Freeman, J. P., ed.; John Wiley & Sons, Inc.: New York, 1990, vol. VII, p. 149.
18. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C.; *Spectrometric Identification of Organic Compounds*; John Wiley & Sons, Inc.: New York, 1991, p. 245.

Received: September 11, 2000
Published on the web: January 11, 2002