REVISTA BOLIVIANA DE QUÍMICA
Bolivian Journal of Chemistry
Received 22 08 2013

Vol. 30, No.1, pp. 60-65, Ene./Jun. 2013
30(1) 60-65, Jan./Jun. 2013
Published 20 09 2013

ENT-KAURANE DITERPENOIDS FROM *BACCHARIS LEPTOPHYLLA*

Lily Salcedo Ortiz[1], Yonny Flores[1], Olov Sterner[2] y Giovanna R. Almanza Vega[*1]*

[1]Laboratorio de Bioorgánica, Instituto de Investigaciones Químicas, Universidad Mayor de San Andrés, Calle 27 Cota-Cota, C.P. 303 La Paz-Bolivia, [2]Organic Chemistry Division, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

Keywords: *Baccharis leptophylla, Bolivian medicinal plants, ent-kaurane diterpenoids, leptophyllin, ent-3β,16β,19-trihydroxykaurane, ent-19-al-17,16β-dihydroxykaurane*

ABSTRACT

A known ent-kaurane diterpenoid, *ent*-19-al-17,16β-dihydroxykaurane (**1**), together with the new one, *ent*-3β, 16β, 19-trihydroxykaurane (**2**)**,** called leptophyllin, were isolated from aerial parts of *Baccharis leptophylla.* Their structures were established on the basis of NMR spectroscopic analysis. Based on this study the ^{1}H and ^{13}C NMR spectral data of compound (**1**) were completed by means of 2D NMR techniques.

*Corresponding author: giovyalmanza@yahoo.com.ar

RESUMEN

Un diterpenoide conocido tipo ent-kaurano, *ent*-19-al-17,16β-dihidroxikaurano (**1**), junto con un diterpenoide nuevo, *ent*-3β,16β,19-trihidroxikaurano (**2**)**,** llamado leptophyllin, fueron aislados de las partes aéreas de *Baccharis leptophylla*. Sus estructuras fueron establecidas en base a técnicas de análisis espectroscópico de RMN. En base a este estudio los datos de RMN ^{1}H y ^{13}C del compuesto (**1**) fueron completados mediante técnicas de RMN 2D.

INTRODUCTION

Baccharis leptophylla is used by the "Alteño" Bolivian ethnia, against the backache, and is commonly known as "*chiñi t'hola*". This species was selected among 150 Bolivian plants, from the "Chacobo", "Moseten" and "Alteño" ethnias. The results of the primary pharmacological screening pointed out interesting antiparasitic activity against *Leishmania amazonensis, L. brazilensis*, *L. donovani*, *Plasmodium falciparum* (97% parasitemic inhibition at 100 μg/ml *in vitro*) and *P. vinckei* (62% parasitemic inhibition at 1000 mg/kg/4J, *in vivo*). In addition, it has shown antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* at a concentration of 1 mg/ml [1]. Continuing our chemical investigations of the Bolivian *Baccharis* [2-4], we have studied the dichloromethane extract of *Baccharis leptophylla*, a Bolivian plant belongs of the large genus *Baccharis* (Compositae, tribe Asteraceae). This genus has a big representation in the Bolivian biodiversity [5,6] and its chemistry is not very uniform, the most widespread compounds are flavonoids [2-4,7] and diterpenoids type clerodane [6,7], but labdane and kauranes have also been isolated [5,7,8]. Here we report the isolation and structure elucidation of two ent-kaurane diterpenoids, a new one *ent*-3β,16β,19-trihydroxykaurane **1** and a known one *ent*-19 aldehyde-17,16β-dihydroxykaurane **2**, isolated of dichloromethane extract from *Baccharis leptophylla* aerial parts**.** The kaurane diterpenoids are a very interesting group of tetracyclic diterpenes constituted by a perhydrophenantrene unit (A, B and C rings) fused with a cyclopentane unit (D-ring) formed by a bridge between C-8 and C-13. They belong from several families such as Asteraceae (*Baccharis*), Annonnaceae, Euphorbiaceae, Celastraceae, Lamiaceae, Erythroxylaceae and Rhyzophoraceae [9] and several showed interesting biological and pharmacological properties. In particular, it is interesting that 16β,17-dihydroxy-ent-kauran-19-oic acid showed significant activity against HIV replication in H9 lymphocyte cells with an EC50 value of 0.8 μg/mL [10] and 16α-hydro-19-al-*ent*-kauran-17-oic acid showed complete inhibitory effects on rabbit platelet aggregation at 200 μM [11] because they have very close structures to compound **1**. So, it should show similar properties or should be used as a precursor of them.

RESULTS AND DISCUSSION

Compound **1** was obtained as white crystals (m.p. 182-183ºC), its EIMS showed the molecular peak at m/z 320 and the ^{13}C NMR spectrum (Table **1**) displayed 20 carbon signals corresponding to four quaternary carbons, four

Vol. 30, No.1, pp. 60-65, Ene./Jun. 2013
30(1) 60-65, Jan./Jun. 2013
Published 20 09 2013

methines, ten methylenes and two methyls, suggesting a diterpenoid skeleton with an elemental composition $C_{20}H_{34}O_3$. Complete structure elucidation of **1** resulted from interpretation of its NMR data. Analyzing the ^{13}C data we can distinguish three signals, a signal at δ 206.0 assigned to CHO at C-19, a quaternary carbon linked to oxygen at δ 81.8 assigned to C-16 and a methylene carbon, also join to oxygen, at δ 66.3 assigned to C-17. The diagnostic of the rest of the signals showed three methines at δ 56.5 (C-5), 55.3 (C-9) and 45.3 (C-13) and three quaternary carbons more at δ 48.4 (C-4), 44.5 (C-8) and 39.4 (C-10) indicating an *ent*-kaurene skeleton for the diterpenoid. The COSY spectrum showed the presence of three proton sequences of -CH_2 (1)-CH_2 (2)-CH_2 (3)-, -CH (5)-CH_2 (6)-CH_2 (7) - and –CH (9)-CH_2 (11)-CH_2 (12)-CH (13)-CH_2 (14)-, as shown in the table **2**. An oxygenated methylene (δ_H 3.79 and 3.67, each 1H, *d*, *J*=11 Hz, δ_C 66.3), which correlates with the protons H-15 (Table 2), was confirmed in C-17, and a methine CHO (δ_H 9.74 *s*, 1H; δ_C 206.0) was located in C-19 based on the HMBC correlations of H-19 with C-4 and C-3. Finally, the long distance correlations from H-17 and H-15 methylene protons with C-16 confirm the location of the last oxygenated substitution in the quaternary carbon (Figure 2).

Figure 1. *Structures of diterpenoids* ***1*** *and* ***2***

Table 1. *^{13}C NMR data to compounds* ***1*** *and* ***2***

C	$\mathbf{1}_{exp}$	$\mathbf{1}_{bib}$ [12]*	2
1	39.7 t	40.9 t	38.2 t
2	18.3 t	19.4 t	27.1 t
3	34.2 t	35.4 t	80.2 d
4	48.4 s	49.0 s	42.2 s
5	55.2 d	56.6 d	55.4 d
6	18.4 t	21.2 t	20.0 t
7	41.9 t	43.2 t	41.9 t
8	44.5 s	45.5 s	44.8 s
9	56.5 d	57.9 d	56.5 d
10	39.4 s	40.6 s	38.5 s
11	20.1 t	21.2 t	18.1 t
12	26.0 t	27.1 t	26.4 t
13	45.3 d	45.5 d	48.2 d
14	37.4 t	38.4 t	37.1 t
15	53.0 t	54.0 t	57.2 t
16	81.8 s	83.0 s	78.8 s
17	66.3 t	66.9 t	23.8 q
18	24.2 q	24.5 q	22.4 q
9	206.0 d	207.0 d	64.0 t
20	16.4 q	17.0 q	18.0 q

$CDCl_3$, 125 MHz; Solvent $CDCl_3$ *CD_3OD

Vol. 30, No.1, pp. 60-65, Ene./Jun. 2013
30(1) 60-65, Jan./Jun. 2013
Published 20 09 2013

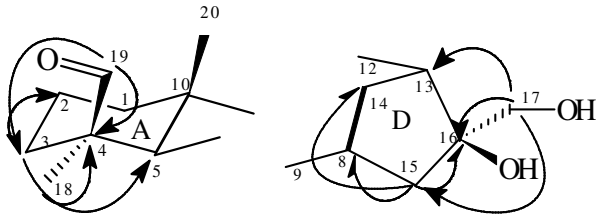

Figure 2. *Some HMBC correlations for compound* ***1***

Table 2. *^{1}H NMR, COSY and NOESY data for compound* ***1***

H	**δ**	*COSY*	*NOESY*
1ax	0.79 *ddd* (13.0, 13.0, 3.9)	H-1eq,H-2ax,H-2eq,H-20	H-1eq, H-2eq, H-3ax, H-5, H-9
1eq	1.81 *brd* (13.0)	H-1ax, H-2ax, H-2eq, H-3eq	H-1ax, H-2ax, H-2eq, H-20
2ax	1,65*	H-1ax, H-1eq, H-2eq, H-3ax, H-3eq	H-2eq, H-1eq, H-3eq
2eq	1,45*	H-1ax, H-1eq, H-2ax, H-3ax, H-3eq	H-1ax, H-1eq, H-2ax, H-3ax, H-3eq
3ax	1,00*	H-2ax, H-2eq, H-3eq	H-1ax, H-5,H-2eq, H-3eq
3eq	2,14 *brd* (13.6)	H-1eq, H-2ax, H-2eq, H-3ax	H-3ax, H-2ax, H-2eq, H-18, H-19
5	1,15 *brd* (12.2)	H-6ax, H-6eq, H-7eq, H-18, H-20	H-1ax, H-3ax, H-6ax, H-6eq, H-9
6ax	1.71*	H-5, H-6eq, H-7ax, H-7eq	H-5, H-6eq, H-7ax, H-7eq, H-19, H-20
6eq	1.89 *brd* (3.5)	H-5, H-6ax, H-7eq, H-7ax	H-5, H-6ax, H-7ax, H-14b, H-18
7ax	1.72*	H-7eq, H-6ax,H-6eq	H-6eq, H-7eq
7eq	1.51*	H-5, H-7ax, H-6ax, H-6eq	H-6ax, H-7ax
9	1.03 *brd* (6.4)	H-11a, H-11b, H-14a, H-20	H-1ax, H-5,H-11a,H-12a, H-15a
11a	1.50*	H-9, H-11b, H-12a, H-12b, H-13	H-9, H-11b, H-20
11b	1.61*	H-9, H-11a, H-12a, H-12b	H-11a, H-13, H-14b, H-20
12a	1,55*	H-11a, H-11b, H-12b, H-13	H-12b, H-9
12b	1,52*	H-11a,H-11b, H-12a, H-13,H-14b	H-12a, H-13, H-11b, H-14b
13	2,05 *br s*	H-11a, H-12a, H-12b, H-14a, H-14b	H-11b, H-12b, H-14b, H-17a
14a	1,64*	H-9, H-13, H-14b	H-14b, H-15b
14b	1,93 *brd* (10.2)	H-12b,H-13,H-14a,H-15a	H-11b, H-12b, H-13, H-14a, H-20
15a	1,58 *brd* (14.9)	H-14b, H-15b	H-9, H-15b, H-17a, H-17b
15b	1,47 *brd* (14.9)	H-15a, H-17b	H-15a
17a	3.67 *d* (11)	H-17b	H-17b, H-15a
17b	3,79 *d* (11)	H-17a	H-13, H-15a, H-17a
18	1.00 s	H-5	H3eq, H-6eq
19	9,74 s		H-3eq, H-6ax
20	0,88 *s*	H-1ax,H-5,H-9	H-1eq, H-6ax, H-11a, H-11b, H-14b

$CDCl_3$ at 500 MHz, δ in ppm *J* in Hz in parenthesis *Overlapping signals

The relative configuration of **1** was established mainly based on NOE effects observed in the NOESY spectrum, where we can observe the follow important NOE correlations: i) H-1ax with H-3ax, H-5 and H-9 showing the binding of the rings **A** and **B** in *trans*; ii) The correlation of H-20 with H-1eq, H-6ax, H-11a, H-11b and H-14b as well as of H-9 with H-1ax, H-5, H-12a and H-15a showing the binding *cis* of the rings **B** and **C** and the relative positions of the rings; iii) finally the coupling of H-15a with the protons H-17 as well as of H-13 with H-17b confirmed the proposed position of the hydroxylated methylene group (Figure 3). Compound **1** was previously reported [12], comparing the ^{13}C NMR data with those reported we found small differences attributed mainly to the change of solvent. In relation

Vol. 30, No.1, pp. 60-65, Ene./Jun. 2013
30(1) 60-65, Jan./Jun. 2013
Published 20 09 2013

of ^{1}H NMR data, in the previous paper not all the protons were assigned neither its relative configuration. Then with this work we give a contribution for the characterization of compound **1**.

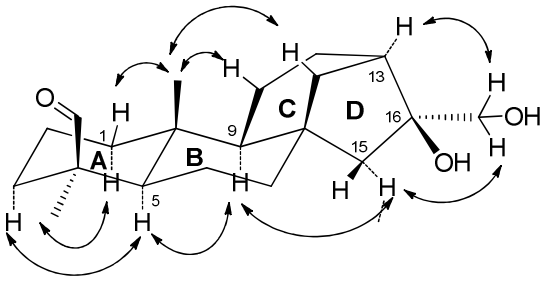

Figure 3. *Some NOE correlations to assign the relative configuration of compound* ***1***

Compound **2** also showed the characteristic signals for an *ent*-kaurane diterpenoid. So, its EIMS showed the molecular peak at *m/z* 322 corresponding to $C_{20}H_{34}O_3$ and its ^{13}C NMR spectrum (Table 1) displayed 20 carbons corresponding to four quaternary carbons, four methines, nine methylenes and three methyls, among them the signals at δ 80.2 (CH), 78.8 (C) and 64.0 (CH_2) suggest three oxygenated substitutions in the skeleton. Complete structure elucidation of **2** resulted from interpretation of its 1D and 2D NMR data. The COSY spectrum showed an oxygenated methylene (δ_H 3.19 and 4.08, each 1H, *d*, *J*=11 Hz, δ_C 64.0) assigned to C-19, because H-19a correlates to long distance W with H-3and H-5. On the other side the oxygenated methine was assigned to C-3 because the double doublet at δ_H 3.25 (*J*= 11 and 5 Hz) showed correlations with the protons H-2 and those with methylene protons H-1 (Table 3). Finally the last oxygenated substitution was located at C-16 based on the HMBC correlations of the H-15 and H-17 protons with the quaternary carbon at δ 78.8 assigned to C-16. The figure **4** shows these and other important long heteronuclear correlations for compound **2**.

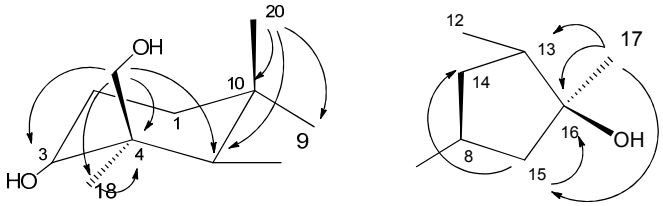

Figure 4. *Some HMBC correlations for compound* ***2***

The relative configuration of **2** was also established mainly through the analysis of NOESY spectrum where we can see the follow relevant correlations: i) H-5 correlates with H-1ax, H-3, H-7ax, H-18 and H-9 showing the binding of the rings **A** and **B** in *trans* and suggesting the location of the oxygenated substitutions in C-3 and C-19 in the other side of the molecule; ii) H-20 correlates with H-1eq, H-6ax, H-11b, H-14b and H-19b determining the binding *cis* of the rings **B** and **C** and confirming the relative position of the CH_2OH-19; iii) finally H-15a correlates with H-7ax, H-9 and H-17 giving the relative configuration of C-16 and determining the β-position of OH in C-16.

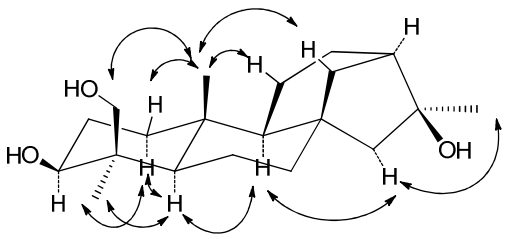

Figure 5. *Some NOE correlations to assign the relative configuration of compound* ***2***

Compound **2** is reported here for first time contributing to the study of *Baccharis sp* in particular of the medicinal *Baccharis* from Bolivia.

Table 3. *^{1}H NMR, COSY and NOESY data for compound* ***2***

H	δ	COSY	NOESY
H-1ax	0,77*	H-1eq, H-2ax, H-2eq, H-20	H-1eq, H-2eq, H-3, H-5, H-9
H-1eq	1,73*	H-1ax, H-2ax,H-2eq	H-1ax, H-2ax, H-2eq, H-20
H-2ax	1,70*	H-1ax, H-1eq, H-2eq, H-3	H-2eq, H-1eq
H-2eq	1,61*	H-1ax, H-1eq, H-2ax, H-3	H-1ax, H-1eq, H-2ax, H-3
H-3	3,25 *dd* (11.3, 5.0)	H-2ax, H-2eq, H-19a	H-1ax, H-2eq, H-5, H-18
H-5	0,74 *brd* (11.4)	H-6ax, H-6eq	H-1ax, H-3, H-6eq, H-7ax, H-9, H-18
H-6ax	1,16 *ddd* (2.8, 12.6 ,2.8)	H-5, H-6a, H-7a, H-7b	H-6eq, H-7eq, H-20, H-19a
H-6eq	1,52 *brs*	H-5, H-6b, H-7ax, H-7eq	H-5, H-6ax, H-7ax, H-7eq, H-18, H-19a
H-7ax	1,34 *ddd* (3.5, 12.7,3.7)	H-6a, H-6b,H-7b	H-5, H-9, H-6eq, H-7eq, H-15a
H-7eq	1,51*	H-6a, H-6b, H-7ax	H-7ax, H-6ax, H-6eq, H-15b
H-9	0,84 *brs*	H-11a, H-11b	H-1ax, H-5, H-7ax, H-11a, H-12a, H-15a
H-11a	1,43*	H-9, H-11b, H-12a, H-12b	H-9, H-11b, H-12a, H-12b
H-11b	1,46*	H-9,H-11a,H-12b	H-20, H-11a, H-14b, H-12b
H-12a	1,43*	H-11a,H-11b,H-12a,H-13	H-9, H-12b, H-17
H-12b	1,47*	H-11a,H-11b,H-12b,H-13	H-12a, H-13, H-14b, H-17
H-13	1,72 *brs*	H-12a, H-12b, H-14a, H-14b	H-12b, H-14a, H-14b, H-17
H-14a	1,45*	H-13	H-13, H-14b
H-14b	1,73*	H-13	H-11b, H-13, H-14a, H-20
H-15a	1,43*		H-7ax, H-9, H-17
H-15b	1,43*		H-7eq, H-17
H-17	1,23 *s*		H-15a, H-15b, H-12a, H-12b
H-18	1,08 *s*	H-19b	H-3, H-5, H-6eq, H-19a
H-19a	3,19 *d* (11.0)	H-19b,H-3	H-6ax, H-6eq, H-18, H-19b
H-19b	4,08 *d* (11.0)	H-19a,H-18	H-2ax, H-19a, H-20
H-20	0,88 *s*	H-1ax	H-1eq, H-6ax, H-11b, H-14b, H-19b

$CDCl_3$ at 500 MHz, δ in ppm *J* in Hz in parenthesis *Overlapping signals

EXPERIMENTAL

General

Mp: uncorr; EIMS were obtained by direct inlet 70 eV. ^{1}H and ^{13}C-NMR were recorder at 500 MHz using TMS and $CDCl_3$ residual signals as references; chemical shift values are reported in δ (ppm) units and coupling constants (*J*) in Hz. Silica gel (E.M. Merck, 70-230 mesh) and silica gel G-60 (E.M. Merck) were used for CC and VLC, respectively, while aluminum plates impregnated with silica gel 60 F_{254} (E.M. Merck) were used for analytical (0.25 mm) and preparative (1 mm) TLC analyses. Spots on chromatograms were detected under UV light (254 and 365 nm) and by spraying the plates with 10% H_2SO_4, followed by heating.

Plant material

The aerial parts of *B. leptophylla* (Asteraceae) were collected at Mizque province (Cochabamba, Bolivia) in September 2006 and identified by Lia de Michel, a botanist of the National Herbarium of Bolivia where a voucher specimen was deposited.

Extraction and isolation

The dry and ground plant material (1900 g) was first extracted with petroleum ether for 10 min at room temperature, the solution was filtered off and evaporated to yield the petroleum ether extract (8 g). The solid residue was macerated for 2 hours with CH_2Cl_2, which after filtering and evaporation of the solvent gave the CH_2Cl_2 extract (48g). Finally, the residue was extracted with EtOH for 48 hours; the ethanolic extract was partitioned between CH_2Cl_2 and H_2O to give a CH_2Cl_2/EtOH crude fraction (12 g). The two extracts and the crude fraction were analyzed by TLC showing the last fraction the most interesting and less complex composition being selected for this study.

The CH_2Cl_2/EtOH crude fraction (12 g) was subjected to VLC on silica gel, eluting with increasing amounts of CH2Cl2 in petroleum ether, followed by increased amounts of MeOH in CH_2Cl_2 finalizing with MeOH, to give nine main fractions. Fractions 2, 5 and 6 were submitted to repeated column chromatography led, after purification by recrystallization in MeOH, the ent-kaurane diterpenoids 1 (42 mg) and 2 (28 mg) isolated together with the oleanolic acid (7 mg) and the flavonoid 3,5,7-trihydroxy-4'-methoxyflavanone (10 mg) previously reported in the same species.

Compound 1 (ent-19-al-17,16β-dihydroxykaurane)

Compound obtained as white crystals (mp. 182–183 ºC) and identified as *ent*-19-al-17,16β-dihydroxykaurane based mainly in the NMR 1D and 2D spectra. ^{1}H NMR, ^{1}H-^{1}H-COSY and HMBC data see Table 3, ^{13}C NMR data see Table 2. MS $[M]^+$ peak at *m/z* 322 corresponding to $C_{20}H_{34}O_3$

Compound 2 *(ent-3β,16β,19-trihydroxykaurane)*

Diterpene obtained as white crystals and identified as *ent*-3β,16β,19-trihydroxykaurane based mainly in the NMR 1D and 2D spectra. ^{1}H NMR, ^{1}H-^{1}H-COSY and HMBC data see Table 1, ^{13}C NMR data see Table 2. MS $[M]^+$ peak at *m/z* 322 corresponding to $C_{20}H_{34}O_3$.

ACKNOWLEDGEMENTS

The authors are grateful to the Swedish Agency SIDA for funding the project "Research on Plant Biodiversity" and also to IFS (International Foundation for Science) for funding the project "Bioguided phytochemical study of *Baccharis leptophylla*" Grant F/2771-1. Finally they want to thank to the experts of National Herbarium of Bolivia LPB for the identification of plant material.

REFERENCES

[1] ALMANZA, G. R., ARDUZ, C., BALDERRAMA, L., OCAÑA, L., FLORES E. "Estudio fitoquímico de *B. leptophylla* biodirigido contra *Neurospora Crasa*". *Rev. Boliviana de Química* 2000, **17**, 1-4.
[2] CALLE, A. YUPANQUI, J., FLORES, Y, ALMANZA G.R. "Flavonoides de *Baccharis boliviensis*" *Revista Boliviana de Química* 2012, **29**, 155-160
[3] TARQUI, S., FLORES, Y, ALMANZA G.R. "Polyoxygenated flavonoids from *Baccharis pentlandii*" *Revista Boliviana de Química* 2012, **29**, 10-14
[4] ESCOBAR, Z., FLORES, Y., TEJEDA, L., ALVARADO, J. A., STERNER, O., ALMANZA G.R. "Phenolic compounds from *Baccharis papillosa* subsp. *papillosa*" *Revista Boliviana de Química* 2009, **26**, 111-117
[5] BOHLMANN, F., KRAMP, W., GRENZ, M., ROBINSON, H., KING, R.M. "Diterpenes from *Baccharis* especies"; *Phytochemistry* 1981, **20**, 1907-1911.
[6] ZDERO, C., BOHLMANN, F., SOLOMON, J.C., KING, R.M., ROBINSON, H. "Ent-clerodanes and other constituyents from bolivian *Baccharis* species"; *Phytochemistry* 1989, **28**, 531-536.
[7] ABAD, M. J., BERMEJO P. "*Baccharis* (Compositae): a review update" *ARKIVOC* 2007, **VII**, 76-96
[8] SIMIRGIOTIS, M.J., GARCIA, M., SOSA, M.E., GIORDANO, O.S., TONN, C.E. "An ent-kaurene derivative from aerial parts of *Baccharis rufescens*" *The journal of the Argentine Chemical Society* 2003, **91**, 109-116
[9] GARCÍA, P. A., BRAGA DE OLIVEIRA, A., BATISTA, R. "Occurrence, Biological Activities and Synthesis of Kaurane Diterpenes and their Glycosides" *Molecules* 2007, **12**, 455-483
[10] WU, Y. C.; HUNG, Y. C.; CHANG, F. R.; COSENTINO, M.; WANG, H. K.; LEE, K. H. "Identification of ent-16,17 dihydroxy kauran-19-oic acid as an anti-HIV principle and isolation of the new diterpenoids annosquamosins A and B from *Annona squamosa*" *Journal of Natural Products* 1996, **59**, 635-637.
[11] YANG Y.L., CHANG, F.R., WU, C.C., WANG, W.Y., WU W.C. "New *ent*-Kaurane Diterpenoids with Anti-Platelet Aggregation Activity from *Annona squamosal*" *Journal of Natural Products* 2002, **65**, 1462–1467
[12] ETSE, J. T.; GRAY, A. T. AND WATERMAN, P. G. "Chemistry in the *annonaceae*. Kaurane and kaur-16-ene-diterpenes from the stem bark of *annona reticulata*". *Journal of Natural Products* 1987, **59**, 979-984.