Artículo original

Secondary metabolites from *Chaptalia meridensis*.

Metabolitos secundarios de *Chaptalia meridensis.*

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RESUMEN

De las hojas de la *Chaptalia meridensis* S. F. Blake (Asteraceae) han sido aislados la furocumarina S-(+)-marmesina y los triterpenoides acetato de lagerenilo, friedelina, friedelan-3β-ol y ácido betulínico. Estos metabolitos fueron identificados por métodos espectroscópicos, incluyendo espectrometría de masas de alta resolución, y experimentos de RMN uni- y bi-dimensionales. Éste es el primer estudio fitoquímico de *Ch. meridensis*.

PALABRAS CLAVE

Chaptalia meridensis, Asteraceae, furocumarina, triterpenoides, S-(+)-marmesina, acetato de lagerenilo.

ABSTRACT

The furocoumarin S-(+)-marmesin and the triterpenoids lagerenyl acetate, friedelin, friedelan-3β-ol and betulinic acid have been isolated from the leaves of *Chaptalia meridensis* S. F. Blake (Asteraceae). These metabolites were identified by spectroscopic methods including high resolution mass spectrometry and 1D- and 2D-NMR experiments. This is the first phytochemical study of *Ch. meridensis*.

KEY WORDS

Chaptalia meridensis, Asteraceae, furocoumarin, triterpenoids, S-(+)-marmesin, lagerenyl acetate.

INTRODUCTION

Chaptalia, one of the seven recognized genera of the *Gerbera*-complex included in tribe Mutisieae, family Asteraceae [1], comprises *ca. 65* species whose distribution range extends from south of United States to central Argentina, through Central America, Caribbean Islands, Andes Mountains and Brazil [2, 3]. Various species of this genus have been recognized in their countries of origin as medicinal plants. Particularly *Ch. nutans* is used in Argentinean traditional medicine as decongestant, anti-depressant, anti-inflammatory, laxant and vulnerary [4,5]; in Brazilian folk medicine, this species is widely appreciated for its anti-fungal, anti-ulcer, anti-thrombotic and anti-inflammatory properties [6,7]. Many of these biological properties have been validated, since several crude extracts of this plant have shown to possess antifungal, antimicrobial and anti-inflammatory activities [6, 8, 9] , and also ability to inhibit edema induced by *Bothrops asper* snake venom [10].

According to literature consulted, few phytochemical works have been carried out with species of *Chaptalia* genus. To date, chemical composition of only two species, *Ch. integerrima* and *Ch. nutans* have been reported; the most common compounds isolated from these species are glucosyl-5-methyl-furanocoumarins [9, 11, 12]; only once it has been detected in *Ch. nutans* the cyanogenic glucoside prunasin [13] as well as some valerolactones such as parasorbic acid and 5-methyl-3α-hydroxyvalerolactone [14].

As a continuation of our research on the phytochemistry of Venezuelan Andean Flora, we tackle the study of *Chaptalia meridensis* (Fig. 1), a small rosulate herb found commonly in Venezuelan Andean moor [15].

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Fig. 1. *Chaptalia meridensis.*

MATERIAL AND METHODS

General Experimental Procedures. Melting points were determined with a Fisher-Johns apparatus and they have not been corrected. Optical activities were measured on 60 Hz-Steeg & Reuter G.m.b.H. polarimeter using methanol or $CHCl_3$ as solvent. IR spectra were recorded on a Perkin-Elmer FT-1725X spectrophotometer as KBr pellets. ^{1}H-, ^{13}C- and two-dimensional NMR spectra were run with a Bruker-Avance DRX-400 instrument, using $CDCl_3$ as solvent. HRMS were acquired on a VG Micromass ZAB-2F. TLC was carried out on 0.25 mm layers of silica gel PF 254 (Merck); spots were visualized using UV light (254 and 365 nm) and subsequently by spraying with a mixture *v/v* CH_3COOH-H_2O-H_2SO_4 (20:4:1) and then heating with air flow at 100 °C for few minutes.VCC was performed with silica gel 60 (70-230 mesh.).

Plant material. Plant material (leaves) was collected in "Paramo Las Nieves between the villages of Estáques and El Molino, Municipio Autónomo Arzobispo Chacón, Estado Mérida, Venezuela" in December 2010. The botanical sample was identified as *Chaptalia meridensis* S. F. Blake by Eng. Juan Carmona Arzola, Department of Pharmacognosy and Organic Medicaments, Faculty of Pharmacy and Bioanalysis, University of Los Andes (ULA). A *voucher specimen* (J. M. Amaro-Luis, Nº 1648) was deposited at Herbarium MERF of this faculty.

Extraction and separation. Dry not crushed leaves (≅ 2.4 Kg) were extracted at room temperature with dichloromethane and then with methanol in a soxhlet to give, respectively, 38 and 52 g of crude extracts. The dichloromethane extract was preadsorbed on silica gel 60 and chromatographed on the same adsorbent, using the Coll & Bowden vacuum liquid chromatography technique [16]. Column was eluted with hexane, dichloromethane, ethyl acetate and methanol in mixtures of increasing polarity. Sixty-four (64) fractions of 0.5 L were collected, concentrated *in vacuo*, and combined according to TLC similarity to afford nineteen (19) major fractions (A-R).

S-(+)-Marmesin (**1**): Combined major fraction "Ñ" (fractions 49-53; ≅ 2.37 g) was rechromatographed on a silica gel column using hexane-ethyl acetate (3:2) to furnish an impure crystalline solid (≅ 0.125 g), that was purified by preparative TLC (silica gel, two development in hexane-ethyl acetate 4:1). Crystallization from methanol provided pure white needles (≅ 78 mg) detected in TLC plates as a blue-green fluorescence spot; m.p. = 187-188 °C; [α]D: + 25.3° (c, 0.38 methanol). IR (KBr), ν_{max} (cm^{-1}): 3448 (O-H), 3044 (=C-H), 2980 (C-H), 1702 (C=O), 1626 (C=C), 1158 and 1128 (C-O), 928 (=C-H). ^{1}H NMR (Fig. 3). ^{13}C NMR (Fig. 3); δ_H and δ_C consistent with those previously reported [17, 18]. HR-EI-MS: *m/z* (%) 246.0906 (55.28) [M^+], 228.0777 (3.53) [M^+-H_2O], 213.0555 (21.43) [M^+-H_2O-CH_3], 188.0464 (79.11), 187.0379 (100) [M^+-HO-C-$(CH_3)_2$], 175.0403 (23.41), 160.0517 (40.45), 159.0445 (14.05), 131.0500 (9.92), 102.0469 (8.01), 77.0391 (13.40), 59-0519 (79.62).

Lagerenyl acetate (**2**): Combined major fraction "H" (fractions 25-26; ≅ 1.91 g) was filtered through a Sephadex LH-20 column eluted with hexane-CH_2Cl_2-MeOH (1:2:2); sub-fraccions "H_6" gave a solid which crystallized from methanol as white flakes (≅ 52.0 mg); m.p. = 122-124 °C; $[\alpha]_D$: +59.2° (c, 0.44 ethanol). IR (KBr) ν_{max} (cm^{-1}): 2944-2364 (C-H), 1736 (C=O), 1712 (C=O), 1464 (C-H), 1244 (C-O) and 1036 (cyclopropil C-H). ^{1}H NMR (Fig. 5), ^{13}C NMR (Fig. 5); δH and δC

comparable with those previously reported [19, 20]. HR-EI-MS: *m/z* (%) 484.0906 (24.06) [M^+], 469.3488 (36.44) [M^+-CH_3], 424.3499 (45.26) [M^+-CH_3-COOH], 409.3275 (100) [M^+-CH_3-COOH/-CH_3], 395.3136 (14.17), 302.2504 (21.02), 297-2484 (17.10), 287.2276 (12.65), 203.1744 (25.82), 187.1437 (20.34), 175.1435 (36.95), 161.1278 (22.00), 147.1120 (23.43), 135.1115 (39.0), 127.1068 (32.80), 107.0807 (32.92), 95.0808 (40.55), 71.0476 (40.46).

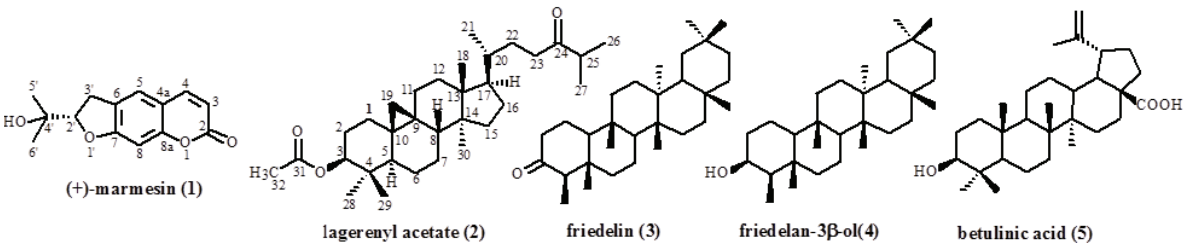

Fig. 2. Secondary metabolites isolated from *Chaptalia meridensis.*

Friedelin (**3**): Isolated from combined major fraction "F" (fractions 18-22; ≅ 2.65 g) by repeated silica gel column chromatography, eluted with different hexane-dichlorometane mixtures.; final purification, was carried out by crystallization from ethyl acetate/ hexane, providing white needles (≅ 160.0 mg); m.p. = 267-267 °C; $[\alpha]_D$: -22.5° (c, 0.41 $CHCl_3$). Physical constants and data of IR, ^{1}H NMR, ^{13}C NMR and MS were comparable with those reported in the literature [21-23].

Friedelan-3β-ol (**4**): Combined major fraction "E" (fractions 16-17; ≅ 3.39 g) was rechromatographed on a dry silica gel column eluted with hexane-dichoromethane 3:2; from subfraction "E_4" a white solid was obtained which was purified by crystallization in methanol, m.p. = 285-287 °C; $[\alpha]_D$: +22.5° (c, 0.38 $CHCl_3$). Its spectral data are in accordance with those described previously [23, 24].

Betulinic acid (**5**): Ethyl acetate solutions of combined major fractions "L", "M" and "N" (fractions 39-48) were pooled and evaporated to dryness to give a green residue (≅ 4.72 g), which was filtered through a Sephadex LH-20 column packed in methanol; the eluted solution obtained was reduced "*in vacuo*" until a solid precipitated and this was then filtered off and recrystallized from methanol yielding pure white flakes; m.p. = 289-290 °C; $[\alpha]_D$: + 8.0 (c, 0.29 methanol). These physical constants and the obtained spectral data are consistent with those previously published for betulinic acid [23, 25].

RESULTS AND DISCUSSION

Dichloromethane extract from leaves of *Ch. meridensis* was purified by standard procedures as detailed in previous section "materials and methods". This methodology led to the isolation and purification of five compounds, whose identity was established by 1D- and 2D-NMR studies and comparison of their spectral data with those reported in the literature.

Compound (**1**) was obtained as white needles (m.p. = 187-188 ºC; $[\alpha]_D$: + 25.3°). It showed in TLC on silicagel GF_{254} plates a blue-green fluorescence spot, typical of a coumarin derivative [26], which is consistent with the presence in its UV spectrum of bands at λ_{max}= 254 and 365 nm [27]. A molecular ion peak at *m/z* 246.0906 [M^+] in its HR-EI-MS, in combination with ^{13}C-NMR spectroscopic data, suggested the molecular formula $C_{14}H_{14}O_4$ with eight indices of hydrogen deficiency.

The IR spectrum showed absorptions for double bonds (3044, 1626 and 928 cm^{-1}), hydroxyl groups (3448 cm^{-1}) and an α,β-unsaturated carbonyl group (1702 cm^{-1}). This latter group was also characterized in the ^{13}C-NMR spectrum, which displayed a carbonyl carbon signal at δ_C 161.6 [-O-C=O (C-2)], that together with the peaks of two substituted aromatic carbon at δ_C 155.8 [-O-C= (C-8a)] and δ_C 112.9 [>C= (C-4a)] and the signals of an olefin system [doublets at δ_H 6.17 and δ_H 7.57; *J* = 9.2 Hz (=C<u>H</u> / H-3 and H-4); HMQC: H-3 ↔ C-3 (δ_C 112.3, =<u>C</u>H); H-4 ↔ C-4 (δ_C 143.8; =<u>C</u>H)], conform the typical α,β-unsaturated δ-lactone of a coumarin α-pyron ring. The following HMBC correlations sequence C-3 ↔ H-4 ↔ C-4a ↔ H-3 ↔ C-2 ↔ H-4 ↔ C-8a (Fig. 3), confirm this structural subunit.

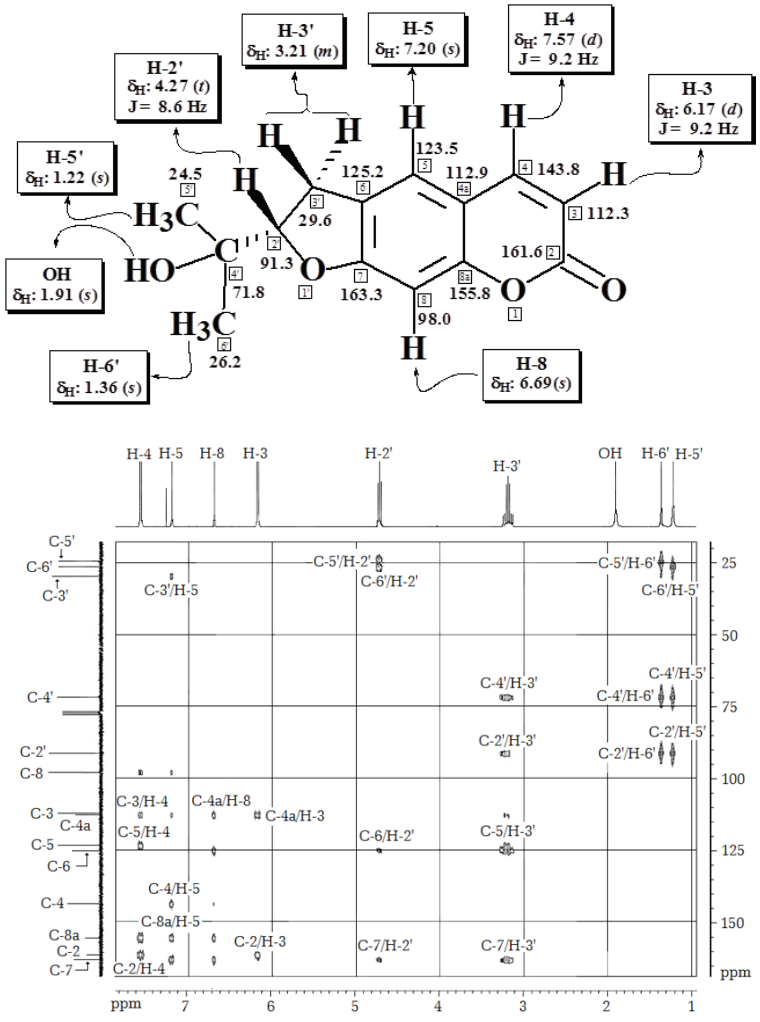

Fig. 3. ^{1}H and ^{13}C chemical shift assignments and HMBC spectrum of S-(+)-marmesin (1).

The α-pyron ring is condensed to a 1,2,4,5 tetrasubstituted benzene nucleus [singlets at δ_H 7.20 and δ_H 6.69 (=CH / H-5 and H-8); HMQC: H-5 ↔ C-5 (δ_C 123.5, =CH); H-8 ↔ C-8 (δ_C 98.0; =CH)] through the carbons C-8a and C-4a, according to HMBC cross-peaks C-8a ↔ H-5 ↔ C-4a ↔ H-8 (Fig. 3). The presence in the molecule of a third structural subunit, integrated by a 2-substituted dihydrofuran ring [δ_H 3.21, *m*, (>CH_2; H-3') and δ_H 4.72, t, *J* = 8.6 Hz (>CH-O-; H-2'); HMQC: H-3' ↔ C-3' (δ_C 29.6, >CH2); H-2' ↔ C-2' (δ_C 91.3; (>CH-O-)], which is linked to benzene nucleus through the carbons C-6 (δ_C 125.2; >C=) and C-7 (δ_C 163.3; -O-C=), assemble a molecular structure of a linear dihydropyranocoumarin.

The substituent at C-2' in dihydrofuran ring was identified as 1-hydroxy-1 methylethyl moiety, which is consistent with the existence of a base peak at *m/z*: 187.0379 [$C_{11}H_7O_3$/ M^+-HO-C-$(CH_3)_2$] in the HR-EI-MS, as well as with NMR data [two methyl singlets at δ_H 1.22 and δ_H 1.36 (H_3C-C(OH)-CH_3), a hydroxyl proton at δ_H 1.91 (*s*, -OH), two methyl carbon peaks at δ_C 24.5 (C-5') and δ_C 26.2 (C-6'), a quaternary oxy-carbon peak at δ_C 71.8 (-O-C<; C-4') and the following HMBC correlations:C-2' ↔ H-5' ↔ C-4'↔ H-6' ↔ C-2' ↔ H-3' ↔ C-4' ; C-5' ↔ H-6' and H-2' ↔ C-6' ↔ H-5'] (Fig 3). Consequently, the above analysis led to the gross structure (**1**) (C-2' ξ) named 2,3-dihydro-2-(1-hydroxy-1-methylethyl)-7*H*-furo[3,2 g][1]

benzopyran-7-one; this structure is assigned in the scientific literature to (+)-marmesin or to (-)-nodakenetin, depending on whether the configuration at C-2' is *S* or *R*, respectively [28]. The isolated compound is dextrorotatory and it is therefore S-(+)-marmesin (**1**), in which the C-2' substituent has an equatorial orientation [29, 30]. This coumarin was isolated for the first time from the bark of *Aegle marmelo* [31] and has subsequently been found in many species, particularly those included in Rutaceae and Apiaceae families [32, 33]. The biological and pharmacological interest of marmesin, as well as that of many other furocoumarins is well illustrated in the literature [18, 34-38].

Compound (**2**): White flakes, m.p. = 122-124 ºC; [α] D: +59.2°. The presence in its HR-EI-MS of an ion molecular peak at *m*/*z*: 484.3734 in conjunction with NMR data, allowed to establish the molecular formula $C_{32}H_{52}O_3$, which includes seven unsaturation degrees. Its IR spectrum showed typical bands of an aliphatic ester [ν_{max}: 1736 (C=O) and ν_{max}: 1244 and 1464 cm^{-1} (C-O-C)], a ketone (1712 cm^{-1}), and a cyclopropane ring (1036 cm^{-1}). The ^1H NMR spectrum shows four angular methyl singlets at δ_H 0.88 (H-28), 0.84 (H-29), 0.89 (H-30) and 0.95 (H-18), two overlapping methyl doublets at δ_H 1.09, J = 6.8 Hz (H-26 and H-27), a third methyl doublet δ_H 0.85, J = 6.4 Hz (H-21) and two mutually coupled proton doublets of a cyclopropane ring at δ_H 0.33 and 0.57, J = 4.2 Hz (>C<u>H</u>$_2$; H-19), suggesting a cycloartane triterpene skeleton. The presence in the molecule of an acetoxy group located at C-3, was made evident in the HR-EI-MS [prominent fragments at *m*/*z*: 424.3499 [M^+-CH_3COOH] and *m*/*z*: 400.3275 [M+-CH3COOH/-CH3, base peak] and also in 1D and 2D-NMR spectra [methyl singlet at δ_H 2.05 (H-32) HMQC: ↔ δ_C 21.5 (C-32); peak at δ_C 171.1 (-O-<u>C</u>=O; C-31); double doublet at δ_H 4.56, J = 11.2 and 5.6 Hz (>C<u>H</u>-O-; H-3) HMQC: ↔ δ_C 80.8 (>C<u>H</u>-O-; C-3) and HMBC correlations: H-3 ↔ C-31 ↔ H-32 (Fig. 5)]; the β-equatorial orientation of this acetoxy group was supported on the multiplicity of H-3 carbinyl hydrogen, which clearly implies axial-axial and axial-equatorial types coupling with C-2 methylene protons. Ketone carbonyl group [δ_C 215.5 (>C=O; C-24) was located in the side chain of cycloartane skeleton, on the carbon adjacent to the terminal isopropy group, as it was inferred from NMR data [^1H,^1H-COSY correlations between a septet at δ_H 2.61, J = 6.8 Hz; (>C<u>H</u> (H-25) and two overlapping methyl doublets at δ_H 1.09, J = 6.8 Hz (H-26 and H-27); ^{13}C NMR: δ_C 40.9 (>C<u>H</u> ; C-25) and δ_C 16.5 (2-C<u>H</u>3; C-26 and C-27) ; HMBC cross coupling: C-24 ↔ H-25, H-26 and H-27; C-25 ↔ H-26 and H-27; C-26 ↔ H-25 and H-27; C-27 ↔ H-25 and H-26] (Fig 5). The β-axial orientation of the side chain was determined through NOESY spectrum, where NOE effects involving H-17(α)/H-30(α); H-17(α)/H-21 and H-18(β)/H-20 were observed; similarly NOESY correlation between H-3(α)/H-5(α), Ha-19/H-29(β), Hb-19/H-8(β) and H-8/H-18(β) confirm a *trans* A/B, cis B/C and *trans* C/D ring junctions, typical in natural cycloartane triterpenes (Fig. 4).

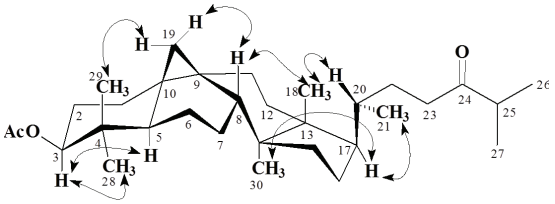

Fig. 4. NOE interactions in lagerenyl acetate (2).

In conclusion, the preceding data confirm that structure (**2**) corresponds to 3β-acetoxy-17α*H*-cycloartan-24-one, which it is commonly known as lagerenyl acetate. Up to now, this compound has been isolated only of two species, both included in genus *Lagerstroemia*: *L. lancasteri* [19] and *L. speciosa* [20] (Lytharaceae). Its 2D NMR spectral study is reported here for first time. Recently a biological study focused on human CYP3A4 promoter activity of this compound and its deacetyl derivative has been published [39].

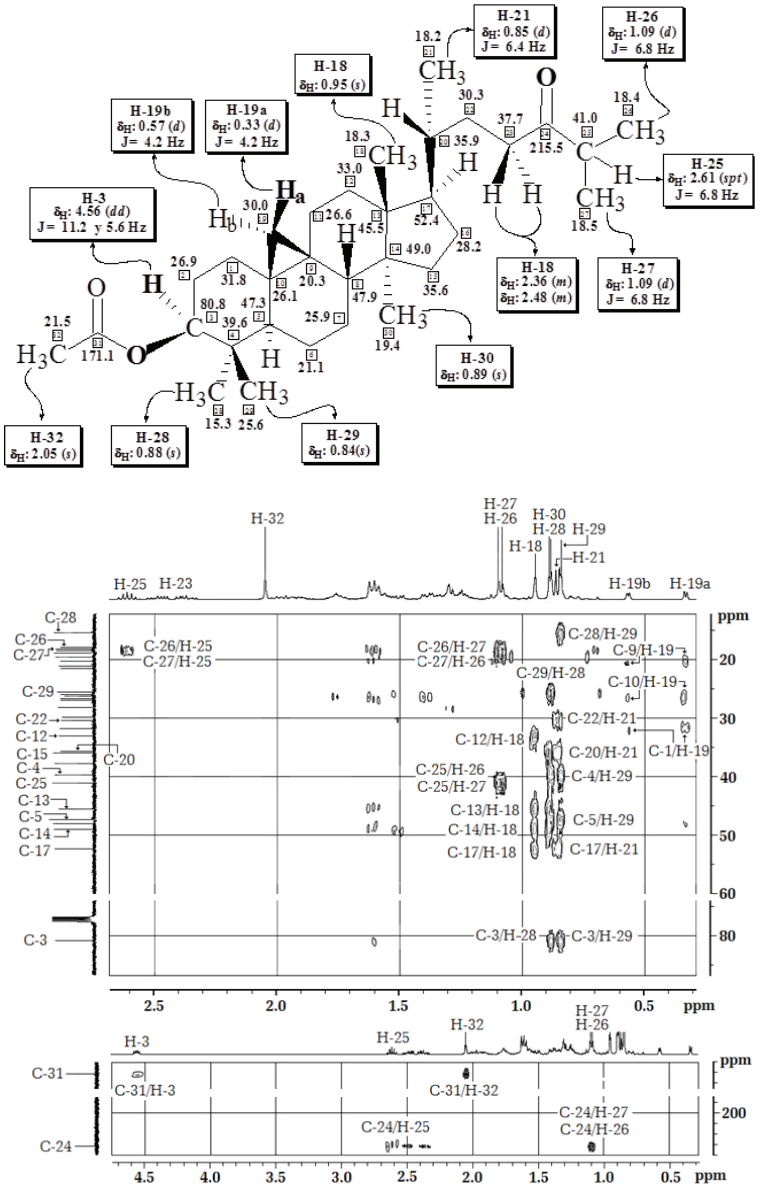

Fig. 5. ^{1}H and ^{13}C chemical shift assignments and HMBC spectrum of lagerenyl acetate (2).

Detailed analysis of spectral data of compounds (**3**), (**4**) and (**5**) (see materials and methods section) indicates that they also are triterpenoids. Comparison of the NMR data with those described in the literature, confirmed the identity of these compounds as friedelin (**3**) [21-23], friedelan-3β-ol (**4**) [23,24] and betulinic acid (**5**) [23,25]. These triterpenoids are widely distributed in plant kingdom [40-43], but up to now they have not been reported in the genus *Chaptalia*. Their potential biological activity is well documented in the literature [23, 24, 44-53].

CONCLUSIONS

In this first phytochemical study of the Venezuelan endemic species *Chaptalia meridensis*, have been isolated and identified four triterpenoids and a linear dihydrofuran-coumarin. It is the first time that this type of compounds is reported in the genus *Chaptalia*. A remarkable aspect of all isolates compounds is their wide range of biological activities.

From a chemotaxonomic point of view, it has

highlighted the presence of 5-methylcoumarins in the subtribe Mutisiinae [54, 55], in which it is included *Chaptalia* [9, 10]. However, this study and other previously reported by Zottis *et al*. [11] show that *Chaptalia* species produce a greater variety of coumarins.

It is highly significant presence in this specie of uncommon cycloartane triterpene lagerenyl acetate (**2**), whose spectral study using two-dimensional NMR techniques is performed here by first time.

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