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CONSTITUENTS OF FRUIT PULP OF *Maytenus salicifolia* AND COMPLETE 1D/2D NMR DATA OF 3β-HYDROXY-D:B-FRIEDO-OLEAN-5-ENE

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*A mixture of long-chain hydrocarbons constituted by nonacosane (29C, 7.5%), hentriacontane (31C, 48.3%), and tritriacontane (33C, 30.1%), the ester 1′-acetyloxymethylpentacosa-20′-enyl 10-hydroxydecanoate (**2**), β-amyrin (**3**), friedelin (**4**), and lupeol (**5**), and 3β-hydroxy-D:B-friedo-olean-5-ene (**6**) were identified as constituents of fruits of* Maytenus salicifolia *Reissek (Celastraceae). The structural formula and the stereochemistry of compound **6** were established by the data obtained through ^{1}H and ^{13}C NMR spectroscopy, including DEPT-135 and 2D (HMQC, HMBC, and NOESY) experiments. By analysis of the spectral data, it was possible to correct seven chemical shift assignments of compound **6**, which were erroneous attributed and published in the scientific literature.*

Keywords: *Maytenus salicifolia*, Celastraceae, 3β-hydroxy-D:B-friedo-olean-5-ene, pentacyclic triterpene, NMR.

Plants of the Celastraceae family comprise 90 genera and 1210 species worldwide, the majority of which are found in tropical and subtropical regions [1]. In Brazil, the members of this family are distributed in four genera: *Maytenus* Juss., *Goupia* Reiss., *Austroplenckia* Lund., and *Franhofera* Mart. [2, 3].

In a continuing research of bioactive metabolites from species of the Celastraceae family, we investigated the chemical constituents of the fruit pulp of *Maytenus salicifolia* Reissek. It is a native plant found mainly in the "cerrado" (Savanna) of the states of Bahia, Minas Gerais, and Rio de Janeiro, Brazil. The leaves of this species have been used in traditional medicine for the treatment of stomach ulcers. The decoction of the leaves made together with other parts of the plant is also traditionally used in the form of a bath to alleviate symptoms of itches and allergies [4]. The pentacyclic triterpenes (PCTTs) 3β-stearyloxy-ursan-12-ene, friedelin, β-friedelinol, α-amyrin, β-amyrin, and lupeol were previously isolated from the leaves of *M. salicifolia* by Miranda et al. [5]. PCTTs of the series friedelane, oleanane, ursane, lupane, and others are compounds that are frequently isolated from other species of the Celastraceae family [5]. Sesquiterpenes [6, 7], alkaloids and flavonoids [8], proanthocyanidins, and glycosylated steroids [9] are also commonly isolated. Pharmacological properties such as anti-inflammatory [10], antitumor [11], and anti-AIDS [12] have been presented for some of these PCTTs compounds. Then, the presence of PCTTs in Celastraceae species or in other plants confers to it an important chemical and therapeutic potential. The traditional medicinal use of *M. salicifolia* and its pharmacological potential induces us to study the phytochemical properties of different parts of this plant, including the pulp fruit. Generally, phytochemical studies of the fruit pulp of Celastraceae species lead to the isolation of sesquiterpenes [13, 14] and alkaloids [15]; nevertheless, the presence of PCTTs in this part of the plant is relatively rare.

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TABLE 1. Comparison of NMR Spectral Data of 3β-Hydroxy-D:B-friedo-olean-5-ene (**6**) with the Literature Data [17, 24]

C atom	δ_C	δ_H	HMBC		Literature δ_C data [9]
			$^2J_{CH}$	$^3J_{CH}$	
1	18.23	1.49 (m)	H-2	H-1, H-23, H-24	18.30
2	27.84	1.67 *eq* (m) 1.86 *ax* (m)			27.93
3	76.35	3.47 (dd, J =2.6, 3.1)			76.44
4	40.83	–			40.90
5	141.65	–	H-10	H-3, H-23, H-24	141.76
6	122.08	5.63 (m)	H-7	H-8, H-10	122.10
7	23.66	1.86 *eq* (m) 1.94 *ax* (m)			23.74
8	47.46	1.52 (m)			47.55
9	34.87	–			34.94
10	49.72	1.99 (m)			49.83
11	34.63	1.40 *eq* (m) 1.53 *ax* (m)			33.24
12	30.38	1.35 (m)			30.45
13	37.86	–			37.95
14	39.33	–			39.40
15	32.11	1.27 *eq* (m) 1.46 *ax* (m)			34.72
16	36.05	1.38 *eq* (m) 1.53 *ax* (m)			35.19
17	29.96	–			30.18
18	43.10	1.58 (m)	H-19	H-16, H-27, H-28	43.21
19	35.11	1.25 *ax* (m) 1.37 *eq* (m)	H-18	H-21, H-29, H-30	35.19
20	28.26	–			28.32
21	33.15	1.25 *eq* (m) 1.46 *ax* (m)	H-22	H-19, H-29, H-30	32.20
22	38.98	0.93 *eq* (m) 1.56 *ax* (m)	H-21	H-16, H-18, H-28	39.05
23	28.96	1.04 (s)	H-24	H-3	29.04
24	25.46	1.14 (s)	H-23	H-3	25.51
25	16.21	0.85 (s)		H-8, H-10, H-11	16.26
26	19.63	1.09 (s)		H-8, H-15	18.47
27	18.42	1.01 (s)		H-12, H-18	19.69
28	32.05	1.16 (s)		H-16, H-18, H-22	32.46
29	32.40	0.99 (s)		H-19, H-21, H-30	32.12
30	34.54	0.95 (s)		H-19, H-21, H-29	34.60

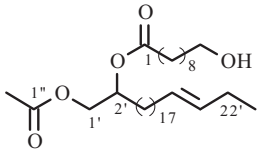
Chemical shifts are expressed in δ (ppm) scale. The multiplicity and J values (Hz) are in parentheses. The terms *ax* and *eq* denote axial and equatorial positions.

During the present study it was possible to isolate from the hexane extract of the *M. salicifolia* fruit pulp, a mixture of long-chain hydrocarbons **1**, which was identified through gas chromatography (GC). The ester (**2**) and a mixture of PCTTs (**3–6**) were later identified by hydrogen and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR).

^{1}H and ^{13}C NMR spectra of **1** (mp 60.5–63.2°C) showed the presence of one singlet at δ_H 1.81 attributed to methylene and methyl hydrogens, and another signal at δ_H 1.28 associated with the terminal methyl group. The ^{13}C NMR and DEPT-135 spectra showed the presence of five carbon signals, one associated with methyl and the other with the methylene group, which suggested the aliphatic nature of **1**. Then, **1** was submitted to GC together with standard linear long-chain hydrocarbons. By this chromatographic process it was possible to determine the presence of nonacosane (29C, 7.5%), hentriacontane (31C, 48.3%), and tritriacontane (33C, 30.1%) as constituents of the solid **1**.

The ^{1}H NMR spectrum of **2** (mp 131.2–136.4°C) showed signals at δ 0.84 and δ 2.40. We also observed signals at δ 4.15 and δ 4.29, which were attributed to hydrogen bonded to carbon attached to oxygen. The multiplicity of the signal at δ 4.29 (dd, H-1′) indicates the presence of diastereotopic hydrogen neighboring the CH-group. This fact was confirmed by HMBC, while the multiplicity of the signal at δ_H 4.15 (dt, H-10) indicates diastereotopic hydrogen of CH_2 bonded to another methylene group. The signal at δ_H 5.34 was attributed to olefin hydrogens (H-20′ and H-21′), and the signal at δ_H 5.26 (H-1′) was attributed to carbinolic hydrogen. From the ^{13}C NMR and DEPT-135 spectra of **2**, it was possible to determine the presence of 22 signals associated with methyl, methylene, methine, and non-hydrogenated carbons. The signal at δ 14.1 attributed to methyl conforms to a terminal methyl of a long-chain hydrocarbon. The signals at δ 62.1, δ 62.4, and δ 68.8 were

attributed to carbons attached to oxygen, and the signals at δ 129.7 and δ 130.0 to methine carbons of the olefin group. The HMQC spectrum showed correlations between the signals of methyl hydrogens at δ_H 2.07 with the carbon signal at δ_C 20.7. The signal at δ_C 20.7 was attributed to methyl carbon located to the side of the carbonyl group (C-1″). The correlation of the terminal methyl hydrogen signal at δ_H 0.88 with the carbon signal at δ_C 14.12 indicates that the hydrogen signal at δ_H 0.88 is associated with only one methyl group. The hydrogen signals at δ_H 4.15, 4.29, and 5.26 showed correlations with the carbon signals at δ_C 62.1, 62.4, and 68.8, respectively, which were associated to three saturated carbons attached to oxygen. The hydrogen signal at δ_H 5.34 showed correlations with the olefin carbon signals at δ_C 129.7 and 130.0. From the HMBC spectrum we observed correlations between the methyl hydrogen signal δ_H 2.07 (H-2″) with the carbon signal at δ_C 170.5, indicating the presence of a carbonyl group, and this carbon signal also showed correlations with the hydrogen signal at δ_H 4.29. In turn, this hydrogen signal showed correlations with another carbonyl signal at δ_C 172.9 (C-1) and with a methine carbon signal at δ_C 68.8 (C-2′). Correlations between the olefin carbons and methylene signals around δ_C 29.1 were not verified, indicating the presence of an isolated double bond. On the other hand, a correlation of the signal at δ_C 172.9 with the methylene hydrogen signal at δ_H 2.31 was observed. Based on the NMR correlations data, it was possible to establish part of the structure of compound **2**. The presence of only one terminal methyl group suggested that the ester has a long-chain hydrocarbon. The multiplicity of the hydrogen signal at δ_H 4.15 (dt) suggested the presence of one terminal hydroxyl group in compound **2**. The double bond position was established through simulations realized through the ACD Labs® program. The spectral data together with the ACD simulation results made it possible to establish that compound **2** has the structure of the ester 1′-acetyloxymethylpentacosa-20′-enyl 10-hydroxydecanoate.



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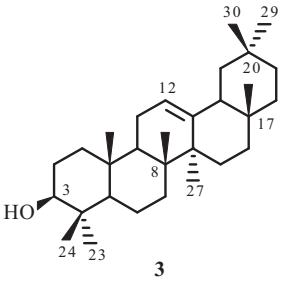
Compounds **3**, **4**, and **5**, when submitted to the Liebermann-Burchard (LB) test, showed a positive result for the pentacyclic triterpene (PCTT) [16].

By comparison of the ^{13}C NMR spectral data obtained with literature data, it was possible to confirm the structure of *β*-amyrin (**3**) [17], 3-oxo-friedelane (**4**) [18, 19], and lupeol (**5**) [20, 21].

Compound **6** was obtained as a white solid (mp 149.5–151.7°C) and when submitted to the LB test showed a positive result for PCTT [16]. From the analysis of ^{13}C NMR spectra, it was possible to detect 30 carbon signals, which by DEPT-135 were classified as 8 methyl, 10 methylene, 5 methine, and 7 nonhydrogenated carbons. The presence of 10 CH_2 and 5 CH groups suggested the structure of an oleanane triterpene. The chemical shift assignment observed for nonsaturated carbons (δ_C 122.08 and δ_C 141.65) led us to suspect that the double bond was located between carbons 5 and 6. These are the typical spectral data observed for a double-bond of the D:B-friedo-oleanane structure [22]. Due to this fact, the signals at δ_C 141.65 (C), δ_H 5.63, and δ_C 122.08 (CH) were respectively attributed to carbons C-5 and C-6.



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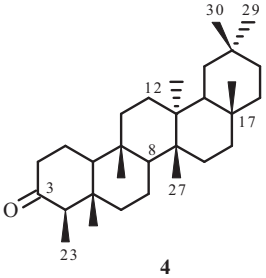

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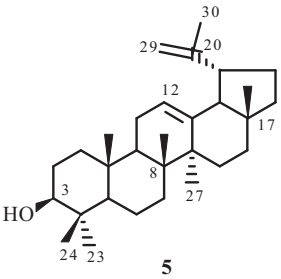

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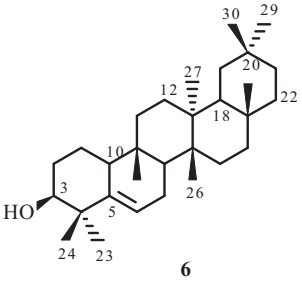

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Through detailed analysis of HMQC and HMBC spectral results associated with the literature data [23], it was possible to assign all hydrogen and carbon chemical shifts of compound **6**. The HMQC spectra allowed establishing the correlations of each carbon with its respective hydrogen, and to associate the signal at δ_C 76.35 to carbon C-3 [(H-C-O), δ_H 3.47] and at δ_C 122.08 to C-6 [(CH), δ_H 5.63]. From the analysis of HMBC spectra it was possible to observe correlations between the signal at δ_H 3.47, attributed to carbinol hydrogen H-3, with the signal of two methyl carbons, [δ_C 28.96 (C-23) and δ_C 25.46 (C-24)], and with the signal at δ_C 141.65 attributed to C-5. The signal at δ_H 5.63 attributed to olefin hydrogen

H-6 correlates with the signal at δ_C 40.83 (C-4), δ_C 23.66 (C-7), δ_C 47.08 (C-8), and δ_C 49.72 (C-10). The signal of C-10 correlates with the signal at δ_H 1.49 (H-1) and δ_H 0.85 (H-25). In turn, correlations were observed between the signal of H-25 with the signal at δ_C 49.72 (C-10), δ_C 34.87 (C-9), δ_C 33.15 (C-11), and δ_C 47.76 (C-8). Agrawal and Jain [22] reported the chemical shift assignments of methyl C-26 and C-27. The NMR values presented in this work are not in accordance with the literature data [22, 24] and indicated a change in the attribution of the spectral data of these methyl groups. This fact was also confirmed here by the analysis of the corresponding methyl correlations observed in the HMBC spectrum. Correlations between the signal at δ_H 1.01 with the signal at δ_C 37.86 (C-13), δ_C 39.33 (C-14), δ_C 30.37 (C-12), and δ_C 43.10 (C-18) were observed. For this reason, the signal at δ_H 1.01 was attributed to H-27. Similarly, correlations between the signal at δ_H 1.09 with the signal at δ_C 37.86 (C-13), δ_C 39.33 (C-14) and at δ_C 47.46 (C-8) were also observed. Then, the signal at δ_H 1.09 was attributed to H-26. Consequently, the chemical shifts at δ_H 1.09 and δ_C 19.63 were attributed to C-26, and δ_H 1.01 and δ_C 18.42 to C-27. We also observed correlations between the signal of H-26 with the carbon signal at δ_C 32.11, which was previously attributed in the literature [23] to C-21. However, this correlation is not possible for hydrogen H-26. Its signal must show a correlation with the signal of C-15. So, the chemical shift attribution of C-21 and C-15 must also change. Considering this change correct, a correlation between C-21 (δ_C 34.63) with the signal at δ_H 0.85 attributed to H-25 was observed in the HMBC spectrum. Nevertheless, this NMR correlation is not possible for methyl C-25, and it is possible to conclude that the signal of C-21 was changed by another carbon signal. Correlations between the signal of H-25 (δ_H 0.85) with the signal at δ_C 47.46 (C-8), δ_C 49.72 (C-10), δ_C 34.86 (C-9), and δ_C 34.63 (C-11) were observed in the HMBC spectrum. Considering the fact that the signal of methyl C-25 (δ_C 16.21) must have correlations with the signal of carbon C-11, it is possible to conclude that the signal at δ_C 34.63 is associated with C-11. Thus, the signal of C-21 was changed by the signal of C-11. Therefore, it was possible to conclude that the signals at δ_C 32.11, δ_C 33.15, and δ_C 34.63 were previously changed and published [23] as corresponding to C-21, C-11, and C-15. By carefully HMBC analysis, these signals are here corrected and adequately attributed to C-15, C-21, and C-11.

The signal at δ_H 1.16 previously attributed to H-29 [23] correlates with the signals at δ_C 29.96 (C-17), δ_C 36.05 (C-16), δ_C 38.98 (C-22), and δ_C 43.10 (C-18). Nevertheless, any of these correlations are possible for H-29 but is adequate for the signal of H-28. Therefore, the signal at δ_H 1.16 attributed before to H-28 was changed by another methyl hydrogen signal, which could only be the signal of H-29 or H-30. Then, the chemical shift assignment of methyl C-28 was established at δ_C 32.05 (δ_H 1.16). Correlations between the signal at δ_C 35.11 (C-19), δ_C 28.26 (C-20), and at δ_C 33.15 (C-21) with those at δ_H 0.95 and δ_H 0.99 were observed, and for this reason were respectively attributed to H-29 and H-30.

The stereochemistry of compound **6** was determined through the NOESY spectra, which provided data adequate to establish the correct attribution of H-29 and H-30. From the analysis of contour plots it was possible to observe the NOE effect between H-3 with H-23, H-24, H-2ax, and H-2eq. Due to the presence of olefin carbons in ring B, the chair conformation of ring A acquires little distortion. NOE effects were observed between H-6 with H-24 and H-7eq; H-26 with H-18, H-25, H-7ax, H-12ax, and H-16ax; H-27 with H-8 and H-12eq, and also between H-18 with H-28, H-26, and H-19ax. In the NOESY spectra we also verified correlations between the signal of H-19eq, H-27, H-21eq, and H-22ax with the signal at δ_H 0.95. By these correlations, it was possible to verify the attribution of H-30 (C-30, δ_C 34.54). The signals of H-19ax and H-21ax correlated with the signal at δ_H 0.99. Therefore, this signal was attributed to H-29 (C-29, δ_C 32.40). The chemical shift assignments of methyl C-29 and C-30 were also inadequately published in previous papers [22, 24]. Some NOE correlations observed for compound **6** are shown in Fig. 1.

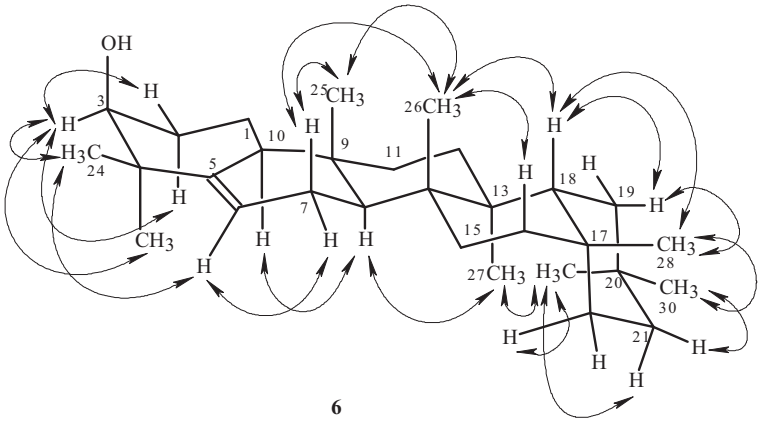

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Fig. 1. NOESY correlations of 3β-hydroxy-D:B-friedo-olean-5-ene (**6**).

The 2D NMR experimental data were very important to establish the correct chemical shift assignments and to determine the stereochemistry of 3β-hydroxy-D:B-friedo-olean-5-ene (**6**). Through 2D techniques, it was possible to establish the correct attributions of five signals published incorrectly. The chemical shift assignments of 3β-hydroxy-D:B-friedo-olean-5-ene (**6**), including the data in the literature, are compiled in Table 1.

EXPERIMENTAL

General Experimental Procedures. Melting points (uncorrected) were determined on a Mettler FP 80 HT apparatus. Plates of silica gel G-60/$F_{254\ nm}$ (0.25 mm, Merck) were previously activated in an oven at 100°C. Spots were visualized on TLC under UV light and exposed to iodine vapor in an iodine chamber, and also by heating the chromatoplates at 100°C in an oven after spraying with a solution of vanillin/perchloric acid [25]. Column chromatography (CC) was run using silica gel of 70–230 mesh (ASTM). The GC analysis was carried out on a Varian CP-3380 chromatograph with an HP1 column, 30 m length, 0.32 mm i.d., column temperature 200°C under isothermal conditions for 1 min followed by a linear temperature program of 10°C/min up to 300°C; injector temperature 300°C, split ratio 1/50; flame ionization detector (FID) at 300°C, 2 mL/min hydrogen flow rate.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DRX-400 spectrometer operating at 400.13 (^{1}H) and 100.62 (^{13}C) MHz and 300 K. A direct-detection 5-mm dual probe $^{1}H/^{13}C$ was used with 90° pulse width (PW) of 6.44 μs and 7.50 μs for ^{1}H and ^{13}C, respectively. The sample was dissolved in $CDCl_3$ (0.5 mL) and transferred to a 5.0 mm i.d. NMR tube. TMS was used as an internal standard ($\delta_H = \delta_C = 0$). The two-dimensional (2D) HSQC spectrum was obtained using a 5 mm multinuclear probe, and data points were recorded with eight transients (ns) for each time increment. The HMBC spectrum was obtained by the gradient-selection method. The 2D NOESY was recorded with 2048 points along f2 and 169 points along f1 with mixing time set to 400 ms. NMR data were processed with Bruker (DRX 400) microprograms and the software XWIN-NMR vs. 3.1 for Windows XP. Infrared (IR) spectra (KBr, ν_{max}) were acquired on a Perkin–Elmer Spectrum One FTIR spectrometer.

Plant Material. The aerial part of *Maytenus salicifolia*, including fruits, was collected in May 2005 from the Ecologic Station of Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil. The material was identified by Dr. Rita Maria Carvalho-Okano of the Universidade Federal de Vicosa, Minas Gerais, Brazil, through comparison with a voucher specimen (No. BHCB 22856) deposited in the Herbarium of the Departamento de Botanica do Instituto de Ciencias Biologicas, UFMG. The pulp of the fruits was immediately separated from the seed and dried over Kraft paper at room temperature.

Compound Isolation. The air-dried and coarsely powdered pulp of *M. salicifolia* fruits (932 g) was submitted to exhaustive extraction with hexane in a Soxhlet apparatus for 24 hours and then filtered. The hexane extract was concentrated in a rotary evaporator to give a crude extract (3.62 g), which was submitted to CC_1 on silica gel (120 g). The column was eluted with hexane and $CHCl_3$ mixtures of increasing polarity to yield 21 fractions (frs. 1–21) according to TLC analysis. Fractions 1–2 (875.12 mg) were subjected to CC_2 on silica gel (30 g), also eluted with a gradient of hexane–$CHCl_3$, furnishing 23 fractions, which were combined into 10 groups (G 1–10). Group G-1 was obtained as a white amorphous solid material (mixture 1) (437.0 mg), which was studied by GC. Group G-2 was submitted to preparative TLC using hexane–$CHCl_3$ (1:1) as eluent, furnishing a white solid material (compound **2**). Group G-9 (85.2 mg) was subjected to successive column chromatography to yield compound **3** (18.4 mg). Fraction 3 (196 mg) obtained from CC_1 was rechromatographed in CC_4 over silica gel (6 g) using hexane–chloroform (7:3) as eluent. Subfractions 6–9 furnished compound **5** (65.7 mg), and subfractions 13–19 gave compound **4** (46.0 mg). Fractions 26–28 furnished **6** (16.0 mg) with a high degree of purity, observed by melting point and TLC.

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