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New Compounds Isolated from *Trichilia pseudostipularis* (Meliaceae)

Renata R. S. Robaina, [a] ***Michel S. Passos,*** [a] ***Raimundo Braz-Filho*** [a,b] ***and Ivo J. C. Vieira*** [a]

[a]*Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 28013-602 Campos dos Goytacazes-RJ, Brazil*

[b]*Departamento de Química, Universidade Federal Rural do Rio de Janeiro, 23897-000 Seropédica-RJ, Brazil*

The genus *Trichilia*, belonging to the Meliaceae family, comprises ca. 102 species, some of which have been the subject of phytochemical studies. Compounds of different classes with cytotoxic, antimicrobial, and antioxidant activities were isolated from *Thichilia*. *Trichilia pseudostipularis*, a native species, is distributed along the entire Brazilian coast, from southern Santa Catarina to Bahia. Due to the inexistence of previous chemical studies, their wood and roots were investigated. Compounds 3,4-dihydro-4-isopropyl-6-methylnaphthalen-1(2*H*)-one (**1**), 3,4-dihydro-7-hydroxy-4-isopropyl-6-methylnaphthalen-1(2*H*)-one (**2**), and pseudostipulariol (**3**) were isolated from wood, while the lactone derivate *rel*-(2*S*,3*S*,4*R*)-3-hydroxy-4-methyl-2-(13"-phenyl-1'-*n*-tridecyl)-butanolide (**4**) was isolated from the roots. Compounds **3** and **4** are reported for the first time.

Keywords: *Trichilia pseudostipularis*, sesquiterpenes, γ-lactone

Introduction

Trichilia P. Browne is one of the most numerous genera in the family Meliaceae.[1,2] According to data from the WFO: Plant List website,[3] there are approximately 102 accepted species of the genus *Trichilia*. Until 2014, 334 different secondary metabolites such as monoterpenes, diterpenes, triterpenes, steroids, limonoids, coumarins, flavonoids, lignans, phenolic acids, amino acids, and lactones had been isolated from species of *Trichilia*.[4] It is worth highlighting that a recent review[5] covering exclusively limonoids between 1996 and 2020 identified 227 of these substances with different skeletons. Studies[6-9] involving extracts and compounds isolated from different species of *Trichilia*, have presented biological activities, such as cytotoxicity, antifeedant, antimicrobial and insecticide.

Trichilia pseudostipularis, a native species from Brazil, is distributed along the entire coast of the country, from southern Santa Catarina to Bahia state. With its smooth grayish-white bark, greenish-white to creamy pink flowers, and yellowish fruits, this species demonstrates more morphological variation than any other member of the genus *Trichilia* present in the coastal forests of Brazil.[10] No papers reporting phytochemical studies of this species were found in the literature. Due to the medicinal[11] importance of the genus *Trichilia* and the lack of any study on the chemical constitution of *T. pseudostipularis*, a study of the wood and roots was carried out aiming to make a chemotaxonomic contribution to the genus. As a result, three sesquiterpenes (**1**-**3**) and one lactone (**4**) were isolated.

*e-mail: renatarobainadasilva@gmail.com
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Experimental

General experimental procedures

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ASCEND 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), using $CDCl_3$ as the solvent. High-resolution electrospray ionization-mass spectrometry (HRESI-MS) data were acquired with a Bruker Daltonics micrOTOF-Q II mass spectrometer, using the positive ion analysis mode. The chromatograms and low-resolution mass spectra were obtained with gas chromatography-mass spectrometry (GC-EIMS) performed in Agilent equipment, model 5975C masses coupled to a 7890A gas chromatograph. Sample insertion was performed using a 10 μL syringe in a model 7693A automatic injector. A capillary column (HP5 ms, 30 m × 250 μm × 0.25 μm of (5% phenyl)-methylpolysiloxane film) was used with helium as a carrier gas at a constant flow of 1.0 mL min^{-1}. The injected volume was 1 μL in split mode 1:2. The

temperature program was set at 50 ºC for 1 min, rising to 180 ºC (2 ºC min^{-1}), subsequently rising to 250 ºC (10 ºC min^{-1}) for 10 min, and finally rising to 280 ºC. Mass detector analysis was performed, applying the following parameters: interface, ionization source and quadrupole analyzer temperature at 280, 230 and 150 ºC, respectively, and electron impact ionization (EI) mode at 70 eV. Column chromatography (CC) was performed in silica gel (0.063-0.200 mm, Merck). Preparative thin layer chromatography (prep-TLC) was carried out using Merck silica gel 60 PF254 on glass plates (20 cm × 20 cm). The solvents used in both the extraction and isolation steps were purchased from Synth (Diadema, Brazil) with an analytical grade. Optical rotations were measured with a Jasco model P-2000 polarimeter using CH_3OH or CH_2Cl_2.

Plant material

The wood and roots of *T. pseudostipularis* were collected in the Vale Natural Reserve, in the municipality of Linhares, Espírito Santo, Brazil. The voucher specimen, code CVRD 622, is in the Vale Natural Reserve.

Extraction and isolation

The dried roots (1.0 kg) and wood (1.2 kg) were powdered and subjected to extraction three times in methanol at room temperature (each for 5 days). The methanol extracts of the wood (45.0 g) and roots (55.0 g) were concentrated under negative pressure, suspended in water, and partitioned using organic solvents in increasing order of polarity. CH_2Cl_2, EtOAc, and ButOH were used in the partitions.

The CH_2Cl_2 partition of the methanol extract of the wood (5.8 g) was fractionated by CC, using silica gel eluted in a CH_2Cl_2:MeOH gradient system (1:0 to 0:1), yielding 22 fractions (TPMD1-TPMD22). The TPMD6 fraction (285.8 mg) was applied to CC with silica gel eluted in a gradient system of hexane:EtOAc (1:0 to 0:1) giving rise to 16 subfractions (TPMD6.1-TPMD6.16). Subfraction TPMD6.6 (67.1 mg) was subjected to CC with silica gel eluted in a gradient system with hexane:EtOAc (1:0 to 1:20) to obtain 3 subfractions (TPMD6.6.1-TPMD6.6.3). TPMD6.6.1 was purified with prep-TLC, obtaining compound **1** (9.0 mg). Fraction TPMD13 (374.3 mg) was purified with silica gel CC eluted with hexane:acetone (1:0 to 0:1), to obtain compound **2** (11.5 mg). TPMD17 (136.2 mg) was subjected to silica gel CC eluted in a gradient system of hexane:EtOAc (1:0 to 0:1), to yield 11 subfractions (TPMD17.1-TPMD17.11). TPMD17.6 (39.6 mg) was purified by CC using silica gel eluted in a CH_2Cl_2:MeOH gradient system (1:0 to 1:20), to yield compound **3** (6.5 mg).

The CH_2Cl_2 partition of the root extract (4.6 g) was fractionated using silica gel CC eluted with CH_2Cl_2:MeOH in gradient (1:0 to 0:1) leading to 22 fractions (TPRD1-TPRD22). The TPRD11 (41.3 mg) fraction was subjected to CC using silica gel with a gradient system of hexane:EtOAc (1:0 to 0:1), giving rise to 23 subfractions (TPRD11.1-TPRD11.23). TPRD11.17 (30.0 mg) was purified by CC with silica gel eluted in a gradual system of CH_2Cl_2:MeOH (1:0 to 1:3), to yield compound **4** (5.9 mg).

Acetylation of compound **3**

Compound **3** was acetylated with a 1:2 mixture of acetic anhydride and pyridine at room temperature for 24 h. The reaction was interrupted by the addition of water. The acetylated product was extracted with CH_2Cl_2 and purified by CC.

Characterization data

Pseudostipulariol (**3**)

White amorphous powder; optical rotation: $[\alpha]_D^{24}$ –13.8° (*c* 0.0026, CH_3OH); spectroscopic data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.62 (d, *J* 7.3 Hz, 2H-1), 4.25 (d, *J* 7.3 Hz, H-2), 3.27 (d, *J* 10.3 Hz, H-3a), 3.44 (d, *J* 10.3 Hz, H-3b), 1.20 (1H-5), 1.43 (t, *J* 8.5 Hz, H-6a), 1.41 (dd, *J* 8.5, 3.8 Hz, H-6b), 1.46 (H-7), 1.55 (2H-8), 1.68 (H-9a), 1.19 (H-9b), 1,76 (s, 3H-12), 4.74 (s, H-13a), 4.72 (s, H-13b), 0.97 (s, 3H-14), 1.14 (s, 3H-15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.0 (C-1), 78.1 (C-2), 72.0 (C-3), 47.9 (C-4), 53.4 (C-5), 27.3 (C-6), 46.4 (C-7), 26.9 (C-8), 41.1 (C-9), 48.4 (C-10), 150.6 (C-11), 20.9 (C-12), 108.5 (C-13), 14.1 (C-14), 20.2 (C-15); GC-EIMS t_R / min 13.707 (81.88%), *m/z*, not available $[M]^+$; (+)-HRESI-MS *m/z*, 261.1781 $[M + Na]^+$ (calc. *m/z* 261.1830).

rel-(2*S*,3*S*,4*R*)-3-Hydroxy-4-methyl-2-(13”-phenyl-1-*n*-tridecyl)-butanolide (**4**)

White amorphous powder; optical rotation: $[\alpha]_D^{24}$ –48.2° (*c* 0.002, CH_2Cl_2); spectroscopic data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.57 (m, H-2), 3.86 (dd, *J* 8.8 and 7.1 Hz, H-3), 4.22 (q, 6.8, H-4), 1.48 (d, 6.3, 3H-5), 1.60 (H-1’a), 1.89 (H-1’b), 1.50 (2H-2’), 1.33-1.28 (2H-3’-2H-11’), 1.63 (m, 2H-12’), 2.62 (t, *J* 7.7 Hz, 2H-13’), 7.29 (d, *J* 7.2 Hz, H-2”/H-6”), 7.19 (t, *J* 7.2 Hz, H-4”), 7.20 (t, *J* 7.2 Hz, H-3”/H-5”); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9 (C-1), 48.7 (C-2), 79.1 (C-3), 79.8 (C-4), 18.3 (C-5), 28.5 (C-1’), 26.8 (C-2’), 29.4-29.7 (C-3’-C-11’), 31.5 (C-12’), 36.0 (C-13’), 143.0 (C-1”), 128.4 (C-2”/C-6”),

125.5 (C-4"), 128.2 (C-3"/C-5"); GC-EIMS t_R / min 23.017 (70.56%), *m/z*, not available $[M]^+$; (+)-HRESI-MS *m/z*, 397.2702 $[M + Na]^+$ (calc. *m/z* 397.2719).

Diacetylpseudostipulariol (**3a**)

Yellow oil; spectroscopic data: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (2H-1), 5.16 (d, *J* 7.5 Hz, H-2), 3.86 (d, *J* 10.3 Hz, H-3a), 3.95 (d, *J* 10.3 Hz, H-3b), 1.34 (H-5), 1.53 (H-6a), 1.36 (H-6b), 1.93 (H-7), 1.58 (2H-8), 1.21 (H-9a), 1.70 (H-9b), 1.77 (s, 3H-12), 4.73 (d, *J* 9.9 Hz, 2H-13), 0.90 (s, 3H-14), 1.08 (s, 3H-15), 2.07 (s, Me-2), 2.08 (s, Me-3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 47.5 (C-1), 79.3 (C-2), 71.5 (C-3), 46.5 (C-4), 53.2 (C-5), 27.0 (C-6), 46.2 (C-7), 26.7 (C-8), 41.8 (C-9), 47.9 (C-10), 150.2 (C-11), 21.1 (C-12), 108.6 (C-13), 14.9 (C-14), 19.2 (C-15), 170.6 (AcO-2), 21.0 (Me-2), 171.3 (AcO-3), 21.2 (Me-3).

Results and Discussion

Three compounds were isolated from the CH_2Cl_2 fraction of methanolic wood extract of *T. pseudostipularis* and identified as sesquiterpenes (**1**-**3**). From the CH_2Cl_2 fraction of methanolic root extract of this one lactone derivate (**4**) was isolated (column and preparative chromatography) and characterized. The chemical structures of the compounds (Figure 1) were determined using nuclear magnetic resonance (^{1}H and ^{13}C NMR 1D and 2D), GC-EIMS and HRESI-MS and comparison with literature data.[12-14]

Two known sesquiterpenes were characterized as 3,4-dihydro-4-isopropyl-6-methylnaphthalen-1(2*H*)-one (**1**)[15] and 3,4-dihydro-7-hydroxy-4-isopropyl-6-methylnaphthalen-1(2*H*)-one (**2**).[16]

Compound **3**, isolated as a white amorphous solid $[\alpha]_D^{24}$ −13.8º (*c* 0.0026, CH_3OH), was identified as a sesquiterpene for which, to date, no reports were found in the literature. The molecular formula $C_{15}H_{26}O_2$ was found from the sodiated *quasi*-molecular ion $[M + Na]^+$ at *m/z* 261.1781 (calcd. *m/z* 261.1830), implying a hydrogen deficiency equal to 3, one referring to unsaturation and two assigned to the two cycles. Fifteen signals were observed in the ^{13}C-DEPTQ (distortionless enhancement by polarization transfer-Q) spectrum (Table S1, Supplementary Information (SI) section): six methylenes ($(CH_2)_6$, including one sp^3 carbon oxygenated at δ_C 72.0 ($HOCH_2$-3) and one sp^2 carbon at δ_C 108.5 (H_2C-13)); three methines ($(CH)_3$, including one oxygenated carbon sp^3 at δ_C 78.1 (HOCH-2)); three methyl groups ($(CH_3)_3$: δ_C 14.1 (CH_3-14), 20.2 (CH_3-15), and 20.9 (CH_3-12)) and three quaternary carbons ($(C)_3$), including one sp^2 at δ_C 150.6 (C-11).

The presence of two hydroxyl groups in the structure of **3** was confirmed by ^{1}H and ^{13}C NMR data of diacetyl derivative **3a** (Table S1). The NMR data of **3** as well as comparison with those of **5**[12] (Table S1) shows a sesquiterpene bearing an additional hydroxyl group at CH-2 (δ_C 78.1/ δ_H 4.25, d, *J* 7.3 Hz). This position was unequivocally deduced by the modifications observed in the comparison of chemical shifts of carbon signals CH_2-3 (δ_C 72.0 (**3**)/73.0 (**5**), CH-5 (δ_C 53.6 (**3**)/47.0 (**5**), C-10 (δ_C 48.5 (**3**)/42.6 (**5**), and CH_3-14 (δ_C 14.1 (**3**)/20.9 (**5**) revealing δ protection effects (Table S1), Δ_C 1.0, 6.6, 5.9, and 6.8 ppm respectively. As expected, β-carbon deshielding effects in C-4 (δ_C 47.9 (3)/42.9 (**5**), and CH_2-1 (δ_C 50.0 (**3**)/34.3 (**5**) were also observed (Table S1). These data revealed that **3**, is closely related to **5**, differing by stereochemistry and the presence of hydroxyl group attached at CH-2.

The stereochemistry of the prop-1-en-2-yl linked at carbon CH-7 (α and β prop-1-en-2-yl, respectively), corroborated with this proposed structure. As observed for compound **5**,[12] the ^{13}C NMR spectrum shows two signals

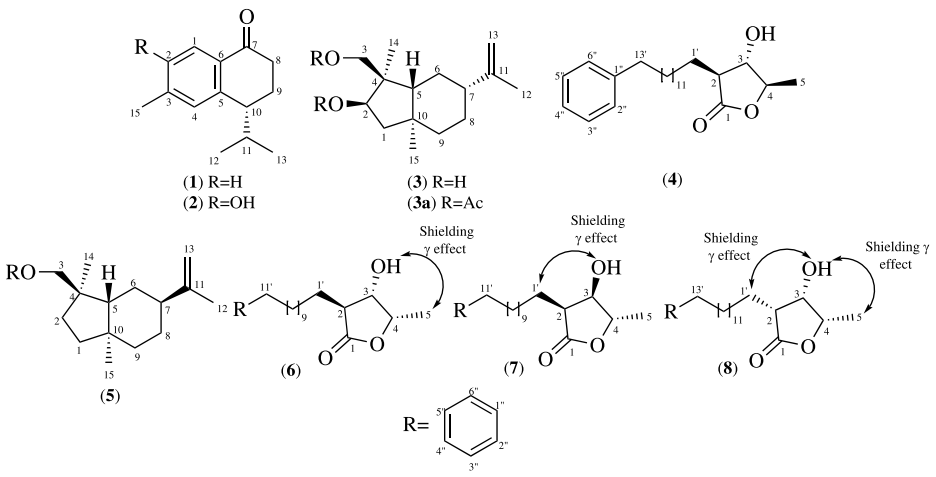

Figure 1. Compounds isolated from *T. pseudostipularis* (**1**-**4**) and prepared derivatives (**3a**). Compounds **6**-**7**,[13] and **8**[14] were used as models for comparison of NMR spectral data.

corresponding to double bond carbons at δ_C 150.6 (C-13) and 108.5 ppm (C-11), as well as signals at δ_C 14.2 and 20.2 ppm, attributed to methyl carbons C-14 and C-15. Also present in the spectral data of **3** and **5**[12] are signals corresponding to the oxygenated carbon in C-3, δ_C 72.0 and 71.5 ppm, respectively. The presence of hydroxyl groups attached to carbons CH-2 and CH_2-3 was confirmed by acetylation of **3** and attainment of the diacetyl derivative **3a** (Figure 1). The comparative NMR (1D and 2D) analysis of **3** and **3a** (Table S1) showed the presence of four additional signals in the ^{13}C NMR spectrum of compound **3a**: two quaternary carbons at δ_C 170.6 and δ_C 171.3 ppm and two methyl carbons at δ_C 21.0 and δ_C 21.2 ppm.

The HMBC (heteronuclear multiple bond correlation) spectrum of **3a** revealed heteronuclear correlations between the hydrogen signals assigned to H-2 (δ_H 5.16, d, *J* 7.5 Hz, $^3J_{HC}$) and the carbon signal at δ_C 170.6 and 3H-Ac-2 (δ_H 2.07, $^2J_{HC}$, correlated in the heteronuclear single quantum coherence (HSQC) ($^1J_{HC}$) with the carbon signal at δ_C 21.0), indicating the presence of the acetyl group attached to CH-2 (Table S1). Correlations were also observed between the 2H-3 hydrogen signals (δ_H 3.95, d, *J* 10.7 Hz, δ_H 3.86, d, *J* 10.7 Hz, $^3J_{HC}$) and signal at δ_C 171.3 (Ac-3) and the singlet signal of 3H-Ac-3 at δ_H 2.08 ($^2J_{HC}$) with the carbon signal at δ_C 21.2, indicating the presence of an additional acetyl group attached to CH_2-3, corroborating with the proposed structure for compound **3** (sesquiterpene diol, Figure 1).

The relative stereochemistry of 3 (*rel*-(2*R*,4*R*,5*R*,7*R*,10*S*)-4-(hydroxymethyl)-4,10-dimetil-7-(prop-1-en-2-yl)-octahydro-1*H*-inden-2-ol) was determined from the coupling constants of relevant hydrogens and from the observed nuclear Overhauser enhancement spectroscopy (^{1}H-^{1}H-NOESY) (**3b**, Figure 2). All the ^1H and ^{13}C NMR chemical shift assignments are summarized in Table S1.

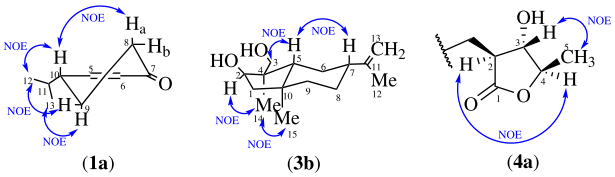

Figure 2. 1a (^{1}H-^{1}H-NOESY of **1** and **2**) **3b** (^{1}H-^{1}H-NOESY of **3**) and **4a** (^{1}H-^{1}H-NOESY of **4**). Key nuclear Overhauser (NOE) correlations.

Consistent with these observations, the ^{1}H-^{1}H-NOESY spectrum of **3b** at 70 ºC showed cross-peaks assigned to the dipolar interaction (spatial proximity, **3b**, Figure 2) of H-5β (axial, δ_H 1.20) with both 2H-3 (δ_H 3.44, d, *J* 10.3 Hz and 3.27, d, *J* 10.3 Hz) and H-7β (axial, δ_H 1.96) and the 3H-14 (δ_H 0.97, s) with both H-2 (δ_H 4.25, d, *J* 7.3 Hz) and 3H-15 (δ_H 1.14, s).

The principal peaks observed in the EIMS (electron ionization mass spectral) spectrum of **3** are in agreement with proposed fragmentation mechanisms summarized in Scheme S1 (SI section).

Compound **4**, obtained as a white solid, presented specific optical rotation of $[\alpha]_D^{24}$ –48.2º (*c* 0.002, CH_2Cl_2). The HRESI-MS exhibited ion peaks at *m/z* 397.2702 $[M + Na]^+$ (calc. *m/z* 397.2719) and 771.5428 [adduct $M + M + Na]^+$ (calc. *m/z* 771.5540)] compatible with the molecular formula $C_{24}H_{38}O_3$. In the low-resolution mass spectrum (EIMS) a peak was observed at *m/z* 356 ($C_{32}H_{36}O_2$) corresponding to the elimination of H_2O of the molecular ion peak at *m/z* 374 (Scheme S3, SI section).

As observed for compounds **6** and **7**,[12] both of which are very similar in structure, the signals at δ_H 1.28-1.33, δ_H 7.20, δ_H 7.19 and δ_H 7.29 in the ^1H NMR spectrum and the ion at *m/z* 91 (Figure S31, SI section) suggest the presence of an alkylphenyl group. The two hydrogen signals attached to oxygenated carbons, H-4 (δ_H 4.22, m) and H-3 (δ_H 3.86, t, *J* 8.3 Hz), one doublet attributed to the methyl group, 3H-5 (δ_H 1.48, d, *J* 6.3 Hz), couple with H-4, revealed by ^{1}H-^{1}H-COSY. Were also observed coupling of H-4 (δ_H 4.22, m) with H-3 (δ_H 3.86, t, *J* 8.3 Hz), H-3 with both H-4 and H-2 (δ_H 2.57, m), and H-2 with 2H-1' (δ_H 1.89, m) compatible with the presence of γ-lactone (Figure 1 and Table S2, SI section), confirmed by heteronuclear correlations observed in the HMBC between carbonyl carbon C-1 (δ_H 175.9) and hydrogen signals H-2 (δ_H 2.57, m, $^2J_{HC}$), H-3 (δ_H 3.86, t, *J* 8.3 Hz, $^3J_{HC}$), H-4 (δ_H 4.22, m, $^3J_{HC}$), and 2H-1' (δ_H 1.89, m, $^3J_{HC}$). Through the correlations between H-2 with C-3 and C-1, and 3H-5 with H-3 and H-4 the structure of this group was defined.

The ^{13}C NMR spectrum of **4** revealed aromatic carbon signals at δ_C 143.0 (C-1''), 128.4 (CH-2''/CH-6''), 128.2 (CH-3''/CH-5''), and 125.5 (CH-4''), characterizing the presence of a monosubstituted aromatic ring, confirmed by HSQC spectrum through direct heteronuclear correlations ($^1J_{HC}$) with hydrogen signals at δ_H 7.29 (d, 7.6 Hz, H-2''/H-6''), 7.20 (d, H-3''/H-5''), and 7.19 (t, 7.2 Hz, H-4''), respectively (Table S2).

In the ^1H and ^{13}C NMR spectra broad and intense signals were also observed, with the chemical shift at δ_H 1.28 and 1.33/δ_C 29.4 and δ_C 29.7 attributed to the hydrogens/carbons of a relatively long methylene chain (CH_2-3' to CH_2-11', compatible with C_6H_5-$(CH_2)_n$-$(CH)_3(CH_3)$(OH)COO = phenyl-$(CH_2)_n$-3-hidroxy-4-methyl-γ-lactone.

The relative stereochemistry of **4**, was determined from the coupling constants of relevant hydrogens and from the observed ^{1}H-^{1}H-NOESY (**4a**, Figure 2), also involving a comparison with stereochemistry described in the literature for compounds **6**-**8**[13,14] (Figure 2). The coupling constant

relative to H-3 of *J* 6.8 Hz, like that observed for the hydrogen in the same position in compound **7**,[13] indicates a *trans* arrangement between H-3 and H-4. Consistent with these observations, the NOESY spectrum of **4** at 70 ºC showed cross-peaks assigned to dipolar interactions (spatial proximity, **4a**, Figure 2) of H-2α (δ_H 2.57) with H-4α (δ_H 4.22) and H-3β (δ_H 3.86) with 3H-5 (δ_H 1.48).

Thus, the structure of the new lactone was established as *rel*-(2*S*,3*S*,4*R*)-3-hydroxy-4-methyl-2-(13"-phenyl-1'-*n*-tridecyl)-butanolide (**4**).

Conclusions

The phytochemical study of *T. pseudostipularis* led to the isolation and identification of 4 new compounds in *Trichilia* genus, 10-isopropyl-3-methyl-8,9-dihydronaphthalen-7(2*H*)ona (**1**), 2-hydroxy-10-isopropyl-3-methyl-8, 9-dihydronaphthalen-7(2*H*)ona (**2**), pseudostipulariol (**3**) and *rel*-(2*S*,3*S*,4*R*)-3-Hydroxy-4-methyl-2-(13"-phenyl-1'-n-tridecyl)-butanolide (**4**). Compounds **3** and **4** are described for the first time in this paper. Only one compound with a similar structure to **4** has been identified in a *Trichilia* species,[14] and the skeleton of sesquiterpene **3** is also new to the genus. Acetylation of pseudostipulariol (**3**) led to diacetylpseudostipulariol (**3a**), a compound also described for the first time in the present paper.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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