



γ -LACTONES FROM *TRICHILIA CLAUSSENII*

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Abstract—The methanol extract of fruits of *Trichilia clausenii* yielded the new γ -lactones, (2*R*,3*S*, 4*S*)-3-hydroxy-4-methyl-2-(13'-phenyl-1'-*n*-tridecyl)-butanolide, (2*R*,3*S*, 4*S*)-3-hydroxy-4-methyl-2-(11'-phenyl-1'-*n*-undecyl)-butanolide, (2*R*,3*S*, 4*S*)-3-hydroxy-4-methyl-2-(1'-*n*-hexadec-7'(Z)-enyl)-butanolide and (2*R*,3*S*, 4*S*)-3-hydroxy-4-methyl-2-(1'-*n*-tetradecyl)-butanolide. The structures of the isolated compounds were determined on the basis of spectral analysis. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The Meliaceae are known to be a rich source of limonoids, which possess interesting biological activities against insects, such as antifeedants, deterrents and inhibitors of ecdysis [1]. As part of our interest in the chemistry of this family, we have recently reported on the isolation of androstane and pregnane steroids from the stems [2] and cycloartane triterpenoids and a mixture of ω -phenyl alkanolic and alkenoic acids from the leaves [3] of *Trichilia clausenii* collected in Brazil. We have also proposed that ω -phenyl alkanolic and alkenoic acids might be precursors of γ -lactones [3] isolated from Myristicaceae species [4]. In continuation of our study of this species, we now report the isolation and identification of γ -lactones from the fruit.

RESULTS AND DISCUSSION

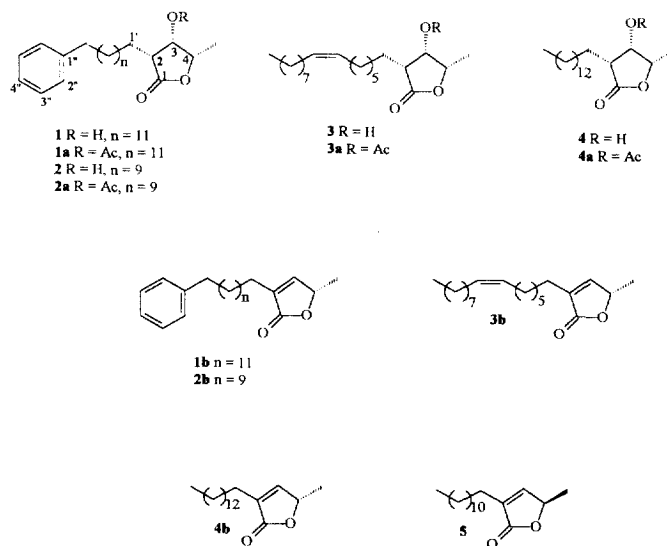
The methanol extract of the fruits of *T. clausenii* was submitted to solvent partition followed by multiple column chromatography. This procedure led to the isolation of four new γ -lactones which were identified as follows.

Compound **1** showed typical IR absorptions of C=O (1738 cm⁻¹) for lactone and hydroxyl to (3440 cm⁻¹) groups. The ¹H NMR spectrum displayed signals for protons of oxygenated carbons (δ 4.45, *dq*, *J* = 3.5, 6.5 Hz; 1H and δ 4.32, *br dd*, *J* = 4.8, 7.8 Hz, 1H) and a methyl signal (δ 1.44, *d*, *J* = 6.5 Hz, 3H) resembling the γ -lactone systems of acetogenins of Annonaceae [5] and grandinolides and juruenolides of

Myristicaceae [4]. In addition, signals (δ 7.17–7.19, *m*, 5H) characterizing a monosubstituted aromatic ring were observed. Three protons, two of them benzylic and one α -carbonyl were represented by the multiplet at δ 2.56–2.62 (3H). Also, the presence of a broad singlet δ 1.25–1.30 was attributed to protons in a long methylene chain in **1**. From the data above, it was possible to assign a structure containing a γ -lactone moiety linked to an aromatic ring through a methylene chain, as observed for grandinolides of Myristicaceae [4]. The signals observed in the ¹³C NMR of **1** and correlation in the ¹H-¹H COSY confirmed the structure of a 2-alkyl-3-hydroxy-4-methyl γ -lactone for this compound. The ¹H-¹H COSY showed couplings between both carbinolic protons. The proton at δ 4.45 showed coupling with the methyl hydrogens δ 1.44. Also, the carbinolic proton at δ 4.32 is coupled to the proton at δ 2.57, attributed to H-2, which is coupled with the protons at δ 1.83, attributed to both H-1'. Two protons showing a very close chemical shift to H-2 at δ 2.60 were assigned to the benzylic protons. GC-mass spectrometry of **1** revealed the presence a minor compound **2** in admixture with **1**. The MS spectra for both compound showed typical fragments of the γ -lactone moiety (*m/z* 129, 116, 111 and 99) and for the aromatic ring (*m/z* 91, base peak). The [*M*]⁺ for **1** (*m/z* 374) and for **2** (*m/z* 346) allowed us to determine the length of the methylene and, thus, establish the structures for both compounds.

Compound **3**, also isolated from the methanol extract, did not exhibit signals for an aromatic ring in the ¹H NMR. However, the presence of a signal (δ 5.35) for olefinic hydrogens and the presence of a triplet (δ 0.88, *J* = 7.2 Hz) of a terminal methyl group, allowed us to propose the structure of a lactone lacking the aromatic ring. The lactone ring signals for **3**

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were the same as those described for **1**. Therefore, the difference between compounds **1** and **3** is the absence of the aromatic ring in the latter and also the presence of unsaturation in its methylene chain. As observed for compound **1**, analysis of **3** revealed the presence of a minor compound **4** in admixture. The presence of a *cis*-double bond in **3** was deduced from ^{13}C NMR spectra, which showed only one signal at δ 31.9. The presence of a *trans*-double bond would require one more signal at ca δ 30.0 for the allylic carbons [6]. The position of the double bond in the side-chain was deduced after preparation of the dimethyl disulfide (DMS) derivative of **3** and further GC-mass spectrometric analysis [7]. The presence of fragments (m/z 414, 241, 193 and 173) allowed us to establish the correct position of the double bond. The difference between **3** and **4** is the absence of the double bond in the side-chain, as well as its length in the latter.

The isolated compounds show three contiguous stereogenic centres, so four diastereomers and their corresponding enantiomers are possible. The relative configuration of the lactones was determined by comparison of their ^{13}C NMR with those described for models in the literature [5]. Therefore, a *cis* configuration for the substituents in the lactone ring is proposed (Table 1).

To determine absolute configuration, compounds **1** and **3** were transformed into their corresponding acetates, which after elimination of acetic acid led to compounds **1b** and **3b** containing only one chiral carbon. Comparison of the optical rotations obtained for these compounds, which were $[\alpha]_{\text{D}}^{26} = +4.2^\circ$ and $[\alpha]_{\text{D}}^{20} = +12.9^\circ$ in dioxane, respectively, with a similar lactone (**5**) from the literature $[\alpha]_{\text{D}}^{22} = -29.8^\circ$ [8], which was assigned the absolute configuration **4R**, indicated that C-4 for both compounds **1** and **3** has

the *S*-configuration and consequently C-3 and C-2 are (*S*) and (*R*), respectively.

A compound having the same structure of **1** is described in the literature from the bark of *Trichilia schomburgkii* [9]. However, the data described for that compound are not in agreement with those we have found for **1** isolated from *T. clausenii*. We believe that a *trans*-stereochemistry for the substituents in the lactone moiety should be assigned to the compound from the literature. Therefore, compound **1** isolated from *T. clausenii* is different from that of *T. schomburgkii*.

A biogenetic proposal for gradinolides and juruenolides involves the condensation of cinnamoyl-CoA (or benzoyl CoA), pyruvyl-CoA and fatty acids, respectively [4]. For γ -lactones without the aromatic ring, the biogenetic proposal also involves the condensation of fatty acids with pyruvyl-CoA. [10]. We have proposed that ω -phenyl alkanolic and alkenolic acids isolated from *T. clausenii* should be the intermediates in the biogenesis of γ -lactones [3], which may occur through the coupling of one $\text{C}_6\text{--C}_3$ unit with polyketide chains of different sizes. In a different approach, the $\text{C}_6\text{--C}_3$ unit could be incorporated in the biosynthesis of fatty acids, leading to the ω -phenyl alkanolic and alkenolic acids, which after condensation with pyruvic acid would afford the γ -lactones. Evidences for another starting material in the biosynthesis of fatty acids were found in the Flacourtiaceae, which produces ω -cyclopentenyl fatty acids from 2-cyclopentenyl carboxylic acid [11]. Therefore, the isolation of these compounds from *T. clausenii* is evidence that they have similar biogenetic route.

Up to the isolation of these γ -lactones from *T. schomburgkii* [9] and *T. clausenii*, this class of compounds was known to occur only in the Myristicaceae [4], Lauraceae [8] and Annonaceae [5].

EXPERIMENTAL

General

IR: KBr. ^1H and ^{13}C NMR: 400 and 100 MHz, respectively, with TMS as int. standard. GC-MS: low resolution.

Plant material

Fruits of *T. clausenii* DC. were collected in Rio Claro, SP, Brazil, and a voucher is deposited in the Herbarium of Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP, Brazil. Fruits were extracted with MeOH.

Isolation of constituents

The MeOH extract (52 g) was suspended in MeOH– H_2O (1:3) and partitioned with CH_2Cl_2 –EtOAc–*n*-BuOH. The CH_2Cl_2 fr. was concd and then partitioned with hexane–MeOH. The MeOH fr. afforded 9 frs after silica gel CC using gradient elution with CH_2Cl_2 – Me_2CO . Fr. 3 (1.78 g) after Florisil cc using gradient elution with CH_2Cl_2 – Me_2CO afforded 14 frs. Fr. 3–6 (110 mg) after silica gel CC (hexane– Me_2CO , 9:1) afforded 12 frs. Fr. 3–6–10 afforded a mixt. of **3** and **4** (2.2 mg) after CC on silica gel (hexane– Me_2CO , 9:1). Fr. 3–6–11 afforded a mixt. of **1** and **2** (4.8 mg) after CC on silica gel (hexane– Me_2CO , 9:1).

Acetylation of **1** and **3**

Compounds **1** and **3** were acetylated (Ac_2O –pyridine) to yield 3 mg of **1a** and 2 mg of **3a**.

Elimination of HOAc from **1a** and **3a**

Compounds **1a** and **3a** were placed on the top of Al_2O_3 (Merck activity I, with 3% of H_2O) column. Elution with pentane and pentane– Me_2CO (19:1) yielded 1.1 mg of **1b** and 1.2 mg of **3b**.

(2R,3S,4S)-3-Hydroxy-4-methyl-2-(13'-phenyl-1'-n-tridecyl)-butanolide (**1**) and (2R,3S,4S)-3-Hydroxy-4-methyl-2-(11'-phenyl-1'-n-undecyl)-butanolide (**2**)

Amorphous solid. $[\alpha]_{\text{D}}^{20}$ –20.9° (CHCl_3 , *c* 0.003, 26°C). GC-MS: (**1**) *R*_t 31.58 min., EIMS *m/z* (rel. int.): 374 [M]⁺ (11), 148 (5), 134 (6), 129 (40), 116 (50), 111 (13), 104 (42), 99 (19), 91 (100), 57 (76). (**2**) *R*_t 28.60 min., EIMS *m/z* (rel. int.): 346 [M]⁺ (12), 134 (6), 129 (41), 116 (50), 111 (14), 104 (38), 99 (19), 91 (100), 57 (70). ^1H and ^{13}C NMR: Table 1.

(4S)-4-Methyl-2-(13'-phenyl-1'-n-tridecyl)-but-2-enolide (**1b**)

$[\alpha]_{\text{D}}^{26} +4.2^\circ$ (dioxane, *c* 0.001). ^1H NMR (CDCl_3): δ 7.17–7.29 (*m*, H-Ar), 6.77 (*m*, H-3), 4.98 (*dq*, H-4), 2.60 (*t*, H-13'), 2.26 (*m*, H-1'), 1.41 (*d*, Me-4), 1.26–1.30 (*m*, CH_2).

(2R,3S,4S)-3-Hydroxy-4-methyl-2-(1'-n-hexadec-7'(Z)-enyl)-butanolide (**3**) and (2R, 3S, 4S)-3-Hydroxy-4-methyl-2-(1'-n-tetradecyl)-butanolide (**4**)

Amorphous solid. $[\alpha]_{\text{D}}^{20}$ –8.30° (CHCl_3 , *c* 0.0037, 26°C). GC-MS: (**3**) *R*_t 24.95 min., EIMS *m/z* (rel. int.): 338 [M]⁺ (1), 129 (58), 116 (51), 111 (25), 99 (23), 57 (100). (**4**): *R*_t 22.19 min., EIMS *m/z* (int. rel.): 312

Table 1. NMR data of γ -lactones **1** and **3** (CDCl_3 , δ)

1			3		
	H	C		H	C
1	—	177.3	1	—	177.0
2	2.57 <i>m</i>	47.6	2	2.57 <i>dt</i> (4.8, 10.0)	47.6
3	4.32 <i>br dd</i> (4.8, 7.8)	71.3	3	4.31 <i>dd</i> (4.8, 8.0)	71.3
4	4.45 <i>dq</i> (3.5, 6.5)	78.6	4	4.45 <i>dq</i> (3.2, 6.4)	78.6
Me-4	1.44 <i>d</i> (6.5)	13.7	Me-4	1.43 <i>d</i> (6.4)	13.7
1'	1.83	23.4	1'	1.80	23.3
2'	1.26–1.30 <i>m</i>	27.6	2'–5'	1.26–1.30 <i>m</i>	26.7–31.9
3'–11'	1.26–1.30 <i>m</i>	29.7–29.4	6'	2.03	26.7–31.9
12'	1.65	31.5	7'	5.35 <i>m</i>	129.7*
13'	2.60	36.0	8'	5.35 <i>m</i>	130.1*
1''	—	143.0	9'	2.03	26.7–31.9
2''	7.17–7.29 <i>m</i>	128.2*	10'–14'	1.26–1.30 <i>m</i>	26.7–31.9
3''	7.17–7.29 <i>m</i>	128.5*	15'	1.26–1.30 <i>m</i>	22.7
4''	7.17–7.29 <i>m</i>	125.9	16'	0.88 <i>t</i> (7.2)	13.7

Coupling constants (Hz, in parentheses).

* Signals may be interchanged.

[M]⁺ (2), 129 (58), 116 (100), 111 (19), 99 (32), 57 (18).
¹H and ¹³C NMR: Table I.

(4S)-4-Methyl-2-(1'-n-hexadec-7' (Z)-enyl)-but-2-enolide (**3b**)

[α]_D²⁰ + 12.9° (dioxane, c 0.0009). ¹H NMR (CDCl₃):
 δ 6.97 (*m*, H-3), 5.35 (*m*, H-7' and H-8'), 2.02 (*m*, H-6' and H-9'), 4.99 (*dq*, H-4), 2.26 (*m*, H-1'), 1.40 (*d*, Me-4), 1.26–1.30 (*m*, CH₂).

REFERENCES

1. Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Towers, G. H. N., *Phytochemistry*, 1992, **31**, 377.
2. Pupo, M. T., Vieira, P. C., Fernandes, J. B., Silva, M. F. G. F. and Rodrigues Fo., E., *Phytochemistry*, 1997, **45**, 1495.
3. Pupo, M. T., Vieira, P. C., Fernandes, J. B. and Silva, M. F. G. F., *Phytochemistry*, 1996, **42**, 795.
4. Vieira, P. C., Yoshida, M., Gottlieb, O. R., Paulino Fo., H. F., Nagem, T. J. and Braz Fo., R., *Phytochemistry*, 1983, **22**, 711.
5. Chaves, M. H. and Roque, N. F., *Phytochemistry*, 1997, **44**, 523.
6. Breitmaier, E., Haas, G. and Voelter, W., *Atlas of Carbon-13 NMR Data*. Heyden, London, 1979.
7. Vincent, M., Guglielmetti, G., Cassani, G. and Tonini, C., *Analytical Chemistry*, 1987, **59**, 694.
8. Takeda, K., Sakurawi, K. and Ishii, H., *Tetrahedron*, 1972, **28**, 3757.
9. Tinto, W. F., Jagessar, P. K., Ketwaru, P., Reynolds, W. F. and McLean, S., *Journal of Natural Products*, 1991, **54**, 972.
10. Etse, J. T. and Waterman, P. G., *Phytochemistry*, 1986, **25**, 1903.
11. Cramer, U. and Spener, F., *Biochimica et Biophysica Acta*, 1976, **450**, 261.