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Antifungal diastereomeric furanones from *Mutisia friesiana*: structural determination and conformational analysis

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Abstract—Two diastereomeric furanones, (4*S*,5*S*)-5-(4′-methyl-3′-pentenyl)-4-hydroxy-5-methyldihydrofuran-2-one **1** and (4*S*,5*R*)-5-(4′-methyl-3′-pentenyl)-4-hydroxy-5-methyldihydrofuran-2-one **2** were isolated for the first time from the shrub *Mutisia friesiana*. The relative stereochemistries of **1** and **2** were ascertained from NOESY NMR data and confirmed by a combination of molecular modeling (molecular mechanics and ab initio molecular orbital calculations) and NMR data. Comparison between experimental and calculated ^1H–^1H vicinal coupling constants revealed that both furanones exist in an equilibrium of two stable conformers of the five-membered ring. Application of Mosher's method suggests that both diastereomeric furanones have the same (*S*)-configuration at C-(4) and are epimers at C-(5). Furanones **1** and **2** showed antifungal activity against the pathogenic fungus *Cladosporium cucumerinum*.

1. Introduction

The South American genus *Mutisia* (Asteraceae, tribe Mutisieae, subtribe Mutisiinae) comprises about 60 species distributed in the Andes from Colombia to S. Argentina and Chile, but it is also present in S.E. Brazil, Paraguay, Uruguay and N.E. Argentina.[1,2] Several species have been investigated chemically. Coumarins,[3–5] related coumaranes,[3] chromones,[3,6] chromenes,[5] mono- and sesquiterpenes,[3,7] flavonoids,[6,9] aromatic glucosides,[8,9] triterpenoids and sterols[4,5,8] have been isolated from aerial parts of the plants, while extracts of roots afforded acetylenic compounds.[3,10]

Mutisia friesiana is a perennial shrub native of S. Bolivia and N.W. Argentina that grows at 3500–4000 m above sea level. The infusion of this species is used in folk medicine as a remedy against chronic coughs, respiratory diseases and stomach pains.[11] Its pleasing and persistent scent prompted us to study its essential oil and we identified more than 100 compounds.[12] Bioassay-guided fractionation of the aqueous extract afforded caffeic acid derivatives and flavonoids as the main bioactive compounds, showing radical scavenging activity.[13] Continuing our search for bioactive compounds from *M. friesiana* we report herein the first isolation of diastereomeric furanones **1** and **2** from a natural source and the structural elucidation of these compounds.

2. Results and discussion

2.1. Isolation, structural determination and antifungal activity

From the chloroform-soluble portion of the residue from the MeOH extract of *M. friesiana*, the diastereomeric furanones **1** and **2** were isolated (Fig. 1). Furanone **1** is a new compound, while **2** has been previously prepared as an intermediate in the synthesis of (–)-vertinolide,[14] a mycotoxin isolated from *Verticillium intertextum*.[15]

Examination of the ^1H and ^{13}C NMR spectra of furanone **1** (Table 1) suggested the presence of an olefinic bond, a γ-lactone carbonyl group and two oxygenated carbons. The IR spectrum of **1** confirmed the presence of the γ-lactone carbonyl group (1759 cm^{-1}) and a hydroxyl function (3432 cm^{-1}). The EIMS spectrum of **1** contained a molecular ion peak at m/z 198, compatible with the molecular composition $C_{11}H_{18}O_3$. The ^{13}C NMR spectrum contained 11 signals, and DEPT analy-

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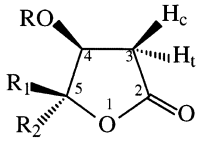

1 R = H, $R_1 = (CH_3)_2C{=}C(CH_2)_2$, $R_2 = CH_3$

1a R = (*R*)-MTPA, $R_1 = (CH_3)_2C{=}C(CH_2)_2$, $R_2 = CH_3$

1b R = (*S*)-MTPA, $R_1 = (CH_3)_2C{=}C(CH_2)_2$, $R_2 = CH_3$

1c R = H, $R_1 = CH_3CH_2$, $R_2 = CH_3$

2 R = H, $R_1 = CH_3$, $R_2 = (CH_3)_2C{=}C(CH_2)_2$

2a R = (*R*)-MTPA, $R_1 = CH_3$, $R_2 = (CH_3)_2C{=}C(CH_2)_2$

2b R = (*S*)-MTPA, $R_1 = CH_3$, $R_2 = (CH_3)_2C{=}C(CH_2)_2$

2c R = H, $R_1 = CH_3$, $R_2 = CH_3CH_2$

Figure 1. Structures of diastereomeric furanones **1** and **2** isolated from *M. friesiana*, MTPA esters **1a**, **1b**, **2a** and **2b** and model compounds **1c** and **2c** for conformational analysis.

Table 1. ^1H and ^{13}C NMR data for furanones **1** and **2** (data were recorded in $CDCl_3$ at 500 and 125 MHz, respectively)

Carbon	Furanone **1**		Furanone **2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	174.49	–	174.16	–
3	38.37	H_c 2.51 (dd, J=17.9, 2.3) H_t 2.95 (dd, J=17.9, 6.1)	38.00	H_c 2.55 (dd, J=17.9, 4.4) H_t 2.91 (dd, J=17.9, 6.9)
4	73.67	4.19 (dd, J=6.1, 2.3)	72.71	4.27 (dd, J=6.9, 4.4)
5	89.26	–	89.32	–
1′	34.05	1.82 m	39.35	1.65 m
2′	22.43	2.13 (dd, J=15.7, 7.3)	22.42	2.10 (dd, J=15.7, 7.3)
3′	123.30	5.16 (tq, J=7.3, 1.4)	123.02	5.08 (tq, J=7.3, 1.4)
4′	133.18	–	132.87	–
5′	17.69	1.64 (d, J=1.4)	17.68	1.61 (d, J=1.4)
6′	25.64	1.71 (d, J=1.4)	25.61	1.69 (d, J=1.4)
1″	23.12	1.35 s	18.48	1.41 s

sis revealed the presence of three methyl groups, three methylenes, two methines (one olefinic and one bearing oxygen) and three quaternary carbons at δ 89.26, 133.18 and 174.49, assigned to C-(5), a vinylic carbon and a lactonic carbonyl carbon, respectively.

The ^1H NMR spectrum of **1** showed signals at δ 2.51 (1H, dd, J=17.9, 2.3, H-(3)), 2.95 (1H, dd, J=17.9, 6.1, H-(3)) and 4.19 (1H, dd, J=6.1, 2.3, H-(4)), which are typical for a γ-butyrolactone with a hydroxyl group at C-(4) and two alkyl substituents at C-(5).[14] The ^1H NMR spectrum also contained two allylic methyl signals at δ 1.64 and 1.71 and one olefinic proton signal at δ 5.16, assigned to a terminal *iso*-butenyl group. The ^{13}C NMR spectrum confirmed the presence of this group, with two methyl group signals at 17.69 and 25.64 (for C-(5′) and C-(6′), respectively) attached to olefinic carbons whose signals are seen at δ 133.18 and 123.30 (C-(4′) and C-(3′), respectively). Analysis of ^1H–^1H COSY and HETCOR data allowed us to assign the methylene groups of the 4′-methyl-3′-pentenyl chain attached to C-(5) (Table 1). From the ^1H–^1H COSY spectrum it was determined that the olefinic proton H-(3′) at δ 5.16 was coupled to the multiplet at δ 2.13, assigned to H_2-(2′), which in turn was coupled to the C-(1′) methylene protons which resonate at δ 1.82.

The IR, EIMS and ^{13}C NMR data of **1** were similar to those published by Schwab et al.[16] for the isomeric 5-(4′-methyl-3′-pentenyl)-3-hydroxy-5-methyldihydrofuran-2-one, obtained as a pair of diastereomers by controlled conversion of 2-methyl-2-hepten-6-one with *Botrytis cinerea*, but their ^1H NMR spectra showed different chemical shifts for H-(3) and H-(4). These differences could arise as a result of the hydroxyl group being attached at C-(4) instead of C-(3). Finally, the position of the hydroxyl group at C-(4) in furanone **1** was deduced from the COLOC crosspeaks at δ 1.35/73.67 for H_3-(1″)/C-(4).

The relative configurations at the stereogenic centers in furanone **1** were assigned on the basis of the 2D NOESY spectrum, which exhibited NOEs, indicating that Me-(1″), H-(4) and the H-(3) appearing at δ 2.95 ppm (H_t) are on the same side of the molecule, while the hydroxyl group has the same orientation as the 4′-methyl-3′-pentenyl chain (Fig. 2). Thus, the furanone **1** was established as (4*S*,5*S*)-5-(4′-methyl-3′-pentenyl)-4-hydroxy-5-methyldihydrofuran-2-one or its enantiomer.

The structure of the known furanone **2** was determined by a combination of one- and two-dimensional NMR techniques, IR, EIMS and comparison with the data published for the intermediate synthesized in the preparation of (−)-vertinolide.[14] Analysis of ^{1}H–^{1}H COSY and HETCOR data allowed assignment of all proton and carbon signals of the molecule for the first time (Table 1). 2D NOESY data allowed us to determine the relative stereochemistry, indicating that Me-(1″), the hydroxyl group at C-(4) and the H-(3) at δ 2.55 ppm (H_c) are on the same side of the molecule, while H-(4) and the H-(3) appearing at δ 2.91 (H_t) have the same orientation as the 4′-methyl-3′-pentenyl chain (Fig. 3), suggesting that **2** is either the (4*S*,5*R*)-5-(4′-methyl-3′-pentenyl)-4-hydroxy-5-methyldihydrofuran-2-one or its enantiomer. On comparison of the specific rotation of **2**, with the $[\alpha]_D$ of −10.0 to the literature value of −4.4 for the known synthetic product with (4*S*,5*R*) absolute configuration,[14] we concluded that furanone **2** has the same absolute configuration. For the purpose of confirming the absolute stereochemistry in compound **2**, we determined the absolute configuration at C-(4) using Mosher's method.[17,18] Furanone **2** was converted into the diastereomeric (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetate (MTPA) **2a** and (*S*)-MTPA **2b** esters (Fig. 1). Comparison of the ^{1}H NMR data of **2a** with those of **2b** (see Section 3) showed a downfield shift ($\Delta\delta$ 0.01) of both methylene C-(3) protons and an upfield shift ($\Delta\delta$ 0.01) of the C-(5) methyl group. These displacements are consistent with (*S*)-configuration at C-(4). However, the small $\Delta\delta$ values may reflect a deviation of the ideal conformation[19] proposed by Mosher for both MTPA esters, thus rendering the $\Delta\delta$ values smaller than those expected for **2a** and **2b**.

The diastereomeric (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetate (MTPA) **1a** and the (*S*)-MTPA **1b** esters (Fig. 1) of diastereomeric furanone **1** showed the same deviations in their ^{1}H NMR spectra (see Section 3) as both MTPA esters of furanone **2**. This fact suggests that both diastereomeric furanones have the

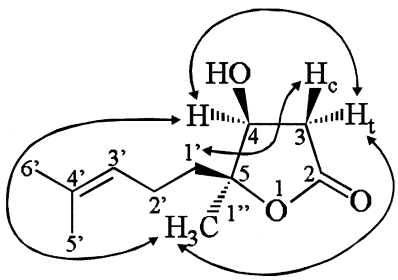

Figure 2. Key NOE interactions for furanone **1**.

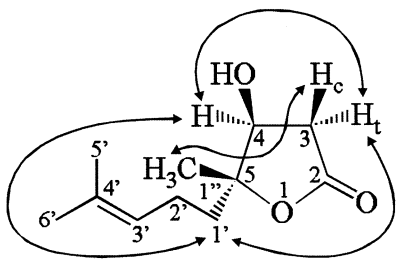

Figure 3. Key NOE interactions for furanone **2**.

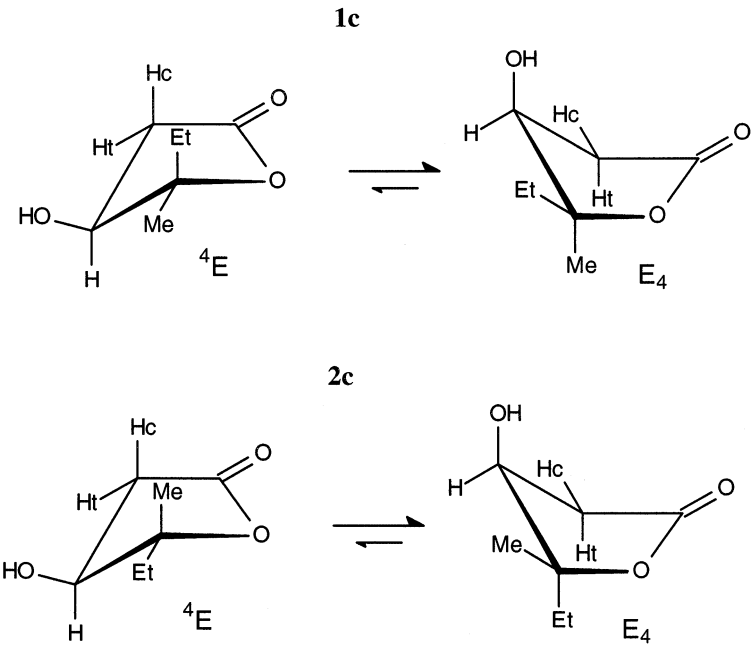

Figure 4. Equilibria in compounds **1c** and **2c**.

same (*S*)-configuration at C-(4) and are epimers at C-(5). Therefore, we propose the (4*S*,5*S*) absolute stereochemistry for furanone **1** as shown in Fig. 2.

Furanones **1** and **2** showed antifungal activity against the pathogenic fungus *Cladosporium cucumerinum*, as evaluated by direct bioautographic assay.[20]

2.2. Conformational and NMR analysis

In previous papers,[21,22] we have shown the value of an accurate conformational assessment of γ-lactones to their configurational assignment. The relative configurations of furanones **1** and **2** were confirmed by molecular modeling of the two analogues, **1c** and **2c**, respectively, in which, for the sake of simplicity, the 4′-methyl-3′-pentenyl chain was replaced by an ethyl group (Fig. 1). Model compounds **1c** and **2c** were analyzed by molecular mechanics (MM3[23]) and ab initio molecular orbital calculations (HF/6-31G), the latter with inclusion of solvent (chloroform) by the polarizable continuum method of Tomasi et al.[24] In either case, two stable conformations of the five-membered ring were found: the envelopes E_4 and ^4E (Fig. 4), sometimes partly distorted. Tables 2 and 3 show the results obtained for analogues **1c** and **2c**, respectively, taking into account the rotation of the side chains, whose consideration was shown not to be trivial.[21] In both cases, the envelope E_4 was found to be more stable. This fact has already been predicted on the grounds of the tendency for the 4-hydroxy group of lactones to adopt an axial orientation.[27–30] However, this effect is not equal for both compounds (Table 4). For compound **1c**, the E_4 envelopes prevail over the ^4E more markedly than for **2c**. In the latter case, the ethyl group has a stronger tendency to stay quasi-equatorial, and thus act as a balance against the drive of the 4-hydroxy axial group (Fig. 4). Table 4 shows the Boltzmann-averaged coupling constants calculated for both compounds. The experimental coupling constants for **1** and **2** show a very good concordance with those calculated, as has been found previously with other

Table 2. Calculated geometries and energies for compound **1c**

Method/conformer	Puckering parameters[a]		Torsion angles of side chains[b] (°)		Relative energies (kcal/mol)	Calculated $^3J_{H,H}$[c] (Hz, °)	
	ϕ (°)	q (Å)	$\theta_{C(5)-C(1')}$	$\theta_{C-(4)O}$		$J_{H-(4),H_c}$	$J_{H-(4),H_t}$
MM3							
E_4	113	0.30	−65	−41	0.00	1.19 (90)	6.10 (−31)
E_4	113	0.30	56	−41	0.13	1.17 (90)	6.24 (−30)
E_4	113	0.29	56	22	0.35	1.17 (90)	6.22 (−30)
E_4	113	0.31	−66	−172	0.99	1.18 (90)	6.12 (−31)
E_4	113	0.30	56	−171	1.15	1.17 (90)	6.23 (−30)
E_4	112	0.28	158	−41	1.61	1.16 (92)	6.46 (−29)
E_4	113	0.30	161	−162	3.02	1.17 (91)	6.28 (−30)
^4E	293	0.31	62	−45	0.92	8.60 (153)	8.28 (31)
^4E	293	0.31	62	41	0.97	8.54 (152)	8.31 (31)
^4E	289	0.25	174	−45	1.88	7.71 (147)	8.79 (26)
^4E	289	0.25	175	41	1.95	7.66 (147)	8.81 (26)
^4E	292	0.31	−49	−44	2.32	8.66 (153)	8.24 (32)
^4E	293	0.31	−49	30	2.50	8.69 (153)	8.22 (32)
^4E	293	0.31	62	−153	3.02	8.88 (154)	8.12 (33)
HF/6-31G + PCM[d]							
E_4	108	0.28	54	42	0.00	1.16 (93)	6.32 (−30)
E_4	107	0.30	−65	−48	0.12	1.19 (90)	5.83 (−33)
E_4	107	0.29	54	−49	0.43	1.16 (91)	6.11 (−31)
E_4	109	0.30	−62	39	0.61	1.17 (91)	6.00 (−32)
$E_4 \rightarrow {}^5T_4$	115	0.29	−66	−173	1.46	1.16 (92)	6.35 (−29)
E_4	108	0.25	154	42	1.62	1.18 (96)	6.91 (−26)
$E_4 \rightarrow {}^5T_4$	114	0.28	53	−172	1.74	1.16 (93)	6.58 (−28)
E_4	106	0.27	154	−48	2.03	1.16 (94)	6.61 (−28)
$^4E \rightarrow {}^4T_5$	294	0.30	60	−45	0.71	8.80 (154)	8.26 (31)
$^4E \rightarrow {}^4T_5$	295	0.29	59	37	0.76	8.65 (153)	8.27 (31)
^4E	292	0.21	172	35	1.36	7.19 (144)	9.08 (23)
^4E	292	0.24	173	−43	1.37	7.85 (148)	8.85 (26)
$^4E \rightarrow {}^4T_5$	294	0.30	−48	−42	1.69	8.82 (154)	8.23 (32)
$^4E \rightarrow {}^4T_5$	295	0.30	−50	34	1.82	8.67 (153)	8.25 (32)
^4E	293	0.28	59	−166	1.88	8.59 (152)	8.40 (30)

[a] According to Cremer and Pople.[25]

[b] $\theta_{C(5)-C(1')} = \theta_{C(1'')-C(5)-C(1')-C(2')}$; $\theta_{C(4)O} = \theta_{H(4)-C(4)-O-H}$.

[c] Calculated using the parameterization of Haasnoot et al.[26] The corresponding torsional angles are in parentheses.

[d] Polarizable continuum solvent model.[24]

Table 3. Calculated geometries and energies for compound **2c**

Method/conformer	Puckering parameters[a]		Torsion angles of side chains[b] (°)		Relative energies (kcal/mol)	Calculated $^3J_{H,H}$[c] (Hz, °)	
	ϕ (°)	q (Å)	$\theta_{C(5)-C(1')}$	$\theta_{C(4)O}$		$J_{H-(4),H_c}$	$J_{H-(4),H_t}$
MM3							
E_4	113	0.29	−61	−41	0.00	1.17 (90)	6.23 (−30)
E_4	113	0.29	−61	23	0.22	1.18 (90)	6.22 (−30)
E_4	113	0.30	59	−41	0.33	1.19 (89)	6.06 (−31)
E_4	113	0.30	59	26	0.52	1.20 (89)	6.03 (−31)
E_4	109	0.23	−177	−40	0.96	1.18 (96)	7.12 (−24)
E_4	114	0.30	−61	−171	1.01	1.17 (90)	6.23 (−30)
E_4	109	0.23	−177	23	1.16	1.18 (96)	7.10 (−24)
E_4	113	0.31	59	−171	1.32	1.19 (89)	6.07 (−31)
E_4	110	0.24	−177	−169	2.14	1.17 (95)	7.01 (−25)
^4E	293	0.31	−57	−45	0.63	8.57 (152)	8.30 (31)
^4E	292	0.30	66	−44	0.66	8.55 (152)	8.31 (31)
^4E	292	0.31	−57	41	0.68	8.50 (152)	8.34 (31)
^4E	292	0.30	−170	−44	0.95	8.44 (152)	8.39 (30)
^4E	292	0.30	−170	42	0.98	8.38 (151)	8.42 (30)
^4E	293	0.31	−57	−155	2.72	8.85 (154)	8.14 (32)
^4E	292	0.30	66	−160	2.74	8.78 (154)	8.19 (32)
^4E	292	0.31	−170	−154	3.05	8.73 (153)	8.23 (32)
HF/6-31G + PCM[d]							
E_4	109	0.27	−60	44	0.00	1.16 (93)	6.48 (−28)
E_4	107	0.29	−60	−50	0.38	1.16 (92)	6.16 (−31)
E_4	108	0.28	56	45	0.61	1.16 (91)	6.15 (−31)
E_4	107	0.29	56	−49	0.90	1.16 (91)	6.09 (−31)
E_4	106	0.20	−176	43	1.11	1.30 (100)	7.49 (−22)
$E_4 \rightarrow {}^3T_4$	101	0.22	−175	−49	1.49	1.21 (97)	7.05 (−25)
$E_4 \rightarrow {}^5T_4$	114	0.28	−60	−172	1.83	1.16 (94)	6.66 (−27)
E_4	113	0.28	56	−173	2.27	1.15 (93)	6.51 (−28)
$^4E \rightarrow {}^4T_5$	294	0.30	−54	−41	0.52	8.75 (153)	8.29 (31)
^4E	293	0.29	66	−42	0.53	8.63 (153)	8.36 (31)
$^4E \rightarrow {}^4T_5$	294	0.28	−54	35	0.70	8.50 (152)	8.37 (30)
$^4E \rightarrow {}^4T_5$	294	0.28	65	27	1.05	8.47 (152)	8.39 (30)
$^4E \rightarrow {}^4T_5$	294	0.29	−168	−40	1.33	8.54 (152)	8.43 (30)
$^4E \rightarrow {}^4T_5$	294	0.28	−168	35	1.46	8.23 (150)	8.55 (29)
^4E	292	0.27	65	−167	1.82	8.41 (151)	8.50 (29)
^4E	292	0.28	−54	−166	1.84	8.54 (152)	8.43 (30)

[a] According to Cremer and Pople.[25]
[b] $\theta_{C(5)-C(1')} = \theta_{C(1'')-C(5)-C(1')-C(2')}$; $\theta_{C(4)O} = \theta_{H(4)-C(4)-O-H}$.
[c] Calculated using the parameterization of Haasnoot et al.[26] The corresponding torsional angles are in parentheses.
[d] Polarizable continuum solvent model.[24]

Table 4. Comparison of coupling constants (Hz) calculated for compounds **1c** and **2c** and experimental data for **1** and **2**

Compound/data	$J_{H-(3c),H-(4)}$	$J_{H-(3t),H-(4)}$	Ratio $E_4/^4E$
1/**1c**			
MM3	2.3	6.5	84:16
HF/6-31G	2.9	6.7	76:24
Experimental	2.3	6.1	
2/**2c**			
MM3	3.4	6.9	70:30
HF/6-31G	4.1	7.2	60:40
Experimental	4.4	6.9	

five-membered ring lactones.[21] In our previous work,[21,22] assignment of diastereomers using the combined ^1H NMR/molecular modeling technique was made mainly by taking into account the different coupling constants of the main conformer (or mixture) of each diastereomer. It is noteworthy that in the present work, the coupling constants calculated for the conformer E_4 of **1c** and **2c** are the same, as both diastereomers differ in the configuration of a distant carbon. However, the calculated Boltzmann-averaged coupling constants still match with the experimental, given the different proportions of conformers present in each diastereomer (Table 4).

It should be mentioned that there is a 4.64 ppm upfield shift for the C-(1″) signal when comparing **1** to **2**, and a corresponding downfield shift of 5.30 ppm for C-(1′) (Table 1). These shifts can be rationalized on the basis of the 1,3-diaxial H–H interactions established by Beierbeck and Saunders.[31] The main envelope conformer for **1c** (E_4) carries the methyl group in a quasi-axial orientation and the ethyl group in a quasi-equato-

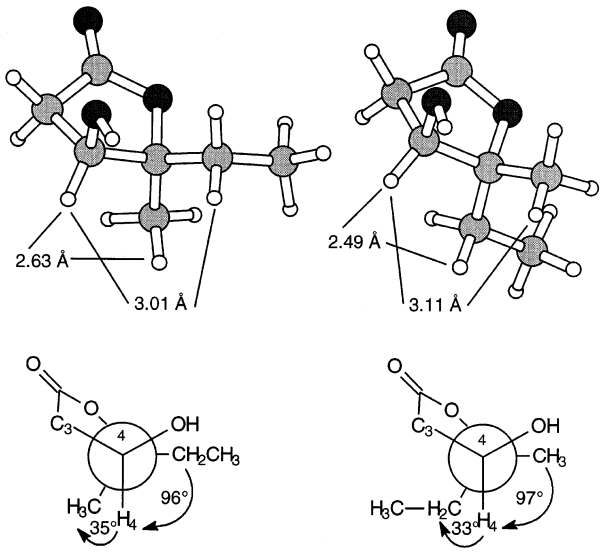

Figure 5. Minimum energy conformations (HF/6-31G level, in $CHCl_3$) of compounds **1c** and **2c**, and some of their selected geometrical features.

rial one. The reverse occurs with **2c**. Although in each carbon there is a hydrogen bearing a *gauche* (±45–70°) relationship with C-(4), the H-4 hydrogen is significantly closer to the hydrogen (Fig. 5) of the quasi-axial carbon (C-(1″) in **1c** and C-(1′) in **2c**), thus leading to clear deshielding of this carbon. This effect is even increased for the minor conformers ^{4}E. A lesser effect of reverse sign is observed for the corresponding hydrogens, which can be explained on the same basis.[31]

There is also a marked difference in chemical shift of the methylene C-(3) protons in diastereomers **1** and **2**. The hydrogen *cis* to the hydroxyl group is 0.34–0.36 ppm upfield with respect to the *trans* hydrogen. In the main conformation, the downfield proton is quasi-axial. This fact can be explained both on the basis of a polar group being *trans* to this proton,[32] or on the basis of the Beierbeck–Saunders interpretation,[31] by which, in some conformers, the quasi-equatorial proton carries a 1,3-diaxial interaction with the hydroxyl proton, and thus should appear shielded.

This work shows again that accurate geometric calculations of five-membered rings in combination with experimental NMR data are useful in the assessment of configurational features of these rings, even with different structural characteristics. Furthermore, those accurate geometries allow the interpretation of chemical shift displacements of proton and carbon atoms due to differences in the spatial disposition of substituents attached to the ring.

3. Experimental

3.1. General

General experimental procedures were as previously reported.[22,33]

3.2. Plant material

Aerial parts of *M. friesiana* (family Asteraceae, tribe Mutisieae) were collected in Jujuy, Departmento de Humahuaca, Argentina at 3500 m altitude in summer. The species was identified by Ing. Novara of the Facultad de Ciencias Naturales, Universidad de Salta. A voucher specimen is deposited at the Herbarium of the Facultad de Ciencias Naturales, Universidad de Salta under the number H.G. 1064.

3.3. Extraction and isolation

Cut, dried and powdered plant material (550 g) was extracted with MeOH (3×1.5 L). The MeOH extract was evaporated under reduced pressure to give a residue (100 g), which was partitioned with *n*-hexane–MeOH–H_2O (10:3:1), yielding a non-polar phase and an aqueous phase. The polar phase was extracted with $CHCl_3$. The extract was evaporated to dryness to yield a chloroform residue (10 g). Part of this residue (2 g) was subjected to vacuum dry-column chromatography on silica gel 60H, eluting with cyclohexane, EtOAc, acetone and MeOH. Fraction 4 (EtOAc) was fractionated by silica gel (40–63 μm) CC with $CHCl_3$–EtOAc (1:1). Fractions 8 and 9 were submitted to repeated reversed-phase HPLC (ODS, 40% CH_3CN–H_2O, flow rate 6 mL/min) to give the pure furanones **1** (3 mg) and **2** (7 mg).

3.4. (4*S*,5*S*)-5-(4′-Methyl-3′-pentenyl)-4-hydroxy-5-methyldihydrofuran-2-one 1

Colorless oil; $[\alpha]_D^{20} = -24.5$ (*c* 0.22, $CHCl_3$); IR (film) ν_{max} (cm^{-1}): 3432 (OH), 1759 (C=O, δ-lactone), 1221 (C–O–C, ester); ^{1}H and ^{13}C NMR: see Table 1; EIMS (70 eV) *m*/*z* (rel. int.%): 198 $[M]^+$ (11), 180 $[M-H_2O]$ (10), 165 [180–15] (2), 111 (12), 109 (47), 82 (23), 71 (19), 69 (62), 68 (77), 43 (100).

3.5. (4*S*,5*R*)-5-(4′-Methyl-3′-pentenyl)-4-hydroxy-5-methyldihydrofuran-2-one 2

Colorless oil; $[\alpha]_D^{20} = -10.0$ (*c* 0.24, $CHCl_3$); IR (film) ν_{max} (cm^{-1}): 3432 (OH), 1759 (C=O, δ-lactone), 1221 (C–O–C, ester); ^{1}H and ^{13}C NMR: see Table 1; EIMS (70 eV) *m*/*z* (rel. int.%): 198 $[M]^+$ (3), 180 $[M-H_2O]$ (6), 165 [180–15] (2), 111 (11), 109 (45), 82 (20), 71 (16), 69 (57), 68 (64), 43 (100).

3.6. Preparation of MTPA esters

(*R*)- or (*S*)-MTPA chloride (5 μL) was added to a solution of furanones **1** and **2** (1.0–1.2 mg) dissolved in dry pyridine (0.1 mL) and the resulting mixture was kept at room temperature. After 2 h the reaction was complete and MeOH was added. The solvent was removed under vacuum and the residue was purified by preparative TLC on silica gel (cyclohexane–EtOAc (7:3)) to give the two C-(4) diastereomeric esters.

3.6.1. (*R*)-MTPA ester 1a. δ_H ($CDCl_3$): 1.40 (3H, s, CH_3-1″), 1.52 (3H, bs, CH_3-5′), 1.55 (3H, bs, CH_3-6′),

2.47 (1H, m, H_c-3), 3.12 (1H, m, H_t-3), 3.90 (1H, s, OCH_3), 5.33 (1H, m, H-4), 7.35–7.64 (5H, m, Ph).

3.6.2. (*S*)-MTPA ester 1b. δ_H ($CDCl_3$): 1.41 (3H, s, CH_3-1″), 1.52 (3H, bs, CH_3-5′), 1.55 (3H, bs, CH_3-6′), 2.46 (1H, m, H_c-3), 3.11 (1H, m, H_t-3), 3.90 (1H, s, OCH_3), 5.33 (1H, m, H-4), 7.35–7.64 (5H, m, Ph).

3.6.3. (*R*)-MTPA ester 2a. δ_H ($CDCl_3$): 1.26 (3H, s, CH_3-1″), 1.60 (3H, bs, CH_3-5′), 1.68 (3H, bs, CH_3-6′), 2.58 (1H, m, H_c-3), 3.11 (1H, m, H_t-3), 3.90 (1H, s, OCH_3), 5.39 (1H, m, H-4), 7.35–7.64 (5H, m, Ph).

3.6.4. (*S*)-MTPA ester 2b. δ_H ($CDCl_3$): 1.27 (3H, s, CH_3-1″), 1.60 (3H, bs, CH_3-5′), 1.68 (3H, bs, CH_3-6′), 2.57 (1H, m, H_c-3), 3.10 (1H, m, H_t-3), 3.90 (1H, s, OCH_3), 5.39 (1H, m, H-4), 7.35–7.64 (5H, m, Ph).

3.7. Bioautographic assays

After application of the samples (100 μg/spot) on a silica gel 60 F_{254} Al sheet (Merck), the plate was sprayed with a suspension of *C. cucumerinum* in a nutritive medium and incubated for 2–3 days in a glass box with a moist atmosphere. Clear inhibition zones (12 mm) appeared against a dark gray background. Benomyl was used as a reference compound.

3.8. Calculation methods and procedures

The calculations were carried out as depicted earlier.[21] The ten possible envelope conformations of **1c** and **2c** were used as starting points for the MM3 calculations. The two final conformers were used as starting points for the ab initio molecular orbital calculations. In the starting conformers, the exocyclic ethyl and hydroxy groups were arranged in a stable conformation, and later the effect of changing both groups was checked by both calculation procedures.

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