One New Prenylated Furanone and Other non Polar Constituents from *Mutisia friesiana*

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In addition to the known furanones **1** and **2**, the aerial parts of the shrub *Mutisia friesiana* afforded a new prenylated furanone, Mutisifuranone A (**3**), together with the known triterpenoids oleanic (**4**) and ursolic (**5**) acids and some sesquiterpenoids and *n*-alkanes. Their structures were elucidated by spectroscopic methods.

Key words: Mutisia friesiana, Furanones, Secondary Metabolites

Introduction

Mutisia friesiana Cabrera (family Asteraceae, tribe Mutisieae, subtribe Mutisiinae) grows in N. W. Argentina and S. Bolivia at 3500 – 4000 m above sea level. Vernacule name of this plant is "chinchircoma colorada" or "romerillo". This species has a pleasant and persistent perfum and is used as a remedy against chronic cough, respiratory diseases and stomach pains [1]. In previous studies on this species we determined the composition of its essential oil and the identification of polyphenolic compounds with antioxidant activity from the aqueous extract [2]. We have also reported the isolation of antifungal methylphenone derivatives and 5-methylcoumarins with acyclic and cyclic terpene residues attached to oxygenated carbocyclic skeletons [3]. These compounds are biosynthetically related to 5-methylcoumaranones also isolated from *M. friesiana* [4]. In addition, we have isolated two antifungal diastereomeric furanones (**1** and **2**) described for the first time from a natural source [5]. In continuation of our studies on *M. friesiana*, we report here the isolation and structure determination of a new prenylated furanone (**3**), together with the known triterpenoids oleanic (**4**) and ursolic (**5**) acids and some sesquiterpenoids and *n*-alkanes from aerial parts of the plant.

Results and Discussion

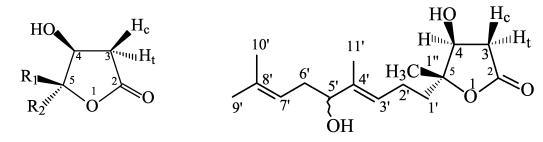
The $CHCl_3$ fraction of the methanolic extract of the aerial parts of *M. friesiana* afforded a new diastereomeric furanone, Mutisifuranone A (**3**), together with the known oleanic (**4**) and ursolic (**5**) acids, a mixture of sesquiterpenes and the known methylphenone derivative mutisiphenone A [3]. Reextraction of the plant residue with $CHCl_3$ rendered a mixture of alkanes that were identified by GC/MS.

The known oleanic (**4**) and ursolic acids (**5**) were identified by comparison of ^{1}H NMR and EIMS data with published results [6, 7] and by TLC analysis with standards.

Mutisifuranone A (**3**) was isolated as a colorless oil. Examination of the ^{1}H and ^{13}C NMR spectra (Table 1) showed the presence of two olefinic bonds, a γ-lactone carbonyl group and three oxygenated carbons. The presence of duplicated signals for certain carbons and H-3 (*t*), H-3', H-5', H-7' and Me-1" (Table 1) indicated that compound **3** was a mixture of diastereomers. The IR spectrum confirmed the presence of the γ-lactone carbonyl group (1751 cm^{-1}) and a hydroxyl function (3448 cm^{-1}). The FABMS showed a pseudomolecular ion at *m/z* 283 [M+H], compatible with the molecular formula $C_{16}H_{26}O_4$. The ^{1}H NMR spectrum showed signals at $\delta = 2.56$ (dd, $J = 17.9$, 4.7), 2.90

Table 1. ^{1}H and ^{13}C NMR spectral data for **3** (data were recorded in $CDCl_3$ at 500 and 125 MHz).

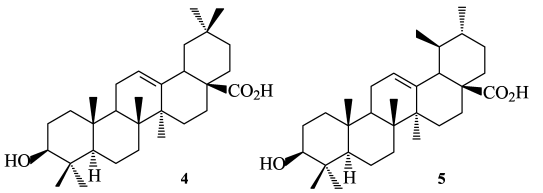
Position	**3**	
	δ_C	δ_H (J in Hz)
2	174.03	–
3	37.98; 38.00	H_c 2.56 (dd, $J = 17.9, 4.7$)
		H_t 2.90 (dd, $J = 17.9, 7.1$);
		2.91 (dd, $J = 17.9, 7.1$)
4	72.72; 72.68	4.28 (dd, $J = 7.1, 4.7$)
5	89.17	–
1’	39.00; 39.03	1.69 m
2’	21.99	2.18 (m)
3’	123.02; 124.64	5.37 (bt, $J = 7.0$); 5.39 (bt, $J = 7.0$)
4’	136.50; 137.60	–
5’	73.65; 73.69	3.97 (bt, $J = 6.1$); 3.98 (bt, $J = 6.1$)
6’	34.34; 34.80	2.24 (m)
7’	119.85; 119.88	5.09 (bt, $J = 7.0$); 5.08 (bt, $J = 7.0$)
8’	138.06	–
9’	25.89	1.72 (bs)
10’	18.00	1.64 (bs)
11’	11.93, 12.13	1.64 (bs)
1”	18.31, 18.54	1.41 s; 1.42 s
5’OH		3.89 (bs)



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

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

3



4 **5**

Fig. 1. Chemical structures of furanones **1 – 3**, oleanic acid (**4**) and ursolic acid (**5**).

(dd, $J = 17.9, 7.1$), 2.91 (dd, $J = 17.9, 7.1$), and 4.28 (dd, $J = 7.1, 4.7$), which are typical for a γ-lactone with a hydroxyl group at C-4 and two alkyl substituents at C-5 [5,8]. Comparison of the NMR spectra of **3** with those of diastereomeric furanones **1** and **2**, previously isolated from *M. friesiana* [5], indicated that Mutisifuranone A shared the same γ-lactone skeleton as **1** and **2** but differed from these compounds in the terpenic side chain. With respect to the relative stereochemistry of the substituents of the γ-lactone ring in **3**, comparison of the chemical shifts of C-4 ($\delta = 72.72$ and 72.68), C-1’ ($\delta = 39.03$ and 39.00) and C-1” ($\delta = 18.31$ and 18.54) with those in furanones **1** ($\delta = 73.67$ (C-4), 34.05 (C-1’), 23.12 (C-1”)) and **2** ($\delta = 72.71$ (C-4), 39.35 (C-1’), 18.48 (C-1”)) indicated that the relative configurations at the two stereogenic centers of the ring were coincident with those in furanone **2**. This was further confirmed by comparison of the chemical shifts of H-4 ($\delta = 4.28$) and Me-1” ($\delta = 1.41$ and 1.42) of **3** with respect to those in furanones **1** ($\delta = 4.19$ (H-4), 1.35 (Me-1”)) and **2** ($\delta = 4.27$ (H-4), 1.41 (Me-1”)). These data indicated that Me-1” and the hydroxyl group at C-4 are on the same side of the γ-lactone ring in **3**, while H-4 ($\delta = 4.28$) has the same orientation as the side chain.

The comparison of the molecular formula of Mutisifuranone A (**3**) ($C_{16}H_{26}O_4$) with that of **2** ($C_{11}H_{18}O_3$), suggested that the difference of 84 amu might correspond to one monohydroxylated and monounsaturated hemiterpenic unit attached to C-4’. This was confirmed by the presence of a secondary hydroxyl group and a trisubstituted double bond in the NMR spectra (Table 1). The location of the hydroxyl group at C-5’ was inferred from the chemical shift and multiplicity of H-5’ ($\delta = 3.97$ and 3.98, bt, $J = 6.1$ Hz) in the ^{1}H NMR spectrum. The ^{1}H-^{1}H COSY experiment confirmed that the secondary hydroxyl proton was coupled to the signal at $\delta = 2.24$ ppm corresponding to the protons attached to C-6’, as deduced from the cross-peaks 2.24/34.34 and 2.24/34.80 in the HETCOR spectrum. Both protons were coupled to the signals at $\delta = 5.08$ and 5.09 ppm, corresponding to the vinylic H-7’ in each diastereomer. This olefinic proton was coupled to the methyl signals at $\delta = 1.64$ and 1.72 ppm in the ^{1}H-^{1}H COSY spectrum. These signals correlated with two vinylic methyl carbons at $\delta = 18.00$ and 25.89 ppm, respectively in the HETCOR spectrum. These data confirmed the presence of the terminal *iso*-butenyl group in the terpenic side chain [9]. *E* geometry of the double bond between C-3’ and C-4’ was deduced from the upfield chemical shift of C-11’ ($\delta = 11.93$ and 12.13) [10]. Due to the chemical shift differences observed for the duplicated signals of C-3’ ($\delta = 1.62$ ppm), C-4’ ($\Delta\delta = 1.10$ ppm), C-6’ ($\delta = 0.46$ ppm), and C-11’ (0.20 ppm) in the ^{13}C NMR spectrum of **3**, and taking into account that both diastereomers share the same relative configurations of the stereogenic centers at the γ-lactone ring, we suggest that Mutisifuranone A is an inseparable mixture of epimers at C-5’.

The side chain of Mutisifuranone A has previously been found in Mutisicoumarin C and as a rest of the sesquiterpenic side chain of Mutisiphenone B

Table 2. Volatiles from chloroformic extracts A and B from *M. friesiana* (% of total volatiles).

Compound[a]	KI[b]	A	B
Sesquiterpenes			–
α-Gurjunene	1410	1.2	–
Germacrene D	1485	0.4	–
(Epi)-cubebol	1492	1.9	–
(Epi)-zonarene	1496	0.7	–
β-Himachalene	1498	0.7	–
γ-Muurolene	1501	7.5	0.6
γ-Cadinene	1516	9.3	0.5
δ-Cadinene	1524	36.0	–
Cubenene	1530	2.7	–
α-Cadinene	1538	2.6	–
α-Calacorene	1545	9.3	–
β-Copaen-4α-ol	1590	0.8	–
Hydrocarbons			–
Octadecane	1800	–	traces
Nonadecane	1900	–	traces
Eicosane	2000	–	traces
Eneicosane	2100	–	0.4
Docosane	2200	–	0.4
Tricosane	2300	–	2.1
Tetracosane	2400	–	0.8
Pentacosane	2500	–	3.2
Hexacosane	2600	–	0.9
Heptacosane	2700	–	12.0
Octacosane	2800	–	1.8
Nonacosane	2900	–	76.9
Triacontane	3000	1.5	–
Entriacontane	3100	–	0.2
Others			
Mutisiphenone A	2360	25.3	–

[a] Compounds of each type are listed in order of elution from HP-1 capillary column; [b] Kovàts indexes are calculated for HP-1 capillary column. Trace $< 0.1\%$.

and Mutisicoumaranone D [3, 4]. The co-occurrence of these compounds and the abundance of mono- and sesquiterpenes in the essential oil of *M. friesiana* [11] induced us to suggest a biosynthetic relationship between 5-methylcoumarins, 5-methylphenones and 5-methylcoumaranones isolated from *M. friesiana* (Mutisieae) [4].

The volatile compounds isolated from the chloroformic extracts A and B (Table 2) were investigated by GC/FID and GC/MS. Each compound was identified by mass spectrometry data [12, 13] and by their Kovàts retention indices [14, 15]. Sesquiterpenes were identified almost exclusively in the cyclohexane fraction obtained from purification of the chloroform extract A. All compounds, with exception of epi-cubebol, epi-sonarene, β-himachalene and β-copaen-4α-ol have been previously identified in the essential oil of *M. friesiana* [11]. Germacrene D, β-selinene, cariofilene oxide and α-bisabolol are the only sesquiterpenes previously reported in plants of the genus *Mutisia* [16]. Besides the sesquiterpenes, we also identified a 5-methylphenone, characterized as 1-(2-hydroxy-6-methylphenyl)-5,9-dimethyl-4,8-decadien-1-one, by comparison of its mass spectrum with data published previously [3].

Analysis by GC/MS of the hydrocarbon mixture isolated from fraction 1 of chloroformic extract B showed the presence of saturated hydrocarbons of 27 C (12.0%) and 29 C (76.9%) as the major components, and minor amounts of saturated hydrocarbons of 18 – 26, 28, and 31 carbons (Table 2). This pattern is in accordance with those observed in higher plants [17].

Experimental Section

General methods

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ for compound **3** and in $CDCl_3$ with addition of CD_3OD drops for compounds **4** and **5** on a Bruker ACE-200 and AM 500 spectrometers. Carbon substitution degrees in ^{13}C NMR spectra were established by DEPT multiple sequence. Mass spectra were measured on a TRIO-2 VG mass spectrometer. IR spectra were obtained on an IRFT Bruker IFS 88 spectrometer. Optical rotation was determined on a Perkin-Elmer 343 polarimeter. Preparative HPLC was carried out on an SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, a Rheodyne manual injector and a refractive index detector using a YMC-Pack ODS-A 5μ column (25 cm $\times$ 20 mm i.d.). TLC was performed on precoated silica gel 60 F_{254} (cyclohexane-EtOAc (6:4)) and ODS reversed-phase plates (65% and 95% MeOH-H_2O). Spots were visualized by spraying with 40% H_2SO_4-EtOH reagent followed by heating.

GC (for volatiles): KNK 3000 G, equipped with a FID and a capillary column HP-1 (50 m $\times$ 0.20 mm, 0.25 μm film). Temperature program: 60 – 230 °C at 6 °C min^{-1} and a 10 min hold. Injector temperature: 250 °C. Detector temperature: 300 °C. Carrier gas H_2, linear velocity 35 cm sec^{-1}. Split injection ratio 1:50.

GC/MS (for volatiles): Hewlett Packard 6890, MS HP 5972A, equipped with a capillary column HP-5MS (30 m $\times$ 0.25 mm, 0.25 μm film). Temperature program: 60 – 280 °C at 4 °C min^{-1} and a 10 min hold. Carrier gas He.

Plant material

Aerial parts of *M. friesiana* 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 Fac-

ultad de Ciencias Naturales, Universidad de Salta under the number H. G. 1064.

Extraction and isolation of non volatiles

Cut dried and powdered plant material (550 g) was extracted with MeOH (3×1.5 l) at room temperature. The MeOH extracts were concentrated in vacuum to give a residue (100 g) which was partitioned with *n*-hexane-MeOH/H_2O (10:3:1 v/v/v), yielding a non-polar and an aqueous phase. The polar phase was extracted with $CHCl_3$. The extract was evaporated to dryness to yield a chloroform residue A (10 g). Part of this residue (3 g) was subjected to vacuum dry-column chromatography on silica gel 60H, eluting with cyclohexane, EtOAc, acetone and MeOH to give ten fractions. Fractions 5 (357 mg) and 6 (166 mg) were joined and subjected to vacuum dry-column on RP-18 with H_2O/MeOH (7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:10 v/v), MeOH and acetone to give 9 fractions (5-6.1 – 5-6.9). Fraction 5-6.5 (62 mg) was submitted to repeated reversed-phase HPLC (ODS, MeOH/H_2O (65:35 v/v), flow rate 6 ml/min) to give Mutisifuranone A (**3**) (2 mg). Fraction 3 (1.114 mg) was subjected to vacuum dry-column on RP-18 with H_2O/MeOH (7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:10 v/v), MeOH and acetone to give 9 fractions (3.1 – 3.9). Fraction 3.8 (180 mg) was submitted to reversed-phase HPLC (ODS, MeOH/H_2O (95:5 v/v), flow rate 6 ml/min) to afford 18 mg of oleanic acid (**4**) and 3 mg of ursolic acid (**5**).

*5-(5-hydroxy-4,8-dimethyl-3,7-nonadienyl)-4-hydroxy-5-methyldihydrofuran-2-one (**3**)*

Colorless oil. $[\alpha]_D = -24.5°$ (*c* 0.24, $CHCl_3$). – IR (KBr) ν = 3448, 2968, 2924, 2867, 1751, 1452, 1382, 1218, 1066 cm^{-1}. – ^1H and ^{13}C NMR see Table 1. – MS (EI, 70 eV): *m/z* (%) = 264 (2) $[M\text{-}H_2O]^+$, 213 (14), 195 (14), 177 (10), 135 (30), 97 (45), 69 (25), 55 (36), 43 (100). – FABMS (positive ion mode): $m/z = 283$ $[M+H]^+$. – HRFABMS: *m/z* $[M+H]^+$: calcd. for $C_{16}H_{27}O_4$, 283.1909; found 283.1914.

*Olean-12-en-3β-hydroxy-28-oic acid (**4**)*

White solid. – ^1H NMR (200.1 MHz, 5% CD_3OD in $CDCl_3$): $\delta = 0.78$ (s, 3 H, 26-H), 0.79 (s, 3 H, 24-H), 0.90 and 0.91 (s, 6 H, 29-H and 30-H), 0.93 (s, 3 H, 25-H), 0.98 (s, 3 H, 23-H), 1.14 (s, 3 H, 27-H), 2.84 (dd, $^2J = 18.8$ Hz, $^3J = 3.6$ Hz, 1 H, 18-H), 3.20 (dd, $J = 9.1$, 6.9 Hz, 1 H, 3-H), 5.28 (br t, $J = 3.3$ Hz, 1 H, 12-H). – MS (EI, 70 eV): *m/z* (%) = 456 (5) $[M^+]$, 248 (74), 235 (1), 203 (73), 189 (15), 165 (1), 139 (2), 137 (3), 119 (21), 69 (46).

*Urs-12-en-3β-hydroxy-28-oic acid (**5**)*

White solid. – ^1H NMR (200.1 MHz, 5% CD_3OD in $CDCl_3$): $\delta = 0.77$ (s, 3 H, 24-H), 0.78 (s, 3 H, 26-H), 0.83 (d, $J = 6.2$ Hz, 3 H, 30-H), 0.93 (s, 3 H, 25-H), 0.95 (d, $J = 6.6$ Hz, 3 H, 29-H), 0.97 (s, 3 H, 23-H), 1.15 (s, 3 H, 27-H), 2.25 (dd, $^2J = 11.5$ Hz, $^3J = 1.5$ Hz, 1 H, 18-H), 3.20 (m, 1 H, 3-H), 5.24 (m, 1 H, 12-H). – MS (EI, 70 eV): *m/z* (%) = 456 (1) $[M^+]$, 248 (48), 235 (1), 203 (33), 189 (11), 165 (2), 139 (2), 137 (3), 119 (15), 69 (29).

Isolation and analysis of volatiles

The non polar cyclohexane fraction (10 mg) obtained from purification of chloroform residue A by vacuum dry-column chromatography on silica gel 60H was analyzed by GC/FID and GC/MS to afford sesquiterpenes and Mutisiphenone A.

The plant residue obtained after extraction with MeOH was reextracted with $CHCl_3$ (1.2 l). The extract was evaporated to dryness to afford a chloroform residue B (3.3 g). Part of this residue (2.5 g) was subjected to vacuum dry-column chromatography on silica gel 60H, eluting with cyclohexane, benzene, EtOAc, acetone and MeOH to give ten fractions. Fraction 1 (68 mg) contained a mixture of *n*-alkanes that was investigated by CG/FID and CG/MS.

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