

5-Methylcoumaranones from *Mutisia friesiana* and Their Bioactivity

Carmen I. Viturro,[†] Juana R. de la Fuente,[‡] and Marta S. Maier^{*,§}

Facultad de Ingeniería, Universidad Nacional de Jujuy, Gorriti 237, 4600 S. S. de Jujuy, Jujuy, Argentina, Facultad de Ciencias Exactas, Universidad Nacional de Salta, Buenos Aires 177, 4400 Salta, Salta, Argentina, and Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

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In addition to the known mutisicoumaranones A (**1**) and B (**2**), the methanolic extract of the aerial parts of the shrub *Mutisia friesiana* afforded two new 5-methylcoumaranones, mutisicoumaranones C (**3**) and D (**4**). Their structures were elucidated by spectroscopic methods. ¹³C NMR data for mutisicoumaranones A and B are reported for the first time. All compounds showed antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum* and bactericidal activity against *Staphylococcus aureus*. The presence of 5-methylcoumaranones A–D is biosynthetically related to 5-methylcoumarins and phenolic derivatives previously isolated from *M. friesiana*.

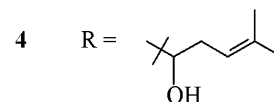
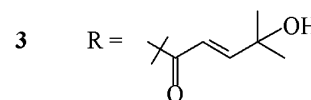
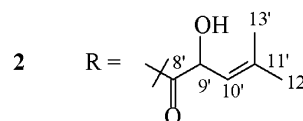
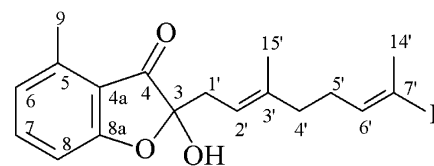
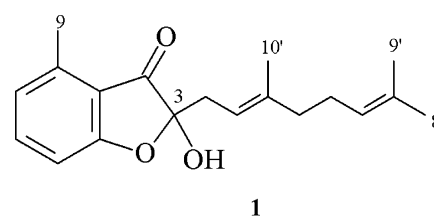
Mutisia friesiana (family Asteraceae, tribe Mutisieae, subtribe Mutisiinae) is a perennial shrub native of south Bolivia and northwest Argentina that grows at 3500–4000 m above sea level.¹ The plant is known under the common names “romerillo”, “chinchircoma”, and “chinchircoma colorada” and is used as a spice.² The infusion of this species is used in folk medicine as a remedy against chronic cough, respiratory diseases, and stomach pains.³ Its pleasing and persistent scent prompted us to study its essential oil composition, identifying more than 100 compounds.⁴ Bioassay-guided fractionation of the aqueous extract of *M. friesiana* afforded caffeic acid derivatives and flavonoids as the main active compounds showing radical-scavenging activity.⁵ Recently, we have reported the isolation and structural elucidation of new antifungal diastereomeric furanones,⁶ 5-methylphenones, 5-methylcoumarins, and a related chromone.⁷

Continuing our search for bioactive compounds from *M. friesiana* we now report the isolation and structural elucidation of four 5-methylcoumaranones (**1**–**4**), as well as the results of their antimicrobial activity and their biosynthetic relation to 5-methylphenones and 5-methylcoumarins, previously isolated from this shrub.

Results and Discussion

The CHCl₃ fraction of the methanolic extract of the aerial parts of *M. friesiana* was sequentially submitted to silica gel column chromatography and C₁₈ vacuum flash chromatography. Final separation and isolation was achieved by C₁₈ HPLC to give two new 5-methylcoumaranones, mutisicoumaranones C (**3**) and D (**4**), together with the known mutisicoumaranone A (**1**) and mutisicoumaranone B (**2**), previously isolated from *M. spinosa*⁸ and *Gypothamium pinifolium*,⁹ respectively.

The known mutisicoumaranone A (**1**) was identified by comparison of IR, EIMS, and ¹H NMR data with published results⁸ and by ¹³C NMR, ¹H–¹H COSY, and HETCOR experiments, which allowed for the first time the unambiguous assignment of all proton and carbon signals (Table 1). ¹³C NMR data for the benzofuranone ring were in



accordance with those published previously for this system.⁹ The allylic coupling of the proton at δ 6.81 (1H, dq, $J = 7.5, 0.7$ Hz) with Me-9 (δ 2.56) allowed us to assign it to H-6. This was confirmed by irradiation of the methyl signal at δ 2.56 ppm. The HETCOR and ¹H–¹H COSY data and the analysis of γ -gauche interactions¹⁰ of the allylic methyl groups (Me-9' and Me-10') permitted assignment of the stereochemistry of the 2'(3')-double bond as *E*. On the basis of the data mentioned above, we reassigned the chemical shifts of Me-8', Me-10', H-6, and H-8 with respect to those published before.⁸

Mutisicoumaranone B (**2**) was identified by comparing IR, EIMS, and ¹H NMR data with those reported¹¹ and by ¹³C NMR, DEPT, ¹H–¹H COSY, and HETCOR experiments. The presence of duplicated signals for most carbons

* To whom correspondence should be addressed. Fax: 54-11-4576-3346. E-mail: maier@qo.fcen.uba.ar.

[†] Universidad Nacional de Jujuy.

[‡] Universidad Nacional de Salta.

[§] Universidad de Buenos Aires.

Table 1. ¹H and ¹³C NMR Data for Mutisicoumaranones A (1) and B (2)

carbon	mutisicoumaranone A (1)		mutisicoumaranone B (2)	
	δ_C mult ^a	δ_H mult ^b (J in Hz)	δ_C mult ^a	δ_H mult ^b (J in Hz)
3	103.3 s		103.5; 103.6 s	
4	199.2 s		201.3; 201.4 s	
4a	114.5 s		117.3 s	
5	143.6 s		140.5 s	
6	123.4 d	6.81 br dq (7.5, 0.7)	123.5; 123.6 d	6.81 br d (7.3)
7	138.2 d	7.46 dd (8.4, 7.5)	138.3 d	7.46 dd (8.2, 7.3)
8	110.2 d	6.84 br d (8.4)	110.2 d	6.82 br d (8.2)
8a	170.7 s		170.6 s	
9	17.8 q	2.56 br s	17.8 q	2.55 br s
1'	34.6 t	2.75 dd (14.5, 8.9)	34.4; 34.5 t	2.71 m; 2.53 m
		2.50 br dd (14.5, 6.5)		
2'	117.5 d	5.22 br dd (8.9, 6.5)	116.1; 116.0 d	5.25 m
3'	140.5 s		140.6; 140.7 s	
4'	39.9 t	2.05 m	38.3 t	2.13 br t (7.3)
5'	26.4 t	2.05 m	27.3 t	2.28 q (7.3)
6'	123.9 d	5.02 br t (7.0)	144.5; 144.6 d	6.52 br q (7.3)
7'	132.1 s		138.0; 138.1 s	
8'	25.6 q	1.65 br s	201.5 s	
9'	17.6 q	1.57 br s	69.8 d	5.28 br dd (9.8, 7.0)
10'	16.3 q	1.69 br s	123.4; 123.3 d	4.98 dq (9.8, 1.4)
11'			134.0 s	
12'			25.8 q	1.73 br s
13'			18.3 q	1.83 br s
14'			11.7 q	1.77; 1.78 br s
15'			16.3; 16.4 q	1.68; 1.67 br s
9' OH				3.96 br s

^a Recorded at 125 MHz in CDCl₃; multiplicity by DEPT. ^b Recorded at 500 MHz in CDCl₃.

Table 2. ¹H and ¹³C NMR Data for Mutisicoumaranones C (3) and D (4)

carbon	mutisicoumaranone C (3)		mutisicoumaranone D (4)	
	δ_C mult ^a	δ_H mult ^b (J in Hz)	δ_C mult ^a	δ_H mult ^b (J in Hz)
3	104.2 s		103.4; 103.2 s	
4	199.9 s		199.2 s	
4a	117.1 s		117.4 s	
5	142.8 s		142.7; 142.6 s	
6	123.4 d	6.81 br d (7.3)	123.4; 123.5 d	6.81 br d (7.3)
7	138.3 d	7.46 dd (8.4, 7.3)	138.2 d	7.45 br dd (8.2, 7.3)
8	110.3 d	6.84 br d (8.4)	110.2; 110.3 d	6.84 br d (8.2)
8a	170.7 s		170.7; 170.6 s	
9	17.8 q	2.56 br s	17.8 q	2.56 br s
1'	34.3 t	2.66 dd (14.5, 8.0)	34.5 t	2.72 m, 2.48 m
		2.57 dd (14.5, 7.0)		
2'	116.5 d	5.39 br dd (8.0, 7.0)	115.1; 115.2 d	5.25 br dd (6.0, 7.2)
3'	140.7 s		140.6 s	
4'	38.4 t	2.23 br t (7.0)	39.5; 39.6 t	2.11 m
5'	26.6 t	2.37 t (7.0)	25.8 t	2.11 m
6'	145.1 d	6.81 m	125.6; 125.3 d	5.32 br t (6.3); 5.34 br t (6.3)
7'	138.5 s		134.5 s	
8'	192.1 s		77.2; 76.9 d	3.94 br t (6.6)
9'	121.1 d	6.81 d (15.3)	34.1 t	2.19 m, 2.79 m, 2.25 m
				2.25 m
10'	151.3 d	7.04 d (15.3)	120.2 d	5.05 br t (6.6), 5.08 br t (6.6)
11'	72.0 s		137.7 s	
12'	29.6 q ^c	1.33 s ^c	25.6 q	1.70 br s, 1.71 br s
13'	29.4 q ^c	1.28 s ^c	18.0 q	1.61 br s
14'	11.8 q	1.83 br s	11.6; 12.0 q	1.59 br s
15'	15.9 q	1.68 br s	16.3 q	1.68 br s

^a Recorded at 125 MHz in CDCl₃; multiplicity by DEPT. ^b Recorded at 500 MHz in CDCl₃. ^c Interchangeable in the same column.

and for the Me-14' and Me-15' protons (Table 1) indicated that compound **2** was a mixture of diastereomers (epimers at C-3- or C-9'). This has been discussed previously on the basis of ¹H NMR data for mutisicoumaranone B.¹¹ ¹³C NMR data for compound **2** are published here for the first time.

Mutisicoumaranone C (**3**) was obtained as a colorless oil and formulated as C₂₄H₃₀O₅ from HRFABMS. The peaks at *m/z* 164, 163, and 135 in the EIMS of **3** and the hydroxyl (3400 cm⁻¹) and ketone (1718 cm⁻¹) bands in its IR spectrum revealed that compound **3** featured the same 5-methylcoumaranone skeleton as **1** and **2**. This was

confirmed by ¹H and ¹³C NMR data (Table 2). The ¹³C NMR spectrum with DEPT displayed signals due to five methyls, three methylenes, two carbonyls (δ 192.1 and 199.9), three oxygen-bearing quaternary carbons (δ 72.0, 104.2, and 170.6), seven methines, and four quaternary carbons. These data suggested the presence of a dioxygenated farnesyl group attached to C-3. Mutisicoumaranones B (**2**) and C (**3**) are isomers. Both structures showed similar ¹³C NMR spectra except for the signals of the terminal hemiterpene group. The presence of a pair of doublets for two *trans*-orientated olefinic protons ($J = 15.3$ Hz) at δ 6.81 and 7.04

ppm as well as two deshielded singlets at δ 1.28 and 1.33 (Me-12' and Me-13') in the spectrum of **3** required a hydroxy group at C-11'.¹² The band at 1657 cm^{-1} in the IR spectrum of **3** together with the signal at δ 192.1 ppm in its ¹³C NMR spectrum indicated that the carbonyl group in the side chain (C-8') was conjugated with both vicinal double bonds.¹³ ¹H-¹H COSY and HETCOR data were in good agreement with the proposed sequence in the sesquiterpene group attached to C-3. The configuration of C-3 remained to be determined.

Mutisicoumaranone D (**4**) was isolated as a colorless oil. The molecular formula was established as C₂₄H₃₂O₄ from HRFABMS. Its IR and EIMS spectra showed characteristic peaks for a 5-methylcoumaranone skeleton. ¹H and ¹³C NMR data of **4** (Table 2) were nearly identical to those of compounds **1-3** with the exception of resonances corresponding to the monooxygenated farnesyl group attached to C-3. On the other hand, the NMR data resembled those of the sesquiterpene unit of mutisiphenone B, isolated previously by us from *M. friesiana*.⁷ The presence of duplicated signals for certain carbons and for H-6', H-10', and Me-12' protons (Table 2) indicated that compound **4** was a mixture of diastereomers (epimers at C-3 or C-8').

The ¹H NMR spectrum of **4** showed the presence of broad vinylic methyl singlets at δ 1.59, 1.61, 1.68, 1.70, and 1.71 and vinylic protons at δ 5.05, 5.08, 5.25, 5.32, and 5.34 ppm. ¹H-¹H COSY and HETCOR data permitted assignment of the sequence and stereochemistry (2'E,6'E) of unsaturations in the farnesyl group attached to C-3. Signals at δ 1.70 and 1.71, 5.05 and 5.08, and 5.32 and 5.34 were assigned to Me-12', H-10', and H-6' of both epimers. The ¹H-¹H COSY experiment revealed that the secondary hydroxyl proton (δ 3.94, 1H, br t, *J* = 6.6 Hz) was coupled to the signals at 2.19 and 2.25 ppm corresponding to the protons attached to C-9', as deduced from the cross-peaks 2.19/34.1 and 2.25/34.1 in the HETCOR spectrum. Both protons were coupled to the signals at δ 5.05 and 5.08 ppm, corresponding to the vinylic H-10' in each diastereomer. The cross-peaks 5.32/125.6 and 5.34/125.3 (H-6'/C-6'), 3.94/76.9 and 3.94/77.2 (H-8'/C-8'), and 1.59/11.6 and 1.59/12.0 (H-14'/C-14') in the HETCOR spectrum of **4** clearly confirmed the presence of two epimers.

The isolation of 5-methylcoumaranones A-D (**1-4**) together with structurally related 5-methylcoumarins and 5-methylphenones⁷ containing mono- and sesquiterpene residues enabled us to suggest a biosynthetic relationship between these three groups of compounds in the shrub *M. friesiana* (Figure 1). 4-Hydroxy-5-methylcoumarin (HMC), biosynthesized from acetyl-CoA and malonyl-CoA,¹⁴ has been postulated as the precursor of 5-methylcoumarins.^{8,15,16} In the first stage, the reaction of HMC with activated mono- and sesquiterpenes present in the essential oil of the plant⁴ would be responsible for the formation of mutisichromone and mutisicoumarins A-C.⁷ In a second stage, hydrolysis of 5-methylcoumarins with elimination of CO₂ and further keto-enol tautomerization would produce mutisiphenones A-E.⁷ Degradation of the pyrone ring and loss of C-2 from a coumarin-terpenoid precursor has already been proposed by Hoeneisen et al.¹⁶ to explain the co-occurrence of triptiliocoumarin and its related 5-methylphenol derivative in *Nassauvia pyramidalis*. In the last stage, oxidation of C-3 of a 5-methylphenone to a diketone would directly lead to the cyclic hemiacetal. Zdero et al.⁸ have proposed that mutisicoumaranone A (**1**), also isolated from *M. spinosa*, is most likely derived from piloselloidan. Our previous isolation of mutisiphenones A-E from *M. friesiana* would confirm the proposed biosynthetic pathway.

The co-occurrence of the three families of compounds as well as the abundance of mono- and sesquiterpenes in the essential oil of *M. friesiana* would suggest the connection between these compounds and the predominance of the acetate route in the secondary metabolism of this species.

Compounds **1-4** were evaluated by a bioautographic technique^{17,18} for their antifungal activity against the plant pathogenic fungus *Cladosporium cucumerinum*. Compounds **1-4** showed inhibition zones of 8, 14, 14, and 10 mm, respectively, at a concentration of 100 $\mu\text{g}/\text{spot}$ (0.25–0.33 $\mu\text{mol}/\text{spot}$). The compounds were less active than benomyl, a commercially available fungicide, which showed an inhibition zone of 20 mm at a concentration of 10 $\mu\text{g}/\text{spot}$.

Antimicrobial activity of compounds **1-4** was evaluated using standard paper sensitivity disk-agar plate methodology.¹⁹ All compounds tested gave a 10 mm zone of inhibition at 100 $\mu\text{g}/\text{disk}$ to *Staphylococcus aureus* and were inactive to *Escherichia coli* and *Candida albicans*. Ampicillin, used as control compound, showed an inhibition zone of 14 mm at 10 $\mu\text{g}/\text{disk}$ against *S. aureus*.

Experimental Section

General Experimental Procedures. ¹H and ¹³C NMR spectra were recorded in CDCl₃ on Bruker ACE-200 and AM 500 spectrometers. Chemical shifts are referenced to solvent peaks: δ_{H} 7.26 (residual CHCl₃) and δ_{C} 77.0. Carbon substitution degrees in ¹³C 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 rotations were determined on a Perkin-Elmer 343 polarimeter. HPLC was performed 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 5 μ column (25 cm \times 20 mm i.d.). TLC was performed on Si gel 60 F₂₅₄ (cyclohexane/EtOAc (6:4)) and C₁₈ reversed-phase plates (MeOH/H₂O (80:10)).

Plant Material. Aerial parts of *M. friesiana* were collected in Jujuy, Departamento 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 (no. H.G. 1064) is on deposit at the Herbarium of the Facultad de Ciencias Naturales, Universidad de Salta, Salta, Argentina.

Extraction and Isolation. Cut, dried, and powdered plant material (550 g) was extracted with MeOH (3 \times 1.5 L) at room temperature. The MeOH extracts were concentrated under reduced pressure to give a residue (100 g), which was partitioned between *n*-hexane/MeOH/H₂O (10:3:1) to yield aqueous MeOH- and *n*-hexane-soluble fractions. The aqueous MeOH fraction was extracted with CHCl₃, and the extract was evaporated to dryness to yield a CHCl₃ residue (10 g). Part of this residue (3 g) was purified by vacuum-dry column chromatography on Si gel 60H, eluting with cyclohexane, EtOAc, acetone, and MeOH to give 10 fractions. Fraction 3 (1.114 g) was subjected to vacuum-dry column chromatography on C₁₈ reversed-phase using H₂O/MeOH (7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:10), MeOH, and acetone to give nine fractions (3.1–3.9). Fractions 3.4 (240 mg) and 3.5 (128 mg) were submitted to repeated reversed-phase HPLC (MeOH/H₂O (8:2), flow rate 6 mL/min) to give 5-methylcoumaranones **3** (4 mg), **2** (6 mg), and **4** (10 mg) from fraction 3.4 and 5-methylcoumaranone **1** (20 mg) from fraction 3.5. Fraction 3.6 (210 mg) was purified by flash chromatography on Si gel H (10–40 μm), eluting with mixtures of increasing polarity of cyclohexane, EtOAc, acetone, and MeOH, to give 28 fractions (3.6.1–3.6.28). Fractions 3.6.21 and 3.6.22 were pooled (56 mg) and purified by reversed-phase HPLC (MeOH/H₂O (8:2), flow rate 6 mL/min) to give 5-meth-

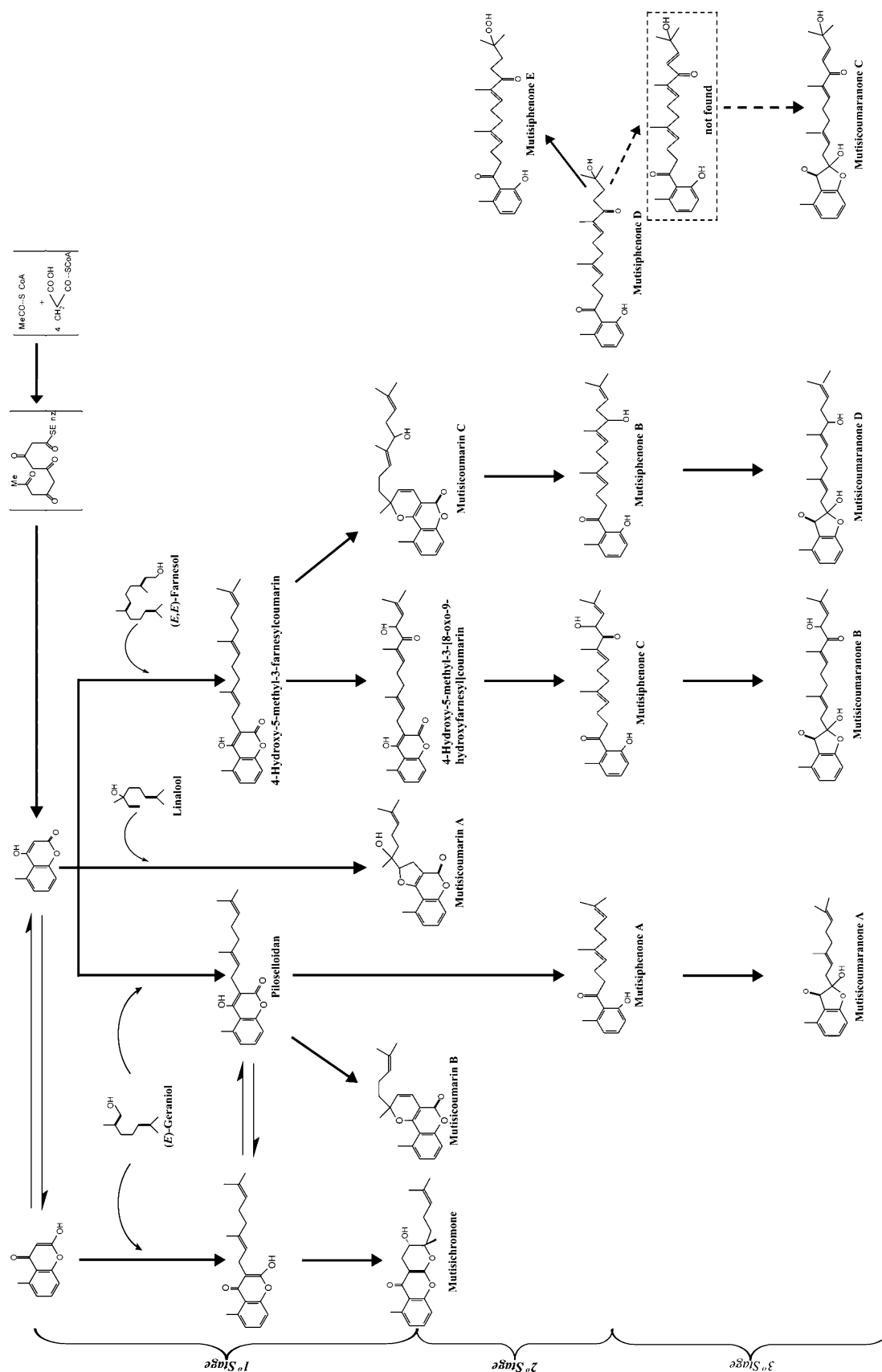


Figure 1. Biogenetic proposal for 5-methylcoumarins, 5-methylphenones, and 5-methylcoumaranones in *M. friesiana*.

ylcoumaranone **4** (6 mg). Fractions 3.6.23 and 3.6.24 were pooled and purified in the same way to give 5-methylcoumaranone **2** (7 mg). Fractions 3.6.27 and 3.6.28 (30 mg) were subjected to reversed-phase HPLC (MeOH/H₂O (8:2), flow rate 6 mL/min) to afford compound **3** (4 mg).

Mutisicoumaranone A (1): colorless oil; $[\alpha]_D^{20} -3.14$ (c 0.87; CHCl₃); UV (MeOH) λ_{max} (log ϵ) 333 (3.3), 260 (3.7), 210 (sh), 205 (4.3) nm; IR (film) ν_{max} 3400, 1720, 1603, 1493, 1454, 1315, 1275, 1153, 955, 931, 777 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; EIMS m/z 300 [M]⁺ (1), 282 [M - H₂O]⁺ (4), 213

[282 – 69]⁺ (1), 164 [M – C₁₀H₁₆]⁺ (64), 163 [M – C₁₀H₁₇]⁺ (30), 137 [M – C₅H₉]⁺ (25), 135 [C₆H₄(OH)CH₃CO]⁺ (27), 107 [135 – CO]⁺ (6), 69 [C₅H₉]⁺ (100).

Mutisicoumaranone B (2): colorless oil; UV (MeOH) λ_{\max} (log ϵ) 335 (3.3), 258 (3.8), 235 (sh), 210 (4.1), 205 (sh) nm; IR (film) ν_{\max} 3400, 1718, 1660, 1601, 1485, 1446, 1315, 1273, 1153, 957, 931, 756 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; EIMS *m/z* 313 [M – CH(OH)CH=C(Me)₂]⁺ (2), 295 [313 – H₂O]⁺ (4), 235 (1), 167 (1), 164 (20), 163 (24), 135 [C₆H₃(OH)CH₃CO]⁺ (88), 107 [135 – CO]⁺ (11), 85 [CH(OH)CH=C(Me)₂]⁺ (18), 83 (55), 82 [167 – 85]⁺ (20), 55 [83 – CO]⁺ (60), 43 (100).

Mutisicoumaranone C (3): colorless oil; [α]_D²⁰ –16.0 (c 0.35; CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 335 (3.4), 258 (4.1), 235 (sh), 212 (4.2), 204 (sh) nm; IR (film) ν_{\max} 3400, 1718, 1657, 1603, 1486, 1445, 1315, 1290, 1153, 980, 955, 931, 775 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; EIMS *m/z* 380 [M – H₂O]⁺ (1), 285 [M – COCH=CHC(OH)(Me)₂]⁺ (1), 163 (12), 164 (49), 150 (10), 135 [C₆H₃(OH)CH₃CO]⁺ (84), 113 [M – 285]⁺ (4), 107 [135 – CO]⁺ (9), 95 [113 – H₂O]⁺ (18), 67 [95 – CO]⁺ (13), 55 (25), 43 (100); HRFABMS *m/z* 399.2175 (calcd for C₂₄H₃₁O₅ [M + H]⁺, 399.2171).

Mutisicoumaranone D (4): colorless oil; UV (MeOH) λ_{\max} (log ϵ) 334 (2.9), 260 (3.5), 235 (sh), 214 (sh), 205 (3.9) nm; IR (film) ν_{\max} 3400, 1718, 1601, 1490, 1450, 1315, 1261, 1153, 955, 931, 758 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; EIMS *m/z* 315 [M – CH₂CH=C(Me)₂]⁺ (5), 297 [315 – H₂O]⁺ (30), 279 (8), 239 (10), 221 (1), 213 (11), 164 (12), 163 (21), 153 [M – C₁₀H₁₇O]⁺ (2), 135 [C₆H₃(OH)CH₃CO]⁺ (100), 107 [135 – CO]⁺ (11), 84 (18); HRFABMS *m/z* 385.2373 (calcd for C₂₄H₃₃O₄ [M + H]⁺, 385.2379).

Bioautographic Assay. Solutions of compounds 1–4 were prepared at a concentration of 10 mg ml⁻¹ in CH₂Cl₂. Of these solutions, 10 μ L was applied on a TLC plate using graduated capillaries. After application of the samples (100 μ g/spot) on a silica gel 60 F₂₅₄ Al sheet (Merck), the plate was sprayed with a suspension of *C. cucumerinum* (DSM 62122) in a nutritive medium²⁰ and incubated for 2–3 days at room temperature in a glass box with a moist atmosphere. Clear inhibition zones appeared against a dark gray background. Benomyl was used as a reference compound.

Antimicrobial Assay. Petri dishes contained 10 mL of agar inoculated with different microorganisms: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), and *Candida albicans* (BE 138/99). Samples (100 μ g/disk) were applied on paper disks (disk diameter, 5 mm) and these on agar plates. The Petri dishes were incubated for 48 h at 37 °C. Clear inhibition zones appeared on the disks.

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