Antiviral Phenylpropanoid Glycosides from the Medicinal Plant *Markhamia lutea*

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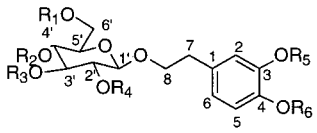
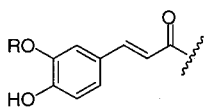
Three new phenylpropanoid glycosides, named luteoside A (**3**), luteoside B (**4**), and luteoside C (**5**), were isolated together with the known compounds verbascoside (**1**) and isoverbascoside (**2**) from the roots of the medicinal plant *Markhamia lutea*. The structures of the new compounds were determined to be 1-*O*-(3,4-dihydroxyphenyl)ethyl *β*-D-apiofuranosyl(1→2)-α-L-rhamnopyranosyl(1→3)-4-*O*-caffeoyl-6-acetyl-*β*-D-glucopyranoside, 1-*O*-(3,4-dihydroxyphenyl)-ethyl *β*-D-apiofuranosyl(1→2)-α-L-rhamnopyranosyl(1→3)-6-*O*-caffeoyl-*β*-D-glucopyranoside, and 1-*O*-(3,4-dihydroxyphenyl)ethyl *β*-D-apiofuranosyl(1→2)-α-L-rhamnopyranosyl(1→3)-6-*O*-feruloyl-*β*-D-glucopyranoside, respectively, on the basis of chemical and spectroscopic data. All five phenylpropanoid glycosides exhibited potent in vitro activity against respiratory syncytial virus.

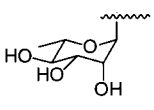
The drug discovery process at Shaman Pharmaceuticals relies on gathering ethnobotanical and ethnomedical information through interactions between western trained botanist−physician scientific teams and traditional healers.[1,2] Our interest has focused on treatments for respiratory syncytial virus (RSV), an important cause of severe lower respiratory tract infections in infants and young children.[3] In a continuation of our antiviral drug discovery program,[4] the plant *Markhamia lutea* Seemann ex Baillor (Bignoniaceae) was identified as a potential treatment for viral respiratory infections, including RSV. In traditional preparations, the roots are soaked in cold water for about 30 min, and the resulting tea is taken three times daily to alleviate symptoms of watery, bloodless diarrhea. When tested in our viral cytopathic effect (CPE) assays,[5,6] we found that several extracts of *M. lutea* exhibited potent and selective inhibition of RSV in cell culture. Bioassay-guided fractionation led to the isolation of five phenylpropanoid glycosides responsible for the antiviral activity of the extract. They were identified by analysis of their spectral data, including 2D-NMR, as the known compounds verbascoside (**1**) and isoverbascoside (**2**), and three new compounds luteoside A (**3**), luteoside B (**4**), and luteoside C (**5**). All of these compounds had potent in vitro activity against RSV, and they appear to inhibit the virus by an intracellular mechanism of action. The new compounds are related to other phenylpropanoid glycosides, including forthysioside B (**6**),[7] myricoside (**7**),[8] and pedicularioside A (**8**),[9] that contain glucose, rhamnose, and apiose units.



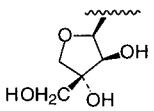
	R_1	R_2	R_3	R_4	R_5	R_6
1	H	Caf	Rha	H	H	H
2	Caf	H	Rha	H	H	H
3	Ac	Caf	Rha	Api	H	H
4	Caf	H	Rha	Api	H	H
5	Fer	H	Rha	Api	H	H
6	Api	Caf	Rha	H	H	H
7	H	Caf	Api(1→3)Rha	H	H	H
8	Rha	Caf	Api	H	H	H
9	Ac	$(Ac)_2$Caf	$(Ac)_3$Rha	Ac	Ac	Ac
10	$(Ac)_2$Caf	Ac	$(Ac)_3$Rha	Ac	Ac	Ac
11	Ac	$(Ac)_2$Caf	$(Ac)_3$Rha	$(Ac)_3$Api	Ac	Ac
12	$(Ac)_2$Caf	Ac	$(Ac)_3$Rha	$(Ac)_3$Api	Ac	Ac
13	(Ac)Fer	Ac	$(Ac)_3$Rha	$(Ac)_3$Api	Ac	Ac
14	H	Caf	Api	H	H	H
15	Caf	H	Api	H	H	H
16	Ac	Caf	$(Ac)_3$Rha	Ac	H	H
17	Caf	Ac	$(Ac)_3$Rha	Ac	H	H



R = H, Caffeoyl
R = CH_3, Feruloyl



Rhamnosyl



Apiosyl

Results and Discussion

The roots of *M. lutea* were ground to a powder and extracted with CH_2Cl_2−*i*-PrOH (1:1) followed by aque-

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Table 1. ^{1}H NMR Assignments (400 MHz) for **3**−**5** (DMSO-d_6 + TFA 0.1%) and **11**−**13** ($CDCl_3$) (Splitting Patterns and *J* Values (Hz) Are Given in Paretheses)

H	3	4	5	11	12	13
caffeoyl						
2	7.03 (d, 2)	7.05 (d, 2)	7.32 (d, 2)	7.37 (d, 2)	7.37 (d, 2)	7.06 (d, 2)
5	6.75 (d, 8)	6.74 (d, 8)	6.77 (d, 8)	7.23 (d, 8)	7.22 (d, 8)	7.07 (d, 8)
6	6.98 (dd, 8, 2)	6.96 (dd, 8, 2)	7.05 (dd, 8, 2)	7.40 (dd, 8, 2)	7.39 (dd, 8, 2)	7.05 (dd, 8, 2)
7	7.46 (d, 16)	7.46 (d, 16)	7.52 (d, 16)	7.65 (d, 16)	7.63 (d, 16)	7.65 (d, 16)
8	6.19 (d, 16)	6.29 (d, 16)	6.50 (d, 16)	6.34 (d, 16)	6.40 (d, 16)	6.40 (d, 16)
OMe			3.80 (s)			3.87 (s)
aglycone						
2_A	6.59 (d, 2)	6.57 (d, 2)	6.58 (d, 2)	7.13 (d, 2)	7.07 (d, 2)	7.07 (d, 2)
5_A	6.62 (d, 8)	6.60 (d, 8)	6.56 (d, 8)	7.07 (d, 8)	7.06 (d, 8)	7.07 (d, 8)
6_A	6.47 (dd, 8, 2)	6.54 (dd, 8, 2)	6.44 (dd, 8, 2)	7.14 (dd, 8, 2)	7.10 (dd, 8, 2)	7.07 (dd, 8, 2)
7_A	2.68 (t, 8)	2.66 (t, 7)	2.66 (t, 7)	2.98 (t, 7)	2.97 (t, 7)	2.97 (t, 7)
8_A	3.80 (m); 3.58 (m)	3.79 (m); 3.55 (m)	3.78 (m); 3.55 (m)	4.03 (dt, 12, 7); 3.76 (dt, 12, 7)	4.03 (dt, 12, 7); 3.76 (dt, 12, 7)	4.03 (dt, 12, 7); 3.77 (dt, 12, 7)
glucose						
1′	4.54 (d, 8)	4.39 (d, 8)	4.39 (d, 8)	4.38 (d, 8)	4.37 (d, 8)	4.37 (d, 8)
2′	3.42 (dd, 9, 8)	3.27 (dd, 9, 8)	3.32 (dd, 9, 8)	3.82 (dd, 8, 10)	3.80 (dd, 8, 9)	3.81 (dd, 8, 10)
3′	3.88 (dd, 10, 9)	3.52 (t, 9)	3.51 (t, 9)	3.97 (t, 10)	3.89 (dd, 10, 9)	3.89 (t, 10)
4′	4.85 (t, 10)	3.31 (t, 9)	3.29 (t, 9)	5.13 (t, 10)	5.03 (t, 10)	5.03 (t, 10)
5′	3.78 (m)	3.49 (m)	3.48 (m)	3.58 (m)	3.55 (ddd, 10, 6, 2)	3.56 (ddd, 10, 6, 2)
6′	4.01 (dd, 12, 5), 3.92 (dd, 12, 3)	4.37 (br d, 12), 4.18 (dd, 12, 5)	4.35 (br d, 12), 4.21 (dd, 12, 6)	4.13 (m)	4.27 (dd, 12, 2), 4.21 (dd, 12, 6)	4.28 (dd, 12, 2), 4.21 (dd, 12, 6)
rhamnose						
1″	4.90 (br s)	4.86 (br s)	4.86 (br s)	5.04 (d, 2)	4.99 (d, 2)	4.99 (d, 2)
2″	3.74 (br d, 3)	3.75 (br d, 3)	3.77 (dr d, 3)	5.19 (dd, 3, 2)	5.18 (dd, 3, 2)	5.18 (dd, 3, 2)
3″	3.29 (dd, 10, 3)	3.49 (dd, 9, 3)	3.47 (dd, 9, 3)	5.07 (dd, 10, 3)	5.12 (dd, 10, 3)	5.12 (dd, 10, 3)
4″	3.12 (t, 10)	3.21 (t, 9)	3.21 (t, 9)	4.98 (t, 10)	4.99 (t, 10)	5.00 (t, 10)
5″	3.34 (dq, 10, 6)	3.89 (dq, 9, 7)	3.88 (dq, 9, 6)	3.87 (dq, 10, 6)	3.90 (dq, 10, 6)	3.90 (dq, 10, 6)
6″	0.97 (d, 6)	1.10 (d, 7)	1.10 (d, 6)	1.12 (d, 6)	1.12 (d, 6)	1.17 (d, 6)
apiose						
1‴	5.06 (d, 2)	5.05 (d, 2)	5.05 (d, 2)	5.27 (br s)	5.32 (br s)	5.32 (br s)
2‴	3.76 (d, 2)	3.75 (d, 2)	3.74 (d, 2)	5.31 (br s)	5.31 (br s)	5.31 (br s)
4‴	3.95, 3.60 (d, 10)	3.92, 3.58 (d, 10)	3.92, 3.58 (d, 10)	4.35, 4.13 (d, 10)	4.34, 4.15 (d, 10)	4.35, 4.15 (d, 10)
5‴	3.39, 3.37 (d, 10)	3.40, 3.37 (d, 10)	3.40, 3.37 (d, 10)	4.69 (s)	4.73, 4.67 (d, 12)	4.74, 4.67 (d, 12)
OAc	1.97 (s)			*a*	*b*	*c*

[a] **11**: 2.32, 2.31, 2.30, 2.86, 2.12, 2.10, 2.08, 2.06, 2.03, 1.94, 1.77 (all s). [b] **12**: 2.32, 2.31, 2.30, 2.86, 2.12, 2.10, 2.08, 2.06, 1.94, 1.77 (all s). [c] **13**: 2.33, 2.27, 2.25, 2.15, 2.10, 2.09, 2.08, 2.04, 2.03, 1.96 (all s).

ous *i*-PrOH or with aqueous EtOH. These extracts exhibited significant inhibition against RSV in the standard antiviral cytopathic effect (CPE) assay.[5,6] The extracts were also active when administered 3 h after infection of the cells with RSV, indicating that they may inhibit the virus by an intracellular mechanism of action. To determine the identity of the compound(s) responsible for the anti-RSV activity of *M. lutea* extracts, bioassay-guided fractionation of these extracts using an RSV CPE assay was conducted. Initially, the extracts were purified using a combination of liquid−liquid extraction, reversed-phase chromatography, centrifugal partition chromatography (CPC), and preparative TLC to isolate the active consitituents **1**−**4**. A method more amenable to larger scale production using reversed-phase chromatography and preparative HPLC was subsequently conducted to give compounds **1**−**4** along with a related minor compound **5**. Compounds **1**−**5** all had potent in vitro activity against RSV. Similar extracts prepared from bark also had anti-RSV activity and were shown to contain compounds **1**−**5**.

Verbascoside (**1**) and isoverbascoside (**2**) were identified from their spectral data, which were identical in all respects to literature values.[10,11] The ^{1}H and ^{13}C NMR assignments for these compounds were determined using 2D NMR experiments (COSY, HMQC, HMBC) and consistent with reported values. As expected, acid hydrolysis of both **1** and **2** gave β-D-glucose and α-L-rhamnose. Acetylation of **1** and **2** gave verbascoside nonaacetate (**9**) and isoverbascoside nonaacetate (**10**), respectively. The spectral data for **9** was also identical to literature values;[12] although the NMR assignments for **10** had not been previously reported, the spectral data for this compound were consistent with the structure.

The spectral data, including UV, IR, MS, and NMR, of luteosides A−C (**3**−**5**) indicated that they were related to **1** and **2**. For example, the UV spectrum of **3** was similar to that of **1**, with λ_{max} at 325, 292, 245, 238, 218, and 203 nm, while the IR spectrum of **3** had absorbances due to hydroxyl groups (ν 3420 cm^{-1}, br), ester (1720, 1690 cm^{-1}), and phenol groups (1636 cm^{-1}). Their molecular formulas, $C_{36}H_{46}O_{20}$ for **3**, $C_{34}H_{44}O_{19}$ for **4**, and $C_{35}H_{46}O_{19}$ for **5**, were determined from their high-resolution negative-ion FAB mass spectra. Comparison of the NMR data, including data from 2D NMR experiments, to similar data for **1** and **2**, identified the presence of a β-D-glucose, an α-L-rhamnose, and a β-(3,4-dihydroxyphenyl)ethoxy group in all three compounds (Tables 1 and 2). In addition, signals due to an acetyl group in **3**, a caffeoyl ester in **3** and **4**, and a feruloyl ester in **5** were observed. All three compounds also contained signals attributed to an β-D-apiose group [e.g., for **3**, δ 5.06 (d, J = 2 Hz, H-1‴), 109.4 (C-1‴); 3.76 (d, J = 2 Hz, H-2‴), 76.7 (C-2‴); 78.9 (C-3‴); 3.95 (d, J = 10 Hz, H-4_a‴), 3.60 (d, J = 10 Hz, H-4_b‴), 73.7 (C-4‴); 3.39 (d, J = 10 Hz, H-5_a‴), 3.37 (d, J = 10 Hz, H-5_b‴), 63.4 (C-5‴)]. Acid hydrolysis of each of the compounds **3**−**5** with 5 N HCl gave three sugars identified as β-D-glucose, α-L-rhamnose, and β-D-apiose. Exact mass

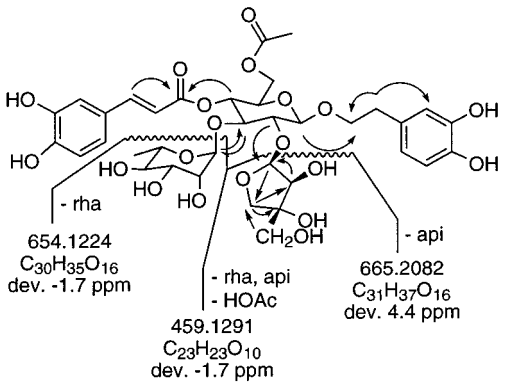
Table 2. ^{13}C NMR Assignments for Phenylpropanoids **3**−**5** (100 MHz, DMSO-d_6 + TFA 0.1%) and Acetates **10**−**13** ($CDCl_3$)

carbon	3	4	5	10	11	12	13
caffeoyl							
1	125.4	125.5	125.5	133.2	132.8	133.1	133.2
2	114.7	115.0	110.9	122.9	122.8	122.8	121.5
3	145.8	145.5	147.9	142.5	142.5	142.4	140.6
4	148.6	145.0	149.3	143.7	143.8	143.6	151.4
5	115.5	115.8	115.4	123.9	124.1	123.9	111.2
6	121.5	121.4	123.4	126.6	126.4	126.6	133.2
7	145.6	148.4	145.0	143.6	144.2	143.5	144.7
8	113.3	113.8	114.3	118.6	118.2	118.6	117.7
CO	165.7	166.5	166.6	166.2	165.0	166.1	166.4
OMe			55.7				56.0
aglycone							
1_A	129.0	129.1	129.0	137.6	136.8	136.8	136.8
2_A	115.8	115.5	115.4	123.1	123.8	123.3	123.3
3_A	145.0	145.3	145.2	140.5	140.6	140.6	141.5
4_A	143.6	143.5	143.5	141.8	141.9	141.9	141.9
5_A	116.2	116.2	116.2	123.8	123.3	123.8	123.8
6_A	119.5	119.5	119.5	127.2	127.0	127.0	127.0
7_A	35.0	35.1	35.1	35.4	35.5	35.5	35.5
8_A	70.7	70.3	70.5	69.8	70.0	69.7	70.0
glucose							
1′	101.2	101.3	101.3	100.7	101.3	101.3	101.4
2′	78.6	77.6	77.6	77.2	76.4	75.5	75.7
3′	78.5	82.1	82.1	81.6	80.4	82.1	82.0
4′	69.3	70.5	68.6	69.8	69.8	70.1	70.7
5′	70.2	73.5	73.4	72.2	72.0	72.1	72.1
6′	62.4	63.3	63.2	62.5	62.6	62.8	62.7
rhamnose							
1″	101.7	101.4	101.4	99.6	98.4	98.8	98.8
2″	70.9	70.6	70.5	69.7	69.5	70.0	69.7
3″	70.5	70.5	70.5	68.8	68.8	68.8	68.8
4″	71.5	71.9	71.9	70.5	70.5	70.7	70.1
5″	68.8	68.7	68.6	67.5	67.3	67.3	67.3
6″	18.1	17.8	17.8	17.3	17.2	17.0	17.1
apiose							
1‴	109.4	109.3	109.2		105.3	105.2	105.2
2‴	76.7	76.6	76.6		75.8	75.9	75.9
3‴	78.9	79.0	79.0		83.9	84.0	84.0
4‴	73.7	73.7	73.7		73.1	73.2	73.2
5‴	63.4	63.7	63.7		63.6	63.7	63.7
$COCH_3$	170.1			*a*	*b*	*c*	*d*
$COCH_3$	20.6			*a*	*b*	*c*	*d*

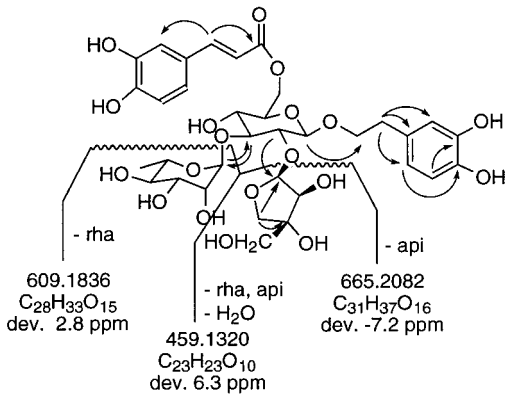
[a] **10**: *C*O, 170.1, 170.0, 169.5, 169.4, 169.4, 168.4, 168.3, 168.0, 167.9; CO*C*H_3, 21.1, 20.9, 20.8, 20.63 (3C), 20.59 (3C). [b] **11**: *C*O, 170.7, 170.6, 170.1, 170.0, 169.8, 169.7, 169.4, 168.3, 168.2, 168.0, 167.9; CO*C*H_3, 21.1, 20.9, 20.79, 20.80, 20.6 (3C), 20.58, 20.52, 20.4. [c] **12**: *C*O, 170.6, 170.0, 169.9, 169.83, 169.77, 169.7, 169.4, 168.32, 168.28, 168.0, 167.9; CO*C*H_3, 21.2, 21.1, 20.9, 20.8 (2C), 20.65 (2C), 20.62 (2C), 20.59 (2C). [d] **13**: *C*O, 170.6, 170.0, 169.9, 169.8, 169.74, 169.70, 169.4, 168.7, 168.3, 168.0; CO*C*H_3, 21.2, 21.1, 20.9, 20.8 (2C), 20.63 (3C), 20.58 (2C).

measurements of fragmentation ions observed in the negative FABMS of **3** and **4** (Figure 1) established that both apiose and rhamnose were terminal units in both **3** and **4** and that the acetate was attached to the glucose in **3** [e.g., for **3**, *m*/*z* 797 ([M − H]$^-$, dev 6.9 ppm), 665 ([M − api − H]$^-$, dev 4.4 ppm), 651 ([M − rha − H]$^-$, dev − 1.7 ppm), and 459 ([M − api − rha − HOAc − H]$^-$, dev − 1.7 ppm)].

The structures of luteosides A−C (**3**−**5**) were determined by careful analysis of 2D NMR data of the natural products and their peracetate derivatives **11**−**13**. For example, in luteoside A (**3**), most of the ^1H NMR signals, including those due to the sugars, were assigned from a COSY spectrum (see Table 1). Heteronuclear $^1H-^{13}C$ correlation experiments (HMQC, HMBC) then allowed assignment of the structure and the remaining NMR signals (Figure 1, Tables 1 and 2). Key HMBC correlations observed for **3** required that the β-(3,4-dihydroxyphenyl)ethoxy group was attached to C-1′ [δ 4.54 (H-1′): 70.7 (C_A-8)], the apiose was attached to C-2′ [δ 3.42 (H-2′): 109.4 (C-1‴)], the rhamnose was attached to C-3′ [δ 3.88 (H-3′): 101.7 (C-1″)], and the caffeoyl group was attached to C-4′ on the glucose [δ 4.85 (H-4′): 165.7 (C-9)]. The chemical shifts at C-6′ (63.9 ppm) and H-6′ [δ 4.01 (dd, J = 12, 5 Hz), 3.92 (dd, J = 12, 3 Hz)] are consistent with acetylation at C-6′ on the glucose. The ^1H NMR chemical shift of the rhamnose secondary methyl group (δ 0.97 ppm) are consistent with attachment of the caffeoyl moeity at C-4′ as in verbascoside (**1**) rather than at C-6′ as in isoverbascoside (**2**).[13] On the basis of these data, the structure of luteoside A was determined to be 1-*O*-(3,4-dihydroxyphenyl)ethyl β-D-apiofuranosyl(1→2)-α-L-rhamnopyranosyl(1→3)-4-*O*-caffeoyl-6-acetyl-β-D-glucopyranoside (**3**).



(**3**)



(**4**)

Figure 1. Structures of luteoside A (**3**) and B (**4**) showing key HMBC correlations ($^1H \rightarrow {}^{13}C$) and HRFABMS fragments.

Analysis of the NMR spectra for luteoside A undecaacetate (**11**) provided further support for the proposed structure. Although some of the sugar protons gave overlapping signals in the ^1H NMR spectrum of **3**, the corresponding signals in **11** were well dispersed and were assigned using 2D NMR, including HMBC and HMQC−TOCSY experiments. Correlations in the HMBC spectrum of **11** from H-1′ to C_A-8, H-2′ to C-1‴, H-3′ to C-1″, H-1″ to C-3′, and H-1‴ to C-2′ confirmed the positions of the sugars. The expected upfield shifts in the ^{13}C NMR spectrum due to the β effect of adjacent acetyl groups and downfield shifts in the ^1H NMR due to the acetyl groups were observed in the NMR spectra of **11**.

The structures of luteoside B and luteoside C were determined to be 1-*O*-(3,4-dihydroxyphenyl)ethyl β-D-apiofuranosyl(1→2)-α-L-rhamnopyranosyl(1→3)-6-*O*-caf-

feoyl-β-D-glucopyranoside (**4**) and 1-*O*-(3,4-dihydroxyphenyl)ethyl β-D-apiofuranosyl(1→2)-α-L-rhamnopyranosyl(1→3)-6-*O*-feruloyl-β-D-glucopyranoside (**5**), respectively, by interpretation of the spectral data for **4**, **5**, and the acetates **12** and **13**, as described in the structure elucidation of **3**. Compounds **4** and **5** are differentiated from **3** in that they are analogues of isoverbascoside (**2**) rather than verbascoside (**1**). The HMBC spectrum of **4** included correlations from H-1′ to C_A-8, H-2′ to C1‴, H-3′ to C-1″, H-1″ to C-3′, and H1‴ to C-2′. All of these HMBC correlations were also observed for **5**, with the exception of the correlation from H-3′ to C-1″, and with an additional correlation from the methoxy signal to C-3. The HMBC correlations observed for **4** (Figure 1) and **5** are consistent with the proposed structures. Key differences in the NMR spectra are signals due to C-4′ and C-6′ on the glucose and C-6″ on rhamnose, with additional signals in **5** due to the methoxy group. The HMQC−TOCSY and HMBC experiments allowed unambiguous assignment of all of the signals in compounds **11**−**13** and provided further support for the proposed structures. The ^{1}H and ^{13}C NMR assignments of **3**−**5** and the acetates **11**−**13** are summarized in Tables 1 and 2, with key HMBC correlations for **3** and **4** shown in Figure 1.

Comparison of the ^{13}C NMR assignments for luteosides A−C (**3**−**5**) with the related compounds **6**−**8**,[7−9] **14**, and **15**[14] revealed that the major differences, due to the attached subunits, are in the signals assigned to C-2′, C-3′, and C-6′ of the glucose. The signals assigned to the apiose group are similar to the apiose signals in **6**−**8**, **14**, and **15**. Compound **4** is isomeric with pedicularioside A (**6**), myricoside (**7**), and forsythioside A (**8**), with the differences being the positions of the rhamnose, apiose, and caffeoyl groups on the central glucose.

The in vitro antiviral activities of the five isolated compounds are summarized in Table 3. Compounds **1**−**4** all have similar or better antiviral activity (EC_{50}) against RSV in the in vitro assay than ribavirin, an approved drug for the treatment of RSV infections in humans; these compounds also have better selectivity against RSV than ribavirin, as demonstrated by the large selective index, or the ratio of cytotoxicity (IC_{50}) to antiviral activity (SI = IC_{50}/EC_{50}). The compounds were active when adminstered 3 h after infection of the cells in a neutral red anti-RSV assay, whereas SP-303, a mixture of proanthocyanidin oligomers known to inhibit RSV via an extracellular mechanism,[5] had no activity against RSV when administered 3 h postinfection (see Table 4). Hence, it is likely that the phenylpropanoid glycosides, like ribavirin, inhibit RSV through an intracellular antiviral mechanism of action. Compound **5** is considerably less active than **1**−**4**; this drop in activity could be due to methylation of the caffeoyl ester. It is noteworthy that there was a considerable drop in the anti-RSV activity of two of the extracts when they were administered postinfection (Table 4). This is probably due to the presence of compounds in the extracts that are active via an extracellular mechanism of action and have no activity in the post infection anti-RSV assay. Other compounds containing caffeoyl ester groups, including caffeic acid, have been reported to have antiviral activity against herpesviruses.[15] However, none of the phenylpropanoid glycosides tested had any activity against the herpesviruses HSV, CMV, or VZV. We prepared some derivatives of verbascoside and isoverbascoside for preliminary SAR studies. The nonaacetates **9** and **10** were hydrolyzed with triethylamine to give the pentaacetates **16** and **17**, respectively. Both **16** and **17** had moderate activity against RSV but were considerably less active than the natural products. These results suggest that the inhibition of RSV by phenylpropanoid glycosides requires two catechol groups and a central sugar unit and that the mechanism of anti-RSV inhibition may be different than the antiherpetic activity reported for other caffeoyl esters. Further details on the anti-RSV activity of related compounds containing a caffeoyl ester group will be published elsewhere.

Table 3. In Vitro Antiviral Activity (EC_{50}) and Cytotoxicity (IC_{50}) of Phenylpropanoids and Various Control Compounds

compd	virus	assay	EC_{50}[a]	IC_{50}[b]	SI[c]	*n*
verbascoside (**1**)	RSV[d]	CPE[e]	0.80	76.9	85	2
1	RSV	plaque[f]	9.7	n.t.		1
1	mCMV[g]	CPE	none	44		1
1	HSV-2[h]	CPE	none	71		1
isoverbascoside (**2**)	RSV	CPE	0.62	51.4	84	1
2	RSV	plaque	1.1	n.t.		1
2	VZV[i]	plaque	none	75		1
2	mCMV	CPE	none	89		1
2	HSV-1[j]	CPE	none	30		1
2	HSV-2	CPE	none	27		1
3	RSV	CPE	0.87	77	89	1
4	RSV	CPE	3.4	>67	>19	1
4	HSV-1	CPE	none	93		1
4	HSV-2	CPE	none	90		1
5	RSV	CPE	15.5	189	12	1
9	RSV	CPE	none	33.7		1
10	RSV	CPE	3.40	11.2	3.3	1
16	RSV	CPE	5.87	26.2	4.4	1
17	RSV	CPE	1.93	15.9	8.2	1
ribavirin	RSV	CPE	1.8 ± 0.2	35 ± 4.6	19	23
ribavirin	RSV	plaque	2.2 ± 0.2	25 ± 1.7	11	21
gancyclovir	mCMV	CPE	5.0 ± 0.4	>100	>20	20
acyclovir	HSV-1	CPE	0.26 ± 0.01	>67	>250	2
acyclovir	HSV-2	CPE	2.3 ± 0.3	>10	>4.3	14
gancyclovir	VZV	plaque	26.7	840	74	12

[a] Antiviral activity, 50% effective concentration (μg/mL). [b] Cytotoxicity, 50% inhibitory concentration (μg/mL). [c] Selective index = IC_{50}/EC_{50}. [d] Respiratory syncytial virus, Long (CPE) or A-2 (plaque) strain in Hep-2 cells. [e] Cytopathic effect. [f] Plaque-neutralization assay. [g] Murine cytomegalovirus, RM-461 strain. [h] Herpes simplex virus type 2. [i] Varicella zoster virus, 3CV-1 strain. [j] Herpes simplex virus type 1.

Table 4. Effect of Time of Administration on Antiviral Activity of Compounds and Extracts

extract or compd	time of administration: before infection (t = 0) EC_{50}[a]	IC_{50}[b]	SI[c]	after infection (t = 3 h) EC_{50}[a]	IC_{50}[b]	SI[c]
A ro E2[d]	0.3	42.8	143	1.7	140	82
A ro E3[e]	8.2	385	46.9	55	>830	>15
B ro E4[f]	2.1	130	62	3.0	130	43
1	0.80	76.9	85	0.26	108	415
2	0.62	51.4	84	0.17	125	735
3	0.87	77	89	11	180	16
4	3.4	>67	>19	0.23	180	780
5	15.5	189	12	12	>330	>28
SP-303	2.9	28	9	none	26	
ribavirin	1.8 ± 0.2	35 ± 4.6	19	2.3 ± 0.2	25 ± 1.8	10.8

[a] Antiviral activity, 50% effective concentration (μg/mL). [b] Cytotoxicity, 50% inhibitory concentration (μg/mL). [c] Selective index = IC_{50}/EC_{50}. [d] First collection, roots, *i*-PrOH−CH_2Cl_2 extract. [e] First collection, roots, *i*-PrOH−H_2O extract. [f] Second collection, roots, EtOH extract.

We have demonstrated that *M. lutea*, a medicinal plant used for symptoms attributed to viral respiratory infections, contains five phenylpropanoid glycosides with antiviral activity against respiratory syncytial virus. The active constituents of *M. lutea* include the commonly occurring compounds[16] verbascoside and isoverbascoside and the new compounds **3**−**5**. It is likely that most of the phenylpropanoid glycosides, a commonly occurring group of plant-derived natural products,[16] have activity against RSV. The phenylpropanoid glycoside echinaside, from *Echinacea pallida*, was reported to have in vitro activity against vesicular stomatitis virus (VSV);[17] it is possible that other phenylpropanoid glycosides are also active against VSV. We report for the first time the isolation of phenylpropanoid glycosides from the genus *Markhamia*.

Experimental Section

General Experimental Procedures. The general experimental procedures have been described previously.[4] NMR data for compounds **16** and **17** were collected using a Varian nanoprobe. ^{13}C multiplicities for all compounds were determined from DEPT spectra. TLC was conducted using EM Separations Si60 and RP-18WFs (0.2 mm, 10 × 10 cm; preparative TLC on 1.0 mm (RP-18WFs) or 2.0 mm (Si60) plates, 20 × 20 mm) HPTLC plates, visualized using UV and/or 5% vanillin/EtOH and 5% H_2SO_4/EtOH with heating (100 °C, 5−10 min). Column chromatography was carried out using HP-20 polystyrene divinylbenzene gel obtained from Mitsubishi Kasei Corp. or Bakerbond C18 obtained from Fisher Scientific. CPC was carried out using a Sanki Series 1000 instrument equipped with a Linear UVIS detector. HPLC was conducted on a Rainin Dynamax system.

Plant Material. The roots of *M. lutea* were collected on Jan 1, 1993, in Misima District, Tanzania, by Dr. Charles Limbach, and on Sep 4, 1993, in Muheza District, Tanzania, by Salehe Waziri. Plant specimens were identified by Charles Mabula of Tanzania and B. Verdcourt, A. Gereau, R. Liesner, and Mary Merello of the Missouri Botanical Garden. Voucher specimens (voucher nos. CL 257, ROA 14, and ROA 33) are deposited in the reference collection, Ethnobotany and Conservation Department, Shaman Pharmaceuticals.

Antiviral and Cytotoxicity Assays. The antiviral activities of compounds **1**−**5** as well as control antiviral compounds were determined using viral cytopathic effect (CPE) assay and the plaque-neutralization (plaque) assay. The procedures used for the antiviral and cytotoxicity assays have been previously described.[5,6] The RSV "post infection" CPE assay was conducted with a neutral red endpoint as follows. The day before the assay was performed, cells were seeded into 96-well plates (10 000 cell in 50 μL/well) in high-glucose containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% or 10% heat-inactivated fetal bovine serum (FBS), L-glutamine, and antibiotics. On the day of the assay, samples were dissolved in an appropriate solvent (10−50 mg/mL), and serial 3-fold dilutions (in 200 μL volumes) were made in DMEM-FBS for a final range of eight concentrations (830−0.38 or 130−0.13 μg/mL). Virus (3000 plaque-forming units in 50 μL of DMEM-FBS with 20 mM HEPES buffer) or medium alone was added to each well and incubated at 37 °C for 3 h to allow virus absorption and penetration. After incubation, diluted sample (50 μL) was added to each well, and plates were incubated at 37 °C for 3 days until complete destruction of the cell monolayer occurred in virus control wells. The plates were then stained with neutral red and the antiviral and cytotoxic activity measured.[18] The antiviral activity of each sample is expressed in μg/mL as 50% effective concentration (EC_{50}), and cytotoxicity is expressed in μg/mL as 50% inhibitory concentration (IC_{50}).

Extraction and Isolation. The roots of the first collection (Jan 1993, 200 g) were air-dried, ground to a powder, and then extracted with *i*-PrOH−CH_2Cl_2 (1:1) for ∼24 h. The resulting extract was evaporated (4.67 g), suspended in water (40 mL), and extracted successively with CH_2Cl_2 (3 × 35 mL) and EtOAc (4 × 35 mL). The two organic-soluble fractions were evaporated to dryness. The aqueous fraction was evaporated to remove residual solvent and purified on an HP-20 column (125 g, 3.5 × 45 cm), eluting with increasing amounts of MeOH in H_2O. The resulting fractions were evaporated to dryness. Samples of all fractions were then submitted for testing in the RSV CPE assay and analyzed by TLC (40% aqueous MeOH, C18).

Fractions eluting from the HP-20 column with 40% aqueous MeOH (318 mg) and 60% aqueous MeOH (208.3 mg) had anti-RSV activity. The 40% aqueous MeOH fraction was purified by column chromatography on C18 (Bakerbond, 60 g, 2 × 45 cm) eluting with 40% aqueous MeOH. Fractions were monitored by TLC (C18, 40% MeOH; SiO_2, CH_2Cl_2−MeOH−H_2O 43:37:20, lower phase) and with the RSV CPE assay. Fractions containing **1** had anti-RSV activity and were combined, evaporated (48.3 mg), and purified by preparative TLC on silica (CH_2Cl_2−MeOH−H_2O 40:40:20, lower phase) to give **1** (12.2 mg, 0.006%). The 60% aqueous MeOH fraction from the HP-20 column was purified by CPC (1200 rpm, 2 mL/min), eluting with the lower phase of CH_2Cl_2−MeOH−H_2O (40:40:20); the eluent was monitored by TLC (C18, 40% aqueous MeOH). Fractions containing **2** and **4** had anti-RSV activity and were combined, evaporated (24.2 mg), and purified by preparative TLC on C18 (40% aqueous MeOH) to give **2** (8.0 mg, 0.004%) and **4** (3.5 mg, 0.002%). Fractions containing **3** had anti-RSV activity and were combined, evaporated (14.9 mg), and purified by preparative TLC on C18 (40% aqueous MeOH) to give **3** (2.0 mg, 0.001%).

The marc was extracted with *i*-PrOH−H_2O (1:1) for ∼24 h to give an extract (5.08 g) that had anti-RSV activity. Purification and analysis of the fractions by TLC and in the RSV CPE assay established that the active constituents in the *i*-PrOH−H_2O extract were compounds **1**−**4**.

A larger collection (Sep 1993, 15 kg) was ground to a powder, extracted with 80% aqueous EtOH (60 L) for 24 h, and evaporated to give an EtOH extract (742.4 g). This was partitioned between CH_2Cl_2 and H_2O to give a dark brown CH_2Cl_2-soluble portion (35.1 g), an insoluble residue (26.3 g), and an aqueous layer. The aqueous layer was purified on an HP-20 column (18 × 70 cm), eluting with increasing amounts of MeOH in H_2O (0−100%); the resulting fractions were evaporated to dryness and samples of all fractions analyzed by TLC (C18, 40% aqueous MeOH). The fraction eluting with

40% aqueous MeOH (52.5 g) was purified on a C18 column (9 × 50 cm) eluting with 40% aqueous MeOH followed by 75% aqueous MeOH (15 mL/min). Two enriched fractions containing **1** and **2** (18 g) and **4** (14 g) eluted with 40% aqueous MeOH and were purified on a second C18 column (9 × 50 cm, 20−100% aqueous MeOH) followed by preparative HPLC on C18 (Prime-sphere, 50 × 250 mm, 20% aqueous MeCN, 45 mL/min) to give **1** (1.7 g, 0.011%), **2** (1.3 g, 0.0087%), and **4** (1.1 g, 0.0073%). A fraction containing **3** and **5** (1.64 g) eluted from the first C18 column with 75% aqueous MeOH; a portion (0.70 g) of this was purified by preparative HPLC on C18 (Kromasil, 20 × 250 mm, 25% aqueous MeCN, 15 mL/min) to give **3** (351 mg, 0.0056%) and **5** (76.5 mg, 0.0012%).

Verbascoside (1): yellowish powder; $[\alpha]_D$ −74.0° (MeOH, *c* 0.424). All spectral data were identical to those in the literature.[10,11]

Isoverbascoside (2): off-white powder; $[\alpha]_D$ −47.2° (MeOH, *c* 0.212). All spectral data were identical to those in the literature.[11]

Luteoside A (3): off-white powder; $[\alpha]_D$ −81.0° (MeOH, *c* 0.648); λ_{max} (log ϵ, MeOH) 203 (4.19), 218 (3.50), 238 (sh, 3.47), 245 (3.53), 292 (3.89), 325 (4.08) nm; IR (KBr) ν_{max} 3420 (br, OH), 2937, 1720, 1690, 1636, 1605, 1522, 1448, 1370, 1280, 1157, 1040, 812 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; negative HRFABMS *m/z* 797.2559 [M − H]$^-$, 665.2111 [M − api]$^-$, 651.1924 [M − rha]$^-$, 459.1283 [M − rha − api − HOAc]$^-$, calcd for $C_{36}H_{45}O_{20}$ 797.2504, for $C_{31}H_{37}O_{16}$ 665.2082, for $C_{30}H_{35}O_{16}$ 651.1925, for $C_{23}H_{23}O_{10}$ 459.1291.

Luteoside B (4): off-white powder; $[\alpha]_D$ −51.5° (MeOH, *c* 0.824); λ_{max} (MeOH) 204 (4.01), 212 (sh, 3.80), 237 (3.58), 291 (3.83), 331 (3.98) nm; IR (KBr) ν_{max} 3420 (br, OH), 2930, 2870, 1700, 1686, 1609, 1522, 1458, 1364, 1284, 1040, 812 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; negative HRFABMS *m/z* 755.2477 [M − H]$^-$, 623.1931 [M − api]$^-$, 609.1836 [M − rha]$^-$, 459.1320 [M − rha − api − H_2O]$^-$, calcd for $C_{34}H_{43}O_{19}$ 755.2399, for $C_{29}H_{35}O_{15}$ 623.1976, for $C_{28}H_{33}O_{15}$ 609.1819, for $C_{23}H_{23}O_{10}$ 459.1291.

Luteoside C (5): off-white powder; $[\alpha]_D$ −42.8° (MeOH, c 0.236); λ_{max} (log ϵ, MeOH) 204 (3.90), 213 (sh, 3.62), 236 (3.55), 289 (3.80), 327 (3.94) nm; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; negative HRFABMS *m/z* 770.2633 [M − H]$^-$; calcd for $C_{35}H_{45}O_{19}$ 769.2555.

Acid Hydrolysis of 3. Luteoside A (1.3 mg) was dissolved in 5 M HCl and heated for 2.5 h at 90 °C. The reaction mixture was cooled to room temperature and then extracted with EtOAc (2 × 200 mL). The aqueous phase was neutralized with 1 M Na_2CO_3, freeze-dried, and extracted with pyridine (100 mL). The pyridine extract was then analyzed by TLC on silica (EtOAc−MeOH−H_2O−HOAc 13:3:3:4; vis. with 1% *p*-anisaldehyde, 2% H_2SO_4 in HOAc, 100 °C). Analysis indicated the presence of β-D-glucose (R_f = 0.65), α-L-rhamnose (R_f = 0.77), and β-D-apiose (R_f = 0.73).

Acid Hydrolysis of 4. Hydrolysis and TLC analysis of luteoside B (**4**) (0.8 mg) using the method described for **3** resulted in the detection of β-D-glucose, α-L-rhamnose, and β-D-apiose in the sample.

Acid Hydrolysis of 5. Hydrolysis and TLC analysis of the luteoside C (**5**) (0.5 mg) using the method described for **3** resulted in the detection of β-D-glucose, α-L-rhamnose, and β-D-apiose in the sample. The presence of ferulic acid was detected by TLC analysis of the EtOAc phase.

Verbascoside Nonaacetate (9). Acetylation of **1** (25.0 mg) [Ac_2O/pyridine (1:1), room temperature, 24 h] and purification on silica gel (CH_2Cl_2−EtOAc) gave verbascoside nonaacetate **9** as a yellow solid (28.0 mg, 70%). All spectral data were identical to those in the literature.[12]

Isoverbascoside Nonaacetate (10). Isoverbascoside (25.5 mg) was acetylated following the procedure used to prepare **9** to give isoverbascoside nonaacetate **10** as an amorphous white powder (27.3 mg, 67%): ^{1}H NMR (DMSO-d_6 + 0.1% TFA) δ 7.65 (d, H-7, J = 16 Hz), 7.42 (dd, H-6, J = 8, 2 Hz), 7.39 (d, H-2, J = 2 Hz), 7.23 (d, H-5, J = 8 Hz), 7.06 (dd, H-6$_A$, J = 8, 2 Hz), 7.05 (d, H-5$_A$, J = 8 Hz), 7.03 (d, H-2$_A$, J = 2 Hz), 6.34 (d, H-8, J = 16 Hz), 5.11 (dd, H-3″, J = 10, 3 Hz), 5.09 (dd, H-2″, J = 3, 2 Hz), 5.08 (dd, H-2′, J = 10, 8 Hz), 5.06 (t, H-4′, J = 10 Hz), 4.80 (d, H-1″, J = 2 Hz), 4.32 (dd, H-6′, J = 12, 3 Hz), 4.25 (dd, H-6′, J = 12, 6 Hz), 4.12 (m, H$_A$-8$_a$), 3.88 (dq, H-5′, J = 10, 6 Hz), 3.79 (t, H-3′, J = 10 Hz), 3.65 (m, H$_A$-8$_b$), 3.62 (m, H-5′), 2.88 (m, H$_A$-7), 2.32 (s, 3 H), 2.31 (s, 3 H), 2.28 (s, 3 H), 2.27 (s, 3 H), 2.14 (s, 3 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 1.96 (s, 3 H), 1.95 (s, 3 H), 1.15 (d, H-6″, J = 6 Hz); ^{13}C NMR see Table 2; negative FABMS *m/z* 1001.9 [M − H]$^-$.

Luteoside A Undecaacetate (11). Acetylation of **3** (35.5 mg) [Ac_2O/pyridine (1:1), room temperature, 24 h] and purification by HPLC on diol (CH_2Cl_2−MeOH) gave the undecaacetate **11** (41.2 mg, 76%) as an amorphous white powder: ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; positive FABMS *m/z* 1241 [M + Na]$^+$, 1219 [M + H]$^+$, 1178, 940.

Luteoside B Undecaacetate (12). Luteoside B (32.6 mg) was acetylated following the procedure used to prepare **11** to obtain the undecaacetate **12** (46.4 mg, 90%) as an amorphous powder: ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; positive FABMS *m/z* 1219 [M + H]$^+$, 1178, 940.

Luteoside C Decaacetate (13). Luteoside C (10.1 mg) was acetylated following the procedure used to prepare **11** to obtain the decaacetate **13** (11.0 mg, 69%) as an amorphous powder: ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; positive FABMS *m/z* 1191 [M + H]$^+$, 1149, 982, 954, 910, 766.

Hydrolysis of 9. Compound **9** (10.0 mg) was dissolved in anhydrous MeOH (1 mL); Et_3N (20 μL) was added and the mixture stirred at room temperature under argon for 90 min. The sample was neutralized by adding formic acid (20 μL) and evaporated in vacuo and the residue purified on a cyano SPE column (0−10% MeOH in CH_2Cl_2) and evaporated under vacuum to give the pentaacetate **16** (6.0 mg, 72%) as an amorphous white powder: ^{1}H NMR ($CDCl_3$) δ 7.60 (d, H-7, J = 16 Hz), 7.12 (br s), 6.95 (br d, J = 8 Hz), 6.90 (d, J = 8 Hz), 6.81 (br s), 6.79 (d, J = 8 Hz), 6.59 (dd, J = 8, 2 Hz), 6.21 (d, H-8, J = 16 Hz), 5.27 (dd, H-2′, J = 10, 8 Hz), 5.13 (dd, H-3″, J = 10, 3 Hz), 5.09 (dd, H-4″, J = 10, 9 Hz), 5.04 (dd, H-2″, J = 3, 2 Hz), 4.96 (t, H-4′, J = 10 Hz), 4.88 (d, H-1″, J = 2 Hz), 4.39 (d, H-1′, J = 8 Hz), 4.19 (m, H-6′), 4.10 (m, H$_A$-8$_a$), 3.91 (t, H-3′, J = 10 Hz), 3.82 (dq, H-5″, J = 9, 7 Hz), 3.68 (m, H-5′), 3.57 (m, H$_A$-8$_b$), 2.75 (m, H$_A$-7), 2.10 (s, 3 H), 2.08 (s, 3 H),

2.04 (s, 3 H), 1.99 (s, 3 H), 1.92 (s, 3 H), 1.04 (d, H-6″, $J = 7$ Hz); ^{13}C NMR ($CDCl_3$) δ 170.1 (s), 169.9 (s), 165.9 (s), 147.5 (s), 146.9 (s), 144.6 (s), 130.8 (s), 126.5 (s), 122.3 (d), 120.7 (d), 116.5 (d), 115.6 (d), 115.1 (d), 114.4 (d), 113.4 (d), 100.6 (d), 98.5 (d), 79.5 (d), 72.8 (d), 71.8 (d), 70.7 (d), 70.5 (t), 70.0 (d), 68.9 (d), 68.6 (d), 67.1 (d), 62.4 (d), 35.1 (t), 20.8 (q), 20.6 (q), 20.5 (q), 17.4 (q); negative FABMS *m/z* 833.227 $[M - H]^-$, 727, 582, 461, 389, 334.

Hydrolysis of 10. Compound **10** (10.0 mg) was hydrolyzed following the procedure used to prepare **16** to give the pentaacetate **17** (8.0 mg, 96%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.53 (d, H-7, $J = 16$ Hz), 7.10 (br s), 6.84 (br s), 6.74 (br s), 6.51 (br d, $J = 8$ Hz), 6.19 (d, H-8, $J = 16$ Hz), 4.96−5.10 (m, 5 H), 4.79 (d, H-1″, $J = 2$ Hz), 4.37 (d, H-1′, $J = 8$ Hz), 4.28 (dd, H-6′$_a$, $J = 12$, 2 Hz), 4.18 (dd, H-6′$_b$, $J = 12$, 5 Hz), 3.92 (m, H-5″), 3.79 (m, H_A-8), 3.77 (t, H-3′, $J = 10$ Hz), 3.61 (m, H-5′), 2.71 (m, H_A-7), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.04 (s, 3 H), 1.99 (s, 3 H), 1.92 (s, 3 H), 1.11 (d, H-6″, $J = 7$ Hz); ^{13}C NMR ($CDCl_3$) δ 170.1 (CO), 170.0 (CO), 169.9 (CO), 169.7 (CO), 169.6 (CO), 167.0 (s), 147.7 (s), 146.0 (d), 144.9 (s), 142.8 (s), 130.2 (s), 126.4 (s), 122.5 (s), 120.6 (s), 116.3 (d), 115.5 (d, 2C), 114.1 (d), 113.8 (d), 100.7 (d), 99.2 (d), 81.0 (d), 71.9 (d), 71.0 (t), 70.4 (d), 69.6 (d), 68.7 (d), 67.3 (d), 62.2 (d), 58.6 (d), 35.2 (d), 21.0 (q), 20.8 (q), 20.7 (q), 20.6 (q), 17.1 (q); negative FABMS *m/z* 833 $[M - H]^-$, 727, 670, 582, 492, 461, 429, 390, 337, 334.

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