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PHENYLPROPANOID GLUCOSIDES FROM *AEGIPHILA OBDUCTA*

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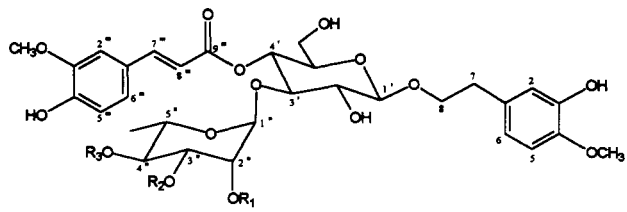
ABSTRACT.—Two new phenylpropanoid glucosides, acetylmartynosides A and B, were isolated from the wood of *Aegiphila obducta* and identified by spectroscopic methods, along with five known phenylpropanoid glucosides.

The genus *Aegiphila* (Verbenaceae) is well represented in the tropical and subtropical regions of Central and South America. In Brazil, plants of this genus are used in folk medicine as a snake bite remedy (1). This investigation on an MeOH extract from the wood of *Aegiphila obducta* Velloso is part of a comparative study on the chemical composition of the various organs of the plant (2). We describe herein the isolation and characterization of two new compounds, acetylmartynosides A [**1**] and B [**2**] as well as the isolation of six known compounds, 4″-*O*-acetylmartynoside, [**3**], 3″-*O*-acetylmartynoside, [**4**], 2″-*O*-acetylmartynoside, [**5**], apigenin, martynoside, and verbascoside.

Compounds **3**–**5**, apigenin, martynoside, and verbascoside were identified by direct comparison with authentic samples using tlc and by comparison of their ^{1}H- and ^{13}C-nmr data with those reported in the literature (3–6). Compounds **1** and **2** were obtained as amorphous powders, and both exhibited a mol wt of 736 (positive fabms *m/z* 759, $[M+Na]^+$), consistent with the molecular formula $C_{35}H_{44}O_{17}$.

Comparison of the ^{1}H- and ^{13}C-nmr data of **1** and **2** with those of martynoside, (5), showed similar structures with characteristic signals for ferulic acid and 3-hydroxy-4-methoxyphenylethanol moieties, and two sugar units (glucose and rhamnose). Additionally, **1** and **2** exhibited signals for two acetyl groups (^{1}H nmr δ 1.90 and 2.03, s, **1**; δ 2.04 and 2.10, s, **2**; ^{13}C nmr δ 20.67, 20.78, 170.22, and 170.42, **1**; δ 20.79, 20.87, 170.22, and 170.60, **2**). A fragment peak in the fabms of **1** and **2** at *m/z* 506 $[M-rha-2Ac]^+$ indicated that both acetyl groups are attached to the rhamnose moiety in the two molecules.

The ^{1}H-nmr spectrum of **1** showed signals for two protons attached to carbons bearing the acetyl groups in the



	R_1	R_2	R_3
1	Ac	Ac	H
2	H	Ac	Ac
3	H	H	Ac
4	H	Ac	H
5	Ac	H	H

rhamnose moiety. The proton at δ 5.32 (H-2″), a doublet of doublets, was coupled to H-1″ (J=1.8 Hz, α-configuration of rhamnose) and to H-3″ (J=3.4 Hz), with a magnitude representing an axial-equatorial coupling which was also observed in the signal at δ 4.92 (H-3″). The latter, also a doublet of doublets, had a J value (9.8 Hz) indicative of an axial-axial relationship with H-4″ (Table 1). These findings were confirmed by the homonuclear ^{1}H-^{1}H correlation (COSY) nmr spectrum of **1**. The starting point was the anomeric proton of rhamnose (δ 5.25) which showed a correlation peak only with the signal at δ 5.32 that, in turn, was coupled to the doublet of doublets at δ 4.92, confirming the acetylation positions at C-2″ and C-3″. The ^{13}C-nmr signals of **1** were assigned by comparison with the analogous data of martynoside, on the basis of chemical shift considerations, analyzing the β-effects due to acetylation (3,7,8). As shown in Table 2, the carbon signals due to the feruloyl, 3-hydroxy-4-methoxyphenylethyl, and

TABLE 1. ^{1}H-Nmr Spectral Data (300 MHz, Me_2CO-d_6, TMS) of Acetylmartynosides (**1–5**) and of Martynoside. J (Hz) Values in Parentheses.

Proton	1	2	3	4	5	Martynoside
2	6.77 d (2.0)	6.77 s	6.77 d (2.0)	6.77 s	6.77 s	6.78 d (2.1)
5	6.84 d (8.2)	6.84 d (8.5)	6.84 d (8.4)	6.84 d (8.2)	6.84 d (8.2)	6.82 d (8.2)
6	6.69 dd (2.0/8.2)	6.69 d (8.5)	6.69 dd (2.0/8.4)	6.69 d (8.2)	6.69 d (8.2)	6.69 dd (2.1/8.2)
7	2.80 t (7.3)	2.80 t (7.3)	2.80 t (7.3)	2.80 t (7.1)	2.80 t (7.1)	2.80 t (7.3)
2‴	7.34 d (1.8)	7.38 d (2.0)	7.37 d (1.7)	7.34 d (1.7)	7. 34 d	7.35 d (2.1)
5‴	6.88 d (8.1)	6.89 d (8.0)	6.89 d (8.2)	6.88 d (8.2)	6.88 d (8.2)	6.87 d (8.2)
6‴	7.15 dd (1.8/8.1)	7.19 dd (2.1/8.0)	7.19 dd (2.0/8.2)	7.15 d (8.0)	7.15 d (8.0)	7.15 dd (2.1/8.2)
7‴	7.66 d (15.8)	7.68 d (16.0)	7.67 d (16.2)	7.66 d (16.0)	7.66 d (16.0)	7.65 d (15.9)
8‴	6.42 d (15.8)	6.45 d (16.0)	6.44 d (16.2)	6.43[a] (16.0)	6.42[a] (16.0)	6.41 d (15.9)
1	4.44 d (7.8)	4.44 d (7.8)	4.45 d (7.8)	4.45 d[a] (7.9)	4.44 d[a] (7.9)	4.45 d (7.8)
4′	4.96 t (9.8)	4.99 t (9.9)	4.96 t (9.3)	4.93 t[a] (9.8)	4.92 t[a] (9.8)	4.90 t (9.5)
1″	5.25 d (1.8)	5.39 d (1.9)	5.43 d (1.5)	5.27 s	5.27 s	5.29 d (1.5)
2″	5.32 dd (1.8/3.4)	5.16 dd (1.9/3.4)	[b] (1.8/3.4)	5.16 dd	[b]	[b]
3″	4.92 dd (3.4/9.8)	[b]	[b]	[b] (3.0/9.8)	4.84 dd	[b]
4″	[b]	4.77 t (9.8)	4.82 t (9.8)	[b]	[b]	[b]
6″	1.18 d (6.7)	1.04 d (5.8)	1.00 d (6.5)	1.12 d[a] (6.5)	1.18 d[a] (6.5)	1.12 d (6.0)
$COCH_3$	1.90 s	2.04 s	1.66 s	1.98 s[a]	2.01 s[a]	—
	2.03 s	2.09 s	—	—	—	—
OCH_3	3.80	3.80	3.80	3.80	3.80	
	3.92	3.92	3.92	3.92	3.92	

[a]Interchangeable.
[b]Overlapped signal.

TABLE 2. ^{13}C-Nmr Spectral Data (75 MHz, Me_2CO-d_6, TMS) of Acetylmartynosides (**1–5**) and of Martynoside.

Carbon	1	2	3	4	5	Martynoside
1	132.53	132.51	132.45	132.56	132.56	132.52
2	116.64	116.63	116.63	116.66	116.66	116.66
3	146.88	146.83	146.77	146.81	146.81	146.81
4	146.77	146.78	146.77	146.78	146.78	146.73
5	112.39	112.38	112.38	112.41	112.41	112.42
6	120.66	120.65	120.63	120.66	120.66	120.64
7	36.15	36.12	36.10	36.17	36.17	36.15
8	72.74	72.64	71.87	71.41	71.41	72.11
1‴	127.28	127.20	127.20	127.35	127.35	127.34
2‴	111.29	111.29	111.29	111.26	111.26	111.25
3‴	148.67	148.76	148.74	148.70	148.70	148.70
4‴	150.18	150.32	150.27	150.19	150.19	150.18
5‴	116.03	116.10	116.11	116.03	116.03	116.03
6‴	124.12	124.10	124.08	124.16	124.16	124.16
7‴	147.18	147.19	147.17	147.24	147.24	147.23
8‴	115.03	114.95	115.00	115.18	115.18	115.25
9‴	166.96	166.84	166.86	167.07[b]	167.02[b]	167.01
1′	103.63	103.61	103.61	103.72	103.72	103.72
2′	75.81	75.63	75.68	75.62[b]	75.84[b]	75.88
3′	80.30	78.41	78.60	80.29[b]	79.24[b]	79.55
4′	70.01	69.80	69.88[a]	70.15	70.15	70.18
5′	75.88	76.05	76.19	75.95	75.95	76.15
6′	62.26	62.17	62.20	62.33[b]	62.28[b]	62.29
1″	99.48	98.34	100.97	99.20	102.22	101.80
2″	70.67	73.04	71.38	73.18	69.60	71.91
3″	70.59	67.22[a]	69.66[a]	70.30[b]	73.18	71.37
4″	71.37	74.75	74.92	73.73	70.60[b]	73.53
5″	69.67	67.79[a]	67.03	69.76[b]	69.71[b]	69.41
6″	18.38	18.10	18.07	18.54[b]	18.36[b]	18.44
$COCH_3$	20.78	20.87	20.59	20.89[b]	21.06[b]	—
$COCH_3$	20.67	20.79	—	—	—	—
$COCH_3$	170.20	170.60	170.80	170.82[b]	170.33[b]	—
$COCH_3$	170.42	170.33	—	—	—	—
OCH_3	56.25	56.30	56.30	56.26	56.26	56.26
	56.25	56.30	56.30	56.26	56.26	56.26

[a]Interchangeable.
[b]Interchangeable values for **4** and **5** (mixture).

glucosyl moieties of **1** appear practically unchanged when compared with the data of martynoside. Differences in chemical shifts between **1** and martynoside C-1″ (−2.29 ppm), C-2″ (−1.22 ppm), C-3″ (−0.81 ppm), and C-4″ (−2.06 ppm) were indicative of the acetyl groups on C-2″ and C-3″ of the rhamnose moiety.

The ^{1}H-nmr spectrum of **2** differed little from that of **1**. Similar to **1**, **2** had an acetyl group on C-2″ as shown by the coupling constants of the signal at δ 5.16 (dd, J=1.8 and 3.4 Hz). The triplet at δ 4.77 was indicative of the attachment of the other acetyl group on a carbon bearing a proton with a diaxial relationship (J=9.8 Hz, Table 1). Hence, the other acetyl group was assigned as being attached to C-4″. The ^{13}C-nmr spectrum of **2** showed a close similarity to those of **1** and martynoside. For compound **2**, it was possible to recognize the sites of acetylation since it is known that upon acylation, carbon signals at the β position are displaced upfield while the carbonyl carbon signal is deshielded (9). Thus, signals at δ 98.42, 73.14, 67.23, 74.75, and 67.83 were assigned to C-1″,

C-2″, C-3″, C-4″, and C-5″, respectively (Table 2). The shielding effect (−3.71 ppm) observed for C-3″ is due to the cumulative β-effect of acetyl groups on C-2″ and C-4″. The heteronuclear ^{1}H-^{13}C correlation (HETCOR) nmr spectrum of **2** confirmed some other signal assignments. The signal at δ 5.40 (d, J=1.8 Hz, H-1″), showed a cross-peak with C-1″ at δ 98.43, while the signal at δ 5.16 (dd, J=1.8 and 3.4 Hz, H-2″) had a corresponding C-2″ peak at δ 73.14. The H-4″ proton (δ 4.74, t, J=9.8 Hz) was correlated to the carbon resonance signal at δ 74.75 (C-4″).

Bioassay studies on the feeding behavior of *Chilo partellus* larvae (Pyralidae, Lepidoptera) to cellulose disks treated separately with acetylmartynoside A [**1**], and 4″-*O*-acetylmartynoside [**3**], indicated that the latter is an antifeedant while the former is a weak stimulant (10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps are uncorrected. ^{1}H- and ^{13}C-nmr spectra (δ, ppm, J(Hz)) were obtained at 300 and 75.5 MHz on a Varian XL-300 (1D and 2D) spectrometer with TMS as internal standard. Fabms were recorded in a positive mode in a 70 EQ instrument (glycerol+NaCl matrix). Si gel 60 (70–270 mesh, Macherey Nagel) was used for cc and Si gel 60 F_{254} (Merck) plates for tlc. The substances were detected by spraying with H_2SO_4-H_2O-Formol (2:1:1) and thymol (0.5%)/sulphuric acid in EtOH (red spots for phenylpropanoid glucosides) reagents followed by heating. Exposure to uv light at 254 and 366 nm was also carried out.

PLANT MATERIAL.—*Aegiphila obducta* was collected in March 1990 in Minas Gerais State, Brazil, and a voucher specimen is deposited at Universidade Federal de Ouro Preto (Departamento de Botânica Herbarium).

EXTRACTION AND ISOLATION.—The wood of *A. obducta* (3.4 kg) was extracted successively with $CHCl_3$ and MeOH. A portion (30.5 g) of the MeOH extract was then extracted with Me_2CO and the soluble part (8.3 g) was chromatographed on a Si gel column (54 cm×2.8 cm) eluting with $CHCl_3$ with increasing amounts of EtOAc, and then EtOAc with increasing amounts of MeOH, to give 250 fractions of 20 ml each. Fractions 7–9 (320 mg, eluted with $CHCl_3$-EtOAc, 7:1) afforded, on evaporation, a solid which was identified by co-tlc and ^{1}H-nmr data (4) as apigenin. Fractions 100–112 (670 mg, eluted with EtOAc) were further purified on a Si gel column (37 cm×1.5 cm) eluting very slowly with EtOAc, affording 70 mg of **1**, 40 mg of **2**, and 350 mg of a mixture of **1** and **2**. Fractions 118–128 (103 mg, eluted with EtOAc) were rechromatographed on a Si gel column (37 cm×1.5 cm) using as solvent system EtOAc-Me_2CO-H_2O (25:4:1, organic layer) yielding 54 mg of **3**. Fractions 129–152 (180 mg; eluted with EtOAc-MeOH, 9:1) were fractionated on a Si gel column (37 cm×1.5 cm) eluted very slowly with EtOAc-Me_2CO-H_2O) (25:4:2, organic layer) furnishing 31 mg of a mixture of **4** and **5**. Fractions 198–212 (356 mg; eluted with EtOAc-MeOH, 9:1) represented pure martynoside and fractions 231-250 (1.2 g eluted with EtOAc-MeOH, 1:1) represented pure verbascoside and these compounds were identified by comparison of their ^{1}H- and ^{13}C-nmr data with those reported in the literature (5,6).

2″,3″-O-Acetylmartynoside [**1**].—Pale beige amorphous powder: uv λ max (MeOH) 217, 230 nm; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; fabms *m/z*, positive peaks at 759 (10) $[M+Na]^+$, 736 (18) $[M]^+$, 569 (30) [M−3-hydroxy-4-methoxyphenylethyl]$^+$, 506 (17) [M−rha−2Ac]$^+$, 339 (98) [glc+feruloyl]$^+$.

2″,4″-O-Acetylmartynoside [**2**].—Pale beige amorphous powder: uv λ max (MeOH) 217, 230 nm; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; fabms *m/z*, positive peaks at 759 (25) $[M+Na]^+$, 736 (26) $[M]^+$; 569 (16) [M−3-hydroxy-4-methoxyphenylethyl]$^+$; 506 (25) [M−rha−2Ac]$^+$; 339 (98) [glc+feruloyl]$^+$.

4″-O-Acetylmartynoside [**3**].—Pale beige amorphous powder: uv λ max (MeOH) 217, 230 nm; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2. Positive identification was obtained by comparison with literature data (3).

3″-O-Acetylmartynoside [**4**] and *2″-O-acetylmartynoside* [**5**].—Pale beige amorphous powder: uv λ max (MeOH) 218, 230 nm; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2. Positive identification was achieved by comparison with literature data (3).

Apigenin.—Yellow crystals. Identified by co-tlc with an authentic sample and by comparison of spectral data with published values (4).

Martynoside.—Pale beige amorphous powder. Identified by co-tlc with an authentic sample and by comparison of spectral data with published values (5).

Verbascoside.—Brownish amorphous powder. Identified by co-tlc with an authentic sample and by comparison of spectral data with published values (6).

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