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TWO IRIDOID GLYCOSIDE CAFFEOYL ESTERS FROM *PREMNA ODORATA*

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Key Word Index—*Premna odorata*; Verbenaceae; iridoid; 6-α-L-rhamnopyranosylcatalpol.

Abstract—Two new iridoid glycosides isolated from *Premna odorata* were shown to be 2″- and 3″-caffeoyl-6-α-L-rhamnopyranosylcatalpol respectively. The position of the acyl moiety in each of these compounds was established by the distinct acylation shifts in the ^{13}C NMR signals of the rhamnose moiety.

INTRODUCTION

In continuation of our studies on Philippine medicinal plants, *Premna odorata* Blanco (Verbenaceae) was investigated. This plant is locally called 'Alagau' and is a small, hairy tree 3–8 m in height. It is endemic in the Philippines and planted around dwellings from the Batan Islands and northern Luzon to Mindanao. In the Philippines, a decoction of the leaves is used for loosening phlegm and as a cough remedy [1].

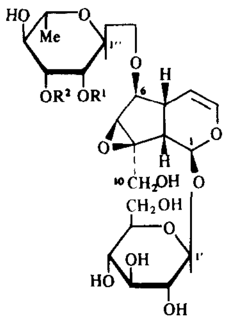
In this paper, we describe the isolation and characterization of two new mono-acylrhamnopyranosylcatalpols from this plant.

RESULTS AND DISCUSSION

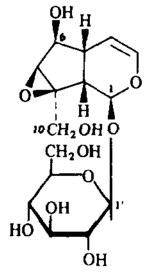
Compounds **1** and **2** were isolated from the methanol extract of the leaves of *P. odorata* by a combination of silica gel CC and droplet counter-current chromatography (DCCC).

Both compounds, analysed for $C_{30}H_{38}O_{17}$ and their ^{13}C NMR spectra were very similar, showing six typical signals for β-glucopyranose and six signals for a substituted α-rhamnopyranose. The presence of these sugar units was confirmed by GC analysis of TMS derivatives of their methanolysates. Among the remaining 18 carbon signals, nine signals were identical with those reported for caffeoyl ester [2].

The rest of the ^{13}C NMR signals showed the presence of a double bond, one acetal, two $-CH<$, two $>CHO-$, one $>CO-$ and one $-CH_2OH$. These indicated that compounds **1** and **2** were iridoid derivatives. The ^{13}C NMR signals of an authentic catalpol (**3**) resembled those of compounds **1** and **2** (see Table 1). However, the ^{13}C NMR of **1** and **2** showed additional signals for rhamnosyl and caffeoyl moieties. Although the signals for the glucose portion were superimposable on those of **3**, the signals for the aglycone moieties showed slight differences. Thus rhamnose appeared to be attached to the C-6 hydroxyl group of the aglycone. This was supported by mild alkaline hydrolysis of compounds **1** and **2**. Both compounds gave the same deacylated derivative (**4**) which has aglycone, glucose and rhamnose moieties. The ^{13}C NMR data (see Table 1, **4** and **5**) and other physico-chemical evidence of this deacylated compound were the same as those of 6-α-L-rhamnopyranosylcatalpol (**5**), which had been isolated from *Scrophularia nodosa* L. [3].

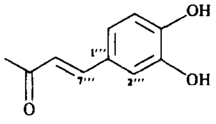


1 R¹ = Caffe R² = H
2 R¹ = H R² = Caffe
4 R¹ = H R² = H
(6-α-L-Rhamnopyranosylcatalpol)



3 Catalpol

Caffe:



When the NMR spectra of **1** and **2** were compared with that of **4**, compounds **1** and **2** were obviously positional isomers in which the caffeoyl group is esterified to different hydroxyl groups of the rhamnose moiety, since no shift was observed in the glucosyl moiety. To decide the esterified position of the rhamnosyl moiety present in **1** and **2**, substitution shift regularity of the esterified sugar was considered [4]. When the ^{13}C NMR chemical shifts of the rhamnose moiety (C-1″–C-6″) of **1** were compared with those of **4**, the C-2″ signal was seen to be significantly shifted downfield by δ1.9, and the C-1″ and C-3″ signals were shifted upfield by δ2.5 and 1.7, respectively. This established that the position of the caffeoyl ester of compound **1** is C-2″ of rhamnose [4]. In compound **2**, the δ3.1 downfield shift of C-3″, and δ1.9 and 2.1 upfield shifts for C-2″ and C-4″, respectively, established that caffeoyl esterification must be at C-3″. Therefore the

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Table 1. ^{13}C NMR data of compounds **1**–**5** (25 MHz, CD_3OD and/or d_6-DMSO, TMS as int. standard)

C	1	2	3	4	5*
Aglycone					
1	95.2	95.2	95.3	95.1 (93.0)	(93.22)
3	142.3	142.2	141.8	142.1 (140.8)	(140.39)
4	103.5	103.6	104.0	103.6 (102.3)	(102.50)
5	37.2	37.2	39.1	37.2 (35.5)	(35.66)
6	84.4	83.8	79.6	83.5 (81.2)	(81.49)
7	59.6	59.3	62.5	59.3 (57.3)	(57.49)
8	66.5	66.6	66.2	66.5 (65.2)	(65.30)
9	43.3	43.2	43.6	43.2 (41.8)	(41.22)†
10	61.5	61.5	61.6	61.4 (58.7)	(58.91)
Glucose					
1′	99.7	99.7	99.7	99.7 (97.7)	(97.91)
2′	74.8	74.8	74.9	74.8 (73.3)	(73.45)
3′	77.6	77.6	77.7	77.6 (77.3)	(77.43)
4′	71.7	71.7	71.7	71.7 (70.4)	(70.33)
5′	78.6	78.5	78.6	78.5 (76.3)	(76.45)
6′	62.9	62.9	62.9	62.9 (61.2)	(61.41)
Rhamnose					
1″	97.8 (−2.5)‡	100.2 (−0.1)§		100.3 (98.7)	(98.89)
2″	74.1 (+1.9)	70.3 (−1.9)		72.2 (70.4)	(70.64)
3″	70.5 (−1.7)	75.3 (+3.1)		72.2 (70.1)	(70.33)
4″	74.2 (+0.4)	71.7 (−2.1)		73.8 (71.8)	(71.97)
5″	70.3 (+0.2)	70.3 (+0.2)		70.1 (68.7)	(68.85)
6″	18.1 (+0.1)	18.0 (±0)		18.0 (17.7)	(17.85)
Caffeoyl					
1‴	127.7	127.5			
2‴	114.9	115.2			
3‴	149.6	149.5			
4‴	146.7	146.7			
5‴	116.5	116.5			
6‴	123.2	123.0			
7‴	147.6	147.1			
8‴	115.3	115.3			
9‴	168.7	168.9			

Chemical shifts in parentheses are in d_6-DMSO.

*Data taken from lit. [3].

†This assignment must be revised. INEPT experiment revealed that this signal is almost overlapped with the lowest field signal of the solvent.

‡ and § are acylation shift values: $\Delta\delta = \delta_1 - \delta_4$ and $\Delta\delta = \delta_2 - \delta_4$, respectively.

structures of the compounds **1** and **2** were elucidated to be 6-α-L-(2″-caffeoyl)rhamnopyranosylcatalpol and 6-α-L-(3″-caffeoyl)rhamnopyranosylcatalpol, respectively.

Concerning related compounds, a Russian group has reported the isolation of saccatoside [6-α-L-(2″-coumaroyl)rhamnopyranosylcatalpol] from *Verbascum saccatum* [5], and from *Verbascum sinuatum*, a German group has isolated diacylrhamnopyranosylcatalpols [6]. The Russian group has also reported the isolation of a positional isomer of saccatoside [6-α-L-(3″-coumaroyl)rhamnopyranosylcatalpol] [7]. Furthermore, the related compound 6-α-L-(3″-coumaroyl)rhamnopyranosylaucubin was isolated from *Verbascum laxum* by the Russian group [8]. Although related compounds have been isolated from several plant sources, compounds **1** and **2** have been isolated for the first time from a natural source. A study of the pharmacological activity of these compounds is in progress.

EXPERIMENTAL

Mp: uncorr. ^{1}H NMR and ^{13}C NMR: 100 and 25 MHz, respectively. MS: 75 eV. Authentic catalpol was purchased from the collection of standard plant constituents of Yoneyama Yakuhin Kogyo Co. Ltd. (Osaka).

Plant material. Premna odorata Blanco was cultivated and harvested at the Department of Chemistry, University of Philippines at Los Baños, Philippines.

Extraction and isolation. Dried and powdered leaves of *P. odorata* (2.12 kg) was extracted with *n*-hexane and MeOH successively. The MeOH extract (222 g) was suspended in H_2O and then extracted with EtOAc followed by *n*-BuOH. The *n*-BuOH extract (105.5 g) was chromatographed on a highly porous polymer (Diaion, HP-20; Mitsubishi Chemical Ind. Co.) with stepwise increase of MeOH content in H_2O (20, 40, 60, 80 and 100%). The 20% MeOH eluent from the Diaion column (11 g) was subjected to silica gel CC (EtOAc–EtOH–H_2O, 100:10:1) to give compound **1** and **2** rich fractions of 0.35 and 1.50 g, respectively. Further purification of these fractions by DCCC (500 columns, ascending method, $CHCl_3$–MeOH–H_2O–*n*-PrOH, 9:12:8:2) gave pure compounds **1** (104 mg) and **2** (376 mg).

Compound **1**[6-α-L-(2″-*caffeoyl*)*rhamnopyranosylcatalpol*]. Amorphous powder, $[\alpha]_D - 120°$ (MeOH; *c* 0.42); IR ν_{max}^{KBr} cm^{-1}: 3350, 1689, 1628, 1600, 1514, 1444, 1265, 1155, 1112, 1060, 915, 831, 810; UV λ_{max}^{MeOH} nm (log ε): 222 (4.11), 245 (3.98), 304 (4.13)sh, 332 (4.26); FABMS *m/z* (rel. int.): 693 $[M+Na]^+$ (4), 309 (10), 163 (83), 43 (100); ^1H NMR (MeOH-d_4) δ: 1.31 (3H, *d*, *J* = 6 Hz, 6″-H), 2.35–2.65 (2H, *m*, 5-H, 9-H), 6.33 (H, *d*, *J* = 16 Hz, 8‴-H), 6.38 (H, *d*, *J* = 6 Hz, 3-H), 6.78 (H, *d*, *J* = 8 Hz, 5‴-H), 6.97 (H, *dd*, *J* = 2, 8 Hz, 6‴-H), 7.07 (H, *d*, *J* = 2 Hz, 2‴-H), 7.60 (H, *d*, *J* = 16 Hz, 7‴-H); ^{13}C NMR: see Table 1. (Found: C, 51.8; H, 5.87. $C_{30}H_{38}O_{17} \cdot H_2O$ requires: C, 52.32; H, 5.85%.).

Compound **2** [6-α-L-(3″-*caffeoyl*)*rhamnopyranosylcatalpol*]. Amorphous powder, $[\alpha]_D - 121°$ (MeOH; *c* 0.38); IR ν_{max}^{KBr} cm^{-1}: 3350, 1694, 1627, 1601, 1518, 1444, 1268, 1157, 1050, 915, 836, 811; UV λ_{max}^{MeOH} nm (log ε): 220 (4.10), 245 (4.01), 303 (4.15)sh, 330 (4.28); FABMS *m/z* (rel. int.): 693 $[M+Na]^+$ (17), 671 $[MH]^+$ (1), 309 (22), 163 (47), 43 (100); ^1H NMR (MeOH-d_4): δ1.31 (3H, *d*, *J* = 6 Hz, 6″-H), 2.35–2.65 (2H, *m*, 5-H, 9-H), 6.36 (H, *d*, *J* = 16 Hz, 8‴-H), 6.38 (H, *d*, *J* = 6 Hz, 3-H), 6.78 (H, *d*, *J* = 8 Hz, 5‴-H), 6.97 (H, *dd*, *J* = 2, 8 Hz, 6‴-H), 7.06 (H, *d*, *J* = 2 Hz, 2‴-H), 7.64 (H, *d*, *J* = 16 Hz, 7‴-H); ^{13}C NMR: see Table 1. (Found: C, 52.5; H, 6.03. $C_{30}H_{38}O_{17} \cdot H_2O$ requires: C, 52.32; H, 5.85%.).

Alkaline hydrolysis of compounds **1** and **2**. Compound **2** (150 mg) was hydrolysed with a stoichiometric amount of 0.01 M NaOH at 20°. The reaction was followed by TLC (silica gel, precoated, EtOAc–EtOH–H_2O, 8:2:1). After disappearance of the starting material, the reaction mixtures were neutralized with Amberlite MB-6, and then concd *in vacuo*. 6-α-L-Rhamnopyranosylcatalpol, thus formed, was purified by DCCC ($CHCl_3$–MeOH–H_2O–*n*-PrOH, 9:12:8:2) and Sephadex LH-20 CC (MeOH) (64 mg). Hydrolysis of a small amount of compound **1** (a few mg) gave the same compound on TLC. Amorphous powder, $[\alpha]_D - 150°$ (MeOH; *c* 0.41); IR ν_{max}^{KBr} cm^{-1}: 3320, 1645, 1050; UV: no absorption between 210–360 nm; FABMS *m/z* 531 $[M+Na]^+$, 323, 173; ^1H NMR (MeOH-d_4) δ: 1.26 (3H, *d*, *J* = 6 Hz, 6″-H), 2.4 (H, *m*, 5-H), 2.56 (H, *t*, *J* = 8, 9-H), 6.37 (H, *dd*, *J* = 1, 6 Hz, 3-H); ^{13}C NMR: see Table 1. (Found: C, 46.0; H, 6.25. Calc. for $C_{21}H_{32}O_{14} \cdot 2H_2O$: C, 46.32; H, 6.66%.).

GC analysis. Compounds **1** and **2** (2 mg each) were treated with 5% HCl in dry MeOH in sealed tubes at 100° for 3 hr. The reaction mixtures were neutralized with Ag_2CO_3, filtered and evapd to dryness. The methanolysates were derivatized with five drops of TMS-imidazole at 60° for 15 min, and then a few drops of H_2O were added. The methylglycoside-TMS ethers were taken up in *n*-hexane and subjected to GC analysis. GC: column 1.5% OV-1 (3 mm × 2 m), N_2 40 ml/min, 180° (isothermal). R_t (min): glc, 9.56 and 10.52; rha, 2.94. Compound **1**: glc, 9.54 and 10.50; rha, 2.92. Compound **2**: glc, 9.55 and 10.52; rha, 2.93.

Compound **1** *nonacetate*. Compound **1** (64 mg) was treated with a mixture of Ac_2O and pyridine. Usual work-up gave an amorphous powder (81 mg). $[\alpha]_D - 35.3°$ ($CHCl_3$, *c* 0.64); IR ν_{max}^{KBr} cm^{-1}: 1750, 1635, 1500, 1420, 1365, 1220, 1040, 900; UV λ_{max}^{MeOH} nm (log ε): 218 (4.14), 280 (4.36); EIMS *m/z* (rel. int.): 697 (< 1), 492 (3), 450 (4), 331 (15), 273 (18), 169 (52), 43 (100); FDMS *m/z* (rel. int.): 1049 $[MH]^+$ (3), 1006 (4), 964 (2), 631 (6), 391 (11), 331 (71), 43 (100); ^1H NMR ($CDCl_3$): δ1.27 (3H, *d*, *J* = 6 Hz, 6″-H_3), 1.99, 2.01, 2.03, 2.04, 2.06, 2.10, 2.13 (3H × 7, *s* × 7, Ac × 7), 2.31 (6H, *s*, Ac × 2), 6.33 (H, *d*, *J* = 6 Hz, 3-H), 6.50 (H, *d*, *J* = 16 Hz), 7.74 (H, *d*, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$); δ17.4, 20.6 (Ac × 9), 35.4, 41.7, 58.0, 61.1, 62.2, 62.4, 66.9, 68.2, 68.9, 70.1, 70.6, 71.1, 72.2, 72.5, 83.5, 94.2, 96.5 × 2, 102.3, 118.2, 123.0, 124.0, 126.6, 132.9, 141.1, 142.5, 143.9, 144.3, 165.6 (C-9‴), 167.9, 168.0, 169.0, 169.2, 169.9 × 2, 170.2, 170.6 × 2 (Ac × 9). (Found: C, 54.7; H, 5.38. $C_{48}H_{56}O_{26}$ requires: C, 54.96; H, 5.38%.).

Compound **2** *nonaacetate*. Compound **2** (94 mg) was treated with the mixture of Ac_2O (1.5 ml) and pyridine (1.5 ml) at 20° overnight. Usual work-up gave an amorphous white powder (140 mg). $[\alpha]_D - 59.7°$ ($CHCl_3$, *c* 0.58); IR ν_{max}^{KBr} cm^{-1}: 1750, 1635, 1500, 1430, 1370, 1220, 1040, 900; UV λ_{max}^{MeOH} nm (log ε): 218 (4.14), 280 (4.36). EIMS: *m/z* (rel. int.): 450 (< 1), 331 (26), 169 (75), 43 (100); FDMS *m/z* (rel. int.): 1049 $[MH]^+$ (26), 331 (65), 43 (100); ^1H NMR ($CDCl_3$): δ1.27 (3H, *d*, *J* = 6 Hz), 2.02 (3H, *s*, Ac), 2.03 (6H, *s*, Ac × 2), 2.05 (3H, *s*, Ac), 2.11 (3H, *s*, Ac), 2.13 (3H, *s*, Ac), 2.17 (3H, *s*, Ac), 2.31 (6H, *s*, Ac × 2), 6.30 (H, *d*, *J* = 16 Hz), 6.34 (H, *d*, *J* = 7 Hz, 7-H), 7.23 (H, *d*, *J* = 8 Hz), 7.59 (H, *d*, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$): δ17.4 (C-6″), 20.6 (Ac × 7), 20.8 (Ac), 20.9 (Ac), 35.4, 41.7, 58.0, 61.1, 62.1, 62.4, 66.9, 68.2, 69.2, 70.1, 70.6, 71.0, 72.2, 72.6, 83.5, 94.3, 96.5 (× 2), 102.4, 118.2, 122.9, 124.0, 126.7, 133.0, 141.1, 142.5, 143.8, 144.0, 165.2 (C-9‴), 167.9, 168.0, 169.0, 169.2, 169.9, 170.0, 170.2, 170.5, 170.6 (9 × acetyl–$\underline{C}$O). (Found:C, 54.7; H, 5.44.$C_{48}H_{56}O_{26}$ requires:C, 54.96; H, 5.38%.).

6-α-L-*Rhamnopyranosylcatalpol octaacetate*. 6-α-L-Rhamnopyranosylcatalpol (25 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) at 20° overnight. Usual work-up and recrystallization from EtOH–H_2O gave colourless needles (28 mg), mp 125–127°. $[\alpha]_D - 72.8°$ ($CHCl_3$; *c* 0.36); IR ν_{max}^{KBr} cm^{-1}: 1750, 1650, 1430, 1370, 1220, 1040; UV: no absorption between 210–360 nm; EIMS *m/z* (rel. int.): 331 (41), 273 (10), 169 (100), 43 (49); FDMS *m/z* (rel. int.): 845 $[MH]^+$ (100), 332 (17), 43 (46); ^1H NMR ($CDCl_3$): δ1.22 (H, *d*, *J* = 6 Hz), 2.00 (3H, *s*, Ac), 2.02 (3H, *s*, Ac), 2.05 (9H, *s*, Ac × 3), 2.11 (3H, *s*, Ac), 2.13 (3H, *s*, Ac), 2.16 (3H, *s*, Ac), 2.40–2.70 (2H), 6.33 (H, *d*, *J* = 6 Hz); ^{13}C NMR ($CDCl_3$): δ17.4, 20.7 (Ac × 7), 20.9 (Ac), 35.4, 41.7, 57.9, 61.1, 62.2, 62.4, 66.9, 68.2, 68.8, 69.9, 70.6, 71.0, 72.2, 72.6, 83.4, 94.2, 96.5 (× 2), 102.3, 141.1 (C-3), 169.0, 169.3, 169.9 (× 2), 170.0, 170.2 (× 2), 170.7 (Ac × 8). (Found: C, 52.9; H, 5.82. Calc. for $C_{37}H_{48}O_{28}$: C, 52.60; H, 5.68%.).

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