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6-*O*-α-L-(2″-*O*- AND 3″-*O*-ISOFERULOYL) RHAMNOPYRANOSYLCATALPOLS FROM *PREMNA JAPONICA*

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Key Word Index—*Premna japonica*; Verbenaceae; iridoid; 6-*O*-α-L-rhamnopyranosylcatalpol; 6-*O*-α-L-(2″-*O*-isoferuloyl) rhamnopyranosylcatalpol; 6-*O*-α-L-(3″-*O*-isoferuloyl) rhamnopyranosylcatalpol.

Abstract—From the methanol extract of the leaves of *Premna japonica*, two new monoacyl 6-*O*-α-L-rhamnopyranosylcatalpols were isolated. The structures of these compounds were determined to be 6-*O*-α-L-(2″-O-isoferuloyl and 3″-*O*-isoferuloyl)rhamnopyranosylcatalpols by NMR spectroscopy and chemical conversions.

INTRODUCTION

In the course of investigation of the Philippine medicinal plant, *Premna odorata* Blanco, several new acylated rhamnopyranosylcatalpols have been isolated [1, 2]. The related plant, *P. japonica* Miq. (= *P. microphylla* Turcz.) (Verbenaceae) (Japanese name: Hamakusagi) is used in China as an antipyretic, hemostasis and for snake venom [3]. This paper describes the isolation and structure determination of new monoacylated iridoid derivatives from this plant, collected at the south eastern part of Tokushima Prefecture, Shikoku Island, Japan.

RESULTS AND DISCUSSION

The methanol extract of the dried leaves of *P. japonica* was separated by a combination of highly porous polymer, Diaion HP-20 and silica gel column chromatography, and droplet counter-current chromatography (DCCC). Compound **1**, $C_{31}H_{40}O_{17}$, was obtained as a colourless amorphous powder, whose M_r was determined by the observation of ions at *m/z* 685 $[MH]^+$, 707 $[M+Na]^+$ and 723 $[M+K]^+$ in the FAB-MS on addition of sodium and potassium iodide, respectively. Its IR spectrum showed the presence of a conjugated ester (1700 and 1625 cm^{-1}) and an aromatic ring (1610 and 1510 cm^{-1}). In the ^{13}C NMR spectrum, a total of 31 signals were observed (Table 1). Comparison of these signals with those of 2″-caffeoylrhamnopyranosylcatalpol (**5**) [1] showed that **1** was most probably a derivative of this compound.

Mild alkaline hydrolysis of **1** in methanol gave a methyl ester of an acyl moiety and 6-*O*-α-L-rhamnopyranosylcatalpol (**7**), which was identified with an authentic sample on TLC. The ^1H NMR spectrum of the methyl ester of the acyl moiety showed the presence of methoxyl and carbomethoxyl groups, *trans*-olefinic protons and three aromatic protons, coupled in an ABX system. These data suggested that this acyl moiety is ferulic acid or isoferulic acid. By comparing the chemical shifts of these aromatic protons with authentic samples of methyl ferulate and methyl isoferulate the acyl portion was identified as isoferulic acid.

Thus the structure of compound **1** was determined to be 6-*O*-α-L-(2″-*O*-isoferuloyl)rhamnopyranosylcatalpol. Acetylation of **1** gave octaacetate (**2**), whose spectroscopic data also supported the structure.

Compound **3**, $C_{31}H_{40}O_{17}$, was obtained as a colourless powder and had similar physical properties to compound **1**. Since differences were observed in the rhamnosyl signals in the ^{13}C NMR spectra (Table 1), compound **3** is an isomer of the esterified position of **1**. This position was determined to be C-3″ by comparison with the ^{13}C NMR data of 3″-caffeoyl-rhamnopyranosylcatalpol (**6**) [1]. The acyl portion was also obtained by mild alkaline hydrolysis in methanol as its methyl ester. The comparison of the ^1H NMR spectral data with that of authentic samples revealed that the acyl moiety is also isoferulic acid. Thus compound **3** is determined to be 6-*O*-α-L-(3″-*O*-isoferuloyl) rhamnopyranosylcatalpol. The spectroscopic data of its octaacetate (**4**) also supported the structure.

Although the isolation of mono-, di- and triacyl-6-*O*-α-L-rhamnopyranosylcatalpols has been reported from several plants of the Verbenaceae and Scrophulariaceae [2], isoferuloyl esters of 6-*O*-α-L-rhamnopyranosylcatalpol were obtained for the first time from natural source.

EXPERIMENTAL

^1H and ^{13}C NMR were measured at 100 and 25 MHz, respectively, except as otherwise stated. EIMS: 70 eV. Ferulic and isoferulic acids were purchased from Tokyo Kasei Chemical Co. Ltd (Tokyo). 6-*O*-α-L-Rhamnopyranosylcatalpol was from our previous experiment [1].

Plant material. The leaves of *P. japonica* were collected in May (1988) at the south eastern part of Tokushima Prefecture (Shikoku Island). A voucher specimen was deposited at the Department of Pharmacognosy, Institute of Pharmaceutical Science, Hiroshima University School of Medicine (88-PJ-Tokushima-1).

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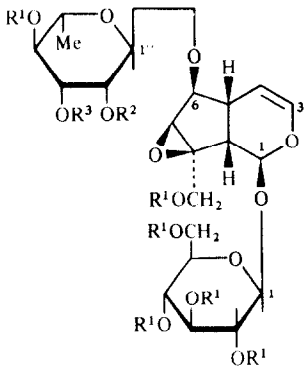

	R^1	R^2	R^3
1	H	IFE	H
2	Ac	IFE	Ac
3	H	H	IFE
4	Ac	Ac	IFE
5	H	CAF	H
6	H	H	CAF
7	H	H	H

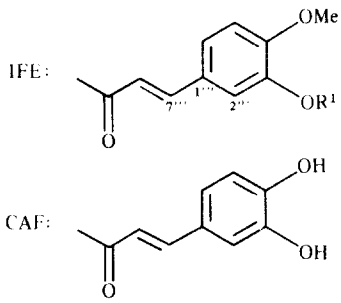

Table 1. ^{13}C NMR data of compounds **1**, **3**, **5** and **6** (CD_3OD)

C	1	5*	3	6*
1	95.3	95.2	95.2	95.2
3	142.3	142.3	142.2	142.2
4	103.5	103.5	103.6	103.6
5	37.3	37.2	37.2	37.2
6	84.5	84.4	83.8	83.8
7	59.6	59.6	59.4	59.3
8	66.6	66.5	66.6	66.6
9	43.4	43.3	43.3	43.2
10	61.5	61.5	61.5	61.5
1′	99.8	99.7	99.7	99.7
2′	74.9	74.8	74.8	74.8
3′	78.7	78.6	78.6	78.5
4′	71.8	71.7	71.7	71.7
5′	77.8	77.6	77.7	77.6
6′	63.0	62.9	62.9	62.9
1″	97.9	97.8	100.2	100.2
2″	74.3	74.1	70.3	70.3
3″	70.6	70.5	75.4	75.3
4″	74.3	74.2	71.4	71.7
5″	70.4	70.3	70.3	70.3
6″	18.1	18.1	18.0	18.0
1‴	129.0	127.7	129.0	127.5
2‴	112.7	114.9	112.5	115.2
3‴	151.7	149.6	151.5	149.5
4‴	148.1	146.7	147.9	146.7
5‴	115.0	116.5	114.8	116.5
6‴	122.9	123.2	122.8	123.0
7‴	147.2	147.6	146.7	147.1
8‴	116.0	115.3	116.4	115.3
9‴	168.5	168.7	168.7	168.9
$-OCH_3$	56.5		56.4	

*Data taken from ref. [1].

Isolation procedure. The dried leaves of *P. japonica* (1.45 kg) were extracted with MeOH (285.0 g). The MeOH extract was dissolved in 95% aq. MeOH and extracted with *n*-hexane. The MeOH layer was evapd and suspended in H_2O, and then extracted with EtOAc and *n*-BuOH, successively. The *n*-BuOH extract (84.38 g) was separated by Diaion HP-20 CC with stepwise increases of MeOH in H_2O (20, 40, 60, 80 and 100%). The 40% MeOH eluent (24.08 g) was chromatographed twice over silica gel ($CHCl_3$–MeOH–H_2O and $CHCl_3$–MeOH) to give 662 and 608 mg of compound **1** and **3** rich fractions, respectively. The final purification of these fractions by droplet counter-current chromatography with the solvent system of $CHCl_3$–MeOH–H_2O–*n*-PrOH (45:60:40:10, ascending method) afforded 297 mg of compound **1** and 237 mg of compound **3**.

Compound **1**. Amorphous white powder; $[\alpha]_D$ −115.5° (MeOH; *c* 0.32); IR ν_{max}^{KBr} cm^{-1}: 3375, 2900, 1700, 1625, 1610, 1510, 1440, 1265, 1125, 1065, 915, 830, 805, UV λ_{max}^{MeOH} nm (log ε): 217 (4.24), 235 (4.09) sh, 243 (4.14), 298 (4.27), 310 (4.27), 326 (4.35); FABMS *m/z*: 685 $[MH]^+$, 707 $[M+Na]^+$ (+NaI), 723 $[M+K]^+$ (+KI); ^1H NMR (CD_3OD, 400 MHz): δ 1.30 (3H, *d*, *J* = 6 Hz), 2.42 (H, *m*), 2.57 (H, *dd*, *J* = 8, 10 Hz), 3.40 (H, *t*, *J* = 9 Hz), 3.49 (H, *t*, *J* = 9 Hz), 3.79 (H, *d*, *J* = 13 Hz), 3.89 (3H, *s*), 4.02 (H, *dd*, *J* = 1, 8 Hz), 4.15 (H, *d*, *J* = 13 Hz), 4.77 (H, *d*, *J* = 8 Hz), 5.02 (H, *d*, *J* = 2 Hz), 5.07 (H, *dd*, *J* = 4, 6 Hz), 5.09 (H, *d*, *J* = 2 Hz), 5.15 (H, *dd*, *J* = 2, 4 Hz), 6.37 (H, *dd*, *J* = 2, 6 Hz), 6.39 (H, *d*, *J* = 16 Hz), 6.94 (H, *d*, *J* = 8 Hz), 7.06 (H, *dd*, *J* = 2, 8 Hz), 7.10 (H, *d*, *J* = 2 Hz), 7.62 (H, *d*, *J* = 16 Hz); ^{13}C NMR: see Table 1. (Found: C, 53.07; H, 6.17. $C_{31}H_{40}O_{17} \cdot H_2O$ requires: C, 52.99; H, 6.02%).

Compound **1** *octaacetate* (**2**). Compound **1** (37 mg) was treated with a mixture of Ac_2O and pyridine at 25° overnight. Usual work-up gave 48 mg of colourless powder. $[\alpha]_D$ −31.7° ($CHCl_3$; *c* 0.46); IR ν_{max}^{KBr} cm^{-1}: 1745, 1630, 1605, 1510, 1430, 1365, 1220, 1120, 1040, 905; UV λ_{max}^{MeOH} nm (log ε): 227 (4.22), 294 (4.42) inf, 309 (4.50); EIMS *m/z*: 331, 177, 169, 110; FABMS *m/z*: 1021 $[MH]^+$, 1043 $[M+Na]^+$ (+NaI), 1059 $[M+K]^+$ (+KI); ^1H NMR ($CDCl_3$): δ 1.26 (3H, *d*, *J* = 6 Hz), 1.99, 2.02, 2.03, 2.05, 2.06, 2.10, 2.13 (3H ea., *s*, alcoholic Ac × 7), 2.33 (3H, *s*, phenolic Ac), 3.88 (3H, *s*), 6.40 (H, *d*, *J* = 16 Hz), 6.92 (H, *d*, *J* = 8 Hz), 7.66 (H, *d*, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$): δ 17.4, 20.6 (Ac × 7), 35.5,

41.7, 56.0, 58.0, 61.1, 62.2, 62.4, 67.0, 68.3, 68.9, 70.0, 70.6, 71.2, 72.3, 72.6, 83.5, 94.3, 96.6 (×2), 102.4, 112.4, 115.7, 122.3, 127.3, 127.9, 140.1, 141.1, 145.0, 153.3, 166.0, 168.7, 169.0, 169.3, 169.9 (×2), 170.2, 170.6 (×2). (Found: C, 54.69; H, 5.47. $C_{47}H_{56}O_{25}\cdot 1/2\ H_2O$ requires: C, 54.81; H, 5.57%).

Compound **3**. Amorphous white powder; $[\alpha]_D$ −117.9° (MeOH; *c* 0.30); IR ν_{max}^{KBr} cm^{-1}: 3375, 2900, 1690, 1625, 1610, 1510, 1265, 1125, 1055, 920, 840, 810; UV λ_{max}^{MeOH} nm (log ε): 217 (4.27), 234 (4.11) sh, 242 (4.15), 296 (4.31), 311 (4.31), 325 (4.37); FABMS *m/z*: 685 $[MH]^+$, 707 $[M+Na]^+$ (+NaI), 723 $[M+K]^+$ (+KI); ^{1}H NMR (CD_3OD, 400 MHz): δ1.31 (3H, *d*, *J* = 6 Hz), 2.45 (H, *m*), 2.57 (H, *dd*, *J* = 8, 10 Hz), 3.14 (H, *t*, *J* = 9 Hz), 3.81 (H, *d*, *J* = 13 Hz), 3.89 (3H, *s*), 4.04 (H, *dd*, *J* = 1, 8 Hz), 4.09 (H, *dd*, *J* = 2, 3 Hz), 4.15 (H, *d*, *J* = 13 Hz), 4.78 (H, *d*, *J* = 8 Hz), 4.97 (H, *d*, *J* = 2 Hz), 5.10 (H, *d*, *J* = 10 Hz), 5.12 (H, *dd*, *J* = 4, 6 Hz), 6.38 (H, *dd*, *J* = 1, 6 Hz), 6.41 (H, *d*, *J* = 16 Hz), 6.94 (H, *d*, *J* = 8 Hz), 7.06 (H, *dd*, *J* = 2, 8 Hz), 7.10 (H, *d*, *J* = 2 Hz), 7.66 (H, *d*, *J* = 16 Hz); ^{13}C NMR: see Table 1. (Found: C, 53.07; H, 6.13. $C_{31}H_{40}O_{17}\cdot H_2O$ requires: C, 52.99; H, 6.02%).

Compound **3** *octaacetate* (**4**). Compound **3** (45 mg) was treated with a mixture of Ac_2O and pyridine at 25° overnight. Usual work-up gave 58 mg of colourless powder (**4**). $[\alpha]_D$ −56.8° ($CHCl_3$; *c* 0.44), IR ν_{max}^{KBr} cm^{-1}: 1750, 1630, 1610, 1510, 1430, 1365, 1220, 1120, 1040, 900; UV λ_{max}^{MeOH} nm (log ε): 227 (4.24), 294 (4.44) inf, 309 (4.50); EIMS *m/z*: 331, 177, 169, 110; FABMS *m/z*: 1021 $[MH]^+$, 1043 $[M+Na]^+$ (+NaI), 1059 $[M+K]^+$ (+KI); ^{1}H NMR ($CDCl_3$): δ1.24 (3H, *d*, *J* = 6 Hz), 2.02 (3H, *s*), 2.03 (6H, *s*), 2.05 (3H, *s*), 2.11 (3H, *s*), 2.13 (3H, *s*), 2.17 (3H, *s*) (alcoholic Ac ×7), 2.32 (3H, *s*, phenolic Ac), 3.87 (3H, *s*), 6.20 (H, *d*, *J* = 16 Hz), 6.32 (H, *br. d*, *J* = 6 Hz), 6.97 (H, *d*, *J* = 9 Hz), 7.56 (H, *d*, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$): δ17.4, 20.6 (Ac×6), 20.8 (Ac), 20.9 (Ac), 35.4, 41.7, 56.0, 58.0, 61.1, 62.1, 62.3, 67.0, 68.3, 68.9, 70.2, 70.7, 71.1, 72.3, 72.6, 83.6, 94.3, 96.5 (×2), 102.4, 112.3, 115.6, 122.2, 127.4, 127.9, 140.1, 141.1, 144.8, 153.2, 165.7, 169.0, 169.2, 169.9, 170.0 (×2), 170.2, 170.6 (×2). (Found: C, 54.69; H, 5.48. $C_{47}H_{56}O_{25}\cdot 1/2\ H_2O$ requires: C, 54.81; H, 5.57%).

Alkaline hydrolysis of compounds **1** *and* **3**. Compounds **1** (50 mg) and **3** (50 mg) were treated with 0.2 M methanolic NaOH and stirred for 2 hr at 15° under a N_2 atmosphere. The solns were neutralized with Dowex 50W×8. The ion exchange resin was removed by filtration, and the filtrates were evapd. The residues were partitioned between EtOAc (50 ml) and H_2O (50 ml). The H_2O layers were evapd to give residues. The EtOAc layers were evapd and purified by prep. TLC (silica gel, 0.5 mm, developed with $CHCl_3$–MeOH, 15:1 and eluted with $CHCl_3$–MeOH, 9:1) to give residues (12 mg, 79% and 11 mg, 73%, respectively). The compounds from the H_2O layers were identified with an authentic sample of 6-*O*-α-L-rhamnopyranosylcatalpol (**7**) by co-chromatography on TLC (silica gel, $CHCl_3$–MeOH–H_2O, 15:6:1 and EtOAc–EtOH–H_2O, 8:2:1). The compounds from the EtOAc layers were also identified with an authentic sample of methyl isoferulate on TLC (silica gel, $CHCl_3$–MeOH, 10:1) and comparison of IR and ^{1}H NMR spectra with those of the authentic samples. Methyl isoferulate from compound **1**, IR ν_{max}^{KBr} cm^{-1}: 3350, 1715, 1630, 1605, 1580, 1505, 1440, 1350, 1310, 1260, 1165, 1125, 1020, 975, 915, 845, 800, 765; ^{1}H NMR (CD_3OD, 400 MHz): δ3.76 (3H, *s*), 3.88 (3H, *s*), 6.31 (H, *d*, *J* = 16 Hz, H-8), 6.93 (H, *d*, *J* = 8 Hz, H-5), 7.04 (H, *dd*, *J* = 2, 8 Hz, H-6), 7.06 (H, *d*, *J* = 2 Hz), 7.56 (H, *d*, *J* = 16 Hz, H-7); HR-EIMS *m/z*: 208.0732 $[M]^+$, 177.0576 $[M-Me]^+$. Methyl isoferulate from compound **3**, IR ν_{max}^{KBr} cm^{-1}: 3350, 1715, 1635, 1610, 1580, 1505, 1440, 1355, 1310, 1265, 1165, 1125, 1020, 975, 920, 850, 805, 765; ^{1}H NMR (CD_3OD, 400 MHz): δ3.76 (3H, *s*), 3.88 (3H, *s*), 6.30 (H, *d*, *J* = 16 Hz, H-8), 6.93 (H, *d*, *J* = 8 Hz, H-5), 7.04 (H, *dd*, *J* = 2, 8 Hz, H-6), 7.06 (H, *d*, *J* = 2 Hz), 7.56 (H, *d*, *J* = 16 Hz, H-7); HR-EIMS *m/z*: 208.0774 $[M]^+$, 177.0568 $[M-OMe]^+$, (208.0735, calcd. for $C_{11}H_{12}O_4$, 177.0553, calcd. for $C_{10}H_9O_3$). Authentic methyl ferulate and isoferulate were obtained by refluxing the corresponding acids in MeOH containing a few drops of conc HCl. Authentic methyl isoferulate ^{1}H NMR (CD_3OD, 400 MHz): δ3.76 (3H, *s*), 3.88 (3H, *s*), 6.31 (H, *d*, *J* = 16 Hz, H-8), 6.93 (H, *d*, *J* = 8 Hz, H-5), 7.04 (H, *dd*, *J* = 2, 8 Hz, H-6), 7.06 (H, *d*, *J* = 2 Hz), 7.56 (H, *d*, *J* = 16 Hz, H-7). Authentic methyl ferulate ^{1}H NMR (CD_3OD, 400 MHz): δ3.76 (3H, *s*), 3.88 (3H, *s*), 6.34 (H, *d*, *J* = 16 Hz, H-8), 6.80 (H, *d*, *J* = 8 Hz, H-5), 7.05 (H, *dd*, *J* = 2, 8 Hz, H-6), 7.16 (H, *d*, *J* = 2 Hz), 7.59 (H, *d*, *J* = 16 Hz, H-7).

Detection of sugar portion. *Ca* 2 mg of each compound (**1** and **3**) was heated in 2 ml HCl in dry MeOH at 95° for 3 hr. The reaction mixture was neutralized with addition of Ag_2CO_3 and then filtered. After the solvent was removed, several drops of TMS–imidazole soln were added and kept at 50° for 15 min. The reaction mixture was partitioned between 1 ml of H_2O and 2 ml of *n*-hexane. The hexane layer was concd and subjected to GLC analysis. Column: 1.5% OV-1 (2 mm × 2 m); Temp: 180° (isothermal); N_2: 40 ml/min. R_t: rham, 2.81 min, gluc, 9.08 and 9.98 min; Compound **1**: rham, 2.81 min, gluc, 9.07 (overlapped by methyl isoferulate-3-OTMS) and 9.97 min; Compound **3**: rham, 2.80 min. gluc, 9.07 (overlapped by methyl isoferulate-3-OTMS) and 10.00 min.

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