Isolation and Structure Elucidation of Mono- and Diacyl-iridoid Diglycosides from Leaves of Premna japonica

Hideaki Otsuka, Yukari Sasaki, Naoko Kubo, Kazuo Yamasaki, Yoshio Takeda, and Tarow Seki

J. Nat. Prod., **1991**, 54 (2), 547-553• DOI: 10.1021/np50074a030 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 2, pp. 547-553, Mar-Apr 1991

ISOLATION AND STRUCTURE ELUCIDATION OF MONO- AND DIACYL-IRIDOID DIGLYCOSIDES FROM LEAVES OF *PREMNA JAPONICA*

HIDEAKI OTSUKA,* YUKARI SASAKI, NAOKO KUBO, KAZUO YAMASAKI,

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

YOSHIO TAKEDA,

Faculty of Pharmaceutical Sciences, The University of Tokushima, 1-79, Shoumachi, Tokushima, Tokushima 770, Japan

and TAROW SEKI

Miyajima Natural Botanical Garden, Faculty of Science, Hiroshima University, Miyajima-cho, Saeki-gun 739-05, Japan

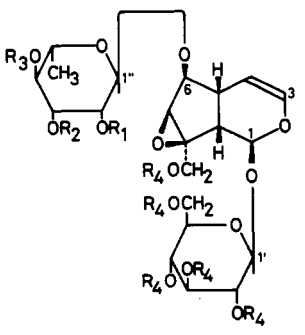
ABSTRACT.—On further phytochemical investigation of *Premna japonica*, four new mono and diacyl 6-α-L-rhamnopyranosylcatalpols were isolated. Their structures were established to be 6-*O*-α-L-(2″-*O*-*p*-methoxycinnamoyl)rhamnopyranosylcatalpol [**1**] and 6-*O*-α-L-(3″-*O*-*p*-methoxycinnamoyl)rhamnopyranosylcatalpol [**3**] and 6-*O*-α-L-(2″-*O*-*p*-methoxycinnamoyl-4″-*O*-acetyl)rhamnopyranosylcatalpol [**5**] and 6-*O*-α-L-(3″-*O*-*p*-methoxycinnamoyl-4″-*O*-acetyl)rhamnopyranosylcatalpol [**6**] from spectroscopic data and the results of acetylation experiments.

From the leaves of the Philippine medicinal plant, *Premna odorata* Blanco (Verbenaceae), two monoacyl and four diacyl 6-*O*-α-L-rhamnopyranosylcatalpols were isolated (1,2). Therefore, constituents of *Premna japonica* Miq., a relative of this plant which grows in southwestern Japan, attracted our attention. In previous papers, we reported the isolation of derivatives of 6-*O*-α-L-rhamnopyranosylcatalpol from the leaves of *P. japonica* (3–5). On further investigation of the plant, two monoacyl and two diacyl 6-*O*-α-L-rhamnopyranosylcatalpols were obtained from the *n*-BuOH-soluble fraction of the MeOH extract. This paper describes the isolation and structure determination of these compounds.

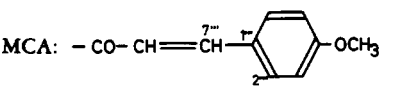
To date, monoacyl derivatives of 6-*O*-α-L-rhamnopyranosylcatalpol have been isolated from *P. odorata* (1), *P. japonica* (3), *Verbascum sinuatum* (Scrophulariaceae) (6), *Verbascum saccatum* (7), and *Verbascum georgicum* (8). Diacyl derivatives have been obtained from *P. odorata* (2) and *V. sinuatum* (9). Only one report has appeared on the isolation of triacyl 6-*O*-α-L-rhamnopyranosylcatalpols and that was from *Scrophularia scopolii* var. *scopolii* (10).

RESULTS AND DISCUSSION

From the MeOH extract of leaves of *P. japonica*, the two monoacyl **1**, **3** and two



1 R_1=*E*-MCA, R_2=R_3=R_4=H
2 R_1=*E*-MCA, R_2=R_3=R_4=Ac
3 R_1=R_3=R_4=H, R_2=*E,Z*-MCA
4 R_1=R_3=R_4=Ac, R_2=*E,Z*-MCA
5 R_1=*E,Z*-MCA, R_2=R_4=H, R_3=Ac
6 R_1=R_4=H, R_2=*E,Z*-MCA, R_3=Ac
7 R_1=R_2=R_3=R_4=H



diacyl **5**, **6** 6-*O*-α-L-rhamnopyranosylcatalpols were isolated as powders by the combination of Diaion HP-20 and Si gel cc and droplet counter-current chromatography (dccc).

Compound **1**, $C_{31}H_{40}O_{16}$, was obtained as a white amorphous powder, whose mol wt was analyzed for 668 by the observation of ion peaks at *m/z* 691 $[M+Na]^+$ (+NaI) and 707 $[M+K]^+$ (+KI) in fabms. The ir spectrum indicated the presence of a conjugated ester (1695 and 1625 cm^{-1}) and an aromatic ring (1600 and 1510 cm^{-1}). Typical trans olefinic proton signals [δ 6.44 (H, d, $J=16$ Hz) and 7.69 (H, d, $J=16$ Hz)] and four protons coupled in an AA'BB' system [δ 6.95 (2H, d, $J=9$ Hz) and 7.64 (2H, d, $J=9$ Hz)] were observed in the ^{1}H-nmr spectrum. The two ^{13}C-nmr signals at δ 115.5 (d) and 131.1 (d), which were of higher intensities, were attributed to the 3,5 and 2,6 positions of 1,4-disubstituted benzene, respectively. From other signals at δ 3.85 (3H, s) in the ^{1}H-nmr spectrum and at δ 128.3 (s), 163.2 (s), 168.5 (s), and 55.9 (q) in the ^{13}C-nmr spectrum, the acyl moiety in **1** was concluded to be *trans-p*-methoxycinnamic acid (10). The remaining ^{13}C-nmr signals suggested that **1** was a de-

TABLE 1. ^{1}H-nmr Data for Compounds **5** and **6**.[a]

Proton	Compound			
	5		**6**	
	E	*Z*	*E*	*Z*
H-1	5.09 (d, 10)	5.08 (d, 10)	5.10 (d, 10)	5.10 (d, 10)
H-3	6.38 (dd, 2, 6)		6.39 (dd, 2, 6)	
H-4	5.04 (dd, 4, 6)		—[b]	
H-5	2.43 (m)		2.47 (m)	
H-6	4.03 (dd, 1, 8)	4.02 (dd, 1, 8)	4.06 (dd, 1, 8)	4.05 (dd, 1, 8)
H-7	3.66 (br s)	3.65 (br s)	3.67 (br s)	
H-9	2.57 (dd, 8, 10)		2.58 (dd, 7, 9)	
H-10a	3.80 (d, 13)		3.82 (d, 13)	
H-10b	4.15 (d, 13)	4.13 (d, 13)	4.27 (d, 13)	
H-1′	4.77 (d, 8)	4.77 (d, 8)	4.78 (d, 8)	4.78 (d, 8)
H-2′	—[b]		—[b]	
H-3′	3.25 (m)		3.25 (m)	
H-4′	3.39 (t, 9)		3.40 (t, 9)	
H-5′	3.25 (m)		3.25 (m)	
H-6′a	3.62 (dd, 7, 12)		3.63 (dd, 7, 12)	
H-6′b	3.91 (dd, 2, 12)		3.92 (dd, 2, 12)	
H-1″	5.07 (br s)		5.02 (d, 1)	5.00 (d, 1)
H-2″	5.19 (dd, 2, 4)	5.16 (dd, 2, 4)	—[b]	
H-3″	4.10 (dd, 4, 10)	4.08 (dd, 4, 10)	—[b]	
H-4″	5.02 (t, 10)	4.91 (t, 10)	—[b]	
H-5″	—[b]		—[b]	
H-6″	1.19 (d, 6)	1.15 (d, 6)	1.20 (d, 6)	1.18 (d, 6)
H-2‴, -6‴	7.59 (d, 9)	7.76 (d, 9)	7.56 (d, 9)	7.77 (d, 9)
H-3‴, -5‴	6.96 (d, 9)	6.90 (d, 9)	6.96 (d, 9)	6.91 (d, 9)
H-7‴	7.71 (d, 16)	6.97 (d, 13)	7.68 (d, 16)	—[b]
H-8‴	6.50 (d, 16)	5.94 (d, 13)	6.38 (d, 13)	5.84 (d, 13)
-OMe	3.83 (s)	3.82 (s)	3.83 (s)	3.82 (s)
MeCO-	2.12 (s)	2.10 (s)	2.01 (s)	1.97 (s)

[a]At 400 MHz, MeOH-d_4. Multiplicities and coupling constants (in Hz) are in parentheses. The same values for the same positions of *E*-*Z*-isomers were actually different, if the third decimal places are taken into consideration.

[b]Signals could not be assigned due to their being hidden by solvent or HDO signals, or due to overlap of several signals.

rivative of 6-O-α-L-rhamnopyranosylcatalpol [7] (1,11). The site of esterification in **1** was determined to be the 2″-position of the rhamnopyranosyl moiety, because on comparison of the ^{13}C-nmr data of **1** with those of **7**, a significant downfield shift (Δ δ 2.0 ppm) was observed at the 2″ position, and the signals on both sides of carbon atoms 1″ and 3″ were shifted upfield by 2.5 and 1.7 ppm, respectively. Thus, the structure of **1** was elucidated to be 6-O-α-L-(2″-*O*-*trans*-*p*-methoxycinnamoyl)rhamnopyranosylcatalpol [**1**]. Acetylation of compound **1** afforded the heptaacetate **2**, which showed seven aliphatic acetyl signals in the ^{1}H-nmr spectrum, and the observed mol wt (*m/z* 962) on fabms indicated an increase of 294 mass units from **1**, which also accounted for seven acetyl groups and helped to confirm the structure of **1**.

TABLE 2. ^{13}C-nmr Data for Compounds **1**, **3**, and **5**–**7**.[a]

Carbon	1	3		5		6		7[b]
		E	*Z*	*E*	*Z*	*E*	*Z*	
Aglycone								
C-1	95.2	95.2		95.2		95.2		95.1
C-3	142.3	142.2		142.5		142.4		142.1
C-4	103.4	103.6		103.3		103.4		103.6
C-5	37.2	37.2		37.3		37.3		37.2
C-6	84.4	83.8		84.8		84.3		83.5
C-7	59.5	59.3		59.6		59.4		59.3
C-8	66.5	66.6		66.6		66.6		66.5
C-9	43.3	43.3		43.3		43.3		43.2
C-10	61.5	61.5		61.5		61.5		61.4
Glucose								
C-1′	99.7	99.7		99.7		99.8		99.7
C-2′	74.8	74.8		74.9		74.9		74.8
C-3′	78.6	78.6		78.7		78.7		78.5
C-4′	71.7	71.7		71.8		71.8		71.7
C-5′	77.7	77.7		77.7		77.7		77.6
C-6′	62.9	62.9		62.9		63.0		62.9
Rhamnose								
C-1″	97.8	100.2		97.8		100.4		100.3
C-2″	74.2	70.3		74.2	73.9	70.2	70.2	72.2
C-3″	70.5	75.4	75.1	68.5	68.4	72.6	72.6	72.2
C-4″	74.2	71.4	71.4	75.4	75.4	73.1	72.8	73.8
C-5″	70.3	70.3		68.2		68.2	68.3	70.1
C-6″	18.0	18.0		17.9	17.9	17.8		18.0
C_6C_3								
C-1‴	128.3	128.4	128.7	128.4	128.7	128.3	128.6	
C-2‴	131.1	131.0	133.6	131.2	133.7	131.2	133.8	
C-3‴	115.5	115.5	114.4	115.5	114.5	115.5	114.5	
C-4‴	163.2	163.1	162.1	163.4	162.2	163.4	162.3	
C-5‴	115.5	115.5	114.4	115.5	114.5	115.5	114.5	
C-6‴	131.1	131.0	133.6	131.2	133.7	131.2	133.8	
C-7‴	146.7	146.4	144.8	147.1	145.9	147.0	145.8	
C-8‴	115.9	116.3	117.6	115.8	117.0	115.7	116.8	
C-9‴	168.5	168.7	167.6	168.4	167.3	168.1	167.1	
-OCH_3	55.9	55.9	55.8	55.9	55.8	55.9	55.8	
CH_3CO				21.0		20.80	20.84	
MeCO				172.4	172.3	171.9	171.8	

[a]In MeOH-d_4. The same values for the same positions of *E*-*Z*-isomers were actually different, if the second decimal places are taken into consideration.

[b]Data in this column are from Otsuka *et al.* (1).

Compound **3**, $C_{31}H_{40}O_{16}$, was also obtained as a white amorphous powder. Although the spectroscopic data for **3** were essentially similar to those for **1**, the ^{1}H- and ^{13}C-nmr spectra showed some complexity. Besides the presence of a trans double bond, which was indicated by the signals at δ 6.46 (d, J = 16 Hz) and 7.73 (d, J = 16 Hz), another set of smaller doublet signals at δ 5.93 (J = 13 Hz) and 7.66 ppm (J = 13 Hz) was observed. Furthermore, two aromatic proton signals at δ 6.95 (d, J = 9 Hz) and 7.56 (d, J = 9 Hz) were accompanied by smaller signals at δ 6.89 (d, J = 9 Hz) and 7.77 ppm (d, J = 9 Hz). In the ^{13}C-nmr spectrum, a set of nine signals and a methoxyl carbon signal were reasonably attributed to *trans-p*-methoxycinnamate (see **1** and **3** in Table 2). But these ten signals also appeared with smaller satellite signals, which were assigned to *cis-p*-methoxycinnamate. From this evidence, the acyl portion of **3** was deduced to be comprised of *trans-* and *cis-p*-methoxycinnamic acids. Other ^{13}C-nmr signals showed a close resemblance to those of **1**, except rhamnopyranosyl carbon signals, indicating that **3** is a positional isomer as to the acyl group in **1**. The site of esterification was determined to be the 3″-hydroxyl group from acylation-induced shifts [2″: Δ δ_{3-7} −1.9 ppm, 3″: Δ δ_{3-7} +3.2 ppm (and +2.9 ppm), and 4″: Δ δ_{3-7} −2.4 ppm]. The structure of **3** was thus established to be a mixture of 6-*O*-α-L-(3″-*O*-*trans-* and *cis-p*-methoxycinnamoyl)rhamnopyranosycatalpols. The trans-cis ratio was calculated to be 67:33 from integrations of the ^{1}H-nmr signals. Compound **3** also afforded its heptaacetate **4** in the acetylation experiment.

Compound **5**, $C_{33}H_{42}O_{17}$, was obtained as a pale yellow amorphous powder. The ^{1}H- and ^{13}C-nmr spectra indicated that **5** has acetyl and *trans-* and *cis-p*-methoxycinnamoyl moieties (*E* to *Z* ratio = 71:29) as the acyl portion. The positions of these acyl moieties were also determined to be 2″ and 4″ from the significant acylation shifts from **7** to **5** [1″: Δ δ_{5-7} −2.5 ppm, 2″: Δ δ_{5-7} +2.0 ppm (and +1.7 ppm), 3″: Δ δ_{5-7} −3.7 ppm (and −3.8 ppm), 4″: Δ δ_{5-7} +1.6 ppm, and 5″: Δ δ_{5-7} −1.9 ppm]. The location of the acyl groups was determined by long-range proton selective decoupling experiments. In the non-decoupled ^{13}C-nmr spectrum of **5**, one carbonyl carbon at δ 168.4 showed a quintet-like (actually ddd) signal, and the other carbonyl carbon signal at δ 172.4 appeared as a multiplet (a quartet of doublet-like signals). This allowed unequivocal assignment of these carbonyl carbon signals. The 2″ and 4″ rhamnosyl ring protons for the *E* isomer were well resolved, appearing at δ 5.19 and 5.02, respectively (Table 1). When the proton at δ 5.02 (H-4″) was irradiated, the carbon signal at δ 172.4 (acetyl-CO) collapsed to a quartet (J_{CCH} = 6.6 Hz), whereas the other signal remained intact. Irradiation of the proton at δ 5.19 (H-2″) removed one of the long-range couplings from the carbonyl carbon signal at δ 168.4 (*E-p*-methoxycinnamoyl-CO) to a doublet of doublets (J_{CCH} = 2.2 and J_{CCCH} = 7.3 Hz). At the same time, as the proton signal at δ 5.16 for H-2″ of the *Z*-isomer was close to that for the *E*-isomer, the signal at δ 167.3 (*Z-p*-methoxycinnamoyl-CO) was also decoupled by the irradiation to a broad doublet [J_{CCCH} = 15.4 Hz (12)]. Thus, the C_6-C_3 moiety was located at the 2″ position, and the acetyl at the 4″ position. Because the signals for the *E*-isomer in the ^{13}C-nmr spectrum of **5** hexaacetate matched well with those of **1** heptaacetate [**2**], this also supported the esterified positions of two acyl groups. Therefore, the structure of **5** was determined to be a mixture of 6-*O*-α-L-(2″-*O*-*trans-* and *cis-p*-methoxycinnamoyl-4″-*O*-acetyl)rhamnopyranosylcatalpols.

Compound **6**, $C_{33}H_{42}O_{17}$, was also obtained as a pale yellow amorphous powder. The ^{13}C-nmr spectrum indicated that compound **6** is a positional isomer as to the acyl groups in **5**. The positions of esterfication were determined to be 3″ and 4″ of rhamnopyranosyl hydroxyl groups from the acylation-induced shift trend [1″: Δ δ_{6-7} +0.1 ppm, 2″: Δ δ_{6-7} −2.0 ppm, 3″: Δ δ_{6-7} +0.4 ppm, 4″: Δ δ_{6-7} −0.7 ppm (and −0.1 ppm), and 5″: Δ δ_{6-7} −1.9 ppm (and −1.0 ppm)]. Attempts to determine the location

of acyl moieties by long-range selective decoupling experiments could not be conducted, because the 3″ and 4″ rhamnose ring protons were not well resolved. Therefore, compound **6** was partially hydrolyzed under mild basic conditions. Two uv-active compounds thus obtained were identified as 2″-*O*- and 3″-*O*-*p*-methoxycinnamoyl derivatives **1** and **3** of 6-*O*-α-L-rhamnopyranosylcatalpol on tlc and by hplc. Because facile acyl migration has been reported between the 2- and 3-hydroxyl groups of rhamnose, but not between 4 and other positions (13), the original location of the *p*-methoxycinnamoyl and acetyl groups should be the 3″ and 4″ positions, respectively. Finally, the structure of **4** was determined to be a mixture of 6-*O*-α-L-(3″-*O*-*trans*- and *cis*-*p*-methoxycinnamoyl-4″-*O*-acetyl)rhamnopyranosylcatalpols (*E* to *Z* ratio = 71:29). This was also confirmed by identity of the ^{13}C-nmr spectrum and other spectroscopic data for its hexaacetate with those for **3** heptaacetate [**4**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Instruments used were as follows: 400 MHz ^{1}H-nmr and 100 MHz ^{13}C-nmr, JEOL GX-400; 100 MHz ^{1}H-nmr and 25 MHz ^{13}C-nmr, JEOL FX-100; optical rotations, Union Giken PM-101; ir, Shimadzu IR-400s; uv, Shimadzu UV-200s; ms, JEOL DX-300; dccc, Tokyo Rikakikai DCC-A; glc, Shimadzu GC-4CMPF with FID; hplc, Tosoh CCPM and UV-8000, and tlc, Merck Si gel F_{254} precoated on aluminum sheets.

PLANT MATERIAL.—Leaves of *P. japonica* were collected in the southeastern part of Tokushima Prefecture (Shikoku Island) in May 1988. The voucher specimen (88-PJ-Tokushima-1) was deposited at the Herbarium of the Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine.

ISOLATION PROCEDURE.—The dried leaves of *P. japonica* (1.45 kg) were extracted with MeOH. The MeOH extract (285.0 g) was dissolved in 95% aqueous MeOH and then extracted with *n*-hexane. The concentrated MeOH layer was suspended in H_2O and then extracted with EtOAc and *n*-BuOH successively. The *n*-BuOH extract (84.38 g) was separated by highly porous polymer (Diaion HP-20) cc with stepwise increases of MeOH content in H_2O (20, 40, 60, 80 and 100%). The 60–80% MeOH eluent (14.0 g) was separated by Si gel cc, and compounds **1** and **3** were eluted in the $CHCl_3$-MeOH-H_2O (15:3:0.1) fraction (5.44 g). This fraction (5.40 g) was repeatedly separated by Si gel cc with $CHCl_3$-MeOH (94:6–85:15) to give 2.24 g of a further purified fraction enriched in compounds **1** and **3**. The final purification of these compounds was performed by dccc. With 3 runs of dccc [$CHCl_3$–MeOH–H_2O–*n*-PrOH (45:60:40:10), 600 mg for each run], totals of 359 mg of compound **1** and 474 mg of compound **3** were obtained. Although compound **1** was obtained in a pure state, compound **3** was still accompanied by some foreign substances. Repeated dccc of compound **3** with the same solvent system afforded 170 mg of an amorphous powder.

From the 80% MeOH eluent (6.48 g) on the Diaion HP-20 cc, compounds **5** and **6** were obtained by repeated Si gel cc. The first Si gel cc gave 438 mg of crude compound **5** in the 5% MeOH in $CHCl_3$ fractions and 574 mg of crude compound **6** in the 6% MeOH in $CHCl_3$ fractions. The second Si gel cc of the crude compound **5** fraction afforded 153 mg of **5** [$CHCl_3$-MeOH (96:4)], and 137 mg of **6** was also obtained on the second Si gel cc [$CHCl_3$-MeOH (95:5)] from the crude compound **6** fraction. The homogeneity of each compound was checked by tlc [Si gel; $CHCl_3$-MeOH-H_2O (15:6:1) and EtOAc-EtOH-H_2O (8:2:1)].

6-O-α-L-(2″-O-trans-p-*Methoxycinnamoyl)rhamnopyranosylcatalpol* [**1**].—White amorphous powder, $[\alpha]_D$ −120.9° (*c* = 0.32, MeOH); ir ν max (KBr) 3325, 2875, 1695, 1625, 1600, 1510, 1250, 1170, 1050, 915, 830 cm^{-1}; uv λ max (MeOH) (log ε) 226 (4.18), 300 (4.45) inf, 311 (4.50) nm; fabms *m/z* $[M+Na]^+$ 691 (+NaI), $[M+K]^+$ (+KI) 707; ^{1}H nmr (100 MHz, CD_3OD) δ 1.31 (3H, d, *J* = 6 Hz, H-6″), ~2.5 (2H, m, H-5, H-9), 3.66 (H, br s, H-7), 3.83 (3H, s, C*H*$_3$O-), 6.44 (H, d, *J* = 16 Hz, H-8‴), 6.46 (H, br d, *J* = 6 Hz, H-3), 6.95 (2H, d, *J* = 9 Hz, H-3‴, -5‴), 7.64 (2H, d, *J* = 9 Hz, H-2‴, -6‴), 7.69 (H, d, *J* = 16 Hz, H-7‴); ^{13}C nmr see Table 2. Found C 54.88, H 6.22; $C_{31}H_{40}O_{16}\cdot\frac{1}{2}H_2O$ requires C 54.95, H 6.10.

6-O-α-L-(2″-O-trans-p-*Methoxycinnamoyl)rhamnopyranosylcatalpol hexaacetate* [**2**].—Compound **1** (39 mg) was treated with a mixture of Ac_2O (1 ml) and pyridine (1 ml) at 20° overnight. The usual workup gave 47 mg of a white amorphous powder: $[\alpha]_D$ −44.5° (*c* = 0.44, $CHCl_3$); ir ν max (KBr) 1750, 1600, 1510, 1365, 1220, 1150, 1040, 830 cm^{-1}; uv λ max (MeOH) (log ε) 227 (4.18), 300 (4.44) inf, 311 (4.50) nm; eims *m/z* 380, 331, 169, 161, 109; fabms *m/z* $[MH]^+$ 963, $[M+Na]^+$ (+NaI) 985, $[M+K]^+$

(+KI) 1001; ^{1}H nmr (100 MHz, $CDCl_3$) δ 1.25 (3H, d, J = 6 Hz, H-6″), 1.99, 2.01, 2.03, 2.04, 2.06, 2.10, 2.12 (Ac × 7), ~2.5 (2H, m, H-5, -9), 3.85 (3H, s, CH_3O-), 6.34 (H, br d, J = 6 Hz, H-3), 6.41 (H, d, J = 16 Hz, H-8‴), 6.92 (2H, d, J = 9 Hz, H-3‴, -5‴), 7.53 (2H, d, J = 9 Hz, H-2‴, -6‴), 7.70 (H, d, J = 16 Hz, H-7‴); ^{13}C nmr (25 MHz, $CDCl_3$) δ 17.4, 20.6 (Ac × 7), 35.5, 41.7, 55.4, 58.0, 61.1, 62.1, 62.4, 67.0 (C-5″), 68.3 (C-4′), 69.0 (C-3″), 69.8 (C-2″), 70.6 (C-4″), 71.2 (C-2′), 72.3 (C-5′), 72.6 (C-3′), 83.5, 94.3, 96.5 (×2), 102.4, 114.4 (×3), 126.9, 130.0 (×2), 141.1, 146.0, 161.8, 166.3, 169.0, 169.2, 169.9 (×2), 170.2, 170.6 (×2). Found C 55.71, H 5.64; $C_{45}H_{54}O_{23}\cdot\frac{1}{2}H_2O$ requires C 55.61, H 5.70.

6-O-α-L-(2″-O-trans- *and* cis-p-*Methoxycinnamoyl)rhamnopyranosylcatalpols* [**3**].—White amorphous powder (*E* to *Z* ratio = 67:33): [α]D −116.8° (c = 0.31, MeOH); ir ν max (KBr) 3300, 2875, 1690, 1620, 1595, 1570, 1510, 1300–1000, 915, 830 cm^{-1}; uv λ max (MeOH) (log ε) 225 (4.17), 300 (4.42) inf, 310 (4.46) nm; fabms m/z $[M + Na]^+$ (+NaI) 691, $[M + K]^+$ (+KI) 707; ^{1}H nmr (100 MHz, CD_3OD) δ (ppm) 1.33 (3H, d, J = 6, H-6″), ~2.5 (m, H-5, -9), 3.67 (br s, H-7), 3.81 (s, *Z*-CH_3O-), 3.83 (s, *E*-CH_3O-), 5.94 (d, J = 13 Hz, *Z*-H-8‴), 6.39 (H, br d, J = 6 Hz, H-3), 6.46 (d, J = 16 Hz, *E*-H-8‴), 6.89 (d, J = 9 Hz, *Z*-H-3‴, -5‴), 6.95 (d, J = 9 Hz, *E*-H-3‴, -5‴), 7.56 (d, J = 9 Hz, *E*-H-2‴, -6‴), 7.66 (d, J = 13 Hz, *Z*-H-7‴), 7.73 (d, J = 16 Hz, *E*-H-7‴), 7.77 (d, J = 9 Hz, *Z*-H-2‴, -6‴); ^{13}C nmr see Table 2. Found C 55.21, H 6.21; $C_{31}H_{40}O_{16}\cdot\frac{1}{2}H_2O$ requires C 54.95, H 6.10.

6-O-α-L-(2″-O-trans- *and* cis-p-*Methoxycinnamoyl)rhamnopyranosylcatalpol heptaacetates* [**4**].—Compound **2** (51 mg) was treated with a mixture of Ac_2O and pyridine at 20° overnight. The usual workup gave 58 mg of a white amorphous powder: [α]D −64.8° (c = 0.54, $CHCl_3$); ir ν max (KBr) 1745, 1625, 1600, 1505, 1365, 1220, 1150, 1040, 975, 905, 803 cm^{-1}; uv λ max (MeOH) (log ε) 226 (4.23), 300 (4.46) inf, 310 (4.49) nm; eims m/z 391, 331, 169, 161, 109; fabms m/z $[M + Na]^+$ (+NaI) 985, $[M + K]^+$ (+KI) 1001; ^{1}H nmr (100 MHz, $CDCl_3$) δ 1.25 (3H, d, J = 6 Hz, H-6″), 2.03 (×4), 2.11, 2.13, 2.17 (Ac × 7), ~2.55 (2H, m, H-5, -9), 3.34 (3H, s, CH_3O-), 5.68 (d, J = 12 Hz, *Z*-H-8‴), 6.18 (d, J = 16 Hz, *E*-H-8‴), 6.34 (H, br d, J = 6 Hz, H-3), 6.90 (2H, d, J = 9 Hz, H-3‴, -5‴), 7.48 (2H, d, J = 9 Hz, H-2‴, -6‴) 7.61 (d, J = 16 Hz, *E*-H-7‴); ^{13}C nmr (25 MHz, $CDCl_3$) δ 17.4, 20.6 (Ac × 7), 35.4, 41.7, 55.4, 58.0, 61.1, 62.1, 62.4, 67.0 (C-5″), 68.3 (C-4′), 68.4 (*Z*-C-3″), 68.7 (*E*-C-3″), 69.9 (*Z*-C-2″), 70.3 (*E*-C-2″), 70.6 (C-4″), 71.2 (C-2′), 72.2 (C-5′), 72.6 (C-3′), 83.5, 94.3, 96.5 (×2), 102.4, 113.5 (*Z*-C-3‴, -5‴), 114.3 (*E*-C-3‴, -5‴ and *E*-C-8‴), 115.6 (*Z*-C-8‴), 126.9 (*E*-C-1‴), 127.1 (*Z*-C-1‴), 130.0 (*E*-C-2‴, -6‴), 132.5 (*Z*-C-2‴, -6‴), 141.1, 145.6, 160.7 (*Z*-C-3‴), 161.7 (*E*-C-3‴), 164.9 (*Z*-C-9‴), 166.0 (*E*-C-9‴), 169.0, 169.2, 169.9, 170.0, 170.2, 170.6 (×2). Found C 55.52, H 5.60; $C_{45}H_{54}O_{23}\cdot\frac{1}{2}H_2O$ requires C 55.61, H 5.70.

6-O-α-L-(2″-O-trans- *and* cis-p-*Methoxycinnamoyl-4″-O-acetyl)rhamnopyranosylcatalpols* [**5**].—Pale yellow amorphous powder (*E* to *Z* ratio = 66:34): [α]D −70.5° (c = 0.40, MeOH); ir ν max (KBr) 3400, 2900, 1720, 1625, 1600, 1510, 1250, 1170, 1135, 1070–1710, 920, 835 cm^{-1}; uv λ max (MeOH) (log ε) 227 (3.97), 303 (4.24) *inf*, 312 (4.29)nm; fabms m/z $[M + Na]^+$ (+NaI) 733, $[M + K]^+$ (+KI) 749; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2. Found C 54.84, H 6.21; $C_{33}H_{42}O_{17}\cdot\frac{1}{2}H_2O$ requires C 55.07, H 6.02.

6-O-α-L-(2″-O-trans- *and* cis-p-*Methoxycinnamoyl-4″-O-acetyl)rhamnopyranosylcatalpol hexaacetates* [**2**].—Compound **5** (40 mg) was treated with a mixture of Ac_2O and pyridine at 20° overnight. The usual workup gave 46 mg of an amorphous powder: [α]D −36.3° (c = 1.33, $CHCl_3$); ir ν max (KBr) 2900, 1345, 1625, 1600, 1510, 1365, 1220, 1050, 1075, 1040, 830 cm^{-1}; uv λ max (MeOH) (log ε) 228 (3.95), 302 (4.24) inf, 313 (4.30) nm; eims m/z 391, 331, 169, 161, 109, 43; fabms m/z $[M + Na]^+$ (+NaI) 985, $[M + K]^+$ (+KI) 1001; ^{1}H nmr (100 MHz, $CDCl_3$) δ 1.21 (d, J = 6 Hz, *Z*-H-6″), 1.25 (d, J = 6 Hz, *E*-H-6″), 1.99, 2.01, 2.03, 2.04, 2.06, 2.10, 2.12 (Ac × 7), ~2.5 (2H, m, H-5, -9), 3.59 (H, br s, H-7), 3.83 (s, *Z*-CH_3O-), 3.85 (s, *E*-CH_3O-), 5.93 (d, J = 13 Hz, *Z*-H-8‴), 6.32 (H, br d, J = 6 Hz, H-3), 6.41 (d, J = 16 Hz, *E*-H-8‴), 6.89 (d, J = 9 Hz, *Z*-H-3‴, -5‴), 6.92 (d, J = 9 Hz, *E*-H-3‴, -5‴), 7.58 (d, J = 9 Hz, *E*-H-2‴, -6‴), 7.70 (d, J = 16 Hz, *E*-H-7‴), 7.75 (d, J = 9 Hz, *Z*-H-2‴, -6‴); ^{13}C nmr (25 MHz, $CDCl_3$) δ 17.4, 20.6 (×7), 35.5, 41.8, 55.3 (*Z*-CH_3O-), 55.4 (*E*-CH_3O-), 58.0, 61.1, 62.2, 62.4, 67.0 (C-5″), 68.3 (C-4′), 69.0 (C-3″), 69.6 (*Z*-C-2″), 69.8 (*E*-C-2″), 70.7 (C-4″), 71.2 (C-2′), 72.3 (C-5′), 72.6 (C-3′), 83.6, 94.3, 96.6 (×2), 102.4, 113.6 (*Z*-C-3‴, -5‴), 114.4 (*E*-C-3‴, -5‴), 114.5 (*E*-C-8‴), 115.7 (*Z*-C-8‴), 126.9 (*E*-C-1‴), 127.2 (*Z*-C-1‴), 130.1 (*E*-C-2‴, -6‴), 132.6 (*Z*-C-2‴, -6‴), 141.1, 145.5 (*Z*-C-7‴), 146.0 (*E*-C-7‴), 160.8 (*Z*-C-4‴), 161.8 (*E*-C-4‴), 165.0 (*Z*-C-9‴), 166.3 (*E*-C-9‴), 169.0, 169.2, 169.9 (×2), 170.2, 170.6 (×2). Found C 56.11, H 5.78; $C_{45}H_{54}O_{23}$ requires C 56.13, H 5.65.

6-O-α-L-(3″-O-trans- *and* cis-p-*Methoxycinnamoyl-4″-O-acetyl)rhamnopyranosylcatalpols* [**6**].—Pale yellow amorphous powder (*E* to *Z* ratio = 71:29): [α]D −109.0° (c = 0.42, MeOH); iv ν max (KBr) 3400, 2900, 1720, 1625, 1600, 1505, 1250, 1160, 1070–1010, 920, 830 cm^{-1}; uv λ max (MeOH) (log ε) 230

(4.00), 302 (4.22) inf, 312 (4.27) nm; fabms *m/z* $[M+Na]^+$ (+NaI) 733, $[M+K]^+$ (+KI) 749; ^1H nmr see Table 1; ^{13}C nmr see Table 2. Found C 54.84, H 6.20; $C_{33}H_{42}O_{17}\cdot\frac{1}{2}H_2O$ requires C 55.07, H 6.02.

6-O-α-L-(3″-O-trans- *and* cis-p-*Methoxycinnamoyl*-4″-O-*acetyl*)*rhamnopyranosylcatalpol hexaacetates* [**4**].—Compound **6** (37 mg) was treated with a mixture of Ac_2O and pyridine at 20° overnight. The usual workup gave 45 mg of an amorphous powder: $[\alpha]_D$ −62.6° (*c* = 1.65, $CHCl_3$); ir ν max (KBr) 2925, 1745, 1630, 1605, 1510, 1370, 1225, 1150, 1040, 980, 830 cm^{-1}; uv λ max (MeOH) (log ε) 227 (3.98), 303 (4.22) inf, 312 (4.28) nm. Eims, fabms, and ^1H- and ^{13}C-nmr data were essentially the same as those of **4**. Found C 56.29, H 5.79; $C_{45}H_{54}O_{23}$ requires C 56.13, H 5.65.

MILD ALKALINE HYDROLYSIS OF **6**.—Compound **6** (5 mg) was treated with 1 ml of 0.01 M NaOH in H_2O at 0° under an N_2 atmosphere for 30 min. The reaction was stopped by the addition of Dowex 50 × 8, when the starting material had disappeared on tlc, and then subjected to tlc and hplc analyses. For tlc [Si gel, $CHCl_3$-MeOH-H_2O (15:6:1)] three new spots along with a faint spot of the starting material were visualized by spraying with 10% H_2SO_4 and heating. Two of them were uv-active and showed the same R_f values as compounds **1** and **3**, while the other was uv-inactive and was expected to be 6-α-L-rhamnopyranosylcatalpol [**7**]. Hplc: column Inertsil-ODS (10 μ, 6.0 mm × 250 mm); solvent 45% aqueous MeOH (1.6 ml/min, 30°); detection uv 311 nm. Retention times: compound **1** 10.5 (*Z*) and 11.7 (*E*) min; compound **3** 7.8 (*Z*) and 8.8 (*E*) min; compound **6** >30 min; hydrolysate of compound **6** 7.8, 8.9, 10.6, and 11.7 min. For compound **1**, a small peak for the *Z* isomer could be seen in a fresh MeOH solution, but thereafter its amount increased until the *Z*-*E* ratio reached approximately 3:7.

ANALYSIS OF THE SUGAR PORTION.—About 2 mg of each sample was treated with 1.5 ml of 5% HCl in dry MeOH at 95° for 3 h. The reaction mixture was neutralized by the addition of Ag_2CO_3 and filtered. The filtrate was evaporated to dryness, and several drops of TMS-imidazole were added. After 15 min at 60°, H_2O (2 ml) and *n*-hexane (2 ml) were added. The partitioned *n*-hexane layer was evaporated and subjected to glc analysis: column 1.5% OV-1 (2 mm × 2 m); temperature 180° (isothermal); N_2 40 ml/min. Retention times: rha 2.81 min, gluc 9.08 and 9.98 min; compound **1** rha 2.81 min, glc 9.08 and 9.98 min; compound **3** rha 2.81 min, glc 9.07 and 10.01 min; compound **5** rha 2.80 min, glc 9.07 and 10.01 min; compound **6** rha 2.80 min, glc 9.06 and 9.99 min.

ACKNOWLEDGMENTS

We are indebted to the late Professor H. Yoshida and Ms. K. Katayama of Hiroshima University for the elemental analyses, to Ms. Y. Yoshioka of The University of Tokushima for the mass spectral measurements, and to the Ministry of Education, Science and Culture for the financial support (Grant No. 0151154).

LITERATURE CITED

1. H. Otsuka, N. Kubo, K. Yamasaki, and W.G. Padolina, *Phytochemistry*, **28**, 513 (1989).
2. H. Otsuka, N. Kubo, K. Yamasaki, and W.G. Padolina, *Phytochemistry*, **28**, 3063 (1989).
3. H. Otsuka, Y. Sasaki, K. Yamasaki, Y. Takeda, and T. Seki, *Phytochemistry*, **28**, 3069 (1989).
4. H. Otsuka, Y. Sasaki, K. Yamasaki, Y. Takeda, and T. Seki, *Chem. Pharm. Bull.*, **38**, 426 (1990).
5. H. Otsuka, Y. Sasaki, K. Yamasaki, Y. Takeda, and T. Seki, *J. Nat. Prod.*, **53**, 107 (1990).
6. M.I. Eribekyan, L.S. Arutyunyan, and V.A. Mnatsakanyan, *Khim. Prir. Soedin.*, 146 (1987).
7. V.A. Mnatsakanyan, L.S. Arutyunyan, and M.I. Eribekyan, *Khim. Prir. Soedin.*, 38 (1983).
8. E.Yu. Agabayan, L.S. Arutyunyan, V.A. Mnatsukanyan, E. Gach-Baitts, and L. Radich, *Khim. Prir. Soedin.*, 446 (1987).
9. G. Falsone, M.D. Laryea, A.E.G. Crea, and E. Finner, *Planta Med.*, **44**, 150 (1982).
10. I. Calis, G.-A. Gross, T. Winkler, and O. Sticher, *Planta Med.*, 168 (1987).
11. K. Weinges, K. von der Eltz, and H. von der Eltz, *Liebigs Ann. Chem.*, 1968 (1978).
12. J.L. Marshall and R. Seiwell, *J. Magn. Reson.*, **15**, 150 (1974).
13. S.A. Abbas and A.H. Haines, *Carbohydr. Res.*, **39**, 358 (1975).

Received 17 September 1990