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10-*O*-ACYLATED IRIDOID GLUCOSIDES FROM LEAVES OF *PREMNA SUBSCANDENS*

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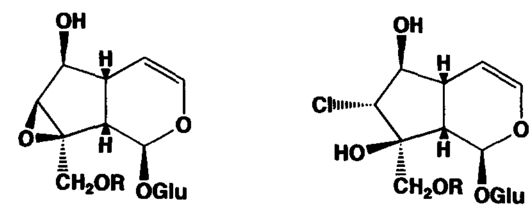
Key Word Index—*Premna subscandens*; Verbenaceae; acylated iridoid glucoside; catalpol; asystasioside E.

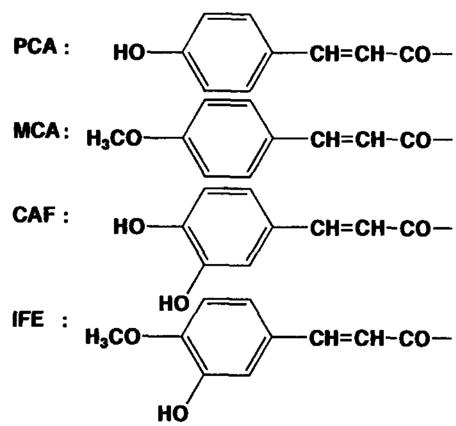
Abstract—From the 1-butanol-soluble fraction of a methanol extract of leaves of *Premna subscandens*, collected on Ishigaki Island, Okinawa, ten 10-*O*-acylated derivatives of catalpol and asystasioside E were isolated. The structures of nine new compounds were elucidated by spectroscopic methods and by chemical conversion. © 1997 Elsevier Science Ltd

INTRODUCTION

Many acylated 6-*O*-α-L-rhamnopyranosylcatalpols have been isolated from *Premna odorata* Blanco, which is cultivated in the Philippines for medicinal use [1, 2]. The positions of acylation are restricted to the hydroxyl groups of the rhamnopyranose moiety. This was found also to be the case when the constituents in leaves of *P. japonica* were investigated [3, 4]. Our current phytochemical study on *P. subscandens* leaves, collected in the southernmost area of the Ryukyu Islands, afforded acylated iridoid glucosides. The hydroxyl groups at the 10-positions of two iridoid glucosides, catalpol and chlorine-containing asystasioside E, were acylated with various kinds of C_6-C_3 units, such as *p*-coumaric, *p*-methoxycinnamic, caffeic and isoferulic acids. This paper deals with their structural determination.



R		R	
1	*E*-PCA (Scutellariosid-II)	7	*E*-MCA
2	*Z*-PCA	8	*Z*-MCA
3	*E*-MCA	9	*E*-PCA
4	*Z*-MCA	10	*Z*-PCA
5	*E*-CAF	12	H
6	*E*-IFE		
11	H		



Structure 1

RESULTS AND DISCUSSION

Compounds **1–9** were isolated from the 1-butanol-soluble fraction of a methanolic extract of leaves of the title plant by the procedures described in the Experimental section.

Compound **1**, on ^{13}C NMR analysis, was found to be an acylated derivative of catalpol (**11**) (Table 1) which was spectroscopically identical with Scutellariosid-II, isolated from *Scutellaria altissima* [5].

Compound **2** was found to have the same elemental

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Table 1. ^{13}C NMR data for compounds **1–10**, catalpol (**11**) and asystasioside E (**12**) (CD_3OD and/or D_2O, 100 MHz)*

C	11†	1	2	3	4	5	6	12‡	7	8		9	10
1	95.3	95.7	95.7	95.6	95.7	95.7	95.7	(92.6)	93.0	(92.5)¶	92.9	93.1	93.0
3	141.8	141.9	141.8	141.8	141.8	141.8	141.8	(139.6)	140.9	(139.8)	140.7	140.9	140.7
4	104.0	103.8	103.7	103.7	103.7	103.8	103.8	(106.1)	105.8	(106.1)	105.9	105.8	105.9
5	39.1	39.1	39.0	39.0	39.0	39.1	39.1	(35.4)	38.1	(35.7)	37.9	38.1	37.9
6	79.6	79.5	79.5	79.5	79.5	79.5	79.5	(81.1)	83.5	(81.3)	83.4	83.5	83.4
7	62.6	62.8	62.8	62.8	62.8	62.8	62.8	(71.6)	73.5	(71.9)	73.5	73.5	73.6
8	66.2	63.7	63.5	63.6	63.5	63.7	63.6	(79.3)	79.5	(78.7)	79.4	79.5	79.4
9	43.6	43.7	43.7	43.6	43.6	43.7	43.7	(47.0)	49.1	(47.3)	49.0	49.2	49.0
10	61.7	63.1	63.1	63.0	63.1	64.2	64.3	(62.4)	66.2	(65.2)	65.7	66.1	65.7
1′	99.7	100.4	100.3	100.3	100.3	100.4	100.4	(98.9)	99.9	(98.9)	99.8	99.9	99.8
2′	74.9	74.9	74.9	74.8	74.9	74.8	74.8	(73.4)	74.8	(73.6)	74.8	74.8	74.8
3′	78.7	78.5	78.6	78.4	78.5	78.5	78.5	(76.4)	78.1	(76.5)	78.1	78.1	78.2
4′	71.8	71.5	71.6	71.4	71.5	71.5	71.5	(70.4)	71.4	(70.4)	71.6	71.4	71.6
5′	77.7	77.9	77.9	77.8	77.9	77.9	77.9	(76.9)	78.0	(77.0)	78.0	78.0	78.0
6′	63.0	63.1	63.1	63.0	63.1	63.0	63.0	(61.5)	62.7	(61.5)	62.8	62.7	62.8
1″		127.2	127.6	128.3	128.7	127.8	128.9		128.4	(127.8)	128.7	127.2	127.6
2″		131.3	133.8	131.1	133.4	114.9	112.5		131.1	(131.1)	133.6	131.3	133.9
3″		116.9	116.0	115.4	114.6	149.6	151.6		115.5	(115.5)	114.5	116.9	115.9
4″		161.4	160.1	163.2	162.1	146.8	148.0		163.3	(162.1)	162.2	161.4	160.2
5″		116.9	116.0	115.4	114.6	116.5	114.9		115.5	(115.5)	114.5	116.9	115.9
6″		131.3	133.8	131.1	133.4	123.1	122.9		131.1	(131.1)	133.6	131.3	133.9
7″		147.0	145.6	146.6	145.0	147.4	146.9		146.5	(146.9)	145.1	146.9	145.6
8″		115.0	116.3	115.9	117.3	115.3	115.9		116.0	(115.4)	117.3	115.0	116.3
9″		169.1	168.0	168.9	167.9	169.1	168.9		168.7	(169.5)	167.6	168.9	167.7
–OMe				55.9	55.8		56.4		55.9	(56.4)	55.8		

* Chemical shifts in parentheses are for D_2O. The 6′-signal was used as an internal standard, δ_C 61.5.
† Data taken from ref. [1].
‡ Data taken from ref. [6].
¶ ran at 50°.

composition ($C_{24}H_{30}O_{12}$ by negative ion HR-FAB mass spectrometry) as that of **1**. The other spectroscopic data were also essentially the same as those of **1**, except for the coupling constants of two olefinic protons, δ_H 5.58 (*d*, $J = 13$ Hz) and 6.88 (*d*, $J = 13$ Hz), from which the geometry of the double bond in the acyl moiety was evidently of the cisoid form. Therefore, the structure of **2** was elucidated to be 10-*O*-*cis*-*p*-coumaroylcatalpol.

Compounds **3** and **4** were found to have the respective functional groups of the foregoing compounds (**1** and **2**) on analyses of the spectroscopic data. However, methoxyl signals were observed in the ^{13}C and ^1H NMR spectra, and the NMR chemical shifts of the *para*-substituted aromatic ring were modified to some extent (Table 1). Therefore, the structures of these compounds were concluded to be 10-*O*-*trans*- and *cis*-*p*-methoxycinnamoylcatalpols, respectively.

Compound **5**, on spectroscopic analyses, was also found to be an acylated derivative of catalpol, and its acyl moiety was expected to have two hydroxyl substitutions. Based on the fact that the three aromatic protons were coupled with each other in an ABX system, and comparison of the NMR data with those reported for *trans*-caffeate [1], the structure of **5** was concluded to be 10-*O*-*trans*-caffeoylcatalpol.

Compound **6** was also a 10-*O*-acylated catalpol. Based on its NMR spectroscopic data, the acyl moiety was expected to have a trisubstituted aromatic ring with hydroxyl and methoxyl functionalities, three protons coupled in an ABX system and a *trans* double bond. Thus, the structure of the acyl moiety was presumed to be that of ferulic or isoferulic acid. On irradiation of the methoxyl protons (δ_H 3.88) in the difference NOE experiment, significant enhancement of the signal intensity of the doublet proton at δ_H 6.94 (H-5″) proved that the acyl group was isoferulic acid. Therefore, the structure of **6** was elucidated to be 10-*O*-*trans*-isoferuloylcatalpol.

Compound **7**, on ^{13}C NMR analysis, was found to contain a β-glucopyranosyl and a *trans*-*p*-methoxycinnamoyl moiety. The remaining nine ^{13}C NMR signals were attributed to those of a typical 11-decarboxylated iridoid skeleton. The DEPT spectrum showed that two of the three carbon atoms with electro-negative substituents in the five-membered ring must each carry one proton [δ_C 83.5 (C-6) and 73.5 (C-7)], and the other no proton [δ_C 79.5 (C-8)]. Highresolution FAB-mass spectral analysis of **7** revealed two quasi molecular ion peaks $[M-H]^-$, *m/z* 559.1406 and 557.1408, in a 1:3 ratio, and the calculation of the elemental composition from the exact masses of the ion peaks indicated that compound **7** contained ^{37}Cl and ^{35}Cl atoms. Therefore, one of the carbon atoms was revealed to carry a chlorine atom as an electro-negative substituent, and the remaining carbons had to bear hydroxyl groups, based on the elemental composition. Mild alkaline treatment gave a single compound (**7a** = **3**), whose spectral data were the same as those of 10-*O*-*trans*-*p*-methoxycinnamoylcatalpol (**3**), which showed that one of the hydroxyl groups was located at the 6-β-position. The formation of an epoxide ring on the β side indicated that a chlorine atom was located at the 7-position in an α-orientation and a hydroxy group at the 8-position in a β-orientation. This was demonstrated by acetylation of **7** under mild condition to give the pentaacetate **7b**. The ^1H NMR spectrum of which showed a significant downfield shift for H-6 (δ_H 3.93 → 4.87), while H-7 remained almost unchanged ($\Delta\delta_H$ 0.17), when compared with the spectrum of **7**. Compound **7a** was hydrolysed under strong alkaline conditions to give catalpol (**11**) itself. This allowed us to confirm the structure of compound **3**. The non-acylated iridoid glucoside (**12**) is a known compound, asystasioside E, isolated from *Asystasia bella* [6]. The ^1H NMR data of **7b** were in good agreement with those reported for asystasioside E hexaacetate [6] and comparison of the ^{13}C NMR spectral data (D_2O) for **7** and **12** also indicated that **7** was the 10-*O*-*trans*-*p*-methoxycinnamoyl ester of **12** (Table 1).

Application of almost the same rationale as that used to derive the structures for the acylated catalpols was used to show that compounds **8**, **9** and **10** are 10-*O*-*cis*-*p*-methoxycinnamoyl, and *trans* and *cis*-*p*-coumaroylasystasioside Es, respectively.

EXPERIMENTAL

General. Mp: uncorr., ^1H and ^{13}C NMR: 400 MHz and 100 MHz, respectively; EI-MS: 70 eV; Reversed-phase open CC (RPCC): Cosmosil (ODS, Nakarai Tesque, Kyoto) (Φ = 50 mm, L = 25 cm), MeOH-H_2O (1:9, 1.5 l) → (7:3, 1.5 l), fractions of 10 g being collected; droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo): 500 columns (Φ = 2 mm, L = 40 cm). The ascending method was used with $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2), and 5 g frs were collected and numbered according to the order of elution with the mobile phase; HPLC: Inertsil (ODS, GL Science, Tokyo) (Φ = 20 mm, L = 20 cm), H_2O–MeOH, flow rate: 6 ml min^{-1}, detection at 254 nm.

Plant material. The plant material used was collected on Ishigaki Island, Okinawa, Japan. It was originally identified as *P. odorata*, but was later revised to *P. subscandens* Merr. by one (A.T.) of the authors. A voucher specimen was deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine (PO-92-Okinawa).

Extraction and isolation. The leaves of *P. subscandens* (840 g) were extracted with MeOH (12 l × 2). The MeOH extract was concd to 1.5 l and 75 ml of H_2O was added to give a 95% aq. soln which was extracted with 1.5 l of *n*-hexane, then the MeOH layer was concd to give a residue. The residue was suspended in 1.5 l of H_2O and then extracted with EtOAc (1.5 l) and 1-BuOH (1.5 l), successively. The 1-BuOH-soluble fr. (56.3 g) thus obtained was subjected to Diaion HP-20 (highly porous synthetic resin, Mitsubishikasei, Tokyo) CC (Φ = 5.5 cm, L = 40 cm) with H_2O–MeOH mixts [H_2O–MeOH (4:1, 3 l, 20%a: frs 1–3 and 20%b: frs 4–7), (3:2, 2.5 l, 40%a: frs 8–11), (2:3, 2.5 l, 60%a: frs 12–13 and 60%b: frs 14–16) and (1:4, 2.5 l, 80%a: frs 17–18 and 80%b: frs 19–23), and MeOH (2.5 l), 500 ml frs being collected]. The residue (13.1 g) of the 40% MeOH eluate was subjected to silica gel (450 g) CC with $CHCl_3$ containing increasing amounts of MeOH [$CHCl_3$ (1.5 l), $CHCl_3$–MeOH (99:1, 2 l), (49:1, 2 l), (24:1, 4 l), (93:7, 4 l), (9:1, 6 l), (7:1, 6 l) (17:3, 6 l), (4:1, 6 l), (3:1, 6 l) and (7:3, 6 l), 500 ml frs being collected]. The residue (887 mg) of the 10% MeOH eluate was sepd by RPCC to give two frs. The residue of frs 95–121 (695 mg) was purified by DCCC (358 mg in frs 35–43) and then by HPLC (135 mg out of 358 mg, H_2O–MeOH, 7:3) to afford compounds **1** (37 min, 105 mg) and **2** (42 min, 25 mg). The residue of frs 122–133 on RPCC (38 mg) was purified by HPLC (H_2O–MeOH, 13:7) to give 20 mg of **6** (29 min). Compounds **10** and **9** were obtained in a similar manner from the residue (831 mg) of the 12.5% MeOH eluate in frs 34–42 on silica gel CC [RPCC (111 mg in frs 127–149), DCCC (54 mg in frs 21–30), and then HPLC (H_2O–MeOH, 7:3), 5 mg (21 min) and 37 mg (26 min), respectively]. Compound **5** was isolated from the residue (2.33 g) of another 12.5% MeOH eluate of frs 43–54 on silica gel CC in a similar manner [RPCC (99 mg in frs 79–89), DCCC (44 mg in frs 22–26), and then HPLC (H_2O–MeOH, 4:1, 27 mg (36 min)].

The residue (10.0 g) of the 60%b MeOH eluate and the residue (5.14 g) of the 80%a MeOH eluate on Diaion HP-20 CC were processed similarly to furnish the following compounds: **4** (59 mg), **8** (26 mg) and **7** (334 mg) from the former residue, and **4** (23 mg) and **3** (566 mg) from the latter residue.

Compound **1** [10-O-trans-p-*coumaroylcatalpol* (*Scutellariosid-II*)]. Amorphous powder, $[\alpha]_D^{24}$ −62.8° (MeOH, *c* 0.92). ^1H NMR (CD_3OD): δ 2.66 (H, *dd*, *J* = 8 and 10 Hz, H-9), 2.96 (H, *ddt*, *J* = 2, 4 and 8 Hz, H-5), 3.20 (H, *dd*, *J* = 8 and 9 Hz, H-2′), 3.49 (H, *d*, *J* = 1 Hz, H-7), 3.67 (H, *dd*, *J* = 6 and 12 Hz, H-6′a), 3.92 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.95 (H, *dd*, *J* = 1 and 8 Hz, H-6), 4.27 (H, *d*, *J* = 13 Hz, H-10a), 4.74 (H, *d*, *J* = 8 Hz, H-1′), 4.98 (H, *d*, *J* = 13 Hz, H-10b), 5.06 (H, *d*, *J* = 10 Hz, H-1), 5.07 (H, *dd*, *J* = 4 and 6 Hz, H-4), 6.35 (H, *dd*, *J* = 2 and 6 Hz, H-3), 6.36 (H, *d*, *J* = 16 Hz, H-8″), 6.80 (2H, *d*, *J* = 9

Hz, H-3″ and 5″), 7.47 (2H, *d*, $J = 9$ Hz, H-2″ and 6″), 7.64 (H, *d*, $J = 16$ Hz, H-7″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 507.1499 ($C_{24}H_{27}O_{12}$ requires 507.1502) [5].

Compound **2** (10-O-cis-p-*coumaroylcatalpol*). Amorphous powder, $[\alpha]_D^{24}$ −70.6° (MeOH, *c* 1.76). IR ν_{max}^{KBr} cm^{-1}: 3325, 2875, 1695, 1600, 1510, 1165, 1070, 1010, 920, 840; UV λ_{max}^{MeOH} nm (log ε): 210 (3.96), 225 (3.88)sh, 300 (4.01)sh, 307 (4.11); ^{1}H NMR (CD_3OD): δ 2.27 (H, *ddt*, $J = 2$, 5 and 8 Hz, H-5), 2.57 (H, *dd*, $J = 8$ and 10 Hz, H-9), 3.22 (H, *dd*, $J = 8$ and 9 Hz, H-2′), 3.42 (H, *d*, $J = 1$ Hz, H-7), 3.66 (H, *dd*, $J = 6$ and 12 Hz, H-6′a), 3.92 (H, *dd*, $J = 2$ and 12 Hz, H-6′b), 3.92 (H, *dd*, $J = 1$ and 8 Hz, H-6), 4.25 (H, *d*, $J = 13$ Hz, H-10a), 4.75 (H, *d*, $J = 8$ Hz, H-1′, 4.93 (H, *d*, $J = 13$ Hz, H-10b), 5.04 (H, *d*, $J = 10$ Hz H-1), 5.06 (H, *dd*, $J = 5$ and 6 Hz, H-4), 5.80 (H, *d*, $J = 13$ Hz, H-8″), 6.35 (H, *dd*, $J = 2$ and 6 Hz, H-3), 6.77 (2H, *d*, $J = 9$ Hz, H-3″ and 5″), 6.88 (H, *d*, $J = 13$ Hz, H-7″), 7.64 (2H, *d*, $J = 9$ Hz, H-2″ and 6″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 507.1532 ($C_{24}H_{27}O_{12}$ requires 507.1502).

Compound **3** (10-O-trans-p-*methoxycinnamoylcatalpol*). Amorphous powder, $[\alpha]_D^{24}$ −62.2° (Me OH, *c* 1.38). IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1685, 1630, 1600, 1570, 1510, 1420, 1250, 1170, 1075, 1015, 925, 830; UV λ_{max}^{MeOH} nm (log ε): 209 (4.01), 226 (4.04), 299 (4.22)sh, 305 (4.25); ^{1}H NMR (CD_3OD): δ 2.30 (H, *ddt*, $J = 2$, 5 and 8 Hz, H-5), 2.67 (H, *dd*, $J = 8$ and 10 Hz, H-9), 3.20 (H, *dd*, $J = 8$ and 9 Hz, H-2′), 3.37 (H, *t*, $J = 9$ Hz, H-3′), 3.50 (H, *d*, $J = 1$ Hz, H-7), 3.68 (H, *dd*, $J = 6$ and 12 Hz, H-6′a), 3.82 (3H, *s*, $-OC\underline{H}_3$), 3.92 (H, *dd*, $J = 2$ and 12 Hz, H-6′b), 3.96 (H, *dd*, $J = 1$ and 8 Hz, H-6), 4.28 (H, *d*, $J = 13$ Hz, H-10a), 4.75 (H, *d*, $J = 8$ Hz, H-1′), 4.99 (H, *d*, $J = 13$ Hz, H-10b), 5.07 (H, *d*, $J = 10$ Hz, H-1), 5.08 (H, *dd*, $J = 5$ and 6 Hz, H-4), 6.36 (H, *dd*, $J = 2$ and 6 Hz, H-3), 6.40 (H, *d*, $J = 16$ Hz, H-8″), 6.95 (2H, *d*, $J = 9$ Hz, H-3″ and 5″), 7.56 (2H, *d*, $J = 9$ Hz, H-2″ and 6″), 7.66 (H, *d*, $J = 16$ Hz, H-7″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 521.1673 ($C_{25}H_{29}O_{12}$ requires 521.1659).

Compound **4** (10-O-cis-p-*methoxycinnmamoylcatalpol*). Amorphous powder, $[\alpha]_D^{24}$ −76.1° (Me OH, *c* 1.38). IR ν_{max}^{KBr} cm^{-1}: 3350, 2890, 1700, 1600, 1510, 1255, 1170, 1075–1015, 920, 840; UV λ_{max}^{MeOH} nm (log ε): 209 (4.06), 223 (3.97)sh, 300 (4.10)sh, 307 (4.13); ^{1}H NMR (CD_3OD): δ 2.26 (H, *ddt*, $J = 2$, 5 and 8 Hz, H-5), 2.56 (H, *dd*, $J = 8$ and 10 Hz, H-9), 3.22 (H, *dd*, $J = 8$ and 9 Hz, H-2′), 3.42 (H, *d*, $J = 1$ Hz, H-7), 3.66 (H, *dd*, $J = 6$ and 12 Hz, H-6′a), 3.81 (3H, *s*, $-OC\underline{H}_3$), 3.92 (H, *dd*, $J = 1$ and 8 Hz, H-6), 3.92 (H, *dd*, $J = 2$ and 12 Hz, H-6′b), 4.21 (H, *d*, $J = 13$ Hz, H-10a), 4.75 (H, *d*, $J = 8$ Hz, H-1′), 4.96 (H, *d*, $J = 13$ Hz, H-10b), 5.04 (H, *d*, $J = 10$ Hz, H-1), 5.05 (H, *dd*, $J = 5$ and 6 Hz, H-4), 5.86 (H, *d*, $J = 13$ Hz, H-8″), 6.35 (H, *dd*, $J = 2$ and 6 Hz, H-3), 6.92 (2H, *d*, $J = 9$ Hz, H-3″ and 5″), 6.93 (H, *d*, $J = 13$ Hz, H-7″), 7.69 (2H, *d*, $J = 9$ Hz, H-2″ and 6″); ^{13}C NMR (CD_3OD): Table 1: HR-FAB-MS (negative centroid): *m/z* 521.1673 ($C_{25}H_{29}O_{12}$ requires 521.1659).

Compound **5** (10-O-trans-p-*caffeoylcatalpol*). Amorphous powder, $[\alpha]_D^{26}$ −52.0° (MeOH, *c* 1.96). IR ν_{max}^{KBr} cm^{-1}: 3350, 1680, 1600, 1510, 1440, 1270, 1160, 1070–1010; UV λ_{max}^{MeOH} nm (log ε): 217 (4.03), 244 (3.89), 308 (4.03), 331 (4.13); ^{1}H NMR (CD_3OD): δ 2.30 (H, *ddt*, $J = 2$, 5 and 8 Hz, H-5), 2.65 (H, *dd*, $J = 8$ and 10 Hz, H-9), 3.19 (H, *dd*, $J = 8$ and 9 Hz, H-2′), 3.37 (H, *t*, $J = 9$ Hz, H-3′), 3.48 (H, *br s*, H-7), 3.67 (H, *dd*, $J = 6$ and 12 Hz, H-6′a), 3.94 (H, *dd*, $J = 2$ and 12 Hz, H-6′b), 3.95 (H, *br d*, $J = 8$ Hz, H-6), 4.27 (H, *d*, $J = 13$ Hz, H-10a), 4.75 (H, *d*, $J = 8$ Hz, H-1′), 4.97 (H, *d*, $J = 13$ Hz, H-10b), 5.06 (H, *d*, $J = 10$ Hz, H-1), 5.07 (H, *dd*, $J = 5$ and 6 Hz, H-4), 6.29 (H, *d*, $J = 16$ Hz, H-8″), 6.35 (H, *dd*, $J = 2$ and 6 Hz, H-3), 6.78 (H, *d*, $J = 8$ Hz, H-5″), 6.96 (H, *dd*, $J = 2$ and 8 Hz, H-6″), 7.06 (H, *d*, $J = 2$ Hz, H-2″), 7.57 (H, *d*, $J = 16$ Hz, H-7″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 523.1441 ($C_{24}H_{27}O_{13}$ requires 523.1452).

Compound **6** (10-O-trans-*isoferuloylcatalpol*). Amorphous powder, $[\alpha]_D^{24}$ −61.2° (MeOH, *c* 1.19). IR ν_{max}^{KBr} cm^{-1}: 3350, 2875, 1685, 1625, 1600, 1505, 1440, 1265, 1160, 1125, 1070–1010, 920, 805, UV λ_{max}^{MeOH} nm (log ε): 216 (4.13), 243 (4.01), 297 (4.12), 324 (4.18); ^{1}H NMR (CD_3OD): δ 2.30 (H, *ddt*, $J = 2$, 5 and 8 Hz, H-5), 2.65 (H, *dd*, $J = 8$ and 10 Hz, H-8), 3.19 (H, *dd*, $J = 8$ and 9 Hz, H-2′), 3.48 (H, *d*, $J = 1$ Hz, H-7), 3.66 (H, *dd*, $J = 6$ and 12 Hz, H-6′a), 3.88 (3H, *s*, $-OC\underline{H}_3$), 3.89 (H, *dd*, $J = 2$ and 12 Hz, H-6′b), 3.95 (H, *dd*, $J = 1$ and 8 Hz, H-6), 4.27 (H, *d*, $J = 13$ Hz, H-10a), 4.74 (H, *d*, $J = 8$ Hz, H-1′), 4.98 (H, *d*, $J = 13$ Hz, H-10b), 5.06 (H, *d*, $J = 10$ Hz, H-1), 5.07 (H, *dd*, $J = 5$ and 6 Hz, H-4), 6.34 (H, *d*, $J = 16$ Hz, H-8″), 6.35 (H, *dd*, $J = 2$ and 6 Hz, H-3), 6.94 (H, *d*, $J = 8$ Hz, H-5″), 7.06 (H, *dd*, $J = 2$ and 8 Hz, H-6″), 7.09 (H, *d*, $J = 2$ Hz, H-2″), 7.59 (H, *d*, $J = 16$ Hz, H-7″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 537.1593 ($C_{25}H_{29}O_{13}$ requires 537.1608).

Compound **7** (10-O-trans-p-*methoxycinnamoylasystasioside* E). Colourless needles, mp 125–127° (H_2O), $[\alpha]_D^{18}$ −119.4° (MeOH, *c* 1.41). IR ν_{max}^{KBr} cm^{-1}: 3300, 1690, 1630, 1510, 1420, 1250, 1195, 1174, 1077, 1020, 960, 845; UV λ_{max}^{MeOH} nm (log ε): 209 (4.02), 227 (4.03), 308 (4.33); ^{1}H NMR (CD_3OD): δ 2.64 (H, *dd*, $J = 4$ and 11 Hz, H-9), 2.72 (H, *dddd*, $J = 2$, 3, 6 and 11 Hz, H-5), 3.22 (H, *dd*, $J = 8$ and 9 Hz, H-2′), 3.68 (H, *dd*, $J = 5$ and 12 Hz, H-6′a), 3.84 (H, *dd*, $J = 2$ and 12 Hz, H-6′b), 3.83 (3H, *s*, $-OC\underline{H}_3$), 3.93 (H, *dd*, $J = 6$ and 8 Hz, H-6), 4.07 (H, *d*, $J = 8$ Hz, H-7), 4.31 (H, *d*, $J = 12$ Hz, H-10a), 4.55 (H, *d*, $J = 12$ Hz, H-10b), 4.63 (H, *d*, $J = 8$ Hz, H-1′), 5.11 (H, *dd*, $J = 3$ and 6 Hz, H-4), 5.62 (H, *d*, $J = 4$ Hz, H-1), 6.23 (H, *dd*, $J = 2$ and 6 Hz, H-3), 6.38 (H, *d*, $J = 16$ Hz, H-8″), 6.96 (2H, *d*, $J = 9$ Hz, H-3″ and 5″), 7.57 (2H, *d*, $J = 9$ Hz, H-2″ and 6″), 7.68 (H, *d*, $J = 16$ Hz, H-7″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 559.1406 ($C_{25}H_{30}O_{12}{}^{37}Cl$ requires

559.1396), 557.1408 ($C_{25}H_{30}O_{12}{}^{35}Cl$ requires 557.1426).

Compound **8** (10-O-cis-p-*methoxycinnamoylasystasioside* E). Amorphous powder, $[\alpha]_D^{18}$ −127.2° (MeOH, *c* 1.84). IR ν_{max}^{KBr} cm^{-1}: 3325, 1700, 1620, 1600, 1510, 1250, 1165, 1070–1015, 960, 825, UV λ_{max}^{MeOH} nm (log ε): 209 (4.00), 223 (3.94)sh, 306 (4.12); ^{1}H NMR (CD_3OD): δ 2.61 (H, *dd*, *J* = 3 and 11 Hz, H-9), 2.69 (H, *dddd*, *J* = 2, 3, 6 and 11 Hz, H-5), 3.20 (H, *dd*, *J* = 8 and 9 Hz, H-2′), 3.67 (H, *dd*, *J* = 5 and 12 Hz, H-6′a), 3.85 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.82 (3H, *s*, –OC$\underline{H}_3$), 3.90 (H, *dd*, *J* = 6 and 8 Hz, H-6), 4.04 (H, *d*, *J* = 8 Hz, H-7), 4.27 (H, *d*, *J* = 12 Hz, H-10a), 4.47 (H, *d*, *J* = 12 Hz, H-10b), 4.62 (H, *d*, *J* = 8 Hz, H-1′), 5.08 (H, *dd*, *J* = 3 and 6 Hz, H-4), 5.59 (H, *d*, *J* = 3 Hz, H-1), 5.85 (H, *d*, *J* = 13 Hz, H-8″), 6.20 (H, *dd*, *J* = 2 and 6 Hz, H-3), 6.89 (2H, *d*, *J* = 9 Hz, H-3″ and 5″), 6.90 (H, *d*, *J* = 13 Hz, H-7″), 7.74 (2H, *d*, *J* = 9 Hz, H-2″ and 6″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 559.1389 ($C_{25}H_{30}O_{12}{}^{37}Cl$ requires 559.1369), 557.1409 ($C_{25}H_{30}O_{12}{}^{35}Cl$ requires 557.1426).

Compound **9** (10-O-trans-p-*coumaruoylasystasioside* E). Amorphous powder, $[\alpha]_D^{18}$ −140.8° (MeOH, *c* 1.14). IR ν_{max}^{KBr} cm^{-1}: 3350, 1685, 1625, 1600, 1510, 1440, 1330, 1260, 1170, 1070–1015, 870, 830; UV λ_{max}^{MeOH} nm (log ε): 209 (4.05), 228 (4.05), 302 (4.30)sh, 309 (4.35); ^{1}H NMR (CD_3OD): δ 2.64 (H, *dd*, *J* = 4 and 11 Hz, H-9), 2.72 (H, *dddd*, *J* = 2, 3, 6 and 11 Hz, H-5), 3.22 (H, *dd*, *J* = 8 and 9 Hz, H-2′), 3.68 (H, *dd*, *J* = 5 and 12 Hz, H-6′a), 3.83 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.94 (H, *dd*, *J* = 6 and 8 Hz, H-6), 4.06 (H, *d*, *J* = 8 Hz, H-7), 4.30 (H, *d*, *J* = 12 Hz, H-10a), 4.55 (H, *d*, *J* = 12 Hz, H-10b), 4.63 (H, *d*, *J* = 8 Hz, H-1′), 5.11 (H, *dd*, *J* = 3 and 6 Hz, H-4), 5.61 (H, *d*, *J* = 4 Hz, H-1), 6.22 (H, *dd*, *J* = 2 and 6 Hz, H-3), 6.33 (H, *d*, *J* = 16 Hz, H-8″), 6.81 (2H, *d*, *J* = 9 Hz, H-3″ and 5″), 7.47 (2H, *d*, *J* = 9 Hz, H-2″ and 6″), 7.66 (H, *d*, *J* = 16 Hz, H-7″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 545.1279 ($C_{24}H_{28}O_{12}{}^{37}Cl$ requires 545.1239), 543.1226 ($C_{24}H_{28}O_{12}{}^{35}Cl$ requires 543.1269).

Compound **10** (10-O-cis-p-*coumaroylasystasioside* E). Amorphous powder, $[\alpha]_D^{18}$ −121.1° (MeOH, *c* 0.38). UV λ_{max}^{MeOH} nm (log ε): 225 (3.90), 301 (4.10)sh, 310 (4.14); ^{1}H NMR (CD_3OD): δ 2.61 (H, *dd*, *J* = 3 and 11 Hz, H-9), 2.67 (H, *dddd*, *J* = 2, 3, 5 and 11 Hz, H-5), 3.21 (H, *dd*, *J* = 8 and 9 Hz, H-2′), 3.66 (H, *dd*, *J* = 5 and 12 Hz, H-6′a), 3.85 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.89 (H, *dd*, *J* = 6 and 8 Hz, H-6), 4.03 (H, *d*, *J* = 8 Hz, H-7), 4.26 (H, *d*, *J* = 12 Hz, H-10a), 4.46 (H, *d*, *J* = 12 Hz, H-10b), 4.62 (H, *d*, *J* = 8 Hz, H-1′), 5.07 (H, *dd*, *J* = 3 and 6 Hz, H-4), 5.58 (H, *d*, *J* = 3 Hz, H-1), 5.80 (H, *d*, *J* = 13 Hz, H-8″), 6.20 (H, *dd*, *J* = 2 and 6 Hz, H-3), 6.75 (2H, *d*, *J* = 9 Hz, H-3″ and 5″), 6.86 (H, *d*, *J* = 13 Hz, H-7″), 7.68 (2H, *d*, *J* = 9 Hz, H-2″ and 6″); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative centroid): *m/z* 545.1227 ($C_{24}H_{28}O_{12}{}^{37}Cl$ requires 543.1239), 543.1277 ($C_{24}H_{28}O_{12}{}^{35}Cl$ requires 543.1269).

Alkaline hydrolysis of compound **7** *to* 10-O-trans-p-*methoxycinnamoylcatalpol* (**3**). Compound **7** (30 mg) was treated with 0.01 M NaOH in 10 ml of MeOH at 0° for 2 hr. The reaction mixt. was neutralized by the addition of Amberlite IR-120B (H^+) and then the filtrate was evapd. The residue was purified by silica gel CC [silica gel (23 g), ϕ = 15 mm, L = 200 mm, $CHCl_3$ (100 ml), $CHCl_3$–MeOH (19:1, 100 ml), (9:1, 100 ml) and (4:1, 200 ml), frs of 12.5 ml being collected] to afford 12 mg of compound **7a** (43%) in frs 23–28. Amorphous powder, $[\alpha]_D^{28}$ −70.8° (MeOH, *c* 0.72). Other spectroscopic data were essentially the same as those of **3**.

Alkaline hydrolysis of compound **7** *to catalpol* (**11**). Compound **7** (51 mg) was treated with 0.1 M NaOH in 10 ml of MeOH. The residue, obtained in a similar manner to that just described, was subjected to silica gel CC [silica gel (23 g), ϕ = 15 mm, L = 200 mm, $CHCl_3$ (50 ml), (9:1, 100 ml), (4:1, 200 ml) and (7:3, 200 ml), frs of 12.5 ml being collected] to give 32 mg of crude catalpol in frs 22–40 as an amorphous powder. This was further purified by prep. HPLC [H_2O–MeOH (4:1), 8.8 min], which afforded 12 mg of **11** (36%). $[\alpha]_D^{16}$ −104.0° (MeOH, *c* 0.42). UV λ_{max}^{MeOH} nm (log ε): 205 (3.60); other spectroscopic data were essentially the same as those reported [1].

Acetylation of compound **7**. Compound **7** (5.0 mg) was acetylated with a mixt. of Ac_2O and pyridine (100 μl each) at 20° for 1.5 hr. The reagents were evapd off under an N_2 stream and the residue was purified by prep. TLC on silica gel [developed with C_6H_6–$(Me)_2CO$ (4:1) and then eluted with $CHCl_3$–MeOH (9:1)] to give 5.8 mg (84%) of a pentaacetate (**7b**). Amorphous powder, ^{1}H NMR ($CDCl_3$): δ 1.99, 2.01, 2.02, 2.06, 2.15 (each 3H, each *s*, C$\underline{H}_3$CO– × 5), 2.66 (H, *m*, H-5), 2.72 (H, *dd*, *J* = 2 and 11 Hz, H-9), 3.70 (H, *ddd*, *J* = 2, 5 and 9 Hz, H-5′), 3.86 (3H, *s*, –OC$\underline{H}_3$), 4.11 (H, *dd*, *J* = 2 and 12 Hz, H-6′a), 4.24 (H, *dd*, *J* = 5 and 12 Hz, H-6′b), 4.24 (H, *d*, *J* = 8 Hz, H-7), 4.42 (H, *d*, *J* = 12 Hz, H-10a), 4.68 (H, *d*, *J* = 12 Hz, H-10b), 4.86 (H, *d*, *J* = 8 Hz, H-1′), 4.87 (H, *dd*, *J* = 4 and 8 Hz, H-6), 4.99 (H, *dd*, *J* = 8 and 9 Hz, H-2′), 5.10 (H, *t*, *J* = 9 Hz, H-3′), 5.22 (H, *t*, *J* = 9 Hz, H-4′), 5.24 (H, *br dd*, *J* = 3 and 6 Hz, H-4), 5.50 (H, *d*, *J* = 2 Hz, H-1), 6.18 (H, *dd*, *J* = 2 and 6 Hz, H-3), 6.26 (H, *d*, *J* = 16 Hz, H-8″), 6.93 (2H, *d*, *J* = 9 Hz, H-3″ and 5″), 7.51 (2H, *d*, *J* = 9 Hz, H-2″ and 6″), H-7.69 (H, *d*, *J* = 16 Hz, H-7″); EI-MS: *m/z* (rel. int.) 770 $[M]^+$ (0.63), 768 $[M]^+$ (1.47), 732 $[M-HCl]^+$ (24), 331 $[Glc(Ac)_4$ oxonium ion$]^+$ (100).

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