

Pentasaccharide Resin Glycosides from *Ipomoea pes-caprae*

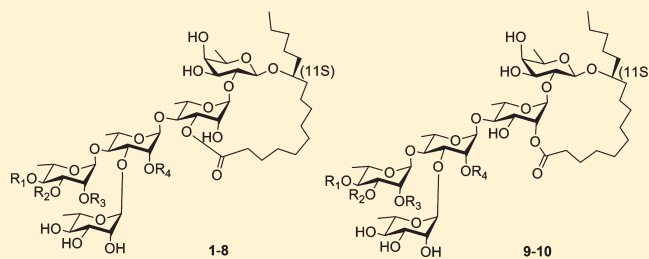
Bang-Wei Yu,^{†,‡} Jian-Guang Luo,[†] Jun-Song Wang,[†] Dong-Ming Zhang,[‡] Shi-Shan Yu,[‡] and Ling-Yi Kong^{*,†}

[†]Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China

[‡]Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xian Nong Tan Street, Beijing, 100050, People's Republic of China

S Supporting Information

ABSTRACT: Pescapreins XXI–XXX (1–10), pentasaccharide resin glycosides, together with the known pescapreins I–IV and stoloniferin III were isolated from the aerial parts of *Ipomoea pes-caprae* (beach morning-glory). The pescapreins are macrolactones of simonic acid B, partially esterified with different fatty acids. The lactonization site of the aglycone, jalapinic acid, was located at C-2 or C-3 of the second saccharide moiety. Their structures were established by a combination of spectroscopic and chemical methods. Compounds 1–10 were evaluated for their potential to modulate multidrug resistance in the human breast cancer cell line MCF-7/ADR. The combined use of these new compounds at a concentration of 5 $\mu\text{g}/\text{mL}$ increased the cytotoxicity of doxorubicin by 1.5–3.7-fold.



Resin glycosides, found mostly in plants of the family Convolvulaceae, have been a focus in natural products research for their diverse structures and various biological activities.¹ The genus *Ipomoea*, containing 300 species distributed from tropical to subtropical regions, was shown to be a rich source of tetra- and pentasaccharide resin glycosides, some of which have potential bioactivities, such as phyto-growth inhibition,² cytotoxicity,^{2a,3} antifungal,⁴ antibacterial,⁵ and bacterial multidrug efflux pumps blocking effects,^{5,6} as well as effects on the central nervous system.⁷

Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae) is a trailing vine distributed worldwide. It is orally used to cure dermatitis caused by jellyfish stings and is externally applied to treat furunculosis, pain, and bedsores in China.⁸ Previous investigations have revealed a series of resin glycosides, pescapreins I–IX, stoloniferin III, and pescaprosides A and B,^{9,10} and pescapreins X–XX^{6c,11} from this species. As a part of our ongoing chemical studies on the resin glycosides from plants in the Convolvulaceae,^{12,13} we have investigated the aerial parts of this plant and obtained 10 new pentasaccharide resin glycosides, pescapreins XXI–XXX (1–10), along with the known pescapreins I–IV⁹ and stoloniferin III.¹⁴ These new compounds had a pentasaccharide core, esterified with different organic acids and lactonized by (11S)-hydroxyhexadecanoic acid (jalapinic acid) to form a macrocyclic lactone. In the present paper, we report the isolation, structure determination, and evaluation of their inhibitory effects against multidrug resistance (MDR) using human breast cancer MCF-7/ADR cells.

RESULTS AND DISCUSSION

The air-dried aerial parts of *I. pes-caprae* were pulverized and refluxed with 95% EtOH. The EtOH extract was evaporated in

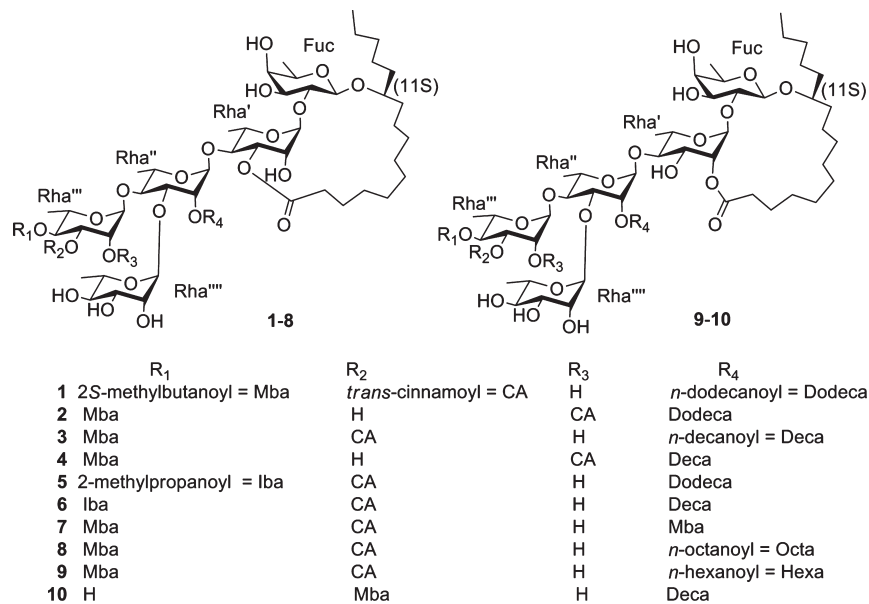
vacuo to remove the solvent and then suspended in H₂O to afford H₂O-soluble and H₂O-insoluble fractions. The H₂O-insoluble fraction was subjected to macroporous resin D101 column chromatography using a gradient of EtOH in H₂O (60:40 to 100:0, v/v) to afford five fractions. The three fractions eluting with EtOH–H₂O (70:30, 80:20, and 90:10) were successively separated over silica gel, MCI gel CHP-20P, Sephadex LH-20, and ODS column chromatography, as well as preparative HPLC to give pescapreins XXI–XXX (1–10), together with the known pescapreins I–IV and stoloniferin III.

Pescapreins XXI (1) was obtained as a white, amorphous powder. Its molecular formula, C₇₂H₁₁₆O₂₅, was established from a quasimolecular ion peak at *m/z* 1425.7785 [*M* + HCOO][−] (calcd for C₇₃H₁₁₇O₂₇, 1425.7787) in the negative-ion HRESIMS. IR peaks at 3445 and 1738 cm^{−1} revealed the presence of hydroxy and ester carbonyl groups, respectively. The NMR spectra exhibited five anomeric signals [δ_{H} 4.79 (d, *J* = 8.0 Hz), 6.30 (d, *J* = 1.5 Hz), 5.62 (d, *J* = 1.5 Hz), 5.91 (br s), 5.60 (br s), and δ_{C} 101.6, 100.2, 99.4, 103.7, 104.2] and signals of long-chain fatty acids, which indicated that 1 was a resin glycoside.^{12,13} The ¹H NMR spectrum exhibited five methyl doublets in the range δ_{H} 1.4–1.7 featuring five 6-deoxyhexose units, and two nonequivalent protons at δ_{H} 2.95 and 2.28 in the aglycone moiety, suggesting its macrocyclic lactone-type structure.^{10,12} A methyl triplet at δ_{H} 0.84 and a methylene triplet at δ_{H} 2.37 suggested a dodecanoyl group; a pair of distinctive *trans*-coupled olefinic protons (δ_{H} 6.52 and 7.81, each *J* = 16.0 Hz) and aromatic protons (δ_{H} 7.31, m, 3H, and 7.42, m, 2H) revealed the presence

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Chart 1. Structures of Compounds Isolated (1–10)



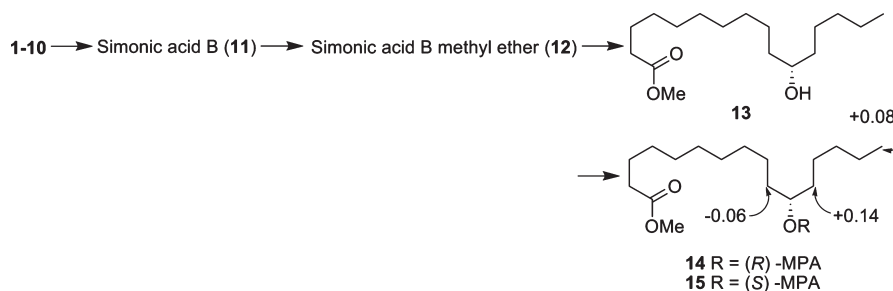
of a *trans*-cinnamoyl moiety; the upfield protons at δ_{H} 0.80 (t, $J = 7.5$ Hz, 3H), 1.12 (d, $J = 7.0$ Hz, 3H), and 2.44 (tq, $J = 7.0, 7.0$ Hz, 1H) composing one spin system in the TOCSY spectrum suggested the occurrence of a 2-methylbutanoyl unit. Alkaline hydrolysis of **1** afforded simonic acid B (**11**),^{12,13} and *n*-dodecanoic, 2-methylbutanoic, and *trans*-cinnamic acids by GC-MS analysis.¹⁰ 2-Methylbutanoic acid was found to have the *S*-configuration by comparison of its specific rotation value with that of an authentic sample. Subsequent acidic hydrolysis of the glycosidic acid methyl ether (**12**) liberated 11-hydroxyhexadecanoic acid methyl ether (**13**) and sugars (Scheme 1). The sugars obtained from the acidic hydrolysates were identified as L-rhamnopyranose and D-fucopyranose by GC-MS analysis of their chiral derivatives.¹⁵ The 11*S*-configuration was assigned on the basis of Mosher's method.^{12,13}

A combination of ¹H and ¹³C NMR and 2D NMR experiments (HSQC, HMBC, and TOCSY) led to the assignment of all proton and carbon signals in **1**, including those of one fucopyranosyl and four rhamnopyranosyl units (Tables 1 and 3). The β -configuration of the D-fucose was suggested by a large coupling constant ($J = 8.0$ Hz) for the anomeric proton (δ_{H} 4.80) in the ¹H NMR spectrum, while the α -configuration for L-rhamnose was revealed by the chemical shift of C-5 of rhamnose in the ¹³C NMR spectrum.¹⁶ The interglycosidic connectivities were determined from correlations of H-1 (δ_{H} 6.30) of Rha' with C-2 (δ_{C} 73.5) of Fuc, H-1 (δ_{H} 5.62) of Rha'' with C-4 (δ_{C} 78.6) of Rha', H-1 (δ_{H} 5.91) of Rha''' with C-4 (δ_{C} 79.6) of Rha'', and H-1 (δ_{H} 5.60) of Rha'''' with C-3 (δ_{C} 80.2) of Rha'' in the HMBC spectrum. The acylation positions were established by the key HMBC correlations between protons of sugars and acyl carbons of the fatty acids, i.e., δ_{H} 5.82 (H-2, Rha'') with δ_{C} 173.0 (*n*-dodecanoyl), δ_{H} 5.80 (H-3, Rha''') with δ_{C} 166.1 (*trans*-cinnamoyl), and δ_{H} 6.01 (H-4, Rha''') with δ_{C} 175.9 (2*S*-methylbutanoyl). The position of the jalapinic acid moiety in the oligosaccharide core was determined by the correlation between H-11 (δ_{H} 3.88) and C-1 (δ_{C} 101.6) of Fuc in the

HMBC spectrum. The site of lactonization was corroborated as C-3 of Rha' by the observed ³J coupling between the carbonyl carbon of the lactone (δ_{C} 174.9) and H-3 of Rha' (δ_{H} 5.59). Thus, the structure of **1** was elucidated as (11*S*)-jalapinic acid 11-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 3)-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)- α -L-rhamnopyranosyl-(1 \rightarrow 4)]-*O*-[2-*O*-*n*-dodecanoyl]- α -L-rhamnopyranosyl-(1 \rightarrow 4)-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-*O*- β -D-fucopyranoside-(1,3''-lactone).

Pescapreïn XXII (**2**) has the same molecular formula, C₇₂H₁₁₆O₂₅, as **1**, inferred by HRESIMS (m/z 1425.7784 [$M + \text{HCOO}^-$]). The NMR spectra (Tables 1 and 3) of **2** were similar to those of **1**, with the only difference being the position of the *trans*-cinnamoyl group, which, combined with GC-MS analysis of the alkaline hydrolysates, suggested that they were positional isomers. In the HMBC spectrum of **2**, H-2 of Rha'''' at δ_{H} 5.95 showed HMBC correlations to the carbonyl group at δ_{C} 166.8 (C-1 of *trans*-cinnamoyl), which suggested that the *trans*-cinnamoyl group was located at C-2 of Rha'''' in **2** rather than at C-3 of Rha'''' in **1**. Therefore, the structure of **2** was identified as (11*S*)-jalapinic acid 11-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 3)-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)- α -L-rhamnopyranosyl-(1 \rightarrow 4)]-*O*-[2-*O*-*n*-dodecanoyl]- α -L-rhamnopyranosyl-(1 \rightarrow 4)-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-*O*- β -D-fucopyranoside-(1,3''-lactone).

Pescapreïn XXIII (**3**) and XXIV (**4**) gave the same quasimolecular ion peak at m/z 1387 [$M + \text{Cl}^-$] in the negative ESIMS. Their negative HRESIMS exhibited a pseudomolecular ion [$M + \text{HCOO}^-$] at m/z 1397.7474 and 1397.7457 (calcd for C₇₁H₁₁₃O₂₇, 1397.7474), 28 mass units less than those of **1** and **2**. Alkaline hydrolysis of **3** and **4** yielded *n*-decanoic acid, 2*S*-methylbutanoic acid, and *trans*-cinnamic acid. Their ¹H NMR spectra were similar to those of compounds **1** and **2**. The major difference between their ¹³C NMR spectra was located in the high-field region (10 to 50 ppm), and it was thus deduced that compounds **3** and **4** were homologues of **1** and **2**, respectively, with a C₁₀ rather than a C₁₂ fatty acid side chain. The key HMBC

Scheme 1. Conversion of 1–10 to the Aglycone Methyl Ester 13 and Its MPA Derivatives (14 and 15)^a

^a $\Delta\delta_{\text{H}} = \delta(\text{S}) - \delta(\text{R})$ values are given in ppm.

correlations from H-2 (δ_{H} 5.84 in 3 and 5.85 in 4) of Rha'' to the acyl carbon (δ_{C} 173.0 in 3 and 172.9 in 4, *n*-decanoyl), H-4 (δ_{H} 6.03 in 3 and 5.73 in 4) of Rha''' to the acyl carbon (δ_{C} 175.9 in 3 and 176.4 in 4, 2*S*-methylbutanoyl), H-3 (δ_{H} 5.82) of Rha''' to the acyl carbon (δ_{C} 166.1, *trans*-cinnamoyl) in 3, and H-2 (δ_{H} 5.96) of Rha''' to the acyl carbon (δ_{C} 166.8, *trans*-cinnamoyl) in 4 confirmed that 3 and 4 were the congeners of 1 and 2 produced by the substitution of *n*-decanoyl units for *n*-dodecanoyl units, respectively. Accordingly, the structures of compounds 3 and 4 were established as (11*S*)-jalapinic acid 11-*O*- α -*L*-rhamnopyranosyl-(1 \rightarrow 3)-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)- α -*L*-rhamnopyranosyl-(1 \rightarrow 4)]-*O*-[2-*O*-*n*-decanoyl]- α -*L*-rhamnopyranosyl-(1 \rightarrow 4)- α -*L*-rhamnopyranosyl-(1 \rightarrow 2)-*O*- β -*D*-fucopyranoside-(1,3''-lactone) and (11*S*)-jalapinic acid 11-*O*- α -*L*-rhamnopyranosyl-(1 \rightarrow 3)-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)- α -*L*-rhamnopyranosyl-(1 \rightarrow 4)]-*O*-[2-*O*-*n*-decanoyl]- α -*L*-rhamnopyranosyl-(1 \rightarrow 4)-*O*- α -*L*-rhamnopyranosyl-(1 \rightarrow 2)-*O*- β -*D*-fucopyranoside-(1,3''-lactone), respectively.

Pescapreins XXV–XXIX (5–9), amorphous, white powders, gave quasimolecular ions of $[\text{M} + \text{HCOO}]^-$ at m/z 1411.7617 ($\text{C}_{72}\text{H}_{115}\text{O}_{27}$), 1383.7295 ($\text{C}_{70}\text{H}_{111}\text{O}_{27}$), 1327.6687 ($\text{C}_{66}\text{H}_{103}\text{O}_{27}$), 1369.7113 ($\text{C}_{69}\text{H}_{109}\text{O}_{27}$), and 1341.6815 ($\text{C}_{67}\text{H}_{105}\text{O}_{27}$), respectively. The ¹³C NMR spectra of 5–9 (Table 3) showed five anomeric signals, and the ¹H NMR spectra of these compounds (Tables 1 and 2) exhibited five methyl doublets for 6-deoxyhexose units and signals attributable to the nonequivalent protons of the methylene group at C-2 of the jalapinic moiety, indicative of the macrocyclic lactone-type structure. Compounds 5–9 were separately saponified with 5% KOH to provide a mixture of organic acids and a glycosidic acid. The organic acids were examined by gas chromatography (GC), while the glycosidic acid was identified from ¹H NMR data. *trans*-Cinnamic acid was found for 5–9, 2-methylpropanoic acid for 5 and 6, and 2*S*-methylbutanoic acid for 7–9. In addition, *n*-dodecanoic acid was obtained from 5, *n*-decanoic acid from 6, 2*S*-methylbutanoic acid from 7, *n*-octanoic acid from 8, and *n*-hexanoic acid from 9. The glycosidic acid obtained was proved to be simonic acid B (11) from ¹H NMR data. The positions of esterification were determined by the correlations in the HMBC spectra: thus, C-3-OH of Rha''' was acylated by *trans*-cinnamoyl in 5–9; C-4-OH of Rha''' was acylated by 2-methylpropanoyl in 5 and 6 and 2*S*-methylbutanoyl in 7–9; C-2-OH of Rha'' was acylated by *n*-dodecanoyl, *n*-decanoyl, 2*S*-methylbutanoyl, *n*-octanoyl, and *n*-hexanoyl, respectively, in 5–9. The location of the jalapinic acid moiety in the oligosaccharide core was determined by the observed HMBC cross-peaks from H-11 of jalapinic acid to C-1 of Fuc and from H-2 of Rha' in 9 and H-3 of

Rha' in 5–8 to the carbonyl of jalapinic acid. Consequently, the structures of 5–9 were determined as shown.

Pescaprein XXX (10), an amorphous, white powder, gave a quasimolecular ion at m/z 1267.7044 $[\text{M} + \text{HCOO}]^-$, which was determined by negative-ion HRESIMS. Basic hydrolysis afforded simonic acid B (11) and 2*S*-methylbutanoic and *n*-decanoic acids. The location of the jalapinic acid moiety was determined by the correlation between H-11 (δ_{H} 3.85) of jalapinic acid and C-1 (δ_{C} 104.3) of Fuc in the HMBC spectrum. The esterification positions of the acyl residues and the lactonization site of the aglycone in the oligosaccharide core were also determined by HMBC long-range correlations. Thus, an *n*-decanoyl unit was attached at C-2 of Rha'', a 2*S*-methylbutanoyl group was bonded at C-3 of Rha''', and the lactonization position of the aglycone was placed at C-2 of Rha'. Accordingly, the structure of 10 was defined as (11*S*)-jalapinic acid 11-*O*- α -*L*-rhamnopyranosyl-(1 \rightarrow 3)-4-*O*-[3-*O*-(2*S*-methylbutanoyl)- α -*L*-rhamnopyranosyl-(1 \rightarrow 4)]-*O*-[2-*O*-*n*-decanoyl]- α -*L*-rhamnopyranosyl-(1 \rightarrow 4)-*O*- α -*L*-rhamnopyranosyl-(1 \rightarrow 2)-*O*- β -*D*-fucopyranoside-(1,2''-lactone).

A common feature of all the resin glycosides is that a glycosidic acid is isolated after mild alkaline hydrolysis. The glycosidic acid is often esterified by various organic acids, mostly short-chain fatty acids, but a few aromatic acids have also been observed. The first example of a resin glycoside with an aromatic acid (*trans*-cinnamic acid) as a component organic acid was reported in 1992.¹⁷ Up to now, only eight macrolactones of simonic acid B as glycosidic acid, lactonized at C-3 of the second monosaccharide unit with *trans*-cinnamic acid, have been reported.^{12,18}

The most potent P-glycoprotein inhibitory activity was reported for the CHCl_3 extracts of *Merremia mammosa* (Lour.) Hallier. F (Convolvulaceae),¹⁹ in which the resin glycosides were the main constituents.²⁰ All the isolates (1–10) were examined for their inhibitory effect against multidrug resistance in MCF-7/ADR cells by the MTT method using verapamil as positive control. The results of the cytotoxicity assay showed that these compounds were not toxic to MCF-7/ADR. Combined use at a concentration of 5 $\mu\text{g}/\text{mL}$ with doxorubicin increased the cytotoxicity of doxorubicin by 1.5–3.7-fold as compared with 21-fold by verapamil (Table 4). The two pairs of regioisomers (1 vs 2, 3 vs 4) showed a large disparity in their MDR reversal ability, which demonstrated that a slight structural difference contributes to a large difference in their MDR reversal activity.¹³

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were obtained

Table 1. ¹H NMR Data of Compounds 1–6 (500 MHz, in pyridine-*d*₅)^a

position ^b	1	2	3	4	5	6
Fuc-1	4.79, d (8.0)	4.79, d (8.0)	4.81, d (7.8)	4.80 d, (7.8)	4.75, d (7.8)	4.81, d (7.8)
2	4.49, dd (9.5, 8.0)	4.50, dd (9.5, 8.0)	4.51, dd (9.5, 7.8)	4.51, dd (9.5, 7.8)	4.47, dd (9.5, 7.8)	4.52, dd (9.5, 7.8)
3	4.16, dd (9.5, 3.0)	4.17, dd (9.5, 3.5)	4.18, dd (9.5, 3.2)	4.18, dd (9.5, 3.4)	4.14, dd (9.5, 3.1)	4.17, dd (9.5, 3.5)
4	3.89, d (3.0)	3.90, d (3.5)	3.91, d (3.2)	3.92, d (3.4)	3.89, d (3.1)	3.91, d (3.5)
5	3.78, br q (6.0)	3.80, br q (6.0)	3.80, br q (6.3)	3.81, br q (6.3)	3.76, br q (6.3)	3.81, br q (6.3)
6	1.49, d (6.0)	1.50, d (6.0)	1.51, d (6.3)	1.52, d (6.3)	1.47, d (6.3)	1.51, d (6.3)
Rha'-1	6.30, d (1.5)	6.32, d (1.5)	6.32, br s	6.33, br s	6.26, br s	6.32, (1.5)
2	5.28, br s	5.30, br s	5.31, br s	5.31, br s	5.25, br s	5.31, br s
3	5.59, dd (10.0, 3.0)	5.60, dd (10.0, 3.0)	5.60, dd (10.0, 3.0)	5.61, dd (9.5, 3.0)	5.58, dd (9.5, 2.5)	5.62 dd (10.0, 3.0)
4	4.60, dd (10.0, 10.0)	4.61, dd (10.0, 10.0)	4.62, dd (10.0, 10.0)	4.62, dd (9.5, 9.5)	4.58, dd (9.5, 9.5)	4.62, dd (10.0, 10.0)
5	5.01, dq (10.0, 6.5)	5.00, dq (10.0, 6.0)	5.03*	5.01*	4.98, dq (9.5, 6.3)	5.03, dq (10.0, 6.3)
6	1.58, d (6.5)	1.58, d (6.0)	1.60, d (6.5)	1.59, d (6.2)	1.59, d (6.3)	1.59, d (6.3)
Rha''-1	5.62, d (1.5)	5.65, br s	5.64, br s	5.66, d (1.2)	5.59, br s	5.65, br s
2	5.82, br s	5.85, br s	5.84, br s	5.85, br s	5.79, br s	5.84, br s
3	4.58, dd (9.0, 3.0)	4.54, dd (10.0, 3.0)	4.60, dd (9.5, 3.0)	4.55, dd (9.1, 3.1)	4.56, dd (9.5, 3.3)	4.60, dd (9.8, 3.0)
4	4.27, dd (9.0, 9.0)	4.31, dd (10.0, 10.0)	4.29, dd (9.5, 9.5)	4.27–4.31*	4.25, dd (9.5, 9.5)	4.29, dd (9.8, 9.8)
5	4.37, dd (9.0, 6.0)	4.33, dd (10.0, 6.0)	4.39, dd (9.5, 6.3)	4.31–4.35*	4.35, dd (9.5, 6.1)	4.39, dd (9.8, 6.2)
6	1.59, d (6.0)	1.61, d (6.0)	1.61, d (6.3)	1.63, d (6.4)	1.61, d (6.1)	1.61, d (6.2)
Rha'''-1	5.91, br s	5.78, d (1.5)	5.93, br s	5.79, d (1.7)	5.86, br s	5.94, br s
2	4.85, br s	5.95, br s	4.87, br s	5.96, br s	4.82, br s	4.85, br s
3	5.80, dd (10.0, 3.0)	4.63, dd (9.5, 3.0)	5.82, dd (10.0, 3.0)	4.64, dd (9.5, 3.2)	5.76, dd (10.0, 3.8)	5.81, d (9.9, 3.8)
4	6.01, t (10.0)	5.72, t (9.5)	6.03, t (10.0)	5.73, t (9.5)	5.96, t (10.0)	6.02, t (9.9)
5	4.44, dd (10.0, 6.5)	4.39, dd (9.5, 6.5)	4.45, dd (10.0, 6.3)	4.40, dd (9.5, 6.5)	4.39–4.41*	4.41–4.44*
6	1.41, d (6.5)	1.49, d (6.5)	1.43, d (6.3)	1.49, d (6.5)	1.40, d (6.2)	1.41, d (6.2)
Rha''''-1	5.60, br s	5.58, br s	5.62, br s	5.59, br s	5.56, br s	5.62, br s
2	4.74, br s	4.89, br s	4.76, br s	4.91, br s	4.71, br s	4.75, br s
3	4.46, dd (9.0, 3.0)	4.42, dd (9.0, 3.5)	4.49, dd (9.0, 3.3)	4.42, dd (9.1, 3.2)	4.45*	4.48, dd (9.8, 3.5)
4	4.18, dd (9.0, 9.0)	4.21, dd (9.0, 9.0)	4.20, dd (9.0, 9.0)	4.22, dd (9.1, 9.1)	4.17, dd (9.1, 9.1)	4.20, dd (9.8, 9.8)
5	4.25, dd (9.0, 6.0)	4.27, dd (9.0, 6.0)	4.27, dd (9.0, 6.1)	4.27 dd (9.1, 6.0)	4.23, dd (9.1, 6.0)	4.28, dd (9.8, 6.1)
6	1.68, d (6.0)	1.69, d (6.0)	1.70, d (6.1)	1.69, d (6.0)	1.64 d (6.0)	1.70, d (6.1)
Ag-2	2.95, m; 2.28, m	2.98, m; 2.26, m	2.97, m; 2.28, m	2.99, m; 2.27, m	2.94, m; 2.28, m	2.96, m; 2.28, m
Ag-11	3.87, m	3.86, m	3.89, m	3.86, m	3.85, m	3.88, m
Ag-16	0.97, t (7.0)	0.97, t (7.0)	0.98, t (7.0)	0.98, t (7.1)	0.96, t (6.1)	0.98, t (6.9)
CA-2	6.52, d (16.0)	6.47, d (16.0)	6.54, d (15.9)	6.48, d (15.9)	6.51, d (16.0)	6.54, d (16.0)
CA-3	7.81, d (16.0)	7.74, d (16.0)	7.83, d (15.9)	7.75, d (15.9)	7.80, d (16.0)	7.83, d (16.0)
CA-2'/6'	7.42, 2H, m	7.27, 2H, m	7.44, 2H, m	7.28, 2H, m	7.39, 2H, m	7.44, 2H, m
CA-3'/5'	7.31, 2H, m	7.27, 2H, m	7.33, 2H, m	7.28, 2H, m	7.31, 2H, m	7.32, 2H, m
CA-4'	7.31, m	7.27, m	7.33, m	7.28, m	7.31, m	7.32, m
Dodeca-2	2.37, t (7.5)	2.40, t (7.5)			2.38, t (7.3)	
Dodeca-12	0.84, t (7.0)	0.84, t (7.0)			0.84, t (6.5)	
Deca-2			2.39, t (7.6)	2.41, t (7.5)		2.37, t (7.3)
Deca-10			0.86, t (7.0)	0.84, t (7.0)		0.85, t (7.2)
Iba-1					2.60, sept (7.0)	2.61, sept (7.0)
Iba-3					1.11, d (7.0)	1.13, d (7.0)
Iba-3'					1.10, d (7.0)	1.12, d (7.0)
Mba-2	2.44, tq (7.0, 7.0)	2.51, tq (7.0, 7.0)	2.46, tq (7.0, 7.0)	2.52, tq (6.8, 6.9)		
Mba-4	0.80, t (7.5)	0.91, t (7.0)	0.83, t (7.5)	0.92, t (6.8)		
Mba-2-Me	1.12, d (7.0)	1.21, d (6.5)	1.14, d (7.0)	1.22, d (6.9)		

^a Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (*J*) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, m = multiplet, q = quartet, sept = septet. All assignments are based on ¹H–¹H TOCSY experiments. ^b Abbreviations: Fuc = fucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Iba = 2-methylpropanoyl; Mba = 2S-methylbutanoyl; CA = *trans*-cinnamoyl; Deca = *n*-decanoyl; Dodeca = *n*-dodecanoyl; Me = methyl.

Table 2. ¹H NMR Data for Compounds 7–10 (500 MHz, in pyridine-*d*₅)^a

position ^b	7	8	9	10
Fuc-1	4.82, d (8.0)	4.81, d (8.0)	4.76, d (7.5)	4.73, d (7.5)
2	4.53, dd (9.0, 8.0)	4.53, dd (9.0, 8.0)	4.17, dd (9.5, 7.5)	4.14, dd (9.6, 7.5)
3	4.18, dd (9.0, 3.0)	4.18, dd (9.0, 3.0)	4.11, dd (9.5, 3.5)	4.08, dd (9.6, 3.5)
4	3.91, d (3.0)	3.91, d (3.0)	3.99, d (3.5)	3.98, d (3.5)
5	3.81, br q (6.5)	3.81, br q (6.5)	3.77, br q (6.5)	3.78, br q (6.4)
6	1.51, d (6.5)	1.49, d (6.5)	1.50, d (6.5)	1.49, d (6.4)
Rha'-1	6.35, br s	6.35, br s	5.49, br s	5.48, br s
2	5.32, br s	5.30, br s	5.96, br s	5.94, br s
3	5.62*	5.59*	5.01*	4.98, dd (9.3, 3.5)
4	4.66, dd (10.0, 10.0)	4.61, dd (10.0, 10.0)	4.20, dd (9.5, 9.5)	4.16*
5	5.03, dq (10.0, 6.0)	5.02*	4.43–4.45*	4.44–4.46*
6	1.58, d (6.0)	1.57, d (6.5)	1.61, d (6.5)	1.57, d (6.0)
Rha''-1	5.62, br s	5.64, br s	6.10, d (1.5)	6.05, br s
2	5.81, br s	5.85, br s	6.01, br s	6.00, br s
3	4.61, dd (9.5, 3.0)	4.61, dd (9.5, 3.0)	4.66, dd (9.0, 3.0)	4.60, dd (9.0, 3.1)
4	4.24, dd (9.5, 9.5)	4.30, dd (9.5, 9.5)	4.33, dd (9.0, 9.0)	4.30, dd (9.0, 9.0)
5	4.38, dd (9.5, 6.0)	4.40, dd (9.5, 6.0)	4.33–4.36*	4.22–4.25*
6	1.60, d (6.0)	1.62, d (6.0)	1.63, d (6.0)	1.54, d (5.9)
Rha'''-1	5.92, br s	5.96, br s	6.02, br s	5.92, br s
2	4.90, br s	4.88, br s	4.93*	4.82, br s
3	5.84, dd (10.0, 3.0)	5.82, dd (10.0, 3.5)	5.92, dd (10.0, 3.0)	5.82, dd (9.0, 9.0)
4	6.05, t (10.0)	6.04, t (10.0)	6.07, t (10.0)	4.38–4.41*
5	4.46, dd (10.0, 6.5)	4.46, dd (10.0, 6.5)	4.47–4.49*	4.41–4.43*
6	1.42, d (6.5)	1.42, d (6.5)	1.42, d (6.5)	1.64, d (5.8)
Rha''''-1	5.66, br s	5.62, br s	5.64, br s	5.60, br s
2	4.77, br s	4.79, br s	4.78, br s	4.75, br s
3	4.43, dd (9.0, 3.0)	4.51, dd (9.5, 3.0)	4.45–4.47*	4.42*
4	4.21, dd (9.0, 9.0)	4.21, dd (9.5, 9.5)	4.22, dd (9.5, 9.5)	4.18, dd (9.0, 9.0)
5	4.27, dd (9.0, 6.0)	4.28, dd (9.5, 6.0)	4.28, dq (9.5, 6.0)	4.27, dd (9.0, 6.0)
6	1.71, d (6.0)	1.69, d (6.0)	1.58, d (6.0)	1.56, d (6.0)
Ag-2	2.83, m; 2.26, m	2.98, m; 2.27, m	2.40, m; 2.22, m	2.38, m; 2.24, m
Ag-11	3.88, m	3.89, m	3.86, m	3.85, m
Ag-16	0.98, t (7.0)	0.84, t (7.0)	0.85, t (7.0)	0.85, t (7.3)
CA-2	6.52, d (16.0)	6.55, d (16.0)	6.55, d (16.0)	
CA-3	7.81, d (16.0)	7.84, d (16.0)	7.81, d (16.0)	
CA-2'/6'	7.44, 2H, m	7.45, 2H, m	7.44, 2H, m	
CA-3'/5'	7.33, 2H, m	7.33, 2H, m	7.33, 2H, m	
CA-4'	7.33, m	7.33, m	7.33, m	
Deca-2				2.40, m; 2.24, m
Deca-10				0.83, t (7.8)
Mba-2	2.45, tq (7.0, 7.0)	2.45, tq (7.0, 7.0)	2.46, tq (7.0, 7.0)	2.40, m
Mba-4	0.80, t (7.5)	0.80, t (7.0)	0.80, t (7.5)	0.87, t (7.4)
Mba-2-Me	1.12, d (7.0)	1.12, d (7.0)	1.13, d (7.0)	1.03, d (6.9)
Octa-2		2.38, t (7.0)		
Octa-8		0.84, t (7.0)		
Mba'-2	2.38, tq (7.0, 7.0)			
Mba'-4	0.88, t (7.5)			
Mba'-2-Me	1.13, d (7.0)			
Hexa-2			2.30, m; 2.26, m	
Hexa-6			0.75, t (7.5)	

^a Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, m = multiplet, q = quartet. All assignments are based on ¹H–¹H TOCSY experiments. ^b Abbreviations: Fuc = fucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Mba = 2S-methylbutanoyl; CA = *trans*-cinnamoyl; Deca = *n*-decanoyl; Hexa = *n*-hexanoyl; Octa = *n*-octanoyl; Me = methyl.

Table 3. ¹³C NMR Data for Compounds 1–10 (125 MHz, in pyridine-*d*₅)^a

position ^b	1	2	3	4	5	6	7	8	9	10
Fuc-1	101.6	101.6	101.7	101.6	101.6	101.7	101.4	101.6	104.1	104.3
2	73.5	73.4	73.5	73.4	73.5	73.5	73.4	73.5	80.0	80.2
3	76.6	76.6	76.6	76.6	76.6	76.7	76.4	76.6	73.1	73.3
4	73.6	73.6	73.6	73.5	73.6	73.6	73.3	73.6	72.7	73.0
5	71.2	71.3	71.3	71.3	71.3	71.3	71.0	71.3	70.6	70.8
5	17.2	17.2	17.2	17.2	17.2	17.2	17.0	17.2	17.1	17.3
Rha'-1	100.2	100.2	100.2	100.2	100.2	100.3	100.0	100.3	98.5	98.7
2	69.8	69.8	69.8	69.8	69.8	69.8	69.6	69.8	73.6	73.9
3	77.7	77.7	77.7	77.7	77.8	77.8	77.7	77.7	69.5	69.7
4	78.6	78.5	78.6	78.5	78.6	78.6	77.1	78.6	80.4	80.6
5	67.9	67.9	67.9	67.9	68.0	67.9	67.7	67.9	68.4	68.6
6	19.2	19.1	19.2	19.1	19.2	19.2	19.0	19.1	19.2	19.4
Rha''-1	99.4	99.2	99.4	99.2	99.4	99.4	98.8	99.3	99.1	99.3
2	73.0	73.0	73.1	73.0	73.1	73.1	72.6	73.0	73.0	73.2
3	80.2	79.7	80.2	79.7	80.2	80.3	79.4	80.1	79.4	79.7
4	79.6	79.6	79.7	79.6	79.6	79.6	79.9	79.5	79.4	79.0
5	68.2	68.4	68.3	68.3	68.3	68.3	68.2	68.2	68.0	68.5
6	18.7	18.7	18.8	18.7	18.8	18.9	18.5	18.7	18.6	18.7
Rha'''-1	103.7	100.4	103.7	100.4	103.7	103.7	103.6	103.6	103.4	103.5
2	70.0	74.1	70.1	74.1	70.1	70.1	69.8	69.9	69.8	70.4
3	73.2	68.1	73.3	68.1	73.3	73.4	73.0	73.2	73.1	75.7
4	71.5	74.8	71.5	74.8	71.6	71.6	71.3	71.3	71.2	71.0
5	68.2	68.4	68.3	68.4	68.2	68.2	68.0	68.2	68.1	71.1
6	17.8	18.0	17.8	17.9	17.7	17.7	17.6	17.8	17.6	18.3
Rha''''-1	104.2	104.3	104.3	104.3	104.3	104.3	104.3	104.3	104.3	104.3
2	72.7	72.2	72.8	72.2	72.8	72.8	72.5	72.6	72.4	72.5
3	72.5	72.6	72.5	72.6	72.5	72.5	72.2	72.5	72.3	72.6
4	73.7	73.7	73.8	73.7	73.8	73.8	73.4	73.7	73.4	73.7
5	70.8	70.9	70.9	70.9	70.9	70.9	70.4	70.8	70.5	70.7
6	18.8	18.8	18.8	18.8	18.8	18.7	18.6	18.2	18.3	18.5
Ag-1	174.9	174.9	174.9	174.9	174.9	174.9	174.5	174.9	172.9	173.1
Ag-2	33.7	33.7	33.8	33.6	33.7	33.7	33.7	33.6	34.0	34.3
Ag-11	79.5	79.4	79.5	79.4	79.5	79.5	79.3	79.5	82.1	82.3
Ag-16	14.5	14.5	14.5	14.4	14.5	14.5	14.3	14.2	14.0	14.3
CA-1	166.1	166.8	166.1	166.8	166.1	166.2	165.9	166.2	166.1	
CA-2	118.5	118.4	118.5	118.4	118.4	118.4	118.2	118.5	118.1	
CA-3	145.4	145.6	145.4	145.6	145.4	145.4	145.1	145.3	145.3	
CA-1'	134.7	134.7	134.7	134.7	134.7	134.7	134.5	134.7	134.4	
CA-2'/6'	128.5	128.6	128.5	128.6	128.5	128.6	128.3	128.5	128.3	
CA-3'/5'	129.3	129.0	129.3	129.0	129.3	129.3	129.0	129.3	129.0	
CA-4'	130.7	130.5	130.7	130.5	130.8	130.8	130.5	130.7	130.6	
Dodeca-1	173.0	172.9			172.9					
Dodeca-2	34.4	34.5			34.4					
Dodeca-12	14.3	14.2			14.3					
Deca-1		173.0	172.9			172.9				172.9
Deca-2		34.4	34.4			34.4				34.3
Deca-10		14.3	14.2			14.3				14.3
Iba-1			176.4	176.4						
Iba-2			34.4	34.4						
Iba-3			19.1	19.1						
Iba-3'			18.9	18.8						
Mba-1	175.9	176.3	175.9	176.4			175.7	175.9	175.7	176.8
Mba-2	41.6	41.6	41.6	41.5			41.3	41.6	41.3	41.3

Table 3. Continued

position ^b	1	2	3	4	5	6	7	8	9	10
Mba-2-Me	16.9	16.9	16.9	16.9			16.6	16.9	16.6	16.5
Mba-4	11.8	11.7	11.8	11.7			11.5	11.8	11.6	11.6
Octa-1								173.0		
Octa-2								34.4		
Octa-8								14.2		
Mba'-1							175.2			
Mba'-2							41.2			
Mba'-2-Me							16.6			
Mba'-4							11.6			
Hexa-1									172.6	
Hexa-2									34.2	
Hexa-6									13.8	

^a Chemical shifts (δ) are in ppm relative to TMS. All assignments are based on HSQC and HMBC experiments. ^b Abbreviations: Fuc = fucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Iba = 2-methylpropanoyl; Mba = 2*S*-methylbutanoyl; CA = *trans*-cinnamoyl; Deca = *n*-decanoyl; Dodeca = *n*-dodecanoyl; Hexa = *n*-hexanoyl; Octa = *n*-octanoyl; Me = methyl.

on a Shimadzu UV-2450 spectrophotometer. IR spectra were measured on a Bruker Tensor-27 spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 NMR instrument using pyridine-*d*₅ as solvent with TMS as internal standard, and chemical shifts were recorded as δ values. The ESIMS experiment was performed on an Agilent 1100 Series LC/MSD ion-trap mass spectrometer [the sample was dissolved in MeOH (10 ppm of NaCl added)], and the HRESIMS experiment was performed on an Agilent TOF MSD G1969A mass spectrometer [drying gas, N₂; flow rate, 9.0 L/min; temperature, 330 °C; nebulizer, 35 psig; capillary, 3000 V; skimmer, 60 V; OCT RFV, 250 V; the sample was dissolved in MeOH–0.1% HCOOH in H₂O (10:1, v/v); analyzed in negative-ion mode; fragment voltage, 120 V]. The GC-MS system consisted of an Agilent 6890 gas chromatograph and an Agilent 5975 mass spectrometer. Absorbents for column chromatography were silica gel (200–300 μ m, Qingdao Marine Chemical Co., Ltd., China), Sephadex LH-20 (75–150 μ m, Pharmacia, Sweden), ODS (40–63 μ m, Fuji, Japan), MCI gel (CHP20P, 75–150 μ m, Mitsubishi Chemical Industries Ltd., Japan), and macroporous resin D101. Preparative HPLC was performed using an Agilent 1100 series instrument with a Shim-Pack RP-C₁₈ column (20 \times 200 mm i.d.) and UV detector at 210 and 280 nm. Thin-layer chromatography was performed on precoated silica gel GF₂₅₄ plates (Qingdao Marine Chemical Co., Ltd.) and detected by spraying with 10% H₂SO₄–EtOH.

Plant Material. The dried aerial parts of *I. pes-caprae* were collected from Hainan Province of China in November 2008. The botanical identification was made by Prof. Min-Jian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. 081108) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The air-dried aerial parts of *I. pes-caprae* (11 kg) were powdered and refluxed with 95% EtOH (90 L \times 3) at 80 °C. After removal of the solvents in vacuo, the residue (1.65 kg) was suspended in H₂O (10 L) to afford H₂O-soluble and H₂O-insoluble fractions. The H₂O-insoluble fraction (900 g) was chromatographed on a macroporous resin D101 column using a gradient of EtOH in H₂O (60:40 to 100:0, v/v) to yield five fractions (A–E).

Fraction B (33 g), a dark green syrup, was subjected to silica gel column chromatography eluting with CH₂Cl₂–MeOH (100:2 to 100:30, v/v) to afford four fractions (B1–B4). Fraction B2 (2.7 g) collected from elution with CH₂Cl₂–MeOH (100:5, v/v) was separated on a Sephadex LH-20 column eluting with CHCl₃–MeOH (1:1, v/v) to

Table 4. Results of Modulating MDR^a Activities in MCF-7/ADR of Compounds 1–10

sample ^b	IC ₅₀ value ^c (μg/mL)	RF ^d value
doxorubicin	5.91 ± 0.34	
doxorubicin + verapamil	0.28 ± 0.03	21.0
doxorubicin + 1	1.76 ± 0.35	3.3
doxorubicin + 2	3.98 ± 0.43	1.5
doxorubicin + 3	2.00 ± 0.45	2.9
doxorubicin + 4	3.20 ± 0.67	1.8
doxorubicin + 5	2.83 ± 0.35	2.1
doxorubicin + 6	1.58 ± 0.15	3.7
doxorubicin + 7	3.12 ± 0.20	1.9
doxorubicin + 8	2.57 ± 0.37	2.3
doxorubicin + 9	1.82 ± 0.21	3.2
doxorubicin + 10	2.60 ± 0.25	2.3

^aMDR: multidrug resistance. ^bSerial dilutions ranging from 0.008 to 25 μg/mL of doxorubicin in the presence or absence of 5 μg/mL sample. ^cValues are expressed as means ± SD. ^dRF = IC₅₀ of doxorubicin alone/IC₅₀ of doxorubicin in the presence of sample.

obtain subfraction B2A (2.1 g), which showed a spot at *R_f* 0.60 by TLC [silica gel, CHCl₃–MeOH (20:1, v/v)] and gave seven main peaks by HPLC analysis [Shimadzu VP-ODS, 4.6 × 150 mm, 5 μm, MeOH–H₂O (90:10, v/v), 1 mL/min; 30 °C; 280 nm]. Then subfraction B2A was further purified on an open ODS column (MeOH–H₂O, 80:20 to 100:0, v/v) to give eight fractions (B2Aa–B2Ah). Compounds 8, 6, and 3 were detected mainly in fractions B2Ac to B2Ae; 4, 1, and 2 in B2Ag to B2Ah; and 5 in B2Af, under the same HPLC conditions. Purification of these compounds was performed by preparative HPLC (Shim-Pack RP-C₁₈, 200 × 20 mm, 5 μm) eluting with MeOH–H₂O (91:9, v/v) at a flow rate of 10 mL/min at 30 °C. Compounds 8 (7 mg), 6 (33 mg), 3 (120 mg), 4 (21 mg), 5 (18 mg), 1 (89 mg), and 2 (56 mg) were thus obtained. Fraction B3 (3.6 g) obtained from elution with CH₂Cl₂–MeOH (100:10, v/v) was further purified on a Sephadex LH-20 column to afford fraction B3A. Then fraction B3A was subjected to passage over an open ODS column (MeOH–H₂O, 80:20 to 100:0, v/v) to give four subfractions (B3Aa–B3Ad). Subfraction B3Aa was separated by preparative HPLC using MeOH–H₂O (90:10, v/v, 10 mL/min) as the mobile phase to give pescapreins I (12 mg) and stoloniferin III (45 mg). Subfractions B3Ab and B3Ac were separated by preparative HPLC using MeOH–H₂O (92:8, v/v, 10 mL/min) as the mobile phase to afford pescapreins II (11 mg) and III (376 mg). Subfraction B3Ad was separated by preparative HPLC using MeOH–H₂O (94:6, v/v, 10 mL/min) to yield pescapreins IV (9 mg).

Fraction C (100 g), a dark green syrup, was subjected to silica gel column chromatography eluting with CHCl₃–MeOH (100:3 to 100:30, v/v) to afford four fractions (C1–C4). Fraction C2 (20 g) was subjected to passage over a silica gel column eluting with CHCl₃–MeOH in a gradient (100:2 to 100:15, v/v), to give three subfractions (C2a–C2c). Subfraction C2a (4.5 g) was purified on an open ODS column (MeOH–H₂O, 70:30 to 100:0, v/v) to give four fractions (C2aa–C2ad). Fraction C2aa was separated by preparative HPLC using MeOH–H₂O (87:13, v/v, 10 mL/min) as mobile phase to yield compound 7 (4 mg). Subfraction C2b (6.4 g) was subjected to passage over an open ODS column (MeOH–H₂O, 70:30 to 100:0, v/v) to give four fractions (C2b1–C2b4). Fraction C2b2 was separated by preparative HPLC using MeOH–H₂O (95:5, v/v, 10 mL/min) as mobile phase to give compound 9 (12 mg).

Fraction D (40 g) was subjected to MCI gel column chromatography eluting with MeOH–H₂O (70:30 to 100:0, v/v) to give five subfractions (D1–D5). Subfraction D2 (9 g) was passed over an open ODS column

(MeOH–H₂O, 80:20 to 100:0, v/v) to give four fractions (D2a–D2d). Fraction D2b was separated by preparative HPLC using MeOH–H₂O (97:3, v/v, 10 mL/min) as the mobile phase to give compound 10 (13 mg).

Pescapreins XXI (1): white, amorphous powder; [α]_D²³ –31.5 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.3), 217 (3.3), 279 (3.4) nm; IR (KBr) ν_{max} 3445, 2930, 2856, 1738, 1638, 1062 cm⁻¹; ¹H NMR see Table 1 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1415 [M + Cl]⁻; negative HRESIMS *m/z* 1425.7785 [M + HCOO]⁻ (calcd for C₇₃H₁₁₇O₂₇, 1425.7787).

Pescapreins XXII (2): white, amorphous powder; [α]_D²³ –46.2 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.2), 217 (3.2), 280 (3.4) nm; IR (KBr) ν_{max} 3443, 2930, 2856, 1739, 1637, 1063 cm⁻¹; ¹H NMR see Table 1 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1415 [M + Cl]⁻; negative HRESIMS *m/z* 1425.7784 [M + HCOO]⁻ (calcd for C₇₃H₁₁₇O₂₇, 1425.7787).

Pescapreins XXIII (3): white, amorphous powder; [α]_D²³ –33.5 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.3), 217 (3.2), 280 (3.3) nm; IR (KBr) ν_{max} 3444, 2932, 2857, 1737, 1637, 1063 cm⁻¹; ¹H NMR see Table 1 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1387 [M + Cl]⁻; negative HRESIMS *m/z* 1397.7474 [M + HCOO]⁻ (calcd for C₇₁H₁₁₃O₂₇, 1397.7474).

Pescapreins XXIV (4): white, amorphous powder; [α]_D²³ –33.7 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.3), 217 (3.3), 280 (3.4) nm; IR (KBr) ν_{max} 3442, 2931, 2856, 1737, 1637, 1063 cm⁻¹; ¹H NMR see Table 1 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1387 [M + Cl]⁻; negative HRESIMS *m/z* 1397.7457 [M + HCOO]⁻ (calcd for C₇₁H₁₁₃O₂₇, 1397.7474).

Pescapreins XXV (5): white, amorphous powder; [α]_D²³ –21.2 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.0), 217 (2.9), 280 (3.2) nm; IR (KBr) ν_{max} 3451, 2931, 2857, 1736, 1635, 1059 cm⁻¹; ¹H NMR see Table 1 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1401 [M + Cl]⁻; negative HRESIMS *m/z* 1411.7617 [M + HCOO]⁻ (calcd for C₇₂H₁₁₅O₂₇, 1411.7631).

Pescapreins XXVI (6): white, amorphous powder; [α]_D²³ –42.3 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.2), 217 (3.1), 279 (3.3) nm; IR (KBr) ν_{max} 3448, 2931, 2857, 1737, 1639, 1062 cm⁻¹; ¹H NMR see Table 1 and ¹³C NMR Table 3; negative ESIMS *m/z* 1373 [M + Cl]⁻; negative HRESIMS *m/z* 1383.7295 [M + HCOO]⁻ (calcd for C₇₀H₁₁₁O₂₇, 1383.7318).

Pescapreins XXVII (7): white, amorphous powder; [α]_D²⁸ –39.2 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.3), 217 (3.3), 279 (3.3) nm; IR (KBr) ν_{max} 3446, 2928, 2855, 1734, 1635 cm⁻¹; ¹H NMR see Table 2 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1317 [M + Cl]⁻; negative HRESIMS *m/z* 1327.6687 [M + HCOO]⁻ (calcd for C₆₆H₁₀₃O₂₇, 1327.6692).

Pescapreins XXVIII (8): white, amorphous powder; [α]_D²⁸ –46.4 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.6), 278 (3.4) nm; IR (KBr) ν_{max} 3443, 2931, 2857, 1737, 1636, 1063 cm⁻¹; ¹H NMR see Table 2 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1359 [M + Cl]⁻; negative HRESIMS *m/z* 1369.7113 [M + HCOO]⁻ (calcd for C₆₉H₁₀₉O₂₇, 1369.7161).

Pescapreins XXIX (9): white, amorphous powder; [α]_D²⁸ –26.7 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.2), 217 (3.2), 280 (3.3) nm; IR (KBr) ν_{max} 3444, 2925, 1735, 1634 cm⁻¹; ¹H NMR see Table 2 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1331 [M + Cl]⁻; negative HRESIMS *m/z* 1341.6815 [M + HCOO]⁻ (calcd for C₆₇H₁₀₅O₂₇, 1341.6848).

Pescapreins XXX (10): white, amorphous powder; [α]_D²³ –26 (c 0.13 MeOH); IR (KBr) ν_{max} 3445, 2929, 2857, 1722, 1065 cm⁻¹; ¹H NMR see Table 2 and ¹³C NMR see Table 3; negative ESIMS *m/z* 1257 [M + Cl]⁻; negative HRESIMS *m/z* 1267.7044 [M + HCOO]⁻ (calcd for C₆₂H₁₀₇O₂₆, 1267.7056).

Alkaline Hydrolysis of 1–10. Compounds 1–10 (1.0 mg each) in 5% KOH (2 mL) were refluxed at 90 °C for 2 h, respectively. The

reaction mixtures were acidified to pH 4.0 with 2 N HCl and extracted with CHCl_3 (2 mL \times 2) and *n*-BuOH (3 mL \times 2). The organic layer was washed with H_2O , dried over anhydrous Na_2SO_4 , concentrated in vacuo, and analyzed by GC-MS on a model 6890 GC interfaced with a model 5975 MS (Agilent) at 70 eV under the following conditions (30 m \times 0.32 mm \times 0.25 μm , DB-5 MS column; He, 0.8 mL/min; 50 $^\circ\text{C}$, 3 min; 50–300 $^\circ\text{C}$, Δ 10 $^\circ\text{C}/\text{min}$). From the GC-MS spectrum and by comparison with authentic samples of *trans*-cinnamic acid (t_{R} 10.6 min): m/z 148 $[\text{M}]^+$ (76), 147 (100), 131 (22), 120 (6), 103 (49), 102 (24), 91 (23), 77 (35), 74 (7), 63 (6), 51 (36), 50 (10), 45 (15); *n*-dodecanoic acid (t_{R} 11.4 min): m/z 200 $[\text{M}]^+$ (8), 183 (1), 171 (8), 157 (27), 143 (10), 129 (36), 115 (17), 101 (12), 87 (15), 85 (26), 83 (14), 73 (90), 71 (26), 60 (100), 57 (55), 55 (60), 43 (77), 41 (66), 29 (27), 27 (14); *n*-decanoic acid (t_{R} 9.9 min): m/z 172 $[\text{M}]^+$ (4), 155 (1), 143 (9), 129 (49), 115 (12), 101 (6), 87 (15), 73 (79), 60 (100), 57 (48), 55 (45), 43 (52), 41 (50), 29 (20), 27 (13); 2-methylbutanoic acid (t_{R} 4.0 min): m/z 87 (24), 74 (100), 73 (15), 57 (71), 55 (11), 45 (21), 41 (60), 39 (38), 29 (45), 27 (23); 2-methylpropanoic acid (t_{R} 3.2 min): m/z 88 $[\text{M}]^+$ (7), 73 (26), 71 (2), 60 (1), 55 (6), 45 (13), 43 (100), 41 (47), 39 (14), 29 (5), 27 (20); *n*-hexanoic acid (t_{R} 5.9 min): m/z 99 (1), 87 (12), 73 (40), 60 (100), 55 (14), 45 (14), 43 (33), 41 (26), 29 (10), 27 (13); and *n*-octanoic acid (t_{R} 8.1 min): m/z 144 $[\text{M}]^+$ (1), 115 (9), 101 (22), 85 (17), 73 (58), 69 (10), 60 (100), 55 (32), 45 (12), 43 (47), 41 (36), 39 (13), 29 (16), 27 (13) were identified. The *n*-BuOH layer was subjected to an open ODS column (MeOH– H_2O , 70:30, v/v) to obtain the glycosidic acid simonic acid B (11).^{12,13} It gave key fragments at m/z 1001 $[\text{M} - \text{H}]^-$, 855 $[\text{M} - \text{H} - \text{C}_6\text{H}_{10}\text{O}_6]^-$, 709 $[855 - \text{C}_6\text{H}_{10}\text{O}_6]^-$, 563 $[709 - \text{C}_6\text{H}_{10}\text{O}_6]^-$, 417 $[563 - \text{C}_6\text{H}_{10}\text{O}_6]^-$, and 271 $[417 - \text{C}_6\text{H}_{10}\text{O}_6]^-$ in the negative ESIMS. The organic fraction (3.2 mg) of the alkaline hydrolysis of **1** was purified on ODS column chromatography eluting with MeOH– H_2O (25:75, v/v) to give 2-methylbutanoic acid (0.5 mg). This was proved to be *S*-configured by comparing the specific rotation ($[\alpha]_{\text{D}}^{25} +18.9$) with that of authentic 2*S*-methylbutanoic acid.^{12,13}

Acid Hydrolysis and Sugar Analysis. The glycosidic acid (15 mg, from alkaline hydrolysis), which was methylated with MeOH, catalyzed with 0.5 N H_2SO_4 gave simonic acid B methyl ester (**12**). Compound **12** was hydrolyzed with 1 N H_2SO_4 and extracted with Et_2O to yield 11-hydroxyhexadecanoic acid methyl ester (**13**).^{12b} The aqueous layer of acidic hydrolysis was concentrated under reduced pressure to yield a residue of the sugars fraction. The protocols applied to determine the configuration of sugars were the same as our previous research, which permitted the identification of the mixture sugars of *L*-rhamnose and *D*-fucose by comparison of their derivatives with those of authentic samples.¹⁵

Preparation of Mosher's Esters. The procedures for the preparation of Mosher's esters to determine the absolute configuration of the aglycone were the same as described previously for resin glycosides from *Ipomoea batatas*.^{12b} The selected $\Delta\delta_{\text{H}}$ values [$\Delta\delta_{\text{H}} = \delta(\text{S}) - \delta(\text{R})$] ($\Delta\delta_{\text{H}} = -0.06$, H-10; $\Delta\delta_{\text{H}} = +0.14$, H-12; $\Delta\delta_{\text{H}} = +0.08$, H-16) of 11-(*R*-MPA)-hexadecanoic acid methyl ester (**14**) and 11-(*S*-MPA)-hexadecanoic acid methyl ester (**15**) (Scheme 1) facilitated assignment of the 11*S* absolute configuration.

11*S*-Hydroxyhexadecanoic acid methyl ester (13): colorless oil (CHCl_3), $[\alpha]_{\text{D}}^{25} +1.3$ (c 0.21, CHCl_3); IR (KBr) ν_{max} 3335, 2923, 2852, 1205 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ_{H} 3.65 (3H, s, OCH_3), 3.57 (1H, m, H-11), 2.28 (2H, t, $J = 7.5$ Hz, H-2), 1.60 (2H, t, $J = 7.0$ Hz, H-10), 1.42 (2H, m, H-12), 0.88 (3H, t, $J = 7.0$ Hz, H-16); HRESIMS m/z 309 $[\text{M} + \text{Na}]^+$.

11-(*R*-MPA)-Hexadecanoic acid methyl ester (14): colorless oil (CHCl_3), $[\alpha]_{\text{D}}^{25} -2.1$ (c 0.11, CHCl_3); IR (KBr) ν_{max} 3443, 2925, 2857, 1742, 1263 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.43 (2H, m, C_6H_5), 7.33 (3H, m, C_6H_5), 4.72 (1H, s, OCH), 4.89 (1H, m, H-11), 3.66 (3H, s, OCH_3), 3.40 (3H, s, OCH_3), 2.29 (2H, t, $J = 7.5$ Hz, H-2),

1.66 (2H, m, H-10), 1.40 (2H, m, H-12), 0.76 (3H, t, $J = 7.0$ Hz, H-16); ESIMS m/z 457 $[\text{M} + \text{Na}]^+$.

11-(*S*-MPA)-Hexadecanoic acid methyl ester (15): colorless oil (CHCl_3), $[\alpha]_{\text{D}}^{25} +1.5$ (c 0.21, CHCl_3); IR (KBr) ν_{max} 3451, 2960, 2927, 2854, 1741, 1260 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.45 (2H, m, C_6H_5), 7.35 (3H, m, C_6H_5), 4.74 (1H, s, OCH), 4.90 (1H, m, H-11), 3.68 (3H, s, OCH_3), 3.41 (3H, s, OCH_3), 2.30 (2H, t, $J = 7.5$ Hz, H-2), 1.60 (2H, m, H-10), 1.54 (2H, m, H-12), 0.84 (3H, t, $J = 7.2$ Hz, H-16); ESIMS m/z 457 $[\text{M} + \text{Na}]^+$.

Cytotoxicity Assays. MCF-7/ADR cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (Clark, Australia), harvested with trypsin, and resuspended in a final concentration of 4.5×10^4 cells/mL. Aliquots (0.1 mL) of cell suspension were seeded evenly into 96-well culture multiplates and incubated in a 37 $^\circ\text{C}$ incubator containing 5% CO_2 for 24 h. A series of concentrations for pure compounds ranging from 400 to 1 $\mu\text{g}/\text{mL}$ in DMSO were added to designated wells. After 48 h, an MTT assay was performed as described previously.²¹

MDR Reversal Assays. MCF-7/ADR cells were distributed into 96-well culture plates at 4.5×10^3 cells per well. Serial dilutions ranging from 0.008 to 25 $\mu\text{g}/\text{mL}$ of the known antitumor agent doxorubicin (Zhejiang Haizheng Pharmaceutical Co., Ltd., China) with or without 5 $\mu\text{g}/\text{mL}$ samples were added to the cells. Verapamil (5 $\mu\text{g}/\text{mL}$) was used as positive control. After 48 h, the MTT assay was performed as described above. IC_{50} values of doxorubicin were calculated from plotted results using untreated cells as 100%. The reversal fold (RF) as potency of reversal was obtained from fitting the data to $\text{RF} = \text{IC}_{50}$ of doxorubicin alone/ IC_{50} of doxorubicin in the presence of sample.²² All assays were performed in triplicate.

■ ASSOCIATED CONTENT

S Supporting Information. ^1H and ^{13}C NMR, ESIMS, and HRESIMS spectra of compounds **1–10**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +86-25-83271405. E-mail: cpu_lykong@126.com.

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