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Pentasaccharide Resin Glycosides from *Ipomoea pes-caprae*

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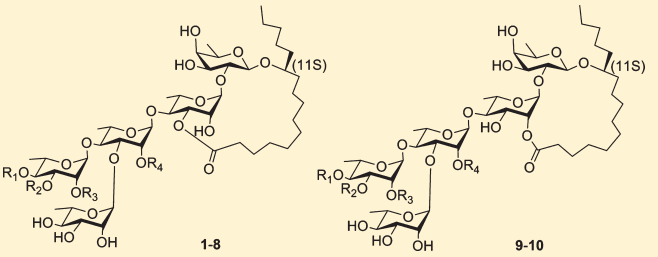
Supporting Information

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, jalapinolic 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 μg/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 phytogrowth 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−IV[9] and stoloniferin III.[14] These new compounds had a pentasaccharide core, esterified with different organic acids and lactonized by (11*S*)-hydroxyhexadecanoic acid (jalapinolic 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.

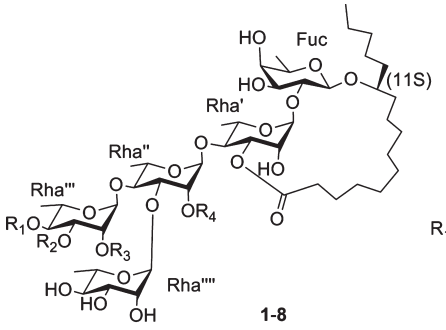
RESULTS AND DISCUSSION

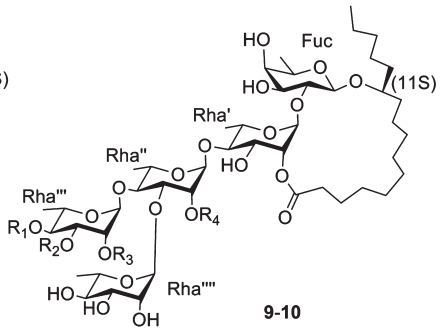
The air-dried aerial parts of *I. pes-caprae* were pulverized and refluxed with 95% EtOH. The EtOH extract was evaporated in vacuo to remove the solvent and then suspended in H_2O to afford H_2O-soluble and H_2O-insoluble fractions. The H_2O-insoluble fraction was subjected to macroporous resin D101 column chromatography using a gradient of EtOH in H_2O (60:40 to 100:0, v/v) to afford five fractions. The three fractions eluting with EtOH−H_2O (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.

Pescaprein XXI (**1**) was obtained as a white, amorphous powder. Its molecular formula, $C_{72}H_{116}O_{25}$, was established from a quasimolecular ion peak at *m*/*z* 1425.7785 [M + HCOO]$^-$ (calcd for $C_{73}H_{117}O_{27}$, 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 ^{1}H 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 macrocylic 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

Received: September 11, 2010
Published: February 21, 2011

Chart 1. Strutures of Compounds Isolated (1−10)





	R_1	R_2	R_3	R_4
1	2*S*-methylbutanoyl = Mba	*trans*-cinnamoyl = CA	H	*n*-dodecanoyl = Dodeca
2	Mba	H	CA	Dodeca
3	Mba	CA	H	*n*-decanoyl = Deca
4	Mba	H	CA	Deca
5	2-methylpropanoyl = Iba	CA	H	Dodeca
6	Iba	CA	H	Deca
7	Mba	CA	H	Mba
8	Mba	CA	H	*n*-octanoyl = Octa
9	Mba	CA	H	*n*-hexanoyl = Hexa
10	H	Mba	H	Deca

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-hydroxyhexdecanoic 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]

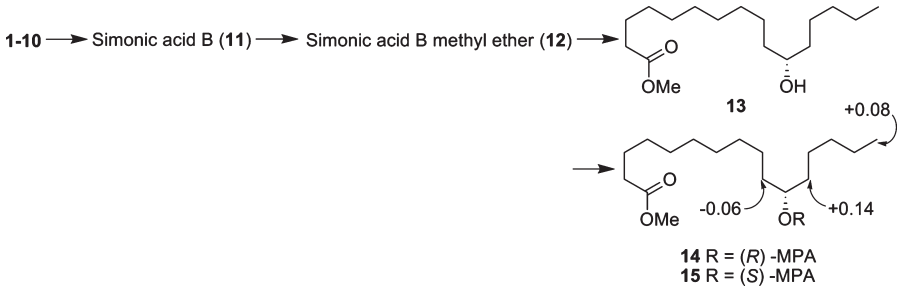
A combination of ^{1}H and ^{13}C NMR and 2D NMR experiments (HSQC, HMBC, and TOCSY) led to the assignment of all proton and carbon signals in **1**, including those of one fucopyranosyl and four rhamnopyranosyl units (Tables 1 and 3). The β-configuration of the D-fucose was suggested by a large coupling constant (J = 8.0 Hz) for the anomeric proton (δ_H 4.80) in the ^{1}H NMR spectrum, while the α-configuration for L-rhamnose was revealed by the chemical shift of C-5 of rhamnose in the ^{13}C 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 jalapinolic 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*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-*n*-dodecanoyl]-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside-(1,3″-lactone).

Pescaprein XXII (**2**) has the same molecular formula, $C_{72}H_{116}O_{25}$, as **1**, inferred by HRESIMS (m/z 1425.7784 [M + 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*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-*n*-dodecanoyl]-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside-(1,3″-lactone).

Pescapreins XXIII (**3**) and XXIV (**4**) gave the same quasimolecular ion peak at m/z 1387 [M + Cl]$^-$ in the negative ESIMS. Their negative HRESIMS exhibited a pseudomolecular ion [M + HCOO]$^-$ at m/z 1397.7474 and 1397.7457 (calcd for $C_{71}H_{113}O_{27}$, 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 ^{1}H NMR spectra were similar to those of compounds **1** and **2**. The major difference between their ^{13}C 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 C_{10} rather than a C_{12} fatty acid side chain. The key HMBC

dx.doi.org/10.1021/np100640f |*J. Nat. Prod.* 2011, 74, 620–628

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



[a] $\Delta\delta_H = \delta(S) - \delta(R)$ 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*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-*n*-decanoyl]-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside-(1,3″-lactone) and (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-*n*-decanoyl]-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside-(1,3″-lactone), respectively.

Pescapreins XXV−XXIX (**5**−**9**), amorphous, white powders, gave quasimolecular ions of $[M + HCOO]^-$ at *m*/*z* 1411.7617 ($C_{72}H_{115}O_{27}$), 1383.7295 ($C_{70}H_{111}O_{27}$), 1327.6687 ($C_{66}H_{103}O_{27}$), 1369.7113 ($C_{69}H_{109}O_{27}$), and 1341.6815 ($C_{67}H_{105}O_{27}$), respectively. The ^{13}C 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 jalapinolic 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 jalapinolic acid moiety in the oligosaccharide core was determined by the observed HMBC cross-peaks from H-11 of jalapinolic 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 jalapinolic 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 $[M + HCOO]^-$, 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 jalapinolic acid moiety was determined by the correlation between H-11 (δ_H 3.85) of jalapinolic 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*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[3-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl-(1→4)]-*O*-[2-*O*-*n*-decanoyl]-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→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 μg/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. ^{1}H 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 ^{1}H−^{1}H TOCSY 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; Me = methyl.

dx.doi.org/10.1021/np100640f |*J. Nat. Prod.* 2011, 74, 620–628

Table 2. ^{1}H 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 ^{1}H−^{1}H TOCSY experiments. [b] Abbreviations: Fuc = fucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Mba = 2*S*-methylbutanoyl; CA = *trans*-cinnamoyl; Deca = *n*-decanoyl; Hexa = *n*-hexanoyl; Octa = *n*-octanoyl; Me = methyl.

dx.doi.org/10.1021/np100640f |*J. Nat. Prod.* 2011, 74, 620–628

Table 3. ^{13}C 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
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, N_2; 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 H_2O (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 μm, Qingdao Marine Chemical Co., Ltd., China), Sephadex LH-20 (75−150 μm, Pharmacia, Sweden), ODS (40−63 μm, FuJi, Japan), MCI gel (CHP20P, 75−150 μm, Mitsubishi Chemical Industries Ltd., Japan), and macroporous resin D101. Preparative HPLC was performed using an Agilent 1100 series instrument with a Shim-Pack RP-C_{18} column (20 × 200 mm i.d.) and UV detector at 210 and 280 nm. Thin-layer chromatography was performed on precoated silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd.) and detected by spraying with 10% H_2SO_4−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 × 3) at 80 °C. After removal of the solvents in vacuo, the residue (1.65 kg) was suspended in H_2O (10 L) to afford H_2O-soluble and H_2O-insoluble fractions. The H_2O-insoluble fraction (900 g) was chromatographied on a macroporous resin D101 column using a gradient of EtOH in H_2O (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 CH_2Cl_2−MeOH (100:2 to 100:30, v/v) to afford four fractions (B1−B4). Fraction B2 (2.7 g) collected from elution with CH_2Cl_2−MeOH (100:5, v/v) was separated on a Sephadex LH-20 column eluting with $CHCl_3$−MeOH (1:1, v/v) to

dx.doi.org/10.1021/np100640f |J. Nat. Prod. 2011, 74, 620–628

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

[a] MDR: multidrug resistance. [b] Serial dilutions ranging from 0.008 to 25 μg/mL of doxorubicin in the presence or absence of 5 μg/mL sample. [c] Values are expressed as means ± SD. [d] RF = IC_{50} of doxorubicin alone/IC_{50} 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, $CHCl_3$−MeOH (20:1, v/v)] and gave seven main peaks by HPLC analysis [Shimadzu VP-ODS, 4.6 × 150 mm, 5 μm, MeOH−H_2O (90:10, v/v), 1 mL/min; 30 °C; 280 nm]. Then subfraction B2A was further purified on an open ODS column (MeOH−H_2O, 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-C_{18}, 200 × 20 mm, 5 μm) eluting with MeOH−H_2O (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 CH_2Cl_2−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−H_2O, 80:20 to 100:0, v/v) to give four subfractions (B3Aa−B3Ad). Subfraction B3Aa was separated by preparative HPLC using MeOH−H_2O (90:10, v/v, 10 mL/min) as the mobile phase to give pescaprein I (12 mg) and stoloniferin III (45 mg). Subfractions B3Ab and B3Ac were separated by preparative HPLC using MeOH−H_2O (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−H_2O (94:6, v/v, 10 mL/min) to yield pescaprein IV (9 mg).

Fraction C (100 g), a dark green syrup, was subjected to silica gel column chromatography eluting with $CHCl_3$−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 $CHCl_3$−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−H_2O, 70:30 to 100:0, v/v) to give four fractions (C2aa−C2ad). Fraction C2aa was separated by preparative HPLC using MeOH−H_2O (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−H_2O, 70:30 to 100:0, v/v) to give four fractions (C2b1−C2b4). Fraction C2b2 was separated by preparative HPLC using MeOH−H_2O (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−H_2O (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−H_2O, 80:20 to 100:0, v/v) to give four fractions (D2a−D2d). Fraction D2b was separated by preparative HPLC using MeOH−H_2O (97:3, v/v, 10 mL/min) as the mobile phase to give compound **10** (13 mg).

*Pescaprein XXI (**1**):* white, amorphous powder; $[\alpha]_D^{23}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1415 $[M + Cl]^-$; negative HRESIMS *m/z* 1425.7785 $[M + HCOO]^-$ (calcd for $C_{73}H_{117}O_{27}$, 1425.7787).

*Pescaprein XXII (**2**):* white, amorphous powder; $[\alpha]_D^{23}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1415 $[M + Cl]^-$; negative HRESIMS *m/z* 1425.7784 $[M + HCOO]^-$ (calcd for $C_{73}H_{117}O_{27}$, 1425.7787).

*Pescaprein XXIII (**3**):* white, amorphous powder; $[\alpha]_D^{23}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1387 $[M + Cl]^-$; negative HRESIMS *m/z* 1397.7474 $[M + HCOO]^-$ (calcd for $C_{71}H_{113}O_{27}$, 1397.7474).

*Pescaprein XXIV (**4**):* white, amorphous powder; $[\alpha]_D^{23}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1387 $[M + Cl]^-$; negative HRESIMS *m/z* 1397.7457 $[M + HCOO]^-$ (calcd for $C_{71}H_{113}O_{27}$, 1397.7474).

*Pescaprein XXV (**5**):* white, amorphous powder; $[\alpha]_D^{23}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1401 $[M + Cl]^-$; negative HRESIMS *m/z* 1411.7617 $[M + HCOO]^-$ (calcd for $C_{72}H_{115}O_{27}$, 1411.7631).

*Pescaprein XXVI (**6**):* white, amorphous powder; $[\alpha]_D^{23}$ −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 ^{13}C NMR Table 3; negative ESIMS *m/z* 1373 $[M + Cl]^-$; negative HRESIMS *m/z* 1383.7295 $[M + HCOO]^-$ (calcd for $C_{70}H_{111}O_{27}$, 1383.7318).

*Pescaprein XXVII (**7**):* white, amorphous powder; $[\alpha]_D^{28}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1317 $[M + Cl]^-$; negative HRESIMS *m/z* 1327.6687 $[M + HCOO]^-$ (calcd for $C_{66}H_{103}O_{27}$, 1327.6692).

*Pescaprein XXVIII (**8**):* white, amorphous powder; $[\alpha]_D^{28}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1359 $[M + Cl]^-$; negative HRESIMS *m/z* 1369.7113 $[M + HCOO]^-$ (calcd for $C_{69}H_{109}O_{27}$, 1369.7161).

*Pescaprein XXIX (**9**):* white, amorphous powder; $[\alpha]_D^{28}$ −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 ^{13}C NMR see Table 3; negative ESIMS *m/z* 1331 $[M + Cl]^-$; negative HRESIMS *m/z* 1341.6815 $[M + HCOO]^-$ (calcd for $C_{67}H_{105}O_{27}$, 1341.6848).

*Pescaprein XXX (**10**):* white, amorphous powder; $[\alpha]_D^{23}$ −26 (*c* 0.13 MeOH); IR (KBr) ν_{max} 3445, 2929, 2857, 1722, 1065 cm^{-1}; ^1H NMR see Table 2 and ^{13}C NMR see Table 3; negative ESIMS *m/z* 1257 $[M + Cl]^-$; negative HRESIMS *m/z* 1267.7044 $[M + HCOO]^-$ (calcd for $C_{62}H_{107}O_{26}$, 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 × 2) and *n*-BuOH (3 mL × 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 × 0.32 mm × 0.25 μm, DB-5 MS column; He, 0.8 mL/min; 50 °C, 3 min; 50−300 °C, Δ 10 °C/min). From the GC-MS spectrum and by comparison with authentic samples of *trans*-cinnamic acid (t_R 10.6 min): *m/z* 148 $[M]^+$ (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 $[M]^+$ (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 $[M]^+$ (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 $[M]^+$ (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 $[M]^+$ (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 aicd B (**11**).[12,13] It gave key fragments at *m/z* 1001 $[M - H]^-$, 855 $[M - H - C_6H_{10}O_6]^-$, 709 $[855 - C_6H_{10}O_6]^-$, 563 $[709 - C_6H_{10}O_6]^-$, 417 $[563 - C_6H_{10}O_6]^-$, and 271 $[417 - C_6H_{10}O_6]^-$ 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 ($[\alpha]_D^{25}$ +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 $\Delta\delta_H$ values [$\Delta\delta_H = \delta(S) - \delta(R)$] ($\Delta\delta_H = -0.06$, H-10; $\Delta\delta_H = +0.14$, H-12; $\Delta\delta_H = +0.08$, 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.

11S-Hydroxyhexadecanoic acid methyl ester (***13***): colorless oil ($CHCl_3$), $[\alpha]_D^{25}$ +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 $[M + Na]^+$.

11-(R-MPA)-Hexadecanoic acid methyl ester (***14***): colorless oil ($CHCl_3$), $[\alpha]_D^{25}$ −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_2), 7.33 (3H, m, C_6H_3), 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 $[M + Na]^+$.

11-(S-MPA)-Hexadecanoic acid methyl ester (***15***): colorless oil ($CHCl_3$), $[\alpha]_D^{25}$ +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_2), 7.35 (3H, m, C_6H_3), 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 $[M + Na]^+$.

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 °C incubator containing 5% CO_2 for 24 h. A series of concentrations for pure compounds ranging from 400 to 1 μg/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 μg/mL of the known antitumor agent doxorubicin (Zhejiang Haizheng Pharmaceutical Co., Ltd., China) with or without 5 μg/mL samples were added to the cells. Verapamil (5 μg/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 RF = IC_{50} of doxorubicin alone/IC_{50} of doxorubicin in the presence of sample.[22] All assays were peformed in triplicate.

■ ASSOCIATED CONTENT

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.

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■ ACKNOWLEDGMENT

This research was financially supported by the National Key Scientific and Technological Special Projects (2009ZX09502-011) and a grant from Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine (Peking Union Medical College); the Ministry of Education of China (2010JZ-W-02) is also acknowledged.

■ REFERENCES

(1) Kinghorn, A. D.; Falk, H.; Kobayashi, J. In *Progress in the Chemistry of Organic Natural Products*; Budzikiewicz, H.; Pereda-Miranda, R.; Rosas-Ramírez, D.; Castañeda-Gómez, J., Eds.; Springerwien: New York, 2010; pp 77−153.

(2) (a) Pereda-Miranda, R.; Mata, R. *J. Nat. Prod.* **1993**, *56*, 571–582. (b) Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997**, *53*, 9007–9022.

(3) (a) León, I.; Mirón, G.; Alonso, D. *J. Nat. Prod.* **2006**, *69*, 896–902. (b) León, I.; Enríquez, R. G.; Gnecco, D.; Villarreal, M. L.; Cortés, D. A.; Reynolds, W. F.; Yu, M. *J. Nat. Prod.* **2004**, *67*, 1552–1556. (c) Cao, S.; Guza, R. C.; Wisse, J. H.; Miller, J. S.; Evans, R.; Kingston, D. G. I. *J. Nat. Prod.* **2005**, *68*, 487–492. (d) ChéRigo, L.; Pereda-Miranda, R. *J. Nat. Prod.* **2006**, *69*, 595–599.

(4) Barnes, C. C.; Smalley, M. K.; Manfredi, K. P.; Kindscher, K.; Loring, H.; Sheeley, D. M. *J. Nat. Prod.* **2003**, *66*, 1457–1462.
(5) Pereda-Miranda, R.; Kaatz, G., W.; Gibbons, S. *J. Nat. Prod.* **2006**, *69*, 406–409.
(6) (a) Cherigo, L.; Pereda-Miranda, R.; Fragoso-Serrano, M.; Jacobo-Herrea, N.; Kaatz, G. W.; Gibbons, S. *J. Nat. Prod.* **2008**, *71*, 1037–1045. (b) ChéRigo, L.; Pereda-Miranda, R.; Gibbons, S. *Phtochemistry* **2009**, *70*, 222–227. (c) Escobedo- Martínez, C.; Cruz-Morales, S.; Fragoso-Serrano, M.; Rahman, M. M.; Gibbons, S.; Pereda-Miranda, R. *Phytochemistry* **2010**, *71*, 1796–1801.
(7) León, I.; Mirón-López, G.; Molina-Salinas, G, M.; Herrera-Ruiz, M.; Estrada-Soto, S.; Gutiérrez, M. C.; Alonso-Cortes, D.; Navarrete-Vázquez, G.; Ríos, M. H.; Said-Fernández, S. *J. Nat. Prod.* **2008**, *71*, 1686–1691.
(8) Zhao, K. F.; Feng, L. T. *Resource of Halophytic Vegetation in China*; Science Press: Beijing, 2001; p 110.
(9) Pereda-Miranda, R.; Escalante-Sánchez, E.; Escobedo-Martínez, C. *J. Nat. Prod.* **2005**, *68*, 226–230.
(10) Escobedo-Martínez, C.; Pereda-Miranda, R. *J. Nat. Prod.* **2007**, *70*, 974–978.
(11) Tao, H. W.; Hao, X. J.; Liu, J. G.; D, J.; Fang, Y. C.; Gu, Q. Q.; Zhu, W. M. *J. Nat. Prod.* **2008**, *71*, 1998–2003.
(12) (a) Yin, Y. Q.; Li, Y.; Kong, L. Y. *J. Agric. Food. Chem.* **2008**, *56*, 2363–2368. (b) Yin, Y. Q.; Huang, X. F.; Kong, L. Y.; Niva, M. *Chem. Pharm. Bull.* **2008**, *56*, 1670–1674. (c) Yin, Y. Q.; Kong, L. Y. *J. Asian. Nat. Prod. Res.* **2008**, *10*, 233–238.
(13) Yin, Y. Q.; Wang, J. S.; Luo, J. G.; Kong, L. Y. *Carbohydr. Res.* **2009**, *344*, 466–473.
(14) (a) Noda, N.; Takakashi, N.; Kawasaki, T.; Miyahara, K.; Yang, C. R. *Phytochemistry* **1994**, *36*, 365–371. (b) Noda, N.; Takakashi, N.; Kawasaki, T.; Miyahara, K.; Yang, C. R. *Phytochemistry* **1998**, *48*, 837–841.
(15) Luo, J. G.; M, L.; Kong, L. Y. *Bioorg. Med. Chem.* **2008**, *16*, 2912–2930.
(16) Sang, S. M.; Lao, A. N.; Leng, Y.; Gu, Z. P.; Chen, Z. L.; Uzawa, J.; Fji-moto, Y. *Tetrahedron Lett.* **2000**, *41*, 9205–9208.
(17) Noda, N.; Yoda, S.; Kawasaki, T.; Miyahara, K. *Chem. Pharm. Bull.* **1992**, *40*, 3163–3168.
(18) Escalante-Sánchez, E.; Pereda-Miranda, R. *J. Nat. Prod.* **2007**, *70*, 1029–1034.
(19) Kim, H. R.; Chung, S. Y.; Jeong, Y. H.; Go, E. J.; Han, A. R.; Kim, N. H.; Sung, M. K.; Song, G.; Jang, J. O.; Nam, J. W.; Lee, H. J.; Seo, E. K. *Nat. Prod. Sci.* **2004**, *10*, 268–271.
(20) (a) Kitagawa, I.; Beak, N. I.; Yokokawa, Y.; Kawashima, K.; Yoshikawa, M.; Ohashi, K.; Shibuya, H. *Chem. Pharm. Bull.* **1996**, *44*, 1680–1692. (b) Kitagawa, I.; Beak, N. I.; Yokokawa, Y.; Yoshikawa, M.; Ohashi, K.; Shibuya, H. *Chem. Pharm. Bull.* **1996**, *44*, 1693–1699. (c) Kitagawa, I.; Ohashi, K.; Beak, N. I.; Sakagami, M.; Yoshikawa, M.; Shibuya, H. *Chem. Pharm. Bull.* **1997**, *45*, 786–794.
(21) Lu, Y. Y.; Luo, J. G.; Huang, X. F.; Kong, L. Y. *Steroids* **2009**, *74*, 95–101.
(22) Ji, B. S.; He, Ling.; Liu, G. Q. *Life Sci.* **2005**, *77*, 2221–2232.