Resin Glycoside Constituents of *Ipomoea pes-caprae* (Beach Morning Glory)

Hongwen Tao,[†] Xiaojiang Hao,[‡] Jinggen Liu,[§] Jian Ding,[§] Yuchun Fang,[†] Qianqun Gu,[†] and Weiming Zhu*[,†]

Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China, State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, and State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China

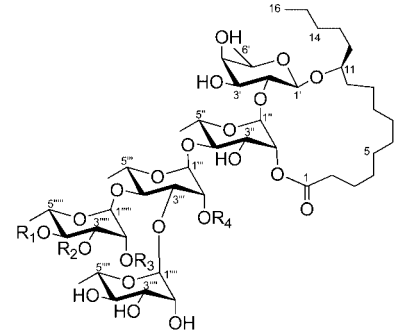
Received June 27, 2008

Eight new resin glycosides, pescapreins X−XVII (**1**−**8**), were isolated from a lipophilic fraction of an ethanol extract of the entire plant of beach morning glory, *Ipomoea pes-caprae*. Their structures were elucidated by spectroscopic data analysis and by chemical transformation. These compounds were evaluated biologically in terms of cancer cell line cytotoxicity, antibacterial and antifungal activity, and effects on the μ-opioid receptor.

Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae) is distributed worldwide and is a trailing vine that colonizes sand dunes along tropical and subtropical coastal beaches and protects dunes from eroding.[1] Besides its ecological importance, *I. pes-caprae* is used to treat fatigue, strain, arthritis, and rheumatism.[1] In mainland China, a decoction of this species is used orally to cure rubella and pruritus caused by jellyfish stings and is externally applied to treat pain and bedsores.[2] It has been demonstrated that extracts of *I. pes-caprae* exhibit antagonistic activity to histamine,[3,4] neutralize the toxic activity of jellyfish venom,[5] have an antinociceptive property,[6] and show inhibitory effects on platelet aggregation and 5-HT release.[7]

Previous chemical investigations on *I. pes-caprae* revealed eugenol, 4-vinylguaiacol,[8] actinidols,[9] β-damascenone, (*E*)-phytol,[10] betulinic acid, isoquercitrin, α- and β-amyrin acetate,[11] and quinic acid esters.[12] In addition, a series of lipophilic resin glycosides, including pescapreins I−IV[13] and VII−IX[14] and stoloniferin III[13] with a pentaglycosidic macrolide core, pescapreins V and VI[14] with a tetraglycosidic macrolide core, and pescaprosides A[13] and B[14] with a pentaglycosidic nonmacrolide core, were also identified from this plant. These compounds exhibited anti-inflammatory,[8,9] antispasmodic,[10] antinociceptive,[11] collagenase-inhibitory,[12] and cytotoxic activities.[13]

The present chemical investigation has afforded eight new resin glycosides (**1**−**8**). These compounds were evaluated for cytotoxicity against several cancer cell lines, antimicrobial activity, and affinity for the μ-opioid receptor.



	R_1	R_2	R_3	R_4
1:	2*S*-methylbutanoyl	cinnamoyl	H	*n*-decanoyl
2:	2*S*-methylbutanoyl	H	cinnamoyl	*n*-decanoyl
3:	2-methylpropanoyl	cinnamoyl	H	*n*-decanoyl
4:	2-methylpropanoyl	H	cinnamoyl	*n*-decanoyl
5:	2-methylpropanoyl	cinnamoyl	H	*n*-dodecanoyl
6:	2-methylpropanoyl	H	cinnamoyl	*n*-dodecanoyl
7:	2*S*-methylbutanoyl	cinnamoyl	H	*n*-dodecanoyl
8:	2*S*-methylbutanoyl	H	cinnamoyl	*n*-dodecanoyl

Results and Discussion

The dried whole plants of *I. pes-caprae* were pulverized and macerated with 95% EtOH. After being subjected to extensive column chromatography over silica gel and Sephadex LH-20, as well as semipreparative HPLC, compounds **1**−**8** were obtained from an EtOAc-soluble portion of the EtOH extract.

Compounds **1**−**8** afforded quasimolecular ions, $[M + H]^+$, at *m*/*z* 1354.1, 1354.1, 1339.9, 1339.9, 1368.0, 1368.0, 1382.0, and 1382.0, respectively, in the positive ESIMS. The similar NMR spectroscopic data to those of the pescapreins indicated that compounds **1**−**8** are resin glycosides.[13,14]

A mixture of compounds **1**−**8** was treated with NaOMe−MeOH to give simonic acid B methyl ester (**9**) (Scheme S1, Supporting Information) with the same specific rotation and NMR data as those reported in the literature.[15] Then, a mixture of these compounds was hydrolyzed by KOH−H_2O to produce an organic fraction and the water-soluble simonic acid B (**10**).[16] GC-MS analysis of the organic fraction was used to identify the acyl groups esterifying the pentasaccharide skeleton as 2-methylpropanoic acid, 2-methylbutanoic acid, *trans*-cinnamic acid, *n*-decanoic acid, and *n*-dodecanoic acid (Scheme S1, Supporting Information).[16] The 2-methylbutanoic acid substituent was identified as the *S*-isomer by analyzing its ester formed with (*R*)-(+)-1-phenylethanol by GC-MS.[17,18] These reaction products showed that compounds **1**−**8** bear the same pentasaccharide skeleton, simonic acid B (**10**).

Pescaprein X (**1**), an amorphous white powder, gave a quasimolecular ion at *m*/*z* 1354.1 $[M + H]^+$ in the positive ESIMS. Its molecular formula was established as $C_{70}H_{112}O_{25}$ by HRESIMS at *m*/*z* 1353.7589 $[M + H]^+$ (calcd 1353.7571). Saponification of **1** liberated simonic acid B (**10**), 2*S*-methylbutanoic acid, *n*-decanoic acid, and *trans*-cinnamic acid.[16] In the positive-ion ESIMS/MS, **1** afforded key fragments at *m*/*z* 1208.0 $[M + H - 146.1]^+$, 807.6 $[M + H - 546.5]^+$, 661.5 $[807.6 - 146.1]^+$, 507.3 ($[661.5 - 154.2]^+$), and 361.2 ($[507.3 - 146.1)]^+$) (Figure S1, Supporting Information). The loss of *m*/*z* 546.5 ($C_{28}H_{50}O_{10}$) indicated a macrocyclic disaccharide unit.[19] The fragment at *m*/*z* 361.2 ($C_{20}H_{25}O_6$) corresponded to a methylpen-

* To whom correspondence should be addressed. Tel: 0086-532-82031268. Fax: 0086-532-82031268. E-mail: weimingzhu@ouc.edu.cn.
[†] Ocean University of China.
[‡] Kunming Institute of Botany, Chinese Academy of Sciences.
[§] Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

10.1021/np800386z CCC: $40.75
Published on Web 12/05/2008

Table 1. ^{1}H NMR Data for Compounds **1**−**8** (600 MHz, in pyridine-d_5)[a]

position [b]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2	2.39, m; 2.23, m	2.39, m; 2.23, m	2.39, m; 2.24, m	2.38, m; 2.24, m	2.39, m; 2.23, m	2.39, m; 2.23, m	2.39, m; 2.23, m	2.39, m; 2.23, m
11	3.86, m	3.85, m	3.86, m	3.85, m	3.86, m	3.85, m	3.86, m	3.86, m
16	0.85, 3H, t (6.4)	0.86, 3H, t (6.9)	0.85, 3H, t (6.3)	0.85, 3H, t (6.9)	0.85, 3H, t (6.4)	0.85, 3H, t (6.8)	0.85, 3H, t (6.9)	0.85, 3H, t (6.4)
1′	4.75, d (7.3)	4.74, d (7.3)	4.76, d (7.3)	4.75, d (7.3)	4.76, d (7.3)	4.74, d (7.3)	4.75, d (7.3)	4.74, d (7.8)
2′	4.17, dd (9.3, 7.3)	4.17, dd (9.6, 7.3)	4.18, dd (9.1, 7.3)	4.18, dd (9.4, 7.3)	4.18, dd (9.3, 7.3)	4.17, dd (9.6, 7.3)	4.17, dd (9.2, 7.3)	4.17, dd (9.6, 7.8)
3′	4.10, dd (9.3, 3.2)	4.09, br.d (9.6)	4.11, br.d (9.1)	4.09, br.d (9.4)	4.10, br.d (9.3)	4.09, dd (9.6, 3.2)	4.11, br.d (9.2)	4.09, dd (9.6, 3.2)
4′	3.98, d (3.2)	3.98, br.s	3.99, d (3.0)	3.98, br.s	3.99, br.s	3.98, br.s	3.99, br.s	3.98, d (3.2)
5′	3.76, br.q (6.4)	3.76, br.q (6.4)	3.77, br.q (6.4)	3.76, br.q (6.2)	3.76, br.q (6.2)	3.76, br.q (6.0)	3.76, br.q (6.4)	3.76, br.q (5.9)
6′	1.49, d (6.4)	1.49, d (6.4)	1.50, d (6.4)	1.48, d (6.2)	1.50, d (6.2)	1.49, d (6.0)	1.49, d (6.4)	1.49, d (5.9)
1″	5.49, s	5.49, s	5.49, s	5.49, s	5.49, s	5.48, s	5.48, s	5.48, s
2″	5.96, br.s	5.97, br.s	5.97, d (3.2)	5.97, br.s	5.97, br.s	5.97, br.s	5.96, br.s	5.97, d (2.0)
3″	5.02, dd (9.2, 3.2)	5.02*	5.03, br.d (9.1)	5.03*	5.03*	5.03, dd (9.6, 3.2)	5.02*	5.03, dd (9.1, 3.2)
4″	4.20, dd (9.2, 9.2)	4.22, dd (9.4, 9.4)	4.21, dd (9.1, 9.1)	4.22, dd (9.2, 9.2)	4.20, dd (8.8, 8.8)	4.21, dd (9.6, 9.6)	4.20, dd (9.6, 9.6)	4.21, dd (9.2, 9.2)
5″	4.44−4.46*	4.47dd (9.4, 6.4)	4.44−4.46*	4.46−4.48*	4.44−4.46*	4.44−4.46*	4.45−4.47*	4.47, dd (9.6, 6.4)
6″	1.61, d (6.4)	1.63, d (6.4)	1.62, d (6.4)	1.62, d (6.0)	1.61, d (6.4)	1.63, d (5.9)	1.61, d (6.0)	1.62, d (6.4)
1‴	6.11, s	6.17, s	6.12, s	6.18, s	6.12, s	6.18, s	6.11, s	6.17, s
2‴	6.03, br.s	6.04, br.s	6.04, d (2.1)	6.04, br.s	6.03, br.s	6.04, br.s	6.03, br.s	6.04, br.s
3‴	4.66, dd (8.3, 2.3)	4.62, dd (8.3, 2.3)	4.66, dd (8.7, 2.1)	4.63, dd (8.4, 2.3)	4.66, br.d (8.3)	4.62, dd (8.3, 2.3)	4.66, br.d (8.3)	4.62, dd (8.3, 2.3)
4‴	4.33, dd (9.2, 9.2)	4.33−4.36*	4.33dd (9.2, 9.2)	4.33−4.36*	4.33dd (9.2, 9.2)	4.33−4.36*	4.33, dd (9.1, 9.1)	4.33−4.36*
5‴	4.34−4.37*	4.33−4.36*	4.34−4.37*	4.33−4.36*	4.33−4.37*	4.33−4.36*	4.33−4.37*	4.33−4.36*
6‴	1.63, d (5.5)	1.66, d (6.4)	1.63, d (5.5)	1.65, d (5.5)	1.63, d (5.7)	1.66, d (5.5)	1.63, d (5.5)	1.66, d (6.4)
1⁗	5.63, s	5.61, s	5.64, s	5.62, s	5.63, s	5.61, s	5.63, s	5.61, s
2⁗	4.78, br.s	4.91, br.s	4.80, br.s	4.91, br.s	4.79, br.s	4.91, br.s	4.78, br.s	4.91, br.s
3⁗	4.47−4.49*	4.44, dd (9.0, 3.2)	4.47−4.49*	4.41−4.45*	4.47−4.49*	4.42−4.46*	4.47−4.50*	4.44, dd (8.7, 3.2)
4⁗	4.21, dd (9.2, 9.2)	4.25, dd (9.0, 9.0)	4.22, dd (9.1, 9.1)	4.25, dd (9.2, 9.2)	4.22, dd (9.0, 9.0)	4.25, dd (9.2, 9.2)	4.21, dd (9.5, 9.5)	4.24, dd (9.2, 9.2)
5⁗	4.28, dq (9.2, 5.9)	4.29, dq (9.0, 6.0)	4.28, dq (9.1, 6.2)	4.29, dq (9.2, 5.6)	4.29, dq (9.0, 5.9)	4.29, dq (9.2, 5.9)	4.29, dq (9.5, 6.0)	4.29, dq (9.2, 5.9)
6⁗	1.58, d (5.9)	1.58, d (6.0)	1.59, d (6.2)	1.60, d (5.6)	1.58, d (5.9)	1.60, d (5.9)	1.58, d (6.0)	1.58, d (5.9)
1⁗′	5.98, s	5.84, s	5.99, s	5.83, s	5.99, s	5.84, s	5.98, s	5.83, s
2⁗′	4.96, br.s	6.03, br.s	4.96, br.s	6.04, br.s	4.96, br.s	6.04, br.s	4.96, br.s	6.02, d (2.0)
3⁗′	5.91, dd (10.1,2.8)	4.71, br.d (9.4)	5.91, dd (9.8, 2.8)	4.71, br.d (9.6)	5.92, dd (10.1,2.8)	4.72, br.d (9.6)	5.91, br.d (9.7)	4.71, dd (9.1, 2.0)
4⁗′	6.09,dd(10.1,10.1)	5.75, dd (9.4, 9.4)	6.06, dd (9.8, 9.8)	5.75, dd (9.6, 9.6)	6.06,dd(10.1,10.1)	5.75, dd (9.6, 9.6)	6.07, dd (9.7, 9.7)	5.75, dd (9.1, 9.1)
5⁗′	4.48−4.50*	4.42 dd (9.4, 6.0)	4.48−4.50*	4.39−4.43*	4.48−4.50*	4.41−4.44*	4.48−4.51*	4.42, dd (9.6, 6.4)
6⁗′	1.42, d (5.9)	1.50, d (6.0)	1.41, d (6.4)	1.49, d (6.4)	1.41, d (5.9)	1.50, d (6.4)	1.42, d (6.4)	1.50, d (5.9)
iba-2			2.62, qq (7.0, 7.0)	2.63, qq (6.9, 6.9)	2.62, qq (7.0, 7.0)	2.62, qq (6.8, 6.8)		
iba-3			1.12, 3H, d (7.0)	1.19, 3H, d (6.9)	1.12, 3H, d (7.0)	1.18, 3H, d (6.8)		
iba-2-Me			1.10, 3H, d (7.0)	1.15, 3H, d (6.9)	1.10, 3H, d (7.0)	1.14, 3H, d (6.8)		
mba-2	2.46, tq (7.3, 6.8)	2.51, tq (6.8, 6.8)					2.46, tq (7.4, 6.8)	2.51, tq (6.9, 6.9)
mba-3	1.68, m; 1.38, m	1.74, m; 1.47, m					1.68, m; 1.38, m	1.74, m; 1.47, m
mba-4	0.79, 3H, t (7.3)	0.90, 3H, t (7.3)					0.79, 3H, t (7.3)	0.89, 3H, t (7.6)
mba-2-Me	1.12, 3H, d (6.8)	1.20, 3H, d (6.8)					1.12, 3H, d (6.8)	1.19, 3H, d (6.9)
CA-2	6.54, d (16.0)	6.50, d (16.0)	6.54, d (16.0)	6.50, d (16.0)	6.55, d (16.0)	6.50, d (16.0)	6.54, d (16.0)	6.48, d (16.0)
CA-3	7.80, d (16.0)	7.73, d (16.0)	7.80, d (16.0)	7.74, d (16.0)	7.81, d (16.0)	7.75, d (16.0)	7.80, d (16.0)	7.73, d (16.0)
CA-2′/ 6′	7.44, 2H, m	7.26, 5H, m	7.44, 2H, m	7.26, 5H, m	7.44, 2H, m	7.26, 5H, m	7.44, 2H, m	7.26, 5H, m
CA-3′/ 5′	7.33, 2H, m		7.33, 2H, m		7.33, 2H, m		7.33, 2H, m	
CA-4′	7.33, m		7.33, m		7.33, m		7.33, m	
deca-2	2.28, t (7.7)	2.30, t (7.8)	2.28, t (7.7)	2.29, t (7.8)				
	2.30, t (7.7)	2.32, t (7.8)	2.30, t (7.7)	2.31, t (7.8)				
deca-10	0.83, 3H, t (7.1)	0.82, 3H, t (6.9)	0.83, 3H, t (7.1)	0.81, 3H, t (7.1)				
dodeca-2					2.28, t (7.8)	2.29, t (7.8)	2.28, t (7.8)	2.29, t (7.8)
					2.30, t (7.8)	2.31, t (7.8)	2.30, t (7.8)	2.30, t (7.8)
dodeca-12					0.83, 3H, t (7.3)	0.83, 3H, t (7.3)	0.83, 3H, t (7.3)	0.83, 3H, t (6.9)

[a] Chemical shifts (δ_H) are in ppm relative to TMS. The spin coupling (J) is given in Hz. Chemical shifts marked with an asterisk (*) indicate overlapped signals. Unless otherwise indicated, all proton signals integrated to ^{1}H. [b] mba: 2-methylbutanoyl; iba: 2-methylpropanoyl; CA: *trans*-cinnamoyl; deca: *n*-decanoyl; dodeca: *n*-dodecanoyl.

tose esterified by 2-methylbutanoic acid and *trans*-cinnamic acid. Analysis of the 2D NMR data allowed the assignment of the pentasaccharide skeleton. The four downfield protons at δ_H 6.09 (H-4⁗′), 6.03 (H-2‴), 5.96 (H-2″), and 5.91 (H-3⁗′) of **1** indicated that etherification occurred at C-4⁗′, C-2‴, C-2″, and C-3⁗′ in simonic acid B (**10**) (Table 1). This deduction was further supported by the key HMBC correlations of H-4⁗′ with an acyl carbon (δ_C 175.9, 2*S*-methylbutanoyl), H-2‴ with an acyl carbon (δ_C 172.9, *n*-decanoyl), H-2″ with an acyl carbon (δ_C 173.1, aglycon), and H-3⁗′ with an acyl carbon (δ_C 166.4, *trans*-cinnamoyl) (Figure 1). Thus, the structure of **1** was elucidated as (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*- decanoyl-α-L-rhamnopyranosyl](1→4)-2-*O*-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside-(1,2″-lactone).

Pescaprein XI (**2**) gave the same molecular formula, $C_{70}H_{112}O_{25}$, by HRESIMS at *m/z* 1353.7563 $[M + H]^+$ (calcd 1353.7571), suggesting it to be an isomer of **1**. Spectroscopic measurements showed compounds **1** and **2** to have similar ESIMS/MS and ^{1}H and ^{13}C NMR spectroscopic data. On comparing with those of **1**, the sugar proton signals H-2⁗′ and H-3⁗′ in **2** were shifted downfield by +1.07 ppm and shifted upfield by −1.20 ppm, respectively. The aromatic proton signals appeared as one multiplet (δ_H 7.26) rather than the two multiplets (δ_H 7.33 and 7.44) found for **1**. These data showed that the esterification location of the *trans*-cinnamoyl group in **2** was $C_{2⁗′}$-OH, which was further supported by the key HMBC correlation between H-2⁗′ (δ_H 6.03) and an acyl carbon (δ_C 166.8, *trans*-cinnamoyl) (Figure 1). Accordingly, the structure of **2** was elucidated as (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*-decanoyl-α-L-rhamnopyranosyl](1→4)-2-*O*-α-L-rhamnopyranosyl (1→2)-β-D-fucopyranoside-(1,2″-lactone).

Pescapreins XII (**3**) and XIII (**4**) showed the same molecular formula, $C_{69}H_{110}O_{25}$, according to their HRESIMS at *m/z* 1361.7253 and 1361.7238 $[M + Na]^+$ (calcd 1361.7234), respectively. They gave the same quasimolecular ion $[M + H]^+$ at *m/z* 1339.9 in the ESIMS and afforded similar fragments at *m/z* 1193.8 $[M + H - 146.1]^+$, 793.5 $[M + H - 546.4]^+$, 647.4 $[793.5 - 146.1]^+$, 493.3 $[647.4 - 154.1]^+$, and 347.2 ($[493.3 - 146.1]^+$) for **3** and at *m/z* 1193.8 $[M + H - 146.0]^+$, 793.6 $[M + H - 546.3]^+$, 647.5 $[793.6 - 146.1]^+$, 493.3 $[647.5 - 154.2]^+$, and 347.2 ($[493.3 - 146.1]^+$) for **4** in the positive ESIMS/MS, which exhibited similar cleavage patterns to those of **1** and **2**. The fragment at *m/z* 347.2 corresponded to a methylpentose esterified by 2-methylpropanoic acid and *trans*-

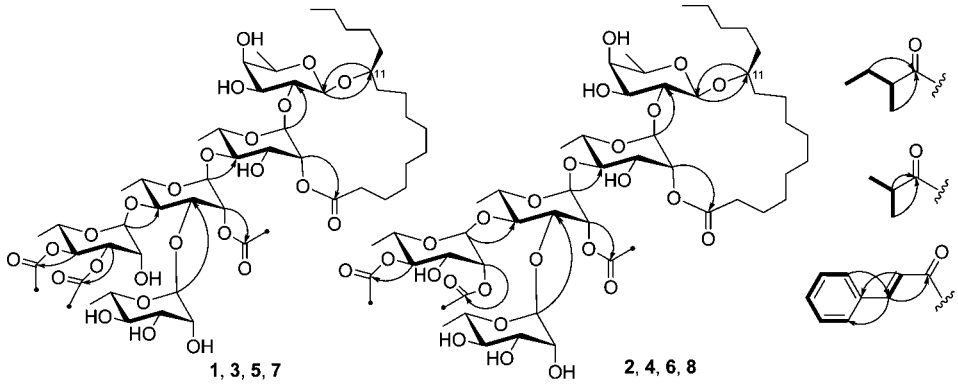


Figure 1. Key HMBC (→) and ^{1}H−^{1}H COSY (—) correlations for compounds **1**−**8**.

cinnamic acid. These data implied that the 2-methylbutanoyl unit in **1** and **2** was replaced by a 2-methylpropanoyl substituent in **3** and **4**. The only difference in the ^{1}H NMR data between **3** and **1**, and between **4** and **2**, was the upfield shift of H-4′′′′′ (Table 1), indicating that the substitution of 2-methylpropanoyl occurs at C-4′′′′′ in both **3** and **4**, which was confirmed by the key HMBC correlation of H-4′′′′′ (δ_H 6.04 for **3** and 5.75 for **4**) with an acyl carbon (δ_C 176.4 for both, 2-methylpropanoyl) (Figure 1). Thus, the structures of compounds **3** and **4** were elucidated as (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2-methylpropanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*-decanoyl-α-L-rhamnopyranosyl](1→4)-2-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside-(1,2′′-lactone) and (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2-methylpropanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*-decanoyl-α-L-rhamnopyranosyl] (1→4)-2-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside-(1,2′′-lactone), respectively.

The molecular formulas of pescapreins XIV (**5**) and XV (**6**) were established as $C_{71}H_{114}O_{25}$ by their positive HRESIMS at *m*/*z* 1367.7706 and 1367.7783 $[M + H]^+$ (calcd 1367.7727), respectively. They showed the same quasimolecular ion at *m*/*z* 1368.0 $[M + H]^+$ leading to a series of fragments at *m*/*z* 1222.0 $[M + H - 146.0]^+$ (in **5**), 1221.9 $[M + H - 146.0]^+$ (in **6**), 821.6 $[M + H - 546.4]^+$, 675.5 $[821.6 - 146.1]^+$, 493.3 $[675.5 - 182.2]^+$, and 347.2 $[493.3 - 146.1]^+$ in the positive ESIMS/MS. These exhibited similar cleavage patterns to those of **3** and **4** except for the loss of a *m*/*z* 182.2 fragment ($C_{12}H_{22}O$). These data indicated that the structures of **5** and **6** were very close to those of **3** and **4** except for the substitution of *n*-dodecanoyl groups for *n*-decanoyl groups. 1D NMR analysis (Tables 1 and 2) and HMBC correlations between H-2′′′ (δ_H 6.03 in **5** and 6.04 in **6**) and the acyl carbon (δ_C 172.9, *n*-dodecanoyl) (Figure 1) confirmed that **5** and **6** are the derivatives of **3** and **4** formed by the substitution of *n*-dodecanoyl units for *n*-decanoyl units, respectively. Thus, the structures of compounds **5** and **6** were elucidated as (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2-methylpropanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*-dodecanoyl-α-L-rhamnopyranosyl](1→4)-2-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside-(1,2′′-lactone) and (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2-methylpropanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*-dodecanoyl-α-L-rhamnopyranosyl](1→4)-2-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside-(1,2′′-lactone), respectively.

Pescapreins XVI (**7**) and XVII (**8**) gave the same quasimolecular ion peak at *m*/*z* 1382.0 $[M + H]^+$ in the positive ESIMS, with the same molecular formula, $C_{72}H_{116}O_{25}$, by positive HRESIMS at *m*/*z* 1381.7867 and 1381.7858 $[M + H]^+$ (calcd 1381.7884), respectively. The positive ESIMS/MS of **7** and **8** exhibited a series of fragments at *m*/*z* 1236.0 $[M + H - 146.0]^+$ (in **7**), 1235.9 $[M + H - 146.1]^+$ (in **8**), 835.6 $[M + H - 546.4]^+$, 689.5 $[835.6 - 146.1]^+$, 507.3 $[689.5 - 182.2]^+$, and 361.2 $[507.3 - 146.1]^+$. Except for the substitution of *m*/*z* 361.2 ($C_{20}H_{25}O_6$) for *m*/*z* 347.2 ($C_{19}H_{23}O_6$), compounds **7** and **8** gave similar cleavage patterns to those of **5** and **6**, suggesting that a 2-methylbutanoyl group in **7** and **8** replaced the 2-methylpropanoyl groups in **5** and **6**. 1D NMR analysis (Tables 1 and 2) and HMBC correlations between H-4′′′′′ (δ_H 6.07 in **7** and 5.75 in **8**) and an acyl carbon (δ_C 175.9 in **7** and 176.4 in **8**, 2-methylbutanoyl) (Figure 1) confirmed that **7** and **8** are the derivatives of **5** and **6**, with the substitution of a 2-methylbutanoyl unit for a 2-methylpropanoyl moiety, respectively. Accordingly, the structures of compounds **7** and **8** were elucidated as (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[3-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*-dodecanoyl-α-L-rhamnopyranosyl](1→4)-2-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside-(1,2′′-lactone) and (11*S*)-*O*-hexadecanoic acid-3-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-[2-*O*-(*trans*-cinnamoyl)-4-*O*-(2*S*-methylbutanoyl)-α-L-rhamnopyranosyl](1→4)-4-*O*-[(2-*O*-*n*-dodecanoyl-α-L-rhamnopyranosyl](1→4)-2-*O*-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside-(1,2′′-lactone), respectively.

It is interesting to note that compounds **2**, **4**, **6**, and **8** each results from the corresponding **1**, **3**, **5**, and **7** via intramolecular 1,2-acyl migration on the third rhamnopyranosyl unit, respectively. This kind of migrations occurs via an *ortho*-acid ester intermediate (Scheme 1).[20,21] In slightly acidic (pH 6.0) and neutral (pH 7.0) aqueous solution, reactions took place between **1** and **2**, between **3** and **4**, between **5** and **6**, and between **7** and **8** (Figures S2 and S3, Supporting Information), showing 1,2-acyl migration to occur smoothly in neutral aqueous solutions. The anhydrous $CHCl_3$ extract and anhydrous petroleum ether extract of the dried whole plants of *I. pes-caprae* both contain the isolates **1**−**8** (Figures S4 and S5, Supporting Information) and indicated that 1,2-acyl migration also took place in the plant. Due to the low energy required for the formation of the cyclic *ortho*-ester intermediate, the migration of the cinnamoyl group on the rhamnopyranosyl moiety to the adjacent *cis*-hydroxyl could take place both in neutral aqueous solutions and under physiological conditions in *I. pes-caprae*.

All the isolates **1**−**8** were evaluated for cytotoxicity against P388 and HL-60 cancer cells by the MTT method,[22] and A549 and BEL-7402 cancer cells by the SRB method.[23] The antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Bacillus subtilis*, and *Candida albicans* were also evaluated by an agar dilution method.[24] Compounds **1**−**8** were evaluated for their affinity to the *μ*-opioid receptor by ligand binding assays.[25] No positive results for compounds **1**−**8** were observed in any of these assays when carried out according to standard protocols.

Experimental Section

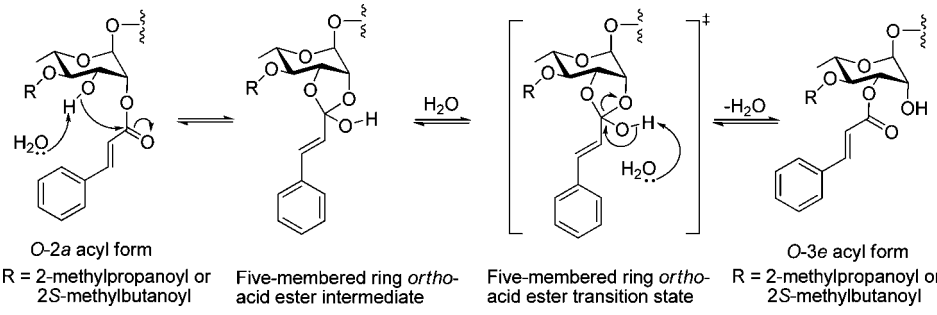
General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were

Table 2. ^{13}C NMR Data for Compounds **1**−**8** (150 MHz, in pyridine-d_5)[a]

position[b]	1	2	3	4	5	6	7	8
1	173.1, qC	173.1, qC	173.1, qC	173.1, qC	173.1, qC	173.1, qC	173.1, qC	173.1, qC
2	34.3, CH_2	34.3, CH_2	34.3, CH_2	34.3, CH_2	34.3, CH_2	34.3, CH_2	34.3, CH_2	34.3, CH_2
11	82.3, CH	82.3, CH	82.3, CH	82.3, CH	82.3, CH	82.3, CH	82.3, CH	82.3, CH
16	14.3, CH_3	14.3, CH_3	14.3, CH_3	14.3, CH_3	14.3, CH_3	14.3, CH_3	14.3, CH_3	14.3, CH_3
1′	104.3, CH	104.3, CH	104.3, CH	104.3, CH	104.3, CH	104.3, CH	104.3, CH	104.3, CH
2′	80.2, CH	80.3, CH	80.2, CH	80.3, CH	80.2, CH	80.2, CH	80.2, CH	80.3, CH
3′	73.3, CH	73.3, CH	73.3, CH	73.3, CH	73.3, CH	73.3, CH	73.3, CH	73.3, CH
4′	73.0, CH	73.0, CH	73.0, CH	73.0, CH	73.0, CH	73.0, CH	73.0, CH	73.0, CH
5′	70.7, CH	70.9, CH	70.7, CH	70.9, CH	70.7, CH	70.9, CH	70.7, CH	70.9, CH
6′	17.4, CH_3	17.4, CH_3	17.4, CH_3	17.4, CH_3	17.4, CH_3	17.4, CH_3	17.4, CH_3	17.4, CH_3
1″	98.7, CH	98.8, CH	98.7, CH	98.8, CH	98.7, CH	98.8, CH	98.7, CH	98.8, CH
2″	73.8, CH	74.1, CH	73.8, CH	74.1, CH	73.8, CH	74.1, CH	73.8, CH	74.1, CH
3″	69.7, CH	69.7, CH	69.7, CH	69.7, CH	69.7, CH	69.7, CH	69.7, CH	69.7, CH
4″	80.6, CH	80.2, CH	80.6, CH	80.2, CH	80.6, CH	80.2, CH	80.6, CH	80.2, CH
5″	68.6, CH	68.6, CH	68.6, CH	68.6, CH	68.6, CH	68.6, CH	68.6, CH	68.6, CH
6″	19.4, CH_3	19.5, CH_3	19.5, CH_3	19.5, CH_3	19.5, CH_3	19.5, CH_3	19.5, CH_3	19.5, CH_3
1‴	99.3, CH	99.0, CH	99.3, CH	99.0, CH	99.4, CH	99.0, CH	99.3, CH	99.0, CH
2‴	73.2, CH	73.2, CH	73.2, CH	73.2, CH	73.2, CH	73.2, CH	73.2, CH	73.2, CH
3‴	79.7, CH	79.6, CH	79.7, CH	79.6, CH	79.7, CH	79.5, CH	79.8, CH	79.6, CH
4‴	79.5, CH	79.5, CH	79.5, CH	79.5, CH	79.5, CH	79.3, CH	79.5, CH	79.5, CH
5‴	68.3, CH	68.4, CH	68.3, CH	68.4, CH	68.3, CH	68.4, CH	68.3, CH	68.4, CH
6‴	18.8, CH_3	18.9, CH_3	18.8, CH_3	18.9, CH_3	18.8, CH_3	18.9, CH_3	18.9, CH_3	18.9, CH_3
1⁗	104.5, CH	104.5, CH	104.5, CH	104.5, CH	104.5, CH	104.5, CH	104.5, CH	104.6, CH
2⁗	72.6, CH	72.2, CH	72.7, CH	72.2, CH	72.6, CH	72.2, CH	72.6, CH	72.2, CH
3⁗	72.5, CH	72.6, CH	72.5, CH	72.6, CH	72.5, CH	72.6, CH	72.5, CH	72.6, CH
4⁗	73.6, CH	73.6, CH	73.6, CH	73.7, CH	73.7, CH	73.6, CH	73.6, CH	73.6, CH
5⁗	70.8, CH	70.8, CH	70.8, CH	70.9, CH	70.8, CH	70.8, CH	70.8, CH	70.8, CH
6⁗	18.6, CH_3	18.7, CH_3	18.6, CH_3	18.7, CH_3	18.6, CH_3	18.7, CH_3	18.6, CH_3	18.7, CH_3
1⁗′	103.5, CH	100.3, CH	103.5, CH	100.3, CH	103.5, CH	100.2, CH	103.5, CH	100.3, CH
2⁗′	70.0, CH	73.8, CH	70.1, CH	73.8, CH	70.0, CH	73.8, CH	70.0, CH	73.8, CH
3⁗′	73.3, CH	68.1, CH	73.3, CH	68.1, CH	73.3, CH	68.1, CH	73.3, CH	68.1, CH
4⁗′	71.4, CH	74.8, CH	71.5, CH	74.9, CH	71.4, CH	74.8, CH	71.4, CH	74.8, CH
5⁗′	68.2, CH	68.4, CH	68.2, CH	68.4, CH	68.2, CH	68.4, CH	68.2, CH	68.4, CH
6⁗′	17.8, CH_3	18.0, CH_3	17.7, CH_3	17.9, CH_3	17.8, CH_3	17.9, CH_3	17.8, CH_3	18.0, CH_3
iba-1			176.4, qC	176.7, qC	176.0, qC	176.4, qC		
iba-2			34.4, CH	34.5, CH	34.4, CH	34.4, CH		
iba-3			19.2, CH_3	19.2, CH_3	19.2, CH_3	19.2, CH_3		
iba-2-Me			18.9, CH_3	19.0, CH_3	18.9, CH_3	19.0, CH_3		
mba-1	175.9, qC	176.4, qC					175.9, qC	176.4, qC
mba-2	41.5, CH	41.5, CH					41.5, CH	41.5, CH
mba-3	26.9, CH_2	27.2, CH_2					26.9, CH_2	27.2, CH_2
mba-4	11.8, CH_3	11.7, CH_3					11.8, CH_3	11.7, CH_3
mba-2-Me	16.9, CH_3	17.0, CH_3					16.9, CH_3	17.0, CH_3
CA-1	166.4, qC	166.8, qC	166.4, qC	166.8, qC	166.4, qC	166.8, qC	166.4, qC	166.8, qC
CA-2	118.3, CH	118.4, CH	118.2, CH	118.4, CH	118.3, CH	118.4, CH	118.3, CH	118.4, CH
CA-3	145.5, CH	145.6, CH	145.5, CH	145.6, CH	145.5, CH	145.6, CH	145.5, CH	145.6, CH
CA-4	134.6, qC	134.7, qC	134.6, qC	134.7, qC	134.6, qC	134.7, qC	134.6, qC	134.7, qC
CA-2′/ 6′	128.5, CH	128.6, CH	128.6, CH	128.6, CH	128.5, CH	128.6, CH	128.5, CH	128.6, CH
CA-3′/ 5′	129.3, CH	129.0, CH	129.3, CH	129.0, CH	129.3, CH	129.0, CH	129.3, CH	129.0, CH
CA-4′	130.8, CH	130.6, CH	130.8, CH	130.6, CH	130.8, CH	130.6, CH	130.8, CH	130.6, CH
deca-1	172.9, qC	172.9, qC	172.9, qC	172.9, qC				
deca-2	34.4, CH_2	34.5, CH_2	34.4, CH_2	34.4, CH_2				
deca-10	14.3, CH_3	14.3, CH_3	14.3, CH_3	14.3, CH_3				
dodeca-1					172.9, qC	172.9, qC	172.9, qC	172.9, qC
dodeca-2					34.4, CH_2	34.5, CH_2	34.4, CH_2	34.5, CH_2
dodeca-12					14.3, CH_3	14.3, CH_3	14.3, CH_3	14.3, CH_3

[a] Chemical shifts (δ_C) are in ppm relative to TMS. [b] mba: 2-methylbutanoyl; iba: 2-methylpropanoyl; CA: *trans*-cinnamoyl; deca: *n*-decanoyl; dodeca: *n*-dodecanoyl.

Scheme 1. Proposed Mechanism for 1,2-Acyl Migrations (*O*-2*a*↔*O*-3*e*) in Compounds **1**−**8**



obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 UV spectrophotometer. 1D-NMR and 2D-NMR spectra were recorded on a JEOL JNM-ECP 600 NMR spectrometer using TMS as internal standard, and chemical shifts are recorded as δ values. ESIMS were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative and analytical HPLC were performed using ODS columns on a Waters 600 multisolvent delivery system equipped with a photodiode array detector (Waters 996). The GC-MS system consisted of an Agilent 6890 gas chromatograph and an Agilent 5973 mass selective detector in the electron-ionization mode.

Plant Material. The medicinal plant *I. pes-caprae* was collected from Hainan Province of the People's Republic of China in April 2004. It was identified by Prof. Hua Peng, and a voucher specimen (KUN-

0071102) has been deposited in the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, People's Republic of China.

Extraction and Isolation. Air-dried and ground entire plants (2.2 kg) were extracted with 95% EtOH (10 L × 3) at room temperature. After removal of the solvents under reduced pressure, the obtained residual material (303.4 g) was distributed between EtOAc and water, and the EtOAc-soluble portion (100.6 g) was subjected to vacuum-column chromatography over silica gel eluted with Me_2CO−petroleum ether in a gradient manner. Two light yellow resinous fractions, A (2.2 g) and B (1.8 g), were collected from the elution of Me_2CO−petroleum ether, 1:9 to 1:8 and 1:5 fractions, respectively. Fractions A and B were separated over Sephadex LH-20 eluted with Me_2CO. Subfraction A1 (2.0 g) showed a spot by TLC on silica gel eluted with $CHCl_3$−CH_3OH (10:1) at R_f 0.50 and gave four main peaks for compounds **3** (t_R 14.9 min), **1** (t_R 17.4 min), **5** (t_R 20.3 min), and **7** (t_R 24.1 min) by HPLC analysis (YMC-Pack ODS-A, 4.6 × 250 mm, 5 μm; H_2O−CH_3OH (2.5:97.5), 1 mL/min; 30 °C; 275 nm). In turn, subfraction B1 (1.8 g) showed a spot at R_f 0.45 on TLC and gave four main peaks for compounds **4** (t_R 13.7 min), **2** (t_R 16.1 min), **6** (t_R 18.8 min), and **8** (t_R 22.4 min), under the same HPLC conditions. Purification of these compounds was performed initially by semipreparative HPLC (YMC-Pack ODS-A, 10 × 250 mm, 5 μm) eluted with H_2O−CH_3OH (2.5:97.5) at a flow of 4 mL/min at 30 °C, respectively. Each subfraction contained two main peaks for compounds **1** and **2**, **3** and **4**, **5** and **6**, and **7** and **8**, respectively. Finally, purification was carried out by semipreparative HPLC eluted with an anhydrous mixture of CH_3OH−CH_3CN (7:3) at a flow rate of 2.8 mL/min at 30 °C by heart cutting and independent reinjection. The pure compounds **3** (15 mg, t_R 18.6 min), **1** (58 mg, t_R 21.1 min), **5** (50 mg, t_R 24.0 min), and **7** (38 mg, t_R 27.9 min) from A1, and **4** (19 mg, t_R 17.4 min), **2** (15 mg, t_R 19.9 min), **6** (30 mg, t_R 22.7 min), and **8** (45 mg, t_R 26.2 min) from B1 were obtained, respectively.

The air-dried and ground entire plant (5 g each) was extracted twice with 80 mL of anhydrous $CHCl_3$ and petroleum ether (dried over anhydrous Na_2SO_4) to afford 250 mg and 180 mg extracts, respectively. The MeOH-soluble portion was applied to HPLC analysis (YMC-Pack ODS-A, 4.6 × 250 mm, 5 μm; H_2O−CH_3OH (2.5:97.5), 1 mL/min; 30 °C; 275 nm).

Pescaprein X (1): amorphous, white powder; $[\alpha]^{20}_D$ −17 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.2), 279 (3.9) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1354.1 ($[M + H]^+$); positive ESIMS/MS *m/z* 1354.1, 1208.0, 807.6, 661.5, 507.3, 361.2; positive HRESIMS *m/z* 1353.7589 ($[M + H]^+$) (calcd for $C_{70}H_{113}O_{25}$, 1353.7571).

Pescaprein XI (2): amorphous, white powder; $[\alpha]^{20}_D$ −12 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.5), 279 (3.8) nm;^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1354.1 ($[M+H]^+$); positive ESIMS/MS *m/z* 1354.1, 1208.0, 807.6, 661.5, 507.3, 361.2; positive HRESIMS *m/z* 1353.7563 ($[M+H]^+$) (calcd for $C_{70}H_{113}O_{25}$, 1353.7571).

Pescaprein XII (3). amorphous white powder; $[\alpha]^{20}_D$ −15 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.7), 279 (3.9) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1339.9 $[M + H]^+$; positive ESIMS/MS *m/z* 1339.9, 1193.8, 793.5, 647.4, 493.3, 347.2; positive HRESIMS *m/z* 1361.7253 ($[M + Na]^+$) (calcd for $C_{69}H_{110}O_{25}Na$, 1361.7234).

Pescaprein XIII (4): amorphous, white powder; $[\alpha]^{20}_D$ −18 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.1), 279 (3.8) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1339.9 $[M + H]^+$; positive ESIMS/MS *m/z* 1339.9, 1193.8, 793.5, 647.4, 493.3, 347.2; positive HRESIMS *m/z* 1361.7238 ($[M + Na]^+$) (calcd for $C_{69}H_{110}O_{25}Na$, 1361.7234).

Pescaprein XIV (5): amorphous, white powder; $[\alpha]^{20}_D$ −26 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (4.3), 279 (4.2) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1368.0 $[M + H]^+$; positive ESIMS/MS *m/z* 1368.0, 1222.0, 821.6, 675.5, 493.3, 347.2; positive HRESIMS *m/z* 1367.7706 ($[M + H]^+$) (calcd for $C_{71}H_{115}O_{25}$, 1367.7727).

Pescaprein XV (6): amorphous, white powder; $[\alpha]^{20}_D$ −13 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (4.0), 280 (3.9) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1368.0 $[M + H]^+$; positive ESIMS/MS *m/z* 1368.0, 1222.0, 821.6, 675.5, 493.3, 347.2; positive HRESIMS *m/z* 1367.7783 ($[M + H]^+$) (calcd for $C_{71}H_{115}O_{25}$, 1367.7727).

Pescaprein XVI (7): amorphous, white powder; $[\alpha]^{20}_D$ −20 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.1), 279 (4.0) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1382.0 $[M + H]^+$; positive ESIMS/MS *m/z* 1382.0,1235.9, 835.6, 689.5, 507.3, 361.2; positive HRESIMS *m/z* 1381.7867 ($[M + H]^+$) (calcd for $C_{72}H_{117}O_{25}$, 1381.7884).

Pescaprein XVII (8): amorphous, white powder; $[\alpha]^{20}_D$ −6 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.9), 280 (3.9) nm; ^1H and ^{13}C NMR, see Tables 1 and 2; positive ESIMS *m/z* 1382.0 $[M + H]^+$; positive ESIMS/MS *m/z* 1382.0, 1235.9, 835.6, 689.5, 507.3, 361.2; positive HRESIMS *m/z* 1381.7858 ($[M + H]^+$) (calcd for $C_{72}H_{117}O_{25}$, 1381.7884).

Intramolecular *trans*-Cinnamoyl Migration under Water. Compound **1** (1 mg) was stirred in a solution of MeOH−H_2O (95:5, 1 mL) for 30 min at room temperature and evaporated to dryness in vacuo. The residue was identified as a mixture of compounds **1** and **2** by HPLC analysis. Compound **2** also gave a mixture of **1** and **2** when subjected to the same procedure. Compounds **3** and **4**, **5** and **6**, and **7** and **8** were treated as above, and similar results were obtained.

Transesterification of the Resin Glycoside Mixture of Fractions A1 and B1 Containing 1−8. A solution of NaOMe (5 mg) in anhydrous MeOH (1 mL) was added dropwise to a solution of fraction A1 (15 mg) and B1 (15 mg) in anhydrous MeOH (4 mL) at room temperature and then refluxed for 2 h. The reaction mixture was adjusted to pH 7.0 with positive ion-exchange resin and filtered. The filtrate was concentrated and subjected to separation over Sephadex LH-20 eluted with MeOH−$CHCl_3$ (1:1) to discard the byproduct in the reaction. Subfractions were collected and evaporated in vacuo. The residue obtained (18 mg) was identified as simonic acid B methyl ester (**9**) by 2D NMR data analysis and comparison of its mass spectrum, ^1H and ^{13}C NMR data, and physical properties (mp 112−114 °C; $[\alpha]^{20}_D$ −81 (*c* 0.76, MeOH)) with the literature values.[15]

Saponification of the Resin Glycoside Mixture of Fractions A1 and B1 Containing 1−8. A solution of fractions A1 (80 mg) and B1 (80 mg) in 5% KOH−H_2O (5 mL) was refluxed for 2 h. The reaction mixture was acidified to pH 4.0 with 4 N HCl and extracted with CH_2Cl_2 (10 mL × 2). The water layer was adjusted to pH 7.0 with 1 N NaOH and evaporated to dryness in vacuo. The residue was subjected to Sephadex LH-20 eluted with MeOH to desalt, and 100 mg of simonic acid B (**10**) was obtained as a colorless oil. It afforded key fragments at *m/z* 1003.4 $[M + H]^+$, 1025.4 $[M + Na]^+$, 857.4 $[M + H - 146.0]^+$, 711.3 $[857.4 - 146.1]^+$, 565.3 $[711.3 - 146.0]^+$, 419.2 $[565.3 - 146.1]^+$, and 273.2 $[419.2 - 146.0]^+$ in the positive ESIMS. Its NMR data were assigned by comparison with those of simonic acid B methyl ester (**9**). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, concentrated in vacuo, and directly analyzed by GC-MS (30 m × 0.32 mm × 0.25 μm HP-5 MS column: He, 1 mL/min; 40 °C, 2 min, 40−250 °C, Δ 15 °C/min, 250 °C, 10 min): 2-methylpropanoic acid (t_R 3.38 min): *m/z* 88 $[M]^+$ (12), 73 (47), 60 (3), 55 (7), 45 (14), 43 (100), 41 (48); 2-methylbutanoic acid (t_R 4.87 min): *m/z* 102 $[M]^+$ (1), 87 (25), 74 (100), 73 (15), 57 (47), 45 (11), 41 (35); *n*-decanoic acid (t_R 10.57 min): *m/z* 172 $[M]^+$ (7), 143 (10), 129 (58), 115 (14), 101 (8), 87 (17), 73 (100), 60 (98), 57 (42), 55 (40), 43 (40), 41 (42); *trans*-cinnamic acid (t_R 11.18 min): *m/z* $^+$ 148 [M] (74), 147 (100), 131 (21), 120 (5) 103 (45), 102 (23), 91(21), 77 (34), 74 (7), 63 (5), 51 (23), 50 (9), 45 (6); and *n*-dodecanoic acid (t_R 12.27 min): *m/z* 200 $[M]^+$ (12), 183 (2), 171 (12), 157 (32), 143 (11), 129 (42), 115 (18), 101 (13), 85 (30), 73 (100), 60 (82), 57 (42), 55 (46), 43 (48), 41 (44).

By the same procedure, pure compound **1** gave 2-methylbutanoic acid [t_R 5.09 min; *m/z* 102 $[M]^+$ (1), 87 (25), 74 (100), 73 (14), 57 (48), 45 (12), 41 (35)], *n*-decanoic acid [t_R 10.62 min; *m/z* 172 $[M]^+$ (6), 143 (11), 129 (60), 115 (14), 101 (8), 87 (20), 83 (13), 73 (100), 71 (38), 60 (98), 57 (40), 55 (39), 43 (38), 41 (40)], and *trans*-cinnamic acid [t_R 11.27 min; *m/z* 148 $[M]^+$ (68), 147 (100), 131 (19), 103 (44), 102 (21), 91 (20), 77 (32), 74 (8), 51 (21), 50 (8), 45 (3)].

Preparation of (*R*)-1-Phenylethyl-(*S*)-2-methylbutanoate. Trace DMAP (1.5 mg) was added to the solution of the above carboxylic acids (a quarter from saponification) in CH_2Cl_2 (2 mL) and cooled to 0 °C. Then (*R*)-1-phenylethanol (7.0 μL) and DCC (15.0 mg) were added. The reaction mixture was stirred at 0 °C for 10 min and room temperature for 12 h and then filtered. The standard (*R*)-1-phenylethyl-(*S*)-2-methylbutanoate was also prepared from (*R*)-1-phenylethanol and (*S*)-2-methylbutanoic acid by the same process. The reaction product was analyzed by GC-MS (30 m × 0.32 mm × 0.25 μm HP-5 MS

column: He, 1 mL/min; 35 °C, 5 min, 35−250 °C, Δ 15 °C/min, 250 °C 10 min): (*R*)-1-phenylethyl-(*S*)-2-methylbutanoate (t_R 14.29 min): *m*/*z* 206 $[M]^+$ (8), 122 (35), 105 (100), 104 (26), 85 (9), 77 (14), 57 (18), 50 (2), 43 (2), 41 (4).

By the same procedure, the carboxylic acids produced by pure compound **1** afforded (*R*)-1-phenylethyl-(*S*)-2-methylbutanoate (t_R 14.29 min): *m*/*z* 206 $[M]^+$ (10), 122 (34), 105 (100), 104 (25), 85 (8), 79 (8), 77 (12), 57 (17), 51 (4), 43 (2), 41 (4).

Sugar Analysis. The glycosidic acid (20 mg from saponification) was refluxed in 1 M H_2SO_4 (2 mL) at 95 °C for 1.5 h. The reaction mixture was diluted with H_2O (2 mL) and extracted with CH_2Cl_2 (5 mL × 3). The aqueous phase was dried in vacuo after neutralization with 4 N NaOH and chromatographed over Sephadex LH-20 eluted with MeOH to desalt. Then, 3 mg of the residue obtained and 2 mg of L-cysteine methyl ester hydrochloride were dissolved in 1 mL of anhydrous pyridine, and the resulting mixture was stirred at 60 °C for 1 h. A 3:1 mixture of HMDS−TMCS (hexamethyldisilazane−trimethylchlorosilane) was then added (300 μL), and the solution was stirred for 30 min. To the solution was then added hexanes (3 mL) and water (1 mL). The hexanes layer was dried over anhydrous Na_2SO_4 and subjected to GC-MS analysis (30 m × 0.32 mm × 0.25 μm HP-5 MS column: He, 1 mL/min; 40 °C 2 min 40−250 °C, Δ 15 °C/min, 250 °C 10 min), which permitted the identification of the 4:1 mixture of the L-rhamnose derivative (t_R 17.99 min) and the D-fucose derivative (t_R 18.24 min), respectively, by coelution with authentic monosaccharide derivatives.[26]

Acknowledgment. This work was supported by a project of the Chinese National Programs for High Technology Research and Development (Nos. 2007AA091502 and 2007AA09Z447).

Supporting Information Available: Chemical transformation scheme, mass spectra, HPLC profiles, GC-MS diagrams, NMR spectra, and bioassay protocols used. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP800386Z