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Full Paper

Two Novel Types of Cardiac Glycosides from *Parepigynum funingense* and the Possible Biogenesis

CAO, Jian-Xin[a,b](曹建新) LUO, Shi-De*[,a](罗士德)

[a] *State Key Laboratory of Phytochemistry and Plant Resouces in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China*

[b] *Department of Chemistry, Zhejiang University, Hangzhou, Zhejiang 310027, China*

Five novel cardiac glycosides with two types of unusual aglycone [funingenin A (**2a**) and B], named funingenosides E—I (**2**—**6**), together with a known compound, funingenoside B (**1**), were isolated from the aerial part of *Parepigynum funingense* Tsiang et P. T. Li (Apocynaceae). The structures of **2**—**6** were elucidated by means of MS, IR, NMR spectral analyses and chemical degradation. The possible biogenetic pathway of the two types of cardiac glycosides was also discussed.

Keywords *Parepigynum funingense*, apocynaceae, cardiac glucoside, funingenoside, biogenesis

Introduction

Parepigynum funingense, an endemic species belonging to the family apocynaceae, is naturally distributed in Yunnan Province, China. In the previous literature, a large number of cardiac glycosides were isolated from various species in this family.[1] However, only four compounds from this plant have been published up till now.[2] As a monotype genus, its unique taxonomic position attracted us to investigate its chemical constituents. In this paper, we report the isolation and structural elucidation of five novel cardiac glycosides from the aerial part of *P. funingense*. The five compounds belonged to two types of cardiac glycosides. The possible biogenetic pathway of the two types of cardiac glycosides was discussed.

Results and discussion

Compound **1** was identified by comparison of the spectral data with reported ones as funingenoside B.[2] But the configuration of cymarosyl of **1** was indicated to *α-D*-cymarosyl by the broad singlet at δ_H 5.14 (H-1') for the anomeric proton of the cymaropyranosyl unit and confirmed by the comparison of spectral data. The ^{1}H and ^{13}C NMR spectral data of the internal sugar of **1** were similar to those of funingenoside A.[2] Furthermore, according to the X-ray crystallographic analysis,[2] the internal sugar of funingenoside A should be *α-D*-cymarosyl. So, the internal sugar of **1** was determined to be *α-D*-cymarosyl. And the *R* configuration of C-20 was indicated by the NOE interactions of δ_H 2.50 (H-20) with δ_H 1.05 (H-18) and δ_H 1.54 (H-15*β*), 1.25 (H-16*β*) with δ_H 4.09, 4.25 (H-21), δ_H 1.05 (H-18) with δ_H 2.80 (H-22). Therefore, the structure of **1** was elucidated as (8*R*)-4*β*-acetoxy-3*β*-[(*O-β-D*-glucopyranosyl-(1 → 6)-*O-β-D*-glucopyranosyl-(1 → 4)-*α-D*-cymaropyranosyl)oxy]-14-oxo-5*α*-20(*R*)-15(14 → 8)-*abeo*-card-20(22)-dihydroenolide (Figure 1).

Funingenoside E (**2**) was obtained as white powder. The molecular formula was determined to be $C_{44}H_{68}O_{19}$ from negative-ion HRFABMS (experimental part). Mild acidic hydrolysis of **2** revealed the presence of oleandrose and glucose by TLC comparison with authentic compounds. The ^{13}C NMR (Table 1) and HMQC-TOCSY spectra of **2** displayed the presence of an unsubstituted glucopyranosyl unit, a substituted glucopyranosyl unit and a substituted oleandropyranosyl unit,[3,4] together with 25 carbon signals for the aglycone. The downfield chemical shifts of C-4' of the oleandropyranosyl unit and C-6'' of the glucopyranosyl unit (δ_C 82.1 and 70.7) proved their substitutions at C-4' and C-6'' respectively. This was supported by the HMBC spectral analysis (Table 2), which displayed significant correlation peaks of H-1'' of the glucopyranosyl unit with C-4' of the oleandropyranosyl unit, and H-1''' of the terminal glucopyranosyl residue with C-6'' of the glucopyranosyl unit. The linkage of saccharide chain to the aglycone was decided by correlation peak between H-1' of the oleandropyranosyl unit with C-3 of the aglycone in HMBC. In the ^{1}H NMR spectrum of **2**, the doublet signals at δ_H 5.16 (H-1''') with coupling constant at 7.9 Hz and δ_H 5.27 (H-1'') with coupling constant at 7.8 Hz for the anomeric protons of the two glucosyl residues respectively indicated the *β* linkages. Moreover, the broad singlet at δ_H 5.31 (H-1') for the anomeric proton of the oleanderopyranosyl unit indicated the *α* linkage.

Acid hydrolysis of **1** and **2** respectively gave the same aglycone, named funingenin A (**2a**). It was neither isolated nor transformed previously. Its NMR data (ex-

* E-mail: jxcao321@hotmail. com; Tel.: 86-871-5223097; Fax: 86-871-5223038
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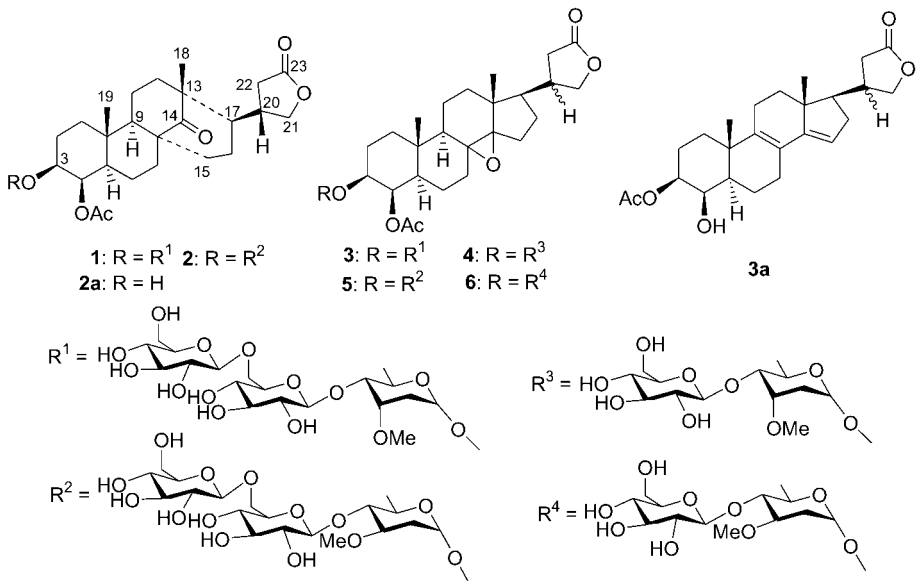

Figure 1 The structures of compounds **1**—**6**, **2a** and **3a**.

Table 1 ^{13}C NMR, DEPT, HMQC and ^{1}H NMR spectral data of **1** and **2** recorded in C_5D_5N

Position	**1**		**2**	
	^{13}C NMR and DEPT	HMQC and ^{1}H NMR	^{13}C NMR and DEPT	HMQC and ^{1}H NMR
1*α*	38.4 (t)	0.81—0.85 (m)	38.5 (t)	0.85—0.89 (m)
1*β*		2.33—2.37 (m)		1.76—1.80 (m)
2*α*	25.3	1.81—1.85 (m)	25.1 (t)	1.68—1.72 (m)
2*β*		1.92—1.96 (m)		1.88—1.92 (m)
3	75.3 (d)	3.75 (brd, *J*=9.8 Hz)	74.0 (d)	3.80 (brd, *J*=9.8 Hz)
4	72.4 (d)	5.38 (brs)	72.0 (d)	5.38 (brs)
5	46.6 (d)	0.08—1.02 (m)	46.5 (d)	1.01—1.05 (m)
6*α*	23.5 (t)	1.23—1.27 (m)	23.6 (t)	1.26—1.30 (m)
6*β*		0.92—0.96 (m)		1.00—1.04 (m)
7*α*	35.0 (t)	0.85—0.89 (m)	35.1 (t)	0.87—0.91 (m)
7*β*		2.35—2.39 (m)		2.33—2.37 (m)
8	49.3 (s)		49.5 (s)	
9	60.6 (d)	1.62—1.66 (m)	60.6 (d)	1.57—1.61 (m)
10	38.5 (s)		38.5 (s)	
11a	20.9 (t)	1.68—1.72 (m)	21.0 (t)	1.70—1.74 (m)
11b		1.89—1.87 (m)		1.94—1.98 (m)
12*α*	42.3 (t)	2.02—2.06 (m)	42.4 (t)	1.97—2.01 (m)
12*β*		1.87—1.89 (m)		1.92—1.96 (m)
13	49.3 (s)		49.7 (s)	
14	220.0 (s)		221.3 (s)	
15*α*	43.4 (t)	1.83—1.87 (m)	43.6 (t)	1.79—1.81 (m)
15*β*		1.60—1.64 (m)		1.52—1.56 (m)
16*α*	23.6 (t)	2.35—2.39 (m)	23.9 (t)	2.33—2.37 (m)
16*β*		1.30—1.33 (m)		1.23—1.27 (m)
17	55.9 (d)	1.96—2.00 (m)	56.1 (d)	1.93—1.97 (m)
18	23.8 (q)	1.05 (s)	24.0 (q)	1.05 (s)
19	16.1 (q)	0.92 (s)	16.2 (q)	0.91 (s)
20	36.4 (d)	2.47—2.51 (m)	36.6 (d)	2.48—2.52 (m)
21a	71.4 (t)	4.07—4.11 (m)	71.8 (t)	4.07—4.11 (m)
21b		4.18—4.22 (m)		4.23—4.27 (m)

Continued

Position	**1**		**2**	
	^{13}C NMR and DEPT	HMQC and ^{1}H NMR	^{13}C NMR and DEPT	HMQC and ^{1}H NMR
22a	36.4 (t)	2.78 (dd, J=7.7, 8.9 Hz)	36.7 (t)	2.80 (dd, J=7.7, 8.9 Hz)
22b		2.34—2.38 (m)		2.34—2.38 (m)
23	177.0 (s)		177.7 (s)	
O-**C**O-Me	170.7 (s)		171.1 (s)	
O-CO-**Me**	21.0 (q)	2.02 (s)	21.1 (q)	1.96 (s)
1'	94.6 (d)	5.14 (brs)	95.1 (d)	5.31 (brs)
2'a	31.8 (t)	1.89—1.93 (m)	35.2 (t)	1.58—1.62 (m)
2'b		2.34—2.38 (m)		2.26—2.30 (m)
3'	73.3 (d)	4.04—4.08 (m)	79.3 (d)	4.26—4.30 (m)
4'	78.7 (d)	4.09—4.28 (m)	82.1 (d)	3.88—3.92 (m)
5'	65.2 (d)	4.63—4.55 (m)	68.2 (d)	4.08—4.12 (m)
6'	18.5 (q)	1.55 (d, J=6.4 Hz)	19.1 (q)	1.77 (d, J=6.4 Hz)
OMe	56.4 (q)	3.41 (s)	57.0 (q)	3.38 (s)
1''	101.8 (d)	5.00 (d, J=7.7 Hz)	105.0 (d)	5.27 (d, J=7.8 Hz)
2''	75.2 (d)	4.06—4.10 (m)	75.9 (d)	4.06—4.10 (m)
3''	78.4 (d)	4.09—4.29 (m)	78.4 (d)	3.93—3.97 (m)
4''	71.9 (d)	4.28—4.32 (m)	72.0 (d)	4.06—4.10 (m)
5''	77.7 (d)	4.03—4.06 (m)	77.4 (d)	4.96—5.00 (m)
6''a	70.4 (t)	4.83—4.85 (m)	70.7 (t)	4.80—4.84 (m)
6''b		4.30—4.31 (m)		4.30—4.33 (m)
1''''	105.5 (d)	5.18 (d, J=7.8 Hz)	105.6 (d)	5.16 (d, J=7.9 Hz)
2''''	75.4 (d)	4.06—4.10 (m)	75.4 (d)	4.10—4.15 (m)
3''''	78.5 (d)	4.09—4.29 (m)	78.5 (d)	4.10—4.15 (m)
4''''	71.8 (d)	3.85—3.89 (m)	71.9 (d)	4.06—4.09 (m)
5''''	78.6 (d)	4.09—4.29 (m)	78.6 (d)	4.18—7.21 (m)
6'''a	62.9 (t)	4.52—4.56 (m)	63.0 (t)	4.43—4.47 (m)
6'''b		4.37—4.41 (m)		4.53—4.57 (m)

Table 2 HMBC and ROESY spectral data for **1** and **2** recorded in C_5D_5N

Position	HMBC (H to C)	ROESY	Position	HMBC (H to C)	ROESY
1α	C-2, C-3, C-5	H-3, H-5, H-9	15β	C-16	H-20
1β		H-19			
2α	C-1, C-3, C-4	H-3	16α	C-17	H-17
2β		H-19			
3	C-1, C-2, C-4, C-1'	H-1α, H-4, H-5, H-1'	16β		H-21, H-17
4	C-2, C-3, C-5, OAc	H-3, H-5, H-6α	17	C-12, C-14, C-16, C-18	H-12α, H-18, H-21
5	C-6, C-7, C-10, C-19	H-9, H-4, H-3, H-1α	18	C-12, C-13, C-14, C-17	H-17, H-20, H-22
6α		H-4	19	C-5, C-9, C-10	H-2β
6β		H-7	20	C-17, C-21, C-22, C-23	H-15β, H-8, H-21, H-22
7α	C-14	H-9	21a	C-20, C-22, C-23	H-16β, H-17
			21b		H-16β
7β	C-14	H-15β	22a	C-20, C-21, C-23	H-18
9	C-5, C-8, C-10, C-11, C-12, C-14, C-19	H-5, H-7α, H-1α	OAc	C-4	
11α	C-9, C-12		1'	C-3, C-5', C-3'	H-3
12α	C-9, C-11, C-14	H-17	1''	C-4', C-5''	
12β	C-18		1'''	C-6'', C-5'''	
15α	C-9, C-14	H-9			

perimental part) was similar to those of aglycone of **1** except for C-3 and C-4, which was explained by glycosylation shifts.

Based on extensive spectral analysis, the structure of **2** was elucidated as (8*R*)-4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-oleandro-pyranosyl)oxy]-14-oxo-5*α*-20(*R*)-15(14 → 8)-*abeo*-card-20(22)-dihydroenolide and named funingenoside E (Figure 1).

Funingenoside F (**3**) was obtained as white powder. The molecular formula was determined to be $C_{44}H_{68}O_{19}$ from negative-ion HRFABMS (experimental part). Mild acidic hydrolysis of **3** revealed the presence of cymarose and glucose by TLC comparison with authentic compounds. Comparison of the NMR spectral data of **3** (Tables 3 and 4) with those of **1** revealed that the saccharide chains of them were the same, and the rings A and E of their aglycones were very similar. Two quaternary carbons δ_C 64.2 and 70.7 appeared in **3** instead of the carbonyl δ_C 221.0 in **1**. The ^{13}C and DEPT spectra of the aglycone of **3** showed the presence of six methines, ten methylenes, three methyl groups, four quaternary carbons and two carbonyl groups. The chemical shifts of the rings B, C and D of aglycone carbons were similar to those of 5*α*-adynerin.[5] The formation of the structure was supported by the correlations of H-18 (δ_H 0.89) with C-14 (δ_C 64.2) and H-19 (δ_H 1.23) with C-8 (δ_C 70.7) and further proved by the product of hydrolysis of **3**.

Funingenin C (**3a**), white powder, was the product of the hydrolysis of **3**. The molecular formula was determined to be $C_{25}H_{35}O_5$ from positive-ion ESI (experimental part). A careful comparison of the ^{1}H and ^{13}C NMR data of **3a** (Tables 3 and 4) with those of aglycone of **3** showed that the two characteristic quaternary carbons (δ_C 64.2 and 70.7) in **3** disappeared and three quaternary carbons (δ_C 150.9, 142.4 and 123.3) and one methine (δ_C 116.9) in **3a** appeared. It was deduced that the 8,14-epoxy bond was destructed to form two conjugate double bonds (Δ^8 and Δ^{14}). This deduction was supported by the correlations of H-19 δ_H 1.48 with C-9 δ_C 142.4, H-18 δ_H 0.81 with C-14 δ_C 150.9 and C-17 δ_C 55.1 in HMBC. The literature also showed the same conversion after the similar hydrolysis.[4] The downfield chemical shift of C-19 from δ_C 15.1 to 22.2 after hydrolysis was explained by the deshielding effect of Δ^8. And it was interesting to note that Ac group migrated from position-4 to position-3 under acidic condition. This was showed by the interactions between H-3 (δ_H 4.94) and H-2 (δ_H 1.85, 2.34) in the ^{1}H-^{1}H COSY spectrum and supported by the coupling constants of H-3 (brd, J=9.8 Hz) and H-4 (brs).

The obvious pair of signals for H-21 of **3a** revealed that a pair of epimer at C-20, which was not separated by HPLC, was present. And it was also the reason of the appearance of doublet signals at C-9, C-12, C-14, C-15, C-16, C-20, C-21, C-22 and C-23 in **3** and **3a**.

Therefore, the structure of **3a** was elucidated as 3*β*-acetoxy-4*β*-hydroxy-5*α*-card-20(22)-dihydro-8,14-dienolide and named funingenin C. The structure of **3** was elucidated as 4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1 → 6)-*O*-*β*-*D*-glucopyranosyl-(1 → 4)-*α*-*D*-cymaropyryl)oxy]-8,14-epoxy-5*α*-card-20(22)-dihydroenolide and named funingenoside F. The aglycone of **3** was named funingenin B (Figure 1).

Funingenosides G (**4**), H (**5**) and I (**6**) were obtained as white powder. Their molecular formulas were deduced from negative-ion HRFABMS respectively (experimental part). The ^{1}D and ^{2}D NMR (Tables 3, 4 and 5) showed that the aglycones of **3**—**6** were the same. The sugar moiety of **4** was similar to that of **3** except that there was one less glucosyl in **4** on the basis of spectroscopic data. Accordingly, **4** was elucidated as 4*β*-acetoxy-3*β*-[*O*-*β*-*D*-glucopyranosyl-(1 → 4)-*α*-*D*-cymaroranosyl]oxy]-8,14-epoxy-5*α*-card-20(22)-dihydroenolide and named funingenoside G (Figure 1). The sugar moiety of **5** was the same to that of **2** on the basis of spectroscopic data. Accordingly, **5** was elucidated as 4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1 → 6)-*O*-*β*-*D*-glucopyranosyl-(1 → 4)-*α*-*D*-oleadropyranosyl)oxy]-8,14-epoxy-5*α*-card-20(22)-dihydroenolide and named funingenoside H (Figure 1). The sugar moiety of **6** was similar to that of **2** except that there was one less glucosyl in **6** on the basis of spectroscopic data. Accordingly, **6** was elucidated as 4*β*-acetoxy-3*β*-[*O*-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-oleadropyranosyl]oxy]-8,14-epoxy-5*α*-card-20(22)-dihydroenolide and named funingenoside I (Figure 1).

Compounds **1**—**6** belonged to two types of cardiac glycosides. A possible pathway of biogenesis of the two types of glycosides has been proposed in Figure 2.[6]

Compounds **1**—**6** all were the cardiac glycosides without the common olefinic bond in ring E. In the previous literature, a large number of cardiac glycosides were isolated from species of apocynaceae.[1] Moreover, the unsaturated lactone at C-17 was the characteristic of cardiac glycosides and associated with cardiotonic activity.[7] However, compounds **1**—**6** instead of normal cardiac glycosides were isolated from *P. funingenese*. This finding supported the view of taxonology that *P. funingenese* is a monotype genus in apocynaceae.[8]

Experimental

Optical rotations were recorded in pyridine and chloroform on a Horiba SEAP-300 spectropolarimeter. IR spectra were taken in KBr pellets on a Bio-Rad FTS-IR spectrophotometer. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz) and ^{2}D NMR spectra were recorded in pyridine-d_5 on a Bruker DRX-500 NMR spectrometer with TMS as an internal standard. MS data were measured by a VG Autospec 3000 mass spectrometer and an API QSTAR Pulsar I system under negative-ion FAB, EI and ESI models respectively. Column chromatogra-

Table 3 ^{13}C NMR spectral data of **3**—**6** and **3a** recorded in C_5D_5N

C	**3**	**4**	**5**	**6**	**3a**
1	37.5 (t)	37.6 (t)	37.5 (t)	37.5 (t)	36.0 (t)
2	25.0 (t)	25.2 (t)	24.7 (t)	24.8 (t)	23.3 (t)
3	75.3 (d)	75.4 (d)	74.0 (d)	74.0 (d)	76.4 (d)
4	72.4 (d)	72.5 (d)	71.7 (d)	71.8 (d)	72.3 (d)
5	47.3 (d)	47.3 (d)	47.0 (d)	47.1 (d)	45.2 (d)
6	23.9 (t)	24.1 (t)	23.9 (t)	23.9 (t)	27.1 (t)
7	32.3 (t)	32.4 (t)	32.3 (t)	32.3 (t)	30.1 (t)
8	64.2 (s)	64.3 (s)	64.2 (s)	64.1 (s)	123.3 (s)
9	51.2 (d)	51.2 (d)	51.1 (d)	51.1 (d)	142.4 (142.5) (s)
10	37.6 (s)	37.7 (s)	37.6 (s)	37.6 (s)	37.4 (s)
11	16.2 (t)	16.3 (t)	16.2 (t)	16.2 (t)	21.4 (t)
12	36.9 (37.3) (t)	37.1 (37.4) (t)	36.9 (37.3) (t)	37.0 (37.3) (t)	35.6 (35.9) (t)
13	40.9 (41.1) (s)	41.0 (41.2) (s)	40.9 (41.1) (s)	40.9 (41.1) (s)	45.7 (s)
14	70.7 (70.8) (s)	70.8 (71.0) (s)	70.8 (70.9) (s)	70.8 (70.9) (s)	150.9 (s)
15	27.6 (t)	27.7 (t)	27.6 (t)	27.6 (t)	116.9 (117.1) (d)
16	25.9 (26.9) (t)	26.1 (27.2) (t)	25.9 (27.0) (t)	25.9 (26.9) (t)	36.1 (36.3) (t)
17	54.7 (d)	54.8 (d)	54.6 (d)	54.6 (d)	55.1 (d)
18	15.8 (15.9) (q)	15.9 (16.0) (q)	15.8 (15.9) (q)	15.8 (15.9) (q)	16.7 (q)
19	15.1 (q)	15.3 (q)	15.1 (q)	15.1 (q)	22.2 (q)
20	37.7 (38.0) (d)	37.8 (38.2) (d)	37.7 (38.0) (d)	37.6 (38.0) (d)	37.3 (37.6) (d)
21	72.5 (72.9) (t)	72.6 (73.0) (t)	72.5 (72.9) (t)	72.4 (72.8) (t)	72.8 (72.9) (d)
22	34.1 (34.2) (t)	34.2 (34.3) (t)	34.1 (34.2) (t)	34.1 (34.2) (t)	34.4 (34.5) (t)
23	177.0 (177.5) (s)	177.0 (177.6) (s)	177.0 (177.5) (s)	177.0 (177.5) (s)	177.1 (177.6) (s)
OCO**Me**	21.0 (q)	21.2 (q)	21.0 (q)	20.9 (q)	21.3 (q)
O**C**OMe	170.7 (s)	171.0 (s)	170.9 (s)	170.9 (s)	170.5 (s)
1'	94.9 (d)	95.0 (d)	94.9 (d)	95.0 (d)	
2'	31.8 (t)	31.8 (t)	35.0 (t)	35.1 (t)	
3'	73.3 (d)	73.3 (d)	79.1 (d)	79.3 (d)	
4'	78.5 (d)	78.9 (d)	82.0 (d)	82.5 (d)	
5'	65.2 (d)	65.1 (d)	68.0 (d)	67.9 (d)	
6'	18.5 (q)	18.6 (q)	18.9 (q)	18.8 (q)	
3'-OMe'	56.4 (q)	56.5 (q)	56.7 (q)	56.7 (q)	
1''	101.8 (d)	102.1 (d)	104.8 (d)	105.2 (d)	
2''	75.4 (d)	75.6 (d)	75.7 (d)	76.0 (d)	
3''	78.3 (d)	78.5 (d)	78.3 (d)	78.3 (d)	
4''	71.9 (d)	72.0 (d)	71.8 (d)	72.1 (d)	
5''	77.7 (d)	78.7 (d)	77.1 (d)	78.2 (d)	
6''	70.4 (t)	63.1 (t)	70.5 (t)	63.2 (t)	
1'''	105.5 (d)		105.4 (d)		
2'''	75.3 (d)		75.2 (d)		
3'''	78.4 (d)		78.2 (d)		
4'''	71.7 (d)		71.7 (d)		
5'''	78.6 (d)		78.5 (d)		
6'''	62.9 (t)		62.8 (t)		

Table 4 ^{1}H NMR spectral data of **3**—**6** and **3a** recorded in C_5D_5N

H	3	4	5	6	3a
1	0.99—1.01 (m) 1.68—1.88 (m)	0.95—0.99 (m) 1.64—1.68 (m)	1.02—1.04 (m) 1.79—1.81 (m)	1.01 (m), 1.66 (m)	0.90—0.94 (m) 1.28—1.32 (m)
2	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	1.84—1.86 (m), 2.32—2.36 (m)
3	3.80 (brd, *J*=9.8 Hz)	3.78 (brd, *J*=9.8 Hz)	3.79 (brd, *J*=9.8 Hz)	3.80 (brd, *J*=9.8 Hz)	4.94 (brd, *J*=9.8 Hz)
4	5.44 (brs)	5.45 (brs)	5.44 (brs)	5.46 (brs)	4.24 (brs)
5	1.97 (m)	1.96—2.00 (m)	1.96—2.00 (m)	1.95—1.99 (m)	1.57—1.61 (m)
6	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	2.19—2.23 (m)
7	1.37—1.39 (m)	1.76—1.79 (m) 1.33—1.37 (m)	1.85—1.89 (m) 1.39—1.43 (m)	1.76—1.80 (m) 1.33—1.37 (m)	
9	1.26—1.30 (m)	1.26—1.30 (m)	1.36—1.40 (m)	1.26—1.30 (m)	
12	1.42—1.46 (m) 1.32—1.36 (m)	1.42—1.46 (m) 1.32—1.36 (m)	1.50—1.54 (m) 1.40—1.44 (m)	1.42—1.46 (m) 1.32—1.36 (m)	1.63—1.67 (m) 1.38—1.42 (m)
15	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	5.37 (brs)
16	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	1.30—2.00 (m)	2.03—2.07 (m)
17	1.31—1.35 (m)	1.31—1.35 (m)	1.34—1.37 (m)	1.29—1.33 (m)	1.73—1.76 (m)
18	0.89 (s)	0.89 (s)	0.90 (s)	0.90 (s)	0.81 (s)
19	1.23 (s)	1.23 (s)	1.23 (s)	1.25 (s)	1.48 (s)
20	2.40—2.44 (m)	2.40—2.44 (m)	2.41—1.45 (m)	2.41—1.45 (m)	2.54—2.58 (m)
21	4.40 (t, *J*=8.1 Hz)	4.40 (t, *J*=8.1 Hz)	4.37 (t, *J*=8.1 Hz)	4.42 (t, *J*=8.1 Hz)	4.47, 4.89 [4.87, 3.82] (t, *J*=8.0 Hz)
22	2.65, 2.50 (dd, *J*=5.8, 7.2 Hz)	2.65, 2.51 (dd, *J*=5.8, 7.2 Hz)	2.67, 2.53 (dd, *J*=5.8, 7.2 Hz)	2.66, 2.52 (dd, *J*=5.8, 7.2 Hz)	2.75 (dd, *J*=5.8, 7.2 Hz) 2.0—2.5, overlap
OAc	2.07 (s)	2.08 (s)	2.05 (s)	2.01 (s)	1.93 (s)
1'	5.14 (brs)	5.14 (brs)	5.31 (brs)	5.31 (brs)	
2'	2.39—2.43 (m) 1.73—1.77 (m)	2.39—2.43 (m) 1.73—1.77 (m)	2.25—2.29 (m) 1.56—1.60 (m)	2.25—2.29 (m) 1.56—1.60 (m)	
3'	3.96 (brs)	3.96 (brs)	3.86—3.90 (m)	3.86—3.90 (m)	
4'	3.99—4.03 (m)	3.99—4.03 (m)	3.79—3.83 (m)	3.79—3.83 (m)	
5'	4.60—4.64 (m)	4.60—4.64 (m)	4.04—4.08 (m)	4.04—4.08 (m)	
6'	1.45 (d, *J*=6.0 Hz)	1.45 (d, *J*=6.0 Hz)	1.72 (d, *J*=6.5 Hz)	1.66 (d, *J*=6.5 Hz)	
OMe	3.43 (s)	3.43 (s)	3.37 (s)	3.40 (s)	
1''	5.16 (d, *J*=7.7 Hz)	5.01 (d, *J*=7.7 Hz)	5.19 (*J*=7.7 Hz)	5.30 (*J*=7.7 Hz)	
6''	4.73—4.77 (m)	4.53—4.57 (m) 3.33—3.37 (m)	4.73—4.77 (m)	4.53—4.57 (m) 3.33—3.37 (m)	
1'''	4.96 (d, *J*=7.7 Hz)		5.07 (d, *J*=7.7 Hz)		
6'''	4.53—4.57 (m)		4.43—4.47 (m)		

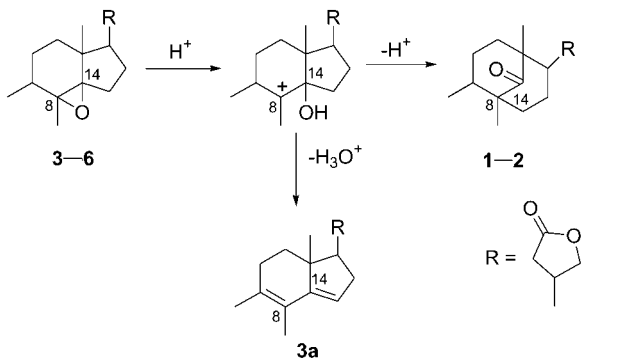

Figure 2 Proposed biogenesis of cardiac glycosides in *Parepigynum funingenese*.

phy and TLC were carried out on silica gel (Qingdao, China), MCI CHP-20P gel, FUJI (ODS-Q_3) gel (Mitsubishi Chemical Co.) and Lobar RP-C18 gel (Merck) using the following solvent systems: $CHCl_3$-MeOH-H_2O and MeOH-H_2O, respectively.

Extraction and isolation procedure

The aerial part of *Parepigynum funingense* Tsiang et P. T. Li (apocynaceae) was collected in Wenshan, Yunnan, China in September 1999 and identified by Yang Zhenghong. A voucher speciemen (No. 90-1297) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany. The dried ae-

Table 5 HMBC and ROESY spectral data for **3** and **3a** recorded in C_5D_5N

H	3		3a	
	HMBC	ROESY	HMBC	ROESY
3	C-1', C-4	H-1', H-5		H-5
4	C-2, C-3, C-5, C-10	H-5, H-3	C-2, C-3, C-5, C-10	H-5
5		H-3, H-9		H-3, H-9
12		H-17		H-17
15			C-16, C-13, C-17, C-8, C-14	
17			C-18, C-21, C-13, C-14, C-16	H-12, H-21, H-22
18	C-12, C-13, C-14, C-17		C-12, C-13, C-14, C-17	H-12, H-15, H-20, H-21
19	C-10, C-5, C-9, C-8		C-10, C-5, C-9	
21	C-20, C-22, C-23		C-20, C-22, C-17, C-23	H-18, H-17

rial part (4.5 kg) of *P. funingenes* was extracted threetimes with 95% EtOH/H_2O at room temperature. After removal of the solvent *in vacuo*, the extract was suspended in H_2O, then extracted with petroleum ether. The aqueous layer was concentrated *in vacuo* to yield a residue, which was subjected to column chromatography on silica gel (550 g, 200—300 mesh), eluting with gradient mixtures of $CHCl_3$-MeOH-H_2O from $CHCl_3$ to $CHCl_3$/MeOH/H_2O (7∶3∶0.3, *V*∶*V*∶*V*) to produce 6 fractions. A 4-g amount of the fraction 3 (7 g) obtained from $CHCl_3$/MeOH (9∶1, *V*∶*V*) was rechromatographed (MCI gel CHP-20P, RP-8, MeOH∶H_2O=60∶40, *V*∶*V*) to afford three fractions. The second fraction (1.2 g) was purified by repeated CC (silica gel, $CHCl_3$/MeOH, 95∶5, 9∶1, *V*∶*V*) to form pure **4** (70 mg) and **6** (270 mg). A 8 g amount of the fraction 6 (17 g) obtained from $CHCl_3$/MeOH/H_2O (8∶2∶0.2, *V*∶*V*∶*V*) was rechromatographed [MCI gel CHP-20P, MeOH∶H_2O=60∶40 (*V*∶*V*)] to afford five fractions. The third fraction (1.4 g) was purified by repeated CC [RP_{18}, FUJI ODS, MeOH∶H_2O=60∶40 (*V*∶*V*); silica gel, $CHCl_3$∶MeOH∶H_2O=8∶2∶0.2 (*V*∶*V*∶*V*)] to yield pure **1** (49 mg), **2** (48 mg), **3** (50 mg) and **5** (240 mg).

Funingenoside B, (8*R*)-4*β*-acetoxy-3*β*-[(O-*β*-*D*-glucopyranosyl-(1→6)-O-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-cymaropyranosyl)oxy]-14-oxo-5*α*-20(*R*)-15(14→8)-*abeo*-card-20(22)-dihydroenolide (**1**): White powder; m.p. 166—168 ℃; $[\alpha]_D^{25.2}$ −56.15 (*c* 0.65, C_5H_5N); ^1H and ^{13}C NMR see Table 1; IR (KBr) *v*: 3430, 2933, 1780, 1699, 1455, 1371, 1245, 1167, 1049, 1024 cm^{-1}; negative ion HRFABMS *m/z*: 899.4270 [M^+−H] (calcd for $C_{44}H_{67}O_{19}$ 899.4277).

Funingenoside E, (8*R*)-4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-oleadropyranosyl)oxy]-14-oxo-5*α*-20(*R*)-15(14→8)-*abeo*-card-20(22)-dihydroenolide (**2**): White powder; m.p. 175—177 ℃; $[\alpha]_D^{25.2}$ −54.44 (*c* 0.90, C_5H_5N); ^1H and ^{13}C NMR see Table 1; IR (KBr) *v*: 3430, 2929, 1777, 1735, 1455, 1370, 1244, 987 cm^{-1}; negative ion HRFABMS *m/z*: 899.4255 [M^+−H] (calcd for $C_{44}H_{67}O_{19}$ 899.4277).

Funingenoside F, 4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-cymaropyranosyl)oxy]-8,14-epoxy-5*α*-card-20(22)-dihydroenolide (**3**): White powder; ^1H and ^{13}C NMR see Tables 3 and 4; IR (KBr) *v*: 3430, 2933, 1780, 1699, 1455, 1371, 1049, 1023 cm^{-1}; negative ion ESI-MS *m/z*: 899.4268 [M^+−H] (calcd for $C_{44}H_{67}O_{19}$ 899.4277); negative ion FAB-MS *m/z*: 899 (M^+, 100), 737 (20).

Funingenoside G, 4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-cymaropyranosyl)oxy]-8,14-eoxy-5*α*-card-20(22)-dihydroenolide (**4**): White powder; ^1H and ^{13}C NMR see Tables 3 and 4; IR (KBr) *v*: 3444, 2943, 1780, 1639, 1453, 1372, 1048, 1019 cm^{-1}; negative ion FAB-MS *m/z*: 737 (M^+, 100), 575 (20).

Funingenoside H, 4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-oleadropyranosyl)oxy]-8,14-epoxy-5*α*-card-20(22)-dihydroenolide (**5**): White powder; ^1H and ^{13}C NMR see Tables 3 and 4; IR (KBr) *v*: 3424, 2941, 1775, 1733, 1455, 1370, 1046, 1019 cm^{-1}; negative ion FAB-MS *m/z*: 899 (M^+, 100), 737 (20).

Funingenoside I, 4*β*-acetoxy-3*β*-[(*O*-*β*-*D*-glucopyranosyl-(1→4)-*α*-*D*-oleadropyrano-syl)oxy]-8,14-epoxy-5*α*-card-20(22)-dihydroenolide (**6**): White powder; ^1H and ^{13}C NMR see Tables 3 and 4; IR (KBr) *v*: 3458, 2937, 1779, 1737, 1455, 1370, 1030, 1020 cm^{-1}; Negative ion FAB-MS *m/z*: 737 (M^+, 100), 575 (20).

Funingenin A, (8*R*)-4*β*-acetoxy-3*β*-hydroxyl-14-oxo-5*α*-15(14→8)-*abeo*-card-20(22)-dihydroenolide (**2a**): White powder; m.p. 198—201 ℃; $[\alpha]_D^{25.2}$ −5.21 (*c* 0.65, $CDCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 5.09 (brs, 1H, 4-H), 4.21—4.25 and 3.80—3.84 (m, 2H, 21-H), 3.66—3.70 (m, 1H, 3-H), 2.68—2.72 (m, 1H, 20-H), 2.08 (s, 3H, OCOMe), 1.03 (s, 3H, 18-H), 0.84 (s, 3H, 19-H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 38.4 (C-1), 25.5 (C-2), 71.4 (C-3), 75.7 (C-4), 46.6 (C-5), 23.5 (C-6), 34.8 (C-7), 49.1 (C-8), 60.6 (C-9), 38.1 (C-10), 20.8 (C-11), 42.0 (C-12), 49.3 (C-13), 220.9 (C-14), 43.2 (C-15), 23.6 (C-16), 55.9 (C-17), 23.6 (C-18), 16.1 (C-19), 36.1 (C-20), 71.2 (C-21), 36.3 (C-22), 176.7 (C-23), 172.2, 21.1 (4-OAc); IR (KBr) *v*: 3428, 2933, 1780, 1699 cm^{-1}; positive ion FAB-MS *m/z*: 433 (M^+, 50), 83 (100).

Acid hydrolysis of Funingenoside E (2)

A solution of compound **2** (20.0 mg) in MeOH (4

mL) was treated with 0.4 mol/L HCl (3 mL) at 70 ℃ for 30 min, 4 mL of H_2O were added and the whole solution was concentrated to 7 mL and then extracted with $CDCl_3$. The organic phase was evaporated *in vacuo* to yield a residue which purified by silica gel column chromatography with petro-ether/acetone (3∶1, *V*∶*V*) to afford an aglycone identified as funingenin A by positive ion HRFABMS, ^{1}H and ^{13}C NMR (in $CDCl_3$). The aqueous layer was neutralized with 5% NaOH. The precipitate was filtered off and the filtrate evaporated to a syrup to give oleandrose and glucose by comparison with authentic compounds.

Acid hydrolysis of compounds **3** gave funingenin C (**3a**). The condition of acid hydrolysis was similar to that of compound **2**.

Funingenin C, 3*β*-acetoxy-4*β*-hydroxy-5*α*-card-20-(22)-dihydro-8,14-dienolide (**3a**): White powder; ^{1}H and ^{13}C NMR see Tables 3 and 4; positive ion ESI-MS *m/z*: 415.2484 [M^+＋H] (calcd for $C_{25}H_{35}O_5$ 415.2488); EI-MS *m/z*: 414 (M^+, 100).

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