Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 6741–6747

Tabularisins A–D, phragmalin *ortho* esters with new skeleton isolated from the seeds of *Chukrasia tabularis*

Cheng-Qi Fan, Xiao-Ning Wang, Sheng Yin, Chuan-Rui Zhang, Fang-Dao Wang and Jian-Min Yue*

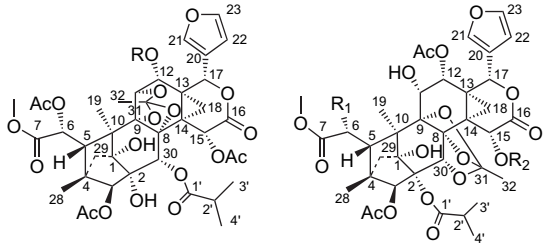
State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai, 201203, PR China

Received 4 March 2007; revised 24 April 2007; accepted 25 April 2007
Available online 29 April 2007

Abstract—Tabularisins A–D (**1**–**4**), a new class of phragmalins incorporating a cyclopropanyl ring, were isolated from *Chukrasia tabularis*. Compounds **1** and **2** are also the first examples of phragmalins with an 8,9,11-*ortho* ester. The structures of **1** and **3** were confirmed by single crystal X-ray studies. The absolute configuration of **2** was determined by CD exciton chirality method on its benzoate (**2a**), and those of **1**, **3**, and **4** were proposed by correlating with **2** chemically and biogenetically.

1. Introduction

Plants of the Meliaceae family are well known for the structurally diversified limonoids with important bioactivities, and have attracted a broad range of interests in both organic chemistry and agrochemistry.[1] *Chukrasia tabularis* A. Juss. (Meliaceae), an economically important timber tree, is native to the tropical areas of Asia, such as India, Malaysia, and South China.[2] Its bark has been applied traditionally in China and India as astringent, antidiarrheal, and anti-influenza agents, and the extract of its leaves also showed moderate antimalarial and antimicrobial activities.[3] Previous studies on this plant have resulted in the isolation of one gedunin derivative and 18 phragmalins, 14 of which are 1,8,9-phragmalin *ortho* esters showing antifeedant activity.[4] Phragmalin-type limonoids have characteristic A and B-rings of tricyclo[3.3.$1^{2,10}$.$1^{1,4}$]decane or tricyclo-[4.2.$1^{10,30}$.$1^{1,4}$]decane, and most of them also bear an *ortho* ester group.[1b,4,5] Up to now, three types of phragmalin *ortho* esters have been reported, and are classified according to the location of the *ortho*-acetate groups as 1,8,9-, 8,9,14-, and 8,9,30-phragmalin *ortho* esters.[1b,5d] In the current investigation, four phragmalin *ortho* esters, namely tabularisins A–D (**1**–**4**), were isolated from the seeds of *C. tabularis* collected from Hainan Island of China. Compounds **1**–**4** represent a new class of phragmalins incorporating a cyclopropanyl ring, and compounds **1** and **2** are also the first reported phragmalins with an 8,9,11-*ortho* ester. Herein, the details of the structural elucidation and proposed biogenetic transformation of these compounds are presented.



1 R = Ac
2 R = H
2a R = benzoyl

3 R_1 = OAc R_2 = Ac
4 R_1 = R_2 = H

2. Results and discussion

Tabularisin A (**1**), colorless plates, had the molecular formula $C_{41}H_{48}O_{20}$ as established by HR-EIMS (*m*/*z* found 860.2746 $[M]^+$, calcd 860.2739) and ^{13}C NMR data. The strong IR absorption at 1758 cm^{-1} showed the presence of ester groups. The ^1H and ^{13}C NMR (with DEPT) spectral data (Table 1) revealed the existence of one methoxyl, one isobutyryl, four acetoxyls, a β-furyl ring, and a typical *ortho*-acetate [δ_H 1.66 (s, 3H); δ_C 16.1 and 119.4]. Two proton signals of hydroxyls at δ_H 3.33 and 2.84 (s, each 1H) were distinguished by HSQC and HMBC spectra. In addition, the remaining 22 carbons in the ^{13}C NMR spectra were observed as two ester carbonyls, eight quaternary carbons (four oxygenated), eight methines (seven oxygenated), two methylenes, and two tertiary methyls. The aforementioned data suggested that compound **1** was a phragmalin-type limonoid

Keywords: Tabularisins A–D; Phragmalins; *Chukrasia tabularis*; Absolute configuration; X-ray diffraction.
* Corresponding author. Tel./fax: +86 21 50806718; e-mail: jmyue@mail.shcnc.ac.cn

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.04.078

Table 1. ^{1}H and ^{13}C NMR data of tabularisins A–D (**1**–**4**)[a] in $CDCl_3$

No.	**1**		**2**		**3**		**4**	
	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)
1	82.9 (s)		83.0 (s)		84.5 (s)		84.6 (s)	
2	76.4 (s)		76.3 (s)		83.0 (s)		83.5 (s)	
3	85.8 (d)	5.46 (s)	85.7 (d)	5.47 (s)	85.5 (d)	5.26 (s)	86.0 (d)	5.24 (s)
4	44.6 (s)		44.6 (s)		44.4 (s)		44.4 (s)	
5	42.9 (d)	2.87 (s)	42.7 (d)	2.76 (s)	43.6 (d)	2.78 (s)	38.7 (d)	2.36 (br s)[b]
6a	70.6 (d)	5.89 (s)	70.9 (d)	5.33 (s)[b]	71.0 (d)	6.15 (s)	33.5 (t)	2.32–2.37 (m)[b]
6b								2.86 (d, 15.9)
7	171.4 (s)		171.2 (s)		171.7 (s)		174.0 (s)	
8	78.0 (s)		77.7 (s)		87.1 (s)		87.6 (s)	
9	90.6 (s)		90.9 (s)		84.9 (s)		84.4 (s)	
10	44.9 (s)		45.1 (s)		49.2 (s)		48.4 (s)	
11	74.7 (d)	4.21 (d, 2.9)	76.2 (d)	4.23 (d, 3.8)	67.2 (d)	4.30 (d, 4.1)	67.6 (d)	4.19 (d, 4.3)
12	66.2 (d)	5.30 (br d, 2.9)	64.8 (d)	4.01 (t-like, 3.8)	68.1 (d)	5.35 (d, 4.1)	68.4 (d)	5.15 (d, 4.3)
13	30.8 (s)		34.5 (s)		29.7 (s)		29.6 (s)	
14	30.5 (s)		31.5 (s)		24.6 (s)		27.0 (s)	
15	69.6 (d)	7.09 (d, 3.0)	69.6 (d)	7.07 (d, 2.9)	70.5 (d)	6.93 (d, 2.4)	67.6 (d)	5.97 (t, 2.5)
16	166.7 (s)		166.9 (s)		165.6 (s)		172.2 (s)	
17	71.3 (d)	6.42 (s)	71.3 (d)	6.41 (s)	71.8 (d)	6.39 (s)	72.2 (d)	6.37 (s)
18a	18.4 (t)	2.65 (dd, 3.0, 7.0)	18.1 (t)	2.46 (dd, 2.9, 6.7)	16.9 (t)	2.94 (dd, 2.4, 6.8)	16.9 (t)	2.78 (dd, 2.5, 6.8)
18b		1.42 (br d, 7.0)		1.36 (m)[b]		1.47 (br d, 6.8)		1.36 (br d, 6.8)
19	15.0 (q)	1.34 (s, 3H)	15.0 (q)	1.33 (s, 3H)[b]	17.4 (q)	1.38 (s, 3H)	16.5 (q)	1.30 (s, 3H)
20	121.9 (s)		121.9 (s)		122.3 (s)		122.4 (s)	
21	141.9 (d)	7.47 (br s)	142.5 (d)	7.68 (br s)	141.8 (d)	7.53 (br s)	141.8 (d)	7.50 (br s)
22	109.6 (d)	6.49 (d, 0.8)	108.7 (d)	6.56 (br s)	109.8 (d)	6.54 (br s)	109.8 (d)	6.54 (br s)
23	143.3 (d)	7.38 (br s)	144.8 (d)	7.52 (br s)	143.3 (d)	7.40 (br s)	143.3 (d)	7.40 (br s)
28	15.1 (q)	0.99 (s, 3H)	15.2 (q)	0.97 (s, 3H)	15.3 (q)	0.91 (s, 3H)	14.3 (q)	0.76 (s, 3H)
29a	39.8 (t)	1.94 (d, 10.8)	39.7 (t)	1.95 (d, 10.7)	40.7 (t)	1.70 (br d, 11.5)	39.8 (t)	1.67 (d, 11.7)
29b		2.05 (d, 10.8)		2.14 (d, 10.7)		2.25 (br d, 11.5)		2.01 (d, 11.7)
30	69.9 (d)	5.37 (s)	69.8 (d)	5.35 (s)[b]	76.7 (d)	5.04 (s)	77.3 (d)	5.66 (s)
31	119.4 (s)		119.2 (s)		116.2 (s)		116.4 (s)	
32	16.1 (q)	1.66 (s, 3H)	16.1 (q)	1.66 (s, 3H)	15.7 (q)	1.72 (s, 3H)	15.7 (q)	1.72 (s, 3H)
7-OMe	53.6 (q)	3.78 (s, 3H)	53.7 (q)	3.80 (s, 3H)	53.6 (q)	3.78 (s, 3H)	52.3 (q)	3.73 (s, 3H)[b]
1′	173.4 (s)		173.4 (s)		175.7 (s)		176.3 (s)	
2′	33.8 (d)	2.50–2.55 (m)	33.8 (d)	2.51–2.56 (m)	34.4 (d)	2.50–2.55 (m)	34.3 (d)	2.63–2.68 (m)
3′	19.3 (q)	1.21 (d, 7.2, 3H)	19.4 (q)	1.20 (d, 6.9, 3H)	18.7 (q)	1.17 (d, 7.0, 3H)	18.7 (q)	1.19 (d, 7.0, 3H)
4′	18.7 (q)	1.19 (d, 7.2, 3H)	18.7 (q)	1.18 (d, 6.9, 3H)	18.7 (q)	1.19 (d, 7.0, 3H)	18.5 (q)	1.19 (d, 7.0, 3H)
3-OAc	169.0 (s)		168.9 (s)		168.5 (s)		168.2 (s)	
	20.9 (q)	2.11 (s, 3H)	21.0 (q)	2.17 (s, 3H)	20.9 (q)	2.32 (s, 3H)	20.9 (q)	2.11 (s, 3H)
6-OAc	169.0 (s)		169.4 (s)		169.1 (s)			
	20.9 (q)	2.08 (s, 3H)	21.0 (q)	2.22 (s, 3H)	20.5[c] (q)	2.19 (s, 3H)		
12-OAc	170.5 (s)				170.9 (s)		170.9 (s)	
	19.5 (q)	1.66 (s, 3H)			19.5 (q)	1.65 (s, 3H)	19.7 (q)	1.62 (s, 3H)
15-OAc	172.2 (s)		172.2 (s)		169.2 (s)			
	21.4 (q)	2.32 (s, 3H)	21.4 (q)	2.32 (s, 3H)	21.0[c] (q)	2.18 (s, 3H)		
–OH		2.84 (s, 1-OH)		2.92 (s, 1-OH)		3.58 (s, 1-OH)		3.68 (s, 1-OH)
		3.33 (s, 2-OH)		3.33 (s, 2-OH)		2.10 (s, 11-OH)		2.15 (s, 11-OH)
				1.85 (d, 4.8, 12-OH)				3.74 (d, 2.5, 15-OH)[b]

[a] Data were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C).
[b] Proton signals were overlapped.
[c] Data might be interchangeable.

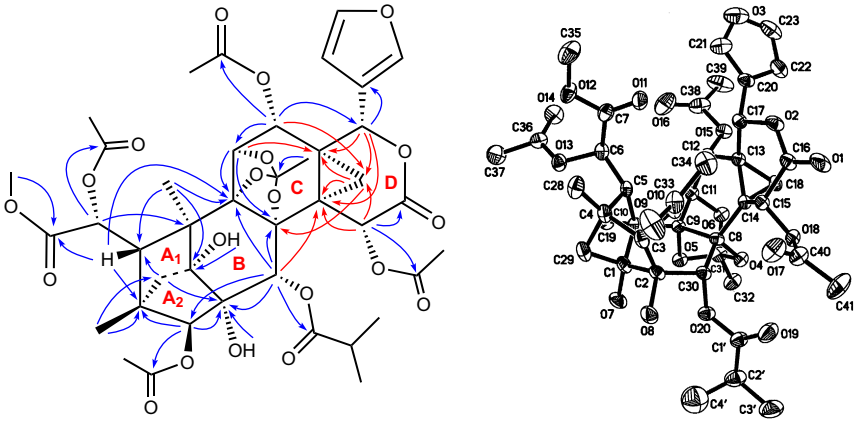


Figure 1. Key HMBC correlations (→) and X-ray structure of **1**.

with 18 degrees of unsaturation, of which, 12 degrees were occupied by seven ester carbonyls, one *ortho*-acetate, and a β-furyl ring, and the remaining 6 degrees required a hexacyclic core for **1**.

A tricyclo[3.3.$1^{2,10}$.$1^{1,4}$]decane was assigned to the A/B-ring system (rings A_1, A_2, and B) based on the HMBC correlations as depicted in Figure 1, which is identical to that of phragmalin 3,30-diisobutyrate that is also isolated in the current study.[4b] An unusual cyclopropanyl ring (C-13, C-14, and C-18) was observed in the HMBC correlations of H-11, H-17, and H-18b/C-13; H-15, H-17, H-30, and H_2-18/C-14; H-18a/C-8; and H-12, H-15, and H-17/C-18 (Fig. 1, in red), and was supported by the up-field shifted quaternary carbon signals at δ_C 34.5 (C-13) and 31.5 (C-14), and methylene signal at δ_C 18.4 (C-18) as compared with those of the phragmalin analogs. Furthermore, the locations of two hydroxyls, one methoxyl, four acetoxyls, an isobutyryloxyl, and a β-furyl ring were determined by HMBC correlations. With the settlement of all the other oxygenated carbons, the 8,9,11-*ortho*-acetate was only attributable to the remaining three oxygenated ones C-8 (δ 78.0), C-9 (δ 90.6), and C-11 (δ 74.7) though there were no direct HMBC correlations available. The planar structure of **1** was thus established, and was further confirmed by a single crystal X-ray diffraction (Fig. 1), which also allowed the determination of its relative configuration.

Tabularisin B (**2**), a white amorphous solid, had a molecular formula $C_{39}H_{46}O_{19}$ as determined by HR-EIMS (*m/z* found 818.2610 $[M]^+$, calcd 818.2633). The NMR data of **2** were very similar to those of **1**. The only difference was the presence of a 12-OH (at δ_H 1.85, d, J=4.8 Hz) in **2**, which caused the H-12 signal at δ_H 4.01 (t-like, J=3.8 Hz) up-field shifted severely as compared with that of **1** bearing a 12-OAc (Table 1). Compound **2** was thus established as a 12-*O*-deacetyl derivative of **1**, which was supported by the HMBC spectrum. The relative configuration of **2** was established by ROESY spectrum. Acetylation of **2** yielded **1** and thus confirmed the structure of **2**.

The absolute configuration of **2** was determined by applying the CD exciton chirality method on its benzoate (**2a**, Table 2). A split CD of **2a** with Cotton effects at 232 nm ($\Delta\varepsilon$−8.26, benzoate) and 209 nm ($\Delta\varepsilon$+10.98, furyl ring) exhibited negative chirality, indicating that the transition dipole moments of two chromophores were oriented in a counterclockwise manner (Fig. 2)[6] corresponding to the absolute configuration of **2a** as depicted. Accordingly, the absolute configuration of **2** was determined. Acetylation of **2** to produce **1** also allowed the assignment of the absolute configuration of **1**.

Tabularisin C (**3**), colorless plates, had the molecular formula $C_{41}H_{48}O_{20}$ as established by HR-EIMS showing an $[M]^+$at *m/z* 860.2788 (calcd 860.2739) and ^{13}C NMR data. The ^1H and ^{13}C NMR (with DEPT) spectral data (Table 1) revealed the existence of one methoxyl, one isobutyryl, four acetoxyls, a β-furyl ring, and one typical *ortho*-acetate [δ_H 1.72 (s, 3H); δ_C 15.7 and 116.2] moiety. Two proton signals of hydroxyls at δ_H 3.58 and 2.10 (s, each 1H) were distinguished by HSQC and HMBC spectra. The remaining 22 carbons at the central core of **3** were resolved in the ^{13}C NMR (with DEPT) as two ester carbonyls, eight quaternary carbons (four oxygenated), eight methines (seven oxygenated), two methylenes, and two tertiary methyls. The spectral data mentioned above suggested that tabularisin C (**3**) was also a phragmalin analog with an *ortho*-acetate group.

A tricyclo[3.3.$1^{2,10}$.$1^{1,4}$]decane was assigned to the A/B-ring system (rings A_1, A_2, and B) based on the HMBC correlations as depicted in Figure 3. A 13,14,18-cyclopropanyl ring was also observed in the HMBC correlations of H-11, H-17, and H-18b/C-13; H-15, H-17, and H_2-18/C-14; H-18a/C-8; and H-12, H-15, and H-17/C-18. Furthermore, the locations of two hydroxyls at C-1 and C-11, one methoxyl group at C-7, four acetoxyls at C-3, C-6, C-12, and C-15, and the β-furyl ring at C-17 were determined by HMBC correlations. The remaining four oxygenated carbons C-2 (δ 83.0), C-8 (δ 87.1), C-9 (δ 84.9), and C-30 (δ 76.7) were left to locate the isobutyryloxyl and the *ortho*-acetate groups. However, there were no direct HMBC correlations available to complete the assignments. Fortunately, a single crystal X-ray diffraction study (Fig. 3) finally enabled us to assign a 2-isobutyryloxyl and an 8,9,30-*ortho*-acetate, and also allowed the determination of the relative configuration of **3**.

Tabularisin D (**4**) had a molecular formula $C_{37}H_{44}O_{17}$ as determined by HR-EIMS (*m/z* found 760.2622 $[M]^+$, calcd 760.2578). Besides one methoxyl, one isobutyryl, a β-furyl

Table 2. ^{1}H and ^{13}C NMR data of **2a** (in $CDCl_3$)[a]

No.	δ_C (multi)	δ_H (multi, *J* in Hz)	No.	δ_C (multi)	δ_H (multi, *J* in Hz)
1	83.0 (s)		23	143.5 (d)	6.75 (br s)
2	76.5 (s)		28	15.2 (q)	1.01 (s, 3H)
3	86.0 (d)	5.49 (s)	29a	39.9 (t)	1.97 (br d, 11.1)
4	44.7 (s)		29b		2.20 (overlapped)
5	43.0 (d)	2.95 (s)	30	69.9 (d)	5.39 (s)
6	70.7 (d)	5.99 (s)	31	119.5 (s)	
7	171.5 (s)		32	16.2 (q)	1.64 (s, 3H)
8	78.0 (s)		7-OMe	53.7 (q)	3.81 (s, 3H)
9	90.8 (s)		1-OH		2.90 (s)
10	45.1 (s)		2-OH		3.46 (s)
11	74.7 (d)	4.37 (d, 3.0)	1′	173.6 (s)	
12	66.5 (d)	5.57 (br d, 3.0)	2′	33.9 (d)	2.52–2.58 (m)
13	31.4 (s)		3′, 4′	19.4, 18.7 (q)	1.19 and 1.21 (d, 7.0, 3H each)
14	30.8 (s)				
15	69.7 (d)	7.13 (d, 2.8)	Acetoxyls	169.2, 169.1, 172.4 (s)	
16	166.9 (s)			21.0, 21.0, 21.5 (q)	2.20, 2.24 and 2.35 (s, 3H each)
17	71.5 (d)	6.49 (s)			
18a	18.4 (t)	2.78 (dd, 3.0, 6.8)	12-OBz	128.2 (s)	
18b		1.48 (br d, 6.8)		130.0 (2C, d)	7.80 (br d, 8.5, 2H)
19	15.1 (q)	1.38 (s, 3H)		127.8 (2C, d)	7.35 (m, 2H)
20	121.7 (s)			133.3 (d)	7.52 (m)
21	141.4 (d)	7.41 (br s)		165.3 (s)	
22	108.9 (d)	6.36 (d, 1.3)			

[a] Data were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C), and were assigned by comparison with those of tabularisins A and B.

ring, and an *ortho*-acetate moiety, only two acetyls were identified from the ^{1}H and ^{13}C NMR (with DEPT) spectra (Table 1). The NMR data of **4** showed high similarity to those of **3**, suggesting that their structures were closely related. A methylene [δ_H 2.32–2.37 (m) and 2.86 (d, J=15.9 Hz); δ_C 33.5] and one hydroxyl at δ_H 3.74 (d, 2.3) in **4** were, respectively, assigned as CH_2-6 and 15-OH by HSQC and HMBC correlations (Fig. 4), suggesting that

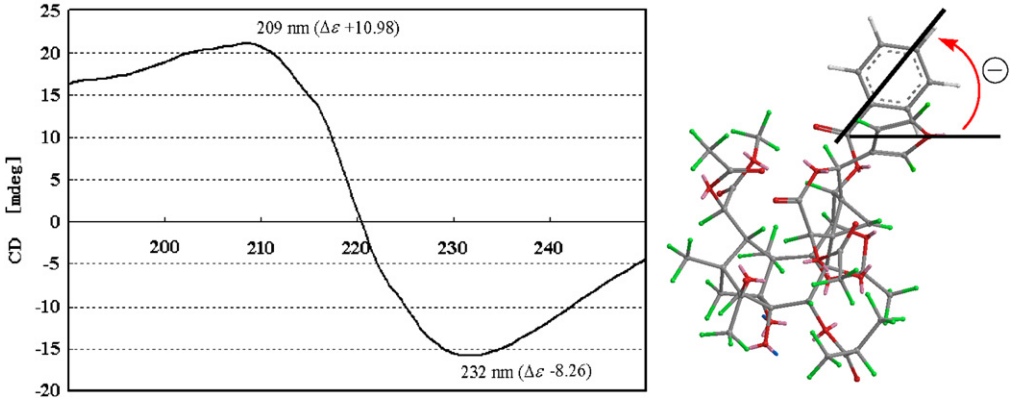


Figure 2. CD spectrum and the exciton chirality of **2a**; the dark lines denote the electric transition dipole of the chromophores.

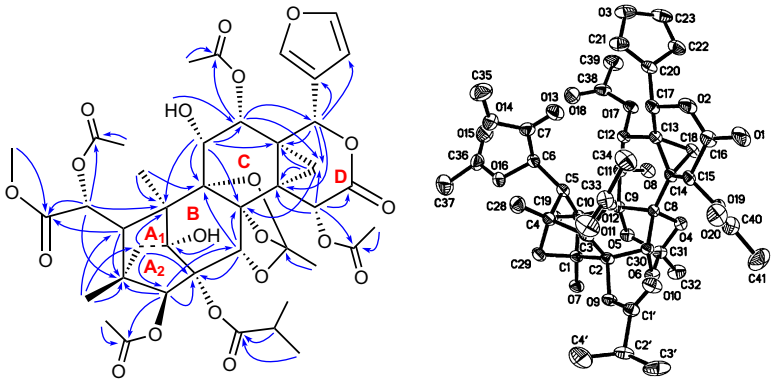


Figure 3. Key HMBC (→) and X-ray structure of **3**.

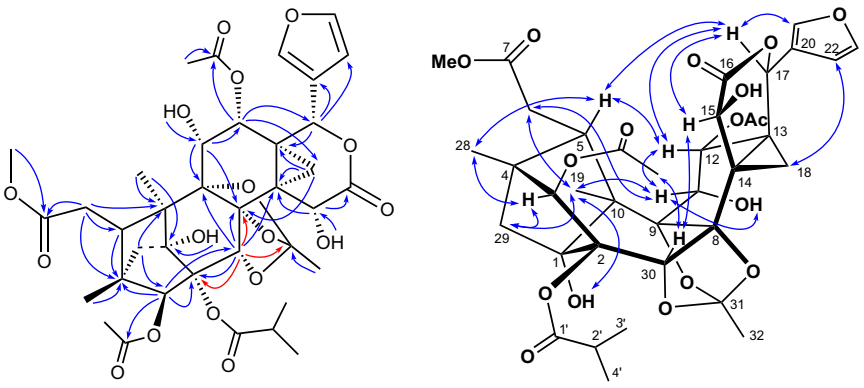


Figure 4. Selectively decoupled signals (→, red) by irradiating H-30, and key HMBC (→, blue), and ROESY (↔) correlations of **4**.

compound **4** was probably a 6-deacetoxy-15-*O*-deacetyl derivative of **3**. As a result, the H-15 proton signal at δ_H 5.97 (t-like, J=2.3 Hz) was severely shielded as compared with that of **3** at δ_H 6.93 (d, J=2.3 Hz). The chemical shifts of C-8, C-9, and C-30 of **4** resembled those of **3**, respectively, and indicated the presence of an 8,9,30-*ortho*-acetate. This was confirmed by a selective decoupling experiment, in which the resonance of C-31 at δ_C 116.4 was significantly increased, when H-30 was selectively irradiated. The isobutyryloxyl was thus only assignable to C-2, and was supported by the chemical shift of C-2 at δ_C 83.5 and HMBC correlations (Fig. 4). The relative configuration of **4** as established by ROESY spectrum (Fig. 4) was consistent with that of **3**.

The biosynthetic origin of **1**–**4** (Scheme 1) could be rationalized to the phragmalin-type limonoids (**A**),[1b,5d] which would undergo a sequence of oxidation and epoxidation to offer an intermediate **i**. Hydrolysis of the 14,15-epoxy group of intermediate **i** via SN_2 mechanism would give an intermediate **ii**. Acid catalyzed dehydration and simultaneous formation of the cyclopropanyl ring of **ii** would afford the key intermediate **iii**.[7] Formation of 8,9,11-*ortho*-acetate or 8,9,30-*ortho*-acetate, and further esterification of **iii** would give **1** and **2** or **3** and **4**, respectively.

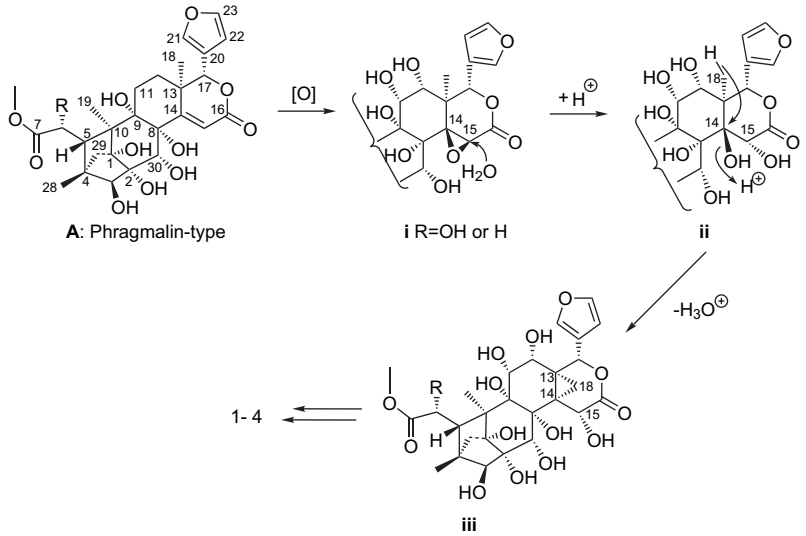
By correlation with tabularisin B (**2**) based on the biosynthetic hypothesis (Scheme 1), the absolute configuration of tabularisins C and D (**3**,**4**) could be proposed as depicted.

In conclusion, the discovery of four phragmalin *ortho* esters, tabularisins A–D, with new skeleton from the seeds of *C. tabularis*, and the proposed biosynthetic pathway for these metabolites are valuable addition to both natural products and synthetic chemistry. Biological evaluation of these isolates is in progress.

3. Experimental section

3.1. General experimental procedures

Melting points were measured on an SGW X-4 melting instrument and are uncorrected. Optical rotations were determined on a Perkin–Elmer 341 polarimeter, CD and UV was obtained on a Jasco 810 spectrometer, and UV was also recorded quantitatively on a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded on a Perkin–Elmer 577 spectrometer. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as an internal standard.



Scheme 1. The plausible biosynthetic origin of **1**–**4**.

EIMS (70 eV) and ESIMS were carried out on a Finnigan MAT 95 mass spectrometer and a Finnigan LCQ^{DECA} instrument, respectively. Semi-preparative HPLC was performed on a Waters 515 pump with a Waters 2487 detector (254 nm) and a YMC-Pack ODS-A column (250×10 mm, S-5 μm, 12 nm). All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd) and Sephadex LH-20 gel (Pharmacia Biotech, Sweden) were used for column chromatography (CC).

3.2. Plant material

The seeds of *C. tabularis* A. Juss were collected from Hainan Island, People's Republic of China, in August 2004 and were identified by Prof. Shi-Man Huang, Department of Biology, Hainan University, P.R. China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (accession number CTS-HN-2004-1Y).

3.3. Extraction and isolation

The air-dried seed powder (5.0 kg) was percolated with 95% ethanol, and the oily extract was dissolved in 2.5 L of petroleum ether (PE) to form a suspension and then extracted with 90% MeOH in water (1.0 L×3). After removal of the fatty components, 790 g of methanolic extract (CT-ME) was obtained. CT-ME was then suspended in water (2.2 L) and partitioned with ethyl acetate (1.6 L×3) to give 425 g of ethyl acetate soluble fraction (CT-EA). CT-EA fraction (400 g) was subjected to a silica gel column eluted with petroleum ether/acetone in gradient (100:0 to 1:1) to afford 14 fractions (Fr1–14) according to TLC monitor. Fr13 (24 g) was chromatographed on a column of silica gel eluted successively with a gradient of $CHCl_3$/*i*-PrOH (100:0 to 30:1) to give five sub-fractions (Fr13a–13e). Fr13b was separated on a silica gel column eluted with $CHCl_3$/*i*-PrOH (60:1 to 30:1) to give two major compounds, each of which was further purified by silica gel column chromatography eluted with petroleum ether/$CHCl_3$/*i*-PrOH (25:25:1 to 15:15:1) in gradient to give **1** (127 mg, 0.0025%) and **2** (910 mg, 0.0182%), respectively. Fr13d was subjected to a column of Sephadex LH-20 eluted with EtOH to afford two major parts Fr13d1 and Fr13d2. Fr13d1 was further purified on a silica gel column eluted with petroleum ether/$CHCl_3$/*i*-PrOH (25:25:1) to give **3** (89 mg, 0.0018%). Fr13d2 was separated by preparative HPLC using CH_3CN/H_2O (60:40, 3 mL/min) as the mobile phase to give **4** (23 mg, 0.00046%).

3.3.1. Tabularisin A (1). Colorless plates (cyclohexane/EtOAc); mp 292–294 °C (decomposed); $[\alpha]_D^{20}$ +27.8 (*c* 0.205, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.88) nm; IR (KBr) ν_{max} 3448, 2974, 1758, 1640 (weak), 1371, 1286, 1217, 1024 cm^{-1}; CD (CH_3CN) 206 ($\Delta\varepsilon$−0.36), 220 ($\Delta\varepsilon$+0.33), 230 ($\Delta\varepsilon$−0.71) nm; ^1H and ^{13}C NMR data, see Table 1; EIMS 70 eV *m/z* (rel int): 859 $[M-H]^+$ (10), 845 $[M-CH_3]^+$ (11), 800 (82), 670 (35), 610 (48), 568 (46), 550 (52), 95 (54), 71 (100); HR-EIMS *m/z*: 860.2746 $[M]^+$ (calcd for $[C_{41}H_{48}O_{20}]^+$: 860.2739); positive ESIMS *m/z* (rel int): 883 $[M+Na]^+$ (100), 861 $[M+H]^+$ (56), 843 $[M+H-H_2O]^+$ (70); negative ESIMS *m/z* (rel int): 859 $[M-H]^-$ (100).

3.3.2. Tabularisin B (2). White amorphous solid; $[\alpha]_D^{20}$ +23.0 (*c* 0.470, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.88) nm; IR (KBr) ν_{max} 3490, 2981, 1755, 1743, 1732, 1640 (weak), 1369, 1219, 1024 cm^{-1}; CD (CH_3CN) 207 ($\Delta\varepsilon$−1.52), 220 ($\Delta\varepsilon$−0.19), 232 ($\Delta\varepsilon$−0.74) nm; ^1H and ^{13}C NMR data, see Table 1; EIMS 70 eV *m/z* (rel int): 818 $[M]^+$ (74), 758 (92), 663 (60), 628 (24), 610 (20), 568 (41), 550 (36), 431 (34), 95 (80), 71 (100); HR-EIMS *m/z*: 818.2610 $[M]^+$ (calcd for $[C_{39}H_{46}O_{19}]^+$: 818.2633); positive ESIMS *m/z* (rel int): 841 $[M+Na]^+$ (92), 819 $[M+H]^+$ (100); negative ESIMS *m/z* (rel int): 817 $[M-H]^-$ (100).

3.3.3. Tabularisin C (3). Colorless plates (cyclohexane/acetone); mp 269–270 °C (decomposed); $[\alpha]_D^{20}$ +25.0 (*c* 0.235, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.69) nm; IR (KBr) ν_{max} 3450, 2976, 1765, 1736, 1637 (weak), 1375, 1286, 1211, 1037 cm^{-1}; CD (CH_3CN) 206 ($\Delta\varepsilon$−1.70), 220 ($\Delta\varepsilon$+0.0069), 232 ($\Delta\varepsilon$−0.86) nm; ^1H and ^{13}C NMR data, see Table 1; EIMS 70 eV *m/z* (rel int): 860 $[M]^+$ (7), 859 $[M-H]^+$ (16), 815 (24), 772 (26), 670 (35), 610 (54), 550 (58), 95 (48), 71 (100); HR-EIMS *m/z*: 860.2788 $[M]^+$ (calcd for $[C_{41}H_{48}O_{20}]^+$: 860.2739); ESIMS *m/z* (positive): 883 $[M+Na]^+$.

3.3.4. Tabularisin D (4). White amorphous solid; $[\alpha]_D^{20}$ −7.0 (*c* 0.260, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.77) nm; IR (KBr) ν_{max} 3444, 2976, 1733, 1633 (weak), 1373, 1209, 1180, 1032 cm^{-1}; CD (CH_3CN) 212 ($\Delta\varepsilon$+1.72), 230 ($\Delta\varepsilon$−0.58) nm; ^1H and ^{13}C NMR data, see Table 1; EIMS 70 eV *m/z* (rel int): 760 $[M]^+$ (2), 742 $[M-H_2O]^+$ (5), 612 (12), 570 (12), 552 (52), 182 (100), 71 (99); HR-EIMS *m/z*: 760.2622 $[M]^+$ (calcd for $[C_{37}H_{44}O_{17}]^+$: 760.2578); ESIMS *m/z* (positive): 783 $[M+Na]^+$.

3.4. Preparation of benzoate (2a) of tabularisin B

To a solution of tabularisin B (**2**, 50 mg) and benzoic acid (50 mg) in 10 mL of CH_2Cl_2, 50 mg of DCC powder and a catalytic amount of DMAP were added, and the reaction mixture was stirred at rt for 72 h. After workup, the crude product was purified by a silica gel column eluted with CH_2Cl_2 to give **2a** (28 mg): a white amorphous solid, CD (CH_3CN) 209 ($\Delta\varepsilon$+10.98), 232 ($\Delta\varepsilon$−8.26) nm; ^1H and ^{13}C NMR, see Table 2; positive ESIMS *m/z* (rel int): 945 $[M+Na]^+$ (100), 923 $[M+H]^+$ (24); negative ESIMS *m/z* (rel int): 921 $[M-H]^-$ (100).

3.5. Transformation of 2 to 1

To a solution of compound **2** (10 mg) in pyridine (1.0 mL), 0.6 mL of Ac_2O was added dropwise, and then the mixture was stirred at rt overnight. After workup, the residue was purified by a silica gel column eluted with $CHCl_3$ to afford 6.7 mg of the desired product (**1**), which was identified by ^1H NMR and EIMS spectra, and specific optical rotation $[\alpha]_D$.

3.6. X-ray crystallographic analysis of 1 and 3

Colorless crystals of **1** and **3** were obtained in the mixture of solvents cyclohexane/EtOAc and cyclohexane/acetone, respectively. Crystal data were obtained on a Bruker SMART

CCD detector employing graphite monochromated Mo Kα radiation (λ=0.71073 Å) at 293(2) K and operating in the ϕ-ω scan mode. The structure was solved by direct methods and refined with full-matrix least-squares calculations on F^2 using SHELX-97.[8] All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. Crystallographic data for **1** and **3** have been deposited in the Cambridge Crystallographic Data Centre with the deposition numbers of CCDC 638610 and 638611, respectively.

3.6.1. X-ray data of 1. $C_{45}H_{56}O_{22}$ ($C_{41}H_{48}O_{20}\cdot CH_3COOEt$), M=948.90, monoclinic, dimensions: 0.503×0.472×0.358 mm, d=1.364 g cm^{-3}, space group $P2_1$, Z=2, a=10.6313(18), b=17.126(3), c=12.745(2) Å, α=90, β=95.153(3), γ=90°, V=2311.1(6) Å^3, reflections collected/unique: 11,378/4637 (R_{int}=0.1264), number of observation [$>2\sigma(I)$] 3455, parameters 621, final R indices [$I>2\sigma(I)$]: $R1$=0.0522, $wR2$=0.1188.

3.6.2. X-ray data of 3. $C_{44}H_{54}O_{21}$ ($C_{41}H_{48}O_{20}\cdot CH_3COCH_3$), M=918.87, orthorhombic, dimensions: 0.516×0.417×0.231 mm, d=1.358 g cm^{-3}, space group $P2(1)2(1)2(1)$, Z=4, a=9.9504(7), b=19.8602(15), c=22.7434(16) Å, $\alpha=\beta=\gamma$=90°, V=4494.5(6) Å^3, reflections collected/unique: 26,543/5451 (R_{int}=0.1369), number of observation [$>2\sigma(I)$] 4102, parameters 578, final R indices [$I>2\sigma(I)$]: $R1$=0.0608, $wR2$=0.1449.

Acknowledgements

Financial support of the Key Project of National Natural Science Foundation (Grant No. 30630072) and the foundation from the Ministry of Science and Technology (Grant No. 2002CB512807) of P.R. China are gratefully acknowledged. We thank Prof. S.-M. Huang for the collection and identification of the plant material.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.04.078.

References and notes

1. (a) Roy, A.; Saraf, S. *Biol. Pharm. Bull.* **2006**, *29*, 191–201; (b) Mulholland, D. A.; Parel, B.; Coombes, P. H. *Curr. Org. Chem.* **2000**, *4*, 1011–1054; (c) Champagne, D. E.; Koul, O.; Isman, M. B.; Scudder, G. G. E.; Towers, G. H. N. *Phytochemistry* **1992**, *31*, 377–394; (d) Akhila, A.; Rani, K. *Progress in the Chemistry of Organic Natural Products*; Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Eds.; Springer: Vienna, 1999; Vol. 78, pp 47–149.
2. Chen, S. K.; Chen, B. Y.; Li, H. *Chinese Flora (Zhongguo Zhiwu Zhi)*; Science: Beijing, 1997; Vol. 43(3), pp 47–49.
3. (a) *Chinese Materia Medica (Zhonghua Benchao)*; Editorial Committee of the Administration Bureau of Traditional Chinese Medicine; Shanghai Science and Technology Press: Shanghai, 1999; Vol. 5, pp 31–32; (b) MacKinnon, S.; Durst, T.; Arnason, J. T.; Angerhofer, C.; Pezzuto, J.; Sanchez-Vindas, P. E.; Poveda, L. J.; Gbeassor, M. *J. Nat. Prod.* **1997**, *60*, 336–341; (c) Nagalakshmi, M. A. H.; Thangadurai, D.; Muralidara Rao, D.; Pullaiah, T. *Fitoterapia* **2001**, *72*, 62–64.
4. (a) Nakatani, M.; Abdelgaleil, S. A. M.; Saad, M. M. G.; Huang, R. C.; Doe, M.; Iwagawa, T. *Phytochemistry* **2004**, *65*, 2833–2841; (b) Connolly, J. D.; Labbe, C.; Rycroft, D. S. *J. Chem. Soc., Perkin Trans. 1* **1978**, 285–288; (c) Ragettli, T.; Tamm, C. *Helv. Chim. Acta* **1978**, *61*, 1814–1831.
5. (a) Abdelgaleil, S. A. M.; Okamura, H.; Iwagawa, T.; Sato, A.; Miyahara, I.; Doe, M.; Nakatani, M. *Tetrahedron* **2001**, *57*, 119–126; (b) Coombes, P. H.; Mulholland, D. A.; Randrianarivelojosia, M. *J. Nat. Prod.* **2003**, *66*, 735–738; (c) Saad, M. M. G.; Iwagawa, T.; Doe, M.; Nakatani, M. *Tetrahedron* **2003**, *59*, 8027–8033; (d) Wu, J.; Xiao, Q.; Huang, J. S.; Xiao, Z. H.; Qi, S. H.; Li, Q. X.; Zhang, S. *Org. Lett.* **2004**, *6*, 1841–1844; (e) Cui, J. X.; Deng, Z. W.; Li, J.; Fu, H. Z.; Proksch, P.; Lin, W. H. *Phytochemistry* **2005**, *66*, 2334–2339; (f) Abdelgaleil, S. A. M.; Doe, M.; Morimoto, Y.; Nakatani, M. *Phytochemistry* **2006**, *67*, 452–458.
6. Stonard, R. J.; Trainor, D. A.; Nakatani, M.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 130–131.
7. Mulholland, D.; Mahomed, H.; Kotsos, M.; Randrianarivelojosia, M.; Lavaud, C.; Massiot, G.; Nuzillard, J.-M. *Tetrahedron* **1999**, *55*, 11547–11552.
8. Sheldrick, G. M. *SHELX-97: Program for X-ray Crystal Structure Solution and Refinement*; University of Göttingen: Göttingen, Germany, 1997.