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Phragmalin limonoids from *Chukrasia tabularis*

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Abstract

Six phragmalin limonoids, named tabulalin and tabulalides A–E, were isolated from the root bark of *Chukrasia tabularis* (Meliaceae). Their structures were determined by spectroscopic methods, and their antifeedant properties evaluated.

Keywords: *Chukrasia tabularis*; Meliaceae; Tabulalin; Tabulalides; Phragmalin limonoids; Insect antifeedants

1. Introduction

In a series of experiments on limonoid constituents from some Meliaceae plants, several types of compounds have been isolated as insect antifeedants from *Trichilia roka* (Nakatani et al., 1981, 1984), *Melia azedarach* (Nakatani, 1999a), *Melia toosendan* (Nakatani, 1999b), *Khaya senegalensis* (Nakatani et al., 2000; Abdelgaleil and Nakatani, 2003), and *Swietenia mahogani* (Saad et al., 2003). *Chukrasia tabularis* A. Juss. is an Indian meliaceous timber tree, the bark of which has been used in Indian traditional medicine as astringent and antidiarrheal drug (Rastogi and Mehrotra, 1993; Kirtikar and Basu, 1981). An extract of the leaves has been reported to exhibit a high antimalarial activity (Mackinnon et al., 1997). Recently, it was also reported that the extracts possessed antifungal and antibacterial activities (Nagalakshmi et al., 2001). *C. tabularis* consists of one (Mabberley et al., 1995) or possibly two species (Lemmens et al., 1995), and the Chinese tree has been grouped into *C. tabularis* A. Juss. var. *velutina* (Wall.) King (Wu, 1986).

Limonoids, being tetranortriterpenoid, have been classified on the basis of which the four rings, designated as A, B, C and D in the intact triterpene nucleus, have been oxidized. Ring B,D-*seco* compounds, in which rings B and D are oxidized to lactones or esters, are common in the mahogany group, and they are divided into sub-groups depending on whether further transformations have occurred. In subgroup (a) rings B and D are opened (angolensates), in subgroup (b) a new ring has been formed between C-2 and C-30 (mexicanolides), while, in subgroup (c), compounds of subgroup (b) are further modified by bridging of ring A. A 2,3,30-trihydroxy,1,8,9-orthoacetate of this type compound is called phragmalin (**1**), and the first limonoids isolated from the wood and seeds of *C. tabularis* were a series of the

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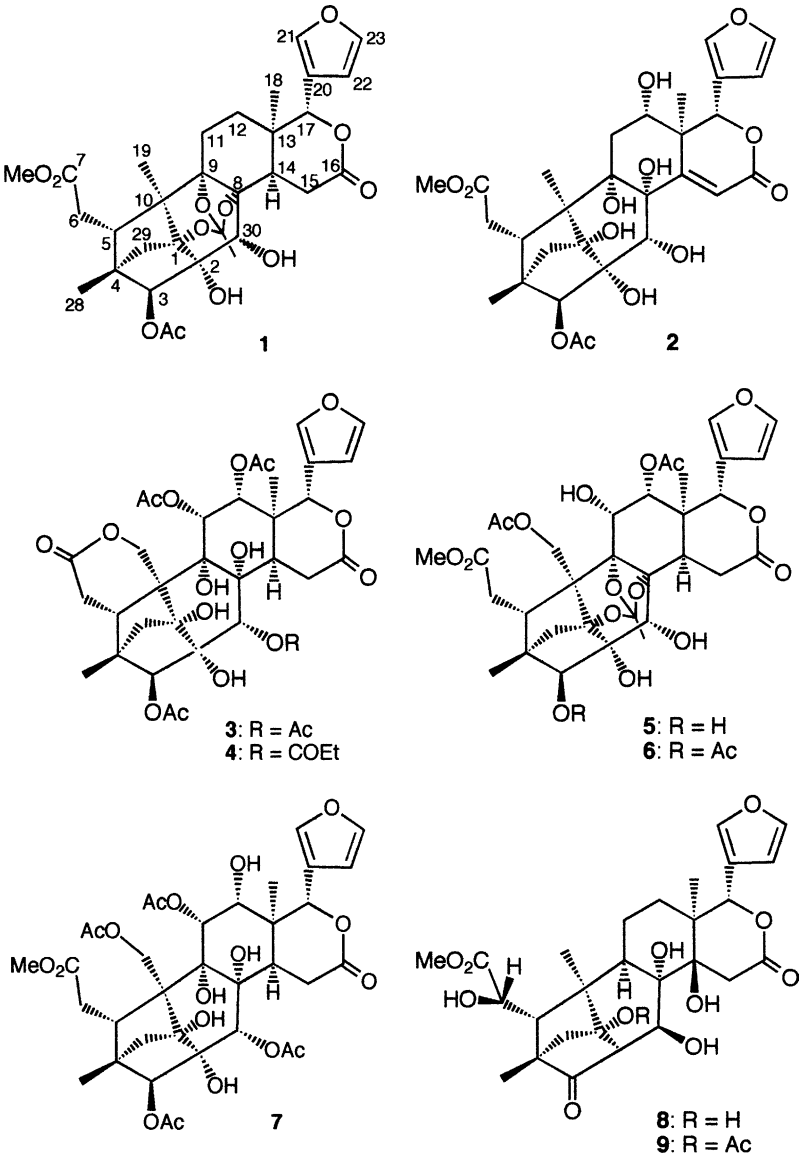
ester derivatives of phragmalin (**1**), having a tricyclo[3.3.1.1]decane ring system (Connolly et al., 1978). Related C-acyl derivatives, the chukrasins, were isolated from the seeds (Ragettli and Tamm, 1978).

In a continuing search for limonoid antifeedants from the family Meliaceae, the diethyl ether extract of the root bark of *C. tabularis* collected at Xiantan, China, displayed potent antifeedant activity against *Spodoptera* insects. The limonoid constituents of the ether extract were studied and six new phragmalin limonoids, named tabulalin (**2**) and tabulalides A (**3**)–E (**7**), were isolated. The isolation, structural elucidation, and antifeeding activity of these limonoids against the third-instar larvae of *Spodoptera littoralis* (Boisduval) are described herein.

2. Results and discussion

After separation and purification by the combined use of droplet countercurrent chromatography (DCCC) and reversed phase HPLC, the diethyl ether extract of the root bark of *C. tabularis* gave six new phragmalins: tabulalin (**2**) possessing an α,β-unsaturated lactone structure and five tabulalides (**3**–**7**) having novel 19-oxygenated structures. The isolates were also divided into two series with a C-7/C-19 lactone bridge (**3** and **4**) and a 19-acetoxy (**5**–**7**) function, and two compounds, **5** and **6**, which contained a 1,8,9-orthoacetate group. The structures of compounds **2**–**7** were elucidated mainly by spectroscopic means using ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY analysis.



Tabulalin (**2**) possessed a molecular formula of $C_{29}H_{36}O_{13}$ as determined from a pseudomolecular ion $[M-1]^-$ at *m/z*: 591.2067 (Δ −0.9 mmu) in the negative HRFAB-MS and from the analysis of ^{13}C NMR spectroscopic data. The IR spectrum revealed absorption bands for hydroxyl (3600–3200 cm^{-1}) and saturated (1740 cm^{-1}) and unsaturated ester carbonyl (1724 cm^{-1}) groups. The UV spectrum indicated the presence of a conjugated system at 215 nm. From the ^1H and ^{13}C NMR spectra, it was clear that six of the twelve elements of unsaturation were present as double bonds: three carbon–carbon (one furan ring) and three CO (as esters).

Table 1
^1H NMR spectral data for tabulalin (**2**) and tabulalides A–E (**3**–**7**)

No.	**2**	**3**	**4**	**5**	**6**	**7**
3	5.04 *s*	5.05 *s*	5.02 *s*	3.60 *s*	4.73 *s*	4.86 *s*
5	2.14 *br d*	2.12 *dd*	2.12 *dd*	3.08 *d*	3.21 *br d*	2.84 *br d*
	(11.6)	(7.7, 7.1)	(9.5, 7.4)	(11.1)	(11.1)	(12.0)
6	2.36 *dd*	2.27 *dd*	2.26 *dd*	2.47 *br dd*	2.48 *dd*	2.46 *dd*
	(16.8, 11.6)	(15.8, 7.1)	(15.5, 9.5)	(17.7, 11.1)	(17.6, 11.1)	(16.6, 11.9)
	2.44 *br d*	2.31 *dd*	2.30 *dd*	2.72 *br d*	2.72 *br d*	2.77 *br d*
	(16.8)	(15.7, 7.7)	(15.5, 7.4)	(17.7)	(17.6)	(16.6)
11α	1.89 *t*					
	(14.4)					
11β	2.07 *br dd*	5.53 *s*	5.54 *d*	4.82 *d*	4.87 *br d*	5.37 *d*
	(14.4, 4.2)		(3.8)	(2.1)	(1.6)	(3.5)
12	3.73 *br dd*	5.53 *s*	5.53 *d*	4.53 *d*	4.53 *br d*	4.22 *br d*
	(14.5, 4.2)		(3.8)	(2.0)	(1.6)	(3.5)
14		2.70 *d*	2.69 *d*	2.74 *d*	2.75 *br d*	2.51 *d*
		(8.8)	(8.8)	(10.0)	(10.2)	(8.7)
15α	6.29 *s*	2.65 *dd*	2.69 *dd*	2.67 *dd*	2.68 *dd*	2.66 *dd*
		(18.9, 8.8)	(18.9, 8.8)	(19.0, 10.0)	(18.9, 10.2)	(19.2, 8.7)
β		3.38 *d*	3.38 *d*	3.27 *d*	3.25 *dd*	3.35 *d*
		(18.9)	(18.9)	(19.0)	(18.9, 0.4)	(19.2)
17	5.87 *s*	5.86 *s*	5.86 *s*	5.60 *s*	5.55 *s*	5.87 *s*
18	1.51 *s*	0.85 *s*	0.85 *s*	1.41 *s*	1.41 *s*	1.00 *s*
19	1.36 *s*	4.16 *d*	4.17 *d*	4.15 *d*	4.15 *d*	4.32 *d*
		(12.6)	(12.7)	(11.6)	(11.6)	(12.6)
		4.84 *d*	4.84 *d*	4.57 *d*	4.58 *d*	4.65 *d*
		(12.6)	(12.7)	(11.6)	(11.6)	(12.6)
21	7.59 *br s*	7.52 *br s*	7.52 *br s*	7.38 *br s*	7.34 *br s*	7.63 *br s*
22	6.57 *br s*	6.48 *br*	6.48 *br s*	6.41 *br s*	6.43 *dd*	6.58 *br d*
		(0.9)			(1.7, 0.9)	(1.1)
23	7.49 *br t*	7.41 *br t*	7.41 *t*	7.36 *t*	7.40 *t*	7.38 *t*
	(1.4)	(1.6)	(1.8)	(1.6)	(1.7)	(1.7)
28	0.74*s*	0.90 *s*	0.90 *s*	0.96 *s*	0.96 *s*	0.80 *s*
$29_{pro\text{-}R}$	1.90 *d*	2.03 *d*	2.01 *s*	1.74 *d*	1.80 *s*	2.08 *d*
	(11.2)	(11.7)		(11.3)		(10.5)
$_{pro-S}$	1.63 *d*	2.01 *d*	2.01 *s*	1.68 *d*	1.80 *s*	1.84 *dd*
	(11.2)	(11.7)		(11.3)		(10.5, 1.3)
30	4.38 *br s*	5.64 *s*	5.67 *s*	4.81 *br s*	4.65 *d*	5.65 *s*
					(7.2)	
OMe	3.71 *s*			3.74 *s*	3.72 *s*	3.64 *s*
3-OAc	2.06 *s*	2.22 *s*	2.24 *s*		2.17 *s*	2.19 *s*
11-OAc		2.10 *s*	2.10 *s*			2.05 *s*
12-OAc		2.08 *s*	2.08 *s*	2.04 *s*	1.67 *s*	
19-OAc				1.66 *s*	2.05 *s*	2.22 *s*
30-OAc		2.09 *s*				2.10 *s*
30-Opropanoyl						
2′			2.34, 2.43 each *dq*			
			(17.1, 7.5)			
3′			1.10 *t*			
			(7.5)			
Orthoesters						
2′				1.71 *s*	1.72 *s*	

All spectra were measured in $CDCl_3$ at 600 MHz.
Chemical shifts are expressed in ppm.
J values in parentheses are in Hz.

Table 2
^{13}C NMR spectral data for tabulalin (**2**) and tabulalides A–E (**3**–**7**)

No.	**2**	**3**	**4**	**5**	**6**	**7**
1	83.5 *s*	85.2 *s*	85.2 *s*	83.9 *s*	83.7 *s*	84.7 *s*
2	76.2 *s*	74.4 *s*	74.4 *s*	78.4 *s*	77.7 *s*	75.1 *s*
3	86.1 *d*	84.2 *d*	84.2 *d*	83.5 *d*	82.5 *s*	84.1 *d*
4	43.7 *s*	44.4 *s*	44.5 *s*	45.8 *s*	45.5 *s*	44.3 *s*
5	38.6 *d*	41.9 *d*	41.9 *d*	35.0 *d*	36.3 *d*	43.1 *d*
6	34.1 *t*	31.4 *t*	31.4 *t*	32.7 *t*	32.9 *t*	33.1 *t*
7	173.8 *s*	172.3 *s*	172.3 *s*	173.2 *s*	172.0 *s*	173.0 *s*
8	72.3 *s*	79.6 *s*	79.6 *s*	87.0 *s*	86.8 *s*	78.4 *s*
9	78.5 *s*	78.9 *s*	78.9 *s*	84.7 *s*	84.7 *s*	77.6 *s*
10	48.0 *s*	51.9 *s*	51.9 *s*	47.1 *s*	47.1 *s*	53.3 *s*
11	34.3 *t*	69.5 *d*	69.5 *d*	70.8 *d*	70.6 *d*	71.8 *d*
12	65.8 *d*	71.0 *d*	71.1 *d*	71.8 *d*	71.7 *d*	71.3 *d*
13	45.2 *s*	38.6 *s*	38.6 *s*	37.8 *s*	37.9 *s*	39.7 *s*
14	163.6 *s*	43.6 *d*	43.7 *d*	41.7 *d*	41.5 *d*	42.2 *d*
15	120.4 *d*	27.2 *t*	27.2 *t*	27.4 *t*	27.4 *t*	27.8 *t*
16	164.9 *s*	168.4 *s*	168.2 *s*	170.6 *s*	170.4 *s*	169.3 *s*
17	78.5 *d*	76.3 *d*	76.2 *d*	77.2 *d*	77.2 *d*	77.2 *d*
18	14.7 *q*	19.2 *q*	19.2 *q*	15.6 *q*	15.7 *q*	19.9 *q*
19	13.9 *q*	69.0 *t*	69.0 *t*	66.5 *t*	66.3 *t*	65.8 *t*
20	121.6 *s*	120.8 *s*	120.8 *s*	121.2 *s*	121.4 *s*	121.2 *s*
21	142.4 *d*	140.6 *d*	140.6 *d*	140.9 *d*	140.4 *d*	140.8 *d*
22	109.8 *d*	108.9 *d*	109.0 *d*	110.0 *d*	109.8 *d*	109.5 *d*
23	144.6 *d*	143.8 *d*	143.8 *d*	142.9 *d*	143.2 *d*	143.3 *d*
28	14.6 *q*	14.4 *q*	14.5 *q*	13.9 *q*	14.0 *q*	15.4 *q*
29	40.2 *t*	39.0 *t*	39.0 *t*	39.4 *t*	39.3 *t*	40.5 *t*
30	68.4 *d*	68.0 *d*	67.6 *d*	68.1 *d*	68.4 *d*	68.6 *d*
OMe	52.3 *q*			52.2 *q*	51.9 *q*	52.0 *q*
3-OAc	170.6 *s*	168.7 *s*	168.6 *s*		170.2 *s*	168.9 *s*
	21.6 *q*	21.0 *q*	21.1 *q*		20.9 *q*	21.2 *q*
11-OAc		170.9 *s*	170.9 *s*			169.6 *s*
		20.7 *q*	20.7 *q*			21.3 *q*
12-OAc		169.4 *s*	169.4 *s*	170.0 *s*	169.9 *s*	
		20.5 *q*	20.6 *q*	21.0 *q*	21.0 *q*	
19-OAc				170.0 *s*	169.9 *s*	171.0 *s*
				20.3 *q*	20.3 *q*	21.6 *q*
30-OAc		170.3 *s*				170.4 *s*
		20.6 *q*				20.6 *q*
30-Pro:						
1′			173.5 *s*			
2′			27.2 *t*			
3′			8.7 *q*			
Orthoester				120.2 *s*	120.3 *s*	
				21.2 *s*	21.1 *q*	

Therefore, the molecule is hexacyclic. The NMR spectroscopic data (Tables 1 and 2) also revealed that compound **2** contained $5CH_3$ (three tertiary, one acetyl and one methoxy), $3CH_2$, 9CH (four olefinic), and 12 quaternary carbons (two olefinic). From the NMR data, the presence of a β-furyl moiety (δ_H 6.57, 7.49 and 7.59, each 1H) and an acetyl group (δ_H 2.06) was also recognized.

All of the protons directly bonded to carbon atoms were assigned by analysis of its HMQC spectrum. The data from decouplings and the subsequent 2D NMR studies using the ^1H–^1H COSY, HMBC, and NOESY spectra, strongly suggested that **2** was a phragmalin-type limonoid (Connolly et al., 1978; Saad et al., 2003). Thus, the H_2-6 methylene protons at δ 2.36 (*dd*, J = 16.8 and 11.6 Hz) and 2.44 (*br d*, J = 16.8 Hz) attached to a carbon adjacent to an ester carbonyl were coupled with the H-5 proton at δ 2.14 (*br d*, J = 11.6 Hz), and the presence of this moiety and a characteristic low-field H-17 singlet at δ 5.87 revealed that **2** was a ring B,D-*seco* limonoid. In addition to this knowledge, the absence of signals due to the two tertiary methyls at 4β (C-29) and 8β (C-30) in the basic limonoid skeleton and the presence of signals to be assigned to 29-methylene protons at δ 1.63 and 1.90 (each *d*, J = 11.2 Hz) suggested that **2** was either a phragmalin having the tricyclo[3.3.$1^{2,10}$.$1^{1,4}$]decane ring system, or a rearranged phragmalin such as the khayanolides possessing a tricyclo[4.2.$1^{10,30}$.$1^{1,4}$]decane structure, as reported from *Khaya senegalensis* (Abdergaleil et al., 2001).

Except for two compounds, **8** and its 1-*O*-acetate **9**, from *K. senegalensis* (Olmo et al., 1997), all of the phragmalin compounds so far isolated have been reported to be present as the 1,8,9- or 8,9,14-orthoacetate derivatives (Taylor, 1984). Compound **2** exhibited similar NMR spectra to those of phragmalin derivatives from the same species *C. taburalis* (Connolly et al., 1978), having six oxygenated sp^3 carbons attributable to C-1, C-2, C-3, C-8, C-9 and C-30. However, compound **2** differed by the lack of any orthoester group and the presence of additional hydroxy group and a trisubstituted double bond at δ_C 120.4 (*d*, C-15) and 163.6 (*s*, C-14).

In the HMBC spectrum of **2** (Fig. 1), the observed long-range C–H correlations of the H-5 signal with the ^{13}C signals at δ 13.9 (*q*), 40.2 (*t*), 48.0 (*s*), 78.5 (*s*), and 86.1 (*d*) led to their assignments as C-19, C-29, C-10, C-9 and C-3, respectively. A downfield shifted singlet methine proton at δ 4.38 assigned to H-30 showed significant HMBC correlations with four quaternary carbons at δ 76.2, 72.3, 78.5 and 83.5 to be assigned to C-2, C-8, C-9 and C-1, and the methine carbon of C-3. The presence of an acetyl group at C-3 was also confirmed from the correlation of the H-3 singlet at δ 5.04 attached to C-3 with an acetyl carbonyl carbon at δ 170.6 (*s*). Further, the 29-methylene protons showed HMBC correlations with C-1-C-5, C-9, C-10, and C-28 (4-Me) at δ 14.6. These findings clearly characterized the first molecular fragment, the left-hand tricyclo[3.3.1.1]decane ring system.

An olefinic proton at δ 6.29 (*s*, H-15) attached to a carbon at δ 120.4 adjacent to a lactone carbonyl carbon at δ 164.9 (C-16), showed correlations with another olefinic carbon at δ 163.6 (C-14) and two quaternary carbons at δ 72.3 (C-8) and 45.2 (C-13). The carbon signal due to C-13 was correlated to two methine protons at δ 3.73 (H-12) and 5.87 (H-17), the methylene protons at δ 1.89 and 2.07 assigned to H_2 -11, and the methyl protons at δ 1.51 (Me-18). Finally, the 11-methylene signals attached to the carbon at δ 34.3 showed correlations with the C-8–C-10, C-12, and C-13 signals. These correlations characterized the second fragment of the molecule, C-8, C-9 and C-11–C-17 of the C and D rings, including 13-Me (C-18) and a furan ring.

The structure of **2**, including its stereochemistry, was fully explained from the NMR data by the consideration of NOE correlations (Fig. 2) using a molecular model. Strong cross-peaks of the H-5 signal with H-12 at δ 3.73 (*br dd*, J = 14.5 and 4.2 Hz) and the H-17 signals, and of H-12 with H-17 indicated the β orientation for these three protons and the folded conformation of **2**. The 29-methylene proton signals at δ 1.63 (*pro-S*) and 1.90 (*pro-R*) showed NOE correlations with the H-3 and 10-Me (Me-19) proton signals. An NOE correlation observed between the H-30 and H-15 signals also clarified the stereochemistry of the ring system in **2**.

Tabulalide A (**3**) was obtained as a white amorphous powder. The molecular formula ($C_{34}H_{40}O_{17}$, 15 unsaturations) was determined by analysis of its HRFAB-MS (*m*/*z*: 743.2159 $[M + Na]^+$, Δ −0.4 mmu) and NMR spectra. The IR spectrum showed similar absorptions to those of **2** at 3600–3200 and 1745 cm^{-1} as broad bands. However, the NMR spectrum showed the change of several functional groups in **3** from **2**, which included the absence of the trisubstituted double bond and the methoxy group in **2**, and the presence of three additional acetate groups in **3**. The most significant difference was the presence of only two methyl groups corresponding to the 4β-Me and the 13α-Me at δ_H 0.90 and 0.85 in **3**. The remaining methyl group (Me-19) observed at δ_H 1.36 in **2**, was converted to an oxymethylene group (δ_C 69.0 *t*; δ_H 4.16 and 4.84, each *d*, J = 12.6 Hz) in **3** and it was correlated in the HMBC spectrum to a lactonic carbonyl carbon at δ 172.3 (C-7), which implied the formation of a six-membered ring. Consequently, the molecule was heptacyclic. A fragmalin structure of **3** having the same stereochemistry as **2** except for the lack of

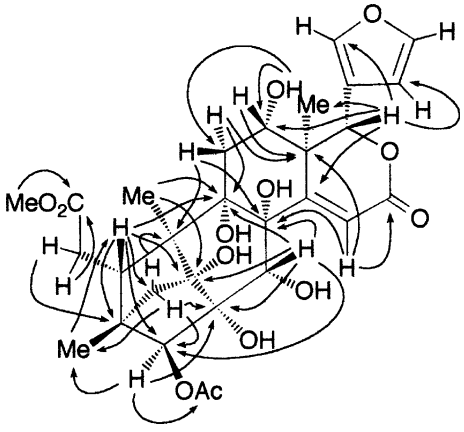


Fig. 1. Selected HMBC correlations in **2**.

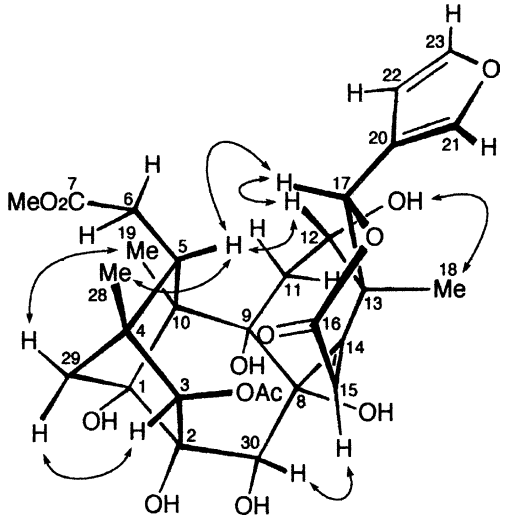


Fig. 2. Significant NOE correlations in **2**.

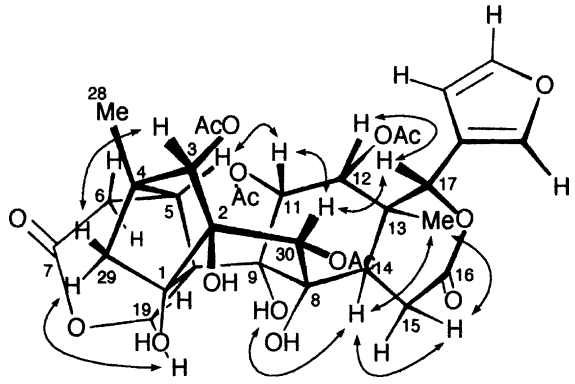


Fig. 3. Selected NOE correlations in **3**.

C-14–C-15 double bond, was confirmed by the ^{1}H and ^{13}C NMR data as presented in Tables 1 and 2 and by the NOE correlations (Fig. 3).

The configuration of H-14 at δ 2.70 (*d*, $J = 8.8$ Hz) coupling with the 15-methylene protons at δ 2.65 (*dd*, $J = 18.9$ and 8.8 Hz) and 3.38 (*d*, $J = 18.9$ Hz) was assigned to be α from the NOE correlation with the 13α-Me (18) signal at δ 0.85. The locations of the four acetate groups were elucidated by the HMBC correlation of the methine protons at δ 5.05 (H-3), 5.53 (H-11), 5.53 (H-12), and 5.64 (H-30) with the acetyl carbonyl carbons at δ 168.7, 170.9, 169.4 and 170.3, respectively, in which the 3-acetoxymethyl protons were observed at low field (δ 2.22), different from δ 2.06 in **2**. This chemical shift change suggested a difference of conformation in **3** from **2**, which was apparent from the NOE correlations of the H-5 signal at δ 2.12 with the H-11 signal, the H-17 signal at δ 5.86 with the H-12 and H-30 signals, and the H-30 signal with the H-11 signal in **3.** The significant low-field shift of H-15β to δ 3.38 by an anisotropic effect of the 16-carbonyl group also supported the proposed conformation.

The molecular formula of tabulalide B (**4**) was determined as $C_{35}H_{42}O_{17}$ by negative HRFAB-MS (*m*/*z*: 733.2336 $[M - H]^-$, Δ −0.7 mmu) suggesting the presence of two additional $-CH_2-$ units compared with compound **3**. The ^{1}H and ^{13}C NMR spectroscopic data of **4** (Tables 1 and 2) were extremely similar to those of **3**, including the 19-oxygenated methylene group (δ_C 69.0 *t*; δ_H 4.17 and 4.84, each *d*, $J = 12.7$ Hz). The only difference was the replacement of the acetoxy group at C-30 in **3** by a propanoyloxy group at δ_H 2.34 and 2.43 (each *dd*, $J = 17.1$ and 7.5 Hz) and 1.10 (*t*, $J = 7.5$ Hz) and at δ_H 173.5 (*s*), 27.2 (*t*) and 8.7 (*q*).

Positive HRFAB-MS of tabulalide C (**5**) gave a pseudomolecular ion at *m*/*z* 693.2418 $[M + H]^+$ (Δ +2.3 mmu), corresponding to the formula $C_{33}H_{40}O_{16}$. Compound **5** was also predicted by the NMR spectra to be an 19-oxygenated phragmalin derivative from the presence of two methyl signals at δ_H 0.96 (Me-28) and 1.41 (Me-18), and oxygenated 19-methylene signals at δ_H 4.15 and 4.57 (each *d*, $J = 11.6$ Hz) due to protons attached to a carbon at δ_C 66.5 (*t*), together with the 29-methylene signals at δ_H 1.68 and 1.74 (each *d*, $J = 11.3$ Hz). This compound, having two acetyl methyl signals at δ 1.66 and 2.04, however, was significantly different from **3** and **4** in the presence of a characteristic downfield shifted methyl signal at δ 1.71, together with one methoxy signal at δ 3.74 assigned to the 7-carbomethoxy group such as in **2**. This methyl was assigned tothe orthoacetate methyl from the HMBC correlation with an orthoester carbon at δ_C 120.2 (*s*). Almost all of the phragmalin group compounds were reported to possess an orthoacetate group and the characteristic orthoester carbon resonance was observed at around δ 119 (Connolly et al., 1978; Saad et al., 2003). This orthoacetate group was located at positions 1,8,9, because the group had been observed either at positions 1,8,9 or 8,9,14 in phragmalins. In **5**, the H-14 signal at δ 2.74 (*d*, $J = 10.0$ Hz) was coupled with one of the characteristic 15-methylene signals at δ 2.67 (*dd*, $J = 19.0$ and 10.0 Hz) and 3.27 (*d*, $J = 19.0$ Hz). On the other hand, the two acetyl carbons at δ 170.0 were correlated in the HMBC spectrum to the 19-methylene protons and the 12-methine proton at δ 4.53 (*d*, $J = 2.0$ Hz) attached to carbon at δ 71.8. The remaining three oxygenated carbons, at δ 78.4 (*s*), 70.8 (*d*), and 68.1 (*d*), to be assigned to C-2, C-11, and C-30, should possess OH groups. The high field shift of one (12-OAc) of the acetyl groups to δ 1.66 was attributed to an anisotropic effect due to the furan ring located on the same side of the molecule (Connolly et al., 1978; Nakatani et al., 1981).

The stereochemistry of **5** was elucidated by the NOE correlations and indicated that all of the asymmetric carbons had the same configurations as those of **3** and **4**. However, as suggested by the large, high-field shift of the 12-acetoxymethyl signal, **3** was present in a different conformation from **3** and **4**, which was also clear from the NOE correlations of the H-5 signal at δ 3.08 with the H-12 and H-30 signals at δ 4.53 and 4.81, and of the H-12 signal with H-17 at δ 5.60 and the H-30 signals, different from those in **3** and **4** (Fig. 4).

The molecular formula of tabulalide D (**6**) was determined to be $C_{35}H_{42}O_{17}$ by a pseudomolecular ion at *m*/*z* 733.2371 $[M - H]^-$ (Δ +2.7 mmu) on negative HRFAB-

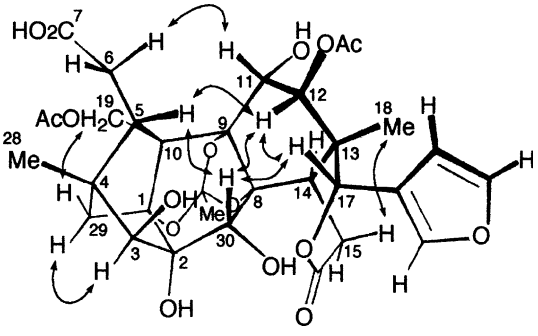


Fig. 4. Significant NOE correlations in **5**.

MS, and the 19-oxygenated phragmalin structure was readily suggested from the comparison of spectral data with **5**. The ^{1}H and ^{13}C NMR data were very similar to those of **5** including one orthoacetate group, and except for the presence of an additional acetyl group at C-3, which was confirmed from the downfield shift of H-3 to δ 4.73. The β configuration of the acetate group was also assigned from a NOE correlation of the H-3 signal with one (*pro-S*) of the 29-methylene signals at δ 1.80. This compound showed characteristic NOE correlations of H-5 at δ 3.21 with H-12β at δ 2.75 and H-30β at δ 4.65, of H-12β with H-30β and H-17 at δ 5.55, and of H-17 with H-30β, in analogy with **5**.

The molecular formula of tabulalide E (**7**) was also determined to be $C_{35}H_{44}O_{18}$ by negative HRFAB-MS (*m*/*z* 751.2421 $[M-H]^-$, Δ −2.9 mmu), and compound **7** was a 19-*O*-acetylphragmalin compound similar to **5** and **6**. Two isolated methylene signals at δ 1.84 and 2.08 (H_2-29) and δ 4.32 and 4.65 (H_2-19) were observed in the ^{1}H NMR spectrum, in which the latter signals were correlated with an acetate carbon at δ 171.0 in the HMBC spectrum. Interestingly, the signal at δ 1.84 assigned to H-$29_{pro\text{-}S}$ showed a W-type long range coupling with the H-5 signal at δ 2.84. Although **7** also showed ten oxygenated sp^3 carbon signals at δ 84.7 (*s*, C-1), 75.1 (*s*, C-2), 84.1 (*d*, C-3), 78.4 (*s*, C-8), 77.6 (*s*, C-9), 71.8 (*d*, C-11), 71.3 (*d*, C-12), 77.2 (*d*, C-17), 66.3 (*t*, C-19), and 52.0 (*q*, OMe) in the ^{13}C NMR spectrum similar to **4** and **5**, the presence of an orthoester group was not observed. The NMR spectra showed the presence of four acetates and their location at C-2, C-11, C-19 and C-30 was elucidated based on the HMBC correlations of the corresponding protons with the acetate carbons. The stereochemistry of **7** was elucidated by the NOESY spectrum to be similar to those of the compounds **3**–**6** (Fig. 5).

Two 19-oxygenated mexicanolides, seneganolide and 2-hydroxyseneganolide, were previously reported from a mahogany species *Khaya senegalensis* (Nakatani et al., 2000; Nakatani et al., 2001), but this is the first report on 19-oxygenated phragmalin compounds.

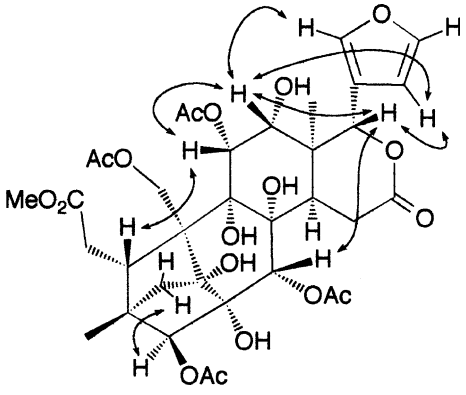


Fig. 5. Significant NOE correlations in **7**.

The antifeedant activity of the isolated compounds, **2**–**7**, was tested by a conventional leaf disk method (Wada and Munakata, 1968) against the third-instar larvae of *Spodoptera littoralis* (Boisduval). Tabulalin (**2**) and tabulalide D (**6**) were strongly active at 500 ppm, with 50 ppm corresponding to a concentration of ca. 1 μg/leaf-cm^2. The activity is weaker than that of well known limonoid antifeedants, ring C-*seco* limonoids of azadirachtins (Ley et al., 1993) and meliacarpinins (Nakatani et al., 1995) from *Azadirachta indica* and *Melia azedarach*, but almost comparable to that of many other limonoid antifeedants (Huang et al., 1995; Mootoo et al., 1996; Abdelgaleil et al., 2000) from Meliaceae plants. Tabulalins A (**3**), B (**4**), and E (**7**) showed weak activity at 1000 ppm, while tabulalide C (**5**) was not active at the same concentration. From this antifeedant assay, some interesting structure-activity correlations were shown. Activity is insensitive to substitution variation in the C-ring, whereas the 30-hydroxyl group on the tricyclo[3.3.1.1]decane ring system has a pronounced effect on the activity, and acylation resulted in reduced activity. In conclusion, the phragmalin compounds isolated here are, in general, somewhat less active than other ring B,C-*seco* limonoids of the mexicanolides (200–1000 ppm) and of the rearranged fragmalins (100–1000 ppm) having a tricyclp[4.2.1.1]decane ring system, isolated from *K. senegalensis* (Nakatani et al., 2000; Abdergaleil et al., 2001; Nakatani et al., 2001).

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were measured at 600 and 150 MHz in $CDCl_3$ on JEOL FX-600 spectrophotometer. IR (KBr) and UV (MeOH) spectra were recorded on JASCO FT/IR 5300 and Shimadzu UV-210A spectrophotometers. Optical rotation was measured at 22° on JASCO DIP-370S spectrophotometer. HPLC was performed on Waters μBondapak C_{18} column by using a gradient of 35–65% H_2O/MeOH as eluant.

3.2. Plant material

Root bark of *C. tabularis* was collected at Xiangtan, China, in December 1995. The plant material was identified by the staff at the Huana Plant Garden, Guangzhou, and voucher specimens are deposited in the herbarium at the Faculty of Science of Kagoshima University.

3.3. Extraction and isolation of compounds 2–7

Air-dried root bark of *C. tabularis* (810 g) was extracted with diethyl ether (2 l) at room temperature for

four weeks to give a crude extract (9.2 g). A sample of the ether extract (4.5 g) was subjected to DCCC using CH_2Cl_2–MeOH–H_2O (5:5:3 v/v) in an ascending mode to give six limonoid fractions of 189, 136, 147, 186, 246 and 188 mg. The first fraction (189 mg) was purified through HPLC with a 40–65% H_2O/MeOH gradient as eluent to give **2** (11.6 mg). The third fraction (147 mg) was subjected to the same purification with a 40–60% H_2O/MeOH gradient eluent to give **2** (13.9 mg), **5** (5.5 mg), and **7** (1.4 mg). From the fourth fraction (186 mg) and the sixth fraction (188 mg), compounds **4** (2.8 mg) and **6** (7.6 mg) were purified with a 35–50% H_2O/MeOH gradient eluent system, respectively.

3.3.1. Tabularin (**2**)

White amorphous powder; $C_{29}H_{36}O_{13}$; HRFAB-MS *m/z*: 591.2067 $[M-1]^-$ (calc. 591.2076); $[\alpha]_D$ +41° (*c* 0.19, MeOH); UV λ_{max} nm (ε): 215 (9000); IR ν_{max} cm^{-1}: 3600–3200, 1740, 1724, 1637, 1024 and 875; for ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.3.2. Tabulalide A (**3**)

White amorphous powder; $C_{34}H_{40}O_{17}$; HRFAB-MS *m/z*: 743.2159 $[M+Na]^+$ (calc. 743.2163); $[\alpha]_D$ −44° (*c* 0.21, MeOH); UV λ_{max} nm (ε): 211 (4000); IR ν_{max} cm^{-1}: 3600–3200, 1745, 1373, 1222, 1064, 1026 and 875; for ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.3.3. Tabulalide B (**4**)

White amorphous powder; $C_{35}H_{42}O_{17}$; (−) HRFAB-MS *m/z*: 733.2336 $[M-1]^-$ (calc. 733.2343); $[\alpha]_D$ −37° (*c* 0.14, MeOH); UV λ_{max} nm (ε): 211 (5000); IR ν_{max} cm^{-1}: 3600–3300, 1745, 1375, 1222 and 875; for ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.3.4. Tabulalide C (**5**)

White amorphous powder; $C_{33}H_{40}O_{16}$; HRFAB-MS *m/z*: 693.2418 $[M+1]^+$ (calc. 693.2395); $[\alpha]_D$ −49° (*c* 0.28, MeOH); UV λ_{max} nm (ε): 212 (3000); IR ν_{max} cm^{-1}: 3600–3350, 1738, 1035 and 875; for ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.3.5. Tabulalide D (**6**)

White amorphous powder; $C_{35}H_{42}O_{17}$; (−) HRFAB-MS *m/z*: 733.2371 $[M-1]^-$ (calc. 733.2344); $[\alpha]_D$ −52° (*c* 0.16, MeOH); UV λ_{max} nm (ε): 211 (4000); IR ν_{max} cm^{-1}: 3600–3300, 1743, 1037 and 873; for ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.3.6. Tabulalide E (**7**)

White amorphous powder; $C_{35}H_{44}O_{18}$; (−) HRFAB-MS *m/z*: 751.2421 $[M-1]^-$ (calc. 751.2440); $[\alpha]_D$ −2.9° (*c* 0.07, MeOH); UV λ_{max} nm (ε): 211 (4000); IR ν_{max} cm^{-1}: 3600–3200, 1739, 1637, 1371, 1221, 1030 and 875; for ^1H and ^{13}C NMR spectral data (see Tables 1 and 2).

3.4. Antifeedant test

The antifeeding potential of the isolated compounds was tested by a conventional leaf disk method against third-instar larvae of *Spodopetra littoralis*. Five leaf disks (diameter 1.2 cm) of Chinese cabbage (*Brassica campestris* var. *chinensis*) were immersed in an acetone solution of the sample for 2 s. The treated disks were arranged alternatively with another five control disks (immersed only in acetone) close to the wall of a Petri dish. Ten larvae were placed in the centre of each Petri dish. The eaten areas of treated and untreated leaf disks were evaluated at appropriate intervals for 2–12 h. The experiment was terminated after the larvae had eaten approximately 50% of the control disks. To determine the minimum antifeedant concentration, this choice test was conducted three times at the concentrations of 300, 500 and 1000 ppm.

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