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PYRANOQUINOLINE ALKALOIDS FROM *ZANTHOXYLUM SIMULANS*

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Key Word Index—*Zanthoxylum simulans*; Rutaceae; bark; alkaloids; pyranoquinoline; simulenoline; peroxysimulenoline; benzosimuline; zanthodioline; anti-platelet aggregation activity.

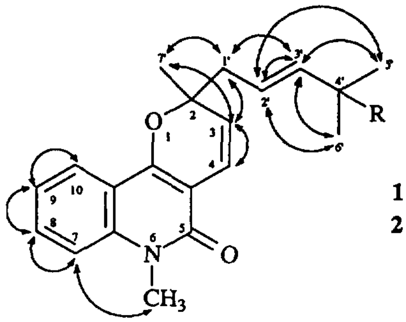
Abstract—Three new pyranoquinoline alkaloids, simulenoline, peroxysimulenoline and benzosimuline, together with 22 known compounds, were isolated from the stem bark of Formosan *Zanthoxylum simulans*. Structures including zanthodioline, a known alkaloid without published data, were elucidated by spectral evidences. Among the isolates, 14 compounds showed strong anti-platelet aggregation activity *in vitro*. © 1997 Elsevier Science Ltd

INTRODUCTION

In continuation of our chemical investigation of Formosan *Zanthoxylum* species, we have isolated nine new alkaloids and 28 known compounds from roots of *Zanthoxylum simulans* [1–5], which is a prickly shrub, distributed in mainland China and Taiwan [6]. Examination of the chloroform-soluble part of the stem bark from this species has resulted in the isolation of 25 compounds, three of which are new pyranoquinoline alkaloids, viz. simulenoline (**1**), peroxysimulenoline (**2**) and benzosimuline (**3**). The structure of another pyranoquinoline alkaloid (**4**) has previously been cited but never published and its spectroscopic data are also presented. The anti-platelet aggregation activities exhibited by 14 compounds, including quinolines, aporphines, acridone, benzo[*c*]phenanthridine and coumarin, are also described.

RESULTS AND DISCUSSION

The molecular formula, $C_{20}H_{23}NO_3$ of simulenoline (**1**), was determined by EI ($[M]^+$, *m/z* 325) and high-resolution mass spectrometry. UV absorptions were similar to those of zanthosimuline (**5**) [1], with no shift on addition of acid, suggesting the presence of a 2-quinolone nucleus [7]. The IR spectrum showed a hydroxyl group at 3400 cm^{-1} and amidocarbonyl absorption at 1630 cm^{-1}. Analyses of the ^1H NMR spectrum of **1** revealed signals for a pyranoquinoline moiety [δ 1.51 (Me-2), 3.69 (*N*-Me), 5.49 (H-3), 6.30 (H-4), 7.23 (H-9), 7.32 (H-7), 7.55 (H-8), 7.94 (H-10)] and a 4-hydroxy-4-methyl-2-pentenyl group [δ 2.47 (2H, H-1′), 5.69 (H-2′), 5.63 (H-3′), 1.18 (6H, *s*, Me-5′,6′)] at the C-2 position. The mass fragments at *m/z* 307 $[M-H_2O]^+$, 292 $[M-H_2O-CH_3]^+$, 226 $[M-CH_2CH{=}CHC(CH_3)_2OH]^+$ also supported the prenyl substitution on C-2. On the basis of the above data, the structure of simulenoline was elucidated as **1**, which was further confirmed by ^{13}C NMR, HETCOR and NOESY (Fig. 1).



1 R = OH
2 R = OOH

Fig. 1. NOESY interaction observed for compounds **1** and **2**.

The UV and IR spectra of peroxysimulenoline (**2**) were similar to those of **1**. The ^1H NMR spectrum was also similar to **1** in the pyranoquinoline moiety, but showed an exchangeable 1H signal at δ 7.25, two

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Table 1. ^1H NMR data for compounds **1** and **2** (400 MHz, in $CDCl_3$)

	Protons	**1**	**2**
Common signal	N-CH_3	3.69 (3H, *s*)	3.69 (3H, *s*)
	H-3	5.49 (1H, *d*, $J = 10.0$ Hz)	5.49 (1H, *d*, $J = 10.0$ Hz)
	H-4	6.80 (1H, *d*, $J = 10.0$ Hz)	6.81 (1H, *d*, $J = 10.0$ Hz)
	H-7	7.32 (1H, *br*, *d*, $J = 7.8$ Hz)	7.32 (1H, *br d*, $J = 7.8$ Hz)
	H-8	7.55 (1H, *ddd*, $J = 7.8$, 7.8, 1.4 Hz)	7.55 (1H, *ddd*, $J = 7.8$, 7.8, 1.4 Hz)
	H-9	7.23 (1H, *t*, $J = 7.8$ Hz)	7.23 (1H, *ddd*, $J = 7.8$, 7.8, 1.4 Hz)
	H-10	7.94 (1H, *dd*, $J = 7.8$, 1.4 Hz)	7.94 (1H, *dd*, $J = 7.8$, 1.4 Hz)
	H-7′	1.51 (3H, *s*)	1.53 (3H, *s*)
Prenyl group	H-1′	2.47 (2H, *d*, $J = 6.4$ Hz)	2.50 (2H, *dd*, $J = 7.0$, 1.0 Hz)
	H-2′	5.69 (1H, *dt*, $J = 16.0$, 6.4 Hz)	5.75 (1H, *dt*, $J = 15.6$, 7.0 Hz)
	H-3′	5.63 (1H, *d*, $J = 16.0$ Hz)	5.62 (1H, *dd*, $J = 15.6$, 1.0 Hz)
	H-5′	1.18 (3H, *s*)	1.17 (3H, *s*)
	H-6′	1.18 (3H, *s*)	1.22 (3H, *s*)
	OH	not observed	7.35 (1H, *br d*)

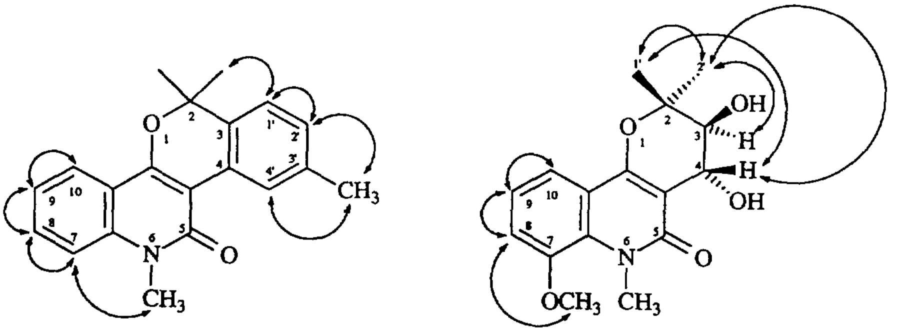
Table 2. ^{13}C NMR data for compounds **1** and **2** (100 MHz, in $CDCl_3$)

	Carbons	**1**	**2**
Common signal	C-1a	155.4	155.4
	C-2	80.7	80.6
	C-3	125.0	124.8
	C-4	118.7	118.8
	C-4a	105.9	105.9
	C-5	160.9	160.9
	N-CH_3	29.2	29.3
	C-7′	26.7	26.9
	C-7	114.0	114.1
	C-7a	139.3	139.4
	C-8	130.9	131.0
	C-9	121.7	121.8
	C-10	123.0	123.0
	C-10a	115.8	115.7
Prenyl group	C-1′	44.1	44.5
	C-2′	120.2	124.7
	C-3′	142.9	137.9
	C-4′	70.6	81.9
	C-5′	29.5	24.1
	C-6′	29.6	24.1

methyl singlets at δ 1.17, 1.22 and a small difference of chemical shift of other signals on the prenyl group comparing with those of **1** (Table 1). In the ^{13}C NMR spectrum (Table 2), δ 70.6 was assigned to C-4′ for connecting an OH group in **1** and δ 81.9 was reasonably assigned to C-4′ for connecting an OOH group to C-4′ in **2** according to reference data [8, 9]. From the above observations, the structure of peroxysimulenoline was reasonably elucidated as **2**, which was further supported by the ion *m/z* 342 $[M+H]^+$ in the FAB mass spectrum. However, the fact that **2** was easily converted into **1** on exposure to air, also gave a good explanation for the presence of an unstable hydroperoxy group in **2**.

The molecular formula of benzosimuline (**3**) was determined to be $C_{20}H_{19}NO_2$ by EI-mass ($[M]^+$, *m/z* 305) and HREI-mass spectrometry. The IR spectrum showed amidocarbonyl absorption at 1640 cm^{-1}. The ^1H NMR spectrum of **3** was similar to that of *N*-methylflindersine, except that the characteristic signals of H-3 and H-4 in pyranoquinolines were replaced by three aromatic protons at δ 7.11 (H-2′), δ 7.13 (H-1′) and δ 8.79 (H-4′), and a methyl group at δ 2.41 (Me-3′). This indicated that C-3 and C-4 were fused with a benzene ring. These observations suggested the structure of benzosimuline as **3**, which was further supported by HETCOR, ^{13}C NMR, HMBC and NOESY (Fig. 2).

The molecular formula, $C_{16}H_{19}NO_5$, of zanthodioline (**4**) was also determined by EI-mass and HREI-mass spectrometry. UV absorptions with no shift after addition of acid indicated the presence of a 2-quinolone skeleton [7]. The IR spectrum showed amidocarbonyl absorption at 1640 cm^{-1} and a hydroxyl group at 3410 cm^{-1}. The ^1H NMR spectrum was close to that of zanthobungeanine (**6**) [1], with regard to the 2-quinolone nucleus. The presence of two hydroxyl groups at δ 3.03, 5.67, two methine protons at δ 3.83 (H-3), 4.73 (H-4) and two singlets of methyl groups at δ 1.30, 1.62, indicated a dihydroxydihydropyran unit fused to a 2-quinolone nucleus. The coupling constant, $J = 7.8$ Hz of H-3 (δ 3.83) and H-4 (δ 4.73) suggested a relative *trans*-configuration in a diol system [10]. With $[\alpha]_D^{23} -20°$, the structure of **4** was reasonably elucidated as *trans*-(−)-3,4-dihydroxy-3,4-dihydrozanthobungeanine, and named as zanthodoline, which was further supported by NOESY experiment (Fig. 2). The structure of **4** has been cited previously but never published [11]; its spectroscopic data are presented for the first time in this study.



3 **4**

Fig. 2. NOESY interaction observed for compounds 3 and 4.

In addition to the four pyranoquinoline alkaloids described above, 21 known compounds were also isolated, including the aporphine alkaloids, (−)-*N*-acetylanonanine (**7**) [1], (−)-*N*-acetylnornuciferine (**8**) [1] and *N*-acetyldehydroanonaine (**9**) [5], the benzo [*c*]phenanthridine and related alkaloids, norchelerythrine (**10**) [1], decarine (**11**) [1] and arnottianamide (**12**) [1], the furoquinoline alkaloids, skimmianine (**13**) [1], γ-fagarine (**14**) [3] and robustine (**15**) [3], the 2-quinolone alkaloids, zanthosimuline (**5**) [1], zanthobugeanine (**6**) [1], huajiaosimuline (**16**) [1], zanthobisquinolone (**17**) [2] and edulitine (**18**) [2], the acridone alkaloid, arborinine (**19**) [12], the coumarin, aesculetin dimethyl ether (**20**) [13], the triterpenoids, β-amyrenone (**21**) [14] and β-amyrin (**22**) [15], the steroids, β-sitosterol (**23**) [2] and β-sitostenone (**24**) [16], and tetracosyl ferulate (**25**) [17]. Compounds **19**, **21**, **22**, **24** and **25** are reported for the first time from this species. These compounds were identified by comparisons of their IR, UV, ^{1}H NMR, TLC and/or mmp with corresponding authentic samples or literature data.

The chloroform-soluble fraction of the stem bark of *Z. simulans* showed strong anti-platelet activity *in vitro* using the turbidimetric method [18]. Bioassay-guided fractionation led to the isolation of simulenoline (**1**), benzosimuline (**3**), zanthobungeanine (**6**), (−)-*N*-acetylanonaine (**7**) [2], (−)-*N*-acetylnornuciferine (**8**), *N*-acetyldehydroanonaine (**9**), decarine (**11**), skimmianine (**13**), γ-fagarine (**14**) [2], robustine (**15**) [2], huajiaosimuline (**16**) [3], edulitine (**18**), arborinine (**19**) and aesculetin dimethyl ether (**20**), as the active principles with anti-platelet aggregation activity (Table 3). The isolated pyranoquinolines except zanthosimuline (**5**) at 100 μg ml^{-1} all showed complete or near inhibitory activity on platelet aggregation induced by arachidonic acid (AA), collagen and PAF in general. But the aggregation induced by thrombin was especially inhibited in the presence of huajiaosimuline (**16**) at 100 μg ml^{-1}. By comparison with 4-methoxy-1-methyl-2-quinolone [19], a selective inhibitor of platelet aggregation induced by AA, the loss of selective activity of pyranoquinolines seemed to be related with the presence of a pyran ring. The phenolic benzo[*c*]phenanthridine alkaloid, decarine (**11**) exhibited the strongest anti-platelet activity induced by AA and showed an IC_{50} value of *ca* 2–5 μg ml^{-1} compared with other isolates. Finally, the cytotoxic activity of the new pyranoquinoline alkaloids, simulenoline (**1**) and benzosimuline (**3**) were also evaluated on cultured human cancer cell lines of BC-1, LU-1, Col-2, KB, KB-VI(+VLB), KB-VI(−VLB) and LNCaP using published methods of bioassay [20]. Both compounds were inactive compared with huajiaosimuline (**16**) [3].

EXPERIMENTAL

General. Mps are uncorr. ^{1}H (400 and 200 MHz) and ^{13}C NMR (100 MHz) were taken in $CDCl_3$. Chemical shifts are given in δ with TMS as int. standard. MS were measured using a direct inlet system. Silica gel (60–230, 230–400 mesh) (Merck) was used for CC and silica gel 60 F-254 (Merck) for TLC.

Plant material. Stem bark of *Z. simulans* Hance was collected from Taichung Hsien, Taiwan in October 1985. A voucher sample (Chen 5364) is deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and isolation. Dried stem bark (1.79 kg) was extracted with MeOH and concd *in vacuo* to a brownish syrup. The total MeOH extract was partitioned between $CHCl_3$–H_2O (1 : 1) to afford a $CHCl_3$-sol. fr. (fr. A, 69.2 g). The H_2O-soluble portion was then partitioned between *n*-BuOH to yield an *n*-BuOH-soluble fr. (fr. B, 35.7 g) and a H_2O-soluble fr. (fr. C, 45.1 g). Fr. A (69.2 g) was chromatographed over silica gel, eluting with $CHCl_3$, gradually increasing the polarity with MeOH, and 19 frs (A1–A19) were collected. Fr. A6 ($CHCl_3$, 7.73 g) was washed with MeOH to afford crude crystals (64 mg). These were purified by prep. TLC (benzene–EtOAc, 20:1), to yield **17** (1.9 mg) and **10** (11 mg). The washings (7.58 g) were rechromatographed on silica gel, using a gradient of *n*-hexane–EtOAc to afford **21** (15 mg), **22** (84 mg), **24** (26 mg), **3** (6.4 mg), **15** (1.9 mg), **23** (39 mg), **25** (4 mg), **5** (139 mg), **9** (55 mg), **20** (18 mg), **2** (9 mg), **14** (38 mg) and **1** (6.5 mg). Fr. A7 ($CHCl_3$, 6.2 g) was washed with MeOH to yield **7** (648 mg) after

Table 3. Inhibitory effects of compounds on aggregation of washed rabbit platelets induced by thrombin, arachidonic acid, collagen and PAF

		Aggregation (%)			
	Concn (μg ml^{-1})	Thrombin (0.1 U ml^{-1})	Arachidonic acid (100 μM)	Collagen (10 μg ml^{-1})	PAF (2 nM)
Control		91.3 ± 1.2 (4)	85.6 ± 1.3 (4)	85.5 ± 2.1 (3)	88.1 ± 1.0 (4)
Simulenoline (**1**)	100	87.2 ± 3.1 (3)	12.8 ± 11.1 (4)***	0.0 ± 0.0 (3)***	23.4 ± 6.8 (3)***
	50		59.6 ± 17.4 (4)		80.1 ± 7.4 (3)
	20		83.4 ± 1.5 (4)		
Benzosimuline (**3**)	100	82.2 ± 1.2 (3)***	0.0 ± 0.0 (4)***	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***
	50		32.9 ± 18.0 (4)*		67.1 ± 6.4 (3)**
	20		43.2 ± 21.6 (4)		82.6 ± 2.0 (3)*
	10		84.4 ± 1.3 (4)		
Zanthobungeanine (**6**)	100	41.9 ± 13.1 (4)**	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***
	50		0.0 ± 0.0 (3)***	6.1 ± 5.0 (3)***	22.8 ± 10.2 (3)***
	20		47.9 ± 15.6 (3)	57.1 ± 19.9 (3)	71.6 ± 3.5 (3)**
	10		79.1 ± 1.1 (3)**	72.8 ± 9.0 (3)	77.6 ± 2.2 (3)**
(−)-*N*-Acetylnornuciferine (**8**)	100	61.0 ± 9.5 (4)**	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***	34.4 ± 17.2 (4)**
	50		0.0 ± 0.0 (3)***	26.2 ± 18.1 (3)**	
	20		37.9 ± 18.9 (3)*	56.5 ± 9.2 (3)**	
	10		70.0 ± 10.9 (3)	74.4 ± 3.2 (3)***	
N-Acetyldehydroanonaine (**9**)	100	80.6 ± 5.2 (3)*	0.0 ± 0.0 (3)***	6.1 ± 5.0 (3)***	0.0 ± 0.0 (4)***
	50		38.4 ± 19.2 (3)*		42.4 ± 13.9 (4)**
	20		84.2 ± 1.3 (3)		87.3 ± 1.5 (4)
Decarine (**11**)	100	44.5 ± 8.6 (3)***	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***
	50				0.0 ± 0.0 (3)***
	20		0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***	73.5 ± 4.3 (3)*
	10		2.7 ± 2.2 (3)***	27.7 ± 19.3 (3)**	78.4 ± 4.9 (3)
	5		30.8 ± 6.6 (3)***	78.4 ± 2.4 (3)*	81.4 ± 4.4 (3)
	2		63.3 ± 1.8 (3)***	84.4 ± 1.7 (3)	
Skimmianine (**13**)	100	81.1 ± 1.2 (4)**	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***	54.5 ± 16.3 (4)**
	50		0.0 ± 0.0 (3)***	11.2 ± 9.1 (3)***	
	20		23.4 ± 14.5 (3)***	54.9 ± 13.8 (3)*	
	10		70.2 ± 2.7 (3)***	77.7 ± 3.8 (3)*	
Edulitine (**18**)	100	85.6 ± 0.9 (4)	7.1 ± 5.8 (3)***	33.6 ± 18.5 (3)**	68.1 ± 9.3 (3)*
	50		73.2 ± 4.2 (3)***		
Arborinine (**19**)	100	88.5 ± 2.6 (3)	0.0 ± 0.0 (3)***	7.9 ± 3.2 (3)***	77.7 ± 5.3 (3)*
	50		73.1 ± 2.1 (3)***		
Aesculetin dimethyl ether (**20**)	100	21.4 ± 13.7 (4)***	0.0 ± 0.0 (3)***	0.0 ± 0.0 (3)***	31.3 ± 16.0 (4)***
	50		31.8 ± 13.5 (3)***	36.1 ± 14.8 (3)**	
	20		60.5 ± 9.2 (3)***	42.7 ± 16.0 (3)**	
	10		69.1 ± 4.4 (3)***	69.1 ± 5.5 (3)**	

Platelets were preincubated with DMSO (0.5%, control) or each compound at 37° for 3 min, and then the inducer was added.

Values are presented as means ± S.E. (*n*).

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared with respective control.

recrystallization. The washings (5.3 g) were rechromatographed on silica gel and eluted with $CHCl_3$ enriched with MeOH to yield **6** (25 mg), **16** (27 mg), **19** (4.2 mg), **8** (8.5 mg) and **4** (5.1 mg). Fr. A8 ($CHCl_3$, 2.2 g) was washed with Et_2O to afford crude crystals (103 mg), which were further purified by silica gel chromatography and recrystallization to obtain **11** (54 mg). Fr. A9 ($CHCl_3$, 3.24 g) was washed with Et_2O to afford **18** (13 mg) after recrystallization. The washings (3.01 g) were rechromatographed on silica gel using benzene–EtOAc (1 : 1) to give 15 frs (frs A9-1–A9-15). Fr. A9-5 (395 mg) was washed with MeOH to afford **12** (10 mg) after recrystallization. Fr. A9-6 (185 mg) was purified by prep. TLC (*n*-hexane–EtOAc, 1 : 1) to yield **13** (133 mg) after recrystallization.

Simulenoline (**1**). Colourless oil. UV λ_{max}^{EtOH} nm (log ε): 228 (4.42), 256 sh (4.02), 336 (3.81), 350 (3.86), 367 (3.69). IR ν_{max}^{neat} cm^{-1}: 3400 (*br* OH), 1630 (C=O). EI-MS *m/z* (rel. int.): 325 $[M]^+$ (5), 307 (10), 264 (9), 228 (13), 227 (67), 226 (100), 200 (20); HREI-MS: $C_{20}H_{23}NO_3$, found: 325.1671, calcd: 325.1678. ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. $[\alpha]_D^{23}$ +6.9° (*c* 0.24, $CHCl_3$).

Peroxysimulenoline (**2**). Colourless oil. UV λ_{max}^{EtOH} nm (log ε): 228 (4.33), 256 sh (3.86), 336 (3.68), 350 (3.73), 367 (3.56). IR ν_{max}^{neat} cm^{-1}: 3250–3350 (*br* OH), 1640

(C=O). FAB-MS *m/z*: 342 $[M+H]^+$. ^{1}H NMR: Table 1. ^{13}C NMR: Table 2.

Benzosimuline (**3**). Colourless oil. UV λ_{max}^{EtOH} nm (log ε): 210 (4.19), 233 (4.42), 250 sh (4.05), 268 (3.95), 310 sh (3.74), 320 sh (3.79), 331 (3.86), 346 (3.94), 365 (3.81). IR ν_{max}^{neat} cm^{-1}: 1640 (C=O). EI-MS *m/z* (rel. int.): 305 $[M]^+$ (39), 291 (32), 290 (100); HREI-MS: $C_{20}H_{19}NO_2$, found: 305.1414, calcd: 305.1416. ^{1}H NMR (400 MHz): δ 1.72 (6H, *s*, CH_3-2), 2.41 (3H, *s*, CH_3-3′), 3.79 (3H, *s*, N-CH_3), 7.11 (1H, *br d*, *J* = 7.9 Hz, H-2′), 7.13 (1H, *br d*, *J* = 7.9 Hz, H-1′), 7.25 (1H, *ddd*, *J* = 7.9, 7.9, 1.1 Hz, H-9), 7.36 (1H, *br d*, *J* = 7.9 Hz, H-7), 7.59 (1H, *ddd*, *J* = 7.9, 7.9, 1.1 Hz, H-8), 8.09 (1H, *dd*, *J* = 7.9, 1.1 Hz, H-10), 8.79 (1H, *br s*, H-4′). ^{13}C NMR: δ 21.5 (CH_3-3′), 27.5 (CH_3-2), 29.7 (N-CH_3), 122.0 (C-1′), 123.6 (C-10), 126.2 (C-4′), 126.8 (C-3′), 128.5 (C-2′), 131.1 (C-8), 134.4 (C-3), 137.4 (C-4), 139.2 (C-7a), 155.9 (C-1a), 161.4 (C-5).

Zanthodioline (**4**). Amorphous solid. UV λ_{max}^{EtOH} nm (log ε): 218 (4.14), 234 (4.21), 254 (4.11), 270 sh (3.56), 280 (3.59), 327 (3.23). IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1640 (C=O). EI-MS *m/z* (rel. int.): 305 $[M]^+$ (11), 235 (49), 234 (100), 206 (14), 205 (65), 204 (30), 191 (16), 190 (18); HREI-MS: $C_{16}H_{19}NO_5$, found: 305.1261, calcd: 305.1263. ^{1}H NMR (400 MHz): δ 1.30 (3H, *s*, CH_3-1′), 1.62 (3H, *s*, CH_3-2′), 3.03 (1H, *br s*, OH, disappeared after addition of D_2O), 3.83 (1H, *d*, *J* = 7.8 Hz, H-3), 3.90 (3H, *s*, OCH_3-7), 3.93 (3H, *s*, N-CH_3), 4.73 (1H, *d*, *J* = 7.8 Hz, H-4), 5.67 (1H, *br s*, OH, disappeared after addition of D_2O), 7.09 (1H, *dd*, *J* = 8.0, 1.4 Hz, H-8), 7.19 (1H, *t*, *J* = 8.0 Hz, H-9), 7.62 (1H, *dd*, *J* = 8.0, 1.4 Hz, H-10). $[\alpha]_D^{23}$ −20.0° (*c* 0.05, $CHCl_3$).

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