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LIMONOIDS FROM SEEDS OF *TOONA CILIATA* AND THEIR CHEMOSYSTEMATIC SIGNIFICANCE

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Key Word Index—*Toona ciliata*; Meliaceae; limonoids; biochemical systematics.

Abstract—From the seeds of *Toona ciliata* were isolated, besides toonacilin, two novel limonoids: 12-deacetoxytoonacilin and 6α-acetoxy-14β,15β-epoxyazadirone. These results do not seem to support the affiliation of *Toona* to the Swietenioideae.

INTRODUCTION

Toona was originally described by Endlicher (1840) as a section of *Cedrela* [1]. However, Roemer later (1846) recognized that they could be separated by a number of sound morphological characters, raising *Toona* to generic rank and most monographers have followed this lead [1]. Thus, the old world species of *Cedrela* were transferred to *Toona* (Endlicher) M. J. Roemer. The two genera were placed by Harms (1940) in the tribe Cedreleae under Cedreloideae [2]. Pennington and Styles, in their more recent monograph, include the Cedreleae in the Swietenioideae [3].

As reported in previous papers [4, 5] genera of Swietenioideae explore limonoid chemistry along only one route which leads to the mexicanolide group. In contrast, genera of Melioideae should form the more primitive taxon, split off from the common ancestral lineage before synthesis of the mexicanolide types. The ring B-seco limonoids then persisted into the Melioideae with development of the ring C-seco limonoids. The known limonoids from *Cedrela* are typical of the Swietenioideae [4, 5]. On the other hand, *Toona* appear to be peripheral Melioideae as well as Swietenioideae [4, 5].

Recently, we have described the isolation and identification of two novel meliacin butenolides, 21-hydroxycedrelonelide and 23-hydroxy-cedrelonelide from the stem of *T. ciliata* [6]. These limonoids are very closely related to those isolated from the melioid genus *Trichilia* [7, 8], showing that the affiliation of *Toona* to the Swietenioideae is still rather problematic. Clearly much more detailed phytochemical investigations of *Toona* species will be essential for a better understanding of its chemotaxonomic position in the Meliaceae.

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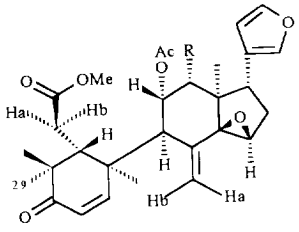
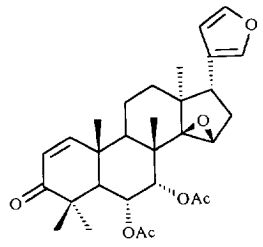
RESULTS AND DISCUSSION

The dichloromethane extract from the seeds of *T. ciliata*, afforded three typically Meliaceous limonoids. The limonoid **1** showed all the spectral data (Tables 1 and 2) of toonacilin which have previously been isolated from the bark of *T. ciliata* [9, 10]. Toonacilin can assume any of several rotations, although the rotamer **1** appears to be sterically the most favourable, since very strong correlations between H-5 and H-11, and between Me-19 and H-30b were revealed from NOESY experiments (Table 3). Moreover, there was a strong correlation between H-9 and H-30b.

The limonoid **2** showed spectral data (Tables 1 and 2) suggestive of a structure similar to toonacilin, but having only one α-acetoxyl substituent in ring C (δ5.23 *br s*, 70.9). The ^1H–^1H COSY spectrum revealed the oxymethine proton to be coupled only to the ^1H signal at δ1.89 (H-12). The signal for H-9 was still visible as a broad singlet at δ2.46 as in **1**, so placing the α-acetoxyl substituent at C-11. This was supported by the NOESY spectrum which showed a very strong correlation between H-11 and H-5 (Table 3). The correlations between H-30b and H-9, and between H-30a and H-15 confirm their assignments and are consistent with the stereochemistry as in **2** (α-H at C-9 and α-H at C-15). A ^1H–^{13}C COSY experiment permitted the assignments of all the protonated carbons including the tertiary methyl groups. The quaternary carbons were assigned on the basis of the ^1H–^{13}C long range correlations listed in Table 4. Thus, the observed



1 R = OAc
2 R = H



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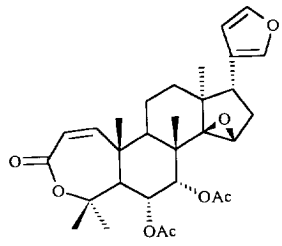

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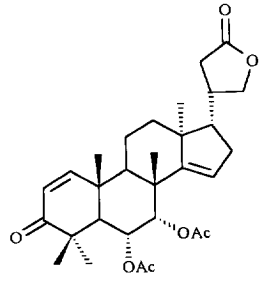

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correlation between the Me-18 at δ0.70 and the ^{13}C signal at δ40.9 (^2J) led unequivocally to the assignment of C-13 to this signal, but not of C-10. The new natural product is, therefore, 12-deacetoxytoonacilin (**2**).

Compound **3** exhibited similar spectral data to surenin (**4**), which has previously been isolated from the leaves of *Toona sureni* (Blume) Merill [11]. The ^{13}C NMR spectrum (Table 2) showed signals at δ157.6 (C-1), 126.1 (C-2) and 204.6 (C-3), characteristic of a ring A 1-en-3-one, as in the model compound **5** [12], instead of an α,β-unsaturated δ-lactone. Moreover, the chemical shifts of the ring B-D carbons were comparable with those reported for surenin (Table 2). From several 2D experiments it was possible to assign the chemical shifts for many protons and all carbons in the molecule (Tables 1 and 2). The assignment of the stereochemistry of the acetoxy groups received further support from NOESY experiments which showed very strong correlations of H-6 and H-7 with Me-30, and H-6 with Me-29. The new natural product is, therefore, 6α-acetoxy-14β, 15β-epoxy-azadirone.

The chemical evidence to hand strongly supports Roemer's taxonomic conclusions. However, limonoids (ring B-seco) of *Toona* can be considered to be biogenetic precursors of the mexicanolide types which are common in *Cedrela*, suggesting a direct derivation of this latter from *Toona*-like ancestors.

EXPERIMENTAL

General. IR: KBr; ^1H and ^{13}C NMR, NOESY and HMQC: 400 and 100 MHz, respectively, ^1H–^{13}C COSY long-range correlation: 200 MHz, $^3J_{CH}$ = 7 Hz, containing TMS as int. standard; GC-MS: low resolution on a HP-2576 instrument.

Isolation of constituents. Toona ciliata was collected in Viçosa, MG, Brazil, and a voucher is deposited in the Herbarium of Departamento de Engenharia Florestal, Universidade Federal de Viçosa, Viçosa, MG. The seeds were dried, powdered and extracted with hexane, CH_2Cl_2 and MeOH. The CH_2Cl_2 extract (77.5 g) was submitted to vacuum chromatography over silica gel using hexane, hexane–CH_2Cl_2 (1:1), CH_2Cl_2, CH_2Cl_2–EtOAc (1:1), EtOAc and MeOH. The CH_2Cl_2–EtOAc fr. was chromatographed on a DCCC (hexane–EtOH–EtOAc–H_2O, 5:4:2:1, ascending method). The organic fr. yielded a ppt. of **1** (150 mg) and **2** (90 mg) which was purified by 2 prep. TLC (silica gel, hexane–CH_2Cl_2–MeOH, 12.5:12.5:0.5; silica gel, hexane–EtOAc–MeOH, 20:5:0.5). The remaining organic fr. was rechromatographed over silica gel using hexane–CH_2Cl_2 (1:1), CH_2Cl_2, EtOAc and MeOH at different ratios of increasing polarity, yielding 25 frs. Frs 8–12 were purified by prep. TLC (silica gel, hexane–CH_2Cl_2–MeOH, 12.5:12.5:0.5) to yield a new fr. containing **3** (20 mg). The latter was then repurified by prep. TLC (silica gel, hexane–CH_2Cl_2–MeOH, 20:5:0.5).

Toonacilin (**1**). Amorphous solid. ^1H NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): Table 2; NOESY (400 MHz, $CDCl_3$): Table 3.

12-*Deacetoxytoonacilin* (**2**). Amorphous solid, mp 165.9–166.6°, $[\alpha]_D$ +25.5° ($CHCl_3$; *c* 0.0047). IR ν_{max}^{KBr} cm^{-1}: 1734, 1679, 876, 841. ^1H NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): Table 2; ^1H–^{13}C COSY long-range corr. (200 MHz, $CDCl_3$):

Table 1. ^{1}H NMR chemical shifts for **1–3** and selected protons in the model compounds **4** and **5**

H	1	2	3	4*	5*
1	7.26 *d* (10.4)	7.28 *d* (10.4)	7.12 *d* (10.4)	6.64 *d* (11.5)	7.12 *d* (10.0)
2	6.18 *d* (10.4)	6.05 *d* (10.4)	5.89 *d* (10.4)	5.93 *d* (11.5)	5.93 *d* (10.0)
5	2.58 *dd* (6.4, 3.2)	2.68 *dd* (6.4, 3.2)	2.47 *d* (12.4)	2.62 *d* (12.0)	2.45 *d* (13.0)
6	2.51 *dd* (16.4, 6.4)	2.52 *dd* (16.4, 6.4)	5.32 *dd* (12.4, 2.4)	5.26 *dd* (12.0, 3.2)	5.40 *m*
6	2.37 *dd* (16.4, 3.2)	2.38 *dd* (16.4, 3.2)			
7	—		4.99 *d* (2.4)	5.08 *d* (3.2)	5.40 *m*
9	2.57 *br s*	2.46 *br s*	2.57 *m*		
11	5.35 *d* (4.4)	5.23 br s	1.87 m		
11	—		1.77 *m*		
12	5.33 *d* (4.4)	1.95 m	1.84 m		
12	—	1.89 *m*	1.81 *m*		
15	3.96 *s*	3.96 *s*	3.40 *s*	3.42 *s*	5.40 *m*
16	2.26 *dd* (14.0, 7.0)	2.26 *dd* (14.0, 7.0)	2.13 *dd* (13.2, 6.5)		
16	1.88 *dd* (14.0, 11.0)	1.85 *m*	1.56 *dd* (13.2, 11.0)		
17	2.94 *dd* (11.0, 7.0)	2.71 *dd* (10.4, 7.0)	2.63 *dd* (11.0, 6.5)	2.63 *m*	
18	0.97 *s*	0.70 *s*	1.14 *s*		
19	1.01 *s*	1.00 *s*	0.92 *s*		
21	7.12 *m*	7.15 *m*	7.06 *m*	7.09 *m*	
22	6.12 *m*	6.17 *m*	6.12 *m*	6.14 *m*	
23	7.31 *m*	7.36 *m*	7.33 *m*	7.35 *m*	
28	1.10 *s*	1.10 *s*	1.21 *s*		
29	1.09 *s*	1.10 *s*	1.12 *s*		
30a	5.41 *br s*	5.34 *br s*	1.19 *s*		
30b	5.23 *br s*	5.18 *br s*			
OCOMe	2.00 *s*	1.98 *s*	2.08 *s*	2.10 *s*	2.03 *s*
OCOMe	1.80 *s*		1.97 *s*	1.96 *s*	1.99 *s*
COOMe	3.65 *s*	3.67 *s*			

Resonances for **1–3** were confirmed by ^{1}H–^{1}H and ^{1}H–^{13}C shift-correlated 2D spectra. Coupling constants (*J* Hz, in parentheses).

* Me = unassigned (**4** = δ0.94, 1.19, 1.51, 1.41, 1.41; **5** = δ1.02, 1.16, 1.16, 1.25, 1.27).

Table 4; NOESY (400 MHz, $CDCl_3$): Table 3. MS *m/z* (rel. int.): 436 $[M-MeCOOH]^{\cdot+}$. (2); 287 [Ring C,D,Furan – fragment]$^+$ (1); 209 [Ring A,B – fragment]$^+$ (13); 248 (3), 162 (9), 86 (3): associated with retro-Diels–Alder cleavage of ring C; 120 (21), 128 (31): associated with retro-Diels–Alder cleavage of ring A from 248; 92 $[120-CO]^{\cdot+}$ (18); 91 $[92-H]^+$ (100); 95 [162 – furan ring]$^+$ (44); 94 $(95-H)^{\cdot+}$ (42); 93 $[94-H]^+$ (46).

6α-Acetoxy-14β,15β-epoxyazadirone (**3**). Amorphous solid, mp 209.9–211.3°, $[\alpha]_D$ + 89.0° ($CHCl_3$; *c* 0.0146). IR ν_{max}^{KBr} cm^{-1}: 1742, 1678, 1244. ^{1}H NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): Table 2; NOESY (400 MHz, $CDCl_3$): Table 3. MS *m/z* (rel. int.): 450 $[M-MeCOOH]^{\cdot+}$ (1); 230 (2), 220 (1): associated with retro-Diels–Alder cleavage of ring B from 450; 136 (7), 94 (42): associated with retro-Diels–Alder cleavage of

Table 2. ^{13}C NMR chemical shifts for **1–3** and the model compounds **4** and **5**

C	1	2	3	4	5
1	151.9	153.4	157.6	160.8	156.9
2	126.4	125.8	126.1	122.5	126.3
3	202.5	203.0	204.6	167.5	204.4
4	45.8	45.9	40.5	84.6	40.8
5	44.0	43.9	48.4	*	48.0
6	31.7	31.8	70.1	69.9†	69.8
7	173.8	173.9	73.5	72.4†	74.4
8	135.3	136.9	42.8	*	42.9
9	57.0	55.4	39.3	*	37.0
10	44.5	41.9	45.1	*	44.9
11	74.5	70.9	16.3	*	16.3
12	70.1	37.2	32.1	*	33.4
13	41.7	40.9	41.7	*	46.5
14	72.2	71.5	72.7	72.3	158.1
15	60.2	60.3	57.1	56.2	119.5
16	32.6	30.7	29.1	*	33.9
17	38.4	39.1	39.0	*	58.1
18	14.1	18.7	21.5	†	†
19	19.8	19.9	21.8	†	†
20	122.7	122.7	123.6	123.4	37.4
21	139.9	139.3	139.5	139.4	72.4
22	111.3	110.7	110.8	110.8	34.8
23	142.2	142.9	142.9	142.8	176.4
28	22.8	23.0	31.5	†	†
29	22.5	22.6	20.1	†	†
30	120.7	119.8	18.8	†	†
OCOMe	168.7		169.9	169.7	170.2
OCOMe	169.6	169.2	169.8	169.4	170.0
OCOMe	20.7	21.2	21.2	21.1	21.3
OCOMe	20.8		21.2	20.8	20.9
COOMe	51.8	51.9			

* Unpublished.
† Unassigned (Me **4** = δ16.1, 18.1, 18.4, 22.8, 26.5; **5** = δ31.6, 26.8, 20.7, 20.4, 20.1).

Table 3. NOESY 2D NMR for limonoids **1–3**

1	2	3
H-11 (5.35)–H-5 (2.58)	H-5 (2.68)–H-28 (1.10)	H-6 (5.32)–H-29 (1.12)
H-30b (5.23)–H-9 (2.57)	H-6b (2.38)–H-19 (1.00)	H-6 (5.32)–H-30 (1.19)
H-30b (5.23)–H-19 (1.01)	H-6a (2.52)–H-29 (1.10)	H-7 (4.99)–H-30 (1.19)
	H-11 (5.23)–H-5 (2.68)	
	H-30b (5.18)–H-9 (2.46)	
	H-30a (5.34)–H-15 (3.96)	

Table 4. Selected long-range $^{1}H-^{13}C$ 2D NMR coupling data for **2**

H	C
9	8 (136.9); C-11 (70.9)
18	13 (40.9)
19	1 (153.4)
22	21 (139.3); 23 (142.9)
28/29	3 (203.0); 4 (45.9)
OCOMe	OCOMe (169.2)
COOMe	7 (173.9)

ring D involving one hydrogen transfer from Me-18 to epoxide with concomitant homolysis of 13-O bond from 230; 107 $[136-CHO]^{+}$ (100).

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