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NORLIMONOIDS FROM SEEDS OF *TOONA CILIATA*

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

Abstract—Further examination of the seeds of *Toona ciliata* led to the isolation of two new norlimonoids which were identified on the basis of spectroscopic analysis as 5α,6β,8α-trihydroxy-28-norisotoonafolin and 5α,6β,8α,12α-tetrahydroxy-28-norisotoonafolin. In addition, the known limonoids cedrelone and toonacilin and the sterols sitosterol, stigmasterol, campesterol and 3β-*O*-β-D-glucopyranosylsitosterol were also isolated and characterised. These results show that *Toona* has a less pronounced relationship to the Swietenioideae.

INTRODUCTION

Toona differs from other genera of Swietenioideae, notably by the absence of limonoids of the mexicanolide group [1]. Recently, we have described the isolation and identification of two novel limonoids, 12-deacetoxytoonacilin and 6α-acetoxy-14β,15β-epoxyazadirone from the dichloromethane extract from the seeds of *Toona ciliata* [2]. Limonoids with an intact carbon skeleton and the B-seco are features that are largely confined to the Melioideae [1]. Thus, *Toona* appears to have a less pronounced relationship to the former.

We have now examined the hexane and methanol extracts of *T. ciliata*. The methanol extract afforded two new pentanortriterpenoids, 5α,6β,8α-trihydroxy-28-norisotoonafolin (**1**) and 5α,6β,8α,12α-tetrahydroxy-28-norisotoonafolin (**2**).

RESULTS AND DISCUSSION

The hexane extract from the seeds of *T. ciliata* gave a mixture of sterols and two limonoids, which were identified by comparison with published data as cedrelone [1] and toonacilin [2]. The mixture of sterols was analysed by GC mass spectrometry, which established that the sterols were sitosterol, stigmasterol and campesterol.

A methanol-soluble fraction of the methanol extract was purified by repeated column chromatography on silica gel to give 3β-*O*-β-D-glucopyranosylsitosterol and two new limonoids **1** and **2**.

The limonoid **1** was identified on the basis of the following data. The ^{1}H NMR spectrum (Table 1) indicated the presence of four tertiary methyl groups (δ 0.99, 1.20, 1.38 and 1.56), a β-substituted furan ring (δ 6.37, 7.45, and 7.57), four signals characteristic of protons attached to a carbon adjacent to an oxygen atom (δ 3.62 *s*; 3.81 *d*, $J = 4.1$ Hz; 4.09 *dd*, $J = 9.5$ and 7.0 Hz; 4.14 *ddd*, $J = 12.0$, 9.6 and 7.2 Hz) and three signals for hydroxyl groups (δ 6.07 *s*; 6.41 *s*; 7.03 *d*, $J = 4.1$ Hz; no correlation in the HMQC spectrum). The ^{13}C NMR (Table 2) and HMBC (Table 3) spectra indicated the five-membered ring D to have a 14β,15β-epoxide by the cross-peak of H-15 (δ 3.62) with C-17 (δ 40.9), C-14 (δ 72.6); H-17 (δ 2.62) with C-20 (δ 122.9), C-21 (δ 140.1) and 3H-18 (δ 0.99) with C-17 and C-14. In the same way, the unsubstituted C-12 emerged from the correlation between the H_3-18 signal and the ^{13}C signal at δ 43.5 (^3J, C-12), which showed one-bond correlation with the ^{1}H signals at δ 1.56 and 2.37, ascribed to H-12a and H-12b, respectively. A second methyl proton at δ 1.20 was attributed to H_3-30 by its correlation with the C-14 signal. The signal for C-9 was established as δ 53.8 (δ_H 3.08, by HMQC) by the existence of a correlation between the H_3-30 signal and this ^{13}C signal. The H_3-30 signal also showed a cross-peak with the singlet resonance at δ 74.6, requiring the presence of a tertiary hydroxyl function at C-8 or a cyclic ether. The shielded resonance observed here (δ 74.6) is not typical of a cyclic ether, when compared with sandoricin (δ 80.3, C-14) [3] and tricoccin S_7 (δ 89.5, C-8) [4], indicating a hydroxyl attached to C-8, rather than a oxygen bridge.

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Table 1. ^{1}H NMR chemical shifts for compounds **1** and **2** and selected protons in the model compound **3**

H	**1**	**2**	**3**
1	4.09 *dd* (9.5, 7.0)	4.14 *dd* (9.1, 7.0)	3.77 *dd* (11.2, 6.4)
2a	2.86 *dd* (15.5, 9.5)	3.40 *dd* (15.6, 9.1)	2.90 *dd* (12, 11.2)
2b	2.61 *dd* (15.5, 7.0)	2.48 *dd* (15.6, 7.0)	2.75 *dd* (12, 6.4)
6	3.81 *d* (4.1)	3.81 *d* (4.1)	
9	3.08 *d* (12.0)	3.07 *d* (12.0)	2.58 *d* (11.8)
11	4.14 *ddd* (12.0, 9.6, 7.2)	4.10 *dd* (12.0, 4.4)	4.14 *ddd* (11.8, 8.8, 5.8)
12a	1.56 *dd* (12.8, 9.6)	4.01 *t* (4.4)	1.79 *dd* (14, 5.8)
12b	2.37 *dd* (12.8, 7.2)		2.37 *dd* (14, 8.8)
15	3.62 *s*	3.61 *s*	
16a	1.84 *dd* (13.2, 11.1)	1.82 *dd* (13.4, 11.0)	
16b	2.09 *dd* (13.2, 6.9)	2.15 *dd* (13.4, 6.7)	
17	2.62 *dd* (11.1, 6.9)	2.65 *dd* (11.0, 6.7)	
18	0.99 *s*	0.87 *s*	
19	1.38 *s*	1.40 *s*	
21	7.45 *m*	7.39 *m*	
22	6.37 *dd* (1.7, 0.8)	6.42 *dd* (1.6, 0.6)	
23	7.57 *t* (1.7)	7.55 *t* (1.6)	
28	1.56 *s*	1.52 *s*	
30	1.20 *s*	1.20 *s*	
OH-5	6.07 *s*	5.99 *s*	
OH-6	7.03 *d* (4.1)	7.01 *d* (4.1)	
OH-8	6.41 *s*	6.32 *s*	
OH-12		5.20 *d* (4.4)	

Resonances for **1** and **2** were confirmed by ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY and for **3** by NOE. Coupling constants (Hz) in parentheses.

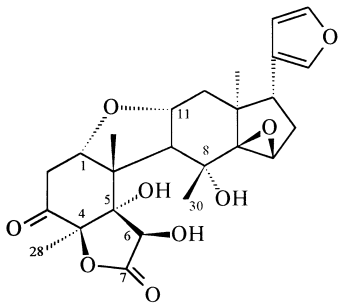
Table 2. ^{13}C NMR chemical shifts for compounds **1** and **2** and selected carbons in the model compound **3**

C	**1**	**2**	**3**
1	84.5	84.9	85.2
2	44.1	42.8	42.0
3	202.3	202.8	213.2
4	90.3	90.1	
5	83.7	83.3	
6	73.8	73.7	
7	173.0	173.0	
8	74.6	74.8	82.2
9	53.8	48.8	62.0
10	50.7	50.6	44.4
11	73.7	77.5	74.2
12	43.5	70.2	39.4
13	41.7	46.6	42.4
14	72.6	72.4	71.0
15	55.5	55.2	56.5
16	31.1	30.9	31.0
17	40.9	41.0	42.2
18	23.1	15.3	22.8
19	17.9	18.2	20.8
20	122.9	123.1	122.4
21	140.1	139.8	139.4
22	111.3	111.4	110.5
23	143.3	142.9	143.2
28	19.7	19.6	
30	22.6	22.6	21.2

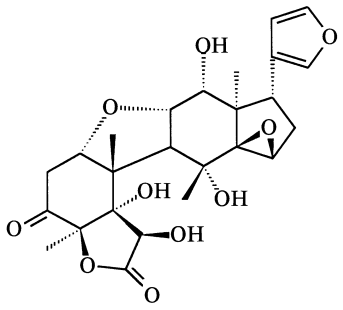
Assignments based on HMQC and HMBC for **1** and **2** and PENDANT for **3**.

Table 3. HMBC for compounds **1** and **2**

	C	
H	**1**	**2**
1	9, 19	
2a	1, 3	
2b	1, 3, 4, 10	3, 4, 10
6	4, 5	4
9	5, 10, 11, 12, 19, 30	
12b	9, 11, 17, 18	9, 17
12a	11, 14, 17	
15	16, 17	16, 17
16b	13, 14, 15	
16a	17, 20	
17	16, 18, 20, 21	13, 18, 20, 21
18	12, 14, 17	14, 17
19	1, 9, 10	1, 10
21	22, 23	22, 23
22	20, 21, 23	20, 21
23	20, 21	20, 21
28	3, 4, 5	3, 4, 5
30	8, 9, 14	8
OH-5	6	
OH-6	5	5
OH-8	9	
OH-12		13



1



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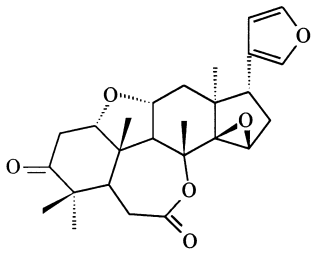

3

The third methyl proton at δ 1.38 showed long-range correlation with the C-9 signal (δ 53.8) and the ^{13}C signal at δ 84.5 (CH), permitting the assignments of these signals to H_3-19 and C-1 (δ_H 4.09 by HMQC), respectively. A second oxymethine proton at δ 4.14 showed one-bond correlation with the ^{13}C signal at δ 73.7 (CH) and was coupled to the ^{1}H signals for H-9, H-12a and H-12b, thus allowing the assignments of these signals to H-11 and C-11, respectively. These correlations resulted in the construction of a cyclic ether between O-1 and O-11, which agreed closely with published data for toonafolin (δ_H 3.77 *dd*, J = 11.2 and 6.4 Hz, H-1, δ_C 85.2, C-1; δ_H 4.14 *ddd*, J = 11.8, 8.8 and 5.8 Hz, δ_C 74.2, C-11, **3**) [5]. Orientation of the cyclic ether followed from the large coupling between H-1 (δ 4.09 *dd*, J = 9.5 and 7.0 Hz) and H-2 axial (δ 2.86 *dd*, J = 15.5 and 9.5 Hz) and between H-11 (δ 4.14 *ddd*, J = 12.0, 9.6 and 7.2 Hz) and H-9α (δ 3.08 *d*, J = 12.0 Hz), which require the oxide ring to be α, as in toonafolin (**3**). Moreover, the existence of correlation between the ^{1}H signals at δ 2.86 and 2.61, assigned to H_2-2, and the ^{13}C signal at δ 202.3 determined a 3-oxo limonoid. These observations and the absence of an exocyclic methylene, indicated that we were not dealing with the more usual B-ring *seco*-limonoid.

A carbonyl resonance at δ 173.0 and a singlet resonance at δ 90.3 were also observed in the ^{13}C NMR spectrum (Table 2) and suggested the presence of a 4–7 lactone ring, since it was the only location left in the nucleus. This implies that compound **1** has only one methyl group at C-4. This was supported by the HMBC experiments, which showed relationship of the H_3-28 signal at δ 1.56 to the C-3 signal at δ 202.3 (^3J) and to the C-4 signal at δ 90.3 (^2J). A hydroxyl must also be connected at C-5 due to the observed correlation between the H_3-28 signal and the ^{13}C signal at δ 83.7. Thus, the fourth oxymethine proton at δ 3.81 (1H, *d*, J = 4.1 Hz) can be attributed to H-6, since it was coupled only to the ^{1}H signal of a hydroxyl group at δ 7.03 (1H, *d*, J = 4.1 Hz) and both signals also showed cross-peaks with the C-5 signal (δ 83.7). The H-6 signal also showed cross-peaks with the C-4 signal (δ 90.3). The relationship of the ^{1}H signal of a hydroxyl at δ 6.07 to the C-6 signal as well as of the ^{1}H signal of a second hydroxyl at δ 6.41 to the C-9 signal led to their assignments as OH-5 and OH-8, respectively.

A model shows that, in compound **1**, ring A is nearer to a chair conformation, in which H-1 and H_3-19 are eclipsed. This was supported by NOESY experiments (Table 4), which showed correlations of the signal of H-1β (δ 4.09) with the signals of H-2β (δ 2.61) and H_3-19 (δ 1.38). Moreover, the existence of a correlation from H_3-19 to H-11 (δ 4.14) confirmed that H-11 is in the β- and the oxide ring in the α-configuration. In addition, the signal of H-2α (δ 2.86) showed cross-peaks with the signal of H_3-28 (δ 1.56), suggesting a spatial proximity of H-2α to H_3-28, which requires the methyl group at C-4 to be in the α-configuration. Thus, the correlations of 5-OH with H_3-28 (α) and H-6, required 5-OH and H-6 on the α-side of the molecule. Moreover, the relationship of 8-OH signal to the H-9, H_3-18 (α) and H-15 signals, indicated

Table 4. NOESY for compounds **1** and **2**

	H	
H	**1**	**2**
1	2b, 19	19
2a	28	
6	19	19
9	18, 28	28
11	12b, 19	19
12b	12a	17
12a	18	
15	16a	30
16b	16a	
16a	18	16b
17	16a, 16b	
21	16a, 17, 18	
22	16a, 17, 18	18
23	22	22
OH-5	6, 28, 30	28, 30
OH-6	6	6, 19
OH-8	9, 15, 18, 28, 30	28, 30

that 8-OH is also in the α-configuration. All these correlations require ring C to be in a boat conformation. These conclusions were supported by several NOEDIFF experiments (Table 5).

The PIDCI mass spectrum showed ions at *m/z* 475 $[M+H]^+$, 457 $[M+H-H_2O]^+$ and 383 (base peak) $[M+H-H_2O-C_2H_2O_3$ (6–7 lactone)$]^+$, confirming the cyclic ether function, the absence of a methyl group, the 4–7 lactone ring, and thus the molecular formula ($C_{25}H_{30}O_9$). This information along with the low field position of the C-4 resonance (δ 90.3) and the lactone vibration frequency at 1797 cm^{-1}, were in agreement with the presence of a γ-lactone ring similar to dregeanin ($\delta_{C\text{-}4}$ 88.6, IR band for γ-lactone 1787 cm^{-1}) [6, 7]. Based in the above evidence we proposed structure **1** for this limonoid.

Table 5. NOEDIFF for compound **1**

Irradiated proton	Observed NOE
Me-18	9, 21, 22, 8-OH
Me-19	1, 6, 11, 5-OH, 6-OH
Me-28	2a, 9, 5-OH
Me-30	5-OH, 8-OH
1	19
2a	28
2b	1
6	19, 6-OH
9	18, 28, 8-OH
11	12b, 19
12a	12b
12b	11, 12a
15	16a
16a	15, 16b, 18, 21, 22
16b	16a, 21, 22
17	21, 22
21	17, 16a, 16b
22	23
23	22
5-OH	6, 28, 30
6-OH	6, 19
8-OH	28, 30

The second limonoid showed spectral characteristics (Tables 1–3) close to those of **1**, except for the presence of an additional signal of a proton attached to a carbon adjacent to an oxygen atom (δ_H 4.01, *t*, $J = 4.4$ Hz; δ_C 70.2). The ^1H–^1H COSY spectrum, in addition to correlation between H-9 and H-11, revealed H-11 (δ 4.10, *dd*, $J = 12.0$ and 4.4 Hz) to be coupled to an oxymethine proton instead of coupling to methylene protons, so placing the hydroxyl substituent at C-12. This was supported by the HMBC experiments with **2** (Table 3), which clearly showed correlations from H-12 (δ 4.01) to the C-17 signal at δ 41.0 and C-9 signal at δ 48.8. The α orientation of the OH-12 was revealed by the small coupling of 4.4 Hz between H-11β and H-12β, as in toonacilin (H-12, δ 5.33 *d*, $J = 4.4$ Hz; H-11, δ 5.35 *d*, $J = 4.4$ Hz) [2]. The NOESY (Table 4) showed correlation of H-12 with H-17, confirming H-12 on the β-side and OH-12 on the α-side of the molecule. The structure of this limonoid was thus established as **2**.

This appears to be the first record of 4–7 lactone limonoids from the *Toona*, a feature typical of *Trichilia* limonoids [1, 2]. Thus the range of limonoids found in *Toona* is still rather typical of the Melioideae [1, 2].

EXPERIMENTAL

NMR: on a Bruker DRX 400, with TMS as int. standard; GC-MS: low resolution on a HP-2576 instrument; ESI-MS, DCI-MS: low resolution on a VG Plataform II (Fisons) instrument; $[\alpha]_D$: Perkin Elmer 241 instrument; IR: Bomen—FT/IR instrument.

Plant material

T. ciliata var. *australis* was collected in Viçosa, M.G., Brazil, and a voucher (DEF 1025) is deposited in the Herbarium of the Departamento de Engenharia Florestal, Universidade Federal de Viçosa, Viçosa, M.G.

Isolation of compounds

The seeds were dried, powdered and extracted with hexane, then CH_2Cl_2 and finally with MeOH. The hexane extract (56 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. 1 was flash chromatographed on silica gel eluting with hexane–EtOAc–MeOH (17:4:1) affording a mixture of sterols

and 3 new frs. The mixture of sterols was analysed by GC mass spectrometry, which established that the sterols were sitosterol, stigmasterol and campesterol. Frs 1–3 were purified by prep. TLC (silica gel; hexane–EtOAc–MeOH, 20:4:1) to yield cedrelone (23 mg). The CH_2Cl_2–EtOAc fr. 2 was chromatographed on florisil eluting with hexane–CH_2Cl_2–MeOH (20:7:1) affording 2 new frs (a and b). Fr. (a) was flash chromatographed on silica gel eluting with hexane–CH_2Cl_2–MeOH (20:7:1) affording a new fr. containing toonacilin (15 mg). The latter was then purified by prep. TLC (silica gel; hexane–CH_2Cl_2–MeOH, 20:7:1). Fr. (b) was twice flash chromatographed on silica gel (hexane–CH_2Cl_2–MeOH, 40:5:1; hexane–CH_2Cl_2–MeOH, 20:3:1) yielding a mixture of sterols, which was analysed as above to give sitosterol, stigmasterol and campesterol. The MeOH extract (64 g) was suspended in MeOH–H_2O (1:3) and partitioned with CH_2Cl_2. The CH_2Cl_2 fr. was concd and then partitioned with hexane–MeOH. The MeOH fr. was flash chromatographed on silica gel eluting with $CHCl_3$–MeOH (24:1) affording 3*β*-*O*-*β*-D-glucopyranosylsitosterol and 2 new frs. Fr. 1 was rechromatographed as above to give **1** (30 mg). Fr. 2 was twice flash rechromatographed as above to give **2** (15 mg).

5α,6*β*,8α-*trihydroxy*-28-*norisotoonafolin* (**1**). Plates, mp 331–333°, $[\alpha]_D$ +152.5° (DMSO; *c* 0.0051). IR ν_{max}^{KBr} cm^{-1}: 3276, 1797, 1735, 873, 826. ^1H NMR (400 MHz, DMSO-d_6): see Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): see Table 2; HMBC (400/100 MHz, DMSO-d_6): see Table 3; NOESY-TPPI (400 MHz, DMSO-d_6): see Table 4; NOEDIFF (400 MHz, DMSO-d_6): see Table 5. PI-DCI-MS *m*/*z* (rel. int.): 475 $[M+H]^+$ (50), 457 $[M+H-H_2O]^+$ (15), 383 $[M+H-H_2O-C_2H_2O_3]^+$ (100), 85 (90).

5α,6*β*,8α,12α-*tetrahydroxy*-28-*norisotoonafolin* (**2**). Plates, mp 329–332°, $[\alpha]_D$ +121.4° (DMSO; *c* 0.0063). IR ν_{max}^{KBr} cm^{-1}: 3250, 1791, 1731, 874, 827. ^1H NMR (400 MHz, DMSO-d_6): see Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): see Table 2; HMBC (400/100 MHz, DMSO-d_6): see Table 3; NOESY-TPPI (400 MHz, DMSO-d_6): see Table 4. PI-ESI-MS *m*/*z* (rel. int.): 513 $[M+Na]^+$ (100); NI-ESI-MS *m*/*z* (rel. int.): 489 $[M-H]^-$ (100).

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