Pergamon

0031-9422(95)00198-0

Phytochemistry, Vol. 40, No. 1, pp. 239–243, 1995

0031 9422/95 $9.50 + 0.00

LIMONOIDS FROM *TURRAEA FLORIBUNDA*

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(*Received in revised form* 28 *December* 1994)

Key Word Index—*Turraea floribunda*; Meliaceae; root bark; limonoids; nortriterpenoids.

Abstract—Extracts of the root bark of *Turraea floribunda* have yielded four new limonoids of the havanensin class: 28-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1,7-diacetate; 28-nor-4α-carbomethoxy-11β-hydroxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate; 18-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate; and 28-nor-4α-carbomethoxy-7-deoxy-7-oxo-11β-acetoxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate. The structures were determined by spectroscopic methods. The structure for the first of these compounds was also confirmed by X-ray diffraction methods.

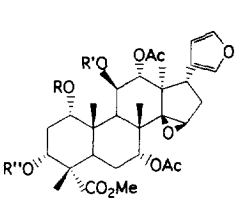
INTRODUCTION

Turraea floribunda (Meliaceae) is a shrub distributed throughout East Africa. In traditional medicine, the bark is used as an emetic, while both the root and the bark are used as a purgative [1]. In an earlier study [2], an isohexane extract of the bark yielded three limonoids (**1–3**) of the havanensin class [3]. These may be viewed as intermediates on the pathway to the B-ring cleaved prieurianins found in the related *T. obtusifolia* [2] and *T. mombasana* [4]. Recently, three limonoids in the prieurianin class have been isolated from the seeds of *T. floribunda* [5]. In continuing our studies of limonoids in this genus [4, 6], we have further explored the chemistry of *T. floribunda* and here report the identification of four new havanensin-type limonoids from the root bark of this plant.

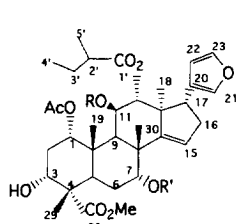
RESULTS AND DISCUSSION

The methanol extract of the air-dried ground rootbark of *T. floribunda* was partitioned between chloroform and water. After various chromatographic steps, the chloroform fraction afforded four new limonoids, **4–7** (Figure 1). ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) are consistent with the assignment of these compounds to the havenensin group of limonoids related to those reported earlier from this plant, but differing mainly in the presence of a 14, 15 double bond rather than an epoxide.

Compound **4** had a molecular formula of $C_{38}H_{52}O_{12}$ (FAB HR-mass spectrometry). ^{1}H and ^{13}C NMR spectra indicated the presence of a double bond, a β-substituted furan, a carbomethoxyl group, three acetate functions, a 2-methyl butanoate residue and four quaternary methyl groups. The mass spectrum displayed fragment peaks corresponding to loss of water, acetic acid, and a C-5 carboxylic acid. Placement of the OH group and the esters was accomplished using ^{1}H COSY and NOESY experiments and X-ray diffraction. COSY correlations of H-1 with H-2 and of H-3 with H-2 established the 1,3-substitution pattern of the A-ring. The proton on the carbon bonded to the OH group absorbed at 3.78 ppm and appeared as a doublet of triplets (10.4, 3 Hz). NOESY correlation of this proton with the 29-Me allowed assignment of the 3α-OH and confirmed the β-stereochemistry of the 3-H indicated by its coupling constant. The coupling constant of the H-1 triplet (3 Hz) as well as its NOESY correlation with 19-Me indicated



1 R=Ac, R'=H, R''=H
2 R=Ac, R'=isobutyrate, R''=H
3 R=H, R'=isobutyrate, R''=Ac



4 R=Ac, R'=Ac
5 R=H, R'=H
6 R=Ac, R'=H

*Author to whom correspondence should be addressed.

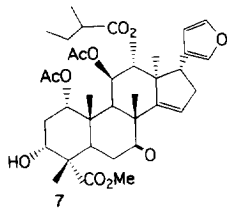


Fig. 1.

an ester at C-1 with the commonly observed α-stereochemistry. An ester was placed at C-7 based on the COSY correlation of H-7 with H-6 and H-6 with H-5. The ester stereochemistry was deduced to be α from the splitting pattern of H-7 (*t*, 3.0 Hz) and a NOESY correlation with 30-Me. COSY correlation of H-9 (2.89 ppm, *d*, 4.4 Hz) with H-11 (5.04 ppm, *m*) and of H-12 (5.13 ppm, *d*, 3.3 Hz) with H-11 established the presence of esters at C-11 and C-12. NOESY correlations between H-17 and H-12 and between H-9 and H-11 demonstrated the 12-ester to be α and the 11-ester to be β. Since we were unable to unambiguously place the 2-methylbutanoate with NMR alone, the structure determination was completed by single crystal X-ray diffraction of **4**, leading to the structure presented in Figure 2.

The ^{1}H and ^{13}C NMR spectra of **5** ($C_{34}H_{48}O_{10}$) were quite similar to those of **4**, with the differences being due to the replacement of two acetate functions with OH groups. Inspection of the ^{1}H COSY spectrum revealed the OH groups to be located at C-11 and C-7. The coupling constant of the H-7 triplet (3 Hz), as well as a H-7 to 30-Me NOESY correlation, proved H-7 to be equatorial and the 7-OH thus axial. A NOESY correla-

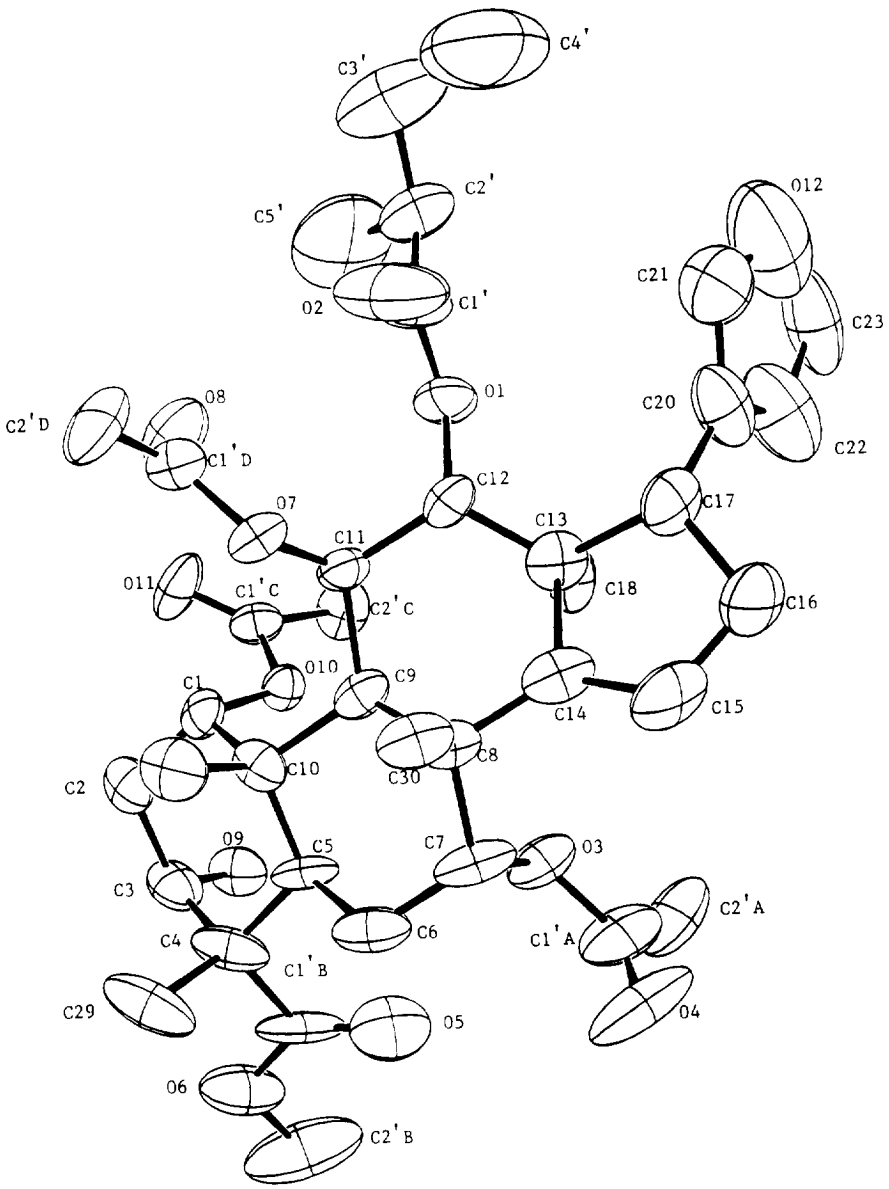


Fig. 2. X-ray diffraction structure of **4**.

Table 1. ^{1}H NMR spectral data of limonoids 4–7[a]

Proton	4	5	6	7
H-1	4.84 *t* (3.0)	5.12 *t* (3.0)	4.80 *t* (3.0)	4.98 *t* (3.0)
2	2.10–2.18 *m*	2.01–2.26 *m*	2.10–2.16 *m*	2.18–2.22 *m*
3	3.78 *dd* (10.4, 3.0)	3.78 *dd* (10.2, 3.0)	3.78 *dd* (10.4, 3.0)	3.84 *dd* (10.0, 3.0)
5	2.93 *d* (4.4)	3.03 *d* (4.4)	3.30 *dd* (12.0, 2.2)	2.80 *dd* (2.7, 2.7)
6	1.88–2.08 *m*	1.88–2.08 *m*	1.88–1.96 *m*	2.52 *m*
7	5.06 *t* (3.0)	3.92 *m*	3.94 *m*	NA
9	2.89 *d* (4.4)	2.65 *d* (4.4)	2.97 *d* (4.7)	2.90 *d* (3.8)
11	5.04 *m*	3.60 *m*	5.04 *m*	5.08 *m*
12	5.13 *d* (3.3)	4.70 *d* (3.3)	5.14 *d* (3.6)	5.15 *d* (3.0)
15	5.65 *t* (3.0)	5.79 *t* (3.0)	5.79 *t* (2.1)	6.23 *t* (3.0)
16	2.30–2.40 *m*	2.36–2.52 *m*	2.48–2.53 *m*	2.45–2.58 *m*
17	3.12 *m*	3.05 *m*	3.09 *m*	3.10 *m*
18	1.11 *s*	1.07 *s*	1.10 *s*	1.08 *s*
19	1.20 *s*	1.27 *s*	1.24 *s*	1.33 *s*
21	7.15 *m*	7.20 *m*	7.16 *m*	7.15 *m*
22	6.24 *m*	6.28 *m*	6.26 *m*	6.26 *m*
23	7.32 *m*	7.35 *m*	7.31 *m*	7.31 *m*
29	1.16 *s*	1.27 *s*	1.13 *s*	1.27 *s*
30	1.47 *s*	1.44 *s*	1.42 *s*	1.63 *s*
2′	2.16 *m*	2.09 *m*	2.09 *m*	2.21 *m*
3′	1.25–1.54 *m*	1.25–1.54 *m*	1.25–1.54 *m*	1.23–1.54 *m*
4′	0.85 *t* (7.2)	0.89 *t* (7.2)	0.85 *t* (7.5)	0.83 *d* (7.2)
5′	0.93 *d* (7.2)	0.96 *d* (7.2)	0.93 *d* (7.0)	0.94 *d* (7.0)
Ac (1-)	2.10 *s*	1.99 *s*	2.07 *s*	2.06 *s*
Ac (3-)	NA	NA	NA	NA
Ac (7-)	2.15 *s*	NA	NA	NA
Ac (11-)	2.02 *s*	NA	2.01 *s*	2.06 *s*
COOMe	3.66 *s*	3.71 *s*	3.71 *s*	3.73 *s*
OH (3-)	2.64 *br d* (10.2)	2.80 *br d* (10.2)	2.72 *br d* (10.2)	2.52 *br d* (10.0)
OH (7-)	NA	3.52 *t* (3.0)	NA	NA

[a]Recorded at 400 MHz in $CDCl_3$ σ (ppm), multiplicity (*J*, Hz).
NA – not applicable.

tion between H-9 and H-11 demonstrated the β stereochemistry of the 11-OH.

Compound **6** ($C_{36}H_{50}O_{11}$) also displayed very similar spectral characteristics to those of **4**, with the differences being due to the replacement of an acetate with an OH. COSY correlations allowed assignment of this OH to the 7-position and the coupling constant of 7-H to 6-H (*t*, 3 Hz). In addition an H-7 to 30-Me NOESY correlation, established α stereochemistry for 7-OH.

The NMR spectra of **7** ($C_{36}H_{48}O_{11}$) were similar to those of **6**, with major differences being the absence of an H-7 absorption in **7**, a shift of the C-7 absorption from 74.4 ppm in **6** to 209 ppm in **7**, a shift of H-15 from 5.79 ppm in **6** to 6.23 ppm in **7**, and a shift of the 30-Me from 1.42 ppm in **6** to 1.63 ppm in **7**. All of these observations were consistent with the presence of a 7-keto group in **7**.

The limonoids reported here are very similar to those reported from bark extracts by Taylor and co-workers [2], differing mainly in the lack of an epoxide functionality at C-14–C-15, and they thus have a close biosynthetic relationship. The possibility cannot be ruled out that one or more of these compounds related by deacetylation is an artefact produced in the methanol isolation procedure. They are, however, closely related to those isolated by Taylor and co-workers *via* isohexane extraction where loss of esters should not be a problem. Furthermore, the presence of a 7-keto function in **7** is consistent with 7-hydroxy intermediates and is not a likely functional group to have been produced in the isolation procedure.

EXPERIMENTAL

Plant material. The root bark of *T. floribunda* was collected in May, 1989, from Kwale National Park in the Longomagadi Forest in Kenya. The plant was identified by Mr. S. G. Mathenge (Department of Botany herbarium, University of Nairobi) and a voucher specimen (89/401) is deposited in that department.

Extraction and isolation. T. floribunda root bark (0.9 kg) was air-dried, powdered and allowed to stand in MeOH at room temperature for 3 weeks. The extract was filtered and evaporated to dryness under vacuum to yield an oil (84 g). A portion of the oil (22 g) was partitioned between water and $CHCl_3$. The $CHCl_3$ phase was evaporated and the residue chromatographed on silica gel (230–400 mesh) using a hexane–EtOAc gradient. Further fractionation by column chromatography on silica gel using 10% acetone–toluene followed by preparative HPLC using a C-18 column (Waters Resolve C-18

Table 2. ^{13}C absorptions for compounds 4–7[a,b]

Carbon	4	5	6	7
1	74.36 (**1**)[b]	74.77 (1)	73.89 (1)	72.98 (1)
2	27.03 (2)	26.10 (2)	27.18 (2)	26.79 (2)
3	73.34 (1)	73.53 (1)	73.31 (1)	73.99 (1)
4	41.68 (0)	41.37 (0)	41.01 (0)	37.83 (0)
5	40.42 (1)	39.66 (1)	39.16 (1)	38.11 (1)
6	25.99 (2)	27.13 (2)	26.95 (2)	26.85 (2)
7	74.45 (1)	73.95 (1)	74.42 (1)	208.98 (0)
8	50.63 (0)	50.11 (0)	50.62 (0)	51.29 (0)
9	41.28 (1)	41.21 (1)	41.26 (1)	41.18 (1)
10	40.77 (0)	43.94 (0)	43.91 (0)	40.38 (0)
11	76.48 (1)	74.71 (1)	74.56 (1)	74.49 (1)
12	84.02 (1)	91.16 (1)	83.78 (1)	83.73 (1)
13	51.51 (0)	51.44 (0)	51.43 (0)	51.75 (0)
14	154.16 (0)	157.35 (0)	156.39 (0)	147.50 (0)
15	123.74 (1)	123.69 (1)	124.21 (1)	130.54 (1)
16	37.26 (2)	36.86 (2)	37.33 (2)	37.66 (2)
17	50.89 (1)	51.57 (1)	50.75 (1)	50.94 (1)
18	16.59 (3)	16.57 (3)	16.79 (3)	16.79 (3)
19	17.15 (3)	17.16 (3)	17.19 (3)	16.72 (3)
20	123.94 (0)	124.15 (0)	123.86 (0)	123.86 (0)
21	140.29 (1)	140.18 (1)	140.29 (1)	140.32 (1)
22	111.41 (1)	111.63 (1)	111.39 (1)	111.45 (1)
23	142.06 (1)	142.07 (1)	142.13 (1)	142.03 (1)
28	169.11 (0)	169.21 (0)	169.13 (0)	169.04 (0)
29	17.35 (3)	18.09 (3)	17.38 (3)	17.27 (3)
30	28.17 (3)	28.59 (3)	28.29 (3)	29.73 (3)
1′	168.42 (0)[c]	169.21 (0)[c]	168.61 (0)[c]	168.33 (0)[c]
2′	32.29 (1)	31.12 (1)	31.21 (1)	44.43 (1)
3′	25.66 (2)	27.59 (2)	25.91 (2)	25.93 (2)
4′	11.85 (3)	11.88 (3)	11.82 (3)	11.76 (3)
5′	15.71 (3)	16.53 (3)	15.61 (3)	15.62 (3)
Ac (Me)	21.45 (3)	21.50 (3)	21.43 (3)	21.59 (3)
Ac (Me)	21.57 (3)	NA	21.68 (3)	21.38 (3)
Ac (Me)	21.44 (3)	NA	NA	NA
Ac (CO)	175.27 (0)[c]	175.50 (0)[c]	175.43 (0)[c]	175.21 (0)[c]
Ac (CO)	175.02 (0)[c]	NA	175.39 (0)[c]	174.67 (0)[c]
Ac (CO)	170.06 (0)[c]	NA	NA	NA
COOMe	52.06 (3)	52.00 (3)	52.13 (3)	52.33 (3)

[a]Recorded at 100.57 MHz in $CDCl_3$.

[b]The numbers in parenthesis indicate number of attached protons as determined by APT.

[c]Assignments with this superscript in same column may be reversed.

NA – Not applicable.

Radial Pak, 25 × 10 cm, 15u) eluted with 65% acetonitrile-water gave pure **4** as colourless crystals from hexane-ethyl acetate (10 mg). Preparative silica gel TLC (toluene–EtOAc, 1:1) of other HPLC fractions yielded **5** as a powder (3 mg) and **7** (2.5 mg) as a powder. Further HPLC (75% MeCN–H_2O) of a fraction from the first column chromatography experiment led to the isolation of **6** (7 mg) as a powder from hexane-ethyl acetate.

Compound **4**. 28-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1,7-diacetate, mp 216–217 °; $(M + Li)^+$ (FAB HR-MS) 707.3594, calc. for $C_{38}H_{52}O_{12}Li$ 707.3619; EIMS, *m/z* (rel. int.): 700 (45%) $[M]^+$, 682 (6%) $[M-H_2O]^+$, 640 (100%) $[M-AcOH]^+$, 598 (19%) $[M-C_5H_{10}O_2]^+$, 580 (99%) $[M-2AcOH]^+$, 538 (38%) $[M-C_5H_{10}O_2 + AcOH)]^+$, 478 (13%) $[M-C_5H_{10}O_2 + 2AcOH]^+$ and 418 (19%) $[M-(C_5H_{10}O_2 + 3AcOH)]^+$; ^1H NMR: Table 1; ^{13}C NMR Table 2. X-ray diffraction: compound **4** crystallized from 65% acetonitrile–water in the orthorhombic crystal system, space group $P2_12_12_1$ with a = 9.665(5), b = 13.70 (1), and c = 29.36(2)A, V = 3888A^3, Z = 4, $C_{38}H_{52}O_{12}$, M_r 700.83, Dc = 1.197 gcm^{-3}, u(MoKa) = 0.8 cm^{-1}. Crystal dimensions: 0.75 × 0.34 × 0.17 mm. A total of 3868 independent reflections were measured on a Syntex $P2_1$ diffractometer, 1359 reflections with $|> 2.5\ \sigma$ (I) were used in the full-matrix refinement (NRCVAX). The structure was solved using the direct methods function of SHELXS [7, 8]. Hydrogen positions were determined from a combination of difference Fourier maps and ideal position

calculation. Final R = 0.101 and Rw = 0.103. The crystallographic data is deposited at Cambridge Crystallographic Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK. Use the full literature citation when ordering material.

Compound **5**. 28-nor-4α-carbomethoxy-11β-hydroxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate, mp 120° dec.; $(M + Li)^+$ (FAB HR-MS) 623.3422, calc. for $C_{34}H_{48}O_{10}Li$ 623.3408; EIMS *m/z* (rel. int.): 616 (11%) $[M]^+$, 598 (17%) $[M-H_2O]^+$, 580 (14%) $[M-2H_2O]^+$, 556 (45%) $[M-AcOH]^+$, 538 (100%) $[M-H_2O-AcOH]^+$, 514 (37%) $[M-C_5H_{10}O_2]^+$, 454 (14%) $[M-(C_5H_{10}O_2-AcOH)]^+$; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Compound **6**. 18-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate, mp 118° dec.; $(M + Li)^+$ (FAB HR-MS) 665.3525, calc. for $C_{36}H_{50}O_{11}Li$ 665.3513; EIMS, *m/z* (rel. int.): 658 (70%) $[M]^+$, 598 (100%) $[M-AcOH]^+$, 580 (63%) $[M-2H_2O-AcOH]^+$, 556 (28%) $[M-C_5H_{10}O_2]^+$, 538 (30%) $[M-H_2O-C_5H_{10}O_2]^+$, 496 (63%) $[M-C_5H_{10}O_2-AcOH)]^+$, 436 (28%) $[M-(C_5H_{10}O_2-2AcOH)]^+$; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Compound **7**. 28-nor-4α-carbomethoxy-7-deoxy-7-oxo-11β-acetoxy-12α-(2-methylbutanoyloxy)-14,15-deoxyhavanensin-1-acetate, $(M + Li)^+$ (FAB HR-MS), 663.3345, calc. for $C_{36}H_{48}O_{11}Li$ 663.3357; EIMS *m/z* (rel. int.): 656 (20%) $[M]^-$, 554 (22%) $[M-C_5H_{10}O_2]^+$, 536 (12) $[M-(C_5H_{10}O_2 + H_2O)]^-$, 494 (100%) $[M-(C_5H_{10}O_2 + AcOH)]^-$, 434 (64%) $[M-(C_5H_{10}O_2 + 2AcOH)]^+$, 419 (40%) $[M-(C_5H_{10}O_2 + 2AcOH + CH_3)]^+$, 401 (48%) $[M-(C_5H_{10}O_2 + 2AcOH + H_2O + CH_3)]^-$, 357 (52%) $[M-(C_5H_{10}O_2 + 2AcOH + H_2O + CH_3 + CO_2)]^+$; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Acknowledgements—The authors thank Mr Edward Nyandat for plant collection and extraction.

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