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7β-Oxygenated limonoids from *Trichilia elegans* ssp. *elegans*

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Abstract

Five 7β- and 7α-oxygenated obacunone-type limonoids were obtained on reinvestigation of the seeds of *Trichilia elegans* ssp. *elegans*: 7-deoxo-7β-acetoxykihadanins A and B, 7-deoxo-7β-hydroxykihadanins A and B and 7-deoxo-7α-hydroxykihadanin A, the last three being isolated after acetylation procedures as their mono- and/or diacetate derivatives. This is the first report of the natural occurrence of C-7 β-substituted limonoids without any oxygenated function at C-6. The structures of these compounds have been established on the basis of 1D- and 2D-NMR spectral techniques, ESI-mass spectrum and X-ray crystallographic data.

Keywords: *Trichilia elegans* ssp. *elegans*; Meliaceae; Limonoids; 7-Deoxo-7β-acetoxykihadanin A; 7-Deoxo-7β-acetoxykihadanin B; 7-Deoxo-7β-hydroxykihadanin A; 7-Deoxo-7β-hydroxykihadanin B; 7-Deoxo-7α-hydroxykihadanin A; X-ray analysis

1. Introduction

In two previous papers we reported the isolation of three *seco*-A-protolimonoids and eight limonoids from the seeds and bark of *Trichilia elegans* ssp. *elegans* A. Juss. (Meliaceae) (Garcez et al., 1996, 1997).

Further investigation of the dichloromethane solubles, obtained from partition of the ethanolic extract from the seeds of that plant, has now allowed the isolation of the new limonoids 7-deoxo-7β-acetoxykihadanins A and B (**1**, **2**). 7-Deoxo-7β-hydroxykihadanins A and B (**3**, **4**) and 7-deoxo-7α-hydroxykihadanin A (**5**) are also new and shown to be constitutive components of this plant, being isolated, after acetylation procedures, as their corresponding mono- and/or diacetate derivatives. To date, the occurrence of 7β-oxygenated substituents has only been described in limonoids from Rutales bearing a concomitant oxygen function at C-6. Therefore, the β-orientation of the C-7 oxygenated groups found in **1**–**4** is being reported for the first time.

2. Results and discussion

Compound **1**, obtained as an amorphous solid, had a molecular formula of $C_{28}H_{34}O_{10}$ as determined from the pseudo-molecular ion at m/z 529 $[M\text{-}H]^-$ in the negative ESI-mass spectrum and elemental analysis. Its ^1H and ^{13}C NMR spectra (Tables 1 and 2) resembled closely those of 7-deoxo-7α-acetoxykihadanin A (**6**), a limonoid previously isolated from this plant (Garcez et al., 1997), showing that they had identical constituting rings and the same γ-hydroxybutenolide side chain at C-17. The latter was characterized in the ^1H NMR spectrum by the two broad singlets at δ6.09 (H-21) and 6.23 (H-22) and by resonances at δ162.7 (C-20), 97.6 (C-21), 123.4 (C-22) and 168.9 (C-23) in the ^{13}C NMR spectra. Differences were, however, observed in the chemical shift and ^1H NMR coupling constant of H-7 and in some resonance values of B, C and D ring carbons. The presence of the acetoxy function at C-7 was inferred by the one-proton double doublet at δ5.13 (J 10.2 and 4.5 Hz), corroborated by a ^1H–^{13}C cross-peak correlation with the carbon resonance at δ75.4 (C-7), as well as by connectivities observed in the ^1H–^1H COSY spectrum between the above mentioned signal and the two H-6

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Table 1
^{1}H NMR spectral data for compounds **1**, **2**, **8**–**11** in $CDCl_3$ (**1**, **2**, **10**, **11**: 200 MHz; **8**, **9**: 300 MHz, TMS $\delta = 0$)[a]

H	1	8	9	2	10	11
1	6.55 *d* (11.8)	6.50 *d* (11.7)	6.52 *d* (11.7)	6.49 *d* (11.9)	6.44 *d* (11.9)	6.45 *d* (11.8)
2	5.94 *d* (11.8)	5.94 *d* (11.7)	5.95 *d* (11.7)	5.90 *d* (11.9)	5.88 *d* (11.9)	5.91 *d* (11.8)
5	2.32 *dd* (12.8; 4.5)	2.30 *dd* (13.2; 4.5)	2.31 *dd* (13.3; 4.5)	2.19–2.35 *m*	2.23–2.31 *m*	2.24–2.33 *m*
6	1.74 *dt* (12.0; 4.5) [$6_{eq.}$]	1.70–1.78 *m* [$6_{eq.}$]	1.71–1.78 *m* [$6_{eq.}$]	1.75–1.87 *m*	1.77–1.88 *m*	1.77–1.85 *m*
	1.85–1.92 *m* [$6_{ax.}$]	1.80–1.91 *m* [$6_{ax.}$]	1.80–1.95 *m* [$6_{ax.}$]	[H_2-6]	[H_2-6]	[H_2-6]
7	5.13 *dd* (10.2; 4.5)	5.12 *dd* (10.8; 4.5)	5.13 *dd* (10.8; 4.4)	5.05 *br t* (7.6)	5.04 *br t* (7.5)	5.04 *br t* (8.0)
9	1.80–1.85 *m*	1.80–1.91 *m*	1.80–1.95 *m*	1.75–1.87 *m*	1.77–1.88 *m*	1.77–1.85 *m*
11	1.60–1.95 *m*	1.80–1.91 *m*	1.80–1.95 *m*	1.21–1.32 *m* [$11_{ax.}$]	1.29–1.43 *m* [$11_{ax.}$]	1.26–1.44 *m* [$11_{ax.}$]
				1.75–1.87 *m* [$11_{eq.}$]	1.77–1.88 *m* [$11_{eq.}$]	1.77–1.85 *m* [$11_{eq.}$]
12	1.85–2.14 *m*	1.70–1.91 *m*	1.71–1.91 *m*	1.75–1.87 *m* [12A]	1.77–1.88 *m* [12A]	1.77–1.85 *m* [12A]
				2.19–2.35 *m* [12B]	2.23–2.31 *m* [12B]	2.24–2.33 *m* [12B]
15	3.68 *s*	3.70 *s*	3.69 *s*	3.67 *s*	3.67 *s*	3.67 *s*
17	5.39 *br s*	5.22 *br s*	5.40 *br s*	5.44 *br s*	5.46 *t* (1.3)	5.49 *br s*
18	1.33 *s*	1.33 *s*	1.43 *s*	1.27 *s*	1.29 *s*	1.26 *s*
19	1.30 *s*	1.29 *s*	1.31 *s*	1.31 *s*	1.30 *s*	1.30 *s*
21	6.09 *br s*	6.88 *br s*	7.01 *br s*	–	–	–
22	6.23 *br s*	6.38 *br s*	6.10 *br s*	7.31 *br s*	7.32 *t* (1.3)	7.33 *d* (0.9)
23	–	–	–	6.21 *br s*	6.89 *t* (1.5)	7.02 *br s*
28	1.45 *s*	1.44 *s*	1.45 *s*	1.44 *s*	1.43 *s*	1.44 *s*
29	1.45 *s*	1.44 *s*	1.45 *s*	1.45 *s*	1.44 *s*	1.44 *s*
30	0.99 *s*	0.99 *s*	0.98 *s*	1.07 *s*	1.06 *s*	1.06 *s*
OAc	2.14 *s*	2.14 *s*	2.13 *s*	2.14 *s*	2.13 *s*	2.13 *s*
		2.19 *s*	2.15 *s*		2.16 *s*	2.15 *s*

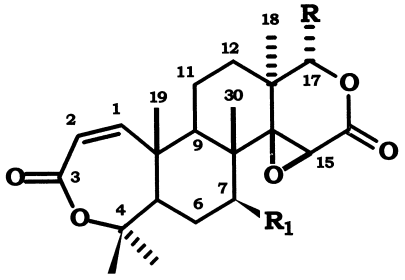
[a] Coupling constants (J in Hz) are given in parentheses. Assignments were confirmed by ^{1}H–^{1}H COSY and HMQC spectra.

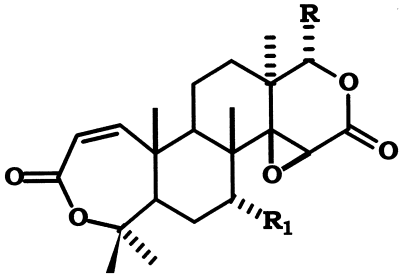
Table 2
^{13}C NMR spectral data for compounds **1**, **2**, **8**–**11** in $CDCl_3$ (**1**, **2**: 50 MHz; **8**–**11**: 75 MHz, TMS $\delta = 0$)[a]

C	1	8	9	2	10	11
1	157.7	157.3	157.5	156.7	157.2	156.6
2	122.7	122.8	121.1	121.9	122.8	122.3
3	167.5	167.2	167.3	167.4	167.9	167.4
4	84.8	84.5	84.6	84.7	85.1	84.5
5	54.7	54.6	54.8	54.2	55.0	54.5
6	30.2	30.2	30.3	29.6[b]	30.2[c]	29.8
7	75.4	75.3	75.5	76.2	76.8	76.2
8	44.3	44.2	44.3	43.8	44.4	43.9
9	48.3	48.0	48.6	47.9	48.6	48.1
10	42.9	42.9	43.0	43.4	44.0	43.4
11	19.5	19.4	19.8	18.8	19.5	19.0
12	32.3	32.3	33.6	29.5[b]	30.3[c]	29.8
13	38.9	39.0	39.4	39.1	39.8	39.3
14	67.3	67.2	67.4	68.7	69.3	68.6
15	51.0	51.1	51.3	52.3	53.1	52.4
16	165.8	165.0	164.7	166.8	166.9	166.3
17	78.5	78.1	78.4	75.7	76.3	76.0
18	21.7	21.5	21.2	20.1	20.9	20.3
19	16.3	16.2	16.4	16.3	16.9	16.3
20	162.7	160.4	158.8	133.3	134.9	134.4
21	97.6	92.3	93.6	169.5	168.9	168.8
22	123.4	124.3	123.1	151.3	149.7	149.6
23	168.9	167.6	168.3	97.5	92.7	92.8
28	32.3	32.3	32.4	32.2	32.9	32.3
29	26.9	26.9	27.1	26.8	27.3	26.8
30	12.1	12.0	12.3	12.9	13.5	12.9
OAc	170.4	170.3, 168.6	170.3, 168.4	170.4	171.1, 169.2	170.4, 168.8
	21.2	21.2, 20.7	21.2, 20.7	21.3	22.0, 21.3	21.4, 20.6

[a] Multiplicities were established from DEPT pulse sequences. Assignments were confirmed by HMQC and/or HMBC spectra.
[b,c] Interchangeable signals.

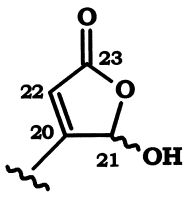
resonances, at δ1.74 (*dt, J* 12.0 and 4.5 Hz, H-6 eq.) and 1.85–1.92 (*m*, H-6 ax.). The magnitude of $J_{6,7}$ (10.2 and 4.5 Hz) was in agreement with axial–axial and axial–equatorial couplings, respectively, thus establishing the axial position of H-7 and indicating that the stereochemistry of the chiral center C-7 in **1** should be opposite to that depicted in **6**. Additional evidence for the α-configuration of H-7 in **1** was deduced from its NOESY spectrum, which revealed correlations between H-7/H_3-18 and H-17/acetate methyl protons. In limonoid **6**, a broad singlet at δ 4.52 determined the equatorial nature of H-7 and the resulting α-orientation of the C-7 acetoxy substituent. This assumption could be confirmed from the X-ray crystallographic data of **6** (Fig. 1) which led, consequently, to the establishment of the β-orientation of the same C-7 substituent in **1**. As already reported for epilimonyl and limonyl acetates, limonoids possessing identical B, C and D rings, as those depicted by, respectively, **1** and **6**, the anisotropic effect of the epoxide group accounts for the inverted order of absorption by the H-7 hydrogens observed in the NMR spectra, wherein the resonance of H-7 equatorial is found further upfield than that due to H-7 axial (Dreyer, 1965). The equatorial orientation of the C-7 acetoxy group in **1** was also responsible by the significant changes observed in the chemical shifts of the B, C and D ring carbons,



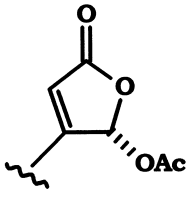


	R	R_1
1	I	OAc
2	IV	OAc
3	I	OH
4	IV	OH
8	II	OAc
9	III	OAc
10	V or VI	OAc
11	VI or V	OAc

	R	R_1
5	I	OH
6	I	OAc
7	IV	OAc



I



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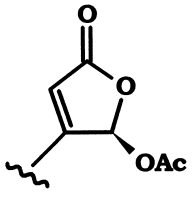

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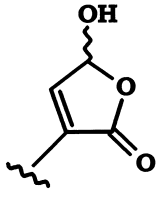

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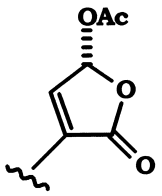
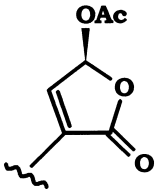


V



VI

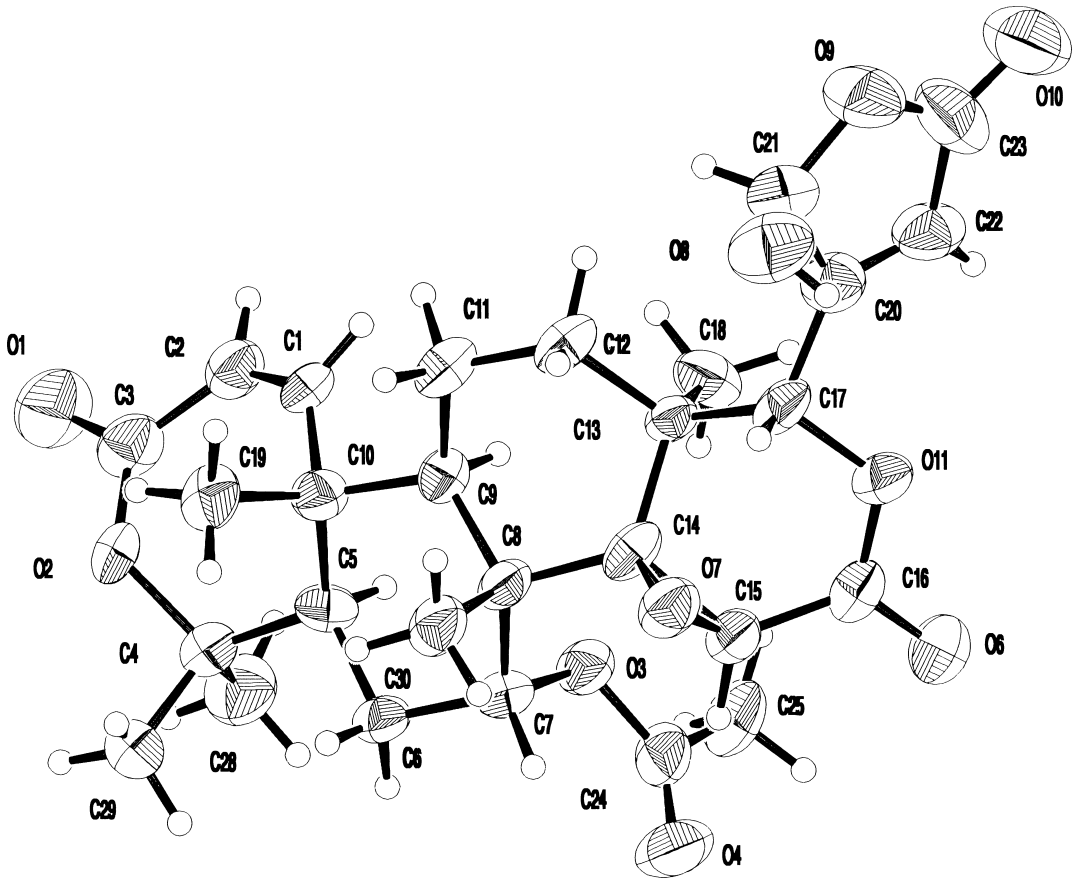


Fig. 1. ZORTEP (Zsolnai and Pritzkow, 1996) drawing of compound **6** and atom labels. Displacement ellipsoids are shown at the 50% probability level.

when compared with those of **6**. As expected, a downfield shift of the C-7 resonance was observed in **1** ($\Delta\delta = 2.8$). Likewise, the equatorial nature of the -OAc group caused a reduction of its γ effect on C-5 and C-9, which were deshielded by 5.8 and 7.3 ppm, respectively. On the other hand, the β-orientation of this substituent accounted for the γ effect observed at C-30 ($\Delta\delta = 5.7$). Further ^{13}C NMR spectral assignments were determined on the basis of HMQC and HMBC data. Thus, compound **1** was characterized as 7-deoxo-7β-acetoxykihadanin A.

The ^{1}H and ^{13}C NMR spectra of compound **2** (Tables 1 and 2) showed striking resemblance with those described earlier for 7-deoxo-7α-acetoxykihadanin B (**7**), a limonoid already shown to occur in this plant (Garcez et al., 1997). The presence of the same 23-hydroxy-20(22)-ene-γ-lactone moiety at C-17 in **2**, as that found in **7**, was inferred on the basis of two broad singlets at δ7.31 (H-22) and 6.21 (H-23) in the ^{1}H NMR spectrum and of carbon resonances at δ133.3, 169.5, 151.3 and 97.5 ascribable to, respectively, C-20, C-21, C-22 and C-23. The remainder of the ^{1}H and ^{13}C NMR spectral data, along with information obtained from ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra, were in agreement with the presence in **2** of identical constituting rings A–D to those reported for **7**. The only outstanding difference in the ^{1}H NMR spectra of **2** and **7** rested in the signal assigned to H-7, the hydrogen on the acetate-bearing carbon, which clearly suggested that these compounds differed only in the stereochemistry of C-7, as already described for limonoids **1** and **6**. The ^{1}H NMR spectrum of **2** displayed a one-proton broad triplet at δ5.05 ($J = 7.6$ Hz), indicative of an axial orientation of H-7, therefore establishing a β-configuration for the acetoxy group at C-7. The same effects attributable to changes in configuration of this acetoxy substituent on the chemical shifts of H-7, C-5, C-9 and C-30 described for compound **1** were shown to operate equally in **2**. The above mentioned data, in addition to the molecular formula $C_{28}H_{34}O_{10}$ consistent with the negative ESI-mass spectrum of **2** [m/z 529 $(M\text{-}H)^-$] and elemental analysis, defined this limonoid as 7-deoxo-7β-acetoxykihadanin B.

A complex limonoid fraction, which could not be effectively separated into individual pure compounds, was acetylated in order to facilitate further separations, after being detected the abscence of acetyl groups in its

^{1}H NMR spectrum. Semi-preparative high performance liquid chromatography (HPLC) of the resultant mixture afforded compounds **1**, **2**, **6**, **8**–**11**.

Compound **8** was shown to be one of the two possible epimeric acetate derivatives of **1**, on the basis of the following lines of evidence. Apart from the downfield shift of the signal of H-21, which was observed at δ 6.88 and of the presence of an additional methyl singlet at δ 2.19 attributable to the acetate function at C-21, no other remarkable differences were observed in the ^{1}H NMR spectrum of **8** when compared with that of **1** (Table 1). Likewise, by comparison of their ^{13}C NMR spectra (Table 2), significant shifts were only seen in the resonances of carbons 20–23, particularly an upfield shifted C-21 at δ 92.3, as a result of the presence of an acetate group at C-21 in **8**. The signals assignable to these acetoxyl carbons were observed at δ 20.7 and 168.6. The stereochemistry at C-21 was established by X-ray crystallographic analysis of **8** (Fig. 2), which also allowed the confirmation of the β-orientation for the acetoxy group at C-7 and, accordingly, in compound **1**.

Analysis of the ^{1}H and ^{13}C NMR spectra of one of the fractions obtained from the aforementioned HPLC separation revealed it to be comprised of a mixture of two limonoids, in which **8** was shown to be the minor component. The signals attributable to the hydrogens and carbons of the major constituent (**9**) showed that it was the C-21 epimer of **8**, since the main differences with respect to the ^{1}H and ^{13}C NMR data of **8** and **9** refer to the resonance values of the γ-acetoxybutenolide moiety (Tables 1 and 2). Considering that **8** and **9** were obtained following acetylation of a fraction which, originally, did not show any acetyl groups in its NMR spectra, it may be concluded that these compounds are acetate derivatives of 7-deoxo-7β-hydroxykihadanin A (**3**), another constitutive limonoid of *T. elegans* ssp. *elegans*.

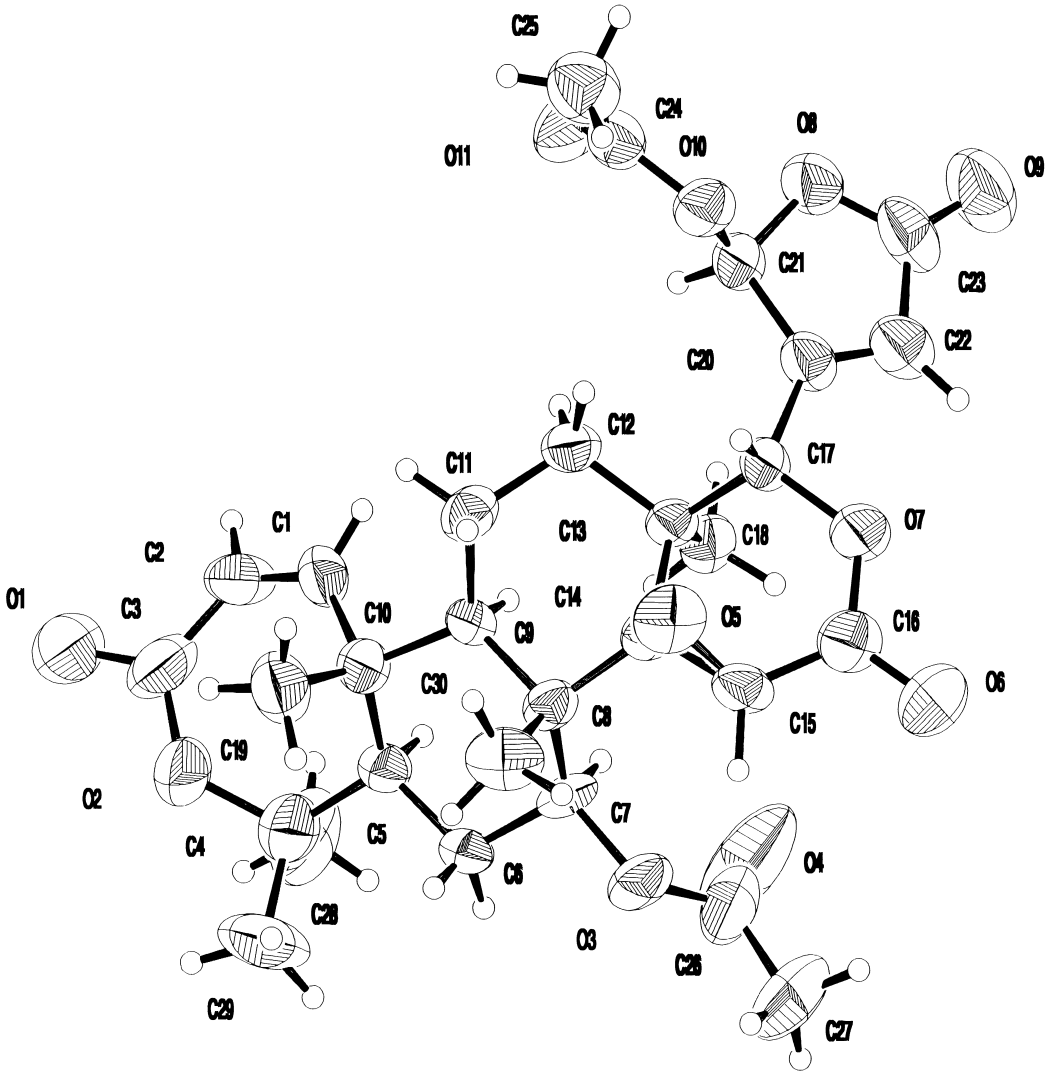


Fig. 2. ZORTEP (Zsolnai and Pritzkow, 1996) drawing of compound **8** and atom labels. Displacement ellipsoids are shown at the 50% probability level.

Therefore, the existing equilibrium between the two epimeric forms at C-21 of the γ-hydroxybutenolide ring of **3** accounts for the obtention of the limonoids **8** and **9** after acetylation procedure.

Compounds **10** and **11**, although being eluted from HPLC separations with different retention times, exhibited almost superimposable ^{1}H NMR spectra. These spectral data were in turn comparable to those of limonoid **2**, except for the presence of an additional methyl acetate singlet and a downfield shift of the signal ascribable to H-23 (δ 6.89 in **10** and δ 7.02 in **11**). These information led to the assumption that **10** and **11** were epimeric C-23 acetyl derivatives of **2**. By comparison of their ^{1}H and ^{13}C NMR spectral data with those of **2** and on the basis of the connectivities discernible from ^{1}H–^{1}H COSY experiments, complete assignment of all hydrogens and carbons of **10** and **11** was carried out (Tables 1 and 2). However, the foregoing information were insufficient to allow a conclusive evidence for determining the stereochemistry at C-23 in either **10** and **11**. These limonoids result from acetylation of 7-deoxo-7β-hydroxykihadanin B (**4**), which therefore is the genuine constitutive secondary metabolite of this plant.

Compound **6** was also isolated from the acetylated fraction above mentioned. It was readily recognized to be 7-deoxo-7α-acetoxykihadanin A (Garcez et al., 1997) on the basis of its ^{1}H and ^{13}C NMR spectral data. These results indicate that 7-deoxo-7α-hydroxykihadanin A (**5**) is, as already described for **3** and **4**, another constituent of *T. elegans* ssp. *elegans*.

The α-orientation of the C-7 acetoxy group depicted by the obacunone-type limonoids **6** and **7** isolated from *T. elegans* ssp. *elegans* (Garcez et al., 1997) is that found in all limonoids so far described in Rutales which bear no oxygenated groups at C-6. The presence of a carbonyl group at this position, however, makes possible an epimerization at C-7, thus leading to limonoids with C-7 β-oriented groups, like those isolated from members of Rutaceae and Simaroubaceae, e.g. *Atalantia monophylla, Evodia glauca, Spathelia sorbifolia* and *Harrisonia abyssinica* (Burke et al., 1972, Sabata et al., 1977; Okorie, 1982; Nakatani et al., 1988). Some synthetic derivatives of 7-keto limonoids also bear a β-orientation of the C-7 substituent, like epilimonol, a β-hydroxy compound obtained by borohydride reduction of limonin (Melera et al., 1957). The aforementioned α-configuration of the C-7 oxygenated substituent is in accordance with the generally accepted biogenetic route for all limonoid structural types, whose carbocyclic skeleton is thought to arise from hypothetical *apo*-tirucallol or *apo*-euphol precursors (Dreyer, 1984; Silva and Gottlieb, 1987) from which C-7 β-oriented hydroxyl limonoid derivatives cannot originate, hence requiring another biogenetic pathway. Therefore, the presence of compounds **1**–**4** in the seeds of *T. elegans* ssp. *elegans* is noteworthy for its biogenetic significance, since it represents the first report of naturally occurring C-7 β-substituted limonoids without any oxygenated function at C-6.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC-200 and DPX-300 spectrometers, with TMS as internal standard. HMQC, HMBC and NOESY experiments were performed at 300 MHz on a Bruker DPX-300 instrument. Standard pulse sequences were used for homo- and heteronuclear correlation experiments. ESI-mass spectrum data were obtained on a Micromass Platform II single quadrupole mass spectrometer operating in the negative ion mode. The samples were introduced into the electrospray source by a loop injection method. Silica gel 60 230–400 mesh was used for flash CC and Sephadex LH-20 for molecular exclusion chromatography. Semiprep. HPLC was performed using RP 18 (25×250 mm, 5 and 7 μm particle sizes) columns, with a flow rate of 10–16 ml min^{-1} and monitoring at 250 nm.

3.2. Plant material

Information concerning the source, identification and voucher number of the botanical material have been reported previously (Garcez et al., 1997).

3.3. Extraction and isolation procedures

As already described (Garcez et al., 1996, 1997), the ethanolic extract of *T. elegans* ssp. *elegans* seeds (225 g) was partitioned between petrol and EtOH–H_2O (1:1) and the aqueous EtOH solution further partitioned with CH_2Cl_2. One of the fractions eluted with EtOAc during silica gel CC of the CH_2Cl_2 phase was initially subjected to CC over Sephadex LH-20 in a gradient solvent system [hexane–CH_2Cl_2 (1:4) followed by CH_2Cl_2–Me_2CO (3:2)] to give six main fractions. Frs. 4 and 5 yielded, after HPLC separations [CH_3CN–MeOH–H_2O (11.5:38.5:50) and (13.5:44.5:42), respectively], **1** (28.0 mg) and **2** (27.0 mg).

A previous fraction named F (Garcez et al., 1997) had proven difficult to purify on subsequent chromatography. In the present work, however, its components could be separated after acetylation of the crude mixture in Ac_2O (0.5 ml) and pyridine (0.5 ml) overnight at room temperature, followed by HPLC [CH_3CN–MeOH–H_2O (13.5:44.5:42)] to afford **8** (13.5 mg), **9** + **8** (2.0 mg), **10** (8.0 mg), **11** (3.5 mg), **6** (0.9 mg) and further amounts of **1** (2.5 mg) and **2** (2.5 mg).

3.4. 7-Deoxo-7β-acetoxykihadanin A (*1*)

Found: C, 63.5; H, 6.3. $C_{28}H_{34}O_{10}$ requires: C, 63.4; H, 6.4). Colorless amorphous solid; $[\alpha]_D^{23}$: +19.8° ($CHCl_3$; *c* 0.092). IR ν_{max}^{KBr} cm^{-1}: 3253, 1735, 1698, 1271, 1236, 1121, 1045. ESI-mass spectrum (negative) *m/z* (rel. int.): 529 $[M\text{-}H]^-$ (100). ^1H and ^{13}C NMR (Tables 1 and 2, respectively).

3.5. 7-Deoxo-7β-acetoxykihadanin B (*2*)

Found: C, 63.5; H, 6.3. $C_{28}H_{34}O_{10}$ requires: C, 63.4; H, 6.4). Colorless amorphous solid; $[\alpha]_D^{24}$: +70.7° ($CHCl_3$; *c* 0.18). IR ν_{max}^{KBr} cm^{-1}: 3240, 1749, 1699, 1234, 1121, 1034;. ESI-mass spectrum (negative) *m/z* (rel. int.): 529 $[M\text{-}H]^-$ (100). ^1H- and ^{13}C NMR (Tables 1 and 2, respectively).

3.6. 7-Deoxo-7β-acetoxykihadanin A-21 S-acetate (*8*)

Colorless needle-like crystals from EtOH; mp. 240°C (dec.). $[\alpha]_D^{23}$: +32.6° ($CHCl_3$; *c* 0.23). IR ν_{max}^{KBr} cm^{-1}: 1757, 1700, 1270, 1235, 1218, 1045. ESI-mass spectrum (negative) *m/z* (rel. int.): 607 $[M\text{-}H + 2H_2O]^-$ (100) ^1H and ^{13}CNMR (Tables 1 and 2, respectively).

3.7. 7-Deoxo-7β-acetoxykihadanin A-21R-acetate (*9*)

Obtained as a mixture with **8**. ^1H and ^{13}C NMR (Tables 1 and 2, respectively).

3.8. 7-Deoxo-7β-acetoxykihadanin B-23ξ-acetate (*10*)

Colorless amorphous solid; $[\alpha]_D^{23}$: +69.6° ($CHCl_3$; *c* 0.80). IR ν_{max}^{KBr} cm^{-1}: 1771, 1750, 1697, 1277, 1220, 1201, 1026. ESI-mass spectrum (negative) *m/z* (rel. int.): 607 $[M\text{-}H + 2H_2O]^-$ (40). ^1H and ^{13}C NMR (Tables 1 and 2, respectively).

3.9. 7-Deoxo-7β-acetoxykihadanin B-23ξ-acetate (*11*)

Colorless amorphous solid; $[\alpha]_D^{23}$: +28.3° ($CHCl_3$; *c* 0.34). IR ν_{max}^{KBr} cm^{-1}: 1771, 1749, 1699, 1220, 1201, 1037. ESI-mass spectrum (negative) *m/z* (rel. int.): 529 $[M\text{-}H + 2\ H_2O]^-$ (40). ^1H and ^{13}C NMR (Tables 1 and 2, respectively).

3.10. X-ray analysis of limonoids *6* and *8*

In both cases irregular colorless crystals were mounted at random on an CAD-4 Enraf–Nonius diffractometer. Cell dimensions were determined by least-squares fitting of the setting angles of 25 reflections ($8.73 < \theta < 13.27°$ for **6** and $8.60 < \theta < 13.83°$ for **8**). Intensity measurements were carried out up to $\theta = 25.5°$, using the $\theta/2\theta$ scan mode and graphite monochromated MoK_α radiation. The crystal data are: for **6** $a = 6.769(1)$, $b = 12.788(2)$, $c = 30.898(4)$ Å, $V = 2674.6(7)$ Å^3, space group $P2_12_12_1$, $Z = 4$, $d_x = 1.362$ g cm^{-3}, 2693 unique reflections were measured, $R_1 = 0.0514$; for **8** $a = 20.399(4)$, $b = 8.835(1)$, $c = 17.608(2)$ Å, $\beta = 101.44°$, $V = 3110.4(8)$ Å^3, space group C2, $Z = 4$, $d_x = 1.223$ g cm^{-3}, 3026 unique reflections were measured, $R_1 = 0.0633$. Lorentz and polarization corrections were applied, but not absorption corrections [μ(MoK_α) = 0.105 and 0.093 mm^{-1} for **6** and **8**, respectively]. The intensities of three standard reflections varied about 0.8% throughout the experiment. The structure was solved by direct methods (Sheldrick, 1986). An E-map based on the solution with the best combined figure of merit revealed the positions of most of the non-hydrogen atoms. The rest of the structure was obtained by difference Fourier calculations. The H-atoms were located on stereochemical grounds, except that of the hydroxyl moiety in **6**. H atoms were refined with fixed geometry, each riding on a carrier atom, with an isotropic displacement parameter amounting to 1.5 (for methyl H atoms) or 1.2 (for the other H atoms) times the value of the equivalent isotropic displacement parameter of the atom they are attached, non-H atoms were refined anisotropically. Refinement was carried on F^2 (Sheldrick, 1997) for all reflections. Lists of atomic fractional coordinates, anistropic thermal parameters for non-hydrogen atoms, interatomic distances and angles and structure have been deposited at the Cambridge Crystallographic Data Center, UK (142610, 142611). In the case of compound **6** there is a water molecule in the crystalline state which is hydrogen bonded to the hydroxyl group: O8...O1W = 2.72(1) Å, H8A...O1W = 1.91 Å, O8–H8A...O1W = 174°.

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References

Burke, B.A., Chan, W.R., Taylor, D.R., 1972. The structure and stereochemistry of spathelin, a new *seco*-ring A tetranortriterpene from *Spathelia sorbifolia*. Tetrahedron 28, 425–430.

Dreyer, D.L., 1965. Citrus bitter principles — II. Application of NMR to structural and stereochemical problems. Tetrahedron 21, 75–87.

Dreyer, D.L., 1984. In: Nes, W.D., Fuller, G., Tsai, L.S. (Eds.), Isopentenoids in Plants, Biochemistry and Function. Marcel Dekker, New York, p. 247.

Garcez, F.R., Garcez, W.S., Rodrigues, E.D., Pott, V.J., Roque, N.F., 1996. *Seco*-protolimonoids from *Trichilia elegans* ssp. *elegans*. Phytochemistry 42 (5), 1399–1403.

Garcez, F.R., Garcez, W.S., Tsutsumi, M.T., Roque, N.F., 1997. Limonoids from *Trichilia elegans* ssp. *elegans*. Phytochemistry 45, 141–148.

Melera, A., Schaffner, K., Arigoni, D., Jeger, O., 1957. Zur Konstitution des Limonins I. Über den Verlauf der Alkalischen Hydrolyse von Limonin und Limonol. Helvetica Chimica Acta XL, 1420–1437.

Nakatani, M., Takao, H., Iwashita, T., Naoki, H., Hase, T., 1988. Glaucin B, a new bitter limonoid from *Evodia glauca*. Phytochemistry 27, 1429–1432.

Okorie, D.A., 1982. Chromones and limonoids from *Harrisonia abyssinica*. Phytochemistry 21, 2424–2426.

Sabata, B., Connolly, J.D., Labbé, C., Rycroft, D.S., 1977. Tetranortriterpenoids and related substances. Part 19. Revised structures of atalantolide and atalantin, limonoids from the root bark of *Atalantia monophylla* Correa (Rutaceae). Journal of the Chemical Society Perkin Transactions I, 1875–1877.

Sheldrick G.M. 1986. SHELXS86, *Program for the Solution of Crystal Structures*. University of Göttingen, Germany.

Sheldrick G.M. 1997. SHELXL97, *Program for the Refinement of Crystal Structures*. University of Göttingen, Germany.

Silva, M.F.G.F., Gottlieb, O.R., 1987. Evolution of quassinoids and limonoids in the Rutales. Biochemical Systematics and Ecology 15, 85–103.

Zsolnai L., Pritzkow H. 1996. ZORTEP, Interactive Graphics Program. University of Heidelberg, Germany.