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Tirucallane triterpenes from the roots of *Ozoroa insignis*

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Abstract

Eight tirucallane triterpenes, methyl 3α,24*S*-dihydroxytirucalla-8,25-dien-21-oate (**2**), methyl 3α-hydroxy-24-oxotirucalla-8,25-dien-21-oate (**3**), methyl 3α-hydroxy-25,26,27-trinor-24-oxotirucall-8-en-21-oate (**4**), 3α,25-dihydroxy-24-(2-hydroxyethyl)-tirucall-8-en-21-oic acid (**5**), 3α,24*S*,25-trihydroxytirucall-8-en-21-oic acid (**6**), 3α,24*R*,25-trihydroxytirucall-8-en-21-oic acid (**7**), 3α,25-dihydroxytirucall-8-en-21-oic acid (**8**), and methyl 3α,25-dihydroxytirucall-8-en-21-oate (**9**), together with α-elemolic acid methyl ester (**1**), were isolated from the roots of *Ozoroa insignis*. Their structures were elucidated on the basis of spectroscopic evidence.

Keywords: *Ozoroa insignis*; Anacardiaceae; Tirucallanes; Tetracyclic triterpenes

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1. Introduction

As part of our phytochemical research on medicinal plants from Guinea-Bissau (Abreu and Pereira, 1998; Abreu et al., 1999; Loukaci et al., 2000; Kayser and Abreu, 2001; Abreu and Relva, 2002) we have investigated *Ozoroa insignis* Del. (*Heeria insignis* Del.) (Anacardiaceae), a species with a wide range of healing properties, namely, in the treatment of diarrhea and venereal diseases, tapeworm and hookworm, schistosomiasis, kidney trouble, and for increasing lactation in women after childbirth (Burkill, 1985; Gelfand et al., 1985; Ndamba et al., 1994; Abreu et al., 1999; Mølgaard et al., 2001; He et al., 2002; Rea et al., 2000). In previous biological screening of *O. insignis* extracts, anthelmintic effect (Mølgaard et al., 2001), cytotoxic activity (Abreu et al., 1999; Rea et al., 2000), and topoisomerase inhibition (Wall et al., 1996) were reported, whereas phytochemical investigation led to the isolation of essential oils, cardanols, and anacardic acids (Ayedoun et al., 1998; He et al., 2002; Rea et al., 2000; Liu and Abreu, 2006). In this communication, we report the isolation of nine tirucallane triterpenes (**1**–**9**) from the roots of *O. insignis*, and their structure elucidation by spectroscopic methods.

2. Results and discussion

Compound **1** was isolated as a white solid with a molecular formula $C_{31}H_{50}O_3$ as indicated by the $[M+H]^+$ peak at *m*/*z* 471.3842 in the HRESIMS. Its IR spectrum showed absorption bands due to hydroxyl ($3445\ cm^{-1}$) and ester (1733 and $1267\ cm^{-1}$) groups. NMR spectra (Table 1, and experimental), including COSY, DEPT, HMQC, and HMBC showed five methyl singlets at δ 0.77, 0.86, 0.88, 0.95, and 0.96, placed on sp^3 carbons, and two additional methyl groups (δ 1.57 and 1.67) linked to a sp^2 carbon (δ_C 132.6), showing $^3J_{H,C}$ correlations with a vinyl carbon at δ_C 123.8, the latter bearing a proton at δ 5.06. The presence of a methyl ester was confirmed by the characteristic resonances at δ 3.65/δ_C 51.1, and the HMBC cross-peak of the methyl protons with a carbonyl at δ_C 176.8. Other relevant NMR data indicated an oxymethine carbon at

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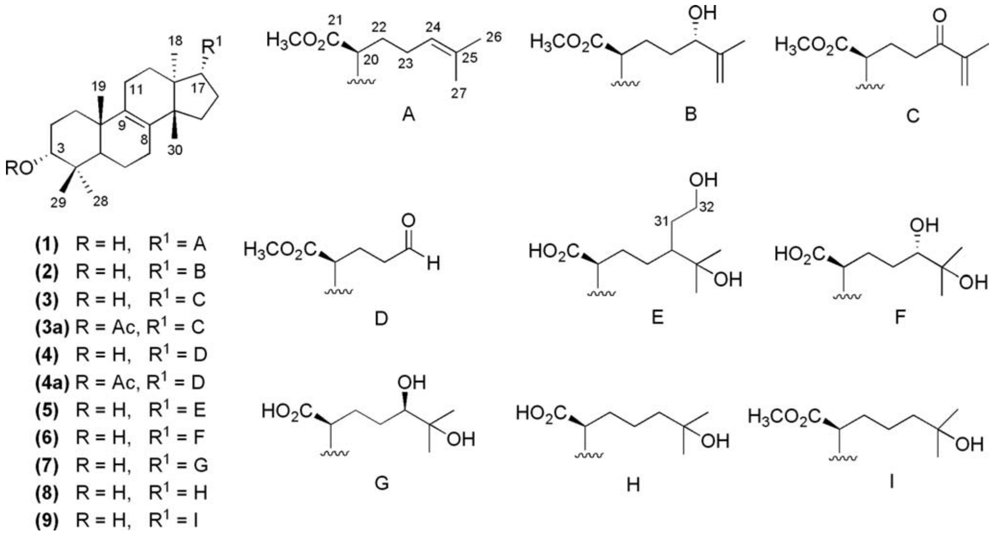

Table 1
^{13}C NMR data of compounds **1**, **2**, **3a**, **4a**, **5**–**9** ($CDCl_3$, 100 MHz)

Position	1	2	3a	4a	5	6	7	8	9
1	31.0	30.9	30.4[a]	30.5[a]	31.0	30.6	30.7	30.7	30.9
2	25.9	25.8	23.4	23.4	25.9	26.0	26.0	26.1	26.8
3	76.0	75.9	78.0	78.0	75.9	75.8	75.9	75.9	75.9
4	37.7	37.7	36.8	36.8	37.7	37.6	37.6	37.6	37.6
5	44.9	44.8	45.8	45.9	44.8	44.5	44.7	44.7	44.8
6	18.8	18.8	18.6	18.6	18.8	18.7	18.7	18.8	18.7
7	26.2	26.5	25.8	26.6	26.5	25.7	25.8	25.8	26.0
8	133.2	132.9	133.1	133.1	133.1	133.1	133.1	133.2	133.1
9	134.5	134.4	134.4	134.4	134.5	134.3	134.3	134.3	134.4
10	37.3	37.2	37.1	37.1	37.3	37.1	37.2	37.7	37.2
11	21.4	21.3	21.3	21.9	21.4	21.3	21.4	21.3	21.4
12	29.9[a]	30.9	30.8[a]	29.8	29.8[a]	30.0[a]	30.0[a]	30.0[a]	29.8[a]
13	44.4	44.8	44.4	44.4	44.4	44.1	44.5	44.4	44.3
14	50.0	49.9	49.9	50.0	49.9	49.7	49.7	49.8	49.9
15	27.3	28.3[a]	27.1	27.1	27.1	27.1	29.8[a]	29.8[a]	27.2
16	29.8[a]	28.7[a]	29.8	29.8	29.7[a]	29.8[a]	27.2	27.1	29.8[a]
17	46.4	46.5	46.3	46.3	46.5	44.7	46.3	46.3	46.5
18	16.1	15.9	16.0	16.0	16.1	15.5	15.6	15.6	16.0
19	20.0	19.9	20.0	20.0	20.0	19.8	19.9	19.9	20.0
20	49.0	49.1	48.5	48.3	48.6	43.2	49.6	43.4	48.5
21	176.8	176.8	176.4	176.0	176.2	175.2	175.2	175.2	176.7
22	31.7	34.8	27.6	30.9[a]	29.7[a]	30.0[a]	30.0[a]	30.0[a]	29.7[a]
23	26.6	32.4	35.0	41.7	28.3	29.7	28.0	32.4	29.0
24	123.8	75.9	201.1	201.2	44.4	83.0	85.7	39.2	44.3
25	132.6	147.1	144.4		73.1	71.0	71.6	71.0	73.1
26	17.7	17.1	17.6		23.2	24.2	23.9	23.9	23.2
27	25.7	111.7	124.5		26.5	26.0	26.1	26.2	26.5
28	28.1	28.1	27.6	27.6	28.1	28.0	28.0	28.1	28.1
29	22.3	22.2	21.9	21.9	22.3	21.7	22.2	22.2	22.2
30	24.5	24.4	24.5	24.5	24.5	24.2	24.3	24.2	24.3
31					29.5[a]				
32					60.0				
$\mathrm{CO_2}\mathit{C}\mathrm{H_3}$	51.1	51.2	51.2	51.3					51.2
$\mathrm{O}\mathit{C}\mathrm{OCH_3}$			170.8	170.8					
$\mathrm{OCO}\mathit{C}\mathrm{H_3}$			21.3	21.3					

[a] The assignments may be exchanged.

δ_C 76.0, showing HMBC correlations with two methyl groups at δ 0.86 and 0.96, and a tetrasubstituted double bond at δ_C 133.2 and 134.5. These data, as well as the mass fragmentation recorded in EIMS (see Section 3) were in agreement with the structure of a 3-hydroxy-Δ^8 tetracyclic triterpenoid, with a side chain bearing a methyl ester at C-

20, and an ending –HC=C(CH_3)$_2$ moiety (Tessier et al., 1982; Rösecke and König, 1999). The hydroxyl group was assigned as axial on the basis of the chemical shift and multiplicity of H-3 (δ 3.43, *bs*) (Lin et al., 1997; Su et al., 2000; Usubillaga et al., 2004). The stereochemistry of the tetracyclic nucleus and orientation of the side chain were deduced from the following correlations observed in the NOESY spectrum: H-3 with 19-CH_3 and 29-CH_3; H-5 with 28-CH_3; H-17 with 30-CH_3; H-20 with 18-CH_3; and 19-CH_3 with 29-CH_3. Nevertheless, when comparing the NMR spectral data of **1** with those reported for tetracyclic triterpenes (Knight, 1974; Ben Harref and Lavergne, 1985; Emmons et al., 1989; Gewal et al., 1990; Keller et al., 1996; Lin et al., 1997; Akihisa et al., 1998; Rösecke and König, 1999; Su et al., 2000; Wang et al., 2003; Usubillaga et al., 2004), it was not possible to confirm its structure as a tirucallane, euphane, or lanostane triterpenoid, since the corresponding carbon resonances are very similar, and occasionally, wrong assignments have been reported in literature (Emmons et al., 1989; Gewal et al., 1990). An essential aid that has been used for the discrimination of euphane (20*R*)/tirucallane (20*S*), is the NOE correlation of H-21 with H-16α,β in euphanes, and with H-12α in tirucallanes (Akihisa et al., 1996a,b, 1998; Mohamad et al., 1999; Wang et al., 2003), but the oxidation of 21-CH_3 in compound **1** precluded the application of this tool. The structure of **1** was confirmed as a tirucallane triterpenoid, from the comparison of its optical rotation with that reported for related 3α-OH tirucallanes, euphanes, and lanostanes, which is positive in euphane and lanostane series, and negative in tirucallanes (Keller et al., 1996; Lin et al., 1997; Rösecke and König, 1999; Mishra et al., 2000). Compound **1** showed a $[\alpha]_D^{25} - 10.7°$, identical to the one reported for the synthetic methyl ester derivative of α-elemolic acid (3α-hydroxy-tirucalla-8,24-dien-21-oic acid), previously isolated from several Burseraceae (Cotterrell et al., 1970; Billet et al., 1971; Pardhy and Bhattacharya, 1978; Tessier et al., 1982; Sawadogo et al., 1985; Lima et al., 2004; Usubillaga et al., 2004). Based on this evidence, the structure of **1** was confirmed as methyl 3α-hydroxy-tirucalla-8,24-dien-21-oate, whose spectroscopic data is here reported for the first time.

The molecular formula of **2** was calculated as $C_{31}H_{50}O_4$ from the HRESIMS pseudomolecular ion peak $[M+Na]^+$ at *m*/*z* 509.3581. As for compound **1**, a 3α-hydroxy-Δ^8 tetracyclic scaffold was assigned on the basis of 1- and 2D NMR experiments, and EI mass spectrum. The presence of a second secondary hydroxyl in the molecule was evidenced by the resonance of a methine proton (δ 4.02) linked to a carbon at δ_C 75.9, and the loss of mass fragments containing two molecules of H_2O, at *m*/*z* 435 $[M-CH_3-2H_2O]^+$, 375 $[M-CH_3-2H_2O-HCO_2CH_3]^+$, and 281 $[M-CH_3-\text{side chain}-H_2O-2H]^+$, recorded in EIMS mode. The following HMBC spectral features indicated that this alcohol was vicinal to an exomethylene and methyl groups: $^3J_{C,H}$ correlations of the methine carbon with two vinyl protons at δ 4.84 (*s*) and 4.92 (*s*), and with a methyl group at δ 1.69; and correlations of these vinyl and methyl protons with a quaternary sp^2 carbon (δ_C 147.1). On the other hand, the olefinic methyl group (δ 1.69) showed strong NOE interactions with the methine and highest field vinyl protons (δ 4.02, H-24; and δ 4.84, H-27a, respectively), whereas H-24 interacted with the lowest field vinyl proton H-27b. A methyl ester group placed on C-20 was assigned as for compound **1**, thus confirming the side chain structure [CH_3O_2C–C_2H_4–C(OH)–C(=CH_2)CH_3]. The configuration at C-24 was suggested as (*S*) by comparison of the methine chemical shifts (δ 4.02/δ_C 75.9) with those of similar (24*R*,24*S*)-hydroxylated side chains (Banskota et al., 2000; Su et al., 2000). These data supported the structure of **2** as methyl methyl 3α,24*S*-dihydroxytirucalla-8,25-dien-21-oate.

Compounds **3** and **4** were obtained from a chromatographic fraction as an inseparable mixture, that was resolved after acetylation to afford **3a** and **4a**. For compound **3a**, HRESIMS showed a pseudomolecular ion peak $[M+H^+]$ at *m*/*z* 527.3734, indicating a chemical composition of $C_{33}H_{50}O_5$. The presence of an axial 3-OAc group was confirmed by the presence of a methyl group at δ 2.07, an oxymethine proton at δ 4.67 (*bs*), and a carbonyl at δ_C 170.8. Other relevant spectra data of **3a** included a carbon resonance for a ketone group (δ_C 201.1) showing HMBC correlations with two vinyl protons (δ 5.92, 1H, *s*; 5.75, 1H, *s*), a methyl group (δ 1.87) placed on a sp^2 carbon (δ_C 144.4), and two methylenic protons at δ 2.62 displaying NOE interactions with the lowest field vinyl proton (δ 5.92). The above data was in agreement with a side chain fragment [–CH_2–C(=O)–C(=CH_2)CH_3], previously found in tetracyclic triterpenes (Leong and Harrison, 1999). As for the case of compounds **1** and **2**, C-21 was oxidized in the form of a methyl ester. DEPT spectrum and bidimensional NMR experiments (COSY, HMQC, HMBC, and NOESY) allowed us to assign the structure of **3a**, which corresponds to the acetylated derivative of methyl 3α-hydroxy-24-oxotirucalla-8,25-dien-21-oate (**3**).

The acetylated derivative **4a**, was analysed for $C_{30}H_{46}O_5$ by positive HRESIMS. Its ^{1}H and ^{13}C NMR showed characteristic signals for an aldehyde (δ 9.75/δ_C 201.2), whose proton showed COSY and HMBC cross-peaks with a methylene group at δ 2.38/δ_C 41.6. The NMR data of the tetracyclic nucleus was identical to those of **3a**, but the resonances of the side chain methyl groups were absent, with exception of those for the methyl ester group at C-20 (δ 3.66/δ_C 51.3). These features indicated that compound **4a** possessed a shorter side chain with an aldehyde at C-24 (Carrera and Seldes, 1987), probably resulting from C-24–C-25 oxidative cleavage. Thus, the structure of **4** was confirmed as methyl 3α-hydroxy-25,26,27-trinor-24-oxotirucall-8-en-21-oate. The molecular formula of **5** was assigned as $C_{32}H_{54}O_5$ by positive HRESIMS. Comparison of its NMR data with those of compounds described above, revealed the characteristic signals for a 3α-hydroxy-Δ^8 tetracyclic nucleus, but relevant differences were observed for the side chain proton and carbon resonances: an oxygenated quaternary carbon at δ_C 73.1 showing HMBC correlations with two methyl groups at δ 1.13 and 1.19; a low-field methylene group (δ 4.14; δ_C 60.0) exhibiting COSY cross-peaks with two protons at δ 1.25, in agreement with a fragment [–CH_2–CH_2OH] (Wang et al., 2004); the signals for the methyl ester were absent, although a carbonyl resonance was displayed at δ_C 176.2. The above spectral data indicated a side chain bearing a hydroxyethyl group at C-24, a C-21 carboxyl, and an ending segment [–$C(CH_3)_2$–OH], which was corroborated by the mass fragmentation recorded in the EI mode (see Section 3), consistent with the structure of 3α,25-dihydroxy-24-(2-hydroxyethyl)-tirucall-8-en-21-oic acid (**5**).

The HRESIMS of compounds **6** and **7** provided pseudomolecular ion peaks $[M+H]^+$ at *m/z* 491.3729 and 491.3741, respectively, corresponding to an identical molecular formula $C_{30}H_{50}O_5$. The EIMS of compound **6** showed fragments at *m/z* 472 $[M-H_2O]^+$, 439 $[M-CH_3-2H_2O]^+$, and 421 $[M-CH_3-3H_2O]^+$, thus indicating the existence of three hydroxyl groups. The ^1H and ^{13}C NMR spectra of **6** and **7** were very similar, both displaying the signals of the 3α-hydroxy-Δ^8 tetracyclic nucleus, and a C-21 carboxylic side chain. The presence of a [–CH(OH)–$C(OH)(CH_3)_2$] side chain ending moiety was inferred from the HMBC correlations of an oxygenated quaternary carbon (δ_C 71.0 and 71.6, for **6** and **7**, respectively) with a low-field methine proton (δ 4.08 and 4.14, for **6** and **7**, respectively), and two tertiary methyl groups at δ 1.18 and 1.25 in compound **6**, and δ 1.21 and 1.27 in compound **7**. NOE interactions were also observed between these two methyl groups with the low-field methine proton. These features were indicative that **6** and **7** were C-24 epimers. Since the low amount of available samples precluded the preparation of the Mosher esters for further stereochemical elucidation, we have compared the side chain NMR resonances of **6** and **7**, with those of 24,25-dihydroxylated tetracyclic triterpenes (Banskota et al., 2000; Ukiya et al., 2003). In these compounds the reported chemical shift of H-24 is 0.05 ppm higher in (24*R*) alcohols, when compared to its epimer, a difference that was also observed between **7** and **6**. Based on this evidence, the structures of compounds **6** and **7** were assigned as 3α,24*S*,25- and 3α,24*R*,25-trihydroxytirucall-8-en-21-oic acid, respectively.

The HRESIMS of compound **8** indicated a molecular formula $C_{30}H_{50}O_4$. As for compounds **6** and **7**, its NMR spectra exhibited the signals of two tertiary methyls (δ 1.12 and 1.19) adjacent to an oxygen-bearing carbon at δ_C 73.1, and a C-21 carboxyl group, but lack any resonances for hydroxyl groups, besides those corresponding to 3α- and 25-OH. From the same chromatographic fraction, a less polar compound of molecular formula $C_{31}H_{52}O_4$ (**9**) was revealed to be the corresponding C-21 methyl ester of **8**. Analysis of COSY, DEPT, HMQC, and HMBC supported the structures of **8** and **9** as 3α,25-dihydroxytirucall-8-en-21-oic acid, and methyl 3α,25-dihydroxy-tirucalla-8-ene-21-oate, respectively.

To the best of our knowledge, compounds **1**–**9** constitute the second reported occurrence of tirucallanes in a plant genus belonging to Anacardiaceae, other than *Pistacea* (Monaco et al., 1974; Caputo et al., 1975, 1977, 1979; Assimopoulou and Papageorggiou, 2005). On the other hand, compound **5** is the second C_{32} tirucallane-type triterpene reported in literature (Schun et al., 1986).

3. Experimental

3.1. General experimental procedures

Mps: uncorrected; TLC: Si gel 60 F_{254}, MN; RP-18 Si gel F_{254}, Merck, detection with Ce_2SO_4 and phosphomolybdic acid. Si gel CC: (35–70 mesh) MN; Flash CC, and LPLC: Si gel (230–400 mesh) MN; LiChroprep RP-18 Si gel (40–63 μm), Merck. Optical rotation was measured with a Perkin–Elmer 241MC polarimeter. UV: Milton Roy Spectronic 1201 spectrophotometer; FTIR: Perkin–Elmer 157G. ^1H NMR, ^{13}C NMR: 400 and 100.61 MHz, respectively (Bruker AC-400), $CDCl_3$ as solvent, with TMS as reference. EIMS (70 eV): Micromass GCTOF spectrometer; ESIMS and HRESIMS: Agilent MSD1100 single quadropole spectrometer, and Agilent ESI-TOF instrument, respectively.

3.2. Plant material

Ozoroa insignis Delile (Anacardiaceae), was collected in January 1994 at Contuboel, Guinea-Bissau, and identified at the Herbarium of Botany Centre (LISC), where the voucher specimen No. 854 was deposited.

3.3. Extraction and isolation

The air dried and powdered roots of *O. insignis* (1.65 kg) were extracted with EtOH (5 l, Soxhlet). Evaporation of the solvent under reduced pressure afforded an oily residue (83.3 g), that was then eluted on a Celite column with hexane, CH_2Cl_2, EtOAc, and MeOH. The hexane and CH_2Cl_2 fractions were combined (34 g), and subjected to Si gel CC, with hexane, hexane–ether (70:10 to 0:100), CH_2Cl_2, and EtOAc elution, to obtain 68 fractions, which were grouped (F1–F7) according their TLC behavior. F2 (18.6 g) was submitted to Si gel flash CC, using a step gradient of hexane–EtOAc (30:1 to 8:1), to yield compound **1** (5.6 mg). F3 (4.61 g) was eluted with hexane–EtOAc (8:1) through a silica gel column, to afford compounds **1** (21 mg) and **2** (3.5 mg), and a subfraction that was further acetylated (Ac_2O/pyridine, overnight, room T) to yield compounds **3a** (2.5 mg) and **4a** (7.0 mg). Repeated RP-18 LPLC of F6 (1.22 g) with MeOH–H_2O (80:20) elution, yielded compounds **5** (4.8 mg), **6** (48 mg), **7** (18 mg), **8** (7.6 mg), and **9** (9 mg), by order of decreasing polarity.

*3.3.1. Methyl 3α-hydroxy-tirucalla-8,24-dien-21-oate (**1**)*

White solid, M.p. 105–106 °C; $[\alpha]_D^{25}-10.7°$ (CH_2Cl_2, *c* 0.2); IR$[v]_{max}^{NaCl}$ cm^{-1}: 3445, 2936, 1733, 1267; EIMS 70 eV, *m/z* (rel. int.): 470 $[M]^+$ (5), 455 $[M-CH_3]^+$ (65), 437 $[M-CH_3-H_2O]^+$ (70), 405 $[M-CH_3-H_2O-OCH_3-H]^+$ (10), 377 $[M-CH_3-H_2O-HCO_2CH_3-H]^+$ (5), 299 $[M-CH_3-\text{side chain}-2H]^+$(5), 281 $[M-CH_3-\text{side chain}-H_2O-2H]^+$ (25), 189 $[C_{14}H_{21}]^+$(50), 149 (100); HRESIMS, *m/z*: 471.3842 $[M+H]^+$ (calcd. for $C_{31}H_{51}O_3$, 471.3838); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (3 H, *s*, H-18), 0.86 (3H, *s*, H-29), 0.88 (3H, *s*, H-30), 0.95 (3H, *s*, H-28), 0.96 (3H, *s*, H-19), 1.57 (3H, *s*, H-26), 1.64 (1H, *m*, H-5), 1.67 (3H, *s*, H-27), 2.07 (1H, *m*, H-17), 2.42 (1H, *m*, H-20), 3.43 (1H, *bs*, H-3), 3.65 (3H, *s*, OCH_3), 5.06 (1H, *t*, $J=7.2$ Hz, H-24); ^{13}C NMR data, see Table 1.

*3.3.2. Methyl 3α,24S-dihydroxytirucalla-8,25-dien-21-oate (**2**)*

White solid, M.p. 120–121 °C; $[\alpha]_D^{25}-16.6°$ (CH_2Cl_2, *c* 0.3); IR$[v]_{max}^{NaCl}$ cm^{-1}: 3445, 2936, 1733, 1656, 1265; EIMS 70 eV, *m/z* (rel. int.): 486 $[M]^+$ (22), 471 $[M-CH_3]^+$ (10), 453 $[M-CH_3-H_2O]^+$ (100), 435 $[M-CH_3-2H_2O]^+$ (70), 421 (25), 375 $[M-CH_3-2H_2O-HCO_2CH_3]^+$, 299 $[M-CH_3-\text{side chain}-2H]^+$(19), 281 $[M-CH_3-\text{side chain}-H_2O-2H]^+$ (22); HRESIMS, *m/z* 509.3599 $[M+Na]^+$ (calcd. for $C_{31}H_{50}O_4Na$, 509.3607); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (6H, *s*, H-18, H-29), 0.94 (3H, *s*, H-28), 0.95 (6H, *s*, H-19, H-30), 1.69 (3H, *s*, H-26), 2.43 (1H, *m*, H-20), 3.46 (1H, *bs*, H-3), 3.65 (3H, *s*, OCH_3), 4.02 (1H, *t*, $J=5.6$ Hz, H-24), 4.84 (1H, *s*, H-27a), 4.92 (1H, *s*, H-27b). ^{13}C NMR data, see Table 1.

*3.3.3. Methyl 3α-acetoxy-24-oxotirucalla-8,25-dien-21-oate (**3a**)*

White solid, M.p. 120–121 °C; $[\alpha]_D^{25}-2.1°$ (CH_2Cl_2, *c* 0.1); IR$[v]_{max}^{NaCl}$ cm^{-1}: 2936, 1733, 1710, 1265; HRESIMS, *m/z* 527.3734 $[M+H]^+$ (calcd. for $C_{33}H_{51}O_5$, 527.3736); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, *s*, H-18), 0.87 (3H, *s*, H-28), 0.91 (6H, *s*, H-29, H-30), 0.97 (3H, *s*, H-19), 1.87 (3H, *s*, H-26), 2.07 (3H, *s*, $OCOCH_3$), 2.42 (1H, *m*, H-20), 2.62 (2H, *t*, $J=7.0$ Hz, H-23), 3.66 (3H, *s*, OCH_3), 4.67 (1H, *bs*, H-3), 5.75 (1H, *s*, H-27a), 5.92 (1H, *s*, H-27b). ^{13}C NMR data, see Table 1.

*3.3.4. Methyl 3α-acetoxy-25,26,27-trinor-24-oxotirucall-8-en-21-oate (**4a**)*

White solid, M.p. 115–116 °C; $[\alpha]_D^{25}-3.1°$ (CH_2Cl_2, *c* 0.1); IR $[v]_{max}^{NaCl}$ cm^{-1}: 2948, 1732, 1246; HRESIMS, *m/z* 487.3419 $[M+H]^+$ (calcd. for $C_{30}H_{47}O_5$, 487.3423); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, *s*, H-18), 0.87 (3H, *s*, H-28), 0.91 (6H, *s*, H-29, H-30), 0.97 (3H, *s*, H-19), 2.06 (3H, *s*, OCOMe), 2.38 (2H, *m*, H-23), 2.46 (1H, *m*, H-20), 3.66 (3H, *s*, OMe), 4.67 (1H, *bs*, H-3), 9.74 (1H, *s*, H-24). ^{13}C NMR data, see Table 1.

*3.3.5. 3α,25-Dihydroxy-24-(2-hydroxyethyl)-tirucall-8- en-21-oic acid (**5**)*

White solid, M.p. 135–136 °C; $[\alpha]_D^{25}-0.6°$ (CH_2Cl_2, *c* 0.4); IR$[v]_{max}^{NaCl}$ cm^{-1}: 3445, 2934, 1716, 1275; EIMS 70 eV, *m/z* (rel. int.): 518 $[M]^+$ (18), 503 $[M-CH_3]^+$ (35), 485 $[M-CH_3-H_2O]^+$ (75), 467 $[M-CH_3-2H_2O]^+$ (100), 439 $[M-CH_3-2H_2O-C_2H_4]^+$ (90), 421 $[M-CH_3-3H_2O-C_2H_4]^+$ (95), 411 $[M-CH_3-H_2O-C_2H_4-HCO_2H]^+$ (40), 393 $[M-CH_3-2H_2O-C_2H_4-HCO_2H]^+$ (90), 299 $[M-CH_3-\text{side chain}-2H]^+$(20), 281 $[M-\text{side chain}-H_2O-2H]^+$ (65), 187 (32); HRESIMS, *m/z* 519.4032 $[M+H]^+$ (calcd. for $C_{32}H_{55}O_5$, 519.4040); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, *s*, H-18), 0.86 (3H, *s*, H-29), 0.90 (3H, *s*, H-30), 0.97 (6H, *s*, H-19, H-28), 1.13 (3H, *s*, H-27), 1.19 (3H, *s*, H-26), 1.25 (2H, *m*, H-31), 2.47 (1H, *m*, H-20), 3.43 (1H, *bs*, H-3), 4.67 (2H, *m*, H-32). ^{13}C NMR data, see Table 1.

*3.3.6. 3α,24S,25-Trihydroxytirucall-8-en-21-oic acid (**6**)*

Light yellow solid, M.p. 138–139 °C; $[\alpha]_D^{25}-9.8$ (CH_2Cl_2, *c* 0.5); IR$[v]_{max}^{NaCl}$ cm^{-1}: 3441, 2941, 1731, 1265; EIMS 70 eV, *m/z* (rel. int.): 472 $[M-H_2O]^+$ (50), 457 $[M-CH_3-H_2O]^+$ (100), 439 $[M-CH_3-2H_2O]^+$ (97), 421 $[M-CH_3-3H_2O]^+$ (70), 393 $[M-CH_3-2H_2O-HCOOH]^+$ (50), 299 $[M-CH_3-\text{side chain}-2H]^+$(20), 281 $[M-\text{side chain}-H_2O-2H]^+$ (50), 187 (45); HRESIMS, *m/z* 491.3729 $[M+H]^+$ (calcd. for $C_{30}H_{51}O_5$, 491.3736); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, *s*, H-18), 0.83 (3H, *s*, H-29), 0.90 (3H, *s*, H-30), 0.94 (6H, *s*, H-19, H-28), 1.18 (3H, *s*, H-27), 1.25 (3H, *s*, H-26), 2.44 (1H, *m*, H-20), 3.41 (1H, *bs*, H-3), 4.08 (1H, *dd*, $J=11.6$, 2.8 Hz). ^{13}C NMR data, see Table 1.

*3.3.7. 3α,24R,25-Trihydroxytirucall-8-en-21-oic acid (**7**)*

Yellow solid, M.p. 131–132 °C; $[\alpha]_D^{25}+5.6°$ (CH_2Cl_2, *c* 0.2); IR$[v]_{max}^{NaCl}$ cm^{-1}: 3445, 2939, 1731, 1264; HRESIMS, *m/z* 491.3741 $[M+H]^+$ (calcd. for $C_{30}H_{51}O_5$, 491.3736); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, *s*, H-18), 0.85 (3H, *s*, H-29), 0.92 (3H, *s*, H-28), 0.95 (6H, *s*, H-19, H-30), 1.21 (3H, *s*, H-27), 1.27 (3H, *s*, H-26), 2.46 (1H, *m*, H-20), 3.43 (1H, b*s*, H-3), 4.14 (1H, *dd*, $J=12.2$, 3.6 Hz, H-24). ^{13}C NMR data, see Table 1.

*3.3.8. 3α,25-Dihydroxytirucall-8-en-21-oic acid (**8**)*

Yellow solid, M.p. 115–116 °C; $[\alpha]_D^{25}-5.3°$ (CH_2Cl_2, *c* 0.7); IR$[v]_{max}^{NaCl}$ cm^{-1}: 3446, 2935, 1733, 1270; HRESIMS, *m/z* 475.3780 $[M+H]^+$ (calcd. for $C_{30}H_{51}O_4$, 475.3787); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (3H, *s*, H-18), 0.84 (3H, *s*, H-29), 0.87 (3H, *s*, H-30), 0.97 (6H, *s*, H-19, H-28), 1.21 (3H, *s*, H-27), 1.25 (3H, *s*, H-26), 2.44 (1H, *m*, H-20), 3.44 (1H, *bs*, H-3). ^{13}C NMR data, see Table 1.

*3.3.9. Methyl 3α,25-dihydroxytirucall-8-en-21-oate (**9**)*

White solid, 110–111 °C; $[\alpha]_D^{25}-0.6°$ (CH_2Cl_2, *c* 0.9); EIMS 70 eV, *m/z* (rel. int.): 488 $[M]^+$ (15), 470 $[M-H_2O]^+$ (50), 457 $[M-OCH_3]^+$ 4(21), 452 $[M-2H_2O]^+$ (100), 439

$[M-OCH_3-H_2O]^+$(55), 421 $[M-OCH_3-2H_2O]^+$(70), 411 $[M-CO_2CH_3-H_2O]^+$(25), 393 $[M-CO_2CH_3-2H_2O]^+$(50), 299 $[M-CH_3-\text{side chain}-2H]^+$(20), 281 $[M-\text{side chain}-H_2O-2H]^+$ (50), 187 (45); HRESIMS, *m/z* 489.3939 $[M+H]^+$ (calcd. for $C_{31}H_{53}O_4$, 489.3944); ^1H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, *s*, H-18), 0.86 (3H, *s*, H-29), 0.90 (3H, *s*, H-30), 0.96 (6H, *s*, H-19, H-28), 1.12 (3H, *s*, H-27), 1.19 (3H, *s*, H-26), 2.48 (1H, *m*, H-20), 3.46 (1H, *bs*, H-3), 3.65 (3H, *s*, OMe). ^{13}C NMR data, see Table 1.

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