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Phytochemistry 66 (2005) 2324–2328
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Tirucallane triterpenes from the leaf extract of *Entandrophragma angolense*

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Received 31 March 2005; received in revised form 25 July 2005
Available online 8 September 2005

Abstract

From the leaves of *Entandrophragma angolense*, three triterpenoidal compounds were isolated and structurally elucidated by mass and NMR spectroscopy. They belong to the tirucallane group but two of them possess the rare seco-ring-A feature. The phytochemical data are discussed from a chemotaxonomic and biogenetic points of view.

Keywords: *Entandrophragma angolense*; Meliaceae; Triterpenoids; Protolimonoids

1. Introduction

Entandrophragma angolense (Welw C. DC) belongs to the family Meliaceae and yields the durable mahogany timbers. Pests avoid feeding on the leaves of the plants of this family. *E. angolense* is used in Western Nigeria as an antimalarial and as an antiulcer (Njar et al., 1995) in ethnomedicine. The meliacins (limonoids) hitherto isolated from *E. angolense* are gedunin (Akisanya et al., 1960) and methyl angolensate (Bevan et al., 1967) from the stem wood and bark as well as from the root (Orisadipe et al., unpublished work). Gedunin has been shown in vitro to possess antimalarial activities (Bray et al., 1990; MacKinnon et al., 1997).

The protolimonoid entandrolide was isolated from the seeds (Okorie and Taylor, 1977).

As far as it is known, there are no reports on the chemistry of leaf extracts of *E. angolense*. In this work, the *n*-hexane extract of the leaves yielded 3,23-dioxotirucalla-7,24-dien-21-al (**1**), 3,4-secotirucalla-23-oxo-4(28), 7,24-trien-21-al-3-oic acid (**2**) and 3,4-secotirucalla-23-oxo-4(28),7,24-trien-3,21-dioic acid (21-methyl ester) (**3**).

2. Results and discussion

The *n*-hexane extract of the leaves was separated by flash column chromatography on RP-18 adsorbent followed by flash chromatography of the mixture B, on normal phase silica gel, to give the known compounds β-sitosterol, stigmasterol and the new compound 3,23-dioxotirucalla-7,24-dien-21-al (**1**). Thereafter, the mixture C was subjected to normal phase HPLC with a gradient of ethanol in hexane as eluents to give two

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doi:10.1016/j.phytochem.2005.07.017

more new compounds: 3,4-secotirucalla-23-oxo-4(28), 7,24-trien-21-al-3-oic acid (**2**) and 3,4-secotirucalla-23-oxo-4(28),7,24-trien-3,21-dioic acid (21-methyl ester) (**3**).

Compound **1** was isolated as an amorphous powder and the UV spectrum showed absorption at 233 nm consistent with an enone moiety.

In the HREIMS, the molecular ion $[M]^+$ at *m/z* 452.3290 established the molecular formula $C_{30}H_{44}O_3$ thus implying nine degrees of unsaturations.

The broadband decoupled and polarization transfer ^{13}C NMR spectra, exhibited signals due to two trisubstituted double bonds, one isolated and one conjugated ketone and one aldehyde carbonyls, thus requiring four rings for compound **1**. Besides, the presence of seven methyls, eight methylenes and four methines suggested a tetracyclic triterpene. The ^1H NMR spectrum of compound **1** showed five methyls on saturated quaternary carbons and one olefinic proton coupled to a vicinal methylene. Proton couplings from 2D experiment (COSY) revealed an isopropylidene group conjugated to a ketone functionality $Me_2C{=}CHCO–$ and a $CHO–CH–(CH)–CH_2–$ spin system, these two fragments can be connected together because of $^1H–^1H$ 2D spatial correlations (NOESY) between the methylene H_2-22 and the vinylic H-24 protons. Finally, proton chemical shifts and coupling pattern suggested the fragment $–CH_2–CH_2–CO–$ which could be further extended to include $–C(Me_2)–CH–$ on the basis of the observed $^1H–^{13}C$ long range correlations (HMBC). The above partial structures closely resemble the side chain and the A ring of the known dymacrin D, previously isolated from the bark of *Dysoxylum macranthum* (Mohamad et al., 1999). In fact, the NMR spectral data of our compound match those reported for dymacrin D, structural differences being limited to an aldehyde group in place of a methyl at C-20 (Table 1).

Compound **2**, which was more polar than compound **1**, gave an $[M^+]$ peak at *m/z* 468.3239 in the HREIMS corresponding to the molecular formula of $C_{30}H_{44}O_4$ and hence nine degrees of unsaturations. Examination of its ^1H- and ^{13}C NMR spectra revealed that the signals for the $–CH_2CH_2–CO–C(Me_2)–CH–$ moiety were absent and the resonances attributable to an isopropenyl and a propanoyl groups emerged. This could be rationalized by admitting an oxidative breakage at the C3–C4 bond. Compound **2** also showed identical NMR spectral frequencies for ring D and the C-17 side chain as those of compound **1**. All proton and carbon resonances of compound **2** were assigned from cross peaks in $^1H–^{13}C$ one bond correlation (HSQC) and COSY experiments. The assignments were fully confirmed from the analysis of extensive HMBC cross peaks in the NMR spectra. The relative configuration at C-20 was deduced from the diagnostic

Table 1
^{13}C NMR data of known dymacrin D and of compounds **1**, **2** and **3**

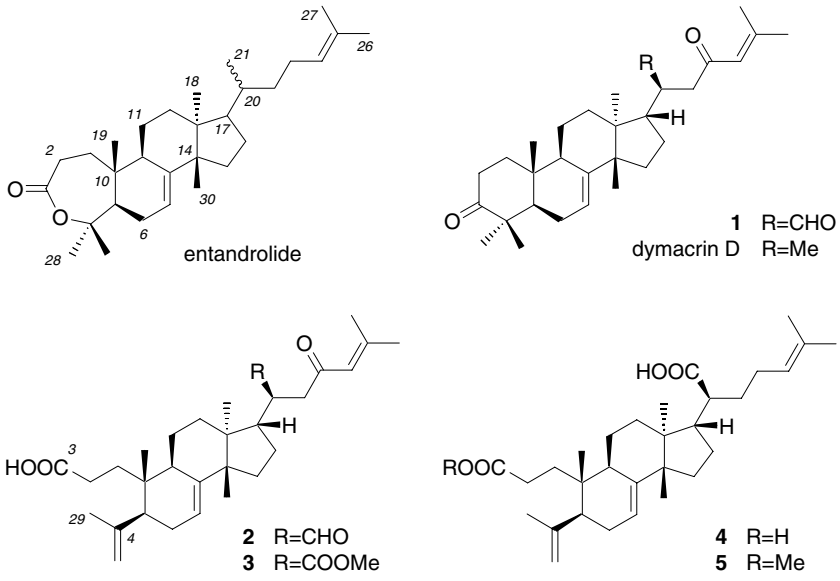
Carbon	Dymacrin D	**1**	**2**	**3**
1	38.6 *t*	38.4 *t*	31.5 *t*	31.5 *t*
2	35.0 *t*	35.0 *t*	28.0 *t*	28.0 *t*
3	216.1 *s*	216.1 *s*	179.9 *s*	179.9 *s*
4	48.0 *s*	47.8 *s*	147.1 *s*	147.2 *s*
5	53.3 *d*	52.2 *d*	49.4 *d*	49.4 *d*
6	24.7 *t*	24.3 *t*	30.1 *t*	30.1 *t*
7	118.0 *d*	118.4 *d*	118.5 *d*	118.3 *d*
8	145.9 *s*	145.1 *s*	145.4 *s*	145.7 *s*
9	48.5 *d*	48.2 *d*	40.6 *d*	40.6 *d*
10	35.1 *s*	34.8 *s*	36.7 *s*	36.6 *s*
11	18.3 *t*	17.9 *t*	18.1 *t*	17.9 *t*
12	33.6 *t*	32.7 *t*	32.7 *t*	29.8 *t*
13	43.7 *s*	43.5 *s*	43.3 *s*	43.1 *s*
14	51.4 *s*	50.7 *s*	50.0 *s*	51.2 *s*
15	34.1 *t*	33.8 *t*	33.9 *t*	33.6 *t*
16	28.5 *t*	27.8 *t*	27.5 *t*	27.3 *t*
17	52.4 *d*	49.0 *d*	49.1 *d*	49.7 *d*
18	22.1 *q*	23.8 *q*	23.9 *q*	21.7 *q*
19	12.9 *q*	12.7 *q*	15.8 *q*	15.8 *q*
20	33.6 *d*	49.2 *d*	49.3 *d*	42.3 *d*
21	19.5 *q*	204.7 *d*	204.7 *d*	176.4 *d*
22	51.6 *t*	45.7 *t*	45.9 *t*	46.8 *t*
23	201.6 *s*	198.5 *s*	198.6 *s*	198.8 *s*
24	124.4 *d*	122.9 *d*	122.9 *d*	123.2 *d*
25	154.8 *s*	156.5 *s*	156.5 *s*	156.9 *s*
26	27.8 *q*	27.8 *q*	27.8 *q*	27.5 *q*
27	20.8 *q*	20.8 *q*	20.8 *q*	20.8 *q*
28	24.7 *q*	24.5 *q*	114.0 *t*	114.0 *t*
29	21.7 *q*	21.6 *q*	22.3 *q*	22.3 *q*
30	27.5 *q*	27.5 *q*	27.4 *q*	27.7 *q*
OMe				51.6 *q*

NOESY correlations between H_3-30 ↔ H-17, H-9 ↔ H_3-18 ↔ H-20 and H-12α ↔ H-21 ↔ H-20 that established compound **2** be a tirucallane triterpene (Akihisa et al., 1996).

The HREIMS of compound **3** gave a mass ion $[M^+]$ at *m/z* 498.3345, which corresponds to the molecular formula $C_{31}H_{46}O_5$. All NMR data were close to those of compound **2** indicating a similar structure. The only difference was the absence of the aldehyde group in compound **2** replaced at the corresponding position by a carbomethoxy group in **3**. The presence of a fragment *m/z* 425 ($C_{28}H_{41}O_3$) suggested that the loss of 73 amu (CH_2CH_2COOH) occurred from the seco ring A. Cross peaks in HMBC between the carbon signal at 176.4 ppm and the protons H-22R and $H_3CO–$ located the ester functionality at C-21.

Ring A-seco protolimonoids structurally similar to compounds **2** and **3**, are entandrolide, isolated from the seeds of *E. angolense* (Okorie and Taylor, 1977) and the carboxylic acids **4** and **5** from the bark extract of *Guarea cedrata* (Akinniyi et al., 1980). The ^{13}C NMR frequencies reported for them are consistent with our assignments.

The limonoids gedunin (Akisanya et al., 1960) and the ring B-seco methyl angolensate (Bevan et al., 1967)



were previously isolated from the stem and root extracts of *E. angolense* thus giving a clear distinction from the protolimonoids entandrolide, contained in the seeds, and these new, identified in the leaves. Our investigation of a Nigerian specimen of *E. angolense*, also suggests a chemotaxonomic differentiation within this genus. In fact, like *E. cylindricum*, *E. angolense* produces protolimonoids. Otherwise, *E. bussei*, *E. candollei*, *E. caudatum*, *E. spicatum* and *E. utile* furnish limonoids of the mexicanolide group (condensed A–C rings, B-seco tetranor-triterpenoids) and of the phragmalin group (A-ring bridged mexicanolide) (Taylor, 1984). *G. cedrata* belongs to the same plant family Meliaceae just as *E. angolense* but to a different genus. The chemotaxonomic difference between these two taxons is the fact that a limonoid co-occurs with the protolimonoids in the bark extracts of *G. cedrata* while in the case of *E. angolense* no limonoid was found co-occurring with protolimonoids.

Apparently, the tirucallane system does not get directly transformed to limonoids. In fact, the biosynthetic studies of the limonoid nimbolide (Ekong et al., 1971; Ekong and Ibiyemi, 1985) had shown that euphol and apo-euphol rather than the tirucallol systems are the preferred intermediates in the biosynthesis of the limonoid. This hypothesis could explain the isolation of the tirucallane and not the euphane system which is biogenetically transformed to limonoids while the tirucallane system does not usually get transformed to limonoids. In this regard, it is noted that dymacrin D from *D. macranthum* was not found co-occurring with limonoids. Secondly and noteworthy is the fact that by far the higher majority of isolated protolimonoids (Connolly and Hill, 1991) belongs to the tirucallane and not the euphane system.

The *n*-hexane extract of *E. angolense* in a preliminary bioassay experiment was shown to be active against *Pseudomonas aeruginosa* and *Bacillus subtilis* resp. at 250 and 300 μg/ml.

3. Experimental

3.1. General

Flash chromatography (FC): Merck-Kieselgel 60 (70–230 mesh), Merck RP-18 LiChroprep (40–65 μm). TLC: Merck-Kieselgel 60 PF_{254}. HPLC: Merck-Hitachi L-7100 pump and L-7400 UV detector, Reinin Dynamax 60A CN (8 μm) 25.1 cm column, solvent flux 3 ml/min. Optical rotation: JASCO-DIP-181, $[\alpha]_D$ in deg ml dm^{-1} g^{-1}. UV: Perkin–Elmer Lambda3 (λ_{max} in nm, ε in mol^{-1} l cm^{-1}). IR: Pye-Unicam SP3-200S (ν_{max} in cm^{-1}). NMR: Bruker AV400 (^1H and 2D-NMR spectra at 400 MHz) and Varian XL-300 (^{13}C NMR spectra at 75.4 MHz), δ in ppm using residual solvent signals as internal standard ($CDCl_3 = 77.0$, $CHCl_3 = 7.26$, $C_6D_6 = 128.7$, $C_6D_5H = 7.15$), J values in Hz, multiplicities and peak assignments from DEPT, ^1H, ^1H-COSY, $^1J_{CH}$- and $^nJ_{CH}$-COSY, chemical shifts of overlapping protons were deduced from COSY and HSQC maps and rounded off ±0.025 ppm. NOESY data are reported as correlation map(s) between protons $^1H \leftrightarrow {}^1H$; HMBC data are reported as $^{13}C \rightarrow$ correlated to ^1H. The subscript symbols R and S stand for *pro-R* and *pro-S*. MS: Kratos MS80 with home-built acquisition system. Molecular mechanics (MM) calculations were carried out with the PCMODEL V7.5 computer program, searching for the global energy minimum (Fig. 1).

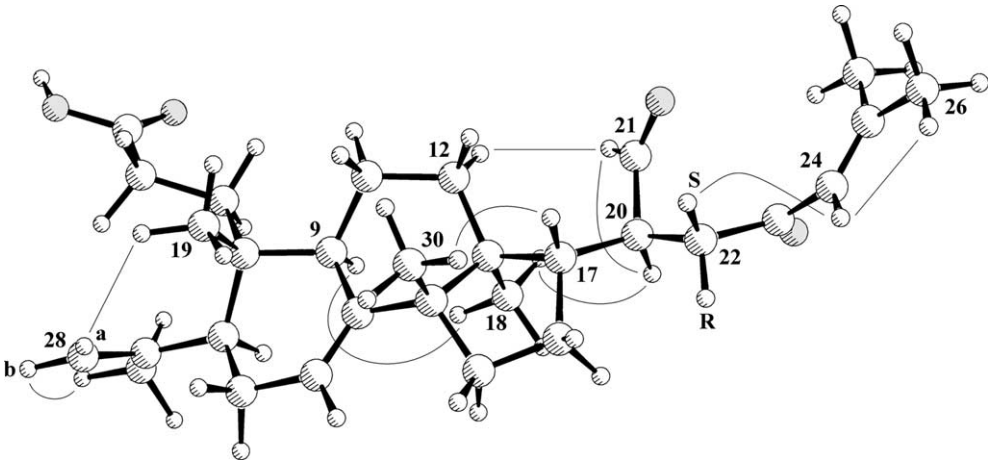


Fig. 1. MM-calculated lowest-energy conformer of compound **2** and main NOESY correlations.

3.2. Plant material

The leaves of *E. angolense* used in this work were collected from Iyere-Owo, Ondo State, Nigeria in November in 1998. Mr. A.O. Ohaeri (now late) of the Herbarium of the National Institute for Pharmaceutical Research and Development, Abuja, FCT, Nigeria authenticated the sample. Herbarium specimen was deposited at the National Institute for Pharmaceutical Research and Development's Herbarium in Abuja, FCT, Nigeria (No. 3606).

3.3. Extraction and isolation

The dried pulverized sample (2.1 kg) was extracted by percolating with refluxing *n*-hexane in an 20 liters aspirator bottle which consists of a modified Soxhlet extractor. The extract was concentrated *in vacuo* to give a gummy solid 56.2 g. A portion of the sample (10 g) was subjected to flash chromatography on RP-18 Merck silica gel and eluted with an increasing gradient of methanol in water. The fractions collected were monitored with TLC (*n*-hexane-ethyl acetate, 4:1) and those with similar R_f were pulled together. Fractions eluted with 60–90% MeOH (3.6 g, mixture B) were further fractionated by normal phase silica gel with a gradient of ethyl acetate in *n*-hexane to give β-sitosterol, stigmasterol and 3,23-dioxotirucalla-7,24-dien-21-al (**1**, 4.7 mg). The fraction eluted with 100% MeOH (2.7 g, mixture C) was subjected to HPLC separation on a CN-column with a gradient of ethanol in *n*-hexane thus furnishing 3,4-secotirucalla-23-oxo-4(28),7,24-trien-21-al-3-oic acid (67 mg, **2**) and 3,4-secotirucalla-23-oxo-4(28),7,24-trien-21-al-3,21-dioic acid (21-methyl ester) (53 mg, **3**).

3.3.1. 3,23-Dioxotirucalla-7,24-dien-21-al (*1*)

Powder. $[\alpha]_D$ −41.7° (EtOH, *c* 0.24). UV λ_{max}^{EtOH} nm (log ε): 233 (3.82). IR ν_{max} (KBr) : 1775, 1740 and 1711. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta \sim 1.45$ and ∼2.00 (two H, H-1), 2.74 (1H, *ddd*, $J_{gem} = 15.0$ Hz, $J_{2\beta,1\alpha} = 14.0$ Hz, $J_{2\beta,1\beta} = 6.0$ Hz, H-2β), ∼2.25 (1H, H-2α), 1.72 (1H, *t*, $J_{5,6} = 8.0$ Hz, H-5), ∼2.10 (2H, H-6), 5.32 (1H, *br.s*, H-7), ∼2.30 (1H, H-9), 1.50–1.60 (2H, H-11), ∼1.40 (1H, H-12α), ∼1.55 (1H, H-12β), ∼1.35 and ∼1.50 (two H, H-15), 1.90–2.00 (2H, H-16), ∼1.95 (1H, H-17), 0.99 (9H, *s*, H-18 and H-19 and H-30), ∼3.00 (1H, H-20), 10.00 (1H, *br.d*, $J_{21,20} = 2.0$ Hz, H-21), ∼2.95 (1H, H-22S), 2.60 (1H, *d*, $J_{gem} = 15.0$ Hz, H-22R), 6.06 (1H, *br.s*, H-24), 1.87 (3H, *d*, $J_{26,24} = 1.0$ Hz, H-26), 2.11 (3H, *d*, $J_{27,24} = 1.0$ Hz, H-27), 1.05 (3H, *s*, H-28), 1.11 (3H, *s*, H-29); (400 MHz, C_6D_6): $\delta \sim 1.00$ and ∼1.45 (two H, H-1), 2.41 (1H, *ddd*, $J_{gem} = J_{2\beta,1\alpha} = 14.0$ Hz, $J_{2\beta,1\beta} = 6.0$ Hz, H-2β), 2.16 (1H, *ddd*, $J_{gem} = 14.0$ Hz, $J_{2\alpha,1} = 4.0$ Hz, $J_{2\alpha,1} = 3.0$ Hz, H-2α), ∼1.80 (1H, H-17), 3.01 (1H, *tt*, $J_{20,17} = J_{20,22S} = 10.0$ Hz, $J_{20,21} = J_{20,22R} = 2.0$ Hz, H-.20), 10.11 (1H, *d*, $J_{21,20} = 2.0$ Hz, H-21), 2.89 (1H, *dd*, $J_{gem} = 17.0$ Hz, $J_{22S,20} = 10.0$, H-22S), 2.33 (1H, *dd*, $J_{gem} = 17.0$ Hz, $J_{22R,20} = 2.0$, H-22R). ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 1. NOESY (400 MHz, $CDCl_3$): H-5 ↔ 3H-28, H-21 ↔ H-12α and H-20, H-24 ↔ H-22R and H-22S and 3H-26. HMBC (400 MHz, $CDCl_3$): C-3 and C-4 → 3H-28 and 3H-29, C-6 and C-19 and C-29 → H-5, C-23 → H-24, C-25 → 3H-26 and 3H-27. EIMS (70 eV) *m/z* (rel. int.): 452 $[M]^+$ (1), 437 $[M - Me]^+$ (4), 424 $[M - CO]^+$ (6), 419 $[M - Me - H_2O]^+$ (5), 409 $[M - Me - CO]^+$ (5), 83 (100). HREIMS *m/z* 452.3289 ± 0.005 $[C_{30}H_{44}O_3]^+$, calc. 452.3290.

3.3.2. 3,4-Secotirucalla-23-oxo-4(28),7,24-trien-21-al-3-oic acid (*2*)

Amorphous powder, mp 79–81 °C. $[\alpha]_D$ +9.2° (EtOH, *c* 0.24). UV λ_{max}^{EtOH} nm (log ε): 236 (3.99). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta \sim 1.65$ (2H, H-1), ∼2.25 and ∼2.40 (two H, H-2), ∼2.40 (1H, H-5), ∼2.05 and ∼2.20 (two H, H-6), 5.26 (1H, *br.s*, H-7), ∼2.45 (1H, H-9), 1.40–1.55 (2H, H-11), ∼1.45 (1H, H-12α), ∼1.60

(1H, H-12β), 1.45–1.60 (2H, H-15), ~1.30 and ~1.95 (two H, H-16), ~1.90 (1H, H-17), 1.02 (3H, *s*, H-18), 0.82 (3H, *s*, H-19), ~3.00 (1H, H-20), 9.99 (1H, *br.d*, $J_{21,20} = 2.0$ Hz, H-21), ~3.00 (1H, H-22S), 2.58 (1H, *d*, $J_{gem} = 15.0$ Hz, H-22R), 6.06 (1H, *qq*, $J_{24,26} = J_{24,27} = 1.0$ Hz, H-24), 1.88 (3H, *d*, $J_{26,24} = 1.0$ Hz, H-26), 2.10 (3H, *d*, $J_{27,24} = 1.0$ Hz, H-27), 4.79 (1H, *br.s*, H-28a), 4.84 (1H, *br.s*, H-28b), 1.77 (3H, *br.s*, H-29), 0.97 (3H, *s*, H-30); (400 MHz, C_6D_6): δ 1.65–1.80 (2H, H-1), ~2.25 and ~2.35 (two H, H-2), ~1.35 (1H, H-12α), ~1.60 (1H, H-12β), ~1.80 (1H, H-17), 3.00 (1H, *tt*, $J_{20,17} = J_{20,22S} = 10.0$ Hz, $J_{20,21} = J_{20,22R} = 2.5$ Hz, H-20), 10.10 (1H, *d*, $J_{21,20} = 2.0$ Hz, H-21), 2.88 (1H, *dd*, $J_{gem} = 17.0$ Hz, $J_{22S,20} = 10.0$ Hz, H-22S), ~2.30 (1H, H-22R). ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 1. NOESY (400 MHz, $CDCl_3$): H-7 ↔ 2H-6 and 2H-15, H-17 ↔ 3H-30, 3H-18 ↔ H-9 and H-20, H-21 ↔ H-12α and H-20, H-24 ↔ H-22R and H-22S and 3H-26, H-28a ↔ H-5 and 3H-19, H-28b ↔ 3H-29. NOESY (400 MHz, C_6D_6): H-21 ↔ H-12α and H-20. HMBC (400 MHz, $CDCl_3$): C-3 → 2H-2, C-4 → H-5 and 3H-29, C-10 → H-5 and 3H-19, C-8 and C-13 and C-14 and C-15 → 3H-30, C-21 and C-23 → H-22R, C-23 and C-25 → 3H-27. EIMS (70 eV) *m/z* (rel. int.): 468 $[M]^+$ (15), 450 $[M - H_2O]^+$ (4), 395 (9), 255 (5), 83 (47), 28 (100). HREIMS *m/z* 468.3244 ± 0.005 $[C_{30}H_{44}O_4]^+$, calc. 468.3240.

*3.3.3. 3,4-Secotirucalla-23-oxo-4(28),7,24-trien-21-al-3,21-dioic acid (21-methyl ester) (**3**)*

Amorphous powder, mp 90–92 °C. $[\alpha]_D$ +6.8° (EtOH, *c* 1.36). UV λ_{max}^{EtOH} nm (log ε): 236 (3.81). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50–1.70 (2H, H-1), ~2.25 and ~2.40 (two H, H-2), ~2.40 (1H, H-5), ~2.00 and ~2.15 (two H, H-6), 5.24 (1H, *br.s*, H-7), ~2.45 (1H, H-9), 1.40–1.55 (2H, H-11), ~1.35 and ~1.60 (two H, H-12), 1.45–1.65 (2H, H-15), ~1.25 and ~1.95 (two H, H-16), ~1.95 (1H, H-17), 0.97 (3H, *s*, H-18), 0.81 (3H, *s*, H-19), 2.75 (1H, *td*, $J_{20,17} = J_{20,22S} = 10.0$ Hz, $J_{20,22R} = 2.0$ Hz, H-20), 3.68 (3H, *s*, MeO), 2.83 (1H, *dd*, $J_{gem} = 16.0$ Hz, $J_{22S,20} = 10.0$ Hz, H-22S), 2.60 (1H, *dd*, $J_{gem} = 16.0$ Hz, $J_{22R,20} = 2.0$ Hz, H-22R), 6.01 (1H, *br.s*, H-24), 1.86 (3H, *d*, $J_{26,24} = 1.0$ Hz, H-26), 2.10 (3H, *d*, $J_{27,24} = 1.0$ Hz, H-27), 4.78 (1H, *br.s*, H-28a), 4.82 (1H, *br.s*, H-28b), 1.76 (3H, *br.s*, H-29), 0.96 (3H, *s*, H-30); ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 1. NOESY (400 MHz, $CDCl_3$): H-7 ↔ 2H-6 and 2H-15, H-24 ↔ H-22R and H-22S and 3H-26, H-28a ↔ H-5 and 3H-19, H-28b ↔ 3H-29. HMBC (400 MHz, $CDCl_3$): C-3 → 2H-2, C-4 → H-5 and 3H-29, C-10 → H-5 and 3H-19, C-8 and C-13 and C-14 → 3H-30, C-21 → H-22R and MeO, C-23 and C-25 → 3H-27. EIMS (70 eV) *m/z* (rel. int.): 498 $[M]^+$ (25), 452 (10), 425 (14), 393 (13), 83 (100). HREIMS *m/z* 498.3343 ± 0.005, $[C_{31}H_{46}O_5]^+$, calc. 498.3345.

Acknowledgements

The authors are grateful to the authorities of the Laboratorio di Chimica Bioorganica, Università degli Studi di Trento, Via Sommarive 14, I-38050 Povo, Trento, Italy for allowing most of the laboratory part of this work to be carried out in their laboratory and to the National Institute for Pharmaceutical Research and Development, P.M.B. 21 Abuja, FCT, Nigeria for supporting A.T.O.'s visit for this work and for study leave.

This work was financially supported by grants from MIUR (PRIN 2003) and Provincia Autonoma di Trento (AGRIBIO).

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