$[M^+]$ 470.3485; $C_{30}H_{46}O_4$ requires: 470.3564. UV λ_{Max}^{MeOH} nm (log ε): 211 (3.65). IR ν_{Max}^{KBr} cm^{-1}: 3584, 3384, 1695. ^1H NMR (400 MHz, pyridine-d_5): δ5.82 (*dd*, 1H, J = 7.2, 3.6 Hz), 4.29 (*ddd*, 1H, J = 10.7, 4.0, 2.6 Hz), 3.77 (*d*, 1H, J = 2.6 Hz), 0.86, 0.93, 0.94, 1.04, 1.13, 1.20 (each 3H, *s*), −0.08 (*dd*, 1H, J = 4.8, 4.8 Hz). For ^{13}C NMR, see Table 1. EIMS (70 ev) *m/z* (rel. int.): 470 $[M]^+$ (82.8), 455 $[M-Me]^+$ (7.5), 452 $[M-H_2O]^+$ (90.9), 425 $[M-COOH]^+$ (7.7), 316 (2.1), 301 (30.5), 232 (1.2), 187 (100), 133 (34.2).

Przewanoic acid B (**2**). Recrystallized from MeOH as white needles, mp 258–259°, $[\alpha]_D$ + 103° (MeOH; *c* 0.465). Found: $[M]^+$ 454.3381; $C_{29}H_{42}O_4$ requires: 454.3252. UV λ_{Max}^{MeOH} nm (log ε): 210 (3.65). IR ν_{Max}^{KBr} cm^{-1}: 3426, 1694, 1650, 905. ^1H NMR (400 MHz $CDCl_3$): δ5.58 (*dd*, 1H, J = 7.4, 4.0 Hz), 5.06 (*s*, 1H), 4.73 (*s*, 1H), −0.08 (*dd*, 1H, J = 4.8 Hz). For ^{13}C NMR, see Table 1. EIMS (70 ev) *m/z* (rel. int.): 454 $[M]^+$ (29.1), 439 $[M-Me]^+$ (5.1), 436 $[M-H_2O]^+$ (34.8), 409 $[M-COOH]^+$ (4.5), 300 (8.2), 285 (8.6), 232 (4.1), 187 (100), 133 (22.1).

Przewanoic acid B acetate (**3**). Compound **2** (6 mg) was dissolved in pyridine (0.4 ml) and Ac_2O (0.6 ml) and left overnight. Normal work-up gave przewanoic acid B acetate (4 mg) as needles. ^1H NMR (90 MHz $CDCl_3$): δ1.98 (*s*, 3H, COMe), 2.07 (*s* 3H, COMe).

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MYRIANTHINIC ACID: A NEW TRITERPENOID FROM *MYRIANTHUS ARBOREUS*

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Key Word Index—*Myrianthus arboreus*; Cecropiaceae; bark; pentacyclic triterpenoids; Myrianthinic acid; 3β,6β-dihydroxyolean-12-en-29-oic acid.

Abstract—A new pentacyclic triterpene acid has been isolated from the stem bark of *Myrianthus arboreus* and its structure has been established as 3β,6β-dihydroxyolean-12-en-29-oic acid and named myrianthinic acid.

INTRODUCTION

Myrianthus arboreus P. Beauv., Cecropiaceae [1] is a small tropical African tree, which grows from Guinea to southern Angola, from southern Sudan to western Tanzania and through Ouganda. The plant is widely used in indigenous medicine: the infusion of bark to treat dysentery; the leaves against heartaches, the accidents during pregnancy and dysmenorrheae; the juice of young leaves against toothaches and bronchitis [2]. Early works on *M. arboreus* reported the isolation of peptide alkaloids from the leaves [3], tormentic acid, 2-acetyl tormentic acid, 3-acetyl tormentic acid and euscaphic acid from the root wood [4, 5], and Myrianthic acid from the same organ [6]. We now report the isolation from the trunk bark of *M. arboreus*, of a new triterpenic acid, myrianthinic acid (**2**) as its methyl ester (**1**).

RESULTS

From the methylated ethyl acetate extracts of ground barks, the methyl ester of myrianthinic acid (**1**) $C_{31}H_{50}O_4$ was obtained which crystallized from methylene chloride as colourless granules, mp 145–147°. The IR spectrum revealed absorptions at ν_{max} 1700 (–COOMe) and 1645 cm^{-1}, and at 3450 and 3525 cm^{-1}, indicative of the presence of two hydroxyl groups. Myrianthinic acid

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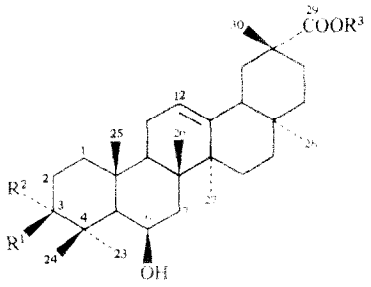


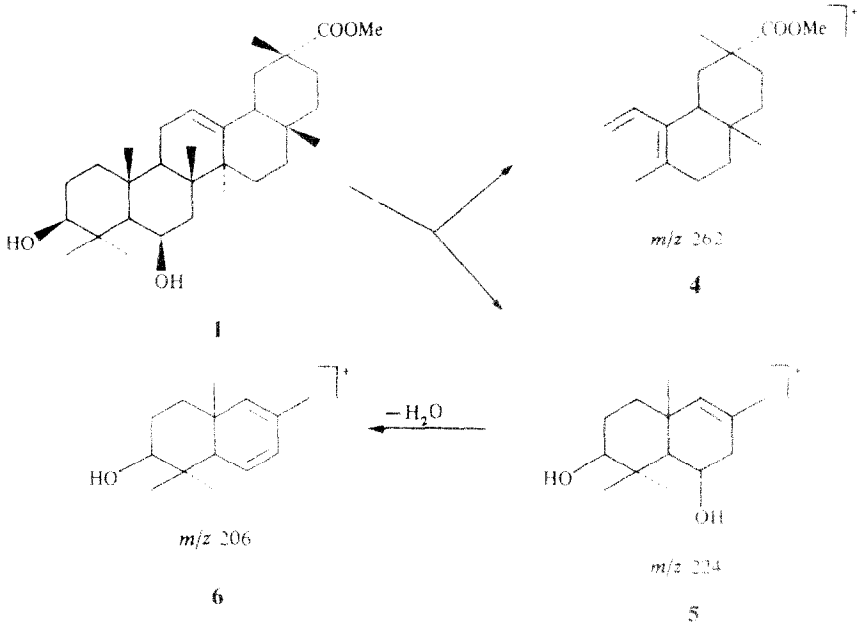
1 R^1 = OH, R^2 = H, R^3 = Me
2 R^1 = OH, R^2 = H, R^3 = H
3 R^1 = OAc, R^2 = H, R^3 = Me
4 R^1, R^2 = O, R^3 = Me

methyl ester (**1**) gave, upon acetylation with acetic anhydride–pyridine, the monoacetate (**3**) which still revealed a hydroxyl absorption at 3525 cm^{-1} in its IR spectrum.

The ^1H NMR spectrum of **1** in $CDCl_3$ showed five three-proton singlets at δ0.68, 0.78, 0.90, 1.20 and 1.24, assigned to the C-24, C-26, C-25, C-27 and C-29 methyl groups, respectively, and a single six-proton singlet at 0.98 attributable to C-23 and C-28. A one-proton multiplet was observed at 2.50–2.70, which could be assigned to the C-3α proton, a three-proton signal at 3.58 which was attributed to a carbomethoxyl group, and another one-proton signal at 5.30 due to the olefinic proton at C-12.

The mass spectrum of **1** showed the molecular ion at *m/z* 486 which agreed with the molecular formula $C_{31}H_{50}O_4$. The base peak occurred at *m/z* 469 (M + 1 − H_2O). The peak at *m/z* 262 (248 + 14) obtained from retro-Diels–Alder fragmentation indicated that the compound was a pentacyclic triterpene of the β-amyrin group with a Δ^{12} double bond [7, 8]. More specifically, the latter showed that the carbomethoxyl group was in the place of C-27, C-28 or C-29/C-30 methyls, which are the only methyl groups attached on the rings C, D and E. In addition, the fragment at *m/z* 262 was consistent with the fact that neither of the two hydroxyl groups was present on the above rings. Consequently, these hydroxyls must be attached on the rings A and/or B. This argument was confirmed by the presence of a fragment at *m/z* 224 (Scheme 1).

The facile loss of 18 mass units (− H_2O) from **5** to give **6** was in good accord with the formation of a stable 1,3-diene in ring B [9]. This suggests the position C-6 for the second hydroxyl group. The location of the second hydroxyl group was established by the results of acetylation of **1** which gave a monoacetyl derivate (**3**). In addition, oxidation of myrianthinic acid methyl ester (**1**) with Jones reagent resulted in the formation of a ketone (**4**) which showed the molecular ion at *m/z* 484 in the mass spectrum, and the IR spectrum revealed a sharp absorption at ν_{max} 3560 cm^{-1}, indicating that only one of the hydroxyl groups of the diol had been oxidized. The difficulty both of acetylation and oxidation of the second hydroxyl group could be explained in two possible ways: firstly, a possible location of the hydroxyl at a tertiary position, and secondly, its location at a hindered environment. The tertiary nature was ruled out on grounds of the presence of a geminal downfield proton. Accordingly, its resistance both to acetylation and oxidation indicated it to be located at a hindered position in ring B, since hydroxyl groups in ring A at positions C-1, C-2 and C-3 are known to undergo acetylation and oxidation readily [10]. Accordingly, the second hydroxyl group was finally suggested to be at C-6, this position being hindered by the C-24, C-25 and C-26 methyl groups. The orientation of



Scheme 1. Mass spectral fragmentation of the methylester of myrianthinic acid (**1**).

this hydroxyl must be β because of its high shielding effect on the C-24, C-25 and C-26 methyl groups. In fact, when an acetoxyl or a hydroxyl group is present at a 6α-position, the C-24, C-25 and C-26 methyl groups appear at δ0.88, 0.98 and 0.98, respectively [11, 12]. However, in myrianthinic acid methyl ester (**1**), the same methyl groups appear upfield at 0.68, 0.78 and 0.90, respectively, because of the 1,3-diaxial interaction [10]. Similarly, it is known from the works of Tursch [11, 13] that when a β-hydroxy group is located at C-3 in triterpenoids, the C-23 methyl group appears at about 1.00 whereas when an acetoxyl group is located at this carbon, then the C-23 methyl group resonates further upfield at 0.86. In myrianthinic acid methyl ester (**1**), the C-23 methyl group appeared at 0.98, and in the 3β-acetate derivative, the C-23 methyl group resonated at 0.84, which is in good accord with Tursch [11, 13], thus confirming the location of the first hydroxyl group in the 3β-position in myrianthinic acid methyl ester (**1**) and the acetoxyl group at the same carbon in the acetyl derivative (**3**).

There were two alternative positions for the location of the carbomethoxyl group: C-28 or C-29/30. From ref. [11], when a COOMe (or COOH) replaces the C-28 methyl group, the C-30 methyl group is expected to appear downfield at δ0.96, and when COOMe (or COOH) is in the place of the C-29 methyl group, the C-30 methyl group resonates at 1.23. In myrianthinic acid methyl ester (**1**), the C-30 methyl group appeared at 1.24. This is in agreement with the location of the COOMe at C-29. To sum up, in the light of the above evidence, myrianthinic acid methyl ester (**1**) is identified as 3β,6β-dihydroxyolean-12-en-29-oic methyl ester and the natural product is 3β,6β-dihydroxyolean-12-en-29-oic acid (**2**), which was obtained from **1** by hydrolysis.

EXPERIMENTAL

Melting points were uncorr. Mass spectra: 70 eV, by chemical ionisation and/or electron impact. IR:KBr discs silica gel GF_{254} (Merck) and silica gel 60 (70–230 mesh ASTM) (Merck) were used for TLC and CC, respectively, and the spots on TLC were visualized by spraying with H_2SO_4 (50%) and heating at 150°.

Extraction and isolation of myrianthinic acid methyl ester (**1**). The plant (stem bark) was collected at Nkoa-Mbang, Yaoundé area, Cameroon, in November 1985, and was identified as *Myrianthus arboreus* P. Beauv. A voucher specimen is deposited in the Cameroon National Herbarium, Yaoundé. The pulverized sun-dried stem bark (6.2 kg) of *M. arboreus* was successively extracted with hot hexane and MeOH. The MeOH extracts were concd under red. pres. to give a brown gummy solid, which was dissolved in 10% aq. MeOH, and extracted with EtOAc. The EtOAc extracts were evapd under red. pres. to furnish a solid (68 g) which was further dissolved in Et_2O and treated with an excess of ethereal soln of CH_2N_2. Chromatography of the EtOAc methylated extract (20 g) and elution with EtOAc–hexane (1:3) yielded a mixture of two compounds (1:9), which gave, from a subsequent chromatography and recrystallization from CH_2Cl_2, myrianthinic acid methyl ester (**1**) (160 mg). The compound gave a positive Liebermann–Buchard test; mp 145–147°. IR ν_{max}^{KBr} cm^{-1}: 3525 and 3450 (OH), 1700 (carbomethoxyl), 1645 disubstituted double bond), 1360 and 1380 (gem dimethyl), 1220 (C–O). ^{1}H NMR ($CDCl_3$): described in the text. MS *m/z*: 486 $[M]^+$, 469 $[M+1-H_2O]^+$, 451 $[M+1-H_2O-H_2O]^+$, 262 [D and E rings]$^+$, 224 [A and B rings with two hydroxyl groups]$^+$.

Myrianthinic acid (**2**). Compound **1** (30 mg) was heated under reflux with 1g KOH in ethylene glycol (10 ml) for 15 hr. Dilution of the reaction medium (20 ml H_2O), acidification with dil. HCl and extraction with Et_2O (25 ml × 3) gave a solid which was recrystallized from CH_2Cl_2 to yield whitish granules of myrianthinic acid (**2**) mp 290–292°. IR ν_{max}^{KBr} cm^{-1}: 3560 and 3350 (OH), 1680 (carboxyl), 1640 (disubstituted double bond), 1370 and 1380 (*gem* dimethyl), 1230 (C–O) MS *m/z*: 472 $[M]^+$, molecular formular $C_{30}H_{48}O_4$.

Myrianthinic acid methyl ester monoacetate (**3**). Compound **1** (50 mg) was treated with a mixture of Ac_2O-pyridine (5 ml each) for 1.5 hr, and the reaction medium evaporated under red. pres. to give the derivative **3** which crystallized from EtOH as colourless granules; mp 200°. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1720 (ester), 166C (carbomethoxyl), 1640 (disubstituted double bond), 1260 (C–O ^{1}H NMR ($CDCl_3$): δ0.68 (3H, *s*, 24-H_3), 0.84 (6H, *s*, 23 and 26-H_3), 0.90 (3H, *s*, 25-H_3), 1.18 (3H, *s*, 27-H_3), 1.22 (6H, *s*, 28 and 30-H_3), 2.00 (3H, *s*, Me–C(=O)–O), 2.58 (1H, *m*, 6α-H) 3.58 (3H, *s* carbomethoxyl), 4.50 (1H, *m*, 3α-H), 5.38 (1H, *m*, 12-H) MS *m/z* 528 $[M]^+$. Molecular formular $C_{33}H_{52}O_5$.

Jones oxidation derivative of myrianthinic acid methyl ester (**4**). Compound **1** (30 mg) was dissolved in Me_2CO (8 ml). Whil cooling, Jones reagent (0.2 ml) was added. After 20 min stirrin the reaction medium was poured into 50 ml of a 10% soln c NaOAc. Extraction with Et_2O and concn of the extracts unde red. pres. gave a solid, which was further purified by CC an recrystallization from hexane to yield the monoketonic derivati **4** (95% yield), mp 202–203, 5° IR ν_{max}^{KBr} cm^{-1}: 3560 (OH), 17 (ketone in a six-membered ring) 1695 (carbomethoxyl), 16 (disubstituted double bond) 1370 and 1385 (*gem* dimethyl), 12 (C–O). MS *m/z*: 484 $[M]^+$ molecular formular $C_{31}H_{48}O_4$, ar traces of the diketonic derivative.

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