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MYRIANTHIC ACID: A TRITERPENE ACID FROM THE ROOTWOOD OF *MYRIANTHUS ARBOREUS*

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Key Word Index—*Myrianthus arboreus*; Urticaceae; triterpenoid; arjunolic acid; myrianthic acid; 2α,3α,24-trihydroxy-19α-hydroxy-urs-12-en-28-oic acid.

Abstract—Arjunolic acid and a new triterpene acid, myrianthic acid, were isolated from the rootwood of *Myrianthus arboreus*. The structures of the two compounds were elucidated by IR, ^{1}H NMR and mass spectroscopy.

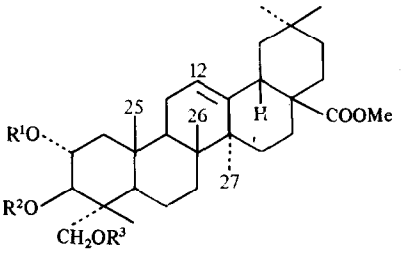
INTRODUCTION

In our previous paper [1], we reported the isolation, from the rootwood of *Myrianthus arboreus*, of tormentic acid, euscaphic acid, 2-acetyl tormentic acid and 3-acetyl tormentic acid as their respective methyl esters as well as a polar solid mixture eluted by Et_2O–MeOH (9:1). We report now the isolation, from the polar material, of arjunolic acid (**1**) and a new triterpene acid, myrianthic acid (**3**), as their methyl esters.

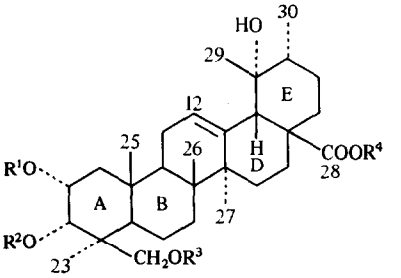
RESULTS AND DISCUSSION

The polar solid mixture was separated by preparative TLC to afford two compounds, **1** and **3**, which were purified on short columns of silica gel. Compound **1**, mp 248–250° (lit. [2]: mp 248–250°), $[M]^+$ at *m/z* 502, was identified by its spectroscopic data (Table 1) as methyl arjunolate [3, 4]. Compound **3**, mp 126°, molecular formula $C_{31}H_{50}O_6$, was assigned its structure on the basis of the following spectroscopic data. The mass spectrum of **3** had $[M]^+$ at *m/z* 518 with prominent peaks at *m/z* 278, 260, 200, 179 and 146 which were quite diagnostic. The fragment ion at *m/z* 278 usually results from retro-Diels–Alder cleavage of ring C of Δ^{12}-pentacyclic triterpenes with a C-19 hydroxy group [1, 5–7], suggesting similar environments in rings C and E of **3**. The IR spectrum showed bands at 3420 cm^{-1} (hydroxyl group), 1720 cm^{-1} (–COOMe) and 1620 cm^{-1} (>C=CH–). The ^{1}H NMR showed a one-proton singlet at δ2.60 which was assigned to H-18 by comparison with similar systems [5, 6]. The singlet nature of the peak confirmed the presence of a hydroxyl group at C-19.

On acetylation, compound **3** gave a triacetate (**4**), $C_{37}H_{56}O_9$, mp 204–205°, whose IR spectrum revealed the presence of a tertiary hydroxyl group (3545 cm^{-1}), acetate groups (1740, 1245, 1225 cm^{-1}), a methyl ester (1730 cm^{-1}) and a trisubstituted double bond (1610 cm^{-1}). The ^{1}H NMR spectrum of **4** revealed three acetoxy groups (3H, each *s*, at δ1.96, 2.01 and 2.06). One of these groups was primary. This was demonstrated by the presence of a two-proton AB quartet between δ3.75 and 4.15 (J_{AB} = 12 Hz). The position and disposition (quartet) of the signal were characteristic of an axial C-24 acetoxymethylene (H_2-24) [8]. The axial H-2, the equatorial H-3 and the olefinic H-12 appeared at δ5.20 (1H, *m*, $W_{\frac{1}{2}}$ = 16 Hz), 4.94 (1H) and 5.3 (1H, *m*), respectively. The mass spectrum of **4** had the $[M]^+$ at *m/z* 644, the $[M - H_2O]^+$ fragment at *m/z* 626, and the diagnostic fragment ions *m/z* 278 (RDA), 260 $[278 - H_2O]^+$, 201 $[260 - MeCOO]^+$ and 179. The mass spectrum of **4** further confirmed that **3** was a Δ^{12}-α-amyrin having C-19



1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = R^3 = Ac$



3 $R^1 = R^2 = R^3 = H, R^4 = Me$
4 $R^1 = R^2 = R^3 = Ac, R^4 = Me$
5 $R^1 = R^2 = R^3 = R^4 = H$

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Table 1. ^{1}H NMR chemical shifts of methyl groups of triterpenes [6, 10, 11]

	H-23	H-24	H-25	H-26	H-27	H-29	H-30
Methyl olean-12-en-28-oate	0.88	0.83	0.93	0.73	1.15	0.93	0.92
Methyl 2α,3α,23-trihydroxyolean-12-en-28-oate*	—	0.91	0.98	0.69	1.13	0.91	0.92
Methyl 2α,3β,23-trihydroxyolean-12-en-28-oate*	—	0.84	0.99	0.71	1.13	0.93	0.93
Methyl arjunolate†	—	0.82	1.00	0.72	1.13	0.93	0.93
Compound 1‡	—	0.83	1.02	0.72	1.14	0.93	0.93
Methyl 2α,3α,24-triacetoxyolean-12-en-28-oate*	1.02	—	1.11	0.71	1.14	0.91	0.92
Methyl 2α,3α,23-triacetoxyolean-12-en-28-oate*	—	0.92	1.09	0.73	1.12	0.89	0.90
Methyl 2α,3β,23-triacetoxyolean-12-en-28-oate*	—	0.90	1.10	0.72	1.12	0.92	0.93
Methyl triacetyl arjunolate†	—	0.89	1.08	0.73	1.11	0.89	0.92
Compound 2‡	—	0.89	1.09	0.73	1.11	0.89	0.91

*Calculated value.
†Data kindly supplied by Professor K. Nakanishi.
‡Observed value.

Table 2. ^{1}H NMR chemical shifts of methyl groups of triterpenes [6, 10, 11]

	H-23	H-24	H-25	H-26	H-27	H-29	H-30
Methyl urs-12-en-28-oate	0.87	0.83	0.93	0.75	1.08	0.8	0.95
Methyl 2α,3β,23-triacetoxy-19α-hydroxyurs-12-en-28-oate*		0.90	1.10	0.69	1.22	1.16	0.94
Methyl 2α,3α,23-triacetoxy-19α-hydroxyurs-12-en-28-oate*		0.92	1.09	0.69	1.22	1.15	0.95
Methyl 2β,3β,23-triacetoxy-19α-hydroxyurs-12-en-28-oate*		1.03	1.20	0.73	1.19	1.18	0.96
Methyl 2α,3β,24-triacetoxy-19α-hydroxyurs-12-en-28-oate*	1.04		1.12	0.68	1.20	1.16	0.94
Methyl 2β,3β,24-triacetoxy-19α-hydroxyurs-12-en-28-oate*	1.03		1.22	0.71	1.21	1.18	0.96
Methyl 2α,3α,24-triacetoxy-19α-hydroxyurs-12-en-28-oate*	1.02		1.11	0.68	1.20	1.15	0.93
Compound 4†	1.02		1.10	0.70	1.22	1.15	0.95

*Calculated value.
†Observed value.

hydroxyl and C-28 carbomethoxy groups [5, 7, 9]. The calculated values (Table 2) for the chemical shift of the angular methyls of the triacetate (4) agreed with the observed values to within 2 Hz, which was in accord with the basic premise of additivity [10, 11]. Methyl myrianthate was therefore assigned structure 3 and myrianthic acid is therefore 2α,3α,19α,24-tetrahydroxy-urs-12-en-28-oic acid (5).

EXPERIMENTAL

Mps were determined on a Reichert Akt microscope hot stage model and are uncorr. ^{1}H NMR spectra were taken at 60 MHz in $CDCl_3$ with TMS as an internal standard, and chemical shift values are in δ. Mass spectra were measured at 70 eV. TLC was on Merck Kieselgel 60, pF 254 + 366 developed with C_6H_6–EtOAc (1:3) and eluted with $CHCl_3$–MeOH (1:1).

Extraction of Myrianthus arboreus. *Myrianthus arboreus* was collected at Choba, Port Harcourt, Nigeria, and the voucher sample was deposited at the University herbarium in the Faculty of Biological Sciences. The powdered rootwood (10 kg) was successively extracted with petrol (60–80°), $CHCl_3$ and EtOH. The concentrates of the extracts gave solids (30 g from $CHCl_3$ and 28 g from EtOH).

Isolation of the triterpene acids. The triterpene acids and the polar solid mixture (400 mg) were isolated from the EtOH extract (5 g) as in ref. [1]. Separation of the components of the mixture (400 mg) eluted by Et_2O–MeOH (9:1) was carried out as follows: TLC of the polar solid revealed two components, 1 and 3. Compound 3 was visible under UV light (254 or 366 nm) while compound 3 (R_f 0.15), was detected by iodine vapour. The R_f of 1 was used to mark out the position of 1 on a prep. TLC plate which was run under the same conditions as the analytical TLC. The polar compound (400 mg) was separated by prep. TLC as outlined above to afford 3 (230 mg) and 1 (100 mg).

Purification of compound 1. Compound 1 (100 mg) was purified on a short column of silica gel (mesh 0.05–0.2 mm) and eluted with 30% MeOH in $CHCl_3$ to give 90 mg 1, which on recrystallization from EtOH gave 1 (85 mg), mp 215–216°, followed by resolidification and final mp 248–250°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (methyl ester), 1610 (trisubstituted double bond). ^{1}H NMR: δ0.72 (3H, *s*), 0.83 (3H, *s*), 0.93 (6H, *s*), 1.02 (3H, *s*), 1.14 (3H, *s*), 3.43–3.93 (4H), 3.60 (3H, *s*, –COOMe), 5.26 (1H, *m*, H-12). MS *m/z*: 502 $[M]^+$, 442 $[M - MeCOOH]^+$, 262 (RDA), 203 $[262 - MeCOO]^+$, 189, 133. (Found: C, 72.38; H, 10.52. $C_{31}H_{50}O_5 \cdot C_2H_5OH$ requires: C, 72.22; H, 10.29%.)

Acetylation of 1 (*methyl arjunolate*). Compound 1 (57 mg) was treated with Ac_2O–pyridine (1:1) at room temp. for 5 days to afford 55 mg crude acetate after work-up in the usual way. The crude acetate was chromatographed on a short column of silica gel, and eluted with petrol–Et_2O (4:1) to yield a solid which crystallized from EtOH to furnish 30 mg 2, mp 223–224°. IR ν_{max}^{KBr} cm^{-1}: 1750, 1735 (acetate), 1720 (methyl ester), 1620 (trisubstituted double bond). ^{1}H NMR: δ0.73 (3H, *s*), 0.89 (6H, *s*), 0.91 (3H, *s*), 1.11 (3H, *s*), 1.09 (3H, *s*), 1.96, 2.01, 2.07 (3H each, *s*, O–COMe), 2.87 (1H, *q*, *J* = 18 Hz, H-18), 3.60 (3H, *s*, –COOMe),

ABq centred at 3.68 (2H, J_{AB} = 12 Hz, H_2-23), 4.93–5.37 (3H, H-2, H-3, H-12). MS *m/z*: 628 $[M]^+$, 568 $[M - MeCOOH]^+$, 262 (RDA), 203 $[262 - MeCOO]^+$, 189, 133.

Purification of compound **3**. Compound **3** (230 mg) was purified on a short column of silica gel to give **3** (200 mg), mp 126°. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1720 (methyl ester), 1620 (trisubstituted double bond). ^1H NMR: δ0.69 (3H, *s*), 0.74 (3H, *s*), 0.99 (3H, *s*), 1.22 (3H, *s*), 1.23 (3H, *s*), 2.60 (1H, *s*, H-18β), 3.9 (1H, *m*, $W_{\frac{1}{2}}$ = 16 Hz, H-2), 3.65 (2H, *q*, H_2-24), 3.4 (1H, H-3), 3.60 (3H, *s*, COOMe), 5.34 (1H, *m*, H-12). MS *m/z*: 518 $[M]^+$, 500 $[M - H_2O]^+$, 458, 278 (RDA), 260 $[278 - H_2O]^+$, 200 $[260 - MeCOOH]^+$, 179, 146.

Acetylation of **3**. Compound **3** (56 mg) was treated with Ac_2O–pyridine (1:1) at room temp. for 5 days, and after work-up in the usual way 47 mg crude acetate was obtained. The product (46 mg) was purified on a short column of silica gel, and eluted with petrol–Et_2O (7:3) to afford 33 mg crystalline **4**, mp 204–205°. IR ν_{max}^{KBr} cm^{-1}: 3545 (tertiary OH), 1740, 1245, 1225 (acetoxy), 1730 (methyl ester), 1610 (trisubstituted double bond). ^1H NMR: δ0.70 (3H, *s*), 0.95 (3H, *s*), 1.07 (3H, *s*), 1.02 (3H, *s*), 1.10 (3H, *s*), 1.15 (3H, *s*), 1.22 (3H, *s*), 1.96, 2.01, 2.06 (3H each, *s*, O–COMe), 3.75, 4.15 (2H, ABq, J_{AB} = 12 Hz, H_2-24), 4.94 (1H, H-3), 5.20 (1H, *m*, $W_{\frac{1}{2}}$ = 16 Hz, H-2), 5.3 (1H, *m*, H-12). UV λ_{max}^{MeOH} nm: 212 (ε = 3488). MS *m/z*: 644 $[M]^+$, 626 $[M - H_2O]^+$, 584 $[M - MeCOOH]^+$, 512 $[584 - C_4H_8O]^+$, 365, 278 (RDA), 260, 219, 201, 179, 146. (Found: C, 68.77; H, 9.32. Calc. for $C_{37}H_{56}O_9$: C, 68.91; H, 8.75%.)

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