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PENTACYCLIC TRITERPENOID RHAMNOSIDES FROM *COMBRETUM IMBERBE* LEAVES

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Key Word Index—*Combretum imberbe*; Combretaceae; triterpenoid rhamnosides.

Abstract—Two new triterpenoid rhamnopyranosides have been isolated from the leaves of *Combretum imberbe* and their structures established as 23-hydroxyimberbic acid 23-*O*-α-L-rhamnopyranoside and 23-hydroxyimberbic acid 23-*O*-α-L-rhamnopyranoside 1-acetate by spectral analysis and chemical transformations.

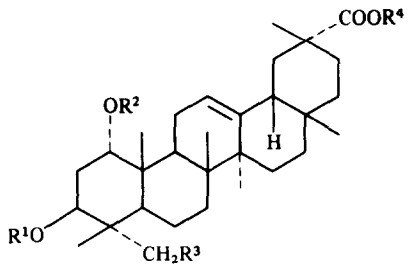
INTRODUCTION

It has been reported previously that the leaves of *Combretum imberbe*, commonly known as the leadwood tree, contain a complex mixture of triterpenoids and their glycosides [1]. Further investigation of the minor triterpenoid constituents of the leaf ether extract has yielded two derivatives of imberbic acid (**1**), the previously isolated and characterized major constituent of the extract [2]. This paper describes the isolation and structure elucidation of these two new acidic triterpenoid rhamnopyranosides, 23-hydroxyimberbic acid 23-*O*-α-L-rhamnopyranoside **(2)** and 23-hydroxyimberbic acid 23-*O*-α-L-rhamnopyranoside 1-acetate **(3)**.

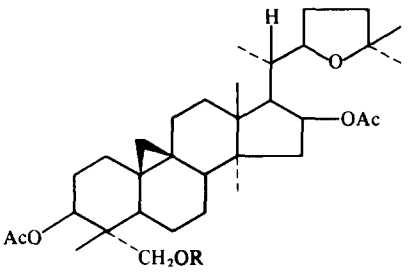
RESULTS AND DISCUSSION

Following the procedure described in a previous paper [2], the air-dried leaves were extracted and the ether extract partially purified. Exhaustive column chromatography of the polar fractions from this ether extract yielded small amounts of the two new compounds, **2** and **3**. Compound **2**, the major compound, has the molecular composition $C_{36}H_{58}O_9$ (elemental analysis and the mass spectrum of the peracetate **2a**).

NMR spectroscopy indicated the presence of a carboxylic acid function, hydroxy substituents, a trisubstituted double bond, an AB system and a sugar moiety. Mildly forcing conditions (heating for 1 hr with Ac_2O in pyridine) were required to prepare **2a**, the peracetate of **2**, which suggested that one (or more) of the hydroxy substituents was hindered. Acid hydrolysis of **2** and a co-chromatographic investigation of the aqueous hydrolysate identified the sugar as rhamnose (assumed to be L-rhamnose on biogenetic grounds). This was confirmed by ^{13}C NMR spectroscopy; the sugar carbon resonances in the spectra of **2** and its peracetate **(2a)** (Table 1) corresponded perfectly with α-rhamnopyranoside signals in known compounds [3, 4]. To facilitate separation, the acid hydrolysis fraction containing the aglycone moiety was esterified with diazomethane and acetylated.



	R^1	R^2	R^3	R^4
1	H	H	H	H
1a	Ac	Ac	H	H
2	H	H	O—Rh	H
2a	Ac	Ac	O—RhAc_3	H
3	H	Ac	O—Rh	H
4	Ac	Ac	O—Ac	Me
5	Ac	H	O—Ac	Me



	R
6	$GlcAc_4$

Rh = α-L-rhamnose
Glc = β-D-glucose

Column chromatography of the resultant mixture produced two products which ^{1}H NMR analysis showed were the triacetate ester (**4**) and the diacetate ester (**5**). IR spectral data showed that **5**, the minor product, contained a free, and therefore hindered hydroxy group. The aglycone moiety of the glycoside **2** must therefore have two free hydroxy groups.

A comparison of the ^{13}C NMR data for **2** and **2a** and the two aglycone derivatives **4** and **5** with that of imberbic acid (**1**) and its diacetate (**1a**) [2] (Table 1) revealed that these compounds differ only in certain of the ring A carbon resonances, i.e. compound **2** has a pentacyclic triterpenoid skeleton with a Δ^{12}-double bond and a C-29 carboxylic acid group. This meant that the hydroxy substituents and the sugar moiety are located on ring A in compound **2**. Since the C-2 and C-10 resonances were almost identical for both imberbic acid (**1**) and compound **2** and for the equivalent acetylated compounds **1a**, **2a**, and the aglycone **4** (Table 1), it follows that one of the hydroxy groups in **2** must be axial and situated at C-1 as in imberbic acid (**1**). The hindered nature of this hydroxy group would account for the reluctance of **2** and **5** to acetylate completely under mild conditions. The presence of a signal (δ4.74, $J_1 = J_2 = 3$ Hz) in the ^{1}H NMR spectrum of **4** identical to that assigned to the equatorial acetoxymethine proton at C-1 in the spectrum of imberbic acid diacetate (**1a**) confirmed the 1α-OH placement.

The AB system in the ^{1}H NMR spectra of **2a** and the aglycone acetates **4** and **5** indicated that one of the remaining oxygen functions in ring A had to be primary and therefore situated at C-4. The absence of the geminal C-23 and C-24 methyl carbon signals from ^{13}C NMR spectrum of **2** was further indication that one of these methyls is oxidised. The stereochemistry at C-4 was established by NMR spectroscopy as follows. Firstly the $1/2[\delta A + \delta B]$ value for the acetoxy methylene protons in the AB system in the ^{1}H NMR spectrum of **4** is δ3.83, which is typical for an equatorial CH_2OAc group at C-4 [5]. Secondly the ^{13}C NMR spectra of compounds **2**–**5** all exhibit a signal at *ca* δ12.8 typical of the resonance for a shielded axial methyl carbon (C-24) adjacent to a carbon containing an oxygen function at C-4 [6]; the equa-

Table 1. ^{13}C NMR spectral data for compounds **1**–**5**

C	1*	1a†	2*	2a†	3*	4†	5†
1	72.8	75.8	71.4	73.5	74.9	73.8	71.4
2	35.9	27.6	35.1	27.2	31.0	27.1	30.4
3	71.9	74.1	66.5	69.5	65.6	69.9	70.2
4	40.1	37.6	42.4	41.0	42.2	40.6	40.6
5	48.6	49.1	40.9	41.2	42.5	42.0	40.6
6	17.4	18.0	18.2	17.8	18.2	17.8	17.8
7	33.0	31.8	32.0	31.3	31.7	31.7	31.6
8	39.8	39.5	39.7	39.5	39.6	39.6	39.4
9	38.4	38.0	38.1	38.0	38.3	38.2	37.8
10	42.7	39.9	42.6	39.7	40.0	39.8	40.6
11	23.8	22.9	23.4	22.9	23.2	23.0	23.0
12	123.7	122.1	122.4	122.4	122.0	122.0	122.3
13	144.7	143.9	144.4	143.7	144.9	144.1	144.1
14	41.7	41.8	41.1	41.7	41.3	41.8	41.9
15	26.7	25.8	25.9	26.0	25.9	25.8	25.8
16	27.5	26.7	27.1	26.7	26.8	26.7	26.8
17	33.0	32.2	32.6	32.2	32.5	32.3	32.3
18	46.8	45.6	46.5	45.6	46.3	45.8	45.9
19	41.8	40.2	41.2	40.1	40.9	40.2	40.4
20	43.0	42.6	42.6	42.4	42.4	42.6	42.6
21	30.0	29.0	29.6	28.7	29.3	29.0	28.9
22	36.7	35.7	36.3	35.7	36.1	35.8	35.7
23	28.6	27.8	71.4	69.8	70.1	65.5	65.5
24	16.8	16.4	12.8	12.8	12.5	12.7	12.7
25	16.6	15.6	16.9	15.8	15.8	16.0	16.6
26	16.6	16.7	17.2	16.7	16.9	16.8	16.8
27	26.3	25.8	26.2	25.5	26.4	25.8	25.8
28	29.0	27.9	28.2	27.9	28.0	28.0	28.1
29	181.4	179.2	181.1	184.3	181.0	179.2	179.2
30	18.9	19.1	19.7	19.0	19.5	19.2	19.2
1′			101.6	97.5	101.4		
2′			72.1	69.6	72.3		
3′			72.8	68.9	72.8		
4′			73.4	71.0	73.5		
5′			69.6	66.4	69.6		
6′			18.4	17.2	18.2		

*Measured in pyridine-d_5 relative to TMS.
†Measured in $CDCl_3$ relative to TMS.

torially oriented methyl group (C-23) at this position would be expected to resonate in the region of δ23 [7].

It is reasonable to assume on biogenetic grounds that the remaining hydroxy substituent is situated at C-3 as in imberbic acid (**1**). This was confirmed by comparing the H-3 signal in the ^{1}H NMR spectrum of imberbic acid diacetate (**1a**) with the signal of the remaining unassigned acetoxymethine proton in **4**. This showed that the signals were superimposable (*dd*, $J_1 = 10$ Hz, $J_2 = 7.2$ Hz), although the signal from the latter compound had shifted downfield by δ0.31, a shift that can be attributed to the deshielding of the H-3 proton by the 23-CH_2OAc function [8]. Furthermore the γ-*gauche* shielding influence of this function on C-3 can explain the difference of δ4.2 between the C-3 ^{13}C NMR resonances in imberbic acid diacetate (**1a**) (δ74.1), and the chemical shift of the third acetoxy methine resonance in **4** (δ69.9). Consequently this hydroxy function is assigned to the equatorial C-3 position in **2**.

The position of the sugar on **2** was adduced to be at C-23 by a comparative analysis of the NMR data from **2**, **2a** and **4** and that presented by thalicosides VII (**6**), a 23-*O*-glucopyranoside [9]. Firstly, of the possible positions, C-1 and C-3 have identical ^{13}C NMR chemical shifts in the spectra of the glycoside **2a** and the aglycone **4**, whereas the shifts for C-23 differed by δ4.3, i.e. it exhibits a glycosylation shift. Secondly, it was clear from the different splitting patterns and chemical shifts observed for the AB system in the ^{1}H NMR spectra of **2a** (δ3.54; δ2.90; $J = 9.8$ Hz; δA-B = 0.64) and **4** (δ3.95; δ3.71 $J = 11.6$ Hz; δA-B = 0.24); that while the environment around the 23-methylene protons was different in these two cases, there was excellent agreement between the values for **2a** and those obtained for thalicoside VII (**6**) (δ3.62; δ3.07; δA-B = 0.55) [9]. The structure of **2** is therefore 1α,3β,23-trihydroxy-olean-12-en-29-oic acid 23-*O*-α-L-rhamnopyranoside.

Compound **3**, the minor constituent, was found to have the molecular formula $C_{38}H_{60}O_{10}$ (elemental analysis and the mass spectrum of its peracetate). ^{13}C NMR spectroscopy showed that this compound was a monoacetate and only differed from **2** in the carbon resonances of ring A. More specifically the carbons most affected were those around C-1 and it was obvious that the differences observed in the chemical shifts in the spectra of **2** and **3**, firstly at C-2 and C-10 and secondly at C-3 and C-5, were typical β-acetylation and γ-*gauche* upfield shifts consistent with the presence of an axial acetate substituent at C-1. The conversion of compound **3** into the peracetate (**2a**) by the usual means was final proof that **3** was 1α-acetoxy-3β,23-dihydroxy-olean-12-en-29-oic acid 23-*O*-α-L-rhamnopyranoside.

EXPERIMENTAL

Mps: uncorr. MS: direct inlet, 70eV. ^{1}H NMR: 90 MHz, TMS as int. standard; ^{13}C NMR: 20 MHz, multiplicities by SFORD and Inversion Recovery (pulse sequence [1.0–180–0.5–90]n), TMS as int. standard. IR: KBr.

Material and extraction. The source of the leaf material and the extraction of the polar compounds has been reported previously [2]. Silica gel CC of the extracts containing the polar compounds (eluent: petrol–EtOAc–EtOH from 1:1:0 to 0:19:1) afforded **2** (250 mg) and **3** (45 mg).

Imberbic acid 23-O-α-L-*rhamnopyranoside* (**2**). Colourless needles from EtOH, mp 212–215°, $[\alpha]_D^{25} + 12.9°$ (pyridine; *c* 1.0). IR ν_{max}cm^{-1}: 3420, 3870, 2925, 2650 (CO_2H dimer), 1695 (carboxy CO) 1457, 1383, 1126, 1049. ^{1}H NMR (pyridine) δ0.59–1.31 (7 × Me), 3.70–5.20 (sugar and aglycone hydroxy-(pyridine-d_5) methine protons) and 5.30 (1H, *m*, H-12); ^{13}C NMR (pyridine-d_5): see Table 1; (Found: C, 66.67; H, 9.24. $C_{36}H_{58}O_9$; H_2O requires C, 66.23; H, 9.26%).

Imberbic acid 23-O-α-L-*rhamnopyranoside pentacetate* (**2a**). Complete acetylation of **1** was achieved by heating (100°) an Ac_2O–pyridine soln of **1** (60 mg) for 2 hr and allowing the soln to stand for 12 hr. The usual work-up provided needles of **2a** crystallized from EtOAc (65 mg) mp 193–194°, $[\alpha]_D^{25} + 22.6°$ ($CHCl_3$; *c* 1.0); EIMS *m/z* (rel. int.) 784 $[M - HOAc]^+$ (2), 724 $[M - 2 \times HOAc]^+$ (1), 451 (2), 273 [sugar] (1), 248 (1), 203 (2), 153 (40), 111 [sugar] (28). IR ν_{max}cm^{-1}: 2935, 1747–1734 (acetate, carboxy CO), 1369, 1250–1222 (acetate), 1082, 1042. ^{1}H NMR ($CDCl_3$): δ0.81–1.25 (6 × Me), 1.19 (*d*, Me rhamnose), 1.96 (OCOMe), 2.00, 2.04, 2.14 (3 × OCOMe rhamnose), 2.17 (OCOMe), 2.90 (1H, *d*, $J = 9.8$ Hz, H-23a), 3.54 (1H, *d*, $J = 9.8$ Hz, H-23b), 3.85 (*m*, H-5′), 4.63 (*br s*, H-1′), 4.75 (1H, *dd*, $J_1 = J_2 = 3$ Hz, H-1β), 4.92–5.40 (*m*, 3H-2′, 3′, 4′), 5.22 (1H, *m*, H-12); ^{13}C NMR ($CDCl_3$): see Table 1.

Acid hydrolysis of **1**. Compound **2** (80 mg) was hydrolysed with 5.0 M HCl in MeOH (10 ml) at reflux temp. for 4 hr, cooled, diluted with H_2O (50 ml) and extracted × 3 with $CHCl_3$. This fraction was treated with CH_2N_2 and then Ac_2O in pyridine in the usual way and the aglycone ester acetates **4** and **5** isolated by silica gel CC using petrol–EtOAc (8:3) as eluant. The H_2O layer was neutralized with $NaHCO_3$, evapd to dryness and the sugar residue extracted with pyridine. TLC analysis on silica gel with AcOEt–MeOH–H_2O–AcOH (19:3:3:4) and also $CHCl_3$–AcOH–H_2O (6:7:1) identified rhamnose as the sugar present in the hydrolysate.

Compound **4**. Colourless glass, EIMS *m/z* (rel. int.): 628 $[M]^+$ (1), 613 (1), 586 $[M - Ac]^+$ (16), 575 (20), 569 $[M - CO_2Me]^+$ (40), 568 $[M - HOAc]^+$ (95), 532 (70), 508 $[M - 2 \times HOAc]^+$ (20), 435 $[M - 2 \times HOAc - CH_2OAc]^+$ (15), 424 (32), 262 (24), 223 (42), 203 (38), 187 (74), 149 (100). IR ν_{max}cm^{-1}: 2920, 2850, 1726, (ester, acetate), 1369, 1249–1237 (acetate), 1109, 1037. ^{1}H NMR ($CDCl_3$): δ0.83, 0.87, 0.98, 1.07, 1.13, 1.19 (3H each, *s*, Me-24, 28, 25, 26, 27, and 30), 1.99, 2.06, 2.09 (3H each, *s*, 3 × OCOMe), 3.65 (3H, *s*, CO_2Me), 3.71 (1H, *d*, $J = 11.6$ Hz, H-23a), 3.95 (1H, *d*, $J = 11.6$ Hz, H-23b) 4.74 (1H, *dd*, $J_1 = J_2 = 3$ Hz, H-1β), 5.13 (1H, *dd*, $J_1 = 6.3$ Hz, $J_2 = 10$ Hz, H-3α) 5.16 (1H, *t*, H-12); ^{13}C NMR ($CDCl_3$): see Table 1.

Compound **5**. Colourless glass; EIMS *m/z* (rel. int.): 586 $[M]^+$ (25), 568 $[M - H_2O]^+$ (31), 540 (16), 526 $[M - HOAc]^+$ (28), 521 (18), 508 $[M - H_2O - HOAc]^+$ (27), 466 (13), 449 $[M - H_2O - HOAc - CO_2Me]^+$ (14), 448 (15), 433 (12), 325 (35), 262 (100), 203 (42), 187 (65). IR ν_{max}cm^{-1}: 3490 (OH), 2920, 2850, 1726 (ester, acetate), 1457, 1369, 1250–1242, 1110, 1039. ^{1}H NMR ($CDCl_3$): δ0.84, 0.86, 0.97, 0.98, 1.16, 1.18 (3H each, *s*, Me-24, 28, 25, 26, 27 and 30), 2.00, 2.06 (3H each, *s*, 3 × OCOMe), 3.58 (1H, signal partially obscured, H-1β), 3.63 (3H, *s*, CO_2Me), 3.70 (1H, *d*, $J = 11.6$ Hz, H-23a), 3.95 (1H, *d*, $J = 11.6$ Hz, H-23b), 5.19 (1H, *dd*, $J_1 = 6.4$ Hz, $J_2 = 10.4$ Hz, H-3α), 5.21 (1H, *t*, H-12); ^{13}C NMR ($CDCl_3$): see Table 1.

Imberbic acid 23-O-α-L-*rhamnopyranoside* 1-*acetate* (**3**). Colourless prisms from EtOH, mp 235–238°, $[\alpha]_D^{25} + 5.6°$ (pyridine; *c* 0.6). IR ν_{max}cm^{-1}: same as for **2** except 1264 (acetate): ^{1}H NMR (pyridine-d_5) δ2.01 (3H, *s*, OCOMe); ^{13}C NMR (pyridine-d_5): see Table 1; (Found; C, 64.06; H, 8.98. $C_{38}H_{60}O_{10}$; $2H_2O$ requires C, 64.04; H, 8.98%).

Base hydrolysis of **3**. Compound **3** (20 mg) was refluxed in 10% methanolic KOH (10 ml) until TLC analysis showed complete conversion of **3** to **2**. Dilution of the reaction mixture with H_2O

and acidification yielded a ppt. of **2** identified by co-chromatography, mp and IR.

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