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THE STRUCTURE OF IMBERBIC ACID, A 1α-HYDROXY PENTACYCLIC TRITERPENOID FROM *COMBRETUM IMBERBE*

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Key Word Index—*Combretum imberbe*; Combretaceae; 1α-hydroxy pentacyclic triterpenoid; ^{13}C NMR.

Abstract—A new 1α-hydroxy pentacyclic triterpenoid acid, imberbic acid, has been isolated from the leaves of *Combretum imberbe* and its structure established as 1α,3β-dihydroxyolean-12-en-29-oic acid by chemical and spectral analysis.

INTRODUCTION

In continuing our chemical investigation of the genus *Combretum*, we have isolated friedelin, epifriedelin and betulinic acid from the bark as well as naringenin and a new triterpenoid, imberbic acid (**1**) from the leaves of *Combretum imberbe*, a tree characterized by its extremely hard and dense, black heartwood. The isolation of imberbic acid is particularly significant, as the 1α-hydroxy substituent is rare in pentacyclic triterpenoids although its has been found to occur in cycloartenoids isolated from the leaves of three other species of the genus *Combretum*, *C. molle* [1], *C. eleaegnoides* [2] and *C. edwardsii* (Rogers, C. B., unpublished results). The presence of a 1α-hydroxy substituent in both tetra- and pentacyclic triterpenoids in Combretaceae is chemotaxonomically important, since it suggests a biogenetic link between these two triterpenoid groups in the genus *Combretum*, and could be a useful chemotaxonomic character. This is also the first reported occurrence of an olean-12-en-29-oic acid system in this genus.

RESULTS AND DISCUSSION

Air-dried leaves of *C. imberbe* were extracted successively with petrol and ether. The precipitate formed during the ether extraction was collected and separated by flash column chromatography on silica gel to yield imberbic acid (**1**) as the major constituent, as well as naringenin and a series of fractions containing complex mixtures of triterpenoid, triterpenoid glycosides and phenolic compounds. Work is continuing on the separation and identification of these minor constituents.

High resolution mass spectral analysis established the molecular formula of imberbic acid (**1**) as $C_{30}H_{48}O_4$. The presence of a carboxylic acid function, two hydroxy groups, and a trisubstituted double bond was indicated by NMR spectroscopy, and confirmed by the formation of a diacetate (**2**) and a diacetate methyl ester (**3**).

^{1}H NMR of these last two compounds showed that **1** has seven tertiary methyl groups, and two secondary hydroxy substituents. Splitting patterns for the acetoxymethine protons showed that one of the hydroxy groups is equatorial (δ4.82, $J_1 = 10$ Hz, $J_2 = 6$ Hz) and the other axial (δ4.73, $J_1 = J_2 = 3$ Hz). One of the tertiary methyl signals (δ1.22) obviously belongs to a methyl group adjacent a carboxy function, since it experiences a slight upfield shift to δ1.18 on esterification of the carboxy function.

The mass spectrum of **1** revealed a pair of diagnostically important mass peaks [*m/z* 248 and 203 (248 − CO_2H)] typical of the retro-Diels–Alder fragmentation in ring C of olean-12-ene derivatives containing a carboxy function in either ring D or E [3]. Acetylation of **1** did not alter the values of these two fragments whereas esterification caused a mass increment of *m/z* 14 in one of the fragments (*m/z* 262). The presence of a Δ^{12} double bond in an oleane system was confirmed by the appearance in all three compounds of a coarse triplet at δ5.15–5.31 (for H-12) and signals in the region of δ144 (*s*, C-13) and 122 (*d*, C-12) in the ^{1}H and ^{13}C NMR spectra respectively. The above data led to the conclusion that imberbic acid (**1**) was a member of the olean-12-ene series with a carboxy group at C-20 and two hydroxy groups situated in rings A/B.

A comparison of the ^{13}C NMR data for **1**–**3** (Table 1) with that of liquiritic acid (**4**) and glycyrrhetic acid (**5**) [4], showed that the resonances of the carbon atoms in ring D and E in imberbic acid (**1**) and its derivatives are in complete agreement with the values obtained for liquiritic acid (**4**), i.e. the C-20 carboxylic acid group in **1** occupies position 29 and is α-equatorially oriented. Furthermore, apart from the resonances of the ring A and C-9 carbons, the resonances of ring B and C carbons in **3** agree with the corresponding assignments in 3β-acetoxy-olean-12-en-29-oic acid methyl ester (**6**) [4], which restricts the positioning of the two hydroxy functions to ring A. On biogenetic grounds, it is reasonable to assume that one must be β-equatorial at C-3 ($J_1 = 10$ Hz, $J_2 = 7.2$ Hz) whereas the other axially oriented hydroxy substituent ($J_1 = J_2 = 3$ Hz) must be attached to C-1 or C-2.

A vicinal glycol can be excluded since Jones oxidation of **1** gave the diketone derivative **7** (M^+ 468) which exhibits characteristic β-diketone infrared absorptions at ν_{max}^{KBr} 1700 and 1725 cm^{-1}; carbonyl carbon resonances at δ208.3 and 210.9 in the ^{13}C NMR spectrum and ^{1}H NMR signals from an AB system centred at δ3.42 (2H, doublets at δ3.64 and 3.20, $J = 18.4$ Hz) attributed to the methylene protons at C-2 [5]. In addition, the presence of a

Table 1. ^{13}C NMR spectral data for compounds **1–3**, **6** [4] and **7**

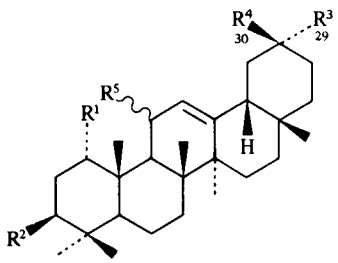
C	1†	2‡	3‡	6‡	7‡
1	72.8 *d*§	75.9 *d*	75.8 *d*	38.4	210.9* *s*
2	35.9 *t*	27.6 *t*	27.6 *t*	23.6	51.2 *t*
3	71.9 *d*	74.1 *d*	74.1 *d*	81.1	208.3* *s*
4	40.1 *s*	37.6 *s*	37.6 *s*	37.8	47.1 *s*
5	48.6 *d*	49.0 *d*	49.1 *d*	55.4	49.2 *d*
6	17.4 *t*	17.9 *t*	18.0 *t*	18.3	19.1 *t*
7	33.0 *t*	31.8 *t*	31.8 *t*	32.7	31.5 *t*
8	39.8 *d*	39.5 *d*	39.5 *d*	39.9	39.8 *d*
9	38.4 *d*	38.0 *d*	38.0 *d*	47.6	37.6 *d*
10	42.7 *s*	39.8 *s*	39.9 *s*	37.0	51.0 *s*
11	23.8 *t*	22.9 *t*	22.9 *t*	23.6	25.8 *t*
12	123.7 *d*	122.2 *d*	122.1 *d*	123.0	123.4 *d*
13	144.7 *s*	143.8 *s*	143.9 *s*	144.4	142.6 *s*
14	41.7 *s*	41.7 *s*	41.8 *s*	41.7	42.0 *s*
15	26.7 *t*	25.8 *t*	25.8 *t*	26.6	26.0 *t*
16	27.5 *t*	26.6 *t*	26.7 *t*	27.0	26.9 *t*
17	33.0 *s*	32.1 *s*	32.2 *s*	32.5	32.4 *s*
18	46.8 *d*	45.6 *d*	45.6 *d*	46.1	46.2 *d*
19	41.8 *t*	40.1 *t*	40.2 *t*	40.6	39.8 *t*
20	43.0 *s*	42.4 *s*	42.6 *s*	42.8	42.4 *s*
21	30.0 *t*	28.7 *t*	29.0 *t*	29.1	28.8 *t*
22	36.7 *t*	35.6 *t*	35.7 *t*	36.0	35.6 *t*
23	28.6 *q*	27.7* *q*	27.8* *q*	28.3	28.8 *q*
24	16.8 *q*	16.4 *q*	16.4 *q*	16.8	20.3 *q*
25	16.6 *q*	15.6 *q*	15.6 *q*	15.6	13.0 *q*
26	16.6 *q*	16.6 *q*	16.7 *q*	16.8	17.4 *q*
27	26.3 *q*	25.8 *q*	25.8 *q*	26.0	25.3 *q*
28	29.0 *q*	27.9* *q*	27.9* *q*	28.2	28.0 *q*
29	181.4 *s*	184.8 *s*	179.2 *s*	179.6	184.7 *s*
30	18.9 *q*	18.9 *q*	19.1 *q*	19.4	19.0 *q*

*Assignments in the same column interchangeable.

†Measured in C_5D_5N relative to TMS.

‡Measured in $CDCl_3$ relative to TMS.

§SFORD and Inversion Recovery [pulse sequence (1.0–180–0.5–90)*n*] multiplicity.



	R¹	R²	R³	R⁴	R⁵
1	OH	OH	CO_2H	Me	H
2	OAc	OAc	CO_2H	Me	H
3	OAc	OAc	CO_2Me	Me	H
4	H	OH	CO_2H	Me	=O
5	H	OH	Me	CO_2H	=O
6	H	OAc	CO_2H	Me	H
7	=O	=O	CO_2H	Me	H

hydroxy or acetoxy function at C-2 would reduce the ^1H NMR signed for H-3 from the observed double doublet to a doublet.

Finally, the ^{13}C resonances of C-5 and C-9 are both strongly shielded relative to the equivalent signals in the model compound (**6**). This shielding is due to typical γ-gauche interactions between these carbons and the axial hydroxy substituent at C-1 [6]. On the basis of this spectral data imberbic acid (**1**) is 1α,3β-dihydroxyolean-12-en-29-oic acid.

EXPERIMENTAL

Plant material. The leaves and bark of *C. imberbe* were collected on Blagdon Farm, Kadoma, Zimbabwe in January 1986. A herbarium specimen has been deposited at the University of Durban-Westville herbarium.

Bark extractives. Powdered bark (250 g) was extracted with boiling petrol (12 hr) followed by boiling Et_2O (12 hr) in a Soxhlet apparatus. Portions (5 g) of both the petrol and Et_2O extracts (18 and 10 g respectively) were subjected to CC separation (silica gel, solvent gradient petrol–EtOAc). Friedelin and epifriedelinol were isolated from the petrol extract and betulinic acid (characterized as the acetate) from the Et_2O extract. These known compounds were identified by TLC, MS, ^1H and ^{13}C NMR spectroscopy.

Leaf extractives. Air-dried leaves (1.3 kg) were milled and defatted with boiling petrol (24 hr) in a Soxhlet apparatus before they were extracted with boiling Et_2O (48 hr). A white solid (46 g) separated from the Et_2O soln on cooling and was collected by filtration. A portion (5 g) of this solid was separated by flash chromatography (silica gel, solvent gradient petrol–EtOAc followed by EtOAc–EtOH) into crude naringenin, imberbic acid (**1**) (563 mg) and several fractions containing polar compounds. Naringenin was purified by prep TLC (silica gel) and identified by IR, MS, ^1H and ^{13}C NMR spectroscopy.

Imberbic acid (**1**). Crystallized from EtOH as needles, mp 286–288°, $[\alpha]_D$ + 70.0° (C_5H_5N; *c* 1.0); EIMS (probe) 70 eV, *m/z* (rel. int): 472.3549 $[M]^+$ [$C_{30}H_{48}O_4$ requires: 472.3552] (1), 454.3444 $[M-H_2O]^+$ (9), 436 $[M-2\times H_2O]^+$ (2), 248. 1770 $[C_{16}H_{24}O_2]^+$ (100), 203.1800 $[C_{15}H_{23}]^+$ (9), 187.1488 $[C_{14}H_{19}]^+$ (62); ^1H NMR (90 MHz, C_5D_5N): δ0.91–1.40 (7 × Me), 3.77 (1H, *dd*, $J_1 = J_2 = 3$ Hz, 1-Hβ), 4.27 (1H, *dd*, $J_1 = 10$ Hz, $J_2 = 7$ Hz, 3-Hα) and 5.30 (1H, *m*, H-12); ^{13}C NMR (20 MHz, C_5D_5N): see Table 1.

Imberbic acid diacetate (**2**). Acetylation of **1** (100 mg) following the usual methods gave **2** as a colourless glass (MeCN–EtOH), mp 150–152°, $[\alpha]_D$ + 51.2° ($CHCl_3$; *c* 1.0); EIMS (probe) 70 eV, *m/z* (rel. int.): 556 $[M]^+$ (1), 496.3539 $[M-HOAc]^+$ requires 496.3553) (100), 451 $[M-HOAc-CO_2H]^+$ (2), 436 $[M-2HOAc]^+$ (18), 248.1776 $[C_{16}H_{24}O_2]^+$ (23), 233.1542 $[C_{15}H_{21}O_2]^+$ (16), 203 (20) 187.1487 $[C_{14}H_{19}]^+$ (35); ^1H NMR (90 MHz, $CDCl_3$): δ0.82 (3H, *s*, Me), 0.87 (3H, *s*, Me), 0.90 (3H, *s*, Me), 0.95 (3H, *s*, Me), 1.01 (*s*, Me-25), 1.12 (*s*, Me-27), 1,20 (s, Me-30), 2.00, 2.06 (3H, *s*, 2 × OCOMe), 4.72 (1H, *dd*, $J_1 = J_2 = 3$ Hz; 1-Hβ), 4.83 (1 H, *dd*, $J_1 = 10$ Hz, $J_2 = 7$ Hz, 3-Hα), 5.15 (1H, *t*, H-12); ^{13}C NMR (20 M Hz, $CDCl_3$): see Table 1.

Methyl imberbate diacetate (**3**). Esterification of (2) (100 mg) with CH_2N_2 gave **3** as colourless needles (EtOH–EtOAc), mp 178–180°, $[\alpha]_D$ + 41.8° ($CHCl_3$; *c* 1.0); EIMS (probe) 70 eV, *m/z* (rel. int.): 570.3910 $[M]^+$ [$C_{35}H_{54}O_6$ requires: 570.3920] (1), 510 $[M-HOAc]^+$ (100), 496.3549 $[C_{32}H_{48}O_4]^+$ (3), 450 $[M-2HOAc]^+$ (25), 262.1931 $[C_{17}H_{26}O_2]^+$ (21), 203, 1796 $[C_{15}H_{23}]^+$ (45), 187 (68); ^1H NMR (90 MHz, $CDCl_3$): δ0.83 (3H, *s*, Me), 0.88 (3H, *s*, Me), 0.91 (3H, *s*, Me), 0.96 (3H, *s*, Me), 1.02 (*s*, Me-25), 1.13 (*s*, Me-27), 1.18 (*s* Me-30), 2.01, 2.07 (3H, *s*, 2 × OCOMe), 3.65

(3H, *s*, CO_2Me), 4.73 (1H, *dd*, $J_1 = J_2 = 3$ Hz, 1 – Hβ), 4.83 (1H, *dd*, $J_1 = 10$ Hz, $J_2 = 7.3$ Hz, 3-Hα), 5.15 (1H, *t*, H-12); ^{13}C NMR (20 MHz, $CDCl_3$): see Table 1.

Imberbic acid diketone (7). The acid (1) (100 mg) was dissolved in Me_2CO and oxidized with Jones reagent. The yellow solid obtained from the work-up was purified by CC (silica gel) to give a colourless glass, mp 132–133°. ν_{max}^{KBr} cm^{-1}: 2980–2860, 1725, 1700, β-diketone, 1610, 1465, 1455, 1380, 1260, 1180, 1035, 810. EIMS (probe) 70 eV, *m/z* (rel. int.): 468 $[M]^+$ (32), 424 $[M - CO_2]^+$ (17), 423 $[M - CO_2H]^+$ (14), 248 (100), 203 (18), 187 (72); ^{1}H NMR (90 MHz, $CDCl_3$): δ0.86–1.24 (21 H, 7 × Me), 3.42 (2H, AB–*q*, $J = 18.4$ Hz, $\delta_{A-B} = 34.06$ Hz), 5.31 (1H, *t*, H-12); ^{13}C NMR (20 MHz, $CDCl_3$): see Table 1.

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REFERENCES

1. Pegel, K. H. and Rogers, C. B. (1985) *J. Chem. Soc. Perkin Trans. I* 1711.
2. Osborne, R. and Pegel, K. H. (1984) *Phytochemistry* **23**, 635.
3. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
4. Duddeck, H., Elgamal, M. H. A., Ricca, G. S., Danieli, B. and Palmisano, G. (1978) *Org. Magn. Reson.* **11**, 130.
5. Pant, P. and Rastogi, R. P. (1977) *Phytochemistry* **16**, 1787.
6. Eggert, H., Van Antwerp, C. L., Bhacca, N. S. and Djerassi, C. (1976) *J. Org. Chem.* **41**, 71.