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CYCLOARTENOID DIENONE ACIDS AND LACTONES FROM *COMBRETUM ERYTHROPHYLLUM*

COLIN B. ROGERS

Department of Chemistry, University of Durban-Westville, Private Bag X54001, Durban 4000, South Africa

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Key Word Index—*Combretum erythrophyllum*; Combretaceae; leaves cycloartenoid dienone acids and lactones; hydrogenolysis.

Abstract—Seven novel triterpenoid acids and lactones with a 9β,19-cyclopropyl-1-en-3-one skeleton have been isolated from the leaves of *Combretum erythrophyllum*. Spectral analysis has shown these compounds to be either hydrated or dehydrated in ring C and to have unsaturated γ- or δ-lactones in their side-chains. Erythrophyllic acid, the representative compound of this series, has been identified as 3-oxo-cycloart-1,11,24-trien-21-oic acid. The most abundant compound, 3-oxo-cycloart-1,11,24-trien-23,21-olide, yielded two hydrogenolysis products when hydrogenated using Adams catalyst.

INTRODUCTION

Combretum erythrophyllum (Burch.) Sonder (the river bushwillow, 1995 South African tree of the year) is a medium sized, spreading, densely foliaged tree up to 12 m in height that has been used by traditional healers for a variety of disorders [1]. This tree is of interest because the fruit is considered poisonous and the practice in Zimbabwe of using material from this tree to reduce the size of the vaginal orifice has recently resulted in the deaths of five women [2]. This practice could be the cause of many more deaths as it appears widespread in rural areas.

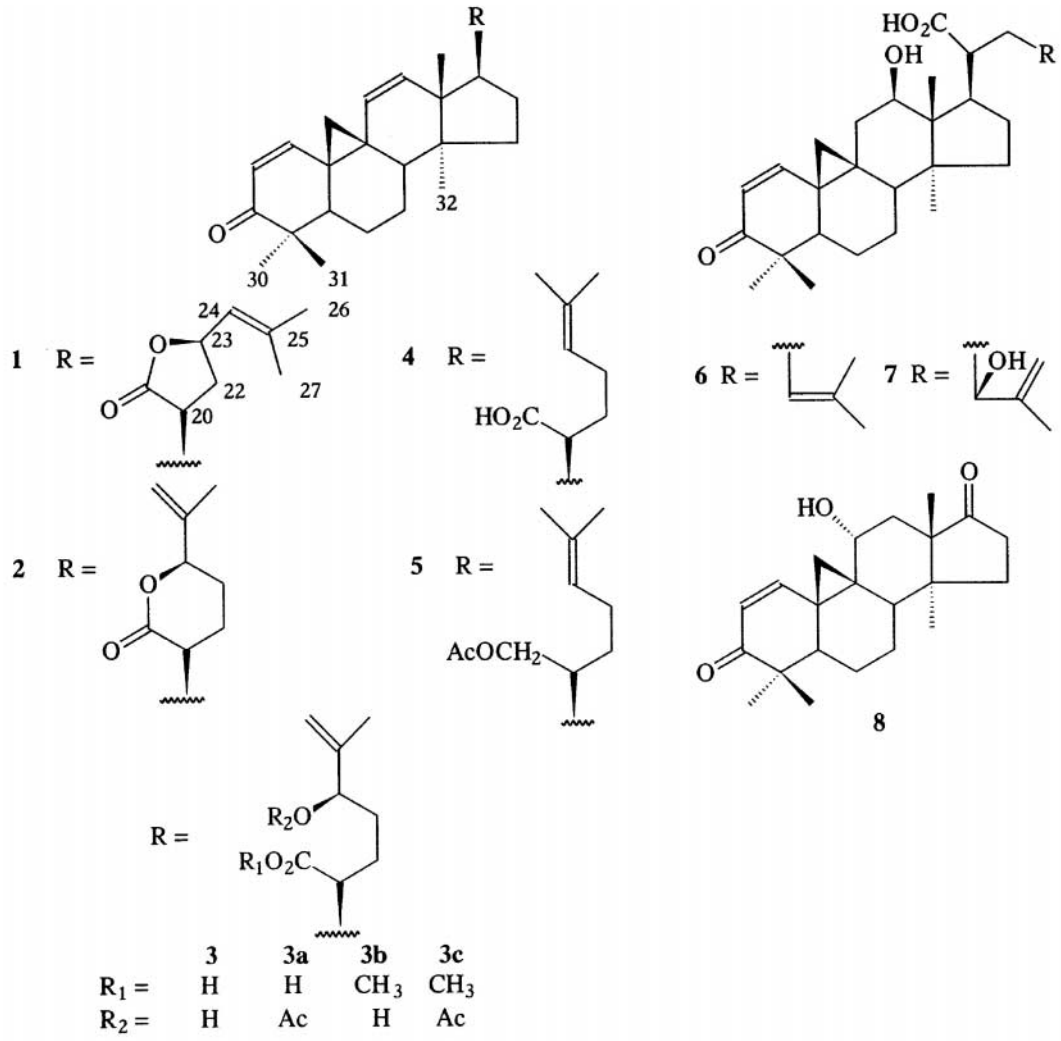
In keeping with all other species of the *Combretum* subgenus *Combretum* [3], scale-like trichomes on the leaf epidermis of *C. erythrophyllum* secrete copious amounts of a complicated mixture of acidic triterpenoids, long chain fatty acids and flavonoids onto the surface of the leaves [4]. Although fatty acids and flavonoids have been isolated from *Combretum* species before, no other species examined thus far has produced them in the quantity and complexity found in *C. erythrophyllum*.

Both the composition of the acidic triterpenoids and the anatomy of the secreting trichomes are species specific [4, 5]. Thus far the triterpenoids isolated from the *Combretum* belong almost exclusively to two distinct groups; *viz*. 30-carboxy-1α-hydroxycyloartanes [6] and 29-carboxy-1α-hydroxyoleanes [7]. This study describes a series of novel cycloartane dienone acids and lactones isolated in the search for the compounds responsible for the toxicity of this plant. The representative compound from this series was given the trivial name erythrophyllic acid (**4**). Attempts to saturate one of the triterpenoids by hydrogenation using Adams catalyst resulted in two unusual hydrogenation products.

Thus far the water soluble sodium salt fraction of these trichome secretions have been found to be active against *Citrobacter, Enterobacter* and *Salmonella* bacteria (Mocktar, personal communication).

RESULTS AND DISCUSSION

Immersion of the leaves in a hot 1% aqueous $NaHCO_3$ solution for several hours followed by acidification, yielded a mixture of triterpenoid acids and lactones, fatty acids, flavonoids and what appears to be an acidic carbohydrate. This carbohydrate, which is common to all *Combretum* leaf extracts, probably binds the trichome secretions to the leaf surface. The triterpenoid constituents of this leaf coating were separated by repeated flash column chromatography into two main groups, which NMR spectroscopy showed had a carbonyl function, six methyl groups and a di-substituted



double bond as common features. High resolution EIMS and NMR established that both groups contained a C_8 side-chain on a pentacyclic skeleton, which indicated the presence of a cyclopropane ring. An additional di-substituted double bond in the ring system of compounds in the major group, Group A, and the presence of an hydroxy function in the minor group, Group B, revealed that compounds in Group A were dehydration products of the Group B compounds. The basic structure common to both groups was established as follows.

The resonances for the common, disubstituted double bond at δ 5.85 and δ 6.57 (as AB doublets, J = 9.9 Hz) in the ^{1}H NMR spectrum and δ 128.7 and δ 158.4 in the ^{13}C NMR spectrum, plus a carbonyl resonance at δ 200.7, are characteristic of the conjugated 1-en-3-one system in ring A [8, 9]; this conjugation was confirmed by a band at 1659 cm^{-1} in the IR and UV absorption at 245 nm (ε = 2196).

The second di-substituted double bond, present in compounds from Group A, exhibits NMR resonances as AB doublets at δ 6.12 (J = 10.2 Hz) and δ 6.56 (J = 10.0 Hz) in the ^{1}H NMR and δ 130.5 and δ 132.1 in the ^{13}C NMR spectra. Therefore this unsaturation is restricted to the C-11(12) position and the conjugated system is extended into ring C forming an exceptionally rigid 9,19-cyclo-1,11-dien-3-one backbone in a cycloartane skeleton. This extended rigidity in the skeletal backbone has interesting manifestations in the NMR of the epimeric mixtures found in compound **1** (see below).

Although the presence of the cyclopropane ring at the 9β,19-position was expected on biogenetic grounds [10], the characteristic C-19 methylene proton signals are not evident in the ^{1}H NMR spectra of all the compounds. This is because they are deshielded firstly by the conjugated systems comprising the 1,11-dien-3-one in the Group A compounds and the 1-en-3-one in the Group B compounds and, secondly, by the deshielding anisotropic effects of both the C-1(2) and C-11(12) double bonds. Consequently, the H-19 protons deshielded by the single C-1(2) double bond in Group B compounds appear at a higher field

Table 1. ^{13}C NMR spectral data for compounds **1**–**7**

C	1	1a	1b	2	3	3a	3b	3c	4	4a	5	6	7
1	158.4	32.3	34.6	158.3	158.6	158.6	158.5	158.4	158.4	158.5	158.6	160.0	160.3
2	128.7	38.6	17.8	128.7	128.6	128.5	128.7	128.7	128.6	128.7	128.7	127.4	127.4
3	200.7	212.1	39.4	200.7	200.9	200.8	200.8	200.8	201.1	200.8	201.0	204.2	204.5
4	49.0	49.0	49.4	49.2	49.1	49.1	49.0	49.0	49.1	49.0	49.4	47.9	47.9
5	45.6	47.0	46.9	45.7	45.6	45.6	45.6	45.6	5.6	45.6	45.7	45.1	45.1
6	19.8	20.3	20.8	19.9	19.9	19.9	19.9	19.9	19.9	19.9	20.0	22.7	22.7
7	24.6	26.5	27.4	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.6	25.8	25.8
8	43.9	42.6	45.1	43.7	43.5	43.4	43.6	43.6	43.4	43.9	43.7	48.8	48.8
9	37.1	38.8	38.8	37.2	37.1	37.2	37.1	37.1	37.1	37.2	37.2	35.7	35.7
10	36.8	34.1	34.3	36.8	36.8	36.8	36.8	36.8	36.8	36.8	36.8	35.1	35.1
11	129.8 (130.0)	131.3	130.9	129.7	130.2	130.3	130.2	130.2	130.3	130.1	130.5	40.1	40.1
12	132.5 (132.1)	132.9	131.4	132.9	131.9	131.8	131.8	131.7	131.9	131.9	133.3	72.3	72.3
13	31.7 (31.5)	30.4	30.5	31.6	31.7	31.7	31.7	31.6	31.7	31.7	31.6	38.1	38.1
14	48.6 (48.1)	47.9	47.9	48.1	47.9	47.9	47.8	47.8	47.9	47.9	47.7	44.2	44.2
15	26.8	25.1	25.1	26.4	27.4	27.4	27.3	27.3	32.4	32.5	27.9	36.2	36.0
16	32.8 (32.6)	32.3	32.5	32.4	32.3	32.3	32.5	32.5	32.6	32.9	32.8	32.5	32.4
17	42.4 (42.3)	42.4	44.8	41.6	44.6	44.5	44.9	44.8	44.5	44.8	42.4	52.1	52.1
18	20.2 (20.0)	18.4	17.8	18.8	18.3	18.3	18.2	18.2	18.3	18.2	18.4	17.8	17.8
19	30.2 (30.0)	29.7	29.6	30.1	30.2	30.0	30.2	30.0	29.9	30.3	30.2	29.7	29.7
20	41.9 (41.3)	47.8	47.7	40.2	47.6	47.7	48.0	47.9	47.7	47.9	39.9	48.2	48.0
21	178.3 (178.4)	179.7	181.6	174.2	180.5	180.0	176.3	176.1	181.0	176.4	64.5	179.6	179.4
22	35.0 (34.7)	27.4	33.2	23.6	29.7	29.9	32.5	30.3	27.5	27.4	30.4	27.2	29.4
23	75.0 (74.8)	24.8	71.8	25.0	28.4	27.9	28.5	27.9	25.9	26.1	24.8	26.0	1.9
24	123.1	38.8	38.8	82.0	75.9	76.4	75.9	76.4	123.6	123.6	124.5	123.8	75.8
25	139.6 (139.4)	27.8	27.8	142.7	147.2	142.5	147.2	142.5	132.3	132.1	131.5	132.0	146.9
26	18.4	22.4	22.4	112.7	111.7	113.6	111.6	113.6	17.7	17.6	17.6	17.7	111.7
27	25.7	22.8	22.7	17.9	17.2	17.7	17.1	17.7	25.7	25.7	25.7	25.7	17.2
30	28.0	22.7	25.5	28.1	28.0	28.0	28.0	28.0	28.1	28.0	28.0	28.0	28.0
31	21.2	20.9	21.2	21.2	21.2	21.2	21.2	21.3	21.2	21.2	20.6	20.6	
32	19.9 (19.2)	19.8	20.4	20.2	19.9	19.9	19.9	19.9	18.3	18.5	20.1	18.5	18.5
						21.2	51.3	21.2		51.2			

(δ 1.15 and 1.26; J = 4 Hz) than the equivalent resonances in Group A compounds (δ 1.21 and 1.38; J = 4 Hz) where both double bonds contribute to the deshielding.

Attempts to hydrogenate these double bonds yielded interesting results; whereas the C-1(2) double bond is hydrogenated readily at room temperature and pressure using Adams catalyst, the C-11(12) double bond is unaffected, presumably because of the combined steric effects of the C-13 axial methyl, the 9β,19-cyclopropyl group and, to a lesser extent, the C-21 carboxy function (see below).

The molecular formula of **1**, the least polar of the three most abundant triterpenoids in Group A, was established as $C_{30}H_{40}O_3$ (by high resolution EIMS); NMR spectroscopy showed the presence of a further tri-substituted double bond plus the signals attributed to the 9,19-cyclo-1,11-dien-3-one backbone. In the ^1H spectrum the presence of the side-chain was indicated by two vinyl methyl resonances at δ 1.72 (H-27) and δ 1.75 (H-26) allylically coupled to a multiplet centred at δ 5.19 (H-24), which was further coupled to a *ddd* at δ 5.02 (H-23). This pattern is typical of a side-chain isopropylidene group with an oxygen function at C-23 [11]; the absence of the doublet typical of the C-21 methyl and a band at 1756 cm^1 in the IR, suggested the presence of a 21,23-γ-lactone moiety in the side-chain. Analysis of the ^{13}C, COSY and HETCOR spectra confirmed this, the C-21 and C-23 resonances appearing at δ 178.3 and δ 75.0, respectively.

Although appearing as a single spot on TLC, the duplicity of side-chain signals and, in particular, of signals from the 9,19-cyclo-1,11-dien-3-one system in the NMR spectra (Table 1 and Fig. 1), indicated that **1** was a roughly 1:2 mixture consistent with different configurations at C-23. Although mixtures of C-23 epimers in cycloartenoids have not been reported before, epimeric mixtures at C-24 are common (see below). Based on the premise that steric crowding results in greater carbon shielding, the less intense resonance at δ 74.8 must belong to the 23(*S*)-epimer, which models show produces the most crowded lactone in the side-chain. The more intense signal at δ 75.0 must then belong to the more sterically favoured 23(*R*)-epimer. Therefore the major component of the mixture is 3-oxo-cycloart-1,11,24-trien-23(*R*),21-olide.

The significant changes in NMR chemical shifts caused by epimerisation at C-23 was unexpected. Changes in configuration at this carbon are transmitted through the lactone ring to the C-21 carbonyl, which is close enough to the C-11(12) double bond to cause it to experience both steric and electronic perturbations. Although changes in ^{13}C signals were confined to carbons in rings C and D and the side-chain (Table 1), the exceptional rigidity of the molecular skeleton plus the extended conjugation causes significant changes in the proton spectrum as far removed as ring A. Figure 1 shows clearly the duplicate signals for each of the H-1,2,11 and 12 AB doublets and that there are significant

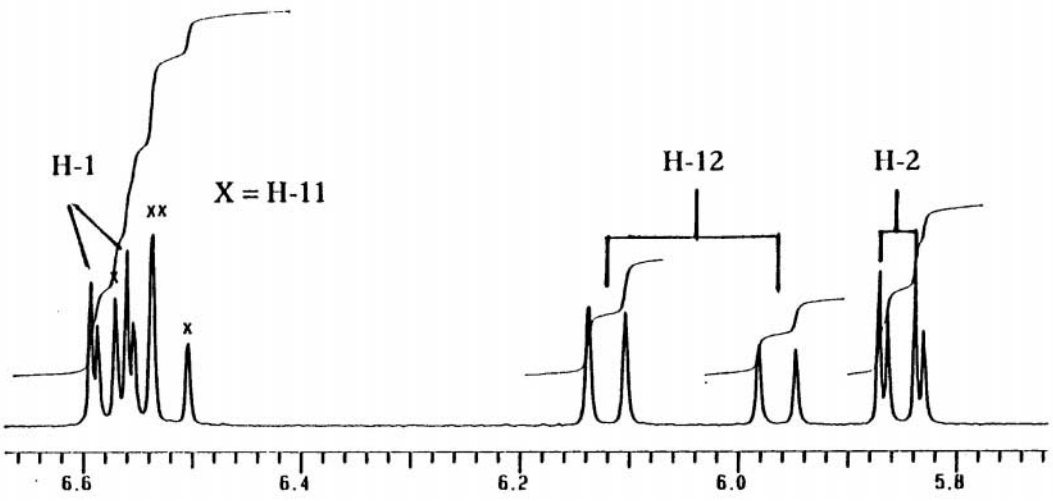


Fig. 1. ^{1}H NMR spectrum of **1** showing duplicity of signals in ring A and ring C due to the long range effect of the 23(*R*/*S*), 21-olide mixture

chemical shift differences between the two doublets for H-11 and, in particular, the two doublets for H-12. These duplications proved helpful in differentiating between the ^{13}C signals for C-9, C-10 and C-13. Whereas two of the signals were not duplicated, the signal at δ 31.7 was and therefore had to be closest to the C-21 carbonyl i.e. at C-13. Attempts to separate the mixture into its constituents were not successful.

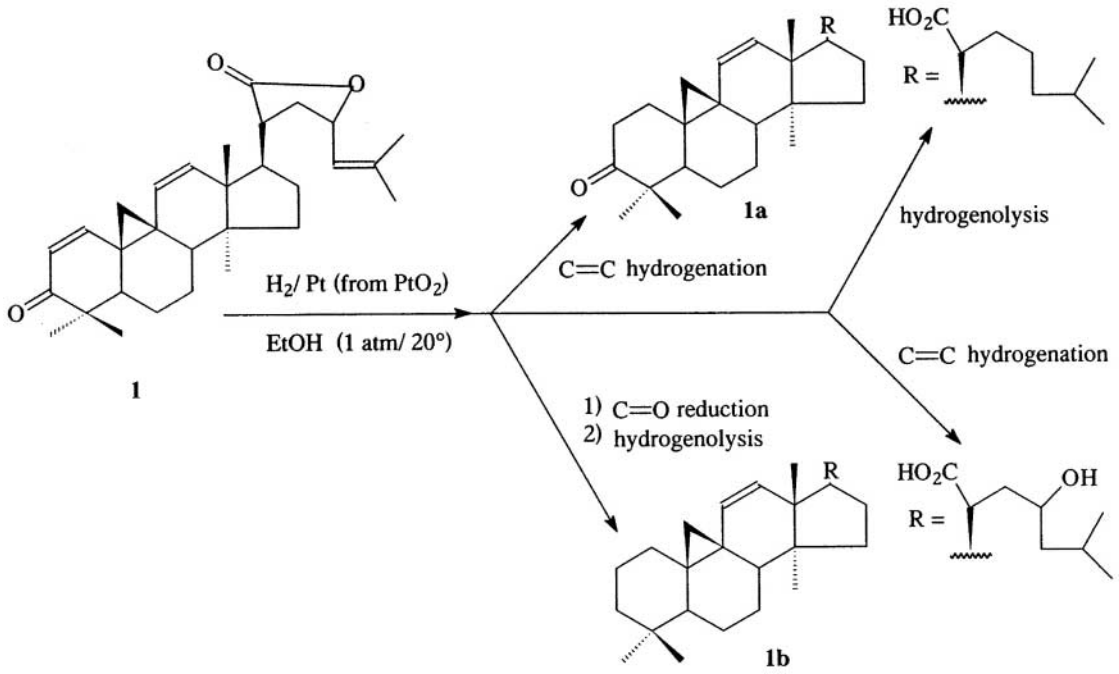
Hydrogenation of **1** with Adams catalyst at room temperature and pressure for 18 h gave two major constituents that were separated by column chromatography. NMR analysis showed that, whereas the C-1(2) and C-24(25) double bonds had been saturated, the C-11(12) double bond [δ 131.3 (C-11) and 132.9 (C-12); AB doublets at δ 5.54 (H-11) and 5.71 (12-H)] plus the C-21 carboxy group (δ 179.7) had been retained in both compounds. In addition, the least polar compound **1a** (established as $C_{30}H_{46}O_3$ by HREIMS) had retained the C-3 carbonyl (δ 212.1), but had lost the C-23 oxygen function by hydrogenolysis (allylic 23,21-lactone). Although the signals for both cyclopropane protons were not visible in the ^{1}H NMR, the presence of only six methyl groups (Me-26 and 27 as doublets) in both **1a** and **1b** showed that this ring was intact.

The spectra of the second compound, **1b** (established as $C_{30}H_{48}O_3$ by HREIMS), showed signals for a secondary hydroxy function (δ 71.8) but not for the C-3 carbonyl. Reduction of this carbonyl did not produce the hydroxy function, because the ^{13}C NMR hydroxy methine signal (δ 71.8) does not correspond with literature values for either a 3α- or 3β-OH carbon in a cycloartane skeleton [12]. Consequently the C-3 carbonyl must have been lost by hydrogenolysis and the C-23 hydroxy function retained. Retention of this hydroxy function would account for the slight difference in the ^{13}C NMR signals for the C-21-carboxy in **1a** (δ 179.7) and **1b** (δ 181.6).

Although unexpected, such reactions are not unusual; hydrogenolysis of allyl alcohols in ring A of sterols [3-hydroxy-4(5)-ene] occurs readily, because the rate of cleavage of the C–O bond is similar to the rate of reduction of the double bond [13]. Therefore the formation of either **1a** or **1b** depends on the competition between the rates of reduction of the 1(2)-double bond and the carbonyl group. Under normal circumstances hydrogenation of the double bond should be more rapid than reduction of the carbonyl and only **1a** should be produced. However, to account for **1b**, hydrogenation of the 1(2)-double bond must be inhibited by the steric effects of the axial 4-methyl and the 9β,19-cyclopropyl groups to the extent that reduction of the C-3 carbonyl occurs preferentially forming the allylic alcohol which then undergoes hydrogenolysis.

The side-chains form as follows. In **1a** hydrogenolysis of the allylic 23-oxygen occurs first followed by hydrogenation of the 24(25)-double bond. In **1b** the 24(25)-double bond is reduced first, the allylic function is thus destroyed and the 23-hydroxy function is retained. Consequently **1a** is 3-oxo-9,19-cycloart-11-en-21-oic acid and **1b** is 23-hydroxy-cycloart-11-en-21-oic acid. The proposed sequence for these reactions is given in Scheme 1.

A second, slightly more polar lactone (**2**) that differed from **1** only in the side-chain from C-23 to C-27 was isolated in pure form in low yield. The two vinyl methyl resonances at δ1.72 (H-27) and δ 1.75 (H-26) have been replaced by a single vinyl methyl at δ1.67 and an exomethylene function [^{1}H NMR: δ 5.03 and 4.96 (2H, *br s*) and ^{13}C NMR: δ 112.8 (t) and δ 143.0 (s)] that is allylically coupled to an oxymethine *dd* at δ 4.73 ($J_1 = 7.5$, $J_2 = 7.6$ Hz). This meant that the side-chain consisted of a C-25(26) double bond and a 21,24-δ-lactone moiety; C-24



Scheme 1.

was assigned the *R*-configuration based on NMR data given for the parent hydroxy acid, compound (**3**), described below. Compound **2** was therefore 3-oxo-cycloart-1,11,25(26)-trien-24(*R*),21-olide.

Compound **3**, the second most abundant triterpenoid (established as $C_{30}H_{42}O_4$ by HREIMS of the acetate **3a** and the methyl ester acetate **3c**), contained C-21 carboxy and C-24 hydroxy functions and a side-chain terminating in an isopropenyl group. Cycloartanoids containing this terminal isopropenyl group frequently occur as mixtures of C-24 epimers in plants and this has led to the suggestion that these compounds are produced by a non-enzymatic process [14]. Compound **3** was no exception although the intensities of the duplicate signals for the protons and carbons of the terminal isopropenyl and 24-hydroxy groups evident in the NMR spectra showed there were only traces of the epimeric impurity. Based on NMR comparisons between the spectra of the acetate (**3a**), ester (**3b**) ester acetate (**3c**) derivatives and known compounds [15], the hydroxy function was assigned the *R*-configuration and it follows that this is the configuration in the corresponding lactone **2**. Similar comparisons between the proton spectra of (**3**) and the ester (**3b**) enabled the C-11 and C-12 resonances in the ^{13}C NMR spectra to be assigned δ 130.2 and δ 131.8, respectively. The proton resonance correlated with the δ 131.8 signal shifted the most on esterification hence this signal must belong to C-12. The carbon assignments for all compounds are give in Table 1. Therefore, compound **3** is 24(*R*)-hydroxy-3-oxo-9,19-cycloart-1,11,25(26)-trien-21-oic acid and the impurity present in trace amounts is the 24(*S*)-hydroxy epimer.

Based on the equilibrium stabilities of lactones, it is not surprising that whereas the free hydroxy acid **3** occurs in greater concentration than its corresponding δ-lactone **2**, the hydroxy acid of the more stable γ-lactone **1** has not been isolated and appears to be absent from the leaf extracts. Concerns that **1** and **2** were lactone artifacts formed during the bicarbonate/acidification extraction procedure were unfounded; extracts obtained by digesting leaves with methanol produced these compounds in similar yields.

The third most abundant triterpenoid in group A, which was given the trivial name erythrophyllic acid (**4**), has the molecular formula, $C_{30}H_{42}O_3$ (by high resolution EIMS). NMR evidence showed resonances typical of a side-chain containing a C-21 carboxy function (δ 181.4) and a terminal isopropylidene group [16], which identified the compound as 3-oxo-cycloart-1,11,24-trien-21-oic acid.

Comparisons of NMR data [17] showed that a further minor constituent, compound (**5**), belonging to Group A differed from erythrophyllic acid in that the carboxyl function at C-21 (δ 181.0) has been replaced by an acetoxy group [δ 64.5 plus 4.33 (1H, *dd*, $J_{1(gem)} = 11.3$, $J_2 = 3.5$ Hz, H-21$_A$) and 4.00 (1H, *dd*, $J_{1(gem)} = 11.3$, $J_2 = 5.4$ Hz, H-21$_B$)]. This compound, part of the fine suspension washed from the leaf surface with the soluble sodium salts, was therefore 21-acetoxy-3-oxo-cycloart-1,11,24-triene.

The two compounds comprising Group B were isolated in low yields. NMR analysis showed that, apart from differences in the side-chain, the com-

pounds were identical. The major compound (**6**) has the same side-chain as that in erythrophyllic acid (**4**), i.e. a carboxy function at C-21 and a terminal isopropylidene function, whereas the minor compound (**7**) has a side-chain corresponding to that in **3**, i.e. 21-carboxy-24(*R*)-hydroxy-25(26)-ene.

Since the Group A compounds are dehydration products of these Group B compounds and the 9,19-cyclopropyl-1-en-3-one system is common to both groups, the hydroxy function in **6** and **7** has to be either on C-11 or C-12. This is supported by NMR evidence; the downfield position of the hydroxy methine carbon in the ^{13}C spectrum (δ 72.3) and the coupling constants for the equivalent proton (δ 4.06; *dd*, J = 2.0 and 10.2 Hz) are indicative of an equatorial configuration for this hydroxy function. The 11α-position was precluded, because of marked differences between the NMR spectra of **6** (and **7**) and 11α-hydroxybuxatenone (**8**) [9]. Whereas the ^{13}C resonances for C-11 and C-12 in (**8**) appeared at δ 67.9 and 35.8, the signals in **6** (and **7**) appeared at δ 72.3 and 40.1, respectively. This confirms the 12β-position for this hydroxy group. Consequently **6** and **7** are identified as 12β-hydroxy-3-oxo-cycloart-1,24-dien-21-oic acid and 12β,24(*R*)-dihydroxy-3-oxo-cycloart-1,25(26)-dien-21-oic acid respectively. Unfortunately, probably due to the vagaries of the African climate, **6** and **7** have not occurred in leaf extracts after 1995 and consequently their physical constants have not been fully characterised. This "disappearance" of extractives has precedents in the *Combretum* [18].

EXPERIMENTAL

General

IR: KBr discs; NMR: 300 MHz in $CDCl_3$; EIMS (probe) 70 eV.

Plant material

Leaves were collected as required from a large tree growing at the entrance to the Department of Chemistry, University of Durban-Westville. Voucher specimens, No. CERTH-1, have been lodged in the Ward Herbarium, University of Durban-Westville.

Extraction and isolation of the leaf coating

Fresh leaves were immersed in hot, 1% aq. $NaHCO_3$ soln and left to stand overnight. The resultant soln was acidified and the precipitated mixture collected by filtration and prepared for CC as described previously [19]. It has been established that the non-acidic, water insoluble constituents of the leaf secretions such as **5** and the flavonoids are intimately mixed with the bicarbonate soluble compounds and are washed off the leaf surface to form a fine suspension that is collected in the final filtration procedure.

Separation procedure

Repeated CC (eluent: varying ratios of petrol, EtOAc and $CHCl_3$) of the extract (5 g) using various grades of silica gel, yielded the compounds **1**–**7** either in pure form or as epimeric mixtures.

Compound **1** *(3-oxo-cycloart-1,11,24-trien-23,21-olide [as a 2:1, 23(R/S)-epimeric mixture]).* Colourless crystalline solid (30 mg), m.p. (epimeric mixture) 191–202°C. Found: $[M]^+$ m/z 448.2963; $C_{30}H_{40}O_3$ requires 448,2977. EIMS m/z (rel. int.) 448 $[M]^+$ (78), 433 (15), 405 (9), 386 (97), 379 (11), 371 (25), 368 (35), 353 (35), 326 (12), 308 (38), 301(50), 291 (18), 279 (31), 273 (38), 255 (41), 241 (23), 231 (28), 213 (53), 199 (28), 185 (26), 171 (32), 159 (33), 149 (53), 147 (58), 129 (100), 111 (49). IR ν_{max} cm^{-1}; 3105 (cyclopropane), 2934, 1756 (unsat. γ-lactone), 1659 (α,β-enone), 1459, 1365, 1166. UV λ_{max}^{EtOH} nm: 245 (ε 2196), 270sh (ε 801), 326 (ε 103). ^1H NMR ($CDCl_3$): δ [epimer] 6.57 (1H, *d*, J = 9.5 Hz, H-1), [6.58 (*d*, J = 9.5 Hz)], 6.52 (1H, *d*, J = 10.0 Hz, H-11), [6.56 (*d*, J = 10.0 Hz)], 5.97 (1H, *d*, J = 10.3 Hz, H-12), [6.12 (*d*, J = 10.2 Hz, H-12)], 5.85 (1H, *d*, J = 9.95 Hz, H-2), [5.86 (*d*, J = 9.95 Hz, H-2)], 5.19 (1H, *d*, J = 8.9 Hz, H-24), 5.02 (1H, *m*, H-23), 2.76 (1H, *m*, H-20), 2.60 (1H, *dd*, J_1 = 7 Hz, J_2 = 10 Hz, H-17), [2.47 (*dd*, H-17)], 2.37 (1H, *m*, H-22), 2.20 (2H, 2 × *dd*, H-5,8), 1.83 (1H, *m*, H-22), 1.74 (3H, *s*, Me-27), 1.72 (3H, *s*, Me-26), 1.63 (2H, *m*, 2 × H-6), 1.38 (2H, *m*, 2 × H-16), 1.11 (3H, *s*, Me-30), 0.98 (3H, *s*, Me-31), 0.92 (6H, *s*, Me-18,32); ^{13}C NMR; Table 1.

Hydrogenation of **1**

Hydrogenation of **1** (50 mg) was carried out in EtOH using Adams catalyst under hydrogen at slight positive pressure (room temp. overnight) and the two major products separated by silica gel CC into **1a** (15 mg) and **1b** (10 mg).

Compound **1*a*** *(3-oxo-cycloart-11-en-21-oic acid).* Colourless crystalline solid, m.p. 206–210°C. Found: $[M]^+$ m/z 454.3847; $C_{30}H_{46}O_3$ requires 454.3861. EIMS m/z (rel. int.) 454 $[M]^+$ (100), 439 (15), 424 (5), 411 (6), 408 (6) 396 (5), 311 (16), 296 (8), 269 (14), 243 (8), 197 (6), 157 (11), 145 (11), 119 (11). IR ν_{max} cm^{-1}; 2932, 2844, 1705–1694 (keto,carboxy C=O), 1467, 1360, 1297. ^1H NMR ($CDCl_3$): δ 5.71 (1H, *d*, J = 10.2 Hz, H-11), 5.54 (1H, *d*, J = 10.0 Hz, H-12), 2.44 (1H, *dt*, J_1 = 14.8, J_2 = 7.8 Hz H-2_A), 2.29 (2H, *m*, H-17,20), 2.13 (1H, *dd*, J_1 = 7.1 Hz, J_2 = 10.2 Hz, H-8), 1.93 (1H, *dd*, J_1 = 3, J_2 = 11.6 Hz, H-5), 1.74 (1H, *m*, J_1 = 14.8, J_2 = 7.8, J_3 = 6 Hz, H-2_B), 1.50 (1H, *m*, H-25), 1.19 (1H, *d*, J = 4.2 Hz, H-19), 0.97 (3H, *s*, Me-18), 0.95 (3H, *s*, Me-31), 0.91 (3H, *s*, Me-30), 0.88 (3H, *s*, Me-32), 0.84 (3H, *d*, J = 2.2 Hz, Me-27), 0.82 (3H, *d*, J = 2.2 Hz, Me-26); ^{13}C NMR; Table 1.

Compound **1b** *(23-hydroxy-cycloart-11-en-21-oic acid)*. Colourless waxy crystalline solid, mp-gradual softening to 169°C. Found: $[M]^+$ *m/z* 456.3616; $C_{30}H_{48}O_3$ requires 456.3603. EIMS *m/z* (rel. int.) 456 $[M]^+$ 456 (76), 442 (64), 438 (28), 424 (27), 413 (16) 401 (15) 385 (12), 383 (11) 311 (22), 295 (20), 257 (18), 213 (19), 174 (30), 159 (41), 119 (44) 55 (100); IR ν_{max} cm^{-1}; 2932, 2844, 1705–1694 (keto, carboxy C=O), 1467, 1360, 1297; IR ν_{max} cm^{-1}; 3456 (OH), 2922, 2844, 1699 (carboxy C=O), 2640 (CO_2H dimer) 1461, 1364, 1175, 1020; ^1H NMR ($CDCl_3$): δ 6.14 (1H, *d*, *J* = 10.2 Hz, H-11), 5.69 (1H, *d*, *J* = 10.1 Hz, H-12), 2.29 (2H, *m*, H-17,20), 1.97 (1H, *dd*, J_1 = 7.1 Hz, J_2 = 10.2 Hz, H-8), 1.51 (1H, *m*, H-25), 1.03 (1H, *d*, *J* = 4.4 Hz, H-19), 0.97 (3H, *s*, Me-18), 0.84 (3H, *d*, *J* = 2.2 Hz, Me-26), 0.83 (3H, *s*, Me-32), 0.82 (3H, *d*, *J* = 2.2 Hz, Me-26), 0.81 (6H, *s*, Me-30,31); ^{13}C NMR; Table 1.

Compound **2** *(3-oxo-cycloart-1,11,25(26)-trien-24(R),21-olide)*. Colourless glassy solid. Found $[M]^+$ *m/z* 448.2989; $C_{30}H_{40}O_3$ requires 448,2977. IR ν_{max} cm^{-1}; 3103 (cyclopropane), 2940, 1745 (unsat. δ-lactone), 1660 (α,β-enone); ^1H NMR ($CDCl_3$): δ 6.56 (1H, *d*, *J* = 9.8 Hz, H-1), 6.49 (1H, *d*, *J* = 10.3 Hz, H-11), 6.08 (1H, *d*, *J* = 10.3 Hz, H-12), 5.84 (1H, *d*, *J* = 9.9 Hz, H-2), 5.03 and 4.91 (2H, *br s*, 2 × H-26), 4.73 (1H, *t*, *J* = 7.6, H-24), 2.56 (1H, *m*, H-20), 2.54 (1H, *m*, H-17), 2.19 (2H, 2 × *dd*, H-5,8), 2.01 (1H, *m*, H-22), 1.84 (1H, *m*, H-23), 1.76, (3H, *s*, Me-27), 1.45 (1H, *m*, H-22), 1.39 (2H, *m*, 2 × H-6), 1.11 (3H, *s*, Me-30), 1.00 (3H, *s*, Me-18), 0.98 (3H, *s*, Me-31), 0.91 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Compound **3** *(24(R/S)-hydroxy-3-oxo-cycloart-1,11,25(26)-trien-21-oic acid)*. Colourless, glassy material. IR ν_{max} cm^{-1}; 3424 (OH), 3103 (cyclopropane), 2940, 1660 (α,β-enone); ^1H NMR ($CDCl_3$): δ 6.56 (1H, *d*, *J* = 9.8 Hz, H-1), 6.43 (1H, *d*, *J* = 10.3 Hz, H-11), 5.83 (1H, *d*, *J* = 9.9 Hz, H-2), 5.76 (1H, *d*, *J* = 10.0 Hz, H-12), 4.91 and 4.81 (2H, *br s*, 2 × H-26), 4.05 (1H, *m*, H-24), 2.56 (1H, *m*, H-20), 2.54 (1H, *m*, H-17), 2.19 (2H, 2 × *dd*, H-5,8), 1.67 (3H, *s*, Me-27), 1.09 (3H, *s*, Me-30), 0.96 (6H, *s*, Me-18,31), 0.86 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Compound **3a** *(24(R/S)-acetoxy-3-oxo-cycloart-1,11,25(26)-trien-21-oic acid)*. Found $[M]^+$ *m/z* 508.3170; $C_{32}H_{44}O_5$ requires 508.3188. EIMS *m/z* (rel. int.) 508 $[M]^+$ (50), 448 (95), 433 (12), 307 (100), 293 (9), 291 (7), 279 (3), 267 (15), 253 (12), 251 (4), 241 (11), 239 (9), 225 (7), 223 (5), 211 (10), 135 (25). ^1H NMR ($CDCl_3$): δ 6.56 (1H, *d*, *J* = 9.9 Hz, H-1), 6.44 (1H, *d*, *J* = 10.3 Hz, H-11), 5.83 (1H, *d*, *J* = 9.9 Hz, H-2), 5.75 (1H, *d*, *J* = 10.3 Hz, H-12), 5.11 (1H, *t*, H-24), 4.93 and 4.89 (2H, *br s*, 2 × H-26), 2.29 (2H, *m*, H-17,20), 2.18 (2H, 2 × dd, H-5,8), 2.03 (3H, *s*, OCOMe), 1.67 (3H, *s*, Me-27), 1.09 (3H, *s*, Me-30), 0.96 (6H, *s*, Me-18,31), 0.85 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Compound **3b** *(methyl-24(R/S)-hydroxy-3-oxo-cycloart-1,11,25(26)-trien-21-oate)*. ^1H NMR ($CDCl_3$): δ 6.57 (1H, *d*, *J* = 9.9 Hz, H-1), 6.46 (1H, *d*, *J* = 10.3 Hz, H-11), 5.84 (1H, *d*, *J* = 10.0 Hz, H-2), 5.52 (1H, *d*, *J* = 10.3 Hz, H-12), 4.90 and 4.81 (2H, *br s*, 2 × H-26), 4.00 (1H, *m*, H-24), 3.68 (3H, *s*, ester Me), 2.30 (2H, *m*, H-17,20), 2.21 (2H, 2 × *dd*, H-5,8), 2.00 (1H, *m*, H-15) 1.67 (3H, *s*, Me-27), 1.10 (3H, *s*, Me-30), 0.97 (3H, *s*, Me-31),0.93 (3H, *s*, Me-18), 0.85 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Compound **3c** *(methyl-24(R/S)-acetoxy-3-oxo-cycloart-1,11,25(26)-trien-21-oate)*. Found $[M]^+$ *m/z* 522.3320; $C_{33}H_{46}O_5$ requires 522.3345. EIMS *m/z* (rel. int.) 522 $[M]^+$ (65), 478 (4), 462 (47), 447 (6), 380 (8), 307 (100), 267 (16), 292 (9), 291 (8), 279 (6), 267 (15), 253 (12), 242 (17), 225 (6), 218 (9), 211 (10). ^1H NMR ($CDCl_3$): δ 6.58 (1H, *d*, *J* = 10.0 Hz, H-1), 6.46 (1H, *d*, *J* = 10.3 Hz, H-11), 5.84 (1H, *d*, *J* = 9.9 Hz, H-2), 5.51 (1H, *d*, *J* = 10.3 Hz, H-12), 4.92 (1H, *t*, H-24), 4.92 and 4.89 (2H, *br s*, 2 × H-26), 3.68 (3H, *s*, ester Me), 2.30 (2H, *m*, H-17,20), 2.19 (2H, 2 × *dd*, H-5,8),2.03 (3H, *s*, OCOMe), 1.66 (3H, *s*, Me-27), 1.10 (3H, *s*, Me-30), 0.97 (3H, *s*, Me-31), 0.93 (3H, *s*, Me-18), 0.85 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Erythrophyllic acid (**4**) *(3-oxo-cycloart-1,11,24-trien-21-oic acid)*. Colourless crystals, m.p. 200–204°C; $[\alpha]_D$ +128.8° ($CHCl_3$; *c* 1.145). Found $[M]^+$ *m/z* 450.3127; $C_{30}H_{42}O_3$ requires 450.3134. EIMS *m/z* (rel. int.) 450 (100), 435 (42), 432 (16), 417 (9), 407 (6), 389 (6), 381 (5), 350 (5), 335 (7), 308 (47) 293 (50), 267 (58), 243 (22), 212 (26), 197 (23), 174 (42), 162 (43), 136 (45), UV λ_{max}^{EtOH} nm: 252 (ε 2056), 270sh (ε 1047), 332 (ε 173). IR ν_{max} cm^{-1}; 3038 (cyclopropyl CH), 2961, 2864, 2563 (CO_2H dimer), 1694 (carboxy C=O), 1670 (α,β-enone C=O), 1452, 1374, 1204, 1156, 1108. ^1H NMR ($CDCl_3$): δ 6.55 (1H, *d*, *J* = 9.9 Hz, H-1), 6.43 (1H, *d*, *J* = 10.3 Hz, H-11), 5.83 (1H, *d*, *J* = 10.0 Hz, H-2), 5.78 (1H, *d*, *J* = 10.3 Hz, H-12), 5.07 (1H, *t*, H-24), 2.32 (2H, m, H-17,20), 2.20 (1H, *dd*, J_1 = 7.0, J_2 = 11.1, H-8), 2.14 (1H, *dd*, J_1 = 3.3, J_2 = 11.5, H-5), 2.01 (4H, *m*, H-22,23), 1.66 (3H, s, Me-27), 1.57 (3H, *s*, Me-26), 1.37 (1H, *d*, *J* = 4 Hz, H-19), 1.25 (1H, *d*, *J* = 4 Hz, H-19) 1.10 (3H, *s*, Me-30), 0.97 (6H, *s*, Me-18,31), 0.88 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Methyl erythropyllate (**4a**). Esterification of **4** (50 mg dissolved in THF) was carried out in an excess of ethereal diazomethane at room temp. to give **4a** (45 mg) as a colourless glass, IR ν_{max} cm^{-1}; 3038 (cyclopropyl CH), 2961, 2854, 1728 (CO_2CH_3 C=O), 1660 (α,β-enone C=O), 1456, 1369, 1235, 1146, 1112. ^1H NMR ($CDCl_3$): δ 5.52 (1H, *d*, *J* = 10.2 Hz, H-12), 3.67 (3H, *s*, ester Me), 1.65 (3H, *s*, Me-27), 1.55 (3H, *s*, Me-26), 1.98 (2H, *m*, H-22), 1.87 (2H, *bdd*, H-23), 1.10 (3H, *s*, Me-30),

0.97 (3H, *s*, Me-31) 0.94 (3H, *s*, Me-18), 0.85 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Compound **5** *(21-acetoxy-3-oxo-cycloart-1,11,24-triene)*. Colourless glass. IR ν_{max} cm^{-1}; 2922, 2844, 1738 (OAc C=O), 1660 (α,β-enone C=O), 1461, 1369, 1233.

EIMS *m/z* (rel. int.) [M$^+$] 478 (26), 463 [M–CH$_3$] (4), 434 (11), 417 (65), 403 (6), 367 (4), 335 (5), 307 (7), 267 (15), 253 (14), 225 (9), 191 (38), 173 (12), 151 (100), 135 (20); ^{1}H NMR ($CDCl_3$): δ 6.58 (1H, *d*, J = 9.9 Hz, H-1), 6.52 (1H, *d*, J = 10.3 Hz, H-11), 5.84 (1H, *d*, J = 9.9 Hz, H-2), 5.76 (1H, *d*, J = 10.3 Hz, H-12), 5.06 (1H, *t*, H-24), 4.33 (1H, *dd*, $J_{1(gem)}$ = 11.3, J_2 = 3.5 Hz, H-21$_A$), 4.00 (1H, *dd*, $J_{1(gem)}$ = 11.3, J_2 = 5.4 Hz, H-21$_B$), 2.06 (3H, *s*, OCOMe), 1.66 (3H, *s*, Me-27), 1.58 (3H, *s*, Me-26), 1.11 (3H, *s*, Me-30), 0.98 (3H, *s*, Me-31), 0.92 (3H, *s*, Me-18), 0.86 (3H, *s*, Me-32); ^{13}C NMR; Table 1.

Compound **6** *(12β-hydroxy-3-oxo-cycloart-1,24-dien-21-oic acid)*. Amorphous solid; IR ν_{max} cm^{-1}; 3450 (OH), 2650 (CO_2H dimer), 1713 (CO_2H carbonyl), 1657 (α,β-enone), 1462, 1374, 1291, 1262, 1110. ^{1}H NMR ($CDCl_3$): δ 6.59 (1H, *d*, J = 9.9 Hz, H-1), 5.81 (1H, *d*, J = 9.9 Hz, H-2), 5.07 (1H, *t*, H-24), 4.08 (1H, *dd*, J_1 = 10, J_2 = 2 Hz, H-12) 2.30 (1H, *dd*, H-5), 2.24(2H, *m*, H-8,20), 2.18 (1H, *dd*, $J_{1(gem)}$ = 14.5, J_2 = 10.3, H-11$_{ax}$), 1.96 (3H, *m*, H-22,23), 1.86 (1H, *dd*, $J_{1(gem)}$ = 14.3, J_2 = 2, H-11$_{eq}$), 1.64 (3H, *s*, Me-27), 1.55 (3H, *s*, Me-26), 1.45 (1H, *m*, H-17), 1.26 (1H, *d*, J = 4 Hz, H-19), 1.18 (3H, *s*, Me-32), 1.15 (1H, *d*, J = 4 Hz, H-19), 1.10 (3H, *s*, Me-30), 1.02 (3H, *s*, Me-31), 0.97 (1H, *s*, Me-18); ^{13}C NMR; Table 1.

Compound **7** *(12β,24(S)-dihydroxy-3-oxo-cycloart-1,25(26)-dien-21-oic acid)*. Amorphous solid; IR ν_{max} cm^{-1}; 3455 (OH), 2650 (CO_2H dimer), 1711 (CO_2H carbonyl), 1657 (α,β-enone), 1462, 1420, 1374, 1290, 1261, 1110. ^{1}H NMR ($CDCl_3$): δ 6.62 (1H, *d*, J = 9.9 Hz, H-1), 5.83 (1H, *d*, J = 9.9 Hz, H-2), 4.91 and 4.82 (2H, *br s*, 2 × H-26), 4.04 (2H, *m*, H-12,24), 2.26 (3H, *m*, H-5, H-8,20), 2.18 (1H, *dd*, $J_{1(gem)}$ = 14.5, J_2 = 10.3, H-11$_{ax}$), 1.96 (3H, *m*, H-22,23), 1.82 (1H, *dd*, $J_{1(gem)}$ = 14.3, J_2 = 2, H-11$_{eq}$), 1.68 (3H, *s*, Me-27), 1.45 (1H, *m*, H-17), 1.18 (3H, *s*, Me-32), 1.11 (3H, *s*, Me-30), 1.03 (3H, *s*, Me-31), 0.97 (1H, *s*, Me-18); ^{13}C NMR; Table 1.

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