Acetylation products of pentacyclic triterpene glucosides from *Combretum Psidioides*

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Dedicated to Professor Georges J. Hoornaert on his 65th birthday

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Abstract

Following acetylation of a mixture of triterpene glycosides extracted from the root bark of *Combretum psidioides* eleven oleanane and ursane triterpene type glycosides were isolated. Their structures were established by spectral analysis and by comparison with related compounds; all acetylated derivatives could be correlated with known triterpene glycosides. Attempted acetylation of the sterically hindered 19α-OH group of arjunglucoside I (**4**) led to introduction of a (3-acetoxy-2-butenoyl) side chain, due to generation of acetylketene as a more reactive acylating agent.

Keywords: *Combretum psidioides*, *Combretaceae*, roots, oleanane, ursane, glucosides, acetylation

Introduction

Combretum psidioides grows widely in the regions of the Democratic Republic of Congo[1] where its roots are used as folk medicine for the treatment of hemorrhoids. Previous chemical investigations of this species have dealt with the amino acid and polysaccharide composition of its gum,[2] and with the identification of several phenanthrene and bibenzyl derivatives isolated from its heartwood.[3] However, until now the triterpene glycosides of this plant have not been examined. In a continuation of our study regarding the chemical constituents of plant species that are used in folk medicine in the Democratic Republic of Congo,[4-6] we herein describe the

structural characterisation of eleven pentacyclic triterpene glycosides resulting from the acetylation of a mixture of triterpene glycosides isolated from *Combretum psidioides*. On the basis of spectroscopic data the acetylated compounds were characterised as genuine acetates or as acetylation artifacts derived from eight known oleanane and ursane triterpene glucosides : arjunglucoside I (**4**),[7] quadranoside III (**6**),[8] arjunetin (**9**),[9] nigaichigoside F1 (**11**),[10] combreglucoside (**13**),[11] chebuloside II (**15**),[12] trachelosperoside E-1 (**17**),[13] and trachelosperoside B-1 (**19**).[14] Although ^{1}H NMR data of some acetylated products isolated in this work have been reported previously, ^{13}C NMR data only were available for combreglucoside heptaacetate (**12**).[11] To our knowledge, there has been no report about the occurrence of the triterpene glycosides arjunetin, chebuloside II, trachelosperoside B-1, and trachelosperoside E-1 in the genus *Combretum*. Peracetylated α-D-glucopyranose, β-D-glucopyranose, and *myo*-inositol were also isolated.

Results and Discussion

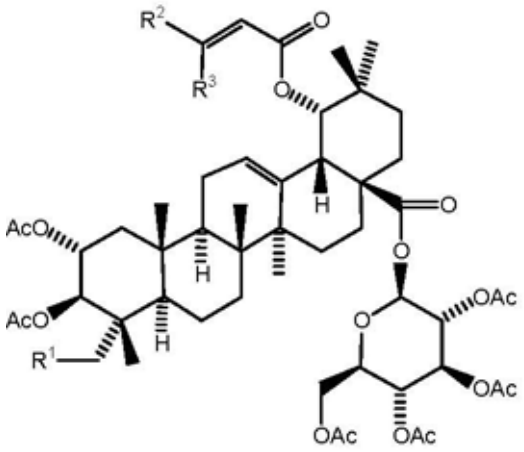
The root bark of *Combretum psidioides* was defatted by treatment with hexanes, and then extracted successively with ethanol and aqueous methanol. Triterpene glycosides were precipitated from the *n*-butanol soluble fraction of each of these alcoholic extracts by addition of diethyl ether. In order to achieve complete acetylation including that of sterically crowded alcohol groups, the acetylation reaction was carried out under forcing conditions, i.e. by heating the mixture of triterpene glycosides with acetic anhydride in pyridine at 120 °C, or by treatment with acetic anhydride and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) at room temperature. Subsequent separation by column chromatography followed by HPLC afforded **1**, **2**, **5**, **12**, **14**, α- and β-D-glucopyranose pentaacetate, and *myo*-inositol hexaacetate as pure compounds. Three chromatographic fractions consisted of a mixture of two components : **3**, **10**; **7**, **8**; and **16**, **18** (Figure 1). All these compounds were isolated from both the ethanolic and methanolic extracts.
The IR spectrum of compound **1** exhibited an absorption band at 1750 cm^{-1} for the ester carbonyl groups. The molecular formula $C_{56}H_{78}O_{21}$ was revealed by DEPT ^{13}C NMR data, and by the positive APCI (atmospheric pressure chemical ionisation) mass spectrum which displayed a pseudo-molecular ion at m/z 1109 ($[M+Na]^+$). The peak at m/z 331 suggested the presence of a tetraacetylated hexopyranosyl moiety. In the ^{13}C and ^{1}H NMR spectra this sugar portion was identified as β-D-glucopyranosyl by characteristic chemical shift values and by the uniformly large coupling constants observed for the *trans*-diaxial ring protons (8-10 Hz).

The ^{13}C NMR broad band spectrum displayed signals for 56 different carbons. Apart from the sixteen peaks corresponding to eight acetyl groups (CH_3CO), the spectrum also showed ^{13}C signals for two further ester carbonyl groups (O-C=O), four ethylenic carbon atoms (two -CH=C< entities), eight oxymethine (>CH-O-), two oxymethylene (-CH_2-O-), three methine (>CH-), and eight methylene (-CH_2-) groups, one allylic methyl (CH_3-C=CH-) and six tertiary methyl (-C-CH_3) groups, and six quaternary carbon atoms (C) (Table 1). One oxymethylene and

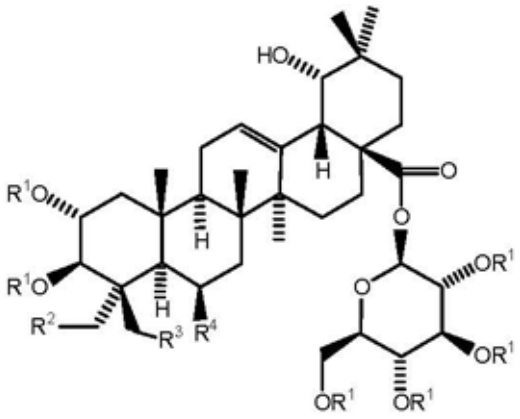
five oxymethine groups belong to β-D-glucose. Therefore the aglycone structure must contain one oxymethylene and three oxymethine groups. Six quaternary carbons absorbing in the range 0-60 ppm and the vinylic carbons detected at 125.8 (CH) and 139.6 (C) ppm are indicative of a Δ^{12}-oleanene triterpene (a β-amyrin derivative). The detection of only six tertiary methyl groups instead of eight for β-amyrin suggested that two methyl groups are transformed either into one oxymethylene and one ester group or into two ester functions. These methyl groups can be identified as Me-23 and Me-28 owing to the absence of their absorption at 28.2 and 28.1 ppm.[15] The oxymethylene peak at 65.3 ppm, the low-field ester signal at 175.5 ppm, and the oxymethine carbon at 74.9 ppm can be respectively attributed to C-23, C-28, and C-3 by comparison with published ^{13}C values for the acetylated hederagenin methyl ester **20**.[15]

In the HETCORR spectrum, the oxymethine carbon at 74.9 ppm is correlated to H-3̩ which appeared as̩ a doublet (*J* 10 Hz) centered at 5.06 ppm in the ^{1}H NMR spectrum (Table 2). The observation of H-3 as a doublet instead of the usual double doublet when C-2 is unsubstituted suggested that C-2 also is an oxymethine carbon. From the magnitude of the coupling constant observed betweeen H-3 and H-2 it is clear that these protons are *trans*-diaxial, and consequently the C-2 substituent has the α-orientation. Inspection of ^{13}C NMR data for a number of oleanane derivatives revealed C-20 as the most upfield quaternary carbon absorbing in the range 30.3-31.4 ppm for unsubstituted oleananes.[16] Such signal was not detected; instead a downfield quaternary carbon signal appeared at δ 34.9 ppm suggesting that C-20 is deshielded by an oxygen atom attached to one of the neighbouring carbons (C-19, C-21, C-29 or C-30). This suggestion was confirmed by a peak at 81.3 ppm assigned to C-19 bearing an α-oxysubstituent.[11,16] This peak is correlated to a doublet centered at 4.96 ppm (*J* 4 Hz) in the ^{1}H NMR spectrum (H-19). This small coupling constant is consistent with either a *cis* or *trans*-eq,eq disposition of H-19 and the vicinal angular proton H-18 but precludes a *trans*-diaxial orientation. A conformational model study of the pentacyclic skeleton (Figure 2) revealed a preferred axial orientation of H-18 relative to ring E in the *cis*-fused bicyclic D-E ring system; hence H-19 is *cis* to H-18 and has the same β-orientation.

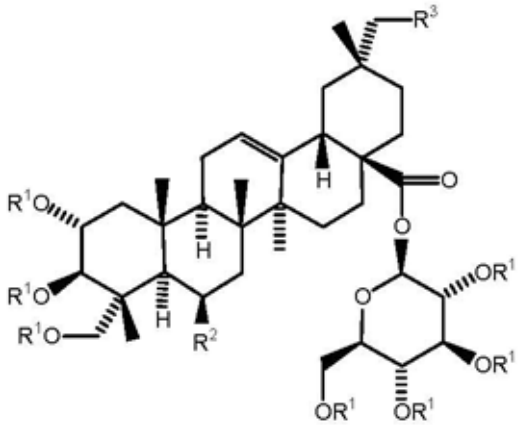
After defining the positions of the four oxygenated carbons in the aglycone and that of the ester group, our remaining task was to determine the site of attachment of the sugar to the aglycone, and the nature and position of the unsaturated carbon chain. As there is only one sugar in the molecule, it must be linked to the aglycone by its anomeric position. The chemical shift of the anomeric proton (5.58 ppm) and that of the anomeric carbon (91.8 ppm) revealed that the sugar is attached to the aglycone by an ester and not an ether linkage. In the ^{13}C NMR spectrum the existence of this ester group is supported by the low-field signal at 175.5 ppm attributed to C-28. The number of ring methylene carbons (eight) observed for compound **1** is in agreement with a trisubstituted Δ^{12}-oleanene triterpene structure. From the above considerations it appeared that compound **1** is an acylated derivative of β-D-glucopyranosyl 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oate, i.e. arjunglucoside I (**4**).



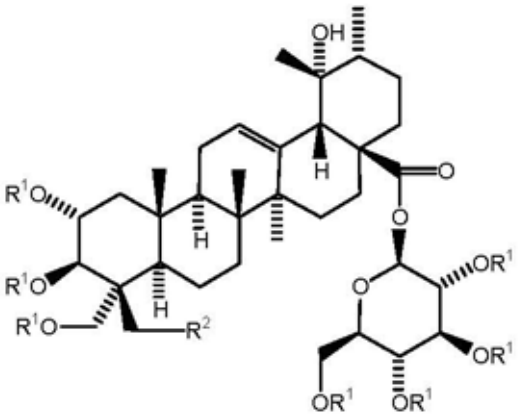
1 : $R^1 = R^2 = OAc$; $R^3 = CH_3$
2 : $R^1 = R^3 = OAc$; $R^2 = CH_3$
7 : $R^1 = H$; $R^2 = OAc$; $R^3 = CH_3$
8 : $R^1 = H$; $R^2 = CH_3$; $R^3 = OAc$



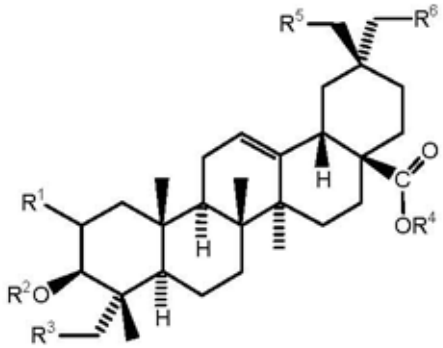
3 : $R^1 = Ac$; $R^2 = OAc$; $R^3 = R^4 = H$
4 : $R^1 = R^3 = R^4 = H$; $R^2 = OH$
9 : $R^1 = R^2 = R^3 = R^4 = H$
12 : $R^1 = Ac$; $R^2 = OAc$; $R^3 = H$; $R^4 = OH$
13 : $R^1 = R^3 = H$; $R^2 = R^4 = OH$
16 : $R^1 = Ac$; $R^2 = R^3 = OAc$; $R^4 = H$
17 : $R^1 = R^4 = H$; $R^2 = R^3 = OH$



5 : $R^1 = Ac$; $R^2 = H$; $R^3 = OAc$
6 : $R^1 = R^2 = H$; $R^3 = OH$
14 : $R^1 = Ac$; $R^2 = OH$; $R^3 = H$
15 : $R^1 = R^3 = H$; $R^2 = OH$
21 : $R^1 = R^2 = R^3 = H$



10 : $R^1 = Ac$; $R^2 = H$
11 : $R^1 = R^2 = H$
18 : $R^1 = Ac$; $R^2 = OAc$
19 : $R^1 = H$; $R^2 = OH$



20 : $R^1 = R^5 = R^6 = H$; $R^2 = Ac$; $R^3 = OAc$; $R^4 = CH_3$
22 : $R^1 = \alpha$-OAc; $R^2 = Ac$; $R^3 = R^6 = H$; $R^4 = CH_3$; $R^5 = OAc$
23 : $R^1 = \beta$-OH; $R^2 = xyl(4\text{-}1)glc$; $R^3 = R^5 = OH$; $R^4 = glc$; $R^6 = H$
24 : $R^1 = \beta$-OH; $R^2 = R^4 = glc$; $R^3 = OH$; $R^5 = OAc$; $R^6 = H$
25 : $R^1 = R^3 = R^5 = H$; $R^2 = ara$; $R^4 = glc$; $R^6 = OH$
26 : $R^1 = \alpha$–OAc; $R^2 = Ac$; $R^3 = R^5 = R^6 = H$; $R^4 = CH_3$

Figure 1

Table 1. ^{13}C NMR data

Carbon No	1	2	3	5	7	8	10	12	14	16	18
1	43.3	43.2	43.3	43.6	43.6	43.6	43.7	45.4	45.7	45.5	45.5
2	69.8	69.8	69.7	69.8	69.9	69.9	69.9	69.8	69.8	69.1	69.2
3	74.9	74.9	74.7	74.8	80.6	80.6	74.9	74.9	74.9	74.2	74.3
4	41.4	41.4	41.9	41.9	39.4	39.4	41.9	42.5	42.4	48.0	48.0
5	47.5	47.5	47.7	47.6	54.9	54.9	47.4	48.5	48.2	48.2	48.2
6	18.0	18.0	17.9	17.9	18.3	18.3	18.0	68.0	67.9	19.3	19.3
7	32.2	32.2	32.2	32.4	32.5	32.5	32.6	41.0	41.0	32.8	33.1
8	39.5	39.5	39.5	39.4	39.5	39.5	40.0	38.9	38.6	39.6	40.0
9	47.6	47.5	47.5	47.5	47.5	47.5	47.2	48.1	47.9	47.4	47.4
10	38.0	38.0	37.9	37.8	38.2	38.3	37.8	37.5	37.4	37.6	37.8
11	23.6	23.5	23.7	23.5	23.6	23.6	23.7	23.7	23.4	23.7	23.8
12	125.8	125.8	124.6	123.0	125.9	122.2	128.9	125.0	122.8	124.5	128.6
13	139.6	139.5	142.2	142.5	139.6	139.4	137.5	141.4	142.0	142.2	137.5
14	41.9	41.9	41.2	41.6	41.9	41.8	41.3	41.9	42.2	41.2	41.0
15	27.7	27.7	27.9	27.5	27.7	27.8	28.2	28.0	27.5	27.3	28.2
16	27.1	27.1	27.1	22.8	27.2	27.1	25.8	27.3	22.9	27.2	25.8
17	45.6	45.6	45.6	46.8	45.7	45.6	47.9	45.6	46.8	45.2	47.4
18	41.7	41.7	43.4	40.0	41.5	41.5	53.0	43.4	41.0	43.4	52.9
19	81.3	81.0	81.4	40.0	81.3	81.0	73.1	81.6	45.7	81.5	73.1
20	34.9	34.8	34.5	34.2	34.9	34.8	41.0	34.7	30.6	34.6	41.0
21	29.3	29.4	27.8	28.7	29.4	29.7	25.2	27.9	33.8	28.0	25.3
22	31.6	31.6	31.7	30.7	31.7	31.7	36.6	31.9	31.8	31.7	36.4
23	65.3	65.3	65.1	65.3	28.4	28.4	65.2	65.2	65.3	63.5	63.5
24	13.8	13.9	13.8	13.9	16.2[a]	16.2[a]	13.9	15.3	15.3	62.5	62.5
25	17.0	17.0	16.9	16.9	17.0[a]	17.0[a]	16.9	18.2	18.3	16.3	16.7
26	16.7	16.7	16.7	16.9	17.6[a]	17.6[a]	16.8	18.0	18.4	16.2	16.5
27	24.6	24.4	24.5	25.5	24.7	24.5	23.9	24.6	25.6	24.3	23.8
28	175.5	175.5	175.8	175.2	175.5	175.5	175.7	175.8	175.6	175.8	175.6
29	27.2	27.2	27.7	74.5	27.2	27.2	27.4	27.8	33.0	27.8	27.3
30	24.5	24.4	24.3	19.1	24.5	24.5	16.0	24.4	23.4	24.4	16.0
Glucose											
1'	91.8	91.8	91.6	91.7	91.9	91.9	91.8	91.9	91.7	91.8	91.8
2'	70.0	70.0	69.8	70.0	70.0	70.0	69.9	70.0	70.0	70.0	69.9
3'	72.9	72.9	72.8	72.8	72.9	72.9	72.9	72.9	72.8	72.8	72.8
4'	68.0	68.0	67.9	68.0	68.1	68.1	68.1	68.0	68.0	68.0	68.0
5'	72.6	72.6	72.4	72.5	72.6	72.6	72.5	72.6	72.5	72.4	72.5
6'	61.5	61.5	61.4	61.5	61.6	61.6	61.5	61.5	61.5	61.5	61.5

Table 1. Continued

Carbon No	1	2	3	5	7	8	10	12	14	16	18
butenoyl											
1”	165.7	163.3	-	-	165.7	163.3	-	-	-	-	-
2”	110.5	108.7	-	-	110.5	108.7	-	-	-	-	-
3”	164.1	160.5	-	-	164.0	160.4	-	-	-	-	-
4”	18.2	21.8	-	-	18.1	21.7	-	-	-	-	-
CH_3*-CO*	21.1	21.1 21.0	21.0	21.1	21.1	21.1 2	21.1	21.1 20.9	21.1	20.9	20.9
	21.1	21.0 20.7	20.8	20.9	21.1	1.0	20.9	20.8 20.7	20.9	20.9	20.9
	21.0	20.7 20.6	20.7	20.9	20.9	20.9	20.8	20.7 20.6	20.8	20.7	20.7
	20.7	20.6 20.6	20.6	20.8	20.7	20.7	20.7	20.5	20.7	20.7	20.7
	20.7		20.5	20.7	20.6	20.6	20.6		20.6	20.7	20.7
	20.6		20.5	20.6	20.5	20.5	20.5		20.6	20.5	20.5
	20.6		20.5	20.6	20.5	20.5	20.5		20.6	20.5	20.5
	20..6			20.6						20.5	20.5
CH*$_3$*-	171.0	171.0	170.8	171.2	170.7	170.7	170.9	170.8	170.8	170.7	170.7
***C*O-**	170.5	170.5	170.5	170.8	170.5	170.5	170.5	170.6	170.5	170.5	170.5
	170.5	170.5	170.4	170.5	170.5	170.5	170.4	174.4	170.4	170.3	170.3
	170.3	170.3	170.3	170.4	170.1	170.1	170.4	170.4	170.3	170.2	170.2
	170.1	170.1	170.0	170.3	169.4	169.4	170.1	170.1	170.1	170.1	170.1
	169.4	169.4	169.3	170.1	168.8	168.8	169.4	169.4	169.4	170.0	170.0
	168.9	168.9	168.8	169.4	167.9	167.5	168.9	168.8	168.9	169.3	169.3
	167.9	167.6		168.9						168.8	168.8

[a] Assignments bearing the same superscript along a vertical column may be interchanged.

Table 2. Selected ^{1}H NMR data

Proton	1	2	3	5	7	8
H-2	5.14, m, 10Hz, -	5.14, m, 10. Hz, -	a	a	a	a
H-3	5.06, d, 10 Hz	5.06, d, 10 Hz	5.08, d, 10 Hz	5.07, d, 10 Hz	4.72, d, 10 Hz	4.72, d, 10 Hz
H-6						
H-12	5.51, br t, 4 Hz	5.51, br t, 4 Hz	5.46, br t, 4 Hz	5.35, br t, 4 Hz	5.52, br t, 4 Hz	5.52, br t, 4 Hz
H-18	3.28, br s	3.27, br s	3.09, br s	2.86, dd, 13, 4Hz	3.26, br s	3.26, br s
H-19	4.96, d, 4 Hz	4.90, d, 4 Hz	3.34, br d, 4Hz		4.96, d, 4 Hz	4.90, d, 4 Hz
H-23a	3.83, d, 12 Hz	3.82, d, 12 Hz	3.85, d, 12 Hz	3.90, d, 12 Hz		
H-23b	3.57, d, 12 Hz	3.57, d, 12 Hz	3.58, d, 12 Hz	3.56, d, 12 Hz		

Table 2. Selected ^{1}H NMR data (continued and concluded)

Proton	1	2	3	5	7	8
H-24a-b						
H-29a,b				b		
-C(H)-CH_3	1.06, s; 1.03, s; 0.96, s; 0.88, s; 0.80, s; 0.66, s	1.06, s; 1.01, s; 0.97, s; 0.88, s; 0.78, s; 0.66, s	1.22, s; 1.08, s; 0.97, s; 0.94, s; 0.90, s; 0.68, s	1.26, s; 1.10, s; 1.09, s; 0.98, s; 0.88, s; 0.74, s	1.25, s; 1.02., s; 1.02, s; 0.90, s; 0.87, s; 0.80, s; 0.65, s	1.21, s; 1.02, s; 1.02, s; 0.90, s; 0.87, s; 0.77, s; 0.65, s
Glucose						
H-1'	5.59, d, 8 Hz	5.59, d, 8 Hz	5.58, d, 8 Hz	5.58, d, 8 Hz	5.59, d, 8 Hz	5.59, d, 8 Hz
H-2'	5.20, t, 8.08, 9.4 Hz	5.20, t, 8.08, 9.4 Hz	a	a	a	a
H-3'	5.25, t, 9.2, 9.16 Hz	5.25, t, 9.2, 9.16 Hz	a	a	a	a
H-4'	5.16, t, 9.56Hz	5.16, t, 9.56Hz	a	a	a	a
H-5'	3.80, ddd, 9.72, 4.6, 2.4Hz	3.80, ddd, 9.72, 4.6, 2.4Hz	3.80, m	b	3.80, ddd, 10, 4, 2 Hz	3.80, ddd, 10, 4, 2 Hz
H-6'a	4.29, dd, 12, 4Hz	4.29, dd, 12, 4Hz	4.30, dd, 12, 4Hz	4.27, dd, 12, 4Hz	4.30, dd, 12.5, 4 Hz	4.30, dd, 12.5, 4 Hz
H-6'b	4.06, dd, 12, 2Hz	4.06, dd, 12, 2Hz	4.06, dd, 12, 2Hz	4.06, dd, 12Hz	4.06, dd, 12.5, 2 Hz	4.06, dd, 12.5, 2 Hz
2-butenoyl						
H-2"	5.69, br q, 0.76Hz	5.58, br	-	-	5.69, d, 1 Hz	5.59, d, 1 Hz
H-4"	2.35, br s	2.23, s	-	-	2.34, br d, 1 Hz	2.23, br d, 1 Hz
Acetyl						
CH_3-CO	2.18, 2.10, 2.07, 20.03, 2.01, 2.01, 2.00, 1.98	2.10, 2.06, 2.03, 2.03, 20.01, 2.01, 2.00, 1.99	2.09, 2.07, 2.03, 2.02, 2.01, 2.00, 1.99	2.08, 2.07, 2.06, 2.02, 2.02, 2.01, 1.98		

a: Overlapping signals at δ 5.1-5.30 ppm; b: overlap of ABq of H-29 and ddd of H-5' at 3.70-3.86 ppm.

Table 2. Selected ^{1}H NMR data (continued and concluded)

	10	12	14	16	18
H-2	a	a	a	a	a
H-3	5.09, d, 10 Hz	5.01, d, 10 Hz	5.01, d, 10 Hz	a	a
H-6		4.36, br s	4.34, br s		
H-12	5.38, br t, 4 Hz	5.50, br t, 3 Hz	5.38, br t, 3 Hz	5.45, br t, 4 Hz	5.38, br t, 4 Hz
H-18	2.53, br s	3.08, br s	2.85, dd, 13, 4 Hz	3.06, br s	2.53, br s
H-19	-	3.35, d, 4 Hz		3.34, br s	-
H-23a	3.87, d, 12 Hz	3.94, d, 12 Hz	3.95, d, 12 Hz	4.17, d, 12 Hz	4.19, d, 12 Hz
H-23b	3.57, d, 12 Hz	3.73, d, 12 Hz	3.70, d, 12 Hz	3.90, d, 12 Hz	3.89, d, 12 Hz
H-24a-b				b	b
H-29a,b					
-C(H)-CH_3	1.26, s; 1.20, s; 1.10, s; 0.94, d, 7 Hz; 0.91, s; 0.72, s	1.46, s; 1.29, s; 1.21, s; 0.99, s; 0.97, s; 0.94, s	1.46, s; 1.27, s; 1.08, s; 1.02, s; 0.92, s; 0.91, s	1.22, s; 1.10, s; 0.97, s; 0.94, s; 0.69, s	1.24, s; 1.20, s; 1.11, s; 0.94, d, 7 Hz; 0.72, s
Glucose					
H-1'	5.52, d, 8 Hz	5.58, d, 8 Hz	5.58, d, 8 Hz	5.56, d, 8 Hz	5.51, d, 8 Hz
H-2'	a	a	a	a	a
H-3'	a	a	a	a	a
H-4'	a	a	5.13, t, 10, 9 Hz	a	a
H-5'	3.78, ddd, 10, 4, 2Hz	3.80, ddd, 10, 4, 2Hz	3.79, ddd, 10, 4, 2Hz	3.79, m	3.79, m
H-6'a	4.27, dd, 12.5, 4Hz	4.29, dd, 12, 4 Hz	4.27, dd, 12, 4 Hz	b	b
H-6'b	4.05, dd, 12.5, 2Hz	4.06, dd, 12, 2 Hz	4.05, dd, 12, 2 Hz	4.06, dd, 12, 2 Hz	4.05, dd, 12 Hz
Acetyl					
CH_3-CO	2.09, 2.06, 2.02, 2.02, 2.01, 2.01, 1.98	2.06, 2.06, 2.03, 2.02, 2.02, 2.01, 1.99	2.06, 2.06, 2.03, 2.02, 2.01, 2.01, 1.98		

a: overlapping signals at δ 5.1-5.30 ppm; b: overlapping signals at 4.25-4.31 ppm.

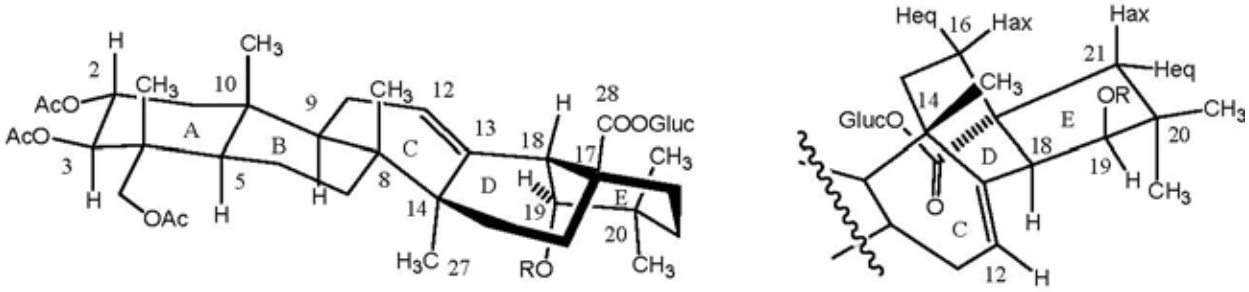


Figure 2

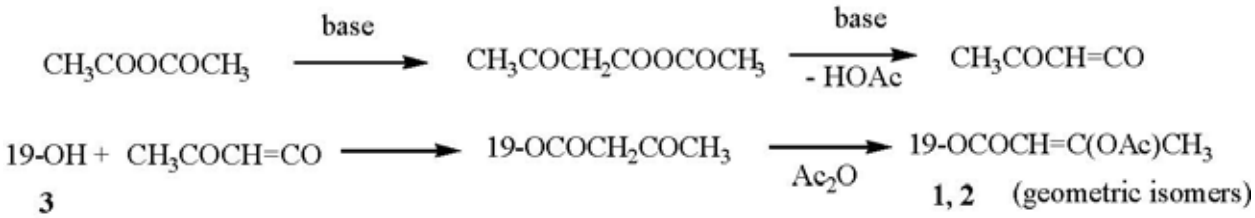
Since four of eight acetyl groups are located on the β-D-glucopyranosyl moiety, the remaining four acetyl groups and an additional C_4 acyl chain must be accommodated on the four hydroxyl groups of the aglycone moiety of **4**. Four signals due to the C_4 acyl group were assigned respectively to the ester carbon atom C-1'' observed as the most upfield CO signal at 165.7 ppm, two ethylenic carbon atoms found at 110.5 (-CH=) and 164.1 (=C<) ppm, and an allylic methyl group at 18.2 ppm. Clearly, one of the four hydroxyl group of the aglycone is substituted with a 2-butenoyl group bearing an acetoxy function, whereas the other three hydroxyl groups are *O*-acetylated. At this stage, it was not possible to determine the exact position of the 2-butenoyl substituent, as well as its geometric configuration.

Finally the latter structural features could be elucidated by comparing the ^{13}C and ^{1}H NMR data of compound **1** with those of its geometric isomer **2** (Tables 1 and 2). In the ^{1}H NMR spectra of both isomers a Δ^{12}-oleanene structure bearing an acyloxy group at C-19 was indicated by the characteristic broad triplet (*J ca* 4 Hz) centered at 5.51 ppm (H-12) and the broad singlet at 3.28 ppm (H-18).[7]Further comparison with the spectra of compound **2** revealed the attachment of the C_4 acyl chain to O-19 by the shielding effects of 0.3 and 0.06 ppm found for C-19 (δ 81.0 for **2** *vs* δ 81.3 for **1**) and H-19β (d, *J* 4 Hz; δ 4.90 for **2** *vs* δ 4.96 for **1**). The ^{1}H NMR spectrum of compound **1** displayed a broad quartet (*J ca* 1 Hz) signal at 5.69 ppm for the olefinic proton H-2'' of the 2-butenoyl group, indicating a long range coupling of H-2'' with a methyl group in the allylic position. This methyl group appeared as a broad doublet at 2.35 ppm. These data are consistent with a (3-acetoxy-2-butenoyl) structure for the C_4 acyl chain located at O-19. The (*E*)-configuration of the trisubstuted double bond was apparent from the upfield shift of 3.6 ppm for the allylic methyl group of **1** (δ 18.2) compared to that of the (*Z*)-isomer **2** (δ 21.8). Such shielding effect is characteristic of allylic carbons having a *cis*-disposed carbon substituent, e.g. the allylic carbon atoms of a *cis*-disubstituted alkene are shifted upfield relative to those of the corresponding *trans*-disubstituted alkene by about 4-6 ppm[17-18]

The mass spectrum of compound **2** revealed a pseudo-molecular ion at m/z 1104 ($[M+NH_4]^+$). The molecular formula deduced from its MS and ^{13}C NMR data is identical to that of compound **1** ($C_{56}H_{78}O_{21}$). The ^{13}C NMR spectrum displayed the same number of peaks and was almost superimposable on that of compound **1** with the exception of signals due to the 2-butenoyl group. The carbon atoms of the unsaturated C_4 acyl group of compound **2** absorbed at

higher field (shielding of 1.8-3.6 ppm), except for the allylic methyl group. Apart from the shielding effect mentioned for C-19, a further shielding also was observed for C-27 in the aglycone part of **2** (0.2 ppm). This effect is due to a severe interaction of the C-27 methyl group (located at C-14) with the 19-*O*-acyl substituent (Figure 2). In the ^{1}H NMR spectrum of **2**, major differences compared to that of **1** also came from the absorptions of the 2-butenoyl group and from H-19. The vinylic proton H-2'' was detected as a broad singlet at 5.58 ppm (overlapping the signal of the anomeric proton), and the allylic methyl group at 2.23 ppm (br s). From the above described data it follows that compounds **1** and **2** are geometric isomers having an unsaturated ester chain at C-19. The structure of compound **1** is 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23-tri-*O*-acetyl-19-*O*-[(*E*)-3-acetoxybut-2-enoyl]-2α,3β,19α,23-tetrahydroxyolean-12-en-28-oate, whereas that of compound **2** is established as 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23-tri-*O*-acetyl-19-*O*-[(*Z*)-3-acetoxybut-2-enoyl]-2α,3β,19α,23-tetrahydroxyolean-12-en-28-oate.

From their occurrence as two geometric isomers compounds **1, 2** were presumed to be artifacts resulting from the base-catalysed acetylation of natural arjunglucoside I (**4**). This presumption was confirmed by the transformation of the structurally related compound **3** having a free 19-OH group into a 3:2 mixture of compounds **1** and **2** by mere heating of **3** with acetic anhydride in pyridine for 2 hours at 120 °C. Under less severe conditions this sterically hindered 19-OH group is not acetylated. Indeed, from inspection of a conformational model of **3** (Figure 2) it appears that the axial 19-OH group is oriented inside the *endo*-face of the *cis*-fused D-E ring system where it experiences severe repulsions with the angular methyl group in position 14, the axial hydrogen atoms H-16ax and H-21ax, and the equatorial 20-methyl group. Since simple acetylation does not occur even under the forcing reaction conditions applied, acylation of 19-OH must involve a more reactive acylating agent, i.e. a sterically more accessible, linear ketene species. Most probably this is not unsubstituted ketene since conversion of acetic anhydride into ketene requires pyrolytic conditions (500-600 °C).[19] Furthermore, reaction of simple ketene with 19-OH is expected to provide the 19-OAc derivative. Hence, the actual reagent presumably is acetylketene that is formed by initial base-catalysed condensation of Ac_2O followed by elimination of HOAc (Scheme 1). Indeed it has been shown that acetylketene can be generated by thermolysis of ethyl and *t*-butyl acetoacetate at 92-106 °C and made to react *in situ* with *n*-BuOH and various tertiary alcohols to give the corresponding acetoacetate esters.[20,21] In contrast to Ac_2O acetylketene now may enter into the interior *endo*-face of the *cis*-fused bicyclic D-E ring system where it reacts with the hindered 19-OH group to form a β-ketoester intermediate; eventually this β-ketoester transforms into the geometric isomers **1**, **2** after further O-acetylation of the corresponding enol forms. Acetylation using acetic anhydride and DMAP at room temperature for 48 hours also resulted in isolation of compounds **1** and **2**. It should be noticed that perchloric acid catalysed acetylation of the hindered 19-OH group was successful in the case of a methyl ester analogue of **4**,[7] probably due to the generation of the small and reactive CH_3CO^+ cation.



Scheme 1

The unexpected 19-*O*-acylation reaction proved to be helpful during the separation process since the inseparable mixture of compounds **3** and **10** could be obtained in different proportions by controlling the reaction conditions. Short reaction times and/or low temperatures furnished a 4:1 mixture of **3, 10**, and only small amounts of **1** and **2**. High temperatures and/or long reaction times led to almost quantitative conversion of compound **3** into **1**, **2**, resulting in isolation of a 1:4 mixture of compounds **3, 10**. Compound **3** was identified as the known arjunglucoside I heptaacetate by comparing its ^{1}H NMR data to those published.[7] Further confirmation showing the absence of the 19-*O*-[2-butenoyl] moiety came from comparison of the ^{13}C NMR data of compound **3** with those of **1**, **2** (Table 1), and those published for combreglucoside heptaacetate **12**.[11]

The APCI mass spectrum of compound **5** exhibited a pseudo-molecular ion at m/z 1020 ($[M+NH_4]^+$). From this result and ^{13}C NMR data the molecular formula $C_{52}H_{74}O_{19}$ was inferred. The ^{13}C NMR broad band spectrum showed signals for 52 carbons. Apart from sixteen peaks attributed to eight acetyl groups, the ^{13}C and DEPT data revealed signals for one ester group (>C=O), two ethylenic carbon atoms (-CH=C<), seven oxymethine (>CH-O-), three oxymethylene (-CH_2-O-), three methine (>CH-), nine methylene (-CH_2-) and five methyl groups (C-CH_3), and six quaternary carbon atoms (>C<). Comparing these data with those obtained for arjunglucoside I heptaacetate **3** revealed identical rings A, B, C, and D; however octaacetate **5** has an additional acetyl group and a different location of the corresponding OAc (OH) substituent in ring E. The disappearance of the signal at δ 81.4 ppm attributed to C-19 of compound **3** and the appearance of an extra methylene signal in the range 0-60 ppm indicated that compound **5** is a derivative of arjunglucoside II (**21**) (19-deoxyarjunglucoside I).[7] The absence of signals at δ 27.7 and 24.3 ppm corresponding to Me-29 and Me-30 in compound **3** and the appearance of oxymethylene and methyl carbon signals at 74.5 and 19.1 ppm indicated that one of these two methyl groups is transformed into an hydroxymethyl substituent. The upfield shift (5.2 or 8.6 ppm) observed for the unsubstituted methyl group is in good agreement with the hydroxylation shift expected for an OH (OAc) group introduced at a γ-carbon.[16] The location of this group at C-29 was apparent from inspection of published ^{13}C NMR data. The C_5D_5N or $CDCl_3$ spectra of compounds **22**,[22] **23**,[23] and **24**[24] possessing an hydroxy or acetoxy group at C-30 revealed a chemical shift value of *ca.* 28 ±0.3 ppm for C-29, and an upfield chemical shift of *ca.* 67 ppm for C-30 (Table 3). On the other hand, for compound **25** having a C-29 hydroxymethyl group the chemical shift values observed (C-29: δ 72.9; C-30: δ 19.7)[25] were comparable to those obtained for compound **5.**

Table 3. Comparison of aglycon ^{13}C NMR data of compound 5 to those published for closely related compounds

Carbon No	20	23 * [a]	24 * [b]	25 * [c]	5	3	22 [d]	26 [d]
1	37.7	44.2	44.0	38.9	43.6	43.3	43.9	44.0
2	23.0	70.9	71.4	26.5	69.8	69.7	70.0	70.1
3	74.3	82.8	82.9	88.8	74.8	74.7	80.6	80.7
4	40.6	42.3	41.1	39.5	41.9	41.9	39.4	39.5
5	47.7	48.5	48.4	56.0	47.6	47.7	54.9	55.0
6	18.1	18.0	17.9	18.7	17.9	17.9	18.2	18.4
7	32.3	32.4	32.8	33.2	32.4	32.2	32.4	32.7
8	39.3	37.0	39.8	40.0	39.4	39.5	39.4	39.8
9	47.7	47.7	47.6	48.1	47.5	47.5	47.5	48.2
10	36.8	35.7	37.0	37.1	37.8	37.9	38.1	38.4
11	23.0	24.0	23.8	23.8	23.5	23.7	23.5	33.5
12	122.0	123.3	123.8	128.8	123.0	124.6	122.5	122.4
13	143.6	144.1	143.5	148.8	142.5	142.2	143.3	144.3
14	41.6	42.8	42.7	42.2	41.6	41.2	41.6	41.9
15	27.7	28.2	28.0	28.3	27.5	27.9	27.5	28.0
16	23.4	23.7	23.5	23.5	22.8	27.1	23.2	23.7
17	46.6	47.0	46.4	47.5	46.8	45.6	46.4	46.9
18	41.3	41.4	43.1	41.0	40.0	43.4	40.7	41.7
19	45.8	41.6	42.1	41.2	40.0	81.4	41.4	46.0
20	30.6	40.6	43.9	36.3	34.2	34.5	33.8	30.8
21	33.8	29.4	29.5	28.3	28.7	27.8	29.2	33.9
22	32.3	32.9	34.0	32.0	30.7	31.7	31.8	32.9
23	65.3	64.7	65.3	28.8	65.3	65.1	28.4	28.3
24	13.1	14.9	14.9	17.5	13.9	13.8	16.8	17.0
25	15.8	17.6	17.4	15.6	16.9	16.9	16.4	16.6
26	16.8	17.3	17.2	16.8	16.9	16.7	17.6	17.8
27	25.8	26.3	26.1	26.1	25.5	24.5	26.0	26.1
28	177.8	176.5	176.8	176.5	175.2	175.8	177.7	177.8
29	33.1	28.3	28.1	72.8	74.5	27.7	27.8	33.1
30	23.6	65.6	67.5	19.7	19.1	24.3	67.7	23.6

* C_5D_5N was used as solvent; a : data taken from reference 23; b: data taken from reference 24; c: data taken from reference 25; d : data taken from reference 22.

In the ^1H NMR spectrum of **5**, the Δ^{12}-oleanene type structure was confirmed by a triplet at

5.35 ppm (H-12) and a double doublet at δ 2.86 ppm (H-18, *J* 13, 4 Hz).[7] The β-configuration of the sugar moiety was apparent from the coupling value found for the anomeric proton (δ 5.58 ppm, d, *J* 8 Hz). The structure of compound **5** can therefore be defined as 29-hydroxyarjunglucoside II octaacetate or 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23,29-tetra-*O*-acetyl-2α,3β,23,29-tetrahydroxyolean-12-en-28-oate. Hence compound **5** is the octaacetyl derivative of the natural triterpene quadranoside III (**6**),[8] β-D-glucopyranosyl 2α,3β,23,29-tetrahydroxyolean-12-en-28-oate.

Compounds **7** and **8** were isolated as an inseparable mixture (3:2), which presented a similar ^{1}H NMR spectrum as that from the mixture **1**, **2** prepared by separate heating of **3** with acetic anhydride in pyridine. From the disappearance of the AB quartets at δ 3.50-3.90 corresponding to the oxymethylene protons H-23 in **1**, **2**, compounds **7**, **8** were presumed to be the 23-H analogues of **1**, **2**. In the ^{13}C NMR spectrum, the 23-deoxy structure was supported by the absence of the signal at δ 65.3 corresponding to the oxymethylene carbon C-23, and the appearence of a peak at δ 28.4 attributed to the C-23 methyl group. Further comparison of the ^{13}C NMR data of **7**, **8** to those of **1**, **2** confirmed their identical structures except for the ring A substituents while comparison to data published for **22** and **26** having a C-23 methyl group (Table 3) revealed identical ring A substituents (Table 1). The structures of compounds **7** and **8** are therefore defined as 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3-di-*O*-acetyl-19-*O*-[(*E*)-3-acetoxybut-2-enoyl]-2α,3β,19α-trihydroxyolean-12-en-28-oate and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3-di-*O*-acetyl-19-*O*-[(*Z*)-3-acetoxybut-2-enoyl]-2α,3β,19α-trihydroxyolean-12-en-28-oate, respectively. These new compounds **7** and **8** are derived from the known natural glucoside arjunetin (**9**),[9] in the same way as compounds **1** and **2** from arjunglucoside I (**4**).

Compound **10** was isolated as an inseparable mixture (4:1) along with compound **3**. The mass spectrum of **10** displayed a pseudo-molecular ion at m/z 978 ($[M + NH_4]^+$). The molecular formula $C_{50}H_{72}O_{18}$ was inferred from the MS and ^{13}C NMR data. The ^{1}H NMR spectrum of **10** showed signals for one secondary (δ 0.94 ppm) and five tertiary (δ 1.26, 1.20, 1.10, 0.91 and 0.72 ppm) methyl groups, and an olefinic proton signal at δ 5.38 ppm (t, *J* 4 Hz). The presence of this olefinic proton and a secondary methyl group suggested that compound **10** is a Δ^{12}-ursene derivative. A broad singlet detected at δ 2.53 corresponds to H-18β of an ursane type structure with 19α-*O*-substitution.[26-28] Compound **10** is therefore a derivative of a 19α-hydroxyurs-12-en type triterpenoid. Further absorptions in the region of 3.50-5.60 ppm were similar to those observed for compound **3** (Table 2). The AB quartet (*J* 12 Hz) centered at 3.87 and 3.57 ppm was due to the oxymethylene protons H-23. The diaxial coupling observed for H-3α (δ 5.09, d, *J* 10 Hz) indicated the presence of an 2α-OAc substitutent. These data suggested that compound **10** is a derivative of 2α,3β,19α,23-tetrahydroxyurs-12-en triterpenoid. The coupling value found for a doublet due to the anomeric proton (δ 5.58, *J* 8 Hz) indicated the presence of a β-hexopyranosyl ester moiety. The sugar attached to C-28 of the aglycone was identified as β-D-glucopyranose by comparison of the ^{13}C NMR data of **10** to those of **1**, **2**, and **3**. The ^{13}C NMR spectrum of **10** provides further support for the ursene type triterpenoid structure of the aglycone. The peaks at δ 128.9 (-CH=) and 137.5 (=C<) ppm are characteristic of a Δ^{12}-ursene

triterpenoid.[16] The signal at δ 73.1 ppm revealed a tertiary carbon attached to an oxygen atom: this corresponds to C-19 bearing an α-hydroxyl group in the Δ^{12}-ursene series.[26-28] Consequently the structure of **10** is defined as 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23-tri-*O*-acetyl-2α,3β,19α,23-tetrahydroxyurs-12-en-28-oate. This is the heptaacetate of the known natural triterpene glucoside nigaichigoside F1 (**11**).[10]

Compound **12** was identified as the known combreglucoside heptaacetate by comparison of its NMR data to those published.[11] Compared to heptaacetate **3** having a single free 19-OH group this compound exhibits an additional sterically hindered and unreactive secondary 6-OH group. According to a model calculation using molecular mechanics the axial 6-OH experiences no less than three severe 1,3-diaxial repulsions with the three β-oriented, axial methyl groups located at the 4-β position and the angular positions 8 and 10.

Compound **14** was found to be closely related to **12** in that it also has an axial 6-OH but not the 19-OH group. The IR spectrum of **14** showed an absorption for the ester groups at 1749 cm^{-1}, while the sterically hindered free hydroxyl group was detected at 3498 cm^{-1}. The APCI mass spectrum displayed a pseudo-molecular ion at m/z 978 ($[M + NH_4]^+$), which together with the ^{13}C NMR data indicated the molecular formula $C_{50}H_{72}O_{18}$. The ^1H NMR spectrum displayed a dd signal centered at δ 2.85 ppm (1 H, *J* 13, 4 Hz), which is characteristic of H-18β in the C-19 unsubstituted oleanane triterpene series.[7] The observation of the triplet at δ 5.38 ppm (1 H, *J* 3 Hz), six tertiary methyl groups (δ 1.46, 1.27, 1.08, 1.02, 0.92 and 0.91 ppm), and the AB quartet at δ 3.70 and 3.95 ppm (*J* 12 Hz) is consistent with a Δ^{12} -oleanene triterpene structure substituted at C-23. Apart from signals due to protons located next to the OAc groups in the β-D-glucopyranosyl moiety, the spectrum also displayed a doublet at δ 5.01 ppm (*J* 10 Hz), and a broad singlet at δ 4.36 ppm. The multiplicity and coupling constant of the doublet signal attributed to H-3α revealed its 1,2-diaxial coupling with a single proton H-2β located near the 2-OAc α-substituent. The broad singlet at δ 4.36 ppm was attributed to H-6α by comparison with ^1H and ^{13}C NMR data of combreglucoside heptaacetate (**12**). Further comparison of the ^1H NMR spectra of **14** and **12** (Table 2) revealed the same absorptions in the region downfield from 3.50 ppm, thus indicating an identical substitution pattern at positions C-2, C-3, C-6, and C-23. The main differences in the spectrum of **14** are the disappearance of the signal at δ 3.35 ppm assigned to H-19β in **12**, and the upfield shift and multiplicity of the signal due to H-18β in **14**. Intercomparison of ^{13}C NMR data indicated that **14** differed from **12** only by the ring E substituents and that signals due to the ring E carbon atoms of **14** were comparable to those published for compounds **20**[15] and **26**[22] having C-19, C-29, and C-30 unsubstituted (Table 3). Compound **14** is therefore identified as 19-deoxycombreglucoside heptaacetate or 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23-tri-*O*-acetyl-2α,3β,6β,23-tetrahydroxyolean-12-en-28-oate. This is the heptaacetate of the known natural glucoside chebuloside II (**15**).[12]

Compounds **16** and **18** were obtained as an inseparable 3:2 mixture. The ^{13}C NMR data of the major compound (**16**) were similar to those of compound **3** except for signals due to the substituents and carbon atoms of ring A. The absence of an absorption at δ 13.8 ppm corresponding to Me-24 in **3**, and the appearance of an extra oxymethylene peak at δ 62.5 ppm

(Table 1) indicated that compound **16** is the 24-OAc analogue of **3**. Further support that C-24 is an oxymethylene carbon was provided by the downfield shift of C-4 and the upfield shift of C-23, which are in good agreement with the hydroxylation shift observed when an hydroxy (acetoxy) group is introduced respectively at a β and a γ-carbon.[16] Therefore the structure of **16** can be defined as 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23,24-tetra-*O*-acetyl-2α,3β,19α,23,24-pentahydroxyolean-12-en-28-oate. It is the octaacetate of the known natural glucoside trachelosperoside E-1 (**17**).[13]

The ^{13}C NMR data of the minor compound (**18**) were similar to those of **10** except for the substituents and carbon atoms of ring A. The absence of the signal at δ 13.9 ppm due to Me-24 in **10**, and the appearence of an extra oxymethylene peak at δ 62.5 ppm (Table 1) indicated that compound **18** is the 24-OAc analogue of **10**. The upfield shift of C-23 and the downfield shift of C-4 confirmed the presence of the extra acetoxy group at C-24. The structure of compound **18** is therefore established as 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23,24-tetra-*O*-acetyl-2α,3β,19α,23,24-pentahydroxyurs-12-en-28-oate. This is the octaacetate of the known natural glucoside trachelosperoside B-1 (**19**).[14]

Conclusions

The triterpene glycosides extracted from the root bark of *Combretum psidioides* were shown to consist of oleanane and ursane triterpene type glycosides. Their structures were established by MS, NMR, and IR analysis and by comparison with related compounds; all acetylated derivatives could be correlated with already known triterpene glycosides. Model calculations using molecular mechanics revealed that the secondary OH-groups located at the axial 6-β or 19-α positions experience severe steric repulsions and are not accessible for base-catalysed acetylation. Even under the forcing reaction conditions applied, i.e. heating in Ac_2O and pyridine at 120 °C or using DMAP, simple acetylation does not occur. Instead acetylketene is generated as a more reactive acylating agent that reacts with the 19α-OH group of arjunglucoside I heptaacetate (**3**) to form the (3-acetoxy-2-butenoyl) substituted derivatives **1**, **2**.

Experimental Section

General Procedures. Melting points were uncorrected. IR spectra were recorded as thin films between NaCl plates on a Perkin-Elmer 297 grating IR spectrophotometer. ^{1}H and ^{13}C NMR were recorded in $CDCl_3$ on Bruker AMX 400 and WM 250 instruments operating at 400 and 250 MHz for ^{1}H and 100 and 62.9 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts are reported in ppm relative to tetramethylsilane as an internal reference. *J* values are reported in Hz. Atmospheric pressure chemical ionisation mass spectra (APCI-MS) were run on a Quattro II Micromass-triple quadrupole instrument. Samples were dissolved in a 1/1 mixture of MeOH-CH_2Cl_2 at a

concentration of 100 ppm, and injected by a syringe at a rate of 20-200 µl/min (cone 20-40 V). The source temperature was 350°C. Ammonium acetate was sometimes added to the solution. Analytical thin layer chromatography was performed using Merck silica gel 60 PF-224. Column chromatography was carried out using 70-230 mesh silica gel 60 (E. M. Merck). Preparative HPLC was carried out using either a Pre-Packed Biosil D90-10 (250 x 10 mm) column (column I) or a Pre-Packed RT 250-25 Lichrosorb Si 60,7 µm column (column II). The flow rate of the solvent was 8 mL/min when using column I, and with column II it was 12 mL/min.

Computational details. Conformational calculations were carried out using *Hyperchem*™ (version 4.5; MM+ force-field).

Plant material

The plant material (root bark of *Combretum psidioides Welw*.) was collected at Kimwenza situated in the vicinity of the campus of the University of Kinshasa. It was authentified by a voucher specimen Mullenders 230 kept at the herbarium of the INERA, Faculty of Sciences, University of Kinshasa.

Extraction

Root bark powder of *Combretum psidioides Welw*. (750 g) was first extracted in a Soxhlet (five portions) respectively with hexanes for 4 hours and EtOH for 15 hours. It was then macerated in 80 % MeOH for 72 hours, and finally was refluxed for 3 hours and filtered. Ethanolic and methanolic extracts were evaporated to dryness separately. Each residue was dissolved in water and the aqueous solution was extracted with *n*-BuOH. The saponins were precipitated from the *n*-BuOH solution by adding a five-fold volume of Et_2O. The precipitate was filtered off, washed with Et_2O, and dried (12.4 g from EtOH and 21.2 g from MeOH extracts).

Acetylation and isolation of arjunetin and nigaichigoside F1 derivatives 7, 8 and 10. To 1.95 g of saponin (from methanolic extract) were added 20 mL of pyridine and 20 mL of acetic anhydride. The resulting mixture was heated at 120 °C for 4 hours after wich period it was cooled to room temperature. Water was added and the solution was extracted with methylene chloride. Evaporation of the dichloromethane solution furnished a residue which was put on the top of a silica gel column chromatography. Fraction A (319 mg) was obtained by eluting the column with a solution of 30 % acetone in hexanes (system A). Further elution with a 1/1 mixture of acetone and hexanes (system B) furnished fraction B (977 mg). HPLC purification of fraction A was performed by elution with 40 % hexanes in ethyl acetate (system C) using column II. Eight fractions (I-VIII) were obtained. Fractions IV (20 mg), VII (25 mg), and VIII (53 mg) were further purified by HPLC with column I using system A. The 3:2 unseparable mixture of compounds **7** and **8** (7 mg) was isolated from fraction IV, whereas 9 mg of the 4:1 unseparable mixture of compounds **10** and **3** were obtained from fraction VII. From fraction VIII was obtained a 3:2 mixture of compounds **1** and **2** (27 mg).

Acetylation and isolation of other saponin derivatives. A mixture of 5 g of saponin (from methanolic or ethanolic extract), 0.5 g of DMAP, and 40 mL of acetic anhydride was stirred at room temperature for 48 hours. Water was added and the solution was extracted with methylene chloride. Evaporation of the dichloromethane solution furnished a residue which was put on the top of a silica gel column chromatography. Fraction A (1.27 g) was obtained by eluting the column with a solution of 30 % acetone in hexanes (system A). Further elution with a 1/1 mixture of acetone and hexanes (system B) furnished fraction B (3.1g). HPLC purification of fraction A with column II using system A as eluent afforded fifteen fractions (retention time) : I (14.34 min), II (16.32 min), III (18.42 min), IV (19.50 min), V (20.70 min), VI (21.72 min), VII (26.58 min), VIII (27.60 min), IX (28.50 min), X (29.82 min), XI (31.26 min), XII (33.60 min), XIII (36.12 min), XIV (37.50 min), and XV (41.30 min). Fractions III, IV, VI, IX, X, XI and XIII represented pure compounds, namely peracetyl α-D-glucopyranose, peracetyl β-D-glucopyranose, peracetyl *myo*-inositol (31.5 mg), **5** (4 mg), **1** (35.3 mg), **2** (12.1 mg), and **12** (10.1 mg), respectively. Fraction VII (87.1 mg) was an unseparable 4:1 mixture of compounds **3** and **10**. Further HPLC purification of fractions VIII (14.8 mg) and XIV with 20 % ethyl acetate in dichloromethane using column I afforded compound **14** (3 mg) and the 3:2 unseparable mixture of compounds **16** and **18**, respectively. From the fraction XII crystallised a pure polyphenol rhamnoside. Its structure will be published together with other polyphenol glycosides isolated from this species.

2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23-tri-*O*-acetyl-19-*O*-[(E)-3-acetoxybut-2-enoyl]-2α,3β,19α,23-tetrahydroxyolean-12-en-28-oate (1). White powder (MeOH), m.p.117-118°C; ν_{max}^{NaCl} (cm^{-1}) 2951, 1750, 1666, 1432, 1372, 1228, 1040; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); APCI-MS m/z 1109 $([M+Na]^+$.

2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23-tri-*O*-acetyl-19-*O*-[(Z)-3-acetoxybut-2-enoyl]- 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oate (2). White powder (MeOH), m.p. 96-98°C; ν_{max}^{NaCl} (cm^{-1}) 2951, 1749, 1669, 1434, 1372, 1232, 1041; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); APCI-MS m/z 1104 $([M+NH4]^+$.

2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23,29-tetra-*O*-acetyl-2α,3β,23,29-tetrahydroxy-olean-12-en-28-oate (5). Unrecrystallised solid , m.p. 67-71°C; ν_{max}^{NaCl} (cm-^{1}) 3057, 2959, 1747, 1373, 1264, 1231, 1042; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); APCI-MS m/z 1020 $([M+NH4]^+$).

2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,3,23-tri-O-acetyl-2α,3β,6β,23-tetrahydroxyolean-12-en-28-oate (14). White powder (MeOH), m.p. 105-107°C; ν_{max}^{NaCl}(cm^{-1}) 3498, 2948, 1749, 1434, 1372, 1230, 1041; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); APCI-MS m/z 978 $([M+NH_4]^+$).

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