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Monoterpene glycosides isolated from *Fadogia agrestis*

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Abstract

Six monoterpene glycosides were isolated from *Fadogia agrestis*. Their structures were elucidated using a combination of mass spectroscopy, 1D- and 2D-homo- and hetero-NMR spectroscopy and chemical analysis, and established as being derivatives of 2,6-dimethyl-2(*E*),6(*Z*)-octadiene-1,8-diol containing from two to four units of rhamnopyranose and, three of them, one or two additional units of glucopyranose. In three of the compounds an acyl group of 8-hydroxy-2,6-dimethyl-2(*E*),6(*Z*)-octadienoyl was found esterifying the *O*-2 position of one of the units of rhamnopyranose.

Keywords: *Fadogia agrestis*; Rubiaceae; Monoterpene glycosides

1. Introduction

Fadogia agrestis Schweinf ex Hiern (Rubiaceae) is a shrub from 30 to 90 cm high, found from Guinea to Sudan. In the African traditional medicine, the decoction of this plant is extensively used as a febrifuge which could be associated with its use as an antimalarial drug. *In vitro* antiplasmodial activity has been reported for extracts from leaves collected in Burkina Faso (Sanon et al., 2003). This shrub is mainly used also as diuretic plant and in the treatment of kidneys pain and convulsions (Adjanohoun et al., 1986, 1989). Aphrodisiac potentials of the aqueous extract of *F. agrestis* stem in male albino rats have been also evaluated (Yakubu et al., 2005).

We here report on the isolation and structural elucidation of six new monoterpene glycosides isolated from *F. agrestis*.

2. Results and discussion

The powdered dried leaves of *F. agrestis* were initially extracted with chloroform, followed by pure methanol to remove less polar products. The solid residue was then treated with 80% methanol and the extract was dried and partitioned between H_2O and 1-butanol. The butanol extract was subjected to Si gel column chromatography, and the fractions obtained were rechromatographed through MPLC to afford compounds **1** and **2**, **4**–**6**. The aqueous extract was also subjected successively to Si gel column and MPLC to yield sucrose and compound **3** (see Fig. 1).

Compound **1** showed a molecular peak at *m*/*z* 608.3 (M^+) in the positive mode ESIMS, suggesting a molecular formula $C_{28}H_{48}O_{14}$. The ^1H NMR spectrum contained *inter alia* two ethylenic protons as broad triplets at 5.43 and 5.36 ppm. A broad multiplet at 2.16 ppm integrating for four protons suggested the presence of two methylene groups. The study of the region at 4.2–3.3 ppm revealed the presence of additional aglyconic protons. Two methyl

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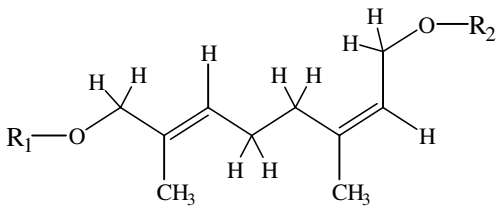

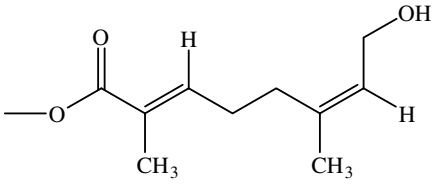

B

1 R_1: α-L-Rha*p*-(1→

R_2: α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→

2 R_1: β-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→

R_2: α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→

3 R_1: β-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→

R_2: β-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→

4 R_1: α-L-Rha*p*-(1→

R_2: α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ 3)-α-L-Rha*p*-(1→
2
↑
Acyl B

5 R_1: α-L-Rha*p*-(1→

R_2: α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ 3)-α-L-Rha*p*-(1→
2 4
↑ ↑
Acyl B CH_3-CO

6 R_1: β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→

R_2: α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→
2
↑
Acyl B

Fig. 1. Monoterpene glycosides structures of *F. agrestis*.

singlets appeared at 1.77 and 1.66 ppm. Three doublets at *ca.* 1.25 ppm integrating for nine protons suggested the presence of 6-deoxy sugars. The existence of three sugar units was revealed by three anomeric doublets (4.99, 4.67, and 4.66 ppm) with coupling constants around 1.7 Hz. The ^{13}C NMR spectrum contained four ethylenic singlets at 142.2, 133.4, 128.7, and 122.4 ppm, which revealed the presence of two double bonds in the molecule. In addition, three signals at 104.1 and 100.3 (two overlapping carbons) corroborated the existence of three sugar units.

The aglyconic structure was established using 2D-homo- (COSY, TOCSY) and hetero-(HMQC, HMBC) experiments, which allowed the assignment of all the protons and carbons (see Tables 1 and 2). From those experiments, the aglycone was deduced to be 2,6-dimethyl-2,6-octadiene-1,8-diol. In order to determine the configuration of the double bonds of the aglycone a 2D ROESY experiment was performed which showed, *inter alia*, crosspeaks connecting H-1a+b with H-3, H-9 with H-4a+b, H-7 with H-10 and H-8a+b with H-5a+b, which revealed configurations 2(*E*) and 6(*Z*) for the double bonds. This compound has been isolated from *Rosa damascena* (Knapp et al., 1998).

The study of the ring protons of the sugars allowed the measurement of most of the proton coupling constants of each sugar unit (Table 3), and also the chemical shifts of all the carbons (Table 4). From these values, the presence of three rhamnose residues was inferred (labelled Rha*p*1-3 according to their anomeric protons from low to high field). Acid hydrolysis of compound **1**, and TLC comparison with an authentic sample of rhamnose corroborated those deductions. Comparison of the carbon chemical shifts with that of model compounds (Bock and Pedersen, 1983) allowed deducing the glycosylation positions (see Table 4). The anomeric configuration of the rhamnose units was derived from a carbon-coupled HMQC experi-

Table 1
^1H NMR data of the aglyconic moieties of compounds **1**–**6**

	1	**2**	**3**	**4**	**5**	**6**
H						
1a	4.01	4.02	4.02	4.00	4.00	4.01
1b	3.83	3.84	3.84	3.84	3.84	3.85
3	5.43	5.44	5.43	5.43	5.43	5.44
4a	2.17	2.16	2.16	2.16	2.16	2.17
4b	2.17	2.16	2.16	2.16	2.16	2.17
5a	2.16	2.16	2.16	2.16	2.16	2.17
5b	2.16	2.16	2.16	2.16	2.16	2.17
7	5.36	5.35	5.35	5.35	5.37	5.35
8a	4.10	4.10	4.10	4.10	4.11	4.09
8b	4.03	4.03	4.03	4.02	4.04	4.03
9	1.66	1.67	1.67	1.66	1.65	1.66
10	1.77	1.77	1.77	1.77	1.76	1.76
B						
3′				6.77	6.78	6.76
4′a				2.33	2.33	2.34
4′b				2.33	2.33	2.34
5′a				2.23	2.23	2.24
5′b				2.23	2.23	2.24
7′				5.42	5.42	5.42
8′a				4.08	4.07	4.09
8′b				4.08	4.07	4.03
9′				1.84	1.84	1.84
10′				1.77	1.76	1.76
Ac.						
2″					2.16	

Table 2
^{13}C NMR data of the aglyconic moieties of compounds **1–6**

	1	2	3	4	5	6
C						
1	74.0	74.0	74.0	74.0	74.1	73.9
2	133.4	133.5	133.4	133.4	133.4	133.3
3	128.7	128.7	128.7	128.7	128.7	129.0
4	27.4	27.4	27.5	27.4	27.4	27.5
5	32.7	32.7	32.6	32.7	32.7	32.6
6	142.2	142.2	142.1	142.2	142.6	142.3
7	122.4	122.4	122.5	122.4	122.2	122.3
8	64.0	64.0	64.3	64.0	64.3	64.0
9	14.2	14.2	14.3	14.2	14.2	14.2
10	23.7	23.7	23.8	23.7[a]	23.7[b]	23.7[c]
B						
1′				168.7	168.7	168.7
2′				129.0	129.0	129.0
3′				143.8	143.8	143.8
4′				28.1	28.1	28.1
5′				31.5	31.5	31.5
6′				138.5	138.5	138.5
7′				126.8	126.7	126.7
8′				59.2	59.3	59.2
9				12.6	12.5	12.6
10′				23.5[a]	23.5[b]	23.5[c]
Ac.						
1″					172.2	
2″					21.1	

[a,b,c]In each column, these values may be interchanged.

ment, which gave carbon–proton anomeric coupling constants $^{1}J_{\text{C-1-H-1}} \approx 170$ Hz, corresponding to α-anomers for the three units (Bock and Pedersen, 1974). Concerning the connections of the rhamnose residues, they were unequivocally deduced from the HMBC experiments. Thus, crosspeaks H-1 of Rha*p*-3 with C-1 of the aglycone, H-1 of Rha*p*-1 with C-3 of Rha*p*-2, and H-1 of Rha*p*-2 with C-8 of the aglycone revealed the arrangement of compound **1**, which was assigned a structure of (2*E*,6*Z*)-2,6-dimethyl-8-[(*O*-α-L-rhamnopyranosyl-(1 → 3)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl α-L-rhamnopyranoside.

Compound **2** exhibited a molecular peak at *m/z* 770.3 (M^+) in the ESI+ spectrum, compatible with a molecular formula $C_{34}H_{58}O_{19}$. The ^{1}H NMR spectrum showed two ethylenic protons as broad triplets at 5.44 and 5.35 ppm, two methyl singlets at 1.77 and 1.67 ppm, and the rest of aglyconic protons showing a pattern very similar to that found in compound **1**. The major difference was the presence of an additional anomeric doublet at 4.45 ppm ($^{3}J_{\text{H-1,H-2}} = 7.9$) which indicated the existence of one more sugar unit. The chemical shifts values for proton and carbons were deduced through 2D NMR experiments analogous to those used for compound **1**. The values found for all the protons and carbons are gathered in Tables 1 and 2. From them, an aglycone identical to that of **1** was deduced. Concerning the nature of the sugars it was inferred from the proton vicinal coupling constants (Table 3), which revealed the presence of three rhamnose and a glucose unit. The linkage positions were deduced from the carbon chemical shifts (Table 4), which indicated that one of the rhamnoses and the glucose units were terminal, and two of the rhamnoses were substituted at positions O-2 and O-3, respectively, and the sequence was unequivocally obtained from an HMBC experiment, which contained crosspeaks connecting H-1 of the Glc*p* moiety with C-2 of Rha*p*-1, H-1 of Rha*p*-1 with C-1 of the aglycone, H-1 of Rha*p*-2 with C-3 of Rha*p*-3, and H-1 of Rha*p*-3 with C-8 of the aglycone. Therefore, the structure of compound **2** was established as (2*E*,6*Z*)-2,6-dimethyl-8-[(*O*-α-L-rhamnopyranosyl-(1 → 3)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl *O*-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

Compound **3** showed a molecular peak at *m/z* 786.3 (M^+) in the ESI+ mass spectrum, indicating a molecular formula $C_{34}H_{58}O_{20}$. The ^{1}H NMR spectrum displayed a general pattern very similar to that of the compounds described above, with two ethylenic protons as broad triplets at 5.48 and 5.35 ppm, a multiplet at 2.16, two methyl singlets at 1.77 and 1.67 ppm, and a doublet at 1.26 (6H). The chemical shifts of protons and carbons (Tables 1 and 2) were deduced from 2D-experiments analogous to those used in compounds **1** and **2**, from which the monoterpene structure established for them was also found in **3**. Four anomeric doublets were also observed in the proton spectrum, at 5.04, 5.02, 4.45, and 4.44 ppm, with coupling constants $^{3}J_{\text{H-1,H-2}} = 1.7$, 1.8, 7.8, and 7.7 Hz, respectively. Most of the ring coupling constants was also measured, from which two α-rhamnopyranoses and two β-glucopyranoses revealed to be present in the molecule (Table 3). Both rha*p* were substituted at positions O-2, while both glc*p* shown to be terminal units. An HMBC experiment gave crosspeaks of H-1 of each glc*p* with C-2 of each rha*p*, and H-1 of each rha*p* with C-1 and C-8 of the aglycone. Thus, the molecular structure of compound **3** was established as (2*E*,6*Z*)-2,6-dimethyl-8-[(*O*-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl *O*-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

A molecular formula $C_{44}H_{72}O_{20}$ was concluded for compound **4**, on the basis of the molecular peak at *m/z* 920.5 (M^+) in the ESI+ mass spectrum. The ^{1}H NMR spectrum was more complex than those of derivatives **1–3**, disclosing four ethylenic protons, at 6.77 (double triplet, $J = 7.7$, 7.3, 1.5 Hz), 5.42 and 5.43 (multiplets), and 5.35 (double triplet, $J = 7.9$, 6.4, and 1.5 Hz). A quartet at 2.33 ppm (2H), a triplet at 2.23 (2H), and a multiplet at 2.16 ppm (4H) appeared at high field. In addition, three methyl signals at 1.84 (3H, $J = 1.5$ Hz), 1.77 (6 H, $J = 1.5$ Hz), and 1.66 (3 H, $J = 1.5$ Hz), and also four doublets ($J = 6$ Hz) at 1.30–1.17 ppm could be observed. The ^{13}C NMR spectrum revealed a carbonyl singlet (168.7 ppm), eight ethylenic singlets between 144 and 122 ppm, corresponding to four double bonds, and four anomeric singlets (104–100 ppm), indicating the presence of four sugar residues. The proton and carbon chemical shifts were deduced through experiments analogous to those used above, and have been gathered in Tables 1–4. From them, the presence of the monoterpene deduced for

Table 3
^{1}H NMR data of the carbohydrate moieties of compounds **1**–**6**

H	**1**	**2**	**3**	**4**	**5**	**6**
	α-Rhap-1	α-Rhap-1	α-Rhap-1	α-Rhap-1	α-Rhap-1	α-Rhap-1
1	4.99 (1.7 *d*)	5.01 (1.8 *d*)	5.04 (1.7 *d*)	5.04 (1.8 *d*)	4.94 (1.6 *d*)	5.04 (1.9 *d*)
2	3.95 (3.4 *dd*)	3.86 (3.4 *dd*)	3.83 (3.6 *dd*)	5.30 (3.3 *dd*)	3.92 (3.4 *dd*)	5.30 (3.0 *dd*)
3	3.75 (9.5 *dd*)	3.72 (9.5 *dd*)	3.72	4.05 (9.6 *dd*)	3.54 (9.5 *dd*)	4.05 (9.4 *dd*)
4	3.37 (9.5 *t*)	3.37 (9.5 *t*)	3.36 (9.5 *t*)	3.50 (9.4 *t*)	3.34 (9.4 *t*)	3.50 (9.2 *t*)
5	3.76 (6.1 *dq*)	3.60 (*m*)	3.58 (*m*)	3.84 (*m*)	3.48 (*m*)	3.85 (*m*)
6a	1.24 (6.1 *d*)	1.26 (6.1 *d*)	1.26 (6.2 *d*)	1.17 (6.1 *d*)	1.16 (6.1 *d*)	ca. 1.26
6b						
	α-Rhap-2	α-Rhap-2	α-Rhap-2	α-Rhap-2	α-Rhap-2	α-Rhap-2
1	4.67 (1.8 *d*)	4.99 (1.8 *d*)	5.02 (1.8 *d*)	4.98 (1.8 *d*)	4.81 (1.8 *d*)	4.99 (1.8 *d*)
2	3.84 (3.4 *dd*)	3.96 (3.4 *dd*)	3.85 (3.6 *dd*)	3.92 (3.3 *dd*)	5.02 (3.4 *dd*)	3.92 (3.3 *dd*)
3	3.72 (9.5 *dd*)	3.76 (9.5 *dd*)	ca. 3.69	3.55 (9.8 *dd*)	4.00 (9.8 *dd*)	3.56
4	3.48 (9.5 *t*)	3.38 (9.5 *t*)	3.37 (9.5 *t*)	3.33 (9.4 *t*)	3.48 (9.4 *t*)	3.54 (9.5 *t*)
5	3.61 (6.1 *dq*)	3.75 (*m*)	3.60 (*m*)	3.58 (*m*)	3.84 (*m*)	3.57 (*m*)
6a	1.25 (6.1 *d*)	1.24 (6.1 *d*)	1.26 (6.2 *d*)	1.25 (6.1 *d*)	1.26 (6.1 *d*)	1.17 (6.2 *d*)
6b						
	α-Rhap-3	α-Rhap-3	β-Glcp-1	α-Rhap-3	α-Rhap-3	α-Rhap-3
1	4.66 (1.7 *d*)	4.67 (1.8 *d*)	4.45 (7.8 *d*)	4.68 (1.8 *d*)	4.72 (1.3 *d*)	4.68 (1.7 *d*)
2	3.77 (3.4 *dd*)	3.84 (3.3 *dd*)	3.25 (9.0 *dd*)	3.84 (3.3 *dd*)	3.86	4.05
3	3.64 (9.4 *dd*)	3.72 (9.5 *dd*)	3.35 (9.5 *dd*)	3.73 (9.5 *dd*)	3.86 (9.7 *dd*)	3.74
4	3.36 (9.5 *t*)	3.48 (9.7 *t*)	3.32 (9.5)	3.53 (9.4 *t*)	5.09 (9.5 *t*)	3.54
5	3.59 (*dq*)	3.62 (*m*)	3.26	3.58 (*m*)	3.75 (*m*)	3.62 (*m*)
6a	1.25 (6.1 *d*)	1.26 (6.1 *d*)	3.83	1.25 (6.1 *d*)	1.15 (6.2 *d*)	*ca.* 1.26
6b			3.65			
		β-Glcp	β-Glcp-2	α-Rhap-4	α-Rhap-4	α-Rhap-4
1		4.45 (7.9 *d*)	4.44 (7.7 *d*)	4.65 (1.8 *d*)	4.65 (1.7 *d*)	4.67 (1.8 *d*)
2		3.26 (9.0 *dd*)	3.25 (9.0 *dd*)	3.77 (3.4 *dd*)	3.76 (3.4 *dd*)	3.84
3		3.35 (>9)	3.35 (9.5 *dd*)	3.64 (9.5 *dd*)	3.63 (9.5 *dd*)	3.75 (9.2 *dd*)
4		3.30 (>8.9)	3.32 (9.5 *t*)	3.36 (9.4 *t*)	3.35	3.54 (9.1 *t*)
5		3.27 (2.4,5.2 *m*)	3.26	3.58 (*m*)	3.57 (*m*)	3.58 (*m*)
6a		3.83 (11.9 *dd*)	3.83	1.24 (6.1 *d*)	1.25 (6.1 *d*)	ca. 1.26
6b		3.66 (11.9 *dd*)	3.65			
						β-Glcp
1						4.51 (7.9 *d*)
2						3.28 (9.4 *dd*)
3						3.34 (>9)
4						3.55
5						3.29
6a						3.82
6b						3.70

compounds **1**–**3** and also the existence of four units of rha*p* were also found here. In addition, it was evident the appearance of a second monoterpene, on the basis of the two new double bonds and two methyl groups. The 2D NMR experiments revealed that the new monoterpene was an acyl group, corroborated by a peak at 1702 in the IR spectrum, corresponding to 8-hydroxy-2,6-dimethyl-2(*E*), 6(*Z*)-octadienecarboxylic acid (labelled B). A (2*E*, 6*E*) isomer of this acid, and an ester with non-reported stereochemistry at C-3 have been isolated from *Radermachia sinica* (Iwagawa et al., 1990) and *Hoffmania strigillosa* (Jaensch et al., 1990), respectively. The HMBC and ROESY experiments indicated that this acid was esterifying the *O*-2 position of the rha*p*-1, the carbon of which and its corresponding geminal proton appeared at very low field, as expected. From all the evidence, the structure of compound **4** was established as being: (2*E*,6*Z*)-2,6-dimethyl-8-[(*O*-α-L-rhamnopyranosyl-(1 → 3)-(2-*O*-((2*E*, 6*Z*)-8-hydroxy-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl α-L-rhamno-pyranoside.

Compound **5** exhibited a molecular peak at *m*/*z* 962.4 (M^+) in the ESI+ mass spectrum, compatible with a molecular formula $C_{46}H_{74}O_{21}$. The ^{1}H NMR spectrum displayed a pattern very similar to that of compound **4**, but a singlet (3H) at *ca.* 2 ppm was observed, suggesting the presence of an acetyl group. The proton and carbon chemical shifts were obtained as described in the cases above (Tables 1–4). Concerning the sugar moieties, the far downfield values of the protons H-2 of rha*p*-2 and H-4 of rha*p*-3 suggested they were acylated. An HMBC experiment showed crosspeaks between H-2 of rha*p*-2 and C-1 of the acyl monoterpene

Table 4
^{13}C NMR data of the carbohydrate moieties of compounds **1**–**6**

C	1	2	3	4	5	6
	α-Rhap-1	α-Rhap-1	α-Rhap-1	α-Rhap-1	α-Rhap-1	α-Rhap-1
1	104.1	99.6	99.6	100.7	104.2	100.7
2	72.1	**82.6**	**82.6**	73.2	72.1	74.0[a]
3	72.2	72.4	72.4	**78.4**	72.4	**78.4**
4	73.9[a]	74.4[a]	74.5	74.0[a]	73.8[a]	73.8[a]
5	70.0[b]	70.1[b]	70.0	69.9[b]	70.4	70.2
6	18.1[c]	18.1[c]	18.2	18.2[c]	17.8[b]	18.1
	α-Rhap-2	α-Rhap-2	α-Rhap-2	α-Rhap-2	α-Rhap-2	α-Rhap-2
1	100.3	104.1	99.8	104.1	100.8	104.1
2	72.2	72.4	**82.6**	72.2	74.0	72.2
3	**79.8**	72.4	72.4	72.3	**78.3**	72.3
4	73.3	74.2[a]	74.5	74.1[a]	73.9[a]	72.7
5	69.9[b]	70.1[b]	70.0	70.5[b]	70.5	70.1[b]
6	18.1[c]	18.0[c]	18.2	17.9[c]	18.0[b]	17.9
	α-Rhap-3	α-Rhap-3	β-Glcp-1	α-Rhap-3	α-Rhap-3	α-Rhap-3
1	100.3	100.3	106.8	100.2	100.3	100.2
2	72.4	72.4	75.6	72.1	72.1	71.6
3	72.5	**79.8**	78.0	**79.5**	**78.8**	**79.5**
4	74.1[a]	73.3	71.3	73.2[a]	74.0[a]	72.7
5	70.1[b]	69.8[b]	78.2	70.6[b]	68.0	69.6[b]
6	18.0[c]	18.0[c]	62.5	18.0[c]	17.8[b]	18.1
		β-Glcp	β-Glcp-2	α-Rhap-4	α-Rhap-4	α-Rhap-4
1		106.8	106.8	100.3	100.3	100.0
2		75.5	75.6	72.4	72.4	71.6
3		77.9	78.0	72.5	72.4	**83.1**
4		71.2	71.3	74.2[a]	73.9[a]	72.1
5		77.9	78.2	70.2[b]	69.9	70.2
6		62.6	62.5	18.0[c]	18.1[b]	18.1
						β-Glcp
1						105.8
2						75.4
3						77.7
4						72.2
5						77.7
6						62.2

[a,b,c]In each column, these values may be interchanged. Bold numbers represent glycosylation sites.

(B), and also between H-4 of rha*p*-3 and C-1 of the acetyl group. All those data led to establish for **5** a structure of (2*E*,6*Z*)-2,6-dimethyl-8-[(*O*-α-L-rhamnopyranosyl-(1 → 3)-(2-*O*-((2*E*,6*Z*)-8-hydroxy-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)-(1 → 3)-4-*O*-acetyl-α-L-rhamnopyranosyl)oxy]-octadien-1-yl α-L-rhamnopyranoside.

Compound **6** was assigned a molecular formula $C_{50}H_{82}O_{25}$ on the basis of a molecular peak observed at *m*/*z* 1082.3 (M^+) in the ESI+ mass spectrum. The ^{1}H and ^{13}C NMR spectra displayed a pattern for the aglyconic moieties very similar to those observed for derivatives **4** and **5** (see Tables 1–4). The glycosidic anomeric region contained five doublets at 5.04, 4.99, 4.68, 4.67 (all with coupling constants *ca.* 1.8 Hz) and 4.51 ($^3J_{H\text{-}1,H\text{-}2} = 7.9$ Hz), indicating the presence of four units of α-rha*p* and one of β-glc*p*. The ROESY and HMBC experiments revealed that rha*p*-1 was linked to the *O*-1 of the aglycone, and substituted at position-3 by glc*p*, the rha*p*-2 was connected to rha*p*-3 at position 3 which, in turn, was linked to position 3 of rha*p*-4. This last was connected to position *O*-8 of the aglycone. As in compounds **4** and **5**, the group B was acylating the *O*-2 of rhap-3. Consequently, compound **6** was assigned the structure: (2*E*,6*Z*)-2,6-dimethyl-8-[(*O*-α-L-rhamnopyranosyl-(1 → 3)-(2-*O*-((2*E*,6*Z*)-8-hydroxy-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl *O*-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

In conclusion, this work constitutes the first contribution to the knowledge of the *F. agrestis* chemistry. In addition, although several related monoterpene derivatives have been described, to the best of our knowledge this is the first time that a monoterpene with 2(*E*), 6(*Z*) configuration for the acyl group B has been found. *F. agrestis* is largely used as antimalarial in African traditional medicine but no study on active compounds has been reported before. We report on six new monoterpene glycosides described for the first time. These compounds presented a moiety related to allylic alcohols. Geraniol, an abundantly available naturally occurring allylic alcohol, has been used as starting material to prepare very promising antimalarial compounds against

multi-drug resistant *Plasmodium* (Singh et al., 2003). The six monoterpene glycosides isolated from *F. agrestis* could be the precursors of compounds which after hydrolysis or photo-oxygenation could exhibit antimalarial activity as demonstrated for geraniol (Singh et al., 2002, 2003). Bioconversions of monoterpene glycosides could explain the formation of active compounds in the traditional extracts by decoction of *F. agrestis*.

3. Experimental

3.1. General procedures

Optical rotations were measured on a Perkin–Elmer 241 polarimeter, in quartz cells (1 dm), with methanol as solvent and using a sodium lamp operating at 598 nm. IR spectra were obtained on a Perkin–Elmer Spectrum One spectrophotometer. NMR spectra were recorded in CD_3OD, on a Varian Unity 500 instrument at 26 °C, with the exception of compound **5**, registered at 30 °C. Chemical shifts were referenced at the methanol-d_4 multiplet (^1H, 3.30 ppm; ^{13}C, 49.0 ppm). Electrospray ionisation (ESIMS) was conducted on a Hewlett–Packard MSD 1100 LC/MS apparatus. Analytical TLC was carried out on Merck silica gel F_{254} aluminum sheets, eluted with *n*-BuOH–HOAc–H_2O (4:1:5), and visualized with 1% vanillin in MeOH–H_2SO_4 (1:1). Carbohydrates were identified by chromatographic comparison with authentic samples of glucose and rhamnose.

3.2. Plant material

Leaves of *Fadogia agrestis Schweinf ex Hiern* (Rubiaceae) were collected in Burkina Faso, in March 2001, and were identified by Alassane Ouedraogo, inspector of National School of Water and Forest at Dinderesso (Burkina Faso). A voucher specimen has been deposited at the laboratory of Pharmacognosy of Marseille as BM-fa 99.

3.3. Extraction and isolation of constituents

Dried leaves of *F. agrestis* (500 g) were powdered and extracted first with chloroform at room temperature, and then with pure MeOH. The solid residue was extracted with 80% MeOH for 16 h and the solvent was evaporated under a vacuum. The aqueous layer was lyophilized, giving a mixture of compounds (98 g). Fifty grams of this mixture were partitioned between H_2O (1 l) and *n*-BuOH (3 × 300 ml). The *n*-BuOH extracts were concentrated to dryness under a vacuum, and were purified first through a Si gel column, using mixtures of CH_2Cl_2/MeOH/H_2O of increasing polarity as eluents, and the fractions obtained were further purified by MPLC (Si gel reversed-phase, eluents: gradient $H_2O \rightarrow$ MeOH, and then 10% MeOH → MeOH. Pres. <20 bars) giving compounds **1** (2.7 mg), **2** (4.2 mg), **4** (33.9 mg), **5** (23.2 mg), and **6** (1.7 mg). The aqueous extract was lyophilized and purified first through a Si gel column, using mixtures of CH_2Cl_2/MeOH/H_2O as above, and the fractions obtained were again purified by MPLC, giving sucrose (154.0 mg) and compound **3** (1.1 mg).

3.4. (2E,6Z)-2,6-Dimethyl-8-[(O-α-L-rhamnopyranosyl-(1 → 3)-α-L-rhamnopyranosyl)-oxy]-octadien-1-yl α-L-rhamnopyranoside (1)

Amorphous powder. $[\alpha]_D$ −92° (MeOH; *c* 1.3). ^1H and ^{13}C NMR (Tables 1–4). ESI+ *m/z* 608.3 (M^+).

3.5. (2E,6Z)-2,6-Dimethyl-8-[(O-α-L-rhamnopyranosyl-(1 → 3)-α-L-rhamnopyranosyl)-oxy]-octadien-1-yl O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside (2)

Amorphous powder. $[\alpha]_D$ −72° (MeOH; *c* 1.0). ^1H and ^{13}C NMR (Tables 1–4). ESI+ *m/z* 770.3 (M^+).

3.6. (2E,6Z)-2,6-Dimethyl-8-[(O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl)-oxy]-octadien-1-yl O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside (3)

Amorphous powder. $[\alpha]_D$ −63° (MeOH; *c* 1.1). ^1H and ^{13}C NMR (Tables 1–4). ESI+ *m/z* 786.3 (M^+).

3.7. (2E,6Z)-2,6-Dimethyl-8-[(O-α-L-rhamnopyranosyl-(1 → 3)-(2-O-((2E,6Z)-8-hydroxy-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl)oxy]-octadien-1-yl α-L-rhamnopyranoside (4)

Amorphous powder. $[\alpha]_D$ −78° (MeOH; *c* 1.2). IR (KBr) ν_{max} cm^{-1}: 3387, 2932, 1702, 1447, 1380, 1266, 1124, 1053, 990. ^1H and ^{13}C NMR (Tables 1–4). ESI+ *m/z* 920.5 (M^+).

3.8. (2E,6Z)-2,6-Dimethyl-8-[(O-α-L-rhamnopyranosyl-(1 → 3)-(2-O-((2E,6Z)-8-hydroxy-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)-(1 → 3)-4-O-acetyl-α-L-rhamnopyranosyl)oxy]-octadien-1-yl α-L-rhamnopyranoside (5)

Amorphous powder. $[\alpha]_D$ −77° (MeOH; *c* 1.1). IR (KBr) ν_{max} cm^{-1}: 3394, 2926, 1701, 1449, 1270, 1127, 1052, 987. ^1H and ^{13}C NMR (Tables 1–4). ESI+ *m/z* 962.4 (M^+).

3.9. (2E,6Z)-2,6-Dimethyl-8-[(O-α-L-rhamnopyranosyl-(1 → 3)-(2-O-((2E,6Z)-8-hy-droxy-2,6-dimethyloctadienoyl)-α-L-rhamnopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl)-oxy]-octadien-1-yl O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside (6)

Amorphous powder. $[\alpha]_D$ −62° (MeOH; *c* 1.2). IR (KBr) ν_{max} cm^{-1}: 3391, 2933, 1710, 1449, 1379, 1232, 1125, 1051, 990. ^1H and ^{13}C NMR (Tables 1–4); ESI+ *m/z* 1082.3 (M^+).

3.10. Acid hydrolysis of compounds **1–6**

Each sample (0.5–1 mg) was refluxed with 10% HCl (4 ml) for 4 h. After extraction with diethyl ether, the aqueous solution was neutralized (10% *N*,*N*-dioctylmethylamine in $CHCl_3$) and concentrated under reduced pressure. The sugars were directly analyzed by TLC. Rhamnose and glucose were identified by TLC comparison with authentic samples (CH_2Cl_2/MeOH/H_2O: 50/25/5 vol. Rf = 0.60 and 0.34, respectively).

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