Triumfettosterol Id and Triumfettosaponin, a New (Fatty Acyl)-Substituted Steroid and a Triterpenoid 'Dimer' Bis(β-D-glucopyranosyl) Ester from the Leaves of Wild *Triumfetta cordifolia* A. RICH. (Tiliaceae)

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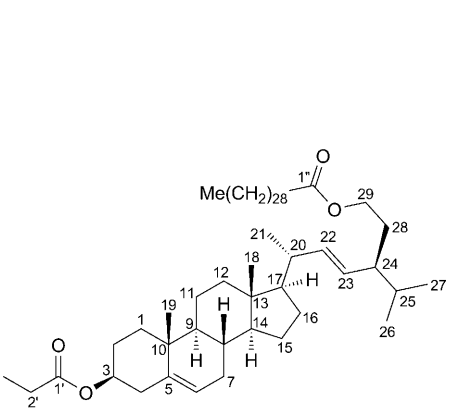
Two new triterpenoid derivatives were isolated from the leaves of wild *Triumfetta cordifolia* A. RICH. and identified to be a (fatty acyl)-substituted steroid **1** and a triterpenoid saponin 'dimer' **2**, named (3β)-stigmasta-5,22-diene-3,29-diol 3-propanoate 29-triacontanoate and (2α,3β,19α)-2,3,19-trimethoxyurs-12-ene-24,28-dioic acid 24-[(2α,3β)-24,28-bis(β-D-glucopyranosyloxy)-2-hydroxy-24,28-dioxours-12-en-3-yl] ester, respectively. These compounds were obtained together with a mixture of known sterols (stigmasterol/β-sitosterol = (3β,22*E*)-stigmasta-5,22-dien-3-ol/(3β)-stigmast-5-en-3-ol) and *trans*-tiliroside (**3**). The structures **1** and **2** were determined on the basis of NMR data (^{1}H-, ^{13}C-, and 2D-NMR analyses) and mass spectrometry and confirmed by chemical transformations. The antimicrobial activities of *trans*-tiliroside (**3**) against eight bacterial and two fungal strains were evaluated. This compound showed weak activities on some bacterial strains.

Introduction. – *Triumfetta cordifolia* A. RICH. (Tiliaceae), located in tropical Africa, is a shrub about 5 meters high [1] used in Cameroon as foodstuff. Its importance in traditional medicine [2][3] prompted us to investigate the stems and the leaves of this plant. In the course of a previous study, two new ceramides were isolated and identified from the stems [4]. Thus, in continuation of this study, four other products were isolated from the leaves of wild *Triumfetta cordifolia*. These products include two new triterpenoids derivatives, **1** and **2**[1]), a mixture of sterols (stigmasterol/β-

[1]) In the index name of **2**, the CIP-preferred substituent at C(4) has lowest locant, *i.e.*, C(23) and C(24), as well as C(23′) and C(24′) must be reversed (see *Exper. Part*).

sitosterol = (3β,22*E*)-stigmasta-5,22-dien-3-ol/(3β)-stigmast-5-en-3-ol) [5], and *trans*-tiliroside (**3**) [6]. This article deals with the isolation and structure elucidation of the new compounds based on spectroscopic methods and some chemical transformations. The antimicrobial activities of compound **3** are also reported.



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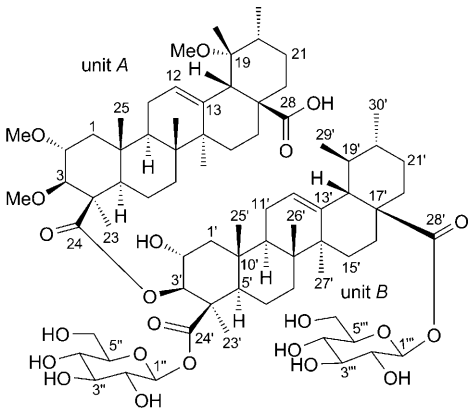
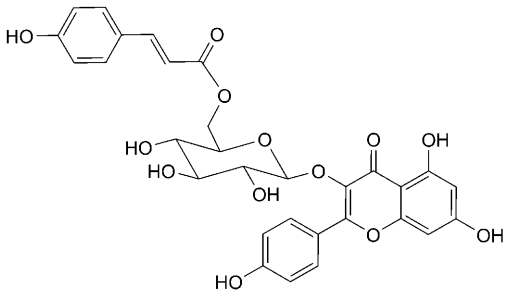


2[1])



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Results and Discussion. – 1. *Structure Elucidation*. The crude extract, subjected to repeated column chromatography (CC), yielded a new steroidal fatty acid ester **1**, a new 'dimeric' triterpene saponin **2**, a mixture of sterols (stigmasterol/β-sitosterol) [5], and *trans*-tiliroside (**3**) [6].

Compound **1** was obtained as a white amorphous solid after CC. Its positive-ion-mode HR-ESI-FT-ICR-MS showed a peak at *m*/*z* 919.8486 corresponding to the molecular formula $[C_{62}H_{110}O_4 + H]^+$. Compound **1** responded to the *Liebermann–Burchard* test of triterpenes. The blue color observed was indicative of a steroid-type triterpenoid. The IR spectrum showed intense absorption bands at 1738 and 1240 cm^{-1}, characteristic of an ester function [7]. It had no UV maximum in the region of λ 210–260 nm indicating the absence of a conjugated system. The ^{1}H- (*Table 1*) and ^{13}C-(APT) (*Table 2*) NMR spectra exhibited three significant signals indicative of a $\Delta^{5,22}$ steroid at δ(H) 5.40 (br. *d*, *J* = 5.0 Hz, H–C(6)), 5.16 (*dd*, *J* = 10.0, 16.7 Hz, H–C(22)), and 5.02 (*dd*, *J* = 10.0, 16.7 Hz, H–C(23)) and δ(C) 122.6 (C(6)), 137.6 (C(22)), and

Table 1. *^{1}H-NMR Data* ($CDCl_3$, 400 MHz) *of Compound* **1**

	δ(H)		δ(H)		δ(H)
CH_2(1)	1.90–2.00 (*m*, H_β), 1.00–1.20 (*m*, H_α)	H–C(14)	1.00–1.05 (*m*)	H–C(25)	1.40–1.60 (*m*)
		CH_2(15)	0.80–0.90 (*m*, H_β), 1.30–1.40 (*m*, H_α)	Me(26)	0.90 (*d*, *J* = 9.7)
CH_2(2)	1.80–1.90 (*m*, H_α), 1.55–1.64 (*m*, H_β)			Me(27)	0.83 (*d*, *J* = 9.7)
		CH_2(16)	1.10–1.30 (*m*, H_β), 1.62–1.75 (*m*, H_α)	CH_2(28)	1.15–1.20 (*m*), 1.35–1.45 (*m*)
H–C(3)	4.55–4.70 (*m*)				
CH_2(4)	1.55–1.65 (*m*, H_β), 2.30–2.34 (*m*, H_α)	H–C(17)	1.12 (br. *s*)	CH_2(29)	4.05 (*t*, *J* = 6.5)
		Me(18)	0.80 (*s*)	*Fatty acyl:*	
H–C(6)	5.40 (br. *d*, *J* = 5.0)	Me(19)	1.01 (*s*)	CH_2(2′), CH_2(2″)	2.27 (*t*, *J* = 4.2)
CH_2(7)	2.01–2.04 (*m*, H_β), 1.42–1.45 (*m*, H_α)	H–C(20)	1.95–2.00 (*m*)	Me(3′), CH_2(3″)	1.62–1.65 (*m*)
		Me(21)	1.00–1.10 (*m*)	$(CH_2)_n$	1.20–1.42 (br. *s*)
H–C(8)	1.30–1.50 (*m*)	H–C(22)	5.16 (*dd*, *J* = 10.0, 16.7)	Me(30″)	0.70 (*t*, *J* = 6.7)
H–C(9)	1.05–1.09 (*m*)	H–C(23)	5.02 (*dd*, *J* = 10.0, 16.7)		
CH_2(11)	1.40–1.50 (*m*, H_α), 1.52–1.55 (*m*, H_β)	H–C(24)	1.59–1.61 (*m*)		
CH_2(12)	1.98–2.20 (*m*, H_β), 1.10–1.20 (*m*, H_α)				

Table 2. *^{13}C-NMR Data* ($CDCl_3$, 100 MHz) *of Compound* **1**

	δ(C)		δ(C)		δ(C)		δ(C)		δ(C)
C(1)	37.0	C(8)	31.9	C(15)	24.3	C(22)	137.6	C(29)	64.4
C(2)	31.9	C(9)	50.0	C(16)	28.2	C(23)	129.4	*Fatty acyl:*	
C(3)	73.7	C(10)	36.6	C(17)	56.0	C(24)	45.8	C(1′), C(1″)	173.3
C(4)	31.9	C(11)	21.0	C(18)	11.8	C(25)	29.1	C(2′), C(2″)	33.9
C(5)	139.7	C(12)	39.7	C(19)	19.3	C(26)	19.8	C(3′), C(3″)	25.1
C(6)	122.6	C(13)	42.3	C(20)	36.1	C(27)	19.0	$(CH_2)_n$	29.1–29.7
C(7)	31.9	C(14)	56.7	C(21)	18.8	C(28)	27.8	C(30″)	14.2

129.4 C(23)) [8]. The deshielded H-atoms at δ(H) 4.55–4.70 (H_α–C(3)) and 4.05 (CH_2(29)) were located on the basis of the multiplicity of their signals, *i.e.*, an unresolved *m* assignable to H–C(3) and a *t* assignable to CH_2(29) considering that C(29) was the unique position of the steroid skeleton where the two H-atoms could appear as *t*. Furthermore, the ^{1}H-NMR spectrum showed two angular Me groups, Me(18) and Me(19), as expected at δ(H) 0.80 (*s*) and 1.01 (*s*), respectively. The ^{13}C-NMR showed characteristic signals of the H–C(9), H–C(14), H–C(17), and H–C(24) groups at δ(C) 50.0, 56.7, 56.0, and 45.8, respectively [8]. From the foregoing data, it was deduced that compound **1** had two ester functions [9]. The downfield shifts of the H–C(3)–O and CH_2(29)–O suggested that these C-atoms are linked to electron-withdrawing groups, *i.e.*, that the corresponding alcohol functions were esterified. Indeed, in each the ^{1}H- and ^{13}C-NMR, four typical signals of aliphatic fatty acids were observed at δ(H) 0.70 (*t*, *J* = 6.7 Hz, Me(30″)), 1.20–1.42 ($(CH_2)_n$), 2.27 (*t*, *J* = 4.2 Hz, CH_2(2′), CH_2(2″)), and 1.62–1.65 (*m*, CH_2(3″), Me(3′)), and δ(C) 14.2 (C(30″)), 29.1–29.7 ($(CH_2)_n$), 33.9 (C(2′), C(2″)), and 25.1 (C(3′), C(3″)) [10].

Some important correlations were observed in the HMBC and COSY plots (*Fig. 1*), which showed HMBCs from both deshielded H-atom signals to the C=O signal at δ(C) 173.3, *i.e.*, H_α–C(3)/C(1′) and CH_2(29)/C(1″) thus confirming the C(3) and C(29) ester linkage. The COSY cross-peaks H–C(6) (δ(H) 5.40)/H_α–C(4) (δ(H) 2.30–2.34) and H_α–C(3) (δ(H) 4.55–4.70)/H_α–C(4) were present. In addition, the correlation H_α–C(3)/H_α–C(4) established the ring *A* of the steroid skeleton, considering the supplementary correlations between H_α–C(3) and both H_α–C(2) and H_β–C(2). For ring *B*, the olefinic H–C(6) correlated with the allylic H_β–C(7) at δ(H) 2.01–2.04. The correlations between CH_2(29) with both H_α–C(28) and H_β–C(28) supported the presence of the primary-alcohol function CH_2(29)O in the steroidal skeleton which is esterified by an aliphatic acid. The above observations were confirmed by a correlation of the $CH_2(\beta)$ group of the fatty acyl moiety with a set of $(CH_2)_n$ group, the latter being correlated also with a terminal Me group.

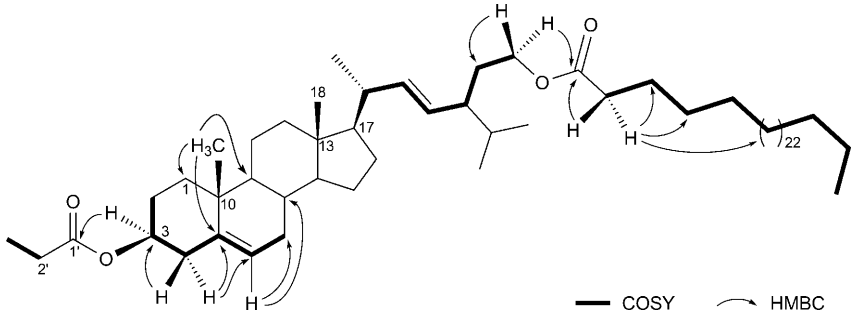


Fig. 1. *COSY Correlations and HMBCs of Compound* **1**

To identify the fatty acid positions at C(3) and C(29), methanolysis (0.9N HCl/MeOH, 70°, 18 h) [11] of compound **1** was carried out which gave a fatty acid methyl ester and a steroid which were identified by LC/MS as triacontanoic acid methyl ester and (3β)-stigmasta-5,22-diene-3,29-diol. The second acyl chain was deduced to be a propanoyl one.

To confirm the exact location of the acyl groups, an oxidative cleavage of the C(22)=C(23) bond [12] of **1** was carried out. The MS analysis by using soft ionization methods (DCI and MALDI-TOF) of the crude organic phase showed peaks at *m*/*z* 419 ($[C_{25}H_{38}O_5+H]^+$), 441 ($[C_{25}H_{38}O_5+Na]^+$), and 457 ($[C_{25}H_{38}O_5+K]^+$) in agreement with the formation of the oxidation product **4** (*Scheme 1*).

From the foregoing data, the structure of compound **1** was determined to be (3β)-stigmasta-5,22-diene-3,29-diol 3-propanoate 29-triacontanoate which was trivially named triumfettosterol Id.

Compound **2** was isolated as a white amorphous solid by CC. It gave positive colorations in the *Liebermann–Burchard* and *Molish* tests suggesting that compound **2** was a triterpenoid saponin. Its molecular formula $[C_{75}H_{116}O_{22}+H]^+$ was determined from the positive-ion-mode HR-ESI-FT-ICR-MS which showed a pseudo-molecular-ion peak at *m*/*z* 1369.8031 consistent with eighteen degrees of unsaturation. The presence of OH groups (3396 cm^{-1}), ester C=O groups (1734 cm^{-1}), and olefinic bonds (1648 cm^{-1}) in **2** was revealed by the IR spectrum. The ^{13}C-NMR spectrum of

Scheme 1. *Oxidative Cleavage of Double Bonds of Compound* **1**

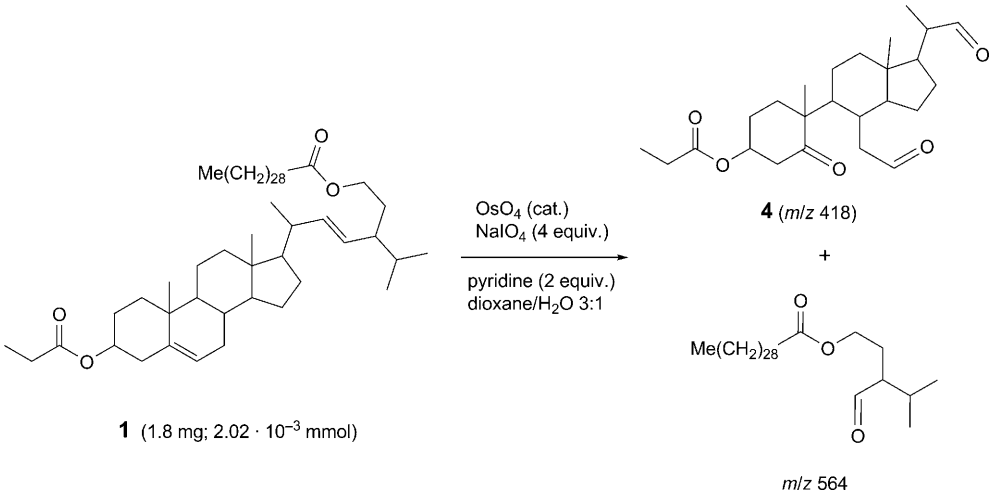


Table 3. *^{13}C-NMR Data* (CD_3OD, 100 MHz) *of Compound* **2**

	δ(C) (unit *A*)		δ(C) (unit *A*)		δ(C) (unit *B*)		δ(C) (unit *B*)
C(1)	47.9	C(18)	55.1	C(1′)	47.1	C(18′)	55.2
C(2)	69.6	C(19)	73.6	C(2′)	69.2	C(19′)	42.8
C(3)	86.0	C(20)	42.8	C(3′)	84.3	C(20′)	42.7
C(4)	50.5	C(21)	27.3	C(4′)	49.7	C(21′)	26.6
C(5)	56.8	C(22)	39.0	C(5′)	56.5	C(22′)	36.0
C(6)	21.4	C(23)[a])[1])	23.8	C(6′)	20.0	C(23′)[a])[1])	24.6
C(7)	34.4	C(24)[1])	180.8	C(7′)	34.3	C(24′)[1])	178.6
C(8)	41.3	C(25)	17.8	C(8′)	41.2	C(25′)	15.2
C(9)	47.2	C(26)	17.7	C(9′)	46.7	C(26′)	16.7
C(10)	39.0	C(27)	25.0	C(10′)	38.3	C(27′)	24.8
C(11)	25.0	C(28)	178.5	C(11′)	25.0	C(28′)	178.6
C(12)	129.6	C(29)	27.2	C(12′)	129.4	C(29′)	16.7
C(13)	139.7	C(30)	16.6	C(13′)	139.7	C(30′)	24.8
C(14)	42.7	*Me*O–C(2)	57.2	C(14′)	42.6		
C(15)	29.6	*Me*O–C(3)	57.3	C(15′)	29.6		
C(16)	27.2	*Me*O–C(19)	57.8	C(16′)	26.5		
C(17)	49.5			C(17′)	49.4		
Glucosyl	δ(C)		δ(C)		δ(C)		δ(C)
C(1″)[a])	95.8	C(4″)[a])	71.3	C(1‴)[a])	95.8	C(4‴)[a])	71.3
C(2″)[a])	73.6	C(5″)[a])	78.7	C(2‴)[a])	73.6	C(5‴)[a])	78.7
C(3″)[a])	78.7	C(6″)[a])	62.4	C(3‴)	78.0	C(6‴)[a])	62.4

[a]) Overlapping signals.

compound **2** (*Table 3*) showed most signals in pairs, and the others appeared with a doubled intensity, suggesting that **2** could be a triterpenoid 'dimer'. Its ^{1}H-NMR spectra (*Table 4*) exhibited eleven *s*, two broad *s*, and one *m* for Me groups; in addition, the

Table 4. *^{1}H-NMR Data* (CD_3OD, 400 MHz) *of Compound* **2**. δ in ppm, J in Hz.

	δ(H) (unit *A*)		δ(H) (unit *A*)		δ(H) (unit *B*)		δ(H) (unit *B*)
CH_2(1)[a])	2.02–2.10 (*m*), 0.92 (*dd*, *J* = 3.8, 15.2)	H–C(18)	2.55 (*s*)	CH_2(1′)[a])	2.00–2.10 (*m*), 0.92 (*dd*, *J* = 3.8, 15.2)	H–C(18′) H–C(19′)	3.05 (*d*, *J* = 9.6) 1.39 (br. *d*, *J* = 3.8)
H–C(2)	3.70–3.80 (*m*)	H–C(20)	1.35–1.40 (*m*)	H–C(2′)	4.09 (*ddd*, *J* = 4.8, 9.6, 9.6)	H–C(20′)	1.31 (br. *d*, *J* = 3.8)
H–C(3)	3.05 (*d*, *J* = 9.6)	CH_2(21)	1.45–1.50 (*m*), 1.78–1.80 (*m*)	H–C(3′)	2.90 (*d*, *J* = 9.6)	CH_2(21′)	1.32–1.40 (*m*)
H–C(5)[b])	0.96–1.05 (*m*)	CH_2(22)	1.74–1.76 (*m*)	H–C(5′)[b])	0.92–1.05 (*m*)	CH_2(22′)	1.30–1.40 (*m*), 1.55–1.65 (*m*)
CH_2(6)	1.75–1.82 (*m*)	Me(23)[1])	2.02 (*s*)	CH_2(6′)	1.29–1.32 (*m*)	Me(23′)[a])[1])	1.43 (*s*)
CH_2(7)	1.29–1.35 (*m*), 1.36–1.42 (*m*)	Me(25)	1.02 (*s*)	CH_2(7′)	1.25–1.40 (*m*), 1.54–1.69 (*m*)	Me(25′)	1.23 (*s*)
H–C(9)	1.90–2.00 (*m*)	Me(26)	0.82 (*s*)	H–C(9′)	1.72–1.80 (*m*)		
CH_2(11)	1.90–2.00 (*m*)	Me(27)	1.35 (*s*)	CH_2(11′)	1.90–2.05 (*m*)	Me(27′)	1.34 (*s*)
H–C(12)	5.32 (*d*, *J* = 2.4)	Me(29)	1.21 (*s*)	H–C(12′)	5.30 (*d*, *J* = 2.4)	Me(29′)	1.01–1.04 (*m*)
CH_2(15)	1.78–1.82 (*m*), 1.25–1.30 (*m*)	Me(30) MeO–C(2)[c])	0.93 (br. *s*) 3.80 (*s*)	CH_2(15′)	1.20–1.30 (*m*), 2.01–2.10 (*m*)	Me(30′)	0.77 (br. *s*)
CH_2(16)	1.64 (*br. d*, *J* = 11.4), 1.00–1.05 (*m*)	MeO–C(3)[c]) MeO–C(19)[c])	3.85 (*s*) 3.87 (*s*)	CH_2(16′)	1.75–1.82 (*m*), 2.10–2.10 (*m*)		
Glucosyl	δ(H)		δ(H)				
H–C(1″)	5.39 (*d*, *J* = 9.6)	H–C(1‴)	5.38 (*d*, *J* = 9.6)				
H–C(2″)[d])	3.35 (*dd*, *J* = 9.6, 9.2)	H–C(2‴)[d])	3.35 (*dd*, *J* = 9.6, 9.2)				
H–C(3″)	3.43 (br. *d*, *J* = 9.2)	H–C(3‴)	3.39 (*dd*, *J* = 9.2)				
H–C(4″)[e])	3.40 (br. *d*, *J* = 9.2)	H–C(4‴)[e])	3.40 (br. *d*, *J* = 9.2)				
H–C(5″)	3.34 (br. *d*, *J* = 9.2)	H–C(5‴)	3.33 (br. *d*, *J* = 9.2)				
CH_2(6″)[c])	3.80–3.90 (*m*), 3.68 (*dd*, *J* = 4.8, 12.0)	CH_2(6‴)[c])	3.80–3.90 (*m*), 3.68 (*dd*, *J* = 4.8, 12.0)				

[a]) [b]) [c]) [d]) [e]) Overlapping signals.

NMR spectra exhibited anomeric signals at δ(H) 5.39 (*d*, $J = 9.6$ Hz, 1 H) and 5.38 (*d*, $J = 9.6$ Hz, 1 H) and δ(C) 95.8 (2 C) [13], and two characteristic olefinic signals at δ(H) 5.32 ($J = 2.4$ Hz, 1 H) and 5.30 ($J = 2.4$ Hz, 1 H) and δ(C) 129.6 and 129.4, indicating the presence of an urs-12-ene-type triterpenoid [14]. These spectra also revealed three MeO groups between δ(H) 3.80 and 3.87 and at δ(C) 57.2, 57.3, and 57.8 which could only be attached to the molecule by an ether function [15] since these signals would appear around δ(C) 51 in case of a methyl ester [13]. In addition, we observed four C=O signals of carboxylic functions at δ(C) 178.5, 178.6, 178.6, and 180.8, an intense quaternary C-atom at δ(C) 139.7 and a set of signals between δ(C) 62.4 and 78.7 suggesting that **2** could be a urs-12-ene 'dimer' substituted with three MeO groups and two β-D-glucopyranosyloxy groups [16].

The HMBC data (*Fig. 2*) presented some important correlations from the signal at δ(H) 2.90 (*d*, $J = 9.7$ Hz, H–C(3′)) to the CH–O at δ(C) 69.2 (C(2′)), the C=O at δ(C) 180.8 (C(24)) and the Me group at δ(C) 24.6 (C(23′)). The signal at δ(H) 3.05 (*d*, $J = 9.7$ Hz, H–C(3)) correlated with the CH–O at δ 69.6 (C(2)) and the Me group at δ 23.8 (C(23)). These informations led us to suggest that both units of the 'dimer' could be a (2α,3β)-2,3-dihydroxyurs-12-ene-24,28-dioic acid derivative [17] linked by an ester function between the C=O at δ(C) 180.8 (C(24)) and the CH–O at δ(C) 84.3 (C(3′)). In addition to these correlations, others were observed between the anomeric H-atoms and both C=O functions at δ(C) 178.6 (C(24′)) and 178.6 (C(28′)), and between the broad *s* at δ(H) 2.55 (H–C(18)) and the quaternary C–O at δ(C) 73.6 (MeO–*C*(19)), in accordance with urs-12-ene structures [17]. The positions of the sugar units were also determined by informations collected from the MS. Indeed, the HR-ESI-FT-ICR-MS showed peaks at *m*/*z* 779.3498 ($[C_{39}H_{54}O_{16} + H]^+$) and 765.3700 ($[C_{39}H_{56}O_{15} + H]^+$)

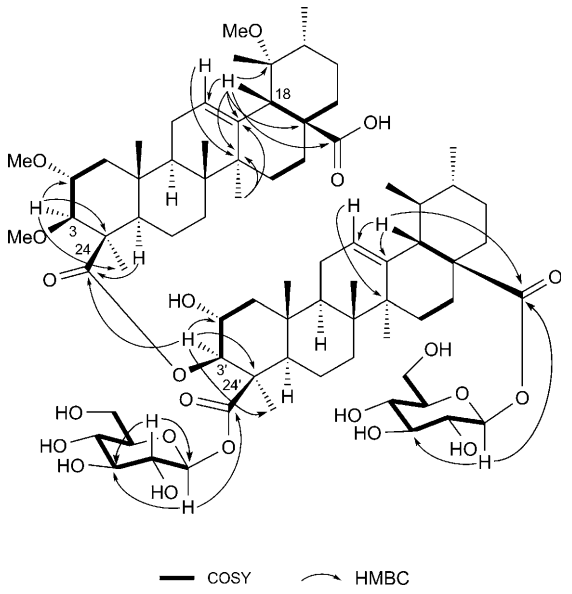
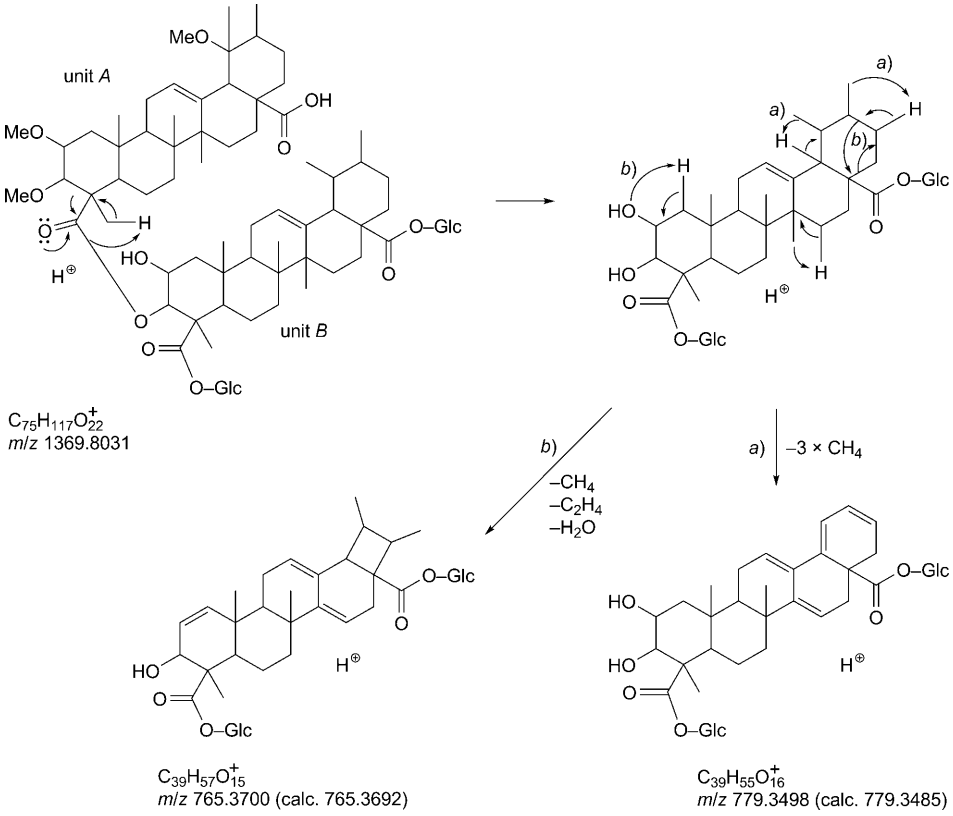


Fig. 2. *COSY Correlations and HMBCs of Compound* **2**

establishing the ester linkages of the glucosyl residues at C(24′) and C(28′) of the same ursene unit (*Scheme 2*). A *retro-Diels–Alder* reaction (*Scheme 3*) gave a fragment ion at *m*/*z* 259.1703 ($C_{17}H_{23}O_2^+$) supporting the location of MeO group at C(19).

Scheme 2. *Important MS Ion Fragments of Compound* **2**



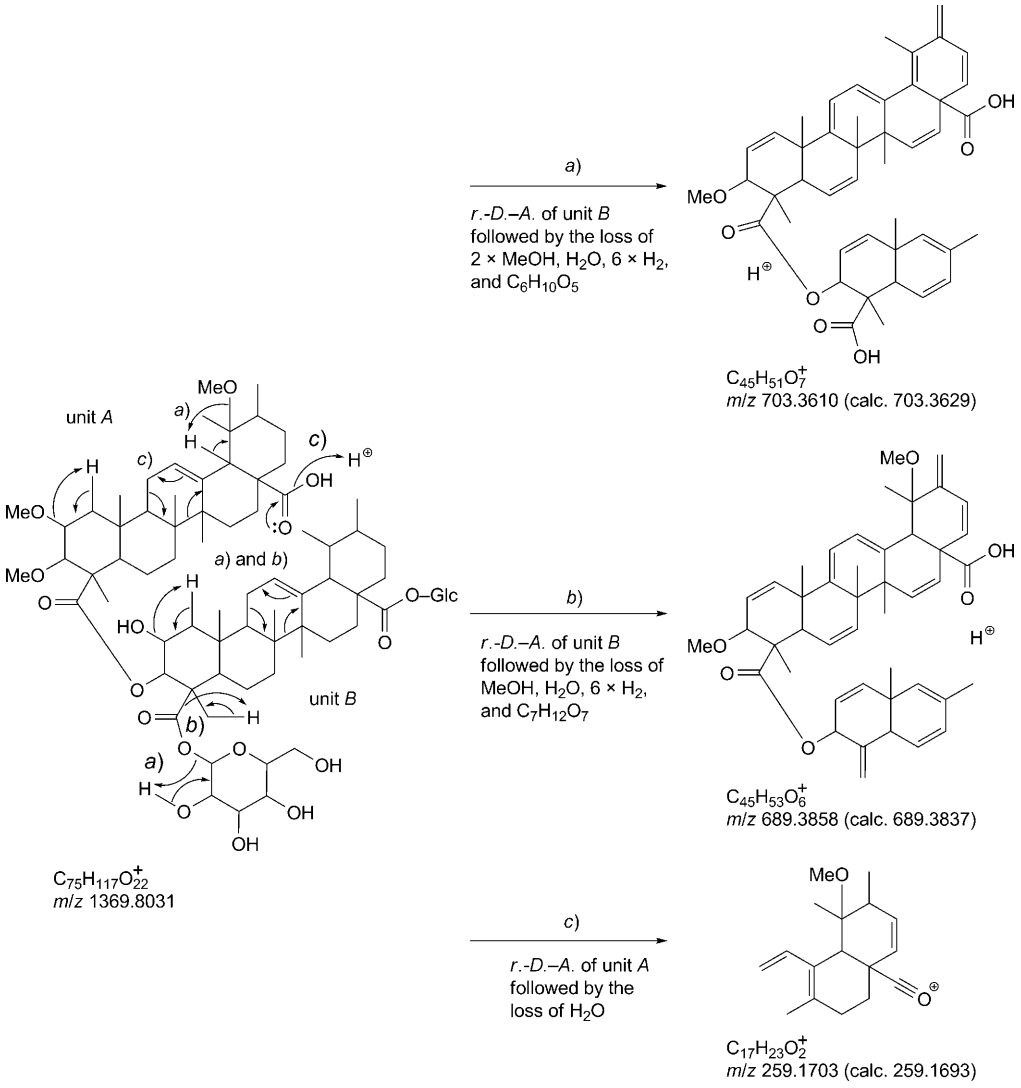
Saponification of compound **2** with an aqueous $LiOH \cdot H_2O$ solution yielded three compounds identified by MALDI-TOF and LC/ESI-MS as the sugar unit, (2α,3β)-2,3-dihydroxyurs-12-ene-24,28-dioic acid and (2α,3β,19α)-2,3,19-trimethoxyurs-12-ene-24,28-dioic acid.

From the foregoing data, the structure of compound **2** was determined to be (2α,3β,19α)-2,3,19-trimethoxyurs-12-ene-24,28-dioic acid 24-[(2α,3β)-24,28-bis(β-D-glucopyranosyloxy)-2-hydroxy-24,28-dioxours-12-en-3-yl] ester[1]) which was trivially named triumfettosaponin.

The NMR data of the two known compounds were in agreement with those reported in [5][6].

2. Antimicrobial Assay. The antimicrobial diffusion test was carried out as described by *Kuete* and co-workers [18][19]. The results of this test showed that *trans*-tiliroside (**3**) was active on six (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*,

Scheme 3. *Proposed MS/MS Fragmentation Mechanism of the Ion at* m/z *1369.8031 of Compound* **2**



Bacillus cereus, *Staphylococcus aureus*, *Streptococcus faecalis*) among the ten microorganisms tested. It showed moderate activity, the *MIC* (minimal inhibition concentration) values being 230.05 μM on *E. coli* and *B. cereus* and 526.10 μM on other sensitive microorganisms. This activity is not significant if compared with that of the reference antibiotic (Gentamycin; *MIC* values from 0.90 to 2.08 μM). However, a cidal effect of **3** could be expected on *E. coli*, *B. cereus*, and *S. faecalis*, as the detectable *MMC* (minimal microbicidal concentration) values on these microbial species were not more than four fold greater than the corresponding *MIC*s [19].

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Experimental Part

General. Flash chromatography (FC): silica gel *60 H* (SiO_2, particle size < 45 μm). Column chromatography (CC): silica gel *60A* (size 70–200 μm). TLC: silica gel *GF 254*; visualization by I_2 vapor. M.p.: *Stuart-Scientific* melting-point apparatus *SMP3*; uncorrected. Optical rotation: *Perkin-Elmer-341* polarimeter at 589 nm. IR Spectra: *Perkin-Elmer* (FT-IR system spectrum *BX* spectrometer); KBr disks; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-DRX-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-FT-ICR-MS and ESI-FT-ICR-MS^n (pos.): *Explorer* high-resolution FT-ICR mass spectrometer (*Varian-IonSpec Corporation*, Palo Alto, CA, USA) fitted with a 9.4-T shielded superconducting magnet and a *Micromass Z-Spray* electrospray source; in *m/z*; high voltage in the range -4000 to -3600 V; N_2 to assist solvent evaporation; temp. of the source and of the probe, 90 and 100°, resp.; ion trapping in the FT-ICR-MS cell with a 2 V trapping potential; under these conditions, the average mass measurement accuracy was typically better than 2 ppm, and the mass resolution close to 150000 ($m/\Delta m$); after calibration, the identification of the ions was ensured by exact-mass measurement and comparison with theoretical isotopic pattern calc. by the 'Omega 8 Exact Mass Calculator' software (*Varian-IonSpec Corporation*, Palo Alto, CA, USA). MS/MS Experiments: in the SORI-CID (sustained off-resonance irradiation collision-induced dissociation) mode; after isolation of the parent ion by ejection of other ions, sustained off-resonance irradiation was performed during 60 ms with an amplitude of 24 V bp.; N_2 was used as collision gas. MALDI-TOF-MS: 1M 2,5-dihydroxybenzoic acid (2,5-DHB) in MeCN/ultrapure H_2O 1:1 (0.1% CF_3COOH) as matrix; *Bruker-Reflex-IV* time-of-flight mass spectrometer (*Bruker-Daltonic*, Bremen, Germany), equipped with the *Scout-384* probe ion source; N_2-pulsed laser (337 nm, model VSD-337ND; *Laser Science Inc.*, Boston, MA) with an energy output of 400 μJ/pulse. DCI-MS: MS apparatus *5989 B* equipped with a simple quadrupole and an electrospray module *59987 A*. LC/MS: HPLC system (LC pump *P4000* and autosampler *AS3000* from *Thermo Separation Products*) coupled to a *LCQ Duo-Ion-Trap* detector (*Thermo Electron*, Zellik, Belgium), equipped with an ESI interface run in the pos.-ion mode; separation of sample components on an *X-Terra* MS *C18* (5 μm particle size, 3.9 × 150 mm; *Waters*, Overijse, Belgium), equipped with an *X-Terra-MS-C18* pre-column (5 μm particle size, 3.9 × 10 mm) and operated at 37°; injection volume 15 μl; mobile phase-mixture of 5 mM $HCOONH_4$ buffer at pH 3.8 (*A*) and MeCN (*B*); separation conditions for all compounds: 0.0–0.50 min, *A/B* 1:1 (*v/v*); 0.50–9.0 min, eluant *B/A* → 97:3; 9.0–12.0 min, *B/A* 97:3; before each run, column equilibration for 6 min with *A/B* 1:1; flow rate for column equilibration and anal. runs, 0.4 ml/min; ionization of the analytes as follows: sheath gas-flow rate (N_2), 47 arbitrary units; auxiliary gas-flow rate (He), 18 arbitrary units; spray voltage 5.0 kV; capillary temp. 200°; capillary voltage 36 V; data acquisition in a time segment between 0.2 and 11.5 min after injection; full MS/MS of $[M+H]^+$ ion for all compounds, isolation width 2.5 *m/z*, normalized collision energy 28.0%.

Plant Material. T. cordifolia A. RICH. was collected in October 2004 from Yaoundé Central Province of Cameroon, and a specimen (No. 12830SRF Cam) has been deposited with the National Herbarium of Yaoundé, Cameroon.

Microbial Strains. The organisms tested included eight clinically antibiotic-resistant bacteria (*Citrobacter freundii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonalla typhi*, *Bacillus cereus*, *Staphilococcus aureus*, and *Streptococcus faecalis*) and two fungi (*Candida albicans* and *Microsporum audorium*) from Yaoundé General Hospital (Cameroon) authentified following standard methods [19].

Extraction and Isolation. Dried leaves of *T. cordifolia* A. RICH. were powdered. Then 1.87 kg of the powder were extracted by maceration at r.t. with CH_2Cl_2/MeOH 1:1 (3 × 10 l). Each extraction lasted 48 h. The extract was concentrated, yielding 93 g of crude extract which was fractionated by FC (cyclohexane, then cyclohexane/AcOEt 3:1 → 1:1 → 1:3, AcOEt, and MeOH). The fraction (9.94 g) obtained with cyclohexane/AcOEt 3:1 was purified by CC (cyclohexane/AcOEt mixtures): *Fractions 1* –

217. From the *Frs. 47–54* eluted with cyclohexane/AcOEt 82.5:7.5, 654 mg of sterol mixture (stigmasterol/β-sitosterol) was isolated. *Frs. 22–32* were mixed and subjected to CC: 12 mg of **1** was eluted with cyclohexane/CH_2Cl_2/AcOEt 92.5:7:0.5. The fraction (8.11 g) obtained with AcOEt was subjected to CC (CH_2Cl_2/MeOH of increasing polarity): *Fractions 1–150. Frs. 53–59* obtained with CH_2Cl_2/MeOH 92.5:7.5 yielded 74.8 mg of *5,7-dihydroxy-2-(4-hydroxyphenyl)-3-{[6-*O*-[(2*E*)-3-(4-hydroxyphenyl)-1-oxoprop-2-en-1-yl]-β-*D*-glucopyranosyl}oxy]-4*H*-1-benzopyran-4-one* (=trans-*tiliroside*; **3**). The MeOH fraction (57 g) was partitioned between H_2O and BuOH, and 8.31 g of the org. extract were purified by CC (CH_2Cl_2/MeOH increasing polarity): 15 mg of triumfettosaponin (**2**) were obtained from the *Frs. 35–70* eluted with CH_2Cl_2/MeOH 87.5:12.5.

(3β)-Stigmasta-5,22-diene-3,29-diol 3-Propanoate 29-Triacontanoate (= *Triumfettosterol*; **1**): White amorphous solid. M.p. 79°. $[\alpha]_D^{20} = -9.5$ ($c = 0.06$, $CHCl_3$). IR: 2917 (=C−H), 2849 (C−H), 1738 (C=O), 1464 (C−O), 1378, 1240, 1179, 1028. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-FT-ICR-MS: 919.8486 ($[C_{62}H_{110}O_4+H]^+$), 845.8121 ($[C_{59}H_{104}O_2+H]^+$), 817.7802 ($[C_{57}H_{101}O_2+H]^+$), 663.6088 ($[C_{46}H_{79}O_2+H]^+$), 393.3549 ($[C_{29}H_{45}+H]^+$). HR-ESI-FT-ICR-MS/MS of *m/z* 845.8121: 393.3528 ($[C_{29}H_{44}+H]^+$), 257.2285 ($[C_{19}H_{28}+H]^+$).

Methanolysis. Compound **1** (15 mg) was refluxed at 70° for 18 h in MeOH (2.5 ml) containing 0.9N HCl (1.5 ml), under magnetic stirring. The medium was neutralized with Na_2CO_3 and extracted three times with $CHCl_3$. The org. layer was concentrated to give a mixture of compounds which were characterized by LC/ESI-MS as (3β)-stigmasta-5,22-diene-3,29-diol (t_R 8.03; *m/z* 429 ($[C_{25}H_{38}O_5+H]^+$)) and triacontanoic acid methyl ester (t_R 6.97; *m/z* 466).

Oxidative Cleavage of the C(22)=C(23) Bond of **1**. To **1** (1.8 mg, $2.02\cdot10^{-3}$ mmol) in dioxane/H_2O 3:1 (4 ml), pyridine (2 equiv.), two drops of 4 wt.-% of an aq. OsO_4 soln., and $NaIO_4$ (4 equiv.) were added. The mixture stirred at r.t. for 3 h (stirring bar). Quenching was done with 10 ml of an aq. $Na_2S_2O_3$ soln. and stirring for *ca.* 20 min. The aq. layer was extracted three times with CH_2Cl_2 (15 ml) and the extract concentrated. The analysis of the residual brown oil by DCI- and MALDI-TOF-MS gave important peaks at *m/z* 419 ($[M+H]^+$), and 441 ($[M+Na]^+$) corresponding to the steroid moiety **4** containing a propanoyloxy group at C(3).

*(2α,3β,19α)-2,3,19-Trimethoxyurs-12-ene-24,28-dioic Acid 24-(2α,3β)-24,28-Bis(β-*D*-glucopyranosyloxy)-2-hydroxy-24,28-dioxours-12-en-3-yl] Ester* (=*(2α,3β,4β,19α)-2,3,19-Trimethoxyurs-12-ene-23,28-dioic Acid 23-[(2α,3β,4β)-23,28-Bis(β-*D*-glucopyranosyloxy)-2-hydroxy-23,28-dioxours-12-en-3-yl] Ester* = *Triumfettosaponin*; **2**): White amorphous solid. $[\alpha]_D^{20} = +12.86$ ($c = 0.14$, MeOH). M.p. 215° (dec.). IR: 3396 (OH), 2932 (C−H), 1734 (C=O), 1648 (C=C), 1508, 1458 (C−O). ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. HR-ESI-FT-ICR-MS: 1369.8031 ($[C_{75}H_{116}O_{22}+H]^+$), 1037.6666 ($[C_{64}H_{93}O_{11}+H]^+$), 779.3498 ($[C_{39}H_{54}O_{16}+H]^+$), 765.3700 ($[C_{39}H_{56}O_{15}+H]^+$), 703.3610 ($C_{45}H_{51}O_7^+$). HR-ESI-FT-ICR-MS/MS of *m/z* 1369: 703.3610 ($C_{45}H_{51}O_7^+$), 689.3858 ($C_{45}H_{53}O_6^+$). HR-ESI-FT-ICR-MS/MS of *m/z* 779: 719.3284 ($C_{37}H_{51}O_{14}^+$), 617.2965 ($C_{33}H_{45}O_{11}^+$), 557.2780 ($C_{31}H_{41}O_9^+$), 539.2679 ($C_{31}H_{39}O_8^+$).

Alkaline Hydrolysis. Compound **2** (2.5 mg, 1.83 μmol) was dissolved in THF/MeOH 6:1 (3.5 ml), and 1N aq. $LiOH\cdot H_2O$ soln. (1 ml) was added. The mixture was stirred at r.t. for 4 h (stirring bar), then neutralized with 1N HCl (1 ml), and extracted with BuOH. Both phases were concentrated and analyzed by LC/ESI-MS for the org. part and by MALDI-TOF for the aq. part. Three compounds were identified as (2α,3β)-2,3-dihydroxyurs-12-ene-24,28-dioic acid (=(2α,3β,4β)-2,3-dihydroxyurs-12-ene-23,28-dioic acid; t_R 8.47 min), (2α,3β,19α)-2,3,19-trimethoxyurs-12-ene-24,28-dioic acid (=(2α,3β,4β)-2,3,19-trimethoxyurs-12-ene-23,28-dioic acid; t_R 10.28 min), and hexose at *m/z* 181 ($[C_6H_{12}O_6+H]^+$).

Antimicrobial Assays: Culture Media. Nutrient Agar (NA) was used for bacteria. *Sabouraud* glucose agar was used for the fungi. The *Mueller-Hinton* broth (MHB) was used to determine the minimal inhibition concentration (*MIC*) of all samples against the tested pathogens. The MHB and *Mueller-Hinton* agar (MHA) were used to determine the minimal microbicidal concentration (*MMC*) of the active samples.

The sensitivity test was carried out following the agar-well diffusion methods as previously described [18][19]. The *MIC*s of samples found to be active following the diffusion assay were determined by the microdilution method [18][19]. The *MIC* was defined as the lowest sample concentration that exhibited complete inhibition of bacterial growth, whilst the lowest concentration that yielded no growth after subculturing was taken as the *MMC* [18][19].

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