65. Saponins with an Unusual Secoursene Skeleton from *Sesamum alatum* THONN.[1])

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Three new saponins, alatoside A–C (**4a**, **5**, and **6a**, resp.), with a 18,19-secours-12-ene skeleton were isolated from the aerial parts of *Sesamum alatum* THONN. (Pedaliaceae) by preparative liquid chromatography. Their structures were elucidated by spectroscopic methods, including X-ray diffraction analysis, and by chemical degradation (acidic and enzymatic hydrolyses). In addition, verbascoside (**1**) and two cyclohexylethanol derivatives, rengyol (**2a**) and isorengyol (**3a**), were isolated and identified.

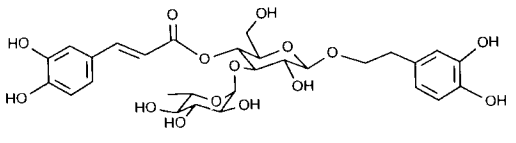
Introduction. – As part of our studies on species of the family Pedaliaceae [1], we have undertaken a phytochemical investigation of *Sesamum alatum* THONN. This plant which grows as a wild herb in tropical Africa is used by traditional healers in Senegal for the treatment of women's sterility [2]. Of the twenty species found in the genus *Sesamum*, only *S. indicum* L. and *S. angolense* WELW. were investigated in some detail. Several lignans were isolated from *S. indicum* seeds [3–5], while naphthoxirenes [6] and iridoid glucosides [7] were found in *S. angolense* root bark. On the other hand, no compound was previously identified in *S. alatum*.

We report here on the isolation and characterization of the main polar constituents from the aerial parts. Emphasis is laid on the structure elucidation of three new saponins containing an unusual 18,19-secours-12-ene aglycone. A comparison of the chemical composition of aerial parts and roots is also discussed.

Results. – The aerial parts of *S. alatum* collected in Niger were extracted successively with CH_2Cl_2 and MeOH. Fractionation of the MeOH extract by a combination of column chromatography on silica gel, centrifugal partition chromatography (CPC; $CHCl_3$/MeOH/i-PrOH/H_2O 5:6:1:4, descending mode) [8], gel filtration on *Sephadex LH-20*, and low-pressure liquid chromatography (LPLC) on *RP* material allowed the isolation of compounds **1**, **2a**, **3a**, **4a**, **5**, and **6a** (see *Exper. Part*).

Compound **1** showed the characteristic UV spectrum of a phenylpropanoid glycoside containing caffeoyl and catechol moieties. It was identified as verbascoside (= acteoside)

[1]) Part of the Ph.D. thesis of *O.P.* [1].



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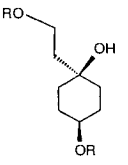

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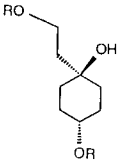
b R = Ac



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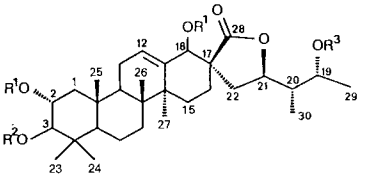
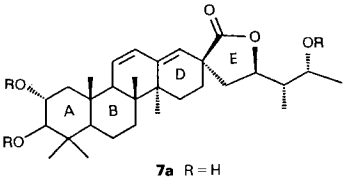
b R = Ac



	R^1	R^2	R^3
4a	H	α-L-Rha-(1→2)-β-D-Xyl	H
b	H	β-D-Xyl	H
c	H	H	H
d	Ac	Ac	Ac
5	H	α-L-Rha-(1→2)-β-D-Xyl	β-D-Glc
6a	H	α-L-Rha-(1→2)-β-D-Glc	β-D-Glc
b	H	α-L-Rha-(1→2)-β-D-Glc	H



7a R = H
b R = Ac

from its ^{1}H- and ^{13}C-NMR data [9] and by chromatographic comparison with an authentic sample isolated from *Sesamum angolense* [7]. Compounds **2a** and **3a** proved to be two stereoisomers derived from cyclohexylethanol. They were shown to have spectroscopic properties identical to those of rengyol and isorengyol, respectively [10]. Characterization of the diacetyl derivatives **2b** and **3b** further confirmed the identity of both compounds. Compounds **4a**, **5**, and **6a** were three saponins; their structures were established as follows.

Acid hydrolysis of saponins **4a**, **5**, and **6a** afforded the same triterpenic derivative **7a** which exhibited strong UV absorption (λ_{max} 250 nm). The EI-MS of **7a** presented a molecular ion at *m/z* 486, corresponding to the molecular formula $C_{30}H_{46}O_5$. Acetylation of **7a** at room temperature provided the crystalline triacetyl derivative **7b**. However, despite detailed NMR investigations, the structures of **7a** and **7b** remained obscure. Suitable crystals of **7b** grown in AcOEt/hexane were, therefore, submitted to an X-ray diffraction analysis (see *Exper. Part*). The structure of **7a** was thus established as (2*R**,3*R**,17*R**,19*R**,20*S**,21*R**)-2,3,19-trihydroxy-18,19-secoursa-11,13(18)-diene-28,21-lactone, a triterpene described here for the first time. A view of **7b** showing the crystallographic atomic numbering scheme is given in the *Figure*. Ring A has a chair conformation with atoms C(2) and C(5) displaced by 0.70(2) and −0.64(2) Å respectively, from the best plane through the remaining four atoms (planar to within 0.01(1) Å). Ring B also has a chair conformation with atoms C(6) and C(9) displaced by 0.71(1) and −0.64(1) Å, respectively from the best plane through the remaining four atoms (planar to within 0.04(1) Å). Ring C is best described as having a sofa conformation with atom C(8) displaced by 0.76(2) Å from the best plane through the remaining five atoms (planar to within 0.08(2) Å). Ring D has a half-chair conformation with atoms C(15) and C(16) displaced by 0.39(2) and −0.30(2) Å, respectively, from the best plane through the remaining four atoms (planar to within 0.02(1) Å). Finally, the five-membered lactone

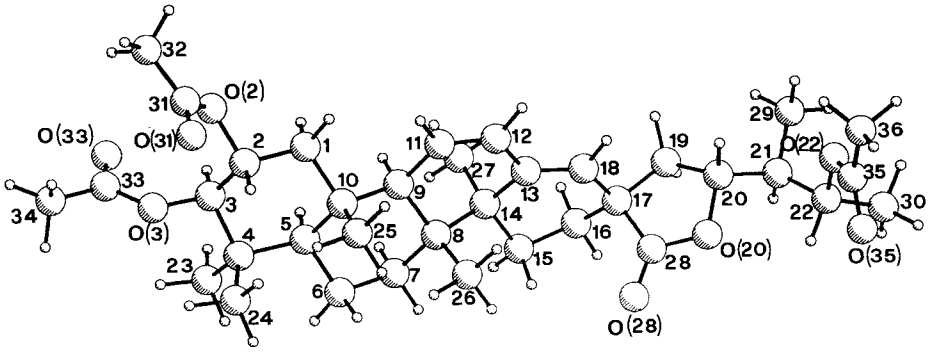


Figure. *PLUTO* [11] *drawing of the molecule* **7b**. Atomic numbering scheme arbitrary for the C(19) to C(22) moiety; for systematic numbering, see *Formulae* **4–6**

ring E has an envelope conformation with atom C(19) (arbitrary numbering, see *Fig.*) displaced by 0.44(2) Å from the best plane through the remaining four atoms (planar to within 0.001(16) Å). Rings A and B are inclined to one another by 13.5(3)°, rings B and C by 8.2(4)°, rings C and D by 11.4(4)°, and finally rings D and E by 94.6(4)°.

Acidic hydrolysis of saponin **4a** afforded, beside **7a**, xylose and rhamnose. The FAB-MS of **4a** (thioglycerol, neg.-ion mode) showed a quasimolecular ion at *m/z* 781 ($[M - H]^-$). Fragments corresponding to successive losses of rhamnosyl and xylosyl units were detected (*m/z* 635 ($[M - H - 146]^-$) and 503 ($[M - H - 146 - 132]^-$)). The mol. wt. of the aglycone appeared thus to be 504, which suggested that the triterpenic derivative **7a** (mol. wt. 486) resulted from elimination of H_2O during acidic hydrolysis. The complete structure of saponin **4a** was established by means of enzymatic degradations. Treatment of **4a** with hesperidinase provided **4b**, while subsequent hydrolysis with β-D-xylosidase afforded aglycone **4c** which was acetylated to tetraacetate **4d**. Extensive spectral analyses of compounds **4a–d** established the structure of the aglycone **4c** to be (2*R**,3*R**, 17*R**,19*R**,20*S**,21*R**)-2,3,18,19-tetrahydroxy-18,19-secours-12-ene-28,21-lactone, a new triterpene named alatogenin. The relative configuration at C(18) could not be determined. Saponin **4a**, called alatoside A, thus is 3-*O*-[*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]alatogenin.

The FAB-MS of **4b** (glycerol, neg.-ion mode) revealed a quasimolecular ion at *m/z* 635 ($[M - H]^-$), as expected after scission of the rhamnosyl residue. In the ^{1}H-NMR spectrum, the anomeric proton H–C(1′) appeared as a *d* at 4.84 ppm ($J = 7.4$ Hz) which proved the β-D-configuration of the xylosyl unit. The signals corresponding to the aglycone moiety were practically identical in the ^{13}C-NMR spectra of **4a** and **4b**. In both saponins, C(3) was in particular observed at low field (see *Table*) which indicated glycosylation of OH–C(3). On the other hand, C(2′) was shifted upfield (−3.9 ppm) in the spectrum of **4b**, whilst C(1′) (+1.9 ppm) and C(3′) (+0.5 ppm) were deshielded. These data demonstrated the presence in saponin **4a** of a *O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl chain attached on OH–C(3) [12]. In the DCI-MS of aglycone **4c** (NH_3, pos.-ion mode), quasimolecular ions were detected at *m/z* 522 ($[M + NH_4]^+$) and 505 ($[M + H]^+$). ^{1}H- and ^{13}C-NMR data confirmed the presence of four OH groups and a double bond. The position of the labile OH function and of the double bond were deduced from ^{13}C-NMR acetylation shifts. Acetylation of OH groups is known to deshield the *ipso* position and to shield the adjacent C-atoms [13]. In the ^{13}C-NMR spectrum of the tetra-*O*-acetyl derivative **4d**, C(18) was shifted downfield by 1–2 ppm (in **4d**, signals of C(18) and C(21) are interchangeable). On the other hand, C(17) (−2–3 ppm) and C(13) (*ca.* −6.5 ppm) were shifted upfield (in **4d**, C(13) is obscured by pyridine, 134.88 ppm in $CDCl_3$). Final evidence was obtained by a long-range HETCOR experiment. Using the FLOCK pulse sequence [14], correlations were detected between H–C(18) and C(13), C(17) and C(28).

Acidic hydrolysis of saponin **5** furnished glucose, rhamnose, and xylose. Treatment of **5** with β-D-glucuronidase afforded a monodesmosidic saponin with spectroscopic properties (^{1}H- and ^{13}C-NMR) identical to those of alatoside A (**4a**). Saponin **5**, called alatoside B, is thus 19-*O*-(β-D-glucopyranosyl)-3-*O*-[*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]alatogenin.

In the FAB-MS of **5** (glycerol, neg.-ion mode), a quasimolecular ion was detected at *m/z* 943 ($[M - H]^-$). Fragments resulting from the elimination of rhamnosyl and glycosyl units were observed at *m/z* 797 ($[M - H - 146]^-$) and 781 ($[M - H - 162]^-$) which demonstrated the terminal position of both these sugars. Consequently, the signal at *m/z* 665 ($[M - H - 132 - 146]^-$) due to the loss of both rhamnosyl and xylosyl residues proved the bidesmosidic nature of **5**. Successive eliminations of rhamnosyl, xylosyl, and glycosyl moieties were also

Table. *^{13}C-NMR Data* ((D$_5$)pyridine) *of the Glycosidic Moieties of Saponins* **4a**, **4b**, **5**, **6a**, *and* **6b**. Chemical shifts of aglycone C-atoms which are affected by the glycosylation are also listed[a]).

	4a	**4b**	**5**	**6a**	**6b**
β-D-Xyl-O–C(3)					
C(1′)	105.28	107.17	105.37		
C(2′)	79.27	75.40	79.34[b])		
C(3′)	78.26	78.76	78.38		
C(4′)	71.02	70.83	71.10		
C(5′)	67.00	67.34	67.10		
β-D-Glc-O–C(3)					
C(1′)				104.76	104.75
C(2′)				79.52[b])	79.51[b])
C(3′)				78.48[c])	78.43[c])
C(4′)				71.94	71.94
C(5′)				78.30[c])	78.24[c])
C(6′)				62.42[d])	62.40
α-L-Rha					
C(1″)	102.23		102.39	102.18	102.13
C(2″)	72.35		72.45	72.47	72.44
C(3″)	72.15		72.23	72.31	72.29
C(4″)	73.92		74.03	74.10	74.08
C(5″)	70.02		70.13	70.05	70.01
C(6″)	18.64[f])		18.57[f])	18.59[f])	18.50[f])
β-D-Glc-O–C(19)					
C(1‴)			103.78	103.79	
C(2‴)			75.18[e])	75.23[e])	
C(3‴)			78.65[d])	78.69[e])	
C(4‴)			71.43	71.52	
C(5‴)			78.00[d])	78.04[e])	
C(6‴)			62.49	62.56[d])	
C(3)	94.30	95.27	94.38	94.81	94.85
C(19)	65.48	65.55	75.12[e])	75.15[e])	65.57
C(20)	46.31	46.42	44.90	44.94	46.41
C(21)	79.27	79.37	79.05[b])	79.10[b])	79.35[b])
C(29)	21.76	21.86	18.74[f])	18.74[f])	21.85

[a]) β-D-Glc = β-D-glucopyranosyl, α-L-Rha = α-L-rhamnopyranosyl, β-D-Xyl = β-D-xylopyranosyl.

[b–e]) Values with the same superscript in each column are interchangeable.

[f]) These values could be interchanged with signals of other Me groups (see complete ^{13}C-NMR data of the aglycone moiety in *Exper. Part*).

observed by tandem MS (FAB-MS-MS) [15]. Compared with saponin **4a**, the ^{13}C-NMR signals attributed to the *O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl chain remained unchanged. On the other hand, C(19) underwent a paramagnetic shift of 9.6 ppm, while C(29) and C(20) were shifted upfield (see *Table*). These data indicated unambiguously glycosylation of OH–C(19).

Acidic hydrolysis of saponin **6a** gave rhamnose and glucose. Enzymatic hydrolysis of **6a** with β-D-glucuronidase afforded the monodesmosidic saponin **6b**. Further attempts to cleave the rhamnosyl residue with hesperidinase remained, however, unsuccessful. Spectral analyses showed the structure of **6a**, called C, to be 19-*O*-(β-D-glucopyranosyl)-3-*O*-[*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]alatogenin.

The FAB-MS (glycerol, neg.-ion mode) of **6a** revealed a quasimolecular ion at *m/z* 973 ($[M - H]^-$). Fragments were also detected at *m/z* 827 ($[M - H - 146]^-$), 811 ($[M - H - 162]^-$), and 665 ($[M - H - 146 - 162]^-$). In the ^{13}C-NMR spectrum, signals of the aglycone moiety were practically identical to those observed in the case of saponin **5** indicating glycosylation of both OH–C(3) and OH–C(19). The FAB-MS of **6b** (glycerol, neg.-ion mode) presented a quasimolecular ion at *m/z* 811 ($[M - H]^-$). The ^{13}C-NMR signal for C(19) in **6b** underwent an upfield shift of 9.6 ppm when compared with the corresponding signal in **6a**, while C(20) and C(29) were more deshielded. On the other hand, in comparison with a terminal glycosyl residue, C(2′) (79.5 ppm) was shifted downfield by *ca.* 4 ppm in the spectra of **6a** and **6b**, while C(1′) and C(3′) were slightly shielded. The glycosylation shifts were very similar to those observed in the case of saponins **4a** and **5**. Moreover, the chemical shifts of C(1′) and C(2′) were in full agreement with the values reported for luperoside H, a dammarane-type saponin containing a *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl chain located at OH–C(3) [16].

The composition of aerial parts and of roots was compared by TLC (detection with *Godin* reagent [17]) and HPLC-UV analysis (see *Exper. Part*). Verbascoside (**1**) was present in both aerial parts and roots, while rengyol (**2a**), isorengyol (**3a**), and alatoside B (**5**) were, together with **1**, the main constituents of the leaves. Neither saponins nor cyclohexylethanol derivatives could be detected in the roots. The roots contained naphthoxirenes [1] and an iridoid glucoside, identified as shanzhiside methyl ester by co-chromatography with an authentic sample. These two classes of compounds were not detected in the aerial parts. The chemical composition of *S. alatum* was confirmed by TLC and HPLC-UV analysis of a second sample collected in Niger in September 1989.

Discussion. – Alatosides A–C (**4a**, **5**, and **6a**, resp.) are, to our knowledge, the first triterpenic glycosides containing a 18,19-secours-12-ene aglycone. Alatosides B (**5**) and C (**6a**) are, moreover, unique examples of bidesmosidic saponins derived from an ursane skeleton in which both glycosyl chains are attached to the aglycone by means of osidic linkages.

Neither cyclohexylethanol derivatives nor saponins were previously found in the Pedaliaceae. From a chemotaxonomical point of view, *S. alatum* differs considerably from the species studied so far in this small plant family. In this context, it is interesting to notice that *S. alatum* belongs to the section *Sesamopteris* ENDL, which is considered as a rather isolated taxon within the family Pedaliaceae [18].

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Experimental Part

General. Enzymatic hydrolyses: β-D-glucuronidase (*G-1512*), hesperidinase (*H-8510*), and β-D-xylosidase (*X*-5375) were purchased from *Sigma Chemical Company*. Centrifugal partition chromatography (CPC): *Ito* multi-layer coil (*P.C. Inc.*; 350 ml; i.d. 2.6 mm); 700 rpm. Prep. low-pressure liquid chromatography (LPLC): *Lobar* columns (40–63 μm; i.d. 2.5 × 27 cm; *Merck*, Darmstadt), equipped with a *Duramat-80* pump (*Chemie und Filter*, Regensdorf). TLC: silica-gel-precoated Al sheets (*Merck*), $CHCl_3$/MeOH/H_2O 65:35:5 (cluent *1*). HPLC-UV: *Spectra-Physics-8750* pump, *Rheodyne* injector, *HP 1040A* diode array detector. M.p.: *Mettler-FP-80/82* hot-stage apparatus; uncorrected. $[\alpha]_D$: *Perkin-Elmer 241MC*. UV: *Varian-DMS-100S* spectrophotometer. ^{1}H- and ^{13}C-NMR: *Varian VXR-200* at 200 and 50.5 MHz, resp.; chemical shifts in ppm rel. to TMS. EI-MS and desorption-chemical-ionization(DCI) MS: *Nermag R 1030*. FAB-MS: *VG Autospec* (compound **4a**), *TSQ-70*, and *MAT-90* spectrometers (*Finnigan MAT*). FAB-MS-MS: *TSQ-70* spectrometer.

Plant Material. S. alatum THONN. was collected in Niger in autumn 1988. A voucher specimen is deposited at the Department of Biology of the University of Niamey, Niger.

Extraction and Isolation. Powdered aerial parts (134 g) were extracted at r.t. with CH_2Cl_2, followed by MeOH. A portion of the MeOH extract (11.7 g) was fractionated on a silica-gel column (63–200 μm; 80 × 5 cm i.d.) with $CHCl_3$/MeOH and $CHCl_3$/MeOH/H_2O mixtures of increasing polarity ($CHCl_3$/MeOH 9:1→$CHCl_3$/MeOH/H_2O 55:45:5). Fourteen fractions were collected (*I–XIV*). Compounds **2a** (165 mg) and **3a** (114 mg) were isolated from *Fraction V* (560 mg) by LPLC on *RP-8* with H_2O. Compounds **1** (147 mg) and **5** (168 mg) were separated from *Fraction XI* (1.2 g) by gel filtration on *Sephadex LH-20* (MeOH). Final purification of **5** was achieved by LPLC on *RP-8* with MeOH/H_2O 4:6. Compound **6a** (19 mg) was obtained from *Fraction XIII* (850 mg) by gel filtration on *Sephadex LH-20* (MeOH), followed by two LPLC steps on *Diol* ($CHCl_3$/MeOH 85:15) and *RP-8* (MeOH/H_2O 6:4). *Fraction VII* (1.25 g) was further separated by CPC with $CHCl_3$/MeOH/i-PrOH/H_2O 5:6:1:4 in the descending mode. Eleven fractions were collected (*A–K*). Compound **4a** (34 mg) was purified from *Fraction B* (282 mg) by gel filtration on *Sephadex LH-20* (MeOH), followed by LPLC on *RP-8* with MeOH/H_2O 6:4.

For the recording of HETCOR-NMR spectra, higher amounts of **4a** were obtained as follows: 16 g of MeOH extract were partitioned between BuOH and H_2O. The org. phase was concentrated under vacuum, and the saponins were precipitated with Et_2O. The precipitate (6 g) was chromatographed on a silica-gel column (63–200 μm; 57 × 4.5 cm i.d.) with $CHCl_3$/MeOH/H_2O 75:25:1. The fraction containing **5** (1.7 g) was further separated by gel filtration on *Sephadex LH-20* (MeOH): **5** (810 mg) in almost pure form. A 410-mg portion of this material was treated with β-D-glucuronidase (120 mg) in acetate buffer (15 h, 37°, pH 5.4). After extraction with BuOH, the crude product was purified by LPLC on *RP-8* (MeOH/H_2O 7:3): 210 mg of **4a**.

HPLC-UV Analysis of MeOH and BuOH Extracts (Aerial Parts and Roots). Analyses were performed on a 5-μm *Nucleosil-RP-8* column (125 × 4 mm i.d.; *Macherey-Nagel*) equipped with a (30 × 4 mm i.d.) precolumn. Samples (10 μl of a MeOH soln.) corresponding to 500 μg of dry extract were injected. Analysis of MeOH extracts with the following gradient: MeCN/H_2O 2:98→7:93 (30 min), then 7:93→37:63 (30 min), all containing 0.05% CF_3COOH; 1 ml/min; detection at 220 nm. For the detection of saponins, BuOH extracts were prepared by partition of MeOH extracts between BuOH and H_2O. Analysis with MeCN/H_2O 2:8→4:6 (30 min) containing 0.05% CF_3COOH; 1 ml/min; detection at 206 nm.

Acidic Hydrolysis. Saponins **4a** (1 mg), and **5** (10 mg), **6a** (1 mg) were refluxed in 2N HCl (10 ml) for 3.5 h. The reaction mixtures were extracted with AcOEt, followed by BuOH. The triterpenic derivative **7a** was detected in the AcOEt extracts by TLC. The aq. layers were adjusted to pH 5 with $NaHCO_3$. After evaporation, the sugars were extracted from the residue with pyridine and analyzed by TLC (silica gel, AcOEt/MeOH/H_2O/AcOH 65:15:15:20, detection with *p*-anisidine phthalate).

X-Ray Analysis. Suitable crystals of **7b** were grown from hexane/AcOEt as colourless prisms. Crystal data: $C_{36}H_{52}O_8 \cdot 0.2\ H_2O$, $M_r = 616.4$, space group $P2_1$, $a = 7.778(1)$, $b = 11.163(2)$, $c = 20.245(3)$ Å, $\beta = 98.17(1)°$, $V = 1739.9$ Å^3, $F(000) = 672$, $Z = 3$, $D_c = 1.177$ gcm^{-3}, MoK_α, $\lambda = 0.71073$ Å, $\mu = 0.08$ cm^{-1}. Size of crystal used for data collection, 0.23 × 0.19 × 0.15 mm. Intensity data were measured using a *Stoe-Siemens-AED2* four-circle diffractometer (graphite monochromated Mo$K\alpha$ radiation) using the ω/θ scan mode for $-8 < h < 8$, $0 < k < 12$, $0 < l < 21$, $\theta_{max} = 22.5°$. Four standard reflections measured every h showed no intensity variation. There were 2425 unique reflections of which 1852 ($I > 2.5\sigma(I)$) were considered observed. Cell parameters were refined from $\pm\omega$ values of 29 reflections and their equivalents in the range $20° < 2\theta < 24°$. No absorption or extinction corrections were applied. The structure was solved by direct methods using the SHELXS-86 system [19]. The NRCVAX program [20] was also used for further calculations. All H-atoms were introduced in idealized positions

(C–H 1.08 Å, $U_{iso} = U$ (C-atom + 0.01)Å^2) and distances were normalized after every second refinement cycle. The anisotropic refinement of 1852 reflexions converged at $R = 0.070$, $wR = 0.086$, $w^{-1} = \sigma^2|F_o| + 0.0020|F_o^2|$. Max. parameter shift/sigma in final cycle, 0.765; height in final difference synthesis, 0.27 to −0.25 eÅ^{-3}. Tables of atomic parameters and bond distances and angles were deposited with the *Cambridge Crystallographic Data Centre*, University Chemical Laboratory, Lensfield Road, GB-Cambridge CB2 1EW. Final observed and calculated structure factors are available from *H. St.-E.*

3-O-[O-α-L-Rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]alatogenin (= *Alatoside A* = *(2R*,3R*,17R*,19R*,20S*,21R*)-2,18,19-Trihydroxy-3-{[O-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]oxy}-18,19-secours-12-ene-28,21-lactone*; **4a**). White amorphous powder. M.p. 219–220°. TLC (SiO_2, eluent *1*): R_f 0.47. $[\alpha]_D^{20} = -39$ ($c = 0.5$, MeOH. ^{1}H-NMR ((D$_5$)pyridine): 6.52 (*s*, H–C(1″)); 6.34 (br. *s*, H–C(12)); 5.25 (*m*, H–C(21)); 4.64 (br. *s*, H–C(18)); 4.48 (*m*, H–C(19)); 3.31 (*d*, $J = 9.3$, H–C(3)); 1.72 (*d*, $J = 6.1$, Me(6″)); 1.33 (2 ×), 1.28, 1.24, 1.02 (4*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.30 (unresolved *d*, Me(29)); 1.04 (*d*, $J = 7.1$, Me(30)). ^{13}C-NMR ((D$_5$)pyridine): sugar moiety, see *Table*; 177.95 (C(28)); 141.27 (C(13)); 118.82 (C(12)); 94.30 (C(3)); 79.27 (C(21)); 75.99 (C(18)); 66.09 (C(2)); 65.48 (C(19)); 55.94 (C(5)); 50.82 (C(17)); 48.27 (C(1)); 47.89 (C(9)); 46.31 (C(20)); 43.86 (C(14)); 42.98 (C(22)); 40.75, 39.92, 37.70 (C(4), C(8), C(10)); 34.67, 31.84, 26.45 (C(7), C(15), C(16)); 28.14 (C(23)); 23.26 (C(11)); 22.81 (C(27)); 21.76 (C(29)); 18.64, 18.35, 18.15, 17.42 (C(24), C(25), C(26), C(6″)); 7.57 (C(30)); C(6) obscured. FAB-MS (thioglycerol, neg.-ion mode): 781 ($C_{41}H_{65}O_{14}$, $[M - H]^-$), 635 ($[M - H - 146]^-$), 503 ($[M - H - 146 - 132]^-$).

Enzymatic hydrolysis of **5**: Saponin **5** (20 mg) and β-D-glucuronidase (25 mg, 12000 units) in acetate buffer (20 ml) of pH 5.4 were stirred at 38° for 24 h. Extraction with BuOH and purification of the crude product (18 mg) by LPLC on *Diol* ($CHCl_3$/MeOH 94:6→9:1) provided 7 mg of **4a**, identical (^{13}C-NMR) with the natural compound.

19-O-(β-D-Glucopyranosyl)-3-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]alatogenin (= *Alatoside B* = *(2R*,3R*,17R*,19R*,20S*,21R*)-19-[(β-D-Glucopyranosyl)oxy]-2,18-dihydroxy-3-{[O-α-L-rhamnopyr-anosyl-(1→2)-β-D-xylopyranosyl]oxy}-18,19-secours-12-ene-28,21-lactone*; **5**). White amorphous powder. M.p. 190°. TLC (SiO_2, eluent *1*): R_f 0.33. $[\alpha]_D^{20} = -36$ ($c = 0.5$, MeOH). ^{1}H-NMR ((D$_5$)pyridine): 6.51 (*s*, H–C(1″)); 6.35 (br. *s*, H–C(12)); 5.40 (*m*, H–C(21)); 3.30 (*d*, $J = 9.5$, H–C(3)); 1.72 (*d*, $J = 6.1$, Me(6″)); 1.31, 1.27 (2 ×), 1.19, 0.96 (4*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.22 (*d*, $J = 6.8$, Me(29)); 0.88 (*d*, $J = 6.9$, Me(30)). ^{13}C-NMR ((D$_5$)pyridine): sugar moiety, see *Table*; 177.85 (C(28)); 140.71 (C(13)); 119.15 (C(12)); 94.38 (C(3)); 79.34, 79.05 (C(2′), C(21)); 76.27 (C(18)); 75.18, 75.12 (C(19), C(2‴)); 66.18 (C(2)); 56.01 (C(5)); 51.32 (C(17)); 48.33 (C(1)); 47.91 (C(9)); 44.90 (C(20)); 43.92 (C(14)); 42.68 (C(22)); 40.83, 39.99, 37.77 (C(4), C(8), C(10)); 34.82, 31.67, 26.50 (C(7), C(15), C(16)); 28.23 (C(23)); 23.30 (C(11)); 22.80 (C(27)); 18.74, 18.57, 18.46, 18.33, 17.47 (C(24), C(25), C(26), C(29), C(6″)); 7.78 (C(30)); C(6) obscured. FAB-MS (glycerol, neg.-ion mode): 943 ($C_{47}H_{75}O_{19}$, $[M - H]^-$), 797 ($[M - H - 146]^-$), 781 ($[M - H - 162]^-$), 665 ($[M - H - 146 - 132]^-$), 503 ($[M - H - 146 - 132 - 162]^-$).

19-O-(β-D-Glucopyranosyl)-3-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]alatogenin (= *Alatoside C* = *(2R*,3R*,17R*,19R*,20S*,21R*)-19-[(β-D-Glucopyranosyl)oxy]-2,18-dihydroxy-3-{[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]oxy}-18,19-secours-12-ene-28,21-lactone*; **6**). White amorphous powder. M.p. 198 200°. TLC (SiO_2, eluent *1*): R_f 0.24. $[\alpha]_D^{20} = -31$ ($c = 0.1$, MeOH). ^{1}H-NMR ((D$_5$)pyridine): 6.54 (*s*, H–C(1″)); 6.35 (br. *s*, H–C(12)): 5.39 (*m*, H–C(21)); 3.32 (*d*, $J = 9.3$, H–C(3)); 1.71 (*d*, $J = 6.1$, Me(6″)); 1.30, 1.26 (2 ×), 1.18, 0.94 (4*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.22 (*d*, $J = 6.6$, Me(29)); 0.89 (*d*, $J = 7.0$, Me(30)). ^{13}C-NMR: sugar moiety, see *Table*; δ's of the aglycone identical to those of **5**. FAB-MS (glycerol, neg.-ion mode): 973 ($C_{48}H_{77}O_{20}$, $[M - H]^-$), 827 ($[M - H - 146]^-$), 665 ($[M - H - 162]^-$).

3-O-(β-D-Xylopyranosyl)alatogenin (*(2R*,3R*,17R*,19R*,20S*,21R*)-2,18,19-Trihydroxy-3-[(β-D-xylopyranosyl)oxy]-18,19-secours-12-ene-28,21-lactone*; **4b**). Saponin **4a** (20 mg) and hesperidinase (200 mg, 54 units) in acetate buffer (20 ml) of pH 5.4 were stirred at 37° for 52 h. Extraction with BuOH and purification of the crude product (19 mg) by LPLC on *Diol* ($CHCl_3$/MeOH 97:3) gave 8 mg of **4b**. Amorphous white powder. TLC (SiO_2, eluent *1*): R_f 0.63. ^{1}H-NMR ((D$_5$)pyridine): 6.36 (br. *s*, H–C(12)); 5.25 (*m*, H–C(21)); 4.84 (*d*, $J = 7.4$, H–C(1′)); 4.65 (br. *s*, H–C(18)); 4.48 (*m*, *dq* after D_2O exchange, H–C(19)); 3.31 (*d*, $J = 9.3$, H–C(3)); 2.39 (*dd*, $J = 12.6, 4.1$, H_β–C(1)); 1.43, 1.34, 1.26, 1.10, 1.02 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.30 (*d*, $J = 6.4$, Me(29)); 1.04 (*d*, $J = 7.0$, Me(30)). ^{13}C-NMR: sugar moiety, see *Table*; δ's of the aglycone identical to those of **4a**. FAB-MS (glycerol, neg.-ion mode): 635 ($C_{35}H_{55}O_{10}$, $[M - H]^-$), 503 ($[M - H - 132]^-$).

3-O-[O-α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]alatogenin (*(2R*,3R*,17R*,19R*,20S*,21R*)-2, 18, 19-Trihydroxy-3-{[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]oxy}-18, 19-secours-12-ene-28, 21-lactone*; **6b**). Saponin **6a** (15 mg) and β-D-glucuronidase (18 mg, 9000 units) in acetate buffer (20 ml) of pH 5.4 were stirred 15 h at 38°. The mixture was extracted with BuOH and the crude product (15 mg) purified by LPLC on *Diol*

($CHCl_3$/MeOH 9:1): 10 mg of **6b**. Amorphous white powder. TLC (SiO_2, eluent *1*): R_f 0.38. ^{1}H-NMR ((D$_5$)pyridine): 6.56 (*d*, *J* = 1.6, H–C(1″)); 6.33 (br. *s*, H–C(12)); 5.24 (*m*, H–C(21)); 3.34 (*d*, *J* = 9.3, H–C(3)); 1.72 (*d*, *J* = 6.1, Me(6″)); 1.32 (2 ×), 1.28, 1.23, 1.00 (4*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.30 (unresolved *d*, Me(29)); 1.04 (*d*, *J* = 6.9, Me(30)). ^{13}C-NMR: sugar moiety, see *Table*; δ's of the aglycone identical to those of **4a**. FAB-MS (glycerol, neg.-ion mode): 811 ($C_{42}H_{67}O_{15}$, $[M - H]^-$).

*(2*R**,3*R**,17*R**,19*R**,20*S**,21*R**)-2,3,18,19-Tetrahydroxy-18,19-secours-12-ene-28,21-lactone* (*Alatogenin*; **4c**; $C_{30}H_{48}O_6$). Saponin **4b** (8 mg) was dissolved in a 1 ml suspension of β-D-xylosidase (2.5 units, buffer pH 5.2). After 4 d incubation at 37°, 1 ml of enzymatic suspension (2.5 units) was added. Acetate buffer (pH 5.4, 2 × 10 ml) was added after 7 and 8 d. After 11 d, the mixture was extracted with AcOEt. Purification of the crude product (9 mg) by LPLC on *Diol* ($CHCl_3$/MeOH 97:3) afforded 5 mg of pure **4c**. Amorphous white powder. ^{1}H-NMR ((D$_5$)pyridine): 6.36 (*m*, H–C(12)); 5.25 (*m*, H–C(21)); 4.64 (br. *s*, H–C(18)); 4.48 (*m*, *dq* after D_2O exchange, H–C(19)); 4.15 (*m*, H–C(2)); 3.43 (*d*, *J* = 9.2, H–C(3)); 2.37 (*dd*, *J* = 12.1, 4.2, H$_\beta$–C(1)); 1.37, 1.31, 1.26, 1.22, 1.09 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.30 (*d*, *J* = 6.4, Me(29)); 1.04 (*d*, *J* = 6.8, Me(30)). ^{13}C-NMR ((D$_5$)pyridine): 178.02 (C(28)); 141.46 (C(13)); 118.93 (C(12)); 83.86 (C(3)); 79.37 (C(21)); 76.10 (C(18)); 68.64 (C(2)); 65.57 (C(19)); 56.16 (C(5)); 50.93 (C(17)); 48.33 (C(1)); 48.12 (C(9)); 46.41 (C(20)); 43.98 (C(14)); 43.09 (C(22)); 40.14, 39.89, 38.47 (C(4), C(8), C(10)); 34.88, 31.94, 26.56 (C(7), C(15), C(16)); 29.45 (C(23)); 23.42 (C(11)); 22.96 (C(27)); 21.86 (C(29)); 18.81, 18.37, 17.83, 17.56 (C(6), C(24), C(25), C(26)); 7.66 (C(30)). DCI-MS (NH_3, pos.-ion mode): 522 ($[M + NH_4]^+$), 505 ($[M + H]^+$), 504 ($C_{30}H_{48}O_6$, M^+, $[M + NH_4 - 18]^+$), 487 ($[M + H - 18]^+$), 469 ($[M + H - 36]^+$), 461, 443, 358.

*2,3,18,19-Tetra-*O*-acetylalatogenin* (**4d**). Treatment of **4c** (5 mg) with Ac_2O (1.5 ml) in pyridine (1.5 ml) for 24 h at r.t. afforded, after the usual workup, 6 mg of **4d**. Amorphous white powder. $[\alpha]_D^{20} = -36$ (*c* = 0.2, AcOEt). ^{1}H-NMR ((D$_5$)pyridine): 5.94 (*m*, H–C(12)); 5.54–5.32 (*m*, H–C(2), H–C(18), H–C(19)); 5.07 (*d*, *J* = 10.3, H–C(3)); 4.44 (*m*, H–C(21)); 2.19, 2.14, 2.11, 2.05 (4*s*, 4 Ac); 1.24, 1.22, 1.02, 0.93, 0.92 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.21 (*d*, *J* = 6.4, Me(29)); 0.90 (*d*, *J* = 6.9, Me(30)). ^{1}H-NMR ($CDCl_3$): 5.59 (*m*, H–C(12)); 5.30 (*m*, H–C(18)); 5.2–5.05 (*m*, H–C(2), H–C(19)); 4.74 (*d*, *J* = 10.3, H–C(3)); 4.14 (*m*, H–C(21)); 2.16, 2.05, 2.01, 1.97 (4*s*, 4 Ac); 1.23 (*d*, *J* = 6.5, Me(29)); 1.17, 1.12, 1.09, 0.92, 0.91 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 0.90 (*d*, *J* = 7.0, Me(30)). ^{13}C-NMR ((D$_5$)pyridine): 176.08 (C(28)); 170.62, 170.44, 170.24, 170.14 (4 CH_3CO); 119.07 (C(12)); 80.69 (C(3)); 78.22, 77.31 (C(18), C(21)); 70.22, 69.78 (C(2), C(19)); 55.10 (C(5)); 48.65 (C(17)); 47.25 (C(9)); 44.49 (C(1)); 44.16, 43.78 (C(20), C(14)); 40.47 (C(22)); 39.89, 39.55, 38.13 (C(4), C(8), C(10)); 34.35, 31.61, 26.11 (C(7), C(15), C(16)); 28.52 (C(23)); 23.04 (C(11)); 22.54 (C(27)); 20.99, 20.96, 20.77, 20.63 (4 CH_3CO); 18.25 (C(6)); 17.98 (2 ×), 17.74, 17.04 (C(24), C(25), C(26), C(29)); 8.33 (C(30)); C(13) obscured by pyridine (134.88 in $CDCl_3$). DCI-MS (NH_3, pos.-ion mode): 690 ($[M + NH_4]^+$), 613, 553, 510, 493, 368, 351.

*(2*R**,3*R**,17*R**,19*R**,20*S**,21*R**)-2,3,19-Trihydroxy-18,19-secoursa-11,13(18)-diene-28,21-lactone* (**7a**). Saponin **5** (40 mg) was refluxed in 5% H_2SO_4 (*v*/*v*; 10 ml) for 1 h. The mixture was extracted with AcOEt and the crude product purified by LPLC (silica gel, $CHCl_3$/MeOH 95:5): 9 mg of pure **7**. Amorphous white powder. UV (MeOH): 257 (sh), 250, 243 (sh). ^{1}H-NMR ((D$_5$)pyridine): 6.05 (*dd*, *J* = 10.0, 2.9, H–C(11)); 5.85 (*d*, *J* = 10.0, H–C(12)); 5.49 (*s*, H–C(18)); 4.92 (*m*, H–C(21)); 4.50 (*m*, H–C(19)); 4.20 (*m*, H–C(2)); 3.44 (*d*, *J* = 9.3, H–C(3)); 2.76 (*m*, not attributed); 2.55 (*dd*, *J* = 12.4, 4.4, H$_\beta$–C(1)); 2.16 (*dd*, *J* = 12.4, 5.3, H$_a$–C(22)); 1.33 (*d*, *J* = 6.5, Me(29)); 1.29, 1.09, 1.07, 1.04, 1.03 (5*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 1.03 (*d*, *J* = 6.9, Me(30)). ^{1}H-NMR ($CDCl_3$): 5.95 (*dd*, *J* = 10.2, 3.1, H–C(11)); 5.70 (*dd*, *J* = 10.2, 1.5, H–C(12)); 5.41 (br. *s*, H–C(18)); 4.55 (*ddd*, *J* = 10.4, 9.5, 5.4, H–C(21)); 4.17 (*dq*, *J* = 6.5, 2.0, H–C(19)); 3.77 (*ddd*, *J* = 9.5, 11.4, 4.6, H–C(2)); 3.03 (*d*, *J* = 9.5, H–C(3)); 1.22 (*d*, *J* = 6.5, Me(29)); 1.04, 0.98 (2 ×), 0.83, 0.82 (4*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 0.88 (*d*, *J* = 7.0, Me(30)). ^{13}C-NMR ((D$_5$)pyridine): 178.94 (C(28)); 144.87 (C(13)); 129.88, 128.93, 122.39 (C(11), C(12), C(18)); 83.82 (C(3)); 78.30 (C(21)); 68.56 (C(2)); 65.60 (C(22)); 55.43, 54.80 (C(5), C(9)); 47.43 (C(1)); 45.59 (C(20)); 45.02 (C(17)); 42.01 (C(22)); 41.16 (2 ×), 39.91, 38.22 (C(4), C(8), C(10), C(14)); 32.39, 28.73, 25.35 (C(7), C(15), C(16)); 29.05 (C(23)); 21.81, 20.28, 19.46, 17.15, 16.88 (C(24), C(25), C(26), C(27), C(29)); 18.66 (C(6)); 7.49 (C(30)). EI-MS: 486 ($C_{30}H_{46}O_5$, 60), 468 (100), 450 (11).

Triacetyl Derivative **7b**. Compound **7a** (9 mg) was treated with Ac_2O (0.8 ml) in pyridine (0.8 ml) for 18 h at r.t. After the usual workup, the crude product was purified by column chromatography (silica gel (40–63 μm) 15 × 1.7 cm i.d., $CHCl_3$/MeOH 99:1): 11 mg of **7b**. Colourless prisms from hexane/AcOEt. M.p. 214–218°. $[\alpha]_D^{20} = -178$ (*c* = 0.1, MeOH). ^{1}H-NMR ($CDCl_3$): 5.95 (*dd*, *J* = 10.0, 2.9, H–C(11)); 5.65 (*dd*, *J* = 10.0, 1.3, H–C(12)); 5.39 (*s*, H–C(18)); 5.25–5.05 (*m*, H–C(2), H–C(19)); 4.78 (*d*, *J* = 10.3, H–C(3)); 4.41 (*ddd*, *J* = 10.4, 8.5, 5.4, H–C(21)); 2.48 (*m*, not attributed); 2.28 (*dd*, *J* = 12.2, 4.6, H$_\beta$–C(1)); 2.14 (*dd*, *J* = 12.4, 5.4, H$_a$–C(22)); 2.07, 2.05, 1.99 (3*s*, 3 Ac); 1.26 (*d*, *J* = 6.4, Me(29)); 1.04, 0.96, 0.91 (2 ×), 0.81 (4*s*, Me(23), Me(24), Me(25), Me(26), Me(27)); 0.95 (*d*, *J* = 7.0, Me(30)). ^{13}C-NMR ($CDCl_3$): 178.24 (C(28)); 170.84, 170.52, 170.35 (3 CH_3CO); 144.86 (C(13)); 129.55,

128.12, 121.31 (C(11), C(12), C(18)); 80.54 (C(3)); 77.05 (C(21)); 70.08, 69.94 (C(2), C(19)); 54.59, 54.15 (C(5), C(9)); 44.45 (C(17)); 43.51 (C(1)); 42.95 (C(20)); 41.33 (C(22)); 40.81 (2 ×), 39.38, 37.76 (C(4), C(8), C(10), C(14)); 31.73, 28.27, 24.82 (C(7), C(15), C(16)); 28.16 (C(23)); 21.28, 21.13, 20.92, 19.97, 18.87, 17.71, 17.12, 16.30 (3 CH_3CO, C(24), C(25), C(26), C(27), C(29)); 17.98 (C(6)); 8.42 (C(30)). DCI-MS (NH_3, pos.-ion mode): 630 ($[M + NH_4]^+$), 613 ($[M + H]^+$).

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