Foetidissimosides C – F, Novel Glycosides from the Roots of *Cucurbita foetidissima*

by **Ghezala Gaidi**[a]), **Tomofumi Miyamoto**[b]), **Holger Lerche**[c]), and **Marie-Aleth Lacaille-Dubois***[a])

[a]) Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique (UMIB EA 3660), Faculté de Pharmacie, Université de Bourgogne, 7 Bd Jeanne d'Arc, BP 87900, F-21079 Dijon Cedex

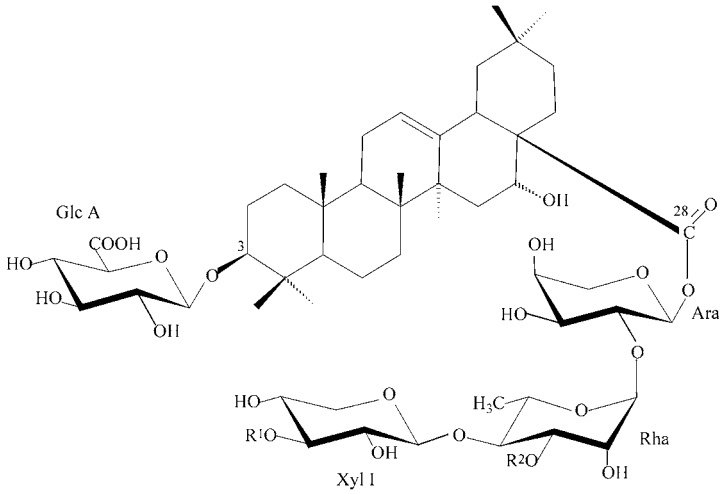
[b]) Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

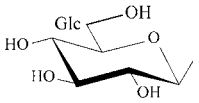
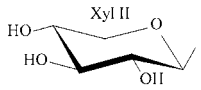
[c]) Department of Pharmacy, Center of Pharmaresearch, University of Munich, D-81377 Munich

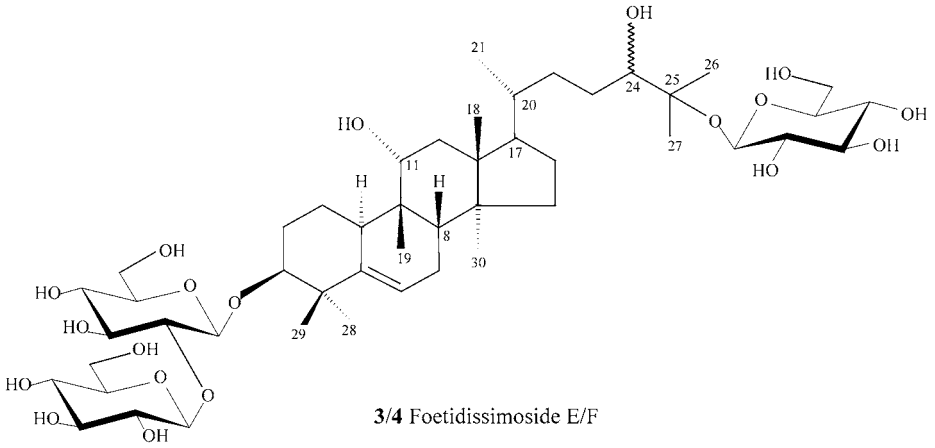
Two novel echinocystic acid (=(3β,16α)-3,16-dihydroxyolean-12-en-28-oic acid) glycosides, foetidissimosides C (**1**), and D (**2**), along with new cucurbitane glycosides, *i.e.,* foetidissimosides E/F (**3/4**) as an 1:1 mixture of the (24*R*)/(24*S*) epimers, were obtained from the roots of *Cucurbita foetidissima.* Their structures were elucidated by means of a combination of homo- and heteronuclear 2D-NMR techniques (COSY, TOCSY, NOESY, ROESY, HSQC, and HMBC), and by FAB-MS. The new compounds were characterized as (3β,16α)-28-{[*O*-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl]oxy}-16-hydroxy-28-oxoolean -12-en-3-yl β-D-glucopyranosiduronic acid (**1**), (3β,16α)-16-hydroxy-28-oxo-28-{{*O*-β-D-xylopyranosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl}oxy}olean-12-en-3-yl β-D-glucopyranosiduronic acid (**2**), and (3β,9β,10α,11α,24*R*)- and (3β,9β,10α,11α,24*S*)-25-(β-D-glucopyranosyloxy)-9-methyl-19-norlanost-5-en-3-yl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (**3** and **4**, resp.).

Introduction. – *Cucurbita foetidissima* H.B.K. (*C. perrenis*, A. GRAY, *Cucumis perrenis* E. JAMES) (Cucurbitaceae) commonly called calabazilla or buffalo gourd is an indigenous plant in Texas, Arizona, New Mexico, and California [1][2]. Our previous phytochemical studies on the MeOH extract of *C. foetidissima* roots allowed the isolation of two new triterpene saponins, foetidissimosides A and B, and the identification of cucurbitacins by HPLC [3][4]. A detailed further investigation of the same extract led to the isolation of two additional triterpene saponins called foetidissimosides C (**1**) and D (**2**), and a pair of cucurbitane glycosides, foetidissimosides E/F (**3/4**) (cucurbitane = (5ξ,9β,10α)-9-methyl-19-norlanostane). Their structures were elucidated mainly by 1D- and 2D-NMR experiments (COSY, TOCSY, ROESY, NOESY, HSQC, and HMBC).

Results and Discussion. – The MeOH extract of the roots of *C. foetidissima* was successively partitioned between $CHCl_3$ and H_2O, then between BuOH and H_2O. The concentrated BuOH-soluble fraction was purified by precipitation with Et_2O and subjected to *Sephadex LH-20* and silica-gel column chromatography to afford a saponin fraction that was further separated by repeated medium-pressure liquid chromatography (MPLC) over silica gel and semi-prep. reversed-phase HPLC (*Si RP 18*), yielding the pure foetidissimosides C (**1**) and D (**2**) and the 1:1 mixture of foetidissimosides E/F (**3/4**). This mixture was homogenous by HPTLC and gaves two overlapped peaks by HPLC, and all attempts to separate them by semi-prep. reversed-phase HPLC (silica gel) were unsuccessful.



	R^1	R^2
1 Foetidissimoside C		H
2 Foetidissimoside D	H	



3/4 Foetidissimoside E/F

The high-resolution (HR) ESI-MS (positive-ion mode) of foetidissimoside C (**1**) exhibited a quasi-molecular-ion peak at *m/z* 1243.6056 ($[M+Na]^+$; calc 1243.6088), consistent with a molecular formula of $C_{59}H_{96}NaO_{26}$. Its negative-ion FAB-MS displayed a quasi-molecular-ion peak ($[M-H]^-$) at *m/z* 1219 indicating a relative molecular mass of 1220, compatible with the molecular formula $C_{59}H_{96}O_{26}$. Other fragment-ion peaks at *m/z* 1057 ($[M-H-162]^-$), 779 ($[M-H-162-132-146]^-$), 647 ($[M-H-162-132-146-132]^-$), and 471 ($[M-H-162-132-146-132-176]^-$) indicated the elimination of one terminal hexosyl, one pentosyl-desoxyhexosyl moiety, one pentosyl, and one hexuronosyl moiety, respectively. The full assignment of all the ^{1}H- and ^{13}C-NMR signals by 2D-NMR experiments of **1** resulted in the establishment of its structure as (3*β*,16*α*)-28-{[*O*-*β*-D-glucopyranosyl-(1 → 3)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl]oxy}-16-hydroxy-28-oxoolean-12-en-3yl *β*-D-glucopyranosiduronic acid. According to several reports on the distribution of echinocystic acid (=(3*β*,16*α*)-3,16-dihydroxyolean-12-en-28-oic acid) glycosides [5–9], **1** appears to be a new natural compound.

Acid hydrolysis of **1** with 2N CF_3COOH yielded arabinose, glucose, xylose, rhamnose (=6-deoxymannose), and glucuronic acid (identified by co-TLC with authentic samples), and an aglycone identified as echinocystic acid on the basis of the DEPT, HSQC, and HMBC NMR spectra of **1** (see *Table 1*). Alkaline hydrolysis of **1** with 5% KOH solution yielded a prosapogenine which furnished, by further acid hydrolysis, glucuronic acid and echinocystic acid (co-TLC with authentic samples) [3][4].

Table 1. *^{13}C-NMR* (150 MHz)[a]) *Data of the Aglycone Parts of* **1** *and* **2** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments.* *δ* in ppm.

	1	**2**		**1**	**2**
CH_2(1)	39.3	37.8	CH (16)	74.2	74.1
CH_2(2)	25.8	25.6	C(17)	49.4	50.4
CH(3)	88.9	89.1	CH(18)	41.3	42.1
C(4)	39.6	40.3	CH_2(19)	46.9	47.9
CH(5)	55.7	56.0	C(20)	30.8	30.9
CH_2(6)	18.5	18.5	CH_2(21)	36.1	36.1
CH_2(7)	33.6	29.1	CH_2(22)	31.9	30.8
C(8)	40.1	40.9	Me(23)	28.1	28.1
CH(9)	47.2	47.1	Me(24)	16.8	17.1
CH(10)	36.9	37.1	Me(25)	15.5	16.5
CH_2(11)	24.6	24.2	Me(26)	16.9	17.9
CH(12)	n.d.	123.0	Me(27)	27.1	27.1
C(13)	144.2	145.2	C(28)	175.7	176.7
C(14)	41.9	42.9	Me(29)	33.4	34.1
CH_2(15)	36.0	36.1	Me(30)	24.6	24.7

[a]) Multiplicities were assigned from DEPT spectra.

Compound **1** was shown to contain five sugar residues from the HSQC spectrum. The anomeric ^{1}H-NMR signals at *δ* 6.48 (br. *s*), 5.65 (br. *s*), 5.13 (*d*, *J* = 7.4 Hz), 5.25 (*d*, *J* = 7.5 Hz), and 4.82 (*d*, *J* = 7.5 Hz) correlated with the anomeric ^{13}C-NMR signals at *δ* 93.3, 100.6, 105.9, 105.3, and 106.9, respectively. Complete assignments of each sugar-proton system were achieved by considering TOCSY and ^{1}H,^{1}H COSY plots, while the C-atoms

were assigned from HSQC and HMBC spectra. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one α-arabinopyranose (Ara), one α-rhamnopyranose (Rha), one β-xylopyranose, (Xyl I), one β-glucopyranose (Glc), and one β-glucopyranuronic acid (GlcA) unit, respectively. The common D-configuration for Xyl, Glc, and GlcA and the L-configuration for Ara and Rha were determined by GC analysis of chiral derivatives of the sugars in the acid hydrolyzate [10].

The cross-peaks in the HMBC plot between δ(H) 4.82 (d, $J = 7.5$ Hz, GlcA H–C(1)) and δ(C) 88.9 (Agly C(3)) showed that the β-D-glucopyranuronic acid moiety was linked to the echinocystic acid at C(3) by a glycosidic bond.

After subtraction of the anomeric signals of the glucopyranuronic acid moiety linked at C(3) from the total NMR spectrum of **1**, the signals of four sugar units linked to the aglycone by an ester linkage remained. A correlation in the HSQC plot at δ(C)/δ(H) 94.3/6.48 (br. s) showed that the arabinose residue was attached to the carboxylic group of the aglycone by an ester linkage. The sugar chain at C(28) was established from HMBC correlations between the following ^{1}H- and ^{13}C-NMR signals: δ(H) 5.25 (d, $J = 7.5$ Hz, Glc H–C(1)) and δ(C) 87.8 (Xyl I C(3)), δ(H) 5.13 (d, $J =$ 7.4 Hz, Xyl I H–C(1)) and δ(C) 83.2 (Rha C(4)), and between δ(H) 5.65 (br. s, Rha H–C(1)) and δ(C) 75.2 (Ara C(2)). The linkages were confirmed by the reverse correlations between δ(H) 4.02 (Xyl I H–C(3)) and δ(C) 105.3 (Glc C(1)), and between δ(H) 4.50 (Ara H–C(2)) and δ(C) 100.6 (Rha C(1)). The ^{1}H- and ^{13}C-NMR data of **1** (see *Tables 1* and *2*) assigned from TOCSY, HSQC, and HMBC experiments were similar to those of (3β,16α)-16-hydroxy-28-oxo-28-{[*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]oxy}olean-12-en-3-yl β-D-glucopyranosiduronic acid (= 3-*O*-(β-D-glucuronopyranosyl)echinocystic acid 28-{*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl} ester = foetidissimoside A) [3], except for the appearance of a set of additional signals, corresponding to a terminal β-D-glucopyranosyl group in **1** attached at the 3-position of the 1,3-linked xylopyranosyl unit (Xyl I).

The HR-ESI-MS (positive-ion mode) of foetidissimoside D (**2**) exhibited a quasi-molecular-ion peak at m/z 1213.5960 $[M + Na]^+$; calc. 1213.5982), consistent with a molecular formula of $C_{58}H_{94}NaO_{25}$. Its negative-ion FAB-MS displayed a quasi-molecular-ion peak ($[M - H]^-$) at m/z 1189 indicating a relative molecular mass of 1190, compatible with the molecular formula $C_{58}H_{94}O_{25}$. Other fragment-ion peaks at m/z 1057 ($[M - H - 132]^-$) and 779 ($[M - H - 132 - 132 - 146]^-$) indicated the elimination of one terminal pentosyl and one pentosyl-desoxyhexosyl moiety, respectively.

The full assignment of all the ^{1}H- and ^{13}C-NMR signals by 2D-NMR experiments of **2** resulted in the establishment of its structure as 3-*O*-(β-D-glucopyranuronosyl)echinocystic acid 28-{*O*-β-D-xylopyranosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl} ester, a new natural compound [5–9].

The NMR data of **2** (see *Tables 1* and *2*) were very similar to those of foetidissimoside A [2], except for the appearance of a set of additional signals, corresponding to a terminal β-D-xylopyranosyl group (Xyl II) in **2** which was attached at the 3-position of Rha. The site of linkage of Xyl II was determinated by the HMBC experiment which showed a long-range correlation between δ(H) 5.15 (d, $J = 7.6$ Hz, Xyl II H–C(1)) and δ(C) 82.5 (Rha C(3)). This linkage was confirmed by the ROESY correlation between δ(H) 5.15 (d, $J = 7.6$ Hz, Xyl II H–C(1)) and δ(H) 4.58 (Rha H–C(3)).

The HR-ESI-MS (positive-ion mode) of the mixture **3**/**4** exhibited a quasi-molecular-ion peak at m/z 985.5360 ($[M + Na]^+$; calc. 985.5348), consistent with a molecular formula of $C_{48}H_{82}NaO_{19}$. Their negative-ion FAB-MS showed a quasi-molecular-ion peak at m/z 961 ($[M - H]^-$), indicating a relative molecular mass of 962. Other significant ion peaks visible at m/z 799 ($[M - H - 162]^-$), 637 ($[M - H - 162 - 162]^-$), and 475 ($[M - H - 162 - 162 - 162]^-$) corresponded to the successive losses of

Table 2. *^1H-* (600 MHz) and *^{13}C-*NMR (150 MHz) *Data of the Sugar Moieties of* **1** *and* **2** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

		1		**2**	
		δ(C)	δ(H)	δ(C)	δ(H)
At C(3): GlcA	H–C(1)	106.9	4.82 (*d*, J = 7.5)	106.7	4.77 (*d*, J = 7.5)
	H–C(2)	75.0	3.99	76.4	3.93
	H–C(3)	78.1	4.52	79.7	4.10
	H–C(4)	74.0	4.25	72.4	4.15
	H–C(5)	78.0	4.21	77.2	4.28
	C(6)	170.0	–	170.0	–
At C(28): Ara	H–C(1)	93.3	6.48 (br. *s*)	94.3	6.48 (br. *s*)
	H–C(2)	75.2	4.50	75.7	4.55
	H–C(3)	69.0	4.50	70.2	4.52
	H–C(4)	65.2	4.35	66.9	4.45
	CH_2(5)	62.4	3.80, 4.02	62.9	3.92, n.d. [b])
Rha	H–C(1)	100.6	5.65 (br. *s*)	101.5	5.73 (br. *s*)
	H–C(2)	71.5	4.50	72.4	4.77
	H–C(3)	72.7	4.52	82.5	4.58
	H–C(4)	83.2	4.32	79.1	4.12
	H–C(5)	68.6	4.35	69.5	4.43
	Me(6)	18.7	1.68 (*d*, J = 6.0)	19.4	1.72 (*d*, J = 6.0)
Xyl I	H–C(1)	105.9	5.13 (*d*, J = 7.4)	105.9	5.44 (*d*, J = 7.4)
	H–C(2)	74.8	3.99	76.2	3.92
	H–C(3)	87.8	4.02	79.2	4.03
	H–C(4)	71.1	4.05	71.7	4.04
	CH_2(5)	67.0	3.40, 4.04	67.9	3.36, 4.10
Glc	H–C(1)	105.3	5.25 (*d*, J = 7.5)		
	H–C(2)	75.6	4.04		
	H–C(3)	78.2	4.22		
	H–C(4)	70.9	4.03		
	H–C(5)	78.4	3.82		
	CH_2(6)	61.9	4.20, 4.51		
Xyl II	H–C(1)			106.7	5.15 (*d*, J = 7.6)
	H–C(2)			76.2	3.92
	H–C(3)			79.1	4.00
	H–C(4)			72.1	4.10
	CH_2(5)			67.9	3.42, 4.05

[a]) Overlapped ^{1}H-NMR signals are reported without designated multiplicity. [b]) n.d. = not determined.

three hexosyl moieties. The detailed analyses of the spectral data of **3/4** lead to the formulation of the structure of the compound pair as *(3β,11α,24*R*)-* and *(3β,11α,24*S*)-25-(β-*D *-glucopyranosyloxy)-11,24-dihydroxycucurbit-5-en-3-yl 2-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranoside* (**3** and **4**, resp.). According to several reports on the distribution of cucurbitane glycosides [9][11–14], **3/4** appear to be novel natural compounds. The ratio of the epimers was approximately 1:1 as determined by the ^{1}H-NMR spectrum.

Acid hydrolysis of **3/4** with 2N CF_3COOH yielded glucose (identified by co-TLC with an authentic sample), and an artifactual aglycone.

Most of the NMR signals of the aglycone of **3/4** (*Table 3*) were assigned through ^2J(H,C) and ^3J(H,C) couplings of the 8 Me groups in the HMBC spectrum allowing the characterization of a cucurbitane skeleton

(*Table 4*). The configurations of rings A–D of the aglycone was confirmed by a NOESY experiment. The following NOEs were observed: H_α–C(3)/Me(28), Me(28)/H_α–C(10) and Me(30), H_β–C(6)/Me(29), Me(19)/H_β–C(8) and H_β–C(11), and Me(18)/H_β–C(8) and H_β–C(11). The ^{13}C-NMR signals of the cucurbitane due to the aglycone were almost superimposable on those of mogrol (=(3*β*,11*α*,24*R*)-cucurbit-5-ene-3,11,24,25-tetrol) isolated from *Fructus Momordicae* [15], while the signals of the side chain (C(24) to C(27)) appeared as double signals (*Table 3*). In the HSQC spectrum, we clearly observed the presence of two pairs of cross-peaks at δ(H) 3.88 (Agly H–C(24))/δ(C) 78.1 (Agly H–C(24)) and at δ(H) 4.02 (Agly H–C(24))/δ(C) 76.3 (Agly H–C(24)) assigned to OH-substituted CH(24). This result suggested that the cucurbitane skeleton existed as two epimers at C(24), one of them corresponding to the (24*R*)-epimer by NMR comparison with mogrol and the other one to the (24*S*)-epimer.

Table 3. *^{13}C-NMR* (150 MHz)[a]) *Data of the Aglycone Parts of* **3/4** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm.

	3	**4**	**3**	**4**	
CH_2(1)	26.0	26.0	CH_2 (16)	28.2	28.2
CH_2(2)	28.6	28.6	CH(17)	50.6	50.6
CH(3)	87.1	87.1	Me(18)	16.6	16.6
C(4)	41.8	41.8	Me(19)	25.9	25.9
C(5)	143.7	143.7	CH(20)	36.4	36.4
CH(6)	118.5	118.5	Me(21)	18.6	18.6
CH_2(7)	24.2	24.2	CH_2 (22)	34.2	34.2
CH(8)	43.2	43.2	CH_2 (23)	28.7	28.7
C(9)	39.8	39.8	CH(24)	78.1	76.3
CH(10)	36.2	36.2	C(25)	80.8	80.4
CH(11)	77.5	77.5	Me(26)	24.2	22.3
CH_2(12)	40.5	40.5	Me(27)	23.8	21.0
C(13)	47.0	47.0	Me(28)	28.5	28.5
C(14)	49.4	49.4	Me(29)	26.8	26.8
CH_2(15)	34.2	34.2	Me(30)	18.9	18.9

[a]) Multiplicities were assigned from DEPT spectra.

Table 4. *Cross-Peaks (*δ [ppm]*) in the ^{1}H-Detected Long-range ^{1}H,^{13}C HMBC Plot Used for Defining the Aglycones of* **3** *and* **4**.

δ(H)	HMBC correlations
5.70 (Agly H–C(6))	C(8) (43.2), C(4) (41.8), C(10) (36.2)
4.07 (Agly H–C(11))	C(10) (36.2), C(19) (25.9)
2.01 (Agly H–C(12))	C(9) (39.8), C(11) (77.5), C(13) (47.0), C(14) (49.4), C(18) (16.6)
0.78 (Agly Me(18))	C(12) (40.5), C(13) (47.0), C(14) (49.4), C(17) (50.6)
1.24 (Agly Me(19))	C(8) (43.2), C(9) (39.8), C(10) (36.2), C(11) (77.5)
0.92 (Agly Me(21))	C(17) (50.6), C(20) (36.4), C(22) (34.2)
1.46 (Agly Me(26))	C(24) (76.3), C(25) (80.4), C(27) (22.3)
1.47 (Agly Me(26))	C(24) (78.1), C(25) (80.8), C(27) (24.2)
1.46 (Agly Me(27))	C(24) (76.3), C(25) (80.4), C(26) (21.0)
1.47 (Agly Me(27))	C(24) (78.1), C(25) (80.8), C(26) (23.8)
1.06 (Agly Me(28))	C(3) (87.1), C(4) (41.8), C(5) (143.7), C(29) (26.8)
1.45 (Agly Me(29))	C(3) (87.1), C(4) (41.8), C(5) (143.7), C(28) (28.5)
0.84 (Agly Me(30))	C(8) (43.2), C(13) (47.0), C(14) (49.4), C(15) (34.2)

The ring protons of the monosaccharide residues of **3/4** were assigned starting from the anomeric protons by means of the COSY, TOCSY, HSQC, and HMBC plots (see *Table 5*), and the sequence of the oligosaccharide chains was obtained from the HMBC and NOESY experiments. Evaluation of spin-spin

Table 5. *^1H- (600 MHz) and ^{13}C-NMR (150 MHz) Data of the Sugar Moieties of* **3**/**4** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

		3		**4**	
		δ(C)	δ(H)	δ(C)	δ(H)
At C(3): Glc I	H–C(1)	104.3	4.75 (d, J = 6.9)	104.3	4.75 (d, J = 6.9)
	H–C(2)	81.1	4.22	81.1	4.22
	H–C(3)	77.6	4.23	77.6	4.23
	H–C(4)	71.0	4.00	71.0	4.00
	H–C(5)	77.6	3.76	77.6	3.76
	CH_2(6)	62.2	4.20, 4.42	62.2	4.20, 4.42
Glc II	H–C(1)	104.3	5.28 (d, J = 7.7)	104.3	5.28 (d, J = 7.7)
	H–C(2)	76.3	4.02	76.3	4.02
	H–C(3)	77.5	4.15	77.5	4.15
	H–C(4)	71.2	4.18	71.2	4.18
	H–C(5)	77.7	3.86	77.7	3.86
	CH_2(6)	62.4	4.25, 4.48	62.4	4.25, 4.48
At C(25): Glc IIIa	H–C(1)	98.2	5.10 (d, J = 7.3)		
	H–C(2)	74.9	3.95		
	H–C(3)	78.1	4.20		
	H–C(4)	71.2	4.08		
	H–C(5)	75.8	3.90		
	CH_2(6)	62.0	4.20, 4.23		
Glc IIIb	H–C(1)			97.3	5.08 (d, J = 7.7)
	H–C(2)			74.8	3.95
	H–C(3)			78.1	4.20
	H–C(4)			71.2	4.08
	H–C(5)			75.8	3.90
	CH_2(6)			62.0	4.20, 4.23

[a]) Overlapped ^{1}H-NMR signals are reported without designated multiplicity.

couplings and chemical shifts allowed the identification of four β-glucopyranosyl units (Glc I , Glc II, Glc IIIa, and Glc IIIb) although the FAB-MS molecular-ion peak corresponds to a cucurbitane triglucoside. The common D-configuration for Glc was determined by GC analysis of chiral derivatives of the sugars in the acid hydrolyzate [10]. The full assignments of all the ^{1}H- and ^{13}C-NMR signals by 2D-NMR experiments allowed the identification of a disaccharide moiety linked to the OH–C(3) of the aglycone. The cross-peaks in the HMBC spectrum of **3/4** between δ(H) 5.28 (d, J = 7.7 Hz, Glc II H–C(1)) and δ(C) 81.1 (Glc I C(2)) and between δ(H) 4.75 (d, J = 6.9 Hz, Glc I H–C(1)) and δ(C) 87.1 (Agly (C–(3)) showed that the disaccharide moiety 2-*O*-β-D-glucopyranosyl-(β-D-glucopyranosyl was bound at C(3) of the aglycone by a glycosidic linkage. This linkage was confirmed by the NOESY correlations between δ(H) 4.75 (d, J = 6.9 Hz, Glc I H–C(1)) and δ(H) 3.56 (Agly H–C(3)), and between δ(H) 5.28 (d, J = 7.7 Hz, Glc II H–C(1)) and δ(H) 4.22 (Glc I H–C(2)). Then the remaining sugars (Glc IIIa and Glc IIIb) of **3/4** must be bound to the genin by a glycosidic linkage at C(25). This was confirmed by the deshielded quaternary ^{13}C-NMR signals of the tertiary OH–C(25) function at δ(C) 80.8 (**3**) and 80.4 (**4**) and by the HMBC correlations between δ(H) 5.10 (d, J = 7.3 Hz, Glc IIIa H–C(1)) and δ(C) 80.8 (Agly C(25)) for **3**, and between δ(H) 5.08 (d, J = 7.7 Hz, Glc IIIb H–C(1)) and δ(C) 80.4 (Agly C(25)) for **4**. These data established that the glucosyl residues Glc IIIa and Glc IIIb were terminal at C(25) in the glycoside pair **3**/**4**.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*). Medium-pressure liquid chromatography (MPLC): silica gel *60* (*Merck,* 15–40 μm), *Gilson* pump *M 305*, *Büchi* column (460 × 25 mm and 460 × 15 mm), *Büchi* precolumn (110 × 15 mm). Anal. HPLC: *Gilson* pumps *M 305* and *306*;

autoinjector *Gilson 234*, *Gilson UV/VIS-151* detector; *Merck-Hitachi D-7500* integrator; column: *Lichrospher RP-18* (5 μm) 125-4 mm; eluent: gradient 30–50% MeCN/H_2O with 0.06% CF_3COOH; detection wavelength 210 nm. Semi-prep. HPLC: column: *Waters Prep Nova-Pak HR C18* (6 mm) 300–7.8 mm; eluent: linear gradient 40–50% MeOH/H_2O during 30 min; flow rate 3 ml/min; detection wavelength 210 nm. TLC and HPTLC: silica gel *60* F_{254} (*Merck*); solvent systems: for saponins, $CHCl_3$/MeOH/AcOH/H_2O 15:8:3:2 (*a*); for sapogenins, $CHCl_3$/MeOH 9:1 (*b*); for monosaccharides, $CHCl_3$/MeOH/H_2O 8:5:1 (*c*); spray reagents: for saponins, *Komarowsky* reagent, a 5:1 solution of 2% 4-hydroxybenzaldehyde in MeOH and 50% H_2SO_4 soln.; for sugars, diphenylamine/phosphoric acid reagent. IR Spectra (KBr): *Perkin-Elmer 281* IR spectrophotometer; in cm^{-1}. 1D- and 2D-NMR Spectra (^1H,^1H-COSY, TOCSY, NOESY, HSQC, and HMBC): at 20°; *Unity-600* spectrometer, *Varian Inova 600* instrument, equipped with a *SUN 4 L-X* computer system (600 MHz for ^1H, 150 MHz for ^{13}C); conventional pulse sequences for COSY, HSQC, HMBC, and TOCSY by using the standard MLEV17 spin-locking sequence and 90 ms mixing time; mixing time in NOESY experiment, 500 ms, ROESY NMR with a 200 ms mixing time; the C-multiplicity (Me, CH_2, CH) was determined by DEPT experiments; chemical shifts δ in ppm, J in Hz; (D_5)pyridine solns. (δ(C) 150.3, 155.9, 123.9). MS: *Q-TOF1-Micromass* (HR-ESI) and *Jeol-SX-102* spectrometer (FAB; neg. mode); in *m*/*z*.

Plant Material. The roots of *Cucurbita foetidissima* H.B.K. were collected in June 1988 and provided by Dr. *Cagiotti* (Perugia , Italy). A voucher specimen (N°5004) has been deposited in the herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, Dijon, France.

Extraction and Isolation. Dried powdered roots of *C. foetidissima* (1000 g) were defatted with hexane and extracted successively with $CHCl_3$ and MeOH. After evaporation, the MeOH extract (50 g) was obtained. This extract was suspended in H_2O (400 ml) and submitted to successive extractions with cyclohexane (3 × 200 ml) and BuOH (3 × 200 ml). After evaporation, 10 g of the BuOH extract was obtained. The BuOH extract was solubilized in MeOH (10 ml) and precipitated in Et_2O (3 × 250 ml) yielding 8.9 g of a crude saponin fraction. This mixture was submitted to CC (*Sephadex LH-20*, MeOH): 8.3 g of a white powder. The latter was fractionated by successive MPLC (silica gel *60*, $CHCl_3$/MeOH/H_2O 8:5:1; *Lichroprep RP-18* (40–63 μm); (50% MeOH/H_2O) and semi-prep. HPLC: **1** (12 mg) and **2** (10 mg); compounds **3**/**4** were purified by MPLC (silica gel, $CHCl_3$/MeOH/H_2O 65:35:10 lower phase) to give **3**/**4** (16 mg).

*(3β,16α)-28-{[*O*-β-*D*-Glucopyranosyl-(1 → 3)*-O*-β-*D*-xylopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl]oxy}-16-hydroxy-28-oxoolean-12-en-3-yl β-*D*-Glucopyranosiduronic Acid* (= *Foetidissimoside C;* **1**). White amorphous powder: TLC (*a*): R_f 0.30. $[\alpha]_D^{25} = -28.8$ ($c = 0.11$, MeOH). IR (KBr): 3500–3300, 2930, 1730, 1610, 1390, 1360. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1* and *2*. HR-ESI-MS (pos.): 1243.6056 ($[M + Na]^+$, $C_{59}H_{96}NaO_{26}^+$; calc. 1243.6088). FAB-MS (neg.): 1219 ($[M - H]^-$), 1057 ($[M - H - 162]^-$), 779 ($[M - H - 162 - 132 - 146]^-$), 647 ($[M - H - 162 - 132 - 146 - 132]^-$), 471 ($[M - H - 162 - 132 - 146 - 132 - 176]^-$).

*(3β,16α)-16-Hydroxy-28-oxo-28-{[*O*-β-*D*-xylopyranosyl-(1 → 3)*-O*-[β-*D*-xylopyranosyl-(1 → 4)]-O-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl}oxy}olean-12-en-3-yl β-*D*-Glucopyranosiduronic Acid* (= *Foetidissimoside D*; **2**). White amorphous powder: TLC (*a*): R_f 0.32. $[\alpha]_D^{25} = -20$ ($c = 0.11$, MeOH). IR (KBr): 3500–3300, 2932, 1734, 1616, 1450. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1* and *2*. HR-ESI-MS (pos.): 1213.5960 ($[M + Na]^+$, $C_{58}H_{94}NaO_{25}^+$; calc. 1213.5982). FAB-MS (neg.): 1189 ($[M - H]^-$), 1057 ($[M - H - 132]^-$), 779 ($[M - H - 132 - 132 - 146]^-$).

*(3β,9β,10α,11α,24*R*)- and (3β,9β,10α,11α,24*S*)-25-(β-*D*-Glucopyranosyloxy)-9-methyl-19-norlanost-5-en-3-yl 2-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranoside* (= *Foetidissimoside E* and *F*, resp.; **3** and **4**, resp.). White amorphous powder: TLC (*a*): R_f 0.6. IR (KBr): 3400, 1687, 1562, 1460, 1078. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 3–5*. HR-ESI-MS (pos.): 985.5360 ($[M + Na]^+$, $C_{48}H_{82}NaO_{19}^+$; calc. 985.5348). FAB-MS (neg.): 961 ($[M - H]^-$), 799 ($[M - H - 162]^-$), 637 ($[M - H - 162 - 162]^-$), 475 ($[M - H - 162 - 162 - 162]^-$).

Determination of Sugar Components. A soln. of glycoside (6 mg) in H_2O (1 ml) and 2N aq. CF_3COOH (5 ml) was refluxed on a water bath for 2 h. Then the mixture was diluted with H_2O (15 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined CH_2Cl_2 extracts were washed with H_2O and evaporated: echinocystic acid for **1** and **2** (co-TLC with an authentic sample) and an artifactual aglycone for **3** and **4**. After repeated evaporations of the aq. layer by adding MeOH to remove the acid, the crude sugar residue was analyzed by TLC (silica gel, *c*) in comparison with standard sugars; the absolute configuration of the sugars was determined according to a method previously described [10].

Alkaline Hydrolysis. The saponin (5 mg) was refluxed with 5% aq. KOH soln. (10 ml) for 2 h. The mixture was adjusted to pH 6 with a dil. HCl soln. and then extracted with H_2O-sat. BuOH (3 × 10 ml). The combined BuOH extracts were washed with H_2O and evaporated: prosapogenin.

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