A New Major Triterpene Saponin from the Roots of *Cucurbita foetidissima*

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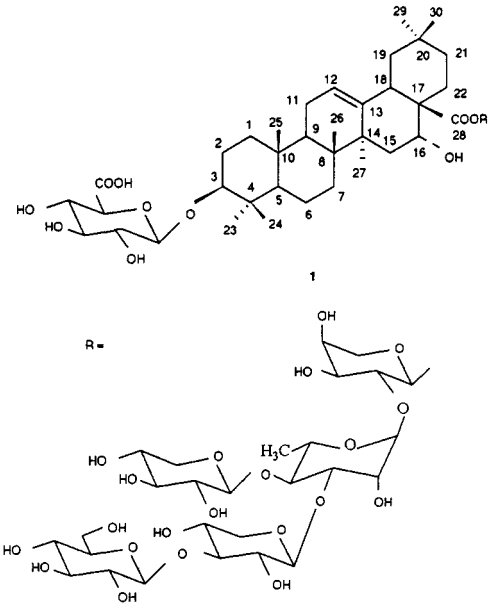
Foetidissimoside B (**1**), a novel triterpene saponin, was isolated from the roots of *Cucurbita foetidissima*. Based on spectroscopic data, especially direct and long-range heteronuclear 2D NMR analysis and on chemical transformations, the structure of **1** was elucidated as 3-*O*-*β*-D-glucuronopyranosyl-echinocystic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl(1→3)-[*β*-D-xylopyranosyl (1→4)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside. Compound **1** did not show any ability to potentiate in vitro cisplatin cytotoxicity in a human colon cancer cell line.

Cucurbita foetidissima H. B. K. (*C. perrenis*, A. Gray; *Cucumis perrenis* E. James) (Cucurbitaceae), commonly called "calabazilla" or "buffalo gourd", is a plant indigenous to Texas, Arizona, New Mexico, and California.[1] It is characterized by a huge root, weighing up to 50 kg, which contains up to 50% starch. The roots were used as a soap substitute and for medicinal purposes by the Indians and Spanish Californians.[1] A juice extract from boiled and pounded roots was used as a disinfectant for wounds and as a treatment for toothache.[1] A preliminary investigation of an alcoholic extract of the roots exhibited some pharmacological properties, such as oxytocic action, spasmolytic effect on the isolated intestine of rat and mouse, constriction of the coronary vessels of the rat heart, and irritating effects.[2] Our previous phytochemical studies on the MeOH extract of *C. foetidissima* roots led to the isolation of a new triterpene saponin, foetidissimoside A, and the identification of cucurbitacins by HPLC.[3] A detailed further investigation of the same extract containing eight saponins furnished a new additional major triterpene−saponin **1**. This paper deals with the isolation and structure elucidation of **1**. The influence of **1** on the potentiation of the cytotoxicity of cisplatin in human colon cancer cells was also investigated.

The concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *C. foetidissima* was purified by precipitation with diethyl ether and subjected to multiple chromatographic steps over Sephadex LH 20 and Si gel to yield foetidissimoside B (**1**). The structure of **1** was elucidated mainly by 500 MHz NMR analysis, including 1D and 2D NMR (^{1}H−^{1}H DQFCOSY, HMQC, HMBC) spectroscopy.

Foetidissimoside B (**1**) was obtained as a white, amorphous powder. The FABMS of **1** (negative-ion mode) displayed a quasimolecular ion peak $[M - H]^-$ at *m*/*z* 1351, indicating a molecular weight of 1352, compatible with the molecular formula $C_{63}H_{100}O_{31}$. Other fragment ion peaks at *m*/*z* 1219 $[(M - H) - 132]^-$, 1057 $[(M - H) - 132 - 162]^-$, 779 $[(M - H) - 132 - 162 - 132 - 146]^-$, 647 $[(M - H) - 132 - 162 - 132 - 146 - 132]^-$, and 471 $[(M - H) - 132 - 162 - 132 - 146 - 132 - 176]^-$ indicated the respective elimination of one terminal pentosyl, one hexosyl, one pentosyl−desoxyhexosyl moiety, one pentosyl, and one hexosyluronic acid moiety.



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Acid hydrolysis of **1** with 2N TFA yielded glucose, arabinose, xylose, and rhamnose in a molar ratio of 1:1:2:1 (estimated by GLC analysis); glucuronic acid (identified by co-TLC with an authentic sample); and an aglycon, which was identified as echinocystic acid on the basis of the DEPT, HMQC, and HMBC NMR spectra of **1**. Most of the signals were assigned through $^2J_{H-C}$ and $^3J_{H-C}$ couplings of the seven methyls and were in good agreement with literature data.[3]

The alkaline hydrolysis of **1** with 5% KOH yielded a prosapogenin, which furnished, by further acid hydrolysis, glucuronic acid and echinocystic acid (co-TLC with authentic samples). The presence of signals at δ 89.2 and 176.1 in the ^{13}C NMR spectrum agreed with the glycosylation at C-3 and at C-28 in accordance with published data.[3] Therefore, **1** has to be a 3,28-bidesmosidic saponin with

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glucuronic acid linked to the C-3 position of the aglycon by a glycosidic linkage, while the five remaining monosaccharides were bound to the C-28 of the aglycon through a glycosidic ester linkage.

Compound **1** was shown to contain six sugar residues from the HMQC spectrum. The anomeric protons at δ 6.50 (br s), 5.62 (br s), 5.42 (d, J = 7.6 Hz), 5.14 (d, J = 7.5 Hz), 5.04 (d, J = 7.4 Hz), and 4.88 (d, J = 7.5 Hz) give correlations with carbon signals at δ 93.4, 101.0, 104.5, 105.4, 105.9, and 106.9, respectively. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one α-arabinopyranose (Ara), one α-rhamnopyranose (Rha), one β-xylopyranose (Xyl), one β-glucopyranose (Glc), one β-xylopyranose (Xyl), and one β-glucuronopyranose (GlcA) unit, respectively. The common D-configuration for Glc, Xyl, and GlcA and the L-configuration for Ara, Rha were assumed, according to those most often encountered among the plant glycosides in each case.

After subtraction of the anomeric signals of the glucuronopyranosyl moiety linked at the C-3 position from the total NMR spectrum of **1**, the signals of five sugars linked to the aglycon by an ester linkage remained and will be assigned. The extensive 2D NMR spectra analysis of **1** showed that the Ara was substituted at position C-2, the Rha was substituted at position C-3 and C-4, and the three remaining sugars were a terminal Glc (T-Glc), a disubstituted 1,3-xylose (1,3-Xyl), and a terminal xylose (T-Xyl). A correlation in the HMQC spectrum at δ_C/δ_H 93.4/6.50 (br s) showed that the arabinose residue was attached to the carboxylic group of the aglycon by an ester linkage. This conclusion was confirmed by the HMBC experiment, which showed a correlation between signals at δ_H (Ara-1) 6.50 and δ_C (Agly−C-28) 176.1. Other HMBC correlations were observed between the following carbon and proton signals in the oligosaccharide ester moiety of **1**: δ_H (Rha-1) 5.62 (s) and δ_C (Ara-2) 75.5, δ_H (Xyl-1) 5.42 and δ_C (Rha-4) 78.1, δ_H (T-Glc) 5.14 and δ_C (1,3-Xyl-3) 88.3, δ_H (1,3-Xyl-1) 5.04 and δ_C (Rha-3) 82.4. These data proved that the 1,3,4-Rha was linked to the 1,2-Ara by a 1→2 linkage, and the 1,3-Xyl and the T-Xyl were bound to the 1,3,4-Rha by a 1→3 and 1→4 linkage, respectively, and the T-Glc was bound to the 1,3-Xyl by a 1→3 linkage. The linkages were confirmed by observation of reverse correlations between the following proton and carbon signals: δ_H (Rha-4) 4.50 and δ_C (T-Xyl-1) 104.5, δ_H (Rha-3) 4.56 and δ_C (1,3-Xyl-1) 105.9, δ_H (1,3-Xyl-3) 4.13 and δ_C (T-Glc-1) 105.4.

At this stage, we observed that compound **1** was different from foetidissimoside A[3] by the presence of two additional sugars (1,3-Xyl at the C-3 of Rha and the T-Glc at the C-3 of 1,3-Xyl). The comparison of the ^{13}C NMR chemical shifts of these two molecules fully supported this observation. With regard to the oligosaccharide ester carbon region, on going from foetidissimoside A to **1**, the signals for C-3 and C-4 of Rha were displayed downfield by +9.8 and upfield by −5.6 ppm, respectively, as a consequence of the glycosylation shift at C-3 of rhamnose.

Based on the above results, the structure of the saponin **1** was represented as 3-*O*-β-D-glucuronopyranosyl-echinocystic acid 28-*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl(1→3)-[β-D-xylopyranosyl (1→4)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside. Echinocystic acid glycosides have been already found in the Labiatae[4] and in the Compositae[5,6] but according to an updated literature search and to previous reviews on triterpene-saponins,[7,8] **1** is a new natural compound. Because cisplatin and digitonin (a steroid saponin) have been shown to interact synergistically to increase tumor cell (ovarian carcinoma 2008 cell line) lethality in vitro,[9] we have tested the effect of foetidissimoside B on the potentiation of cisplatin cytotoxicity in the human cancer colon HT-29 cell line.[10] However, no significant effect could be found in this bioassay.

Table 1. ^{13}C NMR Data of the Aglycon of Compound **1**[a]

position	DEPT	**1**
1	CH_2	38.9
2	CH_2	26.1
3	CH	89.2
4	C	39.6
5	CH	56.0
6	CH_2	18.5
7	CH_2	33.6
8	C	40.1
9	CH	47.2
10	C	37.1
11	CH_2	24.2
12	CH	123.0
13	C	144.8
14	C	42.2
15	CH_2	36.1
16	CH	74.2
17	C	49.5
18	CH	41.3
19	CH_2	47.2
20	C	30.8
21	CH_2	36.1
22	CH_2	32.2
23	Me	28.4
24	Me	17.2
25	Me	15.8
26	Me	17.2
27	Me	27.3
28	C	176.1
29	Me	33.4
30	Me	24.9

[a] ^{13}C chemical shifts are referenced to pyridine-d_5 at δ 123.9, 135.9, 150.3 ppm. Multiplicities were assigned from DEPT spectra.

Experimental Section

General Experimental Procedures. Optical rotations were taken with a Perkin−Elmer 241 polarimeter. IR spectra were measured with a Perkin−Elmer 881 spectrophotometer. UV spectra were recorded on a Kontron Uvicon 939 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. The chemical shifts (δ) were referenced to the solvent peaks (C_5D_5N). FABMS was conducted in the negative-ion mode (thioglycerol matrix) on a JEOL DX 300 instrument with a JMA-3500 system. TLC and HPTLC employed precoated Si gel plates $60F_{254}$ (Merck). The following TLC solvent systems were used: for saponins (a) $CHCl_3$−MeOH−AcOH−H_2O (15:8:3:2); for sapogenins (b) toluene−Me_2CO (4:1); for monosaccharides (c) $CHCl_3$−MeOH−H_2O (8:5:1). Spray reagents for the saponins were Komarowsky reagent, a mixture (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and H_2SO_4 (50%); and for the sugars, diphenylaminephosphoric acid reagent. Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Gilson pump, Büchi column (460 × 25 mm), Si gel 60 (15−40 μm, Merck)]. GLC analysis: Perkin−Elmer 900 B, glass column (200 × 0.3 cm) packed with 0V 225, carrier gas: Ar, 30 mL/min.

Plant Material. The roots of *C. foetidissima* were collected in June 1988, and provided by Dr. Cagiotti (Perugia, Italy). A voucher specimen (no. 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* (500 g) were defatted with *n*-hexane and extracted successively with $CHCl_3$ and MeOH. After removal of the solvent by evaporation, the MeOH extract (30 g) was obtained.

Table 2. ^{13}C and ^{1}H NMR Spectral Data of Sugar Moieties for Compound **1**[a]

position		δ_C	δ_H
3-O-Glc-A	1	106.9	4.88 (d, 7.5)
	2	75.5	4.03
	3	78.4	4.20
	4	73.8	4.36
	5	78.5	4.22
	6	170.0	
28-*O*-Ara	1	93.4	6.5 (br s)
	2	75.5	4.50
	3	69.9	nd[b]
	4	65.7	4.40
	5	62.6	4.46, nd[b]
Rha	1	101.0	5.62 (br s)
	2	71.7	4.74
	3	82.4	4.56
	4	78.1	4.50
	5	68.8	4.40
	6	18.7	1.68 (d, 6.0)
1,3-Xyl	1	105.9	5.04 (d, 7.4)
	2	74.4	3.94
	3	88.3	4.13
	4	69.1	4.55
	5	67.1	3.4, 4.02
Glc	1	105.4	5.14 (d,7.5)
	2	75.5	3.94
	3	78.3	4.02
	4	70.9	4.03
	5	78.4	3.82
	6	61.8	4.20,4.42
T-Xyl	1	104.5	5.42 (d, 7.6)
	2	75.5	3.95
	3	78.1	4.14
	4	71.7	4.10
	5	66.4	4.02, nd[b]

[a] Measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C with reference to δ150.5 in pyridine-d_5. Assignments were made on the basis of $^{1}H-^{1}H$ DQFCOSY, HMQC, HMBC, and DEPT experiments. [b] nd: not determined.

This extract was suspended in H_2O (400 mL) and submitted to successive extractions with cyclohexane (3 × 200 mL) and *n*-BuOH (3 × 200 mL). After evaporation under reduced pressure of the solvent, 10 g of the *n*-BuOH extract was obtained. The *n*-BuOH extract was solubilized in MeOH (10 mL) and precipitated in diethyl ether (3 × 250 mL), yielding 7 g of a crude saponin fraction. This mixture was submitted twice to column chromatography on Sephadex LH-20 eluted by MeOH, to afford 4 g of a white powder. A part of this mixture (600 mg) was separated by flash chromatography on normal-phase Si gel 40−63 μm eluted with a gradient ($CHCl_3$−MeOH−H_2O, 20:8:3→20:9:3) yielding eight fractions. Fraction 7 (22 mg) was further purified by MPLC on a Si gel column ($CHCl_3$−MeOH−H_2O 8:5:1) to give **1** (15 mg; 3 × 10^{-3} %).

Foetidissimoside B (**1**) was obtained as a colorless amorphous powder; $[\alpha]^{25}_D$ −31.8° (*c* 0.110, MeOH); UV (MeOH) λ_{max} 210 nm; IR (KBr) ν_{max} 3500−3300 (OH), 2930 (CH), 1730 (CO ester), 1610, 1450, 1390, 1360 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.81, 0.95, 0.99, 1.05, 1.13, 1.24, 1.79 (each 3H, s, Me at C-25, C-24, C-29, C-26, C-30, C-23, C-27); 5.55 (1H, m, H-12 of the aglycon); ^{13}C NMR (C_5D_5N, 125 MHz), see Tables 1 and 2; long-range correlations in the HMBC spectrum used for defining the aglycon of **1**: δ 0.81 (Me-25) → C-10 (37.1), C-1 (38.9), C-9 (47.2), C-5 (56.0); δ 0.95 (Me-24) → C-23 (28.4), C-4 (39.6), C-5 (56.0), C-3 (89.2); δ 0.99 (Me-29) → C-30 (24.9), C-20 (30.8), C-21 (36.1), C-19 (47.2); δ 1.05 (Me-26) → C-7 (33.6), C-8 (40.1), C-14 (42.2), C-9 (47.2); δ 1.13 (Me-30) → C-20 (30.8), C-29 (33.4), C-21 (36.1), C-19 (47.2); δ 1.24 (Me-23) → C-4 (39.6), C-5 (56.0), C-3 (89.2), δ 1.79 (Me-27) → C-15 (36.1), C-8 (40.1), C-14 (42.2); negative FABMS *m/z* 1351 $[M-H]^-$, 1219 $[(M-H)-132]^-$, 1057 $[(M-H)-132-162]^-$, 779 $[(M-H)-132-162-132-146]^-$, 647 $[(M-H)-132-162-132-146-132]^-$ and 471 $[(M-H)-132-162-132-146-132-176]^-$. TLC R_f 0.2 (system a); gray-violet spots by spraying with Komarowsky reagent.

Acid Hydrolysis of 1. A solution of **1** (3 mg) in 2N aqueous CF_3COOH (5 mL) was refluxed on a water bath for 3 h. After extraction with $CHCl_3$, the aqueous layer was neutralized by repeated evaporation to dryness with MeOH, and then analyzed on Si gel TLC by comparison with standard sugars (solvent system c). A 2-mg quantity of saponin **1** was refluxed in 2N CF_3COOH (2 mL) in a sealed serum vial at 100 °C for 3 h. Sugars in the hydrolysate were converted into the alditol acetates and then subjected to GLC analysis according to the method previously described.[3]

Alkaline Hydrolysis of 1. Compound **1** (7 mg) was refluxed with 5% aqueous KOH (10 mL) for 2.5 h. The reaction mixture was adjusted to pH 6 with dilute HCl, and then extracted with H_2O-saturated *n*-BuOH (3 × 10 mL). The combined *n*-BuOH extracts were washed (H_2O). Evaporation of the *n*-BuOH gave a prosapogenin (4 mg). The acidic hydrolysis of the prosapogenin in 2N aqueous CF_3COOH for 2 h at 120° furnished echinocystic and glucuronic acid (co-TLC with authentic samples).

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