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# Pregnane steroidal glycosides and their cytostatic activities

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**Four new steroidal glycosides such as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside-12-β-tigloyl-14-β-hydroxy-17-β-pregnane (1), 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside-12-β-(2'-amino)-benzoyl-14-β-hydroxy-17-β-pregnane (2), 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside-12-β-14-β-dihydroxy-17-α-pregnane (3) and 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside-12-β-14-β-dihydroxy-17-β-pregnane (4) were isolated from the aerial parts of *Ceropegia fusca* Bolle (Asclepiadaceae), a crassulacean acid metabolism plant, an endemic species to the Canary Islands that has been used in traditional medicine as a cicatrizant, vulnerary and disinfectant. The dichloromethane extract exhibited significant cytostatic activity against HL-60, A-431 and SK-MEL-1 cells, human leukemic, epidermoid carcinoma and melanoma cells, respectively. As shown in Table I, compounds 1 and 2 showed very similar $IC_{50}$ values. The acetylation of 1 to give the diacetate 5 increases 5-fold the cytotoxicity against HL-60 cells. Compounds 3 and 4 did not show cytotoxicity at the assayed concentrations. With respect to the compounds containing only the steroid ring (6–8), the presence of a charged *O*-amino-benzoyl but not a tigloyl group improved the cytotoxicity.**

*Keywords:* apoptosis / cytostatic compounds / glycosides / steroids

## Introduction

In the course of a drug-screening project on medicinal plants for biologically active compounds (Darias et al. 1996; Herrera et al. 1996), we now report the isolation, structure determination and cytostatic activities of tetra-deoxypyranosides (**1**) (4.84%), (**2**) (2.3%), (**3**) (0.9%), (**4**) (0.7%) and their derivatives (**5**), 12-tigloyl-14-hydroxy-17 pregnane (**6**), 12-(2′-amino)-benzoyl-14-hydroxy-17 pregnane (**7**) and ramanone (**8**) in an attempt to establish a structure–activity relationship. The percentages were determined from pure samples (see the "Extraction and isolation" section) drying the compounds in vacuo, at 40°C until achieving a constant weight. This process takes ∼72 h. To assess whether these compounds display cytostatic activities, we also studied their effects on the viability of the human promyelocytic cell line HL-60 and human melanoma SK-MEL-1 cells. The semisynthetic derivative **5**, which we named fuscastatin, showed cytotoxicity against the human myeloid leukemia cell line HL-60, which was caused by the induction of apoptosis as determined by flow cytometry. On the basis of bioassay-directed fractionation using the growth inhibition of tumor cells, we described the extraction and purification of novel pregnane steroidal glycosides from the aerial parts of the endemic plant *Ceropegia fusca*. Similar $IC_{50}$ values for compounds **1** and **5** were obtained on human melanoma SK-MEL-1 cells (31.3 ± 0.5 and 26.2 ± 0.7 μM, respectively).

## Results

The extract showed a positive Liebermann–Burchard reaction, indicating the presence of a steroid skeleton. The structure of the intact glycoside can be established unambiguously by NMR spectroscopic methods (Supplementary data, Tables SI–SIV; Agrawal 1992; Coxon 2009). The chemical shift values for C-2 of the sugar moieties show that the two β-linked cymaroses have the D-configuration ($\delta_C$ 35.4) like oleandrose ($\delta_C$ 35.4). The anomeric carbon of 6-deoxy-3-*O*-methyl-β-D-allopyranoside resonates at $\delta_C$ 104.0 when this sugar is linked to the 4-OH of β-D-cymarose, in contrast resonates at 101.8 in $C_5D_5N$, 101.3 in $Me_2CO$-$d_6$ and 99.1 in $CDCl_3$ (Supplementary data, Table SIV), respectively, when it is linked to the 4-OH of D-oleandrose (Vlegaar et al. 1993; Hayashi et al. 1988). High-resolution $^1$H-NMR spectra of 21-alkylpregnanes very often display well-resolved signals that can be used as starting points in several selective NMR experiments to study scalar (J coupling), and dipolar (NOE) interactions. Selective excitation was carried out using a

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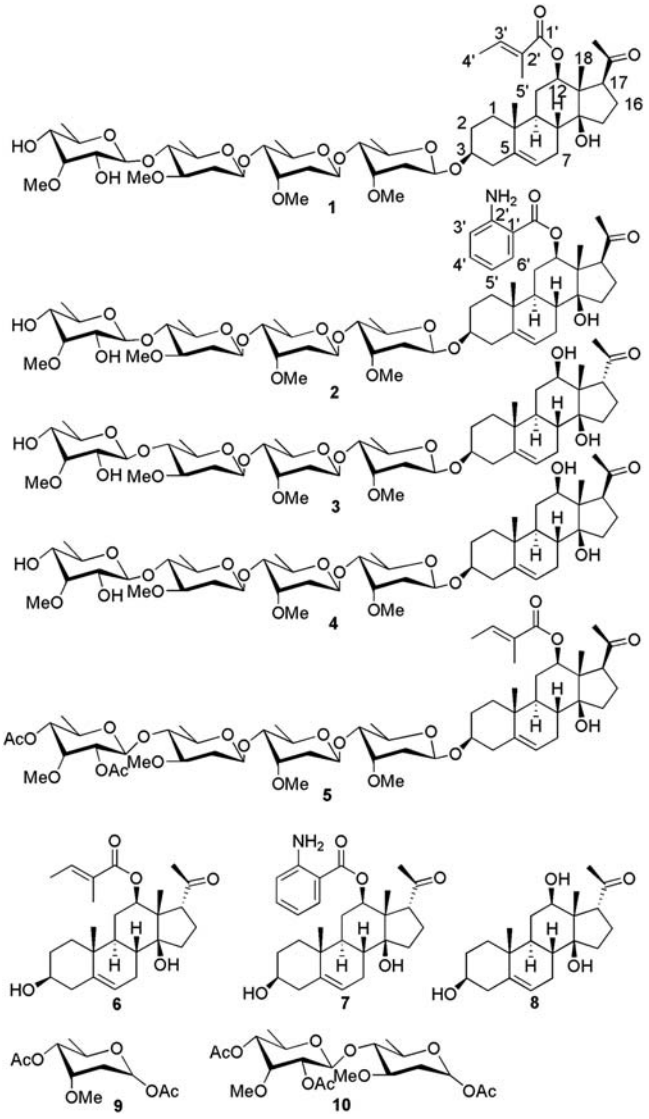

Fig. 1. Natural 21-alkylpregnanes and their derivatives.

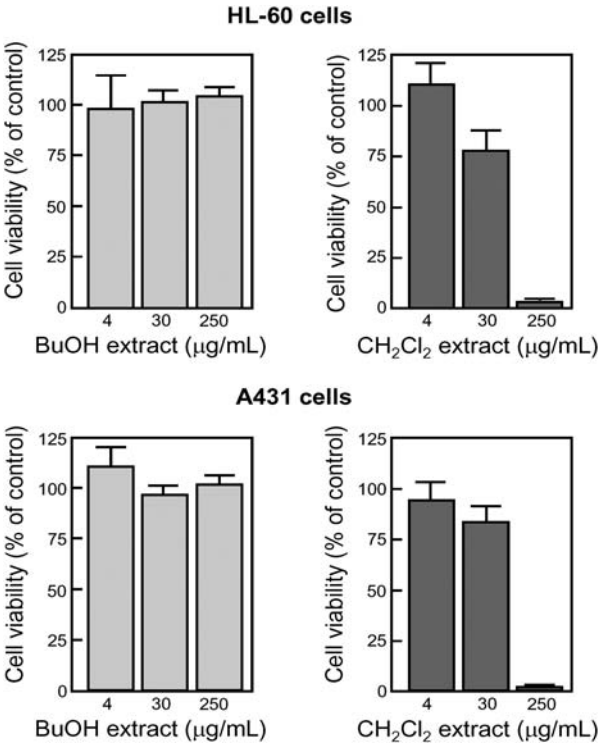


Fig. 2. Effect(s) of extracts on human cell lines viability. HL-60 and A-431 cells were cultured in the presence of the indicated concentrations of extracts for 72 h. Control cells were cultured under the same conditions and received vehicle (DMSO) in the same proportion used in the other groups. Cell viability was determined following the MTT procedure. The results shown are the mean ± SE of triplicate determinations from a single representative experiment and are expressed as the percentage of viability with respect to the control (100%).

double pulsed-field gradient spin-echo sequence in which 180° Gaussian pulses are sandwiched between sine shaped z-gradients (Figure 1). The $CH_2Cl_2$ extract showed cytostatic activity against human leukemia HL-60 cells and human epidermoid tumor A-431 cells, while the butanol extract did not exhibit cytotoxicity (Figure 2).

The tigloyl pregnane glycoside (**1**) was found to have the molecular formula $C_{54}H_{86}O_{18}$ on the basis of its elementary analysis, high-resolution ESIMS and $^{13}C$ DEPT NMR spectroscopy. The acetylation of **1** gave the diacetate **5**. After the acetylation the H-2 of 3-*O*-methyl-6-deoxy-β-D-allopyranosyl moved downfield to 4.67 in the $^1H$-NMR spectrum (dd, $J$ = 8.4, 2.8 Hz, 1H) and the H-4 moved to 4.05 ppm (dd, $J$ = 9.9, 2.7 Hz, 1H). The $^{13}C$-NMR spectrum shows signals at $\delta$ 20.9 (X2), 169.6 and 169.8. On the basis of the above results, the hydroxyl groups at C-2 and C-4 of **1** were acetylated.

The (2′-amino)-benzoyl pregnane glycoside (**2**) was shown to have the molecular formula $C_{56}H_{85}NO_{18}$ on the basis of its elementary analysis, high-resolution ESI, FABMS and $^{13}C$ DEPT NMR.

Acid hydrolysis of **1** and **2** gave the semisynthetic aglycones **6** and **7**, respectively. The hydrolyzation shifts of the aglycone carbon of **1** and **2** were observed at C-2 (+2.0 ppm), C-3 (−6.0) and C-4 (+3.4, +3.3), respectively. The stereochemistry of the 14β-hydroxyl group was further corroborated by synthesis (Mitsuhashi and Nomura 1965). The alkaline hydrolysis and subsequent acid hydrolysis of these two saponins gave a known aglycone, ramanone (**8**) (see Experimental Section in Supplementary data). As shown in Supplementary data, Table SIII, this aglycone had four oxygenated carbons signal at $\delta$ 71.5 (C-3), 68.2 (C-12), 86.9 (C-14) and 214.5 (C-20) in a C/D *cis*-pregnane system. We can find a remarkable difference in the carbon chemical shifts, especially in those assignable to C-13 and C-14, at $\delta$ 53.6–55.2 and 85.6–87.5 in a C/D *cis* junction, while a chemical shift for the same carbons has been reported at $\delta$ 41.6–42.7 and 58.7–59.2 for pregnanes with C/D *trans* junction, respectively (Kimura et al. 1982). The **6**, **7** and **8** aglycones, 17α-epimers, were more stable and were obtained as the main products (Mitsuhashi et al. 1964), whereas the ROESY experiment for 17β compounds showed an H-12α and an H-17α correlation. The 17α configuration was also confirmed by the observation that the carbonyl carbon of α-linked methyl ketone at C-17

appears at $\delta$ 209.2, 209.3 and 214.5 ppm for the aglycones **6**, **7** and **8**, respectively, when compared with $\delta$ 217 ppm for the β configuration (Supplementary data, Table SIII; Warashina and Noro 2000; Pawar et al. 2007).

Studies of aqueous solutions of carbohydrates are complicated because they are present in solution in several forms derived from free sugar mutarotation: a specific optical rotation of aqueous sugar solution changes with time (Junquera et al. 2002). Acid hydrolysis was conducted in 1 M HCl solution (1:1 $H_2O$–MeOH). Acid hydrolysis of the compounds **1** and **2** afforded as main products after the acetylation cymaropyranose diacetate (**9**) and the disaccharide pachibiose triacetate (**10**). The secondary methyl signal in the 500-MHz $^1$H-NMR spectrum of **9** at $\delta$ 1.19 (3H, d, $J = 6.3$) suggest the presence of one 6-deoxy sugar in the molecule. The 2,6-deoxypyranose moiety is a 3-methyl ether, indicated by one singlet signal at $\delta$ 3.49 (3H, s). The disaccharide **10** was suggested to have the molecular formula $C_{20}H_{32}O_{11}$ based on high-resolution FABMS. The $^1$H-NMR spectrum of **10** shows two secondary methyl signals at $\delta$ 1.21 (3H, d, $J = 6.2$) and 1.28 (3H, d, $J = 6.5$), and two 3-methyl ethers at $\delta$ 3.48 (3H, s) and 3.56 (3H, s), respectively (see Experimental Section in Supplementary data). Cymarose and pachibiose were also obtained by Yoshimura et al. (1985) from the acid hydrolysis of dregeoside $D_{p1}$.

The pregnane glycosides **3** and **4** were assigned the molecular formula $C_{49}H_{80}O_{17}$ on the basis of their elementary analysis, high-resolution ESIMS and $^{13}$C DEPT NMR.

We report here on the in vitro activity of natural and synthetic derivatives obtained from *C. fusca* against human HL-60 leukemia cells. Bioassay-directed fractionation using cytotoxicity with human tumor cell lines yielded an active mixture from the aerial parts of the endemic plant *C. fusca*. Fractionation of the $CH_2Cl_2$ extract yielded compounds **1**–**4** and their cytostatic activities against HL-60 cells were assayed. Moreover, semisynthetic derivatives from the above compounds **5**–**8** were also tested. Also, the effect of compound **5** on HL-60 cells viability was studied. Cells were cultured in the presence of the indicated doses of compound **5** for 72 h, and thereafter cell viability was determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide assay as described in Experimental Section in Supplementary data. Antiproliferative studies on **5** indicate that this compound displays cytotoxic properties in human myeloid HL-60 leukemia cells with an $IC_{50}$ value of 10 ± 1.5 µM (Figure 3A). Analysis by phase contrast microscopy at 24 h revealed the evidence of morphological changes and cells appeared to be unhealthy (Figure 3B).

In order to determine whether the decrease in viability observed after treatment with compound **5** occurred by apoptosis (Table I, Figure 3), cells were stained with propidium iodide and subjected to flow cytometry. An increasing number of hypodiploid cells (i.e. apoptosis) were identified in response to compound **5** (Figure 4A). These data suggest that the cytostatic activity of compound **5** in HL-60 cells occurred via apoptosis. The percentage of apoptotic cells increased in a dose- and a time-dependent manner (Figure 4B).

To further explore the molecular mechanisms underlying cell growth inhibition, we conducted a cell cycle analysis

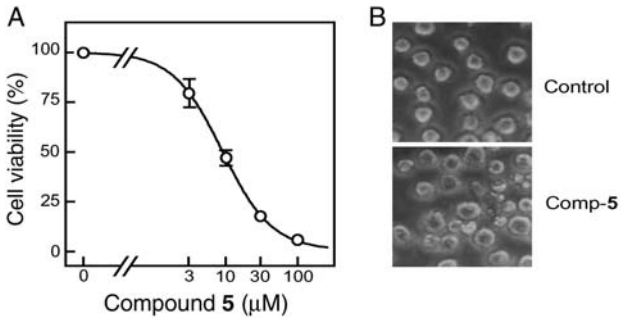


**Fig. 3.** Effect(s) of compound **5** on HL-60 cell viability and morphology. (**A**) Cells were cultured in the presence of the indicated doses of compound **5** for 72 h, and thereafter cell viability was determined by the MTT assay as described in Experimental Section in Supplementary data. The results of a representative experiment are shown. Each point represents the mean ± SE of triplicate determinations. (**B**) Cells were incubated in the absence (control) or the presence of 30 µM of compound **5** for 24 h, and morphology was visualized by phase contrast microscopy. Control cells were cultured under the same conditions and received vehicle (DMSO) in the same proportion used in the other groups.

**Table I.** Effects of the isolated compounds on the growth of HL-60 cell line[a]

| Compound | $IC_{50}$ (µM) |
|---|---|
| **1** | 49 ± 0.6 |
| **2** | 63 ± 0.2 |
| **3** | ND |
| **4** | ND |
| **5** | 10 ± 1.5 |
| **6** | ND |
| **7** | 44 ± 0.6 |
| **8** | ND |

[a]The data shown represent the mean ± SEM of two independent experiments with three determinations in each. The $IC_{50}$ values were calculated from experiments such as those shown in Figure 2A using the methodology described in the Experimental Section in Supplementary data. ND, growth inhibition not detected at the highest dose tested.

which revealed that compound **5** caused G1 arrest in cell cycle progression in a dose- and a time-dependent manner. Concomitant with this increase, a decrease was observed in the percentage of cells in the synthesis (S) and G2/M phases of the cell cycle (Table II).

## Discussion

The results indicate that the natural compounds **1** and **2** showed very similar $IC_{50}$ values (49 and 63 µM, respectively), indicating that the tigloyl group or the *O*-amino-benzoyl group at C-12 of the steroid ring does not play a significant role in cytostatic activity. The aglycon itself is not active. Usually, the glycan moiety has been viewed as a molecular structure that controls the pharmacokinetics of a drug. The introduction of two acetyl groups in the oligosaccharide moiety of compound **1** to give compound **5** increases 5-fold the cytotoxicity against HL-60 cells ($IC_{50}$ 49 µM vs. $IC_{50}$ 10 µM; Table I, Figure 3A). One possibility is that the introduction of the acetyl groups facilitates entry into the cell and

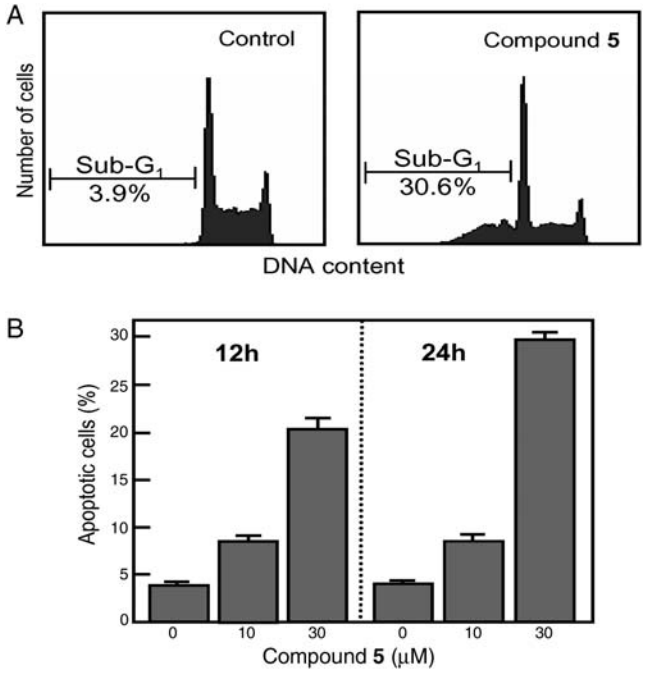


**Fig. 4.** Apoptosis induction by compound **5** on HL-60 cells. (**A**) Cells were cultured in the absence (control) or the presence of 30 μM of compound **5** for 24 h and the measurement of a cellular DNA content was determined by flow cytometry. Percentage of apoptotic cells displaying a sub-G1 DNA content is given. Representative histograms are shown. (**B**) Cells were cultured in the presence of the indicated concentrations of compound **5**, harvested at 12 and 24 h, and the percentage of apoptotic cells were determined by flow cytometry. The results of a representative experiment are shown, and each point represents the mean ± SE of duplicate determinations.

**Table II.** Effects of compound **5** on cell cycle distribution of HL-60 cells[a]

| | %G1 | %S | %G2-M |
|---|---|---|---|
| Control | 48.7 ± 0.7 | 28.3 ± 0.4 | 23.1 ± 0.3 |
| 10 μM comp-**5** | 56.5 ± 1.1 | 22.5 ± 1.4 | 21.0 ± 0.6 |
| 30 μM comp-**5** | 58.4 ± 1.8 | 21.7 ± 0.3 | 19.8 ± 0.7 |

[a]The cells were cultured with the indicated concentrations of compound **5** for 24 h and the cell cycle distribution was determined by flow cytometry. The results of a representative experiment are expressed as the mean ± SE of duplicate determinations.

improves binding to specific targets. When steroidal glycosides are used topically as antineoplasic agents, they enter cells via endogenous lectins that have specific receptors for the sugar moiety of the glycoside (Cham 2007).

Anthracyclines are among the most potent antineoplasic agents, but their use in cancer treatment is limited by their severe chronic cardiotoxicity. It has been reported that anthracyclines which contain several sugars have, in general, fewer side effects than other antitumor antibiotics (Jung et al. 1982; Kremer and Caron 2004; Minotti et al. 2004; Menna et al. 2007). Aclacinomycins A and B are anthracyclines with potent antitumor activity. It has been suggested that aclacinomycins may have different modes of biological activity compared with daunomycin and doxorubicin. The more extensive sugar of aclacinomycins projects further into the solvent region of DNA than the charged monosaccharide daunosamine in daunorubicin and doxorubicin (Yang and Wang 1994). However, the length of the sugar chain did not correlate with in vivo antitumor activity (Matsuzawa et al. 1981).

As shown in Table I, the presence of an ester group on the hydroxyl group of C-12 in the steroid ring of compounds **1** and **2** seems to be important in cytostatic induction since compound **4** did not show cytotoxicity at the assayed concentrations (up to 100 μM). The oligosaccharide moiety is not involved in the cytotoxicity-inducing ability since compounds **2** and **7** display a similar cytotoxic potency. With respect to the compounds containing only the steroid ring (compounds **6**–**8**), 17α-epimers, the presence of a charged *O*-aminobenzoyl (**7**) but not a tigloyl (**6**) group improved the cytotoxicity.

Although the isomerization of C/D *cis* 17β-20-ketopregnane to 17α- by alkaline hydrolysis is well known (Danieli et al. 1962; Hasegawa et al. 1963) to our knowledge, this is the first time that the epimerization of C/D *cis* 17β-20-ketopregnane to the more stable 17α configuration by acid treatment has been reported.

The precise function of the carbohydrate (Křen 2008) and ester moieties in most glycosylated metabolites has not been elucidated.

## Material and methods

### *General experimental procedures*

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Optical rotations were recorded in a Perkin-Elmer model 343 polarimeter. IR spectra were recorded using a Bruker model IFS-55 spectrophotometer and a Bruker model IFS-66/S spectrophotometer for ATR. $^{1}$H-NMR and $^{13}$C-NMR spectra were obtained on a Bruker model AMX-500 spectrometer with the standard pulse sequences operating at 500 MHz in $^{1}$H-NMR and 125 MHz in $^{13}$C-NMR. $CDCl_3$ and CO $(CD_3)_2$ were used as solvents. Chemical shifts were expressed in $\delta$ (ppm) with TMS as an internal standard. FAB and EIMS were taken on a Micromass model Autospec (70 eV) spectrometer. Column chromatography (CC) was carried out on silica gel (70–230 mesh, Merck, Germany) and 0.05 silica gel (Aldrich Chemical Company). Fractions obtained from CC were monitored by TLC (silica gel 60 $F_{254}$).

### *Plant material*

*Ceropegia fusca* Bolle aerial parts were collected in August 2007 at Arico, Tenerife (Canary Islands), and were identified by Dr MC Alfayate (University of La Laguna). Voucher specimens are deposited at the Herbarium of that institution.

### *Extraction and isolation*

The plant aerial parts were macerated in ethanol (8 L) for 30 days at room temperature and then subjected to reverse extraction with dichloromethane (3 × 4 L) and *n*-butanol in a Griffin Flask Shaker (3 × 500 mL). Removal of the solvent from the dichloromethane extraction gave a residue (26.97 g) which was subjected to CC on silica gel using *n*-hexane-ETOAc of

increasing polarity and then with increasing percentages of MeOH. The saponins fractions were re-chromatographed on silica gel with 7:3 *n*-hexane/acetone.

*Cell culture*

Human HL-60 myeloid leukemia cells were grown in RPMI 1640 (Sigma) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Sigma) and 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a humidified atmosphere containing 5% $CO_2$. The cultures were passed twice weekly exhibiting characteristic doubling times of ∼24 h. The cell numbers were counted by a hemocytometer, and the viability was always greater than 95% in all experiments as assayed by the 0.025% trypan blue exclusion method. Stock solutions of 100 mM compounds were made in dimethyl sulfoxide (DMSO), and aliquots were frozen at −20°C. Further dilutions were made in culture media immediately prior to use. In all experiments, the final concentration of DMSO did not exceed 0.5% (v/v), a concentration which is nontoxic to the cells. To ensure exponential growth, cells were resuspended in fresh medium 24 h before each treatment. The A-431 cell line was grown as monolayers in plastic tissue flasks containing Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL of penicillin and 100 µg/mL of streptomycin. Human SK-MEL-1 melanoma cells (DSMZ N° ACC 303, DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were grown in RPMI 1640 (Sigma) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Sigma) and 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a humidified atmosphere containing 5% $CO_2$.

*Flow cytometry analysis*

In order to study the cellular DNA changes, histogram measurements of hypodiploid DNA formation were performed by flow cytometry using a Coulter EPICS™ cytometer (Beckman Coulter). Histograms were analyzed with the Expo 32 ADC Software™ (Beckman Coulter). Briefly, cells ($1 \times 10^6$) were centrifuged for 10 min at $500 \times g$, washed with 1 mL of phosphate buffered saline (PBS) and resuspended in 50 µL of PBS. Following drop-wise addition of 1 mL of ice-cold 75% ethanol, fixed cells were stored at −20°C for 1 h. Samples were then centrifuged at $500 \times g$ for 10 min at 4°C and washed with PBS before resuspension in 1 mL of PBS containing 50 µg/mL of propidium iodide and 100 µg/mL of RNase A and incubation for 1 h at 37°C in the dark. The percentage of cells with decreased DNA staining, composed of apoptotic cells resulting from either fragmentation or decreased chromatin, of a minimum of 10,000 cells per experimental condition was counted. Cell debris was excluded from analysis by selective gating based on anterior and right-angle scattering.

## Supplementary data

Supplementary data for this article is available online at http://glycob.oxfordjournals.org/.

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## Abbreviation

CC, column chromatography; DMSO, dimethyl sulfoxide; PBS, phosphate buffered saline.

## References

Agrawal PK. 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry*. 31:3307–3330.

Cham BE. 2007. Solasodine rhamnosyl glycosides specifically bind cancer cell receptors and induce apoptosis and necrosis. Treatment for skin cancer and hope for internal cancers. *J Biol Sci*. 2:503–514.

Coxon B. 2009. Development in the Karplus Equation as they relate to the NMR coupling constants of carbohydrates. In: Hoston B, editor. *Advances in Carbohydrate Chemistry and Biochemistry*. Washington: Academic Press. p. 17–82.

Danieli N, Mazur Y, Sondheimer F. 1962. The synthesis of digitoxigenin. *J Am Chem Soc*. 84:875–876.

Darias V, Martín D, Abda S, Vivas JM. 1996. Preliminary study of the cicatrizant, haemostatic and chemotherapic activities of *Ceropegia fusca* Bolle. *Phytother Res*. 10:S1–S2.

Hasegawa H, Sato Y, Tsuda K. 1963. Untersuchungen über steroide. XLVI. Über die vier isomere des zum progesterone gehörigen 14,15-diols. *Chem Pharm Bull*. 11:1275–1282.

Hayashi K, Iida I, Nakao N, Nakao Y, Kaneko K. 1988. Four pregnane glycosides, boucerosides AI, AII, BI and BII, from *Boucerosia aucheriana*. *Phytochemistry*. 27:3919–3914.

Herrera RM, Pérez M, Martín-Herrrera DA, López-García R, Rabanal RM, Arias A. 1996. Antimicrobial activity of extracts from plants endemic to the Canary Islands. *Phytother Res*. 10:364–366.

Jung ME, Node M, Pfluger RW, Lyster MA, Lowe JA. 1982. Regiospecific synthesis of bicyclic 6-alkoxy-2-pyrones and their use in the production of tetracyclic intermediates for 11-deoxyanthracycline synthesis. *J Org Chem*. 47:1150–1152.

Junquera E, Olmos D, Aicart E. 2002. Carbohydrate-water interactions of p-nitrophenylglycosides in aqueous solution. Ultrasonic and densitometric studies. *Phys Chem Chem Phys*. 4:352–357.

Kimura M, Hayashi K, Narita H, Mitsuashi H. 1982. Studies on the constituents of Asclepiadaceae plants. LI. Oxidation at the 18-methyl group of C/D-cis-pregnane type steroids and $^{13}$C-Nuclear Magnetic Resonance spectra of 18-oxygenated pregnanes and related compounds. *Chem Pharm Bull*. 30:3932–3941.

Kremer LCM, Caron HN. 2004. Anthracycline cardiotoxicity in children. *N Eng J Med*. 351:120–121.

Křen V. 2008. Glycoside vs. aglycon: The role of glycosidic residue in biological activity. In Fraser-Reid BO, Tatsuta K, Thiem J, editors. *Glycoscience*. Berlin: Springer. p. 2589–2644.

Matsuzawa Y, Oki T, Takeuchi T, Umezawa H. 1981. Structure-activity relationship of anthracyclines relative to cytotoxicity and effects on macromolecular synthesis in L1210 leukemia cells. *J Antibiot*. 34:1596–1607.

Menna P, Minotti G, Salvatorelli E. 2007. *In vitro* modeling of the structure-activity determinants of anthracycline cardiotoxicity. *Cell Biol Toxicol*. 23:49–62.

Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. 2004. Anthracyclines: Molecular advances and pharmacologic development in antitumor activity and cardiotoxicity. *Pharmacol Rev.* 56:185–229.

Mitsuhashi H, Nomura T, Fukuoka M. 1964. Studies on the constituents of Asclepiadaceae plants. XIII. Epimerization at C-17 and optical rotatory dispersion study of C/D cis pregnane-20-one derivatives. *Steroids*. 4:483–493.

Mitsuhashi H, Nomura T. 1965. Studies on the constituents of Asclepiadaceae plants. XVI. On the components of *Metaplexis japonica* Makino. III. The structure of benzoylramanone. *Chem Pharm Bull*. 13:1332–1340.

Pawar RS, Shukla YJ, Khan SI, Avula B, Khan IA. 2007. New oxypregnane glycosides from appetite suppressant herbal supplement *Hoodia gordonii*. *Steroids*. 72:524–534.

Vlegaar R, Van Heerden FR, Anderson LAP, Erasmus GL. 1993. Toxic constituents of the Asclepiadaceae. Structure elucidation of sarcovimiside A-C, pregnane glycosides of Sarcostemma viminale. *J Chem Soc Perkin Trans I*. 1993:483–487.

Warashina T, Noro T. 2000. Steroidal glycosides from the aerial part of *Asclepias incarnata*. *Phytochemistry.* 53:485–498.

Yang D, Wang AH-J. 1994. Structure by NMR of antitumor drugs aclacinomycin A and B complexed to d(CGTACG). *Biochemistry.* 33:6595–6604.

Yoshimura S-I, Narita H, Hayashi K, Mitsuhashi H. 1985. Studies on the constituents of Asclepiadaceae plants. LIX. The structures of five new glycosides from *Dregea volubilis* (L.) Benth. *Chem Pharm Bull*. 33:2287–2293.