# Steroidal Saponins from the Bark of *Dracaena draco* and Their Cytotoxic Activities

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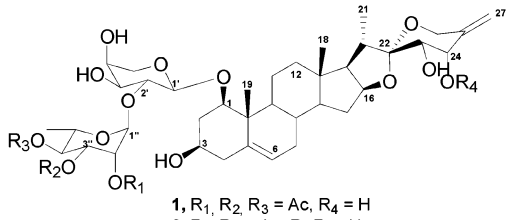
From the stem bark of *Dracaena draco*, three new compounds, namely, draconins A−C (**1**−**3**), were isolated, along with 17 known compounds. The structures of the new compounds isolated were elucidated on the basis of spectroscopic data interpretation. Several of the isolated compounds showed potent cytotoxic activities measured on the human leukemia cell line HL-60 ($IC_{50}$'s from 2.0 to 9.7 $\mu$M at 72 h). The mechanism by which compounds **1** and **2** display their cytostatic properties is through induction of cell death by apoptosis, as evaluated by fluorescence microscopy and DNA fragmentation.

The dragon tree group consists of arborescent taxa of the genus *Dracaena* (Dracaenacea), which are found in both West and East Africa. Two species are found in West Africa: *D. draco*, with subspecies *draco* in the Madeira, Canary, and Cape Verde archipelagoes, and subspecies *ajgal* in southwest Morocco. Dragon's blood is a substance exuded by the dragon trees either naturally or through cuts in trunks and branches, which has an astringent effect and has been used as an antidiarrhetic and as a hemostatic drug. In this paper, we report on the isolation and structure determination of three new steroidal saponins, draconin A (**1**), draconin B (**2**), and draconin C (**3**) along with 17 known compounds including non-triterpenoids and non-saponins. The known compounds were identified by analysis of their $^1$H and $^{13}$C NMR spectra and comparison with published data.

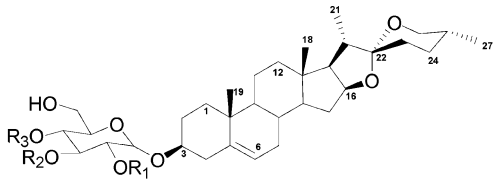
## Results and Discussion

The molecular formulas of the new steroidal saponins draconin A (**1**, $C_{44}H_{64}O_{17}$), draconin B (**2**, $C_{42}H_{62}O_{16}$), and draconin C (**3**, $C_{40}H_{60}O_{15}$) were derived from FABMS, $^{13}$C NMR, and DEPT data.

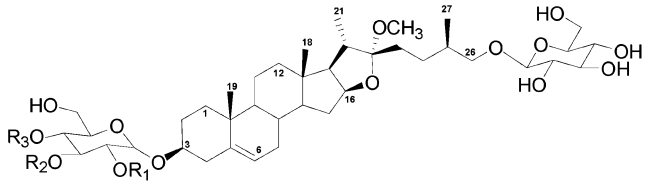
The $^1$H NMR spectrum of draconin A (**1**) showed signals for three steroid methyl groups at $\delta$ 0.85 (3H, s), 0.94 (3H, d, $J$ = 7.0 Hz), and 1.06 (3H, s), an exomethylene group at $\delta$ 5.05 (1H, s) and 5.11 (1H, br s), and an olefinic proton at $\delta$ 5.57 (1H, d, $J$ = 5.8 Hz), which were essentially analogous to those of **5**.[1] The presence of three acetyl groups in **1** was confirmed by IR (1750 cm$^{-1}$), $^1$H NMR at $\delta$ 1.99 (3H, s), 2.05 (3H, s), 2.15 (3H, s), and $^{13}$C NMR at $\delta$ 20.8 × 2 and 20.9 ($CH_3$) and 169.8, 170.1, and 170.7 (C=O). Also, the $^1$H NMR spectrum of **1** displayed signals for two anomeric protons at $\delta$ 4.26 (d, $J$ = 7.6 Hz) and 5.46 (d, $J$ = 1.9 Hz), which gave correlations in the HSQC spectrum with carbon signals at $\delta$ 100.21 (C-1′) and 96.21 (C-1″) (Table 1). A quaternary carbon signal at $\delta$ 112.42 (C-22) in the $^{13}$C NMR spectrum suggested that **1** is a steroid saponin with a spirostanol skeleton.[2] On comparison of the proton and carbon chemical shifts of **1** with those of **(11)**,[1] the structure of the A−E ring part of **1** was shown to be identical to that of **11**, including the orientations of the C-1 and C-3 oxygen atoms and the ring junctions, but significant differences were recognized in the signals from the F ring part by the absence of the C-24 glycosyloxy group. The $^1$H NMR shift and the multiplicities and correlations observed in the ROESY spectrum provided good support for the proposed structures, including the ring junction patterns of the steroid nucleus and the configurations of the oxygen atoms thereon. The $^1$H−$^1$H-COSY and HMBC experiments also allowed the sequential assignment of the resonances for two monosaccharides, starting from the easily distinguished anomeric protons. Multiplet patterns and mea-



**1,** $R_1$, $R_2$, $R_3$ = Ac, $R_4$ = H
**2,** $R_1$, $R_2$ = Ac; $R_3$,$R_4$= H
**3**, $R_1$= Ac; $R_2$, $R_3$, $R_4$ = H
**5,** $R_1$, $R_2$, $R_3$, $R_4$ = H
**7,** $R_1$, $R_2$, $R_4$ = H; $R_3$ = Ac
**11,** $R_1$, $R_2$, $R_3$ = Ac, $R_4$ = β-D-Fuc



**4,** $R_1$, $R_2$ = H; $R_3$ = α-L-Rha
**6,** $R_1$, $R_2$ = H; $R_3$= α-L-Rha
**9**, $R_1$= α-L-Rha; $R_2$ = β-D-Glu; $R_3$ = H



**8,** $R_1$ = α-L-Rha; $R_2$ = β-D-Glu; $R_3$ = H
**10,** $R_1$= α-L-Rha; $R_2$ = H; $R_3$ = α-L-Rha

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**Table 1.** $^{13}C$ NMR and $^{1}H$ NMR Data of Compounds **1**−**3** ($\delta$ ppm)[a,b]

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| no. | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ |
| 1 | 85.07 d | 3.37 dd (4, 11.7) | 84.44 d | 3.34 dd (3.8, 11.5) | 84.32 d | 3.78 m |
| 2 | 39.87 t | 1.84 $dd_{(ax)}$ | 37.24 t | 2.16$_{(ax)}$ | 38.27 t | 2.35$_{(ax)}$ |
| | | 2.19 $m_{(eq)}$ | | 2.62$_{(eq)}$ | | 2.65$_{(eq)}$ |
| 3 | 67.84 d | 3.50 | 68.03 d | 3.45 ddd (2.3, 2.9, 9.5) | 69.00 d | 3.80 m |
| 4 | 42.49 t | 2.23 m | 42.13 t | 2.21 br s | 43.75 t | 1.25 |
| | | 2.34 m | | | | 1.55 |
| 5 | 137.54 s | | 137.72 s | | 140.4 s | |
| 6 | 125.60 d | 5.57 d (5.8) | 125.37 d | 5.57 d (5.8) | 125.6 d | 5.49 d (5.5) |
| 7 | 31.49 t | 1.52 | 31.47 t | 1.52 | 32.87 t | 1.51 |
| | | 1.97 | | 1.97 | | |
| 8 | 32.53 d | 1.55 m | 32.51 d | 1.55 m | 33.85 d | 1.50 |
| 9 | 50.10 d | 1.24 | 49.97 d | 1.18 t (7.0) | 51.24 d | 1.49 |
| 10 | 42.14 s | | 42.13 s | | 44.71 s | |
| 11 | 23.39 t | 1.30 | 23.41 t | 1.30 | 24.89 t | 1.60 |
| 12 | 39.87 t | 1.17 m | 39.86 t | 1.17 m | 40.40 t | 1.26 m |
| | | 1.72 m | | 1.72 m | | 1.55 m |
| 13 | 40.52 s | | 40.53 s | | 41.43 s | |
| 14 | 56.69 d | 1.15 m | 56.70 d | 1.15 m | 57.71 d | 1.07 |
| 15 | 31.86 t | 1.52 | 31.85 t | 1.52 | 33.15 t | 1.35 |
| | | 1.96 | | 1.96 | | 1.90 |
| 16 | 83.32 d | 4.50 dd (7.2, 15.4) | 83.32 d | 4.49 dd (7.2, 15.3) | 84.10 d | 4.52 m |
| 17 | 60.81 d | 1.73 dd (6.7, 8.6) | 60.80 d | 1.73 br d (9.6) | 62.26 d | 1.69 m |
| 18 | 16.54 q | 0.85 s | 16.54 q | 0.85 s | 17.71 q | 0.98 s |
| 19 | 14.31 q | 1.06 s | 14.37 q | 1.06 s | 15.87 q | 1.36 s |
| 20 | 36.08 d | 2.65 m | 36.06 d | 2.65 m | 37.92 d | 2.66 m |
| 21 | 14.03 q | 0.94 d (7.0) | 14.05 q | 0.94 d (7.0) | 15.48 q | 1.06 d (7.0) |
| 22 | 112.42 s | | 112.40 s | | 113.2 s | |
| 23 | 68.42 d | 3.50 | 68.45 d | 3.51 d (3.1) | 70.51 d | 3.88 d (4.9) |
| 24 | 74.08 d | 4.26 m | 74.24 d | 4.24 m | 75.00 d | 4.16 m |
| 25 | 143.06 s | | 143.07 s | | 147.3 s | |
| 26 | 60.15 t | 3.85 $d_{(eq)}$ (12.9) | 60.13 t | 3.85 $d_{(eq)}$ (12.8) | 61.64 t | 3.95 s |
| | | 4.35 $d_{(ax)}$ (12.0) | | 4.34 $d_{(ax)}$ (12.9) | | 3.98 s |
| 27 | 114.21 t | 5.05 br s | 114.13 t | 5.02 s | 113.5 t | 5.04 d (1.4) |
| | | 5.11 br s | | 5.10 br s | | |
| 1′ | 100.21 d | 4.26 d (7.6) | 99.77 d | 4.24 d (7.3) | 101.0 d | 4.62 d (8.0) |
| 2′ | 73.64 d | 3.62 dd (7.7, 8.5) | 74.11 d | 3.61 d (7.7) | 75.79 d | 4.51 m |
| 3′ | 75.10 d | 3.68 dd (7.0, 3.1) | 74.87 d | 3.68 dd (8.8, 3.6) | 76.51 d | 4.07 |
| 4′ | 69.35 d | 3.80 br s | 70.13 d | 3.79 br s | 71.08 d | 4.06 |
| 5′ | 66.20 t | 3.94 dd (12.8, 2.2) | 66.03 t | 3.92 dd (12.8, 1.9) | 68.27 t | 3.58 dd (12.0, 0.5) |
| | | 4.32 m | | 4.32 m | | 4.20 m |
| 1″ | 96.21 d | 5.46 d (1.9) | 96.75 d | 5.36 d (1.9) | 99.5 d | 6.17 d (1.2) |
| 2″ | 69.91 d | 5.21 dd (3.2, 1.8) | 71.81 d | 5.19 d (3.3) | 75.10 d | 5.96 dd (1.5, 3.5) |
| 3″ | 68.78 d | 5.46 dd (10.1, 3.4) | 71.00 d | 5.21 s | 71.25 d | 4.73 dd (8.6, 3.5) |
| 4″ | 71.58 d | 5.05 dd (10.0, 9.8) | 69.27 d | 4.15 m | 75.00 d | 4.19 m |
| 5″ | 66.20 d | 4.30 m | 68.63 d | 4.15 m | 70.28 d | 4.81 m |
| 6″ | 17.35 q | 1.22 d (6.3) | 17.56 q | 1.33 d (6.1) | 19.71 q | 1.70 d (6.1) |
| Ac | 20.81 q | 1.99 s | 20.93 q | 2.11 s | 171.5 | 1.91 s |
| Ac | 20.81 q | 2.05 s | 21.04 q | 2.06 s | | |
| Ac | 20.97 q | 2.15 s | | | | |
| Ac | 169.89 s | | 170.24 s | | 171.5 s | |
| Ac | 170.13 s | | 171.59 s | | | |
| Ac | 170.76 s | | | | | |

[a] Multiplicities were assigned from DEPT spectra. [b] Spectra obtained at 125 MHz for $^{13}C$ and 500 MHz for $^{1}H$ NMR (**1** and **2** in $CDCl_3$ and **3** in $C_5D_5N$). The assignments were based on DEPT, HSQC, HMBC, COSY, and ROESY experiments. Overlapped proton signals are reported without designated multiplicity.

surements of coupling constants confirmed the presence of an α-L-arabinopyranosyl ($^4C_1$) unit and an α-L-rhamnopyranosyl ($^1C_4$) unit in **1** (Table 1). In the HMBC spectrum, the anomeric proton signals of arabinose ($\delta$ 4.26, H-1′) and rhamnose ($\delta$ 5.46, H-1″) showed correlations with C-1 of the aglycone ($\delta$ 85.07) and C-2 of arabinose ($\delta$ 73.64), respectively, leading to an *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopiranosyl structure attached to C-1 of the aglycone. The C-2, C-3, and C-4 hydroxyl groups of rhamnose were presumed acetylated since the proton signals due to H-2″, H-3″, and H-4″ resonated in unexpected lower fields at $\delta$ 5.21 (dd, $J$ = 3.2, 1.8 Hz), 5.46 (dd, $J$ = 10.1, 3.4 Hz), and 5.05 (dd, $J$ = 10.0, 9.8 Hz), respectively. This was confirmed by the observation of correlations from the H-2, H-3, and H-4 protons to the carbonyl carbons of the acetyl groups in the HMBC and ROESY experiments (Figure 1). The 23*S* and 24*S* configurations were revealed by the clear ROESY from H-20 to H-23 and H-23 to H-24 (Figure 1). The structure of **1** was then established as (23*S*,24*S*)-spirosta-5,25(27)-diene-1$\beta$,3$\beta$,23,24-tetrol-*O*-{*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranosyl, named draconin A.

Draconin B (**2**) ($C_{42}H_{62}O_{16}$) exhibited a positive ion FABMS at *m*/*z* 845 $[M + Na]^+$, which differed from that of **1** by 42 mass units. The presence of two acetyl groups in the molecule was shown by the IR (1700 $cm^{-1}$) and the $^{1}H$ and $^{13}C$ NMR spectra (Table 1). Acid hydrolysis of **2** yielded L-arabinose and L-rhamnose as the carbohydrate compounds, together with several unidentified artifactual sapogenols, while no genuine aglycone was obtained. The monosaccharides were identified by TLC with authentic samples of L-arabinose and L-rhamnose. Signals in the $^{1}H$

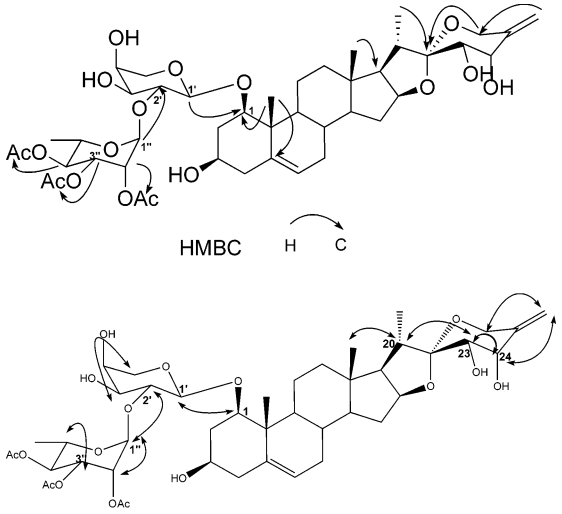


**Figure 1.** Representative HMBC and ROESY correlations of **1**.

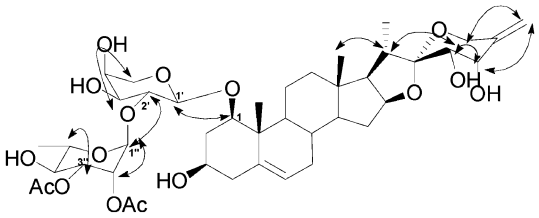

**Figure 2.** Representative ROESY correlations of **2**.

NMR spectrum of **2**, as in that of **1**, also indicated the presence of three steroid methyl groups, an exomethylene group, and an olefinic proton.

The acetyl signal at C-4 of rhamnose disappeared in **2**, but an oxymethine of the hydroxyl group at $\delta$ 4.15 appeared in its $^1$H NMR spectrum. In the $^{13}$C NMR spectrum (Table 1) the chemical shift values were similar to those of the corresponding carbon atoms of **1**, and the absence was noted of the acetyl group at C-4 of rhamnose. On the basis of the above data, **2** was identified as (23*S*,24*S*)-spirosta-5,25(27)-diene-1$\beta$,3$\beta$,23,24-tetrol 1-*O*-{*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranosyl}. The HMBC and ROESY (Figure 2) spectra also supported the structure assignments.

The $^1$H NMR spectrum of draconin C (**3**) ($C_{40}H_{60}O_{15}$) showed signals for three steroid methyls at $\delta$ 0.98 (3H, s), 1.06 (3H, d, $J$ = 7.0 Hz), and 1.36 (3H, s) and two anomeric protons at $\delta$ 4.62 and 6.17. The presence of an acetyl group in the molecule was confirmed by IR (1725 cm$^{-1}$), $^1$H NMR at $\delta$ 2.04 (3H, s), and $^{13}$C NMR at $\delta$ 170.8 (CO) and 2.10 (Me) spectra. Therefore, **3** was found to be a monoacetate, analogously to **2**. In the $^{13}$C NMR spectrum of **3**, the signal of the C-2 in the rhamnose unit was shifted to a lower field than C-2 in the rhamnose unit of **5**, by 2.7 ppm. Comparison of the $^1$H and $^{13}$C NMR signals of **3** with those of **5**[1] led us to conclude that saponin **3** was (23*S*,24*S*)-spirosta-5,25(27)-diene-1$\beta$,3$\beta$,23,24-tetrol 1-*O*-{*O*-(2-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranosyl}, named draconin C.

Compounds **1** and **2**, unlike **3**, were found to inhibit the proliferation of HL-60 cells in a dose-dependent manner, as evaluated by the MTT assay (Figure 3).

The results presented in Table 2 are a summary of several experiments in which the 50% inhibitory concentrations ($IC_{50}$) were measured for the new (**1**−**3**) and known (**4**−**10**) identified compounds. The results obtained show that compounds **1**, **6**, **8**, **9**, and **10** have a similar range of

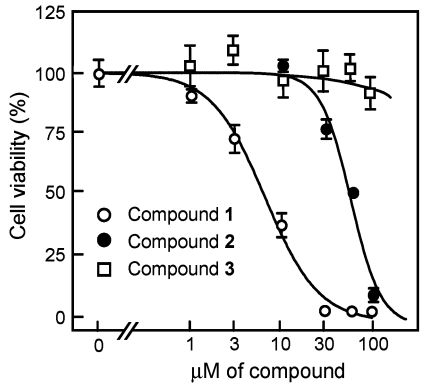


**Figure 3.** Effects of the new compounds on HL-60 cell viability. Cells were cultured in the presence of the indicated doses of products for 72 h, and the percentage of cell viability was determined by the MTT assay as described in the Experimental Section. The results of a representative experiment are shown, and each point represents the mean ± SE of triplicate determinations.

**Table 2.** Effects of the Isolated Compounds on the Growth of HL-60 Cells[a]

| compound | $IC_{50}$ ($\mu$M) |
|---|---|
| **1** | 9.7 ± 2.7 |
| **2** | 39.0 ± 13.8 |
| **3** | >100 |
| **4** | >100 |
| **5** | N.D.[b] |
| **6** | 2.0 ± 0.9 |
| **7** | >100 |
| **8** | 7.2 ± 2.3 |
| **9** | 3.7 ± 0 |
| **10** | 7.3 ± 3.7 |

[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 1 using the methodology described in the Experimental Section. [b]N.D., growth inhibition not detected at the highest dose tested.

cytostatic potency ($IC_{50}$'s from 2.0 to 9.7 $\mu$M), while compound **2** has a somewhat lower potency ($IC_{50}$ 39.0 $\mu$M) than the other compounds. Compounds **3**, **4**, and **7** displayed very weak cytotoxic activity since they showed a significant decrease in cell viability only at the highest concentration tested (100 $\mu$M), and therefore $IC_{50}$'s were always higher than 100 $\mu$M. Only compound **5** did not show cytotoxic activity in the range of concentrations assayed.

To determine whether the two more active compounds, **1** and **2**, induce apoptosis, we incubated HL-60 cells with 30 $\mu$M of these agents for 12 h. Apoptosis was judged by the appearance of typical morphological changes that include chromatin condensation, its compaction along the periphery of the nucleus, and nuclear segmentation into three or more chromatin fragments and visualized by fluorescence microscopy. The results show that compounds **1** and **2** were able to induce apoptosis (Figure 4A). In the case of compound **1** the percentage of apoptotic cells was 35.1 ± 11.2%, while for compound **2** the percentage was 9.4 ± 4.7%, and both were greater than controls (2.7 ± 0.5% of cells were apoptotic). Compound **3** was also included, and in accordance with the above results (see Figure 3 and Table 2), the percentage of apoptotic cells (5.0 ± 1.4%) did not increase significantly compared to control cells. Figure 4B shows the morphological changes associated with the cells in response to compound **1** in comparison with untreated cells.

We also examined whether fragmentation of DNA, which is considered to be the end point of the apoptotic pathway,

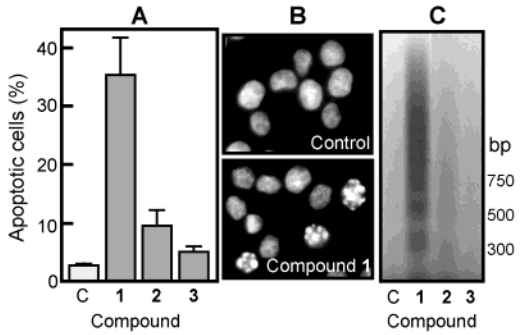


**Figure 4.** Analysis of apoptotic induction on HL-60 cells treated with no drug (C, control) or 30 $\mu$M of the indicated compounds for 12 h. Cells were stained with bisbenzimide trihydrochloride to evaluate nuclear chromatin condensation and quantitated by fluorescence microscopy (panel A). The results of a representative experiment are shown, and each point represents the average ± SE of triplicate determinations. Photomicrographs of representative fields of cells treated and stained as above (panel B). To detect DNA fragmentation, DNA was isolated from the cells, electrophoresed on 2% agarose gels, and stained with ethidium bromide (panel C); bp, base pairs.

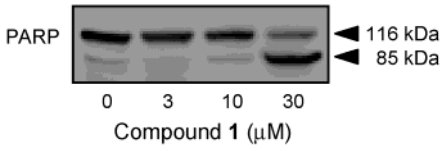


**Figure 5.** Western blot analysis of poly(ADP-ribose) polymerase (PARP) cleavage. Cells were incubated in the presence of the indicated concentrations of compound **1** for 12 h, and cell lysates were subjected to SDS-PAGE. PARP level was determined by immunoblotting with a monoclonal antibody that also recognizes the 85 kDa fragment.

is activated in response to compounds **1**−**3**. As demonstrated in Figure 4C, DNA fragments formed by intranucleosomal hydrolysis of chromatin are observed after 12 h of treatment with 30 $\mu$M of compound **1** and are weakly visible with the same concentration of compound **2**.

We further performed an immunoblot analysis to determine whether compound **1**, the most potent apoptotic inducer among the new compounds identified, induced poly(ADP-ribose) polymerase (PARP) cleavage. This protein plays an important role in chromatin architecture and DNA metabolism. It is considered a hallmark of apoptosis and a caspase substrate whose degradation from 116 kDa to an 85 kDa fragment is indicative of apoptosis.[3] As shown in Figure 5, when cells were incubated with compound **1** for 12 h, PARP cleavage increased in a dose-dependent manner. Hydrolysis of this protein was slightly apparent at 10 $\mu$M of compound **1**, while extensive cleavage was observed at 30 $\mu$M of product.

The results presented herein indicate that the spirostanol saponins show potent antiproliferative activity and that the acetyl group in the sugar moiety plays a crucial role. Compound **1** exhibited the most potent cytotoxic activity among the new isolated compounds ($IC_{50}$: 9.7 ± 2.7 $\mu$M), and similar results were obtained in the case of U937 cells ($IC_{50}$: 6.8 ± 3.0 $\mu$M).

Wang et al.[4] reported that dioscin **6** exerted significant inhibitory effects on the growth of HL-60 human leukemia cells, inducing differentiation and apoptosis, while methyl protodioscin **10**, which possesses a furostanol with 26-*O*-glycopyranoside, had no effect. In contrast to these results we found that methyl protodioscin inhibits the growth of HL-60 cells. We do not know the reason for the discrepancy, although a possible explanation could be the different periods of incubation, 48 h versus 72 h, used in these studies.

Our results are in agreement with those obtained by Mimaki et al.[5] that indicate that furostanol glycosides bearing an α-L-rhamnosyl-(1→2)-$\beta$-D-glucosyl or an α-L-rhamnosyl-(1→2)-[α-L-rhamnosyl-(1→4)]-$\beta$-D-glucosyl at $C_3$ of the aglycone are cytotoxic to HL-60 cells.

Saponins **1** and **2**, with a glycosyl group at C-1 of the aglycone, showed cytotoxic activity on HL-60 cells. The presence of an additional acetyl group increases the effectiveness. The comparison of the $IC_{50}$ of compounds **1**−**3** indicates that the presence of a certain degree of lipophilicity in the sugar moiety is essential for exhibiting not only cytotoxic properties but also apoptosis induction.

In summary, we found that compounds **1** and **2** but not **3** inhibit the growth of HL-60 myeloid leukemia cells in culture, compound **1** displaying a greater cytostatic capacity than **2**. The mechanism by which it achieves this cytotoxic effect is by activating the apoptotic process. Treatment of the cells with these compounds induces the morphological changes and DNA fragmentation characteristic of apoptotic cell death and also associated with PARP cleavage, as demonstrated for compound **1**.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a Perkin-Elmer model 343 polarimeter. UV spectra were recorded using a JASCO model V-560 spectrophotometer. IR spectra were recorded using a Bruker model IFS-55 spectrophotometer. $^1$H and $^{13}$C NMR spectra were obtained on a Bruker model AMX-500 spectrometer with standard pulse sequences, operating at 500 MHz in $^1$H and 125 MHz in $^{13}$C NMR. $CDCl_3$, $C_5D_5N$, and $CO(CD_3)_2$ were used as solvents and TMS as 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), ODS silica gel (Aldrich Chemical Co.), Sephadex LH-20 (Aldrich Chemical Co.), and Diaion HP-20 (Supelco). Fractions obtained from CC were monitored by TLC (silica gel 60 $F_{254}$), and PTLC was carried out on silica gel 60 $PF_{254+366}$ plates (20 × 20 cm, 1 mm thick).

**Plant Material.** The bark of *Dracaena draco* was collected in Parque del Drago, Icod de los Vinos, Tenerife, Canary Islands, in winter 2001. A voucher specimen PDED01 is deposited in the Herbarium of the Parque del Drago, Icod de los Vinos, Tenerife, Canary Islands.

**Extraction and Isolation.** The bark (890 g) of *D. draco* was extracted with 95% EtOH in a Soxhlet apparatus for 72 h. Removal of the solvent gave a residue (30 g), which was subjected to column chromatography on silica gel using *n*-hexane−AcOEt in increasing polarity and then with increasing percentages of MeOH, affording several fractions (I−X). Twenty different compounds were isolated, 17 of which have been identified as known compounds.

Fractions I−III were eluted with *n*-hexane−AcOEt (20:1) and rechromatographed on silica gel with *n*-hexane−AcOEt (20:1), Sephadex LH-20 eluting with *n*-hexane−$CH_2Cl_2$−MeOH (2:1:1), and preparative TLC with *n*-hexane−AcOEt (7:3) to give *trans*-$\beta$-apo-8′-carotenal[6] (2.3 mg), $\beta$-sitosterol[7] (4.0 mg), and diosgenin[2] (5.3 mg). Fractions IV−VI were eluted with *n*-hexane−AcOEt (75:25) and rechromatographed on silica gel with *n*-hexane−AcOEt (8:2), Sephadex LH-20 eluting with *n*-hexane−$CH_2Cl_2$−MeOH (2:1:1), and preparative TLC with toluene−$^i$PrOH (20:1) to give (−)-3′-hydroxy-4′-methoxy-7-hydroxy-8-methylflavan[8] (2 mg), 7-hydroxy-3-(4-hydroxybenzyl)chromane[9] (6 mg), loureirin C[10] (2 mg), (2*S*)-4′,7-dihydroxy-3′-methoxyflavan[11] (2 mg), 4-allylcatecol[12] (4 mg), isoliquiritigenin[13] (8 mg), and sitoindoside I[14] (10 mg). Fractions VII−IX eluted with *n*-hexane−AcOEt (30:70) and AcOEt−MeOH (10:1) and rechromatographed on silica gel with $CH_2Cl_2$−MeOH (10:1), Sephadex LH-20 eluting with *n*-hexane−$CH_2Cl_2$−MeOH (1:1:1), and preparative TLC with $CHCl_3$−MeOH (10:1) gave draconin A (**1**) (3 mg), draconin B (**2**) (15

mg), **4**[15] (15 mg), **5**[1] (38 mg), **6**[4] (50 mg), **7**[1] (15 mg), and draconin C (**3**) (35 mg). Fraction X eluted with MeOH was passed through a Diaion HP-20 column using gradients of MeOH in $H_2O$ and rechromatographed on silica gel with $CHCl_3$−MeOH (8:2), Sephadex LH-20 eluting with MeOH, and on an ODS silica gel column eluting with MeOH−$H_2O$ (2:1) and MeCN−$H_2O$ (1:2) to give **8**[16] (40 mg), **9**[17] (28 mg), and **10**[4] (15 mg).

**Acid Hydrolysis of Compound 2.** Compound **2** (5 mg) was dissolved in 1 M HCl (dioxane−$H_2O$, 1:1.5, 8 mL) and then heated to 80 °C in a water bath for 1 h. After extraction with $CHCl_3$, the aqueous residue was evaporated to dryness. L-Arabinose and L-rhamnose were identified on TLC by comparison with authentic sugar samples, with *n*-butanol−AcOH−ether−$H_2O$ (9:6:3:1, $R_f$ = 0.36 and 0.58, respectively) as the developing solvent.

**Draconin A (1):** amorphous solid; $[\alpha]^{20}_D$ −70° (*c* 1.5, ethanol); IR $\nu_{max}$ (film, KBr) 2380, 1740 (CO), 1230; $^1$H and $^{13}$C NMR, see Table 1; FABMS *m/z* 865 $[M + H]^+$, 847 $[M - Ac]^+$, 592 $[M - Rham]^+$.

**Draconin B (2):** amorphous solid; $[\alpha]^{20}_D$ −100° (*c* 2.6, ethanol); IR $\nu_{max}$ (film, KBr); 3400 (OH), 1740 (CO), 1250, 1100; $^1$H and $^{13}$C NMR, see Table 1; HRFABMS *m/z* 846.3954 $[M + Na + H]^+$ (calcd for $C_{42}H_{63}O_{16}$·Na, 846.4013).

**Draconin C (3):** amorphous solid; $[\alpha]^{20}_D$ −85° (*c* 11.5, ethanol); IR $\nu^{KBr}_{max}$ cm$^{-1}$; 3393 (OH), 2975, 2904, 1730 (CO), 1251, 1052; $^1$H and $^{13}$C NMR, see Table 1; HRFABMS *m/z* 780.3865 (calcd for $C_{40}H_{60}O_{15}$·Na, 780.3932).

**Cell Culture.** The human promyelocytic leukemia HL-60 cell line established by Gallagher et al.[18] was used in this study. Cells were cultured in suspension in RPMI-1640 medium (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin (10 000 units mL$^{-1}$), and streptomycin (10 000 $\mu$g mL$^{-1}$) in a humidified atmosphere of 95% air and 5% $CO_2$ at 37 °C. Cells were maintained at a density of $<1 \times 10^6$ cells/mL. Compounds were dissolved in dimethyl sulfoxide (DMSO) and stored as stock solutions at −20 °C. Further dilutions were made in culture medium immediately prior to use. In all experiments, the final concentration of DMSO did not exceed 1% (v/v), a concentration that was nontoxic to the cells. Cell viability was determined using the trypan blue exclusion test. To ensure exponential growth, cells were resuspended in fresh medium 24 h before each treatment.

**Assay for Growth Inhibition.** Cytotoxic assays were performed using an MTT assay.[19] Cells ($1 \times 10^4$/well) were continuously exposed to different concentrations of the compounds in 96-well plates for 72 h at 37 °C. Compounds were applied in DMSO, and controls were always treated with the same amount of vehicle as used in the corresponding experiments. Surviving cells were detected on the basis of their ability to metabolize 3-[4,5-dimethylthiazol-2-yl-]2,5-diphenyl tetrazolium bromide (MTT) into formazan crystals. Optical density at 570 nm was used as a measure of cell viability. The MTT dye reduction assay measures mitochondrial respiratory function and can detect the onset of cell death earlier than dye-exclusion methods. Cell survival was calculated as the fraction of cells alive relative to control for each point as cell survival (%) = mean absorbance in treated wells/mean absorbance in control wells × 100. Concentrations inducing a 50% inhibition of cell growth ($IC_{50}$) were determined graphically for each experiment using the curve-fitting routine of the computer software Prism 2.0 (GraphPad) and the equation derived by De Lean and co-workers.[20]

**Quantitative Fluorescence Microscopy.** Cells were fixed in 3% paraformaldehyde for 10 min at room temperature and then stained with 10 $\mu$g/mL bisbenzimide trihydrochloride (Hoechst 33258) for 30 min at 37 °C before fluorescence microscopy analysis. Apoptotic cells were identified by their fragmented chromatin.[21] Stained nuclei with condensed chromatin (supercondensed chromatin at the nuclear periphery) or nuclei that were fragmented into multiple smaller dense bodies were considered to be apoptotic. Nuclei with uncondensed and dispersed chromatin were considered to be non-apoptotic. A minimum of 500 cells were counted for each sample, and each experiment was done in triplicate.

**Determination of DNA Fragmentation.** Low molecular weight DNA was extracted following the method described by Colotta et al.[22] Cells ($\sim 2 \times 10^6$) were washed with phosphate buffer saline and incubated in 100 $\mu$L of hypotonic detergent buffer (10 mM Tris-HCl, 1 mM EDTA, 0.2% Triton X-100, pH 7.5) for 30 min at 4 °C. The supernatants obtained after centrifugation were sequentially incubated with 40 $\mu$g of DNase-free RNase and 40 $\mu$g of proteinase K at 37 °C for 30 min. The samples were mixed with 100 $\mu$L of phenol, and the aqueous phase, containing the fragmented DNA, was incubated with 250 $\mu$L of ethanol in the presence of 5 $\mu$g of *t*-RNA for 24 h at −20 °C. After the centrifugation the DNA precipitate was resuspended in 20 $\mu$L of 10 mM Tris-HCl (pH 7.5), and aliquots (10 $\mu$L) were electrophoresed at 40 V for 4 h through a 2% agarose gel in TAE buffer (40 mM Tris-acetate and 1.0 mM EDTA, pH 8.3). DNA bands were visualized under UV light after staining with ethidium bromide (0.5 $\mu$g/mL), and the images were captured by a digital camera (Kodak).

**Western Blot Analysis of PARP Hydrolysis.** Exponentially grown HL-60 cells ($\sim 7 \times 10^5$) were treated with the compounds at the indicated concentrations for different time periods at 37 °C. Cell lysates were prepared in a buffer containing 20 mM Hepes (pH 7.4), 250 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 $\mu$g/mL leupeptin, aprotinin, and pepstatin A. Insoluble materials were removed by centrifugation at 10000g for 10 min. Extracted proteins (50 $\mu$g/well) were separated by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 7.5% polyacrylamide gels and were electrophoretically transferred onto Immobilon-P membrane. The loading and transfer of equal amounts of protein were confirmed by staining the membrane with Ponceau S. Membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h, followed by incubation with anti-PARP monoclonal antibody (BD PharMingen; 1:1000 dilution in TBST supplemented with 3% nonfat milk) overnight. After washing and incubation with anti-mouse antibody conjugated to horseradish peroxidase (HRP) (Amersham Pharmacia Biotech), the antigen−antibody complexes were visualized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) using the manufacturer's protocol. The appearance of an 85 kDa cleavage product was used as a measure of apoptosis.

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## References and Notes

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