Isolation of a Dihydrobenzofuran Lignan from South American Dragon's Blood (Croton spp.) as an Inhibitor of Cell Proliferation

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ISOLATION OF A DIHYDROBENZOFURAN LIGNAN FROM SOUTH AMERICAN DRAGON'S BLOOD (*CROTON* SPP.) AS AN INHIBITOR OF CELL PROLIFERATION[1]

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ABSTRACT.—Dragon's blood is a red viscous latex extracted from the cortex of various *Croton* spp. (Euphorbiaceae), most commonly *Croton lechleri, Croton draconoides* (or *Croton palanostigma*), and *Croton erythrochilus*. It is used in South American popular medicine for several purposes, including wound healing. Bioassay-guided fractionation of dragon's blood, using an in vitro test system for the stimulation of human umbilical vein endothelial cells, has resulted in the isolation of a dihydrobenzofuran lignan, 3′,4-*O*-dimethylcedrusin or 4-*O*-methyldihydrodehydrodiconiferyl alcohol [2-(3′,4′-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7-methoxybenzofuran-5-propan-1-ol] [**1**] as the biologically active principle. A related compound, 4-*O*-methylcedrusin [2-(3′,4′-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7-hydroxybenzofuran-5-propan-1-ol] [**2**], and the alkaloid taspine [**3**], also isolated from dragon's blood, were not active in the same assay. A cell proliferation assay, measuring the incorporation of tritiated thymidine in endothelial cells, showed that compound **1** did not stimulate cell proliferation, but rather inhibited thymidine incorporation, while protecting cells against degradation in a starvation medium.

Dragon's blood, in Spanish "sangre de drago" or "sangre de grado," is used in South American popular medicine for several purposes, including wound healing. It is a red viscous latex produced by slashing the bark of various *Croton* spp. (Euphorbiaceae), most commonly *Croton lechleri* L., *Croton draconoides* (Muell.) Arg., also classified as *Croton palanostigma* Kl., and *Croton erythrochilus* (Muell.) Arg. (1,2). Compounds isolated previously from dragon's blood include the alkaloid taspine (3), which was claimed to be the active cicatrizant principle (4), the dihydrobenzofuran lignan 3′,4-*O*-dimethylcedrusin [**1**] (5), and a series of proanthocyanidins (6). The aim of the present study was the bioassay-guided isolation of the wound-healing principles from dragon's blood.

Since endothelium plays a crucial role in the process of wound healing, and an in vivo guiding test was excluded for practical and ethical reasons, an in vitro test system for the stimulation of endothelial cells was selected (7). The importance of the endothelium in normal and pathological processes such as hemostasis, thrombosis and atherosclerosis, angiogenesis, tissue repair, and tumor growth has long been recognized. Endothelial cell growth is also involved in the process of wound healing. Wound repair requires, among other processes, cell proliferation and the formation of new blood vessels from endothelial cells. The endothelial cell has been considered to be a difficult cell to culture. Human endothelial cells require the presence of high concentrations of serum in the culture medium for optimal growth (8,9). In the present study the characterization of 3′,4-*O*-dimethylcedrusin [**1**] as the in vitro biologically active principle from dragon's blood, the identification of a new, inactive dihydrobenzofuran lignan, 4-*O*-methylcedrusin [**2**], and an evaluation of the biological activity of compound **1** and taspine [**3**] are reported.

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[1]Preliminary results of this work were presented as an oral communication at the International Research Congress on Natural Products, Chicago, Illinois, 21–26 July 1991 [abstract O:26], and at the 39th Annual Congress on Medicinal Plant Research, Saarbrücken, Germany, 3–7 September 1991 [abstract in *Planta Med.* **57** (Suppl. 2), A12].

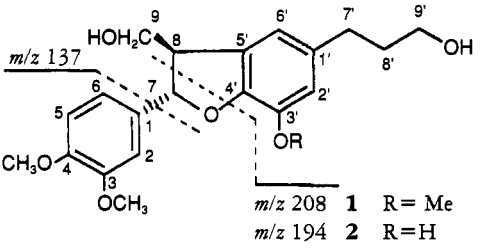
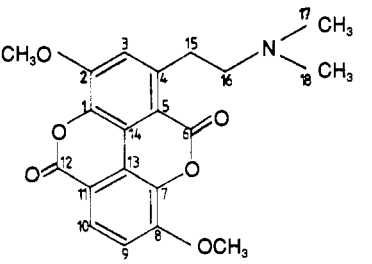


FIGURE 1. Ms fragmentation of **1** and **2**.



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RESULTS AND DISCUSSION

Of all fractions from dragon's blood tested, only the Et_2O extract was found to stimulate human umbilical vein endothelial cells (HUVEC), giving better results than the negative control experiment with only 5% human serum (Table 1). Testing the different subfractions and compounds **1** and **2** obtained by vlc of the (Et_2O) extract from dragon's blood revealed that only compound **1** was biologically active in this assay. At 250 μg/ml it was toxic to the cells, but at 25 and 5 μg/ml cell growth was similar to the positive control experiment with 30% human serum. Compound **2** showed about the same cytotoxicity, but it did not stimulate the growth of endothelial cells. The alkaloid taspine [**3**] was cytotoxic down to a concentration of about 0.5 μg/ml.

Compound **1** was identified by nmr and ms analysis as 3′,4-*O*-dimethylcedrusin or 4-*O*-methyldihydrodehydrodiconiferyl alcohol. In this work ^1H- and ^{13}C-nmr assignments were based on COSY, HETCOR, and long-range HETCOR experiments. Careful analysis of these spectral data showed that some assignments reported previously (5,10),

TABLE 1. Results of the Stimulation of Endothelial Cells (HUVEC)[a].

Compound	Concentration (μg/ml)[b]						
	250	50	25	5	2.5	0.5	0.25
Et_2O extract	+	+	=				
1	T	+	++	++	=		
2	T	=					
3	nt	nt	T	T	T	−	=

[a]++, Similar to the positive control experiment (30% human serum); +, better than the negative control experiment (5% human serum); =, similar to the negative control experiment (no cell growth); −, worse than the negative control experiment (slightly toxic); T, toxic to the cells; nt, not tested.

[b]Concentration (μg/ml) in culture medium of fraction or compound tested.

which were based on reference data for related compounds, had to be revised, and that the existing literature contained some inconsistencies (11). The ^{1}H- and ^{13}C-nmr spectra of **2** were very similar to those of **1**, indicating a related dihydrobenzofuran lignan, but only two MeO groups were observed. Some chemical shift differences between the ^{13}C-nmr spectra of 3′,4-*O*-dimethylcedrusin and of **2** enabled us to locate the MeO groups in the latter compound. C-2, C-5, and C-6 showed about the same chemical shift for both compounds, indicating that both MeO groups might be located in positions 3 and 4, and hence that C-3′ was substituted by a free phenolic OH group. This was confirmed by the downfield shift of 2.2 ppm for C-2′ (from 114.2 ppm in 3′,4-*O*-dimethylcedrusin to 116.4 ppm in **2**), and by the very small downfield shift of only 0.1 ppm for C-6′ (from 117.9 to 118.0 ppm), in agreement with general chemical shift considerations (12). Therefore, compound **2** was identified as 4-*O*-methylcedrusin, or 2-(3′,4′-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7-hydroxybenzofuran-5-propan-1-ol, a new dihydrobenzofuran lignan. The structures of **1** and **2** were also confirmed by ms. Eims of **1** and **2** showed a similar spectral pattern. Compound **1**, 3′,4-*O*-dimethylcedrusin, showed a molecular ion $[M]^+$ at *m/z* 374. Other peaks were observed at *m/z* 356 $[M-H_2O]^+$, *m/z* 344 $[M-CH_2O]^+$, which is characteristic for phenolic MeO groups, and at *m/z* 341 $[M-H_2O-Me]^+$. Corresponding peaks were observed for compound **2**, 4-*O*-methylcedrusin, with a molecular ion at *m/z* 360 and fragment ions at *m/z* 342 $[M-H_2O]^+$, *m/z* 330 $[M-CH_2O]^+$, and *m/z* 327 $[M-H_2O-Me]^+$. High resolution measurements for selected fragment ions of **2** yielded the following expected elemental compositions: *m/z* 360 $C_{20}H_{24}O_6$ $[M]^+$, *m/z* 342 $C_{20}H_{22}O_5$, *m/z* 330 $C_{19}H_{22}O_5$, and *m/z* 327 $C_{19}H_{19}O_5$, in agreement with the fragmentations mentioned above. Ms/ms daughter ion spectra of the molecular ion of both compounds also confirmed this fragmentation pattern and the position of the free phenolic OH group in **2**. Fragment ions observed at *m/z* 208 for **1** and at *m/z* 194 for **2** could be explained by a cleavage in the dihydrofuran ring (see Figure 1). A fragment ion at *m/z* 137, present for both compounds, corresponded to the dimethoxy aryl cation. An additional peak at *m/z* 224 for **2** could be explained as due to the loss of dimethoxybenzyne, with 136 mass units.

The trans configuration of the dihydrofuran ring in **1** and **2** was evident from ^{1}H- and ^{13}C-nmr spectra, but the exact stereochemistry in C-7 and C-8 has not been determined. However, the 7,8 stereochemistry of cedrusin and related compounds was established as depicted for **1** and **2**, 7*S*,8*S* by analogy to compounds with known stereochemistry, such as licarins A and B (13). The structure of **1** was also confirmed by organic synthesis from methylferulate, yielding racemic 3′,4-*O*-dimethylcedrusin (10,14).

For a more precise evaluation of the effects on HUVEC observed for 3,4-*O*-dimethylcedrusin (stimulation) and taspine (cytotoxicity), an in vitro thymidine incorporation assay was performed. An increase in cell density (i.e., growth) should normally correspond to an increase in DNA synthesis, characterized by a higher thymidine incorporation into cellular DNA of the cultured cells. Therefore, the incorporation of tritiated thymidine can be used as a sensitive index of cell proliferation. Results are shown in Figure 2 for 3′,4-*O*-dimethylcedrusin. The first graph (Figure 2a) gives the relative number of cells at the end of the experiment as a function of the concentration, with the negative control experiment at 100%. The maximum number of cells was obtained between 50 and 100 μg/ml, which was higher than observed in the previous experiment (see Table 1). This is due to the fact that in the thymidine incorporation assay only 2% human adult serum was used, instead of 5% as in the cell growth assay, to make the growth conditions even more critical. At higher concentrations (125 μg/ml and 250 μg/ml) the compound was toxic to the cells. It is interesting to note that the actual concentration of 3′,4-*O*-dimethylcedrusin in dragon's blood (0.0014% or about 14 μg/ml, see below) is of the same order of magnitude as the biologically active concentration

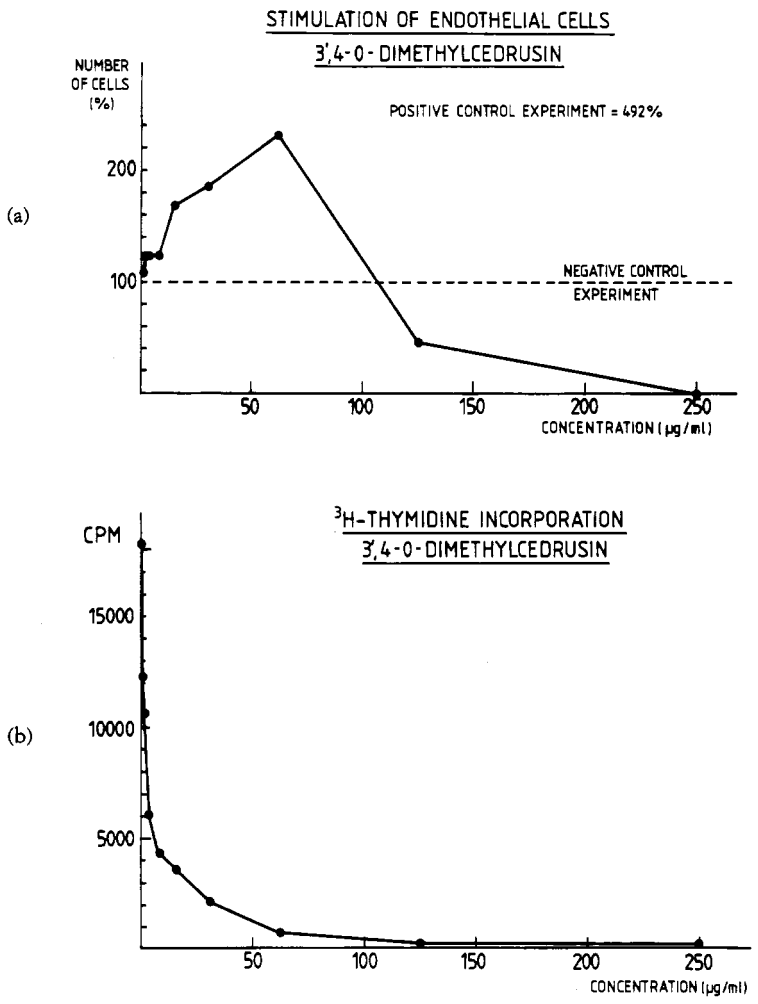

FIGURE 2. 3',4-*O*-Dimethylcedrusin [**1**]: (a) stimulation of endothelial cells (HUVEC), (b) inhibition of thymidine incorporation.

range. Figure 2b gives the incorporation of tritiated thymidine, expressed as cpm, also as a function of the concentration. At first sight these results are contradictory. Normally an increase of thymidine incorporation, or DNA synthesis, was expected in the same concentration range where the number of cells had increased. However, in this experiment an inhibition of thymidine incorporation was observed. This indicated that 3',4-*O*-dimethylcedrusin did not stimulate cell proliferation, but that it had a protective effect against degradation of the cells in a starvation medium with only 2% human adult serum, as in the negative control experiment.

A similar experiment was carried out with taspine [**3**]. Both graphs (number of cells and thymidine incorporation vs. concentration) are shown in Figure 3. As mentioned before, this alkaloid is highly cytotoxic; at all concentrations tested, the number of cells was lower than in the negative control experiment, and it decreased as the concentration increased. In this experiment, both graphs have the same appearance. The decrease of thymidine incorporation was due only to the cytotoxicity.

The inhibition of thymidine incorporation by 3',4-*O*-dimethylcedrusin is calcu-

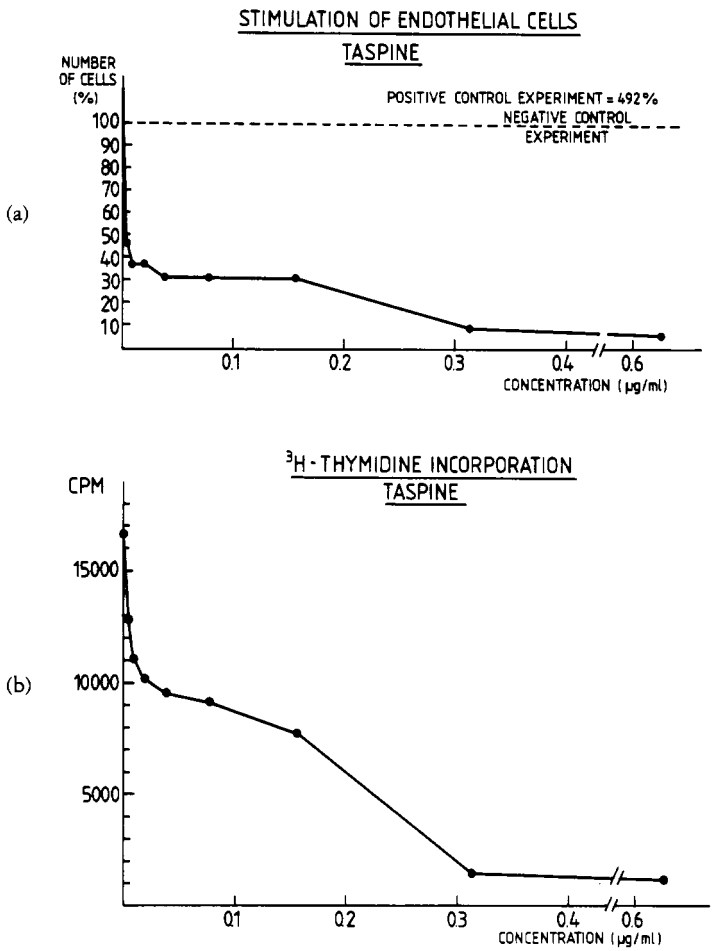

FIGURE 3. Taspine [3]: (a) stimulation of endothelial cells (HUVEC), (b) inhibition of thymidine incorporation.

lated in Table 2. In order to calculate the percentage inhibition of thymidine incorporation, the actual number of cells must be taken into account. Therefore, for each concentration tested Table 2 gives the approximate number of cells per well, the percentage of cells relative to the negative control experiment (=100%), the thymidine incorporation per cell (cpm/number of cells), the percentage thymidine incorporation relative to the negative control experiment (=100%), and the percentage inhibiton of thymidine incorporation. At toxic concentrations (250 and 125 µg/ml) the inhibition of thymidine incorporation is meaningless, since it is due only to cytotoxicity, but at non-toxic concentrations, such as 62.5 µg/ml, about 98% of the thymidine incorporation is inhibited. At only 2 µg/ml, the inhibition is about 50%. Compounds inhibiting thymidine incorporation (or DNA synthesis), which is a sensitive index of cell proliferation, without being toxic may be useful as anti-tumor agents. It is interesting to note that in the traditional South American medicine dragon's blood is not only used for wound healing but also against cancer (15).

At the moment it is not clear if the protective effect against degradation of the cells

TABLE 2. Inhibition of Thymidine Incorporation in Endothelial Cells (HUVEC) by 3′,4-*O*-Dimethylcedrusin [**1**].

Sample concentration (μg/ml)	Number of cells /well	%	cpm/cell	thymidine incorporation (%)	Inhibition (%)
250	0	0	—	—	—
125	3000	46	0.08800	3	97
62.5	15000	231	0.04780	2	98
31.25	12000	185	0.17867	7	93
15.625	11000	169	0.33118	13	87
7.8125	8000	123	0.53850	21	79
3.9062	8000	123	0.75425	29	71
1.9531	8000	123	1.33488	52	48
0.9766	7000	108	1.75357	68	32
0.4883	7000	108	2.60729	101	−1
0.0	6500	100	2.55954	100	0

in a starvation medium as observed for compound **1** is related to the inhibition of thymidine incorporation. In similar experiments compound **2** was also found to inhibit thymidine incorporation, although not to the same extent, but during the bioassay-guided isolation the same protective effect as for **1** was not observed. This may indicate that the 3′-MeO group is important for the specific biological activity of **1** (10).

In conclusion, 3′,4-*O*-dimethylcedrusin [**1**] was isolated as the in vitro biologically active principle from dragon's blood; it showed a protective effect on endothelial cells in a starvation medium. The alkaloid taspine [**3**], which was claimed before to be the cicatrizant principle from dragon's blood by increasing the migration of human foreskin fibroblasts (4), showed no activity in our assays. In vivo experiments in rats to evaluate the wound-healing activity of dragon's blood and its constituents are in progress and will be presented in a separate paper.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—One-dimensional ^{1}H- and ^{13}C-nmr spectra and 2D homonuclear (^{1}H-^{1}H) correlated (COSY) and heteronuclear (^{1}H-^{13}C) correlated (HETCOR) spectra were recorded on a Jeol FX 200 instrument, operating at 199.50 MHz (^{1}H) and 50.10 MHz (^{13}C), using TMS as internal standard. Long-range heteronuclear (^{1}H-^{13}C) correlated spectra were recorded on a Varian Unity instrument (^{1}H, 400 MHz). Eims (70 eV), and ms/ms daughter ion spectra of selected ions were recorded on a VG 70-SEQ hybrid mass spectrometer. Daughter ion spectra were obtained by applying collisional activation with argon gas at low kinetic energy (<30 eV). Hrms measurements were performed for selected fragment ions on the same instrument.

EXTRACTION AND ISOLATION.—Dragon's blood, obtained from Peruvian *Croton* spp., was purchased in liquid form from Quimica Universal, Lima, Peru. Dragon's blood was acidified with citric acid to a final concentration of 2%, filtered, and extracted successively with *n*-hexane, Et_2O, EtOAc, and *n*-BuOH. Alternatively, EtOAc–*n*-BuOH (1:1) may be used. The organic layers were evaporated to dryness under reduced pressure, and the residual aqueous fraction of dragon's blood was brought to pH 9–10 with concentrated NH_3. The voluminous precipitate was filtered off, and the alkaline filtrate was extracted with $CHCl_3$ or alternatively $CHCl_3$-Et_2NH (9:1), which had been used before to wash the residue on the filter. This fraction contained the alkaloidal compounds.

Alternatively, dragon's blood may be freeze-dried, the dry powder extracted with Me_2CO, the Me_2CO extract evaporated to dryness under reduced pressure, and the residue redissolved in 0.1 N HCl-MeOH (1:3). Subsequently, *n*-hexane, Et_2O, EtOAc, and *n*-BuOH extracts may be prepared as well. After extraction of the freeze-dried powder with Me_2CO the residue was extracted exhaustively with $CHCl_3$-Et_2NH (9:1) and

evaporated to dryness under reduced pressure. This residue was dissolved in $CHCl_3$-MeOH-Et_2NH (9:9:2) and then adsorbed on Si gel (particle size 0.040–0.063 mm, Merck) (1). After drying, this quantity of Si gel was transferred to a vlc (16) column to serve as the top layer of a column consisting of Si gel with the same particle size. This column was eluted with $CHCl_3$-Et_2NH (9:1), and the elution was monitored by tlc on Si gel 60 F_{254}, layer thickness 0.2 mm (Merck), with the same mobile phase. The Dragendorff reagent was used for visualization of the spots. All fractions containing a Dragendorff positive spot with R_f 0.50, due to the alkaloid taspine [**3**], were combined, concentrated under reduced pressure, and stored overnight at −25°. Precipitation occurred, and after filtration chromatographically pure **3** was obtained. The structure of **3** was confirmed by ^1H and ^{13}C nmr and eims. Dragon's blood (2340 g), corresponding to about 514 g of dry powder after freeze-drying, yielded about 1.99 g of taspine (0.09%).

The Et_2O extract of crude or freeze-dried dragon's blood was evaporated to dryness under reduced pressure and separated by vlc on Si gel (particle size 0.015–0.040 mm, Merck), using *n*-hexane–iPrOH (2:1) as the mobile phase. Elution was monitored by tlc on Si gel 60 F_{254}, layer thickness 0.2 mm (Merck), with the same solvent system. Fractions showing the same tlc pattern were combined, and a subfraction showing mainly one spot with R_f 0.20 in this tlc system and two spots (R_f 0.15 and 0.20) with $CHCl_3$-Me_2CO (2:1) as the mobile phase was obtained. This subfraction was separated by preparative tlc on Si gel 60 F_{254}, layer thickness 2 mm (Merck), using this second solvent system as the mobile phase. Two almost chromatographically pure compounds, **1** (32 mg) and **2** (6 mg), with R_f 0.20 and 0.15, respectively, were obtained, which corresponded to a yield of 0.0014% w/w (or about 14 μg/ml dragon's blood) and 0.0002% w/w (or about 2 μg/ml), respectively. Both compounds were identified by ^1H and ^{13}C nmr, including 2D techniques, and ms, including eims, ms/ms and high resolution measurements.

STIMULATION OF ENDOTHELIAL CELLS.—All fractions and compounds were tested for stimulation of endothelial cells according to Vanden Berghe *et al.* (7). Briefly, endothelial cells were obtained from human umbilical cord vein (HUVEC, human umbilical vein endothelial cells). The cells were cultured in medium 199 (Gibco), supplemented with 30% heat-inactivated (56°, 30 min) human adult serum, 100 U/ml penicillin, and 20 μg/ml gentamicin, at 37°, under a humidified atmosphere containing 5% CO_2. The cells were fed every 4 to 5 days with a complete change of fresh culture medium until confluence was reached. For subculture, HUVEC was harvested with 0.05% trypsin, 0.02% EDTA solution (trypsin-EDTA solution, Gibco) and split at a ratio 1:3 for inoculation into new culture flasks. HUVEC at passages 2 to 4 was used in the experiments. In all stimulation experiments, cells were subcultured in microtiter plates containing media which did not allow normal growth so that potential stimulation could be easily detected. In such media cells remained alive for several days. In the presence of only 5% human adult serum, without any additional stimulating agent, HUVEC did not multiply in medium 199 (negative control experiment). On the other hand, starting with 4×10^3 cells per well of the microtiter plate, a confluent monolayer containing about 2×10^4 cells per well was usually obtained after 6 days in medium 199 with 30% human adult serum (positive control experiment). For the stimulation experiments, HUVEC was inoculated, after trypsinization, into 96-well microtiter plates (Costar) at a density of 4×10^3 cells per well (0.3 cm^2/well) in medium 199 supplemented with 20% human adult serum and 100 U/ml penicillin. After 8 h, cells were carefully washed twice with medium 199 and then exposed to the same medium containing 5% human adult serum with or without solutions to be tested for stimulation of endothelial cells. Cell growth was evaluated microscopically every other day during 6 days. Samples to be tested were prepared as 0.5% solutions in polyethylene glycol (PEG) 400 33%, and tested as 5, 1, 0.5, 0.1,...% dilutions in the final culture medium. Control experiments with 1 and 2% PEG 400 in the culture medium (corresponding approximately with a 5% dilution of PEG 400 33%, i.e., 1.65%), did not show any toxic effect on the cells.

INCORPORATION OF TRITIATED THYMIDINE.—After trypsinization, HUVEC was resuspended in medium 199 supplemented with 20% human adult serum and 100 U/ml penicillin, and then seeded into 48-well plates (Costar) at a density of 10×10^3 cells per well (1 cm^2/well). After 6 h, cells were washed twice with medium 199, and the samples to be tested, diluted in medium 199 with only 2% human adult serum, were added to the cells (800 μl/well). The plates were incubated in a humidified atmosphere containing 5% CO_2 at 37° for 90 h. At this time, 1 μCi of tritiated thymidine (Amersham) was added to each well, and the plates were reincubated for 6 h at 37°. Next the cells were counted and the DNA extracted. The cells were washed twice with Plaisner medium, in order to remove residual tritiated thymidine, and fixed in 0.5 ml of 10% trichloroacetic acid at 4° for 30 min. Cell densities were estimated by counting the number of cells under an inverted microscope (Nikon). After removal of trichloroacetic acid, 0.5 ml of 0.1% sodium dodecylsulfate in 0.2 N NaOH was added to each well. The plates were incubated at 60° for 2 h to solubilize DNA. The lysate was mixed with 10 ml liquid scintillation cocktail (Instagel, Packard), and the radioactivity was determined in a liquid scintillation counter (Packard) and expressed as cpm (7). Test samples included compound **1** (0.5% or 5 mg/ml in PEG 400 33%) and taspine hydrochloride (0.01% or 0.1 mg/ml in H_2O). For both compounds a series of twofold dilutions was tested starting from a

concentration of 250 μg/ml for **1** (250, 125, 62.5, ..., down to 0.49 μg/ml), and from 2.5 μg/ml for taspine (2.5, 1.25, 0.625, ..., down to 0.0049 μg/ml).

3′,4-O-Dimethylcedrusin (4-O-methyldihydrodehydrodiconiferyl alcohol).—2-(3′,4′-Dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7-methoxybenzofuran-5-propan-1-ol [**1**]: ^{1}H nmr δ (400 MHz, CD_3OD) 6.98 (1H, d, J=1.7 Hz, H-2), 6.94 (1H, dd, J=8.3 Hz, J=1.7 Hz, H-6), 6.91 (1H, d, J=8.3 Hz, H-5), 6.73, 6.72 (2×1H, 2×br s, H-2′, H-6′), 5.53 (1H, d, J=5.9 Hz, H-7), 3.86 (3H, s, 3′-OMe), 3.84 (1H, m, H_a-9), 3.81, 3.79 (2×3H, 2×s, 3-OMe, 4-OMe), 3.70 (1H, m, H_b-9), 3.57 (2H, t, J=6.5 Hz, H-9′), 3.46 (1H, dt, J=6.2 Hz, J=5.9 Hz, H-8), 2.63 (2H, t, J=7.7 Hz, H-7′), 1.82 (2H, tt, J=7.7 Hz, J=6.5 Hz, H-8′); ^{13}C nmr δ (100 MHz, CD_3OD) 150.5, 150.2 (C-3, C-4), 147.5 (C-4′), 145.1 (C-3′), 137.0 (C-1′), 136.1 (C-1), 129.8 (C-5′), 119.5 (C-6), 117.9 (C-6′), 114.2 (C-2′), 113.0 (C-5), 110.8 (C-2), 88.7 (C-7), 65.0 (C-9), 62.2 (C-9′), 56.8 (3′-OMe), 56.5 (2×) (3-OMe, 4-OMe), 55.4 (C-8), 35.7 (C-8′), 32.8 (C-7′); eims *m/z* (%) $[M]^+$ 374 (58), $[M-H_2O]^+$ 356 (100), $[M-CH_2O]^+$ 344 (44), $[M-H_2O-Me]^+$ 341 (30).

4-O-methylcedrusin.—2-(3′,4′-Dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-7-hydroxybenzofuran-5-propan-1-ol [**2**]: ^{1}H nmr δ (200 MHz, CD_3OD) 7.0–6.7 (5H, m, H-2, H-5, H-6, H-2′, H-6′), 5.48 (1H, d, J=6.3 Hz, H-7), 3.84, δ 3.80 (2×3H, 2×s, 2×OMe), 3.8–3.4 (5H, m, H-8, H-9, H-9′), 2.62 (2H, t, J=7.8 Hz, H-7′), 1.81 (2H, m, H-8′); ^{13}C nmr δ (50 MHz, CD_3OD) 149.3, 148.1 (C-3, C-4), 145.2 (C-4′), 136.8 (2×), 134.2 (C-1, C-1′, C-3′), 130.0 (C-5′), 119.8 (C-6), 118.0 (C-6′), 116.4 (C-2′), 114.3 (C-5), 110.7 (C-2), 89.1) C-7), 65.1 (C-9), 62.3 (C-9′), 56.8, 56.4 (2×OMe), 55.4 (C-8), 35.8 (C-8′), 32.9 (C-7′); eims *m/z* (%) $[M]^+$ 360 (31), $[M-H_2O]^+$ 342 (100), $[M-CH_2O]^+$ 330 (64), $[M-H_2O-Me]^+$ 327 (31); hrms *m/z* 360.1580 ($C_{20}H_{24}O_6$, error −0.7 mmu), 342.1460 ($C_{20}H_{22}O_5$, error 0.7 mmu), 330.1455 ($C_{19}H_{22}O_5$, error 1.2 mmu), 327.1227 ($C_{19}H_{19}O_5$, error 0.5 mmu).

Taspine [**3**].—^{1}H nmr δ (200 MHz, $CDCl_3$) 8.18 (1H, d, J=8.8 Hz, H-10), 7.30 (1H, d, J=8.8 Hz, H-9), 7.17 (1H, s, H-3), 4.10 (6H, s, 2×OMe), 3.49 (2H, t, J=7.8 Hz, H-15), 2.63 (2H, t, J=7.8 Hz, H-16), 2.37 (6H, s, 2×OMe); ^{13}C nmr δ (50 MHz, $CDCl_3$) 158.6 (C-12), 157.6 (C-6), 151.2 (C-8), 150.9 (C-2), 144.4 (C-4), 137.7 (C-7), 136.7 (C-1), 126.8 (C-10), 119.1 (C-14), 118.4 (C-13), 116.6 (C-3), 113.6 (C-9), 111.5 (C-11), 109.2 (C-5), 60.3 (C-16), 56.5 (2×) (2×OMe), 45.3 (2×) (2×N-Me), 33.0 (C-15); eims *m/z* (%) $[M]^+$ 369 (23), $[M-CH_3NHCH_3]^+$ 324 (86), $[M-CH_2N(CH_3)_2]^+$ 311 (100).

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