

## The Secalosides, Novel Tumor Cell Growth Inhibitory Glycosides from a Pollen Extract

Jean-Claude Jaton,<sup>\*,†</sup> Karen Roulin,<sup>†</sup> Keith Rose,<sup>†</sup> Francis M. Sirotnak,<sup>‡</sup> Ari Lewenstein,<sup>§</sup> Gérard Brunner,<sup>‡</sup> Catherine P. Fankhauser,<sup>‡</sup> and Ulrich Burger<sup>‡</sup>

Department of Medical Biochemistry, University of Geneva, 1, rue Michel-Servet, CH-1211 Geneva-4, Switzerland, Laboratory of Molecular Therapeutics, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York 10021, Cerbios-Pharma S.A., 6917-Barbengo, Switzerland, and Department of Organic Chemistry, University of Geneva, 30, quai Ernest Ansermet, CH-1211 Geneva-4, Switzerland

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The pollen of rye (*Secale cereale*) was shown to contain a biologically highly active family of glycosides called the secalosides. Secalosides A and B (**1**), both of molecular formula C<sub>46</sub>H<sub>51</sub>NO<sub>24</sub>, were found to be epimeric esters of (2-oxo-3-indolyl)acetic acid (**4**). They are made up, in addition to this heterocyclic aglycon I (**4**), of three hexose building blocks and a carbocyclic aglycon II, which is an indan-derived dicarboxylic acid (**5**). In aqueous solution, secalosides A and B interchanged by epimerization at the chiral center of **4**. A further epimeric pair, secalosides C and D (**2**), contain one additional glucose building block. Secalosides A and B, the racemic aglycon I (**4**), and 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid (**3**), which results from **4** by hydrolytic rearrangement, exhibited significant antitumor activity against S180 sarcoma *in vivo*. IC<sub>50</sub> values obtained were about 5 μg/mouse for the secalosides and 1 μg/mouse for **3** and **4**.

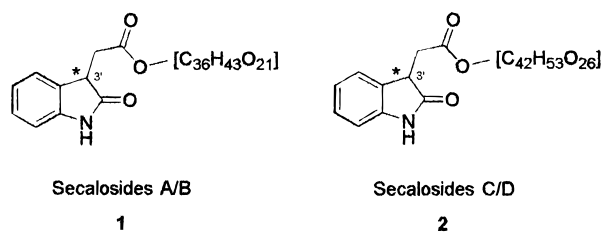
Cernilton is an extract from rye pollen that is produced commercially and used as a phytotherapeutic drug in Europe. This highly heterogeneous extract comprises two main components, namely, the hydrophilic Cernitin T-60 fraction and the hydrophobic Cernitin GBX1 fraction, both of which are devoid of allergens and high-molecular weight substances.

Clinical trials have shown the efficacy of Cernilton in the treatment of benign prostatic hyperplasia<sup>1</sup> and chronic prostatitis<sup>2</sup> without reported side effects. Cernitin T-60 was found to be the active fraction *in vivo*, as it inhibited the growth of prostate cancer cells.<sup>3,4</sup> Recent reports have described the isolation and characterization of a prostate growth inhibitory substance in the Cernitin T-60 fraction, namely, the cyclic hydroxamic acid, 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA).<sup>5,6</sup>

In another series of experiments carried out *in vivo*, the antitumor potential of Cernitin T-60 was demonstrated on Lewis lung carcinoma implanted extraperitoneally in syngeneic mice.<sup>7</sup> Similar results were obtained with mice implanted with S180 sarcoma. The survival time of mice treated with Cernitin T-60 exceeded 100% with the percentage of cured mice being 20–40%. However, synthetic DIBOA, although highly cytotoxic against a large variety of human tumor cell lines in culture, was found not to be inhibitory in *in vivo* Lewis lung carcinoma or S180 sarcoma implanted in mice (Sirotnak, F. M. Unpublished data).

These encouraging data prompted us to identify the ingredients present in Cernitin T-60 that are active *in vivo*. We now report on the isolation and biological evaluation of a family of glycosides shown to be respon-

sible for the *in vivo* biological activity of Cernitin T-60. We propose that these compounds be called secalosides A–D by virtue of their origin from rye pollen (*Secale cereale* L., Gramineae). The structures of the constituent aglycons as well as a partial gross structure for intact secalosides are presented herein. It is considered that one of these aglycons is responsible for most of the biological efficacy of the intact glycosides. The complete structure elucidation of secalosides A and B is the subject of the following paper in this issue by Jaun *et al.*<sup>8</sup>



### Results and Discussion

The strategy for the isolation of active compounds present in the Cernitin T-60 extract is summarized in Figure 1. A functional *in vivo* assay was used to monitor the biological activity through the various fractionation steps (see the Experimental Section). The compounds from peaks 4.51 (secaloside A) and 4.52 (secaloside B) were found to be equally active *in vivo*. NMR spectroscopy in water and HPLC unveiled a slow interconversion of the two compounds suggesting an equilibrium between isomers. Similarly, another pair of isomers (peaks 3.51 and 3.52), which were called secalosides C and D, was isolated from the initial fraction 3 of the gel filtration. This biologically active pair was found to be structurally related to the secalosides A and B (*vide infra*).

FAB-MS gave an identical molecular weight of 1001 Da for secalosides A and B, while that of both the

\* To whom correspondence should be addressed. Tel.: 0041-22-7025495. Fax: 0041-22-3473334. E-mail: Jaton@cmu.unige.ch.

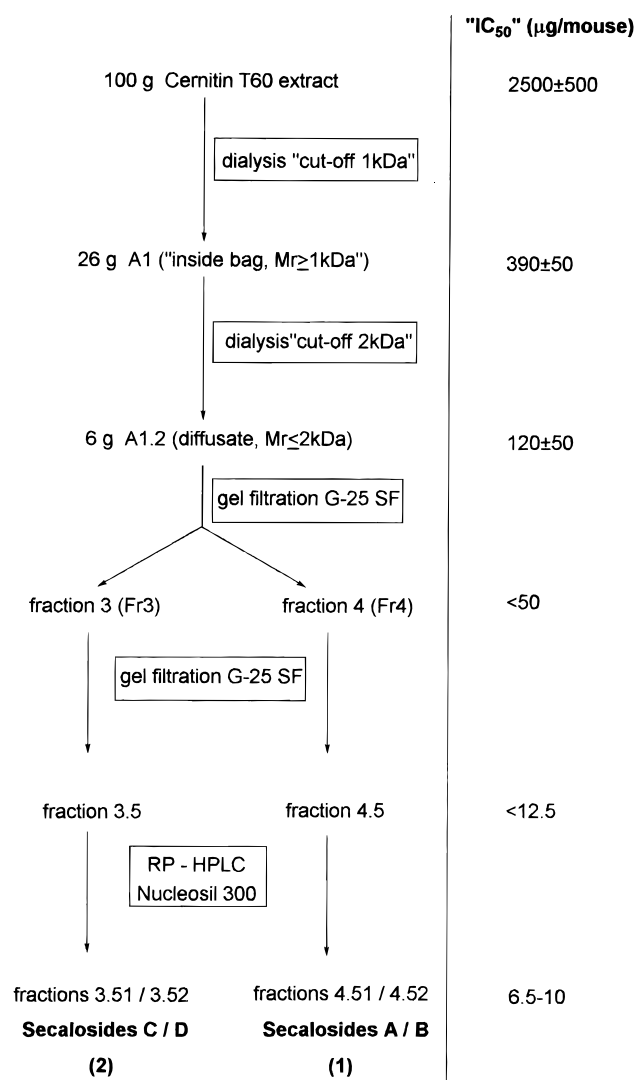
<sup>†</sup> Department of Medical Biochemistry, University of Geneva.

<sup>‡</sup> Memorial Sloan Kettering Cancer Center.

<sup>§</sup> Cerbios-Pharma S.A.

<sup>‡</sup> Department of Organic Chemistry, University of Geneva.

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**Figure 1.** Fractionation steps employed to isolate the secalosides A–D from Cernitin T-60. "IC<sub>50</sub>" values represent the inhibitory concentration of various fractions leading to a 50% reduction of S180 tumor cell growth as compared to untreated mice.

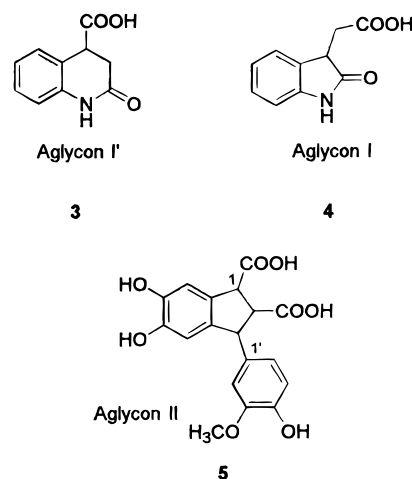
secalosides C and D was 1163 Da. The UV spectra of both compound pairs, recorded in water, were virtually indistinguishable with maxima at 280 nm and minima at 265 nm. Chemical microdeterminations<sup>9,10</sup> suggested that none of the secalosides contained an  $\alpha$ -amino acid, a hydroxamic acid, or a fatty acid as a constituent. They all stained positively for phenolic compounds and for carbohydrates.<sup>9,10</sup> Acid hydrolysis in 1 N HCl for 6 h at 100 °C qualitatively unveiled the presence of D-glucose in both pairs of secalosides, as determined by a specific enzymatic assay. In the enzymatic tests, none of the various  $\alpha$ - and  $\beta$ -glycosidases (see the Experimental Section) hydrolyzed hexoses from secalosides A or B. In contrast, when the mixture of secalosides C and D was digested for 16 h at 37 °C in the presence of  $\alpha$ -glucosidase in phosphate buffer at pH 6.8, D-glucose was released. Quantitative measurements indicated that 0.85 and 1.05 mol of glucose were released per mole of secaloside C and D, respectively. The digest was further analyzed by RP-HPLC, which indicated that the  $\alpha$ -glucosidase treatment yielded a new pair of compounds eluting with retention times indistinguishable from those of the intact secalosides A and B. This result

is consistent with the difference of 162 mass units between the molecular weight of secalosides A and B and secalosides C and D.

The products of the acid hydrolysis mentioned above (1 N HCl, 6 h at 100 °C) were analyzed by RP-HPLC and monitored at 250 nm. Hydrolysates of both pairs of secalosides (A and B and C and D) gave rise to the same elution profile, demonstrating the presence of two aglycons. Aglycon I (**4**) with a UV absorption maximum in water at 250 nm exhibited from its HREIMS the molecular formula C<sub>10</sub>H<sub>9</sub>NO<sub>3</sub>. We consider that the genuine aglycon I underwent, under the hydrolysis conditions, an isomerization into aglycon I' (**3**). For this reason, the HPLC profile showed two peaks for this aglycon in a time-dependent ratio (*vide infra*). Aglycon II (**5**) had a UV absorption maximum in water at 282 nm and a minimum at 256 nm. Its HREIMS led to the apparent molecular formula C<sub>18</sub>H<sub>14</sub>O<sub>7</sub>. Later on it was recognized that aglycon II lost one molecule of water in the spectrometer inlet prior to ionization, so that its definitive formula therefore is C<sub>18</sub>H<sub>16</sub>O<sub>8</sub>.

These findings suggest that the isomeric secalosides A and B are made of five building blocks, namely one molecule each of aglycon I and aglycon II and three hexose subunits. This is consistent with a molecular formula of C<sub>46</sub>H<sub>51</sub>NO<sub>24</sub>, which is in full agreement with the experimental molecular mass of 1001 Da. Secalosides C and D, in addition, contain one extra glucose subunit; they have the molecular formula C<sub>52</sub>H<sub>61</sub>NO<sub>29</sub>, in agreement with the experimentally determined molecular mass of 1163 Da.

An analytical sample (1.2 mg) of the nitrogen-containing aglycon I' was isolated by preparative HPLC from the products obtained by acid hydrolysis of secalosides A and B. The compound was identified by standard spectroscopic means as racemic 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid (**3**). The assignment was confirmed by an independent synthesis following a literature procedure.<sup>11,12</sup> Nevertheless, various obser-



vations suggested that the structure of **3** did not properly reflect the constitution of the corresponding building block within the native glycosides. The HPLC profile of the hydrolysis products had shown next to **3** the peak of a transient compound with a similar retention time. Moreover, the NMR spectra of the glycosides (*vide infra*) are at variance with what one might expect, were the skeleton of **3** to be present. We

therefore considered compound **3** to be an artifact resulting from a rearrangement during the hydrolysis procedure. Its most likely precursor is (2-oxo-3-indolyl)-acetic acid (OXIAA) (**4**). This compound, which is a catabolite of the plant growth hormone (3-indolyl)acetic acid (IAA),<sup>13,14</sup> has indeed been reported to undergo acid-catalyzed ring enlargement to give **3** via an opening/reclosure mechanism.<sup>11</sup> We have prepared racemic compound **4**,<sup>15</sup> which has the same retention time on the HPLC column as the transient species of the hydrolysate mentioned above, and have confirmed that **4** is the genuine aglycon I.

The aglycon II, a markedly air-sensitive compound, was found to be water soluble and to have the molecular composition C<sub>18</sub>H<sub>16</sub>O<sub>8</sub>. Its UV spectrum was similar to that of the secalosides, and it exhibited a complex bathochromic shift when recorded in 0.1 N NaOH. The <sup>1</sup>H-NMR spectrum showed the presence of two benzene rings with a 1,2,4- and a 1,2,4,5-substitution pattern, respectively. Allylic coupling revealed that an aliphatic -CHCHCH- fragment was connected at both ends to the tetrasubstituted aromatic ring. This suggested an indan skeleton, with the additional trisubstituted aromatic ring being attached to a benzylic position. The structure of 5,6-dihydroxy-3-(4-hydroxy-3-methoxyphenyl)indan-1,2-dicarboxylic acid (**5**) accounts best for these observations and for the observed <sup>13</sup>C-NMR spectra. The methoxy group was localized on the basis of long-range couplings. The ease by which compound **5** lost water in the mass spectrometer prior to ionization suggested that the two carboxyl functions were *cis* oriented and, hence, predestined for anhydride formation. However, none of the spectral parameters provided reliable information as to the relative configuration of the adjacent aromatic side chain.

According to their one- and two-dimensional <sup>1</sup>H-NMR spectra including COSY-DQF<sup>16,17</sup> recorded in D<sub>2</sub>O at 400 MHz, secalosides A and B share many structural features. Both were shown to contain the spin systems of aglycon I (**4**) and aglycon II (**5**) in a 1:1 ratio. In addition, each contained one  $\alpha$ -glucopyranose and one  $\beta$ -glucopyranose moiety, readily identified by their respective anomeric protons. The third hexose subunit of the glycosides did not show an anomeric proton, and its constitution was not immediately obvious. <sup>1</sup>H-NMR spectroscopy revealed, besides these constitutional features, that secalosides A and B interchange in solution. In D<sub>2</sub>O the equilibrium was reached within less than 24 h with an A/B ratio of roughly 1.3:1. Initially it was thought that this interchange, which seriously impeded the spectral assignment, might be catalyzed by traces of acid. When the spectra were recorded in a 3:2 mixture of MeOH-*d*<sub>4</sub>/C<sub>5</sub>D<sub>5</sub>N it was found, however, that the interchange was greatly accelerated by the base, and instead of being slowed down, it reached equilibrium in less than 1 h.

Between the two glycosides, the largest chemical shift differences were observed for the AB proton resonances of an ABM-spin pattern appearing at high field. Due to an H/D exchange, this pattern simplified in D<sub>2</sub>O slowly to an AB-appearance at  $\delta$  2.22 and 2.68 for secaloside A and at  $\delta$  2.42 and 2.48 for secaloside B, respectively. The isotopic exchange was faster than the isomerization of the glycosides. We assigned these resonances to the methylene groupings of the (2-oxo-3-

**Table 1.** *In vivo* Biological Activities of T-60 Pollen Extract and Purified Fractions Derived Thereof

extract/compd	dose ( $\mu$ g/mouse)	PCV <sup>a</sup> ( $\mu$ L)	T/C cell growth <sup>b</sup> (fraction of control)
control	0	470	1
T60 batch TMO57	1000	0	0
	500	0	0
	250	43	0.09
	125	270	0.57
<b>1</b>	60	0	0
	40	10	0.02
	20	82	0.17
	10	115	0.24
	5	210	0.45
	2.5	330	0.7
<b>3</b>	20	0	0
	10	0	0
	5	0	0
	2.5	0	0
	1.25	140	0.30
<b>4</b>	0.625	290	0.62
	20	0	0
	10	0	0
	5	0	0
	2.5	0	0
	1.25	75	0.16
	0.625	170	0.36

<sup>a</sup> PCV, packed cell volume. <sup>b</sup> T/C, treated vs control mice cell growth.

indolyl)acetyl fragment (i.e., aglycon I). The <sup>1</sup>H-NMR spectra of the glycosides, recorded in DMSO-*d*<sub>6</sub>, showed a free NH resonance at  $\delta$  10.4. Consequently, aglycon I must be bound via its carboxylic function to the remainder of the molecule. It was concluded, therefore, that secalosides A and B are esters of (2-oxo-3-indolyl)-acetic acid (**1**). They are epimers with respect to the chiral center H-C(3)\* of the nitrogen-containing heterocycle, and their mutual interconversion resulted from epimerization at this site. This is probably also true for secalosides C and D (**2**). The large number of partially overlapping signals observed in both the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of secalosides A and B, being in equilibrium, greatly hampered the assignment. It was not possible to determine the total molecular connectivity of secalosides A and B on the basis of the work done in water or pyridine/methanol. A solution to this structural problem is presented in the following paper in this issue.<sup>8</sup>

Biological experiments carried out so far with the pollen extract Cernitin T-60 have suggested that the latter can inhibit the growth of various tumor cell lines *in vivo* (S180 sarcoma, Lewis lung cancer, and mammary adenocarcinoma EO771) (Sirotnak, F. M. Unpublished data). Biological evaluation of a mixture of secalosides A and B, using the packed cell volume (PCV) assay, as described in the Experimental Section, has shown that these glycosides are highly active *in vivo*. A 10-day treatment of mice with a dose of about 5–10  $\mu$ g/animal sufficed to kill 50% of implanted S180 tumor cells in the peritoneal cavity of the animals (Table 1). The racemic aglycon I (**4**) and the artifactual isomeric aglycon I' (**3**) were included in the evaluation. Both are found to exhibit very strong antitumor activity against S180 sarcoma. Whereas the cell growth inhibitory concentration at 50% (IC<sub>50</sub>) is about 5  $\mu$ g/mouse for secalosides A and B, it is about 1  $\mu$ g/mouse or less for the aglycons I and I' (Table 1). Because of the lack of available material, the activity of aglycon II could not be evaluated.

In *in vitro* cytotoxicity assays (L1210 leukemia, S180 sarcoma, KB cells), neither Cernitin T60 pollen extract nor mixtures of secalosides A and B and C and D showed any activity, in spite of the high activities demonstrated *in vivo*. The data are particularly significant with regard to S180 sarcoma, as secalosides are active when given ip to S180-bearing mice. Putative mechanism(s) of action may involve activation of the animal's immune system for the following reasons: (1) when mice were treated with cyclophosphamide (an inhibitor of cell proliferation) 24 h prior to the implantation of S180 tumor cells, the effect of secalosides was abolished; (2) when secalosides were administered ip together with 2-chloroadenosine (an inhibitor of macrophage activation), no effect was detectable; (3) tumor-transplanted mice strains, immunodeficient in T/B cells (nude, SCID, or beige mice) did not respond to treatment with the secalosides (to be published elsewhere). Perhaps the important observation reported here is that most, if not all, of the biological activity of the secalosides appears to reside in a small moiety of the intact glycosides, i.e., the (2-oxo-3-indolyl)acetyl fragment (**4**) or its tetrahydroquinoline counterpart (**3**). Synthetic samples of the racemic compounds **4** and **3** appear to be as active as the whole intact secaloside pair on a molar basis. A further potentialization of action may be obtained upon attachment of one hexose unit, as predicted from the general gross structure of the glycoside.

## Experimental Section

**General Experimental Procedures.** The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra of the secalosides and of the aglycons were recorded on a Bruker AMX-400 spectrometer (9.4 T) operating at 400 and 100 MHz, respectively. Mass spectra of glycosides were measured by direct insertion on a MS 50 S instrument (Kratos Ltd, Manchester, England) under conditions of electron impact ionization (70 eV) with a probe temperature raised first to 150 °C for about 1 min and then to 350 °C. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were recorded in water on a Varian DMS-80 spectrophotometer within the wavelength range of 400–200 nm. Methods for the detection of biochemical compounds on paper and thin layer chromatograms were carried out as described in ref 9.

**Test Material.** Cernitin T-60 was obtained as a commercial preparation from AB Cernelle, S-4320 Vegeholm, Sweden. The water-soluble T-60 fraction (pH 4.0–4.4) contained about 60% of the total rye pollen.

**Extraction and Isolation** (cf. Figure 1). A portion (400 mL) of the 25% w/v water-soluble T-60 fraction was extensively dialyzed with distilled water for 48 h at 4 °C using Spectra/Por dialysis membrane tubing with a cutoff size of 1000 Da. The diffusate (outside fluid) was changed after 24 h. The "inside bag" content was evaporated to a small volume (100 mL) prior to lyophilization. The weight of this fraction, designated "A1", was 26 g. The diffusate was discarded. A second dialysis step was carried out with a Spectra/Por membrane tubing (cutoff 2000 Da). Fraction A1 (26 g) was redissolved in 100 mL of  $\text{H}_2\text{O}$  and dialyzed for 1 week at 4 °C. Diffusates (2 L) were collected every 2 days, evaporated, and lyophilized. The yield of recovered yellowish powder, a fraction designated A1.2, was 6 g.

This fraction (6 g) was then loaded on three Sephadex G25 (superfine grade, 4.6 cm  $\times$  120 cm) columns equilibrated and developed in distilled water at a flow rate of 20 mL/h at 4 °C; 10 mL fractions, distributed into five distinct zones, were collected and monitored at 280 nm. Fractions under each peak were lyophilized and tested for biological activity (*vide infra*). Material exhibiting *in vivo* activity in mice was contained in peaks 3 and 4. Each peak was rerun on G-25 SF columns (2.6  $\times$  100 cm) under the same elution conditions and yielded four to five well-separated subfractions. Major peak subfractions thus obtained were subjected to the biological activity test. HPLC was performed on a Varian 5000 apparatus using columns packed with a Merck reversed-phase Lichro-Cart  $\text{C}_{18}$ . The analytical column (100 mm  $\times$  4.0 mm i.d.) was operated at 1 mL/min, monitoring at 280 nm. Solvent A was 0.1% aqueous TFA, and solvent B was 0.1% TFA in acetonitrile. A gradient of 5–20% over 20 min followed by a plateau for 5 min at 60% acetonitrile was used. Major peaks were collected in Eppendorf tubes and dried down in a vacuum centrifuge (Savant SVC 200). For preparative runs, purification was achieved with a Macherey-Nagel column (250 mm  $\times$  21 mm i.d.) Nucleosil 300 Å 5- $\mu\text{m}$   $\text{C}_8$  particles. A linear gradient (10–48% acetonitrile in 0.1% aqueous TFA) was applied for 45 min at a flow rate of 5 mL/min.

**Chemical Hydrolysis.** The major, and separately, the minor epimer of glycoside **1** (secalosides A and B) and the major, and separately, the minor epimer of glycoside **2** (secalosides C and D), ca. 1–2 mg each, were hydrolyzed in 1 N HCl (100–200  $\mu\text{L}$ ) at 100 °C for periods ranging from 5 min to 6 h in small sealed glass tubes;<sup>18</sup> hydrolyzates were dried down, washed three times with 300  $\mu\text{L}$  of  $\text{H}_2\text{O}$  and finally redissolved in 0.1% aqueous TFA prior to RP-HPLC, MS, and/or NMR analyses. Each glycoside gave the compounds **3** and **5**. Insoluble material was discarded.

**Enzymatic Hydrolysis.** Glycoside mixtures **1** and **2** were subjected to a series of enzymatic digestions using either exoglycosidases or endoglycosidases, such as  $\alpha$ - and  $\beta$ -glucosidases,  $\alpha$ - and  $\beta$ -amylases,  $\alpha$ -mannosidase, and  $\alpha$ - and  $\beta$ -galactosidases, using procedures recommended by the manufacturer (Boehringer Mannheim). Digests were analyzed by RP-HPLC. The enzymatic determination of glucose was carried out by the Gluco-Quant test, according to the ad-hoc procedure (Boehringer Mannheim).

**Secaloside A (1)** (major epimer) was obtained as a grayish amorphous powder: mp 178–186 °C dec;  $[\alpha]_{\text{D}} +4.6^\circ$  (*c* 0.6, MeOH); UV ( $\text{H}_2\text{O}$ )  $\lambda_{\text{max}}$  320 (sh), 280, strong end absorption; exhibited comparable  $^1\text{H-NMR}$  data in MeOH- $d_4$  (400 MHz) as described in ref 8; FAB-MS  $m/z$   $[\text{M} + \text{H}]^+$  1002 ( $\text{C}_{46}\text{H}_{51}\text{NO}_{24}$ ).

**Secaloside B (1)** (minor epimer) was obtained as a grayish amorphous powder: mp 192–201 °C dec;  $[\alpha]_{\text{D}} +6.8^\circ$  (*c* 0.4, MeOH); UV ( $\text{H}_2\text{O}$ )  $\lambda_{\text{max}}$  320 (sh), 280, strong end absorption; exhibited comparable  $^1\text{H-NMR}$  data in MeOH- $d_4$  (400 MHz) as described in ref 8; FAB-MS  $m/z$   $[\text{M} + \text{H}]^+$  1002 ( $\text{C}_{46}\text{H}_{51}\text{NO}_{24}$ ).

**2-Oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid (3)** (racemic) was obtained as a colorless powder: mp 218–219 °C (lit.<sup>12</sup> mp 220 °C). The UV, IR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , and MS data were identical with those of an authentic sample (refs 11 and 12).

**5,6-Dihydroxy-3-(4-hydroxy-3-methoxyphenyl)-indan-1,2-dicarboxylic acid (5)** was obtained as a colorless, air-sensitive powder: UV (H<sub>2</sub>O)  $\lambda_{\max}$  (log  $\epsilon$ ) 282.6 (0.17), 250 (sh), 201 (1.41) nm; <sup>1</sup>H-NMR (D<sub>2</sub>O, 400 MHz)  $\delta$  3.76 (1 H, dd,  $J = 9.6, 8.8$  Hz, H-2), 3.80 (3 H, s, OCH<sub>3</sub>), 4.42 (1 H, br d,  $J = 8.8$  Hz, H-3), 4.76 (1 H, br d,  $J = 9.6$  Hz, H-1), 6.41 (1 H, d,  $J \leq 0.8$  Hz), 6.84 (1 H, dd,  $J = 8.2; 1.8$  Hz, H-6'), 6.90 (1 H, d,  $J = 8.2$  Hz, H-5'), 6.93 (1H, d,  $J = 1.8$  Hz, H-2'), 6.97 (1H, s); <sup>13</sup>C-NMR (D<sub>2</sub>O, 100 MHz)  $\delta$  54.1 (d, C-3), 55.0 (d, C-1), 58.6 (q, OMe), 59.6 (d, C-2), 114.3 (d), 115.1 (d), 115.4 (d), 118.2 (d), 124.2 (d), 133.5 (s), 138.8 (s), 141.4 (s), 146.4 (s), 146.5 (s), 147.5 (s), 150.2 (s), 179.2 (–CO<sub>2</sub>), 180.1 (–CO<sub>2</sub>); EIMS (70 eV)  $m/z$  342 (70) [M<sup>+</sup> – H<sub>2</sub>O], 314 (38), 270 (100), 169 (32); HREIMS  $m/z$  342.0748 (calcd for [C<sub>18</sub>H<sub>16</sub>O<sub>8</sub> – H<sub>2</sub>O], 342.0739).

**Packed Cell Volume (PCV) Assay.** The measurement of the biological activity of various peak fractions was performed by one of us (F.M.S.) at the Sloan Kettering Cancer Center, New York, NY. Briefly, mice (ca. 20 g) were injected at day 1 with 2–3 × 10<sup>5</sup> cells from S180 sarcoma into the peritoneal cavity. They were then treated at days 3, 5, 7, and 9 with different amounts of the samples to be analyzed. At day 10, mice were sacrificed and weighed. The volume of cells remaining within the peritoneal cavity was determined after 10 000 rpm centrifugation of the collected ascitic fluid, which yielded the percentage of cellular volume (V<sub>c</sub>). After draining of ascitic fluid and drying of the peritoneal cavity, mice were reweighed and the mass difference reflected ascite volume (V<sub>a</sub>) of mice. PCV volume is defined as %V<sub>c</sub> × V<sub>a</sub>. The mean PCV value of treated mice (T) was compared to that of control mice (C). A ratio T/C of, e.g., 0.5, IC<sub>50</sub> (inhibitory concentration at 50%) reflects a cell growth of 50% as compared to control. Data are the average of two experiments using six to seven mice/group (see Table 1).

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