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PLANT COUMARINS. IX.* PHENOLIC COMPOUNDS OF *Ferulopsis hystrix* GROWING IN MONGOLIA. CYTOTOXIC ACTIVITY OF 8,9-DIHYDROFUROCOUMARINS

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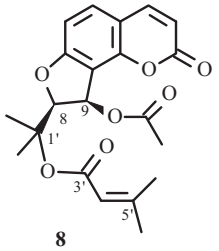
It was shown that plants of the genus Ferulopsis *are valuable sources of coumarins. Nine angular 8-substituted and 8,9-disubstituted 8,9-dihydrofurocoumarins were isolated. The cytotoxicity of these compounds was studied on models of human CEM-13, MT-4, and U-937 tumor cells. X-ray structure data were obtained for peucenidin.*

Keywords: *Ferulopsis hystrix*, coumarins, oroselol, columbianetin, peucenidin, tumor cells, XSA.

Plants of the genus *Peucedanum* are interesting to researchers as sources of coumarins. *P. hystrix* Bunge, which is distributed in South Siberia and in Mongolia, was assigned to the genus *Ferulopsis* and given the systematic name *F. hystrix* (Bunge ex Ledeb.) M. Pimen. [2]. *F. hystrix* previously yielded oroselone (**1**), oroselol (**2**), and 8-(*S*)-*O*-senecioyl-8,9-dihydrooroselol (libanorin) **3** [3]. We studied substances extracted from *F. hystrix* collected near Batkhaan Uul, Bulgan Aimak, Mongolian Republic. The concentrated EtOH extract of the aerial part and subterranean organs of the plant were worked up successively with hexane, $CHCl_3$, Et_2O, and EtOAc. The yields of extracted substances and total coumarin contents in fractions were determined after evaporation of the hexane, $CHCl_3$, and Et_2O using PMR and GC–MS data of the total fractions. The coumarin content in the EtOAc extracts was determined using spectra of $CHCl_3$ extracts of alkaline hydrolysis products. Table 1 presents the results. The total coumarin contents in the aerial part and roots of the plants were 3.9 and 4.6%, respectively. The greatest coumarin content was found in the hexane and $CHCl_3$ extracts of the EtOH extract. The extracted substances were separated by column chromatography over silica gel. 5-Hydroxy-7-methoxy-2-methylchromone (eugenin, **4**, 16% of the extract mass) and coumarins umbelliferone (**5**, 6% of extract mass), oroselone (**1**, 3%), (8,9)-dihydrooroselol (columbianetin, **6**, 3%), 8-*O*-angeloyl-8,9-dihydrooroselol (zosimin, columbianadin, **7**, 7%), (8*S*,9*R*)-9-acetoxy-8-*O*-senecioyl-8,9-dihydrooroselol (peucenidin, **8**, 6%), and (8*S*,9*R*)-8-*O*-angeloyl-9-acetoxy-8,9-dihydrooroselol (libanotin, edultin, **9**, 5%) were isolated from the hexane extract of the EtOH extract of the aerial part. Also, oroselol (**1**) and (8*S*)-2-methylbutanoyl-8,9-dihydrooroselol (**10**) were identified using spectral data. Libanorin (**3**, 8%), columbianetin (**6**, 11%), columbianadin (**7**, 9%), peucenidin (**8**, 9%), and libanotin (**9**, 7%) were isolated from the hexane extract of the EtOH extract of the subterranean organs of *F. hystrix*. Also, dihydrooroselol derivatives such as **10** (1.2% of the extract mass) and (8*S*,9*R*)-8-*O*-acetyl-9-isobutyryloxy-8,9-dihydrooroselol (**11**, 2.2%) were identified.



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*For No. VIII, see Ref. [1].

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TABLE 1. Yield of Extracted Compounds and Total Coumarins of *Ferulopsis hystrix*

Extractant	Aerial part		Roots	
	yield of extracted compounds, %	total coumarin content, %	yield of extracted compounds, %	total coumarin content, %
Hexane	1.8	1.25	2.4	1.5
$CHCl_3$	3.4	2.1	3.0	2.4
Et_2O	2.5	0.45	1.8	0.5
EtOAc	2.2	0.1	2.5	0.2

TABLE 2. Parameters of Intramolecular and Intermolecular H-bonds in the Crystal of **8**

D-H…A	D–H, Å	H…A, Å	D…A, Å	Angle D-H-A, °	Symmetry operator
C9-H9…O3	1.00	2.28	2.688 (2)	103	–
C11-H11A…O1′	0.98	2.33	3.008 (3)	125	–
C11-H11C…O4	0.98	2.55	3.088 (2)	115	–
C12-H12C…O4	0.98	2.39	2.928 (2)	114	–
C16-H16C…O4	0.98	2.18	2.942 (3)	134	–
C3-H3…O3	0.95	2.29	3.157 (3)	152	3 – x, –0.5 + y,0.5 – z
C8-H8…O2	1.00	2.52	3.470 (2)	160	–1 + x, y, z

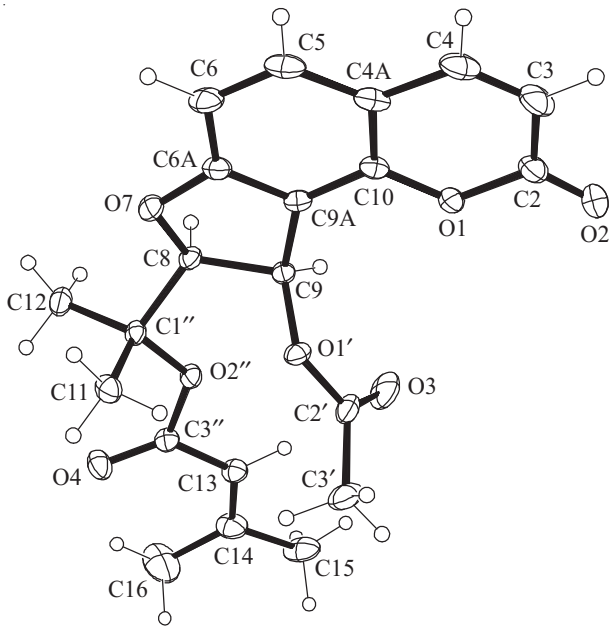

Fig. 1. Molecular structure of peucenidin (**8**) in the crystal.

Chromatography of the $CHCl_3$ extract of the EtOH extract of the aerial part of the plant concentrated and isolated the bulk of umbelliferone (**5**, 10% of the extract mass) and scopoletin (**12**, 6%) in addition to oroselone (**1**, 3%), columbianetin (**6**, 5%), libanorin (**3**, 7%), columbianadin (**7**, 4%), peucenidin (**8**, 6%), and libanotin (**9**, 9%). Angelicin (**13**, 2%), oroselol (**2**, 6%), columbianetin (**6**, 8%), libanorin (**3**, 4%), 8*S*-isovaleryl-8,9-dihydrooroselol (**14**, 3%), columbianadin (**7**, 3%), furocoumarin (**11**, 2%), umbelliferone (**5**, 8%), and (9*R*)-hydroxy-8*S*,9*R*-dihydrooroselol (vaginidiol, **15**, 8%) were isolated successively from the $CHCl_3$ extract of the EtOH extract of the subterranean organs of the plant.

Subsequent work up of the EtOH extract of the aerial part of *F. hystrix* using Et_2O isolated an additional amount of oroselol (**2**, 4% of the extract mass), columbianetin (**6**, 3%), libanorin (**3**, 2%), and peucenidin (**8**, 3%) in addition to vanillic acid (**16**, 12% yield, 23% content) and *trans*-ferulic acid (**17**, 2%, 7% content). Vanillic acid (**16**, 8%) 2′,3′-dihydrooroselol derivatives **3**, **7**, **10**, and **11** (~12%), vaginidiol (**15**, 4%), umbelliferone (**5**, 8%), and libanotin (**9**, 9%) were isolated successively from the Et_2O extract of the EtOH extract of the subterranean organs.

The coumarin contents in the EtOAc extracts of the EtOH extract were minimal (Table 1). The main product from alkaline hydrolysis of these extracts was oroselol (**2**) (according to PMR spectra of the alkaline hydrolysis products).

TABLE 3. Cytotoxicity of Isolated Furocoumarins **2**, **3**, **6**, **7**, **8**, **9**, and **15**

Compound	Tumor cells, $CCID_{50}$, μM		
	CEM-13	U-937	MT-4
2	>100	>100	>100
3	94.1	>100	70.2
6	13.2	89.4	18.1
7	>100	85.3	76.4
8	22.6	62.9	59.2
9	67.3	72.1	95.8
15	14.1	47.1	18.1

TABLE 4. Table 4. Chemical Shifts of C Atoms in ^{13}C NMR Spectra of Furocoumarins **1**–**3**, **6**–**11**, **13**–**15** (δ, ppm)

C atom*	**1**	**2**	**3**	**6**	**7**	**8**
C-2	160.62	160.69	160.76	160.91	160.72	159.70
C-3	113.88	113.95	111.91	112.07	111.86	112.96
C-4	144.32	144.36	143.78	143.83	143.75	143.35
C-4a	113.40	113.42	113.24	113.88	112.88	113.15
C-5	123.79	123.41	128.21	128.61	128.43	131.22
C-6	108.21	108.48	106.48	106.53	106.41	107.46
C-6a	156.90	156.91	163.70	163.56	163.71	163.48
C-8	157.94	164.22	88.84	91.12	89.05	88.46
C-9	99.59	97.77	27.37	27.40	27.35	68.36
C-9a	118.29	117.41	112.75	112.96	112.96	112.42
C-9b	148.08	148.12	151.05	151.14	151.06	151.56
C-1′	132.18	69.11	81.04	71.63	81.56	80.13
1′-$\underline{C}H_3$	19.06	28.58	21.45	24.06	22.46	22.19
		28.58	23.08	26.10	25.48	25.10
C atom*	**9**	**10**	**11**	**13**	**14**	**15**
C-2	159.51	160.55	159.81	160.66	160.28	160.47
C-3	113.21	112.05	113.08	113.82	113.02	112.55
C-4	143.41	143.88	143.53	144.35	144.01	143.85
C-4a	113.24	113.31	113.27	116.75	113.21	113.10
C-5	131.21	127.12	131.32	124.66	127.24	130.77
C-6	107.60	106.43	107.03	108.64	106.56	107.95
C-6a	163.39	163.05	163.52	157.19	163.18	163.50
C-8	88.32	88.68	88.19	145.72	88.59	90.97
C-9	68.11	27.12	68.33	103.92	27.21	69.98
C-9a	112.94	112.88	112.85	113.35	112.91	116.35
C-9b	151.56	152.20	151.82	148.33	152.35	151.52
C-1′	81.02	81.79	78.26		81.93	72.15
1′-$\underline{C}H_3$	23.30	21.18	22.07		21.26	26.33
	25.20	23.46	25.28		22.34	27.49

*Atomic numbering of the coumarin framework given for the structure of **8** was used; *for **1**: 114.46 (C-2′); **3**: 165.61 (C-3′), 116.77 (C-4′), 156.27 (C-5′), 22.03 (C-6′), 21.88 (C-7′); **7**: 167.02 (C-3′), 128.11 (C-4′), 137.31 (C-5′), 20.26 (C-6′), 15.67 (C-7′); **8**: 165.82 (C-3′), 116.41 (C-4′), 156.89 (C-5′), 19.93 (C-6′), 20.75 (C-7′), 168.46 ($\underline{C}OCH_3$), 27.28 (CH_3); **9**: 166.08 (C-2″), 128.46 (C-3″), 137.71 (C-4″), 15.62 (C-5″), 20.81 (C-6″), 170.51 ($\underline{C}OCH_3$), 22.32 (CH_3); **10**: 174.62 (C-3′), 44.21 (C-4′), 26.75 (C-5′), 16.23 (CH_3), 11.31 (CH_3); **11**: 174.62 s (C-2″), 170.41 ($\underline{C}OCH_3$), 42.18 (C-3″), 22.15 (CH_3), 15.58 (CH_3), 11.15 (CH_3); **14**: 173.33 (C-3′), 42.08 (C-4′), 28.46 (C-5′), 16.29 (CH_3), 12.65 (CH_3).

The structures of the compounds were established using spectral data and comparisons with the literature. An x-ray crystal structure analysis (XSA) was performed for peucenidin (**8**). Figure 1 shows the molecular structure of **8** from the XSA. The geometric parameters of **8** agreed within 3σ of the mean-statistical values [4]. Seven structures with an analogous 2′,3′-dihydrofurocoumarin framework, including (2′*S*,3′*R*)-3′-senecioyl-2-*O*-acetyl-2′,3′-dihydrooroselol (isopeucenidin) [6],

were found in the Cambridge Crystallographic Structure Database (CCSD) [5]. However, the 3D coordinates for isopeucenidin are not reported in the CCSD. Therefore, the geometric parameters of isopeucenidin and peucenidin (**8**) could not be compared. The coumarin moiety was practically planar. The mean-square deviation from the plane passing through all nonhydrogen atoms was 0.021 Å. The furan ring had the chair conformation. Atom C(8) deviated by 0.3301(3) Å from the plane of the other four ring atoms. The mean-square deviation from the plane was –0.016 Å. The angeloyl and acetate substituents were planar with mean-square deviations of the atoms from the plane of 0.026 Å (excluding the methyls) and 0.004 Å, respectively. Intramolecular H-bonds of the C–H…O type that were formed by the angeloyl and acetyl substituents were observed in **8** (Table 2). The crystal of **8** also had C–H…O H-bonds between molecules in infinite 3D-frameworks.

It can be seen that *F. hystrix* is a reliable source of furocoumarins. Two groups of dihydrofurocoumarins, i.e., 8,9-dihydrooroselol (columbianetin, **6**) and its esters **3**, **7**, **8**, **10**, **14** and vaginidiol **15** and its 8,9-diesters **8**, **9**, and **11**, were isolated in addition to oroselol (**2**). Metabolites **6**, **7**, **8**, **9**, **10**, **11**, **14**, and **15** are widely represented in plants of the genus *Peucedanum*. However, they were isolated for the first time from *F. hystrix*. 8-Substituted and 8,9-disubstituted 8,9-dihydrofurocoumarins are interesting because of their known antitumor activity. Thus, 8(*S*)-columbianetin sulfate exhibits anti-proliferative activity related to induction of apoptosis of tumor cells through activating Bax, p53, and p21 expression [7]. Dihydrofurocoumarins vaginidiol (**15**) [6, 8] and angelmarin [9] possess antitumor activity. We obtained data on the cytotoxic activity of furocoumarins **2**, **3**, **6**, **7**, **8**, **9**, and **15** for CEM-13 (T-cell leukosis), MT-4 (T-cell leukemia), and U-937 (monocyte leukemia) human tumor-cell models. The standard MTT test according to published recommendations [10] was used to determine the $CCID_{50}$. Table 3 presents the results. Compounds **6** and **15** had the greatest cytotoxicity. The selective cytotoxicity of these compounds against MT-4 and CEM-13 lymphoid tumor cells is noteworthy.

Thus, new data on the metabolite composition of *F. hystrix* (Bunge ex Ledeb.) M. Pimen. were obtained. The plant is a source of 8-substituted and 8,9-disubstituted 8,9-dihydrofurocoumarins. The isolation from *F. hystrix* extracts of the chromone eugenin (**4**) [11] and vanillic (**16**) and *trans*-ferulic (**17**) acids that are widely distributed in plants was interesting.

EXPERIMENTAL

NMR spectra of $CDCl_3$ or CD_3OD solutions were obtained on Bruker AV-300 (operating frequency 300.13 for ^1H and 75.47 MHz for ^{13}C) and AV-600 (600.30 and 150.96 MHz, respectively) spectrometers. Various types of proton–proton and carbon–proton shift correlation spectroscopy (COSY, COXH, COLOC, NOESY) were used to assign resonances in the NMR spectra. The multiplicity of resonances in ^{13}C NMR spectra was determined by recording spectra in J-mode. Table 4 presents ^{13}C NMR spectra of the furocoumarins.

A DFS Thermo Scientific high-resolution mass spectrometer (ionizing electron energy 70 eV, vaporizer temperature 230–280°C) was used to record mass spectra and determine molecular weights and elemental compositions. Melting points were determined on a Stuart SMF-38 stage. Specific rotation $[\alpha]_D^{20}$ was measured on a PolAAr3005 polarimeter.

The x-ray crystal structure analysis (XSA) of **8** was performed using ω–φ scanning (frame width 0.5°) on a Bruker Kappa Apex II diffractometer with a dual-coordinate CCD detector using Mo Kα-radiation (λ = 0.71073 Å).

Purified fractions of extracted substances were studied by GC–MS on a Hewlett–Packard 5890/II MSD GC with an HP MSD 5971 quadrupole mass spectrometer as a detector. We used a quartz column (30 m × 0.25 mm) with HP-5MS (copolymer of 5% diphenyl and 95% dimethylenesiloxane) (0.25 μm thick stationary phase) at 50–280°C (4°/min) and 280°C (15 min). The percent composition of the compounds was calculated from GC peak areas without using correction coefficients.

Pure compounds were isolated by column chromatography over silica gel (Acros, 0.035–0.070 mm) with elution by $CHCl_3$:EtOH. The purity of the isolated compounds was monitored by TLC on Silufol UV-254 plates using $CHCl_3$:EtOH (10:1) and petroleum ether:Et_2O (4:1).

Work up of Plant Material. Air-dried ground roots or aerial parts of *F. hystrix* (500 g) were exhaustively extracted with EtOH (96%) at room temperature. The EtOH extract was evaporated to an aqueous residue, diluted with distilled H_2O (1:1), and filtered. The filtrate was fractionated by solvents of increasing polarity, i.e., hexane, $CHCl_3$, Et_2O, and EtOAc. Extractions were carried out in triplicate (3 × 100 mL) with refluxing. Fractions were condensed in a rotary evaporator. Table 1 presents the yields of extracted substances. Then, the extracts were separated by column chromatography over silica gel.

Separation of Hexane Extract of the Aerial Part of *F. hystrix*. Column chromatography of the hexane extract (0.9 g) of the aerial part afforded successive fractions from which crystallization from Et_2O isolated eugenin (**4**, 144 mg), umbelliferone (**5**, 54 mg), oroselone (**1**, 27 mg), columbianetin (**6**, 27 mg), columbianadin (**7**, 63 mg), peucenidin (**8**, 54 mg),

and libanotin (**9**, 45 mg). Column chromatography of the extract (0.8 g) from the subterranean organs isolated **3** (64 mg), **6** (88 mg), **7** (72 mg), **8** (72 mg), **9** (67 mg), **10** (16 mg), and **11** (20 mg).

Column chromatography of the $CHCl_3$ extract (0.7 g) of the aerial part afforded successive fractions from which crystallization from Et_2O isolated umbelliferone (**5**, 70 mg), scopoletin (**12**, 42 mg), **1** (21 mg), **3** (35 mg), **6** (35 mg), **7** (30 mg), **8** (42 mg), and **9** (63 mg). Column chromatography of the extract (0.8 g) of the subterranean organs isolated angelicin (**13**, 16 mg), **2** (48 mg), **14** (24 mg), **6** (24 mg), **11** (16 mg), **5** (64 mg), and vaginidiol (**15**, 60 mg).

Column chromatography of the Et_2O extract (0.96 g) of the aerial part afforded successive fractions from which crystallization from Et_2O isolated oroselol (**2**, 40 mg), columbianetin (**6**, 30 mg), libanorin (**3**, 20 mg), peucenidin (**8**, 30 mg), vanillic acid (**16**, 116 mg), and *trans*-ferulic acid (**17**, 20 mg). Column chromatography of the extract (0.77 g) of the subterranean organs isolated mixtures of coumarins **3**, **7**, **10** and **11** (92 mg), **15** (30 mg), **5** (60 mg), and **9** (69 mg).

Hydrolysis of EtOAc Extracts. The EtOAc extract (1.5 g) of the EtOH extract of the subterranean organs of *F. hystrix* was dissolved in KOH solution (50 mL, 1.2 N), treated with dioxane (50 mL), heated at 80°C for 2 h, cooled, and neutralized with aqueous H_2SO_4 (10%). Coumarins were extracted by CH_2Cl_2. The solvent was evaporated. The solid was chromatographed over silica gel to isolate coumarins (68 mg) (oroselol content ~50% according to the PMR spectrum).

Characteristics of Pure Compounds. Spectral and analytical data for umbelliferone (**5**) and scopoletin (**12**) agreed with those in a review [12].

Oroselone {8-(prop-1-en-2-yl)-2*H*-furo[2,3-*h*]chromen-2-one} (1), mp 177–180°C (EtOAc), lit. [2] mp 179–180°C. IR spectrum (ν, cm^{-1}): 760, 812, 831, 907, 1020, 1123, 1154, 1374, 1553, 1617, 1680. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.13 (3H, s, CH_3), 5.24 (1H, s, H-2′), 5.82 (1H, s, H-2′), 6.36 (1H, d, J = 9.6, H-3), 6.96 (1H, s, H-9), 7.30, 7.34 (1H each, both d, J = 8.5, H-5,6), 7.77 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 226 (100), 198 (82), 183 (48), 171 (24), 155 (53), 141 (28), 115 (43), 101 (12), 75 (26), 63 (78). $C_{14}H_{10}O_3$.

Oroselol {8-(2-hydroxypropan-2-yl)-2*H*-furo[2,3-*h*]chromen-2-one} (2), mp 150–154°C (Et_2O), lit. [2] mp 149–151°C. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.74 (6H, s, 1′-CH_3), 6.35 (1H, d, J = 9.8, H-3), 6.98 (1H, s, H-9), 7.32 (1H, d, J = 8.5, H-6), 7.37 (1H, d, J = 8.5, H-5), 7.78 (1H, d, J = 9.8, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 244 (32), 229 (100), 212 (5), 201 (15), 187 (8), 171 (14), 115 (15), 101 (18), 43 (23). Found: $[M]^+$ 224.2034. $C_{14}H_{12}O_4$. Calcd: 244.2035.

Libanorin {8(*S*)-(2-senecioyloxypropan-2-yl)-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (3), mp 75–77°C (Et_2O), $[\alpha]_D$ +188.3° (c 0.88, $CHCl_3$), lit. [13] mp 79°C, $[\alpha]_D$ +197° (*c* 2.2, $CHCl_3$). UV spectrum (EtOH, λ_{max}, nm, log ε): 206 (4.11), 237 (3.68), 298 (2.77), 329 (4.13). IR spectrum (ν, cm^{-1}): 806, 832, 930, 980, 1024, 1069, 1263, 1385, 1460, 1506, 1620, 1720, 1736, 3090. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.53, 1.61 (3H each, s, 1′-CH_3), 1.86, 2.10 (3H each, s, 5′-CH_3), 3.36 (2H, m, H-9), 5.21 (1H, dd, J = 7.6, 8.2, H-8), 5.58 (1H, br.s, H-42), 6.23 (1H, d, J = 9.6, H-3), 6.80 (1H, d, J = 8.4, H-6), 7.27 (1H, d, J = 8.4, H-5), 7.62(1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 328 (10), 303 (10), 286 (5), 244 (16), 243 (11), 229 (42), 227 (15), 201 (8), 187 (16), 83 (100), 43 (25). Found: $[M]^+$ 328.1314. $C_{19}H_{20}O_5$. Calcd: 328.1311.

Eugenin (5-hydroxy-2-methyl-7-methoxy-4*H*-chromen-4-one) (4), oil. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.42 (3H, s, CH_3), 3.85 (3H, s, OCH_3), 6.03 (1H, s, H-3), 6.29 (1H, d, J = 2.0, H-6), 6.35 (1H, d, J = 2.0, H-8), 12.67 (1H, s, OH). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 20.59 (CH_3), 55.75 (OCH_3), 92.32 (C-8), 98.22 (C-6), 105.24 (C-4a), 108.65 (C-3), 158.18 (C-8a), 162.54 (C-5), 165.41 (C-7), 166.49 (C-2), 182.44 (C-4). Mass spectrum (*m*/*z*, I_{rel}, %): 206 (100), 189 (35), 177 (20). Found: $[M]^+$ 206.0579. $C_{11}H_{10}O_4$. Calcd: 206.0571.

Columbianetin {8(*S*)-(2-hydroxypropan-2-yl)-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (6), mp 160–163°C (Et_2O), $[\alpha]_D$ +195.36° (c 1.2, $CHCl_3$), lit. [7] mp 163°C, $[\alpha]_D$ +264° (*c* 1.66, CH_3OH). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.22, 1.35 (3H each, s, 12-CH_3), 3.30 (2H, m, H-9), 4.77 (1H, t, J = 8.6, H-8), 6.18 (1H, d, J = 9.6, H-3), 6.73 (1H, d, J = 8.2, H-6), 7.26 (1H, d, J = 8.2, H-5), 7.60 (1H, d, J = 9.6, H-4), 9.50 (1H, s, OH). Mass spectrum (*m*/*z*, I_{rel}, %): 246 (56), 228 (10), 213 (14), 203 (10), 187 (100), 175 (25), 160 (48), 131 (21), 103 (12), 77 (22), 59 (69). Found: $[M]^+$ 246.0964. $C_{14}H_{14}O_4$. Calcd: 246.0969.

Columbianadin {8(*S*)-(2-angeloyloxypropan-2-yl)-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (7), mp 117–120°C (Et_2O), $[\alpha]_D$ +225.6° (c 1.8, $CHCl_3$), lit. [14] mp 118.5–119°C, $[\alpha]_D$ +227° (*c* 2.8, $CHCl_3$). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.62 (3H, br.s, 4′-CH_3), 1.63 (3H each, s, 1′-CH_3), 1.85 (3H, dq, J = 5.0, 0.8, 5′-CH_3), 3.36 (2H, m, H-9), 5.18 (1H, dd, J = 7.8, 8.2, H-8), 5.98 (1H, m, H-5′), 6.20 (1H, d, J = 9.8, H-3), 6.68 (1H, d, J = 8.2, H-6), 7.22 (1H, d, J = 8.2, H-5), 7.60 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 328 (10), 270 (8), 257 (6), 246 (5), 228 (35), 213 (100), 187 (18), 131 (16), 83 (16), 55 (42). Found: $[M]^+$ 328.1314. $C_{19}H_{20}O_5$. Calcd: 328.1311.

Peucenidin {(8*S*,9*R*)-9-acetoxy-8-(2-senecioyloxypropan-2-yl)-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (8), mp 122–125°C (EtOAc), $[\alpha]_D$ –48.2° (c 1.23, $CHCl_3$), lit. [13, 15] $[\alpha]_D$ –46°. UV spectrum (EtOH, λ_{max}, nm, log ε): 207

(5.12), 226 (4.12), 298 (3.77), 322 (4.68). IR spectrum (ν, cm^{-1}): 806, 848, 862, 938, 980, 1005, 1024, 1053, 1075, 1114, 1132, 1320, 1354, 1490, 1576, 1620, 1647, 1727, 1752, 3093. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.61, 1.70 (3H each, s, 1′-CH_3), 1.86, 2.11 (3H each, s, 5′-CH_3), 2.02 (3H, s, C$\underline{H}_3$C=O), 5.15 (1H, d, J = 7.0, H-8), 5.58 (1H, br.s, H-4′), 6.21 (1H, d, J = 9.6, H-3), 6.82 (1H, d, J = 8.5, H-6), 6.97 (1H, d, J = 7.0, H-9), 7.40 (1H, d, J = 8.5, H-5), 7.61 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 386 (15), 344 (10), 326 (12), 311 (18), 286 (22), 261 (12), 244 (28), 243 (15), 229 (40), 227 (32), 201 (24), 186 (22), 158 (29), 128 (25), 102 (26), 83 (100), 55 (26), 43 (29). Found: $[M]^+$ 386.1364. $C_{21}H_{22}O_7$. Calcd: 386.1360.

Libanotin (edultin) {(8*S*,9*R*)-9-(anegloyloxypropan-2-yl)-8-acetoxy-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (9), mp 154–156°C (hexane), $[\alpha]_D$ +78.6° (c 1.3, $CHCl_3$), lit. [6] mp 155–157°C, $[\alpha]_D$ +79.5° (*c* 0.83, $CHCl_3$). UV spectrum (EtOH, λ_{max}, nm, log ε): 207 (5.12), 232 (4.21), 300 (3.36), 318 (4.21). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.59, 1.71 (3H each, s, 1′-CH_3), 1.81 (3H, dq, J = 5.2, 0.8, 4″-CH_3), 1.92 (3H, br.s, 3″-CH_3), 2.01 (3H, s, C$\underline{H}_3$C=O), 5.28 (1H, d, J = 7.0, H-8), 6.08 (1H, m, H-3″), 6.23 (1H, d, J = 9.6, H-3), 6.85 (1H, d, J = 8.5, H-6), 7.02 (1H, d, J = 7.0, H-9), 7.41 (1H, d, J = 8.5, H-5), 7.62 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 386 (18), 311 (12), 287 (12), 261 (8), 243 (32), 227 (18), 187 (73), 83 (100), 43 (54). Found: $[M]^+$ 386.1368. $C_{21}H_{22}O_7$. Calcd: 386.1360.

{(8*S*,11*S*)-[2-(2-Methylbutanoyl)oxypropan-2-yl]-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (10), mp 85–88°C (Et_2O), $[\alpha]_D$ +192.6° (c 1.1, $CHCl_3$), lit. [7] mp 88–91°C, $[\alpha]_D$ +194° (*c* 0.67, $CHCl_3$). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.85 (3H, t, J = 7.0, 5′-CH_3), 1.03 (3H, d, J = 7.0, 4′-CH_3), 1.30, 1.45 (1H each, m, J = 7.0, H-5′), 1.50, 1.56 (3H each, s, 1′-CH_3), 2.89 (1H, m, H-4′), 3.28 (1H, m, J_{gem} = 15.7, H-9), 3.36 (1H, m, J_{gem} = 15.7, H-9), 5.08 (1H, dd, J = 7.6, 9.4, H-8), 6.18 (1H, d, J = 9.6, H-3), 6.71 (1H, d, J = 8.2, H-6), 7.22 (1H, d, J = 8.2, H-5), 7.60 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 330 (16), 305 (8), 259 (6), 231 (30), 213 (100), 187 (31), 176 (28), 85 (32), 57 (46). Found: $[M]^+$ 330.3459. $C_{19}H_{22}O_5$. Calcd: 330. 3467.

{(8*S*,9*R*)-8-(2-Acetoxypropan-2-yl)-9-(2-methylpropanoyloxy)-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (11), mp 153–156°C (Et_2O), $[\alpha]_D$ +100.2° (c 0.6, $CHCl_3$), lit. [8] mp 154–156°C, $[\alpha]_D$ +97.9° (*c* 0.19, $CHCl_3$). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.17 (3H, d, J = 6.8, 3″-CH_3), 1.19 (3H, d, J = 7.0, 3″-CH_3), 1.59, 1.75 (3H each, s, 1′-CH_3), 2.05 (3H, s, C$\underline{H}_3$C=O), 2.53 (1H, m, H-3″), 5.28 (1H, d, J = 6.7, H-8), 6.20 (1H, d, J = 9.6, H-3), 6.84 (1H, d, J = 8.5, H-6), 7.00 (1H, d, J = 6.7, H-9), 7.42 (1H, d, J = 8.5, H-5), 7.62 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 374 (15), 314 (8), 299 (12), 286(15), 261 (18), 244 (33), 229 (100), 213 (22), 187 (38), 158 (20), 71 (54), 43 (61). Found: $[M]^+$ 374.2257. $C_{20}H_{22}O_7$. Calcd: 374.2259.

Angelicin (2*H*-furo[2,3-*h*]chromen-2-one) (13), mp 133–136°C (Et_2O), lit. [16] mp 136–137°C. UV spectrum (EtOH, λ_{max}, nm, log ε): 251 (4.12), 302 (3.85), 326 (4.04). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 6.36 (1H, d, J = 9.6, H-3), 7.10 (1H, dd, J = 2.0, 1.6, H-3′), 7.34 (1H, d, J = 8.5, H-5), 7.41 (1H, dd, J = 8.5, 1.6, H-5), 7.68 (1H, d, J = 2.0, H-2′), 7.79 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 186 (100), 158 (88), 149 (27), 130 (14), 102 (28). Found: $[M]^+$ 186.0312. $C_{11}H_6O_3$. Calcd: 186.0317.

{8*S*-[(2-Isovaleryl)oxypropan-2-yl]-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (14), mp 72–74°C (Et_2O), $[\alpha]_D$ +302.5° (c 1.1, $CHCl_3$), lit. [13] mp 76–77°C, $[\alpha]_D$ +305° (c 0.4, CH_3OH). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.91 (3H, t, J = 7.1, 5′-CH_3), 1.16 (3H, d, J = 7.0, 5′-CH_3), 1.48, 1.62 (1H each, m, H-4′), 1.60, 1.68 (3H each, s, 1′-CH_3), 2.36 (1H, m, H-5′), 3.30 (1H, m, J_{gem} = 15.9, H-9), 3.36 (1H, m, J_{gem} = 15.9, H-9), 5.06 (1H, dd, J = 7.8, 9.6, H-8), 6.19 (1H, d, J = 9.6, H-3), 6.72 (1H, d, J = 8.4, H-6), 7.22 (1H, d, J = 8.4, H-5), 7.61 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 330 (10), 281 (5), 228 (30), 213 (100), 187 (28), 176 (20), 159 (11), 131 (18), 85 (25), 57 (31). Found: $[M]^+$ 330. 3461. $C_{19}H_{22}O_5$. Calcd: 330. 3467.

Vaginidiol {(8*S*,9*R*)-9-hydroxy-8-(2-hydroxypropan-2-yl)-8,9-dihydro-2*H*-furo[2,3-*h*]chromen-2-one} (15), mp 170–173°C (EtOH), $[\alpha]_D$ +224.2° (c 1.2, $CHCl_3$), lit. [17] mp 168–169°C, $[\alpha]_D$ +231° (c 0.01, EtOH). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.48, 1.55 (3H each, s, 1′-CH_3), 4.20 (1H, br.s, OH), 4.35 (1H, d, J = 5.6, H-8), 5.77 (1H, d, J = 5.6, H-9), 6.20 (1H, d, J = 9.6, H-3), 6.82 (1H, d, J = 8.2, H-6), 7.35 (1H, d, J = 8.2, H-5), 7.63 (1H, d, J = 9.6, H-4). Mass spectrum (*m*/*z*, I_{rel}, %): 262 (30), 229 (19), 213 (15), 203 (19), 191 (27), 188 (22), 187 (50), 186 (100), 158 (62), 149 (27), 134 (22), 131 (32), 129 (17), 102 (34), 89 (19), 59 (61). Found: $[M]^+$ 262.0832. $C_{14}H_{14}O_5$. Calcd: 262.0836.

4-Hydroxy-3-methoxybenzoic acid (vanillic acid) (16), mp 210–213°C (Et_2O), lit. [18] mp 213–214°C. Mass spectrum $[M]^+$ 168 (100).

***trans*-Ferulic acid (17)**, mp 165–167°C (Et_2O), lit. [19] mp 168–169°C. Mass spectrum $[M]^+$ 194 (100).

X-ray Crystal Structure Analysis of Peucenidin (8). Crystals of **8** were orthorhombic, space group $P2_12_12_1$, *a* = 8.3816(4), *b* = 13.7298(6), *c* = 17.3074(9) Å, *V* = 1991.7(2) $Å^3$, *Z* = 4, $C_{21}H_{22}O_7$, d_{calc} = 1.289 g/cm^3, μ = 0.097 mm^{-1}, F(000) = 816, crystal size 0.04 × 0.08 × 0.55 mm, 4510 independent reflections. Absorption corrections were applied using

the SADABS program [20] (transmission 0.62–0.75). The structure was solved using the SHELXS-97 program [21] and refined by full-matrix anisotropic least-squares methods (except for H atoms) using the SHELXL-97 program [2]. Positions of H atoms of **8** were calculated geometrically and refined using a rider model (H-atom parameters were calculated in each refinement cycle using the coordinates of the corresponding C atoms). The final structure refinement was performed over all F^2 to wR_2 = 0.1066, S = 1.03. A total of 253 parameters (R = 0.0399 for 3888 $F > 4\sigma$) were refined. The molecular geometry and intermolecular interactions were analyzed using the PLATON program [22]. A CIF-file containing complete information for the structure was deposited in the CCDC, No. 840240, from where it can be accessed by request at the internet site www.ccdc.cam.ac.uk/data_request/cif.

Cell Culture. Human tumor cells MT-4, CEM (human T-cell leucosis), and U-937 (human monocytes) were used. Cells were cultivated in RPMI-1640 medium containing fetal calf serum (10%), L-glutamine (2 mmol/L), gentamicin (80 μg/mL), and lincomycin (30 mg/mL) at 37°C in a CO_2 incubator. Test compounds were dissolved in DMSO and added to the cell culture in the required concentrations. Three wells at each concentration were used. Cells incubated without added test compounds were used as the control.

MTT Test. The standard MTT test [10] was used to determine the $CCID_{50}$ values (doses inhibiting cell viability by 50%).

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