Chemical Modification of Fumagillin. I. 6-*O*-Acyl, 6-*O*-Sulfonyl, 6-*O*-Alkyl, and 6-*O*-(*N*-Substituted-carbamoyl)fumagillols

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The hydroxy group of fumagillol (3), a degradation product of fumagillin (1), was acylated, sulfonylated, alkylated or carbamoylated, and the anti-angiogenic activity of the resulting products was examined. These compounds inhibited the angiogenesis induced by basic fibroblast growth factor in the rat corneal micropocket assay and the growth of vascular endothelial cells *in vitro*. Among them, compound 2 (AGM-1470) was found to show the most potent inhibitory effect on the growth of vascular endothelial cells and was selected from this series as a candidate for further development.

Keywords fumagillin; fumagillol; angiogenesis; anti-angiogenic activity; AGM-1470

Since growth of solid tumors is thought to be dependent on angiogenesis, it has been suggested that regulation of tumor-angiogenesis may provide a new approach for cancer therapy.1) Several angiogenesis inhibitors have been reported in recent years. Among them protamine,2) angiostatic steroids in combination with heparin,3) sulfated polysaccharide–peptidoglycan complex,4) platelet factor-4,5) GPA-1734,6) and D6097) are reported to exhibit anti-tumor activity in addition to anti-angiogenic activity. However, these agents have problems with regard to efficacy, toxicity and structural heterogeneity.

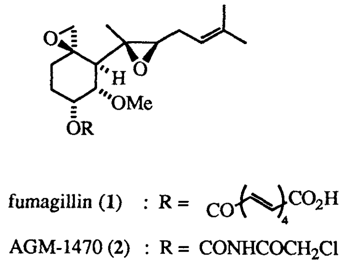
Recently Ingber *et al.*8) reported that fumagillin (**1**), an antibiotic known for its anti-bacteriophage9) and anti-amoeba10) activities, showed potent anti-angiogenic activity. With regard to anti-tumor activity, DiPaolo *et al.*11) reported that fumagillin (**1**) inhibited the growth of Ehrlich ascites and solid carcinoma C3HBA and C-755 in mice. They also reported that these inhibitory effects were accompanied by a 10 to 15% reduction in body weight of the host. Anti-angiogenic activity of **1** may contribute to the inhibitory effect on the growth of murine solid tumors, however, weight loss may be suggestive of toxicity.

Therefore, we started chemical modification of fumagillin (**1**) in an effort to obtain analogues retaining the potent anti-angiogenic activity of **1** but having less toxicity. As reported previously,12) one of the analogues, AGM-1470 (**2**), has been found to show more potent anti-angiogenic activity and to be less toxic than **1**.

In this paper, we describe the preparation of AGM-1470 (**2**) and the related compounds, that is, replacement of the polyenecarboxylic acid group of **1** with other acyl, sulfonyl, alkyl, or carbamoyl groups *via* fumagillol (**3**).13) The anti-angiogenic activity of these compounds is also reported.

Chemistry There have been only a few reports on acylation of fumagillol (**3**) and its analogues without opening the two epoxides. Tarbell *et al.*14) obtained 6-*O*-acetyldihydrofumagillol from dihydrofumagillol using acetic anhydride and pyridine. However, the reaction time was long (36 h) and the yield was relatively low (less than 30%). Corey *et al.*15) used acid chloride and a strong base, methyllithium, in the final step of the total synthesis of fumagillin (**1**); however, the yield was not mentioned in their paper.

As these methods are not convenient for the preparation of various kinds of 6-*O*-acylfumagillols, we tried to find new methods for the acylation of **3**. The hydroxy group at the C-6 position of **3** is axial and consequently in a rather hindered condition. The combination of acid anhydride, pyridine and a catalytic amount of 4-dimethylaminopyridine (DMAP) is a well-known method for the acylation of a hindered hydroxy group. However, the method required a long reaction time (more than 1 d) and the yield was not so high. We then tried acid anhydride and a stoichiometric amount of DMAP in dichloromethane. Under this reaction



fumagillin (1) : R = $\mathrm{CO(CH{=}CH)_4CO_2H}$

AGM-1470 (2) : R = $\mathrm{CONHCOCH_2Cl}$

Chart 1

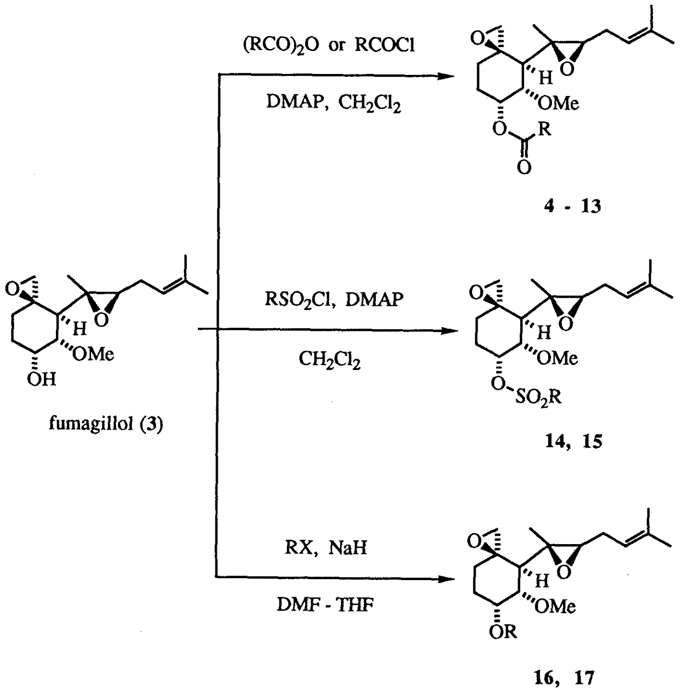


Chart 2

condition, 6-*O*-acetylfumagillol (**4**) was obtained in a 76% yield within 1 h.

We then searched for a suitable base to use with acid chlorides. Benzoyl chloride was employed as the acylation reagent, and pyridine, triethylamine, diisopropylethylamine, or a catalytic amount of DMAP with these bases were examined. However, chlorine-containing compounds,[13] which may be produced by the nucleophilic attack of chlorine on epoxides, were the main products. Only when a stoichiometric amount of DMAP was employed as the base, was 6-*O*-benzoylfumagillol (**6**) obtained in high yield (96%).

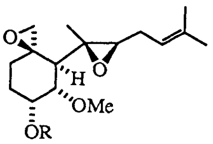
Several other 6-*O*-acylfumagillols were obtained by the methods mentioned above, and 6-*O*-sulfonylfumagillols were obtained by a similar method using DMAP and sulfonyl chloride.

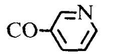
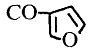
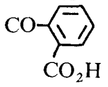
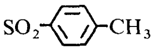
Though there have been no reports on alkylation of fumagillol, 6-*O*-alkylfumagillols were obtained by a method using NaH as a base in a mixed solvent of dimethylformamide (DMF) and tetrahydrofuran (THF).

These results are shown in Chart 2 and Table I.

6-*O*-(*N*-Monosubstituted-carbamoyl)fumagillols were prepared using isocyanates in the presence of DMAP (method A). When it was not easy to obtain isocyanates or 6-*O*-(*N*,*N*-disubstituted-carbamoyl)fumagillols were required, the method *via* 6-*O*-phenoxycarbonylfumagillol (**28**) was used (method B); compound **28**, obtained from **3** using phenyl chloroformate and DMAP, was treated with the corresponding amines. In method B when ammonia water was employed in place of amines, 6-*O*-carbamoylfumagillol

TABLE I. 6-*O*-Acyl, 6-*O*-Sulfonyl and 6-*O*-Alkyl Fumagillols



Compd. No.	R	Yield (%)	$[\alpha]_D$ (°) ($CHCl_3$)	Mass spectrum (*m*/*z*)
4[a)]	$COCH_3$	76	−105.2 (c=0.21, 24°C)	324 (M^+), 292, 256, 223, 213
5[a)]	$CO(CH_2)_2CH_3$	55	−49.8 (c=0.24, 22°C)	352 (M^+), 334, 302, 283, 265
6[b)]	COPh	96	−48.7 (c=0.22, 22°C)	386 (M^+), 354, 317, 301, 285
7[b)]	$CO(CH_2)_6CH_3$	43	−49.4 (c=0.22, 22°C)	408 (M^+), 390, 376, 358, 339
8[b)]	$CO(CH_2)_3Cl$	75	−54.0 (c=0.20, 26°C)	388 (M^++2), 386 (M^+), 354, 317
9[b)]		92	−41.1 (c=0.19, 26°C)	378 (M^+), 318, 302, 290, 247
10[b)]		28	−51.4 (c=0.22, 26°C)	376 (M^+), 344, 307, 275, 258
11[a,c)]	$CO(CH_2)_2CO_2H$	78	−77.7 (c=0.22, 23°C)	382 (M^+), 364, 350, 281, 251
12[a,c)]	$COCH_2OCH_2CO_2H$	71	−41.6 (c=0.22, 23°C)	398 (M^+), 383, 366, 347, 317
13[a,c)]		67	−5.7 (c=0.21, 23°C)	430 (M^+), 361, 343, 329, 317
14		61	−35.6 (c=0.22, 24°C)	436 (M^+), 405, 367, 351, 345
15	SO_2CH_3	87	−61.4 (c=0.22, 24°C)	360 (M^+), 329, 292, 259, 229
16	CH_3	95	−6.2 (c=0.53, 24°C)	296 (M^+), 278, 264, 246, 227
17	CH_2Ph	96	−52.8 (c=0.25, 24°C)	372 (M^+), 354, 303, 273, 241

a) Acid anhydrides were used. *b*) Acid chlorides were used. *c*) Pyridine was used as solvent instead of CH_2Cl_2.

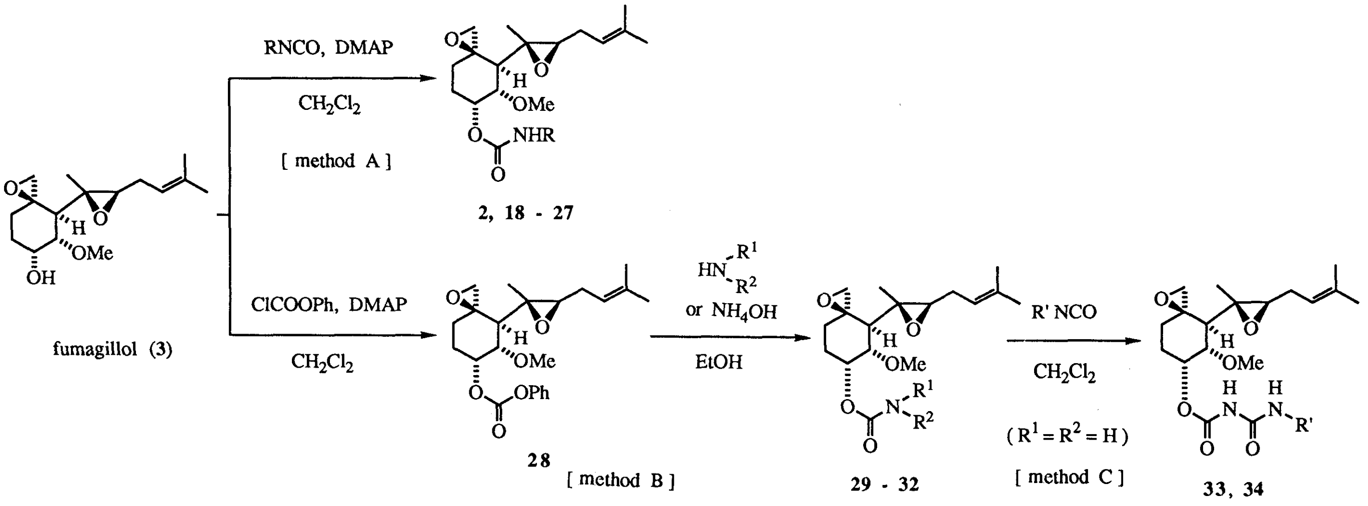
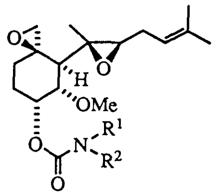
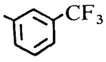
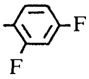
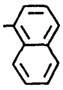


Chart 3

TABLE II. 6-*O*-(*N*-Substituted-carbamoyl)fumagillols



Compd. No.	R^1	R^2	Method[a]	Yield (%)	$[\alpha]_D$ (°) ($CHCl_3$)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
18	H	C_3H_7	A	49	−63.4 (c=0.24, 24°C)	$C_{20}H_{33}NO_5$	65.37 (65.09	9.05 9.09	3.81 3.76)
19	H	CH_2CH_2Cl	A	28	−50.9 (c=0.23, 23°C)	$C_{19}H_{30}NO_5Cl$	58.83 (58.67	7.80 7.51	3.61 3.77)
20	H	Ph	A	39	−55.3 (c=0.25, 24°C)	$C_{23}H_{31}NO_5$	68.80 (68.52	7.78 7.84	3.49 3.60)
21	H		A	82	−48.4 (c=0.22, 24°C)	$C_{24}H_{30}NO_5F_3$	61.40 (61.12	6.44 6.41	2.98 2.92)
22	H		A	63	−41.4 (c=0.21, 23°C)	$C_{23}H_{29}NO_5F_2$	63.15 (62.87	6.68 6.80	3.20 3.15)
23	H		A	46	−54.0 (c=0.10, 24°C)	$C_{27}H_{33}NO_5$	71.82 (71.59	7.37 7.21	3.10 3.07)
24	H	$COCH_3$	A	91	−46.7 (c=0.20, 23°C)	$C_{19}H_{29}NO_6 \cdot H_2O$	59.20 (58.89	8.11 7.90	3.63 3.46)
2	H	$COCH_2Cl$	A	71	−46.6 (c=0.44, 24°C)	$C_{19}H_{28}NO_6Cl \cdot 2/5H_2O$	55.78 (55.97	7.10 6.87	3.42 3.42)
25	H	$COCHCl_2$	A	90	−36.3 (c=0.41, 24°C)	$C_{19}H_{27}NO_6Cl_2$	52.30 (52.01	6.24 6.53	3.21 3.02)
26	H	$COCCl_3$	A	44	−46.0 (c=0.22, 24°C)	$C_{19}H_{20}NO_6Cl_3$	48.48 (48.18	5.57 5.77	2.98 2.85)
27	H	COPh	A	58	−45.4 (c=0.32, 28°C)	$C_{24}H_{31}NO_6 \cdot H_2O$	65.74 (65.85	7.36 7.29	3.19 3.11)
29	H	H	B	84	−73.3 (c=0.21, 22°C)	$C_{17}H_{27}NO_5$	62.75 (62.92	8.36 8.56	4.30 4.35)
30	$-(CH_2)_2-O-(CH_2)_2-$		B	87	−27.2 (c=0.22, 22°C)	$C_{21}H_{33}NO_6$		ND[b]	
31	$-(CH_2)_5-$		B	99	−66.3 (c=0.20, 22°C)	$C_{22}H_{35}NO_5$		ND[b]	
32	H	NH_2	B	50	−69.7 (c=0.20, 23°C)	$C_{17}H_{28}N_2O_5$	59.98 (60.07	8.29 8.45	8.23 7.98)
33	H	$CONHCOCH_2Cl$	C	84	−29.9 (c=0.20, 23°C)	$C_{20}H_{29}N_2O_7Cl \cdot 1/2H_2O$	52.92 (52.83	6.66 6.60	6.17 6.33)
34	H	CONHCOPh	C	34	−63.4 (c=0.24, 24°C)	$C_{25}H_{32}N_2O_7 \cdot 1/2H_2O$	62.36 (62.08	6.91 6.80	5.82 5.72)

a) See experimental. *b*) Not determined (oil); correct $[M^+]$ was observed in MS (see Experimental).

(**29**) was obtained. 6-*O*-(*N'*-Substituted-allophanoyl)fumagillols were prepared from **29** using highly reactive isocyanates (method C) (Chart 3 and Table II).

Biological Activity and Discussion Anti-angiogenic activity of some representative compounds was examined (Table III). At first, the rat corneal micropocket assay was carried out. The effect of the compounds on angiogenesis induced by basic fibroblast growth factor (bFGF) was examined by local administration and the ratio of the number of corneas in which inhibition of angiogenesis was observed per total number of corneas is shown as a percentage. All the compounds examined were found to show anti-angiogenic activity.

TABLE III. Anti-angiogenic Property of Fumagillin Analogues

Compound No.	Corneal assay inhibited/total (%)	Inhibition of HUVE cell proliferation IC_{50} (ng/ml)
1	100	0.20
2	100	0.01
8	80	0.074
19	100	0.016
24	100	0.059
27	75	0.042
33	100	0.09

Angiogenesis is thought to proceed *via* several steps.[1] One of the steps that fumagillin (**1**) inhibits is the proliferation of endothelial cells.[8] Therefore, the inhibitory effect of the analogues on the proliferation of human umbilical vein endothelial (HUVE) cells were examined. All these compounds were found to show more potent inhibitory effect than **1**.

As reported previously,[8] the inhibitory effect of compound **2** (AGM-1470) on the proliferation of HUVE cells is cytostatic at *ca.* 10 pg/ml and cytotoxicity (reduction of cell number to less than the initial plating density) is only observed at much higher concentrations (> 1 μg/ml). The inhibitory effect of the analogues was also cytostatic at the concentration shown in Table III.

Endothelial cells are known to be remarkably quiescent cells under normal conditions.[16] It may therefore be possible that fumagillin (**1**) and its analogues selectively inhibit the proliferation of endothelial cells accelerated under unusual conditions and have little influence on endothelial cells under normal conditions.

AGM-1470 (**2**) showed the most potent cytostatic effect on the proliferation of HUVE cells among the compounds examined. Furthermore, AGM-1470 (**2**) was found to inhibit the tube formation of endothelial cells.[12] Therefore, AGM-1470 (**2**) was selected as a candidate for further development, and its antitumor activity against solid tumors is now under investigation.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DPI-181. Infrared (IR) spectra were taken on a JASCO IR-810 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini-200, and chemical shifts are given in ppm with tetramethylsilane as the internal standard. Mass spectra (MS) were obtained on a Hitachi RMU-6D.

Synthesis of 6-*O*-Acylfumagillols. General Procedure Acid anhydride or acid chloride (0.79 mmol) was added dropwise to a solution of fumagillol (**3**, 0.53 mmol) and DMAP (1.06 mmol) in dry dichloromethane (2 ml). The mixture was stirred for 1 h at room temperature, then diluted with AcOEt and washed with saturated aqueous $NaHCO_3$ and brine. The AcOEt solution was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel using AcOEt : hexane = 1 : 4 as the eluent.

6-*O*-Acetylfumagillol (4) Colorless oil. IR (neat) cm^{-1}: 2950, 1740, 1375, 1240, 1205, 1130, 1105. NMR ($CDCl_3$) δ: 1.09 (1H, m), 1.21 (3H, s), 1.66 (3H, s), 1.74 (3H, s), 1.80—2.45 (5H, m), 1.96 (1H, d, J=11 Hz), 2.10 (3H, s), 2.55 (1H, d, J=4 Hz), 2.59 (1H, t, J=6 Hz), 2.99 (1H, d, J=4 Hz), 3.43 (3H, s), 3.64 (1H, dd, J=3, 11 Hz), 5.21 (1H, m), 5.65 (1H, m).

6-*O*-Butanoylfumagillol (5) Colorless oil. IR (neat) cm^{-1}: 2955, 1735, 1445, 1375, 1250, 1175, 1105. NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7 Hz), 1.08 (1H, m), 1.21 (3H, s), 1.66 (3H, s), 1.74 (3H, s), 1.60—2.50 (7H, m), 1.95 (1H, d, J=11 Hz), 2.34 (2H, dt, J=7, 2 Hz), 2.55 (1H, d, J=4 Hz), 2.59 (1H, t, J=6 Hz), 2.99 (1H, d, J=4 Hz), 3.43 (3H, s), 3.64 (1H, dd, J=3, 11 Hz), 5.21 (1H, m), 5.65 (1H, m).

6-*O*-Benzoylfumagillol (6) Colorless oil. IR (neat) cm^{-1}: 2925, 1720, 1450, 1380, 1270, 1115, 1070, 1025. NMR ($CDCl_3$) δ: 1.18 (1H, m), 1.25 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.50—2.70 (5H, m), 2.08 (1H, d, J=11 Hz), 2.59 (1H, d, J=4 Hz), 2.65 (1H, t, J=6 Hz), 3.04 (1H, d, J=4 Hz), 3.48 (3H, s), 3.76 (1H, dd, J=3, 11 Hz), 5.23 (1H, m), 5.90 (1H, m), 7.30—7.70 (3H, m), 8.00—8.10 (2H, m).

6-*O*-Octanoylfumagillol (7) Colorless oil. IR (neat) cm^{-1}: 2920, 1735, 1440, 1375, 1245, 1160, 1105. NMR ($CDCl_3$) δ: 0.87 (3H, m), 1.08 (1H, m), 1.22 (3H, s), 1.28 (8H, m), 1.66 (3H, s), 1.74 (3H, s), 1.55—2.45 (7H, m), 1.96 (1H, d, J=11 Hz), 2.56 (1H, d, J=4 Hz), 2.59 (1H, t, J=6 Hz), 2.99 (1H, d, J=4 Hz), 3.43 (3H, s), 3.65 (1H, dd, J=2, 11 Hz), 5.22 (1H, m), 5.65 (1H, m).

6-*O*-(4-Chlorobutyryl)fumagillol (8) Colorless oil. IR (neat) cm^{-1}: 2920, 1730, 1375, 1240, 1195, 1170, 1125, 1105. NMR ($CDCl_3$) δ: 1.10 (1H, s), 1.21 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.80—2.45 (7H, m), 2.58 (4H, m), 2.99 (1H, d, J=4 Hz), 3.43 (3H, s), 3.61 (2H, t, J=6 Hz), 3.64 (1H, dd, J=3, 11 Hz), 5.21 (1H, m), 5.68 (1H, m).

6-*O*-Nicotinoylfumagillol (9) Colorless oil. IR (neat) cm^{-1}: 2910, 1715, 1585, 1415, 1275, 1100, 1020, 735. NMR ($CDCl_3$) δ: 1.20 (1H, m), 1.24 (3H, s), 1.67 (3H, s), 1.76 (3H, s), 2.04 (1H, d, J=11 Hz), 1.95—2.47 (5H, m), 2.61 (1H, d, J=4 Hz), 2.63 (1H, t, J=6 Hz), 3.05 (1H, d, J=4 Hz), 3.50 (3H, s), 3.77 (1H, dd, J=11, 3 Hz), 5.22 (1H, m), 5.95 (1H, m), 7.39 (1H, ddd, J=8, 5, 1 Hz), 8.29 (1H, dt, J=8, 2 Hz), 8.78 (1H, dd, J=5, 2 Hz), 9.22 (1H, dd, J=2, 1 Hz).

6-*O*-(3-Furoyl)fumagillol (10) Colorless oil. IR (neat) cm^{-1}: 2920, 1715, 1375, 1300, 1155, 1100, 1070, 870, 755. NMR ($CDCl_3$) δ: 1.15 (1H, m), 1.23 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.97 (1H, d, J=11 Hz), 1.87—2.47 (5H, m), 2.58 (1H, d, J=4 Hz), 2.61 (1H, t, J=6 Hz), 3.02 (1H, d, J=4 Hz), 3.47 (3H, s), 3.71 (1H, dd, J=11, 3 Hz), 5.22 (1H, m), 5.82 (1H, m), 6.72 (1H, dd, J=2, 1 Hz), 7.41 (1H, t, J=2 Hz), 8.00 (1H, dd, J=2, 1 Hz).

6-*O*-(3-Carboxypropionyl)fumagillol (11) Colorless oil. IR (neat) cm^{-1}: 3450, 2930, 1735, 1380, 1160, 1105, 1055, 1005, 925. NMR ($CDCl_3$) δ: 1.08 (1H, m), 1.20 (3H, s), 1.65 (3H, s), 1.75 (3H, s), 1.60—2.20 (5H, m), 2.39 (1H, m), 2.56 (1H, d, J=4 Hz), 2.65 (5H, m), 2.98 (1H, d, J=4 Hz), 3.40 (3H, s), 3.63 (1H, dd, J=11, 3 Hz), 5.22 (1H, m), 5.68 (1H, br s), 7.10 (1H, br s).

6-*O*-Carboxymethoxyacetylfumagillol (12) Colorless oil. IR (neat) cm^{-1}: 3475, 2930, 1735, 1445, 1380, 1220, 1155, 1055, 1025. NMR ($CDCl_3$) δ: 1.10 (1H, m), 1.21 (3H, s), 1.63 (3H, s), 1.72 (3H, s), 1.60—2.60 (8H, m), 2.94 (1H, d, J=4 Hz), 3.41 (3H, s), 3.63 (1H, dd, J=11, 3 Hz), 4.25 (2H, s), 4.30 (2H, s), 5.21 (1H, m), 5.73 (1H, br s), 8.22 (1H, br s).

6-*O*-(2-Carboxybenzoyl)fumagillol (13) Colorless oil. IR (neat) cm^{-1}: 3430, 2920, 1720, 1440, 1380, 1280, 1125, 1065. NMR ($CDCl_3$) δ: 1.08 (1H, m), 1.24 (3H, s), 1.68 (3H, s), 1.77 (3H, s), 1.90—2.50 (5H, m), 2.35 (1H, d, J=12 Hz), 2.60 (1H, d, J=4 Hz), 2.94 (1H, d, J=4 Hz), 3.16 (1H, dd, J=8, 6 Hz), 3.50 (3H, s), 3.75 (1H, dd, J=12, 2 Hz), 5.22 (1H, m), 5.99 (1H, d, J=2 Hz), 7.45—7.65 (3H, m), 7.80—7.90 (1H, m).

Synthesis of 6-*O*-Sulfonylfumagillols. General Procedure Sulfonyl chloride (0.43 mmol) was added dropwise to a solution of fumagillol (**3**, 0.35 mmol) and DMAP (0.53 mmol) in dry dichloromethane (1 ml). The mixture was stirred for 1 h at room temperature, then diluted with AcOEt and washed with saturated $NaHCO_3$ solution and brine. The AcOEt solution was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel using AcOEt : hexane = 1 : 4 as the eluent.

6-*O*-(*p*-Toluenesulfonyl)fumagillol (14) Colorless crystals from diisopropyl ether, mp 123—124 °C. IR (KBr) cm^{-1}: 2950, 2930, 1355, 1190, 1180, 915, 910, 685, 560. NMR ($CDCl_3$) δ: 1.14 (1H, m), 1.16 (3H, s), 1.67 (3H, s), 1.70 (3H, s), 1.84 (1H, m), 1.95 (1H, d, J=11 Hz), 2.04—2.47 (4H, m), 2.44 (3H, s), 2.55 (1H, d, J=4 Hz), 2.56 (1H, t, J=6 Hz), 2.94 (1H, d, J=4 Hz), 3.02 (3H, s), 3.50 (1H, dd, J=11, 3 Hz), 5.07 (1H, m), 5.19 (1H, m), 7.33 (2H, d, J=8 Hz), 7.87 (2H, d, J=8 Hz). *Anal.* Calcd for $C_{23}H_{32}O_6S \cdot 1/5H_2O$: C, 62.76; H, 7.42. Found: C, 62.81; H, 7.50.

6-*O*-Methanesulfonylfumagillol (15) Colorless oil. IR (neat) cm^{-1}: 2950, 1735, 1345, 1175, 1130, 1105, 940, 920, 900. NMR ($CDCl_3$) δ: 1.12 (1H, m), 1.20 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.93 (1H, d, J=11 Hz), 1.85—2.45 (4H, m), 2.58 (1H, t, J=6 Hz), 2.59 (1H, d, J=4 Hz), 2.99 (1H, d, J=4 Hz), 3.14 (3H, s), 3.53 (3H, s), 3.65 (1H, dd, J=2, 11 Hz), 5.20 (1H, m), 5.39 (1H, m).

Synthesis of 6-*O*-Alkylfumagillols. General Procedure Sodium hydride (60% suspension in mineral oil) (1.74 mmol) was added to a solution of fumagillol (**3**, 0.82 mmol) in dry THF (1.5 ml) and dry DMF (1.5 ml) under ice cooling. Alkyl halide (1.60 mmol) was then added dropwise and the mixture was stirred for 20 min. The reaction mixture was poured into water and extracted with ether. The extract was washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel using AcOEt : hexane = 1 : 4 as the eluent.

6-*O*-Methylfumagillol (16) Colorless oil. IR (neat) cm^{-1}: 2920, 1440, 1375, 1205, 1105, 1000, 920. NMR ($CDCl_3$) δ: 1.00 (1H, m), 1.21 (3H, s), 1.65 (3H, s), 1.74 (3H, s), 1.50—1.80 (1H, m), 2.04 (1H, d, J=11 Hz), 1.95—2.25 (3H, m), 2.30—2.50 (1H, m), 2.52 (1H, d, J=4 Hz), 2.55 (1H, t, J=6 Hz), 2.96 (1H, d, J=4 Hz), 3.44 (3H, s), 3.47 (3H, s), 3.59 (1H, dd, J=11, 3 Hz), 3.93 (1H, m), 5.21 (1H, m).

6-*O*-Benzylfumagillol (17) Colorless oil. IR (neat) cm^{-1}: 2930, 1455, 1380, 1205, 1110, 1070, 925. NMR ($CDCl_3$) δ: 1.02 (1H, m), 1.22 (3H, s), 1.68 (3H, s), 1.69 (1H, m), 1.75 (3H, s), 2.00 (1H, m), 2.10—2.25 (3H, m), 2.45 (1H, m), 2.50 (1H, d, J=4 Hz), 2.57 (1H, t, J=6 Hz), 2.98 (1H, d, J=4 Hz), 3.41 (3H, s), 3.59 (1H, dd, J=11, 3 Hz), 4.10 (1H, br s), 4.66 (1H, d, J=13 Hz), 4.78 (1H, d, J=13 Hz), 5.23 (1H, m), 7.20—7.45 (5H, m).

Synthesis of 6-*O*-(*N*-Substituted-carbamoyl)fumagillols. Method A: General Procedure Isocyanate (2.10 mmol) was added dropwise to a solution of fumagillol (**3**, 0.70 mmol) and DMAP (0.70 mmol) in dry dichloromethane (5 ml) under ice cooling. The reaction mixture was stirred for 2 h, then poured into water and extracted with dichloromethane. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel using AcOEt : hexane = 1 : 4 as the eluent.

6-*O*-Chloroacetylcarbamoylfumagillol (2) White amorphous powder. IR (KBr) cm^{-1}: 3280, 2960, 1790, 1755, 1725, 1500, 1380, 1220, 1190, 1105. NMR (CDCl$_3$) δ: 1.10 (1H, m), 1.21 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.93 (1H, d, J=11 Hz), 1.80—2.50 (5H, m), 2.57 (1H, d, J=4 Hz), 2.58 (1H, m), 2.99 (1H, d, J=4 Hz), 3.47 (3H, s), 3.68 (1H, dd, J=11, 3 Hz), 4.44 (2H, s), 5.20 (1H, m), 5.61 (1H, m), 8.33 (1H, br s).

6-*O*-Propylcarbamoylfumagillol (18) White amorphous powder. IR (KBr) cm^{-1}: 3350, 2960, 2930, 1690, 1535, 1270, 1240, 1110. NMR (CDCl$_3$) δ: 0.92 (3H, t, J=7 Hz), 1.07 (1H, m), 1.21 (3H, s), 1.40—2.50 (8H, m), 1.66 (3H, s), 1.75 (3H, s), 2.55 (1H, d, J=4 Hz), 2.57 (1H, t, J=6 Hz), 2.98 (1H, d, J=4 Hz), 3.13 (2H, q, J=7 Hz), 3.45 (3H, s), 3.64 (1H, dd, J=11, 3 Hz), 4.79 (1H, m), 5.21 (1H, m), 5.48 (1H, m).

6-*O*-(2-Chloroethylcarbamoyl)fumagillol (19) White amorphous powder. IR (KBr) cm^{-1}: 3350, 2920, 1720, 1700, 1535, 1445, 1380, 1260, 1205, 1105. NMR (CDCl$_3$) δ: 1.07 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.60—2.50 (6H, m), 2.56 (1H, d, J=4 Hz), 2.57 (1H, t, J=6 Hz), 2.98 (1H, d, 4 Hz), 3.46 (3H, s), 3.40—3.70 (5H, m), 5.20 (2H, m), 5.50 (1H, m).

6-*O*-Phenylcarbamoylfumagillol (20) White amorphous powder. IR (KBr) cm^{-1}: 3330, 2920, 1725, 1705, 1600, 1540, 1440, 1225, 1200. NMR (CDCl$_3$) δ: 1.10 (1H, m), 1.23 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.60—2.40 (6H, m), 2.56 (1H, d, J=4 Hz), 2.58 (1H, t, J=6 Hz), 3.00 (1H, d, J=4 Hz), 3.45 (3H, s), 3.70 (1H, dd, J=11, 3 Hz), 5.21 (1H, m), 5.57 (1H, m), 7.00—7.60 (6H, m).

6-*O*-(3-Trifuluoromethylphenylcarbamoyl)fumagillol (21) White amorphous powder. IR (KBr) cm^{-1}: 3320, 2920, 1730, 1555, 1445, 1325, 1225, 1200, 1120. NMR (CDCl$_3$) δ: 1.12 (1H, m), 1.23 (3H, s), 1.67 (3H, s), 1.75 (3H, s), 1.99 (1H, d, J=11 Hz), 1.80—2.50 (5H, m), 2.59 (2H, m), 3.00 (1H, d, J=4 Hz), 3.48 (3H, s), 3.71 (1H, dd, J=11, 3 Hz), 5.21 (1H, m), 5.60 (1H, m), 7.00 (1H, br s), 7.25—7.60 (3H, m), 7.76 (1H, br s).

6-*O*-(2,4-Difuluorophenylcarbamoyl)fumagillol (22) White amorphous powder. IR (KBr) cm^{-1}: 3300, 2920, 1725, 1530, 1425, 1230, 1195, 1100. NMR (CDCl$_3$) δ: 1.11 (1H, m), 1.23 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.60—2.50 (6H, m), 2.58 (2H, m), 3.00 (1H, d, J=4 Hz), 3.49 (3H, s), 3.70 (1H, dd, J=11, 3 Hz), 5.22 (1H, m), 5.60 (1H, m), 6.80—7.00 (3H, m), 8.05 (1H, br q, 7 Hz).

6-*O*-(1-Naphthylcalbamoyl)fumagillol (23) White amorphous powder. IR (KBr) cm^{-1}: 3330, 2920, 1725, 1530, 1495, 1375, 1220, 1200, 1100. NMR (CDCl$_3$) δ: 1.10 (1H, m), 1.24 (3H, s), 1.67 (3H, s), 1.75 (3H, s), 1.60—2.50 (6H, m), 2.55 (1H, d, J=4 Hz), 2.59 (1H, m), 2.99 (1H, d, J=4 Hz), 3.47 (3H, s), 3.70 (1H, dd, J=11, 3 Hz), 5.22 (1H, m), 5.63 (1H, m), 7.19 (1H, br s), 7.40—8.00 (7H, m).

6-*O*-Acetylcarbamoylfumagillol (24) White amorphous powder. IR (KBr) cm^{-1}: 3270, 2910, 1750, 1700, 1495, 1375, 1215, 1190, 1100. NMR (CDCl$_3$) δ: 1.10 (1H, m), 1.21 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.80—2.50 (6H, m), 2.39 (3H, s), 2.57 (1H, t, J=7 Hz), 2.58 (1H, d, J=4 Hz), 2.99 (1H, d, J=4 Hz), 3.47 (3H, s), 3.68 (1H, dd, J=11, 3 Hz), 5.20 (1H, m), 5.57 (1H, m), 8.03 (1H, br s).

6-*O*-Dichloroacetylcarbamoylfumagillol (25) White amorphous powder. IR (KBr) cm^{-1}: 3280, 2960, 1790, 1760, 1700, 1520, 1380, 1225, 1190, 1100. NMR (CDCl$_3$) δ: 1.11 (1H, m), 1.22 (3H, s), 1.67 (3H, s), 1.75 (3H, s), 1.96 (1H, d, J=11 Hz), 1.60—2.60 (6H, m), 2.58 (1H, d, J=4 Hz), 2.99 (1H, d, J=4 Hz), 3.48 (3H, s), 3.71 (1H, dd, J=11, 3 Hz), 5.20 (1H, m), 5.64 (1H, m), 6.38 (1H, s), 8.50 (1H, s).

6-*O*-Trichloroacetylcarbamoylfumagillol (26) White amorphous powder. IR (KBr) cm^{-1}: 3275, 2920, 1790, 1740, 1500, 1375, 1275, 1245, 1170, 1100. NMR (CDCl$_3$) δ: 1.11 (1H, m), 1.22 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 2.00 (1H, d, J=11 Hz), 1.60—2.70 (6H, m), 2.58 (1H, d, J=4 Hz), 3.01 (1H, d, J=4 Hz), 3.50 (3H, s), 3.73 (1H, dd, J=11, 3 Hz), 5.20 (1H, m), 5.71 (1H, m), 8.68 (1H, br s).

6-*O*-Benzoylcarbamoylfumagillol (27) White amorphous powder. IR (KBr) cm^{-1}: 3330, 2920, 1725, 1705, 1600, 1540, 1440, 1310, 1225, 1200. NMR (CDCl$_3$) δ: 1.09 (1H, m), 1.20 (3H, s), 1.65 (3H, s), 1.74 (3H, s), 1.60—2.45 (6H, m), 2.55 (1H, d, J=4 Hz), 2.56 (1H, t, J=7 Hz), 2.97 (1H, d, J=4 Hz), 3.42 (3H, s), 3.68 (1H, dd, J=11, 3 Hz), 5.19 (1H, m), 6.65 (1H, br s), 7.40—7.60 (3H, m), 7.89 (2H, dd, J=7, 1 Hz), 8.88 (1H, br s).

Method B: General Procedure Phenyl chloroformate (111 mg) was added dropwise to a solution of fumagillol (**3**, 133 mg) and DMAP (115 mg) in dry dichloromethane (3 ml) under ice cooling. The mixture was stirred for 30 min at room temperature, then diluted with AcOEt and washed with saturated aqueous NaHCO$_3$ and brine. The AcOEt solution was dried over anhydrous MgSO$_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel using AcOEt : hexane = 1 : 5 as the eluent to give 6-*O*-phenoxycarbonylfumagillol (**28**) as colorless oil (174 mg, 92%). $[\alpha]_D^{22}$ −35.5° (c=0.24, CHCl$_3$). IR (neat) cm^{-1}: 2925, 1760, 1590, 1490, 1445, 1380, 1270, 1110, 935. NMR (CDCl$_3$) δ: 1.10 (1H, m), 1.22 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.80—2.45 (6H, m), 2.56 (1H, d, J=4 Hz), 2.59 (1H, t, J=6 Hz), 2.99 (1H, d, J=4 Hz), 3.50 (3H, s), 3.69 (1H, dd, J=11, 3 Hz), 5.18 (1H, m), 5.58 (1H, m), 7.15—7.45 (5H, m).

Conc. ammonia water (3 ml) or an amine (3 mmol) was added to a solution of 6-*O*-phenoxycarbonylfumagillol (**28**, 1 mmol) in ethanol (5 ml). The mixture was stirred for 3 h at room temperature, then diluted with AcOEt and washed with brine. The AcOEt solution was dried over anhydrous MgSO$_4$ and concentrated *in vacuo*. The residue was recrystallized from diisopropyl ether or chromatographed on silica gel using AcOEt : hexane = 1 : 4 as the eluent.

6-*O*-Carbamoylfumagillol (29) Colorless crystals, mp 125—126 °C. IR (KBr) cm^{-1}: 3430, 2930, 1725, 1615, 1380, 1325, 1130, 1110, 1070. NMR (CDCl$_3$) δ: 1.07 (1H, m), 1.21 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.60—2.50 (6H, m), 2.55 (1H, d, J=4 Hz), 2.57 (1H, t, J=7 Hz), 2.98 (1H, d, J=4 Hz), 3.45 (3H, s), 3.65 (1H, dd, J=11, 3 Hz), 5.09 (2H, br s), 5.21 (1H, m), 5.46 (1H, m).

6-*O*-Morpholinocarbonylfumagillol (30) Colorless oil. IR (neat) cm^{-1}: 2920, 2850, 1700, 1425, 1375, 1275, 1240, 1200, 1110. NMR (CDCl$_3$) δ: 1.11 (1H, m), 1.21 (3H, s), 1.66 (3H, s), 1.74 (3H, s), 1.60—2.50 (6H, m), 2.55 (1H, d, J=4 Hz), 2.57 (1H, t, J=6 Hz), 2.99 (1H, d, J=4 Hz), 3.46 (3H, s), 3.47 (4H, m), 3.68 (5H, m), 5.21 (1H, m), 5.57 (1H, m). MS m/z: 395 (M$^+$), 352, 326, 310, 256, 233.

6-*O*-Piperidinocarbonylfumagillol (31) Colorless oil. IR (neat) cm^{-1}: 2920, 2850, 1690, 1430, 1375, 1255, 1230, 1150, 1105. NMR (CDCl$_3$) δ: 1.10 (1H, m), 1.22 (3H, s), 1.57 (6H, m), 1.66 (3H, s), 1.74 (3H, s), 1.80—2.50 (6H, m), 2.55 (1H, d, J=4 Hz), 2.59 (1H, t, J=6 Hz), 2.99 (1H, d, J=4 Hz), 3.42 (4H, m), 3.46 (3H, s), 3.64 (1H, dd, J=11, 3 Hz), 5.22 (1H, m), 5.56 (1H, m). MS m/z: 393 (M$^+$), 375, 350, 324, 306, 252, 233.

6-*O*-Carbazoylfumagillol (32) White amorphous powder. IR (KBr) cm^{-1}: 3340, 2920, 1715, 1500, 1440, 1375, 1270, 1125, 1100. NMR (CDCl$_3$) δ: 1.07 (1H, m), 1.21 (3H, s), 1.65 (3H, s), 1.74 (3H, s), 1.60—2.50 (6H, m), 2.55 (1H, d, J=4 Hz), 2.56 (1H, t, J=6 Hz), 2.98 (1H, d, J=4 Hz), 3.47 (3H, s), 3.65 (1H, dd, J=11, 3 Hz), 3.70 (2H, br s), 5.20 (1H, m), 5.55 (1H, m), 6.19 (1H, br s).

Method C: General Procedure Isocyanate (1.2 mmol) was added to a solution of 6-*O*-carbamoylfumagillol (**29**, 0.60 mmol) in dry dichloromethane (4 ml). The mixture was stirred for 4 h at room temperature, then diluted with AcOEt and washed with saturated aqueous NaHCO$_3$ and brine. The AcOEt solution was dried over anhydrous MgSO$_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel using AcOEt : hexane = 1 : 4 as the eluent.

6-*O*-(*N*′-Chloroacetylallophanoyl)fumagillol (33) White amorphous powder. IR (KBr) cm^{-1}: 3250, 2950, 1795, 1710, 1525, 1485, 1220, 1190, 1105. NMR (CDCl$_3$) δ: 1.12 (1H, m), 1.21 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.92 (1H, d, J=11 Hz), 1.90—2.50 (5H, m), 2.57 (1H, d, J=4 Hz), 2.59 (1H, t, J=7 Hz), 2.99 (1H, d, J=4 Hz), 3.48 (3H, s), 3.68 (1H, dd, J=11, 3 Hz), 4.39 (2H, s), 5.20 (1H, m), 5.65 (1H, m).

6-*O*-(*N*′-Benzoylallophanoyl)fumagillol (34) White amorphous powder. IR (KBr) cm^{-1}: 3260, 2920, 1795, 1760, 1725, 1675, 1525, 1495, 1475, 1255. NMR (CDCl$_3$) δ: 1.13 (1H, m), 1.22 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.80—2.50 (5H, m), 1.97 (1H, d, J=11 Hz), 2.58 (1H, d, J=4 Hz), 2.62 (1H, t, J=7 Hz), 3.01 (1H, d, J=6 Hz), 3.50 (3H, s), 3.69 (1H, dd, J=11, 3 Hz), 5.21 (1H, m), 5.72 (1H, m), 7.49—7.71 (3H, m), 7.91 (2H, m).

Rat Corneal Micropocket Assay The rat corneal micropocket assay was carried out as described previously.[12] Both an ethylene vinylacetate copolymer (EVA) pellet containing bovine bFGF (R & D systems) (250 ng) and an empty or a compound-containing EVA pellet were implanted into rat corneas. Ten days later, the anti-angiogenic activity of the compound was examined using a stereomicroscope.

Inhibitory Effect on the Proliferation of HUVE Cells *in Vitro* HUVE cells were isolated by perfusion of an umbilical vein with a trypsin containing medium and were grown in GIT medium (Daigo Eiyo Kagaku) supplemented with 2.5% fetal bovine serum and 2.0 ng/ml of recombinant human basic fibroblast growth factor (rhbFGF: prepared by the Biotechnology Research Laboratories of Takeda Chemical Industries, Ltd.). Compounds to be tested were dissolved in dimethylsulfoxide (DMSO) and then diluted with culture medium so that the final DMSO concentration did not exceed 0.25%.

HUVE cells were seeded onto 96-well microtiter plates at the concentration of 2×10^3 cells per well and were allowed to grow at 37°C in a humidified atmosphere on 5% CO$_2$ and 95% air. On the following day, 100 μl/well of medium containing rhbFGF (final concentration of 2 ng/ml) and compounds at various concentrations were added. After 5 d of incubation, the inhibitory effect on the proliferation was determined using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Cell viability was measured as a function of the ability of the cells to form a blue formazon product, and the concentration required for 50% inhibition (IC_{50}) was estimated from the optical density (590 nm).

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References

1) J. Folkman, *Adv. Cancer Res.*, **43**, 175 (1985); J. Folkman, *J. Natl. Cancer Res.*, **82**, 4 (1990).
2) S. Taylor and J. Folkman, *Nature* (London), **297**, 307 (1982).
3) J. Folkman, R. Langer, R. J. Linhdardt, C. Haudenschild, and S. Taylor, *Science*, **221**, 719 (1983).
4) K. Inoue, H. Korenaga, N. G. Tanaka, N. Sakamoto, and S. Kadoya, *Carbohydrate Res.*, **181**, 135 (1988); N. G. Tanaka, N. Sakamoto, K. Inoue, H. Korenaga, S. Kadoya, H. Ogawa, and Y. Osada, *Cancer Res.*, **49**, 6727 (1989).
5) T. E. Maione, G. S. Gray, J. Petro, A. J. Hunt, A. L. Donner, S. I. Bauer, H. F. Carson, and R. J. Sharpe, *Science*, **247**, 77 (1990); R. J. Sharpe, H. R. Byers, C. F. Scott, S. I. Bauer, and T. E. Maione, *J. Natl. Cancer. Inst.*, **82**, 848 (1990).
6) E. Missirlis, G. Karakiulakis, and M. E. Maragoudakis, *Investigational New Drugs*, **8**, 145 (1990).
7) M. E. Maragoudakis, E. Missirlis, M. Sarmonika, M. Panoutsacopoulou, and G. Karakiulakis, *J. Pharmacol. Exp. Ther.*, **252**, 753 (1990).
8) D. Ingber, T. Fujita, S. Kishimoto, K. Sudo, T. Kanamaru, H. Brem, and J. Folkman, *Nature* (London), **348**, 555 (1990).
9) F. R. Hanson and T. E. Eble, *J. Bacteriol.*, **58**, 527 (1949).
10) M. C. McCowen, M. E. Callender, and J. F. Lawlis, Jr., *Science*, **113**, 202 (1951).
11) J. A. DiPaolo, D. S. Tarbell, and G. E. Moore, *Antibiotics Annual*, **1958—1959**, 541.
12) M. Kusaka, K. Sudo, T. Fujita, S. Marui, F. Itoh, D. Ingber, and J. Folkman, *Biochem. Biophys. Res. Commun.*, **174**, 1070 (1991).
13) J. K. Landquist, *J. Chem. Soc.* (*C*), **1956**, 4237.
14) D. S. Tarbell, R. M. Carman, D. D. Chapman, S. E. Cremer, A. D. Cross, K. R. Huffman, M. Kunstmann, N. J. McCorkindale, J. G. McNally, Jr., A. Rosowsky, F. H. L. Varino, and R. L. West, *J. Am. Chem. Soc.*, **83**, 3096 (1961).
15) E. J. Corey and B. B. Snider, *J. Am. Chem. Soc.*, **94**, 2549 (1972).
16) R. L. Engerman, D. Pfaffenbach, and M. D. Davis, *Lab. Invest.*, **17**, 738 (1967); S. M. Schwartz and E. P. Benditt, *Circ. Res.*, **41**, 248 (1977).