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ANTITUMOR AGENTS, 154.[1] CYTOTOXIC AND ANTIMITOTIC FLAVONOLS FROM *POLANISIA DODECANDRA*

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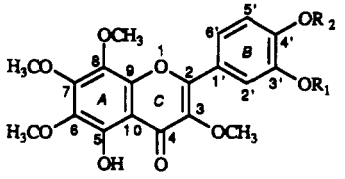
ABSTRACT.—Three flavonols, 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone [**1**], 5,4′-dihydroxy-3,6,7,8,3′-pentamethoxyflavone [**2**], and quercetin 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside [**3**], were isolated from *Polanisia dodecandra*. Compound **1** showed remarkable cytotoxicity in vitro against panels of central nervous system cancer (SF-268, SF-539, SNB-75, U-251), non-small cell lung cancer (HOP-62, NCI-H266, NCI-H460, NCI-H522), small cell lung cancer (DMS-114), ovarian cancer (OVCAR-3, SK-OV-3), colon cancer (HCT-116), renal cancer (UO-31), a melanoma cell line (SK-MEL-5), and two leukemia cell lines (HL-60 [TB], SR), with GI_{50} values in the low micromolar to nanomolar concentration range. This substance also inhibited tubulin polymerization ($IC_{50}=0.83\pm0.2$ μM) and the binding of radiolabeled colchicine to tubulin with 59% inhibition when present in equimolar concentrations with colchicine. Compound **2** also showed cytotoxicity against medulloblastoma (TE-671) tumor cells with an ED_{50} value of 0.98 μg/ml. Compound **1** appears to be the first example of a flavonol to exhibit potent inhibition of tubulin polymerization and, therefore, warrants further investigation as an antimitotic agent.

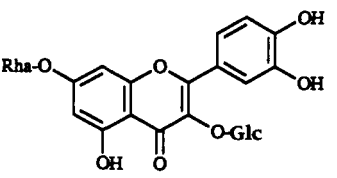
Polanisia dodecandra (L.) DC. (Capparidaceae), Clammy-weed, is native to North America and is abundant from Montana to Wisconsin and from Quebec to Mexico (2). *P. dodecandra* is a branched glandular-viscid herb with a rank odor and grows in sandy places. Except for our previously published work (3), it is a hitherto phytochemically and biologically uninvestigated species and, therefore, attracted our interest for laboratory investigations. In the course of our continuing search for novel cytotoxic agents that are active against slow-growing solid tumors, especially compounds from previously uninvestigated plants, the MeOH extract of the whole plant of *P. dodecandra* was found to show significant in vitro cytotoxicity against various tumor cells. Subsequent

[1]For part 153, see Wang *et al.* (1).

bioassay-guided fractionation and repeated cc on Si gel of the active extract led to the isolation of three flavonols, 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone [**1**], 5,4′-dihydroxy-3,6,7,8,3′-pentamethoxyflavone [**2**], and quercetin 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside [**3**]. These flavonols were identified by their spectral data and the structures of compounds **1** and **2** were confirmed by X-ray diffraction studies. Compound **1** showed remarkable cytotoxicity in vitro against panels of central nervous system cancer, non-small cell lung cancer, small cell lung cancer, ovarian cancer, colon cancer, renal cancer, a melanoma cell line, and leukemia cell lines with GI_{50} values in the low micromolar to nanomolar concentration range. Compound **2** exhibited significant cytotoxicity against medulloblastoma tumor cells. Compound **1** also showed significant inhibition of tubulin polymerization and inhibition of radiolabeled colchicine binding to tubulin. In addition, a dammarane-type triterpene, polacandrin, one of the potent cytotoxic principles, was isolated and has been reported recently (3). We report herein on the isolation, identification, and X-ray analyses of these flavonols and on their cytotoxicity.



1 R_1=H, R_2=CH_3
2 R_1=CH_3, R_2=H



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RESULTS AND DISCUSSION

Compounds **1** and **2** were both obtained as yellow crystals. The ^{1}H-nmr spectrum of each compound exhibited the same B-ring proton signals characteristic for H-2′, -5′, and -6′ of a 3,5,6,7,8,3′,4′-oxygenated flavone with two hydroxy and five methoxy groups. The ^{1}H-nmr spectra (Table 1), nOe studies, and uv behavior of compounds **1** and **2** indicated an OH at position 3′ in **1** and at position 4′ in **2**.

Previously, compound **1** has been isolated from *Gutierrezia microcephala* (4), *Polanisia trachysperma* (5), *Calycadenia truncata* (6), and *Calycadenia mollis* (6), and compound **2** from *Calycopteris floribunda* (7), *Digitalis thapsi* (8), *Baccharis incarum* (9), *Gutierrezia resinosa* (10), *Gutierrezia microcephala* (11), *Calycadenia ciliosa* (6), and *Calycadenia multiglandulosa* (6). The reported ^{1}H-nmr data for **1** (4,5) and **2** (9–11), measured in CCl_4, $CDCl_3$, Me_2CO-d_6 or DMSO-d_6 did not effectively distinguish between OMe-3′ versus OMe-4′ substitution, since these data for both substitution patterns were very similar. In the present study, the spectral data for **1** and **2** measured in $CDCl_3$ were also almost identical (Table 1). However, the spectra measured in C_6D_6 showed good resolution of the two substitution patterns (Table 1).

The previously reported ^{13}C-nmr data measured in $CDCl_3$ did not assign all signals (9). In the present study, HETCOR and long-range HETCOR spectra were used to assign all carbons unequivocally (Table 2). The ^{13}C-nmr data for compounds **1** and **2** measured in $CDCl_3$ are similar to those measured in DMSO-d_6 (5,11).

X-Ray crystallographic analyses confirmed the structures of **1** and **2** and provided details of their solid-state geometries.[2] Corresponding bond lengths in **1** and **2** agree well

[2]Fractional atomic coordinate data and solid-state conformation figures are deposited in A.T. McPhail's laboratory.

TABLE 1. ^{1}H-Nmr Spectral Data for **1** and **2** in Different Solvents.

Proton	Compound **1**		Compound **2**	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
H-2′	7.77, d, J=2.2 Hz	8.03, d, J=2.2 Hz	7.80, s	7.61, d, J=2.0 Hz
H-5′	7.00, d, J=7.0 Hz	6.37, d, J=8.7 Hz	7.07, d, J=7.5 Hz	7.00, d, J=8.6 Hz
H-6′	7.78, dd, J=2.2 and 7.0 Hz	7.79, dd, J=2.2 and 8.7 Hz	7.78, dd, J=1.5 and 7.5 Hz	7.80, dd, J=2.0 and 8.6 Hz
OH-5	12.40, s	13.12, s	12.39, s	13.15, s
OH-3′	5.73, s	5.41, s	—	—
OH-4′	—	—	6.02, s	5.66, s[a]
OMe-3′	—	—	3.98, s	3.21, s[a]
OMe-4′	4.00, s	3.07, s[a]	—	—
OMe-3	3.88, s	3.84, s[b]	3.88, s	3.85, s[b]
OMe-6	3.95, s	3.81, s[b]	3.95, s	3.82, s[b]
OMe-7	4.11, s	3.72, s[b]	4.11, s	3.69, s[b]
OMe-8	3.96, s	3.65, s[b]	3.96, s	3.64, s[b]

[a]In C_6D_6, only the OMe-3′ in compound **1** and OMe-4′ in compound **2** were assigned by the NOESY spectra.
[b]In C_6D_6, the remaining OMe groups were not assigned unambiguously and their assignments may be interchangeable.

in general and are in accordance with expectations (12). The ring A/C atoms O-1–C-10 are approximately coplanar (mean deviation 0.031 Å in **1**, 0.019 Å in **2**), whereas several of the directly bonded atoms are displaced significantly from the least-squares planes through the ring atoms [displacements (Å) in **1**, with corresponding values for **2** in parentheses, follow: O-11 −0.083 (−0.003), O-13 −0.091 (0.105), O-14 0.004 (−0.029), O-15 0.197 (0.013), O-17 0.030 (−0.049), O-19 −0.099 (0.173), C-1′ 0.209 (0.005)]. B-ring atoms are essentially coplanar (mean deviation: 0.005 Å in **1**, 0.004 Å in **2**) while substituent atoms O-7′, O-8′, and C-2 are displaced by small, but significant, amounts from the C-1–C-6′ least-squares plane (ΔO-7 −0.044 Å, ΔO-8 0.038 Å, ΔC-2 0.061 Å in **1**; corresponding values in **2** are −0.017 Å, 0.025 Å, 0.057 Å). The dihedral angle between the O-1–C-10 and C-1′–C-6′ least-squares planes is 17.5° and 7.2° in **1** and **2**, respectively. The OH group at C-5 is intramolecularly hydrogen bonded to O-13 in both compounds [O-14...O-13: 2.591(2) Å in **1**, 2.590(2) Å in **2**]. In the crystals of **1** and **2**, molecules are associated by O-H...O hydrogen bonds involving their B-ring hydroxy groups [O-7′...O-14 (at $-1+x, y, 1+z$) 2.937(3) Å for **1**; O-8′...O-13 (at $x, -1/2-y, 1/2+z$) 2.730(2) Å for **2**].

TABLE 2. ^{13}C-Nmr Spectral Data of **1** and **2**.[a]

Carbon	Compound		Carbon	Compound	
	1	**2**		**1**	**2**
2	155.8	155.9	3′	145.6	146.4
3	138.9	138.6	4′	149.0	148.6
4	179.4	179.3	5′	110.5	114.9
5	149.1	149.2	6′	121.6	122.8
6	136.2	136.2	OMe-3	60.1	60.1
7	152.9	152.9	OMe-6	62.1	62.1
8	132.9	132.8	OMe-7	61.7	61.7
9	144.9	144.9	OMe-8	61.2	61.2
10	107.5	107.5	OMe-3′	—	56.0
1′	123.7	122.5	OMe-4′	56.1	—
2′	114.6	110.8			

[a]Measured in $CDCl_3$.

Previously, no cytotoxic activity has been reported for compound **1**, and only one cell line (KB) has been examined using compound **2** (10). In the present study, compounds **1–3** were initially evaluated against six tumor cell lines, including human epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), melanoma (PRMI-7951), and medulloblastoma (TE-671), as well as one murine leukemia cell line (P-388). Compound **1** exhibited strong cytotoxic effects against these cell lines, while compound **2** showed no significant inhibitory effect (ED_{50}>4.0 μg/ml) for most cell lines tested (Table 3) except for TE-671 (ED_{50}=0.98

TABLE 3. In vitro Cytotoxicity of Compounds **1** and **2**.[a]

Compound	ED_{50} (μg/ml)					
	KB	A-459	HCT-8	P-388	PRMI-7591	TE-671
1	0.045	0.60	4.40	0.055	0.55	0.069
2	8.38	I[b]	I[b]	6.52	5.93	0.98

[a]See Ferguson *et al.* (23) for details.
[b]Inactive.

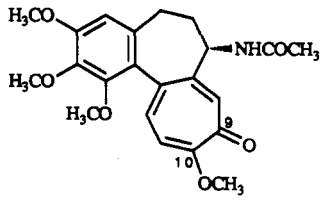
μg/ml). Compound **3** was inactive in all six cell lines. Further testing of the antitumor activity of **1** was performed by the National Cancer Institute (NCI) in their in vitro disease-oriented antitumor screen, which determines a tested agent's effect on growth parameters against a panel of approximately 60 human tumor cell lines (13,14). In this assay, **1** showed strong cytotoxicity against the growth of most of the tumor cell lines, with GI_{50} values (drug concentration required to cause 50% inhibition) in the low micromolar to nanomolar concentration range. Its cytotoxic effect was especially notable on panels of central nervous system cancer, non-small cell lung cancer, small cell lung cancer, ovarian cancer, and leukemia cell lines (Table 4).

The only structural difference between **1** and **2** is in the substitutions at the 2-aryl moiety, which in **1** has a hydroxyl at position 3′ and a methoxy at position 4′, while in **2** these substituent locations are interchanged. Compound **1** was highly active, while **2**

TABLE 4. Inhibition of in vitro Tumor Cell Growth by Compound **1**.[a]

Cell lines[b]	Log_{10} concentration (M)		Cell lines[b]	Log_{10} concentration (M)	
	GI_{50}[c]	TGI[c]		GI_{50}[c]	TGI[c]
HL-60 (TB)	<−8.00	<−8.00	SF-268	<−8.00	−5.55
SR	<−8.00	−6.95	SF-539	<−8.00	<−8.00
HOP-62	−7.32	−5.32	SNB-75	<−8.00	<−8.00
NCI-H226	−7.21	−6.44	U-251	<−8.00	−6.64
NCI-H460	<−8.00	−6.86	SK-MEL-5	<−8.00	−6.00
NCI-H522	<−8.00	<−8.00	OVCAR-3	<−8.00	<−8.00
DMS-114	<−8.00	<−8.00	SK-OV-3	<−8.00	−6.38
HCT-116	<−8.00	−6.91	UO-31	<−8.00	−5.24

[a]Data obtained from the NCI in vitro disease-oriented human cells screen (see Refs. 9 and 10 for details).
[b]HL-60 (TB), SR, leukemia cell lines; HOP-62, NCI-H266, NCI-H460, NCI-H522, non-small cell lung cancer cell lines; DMS-114, small cell lung cancer cell line; HCT-116, colon cancer cell line; SF-268, SF-539, SNB-75, U-251, CNS cancer cell lines; SK-MEL-5, melanoma cell line; OVCAR-3, SK-OV-3, ovarian cancer cell lines; UO-31, renal cancer cell line.
[c]GI_{50}, concentration required to cause 50% inhibition; TGI, concentration required to cause total inhibition.



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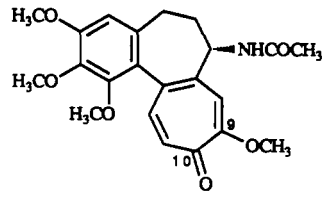

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was almost devoid of activity for most cell lines tested. Therefore, the disposition of the OH and OMe substituents must play a crucial role with regard to the enhanced cytotoxicity. Interestingly, in colchicine [**4**], reversal of the substituents at the 9 and 10 positions yields isocolchicine [**5**] and results in nearly complete loss of activity (15,16).

Computational predictions, derived by use of the NCI-developed program COMPARE (17), suggested that **1** would display antimitotic activity, and this was confirmed experimentally. Compound **1** showed a strong inhibitory effect on in vitro tubulin polymerization, and it was also a potent inhibitor of radiolabeled colchicine binding to tubulin, with 59% inhibition when present in equimolar concentration with colchicine. The effects of polymerization and binding inhibition were comparable with those of colchicine, centaureidin, and podophyllotoxin (Table 5). Accordingly, **1** can be classified as a colchicine site drug, which consists of two biaryl systems connected by a hydrocarbon bridge of variable length (16,18,19). Although we found that **1** interacted with tubulin at the colchicine domain, whether the 3′ and 4′ positions of **1** and the 9 and 10 positions of colchicine share a common binding domain on tubulin is not clear. Therefore, in conclusion, compound **1** appears to be the first example of a flavonol which exhibits potent inhibition of tubulin polymerization and, therefore, warrants further investigation as an antimitotic agent.

TABLE 5. Inhibition of Tubulin Polymerization and Colchicine Binding.[a]

Compound	Inhibition of polymerization IC_{50} (μM)±S.D.	Inhibition of colchicine binding % Inhibition	
		5 μM inhibitor	50 μM inhibitor
Colchicine	1.40±0.3	—	—
Centaureidin	2.00±0.5	35	76
Compound **1**	0.83±0.2	59	89
Podophyllotoxin ..	ND[b]	82	ND[b]

[a]See Li *et al.* (19) for details.
[b]ND=Not determined.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Fisher-Johns melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 1320 spectrophotometer as KBr pellets. Mass spectra were determined on a VG 70-250 SEQ mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were measured on a Bruker AC-300 spectrometer with TMS as internal standard. Si gel (Kieselgel 60, 230–400 mesh, Merck) and reversed-phase Si gel (Bondapak C_{18}, 15–20 μm, Waters) were used for cc. Precoated Si gel plates (Kieselgel 60 F_{254} 0.25 mm, Merck) and C_{18} reversed-phase bonded Si gel plates (Baker Si-C_{18}F, 0.2 mm, J.T. Baker) were used for analytical tlc; Si gel GF (1 mm, Analtech) was used for prep. tlc. Detection of flavonols was performed by spraying with 1% $FeCl_3$ solution in MeOH.

PLANT MATERIAL.—Whole plants of *Polanisia dodecandra* were collected at Fort Sill Military Reservation, Oklahoma, in August, 1990. Identification was made by Dr. J.R. Estes. A voucher specimen is deposited in the Herbarium of the Department of Botany at the University of Oklahoma, Norman, OK.

EXTRACTION AND ISOLATION.—The air-dried herbs (2500 g) were extracted with hot MeOH. The residue after evaporation of the MeOH was digested with H_2O and the aqueous suspension was extracted successively with *n*-hexane, $CHCl_3$, and *n*-BuOH. The *n*-BuOH-soluble fraction was then chromatographed on Si gel in EtOAc with increasing amounts of MeOH. The MeOH fraction was found to show significant in vitro cytotoxicity against KB, A-459, HCT-8, P-388, PRMI-7591, and TE-671 tumor cell lines. The MeOH fraction was further purified by flash chromatography on reversed-phase Si gel employing H_2O-MeOH (1→0 and then 0→1 ratio, respectively) as eluent. Further repeated flash chromatography afforded 150 mg (0.006% yield) of 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone [**1**], 80 mg (0.0032% yield) of 5,4′-dihydroxy-3,6,7,8,3′-pentamethoxyflavone [**2**], and 100 mg (0.004% yield) of quercetin 3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside [**3**], which were purified by recrystallization from Me_2CO.

5,3′-Dihydroxy-3,6,7,8,4′-pentamethoxyflavone [**1**].—Yellow prisms, mp 176–177°; R_f 0.37 ($CHCl_3$-Et_2O, 10:1); uv (MeOH) λ max 257, 278, 345 nm; +NaOMe 280, 396 nm; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* 404.1110 ($C_{20}H_{20}O_9$, calcd 404.1107) (74), 389 (100), 371 (8), 359 (10), 331 (12), 303 (8), 275 (9), 211 (14), 202 (8), 183 (13), 164 (10), 151 (17), 123 (5).

5,4′-Dihydroxy-3,6,7,8,3′-pentamethoxyflavone [**2**].—Yellow crystals, mp 174–175°; R_f 0.40 ($CHCl_3$-Et_2O, 10:1); uv (MeOH) λ max 261, 271, 355 nm; +NaOMe 271, 420 nm; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* 404.1107 ($C_{20}H_{20}O_9$, calcd 404.1107) (73), 389 (100), 375 (16), 359 (11), 331 (12), 303 (8), 275 (9), 211 (16), 202 (8), 183 (16), 164 (11), 151 (22), 135 (9), 123 (5).

Quercetin-3-O-β-D-*glucopyranosyl*-7-O-α-L-*rhamnopyranoside* [**3**].—Yellow needles, mp 194–195°; hydrolysis of **3** with 5% HCl yielded quercetin, glucose, and rhamnose by comparison with authentic samples; ^{1}H nmr (DMSO-d_6) δ 12.63 (1H, s, OH-5), 6.80 (1H, s, H-6), 6.44 (1H, s, H-8), 7.63 (1H, s, H-2′), 9.23 (1H, s, OH-3′), 9.78 (1H, s, OH-4′), 6.89 (1H, d, J=9 Hz, H-5′), 7.62 (1H, d, J=9 Hz, H-6′), 5.56 (1H, br s, H-1-rha), 5.49 (1H, d, J=7.0 Hz, H-1-glc), 3.10–3.85 (10H, sugar protons), 1.12 (3H, d, J=6.0 Hz, Me-6-rha); ^{13}C nmr (DMSO-d_6) δ 155.9 (s, C-2), 133.6 (s, C-3), 177.6 (s, C-4), 160.9 (s, C-5), 99.4 (d, C-6), 161.6 (s, C-7), 94.3 (d, C-8), 156.7 (s, C-9), 105.6 (s, C-10), 121.0 (s, C-1′), 115.2 (d, C-2′), 144.8 (s, C-3′), 148.6 (s, C-4′), 116.3 (d, C-5′), 121.7 (d, C-6′), 100.8 (d, C-1-glc), 74.1 (d, C-2-glc), 76.5 (d, C-3-glc), 70.0 (d, C-4-glc), 77.6 (d, C-5-glc), 61.0 (t, C-6-glc), 98.4 (d, C-1-rha), 70.1 (d, C-2-rha), 70.2 (d, C-3-rha), 71.6 (d, C-4-rha), 69.8 (d, C-5-rha), 17.9 (q, C-6-rha). The ^{1}H-nmr data were similar to those reported (20).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF 5,3′-DIHYDROXY-3,6,7,8,4′-PENTAMETHOXYFLAVONE [**1**] AND 5,4′-DIHYDROXY-3,6,7,8,3′-PENTAMETHOXYFLAVONE [**2**].[3]—*Crystal data for* **1**.—$C_{20}H_{20}O_9$, mol wt=404.38, monoclinic, space group $P2_1/c(C_{2h}^5)$, =8.496(1) Å, b=34.811(2) Å, c=6.634(1) Å, β=110.35(1)°, V=1839.6(7) Å^3, Z=4, D_c=1.460 g cm^{-3}, μ(CuKα radiation, λ=1.5418 Å)=9.4 cm^{-1}; crystal dimensions 0.26×0.40×0.40 mm.

Crystal data for **2**.—$C_{20}H_{20}O_9$, mol wt=404.38, monoclinic, space group $P2_1/c(C_{2h}^5)$, a=8.175(1) Å, b=12.138(1) Å, c=19.397(2) Å, β=100.90(1)°, V=1890.0(6) Å^3, Z=4, D_c=1.421 g cm^{-3}, μ(CuKα radiation)=9.2 cm^{-1}; crystal dimensions 0.24×0.30×0.40 mm.

Preliminary unit-cell parameters and space group information for **1** and **2** were derived from oscillation and Weissenberg photographs. The space group was determined uniquely in each case by the systematic absences: $0k0$ when $k\neq 2n$, $h0l$ when $l\neq 2n$. Intensity data {$+h, +k, \pm l$; θ_{max}=75°; 4106 [**1**] and 4168 [**2**] independent measurements} were recorded on an Enraf-Nonius CAD-4 diffractometer [Cu-Kα radiation; incident-beam graphite monochromator; ω-2θ scans; scanwidth [(0.80+0.14tanθ)° for **1**; (1.00+0.14tanθ)° for **2**]. The intensities of four strong reference reflections, monitored every 2 h during data collection, showed no significant variation (<2.0% overall). Refined unit-cell parameters were derived from the diffractometer setting angles for 25 reflections (36°<θ<47°) widely separated in reciprocal space. The usual Lorentz and polarization corrections were applied to the intensity data. Symmetry equivalent reflections were averaged [R_{merge} (on I)=0.026 for **1**, =0.016 for **2**] to yield 3786 [**1**] and 3896 [**2**] nonequivalent reflections out of which 2572 and 3021 reflections, respectively, with $I>3.0\sigma(I)$ were retained for the analyses.

Both crystal structures were solved by direct methods (MULTAN11/82). Initial coordinates for the non-hydrogen atoms were derived from an *E*-map. Hydrogen atoms were located in difference Fourier syntheses evaluated following several rounds of full-matrix least-squares adjustment of non-hydrogen atom positional and thermal parameters (at first isotropic, then anisotropic). Positional and isotropic thermal parameters of the hydrogen atoms were also refined during the subsequent least-squares iterations; an

[3]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

extinction correction (g) was included as a variable during the later cycles. Hydrogen atoms bonded to C-18 of **2** did not refine to physically acceptable positions and so they were incorporated at calculated positions during the final cycles. The parameter refinements converged (max. shift:esd=0.03) at $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|=0.043$, $R_w=[\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2}=0.060$, $g=4.5(1)\times10^{-6}$, $GOF=[\Sigma w(|F_o|-|F_c|)^2/(N_{observations}-N_{parameters})]^{1/2}=1.57$ for **1**; $R=0.044$ ($R_w=0.065$), $g=7(1)\times10^{-7}$, GOF=2.07 for **2**. Final difference Fourier syntheses contained no unusual features [$\Delta\rho$ (e/Å^{-3}); max, min: 0.18, −0.27 for **1**; 0.34, −0.23 for **2**].

Crystallographic calculations were performed on PDP11/44 and Micro VAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from Ref. (21). In the least-squares iterations, $\Sigma w(|F_o|-|F_c|)^2[w=1/\sigma^2(|F_o|)]$ was minimized.

CYTOTOXICITY ASSAYS.—The in vitro cytotoxicity assay was carried out according to procedures described in Geran *et al.* (22) and Ferguson *et al.* (23). The assay against KB (nasal pharyngeal carcinoma), A-549 (human lung cancer), HCT-8 (human colon carcinoma), PRMI-7951 (human melanoma), TE-671 (human medulloblastoma), and P-388 (murine leukemia) tumor cells was based on a method reported in Lee *et al.* (24).

TUBULIN POLYMERIZATION ASSAY AND DRUG BINDING ASSAYS.—The tubulin polymerization and drug binding assays were carried out according to procedures described by Li *et al.* (19). Briefly, in the tubulin polymerization assay, each 0.24-ml reaction mixture contained 0.8 M monosodium glutamate (pH 6.6 with HCl), 1.0 mg/ml (10 μM) tubulin, and varying drug concentrations, as appropriate to obtain IC_{50} values (drug solvent, DMSO, at 4%). Samples were preincubated for 15 min at 30°, then chilled on ice, and GTP in 10 μl to a final concentration of 0.4 mM was added. Concentrations refer to a final reaction volume of 0.25 ml. Polymerization was followed for 20 min at 30°. In the colchicine binding assay, reaction mixtures contained 1 μM tubulin, 5 μM [^{3}H]colchicine, and the potential inhibitor as indicated. Incubation was for 20 min at 37°.

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