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Cytotoxic 4-Phenylcoumarins from the Leaves of *Marila pluricostata*

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Bioassay-guided fractionation of the CH_2Cl_2 extract of the leaves of *Marila pluricostata* led to the isolation of 17 naturally occurring 4-phenylcoumarins, three of them, 5-hydroxy-8,8-dimethyl-4-phenyl-9,10-dihydro-8*H*-pyrano-[2,3-*f*]chromen-2-one (**1**), 5-hydroxy-8,8-dimethyl-4-phenyl-6-propionyl-9,10-dihydro-8*H*-pyrano-[2,3-*f*]chromen-2-one (**2**), and 5,7-dihydroxy-8-(3-methylbut-2-enyl)-4-phenylchromen-2-one (**3**), are new natural compounds; the remaining (**4**−**17**) are known mammea-type coumarins. Their structures were established by spectroscopic means. All compounds were tested in cytotoxicity assays against the MCF-7, H-460, and SF-268 human cancer cell lines.

Clusiaceae or Guttiferae is a plant family generally confined to the tropics. The genus *Marila* comprises only four species growing in Panama. *Marila pluricostata* is a tree that has a distribution restricted to regions of Panama,[1−3] Costa Rica,[1,2] and Colombia.[4] This species has neither reported common name nor any ethnomedical use. However, *Marila tomentosa* is used in Colombia for the treatment of dysentery.[5]

Previous studies on species of the genus *Marila* resulted in the isolation of betulinic acid,[6] rhamnetin, 3,4-dihydroxybenzoic acid, and several xanthones,[7] in addition to a polyisoprenylated compound, laxifloranone.[8]

As part of a collaborative research program, a CH_2Cl_2 extract of the leaves of *M. pluricostata* showed cytotoxic activitiy against the three human cancer cell lines MCF-7, H-460, and SF-268. Fractionation of these extracts afforded three new 4-phenylcoumarins, 5-hydroxy-8,8-dimethyl-4-phenyl-9,10-dihydro-8*H*-pyrano-[2,3-*f*]chromen-2-one (**1**), 5-hydroxy-8,8-dimethyl-4-phenyl-6-propionyl-9,10-dihydro-8*H*-pyrano-[2,3-*f*]chromen-2-one (**2**), and 5,7-dihydroxy-8-(3-methylbut-2-enyl)-4-phenylchromen-2-one (**3**), and 14 known phenylcoumarins (**4**−**17**).

Results and Discussion

The dried, powdered leaves of *Marila pluricostata* Standl. & L.O. Williams (Clusiaceae) were extracted with CH_2Cl_2. After elimination of fatty compounds by precipitation with MeOH followed by treatment with a saturated solution of urea in MeOH, the soluble part partitioned with a basic aqueous solution yielded a less cytotoxic acidic fraction and a neutral part with enhanced cytotoxicity. Repeated chromatography of the neutral fraction over silica gel and Sephadex LH-20, followed by successive recrystallizations in mixtures of *n*-hexane/ether and $CHCl_3$/MeOH, afforded the new 4-phenylcoumarins **1**−**3**, as well as the known compounds **4**−**17**.

Compound **1** was isolated as a pale yellow amorphous solid. HRFABMS of **1** showed $[M]^+$ at *m/z* 322.1205, corresponding to the molecular formula $C_{20}H_{18}O_4$. Compound **1** gave a green coloration with methanolic ferric chloride, indicating the presence of free phenolic groups; this was supported by an absorption band at 3503 cm^{-1} in its IR spectrum. It showed another absorption at 1712 cm^{-1} corresponding to an α,*β*-unsaturated lactone. Its ^1H NMR spectrum (Table 1) showed one olefinic proton singlet at *δ* 5.94 characteristic of the H-3 of a 4-substituted coumarin. The presence of a monosubstituted phenyl group at C-4 was corroborated by absorptions at 754 and 702 cm^{-1} in the IR spectrum, whereas five aromatic proton signals were evident from its ^1H NMR spectrum. The long-range correlation observed in the HMBC spectrum of **1** between H-3 and the nonprotonated aromatic carbon [*δ* 138.0 (C-1′)] supported the localization of this phenyl group at C-4. The presence in the ^1H NMR spectrum of a pair of two reciprocally coupled triplets (*δ* 1.85, 2H and 2.86 2H, *t*, *J* = 6.8 Hz) together with that of a singlet of a *gem*-dimethyl group (*δ* 1.37) linked to a nonprotonated oxygenated carbon [*δ* 75.9 (C-3‴)] suggested a prenyl group forming a ring involving C-7 and C-8 resulting in a *f*-fused pyran ring. The alternative *g*-fused pyran ring involving C-5 and C-6 was discarded, taking into account the contour map of correlations of the HMBC spectrum. In addition, the ^1H NMR spectrum showed another aromatic singlet (*δ* 6.18) that should be located at C-6. Long-range $^1H-^{13}C$ couplings observed in the HMBC spectrum revealed that the H-3 signal (*δ* 5.94) and the aromatic singlet at *δ* 6.18 both correlated with the same quaternary carbon C-9 (*δ* 101.0), thus supporting the presence of a hydrogen atom at C-6. In addition, H-6 (*δ* 6.18, s) correlated with two aromatic oxygenated carbons (*δ* 153.4 and 158.3). The latter was assignable to C-7, because it was also connected to the methylene H-1‴ (*δ* 2.86). In consequence, the signal at *δ* 153.4 was assigned to C-5. Additionally H-1‴ was connected to another aromatic oxygenated carbon (*δ* 153.6), which was assigned to C-10. These data placed the prenyl substituent at C-8, linked to the oxygen at C-7. The remaining connectivities observed in the HMBC spectrum allowed unambiguous assignment of all of the ^1H and ^{13}C NMR signals (Table 2). Thus, compound **1** was identified as 5-hydroxy-8,8-dimethyl-4-phenyl-9,10-dihydro-8*H*-pyrano-[2,3-*f*]chromen-2-one. The structure of compound **1** was confirmed through its preparation by deacylation[9] of mammeisin (**4**), followed by cyclization of the prenyl chain at position 8. In this way we obtained a substance whose spectroscopic and physical properties were identical to those of the natural compound **1**.

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Chart 1

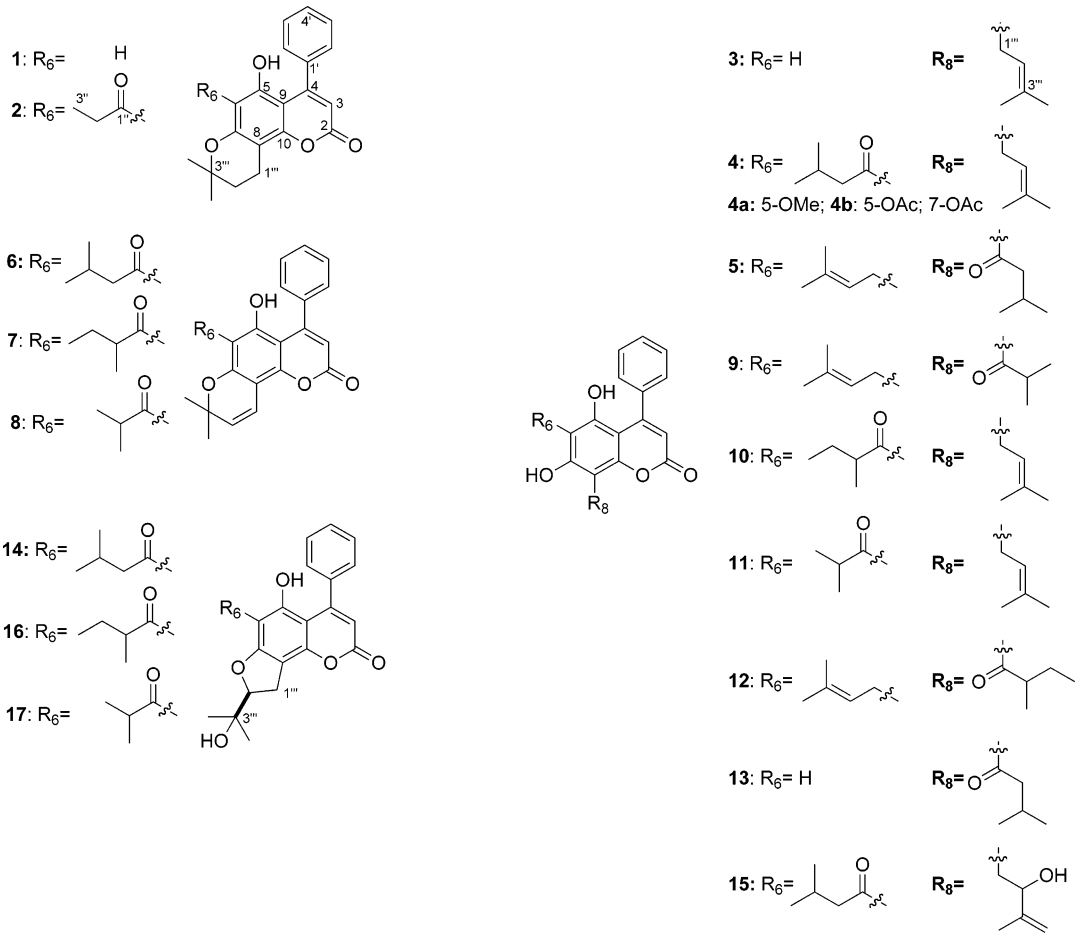

Table 1. ^{1}H NMR Data of Compounds **1**, **2**, and **3**

proton	**1** δ_H (J in Hz)[a]	**1** δ_H (J in Hz)[b]	**2** δ_H (J in Hz)[a]	**3** δ_H (J in Hz)[a]	**4a** δ_H (J in Hz)[a]	**4b** δ_H (J in Hz)[a]
3	5.94 *s*	6.03 s	5.96 *s*	5.96 s	6.10 s	6.24, s
5-OH	5.11 bs		14.72 s	6.45 bs	3.04 s (OMe)	2.29 (OCOMe)
6	6.18 s	7.50 s		6.25 s		
7-OH				6.45 bs	13.14 s	2.29 (OCOMe)
2′,6′	7.45 m	7.45 m	7.31 m	7.37 m	7.40 m	7.33 m
3′, 5′	7.54 m	7.52 m	7.38 m	7.54 m	7.44 m	7.38 m
4′	7.54 m	7.52 m	7.38 m	7.54 m	7.44 m	7.38 m
2″			3.06 q (7.3)		2.82 d (6.8)	2.52 d (6.9)
3″			1.15 t (7.3)		2.21 m	2.14 m
4″					0.91 d (6.8)	0.90 d (6.5)
5″					0.91 d (6.8)	0.90 d (6.5)
1‴	2.86 t (6.8)	3.00 t (6.8)	2.87 t (6.8)	3.56 d (6.8)	3.47 d (7.3)	3.49 d (8.7)
2‴	1.85 t (6.8)	2.06 t (6.8)	1.89 t (6.8)	5.29 t (6.8)	5.13 m	5.27 m
4‴	1.37 s	1.59 s	1.45 s	1.85 s	1.87 s	1.83 s
5‴	1.37 s	1.59 s	1.45 s	1.75 s	1.70 s	1.70 s

[a] δ values obtained in $CDCl_3$ at 200 MHz. [b] δ values were obtained in $MeOD_4$ at 200 MHz. TMS int. std.

Compound **2** was obtained as a white amorphous solid. Its HRFABMS spectra showed a molecular ion *m/z* 378.1454, corresponding to the molecular formula $C_{23}H_{22}O_5$. Its ^{1}H NMR spectrum showed a phenolic hydroxyl signal (δ 14.72) exchangeable with D_2O. The strong deshielding of this signal indicated the existence of an intramolecular hydrogen bond of the phenolic hydrogen in proximity to a carbonyl group. The chemical shift of this signal was characteristic of the hydroxyl group at C-5 for coumarins containing an acyl group at C-6.[10] The IR spectrum, apart from the absorption at 1716 cm^{-1} of the α,β-unsaturated lactone, showed an absorption band at 1618 cm^{-1} corresponding to a chelated acyl group.[11−13] The NMR spectrum of compound **2** differed from that of **1** in the presence of additional signals corresponding to a propionyl group, instead of the aromatic methine of compound **1**. The crowding of signals due to the similarity of several chemical shifts in the ^{13}C NMR spectra (Table 2) of these coumarins made it necessary to rely on 2D HMBC experiments. On the basis of the 2D NMR analysis of **2**, the propionyl residue appeared to be linked via the carbonyl carbon to C-6. These data indicated that it was an almost totally substituted coumarin, with the exception of position C-3. This finding was corroborated by further examination of the HMBC spectrum, which showed a contour map similar to that of compound **1**. Indeed, the singlet at δ 5.96, characteristic for H-3, correlated with the signal of the quaternary carbon C-9 (δ 101.8), and with those of the

Table 2. ^{13}C NMR Data (δ_C[a]) of Compounds **1**−**3**, **5**, **10**, **17**, **4a**, and **4b**

carbon	1	2	3	5	10	17	4a	4b
2	161.2	160.8	160.9	157.1	159.1	159.9	159.4	158.7
3	111.3	112.1	112.0	112.7	112.8	111.9	114.6	118.4
4	155.0	156.5	154.0	154.1	154.5	155.6	154.5	142.4
5	153.4	158.8	153.4	158.6	159.6	163.8	159.8	153.1
6	100.8	107.2	100.8	104.1	107.0	102.4	113.0	122.6
7	158.3	163.3	159.4	166.8	163.1	164.9	163.3	158.7
8	102.0	100.1	108.0	112.1	108.1	105.2	114.3	126.5
9	101.0	101.8	100.8	100.5	101.0	102.4	105.8	111.5
10	153.6	157.6	153.4	156.1	156.6	156.7	156.6	147.8
1′	138.0	139.4	137.0	134.2	135.4	139.1	138.3	137.3
2′,6′	127.4	127.2	127.5	127.5	127.4	127.2	127.6	128.4
3′,5′	128.7	127.5	129.7	129.6	129.2	127.6	127.4	128.1
4′	129.1	128.1	129.9	130.2	129.7	128.3	128.6	128.9
1″		207.7		21.6	211.9	209.6	207.5	199.8
2″		38.1		120.8	46.3	39.1	52.3	52.8
3″		8.9		136.8	11.8	18.6	25.7	23.8
4″				25.7	26.8	19.2	22.6	22.6
5″				17.9	18.1		22.6	22.6
1‴	16.4	16.4	22.1	206.1	21.8	26.7	21.8	23.6
2‴	31.8	31.2	121.1	53.6	120.7	92.9	120.7	119.5
3‴	75.9	77.8	135.5	25.6	137.3	71.5	133.0	133.9
4‴	26.5	26.7	18.1	22.7	16.6	26.2	17.9	18.0
5‴	26.5	26.7	25.9	22.7	25.9	24.9	25.7	25.7
5a							63.9	168.5
5b								19.1
7a								168.5
7b								20.6

[a] ^{13}C spectrum recorded at 400 MHz in $CDCl_3$. Assignments were made on the basis of $^1H-^1H$ COSY, HMQC, and HMBC spectra for compounds **1**−**3**.

lactone carbonyl at C-2 (δ 160.8) and the nonprotonated carbon of the phenyl (δ 139.4) group, which must be attached at C-4. The HMBC spectrum also showed correlations of the methylene signals (δ 1.89 and 2.87) of the prenyl group with that of a nonprotonated oxygenated sp^3 carbon (δ 77.8), supporting a *gem*-dimethyl group (δ 26.7). Both methylene signals (δ 1.89 and 2.87) were connected to C-8 (δ 100.1). Finally, one of these signals (δ 2.87) correlated with those of the two oxygenated aromatic carbons (δ 157.6 and 163.3). In consequence the oxygenated aromatic carbon at δ 158.8 could only be assigned to C-5 and those at δ 157.6 and 163.3 to C-10 and C-7, respectively.

Compound **2** was obtained in good yield by Friedel−Craft acylation[10] from compound **1**, by refluxing with propionyl chloride in a carbon disulfide/nitrobenzene mixture and in the presence of aluminum trichloride. Workup of the crude reaction product led to the isolation of a substance whose spectroscopic properties were identical to those of natural **2**.

Compound **3** was isolated as a white amorphous solid**,** and HRFABMS showed a molecular ion *m/z* 322.1207, corresponding to the molecular formula $C_{20}H_{18}O_4$. The IR spectrum exhibited absorption bands due to −OH (3308 cm^{-1}) and an α,β-unsaturated lactone (1696 cm^{-1}). These data, together with those of the ^1H and ^{13}C NMR spectra, indicated that this compound was another coumarin with a phenyl group at position 4 and phenolic hydroxyl groups at positions C-5 and C-7. Its ^1H NMR spectrum showed the presence of a methine singlet at δ 5.96. It also showed an aromatic singlet at δ 6.25 (H-6 or H-8). The HMBC contour map showed correlations between the H-3 singlet (δ 5.96) and the lactone carbonyl signal (δ 160.9, C-2) and the quaternary carbons (δ 137.0, C-1′ and 100.8, C-9). Other long-range correlations were observed between the proton signal at δ 3.56 and those of C-8, C-7, and C-10, oxygenated carbon signals of the coumarin nucleus. Correlations between H-2‴ (δ 5.29, 1H, t, J = 6.8 Hz), H-4‴ (δ 1.85, 3H, s), and H-5‴ (δ 1.75, 3H, s) were also observed. These data were consistent with a prenyl substituent at position C-8. In consequence, this compound was identified as 5,7-dihydroxy-8-(3-methylbut-2-enyl)-4-phenylchromen-2-one (**3**).

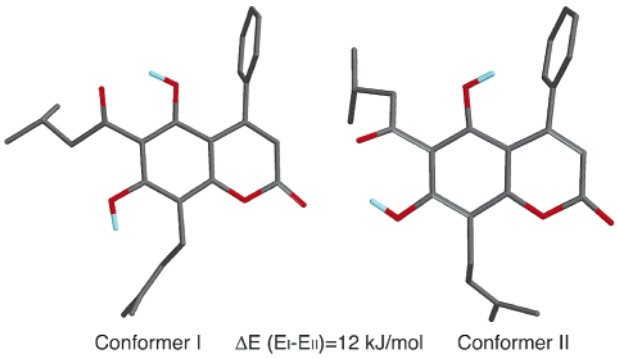

Figure 1. Conformers of mammeisin (**4**).

Coumarins **4**−**17** were identified as known mammeisin (mammea A/AA) (**4**),[14,15] isomammeisin (mammea A/BA) (**5**),[14] mammeigin (mammea A/AA cyclo D) (**6**),[16,17] MAB 5 (mammea A/AB cyclo D) (**7**),[16] mesuagin (mammea A/AD cyclo D) (**8**),[15] isomesuol (**9**)[18] MAB 1 (mammea A/AB) (**10**),[19] mesuol (mammea A/AD) (**11**),[20] (mammea A/BB) (**12**),[15,16] isodispar B (**13**),[21] cyclomammeisin (mammea A/AA cyclo F) (**14**),[13,22] disparinol A (**15**),[21] MAB 3 (mammea A/AB cyclo F) (**16**),[19] and mesuol cyclo F (mammea A/AD cyclo F) (**17**),[19] through the complete analysis of their NMR and MS data and comparison with those reported in the literature for these compounds. ^{13}C NMR data for compounds **5**, **10**, and **17** were not reported before and have, therefore, been included in this paper (Table 2). NMR data of the monomethylated derivative 5-*O*-methylmammeisin (**4a**) and the diacetyl derivative **4b** are also included here.

Signals corresponding to the labile protons of the phenolic groups at positions C-5 and C-7 were very useful for the location of the acyl chain, frequently observed in natural 4-phenylcoumarins and, consequently, for the structural determination of these compounds. In effect, the chemical shifts of the signals of these protons in the ^1H NMR spectrum are strongly influenced by the presence of an acyl group at position C-6 or C-8. To clarify these findings, molecular modeling studies were carried out with mammeisin (**4**), which showed singlets at δ 9.84 and 11.03 in its ^1H NMR spectrum corresponding to both phenolic protons. After a conformational analysis, two main conformers were found for this compound (Figure 1). Relative stabilities were determined by both molecular mechanics and semiempirical methods (AM1).[23] Conformer **I**, which is 12 kJ/mol more stable than conformer **II**, shows an intramolecular hydrogen bond between the phenolic hydrogen 5-OH and the carbonyl of the acyl group of the chain at position C-6, whereas in conformer **II**, the hydrogen bonding appears between the carbonyl and the phenolic group at position 7 (Figure 1). This information is in agreement with the model obtained by X-ray diffraction of this compound (Figure 2). In consequence, the signal at δ 11.03 should be assigned to 5-OH, whereas that at δ 9.84 should correspond to 7-OH.

The original extract and its successive fractions as well as all the purified compounds (**1**−**17**) were screened against three human cancer cell lines, MCF-7, H-460, and SF-268, according to an established protocol. The results are summarized in Table 3. Cytotoxic activity was concentrated in the neutral fraction. Cytotoxic potency of the crude CH_2-

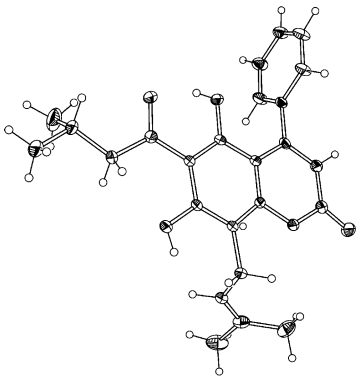

Figure 2. ORTEP drawing of mammeisin (**4**).

Table 3. Cytotoxic Activity of Compounds **1**−**17**[a]

	GI_{50} (μg/mL)		
compound	MCF-7	H-460	SF-268
1	5.7	6.7	7.8
2	4.7	5.7	6.8
3	3.4	4.4	4.8
4	0.2	0.2	0.1
4a	0.4	0.5	0.4
4b	0.6	0.6	0.5
5	0.7	0.8	0.6
6	>10.0	>10.0	>10.0
7	9.6	>10.0	>10.0
8	>10.0	>10.0	>10.0
9	0.7	0.6	0.6
10	0.2	0.1	0.1
11	0.2	0.3	0.1
12	0.2	0.3	0.1
13	1.5	1.9	1.8
14	4.2	4.6	5.2
15	0.9	0.6	0.5
16	3.4	4.4	4.8
17	5.8	6.1	6.4
adriamycin	6.5×10^{-7}	7.2×10^{-7}	8.6×10^{-7}

[a] For cell lines used, see the Experimental Section.

Cl_2 extract was mainly due to the 4-phenylcoumarins, of which, mammesin (**4**), mammea A/AB (**10**), mesuol (**11**), and mammea A/AB (**12**) were the most cytotoxic.

Some 4-phenylcoumarins described previously have revealed cytotoxic[24] and anti-HIV[25,26] activities. Chemopreventive activity against cancer in vitro without cytotoxicity has also been reported for some of these derivatives.[27,28]

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi 510-K melting point apparatus and are uncorrected. IR spectra were recorded (KBr 1%) in a Nicolet Impact 410 spectrophotometer. ^{1}H, ^{13}C NMR, COSY, HMQC, and HMBC were recorded on Bruker AC 200 (200 MHz) and Bruker DRX 400 (400 MHz) instruments. Sephadex LH-20 (Fluka, 25−100 mm) and silica gel 60 (Merck, 230−400 mesh) were used for flash chromatography; precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for TLC analysis. For EIMS and HRFABMS analysis, a VG-TS250 mass spectrometer (70 eV) was used.

Plant Material. Leaves of *Marila pluricostata* were collected from Llano-Cartí in the province of Panama, Republic of Panama, in November 2000. Its taxonomic identity was established by Prof. Mireya Correa, Director of the Herbarium of the University of Panama (PMA), where voucher specimens (F-4740 and F-4937) are deposited.

Extraction and Separation. The dried, powdered leaves (3.4 kg) of *M. pluricostata* were extracted with CH_2Cl_2 (10 L) at room temperature for 5 days. Evaporation of the solvent yielded a crude extract (186.5 g), which was further solubilized with hot *n*-hexane (4 L twice) and cooled (−20 °C) overnight, yielding a soluble fraction (136.1 g), which was defatted successively with MeOH (3 L twice) and a saturated solution of urea in MeOH (3 L twice). The finally soluble part (74.5 g) was partitioned with basic aqueous solution (NaOH 4% × 3 times), yielding an acidic part (3.0 g) and a neutral part (71.1 g). The neutral part was fractionated on a silica gel column with gradient elution using *n*-hexane/EtOAc. Fractions (500 mL each) were combined on the basis of their TLC profiles and after removal of the solvent gave fractions A−J. On the basis of bioactivity data, fractions E−J were subsequently chromatographed on silica gel and Sephadex LH 20 columns, and the compounds were purified through repetitive recrystallization, affording the coumarins **1**−**17**. For chromatographic separations, the appropriate combinations of solvents (*n*-hexane, Et_2O, CH_2Cl_2, EtOAc, and MeOH) were used.

Workup of fraction E by repeated column chromatography using *n*-hexane/ethyl acetate (85:15) led to the isolation of coumarins **4** (2.5 g, 1.340%) and **5** (150 mg, 0.080%). Similarly, fraction F furnished coumarins **6** (242 mg, 0.129%), **7** (56 mg, 0.030%), **8** (164 mg, 0.087%), **9** (118 mg, 0.063%), and **10** (254 mg, 0.136%). Fraction G was chromatographed using CH_2Cl_2/EtO_2 (80:20) and afforded coumarins **11** (220 mg, 0.117%) and **12** (12 mg, 0.0064%). Fraction H, eluting with *n*-hexane/CH_2Cl_2/MeOH (2:1:1), yielded coumarin **13** (15 mg, 0.0080%). Workup of fraction I by repeated column chromatography using *n*-hexane/CH_2Cl_2/MeOH (2:1:1) furnished coumarins **14** (53 mg, 0.030%), **15** (160 mg, 0.085%), **16** (46 mg, 0.024%), **17** (146 mg, 0.078%), and **2** (4 mg, 0.0021%). Finally, fraction J, chromatographed over silica gel using CH_2Cl_2/MeOH (1:1), afforded coumarins **1** (27 mg, 0.014%) and **3** (15.3 mg, 0.0082%). Phenylcoumarins **4**−**17** were identified by comparison of their spectroscopic data with those reported in the literature.[14−20]

5-Hydroxy-8,8-dimethyl-4-phenyl-9,10-dihydro-8*H*-pyrano-[2,3-*f*]chromen-2-one (1): pale yellow amorphous solid; mp 274−275 °C; IR ($CHCl_3$) ν_{max} 3503, 3271, 1712, 1599, 1429, 1362, 1246, 1163, 1111, 1028, 754, 702 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 322 $[M]^+$ (28), 281(20), 267(100), 207(43), 153(12), 139(10), 114(15), 105-(2), 84(9), 77(21), 73(17), 68(18), 65(13), 63(12), 55(14), 50,6); HRFABMS *m/z* 322.1205 (calcd for $C_{20}H_{18}O_4$, 322.1205).

Preparation of Compound 1. Compound **4** (100 mg) was stirred with 1 mL of 75% sulfuric acid at room temperature for 35 h. The mixture was poured onto ice, washed with H_2O, and filtered. The air-dried solid, 0.70 mg, was recrystallized twice, affording a product whose spectroscopic properties were identical to those of the natural compound **1**.

5-Hydroxy-8,8-dimethyl-4-phenyl-6-propionyl-9,10-dihydro-8*H*-pyrano-[2,3-*f*]chromen- 2-one (2): yellow amorphous solid; mp 274−275 °C; IR ($CHCl_3$) ν_{max} 3474, 2975, 2933, 2872, 1746, 1716, 1618, 1599, 1439, 1364, 1246, 1165, 1110, 1030 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 378 $[M]^+$ (64), 349(46), 323(100), 293(86), 265(4), 171(10), 141(5), 115(15), 77(7), 55(5); HRFABMS *m/z* 378.1454 (calcd for $C_{23}H_{22}O_5$, 378.1467).

Preparation of Compound 2. Anhydrous aluminum trichloride (150 mg) was added to a stirred suspension of compound **1** (50 mg) in carbon disulfide (5 mL). Nitrobenzene (2 mL) was then added over 40 min, forming a homogeneous solution with evolution of HCl. The solution was heated under reflux for 30 min, and then propionyl chloride (15 mg) in nitrobenzene (1 mL) was added over 40 min before allowing it to cool with stirring. The mixture was poured onto ice/water and aqueous HCl and was extracted with ethyl acetate (25 mL, twice). Workup of the crude product by chromatography on silica gel led to the isolation of **2** (30 mg), which was identical to the isolated natural product.

5,7-Dihydroxy-8-(3-methylbut-2-enyl)-4-phenylchromen-2-one (3): white amorphous solid; mp 130−132 °C; IR ($CHCl_3$) ν_{max} 3308, 3271, 2959, 2931, 1696, 1559, 1449,1439 1367, 1080 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 322 $[M]^+$ (34), 307(20), 305(4), 279(17), 268(17), 267-(100), 251(7), 238(5), 226(4), 210(2), 197(2), 181(3), 171(4), 165-

(9), 152(7), 139(7), 128(5), 115(14), 105(7), 91(5), 77(10), 69(15), 55(5); HRFABMS found *m/z* 322.1205 (calcd for $C_{20}H_{18}O_4$, 322.1203).

5-*O*-Methylmammeisin (4a). A cooled ethereal diazomethane solution (3 mL) was added dropwise to a cooled solution of mammeisin (10 mg) in ether (2 mL), and the reaction mixture was maintained at 0 °C for 3 h, then concentrated in vacuo to give **4a** (11 mg): white amorphous solid ($CHCl_3$); mp 130−132 °C; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 420 $[M]^+$ (50), 406(24), 405-(91), 377(17), 366(13), 365(55), 363(38), 349(21), 347(12), 308-(22), 307(100), 293(9), 253(16), 205(7), 178(6), 165(13), 152(11), 139(13), 115(13), 105(22), 91(14), 77(16), 69(15), 57(17).

Mammeisin diacetate (4b). Acetic anhydride (0.2 mL) was added to a solution of **4** (15 mg) in dry pyridine (0.5 mL), and the reaction mixture was allowed to stand for 3 h at room temperature, diluted with H_2O (15 mL), extracted with CH_2-Cl_2, and washed with diluted HCl and H_2O four times. Workup led to the isolation of **4b** (17 mg): colorless oil; IR ($CHCl_3$) ν_{max} 2959, 2931, 2871, 1738, 1623, 1581, 1446,1407 1374, 1162, 1123, 1112 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2.

Cytotoxicity Bioassay. The cytotoxicity bioassay was performed against breast (MCF-7), lung (H-460), and CNS (SF-268) human cancer cell lines according to the method of Monks et al.[29] During the isolation process, the activity of all fractions and compounds was monitored using all three cell lines.

Molecular Modeling. Calculations were performed on a Silicon Graphics Indigo computer. Compounds were built using Macromodel v.4.[30] Conformational analysis was performed by a Monte Carlo random search. All freely rotating bonds were searched with MM2[31] minimization to a gradient of less than 0.001 kcal/mol. Full geometry optimization of the two main conformers of each compound was performed using Stewart's AM1 and PM3 Hamiltonian in MOPAC 6.0.

X-ray Analysis of Compound 4. Compound **4**, $C_{25}H_{26}O_5$, crystallizes in orthorhombic space group $P2_12_12_1$, with $Z = 4$, and unit cell parameters $a = 13.675(3)$ Å, $b = 14.243(2)$ Å, $c = 15.810(3)$ Å, $\alpha = 116.46(1)°$, $\beta = 69.46(2)°$, $\gamma = 85.22(1)°$. X-ray diffraction data were collected on a four-circle Seifert XRD 3003 SC diffractometer (Cu Fα, $\lambda = 1.5418$ Å), graphite monochromator, room temperature, $\omega-2\varphi$ scan. The unit cell parameters were determined by least-squares refinement on the 2φ values of 25 strong well-centered reflections in the range $16° < 2\varphi < 40°$. Scattering factors for neutral atoms and anomalous dispersion corrections for C and O were taken from '*International Tables for X-Ray Crystallography* (1995, Vol. C, Kluwer Academic Publishers: Dordrecht). The structure of $C_{25}H_{26}O_5$ was resolved by direct methods and refined in the space group $P2_12_12_1$. Full matrix least-squares refinement with anisotropic thermal parameters for non-H atoms was carried out by minimizing $w(F_o^2 - F_c^2)^2$. Refinement on F^2 for all reflections, weighted R factors (R_w), and all goodness of fit S are based on F^2, while conventional R factors (R) are based on F; R factors based on F^2 are statistically about twice as large as those based on F, and R factors based on all data will be even larger. Resulting absolute structure parameter: 0.36 (155).

All calculations were performed using CRYSOM,[32] software for data collection, XRAY80,[33] and for data reduction, SHELX-TLTM (Siemens SHELXTLTM version 5.0, Siemens Analytical X-ray instruments Inc., Madison, WI, 1995) to resolve and refine the structure and to prepare material for publication. Full crystallographic details have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 2506931).

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