An Antimitotic and Cytotoxic Chalcone from Fissistigma lanuginosum

AN ANTIMITOTIC AND CYTOTOXIC CHALCONE
FROM *FISSISTIGMA LANUGINOSUM*

YATIMAH ALIAS, KHALIJAH AWANG, A. HAMID A. HADI,

*Department of Chemistry, University of Malaya, 59100 Kuala Lumpur, Malaysia*

ODILE THOISON, THIERRY SÉVENET, and MARY PAÏS*

Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette Cedex, France

ABSTRACT.—Bioassay-guided fractionation of an ethyl acetate extract of *Fissistigma lanuginosum* led to the isolation of the known chalcone pedicin [1], which inhibited tubulin assembly into microtubules (IC_{50} value of 300 μM). From the same EtOAc fraction, two new condensed chalcones, fissistin [2] and isofissistin [3], which showed cytotoxicity against KB cells, were also obtained, together with the inactive dihydropedicin [4] and 6,7-dimethoxy-5,8-dihydroxyflavone [5]. In addition, the aminoquinones 6, 8, and 9 were isolated from the alkaloid extract. These compounds were artifacts, prepared by treatment of 1, 4, and 2, respectively, with NH_4OH. The structures of the new compounds were elucidated by spectral methods, especially 2D nmr.

In continuation of our biological screening program to search for new antimitotic drugs, the EtOAc-soluble fraction of *Fissistigma lanuginosum* (Hook. f. & Th.) Merr. (Annonaceae) showed inhibition of tubulin assembly into microtubules and cytotoxic activity against (KB) human nasopharyngeal carcinoma cells. Bioassay-guided fractionation revealed that the compound active against tubulin was pedicin [1], a known chalcone previously extracted from *Didymocarpus pedicellata* (1) and *Polygonum senegalense* (2). In addition, we have isolated from the same fraction two new "condensed" chalcones, 2 and 3, named fissistin and isofissistin, which respectively, were inactive in the tubulin assay but showed cytotoxicity against KB cells. Compounds 2 and 3, which are two positional isomers obtained in an 4:1 ratio, appear to be structurally

2   3
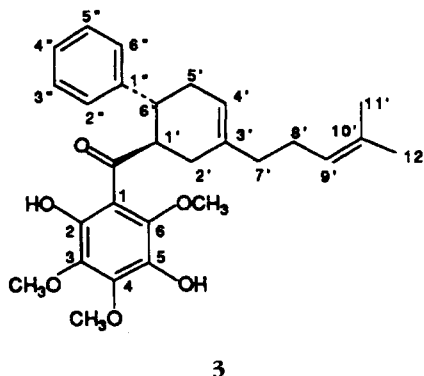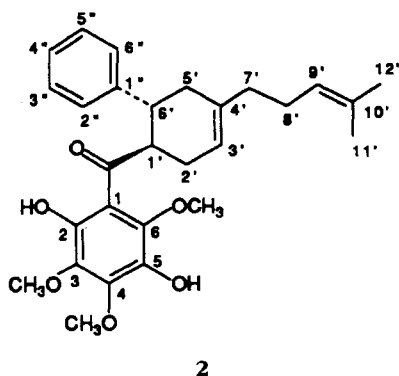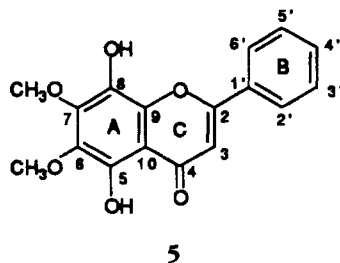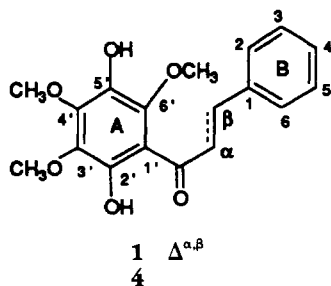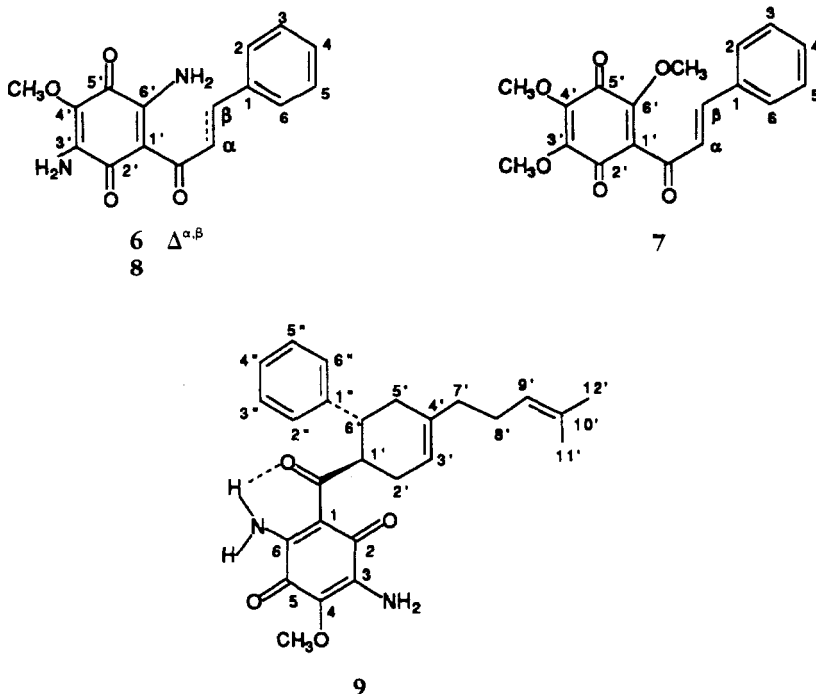
related to schefflerin and isoschefflerin, recently isolated from *Uvaria scheffleri* (3). They can be considered to be biogenetically derived from a Diels-Alder type cyclization between a chalcone and a monoterpenoid diene. The chalcone in the present case is **1**. The diene is β-myrcene instead of β-ocimene for schefflerin and isoschefflerin. Two additional compounds were found in the EtOAc fraction, dihydropedicin [**4**] and 6,7-dimethoxy-5,8-dihydroxyflavone [**5**].

From an alkaloidal extract of the plant, we have isolated the inactive aminoquinone derivatives **6** and **8**. However, these products are artifacts. They were not obtained unless NH$_3$ was used in the alkaloid extraction and could be prepared by treating **1** and **4** with NH$_4$OH. Similarly, treatment of the major "condensed" chalcone **2** gave the corresponding aminoquinone **9**. The latter was also isolated from the plant as a mixture of **9** and a minor positional isomer corresponding to **3**.

RESULTS AND DISCUSSION

Compounds **1**, **4**, and **5** were purified from the EtOAc extract by cc on Si gel. The same chromatographic separation yielded a mixture of fissistin [**2**] and isofissistin [**3**]. Isolation of **2** and **3** was achieved using reversed-phase hplc. The aminoquinones **6**, **8**, and **9** (mixture of isomers) were isolated by cc of the alkaloid extract.

Fissistin [**2**], [α]D 0°, exhibited a M$^+$ peak at *m/z* 466.2368 (calcd 466.2355) in the hreims, corresponding to a molecular formula of C$_{28}$H$_{34}$O$_6$. The ^{1}H- and ^{13}C-nmr spectra showed the signals of a trimethoxy-dihydroxy-benzoyl moiety similar to that of **1**. The HMBC experiment (Figure 1) indicated the same substitution pattern. As for the second moiety, the 1D nmr spectra clearly revealed the presence of a γγ'-dimethylallyl group and a phenyl ring (Table 1). The COSY and HMQC experiments showed two spin-systems as indicated in Figure 1. Analysis of the HMBC spectrum confirmed the assignment and the connectivity of all the protons and carbons as depicted in **2**. The correlations H-3'/C-5', H-2'/C-4' and H-5'/C-4' proved the closure of the cyclohexene ring. The cross-peaks H-1'/CO, H-6'/C-1″ and H-6'/C-2″,6″ supported the proposed
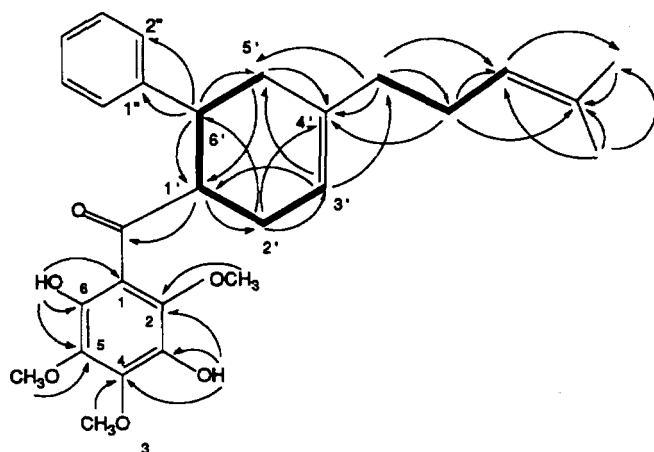
FIGURE 1.   COSY (▬▬) and HMBC (arrows) nmr correlations for
compound **2**.

positioning of the trimethoxy-dihydroxy-benzoyl moiety and the phenyl ring at C-1'
and C-6', respectively. Finally, the position of the aliphatic chain at C-4' was deduced
unambiguously from the correlations H-7'/C-4', H-7'/C-5', and H-3'/C-7'. As for the
relative stereochemistry, the value of the coupling constant ($J = 10$ Hz) between H-1' (δ
4.28) and H-6' (δ 3.25), confirmed by decoupling experiments, indicated their trans
diaxial relationship.

Comparison of the spectral data (ms, 1D nmr, see Table 1) for fissistin {**2**} and
isofissistin {**3**}, [α]D 0°, suggested that **3** is an isomer of **2** of the same structural type.
Since the splitting patterns of H-1' (δ 4.28) and H-6' (δ 3.18) (Table 1) were similar to
those of H-6' and H-1' of **2**, respectively, the two protons were trans diaxial. Therefore,
3 was a positional isomer of **2** which could easily be explained by the postulated
biogenetic origin of the two compounds through a Diels-Alder-like coupling. The
coupling is apparently not enantioselective, since **2** and **3** are racemates. This was also
observed for the schefflerins and other related "condensed" chalcones (3).

The dihydrochalcone **4**, mp 86°, showed uv maxima at 281 (log ϵ 4.14) and 361 nm
(log ϵ 3.68) typical of a flavonoid. The hreims exhibited a molecular ion at m/z 332 that
corresponded to a molecular formula of $C_{18}H_{20}O_6$, which is two mass units greater than
that of the chalcone pedicin {**1**}. Comparison of the nmr data of **1** and **4** showed that the
substitution patterns in rings A and B of both compounds were identical. The spectral
data indicated clearly that the signals of the α and β CH of **1** were replaced by two
methylenes (Table 2) and **4** is thus dihydropedicin. This assumption was confirmed by
conversion of **1** to **4** using catalytic hydrogenation.

Compound **5**, mp 180°, had uv maxima characteristic of a flavone at 284 (log ϵ 4.57)
and 360 nm (log ϵ 3.06). The hreims displayed a molecular ion peak at m/z 314.0787
(calcd 314.0791) that matched the molecular formula $C_{20}H_{20}O_{10}$. In the ir spectrum, the
CO band appeared at 1658 cm^{-1}. The ^{1}H-nmr spectrum exhibited signals of two MeO
groups at δ 3.98 and 4.15, and two multiplets at δ 7.97 (2H) and 7.55 (3H) typical of
an unsubstituted B ring. In addition, the ^{1}H-nmr spectrum showed three singlets (1H)
at δ 12.3, 5.45, and 6.70, assigned to a chelated OH located at C-5, to a second OH, and
to H-3, respectively. The OH group at C-5 showed HMBC correlations with C-6 (δ_C
136.1) and C-10. HMBC correlations were also observed between one MeO and C-6 and
between the second MeO and C-7 (δ_C 146.4). The C-8 position with a typical upfield shift
(δ_C 129.7) thus bears the second OH. This was confirmed by the upfield shift of C-10 (δ_C

TABLE 1. ^{13}C- (62.5 MHz) and ^{1}H-Nmr (300^a or 400^b MHz) Data for Compounds **2**, **3**, and **9** (in CDCl$_3$).

Position	Compound					
	2		**3**		**9**	
	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)
CO.....	210.2		210.1		204.6	
1	111.7		111.7		104.7	
2	150.5		150.6		177.2	
3	135.9		136.0		131.7	
4	142.7		142.7		156.1	
5	133.7		133.7		169.7	
6	146.3		146.3		143.0	
1'......	51.1	4.28 ddd (10,10,5)	51.4	4.30 ddd (10,8,8)	49.0	4.50 ddd (10,10,5)
2'......	30.6	2.50 m	33.5	2.36 m	30.6	2.45 m (17) 2.12 m
3'......	119.2	5.62 br s	136.5		119.5	5.55 d (1.5)
4'......	137.5		120.5	5.56 br s	137.5	
5'......	37.8	2.38 d (8)	34.6	2.36 m	38.2	2.21 m
6'......	44.0	3.25 ddd (10,8,8)	43.7	3.18 ddd (10,10,5)	43.3	3.20 ddd (10,8,8)
7'......	37.4	2.12 m	37.6	2.08 m	37.6	2.02 m
8'......	26.5	2.20 m	26.6	2.14 m	26.6	2.12 m
9'......	124.2	5.20 t (7)	124.2	5.18 t (7)	124.4	5.25 t (7)
10'.....	131.7		131.8		132.5	
11'.....	25.8	1.80 s	25.8	1.73 s	28.8	1.70 s
12'.....	17.8	1.75 s	17.9	1.63 s	17.8	1.62 s
1"......	144.7		144.6		145.9	
2",6" ...	128.3	⎫	128.2	⎫	128.3	⎫
3",5" ...	127.6	⎬ 7.1–7.4 m	127.6	⎬ 7.0–7.3 m	127.5	⎬ 7.1–7.3 m
4"......	126.2	⎭	126.2	⎭	126.0	⎭
3-OMe..	60.9	3.83 s	60.9	3.73 s		
4-OMe..	61.8	4.03 s	61.8	3.93 s	59.7	3.90 s
6-OMe..	61.3	4.17 s	61.4	4.08 s		
2-OH...		11.85 s		11.90 s		
5-OH...		5.20 s		5.28 s		
3-NH$_2$..						5.80 br s
6-NH$_2$..						11.05 s 7.55 s

^{a}For **2** and **3**.
^{b}For **9**.

107.3) indicating the presence of an MeO at C-7 (4). Thus, **5** is 6,7-dimethoxy-5,8-dihydroxyflavone, which is a previously reported compound (5). However, differences between the ^{13}C-nmr chemical shift δ values of **5** and the literature values (5) were observed, especially for C-10 (reported δ_C 103.6). There were also differences in the uv spectra. The previous compound, for which HMBC data were not obtained, is most probably 6,8-dimethoxy-5,7-dihydroxyflavone.

The aminoquinone **6** was obtained as violet-colored crystals, mp 226°, by treatment of **1** with NH$_4$OH in EtOH/CH$_2$Cl$_2$ at room temperature (4 h). Its uv-vis spectrum showed maxima at 334 (log ϵ 4.48) and 508 (log ϵ 2.82) nm. In the ir spectrum, bands at 3375, 1670 (w), and 1543 cm^{-1} were observed. The hreims exhibited a molecular ion peak at *m/z* 298.0955 (calcd 298.2953) corresponding to a molecular formula of C$_{16}$H$_{14}$N$_2$O$_4$. The 1D nmr spectra showed signals characteristic of the α and β CH, the CO, and an unsubstituted B ring of a chalcone similar to those of **1** (Table 2). The remaining part of the molecule corresponded to the formula C$_7$H$_9$N$_2$O$_3$ and contained a MeO group (δ_H 3.90 and δ_C 59.4). These data, coupled with the presence of two downfield CO groups (δ 169.9 and 179.2) in the ^{13}C-nmr spectrum and the fact that **6**

TABLE 2. ^{13}C- (62.5 MHz) and ^{1}H-Nmr (300 MHz) Data for Compounds 4[a], 6[b], and 8[b].

Position	Compound					
	4		6		8	
	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)
CO.....	205.4		189.5		200.1	
α	44.7	3.40 t (7)	129.2	8.10 d (16)	47.7	3.72 t (7)
β	30.2	3.05 t (7)	141.2	8.63 d (16)	31.4	3.28 t (7)
1	141.0		136.9		145.9	
2,6.....	128.1	⎫	129.2	7.70–7.77 m	128.9	7.53–7.58 m
3,5.....	128.1	⎬ 7.20–7.40 m	129.7	⎫ 7.20–7.35 m	129.2	⎫ 7.42–7.50 m
4	125.7	⎭	130.5	⎬	126.3	⎬
1'......	110.0		105.8		104.8	
2'......	151.2		179.2		178.6	
3'......	135.7		133.5		136.5	
4'......	143.0		159.1		156.5	
5'......	133.5		169.9		169.8	
6'......	146.5		146.3		145.8	
OMe-3' .	60.6[c]	3.90 s[c]				
OMe-4' .	60.6[c]	4.10 s[c]	59.7	3.90 s	59.4	4.01 s
OMe-6' .	61.0[c]	3.88 s[c]				
OH-2' ..		12.85 s				
OH-5' ..		5.40 s				

[a]In CDCl$_3$.
[b]In pyridine-d_5.
[c]Within the same column assignments may be reversed.

could be derived from **1** by treatment with NH$_4$OH, supported the proposed structure. Formation of aminoquinones from methoxyquinones by substitution of one or two MeO groups α to the CO with NH$_2$ is well known (6). Obviously, the reaction proceeded by the intermediacy of the quinone corresponding to **1**, the known quinone **7**, which was prepared by treatment of **1** with Ag$_2$O (1,7) and afforded **6** when treated with NH$_4$OH.

Similarly, the aminoquinones **8** and **9** were obtained from **2** and **4**, respectively. The aminoquinone **8**, mp 190°, showed uv maxima at 328 (log ε 4.32) and 512 (log ε 2.78) nm. The ir spectrum revealed bands at 3375, 1670 (w), 1612, and 1562 cm^{-1}. The hreims displayed a molecular ion peak at m/z 300.1107 (calcd 300.1110) corresponding to the molecular formula C$_{16}$H$_{16}$N$_2$O$_4$. The nmr spectra were similar to those of **6** except for the signals at positions α and β which indicated a saturated chalcone (Table 2).

The aminoquinone **9** showed uv maxima at 318 (log ε 4.32) and 511 (log ε 2.89) nm. The ir spectrum exhibited bands at 3370, 1670 (w), and 1570 cm^{-1} similar to those of **6** and **8**. The hreims displayed a molecular ion peak at m/z 434.2193, which corresponded to the molecular formula C$_{26}$H$_{30}$N$_2$O$_4$. The nmr data (Table 1) were similar to those of **6** and **8** for the aminoquinone moiety and to those of **2** for the cyclohexenyl part. The ^{1}H-nmr spectrum recorded in CDCl$_3$ showed two singlets at δ 11.05 and 7.55, assigned to NH. The ^{1}H-^{15}N HMQC spectrum revealed that the two protons correlated with the same nitrogen. The downfield shift of the proton at δ 11.05 was most probably due to chelation between NH$_2$-6 and the non-quinonic CO group.

Pedicin [**1**] inhibited tubulin assembly into microtubules with an IC$_{50}$ value of 300 μM. In the same experimental conditions (8), vinblastine displayed an IC$_{50}$ value of 4 μM. Weak effects on tubulin have been observed previously for calythropsin, a chalcone isolated from *Calythropsis aurea* (9) and a report on structure-activity relationships in a series of synthetic chalcones, some of which are potent inhibitors of the tubulin function, was recently published (10). Pedicin also showed weak cytotoxic activity against KB cells (IC$_{50}$ 7 μg/ml).

Fissistin [**2**] and isofissistin [**3**] both exhibited cytotoxic activity against KB cells with IC_{50} values of 0.15 μg/ml. Such activity has not been found in previously reported condensed chalcones.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a micro hot-stage apparatus and are reported uncorrected. Spectra were recorded as follows: uv, Shimadzu UV-161 uv-visible spectrophotometer; ir, Nicolet 205 Ft-ir spectrometer; eims (70 eV), Kratos MS 50; nmr, Bruker AC 250 and AC 300 or AM 400. Cc was performed using Si gel Merck H60, prep. hplc on a Waters Delta prep 3000 apparatus [Delta-pak C_{18} (100 Å, 15 mm), 47×300 mm, flow rate 50 ml/min, uv detection] and semi-prep. hplc on a Waters RCM Apparatus [Delta-pak C_{18} (100 Å, 15 mm), 10×250 mm, flow rate 10 ml/mn, uv detection].

PLANT MATERIAL.—Leaves of *Fissistigma lanuginosum* were collected at Dungun, Terrenganu, Malaysia in July 1993. The plant identification was made by F. Remy. Voucher specimens have been deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia and the Laboratoire de Phanérogamie, Muséum National d'Histoire Naturelle, Paris, France.

EXTRACTION AND ISOLATION.—The EtOAc-soluble residue of the dried and powdered leaves of *F. lanuginosum* (420 mg, yield 6.4%) was chromatographed on Si gel (cyclohexane/EtOAc) to give a mixture of compounds **2** and **3** (81 mg), the dihydrochalcone **4** (42 mg), pedicin [**1**] (93 mg), and the flavone **5** (35 mg). The mixture of **2** and **3** (28 mg) was further purified by prep. hplc (MeOH-H$_2$O, 6:4 to 8:2) yielding pure **2** (15 mg) and **3** (5 mg).

In addition, a MeOH extract (21.4 g, yield 2.1%) was diluted with CH$_2$Cl$_2$ and reextracted with 5% HCl. The aqueous layer was basified to ca. pH 11 with NH$_4$OH and reextracted with CH$_2$Cl$_2$ until a negative Mayer test was obtained. The CH$_2$Cl$_2$ extracts were pooled, washed with H$_2$O, dried over anhydrous Na$_2$SO$_4$, and evaporated, yielding a crude alkaloid fraction (1.40 g). This crude product (0.90 g) was chromatographed on Si gel with MeOH/CH$_2$Cl$_2$ yielding a mixture of aminoquinones **6** and **7** (10.3 mg), and a mixture of **9** and its positional isomer (16.8 mg), which were not further purified.

Pedicin [**1**].—Orange crystals from CH$_2$Cl$_2$; mp 133–135° [lit. (3) 145°]; uv λ max (MeOH) 240, 315 nm; ir ν max (CHCl$_3$) 1635, 1570 cm^{-1}; eims *m/z* 330 [M]$^+$ (92), 226 (100), 211 (86); ^{1}H nmr (CDCl$_3$) δ 12.7 (1H, s, OH-2′), 7.85 (1H, d, J=15 Hz, H-β), 6.75 (1H, d, J=15 Hz, H-α), 7.45–7.52 (2H, m, H-2′ and H-6′), 7.26–7.32 (3H, m, H-3′, H-5′, and H-6′), 5.30 (1H, s, OH-5′), 4.05 (3H, s, OMe-4′), 3.75 (3H, s, OMe-3′), 3.60 (3H, s, OMe-6′); ^{13}C nmr (CDCl$_3$) δ 193.9 (CO), 126.6 (C-α), 144.3 (C-β), 135.5 (C-1), 129.4 (C-2 and C-6), 128.9 (C-3 and C-5), 130.9 (C-4), 111.2 (C-1′), 152.5 (C-2′), 136.3 (C-3′), 147.5 (C-4′), 134.7 (C-5′), 143.5 (C-6′), 61.4 (MeO-3′), 61.7 (MeO-4′), 62.4 (MeO-6′). [Assignments based on HMQC and HMBC (cross-peaks OH-2′/C-2′, OH-2′/C-1′, OH-2′/C-3′, OH-5′/C-5′, OH-5′/C-4′, OH-5′/C-6′, MeO-3′/C-3′, MeO-4′/C-4′, MeO-6′/C-6′) and nOe difference (OMe-6′/C-α, OMe-6′/C-β) experiments].

Fissistin [**2**].—Yellow gum: uv λ max (MeOH) 288 (log ε 4.00), 367 (log ε 3.43) nm; ir ν max (CHCl$_3$) 3535, 1629 cm^{-1}; eims *m/z* 466 [M]$^+$ (92), 227 (100); ^{1}H- and ^{13}C-nmr data, see Table 1.

Isofissistin [**3**].—Yellow gum: uv λ max (MeOH) 289 (log ε 4.06), 366 (log ε 3.48) nm; ir ν max (CHCl$_3$) 3528 and 1629 cm^{-1}; eims *m/z* 466 [M]$^+$ (100), 227 (100); ^{1}H- and ^{13}C-nmr data, see Table 1.

2′,5′-Dihydroxy-3′,4′,6′-trimethoxydihydrochalcone [**4**].—Yellow crystals from pentane-ether, mp 86°; uv λ max (MeOH) 281 (log ε 4.14), 361 (log ε 3.68) nm; ir ν max (CHCl$_3$) 1650 cm^{-1}; eims *m/z* 332 [M]$^+$ (100), 227 (66), 91 (40); ^{1}H- and ^{13}C-nmr data, see Table 2; *anal.*, calcd for C$_{18}$H$_{20}$O$_6$, C, 65.05, H, 6.07, O, 28.89; found C, 64.68, H, 6.01, O, 29.22. A MeOH solution (10 ml) of **1** (10 mg) and 10% Pd/C (ca. 10 mg) were shaken for 1 h under H$_2$. Removal of the catalyst and the solvent yielded **4** (8 mg), identical to the product extracted from the plant.

5,8-Dihydroxy-6,7-dimethoxyflavone [**5**].—Yellow crystals from MeOH, mp 180°; uv λ max (MeOH) 284 (log ε 4.57), 360 (log ε 3.68) nm; ir ν max (CHCl$_3$) 1658, 1616, 1593 cm^{-1}; eims *m/z* 314 [M]$^+$ (100), 299 (100), 240 (56), 225 (48); ^{1}H nmr (CDCl$_3$) δ 12.30 (1H, s, OH-5), 7.97–7.99 (2H, m, H-2′ and H-6′), 7.52–7.58 (3H, m, H-3′, H-5′, and H-6′), 6.70 (1H, s, H-3), 5.45 (1H, br s, OH-8), 3.98 (3H, s, MeO-6), 4.15 (3H, s, OMe-7); ^{13}C nmr (CDCl$_3$) δ 164.3 (C-2), 107.3 (C-3), 183.3 (C-4), 146.0 (C-5), 136.1 (C-6), 146.4 (C-7), 129.7 (C-8), 139.9 (C-9), 107.3 (C-10), 131.5 (C-1′), 129.2 (C-2′ and C-6′), 126.6 (C-3′ and C-5′), 132.1 (C-4′), 61.1, 61.9 (OMe-6, OMe-7).

Compound **6**.—From pedicin [**1**]: To a solution of **1** (30 mg) in EtOH-CH$_2$Cl$_2$ (50:50, 4 ml) was added NH$_4$OH (0.2 ml) and the mixture was stirred at room temperature for 3 h. The mixture was evaporated to dryness *in vacuo* and dissolved in EtOH-CH$_2$Cl$_2$ (50:50, 6 ml). Subsequent addition of NH$_4$OH (0.2 ml) with

stirring for 2.5 h, and evaporation yielded **6** (26 mg), which crystallized from MeOH/CH$_2$Cl$_2$ as violet-colored crystals, mp 226°; uv λ max (MeOH) 334 (log ε 4.48), 508 (log ε 2.82) nm; ir ν max (CHCl$_3$) 3575, 1670, 1543 cm^{-1}; eims m/z 298 [M]$^+$ (100), 221 (68), 103 (100); ^{1}H- and ^{13}C-nmr data, see Table 2.

From **7**: To a solution of **7** (9 mg) in EtOH-Et$_2$O (50:50, 2 ml) was added NH$_4$OH (0.1 ml). The mixture was stirred at room temperature for 3 h and then evaporated to dryness *in vacuo*. The residue was dissolved in EtOH-CH$_2$Cl$_2$ (50:50, 2 ml), and NH$_4$OH was added. Stirring for 2.5 h and evaporation yielded **6** (8 mg), identical to the product obtained from **1**.

3',4',6'-Trimethoxy-2',5'-quino-chalcone [**7**].—Yellow crystals from ether, mp 112° [lit. (7) 113–114°]. Uv λ max (MeOH) 298 (log ε 4.40), 402 (log ε 3.86) nm; ir ν max (CHCl$_3$) 1681, 1637, 1600 cm^{-1}; eims m/z 328 [M]$^+$ (100), 269 (37), 257 (30), 209 (50); ^{1}H nmr (CDCl$_3$) δ 7.54–7.60 (2H, m, H-2' and H-6'), 7.38–7.42 (3H, m, H-3', H-5', and H-6'), 7.52 (1H, d, J=15 Hz, H-β), 6.95 (1H, d, J=15 Hz, H-α), 3.95, 4.00, 4.08 (3H×3, 3×s, OMe-3', OMe-4', OMe-6'); ^{13}C nmr (CDCl$_3$) δ 191.3 (CO), 128.1 (C-α), 147.0 (C-β), 131.4, 128.1 (C-1, C-4), 129.2 (C-2 and C-6), 128.9 (C-3 and C-5), 121.1 (C-1'), 179.4 (C-2'), 144.6, 143.2 (C-3', C-4'), 182.4 (C-5'), 153.4 (C-6'), 61.0, 61.5 (OMe-3', OMe-4', OMe-6').

Compound **8**.—This compound was prepared from dihydropedicin [**4**] (20 mg) in the same way as compound **6** from pedicin [**1**]. Violet-colored crystals from MeOH, mp 190°: uv λ max (MeOH) 300 (sh), 328 (log ε 4.32), 512 (log ε 2.78) nm; ir ν max (CHCl$_3$) 3575, 1670, 1612, 1562 cm^{-1}; hreims m/z 300.1107 (calcd 300.1110 for C$_{16}$H$_{16}$N$_2$O$_4$) [M]$^+$ (100), 196.0471 (calcd 196.0484 for C$_8$H$_8$N$_2$O$_4$) (72), 91 (60); ^{1}H- and ^{13}C-nmr data, see Table 2.

Compound **9**.—To a solution of **2** (9 mg) in EtOH-Et$_2$O (1:1, 2 ml) was added NH$_4$OH (0.1 ml). The mixture was stirred at room temperature for 3 h and then evaporated to dryness *in vacuo* yielding **9** (8 mg). Violet amorphous solid: uv λ max (MeOH) 300 (sh), 318 (log ε 4.32), 511 (log ε 2.89) nm; ir ν max (CHCl$_3$) 3575, 1670, 1570 cm^{-1}; hreims m/z 434.2193 (calcd 434.2205 for C$_{26}$H$_{30}$N$_2$O$_4$) [M]$^+$ (100), 196.0461 (calcd 196.0484 for C$_8$H$_8$N$_2$O$_4$) [M]$^+$ (100), 196 (46); ^{1}H- and ^{13}C-nmr data, see Table 1.

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