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Full Papers

New Bioactive Flavonoids and Stilbenes in Cubé Resin Insecticide

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Fractionation of cubé resin from *Lonchocarpus utilus* and *L. urucu* roots led to the isolation and identification of 11 minor flavonoids and stilbenes containing the *gem*-dimethylpyran moiety or a dihydrodiol derivative thereof. The eight new compounds were as follows: the isoflavonoid *cis*-4″,5″-dihydro-4″,5″-dihydroxylonchocarpusone (**2**); four (2*S*)-6-(γ,γ-dimethylallyl)-6″,6″-dimethylpyran[2″,3″:7,8]flavanones with substituents of 5-hydroxy-3′,4′-dimethoxy (**3**), 5,3′-dihydroxy-4′-methoxy (**4**), 5,4′-dihydroxy-3′-methoxy (**5**), and 3′,4′-dimethoxy (**6**); and three 6″,6″-dimethylpyran[2″,3″:3′,4′]stilbenes with 4-hydroxy-5′-methoxy (**9**), 3,5′-dimethoxy-4-hydroxy (**10**) and 3,4,5-trimethoxy (**11**) substitution patterns. Structure−activity relationships for inhibition of NADH:ubiquinone oxidoreductase activity (bovine heart electron transport particles) and phorbol ester-induced ornithine decarboxylase activity (cultured MCF-7 cells) generally parallel those for cytotoxicity (MCF-7 and Hepa 1clc7 cells).

Cubé resin is an extract of the roots of *Lonchocarpus utilis* A. C. Smith and *L. urucu* Killip and Smith (Leguminosae) used as an insecticide and piscicide.[1] The principal components are rotenone (44%) and deguelin (22%), which differ structurally only in the isopropenyldihydrofuran versus the *gem*-dimethylpyran moiety. These two rotenoids account for essentially all of the toxicity of cubé resin to insects, fish, and mammals and to mammalian cells in culture.[1,2] Rotenone and deguelin are also the most potent inhibitors in cubé resin for NADH:ubiquinone oxidoreductase activity (the primary target enzyme) and phorbol ester-induced ornithine decarboxylase (ODC) activity (an indicator for antiproliferative effect and candidate cancer chemopreventive action).[3] Further examination of cubé resin with emphasis on the less polar components led to the isolation of 11 minor compounds identified as eight flavonoids (**1**−**8**) and three stilbenes (**9**−**11**) (Chart 1). They all contain the *gem*-dimethylpyran moiety as in deguelin (or a dihydrodiol derivative thereof); the biosynthetic link or coexistence of isoflavones, flavanones, chalcones, and stilbenes with the corresponding rotenoids is often noted in the Leguminosae.[4−7] Eight of the flavonoids are new chemicals with structural assignments presented in this paper. Potency assays in the enzyme systems[3] indicated above allowed comparison with two standards, the flavonoid genistein[8] and the stilbene *trans*-resveratrol.[9]

Results and Discussion

Chromatography of cubé resin (with much of the rotenone removed) on a silica gel column, preparative TLC, and semipreparative HPLC gave lonchocarpusone (**1**) known from the roots of *Lonchocarpus nicou*[4] and 4-hydroxylonchocarpin (**7**) identified by comparison of our spectroscopic data with those reported.[10] 3-Methoxy-4-hydroxylonchocarpin (**8**) has been isolated from the heartwood of *Pongamia glabra* Vent. and obtained by synthesis without reporting the spectral data,[11] which is provided in the Experimental Section.

The *gem*-dimethylpyran moiety was present in 10 of the 11 flavonoids and stilbenes in cubé resin, and the exception (compound **2**) also had the *gem*-dimethylpyran but as a dihydrodiol derivative. Characteristic spectral data for the *gem*-dimethylpyran observed by ^{1}H NMR were a *cis*-ethylenic system (δ 6.34−6.76 and 5.49−5.64) and nearly

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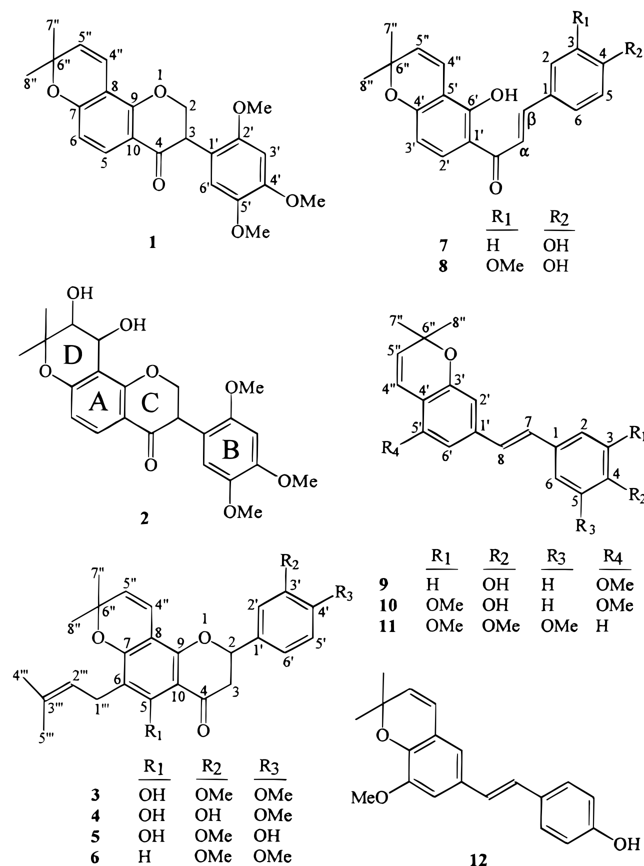
Chart 1



equivalent methyl signals at δ 1.43–1.47, by ^{13}C NMR were the quaternary O-bound C-atom at δ 75.9–78.1 ppm, and by mass spectrometry were the $[M - Me]^+$ fragmentation ions.[5,12]

Compound **2** was the same as **1** except for the D ring. Their isoflavone skeleton was indicated by an intense UV band II absorption at 298 nm and low-intensity band I absorption at 260 nm (Table 1).[13] The molecular formula of **2** $C_{23}H_{24}O_8$, from the HRFABMS, had two more hydroxyl groups than present in **1**, and they were in the dimethylpyran moiety based on the ^1H NMR data (Table 2) since instead of two olefinic doublets at δ 6.85 for H-4″ and 5.72 for H-5″ (each J = 8.8 Hz) in **1** there were two broad doublets with smaller coupling constants (each J = 4.1 Hz) appearing at higher field (δ 4.97 and 3.83 for H-4″ and -5″, respectively) in **2**. These differences and the disappearance of two broad singlets at δ 3.01 and 3.44 on addition of D_2O suggested 4″,5″-dihydroxyl substituents for **2**, an assignment fully supported by the complete ^1H NMR (Table 2) and ^{13}C NMR (Table 3) data and the MS data. Although not detailed here, the stereochemistry at C-4″ and -5″ was assigned as cis by comparison with the ^1H NMR data for the corresponding protons of the *cis*-3,4-diol metabolite of precocene I[14] and of *cis*-4′,5′-dihydro-4′,5′-dihydroxytephrosin[3] (unpublished results). The structure of **2** was therefore *cis*-4″,5″-dihydro-4″,5″-dihydroxylonchocarpusone.

Compounds **3**–**6** had UV spectra characteristic (Table 1) of flavanones with major absorption peaks in the range 264–276 nm as typical band II absorptions.[13] The (2*S*)-flavanone skeleton of **3**–**6** was suggested by the ^1H NMR spectra with characteristic three one-proton double doublets at δ 5.30–5.40 (J = 3.1, 12.8 Hz), 2.78–2.82 (J = 3.1, 16.9 Hz), and 3.01–3.06 (J = 12.3–12.8, 16.4–17.4 Hz) assignable to H-2 (1H) and H-3 (2H) of the flavanone skeleton.[5] Carbon resonances of C-2 (δ 78.7–79.2, d) and C-3 (δ 43.3–44.1, t) in the fully coupled ^{13}C NMR spectra and the UV spectra of **3**–**6** were in agreement with this assignment. Comparisons of ^1H and ^{13}C NMR spectra of **3**–**6** (Tables 2 and 3) with known compound 6-methoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone (NMR data ruled out the [2″,3″:7,6] isomer)[5] suggested that the 6″,6″-dimethylpyran moiety was located on the A ring with positions [2″,3″:7,8] for the four new flavanones **3**–**6**.

Compound **3** exhibited a MS molecular ion peak at *m*/*z* 450 (75%) corresponding to $C_{27}H_{30}O_6$. Its ^1H NMR spectrum showed aromatic signals characteristic for H-2′, H-5′, and H-6′ at δ 7.01 (d, J = 1.5 Hz), 6.89 (d, J = 8.2 Hz), and 6.98 (dd, J = 1.5, 8.2 Hz), respectively, i.e., a 3′,4′-dioxygenation pattern as in the known compound (2*S*)-3′,4′-dimethoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone.[5] Other ^1H NMR signals established two methoxyl groups (δ 3.91, 3H × 2), one chelated hydroxyl group (δ 12.26), and a set of prenyl (γ,γ-dimethylallyl) signals at δ 3.20 (d, J = 7.2, $-CH_2-$), 5.18 (brt, J = 7.2, CH=), 1.66 (Me), and 1.65 (Me).[15] The chelated hydroxyl group must be at C-5, and the two methoxyl groups were therefore placed at C-3′ and C-4′ so the prenyl must be at C-6, assignments consistent with the mass spectral and fully coupled ^{13}C NMR data. Thus, **3** was (2*S*)-6-(γ,γ-dimethylallyl)-5-hydroxy-3′,4′-dimethoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone.

Compound **4** had a very similar structure to **3** with almost identical UV spectra and the same substitution pattern determined from the ^1H and ^{13}C NMR spectra. The only difference was that **3** had two methoxyl groups in the B ring, whereas **4** had one methoxyl group (δ 3.91, s, 3H), and one hydroxyl group (δ 5.76, s, 1H, disappeared after addition of D_2O) at C-4′ and C-3′, respectively, a conclusion confirmed by the M^+ peak at *m*/*z* 436 ($C_{26}H_{28}O_6$) in the mass spectrum of **4**. The observed 2D-NOE between protons at C-5′ and the methoxyl group showed clearly that **4** possessed a 3′-hydroxy-4′-methoxy B-ring. Thus, **4** was identified as (2*S*)-6-(γ,γ-dimethylallyl)-5,3′-dihydroxy-4′-methoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavonone.

Compound **5** was the same as **4** except for the B ring; i.e., they gave the same mass spectra, suggesting one methoxyl and one hydroxyl group, and all ^1H NMR signals of **5** were identical to those of **4** except those for protons in the B ring. Since the signals for three protons of the B ring overlapped in the δ 6.93–6.98 region, it was hard to tell the oxygenation pattern in the B ring from the ^1H NMR data. However, the ^{13}C NMR spectra of compounds **4** and **5** were very similar (Table 3) and were typical of the B ring with two ortho oxygenation points at C-3′ and 4′. In comparison with **4**, these data, including ^1H NMR, fully coupled ^{13}C NMR, and UV, assigned compound **5** as (2S)-6-(γ,γ-dimethylallyl)-5,4′-dihydroxy-3′-methoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone (not 5,5′-dihydroxy-3′-methoxy as previously suggested[3]).

The proposed structures of **3**–**5** were fully supported by the same EIMS fragmentation patterns. They involved cleavage of the intact A- and B-ring fragments, which were the most useful patterns in terms of flavonoid identification.[16] They yielded intense peaks for the molecular ion M^+ (70–82%) and base peaks for $[M - Me]^+$ from loss of the methyl group of the dimethylpyran moiety (D ring). The series of ions derived from the A/C/D system of **3**–**5** were almost identical at *m*/*z* 286 (A_1^+, 5–8%), 271 ($[A_1 - Me]^+$, 22–32%), 258 ($[A_1 - CO]^+$, 5–6%), 243 ($[A_1 - Me - CO]^+$, 15–20%), and 215 ($[A_1 - Me - C_4H_8]^+$, 36–48%). The peaks corresponding to B_3^+ and $[B_3 - Me]^+$ for **4** and **5** were the same at *m*/*z* 150 (4–8%) and 135 (8–10%), respectively. These two ions for **3** were at *m*/*z* 164 and 149,

Table 1. UV Data for Compounds **2**−**11**

compd	γ_{max} (nm) (log ϵ) MeOH	MeOH + NaOMe
2	260 (1.74), 298 (2.25)	260 (1.77), 298 (2.25)
3	276 (2.18), 300 sh (0.65), 314 sh (0.69), 364 (0.18)	276 (2.08), 300 sh (0.65), 314 sh (0.69), 374 (0.22)
4	276 (1.62), 300 sh (0.40), 314 sh (0.49), 364 (0.12)	276 (1.57), 296 sh (0.72), 312 sh (0.52), 368 (0.14)
5	276 (2.12), 300 sh (0.66), 314 sh (0.69), 360 (0.17)	276 (2.14), 300 sh (0.95), 314 sh (0.70), 366 (0.22)
6	264 (2.22), 346 (0.44)	266 (2.20), 346 (0.46)
7	280 (0.64), 372 (1.83)	272 (0.83), 318 (0.55), 436 (1.92)
8	272 (0.96), 380 (1.77)	274 (1.10), 316 (0.65), 456 (1.94)
9	254 (0.62), 340 (2.33)	256 (0.73), 368 (2.32)
10	256 (0.79), 344 (2.42)	256 (0.91), 378 (2.33)
11	276 (1.58), 324 (2.33)	276 (1.59), 324 (2.29)

Table 2. ^{1}H NMR (300 MHz) Data (δ, J (Hz)) in $CDCl_3$ for Compounds **2**−**6**

proton(s)	**2**	**3**	**4**	**5**	**6**
2 (1H)	7.98 s	5.35 dd (J = 3.1, 12.8)	5.30 dd (J = 3.1, 12.8)	5.32 dd (J = 3.1, 12.8)	5.40 dd (J = 3.1, 12.8)
3-cis (1H)		2.81 dd (J = 3.1, 16.9)	2.78 dd (J = 3.1, 16.9)	2.79 dd (J = 3.1, 16.9)	2.82 dd (J = 3.1, 16.9)
3-trans (1H)		3.06 dd (J = 12.8, 16.9)	3.02 dd (J = 12.3, 16.9)	3.05 dd (J = 12.8, 17.4)	3.01 dd (J = 12.8, 16.4)
5 (1H)	8.07 d (J = 8.7)				7.46 s
6 (1H)	6.87 d (J = 8.7)				
2′ (1H)	6.93 s	7.01 d (J = 1.5)	7.03 d (J = 2.1)	6.93−6.98 m (2′, 4′, 5′, 6′)	7.04 d (J = 1.5)
4′ (1H)					
5′ (1H)	6.60 s	6.89 d (J = 8.2)	6.86 d (J = 8.2)		6.90 d (J = 8.2)
6′ (1H)		6.98 dd (J = 1.5, 8.2)	6.92 dd (J = 2.1, 8.2)		7.00 dd (J = 1.5, 8.2)
4″ (1H)	4.97 brd (J = 4.1)	6.63 d (J = 10.3)	6.63 d (J = 9.8)	6.63 d (J = 10.3)	6.61 d (J = 9.8)
5″ (1H)	3.83 brd (J = 4.1)	5.50 d (J = 10.3)	5.49 d (J = 9.8)	5.50 d (J = 9.8)	5.60 d (J = 9.8)
7″-Me	1.50 s	1.45 s	1.44 s	1.45 s	1.45 s
8″-Me	1.32 s	1.44 s	1.43 s	1.43 s	1.44 s
1‴ (2H)		3.20 d (J = 7.2)	3.21 d (J = 7.2)	3.21 d (J = 7.7)	3.33 d (J = 7.2)
2‴ (1H)		5.18 brt (J = 7.2)	5.15 brt (J = 7.2)	5.18 brt (J = 7.7)	5.23 brt (J = 7.2)
Me-4‴		1.66 s	1.67 s	1.66 s	1.69 s
Me-5‴		1.65 s	1.66 s	1.65 s	1.66 s
OMe-3′	3.92	3.91 s		3.92 s	3.92 s
OMe-4′	3.86	3.91 s	3.91 s		3.90 s
OMe-6′	3.76 s				
OH[a]	3.01 brs (OH-5″) 3.44 brs (OH-4″)	12.26 s (OH-5)	5.76 s (OH-3′) 12.26 s (OH-5)	5.76 s (OH-5′) 12.26 s (OH-5)	

[a] On addition of D_2O the signals disappeared for OH-4′ and -5′ and decreased for OH-5.

respectively, which were 14 mass units higher than those for **4** and **5** because of the difference between methoxyl and hydroxyl groups.

Compound **6** had one less oxygen than **3** based on the M^+ at m/z 434 ($C_{27}H_{30}O_5$) in the mass spectrum. Fragment ions derived from the B ring at m/z 164 $[B_3]^+$ and 149 $[B_3 - Me]^+$ indicated that **6** had the same dimethoxyl B ring as that of **3** and, consequently, suggested the absence of a hydroxyl group at C-5. This proposal was supported by the fragment ions from the A/C/D ring system: $[A_1]^+$ at m/z 270 (8%), $[A_1 - Me]^+$ at m/z 255 (33%), $[A_1 - CO]^+$ at m/z 242 (12%), and $[A_1 - Me - CO]^+$ at m/z 227 (16%). The major UV absorption band at 266 nm, 10 nm less than that for **3**, confirmed **6** as the 5-dehydroxyl analogue of **3** because removal of the 5-hydroxyl group from a flavonone caused a 10−15 nm hypsochromic shift of the principal absorption band.[13] Assignment of the 6″,6″-dimethylpyran at positions [2″,3″:7,8] rather than [2″,3″:7,6] was based on comparison of the ^{1}H NMR spectra of the two possible structures for **6** created by the ACD/HNMR Predictor computer program (Advanced Chemistry Development, Inc., Toronto, Canada). The singlet for H-5 was predicted to be at δ 7.606 and 6.897 for the [2″,3″:7,8] and [2″,3″:7,6] isomer, respectively. A singlet (δ 7.46) for H-5 of compound **6** (Table 2) supported the structure **6**, which was also confirmed by the similarity between the observed ^{13}C NMR data (Table 3) and predicted ^{13}C NMR signals from the ACD/CNMR Predictor computer program. These observations and the ^{1}H and fully coupled ^{13}C NMR data assigned compound **6** as (2*S*)-6-(γ,γ-dimethylallyl)-3′,4′-dimethoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone.

Table 3. ^{13}C NMR (75 MHz) Data (δ) for Compounds **2**−**6**

carbon	**2** (CD_3OD)	**3** ($CDCl_3$)[a]	**4** ($CDCl_3$)[a]	**5** ($CDCl_3$)[a]	**6** ($CDCl_3$)[a]
2	156.4	78.7 d	78.5 d	78.9 d	79.2 d
3	123.5	43.4 t	43.3 t	43.5 t	44.1 t
4	178.2	196.2 s	196.3 s	196.3 s	190.9 s
5	127.6	159.8 s	159.8 s	159.8 s	121.6 d
6	117.7	102.9 s	102.8 s	102.9 s	116.9 s
7	158.7	159.3 s	159.3 s	159.3 s	160.5 s
8	112.5	108.6 s	108.7 s	108.6 s	114.5 s
9	159.3	156.6 s	156.6 s	156.6 s	157.3 s
10	119.1	102.7 s	102.7 s	102.7 s	115.6 s
1′	113.7	131.5 s	132.2 s	131.0 s	131.8 s
2′	118.0	109.5 d	110.6 d	108.8 d	109.5 d
3′	144.5	149.4 s	146.8 s	146.7 s	149.1 s
4′	152.0	149.4 s	145.9 s	146.0 s	149.1 s
5′	99.9	111.3 d	112.6 d	119.3 d	111.2 d
6′	154.1	118.5 d	117.8 d	114.5 d	118.4 d
4″	80.5	115.7 d	115.7 d	115.7 d	121.8 d
5″	66.6	125.9 d	125.9 d	125.9 d	129.0 d
6″	75.4	78.1 s	78.1 s	78.1 s	77.4 s
7″	25.2	28.4 q	28.4 q	28.3 q	28.3 q
8″	23.0	28.3 q	28.3 q	28.3 q	28.2 q
1‴		21.5 t	21.5 t	21.5 t	21.9 t
2‴		122.6 d	122.6 d	122.6 d	122.1 d
3‴		131.0 s	131.0 s	130.9 s	131.2 s
4‴		25.8 q	25.8 q	25.8 q	25.6 q
5‴		17.8 q	17.8 q	17.8 q	17.7 q
3′-OMe	57.7	56.0 q		56.0 q	55.8 q
4′-OMe	57.2	56.0 q	56.0 q		55.8 q
6′-OMe	56.9				

[a] Fully coupled spectrum.

Compound **8**, like the known 4-hydroxylonchocarpin (**7**),

Table 4. ^{1}H NMR (300 MHz) Data (δ, J (Hz)) in $CDCl_3$ for Compounds **8**−**11**

proton	8	9	10	11
2	7.12 d (J = 1.5)	7.39 d (J = 8.7)	7.02 d (J = 2.1)	6.70 s
3		6.81 d (J = 8.7)		
5	6.96 d (J = 8.2)	7.39 d (J = 8.7)	6.90 d (J = 8.6)	
6	7.23 dd (J = 2.1, 8.2)	6.81 d (J = 8.7)	7.01 dd (J = 7.2, 2.1)	6.70 s
7 (β)	7.41 d (J = 15.4)	6.84 d (J = 16.4)	6.83 d (J = 15.9)	6.86 d (J = 16.4)
8 (α)	7.82 d (J = 15.4)	7.00 d (J = 16.4)	7.00 d (J = 16.4)	6.94 d (J = 16.4)
2′		6.52 d (J = 1.0)	6.52 br s	7.14 d (J = 2.1)
5′	6.38 d (J = 8.7)			6.76 d (J = 8.2)
6′	7.72 d (J = 9.2)	6.63 d (J = 2.1)	6.64 br s	7.26 dd (J = 2.1, 8.2)
4″	6.76 d (J = 10.3)	6.65 d (J = 9.7)	6.65 d (J = 10.3)	6.34 d (J = 9.8)
5″	5.59 d (J = 10.3)	5.56 d (J = 9.7)	5.56 d (J = 9.9)	5.64 d (J = 9.8)
7″-Me	1.47 s	1.43 s	1.43 s	1.44 s
8″-Me	1.47 s	1.43 s	1.43 s	1.44 s
3-OMe	3.98 s		3.94 s	3.91 s
4-OMe				3.86 s
5-OMe				3.91 s
5′-OMe		3.86 s	3.87 s	
OH-3[a]	5.93 s			
OH-4[a]		5.12 br s	5.68 s	
OH-2′[a]	11.09 s			

[a] On addition of D_2O, the signals disappeared for OH-3 and -4 and decreased for OH-2′.

was recognized as a chalcone by a dominant band I absorption at 380 nm and a relatively minor band II at 272 nm, and the presence of a 4-hydroxyl group was evidenced by the band II bathochromic shift of 76 nm with an increase in intensity on addition of sodium methoxide (Table 1).[13] A set of trans-olefinic protons at δ 7.41 and 7.82 (each, d, J = 15.4 Hz) were at β and α positions, and a chelated hydroxyl group at δ 11.09 was assigned as OH-2′. Comparative analysis of the ^{1}H NMR spectra of **7**[10] and **8** (Table 4) established that the structural difference was in the B ring: a 4-oxygenated B ring for **7** and a 3,4-dioxygenated B ring with one extra methoxyl group for **8**. Since the UV spectrum of **8** indicated a 4-hydroxyl group, the methoxyl substituent should be at C-3. These data, supported by the mass spectrum ($[M]^+$ at m/z 352 corresponding to $C_{22}H_{24}O_4$) and the fully coupled ^{13}C NMR spectrum, assigned **8** as 3-methoxy-4-hydroxylonchocarpin.

Compound **9** gave $[M]^+$ at m/z 308 according to the molecular formula $C_{20}H_{20}O_3$ confirmed by HREIMS. It exhibited the ^{1}H NMR spectrum of a *trans*-stilbene with the typical two one-proton doublets at δ 6.84 and 7.00 ppm (J = 16.4 Hz) indicating the *trans*-ethylenic chain and two pairs of ortho-coupled doublets (d, J = 8.7 Hz) at δ 6.81 (H-3,6) and 7.39 (H-2,5) for the 1,4-substituted aromatic ring (Table 4). Signals for two meta-coupled doublets in the aromatic region for the *gem*-dimethylbenzopyran moiety indicated that compound **9** was a 4,5′-dioxygenated analogue of the known compound lonchocarpene[4] with two substituents at C-5′ and C-4, which must be one methoxyl group (δ 3.86) and one hydroxyl group (δ 5.12 which disappeared after addition of D_2O). An observed NOE between the methoxyl protons and that at C-6′ suggested that the methoxyl group was at C-5′. Now, there were two possible structures: 4-hydroxy-5′-methoxy-6″,6″-dimethylpyran[2″,3″,3′,4′]stilbene (**9**) and 4-hydroxy-5′-methoxy-6″,6″-dimethylpyran[2″,3″:4′,3′]stilbene (**12**). The major difference between the predicted ^{1}H NMR spectra for **9** and **12** created by ACDE/HNMR Predictor are the signals for H-4″ and 5″: two one-proton doublets at δ 6.057 (H-4″) and 5.865 (H-5″) for **12** with dimethylpyran[2″,3″,4′,3′]-stilbene, and at δ 6.638 (H-4″) and 5.588 (H-5″) for **9** with dimethylpyran[2″,3″:3′,4′]stilbene. The signals of H-4″ (d, δ 6.65) and H-5′ (d, δ 5.56) in the ^{1}H NMR spectrum of compound **9** (Table 4) suggested the structure dimethylpyran[2″,3″,3′,4′]stilbene for compound **9**. Also, the structure **9** was supported by similarity between ^{13}C NMR (Table 5) and the predicted ^{13}C NMR from ACD/CNMR Predictor. Therefore, the structure of **9** was 4-hydroxy-5′-methoxy-6″,6″-dimethylpyran[2″,3″:3′,4′]stilbene as previously indicated[3] (although incorrectly shown earlier in the structural formula).

Table 5. ^{13}C NMR (75 MHz) Data (δ) in $CDCl_3$ for Compounds **8**−**11**

carbon	8[a]	9[a]	10	11[a]
1	127.8 s	138.7 s	138.6	137.9 s
2	115.0 d	127.9 d	108.4	103.6 d
3	148.5 s	115.7 d	145.7	153.4 s
4	146.9 s	155.3 s	146.8	133.4 s
5	110.3 d	115.7 d	114.6	153.4 s
6	123.5 d	127.9 d	120.6	103.6 d
7 (β)	144.6 d	126.6 d	126.7	126.4 d
8 (α)	117.9 d	128.3 d	128.7	127.8 d
β′	191.9 s			
1′	109.5 d	130.1 s	130.0	130.1 s
2′	159.7 s	107.3 d	107.3	124.2 d
3′	114.1 s	110.2 s	110.2	121.3 s
4′	160.9 s	153.6 s	153.9	152.8 s
5′	108.2 d	155.4 s	155.4	116.5 d
6′	130.5 d	101.7 d	101.5	122.1 d
4″	115.9 d	116.8 d	116.8	131.1 d
5″	128.1 d	128.7 d	128.7	127.3 d
6″	77.8 s	76.1 s	75.9	76.5 s
OMe-3				56.2 q
OMe-4	56.1 q		55.9	60.9 q
OMe-5				56.2 q
OMe-5′		55.6 q	55.6	
Me-7″	28.4 q	27.7 q	27.8	28.1 q
Me-8″	28.4 q	27.7 q	27.8	28.1 q

[a] Fully coupled spectrum.

Compound **10** had one more methoxyl group than in **9** on the basis of the mass spectral molecular ion peak at m/z 338 (32%) in accordance with the molecular formula $C_{21}H_{22}O_4$. Similar structures for **9** and **10** were suggested by their almost identical UV spectra in methanol and sodium methoxide, and ^{1}H NMR data established their identity except for the substitution pattern of the phenyl ring. A 1,3,4-trisubstituted phenyl ring for **10** was indicated by a typical ABX system of three protons in the aromatic region: signals for H-2 at δ 7.02 ppm (d, J = 2.1 Hz), H-5 at δ 6.90 ppm (d, J = 8.6 Hz), and H-6 at δ 7.01 ppm (dd, J = 8.6, 2.1 Hz) (Table 4). The two one-proton meta-coupled doublets, which were the same as those in **9**, supported the 5′-methoxyl group. An NOE observed between protons at C-2 and the methoxyl group was assignable to 3-meth-

Table 6. Biological Activities of Cubé Resin Constituents **1**−**11**

cmpd	enzyme activity (IC_{50}, μM) NADH:ubiquinone oxidoreductase	enzyme activity (IC_{50}, μM) phorbol ester-induced ornithine decarboxylase	cell growth (IC_{50}, μM) MCF-7	cell growth (IC_{50}, μM) Hepa 1clc7
flavonoids				
1	3.3 ± 0.3[a]	2.6 ± 0.1[a]	1.9	0.34
2	>30	>30	>30	>30
3	2.1 ± 0.4	2.2 ± 0.2	6.6	8.5
4	2.0 ± 0.1	2.1 ± 0.2	6.9	3.9
5	0.68 ± 0.04	1.1 ± 0.1	3.0	3.5
6	2.6 ± 0.4	2.3 ± 0.2	5.7	5.1
7	4.9 ± 0.1	4.7 ± 0.6	27	10
8	4.8 ± 0.2	3.6 ± 0.2	10	5.9
genistein[b]	35 ± 2	26 ± 2	>30	11
stilbenes				
9	5.3 ± 0.3	5.2 ± 0.2	>30	8.5
10	8.1 ± 0.3	7.4 ± 0.5	>30	13
11	4.9 ± 0.1	3.9 ± 0.1	24	7.0
resveratrol[b]	26 ± 1	19 ± 1	>30	11

[a] Mean ± SE, $n = 3$. [b] Comparison compounds.

oxy-4-hydroxyphenyl; the singlet ^{1}H NMR signal for the 4-hydroxyl substituent disappeared after addition of D_2O. The assigned structure for **10**, confirmed by other observed NOE and the fully coupled ^{13}C NMR spectrum, was 3,5′-dimethoxy-4-hydroxy-6″,6″-dimethylpyran[2″,3″:3′,4′]stilbene (see note above relative to the structure for **9**).

Compound **11** had the same skeleton as **9** and **10** with a different aryl substitution pattern evident from the molecular ion $C_{22}H_{24}O_4$ ($[M]^+$ at *m/z* 352) and the ^{1}H and fully coupled ^{13}C NMR spectra. An ABX system of three protons [δ 7.14 (d, $J = 2.1$ Hz), 6.76 (d, $J = 8.2$ Hz), and 7.26 (dd, $J = 2.1$, 8.2 Hz)] was observed for the phenyl substituent of **11** assignable to H-2′, 5′, and 6′, respectively (Table 4). The signal for two equivalent protons (H-2 and -6) at δ 6.70 (s, 2H) indicated a 1,3,4,5-tetrasubstituted phenyl ring for **11**. Three methoxyl groups, evident in the ^{1}H and ^{13}C NMR and mass spectra, were assigned as C-3, C-4, and C-5 in the phenyl ring. Therefore, compound **11** was 3,4,5-trimethoxy-6″,6″-dimethylpyran[2″,3″:3′,4′]stilbene (see note above relative to the structure for **9**).

Stilbenes **9**−**11** gave UV spectra with band I and II absorption maxima like chalcones (Table 1). The 4-hydroxyl groups of **9** and **10** were ionized to some extent by sodium methoxide, resulting in the bathochromic shifts of 28−34 nm. Sodium methoxide had no effect on the UV spectrum of **11**, consistent with the absence of a hydroxyl group.

The biological assays involved inhibition of NADH:ubiquinone oxidoreductase activity in vitro, inhibition of 12-*O*-tetradecanoylphorbol 13-acetate (phorbol ester)-induced ODC activity in cultured MCF-7 cells (6 h assay), and cytotoxicity in MCF-7 and Hepa 1clc7 cells (72 h cell growth assay) (Table 6). The potencies for inhibiting NADH:ubiquinone oxidoreductase activity generally correlated with those for inhibiting phorbol ester-induced ODC activity.[3] The active compounds from cubé resin had the *gem*-dimethylpyran moiety (**1** and **3**−**11**) and conversion to the corresponding dihydrodiol destroyed the activity (**1** vs **2**) (as in the case of the rotenoid tephrosin and its dihydrodiol[3]). The cubé resin flavonoids were generally more active than the stilbenoids. The most potent compound was **5**, suggesting the importance of the hydroxyl substituent at C-4′ in the flavonone series. By comparison, genistein and *trans*-resveratrol were much less active. Some of the compounds are of interest for more advanced biological testing, but they are all very minor constituents in cubé resin and therefore not readily available.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR data at 300 and 75 MHz, respectively, were collected using a Bruker AM-300 spectrometer and $CDCl_3$ as the solvent. Chemical shifts (δ in ppm) are reported for ^{1}H and ^{13}C NMR relative to the internal standards tetramethylsilane and $CDCl_3$, respectively. Fully coupled ^{13}C NMR data were used to assign the resonances. 2D-NOE experiments were carried out at 500 MHz. Electron impact (EI)-MS was accomplished with a Hewlett-Packard 5985B instrument using the Hewlett-Packard 59870C RTE-A data system. The electron energy and source temperature, respectively, were 70 eV and 200 °C. Fast atom bombardment (FAB)-MS (both low and high resolution) was conducted with the Fisons ZAB2-EQ spectrometer.

Plant Materials. The roots of *L. utilis* and *L. urucu* from Peru were extracted in France to obtain the original cubé resin "brittle" for use as a pesticide, as described earlier.[1]

Extraction and Isolation. General procedures for isolation of the cubé resin ingredients were reported previously.[1,3] The first step for separation of minor components was to remove 86% of the rotenone by solvent (MeOH) precipitation. The mixture of other components was fractionated by a silica gel column using a gradient of hexane with 5% EtOAc/MeOH (3:1) to 100% of the latter solvent mixture. Twenty fractions were collected and assayed for NADH:ubiquinone oxidoreductase inhibition and Hepa 1clc7 cytotoxicity. They were further purified by preparative silica gel TLC and C_{18} HPLC.[3] Bioactive materials other than rotenoids appeared in the second, third, and fifth fractions identified as **3**−**6**, **1** and **7**−**11**, and **2**, respectively. Each of these components was individually <0.1% of the resin (as determined by HPLC analysis, not detailed here). The pure compounds were obtained in amounts of 5−30 mg starting with 650 g of cubé resin.

***cis*-4″,5″-Dihydro-4″,5″-dihydroxylonchocarpusone (2):** white powder; mp 228−230 °C; UV data, see Table 1; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; FABMS *m/z* 429 ($[M + 1]^+$, 100), 428 ($[M]^+$, 75); HRFABMS *m/z* found 428.1478, calcd for $C_{23}H_{24}O_8$, 428.1471.

(2*S*)-6-(γ,γ-Dimethylallyl)-5-hydroxy-3′,4′-dimethoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone (3): light yellow powder; UV data, see Table 1; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS *m/z* 450 ($[M]^+$, 79), 435 ($[M - Me]^+$, 100), 286 ($[A_1]^+$, 6), 271 ($[A_1 - Me]^+$, 28), 258 ($[A_1 - CO]^+$, 6), 243 ($[A_1 - Me - CO]^+$, 15), 231 (8), 215 ($[A_1 - Me - C_4H_8]^+$, 40), 164 ($[B_3]^+$, 6), 149 ($[B_3 - Me]^+$, 4).

(2*S*)-6-(γ,γ-Dimethylallyl)-5,3′-dihydroxy-4′-methoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone (4): light yellow powder; mp 110−112 °C; UV data, see Table 1; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS *m/z* 436 ($[M]^+$, 70), 421 ($[M - Me]^+$, 100), 286 ($[A_1]^+$, 5), 271 ($[A_1 - Me]^+$, 22), 258 ($[A_1 - CO]^+$, 5), 243 ($[A_1 - Me - CO]^+$, 15), 231 (8), 215

($[A_1 - Me - C_4H_8]^+$, 36), 164 ($[B_3]^+$, 4), 149 ($[B_3 - Me]^+$, 8). 2D-NOE NMR: observed NOE between protons of H-2 and H-3; H-5′ and OMe-4′; H-4″ and H-5″; H-5″ and diMe-7″,8″ H-1‴ and diMe-4‴, 5‴; H-2‴ and diMe-4‴,5‴; NOE obscured for H-2′, -5′, and -6′.

(2*S*)-6-(γ,γ-Dimethylallyl)-5,4′-dihydroxy-3′-methoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone (5): light yellow powder; UV data, see Table 1; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS *m*/*z* 436 ($[M]^+$, 82), 421 ($[M - Me]^+$, 100), 286 ($[A_1]^+$, 8), 271 ($[A_1 - Me]^+$, 32), 258 ($[A_1 - CO]^+$, 6), 243 ($[A_1 - Me - CO]^+$, 20), 231 (11), 215 ($[A_1 - Me - C_4H_8]^+$, 48), 164 ($[B_3]^+$, 8), 149 ($[B_3 - Me]^+$, 10).

(2*S*)-6-(γ,γ-Dimethylallyl)-3′,4′-dimethoxy-6″,6″-dimethylpyran[2″,3″:7,8]flavanone (6): light yellow powder; UV data, see Table 1; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS *m*/*z* 434 ($[M]^+$, 84), 419 ($[M - Me]^+$, 100), 270 ($[A_1]^+$, 8), 255 ($[A_1 - Me]^+$, 33), 242 ($[A_1 - CO]^+$, 12), 227 ($[A_1 - Me - CO]^+$, 16), 215 (7), 164 ($[B_3]^+$, 11), 149 ($[B_3 - Me]^+$, 4).

3-Methoxy-4-hydroxylonchocarpin (8): orange powder; mp 152−154 °C; UV data, see Table 1; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 5; EIMS *m*/*z* 352 ($[M]^+$, 12), 337 ($[M - Me]^+$, 22), 203 (7), 188 ($[A_1 - Me + 1]^+$, 12), 187 ($[A_1 - Me]^+$, 100).

4-Hydroxy-5′-methoxy-6″,6″-dimethylpyran[2″,3″:3′,4′]-stilbene (9): light yellow powder; UV data, see Table 1; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 5; EIMS *m*/*z* 308 ($[M]^+$, 26), 293 ($[M - 15]^+$, 100); HREIMS *m*/*z* found 308.1412, calcd for $C_{20}H_{20}O_3$, 308.1412. 2D-NOE NMR: observed NOE between protons of H-2,6 and 3,5; H-2,6 and H-7; H-2,6 and H-8; H-7 and H-2′; H-7 and H-6′; H-8 and H-2′; H-8 and H-6′; H-6′ and OMe-5′; H-4″ and H-5″; H-5″ and diMe-7″,8″; NOE obscured for H-7, -8, -2′, and -6′.

3,5′-Dimethoxy-4-hydroxy-6″,6″-dimethylpyran[2″,3″:3′,4′]stilbene (10): light yellow powder; UV data, see Table 1; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 5; EIMS *m*/*z* 338 ($[M]^+$, 34), 323 ($[M - 15]^+$, 100). 2D-NOE NMR: observed NOE between protons of H-2 and OMe-3; H-8 and H-2′; H-8 and H-6′; H-6′ and OMe-5′; H-4″ and H-5″; H-5″ and diMe-7″,8″; NOE obscured for H-2, -5, -6, -7, -8, -2′, -6′, and -4″.

3,4,5-Trimethoxy-6″,6″-dimethylpyran[2″,3″:3′,4′]stilbene (11): light yellow powder; UV data, see Table 1; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 5; EIMS *m*/*z* 352 ($[M]^+$, 92), 337 ($[M - 15]^+$, 100).

NADH:Ubiquinone Oxidoreductase Assays.[3,17] The test compound was incubated with bovine heart electron transport particles (inverted vesicles of the inner mitochondrial membrane) (40 μg of protein) in 50 mM phosphate buffer, pH 7.4 containing 250 mM sucrose (1.0 mL) for 5 min at 25 °C, and NADH (28 μM final concentration) was then added to monitor residual enzyme activity as loss of absorbance at 340 nm for 3 min.[2] The concentration for 50% inhibition (IC_{50}) was determined in three experiments.

Phorbol Ester-Induced Ornithine Decarboxylase Assays.[3,18,19] Human breast cancer cells (MCF-7) were incubated in minimum essential medium with 10% fetal bovine serum and other supplements at 37 °C in a humidified atmosphere of 5% CO_2/95% air.[2,3] Cells (2×10^5/mL) were cultured in 24-well tissue culture plates for 24 h, and then the test compound and phorbol ester (0 or 200 nM final concentration) were added followed by incubation for an additional 6 h. ODC activity was determined as $^{14}CO_2$ liberation from L-[1-^{14}C]ornithine hydrochloride with incubation for 1 h at 37 °C.[3] The $^{14}CO_2$-liberated/mg protein was compared for cells with 0 versus 200 nM phorbol ester in the same 24 well plate to determine the phorbol ester-induced ODC activity, which was generally 3−4-fold relative to the constitutive activity without phorbol ester. IC_{50} values for the induced ODC activity were based on three experiments.

Cytotoxicity Assays. Our reported procedures were used to culture MCF-7 cells[3,19] and mouse liver cancer cells (Hepa 1clc7).[2] The cytotoxicity assays involved addition of the test compound, 72 h incubation, and cell viability determination with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.[2] Each cytotoxicity assay had eight replicates for determining IC_{50} values.

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