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FLAVANONE AND CHALCONE DERIVATIVES FROM *CRYPTOCARYA KURZII*[1]

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ABSTRACT.—Four complex flavanones, kurziflavolactones A [**2**], B [**3**], C [**4**], and D [**5**] and a complex chalcone **6** with an unprecendented carbon side chain on the flavanone or chalcone A ring have been isolated from a Malaysian plant, *Cryptocarya kurzii* (Lauraceae). Their structures were determined by extensive spectroscopic analysis, especially 2D nmr experiments. Compounds **3** and **6** showed slight cytotoxicity against KB cells, with IC_{50} values of 4 and 15 μg/ml, respectively. A biosynthetic pathway for the formation of these compounds is suggested.

Cryptocarya kurzii Hook. f. (Lauraceae) is a Malaysian tree collected from the Mersing area. The EtOAc extract of the leaves of *C. kurzii* showed significant cytotoxicity against KB cells (90% inhibition at 10 μg/ml). Bioactivity- and ^{1}H-nmr-directed studies of this material have led to the isolation and characterization of a new kawa-type lactone, kurzilactone [**1**], which is largely responsible for the cytotoxicity (1). Investigation of the leaves has now afforded four complex flavanones named kurziflavolactones A [**2**], B [**3**], C[**4**], and D [**5**] and a complex chalcone named kurzichalcolactone [**6**]. In this paper, details of the isolation work as well as elucidation evidence leading to these structures are reported.

RESULTS AND DISCUSSION

Kurziflavolactones A [**2**], B [**3**], C [**4**], and D [**5**] were obtained as oils from the EtOAc-soluble fraction of the EtOH extract of the leaves by vlc and tlc followed by reversed-phase hplc. The uv spectra suggested that all four compounds have a flavanone core (2). This proposal was supported by ^{1}H- and ^{13}C-nmr data as discussed below. The salient feature of the ^{1}H-nmr spectra ($CDCl_3$, 400 MHz) was the ABX system, diagnostic for C-2 and C-3 protons of a flavanone nucleus. The C-2 proton, the X part, appeared as a doublet of doublets at δ 5.44±0.04 (J_{AX}=13 Hz; J_{BX}=3 Hz), while the C-3 protons, the AB part, appeared at δ 2.85±0.04 and δ 3.10±0.03 (J_{AB}=17 Hz; J_{AX}=13 Hz; J_{BX}=3 Hz). In the ^{1}H-^{1}H COSY spectrum, these signals were correlated with each other. The HMQC spectrum indicated that the associated carbons (C-2 and C-3) resonated at δ 79.2±0.3 and 43.5±0.3 which are typical of flavanones (3). All four compounds **2**–**5** contain a 5-OH, indicated by their ir and uv spectra. Each of them showed a strong ir absorption at ca. 1640 cm^{-1} attributable to a chelated carbonyl (5-OH and C-4 carbonyl forming an intramolecular hydrogen bond), and a bathochromic $AlCl_3$-induced shift (greater than 20 nm) was observed in the uv spectra. Location of the OH group at C-5 was further substantiated by the nmr spectra, which showed a low field singlet at δ_H ca. 12 (chelated with C-4 carbonyl) and C-4 resonance at δ_C ca. 196 (s). The carbonyl

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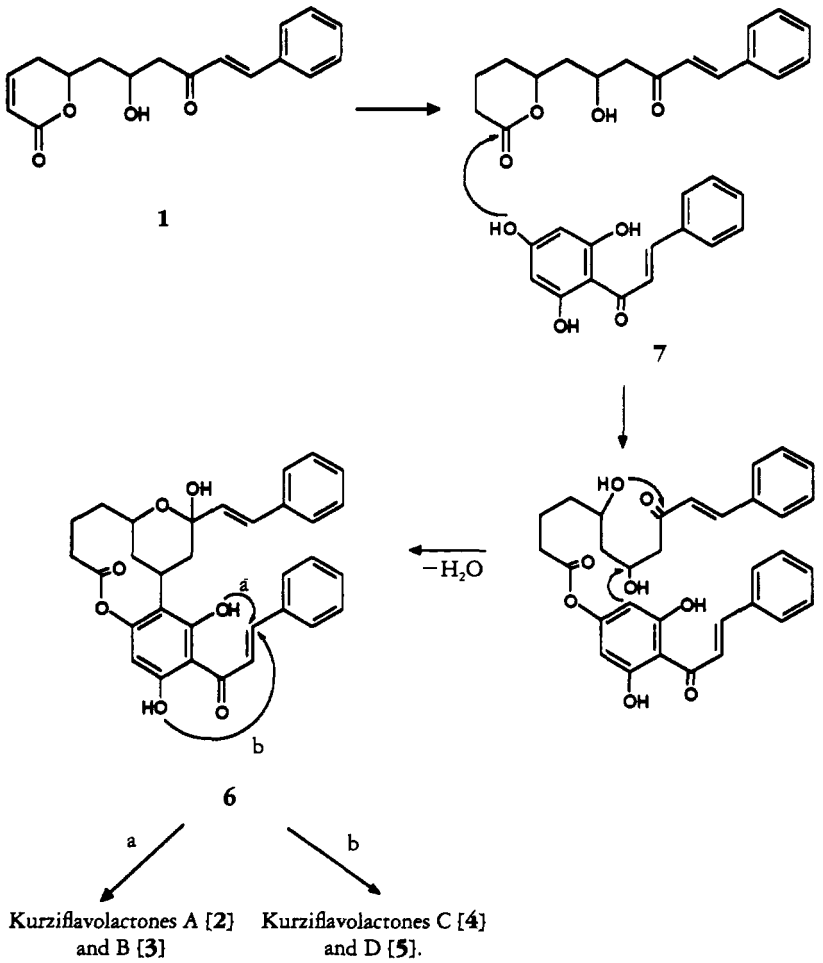
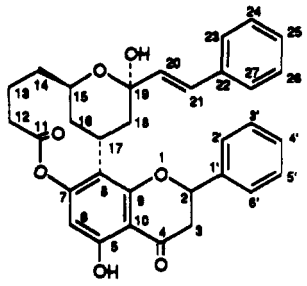


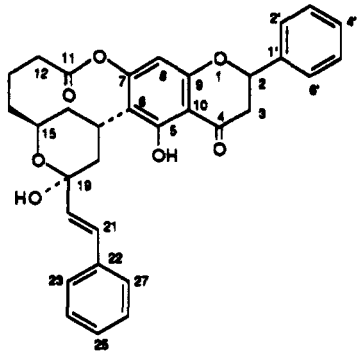
SCHEME 1. Proposed biosynthetic pathway for the formation of kurzichalcolactone [**6**] and kurziflavolactones A [**2**], B [**3**], C [**4**], and D [**5**].

(C-4) resonance exhibited dependence upon the absence or presence of a peri-substituent at the C-5 position. In the case of 5-unsubstituted flavanones, the C-4 resonance absorbs usually at δ 189.7–191.7, whereas in 5-hydroxylated flavanones, it resonates at an appreciably deshielded position (δ 195.6–197.3) because of its involvement in chelation with the OH at the C-5 position (3).

In the following discussion, we will first elucidate the structure of kurziflavolactone B [**3**] in detail, and then the structures of kurziflavolactones A [**2**], C [**4**], and D [**5**] will be assigned by comparison of their spectral data with those of **3**.

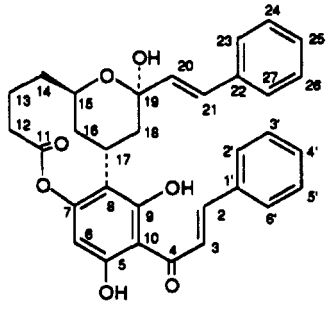
Kurziflavolactone B [**3**], $[\alpha]_D$ −136° ($CHCl_3$, c=0.6), showed a molecular formula of $C_{32}H_{30}O_7$ as established by hreims, m/z $[M]^+$ 526.1985, calcd for $C_{32}H_{30}O_7$, 526.1991; the unsaturation degree is 18. The ir spectrum of kurziflavolactone B showed strong absorptions at 1631 (chelated keto group) and 3400 (OH), 1590 (benzene ring) and 1718 (ten-membered ring lactone) cm^{-1}. On the other hand, ir absorptions at 750 and 690 cm^{-1} and a multiplet at δ 7.20–7.50 (10H) in the ^{1}H-nmr spectrum indicated the presence of two monosubstituted benzene rings, one of which should be a flavanone B ring. The ^{13}C data, which were assigned to the carbons on the B ring (see Table 1), are in good agreement with those of flavanones having an unsubstituted ring B (3). An





2 2*R*
3 2*S*

4 2*S*
5 2*R*



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aromatic proton singlet at δ 6.14 in the ^{1}H-nmr spectrum (correlated with a carbon resonating at δ 96.5 in the HMQC spectrum) and ^{13}C-nmr data assigned to the other carbons on the A ring, by comparison with the ^{13}C data of similar substituted flavanones (3), indicated that the A ring of the flavanone is 5,7-dioxygenated and bears a carbon substituent at C-6 or C-8. The position of the carbon substituent was determined to be at C-8 based on the chemical shift of 5-OH at δ 12.00 (4–6) and was confirmed by the HMBC correlation between the one-proton singlet at δ 12.00 due to 5-OH and the signal at δ 96.5 hence being C-6. All assignments on the A ring of the flavanone core were finally confirmed by the other cross peaks observed in the HMBC spectrum (see Figure 1 and Table 4). The chelated OH group (5-OH) showed further correlations with C-5

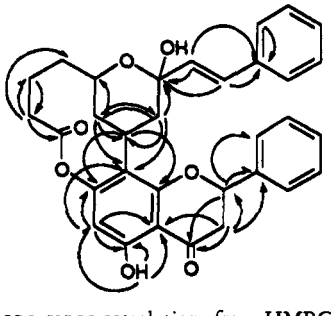

FIGURE 1. Long-range correlations from HMBC experiments of kurziflavolactone B [**3**].

TABLE 1. ^{13}C-Nmr Data for the Isolated Compounds.[a]

Carbon	2	3	4	5	6
C-2	79.5	79.0	79.2	79.2	143.3
C-3	43.7	43.2	43.8	43.4	128.9
C-4	196.0	195.8	196.2	196.1	193.4
C-5	162.4	162.5	160.0	159.9	162.6
C-6	96.7	96.5	106.6	106.6	97.0
C-7	164.2	164.1	164.2	164.1	167.4
C-8	105.5	105.3	95.2	95.2	105.5
C-9	158.4	158.4	161.2	161.1	159.6
C-10	103.4	103.3	103.0	103.1	106.2
C-11	178.6	178.7	178.6	178.4	175.1
C-12	34.1	33.7	33.9	33.8	34.6
C-13	21.0	20.7	20.8	20.7	22.1
C-14	35.2	35.0	35.0	35.0	36.1
C-15	70.0	69.8	70.0	70.0	71.4
C-16	35.9	35.8	35.3	35.4	36.5
C-17	23.5	23.3	22.8	22.7	24.6
C-18	34.1	33.9	33.9	34.0	34.4
C-19	98.7	98.6	98.8	98.8	100.0
C-20	131.3	131.3	131.3	131.2	132.4
C-21	129.3	129.3	129.3	129.2	131.0
C-22	136.2	136.2	136.2	136.1	137.5
C-23	126.0	126.0	126.2	126.2	128.3
C-24	128.9	128.7	129.0	128.9	129.5
C-25	129.0	128.9	129.0	128.9	129.5
C-26	128.9	128.8	129.0	128.9	129.5
C-27	126.0	126.0	126.2	126.2	128.3
C-1′	138.7	138.7	138.7	138.6	136.7
C-2′	127.1	127.5	127.1	127.0	130.0
C-3′	128.7	128.3	128.7	128.6	130.0
C-4′	127.1	127.1	128.3	128.2	130.0
C-5′	128.3	128.3	128.7	128.6	130.0
C-6′	127.1	127.5	127.1	127.0	130.0

[a]The spectra were recorded on a 250 MHz instrument in $CDCl_3$ except for compound **6**, which was recorded in Me_2CO-d_6 on a 400 MHz instrument. Some assignments on the benzene ring, with like multiplicities and very similar chemical shifts, may be reversed.

(δ 162.5) and C-10 (δ 103.3). The aromatic singlet at δ 6.14 (H-6) caused cross peaks with four quaternary carbons assigned to C-5 (δ 162.5), C-7 (δ 164.1), C-8 (δ 105.3), and C-10 (δ 103.3).

The remaining problem was the structure of the side chain residue (C-11–C-27), which was determined as follows. In the ^{1}H-nmr spectrum, a pair of doublets appeared at δ 6.87 and 6.29. They were correlated with each other in the ^{1}H-^{1}H COSY spectrum and assigned to the protons of the C-20,21 double bond. The corresponding carbons resonated at δ 131.3 and 129.3, respectively, as indicated by the HMQC spectrum. The large coupling constant (J=16 Hz) for H-20 and H-21 suggested that the geometry of the C-20,21 double bond is trans. This suggestion was confirmed by the ir absorption at 980 cm^{-1}. The long-range C/H correlation H-20 (δ 6.87)/C-22 (δ 136.2), H-21 (δ 6.29)/C-19 (δ 98.6), H-20/C-19, and H-21/C-22 indicated that the trans-disubstituted double bond must be connected with a benzene ring and C-19 whose ^{13}C resonance at δ 98.6 is characteristic for a hemiketal. The ms peaks at m/z 103 $[C_6H_5CH{=}CH]^+$, m/z 131 $[C_6H_5CH{=}CHCO]^+$, and m/z 395 $[M-131]^+$ confirmed the presence of a cinnamic type moiety. The other spin system in the side chain moiety was apparent from ^{1}H-^{1}H

COSY, DQF COSY, TOCSY, and HMQC, and, therefore, the partial structure from C-12 to C-18 was proposed. Methylene C-12 was connected with a carbonyl group (C-11) since H-12 (δ 2.33, brt, J=7 Hz) showed a long-range coupling with a carbon signal at δ 178.7. Similarly, methine C-17 was connected with C-8 on ring A of the flavanone and methylene C-18 with the hemiketal carbon (C-19) owing to the long-range correlations: H-17 (δ 3.51)/C-7 (δ 164.1) as well as C -9 (δ 158.4); and H-18ax (δ 2.10/C-19 (δ 98.6) as well as H-18eq (δ 1.88)/C-19. C-15 was linked with one of the oxygens at C-19 to form a pyran ring, as the chemical shifts of H-15 (δ 3.70) and C-15 (δ 69.8) were in accord with those of the corresponding proton and carbon in pyran (7). Finally, the carbonyl C-11 was attached to the oxygen at C-7 constituting a ten-membered ring lactone to account for the ^{13}C resonance at δ 178.7 and the ir absorption at 1718 cm^{-1} and to meet the 18 units of unsaturation required for the molecular formula. The observed mass fragmentations at *m/z* 256 and *m/z* 269, which corresponded to the flavanone moiety $[C_{15}H_{10}O_4+2H]^+$ and the side chain $[C_{17}H_{20}O_4-H_2O-H]^+$, respectively, further support the above structure.

The relative stereochemistry of the side chain residue was assigned according to the splitting pattern of H-15 and H-17 in the ^1H -nmr and NOESY spectra. The small coupling constant for H-17 (dd, J=3, 2 Hz in C_6D_6; brs, $W_{1/2}$=10 Hz in $CDCl_3$) indicated that the H-17 must be located at the equatorial position. The peak appearance of H-15 (δ 3.70) was very similar to that of H-3α in a 3β-OH sterol, and the $W_{1/2}$ was equal to about 20 Hz. This indicated that H-15 has an axial orientation. Four prominent cross peaks, H-20/H-18ax, H-21/H-18ax, H-20/H-18eq, and H-21/H-18eq in the NOESY spectrum of kurziflavolactone B [**3**] (Table 4), in combination with the study of a Dreiding model, revealed that 19-OH was most probably located at an axial position. This arrangement places the styryl group far away from the flavanone core, thus avoiding steric hindrance. On the other hand, if it is assumed that 19-OH is at an equatorial postition, the nOe correlations, such as H-15 with H-20 and H-21, H-21 with H-13, and H-21 with H-2, should be shown in the NOESY spectrum; but, in fact, these correlations were not observed.

The absolute stereochemistry in the flavanone skeleton of **3** was determined to be 2*S* from a positive Cotton effect at 330 nm (Δε +1.53) and a negative Cotton effect at 288 nm (Δε −10.7) in the cd spectrum, while kurziflavolactone A [**2**], which is a stereoisomer of **3** (see below), showed a negative Cotton effect at 330 nm (Δε −2.81) and a positive Cotton effect at 282 nm (Δε +12.8), indicating a 2*R* absolute stereochemistry (8). However, the relative stereochemistry between the side chain moiety and the flavanone core (C-2) cannot be defined by nOe and was arbitrarily designated as **3** for convenience. All ^1H and ^{13}C resonances, other than the aromatic methines on two mono-substituted benzene rings, were unambiguously assigned by ^1H-^1H COSY, TOCSY, and HMQC, and were further confirmed by the long-range C/H couplings shown in Figure 1 and Table 4.

Kurziflavolactone A [**2**], $[\alpha]_D$ −53° ($CHCl_3$, c=0.6), showed a molecular ion peak at *m/z* 526 in eims, corresponding to a molecular formula of $C_{32}H_{30}O_7$, the same as kurziflavolactone B [**3**]. The other fragmentation peaks in the eims are almost identical with those of **3** except for their relative intensities. The ir exhibited prominent absorptions at 3400 (br, OH), 1713 (ten-membered ring lactone), 1630 (very strong, chelated keto group), 1594 (benzene ring), 985 (trans-disubstituted double bond), 837 (pentasubstituted benzene ring), and 750 and 687 cm^{-1} (monosubstituted benzene ring), which were similar to those of kurziflavolactone B. Furthermore, the ^1H-nmr (Tables 2 and 3) and ^{13}C-nmr (Table 1) spectra of **2** were also nearly the same as those of **3**. These findings indicated that kurziflavolactone A is a stereoisomer of **3**. Similar nOe

TABLE 2. ^{1}H-Nmr (400 MHz, $CDCl_3$) Data for Compounds **2–5**.

Proton	Compound			
	2	**3**	**4**	**5**
H-2	5.45 dd (13,3)	5.48 dd (13,3)	5.40 dd (13,3)	5.46 dd (13,3)
H-3ax	3.09 dd (17,13)	3.07 dd (17,13)	3.10 dd (17,13)	3.12 dd (17,13)
H-3eq	2.83 dd (17,3)	2.88 dd (17,3)	2.82 dd (17,3)	2.85 dd (17,3)
H-6	6.17 s	6.14 s		
H-8			6.13 s	6.14 s
H-12	2.34 brt (7)	2.33 brt (7)	2.36 brt (7)	2.38 brt (7)
H-13	1.78 m, 1.60 m	1.73 m, 1.63 m		1.83 m, 1.68 m
H-14	1.62 m, 1.55 m	1.60 m, 1.52 m		1.68 m, 1.58 m
H-15	3.72 m ($W_{1/2}$=20)	3.70 m ($W_{1/2}$=20)	3.72 m ($W_{1/2}$=20)	3.70 m ($W_{1/2}$=20)
H-16	1.60 m	1.60 m		1.80 m, 1.72 m
H-17	3.50 brs ($W_{1/2}$=10)	3.51 brs ($W_{1/2}$=10)	3.54 brs ($W_{1/2}$=10)	3.57 brs ($W_{1/2}$=10)
H-18eq	1.84 dd (13,3)	1.88 dd (13,3)	1.87 dd (13,3)	1.86 dd (13,3)
H-18ax	2.08 dd (13,2)	2.10 dd (13,2)	2.12 dd (13,2)	2.13 dd (13,2)
H-20	6.87 d(16)	6.87 d (16)	6.88 d (16)	6.88 d (16)
H-21	6.28 d (16)	6.29 d (16)	6.29 d (16)	6.30 d (16)
Benzene protons	7.23–7.50 m	7.20–7.50 m	7.20–7.50 m	7.30–7.60 m
5-OH	11.93 s	12.00 s	12.33 s	12.40 s

cross peaks were observed in the NOESY spectra of compounds **3** and **2**. In particular, nOe correlations between H-20, H-21 and both protons at C-18 indicated that 19-OH must be located at an axial orientation. The H-15 peak appearance, which resembled that of **3**, as well as the H-17 peak appearance and its small couplings to H-18 ($J_{17eq,18eq}$=3, $J_{17eq,18ax}$=2 in $CDCl_3$) and H-16 ($J_{17eq,16ax}$=3, $J_{17eq,16eq}$=2 in C_6D_6) revealed that H-15 and H-17 were at axial and equatorial positions, respectively. Thus, kurziflavolactone A is an epimer of **3** at C-2 and could be formulated as **2**.

Kurziflavolactone D [**5**], $[\alpha]_D$ −50° ($CHCl_3$, c=1.7), gave $[M]^+$ at m/z 526.1983 in the hreims, which corresponded to a molecular formula $C_{32}H_{30}O_7$ (calcd 526.1991), which was identical with those of kurziflavolactones A [**2**] and B [**3**]. Kurziflavolactone D [**5**] showed almost the same fragmentation pattern as kurziflavolactone B. The ir spectrum gave important absorptions at 3430 (br, OH), 1715 (ten-membered ring lactone), 1649 (very strong, chelated carbonyl), 1576 (benzene ring), 980 (trans-disubstituted double bond), 832 (pentasubstituted benzene ring), and 740 and 699 (monosubstituted benzene ring) cm^{-1}. All these data resembled those of kurziflavolactone B [**3**]. The ^{1}H-nmr spectrum of **5** in $CDCl_3$ was almost the same as that of **3** apart from the chemical shift of the chelated 5-OH (δ 12.40), while the ^{1}H-nmr data (Table 3) of compound **5** in C_6D_6 had some differences from those of **3**, especially H-2, H-6, H-17, H-20, and 5-OH. The ^{13}C-nmr data (Table 1) of **5** were also very close to those of **3** except for the ^{13}C resonances on ring A. The above data strongly indicated that **5** is a regioisomer of **3** due to the position of the alkyl group. The chemical shift of the 5-OH suggested that **5** is a flavanone with a carbon substituent at C-6 (4–6). For confirmation of the structure, the HMBC spectrum was measured, and the position of the carbon substituent was determined to be at C-6. A chelated OH group exhibited cross peaks with three quaternary carbons assigned to C-5 (δ 159.9), C-6 (δ 106.6), and C-10 (δ 103.1). In addition, an aromatic proton based on the A ring, which caused a cross peak with a carbon at δ 95.2 in the HMQC spectrum, also exhibited long-range correlations with four quaternary carbons: C-6 (δ 106.6), C-7 (δ 164.1), C-9 (δ 161.1), C-10 (δ 103.1). The remaining long-range couplings observed in the HMBC spectrum are shown in Figure

TABLE 3. ^{1}H-Nmr (250 MHz, C_6D_6) Data for Compounds **2–5**.

Proton	Compound			
	2	**3**	**4**	**5**
H-2	4.79 dd (13,3)	4.70 dd (13,3)	4.83 dd (13,3)	4.89 dd (13,3)
H-3ax	2.55 dd (17,13)	2.54 dd (17,13)	2.61 dd (17,13)	2.61 dd (17,13)
H-3eq	2.37 dd (17,3)	2.37 dd (17,3)	2.38 dd (17,3)	2.41 dd (17,3)
H-6	6.58 s	6.57 s		
H-8			6.42 s	6.43 s
H-12	2.04 brt (7)	2.01 brt (7)	2.01 brt (7)	2.00 brt (7)
H-13		1.62 m, 1.47 m		
H-14		1.40 m, 1.29 m		
H-15	3.81 m ($W_{1/2}$=20)	3.80 m ($W_{1/2}$=20)	3.80 m ($W_{1/2}$=20)	3.81 m ($W_{1/2}$=20)
H-16		1.40 m, 1.29 m		
H-17	3.25 dd (3,2)	3.30 d (3,2)	3.56 dd (3,2)	3.56 dd (3,2)
H-18eq	1.64 dd (13,3)	1.62 dd (13,3)	1.68 dd (13,3)	1.64 dd (13,3)
H-18ax	1.77 dd (13,2)	1.78 dd (13,2)	1.78 dd (13,2)	1.78 dd (13,2)
H-20	7.22 d (16)	7.22 d (16)	7.07 d (16)	7.07 d (16)
H-21	6.34 d (16)	6.35 d (16)	6.35 d (16)	6.35 d (16)
Benzene protons	7.00–7.30 m	7.00–7.30 m	7.00–7.30 m	7.00–7.30 m
5-OH	12.76 s	12.74 s	13.25 s	13.23 s

2. Consequently, the structure of kurziflavolactone D [**5**] was determined as a C-6 regioisomer of kurziflavolactones A [**2**] and B [**3**]. Cross peaks between H-20, H-21, and both protons at C-18, H-15 peak appearance along with H-17 peak appearance (small coupling with H-18 and H-16, see Tables 2, 3) supported the relative stereochemistry of the kurziflavolactone D [**5**] side chain as shown. A negative Cotton effect at 330 nm ($\Delta\epsilon$ −7.28) and a positive Cotton effect at 282 nm ($\Delta\epsilon$ +12.8) indicated a 2*R* absolute configuration.

Kurziflavolactone C [**4**], $[\alpha]_D$ −53° ($CHCl_3$, c=1.5), showed a molecular ion peak at *m/z* 526 in eims, compatible with the molecular formula of $C_{32}H_{30}O_7$, which is

TABLE 4. 2D Nmr Data for Kurziflavolactone B [**3**].

Proton	LR ^{1}H-^{1}H correlations[a]	LR ^{1}H-^{13}C correlations[b]	NOe correlations[c]
H-2		4, 1′, 2′, 6′	3ax, 3eq
H-3ax		2, 1′, 4, 10	2
H-3eq		2, 1′, 4, 10	2
H-6		5, 7, 8, 10	
H-12	14, 15, 16, 17	11, 13, 14	13, 14
H-13	15, 16, 17	12	12, 14, 15
H-14	12, 16, 17, 18		12, 13, 15
H-15	12, 13, 17, 18		13, 14, 16
H-16	12, 13, 14, 18	8, 15, 17, 18	15, 17, 18ax
H-17eq	12, 13, 14, 15	7, 9	16, 18eq, 18ax
H-18eq	14, 15, 16	8, 16, 17, 19	20, 21
H-18ax	14, 15, 16	8, 16, 17, 19	16, 20, 21
H-20		19, 22	18eq, 18ax, 21
H-21		19, 22	18eq, 18ax, 20
5-OH		5, 6, 10	

[a]From TOCSY.
[b]From HMBC.
[c]From NOESY

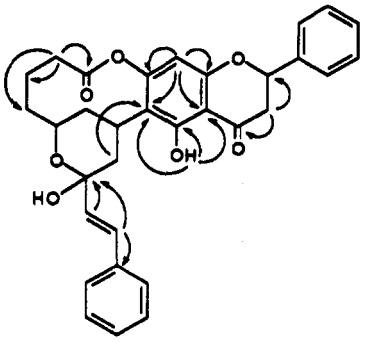

FIGURE 2. Long-range correlations from HMBC experiments of kurziflavolactone D [**5**].

identical with kurziflavolactone D [**5**]. Kurziflavolactone C [**4**] had nearly the same ir, ^{1}H- and ^{13}C-nmr spectra (see Tables 1–3) as **5**. In the cd spectrum of **4**, a positive Cotton effect at 330 nm ($\Delta\epsilon$ +3.75) and a negative Cotton effect at 288 nm ($\Delta\epsilon$ −12.3), characteristic for a 2*S* absolute configuration, were observed. Using reasoning similar to that applied in the structural elucidation of **2**, **3**, and **5**, kurziflavolactone C was deduced as **4**, which is an epimer at C-2 of **5** and a regioisomer of **2** and **3**.

Kurzichalcolactone [**6**] was obtained as an amorphous yellow powder, $[\alpha]_D$ −60° ($CHCl_3$, c=0.57). Its hreims showed a molecular ion peak at *m/z* 526.1974 corresponding to a molecular formula of $C_{32}H_{30}O_7$ (calcd 526.1991). The eims also exhibited fragmentation ion peaks which are similar to those of kurziflavolactones A–D. The ir spectrum disclosed absorption bands at 3400, 3200, 1715, 1610, 1500, 1345, 1220, 970, 950, 745, and 695 cm^{-1}, indicating the presence of OH groups, lactone, conjugated carbonyl group, and aromatic rings. The uv spectrum exhibited maxima at 210, 253, and 347 nm and resembled those of chalcone derivatives (9). The ^{1}H-nmr spectrum (in Me_2CO-d_6) of kurzichalcolactone was similar to that of kurziflavolactones A–D with a few notable exceptions. In kurzichalcolactone [**6**], the signals due to an ABX system which are present in kurziflavolactones A–D were missing; however, a new AB system, which resulted in two doublets at δ 7.72 (J=16) assigned to H-2 and δ 8.22 (J=16) assignable to H-3, appeared. For convenience, the numbering of kurzichalcolactone [**6**] was kept the same as that of kurziflavolactones A–D, though this does not follow the normal rule. In the HMQC spectrum, the doublet at δ 7.72 showed a cross peak with a signal at δ 143.3 attributed to C-2, whereas the one at δ 8.22 correlated with a signal at δ 128.9 assigned to C-3. The ^{1}H-nmr (see Experimental section) and ^{13}C-nmr data (Table 1) also indicated the presence of a side chain moiety which is the same as that of kurziflavolactones A–D. Detailed analysis of the ^{13}C-nmr data of kurzichalcolactone [**6**], by comparing the data of **4** with those of compounds **2–5** as shown in Table 1, suggested a 5,7,9-trioxygenated, 8-C-substituted ring A for **6**. The relative configuration of the side chain moiety was deduced by arguments similar to those presented for kurziflavolactone B [**3**]. Nearly the same peak appearance and coupling constants for H-15 and H-17 and almost the same nOe cross peaks were observable in ^{1}H-nmr and NOESY spectra of kurziflavolactone B [**3**] and kurzichalcolactone [**6**]. Hence, the structure of kurzichalcolactone was determined as **6**. ^{13}C-nmr data were assigned by ^{1}H-^{1}H COSY and HMQC. The long-range couplings in the HMBC spectrum further supported the structure of kurzichalcolactone [**6**] and some ^{13}C assignments (Figure 3).

As a result, the structures of kurziflavolactones A–D and kurzichalcolactone were determined to be **2–6**, respectively, though the stereochemistry of kurziflavolactones A–D could not be established with certainty as nOe correlations are not able to define the

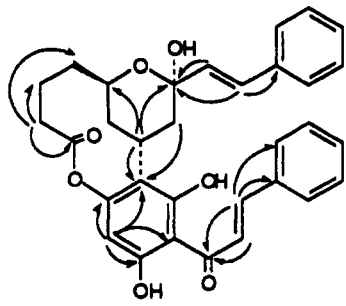

FIGURE 3. Long-range correlations from HMBC experiments of kurzichalcolactone [**6**].

relative stereochemistry of the flavanone core and the side chain moiety, and a suitable crystal for X-ray analysis could not be obtained. To the best of our knowledge, this is the first report of flavonoids found in *Cryptocarya* plants, while kurzichalcolactone **6** is the second chalcone derivative isolated from a species of this genus. The first one, cryptocaryone, was isolated from the roots of *Cryptocarya bourdiloni* (10,11). On the other hand, kurziflavolactones A–D and kurzichalcolactone represent, respectively, the first members of a new class of naturally occurring flavonoids and chalconoids.

It is interesting that the isolated compounds occur in the plant together with compound **1**. A biosynthetic pathway for the formation of kurzichalcolactone [**6**] and kurziflavolactones A [**2**], B [**3**], C [**4**], and D [**5**] starting from **1** and the chalcone **7** is proposed in Scheme 1.

Preliminary bioassays indicated that kurziflavolactone B [**3**] and kurzichalcolactone [**6**] exhibited weak cytotoxicity against KB cells with IC_{50} values of 4 and 15 μg/ml, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a Perkin-Elmer 241 polarimeter in $CHCl_3$. Uv spectra were recorded on a Shimadzu UV-161 uv-visible spectrophotometer; cd on a Jobin-Yvon Mark 5; ir on a Nicolet 205 FT-IR spectrometer; eims (70 eV) on a Kratos MS 50; hreims on a Kratos MS 80; nmr on Bruker AC 250 and AM 400. All nmr spectra were recorded with TMS as internal standard. 2D nmr experiments were carried out with standard pulse sequence. All solvents used were analytical or hplc grade. Tlc grade Si gel H was used for vlc and flash cc. Preparative tlc was performed with Si gel 60 F_{254}. Preparative hplc was carried out on Waters Associate instruments.

PLANT MATERIAL.—Leaves of *C. kurzii* were collected in Mersing Forest by two of us (F.R. and A.H.A. Hadi) on 22 November 1990. Identification was made by one of us (F.R.). Voucher specimens (KL 3978) are deposited at the Museum National d'Histoire Naturelle in Paris and at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

EXTRACTION AND ISOLATION OF COMPOUNDS **2–6**.—The leaves (1.5 kg) were extracted by EtOH. Evaporation of EtOH yielded a sticky residue which exhibited 75% inhibition against KB cells at 10 μg/ml. This residue was re-extracted with EtOAc, and the EtOAc was removed to give an EtOAc-soluble fraction (38 g) which showed 90% inhibition against KB cells at 10 μg/ml. The EtOAc-soluble fraction was subjected to vlc over Si gel (Merck 60 H) with CH_2Cl_2-MeOH (100:0, 99:1, 99:2, 95:5, 90:10) as eluents. Twenty fractions (400 ml/fraction) were collected. Fractions 12 and 13 [CH_2Cl_2-MeOH (97.5:2.5)] were run through preparative tlc on Si gel plates [CH_2Cl_2-MeOH (90:10)] followed by preparative hplc on a Prep-Pak Cartridge (Delta Pak C-18 column 47 mm×350 mm, 15–20 μ) and semipreparative hplc on a Waters RCM C-18 column (25 mm×100 mm) with MeOH-H_2O-HOAc (80:20:0.5) as eluent, flow rate 25 ml/min and 5 ml/min respectively. The four isomeric kurziflavolactones A–D (20.2, 23.9, 15.6, 18.6 mg, respectively) were obtained. Manipulation of fractions 14–15 [CH_2Cl_2-MeOH (95:5)] by flash chromatography (2–5% MeOH in CH_2Cl_2) on Si gel and semipreparative hplc on Waters RCM C-18 column (25 mm×100 mm), eluting with linear gradient from mixed solvent A [MeOH-H_2O-HOAc (70:30:0.2)] to B [MeOH-H_2O-HOAc (85:15:0.2)] within 10 min and then solvent B (flow rate 5 ml/min) afforded kurzichalcolactone [**6**] (34.2 mg).

Kurziflavolactone A [**2**].—An oil: $[\alpha]_D$ −53° ($CHCl_3$, *c*=0.6); ir ($CHCl_3$) ν max cm^{-1} 3400 (br, OH) 1713, 1630, 1594, 1343, 1225, 985, 945, 837, 750, 687; uv λ max (MeOH) nm 292 (ε 38792), 246 (ε 37174), 207 (ε 70353); uv λ max (MeOH+$AlCl_3$) nm 315 (ε 52195), 242 (ε 41473), 207 (ε 77332); eims *m/z* (rel. int. %) $[M]^+$ 526 (98), $[M-OH]^+$ 509 (3), 482 (2), 439 (30), 412 (10), 395 (19), 381 (10), 335 (20), 331 (7), 321 (8), 307 (24), 291 (20), 281 (22), 269 (18), 256 (13), 189 (10), 179 (11), 177 (21), 165 (18), 131 (100), 115 (8), 103 (62), 91 (15), 77 (17); cd λ ext (*c*=0.90, MeOH) nm (Δε) 282 (+12.8), 330 (−2.81); ^1H and ^{13}C nmr see Tables 1–3.

Kurziflavolactone B [**3**].—An oil: $[\alpha]_D$ −136° ($CHCl_3$, *c*=0.6); hreims *m/z* 526.1985 (calcd for $C_{32}H_{30}O_7$, 526.1991); ir ($CHCl_3$) ν max cm^{-1} 3400 (br, OH), 1718, 1631, 1590, 1348, 1220, 980, 945, 835, 750, 690; uv λ max (MeOH) nm 291 (ε 36189), 247 (ε 30403), 209 (ε 61963); uv λ max (MeOH+$AlCl_3$) nm 316 (ε 52442), 242 (ε 40765), 206 (ε 73061); eims *m/z* (rel. int. %) $[M]^+$ 526 (100), $[M-OH]^+$ 509 (3), 482 (1), 439 (22), 412 (7), 395 (15), 381 (7), 335 (15), 321 (5), 307 (17), 291 (17), 281 (20), 269 (9), 256 (12), 189 (9), 179 (13), 177 (17), 165 (12), 131 (100), 115 (9), 103 (62), 91 (15), 77 (24); cd λ ext (*c*=0.55, MeOH) nm (Δε) 288 (−10.7), 330 (+1.53); ^1H and ^{13}C nmr see Tables 1–3.

Kurziflavolactone C [**4**].—An oil: $[\alpha]_D$ −57° ($CHCl_3$, *c*=1.51); ir ($CHCl_3$) ν max cm^{-1} 3400 (br, OH), 1715, 1643, 1580, 1450, 1151, 980, 937, 829, 746, 686; uv λ max (MeOH) nm 291 (ε 29614), 209 (58333); uv λ max (MeOH+$AlCl_3$) nm 313 (ε 36031), 207 (ε 66329); eims *m/z* (rel. int. %) $[M]^+$ 526 (100), $[M-OH]^+$ 509 (6), 439 (10), 412 (10), 395 (42), 382 (27), 335 (6), 307 (10), 291 (17), 281 (23), 269 (29), 256 (58), 183 (44), 179 (27), 177 (9), 165 (13), 152 (17), 131 (100), 115 (8), 103 (52), 91 (17), 77 (25); cd λ ext (*c*=0.73, MeOH) nm (Δε) 288 (−12.3), 330 (+3.75); ^1H and ^{13}C nmr see Tables 1–3.

Kurziflavolactone D [**5**].—An oil: $[\alpha]_D$ −50° ($CHCl_3$, *c*=1.67); ir ($CHCl_3$) ν max cm^{-1} 3430 (br, OH), 1715, 1649, 1576, 1460, 1145, 980, 950, 832, 740, 699; uv λ max (MeOH) nm 290 (ε=24594), 209 (67065); uv λ max (MeOH+$AlCl_3$) nm 315 (ε 28574), 210 (ε 70405); hreims *m/z* 526.1983 (calcd for $C_{32}H_{30}O_7$, 526.1991); eims *m/z* (rel. int. %) $[M]^+$ 526 (100), $[M-OH]^+$ 509 (3), 439 (9), 412 (4), 395 (24), 382 (17), 335 (3), 307 (8), 291 (11), 281 (21), 269 (20), 256 (12), 179 (12), 165 (10), 131 (99), 115 (8), 103 (29), 91 (11), 77 (10); cd λ ext (*c*=0.65, MeOH) nm (Δε) 282 (+10.8), 330 (−7.28); ^1H and ^{13}C nmr see Tables 1–3.

Kurzichalcolactone [**6**].—Amorphous solid: $[\alpha]_D$ −60° ($CHCl_3$, *c*=0.57); hreims *m/z* 526.1974 (calcd for $C_{32}H_{30}O_7$, 526.1991); ir ($CHCl_3$) ν max cm^{-1} 3400 (br, OH), 3200 (br, OH), 1715 (br), 1610 (br), 1500, 1345, 1220, 970, 950, 930, 745, 695; uv λ max (MeOH) nm 347 (ε=43071), 253 (ε=32322), 210 (ε=64645); uv λ max (MeOH+$AlCl_3$) nm 376 (ε 73030), 251 (ε 34456), 209 (ε 53743); eims *m/z* (rel. int. %) $[M]^+$ 526 (100), 509 (7), 439 (9), 425 (11), 412 (9), 395 (43), 382 (27), 335 (5), 307 (10), 291 (18), 281 (23), 269 (29), 256 (59), 183 (45), 179 (27), 165 (14), 152 (18), 131 (100), 103 (51), 91 (18), 77 (25); ^1H-nmr (Me_2CO-d_6) δ 7.72 (d, 16, H-2), 8.22 (d, 16, H-3), 6.11 (s, H-6), 2.32 (brt, 7, H-7), 1.50–1.80 (m, H-13, -14, -16), 3.78 (m, $W_{1/2}$=20, H-15), 3.58 (dd, 3, 2.5, H-17), 2.20 (dd, 13, 3, H-18ax), 2.12 (dd, 13, 2.5, H-18eq), 7.12 (d, 16, H-20), 6.68 (d, 16, H-21), 14.10 (s, 5-OH); ^1H nmr (C_6D_6) δ 7.92 (d, 16, H-2), 8.36 (d, 16, H-3), 6.52 (s, H-6), 2.02 (brt, 7, H-7), 1.20–1.76 (m, H-13, -14, -16), 3.80 (m, $W_{1/2}$=20, H-15), 3.64 (dd, 3, 2.5, H-17), 1.92 (dd, 13, 3, H-18ax), 1.81 (dd, 13, 2.5, H-18eq), 7.05 (d, 16, H-20), 6.48 (d, 16, H-21); ^{13}C nmr see Table 1.

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