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DIHYDROCHALCONES FROM *UVARIA ANGOLENSIS*

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Key Word Index—*Uvaria angolensis*; Annonaceae; uvaretin; isouvaretin; uvangoletin; angoletin; ^{13}C NMR; dihydrochalcones.

Abstract—An investigation of the roots of *Uvaria angolensis* has led to the isolation and identification of two new dihydrochalcones, angoletin and uvangoletin, and the known *C*-benzyldihydrochalcones, uvaretin and isouvaretin. The structures were established from ^{13}C NMR comparisons with known dihydrochalcones.

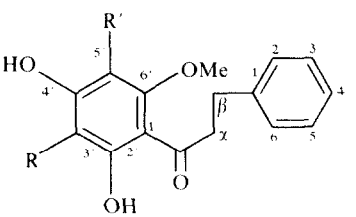
INTRODUCTION

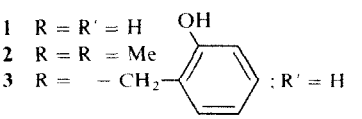
The genus *Uvaria* has been a rich and varied source of new compounds as is evidenced by the isolation of *C*-benzylated flavonoids [1–6], alkaloids [7, 8], and aromatic oils [9]. Our continuing studies of the isolation of antimicrobial and cytotoxic agents from plants has now resulted in the isolation of two new dihydrochalcones, uvangoletin (**1**) and angoletin (**2**), and the known *C*-benzyldihydrochalcones, uvaretin (**3**) and isouvaretin (**4**), from *Uvaria angolensis*.

RESULTS AND DISCUSSION

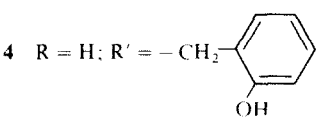
The antimicrobial and cytotoxic activities present in the ethanolic root extract of *Uvaria angolensis* were concentrated in the ethyl acetate fraction of an ethyl acetate–water partition. Silica gel chromatography of this fraction using benzene and ether–benzene mixtures as eluent resulted in the isolation of four dihydrochalcones, **1**–**4**. The antimicrobial and cytotoxic activities of the extracts were traced to uvaretin (**3**), and isouvaretin (**4**), previously reported constituents of *Uvaria* [1, 2, 10].

A substance named chamuvarin has been reported previously as a constituent of *U. chamae* [3] and assigned structure **4**. This identification was based on chemical evidence, the structure **3** being eliminated because of differences in reported spectral data [3]. The structure as proposed for chamuvarin (**4**) also corresponds to that reported for isouvaretin (**4**) [1]. Therefore an authentic sample of chamuvarin [3] was obtained and compared with both uvaretin (**3**) and isouvaretin (**4**). The chamuvarin sample corresponded to uvaretin by TLC and the IR spectra of the two samples were superimposable. The ^{13}C NMR spectra of uvaretin (**3**) and isouvaretin (**4**) were also obtained and the data are given in the Experimental. The ^{13}C NMR spectrum of the chamuvarin sample was identical in all respects to that of uvaretin (**3**). While some of the spectral data for uvaretin (**3**) and isouvaretin (**4**) are similar, they can be easily distinguished by the chemical shift of the methoxyl carbon signal (**3**, 55.9 ppm; **4**, 63.5 ppm). Thus, chamuvarin is identical with uvaretin (**3**) and therefore its name should be dropped from the literature or assigned to another compound. The structure for uvaretin (**3**) was independently deduced [1, 2]. A comparison of these two samples showed them to be identical in all aspects (TLC, mmp, superimposable IR).

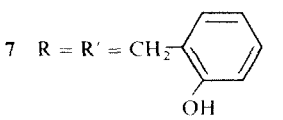




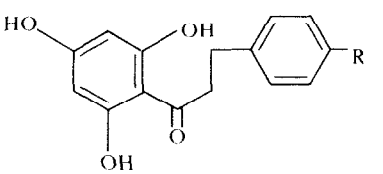
1 R = R′ = H
2 R = R = Me
3 R = $-CH_2-$: R′ = H



4 R = H; R′ = $-CH_2-$



7 R = R′ = CH_2



5 R = OH
6 R = H

Table 1. ^{13}C NMR data of dihydrochalcones*

Carbon assignment†	5	6	1	2
C_1	133.6 *s*	142.9 *s*	142.7 *s*	141.7 *s*
C_2	130.1 *d*	129.0 d^1	129.1 d^1	128.5 *d*
C_3	116.0 *d*	129.2 d^1	129.2 d^1	128.5 *d*
C_4	156.2 *s*	126.5 *d*	126.6 *d*	126.0 *d*
C_5	116.0 *d*	129.2 d^1	129.2 d^1	128.5 *d*
C_6	130.1 *d*	129.0 d^1	129.1 d^1	128.5 *d*
$C_{1'}$	105.3 *s*	105.3 *s*	105.7 *s*	106.6 *s*
$C_{2'}$	165.1 s^1	165.4 *s*	164.5 s^2	159.2 s^1
$C_{3'}$	96.0 *d*	96.0 *d*	96.9 *d*	108.6 s^2
$C_{4'}$	165.3 s^1	165.4 *s*	165.6 s^2	159.2 s^1
$C_{5'}$	96.0 *d*	96.0 *d*	91.9 *d*	109.0 s^2
$C_{6'}$	165.3 s^1	165.4 *s*	168.3 *s*	161.5 s^1
C_α	46.5 *t*	46.1 *t*	46.2 *t*	44.7 *t*
C_β	32.5 *t*	31.4 *t*	31.4 *t*	31.0 *t*
CO	205.6 *s*	205.1 *s*	205.0 *s*	205.2 *s*
OMe	—	—	56.1 *q*	61.9 *q*
$C_{3'}$–Me	—	—	—	7.5 q^3
$C_{5'}$–Me	—	—	—	8.6 q^3

* All data obtained in Me_2CO-d_6 except **2** ($CDCl_3$).

† The assignments are based on chemical shift theory, single-frequency off-resonance decoupling, and by comparisons with the flavanones previously studied [11]. Signals bearing the same numerical superscript in any one column may be reversed.

Uvangoletin (**1**), $C_{16}H_{16}O_4$, had IR and UV spectral data similar to other dihydrochalcones of *Uvaria* [1]. The ^1H NMR showed the characteristic A_2B_2 pattern for dihydrochalcones, a methoxy signal at δ 3.87, a pair of doublets for H-3′ and H-5′ at 5.91 and 5.97 (J = 2 Hz), five aromatic protons as a singlet at 7.20 and a D_2O-exchangeable signal at 13.80. The ^{13}C NMR data for **1** and the known dihydrochalcones, phloretin (**5**) and pinocembrin dihydrochalcone (**6**), are listed in Table 1. The placement of the methoxyl group *ortho* to the carbonyl also follows from ^{13}C NMR. If it were located at $C_{4'}$, the molecule would be symmetrical and, as in **5** and **6**, the signals for $C_{3'}$ and $C_{5'}$ would be equivalent ($C_{2'}$ and $C_{6'}$ would also be equivalent). Thus, the spectral data especially, ^1H and ^{13}C NMR, allow formulation of uvengoletin as shown in **1**.

Angoletin (**2**), $C_{18}H_{20}O_4$, had IR and UV spectral data similar to **1**. The ^1H NMR of **2** was similar to that of **1** except for two 3H's at δ 2.13 and 2.17 and the absence of the pair of doublets for $H_{3'}$ and $H_{5'}$. This suggested that angoletin (**2**) was *C*-dimethyluvangoletin. The ^{13}C NMR data (Table 1) confirmed the presence of the methyl groups at 7.5 and 8.6 ppm. Again, the methoxyl group must be located *ortho* to the carbonyl; otherwise, the molecule would be symmetrical and contain two less carbon signals. Thus, the spectral data allow formulation of angoletin as **2**.

Uvangoletin (**1**) and angoletin (**2**) did not show antimicrobial activity or cytotoxicity when tested as previously described [1, 10].

EXPERIMENTAL

Mps uncorr.; UV spectra were determined in MeOH; ^1H and ^{13}C NMR were recorded at 60 and 15 MHz, respectively, using TMS as int. standard.

Plant. Uvaria angolensis Welv. ex Oliv (syn. *U. cordata*). A voucher specimen is deposited in the Herbarium of the Forest Research Institute of Nigeria (FRIN); the plant material was collected in July 1978, in Oyo State, Nigeria.

Dihydrochalcone isolation. The air-dried ground roots of *U. angolensis* (4.0 kg) were extracted by percolation with EtOH. Evapn of the EtOH extract *in vacuo* at 40° yielded 351.5 g of residue, which was partitioned between 1.5 l. of EtOAc (× 4) and 1.7 l. of H_2O. The combined EtOAc extracts were evapd to yield 130.0 g of residue. A portion of this residue (62.0 g) was chromatographed over silicic acid (1.4 kg) by first absorbing it on to 60.0 g of Celite 545 (Sargent-Welch) and then eluting with C_6H_6. After elution with 7 l. of C_6H_6, the eluent was changed to 1% Et_2O–C_6H_6.

Angoletin (**2**). Elution with 2 l. of 1% Et_2O–C_6H_6 yielded 617 mg of an oily fraction which was further purified by PLC (Si gel G, 1 mm, 33% Et_2O–hexane) to give 195 mg of an oil that was crystallized from $CHCl_3$–hexane to yield 121 mg of **2**: mp 74–76°; UV: λ_{max} nm (log ε): 325 (3.57), 288 (4.10) and 215 (4.13); IR: ν_{max} cm^{-1}: 3350 and 1610; ^1H NMR (Me_2CO): δ 13.53 (1 H, *s*, OH ex. D_2O), 7.30 (5 H, *s*, Ar-H), 3.73 (3 H, *s*, OCH_3), 2.86–3.83 (4 H, *m*, H_α and H_β), 2.17 (3 H, *s*, CH_3) and 2.13 (3 H, *s*, CH_3). (Found: C, 71.76; H, 6.69. $C_{18}H_{20}O_4$ requires: C, 71.98; H, 6.71%).

Uvaretin (**3**). Elution with 4 l. of 2% Et_2O–C_6H_6 yielded 10.4 g fraction which gave 5.2 g of **3** from C_6H_6, mp 164–165°. The spectral data were the same as reported [1] and direct comparison with a previously isolated sample showed no mmp depression and superimposable IR spectra.

Uvangoletin (**1**). Elution with 3 l. of 4% Et_2O–C_6H_6 gave 1.6 g fraction which yielded 236 mg of **1** from C_6H_6: mp 189–191°; UV: λ_{max} nm (log ε): 289 (4.01) and 220 (3.88); IR: ν_{max} cm^{-1}: 3350 and 1625; ^1H NMR (Me_2CO): δ 13.80 (1 H, *s*, OH ex. D_2O), 7.20 (5 H, *s*, Ar-H), 5.97 (1 H, *d*, J = 2 Hz, $H_{3'}$ or $H_{5'}$), 5.91 (1 H, *d*, J = 2 Hz, H_3 or $H_{5'}$), 3.87 (3 H, *s*, OCH_3) and 2.77–3.47 (4 H, *m*, H_α and H_β).

(Found: C, 70.47; H, 5.59. $C_{15}H_{16}O_4$ requires: C, 70.58; H, 6.10%).

Isouvaretin (**4**). Elution with an additional 800 ml of 4% Et_2O C_6H_6 gave a fraction which was further purified by chromatography over alumina (grade V, neutral 60 g). Elution with 300 ml 2% MeOH in $CHCl_3$ gave 250 mg of an oil that yielded 137 mg of **4** upon crystallization from $CHCl_3$–hexane, mp 80–82°. The ^1H NMR, ^{13}C NMR, IR and UV of this compound were identical to those of isouvaretin (**4**) [1].

^{13}C NMR data. The data for **1**, **2**, **5** and **6** are included in Table 1. Phloretin (**5**) was purchased from ICN-K and K, Inc. and pinocembrin dihydrochalcone (**6**) was made as described previously [10]. Uvaretin (**3**), isouvaretin (**4**) and diuvaretin (**7**) have been reported [1]; however, their ^{13}C NMR spectral data were not recorded and are listed here. The spectral conditions are the same as reported for the flavanones [5].

Uvaretin (**3**) (Me_2CO). δ 205.4(*s*), 165.4(*s*), 163.0(*s*), 162.6(*s*), 154.8(*s*), 142.6(*s*), 131.6(*d*), 129.0(*d*), 127.9(*std*), 126.5(*d*), 120.8(*d*), 116.0(*d*), 108.0(*s*), 105.9(*s*), 92.1(*d*), 55.9(*q*), 46.2(*t*), 31.4(*t*) and 22.8(*t*).

Isouvaretin (**4**) (Me_2CO). δ 206.0(*s*), 165.0(*s*), 163.8(*s*), 162.8(*s*), 155.4(*s*), 142.6(*s*), 130.2(*d*), 129.2(*d*), 127.9(*s*), 127.7(*d*), 126.7(*d*), 120.5(*d*), 115.8(*d*), 114.0(*s*), 109.9(*s*), 100.6(*d*), 63.5(*q*), 44.7(*t*), 31.6(*t*) and 23.7(*t*).

Diuvaretin (**7**) (Me_2CO). δ 206.1(*s*), 162.5(*s*), 161.3(*s*), 160.8(*s*), 155.0(*s*), 154.2(*s*), 142.4(*s*), 131.9(*d*), 130.5(*d*), 129.1(*d*), 128.1(*d*), 127.7(*s*), 127.6(*s*), 126.5(*d*), 121.2(*d*), 120.7(*d*), 115.7(*d*), 114.2(*s*), 112.2(*s*), 109.6(*s*), 63.5(*q*), 44.6(*t*), 31.6(*t*), 24.0(*t*) and 23.3(*t*).

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