NEW DIHYDROCHALCONES AND FLAVANONES FROM *UVARIA ANGOLENSIS*

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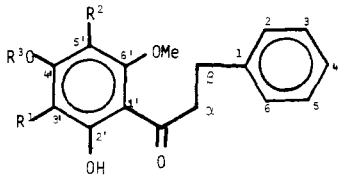
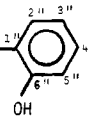
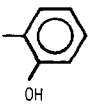
ABSTRACT.—Two flavanones, (±)-chamanetin 5-methyl ether (**5**) and (+)-6,8-C-dimethylpinocembrin 5-methyl ether (**6**) and two dihydrochalcones, anguvetin (**7**) and flavokawin B (**8**) were isolated from the roots of *Uvaria angolensis* and characterized by chemical and spectral methods, particularly ^{13}C-nmr spectroscopy. The antimicrobial activities of these flavonoids are reported.

Antimicrobial and cytotoxic activities of ethanolic root extracts of *Uvaria angolensis* Welw. ex. Oliv. (Annonaceae) have been traced to the known dihydrochalcones, uvaretin (**1**) and isouvaretin (**2**) as previously reported (1). Two additional dihydrochalcones, angoletin (**3**) and uvangoletin (**4**), were also identified and their structures established mainly from ^{13}C-nmr spectral data (1). An investigation of other column fractions has now resulted in the identification of two flavanones, (±)-chamanetin 5-methyl ether (**5**) and (+)-6,8-C-dimethylpinocembrin 5-methyl ether (**6**), and two dihydrochalcones, anguvetin (**7**) and flavokawin B (uvangoletin 4'-methyl ether) (**8**).

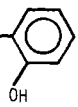


1; R^1 =  , $R^2 = R^3 = H$

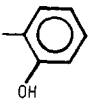
2, $R^1 = R^3 = H$, R^2 = 

3; $R^1 = R^2$ = Me, $R^3 = H$

4; $R^1 = R^2 = R^3 = H$

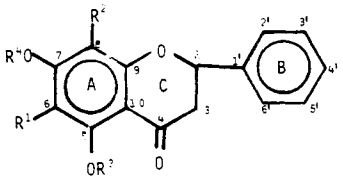
7; R^1 =  , R^2 = Me, $R^3 = H$

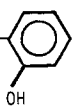
8; $R^1 = R^2 = H$, R^3 = Me

14; $R^1 = R^2$ =  , $R^3 = H$

RESULTS AND DISCUSSION

(±)-Chamanetin 5-methyl ether (**5**) was readily identified as one of the flavanones from its spectral properties, particularly its ^{13}C-nmr spectral data. (±)-Chamanetin 5-methyl ether (**5**) has previously been reported as a constituent



5; R^1 = H, R^2 = -CH_2-  , R^3 = Me, R^4 = H

6; R^1 = R^2 = R^3 = Me, R^4 = H

9; R^1 = R^2 = Me, R^3 = R^4 = H

10; R^1 = R^2 = R^4 = Me, R^3 = H

11; R^1 = R^2 = R^3 = R^4 = H

of *Uvaria chamae* (2), and a direct comparison indicated that the two samples were identical.

The other flavanone, which had the molecular formula $C_{18}H_{18}O_4$, showed ir and uv spectral data similar to other flavnones of *Uvaria* (2, 3). The ^{1}H-nmr (60 MHz, acetone-d_6) showed the characteristic ABX pattern of flavanones, a five proton multiplet (δ 7.30–7.70) characteristic of an unsubstituted B ring, a methoxyl group (3Hs, δ 3.72), two aromatic methyl groups (6Hs, δ 2.11) and one D_2O exchangeable proton. The mass spectrum also confirmed that the B ring must be unsubstituted with peaks at *m/z* 298 (M^+, 14%), 221 (M^+–77, 12%) and 194 (M^+–104, 100%, retro-Diels-Alder). The ^{13}C-nmr data (table 1) confirmed the flavanone ring system (4) and was consistent with a fully substituted A-ring. The ^{13}C-nmr data was similar to that reported for demethoxymatteucinol (6,8-C-dimethylpinocembrin) (**9**) (5) (table 1). Based on the collective spectral data, the

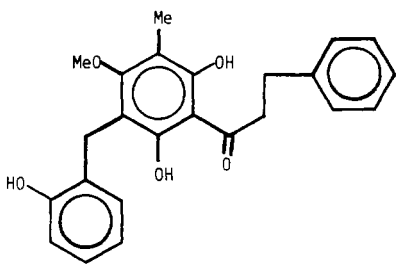
TABLE 1. ^{13}C-nmr spectral data for flavanones (**6**,**9**,**10**).[a]

Carbon	6	9	10
2	77.8	79.3	79.2
3	44.8	43.5	43.7
4	188.5	196.9	197.3
5	160.2[1]	162.4[1]	165.6[1]
6	112.0[2]	103.0[2]	111.4[2]
7	159.0[1]	160.0[1]	159.5[1]
8	108.2[2]	102.9[2]	109.7[2]
9	157.0[1]	158.3[1]	157.9[1]
10	107.5[2]	104.0[2]	105.2[2]
1'	139.5	140.5	139.1
2'	126.0	126.6	125.9
3'	128.5	129.2	128.6
4'	128.1	128.8	128.4
5'	128.5	129.2	128.6
6'	126.0	126.6	125.9
Me	8.4, 8.6	7.1, 7.9	7.9, 8.4
OMe	60.4	—	60.0

[a]The assignments are based upon known chemical shift theories and single-frequency off-resonance decoupling. **6** and **9** were run in dioxane-d_8 while **10** was run in chloroform-d_1. The assignments for C-2'–4' are based on those of pinocembrin (**11**) (7). Signals bearing the same numerical superscript may be reversed. The data for **9** has been previously reported (5) and is listed here for comparison.

new flavanone appeared to be a monomethyl ether derivative of **9**. The methoxyl group must be located at C–5 since the signal for C–4 in **6** appeared at 188.5 ppm.[1] A monomethyl ether of **9** was prepared by methylation with diazomethane and had spectral properties different from **6**. It was assigned structure **10** based on predicted methylation with diazomethane and the ^{13}C-nmr data (C–4, 197.3) (see table 1). Thus, the other flavanone isolated from *U. angolensis* is 6,8-C-dimethylpinocembrin-5-methyl ether (**6**). The absolute stereochemistry at C–2 was determined as R based on the cd data ($[\theta]_{282}+9{,}890$).[2]

Anguvetin (**7**), $C_{24}H_{24}O_5$, was readily identified as a dihydrochalcone from its uv, ir and ^{1}H-nmr data (A_2B_2 system) (3). The ^{13}C-nmr data (table 2) confirmed the presence of a methoxyl group (61.7 ppm), an aromatic methyl (8.8 ppm), and an *ortho*-hydroxylbenzyl moiety. A comparison of the ^{13}C-nmr spectral data for angoletin (**3**) and uvaretin (**1**) (1) with that of **7** clearly indicated that angu-



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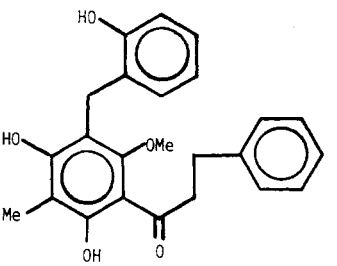

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TABLE 2. ^{13}C-nmr spectral data for dihydrochalcones (**1**, **3**, **7**, **8**, **15**).[a]

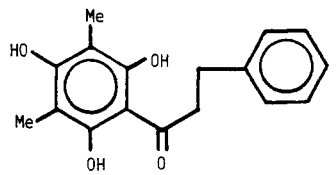
Carbon	7	3	1	8	15
1	141.6	141.6	142.6	142.7	143.0
2	—[b]	128.5	—[c]	129.2	129.2[1]
3	—[b]	128.5	—[c]	129.2	129.1[1]
4	—[b]	126.0	—[c]	126.6	126.6
5	—[b]	128.5	—[c]	129.2	129.2[1]
6	—[b]	128.5	—[c]	129.2	129.1[1]
1′	109.0[1]	106.6[1]	105.9[1]	106.5	106.2[2]
2′	160.9[2]	159.2[2]	165.4[2]	164.1	159.7[2]
3′	110.2[1]	108.6[1]	108.0[1]	94.9	103.2
4′	160.0[2]	159.2[2]	163.0[2]	167.3[1]	160.9[2]
5′	110.8[1]	209.0[1]	92.1	91.5	103.2
6′	160.0[2]	161.5[2]	162.6[2]	168.5	159.7[2]
1″	—[b]	—	—[c]	—	—
2″	152.8	—	154.8	—	—
3″	115.7	—	116.0	—	—
4″	—[b]	—	—[c]	—	—
5″	121.5	—	120.8	—	—
6″	132.0	—	131.6	—	—
Cα	44.3	44.7	46.2	46.3	46.7
Cβ	31.0	31.0	31.4	31.4	31.7
C=O	205.5	205.2	205.4	205.4	206.0
Me	8.8	7.5, 8.6	—	—	8.3
OMe	61.7	61.9	55.9	56.0, 56.3	—
CH_2	22.9	—	22.8	—	—

[a]The assignments are based upon known chemical shift theories and single-frequency off-resonance decoupling. **1**, **8** and **15** were run in acetone-d_6 while **3** and **7** were run in chloroform-d_1. Signals bearing the same numerical superscript may be reversed. The data for **3** and **1** has been reported (1) and is listed here for comparison. [b]Signals for these carbons appear at 128.5, 127.9, 126.4 and 126.2 ppm. [c]Signals for these carbon appear at 129.0, 127.9 and 126.5 ppm.

[1]C–4 appears near 197 ppm in a 5-hydroxyflavanone nucleus and near 188 when a C–5 methoxyl group is present (2).

[2]Optically active flavanones have an intense peak in the cd spectrum and this has been correlated with absolute stereochemistry at C–2 (6).

vetin was a C-methylated *ortho*-hydroxylbenzylated dihydrochalcone methyl ether (table 2). Three possible structures for anguvetin may be drawn (**7**, **12** and **13**) that would be consistent with these data. The choice of structure **7** as representing anguvetin is based upon the following ^{13}C-nmr spectral analyses. The signal for the benzylic carbon in uvaretin (**1**) appears at 22.8 ppm, which is about 1 ppm upfield from that found in isouvaretin (**2**) (23.7 ppm) (1). In diuvaretin (**14**) these benzylic carbon signals appear at 24.0 and 23.3 ppm (1). While these differences are small, they are diagnostic for the location of the benzylic carbon signal between two hydroxyl groups [uvaretin (**1**)] versus one hydroxyl group and one methoxyl group [isouvaretin (**2**)]. Similar differences in these benzylic carbon signals were also noted between chamanetin, isochamanetin, and dichamanetin, isomeric C-benzylated flavanones (4). Only structure **7** has the benzylic carbon located between two phenolic hydroxyl groups[3], and the benzylic carbon signal appears at 22.9 ppm. Similar arguments can be made for the methyl group. The aromatic methyl groups appear at 8.1 and 9.2 ppm in **3** (Acetone-d_6). The assignment of the upfield signal (8.1 ppm) to the methyl group located between two phenolic hydroxyl groups was made by comparing this signal in demethylangoletin (**15**) (8.3 ppm) prepared from angoletin (**3**). These data are summarized in table 3 and strongly favor structure **7** for anguvetin.



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Flavokawin B (**8**) was readily identified as a dihydrochalcone from its spectral data (uv, ir, ^{1}H nmr). The ^{13}C-nmr data (table 2) confirmed all of the structural features for a dihydrochalcone dimethyl ether. Flavokawin B has previously been reported (9, 10), and a sample was prepared from uvangoletin (**4**) by methylation. Uvangoletin-4'-methyl ether (**8**) was identical in all respects to the isolated sample of flavokawin B (**8**).

TABLE 3. Assignments of the methyl and benzylic carbon signals in dihydrochalcones.[a]

Compounds	Me–C3'	Me–C5'	$ArCH_2$–C3'	$ArCH_2$–C5'
Angoletin (**3**)	8.1	9.2	—	—
Demethylangoletin (**15**)	8.3	8.3	—	—
Uvaretin (**1**)	—	—	22.8	—
Isouvaretin (**2**)	—	—	—	23.7
Diuvaretin (**14**)	—	—	23.3	24.0
Anguvetin (**7**)	—	9.3	22.9	—

[a]All data in this table were obtained in acetone-d_6. These values differ slightly depending upon solvent (see Table 2). The data for **1**, **2** and **14** has been previously reported (1) and is listed here for comparison.

The four compounds (**5**, **6**, **7**, **8**) were tested for antimicrobial activity as previously described (**11**), and only anguvetin (**7**) was active. It was active against *Staphylococcus aureus* (1.5 μg/ml), *Bacillus subtilis* (0.2 μg/ml) and *Mycobacterium*

[3]Structure **12** was also not favored on biosynthetic arguments since all previously known O-methylated dihydrochalcones have at least one methoxyl group *ortho* to the carbonyl function (1,3,8).

[4]**7** was not active against other gram-negative bacteria and fungi routinely used for screening (11). Streptomycin sulfate was included at the same time that **7** was tested and it showed minimum inhibitory concentration values of 3.1, 0.2, and 0.8 μg/ml respectively.

smegmatis (1.5 μg/ml). Angoletin (**3**), when initially tested for antimicrobial activity (**1**), was inactive; however, upon retesting it showed values of 12.5, 0.8 and 6.3 μg/ml, respectively. As previously noted (11), the dihydrochalcone derivatives of *Uvaria* were more active than the flavanone derivatives. Based on the results presented here, it would also appear that the dihydrochalcone must contain an alkyl group in the A ring (Me or *o*-hydroxylbenzyl) to have significant antimicrobial activity.

EXPERIMENTAL[5]

PLANT MATERIAL.—The plant material used for this study [roots, *Uvaria angolensis* Welw. ex. *Oliv.* (syn *U. cordata*) (Annonaceae)] was collected in July, 1978, in Oyo State, Nigeria, by the Forest Research Institute of Nigeria (FRIN). A herbarium specimen documenting this collection is deposited in the herbarium of the Forest Research Institute of Nigeria.

EXTRACTION AND CHROMATOGRAPHIC SEPARATION.—The air-dried ground roots of *Uvaria angolensis* (4.0 kg) were extracted, partitioned and chromatographed as previously reported (1).

ANGUVETIN (**7**).—Elution with 8.0 liters of 1% ether in benzene gave a fraction which was purified by preparative layer chromatography (2% ethanol-chloroform; silica gel G precoated plates 2mm). The band corresponding to the major constituent was located under uv, scraped, extracted with chloroform-acetone, and evaporated to dryness, an oily residue was obtained. The residue (211 mg) was further purified by column chromatography over Alumina (grade V, neutral, 50 gm). Elution with 100 ml of 2% methanol in chloroform gave 40.0 mg of (**7**) upon crystallization from chloroform/*n*-hexane, mp 148–150°; uv λ max (MeOH) 340 nm (ε 9.31 x 10^3), 286 nm (ε 8.58 x 10^4), 218 nm (ε 1.69 x 10^2), ir (KBr) ν max: 3350, 3100, 1610, 1595, 1555 cm^{-1}; ^1H nmr (acetone-d_6) δ 13.8 (1H, s, O*H* ex. D_2O), 7.52 (1H; s, O*H* ex. D_2O), 6.67–7.50 (9H, m, Ar-*H*), 4.93 (1H, br. s; O*H* ex. D_2O), 4.00 (2H, s, Ar CH_2Ar), 3.73 (3H, s, OCH_3), 2.83–3.70 (4H, m, Hα and Hβ), 2.13 (3H, s, Ar-CH_3); mass spectrum *m/z* (relative abundance) M^+ 392 (2%), 287 (16%), 260 (4%) and 193 (100%). ^{13}C nmr (See table 1).

Anal. Calc. for $C_{24}H_{24}O_5$: C, 73.45; H, 6.16. Found: C, 73.35; H, 6.17.

(+)-6,8-C-DIMETHYLPINOCEMBRIN 6-METHYL ETHER (**6**).—Elution with 1.0 liters of 8% ether in benzene gave an oily fraction which showed two spots on tlc plates (Alumina neutral: chloroform-methanol 9:1). This fraction (1.2 gm) was purified by chromatography over Alumina (grade V, neutral, 60 gm). Elution with 100 ml of 1% methanol in chloroform gave a residue (120 mg) which after crystallization from ether afforded 40 mg of (**6**), mp 205–207°C; $[\alpha]^{25}D = +2.8$ (c. 0.5, acetone); cd (1.0 x 10^{-4} gm/ml MeOH) $[\theta]_{340}$ +417.2, $[\theta]_{312}$ −298.0, $[\theta]_{252}$ + 9,893; uv λ max (MeOH) 320 nm (ε 3.54 x 10^3) 288 nm (ε 1.30 x 10^4), 218 nm (ε 2.50 x 10^4); ir (KBr) ν max: 3300, 1650, 1590 cm^{-1}; ^1H nmr (acetone-d_6) δ 7.30–7.70 (5H, m, Ar-*H*), 5.47 (X of ABX, dd, 1H, J=6 Hz, 10 Hz), 3.72 (3H, s, OCH_3), 3.15 (1H, br s, O*H* ex. D_2O), 2.68–3.02 (2H, m, AB of ABX), 2.11 (6H, s, 2Ar−CH_3); mass spectrum *m/z* (relative abundance) M^+ 298 (14%), 221 (12%), 195 (13%), 194 (100%) ^{13}C nmr (see table 2).

Anal. Calc. for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.21; H, 6.14.

(±)-CHAMANETIN 5-METHYL ETHER (**5**).—Elution with 4 liters of 32% ether in benzene gave a brown precipitate which on crystallization from acetone yielded 200 mg of (**5**): mp 226–228°C, $[\alpha]^{25}D = 0$ (c 0.48, DMSO). The spectral data were the same as reported (2) and direct comparison with a previously isolated sample showed no mmp depression and superimposable ir spectra.

FLAVOKAWIN B (UVANGOLETIN 4'-METHYL ETHER) (**8**).—A portion of the ethyl acetate solubles (234.0 gm) was chromatographed over silicic acid (2.3 kg) by first adsorbing it onto 230 gm of Celite 545 (Sargent-Welch) and then eluting it with *n*-hexane (10 liters) and 2.5% ether in hexane (14 liters). Elution with 5.0 liters of 5% ether in hexane yielded 37 mg of **8** from hexane, mp 100–103°; uv λ max (MeOH) 330 nm (ε 4.12 x 10^3), 288 nm (ε 2.79 x 10^4), 226 nm (ε 2.77 x 10^4), 213 nm (ε 2.65 x 10^4); ir (KBr) ν max 3450, 2940, 1620, 1590 cm^{-1}; ^1H nmr (acetone-d_6) δ 13.7 (1H, s, O*H* ex. D_2O), 7.17 (5H, br s Ar-*H*) 5.97 (2H, br s, H_3 and H_5), 3.83 (3H, s, OCH_3), 3.78 (3H, s, OCH_3); 2.70–3.50 (4H, m, Hα and Hβ); mass spectrum *m/z* (relative abundance) M^+ 286 (5%), 255 (1%), 182 (9%), 181 (100%), and 154 (35%). ^{13}C nmr (table 1). A direct comparison of this sample with a sample prepared from uvangoletin showed no mixture mp depression, the same mobility on tlc, and superimposable ir spectra.

Anal. Calc. for $C_{17}H_{16}O_4$: C, 71.31; H, 6.34. Found: C, 71.30; H, 6.41.

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[5]Melting points were determined using a Fisher-Digital model 355 melting point apparatus and are uncorrected. Uv spectra were taken on a Beckman model Acta III recording spectrophotometer; ir spectra were determined on either a Beckman IR-33 recording infrared spectrophotometer or a Perkin-Elmer 281B recording spectrophotometer; optical rotations were measured on a Perkin-Elmer 141 automatic polarimeter, ^1H nmr spectra were recorded on a JEOL model C-60 nuclear magnetic spectrometer at 60 MHz using tetramethylsilane (TMS) as internal standard. The ^{13}C nmr spectra were recorded on JEOL-FX60 Fourier Transform nmr spectrometer at 15.03 MHz. Mass spectra were recorded on a Finnigan 3200 mass spectrometer. Elemental analyses were done by Scandinavian Microanalytical Laboratory in Herlev, Denmark. Spot detection on tlc plates were achieved by spraying with 0.5% of either aqueous $KMNO_4$ or Fast Blue B (Aldrich).

METHYLATION OF DESMETHOXYMATTEUCINOL (**9**).—An ethereal solution of **9** (50 mg) was treated with excess diazomethane for 24 hr at room temperature. Evaporation of the solvent gave a crystalline residue from which 38 mg of **10** was obtained, mp 108–109° (hexane); uv λ max (MeOH) 360 nm (ε 2.98 x 10^3), 285 nm (ε 1.30 x 10^4), 218 nm (ε 1.62 x 10^4); ir (KBr) ν max 3060, 2930, 1630, 1590 cm^{-1}, ^1H nmr δ 12.15 (1H, s, O*H* ex. D_2O), 7.36 (5H, s, Ar-H), 5.36 (1H, dd, $J=5$, 10 Hz, x of ABX); 3.73 (3H, s, OCH_3), 2.92–3.08 (2H, m, AB of ABX), 2.12 (6H, s, 2Ar-CH_3); mass spectrum *m/z* (relative abundance M^+ 298 (100%), 221 (23%), 194 (36%), 166 (92%). 123 (47%); ^{13}C nmr (table 1).

METHYLATION OF UVANGOLETIN (**4**).—To 100 mg of **4** dissolved in acetone (6 ml) was added 100 mg of potassium carbonate and 3 ml of methyl iodide. After stirring for 18 hr, the solvent was evaporated, and the resulting residue was partitioned between distilled water (30 ml) and chloroform (4 x 30 ml). The combined, dried (Na_2SO_4) chloroform layers, when evaporated gave a gum which yielded 79 mg of (**8**) from n-hexane, mp 101–103° [flavokawin B, lit. (9,10) mp 103°]. The ^1H nmr, ^{13}C nmr, ir and uv spectral data were consistent with those expected for **8**.

DEMETHYLATION OF ANGOLETIN (**3**).—To 100 mg of **3** dissolved in methylene chloride at −70° was added 0.8 ml of boron tribromide over 2 hr. The solution was stirred at room temperature for 2 hr and the solvent evaporated under high vacuum. The oily residue was partitioned between distilled water (30 ml) and diethyl ether (4 x 30 ml). The combined, dried (Na_2SO_4) ether layers were evaporated *in vacuo* to give a red gum (111 mg) which was chromatographed over silica gel 60 (40 gm). Elution with 20 ml of chloroform gave a precipitate which on crystallization from hexane-ether afforded 26 mg of **15**, mp 122–123° [lit. (10) 125°]; uv λ max (MeOH) 360 nm (ε 5.36 x 10^3), 295 nm (ε 1.32 x 10^4), 225 nm (ε 1.34 x 10^4), 210 nm (ε 1.72 x 10^4); NaOH 336 nm; ir (KBr) ν max 3560, 3420, 1620, 1580 cm^{-1}; ^1H nmr (acetone-d_6) δ 10.90 (1H, s, O*H*, ex. D_2O); 8.00 (1H, s, O*H*, ex. D_2O), 7.17 (5H, br s Ar-*H*), 2.67–3.67 (4H, m, Hα and Hβ), 2.07 (6H, s, 2Ar-CH_3); mass spectrum *m/z* (relative abundance (M^+ 286 (11%), 182 (14%), 181 (100%), and 154 (50%), ^{13}C nmr (acetone-d_6) see table 2.

Anal. Calc. for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 70.45; H, 6.34.

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LITERATURE CITED

1. C. D. Hufford and B. O. Oguntimein, *Phytochemistry*, **19**, 2036 (1980).
2. H. N. El-Sohly, W. L. Lasswell, Jr., and C. D. Hufford, *J. Nat. Prod.*, **42**, 264 (1979).
3. W. L. Lasswell, Jr., and C. D. Hufford, *J. Org. Chem.*, **42**, 1295 (1977).
4. C. D. Hufford and W. L. Lasswell, Jr., *Lloydia*, **41**, 151 (1978).
5. C. D. Hufford, B. O. Oguntimein, and J. K. Baker, *J. Org. Chem.*, **46**, 3073 (1981).
6. W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).
7. H. Wagner, V. M. Chari, and J. Sonnenbichler, *Tetrahedron Letters*, 1799 (1976).
8. J. R. Cole, S. J. Torrance, R. M. Wiedhopf, S. K. Arora and R. B. Bates, *J. Org. Chem.*, **41**, 1852 (1976).
9. R. Haensel, D. Weiss and B. Schmidt, *Planta Med.*, **24**, 1 (1966).
10. F. E. King and A. Robertson, *J. Chem. Soc.*, 403 (1934).
11. C. D. Hufford and W. L. Lasswell, Jr., *Lloydia*, **41**, 256 (1978).