Antimicrobial Compounds from Petalostemum purpureum

Charles D. Hufford, Yimin Jia, Edward M. Croom Jr., Ilias Muhammed, Adewole L. Okunade, Alice M. Clark, and Robin D. Rogers

J. Nat. Prod., **1993**, 56 (11), 1878-1889• DOI: 10.1021/np50101a003 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 11, pp. 1878-1889, November 1993

ANTIMICROBIAL COMPOUNDS FROM *PETALOSTEMUM PURPUREUM*

CHARLES D. HUFFORD,* YIMIN JIA, EDWARD M. CROOM JR., ILIAS MUHAMMED, ADEWOLE L. OKUNADE, ALICE M. CLARK,

Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677

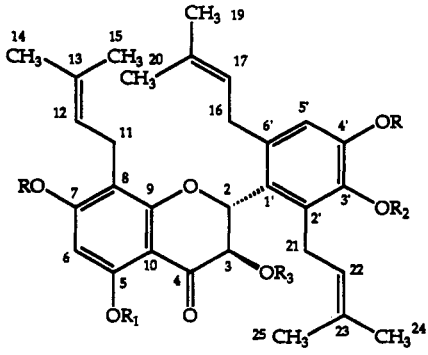
and ROBIN D. ROGERS

Department of Chemistry, Northern Illinois University, DeKalb, Illinois 60115

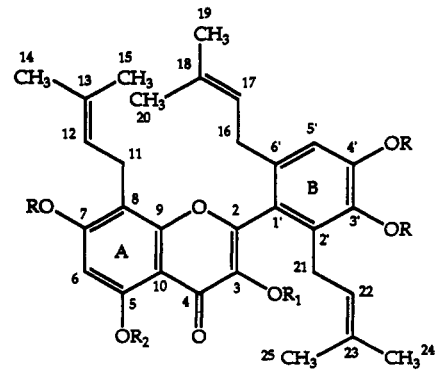
ABSTRACT.—EtOH extracts of *Petalostemum purpureum* demonstrated antimicrobial activity against bacteria and fungi. Bioassay-directed fractionation led to the isolation of petalostemumol [**1**] as the active constituent. Its structure was determined by a single crystal X-ray diffraction analysis. A minor component designated petalostemumol G [**3**] is believed to be an artifact of the isolation procedure. Its structure was confirmed by a single crystal X-ray analysis of its pentamethylether **2**. A number of derivatives of **1** and **3**, including the Me- and benzylethers and acetates, were prepared.

The genus *Petalostemum* (Fabaceae) has received relatively little phytochemical attention and only a few species have been studied. 2-(4-Hydroxybenzyl) malic acid has been found in *Petalostemum gattingeri* (1), the isoflavone, petalostetin, has been isolated from *Petalostemum candidum* (2,3), and some unspecified flavonoids have been found in *Petalostemum villosum* (4). Purple prairie clover, *Petalostemum purpureum* (Vent.) Rydb. has been used as a medicinal plant by North American Indians for treatment of heart trouble, diarrhea, measles, and pneumonia (5), but the chemistry and biological activity of this species have thus far not been investigated.

The EtOH extract of the root of *P. purpureum* showed antimicrobial activity using our standard assay (6–8). Solvent partitioning of the active EtOH extract between EtOAc and H_2O resulted in the activity being located in the EtOAc fraction. Partitioning of the active EtOAc fraction between *n*-hexane and 10% aqueous MeOH concentrated the antimicrobial activity in the aqueous MeOH fraction, which exhibited activity against *Staphylococcus aureus, Bacillus subtilis, Trichophyton mentagrophytes, Myobacterium intracellulare,* and *Cryptococcus neoformans*, and marginal activity against *Candida albicans*.



1 R=R_1=R_2=R_3=H
4 R=R_1=R_2=Me, R_3=H
5 R_1=R_3=H, R=R_2=Me
6 R=Me, R_1=R_2=R_3=H
9 R=R_1=R_2=Me, R_3=COMe



2 R=R_1=R_2=Me
3 R=R_1=R_2=H
7 R_1=R_2=Me, R_1=CH_2Bz
8 R=R_1=Me, R_2=H

There was no activity against Gram-negative bacteria or the filamentous fungus *Aspergillus*. Cc of the active EtOH fraction over Si gel 60 using toluene/EtOAc/HOAc mixtures resulted in seven pooled fractions.

Pooled fraction F-3 was active and yielded petalostemumol [**1**] as the active constituent as pale yellow plates, mp 179–180°, one spot on tlc and one peak by hplcms. A molecular formula of $C_{30}H_{36}O_7$ was determined by combustion analysis and ms. The ^{1}H- and ^{13}C-nmr spectra showed multiple sets of peaks, suggesting that the molecule existed in two forms in solution at room temperature. Heating a sample of **1** to 150° in DMSO collapsed the nmr spectrum into one set of peaks, which was consistent for a molecule ($C_{30}H_{36}O_7$) exhibiting restricted rotation. The combined spectral data suggested a 3-hydroxyflavonol nucleus with four phenolic OH groups and three isoprenyl groups. Careful crystallization provided a suitable crystal of petalostemumol for single crystal X-ray analysis, which showed the structure as indicated by **1**. An ORTEP (9) diagram of petalostemumol [**1**] is shown in Figure 1. The bonding parameters, the hydrogen bonding contact distances, and the crystal data are summarized in Tables 1 and 2.[1] The absolute configuration for **1** was determined as 2*R*,3*R* from cd data, which showed a positive Cotton effect at 335 nm and a negative Cotton effect at 300 nm (10,11).

A careful tlc examination of pooled fraction F-3 and mother liquor fractions of **1** revealed the presence of another compound with R_f very similar to that of **1** but which showed a slightly different color with the spray reagent. Careful flash chromatography of these fractions resulted in the isolation of additional **1** and a small amount of another flavonol derivative, designated petalostemumol G, mp 163–164°, which was unstable. The molecular formula was established as $C_{30}H_{34}O_7$ by hreims. Unlike the data for petalostemumol [**1**], the ^{1}H- and ^{13}C-nmr data for petalostemumol G were all one set of peaks at room temperature. Methylation with MeI produced a stable pentamethyl ether as yellow prisms (mp 97–98°). Attempts to relate petalostemumol G and **1** chemically

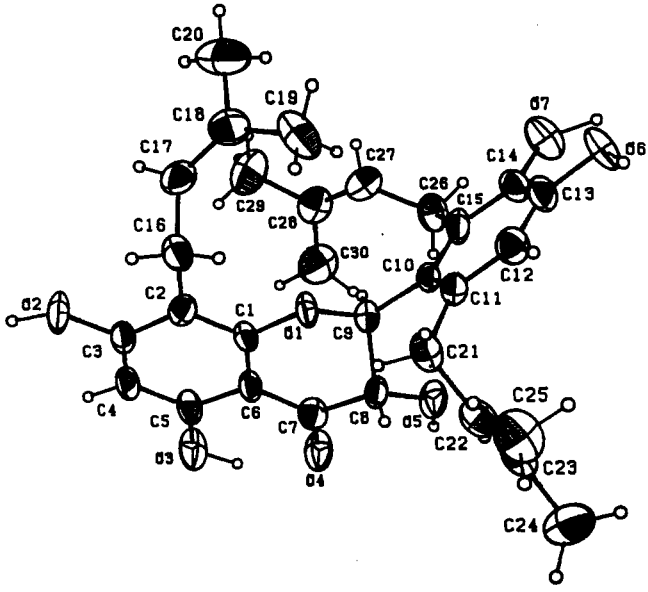


FIGURE 1. ORTEP diagram of petalostemumol **1**.

[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

TABLE 1. Crystal Data and Summary of Intensity Data Collection and Structure Refinement of Petalostemumol [**1**].

Compound	$C_{30}H_{36}O_7$
Color/Shape	Yellow/parallelepiped
Mol. wt	508.61
Space group	$P2_1$
Temperature	20°
Cell constants[a]	
a	8.835 (3) Å
b	9.325 (3) Å
c	16.293 (4) Å
β, deg	95.58 (2)
Cell vol, Å^3	1336
Formula units/unit cell	2
D_{calc}, g·cm^{-3}	1.26
μ_{calc}, cm^{-1}	0.96
Diffractometer/scan	Enraf-Nonius CAD-4/ω–2θ
Radiation, graphite monochromator	MoKα(λ=0.71073)
Max crystal dimensions, mm	0.20×0.33×0.38
Scan width	0.80+0.35 tan θ
Standard reflections	200; 080; 008
Decay of standards	±1%
Reflections measured	2665
2θ range, deg	$2 \leq 2\theta \leq 50$
Range of *h*, *k*, *l*	+10, +11, ±19
Reflections observed [$F_o \geq 5\sigma(F_o)$][b]	1836
Computer programs[c]	SHELX(12)
Structure solution	SHELXS(14)
No. of parameters varied	351
Weights	$[\sigma(F_o)^2+0.00\ 08\ F_o^2]^{-1}$
GOF	1.41
$R=\Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.057
R_w	0.068
R inverse configuration	0.057, 0.068
Largest feature final diff. map	0.4e$^-$ Å^{-3}

[a]Least-squares refinement of $[(\sin\theta)/\lambda]^2$ values for 25 reflections $\theta > 20°$.

[b]Corrections: Lorentz-polarization.

[c]Neutral scattering factors and anomalous dispersion corrections from "International Tables for X-ray Crystallography" (13).

were not successful, so a single crystal X-ray analysis of the pentamethyl ether was performed and showed the structure as indicated by **2**. An ORTEP (9) diagram of **2** is shown in Figure 2. The bonding parameters, the hydrogen bonding contact distances, and the crystal data are summarized in Tables 3 and 4.[1] Thus, structure **3**, the demethylated form of **2**, represents the minor flavonoid isolated by careful chromatography. Since **3** was isolated in very small amounts, it is probable that it is an artifact that arises from air oxidation of **1**. This is further supported by the observation that crude samples of **1**, upon exposure to air, show **3** by tlc.

In our initial attempts at trying to solve these structures a number of derivatives were made, but the results were somewhat confusing until the results of the X-ray analyses of **1** and **3** were obtained.

Methylation of **1** with MeI produced three different methylether derivatives characterized as **4**–**6**. The tetramethylether **4** was the major methylation product (12 hours), while **5** and **6** were minor products formed after relatively short reaction times (3–4 hours). The trimethylether **5** clearly showed three MeO signals in the ^{13}C nmr at

TABLE 2. Final Fractional Coordinates for Petalostemumol [**1**].

Atom	*x/a*	*y/b*	*z/c*	B(eqv)[a]
O-1	0.3136 (4)	0.7500 (6)	0.7275 (2)	2.05
O-2	0.0969 (5)	0.4880 (6)	0.9306 (2)	2.86
O-3	0.0642 (6)	0.9953 (6)	0.9342 (3)	3.40
O-4	0.1721 (6)	1.1296 (6)	0.8143 (3)	3.33
O-5	0.3422 (5)	1.1353 (6)	0.6874 (3)	2.89
O-6	0.7706 (5)	0.7839 (7)	0.4587 (2)	3.20
O-7	0.8841 (5)	0.8930 (8)	0.6001 (3)	3.65
C-1	0.2324 (6)	0.7481 (8)	0.7950 (3)	1.65
C-2	0.2062 (6)	0.6175 (8)	0.8285 (3)	2.05
C-3	0.1256 (6)	0.6176 (7)	0.8976 (3)	2.00
C-4	0.0816 (7)	0.7429 (9)	0.9349 (3)	2.43
C-5	0.1082 (6)	0.8723 (8)	0.8999 (3)	2.10
C-6	0.1833 (6)	0.8787 (8)	0.8275 (3)	1.91
C-7	0.2032 (7)	1.0103 (8)	0.7861 (3)	2.23
C-8	0.2721 (7)	1.0030 (7)	0.7053 (3)	2.07
C-9	0.3899 (6)	0.8837 (7)	0.7117 (3)	1.79
C-10	0.4862 (6)	0.8600 (7)	0.6410 (3)	1.72
C-11	0.4241 (6)	0.8025 (7)	0.5659 (3)	2.01
C-12	0.5193 (7)	0.7758 (8)	0.5058 (3)	2.28
C-13	0.6707 (7)	0.8065 (8)	0.5176 (3)	2.36
C-14	0.7325 (6)	0.8648 (8)	0.5915 (3)	2.32
C-15	0.6419 (6)	0.8910 (7)	0.6549 (3)	2.18
C-16	0.2606 (7)	0.4802 (7)	0.7932 (4)	2.25
C-17	0.4165 (7)	0.4376 (8)	0.8299 (4)	2.77
C-18	0.5425 (8)	0.4286 (8)	0.7912 (4)	2.97
C-19	0.5502 (9)	0.464 (1)	0.7015 (4)	4.17
C-20	0.6915 (9)	0.387 (1)	0.8355 (6)	4.69
C-21	0.2548 (6)	0.7735 (8)	0.5432 (3)	2.42
C-22	0.1845 (6)	0.8944 (8)	0.4933 (3)	2.43
C-23	0.1096 (6)	0.8901 (9)	0.4177 (3)	2.42
C-24	0.0445 (9)	1.0218 (9)	0.3772 (5)	3.81
C-25	0.0856 (9)	0.754 (1)	0.3682 (4)	3.68
C-26	0.7160 (7)	0.9500 (8)	0.7358 (3)	2.59
C-27	0.7152 (7)	0.8461 (8)	0.8071 (3)	2.54
C-28	0.6653 (7)	0.8661 (9)	0.8800 (3)	2.79
C-29	0.667 (1)	0.747 (1)	0.9435 (4)	4.15
C-30	0.596 (1)	1.004 (1)	0.9069 (4)	3.98

[a]$B\ (eqv)=4/3[a^2\beta_{11}+b^2\beta_{22}+c^2\beta_{33}+ab(\cos\gamma)\beta_{12}+ac(\cos\beta)\beta_{13}+bc\ (\cos\alpha)\beta_{23}]$.
[b]Isotropic refinement.

δ 59.9, 56.2, and 56.0. The chelated OH at C-5 was not methylated, as shown by ir and ^{1}H and ^{13}C nmr. The dimethylether also showed the presence of a C-5 chelated OH group, and two MeO signals in the ^{13}C nmr at δ 56.4 and 55.9 mandate structure **6**.

Acetylation of the tetramethylether **4** produced the expected acetate **9**, but benzylation of **4** (reflux, Me_2CO, 48 h) produced compound **7**, apparently formed by air oxidation of **4** followed by benzylation.

Methylation of petalostemumol G [**3**] produced, as the major product, the pentamethylether **2**. A minor methylation product contained four MeO groups as shown by the ^{1}H- and ^{13}C-nmr data; its structure is shown as **8**. The tetramethylether **8** showed two MeO signals near 60 ppm, thus requiring two MeO groups at C-3 and C-3′. A chelated phenolic OH signal at δ^H 12.78 ppm and a comparison of the chemical shift data in Tables 5 and 6 for **2** and **3**, respectively, provided convincing evidence for the tetramethylether as shown in **8**.

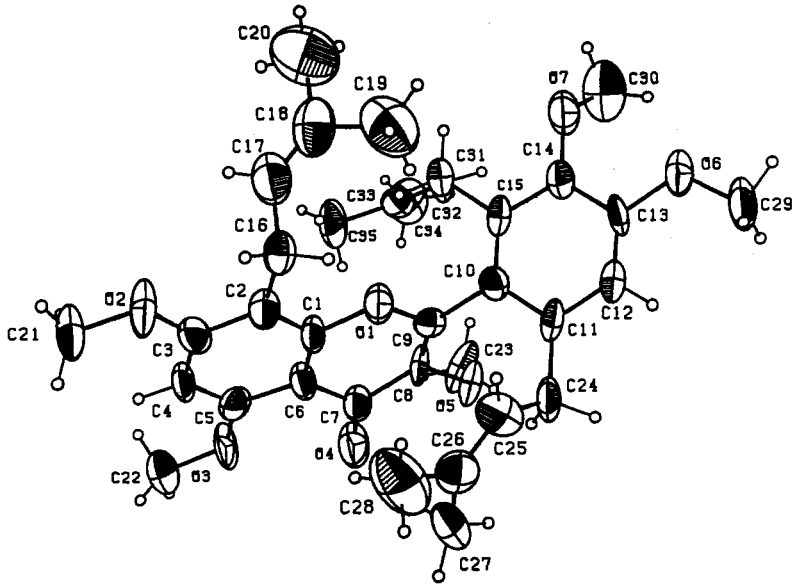


FIGURE 2. ORTEP diagram of petalostemumol G pentamethyl ether.**2**.

With the structures of petalostemumol [**1**], petalostemumol G [**3**], and their derivatives **2**, **4–9** established, the complete ^{1}H- and ^{13}C-nmr assignments were undertaken. Many of the assignments were straightforward based on multiplicity determinations and chemical shift arguments, but a number of 2D nmr experiments were necessary to complete these assignments. The pentamethyl ether [**2**] was chosen for the detailed nmr studies since it was most abundant and most stable. Once these assignments were established (Tables 5 and 7) the remaining assignments for **1**, **3–9** were relatively straightforward using strategies similar to those reported for **2**. These assignments are summarized in Table 6.

Petalostemunol [**1**], petalostemunol G [**3**], and the various derivatives **2**, **4–9** were evaluated for antimicrobial activity in the agar well-diffusion assay. For those compounds that exhibited activity, MIC values were determined by the twofold serial macrobroth dilution assay previously described (6–8). The results of these assays are summarized in Table 8. Petalostemunol [**1**], the most active of the compounds isolated, showed good activity against the Gram-positive bacteria *S. aureus* and *B. subtilis*, as well as moderate activity against the Gram-negative bacterium, *Escherichia coli*. Petalostemumol [**1**] showed only moderate activity against *Ca. albicans* and marginal activity against *Cr. neoformans*. None of the other compounds showed any remarkable activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined either on a Fisher-digital mp analyzer model 355 or in open capillary tubes with Thomas-Hoover capillary mp apparatus and are uncorrected. Ir spectra were recorded in KBr using a Perkin-Elmer 281B infrared spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were obtained on a VXR-300 spectrometer operating at 300 MHz and 75 MHz, respectively. The chemical shift values are reported in ppm, and the coupling constants are in Hz. Standard Varian pulse sequences were used for COSY, HETCOR, DEPTGL, APT, 2D-INADEQUATE, and NOESY experiments. Eims were obtained using an E.I. Finnigan model 3200 (70 eV ionization potential) with INCOS data system. Hreims were carried out at the University of Utah, Salt Lake City, Utah. Fab low and high resolution ms were carried out at the University of Kansas.

CHROMATOGRAPHIC CONDITIONS.—Tlc chromatographic analyses were carried out on precoated Si gel G-25 UV_{254} plates (Macherey-Nagel). Cc was carried out on Si gel 60 (230–400 mesh). Developed plates

TABLE 3. Crystal Data and Summary of Intensity Data Collection and Structure Refinement of Petalostemumol Pentamethylether [**2**].

Compound	$C_{35}H_{44}O_7$
Color/Shape	Colorless/parallelepiped
Mol. wt	576.74
Space group	*P1*
Temperature	22°
Cell constants[a]	
a	11.375 (2) Å
b	12.590 (4) Å
c	12.606 (3) Å
α, deg	88.22 (2)
β, deg	80.58 (1)
γ, deg	66.62 (2)
Cell vol, Å^3	1633.6
Formula units/unit cell	2
D_{calc}, g·cm^{-3}	1.17
μ_{calc}, cm^{-1}	0.87
Diffractometer/scan	Enraf-Nonius CAD-4/ω–2θ
Radiation, graphite monochromator	MoKα(λ=0.71073)
Max crystal dimensions, mm	0.15×0.23×0.40
Scan width	0.80+0.35 tan θ
Standard reflections	200; 030; 004
Decay of standards	±1%
Reflections measured	5754
2θ range, deg	$2 \leq 2\theta \leq 50$
Range of *h*, *k*, *l*	+13, ±14, ±15
Reflections observed [$F_o \geq 5\sigma(F_o)$][b]	1501
Computer programs[c]	SHELX (12)
Structure solution	SHELXS (14)
No. of parameters varied	379
Weights	$[\sigma(F_o)^2+0.002\ F_o^2]^{-1}$
GOF	1.05
$R=\Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.075
R_w	0.095
Largest feature final diff. map	0.3e$^-$ Å^{-3}

[a]Least-squares refinement of $[(\sin\theta)/\lambda]^2$ values for 25 reflections $\theta > 16°$.

[b]Corrections: Lorentz-polarization.

[c]Neutral scattering factors and anomalous dispersion corrections from "International Tables for X-ray Crystallography" (13).

were visualized under uv (short and long wavelength) and sprayed with 5% vanillin in concentrated H_2SO_4 followed by heating at 110°.

PLANT MATERIAL.—The fresh whole plants (6.6 kg) of *P. purpureum* were collected on a limestone ridge in Lee County near Tupelo, Mississippi, in the summers of 1988 and 1989. The whole plant material was air-dried (4.16 kg) and separated into the above-ground parts (1.36 kg) and roots (2.8 kg) before grinding. Voucher specimens (MISS 53,976–53,979) are on deposit at the Herbarium, University of Mississippi. The plants were identified by Dr. E.M. Croom Jr.

ANTIMICROBIAL ASSAYS.—The qualitative assays were performed using an agar well-diffusion assay. Antimicrobial activity was recorded as the width (in mm) of the zone of inhibition, measured from the edge of the agar well to the edge of the zone. The quantitative assay for determination of MIC was performed using a twofold serial dilution technique as previously described (6–8). The MIC, μg/ml, was recorded as the lowest concentration that prevented visible growth. The antifungal agent amphotericin B and the antibacterial agent streptomycin were included as positive controls in each assay.

EXTRACTION AND SOLVENT PARTITIONING.—The dried, ground root material (2 kg) was percolated at room temperature with 95% EtOH for nearly a week until very little residue was obtained upon evaporation. Evaporation of the combined EtOH percolate yielded 220 g of residue.

TABLE 4. Final Fractional Coordinates for Petalostemumol Pentamethylether [2].

Atom	x/a	y/b	z/c	B (eqv)
O-1	−0.4033 (5)	−0.8314 (5)	−0.2414 (4)	3.00
O-2	−0.1448 (6)	−1.1453 (5)	−0.4718 (5)	4.57
O-3	−0.1892 (6)	−0.7591 (5)	−0.5770 (4)	4.00
O-4	−0.3233 (7)	−0.5899 (6)	−0.4259 (5)	4.69
O-5	−0.4981 (6)	−0.5217 (5)	−0.2381 (4)	3.22
O-6	−0.7961 (6)	−0.6359 (6)	0.1874 (4)	4.38
O-7	−0.8624 (6)	−0.6981 (7)	0.0104 (5)	4.78
C-1	−0.321 (1)	−0.865 (1)	−0.3408 (8)	2.76
C-2	−0.270 (1)	−0.987 (1)	−0.3552 (8)	2.80
C-3	−0.190 (1)	−1.028 (1)	−0.4566 (9)	3.34
C-4	−0.162 (1)	−0.956 (1)	−0.5274 (9)	3.20
C-5	−0.213 (1)	−0.837 (1)	−0.5080 (8)	3.24
C-6	−0.295 (1)	−0.7875 (9)	−0.4076 (8)	2.70
C-7	−0.348 (1)	−0.664 (1)	−0.3765 (8)	2.85
C-8	−0.443 (1)	−0.6360 (9)	−0.2739 (8)	2.58
C-9	−0.4623 (9)	−0.7133 (9)	−0.2122 (7)	2.23
C-10	−0.548 (1)	−0.6957 (9)	−0.1052 (8)	2.49
C-11	−0.510 (1)	−0.6624 (9)	−0.0152 (8)	2.65
C-12	−0.592 (1)	−0.6463 (9)	0.0816 (8)	2.88
C-13	−0.707 (1)	−0.653 (1)	0.0911 (8)	3.03
C-14	−0.746 (1)	−0.6876 (9)	0.0030 (8)	2.72
C-15	−0.665 (1)	−0.7061 (9)	−0.0975 (8)	2.63
C-16	−0.304 (1)	−1.0654 (9)	−0.2796 (9)	3.15
C-17	−0.426 (2)	−1.077 (1)	−0.300 (1)	4.88
C-18	−0.523 (2)	−1.066 (1)	−0.235 (1)	4.70
C-19	−0.542 (2)	−1.037 (1)	−0.115 (1)	7.12
C-20	−0.642 (2)	−1.079 (2)	−0.256 (2)	8.24
C-21	−0.063 (1)	−1.193 (1)	−0.572 (1)	5.06
C-22	−0.103 (1)	−0.802 (1)	−0.6751 (9)	4.80
C-23	−0.585 (1)	−0.445 (1)	−0.301 (1)	5.17
C-24	−0.380 (1)	−0.653 (1)	−0.0213 (8)	3.25
C-25	−0.269 (1)	−0.767 (1)	−0.0476 (9)	4.10
C-26	−0.157 (1)	−0.783 (1)	−0.108 (1)	4.08
C-27	−0.123 (1)	−0.695 (1)	−0.164 (1)	6.06
C-28	−0.054 (2)	−0.906 (1)	−0.135 (1)	7.69
C-29	−0.754 (1)	−0.615 (1)	0.2839 (8)	5.17
C-30	−0.900 (2)	−0.767 (2)	0.068 (2)	5.72
C-30'[a]	−0.972 (2)	−0.620 (3)	0.046 (2)	5.57
C-31	−0.713 (1)	−0.7404 (9)	−0.1935 (8)	2.74
C-32	−0.790 (1)	−0.640 (1)	−0.2500 (9)	3.08
C-33	−0.775 (1)	−0.617 (1)	−0.3525 (9)	3.31
C-34	−0.869 (1)	−0.510 (1)	−0.396 (1)	5.69
C-35	−0.668 (1)	−0.695 (1)	−0.4360 (9)	4.99

[a]B (eqv)=A 50/50 disorder of methyl group C-30 was resolved.

The EtOH extract (220 g) was partitioned between EtOAc and H_2O (1 liter each). The aqueous layer was extracted six times (1 liter each) with EtOAc. The combined EtOAc layer was dried over anhydrous Na_2SO_4 and concentrated to dryness to give 150 g of residue which contained the antimicrobial activity. Partitioning of the active EtOAc residue between *n*-hexane and 10% aqueous MeOH (700 ml each) and evaporation of each layer yielded 8 g of *n*-hexane solubles and 100 g aqueous MeOH solubles.

CHROMATOGRAPHIC SEPARATION OF AQUEOUS MeOH EXTRACT.—A 10 g sample of the 10% aqueous MeOH extract was chromatographed over Si gel 60 (120 g, 230–400 mesh) using flash chromatography. The column was eluted initially with toluene by increasing percentages of EtOAc in toluene (each 100 ml of eluent contained 0.1 ml of HOAc). The fractions were analyzed by tlc and combined based on similarities in tlc patterns [EtOAc-hexane (3:7)]. A total of seven pooled fractions were obtained (designated F-1

TABLE 5. ^{1}H- and ^{13}C-nmr Assignments for **2**.[a]

Position	δ_C[b]	δ_H[c]
2	154.0 (0)	
3	142.4 (0)	
4	173.5 (0)	
5	160.3 (0)	
6	92.8 (1)	6.69 s
7	161.4 (0)	
8	109.7 (0)	
9	156.8 (0)	
10	110.3 (0)	
11	22.2 (2)	3.36 m
12	122.9 (1)	5.07 dd (8.0, 8.0)
13	131.6 (0)	
14	25.8 (3)	1.58 s
15	17.7 (3)	1.46 s
16	33.1 (2)	3.25 m
17	124.0 (1)	5.17 dd (7.1, 7.1)
18	132.3 (0)	
19	25.6 (3)	1.53 s
20	17.7 (3)	1.46 s
21	27.2 (2)	3.34 m
22	124.2 (1)	5.04 dd (7.5, 7.5)
23	131.1 (0)	
24	25.6 (3)	1.39 s
25	17.4 (3)	1.46 s
1′	124.0 (0)	
2′	135.5 (0)	
3′	146.0 (0)	
4′	154.5 (0)	
5′	112.0 (1)	6.89 s
6′	137.8 (0)	
3-OMe	59.6 (3)	3.68 s
5-OMe	55.8 (3)	3.95 s
7-OMe	56.4 (3)	4.01 s
3′OMe	60.5 (3)	3.80 s
4′-OMe	56.4 (3)	3.91 s

[a]Data obtained in Me_2CO-d_6.

[b]The number of attached hydrogens determined by APT and DEPTGL experiments.

[c]Multiplicity and J=Hz in parentheses.

through F-7): F-1 (0.33 g, 5% EtOAc-toluene), F-2 (0.15 g, 10% EtOAc-toluene), F-3 (0.53 g, 20% EtOAc-toluene), F-4 (1.6 g, 25% EtOAc-toluene), F-5 (1.5 g, 50% EtOAc-toluene, F-6 (1.4 g, EtOAc), F-7 (0.51 g, Me_2CO wash).

Petalostemumol [**1**].—Crystallization of fraction F-3 from 35% EtOAc/toluene and recrystallization from 20% Et_2O/*n*-hexane yielded 0.2 g of **1** as yellow plates: mp 179–180° [(R_f 0.24, EtOAc–*n*-hexane (3:7)]; $[\alpha]_D$ +6.4° (c=0.032, MeOH); eims *m/z* $[M]^+$ 508 (1%); uv (MeOH) λ max (log ε) 240 sh (4.25), 294 (4.18), (3.63); ir (KBr) γ max 3400, 1685, 1666, 1620, 1505 cm^{-1}; cd (MeOH, c=0.032) $[\theta]_{335}$ +7938, $[\theta]_{297}$ −23,178, $[\theta]_{264}$ + 3175; ^{1}H-nmr (DMSO, 150°) 5.48 (1H, d, 12.3, H-2), 4.30 (1H, d, 12.3, H-3), 6.08 (1H s, H-6), 3.15 (2H, m, H-11), 5.12 (1H, m, H-12), 1.70 (3H, s, H-14), 1.63 (3H, s, H-15), 3.53 (2H, m, H-16), 5.30 (1H, m, H-17), 1.63 (3H, s, H-19), 1.60 (3H, s, H-20), 3.35 (2H, m, H-21), 5.18)1H, m, H-22), 1.54 (3H, s, H-24), 1.60 (3H, s, H-25), 6.64 (1H, s, H-5′) 11.66 (1H, s, 5 OH); ^{13}C nmr see Table 6; calcd for $C_{30}H_{36}O_7$, C 70.84, H 7.13 (found C 70.75, H 7.14).

Petalostemumol G [**3**].—A 200-mg sample of the mother liquors of fraction F-3 was chromatographed over Si gel 60 (230–400 mesh) slurry packed in *n*-hexane. The column was initially eluted with 1000 ml of *n*-hexane followed by increasing percentages of EtOAc in *n*-hexane (with 1% HOAc added). Elution with

TABLE 6. ^{13}C-nmr Assignments for **1**, **3–9**.[a]

Carbon	1[b]	4[b]	5[b]	6[b]	9[d]	3[c]	7[c]	8[c]
C-2	79.8	79.1	80.0	80.9	76.6	149.7	154.6	154.8
C-3	70.5	71.1	71.0	72.1	72.4	138.4	137.9	140.3
C-4	197.3	190.0	199.0	199.2	184.3	177.1	173.7	179.8
C-5	160.7	159.9	162.0	163.3	160.5	160.0	160.3	161.3
C-6	95.6	90.8	92.6	93.1	90.0	98.5	92.8	95.3
C-7	164.0	162.6	165.0	166.4	163.0	161.5	161.4	163.2
C-8	107.0	109.3	107.8	109.3	108.7	106.9	109.7	108.3
C-9	159.5	159.8	158.5	159.9	158.0	156.1	156.7	158.0
C-10	100.2	103.6	101.0	101.7	158.0	104.9	110.1	106.4
C-11	20.5	20.6	20.8	21.7	21.0	22.0	22.1	21.8
C-12	122.3	121.9	122.4	123.2	122.2	123.0	122.9	122.7
C-13	129.2	130.6	130.9	130.3	130.3	131.6	131.5	132.7
C-14	24.4	24.3	25.3	25.8	25.3	25.6	25.7	25.7
C-15	16.8	16.7	17.6	17.8	17.6	17.5	17.6	17.6
C-16	31.3	31.4	32.3	33.2	31.4	32.5	33.0	33.1
C-17	123.8	123.1	122.4	124.5	123.1	123.9	123.9	124.0
C-18	128.8	129.3	130.2	131.1	131.3	131.8	132.4	132.4
C-19	24.4	24.2	25.3	25.6	25.2	25.8	25.5	25.7
C-20	16.8	16.7	17.6	17.8	17.5	17.5	17.6	17.5
C-21	25.1	24.9	25.5	26.7	25.2	26.8	27.1	25.8
C-22	122.7	123.4	122.6	125.6	123.8	124.3	124.2	124.0
C-23	128.9	129.1	129.6	128.7	130.3	129.1	131.0	131.3
C-24	24.4	24.2	25.3	25.6	25.2	25.6	25.5	25.5
C-25	16.6	16.7	17.6	17.6	17.1	17.5	17.3	17.4
C-1′	123.7	125.3	125.4	126.2	124.2	122.7	123.8	123.3
C-2′	129.9	134.8	135.3	131.5	136.0	131.7	135.6	135.4
C-3′	141.0	145.2	145.1	143.3	145.0	142.0	146.0	146.0
C-4′	144.4	151.5	152.0	147.6	152.2	146.7	154.5	154.7
C-5′	114.6	112.9	112.4	111.5	112.4	114.2	111.9	112.1
C-6′	132.2	136.7	137.5	133.5	137.9	133.5	137.9	137.9
3-OMe	—	—	—	—	—	—	—	60.1
5-OMe	—	55.4	—	—	55.8	—	56.3	—
7-OMe	—	55.7	56.2[e]	56.4	56.0	—	56.4	55.8
3′-OMe	—	59.1	59.9	—	59.8	—	60.5	60.5
4′-OMe	—	55.2	56.0[e]	55.9	55.2	—	55.8	56.5
Ac	—	—	—	—	168.5	—	—	—
Ac	—	—	—	—	20.0	—	—	—
CH_2Bz	—	—	—	—	—	73.8	—	
Bz	—	—	—	—	—	128.1,128.6 128.0,141.6	—	

[a]Multiplicity experiments were determined by the APT and DEPTGL pulse sequences.
[b]DMSO-d_6, 150°.
[c]Me_2CO-d_6.
[d]DMSO, room temperature.
[e]Signals may be reversed.

5% EtOAc/*n*-hexane gave 15 mg of **3** from 20% Et_2O/*n*-hexane, mp 162–163°. This compound was unstable even at 0° under N_2; therefore, the spectral data accumulated as rapidly as possible. R_f 0.33 [EtOAc–*n*-hexane (3:7)] (green with spray reagent); uv (MeOH) λ max (log ε) 345 (3.60), 290 sh, 275 sh, 257 (4.00); ir (KBr) γ max 3360, 2980, 2960, 2930, 1630, 1600, 1570, 1520 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) 12.23 (1H, s, 5-OH), 6.37 (2H, s, H-6,-5′), 3.38 (2H, m, H-11), 5.13 (3H, m. H-12,-17,-22), 1.52 (6H, s, H-19,-24), 1.43 (3H, s, H-20), 1.60 (3H, s, H-14), 1.46 (3H, s, H-15), 3.09 (1H, m, H-16), 3.23 (2H, m, H-21), 1.35 (3H, s, H-25); ^{13}C nmr see Table 6; hreims *m/z* $[M]^+$ 506.2296 ($C_{30}H_{34}O_7$ requires 506.2295) thermospray lc-ms $[M+1]^+$ 507. Conversion of petalostemumol G to the more stable methyl ethers alleviated the stability problem. An additional quantity (60 mg) of petalostemumol [**1**] was obtained from later fractions (20% EtOAc/*n*-hexane).

TABLE 7. Summary of 2D Experiments for the Unambiguous Assignments for **2**.

A-ring correlation		B-ring correlation		2D Experiment
H-6	C-5, C-10	H-16	C-6′	LR^{1}H-^{13}C (5Hz)
H-11	C-7, C-8, C-9	H-21	C-2′	
H-6	C-5, C-7	H-5′	C-1′, C-3′	LR^{1}H-^{13}C (10Hz)
H-6	C-8, C-10	H-5′	C-4′	
H-11	C-7, C-8	H-21	C-2′	
H-14	C-12, C-13	H-19	C-17	
H-15	C-13	H-20	C-17	
5-OCH_3	C-5	3′-OCH_3	C-3′	
7-OCH_3	C-7	4′-OCH_3	C-4′	
H-11	H-12	H-21	H-22	COSY
H-12	H-14, H-15			
C-8	C-11	C-6′	C-16	INADEQUATE
C-11	C-12	C-16	C-17	
C-13	C-14	C-18	C-20	
C-13	C-15	C-21	C-2′, C-22	
		C-23	C-24, C-25	
H-6	5-OCH_3	H-5′	H-16, 4′-OCH_3	NOESY
H-6	7-OCH_3	H-12	H-14	
H-11	H-15			
		H-17	H-19	
		H-22	H-24	

METHYLATION OF PETALOSTEMUMOL [**1**].—A 1.4 g sample of **1** was treated with 5 ml of MeI and 8 g of K_2CO_3 in 25 ml of Me_2CO under reflux for 4 h. The reaction suspension was evaporated to dryness and extracted with Et_2O. Evaporation of the Et_2O left 1.45 g of residue which was chromatographed over Si gel 60 (slurry packed in *n*-hexane). Elution with 500 ml of *n*-hexane followed by 750 ml of 10% Me_2CO in *n*-hexane gave 460 mg of fractions containing the dimethylether **6** and trimethylether **5** as a mixture. These ethers were separated by preparative tlc (Si gel G_{254}, 2mm) using multiple development [3×Me_2CO-toluene (3:17)]. Extraction of the separated bands with Et_2O-EtOAc (1:1) gave, after evaporation, 300 mg of **5** and 136 mg of **6**. Continued elution of the column with 20% Me_2CO–*n*-hexane gave 800 mg of the tetramethylether **4**. Refluxing the reaction mixture for 12 h produced **4** in high yield.

Tetramethylether **4** was obtained as an amorphous powder: $[\alpha]_D^{27}$ −30.6 (*c*=0.034, MeOH); uv (MeOH λ max (log ε) 290 (4.09), 320 nmm (3.73); ir (KBr) γ max 3010, 1670, 1600, 1580 cm^{-1}; ^{1}H nmr (DMSO, 150°) 5.42 (1H, d, 12.3 Hz, H-2), 4.63 (1H, d, 12.3, H-3), 6.39 (1H, s, H-6), 6.78 (1H, s, H-

TABLE 8. MIC Values For Petalostemumol and Related Derivatives.

Compound	MIC (μg/ml) vs. Organism[a]					
	Ca	Cn	Mi	Ec	Sa	Bs
1	12.5	25	6.25	6.25	3.12	0.78
2	25	—[b]	25	—	12.5	—
3	—	—	—	—	—	—
4	100	—	12.5	—	12.5	—
5	—	—	—	—	—	—
6	—	—	25	—	—	—
7	—	—	—	—	—	—
Amphotericin B	0.39	0.20	NT[c]	NT	NT	NT
Streptomycin	NT	NT	1.56	3.12	6.25	1.56

[a]Ca=*Candida albicans* NIH B311, Cn=*Cryptococcus neoformans* ATCC 32264, Mi=*Mycobacterium intracellulare* ATCC 23068, Ec=*Escherichia coli* ATCC 10536, Sa=*Staphylococcus aureus* ATCC 6538, Bs=*Bacillus subtilus* ATCC 6633.

[b]—5≥100 μg/ml.

[c]NT=Not tested due to insufficient activity in the qualitative assay.

5′), 3.89, 3.92, 3.75, 3.84 (3H, s, each OMe), 1.59, 1.69, 1.61, 1.51 (18H, s, Me-14, -15, -19, -20, -24, -25), 5.06, 5.12, 5.30 (3H, m, each H-12, -17, -22), 3.17, 3.54 (6H, m, H-11, -16, -21); ^{13}C-nmr see Table 6; hreims *m/z* $[M]^+$ 564.3055 ($C_{34}H_{44}O_7$ requires 564.3075); thermospray lc ms $[M+1]^+$ 565.

Trimethylether **5** was obtained as an amorphous solid: $[\alpha]_D^{27}$ −29.3 (*c*=0.030, MeOH), uv (MeOH) λ max log ε 232 (4.36), 290 (4.26), 342 (3.69); ir (KBr) γ max 3500, 1630, 1590, 1490 cm^{-1}; ^{1}H nmr (DMSO, 90°) 5.49 (1H, d, 12.1, H-2), 4.82 (1H, d, 12.1, H-3), 6.23 (1H, s, H-6), 6.78 (1H, s, H-5′), 3.84, 3.80, 3.77 (3H, s, each OMe) 5.01, 5.30, 5.10 (1H, m, each, H-12, -17, -21), 3.08, 3.63, 3.44 (2H, m, each, H-11, -16, -21), 1.65, 1.56, 1.61, 1.59, 1.50, (18H, s, Me-14, -15, -19, -20, -24, -25); ^{13}C nmr see Table 6; hreims *m/z* $[M]^+$ 550.2927 ($C_{33}H_{42}O_7$ requires 550.2919); thermospray lc ms $[M+1]^+$ 551.

Dimethylether **6** was obtained as a gummy residue; $[\alpha]_D^{27}$ +15.3 (*c*=0.028, MeOH); uv (MeOH) λ max (log ε) 235 (14.28), 290 (4.13), 343 (3.50); ir (KBr) γ max 3400, 1630, 1580, 1490 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) 5.57 (1H, d, 12.3, H-2), 4.94 (1H, d, 12.3, H-3), 6.18 (1H, s, H-6), 6.72 (1H, s, H-5), 3.90, 3.85 (3H, s, each, OMe), 3.17, 3.65 (6H, m, H-11, -16, -21), 5.09, 5.35, 5.24 (1H, m, each, H-12, -17, -22), 1.70, 1.59, 1.63, 1.54 (18H, s, Me-14, -15, -19, -20, -24, -25); ^{13}C nmr see Table 6; hreims *m/z* $[M]^+$ 536.2762 ($C_{32}H_{40}O_7$ requires 536.2763); thermospray lc ms $[M+1]^+$ 537.

ACETYLATION OF PETALOSTEMUMOL TETRAMETHYLETHER [**4**].—Treatment of 200 mg of **4** with Ac_2O (2 ml) in pyridine (15 ml) at room temperature for 24 h yielded 85 mg of the gummy acetate **9** after workup and chromatography over Si gel: R_f 0.53 [EtOAc-toluene (3:2)]; $[\alpha]_D$ −9.1° (*c*=0.0082 g/ml, MeOh); uv (MeOH) λ max (log ε) 233 (4.07), 286 (3.70), 327 (3.36), ir (KBr) γ max 1750, 1690, 1600, 1580 cm^{-1}; ^{1}H nmr (DMSO, 90°) 5.67 (1H, d, 12.6, H-2), 5.84 (1H, d, 12.6, H-3), 6.41 (1H, s, H-6), 6.79 (1H, s, H-5′), 3.88, 3.93, 3.71, 3.83 (3H, s, each, OMe), 3.16, 3.52 (6H total, m, H-11, -16, -21), 5.04, 5.24, (3H total, m, H-12, -17, -22), 1.67, 1.58, 1.60, 1.49 (18H total, s, Me-14, -15, -19, -20, -24, -25), 1.93 (3H, s, Ac); ^{13}C nmr see Table 6; hreims $[M]^+$ 606.3184 ($C_{36}H_{46}O_8$ requires 606.3180).

BENZYLATION OF PETALOSTEMUMOL TETRAMETHYLETHER [**4**].—A sample of 400 mg of **4** with benzyl chloride (6.5 ml) and K_2CO_3 (6.5 g) in Me_2CO (9 ml) was refluxed for 36 h. The suspension was cooled and extracted with Et_2O. The Et_2O solubles were chromatographed over Si gel 60 (slurry packed in hexane). Elution with 33% EtOAc in hexane yielded 40 mg of **7** as a gummy residue: uv (MeOH) γ max (log ε) 251 (4.34), 282 sh, 295 sh, 328 (3.84), ir (KBr) γ max 1640, 1600, 1570 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) 6.71 (1H, s, H-6), 6.89 (1H, s, H-5′), 3.97, 4.02, 3.78, 3.92 (3H, s, each, OMe), 5.09, 5.03, 5.14 (1H, m, each, H-12, -17, -22), 3.37, 3.28, 3.16 (2H, m, ea, H-11, -16, -21), 1.59, 1.43, 1.50, 1.37, 1.49 (18H total, Me-14, -15, -19, -20, -24, -25), 7.10–7.11 (5H, m, Bz), 5.00 (2H, s, CH_2 Bz); ^{13}C nmr see Table 6; hrms *m/z* $[M-CH_2CH=CMe_2]^+$ 583.2674 ($C_{36}H_{39}O_7$ requires 583.2724); thermospray lc ms $[M-1]^+$ 653 (100%), low resolution ms *m/z* 652 (1%).

METHYLATION OF PETALOSTEMUMOL G [**3**].—Treatment of a few mg of **3** with MeI, K_2CO_3 and Me_2CO under reflux for 8 h gave a residue after workup which showed one peak with *m/z* 577 $[M+1]^+$ consistent for five OMe. Tlc showed one spot with R_f 0.37 [Me_2CO-Et_2O (3:17)]. Because the quantity of **3** was limited and the separation from **1** was very difficult, the crude fractions containing **1** and **3** were methylated to the methyl ethers which were easier to separate. Treatment of 1.4 g fraction of **1** and **3** with MeI (5ml) and K_2CO_3 (8 g) in Me_2CO (25 ml) at reflux for 8 h yielded a residue which showed three spots by tlc. The residue was chromatographed over Si gel 60 (230–400 mesh) using flash chromatography and *n*-hexane as initial eluting solvent followed by Et_2O–*n*-hexane (1:1). Increasing percentages of Me_2CO in Et_2O (10, 20, 30, 40; 1000 ml each) gave three pooled fractions containing **4** (400 mg, identical with that prepared from pure **1**, the pentamethyl **2** (70 mg), and the tetramethyl **8** (10 mg) after further purification by centrifugal tlc (Chromatotron Si gel G_{254}, 20% Et_2O/*n*-hexane).

Pentamethyl ether **2** was crystallized from Me_2CO/Et_2O; mp 96–97; $[\alpha]^{27}_D$ +13.3 (*c*=0.026); uv (MeOH) λ max (log ε) 247 (4.30), 281 sh, 295 (3.12), 329 (4.12); ir (KBr) γ max 3400, 1685, 1460 cm^{-1}; ^{1}H mnr and ^{13}C nmr see Table 5; low resolution ms *m/z* $[M]^+$ 576 (1%); thermospray lc ms $[M+1]^+$ 577 (100%).

Tetramethylether **8** was obtained as a gummy residue: uv (MeOH) γ max (log ε) 254 sh, 261 (4.34), 285 sh, 303 sh, 336 (3.95) nm; ir (KBr) γ max 3308, 1650, 1590, 1480, 1450 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) 12.78 (1H, s, ex D_2O, chelated OH at C-5), 6.51 (1H, s, H-6), 6.89 (1H, s, H-5′), 3.73, 3.97, 3.80, 3.92 (3H, each, OMe at C-3, C-7, C-4′, C-3′), 3.33, 3.27 (6H, m, H-11, 16, 21), 5.08, 5.01, 5.15 (1H, m, H-12, 17, 22), 1.59, 1.44, 1.41, 1.50, 1.38, 1.48 (3H, each, Me-14, -15, -19, -20, -24, -25); ^{13}C nmr see Table 6; hrms *m/z* $[M-31]^+$ 531.2750 ($C_{33}H_{39}O_6$ requires 531.2736); thermospray lc ms $[M+1]^+$ 563.

ACKNOWLEDGMENTS

This work was supported in part by the National Institutes of Health, Division of AIDS, grant RO1-AI-27094 and contract NO1-AI-72683.

LITERATURE CITED

1. T.M. Harris and J.V. Hay, *J. Org. Chem.*, **38**, 4457 (1973).
2. D.K. Bhardwaj, R.K. Jain, A. Munjal, and A. Rani, *Indian J. Chem*, **21b**, 493 (1982).
3. S.J. Torrance, R.M. Weidhopf, J.J. Hoffmann, and J.R. Cole, *Phytochemistry*, **18**, 366 (1979).
4. E.W. Wollmann, L.J. Schermeister, and E.G. Schmiess, *Proc. N.D. Acad. Sci.*, **25**, 17 (1972).
5. Daniel E. Moerman, "Medicinal Plants of Native America," University of Michigan Museum of Anthropology, Ann Arbor, 1986, Vol. 1, p. 332.
6. C.D. Hufford, M.J. Funderbunk, J.M. Morgan, and L.W. Robertson, *J. Pharm. Sci.*, **64**, 789 (1975).
7. A.M. Clark, F.S. El-Feraly, and W.S. Li, *J. Pharm. Sci.*, **70**, 951 (1981).
8. J.R. Peterson, J.K. Zjawiony, S. Liu, C.D. Hufford, A.M. Clark, and R.D. Rogers, *J. Med. Chem.*, **35**, 4079 (1992).
9. C.K. Johnson, "ORTEP II. Report on ORNL-5138," Oak Ridge National Laboratory, Tennessee, 1976.
10. W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).
11. H. Takahashi, S. Li, Y. Harigava, and M. Onda, *Chem. Pharm. Bull.*, **36**, 1877 (1988).
12. G.M. Sheldrick, "SHELX76, A System of Computer Programs for X-ray Structure Determination as Locally Modified," University of Cambridge, England, 1976.
13. "International Tables for X-ray Crystallography," Kynoch Press, Birmingham, 1974, pp. 72, 99, 149.
14. G.M. Sheldrick, *Acta Crystallogr.*, **A46**, 467 (1990).

Received 3 February 1993