concentrated to give 2.132 g (96%) of white crystals. The product was recrystallized from dichloromethane–pentane at −78 °C to give 1.269 g of pure **18**. The mother liquors were separated by medium-pressure LC to give an additional 0.383 g of product for a total yield of 1.652 g (75%) of **18**. A portion of the product was recrystallized from methanol to give an analytical sample: mp 93.5–94.5 °C; IR (CCl_4) 1735 cm^{-1}; NMR (CCl_4) δ 1.12 (s, 6), 1.37–2.15 (m, 10), 2.22 (s, 2), 3.05 (s, 4).

Anal. Calcd for $C_{15}H_{22}OS_2$: C, 63.78; H, 7.85; S, 22.70. Found: C, 63.59; H, 7.79; S, 22.80.

5α,8β-Dimethyl-6-methylene-4,4-(ethylenedithio)tricyclo[6.3.0.0^{1,5}]undecane (19). To a stirred suspension of methyl triphenylphosphonium bromide (0.724 g, 2.03 mmol) in dry toluene (2 mL) was added 0.92 mL (2.01 mmol) of a 2.19 M solution of sodium *tert*-amylate in toluene. To the resultant yellow suspension was added a solution of ketone **18** (0.285 g, 1.01 mmol) in 1.5 mL of toluene, and the reaction was refluxed under nitrogen for 3 h. The cool reaction mixture was poured into water (30 mL) and was extracted with pentane (3 × 20 mL). The pentane layers were washed with saturated aqueous sodium chloride (30 mL), dried ($MgSO_4$), and concentrated. The crude product was taken up in THF (5 mL), 1 mL of methyl iodide was added, and the solution was stirred at room temperature for 2 h. The precipitated methyltriphenylphosphonium iodide was filtered, and the pentane was evaporated under reduced pressure to give 0.232 g (82%) of white crystals, mp 76.5–79.5 °C. The product was recrystallized from pentane at −78 °C to give an analytical sample: mp 81.5–82.0 °C; IR (CCl_4) 3080, 1642, 895 cm^{-1}; NMR ($CDCl_3$) δ 0.99 (s, 3), 1.30 (s, 3), 1.33–2.10 (m, 10), 2.20 (m, 2), 3.10 (m, 4), 4.92 (m, 1), 5.08 (m, 1).

Anal. Calcd for $C_{16}H_{24}S_2$: C, 68.51; H, 8.62; S, 22.86. Found: C, 68.74; H, 8.77; S, 22.82.

5α,6,8β-Trimethyl-4,4-(ethylenedithio)tricyclo[6.3.0.0^{1,5}]undec-6-ene (20). To a solution of exocyclic olefin **19** (0.627 g, 2.24 mmol) in dry dichloromethane (40 mL) was added anhydrous *p*-toluenesulfonic acid (0.39 g, 2.3 mmol). The reaction mixture was refluxed under nitrogen for 2 h. GC analysis of a worked up aliquot on a 3% OV-225 column at 210 °C after 1 h and again after 2 h showed a constant isomer ratio of 92:8. The reaction was washed with saturated aqueous sodium bicarbonate (30 mL), and the aqueous layer was extracted with ether (3 × 15 mL). The combined organic layers were washed with saturated aqueous sodium chloride (20 mL), dried ($MgSO_4$), and evaporated to give 0.63 g (101%) of a yellow oil, which slowly crystallized on standing. The semicrystalline product was recrystallized from pentane at −78 °C: mp 54–56 °C; IR (CCl_4) 850 cm^{-1}; NMR ($CDCl_3$) δ 1.10 (s, 3), 1.30 (s, 3), 1.68 (d, J = 1.5 Hz, 3), 1.17–2.37 (m, 10), 3.17 (s, 4), 4.87 (br s, 1).

Anal. Calcd for $C_{16}H_{24}S_2$: C, 68.51; H, 8.62; S, 22.86. Found: C, 68.77; H, 8.52; S, 22.88.

5α,6,8β-Trimethyltricyclo[6.3.0.0^{1,5}]undec-6-en-4-one (21). A stirred mixture of crude olefinic thioacetal **20** (102.6 mg, 0.366 mmol), methyl iodide (2.3 mL, 36.6 mmol), and powdered calcium carbonate (0.110 g, 0.80 mmol) in 80% aqueous acetonitrile (10 mL) was refluxed under nitrogen for 3 days in the dark. The cool reaction was poured into water (20 mL) and was extracted with ether (4 × 20 mL). The ether layers were washed with 2 N aqueous sodium hydroxide (25 mL), water (to neutrality), and saturated aqueous sodium chloride (20 mL), dried ($MgSO_4$), and evaporated to give 85.2 mg (114%) of crude product. The product was chromatographed on a 1-mm Chromatatron plate with 5% ether–hexane as the eluent to give 51.7 mg (69%) of endocyclic olefin **21** and 4.6 mg (6%) of exocyclic olefin **22**. Preparative GC of **21** (5% SE-30 at 150 °C) gave a white waxy solid: mp 84.5–85.0 °C (sealed capillary); IR (CCl_4) 3020, 1735, 847, 832 cm^{-1}; 250-MHz NMR ($CDCl_3$) δ 1.06 (s, 3), 1.17 (s, 3), 1.21–1.66 (m, 6), 1.61 (d, J = 1.34 Hz, 3), 1.85–1.91 (m, 1), 2.03–2.17 (m, 2), 2.37–2.54 (m, 1), 5.09 (br s, 1); ^{13}C NMR ($CDCl_3$) δ 12.3, 15.5, 22.4, 24.0, 28.7, 37.0, 38.6, 42.2, 56.6, 60.0, 65.4, 136.2, 138.7, 220.0; mass spectrum (70 eV), m/e (relative intensity) 204 (M^+, 39), 176 (17), 161 (14), 149 (37), 148 (100), 133 (25), 120 (60), 105 (34), 91 (27); exact mass calcd for $C_{14}H_{20}O$, 204.1514; found, 204.1509.

For exocyclic olefin **22**: IR (CCl_4) 1720, 1655, 908, 892 cm^{-1}; NMR ($CDCl_3$) δ 1.08 (s, 3), 1.17 (s, 3), 1.43–2.73 (series of m, 12), 4.78 (s, 1), 4.89 (s, 1); mass spectrum (70 eV), m/e (relative intensity) 204 (M^+, 100), 175 (62), 162 (67), 148 (57), 147 (50), 133 (52), 120 (43), 119 (91), 105 (54), 91 (45); exact mass calcd for $C_{14}H_{20}O$, 204.1514; found, 204.1513.

Acknowledgment. We thank David Moreland and Molecular Design Limited for their assistance with the MM2 calculations and the SPACFIL and ORTEP drawings.

Registry No. (±)-**1**, 71629-00-0; **4a**, 76251-18-8; **4b**, 76251-19-9; **5**, 33406-25-6; **6**, 76251-20-2; **8a**, 76251-21-3; **9**, 76251-22-4; **10a**, 76251-23-5; (±)-**10b**, 76251-24-6; (±)-**11**, 76251-25-7; (±)-**12**, 76251-26-8; (±)-**18**, 76251-27-9; (±)-**19**, 76251-28-0; (±)-**20**, 76251-29-1; (±)-**21**, 71718-85-9; (±)-**22**, 76251-30-4; ethylene dithiotosylate, 2225-23-2; 2,4-pentanedione, 123-54-6; ethyl 3-bromopropionate, 539-74-2; dimethyl 1,3-acetonedicarboxylate, 1830-54-2; 2,2-dimethylpropane-1,3-diol, 126-30-7; (±)-**3**, 76251-31-5.

Isolation and Structure Determination of Piptocarphins A–F, Cytotoxic Germacranolide Lactones from *Piptocarpha chontalensis*[1]

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Six novel sesquiterpenes, piptocarphins A–F, were isolated from *Piptocarpha chontalensis* Pall. Their structures were elucidated as a series of closely related germacranolide sesquiterpene lactones possessing an unusual conjugated enol lactone and an intramolecular hemiketal functionality on the basis of an extensive spectral analysis. All compounds exhibited cytotoxic activity against the 9KB human nasopharynx carcinoma cells. Piptocarphins A and C also showed borderline activity in the P-388 lymphoid leukemia system.

Piptocarpha chontalensis Pall. (family, Asteraceae; tribe, Vernonieae) is a small, flowering, leafy plant native to tropical America[2] which has not received phytochemical investigation. Our investigation of *P. chontalensis* was prompted by reproducible cytotoxic activity shown by ethanolic extracts in the KB in vitro cell system (human carcinoma of the nasopharanx).[3,4] Six, novel, cytotoxic,

(1) Paper 14 in the series "Potential Antitumor Agents". For paper 13 see: Cassady, J. M.; Abramson, D.; Cowall, P.; Chang, C.-j.; McLaughlin, J. L. *J. Nat. Prod.* **1979**, *42*, 427.

(2) Hoffman, O. In "Die Naturlichen Pflanzenfamilien"; Engler, A., Prantl, K., Eds.; W. Engelman: Leipzig, 1897; Vol. 4, Bands 4–5, pp 118–131.

(3) Significant in vitro activity is shown for crude extracts by an ED_{50} < 20 μg/mL and for pure compounds by ED_{50} < 4 μg/mL. Significant in vivo activity is indicated by a therapeutic index (T/C) ≥130. The protocols followed are detailed in ref 4.

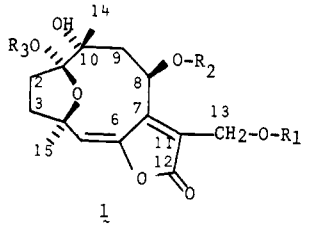
0022-3263/81/1946-1108$01.25/0

Table I. ^{1}H NMR Spectra of Piptocarphins A–F[c]

	A[a]	B	C[a]	D	E[a]	F
H-8	6.54 d, br (10.6)	6.54 d, br (9.5)	6.55 d, br (11)	5.46 ddd (12.0, 10.6, 2.1)	6.41 dd (9.2, 2.3)	6.58 d, br (9.5)
H-3a′	6.28 dq (1.25, 0.8)	7.01 m	6.30 m		6.27 m	6.27 m
H-5	5.91 s	5.90 s	5.88 s	5.87 s	5.91 s	5.82 s
H-3b′	5.68 dq (1.25, 1.5)		5.70 m		5.66 m	5.66 m
H-13a	5.30 d (12.9)	5.28 d (12.8)	4.65 d (13.5)[b]	4.99 d (12.9)	5.18 d (12.9)	4.58 d (12.2)
H-13b	4.89 d (12.9)	4.88 d (12.8)	4.61 d (13.5)[b]	4.79 d (12.9)	4.88 d (12.9)	4.30 d (12.2)
OH	4.14 s, br	4.18 s, br	4.13 s, br	4.79 s, br		4.07 br
OH	3.82 s, br	3.81 s, br	3.78 m, br	4.52 s, br	3.44 s, br	3.77 br
H-9a	2.63 dd (9.8, 10.6)	2.61 dd (9.5, 12.5)	2.63 dd (11, 15.6)		2.53 dd (15.8, 9.2)	
H-2α	2.44 dt (7.0, 12.5)		2.44 dt (7, 12.5)		2.31 m	1.9–2.75 m, c
H-9b	1.8–2.2 c	1.8–2.5 c	2.12 d (15.6)	1.78–2.76 c	1.7–2.2 m, c	
H-2β,3α,3β			1.7–2.2 c			
H-2″	2.07 s	2.05 s		2.08 s	2.05 s	
H-4′	1.94 dd (0.8, 1.5)	1.80 d, br (5)	1.95 dd (1.4, 1.0)		1.94 m	1.93 m
H-15	1.57 s	1.57 s	1.57 s	1.65 s	1.57 s	1.55 s
H-14	1.24 s	1.23 s	1.23 s	1.22 s	1.23 s	1.21 s
C-8 OH				6.12 d (12.0)		
C-13 OH			2.93 m, br			
H-5′		1.83 s			3.93 dq (9.2, 7.0)	
OCH_2CH_3					3.72 dq (9.2, 7.0)	3.57 q (7.0)
OCH_2CH_3					1.17 t (7.0)	1.21 t (7.0)

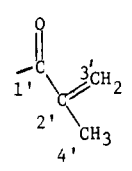
[a] Spectra recorded on both the 80- and 360-MHz instruments. [b] These signals are observable only in the 360-MHz spectrum. The chemical shifts reported correspond to the two inner lines of the AB pattern. [c] Chemical shifts are given in parts per million relative to Me_4Si. Coupling constants in parentheses are in hertz. Multiplicity: d, doublet; t, triplet; br, broadened; m, multiplet; s, singlet; c, complex.

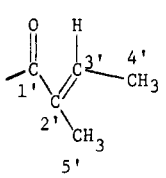
sesquiterpene lactones, piptocarphins A–F (**1a–f**), were isolated, and their structures were elucidated.



1

	R_1	R_2	R_3
1a	Ac	meth	H
1b	Ac	tig	H
1c	H	meth	H
1d	Ac	H	H
1e	Ac	meth	Et
1f	Et	meth	H
1g	H	H	H

meth = 

tig = 

Ac = 

Et = CH_2CH_3

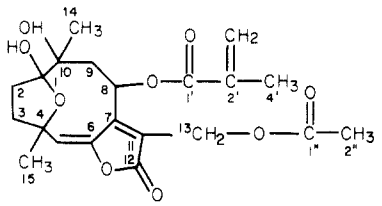
Fractionation of the ethanolic extract, with in vitro activity as a guide for subsequent purification steps, revealed that the cytotoxic activity concentrated in the chloroform extract of a chloroform–water partition. Rapid silica gel column chromatography of the chloroform-soluble material yielded several fractions possessing cytotoxic activity.[3,4] Four of these fractions were separately chromatographed on Sephadex LH-20 followed by low-pressure liquid chromatography (LC) using C-18 reversed-phase silica gel. Continuous UV monitoring of the eluent from the low-pressure LC column visualized the separation of the major and minor constituents. Six compounds were isolated as colorless, homogeneous oils which were characterized directly. The major component of the original extract, piptocarphin A, was isolated in sufficient quantity to allow a thorough spectroscopic analysis. Piptocarphins B–F show similar spectral properties and were characterized by direct comparison with piptocarphin A.

Piptocarphin A (**1a**, $C_{21}H_{26}O_9$) shows a parent ion (chemical-ionization mass spectroscopic analysis) of m/e 423 (MH^+), with m/e 405 ($MH^+ - H_2O$) as the base peak and losses of m/e 86 ($C_4H_6O_2$, methacrylate), m/e 60 ($C_2H_4O_2$, acetate), and two m/e 18 (H_2O). The ^{1}H NMR and ^{13}C NMR spectra (Tables I and II) and ^{1}H–^{1}H decoupling experiments confirm the presence of the acetate and methacrylate ester functionalities[5] and suggest that piptocarphin A is the diester of a sesquiterpenoid lactone. Consistent with this, the IR shows a major carbonyl absorption with a maximum at 1765 cm^{-1} (γ-lactone) and shoulders at 1745 (acetate) and 1720 cm^{-1} (conjugated ester). Interestingly, however, the strong absorption in the UV at 286 nm, indicating extended conjugation in the

(4) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1.

(5) Yoshioka, H.; Mabry, T. J.; Timmerman, B. N. "Sesquiterpene Lactones—Chemistry, NMR, and Plant Distribution"; University of Tokyo Press: Tokyo, 19[illegible].

Table II. ^{13}C NMR Chemical Shifts of Piptocarphin A

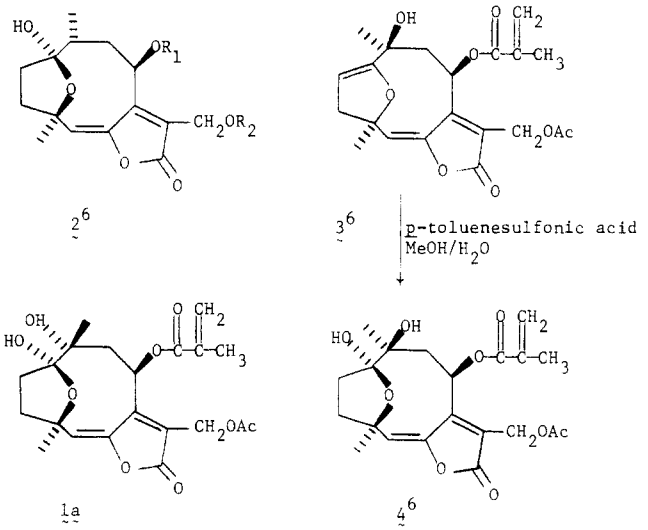


assignment	shift[a]	assignment	shift[a]
C-1''	170.3 (s)	C-10	77.9 (s)
C-12	166.8 (s, satd)	C-8	66.1 (d)
C-1'	165.7 (s)	C-13	55.5 (t)
C-6	149.5 (s, satd)	C-9[b]	37.9 (t)
C-7	143.9 (s, satd)	C-3[b]	37.4 (t)
C-2'	135.6 (s)	C-2[c]	32.0 (t)
C-11	130.9 (s, satd)	C-15	29.1 (q)
C-3'	127.2 (t)	C-14	25.2 (q)
C-5	126.5 (d)	C-2''	20.6 (q)
C-1	108.5 (s)	C-4'	17.9 (q)
C-4	82.1 (s)		

[a] Chemical shifts are given in parts per million relative to Me_4Si. Coupling and peak height information is given in parentheses (satd = partially saturated). [b] These assignments may be reversed. [c] The upfield methylene carbon signal at 32.0 ppm that was assigned to C-2 primarily resulted from the γ-shielding effect of the substituents at C-10.

molecule, is inconsistent with an α-methylene-γ-butyrolactone but is typical of a butadienolide.

A search of the literature revealed the recent isolation of a series of sesquiterpene lactones, **2** and **3**, from *Vernonia* species,[6] which have spectral properties similar to those of piptocarphins A–F. In addition, compound **3** was hydrated (*p*-toluenesulfonic acid in aqueous methanol) to give **4** whose IR, UV, and ^1H NMR data[7] are very similar to those of piptocarphin A (see Table III). From this spectral correlation, we could conclude that piptocarphin A has the same basic structure as the semisynthetic compound **4**. However, our IR and ^1H NMR analysis resulted in several discrepancies in the ^1H NMR peak assignments and differences in the stereochemical assignments at C-10.

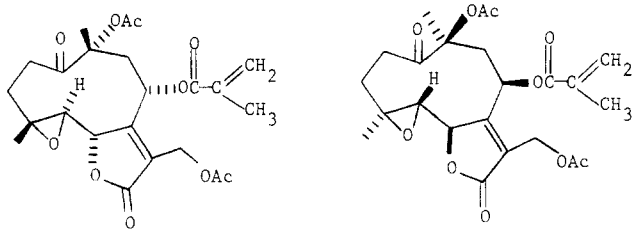


The placement of the acetate at C-13 and the methacrylate at C-8 is further supported on the basis of a comparison of ^1H NMR data for **1a** with glaucolide A (**5b**).[8]

Table III. ^1H NMR Data for Piptocarphin A (1a) and *Vernonia* Lactone 4

1a[a]		4[b]	
atom	shift, ppm (*J*, Hz)	atom	shift, ppm (*J*, Hz)
H-8α	6.54 d, br (10.6)	H-8α	6.48 s, br
H-3a'	6.28 dq (1.25, 0.8)	H-3a'	6.26 s, br
H-5	5.91 s	H-5	5.92 s
H-3b'	5.68 dq (1.25, 1.5)	H-3b'	5.67 dq
H-13a	5.30 d (12.9)	H-13	5.22 d (13)
H-13b	4.89 d (12.9)	H-13	4.95 d (13)
OH	4.14 s, br		
OH	3.82 s, br		
H-9α	2.63 dd (9.8, 10.6)	H-9α	2.61 dd (16, 6)
H-2α[c]	2.44 dt (7.0, 12.5)	H-9β[c]	2.45 ddd (16, 2)
H-9β,2β,3α,3β	1.8–2.2 c	H-2,3	2.0–2.2 m
H-2''	2.07 s	H-2''	2.06 s
H-4'	1.94 dd (0.8, 1.5)	H-4'	1.95 t
H-15[c]	1.57 s	H-14[c]	1.58 s
H-14[c]	1.24 s	H-15[c]	1.25 s

[a] Spectra recorded on 80- and 360-MHz spectrometers in $CDCl_3$. [b] Spectrum recorded on a 270-MHz spectrometer by Bohlmann et al.[6] [c] The assignments of the same positional peaks disagree.



5a Glaucolide A[9,10,11] 5b Glaucolide A[12]

Mabry and co-workers[13] established the placement of acetate in **5b** by reductive cleavage to a vinyl methyl group, consistent with the presence of an allylic acetate. Comparison of the ^1H NMR spectra of **5b** and **1a** shows very similar patterns for the methacrylate portion of the molecule. The C-13 methylene signal is shifted to a more downfield position (5.10 ppm, center of AB quartet) in **1a** as would be expected on the basis of the extended conjugation.

Decoupling experiments differentiated the upfield methylene protons. Irradiation of H-8 at 6.54 ppm resulted in the collapse of only the 2.63-ppm signal (dd C-9 proton) to a doublet with no noticeable effect on the 2.44-ppm (dt) signal or the methylene envelope at 2.0–2.3 ppm. The lack of coupling between H-8 and the other C-9 proton (located in the complex methylene envelope) indicates that the dihedral angle between these protons must be approximately 90°. The assignment of H-9β in *Vernonia* lactone **4**[6] to the 2.44-ppm signal (ddd, Table III) is unlikely since there are only two protons (H-8α and H-9α) adjacent to it. The 2.44-ppm signal (ddd) can thus be assigned to one of the C-2 methylene protons, deshielded because of its

(6) Bohlmann, F.; Brindopke, G.; Rastogi, R. C. *Phytochemistry* **1978**, *17*, 475.

(7) Neither an authentic sample nor spectra of **4** were available for a direct comparison. Therefore only the literature spectral data could be compared.

(8) Padolina, W. D.; Yoshioka, H.; Nakatani, N.; Mabry, T. J.; Monti, S. A.; Davis, R. E.; Cox, P. J.; Sim, G. A.; Watson, W. H.; Wu, I. B. *Tetrahedron* **1974**, *30*, 1161.

cis orientation to the hemiketal hydroxyl group.

The chemical shifts of the skeletal methyl groups in piptocarphin A, made on the basis of ^1H NMR analysis, are reversed from those in *Vernonia* lactone **4**. A 10–14% NOE enhancement of the C-5 olefinic proton is observed upon irradiation of the 1.57-ppm singlet while no appreciable effect is seen when the 1.24-ppm singlet is irradiated. This establishes the chemical shifts of the C-14 and C-15 methyl protons as 1.24 and 1.57 ppm, respectively, and is consistent with the assignments of methyl groups attached directly to the dihydro- and tetrahydrofuran rings in other sesquiterpene lactones.[9] The assignment is also confirmed by the 0.3-ppm downfield shift observed for the 1.24-ppm signal (compared with a 0.08-ppm shift for 1.57-ppm signal) in pyridine solution, indicating the close proximity of the C-14 methyl group to the hydoxyl functionalities.

The stereochemistry of *Vernonia* lactones **2–4** was based on the probable relationship to glaucolide A, enantiomer **5b**, whose relative configuration[10] has been determined by X-ray analysis.[8,11-14] The *Vernonia* lactones and piptocarphin A (**1a**) can be viewed as arising biosynthetically from a precursor of glaucolide A by intramolecular hemiketal formation between the C-4α oxygen and the C-1 carbonyl.[15] These molecules are drawn consistently with other hemiketal sesquiterpene lactones whose structures have been previously determined.[9] Careful examination of Dreiding models does verify the relationship between glaucolide A (**5a**) and piptocarphin A (**1a**; see the supplemental material for photographs of the Dreiding models of **1a** and **4**), which differ only in the configuration at C-8. The configuration of the methacrylate ester at C-8 is consistent with placement of H-8α in the deshielding cone of the tetrahydrofuran ring oxygen, thereby explaining its unusual downfield position (6.54 ppm) in the ^1H NMR.[15] An analogous deshielding effect on H-7α has previously been reported for the C-10 to C-3 hemiketal sesquiterpene lactones.[9b,c,f]

Finally, the relative stereochemistry at C-1,C-10 can be assigned on the basis of the strong intramolecular hydrogen bonding between the C-1 and C-10 hydroxyl groups which is observed in the IR spectrum of piptocarphin A at 3540 cm^{-1}. Intermolecular[16] hydrogen bonding is also seen at 3480 cm^{-1} but disappears upon dilution. Similar intramolecular hydrogen bonding is seen at 3570–3600 cm^{-1} for the rigid *cis*-cyclopentane-1,2-diol system.[17] However, in the *trans*-cyclopentane-1,2-diol isomer in which there is a dihedral angle of approximately 120° between adjacent hydroxyl groups, intramolecular hydrogen bonding cannot occur. Therefore, it follows that the orientation of the hydroxyls about C-1 and C-10 must be cis as shown in **1a** (1*S*,10*R*; see the photograph of the Dreiding model in the supplemental material). This conclusion does not exclude the possibility that **1a** has the 1*R*,10*S* configuration.

In addition to piptocarphin A, several other minor components were isolated from *P. chontalensis*, each showing the typical UV absorption at 280–285 nm and similar IR, mass, and ^1H NMR spectral characteristics. The structures of these compounds, designated piptocarphins B–F (**1b–f**), were assigned by comparison to piptocarphin A. For simplification of the discussion, the parent lactone alcohol will be called piptocarphol (**1g**).

Piptocarphin B (**1b**) was isolated as a minor constituent of column fractions which contain piptocarphin A and was separable from the latter only by low-pressure and LC reversed-phase chromatography. Piptocarphins A and B show almost indistinguishable chromatographic properties in other systems and similar spectral properties. The chemical ionization (CI) mass spectrum of piptocarphin B gives a molecular formula of $C_{22}H_{28}O_9$ (differing from that of piptocarphin A only by a methylene group) and a fragmentation pattern showing losses of *m/e* 60 (acetate), two *m/e* 18 (H_2O), and *m/e* 100 ($C_5H_8O_2$ = methacrylate + CH_2). ^1H NMR spectra of piptocarphins A and B are superimposable except for the signals corresponding to the unsaturated ester side chain. The methyl peaks at 1.83 ppm (s, br) and 1.80 ppm (d, br) and the olefinic proton signal at 7.01 ppm (m) are characteristic of tiglate esters. Therefore it follows that piptocarphin B is the tiglate ester of acetylpiptocarphol.

Piptocarphin C (**1c**, $C_{19}H_{24}O_8$), isolated from a more polar column fraction, has UV, IR, and ^1H NMR spectral characteristics similar to those of the previously isolated compounds, and its CI mass spectrum shows losses of *m/e* 86 (methacrylate) and two *m/e* 18 (H_2O). As would be expected from the CI mass spectrum and molecular formula, the acetate methyl group is absent from the ^1H NMR, and the desacetyl analogue **1c** of piptocarphin A is consistent with the spectral data. The C-13 methylene protons now appear at δ 4.63 (s, br), 0.5 ppm upfield relative to piptocarphins A and B, a shift consistent with that expected for an alcohol and its corresponding ester. The AB system corresponding to the C-13 protons in piptocarphins A and B has also coalesced to a broad singlet in piptocarphin C due to the absence of the bulky acetate group which gives free rotation about the C-11 to C-13 bond.

The expected ABX coupling pattern for the hydroxyl proton at C-13 is observed when the ^1H NMR spectrum is obtained with Me_2SO-d_6 as solvent. The C-13 methylene protons appear as an eight-line system in this spectrum resulting from the additional coupling of the four-line AB pattern with the hydroxyl proton. The hydroxyl proton resonance disappears upon addition of D_2O with the concurrent collapse of the methylene multiplet to an AB quartet.

Decoupling experiments at 360 MHz allow the positions of both C-9 methylene protons of piptocarphin C to be determined. The upfield H-9 resonance which is located at 2.12 ppm in the methylene envelope is therefore not

(9) (a) Raffauf, R. F.; Huang, P.-K. C.; LeQuesne, P. W.; Levery, S. B.; Brennan, R. G. *J. Am. Chem. Soc.* **1975**, *97*, 6884. (b) Herz, W.; Sharma, R. P. *J. Org. Chem.* **1975**, *40*, 3118. (c) Herz, W.; Blount, J. F. *Ibid.* **1978**, *43*, 1268. (d) Pal, R.; Kulshreshtha, D. K.; Rastogi, R. P. *Indian J. Chem., Sect. B* **1977**, *15B*, 208. (e) Pal, R.; Kulshreshtha, D. K.; Rastogi, R. P. *Ibid.* **1976**, *14B*, 77. (f) Herz, W.; Bhat, S. V. *J. Org. Chem.* **1972**, *37*, 906. (g) Chowdury, P. K.; Barua, N. C.; Sharma, R. P.; Thyagarajan, G.; Herz, W. *Ibid.* **1980**, *45*, 535. (h) Torrence, S. J.; Geissman, T. A.; Chedekel, M. R. *Phytochemistry* **1969**, *8*, 2381.

(10) The absolute configuration of many α-methylene sesquiterpene lactones is deduced by analogy to compounds whose structures have been previously determined by rigorous X-ray analysis. The 7α-proton provides a reference point in α-methylene lactones. With the endocyclic α,β-unsaturated lactones, however, this is not possible.

(11) Padolina, W. G.; Nakatani, N.; Yoshioka, H.; Mabry, T. J.; Monti, S. A. *Phytochemistry* **1974**, *13*, 2225.

(12) Watson, W. H.; Wu, I. B.; Monti, S. A.; Davis, R. E.; Mabry, T. J.; Padolina, W. G. *Cryst. Struct Commun.* **1974**, *3*, 697.

(13) Taylor, I. F., Jr.; Watson, W. H.; Betkowski, M.; Padolina, W. G.; Mabry, T. J. *Acta Crystallogr., Sect. B* **1976**, *B32*, 107.

(14) Betkowski, M.; Mabry, T. J.; Taylor, I. F., Jr.; Watson, W. H. *Rev. Latinoam. Quim.* **1975**, *6*, 191.

(15) If the **5a** enantiomer of glaucolide A had been used instead, an analogous argument could be made for a C-8β proton.

(16) Free hydroxyl would appear at approximately 3630 cm^{-1}.

(17) (a) Conley, R. T. "Infrared Spectroscopy"; Allyn and Bacon: New York, 1966; pp 119, 125. (b) Avram, M.; Mateescu, G. D. "Infrared Spectroscopy"; Wiley-Interscience: New York, 1966. (c) Kuhl, L. P. *J. Am. Chem. Soc.* **1954**, *76*, 4323.

observable in piptocarphins A and B due to the acetate methyl peak. In summary, our previous NMR assignments are thus confirmed, and structure **1c** can be assigned to piptocarphin C.

The molecular formula $C_{17}H_{22}O_8$ and comparable IR, UV, ^1H NMR, and CI mass spectra of piptocarphin D are all consistent with structure **1d**, the acetate ester of piptocarphol. The position of the H-8 resonance (5.46 ppm, ddd) and its coupling with the proton at 6.12 ppm (d, exchangeable with D_2O) support the placement of the free hydroxyl on C-8. The observed 1.1-ppm upfield shift of H-8α relative to piptocarphins A and B would also be expected for the corresponding C-8 alcohol. Interestingly, the signals (the pair of doublets corresponding to the AB system) of the C-13 methylene protons are less separated. The absence of the bulky ester group on C-8 apparently allows greater freedom of rotation about the C_{11}–C_{13} bond.

Piptocarphin E ($C_{23}H_{30}O_9$) shows IR and UV spectra analogous to those of the compounds previously discussed but exhibits a much different CI mass spectral fragmentation pattern. The parent ion occurs at m/e 451 (MH^+) with a base peak of m/e of 406 corresponding to loss of 46 mass units (C_2H_6O, ethanol) and with smaller fragments resulting from losses of acetate, methacrylate, and H_2O. The mass spectral data are consistent with the structure of piptocarphin A in which one of the hydroxyl protons has been substituted with an ethyl group. Indeed, the ^1H NMR spectrum (Table I) confirms this structure, showing resonances comparable to piptocarphin A but with additional signals typical of an ethyl ether group. This group must replace either the C-1 or C-10 hydroxyl proton. The ^1H NMR was obtained in pyridine solution in order to observe the anisotropic deshielding effect caused by the association of pyridine solvent molecules with the hydroxyl group. Downfield shifts are seen for the C-10 methyl group (1.23 → 1.42 ppm, $\Delta\delta$ = 0.19 ppm) and for H-9 (2.53 → 2.79 ppm, $\Delta\delta$ = 0.26 ppm), thus indicating the presence of an adjacent hydroxyl group of C-10. If the ethoxy group had instead been substituted at C-10, leaving a free hemiketal hydroxyl on C-1, the deshielding effect would have been expected for the C-2 proton. The C-2 proton, however, is unaffected. Thus structure **1e** can be assigned to piptocarphin E.

Piptocarphin F exhibits spectral properties similar to those of the other *Piptocarpha* lactones. Its molecular formula ($C_{21}H_{28}O_8$) and ^1H NMR spectrum, which lacks the acetate peak and contains instead the ethoxyl resonances, indicate that piptocarphin F is piptocarphyl methacrylate with an ethyl substituent replacing one of the hydroxyl protons. The position of the ethoxyl group must still be determined.

The CI mass spectral fragmentation pattern contains a base peak of $MH^+ - H_2O$ analogous to piptocarphins A–D rather than to piptocarphin E, which has the ethyl group on the hemiketal oxygen and shows a base peak of $MH^+ - C_2H_6O$. This suggests that the ethoxy group of piptocarphin F is not located on C-1. The ^1H NMR taken in Me_2SO-d_6 further allows the placement of the group on C-13. As seen previously in piptocarphin C, a C-13 hydroxymethylene group appears as an eight-line upfield multiplet and a downfield triplet. This pattern, however, is not seen for piptocarphin F. The corresponding downfield triplet is not observed in the spectrum, and the C-13 methylene multiplet is unaffected by proton exchange with D_2O, thus contraindicating the presence of a CH_2OH moiety in the molecule. Therefore it follows that the ethoxy group is located on C-13, and piptocarphin F can be assigned structure **1f**.

The presence of ethoxy substituents in natural products is rare even though an ethyl functionality on the hemiketal hydroxyl oxygen has been reported for phantomolin, a sesquiterpene lactone isolated from *Elephantopus mollis* (Vernonieae).[18] However, our use of ethanol as the original extraction solvent introduces the possibility that piptocarphins E and F may be artifacts of the isolation procedure.

As indicated in the Experimental Section, piptocarphins A–F show cytotoxicities ranging from 0.03 to 7 μg/mL, the most active being piptocarphin E. The α-methylene-γ-butyrolactone has been proposed to be a requirement for significant biological activity, this activity being enhanced by the presence of an OH or *O*-acyl group adjacent to the lactone ring or by other reactive functionalities present in the molecule.[19] One theory to account for the activity is that these compounds serve as potential nonclassical alkylating agents for nucleophilic groups on important biological macromolecules.[20] It is therefore interesting to see the *Piptocarpha* lactones, a series of endocyclic α,β-unsaturated butyrolactones, showing cytotoxicity of similar magnitude to that of related exocyclic compounds.[21] Even though piptocarphol does not contain the exocyclic methylene group conjugated to the lactone carbonyl, an extended dienone system is present in the molecule which could undergo a conjugate addition reaction with nucleophiles. However, this position is highly hindered, and substitution on the methylene carbon has been shown to reduce the cytotoxicity of α-methylene-γ-butyrolactones.[22] It is worthy of note that piptocarphins C and D, the deacetyl and demethacrylyl compounds, appear to possess greater cytotoxic activity than the corresponding esters. These compounds possess a free allylic hydroxyl group, suggesting the importance of this functionality to in vitro activity.

Preliminary testing in vivo against P-388 lymphoid leukemia[3,4] shows dosage-dependent toxicity and slight activity for piptocarphin A (maximum T/C = 122 at 4.6 mg/kg) and piptocarphin C (T/C = 131 at 10 mg/kg). Examination of the structures shows the presence of a novel conjugated dienone system and three allylic oxygen functionalities. The observed borderline activity may, however, result from the reactive ketal center and the C-13 allylic acetoxy/hydroxy functionality, as evidenced by the occurrence of piptocarphins E and F. If these compounds are artifacts of the extraction/isolation procedure, the high reactivity of these moieties toward the weak nucleophil ethanol would be demonstrated. Further work is underway to evaluate the importance of these sites to the biological activity.

Experimental Section

Extraction and Fractionation. *Piptocarpha chontalensis*[23] was extracted, partitioned, and fractionated by using in vitro cytotoxic activity against 9KB cells[3,4] to guide the fractionation

(18) Lee, H.-H.; Ibuka, T.; Huang, H.-C.; Harris D. L. *J. Pharm. Sci.* **1977**, *64*, 1077.
(19) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.* **1971**, *14*, 1147.
(20) Kupchan, S. M.; Fessler, D. C.; Eakin, M. A.; Giacobbe, T. J. *Science* **1970**, *168*, 376. Lee, K.-H.; Eng-Chun Mar, I. H. H.; Starnes, C. P.; ElGabaly, S. A.; Waddell, T. G.; Hadgraft, R. I.; Ruffner, C. G.; Weidner, I. *Ibid.* **1977**, *196*, 533.
(21) Kupchan, S. M.; Ashmore, J. W.; Sneden, A. T. *J. Pharm. Sci.* **1978**, *67*, 865.
(22) Howie, G. A.; Manni, P. E.; Cassady, J. M. *J. Med. Chem.* **1974**, *17*, 840. Howie, G. A.; Stamos, I. K.; Cassady, J. M. *Ibid.* **1976**, *19*, 309.
(23) The dried leaf material of *Piptocarpha chontalensis* (collected in 1976 by Dr. José Saenz-Renauld in Cost Rica) was supplied by the Economic Botany Laboratory of the United States Department of Agriculture (USDA), in accordance with the program developed with the USDA by the Developmental Therapeutics Program, NCI.

procedure. The ground leaf material (5.5 kg) was extracted with 95% EtOH (67 L). The extract was concentrated in vacuo to a brown tar (469 g) of which a 154-g aliquot was partitioned between $CHCl_3$ (4 L) and H_2O (2 L). The $CHCl_3$ solubles were chromatographed on a column of SilicAR CC-7 (2 kg, 7 cm × 100 cm) with $CHCl_3$ and MeOH–$CHCl_3$ mixtures as eluent. Fractions (300 mL) were collected, analyzed by TLC, combined appropriately, and evaporated to yield green tars: fraction A (21.8 L of $CHCl_3$, 48.5 g), B (7 L of $CHCl_3$ and 8 L of 1–2% MeOH–$CHCl_3$, 1.8 g), C (2 L of 2% MeOH–$CHCl_3$, 6.1 g), D (0.9 1 2% MeOH–$CHCl_3$, 14.7 g). Fractions B–D and F possessed significant in vitro activity and were further fractionated to isolate the active constituents. Each of these fractions showed major components which fluoresced on TLC, and the cytotoxic activity conveniently followed these compounds through subsequent separation procedures.

Isolation of Piptocarphins A and B. Because the cytotoxic activity of the fractions appeared to be sensitive to silica gel chromatography, milder separation methods were utilized. Column fraction C was applied to a Sephadex LH-20 column (3.5 cm × 45 cm) prepared in $CHCl_3$/hexane/MeOH (50:50:2) and eluted isocratically; fractions (3 mL) were collected slowly and combined according to TLC results. Repetitive Sephadex chromatography resulted in a KB-active, green, foamy solid (2.9 g) which appeared by TLC analysis to be one major component but by CI mass spectroscopy proved to be a mixture of predominantly two compounds. Small aliquots (30–40 mg) of this solid were then dissolved in $CHCl_3$ and applied to a low-pressure, C-18, reversed-phase column[24] (Lobar type, size B, 2.5 cm × 31 cm, 70–100 psi) equilibrated in MeOH/CH_3CN/H_2O (35:15:50). Continuous UV monitoring of the eluent was used to visualize the emergence of piptocarphins A and B (**1a,b**) from the column, fractions being collected by hand corresponding to the major regions of the elution peaks. Piptocarphins A and B were isolated as colorless oils which produced flocculent solid from CH_2Cl_2–hexane and Et_2O–hexane.

Piptocarphin A (**1a**) was isolated in overall yield of 0.12%:[25] ED_{50} 7.80 μg/mL; T/C 122 (4 mg/kg); UV (MeOH) λ_{max} 286 nm (log ε 4.68); IR (KBr) ν_{max} 3470, 1765, 1745, 1720, 1635 cm^{-1}; high-resolution EI mass spectrum, m/e 422.159 (calcd for $C_{21}H_{26}O_9$, 422.158); CI mass spectrum, m/e 423 (MH^+), 405 (100%, $MH^+ - H_2O$), 387 ($MH^+ - 2\ H_2O$), 363 (MH^+ – HOAc), 345 (MH^+ – HOAc – H_2O), 337 ($MH^+ - C_4H_6O_2$), 319 ($MH^+ - C_4H_6O_2 - H_2O$), 301 ($MH^+ - C_4H_6O_2 - 2\ H_2O$), 277 ($MH^+ - C_4H_6O_2$ – HOAc), 259 ($MH^+ - C_4H_6O_2$ – HOAc – H_2O); ^1H NMR ($CDCl_3$)[26] δ 6.54 (d, br, 10.6, H-8), 6.28 (dq, 0.8, 1.25, H-3a′), 5.91 (s, H-5), 5.68 (dq, 1.5, 1.25, H-3b′), 5.30 (d, 12.9, H-13a), 4.89 (d, 12.9, H-13b), 4.14 (s, br, OH), 3.82 (s, br, OH), 2.63 (dd, 9.8, 10.6, H-9a), 2.44 (dt, 7.0, 12.5, H-2α), 1.8–2.2 (c, H-2α,3α,3β,9β), 2.07 (3 H, s, H-2″), 1.94 (3 H, dd, 0.8, 1.5, H-4′), 1.57 (3 H, s, H-15), 1.24 (3 H, s, H-14); ^1H NMR (pyridine-d_5) δ 6.35 (s, br, H-23a′), 6.3 (m, br, H-8), 6.2 (s, H-5), 5.6 (s, br, H-3b′), 5.2 (s, 2 H, H-13a,b), 4.99 (s, br, OH), 3.1–2.2 (c, br, 6 H, H-2,3,9), 1.97 (s, 6 H, H-4′, H-2″), 1.65 (s, 3 H, H-15), 1.54 (s, 3 H, H-14); ^{13}C NMR ($CDCl_3$) δ 170.3 (s, C-1″), 166.8 (s, C-12), 165.7 (s, C-1′) 149.5 (s, C-6), 143.9 (s, C-7), 135.6 (s, C-2′), 130.9 (s, C-11), 127.2 (t, C-3′), 126.5 (d, C-5), 108.5 (s, C-1), 82.1 (s, C-4), 77.9 (s, C-10), 66.1 (d, C-8), 55.5 (t, C-13), 37.9 (t, C-9 or C-2), 37.4 (t, C-2 or C-9), 32.0 (t, C-3), 29.1 (q, C-15), 25.5 (q, C-14), 20.6 (q, C-2″), 17.9 (q, C-4′).

Piptocarphin B (**1b**) was isolated in overall yield of 0.03%: ED_{50} 5.9 μg/mL; UV (MeOH) λ_{max} 288 nm (log ε 4.13); IR (KBr) ν_{max} 3480, 1765, 1745 (sh), 1715, 1645 cm^{-1}; high-resolution EI mass spectrum, m/e 436.176 (calcd for $C_{22}H_{28}O_9$, 436.173); CI mass spectrum, m/e 437 (MH^+), 419 (100%, $MH^+ - H_2O$), 401 ($MH^+ - 2\ H_2O$), 377 (MH^+ – HOAc), 359 (MH^+ – HOAc – H_2O), 337 ($MH^+ - C_5H_8O_2$), 319 ($MH^+ - C_5H_8O_2 - H_2O$), 301 ($MH^+ - C_5H_8O_2 - 2\ H_2O$), 277 ($MH^+ - C_5H_8O_2$ – HOAc), 259 ($MH^+ - C_5H_8O_2$ – HOAc – H_2O); ^1H NMR ($CDCl_3$) δ 7.01 (m, H-3a′), 6.54 (d, br, 9.5, H-8), 5.90 (s, H-5), 5.28 (d, 12.8, H-13a), 4.88 (d, 12.8, H-13b), 4.18 (s, br, OH), 3.81 (s, br, OH), 2.61 (dd, 9.5, 12.5, H-9a), 1.8–2.4 (5 H, c, 2 H-2, 2 H-3, H-9b), 2.06 (3 H, s, H-2″), 1.83 (3 H, s, H-5′), 1.80 (3 H, d, 5.0, H-4′), 1.57 (3 H, s, H-15), 1.23 (3 H, s, H-14).

Isolation of Piptocarphin C. Fraction F was chromatographed on Sephadex LH-20 as above. One fraction appeared to have only one major component and was applied to the low-pressure LC column which had been reequilibrated in MeOH/CH_3CN/H_2O (55:10:35). Isocratic elution separated piptocarphin C (**1c**) from a closely following, minor component in overall yield of 0.014% as a colorless oil: ED_{50} 0.5 μg/mL; T/C 131 (10 mg/kg); UV (MeOH) λ_{max} 283 nm (log ε 4.25); IR (KBr) λ_{max} 3480, 1760, 1720, 1635 cm^{-1}; high-resolution EI mass spectrum, m/e 380.149 (calcd for $C_{19}H_{25}O_8$, 380.147); CI mass spectrum, m/e 381 (MH^+), 363 (100%, $MH^+ - H_2O$), 345 ($MH^+ - 2\ H_2O$), 295 ($MH^+ - C_4H_6O_2$), 277 ($MH^+ - C_4H_6O_2 - H_2O$), 259 ($MH^+ - C_4H_6O_2 - 2\ H_2O$); ^1H NMR ($CDCl_3$) δ 6.55 (d, br, 11, H-8), 6.30 (m, H-3a′), 5.88 (s, H-5), 5.70 (m, H-3b′), 4.65 (d, 13.5, H-13a), 4.61 (d, 13.5, H-13b), 4.13 (s, br, OH), 3.78 (m, br, OH), 2.93 (m, br, C-13 OH), 2.63 (dd, 11, 15.6, H-9a), 2.44 (dt, 7.0, 12.5, H-2α), 2.12 (d, 15.6, H-9b), 1.8–2.2 (3 H, c, H-2β, 2 H-3), 1.95 (3 H, m, H-4′), 1.57 (3 H, s, H-15), 1.23 (3 H, s, H-14); ^1H NMR (Me_2SO-d_6) δ 6.20 (s, br, H-3a′), 6.14 (s, H-5), 5.9 (m, br, H-8), 5.74 (m, H-3b′), 5.29 (s, OH, exchangeable with D_2O), 5.2 (m, br, OH, exchangeable with D_2O), 5.08 (t, 5.5, C-13 OH, exchangeable with D_2O), 4.30 (dd, 12.9, 5.5, H-13a; d after D_2O exchange), 4.13 (dd, 12.5, 5.5, H-13b; d after D_2O exchange), 2.46–2.16 (m, br, 6 H-2,3,9), 1.92 (s, 3 H, H-4′), 1.52 (s, 3 H, H-15), 1.14 (s, 3 H, H-14).

Isolation of Piptocarphin D. Repetitive column chromatography of fraction D on Sephadex LH-20 by using the procedure detailed above resulted in a fraction containing one component by TLC. Aliquots of 10 mg were applied to the low-pressure LC column equilibrated in MeOH/CH_3CN/H_2O (55:10:35). Isocratic elution gave piptocarphin D (**1d**) as a colorless oil: overall yield 0.0025%; ED_{50} 2.2 μg/mL; UV (MeOH) λ_{max} 280 nm (log ε 4.71); IR (KBr) ν_{max} 3450, 1765, 1740 (sh), 1640, 1225 cm^{-1}; high-resolution EI mass spectrum, no M^+ was observed, $M^+ - H_2O$, m/e 336.124 (calcd for $M^+ - H_2O$, $C_{17}H_{20}O_7$, 336.121), 319 ($MH^+ - 2\ H_2O$), 301 ($MH^+ - 3\ H_2O$), 295 (MH^+ – HOAc), 277 (MH^+ – HOAc – H_2O), 259 (MH^+ – HOAc – $2\ H_2O$); ^1H NMR ($CDCl_3$) δ 6.12 (d, 12.0, C-8 OH, exchangeable with D_2O), 5.87 (s, H-5), 5.46 (dd, 12.0, 10.6, 2.1, H-8), 4.99 (d, 12.9, H-13a), 4.79 (d, 12.9, H-13b), 4.79 (s, br, OH, exchangeable with D_2O), 4.52 (s, br, OH, exchangeable with D_2O), 1.78–2.76 (6 H, c, 2 H-2, 2 H-3, 2 H-9), 2.08 (3 H, s, H-2″), 1.65 (3 H, s, H-15), 1.22 (3 H, s, H-14).

Isolation of Piptocarphins E and F. Fraction B, higher running on silica gel TLC than piptocarphins A–D, was chromatographed on Sephadex LH-20 as above to remove the pigment material. Two column fractions, I and II (224 and 370 mg, respectively), showing significant cytotoxic activity were obtained.

A 120-mg aliquot of I was chromatographed on a Florisil column (prepared in $CHCl_3$) with elution with solvent mixtures containing 0–5% MeOH in $CHCl_3$ in order to remove the residual pigment. The fraction emerging with 1.5% MeOH–$CHCl_3$ contained predominantly piptocarphin E. Low-pressure LC of this fraction eluting with MeOH/CH_3CN/H_2O (50:15:35) separated piptocarphin E (**1e**) as a colorless oil: overall yield 0.0008%; ED_{50} 0.03 μg/mL; UV (MeOH) λ_{max} 284 nm (log ε 4.18); IR (KBr) ν_{max} 3500, 1765, 1735 (sh), 1725, 1635, 1225 cm^{-1}; high-resolution EI mass spectrum, m/e 450.186 (calcd for $C_{23}H_{30}O_9$, 450.189); CI mass spectrum, m/e 451 (MH^+), 405 (100%, $MH^+ - C_2H_6O$), 345 (MH^+ – HOAc – C_2H_6O), 319 ($MH^+ - C_4H_6O_2 - C_2H_6O$), 301 ($MH^+ - C_4H_6O_2 - C_2H_6O - H_2O$), 259 ($MH^+ - C_4H_6O_2 - C_2H_6O$ – HOAc); ^1H NMR ($CDCl_3$) δ 6.41 (dd, 9.1, 2.4, H-8), 6.27 (m, H-3a′), 5.91 (s, H-5), 5.66 (m, H-3b′), 5.18 (d, 12.9, H-13a), 4.88 (d, 12.9, H-13b), 3.93 and 3.72 (1 H each, dq, 9.2, 7.9, OCH_2CH_3), 3.44 (s, br, OH), 2.07–2.75 (c, 6 H, 2 H-9, 2 H-2, 2 H-3), 2.05 (s, 3 H, H-2″), 1.94 (m, 3 H, H-4′), 1.57 (s, 3 H, H-15), 1.23 (s, 3 H, H-14), 1.17 (t, 3 H, 7.0, CH_2CH_3); ^1H NMR (pyridine-d_5) δ 6.59 (br, H-8), 6.44 (m, H-3a′), 6.13 (s, H-5), 5.65 (m, H-3b′), 5.37 (d, 12, H-13a), 5.20 (d, 12, H-13b), 5.11 (s, br, OH, H_2O), 4.23 and 3.92 (1 H each, dq, 9.6, 7.1, OCH_2CH_3), 2.79 (dd, 14.7, 7.0, H-9a; collapses to a doublet upon irradiation of H-8), 2.40–1.8 (m, c, 5 H, H-9b, 2 H-2, 2 H-3), 2.0 (s, 6 H, H-2″, H-4′), 1.55 (s, 3 H, H-15), 1.42 (s, 3 H, H-14), 1.20 (t, 7.1, 3 H, OCH_2CH_3).

A 300-mg aliquot of II was also filtered through Florisil to remove the pigment. The active fraction eluting in 2% MeOH–$CHCl_3$ contained piptocarphin F along with several minor components and was subjected to low-pressure LC (50:15:35

(24) The C-18 reverse-phase silica gel was prepared by using the procedure of: Kingston, D. G. I.; Gerhart, B. B. *J. Chromatogr.* **1976**, *116*, 182.

(25) Yields reported are from the ground plant material.

(26) The integration is assumed to be 1 H unless specified. Coupling information and assignments are in hertz and are given in parentheses.

MeOH/CH_3CN/H_2O). Piptocarphin F (**1f**) was separated as a colorless oil: 0.003% overall yield; ED_{50} 3.2 μg/mL; UV (MeOH) λ_{max} 284 nm (log ε 4.39); IR (KBr) ν_{max} 3500, 1755, 1520, 1630 cm^{-1}; high-resolution EI mass spectrum, *m/e* 408.180 (calcd for $C_{21}H_{28}O_8$, 408.178); CI mass spectrum, *m/e* 409 (MH^+), 391 (100%, $MH^+ - H_2O$), 373 ($MH^+ - 2\ H_2O$), 363 ($MH^+ - C_2H_6O$), 323 ($MH^+ - C_4H_6O_2$), 305 ($MH^+ - C_4H_6O_2 - H_2O$), 287 ($MH^+ - C_4H_6O_2 - 2\ H_2O$), 277 ($MH^+ - C_4H_6O_2 - C_2H_8O$), 259 ($MH^+ - C_4H_6O_2 - C_2H_6O - H_2O$); ^1H NMR ($CDCl_3$) δ 6.58 (d, br, 9.5, H-8), 6.27 (m, H-3a'), 5.82 (s, H-5), 5.66 (m, H-3b'), 4.58 (d, 12.2, H-13a), 4.30 (d, 12.2, H-13b), 4.07 (s, br, OH), 3.77 (s, br, OH), 3.57 (q, 7.0, 2 H, OCH_2CH_3), 2.0–2.75 (m, c, 6 H, 2 H-2, 2 H-3, 2 H-9), 1.93 (m, 3 H, H-4'), 1.55 (s, 3 H, H-15), 1.21 (s, 3 H, H-14), 1.21 (t, 7.0, 3 H, OCH_2CH_3); ^1H NMR (Me_2SO-d_6)[27] δ 6.19 (s, br, 2 H, H-5, H-3a'), 5.76 (m, br, 2 H, H-3', H-8), 5.30 (s, br, 2 H, OH, exchangeable with D_2O), 4.28 (d, 11.7, H-13a), 4.2–4.3 (m, impurity), 4.07 (d, 11.7, H-13b), 3.42 (q, 7.1, OCH_2CH_3), 2.5–2.0 (m, br, H-2,3,9), 1.91 (s, br, 3 H, H-4'), 1.52 (s, 3 H, H-15), 1.24 (br s, m, impurity), 1.15 (s, H-14), 1.08 (t, 7.1, OCH_2CH_3).

The ^1H NMR spectra (Tables I and III) were measured on Varian FT-80, and Nicolet NTC-360 spectrometers. Both the proton-coupled and -decoupled ^{13}C NMR spectra (Table II) were obtained on Varian FT-80. The samples were dissolved in $CDCl_3$ with $CHCl_3$ used as the internal reference unless otherwise noted. All chemical shifts are expressed in parts per million (δ) relative to Me_4Si and are described with the following abbreviations: q, quartet; t, triplet; d, doublet; s, singlet; m, multiplet; br, broad; c, complex. Low-resolution chemical-ionization mass spectra were measured on a Du Pont 21-492B spectrometer using isobutane as the ionizing source. Exact mass measurements were obtained from a CEC 21-110B mass spectrometer. Beckman IR-33 and IR-4230 spectrometers were used to record infrared spectra, and a Perkin-Elmer Coleman 124 was used for ultraviolet spectra.

Thin-layer chromatography (TLC) was performed on Brinkmann EM-F254 0.25-mm precoated silica gel plates which were eluted with acetonitrile/chloroform (1:2). Visualization was accomplished by UV fluorescence (λ = 254 nm) and by charring with 10% H_2SO_4. Column chromatography employed SilicAR CC-7 silica gel, Florisil (Fisher, 60–100 mesh), and Sephadex LH-20 (Pharmacia) as specified. Low-pressure column chromatography (LC) was performed by using reversed-phase (C-18) silica gel prepared from Whatman LF-1 silica gel 80A according to the literature procedure. The low-pressure LC column eluent was monitored continuously by a LKB Uvicord ultraviolet absorbtiometer (control unit Type 8301A, detector unit Type 8300, recorder Type 6520-3).

(27) TLC analysis of the sample after the spectrum was recorded showed a significant amount of decomposition.

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Registry No. **1a**, 76248-63-0; **1b**, 76215-49-1; **1c**, 76215-50-4; **1d**, 76215-51-5; **1e**, 76215-52-6; **1f**, 76215-53-7.

Supplementary Material Available: Photographs of the Dreiding models of **1a** and **4** (2 pages). Ordering information is given on any current masthead page.

Synthesis and Reactions of Spirooxiranes in the 6,5'-Cyclopyrimidine Nucleoside Series: Preparation of 5'-Deoxy-5'-(hydroxymethyl)-6,5'-(*S*)- and -6,5'(*R*)-cyclouridines[1,2]

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Treatment of 2',3'-*O*-isopropylidene-5'-oxo-6,5'-cyclouridine (**6**) with either dimethylsulfonium methylide or dimethylsulfoxonium methylide affords the 5'*R* oxirane **7** stereoselectively in good yield. Under more vigorous conditions (60 °C) both ketone **6** and oxirane **7** react with excess dimethylsulfoxonium methylide to give the spirooxetane **9**. Reduction of **7** with lithium triethylborohydride affords the tertiary alcohol **12** exclusively, whereas hydrogenolysis in the presence of platinum on carbon proceeds with inversion to give the 5'*R* primary alcohol **16**. Hydrogenation of **7** with Raney nickel as catalyst affords two pairs of diastereomers—the alcohols **15** and **16** and the deoxygenated 5'-methyl nucleosides **13** and **14**. The 5'-hydroxymethyl nucleosides **15** and **16**, as well as the title compounds **3** and **4**, undergo base-catalyzed epimerization at C-5' via a carbanion mechanism to give equilibrium mixtures in which the 5'*S* isomers (**3** and **15**) predominate. Ultraviolet irradiation of oxirane **7** affords the aldehyde **18** as the major product. Compound **18** exists predominantly as the (*Z*)-enol form in solution, as shown by nuclear Overhauser studies, but it can be reduced with sodium cyanoborohydride to give selectively the 5'*S*-hydroxymethyl nucleoside **15**. Small amounts of the 5'*S* oxirane **11** are also formed on irradiation of **7**.

Recent investigations in this laboratory have led to the synthesis of a variety of conformationally restricted pyrimidine nucleosides—for example, the 6,5'-cyclouridines[3] **1** (Scheme I) and the ring-expanded 6,6'-cyclohexo-

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(2) This paper is the fourth of a series entitled "Conformationally Restricted Analogs of Pyrimidine Nucleosides". For part 3, see ref 4.

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