Anal. Calcd for $C_{23}H_{18}O_3$: C, 80.68; H, 5.30. Found: C, 80.48; H, 5.43.

1,4,4-Triphenylbut-3-en-1-one (9). A sample of 100 mg (0.292 mmol) of **7** was heated neat at about 200 °C until gas evolution ceased. The brown melt was recrystallized twice from diethyl ether–light petroleum ether to yield 14 mg (0.047 mmol, 16%) of slightly yellow needles, mp 125.0–125.7 °C (lit.[11] 126–126.5 °C). A separate sample of **7** was pyrolyzed neat in an NMR tube to give the spectrum described below.

NMR ($CDCl_3$) showed τ 2.05–2.31 (m, 2 H, benzoyl ortho protons), 2.42–3.0 (m, 13 H, aromatic), 3.59 (t, J = 7.0 Hz, 1 H, H_3), 6.21 (d, J = 7.0 Hz, 2 H, H_2); ir (KBr) 2900, 1670 (lit.[11] 1690), 1435, 1330, 1210, 995, 770, 765, 745, 700, 695, 685 cm^{-1}.

5,7,7-Triphenyl-3,4-diazabicyclo[4.1.0]hept-4-en-2-one (8). To 4.3 ml (4.3 g, 86 mmol) of hydrazine hydrate in 400 ml of absolute ethanol was added 2.47 g (7.22 mmol) of **7**. After stirring for 12 h, a precipitate appeared and was filtered off after another 24 h of stirring to yield 1.77 g (5.22 mmol, 72.3%) of colorless crystals of **8**, mp 244.0–245.0 °C. An analytical sample was obtained on recrystallization from ethanol: mp 244.7–245.2 °C; NMR ($CDCl_3$) τ 1.9 (v br s, 1 H, NH), 1.95–2.13 (m, 2 H, C-5 phenyl ortho protons), 2.40–3.16 (m, 13 H, aromatic), and 6.88 (AB quartet, J_{AB} = 8.0 Hz, 2 H, H_A and H_B) (the upfield half of the AB quartet appears as a doublet of doublets owing to further coupling with the NH proton, J_{BN} = 1.5 and J_{AN} = 0.0 Hz as determined from an HA-100 spectrum); mass spectrum (70 eV) *m/e* (rel intensity) 338 (24), 337 (16), 235 (39), 193 (22), 192 (100), 191 (28), 166 (13), 165 (47), 115 (12), and 77 (13); ir (KBr) 3100, 3000, 2850, 1670, 1495, 1445, 1365, 1320, 1070, 775, 760, 705, and 692 cm^{-1}.

Anal. Calcd for $C_{23}H_{18}N_2O$: C, 81.63; H, 5.36; N, 8.28. Found: C, 79.50, 79.63; H, 5.71, 5.63; N, 8.04. Calcd for $C_{23}H_{18}N_2O \cdot \frac{1}{2}H_2O$: C, 79.51; H, 5.51; 8.07.

2,5,7,7-Tetraphenyl-3,4-diazabicyclo[4.1.0]hepta-2,4-diene (4). Phenyllithium (9.94 mmol) prepared from 197 mg (28.4 mmol) of lithium and 2.23 g (14.2 mmol) of bromobenzene in 60 ml of dry diethyl ether was added dropwise to a magnetically stirred solution of 674 mg (1.99 mmol) of **8** in about 100 ml of freshly distilled tetrahydrofuran (THF). The initial transient red color that appeared as each drop of phenyllithium made contact with the THF solution remained firm after about one-fifth of the addition had been completed.

The deep red reaction mixture was stirred for an additional 30 min and then poured onto an ice–water slush containing 800 mg (13 mmol) of glacial acetic acid. The pale yellow compound was extracted with diethyl ether and chromatographed over basic alumina to yield 374 mg (0.939 mmol, 47.2%) of bright yellow, beautiful needles: mp 227.0–227.5 °C dec; NMR ($CDCl_3$) τ 1.60–2.0 (m, 4 H, ortho protons C-2 and C-5 phenyls), 2.34–2.7 (m, 6 H, meta and para protons C-2 and C-5 phenyls), 2.71 (s, 5 H, exo C-7 phenyl protons), 3.03 (s, 5 H, endo C-7 phenyl protons), and 6.58 (s, 2 H, cyclopropyl protons); mass spectrum (70 eV) *m/e* (rel intensity) 398 (40), 370 (39), 296 (25), 295 (100), 294 (23), 193 (22), 192 (18), and 166 (25); ir (KBr) 2940, 1540, 1495, 1450, 1395, 765, 755, 705, and 690 cm^{-1}.

Anal. Calcd for $C_{29}H_{22}N_2$: C, 87.40; H, 5.57; N, 7.03. Found: C, 87.44; H, 5.58; N, 6.93.

Attempted Base-Catalyzed Rearrangement of 4. A mixture of 93 mg of **4** and 19 mg of sodium hydroxide was refluxed for 75 h in 25 ml of absolute ethanol to give a still yellow, but cloudy mixture which, after filtration and chromatography over basic alumina, yielded 73 mg (78% recovery) of **4**, mp 231–231.5 °C dec. The NMR spectrum of the recovered material was identical with that of authentic **4**.

Registry No.—**3a**, 57694-77-6; **4**, 57694-78-7; **5**, 57694-79-8; **6**, 57694-80-1; **7**, 57694-81-2; **8**, 57694-82-3; **9**, 57694-83-4; hydrazine, 302-01-2; phenyl bromide, 108-86-1; diphenylcadmium, 2674-04-6; phenyllithium, 591-51-5.

References and Notes

(1) (a) Taken in part from the Ph.D. Dissertation presented by R.M.W., March 1972, to the Graduate School of the University of Florida. (b) Financial support of this research, in part, by the National Science Foundation is gratefully acknowledged. (c) Address all correspondence to this author at Clinical Chemistry, North Carolina Memorial Hospital, Chapel Hill, N.C. 27514.
(2) R. G. Amiet and R. B. Johns, *Aust. J. Chem.*, **21**, 1279 (1968).
(3) R. M. White and M. A. Battiste, unpublished results.
(4) J. van Alphen, *Recl. Trav. Chim. Pays-Bas*, **62**, 210 (1943).
(5) R. M. White, M. A. Battiste, G. Palenik, and M. Mathew, results to be published.
(6) D. E. Webster, *J. Chem. Soc.*, 5132 (1960).
(7) G. Maier, *Chem. Ber.*, **98**, 2438 (1965).
(8) G. Maier, *Chem. Ber.*, **101**, 1371 (1968).
(9) H. E. Zimmerman and D. I. Schuster, *J. Am. Chem. Soc.*, **84**, 4527 (1962).
(10) J. B. Miller, *J. Org. Chem.*, **24**, 560 (1959).
(11) H. M. Walborsky and L. Plonsker, *J. Am. Chem. Soc.*, **83**, 2138 (1961).
(12) M. Barfield and B. Chakrabarti, *Chem. Rev.*, **69**, 757 (1969).
(13) S. Ranganathan, C. S. Panda, T. Page, and H. Schecter, *J. Org. Chem.*, **37**, 1071 (1972).
(14) L. Horner and E. Lingnau, *Justus Liebigs Ann. Chem.*, **591**, 21 (1955).
(15) All melting points were obtained using a Thomas-Hoover Unimelt apparatus and are uncorrected. Mass spectra were obtained on a Perkin-Hitachi RMU-9E instrument at 70 eV. Nuclear magnetic resonance spectra were recorded on a Varian A-60A instrument unless otherwise noted. Infrared spectra were procured on either a Perkin-Elmer Model 137 or Model 337.

Pycnolide, a *seco*-Germacradienolide from *Liatris pycnostachya*, and Other Antitumor Constituents of *Liatris* Species[1,2]

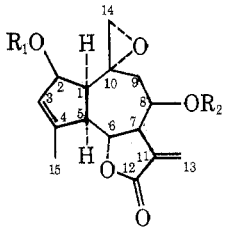
Werner Herz* and Ram P. Sharma

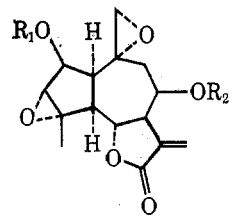
Department of Chemistry, The Florida State University, Tallahassee, Florida 32306

Received October 31, 1975

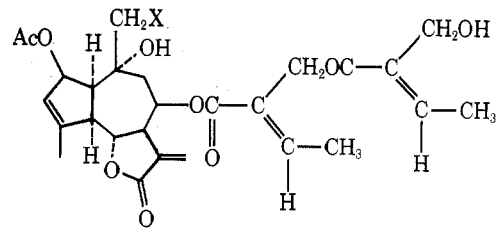
Further study of a *Liatris pycnostachya* Michx. extract yielded pycnolide (**4a**), a 2,3-*seco*-1(10),4,5-germacradienolide, whose structure determination by means of chemical transformations and ^1H and ^{13}C NMR spectrometry is detailed. A chlorine-containing guaianolide, spicatin hydrochloride (**3b**), was also found. The previously known cytotoxic guaianolide spicatin (**1a**), the antileukemic 5,10-epoxygermacranolide chapliatrin (**11**), and euparin (**9**) were isolated from *L. tenuifolia* Nutt. whereas *L. scabra* (Greene) K. Schum. yielded the cytotoxic heliangolide eleganin (**10**). *L. earlei* (Greene) K. Schum. and *L. pauciflora* Pursh gave small amounts of complex lactone mixtures.

In a previous article[3] we reported inter alia the isolation of the complex ester guaianolides spicatin (**1a**) and epoxyspicatin (**2a**) from the less polar fractions of a *Liatris pycnostachya* Michx. extract. Since then, spicatin, also found in *L. spicata*,[3] has been shown to be cytotoxic[5] as were graminiliatrin (**2c**) and deoxygraminiliatrin (**1c**) from *L. graminifolia*[3] and eleganin (**10**) from *L. elegans*.[6] Epoxyspicatin (**2a**) and chapliatrin (**11**, stereochemistry at C-3, C-4, and C-10 tentative)[1] from *L. chapmanii*[7] and *L. gracilis*[1] also exhibited significant in vivo activity against P388 lymphocytic anemia. In the present communication we report isolation and structure determination of spicatin hydrochloride (**3b**) and pycnolide (**4a**) from the more polar fractions of the *L. pycnostachya* extract. Pycnolide, a 2,3-

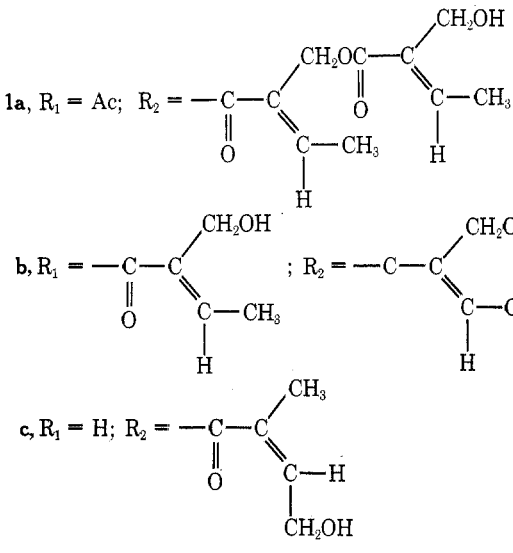




2a, R_1, R_2 as in 1a
b, R_1, R_2 as in 1b
c, R_1, R_2 as in 1c



3a, X = Br
b, X = Cl



1a, R_1 = Ac; R_2 =

b, R_1 =

; R_2 =

c, R_1 = H; R_2 =

seco-1(10),4,5-germacradienolide, is the first *seco*-germacrane derivative encountered in nature. We also describe the results of our examination of *L. tenuifolia* Nutt., *L. scabra* (Greene) K. Schum., *L. pauciflora* Pursh, and *L. earlei* (Greene) K. Schum.[8]

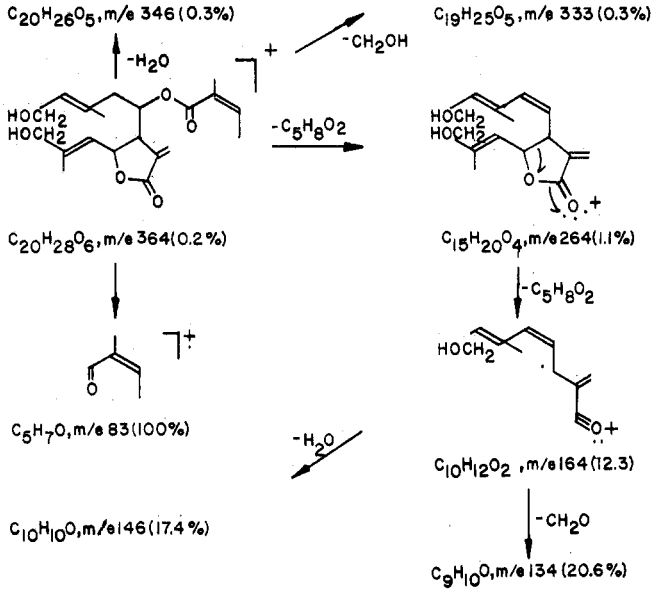
Rechromatography of the more polar fractions of the *L. pycnostachya* extract afforded first a crystalline chlorine-containing sesquiterpene $C_{27}H_{33}O_{10}Cl$ whose physical properties resembled those of spicatin hydrobromide (**3a**); a substance prepared from spicatin for x-ray analytical purposes.[4] Direct comparison with spicatin hydrochloride (**3b**), obtained by treatment of spicatin with hydrochloric acid, established identity.[9] A second, considerably more polar substance could not be induced to crystallize and was named pycnolide.

Pycnolide (**4a**), $C_{20}H_{28}O_6$ (high-resolution mass spectrum and elemental analysis), $[\alpha]^{22}D$ +39.8°, was an α-methylene γ-lactone (ir bands at 1765 and 1650 cm^{-1}, narrowly split NMR doublets at 6.36 and 5.70 ppm); two other oxygen atoms were part of a five-carbon unsaturated ester side chain (ir band at 1720 cm^{-1}) as evidenced by facile loss of a $C_5H_8O_2$ fragment on electron impact (see Scheme I) and by the appearance of an ion C_5H_7O as the base peak. The nature of this ester side chain was revealed by the NMR spectrum (Table I), which had the characteristic peaks of an angelate (vinyl multiplet at 6.1 ppm allylically coupled to a vinyl methyl at 1.83 ppm and vicinally coupled to a vinyl methyl at 1.93 ppm, the latter being long-range coupled to the first vinyl methyl resonance).

Treatment of pycnolide with acetic anhydride–pyridine resulted in formation of a diacetate (**4b**); in the NMR spectrum, this resulted in a downfield shift of two sets of signals, the first an AB system, from 4.16 to 4.54 ppm, and the second a two-proton singlet, from 4.06 to 4.52 ppm. Consequently pycnolide had two primary hydroxyl groups (ir band at 3400 cm^{-1}) and the functionality of all oxygen atoms was established.

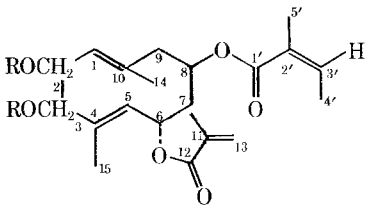
The NMR spectrum of pycnolide also displayed two somewhat broadened three-proton singlets at 1.73 and 1.83 ppm, an observation which indicated the presence of two

Scheme I. Mass Spectral Fragmentation of Pycnolide

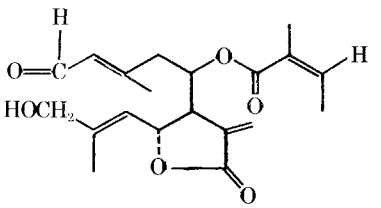


additional vinyl methyl groups. Irradiation at 5.30 ppm sharpened the resonance at 1.73 ppm and irradiation at 5.52 ppm sharpened the resonance at 1.83 ppm. Consequently pycnolide contained two $-C(CH_3)=CH-$ units, a conclusion which in conjunction with the previous deductions and the molecular formula required that pycnolide had no rings other than that of the lactone. Evidence for formula **4a** which seemed biogenetically plausible and where the numbering is that used in the supposed precursor germacradienolide was provided by spin decoupling experiments which will now be detailed.

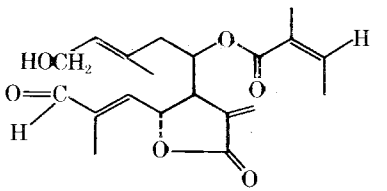
Irradiation at the frequency of H-7 (3.10 ppm) converted the narrowly split doublets of H-13a and H-13b to singlets, collapsed a doublet of doublets at 5.35 ppm (H-6 or H-8) to a doublet (J = 9.5 Hz), and simplified a multiplet at 5.52 ppm (H-8 or H-6). Irradiation at 5.35 ppm simplified the



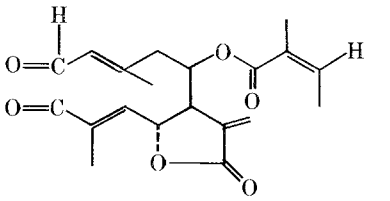
4a, R = H
b, R = Ac
c, R = CPh_3



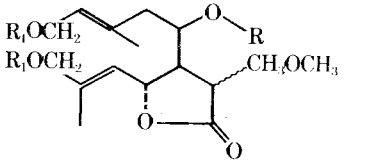
5



6



7



8a, R, R_1 = H
b, R = H; R_1 = Ac
c, R = H; R_1 = CPh

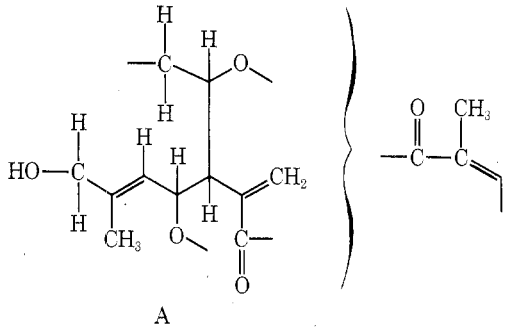
Table I. ^{1}H NMR Spectra of Pycnolide and Derivatives[a]

Compd	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-9	H-13	H-14[b]	H-15[b]	H-3′	H-4′[b]	H-5′[b]	Misc
4a	5.30 t br (6)	4.16[c]	4.06[d]	5.52 d br (9.5)	5.35 dd (9.5, 5.3)	3.10 m (5.3, 2.8, 2.6)	5.52 m (8.5, 6)	2.45 dd (15, 8.5) 2.16 dd (15, 6)	6.30 d (2.8) 5.70 d (2.6)	1.73 br	1.83 br	6.1 m	1.93 m	1.83 br	
4b	5.34 t br (6)	4.54[c]	4.52[d]	5.48 d br (9.5)	5.26 dd (9.5, 5.3)	3.07 m	5.48 m	2.32 dd (15, 8.5) (15, 6) 2.25 dd (15, 6)	6.38 d (2.8) 5.76 d (2.6)	1.79 br	1.85 br	6.0 m	1.93 m	1.85 br	2.01[b], 2.02[b] (Ac)
4c[e]	*f*	3.60[c]	3.65[d]	*f*	*f*	3.17 m	*f*	2.39[c]	6.45 d (2.5) 5.84 d (2.2)	1.58 br	1.78 br	6.14 m	1.93 m	1.85 br	7.8[g]
5	5.80 d br (7.8)	9.94[d] (7.8)	4.10[d]	5.54 d br (10.2)	5.29 dd (10.2, 5.2)	3.1 m	5.54 m	2.55 dd (15, 8.5) 2.42 dd (15, 6)	6.39 d (2.5) 5.75 d (2.2)	2.26 br	1.82 br	6.10 m	1.92 m	1.82 br	
6	5.40 t br (6)	4.10[c]	9.50	6.30 d br (8)	5.45 dd (8, 5.1)	3.2 m	5.45	2.35 dd (15, 8.5) 2.25 dd (15, 6)	6.46 d (2.5) 5.8 t d (2.2)	1.75 br	1.85 br	6.12 m	1.97 m	1.93 br	
7	5.80 d br (7.5)	9.93[d] (7.8)	9.49	6.35 d br (8.5)	5.40 dd (8.5, 5.1)	3.2 m	5.54 m	2.46[c]	6.50 d (2.2) 5.88 d (2.2)	2.24 br	1.83 br	6.14 m	1.92 m	1.90 br	
8a	5.33 t br (7)	4.13[c]	4.02[d]	5.52 d br (9)	5.22 t (9)	2.40 m	3.92 m	2.08[c]	3.66[c]	1.65 br	1.76 br				2.88 (H-11) 3.36 (OMe)
8b	5.38 t br (7)	4.58[c]	4.52[d]	5.54 d br (9)	5.22 t (9)	2.40 m	4.02 m	2.08[c]	3.68 m	1.73 br	1.81 br				3.36 (OMe)

[a] Run at 270 MHz in $CDCl_3$ solution on a Bruker HFX-270 instrument with Me_4Si as internal standard. Values are in parts per million. Multiplicities are indicated by the usual symbols; d, doublet; t triplet; br, broadened singlet; m, multiplet whose center is given. Unmarked signals are singlets. Figures in parentheses are coupling constants in hertz. [b] Intensity three protons. [c] Center of AB system, two protons. [d] Two protons. [e] Run at 60 MHz on Varian HA-60 instrument. [f] Obscured signal. [g] Aromatic protons.

signal of H-7 as well as that of a second proton superimposed on the multiplet at 5.52 ppm which (vide supra) was coupled allylically to the vinyl methyl resonance at 1.83 ppm. Irradiation at 5.52 ppm sharpened this vinyl methyl signal and simplified the H-7 multiplet as expected, converted the doublet of doublets at 5.35 ppm to a doublet (J = 5.3 Hz), and collapsed the AB components of an ABX system at 2.45 and 2.16 ppm to sharp doublets. Hence one of the two protons in the multiplet at 5.52 ppm was adjacent to a methylene group, the other being vinylic. Since a proton under a lactone oxygen usually appears at higher field than a proton under an ester side chain, the protons in the 5.52-ppm multiplet were provisionally identified with H-5 and H-8 and the proton responsible for the 5.35-ppm signal with H-6.

Oxidation of pycnolide with MnO_2 gave two monoaldehydes **5** and **6** and the dialdehyde **7.** In the NMR spectrum of **6,** the signal of H-5, now β to an α,β-unsaturated aldehyde function, had experienced a significant downfield shift to 6.36 ppm and was clearly coupled not only to the vinyl methyl group at 1.85 ppm, but also to a doublet of doublets at 5.45 ppm (H-6) superimposed on the multiplet of H-8. In the NMR spectrum of **7,** the signals of H-6 and H-8 were distinct, a circumstance which permitted unambiguous sequencing, by spin decoupling, of the framework C-3–C-9 as in A.



A

The remaining four-carbon unit, $-C(CH_3)=CHCH_2OH$ in pycnolide and $-C(CH_3)=CHCHO$ in **5** and **7,** was clearly recognizable in the NMR spectra, the vinyl methyl signal being allylically coupled to the vinyl proton (vide supra) and experiencing a significant downfield shift, from near 1.75 to 2.25 ppm, on oxidation of the hydroxymethylene group to the aldehyde. It could be attached to A in one, and only one, way to give **4a** (devoid of stereochemistry) where the only remaining ambiguity was the orientation of the lactone ring, provisionally assumed to be closed to C-6.

This matter was settled as follows. Methanolysis of pycnolide (MeOH, NaOMe, room temperature, 10–12 h) resulted in loss of the angeloyl ester side chain and afforded a substance **8a.** In the NMR spectrum of this compound the H-8 multiplet, formerly near 5 ppm, had undergone a diamagnetic shift to 3.92 ppm. On the other hand the signal near 5.3 ppm had not been affected significantly and was still spin coupled to the H-5 vinyl proton near 5.5 ppm. Consequently the lactone rings of **8a** and of pycnolide are closed to C-6 and the ester side chain is attached to C-8.

The relatively simple fragmentation of pycnolide on electron impact (Scheme I) is fully consonant with this deduction. Initial loss of angelic acid gives rise to an ion of m/e 264 ($C_{15}H_{20}O_4$).

The next significant ion of lower mass is an ion $C_{10}H_{12}O_2$ (m/e 164) which could arise as shown in Scheme I; corresponding ions of mass 206, 162, 164, and 162, respectively, appear in the mass spectra of **4b, 5, 6,** and **7.** This ion further loses H_2O to give an ion of mass 146 ($C_{10}H_{10}O$) or CH_2O to give an ion of mass 134 ($C_9H_{10}O$); both ions are present in the mass spectra of **4b** and **6,** but a corresponding ion of mass 144 is absent from the mass spectra of **5** and **7,** thus confirming the proposed scheme.

Table II. ^{13}C NMR Spectrum of Pycnolide[a]

Signal no.	4a	Assignment
1	169.7	C-1′
2	166.8	C-12
3	142.7	C-10
4	138.8 d	C-3′
5	135.9	C-4
6	132.3	C-11
7	128.9 d	C-5
8	127.2	C-2′
9	123.3 t	C-13
10	121.6 d	C-1
11	74.6 d	C-6
12	71.3 d	C-8
13	66.3 t	C-3
14	58.7 t	C-2
15	47.7 d	C-7
16	41.9 t	C-9
17	20.3 q	C-14
18	15.8 q	C-15
19	15.6 q	C-4′
20	14.0 q	C-5′

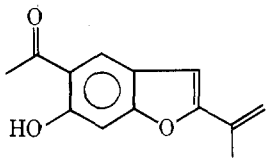
[a] Run in $CDCl_3$ on a Bruker HX-270 MHz instrument with Me_4Si as internal standard.

The ^{13}C NMR spectrum of pycnolide (Table II) is fully in accord with the proposed structure and was helpful in ruling out other possibilities prior to the work discussed in the previous paragraphs. Tentative assignments are based on predicted shifts and comparisons with data in the literature[1] and in our files.

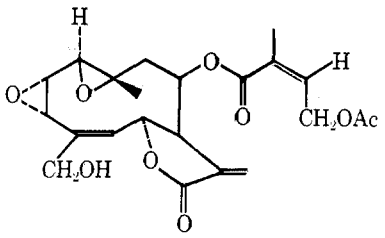
The stereochemistry of pycnolide was established in the following manner. The pronounced paramagnetic shift of the C-10 methyl signal on going from **4a** to **5** or **7** clearly demonstrated trans, or E, stereochemistry around the 1,10 double bond.[13,14] The trans or E configuration of the 4,5 double bond was evident from the NMR spectra of **6** and **7.** The shift of the aldehyde proton on C-1 (near 9.5 ppm) is indicative of a cis relationship between the aldehyde function and the vinylic hydrogen, trans aldehydes with this substitution pattern giving rise to signals near 10 ppm or higher.[16]

The absolute configuration at C-8 was determined by Horeau's method. Initially, the primary hydroxyls of pycnolide were protected by conversion to the bis trityl ether **4c** which was converted to **8c** by methanolysis (NMR spectrum). However, attempts at purification resulted in partial cleavage of **8c** to **8a,** previously prepared more directly by methanolysis of **4a.** Selective acetylation of **8a** (acetic anhydride–pyridine, 10 min) gave the diacetate **8b** which was esterified with excess (+)-α-phenylbutyric anhydride. The recovered α-phenylbutyric acid was dextrorotatory (14.2% optical yield). Hence the configuration at C-8 is R; i.e, the ester function is β if the structure is depicted as in the formulas.

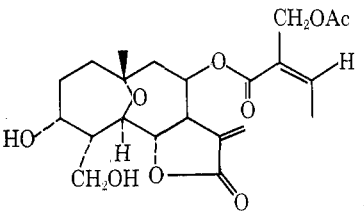
The value of $J_{6,7}$ in **4a–7** (~5.2, see Table I) would be appropriate for either a cis or a trans lactone ring closure (models). However, $J_{6,7}$ = 9 Hz in **8a** and **8b** indicates that the lactone ring is trans fused. Since $J_{7,13}$ < 3 Hz (see Table I), Samek's rule ($J_{7,13} \geq 3$ Hz for trans-fused lactones)[17] is therefore apparently not applicable to *seco*-germacradienolides of the type represented by pycnolide.[18] If the C-7 side chain is β (i.e., 7 R) as in all sesquiterpene lactones of established stereochemistry, the hydrogen at C-6 must be β (6R). If this be so, the observed positive Cotton effect (θ_{240} +990) is not in agreement with the empirical rule of Stöcklin et al.[19,20] Unfortunately, all compounds en-



9



10



11

countered in the present work were noncrystalline; means to confirm the stereochemistry at C-6 by x-ray analysis and to relate the chirality of the lactone system of pycnolide to the Cotton effect are therefore lacking at present.[21]

Pycnolide represents a new type of sesquiterpene structure, a *seco*-germacrane, whose biogenesis obviously involves cleavage of the 2,3 bond of a germacrane precursor. However it is highly unusual for the following reason. In other seco-type structures, whether sesquiterpenoid, diterpenoid, or triterpenoid, the oxidation states of the carbon atoms at the points of scission are higher than prior to cleavage. In pycnolide, on the other hand, enzymatically induced scission must be followed by two simultaneous or consecutive reduction steps.

Examination of *Liatris tenuifolia* gave the benzofuran derivative euparin (**9**) and, as the only components of a complex lactone mixture which could be separated and identified, spicatin (**1a**) and the 5,10-epoxygermacranolide chapliatrin (**11**) previously[1] isolated from *L. chapmanii* and *L. gracilis* Pursh. *L. scabra* (Greene) K. Schum. also yielded a mixture of lactones from which the heliangolide eleganin (**10**) previously[6] found in *L. elegans* (Walt.) Michx. could be separated by extensive chromatography. Attempts to separate lactone mixtures obtained in small amount only by extraction of *L. earlei* (Greene) K. Schum. and *L. pauciflora* Pursh have so far not been successful.

Experimental Section[22]

Isolation of Spicatin Hydrochloride and Pycnolide. The extraction of *L. pycnostachya* has been described previously.[3] The $CHCl_3$ and $CHCl_3$–MeOH eluates (10:1) gave a mixture, wt 5.5 g, which was rechromatographed over 200 g of silicic acid, 200-ml fractions being collected in the following order: $CHCl_3$ (10 fr), $CHCl_3$–MeOH (99:1, 10 fr), $CHCl_3$–MeOH (49:1, 10 fr), $CHCl_3$–MeOH (99:1, 10 fr), $CHCl_3$–MeOH (9:1, 10 fr) and $CHCl_3$–MeOH (4:1, 10 fr). Fractions 5–8 yielded 0.5 g of spicatin hydrochloride (**3b**), identical with material prepared from spicatin (see below). Fractions 34–39 gave 2 g of pycnolide (**4a**) which was purified by preparative TLC on silica gel $PF_{254-355}$ ($CHCl_3$–MeOH, 8:1). The noncrystalline substance had $[\alpha]^{22}D$ +39.8° (*c* 1.1, $CHCl_3$); CD curve $[\theta]_{240}$ +990; ir bands at 3400, 1765, 1720, 1650, 1230, 960, and 850 cm^{-1}.

Anal. Calcd for $C_{20}H_{28}O_6$: C, 65.92; H, 7.74; O, 26.34; mol wt, 364.1884. Found: C, 65.44; H, 7.79; O, 26.76; mol wt (MS), 364.1890.

Spicatin Hydrochloride. A solution of 0.1 g of spicatin in 40 ml of absolute ethanol and 0.01 ml of 38% hydrochloric acid was stirred overnight at room temperature and evaporated in vacuo. A solution of the gummy residue in $CHCl_3$ exhibited two main spots on TLC. Preparative TLC (silica gel, $CHCl_3$–ether–MeOH, 30:30:1) developed three times gave 40 mg of starting material and 44 mg of solid spicatin hydrochloride which was recrystallized from ethyl acetate–hexane: mp 122–123 °C dec; ir bands at 3480, 3405, 1770, 1715, and 1655 cm^{-1}, high-resolution mass spectrum peaks at *m/e* (composition, %) 552 ($C_{27}H_{33}O_{10}Cl$, M^+, 0.23), 492 ($C_{25}H_{29}O_8Cl$, 24.2), 474 ($C_{25}H_{27}O_7Cl$, 0.54), 456 ($C_{25}H_{25}O_6Cl$, 0.55), 395 ($C_{20}H_{24}O_6Cl$, 0.65), 278 ($C_{15}H_{15}O_3Cl$, 7.9), 260 ($C_{15}H_{13}O_2Cl$, 7.4), 115 ($C_5H_2O_3$, 24.1), 99 ($C_5H_7O_2$, 100), 81 (C_5H_5O, 55.6); NMR signals ($CDCl_3$, 90 MHz) at 7.06 q and 6.88 q (*J* = 7 Hz, H-3′ and H-8′), 6.24 d (3.6) and 5.48 d (3.1, H-13), 5.81 m (H-3), 5.75 m (H-8), 5.41 m (H-2), 4.87 (H-5′), 4.56 dd (10, 8, H-6), 4.30 (H-10′), 3.94 m (H-7), 3.57 m (H-14), 2.77 dd (10,.9, H-5), 2.37 m (H-9), 2.05 (Ac), 2.03 (H-15), 1.93 d and 1.89 d (7, H-4′ and H-9′).

Anal. Calcd for $C_{27}H_{33}O_{10}Cl$: Cl, 6.41. Found: Cl, 6.28.

Spicatin Hydrobromide (3a). The reaction of 0.1 g of spicatin in 40 ml of absolute ethanol with 0.01 ml of 48% HBr was carried out and worked up as described in the previous paragraph. Preparative TLC (silica gel, $CHCl_3$–ether–MeOH, 30:30:1) developed three times gave yellowish crystals of hydrobromide (wt mg) and 40 mg of starting material. Recrystallization from MeOH–H_2O gave colorless crystals: mp 95–98 °C dec; ir bands at 3485, 3410, 1770, 1715, and 1655 cm^{-1}; NMR signals ($CDCl_3$, 90 MHz) at 7.05 q and 6.87 q (*J* = 7 Hz, H-3′ and H-8′), 6.23 d (3.6) and 5.47 d (3.1, H-13), 5.81 m (H-3), 5.75 m (H-8), 5.41 m (H-2), 4.87 (H-5′), 4.56 dd (10, 8, H-6), 4.30 br (H-10′) 3.94 m (H-7), 3.52 m (H-14), 2.73 dd (10, 9, H-5), 2.42 m (H-9), 2.03 (H-15) 2.01 (Ac), 1.93 d and 1.87 d (7, H-4′ and H-9′).

Anal. Calcd for $C_{27}H_{33}O_{10}Br$: Br, 13.37. Found: Br, 12.60.

Diacetylpycnolide (4b). Reaction of 50 mg of pycnolide with 1 ml of acetic anhydride and 0.5 ml of pyridine followed by the usual work-up gave **4b** as a gum which had in bands at 1765, 1735, 1720, 1650, 1250, 960, and 850 cm^{-}.

Anal. Calcd for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19; O, 28.54; mol wt, 448.2095. Found: C, 64.10; H, 7.06; O, 27.78; mol wt (MS), 448.2121.

Other significant peaks in the high-resolution mass spectrum were at *m/e* (composition, %) 348 ($C_{19}H_{24}O_6$, 1.0), 306 ($C_{17}H_{22}O_5$, 1.7), 288 ($C_{17}H_{20}O4$, 2.0), 246 ($C_{15}H_{18}O_3$, 2.8), 228 ($C_{15}H_{16}O_2$, 5.3), 206 ($C_{12}H_{14}O_3$, 4.3), 178 ($C_{11}H_{14}O_2$, 1), 164 ($C_{10}H_{12}O_2$, 6.5), 146 ($C_{10}H_{10}O$, 54.3), 134 ($C_9H_{10}O$, 3.1), 83 (C_5H_7O, 100).

MnO_2 Oxidation of Pycnolide. A solution of 0.2 g of pycnolide in 10 ml of AR $CHCl_3$ was stirred with 0.5 g of active MnO_2, the reaction being monitored by TLC. After 4 h, when starting material had disappeared, the mixture was filtered and the residue thoroughly washed with $CHCl_3$. The combined filtrate and washings were evaporated; the residue was purified by preparative TLC (silica gel $PF_{254-355}$, solvent benzene–ethyl acetate, 1:1). One major and two minor products were isolated. The major product (R_f 0.4) was identified as the monoaldehyde **5**, wt 0.105 g; the minor products (R_f 0.5 and 0.6) were **6** (0.025 g) and **7** (0.020 g), respectively. Attempts to obtain **5**, **6**, and **7** in crystalline form were unsuccessful. Longer reaction time or excess MnO_2 did not give better yields of **6** and **7**, but when pure **5** or **6** was exposed to fresh MnO_2, the reaction proceeded quickly to give **7** in 100% yield. Similiar observations were recorded in the case of ligantrol.[23]

Substance **5** had ir bands at 3400, 1760, 1710, 1660, 1230, 970, and 850 cm^{-1}.

Anal. Calcd for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23; O, 26.49; mol wt, 362.1728. Found: C, 65.27; H, 7.14; O, 26.98; mol wt (MS), 362.1726.

Other significant peaks in the high-resolution mass spectrum were at *m/e* (composition, %) 262 ($C_{15}H_{18}O_4$, 1.5), 244 ($C_{15}H_{16}O_3$, 1.9), 233 ($C_{14}H_{17}O_3$, 1.4), 232 ($C_{14}H_{16}O_3$, 1.4), 231 ($C_{14}H_{15}O_3$, 1.2), 162 ($C_{10}H_{10}O_2$, 5.6), 161 ($C_{10}H_9O_2$, 11.8), 134 ($C_9H_{10}O$, 5.4), 133 (C_9H_9O, 21), and 83 (C_5H_7O, 100).

Substance **6** exhibited ir bands at 3400, 1760, 1710, 1660, 1240, 980, and 850 cm^{-1}. Its low-resolution mass spectrum had significant peaks at *m/e* 362 (M^+), 344 ($M - H_2O$), 262 ($M - C_5H_8O_2$), 164 ($M - C_5H_8O_2 - C_5H_6O_2$), 146 ($M - C_5H_8O_2 - C_5H_6O_2 - H_2O$), 134 ($M - C_5H_8O_2 - C_5H_6O_2 - CH_2O$), and 83 ($C_5H_7O$, base peak).

Substance **7** had ir bands at 1760, 1710, 1660, 1240, 1050, and 850 cm^{-1}.

Anal. Calcd for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71; O, 26.64; mol wt, 360. Found: C, 65.80; H, 7.16; O, 26.88; mol wt (MS), 360.

Other significant peaks in the low-resolution mass spectrum were at *m/e* 331 ($M - CHO$), 260 ($M - C_5H_8O_2$), 231 ($M - C_5H_8O_2 - CHO$), 162 ($M - 2C_5H_8O_2$), 134 ($M - 2C_5H_8O_2 - CO$), 83 (C_5H_7O, base peak).

Methanolysis of Pycnolide. A solution of 0.2 g of pycnolide in 10 ml of anhydrous MeOH was stirred with 0.3 g of NaOMe under nitrogen. After 0.5 h, all starting material had disappeared (TLC).

The mixture was acidified with acetic acid, extracted with $CHCl_3$, washed, dried, and evaporated. The residue gave a single spot on TLC; the NMR spectrum indicated retention of the angeloyl ester side chain and addition of MeOH to the α,β unsaturated lactone function. Extension of the time of methanolysis to 12 h followed by the usual work-up resulted in isolation of gummy **8a** which was purified by preparative TLC (solvent $CHCl_3$–MeOH, 10:1), ir bands at 3400, 1770, 1230, 1050, and 950 cm^{-1}.

Anal. Calcd for $C_{16}H_{26}O_6$: C, 61.13; H, 8.34; O, 30.53; mol wt, 314. Found: C, 60.43; H, 8.25; O, 31.19; mol wt (MS), 314.

Other significant peaks in the low-resulting mass spectrum were at *m/e* 282 (M − MeOH), 265 (M − MeOH − OH) 264 (M − MeOH − H_2O).

The gummy diacetate **8b** was prepared by allowing 0.08 g of **8a** to stand with 1 ml of acetic anhydride and 0.5 ml of pyridine for 10 min at 10 °C. The usual work-up gave 0.075 g of **8b** which was purified by preparative TLC (solvent Bz–ethyl acetate, 1:2).

Anal. Calcd for $C_{20}H_{30}O_8$: C, 60.29; H, 7.59; O, 32.12; mol wt, 398. Found: C, 60.03; H, 7.18; O, 32.31; mol wt (MS), 398.

Other significant peaks in the low-resolution mass spectrum were at *m/e* 314 (M − $2C_2H_2O$), 282 (M − $2C_2H_2O$ − MeOH), and 264 (M − $2C_2H_2O$ − MeOH − H_2O).

A solution of 0.203 g (6.55×10^{-4} mol) of (±)-α-phenylbutyric anhydride and 0.075 g of **8b** in 1 ml of pyridine was kept at room temperature for 48 h. Excess anhydride was destroyed by 2 ml of water. The solution was extracted with ethyl acetate and the extract washed thoroughly with water, 5% $NaHCO_3$ solution, and again with water. The combined aqueous layers were extracted with $CHCl_3$, acidified with 1 N H_2SO_4, and extracted again with $CHCl_3$. Evaporation of the last $CHCl_3$ extracts gave 0.140 g of pure (by TLC criteria) α-phenylbutyric anhydride, $[\alpha]^{24}D$ 2.55°. This corresponded to an optical yield of 14.2%.

Extraction of *Liatris tenuifolia*. Above-ground parts of *L. tenuifolia Nutt.* collected by R. Lazor on Sept 23, 1971, 3 miles east of the junction of U.S. route 319 and State road 155, Thomas County, Ga. (Lazor no. 5587 on deposit in herbarium of Florida State University) was extracted with $CHCl_3$ and worked up in the usual manner. The crude gum, 87 g, was chromatographed over 1.1 kg of silicic acid, 1-l. fractions being collected in the following order: benzene (15 fr), Bz–$CHCl_3$ (4:1, 15 fr), Bz–$CHCl_3$ (1:1, 15 fr), Bz–$CHCl_3$ (1:4, 15 fr), $CHCl_3$ (35 fr), $CHCl_3$–MeOH (99:1, 8 fr), $CHCl_3$–MeOH (49:1, 9 fr), and $CHCl_3$–MeOH (19:1, 9 fr). Fraction 3, wt 0.22 g, consisted of yellow crystals which were identified as euparin (**9**), mp 120 °C, identical with an authentic sample. Fractions 51 and 52, wt 2.2 g, exhibited two spots. These were separated by rechromatography into two apparently homogeneous fractions which polymerized on standing. Fractions 53–57, wt 5.8 g, also exhibited two spots and were combined. Rechromatography afforded 3.4 g of spicatin (**1a**), and 0.8 g of chapliatrin (**11**) as gums, ir, NMR, and TLC behavior identical with those of authentic samples. The later fractions were complex mixtures.

Extraction of *Liatris scabra*. Above-ground parts of *L. scabra* (Greene) K. Schum., wt 2.9 kg, collected by Dr. S. McDaniel on Oct 12, 1974, 1 mile northwest of Wahalak, Kemper County, Miss. (McDaniel no. 19406 on deposit in herbarium of Mississippi State University), was extracted with $CHCl_3$ in the usual manner. The crude gum, wt 15 g, was chromatographed over 600 g of silicic acid. Fractions 1–10 (benzene) eluted nothing. Fractions 11–18 (benzene–$CHCl_3$, 1:1) gave a complex mixture (TLC) whose components were not identified. Fractions 19–25 ($CHCl_3$) eluted 3.8 g of gummy material which exhibited one major spot on TLC. Further purification by TLC on silica gel ($PF_{254-355}$, solvent benzene–ethyl acetate, 1:1) gave a solid, wt 2.5 g, which was recrystallized from ethyl acetate and identified as eleganin (**10**) by melting point, mixture melting point, ir, and NMR spectrum. Fractions 26–35 ($CHCl_3$–MeOH) eluted nothing.

Extraction of *Liatris earlei*. Above-ground parts of *L. earlei* (Greene) K. Schum., wt 4.4 kg, collected by Mr. Robert Stewart on Oct 20, 1974, near Hobolochitto Creek 11 miles northwest of Polarville, Pearl River County, Miss. (Stewart no. 100 on deposit in herbarium of Mississippi State University), were extracted in the usual manner. The crude gum weighed only 7 g and exhibited several spots on TLC. Since different lactones from *Liatris* species frequently exhibit the same R_f, it was not possible to identify any lactone constituents in this way. Attempts to separate the components by preparative TLC using various solvent systems did not yield any homogeneous material.

Extraction of *Liatris pauciflora*. Above-ground parts of *L. pauciflora* Pursh, wt 5.9 kg, collected by Dr. R. K. Godfrey on Sept 8, 1972, 3 miles southeast of Orangedale along State Road 16, St. Johns County, Fla. (Godfrey no. 71997 on deposit in herbarium of Florida State University), was extracted with $CHCl_3$ in the usual fashion but gave a disappointingly low yield (8 g) of gum which contained a mixture of sesquiterpene lactones as revealed by TLC analysis. Column chromatography manner over silicic acid (Mallinckrodt 100 mesh) using benzene, benzene–$CHCl_3$, (3:1 and 1:1), $CHCl_3$, and $CHCl_3$–MeOH (2%) did not yield any homogeneous fractions.

Registry No.—**1a,** 53142-46-4; **3a,** 57576-26-8; **3b,** 58074-48-9; **4a,** 55306-05-3; **4b,** 55186-22-6; **4c,** 58074-49-0; **5,** 55051-66-6; **6,** 55051-67-7; **7,** 55051-68-8; **8a,** 55051-69-9; **8b,** 55051-70-2; **9,** 532-48-9; **10,** 57498-84-7; **11,** 58074-50-3.

References and Notes

(1) Part XI in a series "Constituents of *Liatris* Species". For part X, see W. Herz, I. Wahlberg, C. S. Stevens, and P. S. Kalyanaraman, *Phytochemistry*, **14,** 1803 (1975).

(2) This work was supported in part by Grant CA-13121 from the U.S. Public Health Service through the National Cancer Institute.

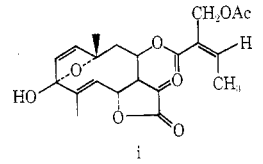
(3) W. Herz, J. Poplawski, and R. P. Sharma, *J. Org. Chem.*, **40,** 199 (1975). The tentative structures **1b** and **2b** suggested in this report were subsequently revised to **1a** and **2a** as the result of an x-ray analysis of spicatin hydrobromide (**3a**).[4]

(4) B. Karlsson, A.-M. Pilotti, A.-C. Wiehager, I. Wahlberg, and W. Herz, *Tetrahedron Lett.*, 2245 (1975). The preparation of **3a** is described in the Experimental Section.

(5) Assays in the KB (cytotoxicity) and PS (P-388 lymphocytic leukemia) systems were carried out under the auspices of Drug Research and Development, Chemotherapy, National Cancer Institute.

(6) W. Herz and R. P. Sharma, *Phytochemistry*, **14,** 1561 (1975).

(7) An earlier study of *L. chapmanii* yielded the cytotoxic and antileukemic sesquiterpene lactone liatrin (i): S. M. Kupchan, V. H. Davis, T. Fujita,



i

M. R. Cox, R. J. Restivo, and R. F. Bryan, *J. Org. Chem.*, **38,** 1853 (1973).

(8) A preliminary communication on pycnolide has appeared: W. Herz and R. P. Sharma, *J. Org. Chem.*, **40,** 392 (1975). In this article as well as in ref 3, *L. pycnostachya* Michx. was erroneously referred to as *L.pycnostachya* (Michx.) Kuntze. Similarly, the correct reference to *L. spicata* is *L. spicata* (L.) Willd., that to *L. graminifolia* (ref 3) is *L. graminifolia* (Walt.) Willd., that to *L. secunda* (ref 6) is *L. secunda* Ell., and that to *L. chapmanii* (ref 7) is *L. chapmanii* T & G.

(9) It is not clear whether spicatin hydrochloride occurs naturally or is an artifact formed during the extraction process which employs refluxing chloroform. Other chlorine-containing guaianolides which have been isolated and could have arisen by similar opening of an epoxide ring are eupachlorin,[10] eupachlorin acetate,[10] eupachloroxin,[10] chlorohyssopifolin[11] (identical with centaurepensin),[12] and graminichlorin.[3]

(10) S. M. Kupchan, J. E. Kelsey, M. Maruyama, J. M. Cassady, J. C. Hemingway, and J. R. Knox, *J. Org. Chem.*, **34,** 3876 (1969).

(11) A. G. Gonzalez, J. Bermejo, J. L. Breton, and J. Triana, *Tetrahedron Lett.*, 2017 (1972); A. G. Gonzalez, J. Bermejo, J. L. Breton, G. M. Massenet, and J. Triana, *Phytochemistry*, **13,** 1193 (1974).

(12) J. Harley-Mason, A. T. Hewson, D. Kennard, and R. C. Pettersen, *J. Chem. Soc., Chem. Commun.*, 460 (1972).

(13) See ref 17 of W. Herz and R. P. Sharma, *J. Org. Chem.*, **40,** 192 (1975).

(14) In **4a, 6,** or **8b,** the C-10 methyl resonance exhibits a chemical shift (1.73–1.75 ppm) characteristic of the *Z* rather than the *E* configuration if geraniol and nerol are taken as models.[15] This is clearly inappropriate; the downfield shift of ca. 0.1 ppm must be due to deshielding by the ester side chain on C-8 (compare **4a** with **8a**). Acetylation of the primary hydroxyl groups results in a small paramagnetic shift of the vinyl methyl signal (compare **4a** with **4b** and **8a** with **8b**), as might be expected for an *E* isomer. On the other hand, conversion to a trityl derivative produces a pronounced diamagnetic shift (compare **4a** with **4c**).

(15) See ref 16 of paper cited in ref 13.

(16) See ref 19 of paper cited in ref 13.

(17) Z. Samek, *Tetrahedron Lett.*, 671 (1970).

(18) For other exceptions, see (a) W. Herz and I. Wahlberg, *J. Org. Chem.*, **38,** 2485 (1973); (b) W. Herz and R. P. Sharma, *J. Org. Chem.*, **40,** 3118 (1975).

(19) W. Stöcklin, T. G. Waddell, and T. A. Geissman, *Tetrahedron*, **26,** 2397 (1970).

(20) For exceptions to this rule, see (a) P. Sundararaman, R. S. McEwen, and W. Herz, *Tetrahedron Lett.*, 3809 (1973); (b) W. Herz and S. V. Bhat, *Phytochemistry*, **12,** 1731 (1973); (c) ref 6 and 18b; (d) W. Herz and R. P. Sharma, *J. Org. Chem.*, **41,** 1015 (1976).

(21) (a) A. T. McPhail and G. A. Sim, *Tetrahedron*, **29,** 1751 (1973); (b) P. J. Cox, G. A. Sim, and W. Herz, *J. Chem. Soc., Perkin Trans. 2,* 459 (1975); (c) P. Sundararaman and R. S. McEwen, *ibid.*, 440 (1975).

(22) Experimental details were specified previously: W. Herz, A. Srinivasan, and P. S. Kalyanaraman, *Phytochemistry*, **14,** 233 (1975).

(23) See paper cited in ref 13.