will be included in the next paper of this series; cf. D. C. Shew and R. A. Manning, Ph.D. Dissertations, University of Arkansas, 1969 and 1971, respectively.
(17) E. Wenkert and D. K. Roychaudhuri, *J. Am. Chem. Soc.*, **80**, 1613 (1958), and references cited therein.
(18) N. Kornblum in "Organic Reactions", Vol. 2, R. Adams, Ed., Wiley, New York, N.Y., 1944, Chapter 7.
(19) W. R. Brode, L. Seldin, P. E. Spoerri, and G. M. Wyman, *J. Am. Chem. Soc.*, **77**, 2762 (1955).
(20) We are grateful to Professor E. Wenkert for samples of (+)-carnosic acid dimethyl ether and (+)-carnosol dimethyl ether.
(21) F. C. Chang and N. F. Wood, *Tetrahedron Lett.*, 2969 (1964).
(22) G. A. Russell and G. J. Mikol, *J. Am. Chem. Soc.*, **88**, 5498 (1966).
(23) J. D. McChesney, Ph.D. Dissertation, Indiana University, 1965.
(24) The hydrogenation by-product mentioned in footnote 21 of ref 12 is also **31** and not the still unknown *cis*-decalone.
(25) W. L. Meyer, D. L. Davis, L. Foster, A. S. Levinson, V. L. Sawin, D. C. Shew, and R. F. Weddleton, *J. Am. Chem. Soc.*, **87**, 1573 (1965).
(26) W. L. Meyer, D. L. Davis, A. W. McCollum, J. W. Morgan, N. S. Starr, and D. A. Templer, *J. Am. Chem. Soc.*, **94**, 466 (1972).
(27) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).
(28) Saponification of **30** and acid-catalyzed lactonization of the resulting hydroxy acid also affords **32**, but this result is less useful as evidence for the configuration of **30** in view of the possible intervention of an allylic 7-carbonium ion during lactonization (R. W. Huffman and W. L. Meyer, unpublished results).
(29) E. W. Garbisch, Jr., *J. Am. Chem. Soc.*, **86**, 5561 (1964).
(30) M. Verzele, M. Acke, and M. Anteunis, *J. Chem. Soc.*, 5598 (1963); S. Nishimura, T. Itaya, and M. Shiota, *Chem. Commun.*, 422 (1967).
(31) The alternative possibility that some or all of **28–31** are formed through intermediacy of a Δ^6 enol which results from initial 1,4 reduction of the enone should not be discounted, however; cf. R. L. Augustine, D. C. Migliorini, R. E. Foscante, C. S. Sodano, and M. J. Sisbarro, *J. Org. Chem.*, **34**, 1075 (1969), and references cited therein.
(32) The observation that **33** was reduced only very slowly, if at all, at 1 atm over the limited number of Pd/C samples which were examined suggests that hydrogenolysis of **30** may proceed through a π-allyl adsorbed intermediate[33] to form a mixture of **33** and its unisolated Δ^6 isomer, with the latter rather than **33** being the precursor of decalin **34**.
(33) Cf. W. R. Moore, *J. Org. Chem.*, **84**, 3788 (1962); J. J. Rooney, F. G. Gault, and C. Kemball, *J. Catal.*, **1**, 255 (1962).
(34) Hydrogenation of 10-carbethoxy-4,4-dimethyl-Δ^5-octalin derivatives to afford mixtures of trans- and cis-fused products has been observed, however, with systems other than the 7-ketone, including **33** itself; cf. R. F. C. Brown, *Aust. J. Chem.*, **17**, 47 (1964); R. S. Schroeder, Ph.D. Dissertation, Indiana University, 1970.
(35) The low extinction coefficients indicate that the sample used for these uv determinations had undergone partial decomposition.
(36) This ethoxyl resonance shows distinct ABC_3 character with $J_{AB} = J_{AC} = 7$ Hz, but was not precisely analyzed; these chemical shifts are only estimated visually, ±0.02 ppm; cf. ref 25.
(37) We are grateful to the Eastman Chemical Co. for a generous gift of this material.
(38) We were unable to find conditions which would bring about this hydrogenolysis without sequential exposure to two fresh batches of catalyst.
(39) It is possible that the recovered **6** is formed by retro-Michael reaction during the alkaline extraction process, and it is therefore in general desirable to avoid this step in the isolation sequence.
(40) T. L. Moore, *J. Org. Chem.*, **32**, 2786 (1967).
(41) The ethyl isovalerate was prepared from isovaleryl chloride obtained from K and K Laboratories, Inc., Plainview, N.Y. Although the ester distills sharply at 134 °C it contains ca. 10% of an impurity (τ 8.85, d, J = 7 Hz) which originated in the acid chloride and could not be removed by careful distillation of either the acid chloride or the ester.[1a] A related impurity (τ 8.88) is present in the distilled β-keto sulfoxide.
(42) R. E. Ireland and J. A. Marshall, *J. Org. Chem.*, **27**, 1620 (1962); R. F. Church and R. E. Ireland, *ibid.*, **28**, 17 (1963).
(43) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).
(44) This experiment was conducted by Mr. B. H. Fahoum; microanalysis by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Sesquiterpene Lactones of *Eupatorium hyssopifolium*. A Germacranolide with an Unusual Lipid Ester Side Chain[1]

Werner Herz* and Ram P. Sharma

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

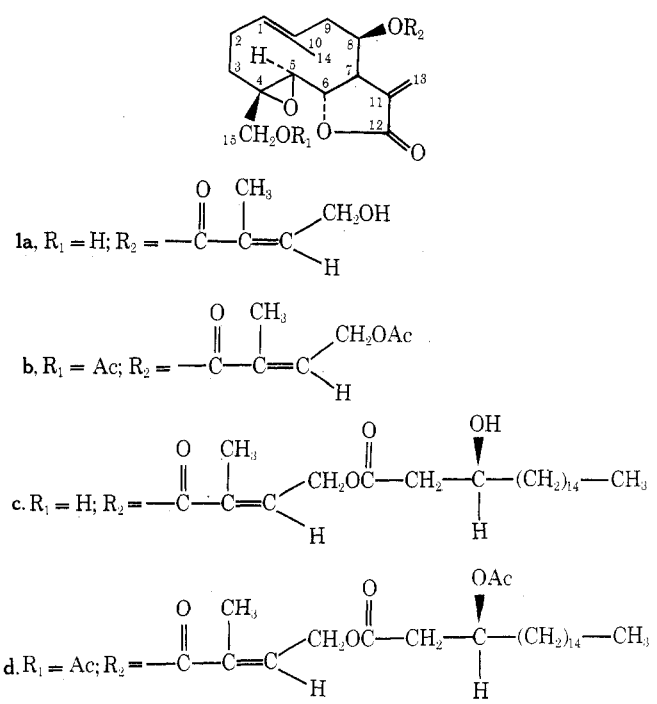
Received October 6, 1975

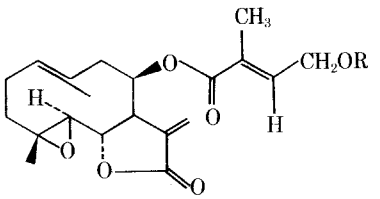
The isolation and structure determination of three new closely related germacranolides, eupassopin, eupassopilin, and eupassofilin, from *Eupatorium hyssopifolium* L. are reported. Eupassofilin is highly unusual in being the first ester of D(−)-3-hydroxyoctadecanoic acid isolated from a higher plant. Generalizations for the ease of hydrolysis of five-carbon unsaturated ester side chains in germacranolides are presented.

Chemical examination of several *Eupatorium* species *sensu stricto*[2] has produced a number of cytotoxic and antitumor germacranolides and guaianolides.[3-7] In the present communication, we report the isolation and structure determination from *Eupatorium hyssopifolium* L. of three new noncrystalline germacranolides, eupassopin, eupassopilin, and eupassofilin, the last of which is linked in an unprecedented way to a D(−)-β-hydroxystearoyl ester side chain.[8]

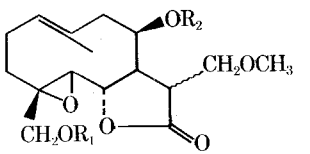
For the sake of convenience we discuss first the structure of eupassopin (**1a**), $C_{20}H_{26}O_7$ (high-resolution mass spectrum), $[\alpha]D$ −137.5°, which was a conjugated γ-lactone of the type partially shown in A (ir bands at 1760 and 1650 cm^{-1}), as evidenced by the usual criteria of narrowly split doublets at 6.25 and 5.67 ppm (H_a and H_b) in the ^1H NMR spectrum (Table I) and the appropriate signals in the ^{13}C NMR spectrum, particularly the triplet at 122.8 ppm (Table II). D_2O exchange sharpened a two-proton AB system at 3.89 d and 3.75 d (J = 12 Hz) and a two-proton broad doublet at 4.24 ppm (J = 6 Hz); hence eupassopin appeared to contain two primary hydroxyl groups.

Acetylation of eupassopin indeed furnished a diacetate **1b** (two new acetate signals at 2.14 and 2.08 ppm), but while the two-proton broad doublet had moved downfield from 4.24 to 4.80 ppm as expected (see Table I), only one of

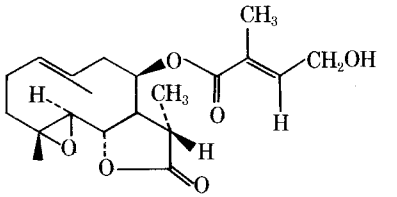




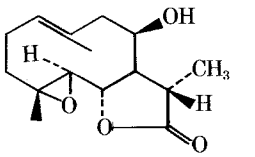
2a, R = H
b, R = Ac



3a, R_1, R_2 = H
b, R_1, R_2 = Ac



5

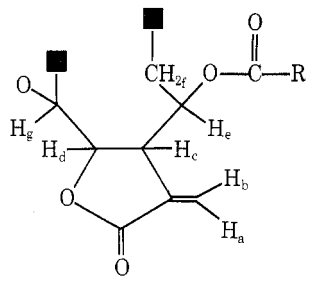


6

Table I. ^{1}H NMR Spectra of *E. Hyssopifolium* Constituents and Derivatives[a]

Compd	H-1	H-5	H-6	H-7	H-8	H-9	H-13	H-14[b]	H-15	H-3′	H-4′[c]	H-5′[b]	Misc
1a	5.29 dd br (11, 2)	2.97 d (9)	4.77 dd (9, 9)	3.12 m	5.67 d br (5, <1, <1)	2.70 dd (15, 5) 2.33 d br (15, <1)	6.25 d (3.5) 5.67 d (3.2)	1.65 br	3.89 d (12) 3.75 d (12)	6.71 m	4.24 d br (6)	1.72 br	
1b	5.39 dd br (11, 2)	3.02 d (9)	4.5 dd (9, 9)	3.3 m	5.76 d br (5, <1, <1)	2.80 dd (15, 5) 2.46 d br (15, <1)	6.37 d (3.5) 5.76 d (3.2)	1.74 br	4.80 d (12) 3.80 d (12)	6.70 m	4.80 d br (6)	1.88 br	2.08 (Ac) 2.14 (Ac)
1c	5.33 dd br (11, 2)	2.98 d (9)	4.88 dd (9, 9)	3.11 m	5.74 d br (5, <1, <1)	2.77 dd (15, 5) *d*	6.36 d (3.5) 5.74 d (3.2)	1.70 br	3.89 d (12) 3.79 d (12)	6.69 m	4.77 d br (6)	1.84 br	4.03 m (OH) 1.24 (~24 H) 0.86 t (7)[b]
1d	5.36 dd br (11, 2)	2.97 d (9)	4.46 dd (9, 9)	3.17 m	5.74 d br (5, <1, <1)	2.80 dd (15, 5) 2.44 d br (15, <1)	6.36 d (3.5) 5.74 d (3.2)	1.73 br	4.73 d (12) 3.79 d (12)	6.70 m	4.75 d br (6)	1.86 br	2.01, 2.12 (Ac) 5.21 q (6.5) 1.24 (~24 H) 0.87 t (7)[b]
2a	5.28 dd br (11, 2)	2.82 d (9)	4.41 dd (9, 9)	3.17 m	5.70 d br (5, <1, <1)	2.73 dd (15, 5) 2.35 d br (15)	6.31 d (3.5) 5.70 d (3.2)	1.67 br	1.33[b]	6.88 m	4.30 d br (6)	1.75 br	
2b	5.33 dd br (11, 2)	2.85 d (9)	4.41 dd (9, 9)	3.21 m	5.77 d br (5, <1, <1)	2.85 dd (15, 5) 2.40 d br (15)	6.36 d (3.5) 5.73 d (3.2)	1.70 br	1.35[b]	6.66 m	4.71 d br (6)	1.84 br	2.07 (Ac)
3a	5.25 dd br (11, 2)	2.92 d (9)	4.71 dd (9, 9)	*d*	4.33 d br (5, <1, <1)	*d*	3.70 dd (10.5, 3.5) 3.60 dd (10.5, 3.5)	1.83 br	3.90 d (12) 3.71 d (12)				3.35 (OMe)
3b	5.37 dd br (11, 2)	2.87 d (9)	4.20 dd (9, 9)	*d*	5.37 d br (5, <1, <1)	*d*	3,6 m[c]	1.71 br	4.64 d (12) 3.74 d (12)				3.34 (OMe) 2.09, 2.11 (Ac)
5	5.27 dd br (11, 2)	2.76 d (9)	4.33 dd (9, 9)	*d*	5.40 d br (5, <1, <1)	*d*	1.26 d[b] (6.8)	1.68 br	1.35[b]	6.83 m	4.35 d br (6)	1.82	
6[e]	5.26 dd br (11, 2)	2.95 d (9)	4.79 dd (9, 9)	*d*	4.43 t br[f] (5)	*d*	1.29 d[b] (6.8)	2.01 br	1.39[b]				

[a] Run in $CDCl_3$ at 270 MHz on a Bruker HX-270 instrument with Me_4Si as internal standard, unless otherwise specified. Values are in parts per million: d, doublet; t, triplet; q, quintet; br, broadened singlet. Unmarked signals are singlets. Values in parentheses are coupling constants in hertz. [b] Intensity three protons. [c] Intensity two protons. [d] Signal obscured. [e] Run in pyridine d_5. [f] Changes to broad doublet on D_2O exchange.

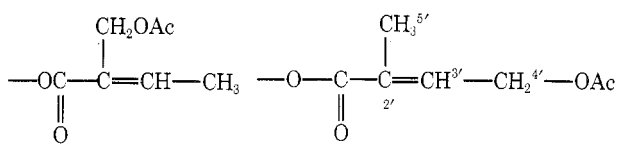


A

the protons in the AB system originally near 3.8 ppm had experienced the expected paramagnetic shift, the other signal remaining at 3.80 ppm. Consequently, it appeared initially that eupassopin contained one primary and one secondary hydroxyl group. However, the ^{13}C NMR spectrum (Table II) displayed two low-field triplets at 59.6 and 60.8 ppm which could only be assigned to two primary carbon atoms carrying hydroxyl groups. Decoupling experiments on **1b** which confirmed this assignment are described below. A possible explanation for the unusual behavior on acetylation of the AB system representing $-CH_2OH$ will be presented later.

Spin-decoupling experiments on **1b** involving H_a and H_b established the location of the H_c multiplet at 3.30 ppm. Irradiation at the frequency of H_c collapsed H_c and H_b into singlets, converted a doublet of doublets at 4.50 ppm (J_1 = J_2 = 9 Hz) into a doublet, and affected a broadened doublet at 5.76 ppm (partially obscured by H_b). Thus H_d and H_e were at 4.50 and 5.76 ppm, respectively, or the reverse. The chemical shift of the lower field proton suggested that it was under an ester rather than under a lactone oxygen, especially since the ir spectrum indicated the presence of an additional carbonyl function usually associated with a conjugated ester (at 1710 cm^{-1} in **1a**, 1715 cm^{-1} in **1b**). Hence the signal at 4.50 ppm was provisionally assigned to H_d and the signal at 5.76 ppm to H_e.

On the basis of the molecular formula, the unsaturated ester function had to consist of five carbon atoms. Since the low-resolution mass spectrum of **1a** exhibited prominent peaks at *m/e* 262 ($M^+ - C_5H_8O_3$) and 99 ($C_5H_7O_2$, base peak), it was concluded that an ester side chain of type B or C was present in **1b**; a clear decision in favor of C was possible on inspection of the NMR spectrum, which revealed the presence of a vinyl multiplet at 6.70 ppm (H-3′) coupled to a two-proton doublet at 4.80 ppm (H-4′) and the broad vinyl methyl of H-5′ at 1.88 ppm.



B C

Irradiation at 5.76 ppm (H_b and H_e) affected the H_c multiplet, collapsed a doublet of doublets at 2.80 ppm (J_1 = 5, J_2 = 15 Hz) to a doublet (J = 15 Hz), and sharpened a broadened doublet (J = 15 Hz) at 2.46 ppm. Irradiation at 2.80 ppm converted the broadened doublet of H_e to a broadened singlet and the broadened doublet at 2.46 ppm to a broad singlet. Analogously, irradiation at the frequency corresponding to 2.46 ppm converted the signal at 2.80 ppm to a doublet (J = 5 Hz) and affected the signal at 5.76 ppm. Consequently, H_e was adjacent to a methylene group (H_f of A) which was in turn adjacent to a quaternary center.

Irradiation at the frequency of H_d collapsed a sharp doublet at 3.02 ppm (J = 9 Hz) to a singlet. The proton responsible for this signal (H_g) was undoubtedly epoxidic because of its chemical shift, the empirical formula of eupassopin, which required one extra oxygen, and because the signal did not undergo a paramagnetic shift on acetylation of **1a** to **1b**. This assignment was also in agreement with the presence in the ^{13}C NMR spectrum of three doublets in the range 66–75 ppm, the doublets of C_d and C_e appearing at 75.0 and 74.0 ppm, respectively, and that of the carbon carrying H_g at 66.8 ppm (single frequency off-resonance decoupling). The other terminus of the epoxide ring was represented by a carbon singlet at 64.5 ppm.

Table II. ^{13}C NMR Spectra of Constituents of *E. Hyssopifolium*[a]

Carbon atom[b]	1a	1c	2a
1	128.8 d	128.8 d	128.5 d
2	31.8 t	32.0 t	35.6 t
3	23.9 t	23.9 t	23.9 t
4	64.5	64.4	61.8
5	66.8 d	67.0 d	66.2 d
6	75.0 d	74.8 d	75.6 d
7	49.8 d	49.8 d	49.1 d
8	74.0 d	74.2 d	74.0 d
9	44.0 t	44.1 t	43.5 t
10	132.2	132.1	131.2
11	127.0	129.3	126.7
12	169.0	168.5	168.5
13	122.8 t	122.7 t	122.3 t
14	12.8 q	12.9 q	12.4 q
15	60.8 t	60.8 t	16.9 q
1′	166.2	165.7	165.9
2′	136.4	136.3	136.0
3′	142.9 d	137.3 d	142.6 d
4′	59.6 t	61.2 t	59.1 t
5′	19.6 q	19.6 q	19.4 q
1″		172.5	
2″		41.6 t	
3″		60.2 d	
4″		37.0 t	
5″–16″		32.1 t 32.0 t 29.8 t (8 by difference) 29.4 t 25.6 t	
17′		22.8	
18′		14.2 q	

[a] Run in $CDCl_3$ on a Bruker HX-270 instrument. Unmarked signals are singlets. [b] Assignments based on predicted shifts, comparisons with data in our files and in the literature, and by single frequency off-resonance decoupling.

To complete the empirical formula, the following additional facts had to be accommodated. (1) The presence of a second primary hydroxyl group whose protons were mutually coupled but not coupled to any of the remaining eight protons indicated by the ^1H NMR spectrum and by molecular spectrometry. (2) The presence of the function $-CH{=}C{-}CH_3$ revealed by a slightly broadened three-proton resonance at 1.74 ppm and a broad doublet at 5.39 ppm in the ^1H NMR spectrum of **1b** and confirmed by a singlet at 132.2 ppm, a doublet at 128.8 ppm, and a quartet at 12.8 ppm in the ^{13}C NMR spectrum of **1a**.

Irradiation at the frequency of the vinyl proton not only sharpened the vinyl methyl signal, but caused some changes in the methylene region. Conversely, irradiation at 1.74 ppm converted the vinyl proton resonance to a doublet of doublets which required that the vinyl proton be next to a methylene group. The remaining two protons of the empirical formula must also be part of a methylene group since the ^{13}C NMR spectrum exhibited three methylene triplets at 44.0 (C-9), 31.8 (C-2), and 23.9 ppm (C-3). Consequently, the gross formula of eupassopin must be represented by **1a**.

Before taking up the stereochemistry of eupassopin, we would like to discuss the structure of eupassofilin (**1c**), whose NMR spectrum was very similar to that of **1a.** In addition to the signals present in **1a,** eupassofilin had an additional one-proton multiplet at 4.03 ppm, a triplet at 0.86 ppm, and a very strong broad peak centered at 1.24 ppm integrating for more than 24 protons, as well as some more protons in the methylene region between 2 and 2.5 ppm. The two-proton H-4′ signal of **1a** was shifted downfield to 4.77 ppm, thus suggesting that the primary hydroxyl group of the five-carbon side chain was now esterified. This conclusion was reinforced by the observation that the ir spectrum of eupassofilin displayed a double-strength peak at 1715 cm^{-1}. Extended purification did not alter the spectral characteristics of **1c;** hence it was suspected that the five-carbon side chain of **1a** might be esterified in eupassofilin by a lipid moiety. Unfortunately, conventional electron impact mass spectrometry on **1c** resulted in loss of the entire side chain and did not permit determination of the nature of the lipid fragment. On the other hand, chemical ionization mass spectrometry yielded a significant peak at *m/e* 300 which, it was believed, could correspond to a fragment of formula $C_{18}H_{36}O_3$.

Acetylation of **1c** afforded a diacetate **1d,** partially as the result of acetylation of the primary hydroxyl group present at C-15 of **1a** and **1c** (NMR spectrum). Simultaneously, however, the multiplet of **1c** at 4.03 ppm had moved downfield to 5.21 ppm and now appeared as a quintet (J = 6.5 Hz), an observation which suggested that the newly formed acetoxyl was adjacent to two methylene groups.

The ^{13}C NMR spectrum of eupassofilin (Table II) exhibited all the frequencies of **1a** as well as a carbonyl carbon frequency at 172.5 ppm due to the additional ester function, a doublet at 68.2 ppm undoubtedly associated with the secondary hydroxyl group, a quartet at 14.2 ppm, presumably the terminal methyl of the lipid side chain and associated with the protonic methyl triplet at 0.86 ppm, a very strong triplet at 29.8 ppm due to at least six methylene groups, and seven well-separated triplets at 22.8, 25.6, 29.4, 32.0, 32.1, 37.0, and 41.6 ppm. Analysis of these chemical shifts[9] suggested that the hydroxyl group might possibly be located at C-3 of the lipid side chain, i.e., that eupassofilin might be the 3-hydroxystearoyl ester **1c** of eupassopin, if the chemical ionization results could be given credence.

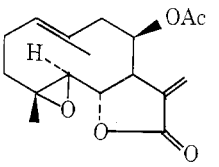
This conclusion was placed on a secure footing by hydrolysis of eupassofilin with methanolic sodium methoxide, a procedure which permitted isolation of the lipid fragment as the methyl ester. Two products were obtained and isolated by preparative TLC. The more polar material was identified as **3a,** also obtained by hydrolysis of **1a** with potassium carbonate in aqueous methanol (vide infra). The less polar substance was methyl D(−)-3-hydroxyoctadecanoate (**4**): mp 55 °C; $[\alpha]_D$ −12.5° (lit. mp 55.5–56.5 °C, $[\alpha]_D$ −15°);[10] molecular formula $C_{19}H_{38}O_3$ (high-resolution mass spectrum); low-resolution mass spectrum identical with that reported for methyl (±)-3-hydroxyoctadecanoate[11] and highly characteristic of a β-hydroxy fatty acid ester (see Experimental Section); ^1H NMR spectrum fully consonant with the proposed formula (see Experimental Section).

The isolation of D(−)-3-hydroxystearic acid as an ester component of a secondary metabolite produced by a higher plant is very unusual. The substance has been isolated previously[9] from species of the red yeast *Rhodotorula* which produce extracellular glycolipids consisting of a mixture of mannitol and pentitol esters of D(−)-3-hydroxypalmitic and D(−)-3-hydroxystearic acids, one molecule of the long-chain acid being attached to each polyol molecule and most of the remaining hydroxyl groups, including the one in the fatty acid moiety, being acetylated. The only higher plant source reported so far as we know is *Cistus ladaniferus* L.,[10] the gum resin of which contains a mixture of D(−)-β-hydroxy acids in the range C_{18}–C_{30}.

The molecular formula of eupassopilin, the third constituent of *E. hyssopifolium,* contained one oxygen less than that of **1a.** In the NMR spectrum, the two-proton AB system at C-15 was absent and replaced by a methyl singlet at 1.33 ppm. The remaining signals were essentially identical with those of **1a** (Table I); hence formula **2a** could be written for eupassopilin. This was also in full agreement with the ^{13}C NMR spectrum (Table II).

We now turn to the stereochemistry of the three new germacranolides. If it be assumed that H-7 is α as in all compounds whose absolute stereochemistry has been established by x-ray analysis or chemical correlations, the large value of $J_{6,7}$ (9 Hz) and $J_{5,6}$ (9 Hz) requires that the H-6 be β and H-5 be α. That the lactone ring is trans fused is also supported by the magnitude of $J_{7,13a}$ and $J_{7,13b}$ (>3 Hz).[12] Furthermore, in going from **1a** to **1b,** or from **1c** to **1d,** H-6 moves upfield which means that it comes into the shielding cone of the acetate carbonyl at C-15. Molecular models show that this is possible only if H-6 and the C-4, C-15 bond point in the same direction, i.e., the C-4, C-15 bond must be β as well. The models further suggest that this is possible only if the epoxide is derived from a C-4, C-5 trans double bond. Lastly, the very small value of $J_{7,8}$ (<1 Hz) requires that the ester side chain be β oriented; the absence of an NOE between H-1 and the C-10 methyl group suggests that the 1,10 double bond is trans.

Unequivocal proof for the proposed stereochemistry was obtained in the following manner. Since eupassopilin (**2a**) was available in relatively large quantity, it was hydrolyzed with K_2CO_3 in aqueous methanol to **3a** and the crystalline product acetylated to **3b.** The chemical shifts of C-1, C-5, C-6, C-7, and C-8 and the coupling constants $J_{5,6}$, $J_{6,7}$, and $J_{7,8}$ were very similar to those of lipiferolide (**7**), a sub-



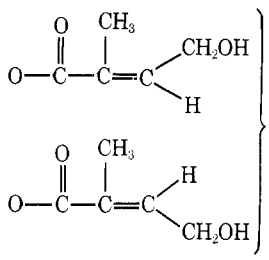
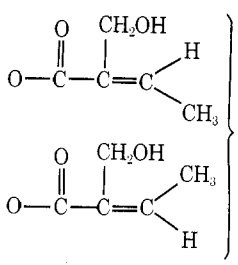
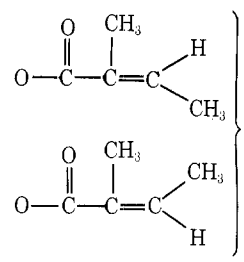
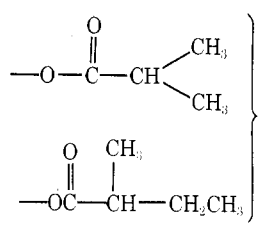
7

stance of established stereochemistry which was reported[14] at the time when our work was in progress. In order to establish a direct correlation with lipiferolide, eupassopilin (**2a**) was reduced with sodium borohydride and the product **5** hydrolyzed with potassium carbonate in aqueous methanol to **6.** The melting point, rotation, and spectroscopic properties of this material were identical in all respects with those of 11,13-dihydrodeacetyllipiferolide.[14,15]

With the stereochemistry of the new substances established, a comment on the CD curves is in order. Eupassopilin and eupassofilin exhibit a negative Cotton effect in the 250-nm region, whereas eupassopin displays a positive Cotton effect. Thus it appears that the side chain attached to C-8 has some effect on the CD curve, a possibility to which we have referred earlier[16] and which obviously interferes with application of the Stöcklin–Waddell–Geissman rule.[17]

It has been mentioned earlier that on acetylation of **1a** (and of **1c**) only one of the protons on C-14 exhibits the expected downfield shift. The most probable explanation, derived from inspection of molecular models, is that restricted rotation around the C-4, C-15 bond results in a fixed conformation which places one of the protons in the shielding cone of the carbonyl attached to C-8 (or possibly the epoxide function[18]).

Our experience with the compounds from *E. hyssopifolium* and a large number of other sesquiterpene lactones[19] containing five-carbon unsaturated ester side chains most of which are gummy leads us to make some concluding remarks about the ease with which such side chains can be removed. These procedures, which generally lead to crystalline substances, may be useful to other workers and are listed below.

	Hydrolyzed by
	K_2CO_3—MeOH—H_2O for 20—30 min at room temperature
	K_2CO_3—MeOH—H_2O extremely slow at room temperature $NaOCH_3$—MeOH for 2—4 h at room temperature
	K_2CO_3—MeOH—H_2O no reaction at room temperature $NaOCH_3$—MeOH for 6—12 h at room temperature
	K_2CO_3—MeOH—H_2O no reaction at room temperature $NaOCH_3$—MeOH no reaction at room temperature

Experimental Section

Experimental details have been specified previously.[20]

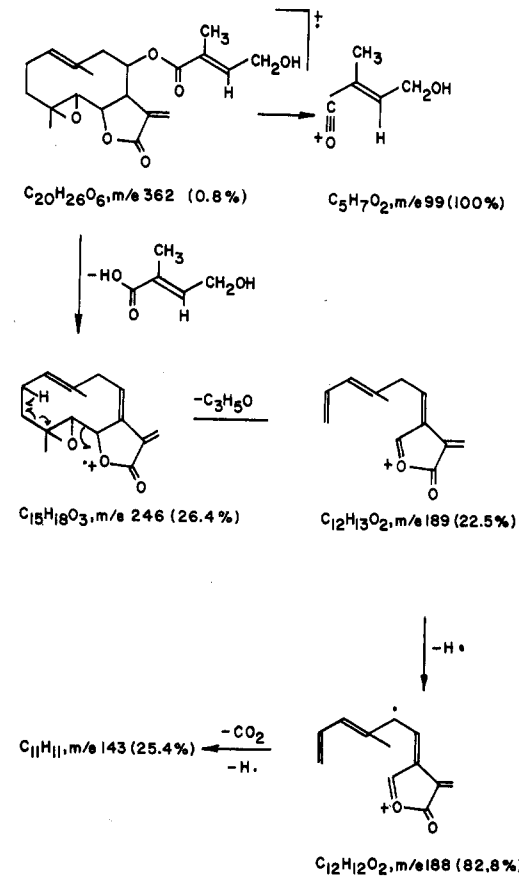
Extraction of *Eupatorium hyssopifolium*. Above-ground parts of *E. hyssopifolium* L., wt 11.5 kg, collected by Mr. R. F. Doren on August 9, 1972, in Wakulla County, Florida, between Wakulla and Newport, was extracted with chloroform and worked up in the usual manner.[21] The crude gum, wt 80.5 g, was chromatographed over 1.15 kg of silicic acid (Mallinckrodt 100 mesh) packed in benzene. The column was eluted with solvents of increasing polarity, 1-l. fractions being collected.

Elution with benzene (fractions 1–10) gave an oil (wt 10.2 g) which appeared to be a complex mixture of several compounds. Elution with benzene–$CHCl_3$ (10:1, fractions 11–20) gave a pale yellow gum (1.5 g) which on further purification over silica gel (Merck $PF_{254-356}$, solvent benzene–ethyl acetate, 6:1) gave 1.1 g of eupassofilin (**1c**) which could not be induced to crystallize: $[\alpha]^{22}D$ −143° (*c* 0.14, MeOH); CD curve $[\theta]_{275}$ 0, $[\theta]_{250}$ +954 (max); uv end absorption; ir bands (film) at 3450 (–OH), 1765 (lactone), 1715 (double intensity, esters), 1650 (exocyclic double bond), 1250, 1140, and 1080 cm^{-1}.

Anal. Calcd for $C_{38}H_{60}O_9$: C, 69.06; H, 9.15; O, 21.79. Found: C, 68.51; H, 9.15; O, 22.55.

The low-resolution mass spectrum failed to give the molecular ion; the first major peak appeared at *m/e* 262 which corresponds to loss of the entire side chain of 23 carbon atoms. Other significant peaks appeared at *m/e* 244 (M^+ − side chain − H_2O), 232 (M^+ − side chain − CH_3OH), and 99 (base peak). The chemical ionization spectrum gave a significant peak at *m/e* 300 which could correspond to $C_{18}H_{36}O_3$.

Scheme I. Mass Spectral Fragmentation of Eupassopilin



Acetylation of 0.11 g of **1c** with 1 ml of acetic anhydride in 0.5 ml of pyridine at room temperature overnight gave after the usual work-up 0.10 g of the diacetate **1d** as a gum: ir bands at 1770, 1715, 1650, 1730, 1140, and 1040 cm^{-1}.

Anal. Calcd for $C_{42}H_{64}O_{11}$: C, 67.60, H; 8.60; O, 23.65. Found: C, 66.94; H, 8.70; O, 23.91.

Further elution of the column with benzene–$CHCl_3$ (10:1, fractions 21–30) gave 3.5 g of gum which showed a major spot on TLC. Purification by preparative TLC (silica gel PF 254–356, solvent benzene–ethyl acetate, 5:1) yielded eupassopilin (**2a**) as a noncrystallizable gum: $[\alpha]^{22}D$ −161° (*C* 0.31, MeOH); CD curve $[\theta]_{290}$ 0, $[\theta]_{235}$ −7590 (minimun); ir bands (film) at 3450 (OH), 1765 and 1650 (conjugated lactone), 1710 (ester), 1250, 1140, 1030, 740 cm^{-1}; high-resolution mass spectrum *m/e* (composition, %) 362 (M^+, $C_{20}H_{26}O_6$, 0.8), 263 (M^+ − $C_5H_7O_2$, $C_{15}H_{19}O_4$, 8.1), 262 (M^+ − $C_5H_8O_2$, $C_{15}H_{18}O_4$, 2.7), 247 (M^+ − $C_5H_7O_3$, $C_{15}H_{19}O_3$, 4.6), 246 (M^+ − $C_5H_8O_3$, $C_{15}H_{18}O_3$, 26.4), 245 (M^+ − $C_5H_9O_3$, $C_{15}H_{17}O_3$, 17.9), 228 ($C_{15}H_{16}O_2$, 15.1), 189 ($C_{12}H_{13}O_2$, 22.5), 188 ($C_{12}H_{12}O_2$, 81.8), 143 ($C_{11}H_{11}$, 25.4), 142 ($C_{11}H_{10}$, 11.9), 99 ($C_5H_7O_2$, 100). Scheme I is a rationalization of the major peaks.

Anal. Calcd for $C_{20}H_{26}O_6$: mol wt, 362.1728. Found: mol wt, 362.1744 (MS).

Acetylation of 0.1 g of **2a** at room temperature overnight gave, after the usual work-up, the monoacetate **2b** as a gum: yield 0.094 g; ir bands ($CHCl_3$) at 1770 and 1650 (conjugated lactone), 1735 (acetate), 1720 (esters), 1230, 1140, and 900 cm^{-1}. The low-resolution mass spectrum gave M^+ at *m/e* 404 ($C_{22}H_{28}O_7$); other major peaks were at *m/e* 362 (M^+ − C_2H_2O), 345 (M^+ − $C_2H_3O_2$), 263 (M^+ − $C_7H_9O_3$), 246 (M^+ − $C_7H_{10}O_4$), 188 ($C_{12}H_{12}O_2$), and 99 (base peak).

Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98; O, 27.69. Found: C, 64.58; H, 6.79; O, 27.99.

Elution of the column with benzene–$CHCl_3$ (1:1, fractions 31–40) gave the major component (15.1 g). Purification by preparative TLC over silica gel (solvent benzene–ethyl acetate, 1:1) gave eupassopin (**1a**) as a gum: $[\alpha]^{22}D$ −137.5° (*c* 0.4, MeOH); CD curve $[\theta]_{290}$ 0, $[\theta]_{235}$ −6038 (minimum); ir bands (film) at 3400 (OH), 1760 and 1650 (conjugated lactone), 1710 (ester), 1250, 1140, and

1030 cm^{-1}. The low-resolution mass spectrum gave the molecular ion at *m/e* 378 ($C_{20}H_{26}O_7$); other major peaks were at *m/e* 346 ($M^+ - CH_3OH$), 262 ($M^+ - C_5H_8O_3$), 244 ($M^+ - C_5H_8O_3 - H_2O$), 231 ($M^+ - C_5H_8O_3 - CH_3OH$), and 99 ($C_5H_7O_2$, base peak).

Anal. Calcd for $C_{20}H_{26}O_7$: mol wt, 378.1678. Found: mol wt, 378.1680 (MS).

Acetylation of 0.15 g of **1a** with 1 ml of acetic anhydride in 0.5 ml of pyridine at room temperature overnight gave the noncrystalline diacetate **1b**, ir bands (film) at 1770 and 1655 (lactone), 1740, 1735 (two acetates), 1710 (ester), 1250, 1140, 1040, and 740 cm^{-1}. The low-resolution mass spectrum gave the molecular ion at *m/e* 462 ($C_{24}H_{30}O_9$); other major peaks were at *m/e* 470 ($M^+ - C_2H_2O$), 402 ($M^+ - CH_3CO_2H$), 360 ($M^+ - C_2H_2O - CH_3CO_2H$), 342 ($M^+ - 2CH_3CO_2H$), 304 ($M^+ - C_7H_{10}O_4$), 267 ($M^+ - C_2H_2O - C_7H_{10}O_4$), 244 ($M^+ - CH_3CO_2 - C_7H_{10}O_4$), 188, and 99 (base peak).

Anal. Calcd for $C_{24}H_{30}O_9$: C, 62.33; H, 6.54; O, 31.13. Found: C, 62.08; H, 6.24; O, 30.59.

Preparation of 3a and 3b. A solution of 0.15 g of **1a** in 15 ml of MeOH, 2 ml of water, and 0.5 g of K_2CO_3 was stirred in a nitrogen atmosphere for 25 min when TLC indicated that all of **1a** had reacted. The mixture was diluted with water and extracted with $CHCl_3$. The washed and dried extract was evaporated; the solid residue was purified by preparative TLC on silica gel (solvent benzene–ethyl acetate, 1:1) to give 0.05 g of **3a:** mp 145°; ir bands at 3440, 3400, 1750, 1050, and 980 cm^{-1}; low-resolution mass spectrum peaks at *m/e* 312 (M^+), 281 ($M^+ - CH_3O$), 249 ($M^+ - CH_3O - CH_3OH$), 231 ($M^+ - OCH_3 - CH_3OH - H_2O$).

Anal. Calcd for $C_{16}H_{24}O_6$: C, 61.52; H, 7.74; O, 30.73. Found: C, 61.67; H, 7.68; O, 31.06.

Acetylation of 0.062 g of **3a** at room temperature for 24 h gave a solid which was recrystallized from ethyl acetate and methanol to yield **3b:** wt 0.06 g; mp 180 °C; ir bands (Nujol) at 1790, 1725, 1250, 1270, 1170, 1050, and 990 cm^{-1}; low-resolution mass spectrum peaks at *m/e* 396 (M^+), 354 ($M^+ - C_2H_2O$), 336 ($M^+ - CH_3CO_2H$), 294 ($M^+ - C_2H_2O - CH_3CO_2H$), 276 ($M^+ - 2CH_3CO_2H$).

Anal. Calcd for $C_{20}H_{28}O_8$: C, 60.59; H, 7.12; O, 32.29. Found: C, 61.29; H, 7.07; O, 32.07.

Methanolysis of Eupassofilin. A solution of 0.2 g of **1c** in 10 ml of MeOH and 0.1 g of sodium methoxide was stirred in a nitrogen atmosphere for 30 min (TLC control). The mixture was worked up as described in the previous section. The crude product was separated by preparative TLC (silica gel, solvent benzene–ethyl acetate, 2:1) into two major bands. The less polar material (R_f 0.8) was recrystallized from ethyl acetate and characterized as the methyl ester of D(−)-3-hydroxyoctadecanoic acid (**4**): yield 0.020 g; mp 55 °C; $[\alpha]D$ −12.5° (*c* 1.0, $CHCl_3$); ir bands at 3500, 1725, 1250, 1180, and 1000 cm^{-1}. The low-resolution mass spectrum gave the molecular ion peak at *m/e* 314 ($C_{19}H_{38}O_3$); other major peaks were at *m/e* 296 ($M^+ - H_2O$), 283 ($M^+ - CH_3O$), 264 ($M^+ - CH_3OH - 2H_2O$), 222, 103 ($C_4H_7O_3$, base peak), and 74. The ^1H NMR spectrum exhibited a one-proton multiplet at 4.00 ppm, a methoxyl singlet at 3.72 ppm, a methyl triplet (J = 7 Hz) at 0.88 ppm, and a large peak centered at 1.24 ppm integrating for more than 24 protons. In addition there was a doublet of doublets at 2.52 ppm (J = 16, 4 Hz) and at 2.41 ppm (J = 16, 8.5 Hz) in the form of an AB system, characteristic of two nonequivalent protons adjacent to a carbonyl group. Irradiation at 4.00 ppm collapsed each doublet of doublets to a doublet (J = 16 Hz).

Anal. Calcd for $C_{19}H_{38}O_3$: mol wt, 314.2821. Found: mol wt, 314.2823 (MS).

The more polar band (R_f 0.35) was identical with **3a.**

Correlation of Eupassopilin with Lipiferolide. A. A solution of 0.2 g of **2** in 10 ml of MeOH was cooled to 0°C and stirred with 0.1 g of $NaBH_4$ in 10 ml of MeOH until TLC indicated consumption of starting material (4 h). The mixture was diluted with water, acidified with acetic acid, and extracted with $CHCl_3$. The washed and dried extracts were evaporated and the residue purified by preparative TLC (solvent benzene–ethyl acetate, 6:1). The major band was eluted with $CHCl_3$; evaporation of the solvent furnished **5** as a gum, wt 0.15 g, ir bands (film) at 3450, 1770, 1710, 1650, 1250, 1130, and 740 cm^{-1}. The high-resolution mass spectrum gave the molecular ion (0.4%); other major peaks were at *m/e* (composition, %) 346 ($M^+ - H_2O$, $C_{20}H_{26}O_5$, 4), 265 ($M^+ - C_5H_7O_2$, $C_{15}H_{21}O_4$, $C_{15}H_{20}O_3$, 20.1), 190 ($M^+ - C_5H_8O_3 - C_3H_6O$, $C_{12}H_{14}O_2$, 14), 175 ($C_{12}H_{15}O$, 13.9), 145 ($C_{11}H_{13}$, 17.7), and 99 ($C_5H_7O_2$, 100).

Anal. Calcd for $C_{20}H_{28}O_6$: mol wt, 364.1884. Found: mol wt, 364.1902 (MS).

B. A solution of 0.15 g of **5** in 12 ml of MeOH containing 0.25 g of K_2CO_3 in 1.5 ml of water was stirred at room temperature in a nitrogen atmosphere, the reaction being followed by TLC. The reaction was complete after 30 min. The product was worked up as usual, purified by preparative TLC (solvent benzene–ethyl acetate, 4:1), and recrystallized from ethyl acetate: yield 0.06 g; mp 166–167 °C (lit. mp 166–167 °C);[14] $[\alpha]D$ −109° (*c* 0.24, MeOH); ir bands (KBr) at 3500, 1735, 1190, 1070, 990, and 875 cm^{-1}. The ir and NMR traces were identical with similar traces of 11,13-dihydrodeacetylipiferolide (α-oriented C-13 methyl group)[14] supplied by Professor Doskotch. The high-resolution mass spectrum gave the molecular ion (9%); other major peaks were at *m/e* (composition, %) 248 ($M^+ - H_2O$, $C_{15}H_{20}O_3$, 62.9), 230 ($M^+ - 2H_2O$, $C_{15}H_{18}O_2$, 23.2), 208 ($M^+ - C_3H_6O$, $C_{12}H_{16}O_3$, 44.1), 190 ($M^+ - H_2O - C_3H_6O$, $C_{12}H_{14}O_2$, 81.4), and 175 ($C_{12}H_{15}O$, 100).

Anal. Calcd for $C_{15}H_{22}O_4$: mol wt, 266.1517. Found: mol wt, 266.1510 (MS).

Registry No.—**1a,** 57718-77-1; **1b,** 57718-78-2; **1c,** 57718-79-3; **1d,** 57718-80-6; **2a,** 57718-81-7; **2b,** 57718-82-8; **3a,** 57718-83-9; **3b,** 57718-84-0; **4,** 57793-27-8; **5,** 57718-85-1; **6,** 56064-69-8.

References and Notes

(1) Work supported in part by U.S. Public Health Service Grant CA-13121 through the National Cancer Institute.

(2) For a recent redefinition of the genus *Eupatorium,* see R. M. King and H. Robinson, *Taxon,* **19,** 769 (1970).

(3) *E. cannabinum* L.: F. Gyzicki, *Pharmazie,* **6,** 686 (1950); L. Dolejs and V. Herout, *Collect. Czech. Chem. Commun.,* **27,** 2654 (1962); R. W. Doskotch and F. S. El Feraly, *J. Org. Chem.,* **35,** 1928 (1970); B. Drozdz, H. Graborzyk, Z. Samek, M. Holub, V. Herout, and F. Sorm, *Collect. Czech. Chem. Commun.,* **37,** 1546 (1972); W. Herz and I. Wahlberg, *J. Org. Chem.,* **38,** 2485 (1973).

(4) *E. cuneifolium* Willd.: S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, T. Fujita, P. D. Cradwick, A. D. V. Hardy, and G. A. Sim, *J. Am. Chem. Soc.,* **93,** 4914 (1971); S. M. Kupchan, T. Fujita, M. Maruyama, and R. W. Britton, *J. Org. Chem.,* **38,** 1260 (1973); S. M. Kupchan, M. Maruyama, R. J. Hemingway, S. Shibuya, and T. Fujita, *ibid.,* **38,** 2189 (1973).

(5) *E. formosanum* Hoyata: K. H. Lee, H. C. Huang, E. S. Huang, and H. Furukawa, *J. Pharm. Sci.,* **61,** 629 (1972); A. T. McPhail, K. D. Onan, K. H. Lee, T. Ibuka, and H. C. Huang, *Tetrahedron Lett.,* 3203 (1974).

(6) *E. rotundifolium* L.: S. M. Kupchan, J. C. Hemingway, J. M. Cassidy, J. R. Knox, A. T. McPhail, and G. A. Sim, *J. Am. Chem. Soc.,* **89,** 465 (1967); S. M. Kupchan, J. E. Kelsey, M. Maruyama, and J. M. Cassidy, *Tetrahedron Lett.* 3517 (1968); S. M. Kupchan, J. E. Kelsey, M. Maruyama, J. M. Cassidy, J. C. Hemingway, and J. R. Knox, *J. Org. Chem.,* **34,** 3876 (1969).

(7) *E. semiserratum* DC.: S. M. Kupchan, T. Fujita, M. Maruyama, and R. W. Britton, *J. Org. Chem.,* **38,** 1260 (1973).

(8) Flavonoid constituents of this and other *Eupatorium* species have been reported previously: H. Wagner, M. A. Iyengar, L. Hörhammer, and W. Herz, *Phytochemistry,* **11,** 1504 (1972); H. Wagner, M. A. Iyengar, P. Dull, and W. Herz, *ibid.,* **11,** 1506 (1972); H. Wagner, M. A. Iyengar, O. Seligmann, L. Hörhammer, and W. Herz, *ibid.,* **11,** 2630 (1972); W. Herz, S. Gibaja, S. V. Bhat, and A. Srinivasan, *ibid.,* **11,** 2859 (1972); S. M. Kupchan, W. Sigel, J. R. Knox, and M. S. Udayamurthy, *J. Org. Chem.,* **34,** 1460 (1969); S. K. Talapatra, D. S. Bhar, and B. Talapatra, *Aust. J. Chem.,* **27,** 1137 (1974).

(9) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N.Y., 1972.

(10) A. P. Tulloch and J. F. T. Spencer, *Can. J. Chem.,* **42,** 830 (1964).

(11) G. Eglinton, D. H. Hunneman, and A. McCormick, *Org. Mass Spectrom.,* **1,** 593 (1968).

(12) J. de Pascual Teresa and B. Bermejo Jimenez, *An. Fis. Quim.,* **B62,** 569 (1966).

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

(14) R. W. Doskotch, S. L. Keely, C. D. Hufford, and F. S. El-Feraly, *Phytochemistry,* **14,** 769 (1975).

(15) We wish to express our thanks to Professor R. W. Doskotch for copies of the NMR and ir spectra of this substance.

(16) P. J. Cox, G. A. Sim, and W. Herz, *J. Chem. Soc., Perkin Trans. 2,* 459 (1975).

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

(18) W. Herz and A. L. Hall, *J. Org. Chem.,* **39,** 11 (1974).

(19) E.g., W. Herz, S. V. Bhat, and A. Srinivasan, *J. Org. Chem.,* **37,** 2532 (1972); W. Herz, and I. Wahlberg, *Phytochemistry,* **12,** 1421 (1973); *J. Org. Chem.,* **38,** 2485 (1973); W. Herz and R. P. Sharma, *ibid.,* **40,** 199 (1975); W. Herz and R. P. Sharma, *ibid.,* **40,** 392 (1975); B. Karlsson, A.-M. Pilotti, A.-C. Wiehager, I. Wahlberg, and W. Herz, *Tetrahedron Lett.,* 2245 (1975); W. Herz and R. P. Sharma, *Phytochemistry,* **14,** 1561 (1975); W. Herz, I. Wahlberg, C. S. Stevens, and P. S. Kalyanaraman, *Phytochemistry,* **14,** 1803 (1975); W. Herz and R. P. Sharma, *J. Org. Chem.,* **40,** 3118 (1975); W. Herz and P. S. Kalyanaraman, *J. Org. Chem.,* **40,** 3486 (1975).

(20) W. Herz, A. Srinivasan, and P. S. Kalyanaraman, *Phytochemistry,* **14,**

(21) W. Herz and G. Högenauer, *J. Org. Chem.,* **27,** 905 (1962).