rated to a small volume and acidified to pH 4 using 0.1 *N* HCl. The precipitate thus formed was collected by centrifugation, washed several times with water, and dried *in vacuo* to give 30% yield of the product, **6**, as a brown powder, mp >300°. In 0.1 *N* NaOH λ_{max} were found at 380 nm (ϵ 10,117) and 275 (23,690), and in 0.1 *N* HCl at 346 and 271 nm; nmr 9.10 (s, one proton, H_7), 7.75 (d, two protons, J = 8 cps, $H_{2',6'}$), 7.48 (d, two protons, J = 8 cps, $H_{3',5'}$), 4.62 (t, α proton of glutamic acid), and 2.65 ppm (c, four protons of glutamic acid). Hydrolysis of **6** by HCl in glacial HOAc gave P-6-COOH, *p*-aminobenzoic acid, and glutamic acid. Compound **5** was prepared in the same manner using *p*-aminobenzoic acid as the nucleophile. The product which eluted at 0.43 *M* NaCl showed λ_{max} in 0.1 *N* NaOH at 380 nm (ϵ 10,507) and 275 (22,809), and in 0.1 *N* HCl at 350 and 275 nm. On acid hydrolysis **5** gave P-6-COOH and *p*-aminobenzoic acid.

Synthesis of 9-Oxoisohomopterioc Acid (11). A. Preparation of α-Amino-*p*-toluic Acid.—*p*-Carboxybenzaldehyde (1.5 g) and 1.2 g of hydroxylamine hydrochloride were dissolved in 20 ml of EtOH and brought to reflux; a clear solution was obtained. To this was added dropwise 1.7 g of NaOH dissolved in 7 ml of water, over a period of 0.5 hr. Water (0.5 ml) was then added to bring the suspension into solution. Refluxing was continued for an additional 15 min and the reaction mixture was poured into 100 ml of ice-cold 20% HCl. The precipitate was collected by filtration and recrystallized from MeOH: yield 1.1 g; mp 223–224°; nmr (DMSO) 7.9 (q, four protons, J = 7 cps, aromatic), 8.3 (s, one proton), and 10.2 ppm (s, one proton, carboxyl).

The above oxime (1 g) was dissolved in 100 ml of 95% EtOH, 100 mg of 5% Pd/C was added, and hydrogenation was carried out for 18 hr at 30 psi. Filtration and washing the residue with two 20-ml portions of hot glacial HOAc gave a solution which was evaporated to dryness. The solid thus obtained was triturated with absolute EtOH and filtered, producing 850 mg of solid. This was crystallized from water to give the white crystalline α-amino-*p*-toluic acid: mp 294–295°; λ_{max} 234 nm (H_2O); nmr (TFA) 4.15 (q, J = 6 cps), 7.3 (d, J = 8 cps, two protons adjacent to the aminomethyl group), and 7.9 ppm (d, J = 8 cps, two protons adjacent to the carboxyl group).

Treatment of this material with diazomethane gave the corresponding methyl ester, whose high-resolution mass spectrum showed the molecular ion at 164.0708 (calculated for $C_9H_{10}NO_2$, 164.0711), again representing the loss of a hydrogen from the benzylic position.

B. Synthesis of 9-Oxoisohomopteroic Acid (11).—α-Amino-*p*-toluic acid and **8** (1 mmol of each) were treated as usual. After deprotection and chromatography on DEAE cellulose Cl^-, **11** eluted as a single band at 0.22 *M* NaCl, and was recovered from the pooled peak in about 36% yield. The uv spectral data revealed λ_{max} at 370 nm (ϵ 10,750) and 270 (25,240) and λ_{min} at 320 and 250 nm in 0.1 *N* NaOH; nmr 9.25 (s, one proton, H_7), 8.15 (d, two protons, J = 8 cps, $H_{2',6'}$), 7.7 (d, two protons, J = 8 cps, $H_{3',5'}$), and 4.67 ppm (s, two protons, benzylic). Hydrolysis of **11** with 6 *N* HCl in glacial HOAc cleanly gave P-6-COOH and α-amino-*p*-toluic acid, identified by comparison with authentic samples.

Registry No.—**1**, 39707-60-3; **2**, 39707-61-4; **3**, 39707-62-5; **5**, 39707-63-6; **6**, 39707-65-8; **8**, 39707-64-7; **11**, 39707-66-9; **15**, 39707-67-0; **16b**, 39707-68-1; P-6-COOH, 948-60-7; trifluoroacetic anhydride, 407-25-0; *p*-aminobenzoic acid, 150-13-0; *p*-aminobenzoyl-L-glutamic acid, 4271-30-1; *p*-bromoaniline, 106-40-1; α-amino-*p*-toluic acid, 56-91-7; *p*-carboxybenzaldehyde, 619-66-9; hydroxylamine hydrochloride, 5470-11-1; diazomethane, 334-88-3; α-amino-*p*-toluic acid methyl ester, 18469-52-8.

Structural Elucidation of Novel Tumor-Inhibitory Sesquiterpene Lactones from *Eupatorium cuneifolium*[1,2]

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Five new cytotoxic germacranolide lactones have been isolated from *Eupatorium cuneifolium* Willd. The structures of eupacunin (**1**) and eupacunoxin (**2**) were elaborated by chemical and spectral arguments, and confirmed by X-ray crystallographic analysis of their *o*-bromobenzoate (**4**) and *m*-bromobenzoate (**5**) derivatives, respectively. Eupatocunin (**6**) was interrelated with **1** by conversion of each to the epoxy ketone **9**, and spin-decoupling studies of **6** and **9** confirmed the structural assignments. Eupatocunoxin, isomeric with eupacunoxin (**2**), was assigned structure **7** on the basis of spectral arguments. Eupacunolin (**19**) has been characterized as a hydroxy eupacunin. Eupacunin (**1**) and its companions **2** and **19** appear to be the first recognized naturally occurring germacranolide *cis,cis*-dienes. The most abundant lactone, eupacunin, was tested *in vivo* and was found to show inhibitory activity against the P-388 leukemia in mice and the Walker 256 carcinosarcoma in rats.

In the course of a continuing search for tumor inhibitors of plant origin,[4] an alcoholic extract of *Eupatorium cuneifolium* Willd. (Compositae)[5] was found to show significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) carried in tissue culture.[6] Consequently, a systematic study aimed at the isolation of the KB inhibitory principles of *E. cuneifolium* was undertaken.

A preliminary communication[7] described the isolation and structural elucidation of the novel antileukemic germacranolide eupacunin (**1**), and of two other cytotoxic germacranolides, eupacunoxin (**2**) and eupatocunin (**6**). It is the purpose of this paper to present in detail the structural elucidation of these materials and of the companion germacranolides, eupatocunoxin (**7**)

(1) Tumor Inhibitors. LXXXV. Part LXXXIV is ref 3.

(2) This investigation was supported by grants from the National Cancer Institute (CA-11718) and the American Cancer Society (IC-57), and a contract with the Division of Cancer Treatment, National Cancer Institute (NIH-NCI-C-71-2099).

(3) S. M. Kupchan, T. Fujita, M. Maruyama, and R. W. Britton, *J. Org. Chem.*, **38**, 1260 (1973).

(4) S. M. Kupchan, *Trans. N. Y. Acad. Sci.*, **32**, 85 (1970).

(5) Whole plant gathered in Florida in 1966 and 1969. The authors acknowledge receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the USDA by the National Cancer Institute.

(6) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the National Cancer Institute. The procedures were those described in *Cancer Chemother. Rep.*, **25**, 1 (1962).

(7) S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, and T. Fujita, *J. Amer. Chem. Soc.*, **93**, 4914 (1971).

and eupacunolin (19). A sixth germacranolide, eupaserrin, was identical with a cytotoxic lactone which was characterized in the course of a parallel study of the active principles of *Eupatorium semiserratum*.[3]

Fractionation of the ethanol extract (Chart I), guided by assay against KB (Table I), revealed that the

CHART I

FRACTIONATION OF THE CYTOTOXIC PRINCIPLES OF *Eupatorium cuneifolium* WILLD.

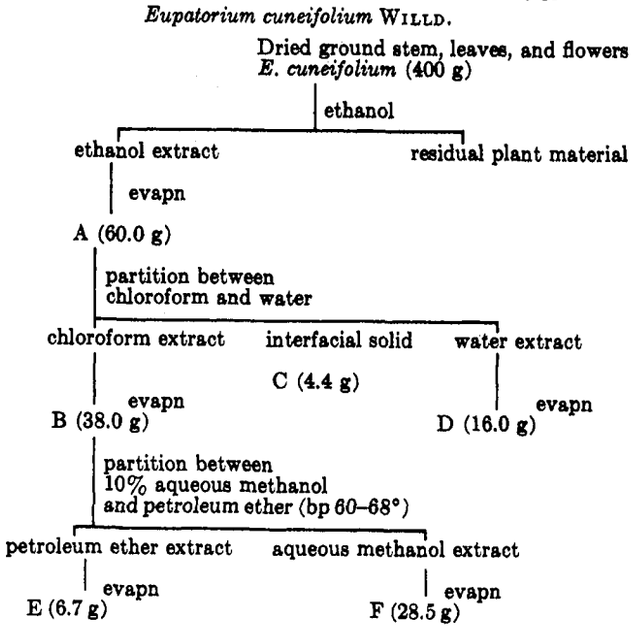


TABLE I

ACTIVITY OF FRACTIONS FROM *E. cuneifolium* AGAINST KB TISSUE CULTURE

Fraction	ED_{50}, μg/ml	Fraction	ED_{50}, μg/ml
A	1.8	L	0.5
B	2.8	M	3.9
C	19	N	2.4
D	>100	O	1.7
E	>100	P	5.6
F	1.8	1	2.1
G	1.6	2	2.1
H	2.3	6	0.11
I	6.7	7	1.7
J	3.7	19	3.7
K	7.0		

active principles were concentrated, successively, in the chloroform layer of a chloroform–water partition and in the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition. The active fraction was chromatographed on silicic acid. Eupacunin (1) and eupatocunin (6) were eluted with chloroform in two separate fractions. Eupacunoxin (2) and eupatocunoxin (7) were eluted together with 1% methanol in chloroform. They were subsequently separated by rechromatography on silicic acid and silica gel and purified by crystallization. Eupacunolin (19) was eluted with 2% methanol in chloroform and further purified by silica gel chromatography. All five compounds showed ultraviolet high intensity end absorption and infrared bands near 5.7 and 6.1 μ, which was suggestive of the presence of an α,β-unsaturated γ-lactone, a structural feature common in other sesquiterpenoids of *Eupatorium* species.[8-10] All of the lactones showed *in vitro* cytotoxicity against KB cell culture, but only eupacunin has significant *in vivo* activity against lymphocytic leukemia (PS) in mice and Walker 256 intramuscular carcinosarcoma in rats.

Elemental analysis and mass spectrometry established that eupacunin (1) and eupatocunin (6) had the same molecular formula, $C_{22}H_{28}O_7$. Similarities in spectral data indicated a close structural relationship. Spectra for eupatocunin (6) indicated the presence of a hydroxyl group (ir 2.86 μ), an acetate [ir 5.73 and 8.08 μ, nmr, τ 7.97 (3 H, s)], an α,β-exocyclic methylene γ-lactone [uv 212 nm (ε 28,000), ir 5.68 μ, and characteristic nmr signals at τ 3.76 (d, J = 2.5 Hz) and τ 4.04 (d, J = 2 Hz)] and an ester (ir 5.82 μ). These groups accounted for all of the oxygen functions of 6. The same functionalities were also indicated as present in eupacunin (1).

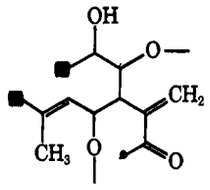
The nature of the ester group of eupatocunin was revealed by its nmr spectrum, which showed characteristic signals for the methyl [τ 8.10 (6 H)] and vinyl [τ 3.91 (1 H)] protons of an angeloyl residue. The corresponding signals for the isomeric tiglyoyl residue occur at somewhat different chemical shifts.[11] Methanolysis of eupatocunin yielded methyl angelate.

While the signals in the 100-MHz nmr spectrum for eupacunin (1) were not all well resolved and could not be specifically assigned (see Experimental Section), the nmr spectrum of eupatocunin (6) (see Table II) was clearly resolved and by utilizing double-resonance studies a structure for eupatocunin could be postulated. The typical pair of doublets (J = 2 and 2.5 Hz) characteristic of exocyclic (C-13) methylene protons in germacranolides[12] was collapsed to two singlets on irradiation of the multiplet at τ 6.70, which could therefore be assigned as the C-7 proton. The two well-resolved signals at τ 4.22 (dd, J = 2.5 and 11 Hz) and 4.36 (dd, J = 1 and 3 Hz), each corresponding to one proton, could be assigned to either the C-6 or C-8 proton, since the signals collapsed to doublets (J = 11 and 3 Hz, respectively) on irradiation of the C-7 proton signal. The proton giving rise to the signal at τ 4.36 was further coupled to a proton on carbon bearing hydroxyl which appeared as a multiplet at τ 5.54 and collapsed to a doublet (J = 3 Hz) on addition of D_2O. In addition the proton giving rise to the signal at τ 4.22 was further coupled to an olefinic proton which appeared as a doublet of quartets at τ 4.82 (J = 1.5 and 11 Hz). The olefinic proton was also coupled to a methyl group, τ 8.21 (d, J = 1.5 Hz). These facts supported postulation of partial structure A for eupatocunin.

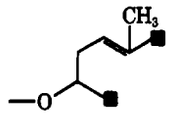
A complex signal at τ 8.10, integrating for nine protons, was assigned to the two methyl groups of the angelate ester, and the other germacrane methyl group. The remaining high-field signals were two doublets of doublets at τ 7.24 (J = 2.5, 10, 14 Hz) and 7.62 (J = 4, 7, 14 Hz). On irradiation of the proton signal appearing as a doublet of doublets at τ 4.80

(8) S. M. Kupchan, J. C. Hemingway, J. M. Cassady, J. R. Knox, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **89**, 465 (1967).
(9) S. M. Kupchan, J. E. Kelsey, M. Maruyama, and J. M. Cassady, *Tetrahedron Lett.*, 3517 (1968).
(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) H. Morimoto, Y. Sanno, and H. Oshio, *Tetrahedron*, **27**, 3173 (1966).
(12) Z. Samek, *Tetrahedron Lett.*, 671 (1970).

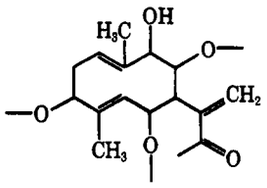
(J = 2.5, 4 Hz), these complex signals were simplified to doublets of doublets. Furthermore, irradiation at τ 7.24 resulted in collapse of the signals at τ 4.80 and 4.60 (m) to a doublet and narrower multiplet, respectively. These decoupling experiments suggested partial structure B for eupatocunin. Partial structures A and B could only be linked as in partial structure C, a biogenetically reasonable germacrane skeleton.



A



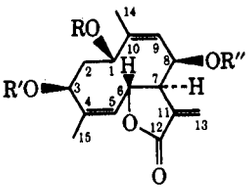
B

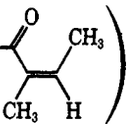


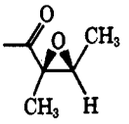
C

Eupatocunin (6) was further characterized by hydrolysis. Treatment of 6 with sodium methoxide gave a product (8) which still retained an acetyl group, but had lost an angeloyl group. This suggested that the angeloyl group in 6 was vicinal[13] to the C-9 hydroxyl group, and therefore at C-8. Consequently, the acetyl group in 6 could be assigned to C-3 and eupatocunin represented as structure 6. Additional support for this structure was furnished by its interrelationship with eupacunin (1). Oxidation of 1 and 6 with Jones reagent afforded the same product (9). The nmr spectrum of 9 was similar to that of eupatocunin. The significant changes were the disappearance of one of the vinyl methyl signals at τ 8.10 and an olefinic proton multiplet at τ 4.60, and the appearance of a methyl singlet at τ 8.53 and a doublet of doublets at τ 6.07 (J = 2, 11 Hz). These changes suggested formation of a C-1, C-10 epoxide ring in 9. Reactions of this type, involving epoxidation of allylic alcohols, have been observed previously with chromate oxidations.[14,15] The nmr spectrum of 9 also lacked the doublet at τ 5.54 (J = 3 Hz) assigned to the C-9 proton in 6, and had a new doublet at τ 4.36 (J = 3.5 Hz) which corresponded to the doublet of doublets at τ 4.36 (J = 1, 3 Hz) in the spectrum of 6. This was in good accord with the formation of a carbonyl group at C-9. Therefore the common oxidation product of 1 and 6 possessed a 1,10-epoxy 9-ketone structure. This was a reasonable proposition based on the postulated structure of 6 and suggested that 1 and 6 differed only at C-1, C-9, and C-10. Most reasonably, eupacunin (1) was presumably converted to 9 by an allylic rearrangement followed by epoxidation and oxidation.

In addition to 9, the Jones oxidation of eupacunin (1) afforded a second, more polar product 11. Elemental analysis and mass spectrometry supported a $C_{22}H_{28}O_8$



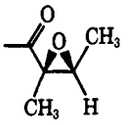
1, R = H; R′ = Ac; R″ = Ang (= )



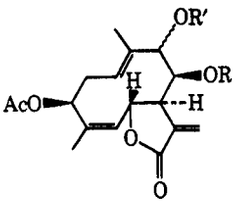
2, R = H; R′ = Ac; R″ =

3, R = R′ = H; R″ = Ang

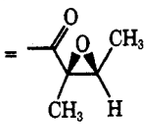
4, R = *o*-bromobenzoyl; R′ = Ac; R″ = Ang



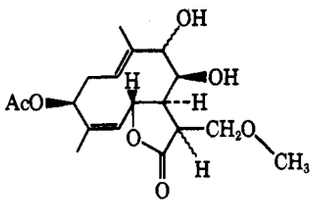
5, R = *m*-bromobenzoyl; R′ = Ac; R″ =



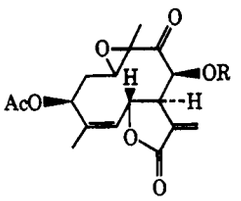
6, R = Ang; R′ = H



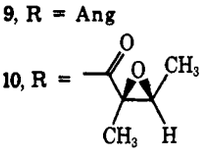
7, R = H; R′ =



8



9, R = Ang



10, R =

formula for 11. The nmr spectrum of 11 showed the absence of one of the vinyl methyl signals at τ 8.10 and the presence of a methyl singlet at τ 8.66. The uv spectrum of 11 exhibited a maximum at 210 nm with an extinction somewhat lower than that for eupacunin, and the ir spectrum still showed a hydroxyl group at 2.86 μ. These spectral data were most consistent with a structure for a product which arose from 1 by an allylic rearrangement followed by epoxidation.

The postulated structures for the oxidation products of 1 and 6 were supported by the methanolysis[10,16,17] of eupacunin (1), which yielded deacetyldeangeloyl-13-methoxydihydroeupacunin (12). The C-10 methyl

(13) S. M. Kupchan, P. Slade, R. J. Young, and G. W. A. Milne, *Tetrahedron*, **18**, 499 (1962).

(14) J. Iriarte, J. N. Shoolery, and C. Djerassi, *J. Org. Chem.*, **27**, 1139 (1962).

(15) E. Glotter, S. Greenfield, and D. Lavie, *J. Chem. Soc. C*, 1646 (1968).

(16) G. H. Kulkarni, A. Paul, A. S. Rao, G. R. Kelkar, and S. C. Bhattacharya, *Tetrahedron*, **12**, 178 (1961).

(17) S. M. Kupchan, J. M. Cassady, J. E. Kelsey, H. K. Schnoes, D. H. Smith, and A. L. Burlingame, *J. Amer. Chem. Soc.*, **88**, 5292 (1966).

TABLE II

NUCLEAR MAGNETIC RESONANCE DATA[a]

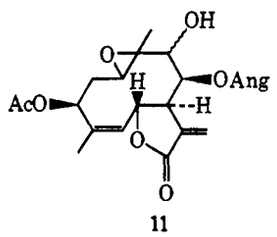
Compd	C-1	C-2	C-3	C-5	C-6	C-7	C-8	C-9	C-13	C-14	C-15	OCOR, OH, other
6	4.60 m	7.24 ddd (2.5, 10, 14) 7.62 ddd (4, 7, 14)	4.80 dd (2.5, 4)	4.82 dq (1.5, 11)	4.22 dd (2.5, 11)	6.70 m	4.36 dd (1, 3)	5.54 d (3)	3.76 d (2.5) 4.04 d (2)	8.10 s	8.21 d (1.5)	OH, 6.77 m 1-H, 3.91 m 2-CH₃, 8.1 m Ac, 7.97 s
7	4.8 m	7.64 ddd (3.7, 7, 15) 7.27 ddd (3, 9, 15)	4.8 m	4.8 m	4.21 dd (2, 10.5)	6.9 m	5.85 m	4.72 d (2)	3.81 d (2.5) 4.20 d (2)	8.17 br s	8.27 d (1.5)	1-H, 6.90 q (5.5) CH₃, 8.73 d (5.5) CH₃, 8.46 s Ac, 7.98 s
9[b]	6.07 dd (2, 11)	7.55 ddd (2, 5, 15) 8.30 ddd (3, 11, 15)	4.7 m	4.67 dd (1.5, 11)	4.38 dd (8, 11)	6.7 m	4.36 d (3.5)		3.63 d (3) 4.21 d (3)	8.53 s	8.11 d (1.5)	1-H, 3.80 q (7) CH₃, 8.13 br s CH₃, 8.05 br s Ac, 7.91 s
10[b]	6.29 dd (2.2, 11)	7.46 ddd (2.2, 5, 15) 8.36 ddd (2, 11,15)	4.64 dd (2, 5)	4.60 br d (11)	4.52 dd (7, 11)	6.7 m	4.22 d		4.60 d (3) 4.06 d (2.8)	8.46 s or 8.44 s	8.06 br s	1-H, 6.95 q (5.5) CH₃, 8.67 d (5.5) CH₃, 8.44 s or 8.46 s Ac, 7.85 s
12	5.61 br d (5)	8.17 m	4.19 dd (2, 5, 11)	4.92 br d (10)	3.56 t (10)	7.8 m	5.49 br d (5)	4.90 dd (1.5, 5)	6.37 m	8.35 m	8.35 m	OCH₃, 6.22 s C-11, 7.06 dt (3.5, 11.5)
18[c]	4.40 m	7.50 m 8.30 m	4.73 m	4.73 m	4.40 m	7.05 m	4.40 m	5.70 d	8.87 d (7)	8.12 br s	8.19 d (1.5)	OH, 7.05 m; H, 3.95 m Ac, 7.98 s CH₃, 8.12 s; CH₃, 8.15 s

[a] Spectra were determined on a Varian HR-100 spectrometer in acetone-d_6 unless otherwise indicated. [b] Spectra measured in deuteriochloroform. [c] Spectrum measured in deuteriochloroform on a Varian A-60A spectrometer. Values are given in τ units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet; br, broad. Absence of designation implies that signal is overlapped with another signal and multiplicity is unclear. Numbers in parentheses denote coupling constants in hertz.

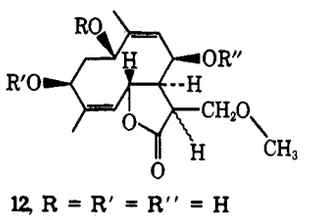
(τ 8.35) of **12** was coupled to both the C-8 [τ 5.49 (br d, J = 5 Hz)] and C-9 [τ 4.90 (dd, J = 1.5, 5 Hz)] protons. This fact could be explained by a homoallylic arrangement[18,19] and supported the location of a double bond at C-9, C-10. Location of the hydroxyl group at C-1 was thereby substantiated also.

The nmr spectrum of **12** measured with addition of trichloroacetylisocyanate showed downfield shifts of 1.1, 0.87, and 1.36 ppm for the signals assigned to the C-1, C-3, and C-8 protons, respectively, while the C-6 proton signal was shifted upfield as much as 0.25 ppm.[20] Accordingly, the three hydroxyl groups in **12** could be located at C-1, C-3, and C-8, thus showing that the γ-lactone closure of **12**, and consequently of **1** and **6**, was oriented at C-6.

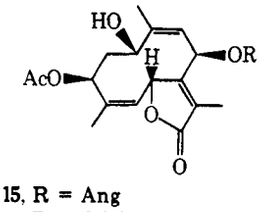
Eupacunin (**1**) was further characterized by its saponification products. Treatment of **1** with 2% sodium hydroxide in aqueous dioxane gave deacetyleupacunin (**3**), while treatment with 2% sodium hydroxide in aqueous methanol gave deacetyl-13-methoxydihydroeupacunin (**13**). The latter product was acetylated to give the diacetate **14**.



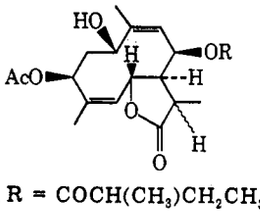
11



12, R = R′ = R″ = H
13, R = R′ = H; R″ = Ang
14, R = R′ = Ac; R″ = Ang



15, R = Ang
16, R = $COCH(CH_3)CH_2CH_3$



17, R = $COCH(CH_3)CH_2CH_3$

All of these studies provided firm chemical support for the postulation of structures **1** and **6** for eupacunin and eupatocunin, respectively. Unequivocal proof of the structure, stereochemistry, and absolute configuration of eupacunin shown in **1** was achieved by X-ray crystallographic analysis[21] of eupacunin *o*-bromobenzoate (**4**).

Hydrogenation of eupacunin using 10% palladium on charcoal as catalyst yielded several products, three of which were isolated. In one of these, an isomeric compound, the nmr spectrum showed peaks for five vinyl methyl groups, two at τ 7.95, two at τ 8.10, and one at τ 8.30. This suggested that the exocyclic double bond had been isomerized to the endo position and that the product could be assigned structure **15**.[22] The second product was shown by its mass spectrum [*m/e* 305, M−101 ($C_5H_9O_2$)] and nmr spectrum to be the dihydro isomeric compound, **16**, in which the double bond of the angelate group was reduced. The six-proton methyl singlet at τ 8.05 of eupacunin was no longer present, but was replaced in **16** by a three-proton doublet at τ 8.82 (J = 7 Hz) and a three-proton triplet (J = 7 Hz) at τ 9.07. In addition, signals for three vinylic methyl groups remained. The final hydrogenation product was the tetrahydro compound **17**. The intensity of absorption in the uv spectrum of **17** was substantially lessened, indicative that the conjugated double bonds of the ester and lactone had been reduced.

Dihydroeupatocunin (**18**) was obtained by the treatment of eupatocunin with sodium borohydride.[23]

(18) J. T. Pinhey and S. Sternhell, *Tetrahedron Lett.*, 275 (1963).
(19) J. T. Pinhey and S. Sternhell, *Aust. J. Chem.*, **18**, 543 (1965).
(20) V. W. Goodlet, *Anal. Chem.*, **37**, 431 (1965).
(21) X-Ray crystallographic analysis was carried out by Dr. G. A. Sim, P. D. Cradwick, and A. D. U. Hardy. A complete description of the X-ray results will be published separately by Professor Sim.
(22) *E.g.*, L. A. P. Anderson, W. T. deKock, W. Nel, K. G. R. Pachler, and G. van Tonder, *Tetrahedron*, **24**, 1687 (1968).
(23) W. Renold, H. Yoshioka, and T. J. Mabry, *J. Org. Chem.*, **35**, 4264 (1970).

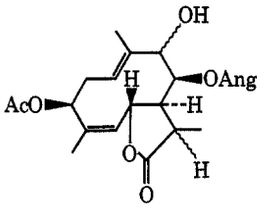
For **18** the intensity of the uv end absorption was decreased, and the nmr spectrum no longer showed the doublets typical of the exocyclic methylene. A new doublet methyl appeared at τ 8.87 (J = 7 Hz).

The molecular formula $C_{22}H_{28}O_8$ was assigned for eupacunoxin (**2**) on the basis of elemental analysis and mass spectrometry. The presence of an α-methyl-α,β-epoxybutyrate side-chain ester was indicated by the nmr spectrum, which showed two methyl signals at τ 8.82 (d, J = 5.5 Hz) and 8.38 (s), and a signal at τ 6.95 (q, J = 5.5 Hz) which could be assigned to a proton on an epoxide ring. Otherwise, the spectrum of **2** was very similar to the spectrum of **1**. The uv extinction coefficient of **2** was less than that of eupacunin (**1**), suggesting the absence of one of the two conjugated systems present in the latter. Methanolysis of **2** gave methyl α-methyl-*trans*-α,β-epoxybutyrate and deacetyldeangeloyl-13-methoxydihydroeupacunin (**12**). Consequently, structure **2** was proposed for eupacunoxin. Jones oxidation of **2** yielded the epoxy ketone **10**, the nmr of which was very similar to that of **9** except for the signals due to the side chain at C-8. Proof of structure **2** was furnished by the X-ray crystallographic structure[21] of eupacunoxin *m*-bromobenzoate (**5**), $C_{29}H_{31}O_9Br$.

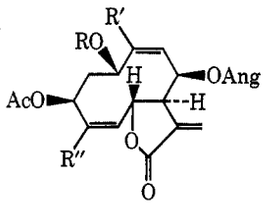
The uv spectrum of eupatocunoxin (**7**), $C_{22}H_{28}O_8$, was essentially the same as that of **2**. A methyl doublet at τ 8.73 (J = 5.5 Hz), a methyl singlet at τ 8.46, and a quartet at τ 6.90 (J = 5.5 Hz) in the nmr spectrum of **7** suggested the presence of an α-methyl-*trans*-α,β-epoxybutyrate as in **2**. Location of the epoxy ester at C-9 was supported by the downfield shift of the signal assigned to the C-9 proton [τ 4.72 (d, J = 2 Hz)] and the upfield shift of the signal assigned to the C-8 proton [τ 5.85 (m)] relative to the signals for the C-9 proton [τ 5.54 (d, J = 3 Hz)] and C-8 proton [τ 4.36 (dd, 1 H, J = 3 Hz)] in the nmr spectrum of eupatocunin (**6**). Otherwise the spectrum of **7** was very similar to the spectrum of **6**. In contrast to other sesquiterpene lactones in this series, eupatocunoxin (**7**) was resistant to Jones oxidation, giving additional support to the absence of a C-9 hydroxyl group. This evidence suggested that eupatocunoxin had the same germacrane skeleton as **6** and differed in the nature and location of the side-chain ester as shown by structure **7**.

Eupacunolin (**19**), $C_{22}H_{28}O_8$, showed the same uv spectrum as eupacunin (**1**) and a stronger hydroxyl absorption band at 2.88 μ in the ir than **1**. Many features of the nmr spectrum of **19** were similar to those of the spectrum of **1**. However, **19** lacked one of the vinyl methyl signals at τ 8.10, showing instead a two-proton signal at τ 5.90. In addition, the nmr spectrum exhibited a two-proton signal at τ 7.25 which was D_2O exchangeable. It therefore appeared that compounds **1** and **19** were similar, and the extra oxygen in **19** was present as a hydroxymethyl group.

Further evidence for the diol structure was obtained by acetylation, which afforded two oily products. The nmr spectra of these were consistent with the mono- and diacetate structures **20** and **21**. The nmr spectrum of compound **20** showed one new acetate signal at τ 7.86, and the signal for the methylene protons at τ 5.90 in **19** were shifted to τ 5.40. Since the remainder of the low-field signals were essentially the same in compounds **19** and **20**, compound **20** could be assigned a primary acetate function. In the nmr spectrum of **21**, resonance for three acetate methyl groups appeared between τ 7.82 and 7.88 and the signal for the methylene protons (at τ 5.90 in **19**) again resonated at lower field (τ 5.45), indicative of a primary acetate. This spectrum showed no change on addition of D_2O. Oxidation of **19** with manganese dioxide gave, in good yield, an oily product, **22**. The nmr spectrum of this material lacked the methylene signal at τ 5.90, but showed instead a singlet at τ 0.54 characteristic of an aldehyde proton. In other respects the nmr spectrum of **19** and **22** were similar. The aldehyde was characterized as the *p*-bromophenylhydrazone **23**.



18



19, R = H; R′ = CH_3; R″ = CH_2OH or R′ = CH_2OH; R″ = CH_3
20, R = H; R′ = CH_3; R″ = CH_2OAc or R′ = CH_2OAc; R″ = CH_3
21, R = Ac; R′ = CH_3; R″ = CH_2OAc or R′ = CH_2OAc; R″ = CH_3
22, R = H; R′ = CH_3; R″ = CHO or R′ = CHO; R″ = CH_3
23, R = H; R′ = CH_3; R″ = CH=NNH-*p*-C_6H_4Br or R′ = CH=NNH-*p*-C_6H_4Br; R″ = CH_3

To confirm the suspected relationship between **1** and **19**, a sample of eupacunolin (**19**) was hydrogenated using 10% palladium on charcoal as catalyst. The reaction yielded a complex mixture of products from which dihydroisoeupacunin (**16**) and tetrahydroeupacunin (**17**) were obtained by chromatography on silicic acid. This experiment confirmed that **19** was a hydroxy eupacunin. Whether that hydroxyl group is at C-14 or C-15 remains to be established.

Eupacunin (**1**) and its companions **2** and **19** appear to be the first recognized naturally occurring germacranolide *cis,cis*-dienes. Furthermore, the unique location of the double bonds (Δ^4, Δ^9) renders the skeletal structures essentially symmetrical about the C-2, C-7 axis. In an earlier communication, we noted the ambiguity of prior practices for describing germacranolides, and proposed the convention that each germacranolide be represented with the alkyl group β at C-7 and with the ring numbering running counterclockwise.[24] However, it was subsequently noted that the numbering produced by these rules for melampodin was ambiguous until the absolute configuration was

(24) S. M. Kupchan, J. E. Kelsey, and G. A. Sim, *Tetrahedron Lett.*, 2863 (1967).

known.[25] Consequently, the proposal (rule 1) was advanced that the distinction between the α and β faces should be based not on the configuration at C-7, but on other evidence which can be firmly related to the asymmetry of the molecule's mode of biogenesis.[24] Such features as positions of double bonds or their equivalents (*e.g.*, epoxides) or patterns of oxygen functions that are indicative of the former positions of the double bonds were proposed. The symmetry of the structures of eupacunin (**1**) and its companions **2** and **19** makes the numbering produced for these molecules by the newly proposed convention ambiguous without a prior knowledge of the absolute configuration. We applaud the efforts of Rogers, *et al.*, to eliminate the ambiguity and confusion in the representation of germacranolides, and approve of their proposed rules 2, 3, and 4. However, for the distinction of the α and β faces, we favor adoption of a generally applicable rule which includes the configuration at tetrahedral C-7 as *one of the primary asymmetric features* to be considered.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Infrared spectra were determined on a Beckman Model IR-9 and Perkin-Elmer 257 recording spectrophotometers. Ultraviolet absorption spectra were determined on Beckman DK2A and Coleman EPS-3T spectrophotometers. Nmr spectra were determined on Varian A-60, Varian HA-100, and Hitachi H20 spectrometers using tetramethylsilane as internal standard. Nmr data are listed in Table II for compounds with well-resolved signals which were assignable to specific protons. Otherwise, partial data are reported under the compound in the Experimental Section. Gas-phase chromatography (glpc) was carried out on a Varian Aerograph Model 1860 gas chromatograph. Specific rotations were determined on a Zeiss Winkel polarimeter and are approximated to the nearest degree. The petroleum ether used was Skellysolve B, bp 60–68°. Evaporations were carried out at temperatures less than 40°. Tlc was carried out on silica gel (E. Merck) plates and chromatograms were visualized by spraying with a 3% $Ce(SO_4)_2$–3 *N* H_2SO_4 solution followed by heating. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Extraction and Preliminary Fractionation.—The dried ground stems, leaves, and flowers (400 g) of *Eupatorium cuneifolium* were continuously extracted with 95% ethanol for 16 hr, and the ethanol extract was evaporated under reduced pressure to yield a dark green gum, A (60 g). Fraction A was partitioned between water (500 ml) and three 500-ml portions of chloroform; the combined chloroform layers were finally washed with water (200 ml) and evaporated under reduced pressure to yield a dark green foam, B (38.0 g). Evaporation of the combined aqueous layer and washings under reduced pressure yielded a brown syrup, D (16.0 g). The interfacial material, after drying, yielded the solid C (4.4 g).

The chloroform-soluble fraction (B) was partitioned between 10% aqueous methanol (300 ml) and three 200-ml portions of petroleum ether. The combined petroleum ether layers, after washing with three 100-ml portions of 10% aqueous methanol, were evaporated to dryness under reduced pressure to yield a green oil, E (6.7 g). Evaporation of the aqueous methanol layer and washings under reduced pressure yielded a green gum, F (28.5 g).

Isolation of Sesquiterpene Lactones.—Fraction F (7.5 g) was fractionated by chromatography on silicic acid by elution with chloroform (20 l.). After 10 l. of eluate, fractions (20 ml) were collected and analyzed by tlc. Tubes 176–205 were combined to yield fraction G (0.273 g) and tubes 231–275 formed fraction H (0.159 g). Continued elution with 1% methanol in chloroform (11 l.) afforded fraction I (1.21 g). Subsequent elution with 2% methanol in chloroform (10 l.) yielded fraction J (2.58 g). Repeated chromatographies, as described above, yielded larger quantities of each fraction. A larger batch of fraction G (2.6 g) was crystallized from methanol and ether to yield eupacunin (**1**, 1.8 g) as colorless needles: mp 166–167°; $[\alpha]^{26}D$ +55° (*c* 1.24, acetone); uv max (MeOH) 211 nm (ϵ 23,900); ir ($CHCl_3$) 2.77, 3.40, 5.70, 5.75, 5.84, 6.10, 7.26, 7.32, 8.02, 8.78, and 9.60 μ; nmr (CD_3COCD_3) τ 6.42 (1 H, br d, J = 3 Hz), 6.90 (1 H, m), 7.25 (1 H, br s), 7.58 (1 H, dd, J = 3, 15 Hz), 7.94 (3 H, s), 7.96 (3 H, br s), 8.05 (3 H, br s), 8.17 (3 H, br s), 8.26 (3 H, br s); mass spectrum *m/e* 404 (M^+), 345, 313, 305, 283, 263, 253, 245, 227, 163, and 111.

Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.88; H, 7.08.

A fraction equivalent to H (0.485 g) was crystallized from methanol–ether to yield eupatocunin (**6**, 0.224 g) as colorless prisms: mp 163–164°; $[\alpha]^{26}D$ −129° (*c* 1.36, acetone); uv (MeOH) end absorption 212 nm (ϵ 28,000); ir ($CHCl_3$) 2.86, 3.30, 5.68, 5.73, 5.82, 6.06, 7.24, 7.36, 8.08, 8.67, and 9.60 μ; mass spectrum *m/e* 404 (M^+), 387, 362, 344, 321, 305, 261, 244, 214, 165, 149, 137, 83, and 55.

Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.51; H, 7.13.

Silicic acid chromatography of fraction I by elution with 0.5% methanol–chloroform afforded fraction K (6.09 g), which appeared mainly as a large yellow-brown spot on tlc after visualization. A portion of this fraction (5.0 g) was rechromatographed on a silica gel column by elution with acetone–methanol–chloroform (10.0:1.5:88.5) to afford fraction L (2.71 g). This material (200 mg) crystallized from ether to yield eupacunoxin (**2**, 23 mg) as colorless needles: mp 171–172°; $[\alpha]^{26}D$ +27° (*c* 1.0, acetone); uv (MeOH) end absorption 209 nm (ϵ 17,000); ir (KBr) 2.84, 5.67, 5.71, 7.88, 8.03, 8.66, 10.20, 10.44, 10.55, and 10.99 μ; nmr (CD_3COCD_3) τ 3.82 (1 H, d, J = 4 Hz), 7.23 (2 H, br s), 7.92 (3 H, s), 8.15 (3 H, d, J = 1.5 Hz), 8.25 (3 H, d, J = 1.0 Hz), 8.38 (3 H, s), 8.82 (3 H, d, J = 5.5 Hz); mass spectrum *m/e* 420 (M^+), 361, 305, 263, 245, 163, 95, and 43.

Anal. Calcd for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 62.52; H, 6.54.

Further elution of the fraction I column gave fraction M (2.25 g), a two-component mixture by tlc. Rechromatography of M on silica gel by elution with acetone–methanol–chloroforom (10.0:1.5:88.5) gave fraction N (267 mg), which crystallized from acetone to yield eupatocunoxin (**7**, 81 mg) as colorless needles: mp 200–201°; $[\alpha]^{26}D$ −209° (*c* 1.0, acetone); uv (MeOH) end absorption 210 nm (ϵ 15,500); ir (KBr) 2.92, 5.76, 8.66, 9.25, 9.66, 10.31, and 10.47 μ; mass spectrum *m/e* 420 (M^+), 403, 361, 360, 305, 262, 244, 237, 165, 137, 97, 71, and 43.

Anal. Calcd for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 62.87; H, 6.66.

Further elution of the fraction M column gave fraction O (200 mg), which crystallized from methanol–ether to yield eupaserrin (25 mg) as colorless needles, mp 153–154°. The material was characterized by melting point, mixture melting point, ir, nmr, and mass spectral comparison with a sample isolated from *Eupatorium semiserratum*.[3]

A sample of fraction J (1.07 g) from the initial silicic acid column was rechromatographed on a second silicic acid column to afford fraction P (147 mg). Finally, fraction P was chromatographed on a silica gel column by elution with acetone–methanol–chloroform (10:2:88) to afford a fraction (84 mg) which crystallized to yield eupacunolin (**19**) as colorless needles: mp 164–165°; $[\alpha]^{26}D$ +46° (*c* 1.02, acetone); uv max (MeOH) 211 nm (ϵ 23,800); ir ($CHCl_3$) 2.88, 3.40, 5.66, 5.72, 5.80, 6.06, 8.02, and 8.88 μ; nmr ($CDCl_3$) τ 3.68 (1 H, d, J = 4 Hz), 5.90 (2 H, br s), 7.25 (2 H, m), 7.90 (3 H, s), 7.92 (3 H, s), 8.02 (6 H, s), 8.28 (3 H, s); mass spectrum *m/e* 420 (M^+), 361, 321, 303, 261, 242, 215, 95, 83, 58, and 43.

Anal. Calcd for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 63.11; H, 6.87.

1,10-Epoxyeupacunin (11) and 1,10-Epoxy-9-dehydroeupacunin (9).—A solution of eupacunin (**1**, 240 mg) in acetone (8 ml) at 0° was treated with Jones reagent (1 ml) and allowed to react for 6 min. The reaction mixture was poured into ice water (40 ml) and extracted with chloroform. The chloroform extract was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield an oily residue. Chromatography on SilicAR CC-7 (Mallinckrodt, 100–200 mesh) by elution with chloroform gave three fractions, a (50 mg), b (55 mg), and c (60 mg). Crystallization of fraction b from methanol–ether gave **11** as colorless prisms: mp 195–196°; $[\alpha]^{26}D$ +54° (*c* 0.90, acetone); uv max (MeOH) 210 nm (ϵ 15,400); ir ($CHCl_3$) 2.86, 3.30, 5.67, 5.72,

(25) D. Rogers, G. P. Moss, and S. Neidle, *J. Chem. Soc., Chem. Commun.*, 142 (1972).

6.10, 8.06, and 8.76 μ; nmr ($CDCl_3$) τ 3.59 (1 H, d, J = 3 Hz), 4.60 (1 H, dm, J = 12 Hz), 4.66 (1 H, dd, J = 2, 3 Hz), 6.00 (1 H, d, J = 4 Hz), 6.08 (1 H, dd, J = 3.5, 11 Hz), 7.00 (1 H, m), 7.89 (3 H, s), 8.10 (9 H, s), 8.66 (3 H, s); mass spectrum m/e 420 (M^+), 378, 360, 316, 279, 260, 177, 165, 147, 109, 83, 55, and 43.

Anal. Calcd for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 62.83; H, 6.36.

Crystallization of fraction a afforded **9** (30 mg) as colorless prisms: mp 200–201°; $[\alpha]^{26}D$ +66° (*c* 1.11, acetone); uv max (MeOH) 210 nm (ε 14,600); ir ($CHCl_3$) 3.29, 5.65, 5.75, 6.00, 6.08, 8.04, and 8.78 μ; mass spectrum m/e 418 (M^+), 376, 334, 319, 277, 275, 259, 165, 151, 109, 83, 55, and 43.

Using a procedure identical with the one above, eupatocunin (**2**, 40 mg) was treated with the Jones reagent to afford (after normal work-up) 1,10-epoxy-9-dehydroeupacunin (**9**), shown to be identical with the material obtained from eupacunin (**1**) by melting point, mixture melting point, and ir and nmr spectral comparison.

Eupacunin *o*-Bromobenzoate (**4**).—A solution of eupacunin (**1**, 50 mg) in pyridine (1 ml) was treated with *o*-bromobenzoyl chloride (100 mg). The reaction mixture was allowed to stand at room temperature for 24 hr, and was then poured into ice water, acidified with HCl, and extracted with chloroform. The organic layer was washed with sodium bicarbonate solution and water, dried (Na_2SO_4), and evaporated under reduced pressure to give an oily residue. Preparative thin layer chromatography on silica gel afforded crystals (80 mg), which were recrystallized from methanol to afford **4** (29 mg) as colorless prisms: mp 184.5–186°; uv (MeOH) end absorption 210 nm (ε 42,000), 284 (1000); ir (KBr) 5.65, 5.73, 5.76, 5.84, 5.95, 6.08, and 6.28 μ.

Anal. Calcd for $C_{29}H_{31}O_9Br$: C, 59.28; H, 5.31; Br, 13.62. Found: C, 59.03; H, 5.43; Br, 13.67.

Deacetyldeangeloyl-11,13-dihydro-13-methoxyeupacunin (**12**). **A.**—A solution of eupacunoxin (**2**, 70 mg) in methanol (2 ml) was treated with a 2% solution of sodium hydroxide in 10% aqueous methanol (7 ml) and allowed to react at room temperature for 16 hr. The reaction mixture was acidified with 5% sulfuric acid and extracted with chloroform. The chloroform extract was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield a colorless oil (14 mg). Crystallization from methanol–ether yielded **12** as colorless prisms: mp 202–203°; uv (MeOH) end absorption 210 nm (ε 4368); ir (KBr) 2.84, 2.91, 5.66, 8.13, 8.27, 9.23, 10.36, 10.99, and 11.49 μ; mass spectrum m/e 312 (M^+), 294, 287, 277, 263, 192, 166, 149, 100, 95, 71, and 45.

Anal. Calcd for $C_{16}H_{24}O_6$: C, 61.52; H, 7.75. Found: C, 61.50; H, 7.84.

B.—A solution of deacetyl-11,13-dihydro-13-methoxyeupacunin (**13**, 70 mg) in methanol was hydrogenated in the presence of 10% palladium on charcoal (20 mg) at atmospheric pressure and room temperature. Uptake of hydrogen stopped after 15 min, at which time 1 molar equiv of hydrogen had been absorbed. The suspension was filtered and concentrated to give a colorless oil (60 mg). A solution of the product (50 mg) in methanol (2 ml) was treated by the above procedure (conversion of **2** to **12**) to yield a colorless glass (10 mg) which crystallized to give **12** as colorless prisms (7 mg) shown to be identical with the material obtained from eupacunoxin (**2**) by mixture melting point and infrared and mass spectral comparison.

C.—A solution of eupacunin (**1**, 250 mg) in anhydrous methanol (25 ml) was treated with sodium methoxide (100 mg) and kept at room temperature for 7 days. The reaction mixture was acidified with acetic acid, evaporated under reduced pressure, dissolved in water, and extracted with chloroform. The organic solution was washed with water, dried (Na_2SO_4), and evaporated to give an oil. Preparative thin layer chromatography on silica gel afforded a material which crystallized from methanol–water to afford **12** as colorless prisms. This material was shown to be identical with the product obtained from eupacunoxin (**2**) by mixture melting point.

Deacetyleupacunin (**3**).—A solution of eupacunin (**1**, 50 mg) in dioxane (3 ml) was treated with 2% potassium hydroxide in water (2.5 ml) and kept at room temperature for 16 hr. The reaction mixture was diluted with water (10 ml), acidified with 5% hydrochloric acid, and extracted with ether. The ether was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield the crude product (25 mg). Crystallization from ether–hexane afforded deacetyleupacunin (**3**) as colorless crystals: mp 155–156°; $[\alpha]^{25}D$ +114° (*c* 1.50, acetone); uv (MeOH) end absorption 208 nm (ε 14,100); ir (KBr) 2.87, 5.71, 5.76, 8.00, 8.69, 10.00, 10.34, and 10.47 μ; nmr ($CDCl_3$–CD_3COCD_3) τ 3.77 (1 H, d, J = 3.5 Hz), 5.45 (1 H, m), 8.05 (3 H, s), 8.25 (9 H, br s).

Anal. Calcd for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23. Found: C, 66.61; H, 7.26.

Deacetyl-11,13-dihydro-13-methoxyeupacunin (**13**).—A solution of eupacunin (**1**, 250 mg) in 20% aqueous methanol (5 ml) containing sodium hydroxide (100 mg) was kept at room temperature for 16 hr. The reaction mixture was cooled, diluted with water (15 ml), acidified with concentrated hydrochloric acid, and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), filtered, and evaporated to afford an oil (260 mg). Chromatography on silicic acid by elution with methanol–chloroform (1:99) gave a fraction (127 mg) which crystallized from ether–petroleum ether to afford **13** as colorless prisms (91 mg): mp 150–151°; $[\alpha]^{26}D$ +76.5° (*c* 0.63, acetone); uv max (MeOH) 217 nm (ε 11,000); ir ($CHCl_3$) 2.77, 2.90, 5.65, 5.83, 6.08, and 8.66 μ; nmr ($CDCl_3$) τ 6.62 (3 H, s), 7.96 (6 H, br s), 8.28 (6 H, br s); mass spectrum m/e 394 (M^+), 377, 364, 363, 362, 361, 360, 310, 293, 275, 231, 215, 199, 191, 173, 149, 135, 95, 83, and 55.

Anal. Calcd for $C_{21}H_{30}O_7$: C, 63.94; H, 7.66. Found: C, 64.20; H, 7.87.

11,13-Dihydro-13-methoxyeupacunin Acetate (**14**).—A solution of deacetyl-11,13-dihydro-13-methoxyeupacunin (**13**, 48 mg) in isopropenyl acetate (2 ml) containing *p*-TsOH (2 mg) was kept at 40° for 16 hr, after which the reaction mixture was evaporated to dryness under reduced pressure. Chromatography on silicic acid by elution with chloroform yielded one main fraction (61 mg) which crystallized from ether–petroleum ether to yield **14** (32 mg) as colorless prisms: mp 136–137°; $[\alpha]^{30}D$ −12° (*c* 1.53, acetone); uv max (MeOH) 216 nm (ε 10,900); ir ($CHCl_3$) 3.23, 3.42, 5.65, 5.77, 6.05, 7.23, 7.32, and 8.00 μ; nmr ($CDCl_3$) τ 6.65 (3 H, s), 7.82 (3 H, s), 8.00 (6 H, br s), 8.10 (3 H, s), 8.22 (3 H, d, J = 2 Hz), 8.26 (3 H, d, J = 2 Hz); mass spectrum m/e 478 (M^+), 419, 370, 337, 327, 259, 245, 231, 227, 215, 199, 173, 83, and 55.

Anal. Calcd for $C_{25}H_{34}O_9$: C, 62.75; H, 7.16. Found: C, 63.06; H, 7.24.

Partial Methanolysis of Eupatocunin (**6**).—A solution of eupatocunin (**6**, 81 mg) and sodium methoxide (11 mg) in anhydrous methanol was kept at room temperature for 21 hr. The reaction mixture was neutralized with acetic acid and evaporated. The residue was dissolved in chloroform, washed with 5% sodium carbonate solution and water, dried (Na_2SO_4), and evaporated to afford an amorphous solid (53 mg). Preparative thin layer chromatography yielded homogeneous **8**: ir ($CHCl_3$) 2.79, 2.81, 5.72, 5.76, 6.01, 6.91, 7.28, 7.32, 8.04, 8.37, 9.17, 9.69, and 10.30 μ; mass spectrum m/e 354 (M^+), 337, 309, 295, 294, 275, 262, 69, 46, and 43.

Isoeupacunin (**15**).—A solution of eupacunin (**1**, 200 mg) in methanol (20 ml) was hydrogenated using 10% palladium on charcoal (50 mg) as catalyst at atmospheric pressure and room temperature. Uptake of hydrogen stopped after 11 min, at which time 1 molar equiv of hydrogen had been absorbed. The suspension was filtered and concentrated to give a colorless oil (180 mg). Chromatography on silica gel and elution with acetone–methanol–chloroform (10:1:89) yielded three fractions. The major fraction (100 mg) crystallized from ether–hexane to afford isoeupacunin (**15**): mp 144–145°; $[\alpha]^{26}D$ −66° (*c* 0.77, acetone); uv (MeOH) end absorption 213 nm (ε 15,800); ir (KBr) 2.93, 5.70, 5.72, 5.81, 8.08, and 9.00 μ; nmr ($CDCl_3$) τ 2.70 (1 H, br d, J = 10 Hz), 3.77 (1 H, br d, J = 10 Hz), 4.44 (1 H, m), 4.85 (1 H, br d, J = 10 Hz), 5.22 (1 H, br d, J = 10 Hz), 7.95 (6 H, s), 8.10 (6 H, s), 8.30 (3 H, s).

Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.50; H, 7.10.

Dihydroisoeupacunin (**16**). **A.**—A solution of eupacunin (**1**, 200 mg) in methanol (10 ml) was hydrogenated using 10% palladium on charcoal (80 mg) as catalyst at atmospheric pressure and room temperature. Uptake of hydrogen stopped after 20 min, at which time 3 molar equiv of hydrogen had been absorbed. The suspension was filtered and concentrated to give a colorless oil. Chromatography on silic acid by elution with chloroform yielded two main fractions, a (53 mg) and b (59 mg), respectively. Recrystallization of fraction a from ether–petroleum ether yielded dihydroisoeupacunin (**16**) as colorless prisms: mp 130–131°; $[\alpha]^{28}D$ −45° (*c* 1.11, acetone); ir ($CHCl_3$) 2.87, 3.36, 5.72, 5.96, 8.04, 8.94, and 9.90 μ; nmr ($CDCl_3$) τ 2.71 (1 H, br d, J = 10 Hz), 3.79 (1 H, br d, J = 10 Hz), 4.42 (1 H, m),

4.88 (1 H, br d, J = 10 Hz), 5.21 (1 H, br d, J = 10 Hz), 7.94 (6 H, s), 8.10, (3 H, d, J = 2 Hz), 8.30 (3 H, d, J = 2 Hz), 8.82 (3 H, d, J = 7 Hz), 9.07 (3 H, t, J = 7 Hz).

Anal. Calcd for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 65.20; H, 7.63.

B.—A solution of eupacunolin (**19**, 140 mg) in methanol (15 ml) was hydrogenated using 30% palladium on charcoal (50 mg) as catalyst at atmospheric pressure and room temperature. Uptake of hydrogen stopped after 100 min, at which time 3 molar equiv of hydrogen had been absorbed. The suspension was filtered and concentrated to give a colorless oil. Careful chromatography on SilicAR CC-7 by elution with methanol–chloroform (1:99) yielded two fractions, a (15 mg) and b (30 mg). Crystallization of fraction a from ether–hexane yielded dihydroisoeupacunin (**16**, 7 mg) as prism rosettes shown to be identical with the material obtained from eupacunin by melting point, mixture melting point, and infrared spectral comparison.

Tetrahydroeupacunin (17). A.—Crystallization of the fraction b (from the hydrogenation of eupacunin to give dihydroisoeupacunin) from ether–petroleum ether yielded tetrahydroeupacunin (**17**, 45 mg) as colorless prisms: mp 152–153°; $[\alpha]^{25}D$ +99° (c 1.00, acetone); uv max (MeOH) 209 nm (ϵ 3600); ir ($CHCl_3$) 2.87, 3.35, 5.64, 5.75, 6.06, 7.24, 8.03, and 8.70 μ; nmr ($CDCl_3$) τ 7.92 (3 H, s), 8.10 (3 H, br s), 8.30 (3 H, br s), 8.60 (3 H, d, J = 7 Hz), 8.88 (3 H, d, J = 7 Hz), 9.07 (3 H, t, J = 7 Hz); mass spectrum m/e 408 (M^+), 349, 307, 265, 246, 239, 219, 201, 191, 173, 165, 122, 119, 95, 85, and 57.

Anal. Calcd for $C_{22}H_{32}O_7$: C, 64.68; H, 7.90. Found: C, 64.74; H, 8.13.

B.—Crystallization of fraction b (from hydrogenation of eupacunolin) from ether–hexane yielded tetrahydroeupacunin (**17**, 19 mg) shown to be identical with the material obtained from 1 by mixture melting point and infrared spectral comparison.

11,13-Dihydroeupatocunin (18).—A solution of eupatocunin (**6**) (90 mg) in methanol (3 ml) was treated with a methanolic solution (30 ml) of sodium borohydride (90 mg) at room temperature for 3 hr. The reaction mixture was diluted with water (20 ml) and extracted with chloroform. The chloroform extract was washed with water and dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield 70 mg of product which was crystallized from ether–hexane to yield dihydroeupatocunin (**18**, 40 mg) as colorless crystals: mp 184–185°; $[\alpha]^{25}D$ −68° (c 0.93, acetone); uv (MeOH) end absorption 210 nm (ϵ 10,500); ir (KBr) 2.89, 5.67, 5.78, 10.08, 10.44 μ; mass spectrum m/e 406 (M^+), 323, 307, 263, 245, 165, 95, 83, 55, and 43.

Anal. Calcd for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 65.16; H, 7.23.

1,10-Epoxy-9-dehydroeupacunoxin (10).—A solution of eupacunoxin (**2**, 40 mg) in acetone (3 ml) at 4° was treated with Jones reagent (1 ml) and stirred for 8 min. The reaction mixture was poured into ice water (20 ml) and extracted with chloroform. The chloroform extract was dried (Na_2SO_4), filtered, and evaporated. The residue was chromatographed on silica gel by elution with acetone–methanol–chloroform (20:1:79). The main fraction (34 mg) crystallized to afford colorless needles (20 mg) of **10**: mp 205–206°; $[\alpha]^{26}D$ +82.5° (c 0.94, acetone); uv max (MeOH) 210 nm (ϵ 15,600); ir (KBr) 5.64, 5.60, 5.76, 6.90, 7.23, 8.05, 8.12, 8.63, and 10.20 μ; mass spectrum m/e 434 (M^+), 335, 319, 275, 259, 109, 97, 95, 69, 55, and 43.

Anal. Calcd for $C_{22}H_{26}O_9$: C, 60.82; H, 6.03. Found: C, 60.78; H, 6.01.

Eupacunoxin *m*-Bromobenzoate (5).—To a solution of eupacunoxin (**2**, 71 mg) in pyridine (0.5 ml) was added a mixture of *m*-bromobenzoyl chloride (0.5 ml) in pyridine (0.5 ml). The reaction mixture was kept for 24 hr at room temperature, poured into ice water, stirred for 3 hr, and extracted with chloroform. The chloroform layer was washed with sodium bicarbonate solution, and water, dried (Na_2SO_4), and evaporated to dryness *in vacuo*. Preparative thin layer chromatography on silica gel plates afforded 117 mg of crystals, which were recrystallized from methanol to yield the *m*-bromobenzoate **5** as needles: mp 191–192°; ir (KBr) 5.72, 5.75, 5.82, 5.95, 6.30, 8.00, 8.13, 8.82, and 12.60 μ.

Anal. Calcd for $C_{29}H_{31}O_9Br$: C, 57.77; H, 5.17; Br, 13.24. Found: C, 57.82; H, 5.18; Br, 13.16.

Eupacunolin Acetate (20) and Eupacunolin Diacetate (21).—To a solution of eupacunolin (**19**, 100 mg) in dry pyridine (4 ml) was added acetic anhydride (200 mg) and the solution was kept at room temperature for 12 hr. The reaction mixture was evaporated at room temperature *in vacuo*, and the residue was chromatographed on SilicAR CC-7 by elution with chloroform and 0.5% methanol in chloroform. Two main fractions, a (90 mg) and b (48 mg), were obtained. Spectral data for a were consistent with the structure proposed for eupacunolin diacetate (**21**): ir (neat) 3.30, 3.40, 5.65, 5.73, 6.06, 7.30, 8.00, and 8.78 μ; nmr ($CDCl_3$) τ 3.65 (1 H, d, J = 3 Hz), 5.45 (2 H, br s), 7.82 (3 H, s), 7.88 (6 H, s), 8.07 (6 H, br s), 8.22 (3 H, br s). Spectral data for b were consistent with the structure proposed for eucapunolin acetate (**20**): ir (neat) 2.72, 3.30, 3.40, 5.65, 5.73, 6.06, 7.30, 8.08, and 8.78 μ; nmr ($CDCl_3$) τ 3.62 (1 H, d, J = 3 Hz), 5.40 (2 H, br s), 7.86 (9 H, br s), 8.00 (3 H, s), 8.22 (3 H, br s).

Dehydroeupacunolin (22).—To a solution of eupacunolin (**19**, 120 mg) in chloroform (5 ml) was added manganese dioxide (1.0 g) and the suspension was stirred at 35° for 36 hr. The suspension was filtered and concentrated to give an oily product which was chromatographed on silicic acid to afford a homogeneous, colorless oil (80 mg). Spectral evidence was consistent with structure **22**: uv max (MeOH) 212 nm (ϵ 22,100); ir (neat) 2.86, 3.38, 5.63, 5.71, 5.80, 5.91, 6.05, 8.17, and 8.80 μ; nmr ($CDCl_3$) τ 0.54 (1 H, s), 3.61 (1 H, d, J = 3 Hz), 6.85 (1 H, m), 7.91 (6 H, s), 8.03 (3 H, s), 8.43 (3 H, br s).

Dehydroeupacunolin *p*-Bromophenylhydrazone (23).—A solution of dehydroeupacunolin (**12**, 54 mg) in 50% aqueous methanol (2 ml) was treated with a solution of *p*-bromophenylhydrazine hydrochloride (75 mg) in 50% aqueous methanol (1 ml). The reaction mixture was cooled, diluted with water, and extracted with chloroform. The chloroform was dried (Na_2SO_4), filtered, and evaporated to give a brown oil (60 mg). Chromatography on silicic acid by elution with methanol–chloroform (1:99) yielded one main fraction (47 mg). Recrystallization from methanol–ether–hexane afforded the hydrazone **23** (23 mg) as pale yellow crystals: mp 173–180° dec; ir (KBr) 2.83, 3.03, 3.38, 5.67, 5.73, 6.06, 6.26, 6.37, 6.72, 8.16, 8.73, 9.58, 10.25, and 12.20 μ.

Anal. Calcd for $C_{23}H_{31}BrN_2O_7$: C, 57.24; H, 5.32; Br, 13.61. Found: C, 57.05; H, 5.33; Br, 13.69.

Methanolysis of Eupatocunoxin.—A solution of eupatocunoxin (**7**, 50 mg) and sodium methoxide (10 mg) in absolute methanol (50 ml) was refluxed for 6 days. The mixture was acidified with acetic acid, diluted with water (50 ml), and extracted with ether. The ether layer was washed with water, dried (Na_2SO_4), and evaporated. Glpc of the residue showed one short retention time peak which was identified as methyl 2-methyl-*trans*-2,3-epoxybutyrate by comparison of retention times with an authentic sample.

Methanolysis of Eupatocunin.—A solution of eupatocunin (**6**, 165 mg) and sodium methoxide in absolute methanol (16 ml) was kept at room temperature for 11 days. The reaction mixture was acidified with concentrated HCl, diluted with water (60 ml), and extracted with ether. The ether layer was separated into acid and neutral fractions in the usual way. The neutral fraction was dried (Na_2SO_4) and evaporated. Glpc of the residue showed a peak which was identified as methyl angelate by comparison of its retention time with that of an authentic sample. Methyl tiglate had a different retention time.

Registry No.—**1**, 33854-15-8; **2**, 33853-88-2; **3**, 33853-82-6; **4**, 33853-89-3; **5**, 33853-90-6; **6**, 33853-87-1; **7**, 39204-36-9; **8**, 39204-37-0; **9**, 33853-86-0; **10**, 39204-39-2; **11**, 33911-41-0; **12**, 33853-85-9; **13**, 33853-83-7; **14**, 33853-84-8; **15**, 39266-89-2; **16**, 39204-44-9; **17**, 39204-45-0; **18**, 39204-46-1; **19**, 39152-57-3; **20**, 39152-58-4; **21**, 39152-59-5; **22**, 39152-60-8; **23**, 39152-61-9.