Tumor Inhibitors. XLI.[1a] Structural Elucidation of Tumor-Inhibitory Sesquiterpene Lactones from *Eupatorium rotundifolium*[1b]

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The structure and stereochemistry of eight cytotoxic sesquiterpene lactones from *Eupatorium rotundifolium* have been determined by relating them to the X-ray-defined structure of euparotin bromoacetate (**3**). Three of the compounds, eupachlorin (**6**), eupachlorin acetate (**5**), and eupachloroxin (**13**), are the first reported chlorine-containing sesquiterpenes. Chemical and spectroscopic evidence is advanced for assignment of stereochemically complete structures for these compounds and euparotin (**2**), euparotin acetate (**1**), eupatoroxin (**7**), eupatundin (**8**), and 10-epieupatoroxin (**12**). Two of the more abundant compounds, **1** and **5**, were also tested *in vivo* and were found to show tumor-inhibitory activity.

In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of *Eupatorium rotundifolium* L. (Compositae)[5] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[6] Our preliminary reports[7,8] have described the isolation and structural elucidation of eight novel growth-inhibitory guaianolide sesquiterpene lactones. It is the purpose of this paper to present in detail the structural elucidation of these materials.

Fractionation of the ethanol extract (Chart I), guided by assay against KB (Table I), revealed that the 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. Silicic acid column chromatography of the aqueous methanol soluble material yielded six fractions (G–L) possessing cytotoxic activity. Further separations yielded eight new cytotoxic sesquiterpene lactones, euparotin acetate (**1**), euparotin (**2**), eupachlorin acetate (**5**), eupachlorin (**6**), eupatoroxin (**7**), eupatundin (**8**), 10-epieupatoroxin (**12**), and eupachloroxin (**13**). While all of the lactones showed *in vitro* cytotoxicity against KB cell culture, only euparotin acetate and eupachlorin acetate were isolated in sufficient quantity for *in vivo* assay. Both compounds showed reproducible inhibitory activity against the Walker 256 intramuscular carcinosarcoma in rats.

The molecular formula, $C_{20}H_{24}O_7$, was assigned for euparotin (**2**) on the basis of elemental analysis. The presence of high-intensity end absorption in the ultraviolet spectrum and of bands at 5.68 and 6.05 μ in the infrared spectrum suggested the presence of an α,β-unsaturated lactone group. The infrared spectrum also indicated the presence of hydroxyl (2.90 μ) and α,β-unsaturated ester (5.86 μ) groupings.

Acylation of euparotin with bromoacetic anhydride gave a monobromoacetate, which was crystallized from benzene–petroleum ether as a benzene solvate, $C_{22}H_{25}BrO_8 \cdot {}^1/_2C_6H_6$. X-Ray crystallographic analysis[9] established that the bromoacetate has structure **3**. It therefore follows that euparotin has structure **2**. Acetylation of euparotin yielded euparotin acetate (**1**), identical with the natural product, which was assigned the molecular formula $C_{22}H_{26}O_8$ on the basis of elemental analysis and high-resolution mass spectrometry.[10] In addition to the infrared bands present in euparotin (**2**), euparotin acetate (**1**) showed bands at 5.74 and 7.95 μ, attributable to the C-2 acetate function. Furthermore, the nmr spectrum showed a singlet at τ 7.98 (3 H) corresponding to the acetate methyl group.

Spectral studies indicated that the six additional cytotoxic natural products isolated from the plant were members of the same chemical series. A detailed correlation between infrared and nmr spectral signals and functional groupings in euparotin acetate (**1**) and euparotin (**2**) facilitated assignment of partial structures to the remaining lactones and led to the design of experiments aimed at correlation of the new members of the series with the X-ray-defined compounds. Double-resonance studies of euparotin acetate (**1**) (Figure 1)[11] made possible the assignment of signals for the angelate ester at τ 8.20 (β-methyl signals, J = 1.5 and 7 Hz), 8.25 (α-methyl signals, J = 1.5 Hz), and 3.95 (vinyl proton signal,[12,13] J = 7 and 1.5 Hz). These assignments were supported by irradiation of the vinyl proton signal, which resulted in the collapse of both methyl signals to broad singlets, and by irradiation of the methyl signal region, which resulted in the collapse of the vinyl proton multiplet to a singlet.

Irradiation of the C-7 proton signal (τ 5.82) resulted

(1) (a) Part XL: S. M. Kupchan, Y. Aynehchi, J. M. Cassady, H. K. Schnoes, and A. L. Burlingame, *J. Org. Chem.*, **34**, 3867 (1969). (b) The investigation at the University of Wisconsin was supported by grants from the National Institutes of Health (CA-04500) and the American Cancer Society (T-275), and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH 43-64-551).

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(5) Leaves, stems, flowers, and roots were collected in Florida, Sept 1960. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center (CCNSC).

(6) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the CCNSC by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(7) 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).

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(9) X-Ray crystallographic analysis was carried out by Dr. G. A. Sim and Dr. A. T. McPhail. A complete description of the X-ray results will be published separately by Professor Sim.

(10) For the mass spectral data, the authors thank Dr. G. Van Lear and Dr. F. W. McLafferty of the Purdue Mass Spectrometry Center, and Dr. H. K. Schnoes and Dr. A. L. Burlingame, University of California, Berkeley.

(11) The double-resonance studies were done at 100 MHz, and the values differ slightly from those recorded in Table II, which were obtained from 60-MHz studies. We thank Dr. P. Bender and Miss M. Petri for measuring the 100-MHz spectra.

(12) R. R. Frazer, *Can. J. Chem.*, **38**, 549 (1960).

(13) W. Herz and M. V. Lakshmikantham, *Tetrahedron*, **21**, 1711 (1965).

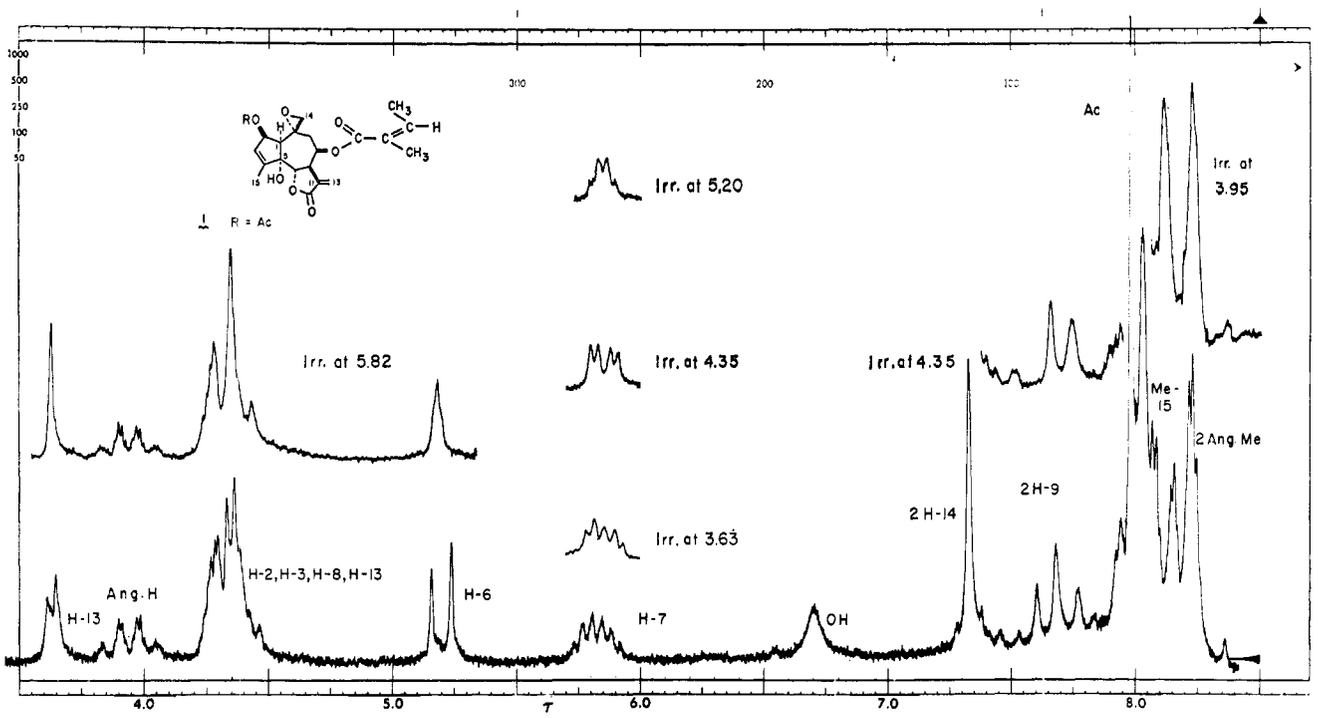

Figure 1.—Double-resonance (100 MHz) nmr spectrum of euparotin acetate (1).

CHART I
FRACTIONATION OF THE CYTOTOXIC EXTRACT FROM *E. rotundifolium*

concentrated ethanol extract from *E. rotundifolium*
A, 500 g

partition between chloroform and water

aqueous extract B, 150 g

chloroform extract C, 283 g

partition between aqueous methanol (1:9) and petroleum ether

aqueous methanol extract E, 170 g

petroleum ether extract D, 88 g

silicic acid chromatography

fractions F–L

TABLE I
CYTOTOXICITY OF FRACTIONS FROM *E. rotundifolium*

Fraction	ED_{50}, μg/ml	Fraction	ED_{50}, μg/ml
A	2.6	G	0.29
B	100	H	0.80
C	2.1	I	2.6
D	30	J	2.9
E	1.9	K	2.4
F	99	L	2.6

in the collapse of one of the C-13 proton doublets (at τ 3.65) to a broad singlet,[14] the sharpening of part of the τ 4.3 region (containing the other C-13 proton), and the collapse of the C-6 proton doublet (at τ 5.20) to a singlet. Irradiation at τ 4.35 resulted in collapse of the C-7 proton signal (τ 5.82) to a doublet of doublets ($J_{7,13a}$ = 3 Hz and $J_{6,7}$ = 8 Hz), collapse of the C-13 proton doublet at τ 3.65, and collapse of the multiplet centered at τ 7.70 (2 H) to an AB quartet [τ 7.59 (d), τ 7.81 (d, J = 15 Hz)]. Apparently, the τ 7.70 region contained the AB signals of the ABX spin system attributable to protons on carbon atoms 8 and 9. Additional spin-decoupling studies are summarized in Figure 1.

Treatment of euparotin acetate (1) with sodium methoxide in methanol gave 13-methoxydihydroeuparotin (4).[15,16] The intensity of absorption in the ultraviolet spectrum was substantially lessened, the infrared spectrum indicated loss of the acetate carbonyl, and the nmr spectrum (Table II) showed signals corresponding to $-CH_2OCH_3$, angelate ester, exocyclic epoxide, and allylic alcohol groupings. The τ 3.0–5.0 region of the spectrum lacked the two C-13 proton signals and the C-2 proton signal present in the spectrum of the precursor.

Eupachlorin acetate (5), $C_{22}H_{27}ClO_8$, showed spectral characteristics indicative of the presence of an angelate ester, an α,β-unsaturated lactone with an exocyclic methylene group, and an allylic acetate, and the absence of an exocyclic epoxide methylene grouping. Location of the chlorine atom on the C-14 methylene group was supported by the downfield shift of the signal assigned to the C-14 methylene protons in 5 (τ 6.47) relative to the signal for the C-14 methylene protons (τ 7.32) in the nmr spectrum of euparotin acetate (1). Two D_2O-exchangeable hydroxyl proton signals were detected in contrast to one in euparotin acetate (1). The tertiary nature of these hydroxyl functions was indicated by their resistance to acetylation upon treatment with acetic anhydride and pyridine. These data were consistent with structural formula 5 for eupachlorin acetate, apart from stereochemistry. Chroma-

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TABLE II

NUCLEAR MAGNETIC RESONANCE DATA FOR *Eupatorium rotundifolium* DERIVATIVES[a]

Compd	C-1	C-2	C-3	C-6	C-7	C-8	C-9	C-13	C-14	C-15	Angelate	Other
Euparotin acetate (1)	7.9	4.3 m	4.3 m	5.20 d (8)	5.8 m	4.3 m	7.59 dd (3, 10) 7.82 dd (4, 10)	3.63 d (3) 4.35 d (3)	7.32 s	8.0	8.2 m 8.0 m 3.9 m	OAc 7.98 s 1 OH
Euparotin (2)	7.35	5.2 m	4.2 m	5.20 d (8)	5.8 m	4.3 m	7.25 dd (7, 15) 7.75 dd (8, 15)	3.62 d (3) 4.35 d (3)	7.30 s	8.0	8.2 m 8.1 m 3.9 m	2 OH
Euparotin bromoacetate (3)	7.60	4.2 m	4.3 m	5.20 d (8)	5.80	4.30	7.5 m	3.60 d (3) 4.35 d (3)	7.30 s	8.0	8.2 m 8.0 m 3.9 m	1 OH CH_2Br 6.21 s
13-Methoxy dihydro-euparotin (4)	7.60 d (6)	5.2 m	4.3 m	5.24 d (9)	7.1–7.8	4.7 m	7.6 m	6.25 m	7.27 s	8.1	8.1 m 8.1 m 3.9 m	2 OH OCH_3 6.60 s
Eupachlorin acetate (5)	7.29 d (5)	4.2 m	4.2 m	5.20 d (8)	5.4 m	4.4 m	7.6 m	3.68 d (3) 4.45 d (3)	6.47 s	8.0	8.2 m 8.0 m 3.9 m	OAc 8.02 s 2 OH
Eupachlorin (6[b])	7.30 m	5.5 m	4.3 m	5.72 d (8)	5.5 m	4.3 m	7.8 m	3.87 d (3) 4.52 d (3)	6.14 d (11) 6.37 d (11)	8.1	8.1 m 8.3 m 3.9 m	3 OH
Eupatoroxin (7[c])	8.4 m	5.76 d (5)	6.64 br s	4.96 d (7)	5.7 m	4.45 m	7.7 m	3.75 d (3) 4.21 d (3)	7.32 d (5) 7.43 d (5)	8.42 s	8.2 m 8.1 m 3.9 m	2 OH
Eupatundin (8)	7.32 d (5)	5.60 br d (5)	6.58 br s	5.08 d (8)	6.2 m	4.5 m	7.06 d (8)	3.70 d (3) 4.45 d (3)	4.94 br s	8.35 s	8.2 m 8.1 m 3.9 m	2 OH
Eupatundin acetate (9)	...	4.60 d (7)	6.50 br s	5.17 d (8)	6.2 m	4.5 m	7.1 m	3.68 d (3) 4.42 d (3)	4.90 s	8.35 s	8.2 m 8.1 m 3.9 m	OAc 7.92 s 1 OH
13-Methoxy dihydro-eupatundin (10)	7.25 d (5)	5.52 br d (5)	6.52 br s	5.17 d (9)	7.2 m	4.8 m	7.2 m	6.30 d (4)	4.92 s	8.40 s	8.1 m 8.0 m 3.9 m	OCH_3 6.60 s C-11 6.62 2 OH
Dehydro-eupatundin (11)	6.55 s	...	6.31 s	5.46 d (8)	6.3 m	4.45 m	6.9 m 8.1 m	3.65 d (3) 4.40 d (3)	4.90 br s 4.75 br s	8.25 s	8.2 m 8.2 m 3.9 m	1 OH
10-Epieupator-oxin (12[c])	8.52 d (5)	5.5 m	6.62 br s	4.92 dd (8, 1)	5.9 m	4.35 m	6.7 m	3.76 d (3) 4.35 d (3)	7.3 m 7.24 d (5)	8.41 s	8.0 m 8.2 m 3.9 m	2 OH 5.23 d (3) 5.49 d (1)
Eupachloroxin (13[c])	7.5 m	5.6 m	6.63 br s	4.98 d (8)	5.2 m	4.2 m	7.4 m	3.80 d (4) 4.47 d (3)	6.19 d (12) 6.34 d (12)	8.40 s	8.2 m 8.1 m 3.9 m	3 OH
Chlorohydrin 14[d]	6.59 d (5)	5.7 m	5.22 d (3)	4.60 d (8)	6.0 m	4.3 m	6.6–6.9	3.66 d (3) 4.44 d (3)	4.7 m 5.0 m	7.80 s	8.2 m 8.3 m 4.0 m	3 OH
Chlorohydrin 15[c]	7.06 d (8)	5.69 t (8)	5.39 d (8)	4.50 d (8)	6.3 m	4.3 m	7.32 d (4)	3.78 d (3) 4.31 d (3)	4.8 m	8.30 s	8.1 m 8.2 m 3.9 m	3 OH
Chlorohydrin diacetate 16[c]	6.60 d (8)	4.76 dd (8, 7)	3.94 d (7)	4.40 d (9)	6.3 m	4.3 m	7.4 m	3.70 d (4) 4.22 d (3)	4.7 m	8.28 s	8.1 m 8.1 m 3.8 m	OAc 7.82 s 7.98 s 1 OH
Epoxide 17	6.7 m	5.9 m	5.9 m	5.04 d (10)	6.3 m	4.2 m	7.36 br s	3.76 m 4.26 d (3)	4.79 4.95	8.42 s	8.1 m 8.2 m 3.9 m	2 OH
Epoxide diacetate 18	6.42 d (8)	5.21 t (8)	4.2 m	5.00 d (9)	6.4 m	4.25 m	7.4 m	3.62 d (3) 4.3 m	4.97 4.83	8.44 s	8.1 m 8.1 m 3.9 m	OAc 8.00 s 7.79 s no OH

[a] Spectra were determined on a Varian A-60A spectrometer in deuteriochloroform unless otherwise indicated. Values are given in τ units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet. Numbers in parentheses denote coupling constants in hertz. [b] Dimethyl sulfoxide-d_6. [c] Acetone-d_6. [d] Pyridine-d_5.

tography of eupachlorin acetate (5) upon acid-washed alumina resulted in transformation to euparotin acetate (1) in 65% yield.[17,18] This interrelation of eupachlorin acetate (5) with X-ray-defined euparotin bromoacetate (3) proved the functional group pattern, stereochemistry, and absolute configuration of eupachlorin acetate (5). In accord with expectation, treatment of eupachlorin acetate (5) with sodium methoxide in methanol gave 13-methoxydihydroeuparotin (4).

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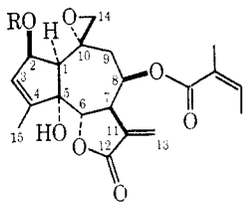
(18) I. Mochida, I. Take, Y. Saito, and Y. Yoneda, *ibid.*, **32**, 3894 (1967).

Eupachlorin (**6**) was assigned the molecular formula $C_{20}H_{25}ClO_7$ on the basis of elemental analysis. The nmr spectrum showed signals assignable to an α,β-unsaturated lactone with an exocyclic methylene, angelate ester and allylic alcohol groupings, and an AB quartet at τ 6.14 and 6.37 (2 H, doublets, J = 11 Hz) assignable to the C-14 methylene protons. Acetylation of eupachlorin (**6**) yielded eupachlorin acetate (**5**). Acid-washed alumina chromatography of eupachlorin (**6**) gave (in 80% yield) euparotin (**2**).

Since there have been no apparent prior reports of isolation of chloro-terpene natural products (although numerous other halogenated compounds occur naturally[19-21]), the possibility was considered that eupachlorin acetate (**5**) may have been formed during the isolation procedure, by addition of the elements of hydrogen chloride to euparotin acetate (**1**). However, when a separation procedure was devised which rigorously excluded chlorine-containing salts and solvents, crude eupachlorin acetate (**5**) was isolated in good yield. This fact, and the well documented cooccurrence in the Compositae of polyacetylenic epoxides, chlorohydrins, and chlorohydrin acetates,[22] support the view that the chloro sesquiterpenes are, indeed, naturally occurring compounds.



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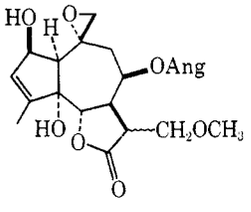
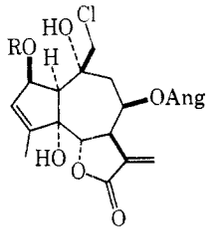
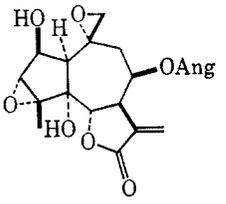
2, R = H
3, R = $COCH_2Br$



4



5, R = Ac
6, R = H



7

The fifth naturally occurring sesquiterpene lactone from *E. rotundifolium* was designated eupatoroxin (**7**). The nmr spectrum revealed the presence of an angelate ester grouping, an α,β-unsaturated lactone with an exocyclic methylene group, and two D_2O-exchangeable hydroxyl protons. The signal for the exocyclic epoxide methylene at C-14 appeared as an AB quartet. The signals for the C-3 vinyl proton and C-15 vinyl methyl protons in the euparotin (**2**) spectrum were replaced by a broad singlet at τ 6.64 and a sharp methyl singlet at τ 8.42. These data, and the fact that eupatoroxin (**7**), $C_{20}H_{24}O_8$, upon elemental analysis, was found to contain one more oxygen than euparotin (**2**) indicated the presence of a 3,4-epoxide (*cf.* calocephalin,[23] C-3 H, τ 6.55; C-15 H, τ 8.55). To confirm the proposed structure of **7**, a study of its preparation *via* expoxidation of **2** was undertaken. Treatment of a chloroform solution of euparotin (**2**) with *m*-chloroperbenzoic acid yielded a mixture with spectral properties indicative of epoxidation of the C-3,4 double bond and the angelate double bond; there was no detectable epoxidation of the α,β-unsaturated lactone double bond. Temperature, reaction time, and molar ratios were varied until an optimum yield of the desired 3,4-epoxide **7** was obtained. Epoxidation of euparotin (**2**) to eupatoroxin (**7**) defined the latter's structure and the relative and absolute configurations of seven of the nine asymmetric centers (apart form the 3,4-epoxide stereochemistry). The 3,4-epoxide was assigned the α configuration on the basis of interrelation with eupatundin (**8**), a sixth sesquiterpene lactone isolated from the plant (see below).

Preliminary characterization of eupatundin (**8**) indicated a close relationship to euparotin (**2**); the infrared and ultraviolet spectral characteristics were similar, and both formed monoacetates (**9** and **1**, respectively) upon acetylation in acetic anhydride–pyridine. Elemental analysis supported assignment of molecular formula $C_{20}H_{24}O_7$, indicative of an isomeric relationship to euparotin (**2**). The nmr spectrum of eupatundin (**8**) showed signals assignable to an angelate group and an α,β-unsaturated lactone with an exocyclic methylene group, and showed two D_2O-exchangeable hydroxyl protons. Signals assignable to the C-15, C-3, and C-2 protons appeared at positions near those in the spectrum of eupatoroxin (**7**), which indicated the presence of an epoxide at C-3,4. Accordingly, a broad doublet at τ 5.60 (J = 5 Hz), assignable to the C-2 proton, was at higher field than the corresponding signal in euparotin (**2**, τ 5.20). A two-proton broad singlet at τ 4.94 was indicative[24] of the presence of a second exocyclic methylene grouping. This exocyclic methylene was tentatively placed at C-10 because of the absence of the usual signals for exocyclic epoxide methylene protons and the selective deshielding of one of its protons in the corresponding 2-keto derivative, dehydroeupatundin (**11**), obtained by Jones oxidation of **8**. Treatment of eupatundin (**8**) with methanolic sodium methoxide gave crystalline 13-methoxydihydroeupatundin (**10**), isomeric with **4**.

Confirmation of structure **8** for eupatundin was obtained by direct interrelation with eupatoroxin (**7**) *via* epoxidation of the C-10,14 double bond of eupatundin (**8**). Initial epoxidation attempts yielded large amounts of angelate side-chain epoxidation products, with chromatographic behavior so similar to the desired C-10,14 epoxides that separation could not be achieved. However, oxidation for 0.5 hr in refluxing chloroform with a slight molar excess of *m*-chloroperbenzoic acid, followed by column chromatography, gave a mixture containing two C-10,14 epimeric epoxides of eupatundin (**8**), in addition to a 78% recovery of starting material. Silica gel chromatography of the product mixture gave partial separation of the major product from the minor product. The major

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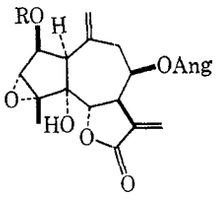
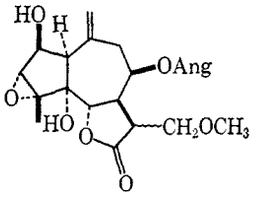
(21) H. Matsuda, Y. Tomiie, S. Yamamura, and Y. Hirata, *Chem. Commun.*, 898 (1967).
(22) F. Bohlmann, *Progr. Chem. Org. Nat. Prod.*, **25**, 1 (1967).
(23) T. J. Batterham, N. K. Hart, and J. A. Lamberton, *Aust. J. Chem.*, **19**, 143 (1966).
(24) W. Herz and S. Inayama, *Tetrahedron*, **20**, 341 (1964).

fraction yielded crystalline eupatoroxin (**7**). Correlation of eupatundin (**8**) with eupatoroxin (**7**) defined the structure and the stereochemistry and absolute configuration of all centers in **8** excepting C-3 and C-4. The column fraction rich in the minor epoxide was found to yield, upon further chromatography, material identical with the naturally occurring 10-epieu patoroxin (**12**), $C_{20}H_{24}O_8$. 10-Epieupatoroxin was thus characterized as the C-10,14 epimer of eupatoroxin (**7**).

Eupachloroxin (**13**) was isolated as a chromatographically homogeneous amorphous solid. Mass spectral analysis supported the molecular formula $C_{20}H_{25}ClO_8$, while the nmr spectrum showed signals which supported the presence of a 3,4-epoxide (τ 6.63, 1 H, br s), a chloromethyl grouping attached to C-10 (τ 6.19 and 6.34, 2 H, doublets, J = 12 Hz), two tertiary hydroxyl groups (τ 5.67, 1 H, singlet, and τ 6.03, 1 H, singlet), and one secondary hydroxyl group (τ 5.22, doublet, J = 4 Hz). Eupachloroxin (**13**) was converted to eupatoroxin (**7**) upon chromatography on alumina, to confirm the functional group pattern and all stereochemical assignments but the 3,4-epoxide orientation.



8, R = H
9, R = Ac



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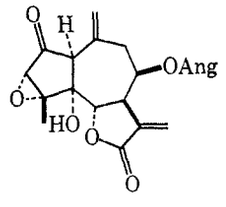
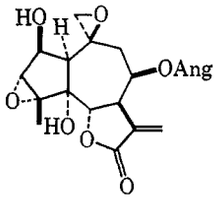


11



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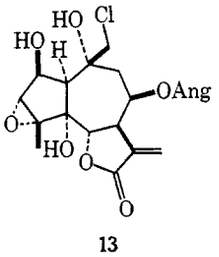
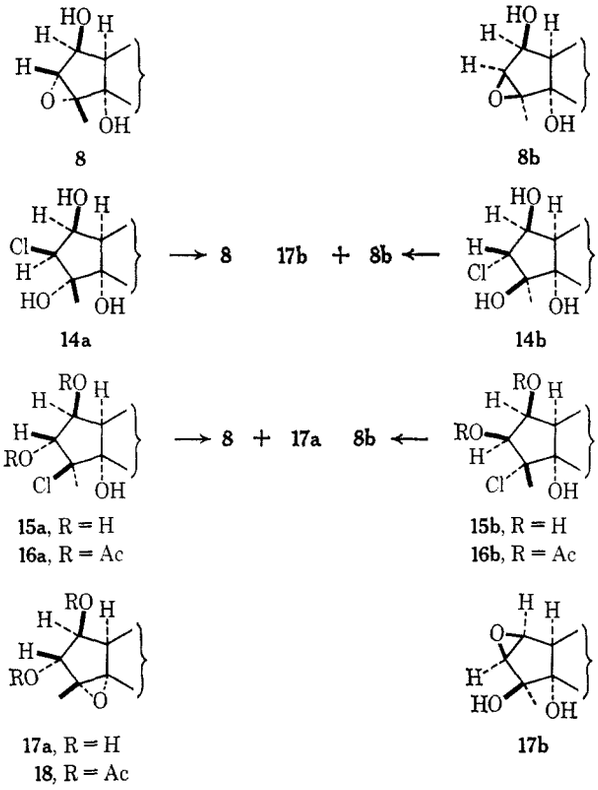


13

Eupatundin (**8**), eupatoroxin (**7**), 10-epieupatoroxin (**12**), and eupachloroxin (**13**) were interrelated in a manner which demonstrated their identical 3,4-epoxide stereochemistry. The stereochemistry of the 3,4-epoxide was determined by a series of epoxide–chlorohydrin interconversion reactions. Treatment of eupatundin (**8**) with hydrogen chloride gave a mixture of chlorohydrin **14** (72% yield) and chlorohydrin **15** (20% yield). A compound with β-3,4-epoxide stereochemistry (**8b**) would be expected to yield chlorohydrins **14b** and **15b**; one with an α-3,4-epoxide (**8**) would yield **14a** and **15a**. Analysis and mass spectral data of each isomer fit the molecular formula $C_{20}H_{25}ClO_7$ while the nmr spectral data indicated that the α,β-unsaturated lactone, the angelate, and the exocyclic C-10,14 methylene functions were still present. Each spectrum showed a change in the chemical shift and multiplicity of the C-3 proton signal, a change in the chemical shift of signal for the C-15 methyl protons, and the appearance of a third D_2O-exchangeable hydroxyl proton. Acetylation of the chlorohydrin **15** afforded diacetate **16**, indicated by the appearance of two acetate methyl signals in the nmr, and by the mass spectral molecular ion and peaks which indicated loss of one and two acetate functions. The nmr spectrum indicated that both acylated hydroxyl groups were secondary, since the two signals for protons on ester-bearing carbons appeared at appropriately lower field. The chlorohydrin possessing two secondary hydroxyl functions could be assigned either structure **15a** or **15b**.



8 **8b**

14a → **8** **17b** + **8b** ← **14b**

15a, R = H
16a, R = Ac

→ **8** + **17a** **8b** ←

15b, R = H
16b, R = Ac

17a, R = H
18, R = Ac

17b

Acetylation of the chlorohydrin **14** in either acetic anhydride–pyridine or acetic anhydride–*p*-toluenesulfonic acid yielded only eupatundin acetate (**9**). Acid-washed alumina chromatography catalyzed epoxide formation from chlorohydrins **14** and **15**. Chlorohydrin **14** gave only eupatundin (**8**), in 89% yield, in accord with the expected *trans* opening of eupatundin (**8**) upon treatment with hydrogen chloride and expected *trans* closure of the chlorohydrin upon treatment with acid-washed alumina; a *cis* reaction in either case would preclude re-formation of eupatundin (**8**). Similar treatment of chlorohydrin **15** gave eupatundin (**8**) in 6.5% yield and a new substance in 90% yield. Mass spectral analysis of the new material indicated that it was an isomer of eupatundin (**8**). Angelate, α,β-unsaturated lactone exocyclic methylene, and two D_2O-exchangeable hydroxyl protons were detected by nmr spectroscopy. There was a signal for a methyl group attached to a carbon bearing

an epoxide grouping, but none for a proton on carbon bearing an epoxide. This information indicated that the new substance was the 4,5-α-epoxide **17a**. Acetylation of **17a** gave the diacetate, $C_{24}H_{28}O_9$, whose nmr spectrum showed two acetate methyl signals and the C-2 and the C-3 proton signals in the expected deshielded chemical shift region. The diacetate showed neither D_2O-exchangeable hydroxyl proton nmr spectral signals nor hydroxyl stretching absorption in the infrared spectrum. With this evidence, the diacetate could be assigned structural formula **18**. These experiments clearly distinguished between the α and the β stereochemistry of the 3,4-epoxide in eupatundin (**8**). Had the 3,4-epoxide been β, treatment with hydrogen chloride would have been expected to yield the 3α-chloro derivative **14b** and the 4α-chloro derivative **15b**. Compound **15b** would have been expected to yield only **8b**, while **14b** would have been expected to close, in part, to the unacylable 2,3-β-epoxide **17b**. However, as noted above, the re-formed epoxide was readily acetylated. Hence, the experimental findings strongly support the view that the 3,4-epoxide is α oriented (3*R*,4*R*) in eupatundin (**8**), and therefore also in eupatoroxin (**7**), 10-epieupatoroxin (**12**), and eupachloroxin (**13**).

The eight new sesquiterpene lactones described herein all showed growth-inhibitory activity against KB[6] (see Table III). The only substances tested *in vivo*,

TABLE III

BIOLOGICAL ACTIVITY[a]

A. Cytotoxicity of Compounds against Eagle's KB Strain of Human Carcinoma of the Nasopharynx

Compd	ED_{50}, μg/ml
1	0.21
2	0.21
5	0.18
6	0.21
7	2.8
8	0.39
12	2.6
13	3.6

B. Tumor-Inhibitory Activity against the Walker 256 Intramuscular Carcinosarcoma in Rats[b]

Compd	Dose, mg/kg	Survivors	Animal wt change difference, g T − C	Tumor wt, mg T/C	T/C × 100
1	100	2/4	−4	1800/7800	Toxic
	75	4/4	−10	1800/7800	23
	55	4/4	−5	9100/12800	71
5	400	3/4	−20	2100/5000	42
	300	3/4	−12	1900/5000	38
	200	4/4	−15	4400/5000	88

[a] Reference 6. [b] T, treated animals; C, control animals.

euparotin acetate (**1**) and eupachlorin acetate (**5**), showed significant tumor-inhibitory activity. It is noteworthy that these two compounds, like other unsaturated lactones found to show significant *in vivo* tumor-inhibitory activity,[25,26] possess at least two "alkylating" functions, *i.e.*, structural moieties sensitive to attack by nucleophiles. Investigations are in progress which are aimed at evaluation of the significance of the α,β-unsaturated lactone, the epoxide, the allylic acetate, and other structural features in relation to tumor-inhibitory activity.

(25) S. M. Kupchan, Y. Aynehchi, J. M. Cassady, A. T. McPhail, G. A. Sim, H. K. Schnoes, and A. L. Burlingame, *J. Amer. Chem. Soc.*, **88**, 3674 (1966); *J. Org. Chem.*, in press.

(26) S. M. Kupchan, R. J. Hemingway, D. Werner, A. Karim, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **90**, 3596 (1968); *J. Org. Chem.*, in press.

Experimental Section

Melting points were determined on a Thomas–Hoover capillary melting point apparatus which had been calibrated with standard samples. Values of $[\alpha]D$ were determined on a Zeiss–Winkel polarimeter and have been approximated to the nearest degree. Optical rotatory dispersion curves were determined on a Cary 60 recording spectropolarimeter. Ultraviolet absorption spectra were determined on a Beckman Model DK-2A recording spectrophotometer. Infrared absorption spectra were determined on Beckman Model 5A and Model 9 recording spectrophotometers. Nuclear magnetic resonance spectra were determined on Varian A-60A and HA-100 spectrometers. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. Petroleum ether refers to the fraction with bp 60–68°. Thin layer chromatography (tlc) was carried out on precoated silica gel (Brinkmann) plates developed with 5% methanol in chloroform. Spots were visualized by heating plates sprayed with a 3% solution of ceric sulfate in 3 *N* sulfuric acid. Evaporations were carried out at reduced pressure under 40°.

Isolation Procedure.—The concentrated alcoholic extract from 17 kg of dried plant (A, 500 g) was partitioned between 4 l. of chloroform and 2 l. of water. The water layer was washed with chloroform (1 l.) and the combined chloroform extract was washed with water. The water layer was evaporated to a red oil (B, 150 g); evaporation of the chloroform gave a dark green tar (C, 283 g) which contained most of the KB activity present in the alcoholic extract. The green tar was partitioned between 10% aqueous methanol (3 l.) and petroleum ether (3 l.). The petroleum ether fraction was washed with 10% aqueous methanol (1 l.) and the combined methanolic fractions were washed with petroleum ether (three 2-l. portions). The petroleum ether layer was evaporated to a green tar (D, 88 g), while the aqueous methanol solution was concentrated to a dark brown tar (E, 170 g) which showed significant KB activity. Chromatography of 70 g of the methanol-soluble material on silicic acid (3 kg, Mallinckrodt, 100 mesh) gave fractions F–L upon elution with successively more polar chloroform–methanol mixtures. Further fractionation as described below gave pure sesquiterpene lactones.

Silicic acid column chromatography (elution with chloroform–methanol mixtures) of fraction G (13 g) gave crude euparotin acetate (**1**, 6 g). Trituration with ether (50 ml) gave crystalline material. Recrystallization from a chloroform–benzene–petroleum ether mixture gave 4.35 g of euparotin acetate (**1**): R_f 0.50; mp 156–157° *in vacuo*; $[\alpha]^{30}D$ −191° (*c* 0.54, EtOH); uv λ^{EtOH} end absorption 210 mμ (ϵ 18,400); ir λ^{KBr}_{max} 2.91, 5.67 (lactone carbonyl), 5.74 (acetate carbonyl), 5.85 (angelate carbonyl), 6.03, 6.04 (C=C), and 7.95 μ; mass spectrum *m/e* 418 (M^+).

Anal. Calcd for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26. Found: C, 63.08; H, 6.14.

Further elution of the fraction G column gave crude eupachlorin acetate (**5**, 7 g). Trituration with ether gave crystalline material, mp 197–198° dec. Fractional recrystallization from benzene gave colorless needles of eupachlorin acetate (**5**): R_f 0.44; mp 161–164° *in vacuo*, dec; $[\alpha]^{29}D$ −192° (*c* 0.63, MeOH); uv λ^{MeOH}_{max} 212 mμ (ϵ 15,800); ir λ^{KBr}_{max} 2.94, 5.65, 5.75, 5.81, 6.01, and 8.14 μ. The material gave a blue-green Beilstein test for halogen.

Anal. Calcd for $C_{22}H_{27}ClO_8$: C, 58.10; H, 5.95; Cl, 7.71. Found: C, 58.16; H, 5.93; Cl, 7.80.

Trituration of fraction H (5 g) with ether gave crystalline eupatundin. Recrystallization from chloroform–benzene–petroleum ether gave colorless needles of eupatundin (**8**, 3.90 g): R_f 0.36; mp 188–189°; $[\alpha]^{29}D$ −80° (*c* 0.44, EtOH); uv λ^{MeOH}_{max} 209 mμ (ϵ 16,100); ir λ^{KBr}_{max} 2.87, 5.67, 5.86, 6.02, and 6.13 μ.

Anal. Calcd for $C_{20}H_{24}O_7$: C, 63.82; H, 6.43. Found: C, 63.84; H, 6.47.

Fraction I (1 g) was recrystallized from methanol to give colorless plates of eupachlorin (**6**, 0.95 g): R_f 0.30; mp 219–221° dec; $[\alpha]^{27}D$ −110° (*c* 0.35, EtOH); uv λ^{MeOH}_{max} 212 mμ (ϵ 16,000); ir λ^{KBr}_{max} 2.83, 2.98, 5.68, 5.86, 6.08, and 8.04 μ.

Anal. Calcd for $C_{20}H_{25}ClO_7$: C, 58.19; H, 6.06; Cl, 8.61. Found: C, 58.35; H, 6.22; Cl, 8.65.

Silica gel (Merck) chromatography of fraction J (3 g), upon elution with chloroform–acetone–ethanol (80:20:1), gave crude euparotin (1.2 g), which was recrystallized from ethyl acetate–petroleum ether to give needles of euparotin (**2**, 0.95 g): R_f 0.25; mp 199–200° *in vacuo;* $[\alpha]^{32}D$ −124° (*c* 1.25, EtOH); uv λ^{EtOH} end absorption 213 mμ (ε 17,800); ir λ_{max}^{KBr} 2.90, 5.68, 5.86, 6.05, and 6.08 μ; mass spectrum *m/e* 376 (M^+).

Anal. Calcd for $C_{20}H_{24}O_7$: C, 63.82; H, 6.43. Found: C, 63.83; H, 6.59.

Continuing silica gel chromatography of fraction J gave crude eupatoroxin (1.5 g), which was crystallized from acetone–petroleum ether to give colorless needles of eupatoroxin (**7**, 1.1 g): R_f 0.22; mp 197–200°; $[\alpha]^{26}D$ −98° (*c* 1.08, MeOH); uv λ_{max}^{MeOH} 213 mμ (ε 12,900); ir λ_{max}^{KBr} 2.89, 5.65, 5.83, and 6.02 μ.

Anal. Calcd for $C_{20}H_{24}O_8$: C, 61.21; H, 6.17. Found: C, 61.23; H, 6.16.

Further silica gel chromatography of fraction J gave crude eupachloroxin (0.25 g), which was rechromatographed on silica gel to give eupachloroxin (**13**, 100 mg) as a chromatographically homogeneous amorphous solid: R_f 0.23; ir λ_{max}^{KBr} 2.90, 5.65, and 5.85 μ; mass spectrum *m/e* 428 and 430 (3:1, M^+, $C_{20}H_{25}ClO_8$).

Fraction K (0.8 g) was crystallized from acetone–chloroform–petroleum ether to give needles of 10-epieupatoroxin (**12**, 0.68 g): R_f 0.22; mp 230–232°; $[\alpha]^{26}D$ −109° (*c* 0.33, MeOH); uv λ_{max}^{MeOH} 213.5 mμ (ε 15,000); ir λ_{max}^{KBr} 2.88, 5.63, 5.88, 5.91, and 6.07 μ.

Anal. Calcd for $C_{20}H_{24}O_8$: C, 61.21; H, 6.17. Found: C, 61.22; H, 6.07.

Conversion of Euparotin (2) to Euparotin Acetate (1).—Treatment of euparotin (**2**, 50 mg) in pyridine (5 ml) with acetic anhydride (1 ml) gave, after normal work-up, euparotin acetate (**1**, 46 mg) characterized by melting point, mixture melting point, and ir spectral comparison with an authentic sample.

Euparotin Bromoacetate (3).—A solution of euparotin (**2**, 52 mg) in benzene (20 ml) was treated with bromoacetic anhydride at 80° for 10 min. After addition of pyridine (1 ml), the mixture was allowed to stand for 42 hr at 25°. Normal work-up yielded monobromoacetate **3** (47 mg), which was crystallized from benzene–petroleum ether as a benzene solvate, $C_{22}H_{25}BrO_8 \cdot {}^1/_2C_6H_6$: mp 156–157°; $[\alpha]^{32}D$ −142° (*c* 0.38, EtOH); uv λ^{MeOH} end absorption 212 mμ (ε 12,000); ir λ_{max}^{KBr} 2.93, 5.66, 5.71, 5.90, and 6.02 μ.

Anal. Calcd for $C_{22}H_{25}BrO_8 \cdot C_6H_6$: C, 55.98; H, 5.26. Found: C, 56.27; H, 5.31.

Conversion of Euparotin Acetate (1) to 13-Methoxydihydroeuparotin (4).—A solution of euparotin acetate (**1**, 300 mg) and sodium methoxide (200 mg) in methanol (50 ml) was allowed to stand at 25° for 12 hr. The pale yellow solution was diluted with ice–water (500 ml), acidified to pH 1 with 2 *N* sulfuric acid solution, and extracted with chloroform (two 300-ml portions). The combined chloroform extract was washed with water (300 ml), dried (Na_2SO_4), filtered, and evaporated to give colorless crystals (270 mg). Recrystallization from chloroform–petroleum ether gave 13-methoxydihydroeuparotin (**4**): mp 162–163° *in vacuo;* $[\alpha]^{29}D$ −123° (*c* 0.90, $CHCl_3$); uv λ_{max}^{MeOH} 218 mμ (ε 6340); ir $\lambda_{max}^{CHCl_3}$ 2.76, 2.79, 2.86, 5.62, 5.81, and 6.06 μ.

Anal. Calcd for $C_{21}H_{28}O_8$: C, 61.75; H, 6.91. Found: C, 61.46; H, 6.98.

Treatment of Eupachlorin Acetate (5) with Acetic Anhydride–Pyridine.—Treatment of a pyridine solution (2 ml) of eupachlorin acetate (**5**, 182 mg) with acetic anhydride (1 ml) for 18 hr at 25°, followed by normal work-up, gave unchanged eupachlorin acetate (**5**, 173 mg, 95% recovery).

Conversion of Eupachlorin Acetate (5) to 13-Methoxydihydroeuparotin (4).—A solution of eupachlorin acetate (**5**, 100 mg) in methanol (30 ml) containing sodium methoxide (100 mg) was allowed to stand at 25° for 12 hr. The solution was diluted with ice–water (200 ml), acidified to pH 1 with 2 *N* sulfuric acid, and extracted with chloroform (two 200-ml portions). The combined chloroform extract was washed with water (200 ml), dried (Na_2SO_4), filtered, and evaporated to give colorless crystals (85 mg). Recrystallization from chloroform–petroleum ether gave pure 13-methoxydihydroeuparotin (**4**), shown to be identical with the material obtained from euparotin acetate (**1**) by melting point, mixture melting point, and ir and nmr spectral comparisons.

Conversion of Eupachlorin (6) to Eupachlorin Acetate (5).—Treatment of a solution of eupachlorin (**6**, 36 mg) in pyridine (3 ml) with an excess of acetic anhydride at 25° for 12 hr yielded eupachlorin acetate (**5**, 33 mg), characterized by melting point, mixture melting point, mixture tlc, and ir spectral comparisons with the authentic sample.

Conversion of Eupachlorin (6) to Euparotin (2).—Adsorption of eupachlorin (**6**, 41 mg) on Celite (1 g), followed by addition of this mixture to a column of acid-washed alumina (20 g, Merck) and elution with 5% ethanol in chloroform, gave euparotin (**2**, 33 mg), characterized by mixture melting point, $[\alpha]D$, and ir and nmr spectral comparisons with the authentic sample.

Conversion of Eupachloroxin (13) to Eupatoroxin (7).—Adsorption of eupachloroxin (**13**, 46 mg) on Celite (1 g), followed by addition of this mixture to a column of acid-washed alumina (20 g) and elution with 5% ethanol in chloroform, gave eupatoroxin (**7**, 30 mg), characterized by melting point, mixture melting point, mixture tlc, and ir and nmr spectral comparisons with the authentic sample.

Conversion of Eupachlorin Acetate (5) to Euparotin Acetate (1).—Chromatography of eupachlorin acetate (**5**, 270 mg) on acid-washed alumina (200 g) gave two crystalline products upon elution with chloroform–methanol mixtures. The higher R_f component (170 mg) was recrystallized from chloroform–benzene–petroleum ether to give euparotin acetate (**1**, 153 mg, 65% yield), identified by melting point, mixture melting point, and ir spectral comparisons with the authentic sample. The lower R_f component (48 mg) was recrystallized from ethyl acetate–petroleum ether to give euparotin (**2**, 35 mg, 15% yield), identified by melting point, mixture melting point, mixture tlc, and ir spectral comparisons with the authentic sample.

Conversion of Euparotin (2) to Eupatoroxin (7).—Euparotin (**2**, 50 mg, 0.13 mmol) was added to a boiling chloroform (50 ml) solution of 85% *m*-chloroperbenzoic acid (25 mg, 0.12 mmol, FMC Corp.) and the solution was heated under gentle reflux for 90 min. The solution was cooled to 25°, concentrated to a volume of 10 ml, and chromatographed upon neutral alumina (Woelm, activity I, 50 g). Elution with 2% methanol in chloroform gave 43 mg of two-spot material (tlc). The mixture was dissolved in acetone (10 ml) and adsorbed on Celite (1 g) by evaporation of the acetone and drying *in vacuo*. The dried powder was added to a silica gel column (25 g) packed in ethanol-free chloroform. Elution with a chloroform–acetone–ethanol mixture (80:20:1) gave colorless, homogeneous, higher R_f material (25 mg). Crystallization from acetone–petroleum ether gave eupatoroxin (**7**), characterized by mixture melting point, mixture tlc, and ir and nmr spectral comparisons with the authentic sample. The lower R_f material (12 mg) was a mixture which appeared, from nmr spectral data, to contain angelate epoxides.

13-Methoxydihydroeupatundin (10).—Eupatundin (**8**, 200 mg) was added to a solution of sodium methoxide (200 mg) in methanol (40 ml). After 24 hr at 25°, the pale yellow solution was poured into ice–water (400 ml), acidified to pH 1 with 2 *N* sulfuric acid solution, and extracted with chloroform (two 300-ml portions). The chloroform extract was washed with water (200 ml), dried (Na_2SO_4), filtered, and evaporated to a pale yellow solid (125 mg). Crystallization from chloroform–petroleum ether gave 13-methoxydihydroeupatundin (**10**, 80 mg, 41% yield): mp 136–137°; $[\alpha]^{27}D$ −98° (*c* 0.81, MeOH); uv λ_{max}^{MeOH} 217 mμ (ε 11,200); ir λ_{max}^{KBr} 2.94, 5.65, 5.85, and 6.10 μ.

Anal. Calcd for $C_{21}H_{28}O_8$: C, 61.75; H, 6.91. Found: C, 61.82; H, 6.83.

Dehydroeupatundin (11).—Dropwise addition of an excess of 8 *N* chromic acid solution to an ice-cold solution of eupatundin (**8**, 300 mg) in acetone (40 ml) gave, after 12 min at 0°, a red-brown suspension. The suspension was diluted with ice–water (200 ml) and extracted with chloroform (two 200-ml portions). The chloroform solution was dried (Na_2SO_4), filtered, and evaporated to give a pale yellow homogeneous (tlc) solid (255 mg). Chromatography of this material on silicic acid and elution with chloroform–benzene (1:1) gave a colorless oil (220 mg), which was crystallized from chloroform–petroleum ether to give dehydroeupatundin (**11**, 200 mg) as needles: mp 154–155° *in vacuo;* $[\alpha]^{25}D$ −87° (*c* 0.12, MeOH); uv λ_{max}^{EtOH} 213 (ε 18,600) and 296 mμ (ε 52); ir λ_{max}^{KBr} 2.85, 5.63, 5.75 (ketone), 5.85, 6.00, and 6.06 μ; ORD (*c* 0.1235, MeOH) $[\alpha]_{600}^{25}$ −85°, $[\alpha]_{589}$ −87°, $[\alpha]_{320}$ −1101° (trough), λ_0 296 mμ, $[\alpha]_{284}$ +268° (peak), $a = 13.7 \times 10^2$.

Anal. Calcd for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 64.23; H, 5.88.

Eupatundin Acetate (9).—Treatment of a solution of eupatundin (**8**, 200 mg) in pyridine (5 ml) with acetic anhydride (0.5 ml)

at 25° for 24 hr, followed by normal work-up, gave a yellow oil (192 mg). Silica gel column chromatography yielded colorless eupatundin acetate (**9**, 188 mg): ir $\lambda_{max}^{CHCl_3}$ 2.89, 5.67, 5.74, 5.83, 6.03, 6.05, 6.11, and 8.11 μ; mass spectrum *m/e* 418 (M^+, $C_{22}H_{26}O_8$).

Conversion of Eupatundin (8) to Eupatoroxin (7) and 10-Epieupatoroxin (12).—Eupatundin (**8**, 300 mg, 0.80 mmol) was added to a chloroform (25 ml) solution of 85% *m*-chloroperbenzoic acid (190 mg, 0.93 mmol). Epoxidation was allowed to proceed for 0.5 hr under reflux. The solution was cooled to 25°, concentrated to 10 ml, and chromatographed on neutral alumina (300 g). Elution with chloroform gave unchanged eupatundin (**8**, 235 mg, 78% recovery). Elution with 2% methanol in chloroform gave a mixture (60 mg) rich in the higher R_f component of the two major reaction products. Repeated silica gel column chromatography and crystallization from acetone–chloroform–petroleum ether gave 10-epieupatoroxin (**12**), characterized by melting point, mixture melting point, $[\alpha]D$, and ir and nmr spectral comparisons with authentic material. Elution with 2% methanol in chloroform gave a mixture (28 mg) rich in the lower R_f product. This material was dissolved in acetone (10 ml), adsorbed on Celite (1 g), and added to a silica gel column (26 g, packed in ethanol-free chloroform). Elution with chloroform–acetone–ethanol (80:20:1) gave 12 mg of colorless, homogeneous material, which was crystallized from acetone–petroleum ether to give eupatoroxin (**7**, 7 mg), characterized by melting point, mixture melting point, mixture tlc, and ir spectral comparisons with authentic material.

Treatment of Eupatundin (8) with Hydrogen Chloride.—An excess of hydrogen chloride was bubbled into a solution of eupatundin (**8**, 1.0 g) in dioxane–water (9:1, 20 ml) at 25° over the course of 2 min. The solution was diluted with ice–water (400 ml) and extracted with chloroform (two 400-ml portions). The chloroform solution was washed with water (100 ml), dried (Na_2SO_4), filtered, and evaporated to give a pale yellow two-spot (tlc) solid. Silica gel chromatography gave two crystalline chlorohydrins upon elution with chloroform–acetone–ethanol (80:20:1). The higher R_f chlorohydrin was crystallized from chloroform to give colorless needles (**14**, 788 mg, 72% yield): mp 260–262° dec; $[\alpha]^{27}D$ +50° (*c* 0.10, MeOH); uv λ_{max}^{MeOH} 212 mμ (ϵ 15,300); ir λ_{max}^{KBr} 2.92, 5.68, 5.80, 5.99, and 6.05 μ; mass spectrum *m/e* 412 and 414 (3:1, M^+).

Anal. Calcd for $C_{20}H_{25}ClO_7$: C, 58.17; H, 6.10. Found: C, 58.01; H, 6.13.

The lower R_f chlorohydrin was crystallized from acetone–petroleum ether to give colorless needles of **15** (220 mg, 20% yield): mp 190–192° dec; $[\alpha]^{27}D$ −50° (*c* 0.86, MeOH); uv λ_{max}^{MeOH} 212 mμ (ϵ 13,700); ir λ_m^{KBr} 2.90, 5.68, 5.83, 5.99, and 6.06 μ; mass spectrum *m/e* 412 and 414 (3:1, M^+).

Anal. Calcd for $C_{20}H_{25}ClO_7$: C, 58.17; H, 6.10. Found. C, 57.95; H, 6.03.

Acetylation of Chlorohydrin 15.—Treatment of a solution of chlorohydrin **15** (30 mg) in pyridine (1.5 ml) with acetic anhydride (0.5 ml) for 12 hr at 25°, followed by normal work-up, gave a single-spot (tlc) oil (30 mg). Silica gel (3 g) chromatography with chloroform–acetone–ethanol (90:10:1) gave noncrystalline homogeneous diacetate **16** (24 mg): ir $\lambda_{max}^{CHCl_3}$ 2.80, 2.95, 5.65, 5.70, 5.80, 5.99, 6.05, and 8.00 μ; mass spectrum *m/e* 496 and 498 (3:1, M^+, $C_{24}H_{29}ClO_9$).

Acetylation of Chlorohydrin 14.—A solution of chlorohydrin **14** (100 mg) in pyridine (5 ml) was treated with acetic anhydride (1 ml) for 60 hr at 25°, followed by normal work-up, to give eupatundin acetate (**9**, 102 mg), characterized by mixture tlc and ir and nmr spectral comparisons with authentic material.

Conversion of Chlorohydrin 14 to Eupatundin (8).—A solution of chlorohydrin **14** (50 mg) in chloroform was added to a column of acid-washed alumina (30 g). Elution with 2% methanol in chloroform gave only eupatundin (**8**, 40 mg, 89% yield), characterized by mixture melting point, mixture tlc, and ir and nmr spectral comparisons with authentic material. No other products were detected.

Conversion of Chlorohydrin 15 to Eupatundin (8) and Epoxide 17.—Chlorohydrin **15** (150 mg), adsorbed on Celite (1 g) was placed on a column of acid-washed alumina (30 g). Elution with chloroform–methanol mixtures gave two products. Elution with 2% methanol in chloroform gave the higher R_f product (9 mg), which was crystallized from benzene and characterized as eupatundin (**8**) by melting point, mixture melting point, mixture tlc, and ir spectral comparisons with authentic material. Elution with 10% methanol in chloroform gave the second product (**17**, 124 mg), which resisted crystallization: ir $\lambda_{max}^{CHCl_3}$ 2.89, 5.61, 5.81, and 6.06 μ; mass spectrum *m/e* 376 (M^+, $C_{20}H_{24}O_7$).

Acetylation of Epoxide 17.—Treatment of a solution of epoxide **17** (40 mg) in pyridine (5 ml) with acetic anhydride (1 ml) for 12 hr at 25° gave, upon normal work-up, pale yellow noncrystalline homogeneous diacetate **18** (30 mg): ir $\lambda_{max}^{CHCl_3}$ 5.62, 5.71, 5.81, 6.06, and 8.03 μ; mass spectrum *m/e* 460 (M^+, $C_{24}H_{28}O_9$).

Registry No.—**1**, 10215-89-1; **2**, 10191-01-2; **3**, 21893-24-3; **4**, 21893-25-4; **5**, 20501-52-4; **6**, 20071-50-5; **7**, 20071-51-6; **8**, 20071-53-8; **9**, 21893-29-8; **10**, 21893-30-1; **11**, 21893-31-2; **12**, 20071-54-9; **13**, 20071-52-7; **14a**, 20071-55-0; **15a**, 21893-35-6; **16a**, 21893-36-7; **17a**, 20071-57-2; **18**, 21893-38-9.