hydrochloric acid (3%), sodium bicarbonate, and water, and dried and the solvent was removed. The residue (0.575 g) was passed through a column of silica gel (50 g) to yield a product (0.290 g) homogenous by tlc. On keeping, some crystals appeared which were separated with ice-cold hexane. Based on the nmr (Table II) and ir data (Table III) the crystalline benzoate was assigned structure **15**, and the oily benzoate was assigned structure **13**. The acetylation of the mixture of benzoates (0.210 g) obtained above in acetic anhydride (3.9 ml) and dry pyridine (1.7 ml) yielded after the usual work-up the crude acetate (0.2 g) as two spots on tlc. Chromatographic separation on silica gel yielded the major product (0.129 g) in the pure form, which was assigned the structure **14**. An ir showed no absorption in the NH region, and bands at 1775, 1750, 1700, 1633, and 1590 cm^{-1}. The nmr showed signals at δ 7.21 (4 H, q, aromatic), 5.55 (2 H, q, vinylic), 5.22 (1 H, d, carbinolic at C-3), 4.5 (3 H, m, carbinolics C-2 hydromethyl and C-4), 2.03 (3 H, s, acetyl methyl), and 0.88 (3 H, t, terminal methyl). A mass spectrum exhibited M^+ (*m/e* 590), $M^+ - 127$ (*m/e* 463), *m/e* 184 $(-COC_6H_4Br)^+$.

The minor products of acetylation of benzoate from two experiments were pooled and purified by repeated chromatography. The structure **16** for this product follows from the following data. An ir spectrum showed bands at 3400 (NH), 1776, 1750, 1705, 1670, and 1590 cm^{-1}. An nmr had signals at δ 7.61 (4, H, s, aromatic), 6.81 (1 H, s, NH), 5.83 (1 H, d, carbinolic at C-3), 5.50 (2 H, m, vinylic), 4.75 (1 H, m, carbinolic at C-4), 4.61 (2 H, s, carbinolic C-2 hydroxymethyl), 2.08, 1.88 (3 H each, s, acetyl methyl), and 0.88 (3 H, t, terminal $-CH_3$). A mass spectrum exhibited the following peaks: M^+ (*m/e* 650), $M^+ - 60$ (*m/e* 590), $M^+ - 127 + 60$) (*m/e* 463), $M^+ - (184 + 127 + 60)$ (*m/e* 279), and *m/e* 184 $(COC_6H_4Br)^+$.

Mesylation of Diacetate 18.—To a solution of diacetate **18** (0.117 g) in methylene chloride (4 ml) was added triethylamine (0.152 g) followed by methanesulfonyl chloride (0.126 g). The mixture was stirred overnight at room temperature. The reaction mixture was diluted with methylene chloride and washed with sodium bicarbonate, followed by dilute hydrochloric acid and water. The organic liquor was dried and the solvent was removed to yield the crude product (0.155 g). Chromatographic purification gave a pure sample of **21** (0.075 g).

Anal. Calcd for $C_{26}H_{43}NO_9S$ (545): C, 57.0; H, 7.89; N, 2.57; S, 5.86. Found: C, 56.84; H, 7.99; N, 2.59; S, 5.85.

An ir showed bands at 3400 (NH), 1780, 1750, 1690 (carbonyl); nmr δ 6.62 (1 H, s, NH), 5.55 (3 H, m, 2 H vinylic and 1 H C-3 carbinolic), 4.75 (1 H, m, C-4 carbinolic), 4.56 (2 H, d, C-2, CH_2O-), 3.05 (3 H, s, CH_3SO_2), 2.08 (6 H, s, $2CH_3CO-$), 0.89 (3 H, t, terminal $-CH_3$).

Sodium Acetate Treatment of the Mesylate 21.—The mesylate **21** (0.535 g) was dissolved in absolute ethanol (12 ml) in the presence of sodium acetate (0.3 g) and the mixture was refluxed overnight. The reaction mixture was cooled, diluted with ether, washed with water, and dried and the solvent was evaporated. The resulting residue after one crystallization from ether–petroleum ether gave a solid (0.270 g, 73%), mp 87–91°. An analytical sample had mp 94–96°.

Anal. Calcd for $C_{22}H_{35}O_4N$ (377): C, 69.99; H, 9.35; N, 3.71. Found: C, 70.16; H, 9.38; N, 3.64.

An ir showed bands at 3385, 3300 (NH), 1742, 1698, and 1650 cm^{-1} (carbonyl); nmr δ 7.95 (1 H, broad, NH), 7.4 (1 H, d, J = 2 Hz, vinylic), 5.45 (2 H, q, vinylic), 5.02 (1 H, d of t, J = 2 Hz, carbinolic), 2.2 (3 H, s, CH_3CO), 0.9 (3 H, t, terminal $-CH_3$); uv λ_{max}^{EtOH} 246 nm (ϵ 5400).

Treatment of Oxazoline 20 with Sodium Acetate.—To a solution of **20** (30 mg) in absolute ethanol (2.2 ml) was added sodium acetate (60 mg). After refluxing overnight the product was put through a small column of silica gel to yield a product (10 mg) which showed a major spot corresponding to alcohol **19** and a minor spot corresponding to diol **17**. These were separated on a thick layer plate[16] and were shown to be identical with **19** and **17** by comparison of mass spectra with those of the authentic samples.

Registry No.—**1**, 35891-70-4; **1** (tetrahydro tetraacetate), 38223-34-6; **2**, 35891-69-1; **2** (HCl), 38223-36-8; **3**, 38223-37-9; **3** (dihydro), 38223-38-0; **4**, 38223-39-1; **8**, 38223-40-4; **11**, 38223-41-5; **13**, 38223-42-6; **14**, 38223-43-7; **15**, 38223-44-8; **16**, 38337-05-2; **17**, 38223-46-0; **18**, 38223-47-1; **19**, 38223-48-2; **20**, 38223-59-5; **21**, 38223-60-8; **23**, 38223-61-9.

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(16) The authors wish to thank Dr. G. Schilling for carrying out this separation and performing the mass spectral comparison.

The Isolation and Structural Elucidation of Eupaserrin and Deacetyleupaserrin, New Antileukemic Sesquiterpene Lactones from *Eupatorium semiserratum*[1,2]

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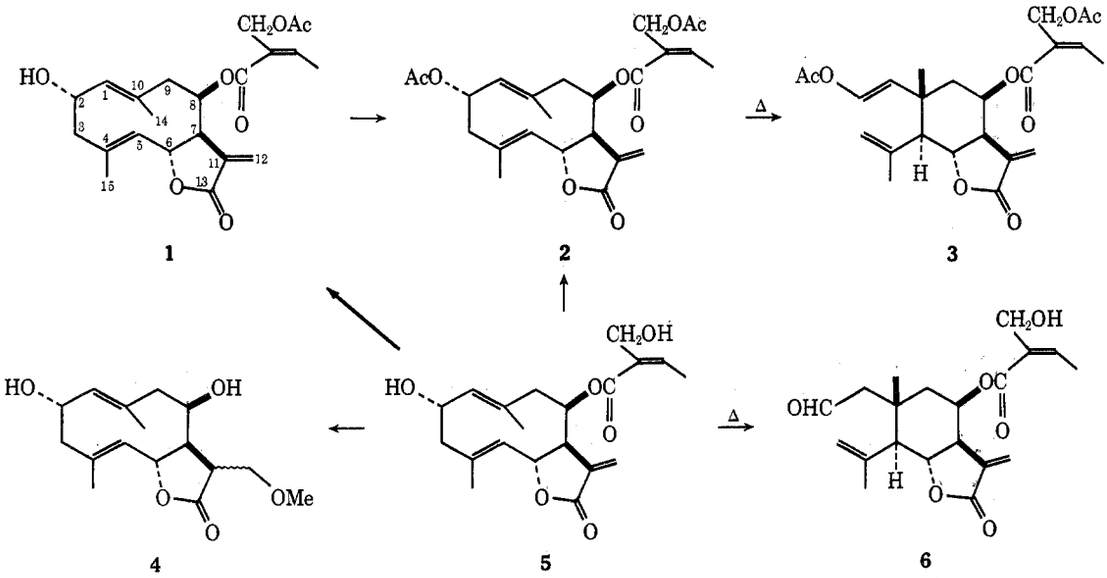
Evidence is presented for the assignment of structures for eupaserrin (**1**) and deacetyleupaserrin (**5**), two antileukemic sesquiterpene lactones from *Eupatorium semiserratum* DC. Elemental analysis and high resolution mass spectrometry supported a $C_{22}H_{28}O_7$ molecular formula for eupaserrin (**1**) and a $C_{20}H_{26}O_6$ molecular formula for deacetyleupaserrin (**5**). Acetylation of **5** gave **1** and acetyleupaserrin (**2**), whereas alkaline hydrolysis of **5** gave sarracinic acid. Chemical and spectral evidence indicated the presence of α-methylene-γ-lactone, α,β-unsaturated ester, secondary hydroxyl, and two vinyl methyl groupings in **1** and **5** and suggested that both **1** and **5** were germacranolide dienes. Pyrolysis of **5** gave an oily aldehyde lactone (**6**), and pyrolysis of **2** gave an enol acetate (**3**). Chemical and spectral arguments are advanced for assignment of structure and stereochemistry for **2** and **3** and therefore **1** and **5**.

In the course of a continuing search for tumor inhibitors from plant sources, an alcoholic extract of *Eupatorium semiserratum* DC (Compositae)[3] was found to show significant inhibitory activity *in vivo* against the P-388 leukemia in the mouse and *in vitro* against cells derived from human carcinoma of the nasopharynx

(1) Tumor Inhibitors. LXXXIV. Part LXXXIII: S. M. Kupchan, G. Tsou, and C. W. Sigal, *J. Org. Chem.*, **38**, 1420 (1973).

(2) This investigation was supported in part 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-2099).

(3) Leaves, stems, flowers, and fruits were collected in Florida in Sept 1967. We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material.



(KB).[4] Consequently, a systematic study aimed at the isolation of the active principles was undertaken. It is the purpose of this paper to present in detail the fractionation of the active extract of *E. semiserratum* and the isolation and structural elucidation of the active constituents eupaserrin (**1**) and deacetyleupaserrin (**5**).[5]

Fractionation of the ethanol extract, guided by assay against KB, revealed that the activity was concentrated, successively, in the chloroform layer of a chloroform–water partition, in the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition, and in the propylene glycol layer of a propylene glycol–benzene partition. Aqueous sodium bicarbonate was added to the propylene glycol layer and the combined fraction was extracted with ethyl acetate. By this process all of the activity was concentrated in the final ethyl acetate layer (fraction H). Rapid column chromatography of the ethyl acetate soluble material on silica gel gave two cytotoxic fractions (I and J). Careful rechromatography of fraction I on alumina and elution with chloroform gave crystalline eupaserrin (**1**). Rechromatography of the more polar fraction J on silica gel gave, on elution with 5% methanol in chloroform, deacetyleupaserrin (**5**) as a colorless brittle foam, which resisted all attempts at crystallization.

The molecular formula for eupaserrin (**1**), $C_{22}H_{28}O_7$, was assigned on the basis of high resolution mass spectrometry and elemental analysis. The ultraviolet high end absorption, infrared bands at 5.66 and 6.14 μ, and nmr signals at τ 3.70 and 4.40 (a pair of doublets, J = 3.5 and 3.0 Hz, respectively) suggested the presence of an exocyclic methylene γ-lactone. In addition, the presence in the nmr spectrum of **1** of a three-proton singlet at τ 8.02, a three-proton doublet at τ 7.88 (J = 7 Hz), which was shown to be coupled to an olefinic one-proton quartet at τ 3.48 (J = 7 Hz), and an AB quartet with ν_A τ 5.16 and ν_B 5.51 (J = 12 Hz) indicated the likelihood of an acetylsarracinate residue. These signals in the nmr spectrum were almost identical in chemical shift and multiplicity with those assigned to the acetylsarracinate moiety in liatrin.[6] In addition, infrared bands at 5.78, 5.82, and 8.00 μ and a large peak in the mass spectrum at m/e 141 ($C_7H_9O_3$) of **1** attested to the presence of the acetylsarracinate residue. Subtracting this residue from the molecular formula left $C_{15}H_{19}O_4$ for the sesquiterpene skeleton and, since two oxygen atoms were accounted for by the γ-lactone moiety and one more by the ester linkage, only one oxygen atom remained unassigned. The infrared spectrum of **1** showed a sharp peak at 2.90 μ indicating the presence of a hydroxyl group, and indeed acetylation of **1** with acetic anhydride in pyridine gave in good yield amorphous acetyleupaserrin (**2**). The nmr spectrum of **2** was very similar to that of eupaserrin (**1**) except for the presence of a new acetyl methyl at τ 7.90 and a one-proton doublet of triplets at τ 4.38 (J = 5, 9 Hz), which in the case of **1** had appeared at τ 5.28 (J = 6, 9 Hz). This result indicated that the remaining oxygen was present as a secondary alcohol adjacent to three other protons. The nmr spectrum of eupaserrin (**1**) also showed the presence of two tertiary vinyl methyl groups as broad singlets at τ 8.46 and 8.20, which were shown to be coupled to a two-proton multiplet at τ 5.0. The combined data suggested that eupaserrin (**1**) is bicyclic, since this would account for all of the degrees of unsaturation allowed by the molecular formula. The γ-lactone accounted for one ring, and, since the nmr of **1** clearly showed signals for two vinyl tertiary methyl groups, it was apparent that eupaserrin (**1**) was probably a member of the germacranolide diene class of sesquiterpenes.

The molecular formula for deacetyleupaserrin (**5**), $C_{20}H_{26}O_6$, was assigned on the basis of high resolution mass spectrometry. The nmr spectrum of **5** was very similar to that of eupaserrin (**1**) except that it lacked the acetyl methyl singlet at τ 8.02. In addition, an AB quartet with ν_A τ 5.16 and ν_B 5.51 (J = 12 Hz) in the spectrum of **1** was replaced by an apparent triplet centered at τ 5.80 (J = 13 Hz) in the spectrum of **5**. These results indicated that **5** was probably the deacetyl derivative of **1**. In fact, acetylation of **5** with

(4) Cytotoxicity and *in vivo* activity were assayed as in *Cancer Chemother. Rep.*, **25**, 1 (1962).

(5) Eupaserrin and deacetyleupaserrin showed significant antileukemic activity against the P-388 leukemia in mice at 30 and 18 mg/kg, respectively, and cytotoxicity against KB cell culture (ED_{50} = 0.23 and 0.29 μg/ml, respectively).

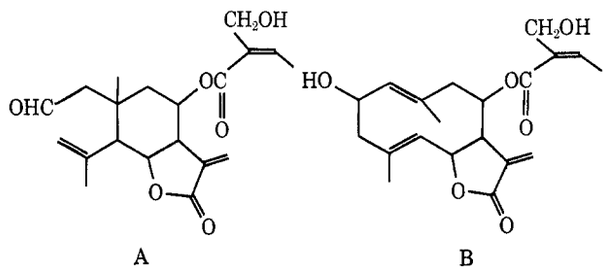
(6) S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, and R. F. Bryan, *J. Amer. Chem. Soc.*, **93**, 4916 (1971).

acetic anhydride in pyridine gave a mixture of eupaserrin (**1**) and acetyleupaserrin (**2**). Alkaline hydrolysis of deacetyleupaserrin (**5**) gave sarracinic acid;[6-8] thus the side-chain ester of **5** and, therefore, **1** was firmly established as the sarracinate and acetylsarracinate, respectively.

Careful inspection of the nmr spectra of **1** and **5** combined with several decoupling experiments revealed additional structural features of the two molecules. For example, irradiation of a multiplet centered at τ 7.0 in the spectrum of **1** caused the two one-proton doublets at τ 3.70 and τ 4.40, characteristic of the C-13 protons of an exocyclic conjugated methylene lactone,[9] to collapse to singlets. Thus the multiplet at τ 7.0 could be assigned to the C-7 proton. In addition, irradiation of the C-7 proton also caused a one-proton multiplet at τ 4.2 to collapse to a triplet which could thus be assigned as the C-8 (or C-6) proton signal. Furthermore, irradiation of the signal at τ 4.2 caused two one-proton doublets of doublets at τ 7.13 (J = 14, 6 Hz) and 7.63 (J = 14, 2 Hz) to collapse to doublets (J = 14 Hz), which could thus be assigned to an isolated methylene group with no other adjacent protons. Although the signal for the C-6 proton was not readily apparent in the nmr spectrum of **1**, in deacetyleupaserrin (**5**) it appeared clearly as a doublet of doublets at τ 4.90 (J = 8, 10 Hz). Irradiation of the C-6 proton caused a sharpening in the multiplet at τ 6.96 which was assigned to the C-7 proton. Irradiation of the C-7 proton not only collapsed the C-8 multiplet at τ 4.16 to an apparent triplet but also caused the C-6 doublet of doublets to collapse to a doublet (J = 8 Hz).

On treatment with sodium methoxide in methanol, deacetyleupaserrin (**5**) gave the oily methanol adduct **4**. In the nmr spectrum of **4**, the C-6 proton was evident as a triplet at τ 4.90 (J = 9 Hz), but the C-8 proton multiplet, which appeared at τ 4.16 in **5**, now appeared at τ 5.64. The large change in position of the C-8 multiplet indicated that the sarracinate ester moiety of **5** and therefore also of **1** must be located at C-8.

It has been shown that germacranolide dienes such as dihydrotamaulipin A (**8**)[10] undergo a Cope rearrangement on heating at 180–200° for a short period of time. It appeared that both **1** and **5** were probably germacranolide dienes of this type, and consequently deacetyleupaserrin (**5**) was subjected to the Cope rearrangement conditions. When **5** was heated at 180° for 3 min, the major product isolated was characterized as the oily aldehyde lactone **6**. The nmr spectrum of **6** exhibited a one-proton triplet (J = 2 Hz) at τ 0.12 characteristic of an aldehyde proton, which was shown to be coupled to a two-proton doublet at τ 7.45 (J = 2 Hz). The appearance of a three-proton singlet at τ 8.73, a new vinyl methyl singlet at τ 8.07, and two new vinyl protons at τ 4.85 and 5.19 indicated that a Cope rearrangement[10-13] had taken place in the desired manner and allowed us to postulate a partial structure A for the



aldehyde lactone, and thus B for deacetyleupaserrin. This result placed the secondary hydroxyl group of eupaserrin (**1**) and deacetyleupaserrin (**5**) at C-2. In order to determine the relative stereochemistry of the C-2 hydroxyl, it was necessary to repeat the pyrolysis reaction with acetyleupaserrin (**2**), which gave a 1:1 mixture of the enol acetate **3** and starting material **2**. The trans nature of the enol acetate double bond was indicated by the presence in the nmr spectrum of **3** of two one-proton doublets at τ 2.97 (J = 13 Hz) and 4.58 (J = 13 Hz) for the C-2 and C-1 vinyl protons, respectively. The corresponding coupling constant for enol ester cis double bonds has been found to be on the order of 7 Hz.[14] By analogy with similar studies on dihydrotamaulipin A (**8**) and dihydrotamaulipin A acetate (**9**),[10] the hydroxyl group at C-2 of eupaserrin (**1**) and deacetyleupaserrin (**2**) could now be assigned the α configuration as shown. In addition, the nmr spectrum of **3** clearly showed the C-5 proton as a sharp doublet at τ 7.54 (J = 12 Hz), which was coupled to a one-proton triplet at τ 5.42 (J = 12 Hz). The τ 5.42 peak, which could now be assigned to the C-6 proton, was also coupled to a doublet of multiplets at τ 7.12 (J = 12 Hz), which could thus be assigned to the C-7 proton. Comparison of the coupling constants and chemical shifts for the C-5, C-6, C-7, and C-8 protons in the aldehyde lactone **6** and the enol acetate **3** with the litera-

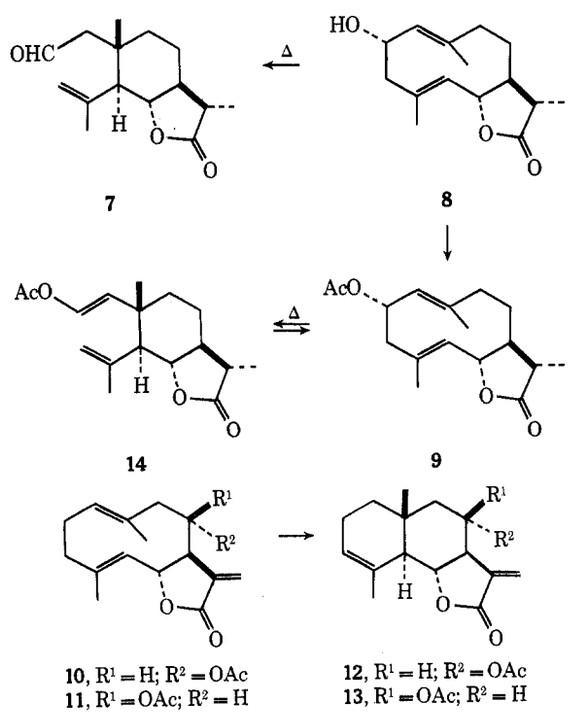


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TABLE I

NUCLEAR MAGNETIC RESONANCE DATA[a]

Compd	C-1	C-2	C-5	C-6	C-7	C-8	C-9	C-13	C-14	C-15
1	5.0 m	5.28 dt (6, 9)	5.0 m	5.0 m	7.0 m	4.2 m	7.13 dd (14, 6) 7.63 dd (14, 2)	3.70 d (3.5) 4.40 d (3)	8.46 br s	8.20 br s
2	4.95 m	4.38 dt (5, 9)	4.95 m		7.0 m	4.13		3.69 d (3) 4.38 d (3)	8.32 br s	8.12 br s
3	4.58 d (13)	2.97 d (13)	7.54 d (12)	5.42 dd (12, 12)	7.12 br d (12)	4.20 m		3.83 d (3) 4.50 d (3)	8.72 s	8.12 br s
4	5.00 m	5.17 dt (6, 10)	5.00 m	4.90 t (9)		5.62 m		6.32 br d (4) 3.56 s (C-13 OCH_3)	8.31 br s	8.24 br s
5	5.00 m	5.28 dt (5.5, 9)	5.00 m	4.9 dd (8, 10)	6.96 m	4.16 m	7.10 dd (14, 4) 7.62 dd (14, 2)	3.70 d (3.5) 4.47 d (3)	8.44 br s	8.20 br s
6	7.45 d (2)	0.12 t (2)	7.18 d (12)	5.42 t (12)	7.05 br d (12)	4.20 m	7.82 d (3)	3.83 d (3) 4.78 d (3)	8.73 s	8.07 br s
7[b]	7.53 d (3)	0.18 t (3)	7.53 d (12)	5.89 br dd (12, 10)				8.78 d (7)	8.90 s	8.16 br s
8[b]	5.1 m	5.40 m	5.1 m	5.45 m				8.76 d (6.5)	8.52 br s	8.28 d (1.1)
9[b]	5.09 br	4.37 dt (5.5, 10)	5.09 br	5.46 dd (7.5, 10)				8.78 d (7)	8.44 br s	8.25 br s
10[c]			5.15 br d (10)	5.44 dd (8, 10)	7.45 br m	4.8–5.2 obsc		3.77 d (3.5) 4.45 d (3)	8.58 br s	8.30 br s
11[c]			5.22 dd (1.3, 10)	4.87 dd (8.1, 10)	7.07 br m	4.28 m		3.72 d (3.5) 4.41 d (3.1)	8.48 br s	8.24 d (1.3)
12[c]				6.02 dd (10.8, 11.3)	7.20 m	4.70 m		3.89 d (3.1) 4.49 d (2.9)	9.01 s	8.13 br s
13[c]				5.60 dd (11, 11)	7.20 m	4.30 m		3.85 d (3.3) 4.56 d (3.1)	8.92 s	8.11 br d (1.5)
14[b]	4.51 d (13)	2.95 d (13)	7.77 d (10)	5.89 br dd				8.78 d (6.5)	8.88 s	8.22 br s

[a] Spectra were determined on a Varian HA-100 spectrometer in deuteriochloroform solutions unless otherwise indicated. 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; dt, doublet of triplets; m, multiplet; obsc, obscured; br, broad. Numbers in parentheses denote coupling constants in hertz. [b] Reference 10. [c] Reference 15.

ture values for the corresponding compounds from dihydrotamaulipin A (**8** → **7**) and dihydrotamaulipin A acetate (**9** → **14**),[10] as well as the rearrangement products of tulipinolide (**10** → **12**) and epitulipinolide (**11** → **13**)[15] allowed us to assign the relative stereochemistry of all four centers (see Table I). In particular, a comparison of the relevant data for **6** with **7** and **3** with **14** offered convincing proof of the relative stereochemistry at C-2, C-5, C-6, and C-7 of these compounds. Furthermore, the chemical shift and multiplicity of the C-8 proton in eupaserrin (**1**) and its derivatives (**5**, **2**, **6**, and **3**) were very similar to those observed in epitulipinolide (**11**) and its derivative (**13**). This was in marked contrast to the corresponding data for tulipinolide (**10**) and its derivative **12** and strongly suggests that eupaserrin (**1**) and, therefore, deacetyleupaserrin (**5**) have the β configuration at this center as depicted above.[16]

Experimental Section

Melting points were determined on a Mettler Model FP2 hot stage and are corrected. Ultraviolet absorption spectra were determined on Beckman Model DK-2A and Coleman Hitachi Model EPS-3T recording spectrophotometers. Infrared spectra were determined on Beckman Model IR-9, Perkin-Elmer Model 257, and Perkin-Elmer Model 337 recording spectrophotometers. Nuclear magnetic resonance spectra were determined on a Perkin-Elmer Model R-20 spectrometer at 60 MHz and on a Varian HA-100 spectrometer in deuteriochloroform solution with tetramethylsilane as an internal standard. Mass spectra were obtained from Hitachi Perkin-Elmer Model RMU-6A (RMU-6E) and AEI Model MS-902 spectrometers. Values of $[\alpha]_D$ were determined on a Perkin-Elmer Model 141 automatic polarimeter. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. Analytical thin layer chromatography (tlc) was carried out with 5% methanol in chloroform on silica gel plates (supplied by E. Merck), which were visualized with either concentrated sulfuric acid–vanillin–ethanol (20:1:3) spray or 2% ceric sulfate spray, unless otherwise specified. Evaporations were carried out under reduced pressure at less than 40°. Petroleum ether refers to the fraction with bp 60–68°.

Isolation Procedure.—The dried ground stems, leaves, flowers, and fruit of *Eupatorium semiserratum* (2.85 kg) were continuously extracted with hot ethanol for 48 hr, and the ethanol extract was evaporated under reduced pressure to yield a dark green residue (A, 510 g). Fraction A was partitioned between chloroform (2 l.) and water (1 l.). The chloroform layer was evaporated to give a green residue (D, 169 g) and the aqueous layer gave a dark tar (B, 213 g). Fraction D was partitioned between petroleum ether (4 l.) and 10% aqueous methanol (1.5 l.). Evaporation of the petroleum ether fraction gave a green tar (E, 61 g) and the 10% aqueous methanol layer gave a dark tar (F, 99 g). Fraction F was partitioned between benzene (2 l., G, 16 g) and propylene glycol (0.5 l.), and then the propylene glycol layer was diluted with saturated sodium bicarbonate solution (1.2 l.) and water (2 l.) and extracted with ethyl acetate (1 l.). Evaporation of the final ethyl acetate layer gave a dark brown gum (H, 39 g), which contained almost all of the original KB activity. Fraction H was chromatographed on 200 g of silica gel. Eupaserrin (**1**) and deacetyleupaserrin (**5**) were eluted with chloroform (fractions I and J). Fraction I was rechromatographed on alumina (50 g) to give on elution with chloroform a fraction, which was crystallized from ether–methanol, affording eupaserrin (**1**, 190 mg): mp 153–154°; $[\alpha]^{25}D$ +71.2° (*c* 0.94, MeOH); uv λ_{end}^{EtOH} 210 nm (ϵ 27,270); ir λ_{max}^{KBr} 2.9, 5.66, 5.78, 5.82, 6.14, and 8.00 μ; mass spectrum *m/e* 404.1830 (M^+, calcd for $C_{22}H_{28}O_7$, 404.1841), 386, 246, 202, 141, and 99; R_f 0.55.

Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.27; H, 7.10.

Rechromatography of fraction J on silica gel (200 g) on elution with 5% methanol in chloroform gave deacetyleupaserrin (**5**, 6.6 g), as an amorphous brittle white foam. Although **5** appeared to be homogeneous by tlc, it could not be crystallized and did not give satisfactory analytical data. Deacetyleupaserrin appeared to be quite unstable and even freshly prepared samples quickly decomposed on standing. Spectral data were obtained on freshly prepared samples: $[\alpha]^{25}D$ +75.0° (*c* 0.92, MeOH); uv λ_{end}^{EtOH} 209 nm (ϵ 23,200); ir $\lambda_{max}^{CHCl_3}$ 2.78, 2.90, 5.66, 5.82, 6.04, 8.12, and 8.73 μ; mass spectrum *m/e* 362.1710 (M^+, calcd for $C_{20}H_{26}O_6$, 362.1730), 264, 246, 202, and 99; R_f 0.37.

Acetyleupaserrin (2)—Eupaserrin (**1**, 195 mg) was dissolved in pyridine (4 ml), and acetic anhydride (2 ml) was added at 0°. The reaction mixture was stirred for 1.5 hr at room temperature, then diluted with water, and extracted with chloroform. The chloroform extract was dried over magnesium sulfate and evaporated to afford a yellow oil (220 mg). The total crude product was applied to 10 ChromAR 7GF (20 × 20 cm × 0.25 mm) tlc

(15) R. W. Doskotch and F. S. El-Feraly, *J. Org. Chem.*, **35**, 1928 (1970).

(16) We have also isolated eupaserrin (**1**) from *Eupatorium cuneifolium Willd.*

plates and developed with 1:1 ether in benzene. The major band was removed from the plates and eluted with chloroform to afford acetyleupaserrin (**2**) as a viscous colorless glass. Various attempts at crystallization of **2** were unsuccessful and so it was characterized as a foam: $[\alpha]^{25}D$ +83° (*c* 0.95, $CHCl_3$); ir λ_{max}^{KBr} 5.68, 5.78, 5.82, 6.10, 8.10, 8.62, and 8.77 μ; mass spectrum *m/e* 446.1931 (M^+, calcd for $C_{24}H_{30}O_8$, 446.1939), 386, 246, 228, 213, and 141; R_f 0.70.

Acetylation of Deacetyleupaserrin (5).—To a solution of deacetyleupaserrin (**5**, 300 mg) in acetic anhydride (10 ml), powdered potassium carbonate (20 mg) was added and the mixture stirred at room temperature for 2 hr. The reaction mixture was poured into ice–water, stirred for a further 3 hr, and extracted with chloroform. The organic extract was washed with aqueous sodium bicarbonate and then water, dried over sodium sulfate, and evaporated to give a colorless residue (300 mg), which yielded two major components on preparative tlc. The band of higher R_f, eluted with 10% methanol in chloroform, was crystallized from methanol to yield eupaserrin (**1**, 85 mg): mp 153–154° (mixture melting point, tlc, ir, and nmr identical with those of the material described above). The lower R_f band was extracted in the same manner to give acetyleupaserrin (**2**, 122 mg) as a colorless foam (tlc, ir, and nmr identical with those of the material described previously).

Hydrolysis of Deacetyleupaserrin (5).—Deacetyleupaserrin (**5**, 300 mg) was dissolved in 5 *N* aqueous sodium hydroxide (25 ml) and heated under nitrogen at 60° for 30 min. The reaction mixture was then acidified with concentrated hydrochloric acid, saturated with sodium chloride, and extracted with ether (6 × 80 ml). The ether layer was extracted with 5% sodium carbonate solution (3 × 10 ml), which was acidified, saturated with sodium chloride, and extracted with ether (3 × 30 ml). The final ether layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to give a foam (84 mg). This material was applied to ten Cellulose F precoated plates (20 × 20 cm × 0.2 mm) and developed with 20:80 2 *N* aqueous ammonia in *sec*-butyl alcohol. The acidic band (visualized with bromophenol blue) was scraped from the plates and extracted with methanol. Evaporation of the methanol afforded 16 mg of oily crystals which were recrystallized from ether–petroleum ether to give sarracinic acid, mp 51.4–52.1°. The infrared spectrum of this sample was identical with that of an authentic sample and the mixture melting point was undepressed.

Methanolysis of Deacetyleupaserrin (5).—To a solution of sodium methoxide (65 mg) in 4 ml of anhydrous methanol was added deacetyleupaserrin (**5**, 200 mg). The reaction mixture was stirred for 1 hr at room temperature, heated for 10 min at 60°, cooled and acidified with dilute hydrochloric acid, and then extracted with chloroform. The chloroform extract was dried over magnesium sulfate and evaporated to give 200 mg of yellow foam. This material was applied to eight ChromAR 7GF plates (20 × 20 cm × 0.25 mm) and developed with 5% MeOH in chloroform. The major band was removed and eluted with chloroform to afford 86 mg (50%) of the methanol adduct **4** as a viscous oil: $[\alpha]^{25}D$ +72.5° (*c* 0.99, $CHCl_3$); ir $\lambda_{max}^{CHCl_3}$ 2.78, 2.90, 5.70, 6.05, 8.55, 8.85, 9.00, and 10.30 μ; mass spectrum *m/e* 296.1614 (M^+, calcd for $C_{16}H_{24}O_5$, 296.1617), 278, 264, 246, 233, 195, 152, 122, 113, 107, 95; R_f 0.31.

Pyrolysis of Deacetyleupaserrin (5).—Pyrolysis of deacetyleupaserrin (**5**, 85 mg) at 180–200° for 3 min under aspirator pressure gave quantitatively a yellow glass. This material was separated on four silica gel plates (20 × 20 cm × 0.25 mm) using 5% methanol in chloroform to give 69 mg (81%) of the aldehyde lactone **6** as a colorless foam: $[\alpha]^{25}D$ +9.3° (*c* 0.71, $CHCl_3$); ir $\lambda_{max}^{CHCl_3}$ 2.90, 3.68, 5.67, 5.83, 6.12, 8.70, and 9.90 μ; mass spectrum *m/e* 362.1719 (M^+, calcd for $C_{20}H_{26}O_6$, 362.1722), 347, 344, 300, 298, 264, 246, 202, 163, 135, 107, 99; R_f 0.59.

Pyrolysis of Acetyleupaserrin (2).—Pyrolysis of acetyleupaserrin (**2**, 90 mg) at 200° for 4 min under aspirator pressure gave an approximately 1:1 mixture of starting **2** and the enol acetate **3**. The crude product was applied to five silica gel plates (20 × 20 cm × 0.25 mm) and eluted with 1:1 ether in benzene giving in the band of higher R_f enol acetate **3** as a colorless foam (31 mg, 34%). The lower R_f band, corresponding to acetyleupaserrin (**2**), was eluted to give **2** as a pale yellow glass (28 mg, 31%), which was shown to be identical with **2** described above. The enol acetate **3** was unstable and was characterized by nmr (see Table I); ir λ_{max}^{KBr} 2.90, 3.25, 5.67, 5.75, 5.82, 6.00, 6.08, 8.15, and 8.65 μ; mass spectrum *m/e* 446.1942 (M^+, calcd for $C_{24}H_{30}O_8$, 446.1939), 386, 288, 246, 213, 141, 99, and 81; R_f (ChromAR, 1:1 ether–benzene) 0.25.

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Novel Tricyclic Compounds from Alkylated Hydroquinones and C-10 Terpenes

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The novel hydroxylated 7-oxatricyclo[6.4.0.$1^{2,6}$]trideca-8,10,12-trienes **9** and **10** and the spiro[cyclohexane-1,2′-chroman] **11** have been synthesized in 5–31% yields *via* the acid-catalyzed condensation of alkylated hydroquinones with linalool **6** and myrcene **7**. Their structures were assigned on the basis of nmr and mass-spectral data and were mechanistically rationalized. Yields of the type **9** structures were substantially increased with *d*-limonene (**15**) or α-phellandrene (**16**), supporting the idea that a cyclized monoterpene is involved in the formation of both **9** and **10**.

The acid-catalyzed condensation of open-chain monoterpenes with phenolic compounds in general leads to alkenyl-substituted chromans, but in several cases tricyclic compounds have been reported as a result of further cyclization under the acidic conditions. Green and McHale cited the formation of tricyclic chromanols[1] from trimethylhydroquinones and geraniol and linalool, but their materials were not characterized unequivocally. More recently, Ichikawa and Kato[2] isolated the tricyclic compound **1** as a by-product in the synthesis of chromanol **2**, and Kane[3] characterized the product from phloroglucinol dimethyl ether and citral (mixture of neral and geranial) as the tetracyclic **3a**. Tricyclic chromanols **3b**, **3c**, and **3d** have also been synthesized in cannabinoid studies. Mechoulam and Yagen[4] prepared **3b** from olivetol and geraniol *via* the stereoselective cyclization of cannabigerol, while Crombie and Ponsford[5] obtained

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