[s, 24 $^{+}N(CH_3)_4$], 4.50 (s, 2, OCH_2Ph), and 7.10–7.27 (m, 15, 3 C_6H_5); ^{13}C NMR [D_2O–dioxane (67:41)] decoupled, δ 56.1 (CH_3), 65.7 (OCH_2), 72.7 (OCH_2Ar), 77.8 (OCH), 120.8, 121 (C-2, 6 of PhO), 1.8 (C-4 of PhO), 128.8, 129, 129.2 (C-2, 4, 6 of Ph), 130.5 (C-3 of Ph), 138.2 (C-1 of Ph), and 152.9 (C-1 of PhO).

Anal. Calcd for $C_{30}H_{46}N_2O_9P_2$: H, 7.24; N, 4.37. Found: H, 7.49; N, 4.25.[22]

A 3-mL sample of Dowex 50 W-X8 (1.7 mg/mL resin bed, 20–50 mesh) hydrogen form was exchanged with cyclohexylamine and then washed with distilled water. A solution of 500 mg of **8h**, obtained above, in 5 mL of water was passed through a column of the cyclohexylamine treated resin. The column was eluted with an additional amount of water to collect the salt **8i**. The aqueous fractions containing the product were collected and evaporated to dryness under reduced pressure. Crystallization of the residue from methanol and acetone yielded crystals, mp 174–178 °C: R_f 0.26 [NaOAc–EtOH–H_2O (0.05:1:1)]; ^1H NMR δ 1.13–2.18 [m, 20, $2(CH_2)_5$], 3.78–4.20 (m, 5, OCH_2CHCH_2O), 4.62 (s, 2, OCH_2Ph), and 7.07–7.35 (m, 15, 3 C_6H_5); ^{31}P NMR (D_2O) (H_3PO_4, δ 56.46) δ 54.94 (1400 transients with proton decoupling), a partially resolved triplet was found when decoupler was turned off.

Anal. Calcd for $C_{34}H_{50}N_2O_9P_2$: C, 58.95; H, 7.28; N, 4.04; P, 8.95. Found: C, 56.60; H, 7.38; N, 4.30; P, 8.79.[24]

***rac*-1-Stearoyl-2-arachidonoyl-*sn*-glycerol-3-phosphoryl-(*N*,*N*-dimethyl)ethanolamine (2c).** Arachidonic acid anhydride was prepared in the following way. A solution of 10.7 g (35 mmol) of arachidonic acid (90% purity from Hormel Institute) in 200 mL of dry carbon tetrachloride was added, with stirring over a period of 5 min and in an atmosphere of nitrogen, to a solution of 3.72 g (18 mmol) of dicyclohexylcarbodiimide in 110 mL of carbon tetrachloride. After stirring in the dark for 6 h, the mixture was filtered through a Celite filter pad. The filtrate was distilled to dryness under reduced pressure at 50 °C. The oil 11 which remained weighed 10.7 g [R_f 0.89, MeOH–$CHCl_3$ (1:19)] and was utilized without further purification.

A mixture of 3.90 g (8.1 mmol) of the lysolipid **2b**, prepared from **8j** by the method of Van Deenan,[22,24] 10.7 g (17 mmol) of freshly prepared 11, 29 mL of pyridine (distilled over CaH), and 290 mg of 4-dimethylaminopyridine was stirred at 23 °C for 18 h. A TLC indicated that the reaction was complete. The solution was poured into 250 mL of toluene and concentrated by distillation to remove the pyridine. The concentrated solution was evaporated to dryness at 50 °C (0.3 mm). The crude product weighed 16.1 g. It was dissolved in methanol–chloroform (1:19) and purified by chromatography on 1400 g of CC-7 silica gel. Elution with methanol–chloroform (1:19) gave a forefraction which was discarded. Elution with methanol–chloroform (1:9) yielded **2c** weighing 4.38 g (70%); TLC R_f 0.47 on CC-7 silica gel; 95 $CHCl_3$: 35 MeOH:6 H_2O δ 0.80–1.02 (t, 6, 2 CH_3), 1.30 [s, broad $(CH_2)_{13}$, $(CH_2)_4$, $(CH_2)_3$], 1.67–2.41 (m, 10, $C{=}CCH_2$, 2 CH_2CO), 4.43–4.64, [m, s, 12, $(C{=}CCH_2C{=}C)_3$, $N(CH_3)_2$], 3.20–3.33 (m, hidden with D_2O, NCH_2), 3.97–4.43 (m, 7, OCH_2CHCH_2O, OCH_2O), and 5.33, 5.41, 5.50 (m, 8, 4 cis CH=CH).

Anal. Calcd for $C_{45}H_{82}NO_8P$: H, 10.24; N, 1.82. Found: H, 10.03; N, 1.66.[24]

Acknowledgments. The authors acknowledge V. Coker and B. Coleman for their technical help in synthesis, and R. Bible, Jr., and L. Swenton for their consultation in NMR analysis.

Registry No.—**1b,** 14690-00-7; **2b,** 19491-37-3; **2c,** 62085-19-2; **3,** 62067-16-7; **4,** 62067-17-8; **5,** 42320-93-4; **6,** 42320-37-6; **8a,** 62067-18-9; **8b,** 62106-90-5; **8c,** 18679-03-3; **8d,** 62067-19-0; **8e,** 62067-20-3; **8f,** 62067-21-4; **8g,** 62085-20-5; **8h,** 62085-22-7; **8i,** 62067-23-6; **9,** 62067-24-7; **10,** 62067-25-8; **11,** 55726-28-8; dimethyl sulfite, 616-42-2; dichlorophenyl phosphate, 770-12-7; palmitic acid, 57-10-3; potassium stearate, 593-29-3; *p*-chlorophenol, 106-48-9; phenylphosphoric acid, 701-64-4; tetramethylammonium hydroxide, 75-59-2; cyclohexylamine, 108-91-8; arachidonic acid, 506-32-1.

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Sesquiterpene Lactones of *Eupatorium perfoliatum*[1,2]

Werner Herz,* Palaiyur S. Kalyanaraman, and Ganapathy Ramakrishnan

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

John F. Blount

Research Division, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

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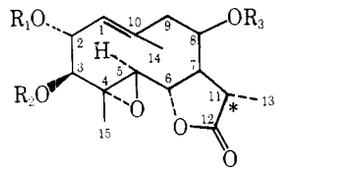
Structures of four new sesquiterpene lactones isolated from *Eupatorium perfoliatum* L. were determined by a combination of chemical and physical techniques. Three are esters of tiglic acid with the lactone ring closed to C-6. Two, euperfolin and euperfolitin, are 8-tigloyl-4,5-epoxy-1(10)-germacranolides with hydroxyl groups at the 3 and the 2 and 3 positions of the ring, respectively. The third, eufoliatin, is a 3-tigloyl-4,5,9,10-diepoxyguianolide with the lactone ring closed to C-6. The fourth, eufoliatorin, is a novel dilactone of the guaiane series whose stereochemistry was established by x-ray crystallography.

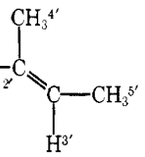
In the present paper we continue our reports on the constituents of *Eupatorium* species sensu stricto,[5] which have produced a number of cytotoxic and antitumor sesquiterpene lactones,[6] and describe the isolation of four polar new

sesquiterpene lactones in rather small quantity from *Eupatorium perfoliatum* L.[7] Two of these were the germacranolide euperfolitin (**1a**), of interest as a possible precursor of the secogermacradienolide pycnolide (**7**) from *Liatris pycnostachya* Michx.,[8] and the related germacranolide **4a** (euperfolin). The other two substances were the diepoxyguaianolide eufolitin (**5a**) and the interesting dilactone eufoliatorin (**6**), which appears to represent a new type of sesquiterpene dilactone.

We discuss first the structure elucidation of euperfolitin



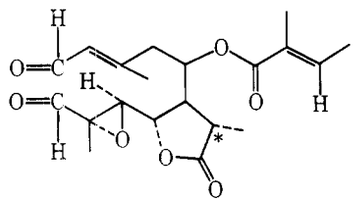
1a, R_1, R_2 = H; R_3 =



b, R_1, R_2 = Ac; R_3 same as in **1a**

c, R_1, R_2, R_3 = H

d, R_1, R_2 = —C(=O)—Ph; R_3 same as in **1a**



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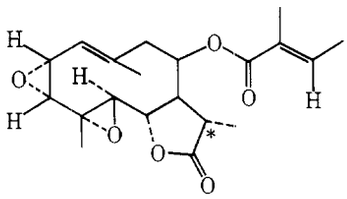

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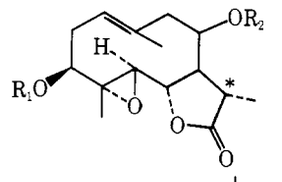
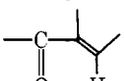
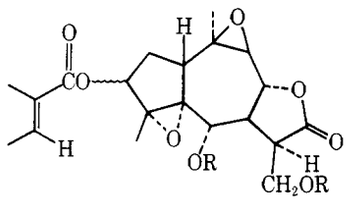


4a, R_1 = H; R_2 =



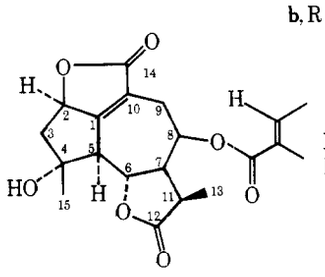
b, R_1 = Ac; R_2 same as in **4a**

c, R_1, R_2 = H

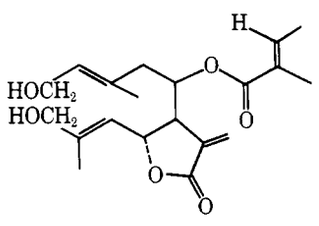


5a, R = H

b, R = Ac



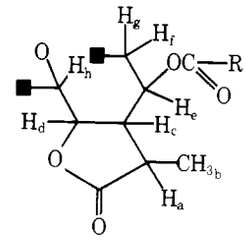
6



7

* Assignment dubious. Methyl may be β by analogy with **6**.[8]

(**1a**), $C_{20}H_{28}O_7$ (high-resolution mass spectrum and elemental analysis), mp 190–192 °C (amorphous), $[\alpha]_D$ −5.81°, which we have so far been unable to convert to a substance suitable for x-ray analysis. It was a γ-lactone of the type partially shown in A (IR band at 1765 cm^{-1}, presence of a methyl doublet at 0.95 ppm instead of the usual two doublets characteristic of an exocyclic methylene group). The remaining part structure shown in A (where ■ represents a quaternary

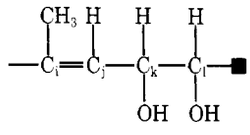


A

center) was evident from spin-decoupling experiments in Me_2SO-d_6 (Table I). H_d was conveniently located at 4.57 ppm as a doublet of doublets. Irradiation at the frequency of H_d converted a doublet at 3.05 ppm (H_h) to a singlet and a slightly broadened triplet at 2.80 ppm (H_c) to a broadened doublet. In the reverse experiment, irradiation at the frequency of H_h collapsed H_d to a doublet. Irradiation at the frequency of H_c converted H_d to a doublet, affected a multiplet at 2.81 ppm (H_a), and sharpened a broadened doublet at 5.28 ppm (H_e); conversely, irradiation at the frequency of H_a reduced H_c to a broadened doublet and collapsed the methyl doublet at 0.95 ppm to a singlet. Irradiation at the frequency of H_e slightly sharpened the signal of H_c and simplified what was obviously the AB part of an ABX system at 2.58 (H_f) and 2.22 ppm (H_g) (J_{AX} = 6, J_{BX} < 1, J_{AB} = 14 Hz), as could be demonstrated further by the results when H_f and H_g were irradiated. Chemical shift and multiplicity of H_h suggested that it was on carbon vicinal to a quaternary center and carrying an epoxidic oxygen; similarly, the behavior of the ABX system indicated that H_f and H_g represented a methylene group next to a quaternary center and that H_e was on carbon carrying an ester function which, because of the empirical formula, the NMR spectrum (vinyl multiplet at 6.87 ppm, two superimposed methyl multiplets at 1.74 ppm), and the mass spectrum [prominent peak at *m/e* 280 ($M^+ - C_5H_8O_2$), base peak at *m/e* 83 (C_5H_7O)], was a tiglate. The presence of a five-carbon ester was also established by hydrolysis of **1a** (NaOMe, MeOH)[9] to **1c**, $C_{15}H_{22}O_6$.

The assignments of H_d, H_e, and H_h were confirmed by single frequency decoupling of the off-resonance ^{13}C NMR spectrum of **1a** (Table II) which displayed the doublets of C_d, C_e, and C_h at 77.5, 72.8, and 83 ppm, respectively. The other terminus of the epoxide group attached to C_h was represented by a carbon singlet at 64.2 ppm.

The ^1H NMR spectrum of euperfolitin also displayed a doublet at 5.39 ppm (H_j of partial structure B). Irradiation at



B

this frequency sharpened a somewhat broadened methyl resonance at 1.57 ppm and collapsed a doublet of doublets at 4.08 ppm (H_k) to a doublet. Conversely, irradiation at the frequency of H_k collapsed the doublet of H_j to a broad singlet and a doublet at 2.90 (H_l) to a singlet which must be adjacent to a quaternary center. Since acetylation of **1a** gave a diacetate **1b** (new double strength IR band at 1740 cm^{-1}, new 3-proton

Table I. ^{1}H NMR Spectra of Sesquiterpene Lactones from *Eupatorium perfoliatum* and Derivatives[a]

Compd	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-9	H-11	H-13	H-14	H-15	H-3′	H-4′, H-5′[h]	Misc
1a[b]	5.39 dbr (9,1)	4.57 dd (9,9)	2.89 d (9)	3.05 d (9)	4.57 dd (9,9)	2.80 m	5.28 dbr (5, <1, <1)	2.58 dd (14,5) 22.2 dbr (14, <1)	2.80 m	0.95 d[g] (7)	1.55	1.20	6.87 m	1.74 m	
1b	5.30 dbr (10,1)	5.60 dd (10,10)	4.82 d (9)	3.03 d (9)	4.54 dd (9,9)	2.75 m	5.43 dbr (5, <1, <1)	2.94 dd (14,5) 2.27 dbr (14, <1)	2.75 m	1.12 d[g] (7)	1.82 m	1.42	6.86 m	1.82 m	2.90 (Ac) 2.14 (Ac)
1c[c]	5.80 dbr (9,1)	5.00 dd (9,9)	3.85 d (9)	3.25 d (9)	4.90 dd (9,9)	2.37 m	4.50 dbr (5, <1, <1)	2.72 dd (14,5) 2.37 dbr (14, <1)	3.07 m	1.33 d[g] (7.5)	2.01	1.70			
1e	5.30 d (9)	5.91 dd (9,9)	5.45 d	3.17 dbr	4.61 br	2.86 m	5.45 d	2.86 m 2.30 dbr (14,<1)	2.86 m	1.16 d[g] (7)	1.99	1.60	6.89	1.87 m	10 arom protons
2	5.80 d (8)	9.92 d (8)	8.87	3.25 d (4.5)	4.80 dd	2.65 m	5.33 m	2.65 m	2.65 m	1.20 d[g] (7.5)	2.28 br (1)	1.58	6.81 m	1.77 m	
3	5.42 m[e]	4.57 m[e]	3.46 d (9)	3.00 d (9)	4.57 m	2.80 m	5.42 dbr	2.90 dd (14,6) 2.30 dbr (14, <1)	2.80 m	1.15 d[g] (7)	1.73	1.40	6.8 m	1.84 m	
4a	5.20 dbr (12,1)	2.40 m[f]	3.46 dd (10, 7)	2.87 d (9)	4.57 dd (9,9)	2.72 m	5.40 dbr (6, <1, <1)	2.72 m 2.16 dbr (15, <1)	2.72 m	1.10 d[g] (8)	1.61	1.34	6.84	1.77 m	2.87 bs[j] (−OH)
4b	5.24 dbr (12,1)	2.30 m[f]	4.52 dd (10, 6)	2.89 d (9)	4.58 dd (9,9)	2.78 m	5.40 dbr (6, <1, <1)	2.78 m 2.22 dbr (14, <1)	2.78 m	1.13 d[g] (7)	1.65	1.43	6.86 m	1.84 m	2.10 (Ac)
4c	5.26 dbr (12,1)	2.67 m[f]	3.76 dd (10,7)	3.05 d	4.78 dd (9,9)	2.31 m	4.48 dbr (4, <1, <1)	2.67 m 2.31[e]	3.05[e]	1.39 d[g] (7)	1.99	1.58			
5a[b]	2.40 m	2.11 ddd (15,5,2 1.90 ddd (15,5,2))	5.30 br		3.34	2.74 m	4.81 dd (10,10)	2.74 m	2.40 m	3.83 m	1.44	1.05	6.93 m	1.80 m	6.97 br (OH)[j] 5.96 br (OH)[j]
5b	2.71 m	2.30 ddd (15,5,2) 1.83[e]	5.50 br		4.2	2.90 m	4.68 dd (12,9)	2.93 d (10)	2.90 m	4.10[i]	1.62	1.32	6.81	1.80 m	2.04 (Ac) 2.11 (Ac)
5b[d]	2.41 m (4,3)	1.88 ddd (15,4,3) 1.09 ddd (15,3,2)	5.47 br (~3,2)		4.53	2.98 dd (12,10) 10)	4.66 dd (12,9)	2.95 d (9)	2.41 m	3.95[i]	1.61[e]	1.53	6.54	1.30 m	1.68 (Ac) 1.61 (Ac)
6		5.40 m	2.39[e] 1.36 dd (12,12)	3.19 d (9)	4.62 dd (9,9)	2.70 m	5.54 m	2.98 dd (17.4) 2.44 m	2.70 m	1.14 d[g] (8)		1.61	6.70 m	1.74 m	

[a] Run in $CDCl_3$ at 270 MHz on a Brucker HFX-270 instrument with Me_4Si as internal standard unless otherwise specified. Values are in parts per million: d, doublet; t, triplet; br, broadened singlet; m, multiplet. Unmarked signals are singlets. Figures in parentheses are coupling constants in hertz. [b] Run in Me_2SO-d_6. [c] Run in pyridine-d_5. [d] Run in benzene-d_6. [e] Signal partially obscured or superimposed. [f] Intensity two protons. [g] Intensity three protons. [h] Intensity six protons. [i] Center of AB of an ABX system. [j] Exchanges with D_2O

Table II. ^{13}C NMR Spectra of Lactones from *E. perfoliatum*

Carbon atom	1a[a]	4a[b]	6[b]
1	130.6 d	125.1 d	165.9
2	72.6 d	33.0 t	81.5 d
3	65.6 d	64.9 d	29.8 t
4	64.2	65.1	83.1
5	83.0	77.0 d	38.8 d
6	77.5 d	76.2 d	75.8 d
7	47.9 d	47.4 d	48.2 d
8	72.8 d	72.0 d	66.9 d
9	44.1 t	43.5 t	45.6 t
10	133.3	133.2	128.3
11	40.9 d	40.3 d	56.9 d
12	178.5	178.3	177.7
13	12.0 q	11.1 q	11.6 q
14	20.5 q	19.7 q	175.0
15	12.8 q	12.2 q	26.2 q
1′	166.8	166.3	166.7
2′	129.1	128.3	122.7
3′	139.3 d	138.8 d	138.8 d
4′	14.5 q	14.5 q	14.4 q
5′	12.3 q	11.8 q	12.0 q

[a] Run in Me_2SO-d_6 on a Bruker HFX-270 instrument operating at 67.905 MHz. Unmarked signals are singlets. Assignment of all multiplets made by single frequency off-resonance spin decoupling. Assignments of C-4, C-5, C-10, and C-2[1] based on predicted shifts and other spectra in our files. [b] Run in $CDCl_3$. Assignments based on comparison with spectrum of **1a** and predicted shifts.

NMR signals at 2.00 and 2.10 ppm) in whose NMR spectrum the resonances at 4.08 and 2.90 ppm were shifted downfield to 5.60 and 4.82 ppm, the presence of partial structure B was inferred and confirmed by the single frequency off-resonance decoupled ^{13}C NMR spectrum, which showed C_i and C_j as a singlet and a doublet at 133 and 131 ppm, respectively, and C_k and C_l as two doublets at 72.6 and 65.6 ppm.

The remaining signal in the NMR spectrum of **1a** was a methyl resonance at 1.21 ppm. Combination of A and B with this information led to gross structure **1a.**

As for the stereochemistry, if it be assumed that H-7 of euperfolitin is α as in all sesquiterpene lactones of established absolute stereochemistry, the large values of $J_{6,7}$ (9 Hz) and $J_{5,6}$ (8 Hz) require that H-6 be β- and that H-5 be α-oriented. Hence the lactone ring is trans fused. The magnitude of $J_{7,11}$ (9 Hz) is somewhat more appropriate for H-11β (models), but does not completely exclude α orientation of H-11.[10] The very small value of $J_{7,8}$ (<1 Hz) requires that the tigloyl side chain be β oriented.

We now consider the stereochemistry of the 1,10 double bond and the 4,5-epoxide. Oxidation of **1a** with sodium metaperiodate in methanol yielded a dialdehyde **2a,** thereby furnishing chemical proof for the presence of the vicinal diol system. The NMR spectrum of **2a** exhibited a doublet at 9.92 ppm due to the aldehyde proton of C-2 and the resonance of H-1 now appeared at 5.80 ppm. Furthermore, the C-14 methyl resonance was shifted downfield to 2.28 ppm due to deshielding by the aldehyde carbonyl; hence the 1,10 double bond is trans.[11] As for the stereochemistry of the epoxide ring, Dreiding models demonstrated that the large value of $J_{5,6}$ cannot be satisfied by a cis epoxide whereas the values of $J_{5,6}$ and $J_{6,7}$ are appropriate for a trans 4,5-epoxide derived from a trans,trans germacradiene as shown in the formulas.

In discussing the remaining problem, i.e., the configurations of euperfolitin at C-2 and C-3, we assume first that, as has been shown in numerous instances,[12] those conformations of trans-1(10),trans-4,5 germacradienes or the corresponding

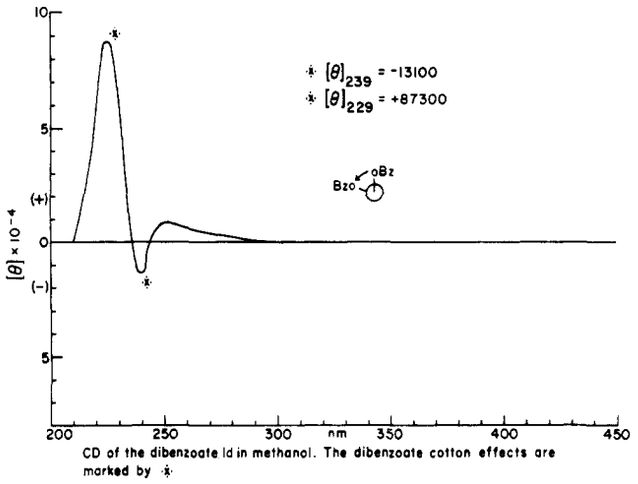
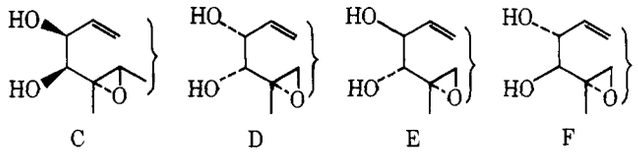


Figure 1. Application of dibenzoate rule to **1d.**

4,5-epoxides are preferred in which the 1,10 double bond is crossed with the 4,5 bond so that the C-4 and C-10 methyl groups are syn to each other. Support for this inference was the absence of an NOE between H-5 and the C-14 methyl group; other experiments along this line confirmed that the 1,10 double bond was trans (no NOE between H-1 and H-14) and suggested that H-3 was α oriented (no NOE between H-3 and H-15).

If this last bit of evidence be neglected, four possible arrangements, C, D, E, and F, are possible for the diol moiety.



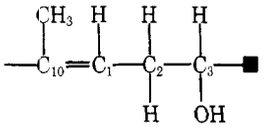
Preparation of the dibenzoate **1a,** determination of the CD curve (Figure 1), and application of the dibenzoate rule[13] show that the glycol system possesses negative chirality. Inspection of the Dreiding models then eliminated partial formulas C and D which give rise to positive chirality. Lastly, while the model of E displays negative chirality, the H-2, H-3 dihedral angle approximates 90° which clashes with the observed large value of $J_{2,3}$ (9 Hz). Consequently, the correct stereochemistry is that of F which gives rise to negative chirality and an H-2, H-3 dihedral angle of ~170° as well as satisfying all the other requirements.

Treatment of **1a** with $SOCl_2$–pyridine surprisingly effected transformation to a 2,3-epoxide **3** which $J_{2,3}$ and inspection of Dreiding models required to be α-cis.[14] We suggest that this is the result of S_Ni displacement of C-3 hydroxyl by $SOCl_2$–pyridine followed by intramolecular displacement of the β-oriented halogen by the α-oriented hydroxyl group on C-2.

The C-2, C-3 stereochemistry deduced for euperfolitin (**1a**) is identical with that of 2β,3α-dihydroxy-1(10),4,5,11,13-germacatriene, a stress metabolite isolated[15] from *Datura stramonium* infected with various fungi. The observed coupling constants for the diol systems are remarkably similar and further support our assignments. Unfortunately, the crystals of euperfolitin or its derivatives were unsuitable for x-ray analysis which is needed to settle the stereochemistry at C-11.

The second substance euperfolin (**4a**), $C_{20}H_{28}O_6$ (high-resolution mass spectrum, elemental analysis), mp 173 °C, $[\alpha]_D$ − 13.9°, exhibited IR, UV, and NMR absorption similar to that of **1a,** thus suggesting a close structural relationship. Spin-decoupling experiments (Table I) reinforced this conclusion, partial structure A being established in the manner

discussed for euperfolitin. On the other hand, the C-2 hydroxyl group of euperfolitin was missing in **4a;** the vinylic proton (H-1) of **4a** appeared as a broad doublet at 5.20 ppm which was allylically coupled to a vinyl methyl group and vicinally coupled to a two-proton multiplet centered at 2.40 ppm (H-2a and H-2b). These in turn were coupled to a doublet of doublets at 3.46 ppm which moved downfield to 4.52 ppm on acetylation of **4a** to **4b.** Hence **4a** contains partial structure G where C-3 is again joined to a quaternary carbon atom.

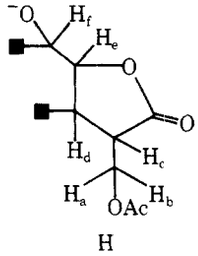


G

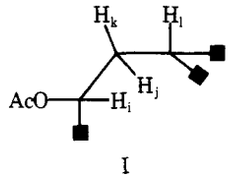
Comparison of the ^{13}C NMR spectrum of **4a** with that of **1a** (Table II) confirmed the absence of the 2-hydroxyl group (Δ C-2 = 4.26 ppm).[16] Since the various coupling constants of **1a** and **4a** were very similar (Table I), the stereochemistry of euperfolitin and euperfolin at the various centers must be the same. The C-3 hydroxyl group of euperfolin was deduced to be β as in **1a** because of the absence of an NOE between H-3 and H-15,[11] by analogy with **1a,** and by the correspondence of $J_{2a,3}$ and $J_{2b,3}$ (10, 7 Hz) with those of 11,13-dihydronovanin, which has a β-oriented acetate function at C-3.[10f,17] Hydrolysis (NaOMe, MeOH)[9] furnished **4c** which exhibited the expected spectroscopic properties; but further work was negated by lack of material.

The third substance, eufoliatin (**5a**) from *E. perfoliatum*, $C_{20}H_{26}O_8$ (high-resolution mass spectrum, elemental analysis), mp 227–229 °C (amorphous), $[\alpha]_D$ −27.5°, was available in small quantity only. Like euperfolin, it contained a hydroxyl group, a γ-lactone, and a tigloyl ester (IR bands at 3420, 1765, 1700, and 1640 cm^{-1}; NMR signals typical of tiglate—see Table I—and mass spectrum which exhibited significant peaks at *m/e* 311 ($M^+ - C_5H_7O$), 294 ($M^+ - C_5H_8O_2$), and 83 (base peak, C_5H_7O). However, two methyl singlets at 1.44 and 1.05 ppm suggested that at least one of the methyl groups was attached to carbon carrying an ether or hydroxyl; of two protons in the 4.8–5.3 ppm region, the one at lower field, a somewhat broadened singlet at 5.30 ppm, was provisionally assigned to the proton under the ester and the one at higher field, a doublet of doublets at 4.81 ppm, to hydrogen under the lactone.

Acetylation yielded a diacetate **5b** whose NMR spectrum in benzene-d_6 afforded excellent separation of signals and permitted deduction, by spin decoupling, of the sequence shown in partial structure H, where H_a, H_b, H_c, H_d, H_e, and H_f could be located at 4.14, 3.77, 2.41, 2.98, 4.66, and 2.95 ppm, respectively. H_a and H_b represented the AB part of an ABX system whose chemical shift, when compared with those in **5a,** indicated that the carbon atom to which they were attached carried one of the two new acetate groups. H_d, which was next to H_c, and H_e, the lactonic proton, was next to a quaternary center as was H_f whose chemical shift (compare with shift of H_c and H_f in $CDCl_3$) indicated that it was attached to carbon carrying an oxygen atom. A second sequence determined by spin decoupling was I, where H_i, H_j, H_k, and H_e were found at 5.47 (hydrogen under tigloyl ester), 1.88, 1.09 (two geminate-coupled protons each coupled to H_i and H_e), and 2.41, respectively, the last apparently to two quaternary centers.



H



I

Since hydroxyl groups, double bonds, and carbonyl groups other than those represented in the ester and lactone functions were absent, the molecular formula of eufoliatin required a bicyclic carbon skeleton and the incorporation of two ether functions, one of which is included in H. In view of the chemical shift of the two methyl singlets, these requirements could be met most satisfactorily by writing gross structure **5b** for the diacetate, although scarcity of material prevented further verification by chemical methods. The tentative stereochemistry assigned to **5a** and **5b** is based on the usual assumption that the C-11 side chain is equatorial and β. In that case, the large coupling constants $J_{7,8}$ and $J_{8,9}$ require that H-8 be β and H-9 be α. The large coupling constant $J_{7,11}$ suggests but does not prove that H-11 is β[10] and the lack of coupling between H-6 and H-7 requires that H-6 be β oriented. In this arrangement the dihedral angle between H-6 and H-7 would be about 105°.

The further assumption can be made that H-1 is α as in all naturally occurring guaianolides produced by cyclization of a trans-1(10)-trans-4,5 germacradiene precursor[18] (this seems likely because of the cooccurrence of **1a** and **4a**) where subsequent epimerization due to the presence of a ketone at C-2 or a vinylogous ketone at C-3 is not possible.[19] However, the observed coupling constants $J_{1,2a}$, $J_{1,2b}$, $J_{2d,3}$, and $J_{2b,3}$ (~4, 3, 3, and 2 Hz) leave uncertain the orientation of the C-3 ester function even after the knotty question of the configuration of the two epoxide rings has been considered.

Models incorporating a trans-9,10- or an α-cis-9,10-epoxide did not satisfy the requirement for a large value of $J_{8,9}$; hence the 9,10-epoxide must be β-cis. Models incorporating a trans 4,5-epoxide could not be constructed; biogenetic considerations suggest that the 4,5-epoxide is α-cis, which satisfies the requirement that $J_{6,7}$ vanish, whereas models with the 4,5-epoxide β-cis display a H-5, H-7 dihedral angle such that $J_{6,7}$ should be appreciable. Consequently we prefer the stereochemistry shown in **5a** and **5b,** where the stereochemistry at C-3 remains in doubt. Unfortunately, we have so far been unable to obtain crystals suitable for x-ray analysis.

Only a small amount of the crystalline fourth substance eufoliatorin (**6**), $C_{20}H_{24}O_7$, mp 224 °C, was isolated. It had a γ-lactone function of the type present in **1a** and **4a** (IR band at 1780 cm^{-1}, methyl doublet at 1.14 ppm) and a tigloyl ester [IR bands at 1685 and 1640 cm^{-1}, typical NMR signals at 1.61 and 1.74 ppm (two vinyl methyl multiplets), significant mass spectral peaks at *m/e* 277 ($M^+ - C_5H_7O_2$), 276 ($M^+ - C_5H_8O_2$), and 83 (base peak, C_5H_7O)]. The IR spectrum suggested the presence of another α,β-unsaturated γ-lactone (band at 1753 cm^{-1}), especially since there was significant UV absorption at 219 and 210 nm (ϵ 26 100 and 24 600).

Spin decoupling experiments on eufoliatorin demonstrated the pattern shown in partial structure J. H_d was located as a doublet of doublets at 4.62 ppm. Irradiation at this frequency collapsed a doublet at 3.19 ppm (H_e) to a singlet and affected a two-proton multiplet at 2.70 ppm. Conversely, irradiation at the frequency of H_e collapsed the H_d signal to a doublet; hence H_e must be adjacent to two quaternary carbon atoms. Irradiation at the frequency of the 2.70-ppm multiplet collapsed H_d to a doublet and the methyl doublet (H_b) at 1.14 ppm to a singlet and affected a multiplet at 5.54 ppm (H_f), obviously the proton under the tiglate. Consequently, it became evident that the signals of H_a and H_c overlapped at 2.70 ppm. Irradiation at the frequency of H_f simplified what appeared to be the AB part of an ABX system where A, geminate-coupled to B, was a doublet of doublets at 2.98 ppm (H_g) and B a multiplet at 2.44 ppm (H_h). The reverse experiments

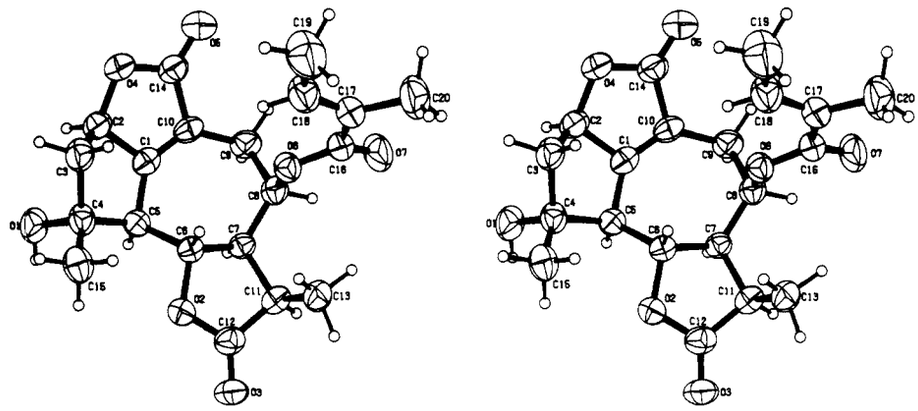
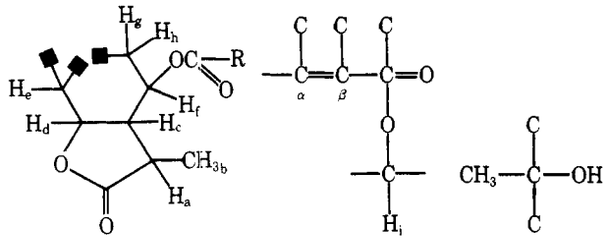


Figure 2. Stereoscopic view of **6**.

confirmed these conclusions and showed that the methylene group including H_g and H_h was next to a quaternary center.



J K L

The ^{13}C NMR off-resonance spectrum of **6** (Table II) displayed, as surmised, three carbonyl frequencies in the region characteristic of lactone and ester carbonyls, two of these being identifiable with the carbonyl groups of J; the third was assigned to the α,β-unsaturated lactone partially shown in K, since the off-resonance spectrum also displayed two vinylic carbon singlets at 128 (α) and 165.9 (β) in addition to the vinylic carbons of the tigloyl residue (122.7 and 138.8 d). Three doublets at 81.5, 75.8, and 66.9 ppm and a singlet at 83.1 ppm represented carbons carrying ethereal, alcohol, and ester oxygen. Failure to achieve acetylation by the acetic anhydride–pyridine method indicated that the hydroxyl group was tertiary and therefore attached to the carbon represented by the singlet at 83.1 ppm. Furthermore, since the ^1H NMR spectrum contained a methyl singlet at 1.61 ppm, the tertiary hydroxyl group was incorporated in partial structure L. Two of the three CHO doublets could be identified with C_d and C_f of J; hence K was closed to a secondary carbon as shown.

The ^1H NMR spectrum displayed three additional signals not so far accounted for. A multiplet at 5.40 ppm was logically assigned to H_i of K. Irradiation at the frequency of H_i changed a multiplet at 2.39 ppm (H_j) to a broad doublet and collapsed the third remaining signal, a doublet of doublets at 1.36 ppm (H_k), to a doublet. Conversely, irradiation at the frequency of H_j converted the H_i and K_k signals to doublets, while irradiation at the frequency of H_k affected the multiplets of H_i and H_j.

The molecular formula of eufoliatorin required that it must be bicyclic apart from its two lactone rings. A structure which incorporates J, K, and L and satisfies all requirements was **6**, exclusive of stereochemistry. If H-7 is α as usual, the large coupling constants $J_{6,7}$ and $J_{5,6}$ require that H-6 be β and H-5 be α oriented. Again while $J_{7,11}$ is large, a decision in favor of α or β orientation of C-13 was difficult and could not be achieved by attempts to assess the effect of paramagnetic shift reagents on the shift of C-13. The very small value of $J_{7,8}$ (<1 Hz) showed that the tigloyl ester was β. On biogenetic grounds, it was assumed that H-5 and the hydroxyl group at C-4 were α oriented, but since determination of the configuration at the various centers was limited by the amount of material available and since the structure was novel, eufoliatorin was submitted to x-ray analysis.

Table III. Crystal Data for 6

Formula:	$C_{20}H_{24}O_7$	
Orthorhombic		
Space group	$P2_12_12_1$	$Z = 4$
a = 8.124 (5) Å		
b = 13.041 (7) Å		
c = 17.840 (10) Å		
d_{calcd} 1.322 g cm^{-1}		

Crystal data for **6** are listed in Table III. Figure 2 is a stereoscopic drawing of the molecule which represents the absolute configuration if our inference about the absolute stereochemistry at H-7 is correct. It confirms our conclusions about the gross structure, the seven-membered ring being a deformed chair with C-6 as apex, and about the configurations at C-6, C-7, and C-8, supports our speculative assignment of stereochemistry on biogenetic grounds to C-4 and C-5, and establishes the previously unknown configurations at C-2 and C-11. Because of the cooccurrence of **1a, 4a, 5a,** and **6** in the same plant it is conceivable that the stereochemistry of **1a, 4a,** and **5a** is the same as that of **6**. Tables IV, V, and VI containing bond lengths, bond angles, and torsion angles are available as supplementary material.

Experimental Section[20]

Extraction of *Eupatorium perfoliatum*. Above-ground parts of *E. perfoliatum* L., collected by Mr. R. Lazor (Lazor no. 1218) on Sept 20, 1968 at the intersection of Florida State Road 20 with a side road 7 miles east of Hosford, Fla., wt 45 lb, was extracted with $CHCl_3$ and worked up in the usual manner.[21] The dark brown gum, wt 180 g, was chromatographed over a column of 1.2 kg of silicic acid (Mallinckrodt 100 mesh) packed in benzene, the column being eluted with fractions of increasing polarity, 200-mL fractions being collected. Fractions 15–26 ($CHCl_3$), 27–54 ($CHCl_3$–MeOH, 49:1), and 55–67 ($CHCl_3$–MeOH, 19:1) eluted a complex mixture (TLC), mixed and evaporated to give a gum. This gum (80 g) was rechromatographed over a column of 1.2 kg of silicic acid, the column being eluted with solvent of increasing polarity beginning with benzene. The $CHCl_3$ eluates on preparative TLC (solvent system benzene–ethyl acetate, 1:2) gave solid euperfolin (**4a**) which was recrystallized from ethyl acetate–hexane to give colorless material: wt 0.40 g; mp 173 °C; $[\alpha]^{22}D$ −13.9° (c 0.0228, MeOH); CD curve $[\theta]_{290}$ 0, $[\theta]_{260}$ + 554, $[\theta]_{250}$ +998, $[\theta]_{240}$ + 1164, $[\theta]_{231}$ +44 (last reading); IR bands (KBr) at 3410 (–OH), 1775 (γ-lactone), 1708 and 1642 cm^{-1} (α,β-unsaturated ester); strong UV end absorption. Elemental analysis consistently indicated what was probably stubborn retention of solvate, but the high-resolution mass spectrum and the derivatives (vide infra) confirmed the proposed empirical formula.

Anal. Calcd for $C_{20}H_{28}O_6$: mol wt, 364.1884. Found: mol wt (MS), 364.1896.

The high-resolution mass spectrum showed other significant peaks at *m/e* (composition, %) 349 ($M^+ - CH_3$, 3.2), 346 ($M^+ - H_2O$, 3.0), 334 ($M^+ - CH_2O$, 3.6), 333 ($C_{15}H_{22}O_5$, 10.5), 281 ($M^+ - C_5H_7O$, 1.6), 265 ($M^+ - C_5H_7O_2$, 3.3), 264 ($M^+ - C_5H_8O_2$, 14.9), 247 ($M^+ - C_5H_7O_2 - H_2O$, 6.2), 246 ($M^+ - C_5H_8O_2 - H_2O$, 13.4), 236 ($M^+ - C_5H_8O_2 - CO$, 1.4), 231 ($M^+ - C_5H_9O_2 - H_2O, CH_3$, 6.7), 221 ($M^+ - C_5H_8O_2 - CO - CH_3$, 17.9), 203 ($M^+ - C_5H_8O_2 - H_2O - CO$, 1.4), 191 ($C_{13}H_{15}O_3$, 52.7), 100 ($C_5H_8O_2$, 24.4), and 83 ($C_5H_7O$, base peak).

The $CHCl_3$–MeOH (19:1) eluate yielded a gum which showed a major spot on TLC. This component (eufoliatorin) was separated by repeated preparative TLC (solvent system benzene–ethyl acetate, 1:1) as a gum which eventually solidified and was recrystallized from ethyl acetate–hexane as colorless needles (**6**) which were suitable for x-ray analysis: wt 0.15 g; mp 224 °C; CD curve $[\theta]_{315}$ 0, $[\theta]_{300}$ +677, $[\theta]_{290}$ +1692, $[\theta]_{280}$ +2370, $[\theta]_{269}$ +2540, $[\theta]_{255}$ +6770, $[\theta]_{245}$ +11 340, $[\theta]_{237}$ 0, $[\theta]_{230}$ − 54 950, $[\theta]_{220}$ − 116 100, $[\theta]_{210}$ −63 400 (last reading); IR bands (KBr) at 3490 (–OH), 1780 (γ-lactone), 1753 (α,β-unsaturated γ-lactone), 1685 and 1640 cm^{-1} (α,β-unsaturated ester); UV spectrum (MeOH) λ_{max} 282 nm (ε 2990), 250 (6840), 219 (26 100), and 210 (24 600). As in most other compounds encountered in this work, the carbon analysis remained consistently low.

Anal. Calcd for $C_{20}H_{24}O_7$: C, 63.82; H, 6.43; O, 29.75; mol wt, 376.1521. Found: C, 63.06; H, 6.42; O, 29.71; mol wt (MS), 376.1525.

The high-resolution mass spectrum showed other significant peaks at *m/e* (composition, %) 277 ($M^+ - C_5H_7O_2$, 8.4), 258 ($M^+ - C_5H_8O_2 - H_O$, 13.4), 249 ($M^+ - C_5H_7O_2 - Co$, 3.6), 100 ($C_5H_8O_2$ 5.5), 99 ($C_5H_7O_2$, 5.6), and 83 (base peak, C_5H_7O).

Fractions 68–70 of the original chromatogram ($CHCl_3$–MeOH, 19:1) gave 10 g of a gum which showed two major spots on TLC. This was rechromatographed over 300 g of silica gel. $CHCl_3$–MeOH (3%) eluted solid material (eufoliatin) which was recrystallized from MeOH–$CHCl_3$ to give a colorless powder (**5a**): wt 0.10 g; mp 227–229 °C; $[\alpha]_D$ − 27.5° (*c* 0.0121, MeOH); IR bands (KBr) 3420 (–OH), 1765 (γ-lactone), 1700 and 1640 cm^{-1} (α,β-unsaturated ester); UV spectrum λ_{max} 220 nm (ε 12 800). The carbon analysis was consistently low.

Anal. Calcd for $C_{20}H_{26}O_8$: C, 60.90; H, 6.64; O, 32.45; mol wt, 394.1627. Found: C, 60.18; H, 6.57; O, 33.02; mol wt (MS), 394.1618.

The high-resolution mass spectrum also exhibited significant peaks at *m/e* (composition, %) 311 ($M^+ - C_5H_7O$, 1.7), 295 ($M^+ - C_5H_7O_2$, 3.1), 294 $M^+ - C_5H_8O_2$, 2.4), 277 ($M^+ - C_5H_7O_2 - H_2O$, 1.7), 276 ($M^+ - C_5H_8O_2 - H_2O$, 1.4), 248 ($M^+ - C_5H_8O_2 - H_2O - H_2O - CO$, 2.3), 100 ($C_5H_8O_2$, 6.8), and 83 ($C_5H_7O$, base peak).

Fractions 71–73 of the original chromatogram ($CHCl_3$–MeOH, 8%) gave a gum which was rechromatographed over 200 g of silica gel. The $CHCl_3$–MeOH (5%) eluates gave euperfolitin (**1a**) as a solid which was recrystallized from ethyl acetate–hexane to give **1a:** mp 190–192 °C; $[\alpha]^{82}_D$ −5.81° (*c* 0.0119, MeOH); CD curve $[\theta]_{290}$ 0, $[\theta]_{265}$ +490, $[\theta]_{255}$ 982, $[\theta]_{243}$ +1470, $[\theta]_{235}$ +1150, $[\theta]_{230}$ 0, $[\theta]220$ −982, $[\theta]_{210}$ −1310 (last reading); IR bands (KBr) 3450 (–OH), 1765 (γ-lactone), 1700 and 1650 cm^{-1} (α,β-unsaturated ester); UV spectrum 220 nm (ε 7600). The carbon analysis remained consistently low.

Anal. Calcd for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42; O, 29.44; mol wt, 380.1835. Found: C, 61.85; H, 7.49; O, 29.77; mol wt (MS), 380.1856.

Other significant peaks in the high-resolution mass spectrum which, except for the molecular ion (1%), showed only rather weak peaks above *m/e* 200 were at *m/e* (composition, %) 362 ($M^+ - H_2O$, 0.1), 280 ($M^+ - C_5H_8O_2$, 0.3), 262 ($M^+ - C_5H_8O_2 - H_2O$, 1.1), 244 ($M^+ - C_5H_3O_2 - 2H_2O$, 0.5), 100 ($C_5H_8O_2$, 2.5), and 83 (C_5H_7O, base peak).

Preparation of Acetates 1b, 4b, and 5b. A. Acetylation of 0.1 g of **1a** in 1 mL of pyridine and 0.5 mL of acetic anhydride at room temperature overnight followed by the usual workup gave a gummy diacetate which was purified by preparative TLC (silica gel, solvent system $CHCl_3$–MeOH, 6%), but could not be induced to crystallize: IR bands (film) 1780, 1740 (very strong), 1640, 1375, and 1240 cm^{-1}. The low-resolution mass spectrum exhibited no parent peak, but other significant peaks at *m/e* 422 ($M^+ - C_2H_2O$), 379 ($M - C_2H_2O - C_2H_3O$), 364 ($M^+ - C_5H_8O_2$), and 83 (base peak, C_5H_7O). The carbon analysis was consistently low.

Anal. Calcd for $C_{24}H_{32}O_9$: C, 62.06; H, 6.94; O, 31.00; mol wt, 422.1941. Found: C, 60.31; H, 6.96; O, 31.87; mol wt (MS), 422.1946.

B. Acetylation of 0.050 g of **4a** in the above manner and purification of the crude product by preparative TLC (silica gel, solvent system benzene–ethyl acetate, 1:2) followed by recrystallization from benzene–hexane gave **4b:** mp 130 °C; IR bands (KBr) 1778, 1738, 1709, 1640, 1255, 1190, and 1040 cm^{-1}.

Anal. Calcd for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44; O, 27.55; mol wt, 406.1990. Found: C, 64.13; H, 7.55; O, 28.04; mol wt (MS), 406.1995.

Other significant peaks in the high-resolution mass spectrum were at *m/e* (composition, %) 364 ($M^+ - C_2H_2O$, 5.5), 346 ($M^+ - C_2H_4O_2$, 1.1), 306 ($M^+ - C_5H_8O_2$, 1.3), 264 ($M^+ - C_2H_2O - C_5H_8O_2$, 2.5), 246 ($M^+ - C_2H_4O_2 - C_5H_8O_2$, 10.4), and 83 ($C_5H_7O$, base peak).

C. Acetylation of 0.030 g of **5a** in the above manner and purification of the crude product by preparative TLC (silica gel, $CHCl_3$–MeOH, 5%) gave **5b** as a gum, wt 0.020 g, which had IR bands (film) at 1785, 1741, 1440, 1370, 1240, and 1015 cm^{-1}. The low-resolution mass spectrum exhibited significant peaks at *m/e* 436 ($M^+ - C_2H_2O$), 394 ($M - 2C_2H_2O$), 376 ($M - C_2H_4O_2 - C_2H_2O_2$), 294 ($M - 2C_2H_2O - C_5H_8O_2$), and 83 (base peak, C_5H_7O).

Anal. Calcd for $C_{24}H_{30}O_{10}$: mol wt, 478.1830. Found: mol wt (MS), 478.1839.

Hydrolysis of 1a and 4a. A. A solution of 0.1 g of **1a** in 10 mL of MeOH was stirred with 80 mg of NaOMe at room temperature (N_2 atmosphere) for 4 h. The reaction remained incomplete (TLC). The solution was acidified with dilute acetic acid, the methanol removed at reduced pressure, and the residue diluted with water. The mixture was extracted with ethyl acetate; the washed and dried extract was evaporated and the residue separated by preparative TLC (silica gel, $CHCl_3$–MeOH, 15%). The more polar band yielded a solid (**1c**) which was recrystallized from acetone–hexane to give a colorless powder: yield 10 mg; mp 210–211 °C; IR bands (KBr) 3400, 1760, 1640, 1445, 1380, 1180, 1000, and 850 cm^{-1}.

Anal. Calcd for $C_{15}H_{22}O_6$: mol wt, 298.1415. Found: mol wt (MS), 298.1392.

Other significant peaks in the high-resolution mass spectrum were *m/e* (composition, %) 280 ($M^+ - H_2O$, 9.3), 265 ($M^+ - CH_3 - H_2O$, 11.9), 262 ($M^+ - 2H_2O$, 24.5), 247 ($M^+ - 2H_2O - CH_3$, 6.1), 244 ($M^+ - 3H_2O$, 9.7), 219 ($M^+ - CH_3 - CO - 2H_2O$, 36.6).

The less polar band (wt 50 mg) represented starting material.

B. Hydrolysis of 0.050 g of **4a** in the above manner, purification of the crude product by preparative TLC (silica gel, benzene–ethyl acetate, 1:2), and recrystallization from acetone–hexane gave 8 mg of **4c** as an amorphous powder: IR bands (KBr) 3496, 3392, 1755, 1435, 1240, and 1125 cm^{-1}. The low-resolution mass spectrum exhibited the molecular ion at *m/e* 282 ($C_{15}H_{22}O$) and other significant peaks at *m/e* 264 ($M^+ - H_2O$, 246 ($M^+ - 2H_2O$), and 221 ($M^+ - H_2O - CH_3 - CO$).

Anal. Calcd for $C_{15}H_{22}O_5$: mol wt, 282.1467. Found: mol wt (MS), 282.1494.

Periodate Cleavage of 1a. A solution of 0.10 g in 2 mL of MeOH was stirred with a solution of 0.1 g of sodium metaperiodate in 2 mL of MeOH and 3 mL of water overnight, diluted with water, and extracted with ethyl acetate. The washed and dried extracts were evaporated at reduced pressure and purified by preparative TLC (silica gel, $CHCl_3$–MeOH, 10%) to give 46 mg of gummy **2** which had IR bands (film) at 1770, 1750, 1700 (br), 1645, 1450, and 1380 cm^{-1}. The low-resolution mass spectrum exhibited the molecular ion at *m/e* 378 ($C_{20}H_{26}O_7$) and other significant peaks at *m/e* 349 ($M^+ - CHO$), 295 ($M^+ - C_5H_7O$), 249 ($M^+ - CHO - C_5H_8O_2$), and 83 (base peak, C_5H_7O).

Anal. Calcd for $C_{20}H_{26}O_7$: mol wt, 378.1678. Found: mol wt (MS), 378.1684.

Dibenzoate of 1a. A solution of 0.025 g of **1a** in 0.5 mL of pyridine and 0.2 mL of benzoyl chloride was allowed to stand at room temperature for 24 h and worked up in the usual manner. The crude gummy product solidified on triturating with hexane. The amorphous product was filtered, washed with hexane, and dried, yield 0.020 g, mp 145–147 °C. The CD curve is reproduced in Figure 1. The low-resolution mass spectrum exhibited the molecular ion at *m/e* 588 ($C_{34}H_{36}O_9$) and other significant peaks at *m/e* 505 ($M^+ - C_5H_7O$), 488 ($M^+ - C_5H_8O_2$), 483 ($M^+ - C_7H_5O$), 383 ($M^+ - C_5H_8O_2 - C_7H_5O$), and 83 ($C_5H_7O$). The 270-MHz NMR spectrum was practically superimposable on that of **1b** (chemical shifts, coupling constants), except for additional signals due to the ten aromatic protons.

Anal. Calcd for $C_{34}H_{36}O_9$: C, 69.37; H, 6.16; O, 24.46. Found: C, 69.98; H, 6.29; O, 24.23.

Reaction of 1a with Thionyl Chloride. A solution of 0.10 g of **1a** in 2 mL of pyridine was cooled to 0 °C, mixed with 0.2 mL of thionyl chloride, and stirred, the reaction being monitored by TLC. After 2.5 h the mixture was decomposed with ice water and extracted with $CHCl_3$. The washed and dried extract was evaporated and purified by preparative TLC (silica gel, $CHCl_3$–MeOH, 10%). The gummy product **3**, wt 21 mg, had IR bands ($CHCl_3$) at 1770, 1705, 1640, 1380, and 1085 cm^{-1}. The high-resolution mass spectrum did not exhibit

the molecular ion, but a peak (1.5%) at *m/e* 279.1231 (calcd for $C_{20}H_{26}O_6$, C_5H_7O, 279.1243). Other significant peaks were at *m/e* (composition, %) 263 ($M^+ - C_5H_7O_2$, 22.8), 262 ($M^+ - C_5H_8O_2$, 26.8), 245 ($M^+ - C_5H_7O_2 - H_2O$, 3.4), 244 ($M^+ - C_5H_8O_2 - H_2O$, 49.6), 229 ($M^+ - C_5H_8O_2 - H_2O - CH_3$, 6.3), 100 ($C_5H_8O_2$, base peak), and 99 ($C_5H_7O_2$, 31.3).

X-Ray Analysis of Eufoliatorin. Intensity data were measured on a Hilger-Watts diffractometer (Ni filtered Cu Kα radiation, θ–2θ scans, pulse height discrimination). A crystal measuring approximately 0.15 × 0.4 × 0.5 mm was used for data collection; no absorption correction was made (μ = 8.4 cm^{-1}). A total of 2210 reflections were measured for $\theta < 76°$, of which 1992 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple solution procedure[22] and was refined by full-matrix least squares. In the final refinement anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are $R = 0.055$ and $wR = 0.076$ for the 1992 observed reflections. The final difference map has no peaks greater than ±0.3 e $Å^{-3}$.

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Registry No.—**1a,** 62197-54-0; **1b,** 62197-55-1; **1c,** 62197-56-2; **1d,** 62197-57-3; **2,** 62197-58-4; **3,** 62197-59-5; **4a,** 62197-60-8; **4b,** 62197-61-9; **4c,** 62197-64-2; **5a,** 62197-62-0; **5b,** 62197-63-1; **6,** 62197-65-3.

Supplementary Material Available. Tables IV, V, and VI listing bond distances, bond angles, and torsion angles of compound **6** (3 pages). Ordering information is given on any current masthead page.

References and Notes

(1) This article is dedicated to the memory of S. Morris Kupchan, a long-time friend who published several noteworthy papers on constituents of *Eupatorium* species in the decade before his untimely death in 1976.
(2) Work at Florida State University supported in part by USPH Grant CA-13121 through the National Cancer Institute.
(3) W. Herz and R. P. Sharma, *J. Org. Chem.*, **41,** 1015 (1976).
(4) W. Herz and R. P. Sharma, *J. Org. Chem.*, **41,** 1021 (1976).
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(6) For references to earlier work, on *Eupatorium* species, see ref 3, which also includes citations concerning the flavonoids of *E. perfoliatum* and related species.
(7) The crude extract as a whole was rather polar and difficult to separate. The four substances we were successful in purifying represented a relatively small portion of the total extract.
(8) W. Herz and R. P. Sharma, *J. Org. Chem.*, **41,** 1248 (1976).
(9) See ref 3 for reasons governing choice of this reagent combination.
(10) Use of paramagnetic shift methods which can occasionally be used to solve this vexing problem was ineffective in this case and that of the other substances described in this report because of line broadening, overlapping of signals, etc. The applicability of the solvent shift method of C. R. Narayanan and N. K. Venkatasubramaniam [*Tetrahedron Lett.*, 5865 (1966); *J. Org. Chem.*, **33,** 3156 (1968)], which gives good results with 11,13-dihydroeudesmanolides, to 11,13-dihydrogermacranolides and 11,13-dihydroguaianolides of known stereochemistry is currently being tested.
(11) For leading citations, see ref 8.
(12) See, for example, (a) N. S. Bhacca and N. H. Fischer, *Chem. Commun.*, 68 (1969); (b) F. Sorm, M. Suchý, M. Holub, A. Linek, I. Hadinec, and C. Novák, *Tetrahedron Lett.*, 1893 (1970); (c) S. K. Talapatra, A. Patra, and B. Talapatra, *Chem. Commun.*, 1534 (1970); (d) E. J. Gabe, S. Neidle, D. Rogers, and C. E. Nordman, *ibid.*, 559 (1971); (e) K. Tori, I. Horibe, Y. Tamura, and H. Tada, *J. Chem. Soc., Chem. Commun.*, 620 (1973); (f) M. A. Irwin and T. A. Geissman, *Phytochemistry,* **12,** 875 (1973); (g) R. W. Doskotch, S. L. Keely, and C. D. Hufford, *J. Chem. Soc., Chem. Commun.*, 1137 (1972); (h) A. T. McPhail and K. D. Onan, *J. Chem. Soc., Perkin Trans. 2,* 1798 (1975); (i) K. Tori, I. Horibe, Y. Tamura, K. Kuriyama, H. Tada, and K. Takeda, *Tetrahedron Lett.*, 387 (1976); (j) A. Quick and D. Rogers, *J. Chem. Soc., Perkin Trans. 2,* 465 (1976).
(13) N. Harada and K. Nakanishi, *J. Am. Chem. Soc.,* **91,** 3990 (1969). The exciton split CD curves of dibenzoates invariably have either a stronger first extremum and weaker second or they are equal. Figure 1 shows some anomalies: (1) the extrema are shifted by 7–8 nm to the red and (2) the second extremum is much more intense. A reviewer has commented that the strong intensity of the second Cotton effect is probably due to the overlap of the positive CD maximum arising from the tiglate group.
(14) A Dreiding model incorporating a 2,3-trans epoxide and the other structural features whose presence had already been demonstrated proved to be impossible to construct.
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(16) Since the ^{13}C NMR spectra of **1a** and **4a** were run in different solvents, no attempt is made to interpret the chemical shifts exhibited by C-1, C-5, and C-8, although that of C-1 is in the right direction.
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Grisabine and Grisabutine, New Bisbenzylisoquinoline Alkaloids from *Abuta grisebachii*

Roshan Ahmad and Michael P. Cava*

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174

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Grisabine and grisabutine, new bisbenzylisoquinoline alkaloids from *Abuta grisebachii,* Triana & Planchon, have been assigned structures **1** and **2,** respectively, on the basis of spectroscopic and chemical evidence.

Recent studies from our laboratory have led to the isolation of a number of new alkaloids from plants of the tropical American genus *Abuta* (Menispermaceae).[1] We now wish to report the isolation and structure determination of two new bisbenzylisoquinoline alkaloids, grisabine (**1**) and grisabutine (**2**), from the Amazonian species *Abuta grisebachii.*[2]

Grisabine (**1**) was obtained as white needles, mp 148–149 °C. The composition $C_{37}H_{42}N_2O_6$ was determined by high-resolution mass spectrometry.

The infrared spectrum (KBr) of grisabine showed a band at 3400 cm^{-1}, attributable to a nonassociated phenolic group. The NMR spectrum of grisabine showed the presence of three superimposed aromatic methoxyls at δ 3.83, two nonequivalent methylimino groups at δ 2.43 and 2.48, and a band of 11 unresolved aromatic protons in the range of δ 6.38–7.01.

The mass spectrum of grisabine is typical of that of a bisbenzylisoquinoline alkaloid containing only a single tail-to-tail ether bridge. Thus, the molecular ion at *m/e* 610 was quite weak (4%), while the two identical head fragments **13** formed the base peak at *m/e* 192.