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NEW SESQUITERPENE LACTONES FROM *LIRIODENDRON TULIPIFERA**

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Key Word Index—*Liriodendron tulipfera*; Magnoliaceae; sesquiterpene lactones; lipiferolide, epitulipinolide diepoxide; γ-liriodenolide; epitulipdienolide; stereochemistry; cytotoxicity.

Abstract—Four new sesquiterpene lactones were obtained from *L. tulipifera*; two germacranolides, lipiferolide (**4**) and epitulipinolide diepoxide (**5**) from the leaves, an elemanolide, epitulipdienolide (**7**), and an eudesmanolide, γ-liriodenolide (**6**) from the root bark. Structural determination was by physical and chemical methods and by direct correlation to the known epitulipinolide (**3**). Lipiferolide and epitulipinolide diepoxide possess cytotoxic activity against KB cells.

INTRODUCTION

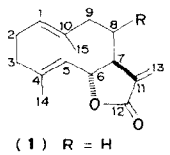
The tulip poplar, *Liriodendron tulipifera* L., a valuable timber tree of eastern North America, has already been reported to contain, in the root bark, three germacranolide cytotoxic agents, costunolide (**1**), tulipinolide (**2**), and epitulipinolide (**3**) [1–3]. Subsequent studies, herein reported, have revealed the presence of two cytotoxic† sesquiterpenes, lipiferolide (**4**) and epitulipinolide diepoxide (**5**) in the leaves and two additional sesquiterpene lactones, γ-liriodenolide (**6**) and epitulipdienolide (**7**) in the root bark. A preliminary report on lipiferolide (**4**) and γ-liriodenolide (**6**) has been published [4].

RESULTS AND DISCUSSION

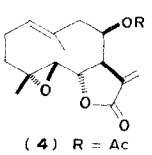
The leaf ethanolic extract was found to inhibit KB cells in tissue culture and on partitioning between chloroform and H_2O, the chloroform residue was toxic. Partitioning the chloroform-soluble material between petroleum ether and 10% aqueous methanol gave an active methanol-soluble fraction, which was chromatographed on several columns to yield lipiferolide (**4**) and epitulipinolide diepoxide (**5**). Lipiferolide (**4**), m.p. 118–119°, showed IR bands for an α-methylene-γ-lactone and an acetate, while the UV spectrum exhibited only end absorption for the unsaturated lactone. Elemental analysis established the formula as $C_{17}H_{22}O_5$, but the MS gave only fragment ions at *m/e* 264 (M-42) and 246 (M-60) consistent with the presence of an acetoxy group. The NMR spectrum showed a pair of doublets for the exocyclic methylene of the unsaturated lactone at δ 5·73 (*J* 3·2 Hz) and 6·38 (*J* 3·6 Hz), a 3-proton singlet at δ 2·07 for the acetate and a broadened singlet at δ 1·77 for the olefinic methyl. Preparation of deacetyllipiferolide (**8**) by hydrolysis confirmed the presence of an acetate function. A 3-proton singlet at δ 1·38 was assigned to a methyl on a carbon of an oxirane ring based on the following evidence. The fifth oxygen required by the molecular formula was chemically inert suggesting an ether and the NMR spectrum supported a C-4, C-5 epoxide because the C-5 proton appeared as a doublet (*J* 8·2 Hz) at

* Part 8 in the series 'Antitumor Agents'. For Part 7 see Ref. 4.

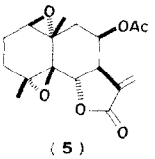
† The cytotoxicity was tested in KB cells through the courtesy of the Cancer Chemotherapy National Service Center following the protocol as given in *Cancer Chemother. Rep.*, **25**, 22 (1962). Lipiferolide and epitulipinolide diepoxide showed ED_{50} values of 0·16 and 0·34 μg/ml, respectively. Epitulipdienolide was inactive and γ-liriodenolide marginally effective with ED_{50} = 4·1 μg/ml. Some of these compounds possess antifeeding activity against the Gypsy moth larvae (*Porthetria dispar* L.).



(1) R = H
(2) R = α-OAc
(3) R = β-OAc



(4) R = Ac
(8) R = H



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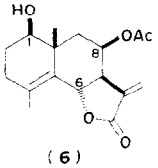

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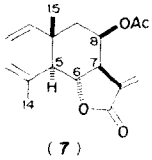

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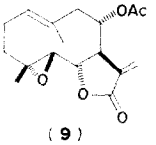

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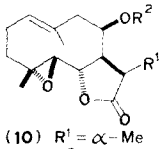
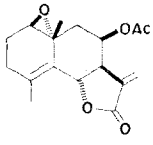


(10) R^1 = α-Me, R^2 = Ac
(11) R^1 = β-Me, R^2 = Ac
(19) R^1 = α-Me, R^2 = H



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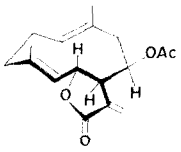
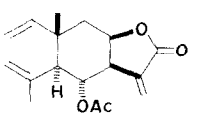


(13)

δ 2·84 that collapsed to a singlet when the triplet (*J* 8·2 Hz) assigned to the C-6 proton was irradiated in a double resonance experiment. In addition, during this irradiation the multiplet at δ 3·18 due to the C-7 proton was simplified. However, irradiation of the C-7 proton did not affect the doublet at δ 2·84 but converted the C-6 proton triplet to a doublet and simplified the multiplet at δ 5·72 for the C-8 proton. The relative order of substituents from positions C-4 to C-8 as shown in formula **4** was substantiated by additional double irradiation experiments.

The properties of lipiferolide suggested a germacranolide structure and specifically an epoxy derivative of tulipinolide (**2**) or of epitulipinolide (**3**). The former is 11,13-dehydrolanuginolide (**9**), a constituent of *Michelia lanuginosa* [5], with reported properties different from lipiferolide. Also (11*S*)-11,13-dihydrolipiferolide (**10**) produced by $NaBH_4$ reduction of lipiferolide and the (11*R*)-dihydro compound (**11**) from catalytic hydrogenation were also different from lanuginolide. Stereochemical assignments at C-11 for compounds (**10**) and (**11**) were made on the basis of analogies with similar compounds [2, 3]. Epoxidation of both lipiferolide (**4**) and epitulipinolide (**3**) with *m*-chloroperoxybenzoic acid produced a common product, epitulipinolide diepoxide (**5**), establishing for lipiferolide the absolute stereochemistry at C-6, C-7 and C-8, and confirming the nature of the fifth oxygen.

Attempts at epoxidation of epitulipinolide (**3**) with 1 mol. equiv. of reagent gave a single monoepoxide different from the natural product and characterized as epitulipinolide-1,10-epoxide (**12**) on the basis of the NMR spectrum which showed clearly the split AB quartet pattern characteristic of the C-4 and C-5 protons [2] with loss of the C-1 proton at δ 4·9. Clearly, lipiferolide is the 4,5-epoxide. The stereochemistry at carbons 1, 4, 5 and 10 of epitulipinolide was assigned on the basis that the solution conformation of epitulipinolide as in (**13**) is similar to that of costunolide. It is supported by the CD peak at 222 nm ($[\theta]$ + 146000) the one peak observed (instrument limitations) of the two having their origin in the exciton splitting of the transannularly interacting $\pi - \pi$ orbitals[6–8] when the double bonds are "crossed". The conformation of costunolide ($[\theta]_{220}$ + 110000) is supported by X-ray analysis of the silver ion adduct [9] in the solid state and by Nuclear Overhauser Effect studies [10] in solution. N.O.E. experiments [8] with the germacranolide, laurenobiolide (isomeric with tulipinolide, but with a 7,8-*trans*-lactone) revealed the most stable conformation to be also the "crossed" diolefin with methyl groups *syn*. As a consequence, diepoxide formation results in the *R* configuration at carbons 1, 4, 5 and 10. Subsequent work revealed epitulipinolide diepoxide to be a natural product with cytotoxic activity.



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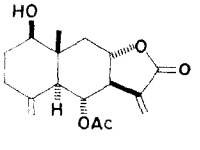

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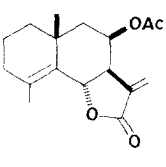

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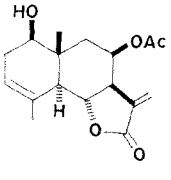

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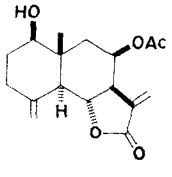

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Chromatography of the root bark extract produced epitulipdienolide (**7**) and γ-liriodenolide (**6**). Epitulipdienolide showed spectral characteristics

for an α-methylene-γ-lactone, an acetate and olefinic functions. The conjugated exocyclic methylene protons appeared in the NMR spectrum as a pair of doublets at δ 5·50 (J 3·0 Hz) and 6·18 (J 3·2 Hz) while the other olefinic protons gave non-first order patterns between δ 4·4 and 6·2. A one-proton triplet at δ 4·6 (J 11·3 Hz) for the C-6 proton, a doublet at δ 2·46 (J 11·3 Hz) for the C-5 proton and a doublet of quartets at δ 2·83 (J 11·3, 3·2, 3·0 and 2·2 Hz) for the C-7 proton suggested that the C-5, C-6 and C-7 protons were axially and the C-8 proton was equatorially disposed, in agreement with a stereochemistry established for epitulipinolide (**3**) at these positions.

Confirmation of the spectral assignments was made by preparation of *iso*epitulipdienolide (**14**) by base hydrolysis of epitulipdienolide (**7**) followed by lactone formation under acid conditions and then acetylation. Notable changes in consonance with structural changes included the downfield shift of the C-6 proton pattern, now a pair of doublets, to δ 5·28 (J 10·6 and 9·3 Hz) and the upfield shift of the C-8 proton multiplet from δ ~ 5·7 to δ 4·67.

Conversion of epitulipinolide (**3**) to the diene (**7**) under conditions of the Cope rearrangement (refluxing *n*-propanol for 24 hr) established its structure in total including the stereochemistry at C-10, on the basis that a *trans*-1,2-divinyl product is formed from a *trans, trans*-cyclodeca-1,5-diene system [11]. Epitulipinolide (**3**) has *trans*-1,10 and *trans*-4,5 stereochemistry on the basis of the conversions of epitulipinolide (**3**) to laurenobiolide via tulipinolide (**2**) [3] and laurenobiolide to pyrethrosin [12], which had its absolute stereochemistry established by X-ray crystallography [13]. Epitulipdienolide (**7**) does not appear to be an artifact of isolation as conditions for plant drying, extraction and subsequent purification did not exceed 40° while the Cope rearrangement required a temperature of at least 97°. Also epitulipinolide (**3**) in refluxing ethanol at 78° yielded only the starting material.

The second terpene from the root bark, γ-liriodenolide (**6**), exhibited spectral characteristics (IR, UV, NMR and MS) of an α-methylene-γ-lactone, an acetate ester and an hydroxyl group. Analytical and mass spectral data supported the molecular formula $C_{17}H_{22}O_5$. The NMR spectrum also revealed the presence of a vinyl methyl group as a broadened singlet at δ 1·91 and patterns that fit portions of the eudesmanolide structure (**6**); a multiplet at δ 5·76 (J 3·6, 2·6 and 2·4 Hz) for the C-8 proton, a broadened doublet at δ 5·13 (J 11·6 Hz) for the C-6 proton, and a broadened pair of doublets at δ 3·57 (J 8·4 and 6·6 Hz) for the C-1 proton. The positions and patterns for the C-1 and C-6 protons were similar to those observed respectively for the equivalent protons in β-cyclopyrethrosin (**15**) [14] and γ-cycloepitulipinolide (**16**) [2]. Ludalbin, a C-1 α-hydroxy eudesmanolide (*S* configuration) showed a very different pattern for the C-1 proton [15].

Confirmation of the structure of γ-liriodenolide (**6**) was obtained by synthesis as one of the three isomeric crystalline cyclo products, α-(**17**), β-(**18**) and γ-liriodenolide (**6**), from epitulipinolide-1,10-epoxide (**12**). The assignment of the hydroxyl group at C-1 as β (*R*) in the cyclo products from the values of the coupling constants for the C-1 proton to the C-2α and C-2β protons lends further support to the assigned stereochemistry at C-1 and C-10 in the starting material. A logical biosynthetic precursor for γ-liriodenolide (**6**) is epitulipinolide-1,10-epoxide (**12**) but a search in the expected fractions did not reveal its presence suggesting it either is not a biosynthetic intermediate or its steady state concentration is too low to be detected by our method.

EXPERIMENTAL

Mps are uncorrected. UV spectra were determined in MeOH. NMR spectra were measured at 60 MH_2 with TMS added as internal standard. Elemental analyses were by courtesy of A. H. Robins Co., Richmond, Va. Si gel G (Merck) used for column chromatography was TLC grade material which was mixed with water, dried, powdered, seived (50 mesh) and activated before use. This method allowed direct transfer of TLC solvent systems to column work. TLC detection reagents were 1% $KMnO_4$ or H_2SO_4–Et_2O (4:1) followed by heating at 110°.

Isolation of lipiferolide (**4**) *and epitulipinolide diepoxide* (**5**). The dried and powdered leaves (2 kg) of *Liriodendron tulipifera* L. collected in Ohio. Pennsylvania and Mississippi were exhaustively extracted by percolation with 95% EtOH. The residue (343 g) remaining after evaporation of the solvent at red pres and at 40° was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ residue (100 g) was partitioned between hexane and 10% aq MeOH to give MeOH soluble material (75 g). Chromatography of the MeOH fraction (20 g) on silicic acid (Mallinckrodt) or Si gel 60 (Merck-Darmstadt) (1·5 kg) with $CHCl_3$ as eluting solvent gave 2·5 g of residue from the first band. Partition chromatography [16] of this material on a column of Celite 545 (200 g) with $HCONH_2$ (120 ml) as stationary phase and C_6H_6–hexane (2:1) as mobile phase yielded crude lipiferolide (700 mg). Chromatography of this fraction on Si gel G (200 g) eluted with Et_2O–$CHCl_3$–C_6H_6 (3:1:1) produced a crystalline product (430 mg) which was recrystallized from

EtOH–hexane to give lipiferolide (**4**): mp 118–119°, $[\alpha]_D^{22}$ − 125° (*c*, 0·060 in MeOH): UV: end abs. 210 nm, log ε = 4·07; IR ($CHCl_3$) ν_{max}: 1770, 1745, 1660, 1245 and 1230 cm^{-1}; NMR ($CDCl_3$): δ 6·38 (1H, *d*, *J* 3·6 Hz C-13), 5·73 (1H, *d*, *J* 3·2, C-13), 5·72 (1H, *m*, C-8), 5·32 (1H, *br m*, C-1), 4·40 (1H, *t*, *J* 8·2, C-6), 3·18 (1H, *m*, C-7), 2·84 (1H, *d*, *J* 8·2, C-5), 2·07 (3H, *s*, Ac), 1·77 (3H, *br s*, C-15) and 1·38 (3H, *s*, C-14); MS: *m/e* 264·1372 (0·1%, M–CH_2CO, $C_{15}H_{20}O_4$ requires 264·1362), 246·1276 (0·7%, M–$MeCO_2H$, $C_{15}H_{18}O_3$ requires 246·1256) and 43 (100%, MeCO) (*Anal.* Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24. Found: C, 66·50; H, 7·18%).

The band following the lipiferolide fraction from the silicic acid column chromatography was further chromatographed on Si gel G (20 g for each 0·45 g of material) and eluted with 3·5% EtOH in $CHCl_3$. The first fractions yielded an oil (0·29 g) that on several crystallizations from EtOH–Et_2O gave 54 mg of epitulipinolide diepoxide (**5**): mp 214–215°; $[\alpha]_D^{25}$ − 55·7° (*c*, 0·525 in $CHCl_3$); UV: end abs. 210 nm, log ε = 3·98; IR ($CHCl_3$) ν_{max}: 1770, 1745, 1660 and 1245 cm^{-1}; NMR ($CDCl_3$): δ 6·38 (1H, *d*, *J* 3·4, C-13), 5·72 (1H, *d*, *J* 3·2, C-13), 5·7 (1H, *m*, C-8), 4·47 (1H, *t*, *J* 8·4, C-6), 2·99 (1H, *d*, *J* 8·4, C-5), 2·09 (3H, *s*, Ac), 1·44 (3H, *s*, C-15) and 1·38 (3H, *s*, C-14). Found: C, 63·16; H, 6·86. Calc. for $C_{17}H_{22}O_6$: C, 63·34; H, 6·88%.

Deacetyllipiferolide (**8**). A sample (190 mg) of lipiferolide (**4**) was stirred overnight in a soln of KOH (130 mg) in H_2O (10 ml) at ambient temp. The soln was acidified to pH 1–2 with 20% aq H_2SO_4 then treated with NaCl to satn and stirred 1 hr. The coagulated product was extracted with $CHCl_3$ and the extract washed with 1% aq $NaHCO_3$ and H_2O, dried over anhyd Na_2SO_4 and evaporated to leave an oily residue (115 mg) that gave from EtOH–*i*-Pr_2O 54 mg of deacetyllipiferolide (**8**) as feathery needles: mp 162–163°; $[\alpha]_D^{25}$ −295° (*c*, 0·20 in MeOH); IR (KBr) ν_{max}: 3500, 1740 and 1650 cm^{-1}; NMR (Pyr-d_5): δ 6·94 (1 H, *d*, *J* 4·6, OH, lost in D_2O), 6·46 (1H, *d*, *J* 3·6, C-13), 5·74 (H, *d*, *J* 3·1, C-13), 5·30 (1 H, *m*, C-1), 5·03 (1 H, *dd*, *J* 8·7 and 7·4, C-6), 4·8 (1 H, *m*, C-8), 3·27 (1 H, *m*, C-7), 3·03 (1 H, *d*, *J* 8·7, C-5) (Found: C, 68·21; H, 7·49. Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63%).

(11*S*)-11,13-*Dihydrolipiferolide* (**10**). Lipiferolide (**4**) (100 mg) in abs EtOH (5 ml) was treated with $NaBH_4$ (20 mg) for 30 min with stirring. After acidification to pH 5 with 10% aq. AcOH and addition of H_2O (4 ml) the EtOH was removed by evaporation. The residue was taken up in $CHCl_3$ and the soln washed with H_2O, dried and evaporated to leave an oil (92 mg) which showed two spots (R_f 0·60 and 0·20) on TLC with Si gel G and 8% EtOH in $CHCl_3$ as solvent. Column chromatography of the oil on the same absorbent (21 g) with $CHCl_3$ as solvent gave the R_f 0·60 material as an oil (74 mg) which was a single component by TLC and gave a clean NMR spectrum, but resisted crystallization. It was characterized as (11*S*)-dihydrolipiferolide (**10**): $[\alpha]_D^{25}$ − 121·8° (*c*, 0·64 in MeOH); IR ($CHCl_3$) ν_{max}: 1780, 1740 and 1250 cm^{-1}; NMR ($CDCl_3$): δ 5·38 (1 H, *br dd*, *J* 5·4 and 1·8, C-8), 5·3 (1 H, *br m*, C-1), 4·26 (1 H, *t*, *J* 8·5, C-6), 2·75 (1H, *d*, *J* 8·5, C-5), 2·13 (3 H, *s*, Ac), 1·76 (3 H, *br s*, C-15), 1·36 (3H, *s*, C-14) and 1·31 (3 H, *d*, *J* 5·5, C-13), the C-13 doublet was located at δ 1·16 in C_6D_6 and at δ 1·29 in Pyr-d_5; MS: *m/e* 308 (0·5%).

Elution of the column with 5% abs EtOH in $CHCl_3$ produced the more polar product (19 mg) which crystallized from EtOH–$CHCl_3$ and was characterized as (11*S*)-11,13-dihydrodeacetyllipiferolide (**19**): m.p. 166–167°; $[\alpha]_D^{25}$ −107° (*c*, 0·22 *in* MeOH); IR (KBr) ν_{max}: 3500 and 1735 cm^{-1}; NMR (Pyr-d_5): δ 6·78 (1 H, *d*, *J* 4·8, OH, lost in D_2O), 5·28 (1 H, *br m*, *J* 11, C-1), 4·78 (1 H, *t*, *J* 8·8, C-6), 4·43 (1 H, *br t*, *J* ~ 5, pattern changes to *br d* in D_2O, *J* 6, C-8), 2·94 (1 H, *d*, *J* 8·8, C-5), 2·01 (3 H, *br s*, C-15), 1·40 (3 H, *s*, C-14) and 1·29 (3 H, *d*, *J* 6·8, C-13) (Found: C, 67·57; H, 8·24 Calc. for $C_{15}H_{22}O_4$: C, 67·64; H, 8·33%).

(11*R*)-11,13-*Dihydrolipiferolide* (**11**). Lipiferolide (**4**) (100 mg) in abs EtOH (8 ml) was added to 5% Pd on C (20 mg) in abs EtOH (5 ml) presaturated with H_2 and hydrogenated at ambient temp. and atm. pres. Uptake of H_2 stopped in 12 min. Filtration through diatomaceous earth and evaporation left an oil (107 mg) that crystallized from *i*-Pr_2O–EtOH to give 51 mg of dihydrolipiferolide (**11**): mp 171–172°; $[\alpha]_D^{25}$ −82·7° (*c*, 0·29 in MeOH). IR ($CHCl_3$) ν_{max}: 1775 and 1745 cm^{-1}; NMR ($CDCl_3$): δ 5·38 (1 H, *br dd*, *J* 5·6 *and* 1·6, C-8), 5·35 (1 H, *br m*, C-1), 4·48 (1 H, *t*, *J* 9·0, C-6), 2·79 (1 H, *d*, *J* 9·0, C-5), 2·12 (3 H, *s*, Ac), 1·72 (3 H, *br s*, C-15), 1·37 (3 H, *s*, C-14) and 1·18 (3 H, *d*, *J* 7·0, C-13) with the C-13 doublet at δ 0·93 in C_6D_6 and at δ 1·17 in Pyr-d_5 (Found: C, 66·21; H, 7·88 Calc. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·85%).

Conversion of lipiferolide (**4**) *to epitulipinolide diepoxide* (**5**). To a soln of 77 mg of lipiferolide (**4**) in CH_2Cl_2 (10 ml) was added *m*-chloroperoxybenzoic acid (85% tech. grade, 70 mg). After reacting for 16 hr at ambient temp. the soln was washed successively with 3% aq. Na_2SO_3, 5% aq. Na_2CO_3 and H_2O. The residue from the CH_2Cl_2 soln gave 72 mg of crystalline diepoxide (**5**) identical with the material isolated from *L. tulipifera*.

Conversion of epitulipinolide (**3**) *to epitulipinolide diepoxide* (**5**). A soln of 290 mg of epitulipinolide (**3**) in CH_2Cl_2 (10 ml) was treated, dropwise with *m*-chloroperoxybenzoic acid (1 g) in CH_2Cl_2 (20 ml). After several days, the soln was processed as described above. The product (274 mg) was identical with the diepoxide (**5**) isolated from the natural source.

Epitulipinolide 1,10-epoxide (**12**). Epitulipinolide (**3**) (290 mg) in CH_2Cl_2 (25 ml) was treated dropwise, while stirring, with *m*-chloroperoxybenzoic acid (260 mg) in CH_2Cl_2 (3 ml). After 15 min the soln was treated as described above and the residue was crystallized from abs EtOH–hexane to give the epoxide (**12**): mp 148–149°; $[\alpha]_D^{22}$ +28° (*c*, 0·06 in MeOH); UV: end abs 210 nm log ε = 4·36; IR ($CHCl_3$) ν_{max}: 1760, 1745, 1665 and 1230 cm^{-1}; NMR: ($CDCl_3$) δ 6·30 (1 H, *d*, *J* 3·4, C-13), 5·71 (1 H, *br d*, *J* 6, C-8), 5·58 (1 H, *d*, *J* 3·0, C-13), 5·37 (1 H, *br d* of AB *q*, *J* 9·8, C-5), 5·12 (1 H, *dd* of AB *q*, *J* 9·8 and 8·4, C-6), 2·09 (3 H, *s*, Ac), 1·90 (3 H, *d*, *J* 1·3, C-14) and 1·18 (3 H, *s*, C-15) (Found: C, 66·55; H, 7·27. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24%).

Isolation of epitulipdienolide (**7**) *and γ-liriodenolide* (**6**). The dried and powdered root bark (3·5 kg) of *L. tulipifera* collected in N. Carolina in 1968 was percolated with commercial hexane to exhaustion giving 60 g of residue. Chromatography of this residue (94 g) on silicic acid (Mallinckrodt) (1·5 kg) with C_6H_6 as initial solvent, and followed by increasing amounts of $CHCl_3$ in C_6H_6, and then MeOH in $CHCl_3$ gave from the $CHCl_3$–C_6H_6 (1:1) fraction a residue that yielded 214 mg of crystalline epitulipdienolide (**7**) from C_6H_6–hexane: mp 134–135°; $[\alpha]_D^{22}$ + 6° (*c*, 0284 in MeOH): UV: end abs. 210 nm, log ε = 4·07; IR ($CHCl_3$) ν_{max}: 1770, 1740, 1675, 1640 and 1210–1260 cm^{-1}; NMR ($CDCl_3$): δ 6·18 (1 H, *d*, *J* 3·2, C-13), 5·75 (1 H, *m*, C-8), 5·50 (1 H, *d*, *J* 3·0, C-13), 4·63 (1 H, *t*, *J* 11·3, C-6), 2·83 (1 H, *d q*, *J* 11·3, 3·2, 3·0 and ~ 3, C-7), 2·46 (1 H, *d*, *J* 11·3, C-5), 2·08 (3 H, *s*, Ac), 1·85 (3H, *m*, C-14) and 1·20 (3H, *s*, C-15); MS: *m/e* 290 (< 1%, M^+), 248 (3%, M–CH_2CO) and 230·1315 (56%, M–$MeCO_2H$, $C_{15}H_{18}O_2$ requires 230·1307) (Found: C, 70·63; H, 7·69. Calc. for $C_{17}H_{22}O_4$: C, 70·32; H, 7·64%).

The next more polar column fraction yielded 10·4 g of crystalline epitulipinolide (**3**). Elution with MeOH–$CHCl_3$ (1:50) gave a fraction that produced 604 mg of γ-liriodenolide (**6**) from Et_2O–hexane: mp 179–180°; $[\alpha]_D^{25}$ −4° (*c*, 0·252 in MeOH); IR ($CHCl_3$) ν_{max}: 3620, 3520, 1770, 1740, 1675, 1630 and 1210–1250 cm^{-1}; NMR ($CHCl_3$): δ 6·25 (1H, *d*, *J* 3·3, C-13), 5·76 (1H, *m*, C-8), 5·53 (1H, *d*, *J* 3·0, C-13), 5·13 (1H, *br d*, *J* 11, C-6), 3·57 (1H, *br dd*, *J* 8·4 and 6·6, C-1), 2·90 (1H, *dq*, *J* 11·6, 3·3, 3·0 and 2·4, C-7), 2·06 (3H, *s*, Ac), 1·91 (3H, *br s*, C-14), 1·7 (1H, *br s*, OH,

lost in D_2O) and 1·25 (3H, *s*, C-15); *br s*, C-14), 1·7 (1H, *br s*, OH, lost in D_2O) and 1·25 (3H, *s*, C-15); MS: *m/e* 306·1475 (2%, M^+, $C_{17}H_{22}O_5$ requires 306·1467), 289 (3%, M-OH) and 246 (73%, M-$MeCO_2H$) (Found: C, 66·59; H, 7·24. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24).

Iso*epitulipdienolide* (**14**). A sample (60 mg) epitulipdienolide (**7**) was suspended in H_2O (20 ml) containing KOH (270 mg) and stirred for 21 hr. The soln was acidified with 1N HCl, extracted with $CHCl_3$ and the $CHCl_3$ residue treated with dil ethanolic HCl for 2 hr. The residue, after solvent removal, was dissolved in $CHCl_3$, washed with dil aq $NaHCO_3$ and H_2O and the residue (65 mg) was acetylated with Ac_2O–Pyr. The product was crystallized from EtOH–hexane to give 29 mg of diene (**14**): m.p. 90–91°; $[\alpha]_D^{22}$ − 18° (*c*, 0·084 in MeOH); IR ($CHCl_3$) ν_{max}: 1775, 1750, 1675, 1640 and 1230 cm^{-1}; NMR ($CDCl_3$): δ 6·19 (1H, *d*, *J* 1·4, C-13), 5·58 (1H, *d*, *J* 1·4, C-13), 5·28 (1H, *dd*, *J* 10·6 and 9·3, C-6), 4·7 (1H, *m*, C-8), 3·0 (1H, *m*, C-7), 2·21 (1H, *d*, *J* 10·7, C-5); 2·08 (3H, *s*, Ac), 1·70 (3H, *m*, C-14) and 1·18 (3H, *s*, C-15); MS: *m/e* 290 (0·6%, M^+) and 230·1308 (17%, M–MeCOOH, $C_{15}H_{18}O_2$ requires 230·1307) (Found: C, 70·45; H, 7·65. Calc. for $C_{17}H_{22}O_4$: C, 70·32; H, 7·64%).

Cope rearrangement of epitulipinolide (**3**). Epitulipinolide (**3**) (458 mg) was refluxed in *n*-PrOH (10 ml) under N_2 for 24 hr. The residue was chromatographed on a partition column [16] made from Celite 545 (100 g) with $HCONH_2$ (60 ml) as stationary phase and C_6H_6–hexane (1:15) as mobile phase. The Cope product (**7**) was eluted first to give crystals (23 mg) from C_6H_6–hexane, mp 133–134° identical (IR, NMR, mmp and TLC) with the natural product, epitulipdienolide (**7**). Later fractions contained epitulipinolide (383 mg).

Cyclization of epitulipinolide-1,10-*epoxide* (**12**). 500 mg compound (**12**) in $CHCl_3$ (5 ml) was treated with $CHCl_3$ (1 ml) through which HCl gas was bubbled for 30 sec. After 15 min the soln was dil. with $CHCl_3$ and extracted with 1% aq. $NaHCO_3$ and H_2O. The dried soln yielded the cyclized mixture (509 mg) which was dissolved in a few drops of $CHCl_3$ and dil with warm *i*-Pr_2O (2 ml) to deposit on cooling 84 mg of γ-liriodenolide (**6**) as silky needles, mp 178–179°. The substance was identical (IR, NMR, TLC, mmp and $[\alpha]_D$) with the natural product. TLC of the non-crystalline residue on 5% aq. $AgNO_3$–Si gel G developed with Et_2O showed 3 spots (R_f 0·50, 0·40 and 0·12) corresponding to γ-(**6**), α-(17) and β-lipiferolide (**18**) respectively. The starting material has R_f 0·20 and was not present.

A sample (300 mg) of the mother liquor residue in Et_2O (2 ml) was separated on a column of 5% $AgNO_3$–Si gel G (14 g). Effluent fractions, monitored by TLC, yielded first γ-liriodenolide (37 mg), then an oil (107 mg) that crystallized from Et_2O–*i*-Pr_2O to give 59 mg of α-liriodenolide (**17**): m.p. 74–75; $[\alpha]_D^2$ + 42° (*c*, 0·45 in MeOH); IR ($CHCl_3$) ν_{max}: 3620, 3520, 1740, 1680, 1605 and 1210–1250; NMR ($CDCl_3$): δ 6·19 (1H, *d*, *J* 3·3, C-13), 5·76 (1H, *m* C-8), 5·47 (1H, *d*, *J* 3·0, C-13), 5·40 (1H, *br m*, C-3), 4·47 (1H, *t*, *J* 11·0, C-6), 3·69 (1H, *br t*, C-1, in D_2O forms *dd*, *J* 9·0 and 6·8), 2·78 (1H, *dq*, *J* 11·0, 3·3, 3·0 and 3, C-7), 2·07 (3H, *s*, Ac), 1·89 (3H, *br s*, C-14), 1·8 (1H, *br s*, OH, lost in D_2O) and 1·07 (3H, s, C-15); MS: *m/e* 263·1281 (0·7%, M-MeCO, $C_{15}H_{19}O_4$ requires 263·1283) and 246 (3%, M-$MeCO_2H$) (Found: C, 66·86; H, 7·33. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24%).

Continued elution of the column with ether gave an oil (19 mg) rich in β-isomer, which with the original mother liquor residue (290 mg) was rechromatographed first on 5% $AgNO_3$ impregnated Si gel G as before and the oily β-isomer fraction then separated on Si gel G with Et_2O as solvent to give 27 mg of pure β-liriodenolide (**18**) from *i*-Pr_2O: mp 119–120°; $[\alpha]_D^{25}$ + 53° (*c*, 0·44 in MeOH); IR ($CHCl_3$ ν_{max}: 3610, 3510, 1770, 1740, 1675, 1650 and 1210–1250 cm^{-1}; NMR ($CDCl_3$): δ 6·18 (1H, *d*, *J* 3·3, C-13), 5·74 (1H, *m*, C-8), 5·47 (1H, *d*, *J* 3·0, C-13), 5·04 (1H, *br s*, C-14), 4·97 (1H, *br s*, C-14), 4·55 (1H, *t*, *J* 11·0, C-6), 3·53 (1H, *dd*, *J* 10·2 and 5·2, sharpens in D_2O, C-1), 2·81 (1H, *d q*, *J* 11·0, 3·3, 3·0 and ~ 3, C-7, 2·07 (3H, *s*, Ac), 1·67 (1H, *s*, OH, lost in D_2O) and 0·98 (3H, *s*, C-15); MS: *m/e* 306·1461 (0·1%, M^+, $C_{17}H_{22}O_5$ requires 306·1467) (Found: C, 66·50; H, 7·25. Calc. for $C_{17}H_{22}O_5$: C, 66·65; H, 7·24%).

A small quantity of a fourth more polar product (R_f 0·10) was obtained as cubic crystals mp 189–190·5° from Et_2O–*i*-Pr_2O and its spectral properties were suggestive of a hydrated cyclo product with OH at C-4, but insufficient material prevented its full characterization.

The cyclo products could also be separated by partition chromatography; 625 mg of mixture on a column of Celite (100 g) with $HCONH_2$ (60 ml) as stationary phase, and C_6H_6 as mobile phase gave the same order of elution as for the adsorption column but recovery was poor.

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