The Structure of Tulipinolide and Epitulipinolide. Cytotoxic Sesquiterpenes from *Liriodendron tulipifera* L.[1]

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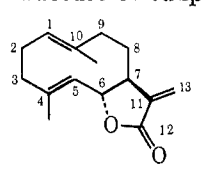
The structures for two new sesquiterpenes, tulipinolide (**2**) and epitulipinolide (**3**), were shown to be 8α-acetoxy-6(βH),7(αH)-germacra-1(10)-*trans*,4(5)-*trans*,11(13)-trien-6,12-olide and the C-8 epimer, respectively, by cyclization to conformationally rigid decalin products and their study by physical methods. Eupatoriopicrin must be revised to **38** on the basis of yielding degradation products identical with those obtained from epitulipinolide. The application of the α-phenylbutyric anhydride (Horeau) method for establishing the configuration of the hydroxyl at C-8 in deacetylepitulipinolide (**26**), deacetyldihydro-β-cyclotulipinolide (**13**), and deacetyldihydro-β-cycloepitulipinolide (**30**) gave anomalous results, but Brewster's benzoate method yielded values consistent with the configuration assignments made from nmr studies.

We had recently reported the isolation of two cytotoxic substances, costunolide (**1**) and a new sesquiterpene, tulipinolide $C_{17}H_{22}O_4$, mp 181° dec, from *Liriodendron tulipifera* L.[2] Evidence is presented here for the structure of tulipinolide (**2**) and epitulipinolide



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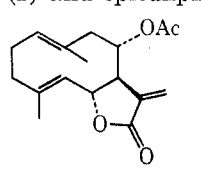

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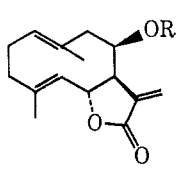
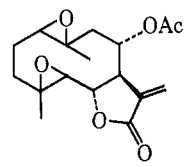


3, R = Ac
26, R = H



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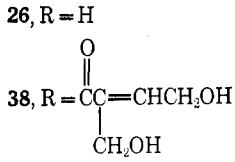
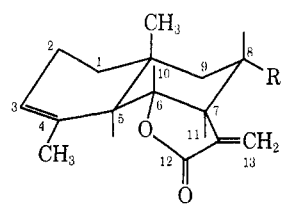


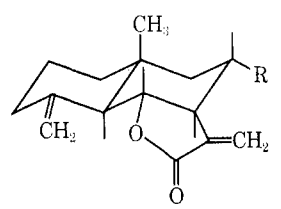
38, R = CC(=O)C=CHCH$_2$OH, CH$_2$OH

(**3**), a third cytotoxic[3] germacranolide from the same source. The nmr peaks for the substances to be discussed are in Table I.

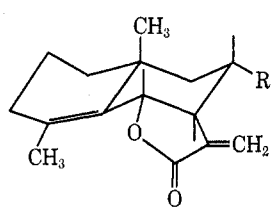
The presence in tulipinolide of an acetate and an α,β′-unsaturated γ-lactone function was previously established.[2] The gross germacranolide structure was indicated by the functional groups as interpreted from the nmr spectrum and supported by the isolation of levulinic acid on ozonolysis and of the diepoxide **4** on peroxidation. Cyclization of tulipinolide (**2**) afforded α-(**5**) and β-cyclotulipinolide (**6**), but surprisingly the γ isomer (**7**) was not detected. These "rigid" decalin compounds allowed the use of nmr to establish their structure and configuration. The stereochemistry at carbons 6, 7, and 8 in the β isomer **6** was determined in this way, for the H_6 proton appears as a triplet at δ 4.07, the X of an ABX pattern (J_{BX} = J_{BX} = 10.9



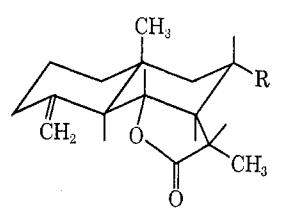
5, R = OAc
8, R = H



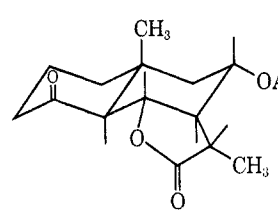
6, R = OAc
9, R = H



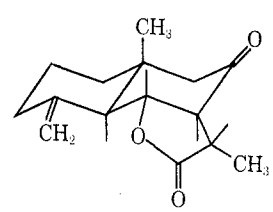
7, R = OAc
10, R = H



11, R = OAc
13, R = H



12



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Hz), and the large coupling constants support axial positioning of the H_5, H_6, and H_7 protons. The H_8 proton found at δ 5.23 was placed axial on the basis of the six-peak pattern, the X of an ABMX (J_{AX} = J_{MX} = 10.7 and J_{BX} = 4.5 Hz) system, the result of interaction of the neighboring two axial and one equatorial protons. The spectral interpretations were aided by comparison with the costunolide cyclized products, α-**8**, β-**9**, and γ-**10**.[4]

The *trans*-decalin system for **6** was established by ozonolysis of dihydro-β-cyclotulipinolide (**11**) to the ketone **12** which exhibited a strong negative Cotton effect peak in the CD at 290 mμ ([Θ] −4670), a result predictable from the octant rule.[5] The C_{11} methyl

(1) Antitumor Agents. IV. Previous paper: R. W. Doskotch and C. D. Hufford, *J. Org. Chem.*, **35**, 486 (1970). This investigation was supported by Public Health Service Research Grant No. CA-08133 from the National Cancer Institute and No. FR-03328 from Special Research Resources for purchase of the nmr spectrometer (Varian A-60A) and accessories. Taken in part from the Ph.D. Thesis of F. S. E., June 1969.

(2) R. W. Doskotch and F. S. El-Feraly, *J. Pharm. Sci.*, **58**, 877 (1969).

(3) Tested in the KB cell culture assay through the courtesy of the Cancer Chemotherapy National Service Center (CCNSC) according to the method in *Cancer Chemother. Rep.*, **25**, 22 (1962). Tulipinolide and epitulipinolide showed ED_{50} values of 0.46 and 2.1 μg/ml, respectively.

(4) The α isomer was first obtained crystalline by G. H. Kulkarni, G. R. Kelkar, and S. C. Bhattacharya, *Tetrahedron*, **20**, 2639 (1964), and recently T. C. Jain and J. E. McCloskey, *Tetrahedron Lett.*, 2917 (1969), reported the properties of the β isomer.

(5) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p 178. For studies of ketones in the eudesmane series, see D. C. Humber, A. R. Pinder, and S. R. Wallis, *J. Chem. Soc., C*, 2941 (1968).

group in **11** was assigned as α on the basis that the methine proton H_{11} appeared as a six-peak multiplet, the result of the overlap (J = 12.0 Hz) of a pair of quartets (J = 6.7 Hz). In addition, the C_{11} methyl group showed an upfield shift of only 0.09 ppm in deuteriobenzene relative to deuteriochloroform. These two conditions are in accord with observations on other *trans*-fused γ-lactones bearing a pseudoaxial H_{11} proton.[6]

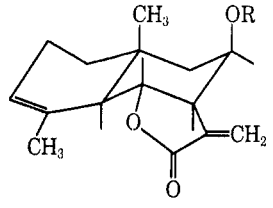
Saponification of dihydro-β-cyclotulipinolide (**11**) produced the alcohol **13**. That no other changes had occurred during hydrolysis was shown by the regeneration of the starting material on reacetylation. Oxidation of the alcohol **13** gave the ketone **14** that gave a negative Cotton effect curve ([θ] −8560 at 290 mμ), as predicted by the use of the octant rule. Other spectral (nmr and ir) features of these derivatives were in agreement with the assignment of the functional groups and their locations in this series.

In repeat collections of plant material tulipinolide was not present; yet the column fraction from which it was originally obtained did not appreciably decrease. The difference was due to the presence of another compound which could not be distinguished from tulipinolide by any number of chromatographic methods yet was separated from it by crystallization from petroleum ether–ethanol mixtures. This new substance, epitulipinolide (**3**), mp 91–92°, $[\alpha]_D$ +76°, was shown by the following evidence to be epimeric to tulipinolide at position 8.

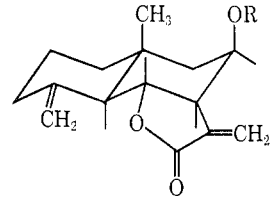
The spectral properties (ir, uv, mass spectrum, and nmr) of epitulipinolide (**3**) were similar to those of tulipinolide. A notable difference was the position of a one-proton multiplet in the nmr at δ 5.72 due to H_8 which in tulipinolide is located between δ 4.8 and 5.2 as part of a four-proton (H_1, H_5, H_6, and H_8) envelope. The corresponding region in the epitulipinolide spectrum was consequently much simplified and now analyzable by first-order methods, the results of which are found in Table I.

Cyclization of epitulipinolide produced the isomers α-(**15**), β-(**16**), and γ-cycloepitulipinolide (**17**), readily characterized by their nmr spectra. The major difference in the spectra between the β isomers of cyclotulipinolide and cycloepitulipinolide occurs for the H_8 multiplet. The six-peak pattern at δ 5.23 in the former is replaced by a four-peak signal at δ 5.71, the X of an ABMX pattern where the three J values are about 3 Hz. This is the consequence of the interaction of an equatorial proton with the neighboring two axial and one equatorial protons. A change in the pattern for the H_7 absorption from a trio of triplets (J values of 2.9, 3.1, 10.7, and 10.9) to a pair of quartets (J values of 3.0, 3.0, 3.3, and 11.0) was a predictable consequence. The downfield chemical shift and the change in coupling constants for H_8 was as anticipated in going from an axial to an equatorial proton.[7]

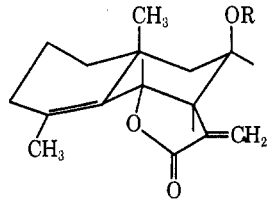
Hydrogenation of β-cyclopitulipinolide (**16**) afforded the dihydro derivative **18** which on treatment with sodium methoxide gave deacetyldihydro-β-cycloisoepitulipinolide (**19**). That the lactone ring was reclosed to C_8 was evidenced by the sharpening of the ABX triplet (originally the lactonic proton in the starting material **18**) at δ 3.68 (J = 10.5, 10.5 Hz) after treatment with D_2O and formation of dihydro-β-cycloisoepitulipinolide (**20**) on acetylation. In addition, the triplet for the H_6 proton of **19** was lost in forming the oxidation (Moffatt reagent[8]) product **21**.



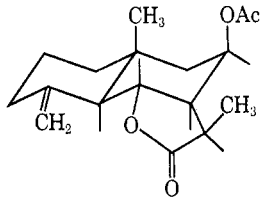
15, R = Ac
27, R = H



16, R = Ac
28, R = H



17, R = Ac
29, R = H



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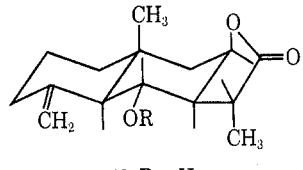
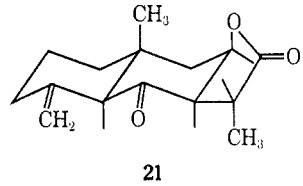
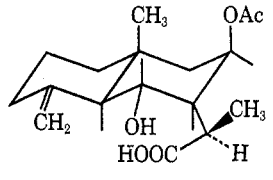


19, R = H
20, R = Ac



21

Hydrolysis of **18** under milder conditions (sodium carbonate) easily opened the lactone ring but left the acetate group intact to give the hydroxy acid **22**. A similar lactone opening was possible with γ-cycloepitulipinolide (**17**) to form the hydroxy acid **23**.



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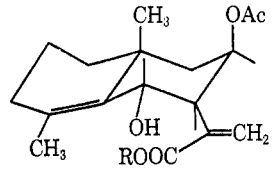
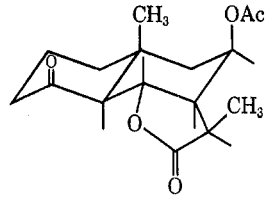


23, R = H
24, R = CH_3



25

Oxidation of its methyl ester **24** to the corresponding α,β-unsaturated ketone was not successful with man-

(6) C. R. Narayanan and N. K. Venkatasubramanian, *J. Org. Chem.*, **33**, 3156 (1968).

(7) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, Inc., New York, N. Y., 1969, pp 77 and 132.

(8) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **87**, 5661, 5670 (1965), and references therein.

TABLE I

NMR PEAKS OF COSTUNOLIDE, TULIPINOLIDE, EPITULIPINOLIDE, AND DERIVATIVES[a]

Compound	C_4 —Me or =CH_2	C_{10} —Me	H_6	H_7	H_8	C_{11} =CH_2 or —Me	Miscellaneous
1	1.70 (br)	1.42 (br)	4.56[b]	2.55 (brm)		5.55 (d, 3.0)	4.85 (brd, 10.0, H_6)
						6.23 (d, 3.5)	
2	1.71 (br)	1.58 (br)		3.08 (brm)		5.84 (dd, 1.5, 3.0)	2.08 ($COCH_3$)
						6.34 (dd, 1.5, 3.5)	4.8–5.2 (H_1, H_5, H_8, and H_9)
3	1.76 (d, 1.3)	1.52 (br)	5.13[c]	2.93 (brm)	5.72 (m)	5.59 (d, 3.1)	2.06 ($COCH_3$)
						6.28 (d, 3.5)	4.78 (dd, 1.3, 10.0, H_6)
4	1.42	1.42	4.6 (m)		4.6 (m)	5.71 (d, 3.0)	2.00 ($COCH_3$)
						6.32 (d, 3.5)	
5	1.87 (br)	0.99	3.98 (dd, 10.8, 11.3)	2.80 (m)	5.30 (m,[d] 4.6, 10.6, 10.7)	5.51 (d, (2.9)	2.13 ($COCH_3$)
						6.11 (d, 3.1)	5.43 (m, H_3)
6	4.85 (br)	0.93	4.07 (dd, 10.9, 10.9)	2.86 (m)	5.23 (m,[d] 4.5, 10.7, 10.7)	5.55 (d, 2.9)	2.13 ($COCH_3$)
	4.99 (br)					6.13 (d, 3.1)	
8	1.85 (br)	0.92	3.88 (dd, 10.0, 11.5)	2.46 (m)		5.38 (d, 3.0)	5.4 (brm, H_3)
						6.05 (d, 3.0)	
9	4.79 (br)	0.85	3.95 (dd, 10.5, 10.5)	~2.6 (m)		5.38 (d, 3.0)	
	4.92 (br)					6.03 (d, 3.2)	
10	1.88 (br)	1.12	4.55 (brd, 11)	2.6 (m)		5.43 (d, 3.0)	
						6.13 (d, 3.3)	
11	4.80 (br)	0.93	4.08 (dd, 10.6, 10.6)		5.15 (m[d] 4.5, 10.6, 10.6)	1.24 (d, 6.7)	2.08 ($COCH_3$)
	4.97 (br)					1.15 (d)[e]	2.59 (dq, 6.7, 12.0, H_{11})
12		0.95	4.18 (dd, 10.6, 10.8)		5.11 (m)[d] 4.6, 10.6, 10.6)	1.23 (d, 6.8)	2.10 ($COCH_3$)
13	4.78 (br)	0.88	4.02 (dd, 10.6, 10.6)		3.98 (m)[f]	1.39 (d, 7)	1.97 (d, 5, OH[g])
	4.96 (br)						2.58 (dq, 7, 12, H_{11})
14	4.88 (br)	0.84	4.15 (dd, 11, 11)			1.28 (d, 6.5)	
	5.04 (br)						
15	1.89 (brd, 1.5)	1.08	4.40 (dd, 11.0, 11.0)	2.80 (m)	5.70 (m)[h]	5.44 (d, 3.1)	2.05 ($COCH_3$)
						6.15 (d, 3.3)	5.42 (br, H_3)
16	4.92 (br)	1.01	4.51 (dd, 11.0, 11.0)	2.88 (m)	5.71 (m)[h]	5.46 (d, 3.0)	2.06 ($COCH_3$)
						6.18 (d, 3.3)	
17	1.90	1.27	5.11 (brd, 12)	2.92 (m)	5.72 (m)[h]	5.51 (d, 3.1)	2.07 ($COCH_3$)
						6.23 (d, 3.4)	
18	5.04 (br)	1.03	4.91 (dd, 11.4, 11.4)		5.65 (m)[h]	1.29 (d, 7.6)	2.11 ($COCH_3$)
	5.15 (br)						2.83 (dq,[i] 7.6, 7.8, H_{11})
19	4.67 (br)	0.88	3.67 (dd, 8.5, 11.0)		4.80 (m,[d] 2.5, 4.5, 4.5)	1.35 (d, 7.5)	2.67 (br, OH[g])
	5.03 (br)						2.93 (q, 7.5, H_{11})
20	4.43	0.91	5.08 (dd, 9.0, 11.4)		4.78 (m,[d] 2.1, 4.8, 4.8)	1.23 (d, 7.6)	2.02 ($COCH_3$)
	4.83						2.9 (q, 7.6, H_{11})
21	5.07 (br)	0.86		2.83 (brd, 6.3)	5.10 (m,[d] 2.5, 4.5, 6.3)	1.31 (d, 7.8)	3.06 (br, H_5)
	5.91 (br)						3.46 (brq, 7.8, H_{11})
22	4.77 (br)	0.87	4.35 (dd, 10.5, 10.5)		5.29 (m)[h]	1.26 (d, 7.3)	1.98 ($COCH_3$)
	5.02 (br)						3.02 (m, H_{11})
							6.70 (br,[g] OH and COOH)
23	1.95 (br)	1.23	5.00 (brd, 11)	3.17 (dd, 4.0, 11.2)	5.17 (m)	5.82 (br)	2.00 ($COCH_3$)
						6.52 (br)	6.67 (br,[g] OH and COOH)
24	1.96 (br)	1.23	5.02 (brd, 11)	3.17 (dd, 4.0, 11.2)	5.15 (m)	5.73 (br)	1.98 ($COCH_3$)
						6.38 (br)	2.1 (br,[g] OH)
							3.78 (OCH_3)
25		1.01	4.82 (dd, 11.0, 11.0)		5.43 (m)[h]	1.21 (d, 7.5)	2.05 ($COCH_3$)
26	1.73 (d, 1.3)	1.63 (br)	5.27 (m)	2.8 (m)	4.6 (m)	5.58 (d, 3.1)	4.6 (m, H_1)
						6.21 (d, 3.5)	
26[j]	1.72 (d, 1.5)	1.69 (d, 1.0)	5.28[k]	2.9 (m)	4.7 (m)	5.65 (d, 3.2)	4.20 (d, 4.5,[g] OH)
							4.7 (m, H_1)
						6.19 (d, 3.5)	4.88 (dd, 1.5, 10.0, H_5)
28	4.88 (br)	1.10	4.58 (dd, 11.1, 11.1)	2.73 (m)	4.60 (m)	5.54 (d, 3.1)	2.23 (br,[g] OH)
	4.96 (br)					6.21 (d, 3.3)	
29	1.88 (br)	1.34	5.20 (brd, 11.4)	2.80 (m)	4.60 (m)	5.55 (d, 3.1)	1.9 (br,[g] OH)
						6.31 (d, 3.4)	
30	4.80 (br)	1.08	4.51 (dd, 10.8, 10.8)		4.28 (m)	1.20 (d, 6.8)	2.38 (br,[g] OH)
	4.91 (br)						2.76 (dq, 6.8, 12.8,[l] H_{11})
31	4.84 (br)	1.00	4.42 (dd, 10.8, 10.8)		5.30 (m)[h]	1.22 (d, 6.7)	2.10 ($COCH_3$)
	4.95 (br)					1.15 (d, 6.8)[e]	2.4 (m, H_{11})
32	5.0 (br, 2H)	1.18	5.22 (brd, 11.2)		5.0 (m)	1.87 (d, 1.8)	2.13 (br,[g] OH)

TABLE I
(*Continued*)

Compound	C_4—Me or $=CH_2$	C_{10}—Me	H_6	H_7	H_8	$C_{11}=CH_2$ or —Me	Miscellaneous
33	5.0 (br, 2H)	1.12	5.1 (brd, 11)		5.90 (dd, 2.2, 4.3)	1.98 (d, 1.9)	2.08 ($COCH_3$)
34	1.70 (d, 1.0)	1.63 (d, 0.5)	5.20[m]		4.35 (m)		2.75 (br,[g] OH) 3.38 (OCH_3) 4.72 (dd, 1.5, 10, H_8)
35	1.55 (br)	1.55 (br)					3.33 (OCH_3) 4.6–5.4 (m, H_1, H_8, H_6)
36	1.58 (br)	1.58 (br)	4.83[n]	4.08 (dt, 2.9, 3.2, 8.0)		5.48 (d, 2.9) 6.34 (d, 3.2)	2.89 (d, 10, H_9) 3.50 (d, 10, H_9) 5.15 (brd, 10.5, H_8) 5.2 (br, H_1)

[a] Spectra were determined as given,[25] with chemical shifts in δ (parts per million) and coupling constants shown in parentheses in hertz; singlets are unmarked, d = doublet, m = multiplet with center given, q = quartet, t = triplet, and br = broadened signal. [b] In the 100-MHz spectrum this proton appears as a pair of doublets of unequal height, the A of an ABX pattern where $J_{AB}/\Delta\nu_{AB} = 0.57$, $J_{AB} = 10.0$, and $J_{AX} = 8.0$. [c] As in *b* but at 60 MHz, $J_{AB}/\Delta\nu_{AB} = 0.48$, $J_{AB} = 10.0$, and $J_{AX} = 8.1$. [d] The multiplet is a split triplet, the X of an ABMX pattern. [e] In C_6D_6 as solvent. [f] Sharpens to a split triplet (J = 4.4, 10.2, and 10.2) after D_2O exchange. [g] Lost in D_2O. [h] A tight "quartet" pattern X of an ABMX where all J values are ~3. [i] A quintet formed by a pair of overlapping quartets stands out clearly in the 100-MHz spectrum. [j] Determined in acetone-d_6 because of greater solubility and more reliable δ and J values. [k] As in *c*, $J_{AB}/\Delta\nu_{AB} = 0.42$, $J_{AB} = 10.0$, and $J_{AX} = 8.0$. [l] Clearly seen as a six-peak pattern in the 100-MHz spectrum. [m] As in *c*, $J_{AB}/\Delta\nu_{AB} = 0.35$, $J_{AB} = 10$, and $J_{AX} = 9.0$. [n] As in *c*, $J_{AB}/\Delta\nu_{AB} = 0.35$, $J_{AB} = 10$, and $J_{AB} = 9.0$.

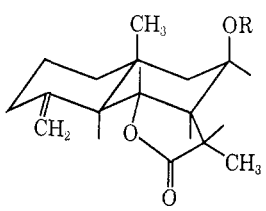
ganese dioxide[9] or Jones,[10] Sarett,[11] or Moffatt reagents.[8] Many reaction products were formed but no ketones were detected (ir). The formation of deacetyldihydro-β-cycloisoepitulipinolide (**19**) from dihydro-β-cycloepitulipinolide was further evidence for placing the C_8 substituent in the axial position, as the resulting *cis*-lactone is more stable than the *trans* isomer.[12]

The stereochemistry at C_{11} was established for deacetyldihydro-β-cycloisoepitulipinolide (**19**) from the presence of a one-proton quartet at δ 2.93 (J = 7.5 Hz) for H_{11}. Double irradiation experiments in which the C_{11} methyl doublet at δ 1.35 was saturated collapsed the quartet to a singlet. Since the coupling constant between H_7 and H_{11} is virtually zero, then H_{11} must be pseudoequatorial in the *cis*-lactone[6] and the C_{11} methyl group is therefore positioned α in **19** and β in dihydro-β-cycloepitulipinolide (**18**).

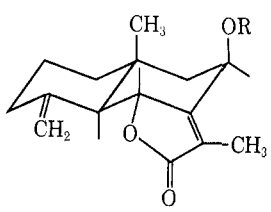
Dihydro-β-cycloepitulipinolide (**18**) was ozonized to the nor ketone **25** which exhibited, as predicted for a *trans*-decalin system, a negative Cotton effect curve at 290 mμ ([θ] −3870).

Since it was not possible to remove the acetate group from dihydro-β-cycloepitulipinolide (**18**) without changing the position of lactone closing, cyclization of deacetylepitulipinolide (**26**) was necessary if a common product was to be derived from the two tulipinolides. Treatment of epitulipinolide (**3**) with potassium hydroxide yielded the deacetyl compound **26**, mp 186–188°, $[\alpha]^{25}D$ +29.7°. The lactone ring was not altered, for reacetylation gave epitulipinolide. Apparently, the stability difference between *cis*- and *trans*-lactones in the conformationally less rigid germacranolide ring system is not so great as was observed in the eudesmanolide system.

Cyclization of deacetylepitulipinolide (**26**) produced the α-(**27**), β-(**28**), and γ-cyclo-(**29**) isomers of which only the latter two were obtained in pure form. The acetylation of the β isomer **28** yielded β-cycloepitulipinolide (**16**), establishing that the cyclization proceeds in a manner analogous to that for epitulipinolide (**3**). Deacetyl-β-cycloepitulipinolide (**28**) was hydrogenated over palladium-on-charcoal catalyst to give the dihydro compound **30**. The configuration of the newly established asymmetric center (C_{11}) was assigned from the presence of a well-defined sextet for H_{11} in the 100-MHz nmr spectrum and is the same as found for dihydro-β-cyclotulipinolide (**11**). Acetylation of **30** gave compound **31**, the C_{11} epimer of dihydro-β-cycloepitulipinolide (**18**). Apparently, in the hydrogenation the C_8 axial hydroxyl group does not introduce a significant steric hindrance relative to that of the H_8 proton in β-cyclotulipinolide (**6**) to influence the manner of hydrogen transfer, yet an acetate group as in **28** reverses the manner. A minor product of the hydrogenation of **28** was the isomer **32** in which the conjugated exocyclic methylene was converted into a vinyl methyl group. Reexamination of the reduction by-products from β-cycloepitulipinolide (**16**) hydrogenation revealed the presence of the corresponding isomeric acetate **33**. Migration of the double bond in this manner on a catalyst surface has been well established for other unsaturated γ-lactones, especially in the pseudoguaianolide series.[13]



30, R = H
31, R = Ac



32, R = H
33, R = Ac

(9) S. Ball, T. W. Goodwin, and R. A. Morton, *Biochem. J.*, **42**, 516 (1948).

(10) B. Tursch, I. S. deS. Guimaraes, B. Gilbert, R. T. Aplin, A. M. Duffield, and C. Djerassi, *Tetrahedron*, **23**, 761 (1967).

(11) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Amer. Chem. Soc.*, **75**, 422 (1953).

(12) G. H. Kulkarni, G. E. Kelkar, and S. C. Bhattacharyya, *Tetrahedron*, **20**, 2639 (1964); C. D. Hufford and R. W. Doskotch, unpublished results on studies connected with the *cis*-lactone pseudoguaianolide damsin.

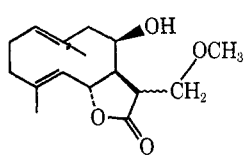
(13) Numerous examples can be found in the review by J. Romo and A. Romo de Vivar, *Progr. Chem. Org. Natur. Prod.*, **25**, 90 (1967).

Oxidation of dihydrodeacetyl-β-cycloepitulipinolide (**30**) with Jones reagent yielded a product identical with the ketone **14** obtained from β-cyclotulipinolide (**6**). It follows that epitulipinolide (**3**) differs from tulipinolide (**2**) in the stereochemistry at C_8 and the only remaining uncertainty in their structures was the stereochemistry of the two endocyclic double bonds. Snatzke and coworkers[14] have studied the CD characteristics of a series of germacranolides and concluded that the "optically active" absorption bands at about 220 and 200 mμ are due to the exciton splitting of the homoconjugated transannular double bonds. These in turn reflect the chirality of that system. Costunolide with two *trans* endocyclic double bonds[15] exhibited a positive Cotton effect curve at 220 mμ ([θ] +110,000), but the lower wavelength peak was not reached. Tulipinolide and epitulipinolide gave similar results with positive peaks at 221 ([θ] +121,000) and 222 mμ ([θ] +146,000), respectively. The two new germacranolides consequently possess two *trans* trisubstituted double bonds.[16] There was also observed in the CD a small negative Cotton effect curve at about 265 mμ for these three compounds. This absorption has been related recently to the stereochemical effect on the n → π* transition of the lactone carbonyl.[18] Our assignments are in complete agreement.

With the structures for tulipinolide and epitulipinolide thus established, it was possible to resolve a number of inconsistencies that appeared early in the study. Epitulipinolide (**3**) on hydrolysis with sodium methoxide gave the alcohol **34** which on oxidation afforded the ketone **35**. The conditions of the oxidation were the same as those used to convert deacetylepitulipinolide (**26**) into the corresponding ketone **36**, which showed no major uv absorption peaks above 210 mμ and which gave a positive Zimmerman's test. The three derivatives **26**, **34**, and **35** have physical properties (melting point, specific rotation, and characteristic bands in the ir and uv) like those of substances reported to be derived in a similar manner from eupatoriopicrin (**37**).[19] On the basis of our results the structure for eupatoriopicrin should be revised to **38** and the derivatives thereof changed accordingly.

In order to verify the stereochemical assignment of the acetate group in epitulipinolide by another procedure the Horeau "partial resolution" method[20] was employed and deacetylepitulipinolide (**26**) was esterified with α-phenylbutyric anhydride. The α-phenylbutyric acid isolated after acylation was negative in an optical yield of 39.7%, thus requiring that the C_8 carbon have an *S* configuration. Since the nmr and CD studies supported an *R* configuration, this unexpected result needed explanation. When the Horeau method was applied to deacetyldihydro-β-cyclotulipinolide (**13**) and deacetyldihydro-β-cycloepitulipinolide (**30**) the isolated acid was positive (32.8%) and negative (19.4%), respectively, and requiring again configurations opposite to previous assignments. Application of Brewster's benzoate method,[21] however, did yield results consistent with the initial designations, for the difference in molecular rotation between the benzoate of deacetyldihydro-β-cyclotulipinolide and the carbinol **13** was +176° (suggesting as *S* configuration) and that for the benzoate of deacetyldihydro-β-cycloepitulipinolide and **30** was −195° (suggesting an *R* configuration).

The Horeau method has been applied to a number of natural products[22] including some sesquiterpene lactones of the pseudoguaianolide series.[23] The correct configuration was obtained for these examples where assignment of relative bulk for groups on carbons adjacent to the hydroxyl-bearing carbon was made according to the established order: $R_3C- > R_2CH- > RCH_2-$. In cases where the shape of the entire molecule could influence the shielding of the hydroxyl, the simple rules break down. Examples of such cases have been considered[24] and to these can now be added the eudesmanolide alcohols **13** and **30**. The steric hindrance from the C_{10} methyl, though one methylene group away from the hydroxyl-bearing carbon (C_8), appears to be greater than from the adjacent trisubstituted carbon (C_7). For the more flexible deacetylepitulipinolide (**26**) the answer is less obvious, unless the C_{10} methyl group is considered to be held by a ring conformation in a manner which crowds the hydroxyl group. Studies with deacetyltulipinolide might have contributed to a better understanding of this anomaly, but, unfortunately, the paucity of starting material prevented the development of hydrolysis conditions to yield that product.



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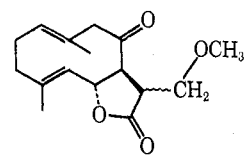

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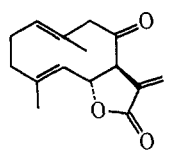

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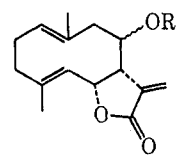
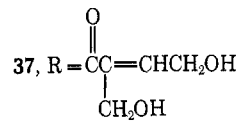




(14) G. Snatzke, *Riechs. Aromen, Koerperpflegem.*, **19**, 1 (1969); M. Suchy, L. Dolejs, V. Herout, F. Sorm, G. Snatzke, and J. Himmelreich, *Collect. Czech. Chem. Commun.*, **34**, 229 (1969).

(15) R. B. Bates and D. M. Gale, *J. Amer. Chem. Soc.*, **82**, 5749 (1960).

(16) A substance having the same general structure as tulipinolide [mp 180° dec, [α]D +249° (*c* 4.8, $CHCl_3$), and identical stereochemistry at C_6, C_7, and C_8] has been proposed for acetoxycostunolide [mp 98°, [α]D −37.4° (*c* 5.9, $CHCl_3$)], a crystalline acetate of an oily alcohol from *Artemisia balchanorum* H. Krasch.[17] The latter substance most probably differs from tulipinolide in the stereochemistry of the double bonds.

(17) M. Suchy, V. Herout, and F. Sorm, *Collect. Czech Chem. Commun.*, **28**, 1618 (1963).

(18) T. G. Waddell, W. Stocklin, and T. A. Geissman, *Tetrahedron Lett.*, 1313 (1969).

(19) L. Dolejs and V. Herout, *Collect. Czech. Chem. Commun.*, **27**, 2654 (1962).

(20) A. Horeau and H. B. Kagan, *Tetrahedron*, **20**, 2431 (1964).

(21) J. H. Brewster, *ibid.*, **13**, 106 (1961), and references therein, as well as M. Miyamoto, K. Morita, Y. Kawamatsu, Y. Kawashima, and K. Nakanishi, *ibid.*, **23**, 411 (1967).

(22) References to terpene and steroid examples are given in ref 23. A mold metabolite, caldariomycin, in which the hydroxyl is flanked by α-chloro groups was studied by S. M. Johnson, I. C. Paul, K. L. Rinehart, Jr., and R. Srinivasan, *J. Amer. Chem. Soc.*, **90**, 136 (1968).

(23) W. Herz and H. B. Kagan, *J. Org. Chem.*, **32**, 216 (1967).

(24) A. Marquet and A. Horeau, *Bull. Soc. Chim. Fr.*, 124 (1967).

Experimental Section[25]

Isolation of Tulipinolide (2) and Epitulipinolide (3).—The method for isolating tulipinolide (**2**), mp 181° dec, $[\alpha]^{28}D$ +260° (*c* 1.0, C_6H_6), $[\alpha]^{25}D$ +249° (*c* 4.8, $CHCl_3$), CD (*c* 0.046 and 0.0023, MeOH), 25°, $[\Theta]_{264}$ −4780, $[\Theta]_{221}$ +121,000, has been reported.[2] Epitulipinolide (**3**) was obtained in a similar manner from later collections of *Liriodendron tulipifera* L.,[26] and from the mother liquors of tulipinolide by crystallization from petroleum ether (40–60°)–C_2H_5OH. In one collection simply concentrating the petroleum ether percolate at reduced pressure and adding an equal volume of isopropyl ether gave a 0.5% yield of crystalline epitulipinolide (**3**): mp 91–92°; $[\alpha]^{25}D$ +76° (*c* 3.2, $CHCl_3$); CD (*c* 0.040 and 0.0020, MeOH), 25°, $[\Theta]_{264}$ −7180, $[\Theta]_{222}$ +146,000; uv end absorption 210 mμ (log ε 4.36); ir 1767 and 1673 cm^{-1} (α,β-unsaturated γ-lactone), 1735 and 1250 cm^{-1} (acetate). The mass spectrum showed M^+ 290 (0.1%) and other peaks at *m/e* 248 (1.3), 230 (20), and 43 (100).

Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.28; H, 7.81.

Epoxidation of Tulipinolide (2).—To 300 mg of *m*-chloroperbenzoic acid in 20 ml of $CHCl_3$ was added 145 mg of **2**. After 24 hr at 5° the solution was shaken with 10-ml portions of 5% aqueous Na_2SO_3 until the $CHCl_3$ layer gave a negative starch-iodide test and then extracted with dilute $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated. The residue gave 121 mg (absolute C_2H_5OH) of the diepoxide **4**: mp 180–181°; $[\alpha]^{25}D$ +81° (*c* 0.34, CH_3OH); ir 1775 (γ-lactone), 1749, 1240 (acetate), and 1662 cm^{-1} (exocyclic olefin).

Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.55; H, 6.82.

Ozonolysis of Tulipinolide (2).—A solution of 290 mg of **2** in 40 ml of ethyl acetate at 0° was treated with 1% ozone in oxygen for 30 min. The residue on evaporation at reduced pressure and 30° was treated with 25 ml of water, warmed for 1 hr on a steam bath, and steam distilled. The distillate collected in 25 ml of 2.5% 2,4-dinitrophenylhydrazine in 2 *N* H_2SO_4 gave 139 mg of an orange-red precipitate which crystallized from chloroform as yellow-orange needles (107 mg), mp 206° which showed no depression when admixed with an authentic sample of the 2,4-dinitrophenylhydrazone of levulinic acid. Both gave the same ir spectrum.

Cyclization of Costunolide (1).—A solution of 250 mg of costunolide (**1**) {CD (*c* 0.020 and 0.0010, CH_3OH), 25°, $[\Theta]_{262}$ −6660, $[\Theta]_{220}$ + 110,000} in 50 ml $CHCl_3$ containing 0.1 ml of $SOCl_2$[27] was kept at room temperature for 30 min. The oily residue (266 mg) remaining after evaporation of solvent at reduced pressure and 40° showed one spot (R_f 0.6) on tlc (isopropyl ether as solvent). On tlc plates [petroleum ether–isopropyl ether (3:1)] poured with 5% $AgNO_3$,[28] three spots were obtained but better resolved with 15% $AgNO_3$, giving R_f 0.10 [β-cyclocostunolide (**9**)], 0.22 [α-cyclocostunolide (**8**)], and 0.39 [γ-cyclocostunolide (**10**)].

The mixture of cyclocostunolides was applied to a column of 14 gm of silica gel G (15% $AgNO_3$) and elution with the solvent system of petroleum ether–isopropyl ether (3:1) gave 26 mg of a γ-cyclocostunolide fraction which from C_2H_5OH–H_2O yielded **10** as needles: mp 87–88°; $[\alpha]^{25}D$ +22.2° (*c* 0.135, CH_3OH); uv end absorption 210 mμ (log ε 4.43); ir 1765, 1670, 1257, and 825 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}O_2$: C, 77.55; H, 6.86. Found: C, 77.31; H, 8.71.

After the γ-cyclocostunolide was eluted, the solvent was changed to isopropyl ether which gave the α-cyclocostunolide fraction (111 mg). On crystallization from C_2H_5OH–H_2O, **8** was obtained: mp 82–83° $[\alpha]^{25}D$ +112° (*c* 0.25, CH_3OH); uv end absorption 210 mμ (log ε 4.07) {lit.[4] mp 83–84°; $[\alpha]^{25}D$ +118° ($CHCl_3$); uv end absorption 210 mμ (log ε 4.00)}.

Anal. Calcd for $C_{15}H_{20}O_2$: C, 77.55; H, 8.68. Found: C, 77.48; H, 8.84.

The β-cyclocostunolide fraction (106 mg) was obtained by continued washing of the column with the same solvent until the effluent no longer contained a $KMnO_4$ reducing solute. Crystallization from C_2H_5OH–H_2O yielded **9**: mp 68–69°; $[\alpha]^{25}D$ +166° (*c* 0.235, CH_3OH); uv end absorptions 210 mμ (log ε 3.89) {lit.[4] mp 66.5–67°; $[\alpha]^{27}D$ +179° ($CHCl_3$); uv end absorption 205 mμ (log ε 4.18)}; ir 1762, 1673, 1651 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}O_2$: C, 77.55; H, 8.68. Found: C, 77.39; H, 8.52.

Cyclization of Tulipinolide (2).—To 534 mg of **2** in 50 ml of chloroform was added 1.0 ml of $SOCl_2$. After 30 min the solvent was evaporated at reduced pressure and the residue crystallized from isopropyl ether to give 239 mg of needles, mp 110–111°, which gave two spots on tlc with 5% $AgNO_3$-impregnated adsorbent. Separation of these crystals was accomplished on a column with 14 g of the same adsorbent using isopropyl ether–$CHCl_3$ (4:1) as solvent. The first eluted material was the α-cyclotulipinolide fraction (49 mg) from which 40 mg of **5** crystallized (isopropyl ether) as prisms: mp 110–111°; $[\alpha]^{25}D$ +187° (*c* 0.685, MeOH); uv end absorption 210 mμ (log ε 4.20); ir 1767 (γ-lactone), 1739 (acetate), 1671 (olefin), and 1200–1255 cm^{-1} (C–O–C stretching).

Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.33; H, 7.77.

The second eluted fraction (160 mg) crystallized as plates from isopropyl ether to give β-cyclotulipinolide (**6**) (140 mg): mp 136–137°; $[\alpha]^{25}D$ +213° (*c* 0.305, CH_3OH); uv end absorption 210 mμ (log ε 3.83); ir 1767, 1737, 1668, 1645, and 1200–1255 cm^{-1}.

Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.43; H, 7.75.

An additional proportionate amount of compounds **5** and **6** could be obtained from the cyclization reaction mother liquors. The presence of γ-cyclotulipinolide was not observed.

Dihydro-β-cyclotulipinolide (11).—β-Cyclotulipinolide (**6**) (81 mg) in 13 ml of absolute C_2H_5OH was hydrogenated over 66 mg of 5% Pd on charcoal at atmospheric pressure and ambient temperature. After uptake (10 min) of 1 mol equiv of hydrogen, the catalyst was removed and the solvent evaporated. The oily residue (82 mg) showed two spots on tlc (isopropyl ether–$CHCl_3$ (4:1)) at R_f 0.55 and 0.40 utilizing $AgNO_3$-treated plates. A column separation utilizing the same adsorbent (14 g) and solvent system gave 20 mg of the first less-polar fraction which was shown by nmr to be a mixture of at least two substances and was not further studied. Decreasing the amount of catalyst by about one-half eliminated this first fraction.

The second fraction (54 mg) on crystallization from isopropyl ether gave pure dihydro-β-cyclotulipinolide (**11**) as plates (40 mg): mp 139–140°; $[\alpha]^{25}D$ +136° (*c* 0.415, CH_3OH); ir 1775 (γ-lactone), 1735 (acetate), and 1648 cm^- (olefin).

Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 70.17; H, 8.44.

Ozonolysis of Dihydro-β-cyclotulipinolide (11) to the Ketone 12.—A solution of 50 mg of **11** in 10 ml of acetic acid at 10° was treated with a stream of oxygen containing about 3% ozone for 10 min. The reaction solution at ambient temperature was shaken with 0.25 g of Zn dust for 30 min and then filtered. The residue remaining after evaporation of the solvent was crystallized from isopropyl ether–C_2H_5OH to give 22 mg of the ketone **12**: mp 178–179°; $[\alpha]^{25}D$ +63.7° (*c* 0.245, CH_3OH); CD (*c* 0.105, CH_3OH), 25°, $[\Theta]_{290}$ −4670; uv max 285 mμ (ε 35); ir 1780 (γ-lactone) and 1730 cm^{-1} (a broad double-intensity band for acetate and cyclohexanone) and no olefinic bands.

(25) Melting points were taken in capillaries on a Thomas–Hoover apparatus and are uncorrected. Elemental analyses were by Mr. Joseph F. Alicino, Metuchen, N. J., and by Dr. Alfred Bernhardt, Germany. Infrared spectra were taken in $CHCl_3$ on a Perkin-Elmer Model 237 or 257 spectrophotometer and ultraviolet spectra were obtained in CH_3OH on a Cary Model 15 spectrophotometer. The nmr spectra were measured in $CDCl_3$ on a Varian A-60A instrument with $(CH_3)_4Si$ as internal standard unless otherwise stated, and chemical shifts reported in δ (ppm) units. The ORD, CD, and optical rotation values were determined on a Jasco ORD/UV-5 spectropolarimeter or the latter on a Zeiss polarimeter. Mass spectra were obtained on an AEI MS-9 double-focusing instrument and samples were introduced *via* the direct inlet probe. Thin layer chromatography (tlc) was performed on silica gel G (Merck) with detection by iodine vapor or spraying with 0.3% $KMnO_4$ solution. Plates incorporating $AgNO_3$ were poured as a slurry with the per cent (w/v) of complexing agent indicated. Columns run with such adsorbents were made from thep owdered (through 100-mesh), dried (110°) slurries prepared for the plates and were continuously protected from light.

(26) Collections of root bark were obtained from trees grown in Virginia through the courtesy of Dr. Robert E. Perdue, Jr., of the U. S. Department of Agriculture, Beltsville, Md., under agreement with the CCNSC. The 1964 collection contained 0.4% tulipinolide and 0.1% epitulipinolide, but the 1965 and 1968 supply yielded only epitulipinolide.

(27) The use of thionyl chloride for cyclization is unusual and was discovered accidentally. Its exact role has yet to be determined, but, since the yields obtained with it were better than those from the use of boron trifluoride etherate or hydrochloric acid, it was employed exclusively.

(28) A collection of references to separations of unsaturated substances on $AgNO_3$-treated adsorbents is found in E. Heftmann, "Chromatography," 2nd ed. Reinhold Publishing Corp., New York, N. Y., 1967, pp 485–488.

Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 62.59; H, 7.73.

Deacetyldihydro-β-cyclotulipinolide (13).—To a 2.5-ml solution of $NaOCH_3$ from 12 mg of Na was added 60 mg of dihydro-β-cyclotulipinolide (**11**). After 20 hr at ambient temperature the solution was acidified with acetic acid, diluted with water, and extracted with $CHCl_3$ several times. The $CHCl_3$ extract was washed with water and dried (Na_2SO_4), and the residue after evaporation was crystallized as long needles from isopropyl ether–$CHCl_3$ to give 43 mg of **13**: mp 218–219°; $[\alpha]^{25}D$ +172° (*c* 0.38, CH_3OH), $[M]D$ +432°; ir 3593 and 3443 (hydroxyl), 1765 (γ-lactone), 1650 and 873 cm^{-1} (olefin).

The product **13** was reacetylated by treatment of 20 mg with 0.5 ml each of acetic anhydride and pyridine for 24 hr followed by the usual work-up to give a substance (20 mg) identical (melting point, mixture melting point, and ir) with dihydro-β-cyclotulipinolide **11**.

Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.31; H, 9.05.

The benzoate ester of **13** was prepared by treatment of 40 mg of **13** with 0.50 ml of pyridine and 0.10 ml of benzoyl chloride for 24 hr; 5 ml of H_2O was then added followed by 100 ml of ether. The reaction mixture was extracted successively with 1 *N* H_2SO_4, H_2O, 5% $NaHCO_3$, and H_2O, then dried (Na_2SO_4). Removal of solvent left 39 mg of an oil that was purified on 7 g of 5% $AgNO_3$-prepared adsorbent, eluting with isopropyl ether–$CHCl_3$ (4:1). The first fraction (22 mg) was crystallized from isopropyl ether–CH_3OH to give 13 mg of the benzoate as needles: mp 106–107°, $[\alpha]^{25}D$ +171.4° (*c* 0.28, CH_3OH), $[M]^{25}D$ +607°. The ir lacked –OH absorption but showed peaks at 1715 (benzoate ester), 1603, and 1583 cm^{-1} (aromatic). The difference in $[M]D$ between the benzoate and the alcohol ($[M]D$ +432°) is +175°, indicating an alcohol with an *S* configuration.[21]

In the Horeau procedure, 96.0 mg of α-phenylbutyric anhydride and 21.0 mg of **13** in 0.8 ml of pyridine were allowed to react for 16 hr. The optically active α-phenylbutyric acid {71.0 mg, $[\alpha]^{25}D$ +4.93° (*c* 1.42, C_6H_6)} was isolated as already described.[23] The optical yield was (+) 32.8% suggesting an *R* configuration for the alcohol. The examination of the neutral fraction by ir showed no starting material.

Oxidation of Deacetyldihydro-β-cyclotulipinolide (13) to the Ketone 14.—Compound **13** (20 mg) in 2 ml of acetone was stirred magnetically and 0.05 ml of Jones reagent[10] was added. After 5 min at ambient temperature, 2 ml of CH_3OH was added followed by 50 ml of diethyl ether. The ether solution was extracted with 1% $NaHCO_3$ and water and then dried (Na_2SO_4). Evaporation of the solvent left an oil that crystallized from isopropyl ether as prisms to give 15 mg of ketone **14**: mp 141–142°; $[\alpha]^{25}D$ −27.3° (*c* 0.128, CH_3OH); CD (*c* 0.128, CH_3OH), 25°, $[\Theta]_{290}$ −8560; uv max 280 mμ (ε 29); ir 1769 (γ-lactone), 1725 (cyclohexanone), 1651, and 875 cm^{-1} (olefin).

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.33; H, 8.02.

Cyclization of Epitulipinolide (3).—A solution of 1.0 g of **3** in 30 ml of chloroform containing 1.0 ml of $SOCl_2$ was kept at ambient temperature for 30 min with occasional stirring. Removal of solvent by evaporation left 1.05 g of an oily residue that crystallized on addition of isopropyl ether to give 680 mg of a mixture of needles and prisms (mp 121–122°). Tlc on 5% $AgNO_3$-impregnated plates [isopropyl ether–$CHCl_3$ (4:1)] revealed three spots, R_f 0.53 [γ-cycloepitulipinolide (**17**)], 0.40 [α-cycloepitulipinolide (**15**)], and 0.15 [β-cycloepitulipinolide (**16**)]. Separation of the isomers was performed on columns using the same solvent system and adsorbent, 14 g for each 200 mg of the mixture (crystalline and mother liquor residue). Typical results are as follows.

The first fraction (tlc-monitored) crystallized from isopropyl ether–C_2H_5OH to give glistening needles (29 mg) of the γ isomer **17**: mp 151–152°; $[\alpha]^{25}D$ −47.0° (*c* 0.50, CH_3OH); uv end absorption 210 mμ (log ε 4.22); ir 1768 (γ-lactone), 1731 (acetate), 1650 (olefin), and 1250 cm^{-1} (C–O–C).

Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.20; H, 7.40.

The second fraction crystallized slowly from isopropyl ether to give 39 mg of feathery needles of the α isomer **15**: mp 75–76°; $[\alpha]^{25}D$ +45.6° (*c* 0.34, CH_3OH); uv end absorption 210 mμ (log ε 4.10); ir 1768, 1740, 1650, 1250 cm^{-1}.

Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.44; H, 7.92.

The third fraction crystallized from isopropyl ether to give prisms (91 mg) of the β isomer **16**: mp 127–128°; $[\alpha]^{25}D$ +42.5° (*c* 0.27, CH_3OH); uv end absorption 210 mμ (log ε 4.02); ir 1769, 1739, 1667, 1653, and 1251 cm^{-1}.

Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.29; H, 7.67.

Dihydro-β-cycloepitulipinolide (18) and β-Isocycloepitulipinolide (33).—β-Cycloepitulipinolide (**16**) (120 mg) in 10 ml of absolute C_2H_5OH was hydrogenated over 60 mg of 5% Pd on charcoal as catalyst at atmospheric pressure and ambient temperature. The reduction was terminated after uptake of 1 mol equiv of hydrogen, the catalyst was removed by filtration, and the residue remaining after evaporation of solvent was crystallized from isopropyl ether to give prisms (42 mg) of **18**: mp 160–161°; $[\alpha]^{25}D$ +65.5° (*c* 0.625, CH_3OH); ir 1774 (γ-lactone), 1740 (acetate), 1650 (olefin), and 1240 cm^{-1} (C–O–C).

Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 69.95; H, 8.20.

The mother liquor residues (260 mg) from a number of hydrogenations were chromatographed on columns employing 5% $AgNO_3$-treated plates and ether–isopropyl ether (1:9) as solvent. The first few fractions gave an oil (37 mg) lacking olefin bands in the ir and were not further investigated. The second fraction (179 mg) crystallized from isopropyl ether as flattened cubes of β-isocycloepitulipinolide (**33**): mp 111–112°; $[\alpha]^{25}D$ +103° (*c* 0.31, CH_3OH); uv max 218 mμ (log ε 3.99); ir 1750 (γ-lactone), 1685, and 1650 cm^{-1} (olefins).

Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.01; H, 7.29.

Hydrolysis of Dihydro-β-cycloepitulipinolide (18) to the Hydroxylactone 19.—A solution of 140 mg of **18** in 5 ml of CH_3OH originally treated with 23 mg of Na was kept at ambient temperature for 20 hr. The solution was then diluted with 10 ml of H_2O, acidified with acetic acid, and extracted with three 50-ml portions of $CHCl_3$. The $CHCl_3$ extract was washed with 5% $NaHCO_3$ and H_2O and dried (Na_2SO_4). On solvent evaporation a residue was left (101 mg) that crystallized from isopropyl ether to give 71 mg of **19** as needles: mp 154–156°; $[\alpha]^{25}D$ +75.3° (*c* 0.465, CH_3OH); ir 3581 and 3410 (hydroxyl), 1768 (γ-lactone), and 1648 cm^{-1} (olefin). In other runs, the same product was obtained in the same yield when the acidification step was omitted.

Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.43; H, 8.68.

β-Cycloisoepitulipinolide (20).—The hydroxylactone **19** (49 mg) in 0.75 ml of pyridine was treated with the same volume of acetic anhydride. About 20 hr later the solution was diluted with 180 ml of ether and extracted successively with 25-ml portions of 5% oxalic acid, 5% $NaHCO_3$, and water. The dried (Na_2SO_4) ether solution on evaporation left 50 mg of a residue that crystallized from isopropyl ether as feathery needles of **20** (41 mg): mp 150–151°; $[\alpha]^{5}D$ +63.3° (*c* 0.30, CH_3OH); ir 1773 (γ-lactone), 1729 (acetate), 1650 (olefin), and 1250 cm^{-1} (C–O–C).

Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 69.56; H, 8.60.

Oxidation of the Hydroxylactone 19 to the Ketone 21.—To the hydroxylactone **19** (49 mg) in 0.2 ml of $(CH_3)_2SO$ was added 0.5 g of dicyclohexylcarbodiimide, 0.2 ml of 1 *M* H_3PO_4 in $(CH_3)_2SO$, and 2 ml of C_6H_6. After 30 hr at ambient temperature the mixture was diluted with 50 ml of ethyl acetate and 0.5 gm of oxalic acid in 2 ml of CH_3OH was added. The precipitated dicyclohexylurea was collected after 30 min and the filtrate was washed with two 10-ml portions of water, dried (Na_2SO_4), and evaporated. The small amount of the urea still contaminating the residue was removed by mixing with diethyl ether and filtering. The filtrate residue (31 mg) crystallized from isopropyl ether as prisms (15 mg) of **21**: mp 152–154°; $[\alpha]^{25}D$ +110° (*c* 0.10, CH_3OH); uv max 285 mμ (ε 31); ir 1775 (γ-lactone), and 1721 cm^{-1} (cyclohexanone).[29]

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.71; H, 8.39.

Hydrolysis of Dihydro-β-cycloepitulipinolide (18) to the Hydroxy Acid 22.—A solution of 206 mg of **18** in 40 ml of C_2H_5OH was stirred with 300 mg of Na_2CO_3 in 60 ml of H_2O for 20 hr. Acidification with acetic acid was followed by extraction with three 50-ml portions of $CHCl_3$ and drying of the extract with Na_2SO_4. The residue (150 mg) from the $CHCl_3$ extract crystallized to give 110 mg of silky needles of **22**: mp 170–171°; $[\alpha]^{25}D$ −33.3° (*c* 0.18, CH_3OH); ir 3587 (hydroxyl), 1734 (acetate),

1701 cm^{-1} (carboxyl), as well as the characteristic broad absorption between 3400 and 3000 cm^{-1} for carboxylic acids.

Anal. Calcd for $C_{17}H_{26}O_5$: C, 65.78; H, 8.44. Found: C, 65.49; H, 7.68.

Hydrolysis of γ-Cycloeptiulipinolide (17) to the Hydroxy Acid 23.—A solution of **17** (265 mg) in 50 ml of C_2H_5OH and a solution of 300 mg of Na_2CO_3 in 75 ml of H_2O were stirred together for 20 hr. The reaction mixture was acidified with acetic acid and extracted with chloroform, and the solvent was evaporated after drying (Na_2SO_4) to leave a 200 mg residue. Crystallization from isopropyl ether gave fine needles (150 mg) of **23**: mp 147–149°; $[\alpha]^{25}D$ −21.8° (*c* 0.55, CH_3OH); ir 3600 (hydroxyl), 1735 (acetate), and 1694 cm^{-1} (α,β-unsaturated carboxylic acid), as well as the typical broad band between 3400 and 3050 cm^{-} for carboxylic acids.

Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85. Found: C, 66.43; H, 7.89.

Methylation of Hydroxy Acid 23 to the Ester 24.—The hydroxy acid **23** (50 mg) dissolved in 3 drops of C_2H_5OH was diluted with 10 ml of diethyl ether and cooled to 5°. An ethereal solution of CH_2N_2 was added while stirring until a persistent yellow color was reached. Evaporation of the solvent left a crystalline mass (55 mg) that was recrystallized from isopropyl ether to give 30 mg of the ester **24** as needles: mp 131–131.5°; $[\alpha]^{25}D$ −21.8° (*c* 0.505, CH_3OH); ir 3600 and 3500 (hydroxyl), 1732 (acetate), and 1727 cm^{-1} (methyl ester).

Anal. Calcd for $C_{18}H_{26}O_5$: C, 67.06; H, 8.13. Found: C, 66.89; H, 8.41.

Ozonolysis of Dihydro-β-cycloepitulipinolide (18) to the Ketone 25.—Oxygen containing about 3% ozone was passed through an acetic acid solution of 200 mg of **18** for 25 min while cooling at 10°. The reaction mixture was diluted with 100 ml of ether and shaken with 1.0 g of Zn dust for 30 min. Removal of the Zn and evaporation of the filtrate gave a crystalline residue (125 mg) which was recrystallized from absolute C_2H_5OH to give fine needles (97 mg) of ketone **25**: mp 210° after yellowing at 190°; $[\alpha]^{25}D$ +27.3° (*c* 0.55, CH_3OH); CD (*c* 0.095, CH_3OH), 25°, $[\Theta]_{290}$ −3870; uv max 285 mμ (ε 26.5); ir 1776 (γ-lactone), 1739 (acetate), and 1720 cm^{-1} (cyclohexanone).

Anal. Calcd for $C_{15}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.49; H, 7.68.

Deacetylepitulipinolide (Eupatolide) (26).—Epitulipinolide (**3**) (100 mg) was stirred in 20 ml of 60% aqueous CH_3OH containing 100 mg of KOH. After 20 hr at ambient temperature the CH_3OH was removed by evaporation and the aqueous solution acidified with 1 *N* H_2SO_4 to give a cloudy suspension. The mixture was extracted with three 50-ml portions of diethyl ether and the combined extract washed with 5% $NaHCO_4$ and H_2O and then dried (Na_2SO_4). Evaporation of the solvent and crystallization of the residue from isopropyl ether gave fine prisms of deacetylepitulipinolide (**26**): mp 186–188° (lit.[19] mp 182–188° as eupatolide); $[\alpha]^{25}D$ +29.7° (*c* 1.88, acetone); ir 3400 (hydroxyl), 1757 (γ-lactone), and 1652 cm^{-1} (olefin).

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.34; H, 8.46.

It was not possible to apply Brewster's method[21] for determining the configuration of the hydroxyl in **26** as the benzoate ester could not be made by treating **26** with benzoyl chloride in pyridine or by refluxing with benzoic anhydride in pyridine.

In applying the Horeau procedure, 240.7 mg of α-phenybutyric anhydride and 72.0 mg of **26** in 2.5 ml of pyridine were allowed to react for 16 hr, then 1 ml of H_2O was added, and 141.0 mg of α-phenylbutyric acid, $[\alpha]^{25}D$ −8.7° (*c* 2.82, C_6H_6), was isolated as previously described.[23] The optical yield of (−) 39.7% would indicate an *S* configuration; see discussion for analysis of this result. The neutral crystalline fraction (103 mg) from the esterification contained no starting material as shown by the ir spectrum.

Cyclization of Deacetylepitulipinolide (26).—To a solution of 600 mg of **26** in 70 ml of $CHCl_3$ was added 1.0 ml of $SOCl_2$ and after 30 min at ambient temperature the solution was evaporated at reduced pressure. The residue deposited silky needles (150 mg) from isopropyl ether as a first crop (fraction A) and mostly prisms (250 mg) as a second crop (fraction B). Both fractions were mixtures as evidenced by tlc and nmr. Recrystallization of A (mixture of α and γ isomers) from isopropyl ether–C_2H_5OH gave pure deacetyl-γ-cycloepitulipinolide (**29**) (50 mg): mp 202–203°; $[\alpha]^{25}D$ −63.2° (*c* 0.245, CH_3OH); ir 3600 and 3400 (hydroxyl), 1776 (γ-lactone), and 1670 cm^{-1} (olefin).

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.19; H, 8.23.

Chromatography of fraction B on 14 gm of 5% $AgNO_3$-impregnated adsorbent and elution with ether resulted in two fractions. The first a mixture of α and γ isomers yielded more (51 mg) of the crystalline γ isomer **29**. The second (93 mg) was essentially pure deacetyl-β-cycloepitulipinolide (**28**) which crystallized from isopropyl ether–C_2H_5OH as prisms: mp 140–141°; $[\alpha]^{25}D$ +67.3° (*c* 0.26, CH_3OH); ir 3600 and 3500 (hydroxyl), 1762 (γ-lactone), 1675, and 1652 cm^{-1} (olefins).

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.33; H, 8.02.

Although the α isomer was formed in the reaction as evidenced from the nmr spectra of the less polar mother liquors, a pure sample was not available either through repeated crystallization or by column chromatography. Additional quantities of the pure β and γ isomers were obtained by chromatography of mother liquor residues.

Acetylation of Deacetyl-β-cycloepitulipinolide (28) to 16.—A 25-mg sample of **28** was added to 0.5 ml of pyridine and 0.5 ml of acetic anhydride. After 20 hr the reaction mixture was diluted with 50 ml of diethyl ether and extracted successively with 10-ml portions of 5% oxalic acid solution, 5% $NaHCO_3$ solution, and H_2O. The dried (Na_2SO_4) ether solution on evaporation left a residue that from isopropyl ether gave prisms (20 mg) of β-cycloepitulipinolide (**16**), mp 127–128° (undepressed when admixed with authentic **16**); the compound gave the same ir spectrum as that of authentic **16**.

Dihydrodeacetyl-β-cycloepitulipinolide (30) and Deacetyl-β-isocycloepitulipinolide (32).—Deacetyl-β-cycloepitulipinolide (**28**) (76 mg) was hydrogenated in 17 ml of absolute C_2H_5OH over 50 mg of 5% Pd on charcoal as catalyst at ambient temperature and atmospheric pressure. The uptake of 1 mol equiv of hydrogen was rapid (~9 min) and the reaction was stopped; the catalyst was removed by filtration. The residue (64 mg) remaining after evaporation of the solvent gave two spots (R_f 0.35 and 0.25) on tlc with ether as solvent. The major product (R_f 0.35) crystallized from isopropyl ether–absolute C_2H_5OH to give 33 mg of dihydrodeacetyl-β-cycloepitulipinolide (**30**): mp 169–170°; $[\alpha]^{25}D$ +87.5° (*c* 0.24, CH_3OH), $[M]^{25}D$ +219°; ir 3400 (hydroxyl), 1760 (γ-lactone), and 1652 cm^{-1} (olefin).

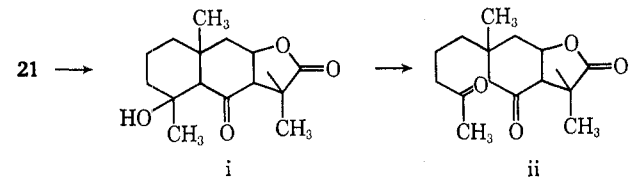
Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.86; H, 8.80.

The mother liquor residue (30 mg) was streaked on 1-mm-thick preparative tlc plates and developed by diethyl ether. The R_f 0.25 zone was removed and extracted with $CHCl_3$, and the residue (23 mg) remaining after evaporation of solvent was crystallized as needles from isopropyl ether–C_2H_5OH to give 15 mg of deacetyl-β-isocycloepitulipinolide (**32**): mp 147–148°; $[\alpha]^{25}D$ +100° (*c* 0.15, CH_3OH); ir 3590 and 3440 (hydroxyl), 1750 (γ-lactone), 1685, and 1650 cm^{-1} (olefins).

Anal. Calcd for $C_{14}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.59; H, 8.09.

The benzoate ester of **30** was prepared from 39 mg of **30**, 0.5 ml of pyridine, and 0.1 ml of benzoyl chloride. After 24 hr the reaction mixture was treated with 5 ml of H_2O, diluted with 100 ml of ether, and extracted successively with 1 *N* H_2SO_4, H_2O, 5% $NaHCO_3$, and H_2O. The dried (Na_2SO_4) ether phase gave a residue (31 mg) that crystallized from isopropyl ether to give 24 mg of the benzoate as needles: mp 174–174.5°; $[\alpha]^{25}D$ +6.7° (*c* 0.30, CH_3OH), $[M]^{25}D$ +23.7°. The ir lacked -OH absorption but instead had peaks at 1716, 1603, and 1583 cm^{-1}, characteristic of benzoates. The difference in $[M]D$ between the benzoate and the alcohol ($[M]D$ +219°) is −195°, indicating an alcohol with an *R* configuration.[21]

(29) Ketone **21** gave a strongly positive Zimmerman's test[30] and was interpreted as resulting from the production of the diketone ii by the retro-aldol opening of the β-hydroxy ketone i formed under the strongly alkaline conditions of the test. The generated α-methylene keto groups in ii would then give the positive result.



(30) R. Neher, "Steroid Chromatography," Elsevier Publishing Co., New York, N. Y., 1964, p 125.

In the Horeau procedure, 83.0 mg of α-phenylbutyric anhydride and 25.0 mg of **30** in 1 ml of pyridine were allowed to react for 16 hr. The α-phenylbutyric acid, 82.0 mg, $[\alpha]^{25}D$ −3.96° (*c* 1.64, C_6H_6), was isolated as already described.[23] The optical yield of (−) 19.4% suggested an *S* configuration. The neutral fraction on examination in the ir showed no starting material.

Acetylation of Dihydrodeacetyl-β-cycloepitulipinolide (30) to 31.—A 43-mg sample of **30** was dissolved in 0.5 ml of pyridine and 1.0 ml of acetic anhydride added. After 20 hr about 5 g of ice was added followed by 5 ml of 5% $NaHCO_3$ solution. After 1 hr the mixture was extracted with three 25-ml portions of diethyl ether and the extract was washed successively with 1 *N* H_2SO_4, H_2O, 5% $NaHCO_3$, and H_2O again. The dried (Na_2SO_4) ether solution left a residue (50 mg) on evaporation that crystallized from petroleum ether–isopropyl ether as fine rods (33 mg): mp 84–85°; $[\alpha]^{25}D$ +62.6° (*c* 0.335, CH_3OH); ir 1775 (γ-lactone), 1740 (acetate), 1655 (olefin), and 1245 cm^{-1} (C–O–C).

Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 70.28; H, 7.90.

Hydrolysis of Epitulipinolide (3) to 34.—To a 2.5 ml of $NaOCH_3$ (from 23 mg of Na) solution in CH_3OH was added 100 mg of **3**. After 20 hr, 5 ml of H_2O was added and the solution was acidified with acetic acid then extracted with three 50-ml portions of $CHCl_3$. The $CHCl_3$ extract was washed with 5% $NaHCO_3$ solution and H_2O and then dried (Na_2SO_4). The residue (87 mg) after removal of solvent was recrystallized from isopropyl ether–C_2H_5OH forming feathery needles (71 mg) of **34**: mp 138–138.5°; $[\alpha]^{25}D$ +65.0 ± 2.5° (*c* 2.4, $CHCl_3$) {lit.[19] mp 138–139.5°; $[\alpha]^{25}D$ +60.3° (*c* 3.1, $CHCl_3$)}; ir 3600 and 3450 (hydroxyl) and 1757 cm^{-1} (γ-lactone).

Anal. Calcd for $C_{16}H_{24}O_4$: C, 68.54; H, 8.63. Found: C, 68.64; H, 8.63.

Oxidation of the Hydroxylactone 34 to the Ketone 35.—The lactone **34** (40 mg) was added to 1 ml of Sarett's reagent (120 mg of CrO_3 in 1 ml of pyridine) and after 24 hr at ambient temperature the mixture was diluted with 45 ml of diethyl ether. The resultant mixture was extracted successively with four 10-ml portions of 2% tartaric acid, 5% $NaHCO_3$, and H_2O, and then dried (Na_2SO_4). Removal of solvent gave an oil (32 mg) that crystallized from isopropyl ether as needles (19 mg) of **35**: mp 87–87.5°; $[\alpha]^{25}D$ −409.5° (*c* 0.21, CH_3OH) (lit.[19] mp 87–87.5°); uv max 303 mμ (ε 456) and end absorption 210 (log ε 3.88); ir 1775 (γ-lactone) and 1707 cm^{-1} (ketone). The product **35** gave a positive Zimmerman's test.[30]

Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97. Found: C, 68.94; H, 7.95.

Oxidation of the Hydroxylactone 26 to the Ketone 36.—The lactone **26** (124 mg) was dissolved in 20 ml of acetone and after cooling the solution to −5°, Jones reagent[10] (0.20 ml) was added while stirring. The reaction was stopped after 6 min by the addition of 2 ml of CH_3OH. The mixture was filtered and the filtrate diluted with 50 ml of H_2O and extracted with two 250-ml portions of diethyl ether. The ether extract was washed with 5% $NaHCO_3$ and H_2O and then dried (Na_2SO_4). The crystalline residue (85 mg) remaining on evaporation of the solvent was recrystallized from petroleum ether–C_2H_5OH to give 74 mg of needles of **36**: mp 127–128°; $[\alpha]^{25}D$ −563° (*c* 0.37, CH_3OH); uv max 308 mμ (ε 338) and end absolution at 210 (log ε 4.14); ir 1774 (γ-lactone), 1703 (ketone), and 1660 cm^{-1} (olefin). Ketone **36** gave a positive Zimmerman's test.[30]

Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.14; H, 7.37. Found: C, 73.20; H, 7.44.

Registry No.—**1**, 553-21-9; **2**, 24164-12-3; **3**, 24164-13-4; **4**, 24164-14-5; **5**, 24164-15-6; **6**, 24164-16-7; **8**, 2221-81-0; **9**, 2221-82-1; **10**, 24164-19-0; **11**, 24164-20-3; **12**, 24164-21-4; **13**, 24164-22-5; **13** benzoate, 24164-23-6; **14**, 24164-24-7; **15**, 24215-66-5; **16**, 24164-25-8; **17**, 24164-26-9; **18**, 24164-27-0; **19**, 24164-28-1; **20**, 24164-29-2; **21**, 24164-30-5; **22**, 24164-31-6; **23**, 24164-32-7; **24**, 24164-33-8; **25**, 24164-34-9; **26**, 24164-35-0; **28**, 24164-36-1; **29**, 24164-37-2; **30**, 24164-38-3; **30** benzoate, 24165-30-8; **31**, 24165-31-9; **32**, 24165-32-0; **33**, 24165-33-1; **34**, 24165-34-2; **35**, 24165-35-3; **36**, 24165-36-4.

Dimethyl Sulfoxide Oxidation of the Hydroxy Group in Steroids

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The acid-catalyzed reactions between diphenylketene-*p*-tolylimine and DMSO, N,N-diethylaminoprop-1-yne and DMSO, and N,N-dimethylaminophenylacetylene and DMSO have been used to effect the oxidation of the hydroxy group in a number of steroids. These reactions illustrate some interesting variations of the well-known oxidation procedure of Moffatt, *et al.*, involving dicyclohexylcarbodiimide and DMSO. The mechanism of the ynamine–DMSO oxidation has been investigated.

The acid-catalyzed dimethyl sulfoxide (DMSO)–dicyclohexylcarbodiimide (DDC) oxidation of alcohols to the corresponding aldehydes and ketones has been reported by Moffatt, *et al.*[1,2] In this connection, our preliminary investigation demonstrated the application of diphenylketene-*p*-tolylimine–dimethyl sulfoxide[3] and N,N-diethylaminoprop-1-yne–dimethyl sulfoxide[4] for the oxidation of the hydroxy group in steroids. Recently, we have also reported on the mechanism of ketenimine–DMSO and carbodiimide–DMSO oxidations.[5] Our results based on nuclear magnetic resonance spectroscopy using hexadeuteriodimethyl sulfoxide (DMSO-d_6) substantiated the stepwise mechanism for the DCC–DMSO oxidation as proposed by Moffatt, *et al.*,[6] and refuted Torsell's three-body concerted mechanism.[7] In this paper, we wish to illustrate the usefulness of the reagents ynamine–DMSO and ketenimine–DMSO in the oxidation of the hydroxy group in steroids and propose a mechanism for the ynamine–DMSO oxidation.

During the past 2–3 years, interest in the chemistry and application of ynamines has increased considerably. It has been shown that they undergo some very interesting reactions. For instance, they have been reported to undergo reactions analogous to carbodiimides and ketenimines.[8–11] Based on these observations, we re-

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