mately the same (3.4 Hz) for each methyl carbon. Similar techniques may find application in the analysis of the conformations of *N*-formyl derivatives of greater complexity.

Conclusion

One of the most important carbon-13 chemical shift effects seen in the amides is a proximity effect associated with the carbonyl oxygen. Thus, *N*-alkyl carbons which are syn to this oxygen are strongly shielded. While at present this effect has only been recognized for carbon nuclei attached to the nitrogen of amides or the ether oxygen of esters,[6] there appears to be no reason why a similar effect cannot occur at the β carbon of amino acids. Such an effect could have important application to the conformational analysis of such systems.

Carbon–proton coupling appears to hold promise of useful applications in the conformational analysis and peak assignment for at least the simple amides, and may be useful in further investigations into the shielding effect of the carbonyl group in small molecules. Such experiments are currently in progress.

Carolenin and Carolenalin, Two New Guaianolides in *Helenium autumnale* L. from North Carolina[1,2]

HIROSHI FURUKAWA, KUO-HSIUNG LEE,*[3,4] TETSURO SHINGU,[5] RONALD MECK,[4,6] AND CLAUDE PIANTADOSI[4]

Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514, and Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Received December 5, 1972

The major sesquiterpene lactones found in *Helenium autumnale* L. collected during the summer in North Carolina were not the pseudoguaianolide helenalin or the norsesquiterpene lactone, dihydromexicanin E, but were the new guaianolides, carolenin and carolenalin. The structures of carolenalin and carolenin have been shown to be **1** and **13** on the basis of chemical transformations and spectral evidence.

The constituents of *Helenium autumnale* L. collected from different populations were previously examined and reported to contain helenalin,[7-10] dihydromexicanin E,[11-12] helenium lactone,[13] 2-acetyl flexuosin A,[14] autumnolide,[14] tenulin,[15] mexicanin I,[15] and flexuosin A.[15] In the course of a search for convenient supply of the pseudoguaianolide helenalin for investigation of the relationship between the sesquiterpene lactone structure and the antitumor or cytotoxic activity,[16-19] we had occasion to extract a batch of *Helenium autumnale* L., collected during the summer in the vicinity of Durham, N. C. We report herein the isolation and structural elucidation of two new guaianolides, carolenalin and carolenin.[20]

Carolenalin and carolenin, isolated from a chloroform extract of the finely ground plant material by fractionation involving successive solvent partitions and silica gel chromatography, were assigned structure **1** and **13**, respectively, on the basis of the following chemical transformations and spectral evidence.

Carolenalin (**1**) was isolated as an oil in 0.4% yield and had infrared bands at 3500, 1760, and 1640 cm^{-1}, thus indicating the presence of a hydroxyl group, a γ-lactone carbonyl, and a carbon–carbon double bond. The nmr spectrum of carolenalin (Table I) is in accord with the structure **1**. The vinyl methyl protons at C-10 appeared as a broad singlet at δ 1.75 and the methyl groups at C-11 and C-4 were seen, respectively, as a doublet (J = 7.5 Hz) at 1.19 and a sharp singlet at 1.08. Other nmr signals were seen at δ 5.35 (1 H, m, H-9), 5.09 (1 H, m, H-8), and 3.74 (1 H, t, J = 3.0 Hz, H-3) which was shifted downfield to 4.79 (q, J = 3.0, 5.25 Hz) in the monoacetate **2** and 5.45 (t, J = 3.0 Hz) in the diacetate **3** as described below.

Acetylation of **1** with acetic anhydride in pyridine for 3 days at room temperature yielded approximately equal amounts of a monoacetate **2** (mp 160–161°, $C_{17}H_{24}O_5$) and a diacetate **3** (mp 146–148°, $C_{19}H_{26}O_6$),[21] indicating the presence of two hydroxyl groups. Mass spectral peaks at m/e 308 (M^+), 290 (M − 18) (M − H_2O), 248 (M − 60) (M − AcOH), and 230 (M − 78) (M − H_2O and AcOH), and ir absorption at 3580 (OH) and 1740 cm^{-1} (acetyl C=O) showed that **2** was a

(1) This investigation was supported by U. S. Public Health Service Research Grant No. CA12360-02 from the National Cancer Institute.

(2) Presented in part at the 13th Annual Meeting of the American Society of Pharmacognosy, The Ohio State University, Columbus, Ohio, July 21, 1972.

(3) Address correspondence to this author.

(4) University of North Carolina.

(5) Kyoto University.

(6) Medicinal Chemistry Trainee; supported by Public Health Training Grant 5T01-AM01770-03 from the National Institute of General Medical Sciences, National Institutes of Health.

(7) E. P. Clark, *J. Amer. Chem. Soc.*, **58**, 1982 (1936).

(8) R. Adams and W. Herz, *ibid.*, **71**, 2546 (1949), and related papers.

(9) W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *ibid.*, **85**, 19 (1963).

(10) M. T. Emerson, C. N. Caughlan, and W. Herz, *Tetrahedron Lett.*, 621 (1964).

(11) R. A. Lucas, R. G. Smith, and L. Dorfman, *J. Org. Chem.*, **29**, 2101 (1964).

(12) A. Romo de Vivar and J. Romo, *J. Amer. Chem. Soc.*, **83**, 2326 (1961); J. Romo, A. Romo de Vivar, and W. Herz, *Tetrahedron*, **19**, 2317 (1963).

(13) H. Hikino, D. Kuwano, and T. Takemoto, *Chem. Pharm. Bull.*, **16**, 1601 (1968).

(14) W. Herz, P. S. Subramaniam, and N. Dennis, *J. Org. Chem.*, **34**, 2915 (1969).

(15) W. Herz and P. S. Subramaniam, *Phytochemistry*, **11**, 1101 (1972).

(16) K. H. Lee, E. S. Huang, C. Piantadosi, J. S. Pagano, and T. A. Geissman, *Cancer Res.*, **31**, 1649 (1971).

(17) K. H. Lee, D. C. Anuforo, E. S. Huang, and C. Piantadosi, *J. Pharm. Sci.*, **61**, 626 (1971).

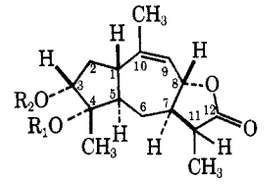
(18) K. H. Lee, H. Furukawa, and E. S. Huang, *J. Med. Chem.*, **15**, 609 (1972).

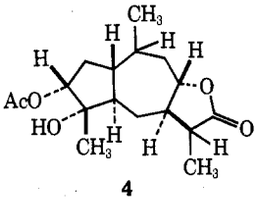
(19) K. H. Lee, R. Meck, C. Piantadosi, and E. S. Huang, *ibid.*, **16**, 299 (1973).

(20) The plant material used in the present work contained neither helenalin nor dihydromexicanin E, although the latter compound was isolated before from the same plant species in almost the same season and at the same site.[11]

(21) The formation of a diacetate (**3**) with acetic anhydride–pyridine suggested that a vicinal diol moiety was present, since under this mild reaction condition **3** could only be formed *via* a neighboring group participation mechanism.

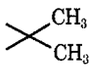
hydroxy monoacetate. That the remaining hydroxyl group in **2** was tertiary was evidenced by the fact that **2** showed a sharp D_2O-exchangeable one-proton singlet at δ 4.42 in the nmr spectrum (DMSO-d_6). The structure of **2** and **3** was also verified by the appearance of the three-proton singlet for the acetyl methyl groups in **2** and **3** at δ 2.10 and 2.00 and 2.03, respectively.

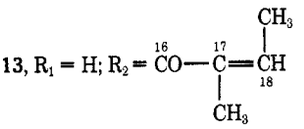


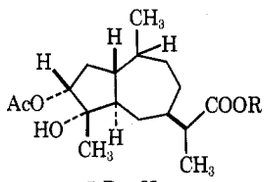


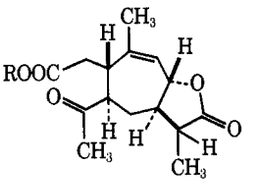
4

1, $R_1 = R_2 = H$
2, $R_1 = H$; $R_2 = Ac$
3, $R_1 = R_2 = Ac$

9, $R_1, R_2 =$ 

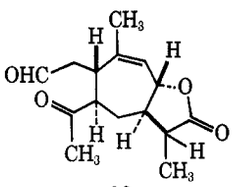
13, $R_1 = H$; $R_2 =$ 

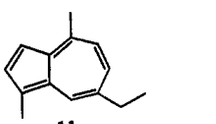




5, R = H
6, R = CH_3

7, R = H
8, R = CH_3





10 **11**

Catalytic hydrogenation of **2** with platinum oxide in ethyl acetate afforded a mixture of the corresponding dihydro compound **4** (mp 221–222°, $C_{17}H_{26}O_5$) whose nmr spectrum showed a new secondary methyl group at C-10 as a doublet (J = 6.0 Hz) at δ 0.95, and an acid **5** (mp 125–126°, $C_{17}H_{28}O_5$) whose nmr spectrum showed the disappearance of the characteristic lactonic (H-8) and vinylic (H-9) protons in the low-field region. Methylation of **5** with diazomethane gave a methyl ester (**6**, oil) which showed a carboxymethyl singlet at δ 3.63. The ready formation of the acid **5** by hydrogenolysis indicated an allylic disposition for the oxygen atom of the lactone ring. Support for this suggestion was found in the nature of the signal for the lactonic proton at H-8 in the nmr spectra of **2** and **4**. The H-8 proton in **4** (4.53, 1 H, m) is upfield shifted compared to the H-8 signal in **2** (5.08, 1 H, m).

Oxidation of **1** with Jones reagent at room temperature furnished a keto acid **7** (mp 171–172°, $C_{12}H_{20}O_5$). The nmr spectrum of **7** exhibited, instead of a tertiary methyl singlet at δ 1.08 (C-4 CH_3) as seen in **1**, a new sharp three-proton singlet at 2.14 attributable to the methyl ketone. Methylation of **7** yielded the corresponding ester **8**. The facile reaction of **1** to form acetonide (**9**) from acetone and *p*-toluenesulfonic acid, and

TABLE I

NMR SPECTRAL DATA FOR CAROLENALIN, CAROLENIN, AND DERIVATIVES[a]

Compd	H-3	H-8	H-9	C-11 CH_3	C-10 CH_3	C-4 CH_3	Ac	Misc
1	3.74	5.09	5.35	1.19	1.75	1.08		
	t (3.0)	m	m	d (7.5)	m	s		
2	4.79	5.08	5.35	1.19	1.75	1.13	2.10	
	dd (3.0, 5.25)	m	m	d (7.5)	m	s	s	
3	5.45[b]	5.15	5.45	1.22	1.78	1.32	2.00	
	t (3.0)	m	m	d (7.5)	m	s	s	
							2.03	
							s	
4	4.80	4.53		1.15	0.95	1.22	2.08	
	dd (6.0, 9.75)	m		d (7.5)	d (6.0)	s	s	
5	4.78			1.14	0.89	1.19	2.10	
	dd (4.5, 7.5)			d (7.5)	d (6.0)	s	s	
6	4.80			1.11	0.88	1.18	2.08	3.63[c]
	dd (5.25, 7.5)			d (7.5)	d (6.0)	s	s	s
7		5.42	5.20	1.20	1.82	2.14		
		m	m	d (6.75)	m	s		
8		5.45	5.25	1.20	1.82	2.14		3.67[c]
		m	m	d (6.75)	m	s		s
9	4.20	5.40	5.15	1.18	1.78	1.45		1.38[d]
	dd (1.5, 6.0)	m	m	d (6.75)	m	s		s
10		5.49	5.25	1.20	1.80	2.15		9.84[e]
		m	m	d (6.75)	m	s		
13	4.88	5.14	5.40	1.22	1.78	1.18		6.18[f]
	dd (3.0, 4.5)	m	m	d (6.75)	m	s		m
								1.92[g]
								s

[a] These spectra were measured in $CDCl_3$ with a Jeolco C-60 HL nmr spectrometer using TMS as an internal standard. All chemical shifts were reported in δ (ppm) values and the coupling constants (figures in parentheses) in hertz. Signals are characterized in the usual way: s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. H-3, H-8, and H-9 each integrated for one proton. Methyl as well as acetyl signals had three proton intensities. [b] Overlapped with H-9. [c] Carboxymethyl group. [d] Geminal dimethyl groups (6 H) of the acetonide. [e] Aldehyde (1 H). [f] H-18. [g] C-17 and C-18 methyl groups (6 H).

aldehyde[22] [**10**, nmr δ 9.84 (aldehyde) and 2.15 (methyl ketone)] by treatment with sodium periodate, indicated the presence of the partial structure –CH(OH)C(OH)-(CH_3)– in carolenalin. Dehydrogenation of **1** with 30% palladium on carbon led to the expected chamazulene (**11**), which was characterized as the trinitrobenzene adduct (mp 128–129°, $C_{14}H_{16} \cdot C_6H_3O_6N_3$) and was shown to be identical with an authentic sample of chamazulene trinitrobenzene adduct[23] by mixture melting point determination and direct infrared spectral comparison.

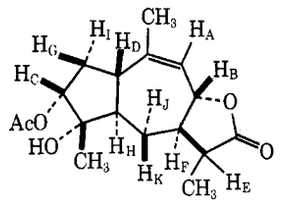
Added confirmation for the assignment of structure **1** to carolenalin was obtained by detailed double resonance experiments of monoacetate **2**. When the C-8 lactonic proton (H_B) at 515 Hz was irradiated, the vinyl proton at C-9 (H_A) collapsed to a broad singlet. Irradiation of the C-1 proton (H_D) at 322 Hz caused the signal for the lactonic proton (multiplet) at C-8 (H_B) to collapse to a quartet with J_{AB} = 4.0 and J_{BF} = 7.2 Hz. The coupling constant of the protons at C-7 and C-8 ($J_{7,8}$ = 7.2 Hz) suggested the trans-fused lactone ring. Dreiding models of this compound indicate the feasibility of this suggestion, since the conformation of the seven-membered ring is very rigid. Additional spin-decoupling studies are summarized in Table II.

The conformation of carolenalin monoacetate (**12**) was determined by nuclear Overhauser effect (NOE)

(22) The attempted purification of this compound was unsuccessful.

(23) The authors are indebted to Dr. Ken-ichi Takeda, Shionogi Research Lab, Osaka, Japan, for a generous sample of chamazulene trinitrobenzene adduct.

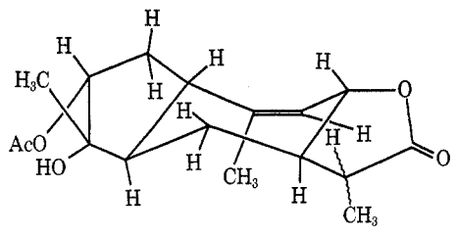
TABLE II
DOUBLE-RESONANCE (100 MHz) NMR DATA FOR CAROLENALIN MONOACETATE[a]



Original proton signal	Position of irradiation, Hz	Signal change after irradiation
H_A m	265 (H_E, H_F)	dd (2.3, 4.0)
	322 (H_D)	d (4.0)
	515 (H_B)	s (broad)
H_B m	265 (H_E, H_F)	dd (4.0, 1.8)
	322 (H_D)	dd (7.2, 4.0)
H_C dd (2.8, 5.0)	220 (H_G, H_I)	s
H_D m	210 ($H_{H,G,I}$)	s (broad)

[a] The double-resonance studies were done on a Varian HA-100 nmr spectrometer in $CDCl_3$ and the values differ slightly from those recorded in Table I, which were obtained from 60-MHz studies.

studies. Irradiation of the C-4 methyl signal at 113 Hz increased the intensity of the H-3 to 12%, suggesting that the C-4 methyl and the H-3 are cis to each other.



12

Irradiation of the H-1 proton at 320 Hz also produced a positive response (11% increase) on the H-8 proton signal, indicating a 1,4-diaxial relationship between these two protons. Thus, if the C_8 H configuration is β, as is likely, the C_1 H configuration is then β. Since the biogenetic evidence indicates that the naturally occurring guaianolide-type sesquiterpene lactones isolated so far all possess a β-oriented C-4 methyl group and an α-oriented C-7 proton, this consideration, coupled with the above observations, leads to the conclusion that the stereochemistry of carolenalin is as depicted in structure **1** (or **12**) in which the configuration of the C-11 methyl group remains to be determined. The configuration of C_5 H is assigned α since an alternative is not supported by models on the basis of a 1,4-diaxial relationship between H-1 and H-8.

The other new guaianolide carolenin (**13**) was also isolated as an oil. Carolenin showed a molecular ion peak at *m/e* 348 and a prominent peak at *m/e* 330 (M − 18) in the mass spectrum. The presence of an α,β-unsaturated ester group was revealed by an ir band at 1713 cm^{-1}, and by a characteristic ion of *m/e* 83 (base peak) [$COC(CH_3)=CH(CH_3)$] (*i.e.*, the cleavage of esters of angelic, tiglic, and senecioic acids).[24] That carolenin (**13**) is probably an angelate ester of carolenalin (**1**) was indicated by the nmr spectrum, in which the vinyl proton at C-18 of the ester moiety appeared as a one-proton multiplet at δ 6.10, a value which is characteristic of an angeloyl residue.[23,25] The two vinyl methyl groups (C-17 and C-18) appeared as one sharp singlet at δ 1.92 (6 H). Other nmr signals of **13** (Table I) are nearly identical in form and multiplicity with the signals for the corresponding groups of carolenalin (**1**). The downfield shift of the proton at C-3 (4.88, q, J = 3.0, 4.5 Hz) in **13** compared with that in the nmr spectrum of **1**, in which the H-3 signal appeared at δ 3.73 (t, J = 3.0 Hz), further indicated that this angeloyl side chain is located at the C-3 position. To further confirm the structure of carolenin, it was hydrolyzed with potassium hydroxide in methanol, with the formation of carolenalin (**1**) (identified with an authentic sample by ir comparison), a C-11 epimer of carolenalin,[26] and an angelic acid which was isolated as *p*-phenylphenacyl ester and was shown to be identical with an authentic sample of *p*-phenylphenacyl angelate by mixture melting point determination. The foregoing evidence leads to the assignment of structure **13** for carolenin beyond doubt.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and were corrected. Unless otherwise specified, optical rotations were determined on a Perkin-Elmer 141 polarimeter. Ultraviolet (uv) spectra were determined on a Cary Model 15 spectrophotometer. Infrared (ir) spectra were measured in chloroform with a Perkin-Elmer 257 grating infrared spectrophotometer. Mass spectra were determined on an A. E. I. MS-902 instrument at 70 eV using a direct inlet system. We thank Mr. F. Williams of the Research Triangle Center for Mass Spectrometry for these determinations. Silica gel and neutral alumina for column chromatography refers to Baker A. R. No. 3405 and Bio-Rad neutral alumina AG-7 (100–200 mesh), respectively; and silica gel for thin layer chromatography (tlc) refers to Merck silica gel G developed with chloroform–acetone (3:1) and visualized by spraying with concentrated sulfuric acid and heating. Silica gel for preparative tlc refers to Merck silica gel GF-254. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga.

Isolation of Carolenin (13) and Carolenalin (1).—The *Helenium autumnale* L. (*Compositae*) used was from a collection made on July 26, 1971, along the meadow approximately 0.4 mile south of Tar River station and east of US 15 in Granvilk County, N. C.[27] The ground, air-dried, whole plant material (40 lb) was exhaustively extracted with chloroform and worked up in the usual manner.[28] This gave 359 g of a dark-brown syrup. Tlc showed that this crude extract was a mixture of two major components. This extract was then dissolved in a minimum amount of benzene and chromatographed over 1.5 kg of silica gel, using benzene, ethyl acetate, and ethyl acetate–methanol as eluents. The first 3.5 l. of benzene (fractions 1–7) eluted minor quantities of nonpolar substances. The subsequent 10 l. of benzene eluate (fractions 8–28) afforded a mixture of the above-mentioned two components (carolenin and carolenalin). The polar fractions 29–35

(24) T. A. Geissman and T. S. Griffin, *Rev. Latinoamer. Quim.*, **2**, 81 (1971), and references cited therein.

(25) (a) It should be noted that the formation of the tiglate is due to the isomerization of the angelate during the alkaline hydrolytic procedure. We assigned the vinyl proton signal of carolenin to an angelate instead of a tiglate because the observed characteristic chemical shift of the former (δ 6.18) is comparable with those of the angeloyl esters of tomentosin,[24] eriofertin,[25b] and fastigilin A[25c] in the nmr spectra. (b) T. Saitoh, T. A. Geissman, T. G. Waddell, W. Herz, and S. V. Bhat, *Rev. Latinoamer. Quim.*, **2**, 69 (1971), and references cited therein. (c) W. Herz, S. Rajappa, S. K. Roy, J. J. Schmid, and R. N. Mirrington, *Tetrahedron*, **22**, 1907 (1966).

(26) The cooccurrence of carolenalin and carolenin, coupled with the fact that this epimeric compound was only isolated in small quantity, led to the conclusion that carolenin is the angelate ester of carolenalin and not of the C-11 epimer of carolenalin.

(27) The authors wish to thank Mr. S. W. Leonard, Department of Botany, University of North Carolina at Chapel Hill, for collecting and identifying the plant material. A Voucher specimen has been placed in the herbarium of the Botany Department, UNC-CH.

(28) K. H. Lee, S. Matsueda, and T. A. Geissman, *Physochemistry*, **10**, 405 (1971).

(ethyl acetate, 3 l.) and 36–40 (ethyl acetate–methanol, 6:1, 2 l.) yielded a mixture of mainly carolenalin and very small amounts of more polar substances whose structures are now under investigation.

The brown syrup obtained from fractions 8–28 (192 g) was rechromatographed on silica gel (1 kg). Elution with benzene (19.5 l.) gave 71.8 g of brown oil (crude carolenin). The tlc analysis of this oil showed only a faster moving single spot. One rechromatography of this oil (20 g) on silica gel (200 g) using benzene and benzene–chloroform as eluents afforded the pure carolenin (0.97 g)[29] (**13**): $[\alpha]^{25}D$ − 101.9° (*c* 0.598, MeOH); ir bands at 3586 (hydroxyl), 1765 (γ-lactone), 1713 (α,β-unsaturated ester carbonyl), and 1643 cm^{-1} (unsaturation); nmr data listed in Table I.

The materials remaining in the column after the removal of carolenin were further eluted with chloroform (6 l.) to yield carolenalin (**1**)[29] as a brown, viscous oily substance (100 g) which gave primarily one slower moving spot on the thin layer chromatogram using different solvent systems.

Carolenalin Monoacetate (**2**).—Carolenalin (5 g) was acetylated with acetic anhydride and pyridine for 24 hr at room temperature to yield after two crystallizations from ethyl acetate 3.53 g of monoacetate **2**: mp 160–161°; $[\alpha]^{25}D$ − 91.7° (*c* 1.002, MeOH); uv (EtOH) end absorption; ir bands at 3580 (hydroxyl), 1760 (γ-lactone), 1740 (acetyl), and 1640 cm^{-1} (unsaturation).

Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85. Found: C, 66.23; H, 7.69.

Carolenalin Diacetate (**3**).—Carolenalin (5 g) was acetylated with acetic anhydride and pyridine at room temperature for 72 hr to give a mixture of mono- and diacetates **2** and **3**. The latter was isolated by column chromatography on silica gel (100 g) using benzene and benzene–chloroform as eluents. Appropriate fractions were combined to yield after recrystallization from chloroform–*n*-hexane 1.2 g of the diacetate **3**: mp 146–148°; uv (EtOH) end absorption; ir bands at 1762 (γ-lactone) and 1740 cm^{-1} (double strength, acetyl).

Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.12; H, 7.48. Found: C, 64.77; H, 7.48.

Catalytic Hydrogenation of Carolenalin Monoacetate (**2**). **A. Dihydrocarolenalin Monoacetate** (**4**).—A solution of carolenalin monoacetate (200 mg) in methanol (15 ml) was hydrogenated at room temperature and atmospheric pressure using platinum oxide as catalyst. After 2 hr the catalyst was removed by filtration and the solvent was evaporated *in vacuo* to yield a residue. This residue was crystallized from *n*-hexane–ether (1:1) and recrystallized from ether containing a small amount of *n*-hexane to give the pure dihydrocarolenalin monoacetate (**4**) as colorless prisms (13 mg), mp 221–222°.

Anal. Calcd for $C_{17}H_{26}O_5 \cdot {}^1/_2H_2O$: C, 63.95; H, 8.15. Found: C, 64.19; H, 8.18.

B. Acid 5.—The filtrate after the removal of **4** was further concentrated to give another crystalline product which, upon recrystallization from *n*-hexane containing a small amount of ether, afforded the acid **5** as colorless needles (136 mg): mp 125–126°; ir bands at 3590 (hydroxyl), 1725 (acetyl), and 1700 cm^{-1} (acid).

Anal. Calcd for $C_{17}H_{28}O_5$: C, 65.36; H, 9.03. Found: C, 65.29; H, 9.05.

Methylation of Acid 5.—The acid **5** (54 mg) was methylated with diazomethane in the usual manner. The product crystallized and was collected and recrystallized from *n*-hexane–ether (1:1) to give 50 mg of the methyl ester **6** as colorless needles: mp 87–88°; ir bands at 3590 (hydroxyl) and 1725 cm^{-1} (double strength, ester and acetyl carbonyl).

Anal. Calcd for $C_{18}H_{30}O_5$: C, 66.26; H, 9.20. Found: C, 66.07; H, 9.32.

Oxidation of Carolenalin (**1**). **Keto Acid 7**.—A solution of carolenalin (250 mg) in acetone (20 ml) was cooled to 10–12° with stirring and treated with 0.5 ml of Jones reagent. After 10 min the solution was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dried (Na_2SO_4), and evaporated to afford a crystalline residue which was recrystallized from ether to give **7** as colorless prisms (190 mg): mp 171–172°; ir bands at 3500 (broad, hydroxyl), 1765 (γ-lactone), and 1710 cm^{-1} (acid).

(29) Although carolenin and carolenalin could not be induced to crystallize, purification has been achieved by repeated column chromatography over silica gel.

Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.57; H, 7.46.

Methylation of Keto Acid 7.—The keto acid **7** was methylated in the manner described for compound **6**. The product formed colorless oil **8**: ir bands at 1765 (γ-lactone), 1730 (ester), and 1710 cm^{-1} (methyl ketone).

Carolenalin Acetonide (**9**).—To a solution of carolenalin (100 mg) in acetone (10 ml) was added *p*-toluenesulfonic acid (5 mg). After 5 min at room temperature the reaction mixture was diluted with water and extracted with chloroform. The dried chloroform extract was evaporated to give an oil which was passed through a column of neutral alumina (3 g) and eluted with ether to yield **9** as a colorless oil (80 mg). Tlc analysis of this oil showed a single spot, although it could not be crystallized. It showed ir absorption at 1760 (γ-lactone) and 1655 cm^{-1} (unsaturation).

Treatment of Carolenalin (**1**) **with Sodium Periodate. Keto Aldehyde 10**.—A solution of carolenalin (418 mg) in ethanol (20 ml) was added to a solution of sodium periodate (500 mg) in water (2 ml) containing concentrated sulfuric acid (0.5 ml). After standing at 38–40° for 30 min the reaction mixture was diluted with water, extracted with chloroform, and dried (Na_2SO_4). The dried chloroform extract was evaporated to furnish a yellowish, oily substance (**10**) which could not be induced to crystallize. Compound **10** showed a single spot on tlc (ethyl acetate–acetone, 3:1) and had ir absorption at 1765 (γ-lactone), 2730, 2830, 2880 (weak), and 1720 cm^{-1} (aldehyde).

Dehydrogenation of Carolenalin (**1**).—A mixture of carolenalin (3.3 g) and 30% palladium on carbon (500 mg) was heated at 200–300° for 10 min and then at 300–320° for 7 min. After cooling, the reaction mixture was extracted with ether, and the ether extract was chromatographed on neutral alumina. The first 30 ml of the ether eluate was further rechromatographed on neutral alumina. Elution with *n*-hexane (50 ml) afforded **11** as a dark blue oil (37 mg) which was converted to the crystalline trinitrobenzene complex, mp 128–129° (ethanol), ir (Nujol) bands at 1615 and 1535 cm^{-1} (unsaturation).

Anal. Calcd for $C_{20}H_{19}O_6N_3$: C, 60.45; H, 4.82. Found: C, 60.24; H, 4.92.

A mixture melting point with authentic chamazulene–trinitrobenzene adduct[23] showed no depression and the ir spectra were identical.

Hydrolysis of Carolenin (**13**).—A solution of carolenin (130 mg) in methanol (3 ml) and 10% potassium hydroxide (0.6 ml) was refluxed for 40 min. The reaction mixture was diluted with water, acidified with concentrated hydrochloric acid, and extracted with chloroform. The chloroform extract was further washed with 5% sodium bicarbonate solution in order to separate the acidic and the neutral fractions. The neutral fraction (the chloroform extract) was dried (Na_2SO_4) and evaporated *in vacuo* to give an oil which showed a mixture of two compounds on a thin layer chromatogram. The separation of this mixture of compounds was achieved by preparative tlc (silica gel, ethyl acetate–acetone, 6:1). One of them (90 mg) was identified as carolenalin (**1**) by tlc and ir and nmr spectral comparison with the authentic sample. The other was obtained as a colorless oil (35 mg) which showed an nmr spectrum virtually identical with that of carolenalin except for the slight differences of the chemical shifts and coupling constants in the methyl group at the C-11 position. The C-11 methyl group, which was seen as a three-proton doublet at δ 1.19 (J = 7.5 Hz) in carolenalin, was shifted to 1.23 (J = 6.0 Hz). This oily substance was believed to be the C-11 epimer of carolenalin.

The acidic fraction (the aqueous sodium bicarbonate layer) was acidified with dilute hydrochloric acid and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4) and evaporated to yield an oil, which was converted to the crystalline *p*-phenylphenacyl ester in the usual manner. The crude crystalline product was purified by preparative tlc (silica gel, benzene) to give *p*-phenylphenacyl angelate (mp 81–83°, 20 mg) and *p*-phenylphenacyl tiglate (mp 87–90°, 4 mg),[25a] which were identified by direct ir comparison, respectively.

Registry No.—**1**, 38769-25-4; **2**, 38769-26-5; **3**, 38769-27-6; **4**, 38769-28-7; **5**, 38769-29-8; **6**, 38769-30-1; **7**, 38769-31-2; **8**, 38769-32-3; **9**, 38769-33-4; **10**, 38769-34-5; **11** trinitrobenzene salt, 4955-13-9; **13**, 38769-36-7; *p*-phenylphenacyl angelate, 16193-68-3; *p*-phenylphenacyl tiglate, 19451-66-2.