[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE FLORIDA STATE UNIVERSITY]

The Structure of Tenulin

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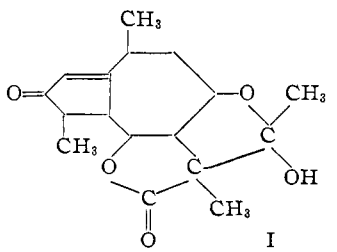
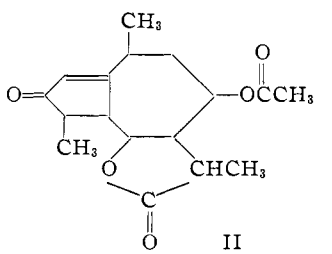
Evidence is presented for structures of tenulin and isotenulin which differ in one detail from formulas proposed recently. The relationship between isotenulin and helenalin is discussed.

Tenulin, $C_{17}H_{22}O_5$, the bitter principle of *Helenium tenuifolium* and several other *Helenium* species, has been investigated by several authors,[2,3] but little headway has been made toward elucidating its structure. A close relationship to helenalin, $C_{15}H_{18}O_4$, the azulenogenic bitter principle[4] of *Helenium autumnale*, was suspected, and since the plant occurs in the vicinity of Tallahassee, we initiated a study of its properties some time ago. A recent communication by Barton and de Mayo[5] in which structures I and II are proposed for tenulin and its isomeride isotenulin prompts us to record our findings at this time.



I



II

In many respects our observations and conclusions duplicate the results of the English workers and will therefore not be discussed in detail. Ultraviolet[3] and infrared spectra of tenulin and its derivatives indicated a monosubstituted cyclopentenone system, a γ-lactone and a tertiary hydroxyl group which disappeared on isomerization of tenulin to isotenulin.[2,6] Isotenulin had the same cyclopentenone system as tenulin but had a new acetate group. That the rearrangement did not involve the five-membered carbonyl group and double bond of tenulin was shown in the manner described also by the English workers,[5] and we therefore postulated the existence of the interesting hemiketal structure also shown in I which is converted to II by alkali. Oxidation of desacetyldihydroisotenulin (III) which showed infrared bands ($CHCl_3$) at 1770 (γ-lactone) and 1732 cm.$^{-1}$ (cyclopentanone) and typical hydroxyl absorption near 3500 cm.$^{-1}$ gave a diketone, dehydrodesacetyldihydroisotenulin (IV) (also prepared by catalytic reduction of dehydrodesacetylisotenulin), whose infrared spectrum ($CHCl_3$) showed bands at 1778 (γ-lactone), 1758 (somewhat displaced cyclopentanone) and 1710 cm.$^{-1}$ (normal aliphatic or alicyclic ketone). This indicated that the hydrolyzable acetyl function of isotenulin esterifies a secondary hydroxyl group which is not located in the cyclopentenone ring. Its assignment to C_6 of the guaiane system[7] is discussed in the sequel.

We next turned our attention to the determination of the carbon skeleton of tenulin which, in analogy to that of helenalin,[8] was assumed to be derived from guaiane. Pyrolysis of tenulin (under conditions which according to Clark[2] also yield pyrotenulin) indeed furnished chamazulene, but the small yield (7 mg. of TNB complex from 3.5 g. of tenulin) suggested caution in placing too much emphasis on this result since a small amount of an azulenogenic impurity associated with tenulin might have been responsible.[9] Consequently considerable effort was devoted to the stepwise removal of functional groups from the tenulin nucleus in order to obtain substances more amenable to dehydrogenation. These efforts failed to produce azulenes but resulted in the isolation of several compounds which may prove to be of importance in relating tenulin to other azulenogenic sesquiterpenes. At the same time they permitted assignment of the acetoxy group to C_6.

The hydrolysis of dihydroisotenulin according to Clark[2] furnishes desacetyldihydroisotenulin (III), m.p. 196–197°, $[\alpha]D$ +150.7°. We were able to isolate considerable quantities of an isomeric substance V, m.p. 155–156°, $[\alpha]D$ +3.0, infrared bands ($CHCl_3$) at 3550 (hydroxyl), 1778 (γ-lactone) and 1756 cm.$^{-1}$ (cyclopentanone; the mull spectrum showed this at a more normal position of 1743

(1) To whom inquiries concerning this article should be sent.

(2) E. P. Clark, THIS JOURNAL, **61**, 1836 (1939); **62**, 597, 2154 (1940).

(3) H. E. Ungnade and E. C. Hendley, *ibid.*, **70**, 3921 (1948); H. E. Ungnade, E. C. Hendley and W. Dunkel, *ibid.*, **72**, 3818 (1950).

(4) R. Adams and W. Herz, *ibid.*, **71**, 2546, 2551, 2554 (1949).

(5) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 142 (1956).

(6) In our hands the isomerization of tenulin to isotenulin is carried out most smoothly (in yields ranging from 55–70%) by adding a methanolic solution of tenulin heated to 60° to water maintained at the same temperature, followed by addition of a 1% solution of sodium hydroxide. The various methods described by Clark gave quite erratic results.

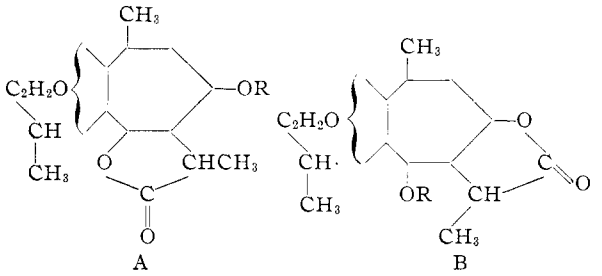
(7) For a recent review of azulenogenic sesquiterpenes see A. J. Haagen-Smit, "Sesquiterpenes and Diterpenes," Fortschritte der Chemie Organischer Naturstoffe, Vol. XII, Springer Verlag, Vienna, 1955, pp. 1–43.

(8) We wish to thank Dr. G. Büchi of the Massachusetts Institute of Technology for informing us of his work on helenalin prior to publication.

(9) Barton and de Mayo obtained similarly small amounts of chamazulene (from the crude potassium borohydride reduction product of isotenulin) and linderazulene (from the crude lithium aluminum hydride reduction product of dihydroisotenulin). In our hands the dehydrogenation of the latter did not furnish any azulene. A crystalline triol prepared by us from desoxodesacetyldihydroalloisotenulin (VII) also could not be dehydrogenated satisfactorily.

cm.$^{-1}$), which was at first thought to be an epimer of III. Acetylation converted V to VI, an isomer of dihydroisotenulin, m.p. 176.5–178°, $[\alpha]_D$ +3.2, bands ($CHCl_3$) at 1779 (γ-lactone) and 1747 cm.$^{-1}$ (combined acetoxy and cyclopentanone). Wolff–Kishner reduction converted dihydroisotenulin, IV and V to the same hydroxylactone $C_{15}H_{24}O_2$ (VII), m.p. 157–158°, $[\alpha]_D$ −48.4, infrared bands at 3600 (hydroxyl) and 1760 cm.$^{-1}$ (γ-lactone) which gave an acetate VIII.

That V and VI are in fact not epimers of the corresponding compounds of the isotenulin series (partial structure A) but have the partial structure B was shown in the following manner

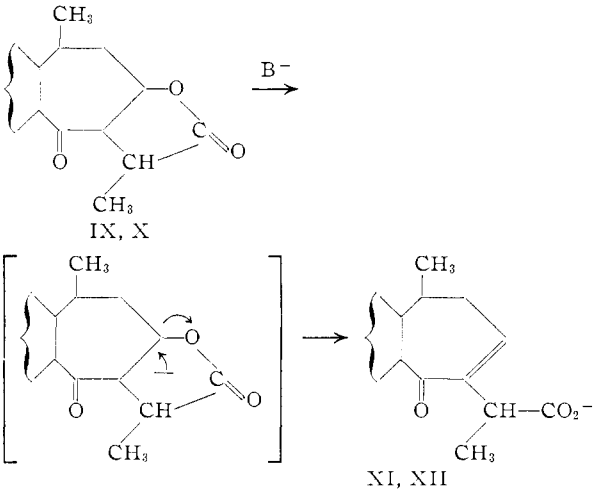


A B

Dihydroisotenulin and desacetyldihydroisotenulin (III) contain the grouping $-CH_2C-$ (infrared band at 1410 cm.$^{-1}$[10] formation of a monopiperonylidene derivative from III). The isomer V (B, R = H) also has one active methylene group (band near 1410 cm.$^{-1}$, monopiperonylidene adduct), but whereas III (A, R = H) contains *two* active methylene groups as expected (relatively stronger band, dipiperonylidene derivative), compound IX, obtained from V by oxidation with chromic acid, has only *one* such function (monopiperonylidene derivative), infrared bands ($CHCl_3$) at 1778 (γ-lactone), 1744 (cyclopentanone), 1710 (cycloheptanone) and 1412 cm.$^{-1}$($-CH_2C-$). The hydroxylactone VII, as expected, has no 1410 cm.$^{-1}$ band and does not condense with piperonal, but *neither does* a ketolactone $C_{15}H_{22}O_3$ (X), infrared bands ($CHCl_3$) at 1762 (γ-lactone) and 1707 cm.$^{-1}$ (cycloheptanone), prepared from VII by chromic acid oxidation. A negative Zimmermann[11] test provided confirmatory evidence for the absence of the active methylene group in X.

These observations demonstrate that V, VII, IX, X and the compounds derived from them must be assigned to a new series (the so-called alloisotenulin series[12]) which differs from the isotenulin series in that the free hydroxyl group and the hydroxyl group involved in lactone formation have been interchanged. The carbon atoms carrying the hydroxyl groups must occupy the relative positions shown in the partial formulas since the infrared spectra of both series demand a γ-lactone ring.

Further evidence for this relationship was provided when the monoketolactone X and the diketolactone IX were subjected to the action of sodium carbonate. X furnished an acidic, α,β-unsaturated ketone (XII, λ_{max} 231 mμ, ϵ_{max} 8100) whose infrared bands ($CHCl_3$) occurred at 1705 (carboxyl) and 1675 cm.$^{-1}$ (cycloheptenone), while IX yielded an analogous acidic diketone (XI, λ_{max} 242 mμ, shoulder at 292 mμ presumably due to a cyclopentanone group, ϵ_{max} 5500) whose infrared spectrum exhibited bands at 1740 (cyclopentanone) 1711 (carboxyl) and 1676 cm.$^{-1}$ (cycloheptenone).[13] The results are consistent with the formulation shown below[14,15]



IX, X

XI, XII

Catalytic reduction of XII gave two optically active crystalline substances. The first (XIII) was a ketoacid $C_{15}H_{24}O_3$ (carboxyl and cycloheptanone frequency at 1700 cm.$^{-1}$, negative Zimmermann test). The second was a saturated lactone of formula $C_{15}H_{24}O_2$ (XIV, lactone band at 1764 cm.$^{-1}$).

(10) R. N. Jones and A. R. H. Cole, THIS JOURNAL, **74**, 5648 (1952).

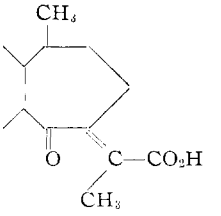
(11) W. Zimmermann, *Z. physiol. Chem.*, **233**, 257 (1935). Compounds III, IV, V, VI and IX gave a positive test.

(12) This name seems preferable to the simpler term "allotenulin" because the parent compound differs from tenulin in two gross structural features and from isotenulin in only one.

(13) The Nujol mull spectrum of this substance had only two bands at 1740 (cyclopentanone and carboxyl) and at 1648 cm.$^{-1}$ (bonded cycloheptenone).

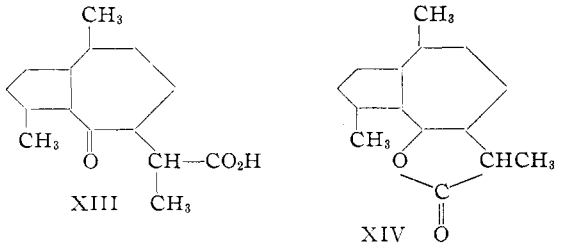
(14) An analogous reaction has been observed recently by W. G. Dauben and P. D. Hance, THIS JOURNAL, **77**, 606 (1955).

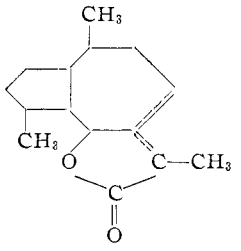
(15) E. J. Corey, H. J. Burke and W. A. Remers, *ibid.*, **78**, 180 (1956), report a maximum of 239.5 mμ for γ, δ-dihydroeucarvone, an analogous compound. Woodward's rule predicts a value near 235 mμ. The difference in the ultraviolet maxima of XI and XII might conceivably be explained by assuming that the double bond in one of the two compounds is exocyclic, as shown in the partial formula. Alter-



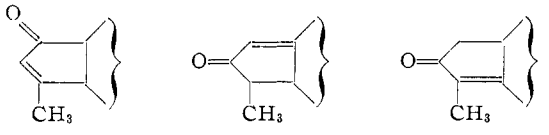
natively, the double bond might be exocyclic in both XI and XII, with *cis-trans* isomerism around the double bond accounting for the shift. This latter possibility might be expected to affect the infrared spectrum of one of the isomers due to intramolecular hydrogen bonding between carboxyl and keto groups; this was not observed. Published spectra of certain α,β-unsaturated-γ-ketoacids (E. Kyburz, B. Riniker, H. R. Schenk, H. Heusser and O. Jeger, *Helv. Chim. Acta*, **36**, 1891 (1953); L. F. Fieser, THIS JOURNAL, **75**, 4386 (1953)) are not very helpful since the maxima coincide with those of the α,β-unsaturated ketones; in general, however, the extinction coefficients are considerably higher than those of XI and XII. It is hoped that his question can be decided satisfactorily when more material becomes available.

Compound XIV is of interest because, to our knowledge, it represents the first crystalline guaianolide isolated from a natural source. Structures XIII and XIV have been assigned to oils obtained in the course of work on carpesia lactone[16]; another oil of probable formula XIV was obtained by reduction of a substance $C_{15}H_{20}O_3$ which occurs in *Artemisia absinthium*.[17] Reduction of XII with sodium borohydride resulted in an oily, unsaturated γ-lactone whose infrared spectrum (lactone band at 1750, double bond at 1667 cm.$^{-1}$) does not permit a distinction between the isomeric structures shown but would appear to rule out a β,γ-unsaturated γ-lactone structure in which the double bond is endocyclic.[18]





There remained the problem of locating the carbonyl group in the unsaturated five-membered ring of tenulin and isotenulin. The evidence presented so far allows of only two possible formulations, C and D. We selected C for the following reason.



Several years ago, one of us[19] discovered that dehydrotetrahydrohelenalin,[4] $C_{15}H_{20}O_4$, underwent a remarkable transformation under the influence of base. Analysis of the crystalline, optically active acidic product XV and of its derivatives indicated the formula $C_{15}H_{22}O_5$, but it quickly developed that this compound was not merely the hydroxy acid formed by the opening of the lactone ring. Titration and the preparation of esters established the presence of two carboxyl groups. The ultraviolet spectrum (λ_{1max} 236 mμ, λ_{2max} 318 mμ, ϵ_1 6650, ϵ_2 54) suggested the presence of a dialkyl-substituted straight chain or monoalkylated ring α,β-unsaturated ketone chromophore different from the cyclopentenone system of helenalin. The infrared spectrum (Nujol mull) had two significant bands at 1703 (assigned to the carboxyl groups since the esters exhibit instead the usual ester frequency near 1740 cm.$^{-1}$) and at 1648 cm.$^{-1}$. The latter undoubtedly represents the conjugated keto group; its low frequency is probably caused by intramolecular hydrogen bonding. For when the carboxyl groups are protected, as in the esters, the ketone band moves up to 1666 cm.$^{-1}$ and when the conjugated double bond is reduced, the spectrum contains a normal ketone band at 1700 cm.$^{-1}$. Further work dealing with this acid is described in the Experimental Part.

We have now found that *dehydrodesacetyldihydroisotenulin (IV) undergoes the same transformation* on treatment with sodium carbonate solution. The product XVI was amorphous, possibly a mixture of stereoisomers, λ_{max} 235 mμ (monosubstituted alicyclic α,β-unsaturated ketone) whose infrared spectrum (Nujol mull) had bands at 1703 (carboxyl) and 1648 cm.$^{-1}$ (hydrogen-bonded conjugated ketone), bands in $CHCl_3$ at 1709 and 1663 (normal α,β-unsaturated ketone). The dimethyl ester, $C_{17}H_{26}O_5$ (λ_{max} 235 mμ, ϵ_{max} 8600), infrared bands at 1736 (carbomethoxy) and 1666 cm.$^{-1}$ (α,β-unsaturated ketone, moves up to 1705 cm.$^{-1}$ on hydrogenation) was directly comparable to the corresponding dimethyl ester of XV.

Now neither tetrahydrohelenalin nor desacetyldihydroisotenulin undergo such a change on treatment with base, and treatment of the dehydro compounds of the alloisotenulin series with sodium carbonate (*vide supra*) does not result in cleavage of the cyclopentanone ring. Therefore the conversions dehydrotetrahydrohelenalin → XV and dehydrodesacetyldihydroisotenulin → XVI are bound up with the position of the new cycloheptanone carbonyl, situated at C_6 of the tenulin skeleton,[20] relative to the old ketone group which is part of the five-membered ring. The interpretation which is presented in the formula chart on the next page does justice to all these requirements, places the cyclopentanone carbonyl uniquely at C_3 and requires formula XVII for tenulin.

The initial step requires no further comment since it already has been encountered in the allo series. The isomerization of XVIII to XIX is but one of several which permits the formation of a vinylogous β-diketone system that should be readily susceptible to cleavage under the influence of dilute base. XVIa has been selected as a possible structure which best meets the spectral characteristics of the products XV and XVI.

Barton and de Mayo[5] based their argument in favor of I on the isolation of a new substance, desacetylneotenulin, which is formed when tenulin is

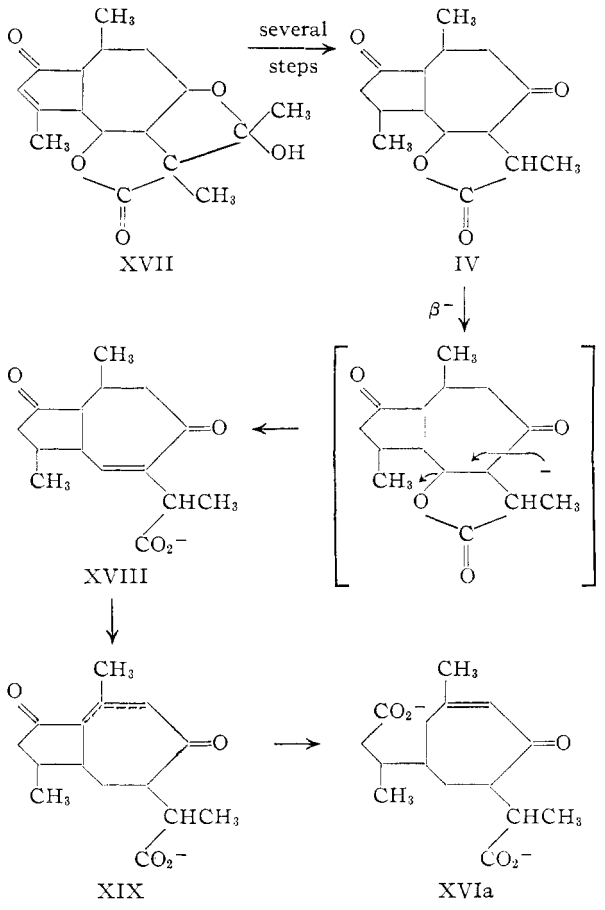
(16) T. Kariyone and S. Naito, *J. Pharm. Soc. Japan*, **75**, 39 (1955); S. Naito. *ibid.*, **75**, 93 (1955).

(17) V. Herout and F. Sorm, *Coll. Czech. Chem. Commun.*, **19**, 927 (1954).

(18) W. G. Dauben and P. D. Hance, THIS JOURNAL, **75**, 3352 (1953).

(19) We wish to thank Prof. Roger Adams for permission to publish the results of work carried out at the University of Illinois during the years 1947–1949 under his guidance.

(20) While there is ample evidence for locating the hydroxyl group of tenulin and isotenulin at C_6, the published work on helenalin offers no clues for doing the same. However, if the hydroxyl group of helenalin be placed at C_5, there is no clear mechanism by which the above transformation might occur, and it would be difficult to understand the close similarity between dehydrotetrahydrohelenalin and IV. It would appear, therefore, that dihydroisotenulin and acetyltetrahydrohelenalin are stereo- rather than structural isomers.

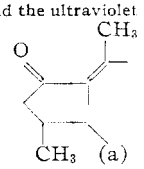


treated with sodium bicarbonate.[21] Desacetylneotenulin had a spectrum in harmony with partial structure E[22] and on prolonged ozonolysis furnished acetic acid. If desacetylneotenulin indeed possesses a structure based on E, its formation would be difficult to explain on the basis of our structure XVII. On the other hand, Barton and de Mayo's structure I appears to offer no simple *rationale* for the transformations which we have observed. We are presently engaged in trying to resolve this discrepancy.

Acknowledgments.—This work was supported in part by a grant from the National Science Foundation, for which we express our thanks. We also wish to thank Mr. Terry Shaw for his help in collecting and extracting the plant material.

(21) We had not encountered this substance in the course of our work. On attempting to repeat the procedure described by the English workers we isolated only desacetylisotenulin. Carrying out the reaction in a homogeneous medium formed by adding ethanol gave 45% of crude desacetylneotenulin.

(22) The infrared band at 1628 cm.$^{-1}$ has a frequency which is unusually high for conjugated C=C in a cyclopentenone ring. In the absence of infrared data for model compounds such as bicyclo-(5,3,0)dec-1(7)-en-8-one and 9-alkylbicyclo(5,3,0)-1(10)-en-9-ones, the conclusion that a trisubstituted cyclopentenone of type E absorbs in this range would be premature (however, see D. Arigoni, R. Viterbo, M. Dunnenberger, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **37**, 2306 (1954) for an analogous case). On the other hand the ultraviolet spectrum makes improbable structure (a), which satisfies the infrared spectrum and whose ozonolysis could conceivably give rise to acetic acid through oxidation of the hydroxyl group and cleavage of the resultant diketone. However, see A. Sandoval, J. Romo, G. Rosenkranz, St. Kaufmann and C. Djerassi, THIS JOURNAL, **73**, 382 (1951), for a comparable compound (their formula VII) which absorbs at 236 mμ.



(a)

Experimental[23]

Tenulin.—The procedure of Clark,[2] modified in several minor details, furnished tenulin in yields of 2.1–2.7%, m.p. in the range 185–190°, which was satisfactory for further work. Recrystallization from benzene furnished material which stubbornly retained solvent; recrystallization from butyl alcohol–butyl ether as described by Clark gave crystals melting in the range 185–206° and having $[\alpha]_D$ −24.5° to −25.8°. (Clark reports erratic m.p.'s in the range 175–196° and $[\alpha]_D$ −21.6.) The infrared and ultraviolet spectra (λ_{max} 226.5, 322 mμ, λ_{min} 275 mμ, log ϵ_{max} 3.90 and 1.63, log ϵ_{min} 1.23) of all samples were identical and corresponded to those reported in the literature.

Ungnade[3] has suggested that isotenulin as well as tenulin exists in the plant. Infrared spectra of the first crude chloroform extract and of the chloroform extract obtained after treatment of the crude extract with lead acetate indicated that other compounds contaminate tenulin, but they show no trace of the strong band at 1750 cm.$^{-1}$ which is characteristic of isotenulin.

Dihydrotenulin.—Reduction according to Clark gave an 80% yield of dihydrotenulin which formed a solvate when crystallized from benzene. Drying under a heat lamp removed the solvent and gave crystals, m.p. 178–181°, $[\alpha]_D$ +101.7° (95% ethanol, *c* 4.905), λ_{max} 290 mμ, λ_{min} 259 mμ, log ϵ_{max} 1.61, log ϵ_{min} 1.38. The substance was inert to Tollens reagent, contrasting with tenulin in this respect.

Lithium aluminum hydride reduction of dihydrotenulin gave two amorphous fractions, one chloroform-soluble whose infrared spectrum still indicated the presence of a carbonyl group and one chloroform-insoluble which was apparently a mixture of polyhydroxy derivatives. Attempts to dehydrate these fractions gave intractable material and several small-scale dehydrogenations did not result in the formation of an azulene.

Dehydrodihydrotenulin.—Oxidation of 3.1 g. of dihydrotenulin dissolved in 30 ml. of acetic acid with 0.7 g. of chromic acid was complete in 15 minutes on the steam-cone. The mixture was concentrated to a thick sirup, the sirup extracted with acetone, the acetone washings taken to dryness, the residue thoroughly washed with water and extracted with a small amount of acetone. The insoluble material, wt. 0.6 g., was crude dehydrodihydrotenulin. Recrystallization from ethanol furnished colorless crystals, m.p. 244–248.5° dec., $[\alpha]_D$ +27.6° (95% ethanol, *c* 0.545), +26.9° (*c* 0.26), infrared bands at 1770 (lactone), 1755 (double strength), 1702 and 1414 cm.$^{-1}$ (–CH_2C–). This substance is probably the dihydro derivative of a dehydrotenulin which Barton and de Mayo[5] obtained by chromic acid oxidation of tenulin, but comparison of the infrared spectra of dehydrodihydrotenulin and dehydrotenulin[5] raises some doubt as to the correctness of the formulation of the latter (their formula XXVII).

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.94; H, 7.23.

From the water washings there was obtained 0.75 g. of crude dehydrotenulin. The acetone washings yielded 0.9 g. of a mixture of starting material and product. The recovery of starting material suggested the use of more chromic acid. Oxidation of 1.5 g. of dihydrotenulin with 1 g. of chromic acid furnished 0.75 g. (50%) of crude product and no dihydrotenulin.

Isotenulin.—Of the several procedures described by Clark, the method which gave the most consistent results involved addition of a 1% sodium hydroxide solution to a solution of tenulin, recrystallized from alcohol–water, in aqueous methanol. The yields of isotenulin, m.p. 156–157.8° after recrystallization from butyl alcohol–butyl ether, averaged 55–70%. Further recrystallization raised

(23) Melting points and boiling points are uncorrected. Analyses by Drs. Weiler and Strauss, Oxford. Infrared spectra were run in chloroform solution, unless otherwise specified, on a Perkin–Elmer Model 21 instrument. Ultraviolet spectra were determined by Mrs. Shirley Ann Pinner on a Beckman Model DK recording spectrophotometer.

the m.p. to 156.5–158°, $[\alpha]_D$ +8.7° (95% ethanol, *c* 3.555), λ_{max} 225.5, 324 mμ, λ_{min} 270 mμ, log ϵ_{max} 3.93, 1.66, log ϵ_{min} 1.18, essentially as given by Ungnade.[3] The substance gave a positive Tollens and Zimmermann test.

The mother liquors of the isomerization yielded desacetylisotenulin in varying amounts. In one large-scale run, using 25.8 g. of tenulin, the aqueous mother liquor was strongly acidified, heated to boiling (to hydrolyze the mixture of isotenulin and desacetylisotenulin usually obtained at this point) and allowed to stand. The crystals which separated were washed with cold methanol; dilution of the filtrate with water furnished a colorless substance whose m.p. and high rotation indicated that it was different from tenulin and desacetylisotenulin. Repeated crystallization from aqueous methanol furnished a solid, m.p. 124–125° (with previous sintering), $[\alpha]_D$ +250°. Analysis and behavior above the m.p. indicated that it was a solvate. Drying at elevated temperatures caused softening.

Anal. (air-dried sample). Calcd. for $C_{15}H_{20}O_4 \cdot H_2O$: C, 63.80; H, 7.85. Found: C, 63.74; H, 7.65.

The ultraviolet spectrum (λ_{max} 225 mμ, 320 mμ, ϵ_{max} 9850) and the infrared bands at 3350 (hydroxyl), 1762 (lactone), 1695 (cyclopentenone) and 1592 cm.$^{-1}$ (C=C) indicated that this substance is probably an epimer of desacetylisotenulin. An attempted acetylation was not successful.

Catalytic reduction of 0.175 g. of this material in ethyl acetate (catalyst platinum oxide) showed the presence of one double bond. The product was recrystallized from aqueous methanol, m.p. 137–138°, $[\alpha]_D$ +13.5, infrared bands at 3400 (hydroxyl), 1770 (lactone), 1730 (cyclopentanone) and 1408 cm.$^{-1}$ ($-CH_2\overset{\|}{C}-$).

Anal. Calcd. for $C_{15}H_{22}O_4 \cdot H_2O$: C, 63.36; H, 8.51. Found: C, 63.17; H, 8.55.

Dehydrodesacetylisotenulin.—This compound, obtained by chromic acid oxidation of desacetylisotenulin, has also been described by Barton and de Mayo. In our hands it melted at 227–228°, $[\alpha]_D$ − 178° (95% ethanol, *c* 0.3985), infrared bands at 1700 (weak), 1745 and 1790 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.54; H, 6.85.

Catalytic reduction of this substance furnished dehydrodesacetyldihydroisotenulin (IV).

Dihydroisotenulin.—Reduction of isotenulin according to Clark furnished 90% of dihydroisotenulin, m.p. 145–150° after one recrystallization from acetone–cyclohexane. Further recrystallizations raised the m.p. to 149–150°, $[\alpha]_D$ +119.5° (95% ethanol, *c* 4.985, λ_{max} 290 mμ, λ_{min} 249 mμ, log ϵ_{max} 1.50, log ϵ_{min} 0.80). The substance gave a positive Zimmermann and a negative Tollens test.

Lithium aluminum hydride reduction of dihydroisotenulin gave a viscous glass. Dehydrogenation on a small scale did not furnish an azulene.

Hydrolysis of Dihydroisotenulin.—A mixture of 16 g. of dihydroisotenulin and 320 ml. of hot 10% sodium hydroxide solution was warmed until solution was complete. About 10 minutes were required. After addition of 450 ml. of cold water, the solution was cooled to room temperature, acidified with dilute sulfuric acid and chilled overnight. This precipitated 6.3 g. (45%) of crude desacetyldihydroisotenulin, m.p. 191.5–195°. The filtrate was extracted with five 40-ml. portions of chloroform.

Recrystallization of the crude product from 60 ml. of methanol and 60 ml. of water gave 5.6 g. of crystals, m.p. 196–197.8° (lit.[2] 203°), $[\alpha]_D$ +150.7° (95% ethanol, *c* 1.005).

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.60; H, 8.34.

Acetylation at room temperature with acetic anhydride–pyridine furnished dihydroisotenulin.

The initial chloroform extract was concentrated *in vacuo*. The residue, wt. 6.2 g. (45%), on being taken up in acetone and diluted with cyclohexane furnished 3.8 g. of crude desacetyldihydroalloisotenulin (V), m.p. 152–155°. Further crystallizations raised the m.p. to 154.8–156.5°, $[\alpha]_D$ +3.0 ± 1.2° (95% ethanol, *c* 1.68).

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.33; H, 8.31.

A solution of 100 mg. of desacetyldihydroisotenulin, 65 mg. of piperonal, 5 ml. of ethanol and 5 ml. of ethanolic hydrogen chloride was allowed to stand overnight. Dilution with water gave 140 mg. of crystals, m.p. 185.5–187°. Recrystallization from dilute ethanol resulted in colorless needles, m.p. 186.2–188°. The analysis showed association of the piperonylidene group with one-half a molecule of ethanol which appears to be characteristic of this series of compounds.

Anal. Calcd. for $C_{23}H_{26}O_6 \cdot {}^1/_2C_2H_6O$: C, 68.54; H, 6.90. Found: C, 68.55; H, 7.01.

A run with two equivalents of piperonal gave the same product. Reaction of V with piperonal in the manner described above gave, on repeated recrystallization from dilute ethanol, material which sintered at 127° and melted unsharply at 132–134° (capillary), 127° (Kofler). Attempts to improve the m.p. by changing the solvent failed. The analytical sample was recrystallized from alcohol–water and again stubbornly retained solvent. Excess piperonal gave the same compound.

Anal. Calcd. for $C_{23}H_{26}O_6 \cdot {}^1/_2C_2H_6O \cdot H_2O$: C, 65.58; H, 7.11. Found: C, 65.73; H, 6.80.

When 5% sodium hydroxide was used for the hydrolysis, the yield of desacetyldihydroisotenulin was raised to 75% and little V was formed.

Dihydroalloisotenulin (VI).—Acetylation of desacetyldihydroalloisotenulin (V) with acetic anhydride in pyridine gave, on dilution with water, an oil which solidified on scratching. Recrystallization from acetone–cyclohexane resulted in material of m.p. 142–143.5°, $[\alpha]_D$ +3.2 ± 1.3° (95% ethanol, *c* 1.545).

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85. Found: C, 66.21; H, 7.96.

Dehydrodesacetyldihydroisotenulin (IV).—A solution of 5.5 g. of desacetyldihydroisotenulin in a little acetic acid was treated with 1.6 g. of chromic acid. Dilution with water furnished crystals which were washed thoroughly with water. The filtrate was extracted with chloroform and evaporation of the chloroform extracts furnished additional material. Recrystallization from acetone–cyclohexane gave 4.2 g. of product, m.p. 144–146.5°. The analytical sample melted at 145.5–147°, $[\alpha]_D$ +10.1 ± 2.9° (95% ethanol, *c* 0.695). Recrystallization from alcohol–water gave a solvate which must be dried at 120° *in vacuo* to give satisfactory analytical values.

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.29; H, 7.91.

The piperonylidene derivative was prepared by using two moles of piperonal per mole of diketone, yield 109 mg. of colorless needles (from 100 mg. of starting material), m.p. 264–265°. Again, each piperonylidene group appeared to be associated with half a molecule of ethanol.

Anal. Calcd. for $C_{31}H_{28}O_8 \cdot C_2H_6O$: C, 68.97; H, 5.96. Found: C, 68.75; H, 5.89.

Dehydrodesacetyldihydroalloisotenulin (IX).—Oxidation of 3.56 g. of V with 1 g. of chromic acid gave 3.45 g. of crude material which was recrystallized twice from ethanol, yield 2.66 g., m.p. 175.5–178.2°. The analytical specimen melted at 176.5–178.2°, $[\alpha]_D$ −105.4° (95% ethanol, *c* 1.06).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.37; H, 7.74.

The piperonylidene derivative was prepared in the usual manner, using one equivalent of piperonal. Repeated crystallization from ethanol gave crystals which began to melt at 167–168° (Kofler) but did not lose their shape until the temperature reached 170°. Use of excess piperonal also resulted in the above compound.

Anal. Calcd. for $C_{23}H_{24}O_6 \cdot {}^1/_2C_2H_6O$: C, 68.72; H, 6.49. Found: C, 68.51; H, 6.45.

Reaction of Dehydrotetrahydrohelenalin with Sodium Carbonate.—A mixture of 13.6 g. of dehydrotetrahydrohelenalin,[4] 28 g. of sodium carbonate and 280 ml. of water was warmed on the steam-bath for 2 hr. with occasional shaking to dissolve the diketoue. The mixture was chilled, acidified cautiously with hydrochloric acid and placed in the refrigerator. The solid was filtered and washed with water, yield 10.2 g., m.p. 122–130°. It was taken up in 50 ml. of hot ethyl acetate, treated with charcoal and chilled. Chilled

pentane was added in small portions until the mixture was faintly cloudy, a little ethyl acetate was added to remove the cloudiness and the flask was placed in the refrigerator. Chunky white crystals separated gradually, yield 7.1 g. (48%), m.p. 139–141°. Further purification yielded crystals of m.p. 142.5–143°, $[\alpha]_D$ +64.2 (95% ethanol, *c* 3.350).

Anal. Calcd. for $C_{15}H_{22}O_5$: C, 63.82; H, 7.86; neut. equiv., 141. Found: C, 63.85; H, 7.96; neut. equiv., 139.

The ethyl acetate–pentane mother liquors, on further dilution with pentane, deposited an oil which could not be crystallized although infrared analysis indicated that it consisted primarily of the dibasic acid. Ether extraction of the original aqueous filtrate yielded viscous material which also contained a large proportion of the desired product. The oils were combined, dissolved in ether and converted to the dimethyl ester by treatment with diazomethane. Distillation furnished a fraction, b.p. 155–160° (0.1 mm.), wt. 3.5 g., identical with material prepared directly from the pure acid. The infrared spectrum had bands at 1736 (carbomethoxy) and 1666 cm.$^{-1}$ (conjugated ketone). The ultraviolet spectrum (λ_{max} 236, 306 mμ, ϵ_{max} 7620, 76) resembled that of the parent acid.

Anal. Calcd. for $C_{17}H_{26}O_5$: C, 65.73; H, 8.45. Found: C, 65.85; H, 8.43.

An attempt to reduce the ester by refluxing with aluminum isopropoxide in isopropyl alcohol did not liberate acetone. Decomposition of the reaction mixture and distillation in a high vacuum (bath temperature 150–170°) yielded the diisopropyl ester of the acid.

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.83; H, 9.50.

The di-*p*-bromophenacyl ester of the acid was recrystallized from methanol and melted at 124–125°.

Anal. Calcd. for $C_{31}H_{32}O_7Br_2$: C, 55.04; H, 4.77; Br, 23.63. Found: C, 54.82; H, 4.82; Br, 23.34.

Reduction of the acid in ethyl alcohol, ethyl acetate or acetic acid, using palladium–charcoal, platinum oxide or Raney nickel, stopped after absorption of slightly less than one mole-equivalent of hydrogen. Removal of solvent yielded a sirup which could not be induced to crystallize and gave no crystalline derivatives with dinitrophenylhydrazine, hydroxylamine and *p*-bromophenacyl bromide. The double bond region of the infrared spectrum had only one band at 1700 cm.$^{-1}$. Treatment with diazomethane converted the acid to the dimethyl ester, b.p. 140–150° (bath temperature, 0.1 mm.), positive test with Brady's reagent, infrared bands at 1735 (carbomethoxy) and 1700 cm.$^{-1}$ (ketone). General absorption throughout the ultraviolet region masked a band appearing as a shoulder at approximately 285 mμ (ϵ 82) characteristic of a ketone group.

Anal. Calcd. for $C_{17}H_{28}O_5$: C, 65.36; H, 9.03. Found: C, 65.09; H, 8.96.

Sodium Carbonate Treatment of Dehydrodesacetyldihydroisotenulin.—A mixture of 2.0 g. of IV and 30 ml. of 10% sodium carbonate solution was heated on the steam-bath for 2 hr. with occasional shaking. The solution was cooled and extracted with chloroform. Acidification gave a gum which was extracted with chloroform. The washed extract was concentrated *in vacuo* and dried. The residual gum solidified gradually but could not be recrystallized satisfactorily. Its ultraviolet spectrum (λ_{max} 235, ϵ_{max} 6025) and positive test with Brady's reagent indicated the presence of an α,β-unsaturated ketone; the infrared spectrum has been discussed previously. Titration of the crude material gave a neutral equivalent of 160, calcd. for a dibasic acid, 141; $[\alpha]_D$ +44.2° (95% ethanol, *c* 0.754). Derivatives with dinitrophenylhydrazine, *p*-bromo- and *p*-phenylphenacyl bromide did not crystallize satisfactorily.

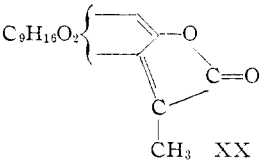
A solution of 0.63 g. of the gum in anhydrous ether was converted to the dimethyl ester with diazomethane. The product distilled at 170–180° (bath temperature, 0.8 mm.), $[\alpha]_D$ +60.6° (95% ethanol, *c* 0.429). The ultraviolet and infrared spectra of this substance have been discussed previously.

Anal. Calcd. for $C_{17}H_{26}O_5$: C, 65.73; H, 8.45. Found: C, 66.13; H, 8.50.

Catalytic reduction of the dimethyl ester established the presence of one double bond. The product, infrared bands at 1736 (double strength carbomethoxy) and 1705 cm.$^{-1}$ (normal ketone), boiled at 170–175° (bath temperature, 1.2 mm.), $[\alpha]_D$ −22.7° (95% ethanol, *c* 0.796).

Anal. Calcd. for $C_{17}H_{28}O_5$: C, 65.36; H, 9.03. Found: C, 65.51; H, 9.20.

In an attempt to purify the crude dibasic acid, it was distilled in a high vacuum. The main fraction boiled at 225–230° (0.8 mm.) as a highly viscous light-brown oil. The infrared spectrum and analysis indicated that lactonization had taken place (acid –OH at 3000–3600, lactone at 1750, shoulder near 1720 due to carboxyl, C=C at 1663 and 1625 cm.$^{-1}$). The ultraviolet spectrum (λ_{max} 290 mμ, ϵ 14250) was characteristic of a highly conjugated enol-lactone, possibly XX.



XX

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.74; H, 7.50.

Reaction of Dehydrodesacetyldihydroalloisotenulin with Sodium Carbonate.—A mixture of 0.5 g. of IX, 0.33 g. of sodium carbonate and 3.5 ml. of water was warmed in a stoppered flask until solution was complete. Acidification of the cooled solution precipitated an oil which was extracted with chloroform. The residue, wt. 0.5 g., crystallized on trituration with petroleum ether. Several recrystallizations from ethyl acetate–petroleum ether furnished material melting at 142.4–143.5°, $[\alpha]_D$ +92.3° (95% ethanol, *c* 1.38), +90.0° (*c* 3.405).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.19; H, 7.81.

The *p*-phenylphenacyl ester melted at 113–114°.

Anal. Calcd. for $C_{29}H_{30}O_5$: C, 75.96; H, 6.59. Found: C, 76.01; H, 6.76.

Excess dinitrophenylhydrazine was necessary to prepare a solid dinitrophenylhydrazone, possibly because two carbonyl groups were present, but only one dinitrophenylhydrazine residue was introduced. The orange product which separated from benzene–petroleum ether (b.p. 60–105°) decomposed at 218–220°.

Anal. Calcd. for $C_{21}H_{24}N_4O_7$: C, 56.75; H, 5.44. Found: C, 56.51; H, 5.18.

Desoxodesacetyldihydroalloisotenulin (VII).—By modifying the usual procedure[24] in a slight detail a much purer product was obtained. A mixture of 10 g. of dihydroisotenulin and 15 ml. of hydrazine hydrate was heated for 1 hr., diluted with 130 ml. of triethylene glycol containing 10 g. of potassium hydroxide and refluxed again for 1 hr. The temperature was raised to 200° and maintained at this level for 4 hr. Cooling and dilution with water furnished 4.2 g. (51%) of VII, m.p. 156–157°. Recrystallization from ligroin (b.p. 65–110°) raised the m.p. to 157–158°, $[\alpha]_D$ −48.4° (95% ethanol, *c* 0.785). The Zimmermann test was negative.

Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.73; H, 9.73.

Wolff–Kishner reduction of desacetyldihydroalloisotenulin (V) and desacetyldihydroisotenulin (III) also gave VII in 40–45% yield.

Desoxodihydroalloisotenulin (VIII).—Acetylation of VII with acetic anhydride–pyridine at room temperature gave an oil which solidified on scratching. Recrystallization from 50% ethanol gave the analytical sample, m.p. 123.2–124.5°, $[\alpha]_D$ −31.7° (95% ethanol, *c* 1.07), infrared bands at 1767 (lactone) and 1737 cm.$^{-1}$ (acetoxy).

Anal. Calcd. for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.49; H, 8.96.

6,8-Dihydroxy-1,4-dimethyl-7-(2-hydroxyisopropyl)-decahydroazulene.—A solution of 1.82 g. of VII in 100 ml. of ether was added gradually to 1.5 g. of lithium aluminum hydride in 500 ml. of ether. The reaction mixture was worked up in the usual manner and the ethereal layer was dried and stripped. The residue was stirred with petroleum ether. Evaporation of the latter furnished a few seed

(24) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

crystals which were used in recrystallizing the gum from acetone–heptane; yield 1.54 g. Several additional recrystallizations furnished fluffy needles, m.p. 105.8–106.8°, $[\alpha]_D$ −17.4° (95% ethanol, *c* 1.61). The infrared spectrum had no bands in the double bond region.

Anal. Calcd. for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 69.91; H, 11.36.

An attempt to oxidize this substance with lead tetraacetate failed; saponification of the recovered gum which had been partially acetylated (infrared spectrum) resulted in recovery of pure starting material. Acetylation, benzoylation and tosylation gave gums and no azulene was encountered when 0.1-g. quantities of the triol were subjected to dehydrogenation under various conditions.

Dehydrodesoxodesacetyldihydroalloisotenulin (X).—Oxidation of 8 g. of VII with 2.3 g. of chromic oxide in aqueous acetic acid was complete after 3 hr. at 60–70°. The volume was reduced to 15 ml. *in vacuo* and the residue was diluted with water; yield 7.8 g. (96%), m.p. 127°. Recrystallization from aqueous methanol furnished needles, m.p. 130.5–131.5°, $[\alpha]_D$ −121.3° (95% ethanol, *c* 1.05). The Zimmermann test was negative. The substance did not furnish a derivative with piperonal.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.71; H, 8.80.

Reaction of X with Sodium Carbonate.—A mixture of 7.8 g. of X and 100 ml. of 10% sodium carbonate solution was warmed on the steam-bath until it was homogeneous. The cooled solution was filtered, extracted with chloroform, heated to boiling, cooled and acidified. There separated 6.7 g. of crude XII, m.p. 118–120°. Recrystallization from aqueous methanol yielded colorless material, m.p. 125.5–126°, $[\alpha]_D$ −53.4° (95% ethanol, *c* 0.665).

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.95; H, 8.95.

A solution of 0.80 g. of XII in 50 ml. of ethyl acetate was reduced catalytically (platinum oxide) until hydrogen uptake ceased. The residue obtained after removal of solvent *in vacuo* was taken up in hot 10% sodium carbonate solution. The solid which separated on cooling was filtered, the filtrate was extracted with ether, acidified and cooled. There separated 0.53 g. of the colorless keto acid XIII which was recrystallized from aqueous methanol to a constant m.p. of 120–120.5°, $[\alpha]_D$ +51.8° (95% ethanol, *c* 0.554), infrared bands at 3500 (acid–OH) 3000–3500 (broad, bonded acid–OH), 1750 (weak, monomeric carboxyl) and 1700 cm.$^{-1}$ (cycloheptanone, carboxyl).

Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.49; H, 9.17.

The oxime, m.p. 178–180°, was recrystallized from benzene–acetone–petroleum ether (b.p. 30–60°).

Anal. Calcd. for $C_{15}H_{25}NO_3$: C, 67.38; H, 9.43; N, 5.2. Found: C, 67.37; H, 9.41; N, 4.7.

The neutral fractions, wt. 0.2 g., from the above reduction were combined and recrystallized from aqueous methanol. Lactone XIV melted at 90.5–91°, $[\alpha]_D$ +13.1° (95% ethanol, *c* 0.738), 13.8° (*c* 0.897), infrared band at 1764 cm.$^{-1}$ (lactone).

Anal. Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found: C, 76.38; H, 9.88.

Lithium aluminum hydride reduction of 1.0 g. of XII in boiling ether furnished a diol, b.p. 140–145° (bath temperature, 1 mm.). The diol rapidly decolorized bromine.

Anal. Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.55; H, 10.99.

Acetylation resulted in the formation of an oily acetate. A mixture of 510 mg. of the diol, 500 mg. of *p*-toluenesulfonic acid and 10 ml. of xylene was refluxed for 5 minutes. The neutral fraction was distilled at 90–120° (0.8 mm.). Redistillation furnished a colorless oil, b.p. 93–95° (0.5 mm.), whose infrared spectrum showed the absence of hydroxyl group and had a strong ether band at 1060 cm.$^{-1}$. The analysis suggested contamination of the unsaturated ether by a small amount of diene.

Anal. Calcd. for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 82.79; H, 10.54.

An attempt to dehydrogenate 133 mg. of the ether with sulfur did not result in the isolation of an azulene. Likewise, potassium acid sulfate dehydration of the diol followed by dehydrogenation or direct dehydrogenation of the diol with palladium–charcoal did not furnish an azulene. Dehydrogenation of 774 mg. of the diol with 575 mg. of sulfur at 185–195° furnished, on extraction of the cold residue with petroleum ether and chromatographing the extracts over alumina, a non-azulenic fraction which gave a picrate (*ca.* 3 mg.), dec. above 320°. Decomposition of the picrate with 10% ammonium hydroxide solution gave a light yellow compound, m.p. 237–240°, in quantity insufficient for analysis whose ultraviolet spectrum (95% ethanol), exhibited a maximum at 358 mμ and a minimum at 293.5 mμ.

A solution of 500 mg. of XII in 25 ml. of methanol was added to 0.4 g. of sodium borohydride in 25 mg. of methanol. The mixture was decomposed in the usual way, and the alcoholic layer was filtered and concentrated. The residue was dissolved in ether and extracted with sodium bicarbonate. Acidification furnished starting material. The neutral fraction, wt. 138 mg., was distilled, b.p. 159–164° (bath temperature, 0.8 mm.), infrared bands at 1750 (lactone) and 1670 cm.$^{-1}$ (C=C).

Pyrolysis of Tenulin.—Pyrolysis of 3.5 g. of tenulin according to Clark[2] gave a yellow distillate which was shaken with petroleum ether. When the extract was chromatographed over alumina, a small blue zone began to develop. Repeated chromatography of the eluate corresponding to this zone finally resulted in a blue solution whose visible and ultraviolet absorption spectrum corresponded to that of chamazulene. The intensity indicated the presence of about 3 mg. of azulene. The solvent was evaporated and the residue was treated with 4 mg. of trinitrobenzene in the minimum amount of alcohol. After one recrystallization, the needles melted at 128–130°; a second crystallization furnished one crystal whose m.p. was 131–132° (capillary); lit. 133–133.5°.

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