exchange oxygen with the solvent under the conditions of the solvolysis.

(C) **Exchange between Benzhydryl *p*-Nitrobenzoate and *p*-Nitrobenzoic Acid-7-^{14}C.**—Four 30-ml. portions of a 90% aqueous acetone solution 0.0495 *M* in benzhydryl *p*-nitrobenzoate and 0.0294 *M* in ^{14}C-labeled *p*-nitrobenzoic acid ($580\pm13 \times 10^{-4}$ μc. mmole^{-1}) were sealed (under nitrogen) in heavy walled glass ampules. The ampules were placed in a 118.6° constant temperature bath for appropriate time intervals after which the unsolvolyzed ester was isolated and purified as described in the preceding section except that silicic acid was used for the chromatographic separation. The radioactivity of the ester was determined by scintillation counting.[16] The activity of the labeled acid was determined by converting it to its benzhydryl ester derivative which after purification was counted.[16] The results of two exchange experiments are summarized in Table III.

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

Constituents of Helenium Species. X. Revised Structure of Tenulin[1,2]

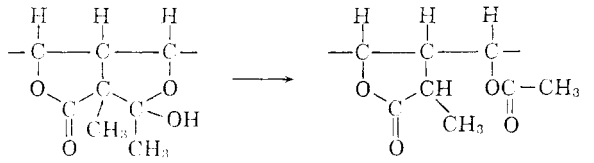
BY WERNER HERZ, W. A. ROHDE,[3] K. RABINDRAN, P. JAYARAMAN AND N. VISWANATHAN

RECEIVED MAY 2, 1962

The structure of tenulin is shown to be V.

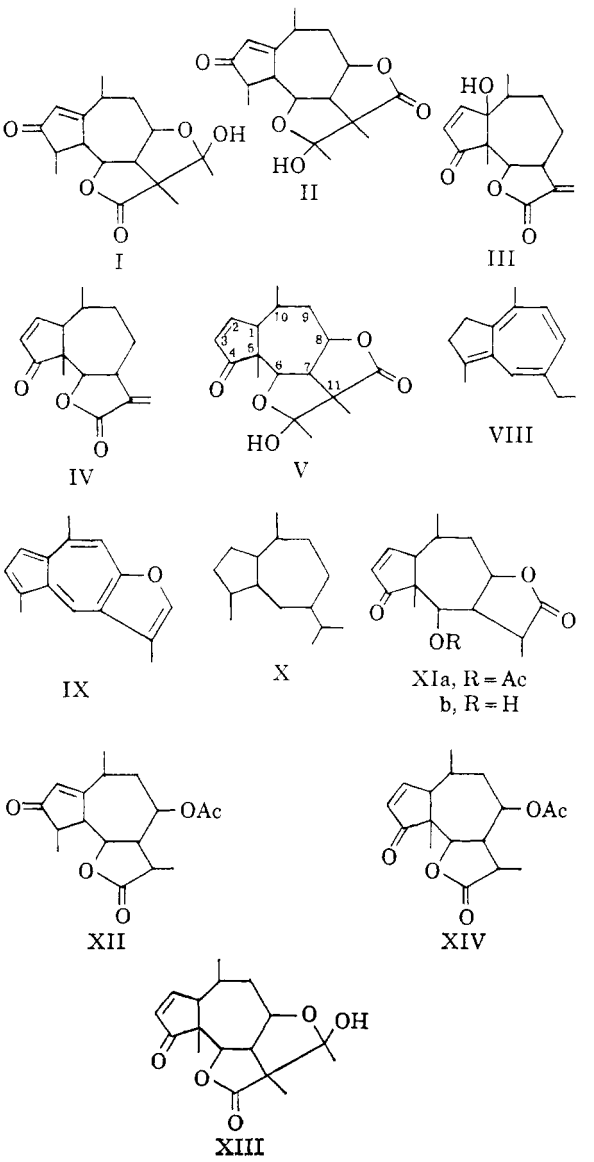
Tenulin, the bitter principle of *Helenium amarum* (Raf.)[4] and several other *Helenium* species, has been the subject of two recent investigations[6-8] which led to its formulation as a guaianolide. However, some uncertainties remained which could not be resolved satisfactorily by the generally accepted formula I or its alternative II.[9,10]

The recent demonstration[11,12] that parthenin (III) and ambrosin (IV) were not guiananolides also raised doubts about the carbon skeletons of tenulin and other sesquiterpene lactones of *Helenium* species such as helenalin,[13-16] balduilin[17] and the mexicanins.[17,18]



VI VII

In the following we show that these doubts were indeed well founded and present proof for the formulation of tenulin as V. The new formula not only accommodates all work previously published on



I II III IV V VIII IX X XIa, R = Ac b, R = H XII XIII XIV

(1) Previous paper, W. Herz, P. Jayaraman and H. Watanabe, *J. Am. Chem. Soc.*, **82**, 2276 (1960).

(2) Supported in part by grants from the National Science Foundation (NSF-G-14396), Research Corporation, and Eli Lilly and Co., Inc.

(3) Ethyl Corporation Fellow, 1957–1958; United States Public Health Fellow, 1958–1959. Abstracted in part from the Ph.D. thesis of W. A. Rohde, June, 1960.

(4) While *H. tenuifolium* Nutt. is the name by which this species is commonly known, there is a prior designation which must be used.[5]

(5) H. F. L. Rock, *Rhodora*, **59**, 128 (1957).

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

(7) B. H. Braun, W. Herz and K. Rabindran, *J. Am. Chem. Soc.*, **78**, 4423 (1956).

(8) C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.*, **22**, 1361 (1957).

(9) For comments on this, see D. H. R. Barton and P. de Mayo, *Quart. Revs.*, **11**, 189 (1957).

(10) W. A. Rohde, Ph.D. Dissertation, Florida State University, June, 1960.

(11) W. Herz, M. Miyazaki and Y. Kishida, *Tetrahedron Letters*, No. **2**, 82 (1961).

(12) W. Herz, H. Watanabe, M. Miyazaki and Y. Kishida, *J. Am. Chem. Soc.*, **84**, 2601 (1962).

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

(14) G. Büchi and D. Rosenthal, *ibid.*, **78**, 3860 (1956).

(15) V. Herout, M. Romanuk and F. Sörm, *Coll. Czechoslov. Chem. Commun.*, **21**, 1359 (1956).

(16) W. Herz and R. B. Mitra, *J. Am. Chem. Soc.*, **80**, 4876 (1958).

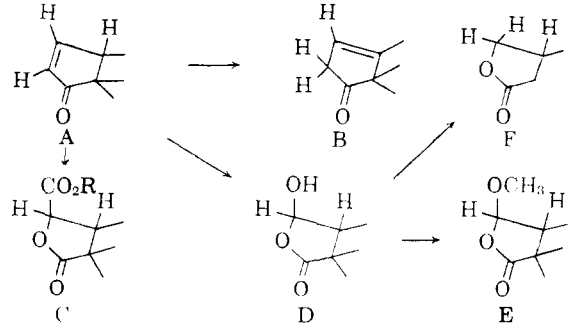
(17) W. Herz, R. B. Mitra and P. Jayaraman, *ibid.*, **81**, 6061 (1959).

(18) A. Romo de Vivar and J. Romo, *Chemistry & Industry*, 882 (1959); *Ciencia (Mex.)*, **21** (1), 33 (1961); *J. Am. Chem. Soc.*, **83**, 2326 (1961).

this subject but removes the many discrepancies which have since been noted.

Before proceeding to the new evidence, we briefly review the salient facts concerning the functional groups present in tenulin and in its most important transformation product, isotenulin. It has been clearly established[6,7] that these substances are cyclopentenones and α-methyl-γ-lactones and contain the partial structures VI and VII, the masked acetyl group of tenulin (VI) being converted to the acetate of isotenulin (VII) on very mild treatment with base. Both potential hydroxyl groups are secondary and attached to six-membered or larger alicyclic rings. The presence of these groups and the formula $C_{17}H_{22}O_5$ require that tenulin and isotenulin possess a bicyclic carbon skeleton which, because of the formation of chamazulene (VIII)[6,7] and linderazulene (IX)[6] on reduction and dehydrogenation of isotenulin and dihydroisotenulin, respectively, was assumed to be based on that of guaiane (X).

The n.m.r. spectra[19] of tenulin and isotenulin (XIa) displayed, in the vinyl proton region, two sets of quadruplets characteristic of the A and B protons of an ABX spectrum (J_{AB} = 5, J_{AX} = 2.5, J_{BX} = 1.5 and 2). The presence of *two* vinyl protons spin-coupled to a third is clearly incompatible with formulas I or II but may be rationalized in terms of partial structure A, since the presence of an α,β-unsaturated cyclopentenone system unsubstituted at the α-position is well documented.[6,7] The chemical shift exhibited by these protons (see Table I) is the same as that displayed by the α- and β-protons of parthenin (III) and ambrosin (IV).[11,12] Pyrotenulin, a substance in which the conjugated double bond has migrated to the β,γ-position,[6] must have partial formula B, since its n.m.r. spectrum exhibits, *inter alia*, an unresolved vinyl multiplet at 5.96 and a complex signal, intensity two protons, at 3.00 p.p.m., thus duplicating exactly the spectrum of an analogous compound of the parthenin series.[12]

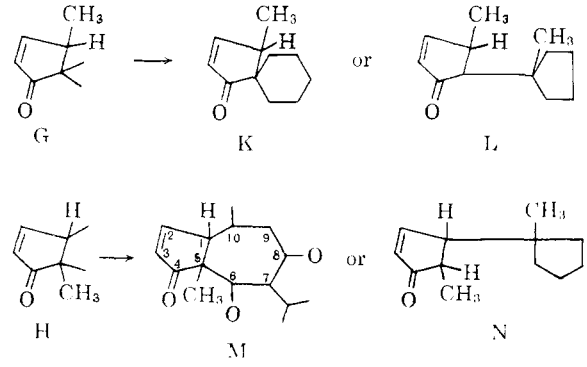


Tenulinic acid and its relatives which are obtained by permanganate or alkaline peroxide oxidation of tenulin[6,20] would then have partial structure C, a mode of oxidation which is not unprecedented.[21] Likewise, a lactol, $C_{16}H_{22}O_7$, obtained[6] in small amount by ozonolysis of isotenulin, should now be represented by D. We have improved the yield and have prepared the lactol by a second route—osmylation of isotenulin followed by periodate oxidation of the resulting gummy ketodiol—thus excluding a skeletal rearrangement.

In accordance with the postulated formula, the lactol gave a positive Tollens test, furnished a methyl ether (E) on treatment with diazomethane, and was reduced to an acetoxydilactone (F) with sodium borohydride. The n.m.r. spectrum of E exhibited, besides the signals characteristic of H_6 and H_8 (*vide infra*), *an additional low-field doublet at 5.20 p.p.m.* (J = 4.8, intensity one proton). The chemical shift and splitting indicate that this signal is generated by the proton on the carbon atom, carrying the methoxyl and lactone ether functions, and that there is spin coupling to one adjoining proton, an observation which confirms the assigned partial structures A-E. Moreover, the acetoxydilactone has the additional two-proton signal near 4 p.p.m. expected from partial formula F.

Four methyl groups are present in the tenulin structure and appear at the frequencies: doublet at 1.27 (J = 5), singlet at 1.32 (intensity six protons) and singlet at 1.60 p.p.m. The low-field signal is that of the masked acetyl group,[6,7] since it is shifted to 2.17 p.p.m. in the n.m.r. spectrum of isotenulin (XIa). Isotenulin has three other methyl signals, two superimposed doublets (J = 4) at 1.20 and a sharp singlet at 1.20 p.p.m. The presence of a sharp methyl singlet in the n.m.r. spectra of isotenulin and all of its derivatives (see Table I) is again incompatible with the old formula XII. It requires the presence of a tertiary methyl group and recalls the situation prevailing in the parthenin and ambrosin series[11,12] where the tertiary methyl group is definitely located at C_5.

The formation of chamazulene and linderazulene on dehydrogenation requires that one of the methyl groups be located on the five-membered ring. Hence A can be expanded to G or H. In considering further expansion, the necessity for including



(19) N.m.r. spectra were run by Mr. Fred Boerwinkle of our Department and Mr. L. F. Johnson of Varian Associates on a Varian HR-60 instrument in deuteriochloroform solution. Tetramethylsilane served as internal standard, and frequencies were calibrated by the sideband technique. We are grateful to Dr. M. T. Emerson and Mr. Johnson for assistance with the assignments and to Mr. Gerald Caple for help with the early work. The spectrometer at Florida State University was purchased with funds provided by the Institute of Molecular Biophysics.

(20) E. P. Clark, *J. Am. Chem. Soc.*, **61**, 1836 (1939); **62**, 2154 (1940).

(21) K. Brückner, K. Irmscher, F. v. Werder, K. H. Bork and Harald Metz, *Ber.*, **94**, 2877 (1961). The n.m.r. spectrum of methyl dehydrotenulinate (see Experimental), also supports this formulation.

TABLE I

N.M.R. PEAKS OF TENULIN DERIVATIVES[a]

Compound	H_2	H_3	H_6	H_8[b]	C_5-Me	C_{10}-Me and/or C_{11}-Me		Acetyl
V	7.54dd(5,1.5)	6.04dd(5,2.5)	4.45d(5.5)	5.33t(11)	1.32	1.27d(5)	1.31	1.32
XIa	7.57dd(6,2)	6.12dd(6,2.5)	5.60d(4)	4.70t(11)	1.20	1.20d(4)	1.20d(4)	2.17
XVa			5.47d(4.5)	4.49t(11)	1.03	1.22d(7.5)	1.05d(5.5)	2.15
XVb			3.98dd(8,2.7)	4.58t(11)	1.03	1.20d(7)	1.04d(4)	
Dehydrodesacetylisotenulin	7.70dd(6.2, 1.8)	6.12dd(6.2,3)		4.2br	1.38	1.25d(6.8)	1.23d(7.5)	
XVIa			4.66d(8.5)	5.12t(10)	1.025	1.27d(6.7)	0.94d(4)	2.02
XVIb			4.61d(8)	3.94t(10)	1.05	1.19d(7)	1.09d(7)	
XVII				4.30t(11)	1.215	1.17d(5.5)	1.17d(5.5)	
XVIII			4.93d(10)		0.78	1.24d(9)	1.13d(7)	
XXI			4.45d(8)	5.36c[c](2H)	.975	1.23d(7.5)	1.03d(7.5)	
XXIIb			3.75c	4.61t(11)	.995	1.27d(6.5)	1.04d(6.5)	
XXIII				4.25t(10)	1.06	1.23d(7)	1.05d(4)	
XXIVa			4.33d(10)	5.25t(9)	0.867	1.22d(7)	0.91d(5)	2.07
XXIVb			4.35d(9)	4.14t(9)	.81	1.39d(6)	0.945d(6)	
XXV			4.75d(8)		.60	1.25d(6.5)	1.03d(5)	
XXVIII	5.96c	3.00c (2 protons)	4.33d(9)	4.43t(11)	1.29	1.295d(7)	1.39	4.45d(2)[d] 4.15d(2)
XXXIII			5.46t(2.5)	4.02t(10.5)	1.75d(1.5)	1.25d(6.5)	1.17d(7)	1.98
XXXV			5.36t(2.2)	4.06t(10.2)	1.69d(1)	1.25d(6.8)	1.04d(7.2)	2.02
XXXVI	3.02br (2H on C_4)	3.45c(H_7?)	5.56br	4.1c	1.82[e]	1.36d(6)	1.30d(6.5)	
XXXVIII			5.36t(2.5)	4.05t(10)	2.11[f]	1.25d(6.5)	1.14d(7.5)	2.11
XL	3.80t(6,H_8)			4.44t(10)	2.36[f]	1.37d(6.8)	1.37d(6.8)	
XLI	3.85d(2)H_8 3.78d(2)			4.12c	1.72[e]	1.29d(7)	1.22d(6)	

[a] Values are given in p.p.m. relative to tetramethylsilane as internal reference. All signals in first four columns correspond to one proton; all signals in last four to three, unless otherwise specified. Singlets are unmarked; multiplets are described as follows: d doublet, dd doublet of doublets, t triplet, br somewhat broadened singlet or ill-defined doublet, c complex signal whose center is given. Numbers in parentheses denote coupling constants in c.p.s. [b] Each of the triplet components is split again, generally into a doublet ($J \sim 2$–4). The center of this band system is frequently ill-defined. [c] Signal corresponds to protons at C_8 and C_9. [d] Two protons of methylene group. [e] Located at C_1 in neotenulin series. [f] Methyl ketone group.

partial formula VI in a six-membered or larger ring must be borne in mind. This immediately excludes G, since the two partial structures K or L derived from it violate either this requirement (L) or the requirement for including a tertiary methyl group in the complete isotenulin skeleton (K). Of the two partial structures derived from H, only M satisfies all conditions which we have laid down previously and, gratifyingly enough, leads to a formulation completely analogous to that of parthenin and ambrosin. (For a more direct proof of the correctness of H, *vide infra*.) Although degradative proof for the placement of the third methyl at C_{10} and the potential isopropyl group at C_7 has not been obtained, the isolation of chamazulene and linderazulene provides quite satisfactory evidence for their attachment to C_7 and C_{10} as in M.

Tenulin is thus another member of the "abnormal" class of sesquiterpene lactones which can be derived formally from a guaianolide by migration of a methyl group from C_4 to C_5. The formation of chamazulene and linderazulene on dehydrogenation of tenulin derivatives is obviously due to a 1,2-methyl shift, perhaps the reversal of a migration occurring during biogenesis.

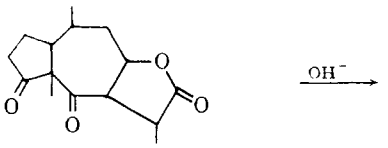
A clear decision between the two remaining possibilities V and XIII (for tenulin) and XIa and XIV (for isotenulin) is again made possible by consideration of the n.m.r. spectra. In the region characteristic of protons on carbon carrying lactone or ester oxygen, there are found two signals (see Table I). The first, a sharp doublet near 5.5 p.p.m., moves upfield to 4.2 p.p.m. on deacetylation and disappears altogether in the n.m.r. spectra of dehydrodesacetylisotenulin and dehydrodesacetyldihydroisotenulin (XVII). Hence hydrogen on carbon carrying the acetate or masked acetyl group[22] is coupled to only one hydrogen and must be placed at C_6. The lactonic hydrogen, on the other hand, is signaled by a complex band whose appearance indicates coupling to three hydrogens. Two of these spin couplings are large (J = 9–11), while one is small (J = 2–4), thus producing a triplet with smaller doubling. Hence the lactone ring of tenulin and isotenulin is closed to C_8.

We defer discussion of the chemical evidence which substantiates this assignment in order to comment briefly on the "allo" series of compounds isolated by us previously.[7] Depending on the conditions, hydrolysis of dihydroisotenulin, now known to be XVa, yielded either desacetyldihydroisotenulin (XVb) or an isomer, desacetyldihydroalloisotenulin (XVIb), which was subjected to a number of transformations. It was inferred that the allo series differed from the parent series in orientation of the lactone ring. Although the previous assignments will now have to be reversed, the n.m.r. spectra clearly show that reorientation of the lactone ring has accompanied the conversion of XVa to XVIb. In compounds of the allo series (Table I), the lactone hydrogen is represented by a sharp doublet which requires lactone ring closure to C_6. On the other hand, hydrogen on carbon carrying the hydroxyl appears as a triplet which moves to lower field on acetylation and on which spin coupling to H_7 has been superimposed, thus requiring its placement at C_8.

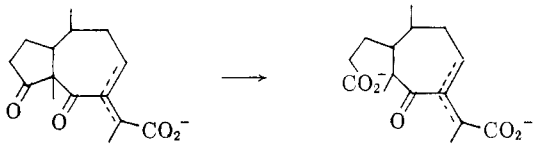
The chemical evidence for these assignments is clear-cut. The "remarkable" base-catalyzed

(22) In accordance with expectations, the greater shielding of this proton in a substance of structure V shifts the doublet to 4.41 p.p.m.

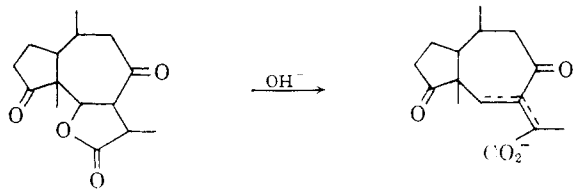
conversion of dehydrodesacetyldihydroisotenulin (XVII) to a dibasic α,β-unsaturated ketoacid[7] may now be interpreted quite simply as being due to the cleavage of a β-diketone.[23] This reaction, which was difficult to rationalize formerly, now



XVII



provides powerful chemical evidence for the relationship of the two ketone groups deduced on the basis of n.m.r. spectra. On the other hand, dehydrodesacetyldihydroalloisotenulin (XVIII), having the lactone ring closed to C_6, can undergo only the first of the postulated transformations, the product being an α,β-unsaturated diketoacid.[7]



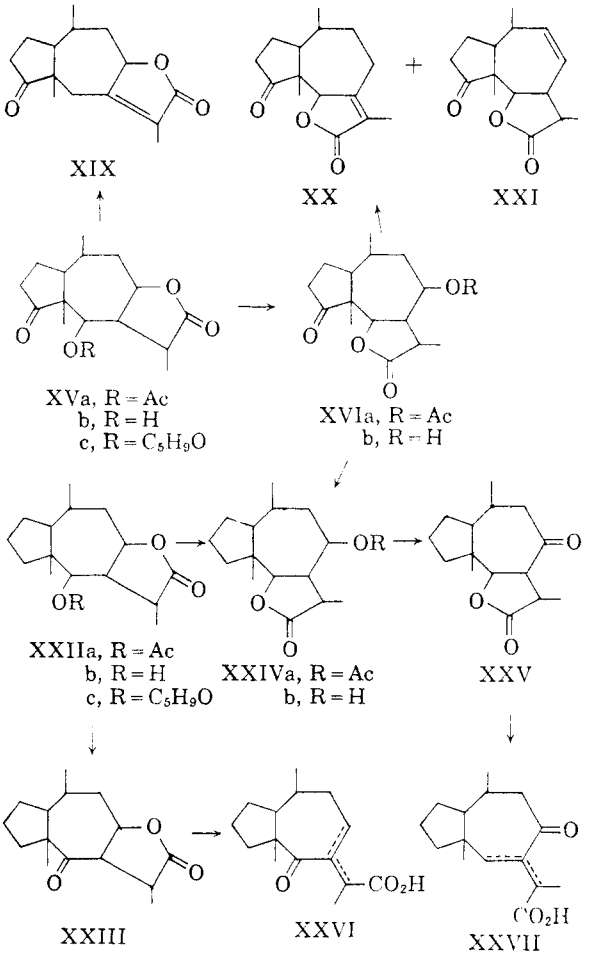
XVIII

While desacetylisotenulin did not furnish a mesylate or tosylate, the mesylates of XVb and XVIb on treatment with lutidine afforded different anhydro derivatives, thus providing additional chemical evidence for the different orientation of the lactone groups in XVb and XVIb. In view of the ultraviolet and infrared spectra (see Experimental), the products are formulated as XIX and XX. An unconjugated double-bond isomer (XXI), which is not an enol lactone, was also obtained from XVIb, an observation which corroborates the lactone ring orientation.

Desulfurization of the thioketal of XVa gave an oily acetoxylactone, desoxodihydroisotenulin (XXIIa), which was hydrolyzed under mild conditions to a solid desacetyl derivative, XXIIb. That no isomerization had accompanied the hydrolysis was shown in the following manner: XVb was converted to a thioketal which was desulfurized directly to XXIIb. Reacetylation of XXIIb regenerated XXIIa. When XXIIb was oxidized with chromic acid there was obtained desoxodehydrodesacetyldihydroisotenulin (XXIII). This substance, like the previously reported[7] allo isomer XXV, gave no Zimmermann test[24] and could not be induced to condense with aromatic aldehydes.[25] Base-catalyzed cleavage of the ketolactone XXIII gave an α,β-unsaturated ketoacid XXVI whose properties differed from those of the ketoacid XXVII previously prepared from dehydrodesoxodesacetyldihydroalloisotenulin (XXV)[7] and from those of similar compounds of the helenalin series.[16]

Now, although XVb and XXIIb could be converted directly to the allo isomers XVIb and XXIVb on treatment with base, the corresponding dihydropyranyl ethers XVc and XXIIc resisted isomerization. This finding buttresses our conclusion that isomerization of the normal to the allo series involves reorientation of the lactone ring. However, this is not the only feature which distinguishes the two series. Lithium aluminum hydride reduction of XXIIb yielded a triol which differed from the triol previously prepared[7] from XXIVb in m.p. and rotation. Thus the normal and the allo series differ from each other, not only in orientation of the lactone ring, but also in absolute configuration at C_{11}.

The Experimental section also describes additional previously unrecorded transformations of tenulin and its derivatives. Particularly pertinent



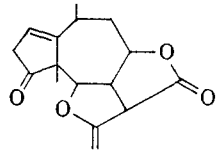
(23) The sequence of events is assumed arbitrarily.

(24) The Zimmermann test in the allo series seems to have no diagnostic value. See also L. F. Fieser and M. Fieser, Steroids, Reinhold Publishing Corporation, p. 522 (1960). Steric hindrance may be responsible for this as for the lack of reactivity toward aromatic aldehydes.

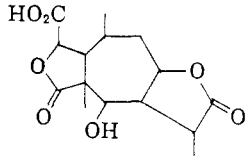
(25) Reanalysis and spectroscopic investigation of a substance previously thought to be the dipiperonylidene derivative of XVII, which was taken[7] to indicate the presence of two active methylene groups and thus to require lactone ring closure to C_4, showed it to be a monopiperonylidene derivative.

are the results of peracetic acid oxidation, undertaken with a view to demonstrating the environment of the cyclopentanone carbonyl. The dilactones resulting from oxidation of XVa, XVb and XVIb (the latter being acetylated in the course of the reaction) exhibited no additional n.m.r. signals in the region 3.5–5.5 p.p.m. Hence, the cyclopentanone carbonyl adjoins a quaternary carbon atom, and the tenulin skeleton is confirmed.[26]

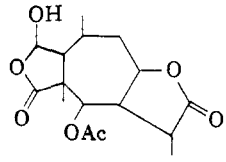
Having satisfied ourselves as to the structure of tenulin (V) and its principal transformation products, isotenulin (XIa), pyrotenulin (XXVIII), tenulinic acid (XXIX) and the lactol XXX, we faced the problem of arriving at a rational structure for desacetylneotenulin. This compound is formed, besides desacetylisotenulin, on treatment of tenulin with sodium bicarbonate solution.[6] Its properties, λ_{max} 240 mμ (ϵ 16000), infrared bands (Nujol) at 1765 (γ-lactone), 1682 and 1628 (cyclopentenone) and 3300 cm.$^{-1}$ (secondary hydroxyl), coupled with the formation of acetic acid on ozonolysis, indicate that it is an α-methyl-β,β-disubstituted-α,β-unsaturated cyclopentenone. Because oxidation furnished an unsaturated diketolactone, dehydrodesacetylneotenulin, which was not a 1,4-enedione, Barton and de Mayo[6] formulated desacetylneotenulin as XXXI and tenulin as I.[27]



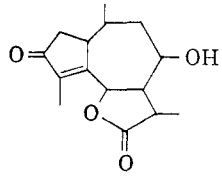
XXVIII



XXIX



XXX

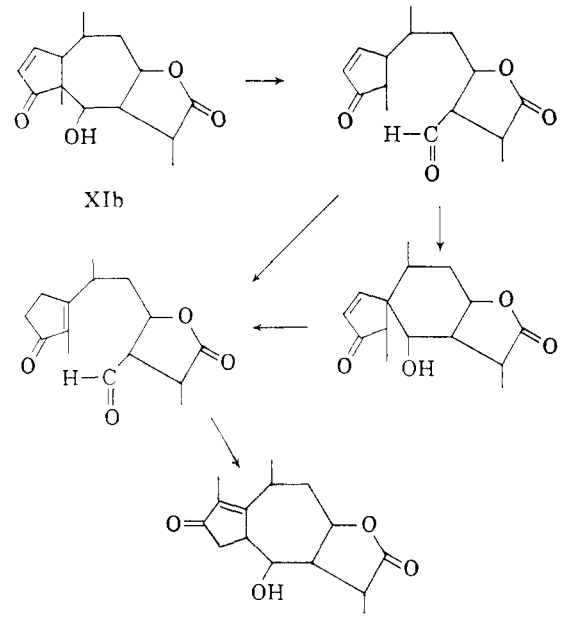


XXXI

A plausible route by which a compound with the properties of desacetylneotenulin could be generated from V appeared to be initial isomerization to isotenulin (XIa), hydrolysis of the latter to desacetylisotenulin (XIb), a substance reported as a by-product, and base-catalyzed retroaldol ring opening of XIb as illustrated below. Double-bond isomerization, directly or perhaps through the intermediacy of a spiro derivative, would permit aldol ring closure to the newly vinylogous γ-position and lead to a compound of formula XXXII.

(26) Compounds XXIII and XXV were not affected by peracetic acid, nor was it possible to oxidize the cycloheptanone carbonyl group of XVII (see Experimental).

(27) However, the argument (footnote on p. 145 of ref. 6) that the lactone ring of desacetylneotenulin is closed to the same position as the lactone ring of tenulin because desacetylneotenulin is obtained from bicarbonate solution *without* acidification is not necessarily valid. Thus both XVIb and XXIVb separated from basic solution without acidification, although their formation is accompanied by reorientation of the lactone ring. See also the lactonization of an acid to a guaianolide in weakly basic solution reported by S. Naito, *J. Pharm. Soc. Japan*, **75**, 325 (1955).



XIb

XXXII

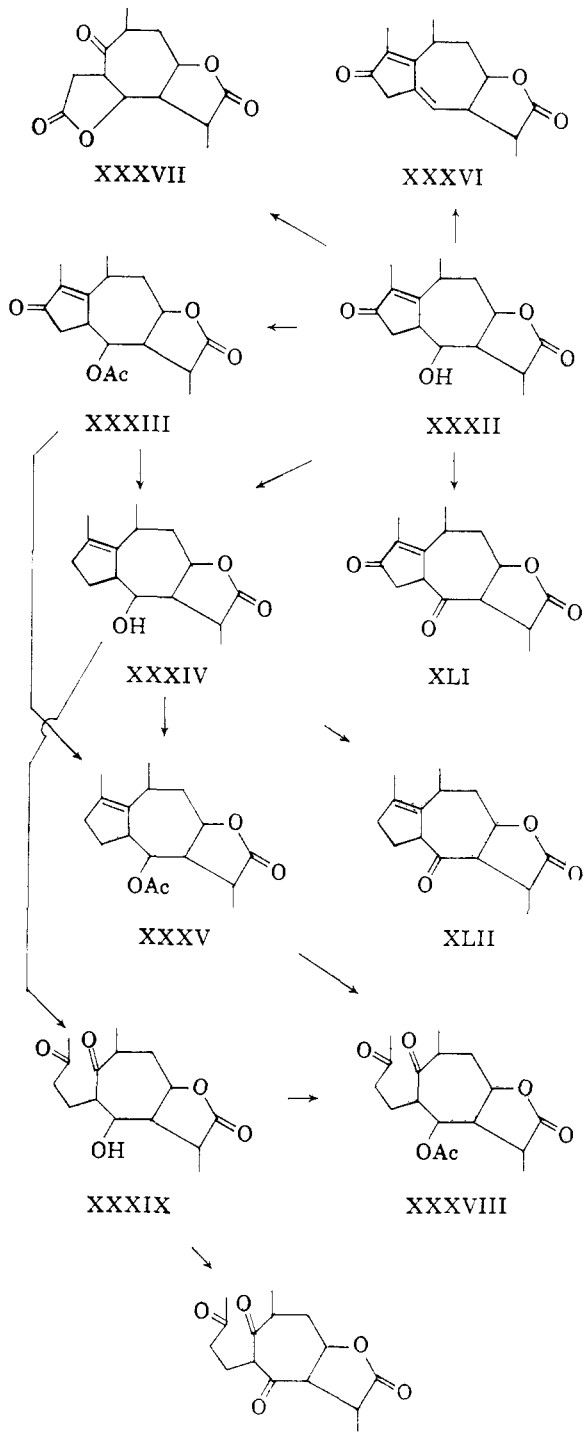
SCHEME I

Support for this hypothesis was found in the inevitable loss of the acetyl group during the conversion of tenulin or isotenulin to desacetylneotenulin and in the discovery that desacetylisotenulin could be converted directly to desacetylneotenulin by treatment with potassium carbonate solution or *p*-toluenesulfonic acid in methanol. On the other hand, the last stage of this scheme, involving as it does a remarkably facile ring closure to a seven-membered ring, seemed somewhat questionable.

Desacetylneotenulin, whose preparation from tenulin is accompanied by the formation of several minor products (*vide infra*), has in fact structure XXXII. Its transformations are outlined in scheme II, with the aid of which the evidence leading to this conclusion will be discussed.

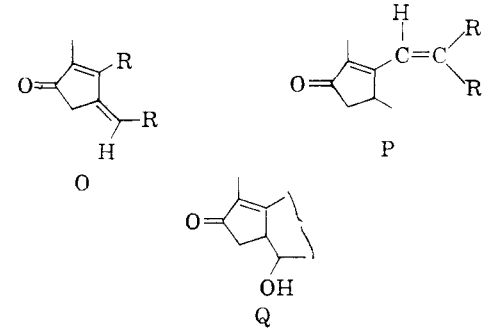
The double bond of XXXII was resistant to catalytic reduction. Hydrogenation under acid conditions resulted in desoxodesacetylneotenulin (XXXIV), the infrared spectrum of which indicated the loss of the ketone function and retention of a hydroxyl group (infrared bands at 3490, 1755 and 1645 cm.$^{-1}$). That the cyclopentenone carbonyl and not the hydroxyl group had undergone hydrogenolysis was shown as follows. Hydrogenation of neotenulin (XXXIII) gave desoxoneotenulin (XXXV), infrared bands at 1775 (γ-lactone), 1760 (acetate) and 1655 cm.$^{-1}$ (double bond), which could also be prepared by acetylation of XXXIV.

In the n.m.r. spectra of XXXIII and XXXV (see Table I) are found the typical lactone hydrogen triplet centered near 4 p.p.m. spin-coupled to one other hydrogen, an acetate resonance near 2 p.p.m., a vinyl methyl signal split by long range coupling (J = 1) near 1.7 p.p.m., and two normally split methyl doublets. Hydrogen carrying the acetoxy group appears as a narrowly spaced triplet indicating spin coupling to *two* hydrogens, an observation which immediately eliminates formula XXV.



SCHEME II

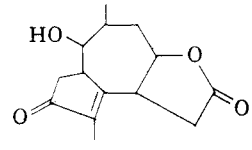
The mesylate of XXXII on treatment with lutidine furnished a dienone XXXVI, λ_{max} 214 and 287 mμ (ε 9000 and 16000), infrared bands at 1765, 1680, and 1590 cm.$^{-1}$, the n.m.r. spectrum of which indicated the presence of one vinyl proton (signal broadened by coupling to H_7) at 5.56 p.p.m., one vinyl methyl singlet and two methyl doublets. Ozonolysis of the dienone gave acetic acid. Either of the chromophores shown below (where R is not methyl) satisfies these properties but O is much more reasonable, since desacetylneotenulin is not oxidized by manganese dioxide nor reduced by zinc and acetic acid.



Positive proof for this conclusion and for the placement of the hydroxyl group at C_6 was furnished by the ozonolysis of XXXII. The initial product, a gummy α-diketone, was not purified, but oxidized with periodic acid to a crystalline ketodilactone $C_{13}H_{16}O_5$ (XXXVII) which exhibited infrared bands at 1785 (double strength, two γ-lactones) and 1708 cm.$^{-1}$ (cycloheptanone). The presence of two lactone groups was confirmed by titration. Formation of a new γ-lactone function in the course of this reaction sequence requires that the hydroxyl group of desacetylneotenulin be γ- to the cyclopentenone carbonyl along a chain including no double bonds, *i.e.*, partial formula Q. Structure XXXII follows.[28,29]

Oxidation of XXXIV furnished the ketone XLII which had the high ultraviolet absorption characteristic of certain β,γ-unsaturated ketones.[30] Ozonolysis of XXXIV and XXXV yielded the diketones XXXVIII and XXXIX which could be interrelated. Dehydration of XXXVIII which gave a positive iodoform test but did not react with periodic acid and lead tetraacetate was not successful, but it was readily converted to the triketone XL. Compound XL and XLI did not give a positive ferric chloride reaction, a fact which initially caused us some concern until the transformation of desacetylneotenulin to XXXVII settled the structural problem. The lack of enolization in XLI is perhaps understandable on steric grounds since the conversion of XXXII to XXXVI required relatively stringent conditions, but that of XL is less so. However, the n.m.r. spectra of

(28) Strictly speaking, these experiments do not exclude expression i for desacetylneotenulin. However, there is no *rationale* for the formation of such a substance from V.



i

(29) We are not ready to grant that the facile lactonization to XXXVII necessarily implies a *cis* relationship of the side chain with the C_8-hydroxyl group (model experiments with Mr. L. Glick). Moreover, the oxidation affords ample opportunity for equilibration of the less with the more stable isomer, *i.e.*, epimerization at C_8.

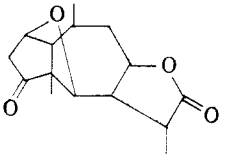
(30) R. C. Cookson and N. S. Wariyar, *J. Chem. Soc.*, 2302 (1956); see also S. F. Mason, *Quart. Revs.*, **15**, 287 (1961).

XL and XLI displayed signals in the region 3.7–4.5 p.p.m. which revealed the presence of *two* deshielded protons. One of these, characterized by the usual doubly split triplet (centered at 4.39 p.p.m. in the spectrum of XL) is obviously the hydrogen atom at C_8. The other (multiplet centered at 3.80 p.p.m.) exhibits the chemical shift to be expected of a methine hydrogen deshielded by two adjacent carbonyl groups, as required by formulas XL and XLI.[31]

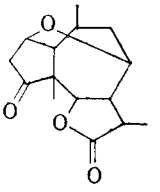
The isolation of several other substances from large scale isomerizations of tenulin to desacetylneotenulin is described in the Experimental section. One of these, m.p. 145°, was a saturated ketoepoxylactone (infrared bands at 1775 (γ-lactone) and 1745 cm.$^{-1}$ (cyclopentanone); λ_{max} 298 mμ, ϵ 32), whose n.m.r. spectrum (see Experimental) did not permit ready differentiation between formulas XLIII and XLIV. Treatment with acetic anhydride–*p*-toluenesulfonic acid converted this substance to an isomer of tenulin or alloisotenulin, infrared bands at 1775, 1707 (shoulder at 1740) and 1590 cm.$^{-1}$, λ_{max} 223.5 mμ (ϵ 9600) which was reduced catalytically to a stereoisomer of XVa or XVIa. A second substance, m.p. 208–209°, was isomeric with desacetylisotenulin, infrared bands at 3600, 3300, 1760, 1700 and 1590 cm.$^{-1}$, λ_{max} 225 and 320 mμ, (ϵ 7300 and 99).[33]

Since tenulin has been correlated[16] with helenalin, and the latter with balduilin and mexicanin C[17], helenalin, balduilin, and the mexicanins possess the same "abnormal" carbon skeleton as tenulin. Evidence bearing on the detailed structure and stereochemistry of these sesquiterpene lactones will be presented in future communications.

Acknowledgment.—We wish to thank Dr. Reuben G. Jones of Eli Lilly and Co., Inc., for arranging a large scale extraction of *H. amarum* (Raf.).



XLIII



XLIV

Experimental[34]

Tenulin.—Tenulin was isolated from *H. amarum* (Raf.) essentially as previously described.[7,20] It was found expedient to purify tenulin, after initial crystallization from benzene, by refluxing an ethanolic solution of tenulin to remove benzene of crystallization by azeotropic distillation. Concentration of the solution then yielded white needles of tenulin, m.p. 196–198°.

Anhydrodihydrotenulin.—Dihydrotenulin, carefully dried and free of solvent of crystallization, wt. 5.5 g., and 100 ml. of practical grade acetic anhydride and 6 g. of anhydrous sodium acetate was refluxed for 7 hours. The solvent was removed at reduced pressure, and the residue cooled, diluted with water, and chilled. After several hours, the solid was filtered, washed and dried; wt. 4.65 g., m.p. 169°. The success of the preparation seems to depend on the purity of dihydrotenulin and the grade of acetic anhydride. Distilled acetic anhydride proved unsuitable. The n.m.r. spectrum had signals at 4.50 (doublet, H_6), 4.46 and 4.09 (two doublets, J = 2.5, = CH_2), 4.41 (center of complex band, H_8), 1.37 (singlet methyl), 1.07 (singlet methyl) and 1.06 (doublet methyl, J = 6).

An attempt to prepare anhydrotenulin by the method of Clark resulted in a 13% yield of material, m.p. 155–156°, identified as isotenulin by mixed m.p. and infrared spectrum.

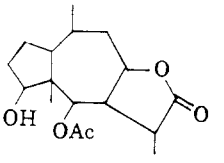
Methyl Dehydrotenulinate.[6]—Satisfactory analyses for this compound, obtained in 90% yield from methyl tenulinate, were obtained, m.p. 228° after recrystallization from aqueous solution. It gave a negative Zimmermann test and had infrared bands at 1770 (wide, two γ-lactones), 1740 (ester carbonyl) and 1700 (cycloheptanone); *cf.* the somewhat contrasting results of Barton and de Mayo.[6] The n.m.r. spectrum had the expected signals at 3.92 (methoxyl), 1.55 (C_5–methyl deshielded by two adjacent carbonyl groups), 1.18 (doublet, J = 7.1) and 1.10 (doublet, J = 7.3); (C_{10} and C_{11}-methyls), but the H–C–O signals were not clearly discernible, due to low solubility. However, there appeared to be a doublet at 4.58 p.p.m. (J = 9, H_2 split by neighboring H_1) characteristic of the assigned structure.

Anal. Calcd. for $C_{16}H_{20}O_7$: C, 59.25; H, 6.20. Found: C, 59.63; H, 6.29.

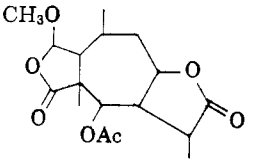
Tetrahydroisotenulin.—When the usual method of catalytic reduction of isotenulin[7] was modified by carrying out the hydrogenation in ethyl acetate with platinum oxide, the keto group was reduced as well. From 1 g. of isotenulin, there was obtained a quantitative yield of a hydroxy acetoxylactone XLV, m.p. 136°, which crystallizations from aqueous methanol raised to 145°; infrared bands at 3600, 1770 (γ-lactone) and 1725 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{17}H_{26}O_5$: C, 65.77; H, 8.44. Found: C, 65.74; H, 8.27.

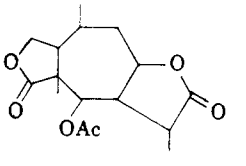
Oxidation of 0.5 g. of this substance with chromic acid in acetic acid furnished 0.2 g. of dihydroisotenulin, m.p. 149°, identical with authentic material by mixed m.p. and infrared spectrum.



XLV



XLVI



XLVII

Ozonolysis of Isotenulin.—A solution of 0.8 g. of isotenulin in chloroform was ozonized at −5°. The ozonide was decomposed with water, and the chloroform layer dried and evaporated. The residual gum was digested with sodium bicarbonate solution for several hours and then extracted with methylene chloride. The aqueous layer was acidified and extracted with methylene chloride. Crystallization of the residue from aqueous methanol yielded 0.2 g. of the lactol XXX, m.p. 233–234°, positive Tollens test, infrared bands

(31) In a quite similar way, signals near 3.85 p.p.m. in the n.m.r. spectra of narbomycin transformation products permitted the identification of these compounds as β-ketolactones, notwithstanding their negative ferric chloride tests.[32]

(32) V. Prelog, A. M. Gold, G. Talbot and A. Zamojski, *Helv. Chim. Acta*, **45**, 4 (1962).

(33) The acetate of this substance could not be purified satisfactorily. Its n.m.r. spectrum indicated that it was not derived from the spiro compound suggested as a possible intermediate in the conversion of tenulin to desacetylneotenulin.

(34) M.p.'s and b.p.'s are uncorrected. Analyses were carried out by Drs. Weiler and Strauss, Oxford, and Dr. F. Pascher, Bonn. Ultraviolet spectra were determined in 95% ethanol solution on Cary model 14, Beckman DK1 or DV spectrophotometers. Infrared spectra were run in chloroform solution, unless otherwise specified, on Perkin-Elmer model 221, 21, or InfraCord instruments. Unless otherwise specified, Alcoa alumina (F-20) was used as chromatographic adsorbent. Eluents were in the order: petroleum ether (b.p. 30–60°), benzene, chloroform, ether, and methanol.

(KBr) at 3500 (strong bonded hydroxyl), 1780 (double strength, two lactones) and 1725 cm.$^{-1}$ (acetate). The neutral fraction yielded additional crystalline lactol on standing. Attempts to scale up the preparation reduced the yields somewhat; in one run using 3 g. of isotenulin there was obtained 0.24 g. of lactol from the acid and 0.4 g. from the neutral portion.

The lactol was also obtained in the following manner: To 0.4 g. of isotenulin in 7 ml. of dry dioxane was added 0.55 g. of osmium tetroxide. After 5 days at room temperature, the complex was decomposed with hydrogen sulfide. The filtrate and washings (dioxane) were concentrated and the residue washed with petroleum ether and benzene to remove unreacted isotenulin. The material gave a positive periodate test and could not be induced to crystallize, but the infrared spectrum indicated conversion of the cyclopentenone to a cyclopentanone carbonyl and strong hydroxyl absorption. Oxidation of the gum, wt. 0.22 g., in 5 ml. of methanol with 450 mg. of periodic acid in 25 ml. of water with stirring overnight followed by extraction with chloroform, evaporation of the organic layer, and crystallization from chloroform–petroleum ether yielded 0.102 g. of the lactol, m.p. 233–235°, mixed m.p. with material from the ozonolysis undepressed. An attempt to prepare an oxime or a dinitrophenyl hydrazone was not successful.

A solution of 0.3 g. of XXX in 10 ml. of methanol was mixed with ethereal diazomethane (from 2.13 g. of EXR 101) and allowed to stand at room temperature overnight. Solvents were removed, the residue dissolved in 5 ml. of benzene, filtered, and concentrated. The product XLVI, wt. 0.185 g., was recrystallized from benzene–petroleum ether; m.p. 213–215°, infrared bands at 1778 (double strength, γ-lactones) and 1752 cm.$^{-1}$ (acetate), but no hydroxyl; n.m.r. signals at 1.11 and 1.21 (two methyl doublets, J = 5.2 and 7.8), 1.48 (C_{10}-methyl singlet), 2.24 (singlet, acetate methyl), 3.60 (singlet, methoxyl), 4.58 (center of broad triplet, H_8), 5.20 (doublet, J = 4.8, H_1) and 5.52 p.p.m. (doublet, J = 5.6, H_6).

Anal. Calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11; O, 32.91. Found: C, 60.07; H, 7.14; O, 33.36.

The methyl ether was also obtained in slightly less pure form (m.p. 200–203°) by acid-catalyzed treatment of the lactol with methanol.

Sodium Borohydride Reduction of the Lactol XXX.—A solution of 0.1 g. of XXX in 8 ml. of dioxane was refluxed for 1.5 hours with 0.1 g. of sodium borohydride and left overnight at room temperature. Removal of solvent *in vacuo*, decomposition with water and a small amount of acetic acid and extraction with chloroform furnished, on evaporation of the chloroform, a gum which solidified on adding ether. Recrystallization from chloroform–petroleum ether furnished XLVII, m.p. 215–217°, infrared bands (KBr) at 1775^{-1} (combination of two γ-lactone groups and acetate), but no hydroxyl, negative Tollens test.

Anal. Calcd. for $C_{16}H_{22}O_6$: C, 61.92; H, 7.15; O, 30.93. Found: C, 61.62; H, 7.30; O, 31.05.

Ethylenethioketal of Dihydroisotenulin.—To a mixture of 0.100 g. of dihydroisotenulin and 0.2 ml. of ethane dithiol was added 0.2 ml. of boron trifluoride etherate. After 5 minutes the mixture was diluted with water and extracted with ether. The ether layer was washed, dried, and the solid obtained on removal of solvent was recrystallized repeatedly from aqueous methanol; yield 0.095 g., m.p. 144–145°, infrared bands at 1770 (lactone) and 1745 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{19}H_{28}O_6S_2$: C, 59.36; H, 7.34; S, 16.6. Found: C, 59.35; H, 7.31; S, 16.4.

Desoxodihydroisotenulin (XXIIa).—A solution of 1.0 g. of the previous substance in 20 ml. of absolute ethanol was mixed with 8 g. of Ranev nickel and refluxed for 8 hours with stirring. Removal of solvent gave an oil which could not be induced to crystallize. A sample, purified by chromatography over alumina, was analyzed. The infrared spectrum had bands at 1770 (lactone) and 1745 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.53; H, 9.01.

The same substance was also prepared by acetylation of XXIIb (see below). The infrared spectra of the two samples were superimposable.

Desacetyldihydroisotenulin (XVIb).—The following modified procedure gave excellent yields of the above compound. To a solution of 33.6 g. of potassium carbonate in 67 ml. of water and 225 ml. of boiling methanol was added 21.0 g. of dihydroisotenulin. The mixture was refluxed for 15 minutes, poured into 680 ml. of water, and placed in the refrigerator overnight. Filtration yielded 15 g. of crystalline desacetyldihydroisotenulin, m.p. 195–196°. Extraction of the filtrate with methylene chloride provided an additional 2 g. of product (94%).

When desacetyldihydroisotenulin was refluxed with Raney nickel under the conditions generally used for desulfurization, starting material was recovered in 95% yield.

Conversion of Desacetyldihydroisotenulin to Desacetyldihydroalloisotenulin.—A mixture of 4 g. of XVb and 100 ml. of 10% sodium hydroxide solution was heated on the steam-bath until solution was complete, diluted to 200 ml., treated with carbon, filtered and acidified. A small amount of solid which separated on cooling was removed by filtration. The filtrate was extracted thoroughly with chloroform, the solvent layer washed to neutrality, dried, and evaporated. The residual oil solidified. Crystallization from benzene–petroleum ether gave 3.0 g. of colorless crystals, m.p. 156°, which did not depress the m.p. of pure XVIb obtained earlier.

When XVb was treated with methanolic sodium hydroxide by the procedure described for XXIIb (*vide infra*) the product which separated *without acidification* consisted of a mixture of starting material (approximately 60%) and XXIVb (approximately 40%).

Ethylenethioketal of Desacetyldihydroisotenulin.—Reaction of 1.0 g. of XVb with ethanedithiol in the usual manner gave, on recrystallization from aqueous methanol, 0.75 g. of colorless needles, m.p. 163–164°. Subsequent recrystallization raised the m.p. to 165–166°, infrared band (CCl_4) at 1780 cm.$^{-1}$ (lactone).

Anal. Calcd. for $C_{17}H_{26}O_3S_2$: C, 59.63; H, 7.65; S, 18.7. Found: C, 59.58; H, 7.87; S, 18.7.

A blank experiment with desacetyldihydroisotenulin, using boron trifluoride but no ethanedithiol resulted in almost quantitative recovery of starting material.

Desoxodesacetyldihydroisotenulin (XXIIb).—Desulfurization of 0.47 g. of the preceding thioketal in the usual manner furnished 0.36 g. of solid, m.p. 106°, after one recrystallization from benzene–petroleum ether. Further crystallizations raised the m.p. to 110–111°, $(\alpha)_D$ 45.8° (95% ethanol, c 1.05). The infrared spectrum (CCl_4) had a band at 1780 cm.$^{-1}$ (lactone).

Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.72; H, 9.66.

A solution of 1 g. of the above in 10 ml. of methanol and 1 ml. of 10% sodium hydroxide solution was heated on the steam-bath for 5 minutes, diluted with 10 ml. of water, and most of the methanol was evaporated on the steam-bath. The crystals which separated on cooling were filtered, washed, and dried, m.p. 156–158° without further purification. A mixed m.p. with XXIVb was undepressed. This procedure is more convenient for the preparation of XXIVb than the one reported previously.[7]

Treatment of desoxodesacetyldihydroisotenulin with sodium bicarbonate did not result in isomerization.

Dehydrodesoxodesacetyldihydroisotenulin (XXIII).—A solution of 0.10 g. of desoxodesacetyldihydroisotenulin in 5 ml. of acetic acid was oxidized at 5° with 1.8 ml. of a 2% solution of chromic acid in acetic acid (36 mg. of CrO_3, theory requires 26.4 mg.). After 12 hours, excess oxidizing agent was destroyed by adding ethanol. The solvents were removed *in vacuo;* the residue was diluted with water and filtered. Crystallization from aqueous methanol gave colorless needles, wt. 0.087 g., m.p. 75–76°, $(\alpha)_D$ −56.3° (95% ethanol, c 0.88). The infrared spectrum (CCl_4) had bands at 1790 (lactone) and 1705 cm.$^{-1}$ (cycloheptanone) but no band near 1410 cm.$^{-1}$ indicative of $-CH_2-\overset{\overset{O}{\|}}{C}-$. The Zimmermann test was negative. All efforts to prepare a derivative by condensation with benzaldehyde, piperonal and anisaldehyde failed. Starting material was recovered.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.99; H, 8.56.

Monoethylenethioketal of Dehydrodesacetyldihydroalloisotenulin.—Reaction of 0.35 g. of XVIII with excess ethanedithiol in the customary manner gave 0.32 g. of crude product, m.p. 120–130°. The m.p. rose to 135–136° on

recrystallization from benzene–petroleum ether. Analysis and infrared spectrum (lactone at 1770, cycloheptanone at 1710 cm.$^{-1}$) showed that only the cyclopentanone carbonyl had reacted.

Anal. Calcd. for $C_{17}H_{24}O_3S_2$: C, 59.99; H, 7.10; S, 18.8. Found: C, 60.40; H, 6.87; S, 18.7.

Desulfurization of the above in the usual manner gave an 85% yield of XXV, m.p. 129–130°, undepressed on admixture of an authentic sample.

Exchange Studies.—An attempt was made to determine the number of hydrogen atoms α- to the keto groups of XXIII and XXV by acid-catalyzed deuterium exchange (since these substances undergo cleavage by base, it was impossible to carry out the equilibration under basic conditions). This resulted in the introduction into XXIII of one deuterium atom per molecule, as expected. Almost two atoms of deuterium were introduced into XXV. Apparently enolization was incomplete under acid conditions.

A mixture of 100 mg. of XXIII, 15 ml. of dioxane, 2 ml. of deuterium oxide and 6 drops of deuterium chloride solution was heated on the steam-bath for 24 hours under nitrogen, the solvent was removed *in vacuo*, and the residue recrystallized from ligroin; yield 60 mg., m.p. 76–77°. There were some changes in the fingerprint region of the infrared spectrum.

Anal. (by Mr. J. Nemeth, University of Illinois). Calcd. for $C_{15}H_{21}DO_3$: 4.54 atom % D. Found: 3.73 atom % D which is equivalent to 0.82 atoms of deuterium per molecule.

When refluxing was continued for 72 hours, a slightly greater amount of deuterium was introduced.

Anal. Found: 4.24 atom % D which is equivalent to 0.92 atom of deuterium per molecule.

Deuteration of XXV in the same manner (reflux period 3 days) yielded a product, m.p. 131°. There were some changes in the fingerprint region of the infrared spectrum.

Anal. Calcd. for $C_{15}H_{20}D_2O_3$: 9.09 atom % D. Found: 8.70 atom % D, which is equal to 1.91 atoms of deuterium per molecule.

Bromination of XXV.—A solution of 200 mg. of XXV in 10 ml. of acetic acid was mixed with 10 ml. of acetic acid containing 160 mg. of bromine. Another 5 ml. of bromine solution was added after 4 hours; further additions resulted in no discharge of the bromine color. After 12 hours at room temperature, acetic acid was removed in an air stream. The residue crystallized on stirring with water. Several recrystallizations from ethanol–water gave material melting at 119–119.5°, infrared bands at 1780 (lactone) and 1710 cm.$^{-1}$ (cycloheptanone).

Anal. Calcd. for $C_{15}H_{21}BrO_3$: Br, 24.27. Found: Br, 23.54.

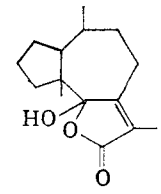
Treatment of XXIII in an analogous manner resulted in quantitative recovery of starting material. An attempt to induce bromination by adding perchloric acid also failed.

Treatment of XXIII with Base.—A mixture of 0.6 g. of XXIII and 20 ml. of 5% sodium hydroxide solution was warmed on the steam-bath until solution was complete (when 10% potassium carbonate solution was used as in earlier experiments[7] solution remained incomplete, and 40–50% of starting material was recovered). The solution was cooled, filtered, cautiously acidified, and extracted with methylene chloride. Removal of the organic solvent yielded an oil which was taken up in 5 ml. of petroleum ether and refrigerated. There separated 0.35 g. of crystalline material, m.p. 56–58°, unchanged on further crystallization from petroleum ether, $(\alpha)_D$ −3.49° (95% ethanol, *c* 0.516), λ_{max} 234 mμ, ϵ 3410 (isoöctane). The infrared spectrum was indicative of dimerization or an equilibrium between keto acid and lactol forms, bands being found at 3500 (hydroxyl), 3000–3500 (broad, bonded acid –OH), 1750 (strong, monomeric carboxyl or lactone), 1705 (strength increases with concentration, cycloheptanone, dimeric carboxyl), 1655 (relatively strong, cycloheptenone or conjugated double bond) and 1635 cm.$^{-1}$ (C=C).

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.97; H, 9.22.

When an attempt was made to hydrogenate the ketoacid (solvent ethanol, catalyst platinum oxide), a neutral substance, m.p. 186–187°, was obtained after removal of solvent. The same substance was also isolated on attempting to prepare the *p*-bromophenacyl ester of the ketoacid and on subjecting it to further treatment with sodium hydroxide. The ultraviolet (λ_{max} 213 mμ, ϵ 10,000) and infrared spectrum (sharp –OH band at 3550, lactone at 1750 and conjugated C=C at 1670) showed its structure to be XLVIII.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.25; H, 8.81.



XLVIII

Piperonylidine Derivatives.—The piperonylidine derivative of desacetyldihydroisotenulin, reported previously,[7] had ultraviolet maxima (95% ethanol, *c* 3.82 × 10^{-5}) at ϵ_{345} 21,500, and ϵ_{306} 10,400. The piperonylidine derivative of desacetyldihydroalloisotenulin exhibited the extinction coefficients (95% ethanol, *c* 4.02 × 10^{-5}): ϵ_{345} 20,700, ϵ_{306} 10,000.

The piperonylidine derivative of dehydrodesacetyldihydroisotenulin was dried at 120° for several days. The m.p. was unchanged, but the analysis and the ultraviolet spectrum (95% ethanol, *c* 3.28 × 10^{-5}), ϵ_{345} 19,500, ϵ_{304} 11,600, indicated a monopiperonylidine derivative.

Anal. Calcd. for $C_{23}H_{24}O_6$: C, 69.68; H, 6.10. Calcd. for $C_{31}H_{28}O_8$: C, 70.44; H, 5.34. Found: C, 69.90; H, 6.40.

Lithium Aluminum Hydride Reduction of XXIIb.—Reduction of 1.5 g. of XXIIb with 1 g. of lithium aluminum hydride by the procedure used with the allo isomer[7] resulted in 0.95 g. of material, m.p. 164–165°. Recrystallization from ethanol–water raised the m.p. to 176–177°, $(\alpha)_D$ −34.8° (95% ethanol, *c* 0.500). Analysis showed that this material was not the expected triol but a lactal IL (positive Tollens test, infrared band at 3300 cm.$^{-1}$ but no carbonyl group) of the type encountered also in the matricin series.[35]

Anal. Calcd. for $C_{15}H_{26}O_3$: C, 70.87; H, 10.30. Found: C, 71.13; H, 10.27.

The reduction to the triol L was carried out by adding 1.5 g. of the hydroxylactone dissolved in 50 ml. of purified N-ethylmorpholine dropwise to a slurry of 1 g. of lithium aluminum hydride in 100 ml. of the tertiary base. After refluxing for 2 hours and working up in the usual manner there was obtained an oil which crystallized on triturating with petroleum ether. Recrystallization from acetone–petroleum ether furnished needles, m.p. 119–119.5°, $(\alpha)_D$ 9.8° (95% ethanol, *c* 0.510).

Anal. Calcd. for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 70.00; H, 10.87.

Reduction of XXIVb with lithium aluminum hydride in N-ethylmorpholine yielded the previously reported[7] triol of m.p. 106°. The identity was established by mixed m.p. and infrared spectrum.

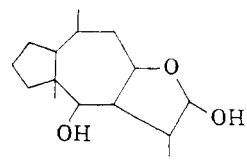
An attempt was made to protect the primary hydroxyl group of the triol L prior to subsequent oxidation by selective tosylation. Treatment of 0.12 g. of the triol in 5 ml. of dry pyridine with 0.095 g. of toluenesulfonyl chloride in the usual fashion yielded a yellow oil, wt. 0.11 g., which was dissolved in benzene and chromatographed over 7 g. of acid-washed alumina. Benzene eluted 0.06 g. of an oil which distilled at 130–140° (2 mm.). Analysis and infrared spectrum showed this to be the ether LI or LII.

Anal. Calcd. for $C_{15}H_{26}O_2$: C, 75.56; H, 11.00. Found: C, 75.07; H, 10.85.

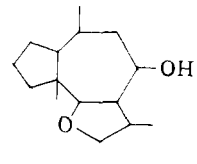
Elution of the column with benzene–ethanol yielded 0.012 g. of starting material.

Selective tritylation of the primary hydroxyl group was achieved in the following way, the secondary hydroxyls being acetylated in the course of the reaction. A mixture of 0.49 g. of the triol V and 0.55 g. of trityl chloride in dry pyridine was kept on the steam-bath for 3 hours. After cooling, 6 ml. of acetic anhydride was added, and the mixture kept at room temperature for 3 days, decomposed with crushed ice, and extracted with chloroform. The extract was washed, dried,

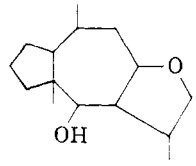
(35) Z. Čekan, V. Herout and F. Šorm, *Coll. Czechoslov. Chem. Commun.*, **22**, 1921 (1957); *Chemistry & Industry*, 1234 (1956).



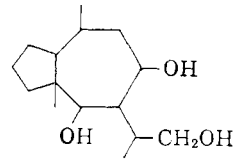
IL



LI



LII



L

and evaporated. The residue, wt. 0.64 g., was dissolved in benzene and chromatographed over alumina. Benzene eluted the diacetate trityl ether, wt. 0.46 g., m.p. 160–161° after crystallization from benzene–petroleum ether, infrared bands at 1730 and 1600 cm.$^{-1}$.

Anal. Calcd. for $C_{38}H_{46}O_5$: C, 78.31; H, 7.96. Found: C, 78.54; H, 7.74.

Attempts to displace the trityl group by treatment with phosphorus tribromide for eventual removal of functional groups resulted in recovery of starting material in 50% yield.

Sodium Borohydride Reduction of XXIII.—To a solution of 0.8 g. of sodium borohydride in 10 ml. of ethanol was added dropwise, with stirring (magnetic stirrer), a solution of 1 g. of the ketolactone in 10 ml. of ethanol. Stirring was continued for 2 hours and dilute hydrochloric acid was added to hydrolyze the reaction mixture. The solvents were removed, and the residual oil was extracted with ether. Removal of the ether gave an oil which was dissolved in benzene and chromatographed over 20 g. of alumina. Elution with benzene–chloroform (3:1) gave an oil (0.2 g.) which resisted crystallization. Elution with benzene–chloroform (1:1) gave 0.57 g. of an oily residue which solidified on trituration with ligroin. The crude material melted at 102–105°. Fractional crystallization from ethanol–water gave in almost equal proportions platelets of XXIIb, m.p. 109–110° (less soluble component), and C_6-epidesoxodesacetyldihydroisotenulin as the more soluble component, long needles, m.p. 131–132°, $(\alpha)_D$ 8.96° (95% ethanol, *c* 0.625), infrared bands at 3585 (non-bonded -OH) and 1755 cm.$^{-1}$ (lactone).

Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 70.68; H, 8.99.

Oxidation of the C_6-epimer with chromium trioxide in the usual manner regenerated XXIII.

Treatment with sodium acetate and acetic anhydride in the usual manner gave C_6-epidesoxodihydroisotenulin, m.p. 149–150° from ethanol–water, $(\alpha)_D$ 11.2° (95% ethanol, *c* 0.445), infrared bands at 1755 (lactone) and 1730 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: 69.18; H, 8.68.

Sodium Borohydride Reduction of XXV.—Reduction of 0.5 g. of XXV with sodium borohydride in the same manner gave a mixture which was separated by fractional crystallization from ligroin into XXIVb, m.p. 156–158°, and its more soluble C_6-epimer, m.p. 157.5–158°, $(\alpha)_D$ −90° (95% ethanol, *c* 0.500) infrared bands at 3600 (non-bonded OH) and 1750 cm.$^{-1}$ (lactone). The two substances gave a sharp m.p. depression. The new epimer was identical with desoxodesacetyltetrahydrobalduilin.[17]

Anal. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.15; H, 9.45.

Oxidation of C_6-epidesoxodesacetyldihydroalloisotenulin regenerated XXV. Its acetate, C_6-epidesoxodihydroalloisotenulin, was prepared in the conventional manner with sodium acetate and acetic anhydride; m.p. 75–76° after recrystallization from ethanol–water, infrared bands at 1762 (lactone) and 1740 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 68.85; H, 8.73.

Ethylene Ketal of Dihydroisotenulin.—A mixture of 1.0 g. of dihydroisotenulin, 0.2 g. of *p*-toluenesulfonic acid and 1 ml. of ethylene glycol was refluxed with 100 ml. of benzene overnight, using a Dean–Stark trap. The benzene layer was washed with sodium bicarbonate solution and water; removal of the solvent *in vacuo* gave a mixture of ketal and starting material which was separated by chromatography over alumina (solvent and eluent benzene). Crystallization of the benzene fraction from benzene–petroleum ether gave colorless crystals, m.p. 126–128°, infrared bands at 1770 (lactone) and 1745 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{19}H_{28}O_6$: C, 64.75; H, 8.01. Found: C, 65.09; H, 8.11.

Ethylene Ketal of Desacetyldihydroisotenulin.—Treatment of 2.0 g. of XVb in the same manner furnished, on removal of benzene, a gum which was triturated with petroleum ether whereupon 1.2 g. of yellow crystals, m.p. 158–165°, separated. Recrystallization from benzene–petroleum ether gave colorless needles, m.p. 166–167°, infrared bands at 3300 (bonded hydroxyl) and 1770 cm.$^{-1}$ (lactone).

Anal. Calcd. for $C_{17}H_{26}O_5$: C, 65.78; H, 8.44. Found: C, 66.22; H, 8.56.

Monoethylene Ketal of Dehydrodesacetyldihydroisotenulin.—To 0.5 g. of the previous substance, dissolved in 10 ml. of anhydrous pyridine, was added, with stirring and cooling, 0.5 g. of chromic oxide in small portions (nitrogen atmosphere). After standing overnight the mixture was poured into 100 ml. of water and extracted with a 1:1 mixture of benzene and petroleum ether. Drying and removal of solvents gave an oil which solidified. The product was recrystallized from acetone–petroleum ether and then from aqueous methanol; yield 0.35 g., m.p. 121–122°, infrared bands at 1778 (lactone), 1710 (cyclopentanone) and bonded hydroxyl near 3250 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85. Found: C, 66.11; H, 8.03.

Monoethylenethioketal of Dehydrodesacetyldihydroisotenulin.—A mixture of 100 mg. of the ketal ketone, 0.2 ml. of ethane dithiol and 0.1 ml. of boron trifluoride etherate was allowed to stand for 5 minutes. On working up in the usual fashion there was obtained 85 mg. of colorless needles, m.p. 200–201°, recrystallized from benzene–petroleum ether, infrared bands at 1780 (lactone) and 1710 cm.$^{-1}$ (cycloheptanone). This and the analysis indicated that the ethylene ketal had been converted to the monothioketal.

Anal. Calcd. for $C_{17}H_{24}O_3S_2$: C, 59.99; H, 7.11; S, 18.8. Found: C, 59.94; H, 7.11; S, 18.8.

The substance was also obtained by treatment with excess ethanedithiol and boron trifluoride or perchloric acid. In an attempt to isomerize it or to open the lactone ring, the monothioketal was refluxed with 10% sodium carbonate solution for 3 hours. The substance did not appear to go into solution, and 99% of starting material was recovered.

Pyranyl Ether of Desoxodesacetyldihydroisotenulin (XXIIc).—To 10 ml. of dihydropyran was added 1 g. of XXIIb and 2 drops of concd. hydrochloric acid. The mixture was shaken vigorously, allowed to stand for 22 hours, diluted with ether, neutralized with sodium carbonate, filtered, and concentrated in an air stream. The crystalline product was washed with cyclohexane; yield 0.6 g., m.p. 162–163°. Crystallization from ethanol raised the m.p. to 168.5–169°, infrared band at 1760 cm.$^{-1}$ (lactone) but no hydroxyl.

Anal. Calcd. for $C_{20}H_{32}O_4$: C, 71.38; H, 9.59. Found: C, 71.37; H, 9.69.

This material was recovered without change on treatment with hot methanolic sodium hydroxide.

Attempts to prepare the pyranyl ether of XVIb and XXIVb led to recovery of starting material. Desacetyldihydroisotenulin yielded a glassy product, infrared band at 1760 cm.$^{-1}$ (lactone), no hydroxyl, which did not appear to undergo any change on treatment with methanolic alkali.

Anhydrodesacetyldihydroisotenulin (XIX).—A solution of 0.55 g. of XVb in 5 ml. of dry pyridine was chilled, mixed with 1 ml. of methanesulfonyl chloride at ice-bath temperature, kept in the refrigerator for 12 hours, and poured over crushed ice. The product was extracted with chloroform, and the extracts washed, dried, and evaporated. The residue crystallized; yield 90% of light tan rods from chloroform–petroleum ether, m.p. 180–181°.

Anal. Calcd. for $C_{16}H_{24}O_6S$: C, 55.80; H, 7.02. Found: C, 55.40; H, 6.79.

A solution of 1 g. of the mesylate in 12 ml. of lutidine was refluxed for 8 hours, cooled, poured over crushed ice–hydrochloric acid, and extracted with methylene chloride. The organic layer was washed, dried, and evaporated *in vacuo*. The remaining material was taken up in benzene and chromatographed over 40 g. of acid-washed alumina. Benzene eluted 0.45 g. of XIX, m.p. 143.5–144°, λ_{max} 219 mμ (ε 13600), infrared bands at 1750 (γ-lactone and cyclopentanone) and 1665 cm.$^{-1}$ (conjugated double bond).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.62; H, 8.05.

A solution of the mesylate was refluxed with sodium iodide in dry acetone for 14 hours. Workup in the usual manner gave a quantitative recovery of starting material. Attempted solvolysis of the mesylate with sodium acetate–acetic acid resulted in recovery of desacetyldihydroisotenulin.

Reduction of 0.4 g. of the anhydro-derivative with 0.35 g. of lithium aluminum hydride in tetrahydrofuran followed by the usual workup yielded 0.48 g. of an oil whose infrared spectrum indicated the presence of a small amount of a carbonyl-containing impurity. Dehydrogenation of this material in a nitrogen atmosphere in Nujol solution with 10% palladium–charcoal at 310–320° for 2 hours resulted in a bluish color. The mineral oil was diluted with petroleum ether, and the azulene extracted with 60% phosphoric acid. Dilution of the acid extract with crushed ice followed by extraction with petroleum ether and evaporation resulted in a blue oil which was chromatographed over alumina; yield 20 mg., but could not be induced to form a trinitrobenzene complex. The visible spectrum had peaks at 570 and 582 mμ, but the intensity (log ε 1.9) indicated the presence of non-azulenic impurities. Paper chromatography indicated the presence of a single azulenic component, R_f 0.85. Guaiazulene under similar conditions had an R_f value of 0.84.

Anhydrodesacetyldihydroalloisotenulin (XX and XXI).—A solution of 0.55 g. of XVIb in 5 ml. of dry pyridine yielded 0.7 g. of the mesylate, m.p. 164–165° after recrystallization from chloroform–petroleum ether.

Anal. Calcd. for $C_{16}H_{24}O_6S$: C, 55.80; H, 7.02; S, 9.31. Found: C, 55.61; H, 6.99; S, 9.27.

Treatment of 0.6 g. of the mesylate with 2,6-lutidine yielded 0.55 g. of an oil which was chromatographed over 30 g. of acid-washed alumina. Elution with benzene–ether (4:1) gave an oil which gradually crystallized and was recrystallized from benzene–petroleum ether; m.p. 138–139°, λ_{max} 218 mμ (ε 12300), infrared bands at 1750 (γ-lactone and cyclopentanone) and 1665 cm.$^{-1}$ (conjugated double bond).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.41; H, 8.11.

Repetition of this experiment on a large scale, using 4 g. of mesylate, gave two fractions. Fraction 1, eluted with benzene, was a new anhydro derivative, apparently XXI, yield 1.93 g., m.p. 101°, $[\alpha]^{23}D$ −100° ($CHCl_3$, *c* 0.33), λ_{max} 296 mμ (ε 29), infrared bands at 1745–1755 (γ-lactone and cyclopentanone) and 1660 cm.$^{-1}$ (rel. weak, unconjugated double bond.

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.56; H, 8.12. Found: C, 72.39; H, 8.46.

The second fraction, eluted with ether, wt. 0.17 g., was XX.

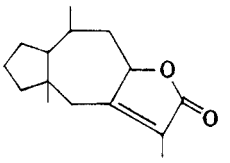
Anhydrodesoxodesacetyldihydroisotenulin.—Reaction of 0.9 g. of XXIIb with methanesulfonyl chloride in the usual manner gave 1.0 g. of solid, m.p. 113–113.5° after recrystallization from chloroform–ligroin.

Anal. Calcd. for $C_{16}H_{26}O_5S$: C, 58.16; H, 7.93; S, 9.71. Found: C, 58.45; H, 8.20; S, 9.75.

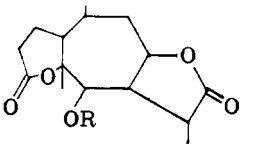
Treatment of 1 g. of the mesylate with refluxing lutidine resulted in 0.6 g. of oil which was dissolved in petroleum ether and chromatographed over 40 g. of acid-washed alumina. Elution with benzene–petroleum ether gave a colorless viscous liquid, yield 0.5 g., b.p. (bath temperature) 135° (0.05 mm.), $(\alpha)^{21}D$ + 49.3°, λ_{max} 219 mμ (ε 13300), infrared bands at 1748 (γ-lactone) and 1665 cm.$^{-1}$ (conjugated double bond). Based on the spectroscopic evidence, the structure is LIII.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 76.88; H, 9.46. Found: C, 76.67; H, 9.75.

Peracid Oxidation of Dihydroisotenulin.—A solution of 1.5 g. of dihydroisotenulin in 10 ml. of acetic acid and 7.5 ml. of 40% peracetic acid was allowed to stand at room temperature for 4 days with a trace of *p*-toluene-sulfonic acid.[36] The solution was concentrated to 10 ml. *in vacuo*, diluted with water, neutralized with sodium bicarbonate, and extracted with chloroform; 1.4 g. of a solid was obtained. Recrystallization from benzene–petroleum ether furnished hexagonal prisms of LIVa, m.p. 202°, negative iodoform test, infrared bands at 1770 (γ-lactone) and 1735 cm.$^{-1}$ (δ-lactone and acetate). The n.m.r. spectrum exhibited an unresolved doublet at 5.41 (H_5), complex band at 4.39 (H_8) and methyl signals at 2.22 (acetate), 1.35 (singlet), 1.32 (doublet, J = 4) and 1.11 (doublet, J = 6.5), but no new proton signal in the region 3.5–5.5 p.p.m. compared with the spectrum of dihydroisotenulin.



LIII



LIVa, R = Ac
b, R = H

Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 62.31; H, 7.09.

This substance could not be reduced satisfactorily with lithium aluminum hydride in tetrahydrofuran or N-ethylmorpholine, or in ether by the Soxhlet technique.

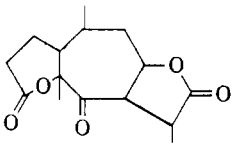
Peracid Oxidation of Desacetyldihydroisotenulin.—Oxidation of 2 g. of XVb with 40% peracetic acid using *p*-toluenesulfonic acid as catalyst yielded 1.85 g. of crude hydroxydilactone LIVb, m.p. 165–170°, which recrystallization from ethyl acetate raised to 174.5–177°, $(\alpha)^{22}D$ ($CHCl_3$, *c* 0.573) +16.1°, negative iodoform test, no absorption in the ultraviolet, infrared bands at 3430, 1765 (γ-lactone) and 1725 (δ-lactone), n.m.r. signals at 4.39 (complex triplet, H_8), 4.16 (doublet, J = 11, H_6), 1.38 (singlet methyl), 1.19 (doublet methyl, J = 6.5) and 1.01 (doublet methyl, J = 4.5).

Anal. Calcd. for $C_{15}H_{22}O_5$: C, 63.83; H, 7.86. Found: C, 63.86; H, 7.86.

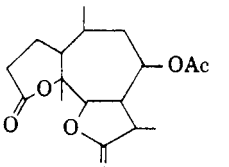
Oxidation of 0.25 g. of the above with chromic acid–acetic acid yielded 0.17 g. of a ketodilactone LV, m.p. 197–198° from absolute ethanol, λ_{max} 296 mμ (ε 36), infrared bands (Nujol) at 1780 (γ-lactone), 1740 (δ-lactone) and 1720 cm.$^{-1}$ (cycloheptanone).

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.25; H, 7.20. Found: C, 64.34; H, 7.23.

The same substance was also obtained in 40% yield by oxidation of XVIII, the cycloheptanone carbonyl proving inert toward peracetic acid. Similarly, XXIII and XXV were recovered from attempted oxidations.



LV



LVI

Peracid Oxidation of Desacetydihydroalloisotenulin.—The above compound, wt. 0.35 g., was oxidized as usual with excess peracetic acid, weight of crude product 0.27 g. Recrystallization from 95% ethanol yielded fine needles LVI, m.p. 213–214°, $(\alpha)D$ −91.8° (95% ethanol, *c* 0.485), infrared bands at 1760 (γ-lactone) and 1730 cm.$^{-1}$ (broad, δ-lactone and acetate), n.m.r. signals at 4.96 (broad, H_3), 4.78 (doublet, J = 9.5, H_6), 2.03 (singlet, C_3-acetate), 1.28 (methyl doublet, J = 6) and 1.10 p.p.m. (broad methyl). It was evident that acetylation had taken place spontaneously.

Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 63.01; H, 7.66.

(36) D. H. R. Barton, A. da S. Campos-Neves and A. I. Scott, *J. Chem. Soc.*, 2699 (1957).

The same substance was obtained in 50% yield by peracid oxidation of dihydroalloisotenulin.

Desacetylneotenulin. (A) From Tenulin.—In our hands the procedure described by Barton and de Mayo[6] yielded only desacetylisotenulin. Carrying out the reaction in a homogeneous medium by adding methanol gave 55–65% yields of desacetylneotenulin. A boiling solution of 20 g. of tenulin in 400 ml. of methanol was diluted with 1 l. of warm water, and 160 ml. of saturated sodium bicarbonate solution was added. The clear yellow solution was heated on the steam-bath for 4 hours, concentrated to 600 ml. at reduced pressure, and allowed to stand for 2 days. There precipitated 6.8 g. of desacetylneotenulin, m.p. 239–240°. The filtrate was extracted with methylene chloride. The organic extract on washing, drying and concentrating yielded 5 g. of gum, which on stirring with benzene–chloroform gave 2 g. of XXXII, m.p. 236–237°. A further quantity of XXXII, wt. 0.5 g., separated on standing. The benzene–chloroform mother liquor was concentrated to dryness *in vacuo*, the residue redissolved in benzene and chromatographed over acid-washed alumina. The first fractions were gummy. Later fractions (benzene) gave colorless crystals, wt. 0.38 g., m.p. 194–196°, identified as desacetyldihydroisotenulin. Since this substance was obtained quite consistently, it appears that tenulin, as purified for ordinary use, is accompanied by small quantities (3–5%) of dihydrotenulin or dihydroisotenulin. In one run, desacetylisotenulin was followed on the chromatogram by another substance, m.p. 124–125°, wt. 0.25 g., which appeared to be isomeric with it, λ_{max} 224 and 320 mμ (ε 8900 and 50); infrared bands at 3650 and 3450 (-OH), 1760 (γ-lactone), 1695 and 1600 (cyclopentenone).

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.68; H, 8.10.

The aqueous layer, after extraction with methylene chloride, was acidified and extracted with chloroform. Removal of chloroform yielded a gum which was taken up in hot benzene. Cooling precipitated 1.5 g. of desacetylneotenulin. The benzene mother liquor was chromatographed over 15 g. of acid-washed alumina. Two substances were eluted. The less polar material (eluent benzene–ether 4:1) was the keto-epoxylacetone XLIII or XLIV, wt. 0.35 g., m.p. 144–145° after recrystallization from ether–ligroin; n.m.r. signals at 4.59 (1 H, unresolved multiplet), 4.33 and 4.25 (1 H, doublet), 3.92 (1 H, unresolved multiplet), 1.38 (singlet methyl), 1.19 (doublet methyl) and 1.18 p.p.m. (doublet methyl). Further transformations of this substance will be described subsequently.

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.15; H, 7.63; O, 24.21. Found: C, 67.98; H, 7.53; O, 24.69.

The more polar substance was not always obtained in pure form. In one run it was isolated in 0.35 g. yield, m.p. 208–209° from benzene–petroleum ether, $(\alpha)^{25}D$ + 102.9° ($CHCl_3$, *c* 0.345).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.62; O, 24.22. Found: C, 67.80; H, 7.63; O, 24.77.

(B) From Isotenulin.—A mixture of 0.1 g. of isotenulin and 10 ml. of saturated sodium bicarbonate solution was heated on the steam-bath for 2 hours. The yellow solution was cooled, filtered, and extracted with chloroform. Recrystallization of the chloroform extract from benzene gave a 40% yield of XXXII.

(C) From Desacetylisotenulin.—A solution of 0.5 g. of desacetylisotenulin in 100 ml. of methanol (homogeneity was essential for this reaction) was isomerized exactly as described for tenulin. Removal of solvent after neutralization with acetic acid yielded 45% of XXXII.

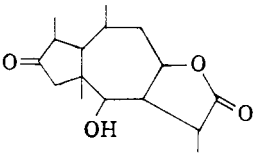
A solution of 2 g. of XIb in 125 ml. of absolute ethanol containing 1.75 g. of *p*-toluenesulfonic acid was refluxed for 18 hours, concentrated to 25 ml. *in vacuo* and diluted with 100 ml. of benzene. On cooling, 1.0 g. of XIb was recovered. The filtrate was evaporated to dryness, the residue taken up in benzene and chromatographed over 40 g. of alumina. Elution with benzene–ether (4:1) gave a small amount of the substance, m.p. 124–125°, previously described. Elution with chloroform yielded 0.7 g. of desacetylneotenulin (80% based on unrecovered XIb). The infrared ultraviolet spectrum of a sample, m.p. 240–241°, agreed with that reported previously[6]; $(\alpha)^{22}D$ −24° ($CHCl_3$, *c* 0.50).

The dinitrophenylhydrazone melted at 286°, λ_{max} 389 and 385 mμ (ε 28400 and 28100).

Anal. Calcd. for $C_{21}H_{24}N_4O_7$: C, 56.75; H, 5.44. Found: C, 56.29; H, 5.03.

Dihydrodesacetylneotenulin.—Desacetylneotenulin, wt. 2 g., was stirred into liquid ammonia and 0.22 g. of lithium wire was added in small pieces until the solution became blue. After stirring for 1 hour, solid ammonium chloride was added until the solution became colorless. The ammonia was allowed to vaporize, and water was added. After neutralization with acid, the solution was extracted with chloroform. The organic layer yielded 1.1 g. of a reddish gum which was taken up in 10 ml. of benzene and chromatographed over acid-washed alumina. Benzene eluted 0.25 g. of an oil which crystallized slowly and was recrystallized from benzene–petroleum ether; m.p. 180°, $(\alpha)^{23}D$ 43.8 ($CHCl_3$, 0.625); infrared bands at 3450 (hydroxyl), 1760 (γ-lactone), 1745 (cyclopentanone) and 1400 cm.$^{-1}$ (-CH_2-C_{11}-); n.m.r. signals at 4.82 (triplet of doublets, H_8), 3.80 (triplet, J = 5.8, H_6), 1.21, 1.03 and 0.99 (methyl doublets, J = 6.7, 2 and 3.3). The structure is LVII.

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33; O, 24.03. Found: C, 67.66; H, 8.26; O, 24.11.



LVII

Desoxodesacetylneotenulin (XXXIV).—Catalytic hydrogenation of desacetylneotenulin in neutral solution did not proceed. A solution of 1.0 g. of XXXII in 150 ml. of absolute ethanol and 5 ml. of ethanol saturated with hydrogen chloride was reduced catalytically with platinum oxide until hydrogen uptake ceased. The solvent was evaporated; the residue was dissolved in benzene–petroleum ether (1:1) and chromatographed over 20 g. of acid-washed alumina. Methylene chloride eluted 0.45 g. of solid, m.p. 96–98°. Recrystallization from ligroin and ethanol–water gave crystals, m.p. 102.8–103.5° (Kofler), $(\alpha)^{25}D$ −29.5° (95% ethanol, *c* 0.550), infrared bands at 3500 (-OH), 1765 (γ-lactone) and 1640 cm.$^{-1}$ (very weak, C=C). The ultraviolet spectrum exhibited end absorption near 210 mμ characteristic of the tetrasubstituted double bond.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.83; H, 8.79.

Mesylation of XXXIV in the manner described earlier for XXXII gave the mesylate, m.p. 142–143°.

Anal. Calcd. for $C_{16}H_{24}O_5S$: C, 58.57; H, 7.37. Found: C, 58.69; H, 7.30.

Desoxoneotenulin (XXXV).—Catalytic reduction of 1.0 g. of neotenulin in acid solution and chromatography over 30 g. of acid-washed alumina gave, in the benzene–petroleum ether (1:1) fraction, 0.6 g. of solid, m.p. 114–118.°

Recrystallization from petroleum ether–ligroin raised the m.p. to 122–122.5°, $(\alpha)D$ − 33.7° ($CHCl_3$, *c* 0.525), infrared bands at 1765 (γ-lactone), 1730 (acetate) and 1655 cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 70.11; H, 8.33.

This substance was also formed by acetylation of XXXIV with acetic anhydride in pyridine. Chromatography of the material resulting from 0.8 g. of XXXIV over acid-washed alumina (solvent benzene) yielded in the benzene–petroleum ether eluates 0.35 g. of XXXV. Elution with benzene–ether (10%, then 20%) gave a new isomer of XXXIV, which after crystallization from ether-petroleum ether melted at 148–150°, infrared bands at 3620 (-OH) and 1800 cm.$^{-1}$ (γ-lactone). It did not exhibit any double bond frequency in the infrared and stubbornly retained solvent of crystallization.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 72.01; H, 8.80; O, 19.19. Found: C, 71.09; H, 9.52; O, 19.52.

Anhydrodesacetylneotenulin (XXXVI).—A solution of 1.5 g. of desacetylneotenulin methanesulfonate in 20 ml. of lutidine was refluxed for 4 hours and worked up in the usual manner (use of β-collidine did not effect elimination). Chromatography over acid-washed alumina (eluent 9:1

benzene–petroleum ether) furnished 0.465 g. of solid, m.p. 145–147°, which was recrystallized from ether and ligroin to a constant m.p. of 155–156°, $(\alpha)^{25}D$ −21° (95% ethanol, *c* 0.52); λ_{max} 287 and 213 mμ, ϵ_{max} 16000 and 9200, infrared bands at 1770, 1690 (shoulder near 1650) and 1597 mμ, λ_{max} (alkali) 297 mμ (ε 18600).

Anal. Calcd. for $C_{16}H_{18}O_3$: C, 73.14; H, 7.37. Found: C, 72.86; H, 7.40.

The dinitrophenylhydrazone was recrystallized from chloroform–ligroin. The dark-red crystals melted at 230–231°; λ_{max} 395, 310, 270 and 243 mμ; ϵ_{max} 37300, 13000, 18600 and 18200.

Anal. Calcd. for $C_{21}H_{22}N_4O_6$: C, 59.15; H, 5.20; N, 13.14. Found: C, 58.96; H, 5.10; N, 13.29.

From the above chromatogram, petroleum ether–benzene (1:4) eluted 0.05 g. of gum, infrared bands at 1750 (shoulder near 1770), 1690, 1635 (rel. strong) and 1600 cm.$^{-1}$. This indicated the possibility of an isomer containing an α,β-unsaturated lactone system.

Further elution of the column gave 0.23 g. of the starting mesylate.

Compound XXXVI was also prepared in lower yield by refluxing 1 g. of the methanesulfonate in 25 ml. of dimethylformamide[37] for 24 hours. The dark brown reaction mixture was cooled and diluted with ether. The organic layer was washed in the usual manner, evaporated, taken up in benzene, and chromatographed. Elution with benzene–petroleum ether (9:1) yielded 0.15 g. of a solid which was recrystallized from ether to give XXXVI, m.p. 152–154°.

Compound XXXVI was first prepared accidentally in the course of a reaction designed to lead to the ethylene ketal of XXXII. A solution of 1.5 g. of XXXII, 0.2 g. of *p*-toluenesulfonic acid, 1 ml. of ethylene glycol and 100 ml. of benzene was refluxed for several days. The usual workup furnished a glass which was chromatographed over alumina, solvent and eluent benzene. The eluate on recrystallization from benzene yielded a few mg. of XXXVI and unsharply melting material which was not further characterized.

A solution of 0.1 g. of XXXVI in 25 ml. of chloroform was ozonized at 0° for 1.5 hours. The mixture was extracted, with prolonged shaking, with water, the aqueous layer steam distilled, the distillate neutralized, concentrated *in vacuo*, and converted to the *p*-bromophenacyl ester; yield of *p*-bromophenyl acetate 21 mg.

Anhydrodesoxodesacetylneotenulin.—A solution of 1 g. of the mesylate of desoxodesacetylneotenulin in 10 ml. of dry lutidine was refluxed for 4 hours (drying tube). The brown solution was diluted with water and extracted with ether. The combined ether extracts were washed with dilute acid, water, sodium bicarbonate, and water, dried and evaporated *in vacuo*. The residual oil was taken up in benzene–petroleum ether and chromatographed over 30 g. of acid-washed alumina which had been placed on the column with petroleum ether. The benzene eluate yielded a colorless viscous oil, wt. 0.58, which was distilled *in vacuo* (bath temp. 125–130°, 0.05 mm.). The distillate crystallized on attempted transfer and was recrystallized from ethanol–water and ligroin; m.p. 96–97°, $(\alpha)^{25}D$ − 12.0° (95% ethanol, *c* 0.560), λ_{max} 249 mμ (ε 10000), infrared bands at 1760 (γ-lactone) and rather broad, moderate-strength band at 1645–1620 cm.$^{-1}$ (double bonds). The substance decomposed rapidly on standing in air, more slowly in a sealed vial, and could not be analyzed satisfactorily.

Degradation of Desacetylneotenulin to XXXVII.—A solution of 1.1 g. of XXXII in 40 ml. of ethyl acetate and 30 ml. of chloroform was ozonized at −10°. Ozone absorption stopped after 1.5 hours, but ozone was passed through for an additional 15 minutes. Excess ozone and chloroform was removed by passing a stream of air through the solution, 0.2 g. of 10% palladium–charcoal was added, and the solution hydrogenated at 24 lb. for 0.5 hour. The solution was filtered and concentrated in an air stream. The residual gum could not be induced to crystallize and exhibited infrared bands at 1725 and 1707 cm.$^{-1}$ (α-diketone) in addition to the lactone peak at 1780 cm.$^{-1}$.

The crude ozonolysis product was dissolved in 10 ml. of methanol, mixed with 0.8 g. of sodium periodate in 25 ml. of water and stirred overnight at room temperature. The solution was acidified with a few drops of concd. hydrochloric acid and extracted thoroughly with methylene chloride. The organic extract was washed, dried and concentrated. The residue solidified on triturating with petroleum ether. Crystallization from chloroform–petroleum ether yielded 0.25 g. of dilactone XXXVII, m.p. 206–209°, infrared bands at 1785 (double strength, γ-lactones) and 1708 cm.$^{-1}$ (cycloheptanone), negative Zimmermann test, $(\alpha)^{22}D$ +77.4° ($CHCl_3$, *c* 1.46).

Anal. Calcd. for $C_{13}H_{16}O_5$: C, 61.89; H, 6.39; O, 31.71; neut. equiv., 126. Found: C, 61.80; H, 6.40; O, 31.73; neut. equiv., 123.

An attempt to prepare the oxime resulted in recovery of starting material.

Ozonolysis of Desoxodesacetylneotenulin.—A solution of 0.8 g. of XXXIV in 35 ml. of dry redistilled ethyl acetate was ozonized at 5° for 1.5 hours. The cooling bath was removed, excess ozone driven off by a stream of oxygen, the solution transferred to a citrate bottle and hydrogenated with 0.12 g. of 10% palladium–charcoal at 25 lb. pressure. It was filtered, concentrated *in vacuo*, and the residue rubbed with a benzene–ether–petroleum ether mixture until crystalline; total yield from two runs 0.9 g., m.p. 195–198°. One recrystallization from ethyl acetate containing a little ethanol gave needles (XXXIX), m.p. 204–206°, infrared bands at 3550 (–OH), 1775 (γ-lactone) and 1705 cm.$^{-1}$ (methyl ketone and cycloheptanone). The substance gave a positive iodoform test and a negative periodic acid test. Before analysis, the sample was dried at 80° for 20 hours and then exhibited a m.p. of 186–189°, but the infrared spectrum had not changed.

Anal. Calcd. for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85; O, 28.34; active H, 0.355. Found: C, 63.81; H, 7.77; O, 28.64; active H, 0.274.

Oxidation of 0.14 g. of XXXIX in 4 ml. of acetic acid with 0.35 g. of freshly prepared lead tetraacetate in 8 ml. of acetic acid for 15 hours gave a gum on removal of solvent. Trituration with ethyl acetate–petroleum ether resulted in recovery of 22 mg. of XXXIX. The infrared spectrum of the remaining material indicated partial acetylation (weak –OH band, 1775 lactone, 1730 acetate and 1705 ketone. Reacetylation with acetic anhydride–pyridine caused disappearance of the hydroxyl, strengthening and broadening of the 1770 band, and weakening of the ketone band (perhaps because of formation of an enol acetate) but did not result in crystalline material. The material was washed free of acidic products with sodium bicarbonate solution; the neutral fraction weighed 0.135 g., which indicated that no acidic product had been formed and that XXXIX was not an α-ketol.

Acetylation of 0.2 g. of XXXIX with acetic anhydride–pyridine yielded solid material XXXVIII, which was crystallized from ethyl acetate–petroleum ether; yield 0.11 g., m.p. 142–143°, positive iodoform and Zimmermann test, λ_{max} 288 mμ; infrared bands at 1777 (γ-lactone), 1745 (acetate) and 1706 cm.$^{-1}$ (methyl ketone and cycloheptanone).

Anal. Calcd. for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46; acetyl, 13.27. Found: C, 63.13; H, 7.38; acetyl, 13.27.

The same substance was also obtained by ozonolysis of 0.8 g. of desoxoneotenulin (XXXV) in ethyl acetate at −10°. Hydrogenation of the solution and crystallization from ethyl acetate–petroleum ether yielded 0.58 g. of XXXVIII.

Treatment of 0.25 g. of XXXIX with methanesulfonyl chloride in pyridine furnished, after the usual workup, 0.17 g. of the mesylate, m.p. 150–151° after crystallization from chloroform–petroleum ether; infrared bands at 1775 (γ-lactone), 1705 (methyl ketone and cycloheptanone) and the usual sulfonate frequencies.

Anal. Calcd. for $C_{16}H_{24}O_7S$: C, 53.33; H, 6.67. Found: C, 53.28; H, 6.85.

An attempt to carry out an elimination reaction by refluxing the mesylate with lutidine resulted in a 15% recovery of starting material and unidentifiable oils whose spectra indicated the appearance of some conjugation (α,β-unsaturated ketone or lactone).

Oxidation of XXXIX.—A solution of 0.25 g. of XXXIX in 2 ml. of pyridine was added to 0.17 g. of chromic oxide in 2 ml. of pyridine at 5°. After 12 hours at 5°, the mixture was extracted with ether, then poured into water and extracted with chloroform. The combined organic extracts

(37) F. C. Chang and R. T. Blickenstaff, *J. Am. Chem. Soc.*, **80**, 2906 (1958); H. R. Nace, *Chem. and Ind.*, 1679 (1958).

were dried, concentrated, and chromatographed over acid-washed alumina (solvent and eluent chloroform). The eluate was concentrated, and the residue crystallized from benzene–petroleum ether; yield 0.035 g. of XL, m.p. 115–117°, infrared bands at 1780 (γ-lactone) and 1705 cm.$^{-1}$ (composite of three ketones); ultraviolet spectrum λ_{max} 282 and 312.5 (ϵ 1220 and 1100), after addition of base λ_{max} 306 (ϵ 17500).

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.28; H, 7.14. Found: C, 64.72; H, 7.03.

Dehydrodesacetylneotenulin (XLI).—A solution of 2 g. of desacetylneotenulin in 50 ml. of acetic acid was treated dropwise, with stirring, with a solution of 0.8 g. of chromic acid (1.6 equivalents) in 80 ml. of acetic acid at such a rate that there was no excess of chromic acid. This required about 4.5 hours. The solution was concentrated to 10 ml. at reduced pressure, diluted with ice-water and thoroughly extracted with methylene chloride. The organic layer was washed, dried and evaporated, the gummy residue taken up in methylene chloride, diluted with benzene (ratio benzene–CH_2Cl_2, 4:1) and chromatographed over 60 g. of acid-washed alumina. Benzene–ether (9:1) eluted a light-yellow band which gave a colorless solid (XLI), wt. 0.5 g., m.p. 145–160° (no infrared absorption at 3300–3500 cm.$^{-1}$). Two crystallizations from ethanol yielded 110 mg. of rods, m.p. 178.5–179.5°, $(\alpha)^{25}D$ − 139° (95% ethanol, *c* 0.525). Barton and de Mayo[6] report m.p. 178–182° (somewhat variable), $(\alpha)D$ − 164° (chloroform, *c* 1.3). The infrared spectrum had bands at 1780, 1725 (strong shoulder on next band), 1705 and 1630 cm.$^{-1}$. The ultraviolet spectrum had λ_{max} 244 mμ (ϵ 11700) and 285 mμ (ϵ 700, plateau); 12 hours after addition of three drops of 8 *N* KOH λ_{max} 305 mμ (ϵ 4700), after acidification λ_{max} 280 and 320 mμ (ϵ 3500 and 3100).

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.70; H, 6.90. Found: C, 68.03; H, 6.40.

The ethanolic mother liquors yielded material, m.p. 132–135°. Recrystallization raised the m.p. to 134–135°, $(\alpha)^{25}D$ − 66° (95% ethanol, *c* 0.515), $(\alpha)D$ − 39° ($CHCl_3$, *c* 0.675). The infrared spectrum resembled that of the higher-melting isomer except in the intensity of certain bands in the fingerprint region. The ultraviolet spectrum also was similar, λ_{max} 244 and 285 mμ (ϵ 11300 and 550). This substance may be a C_5- or C_{11}-epimer of XL.

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.70; H, 6.90. Found: C, 68.79; H, 6.47.

Contact of any basic solid (alumina or boiling chip) with an ethanolic solution of these substances gave a bright yellow color.

Further elution of the chromatogram with benzene–ether yielded 0.2 g. of non-crystallizable gum; chloroform gave 0.7 g. of desacetylneotenulin; and methanol containing 1% of acetic acid gave 0.5 g. of a yellow acidic gum which on further purification melted at 172–175°, infrared bands at 3500–3200 (carboxyl OH), 1775 (γ-lactone), 1735, 1700 and 1625 cm.$^{-1}$. Attempts to prepare a sharply melting analytical sample were not successful.

Dehydrodesoxodesacetylneotenulin (XLII).—A solution of 0.25 g. of XXXIV in 2.5 ml. of pyridine was added to 0.3 g. of chromium oxide in 2.5 ml. of pyridine at 5°. After 40 hours at room temperature, the mixture was extracted twice with ether, the residue poured into water and extracted with chloroform. The combined organic extracts were washed, dried, and concentrated. The gummy residue was dissolved in benzene and chromatographed over acid-washed alumina. Fraction 1 (benzene) and fraction 2 (benzene–ether 3:1) had the same infrared spectrum and were combined; wt. 0.14 g., infrared bands at 1775 (γ-lactone) and 1703 cm.$^{-1}$ (cycloheptanone), negative Zimmermann test, $(\alpha)^{22}D$ 29.5° ($CHCl_3$, *c* 3.29), λ_{max} 287 mμ (ϵ 230, no change in alkali). The material could not be induced to crystallize. An attempt to isomerize it by refluxing with methanol containing two drops of concd. hydrochloric acid resulted in recovery of starting material. Sublimation furnished a yellowish, viscous gum which exhibited the usual tendency of compounds of this series to decompose on standing (water or oxygen uptake).

Anal. Calcd. for $C_{15}H_{20}O_3 \cdot 0.5H_2O$: C, 70.04; H, 8.17; O, 21.79. Found: C, 70.27; H, 8.24; O, 21.85.

Reactions of the Ketoepoxylactone XLIII or XLIV.—A solution of 0.2 g. of the ketoepoxylactone and 0.5 g. of *p*-toluenesulfonic acid in 8 ml. of acetic acid and 4 ml. of acetic anhydride was warmed at 60–65° for 3 hours and left overnight at room temperature. The solvents were removed *in vacuo* and the residue decomposed with water and extracted with methylene chloride. Concentration of the dried extract yielded solid material which was recrystallized from benzene–petroleum ether, m.p. 214–215°; infrared bands at 1775 (γ-lactone), 1745 (acetate), 1707 and 1590 cm.$^{-1}$ (cyclopentenone); ultraviolet maximum at 223.5 mμ (ϵ 9600); n.m.r. signals at 7.73 (doublet, J = 3.3) and 7.64 (doublet, J = 3.3)-H_2, 6.44 (doublet, J = 1.5) and 6.34 (doublet, J = 1.5)-H_5, 4.79 (doublet, J = 3.8, H_6) superimposed on triplet of doublets centered at 4.79 (H_8), 1.98 (singlet, acetate), 1.38 (singlet, C_5-methyl), 1.29 (doublet, J = 6.5, C_{10}- or C_{11}-methyl) and 1.10 (doublet, J = 7.1, C_{10}- or C_{11}-methyl). The structure is XIa or XIV.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.67; H, 7.19; O, 26.14. Found: C, 66.84; H, 7.29; O, 25.54.

Treatment of the ketoepoxylactone with toluenesulfonic acid in acetic acid or with boron trifluoride etherate in benzene ether resulted in recovery of starting material.

A solution of 0.12 g. of the preceding α,β-unsaturated ketone in 5 ml. of ethyl acetate was reduced with prereduced platinum oxide; calculated uptake 9.8 ml. of H_2, found 9.8 ml. The solution was filtered, concentrated, and the solid recrystallized from ethyl acetate–petroleum ether; yield 0.09 g., m.p. 177–179°, infrared bands at 1775 (γ-lactone) and 1735–1740 cm.$^{-1}$ (combination of cyclopentanone and acetate), λ_{max} 293 mμ (ϵ 44); n.m.r. signals at 4.76 (doublet, J = 7.6, H_6) superimposed on broad multiplet (H_8), 2.03 (singlet, acetate), 1.25 (singlet), 1.09 (doublet, J = 5) and 1.00 p.p.m. (doublet, J = 6.7), the last three signals representing the three methyl groups. The structure is XVa or XVIa.

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.22; H, 7.79. Found: C, 66.31; H, 7.97.