27. *Sesquiterpenoids. Part VII.* The Constitution of Tenulin, a Novel Sesquiterpenoid Lactone.*

By D. H. R. Barton and P. de Mayo.

The sesquiterpenoid lactone tenulin has been shown to contain a masked acetyl grouping which rearranges very easily to a true acetate residue in the derived *iso*tenulin. Inter-relation of the functional groups of tenulin has been effected. Dehydrogenation of suitable derivatives has afforded chamazulene and linderazulene. Based on these and earlier observations, constitutions have been deduced for tenulin and *iso*tenulin, and their derivatives.

The crystalline bitter principle tenulin, $C_{17}H_{22}O_5$, was isolated by Clark (*J. Amer. Chem. Soc.*, 1939, **61**, 1836; 1940, **62**, 597) from various *Helenium* species (*H. tenuifolium*, *H. elegans*, *H. badium*, and *H. montanum*). The compound has interesting physiological properties and was studied in some detail by this author (*locc. cit.*; also *ibid.*, 1940, **62**, 2154) and later by Ungnade *et al.* (Ungnade and Hendley, *ibid.*, 1948, **70**, 3921; Ungnade, Hendley, and Dunkel, *ibid.*, 1950, **72**, 3818). The more important features of Clark's

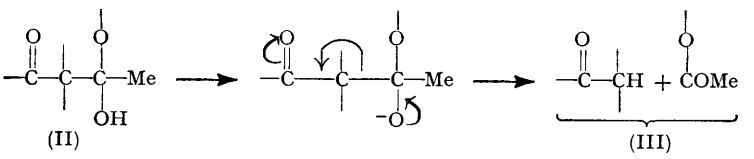
* Part VI, *J.*, 1954, 4665.

work can be summarised as follows. Tenulin is isomerised by mild alkali to *iso*tenulin. Both compounds are smoothly hydrogenated to dihydro-derivatives, both of which afford phenylhydrazones. Both compounds give dibromides which easily evolve one mol. of hydrogen bromide. Pyrolysis of tenulin affords, first, anhydrotenulin, $C_{17}H_{20}O_4$, and then pyrotenulin, formulated as $C_{13}H_{16}O_3$. Treatment of *iso*tenulin with sulphuric acid gave deacetyl*iso*tenulin (from which *iso*tenulin was regenerated by acetylation) and one mol. of acetic acid. Similar hydrolysis of dihydro*iso*tenulin with either acid or alkali gave comparable results. Oxidation of tenulin or *iso*tenulin with alkaline hydrogen peroxide gave tenulinic acid, $C_{15}H_{20}O_7$, characterised as its methyl ester. Acetylation of the acid afforded acetyltenulinic acid, $C_{17}H_{22}O_8$, which was the direct product of the oxidation of tenulin or *iso*tenulin with potassium permanganate. On the basis of these experiments Clark concluded that the functional groups of tenulin could be symbolised as in (I).

$C_{15}H_{18}O$ { -OH, =O, O·COMe } (I)

Ungnade and his collaborators (*locc. cit.*) reported that the ultraviolet absorption spectra of tenulin and *iso*tenulin showed them to be αβ-unsaturated ketones. All compounds were regarded as lactones on the basis of their infrared spectra and it was shown specifically that *iso*tenulin consumed two mols. of alkali, one for hydrolysis of an acetate residue (see above) and the other for the opening of a lactone ring. Now if Clark's partial symbol (I) be accepted, there is insufficient uncharacterised oxygen left for the formation of a lactone ring. It was this paradox which first attracted us to the chemistry of tenulin. Our own analysis of the problem is presented in the sequel.

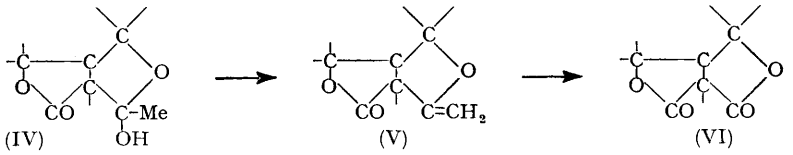
Extraction of *H. tenuifolium* afforded, without difficulty, tenulin. This showed $\lambda_{max.}$ 226 mμ (ε 7000) and gave bands in the infrared as follows: (*a*) (in $CHCl_3$) 1772 (γ-lactone), 1708 and 1595 (*cyclo*pentenone), and 3620 (hydroxyl); (*b*) (in Nujol) 1778 (γ-lactone), 1695 and 1590 (*cyclo*pentenone), and 3450 cm.$^{-1}$ (hydroxyl). The position of the ultraviolet absorption maximum of tenulin [accepted as a *cyclo*pentenone (see above)] indicates only two substituents on the double bond (Woodward, *ibid.*, 1941, **63**, 1123; 1942, **64**, 76; Gillam and West, *J.*, 1942, 486). A choice between the arrangements (*A*) O:Ċ·CH:C< and (*B*) O:Ċ·Ċ:CH· was made by ozonolysis. Under neutral conditions this afforded formic acid indicative, unless some extra complication is introduced, of grouping (*A*), not (*B*). On brief treatment with boiling (London) tap-water tenulin was smoothly isomerised to *iso*tenulin. This showed $\lambda_{max.}$ 226 mμ (ε 7400) and gave infrared bands in Nujol at 1778 (γ-lactone), 1705 and 1588 (*cyclo*pentenone), 1748 and 1238 cm.$^{-1}$ (acetate). Clearly *iso*tenulin has the same *cyclo*pentenone system as tenulin; this was confirmed by ozonolysis which also gave formic acid. The most interesting aspect of these results is that tenulin contains no acetate grouping, but does possess a hydroxyl group, whereas the converse holds for *iso*tenulin.* In agreement, tenulin gave no acetic acid on acid hydrolysis, but 1 mol. of this acid if treated first with alkali. Also *iso*tenulin gave 1 mol. of acetic acid under acid conditions according to the usual method of determination. The simplest explanation for these remarkable facts is that tenulin contains the masked acetyl system (II), cleavage of which by base would afford *iso*tenulin (III).



The carbonyl group which triggers this rearrangement could be either the ketone of the *cyclo*pentenone or the carbonyl of the lactone grouping. A distinction was made as follows. Dihydrotenulin, which showed the expected bands (in $CHCl_3$) at 1770 (γ-lactone), 1738

* When investigating the conversion of tenulin into *iso*tenulin we studied, first, one of Clark's methods, *viz.*, trituration with 5% aqueous sodium carbonate. To test if an enolic intermediate was formed [as is indeed the case; see scheme (II) ⟶ (III)] deuterium oxide was used. Although a product of the correct m. p. resulted it contained no deuterium. The enigma was resolved when it was found that the product was impure tenulin (isolated by chromatography). This particular method for making *iso*tenulin is not therefore reliable.

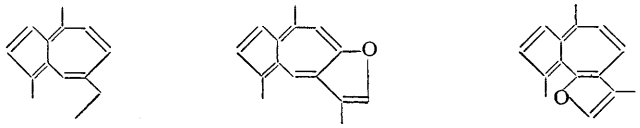
(*cyclo*pentanone), and 3500 cm.$^{-1}$ (hydroxyl), was smoothly rearranged to dihydro*iso*tenulin; the masked acetate grouping, therefore, cannot be attached vinylogously β with respect to the ketone group. Further, dihydrotenulin oxime also rearranged smoothly to dihydro*iso*tenulin oxime; hence ketonic carbonyl does not induce the rearrangement and the masked acetate grouping must, by exclusion, be attached β to the lactone-carbonyl group, as in (IV). This was confirmed in the following way. Pyrotenulin, which Clark had formulated as $C_{13}H_{16}O_3$ (see above), consumes only 1 mol. of alkali (Ungnade, Hendley, and Durkel, *loc. cit.*) and shows only end-absorption in the ultraviolet region. We have shown that pyrotenulin is identical with a compound obtained by Clark by the action of acetic anhydride–sodium acetate on tenulin. Clark tentatively formulated this compound as $C_{22}H_{26}O_5$ but noted that it contained no acetate residue. The correct formula for pyrotenulin is $C_{17}H_{20}O_4$ with the partial formulation as in (V).



(IV) (V) (VI)

In the infrared spectrum pyrotenulin showed the following bands : (*a*) (in $CHCl_3$) 1778 (γ-lactone), 1754 (*cyclo*pentanone), 1656 and 1605 (C:C of vinyl ether); (*b*) (in Nujol) 1772 (γ-lactone), 1752 (*cyclo*pentanone), 1673, 1630, and 810 cm.$^{-1}$ (·O·C:CH_2) (see Meakins, *J.*, 1953, 4170). Treatment of dihydrotenulin with acetic anhydride–sodium acetate gave anhydrodihydrotenulin, $C_{17}H_{22}O_4$ [as (V)], which on ozonolysis afforded formaldehyde and the expected bis-γ-lactone (VI). Anhydrodihydrotenulin showed bands (in $CHCl_3$) at 1784 (γ-lactone), 1738 (*cyclo*pentanone), and 1670 and 1652 cm.$^{-1}$ (C:C of vinyl ether). The bis-γ-lactone gave bands (in $CHCl_3$) at 1800 and 1765 (γ-lactones attached to the same carbon atom; cf. Fox and Martin, *Proc. Roy. Soc.*, 1938, *A*, **167**, 257; Bergmann and Pinchas, *Rec. Trav. chim.*, 1952, **71**, 161) and at 1740 cm.$^{-1}$ (*cyclo*pentanone).

We shall next abbreviate further discussion by deducing complete structures for tenulin and *iso*tenulin and show how these explain all significant prior knowledge. Reduction of *iso*tenulin with potassium borohydride and dehydrogenation of the product gave chamazulene (VII) (Meisels and Weizmann, *J. Amer. Chem. Soc.*, 1953, **75**, 3865; Šorm, Herout, and Takeda, *Chem. Listy*, 1954, **48**, 281; Novak, Šorm, and Sicher, *ibid.*, p. 1648). This establishes the position of fourteen of the fifteen carbon atoms of the basic skeleton. Reduction of dihydro*iso*tenulin with lithium aluminium hydride and dehydrogenation of the product gave linderazulene (VIII) [not (IX)] [Takeda and Nagata, *Pharm. Bull.* (*Japan*) 1953, **1**, 164; cf. Takeda and Shimada, *J. Pharm. Soc. Japan*, 1944, **64**, 32; Kondo



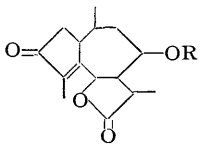
(VII) (VIII) (IX)

and Takeda, *ibid.*, 1939, **59**, 162; Herout and Šorm, *Chem. Listy*, 1954, **48**, 706], thus establishing the complete carbon skeleton and showing the position of the lactonic carbonyl group lost in the dehydrogenation to chamazulene. Two possible *cyclo*pentenone formul-

(X) (XI) (XII) (XIII)

ations must be considered, (X) and (XI). The correctness of the former was shown as follows. Treatment of tenulin with aqueous sodium hydrogen carbonate gave, besides

the known deacetyl*iso*tenulin (see above), a new conjugated ketone which we designate deacetyl*neo*tenulin. This showed $\lambda_{max.}$ 240 mμ (ε 16,000) and gave infrared bands (in Nujol) at 1765 (γ-lactone), 1682 and 1628 (*cyclo*pentenone), and at 3300 cm.$^{-1}$ (hydroxyl) and furnished *neo*tenulin on acetylation. These compounds must be represented by either (XII), based on (X), or (XIII), based on (XI). That (XII) was correct was shown by ozonolysis of deacetyl*neo*tenulin which afforded, under neutral conditions, acetic acid. Deacetyl*neo*tenulin must therefore be represented by either (XIV or XV; R = H).* Chromic acid oxidation gave deacetyldehydro*neo*tenulin, (XVI) or (XVII). This showed $\lambda_{max.}$ 245 mμ (ε 12,000) and had infrared bands as follows: (*a*) (in $CHCl_3$) 1785 (γ-lactone), 1708 and 1635 (*cyclo*pentenone), and 1730 cm.$^{-1}$ (somewhat displaced *cyclo*heptanone);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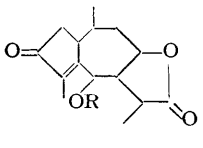
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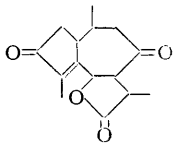
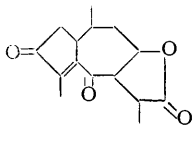
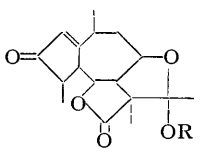

(XVI)


(XVII)

(*b*) (in Nujol) 1768 (γ-lactone), 1698 and 1628 (*cyclo*pentenone), and 1730 cm.$^{-1}$ (somewhat displaced *cyclo*heptanone). The data are not consistent with the ene-1 : 4-dione structure (XVII), which would have shown an infrared band between 1690 and 1670 cm.$^{-1}$ as well as the usual *cyclo*pentenone bands. Also the shape of the ultraviolet absorption curve was quite different (see Experimental section) from that of cholest-4-ene-3 : 6-dione. Dehydrodeacetyl*neo*tenulin was not reducible by zinc and acetic acid, as would have been expected if the ene-1 : 4-dione structure (XVII) were correct. The position of the *cyclo*heptanone infrared band in the compounds mentioned above is somewhat displaced (expected position about 1700 cm.$^{-1}$). This feature is common to all the corresponding ketonic derivatives of tenulin that we have examined. For example, oxidation of deacetyl-*iso*tenulin afforded deacetyldehydro*iso*tenulin. This showed infrared maxima (in $CHCl_3$) at 1780 (γ-lactone), 1738 (displaced *cyclo*heptanone), and 1692 and 1596 cm.$^{-1}$ (*cyclo*-pentenone).

In summary, on the basis of all this evidence, tenulin is formulated as (XVIII; R = H), *iso*tenulin as (XIX), pyrotenulin as (XX) (or an equivalent βγ-unsaturated structure), anhydrotenulin as (XXI), anhydrodihydrotenulin as (XXII), the derived bis-γ-lactone as (XXIII), and *neo*tenulin as (XIV; R = Ac).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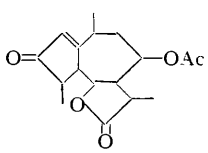
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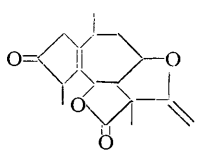
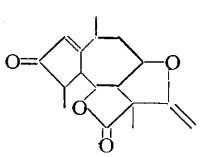
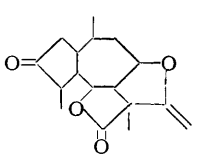

(XX)


(XXI)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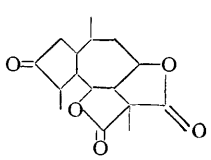
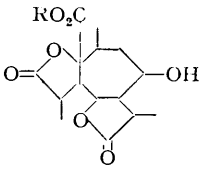
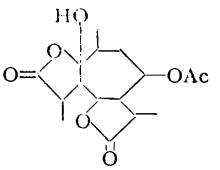

(XXIII)

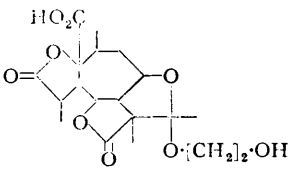
Tenulinic acid is readily formulated as (XXIV; R = H). In agreement the methyl ester (XXIV; R = Me) showed infrared bands in $CHCl_3$ at 1774 (γ-lactones and ester; broad unsymmetrical band, strength indicative of three carbonyl groups), and 3600 cm.$^{-1}$

* In principle the position of the lactone ring could be different in deacetyl*neo*tenulin from that in tenulin. However deacetyl*neo*tenulin can be obtained from the sodium hydrogen carbonate solution *without* acidification; hence the lactone ring is not opened in the transformation and thus has no opportunity to close in the alternative direction. Even if the lactone ring had opened, the work on tenulinic acid discussed in the text shows that it would close back again to its original position.

(hydroxyl). Oxidation of the methyl ester afforded methyl dehydrotenulinate which gave infrared maxima in $CHCl_3$ at 1800 (γ-lactones), 1760 (somewhat displaced methoxycarbonyl), and 1722 cm.$^{-1}$ (*cyclo*heptanone; cf. above). An analogue of tenulinic acid was

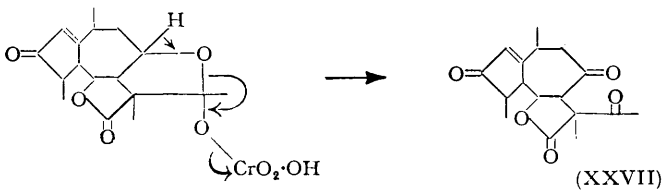

(XXIV)


(XXV)


(XXVI)

obtained in the ozonolysis (cf. above) of *iso*tenulin. This compound, $C_{16}H_{22}O_7$, was acidic but dissolved only slowly in sodium hydrogen carbonate solution. It showed infrared maxima (in Nujol) at 1764 (γ-lactones; strength of band indicative of two groupings), at 1712 and 1255 (acetate), and at 3400 cm.$^{-1}$ (hydroxyl) and is therefore formulated as the lactol (XXV).

In his last paper on tenulin Clark (*loc. cit.*) noted that it reacted very easily with ethylene glycol to furnish an acylable compound, $C_{19}H_{22}O_6$, readily cleaved by acid treatment to regnerate its precursors. This would be an expected reaction for our hemiketal tenulin structure; the ether may be formulated as (XVIII; R = $[CH_2]_2$·OH). Oxidation of the ether with *either* potassium permanganate *or* alkaline hydrogen peroxide afforded the *same* acid, $C_{19}H_{26}O_9$. This acid may be formulated as (XXVI); the failure to lose the acetic acid generating residue (see Clark, *loc. cit.*) under alkaline conditions (contrast tenulinic acid) is unexceptional.


(XXVII)

An interesting observation incidental to our work is that, whilst *iso*tenulin is stable to chromic acid at room temperature, tenulin itself is easily oxidised to dehydrotenulin. This is formulated as (XXVII), being formed by the mechanism indicated. In agreement with this structure dehydrotenulin gave 1 mol. of acetic acid on treatment with alkali and showed infrared maxima as follows: (*a*) (in Nujol) 1760 (γ-lactone), 1728 (methyl ketone and *cyclo*heptanone), 1700 and 1590 (*cyclo*pentenone), and 1354 cm.$^{-1}$ (methyl of methyl ketone); (*b*) (in CCl_4) 1780 (γ-lactone), 1742 (methyl ketone and *cyclo*heptanone), and 1710 cm.$^{-1}$ (*cyclo*pentenone).

Experimental

For general experimental directions, see *J.*, 1952, 2339. Infrared spectra were kindly determined by Messrs. Glaxo Laboratories Limited. Unless specified to the contrary, $[\alpha]_D$ are in $CHCl_3$; ultraviolet absorption spectra were determined in ethanol on the Unicam S.P. 500 Spectrophotometer. Unless stated to the contrary, the light petroleum used was of b. p. 40—60°.

Tenulin.—This compound was isolated by Ungnade and Hendley's method (*J. Amer. Chem. Soc.*, 1948, **70**, 3921). Recrystallised from benzene, tenulin had m. p. about 130—140° with evolution of benzene (isolated and identified spectroscopically). The partial melt rapidly resolidified and the final m. p. depended on the rate of heating and the original form of the tenulin crystals. Tenulin was obtained in at least three different crystalline forms: solvated square heavy plates (from hot benzene), solvated small prisms [from hot benzene–light petroleum (b. p. 60—80°)] and needles (from the same solvent mixture in the cold). The needles are unsolvated and rapidly change in contact with solvent into the small prisms. The m. p. of

tenulin, which may be as high as 215°, is indefinite. In contrast, the rotation of all specimens was the same: $[\alpha]_D$ −20° (*c* 2·18 in EtOH), −21° (*c* 1·99 in EtOH), −22° (*c* 1·86 in EtOH), −21° (*c* 1·46), −24° (*c* 0·88). $\lambda_{max.}$ was at 226 mμ (ε 7000). Tenulin was recovered unchanged after treatment with pyridine–acetic anhydride overnight at room temperature.

Acetyl determinations. (*a*) Acetyl determination in the usual way with 30% sulphuric acid (H. Roth, " Pregl's Quantitative Organic Microanalysis," J. and A. Churchill Ltd., London, 1937) gave no volatile acid. (*b*) Hydrolysis by concentrated sulphuric acid at 90° for 3 min., followed by dilution, partial neutralisation, and distillation, afforded 0·3 mol. of volatile acid. (*c*) Hydrolysis with 5N-sodium hydroxide in the usual way (*op. cit.*, p. 197) gave 1·0 mol. of volatile acid.

Trituration of tenulin with 5% aqueous (protium or deuterium) sodium carbonate (Clark, *J. Amer. Chem. Soc.*, 1939, **61**, 1836) afforded material (from benzene–light petroleum), m. p. 160—161° (preliminary partial melt at 130° with solvent evolution), $[\alpha]_D$ −24° (*c* 0·98 in EtOH). Chromatography over silica gel yielded only tenulin (m. p., mixed m. p., $[\alpha]_D$).

Dehydrotenulin.—Tenulin (240 mg.) was treated with chromic acid solution (0·05N in " AnalaR " acetic acid; 50 ml.) for 40 min. at room temperature (0·8 atom of " oxygen " consumed). Chromatography of the product over deactivated neutral alumina (10 g.) (13 fractions) gave *dehydrotenulin*, 4 fractions eluted with benzene–ether (19 : 1) (50 mg.), m. p. (prisms from benzene) 203—205° (effervescence), $[\alpha]_D$ −83° (*c* 1·10), −81° (*c* 0·97), −82° (*c* 0·94) in EtOH, $\lambda_{max.}$ 226 mμ (ε 7500) (Found: C, 66·95; H, 6·55. $C_{17}H_{20}O_5$ requires C, 67·1; H, 6·6%). Acetyl determination by the alkaline method (see above) gave 0·88 mol. of volatile acid. From the same chromatogram elution with benzene–ether (7 : 3) (four fractions) gave unchanged tenulin (85 mg.). Increased uptake of chromic acid did not increase the yield of dehydrotenulin.

Pyrotenulin.—(*a*) (cf. Clark, *ibid.*, 1940, **62**, 597) Tenulin (250 mg.) was heated under nitrogen to 200°. After the evolution of benzene had ceased, the flask was removed, the bath heated to 300°, and the flask again heated at this temperature until a further evolution of gas had ceased (10 min.). The product was chromatographed over neutral deactivated alumina. Elution with benzene–light petroleum (7 : 3) gave pyrotenulin, m. p. (from methanol) 235—237°, $[\alpha]_D$ −34° (*c* 1·10), ε 6400 at 205 mμ, ε 5200 at 210 mμ, ε 2100 at 220 mμ [Clark (*loc. cit.*) gives m. p. 235—236°, but Ungnade, Hendley, and Dunkel (*ibid.*, 1950, **72**, 3818) record m. p. 209°].

(*b*) (cf. Clark, *ibid.*, 1939, **61**, 1836) Tenulin (100 mg.) was refluxed in acetic anhydride (1 ml.) containing sodium acetate (25 mg.) for 50 min. The acetic anhydride was removed *in vacuo* and the product, worked up in the usual way, was crystallised to give pyrotenulin, m. p. (from methanol) 233—235°, $[\alpha]_D$ −31° (*c* 0·60). According to Clark (*loc. cit.*) this procedure affords a compound, $C_{22}H_{26}O_5$, m. p. 240°. The identity of the compounds prepared under (*a*) and (*b*) was confirmed by mixed m. p. and infrared spectra (in $CHCl_3$).

Dihydrotenulin.—This compound, prepared by Clark's method (*loc. cit.*), had m. p. 184—186° (Clark gives m. p. 182° and 172°), $[\alpha]_D$ +86° (*c* 1·38), +76° (*c* 1·24 in EtOH), +76° (*c* 1·31 in EtOH). Treatment with pyridine–hydroxylamine hydrochloride overnight at room temperature afforded *dihydrotenulin oxime*, m. p. (from chloroform–benzene) 210—211°, $[\alpha]_D$ +54° (*c* 1·38), +54° (*c* 1·02) (Found: N, 4·05. $C_{17}H_{25}O_5N$ requires N, 4·35%).

iso*Tenulin* (cf. Ungnade and Hendley, *loc. cit.*).—Tenulin (600 mg.) was added to boiling (London) tap-water and the solution boiled for 2 min. Cooling furnished *iso*tenulin (370 mg.) as long needles, m. p. 160—161°, $[\alpha]_D$ +6° (*c* 1·57), +4° (*c* 1·44), +9° (*c* 1·14 in EtOH), +8° (*c* 0·83 in EtOH), $\lambda_{max.}$ 226 mμ (ε 7400). An acetyl determination with diluted sulphuric acid in the usual way (Roth, *op. cit.*) gave 0·96 mol. of volatile acid.

*Dihydro*iso*tenulin.*—This compound, prepared by Clark's method (*loc. cit.*), had m. p. 148—149°, $[\alpha]_D$ +111° (*c* 1·80). It gave an *oxime*, m. p. (from ethyl acetate–light petroleum) 174—175·5°, $[\alpha]_D$ +109° (*c* 1·63) (Found: C, 63·4; H, 7·35; N, 3·85. $C_{17}H_{25}O_5N$ requires C, 63·15; H, 7·8; N, 4·35%).

Dihydrotenulin (30 mg.) was refluxed in (London) tap-water (3 ml.) for 2 min. Cooling and crystallisation from ether–light petroleum afforded dihydro*iso*tenulin, identified by m. p., mixed m. p., and rotation {$[\alpha]_D$ +114° (*c* 0·83)}.

Similarly dihydrotenulin oxime (30 mg.) was treated as above. Cooling and crystallisation from ethyl acetate–light petroleum afforded dihydro*iso*tenulin oxime, identified by m. p., mixed m. p. and rotation {$[\alpha]_D$ +109° (*c* 0·97)}.

Ozonolysis of iso*Tenulin.*—(a) *iso*Tenulin (290 mg.) in chloroform (25 ml.) was ozonised at 0° for 45 min. (disappearance of ultraviolet maximum). Water (15 ml.) was added and the chloroform evaporated. The product was again taken up in chloroform. Extraction with

sodium hydrogen carbonate solution afforded the lactol (XXV), m. p. (from aqueous methanol) 233—236°, $[\alpha]_D$ +64° (*c* 1·00) (Found : C, 59·2; H, 6·75. $C_{16}H_{22}O_7$ requires C, 58·9; H, 6·8%). The compound dissolved only slowly in aqueous sodium hydrogen carbonate.

(b) *iso*Tenulin (100 mg.) in carbon tetrachloride (10 ml.) was ozonised at 0° until the ultraviolet absorption maximum had disappeared. Water (10 ml.) was added and the mixture distilled. The total distillate was neutralised with aqueous sodium hydroxide, and the aqueous layer treated with ethanolic *p*-bromophenacyl bromide in the usual way. Chromatography of the product over alumina gave *p*-bromophenacyl formate (8 mg.), identified by m. p. and mixed m. p. Ozonolysis of tenulin under the same conditions yielded a similar result.

Anhydrodihydrotenulin.—Dihydrotenulin (250 mg.) in acetic anhydride (30 ml.) containing sodium acetate (250 mg.) was refluxed for 7 hr. The acetic anhydride was removed *in vacuo* and the product worked up in the usual way. Chromatography over neutral deactivated alumina (8 g.) and elution with benzene gave *anhydrodihydrotenulin*, m. p. [from benzene–light petroleum (b. p. 60—80°)] 173—175°, $[\alpha]_D$ +55° (*c* 1·50) (Found : C, 70·05; H, 7·7. $C_{17}H_{22}O_4$ requires C, 70·3; H, 7·65%). This compound gave a strongly positive tetranitromethane test.

Ozonolysis of Anhydrodihydrotenulin.—Anhydrodihydrotenulin (130 mg.) in chloroform (5 ml.) was ozonised at 0° until the solution gave no colour with tetranitromethane (10 min.). Water (10 ml.) was added and the chloroform and water were distilled off. The crystalline residue readily gave the *dilactone* (XXIII), m. p. (from methanol) 256—258°, $[\alpha]_D$ +82° (*c* 1·49) (Found : C, 65·65; H, 6·9. $C_{16}H_{20}O_5$ requires C, 65·75; H, 6·9%). To the combined distillate was added dimedone (100 mg.) in methanol (5 ml.). The chloroform and most of the methanol were removed on the steam-bath, whereupon the dimedone–formaldehyde derivative crystallised (6 mg.; identified by m. p. and mixed m. p.).

Tenulinic Acid and its Derivatives.—Tenulinic acid was prepared with alkaline hydrogen peroxide according to Clark (*J. Amer. Chem. Soc.*, 1940, **62**, 597) and converted into the methyl ester {m. p. 206—208°, $[\alpha]_D$ +81° (*c* 1·00)} with diazomethane.

Methyl tenulinate (333 mg.) was treated with chromic acid in " AnalaR " acetic acid (25 ml.; 0·12N) at room temperature. After 1½ hr. the uptake of chromic acid had reached 1·0 atom of " oxygen " and was unchanged later. Working up in the usual way gave *methyl dehydrotenulinate*, m. p. (from benzene–light petroleum) 228—229°, $[\alpha]_D$ −9° (*c* 2·04) (Found : C, 58·6; H, 6·6. $C_{16}H_{20}O_7$ requires C, 59·25; H, 6·2%; several samples of this compound, after repeated crystallisation, were analysed by different analysts with very widely different results on the same specimen).

neo*Tenulin and Derivatives.*—Tenulin (250 mg.) in distilled water (25 ml.) and saturated aqueous sodium hydrogen carbonate solution (2 ml.) was heated on the steam-bath for 2½ hr. (prior investigation had shown that this was the optimum time). Extraction with chloroform afforded *deacetyl*neo*tenulin*, m. p. (from chloroform–benzene–light petroleum) 239—242°, $[\alpha]_D$ −27° (*c* 1·04), $\lambda_{max.}$ 240 mμ (ε 16,000) (Found : C, 67·85; H, 7·55. $C_{15}H_{20}O_4$ requires C, 68·15; H, 7·65%). Acetylation with pyridine–acetic anhydride overnight at room temperature afforded neo*tenulin*, m. p. (from aqueous methanol) 193—193·5°, $[\alpha]_D$ −23° (*c* 0·93), $\lambda_{max.}$ 239 mμ (ε 15,000) (Found : C, 66·2; H, 7·45. $C_{17}H_{22}O_5$ requires C, 66·65; H, 7·25%). Chromatography of the combined mother-liquors from the preparation of deacetyl*neo*tenulin over neutral deactivated alumina (2 g.) gave, on elution with benzene, deacetyl*iso*tenulin, m. p. 251—253°, $[\alpha]_D$ −20° (*c* 1·09), $\lambda_{max.}$ 225 mμ (ε 8500), identical (mixed m. p. and the same physical properties) with a specimen prepared (Clark, *loc. cit.*) from *iso*tenulin by acid hydrolysis.

Deacetyl*neo*tenulin (96 mg.) in chloroform (25 ml.) was ozonised at 0° for 4 hr. (disappearance of ultraviolet absorption maximum). Water (25 ml.) was added and the mixture distilled. Neutralisation (aqueous sodium hydroxide) of the distillate, removal of the chloroform, and treatment with *p*-bromophenacyl bromide in the usual way gave after chromatography *p*-bromophenacyl acetate (27 mg.), identified by m. p., mixed m. p., and analysis (Found : C, 46·9; H, 3·6. Calc. for $C_{10}H_9O_3Br$: C, 46·7; H, 3·5%).

Deacetyl*neo*tenulin (220 mg.) was treated with chromic acid in " AnalaR " acetic acid (20 ml.; 0·25N) at room temperature. After 1 hr. the uptake of chromic acid corresponded to 0·9 atom of " oxygen." Isolation of the product in the usual way gave *deacetyldehydro*neo*tenulin*, m. p. (from benzene–light petroleum) 178—182° (somewhat variable), $[\alpha]_D$ −164° (*c* 1·30), −164° (*c* 1·28), $\lambda_{max.}$ 245 mμ (ε 12,000), ε 6000 at 229 and 257 mμ (Found : C, 68·8; H, 7·35. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%). Cholest-4-ene-3 : 6-dione has $\lambda_{max.}$ 254 mμ (ε 10,000), ε 5000 at 226 and 270 mμ : the latter compound had a much wider band, apart from a maximum at longer wavelength, as shown by the band width at half the maximum intensity.

The diketone (5 mg.) was dissolved in acetic acid (0·5 ml.), zinc (200 mg.) added, and the

mixture heated on the steam-bath, with occasional stirring, for 1 hr. At the end of this time the spectrum of the isolated material was essentially unchanged.

Deacetyldehydroisotenulin.—Deacetyl*iso*tenulin (see above) (109 mg.) was treated with chromic acid in "AnalaR" acetic acid (11 ml.; 0·13N) at room temperature for 90 min. Crystallisation of the product from benzene–light petroleum gave *deacetyldehydroisotenulin*, plates, m. p. 223—224°, $[\alpha]_D$ −209° (*c* 0·90), $\lambda_{max.}$ 228 mμ (ε 8000) (Found: C, 68·45; H, 7·2. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%).

Deacetyldihydroisotenulin.—This compound was best prepared as follows (cf. Clark, *loc. cit.*). Dihydro*iso*tenulin (see above) (480 mg.) was refluxed with water (80 ml.) and saturated aqueous sodium hydrogen carbonate (20 ml.) for 2 hr. On cooling, deacetyldihydro*iso*tenulin (280 mg.) crystallised, m. p. 202—203°, $[\alpha]_D$ +151° (*c* 0·80). The 2 : 4-*dinitrophenylhydrazone*, prepared in the usual way and chromatographed over kieselguhr–bentonite (Elvidge and Whalley, *Chem. and Ind.*, 1955, 589), had m. p. (from methanol) 276—277° (Found: C, 56·9; H, 5·9; N, 12·0. $C_{21}H_{26}O_7N_4$ requires C, 56·5; H, 5·85; N, 12·55%). The *oxime*, prepared with pyridine–hydroxylamine hydrochloride overnight at room temperature, had m. p. (from methanol) 246—248° (Found: N, 5·65. $C_{15}H_{23}O_4N$ requires N, 5·0%).

Dehydrogenations.—(*a*) *iso*Tenulin (1·5 g.) in ethanol (10 ml.) was treated with potassium borohydride (1·0 g.) in water (2 ml.) for 1 hr. The total product was dehydrogenated in 100-mg. portions by 10% palladised charcoal (100 mg.) under nitrogen at 320° for 15 min. The combined dehydrogenated product was filtered in light petroleum (b. p. 60—80°) solution through neutral deactivated alumina (3 g.) and the azulene fraction was extracted with 90% phosphoric acid. Recovery from phosphoric acid into light petroleum solution by addition of water gave (by the absorption spectrum) about 3 mg. of azulene. The theoretical amount of 1 : 3 : 5-trinitrobenzene was added and the adduct, crystallised from light petroleum (b. p. 60—80°) and then from methanol, was identified as the chamazulene derivative by crystal form, m. p., mixed m. p., and ultraviolet and visible absorption spectra (identical at all wavelengths) (for spectra of azulenes see Takeda, Kubota, and Nagata, *Pharm. Bull.*, 1953, **1**, 241).

(*b*) *iso*Tenulin (1·7 g.) was converted into dihydro*iso*tenulin (see above), and the total product reduced with lithium aluminium hydride (900 mg.) in dry ether (200 ml.) under reflux for 2 hr. Decomposition of the excess of lithium aluminium hydride with ethyl acetate and dilute aqueous hydrochloric acid, followed by extraction with chloroform (5 × 10 ml.) and ether (5 × 10 ml.), gave a semicrystalline product (1·3 g.). This was dehydrogenated in 200-mg. portions by 10% palladised charcoal (200 mg.) under nitrogen at 320° for 15 min. The dehydrogenated product was worked up as above to give approx. 1·5 mg. of azulene. The derived 1 : 3 : 5-trinitrobenzene adduct had m. p. 155—157°. It was largely depressed (25°) with the S-guaiazulene adduct (m. p. 150—152°), but did not depress the m. p. of linderazulene 1 : 3 : 5-trinitrobenzene adduct (m. p. 153—157°).

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