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CONSTITUENTS OF *Polygala* SPECIES

THE STRUCTURE OF TENUIFOLIN, A PROSAPOGENIN FROM *P. SENEGA* AND *P. TENUIFOLIA*

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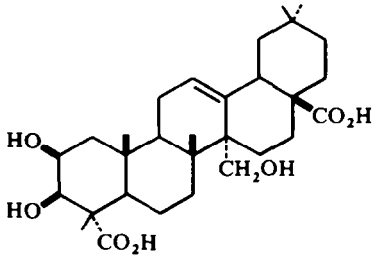
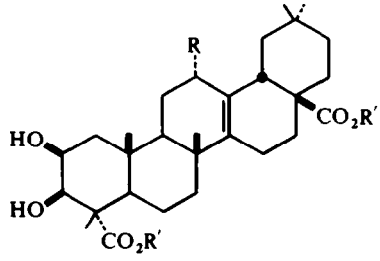
Abstract—Tenuifolin (V), a prosapogenin isolated from the roots of *P. tenuifolia* and *P. senega*, has been shown to be 2β,27-dihydroxy-23-carboxyoleanolic acid 3β-O-glucoside, on the basis of physical data of tenuifolin and several of its degradation products, by direct correlation with presenegenin (I) and by the isolation of glucose from the acid hydrolysate of tenuifolin dimethyl ester.

RECENTLY[1] mild hydrolysis of the saponin mixture ("senegin") of *Polygala senega* with soil bacteria has demonstrated that the major aglucone is presenegenin (I), a normal triterpenoid previously isolated from the same source by a combination of oxidative cleavage and alkaline hydrolysis.[2] The acidic hydrolysis[3,4] of senegin gives rise to the artifacts, senegenin (II), hydroxy senegenin[5] (III), and senegenic acid[6] (IV) (polygalic acid),[4] the nature of the product being determined by the hydrolytic conditions employed.



I



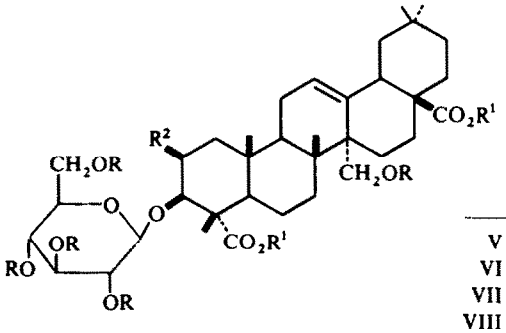
II: R = CH_2Cl; R′ = H
IIa: R = CH_2Cl; R′ = CH_3
III: R = CH_2OH; R′ = H
IV: R = R′ = H
IVa: R = H; R′ = CH_3

A report[7] that hydrolysis of the saponins from *P. tenuifolia* gives a senegenin-like sapogenin led us to examine the sapogenins of this plant.[8] Hydrolysis of this saponin mixture with ethanolic hydrochloric acid gave a compound, m.p. 265–267°, which was identified as senegenin by mixture mp and comparison of IR spectra.* The oxidative and hydrolytic cleavage of the saponin from *P. tenuifolia* afforded presenegenin together with 4-methoxycinnamic acid. The behavior on TLC of the saponin mixtures isolated from *P. senega* and *P. tenuifolia* was almost identical. Thus it is

* Tenuifolic acid[9] from *P. tenuifolia* has been found to be identical with senegenin from *P. senega* by Professor Tschesche's group[10,11] also.

evident that the compositions of the saponins from *P. senega* and *P. tenuifolia* are similar.*

In this paper we wish to describe the isolation and structure elucidation of a new prosapogenin, named *tenuifolin* (V) obtained from the basic hydrolysate of the saponin of either *P. senega* or *P. tenuifolia*. Tenuifolin, m.p. 298–300† is a polyhydroxy acidic compound as is evident from its IR spectrum. On treatment with diazomethane, it yielded a dimethyl ester (VI), m.p. 270–272. Acetylation of VI with acetic anhydride and pyridine at room temperature gave a pentaacetate (VII), m.p. 220–222° which showed in its NMR spectrum a vinyl proton signal at $\delta = 5{\cdot}55$ (1H, m), five tertiary alkyl Me signals, five acetyl Me signals, and two ester Me signals. In its IR spectrum VII showed a band at 3540 cm^{-1} indicating incomplete acetylation. However, acetate VII resisted further acetylation with acetic anhydride and pyridine even under refluxing conditions. Oxidation of VII with chromium trioxide in pyridine gave a ketone (VIII), m.p. 217·5–219·5°, ν_{max} 1720 cm^{-1}, that showed no OH absorption. Reduction of VIII with sodium borohydride in ethanol, followed by acetylation with acetic anhydride-pyridine regenerated tenuifolin dimethyl ester pentaacetate



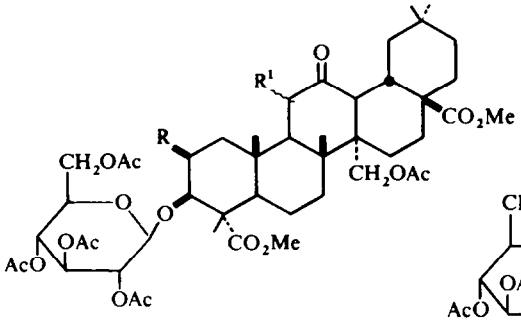
	R	R^1	R^2
V	H	H	OH
VI	H	Me	OH
VII	Ac	Me	OH
VIII	Ac	Me	=O
XIII	Ac	H	OH

(VII). These reactions indicate that one of the OH groups in tenuifolin is in a sterically hindered position. Of the twelve oxygen functions in V, four are accounted for in two carboxyl groups and six in the six OH groups (secondary or primary).

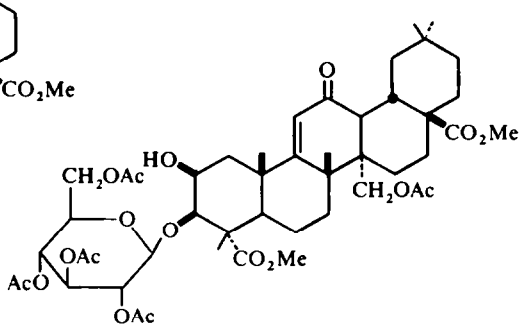
Information about the nature of the trisubstituted double bond and about the functional groups in its vicinity was gained as follows: Oxidation of the acetate (VII) with *m*-chloroperbenzoic acid in chloroform gave a mixture of two products, the corresponding epoxide, m.p. 126–129°, and a ketone (IX). In the NMR spectrum of the epoxide, the signal due to the proton on the epoxide ring appeared as a broad singlet at $\delta = 3{\cdot}15$ and there was no signal due to a vinylic proton. Treatment of the epoxide with boron trifluride etherate in ether at room temperature yielded the ketone (IX), m.p. 280–283°, manifesting CO absorption at ν_{max} 1703 cm^{-1} indicative of the presence of a 6-membered ring ketone. Oxidation of ketone IX with chromium trioxide gave a diketone (X), m.p. 210–212°. Treatment of ketone IX with bromine in acetic acid gave a bromoderivative (XI), m.p. 199–203°, which on debromination with lithium chloride in dimethyl formamide afforded a debromo compound (XII).

* Other previous work on *P. tenuifolia* includes a report[12] on the isolation of an indole alkaloid, tenuidine, m.p. 256°, and of a sugar,[13] polygalitol.

† The melting point of 249–253° reported for tenuifolin in our previous paper is in error.

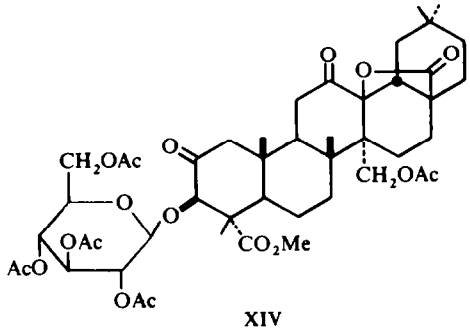


IX: R = βOH, H; R^1 = H
X: R = =O; R^1 = H
XI: R = βOH, H; R^1 = Br



XII

m.p. 190–191°. That XII is a trisubstituted enone was evidence by its ν_{max} at 1665 cm^{-1} and λ_{max} at 240 nm (ε = 7200). The NMR spectrum of the debromo compound showed a singlet at δ = 5·20 attributable to a vinylic proton. Tenuifolin on acetylation gave a pentaacetate (XIII), as an amorphous powder which on methylation with diazomethane afforded the crystalline dimethyl ester pentaacetate (VII) of tenuifolin. The amorphous acetate (XIII), when oxidized with hydrogen peroxide in acetic acid, gave, after methylation with diazomethane, an amorphous lactonic ester, ν_{max} 1775 cm^{-1} (γ-lactone). Oxidation of the latter with chromium trioxide in pyridine gave a ketolactone, (XIV), m.p. 255–256°; ν_{max} 1775, 1720 cm^{-1} (γ-lactone and six-membered ring carbonyl). Its NMR spectrum showed signals attributable to five tertiary alkyl methyls, five acetyl methyls and one ester methyl. These reactions



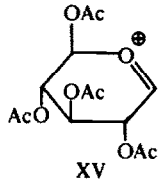
XIV

indicate that the trisubstituted double bond in tenuifolin is in a 6-membered ring with a carboxyl group situated on either an α- or β-C atom. These reactions closely resemble those of oleanolic acid[14] and other pentacyclic triterpenoids of the β-amyrin group.

The tertiary alkyl Me signals in the NMR spectra of tenuifolin dimethyl ester pentaacetate (δ = 0·66, 0·84, 0·91, 1·22, 1·30) and of presenegenin dimethyl ester triacetate (δ = 0·68, 0·83, 0·90, 1·17, 1·37) followed the same pattern.* This behavior, coupled with the fact that presenegenin is a component of the saponins of *P. tenuifolia*

* Substituent effects on the methyl signals of olean-12-ene type pentacyclic triterpenes have been well documented[15,16,17] and can be used for structural studies of the unknown members of the class.

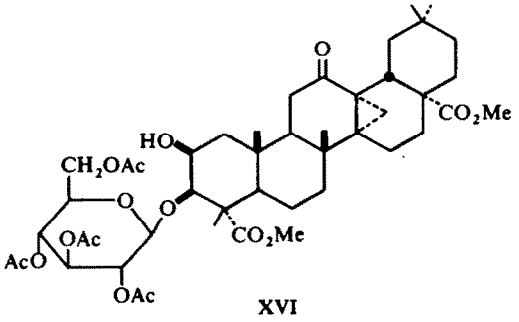
and *P. senega*, led us to the conclusion that the carbon skeleton of tenuifolin is probably the same as that of presenegenin. This conclusion was reinforced by the observation that the reactions of the trisubstituted double bond in both compounds are similar. At this point tenuifolin appeared to involve the presenegenin moiety substituted with a hexose unit on one of its three hydroxyl groups. This idea was supported by the fact that the mass spectrum of tenuifolin dimethyl ester pentaacetate showed a prominent peak at *m/e* 331 which is characteristic[18] of glycosides and is attributed to structure XV.



XV

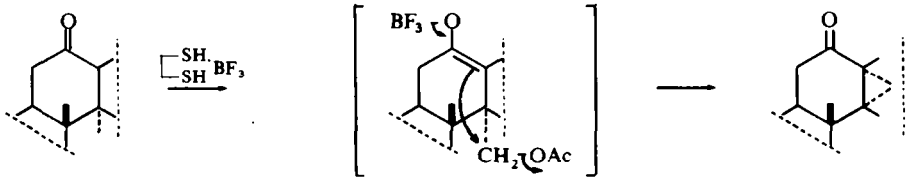
Tenuifolin dimethyl ester was hydrolysed with hydrochloric acid in dioxane. the hydrolysate was diluted with water and was extracted with ether. The ether extract on workup gave two compounds: dimethyl senegenin (IIa), senegenic acid dimethyl ester (IVa), both identified by comparison by TLC and IR spectra. The aqueous solution was deacidified by passing through a column of anion exchange resin. Dowex-IX8 (CO_3^- form). The deacidified solution showed a positive Fehlings test. whereas. tenuifolin dimethyl ester did not reduce Fehlings' solution. The eluates were freed from water. and the dry residue acetylated with acetic anhydride-sodium acetate to give β-D-glucose pentaacetate. m.p. 131–132°. identified by TLC. mixture m.p.. and IR spectral comparison. Since it is known that under acidic hydrolysis conditions. presenegenin rearranges to senegenic acid and senegenin. these reactions suggest that tenuifolin is in fact the glucosidc of presenegenin. This point was confirmed as follows: Tenuifolin dimethyl ester was oxidized with sodium metaperiodate in aqueous methanol and the resulting amorphous dialdehyde was hydrolysed with sodium hydroxide to afford a crystalline compound identified as presenegenin dimethyl ester by mixture m.p.. TLC and comparison of IR spectra of the triacetates.

The glucose moiety could be attached to any one of the three OH groups present in presenegenin. The position of linkage was determined as follows: 12-ketotenuifolin dimethyl ester pentaacetate (IX) when treated with boron trifluoride etherate and ethane dithiol gave as the major product a tetraacetate (XVI), instead of the expected



XVI

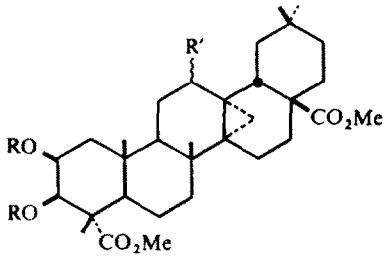
thioketal. The tetraacetate (XVI), m.p. 248–250°, showed in its IR spectrum absorption at 1695 cm^{-1} and in the UV absorption at λ_{max} 213·5 nm (ε = 4450), indicating the presence of a 6-membered ring CO group conjugated with a cyclopropane[19] ring. It did not show any bands due to unsaturation in its IR spectrum and neither did it give any coloration with tetranitromethane. A possible rationalisation for the formation of this structure feature is suggested below: The NMR spectrum of XVI



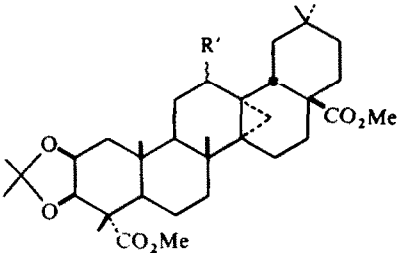
showed signals attributable to five tertiary alkyl methyls, four acetate methyls and two ester methyls. Due to the proximity of the CO group, the cyclopropyl ring protons are deshielded and the signals due to them are submerged under the Me signal as determined by integration. In its mass spectrum, XVI showed two prominent peaks, one at *m/e* 331 attributable[18] to the oxonium ion (XV), and the second at *m/e* 543 attributable to the residual 30-carbon hexacyclic system. The formation of XVI in which the glucose moiety is still present indicates that this hexose unit is not attached to the C-27 OH group of tenuifolin.

Strong evidence for structure XVI was obtained by the isolation of the hexacyclic diol (XVII) from the acidic hydolysate of XVI. The diol (XVII) could also be obtained by the acidic hydrolysis of 12-keto-tenuifolin dimethyl ester pentaacetate and by the base hydrolysis of 12-ketopresenegenin dimethyl ester triacetate. The diol (XVII), m.p. 263–265°; λ_{max} 213 nm (ε = 4760) and ν_{max} 1693 cm^{-1} showed in its mass spectrum the molecular ion peak at *m/e* 554. Its NMR spectrum showed signals attributable to five tertiary alkyl methyls, two ester methyls and an unresolved multiplet at δ = 4·1, attributable to the 2 protons on carbons bearing the glycol group. Acetylation of the diol (XVII) with acetic anhydride and pyridine gave a diacetate (XVIII), m.p. 250–252°; λ_{max} 212·5 nm (ε = 3419); ν_{max} 1690 cm^{-1}. Its NMR spectrum showed five tertiary alkyl Me signals, two ester Me signals, two acetate Me signals and a multiplet centered at δ = 5·4 attributable to the protons on C-2 and C-3. The diol (XVII), when treated with acetone and anhydrous cupric sulfate gave an acetonide (XIX), m.p. 179–181°.

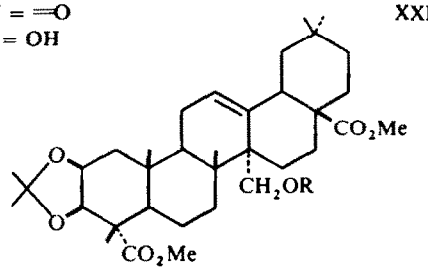
Further proof for the structure of the diol itself was obtained by the isolation of the diol (XVII) (as the acetonide) from the solvolysis of presenegenin dimethyl ester 2,3-0-acetonide-27-tosylate.[20] Treatment of presenegenin diethyl ester 2,3-0-acetonide (XX), m.p. 158–159°, with *p*-toluenesulfonyl chloride in pyridine gave the C-27 tosylate (XXI), m.p. 151–153;° ν_{max} 1632, 1123, 1038, 1012 cm^{-1}. The tosylate showed in its NMR spectrum signals attributable to five tertiary alkyl methyls, two acetonide methyls, two ester methyls, one aromatic ring methyl and an AB-type quartet centered at δ = 7·35 (4H) attributable to aromatic protons. Solvolysis of the tosylate with sodium acetate in aqueous acetone gave an alcohol (XXII), m.p. 197–198°; ν_{max} 3050 cm^{-1}, as the major product. Its NMR spectrum showed seven tertiary



XVII: R = H; R′ = =O
XVIII: R = Ac; R′ = =O
XXIII: R = H; R′ = OH



XIX: R′ = =O
XXII: R′ = OH



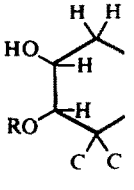
XX: R = H
XXI: R = Ts

alkyl Me signals, two ester Me signals, a multiplet at $\delta = 4{\cdot}32$ for the 3 protons attached to carbons carrying O atoms (C-2, C-3, and C-12) and two doublets at $\delta = 0{\cdot}40$ and $0{\cdot}03$ ($J = 3$ c/s) attributable to the cyclopropyl ring protons. Oxidation of XXII with chromium trioxide-pyridine afforded the ketone (XIX), m.p. 179–181°, λ_{max} 214 nm (ε, 4200); ν_{max} 1698 cm^{-1}, identical in all respects with the same compound obtained from 12-keto-13,27-cyclotenuifolin dimethyl ester tetraacetate.

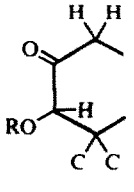
The facts presented above clearly show that the C-27 carbon is involved in cyclopropane ring formation in XVI. Since the analytical and spectral data indicate that XVI contains the glucose unit, the latter must be attached to either the C-2 or C-3 OH group. A distinction can be made from the following evidence:

1. The NMR spectrum of the acetate (VII) has a doublet at $\delta = 4{\cdot}50$ (1H, $J = 8$ c/s) attributable to R—O—C—H and a multiplet at $\delta = 5{\cdot}00$ attributable to HO—C—H. In the spectrum of the ketone (VIII), this doublet signal appears as a singlet at $\delta = 4{\cdot}73$ while the multiplet signal at $\delta = 5{\cdot}00$ has disappeared.

2. Similarly, the NMR spectrum of 12-ketotenuifolin dimethyl ester pentaacetate shows a doublet at $\delta = 4{\cdot}55$ (1H, $J = 8$ c/s), whereas the spectrum of the corresponding diketone (X) lacks this doublet. Instead a singlet appears at $\delta = 4{\cdot}75$ (1H).



A



B

These spectral data indicate that in acetate (VII) the partial structure **A** with the glucose moiety on the 3-β-hydroxyl, is oxidised to ketone **B**.

The nature of the glycosidic linkage is clearly shown to be β by the fact that the NMR spectrum of the acetate (VII) shows a doublet at $\delta = 5{\cdot}22$ (1H, $J = 9$ c/s) due to the axial anomeric proton of the glucose moiety.[1,21] This doublet appears more or less at the same field in other tenuifolin derivatives also.

EXPERIMENTAL

General. M. ps are corrected and were taken on a Kofler hot block. UV spectra were determined in 95% EtOH on a Perkin-Elmer Model 202 spectrophotometer and IR spectra on an Infracord 237B spectrophotometer. PMR spectra were taken on a Varian A-60 spectrometer in $CDCl_3$ with TMS as an internal standard and are reported as δ-values in ppm. Specific rotations were measured at room temp (27°) in chloroform soln on a Perkin-Elmer Model 141 polarimeter.

Isolation of saponins. The dry powdered roots of the plant were defatted by percolation with n-hexane in the cold several times. The defatted root powder was then refluxed in 95% EtOH several times and the extract filtered hot. The EtOH extract was concentrated and dissolved in water. The dark brown aqueous soln was extracted with ether and then with n-BuOH. The BuOH extract was concentrated and the saponins were precipitated with ether. The crude saponin mixture was collected by filtration and dried.

The yield of saponins from *P. senega* and *P. tenuifolia* was 12-13% and 9-10% respectively. The crude saponin mixture from *P. senega* when dissolved in hot EtOH and cooled, slowly deposited white crystals which were collected and recrystallized to m.p. 141-142°: $[\alpha]_D$ +39·6° (*c*, 1·2; EtOH). This product was identified as polygalitol[22,23] (1,5-anhydro-D-sorbitol) from a study of the spectral data of the alcohol as well as its acetate, m.p. 71-72° $[\alpha]_D$ +34·1° (*c*, 1·56).

The inverted dry column chromatography[24] of the saponin esters (esterified with diazomethane) from *P. senega* gave another crystalline compound, m.p. 181-182°, which was identified as sucrose by mixture m.p. and IR spectrum.

TLC *of the crude saponin esters.* TLC (Silica gel G: solvent: lower layer of chloroform-MeOH-water (65:35:10): sprayed with H_2SO_4) showed that the crude saponin esters of both *P. senega* and *P. tenuifolia* contained at least six components each. Although the R_f values of the components of the saponin obtained from one plant corresponded with those of the other, there were some minor differences in the intensities of the spots. The TLC spots of two components with lowest R_f values corresponded with those of polygalitol and sucrose.

Hydrolysis of the crude saponins. The tenuifolia saponin on hydrolysis with dil HCl gave mostly senegenin and on hydrolysis with phosphoric acid gave hydroxysenegenin. The same saponin on oxidation with sodium metaperiodate followed by hydrolysis gave presenegenin and 4-methoxycinnamic acid. All these sapogenins had earlier been obtained from *P. senega* saponin using the same hydrolytic conditions.

Isolation of tenuifolin (V). (1) The saponing from *P. tenuifolia* (5 g) was dissolved in NaOH (5%: 60 ml) and refluxed for 1·5 hr. The resulting dark brown soln was extracted with ether–EtOH (9:1). The aqueous portion was acidified with phosphoric acid and on storage deposited white crystalline material (600 mg). This material after several recrystallizations gave tenuifolin (210 mg), m.p. 298-300°: $[\alpha]_D$ +49° (*c*, 0·8: EtOH): ν_{max} 3400, 1075, 1015 cm^{-1} (OH), 2700, 1705, 1250 cm^{-1} (COOH). (Found: C, 62·65; H, 8·48. Calcd for $C_{36}H_{56}O_{12}\cdot 1\,C_2H_5OH$: C, 62·81; H, 8·54%).

(2) The saponin from *P. senega* (45 g) dissolved in NaOH (5%: 600 ml) was kept at 75-80° for 18 hr. The resulting soln was then cooled to 0°, and acidified with phosphoric acid to pH 2. The acidified soln was extracted twice with ether–EtOAc (9:1) and then several times with ether–EtOH (85:15). The ether-EtOH extract on concentration gave crystalline deposits (8 g) which were combined and recrystallized from EtOH to afford tenuifolin (5·1 g), m.p. 298-300°, identical with the sample obtained from *P. tenuifolia* (mixture m.p., IR).

The filtrate remaining after collecting the deposits from the ether–EtOH extract was mixed with the earlier ether–EtOAc extract and the total extract was esterified with diazomethane to give a dark brown residue (6·3 g). This residue was passed through a column of silica gel (110 g) using $CHCl_3$–MeOH (98:2) as eluent to give a pale brown viscous liquid (Fraction A; 4·2 g). Further elution with $CHCl_3$–MeOH (95:5) gave a crystalline solid (Fraction B: 810 mg).

Fraction A was rechromatographed over silica gel using benzene containing increasing proportions of

EtOAc as eluent to give methyl 4-methoxycinnamate, m.p. 88–89°, and methyl-3,4-dimethoxycinnamate, m.p. 68–69° (1·7 g), both identified by comparison of IR spectra and mixture m.p.

Fraction B on recrystallization from EtOAc gave VI (0·67 g), m.p. 270–272°; $[\alpha]_D$ +41·5° (*c*, 1·52); ν_{max} 3350, 1075, 1030 cm^{-1} (OH), 1730, 1175 cm^{-1} (ester CO). (Found: C, 64·60; H, 8·59. Calcd for $C_{38}H_{60}O_{12}$: C, 64·38; H, 8·53%).

Dimethyl tenuifolin pentaacetate (VII). A soln of dimethyl tenuifolin (1·6 g) in pyridine (8 ml) and Ac_2O (15 ml) was kept at room temp for 18 hr. Excess pyridine and Ac_2O were distilled off in a rotary evaporator and the residue was crystallized from benzene–pentane to give dimethyl tenuifolin pentaacetate (1·8 g), m.p. 220–222°; $[\alpha]_D$ +70·2° (*c*, 0·63); ν_{max} 1770, 1753, 1745, 1730, 1240 cm^{-1} (acetates, esters), 3540 cm^{-1} (OH); NMR δ = 0·66, 0·84, 0·91, 1·22, 1·30 (singlets, 3H each, five tertiary alkyl methyls), 1·96 (s, 3H), 1·99 (s, 6H), 2·02 (s, 3H), 2·05 (s, 3H) (five acetyl methyls), 3·67 (s, 3H), 3·60 (s, 3H) (two ester methyls), 4·50 (1H, d, J = 8 c/s) (H—C3), 5·00 (1H, m) (H—C2), 5·22 (1H, d, J = 9 c/s) (glycosyl H), 5·55 (1H, m) (vinyl H) (Varian HA-100 spectrum). (Found: C, 62·71; H, 7·59. Calcd for $C_{48}H_{70}O_{17}$: C, 62·74; H, 7·62%).

Dimethyl tenuifolin pentaacetate (100 mg) was refluxed in a mixture of pyridine (5 ml) and Ac_2O (10 ml) for 8 hr and on workup gave the unchanged pentaacetate (95 mg).

Oxidation with chromium trioxide. A freshly prepared complex of CrO_3 (120 mg) in pyridine (2 ml) was added to a soln of tenuifolin dimethyl ester pentaacetate (80 mg) in pyridine (2 ml) and the mixture was stirred at room temp for 4 hr. The mixture was then poured on to ice and the product extracted with ether. The ethereal extract was washed with 5N H_2SO_4, water, and $NaHCO_3$ aq, dried (Na_2SO_4) and was freed from solvent. The residue was crystallized from 95% EtOH to give VIII (62 mg), m.p. 218–220°; $[\alpha]_D$ +48° (*c*, 1·0); ν_{max} 1720 cm^{-1} (6-membered ketone), 1730, 1750, 1760 cm^{-1} (acetate, ester); NMR δ = 0·65, 0·85, 0·80, 1·01, 1·10 (singlets, 3H each, five tertiary alkyl methyls), 1·98, 1·99, 2·00, 2·01, 2·03 (singlets, 3H, five acetyl methyls), 3·60, 3·75 (singlets, 3H, each, two ester methyls), 4·11 (s, 2H, C(27)H_2), 4·35 (m, 2H, C(6′)H_2), 4·73 (s, 1H, C(3)H) 5·08 (m, 4H,H's on C(1′)—C(4′)), 5·51 (m, 1H, vinyl proton) (Varian HA-100 spectrum). (Found: C, 63·10; H, 7·58. Calcd for $C_{48}H_{68}O_{17}$: C, 62·89; H, 7·42%).

Sodium borohydride reduction of ketone. A soln of 2-Ketotenuifolin dimethyl ester pentaacetate (80 mg) and $NaBH_4$ (100 mg) in 8 ml of EtOH was stirred for 3 hr at room temp. The excess borohydride was decomposed with acetone and the product was extracted with EtOAc. The extract was washed with water, dried (Na_2SO_4) and was freed from solvent. The amorphous residue (58 mg) on acetylation with Ac_2O–pyridine at room temp, workup and crystallization from 95% EtOH gave tenuifolin dimethyl ester pentaacetate (43 mg) identified by mixed m.p. and IR spectra.

Oxidation with m-chloroperbenzoic acid. Tenuifolin dimethyl ester pentaacetate (5·4 g) in chloroform (200 ml) and *m*-chloroperbenzoic acid (5·5 g) in chloroform (50 ml) were mixed and refluxed for 4 hr. The chloroform soln was washed with Na_2SO_3 aq (10%) and $NaHCO_3$ aq and dried over Na_2SO_4 and finally freed from solvent. The residue on crystallization from benzene–pentane gave IX, m.p. 275–280° (1·02 g), recrystallized to m.p. 281–283°; $[\alpha]_D$ +20° (*c*, 2·2); ν_{max} 3540 cm^{-1} (OH), 1710 cm^{-1} (6-membered ketone), 1767, 1745, 1730, 1250, 1235 cm^{-1} (ester, acetate); δ = 0·87, 0·95, 1·00, 1·23, 1·35 (singlets, 3H each, five tertiary alkyl methyls), 1·95, 2·02, 2·03, 2·07, 2·08 (singlets, 3H each, acetate methyls), 3·72, 3·73 (singlets, 3H each, ester methyls), 5·1 (d, 1H, J = 9), 4·55 (d, 1H, J = 8). (Found: C, 61·59; H, 7·67. Calcd for $C_{48}H_{70}O_{18}$: C, 61·65; H, 7·54%). The mother liquors left after the crystallization of the 12-keto derivative were chromatographed over a column of silica gel and eluted with benzene containing increasing portions of EtOAc to give the 12,13-epoxide as an amorphous powder (TLC, single spot) (3·2 g), m.p. 126–129° $[\alpha]_D$ +16·6° (*c*, 0·63). In its NMR spectrum, the epoxide proton appears as a broad singlet at δ = 3·15 (1H). The epoxide on treatment with BF_3-etherate (0·5 ml) in ether (60 ml) at room temp for 1 hr gave 2·75 g of the 12-keto derivative as a crystalline deposit, m.p. 275–281°, identical with the earlier sample (mixture m.p., IR).

Diketone (X). Compound IX (350 mg) was oxidised with CrO_3 (400 mg) in pyridine (10 ml) at room temp for 4 hr. After workup, the residue (125 mg) was crystallized from 95% EtOH to give 2,12-diketotenuifolin dimethyl ester pentaacetate (80 mg), m.p. 210–212°; $[\alpha]_D$ +5·5° (*c*, 1·4); ν_{max} 1703 cm^{-1} (6-membered ketone), 1725, 1730, 1750 cm^{-1} (acetates, esters); δ = 0·85, 0·91, 0·95, 1·00, 1·04, 1·94, 1·99, 2·00, 2·01, 2·02, 3·72, 3·82 (singlets, 3H each, 5 tertiary alkyl methyls, 5 acetyl methyls and 2 ester methyls), 4·75 (s, 1H, C(3)H). (Found: C, 61·21; H, 7·38. Calcd for $C_{48}H_{68}O_{18}$: C, 61·27; H, 7·29%).

Enone (XII). Compound IX (250 mg) in AcOH (20 ml) was stirred at 40° with a few drops of a soln (1%) of Br_2 in AcOH and a drop of HBr. Addition of Br_2 and stirring was continued until decolorization ceased. The mixture was freed from solvents and the residue was dissolved in ether. The ethereal soln was washed with $NaHCO_3$ aq (10%), dried (Na_2SO_4), and concentrated to give the 11-bromo derivative (204 mg) which was recrystallised to mp 199–204°; $[\alpha]_D$ +47·5° (*c*, 0·57). This bromo compound (200 mg) in DMF

(20 ml) and LiCl (300 mg) was kept at 153° for 5 hr. The reaction mixture was then diluted with water and extracted with ether. The ethereal soln was washed with water, dried (Na_2SO_4) and concentrated to give an oil (140 mg) which on chromatography over silica gel afforded 67 mg of an amorphous solid. Crystallization of the material gave XII. (33 mg). mp 190–191°; λ_{max}^{EtOH} 240 nm (ε = 7200); ν_{max} 3550 cm^{-1} (OH). 1665 cm^{-1} (conjugated ketone). 1750. 1225 cm^{-1} (ester. acetate). (Found: C. 60·98; H. 7·55. Calcd for $C_{48}H_{68}O_{18}$: C. 61·27; H. 7·29%).

Ketolactone (XIV). Tenuifolin (1·4 g). pyridine (25 ml) and Ac_2O (25 ml) were kept at room temp for 48 hr and then free from excess pyridine and Ac_2O by evaporation on a rotary evaporator. The residue was dissolved in benzene and precipitated with n-pentane to give tenuifolin pentaacetate as an amorphous powder (1·64 g). This product XIII (1·3 g). AcOH (30 ml) and H_2O_2 (12 mL 30%) were kept at 80–90° for 2 hr. This mixture was then diluted with water and extracted with ether. The ethereal extract on workup gave an amorphous solid (752 mg) which, after esterification with diazomethane, was chromatographed over silica gel to give a pure white amorphous solid (350 mg; TLC. single spot). This solid was dissolved in a mixture of CrO_3 (500 mg) and pyridine (20 ml) and was kept at room temp for 4 hr. Thie mixture was then diluted with water and extracted with ether. The ethereal extract on workup gave a residue (290 mg) which was recrystallized from 95% EtOH to give XIV. mp 255–256°; $[\alpha]_D$ +3° (*c*. 0·9): ν_{max} 1775 cm^{-1} (γ-lactone). 1720 cm^{-1} (6-membered ketone). 1750. 1225 (ester. acetate); δ = 0·92. 0·93. 0·97. 1·04. 1·26. 1·99 (6H). 1·98. 2·01. 2·08 (s. 3H each; 5 tertiary alkyl methyls and 5 acetyl methyls). (Found: C. 61·41; H. 6·85. Calcd for $C_{47}H_{64}O_{18}$: C. 61·57; H. 6·99%).

Hydrolysis of tenuifolin dimethyl ester. Tenuifolin (700 mg). after esterification with diazomethane, was dissolved in a soln of EtOH (50 ml). water (10 ml) and conc HCl (5 ml) and the soln was refluxed for 18 hr. The mixture was freed from most of the EtOH under vacuum, diluted with water (40 ml) and extracted with ether. The ethereal extract was washed with water, dried (Na_2SO_4). esterfified with diazomethane, and freed from solvent to give an oily residue (610 mg). This residue consisted mainly of dimethyl esters of senegenin and senegenic acid (TLC) which were separated by column chromatography over silica gel (eluents: benzene–EtOAc) and identified by the usual means.

In another experiment, tenuifolin dimethyl ester (320 mg) was dissolved in a mixture of dioxane (25 ml). water (45 ml) and conc HCl (6 ml) and the soln refluxed for 4 hr. The mixture was extracted with ether and the ethereal extract when worked up as in the previous case gave the dimethyl ester of senegenic acid. The aqueous portion was concentrated and passed through a column of 300 g Dowex 1-X8 (CO_3^- form) (column: 30 × 4·8 cm) and eluted with water (2 liters). The eluates were evaporated to dryness *in vacuo* to give 80 mg syrupy residue. (TLC: single spot corresponding to glucose; solvent: MeOH–$CHCl_3$ 60:40). This residue was acetylated with Ac_2O (3 ml) and fused NaOAc (160 mg) at 100° for 3 hr. After the usual workup, the ppt obtained (45 mg) was crystallised from MeOH to mp 131–132°. The crystalline product was identified as β-D-glucose pentaacetate by mixture mp, IR and TLC.

Periodate cleavage of tenuifolin. Tenuifolin dimethyl ester (275 mg; 0·0004 mole) in MeOH (40 ml) was mixed with sodium metaperiodate (190 mg in 2 ml water; 0·0009 mole) under a N_2 atmosphere at 0°. The mixture was kept in the cold for 4 hr and at room temp overnight, and then concentrated under vacuum to 10 mL diluted with water (50 ml) and extracted with ether. The ether extract was freed from solvent and the residue was dissolved in a mixture of EtOH (20 ml), water (10 ml) and KOH (200 mg). The mixture was refluxed for 4 hr under N_2, cooled, diluted with water (30 ml). acidified with phosphoric acid to pH 3 and extracted with ether. The ether extract after the usual workup gave a residue which was acetylated with Ac_2O (1·5 ml) and pyridine (1 ml) at room temp for 16 hr. Excess Ac_2O and pyridine were removed under vacuum and the residue on chromatography over silica gel (30 g) (eluents: benzene–EtOAc) gave two crystalline compounds **A** and **B**. Compound **A** (65 mg; mp 218–220°) was identified as presenegenin dimethyl ester triacetate by TLC. mixture mp and IR. Compound **B** (16 mg: mp 220–222°) was identified as (unreacted) tenuifolin dimethyl ester pentaacetate by TLC. mixture mp and IR.

12-Keto-13.27-cyclotenuifolin dimethyl ester tetraacetate (XVI). 12-Ketotenuifolin dimethyl ester pentaacetate (3·35 g). ethanedithiol (12 ml) and BF_3-etherate (8 ml) were mixed and stirred at room temp for 1 hr. To the mixture was added MeOH (50 ml) and benzene (150 ml) and the mixture was washed with water and 10% $NaHCO_3$ aq· dried and concentrated to give 2·7 g of a crystalline residue. mp 232–237°. Recrystallization of this residue from benzene–pentane gave 1·15 g of XVI. m.p. 248–250°: $[\alpha]_D$ + 29·5 (*c*, 0·83): ν_{max} 3520 cm^{-1} (OH). 1765. 1745. 1250 (ester. acetate). 1695 cm^{-1} (6-membered ring CO with cyclopropyl conjugation): λ_{max} 213·5 nm (ε. 4450): δ = 0·68. 0·83. 1·00. 1·13. 1·30. 1·95. 1·97. 2·00. 2·01. 3·60. 3·67 (singlets. 3H each. 5 tertiary alkyl-4-acetate-. and 2 ester methyls). (Found: C. 63·17: H. 7·58. Calcd for $C_{46}H_{66}O_{16}$: C. 63·16; H. 7·55%).

The mother liquors remaining from the above crystallization on column chromatography (silica gel)

gave besides XVI. an unidentified compound (63 mg). m.p. 239–240°; λ_{max} 208·5 nm (ε = 1250): ν_{max} 3510. 1080 cm^{-1} (OH). 1765. 1730. 1245 cm^{-1} (ester. acetate). 1710 cm^{-1} (6-membered ring C=O). (Found: C. 62·75; H. 7·75. Calcd for $C_{46}H_{68}O_{16}$: C. 63·01; H. 7·76%).

12-*Keto*-13.27-*cyclopresengenin dimethyl ester* (XVII). Compound. XVI (900 mg). EtOH (150 ml). water (20 ml) and conc H_2SO_4 (5 ml) were mixed and boiled under reflux for 48 hr. The mixture was cooled. excess acid was destroyed with $BaCO_3$. and the product chromatographed over silica gel to give 230 mg of XVII. m.p. 263–265°; λ_{max} 213 nm (ε. 4760); ν_{max} 3510, 1100 cm^{-1} (OH). 1737, 1725, 1270 cm^{-1} (ester), 1690 cm^{-1} (6-membered ring C=O with cyclopropyl conjugation): δ = 0·7. 0·85. 1·00. 1·17. 1·32. 3·62. 3·72 (singlets. 3H each. 5 tertiary alkyl methyls. 2 ester methyls). 5·9 (m. 2H. C-2 and C-3H). (Found: C. 70·49: H. 8·92. Calcd for $C_{32}H_{48}O_7$: C. 70·56: H. 8·88%).

The acetate XVIII of XVII prepared by acetylation in Ac_2O-pyridine had m.p. 250–252°: λ_{max} 212·5 nm (ε. 3419). ν_{max} 1690 cm^{-1} (6-membered ring C=O with cyclopropyl conjugation). 1760. 1752. 1744. 1730. 1245 cm^{-1} (ester. acetate): δ = 0·77. 0·92. 1·05. 1·20. 1·42. 2·00. 2·10. 3·67. 3·73 (singlets. 3H each. 5 tertiary alkyl-. 2-acetate-. and 2 ester methyls). 5·4 (m. 2H. C-2 and C-3H). (Found: C. 68·62: H. 8·51. Calcd for $C_{36}H_{52}O_9$: C. 68·76: H. 8·34%).

The acetonide XIX of XVII prepared by stirring an acetone soln with anhydrous cupric sulfate (28°, 20 hr) had m.p. 179–181°: λ_{max} 214 nm (ε. 4200); ν_{max} 1698 g^{-1} (conjugated C=O), 1730, 1250 (ester). (Found: C. 71·64: H. 8·87. Calcd for $C_{35}H_{52}O_7$: C. 71·88; H. 8·96%).

(2) 12-Ketotenuifolin dimethyl ester pentaacetate (4·54 g). EtOH (200 ml). water (60 ml) and HCl (25 ml) were refluxed for 8 hr. The mixture was diluted with ether (1400 ml). the ether soln washed with water dried ($MgSO_4$) and concentrated to give a residue (4·0 g) which was chromatographed over a column of silica gel (eluent: chloroform–EtOAc). Two products. **C** and **D** were isolated. Compound **C**. (630 mg). m.p. 262–264°. was identified as 12-ketopresenegenin dimethyl ester 27-acetate. ν_{max} 3500. 1065 cm^{-1} (OH). 1750. 1724. 1245 (ester. acetate). 1700 cm^{-1} (6-membered C=O): δ = 0·83. 0·92. 0·97. 1·22. 1·34. 1·98. 3·68. 3·73 (5 tertiary alkyl-. 1 acetate-. and 2 ester methyls). 4·13 (broad s. 2H. —C<u>H</u>$_2$OAc). 4·0 (m. 2H. C-2 and C-3H). (Found: C. 67·25: H. 8·65. Calcd for $C_{34}H_{52}O_9$: C. 67·52: H. 8·67%). Compound **D** (1·5 g) was recrystallised from MeOH to m.p. 263–265° and was found to be identical with XVII by TLC. mixture m.p. and IR.

(3) 12-Ketopresenegenin dimethyl ester triacetate (200 mg). EtOH (15 ml) water (5 ml) and NaOH (800 mg) were kept at 50° for 4 hr. The mixture was acidified and worked up to give a yellow oil (191 mg) which on column chromatography over silica gel (12 g) (eluent: $CHCl_3$–EtOAc) gave a homogeneous amorphous powder (82 mg) which on crystallization afforded a product. m.p. 259–263°. identical (TLC. mixture m.p.. IR) with (XVII).

Presenegenin dimethyl ester acetonide (XX): Presenegenin dimethyl ester (500 mg) dissolved in anhyd acetone (25 ml) was mixed with anhyd cupric sulfate (3 g) and stirred at 28° for 2·5 hr. The mixture was freed from cupric sulfate by filtration and the filtrate on concentration gave an oily material (612 mg). The oil was chromatographed over a column of alumina (neutral) (eluent: ether) to give the pure acetonide (354 mg). m.p. 158–159°. $[\alpha]_D$ +91° (*c*. 1·0); ν_{max} 3640. 1030 cm^{-1} (OH). 1730. 1250 (ester C=O): δ = 0·65. 0·88. 0·92. 1·10. 1·15. 1·29. 1·46. 3·72. 3·79 (9 singlets. 3H each. 5 tertiary alkyl methyls. 2 acetonide methyls. 2 ester methyls). 3·15 (2H. AB-type. C<u>H</u>$_2$OH). 4·42–4·00 (m. 2H. C-2 and C-3H). 5·90 (m. 1H. vinylic H). (Found: C. 71·60; H. 9·28. Calcd for $C_{35}H_{54}O_7$: C. 71·64; H. 9·28%).

Presenegenin dimethyl ester 2.3-O-*acetonide* 27-*tosylate* (XXI). A soln of XX (558 mg) and *p*-toluenesulfonyl chloride (1 g) in pyridine (5 ml) was allowed to stand at room temp for 3 days. The mixture was then poured onto ice. extracted with benzene. and the benzene soln washed with water. dried and freed from solvent to give 450 mg of a solid which was recrystallized to m.p. 151–153°: $[\alpha]_D$ +51·8° (*c*. 1·0): ν_{max} 1720. 1255 cm^{-1} (ester C=O). 1632. 1213. 1188. 1012 (tosyl group): δ = 0·37. 0·64. 0·82. 1·02. 1·44. 1·18. 1·24. 2·28 (singlets. 3H each. 5 tertiary alkyl-. 2 acetonide-. and 1 aromatic methyls). 3·64 (s. 6H. 2 ester methyls). 7·35 (AB-type. 4H. J = 8 c/s. aromatic protons). (Found: C. 68·60: H. 8·00. Calcd for $C_{42}H_{60}O_9S$: C. 68·36: H. 8·16%).

Solvolysis of p-*toluenesulfonate*. The tosylate XXI (343 mg) dissolved in acetone (75 ml) was mixed with a soln of NaOAc (1·8 g) in water (50 ml) and the mixture was refluxed for 20 hr. Most of the acetone was removed by distillation and the product which precipitated from the concentrated soln was collected, washed, and dried (232 mg). This was chromatographed over a column of alumina (50 g, neutral) using ether containing increasing amounts of EtOH as eluent. Three crystalline compounds. **E**. **F** and **G** were isolated. Compound **E**. 42 mg. m.p. 189–191° was identified as dimethyl 2.3-isopropylidencdioxy-13.27-cyclo-Δ^{11}-oleanene-23.28-dioate[8] by mixture m.p.. TLC and IR.

Compound **F** (80 mg). m.p. 197–198°. $[\alpha]_D$ +41·3° (*c*. 1·21) is shown to be XXII by the following spectral

data: ν 3500 cm^{-1} (OH). 1710. 1725. 1240 cm^{-1} (ester C=O); δ = 0·77. 0·85. 0·90. 1·02. 1·46. 1·17. 1·26. 3·40. 3·60 (singlets. 3H each. 5 tertiary alkyl-. 2 acetonide-. and 2 ester methyls) 0·40. 0·03 (2H. cyclopropane) 4·35 (m. 3H. C-2. C-3. and C-12 protons). (Found: C. 71·41; H. 9·36. Calcd for $C_{35}H_{54}O_7$: C. 71·64: H. 9·22%).

Compound **G** (28 mg). m.p. 235–236°: $[\alpha]_D$ +68·6° (*c*. 1·0) is shown to be **XXIII** by the following data: ν_{max} 3450. 1050 cm^{-1} (OH). 1723. 1726. 1245 cm^{-1} (ester C=O); δ = 0·80. 0·85. 0·88. 1·17. 1·32. 3·63. 3·67 (singlets. 3H each. 5 tertiary alkyl-. and 2 ester methyls). 4·05 (m. 3H. C-2. C-3. and C-12 protons). 0·17. 0·44 (1H each. d. J = 3·5 cyclopropane). (Found: C. 70·16: H. 8·99. Calcd for $C_{32}H_{50}O_7$: C. 70·30: H. 9·22%).

Oxidation of 12-hydroxy-13,27-cyclopresenegenin acetonide dimethyl ester. The alcohol **XXII** (36 mg) dissolved in pyridine (3 ml) was treated with a freshly prepared complex of CrO_3 (50 mg) in pyridine (3 ml) at room temp for 18 hr. The mixture was poured into ice water. extracted with ether. the ether extract washed with 5N H_2SO_4 and dried. and on concentration gave the 12-keto compound (28 mg). m.p. 179–181°: $[\alpha]_D$ +63·6° (*c*. 0·98). identical with (TLC. mixture m.p.. IR) **XIX** described earlier.

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