Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

TERPENOIDS

X. PRE-SENEGENIN, A QUITE NORMAL TRITERPENOID

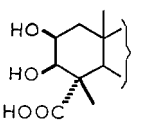
J. J. DUGAN AND P. DE MAYO
Department of Chemistry, University of Western Ontario, London, Ontario
Received February 22, 1965

ABSTRACT

Pre-senegenin has been obtained by a combination of oxidative and hydrolytic cleavage of the saponin, and has been shown to be a member of the normal and unrearranged oleanolic acid series: 2β,27-dihydroxy-23-carboxyoleanolic acid (XXII). On treatment with ethanolic hydrochloric acid it is converted quantitatively to a mixture of polygalic acid (XXI) and senegenin.

Previous work (1) has shown that senegenin is a triterpenoid of empirical formula $C_{30}H_{45}O_6Cl$ with a 2β,3β-glycol system and a 4α-carboxyl group. A second carboxyl group is also present. It has now been found to be a member of the β-amyrin-oleanolic acid series in which a carbon atom at C_{14} has migrated to C_{12} with concomitant movement of the double bond to the C_{13}—C_{14} position, and that it has the structure (XX).*

In our earlier study of the triterpenoid senegenin, we showed that, surprisingly, it contained chlorine and that it had the empirical formula $C_{30}H_{45}O_6Cl$. Four of the oxygen atoms were found to be present as shown in the part-structure (I), and the remaining two, in agreement with the conclusions of previous workers (3, 4), formed part of a carboxyl function.



(I)

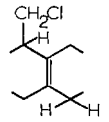
By heating senegenin with quinoline a substance, de(hydrochloro)senegenin, was obtained which showed the ultraviolet absorption spectrum of a conjugated diene. This compound could also be obtained, under defined conditions, as the product of the alkali treatment of senegenin, the "extra", and third, equivalent of alkali required in the titration of senegenin being consumed by the liberated hydrochloric acid.

The ultraviolet absorption spectrum of de(hydrochloro)senegenin (λ_{max} 243, 249, 256 mμ) was clearly indicative of a heteroannular diene and the nuclear magnetic resonance (n.m.r.) spectrum, which showed a multiplet (one proton) at τ 4.65 and a broad singlet (ca. three protons) at τ 8.19 suggested that a methyl group and a proton were substituents on this diene. However, none of the methyl groups of senegenin, of which there were clearly five, provided the methyl group of the diene, and indeed the mere presence of but five methyl groups in senegenin derivatives suggested that the biogenetically to be expected sixth bore the chlorine atom. This could give rise, eventually, to the vinylic methyl group.

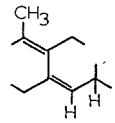
The n.m.r. spectrum of senegenin esters was obscured by the signals from the methoxyl groups in the region where —CH_2Cl signals were to be expected. The spectrum of senegenin itself, determined in pyridine, showed a multiplet in the expected region of about two

**Part of this material formed a preliminary communication (2).*

protons. The fact that a complex pattern was observed further suggested that there was at least one proton attached to the terminus of the chloromethyl group. Since senegenin derivatives showed no vinyl proton signal in their n.m.r. spectra, these observations suggested, though subject to other interpretations in detail, that the changes observed in the conversion of senegenin to de(hydrochloro)senegenin could be represented as (II) → (III). An obvious point requiring clarification was the nature of the prototropic shift apparently occurring under basic conditions. This will be returned to later.



(II)



(III)

These experiments at least indicated the proximity of the chlorine to the one double bond present in senegenin. It was also necessary to relate these functions, spatially, to the remaining carboxyl group.

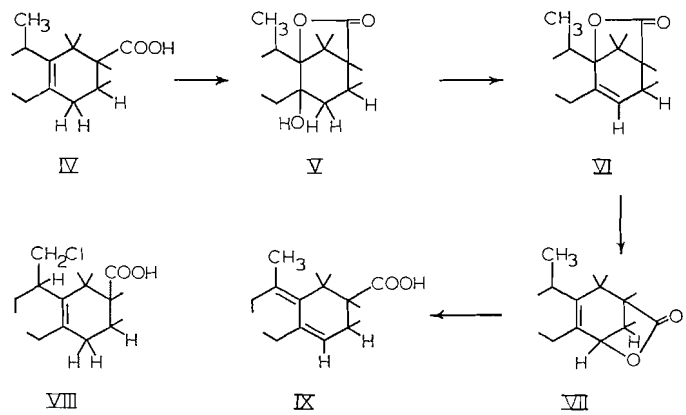
For these experiments dechlorosenegenin (1), obtained from senegenin monoacetate by reduction with lithium and liquid ammonia, was used.

Oxidation of dechlorosenegenin diacetate with hydrogen peroxide in acetic acid gave, after esterification with diazomethane, an oily lactonic ester. This showed absorption in the infrared compatible with the presence of a hydroxyl group (3 500 cm^{-1}) and of a γ-lactone (1 767 cm^{-1}). The tertiary alcohol in this compound (no methine proton on carbon bearing an oxygen) could be dehydrated with thionyl chloride in pyridine to give an anhydro lactone (VI) which appeared to contain a vinylic proton.

Merely on heating in methanol, this unsaturated lactone was isomerized into a new lactone (VII), likewise a γ-lactone (ν_{max} 1 773 cm^{-1}). This isomeric lactone, however, had no vinylic proton, but had, instead, a doublet (one proton) at τ 5.19 suggestive of the presence of a proton on carbon bearing an oxygen.

The mildness of the isomerism conditions (the isomerism was also achieved by hydrolysis of the anhydro lactone with base followed by reacetylation) seemed only compatible with an allylic rearrangement. This required that both ends of the allylic system be γ to the carboxyl group. The sequence of reactions could then be represented as in (IV) → (VII).

The ready isomerism of VI to VII, presumably through an allylic cation, suggested that if this cation could be induced to lose a proton the familiar chromophore of III would

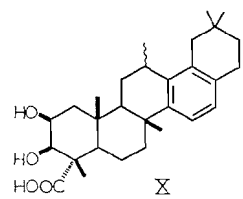


Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

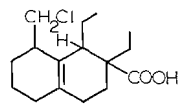
be obtained. When the unacetylated ester of (VII) was allowed to stand in 5% methanolic hydrochloric acid at room temperature the diene was indeed rapidly formed and the monocarboxylic acid (IX) could be obtained in quantitative yield. This, when converted to the ester with diazomethane, gave the dimethyl ester of de(hydrochloro)senegenin.

The spatial relationship of the three functional groups in senegenin under discussion appeared to be as represented in (VIII). It was in addition, because of the difficulty of hydrolysis of the corresponding ester, probable that the carboxyl was attached to quaternary carbon. However, the mode of fusion of the remaining ring systems onto the ring in (VIII) remained to be ascertained.

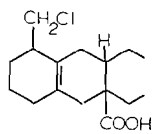
One method by which information could be obtained appeared to be, in principle, by partial aromatization. Should the carboxyl be the only blocking group, then its removal should engender ready aromatization. The route selected was that used by Barton in the study of tomentosolic acid (6) and for this purpose it was desirable to have an unsaturated derivative of senegenin in which the ring A carboxyl was esterified and the other was not. Partial hydrolysis of a diester could not serve since the ring A carboxyl was the more readily hydrolyzed, but such a compound was available, by a devious route, in IX, which after acetylation gave de(hydrochloro)senegenin diacetate monomethyl ester. Conversion of this to the acid chloride, followed by pyrolysis, with loss of carbon monoxide and hydrogen chloride, gave the desired aromatic product represented now in terms of the completed structure as (X). Some difficulty was experienced in the purification of X until it was found that brief treatment with ozone after partial separation removed some olefinic contaminant.



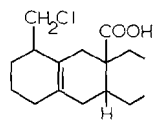
X



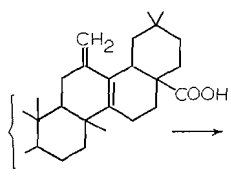
XI



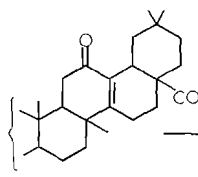
XIIa



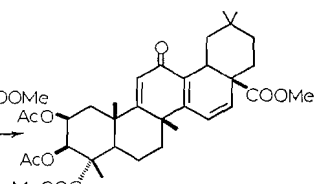
XIIb



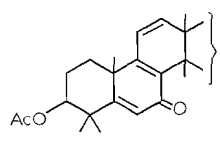
XIII



XIV



XV



XVI

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

The nature of X rests on the following evidence, other than its mode of genesis. It has the ultraviolet absorption of an alkylated benzene (λ_{max} 269 mμ). Two, and only two, aromatic protons are present in the n.m.r. spectrum as a near singlet at τ 3.05 (indicating their nearly identical environment). All four remaining positions of the benzenoid ring must be occupied by carbon (there is no aromatic methyl group) and represent the points of fusion of the adjacent rings. Part-structure (VIII) can therefore be expanded to (XI) or (XII*a*, *b*). Of these (XII*a*) may be excluded since (IX), aside from the mechanistic requirements of its genesis, does not have the properties of a β,γ-unsaturated acid.

Although there were suggestions that (XI), and therefore (X), was correct (the vinylic proton in (IX) was not a clean doublet, for instance) this sequence of reactions did not provide unequivocal evidence which allowed a decision between (XI) and (XII*b*) to be made.

Whilst the conversion of (II) to (III) under the influence of quinoline at high temperatures could be envisaged (the quinoline hydrochloride could supply a proton for a prototropic shift) the same change under the action of dilute sodium hydroxide would be impressive. The action of base was re-investigated and, indeed, in alkaline solution a new heteroannular diene could be observed. This was partly, or wholly, destroyed by casual acidification but by careful adjustment of pH to near 6 with phosphate buffer and extraction followed by esterification, an isode(hydrochloro)senegenin dimethyl ester could be obtained. Rearrangement into the de(hydrochloro) series was easily effected at room temperature with hydrochloric acid in dioxane.

Isode(hydrochloro)senegenin (XIII) showed the presence of the expected exocyclic methylene group by exhibiting bands at τ 5.13, 5.40. Ozonolysis gave, in agreement, an α,β-unsaturated ketone (λ_{max} 253 mμ) (XIV). Reduction of this gave an allylic alcohol which was readily dehydrated to a heteroannular diene absorbing at shorter wavelength than in the de(hydrochloro) series. Acetylation of the unsaturated ketone gave the expected keto-diacetate which was characterized as the allylic alcohol.

This conjugated ketone was a suitable substance for the introduction of further unsaturation in a classical procedure for structural elucidation. Bromination of the keto-diacetate resulted in the uptake of two atoms of bromine. Direct dehydrobromination of the crude dibromo compound with lithium chloride in dimethyl formamide then gave a trienone (XV).

The ultraviolet absorption spectrum of XV was characteristic of a cross-conjugated carbonyl system which, to a first approximation, shows the absorption of the separate chromophores. In the present case, excellent analogy was available in the lanosterol derivative (XVI) (5) which showed λ_{max} 258, 326 mμ (ϵ 8 500 and 8 500) as against λ_{max} 260, 320 mμ (ϵ 8 200, 7 200) for (XV) (see also (14)). The n.m.r. spectrum of (XV) showed all the signals to be expected of such a molecule. In particular, five singlets for the methyl groups were observable (Fig. 1) as were appropriate signals for three vinyl protons, two of which were strongly coupled. One of the latter shows weak long-range coupling probably with the methine proton at C_{18}.

The existence of the chromophore also requires that a blocking group, represented in (XV) by the C_8 methyl group, be present to prevent the formation, by tautomerism, of a phenol.

The summation of the part-formula (1) and the requirements for senegenin itself implied by (XV) leads, most plausibly, to the structure (XVII) for senegenin, where the weaker lines are those for which no direct evidence has been adduced. It should be noted that, in fact, only six carbon atoms have not been specifically located, and that of these three are

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

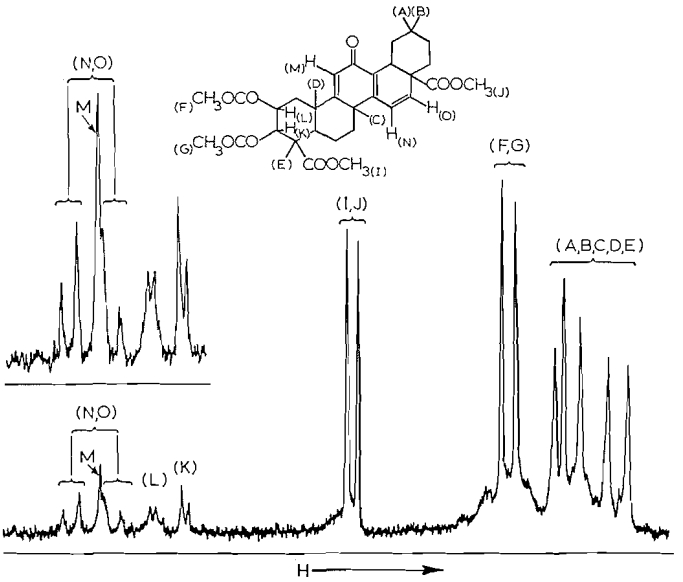
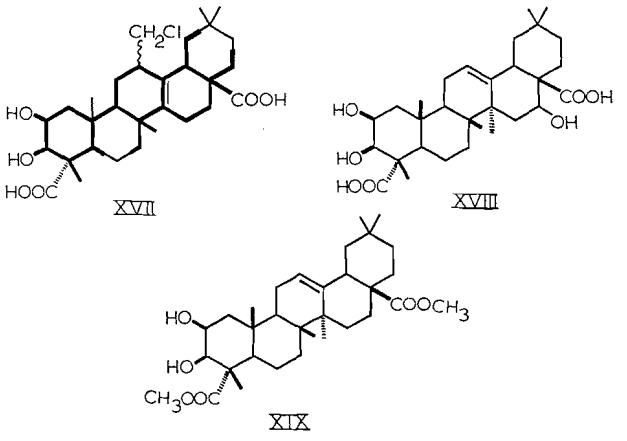


FIG. 1. Nuclear magnetic resonance spectrum of trienone (XV), with expanded inset of the vinyl region.

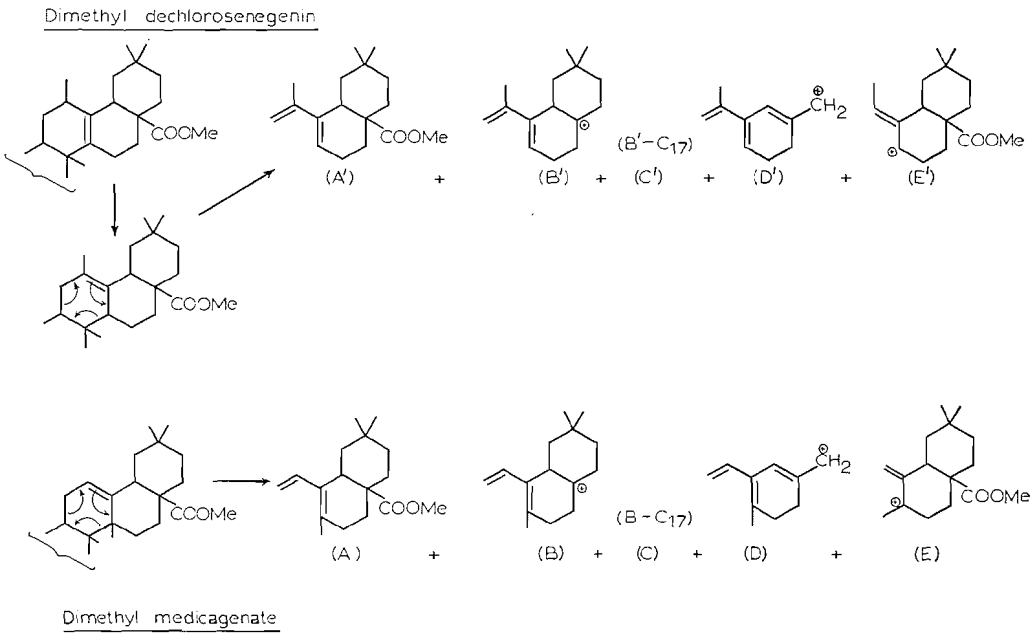
methyl groups attached to quaternary carbon. The formulation (XVII), that of a simply modified oleanane derivative, acquired added verisimilitude with knowledge that polygalacic acid, (XVIII) another oleanane derivative, has recently been isolated and characterized from a related species of polygala, *Polygala paenea* L., by Polonsky and her collaborators (7).



In the hope of obtaining direct evidence for the original ring system in senegenin, and in particular the location of the double bond in it, the mass spectrum of dechlorosenegenin dimethyl ester was obtained. For comparison that of dimethyl medicagenate (XIX) was also recorded.

Dimethyl medicagenate showed peaks at *m/e* values to be expected for an oleanolic acid derivative. These peaks, following the interpretation of Budzikiewicz, Wilson, and Djerassi (8), and irrespective of the accuracy of the interpretation of the sources of certain

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.



SCHEME 1. Breakdown scheme for dimethyl dechlorosenegenin and dimethyl medicagenate.

of these peaks, are to be attributed to the fragments illustrated in Scheme 1 (A–E). In this sense the pattern fully confirms the suggested importance of the retro-Diels–Alder reaction in this series, with the retention of the charge in the D/E system.

The spectrum of dechlorosenegenin dimethyl ester up to $m/e \sim 300$ is shown in Fig. 2. Comparison with the spectrum of dimethyl medicagenate shows a striking similarity in the ratios and, with one exception, an identity of position of the more important peaks. The only important difference is the presence, in dechlorosenegenin dimethyl ester, of an additional peak at 241.

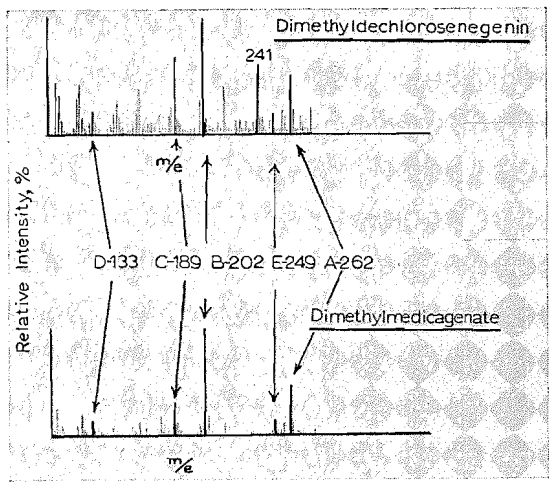
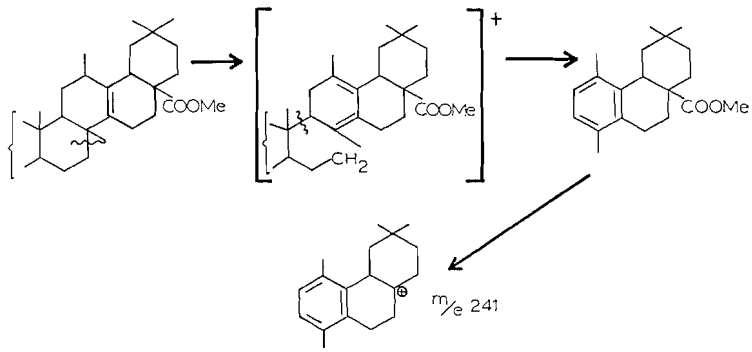


FIG. 2. Mass spectra of dimethyl dechlorosenegenin and dimethyl medicagenate.

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

This impressive identity would suggest (8) that senegenin belonged to the Δ^{12}-unsaturated oleanene or ursene series, a conclusion which would be, in view of the facts already presented, totally unacceptable. This seeming contradiction, however, serves to complete the structure of senegenin.

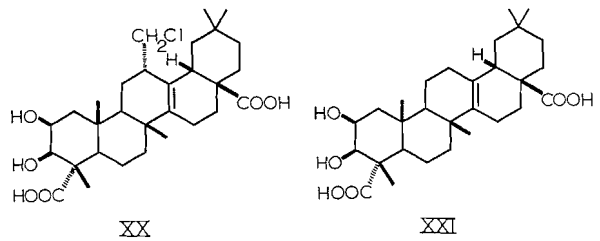
If it be assumed that after impact the ion produced may transfer the hydrogen atom at C_{12}, either in one or two stages, to C_{14} then (without charge indication) a series of fragments (A′–E′) may be written differing from, but isomeric with, the series (A–E) from dimethyl medicagenate, which will produce a series of peaks of identical *m/e* with those of that series.* If it be assumed, as appears reasonable, that the retro-Diels–Alder is energetically the most desirable mode of fragmentation other modes may be sufficiently slow to permit hydrogen transfer and breakdown by this route.



SCHEME 2. Possible origin of the peak at *m/e* 241 in the mass spectrum of dimethyl dechlorosenegenin.

The remaining feature requiring explanation is the peak at *m/e* 241 in the spectrum of dechlorosenegenin dimethyl ester. Since this has no equivalent in dimethyl medicagenate it could arise from fragmentation in the unrearranged molecule. Such a scheme is represented in Scheme 2. If this interpretation be correct the peak at *m/e* 241 should be independent of substitution in ring A. The spectrum of dechlorosenegenin diacetate was accordingly obtained and the same series of peaks, including that at *m/e* 241 was observed.†

These observations suffice, therefore, to permit the attribution of the structure (XX) to senegenin. No evidence has been provided for the stereochemistry of the methyl group at C_8, but it seems probable that it is β as in all known members of the oleanene series.



XX XXI

The stereochemistry of the chloromethyl group will have been determined by the mechanism of transfer of what was evidently the C_{14} methyl in a precursor to C_{12}. On the assumption, for the moment, that the migration is intramolecular, then the chloromethyl

Thermal rearrangement prior to ionization is also conceivable.
†*Mass spectra by Dr. R. Ryhage, Karolinska Institutet, Stockholm, Sweden.*

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

group surely lies on the same face of the molecule as did the original methyl group (or equivalent); that is, α. The configuration at C_{18} follows from the relationship to polygalic acid (see below).

At this point it was possible to direct attention to the structure of pre-senegenin the presumed precursor of senegenin. The structure of polygalic acid (XXI), the substance (as the monoethyl ester) accompanying senegenin when prepared by the normal isolation procedure, has been determined by conversion into the unsaturated ketone (XIV) (9). From other evidence Professor Pelletier and his colleagues have arrived at the same conclusion (10).* It seemed most probable that the carbon atom transferred in the formation of senegenin from pre-senegenin was lost in the concomitant formation of polygalic acid by a reverse Prins reaction or some equivalent on a different oxygen level.

The saponin from *polygala senega*, as already demonstrated, contains no halide (1), but attempts to hydrolyze the saponin under milder acid conditions in an attempt to isolate pre-senegenin were, in our hands, unavailing. It seemed possible, as an alternative approach, that preliminary cleavage with periodic acid might produce carbonyl functions in a suitable position for activated release of the ethereal linkage retaining the sapogenin. The cleavage might then be induced by the action of base through some process such as β-elimination. Even if not suitably placed, then, by ketol equilibration, the carbonyl function might reach such a position.

Whilst the details of the reaction are unknown the overall result was that desired. The saponin, after cleavage with sodium metaperiodate (the excess oxidant being destroyed reductively) was heated with 5% potash under nitrogen. From this medium, after adjustment to pH $\sim$ 3, the sapogenin could be extracted and was obtained crystalline as the dimethyl ester. It had the empirical formula $C_{32}H_{50}O_7$ and on acetylation gave a triacetate. By another chromatographic procedure, pre-senegenin was itself obtained crystalline. The material was accompanied by *p*-coumaric acid identified by comparison with an authentic specimen.†

When pre-senegenin was heated with ethanolic hydrochloric acid it was converted in high yield into a mixture of senegenin and polygalic acid which was separated, after methylation, and the individual components identified spectroscopically and, in the case of dimethyl polygalate, also by mixed melting point. The new sapogenin was, therefore, the sought-for precursor.

Pre-senegenin differed in composition from polygalic acid by the elements of formaldehyde. If the concept of a reverse Prins were correct, formaldehyde itself should be eliminated during the conversion of pre-senegenin into polygalic acid. By conducting the acid treatment in the presence of 2,4-dinitrophenylhydrazine the 2,4-dinitrophenylhydrazone of formaldehyde could, in fact, be isolated. The yield of polygalic acid remained unaffected by the presence of the reagent as it was when the acid treatment was conducted in the presence of a large excess of formaldehyde. The structure of pre-senegenin could then be represented by (XXII) or (XXIII), the former being a normal derivative of the oleanene series whilst (XXIII) would be that of a member of taraxerene series. In either case, the geometry of ring C, it was considered, should be such as to facilitate a Cope rearrangement through a six-center transition state including C_{12}, C_{13}, C_{14}, and the primary hydroxyl group (as in (XXIV)) with the eventual transfer of the hydroxyl proton to C_{12} and the

Professor Pelletier has used the term 'senegenic acid' for polygalic acid. Aside from observing the slight confusion possibly engendered between, for instance, dimethyl senegenin and dimethyl senegenate, we have no particular preference. The term 'polygalic acid' coined by Shamma and Irwin (18) was used in our earlier publication and we use it here for simplicity.

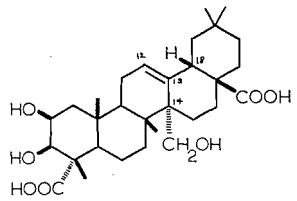
†*We thank Dr. A. Stoessl (Department of Agriculture) for a specimen of this substance.*

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

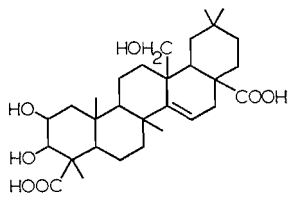
liberation of formaldehyde. Under these conditions untoward migration of the double bond prior to the reverse Prins could be discounted, a possibility which could not be excluded in the more vigorous acid treatment.

At 220° the dimethyl ester of pre-senegenin smoothly underwent the desired rearrangement and entrainment of the volatile material into aqueous dimedone gave a 73% yield of the dimedone derivative of formaldehyde. The other product was identified as dimethyl polygalate. Since, in this transformation, no possibility of inversion at C_{18} is presented this must be the same in polygalic acid as in pre-senegenin.

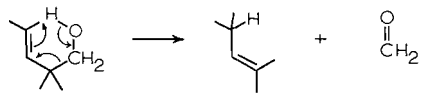
The identity of pre-senegenin as either (XXII) or (XXIII) was thus confirmed. In agreement there was a single vinyl proton in the n.m.r. spectrum of the pre-senegenin esters. The rearrangement just discussed offered a simple means of distinguishing between these formulations. Equilibration of pre-senegenin dimethyl ester with deuterium oxide in dioxane converted the hydroxyl proton in (XXIV) to a deuteron. Removal of the solvents and pyrolysis in a sealed tube under high vacuum gave after crystallization dimethyl polygalate, as previously, in which deuterium (1.4% excess) had been incorporated. Since the incorporation of one deuterium atom (at C_{12} or C_{15}) should have resulted in about 2% excess deuterium, inefficient exchange or insufficiently vigorous exclusion of water may be responsible for its incompleteness.



XXII



XXIII



XXIV

To distinguish between structures (XXII) and (XXIII) for pre-senegenin it was necessary only to ascertain the position of the introduced deuteron. Oxidation of the deuteriopolygalate ester diacetate to the unsaturated ketone (XIV) gave material containing no excess deuterium. The deuteron was therefore required to be at C_{12} unless during the oxidation, by equilibration with the solvent, a deuteron had been removed from C_{15}. This was intrinsically unlikely since this would require enolization, and such an enol would be expected to be trapped by the oxidant to give the transoid cross-conjugated ene-dione. However, the possibility was rendered even more remote by performing an equivalent oxidation of dimethyl polygalate in chromium trioxide – acetic acid-*d*. No deuterium was incorporated into the unsaturated ketone obtained. The structure of pre-senegenin is thus (XXII); bathetically, that of a normal oleanene triterpenoid.

Three points remain for discussion. Under the original isolation procedure (4) senegenin was obtained largely as the free dicarboxylic acid whilst polygalic acid was obtained in a partly esterified condition. Since normal oleanane derivatives would not be expected to be

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

esterified significantly under Fischer-Speier conditions, some explanation is required. The simplest is that the change in geometry implied by the introduction of a C_{13-14} ethylenic linkage may reduce hindrance to a point where partial esterification may take place under, what are for this type of procedure, forcing conditions. In agreement, use of milder conditions for the transformation of pre-senegenin gave senegenin and polygalic acid which were only $\sim 10\%$ esterified.

Treatment of polygalic acid diacetate and senegenin monoacetate with refluxing methanolic hydrochloric acid (2.4 *N*) for 6 h resulted in $\sim 100\%$ and 24% esterification respectively, as estimated by n.m.r. measurements of the methoxyl signal. Under the same conditions oleanolic acid was 7% esterified. The difference in rate between senegenin and polygalic acid may be attributed to interaction of the bulky chloromethyl group with ring E resulting in slight torsional movement of the 17-carboxyl towards ring C and somewhat increased difficulty of steric access.

Secondly, since the configuration at C_{18} in pre-senegenin is presumably β as in other oleanene derivatives this configuration must obtain in polygalic acid (because of relationship through the Cope rearrangement) and in senegenin (because of its relationship to polygalic acid through the unsaturated ketone (XIV)).

The last point, the mechanism of the evidently intramolecular conversion of pre-senegenin to senegenin is discussible in terms of cyclobutonium ions, but such discussion will be deferred until present investigations in this area are completed. It should be noted that the solvolysis of the system present in pre-senegenin has been studied (for instance refs. 11, 12) and has in fact been used for the synthesis of the triterpenoid phyllanthol (13). However, these studies were performed under essentially irreversible conditions.

The isolation of substances apparently closely related to senegenin (15–17) from *Polygala* species suggests that these, too, contain substances of the type of pre-senegenin.

NOTE ADDED IN PROOF: Professor Pelletier has very recently reported* the formation of a cyclosenegenin by the action of alkali on senegenin: a reaction with ample analogy. With hydrochloric acid senegenin (but not polygalic acid) is formed. The title of the communication requires that cyclosenegenin be present in *Polygala senega*, and the text suggests that it may be the precursor. We have also noted the formation of cyclosenegenin, but an n.m.r. study of the saponin failed to show any signals near τ 10.0 of an intensity sufficient to render possible the presence of a combined cyclosenegenin.

EXPERIMENTAL

Unless otherwise stated the following are implied: light petroleum refers to the fraction of b.p. 60–80°, rotations and infrared spectra are in chloroform solution, ultraviolet spectra are in 95% ethanol solution, and melting points were determined in evacuated capillaries. Thin-layer chromatography was done using Camag silica gel DF-5 with fluorescent indicator. The n.m.r. spectra were determined in deuteriochloroform using 1% tetramethylsilane as internal standard using a Varian D.P. 60 spectrometer (peak positions being determined using an audio oscillator calibrated with a Hewlett Packard 522-B frequency counter) or a Varian A-60. Band positions are in tau (τ) units.

Senegenin

The Anhydro Lactone Methyl Ester Diacetate (VI)

Dechlorosenegenin diacetate (290 mg) was heated in acetic acid (10 ml) containing hydrogen peroxide (4.5 ml, 30%) at 85° for 1.5 h. Dilution with water and extraction into ether followed by methylation (diazomethane) gave, after thin-layer chromatography (t.l.c.) (eluant: ether : isopropyl ether, 3:7) and extraction of the appropriate band, the hydroxy lactone (V) as an oil (250 mg), $[\alpha]_D$ +38° (*c*, 2.05), ν_{max} 3 500, 1 767, 1 742 cm^{-1}, n.m.r. bands at 9.09, 9.05, 8.85, 8.63, 8.07 (3H, singlet), 7.96 (3H, singlet), 6.32 (3H, singlet), 4.64 (2H, multiplet), which was used directly for dehydration.

The hydroxy lactone (115 mg) was dissolved in dry pyridine (8 ml), thionyl chloride (0.5 ml, purified)

* *Y. Shimizu and S. W. Pelletier. J. Am. Chem. Soc. 87, 2065 (1965).*

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

added dropwise with stirring, and the solution allowed to stand at room temperature for 1 h. Pouring into sodium acetate (75 ml, 10%) with stirring, extraction into ether, and washing with dilute hydrochloric acid to remove pyridine followed by careful evaporation in the cold gave crystalline anhydro lactone. Crystallization from methanol (in the cold) gave pure material, m.p. 187–192° (decomposition, rapid heating), m.p. 180–185° (decomposition, slow heating), $(\alpha)_D$ +50° (*c*, 1.94), ν_{max} 1 742 cm^{-1} (broad band), λ_{max} 200 (ϵ 5 000), n.m.r. bands at 8.98, 8.77, 8.57, 8.04 (3H, singlet), 7.94 (3H, singlet), 6.34 (3H, singlet), and near 4.7 (3H, complex multiplet).

Anal. Calcd. for $C_{35}H_{50}O_8$: C, 70.20; H, 8.42. Found: C, 69.77; H, 8.31.

Isoanhydro Lactone Ester Diacetate (VII)

Anhydro lactone ester diacetate (60 mg) was refluxed for 35 min in methanol (25 ml). Evaporation of the solvent and crystallization from methanol, after separation by t.l.c. (eluant: ether:benzene, 1:1), gave the isoanhydro lactone ester diacetate (30 mg), m.p. 223–225°, $[\alpha]_D$ +10° (*c*, 1.20), ν_{max} 1 745 cm^{-1} (broad band), ν_{max} 1 773, 1 745 cm^{-1} (dioxane), n.m.r. bands at 9.05, 8.93, 8.88, 8.76, 8.61, 8.05 (3H, singlet), 7.96 (3H, singlet), 6.35 (3H, singlet), 5.19 (1H, doublet $J \sim 5$ c.p.s.), 4.62 (2H, multiplet).

Anal. Calcd. for $C_{35}H_{50}O_8$: C, 70.20; H, 8.42. Found: C, 69.98; H, 8.34.

Isoanhydro Lactone Ester

Anhydro lactone ester diacetate (115 mg) was refluxed for 10 min in methanolic potassium hydroxide (15 ml, 5%) under nitrogen. Dilution with water and extraction into ether followed by crystallization from acetone gave the isoanhydro lactone ester (90 mg), m.p. 215–219°, $[\alpha]_D$ −4° (*c*, 1.32), ν_{max} 3 500, 1 760, 1 721 cm^{-1}, n.m.r. bands at 9.02, 8.91, 8.85, 8.81, 8.75, 8.65, 6.28 (3H, singlet), 5.90 (2H, multiplet), 5.17 (1H, doublet $J \sim 5$ c.p.s.).

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 71.70; H, 8.63.

Isoanhydro lactone ester (50 mg) was acetylated (pyridine – acetic anhydride, 3:1) by heating for 2.5 h at 90° under nitrogen. Isolation of the product and crystallization from methanol gave a diacetate, m.p. 223–225°, $[\alpha]_D$ +10° (*c*, 1.20) showing no melting point depression on admixture with a sample prepared by refluxing the unrearranged lactone in methanol and having an n.m.r. spectrum identical with that sample

Monomethyl(A) De(hydrochloro)senegenin (IX)

Isoanhydro lactone ester (30 mg) was allowed to stand at room temperature for 15 min in methanol – hydrochloric acid (5%). Isolation of the product and crystallization from acetone gave a quantitative yield of monomethyl (A) de(hydrochloro)senegenin. Recrystallization from acetone gave material which softens at 200°, resolidifies, and melts at 299–301°, $[\alpha]_D$ +71° (*c*, 1.05), λ_{max} 242, 249, 258 mμ (ϵ 14 000, 16 100, 10 100), n.m.r. bands at 9.21, 9.06, 8.95, 8.75, 8.63, 8.23, 6.31 (3H each, all singlets), 5.96 (2H, multiplet), 4.66 (1H, multiplet).

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 71.74; H, 8.82.

Methylation with diazomethane and crystallization from acetone gave de(hydrochloro)senegenin dimethyl ester, m.p. 234–235°, $[\alpha]_D$ +82° (*c*, 1.12), showing no melting point depression on admixture with an authentic sample and having an n.m.r. spectrum identical with that of the authentic material.

Monomethyl (A) de(hydrochloro)senegenin (140 mg) was acetylated (pyridine – acetic anhydride, 3:1) by heating 2.5 h at 95° under nitrogen. Isolation of the product and crystallization from acetone – light petroleum gave a diacetate, m.p. 175–180°, $[\alpha]_D$ +60° (*c*, 1.43), ν_{max} 1 739, 1 695 cm^{-1}, λ_{max} 242, 249, 256 mμ (ϵ 15 000, 16 900, 11 700), n.m.r. bands at 9.22, 9.08, 8.97, 8.80, 8.58, 8.25, 8.05, 7.95, 6.35 (3H each, all singlets), 4.56 (3H, multiplet).

Anal. Calcd. for $C_{35}H_{50}O_8$: C, 70.20; H, 8.42. Found: C, 69.60; H, 8.11.

Acid Chloride (IX, COCl Instead of COOH) Formation and Pyrolysis

Monomethyl (A) de(hydrochloro)senegenin diacetate (57 mg) was refluxed in a dry atmosphere for 45 min in carbon tetrachloride (10 ml, dried) containing thionyl chloride (1 ml). Evaporation to dryness gave an oily acid chloride, ν_{max} 1 745, 1 792 cm^{-1}, which on contact with wet solvents was hydrolyzed to the corresponding acid. The freshly made acid chloride (350 mg total) was heated in five equal portions under nitrogen at 265° for 30 min. Separation by t.l.c. (eluant: ether:benzene, 3:7) gave a major portion (130 mg) (R_f near 0.5) which was treated in ethyl acetate solution with a stream of ozone for 5 min at −70°. Nitrogen was then blown through (15 min) to remove excess ozone. Reseparation by t.l.c. and crystallization from methanol gave the pure benzene derivative (X) (53 mg), m.p. 302–303°, ν_{max} 1 739 cm^{-1}, $[\alpha]_D$ +50° (*c*, 0.91), λ_{max} 269 (ϵ 535), n.m.r. bands at 9.06, 9.00, 8.89, 8.85, 8.58, 8.02, 7.91, 6.35 (3H each, all singlets), 4.6 (2H, multiplet), 3.05 (2H, broad singlet).

Anal. Calcd. for $C_{34}H_{48}O_6$: C, 73.88; H, 8.75. Found: C, 73.49; H, 8.96.

Isode(hydrochloro)senegenin Dimethyl Ester

Senegenin monoacetate (495 mg) was refluxed for 18 h under nitrogen in aqueous alcoholic (1:3) sodium hydroxide (85 ml, 5%). Dilution with water and neutralization with saturated KH_2PO_4 solution to pH ~ 6 and extraction into ether gave material which, after methylation (diazomethane) and crystallization from methanol, gave isode(hydrochloro)senegenin dimethyl ester (XIII) (250 mg), m.p. 228–230°, $[\alpha]_D$ +23° (*c*, 1.17), λ_{max} 249 mμ (ϵ 13 100), ν_{max} 3 500, 1 715, 1 616, 1 592 cm^{-1}, n.m.r. bands at 9.13, 8.98, 8.83, 8.70, 6.48 (3H, singlet), 6.36 (3H, singlet), 6.0 (2H, multiplet), 5.40 (1H, singlet), 5.13 (1H, singlet).

Anal. Calcd. for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15. Found: C, 72.75; H, 9.43.

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

Rearrangement of Isode(hydrochloro)senegenin Dimethyl Ester

The ester (50 mg) was allowed to stand for 15 min at room temperature in dioxane (5 ml) containing hydrochloric acid (0.5 ml, concentrated). Isolation of the product and crystallization from methanol gave de(hydrochloro)senegenin dimethyl ester, melting point and mixed melting point 221–223°, λ_{max} 243, 249, 258 mμ (ϵ 12 700, 14 700, 10 300). The n.m.r. spectrum was identical with that of an authentic sample.

The α,β-Unsaturated Ketone (XIV)

Isode(hydrochloro)senegenin dimethyl ester (99 mg) was dissolved in methanol (5 ml), the solution cooled to −70°, and ozone bubbled in for 2 min. Nitrogen was blown through the solution for 15 min and acetic acid (0.5 ml) and potassium iodide (0.5 ml, 50% aqueous) was added. The solution was then allowed to warm up to room temperature and after 30 min it was poured into dilute sodium thiosulfate (5%) and extracted into ether. Separation by t.l.c. (eluant: ether:benzene, 3:7) and crystallization from methanol gave the α,β-unsaturated ketone (32 mg), m.p. 228–230°, $[\alpha]_D$ +41° (*c*, 1.38), λ_{max} 253 mμ (ϵ 9 400), ν_{max} 3 500, 1 721, 1 653, 1 613 cm^{-1}.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74. Found: C, 70.81; H, 8.50.

The ketone (90 mg) was acetylated (pyridine – acetic anhydride, 3:1) by heating for 3 h at 95° under nitrogen. Extraction into ether and crystallization from methanol gave a diacetate (85 mg), m.p. 245–247°, $[\alpha]_D$ +46° (*c*, 1.88), λ_{max} 253 mμ (ϵ 9 400), ν_{max} 1 745, 1 661, 1 613 cm^{-1}, n.m.r. bands at 8.97, 8.78, 8.62, 8.06, 7.94, 6.44, 6.33 (3H each, all singlets), 4.6 (2H, multiplet).

Anal. Calcd. for $C_{35}H_{50}O_9$: C, 68.38; H, 8.20. Found: C, 68.15; H, 7.89.

Reduction of the α,β-Unsaturated Ketone

The unsaturated ketone dimethyl ester diacetate (72 mg), in three equal portions, was allowed to stand at room temperature for 20 min in methanol (5 ml) containing sodium borohydride (120 mg). The product was poured into dilute acetic acid, neutralized with sodium carbonate (5%), and extracted into ether. Crystallization from methanol gave an alcohol, m.p. 223–224°, $[\alpha]_D$ +33° (*c*, 1.17), λ_{max} 205 mμ (ϵ 7 000), ν_{max} 3 450, 1 733 cm^{-1}. The compound had n.m.r. bands at 9.06 (~ 9H, singlet), 8.86 (3H, singlet), 8.66 (3H, singlet), 8.10 (3H, singlet), 7.97 (3H, singlet), 6.36 (6H, singlet), 5.84 (1H, multiplet), 4.58 (2H, multiplet).

Anal. Calcd. for $C_{35}H_{52}O_9$: C, 68.15; H, 8.50. Found: C, 68.56; H, 8.61.

Norde(hydrochloro)senegenin Dimethyl Ester

The unsaturated ketone dimethyl ester (19 mg) was allowed to stand at room temperature for 15 min in methanol (4 ml) and sodium borohydride (89 mg). The product was isolated as above and dehydrated by warming on a steam bath for 15 min in methanolic hydrochloric acid (10 ml, 5%). Isolation and crystallization from methanol gave the diene (15 mg), m.p. 200–210°, $[\alpha]_D$ +58° (*c*, 1.40), λ_{max} 238, 246, 254 mμ (ϵ 11 800, 13 800, 9 750), n.m.r. bands at 9.17, 9.10, 9.02, 8.77, 8.65, 6.46, 6.31 (3H each, all singlets), 5.99 (2H, multiplet), 5.60 (2H, multiplet).

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 71.91; H, 9.00.

Bromination of the Unsaturated Ketone

The unsaturated ketone dimethyl ester diacetate (16.0 mg) was dissolved in 10 ml of a bromine solution (232 mg Br_2 / ml of acetic acid containing 0.05% HBr). Aliquots (1 ml) were withdrawn and dropped into a solution containing potassium iodide (1 ml, 5%), water (5 ml), thiodene indicator (1 ml, 5% aqueous) and titrated with standard *N*/100 sodium thiosulfate. After 90 min the uptake was 1.99 atoms of bromine which increased to 2.05 atoms after a further 90 min.

Preparation of the Trienone (XV)

The unsaturated ketone dimethyl ester diacetate (175 mg) was brominated by allowing it to stand for 2.25 h at room temperature in a mixture of acetic acid (10 ml) and a bromine solution (4 ml, acetic acid containing 46 mg Br_2 / ml and 0.05% HBr). Pouring into sodium thiosulfate solution (25 ml, 10%) and neutralization with bicarbonate (5%), followed by extraction into ether and evaporation in the cold gave the crude dibromo compound (212 mg).

The material so obtained was heated at 105–110° under nitrogen for 5.25 h in dimethyl formamide (12 ml) containing lithium chloride (180 mg). Dilution with water, extraction into ether, and separation by t.l.c. (eluant: ether:benzene, 1:3) followed by crystallization from methanol gave the trienone (88 mg), m.p. 312–314°, $[\alpha]_D$ +136° (*c*, 1.44), λ_{max} 260, 320 mμ (ϵ 8 200, 7 200), ν_{max} 1 742, 1 650, 1 623, 1 570 cm^{-1}. The n.m.r. spectrum showed bands at 9.16, 8.97, 8.68, 8.52, 8.43, 8.05, 7.91, 6.47, 6.36 (3H each, all singlets), 4.73 (1H, doublet $J \sim 4.5$ c.p.s.), 5.40 (1H, complex multiplet), 3.88 (1H, singlet), 3.77 (2H, AB pattern, $J_{AB} = 10$ c.p.s., $\delta_B - \delta_A = 20.7$ c.p.s.).

Anal. Calcd. for $C_{35}H_{46}O_9$: C, 68.83; H, 7.59. Found: C, 69.11; H, 7.55.

Pre-senegenin

Cleavage of the Saponin and Isolation of the Sapogenin

The saponin (30 g) was dissolved in water (1 l) and sodium metaperiodate (41 g) was added over a period of ½ h while the solution was being stirred and cooled in an ice bath. The solution was allowed to stand in the dark at room temperature for 24 h. Then while the solution was stirred, potassium iodide was added (20 g) and then sodium arsenite until the iodine color disappeared. The mixture was neutralized with solid potassium hydroxide, and then an additional 50 g were added. The solution was heated at 100° under

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

nitrogen with stirring for 1 h, cooled, and carefully acidified with hydrochloric acid to pH ~ 3. Extraction with ether gave 4.5 g of crude sapogenin. Methylation (diazomethane) and chromatography on silica gel (100 g, BDH) gave a crystalline sapogenin ester (1.5 g). Several recrystallizations from ethyl acetate gave the sapogenin dimethyl ester, m.p. 214–215°, $[\alpha]_D$ +78° (*c*, 2.22), ν_{max} 3 500, 1 718 cm^{-1}, n.m.r. bands at 9.37, 9.12, 9.07, 8.82, 8.68, 6.38, 6.32 (3H each, all singlets), 5.98 (2H, multiplet), 4.16 (1H, multiplet).

Anal. Calcd. for $C_{32}H_{50}O_7$: C, 70.30; H, 9.22. Found: C, 70.42; H, 9.28.

Acetylation of Pre-senegenin Dimethyl Ester

The ester was acetylated (pyridine – acetic anhydride, 3:1) by heating at 95° for 3 h under nitrogen. Evaporation and extraction into ether gave a triacetate which, on crystallization from methanol, had m.p. 219–220°, $[\alpha]_D$ +110° (*c*, 2.32), ν_{max} 1 727, 1 739 cm^{-1}, n.m.r. bands at 9.30, 9.13, 9.07, 8.80, 8.60, 8.07, 7.98, 7.96, 6.26, 6.23 (~ 3H each, all singlets), 5.90 (2H, broad singlet), 4.56 (3H, complex multiplet).

Anal. Calcd. for $C_{38}H_{50}O_{10}$: C, 67.83; H, 8.39. Found: C, 67.85; H, 8.45.

Isolation of Pre-senegenin

The crude sapogenin (1.4 g) was separated by t.l.c. (eluant: acetic acid : chloroform, 1:4) to give 450 mg of sapogenin. Crystallization from aqueous alcohol (care being taken not to heat the solvent excessively) gave pure pre-senegenin, m.p. 310–311°, $[\alpha]_D$ +91° (*c*, 1.16, MeOH).

Anal. Calcd. for $C_{30}H_{46}O_7 \cdot 0.5H_2O$: C, 68.28; H, 8.98. Found: C, 67.95; H, 8.82.

Identification of p-Methoxy Cinnamic Acid

Chromatography of the methylated crude sapogenin mixture on silica gel (BDH) yielded, besides the terpenoid ester, *p*-methoxy methyl cinnamate as identified by melting point, mixed melting point, and infrared spectrum. Separation of the crude sapogenin gave, besides the sapogenin, *p*-methoxy cinnamic acid as identified by melting point and mixed melting point.

Conversion of Pre-senegenin to Senegenin and Polygalic Acid

The sapogenin (167 mg) was refluxed for 1.25 h in a mixture of ethanol (10 ml), water (3 ml), and hydrochloric acid (1.35 ml, concentrated). Dilution with water and extraction into ether followed by re-extraction into aqueous sodium bicarbonate (5%) gave neutral (17 mg) and bicarbonate-soluble material (140 mg). Methylation of the latter with diazomethane and separation on t.l.c. (eluant: ether:benzene, 3:1) gave senegenin dimethyl ester (61 mg), $[\alpha]_D$ +14° (*c*, 1.12) and dimethyl polygalate (50 mg), m.p. 211–212°, $[\alpha]_D$ +23° (*c*, 1.11). The dimethyl senegenin was identified by comparison of the n.m.r. spectrum, infrared spectrum, and R_F (t.l.c.) with an authentic specimen. The dimethyl polygalate was identified by melting point and mixed melting point determination with an authentic specimen prepared from polygalic acid by esterification with diazomethane. Crystallized from methanol this had m.p. 211–212°, $[\alpha]_D$ +22° (*c*, 1.00).

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.62; H, 8.93.

No other product could be detected by t.l.c.

Treatment of Pre-senegenin Dimethyl Ester with HCHO:HCl

(*a*) The ester (4 mg) was refluxed 1.25 h in a mixture of ethanol (1.5 ml), water (0.5 ml), and hydrochloric acid (0.2 ml, concentrated). The product was diluted with water and extracted into ether.

(*b*) The ester (4 mg) was refluxed 1.25 h in a mixture of ethanol (1.5 ml), water (0.18 ml), formaldehyde (0.32 ml, 3.7% aqueous solution), and hydrochloric acid (0.2 ml, concentrated). The product was isolated as above.

Running a series of spots of various concentrations, from the above experiments, using t.l.c. (eluant: ether:benzene, 3:1) indicated no detectable difference in the ratio of senegenin ester to polygalic acid ester in the two experiments (the plates were sprayed with 50% H_2SO_4 and charred).

Pyrolysis of Pre-senegenin Dimethyl Ester

The ester (75 mg) was heated at 220° for 30 min under a stream of nitrogen that bubbled into an aqueous dimedone solution (40 ml, 0.45%). A precipitate formed immediately. The solution was allowed to stand for 2 h and the precipitate collected. The product (29 mg, 73% yield) was identified as the dimedone derivative of formaldehyde by melting point and mixed melting point comparison with an authentic sample; t.l.c. (eluant: ether:benzene, 3:1) showed only one pyrolysis product which, on crystallization from methanol, was identified as dimethyl polygalate (57 mg) by melting point, mixed melting point, and rotation.

Acid Treatment of Pre-senegenin Dimethyl Ester in the Presence of 2,4-Dinitrophenylhydrazine

Pre-senegenin dimethyl ester (86 mg) and 2,4-dinitrophenylhydrazine (89 mg) were refluxed for 2.5 h in a mixture of methanol (13 ml), water (4 ml), and hydrochloric acid (2 ml, concentrated). Isolation of the products and separation by t.l.c. (eluant: chloroform) gave formaldehyde 2,4-dinitrophenylhydrazone (4.7 mg) identified by melting point and mixed melting point comparison with an authentic sample.

*Deuterium Experiments**

Pre-senegenin dimethyl ester (215 mg) was dissolved in dried dioxane (1 ml), D_2O (10 drops, 99.65%) added, and the mixture evaporated to dryness. This process was repeated three times, the tube sealed under high vacuum and heated at 220° for 20 min. The product was separated by t.l.c. (eluant: ether:benzene, 3:1) and crystallized from methanol to give dimethyl polygalic acid-d_1 (106 mg).

**Deuterium analyses by Josef Nemeth, 303 Washington St., Urbana, Illinois.*

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.

Anal. Calcd. for $C_{31}H_{47}O_6D$: 2.08% excess deuterium. Found: 1.40% excess deuterium.

Dimethyl polygalate-d_1 (80 mg) was acetylated (pyridine – acetic anhydride) to give the diacetate. The product was dissolved in acetic acid (1 ml), chromic acid (5 ml, 0.4 *N* in acetic acid) added, and the solution allowed to stand for 1 h at room temperature. The product was poured into a bisulfite solution and extracted into chloroform. Separation by thin-layer chromatography and crystallization gave the α,β-unsaturated ketone (XIV) (10 mg), m.p. 245–247°.

Found: 0.00% excess deuterium.

Dimethyl polygalate diacetate (87 mg) was allowed to stand 1 h at room temperature in 5 ml of 0.5 *N* chromic acid in acetic acid-d_1. The solution was poured into D_2O (30 ml), bisulfite added, and the solution extracted into chloroform. Purification in the usual way gave the α,β-unsaturated ketone (XIV) (16 mg).

Found: $< 0.04\%$ excess deuterium.

Esterification Experiments

Polygalic acid diacetate (113 mg) was refluxed for 6 h in a mixture of methanol (20 ml) and hydrochloric acid (5 ml, concentrated). This was poured into water and extracted into ether. The n.m.r. was taken in pyridine indicating $\sim 100\%$ methylation.

Senegenin monoacetate (87 mg) was treated under exactly the same conditions as above and the n.m.r. spectrum of the crude product was taken in pyridine showing $\sim 24\%$ methylation.

Oleanonic acid (91 mg) was treated as above and the n.m.r. spectrum of the crude product, taken in deuteriochloroform, indicated $\sim 7\%$ methylation.

ACKNOWLEDGMENTS

The authors wish to thank the National Research Council of Canada for financial support and for a scholarship to one of us (J. J. D.).

REFERENCES

1. J. J. DUGAN, P. DE MAYO, and A. N. STARRATT. Can. J. Chem. **42**, 491 (1964).
2. J. J. DUGAN, P. DE MAYO, and A. N. STARRATT. Proc. Chem. Soc. 264 (1964).
3. M. SHAMMA and L. P. RIEFF. Chem. Ind. London, 1272 (1960).
4. W. A. JACOBS and O. ISLER. J. Biol. Chem. **119**, 155 (1937).
5. D. H. R. BARTON and B. R. THOMAS. J. Chem. Soc. 1842 (1953).
6. D. H. R. BARTON, H. T. CHEUNG, P. J. L. DANIELS, K. G. LEWIS, and J. F. MCGHIE. J. Chem. Soc. 5163 (1962).
7. J. RONDEST and J. POLONSKY. Bull. Soc. Chim. France, 1253 (1963).
8. H. BUDZIKIEWICZ, J. M. WILSON, and C. DJERASSI. J. Am. Chem. Soc. **85**, 3688 (1963).
9. J. J. DUGAN, P. DE MAYO, and A. N. STARRATT. Tetrahedron Lettors, **37**, 2567 (1964).
10. S. W. PELLETIER, N. ADITYACHAUDHURY, M. TOMASZ, J. J. REYNOLDS, and R. MECHOULAM. Tetrahedron Letters, **41**, 3065 (1964).
11. J. W. ROWE, A. MELERA, D. ARIGONI, O. JEGER, and L. RUCZICKA. Helv. Chim. Acta, **40**, 1 (1957).
12. W. G. DAUBEN and J. B. ROGAN. J. Am. Chem. Soc. **79**, 5002 (1957).
13. A. ZURCHER, O. JEGER, and L. RUCZICKA. Helv. Chim. Acta, **37**, 2145 (1954).
14. A. I. SCOTT. The ultraviolet spectra of natural products. Pergamon Press. 1964. p. 59.
15. T. Q. CHOU, J. H. CHU, and P. F. MEI. J. Am. Pharm. Assoc. Sci. Ed. **36**, 241 (1947).
16. R. TSCHESCHE and A. K. SEN GUPTA. Chem. Ber. **93**, 1903 (1960).
17. M. FUJITA and H. ITOKAWA. Chem. Pharm. Bull. Tokyo, **9**, 1006 (1961).
18. W. E. IRWIN. Ph.D. Thesis, The Pennsylvania State University. 1962.

Can. J. Chem. Downloaded from www.nrcresearchpress.com by SUNY AT STONY BROOK on 11/12/14
For personal use only.