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TERPENOIDS

V. SENEGENIN: FUNCTIONAL GROUPS AND PART STRUCTURE

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ABSTRACT

Senegenin, the sapogenin from *Polygala senega* has been shown to contain chlorine; it has the empirical formula $C_{30}H_{45}O_6Cl$. The oxygen atoms, contrary to earlier work, are contained in two carboxyl groups and two secondary hydroxyl groups. There is one isolated ethylenic linkage. The environment of the hydroxylic functions and one carboxyl group has been shown to be as in (XI).

Of the known triterpenoids of undetermined constitution remaining, senegenin has a claim to outstanding interest. Investigations directed to a study of the saponin of *Polygala senega* have spread over many years, but, as mentioned by Simonsen and Ross (1), such "investigations . . . have given rise to very divergent results". It is this confusion, and its present clarification, which exculpates us from a charge of literary proliferation in the presentation of incomplete work.

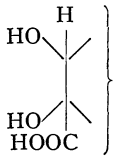
Although senega extract has been used medicinally for some time, chiefly as an expectorant (however, see ref. 2), the first results of chemical importance were obtained by Wedekind and Krecke (3). They isolated, by acid hydrolysis of the saponin, a product to which they assigned the formula $C_{26}H_{44}O_6$. It contained two hydroxyl groups, as indicated by the formation of a diacetate, and gave a dimethyl ester.

A more extensive study was later reported by Jacobs and Isler (4), who observed the presence of two crystalline sapogenins. That of lower solubility appeared to be senegenin, although the constants differed somewhat from those of Wedekind and Krecke: the diacetate, in particular, melted at a much higher temperature. The empirical formula $C_{30}H_{46}O_8$ or $C_{30}H_{44}O_8$ was proposed, and evidence for an isolated ethylenic linkage found in a weak tetranitromethane test. Titration with alkali indicated the presence of two carboxyl groups (again confirmed by the preparation of a non-crystalline dimethyl ester) and the presence of a lactone was inferred by the consumption of a third equivalent of alkali on heating. Senegenin could not be recovered from the acidified hydrolysis mixture.

The second sapogenin isolated from senega root by Jacobs and Isler was an acid, m.p. 257°, which appeared to have the empirical formula $C_{31}H_{50}O_6$ or $C_{31}H_{48}O_6$. This substance also gave a positive reaction with tetranitromethane, and was shown to give a diacetate. Hydrolysis revealed it to be the monoethyl ester of a dibasic acid. Both the sapogenins gave, on selenium dehydrogenation, the same substituted picene obtained previously from such triterpenoids as oleanolic acid, and shown to be (5) 1,8-dimethylpicene: results which strongly supported the supposition of Jacobs and Isler that they were dealing with a triterpenoid.

More recently senegenin has been the subject of study by Shamma and Reiff (6). The allocation of oxygen functions previously proposed (4) was accepted by these authors with the added qualification, because of the absence of an appropriate band for a γ-lactone, that the lactone be six (or more) membered. Confirmation of the presence of the double bond led them to assign the molecular formula $C_{30}H_{44}O_8$ to senegenin, an interpretation requiring that the substance be tetracyclic. Senegenin gave on lithium aluminum hydride reduction a product believed to be a hexaol from which a pentaacetate could be prepared. An important observation was that senegenin gave an isopropylidene derivative.

Senegenin consumed (6) 2 molecules of lead tetraacetate whereas the dimethyl ester consumed but 1 molecule. No crystalline or characterized compounds were, however, isolated from the cleavage. From their observations the conclusion was drawn that senegenin contained the grouping (I) which required it to be of a carbon skeleton different from that of the then known triterpenoids. It could, however, be incorporated into the carbon skeleton, recently established, of ceanothic acid (7) (II) and our interest in this substance led us to a study of senegenin.



(I)

A number of triterpenoids have been reported as having been isolated for *Polygala* species. *Polygala tenuifolia* Willd. has been shown to contain (8, 9) an acid, tenuifolic acid, to which the empirical formula $C_{30}H_{44-46}O_8$ has been attributed: the same substance has been isolated from *Bredemeyera floribunda* Willd. A similar substance has been isolated from *Polygala senega* L. var. *latifolia* Torr. and Gray (10) which may, however, be identical with senegenin. From *Polygala paenea* Polonsky has isolated polygalacic acid (11) and shown it to have the structure (III) whilst very recently, after the completion of the work to be described, Tschesche and his collaborators have modified their original findings and presented (IV) (12) as the structure of bredemolic acid, a substance found together with tenuifolic acid. The relevance of this related work will become apparent in the sequel.

The isolation procedure of Jacobs and Isler was modified (see Experimental), the crude sapogenin, obtained from the hydrochloric acid hydrolysis of the prosapogenin, being acetylated directly. The material extracted with aqueous bicarbonate solution gave senegenin monoacetate.* Acid hydrolysis afforded senegenin (see Table I).

Our first endeavor was to confirm the nature of the functional groups. The ultraviolet absorption (λ_{max} 205 mμ, ϵ = 6900) suggested the presence of an isolated ethylenic linkage (4), but no indication whatsoever could be found for the presence of a vinylic proton (6) in the n.m.r. spectra of several derivatives. The double bond was, therefore, fully substituted.

The n.m.r. spectrum of dimethyl senegenin showed two singlets (3H each) at τ 6.29 and 6.41 in agreement with the presence of two carbomethoxyl groups. In addition there was absorption at τ 5.81 and 6.10 (1H each). Senegenin monoacetate dimethyl ester, obtained by brief exposure to mild acetylating conditions, had two bands at τ 5.74 (1H, multiplet) and 4.77 (1H, doublet) whilst in the diacetate both bands had moved

**Professor W. D. Ollis (Sheffield) has isolated senegenin in a similar manner, and we would like to present our sincere thanks for a gift of a generous sample.*

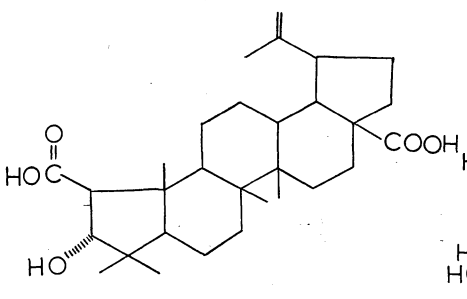
TABLE I

Compound	Present work	Jacobs and Isler (4)	Reiff (22)
Senegenin	M.p. 281–283°* $[\alpha]_D$+19° (EtOH)	290–292° $[\alpha]_D$+19° (EtOH)	280–283° $[\alpha]_D$+16° (EtOH)
Senegenin diacetate	M.p. 257–263° $[\alpha]_D$+29° (MeOH)	260–270°	225–230° $[\alpha]_D$+26° (EtOH)

*The melting point was determined on the Kofler block by placing the compound on the block about 15° below the melting point. This is the procedure indicated to us by Dr. S. W. Pelletier (Athens) as giving the recorded melting point on Dr. Jacob's samples. We are grateful to Dr. Pelletier for this information. Melting points determined on the block in the usual way were in the region 265–270° and varied with the rate of heating.

A direct comparison of the senegenin used in these experiments and that of Jacobs and Shamma has established identity in melting point, mixed melting point, infrared spectrum, and behavior on thin-layer chromatography. We are grateful to Professors Pelletier and Shamma for making the material available.

down field to τ 4.47 (1H, multiplet) and 4.68 (1H, doublet). These changes are those to be expected in the stepwise acetylation of two secondary hydroxyl groups. In confirmation, a diformate could be prepared by the action of formic acid. Further, since one of the methine hydrogens was a doublet, and senegenin dimethyl ester consumed one mole of periodic acid, the part structure (I) (6) must be rejected and replaced by (V), where $(HO)_b$ is the hydroxyl group acetylated first.



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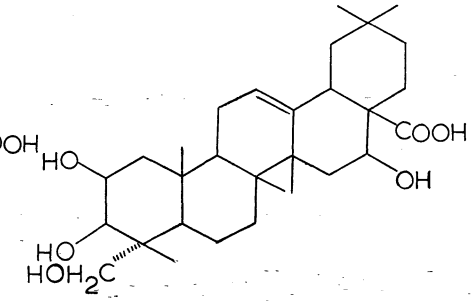

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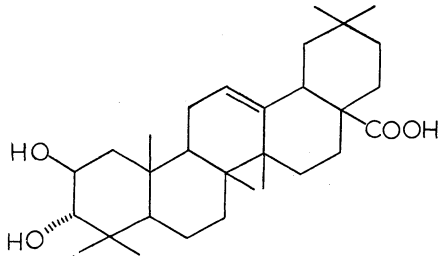
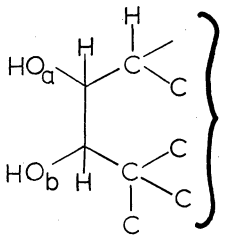


IV



V

Six of the oxygen atoms in senegenin were thereby satisfactorily accounted for. The remaining two were those attributed by previous workers to a lactonic function essentially because of the consumption of an extra equivalent of alkali. This lactone was presumed to suffer ring change to a γ-lactone with perbenzoic acid (6) the exact nature of the

transformation being, however, unspecified. In an attempt to obtain direct spectral evidence for the lactone the sodium salt of senegenin was prepared and shown, by analysis, to be a disodium salt. However, the infrared spectrum of this salt showed no carbonyl absorption above 1550 cm^{-1} (carboxylate anion). The presence of a lactone in senegenin was, therefore, precluded.

The obstinate fact remained that one equivalent of alkali was consumed by senegenin, beyond the requirements of its carboxyl functions. Yet this potentially acidic function was generated from a non-carbonylic precursor. Whilst such a system, as an improbable grotesquerie, can be devised, the interpretive problem was considerably simplified when it was discovered that senegenin contained chlorine. The atomic weight of chlorine renders distinction from two oxygen atoms by carbon and hydrogen analysis alone a subtle one, and the presently proposed empirical formula, $C_{30}H_{45}O_6Cl$, has requirements close to the O_8 formula previously entertained.

For the further study of senegenin, and in particular for the investigation of the environment of the vicinal hydroxyl groups, the removal of the chlorine was required. This was achieved in two ways.

In the first, senegenin was refluxed in quinoline when de(hydrochloro)senegenin was obtained. This compound could also be obtained, under defined conditions, as the product of the alkali treatment of senegenin, the "extra" equivalent of alkali being consumed by the liberated hydrochloric acid. De(hydrochloro)senegenin was characterized as a diacetate and a dimethyl ester. From the ester a mono- and di-acetate were prepared. The ultraviolet absorption (see Experimental) showed the presence of a heteroannular diene. In the n.m.r. spectrum the de(hydrochloro)senegenin dimethyl ester showed the two bands (3H each) at τ 6.49 and 6.32 for the ester methyl groups and at 6.11 (doublet) and 5.92 (multiplet) for the continuing presence of the glycol system. There was also a band at 4.70 (1H) for a vinyl hydrogen. In addition it was still possible to prepare an isopropylidene derivative. Treatment of senegenin dimethyl ester with quinoline afforded a product identical with that obtained by the methylation of the product from senegenin. The molecular weight, 528, determined mass spectroscopically* further showed that only hydrogen chloride had been lost during the treatment with alkali or with quinoline.

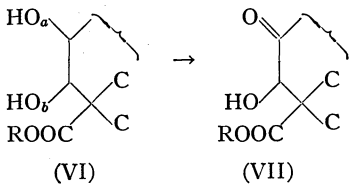
De(hydrochloro)senegenin dimethyl ester was hydrogenated over platinum oxide, with the uptake of one molecule of hydrogen, to give a dihydro compound, termed isodechlorosenegenin dimethyl ester, showing end absorption (λ_{max} 199 mμ, ϵ = 6700) only, and which was characterized as a mono- and di-acetate. De(hydrochloro)senegenin, similarly, gave isodechlorosenegenin.

In the second method of eliminating the chlorine direct reduction of the monoacetate with lithium in liquid ammonia was employed; the product, dechlorosenegenin, was characterized as the diacetate.

A difference in the reactivity of the two carboxyl groups was noted when de(hydrochloro)senegenin dimethyl ester was heated for 2 hours in refluxing 5% alcoholic potassium hydroxide. The product proved to be a monomethyl ester. It has previously been observed in other triterpenoids (13, 14, 15, 16) that the presence of an appropriately situated oxygen function can greatly facilitate the hydrolysis of an otherwise very resistant ester. This observation suggested that the carbomethoxyl group which underwent hydrolysis should be placed as in (VI, R = Me), a part structure which already assumed a general familiarity, but a more direct demonstration of this relationship was required.

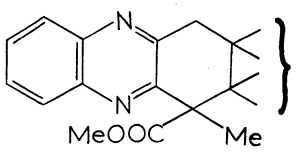
Oxidation of isodechlorosenegenin dimethyl ester with chromic acid in pyridine gave a

*We are grateful to Professor K. Biemann for this determination.

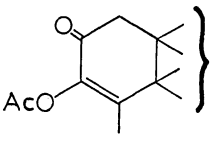


(VI) (VII)

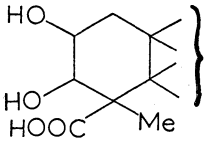
ketol showing hydroxyl absorption at 3495 cm^{-1} in the infrared and a band in the n.m.r. spectrum at τ 5.51 moved to 4.57 on acetylation. This band was a singlet, and therefore it is hydroxyl (*a*) which has been oxidized to the ketone (as in (VII, R = Me)). Since



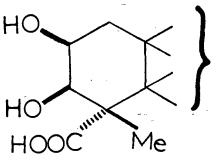
VIII



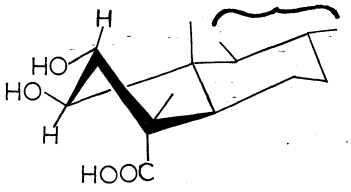
IX



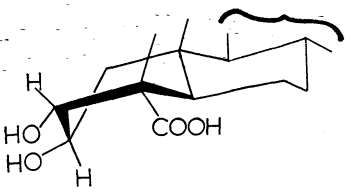
X



XI



XII



XIII

the carbonyl absorption of the keto-acetate was at 1728 cm^{-1} the possibility of a five-membered ring could not, at this stage, be dismissed. Attempts to hydrolyze the ester grouping in (VII, R = Me) were abortive, however, since the ketol underwent air-oxidation (17) and yielded no tractable product. (VII, R = Me) was converted by bismuth oxide (18) to the non-crystalline α-diketone characterized as the crystalline quinoxaline derivative (VIII).

An alternative approach to the establishment of the relationship of the hydroxyl and carboxyl functions was therefore sought. Isodechlorosenegenin dimethyl ester was converted to the monomethyl ester by alkaline hydrolysis. Oxidation with chromic acid in pyridine gave a gum. On warming in alkali, and acidification, a compound was obtained having the properties of a diosphenol and which had decarboxylated. It was characterized

as the crystalline acetyl derivative (IX) (λ_{max} 247 mμ).* Since this compound showed ν_{max} for the unsaturated ketone at 1675 cm^{-1} a six-membered (or larger) ring was indicated.

The decarboxylation, that of a β-keto acid, confirms the position of the carboxyl function and the presence of a singlet (3H) at τ 7.76 in the diosphenol acetate indicates the position of a methyl group at this point. The part structure (V) may thus be reasonably expanded to (X)—that is, the normal triterpenoid ring A carbon skeleton. No evidence is presently available for the presence of the C_{10} methyl group.

The carboxyl group must be placed at the 4α, equatorial, position since the resistance of 4β carboxylic ester groups to hydrolysis at this position is well known (for example, ref. 19). Of the four possible arrangements of the glycol one, 2α,3β, can be immediately dismissed since this would require a large trans diaxial coupling of the methine protons ($J \sim$ 8–10 c.p.s.) (20) whereas that observed is ~3.8 c.p.s. Of those remaining only the 2α, 3α, and 2β,3β would be expected to give an isopropylidene derivative easily (21).

Since in senegenin monoacetate the low field (τ 4.83) methine proton is the doublet it is the C_3 hydroxyl that acetylates first. If the arrangement were 2α,3α then this would require the preferential acetylation of an axial 3-hydroxyl group over the far less hindered equatorial 2-hydroxyl group. Since this cannot be accepted the remaining 2β,3β stereochemistry follows (XI), and is consistent with the preferred oxidation of the 2β hydroxyl. It will be noted that a 2β configuration of a hydroxyl group requires a 4α configuration of the carboxyl group since no evidence of lactonization under a variety of conditions has been observed. The structure of what is presumably ring A of senegenin is therefore identical with that observed in medicagenic acid (13).†

The above discussion requires that ring A be a chair, but the possibility of twist or boat forms requires consideration. Of the three remaining possible assignments of glycol stereochemistry rejected, two, namely 2α,3β and 2α,3α, should show no tendency to assume conformations other than chair and so remain unacceptable. The third, 2β,3α, because of the three 1:3 hydroxyl and methyl interactions (present also in (XI)) and because of two 1:3 hydroxyl–hydrogen interactions could invert into a boat or twist form of which (XII) and (XIII) represent extremes. Whilst this might account for acetonide formation and the order of acetylation and oxidation of the hydroxyl groups, in (XII) and (XIII) or all conformations in between the methine protons at C_2 and C_3 are essentially trans-diaxial. The coupling constant here, as in the 2α,3β chair form, would therefore be larger than that actually observed.

Since the hydrolysis of the saponin was effected by all previous workers using hydrochloric acid it seemed probable that the introduction of chlorine into the senegenin moiety took place at this point. Isolation and halogen analysis of the crude saponin indicated the total absence of chlorine. Analysis of the crude prosapogenin on the other hand indicated the presence of 1.57% chlorine not removed by repeated precipitation from ethanol. It is therefore at this stage that the halide is most probably introduced.

Tenuifolic acid, previously referred to (9), has two unassigned oxygen atoms in the formula proposed. On treatment with quinoline it is converted to anhydrotenuifolic acid which, although not a conjugated diene as in the case of de(hydrochloro)senegenin, has analytical figures closely corresponding. It is possible therefore that tenuifolic acid may also contain chlorine, as may other "O_8 substances" from *Polygala* species (10).

Such a sequence has been reported in the chemistry of medicagenic acid (13).

†*That dechlorosenegenin is not medicagenic acid has been shown by a comparison of the acids. We thank Dr. R. J. Morris (Nevada) for a generous specimen of medicagenic acid.*

EXPERIMENTAL*

Extraction of Ground Root of Polygala senega

The ground root (6 kg, supplied by S.B. Penick and Co.) was extracted in methanol for approximately 1 day, after which time the solvent was drained off. This was repeated three times and the combined extract was concentrated. The saponin was precipitated by the addition of ethanol and removed by filtration. Crude saponin (2 g) was dissolved in methanol and treated with activated charcoal. After filtration the solution was concentrated to one-third volume and the saponin precipitated with acetone. Analysis showed the absence of chlorine.

The saponin (156 g) was dissolved in alcohol–water (1:1; 480 ml) and warmed on the steam bath for 1 hour with concentrated hydrochloric acid (320 ml). The solution was cooled and the precipitated prosapogenin filtered off.

Upon standing, a crystalline material was slowly deposited from the concentrated saponin mother liquors, m.p. 183–186° (alcohol), $[\alpha]_D$ +67° (*c*, 1.21; water). This compound was identified as sucrose by mixed melting point and infrared spectrum.

Prosapogenin (1 g) was dissolved in alcohol and treated with activated charcoal. After filtration the solution was concentrated and the prosapogenin precipitated with water. This process was repeated six times (with omission of treatment with activated charcoal) at which time the mother liquors no longer gave a positive test for chloride ion. The dried material contained 1.57% chlorine.

Without drying, the total prosapogenin was further hydrolyzed by refluxing 6 hours in a mixture of alcohol (960 ml), water (320 ml), and concentrated hydrochloric acid (320 ml). The solution was cooled and the precipitate collected. The precipitate was extracted exhaustively with ether and the ether extract washed with water and concentrated to dryness.

Acetylation of the Crude Sapogenin Mixture

To the above ether crude extract in pyridine (100 ml) was added acetic anhydride (50 ml) and the solution allowed to stand at room temperature for 6 hours. After isolation the product was dissolved in ether and extracted with sodium bicarbonate solution (5%). After acidification with dilute hydrochloric acid, the acidic product was crystallized from ether–chloroform solution to yield a crude monoacetate (6.8 g). Additional crystalline material (1.6 g) was obtained as a second crop. Recrystallization from methanol–chloroform gave senegenin monoacetate, m.p. 209–211°, $[\alpha]_D$ +23° (*c*, 1.32), ν_{max} 1743, 1700 cm^{-1} (Nujol mull), λ_{max}^{MeOH} 205 mμ (ϵ 6400). Calc. for $C_{32}H_{47}O_7Cl$: C, 66.26; H, 8.18; Cl, 6.12%. Found: C, 66.05; H, 7.95; Cl, 6.01%. Methylation with diazomethane yielded a non-crystalline ester, ν_{max} 1722 cm^{-1}, n.m.r. bands at τ 9.13, 9.07, 8.97, 8.80, 8.64, 7.95, 6.43, 6.37, 5.78 (multiplet), 4.83 (doublet).

On one occasion, the material not extracted by bicarbonate solution was purified by filtration through silica gel. A crystalline product was obtained which was recrystallized from alcohol to give an acetate, m.p. 164–166°, $[\alpha]_D$ +25° (*c*, 1.10), λ_{max} 200 mμ (ϵ 5560), n.m.r. bands of methyl ester at τ 7.98, 6.41, 5.98 (quartet), 4.97 (doublet). Found: C, 70.74; H, 8.97%.

Hydrolysis of the latter acetate (100 mg) by refluxing 30 minutes under nitrogen in methanolic potassium hydroxide solution (5%; 10 ml) afforded, upon isolation, a substance recrystallized from aqueous alcohol to give a product, m.p. 214–217°, $[\alpha]_D$ +20° (*c*, 0.70; alcohol). Found: C, 71.09; H, 9.32%. This compares favorably with the dihydroxy dicarboxylic acid monoethyl ester isolated together with senegenin by Jacobs and Isler (4) and reported to have m.p. 215–218°, $[\alpha]_D$ +24.5° (alcohol). This compound was found to contain no chlorine.

Senegenin

The monoacetate (1.33 g), m.p. 209–211°, was refluxed for 1 hour with methanol (83 ml), water (6 ml), and concentrated hydrochloric acid (18 ml). Isolation of the product and recrystallization from aqueous alcohol gave a product (962 mg) further recrystallized from aqueous alcohol and acetone–water to yield senegenin, m.p. 265–267° (gas evolution) (see Table I), $[\alpha]_D$ +19° (*c*, 0.74; alcohol), ν_{max} 3400, 1700 cm^{-1} (Nujol mull), λ_{max} 205 mμ, (ϵ 6950). The dimethyl ester (glass) exhibited n.m.r. bands at τ 9.15, 9.09, 8.98, 8.85, 8.62, 6.41, 6.29, 6.10, and 5.81. Calc. for $C_{30}H_{45}O_6Cl$: O, 17.87; Cl, 6.60%. Found: O, 17.87; Cl, 6.56%.

Senegenin Diacetate

Acetylation of senegenin at room temperature for 46 hours (acetic anhydride – pyridine) gave senegenin diacetate, m.p. 257–263° (decomposition), $[\alpha]_D$ +29° (*c*, 1.09; methanol), ν_{max} 1736, 1698 cm^{-1}. Calc. for $C_{34}H_{49}O_8Cl$: C, 65.73; H, 7.95; Cl, 5.71%. Found: C, 65.94; H, 7.99; Cl, 5.90%. The non-crystalline methyl ester showed n.m.r. bands at τ 9.14, 9.05, 9.00, 8.87, 8.62, 8.06, 7.93, 6.40, 6.34, 4.68 (doublet), 4.48 (multiplet), and 6.77, 6.58.

Acetonide of Senegenin

Senegenin (48 mg) in acetone (10 ml) was shaken 70 hours with anhydrous copper sulphate. The solution was filtered and the solvent evaporated to give a product which was recrystallized from aqueous alcohol

**For general procedure, see part IV (23).*

to yield the acetonide of senegenin, m.p. 282–284°, (Reiff (22) reports m.p. 261–264°) $[\alpha]_D$ +28° (*c*, 0.93; methanol), ν_{max} 1697 cm^{-1} (Nujol mull). Calc. for $C_{33}H_{49}O_6Cl$: C, 68.64; H, 8.56%. Found: C, 68.86; H, 8.53%.

Sodium Salt of Senegenin

Senegenin was titrated with sodium hydroxide solution (*N*/100 in methanol:water (1:1)). The solvent was evaporated and the residue, dissolved in methanol, was precipitated with ether. The product, $[\alpha]_D$ +5° (*c*, 1.16; methanol), decomposed over a wide range starting at about 260° and showed ν_{max} 1550 cm^{-1} (KBr disk) (carboxylate group). No absorption attributable to a γ or δ lactone was detected between 1550 and 1800 cm^{-1}. Calc. for $C_{30}H_{43}O_6ClNa_2$: Na, 7.92%. Found: Na, 7.34%.

Dechlorination of Senegenin Monoacetate

Senegenin monoacetate (1.2 g) dissolved in anhydrous ether (200 ml) was added over a period of 5 minutes to a stirred solution of an excess of lithium metal in liquid ammonia (200 ml) cooled by immersion in a dry ice – acetone bath. Stirring was continued for 20 minutes and then ammonium chloride was added carefully until the disappearance of the blue color. The ammonia was allowed to evaporate, water added, and the mixture acidified with hydrochloric acid. Isolation of the product by ether extraction and crystallization from methanol afforded dechlorosenegenin (760 mg), m.p. 279–281, $[\alpha]_D$ −15° (*c*, 1.57, methanol). Calc. for $C_{30}H_{46}O_6$: C, 71.68; H, 9.22; O, 19.10%. Found: C, 71.48; H, 9.14; O, 18.58%. The compound was converted into the non-crystalline dimethyl ester with diazomethane and the molecular weight determined by mass spectroscopy. Mol. wt. calc. for $C_{32}H_{50}O_6$: 530.7. Found: 530.*

Dechlorosenegenin Diacetate

Dechlorosenegenin (57 mg) was acetylated (acetic anhydride – pyridine) by standing 3 days at room temperature. Isolation of the product and recrystallization from ether – light petroleum gave dechlorosenegenin diacetate (45 mg), m.p. 281–285°, $[\alpha]_D$ −2° (*c*, 1.61). Calc. for $C_{34}H_{50}O_8$: C, 69.59; H, 8.59%. Found: C, 69.76; H, 8.44%.

De(hydrochloro)senegenin

Senegenin (290 mg) was refluxed 3 hours in quinoline (6 ml; freshly distilled from zinc dust) under nitrogen. The reaction mixture was poured into dilute acid and extracted with ether. The ether extract was then extracted with sodium bicarbonate solution (5%) and the bicarbonate-soluble material isolated (180 mg). This gave a crystalline product (146 mg), found to be free of chlorine, from aqueous alcohol. Further recrystallization gave de(hydrochloro)senegenin, softened ~240°, m.p. 294–296° (gas evolution; evacuated capillary), $[\alpha]_D$ +80° (*c*, 0.75; methanol), ν_{max} 3320, 1697 cm^{-1} (Nujol mull). Calc. for $C_{30}H_{44}O_6.0.5H_2O$: C, 70.68; H, 8.90%. Found: C, 70.61; H, 8.67%.

De(hydrochloro)senegenin Diacetate

De(hydrochloro)senegenin (60 mg) in pyridine (2 ml) was treated overnight at room temperature with acetic anhydride (1 ml). After isolation, the product was placed on a silica gel column (Davison) and eluted with benzene–ether (4:1) to yield a crystalline product which when recrystallized from aqueous alcohol gave de(hydrochloro)senegenin diacetate, m.p. 215–218°, $[\alpha]_D$ +84° (*c*, 0.72; methanol). Calc. for $C_{34}H_{48}O_8.0.5H_2$: C, 68.77; H, 8.32%. Found: C, 68.61; H, 8.64%.

Dimethyl Ester of De(hydrochloro)senegenin

Dehydrochlorosenegenin (93 mg) was methylated with diazomethane and chromatographed on alumina (3 g; Woelm). Elution with benzene:ether (19:1) and recrystallization from alcohol gave the dimethyl ester of de(hydrochloro)senegenin, m.p. 228–231° (evacuated capillary), $[\alpha]_D$ +78° (*c*, 0.99), ν_{max} 3500, 1716 cm^{-1}, λ_{max} 243, 249, 256 mμ (ϵ = 13,200; 14,900; 11,800), n.m.r. bands at τ 9.22, 9.12, 8.97, 8.79, 8.65, 6.49, 6.32, 6.11 (doublet), 5.92 (multiplet), 4.70 (multiplet). Calc. for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15%. Found: C, 72.11; H, 8.99%. Molecular weight determined by mass spectroscopy 528. Mol. wt. calc. for $C_{32}H_{48}O_6$: 528.7.

Treatment of Senegenin Dimethyl Ester with Quinoline

Senegenin dimethyl ester (53 mg) in quinoline (2.5 ml) was refluxed 0.5 hour under nitrogen. The mixture was poured into dilute sulphuric acid and extracted by ether. The ether extract was washed with dilute hydrochloric acid and then with sodium bicarbonate solution (5%). Removal of the ether gave a crude product (48 mg) which was chromatographed on alumina (2 g; Woelm). Elution with benzene:ether (9:1) afforded a substance recrystallized from alcohol and shown by ultraviolet and infrared spectra and mixed melting point to be identical with de(hydrochloro)senegenin dimethyl ester obtained above.

Acetylation of De(hydrochloro)senegenin Dimethyl Ester

The dimethyl ester of de(hydrochloro)senegenin (97 mg) in pyridine (2 ml) was treated overnight with acetic anhydride (1 ml). Upon isolation, the product was filtered through alumina (Merck). Benzene eluted a crystalline fraction recrystallized from alcohol to give the dimethyl ester of de(hydrochloro)senegenin

**We wish to thank Professor K. Biemann (M.I.T.) for this determination.*

diacetate, m.p. 179–181° (evacuated capillary), $[\alpha]_D$ +64° (*c*, 1.03), ν_{max} 1732 cm^{-1} ($CHCl_3$), n.m.r. bands at τ 9.21, 9.09, 8.94, 8.83, 8.69, 8.09, 7.96, 6.51, 6.37, 4.76 (broad composite band). Calc. for $C_{36}H_{52}O_8$: C, 70.55; H, 8.55%. Found: C, 70.49; H, 8.50%.

Elution with ether gave a product recrystallized from alcohol to yield the monoacetate of the dimethyl ester of de(hydrochloro)senegenin, m.p. 218–221° (evacuated capillary), $[\alpha]_D$ +75° (*c*, 1.02), ν_{max} 3500 1720 cm^{-1}, n.m.r. bands at τ 9.21, 9.07, 8.92, 8.87, 8.74, 7.95, 6.49, 6.42, 6.32, 5.98 (multiplet), 4.80 (composite band). Calc. for $C_{34}H_{50}O_7$: C, 71.55; H, 8.83%. Found: C, 71.25; H, 8.79%.

Acetonide of De(hydrochloro)senegenin

De(hydrochloro)senegenin (24 mg) was shaken 64 hours in acetone (6 ml) with anhydrous copper sulphate. Paper chromatography using Tschesche's mixture B (9) indicated reaction to be complete at this time. The copper sulphate was filtered off and the acetone evaporated to yield a product which upon recrystallization from alcohol gave the acetonide of de(hydrochloro)senegenin, m.p. 281–295° (gas evolution), $[\alpha]_D$ +89° (*c*, 0.64; methanol). Calc. for $C_{33}H_{48}O_6 . 0.5H_2O$: C, 72.07; H, 8.98%. Found: C, 72.52; H, 8.76%.

Dimethyl De(hydrochloro)senegenin Diformate

Senegenin (59 mg) was refluxed 4 hours with formic acid (15 ml; 97%). The formic acid was removed under reduced pressure on the steam bath and the residue filtered through silica gel with ether. The product furnished crystals from alcohol when methylated with diazomethane. The crude methyl ester was chromatographed on silica gel (1.5 g; Davison). Elution with benzene afforded crystalline fractions (41 mg) which were combined and recrystallized from alcohol to give the dimethyl ester diformate of de(hydrochloro)-senegenin, m.p. 235–240° (evacuated capillary), $[\alpha]_D$ +72° (*c*, 0.95), ν_{max} 1720 cm^{-1} (broad band), λ_{max}^{MeOH} 240, 248, 256 mμ, (ϵ 11,910; 13,850; 9440), n.m.r. bands at τ 6.34, 6.22, 1.82, 1.65. Calc. for $C_{34}H_{48}O_8$: C, 69.83; H, 8.27%. Found: C, 69.16; H, 8.39%.

Alkaline Hydrolysis of Senegenin Monoacetate

(*a*) Senegenin monoacetate (135 mg) was refluxed under nitrogen with methanolic potassium hydroxide solution (10 ml; 5%) for 15 minutes. Upon cooling, crystals of the potassium salt deposited on the surface of the flask. Isolation of the product after acidification and recrystallization from alcohol afforded senegenin showing no melting point depression on admixture with material obtained by acid hydrolysis of the monoacetate.

(*b*) Senegenin monoacetate (30 mg) was refluxed 24 hours under nitrogen with an aqueous alcoholic (1:3, water:ethanol) sodium hydroxide solution (10 ml; 5%). After acidification, isolation of the product, followed by methylation and recrystallization from alcohol, the dimethyl ester of de(hydrochloro)senegenin was obtained, m.p. 234–237° (evacuated capillary), which was undepressed on admixture with that obtained above and which exhibited identical behavior upon thin-layer chromatography.

Isodechlorosenegenin

Dehydrochlorosenegenin (28 mg) was hydrogenated in glacial acetic acid (5 ml) over platinum oxide catalyst for a period of 2.5 hours. Recrystallization of the product from aqueous alcohol gave isodechlorosenegenin, m.p. 289–292°, $[\alpha]_D$ +58° (*c*, 0.96; methanol), λ_{max} 199 mμ, (ϵ 6680). Calc. for $C_{30}H_{46}O_6 . 0.5H_2O$: C, 70.42; H, 9.26%. Found: C, 69.96; H, 9.22%.

Isodechlorosenegenin Dimethyl Ester

The dimethyl ester of dehydrochlorosenegenin (31 mg) in ethyl acetate was hydrogenated using palladium on charcoal (5%) catalyst. In 7 minutes 0.9 mole of hydrogen was absorbed. The product was recrystallized from alcohol to give the dihydro derivative, isodechlorosenegenin dimethyl ester, m.p. 221–223° (evacuated capillary), $[\alpha]_D$ +41° (*c*, 1.30), n.m.r. bands at τ 5.86 (multiplet), 6.05 (doublet), 6.29, 6.40 ($COCH_3$). Calc. for $C_{32}H_{50}O_6 . 0.5H_2O$: C, 71.20; H, 9.52%. Found: C, 71.41; H, 9.48%.

Acetylation of Isodechlorosenegenin Dimethyl Ester

Isodechlorosenegenin dimethyl ester (43 mg) in pyridine (2 ml) was treated with acetic anhydride (1 ml) for 6 hours. The product consisting of monoacetate together with some diacetate (see below) was separated by (silicic acid) thin-layer chromatography (3% methanol in chloroform). Recrystallization of the slower moving compound from methanol gave the monoacetate of isodechlorosenegenin dimethyl ester, m.p. 173–176°, $[\alpha]_D$ +33° (*c*, 1.15), ν_{max} 3490, 1720 cm^{-1}. Calc. for $C_{34}H_{52}O_7 . 0.5H_2O$: C, 70.19; H, 9.18%. Found: C, 70.55; H, 8.79%.

Isodechlorosenegenin dimethyl ester (74 mg) was allowed to react with acetic anhydride – pyridine for a period of 50 hours and yielded a product which was recrystallized from alcohol to give the dicaetate, m.p. 192–196° (evacuated capillary), $[\alpha]_D$ +53° (*c*, 0.96), ν_{max} 1725 cm^{-1}, n.m.r. bands at τ 8.04, 7.94, 6.39, 6.34, 4.66 (broad composite band) ($CDCl_3$). Calc. for $C_{36}H_{54}O_8$: C, 70.33; H, 8.85%. Found: C, 70.65; H, 8.77%.

De(hydrochloro)senegenin Monomethyl Ester

De(hydrochloro)senegenin dimethyl ester (34 mg) was refluxed for 2 hours under nitrogen with alcoholic potassium hydroxide solution (5%; 10 ml). The product was isolated by ether extraction after acidification

and dilution with water. The acid obtained was recrystallized from aqueous alcohol to give the de(hydrochloro)senegenin monomethyl ester, m.p. 249–252° (evacuated capillary; gas evolution), $[\alpha]_D$ +58° (*c*, 0.79; methanol). Calc. for $C_{31}H_{46}O_6.H_2O$: C, 69.89; H, 9.08%. Found: C, 70.38; H, 9.03%.

Remethylation of the monoacid (6 mg) gave a crystalline compound with identical infrared spectrum and behavior upon thin-layer chromatography (silica gel) as was observed with de(hydrochloro)senegenin dimethyl ester.

Periodic Acid Titration of Senegenin Dimethyl Ester

Senegenin dimethyl ester (36 mg), dissolved in methanol (8 ml) and water (1 ml), was titrated with periodic acid (1 ml; approx. 0.32 *N*). After 1.25 hours, the uptake of oxidant had ceased with consumption of 1 mole. The product was isolated but could not be induced to crystallize.

Dehydroisodechlorosenegenin Dimethyl Ester

Isodechlorosenegenin (109 mg) in pyridine (1.1 ml) was added to chromic anhydride (108 mg) in pyridine (1.1 ml) and let stand at room temperature for 10 hours. The reaction mixture was dissolved in alcohol and dilute hydrochloric acid, diluted with water, and extracted with ether to yield the crude ketol (92 mg). This was recrystallized from alcohol to give dehydroisodechlorosenegenin dimethyl ester, m.p. 186–188° (evacuated capillary), $[\alpha]_D$ +31° (*c*, 0.97), ν_{max} 3495, 1723 cm^{-1} (CCl_4), n.m.r. bands at τ 6.43, 6.27, 5.51. Calc. for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15%. Found: C, 73.11; H, 9.03%.

The above ketol (20 mg) was acetylated (acetic anhydride – pyridine; room temperature) to give a non-crystalline acetate, ν_{max} 1758, 1728 cm^{-1} (CCl_4), n.m.r. bands at τ 7.95, 6.46, 6.32, 4.57 (singlet).

Diketone from Isodechlorosenegenin Dimethyl Ester

The above ketol (42 mg) was refluxed 10 hours under nitrogen in glacial acetic acid (4 ml) with bismuth oxide (53 mg). The solvent was evaporated and the residue dissolved in ether and dilute hydrochloric acid. The ether solution was washed, dried, and evaporated, yielding a crude product, which appeared as mainly one spot upon thin-layer chromatography. This crude, non-crystalline, diketone in absolute alcohol (3 ml) and acetic acid (0.5 ml) was refluxed 3 hours under nitrogen with *o*-phenylenediamine. The product was isolated by ether extraction and the ether was washed with dilute hydrochloric acid. Recrystallization from alcohol afforded the quinoxaline derivative, m.p. 260–263° (evacuated capillary), $[\alpha]_D$ +40° (*c*, 0.99), ν_{max} 1728 cm^{-1} (CCl_4), λ_{max} 238, 322, 312 (shoulder) (ϵ 34,300; 10,200; 7900). Calc. for $C_{38}H_{48}O_4N_2.0.5H_2O$: C, 75.34; H, 8.15; N, 4.63%. Found: C, 75.61; H, 8.44; N, 4.79%.

Isodechlorosenegenin Monomethyl Ester

Isodechlorosenegenin dimethyl ester (40 mg) was refluxed 2 hours under nitrogen with alcoholic potassium hydroxide solution (5%; 5 ml). The acidic material was isolated and recrystallized from aqueous alcohol to give isodechlorosenegenin monomethyl ester, m.p. 284–286° (evacuated capillary), $[\alpha]_D$ +57° (*c*, 1.09; methanol). Calc. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36; O, 18.58%. Found: C, 71.82; H, 9.20; O, 18.61%.

Nor-diosphenol Acetate

Isodechlorosenegenin monomethyl ester (80 mg) in pyridine (0.8 ml) was added to chromic anhydride (88 mg) in pyridine (0.8 ml) and let stand 9 hours at room temperature. The oxidation product (53 mg) was isolated as previously but could not be induced to crystallize. The gum was then refluxed 1 hour with methanolic potassium hydroxide (10%; 10 ml). The crude product (49 mg) was isolated and found to give a positive ferric chloride test. It exhibited ν_{max} 3460 (hydroxyl), 1725 (methyl ester and ketone), 1675 (unsaturated ketone), 1648 (double bond) cm^{-1} (CCl_4), λ_{max}^{MeOH} 279 mμ (ϵ 4600) (band showed characteristic change to higher wavelength upon addition of alkali). It was then acetylated (acetic anhydride – pyridine, overnight). The crude acetate was chromatographed on a column of silica gel (Davison). Elution with benzene:ether (19:1) afforded the crystalline nor-diosphenol acetate, m.p. 190–192° (evacuated capillary), $[\alpha]_D$ +83° (*c*, 0.85), ν_{max} 1752 (acetate), 1718 (methyl ester), 1674 (unsaturated ketone), 1640 (double bond) cm^{-1}, λ_{max}^{MeOH} 246 mμ (ϵ 9630). Calc. for $C_{32}H_{46}O_5$: C, 75.26; H, 9.08%. Found: C, 74.87; H, 9.08%.

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