the identity of eburnamylpleiocarpinine and pleiomutine. This investigation was supported by a Public Health Service fellowship (5-F1-GM-21, 624-03) from the National Institute of General Medicine Sciences (to D. W. T.) and a research grant from the National Science Foundation (GP-3734).

# Elucidation of the Structures of the Sapogenins of *Polygala senega* by Correlation with Medicagenic Acid[1,2]

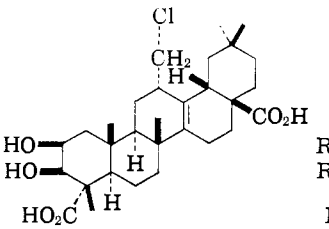
**Y. Shimizu and S. W. Pelletier**[3]

*Contribution from the Department of Chemistry, The University of Georgia, Athens, Georgia 30601.* *Received November 8, 1965*

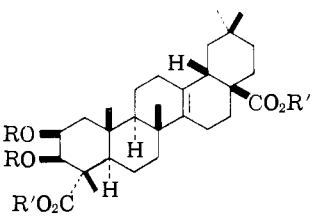
**Abstract:** A postulated precursor of senegenic acid (**2**), "hydroxysenegenin" (**6**), was isolated by the dilute sulfuric acid hydrolysis of senegin, but proved not to be a precursor. Another presumed precursor of senegenin (**1**), cyclosenegenin (**9**), was obtained by the action of alkali on senegenin. Hydrolysis of cyclosenegenin with hydrochloric acid affords senegenin as predicted, but its presence in the saponin, senegin, is not indicated. The structures of the real precursor, presenegenin (**17**), and its artifacts were confirmed by a direct correlation with medicagenic acid by a five-step sequence. This correlation establishes certain stereochemical features which were based on biogenetic analogy to other terpenes.

Extracts of *Polygala senega* L. (Polygalaceae) have been used as an expectorant for centuries. The main constituent is the saponin "senegin," which on hydrolysis with hydrochloric acid affords two crystalline sapogenins: senegenin and senegenic acid.[4a] Recent work has resulted in the assignment of structures[4b] **1** and **2** to senegenin and senegenic acid, respectively.[5-7] Since senegenin contains chlorine and senegenic acid has one less carbon than **1**, it was suspected that both compounds are artifacts produced during the hydrochloric acid treatment. In the course of a search for the precursor of these artifacts two new senega compounds were isolated. This paper describes the isolation and structure determination of these compounds as well as the correlation of the genuine precursor, presenegenin, with medicagenic acid.

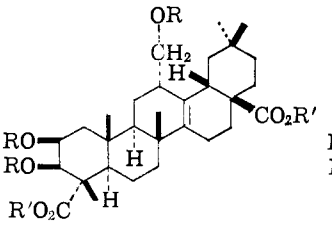
In an effort to explore the effect of milder hydrolytic conditions on senegin, the saponin was treated briefly with 2 *N* aqueous sulfuric acid. The water-insoluble product was acetylated and chromatographed on a silica gel column. Two crystalline acetates were isolated,[8] one of which was identified as senegenic acid diacetate (**3**).[6,7] The other acetate is assigned structure **5** on the following evidence. It has the formula $C_{36}H_{52}O_{10}$, and is hydrolyzed to an acetyl-free compound, $C_{30}H_{46}O_7$ (**6**) which regenerates (**5**) upon reacetylation. The three hydroxyl groups of **6** are acetylable, since **5** shows no hydroxyl absorption in the infrared. The pmr spectrum of **5** exhibits three acetyl groups at $\tau$ 8.04, 7.94, 7.92 besides five C-methyl signals. Signals at $\tau$ 4.66 (1 H, doublet, $J$ = 4 cps) and 4.44 (1 H, broad) are very similar to those exhibited by the diacetates of senegenin and senegenic acid, suggesting the presence of 2$\beta$,3$\beta$-diacetoxy groups[5a,6] in compound **5**. The ill-defined AB system at $\tau$ 6.10 and 5.65 ($J$ = 11 cps) is probably due to an acetoxy methylene group attached to an asymmetric center.[9] Methylation of **5** with diazomethane gave a noncrystalline dimethyl ester (**7**) which shows two



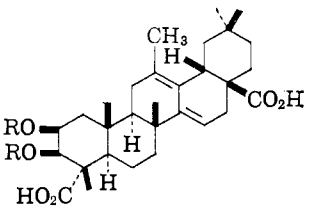
**1**



**2**, R = R′ = H
**3**, R = Ac; R′ = H
**4**, R = H; R′ = $CH_3$



**5**, R = Ac; R′ = H
**6**, R = R′ = H
**7**, R = Ac; R′ = $CH_3$



**8**, R = H
**14**, R = Ac

(1) This investigation was supported in part by Grant GM 10966 from the National Institutes of Health, U. S. Public Health Service.

(2) Preliminary accounts of this work were outlined in communications: (a) Y. Shimizu and S. W. Pelletier, *J. Am. Chem. Soc.*, 87, 2065 (1965); (b) *Chem. Ind.* (London), 2098 (1965).

(3) To whom inquiries regarding this paper should be addressed.

(4) (a) W. A. Jacobs and O. Isler, *J. Biol. Chem.*, **119**, 155 (1937). (b) The complete stereochemistry shown for these compounds anticipates the results of the correlation of presenegenin with medicagenic acid which is described in this paper.

(5) (a) J. J. Dugan, P. de Mayo, and A. N. Starratt, *Can. J. Chem.*, **42**, 491 (1964); (b) *Tetrahedron Letters*, 2567 (1964); (c) *Proc. Chem. Soc.*, 264 (1964).

(6) S. W. Pelletier, N. Adityachaudhury, M. Tomasz, J. J. Reynolds, and R. Mechoulam, *Tetrahedron Letters*, 3065 (1964).

(7) S. W. Pelletier, N. Adityachaudhury, M. Tomasz, J. J. Reynolds, and R. Mechoulam, *J. Org. Chem.*, **30**, 4234 (1965).

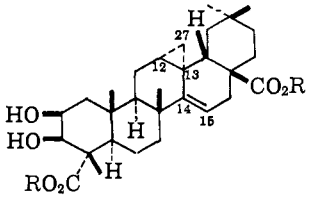
(8) A very small amount of nonterpenoid crystals was also obtained. This proved to be a mixture of 4-methoxycinnamic acid and 3,4-dimethoxycinnamic acid (see the Experimental Section).

(9) L. M. Jackmann, "Nuclear Magnetic Resonance Spectroscopy," Pergamon Press Inc., New York, N. Y., 1959, p 102.

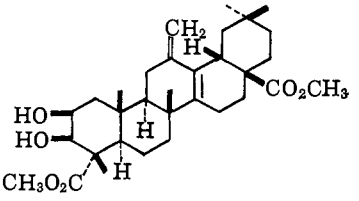
methyl ester signals at $\tau$ 6.33 and 6.40 in addition to the peaks present in **5**.

Treatment of **6** with dilute sulfuric acid at reflux for 18 hr afforded a crystalline compound, which proved to be the heteroannular diene (**8**) obtained earlier by dehydrochlorination of **1** with quinoline.[5a] Clearly, compound **6** may be assigned the structure of 2$\beta$,3$\beta$-dihydroxy-12$\alpha$-hydroxymethyl-27-nor-$\Delta^{13(14)}$-oleanene-23,28-dioic acid and is therefore the "hydroxysenegenin" proposed by Dugan, de Mayo, and Starratt as a possible precursor which could lead to senegenic acid (**2**) by a reverse Prins reaction followed by double-bond migration.[5b] Since treatment of "hydroxysenegenin" (**6**) with either dilute sulfuric or hydrochloric acid led only to the diene **8**, with no evidence for the formation of **1** or **2**, it is certain that **6** is not a precursor of senegenic acid or senegenin.

With the intention of correlating "hydroxysenegenin" (**6**) with senegenin (**1**), alkaline solvolysis of the chlorine of **1** was explored. Dugan, de Mayo, and Starratt have reported that solvolysis of **1** with alcoholic potassium hydroxide furnishes de(hydrochloro)senegenin (**8**).[5a] In our case, the reaction was carried out in 2 *N* aqueous sodium hydroxide solution. Work-up of the reaction mixture gave a crystalline compound designated as cyclosenegenin (**9**), mp 302–305°, with absorption consistent with the presence of a conjugated cyclopropane,[10] $\lambda_{max}$ 209 m$\mu$ ($\epsilon$ 6870); $\lambda$ 230 m$\mu$ ($\epsilon$ 2700). The mother liquor of **9** afforded a crystalline mixture, the ultraviolet spectrum of which is a composite of a diene ($\lambda_{max}$ 249 m$\mu$) and cyclosenegenin (**9**) ($\lambda_{max}$ 209 m$\mu$).[11]

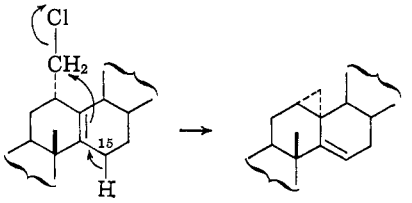


**9**, R = H
**11**, R = $CH_3$



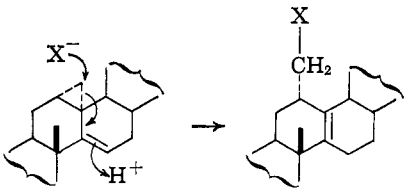
**10**

The pmr spectrum of cyclosenegenin shows two complex signals at $\tau$ 9.94 and 9.47 (in pyridine).[12] The noncrystalline methyl ester (**11**) also has two complex peaks at $\tau$ 10.11 and 9.61, besides a broad signal at 4.47 for one vinylic proton and others at $\tau$ 9.09 (9 H), 8.76 (3 H), 8.67 (3 H) for five methyl groups and at $\tau$ 6.39 (3 H), and 6.30 (3 H) for two methyl esters. The signals at high field near $\tau$ 10.00 are characteristic of cyclopropane hydrogens.[13] These data are consistent with a 12$\alpha$,13$\alpha$-methylen-14-ene structure, which results from the solvolytic removal of chlorine with the concomitant formation of the cyclopropane ring and abstraction of a hydrogen at C-15[14] (**12** → **13**). Chemical evidence also supports this structure. When **9** was treated with dilute hydrochloric acid, reintroduction of the chlorine occurred to give senegenin (**1**) in a high yield.[2a,15] Moreover, when **9** was treated with



**12** **13**

dilute sulfuric acid, "hydroxysenegenin" (**6**) and a small amount of the diene **8** were formed and characterized as the acetates **5** and **14**. These reactions involve non-Markovnikov cleavage of the cyclopropane ring caused by the protonation of the double bond as shown[16] (**15** → **16**).



**15** **16**

Cyclosenegenin (**9**), a derivative of isooleanane, has been postulated as a precursor of senegenin[2a,5c] and indeed has been transformed to **1** in high yield.[2a] However, no direct evidence for its presence in senegin

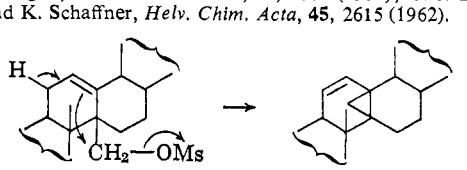
(10) 3$\alpha$,5-Cyclo-$\Delta^6$-cholestene absorbs at $\lambda_{max}$ 207.5 m$\mu$ (R. A. Micheli and T. H. Applewhite, *J. Org. Chem.*, **27**, 245 (1962)) and several other vinylcyclopropanes are reported to have maxima from 206 to 215 m$\mu$ with extinctions between 5300 and 12,000; *cf.* A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," The Macmillan Co., New York, N. Y., 1964, p 49, and also O. L. Chapman, G. W. Borden, R. W. King, and B. Winkler, *J. Am. Chem. Soc.*, **86**, 2660 (1964).

(11) When **1** was treated with alcoholic alkali, more diene was formed. Crystallization from methanol–water gave the diene **8**, but crystallization after treatment with diazomethane gave a dimethyl ester of a different diene, mp 221–224°, $\lambda_{max}$ 249 m$\mu$ ($\epsilon$ 13,800), $\tau$ 5.28, 5.02 (1 H each, singlets), to which an exomethylene structure **10** was assigned. Recrystallization of **10** from methanol with a trace of HCl gave the methyl ester of the diene **8**. This result is consistent with that reported recently by Dugan and de Mayo.[17]

(12) The measurement was done with and without tetramethylsilane as an internal standard.

(13) N. S. Bhacca and D. H. Williams, "Application of N.M.R. Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 190. As to trisubstituted cyclopropanes, 20-methylamino-12$\beta$,18-cyclopregnan-3$\beta$-ol was reported to have two complex peaks at $\tau$ 10.08 and 9.67 by V. Georgian, J. F. Kerwin, M. E. Wolff, and F. F. Owings, *J. Am. Chem. Soc.*, **84**, 3594 (1962), and 4$\alpha$,5-methylenecholestane a complex peak at $\tau$ 10.00 by K. Kocsis, P. G. Ferinni, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **43**, 2178 (1960).

(14) A similar transformation has been reported in the synthesis of an $\alpha$-amyrin type triterpene, phyllanthol: A. Zürcher, O. Jeger, and L. Ruzicka, *ibid.*, **37**, 2145 (1954). See also, J. W. Rowe, A. Melera, D. Arigoni, O. Jeger, and L. Ruzicka, *ibid.*, **40**, 1 (1957); W. G. Dauben and J. B. Rogan, *J. Am. Chem. Soc.*, **79**, 5002 (1957); J. J. Bonet, H. Wehrli, and K. Schaffner, *Helv. Chim. Acta*, **45**, 2615 (1962).

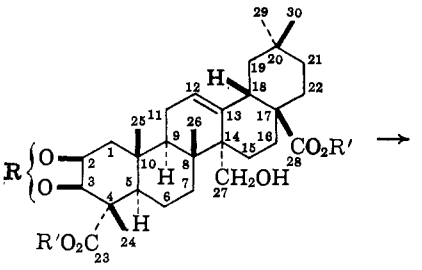


(15) This interconversion of senegenin and cyclosenegenin explains the contradictory experiments concerning the recovery of senegenin after boiling with alkali. Jacobs and Isler[4] reported that senegenin was not recovered after consumption of 3 equiv of alkali but in some unpublished experiments in our laboratory it was recovered.

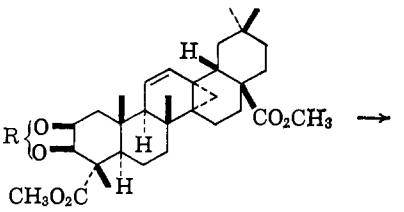
(16) Cyclopropanes usually open according to Markovnikov's rule (D. H. R. Barton, J. E. Page, and E. W. Warnhoff, *J. Chem. Soc.*, 2715 (1954)), but with a conjugated cyclopropane protonation takes place first on the double bond, which leads to non-Markovnikov opening; *cf.* Georgian, Kerwin, Wolff, and Owings, ref 13.

is available.[17] Recently both senegenin and senegenic acid have been shown to arise from a common precursor designated as "presenegenin" and having the structure[4b] of 2β,27-dihydroxy-23-carboxyoleanolic acid (**17**).[17] When heated with ethanolic hydrochloric acid presenegenin is converted in high yield to a mixture of senegenin (**1**), senegenic acid (**2**), and formaldehyde.[17] Though the gross structure of presenegenin (**17**) and its various artifacts rests upon solid chemical evidence,[5,6,7,17] certain stereochemical points and skeletal features are based entirely on biogenetic analogy to other triterpenes. For example, the configuration of the methyl group at C-8 and the D/E ring juncture is deduced entirely by analogy. In view of this situation, an attempt was made to correlate presenegenin (**17**) with medicagenic acid,[18] whose structure and stereochemistry are known by a direct correlation with arjunolic acid,[18b] which in turn has been related to a degradation product of hederagenin.[19] The correlation of presenegenin with medicagenic acid was effected as follows.

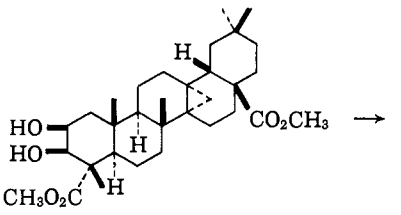
Presenegenin dimethyl ester (**18**), prepared by a modification of the published procedure,[17] was converted to an amorphous acetonide (**19**) by *brief* treatment with cupric sulfate in acetone.[20] Presenegenin dimethyl ester is very acid labile, since longer treatment with cupric sulfate afforded the acetonide of dimethyl senegenate (**4**). Treatment of the acetonide **19** with tosyl chloride in pyridine at reflux gave the cyclopropyl derivative **20**, mp 181–185°, in 75% yield. The compound shows a maximum at 221 mμ (ε 5900), implying the formation of a conjugated cyclopropane.[21] The pmr spectrum has signals at τ 9.67 (1 H, doublet, $J$ = 5 cps) for the higher half of the AB system derived from the cyclopropane hydrogens.[22] The presence of a disubstituted double bond was revealed by an AB system at τ 4.78, 4.10 (2 H, $J$ = 11 cps). Since this reaction is analogous to one used by Zürcher, Jeger, and Ruzicka in the synthesis of phyllanthol,[14] the product may be assigned the structure of 13,27-cyclo-$\Delta^{11}$-oleanene (**20**).[23] Here again, homoallylic participation of a double bond in the solvolysis was observed. It is rather interesting that while strong acid treatment of presenegenin (**17**) leads to a 1,3-shift of the C-27 carbon,[17] ordinary solvolysis conditions give a cyclopropane derivative.



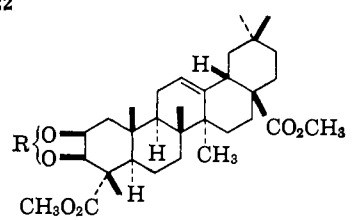
**17**, R = H, H; R′ = H
**18**, R = H, H; R′ = $CH_3$
**19**, R = $(CH_3)_2C$; R′ = $CH_3$



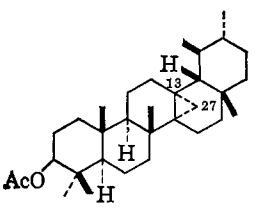
**20**, R = $(CH_3)_2C$
**21**, R = H, H



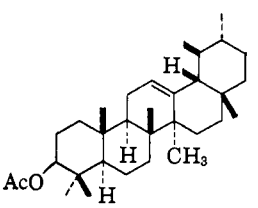
**22**



**23**, R = $(CH_3)_2CH$, H
**26**, R = H, H
**27**, R = Ac, Ac



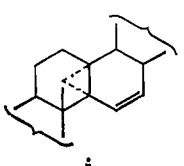
**24**



**25**

Hydrolysis of the acetonide group of **20** was effected smoothly by brief warming in aqueous acetic acid to give compound **21**, mp 223–227°, $\lambda_{max}$ 220 mμ (ε 5250).[21] Hydrogenation[24] of **21** afforded the dihydro derivative **22**, which shows no double-bond absorption in ultraviolet region but exhibits an AB system at τ 10.01, 9.49 (2 H, $J_{AB}$ = 5 cps) characteristic of the cyclopropane hydrogens.[13] Since phyllanthol acetate (**24**), a 13,27-cycloursane derivative, is known to be cleaved between C-13 and C-27 with hydrochloric acid

(17) J. J. Dugan and P. de Mayo, *Can. J. Chem.*, **43**, 2033 (1965).
(18) (a) E. D. Walter, G. R. Van Atta, C. R. Thompson, and W. D Maclay, *J. Am. Chem. Soc.*, **76**, 2271 (1954); (b) C. Djerassi, D. B. Thomas, A. L. Livingston, and C. Ray Thompson, *ibid.*, **79**, 5292 (1957); (c) R. J. Morris and E. W. Hussey, *J. Org. Chem.*, **30**, 166 (1965).
(19) F. E. King, T. J. King, and J. M. Moss, *J. Chem. Soc.*, 3995 (1954).
(20) It has been reported that acetonide formation of senegenin and senegenic acid requires an extended period of time.[5a,7]
(21) Usually, vinylcyclopropanes absorb near 210 mμ, but some absorb at higher wavelength. 3β,28-Diacetoxy-13,27-cyclo-$\Delta^{11}$-ursene has an absorption at 224 mμ (log ε 3.64): Zürcher, Jeger, and Ruzicka, ref 14.
(22) Because of the conjugation, the lower half of the AB system is covered under the methyl peaks.
(23) If presenegenin had a 27-hydroxy-$\Delta^{14}$-isooleane structure, a 13,27-cyclo-$\Delta^{15}$-oleanene structure (i) would be possible for this product. However, such a possibility was disproved by a deuterium study.[17]



i

(24) Direct hydrogenation of the acetonide (**20**) was accompanied by hydrogenolysis of the isopropylidine group to give, after hydrochloric acid treatment, an isopropyl ether derivative (**23**).

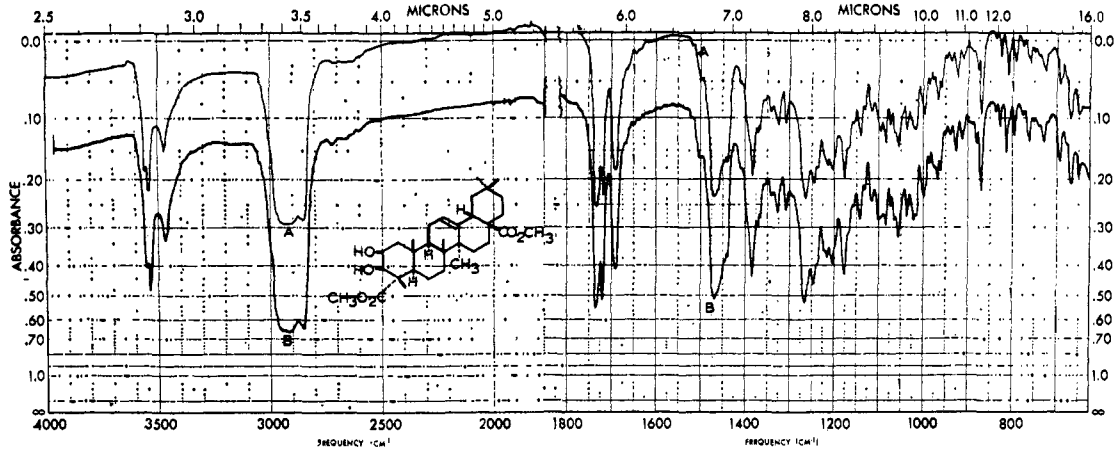



Figure 1. Infrared spectra of dimethyl medicagenate (**26**): A, authentic sample; B, sample prepared by degradation of presenegenin.

to give $\alpha$-amyrine acetate (**25**),[25] we expected compound **22** to give an $\Delta^{12}$-oleanene type compound upon hydrochloric acid treatment. This expectation was born out for when treated at reflux, with concentrated hydrochloric acid in acetic acid, **22** yielded a single product, mp 229–237°, $[\alpha]^{26.5}D$ +88.5°, which proved to be identical with dimethyl medicagenate (**26**)[18] as judged by mixture melting point, behavior on thin layer chromatography, and the infrared spectrum in Nujol (Figure 1). The identity was further confirmed by the comparison of its acetate **27**, mp 238–241°, with an authentic sample. This correlation unequivocally demonstrates that presenegenin (**17**) has the structure of 2$\beta$,3$\beta$,27-trihydroxy-$\Delta^{12}$-oleanene-23,28-dioic acid and the various artifacts have structures and stereochemistry as shown.

## Experimental Section

**General Procedures.** Melting points are corrected and were taken on a hot stage equipped with a microscope and polarizer. Finely powdered samples were placed on the stage 15° below the melting point and the temperature was raised at a rate of about 4°/min. Ultraviolet spectra were determined in 95% ethanol on a Perkin–Elmer Model 202 spectrophotometer and infrared spectra on Infracord 137 and 237B spectrophotometers. Proton magnetic resonance (pmr) spectra were taken on a Varian A-60 spectrometer in deuteriochloroform with tetramethylsilane as an internal standard. The removal of solvents *in vacuo* was accomplished with a Craig-type rotating flash evaporator at 15–20 mm and with the water bath at 35–50°. Thin layer chromatography (tlc) was effected with Brinkmann HF(254 + 366) silica gel with the system chloroform–ethyl acetate.

**Hydrolysis of Senegin with Dilute Aqueous Sulfuric Acid.** A solution 5 g of senegin in 50 ml of water was heated to the boiling point and treated with 50 ml of 4 *N* sulfuric acid, preheated to 90°. After boiling for 10 min, a voluminous precipitate began to appear. The mixture was cooled after 2 hr and diluted to 1 l. The solid which separated was collected and washed well. The semidried material was dissolved in methanol containing 1 ml of pyridine and dried *in vacuo*. This crude material was acetylated with 40 ml of pyridine–acetic anhydride (1:1) at room temperature for 48 hr. The crude acetate obtained after work-up was separated into acidic and "neutral" fractions with bicarbonate solution. An acidic fraction of 630 mg was obtained by extraction with ether after acidification with dilute hydrochloric acid. The "neutral" part was discarded. The acidic fraction was chromatographed over 20 g of silicic acid (Mallinckrodt) (see Table I).

Fraction 3 consisted of a mixture of aromatic acids, mp *ca.* 180°, which later proved to be 4-methoxycinnamic acid and 3,4-dimethoxycinnamic acid (see below).

(25) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 2178 (1953).

**Senegenic Acid Diacetate (3).** Fractions 6 and 7 were combined and crystallized from ethyl acetate-benzene and from acetone to give crystals, mp 270–276°; $\nu_{max}$ (Nujol) 3200, 2650, 1750, and 1700 cm$^{-1}$, identical with authentic senegenic acid diacetate (**3**)[6,7] as judged by infrared spectra, and mixture melting point.

Table I

| Solvent | Fractions | Ml | Wt, mg | |
|---|---|---|---|---|
| $CHCl_3$ | 1,2 | 200 | ... | Oil |
| $CHCl_3$ | 3 | 100 | 7.0 | Cryst |
| $CHCl_3$–EtOAc (20:1) | 4,5 | 200 | 56.9 | Cryst |
| $CHCl_3$–EtOAc (20:1) | 6,7 | 200 | 161.2 | Cryst |
| $CHCl_3$–EtOAc (20:1) | 8 | 100 | 43.4 | Cryst |
| $CHCl_3$–EtOAc (20:1) | 9–11 | 300 | 80.9 | Cryst |
| $CHCl_3$–EtOAc (20:1) | 12–14 | 300 | 76.1 | Cryst |
| $CHCl_3$–EtOAc (20:1) | 15,16 | 200 | 37.7 | Cryst |
| $CHCl_3$–EtOAc (7:1) | 17,18 | 200 | 140.0 | Noncryst |

**"Hydroxysenegenin" Triacetate (5).** Fractions 9 to 11 were combined and crystallized from ethyl acetate–benzene and from acetone–benzene to give needles, mp 329–332° (with effervescence); $[\alpha]^{22}D$ +21.3° (*c* 0.94, EtOH). Recrystallization from acetone–water gave plates of the same melting point; $\nu_{max}$ (Nujol) 2600, 1750, and 1700 cm$^{-1}$; $\tau$ 9.12, 9.08, 8.99, 8.88, and 8.61 (3 H each, all singlets (C-methyls)), 8.04, 7.94, 7.92 (3 H each, all singlets (3-OCOC$H_3$)), 6.10, 5.65 (2 H, AB pattern, $J_{AB}$ = 11 cps (C$H_2$–OAc)), 4.66 (1 H, doublet, $J$ = 4 cps (C$H$–OAc)), 4.44 (1 H, multiplet (C$H$–OAc)).

*Anal.* Calcd for $C_{36}H_{52}O_{10}$: C, 67.06; H, 8.13. Found: C, 66.81; H, 8.21.

**"Hydroxysenegenin" (6).** A solution of 200 mg of "hydroxysenegenin" triacetate (**5**) in 20 ml of 1 *N* sodium hydroxide was refluxed for 4 hr under nitrogen. After cooling, the mixture was acidified with dilute hydrochloric acid and extracted with a mixture of chloroform and ethanol (4:1). The organic layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue was crystallized from acetone–water to give solvated needles of "hydrosenegenin," mp 246–253°, $[\alpha]^{22}D$ +5.7° (*c* 0.71, EtOH, $\nu_{max}$ (Nujol) 3500 and 1695 cm$^{-1}$.

*Anal.* Calcd for $C_{30}H_{46}O_7 \cdot H_2O$: C, 67.13; H, 9.02. Found: C, 66.86; H, 8.63.

**Acetylation of "Hydroxysenegenin."** A solution of 10 mg of "hydroxysenegenin" (**6**) in a mixture of 0.8 ml of pyridine–acetic anhydride (5:3) was left at room temperature for 48 hr. Ice was added to the mixture and the crystals which separated were collected. Recrystallization from acetone–water gave plates, mp 321–332° dec, identical with "hydroxysenegenin" triacetate (**5**) (see above) by infrared spectra and mixture melting point.

**"Hydroxysenegenin" Triacetate Dimethyl Ester (7).** "Hydroxysenegenin" triacetate was converted to the dimethyl ester by treatment with diazomethane in ether. The compound did not crystal-

lize from the usual solvents, but showed one spot by tlc, $\nu_{max}$ (film) 1740 cm$^{-1}$, no OH; $\tau$ 9.13, 9.11, 9.00, 8.88, 8.69 (3 H each, all singlets (C-methyls)), 8.07, 7.95, 7.93 (3 H each, all singlets (3-OCOC$H_3$)), 6.33, 6.40 (3 H, each, all singlets (2-COOC$H_3$)), 6.10, 5.65 (2 H, AB pattern, $J_{AB}$ = 12 cps (C$H_2$-OAc)).

**Treatment of "Hydroxysenegenin" with Sulfuric Acid to Give De(hydrochloro)senegenin (8).** A solution of 140 mg of "hydroxysenegenin" in 3.5 ml of dioxane was treated with 4.5 ml of 4 *N* sulfuric acid and heated under reflux for 18 hr. After cooling, the crystals which separated were collected and washed well with water. Crystallization from ethanol–water gave 110.9 mg of needles (**8**), mp 239–241° then 295–300° (lit[5a] 240°/294–297°); $\lambda_{max}$ 249 m$\mu$ ($\epsilon$ 14,200), 242 (12,400), 258 (9500); $\nu_{max}$ (Nujol) 3670, 3460, 1700, and 1650 cm$^{-1}$. Identity with an authentic sample of de(hydrochloro)senegenin[5a] was confirmed by mixture melting point, infrared spectra, and tlc.

**Treatment of "Hydroxysenegenin" with Hydrochloric Acid.** "Hydroxysenegenin" (30 mg) was heated at reflux in a mixture of ethanol (1.5 ml) and 6 *N* hydrochloric acid (1.5 ml) for 13 hr. The mixture was extracted with ether, and the ethereal solution was washed with saturated sodium chloride solution, dried, and evaporated to give a crystalline residue, which showed a negative Beilstein test. Recrystallization from ethanol–water gave needles, mp 237–240°, 294–298°, which were identical with an authentic sample of de(hydrochloro)senegenin[5a] in all respects (ultraviolet, infrared spectra, tlc, and mixture melting point).

**Cyclosenegenin (9).** A solution of 300 mg of senegenin in 20 ml of 2 *N* aqueous sodium hydroxide solution was heated at reflux under nitrogen for 16 hr. The solution was acidified carefully with dilute phosphoric acid with cooling in ice and then extracted with a mixture of chloroform and ethanol (4:1). The organic layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to dryness *in vacuo*. When crystallized from ethanol–water (3:2) the residue gave needles (105 mg), which were recrystallized from ethanol to provide an analytical specimen of cyclosenegenin (**9**), mp 302–305° (with effervescence); $[\alpha]^{22}$D +57.8° (*c* 0.81, EtOH); $\lambda_{max}$ 209 m$\mu$ ($\epsilon$ 6780), $\lambda$ 230 m$\mu$ ($\epsilon$ 2700); $\nu_{max}$ (Nujol) 2650 and 1710 cm$^{-1}$; $\tau$ 9.94 (1 H, unresolved triplet), 9.47 (multiplet) (in pyridine).

*Anal.* Calcd for $C_{30}H_{44}O_6$: C, 71.97; H, 8.86. Found: C, 71.77; H, 9.14.

**Cyclosenegenin Dimethyl Ester (11).** Cyclosenegenin was methylated in methanol–ether with diazomethane. The glassy ester did not crystallize from the usual solvents. Tlc ($CHCl_3$–ethyl acetate, 2:1) showed one spot, $\nu_{max}$ (film) 3570 and 1730 cm$^{-1}$; $\tau$ 10.11 (1 H, unresolved triplet), 9.61 (broad multiplet), 9.09 (9 H, singlet (3 C-methyls)), 8.76, 8.67 (3 H each, singlets (2 C-methyls)), 6.39, 6.30 (3 H each, all singlets (2-COOC$H_3$)), 4.47 (1 H, multiplet (vinyl proton)); not analyzed.

**Treatment of Cyclosenegenin (9) with Hydrochloric Acid to Give Senegenin (1).** Cyclosenegenin (100 mg) was heated at reflux in a mixture of ethanol (1.5 ml) and 6 *N* hydrochloric acid (1.5 ml). Instantly, needles began to appear. After 3 hr of reflux, the crystals which separated were collected and washed with water. Recrystallization from ethanol–water gave 62 mg of needles, mp 280–287°, Beilstein test (+), $\nu_{max}$ (Nujol) 3500, 2700, 1695, and 1650 cm$^{-1}$. The product was identical with an authentic sample of senegenin in all respects (infrared spectra, mixture tlc, and melting point).

**Treatment of Cyclosenegenin (9) with Sulfuric Acid.** A solution of 50 mg of cyclosenegenin (**9**) in a mixture of dioxane (3.5 ml) and 4 *N* sulfuric acid (4 ml) was maintained at reflux for 3 hr. After dilution with water, the mixture was extracted with a mixture of chloroform and ethanol (4:1). The organic layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to dryness to give a residue (48 mg). A small portion of the residue, after methylation with diazomethane, showed two spots by tlc ($CHCl_3$–ethyl acetate, 3:1) corresponding to "hydroxysenegenin" (**6**) and de(hydrochloro)senegenin (**8**).

The remainder of the product was acetylated with pyridine–acetic anhydride at room temperature for 48 hr. The mixture was poured into ice water and the solid which separated was collected and chromatographed in chloroform–ethyl acetate over 2 g of silicic acid (Mallinckrodt).

Elution with mixtures of chloroform and ethyl acetate gave two fractions. The first (11 mg) crystallized from methanol as needles, mp 214–219°, $\nu_{max}$ (Nujol) 2650, 1750, 1700, and 1245 cm$^{-1}$, and was identical with de(hydrochloro)senegenin diacetate (**14**)[5a] as shown by mixture melting point, infrared spectra, and tlc. The second fraction (26 mg) crystallized from acetone–water as plates, mp 325–333° dec. The mixture melting point with "hydroxysenegenin" triacetate (**5**) showed no depression and the infrared spectrum was identical with an authentic sample of **5**.

**Presenegenin Dimethyl Ester (18).** To a solution of 40 g of senegin in 1 l. of water was added, with stirring, 50 g of solid sodium metaperiodate over a period of 30 min at room temperature. After 30 min, precipitation began. The mixture was stirred overnight at room temperature. The precipitated material (30 g) was collected, washed several times, and dissolved in 1 l. of 5% sodium hydroxide solution. After heating on a steam bath for 1 hr under nitrogen, the solution was cooled in ice and neutralized with cold, dilute phosphoric acid to pH 3. The mixture was extracted with ether–ethanol (5:1). The emulsion which formed was broken by passing through Celite. The organic layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated *in vacuo* to give a gummy residue (12 g), which was immediately methylated with diazomethane in methanol. The product was chromatographed on silicic acid (180 g, Mallinckrodt). Elution with mixtures of chloroform and ethyl acetate gave three fractions, A, B, and C in the order of elution.

Fraction A (2.3 g) crystallized from methanol as needles, mp 89–90° (lit[26] mp 89°, which proved to be methyl 4-methoxycinnamate[27] by pmr; $\tau$ 6.20, 6.17 (3 H each, singlets, OC$H_3$, COOC$H_3$), 3.70, 2.33 (2 H total, AB pattern, $J_{AB}$ = 16 cps (C$H$=C$H$)), 3.12, 2.52 (4 H total, AB pattern, $J_{AB}$ = 9 cps (four aromatic protons)).

Fraction B (0.94 g) crystallized as prisms, mp 68–69° (lit[28] mp 64°), which proved to be methyl 3,4-dimethoxycinnamate[27] by pmr; $\tau$ 6.19 (3 H, singlet (COOC$H_3$)), 6.10 (6 H, singlet (2OC$H_3$)), 3.69, 2.35 (2 H total, AB pattern, $J_{AB}$ = 16 cps (CH=CH)), 3.14, 2.84 (2 H total, AB pattern, $J_{AB}$ = 9 cps (two aromatic protons)), 2.94 (1 H, singlet (one aromatic proton)).

Fraction C (1.5 g) was crystallized once from ether and then twice from methanol–water to give needles of presenegenin dimethyl ester (**18**), mp 223–225°; $[\alpha]^{28}$D +80.0° (*c* 0.5, $CHCl_3$), (lit[17] mp 214–215°, $[\alpha]$D +78°); $\nu_{max}$ (Nujol) 3560 and 1723 cm$^{-1}$; $\tau$ 9.33, 9.10, 9.07, 8.77, 8.66 (3 H each, all singlets (**5** C-methyls)), 6.80 (2 H, unresolved AB pattern), 6.35, 6.28 (3 H each, singlets (2COOC$H_3$)), 5.84 (2 H, multiplet), 4.13 (1 H, unresolved triplet (vinyl proton)).

*Anal.* Calcd for $C_{32}H_{50}O_7$: C, 70.30; H, 9.22. Found: C, 70.27; H, 9.08.

**Presenegenin Dimethyl Ester Acetonide (19).** To a solution of 100 mg of presenegenin dimethyl ester (**18**) in 10 ml of dry acetone was added 3 g of freshly dried copper sulfate and the mixture was stirred at room temperature. The reaction was followed by tlc (benzene–ethyl acetate, 4:1). After 2 hr, the reaction was complete and 1 g of anhydrous potassium carbonate was added to the reaction mixture. After filtration, evaporation of the filtrate gave a noncrystalline residue (102 mg) which was homogeneous by tlc; $\nu_{max}$ (film) 3640 (small, sharp), 1730, 1030, 915, 878, and 835 cm$^{-1}$.

**Dimethyl 2$\beta$,3$\beta$-Isopropylidenedioxy-13,27-cyclo-$\Delta^{11}$-oleanene-23,28-dioate (20).** Presenegenin dimethyl ester acetonide (**19**, 202 mg) was dissolved in dry pyridine (10 ml) containing tosyl chloride (500 mg) and heated at reflux for 5 hr under nitrogen. The mixture was poured into ice water and the crystals which separated were collected. The product in benzene was passed through a Florisil column to remove brown material. The eluate showed one spot on tlc (benzene–ethyl acetate, 4:1) and crystallized from aqueous methanol to give 145 mg of fine needles of compound **20**, mp 181–185°; $[\alpha]^{23.5}$D +63.5° (*c* 0.62, $CHCl_3$); $\lambda_{max}$ 221 m$\mu$ ($\epsilon$ 5900); $\nu_{max}$ (Nujol) 1730, 1020, 915, 870, and 835 cm$^{-1}$; $\tau$ 9.67 (1 H, half of AB pattern, $J_{AB}$ = 5 cps (cyclopropyl proton)), 9.33, 9.08, 9.05, 8.88, 8.76, 8.68, 8.49 (3 H each, all singlets (7 C-methyls)), 6.28, 6.35 (3 H each, singlets (2COOC$H_3$)), 5.55 (2 H, a broad singlet), 4.78, 4.10 (2 H, AB pattern, $J_{AB}$ = 11 cps (CH=CH)).

*Anal.* Calcd for $C_{35}H_{52}O_6$: C, 73.91; H, 9.22. Found: C, 74.01; H, 9.02.

**Dimethyl 2$\beta$,3$\beta$-Dihydroxy-13,27-cyclo-$\Delta^{11}$-oleanene-23,28-dioate (21).** A solution of 130 mg of compound **20** in 10 ml of 80% acetic acid was heated on a steam bath and the reaction was followed by tlc (benzene–ethyl acetate, 4:1). After 10 min, 4 ml of water was added and the mixture was allowed to cool. The needles

(26) W. H. Perkin, *J. Chem. Soc.*, **39**, 409 (1881).

(27) Dugan and de Mayo[17] reported the isolation of 4-methoxycinnamic acid but we also isolated 3,4-dimethoxycinnamic acid.

(28) F. Tiemann and W. Will, *Ber.*, **14**, 946 (1881).

which separated were collected and crystallized from ethanol–water (4:1) to give 110 mg of needles of compound **21**, mp 223–227°; $[\alpha]^{29}D$ +42.5° (*c* 0.86, $CHCl_3$); $\lambda_{max}$ 220 mμ (ε 5250); $\nu_{max}$ (Nujol) 3640, 1730, and 1695 $cm^{-1}$.

*Anal.* Calcd for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15. Found: C, 72.33; H, 8.84.

**Dimethyl 2β,3β-Dihydroxy-13,27-cyclooleanene-23,28-dioate (22).** Compound **21** (90 mg) was added to prereduced $PtO_2$ (100 mg) in acetic acid (10 ml) and stirred under a hydrogen atmosphere. After 10 min, 3.6 ml of hydrogen (at 26°) was absorbed. After 30 min, the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue crystallized from methanol–water as needles (72 mg), mp 221°, then 237°; $[\alpha]^{28.5}D$ +58.5° (*c* 0.40, $CHCl_3$); $\nu_{max}$ (Nujol) 3700, 1735, and 1695 $cm^{-1}$; τ 10.01, 9.49 (2 H, AB pattern, $J_{AB}$ = 5 cps (cyclopropyl protons)), 9.10 (9 H, singlet (3 C-methyls)), 8.80, 8.66, (3 H each, singlets (2 C-methyls)), 6.32, 6.28 (3 H each, singlets ($2COOCH_3$)). Though this compound showed one spot on tlc; the pmr spectrum indicated a small amount of a contaminant probably due to the 1,4-addition product.

*Anal.* Calcd for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.36; H, 9.36.

**Dimethyl 2β,3β-Dihydroxy-Δ$^{12}$-oleanene-23,28-dioate (Dimethyl Medicagenate) (26).** A solution of 54 mg of compound **22** in a mixture of 10 ml of acetic acid and 1.6 ml of concentrated hydrochloric acid was heated at reflux for 1 hr. Dilution of the reaction mixture with water afforded a crystalline solid which showed the presence of partially acetylated materials by tlc ($CHCl_3$–ethyl acetate, 3:1). The solid was heated in a solution of 100 mg of potassium carbonate in 10 ml of methanol and 4 ml of water for 1 hr. Upon cooling, crystals separated (42 mg). Crystallization from methanol afforded needles of dimethyl medicagenate, mp 229–237°; $[\alpha]^{26.5}D$ +88.5° (*c* 0.45, $CHCl_3$); $\nu_{max}$ (Nujol) 3540, 3470, 1735, 1720, and 1690 $cm^{-1}$; identical with an authentic sample[18] in all respects (mixture melting point, infrared spectra, and tlc) (Figure 1).

*Anal.* Calcd for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.57; H, 9.55.

**Dimethyl 2β,3b-Acetoxy-Δ$^{12}$-oleanene-23,28-dioate (Dimethyl Medicagenate Diacetate) (27).** A sample of 10 mg of dimethyl medicagenate (**26**), obtained by degradation of presenegenin, was acetylated at room temperature in a mixture of 0.5 ml of pyridine and 0.25 ml of acetic anhydride. After 72 hr, the mixture was poured into ice water. The product which separated crystallized from methanol as rhombs (6 mg), mp 238–241°, $\nu_{max}$ (Nujol) 1760 (with inflection at 1770), 1730, 1740 $cm^{-1}$, identical with a sample of dimethyl medicagenoate diacetate prepared from authentic medicagenic acid diacetate.[18c]

**Catalytic Hydrogenation of Dimethyl 2β,3β-Isopropylidenedioxy-13,27-cyclo-Δ$^{11}$-oleanene-23,28-dioate (20).** The title compound (55 mg) was shaken with 100 mg of prereduced $PtO_2$ in 10 ml of acetic acid for 1 hr. The hydrogen uptake amounted to 1.6 equiv. The catalyst was removed by filtration and evaporation of the solvent *in vacuo* gave an oily residue, which was heated in a mixture of acetic acid (10 ml) and hydrochloric acid (1.6 ml) for 1 hr under reflux. The mixture was extracted with ether, the extract was evaporated, and the residue was heated for 40 min on a steam bath with a solution of 100 mg of potassium carbonate in 10 ml of 90% methanol. Dilution with water gave an amorphous substance. Tlc (benzene–ethyl acetate, 4:1) showed one major and one minor spot, separated on preparative tlc to give fraction A (31 mg) and fraction B (6 mg). Fraction A is an oily substance (**23**), $\nu_{max}$ (film) 3650 and 1740 $cm^{-1}$; τ 9.27 (3 H), 9.08 (6 H), 8.93, 8.87, 8.84, 8.78, 8.73, 6.37, 6.28 (3 H each), 4.70 (1 H, triplet).

Fraction B crystallized from methanol–water as needles, mp *ca.* 221°, but was not purified further.

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# Purine Nucleosides. XIV. Unsaturated Furanosyl Adenine Nucleosides Prepared *via* Base-Catalyzed Elimination Reactions of 2′-Deoxyadenosine Derivatives[1]

**James R. McCarthy, Jr., Morris J. Robins, Leroy B. Townsend, and Roland K. Robins**

*Contribution from the Department of Chemistry, University of Utah, Salt Lake City, Utah 84112.* *Received December 13, 1965*

**Abstract:** 3′-O-Tosyl-2′-deoxyadenosine (I) has been treated with sodium methoxide in dimethylformamide to provide the first reported synthesis of 2′,3′-dideoxy-2′,3′-didehydroadenosine (II). Studies have been made which support a simple E2 mechanism for the general introduction of a 2′,3′ double bond from the corresponding 3′-O-tosyl-2′-deoxyadenosine derivative under these conditions. In the presence of potassium *t*-butoxide in dimethyl sulfoxide further elimination occurs with 5′-S-ethyl-3′-O-tosyl-5′-thio-2′,5′-dideoxyadenosine (V) to yield 9-(5′-methyl-2′-furyl)adenine (X) as a final product. A mechanism has been proposed for the formation of X consistent with the present work. The preparation of these unsaturated adenine nucleosides has provided a new route to the synthesis of 2′,3′-dideoxyadenosine (III) and 2′,3′,5′-trideoxyadenosine (VIII) by direct hydrogenation procedures. The direct utilization of these novel unsaturated nucleoside derivatives as reaction intermediates offers a unique opportunity for future synthetic studies.

The interest in the synthesis of 2′,3′-unsaturated nucleoside derivatives has been stimulated by the proposal that unsaturation at the 2′,3′ position is an intermediate step in the biosynthesis of 2′-deoxyribonucleotides.[2–4] Additional interest in unsaturated nucleosides has resulted from the recent elucidation of the structure of the antibiotic blasticidin S as a 2′,3′-unsaturated pyranosyl derivative of cytosine.[5] Decoy-

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