Hydrogenation of XIX.—Hydrogenation of 50 mg. of XIX in 10 ml. of hexane with 12 mg. of platinum oxide as a catalyst at atmospheric pressure provided 42 mg. of XVI, identified by infrared spectra comparison with that of the previously described sample of XVI.

Pyrolysis of IV.—A 3 × 35 cm. Pyrex tube filled with $^3/_{16}$-in. glass helices and equipped with a micro addition funnel and a nitrogen inlet tube at the top and a round-bottom flask immersed in a Dry Ice–acetone bath at the bottom was used for the pyrolysis. One gram of IV in 10 ml. of hexane was passed through the column heated at 340° over 2.5 hr. with a nitrogen flow rate of 20 cc./min. The condensate was allowed to warm to room temperature with no appreciable amounts of volatile materials distilling. The hexane was removed by vacuum evaporation at 25° to leave 790 mg. of oil. Analysis by g.l.p.c. on a 0.25 in. × 10 ft. column packed with 20% DC-550 silicone oil on Diatoport S at 200° showed a number of very weak, low retention time peaks and three major peaks at 7.9, 12.3, and 16 min. Samples of the major peaks were isolated by preparative g.l.p.c. using the same column.

The 7.9-min. R_t component was shown to be 1-formyl-3,6,6-trimethyl-1,3-cyclohexadiene (XXI): $\lambda_{max}^{CCl_4}$ 3.69, 5.95, 6.09 μ; $\lambda_{max}^{CH_3OH}$ 310 mμ (ϵ 9800) [lit.[19] $\lambda_{max}^{C_6H_{14}}$ 309 mμ (ϵ 9000)]; δ_{CDCl_3} = 1.21 (6H, singlet), 1.92 (3H, narrow multiplet), 2.12 (2H, narrow multiplet), 6.00 (1H, broad multiplet), 6.63 (1H, doublet, J = 5.5 c.p.s.), 9.44 (1H, singlet) p.p.m.; semicarbazone m.p. 211–213° (lit.[19] m.p. 213°). The infrared spectra of an authentic sample of the aldehyde prepared from 3-methylcrotonaldehyde was identical with the infrared spectra of the above sample.[20]

The 12.3-min. R_t component was identified as isopiperitenone (XX): $\lambda_{max}^{CH_3OH}$ 236 mμ (ϵ 10,800); $\lambda_{max}^{CCl_4}$ 3.26, 5.97, 6.10, 11.23 μ; δ_{CDCl_3} = 1.77 (3H, narrow multiplet), 1.97 (3H, narrow multiplet), 2.00–2.50 (4H, broad multiplet), 2.96 (1H, triplet, J = 8 c.p.s.), 4.78 (1H, narrow multiplet), 4.96 (1H, narrow multiplet), 5.92 (1H, narrow multiplet) p.p.m.; 2,4-dinitrophenylhydrazone m.p. 155–156° (lit.[18] m.p. 155–156°). One drop of 0.1 N sodium hydroxide added to a methanolic solution of XX quantitatively isomerized it to piperitenone (II) as shown by the change in the ultraviolet spectra of the solution.

The 16.0-min. R_t component was identified as piperitenone (II): 2,4-dinitrophenylhydrazone m.p. 184–185°.

When the pyrolysis was conducted at lower temperatures (250°), considerable starting material was recovered along with XXI and XX but no II. At higher temperatures (400°) II and m-xylene were the only products isolated. Both piperitenone (II) and V were recovered unchanged when they were pyrolyzed at 340°.

Lactonization of the Methyl Ester VII.—A solution of 1 g. of VII and 1 g. of p-toluenesulfonic acid in 45 ml. of toluene was refluxed for 16 hr. The solution was washed with water, 5% sodium hydroxide, and water. Evaporation of the toluene provided 940 mg. of oil that was a mixture of the starting methyl ester (30%) and the two isomeric γ-lactones XII and XIII (70%); the identity of the γ-lactones was established by infrared spectra comparison of preparative g.l.p.c. isolated samples.

Lactonization of α-Fencholenic Acid (VI).—Under the conditions described above, 1 g. of VI provided 360 mg. of starting material and 610 mg. of crude δ-lactone XIV, which was crystallized from pentane to give 440 mg. of crystalline product, m.p. 77–78°. A sample of XIV, m.p. 77–78°, prepared from VI and concentrated sulfuric acid,[18a] was identical by infrared spectra and admixture melting point determinations.

Acknowledgment.—The author is indebted to Professor G. Büchi for many stimulating discussions and to Mr. L. Lackey for technical assistance.

(20) Considerable care had to be exercised in isolating this component to avoid contamination with an impurity that appeared as a weak peak at 8.7 min. Insufficient amounts of this component were available for positive identification, but from the infrared and n.m.r. spectra of the mixture of the two components we believe it to be the nonconjugated aldehyde 1-formyl-4,6,6-trimethyl-2,4-cyclohexadiene.

The Structure of Senegenic Acid, a Nortriterpene Artifact from *Polygala senega*[1]

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Hydrolysis of senegin, the partially purified saponin from *Polygala senega* root, with ethanolic hydrochloric acid gave a complex mixture from which senegenin and a new triterpene acid, senegenic acid, and its monoethyl ester were isolated. Chemical and spectroscopic evidence led to assignment of structures **22** and **24** to senegenic acid and its monoethyl ester, respectively. Senegenin and senegenic acid are artifacts produced during hydrolysis of the senega saponins.

Although the extracts of *Polygala senega* L. (*Polygalaceae*) have been used as an expectorant for centuries, little work has appeared in the literature regarding the chemistry of the saponins of this plant. Quevenne[2] first isolated a saponin from *Polygala senega* which he named "senegin." At a later period Kobert[3] designated a neutral saponin as "senegen" and an acidic saponin as "polygalic acid."

The first chemically significant results on this problem were reported by Wedekind and Krecke[4] who, from the acid hydrolysate of commercial senegin, isolated a sapogenin, which they called senegenin and to which they assigned the formula, $C_{26}H_{46}O_6$. This compound was dibasic, contained two hydroxyl groups as indicated by formation of a diacetate, and gave a dimethyl ester. A more extensive chemical investigation of the sapogenins from *P. senega* was later reported by Jacobs and Isler.[5] The partially purified saponin was hydrolyzed with ethanolic hydrochloric acid to give a sapogenin mixture from which two crystalline sapogenins were isolated on the basis of solubility differences in ethanol. The less soluble compound appeared to be the same as the senegenin of Wedekind and Krecke,[4] although the physical constants differed somewhat. Analytical figures agreed with the molecular formula $C_{30}H_{46}O_8$ or $C_{30}H_{44}O_8$. An inert double bond was suggested by a positive tetranitromethane test and resistance to hydrogenation. Titration indicated the presence of two carboxyl groups and the presence of a lactone was inferred by consumption of a third equivalent of alkali on heating.

(1) Early phases of this work were carried out in the laboratories of the Rockefeller Institute, New York, N. Y.

(2) P. Quevenne, *J. prakt. Chem.*, **12**, 427 (1837); *Ann. Chem.*, **20**, 34 (1836); **28**, 248 (1838).

(3) R. Kobert, *Pharm. Zentralhalle*, 1631 (1885).

(4) E. Wedekind and R. Krecke, *Ber.*, **57**, 118 (1924).

(5) W. A. Jacobs and O. Isler, *J. Biol. Chem.*, **119**, 155 (1937).

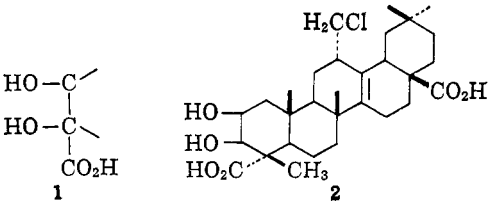
From the more soluble sapogenin fraction, a second crystalline sapogenin, m.p. 215–218° and 257°, $[\alpha]^{20}D$ +24.5° (EtOH), was obtained which had an analysis corresponding to $C_{31}H_{48}O_6$ or $C_{31}H_{50}O_6$.[5] Further investigation showed that this material was the monoethyl ester of a dibasic acid of formula $C_{29}H_{46}O_6$ or $C_{29}H_{44}O_6$. This dibasic acid, m.p. 230°, could be obtained by vigorous alkaline hydrolysis of the monoethyl ester. The authors[5] attributed the presence of the ester to partial esterification of the dibasic acid during hydrolysis with ethanolic hydrochloric acid. These workers, however, were not able to detect the presence of the free dibasic acid in the saponin hydrolysate.

The monoethyl ester of the dibasic acid[5] easily formed an amorphous neutral methyl ethyl diester and a crystalline diacetate, thus confirming the presence of two carboxyl and two hydroxyl groups. The monoethyl ester gave a positive test with tetranitromethane, but no double bond could be detected by hydrogenation.

On selenium dehydrogenation both senegenin and the monoethyl ester of the dibasic acid gave 1,8-dimethylpicene and a chrysene homolog[5] similar to those obtained from hederagenin—results which suggested that these sapogenins were triterpenoids.

More recently senegenin has been studied by Shamma and Reiff.[6] The allocation of oxygen functions previously proposed was accepted by these workers. They also assumed that in the absence of an appropriate band in the infrared spectrum for a γ-lactone, the lactone function in senegenin must be six (or more) membered. The p.m.r. and ultraviolet spectra of senegenin derivatives were interpreted in favor of a trisubstituted double bond. These authors concluded that senegenin is tetracyclic and assigned to it the molecular formula, $C_{30}H_{44}O_8$. The formation of an isopropylidene derivative and the results of lead tetraacetate oxidation led them to suggest that senegenin contained the partial structure **1**, which required it to possess a skeleton different from that of any of the then known triterpenes. That a tetracyclic formulation is incorrect and that senegenin does not contain a lactone function has been demonstrated recently by Dugan, de Mayo, and Starratt,[7] who showed that senegenin contains an atom of chlorine and has the molecular formula, $C_{30}H_{45}ClO_6$. Thus, it is an artifact formed during hydrolysis of the saponin by ethanolic hydrochloric acid. Structure **2** has been assigned to senegenin by Dugan, de Mayo, and Starratt.[7,8]

A careful reinvestigation of the hydrochloric acid hydrolysis of *Polygala senega* in this laboratory has resulted in the isolation of senegenin, a new triterpene acid



named *senegenic acid*[14] and the monoethyl ester of the latter. The properties of this monoethyl ester compare favorably with the dihydroxy dicarboxylic acid monoethyl ester[5] reported earlier by Jacobs and Isler. In a preliminary communication[14] we have reported the structure elucidation of senegenic acid.[15] This paper discloses the details of the isolation, purification, and structure elucidation of this compound.

Isolation and Purification.—Senegin, the partially purified saponin from *P. senega* root, on hydrolysis with ethanolic hydrochloric acid gave two crude sapogenin fractions which were separated roughly by their different solubilities in aqueous ethanol. On cooling the hydrolysate to 60° and filtering the solution at this temperature, the first triterpenoid fraction was obtained. This material was further purified by fractional precipitation from boiling aqueous ethanol, giving some material which was insoluble in the boiling solvent mixture and senegenin, which crystallized from the chilled solution. The insoluble material consisted largely of senegenin and senegenic acid together with unidentified components. Fractional crystallization of this fraction furnished senegenic acid, m.p. 282–286°,[26] identical with a sample prepared by hydrolysis of senegenic acid monoethyl ester (*vide infra*).

The hydrolysate solution remaining after filtration at 60° was allowed to stand at 0° for a few days, when a second batch of crude triterpenoid mixture was obtained. The latter was found by thin layer chromatography to consist of senegenin, senegenic acid, and senegenic acid monoethyl ester with traces of minor

(6) M. Shamma and L. P. Rieff, *Chem. Ind.* (London), 1272 (1960).

(7) J. J. Dugan, P. de Mayo, and A. N. Starratt, *Can. J. Chem.*, **42**, 491 (1964); *Proc. Chem. Soc.*, 264 (1964).

(8) Several other triterpenoids have been isolated recently from various *Polygala* species. *E.g.*, *Polygala tenuifolia* Willd. contains tenuifolic acid,[9,10] $C_{30}H_{44-46}O_8$, which is also present in *Bredemeyera floribunda* Willd. A substance similar to senegenin and tenuifolic acid has been isolated from *P. Senega* L. var. *latifolia* Torr. and Gray.[11] Structures have been assigned to polygalacic acid (*P. paenea*) by Polonsky[12] and to bredemolic acid, a companion of tenuifolic acid by Tschesche, *et al.*[13]

(9) T. Q. Chou, J. H. Chu, and P. F. Mei, *J. Am. Pharm. Assoc., Sci. Ed.*, **36**, 241 (1947).

(10) R. Tschesche and A. K. Sen Gupta, *Ber.*, **93**, 1903 (1960).

(11) M. Fujita and H. Itokawa, *Chem. Pharm. Bull.* (Tokyo), **9**, 1006 (1961).

(12) J. Rondest and J. Polonsky, *Bull. soc. chim. France*, 1253 (1963).

(13) R. Tschesche, E. Henckel, and G. Snatzke, *Tetrahedron Letters*, 613 (1963).

(14) S. W. Pelletier, N. Adityachaudhury, M. Tomasz, J. J. Reynolds, and R. Mechoulam, *ibid.*, **No. 41**, 3065 (1964).

(15) This compound has been studied by J. J. Dugan, P. de Mayo, and A. N. Starratt [*ibid.*, **No. 37**, 2567 (1964)], who have independently derived the same structure as in this paper. We thank Professor de Mayo for providing us with a copy of their manuscript prior to publication. The Canadian authors, following the nomenclature of Shamma and Irwin,[16] designate the dihydroxydicarboxylic acid as *polygalic acid*. However, this name is already firmly established in the literature[9,11,17–25] to designate the crude acidic *saponin* (mixture) from *Polygala senega*. To avoid the confusion which would result from now using the name polygalic acid for a sapogenin, we have named this compound *senegenic acid*.[14]

(16) W. E. Irwin, Ph.D. Thesis, The Pennsylvania State University, 1962.

(17) C. F. Millspauch, "American Medicinal Plants," Boericke and Tafel, New York, N. Y., 1887, p. 45-3.

(18) A. Funaro, *J. Chem. Soc.*, **58**, 262 (1890).

(19) H. Kraemer, "Scientific and Applied Pharmacognosy," Kraemer, Philadelphia, Pa., 1915, p. 464.

(20) E. N. Gathercoal and E. H. Wirth, "Pharmacognosy," Lea and Febiger, Philadelphia, Pa., 1936, p. 432.

(21) N. L. Allport, "Chemistry and Pharmacy of Vegetable Drugs," George Newnes, London, 1943, p. 107.

(22) B. E. Hebert and K. W. Ellery, "Practical Pharmacognosy," Bailhere, Tindall and Cox, London, 1948, p. 56.

(23) H. W. Youngken, "Textbook of Pharmacognosy," 6th Ed., Blakiston, Philadelphia, Pa., 1950, p. 516.

(24) T. E. Wallis, "Textbook of Pharmacognosy," 2nd Ed., J. and A. Churchill Ltd., London, 1951, p. 390.

(25) T. Kariyone and S. Shibata, "Dictionary of Medicinal Plants," Hirokawa, Tokyo, 1963, p. 190.

(26) The melting point of senegenic acid varies greatly depending on the temperature at which it is placed on the hot stage. The following results are typical where the value in parenthesis is the temperature at which the sample was placed on the hot stage: 248–250° (30°); 272–278° (250°); 282–286° (265°). Dugan, *et al.*,[7] reported m.p. 299–301° in an evacuated capillary.

components. It was observed at an early stage of our work that both senegenin and senegenic acid are practically insoluble in chloroform, whereas senegenic acid ethyl ester is very soluble. By virtue of this difference in solubility the triterpenoid mixture was separated roughly into equal parts of chloroform-soluble and chloroform-insoluble materials. Methylation of the chloroform-insoluble material with diazomethane afforded a mixture of the dimethyl esters of senegenin[27] and senegenic acid from which crystalline dimethyl senegenate, m.p. 135–170°, 194–196°, was obtained in a pure state by fractional crystallization from methanol.

The *chloroform-soluble* material yielded crude senegenic acid monoethyl ester which was purified by fractional crystallization from aqueous ethanol. The pure monoethyl ester showed m.p. 221–224°, $[\alpha]D$ +23.4° (EtOH), and gave a crystalline ethyl methyl diester, m.p. 193–195°, $[\alpha]D$ +20.3°, on treatment with diazomethane. Impure senegenic acid monoethyl ester containing traces of senegenin is best purified by methylation and subsequent chromatography of the methylated products over acid-washed alumina or by crystallization of the diethylamine salt, m.p. 221–224°, from ethyl acetate.

Hydrolysis of either senegenic acid monoethyl ester or dimethyl senegenate with amyl alcoholic potassium hydroxide gave senegenic acid, m.p. 284–288°,[27] $[\alpha]D$ +23° (EtOH), identical with a sample isolated directly from the hydrolysis mixture. The acid so isolated had the properties ascribed to it by Jacobs and Isler except for the melting point,[28] but as will be seen in the sequel, the empirical formula requires modification to $C_{29}H_{44}O_6$, a point difficult to establish by analyses alone.

Structure of Senegenic Acid.—Mass spectrometric molecular weight determinations[29] of the monoethyl, ethyl methyl, and dimethyl esters of senegenic acid demonstrated that the correct molecular formula for the acid is $C_{29}H_{44}O_6$. Senegenic acid is, therefore, a nortriterpene acid. It forms a crystalline diacetate, dimethyl ester, and dimethyl ester diacetate (see Table I for derivatives), thus accounting for the six oxygen functions as two hydroxyl and two carboxyl groups. The presence of at least one double bond in senegenic acid is indicated by the yellow color given with tetranitromethane and also by the ultraviolet end absorption [λ_{max} 205 mμ (ϵ 5000)]. The unsaturated center is inert to catalytic hydrogenation. The formation, by the action of hydrogen chloride on senegenic acid diacetate, of a saturated lactone diacetate (isolated as the methyl ester) which involves one of the carboxyl groups and the unsaturated center, confirms the presence of one double bond and moreover suggests that this double bond is either β,γ or γ,δ to one of the carboxyl groups. The presence of a single double bond was also confirmed by titration of senegenic acid derivatives with perbenzoic acid. That the double bond is tetrasubstituted is indicated by the absence of a vinylic proton in the p.m.r. spectra of various senegenic acid derivatives.

Kuhn–Roth oxidation[30,31] of senegenic acid monoethyl ester gave only acetic acid and no trace of higher fatty acids; thus, no straight alkyl side chain other than C–CH_3 is present. This evidence, coupled with earlier selenium dehydrogenation studies,[5] strongly suggested a pentacyclic skeleton for this nortriterpene acid.

Selective saponification of dimethyl senegenate diacetate with 5% potassium hydroxide leads to crystalline dimethyl senegenate. Since these mild conditions are usually not sufficient to saponify the conventional 3β-acetoxy group of a pentacyclic triterpene, the presence of additional activating substituents in ring A is suggested. The p.m.r. spectra (Table II) of both the ethyl methyl and dimethyl esters of senegenic acid show bands at τ 5.84 (1H multiplet) and 6.10 (1H doublet) which shift to τ 4.5 (1H multiplet) and 4.68 (1H doublet) on acetylation, indicating that the two hydroxyls are secondary in nature. Moreover, the appearance of one of the *H*–C–OH protons as a doublet in the p.m.r. spectra of the above derivatives indicates that the carbon adjacent to the one bearing the proton is quaternary, and the glycol system **3** is present in the A ring of senegenic acid. The presence of the 1,2-glycol

TABLE I

SENEGENIC ACID DERIVATIVES

Compound	M.p., °C.	$[\alpha]D$, deg.
Senegenic acid (**22**)	284–288	+23 (EtOH)
Diacetate **23**	274–275	+34
Monoethyl ester **24**	221–224	+23.4 (EtOH)
Monoethyl ester monoacetate **30**	160–161	+25.5
Monoethyl ester diethylamine salt	221–224	
Dimethyl senegenate (**26**)	135–137, 194–196	+20.5
Dimethyl senegenate diacetate (**27**)	174–176	+31
Dimethyl senegenate acetonide (**17**)	161–163	+40.2
Monomethyl senegenate (**25**)	257–261	+21.1
Ethyl methyl diester **28**	193–195	+20.3
Ethyl methyl diester diacetate **29**	142–145	
α-Ketol acid ethyl ester **5**	191–196	+10
α-Ketol acid ethyl ester acetate **6**	230–235	+30.2
Diosphenol acetate **8**	194–196	+13.2
α,β-Unsaturated ketone DNPH	204–206, 218–219	
Decarboxysenegenic acid	274–279	
α-Ketol ester **11a** and **11b**	(A) 117–120 (B) 118–127 (C) 128–135	
α-Ketol ester acetate **12a** and **12b**	(B) 143–150 (C) 160–163, 185–190	
Ketone **14**	117–121	
Ketone DNPH	136–140	
Bromolactone **31**	284–289	+49 (EtOH)
Bromolactone diacetate **32**	178–181	
Dehydrosenegenic acid (**33**)	223–225, 279–284	+59 (EtOH)
Dimethyl dehydrosenegenate (**34**)	144–146	+54.4
Lactone diacetate methyl ester **38**	274–278	+28.2

(27) Senegenin forms an amorphous dimethyl ester which is very soluble in methanol.

(28) It was reported to have m.p. 230°.

(29) We are grateful to Professor K. Biemann and Dr. B. Das, Massachusetts Institute of Technology, Cambridge, Mass., for the mass spectral determinations.

(30) C. F. Garbers, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **37**, 1336 (1954).

(31) B. Lindqvist and T. Storgards, *Acta Chem. Scand.*, **7**, 87 (1953).

TABLE II
P.M.R. SPECTRA[a]

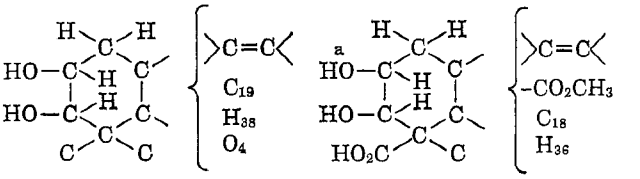
Senegenic acid derivative	H–C–OAc	H–C–OH	$-COOCH_2CH_3$	$-COOCH_3$	$-COCH_3$	$-COOCH_2CH_3$	$-C-CH_3$ C-4	C-10	C-8	C-20 (gem-dimethyl)
Monoethyl ester (24)	...	5.83[b] (2H)	5.92 q $J = 6.6$	...	...	8.80 t $J = 6.6$	8.67	8.82	9.06	9.09 (6H)
Ethyl methyl diester (28)	...	5.84[b] (2H)	5.92 q $J = 6.6$	6.32	...	8.80 t $J = 6.6$	8.63	8.83	9.07	9.12 (6H)
Ethyl methyl diester diacetate (29)	4.55 m (1H) 4.68 d (1H) $J = 4.2$	...	5.92 $J = 6.6$	6.36	7.95 8.06	8.80 t $J = 6.6$	8.63	8.87	9.08	9.11 (6H)
Dimethyl ester (26)	...	5.84 m (1H) 6.10 d (1H) $J = 3.5$	...	6.31 6.41	...	...	8.67	8.84	9.08	9.10, 9.11
Dimethyl ester diacetate (27)	4.51 m (1H) 4.68 d (1H) $J = 4.2$	...	...	6.36 6.41	7.95 8.06	...	8.62	8.87	9.07	9.10, 9.11
Ethyl ester monoacetate (30)	4.75 d (1H) $J = 3.8$	5.83[b] (1H)	5.92 q $J = 6.6$	...	7.92	8.80 $J = 6.0$	8.60	8.80	9.07	9.09 (6H)
Dimethyl dehydro (34)	...	5.87 m (1H) 6.04 d (1H) $J = 3.8$	...	6.34 6.44	...	...	8.65	8.75	8.99	9.09, 9.15
Lactone diacetate methyl ester (38)	4.57 m (1H) 4.72 d (1H) $J = 3.8$	...	...	6.37	7.97 8.07	...	8.64	8.82	9.00	9.02, 9.09

[a] d = doublet, m = multiplet, q = quartet, t = triplet, J value given in cycles per second. [b] Protons overlapped in the quartet region.

system has been confirmed by the consumption of 1 equiv. of periodate by either the monoethyl ester or the ethyl methyl diester. Moreover, the cleavage product of senegenic acid ethyl methyl diester shows the retention of both the methyl and ethyl ester groups in the p.m.r. spectrum, confirming that the glycol system is part of a ring.

With a view to providing further information as to the environment of the vicinal hydroxyl groups, attention was directed toward locating the positions of the two carboxyl groups. Senegenic acid readily forms a crystalline bromolactone,[32] m.p. 284–289°, showing infrared adsorption consistent for a γ-lactone (1773 cm.$^{-1}$). Treatment of the bromolactone with zinc dust in acetic acid regenerates senegenic acid, demonstrating that no rearrangement has occurred during bromolactone formation. Senegenic acid monoethyl ester, on the other hand, remains unaffected when treated with bromine under the same conditions. This implies that one of the angular carboxyl groups must be situated either β,γ or γ,δ to the tetrasubstituted double bond. Moreover, the above evidence suggests that the carboethoxyl group of senegenic acid monoethyl ester, which is formed by esterification of senegenic acid (see Experimental Section) is most probably situated near the double bond.

Again, a difference in the reactivity of the two carboxyl groups was noted when dimethyl senegenate was boiled with 10% methanolic potassium hydroxide. In contrast to methyl oleanolate and related triterpenes in which the methyl esters of C-17 carboxylic acids require severe conditions (diethylene glycol or sealed tube) for saponification,[33] dimethyl senegenate gave a monomethyl ester, m.p. 257–261°, $[\alpha]D$ +21°. This ester, like senegenic acid monoethyl ester, did not form a bromolactone under the conditions[32] successfully employed with senegenic acid. These observations suggest that the carbomethoxyl group which undergoes smooth hydrolysis is most probably situated adjacent to the glycol moiety as in **4**. This partial structure for the monomethyl ester **4** is supported by the following sequence of reactions.



3 **4**

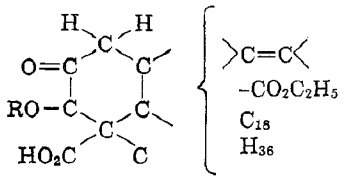
Oxidation of senegenic acid monoethyl ester with Jones' reagent[34] afforded a crystalline α-ketol acid (**5**), m.p. 191–196°, $[\alpha]D$ +10°, showing appropriate absorption for hydroxyl and carbonyl groups (ν 3448, 1709 cm.$^{-1}$) and a band in the p.m.r. at τ 5.32 which shifts to τ 4.36 in the corresponding acetate **6**. The appearance of the *H*–C–OAc signal as a singlet indicates that the hydroxyl function labeled a in **4** is that oxidized to a ketone as in the partial formula **5**. Treatment of the ketol acid **5** with 10% methanolic potassium hydroxide and acidification gave rise to a diosphenol (**7**) [ν_{max} 3413, 1724, 1672, 1645 cm.$^{-1}$; λ_{max} 281 mμ; λ_{max} (EtOH–KOH) 329 mμ] which was characterized as an enol acetate (**8**): m.p. 194–196°; $[\alpha]D$ +13.2°; ν_{max} 1764, 1718, 1672, 1645 cm.$^{-1}$; λ_{max} 249 mμ (ϵ 9822). The appearance of a vinylic methyl signal (τ 8.24, 3H singlet) defines the position of the methyl group in **7** and **8**.

While studying the behavior of the α-ketol carboxylic acid **5** toward methanolic potassium hydroxide, several interesting reactions were observed which will now be

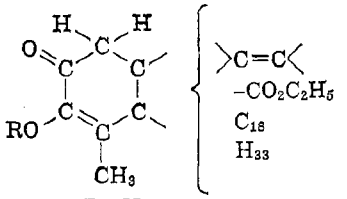
(32) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 887 (1954).

(33) C. Djerassi and A. W. Lippman, *J. Am. Chem. Soc.*, **77**, 1825 (1955); C. Djerassi and H. G. Monsimer, *ibid.*, **79**, 2901 (1957).

(34) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).



5, R = H
6, R = Ac



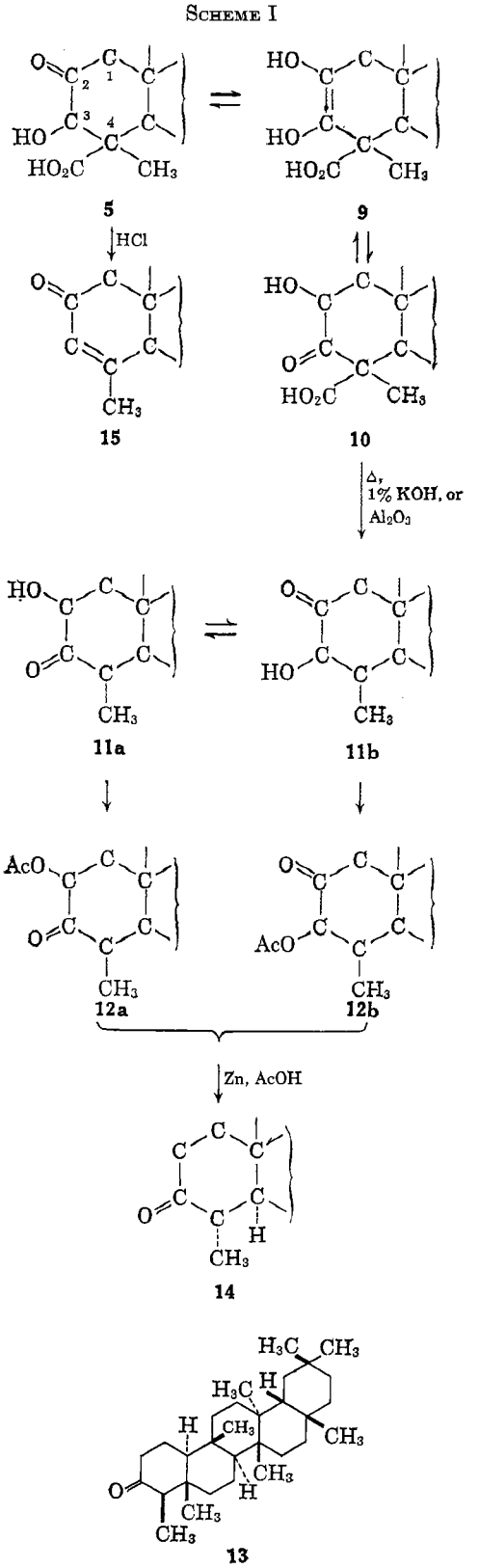
7, R = H
8, R = Ac

described. The α-ketol acid could be easily decarboxylated by (a) pyrolysis under nitrogen; (b) by refluxing in 1% methanolic potassium hydroxide; or (c) by standing overnight in contact with acid-washed alumina. The products of these reactions appear to be a mixture of the α-ketols **11a** and **11b** which arise from the γ-keto acid **5** *via* the enediol **9** and β-keto acid **10**. The mass spectrum of the product showed a molecular weight of 470.[29] The cleanest product results from alumina treatment and shows m.p. 128–135° (uncor.). It is readily converted into the previously cited diosphenol **7** either by percolation over an alumina column or by oxidation with cupric acetate.[35] If the reaction of the α-ketol acid **5** with base and also the pyrolysis of the same compound were not conducted under nitrogen, simultaneous oxidation and decarboxylation occurred to give a mixture of the above α-ketol derivatives **11a** and **11b** and the diosphenol **7**. The latter could be obtained in pure state by percolating this mixture through acid-washed alumina.

The mixture of isomeric α-ketols **11a** and **11b** on acetylation gave a mixture of α-acetoxy ketols **12a** and **12b**. This mixture undergoes reduction with zinc dust in acetic acid to afford a saturated six-membered ketone, m.p. 117–121° (uncor.). The O.R.D. curve of this ketone is significant in that it exhibits a positive Cotton effect and its shape[36] is very similar to that of friedelin (**13**) (which possesses a negative Cotton effect), suggesting that the above six-membered ketone might be represented by **14**. A low yield of an amorphous enone (**15**) was obtained when the α-ketol acid **5** was refluxed in ethanolic hydrochloric acid for a few hours. (See Scheme I.)

The evidence for the stereochemistry of the glycol system in ring A will now be presented. The smooth hydrolysis of the 4-carbomethoxy group of dimethyl senegenate indicates that the conformation of this group is α-equatorial (**16**). The axial position is excluded since such axial methyl esters of β-boswellic acid[37] can be saponified only with great difficulty. Dimethyl senegenate forms a crystalline acetonide

SCHEME I



(**17**), m.p. 161–163°, [α]D +40.2°. Of the four stereochemical possibilities for the 1,2-glycol system, the 2β,3α and 2α,3β may, therefore, be eliminated.[38] Moreover, the 2α,3β isomer would require a large *trans*-diaxial coupling (J = 8–10 c.p.s.) of the methine protons,[39] whereas the observed values range from 3.5 to 4.2 c.p.s. It has already been noted that the 2-hy-

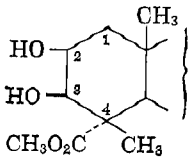
(35) N. L. Wendler, D. Taub, and R. P. Gaber, *Tetrahedron*, **7**, 173 (1959).

(36) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 99.

(37) P. Bilham, G. A. R. Kon, and W. C. J. Ross, *J. Chem. Soc.*, **35** (1942); A. Vogel, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **34**, 2321 (1951); J. L. Beton, T. G. Halsall, and E. R. H. Jones, *J. Chem. Soc.*, 2904 (1956).

(38) N. L. Wendler and H. L. Slates, *Chem. Ind.* (London), 167 (1955).

(39) R. W. Lemieux, R. N. Kullnig, H. J. Berstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **79**, 1005 (1957); M. Karplus, *ibid.*, **85**, 2870 (1963).



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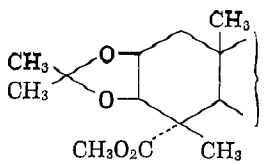

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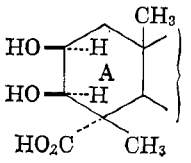


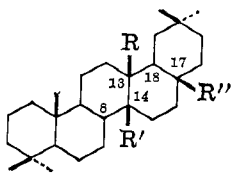
18

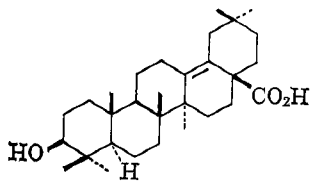
droxyl of senegenic acid is the one oxidized to a ketone. If the glycol configuration were $2\alpha,3\alpha$, this would require preferential oxidation of an equatorial over an axial hydroxyl group. Moreover, senegenic acid monoethyl ester monoacetate shows a 1-H doublet at τ 4.75 and a 1-H multiplet at τ 5.83—clear evidence that the acetate group is located on the C-3 carbon. Thus, if the glycol configuration were $2\alpha,3\alpha$, this would require preferential acetylation of an axial 3-hydroxyl over the less hindered equatorial 2-hydroxyl. It is thus clear that a $2\beta,3\beta$ configuration (**18**) is present. Incidentally, a 2β-hydroxyl (axial) requires a 4α (equatorial) configuration of the carboxyl group since lactonization is not observed under a variety of conditions.

The p.m.r. spectra of several senegenic acid derivatives (Table II) reveal the presence of five quaternary methyl groups. The quaternary methyl groups at C-4 and C-10 are discernible in the p.m.r. spectra by a 3-H singlet at τ 8.60–8.67 (deshielded by the 4α-equatorial CO_2Me) and 8.80–8.87 (deshielded by the 2β-axial hydroxyl), respectively. The above reactions indicate that the A ring of senegenic acid (**18**) is identical with that described for medicagenic acid[40] and senegenin.[7]

There now remains to be considered the position of the inert ethylenic linkage and the second carboxyl group of senegenic acid. The presence of five quaternary methyl groups in senegenic acid is significant in the sense that it gives a clue as to the class to which this nortriterpene acid belongs. A dihydroxydicarboxylic acid belonging to the β-amyrin class possesses six methyl groups. Senegenic acid being a nordihydroxydicarboxylic acid can, therefore, be easily accommodated in such a class. The reactions centered around the inert tetrasubstituted double bond and the second carboxyl group throw light on the structure of the acid.

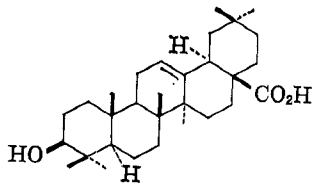
The location of the tetrasubstituted double bond on a pentacyclic triterpene skeleton at either $\Delta^{8(14)}$ with a COOH group at C-13 (**19a**) or a $\Delta^{13(18)}$ with a COOH group at C-14 (**19b**) or C-17 (**19c**) were excluded on the ground that senegenic acid remains unchanged when it is heated above its melting point. This stability implies that the second carboxyl group is situated at least γ,δ to the tetrasubstituted double bond. The ease of bromolactone and lactone formation (*vide supra*) supports this conclusion. Furthermore, the behavior of dimethyl senegenate, dimethyl senegenate diacetate, and senegenic acid diacetate toward hydrogen chloride furnishes interesting clues to the stability of this inert tetrasubstituted double bond. Under these conditions, dimethyl senegenate and dimethyl senegenate diacetate remain unaffected, showing that no isomerization[41] of the double bond takes place during such treatment. Senegenic acid diacetate, however, forms a very stable γ-lactone, characterized as its methyl ester, m.p. 274–278°, $[\alpha]D$ 28.2°, in almost quantitative yield. The stability of dimethyl senegenate and its acetate suggests that the double bond is situated at a very stable position. Moreover, the ready ease of lactonization of the second COOH group on the unsaturated center indicates that the COOH function must be axial. Such a behavior is encountered in δ-oleanolic (**20**) and 18-isooleanolic acids (**21**).[42] A C_{29} formulation with a double bond and second carboxyl group with the above characteristics can be readily rationalized on the basis of a pentacyclic triterpene containing a Δ^{13}–17-COOH system. Since it will subsequently be demonstrated that this system is present in senegenic acid (**22**), the following transformations will be interpreted on the basis of a pentacyclic triterpene containing a Δ^{13}–17-COOH moiety.





20

19a, $\Delta^{8(14)}$, R = CO_2H
b, $\Delta^{13(18)}$, R′ = CO_2H
c, $\Delta^{13(18)}$, R″ = CO_2H



21

Digestion of senegenic acid bromolactone (**31**) with pyridine[43] affords a crystalline dehydro acid (**33**) which exhibits absorption maxima [λ_{max} 237 mμ (ϵ 12,400), 246 (14,922), 254 (11,500); the p.m.r. spectrum of the dimethyl ester **34** shows two vinylic protons at τ 4.45] characteristic of a heteroannular diene system.[44–46] This dehydro acid has also been obtained by another route. Treatment of either dimethyl senegenate (**26**) or diacetoxydimethyl senegenate (**27**) with perbenzoic acid gives a very labile oxide (**36** or **37**) which undergoes dehydration[47] when attempts are made to purify either of the "oxides" by crystallization or chromatography over alumina to afford the corresponding dehydro de-

(40) C. Djerassi, D. B. Thomas, A. L. Livingston, and C. R. Thompson, *J. Am. Chem. Soc.*, **79**, 5292 (1957).

(41) A Δ^8–13β-COOH–14α-H system could undergo isomerization under such treatment to give the thermodynamically stable $\Delta^{8(14)}$ isomer: see Simonsen and Ross, "The Terpenes," Vol. IV, Cambridge University Press, London, 1957, p. 49.

(42) D. H. R. Barton and N. J. Holness, *J. Chem. Soc.*, 78 (1952).

(43) H. Wieland and F. Hoshino, *Ann.*, **479**, 179 (1930); H. Wieland and S. Utzino, *ibid.*, **488**, 242 (1931).

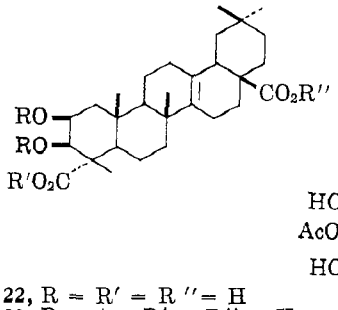
(44) L. J. Bellamy and C. Dorée, *J. Chem. Soc.*, 172 (1941).

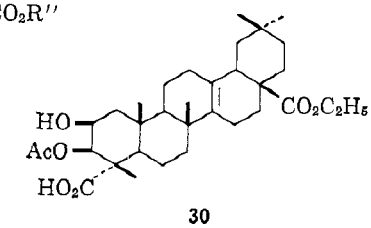
(45) E. R. H. Jones and T. G. Halsall, in "Fortschritte der Chemie Organischer Naturstoffe," Vol. 12, Springer-Verlag, Vienna, 1958, p. 104.

(46) L. Ruzicka and A. Marxer, *Helv. Chim. Acta*, **25**, 1561 (1942).

(47) M. J. Birchenough and J. F. McGhie, *J. Chem. Soc.*, 2038 (1949).

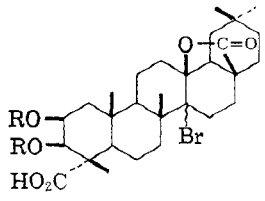
rivative **34** or **35**. Saponification of dimethyl dehydrosenegenate (**34**) with alkali gives the same dehydrosenegenic acid (**33**) obtained by the earlier-mentioned transformation.



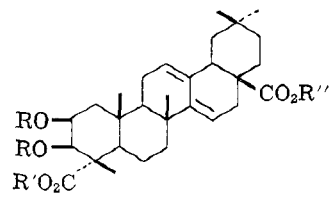


22, R = R′ = R″ = H
23, R = Ac; R′ = R″ = H
24, R = R′ = H; R″ = C_2H_5
25, R = R′ = H; R″ = CH_3
26, R = H; R′ = R″ = CH_3
27, R = Ac; R′ = R″ = CH_3
28, R = H; R′ = CH_3; R″ = C_2H_5
29, R = Ac; R′ = CH_3; R″ = C_2H_5

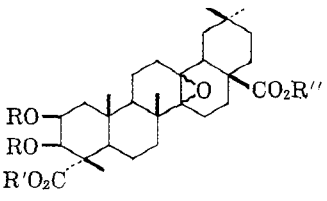
30

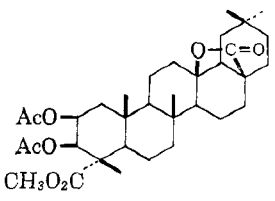


31, R = H
32, R = Ac



33, R = R′ = R″ = H
34, R = H; R′ = R″ = CH_3
35, R = Ac; R′ = R″ = CH_3





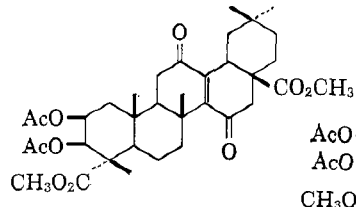
36, R = H; R′ = R″ = CH_3
37, R = Ac; R′ = R″ = CH_3

38

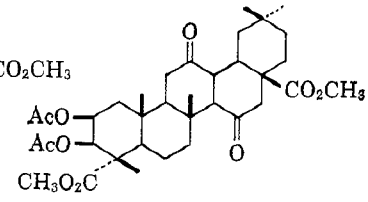
Although the ultraviolet absorption maxima of these dehydro derivatives closely resemble the characteristic triple maxima exhibited by the triterpenoids belonging to the lanosterol series,[44,45] their behavior toward catalytic hydrogenation[48,49] is not compatible with a $\Delta^{7,9(11)}$ diene chromophone derivable from a Δ^{8}-13β-COOH-14α-H system. Support is lent to this view by the fact that both dehydrosenegenic acid (**33**) and dimethyl dehydrosenegenate (**34**) undergo catalytic hydrogenation to regenerate senegenic acid (**22**) and dimethyl senegenate (**26**), respectively, in almost quantitative yield. This reaction is readily interpretable on the basis of 1,4 addition of hydrogen to the α face of a $\Delta^{12,14}$ diene, but not applicable in the case of a $\Delta^{7,9(11)}$ diene exhibiting the same type of absorption maxima.[48,49]

The p.m.r. spectra of dimethyl dehydrosenegenate (**34**) and senegenic acid lactone diacetate methyl ester (**38**) were very helpful in disclosing the presence of five quaternary methyl groups in senegenic acid. Each of these five $-CCH_3$ groups appeared as a 3-H singlet. The deshielded nature of the two $-CCH_3$ groups at C-4 and C-10 have already been discussed (*vide supra*). From their respective signals, it was possible to make a probable assignment of the three $-CCH_3$ groups at C-8 and C-20 (*gem*-dimethyl) which are shown in Table II. The presence of a *gem*-dimethyl group[50,51] was also discernible in the infrared spectrum of dehydrosenegenic acid (**33**) (ν_{max}^{KBr} 1385, 1365 cm.$^{-1}$).

Further evidence as to the location of the double bond at Δ^{13} has been gathered by a sequence of reactions analogous to that carried out with pyroquinovic acid[52] having a Δ^{13} double bond. Oxidation of dimethyl senegenate diacetate (**27**) with chromium trioxide in acetic acid gives a pale yellow amorphous enedione (**39**) in poor yield which exhibits characteristic



39

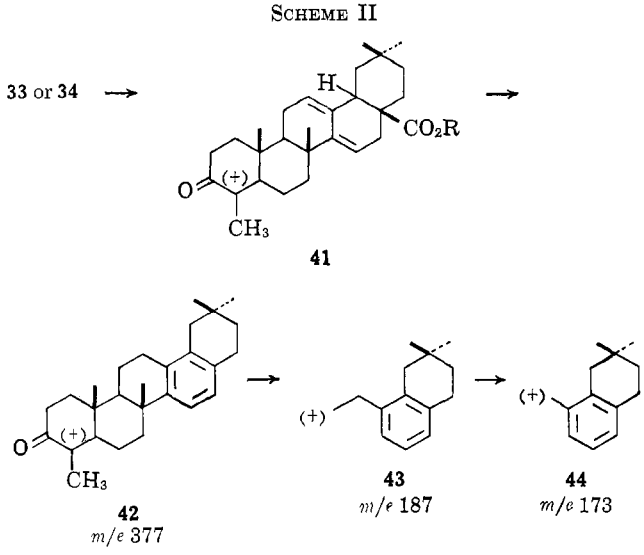


40

spectral data [λ_{max} 270 mμ (ϵ 9574), $\nu_{max}^{CHCl_3}$ 1669 cm.$^{-1}$ (O=C—C=C—C=O)] for such a system.[52] This enedione undergoes reduction to give the corresponding saturated diketone **40**, showing no high intensity absorption in the ultraviolet spectrum. The mass spectral fragmentation pattern of dehydrosenegenic acid (**33**), dimethyl dehydrosenegenic acid (**34**), senegenic acid monoethyl ester (**24**), and senegenic acid ethyl methyl diester (**28**) supports the above findings and is discussed below.

The mass spectrum[29] of dehydrosenegenic acid (**33**) (Figure 1) shows significant peaks at *m/e* 486 (M^+), 377 (M − 109), 187, and a base peak at *m/e* 173. Similar peaks are also present in the spectrum of dimethyl dehydrosenegenate (**34**) (Figure 2) at *m/e* 514 (M^+), 377 (M − 137), 187, and 173 (base peak). These fragments may be derived from **33** or **34** *via* species such as **41** and **42** (see Scheme II). Senegenic

SCHEME II



41

42
m/e 377

43
m/e 187

44
m/e 173

(48) L. Ruzicka, R. Denss, and O. Jeger, *Helv. Chim. Acta*, **29**, 204 (1946).
(49) R. M. Gascoigne, A. Robertson, and J. J. H. Simes, *J. Chem. Soc.*, 1830 (1953).
(50) W. Bottomley, A. R. H. Cole, and D. E. White, *ibid.*, 2624 (1955).
(51) G. Snatzke, F. Lampert, and R. Tschesche, *Tetrahedron*, **18**, 1417 (1962).
(52) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 3111 (1953).

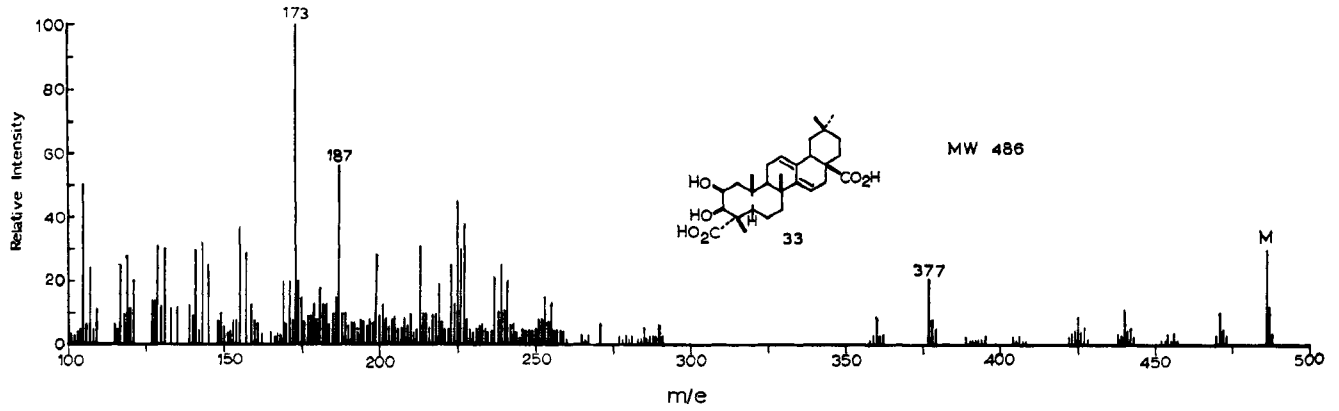


Figure 1.—Mass spectrum of dehydrosenegenin acid (**33**).

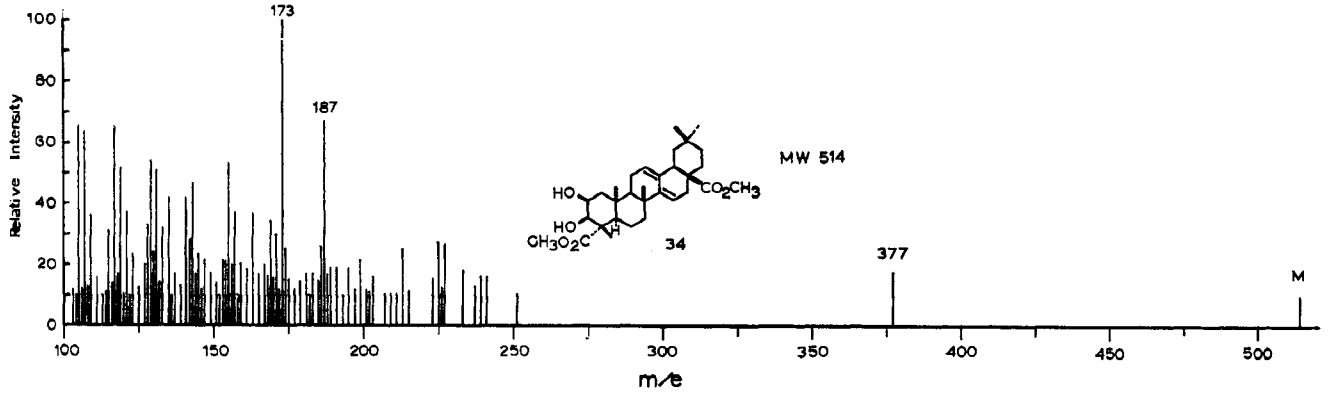
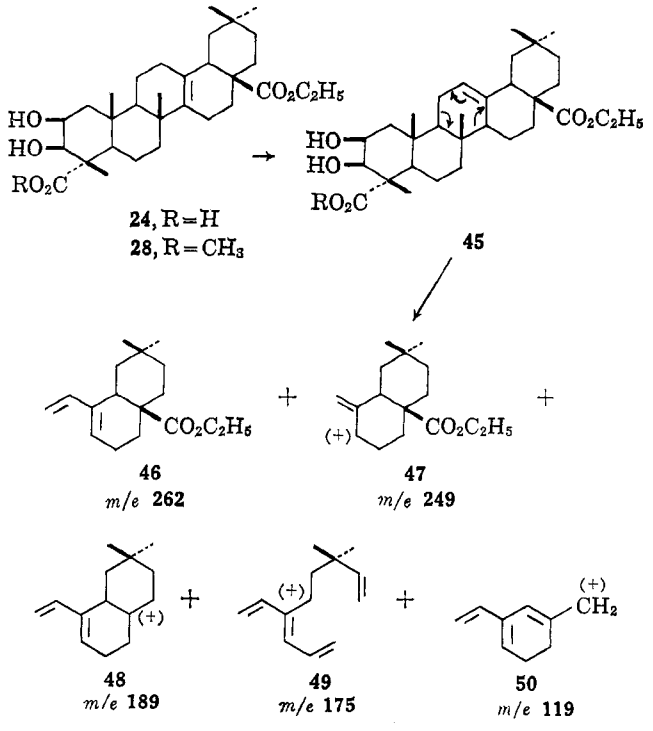


Figure 2.—Mass spectrum of dimethyl dehydrosenegenate (**34**).

acid monoethyl ester (**24**) (Figure 3) exhibits strong peaks at *m/e* 516 (M^+), 442 (M − 74), 427, 189, and a base peak at 175. The ethyl methyl diester (Figure 4) shows corresponding peaks at 530 (M^+), 456 (M − 74), 441, 189, and 175 (base peak). The presence of an M − 74 peak in the spectra of both **24** (*m/e* 442) and **28** (*m/e* 456) is probably due to the cleavage of a $C_3H_6O_2$ fragment, containing the C-1, C-2, C-3 carbon atoms and glycol grouping, from ring A.

Budzikiewicz, Wilson, and Djerassi[53] have shown that the most characteristic fragmentation of Δ^{12}-triterpenes of the α- and β-amyrin class involves a retro-Diels–Alder reaction in ring C. If, owing to either electron impact or thermal rearrangement, the Δ^{13} double bond in **24** and **28** rearranges to a Δ^{12} double bond as in **45**, then a fragmentation pattern involving species **46–50** might be expected.[54] As a matter of fact, the mass spectra of both **24** and **28** do show peaks for **46** (*m/e* 262), **47** (*m/e* 249), **48** (*m/e* 189), **49** (*m/e* 175), and **50** (*m/e* 119). Whether such an isomerization of **24** and **28** to **45**, followed by a retro-Diels–Alder reaction, actually occurs is unknown. The significant point is that all the species mentioned above can be derived by rational processes from the D/E ring system of a $\Delta^{12,14}$ diene (species **41–44**) or a Δ^{13} system (species **46–50**), but not from a $\Delta^{7,9(11)}$ diene or Δ^8 system. (See Scheme III.)

SCHEME III



The above evidence leads to structures **22** and **24** for senegenic acid and senegenic acid monoethyl ester, respectively. These compounds are thus members of the β-amyrin–oleanolic acid series in which a carbon atom at C-14 has been eliminated with concomitant shift of the double bond to the Δ^{13} position. The nor structure of senegenic acid recently has been shown to arise from a precursor designated as presenegenin and

(53) H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).

(54) de Mayo has invoked such a rearrangement of a Δ^{13} to a Δ^{12} system to explain the correspondence of peaks in the mass spectra of dimethyl dechlorosenegenin and dimethyl medicagenate.[55]

(55) Private communication, P. de Mayo. We thank Dr. de Mayo for sending us a copy of his manuscript prior to publication [NOTE ADDED IN PROOF.—J. J. Dugan and P. de Mayo, *Can. J. Chem.*, **43**, 2033 (1965)].

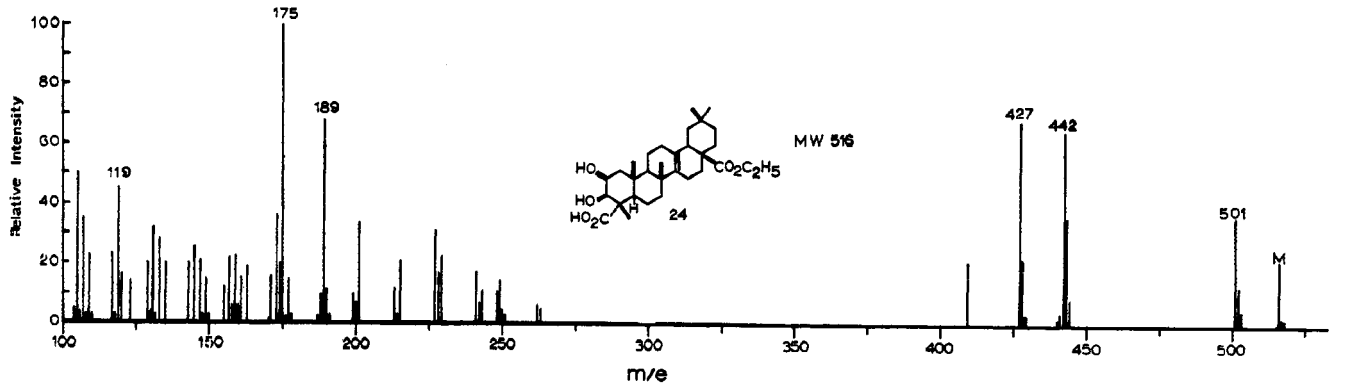


Figure 3.—Mass spectrum of senegenic acid monoethyl ester (24).

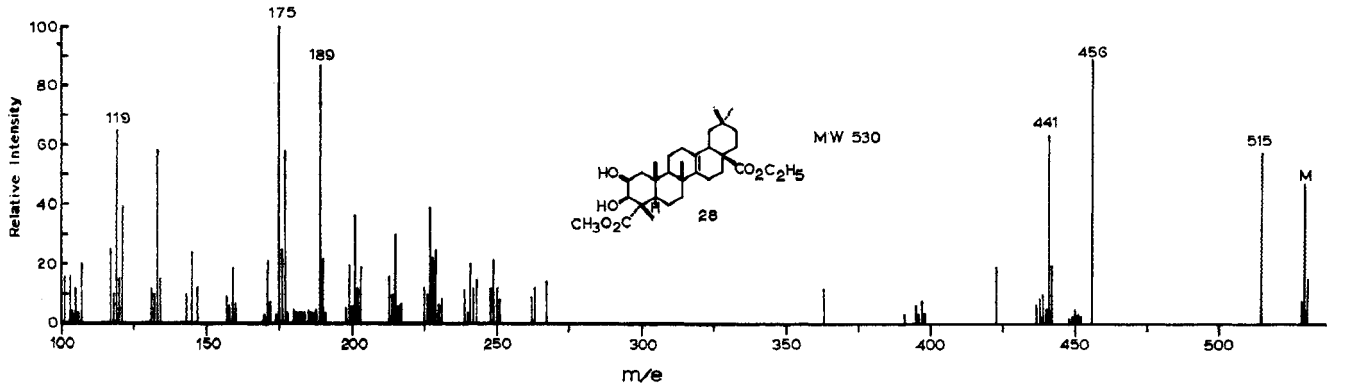
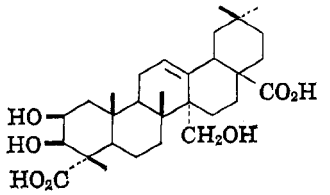


Figure 4.—Mass spectrum of senegenic acid ethyl methyl diester (28).

having the structure of 2β,27-dihydroxy-3-carboxyoleanolic acid (**51**).[55] When presenegenin was heated



51

with ethanolic hydrochloric acid it was converted in high yield to a mixture of senegenin (**2**), senegenin acid (**22**), and formaldehyde.[55] Moreover, on heating the dimethyl ester of presenegenin at 220°, it underwent smooth rearrangement to dimethyl senegenate (**26**) and formaldehyde.[55]

It should be noted that no evidence is available for the stereochemistry of the C-8 methyl or C-18 hydrogen. It is likely that both groups possess the β configuration as in all known members of the oleanene series. Other products arising from the mild hydrolysis of senegenin are discussed in our preliminary communication.[56]

Experimental Section

General Procedure.—Melting points are corrected except where otherwise noted and were determined on a hot stage equipped with a microscope and polarizer. Finely powdered samples were placed on the stage at 15° below the melting point and the temperature was raised at a rate of about 4°/min. Unless otherwise noted, rotations were taken in chloroform, ultraviolet absorption spectra in 95% ethanol on a Perkin-Elmer 202 spectrometer, and infrared absorption spectra as Nujol mulls on an Infracord spectrometer. Proton magnetic resonance spectra (p.m.r.) were taken on an A-60 spectrometer in deuteriochloroform solution with tetramethylsilane as an internal reference. Petroleum ether refers to a fraction of b.p. 30–60°. As a routine check on the purity of senegenic acid ethyl ester and various senegenic acid derivatives, thin layer chromatography (t.l.c.) on silica gel G was employed. Separation of many of the mixtures encountered was effected by the system, *n*-butyl acetate–methanol–diethylamine (25:12:1) hereafter designated as system P.

Isolation of Triterpenoids from *Polygala senega*.—Senegenin, the saponin from *Polygala senega* root,[57] was extracted and purified according to the procedure of Jacobs and Isler.[5] Powdered senegenin (350 g.), dissolved in ethanol–water (1:1, 2.8 l.), was refluxed with 700 ml. of concentrated hydrochloric acid for 30 min. After cooling, the voluminous precipitate of prosapogenin was filtered through thick filter paper covered with several layers of muslin. The moist precipitate was dissolved in 2.8 l. of boiling ethanol–water (3:1) and refluxed with 700 ml. of concentrated hydrochloric acid for 4 hr. The hydrolysate was cooled to 60° and filtered at this temperature to give a solid (A, 50 g.). The mother liquor was left at 0° for 5 days when another crop of solid (B, 50 g.) was obtained after filtration. The final mother liquor was discarded.

Senegenin.—Solid A (50 g.) was dissolved in 1.5 l. of boiling ethanol. Water was added slowly, and the boiling solution was filtered from a sparingly soluble solid (A_1, 34 g.). The filtrate was left to cool overnight and the crystals were collected (C). The latter was dissolved in 500 ml. of ethanol and boiled with Norit. The ethanol solution was concentrated to 150 ml. and then to the boiling solution water was added until turbidity appeared. The solution was left to cool and the crystals of senegenin (**2**) were collected (4.83 g.). A portion of this was fractionally crystallized from aqueous ethanol to give the analytical sample, m.p. 278–284°, $[\alpha]^{25}D$ +19° (*c* 1.5, EtOH).

Anal. Calcd. for $C_{30}H_{45}ClO_6$: C, 67.10; H, 8.38; Cl, 6.60. Found: C, 67.50, 67.27; H, 8.23, 8.46; Cl, 6.82.

Fractionation of Sapogenin B Mixture.—A solution of 50 g. of solid B in 500 ml. of ethanol was boiled twice with 8-g. portions of Norit and then concentrated to *ca.* 150 ml. Water was added slowly to the boiling solution until precipitation began. On cooling, a crystalline sapogenin mixture was deposited (30 g.);

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

(57) *Polygala senega* root was obtained from S. B. Penick and Co., New York, N. Y.

t.l.c. system P indicated three components. The sapogenin mixture was stirred with 1.8 l. of chloroform for 6 hr., which separated the sapogenin mixture into about equal parts of chloroform-soluble and chloroform-insoluble fractions. This mixture was filtered by suction through a large sintered funnel with Celite. The chloroform-insoluble waxy solid (D) remained on the Celite bed. The filtrate (E) contained mainly senegenic acid monoethyl ester (**24**) with traces of senegenin (**2**) and senegenic acid (**22**) (t.l.c. system P).

Isolation of Senegenic Acid Monoethyl Ester (24).—Evaporation of the solution E gave a solid F (14 g.) which was stirred with 1 l. of benzene–ether (9:1) for 1 hr. and filtered. The filtrate (G) was reserved. The residue (8 g.) was stirred with 250 ml. of cold chloroform for 2 hr. The filtrate on evaporation gave 5.5 g. of crude monoester. The latter was treated with Norit in boiling ethanol, filtered, diluted, and allowed to crystallize. The 3.35 g. of product was crystallized three times from aqueous ethanol to give 2.25 g. of pure senegenic acid ethyl ester (**24**): m.p. 221–224°[58]; $[\alpha]^{28}D$ +23.4° (*c* 0.96, EtOH); ν_{max} 3448, 1718, 1704 cm.$^{-1}$; homogeneous with t.l.c. system P; negative Beilstein test. This derivative sublimes at 220–225° at 0.05 mm.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36; mol. wt., 516. Found: C, 71.52, 72.04; H, 9.36, 9.28; mol. wt., 516 (mass spectrum).[29]

The mother liquors from triangular crystallization afforded another crop (0.6 g.) of the monoethyl ester, m.p. 216–219°. The filtrate (G) on evaporation afforded 6 g. of solid which was stirred with 300 ml. of benzene–ether (9:1) and filtered. The residue (0.5 g.) was substantially pure monoester, m.p. 216–219°. The solution on percolation through silica gel gave fractions which were fractionally crystallized from aqueous ethanol to to give 1.71 g. of monoester, m.p. 218–224°. The total yield of pure monoethyl ester was 5.06 g.

Impure senegenic acid monoethyl ester from the mother liquors was usually accompanied by *ca.* 5% of senegenin (**2**) and senegenic acid (**22**) but could be purified by methylation and subsequent chromatography of the ethyl methyl diester over acid-washed alumina to give pure senegenic acid ethyl methyl diester (**28**).

Purification of Senegenic Acid Monoethyl Ester *via* Its Diethylamine Salt.—Senegenic acid ethyl ester (100 mg.) was dissolved in 5 ml. of ethyl acetate, and then 2 ml. of diethylamine was added to the boiling solution. On cooling and scratching crystals separated which on recrystallization from the same solvent mixture gave the pure diethylamine salt of senegenic acid monoethyl ester: m.p. 221–224°; ν_{max} 1712, 1629, 1550 cm.$^{-1}$.

Anal. Calcd. for $C_{35}H_{59}NO_6$: C, 71.31; H, 10.02. Found: C, 71.48, 71.12; H, 9.47, 10.01.

In some preparations when senegenic acid monoethyl ester was found to contain traces of senegenin and senegenic acid (t.l.c. system P), the above method was used to purify the ethyl ester. Acidification of the diethylamine salt, filtration, and subsequent crystallization from aqueous ethanol gave pure senegenic acid monoethyl ester (t.l.c. system P).

Isolation of Senegenic Acid (22) from Saponin Hydrolysate. A. As the Free Acid.—A portion of the chloroform-insoluble material (fraction D) was suspended in hot chloroform and the mixture was filtered. The precipitate was crystallized from aqueous ethanol to give a crop of crude senegenin. Concentration of the mother liquors gave second and third crops of crystals melting at 280–282 and 257°, respectively. Recrystallization of the 257° material gave material melting at 280–285°. Recrystallization from aqueous ethanol gave needles of the hemihydrate of senegenic acid, m.p. 282–286°,[26] with an infrared spectrum identical with that of a sample of senegenic acid prepared by hydrolysis of senegenic acid monoethyl ester (**24**). An analytical sample melted at 284–288°.

Anal. Calcd. for $C_{29}H_{44}O_6 \cdot 0.5H_2O$: C, 70.01; H, 9.11. Found: C, 69.62; H, 9.03.

B. As the Dimethyl Ester.—The Celite bed containing the chloroform-insoluble waxy solid (D) was treated with methanol in a Soxhlet apparatus for 3 hr. Evaporation of the solvent afforded a crystalline sapogenin mixture (12 g.) which was found by t.l.c. system P to contain mainly senegenin, senegenic acid, and traces of senegenic acid monoethyl ester with another less polar material. The 12 g. of sapogenin mixture was dissolved in methanol–ether and treated with an excess of diazomethane in ether. Removal of ether gave a viscous yellow oil. The latter on crystallization from methanol afforded fine needles (5 g.), m.p. 136–138°, 195–198°. Three more recrystallizations from the same solvent furnished 3.8 g. of pure **dimethyl senegenate** (**26**): m.p. 135–137°, 194–196°; $[\alpha]^{25}D$ +20.5° (*c* 1.84); ν_{max} 3571, 3425, 1724, 1706 cm.$^{-1}$.

Anal. Calcd. for $C_{31}H_{48}O_6 \cdot 0.5CH_3OH$: C, 71.57; H, 9.42. Found: C, 71.50; H, 9.31; mol. wt., 516 (mass spectrum,[29] lost CH_3OH).

Dimethyl senegenate obtained from the saponin hydrolysate by methylation was found to be identical in all respects (melting point, mixture melting point, and infrared and p.m.r. spectra) with dimethyl senegenate obtained by methylation of senegenic acid (*vide infra*). The mother liquor from fractional crystallization of crude dimethyl senegenate (*vide supra*) contained impure senegenin dimethyl ester and dimethyl senegenate (t.l.c.).

Dimethyl Senegenate (26).—Senegenic acid (100 mg.) was methylated with an excess of diazomethane in ether. Evaporation of the ether gave a crystalline residue which was crystallized from methanol to give 87 mg. of the dimethyl ester as needles: m.p. 135–137°, 194–196°; homogeneous by t.l.c.; ν_{max}^{Nujol} 3650–3289 (s), 1724 (s), 1706 cm.$^{-1}$ (s). This sample was identical with that obtained by methylation of fraction D, as described above.

Anal. Calcd. for $C_{31}H_{48}O_6 \cdot 0.5CH_3OH$: C, 71.57; H, 9.42. Found: C, 71.50; H, 9.31.

Dimethyl Senegenate Acetonide (17).—A solution of 50 mg. of dimethyl senegenate (**26**) in 10 ml. of dry acetone was shaken with 200 mg. of anhydrous copper sulfate for 96 hr. at room temperature. After filtration, the solution was evaporated to give a product which crystallized from methanol (45 mg.). A benzene solution of the compound was chromatographed over 6 g. of neutral alumina (Woelm, activity I) and eluted with 100 ml. of benzene–petroleum ether (1:1). The eluates on evaporation gave a residue which on crystallization from methanol afforded 25 mg. of the acetonide **17** as needles, m.p. 161–163°, $[\alpha]^{26}D$ +40.2° (*c* 0.52), ν_{max} 1724 cm.$^{-1}$ (no OH absorption).

Anal. Calcd. for $C_{34}H_{52}O_6$: C, 73.34; H, 9.41. Found: C, 73.87; H, 9.50.

Partial Saponification of Dimethyl Senegenate to Give Monomethyl Senegenate (25).—A solution of 50 mg. of **26** in 8 ml. of 10% methanolic potassium hydroxide was heated under reflux for 18 hr. After dilution with water and extraction with ether (discarded), the aqueous phase was acidified and the crude monomethyl ester was extracted with ether. Evaporation of the solvent gave a residue (45 mg.) which crystallized from aqueous ethanol to give needles of the monomethyl ester **25**: m.p. 257–261°; $[\alpha]^{25}D$ +21.1° (*c* 1.02); ν_{max} 3663, 3571, 1695 (broad) cm.$^{-1}$.

Anal. Calcd. for $C_{30}H_{46}O_6$: C, 71.68; H, 9.22. Found: C, 71.45; H, 9.52.

Hydrolysis of Dimethyl Senegenate to Senegenic Acid (22).—A solution of 100 mg. of **26** in 20 ml. of isoamyl alcohol containing 1.5 g. of potassium hydroxide was refluxed under nitrogen for 24 hr. Work-up under the usual conditions afforded 70 mg. of acid which crystallized from aqueous ethanol to give needles of pure senegenic acid (**22**), m.p. 280–284°,[26] identical (melting point, infrared) with senegenic acid obtained by saponification of senegenic acid ethyl ester (**24**), *vide infra*.

Attempted Hydrogenation of Senegenic Acid (22).—A solution of 40 mg. of **22** in 5 ml. of absolute ethanol was treated with hydrogen in the presence of 50 mg. of Adams catalyst for 24 hr. The product was isolated and crystallized from ethyl acetate to give 35 mg. of needles with an infrared spectrum identical with that of authentic senegenic acid. The dimethyl ester (**26**), prepared by methylation with diazomethane, showed m.p. 194–196°.

Conversion of Senegenic Acid into Senegenic Acid Monoethyl Ester (24).—A solution of 40 mg. of **22** in a mixture of 25 ml. of ethanol–water–hydrochloric acid (3:1:1) was refluxed for 4 hr. and then allowed to stand in a refrigerator for 24 hr. The separated crystals consisted of a mixture of senegenic acid and senegenic acid monoethyl ester (t.l.c. system P). This mixture was stirred in hot chloroform and then filtered. The filtrate on evaporation yielded a solid (20 mg.) which on crystallization from aqueous ethanol afforded senegenic acid monoethyl ester.

(58) The melting point of senegenic acid monoethyl ester is very dependent on the rate of heating the sample. Reproducible melting points could only be obtained when the substance was introduced at 15–20° below the melting point on a preheated hot stage. If the sample was heated slowly on the hot stage, slow decomposition occurred and the compound melted at a lower temperature.

m.p. 216–221°,[58] identical with authentic senegenic acid monoethyl ester (**24**) as shown by infrared and t.l.c. behavior.

Senegenic Acid Ethyl Ester Monoacetate (30).—Senegenic acid ethyl ester (**24**) (200 mg.) was treated with acetic anhydride–pyridine previously cooled to 0° and kept in a refrigerator for 3 hr. The mixture was poured into ice–water and extracted with ether. After washing with dilute hydrochloric acid and water and drying, evaporation of the ether layer gave a yellow gum. Chromatography in benzene over 15 g. of silica gel (Davison) gave an oil which crystallized from aqueous ethanol. Crystallization from aqueous ethanol gave 154 mg. of senegenic acid monoethyl ester monoacetate (**30**) as needles: m.p. 160–161°; $[\alpha]^{30}D$ +25.5° (*c* 1.0); ν_{max} 3571, 1748, 1730, 1712, 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{33}H_{50}O_7$: C, 70.93; H, 9.02. Found: C, 70.75; H, 9.20.

Senegenic Acid Ethyl Methyl Diester (28).—A solution of 180 mg. of senegenic acid monoethyl ester (**24**) in 10 ml. of ether–methanol (1:1) was treated with an excess of ethereal diazomethane. Removal of the solvent gave an oil, which was chromatographed in benzene on Woelm acid-washed alumina (activity I). The center fractions crystallized from acetone–petroleum ether to give 139 mg., m.p. 184–185°. Two more crystallizations gave 90 mg. of feathery needles of senegenic acid ethyl methyl diester (**28**), m.p. 193–195°, $[\alpha]^{25}D$ +20.3° (*c* 0.53).

Anal. Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50; mol. wt., 530. Found: C, 72.36; H, 9.48; mol. wt., 530 (mass spectrum).[29]

Senegenic Acid Ethyl Methyl Diester Diacetate (29).—A solution of 470 mg. of senegenic acid ethyl methyl diester (**28**) was treated with 10 ml. of acetic anhydride and 10 ml. of dry pyridine and allowed to stand for 3 days. The reaction mixture was concentrated to dryness *in vacuo* and the residue was chromatographed in benzene over 50 g. of alumina. Elution with benzene–ether (19:1) gave a colorless gum which crystallized from methanol as feathery needles, m.p. 144° (uncor.). Further recrystallization did not alter the melting point.

Anal. Calcd. for $C_{36}H_{54}O_8$: C, 70.33; H, 8.85. Found: C, 70.27; H, 8.84.

In another experiment the crude product was purified by preparative t.l.c. (chloroform) to give needles: m.p. 142–145° (uncor.); ν_{max} 1736 (s), 1718 (sh), 1236 cm.$^{-1}$.

Saponification of Senegenic Acid Ethyl Methyl Diester Diacetate (29).—A solution of 60 mg. of **29** in 4 ml. of 1% methanolic KOH was boiled for 2 hr. under nitrogen. Work-up gave 50 mg. of oil which was purified by preparative t.l.c. (2% EtOH in $CHCl_3$ on silica gel). The product (30 mg.) was crystallized from petroleum ether to give 20 mg. of the ethyl methyl diester (**28**), m.p. 187–189° (uncor.). The infrared spectrum in Nujol was identical with that of an authentic sample of **28**.

Senegenic Acid Diacetate (23).—Senegenic acid (90 mg.) was acetylated in 2.5 ml. of acetic anhydride and 1.0 ml. of pyridine at room temperature for 72 hr. Isolation of the product and crystallization from aqueous ethanol gave 90 mg. of **23**, m.p. 269–272°. Recrystallization afforded material of m.p. 274–275°; $[\alpha]^{30}D$ +34° (*c* 1.0); ν_{max} 1739, 1689, 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{33}H_{48}O_8$: C, 69.20; H, 8.45. Found: C, 69.07; H, 8.71.

Dimethyl Senegenate Diacetate (27). A. From Dimethyl Senegenate (26).—A solution of 1.117 g. of dimethyl senegenate in 15 ml. of acetic anhydride and 10 ml. of dry pyridine was allowed to stand for 3 days. Work-up in the usual way afforded 1.0 g. of **27**: m.p. 174–176°; $[\alpha]^{28}D$ +31° (*c* 1.03); ν_{max} 1739 (broad), 1250 cm.$^{-1}$.

Anal. Calcd. for $C_{35}H_{52}O_8$: C, 69.97; H, 8.72. Found: C, 69.75; H, 8.81.

B. From Senegenic Acid Diacetate.—A solution of 100 mg. of senegenic acid diacetate (**23**) in ether–methanol was treated with an excess of diazomethane in ether. Evaporation of the solvent and crystallization of the residue from methanol afforded 90 mg. of **27** as needles, m.p. 174–176°.

Anal. Calcd. for $C_{35}H_{52}O_8$: C, 69.97; H, 8.72. Found: C, 69.86; H, 8.65.

Saponification of Dimethyl Senegenate Diacetate (27) to Dimethyl Senegenate (26).—A solution of 50 mg. of **27** in 10 ml. of 5% methanolic potassium hydroxide solution was refluxed for 3 hr. The residue after removal of solvent was suspended in water and extracted with ether; the ether solution was washed with water, dried, and evaporated to give a colorless residue. The latter on crystallization from methanol afforded needles (30 mg.), m.p. 133–137°, 194–196°. There was no depression in melting point on admixture with **26** and the infrared spectra were identical.

Saponification of Senegenic Acid Monoethyl Ester (24).—**24** (0.5 g.) dissolved in 70 ml. of amyl alcohol containing 4 g. of potassium hydroxide was refluxed for 20 hr. under nitrogen. The solution was poured into 10% hydrochloric acid and extracted thoroughly with ether. The ether phase was extracted with 7% sodium carbonate solution, and the latter was then acidified with 10% hydrochloric acid and extracted with ether; the ether solution was washed, dried, and evaporated to give a colorless solid. The latter was stirred with chloroform (100 ml.) to free it from any unreacted material and filtered. The residue on crystallization from aqueous ethanol afforded senegenic acid (**22**) as solvated needles (280 mg.): m.p. 282–286°[26]; $[\alpha]^{24}D$ +23° (*c* 1.02, EtOH); λ_{max} 205 mμ (ε 5000); ν_{max} 3704, 3571, 1695 (broad) cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{44}O_6 \cdot 0.5H_2O$: C, 70.01; H, 9.11. Found: C, 69.95, 79.85; H, 9.20, 9.32.

Senegenic acid, when recrystallized from dry methyl acetate, gave anhydrous needles, m.p. 284–288°.[26]

Anal. Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found: C, 71.35; H, 9.06.

Decarboxysenegenic Acid.—In another experiment, involving the hydrolysis of 1.10 g. of senegenic acid ethyl ester, the ether layer which had been extracted with sodium carbonate solution was evaporated to give an oily residue. This was suspended in benzene and filtered. The residue (370 mg.) was crystallized from aqueous methanol to give 100 mg. of the "decarboxy" compound, m.p. 274–279° dec. Recrystallization from methanol gave 56 mg. of the pure decarboxy derivative: m.p. 274–279° dec.; ν_{max}^{Nujol} 3448 (s), 1701 cm.$^{-1}$ (s).

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.87; H, 10.01.

Modified Kuhn–Roth Oxidation of Senegenin (2) and Senegenic Acid Monoethyl Ester (24). General Procedure.—Five milligrams of the compound under investigation was dropped into 2 ml. of boiling CrO_3–H_2SO_4 solution[30] and boiled gently for 5–10 min. The distillate was collected in a flask containing 10–15 ml. of water. The aqueous distillate was neutralized with a few drops of 20% aqueous diethylamine solution and then concentrated to a few drops, a portion of which was spotted on Whatman filter paper No. 1 for chromatographic separation.[31] Standard solutions were made up of known fatty acids, and these were each converted to the ethylamine salts before use. The descending mobile phase technique was used for paper chromatography. The solvent used for the mobile phase was water saturated with butanol. The phase in the bottom of the tank was butanol saturated with diethylamine made by taking 20% aqueous ethylamine and diluting this with butanol saturated with water until the concentration of ethylamine was 0.025 *N*. It was found convenient to run the chromatograms overnight and to develop them with a solution of 250 mg. of bromocresol purple in 50 ml. each of ethanol and butanol. The minimum amount of acid that could be detected by this method was of the order of 0.03 mg. of acetic acid.

Senegenin (5 mg.) and senegenic acid monoethyl ester (5 mg.) were each oxidized and analyzed by the above method. Only a strong spot corresponding to acetic acid and no traces of any higher fatty acids could be detected on either paper chromatogram.

Periodic Acid Titration of Senegenic Acid Derivatives.—The following general procedure was adapted. The compound (*ca.* 0.1 mmole) was added to 10 ml. of metaperiodic acid solution (made by dissolving 5 g. of crystalline metaperiodic acid in 200 ml. of water and 800 ml. of acetic acid) and at suitable time intervals 1-ml. aliquots were removed and titrated against standard sodium thiosulfate solution. A blank was run at the same time and the uptake of periodic acid was measured by the difference in titration values between the blank and the solution of the compound under investigation.

By the above procedure it was found that senegenic acid monoethyl ester (**24**) and senegenic acid ethyl methyl diester (**28**) rapidly consumed 1 mole of periodic acid in less than 1 hr. Senegenic acid ethyl methyl diester diacetate (**29**) and the dimethyl ester diacetate (**27**) did not consume periodate over a period of several hours.

Periodate Oxidation of Senegenic Acid Ethyl Methyl Diester (28).—**28** (120 mg.) and sodium metaperiodate (120 mg.) were dissolved in ethanol (15 ml.) and 1 *N* sulfuric acid (12 ml.). The solution was left at room temperature for 70 min. and then

poured into 150 ml. of water and 400 ml. of ether. After equilibration of the phases, the ether solution was dried and evaporated to give a gum (216 mg.). A benzene solution of the gum was chromatographed over 22 g. of acid-washed alumina (Merck). The column was eluted with benzene–ether. The following fractions were collected: 50 ml., 11.4 mg.; 100 ml., 54.8 mg.; 150 ml., 56 mg.; and 500 ml., 25.5 mg. None of these fractions could be crystallized, nor could any pure 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives be prepared.

The third fraction obtained above (56 mg.) was used for a p.m.r. spectrum. It was found from the spectrum that the ethyl and methyl esters were still present in the product, showing that the glycol function must be part of a ring system.

Oxidation of Senegenic Acid Monoethyl Ester (24) with Jones Reagent.—Jones reagent[34] (3.8 ml., 15% excess) was added dropwise with stirring and cooling (ice bath) to a solution of 500 mg. of **24** in 70 ml. of acetone. The mixture was stirred for 7 min. and then poured into a mixture of saturated aqueous sodium chloride–ether. After being shaken and separated, the aqueous phase was extracted with ether. The ether phase was dried over magnesium sulfate and evaporated. The oily residue (450 mg.) on trituration with ether gave a crystalline material (286 mg.). The latter, when crystallized from ethyl acetate, gave 210 mg. of the α-ketol acid ethyl ester **5**: m.p. 191–196°; $[\alpha]^{28}D$ +10° (*c* 0.94); ν_{max} 3448, 1709 (broad) cm.$^{-1}$.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.46; H, 8.92.

Oxidation of Senegenic Acid Monoethyl Ester with Chromium Trioxide–Pyridine.—A solution of 100 mg. of **24** in 6 ml. of dry pyridine was oxidized with 120 mg. of chromium trioxide in 6 ml. of dry pyridine at room temperature overnight. The reaction mixture was diluted with water and extracted thoroughly with a large volume of ether. The ethereal extract was washed once with dilute hydrochloric acid and then with water. The ether phase was dried and evaporated to give an oily residue. The latter on trituration with petroleum ether furnished 50 mg. of crystals, m.p. 186–196° dec. Recrystallization from ethyl acetate gave a pure sample, m.p. 191–196° dec. This product was identical with the α-ketol acid derivative **5** obtained by oxidizing senegenic acid monoethyl ester with Jones reagent (melting point, mixture melting point), *vide supra*.

Acetate of the α-Ketol Acid Ethyl Ester 6.—A crystalline acetate was prepared by treating the above compound **5** with acetic anhydride–pyridine. The product crystallized from aqueous alcohol as leaflets: m.p. 230–235°; $[\alpha]^{26}D$ +30.2° (*c* 1.0); ν_{max} 1757, 1232, 1724, 1701 cm.$^{-1}$.

Anal. Calcd. for $C_{33}H_{48}O_7$: C, 71.19; H, 8.69. Found: C, 71.02; H, 8.67.

Conversion of the α-Ketol Acid Ethyl Ester 5 to the Diosphenol Acetate 8.—A solution of 100 mg. of **5** in 10 ml. of 10% methanolic potassium hydroxide was heated under reflux for 1.5 hr. The mixture was diluted with water, acidified, and extracted with ether, and the ether was removed by distillation with benzene. An amorphous residue was obtained (96 mg.) which failed to crystallize from any solvent: ν_{max} 3413, 1724, 1672, 1645 cm.$^{-1}$; λ_{max} 281 mμ; λ_{max} (EtOH–KOH) 329 mμ; dark green color with ferric chloride. The above diosphenol **7** (95 mg.) was acetylated at room temperature overnight with acetic anhydride–pyridine. Working up in the usual way gave an oil which on trituration with ether afforded clusters of needles (80 mg.), m.p. 186–191°. Further purification was effected by passing through a column of Florisil (6 g.). Elution with benzene–petroleum ether (1:1) gave 60 mg. of the diosphenol acetate (**8**) which crystallized from methanol containing a few drops of ether as needles, 37 mg., m.p. 191–196°. Recrystallization afforded an analytical sample: m.p. 194–196°; $[\alpha]^{30}D$ +13.2° (*c* 0.5); ν_{max} 1764, 1718, 1672, 1645 cm.$^{-1}$; λ_{max} 249 mμ (ε 9822); τ 7.78 (–OCOCH_3), 8.24 (vinylic CH_3).

Anal. Calcd. for $C_{32}H_{46}O_5$: C, 75.26; H, 9.08. Found: C, 75.25; H, 9.05.

Conversion of the α-Ketol Acid Ethyl Ester 5 to the α,β-Unsaturated Ketone 15.—A solution of 90 mg. of **5** in 40 ml. of ethanol and 4.4 ml. of hydrochloric acid (1:1) was heated under reflux under nitrogen for 8 hr. and then allowed to stand at room temperature overnight. After removal of solvent and addition of petroleum ether, crystals of impure starting material (14 mg., m.p. 170–180°), as indicated by the infrared spectrum and t.l.c., separated. The mother liquor (69 mg.) was chromatographed over 6 g. of Merck acid-washed alumina. Elution was carried out with benzene–petroleum ether (1:1) and each fraction was checked by t.l.c. A middle fraction (11 mg.) behaving as a homogeneous oil (**15**) showed $\nu_{max}^{CHCl_3}$ 1718, 1661, 1613 cm.$^{-1}$; λ_{max}^{EtOH} 251 mμ (ε 8730); no ferric chloride color. This enone (**15**) gave a red crystalline DNPH derivative which crystallized from ethanol as prisms, m.p. 204–206° (uncor.), 218–219° (uncor.).

Anal. Calcd. for $C_{36}H_{48}N_4O_6$: C, 68.33; H, 7.65. Found: C, 68.69; H, 7.35.

Decarboxylation of the α-Ketol Acid Ethyl Ester (5). A. By Heating.—**5** (100 mg.) was heated under nitrogen in a metal bath. At a bath temperature of 210–230°, vigorous gas evolution occurred which subsided after 5 min. After cooling, the residual oil crystallized on adding petroleum ether and scratching. T.l.c. (0.5% EtOH–$CHCl_3$) indicated one component (neutral) with traces of impurities. On recrystallization from petroleum ether, 59 mg. of needles of the decarboxylated material (**11a** and **11b**) was obtained: m.p. 117–120° (uncor.); ν_{max}^{Nujol} 3472, 1712 cm.$^{-1}$. It sublimed at 130–170° at 0.2 mm.

Anal. Calcd. for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85; mol. wt., 470. Found: C, 76.62; H, 9.79; mol. wt., 470 (mass spectrum).[29]

B. By 1% Methanolic Potassium Hydroxide.—A solution of 67 mg. of **5** in 6 ml. of 1% methanolic potassium hydroxide was refluxed for 2.5 hr. under nitrogen. It was then acidified with an excess of dilute acetic acid, poured into water, and extracted with ether; the ether phase was washed once with saturated sodium carbonate solution and once with water, dried over magnesium sulfate, and evaporated. The residue crystallized on adding petroleum ether and scratching, m.p. 110–118°. The crystals and mother liquor were combined and chromatographed in chloroform over 4 g. of Merck acid-washed alumina. The decarboxylated material crystallized from petroleum ether as needles (50 mg.), m.p. 118–127° (uncor.), t.l.c. homogeneous (same as product in A).

Acetate.—The above α-ketol derivative (**11a** and **11b**) readily gave a crystalline acetate (**12a** and **12b**) on treatment at room temperature with acetic anhydride and pyridine. It crystallized from petroleum ether as needles: m.p. 143–150° (uncor.); $\nu_{max}^{CCl_4}$ 1751, 1724, 1248 cm.$^{-1}$, no –OH absorption.

Anal. Calcd. for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.91; H, 9.51.

C. By Adsorption on Acid-Washed Alumina.—A chloroform solution of **5** was poured onto a column of Merck acid-washed alumina and left overnight. Elution with 70 ml. of chloroform gave 100 mg. of crystalline material, m.p. 124–127° (uncor.), t.l.c. with 1% EtOH–$CHCl_3$ homogeneous (same as in A and B). It was recrystallized from petroleum ether: first crop, 38 mg., m.p. 128–135° (uncor.); second crop, 18 mg., m.p. 118–135° (uncor.). The infrared spectra and t.l.c. of the two crops were identical, as in A, except some bands in A seemed less sharp. Crystalline acetates **12a** and **12b** were prepared from the two crops separately by treating each overnight with acetic anhydride–pyridine. The products proved to be identical: m.p. 160–163° (uncor.), 185–190° (uncor.); mixture melting point was same; t.l.c. (1% EtOH–$CHCl_3$) homogeneous, same as the acetate (**12a** and **12b**) in B; ν_{max} 1751, 1733, 1712, 1248 cm.$^{-1}$ (all strong).

Oxidation of the α-Ketol Derivative 11a and 11b to the Diosphenol 7.—To a solution of 50 mg. of the α-ketol derivative prepared by method C (**11a** and **11b**) in 0.3 ml. of methanol was added 70 mg. of powdered cupric acetate and 1.3 ml. of 70% acetic acid. The mixture was immersed in a hot oil bath. A red precipitate of cuprous oxide appeared after 3 min. The mixture was heated under reflux for 5 min., poured into water, and extracted with ether. The ether solution was washed once with dilute sodium bicarbonate solution and once with saturated sodium chloride, dried, and evaporated to give an oil (45 mg.). The latter was purified by t.l.c. to give 22 mg. of an oily diosphenol (**7**) which could not be induced to crystallize (homogeneous on t.l.c.). It gave a dark violet color with ferric chloride: $\nu_{max}^{CCl_4}$ 3521, 1724, 1672, 1645 cm.$^{-1}$; λ_{max}^{EtOH} 282 mμ; λ_{max} (EtOH–KOH) 330 mμ.

This diosphenol (**7**) was characterized as its enol acetate (**8**) by treating with acetic anhydride–pyridine. Crystallization from methanol containing a little ether afforded needles, m.p. 186–190°. There was no depression in melting point on admixture with the diosphenol acetate obtained by autoxidation.

Autoxidation of the α-Ketol Acid 5.—When reactions involving the α-ketol acid **5** were not conducted under nitrogen, ferric chloride positive spots, due to formation of the diosphenol **7**,

appeared on t.l.c., in particular A–B. (A) Decarboxylation of α-ketol acid **5** carried out by base treatment in the presence of air yielded oily mixtures which were strongly positive to ferric chloride. (B) The α-ketol acid **5** in acetic acid overnight yielded mostly the diosphenol (t.l.c., infrared, $FeCl_3$ test), presumably *via* successive decarboxylation and oxidation. (C) An attempt was made to obtain the α-ketol derivative (**11a** and **11b**) by heat decarboxylation of the α-ketol acid **5**. The crude mixture was heated at 230–250° at 0.1 mm. in a sublimation tube but the sublimate proved to consist mostly of the above diosphenol (**7**). The diosphenol **7** was purified by passing its chloroform solution through acid-washed alumina.

Removal of the Acetoxy Group from the α-Ketol Acetate 12a and 12b.—To a solution of 52 mg. of the α-ketol acetate **12a** and **12b**, prepared by the alumina decarboxylation method, in 7 ml. of glacial acetic acid was added 1.8 g. of zinc dust and the mixture was stirred under reflux for 7 hr. During this period *ca.* 300 mg. of fresh zinc dust was added every hour. The mixture was filtered and washed with ether. The ether extract was washed successively with aqueous saturated sodium bicarbonate and sodium chloride solution. The dried ether solution was evaporated to give an oil which crystallized on addition of methanol and scratching. The resulting ketone (**14**) was recrystallized from methanol, yielding 22 mg. of plates, m.p. 115–120° (uncor.). Work-up of material from the mother liquors gave an additional 20 mg. which was combined with the 22-mg. fraction and recrystallized from methanol to give 19 mg.: m.p. 117–121° (uncor.); ν_{max} 1721 (s), 1706 (s) cm.$^{-1}$; O.R.D. in dioxane (*c* 0.174): $[\alpha]_{589}$ +11°, $[\alpha]_{321}$ +809°, $[\alpha]_{315}$ +601° (trough), $[\alpha]_{312}$ +676°, $[\alpha]_{275}$ −772° (trough).

Anal. Calcd. for $C_{30}H_{46}O_3$: C, 79.24; H, 10.20. Found: C, 79.25; H, 10.17.

The ketone formed a light orange precipitate with 2,4-DNP reagent: m.p. 136–140° (uncor.), ν_{max} 1724 cm.$^{-1}$.

Perbenzoic Acid Titration of Dimethyl Senegenate (26) and Dimethyl Senegenate Diacetate (27).—**26** (50.25 mg.) and **27** (50.65 mg.) in dry chloroform (2 ml.) were separately treated with 2 ml. of 1.32 *N* perbenzoic acid[59] in chloroform, and left at 0°. After 24 hr. 95% of the amount of peracid required for one double bond had been consumed; after 48 hr. the titer corresponded to 1.07 and 1.09 double bonds for **26** and **27**, respectively.

Senegenic Acid Bromolactone (31).—To a solution of 95 mg. of senegenic acid (**22**) in 20 ml. of 90% acetic acid containing 180 mg. of sodium acetate trihydrate was added dropwise and with stirring 0.20 ml. of a solution containing 0.5 ml. of bromine in 10 ml. of glacial acetic acid. The color of bromine was immediately discharged. The mixture was poured into water containing a little sodium bisulfite and the precipitate was extracted thoroughly with chloroform. The chloroform solution was washed with water, dried, and evaporated to give a solid which on crystallization from methanol afforded 65 mg. of bromolactone **31**: m.p. 284–289°, $[\alpha]^{30}D$ +49° (*c* 1.0, EtOH); ν_{max} 1773 (γ-lactone), 1709 (COOH) cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{43}BrO_6$: C, 61.31; H, 7.57. Found: C, 61.47; H, 7.64.

Under similar conditions of bromolactone formation, senegenic acid monoethyl ester (**24**) was recovered unchanged.

Diacetate 32.—The above bromolactone (**31**) (30 mg.) was acetylated with 1.0 ml. of acetic anhydride and 0.5 ml. of pyridine at room temperature for 72 hr. Upon isolation, senegenic acid bromolactone diacetate (**32**) separated from dilute methanol as microcrystals: 22 mg.; m.p. 178–181°; ν_{max} 1779 (γ-lactone), 1745, 1248 (OAc), 1709 cm.$^{-1}$ (COOH), no hydroxyl absorption. This product was used directly for the next reaction.

Action of Zinc Dust and Acetic Acid on Senegenic Acid Bromolactone Diacetate (32).—Finely powdered zinc dust (5 g.) was added during the course of 2 hr. to a boiling acetic acid solution of **32** (50 mg.). The mixture was filtered, washed with acetic acid, and evaporated to give a white residue. The latter furnished 45 mg. of flakes of senegenic acid diacetate (**23**) on crystallization from aqueous ethanol, m.p. 272–276°. There was no depression in melting point on admixture with authentic senegenic acid diacetate (**23**) and the infrared spectra were identical.

Dehydrosenegenic Acid (33).—Senegenic acid bromolactone (**31**) (100 mg.) was treated with dry pyridine (0.5 ml.) and heated in an oil bath at 125–130° for 6 hr. The residue after evaporation of the solvent was suspended in dilute hydrochloric acid and extracted thoroughly with ether. The ether solution was washed with water, dried, and evaporated to give a colorless solid (90 mg.) which crystallized from ethyl acetate as needles (**33**): 57 mg.; m.p. 223–225°, 279–284°; $[\alpha]^{28}D$ +59° (*c* 1.0, EtOH); λ_{max} 237 mμ (ε 12,460), 246 (14,922), 254 (11,500); p.m.r. of dimethyl ester **34**, two vinylic protons at τ 4.54.

Anal. Calcd. for $C_{29}H_{42}O_6$: C, 71.57; H, 8.70; mol. wt., 486. Found: C, 71.70; H, 8.94; mol. wt., 486 (mass spectrum).[29]

Treatment of Dimethyl Senegenate (26) with Perbenzoic Acid to Give Dimethyl Dehydrosenegenate (34).—A solution of 200 mg. of **26** in 5 ml. of chloroform was treated with 2 ml. of 1.32 *N* perbenzoic acid[59] in chloroform and left at 0°. After 48 hr. the excess oxidizing agent was decomposed with 20 ml. of 1% potassium iodide, the mixture was acidified, and the liberated iodine was removed by shaking with 0.1 *N* sodium thiosulfate solution. The chloroform layer was separated and washed successively with dilute sodium hydroxide solution and water. The chloroform solution was dried and evaporated to give an oil (200 mg.). The latter on trituration with methanol furnished colorless needles (180 mg.), m.p. 204–208°. Since this product did not exhibit end absorption in the ultraviolet spectrum, it was assumed to be the "oxide" **36** of dimethyl senegenate. Attempts to crystallize the "oxide" from a mixture of methanol–chloroform (9:1) gave 160 mg. of the corresponding crystalline dehydro derivative **34**, m.p. 139–141°, which showed significant maxima in the ultraviolet spectrum (λ_{max} 238, 246, 254 mμ). Compound **34** could also be obtained in a pure state by passing a benzene solution of the "oxide" **36** through a column of neutral alumina (Woelm activity I) and eluting with chloroform–methanol (9:1). Dimethyl dehydrosenegenate (**34**) crystallized from a mixture of methanol–chloroform (9:1) as fine needles: m.p. 144–146°; $[\alpha]^{30}D$ +54.4° (*c* 1.23); λ_{max} 238 mμ (ε 10,237), 246 (12,187), 254 (9165); p.m.r., two vinylic protons at τ 4.53.

Anal. Calcd. for $C_{31}H_{46}O_6 \cdot CH_3OH$: C, 70.30; H, 9.22; mol. wt., 546. Found: C, 70.50; H, 9.22; mol. wt., 514 (mass spectrum,[29] lost CH_3OH).

Treatment of Dimethyl Senegenate Diacetate (27) with Perbenzoic Acid.—A solution of 150 mg. of **27** in 5 ml. of chloroform was treated with 3 ml. of 1.32 *N* perbenzoic acid[59] in chloroform and kept in a refrigerator for 48 hr. The excess oxidizing agent was decomposed by potassium iodide, the mixture was acidified, and the liberated iodine was removed by shaking with excess sodium thiosulfate solution. The colorless chloroform layer was separated and washed successively with dilute sodium carbonate solution and water. The chloroform solution was dried and evaporated to give an oil. A benzene solution of this oil (no diene absorption in ultraviolet, this is the intermediate "oxide" **37**) was passed over 10 g. of neutral alumina (Woelm, activity I) and eluted successively with benzene–petroleum ether (1:1), benzene, and benzene–ether (9:1). The benzene eluates on evaporation gave the desired dehydro derivative **35** as an amorphous solid (80 mg.). This could not be induced to crystallize from the usual solvents [λ_{max} 238 mμ (ε 8810), 246 (10,468), 255 (7212)].

Attempted Oxidation of Dimethyl Senegenate Diacetate (27) with Selenium Dioxide.—A solution of 120 mg. of **27** in 20 ml. of glacial acetic acid was heated under reflux for 48 hr. with 24 mg. of freshly sublimed selenium dioxide. The reaction mixture was filtered and washed with benzene, and the solvent was evaporated to leave a gum. Trituration of this gum with petroleum ether afforded 80 mg. of crystals. Two crystallizations of this material from the same solvent gave needles, m.p. 174–176°. There was no depression in melting point on admixture with the starting material. The main filtrate was evaporated to give a gum (30 mg.). A benzene solution of this gum was chromatographed over 4 g. of acid-washed alumina (Woelm, activity I), elution being effected with benzene–petroleum ether (1:1) and benzene. Evaporation gave a glassy mass (7 mg.) which failed to crystallize from any solvent. This product exhibited λ_{max} 237 mμ (ε 8034), 245 (9126) and 255 (6269), indicating a diene chromophore like dimethyl dehydrosenegenate (**34**). Evaporation of the benzene eluates gave another fraction (15 mg.) which proved to be a mixture of dimethyl senegenate diacetate (**27**) and dimethyl dehydrosenegenate diacetate (**35**). With a large excess of selenium dioxide and the above vigorous conditions **27** gave a very complex mixture.

Attempted Oxidation of Dimethyl Senegenate Diacetate (27) with N-Bromosuccinimide.—A solution of 60 mg. of **27** in 6 ml. of dry carbon tetrachloride was refluxed with 18 mg. of N-

(59) J. R. Mayer and N. C. Manley, *J. Org. Chem.*, **29**, 2099 (1964).

bromosuccinimide for 4 hr. Succinimide was collected and the solvent was washed successively with dilute sodium hydroxide solution and water. Evaporation of the dried carbon tetrachloride gave a gum (50 mg.) which was chromatographed over 4 g. of acid-washed alumina (Woelm, activity I). Elution with benzene–petroleum ether furnished a glassy product (10 mg.) which exhibited ultraviolet maxima corresponding to dimethyl dehydrosenegenate diacetate (**35**) [λ_{max} 237 mμ (ϵ 8133), 245 (9524), 255 (6353)].

Saponification of Dimethyl Dehydrosenegenate (34).—A solution of 170 mg. of **34** in 30 ml. of 5% amyl alcoholic potassium hydroxide was heated under reflux under nitrogen for 24 hr. The solution was poured into 10% hydrochloric acid and extracted with ether. The ether solution was then shaken with 7% sodium carbonate solution; the aqueous alkaline solution was acidified with 10% hydrochloric acid and extracted with ether. The ether phase was washed, dried, and evaporated to give a colorless solid (70 mg.). The latter on crystallization from ethyl acetate afforded dehydrosenegenic acid (**33**), m.p. 223–225°, 276–284°. The latter was identical (mixture melting point, infrared) with dehydrosenegenic acid obtained by digestion of senegenic acid bromolactone (**31**) with pyridine (*vide supra*).

Catalytic Hydrogenation of Dehydrosenegenic Acid (33).—A solution of 50 mg. of **33** in 7 ml. of absolute ethanol was hydrogenated with 50 mg. of platinum oxide at room temperature and atmospheric pressure for 24 hr. The resulting compound (45 mg.) after crystallization from aqueous ethanol showed m.p. 279–284° and was identical in all respects (mixture melting point, infrared, ultraviolet) with senegenic acid (**22**).

Catalytic Hydrogenation of Dimethyl Dehydrosenegenate (34).—A solution of 28.6 mg. of **34** in 5 ml. of absolute ethanol was hydrogenated in the presence of 50 mg. of Adams catalyst for 3 hr. After filtration and evaporation of the solvent, the residue was crystallized from methanol to give 18 mg. of dimethyl senegenate (**26**), m.p. 137°, 194–196°, with an infrared spectrum identical with that of an authentic sample. The ultraviolet spectrum did not exhibit any diene absorption.

Attempted Pyrolysis of Senegenic Acid (22).—**22** (50 mg.) was heated at 0.1 mm. in a metal bath at 290–295° for 7 min. After cooling, the residue was crystallized twice from aqueous ethanol. Senegenic acid, m.p. 279–284°, was recovered unchanged in good yield (40 mg.). In another pyrolysis experiment the ultraviolet spectrum of the crude product was examined: λ_{max}^{EtOH} 207 mμ, no diene absorption.

Chromic Acid Oxidation of Dimethyl Senegenate Diacetate (27).—A solution of 150 mg. of **27** in 10 ml. of glacial acetic acid was oxidized by dropwise addition over a 1-hr. period of 150 mg. of chromium trioxide in 5 ml. of 90% acetic acid. The reaction mixture was stirred for another hour at room temperature and left overnight. Excess chromic acid was destroyed with methanol. The resulting dark green solution was poured into water and extracted with ether, and the ether was successively washed with dilute sodium carbonate solution and water. Evaporation of the dried ether phase gave a yellow resin from which an amorphous pale yellow solid (145 mg.) was obtained by diluting its methanolic solution with water. The amorphous material was chromatographed over 15 g. of neutral alumina (Woelm, activity-1) as described in Table III. Attempts to crystallize fractions 11–14 from various solvents met with failure. The amorphous product was characterized as the enedione **39** [λ_{max} 270 mμ (ϵ 9574); $\nu_{max}^{CHCl_3}$ 1739, 1242 (OAC), 1724 (COOMe), 1669 cm.$^{-1}$ (O=C—C=C—C=O)].

Anal. Calcd. for $C_{35}H_{48}O_{10}$: C, 66.86; H, 7.70. Found: C, 66.90; H, 8.20.

The above enedione from **27** was easily hydrogenated in acetic acid with platinum oxide at room temperature and pressure. The resulting product, isolated as an amorphous solid, could not be crystallized: λ_{max} 284 mμ (ϵ 84); ν_{max} 1745, 1242 (OAc), 1724 (COOMe) cm.$^{-1}$. The spectral data were fully consistent with the saturated diketone formulation **40**.

TABLE III

Solvent	Fraction	Vol., ml.	Product
Benzene–ether (19:1)	1–4	200	Unreacted substance, *ca.* 20 mg.
Benzene–ether (9:1)	5–10	300	Enedione and trace of unreacted material, 25 mg.
Benzene–ether (9:1)	11–12	100	Enedione (amorphous), 10 mg.
Benzene–ether (9:1)	13–14	100	Enedione, 15 mg.
Benzene–ether (85:15)	15–18	200	Enedione and trace of another component, 20 mg.

Action of Hydrogen Chloride on Dimethyl Senegenate (26).—**26** (100 mg.) in 7 ml. of chloroform was treated with a stream of dry hydrogen chloride at room temperature for 3 hr. The solvent was removed *in vacuo* and the residue was crystallized from methanol. The product, 80 mg., m.p. 176–196°, $[\alpha]^{23}D$ +23°, appeared to be unchanged dimethyl senegenate.

Action of Hydrogen Chloride on Dimethyl Senegenate Diacetate (27).—A solution of 200 mg. of **27** in 10 ml. of refluxing chloroform was treated with a stream of dry hydrogen chloride for 3 hr. After removal of the solvent *in vacuo*, the residue crystallized from methanol as needles, 120 mg., m.p. 170–172°, $[\alpha]^{26}D$ +28.2° (*c* 1.03). The product was unchanged starting material. The mother liquor furnished an additional quantity of **27**, m.p. 170–172°.

Senegenic Acid Lactone Diacetate Methyl Ester (38).—A stream of dry hydrogen chloride was passed into a solution of 150 mg. of senegenic acid diacetate (**23**) in 15 ml. of chloroform for 4 hr. The solvent was evaporated to dryness *in vacuo*. The residue in ether was treated with diazomethane in ether. Evaporation of the solvent afforded a colorless solid which crystallized from petroleum ether containing a few drops of methanol as prisms (140 mg., m.p. 259–268°). Further purification was effected by passing a benzene solution of 80 mg. of this product through 4 g. of acid-washed alumina (Woelm, activity 1) and eluting with benzene–ether (1:1). Evaporation of the benzene–ether eluates and crystallization from petroleum ether containing a few drops of methanol afforded the pure lactone **38**: m.p. 274–278°; $[\alpha]^{26}D$ +28.2° (*c* 1.34); ν_{max} 1779 (γ-lactone), 1751, 1250 (OAc), 1739 cm.$^{-1}$ (COOMe); no end absorption in the ultraviolet spectrum; see Table II for p.m.r. data.

Anal. Calcd. for $C_{34}H_{50}O_8$: C, 69.59; H, 8.59. Found: C, 69.50; H, 8.45.

A solution of the above lactone in chloroform was treated with hydrogen chloride for 4 hr. Evaporation of the solvent and crystallization of the residue from petroleum ether containing a few drops of methanol gave back the lactone **38**, m.p. 269–274°, undepressed with starting material. The infrared spectrum of the product was identical with that of an authentic sample of **38**.

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