dried and the solvent removed under reduced pressure. The residual oil was crystallized from isopropyl alcohol, 1.75 g. (71%), m.p. 68–71°. A mixed melting point determination with authentic 3-(2,4-dichlorobenzyl)-2-oxazolidone was not depressed.

B.—A solution of 5.1 g. (0.01 mole) of N-(2,4-dichlorobenzyl)-N,O-bis-(trichloroacetyl)-ethanolamine and 0.5 g. (0.003 mole) of anhydrous *p*-toluenesulfonic acid in 50 ml. of anhydrous methanol was refluxed for six hours. The resulting solution was concentrated to about 10 ml. and cooled. The solid which separated was collected, washed with ether and recrystallized from isopropyl alcohol, 1.1 g. (94%), m.p. 145–146.5°. The product was identified as the *p*-toluenesulfonic acid salt of N-(2,4-dichlorobenzyl)-ethanolamine by a mixed melting point determination with an authentic sample.

Anal. Calcd. for $C_9H_{11}Cl_2NO \cdot C_7H_8SO_3$: Cl, 18.08. Found: Cl, 18.35.

The filtrate and ether washings from above yielded 3.5 g. (68%) of the starting amine.

RENSSELAER, NEW YORK

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

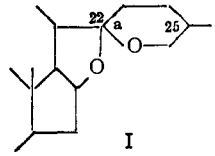
The C-25 Isomerism of Smilagenin and Sarsasapogenin[1]

BY IRVING SCHEER, ROBERT B. KOSTIC AND ERICH MOSETTIG

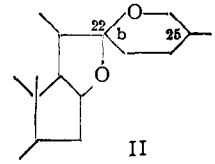
RECEIVED MAY 26, 1954

It is shown that pseudosmilagenin and pseudosarsasapogenin are different compounds. In acidic media they are recyclized to the original genins, smilagenin and sarsasapogenin, respectively. Contrary to previous reports in the literature, dihydrosmilagenin and dihydrosarsasapogenin, and tetrahydrosmilagenin and tetrahydrosarsasapogenin are not identical. The oxidation of pseudosmilagenin and pseudosarsasapogenin yielded the side chain fragments (−)-α-methylglutaric acid and (+)-α-methylglutaric acid, respectively. By the reduction of both dihydrosmilagenin 26-tosylate and dihydrosarsasapogenin 26-tosylate 16,22-epoxycoprostan-3β-ol is obtained. By these experiments it is established that smilagenin and sarsasapogenin are isomeric at C-25.

The so-called "iso" (I) and "normal" (II) ("22a" and "22b") epimeric steroidal sapogenins differ, according to Marker's concepts,[2] in their



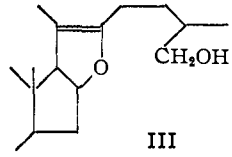
I



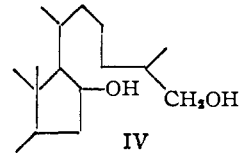
II

configuration at the asymmetric C-22 position. The "22b"-sapogenins can be converted irreversibly to the "22a" epimers by refluxing with alcoholic hydrochloric acid.

The main support for the assumption that the configurational differences accounting for the epimeric "22a" and "22b" series are located at C-22 was the observation that smilagenin and sarsasapogenin gave, with acetic anhydride at 200°,[3] the same pseudosapogenin[4] (III) and, in the Clemmensen reduction, the same tetrahydrosapogenin[5] (IV). In both procedures the asymmetric center at C-22 was destroyed.



III



IV

While preparing a number of pseudosapogenins by rearrangement in acetic anhydride we observed that smilagenin (V) and sarsasapogenin (VI) gave different compounds, a pseudosmilagenin (VII) and a pseudosarsasapogenin (VIII), respectively. This was established by the direct comparison (melting points, rotations, infrared spectra) of the alcohols and the diacetates and the di-3,5-dinitrobenzoates. In acidic media pseudosmilagenin (VII) was recyclized to smilagenin (V), and pseudosarsasapogenin (VIII) gave sarsasapogenin (VI),[6] in quantitative yields. The large positive shift in rotation[7] from $[\alpha]^{20}D$ −66° of V to $[\alpha]^{20}D$ +24° of VII and the ease of recyclization of VII to the original structure V indicated that VII was a pseudosapogenin. The presence of a band of moderate intensity at 1695 cm.$^{-1}$ in the infrared spectrum of VII was a further indication of its pseudosapogenin nature, for it has been shown[8] that the appearance of a band in this region is a characteristic of pseudosapogenins and does not occur with the sapogenins or their derivatives which do not have a C-20—C-22 double bond. The chromic acid oxidation and subsequent hydrolysis of VII and VIII led to the same product, Δ^{16}-pregnene-3,20-dione (IX),[3] and the oxidation of the acetates of VII and VIII, followed by catalytic reduction and hydrolysis, gave the same triol, pregnane-3β,16β,20β-triol (X).[9] The results of these oxidations furnished strong evidence that the pseudosapogenins VII and VIII were epimeric at C-25. Reduction (PtO_2, acetic acid, 25°) of VII and VIII yielded a dihydropseudosmilagenin (XI) and a dihydropseudosarsasapogenin[10] (XII), respectively. The direct comparison (melting points, mixed melting points, rotations, infrared spectra) of the alcohols and the diacetates, and the dibenzoates showed that XI and XII were different.

In view of these findings it appeared pertinent

(1) A preliminary report of this work has appeared earlier: I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **75**, 4871 (1953).

(2) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, *ibid.*, **68**, 2167 (1947).

(3) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 518 (1940).

(4) R. E. Marker, E. Rohrmann and E. M. Jones, *ibid.*, **62**, 648 (1940).

(5) R. E. Marker and E. Rohrmann, *ibid.*, **61**, 846 (1939).

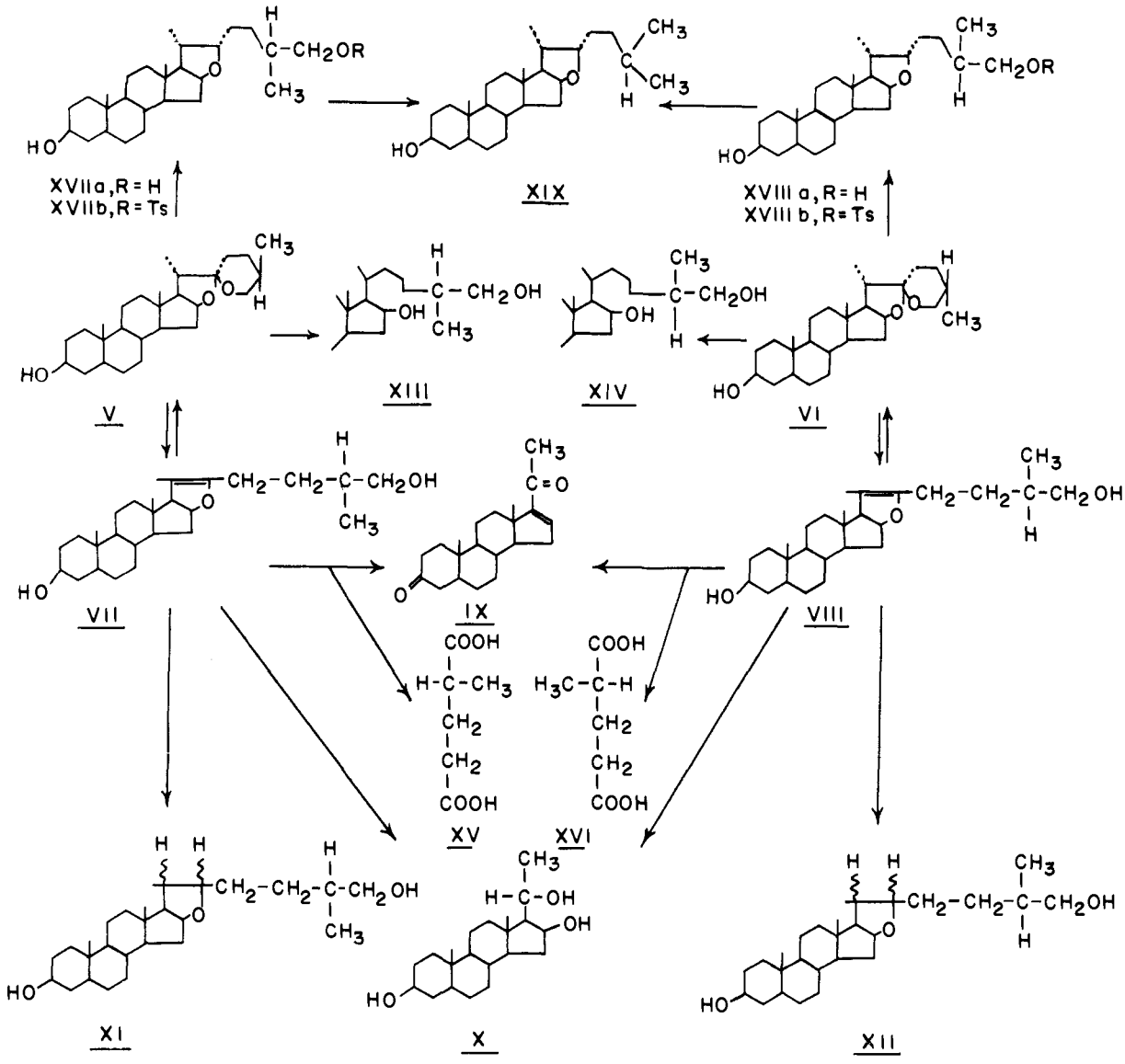
(6) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 896 (1940).

(7) C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 303 (1951).

(8) A. Hayden, P. Smeltzer and I. Scheer, *Anal. Chem.*, **26**, 550 (1954).

(9) R. E. Marker, D. L. Turner, R. B. Wagner, P. R. Ulshafer, H. M. Crooks, Jr., and E. L. Wittle, THIS JOURNAL, **63**, 779 (1941)

(10) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 521 (1940).

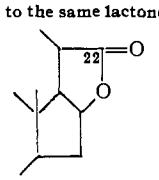


to reinvestigate the Clemmensen reduction of smilagenin (V) and sarsasapogenin (VI). Reduction of V yielded a tetrahydrosmilagenin (XIII) whereas the identical treatment of VI yielded tetrahydrosarsasapogenin (XIV) as previously reported.[5] The melting points of XIII and XIV were very similar and were not depressed when the two tetrahydrosapogenins were mixed. Acetylation of XIII and XIV afforded oily triacetates with similar rotations. The tribenzoates[11] differed slightly in their melting points, but a mixture of the tribenzoates gave a marked melting point depression. The melting points of the tri-*p*-chlorobenzoates of XIII and XIV were considerably different.

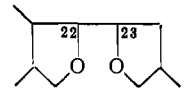
It was now evident that smilagenin and sarsasapogenin and their respective isomeric derivatives were different with respect to their configuration at the C-25 asymmetric center.[12] In order to establish definitely the isomerism as being at C-25, we isolated and characterized the side chain moieties of the pseudosapogenins VII and VIII. Following the experimental procedure of Marker's group,[9] we obtained, from the oxidation mixture of pseudosarsasapogenin (VIII), α-methylglutaric

(11) Marker and Rohrmann (ref. 5) reported that benzoylation of XIV gave a dibenzoate (m.p. 149°) only. In our hands benzoylation (benzoyl chloride–pyridine, 25°, 18 hours) of XIV gave only a tribenzoate (m.p. 135–136°) as shown by infrared and combustion analyses. No attempt was made to prepare a dibenzoate.

(12) In a recent preliminary communication, V. H. James [*Chem. and Ind.*, 1388 (1953)] states that S. N. Farmer and G. A. R. Kon [*J. Chem. Soc.*, 414 (1937)] believed that sarsasapogenin and smilagenin differed in their configuration at C-25. After studying the above paper of Farmer and Kon, we are unable to see how James arrived at this conclusion. Farmer and Kon oxidized sarsasapogenin and smilagenin to the same lactone



and concluded that these compounds differed in the configuration of the asymmetric carbon atom contained in the last ring of the side chain whose structure was represented as



Therefore, the isomerism could just as well have been at C-23. It also seems that the possibility of isomerism at C-22 was overlooked entirely.

acid (XVI) of melting point 78.5–81°, and found its rotation to be $[\alpha]^{20}D$ +18°. In an analogous manner we isolated from the oxidation mixture of pseudosmilagenin (VII) a levorotatory α-methylglutaric acid (XV) of melting point 78.5–81°, which had a rotation of $[\alpha]^{20}D$ −20°. The mixed melting point curve of XV and XVI was similar to that obtained by Berner and Leonardsen.[13] The infrared spectra of chloroform solutions of XV and XVI and of an authentic sample of racemic α-methylglutaric acid were identical. The isomerism at C-25 thus was established.[14]

By hydrogenating (PtO_2, acetic acid, 75°) the sapogenins, Marker obtained dihydrosapogenins which were isomers of the dihydropseudosapogenins obtained by the reduction of the pseudosapogenins. Thus Marker and Rohrmann[5] obtained dihydrosarsasapogenin from sarsasapogenin. They also reported that smilagenin was reduced to dihydrosarsasapogenin. This unexpected result was never adequately explained. In contrast to this work, we obtained two distinctly different dihydrosapogenins, XVIIa and XVIIIa, from smilagenin (V) and sarsasapogenin (VI), respectively. It is worth mentioning that these dihydrosapogenins showed a difference of only 2° in melting points and when mixed did not give a melting point depression. The difference between these two compounds was established by direct comparison of their rotations and infrared spectra and by a comparison (melting points, infrared spectra, rotations) of the diacetates and the dibenzoates.

Many infrared spectral determinations were made, during the course of this work, on the epimeric alcohols and their esters, *i.e.*, the pseudosapogenins, the dihydropseudosapogenins, the dihydrosapogenins, the tetrahydrosapogenins. In most instances the spectra of solutions (in chloroform or carbon disulfide) of the epimers were very similar while the spectra of their Nujol mulls showed distinct differences, *e.g.*, Fig. 1.

We proved that configurational differences at the asymmetric C-22 center were not involved in the stereochemistry of the *dihydrosapogenins* XVIIa and XVIIIa by converting these compounds to the respective 26-tosyl esters XVIIb and XVIIIb and, according to the procedure of Schmid and Karrer,[15] subsequently reducing these esters with lithium aluminum hydride to 16,22-epoxycoprostan-3β-ol (XIX). Moreover, it appears unlikely to us that the original sapogenins V and VI differ at C-22. Our experiments, however, do not exclude the possibility of such isomerism. Evidence for definitely settling this point is yet to be obtained.

Other epimeric pairs of sapogenins, such as diosgenin and yamogenin, tigogenin and neotigogenin, and yuccagenin and lilagenin, for which Marker[2] has postulated the existence of isomerism at C-22,

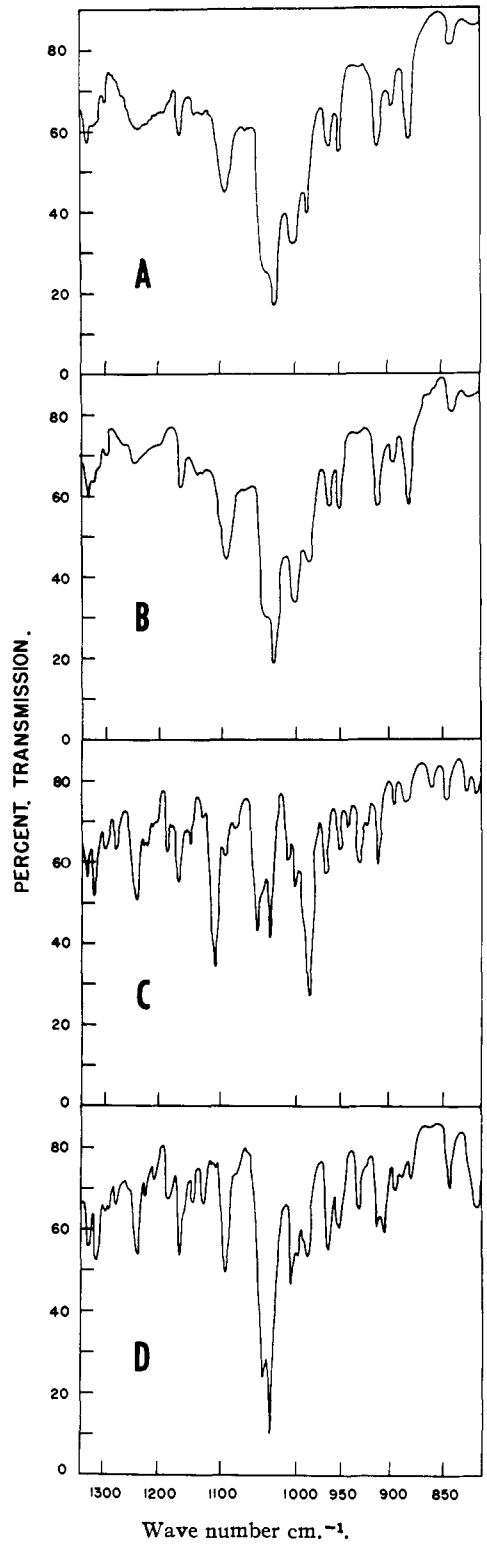


Fig. 1.—Infrared absorption spectra of A, dihydrosmilagenin in chloroform; B, dihydrosarsasapogenin in chloroform; C, dihydrosmilagenin in Nujol; D, dihydrosarsasapogenin in Nujol.

(13) E. Berner and R. Leonardsen, *Ann.*, **538**, 1 (1939).

(14) James (ref. 12) has obtained, in an analogous manner, (−)α-methylglutaric acid from pseudodiosgenin and pseudohecogenin, thereby relating diosgenin and hecogenin to smilagenin in respect to the configuration at C-25. (These three sapogenins were regarded as belonging to the same "22a" or "iso" series in respect to their configuration at C-22.) Furthermore, James has related the last 5 carbon atoms of the side chain of hecogenin and diosgenin, and thereby of smilagenin, to (+)α-methylsuccinic acid, which had been assigned previously the D configuration.

(15) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949).

have been related by various transformations[2,16] to smilagenin and sarsasapogenin. On the basis of these correlations we *assume* that all of these sapogenins have the identical steric arrangement at C-22 and that these so-called "C-22a" and "C-22b" sapogenins are actually C-25a and C-25b isomers.[17,18]

TABLE I

Compound	Smilagenin series M.p., °C.	$[\alpha]^{20}D$	Sarsasapogenin series M.p., °C.	$[\alpha]^{20}D$
Sapogenin	187–188	−66°	198–199	−75°
Acetate	151–151.5	−60	143–144	−70
Pseudo-	158–161	+24	167–169	+12
Diacetate	Oil	+ 8	Oil	− 6
Di-3,5-dinitrobenzoate	176–178	+19	192–194	+30
Dihydropseudo-	159–161	− 2	167–170	− 8
Diacetate	97–98	− 4	93.5–95.5	− 2
Dibenzoate	153–155	± 0	129–131	+ 3
Tetrahydro-[a]	193–195	+39	192.5–194	+33
Triacetate	Oil	+58	Oil	+56
Tribenzoate	130–131	+38	135–136	+40
Tri-*p*-chlorobenzoate	182–183.5	+43	127–129	+38
Dihydro-[a]	164–165.5	+ 3	166–168	− 4
Diacetate	91–93	− 1	67.5–68.5	− 3
Dibenzoate	138–140.5	+ 2	95.5–98	+ 3
26-Tosylate	134–135.5	− 5	131–133	± 0

[a] A mixture of the epimers did not show a melting point depression.

Experimental[19,20]

Purification of Sarsasapogenin (25b-Spirostan-3β-ol)[21] (VI).—Thirty grams of VI (m.p. 189–194°, $[\alpha]^{20}D$ −71°) was acetylated with 500 ml. of pyridine and 120 ml. of acetic anhydride for 18 hours at room temperature. The solution was concentrated to dryness *in vacuo* and the residue was crystallized from 750 ml. of 95% ethanol; yield 28.0 g., m.p. 143–144°, $[\alpha]^{20}D$ −70° (lit.[22] m.p. 145°, $[\alpha]^{25}D$ −70°). The acetate was hydrolyzed by refluxing with 1.4 l. of 2% alcoholic potassium hydroxide solution for 50 min. Crystallization from ethyl acetate–petroleum ether (30–60°) afforded 22.2 g. of pure sarsapogenin (VI), m.p. 198–199°, $[\alpha]^{20}D$ −75° (lit.[22] m.p. 200°, $[\alpha]^{25}D$ −78°).

Smilagenin (25a-Spirostan-3β-ol) (V).—The saponification of smilagenin acetate (from Nutritional Biochemicals Corp., Cleveland, Ohio) afforded smilagenin (V) of such purity that further purification was not necessary. The material,[23] after crystallization from ethanol–water, melted at 187–188°, $[\alpha]^{20}D$ −66° (lit.[22] m.p. 184°, $[\alpha]^{25}D$ −73°).

Pseudosarsasapogenin (16,22-Epoxy-25b-coprost-20(22)-ene-3β,26-diol) (VIII).—In the manner described by Marker and Rohrmann[3] a solution of 5.0 g. of sarsasapogenin (VI) in 25 ml. of acetic anhydride was heated in a bomb tube at 195–200° for eight hours. The acetic anhydride was evaporated *in vacuo* and the oily residue was refluxed for 50 min. with 250 ml. of 2% methanolic potassium hydroxide solution. The solution was diluted with water and the mixture ether extracted. The ether solution was washed with water, dried and evaporated. The residue was crystallized from ethyl acetate containing a few drops of pyridine (added to prevent recyclization due to possible traces of acid in the solvent) to yield 2.04 g. (40.8%) of thick white needles, m.p. 167–169°, $[\alpha]^{20}D$ + 12° (lit.[3] m.p. 171–173°, no rotation given), $\nu_{max}^{CHCl_3}$ 3600 cm.$^{-1}$ (hydroxyl) and 1692 cm.$^{-1}$ (—C=C—O—).

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.86; H, 10.32.

The diacetate was obtained as an oil. Distillation of the oil at 0.001 mm. and 150° gave analytically pure material, $[\alpha]^{20}D$ −5.8°, $\nu_{max}^{CS_2}$ 1742 cm.$^{-1}$ (acetoxy) and 1695 cm.$^{-1}$ (—C=C—O—).

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.11; H, 9.51.

The di-3,5-dinitrobenzoate of VIII, prepared in the usual manner (3,5-dinitrobenzoyl chloride–pyridine, 5 hours, steam-bath) was obtained crystalline from acetone–methanol, m.p. 192–194°, $[\alpha]^{20}D$ +29°, $\nu_{max}^{CHCl_3}$ 1730 cm.$^{-1}$ (ester), 1698–1695 cm.$^{-1}$ (—C=C—O), 1631 and 1597 cm.$^{-1}$ (substituted benzene), and 1546 cm.$^{-1}$ (nitro groups).

Anal. Calcd. for $C_{41}H_{50}N_4O_{13}$: C, 61.02; H, 6.25; N, 6.94. Found: C, 60.80; H, 6.00; N, 6.73.

Pseudosmilagenin (16,22-Epoxy-25a-coprost-20(22)-ene-3β,26-diol) (VII).—Treatment of 4.0 g. of smilagenin as described above for the preparation of VIII yielded 1.0 g. (25%) of VII as thin white needles, m.p. 158–161°, $[\alpha]^{20}D$ +24°, $\nu_{max}^{CHCl_3}$ 3600 cm.$^{-1}$ (hydroxyl) and 1695 cm.$^{-1}$ (—C=C—O—).

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.53; H, 10.55.

The diacetate was obtained as a clear colorless oil. Distillation of the oil at 0.001 mm. and 140° gave analytically pure material, $[\alpha]^{20}D$ +8.3° $\nu_{max}^{CS_2}$ 1742 cm.$^{-1}$ (acetoxy) and 1698 cm.$^{-1}$ (—C=C—O—).

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.25; H, 9.55.

The diacetates of VII and VIII in chloroform solutions exhibited very similar infrared spectra. Liquid smears of the diacetates exhibited significantly different infrared spectra.

The di-3,5-dinitrobenzoate crystallized from acetone–methanol, m.p. 176–178°, $[\alpha]^{20}D$ +19°, $\nu_{max}^{CHCl_3}$ 1730 cm.$^{-1}$ (ester), 1698 cm.$^{-1}$ (—C=C—O), 1631 and 1600 cm.$^{-1}$ (substituted benzene), 1546 cm.$^{-1}$ (nitro groups).

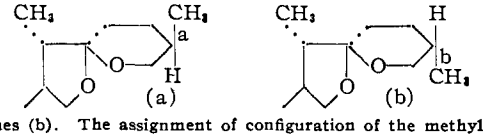
Anal. Calcd. for $C_{41}H_{50}N_4O_{13}$: C, 61.02; H, 6.25; N, 6.94. Found: C, 61.17; H, 6.42; N, 6.97.

Recyclization of VII and VIII.—A solution of 0.1 g. of VIII in 10 ml. of chloroform and one drop of 12 *N* hydro-

(16) R. E. Marker and J. Lopez, THIS JOURNAL, **69**, 2375, 2383, 2393, 2397 (1949).

(17) Marker (ref. 2) claimed to have related kryptogenin to sarsasapogenoic acid by the oxidation of each to the same 3-dehydro-Δ^4-sarsasapogenic acid. Since kryptogenin has been reduced to diosgenin (ref. 2; St. Kaufmann and G. Rosenkranz, THIS JOURNAL, **70**, 3502 (1948)) the triketo acids obtained in the oxidation of kryptogenin and sarsasapogenoic acid must be C-25 epimers. Marker established the sameness of these acids by a comparison of their melting points. However, the triketo acid from sarsasapogenoic acid reportedly melted at 199–201° and the one from kryptogenin melted at 208–210°. We are reinvestigating these experiments.

(18) Based on James' deductions (ref. 12) and on considerations of molecular models (taking into account the fact that bromination leads to a 23-dibromosarsasapogenin and a 23-monobromosmilagenin (ref. 7)) we are tentatively and as a working hypothesis assigning the side chain arrangement (a) to smilagenin. Accordingly, sarsasapogenin



becomes (b). The assignment of configuration of the methyl group at C-20 also corresponds with that in the cholesterol side chain as postulated recently by B. Riniker, D. Arigoni and O. Jeger, [*Helv. Chim. Acta*, **37**, 546 (1954)].

(19) All melting points were determined on the Kofler block. Unless otherwise noted, rotations were determined in approximately 1% solutions in chloroform. Ultraviolet absorption spectra were determined in isoöctane solutions. Infrared spectra were obtained with a Perkin–Elmer model 21 double beam spectrophotometer with sodium chloride prism and cells.

(20) Acetates were prepared by treatment of the alcohol with acetic anhydride and pyridine at room temperature for 18 hours or by refluxing the alcohol with acetic anhydride for 30 min.

(21) The names given in parentheses conform to the suggested rules of the NRC Subcommittee on Steroid Nomenclature and are derived from the structure VI.

(22) M. E. Wall, M. M. Krider, E. S. Rothman and C. R. Eddy, *J. Biol. Chem.*, **198**, 533 (1952).

(23) The smilagenin obtained in this manner was identical with smilagenin obtained from sarsasapogenin by refluxing for 5 days in alcoholic hydrochloric acid.

chloric acid was allowed to stand at room temperature for 45 min. The solution was washed with 2% aqueous sodium bicarbonate, water, dried over sodium sulfate and concentrated *in vacuo*. The residue was crystallized from ethyl acetate to yield 0.09 g. (90%) of sarsasapogenin (VI), m.p. 194–197°, $[\alpha]^{20}D$ −73°. The infrared spectrum was identical with that of an authentic sample of sarsasapogenin.

Refluxing VIII with a mixture of ethanol and concentrated hydrochloric acid for 2 hours, as described by Marker and Rohrmann,[6] gave similar results.

Recyclization of VII gave smilagenin (V) only, in quantitative yield, m.p. 186–188°. The infrared spectrum was identical with that of an authentic sample of smilagenin. A mixture of this product with the one obtained from the recyclization of VIII melted at 180–183°.

Oxidation of Pseudosmilagenin.—To a solution of 1.0 g. of pseudosmilagenin (VII) in 50 ml. of glacial acetic acid at 20° was added, with rapid stirring, a solution of 2.4 g. of chromic acid in 20 ml. of 80% acetic acid. At the end of the addition (3 min.) the temperature had risen to 31°. The mixture was kept at 30° for 45 min., diluted with water and ether extracted. The ethereal solution was washed almost neutral with ice-cold 2% aqueous bicarbonate, then washed with water, dried over sodium sulfate and concentrated to a green oil *in vacuo*. The oily intermediate was not isolated. It was hydrolyzed at room temperature with a solution of 1.0 g. of potassium hydroxide in 50 ml. of wet *t*-butyl alcohol[24] for three hours with vigorous stirring. The mixture was diluted with water and ether extracted. The ether solution was washed with water, dried and evaporated to dryness *in vacuo*.

The residue was crystallized from acetone to yield 0.11 g. (14.7%) of **Δ^{16}-pregnene-3,20-dione (IX)**, m.p. 194–196° (lit.[3] m.p. 200–202°), λ_{max} 233 mμ, log ε 4.0. This compound was identical (melting points, ultraviolet and infrared spectra) with that obtained from the oxidation of pseudosarsasapogenin.

The water washes of the ether solution were combined with the alkaline aqueous fraction and acidified with 5% hydrochloric acid and ether extracted. The ether was removed *in vacuo* and the orange oily residue was sublimed at 90–100° and 0.07 mm. The sublimate was crystallized from ether–pentane to yield 0.021 g. (6.0%) of **(−)-α-methylglutaric acid (XV)**, m.p. 78.5–81.0°, $[\alpha]^{20}D$ −19.9° (EtOH).

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found: C, 49.79; H, 7.03.

Oxidation of Pseudosarsasapogenin.—From 1.0 g. of pseudosarsasapogenin (VIII), oxidized as described above for pseudosmilagenin, was obtained 0.20 g. of **Δ^{16}-pregnene-3,20-dione (IX)**, m.p. 188–192°. Recrystallization from acetone afforded 0.07 g. of pure material, m.p. 194–197° (lit.[3] m.p. 200–202°), λ_{max} 233 mμ, log ε 4.0.

Extraction, sublimation and crystallization of the side chain fragment as described above gave 0.015 g. (4.3%) of **(+)-α-methylglutaric acid (XVI)**, m.p. 78.5–81°, $[\alpha]^{20}D$ +18° (EtOH) [lit.[13] m.p. 81°, $[\alpha]^{20}D$ + 21.1° (EtOH)].

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found: C, 49.40; H, 6.76.

A mixture of the (+)- and (−)α-methylglutaric acids melted at 66–68°. A mixture of equal parts of XV and XVI was recrystallized from ether–pentane and melted at 74–75° (Berner and Leonardsen[13] reported 77° as the melting point of racemic α-methylglutaric acid). These melting points conform to the melting point diagram obtained by Berner and Leonardsen.[13]

The infrared spectra of chloroform solutions of XV and XVI were identical with the spectrum of an authentic sample of racemic α-methylglutaric acid.

Pregnan-3β-16β,20β,triol (X).—This material was prepared from pseudosmilagenin (VII) and from pseudosarsasapogenin (VIII) according to the procedure of Marker, *et al.*,[9] in approximately equal yield. Thus from 0.9 g. of VII was obtained 0.16 g. (21.9%) of X, m.p. 236–240°, $[\alpha]^{20}D$ +31.1° (EtOH) (lit.[9] m.p. 236–240°, no rotation given). From 0.9 g. of VIII was obtained 0.18 g. (24.7%) of X, m.p. 233–239°, $[\alpha]^{20}D$ +31.5° (EtOH).

Dihydropseudosmilagenin (16,22-Epoxy-20ξ,22ξ,25a-coprostane-3β,26-diol (XI).—A mixture of 0.2 g. of pseudosmilagenin (VII), 0.04 g. of Adams catalyst and 20 ml. of acetic acid was shaken with hydrogen at room temperature and pressure. Hydrogenation was complete in 15 min. and afforded 0.2 g. of XI (from ethyl acetate) melting at 145–153°. Two recrystallizations from ethyl acetate gave 0.06 g. (30.0%) of analytically pure material, m.p. 159–161°, $[\alpha]^{20}D$ −1.8°, $\nu_{max}^{CHCl_3}$ 3571 cm.$^{-1}$ (free hydroxyl), no —C=C—O— band present. A second crop of 0.09 g. melted at 155–157°. A mixture with dihydrosmilagenin (164–166°) melted at 157–159°.

Anal. Calcd. for $C_{27}H_{46}O_3 \cdot H_2O$: C, 74.26; H, 11.08. Found: C, 74.04; H, 11.22. Calcd. for $C_{27}H_{46}O_3$: C, 77.47; H, 11.07. Found: C, 77.14; H, 11.02; after drying for 7 hours at 115° and 1 mm., wt. loss 4.10; calcd. for 1 mole of H_2O, 4.12.

The diacetate was crystallized from methanol, m.p. 97–98°, $[\alpha]^{20}D$ −4.3°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 74.07; H, 10.02.

A mixture with dihydrosmilagenin diacetate (91–93°) melted at 76–79°.

Benzoylation (benzoyl chloride–pyridine, 1 hour, steam-bath) and recrystallization from ether–methanol afforded **the dibenzoate** as white needles, m.p. 153–155°, $[\alpha]^{20}D$ ±0°.

Anal. Calcd. for $C_{41}H_{54}O_5$: C, 78.55; H, 8.68. Found: C, 78.59; H, 8.85.

Dihydropseudosarsasapogenin (16,22-Epoxy-20ξ,22ξ-25b-coprostane-3β,26-diol) (XII).—Hydrogenation of 1.0 g. of pseudosarsasapogenin (VIII), as described above for the preparation of XI, gave 0.64 g. (64%) of crystalline **dihydropseudosarsasapogenin (XII)**, m.p. 167–170°, $[\alpha]^{20}D$, −7.5° (lit.[10] m.p. 168–170°, no rotation given), $\nu_{max}^{CHCl_3}$ 3570 cm.$^{-1}$ (free hydroxyl), no —C=C—O band present.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.53; H, 11.01.

A mixture with dihydrosarsasapogenin (165–167°) melted at 159–162°; with dihydrosmilagenin (164–166°) 152–160°; with dihydropseudosmilagenin (159–161°) 153–157°.

The diacetate melted at 93.5–95.5°, $[\alpha]^{20}D$ −1.8° (lit.[10] m.p. 95–97°, no rotation given).

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 73.97; H, 9.93.

A mixture with dihydropseudosmilagenin diacetate (97–98°) melted at 82–95°; with dihydrosmilagenin diacetate (91–93°) 77–80°.

The dibenzoate melted at 129–131°, $[\alpha]^{20}D$ +3.1°.

Anal. Calcd. for $C_{41}H_{54}O_5$: C, 78.55; H, 8.68. Found: C, 78.49; H, 8.48.

Dihydrosmilagenin (16,22-Epoxy-25a-coprostane-3β,26-diol) (XVIIa).—A mixture of 1.25 g. of smilagenin (V), 0.25 g. of Adams catalyst and 125 ml. of acetic acid was shaken with hydrogen at room temperature and pressure. Hydrogenation was complete in 90 min. Crystallization from ethyl acetate gave 0.78 g. of white needles of XVIIa melting at 157–164°. Recrystallization from benzene gave 0.73 g. (58.4%) melting at 160–164°. For analysis a portion of this material was chromatographed on ethyl acetate-washed alumina and the fraction eluted with benzene–chloroform 1:1 was crystallized from ethyl acetate; m.p. 164–165.5°, $[\alpha]^{20}D$ +3.0°, $\nu_{max}^{CHCl_3}$ 3559 cm.$^{-1}$ (free hydroxyl).

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.28; H, 11.15.

The diacetate was crystallized from methanol at 0°, m.p. 91–93°, $[\alpha]^{20}D$ −1°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 73.90; H, 10.05.

The dibenzoate (benzoyl chloride–pyridine, 16 hours, 25°) was obtained as white needles from ether–methanol, m.p. 138–140.5°, $[\alpha]^{20}D$ +2.1°.

Anal. Calcd. for $C_{41}H_{54}O_5$: C, 78.55; H, 8.68. Found: C, 78.33; H, 8.58.

Dihydrosarsasapogenin (16,22-Epoxy-25b-coprostane-3β,26-diol) (XVIIIa).—Hydrogenation of 2.0 g. of sarsasapogenin (VI), as described above for the preparation of XVIIa gave 1.23 g. (61.5%) of **dihydrosarsasapogenin (XVIIIa)** as white needles, m.p. 165–168°. For analysis a

(24) M. E. Wall, H. E. Kenney, H. W. Jones and E. S. Rothman, Fifth Meeting-in-Miniature, Phila. Section, A.C.S., Jan. 29, 1953, abstracts of papers, page 10.

portion was purified as described for XVIIa to afford material melting at 166–168° (lit.[5] m.p. 165°), $[\alpha]^{20}D$ −4° (lit.[25] $[\alpha]^{20}D$ −2°), $\nu_{max}^{CHCl_3}$ 3571 cm.$^{-1}$ (free hydroxyl).

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.62; H, 11.16.

Admixture with dihydrosmilagenin (164–165.5°) did not depress the melting point.

The diacetate was obtained as white needles from methanol at −20°, m.p. 68–69°, $[\alpha]^{20}D$ −3.1°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 74.02; H, 9.86.

The dibenzoate was obtained as white needles from ether–methanol, m.p. 95.5–98°, $[\alpha]^{20}D$ +3.0.

Anal. Calcd. for $C_{41}H_{54}O_5$: C, 78.55; H, 8.68. Found: C, 78.37; H, 8.85.

Dihydrosmilagenin 26-Tosylate (16,22-Epoxy-25a-coprostane-3β,26-diol 26-Tosylate) (XVIIb).—A solution of 1.5 g. of dihydrosmilagenin (XVIIa) in 15 ml. of dry pyridine was cooled to −6° and to it was added a solution of 0.78 g. of *p*-toluenesulfonyl chloride in 7 ml. of dry pyridine over a 1-hour period. The temperature of the reaction mixture was maintained at −6 to −10° during this addition then kept at −5 to 0° for 1 hour. The mixture was allowed to stand overnight at room temperature. The colorless solution was poured into ice and water and the oily precipitate was ether extracted. The ethereal solution was washed with cold 5% hydrochloric acid, water, 2% sodium bicarbonate solution, water and dried over sodium sulfate. The solution was concentrated to dryness *in vacuo* to leave a clear, colorless oily tosylate, **XVIIb**. For characterization and analysis a portion of the oil was chromatographed on Florisil. Crystallization from ether–petroleum ether (30–60°) of the fraction eluted with benzene–chloroform 3:1 gave white needles, m.p. 134–135.5°, $[\alpha]^{20}D$ −4.6°.

Anal. Calcd. for $C_{34}H_{52}O_5S$: C, 71.29; H, 9.15. Found: C, 71.24; H, 9.05.

Dihydrosarsasapogenin 26-Tosylate (16,22-Epoxy-25b-coprostane-3β,26-diol 26-Tosylate) (XVIIIb).—From dihydrosarsasapogenin (XVIIIb) in exactly the same manner as described above for the preparation of XVIIb, an oily **26-tosylate (XVIIIb)** was obtained. For characterization and analysis a portion of this oil was chromatographed on Florisil. Crystallization from ether–petroleum ether (30–60°) of the fraction eluted with benzene–chloroform 3:1 yielded white needles, m.p. 131–133°, $[\alpha]^{20}D$ ±0°. A mixture with XVIIb melted at 114–118°.

Anal. Calcd. for $C_{34}H_{52}O_5S$: C, 71.29; H, 9.15. Found: C, 71.43; H, 9.02.

16,22-Epoxycoprostan-3β-ol (XIX).—The crude oily dihydrosmilagenin 26-tosylate was dissolved in 50 ml. of benzene and the solution concentrated to a volume of 30 ml. (to remove water). After the addition of 30 ml. of dry ether and 10 ml. of a 1.5 *M* solution of lithium aluminum hydride in ether the reaction mixture was refluxed overnight. The mixture was cooled, a few drops of ethyl acetate added to it and then treated with 25 ml. of 6 *N* hydrochloric acid. The aqueous layer was separated, extracted with ether and the extracts combined with the benzene–ether layer. The non-aqueous layer was washed with 2% sodium bicarbonate solution and with water, dried over sodium sulfate and concentrated to dryness *in vacuo*. The oily residue was chromatographed on ethyl acetate-washed alumina. The fraction eluted with benzene was crystallized from ethanol–water and recrystallized from acetone–water to yield 0.49 g. of white needles, m.p. 137–139°, $[\alpha]^{20}D$ −4.0°. A second crop weighing 0.19 g. melted at 134–137°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.53; H, 11.52. Found: C, 80.39; H, 11.73.

Reduction of the crude oily dihydrosarsasapogenin 26-tosylate (XVIIIb) in the same manner gave similar results. The product obtained was identical with the one from XVIIb in every respect (melting point, rotation, infrared spectra, derivatives).

(25) H. M. Doukas and T. K. Fontaine, THIS JOURNAL, **75**, 5355 (1953).

The acetate of XIX crystallized from methanol–water, m.p. 91–93°, $[\alpha]^{20}D$ −5.0°.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.44; H, 10.79.

The benzoate (benzoyl chloride–pyridine, 1 hour, steam-bath) crystallized from ether–methanol, m.p. 138–140°, $[\alpha]^{20}D$ +1°.

Anal. Calcd. for $C_{34}H_{51}O_3$: C, 80.42; H, 10.13. Found: C, 80.57; H, 10.27.

Tetrahydrosmilagenin (25a-Coprostane-3β,16β,26-triol[26]) (XIII).—The Clemmensen reduction of 1.0 g. of smilagenin (V), as described by Marker and Rohrmann[5] for the preparation of tetrahydrosarsasapogenin from sarsasapogenin, afforded 0.6 g. (59.4%) of thick crystals, m.p. 193–195°, $[\alpha]^{20}D$ +39.4° (EtOH), $[\alpha]^{20}D$ +44.5° (pyridine).

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.09; H, 11.50. Found: C, 77.35; H, 11.35.

The triacetate was obtained as a clear, colorless oil, $[\alpha]^{20}D$ +58.4°.

Anal. Calcd. for $C_{33}H_{54}O_6$: C, 72.49; H, 9.96. Found: C, 72.51; H, 10.15.

The tribenzoate (benzoyl chloride–pyridine, 18 hours, 25°) was crystallized from ether–methanol, m.p. 130–131°, $[\alpha]^{20}D$ +37.8°.

Anal. Calcd. for $C_{48}H_{60}O_6$: C, 78.65; H, 8.25. Found: C, 78.51; H, 8.17.

The tri-*p*-chlorobenzoate (*p*-chlorobenzoyl chloride–pyridine, 18 hours, 25°) crystallized from ether–methanol, m.p. 182–183.5°, $[\alpha]^{20}D$ +43°.

Anal. Calcd. for $C_{48}H_{57}O_6Cl_3$: C, 68.93; H, 6.87. Found: C, 68.70; H, 6.77.

Tetrahydrosarsasapogenin (25b-Coprostane-3β,16β,26-triol) (XIV).—From 1.0 g. of sarsasapogenin (VI), by Clemmensen reduction as described by Marker and Rohrmann,[5] was obtained 0.6 g. (59.4%) of thick crystals, m.p. 192.5–194°, $[\alpha]^{20}D$ +33.6° (EtOH), $[\alpha]^{20}D$ +36.5° (pyridine) (lit.[5] m.p. 193°, no rotation given).

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.09; H, 11.50. Found: C, 77.06; H, 11.39.

Admixture with tetrahydrosmilagenin did not depress the melting point.

The triacetate was obtained as a clear, colorless oil, $[\alpha]^{20}D$ +55.7°.

Anal. Calcd. for $C_{33}H_{54}O_6$: C, 72.49; H, 9.96. Found: C, 72.35; H, 9.80.

The tribenzoate crystallized from ether–methanol, m.p. 135–136°, $[\alpha]^{20}D$ +40.4°.

Anal. Calcd. for $C_{48}H_{60}O_6$: C, 78.65; H, 8.25. Found: C, 78.50; H, 8.39.

A mixture with tetrahydrosmilagenin tribenzoate (130–131°) melted at 120–124°.

The tri-*p*-chlorobenzoate was obtained crystalline from ether–methanol, m.p. 127–129°, $[\alpha]^{20}D$ +37.7°.

Anal. Calcd. for $C_{48}H_{57}O_6Cl_3$: C, 68.93; H, 6.87. Found: C, 68.80; H, 6.80.

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(26) For a discussion of the assignment of configuration at position C-16 see H. Hirschmann, F. B. Hirschmann and M. A. Daus, *J. Biol. Chem.*, **178**, 751 (1949).