[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. XVII. Stereochemistry of the Spiroketal Side Chain[2]

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Pseudosarsasapogenin and pseudosmilagenin were converted by acetic acid to a new series isomeric at C_{20} with natural sapogenins. On catalytic hydrogenation, the 20-isosapogenins form dihydro-20-isosapogenins in a manner analogous to natural sapogenins. Oxidation of 20-isosapogenins with CrO_3–acetic acid ruptures the C_{20}–C_{22}-linkage and leads to the Δ^{16}-20-keto-pregnenes. This reaction and others indicate that ring E is under strain in 20-isosapogenins and thus enables assignment of the configuration at C_{20} of natural and 20-isosapogenins. The configurations at C_{20} and C_{22}, and configurations and conformations at C_{25} of natural and 20-iso sarsasapogenin, smilagenin and their dihydro analogs are discussed and evidence presented accounting for the proposed configurations and conformations.

The basic structures of the various steroidal sapogenins have been well established, largely as a result of the researches of Marker and his co-workers,[3] and are shown in Fig. 1. However, the problem of the configuration of the spiroketal side chain which contains three asymmetric carbon atoms at C_{20}, C_{22} and C_{25}, has not been attacked until quite recently.

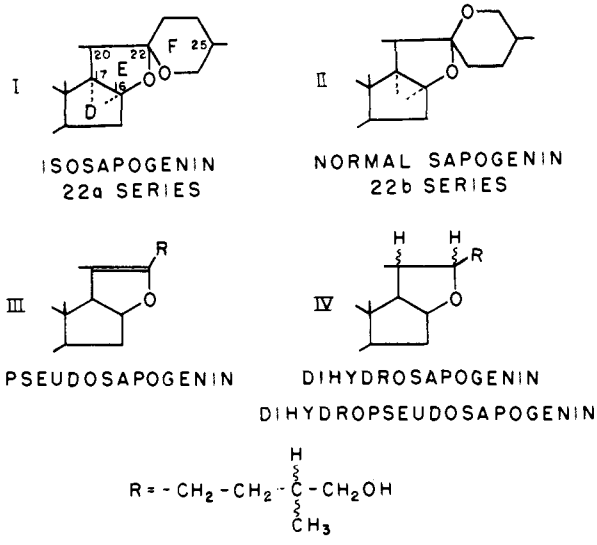


Fig. 1.—Current concepts of the steroidal sapogenin spiroketal side chain.

We became interested in this problem as a result of our researches on pseudomarkogenin and pseudosamogenin, probable dihydroxy analogs of sarsasapogenin and smilagenin, respectively.[4] On treatment of these compounds with hydrochloric acid we obtained markogenin and samogenin, respectively, whereas from the work of Marker, Rohrmann and Jones[5] we would have expected to get only one of these.

These researches were continued with the more available isomers sarsasapogenin and smilagenin. Pseudosarsasapogenin (V) and pseudosmilagenin (VI), *cf.* Fig. 2, were prepared by a slight modification of Marker's usual procedures.[3] In contrast to Marker's findings[5] we observed that pseudosapogenins V and VI were distinctly different, particularly in regard to melting point and optical rotation (Table I), and X-ray diffraction patterns. The infrared spectra of the two compounds in CS_2 were identical (Fig. 3). Refluxing V and VI with alcoholic hydrochloric acid gave high yields of sarsasapogenin (VII) and smilagenin (VIII), respectively. The data thus clearly show that pseudosarsasapogenin (V) and pseudosmilagenin (VI) are different, presumably at carbon 25. This was demonstrated unequivocally by Scheer, Kostic and Mosettig.[6] In a preliminary communication which appeared while these researches were in progress, these work-

TABLE I

PHYSICAL CONSTANTS OF SARSASAPOGENIN, SMILAGENIN AND THEIR DERIVATIVES

Compound	M.p., °C.	$[\alpha]^{25}D$, $CHCl_3$	MD
Sarsasapogenin	200	−85	−354
Acetate	145	−71	−320
Smilagenin	183	−74	−306
Acetate	150	−64	−292
3-Desoxysarsasapogenin	218	−73	−292
3-Desoxysmilagenin	135	−61	−244
20-Isosarsasapogenin	176	+32	+133
Acetate	167	+30	+137
20-Isosmilagenin	185	−60	−248
Acetate	160	−49	−224
Sarsasapogenone	223	−70	−290
Smilagenone	188	−60	−248
20-Isosarsasapogenone	151	+50	+206
20-Isosmilagenone	162	−55	−228
Pseudosarsasapogenin	169	+12 (dioxane)	+ 50
Pseudosmilagenin	161	+20 (dioxane)	+ 83
3-Desoxypseudosarsasapogenin	167	+ 2 (dioxane)	+ 8
Dihydrosarsasapogenin	164	− 7	− 29
Diacetate	Oil	− 4	− 18
Dihydrosmilagenin	162	+ 1.5	+ 6
Diacetate	93	+ 2	+ 9
Dihydro-20-isosarsasapogenin	167	− 8	− 34
Diacetate	96	− 3	− 14
Dihydro-20-isosmilagenin	161	+ 3	+ 13
Diacetate	96	− 4	− 18
16-Pregnen-3,20-dione	202	+88	+277
16,22-Epoxycoprostan-3β-ol-3(3,5)-dinitrobenzoate	237	+ 6.3	+ 38

(1) A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture. Article not copyrighted.

(2) Paper XVI, M. M. Krider and M. E. Wall, THIS JOURNAL, **76**, 3515 (1954). A preliminary report of these researches has appeared earlier: (a) M. E. Wall, C. R. Eddy and S. Serota, *ibid.*, **76**, 2849 (1954), and (b) M. E. Wall and S. Serota, *ibid.*, **76**, 2850 (1954).

(3) For pertinent references see R. E. Marker, *et al.*, *ibid.* (1939-1947); in particular **69**, 2167 (1947).

(4) M. E. Wall, C. R. Eddy, S. Serota and R. F. Mininger, *ibid.*, **75**, 4437 (1953).

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

(6) I. Scheer, R. B. Kostic and E. Mosettig, *ibid.*, **75**, 4871 (1953).

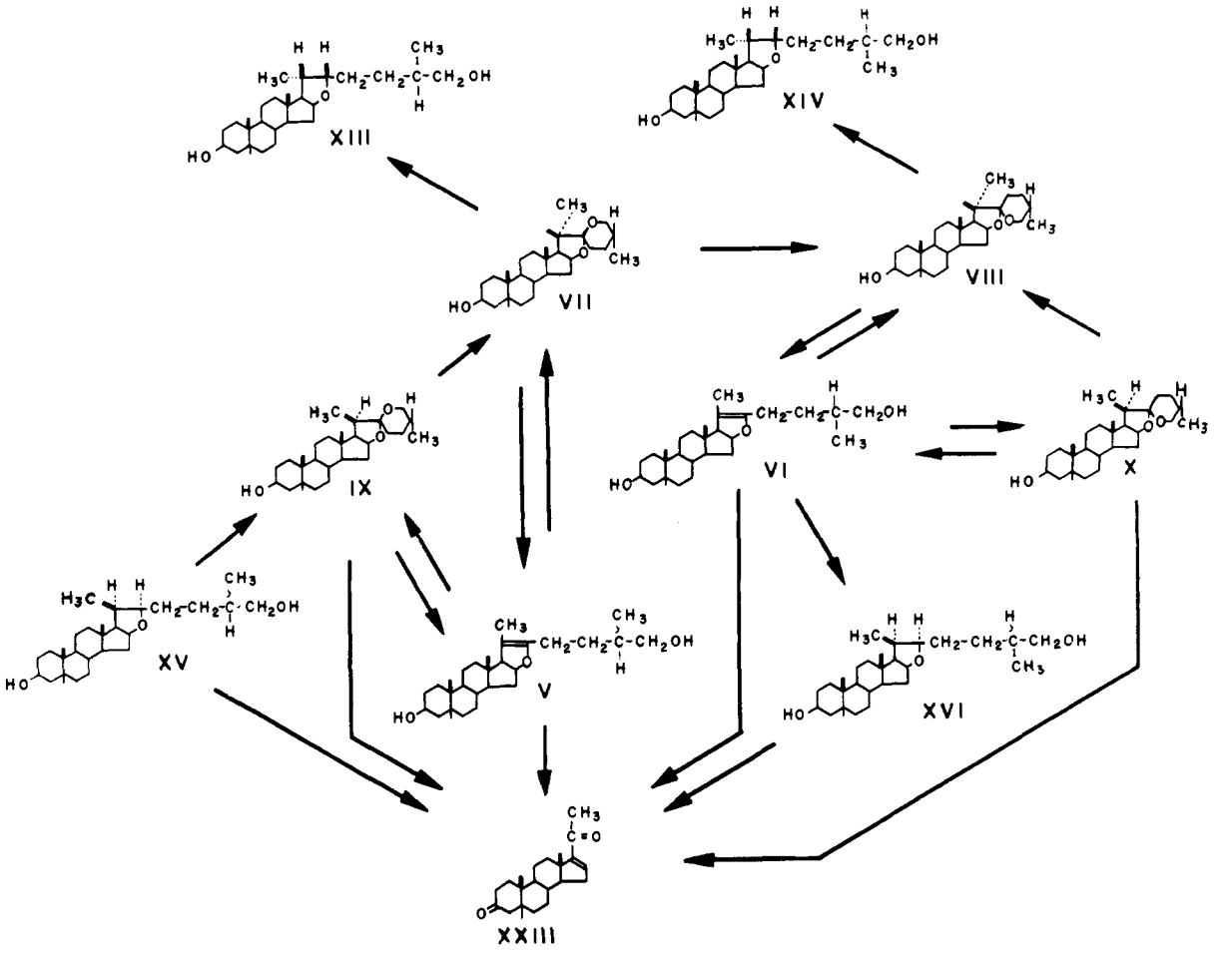


Fig. 2.—Reactions of sapogenins and 20-isosapogenins.

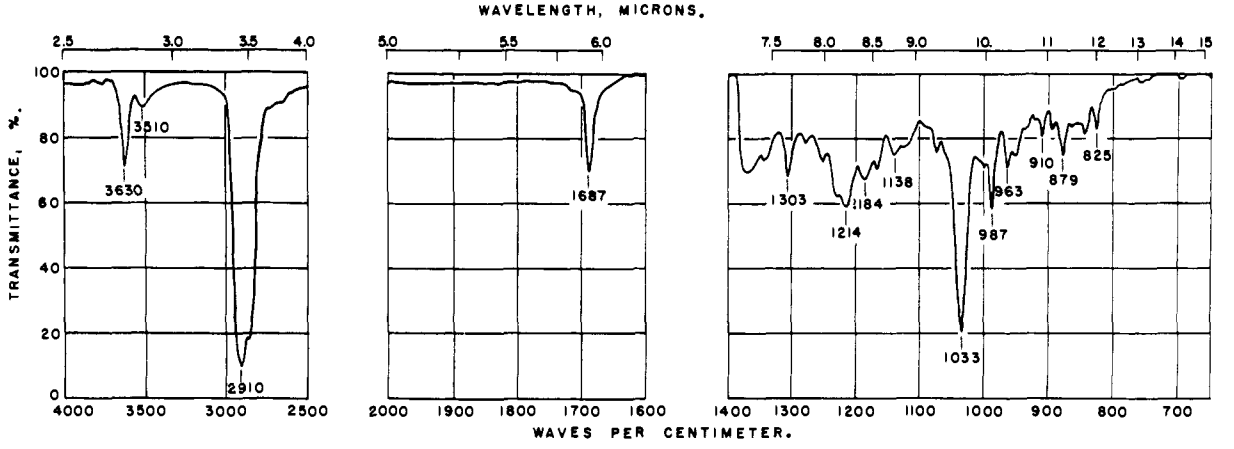


Fig. 3.—Infrared spectrum of pseudosarsasapogenin, 3 g. per liter in CS_2, 3-mm. cell. Pseudosmilagenin has an identical spectrum.

ers showed that V and VI were different at carbon 25 by obtaining, on oxidation followed by alkaline cleavage, the corresponding (+)- and (−)-α-methylglutaric acids.

Isomerization of pseudosapogenins V and VI under mild acidic conditions resulted in formation of a new series of sapogenin derivatives with characteristic infrared spectra shown in Figs. 4 and 5. With hydrochloric acid (1–10% by volume in ethanol) at room temperature immediate conversion to the new derivatives occurred followed by the much slower (4–5 hours) formation of sarsasapogenin and smilagenin. Treatment of pseudosapogenins V and VI with a 50–50 mixture of glacial acetic acid and ethanol at room temperature for periods up to 24 hours resulted only in formation of the new derivatives and is thus the preferred way to make these compounds. For reasons to be developed sub-

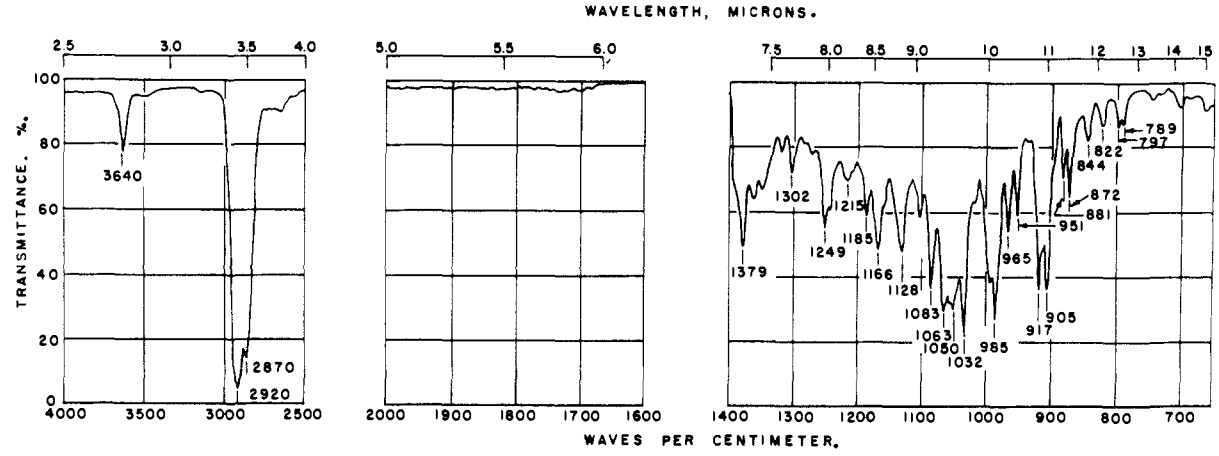


Fig. 4.—Infrared spectrum of 20-isosarsasapogenin, 10 g. per liter in CS_2, 1.0-mm. cell.

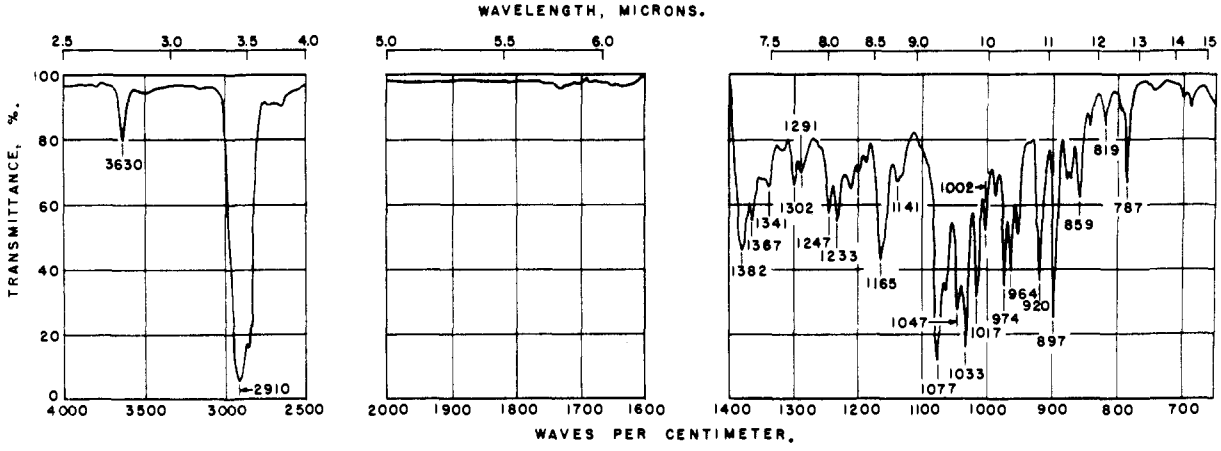


Fig. 5.—Infrared spectrum of 20-isomilagenin, 10 g. per liter in CS_2, 1.0-mm. cell.

sequently, we believe that the new compounds formed from pseudosarsasapogenin (V) and pseudosmilagenin (VI) are C_{20}-isomers of sarsasapogenin and smilagenin, respectively. In accordance with the nomenclature proposed by Fieser and Fieser[7] we will call these compounds 20-isosarsasapogenin (IX) and 20-isosmilagenin (X).[8]

In addition to the difference in infrared spectra, 20-isosarsasapogenin has unusually high positive molecular rotation values as compared with 20-isosmilagenin (*cf.* Table I).

The 20-isosapogenins IX and X are isomeric with pseudosapogenins V and VI and sapogenins VII and VIII. As outlined in Fig. 2 all these isomers have been inter-related.

Room temperature or hot acetylation of IX and X with pyridine–acetic anhydride gave the 3-monoacetates, 20-isosarsasapogenin acetate (XI) and 20-isosmilagenin acetate (XII), respectively. On alkaline hydrolysis XI and XII formed IX and X. When the 20-isosapogenins IX and X were refluxed with acetic anhydride for several hours and the oily product hydrolyzed with alcoholic KOH, pseudosarsasapogenin (V) and pseudosmilagenin (VI) were obtained. Heating IX and X for short periods at their melting points also resulted in formation of V and VI. Refluxing IX and X for short periods with alcoholic hydrochloric acid resulted in rapid formation of sapogenins VII and VIII.

Catalytic hydrogenation of sarsasapogenin (VII) and smilagenin (VIII) in acetic acid solution resulted in formation of dihydrosarsasapogenin (XIII) and dihydrosmilagenin (XIV). Although XIII and XIV have similar melting points and identical infrared spectra in CS_2 solution (Fig. 6), the compounds have distinctly different X-ray diffraction patterns and hence are not identical. This differs from the results of Marker and Rohrmann[9] and is in accord with Scheer, *et al.*,[6] who demonstrated that XIII and XIV are different only at C_{25} by converting them to the identical 16,22-epoxycoprostan-3β-ol.[10]

(7) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Co., 3rd ed., New York, 1949, pp. vi-viii.

(8) After submission of this paper to THIS JOURNAL a number of preliminary communications have appeared in which several pseudosapogenins have been isomerized to products which we believe have the 20-isosapogenin configuration: (a) R. K. Callow and V. H. T. James, *Chemistry & Industry*, June 12, 691 (1954). (b) D. H. W. Dickson, *et al.*, *ibid.*, June 12, 692 (1954). (c) J. B. Ziegler, W. E. Rosen and A. Shabica, THIS JOURNAL, **76**, 3865 (1954). Further, one of the reviewer's of the paper believes that Marker and Lopez, *ibid.*, **69**, 2373 (1947), may have actually been the first group to prepare what we call 20-isosapogenins, although these workers obviously were under the impression that their neosapogenins were "normal" or 22b-sapogenins.

(9) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846 (1939).

(10) We have confirmed this important observation by converting XIII and XIV to the identical 16,22-epoxycoprostan-3β-ol-3-dinitrobenzoate, *cf.* Experimental section.

Similar hydrogenation of 20-isosapogenins IX and X gave dihydro-20-isosarsasapogenin (XV) and dihydro-20-isosmilagenin (XVI). As with dihydrosapogenins XIII and XIV, dihydro-20-isosapogenins XV and XVI had similar physical properties including identical infrared spectra, but as shown by X-ray data were distinctly different. Catalytic hydrogenation of pseudosarsasapogenin (V) and pseudosmilagenin (VI) *as the 3,26-diacetates* followed by alkaline hydrolysis yielded the corresponding dihydropseudosapogenins.[11] These were *identical* with the dihydro-20-isosapogenins XV and XVI. In order to avoid confusion we will use the term dihydro-20-isosapogenin to refer to the hydrogenation products of both 20-iso- and pseudosapogenins.[12] Acetylation of XV and XVI gave the corresponding dihydro-20-isosapogenin-3,26-diacetates XVII and XVIII. The dihydro-20-isosapogenins XV and XVI were not affected by refluxing with alcoholic hydrochloric acid for several hours.

The evidence accumulated at this stage pointed to the formation of a new type of steroid sapogenin. The conversion of the 20-isosapogenins to pseudosapogenins or to dihydro-20-isosapogenins was analogous to the reactions of sapogenins. The infrared spectra had many similarities to those obtained with natural steroidal sapogenins. After hydrogenation, relatively simple spectra similar to that of dihydrosapogenins were obtained. From observations on the infrared spectra from this Laboratory[13,14] and from the work of Jones and his associates[15,16] we concluded that 20-isosapogenins must have a spiroketal side chain similar to that of their naturally occurring analogs.[17]

A study of the oxidative behavior of sarsasapogenin VII and smilagenin VIII, as compared with their 20-iso analogs IX and X, revealed differences which permit assignment of configuration at C_{20}. Oxidation of VII and VIII with the mild oxidizing agent CrO_3 in pyridine[18] gave the known 3-ketones, sarsasapogenone (XIX) and smilagenone (XX).[19] Similar oxidation of the 20-isosapogenins IX and X gave 20-isosarsasapogenone (XXI) and 20-isosmilagenone (XXII), respectively. Refluxing ketones XXI and XXII with hydrochloric acid resulted in formation of XIX and XX, respectively.

Oxidation of sarsasapogenin and smilagenin with CrO_3–acetic acid at room temperature gave only the 3-ketones XIX and XX. Similar oxidation of 20-isosarsasapogenin (IX) and 20-isosmilagenin (X) resulted in formation of amorphous acids. On treating these with KOH in *t*-butyl alcohol,[20] 16-pregnen-3,20-dione (XXIII) was obtained in each case in good yield.

Oxidation of dihydro- and dihydro-20-isosapogenins revealed similar differences. Thus dihydrosapogenins XIII and XIV on CrO_3–acetic acid oxidation formed three keto-26-acids[19,21] unchanged by alkaline treatment. Similar oxidation and alkaline cleavage of the dihydro-20-isosapogenins XV and XVI gave 16-pregnen-3,20-dione (XXIII).

From the acidic fractions obtained from the alkaline hydrolysis of the oxidation products of 20-isosarsasapogenin (IX) and 20-isosmilagenin (X) we isolated (+)- and (−)-α-methylglutaric acids, respectively. Thus the 20-isosapogenins IX and X differ at C_{25} in the same manner as was demonstrated for the sapogenins VII and VIII by Scheer, *et al.*[6]

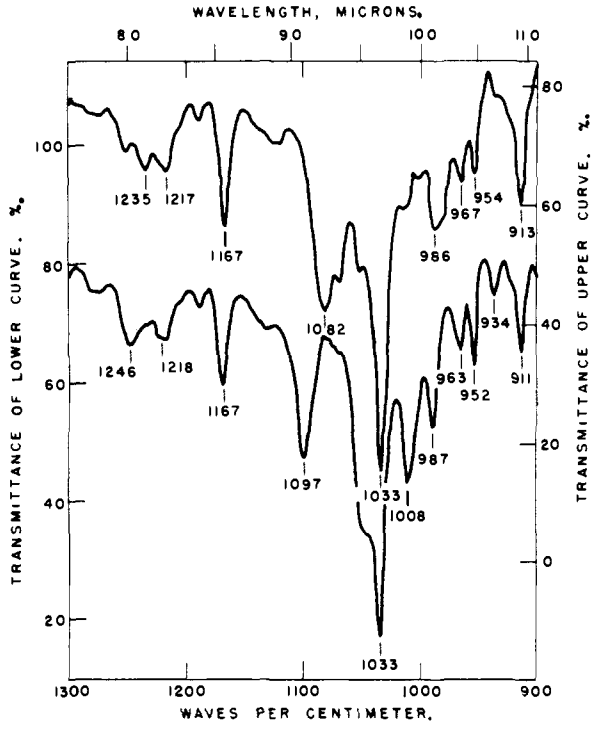


Fig. 6.—Upper curve: dihydro-20-isosarsasapogenin, 4 g. per liter in CS_2, 3-mm. cell. The smilagenin analog has an essentially identical spectrum. Lower curve: dihydrosarsasapogenin, 4 g. per liter in CS_2, 3-mm. cell. The smilagenin analog has an essentially identical spectrum.

(11) R. E. Marker and E. S. Rohrmann, THIS JOURNAL, **62**, 518, 521 (1940).

(12) Since the products of hydrogenation of sapogenins are called dihydrosapogenins, it is logical to give the name dihydro-20-isosapogenins to products from similar hydrogenation of 20-isosapogenins. Since hydrogenation of pseudosapogenins also gives products now identified as dihydro-20-isosapogenins, we propose that the earlier term dihydropseudosapogenin be abandoned.

(13) C. R. Eddy, M. E. Wall and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).

(14) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *ibid.*, **24**, 1337 (1952).

(15) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953).

(16) R. N. Jones, E. Katzenellenbogen and K. Dobriner, "Collected Infrared Absorption Spectra of Steroid Sapogenins," Div. Inf. Serv. Nat. Res. Council, Ottawa, Canada, N.R.C. No. 2929 (1953).

(17) The changes in infrared spectra are best illustrated with desoxysapogenins. Thus 3-desoxysarsasapogenin has an infrared absorption spectrum in which all hydroxyl bands are lacking and with the typical 22b-spiroketal bands.[14,15] Conversion to 3-desoxypseudosarsasapogenin results in loss of the spiroketal bands, but a new hydrogen-bonded C_{26}-hydroxyl is formed. On treatment of the pseudosapogenin with dilute hydrochloric or in ethanol-acetic acid to form 3-desoxy-20-isosarsasapogenin the hydroxyl bands disappear and spiroketal bands similar in complexity but distinctly different from those in 3-desoxysarsasapogenin are found. On refluxing the 20-isosapogenin with hydrochloric acid, 3-desoxysarsasapogenin is obtained completing the cycle.

(18) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(19) L. F. Fieser and R. P. Jacobsen, *ibid.*, **60**, 28 (1938).

(20) 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, p. 10.

(21) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 3477 (1939).

Carbon 20 Configuration.—Steroidal sapogenins and their 20-iso analogs must have one or the other of the configurations shown below in Fig. 7, A and B.

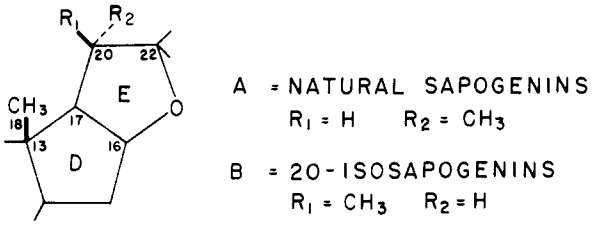


Fig. 7.—Configuration of sapogenins and 20-isosapogenins at carbon 20.

Fisher–Taylor–Hirschfelder models constructed in accordance with these formulations indicate that in B, the methyl groups attached to carbons 13 and 20 put a tremendous strain on ring E[22] whereas models of formulation A indicate relatively much less ring strain. Formulation B is assigned to 20-isosarsasapogenin (IX), 20-isosmilagenin (X) and 20-isosapogenins in general. It is in accord with the *facile* oxidative cleavage of IX and X and their dihydro-20-iso-analogs XV and XVI[23] and rationalizes the previously inexplicable oxidative cleavage of dihydropseudosapogenins (dihydro-20-isosapogenins).[3,11] It is also in accord with the greater lability of IX and X toward acetic anhydride, hydrochloric acid and heat as compared with sapogenins VII and VIII.[24] Accordingly, sarsasapogenin (VII), smilagenin (VIII), and all natural sapogenins have C_{20}-configuration as shown in A.

As we have noted in a previous communication[2a] the establishment of the configuration of naturally occurring steroidal sapogenins at C_{20} is confirmation of the 20-α-configuration of the cholesterol side chain. Recent papers have established the absolute configuration of the cholesterol side chain.[26,27] Our assignment of C_{20}-configuration of naturally occurring steroidal sapogenins is in complete agreement with these recent findings and also with the conversion of diosgenin to cholesterol reported by Marker and Turner.[28]

Configurations at C_{22} and C_{25}.—Ring F in steroidal sapogenins can theoretically have two configurations at C_{22} and two at C_{25}. The researches of Scheer, *et al.*,[6] and James' deductions[29] permit assignment of configuration at C_{25} for sarsasapogenin and smilagenin. Since 20-isosarsasapogenin and 20-isosmilagenin on oxidation also yield (+)- and (−)-α-methylglutaric acids, respectively, these 20-isosapogenins and their open chain analogs must have the same C_{25}-configuration as the corresponding natural sapogenins.

Molecular models of rings E and F may be constructed with ring F in a variety of planar chair forms. The various possibilities for sarsasapogenin and smilagenin are shown in Fig. 8 with configurations at C_{20} according to our deductions and at C_{25} according to James.[29]

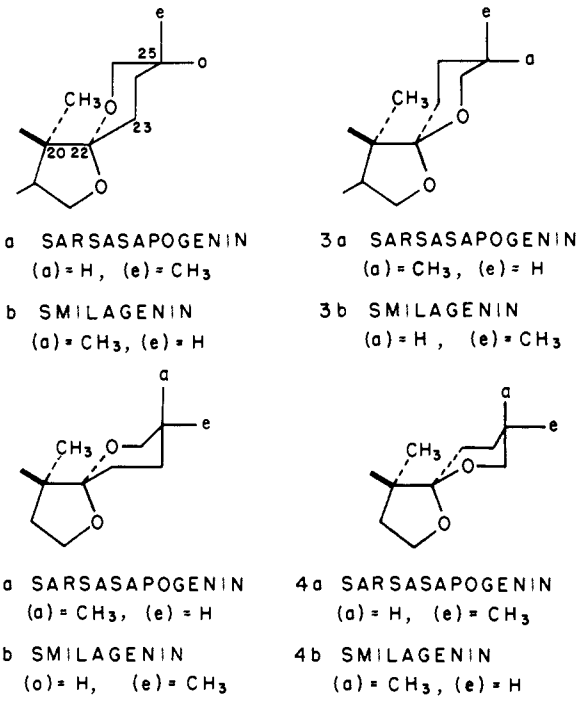


Fig. 8.—Configuration at C_{22}, configuration and conformation at C_{25}, of sarsasapogenin and smilagenin.

Inspection of models of formulations 1a,b and 3a,b (Fig. 8) indicate that these formulations are improbable due to steric interaction between the C_{21}-methyl and C_{23}-methylene or C_{26}-oxygen groups. Conformational analysis allows some selection from the remaining alternatives.[30–32] On prolonged

(22) Formulation B appears improbable from molecular models due to steric hindrance of the C_{18}- and C_{21}-methyl groups. The difficulty of constructing steroid models, particularly fusing rings C and D, is well known. Hence the models must be used more to indicate relative differences between two possible forms, *i.e.*, B, if its exists, would be less stable than A rather than the reverse.

(23) Recently, several research groups have reported on the isomerization of pseudotigogenin and pseudohecogenin to give new sapogenins[8a,8b] which we believe are identical to 20-isotigogenin and 20-isohecogenin prepared in our laboratory. These workers postulated that their new sapogenins differed from natural sapogenins at C_{22} because of the improbability of models showing C_{20} isomerism (*cf.* ref. 22). In researches which will be reported in detail in another paper we have found that the side chain of these compounds as well as that of 20-isodiosgenin is easily cleaved by CrO_3 in acetic acid. We feel that the facile oxidative cleavage of 20-isosapogenins and their hydrogenated analogs is best explained by C_{20} isomerism.

(24) The formation of the unstable 20-isosapogenins from pseudosapogenins by the action of dilute hydrochloric acid may be explained as follows: Addition of H^+ on the unhindered rear face of carbon 20 is favored on steric grounds. This is clearly demonstrated by molecular models. Accordingly, this reaction is rapid or in terms used by Corey,[25] 20-isosapogenins are the product of kinetic control. Owing to their greater energy resulting from steric repulsions, the 20-isosapogenins are unstable. The more stable sapogenins which are formed slowly are thus the product of thermodynamic control. At reflux temperatures or on long standing at room temperature, only the thermodynamically stable isomer will be found. Recently Callow and James[8a] and Dickson, *et al.*,[8b] have observed that pseudohecogenin and neohecogenin probably = anahecogenin = 20-isohecogenin mutarotate to the same equilibrium mixture in the presence of dilute HCl. These observations favor the concept that pseudosapogenin is the intermediate in the hydrochloric acid conversion of 20-isosapogenins to sapogenins.

(25) E. J. Corey, THIS JOURNAL, **76**, 175 (1954).

(26) O. Jeger, *et al.*, *Helv. Chim. Acta*, **37**, 546 (1954).

(27) J. W. Cornforth, *et al.*, *Nature*, **173**, 536 (1954).

(28) R. E. Marker and D. L. Turner, THIS JOURNAL, **63**, 767 (1941).

(29) V. H. T. James, *Chem. and Ind.*, 1388 (1953).

(30) D. H. R. Barton, *Experientia*, **6**, 316 (1950).

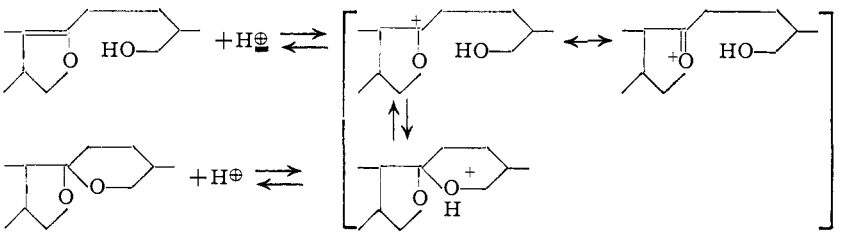
(31) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(32) J. A. Mills, *Chemistry and Industry*, 633 (1954).

heating with hydrochloric acid, sarsasapogenin is converted to smilagenin.[9] Accordingly formulation 2b in which the methyl group attached to C_{25} is *equatorial* and in which the *smaller* C_{26}-oxygen atom is in proximity to the C_{21}-methyl is much more probable than 4b in which the methyl group is *axial* and the *larger* C_{23}-methylene group is in proximity to the C_{21}-methyl. The choice between formulations 2a and 4a for sarsasapogenin is not so clear cut. It is our tentative hypothesis that formulation 4a accounts better for the great differences in infrared spectra between sarsasapogenin and smilagenin[13,15] than does 2a.

The evidence at hand does not permit assignment of C_{22}-configuration or C_{25}-conformation to the 20-isosapogenins. However, the structure and configuration of dihydro-20-isosarsasapogenin XV and dihydro-20-isosmilagenin XVI seem well established and are shown in Fig. 2. The evidence for the configurations of XV and XVI at C_{20} and C_{25} have been presented earlier in this paper. Since both steric conditions and theory strongly favor *cis* hydrogenation,[33] the hydrogen attached to C_{22} should have the same configuration as that at C_{20}. Similarly we assign structures XIII and XIV to dihydrosarsasapogenin and dihydrosmilagenin, respectively.

A mechanism for forming a resonance stabilized, planar C_{22}-carbonium ion[34,35] from sarsasapogenin, smilagenin or their pseudo analogs is



This mechanism is attractive because practically all spiroketal reactions take place only under acidic conditions. In this manner sarsasapogenin and smilagenin will give dihydro derivatives identical at C_{22}[6] regardless whether the original sapogenins were the same or different at C_{22}. Similarly ring closure of pseudosapogenins may occur to give the same or different C_{22} configurations and may be influenced by steric factors at C_{25} discussed previously.

Experimental

Melting points were obtained on a Kofler micro hot stage. Optical rotations were determined in chloroform, except with the acid-unstable pseudosapogenins, in which case dioxane was used, with concentrations of 25 mg. per 3 ml. in a 4-decimeter micro-polarimeter tube. Ultraviolet absorption spectra were obtained in methanol solution with a Cary recording spectrophotometer. Infrared absorption spectra were for the most part obtained in CS_2 solution, concentration 3–10 g. per liter with a Perkin–Elmer model 21 spectrophotometer.

The following extraction operation was performed repeatedly. It is presented in detail at this point and afterward will be termed the "usual procedure"; any exceptions will be specified. After completion of any reaction in water-soluble solvents, 2 volumes of water was added. The steroid then was extracted repeatedly with ether until a test portion of solvent indicated extraction was complete. Usually, 3 or 4 extractions were sufficient. When necessary, the ethereal solution was washed with dilute hydrochloric acid (10 ml. of concentrated HCl per 100 ml. of water) to remove bases such as KOH and pyridine, then with 10% aqueous $NaHCO_3$ to remove acids, and finally with distilled water until neutral. After drying over anhydrous sulfate, the ethereal solution was evaporated on the steam-bath.

Preparation of Pseudosarsasapogenin (V) from Sarsasapogenin (VII).[36]—A mixture of 100 g. of sarsasapogenin acetate and 500 ml. of acetic anhydride was heated in a sealed tube for 10 hours at 200°. After cooling, the tube was opened and the acetic anhydride concentrated to a sirup *in vacuo*. Water was added, and then the "usual procedure" was followed. The crude V diacetate thus obtained was hydrolyzed by refluxing for 1 hour in 1 liter of methanol–10% KOH; the usual procedure was then used, with the following exceptions. Pseudosapogenins but not their diacetates are extraordinarily susceptible to alteration in the presence of even trace amounts of acid. Hence, the acidic wash was omitted. Most of the alkali was removed from the ethereal pseudosapogenin solution with distilled water. Moreover, to obtain *consistent* results, all pseudosapogenin solutions were always concentrated in the presence of KOH. Concentration of the crude ethereal solution yielded several crops of crystalline V, which were further purified by crystallization from ether. The yield was 71 g., 78% of a product with m.p. 163–170°. The analytical sample was crystallized from ether and then ethyl acetate; white, rectangular rods, m.p. 171–172°, $[\alpha]^{25}D$ +12°. The infrared spectrum is shown in Fig. 3. The absorption band near 1690 Kaysers is characteristic of all pseudosapogenins.[37,38]

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.79; H, 10.63.

Important Note: Chromatography is permissible with pseudosapogenin acetates but *must not be used* on the pseudosapogenin diols. Numerous rearrangements take place, which will be reported at a later date.

Pseudosmilagenin (VI).—In a similar manner, smilagenin acetate (VII),[39] 100 g., gave 40 g. of VI, m.p. 155–160°, yield 44%. The analytical sample was crystallized from ethyl acetate as white, rectangular rods, m.p. 161–162°, $[\alpha]^{25}D$ +20°. The infrared spectrum was identical with that shown in Fig. 3. The X-ray powder diffraction patterns of V and VI were completely different; VI is much more soluble in ether and methanol than V.

3-Desoxypseudosarsasapogenin.—This product was prepared from 2 g. of 3-desoxysarsasapogenin in the manner described above. It was obtained as a viscous oil, which crystallized from concentrated ethyl acetate solution after standing for 6 months in the refrigerator. Recrystallization from a mixture of 92% acetone–8% water yielded 0.5 g. of white, needles, m.p. 167–168°,[40] $[\alpha]^{25}D$ +2.1°. The infrared spectrum included the typical 1690 Kaysers olefinic band and C_{26}-hydroxyl bands at 1031, 3520 and 3630 Kaysers. Except for the 1031 band, there were no strong bands between 650 and 1400 Kaysers. Although the infrared spectrum and subsequent reactions of this compound were in accord with its designation as 3-desoxypseudosarsasapogenin, the analytical values were poor.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Calcd.

(33) G. W. Wheland, "Advanced Organic Chemistry," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 297–298.

(34) E. R. Alexander, "Principles of Ionic Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 34–38, 213–218.

(35) D. H. Gould, H. Staeudle and E. B. Hershberg, THIS JOURNAL, **74**, 3685 (1952).

(36) Prepared from *Yucca schidigera* leaves by the procedure of Wall, *et al.*, *J. Biol. Chem.*, **198**, 533 (1952).

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

(38) For spectroscopic symbolism see *J. Opt. Soc. Am.*, **43**, 410 (1953).

(39) Prepared from *Agave lechegvilla*.[42]

(40) R. E. Marker, *et al.*, THIS JOURNAL, **62**, 2532 (1940), give m.p. 130°.

for $C_{27}H_{44}O_2 \cdot H_2O$: C, 77.46; H, 11.08. Found: C, 77.73; H, 10.40.

Conversion of Pseudosapogenins to Sapogenins.—One hundred mg. of V was refluxed for 1 hour in 10 ml. of ethanol containing 1 ml. of concentrated HCl. After cooling, the "usual procedure" was applied. Infrared absorption spectra of the residue from ether indicated that V had been completely converted to VII. Recrystallization of the residue gave crystals with m.p. 196–198°.

In a similar manner, VI yielded only VIII, m.p. 177–178°, and 3-desoxypseudosarsasapogenin gave 3-desoxysarsasapogenin, m.p. 214–217°.[41]

Conversion of Pseudosapogenins to 20-Isosapogenins.—Although this reaction can be carried out by treating pseudosapogenins with ethanolic hydrochloric acid for short periods, use of ethanol–acetic acid is preferable, as the reaction stops at the desired 20-isosapogenins.

V, 10 g., was dissolved with gentle warming in 300 ml. of absolute ethanol. After cooling to room temperature, 300 ml. of glacial acetic acid was added, and the mixture was allowed to stand overnight (17 hours). Crude 20-isosarsasapogenin (IX) was obtained by ethereal extraction in the usual manner. Crystallization from methanol gave 9.4 g. (94%) of white plates with a double m.p. 100°, followed by resolidification and final m.p. 170–175°. The analytical sample was recrystallized three times from acetone as plates, single m.p. 176–177°, $[\alpha]^{25}D$ +31.9°. The infrared absorption spectrum is shown in Fig. 4.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.70; H, 10.62.

In a similar manner VI, 15 g., yielded 12 g. (80%) of 20-isosmilagenin (X); rectangular rods from methanol, double m.p. 90° and 175–180°. The analytical sample was crystallized three times from methanol, double m.p. 90° and 185°, $[\alpha]^{25}D$ −60.3°. The infrared spectrum is shown in Fig. 5.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.92; H, 10.74.

Treatment of 3-desoxypseudosarsasapogenin in the manner described above yielded 3-desoxy-20-isosarsasapogenin as an oil. The product had an infrared absorption spectrum typical of IX lacking the 3-hydroxyl.

Conversion of 20-Isosapogenins to Sapogenins.—Refluxing IX and X in ethanol–hydrochloric acid, as described previously, gave quantitative yields of VII and VIII, respectively.

Effect of Acetic Anhydride on 20-Isosapogenins. (a) **Heating at 200°.**—Five grams of IX were heated with 25 ml. of acetic anhydride at 200° in a sealed tube for 10 hours. After a work-up identical with that given for VII to V, 3.0 g. of V, m.p. 167–169°, was obtained. In a similar manner, X, 3 g. yielded 1.8 g. of VI, m.p. 158–160°.

(b) **Reflux.**—After completing part (a) above, it was found that IX and X were converted to V and VI by simply refluxing for 1 hour in acetic anhydride followed by alkaline hydrolysis in methanol containing 10% KOH.

(c) **In Pyridine at Room Temperature.**—IX, 2 g., was dissolved in 10 ml. of pyridine. To this was added 10 ml. of acetic anhydride, and the mixture was allowed to stand overnight. After the usual ethereal work-up, the product, 20-isosarsasapogenin-3-acetate (XI), was crystallized from methanol; the yield was 1.4 g. of thick rods. The analytical sample was recrystallized several times from methanol, m.p. 167–168°, $[\alpha]^{25}D$ +30.3°. The infrared spectrum showed (a) no OH bands at 3000–4000 Kaysers, (b) an acetate band at 1732 Kaysers of strength corresponding to a monoacetate, (c) bands at 1200-1275 Kaysers of the same type as observed with sarsasapogenin acetate[3,16] indicating *cis* A/B ring fusion,[42] and (d) strong sharp bands at 850–1200 Kaysers, very similar to, although not identical with, those in Fig. 4.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.95; H, 10.11. Found: C, 75.94; H, 9.94.

In a similar manner, X was acetylated to form 20-isosmilagenin-3-monoacetate (XII), crystallizing as plates from methanol, m.p. 160°, $[\alpha]^{25}D$ −48.7°. The infrared spectrum was similar to that of 20-isosarsasapogenin acetate above except that the bands at 850–1200 Kaysers were similar to those shown in Fig. 5.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.95; H, 10.11. Found: C, 75.77; H, 10.05.

Catalytic Hydrogenation.—The same general method was used in all cases. One gram of sapogenin, or in the case of pseudosapogenins the diacetate, was dissolved in glacial acetic acid with warming if necessary. After cooling, an equal weight of Adams catalyst (PtO_2) was added. The suspension was hydrogenated, with shaking, for 17 hours at 50 pounds pressure. After the catalyst was removed, the steroids were extracted in the usual manner, and then saponified with methanol containing 10% KOH. This latter treatment was applied in all cases to hydrolyze acetates. After saponification, the usual work-up was carried out followed by recrystallization from appropriate solvents.

Dihydrosarsasapogenin (XIII) from VII.—The compound formed white plates from acetone, m.p. 164–165°, $[\alpha]^{25}D$ −6.9°. The infrared spectrum is shown in Fig. 6, lower curve.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.60; H, 11.21.

Dihydrosmilagenin (XIV) from VIII.—The compound was obtained as plates from acetone, m.p. 162–163°, $[\alpha]^{25}D$ +1.5°. The infrared spectrum was essentially identical with that of XIII (Fig. 6, lower curve). The X-ray powder diffraction patterns of XIII and XIV were markedly different.

Anal. Found: C, 77.36; H, 11.14.

Dihydro-20-isosarsasapogenin (XV) from IX.—The compound crystallized as plates from acetone and then ethyl acetate, m.p. 167–168°, $[\alpha]^{25}D$ −8.1°. The infrared spectrum is shown in Fig. 6, upper curve.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.57; H, 10.98.

Dihydro-20-isosmilagenin (XVI) from X.—Recrystallization from acetone gave needles, m.p. 161–162°, $[\alpha]^{25}D$ +2.7°. The infrared spectrum was essentially identical with that of XV (Fig. 6, upper curve). The X-ray powder diffraction patterns of XV and XVI were distinctly different.

Anal. Found: C, 77.73; H, 11.02.

Refluxing XV and XVI with an HCl solution (10 ml. of concentrated HCl + 90 ml. of ethanol) gave only *unchanged* starting material.

XV from Diacetate of V.—Recrystallization from acetone gave plates, m.p. 166–167°, $[\alpha]^{25}D$ −9.0°. The infrared spectrum and the X-ray powder diffraction pattern were identical with those of XV from IX.

Anal. Calcd. for $C_{27}H_{47}O_3$: C, 77.46; H, 11.08. Found: C, 77.57; H, 10.98.

XVI from Diacetate of VI.—Recrystallization from acetone yielded needles, m.p. 161–162°, $[\alpha]^{25}D$ +1.8°. The infrared spectrum and the X-ray powder diffraction patterns were identical with those of XVI from X.

Anal. Found: C, 77.47; H, 10.93.

Acetylation of XIII, XIV, XV, XVI.—In all cases, the starting material was dissolved in pyridine to which was added an equal volume of acetic anhydride. After standing for 17 hours at room temperature, the usual ethereal extraction was applied.

The diacetate of XIII could not be crystallized, $[\alpha]^{25}D$ −3.9°.

The diacetate of XIV was crystallized from methanol, m.p. 93–94°, $[\alpha]^{25}D$ +2.1°.

The diacetate of XV (XVII) was crystallized from methanol, m.p. 95.5–96.5°, $[\alpha]^{25}D$ −2.6°. *Anal.* Calcd. for $C_{29}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 74.08; H, 10.17.

The diacetate of XVI (XVIII) was crystallized from methanol, m.p. 96–97°, $[\alpha]^{25}D$ −3.8°. *Anal.* Calcd. for $C_{29}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 74.36; H, 10.09.

Oxidation with CrO_3–Pyridine.—IX and X were oxidized at room temperature for 17 hours as described by Sarett and co-workers.[18] After the usual ethereal extraction (acid wash was omitted), the residual crude product was freed of solvent and pyridine by drying *in vacuo*. Two grams of IX oxidized in this manner gave 1.5 g. of crude 3-ketone-

(41) R. E. Marker, *et al.*, THIS JOURNAL, **61**, 1284 (1939), give m.p. 214–216°.

(42) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *ibid.*, **73**, 3215 (1951).

20-isosarsasapogenone (XXI). Crystallization from methanol gave 1.0 g. of rods, m.p. 151–152°, $[\alpha]^{25}D$ +49.5°. The infrared spectrum shows (a) absence of hydroxyl bands at 3000–4000 Kaysers, (b) presence of a ketonic band at 1714 Kaysers, (c) strong, sharp bands at 850–1350 Kaysers resembling those in Fig. 4, the major difference being the absence of the 1032 Kayser hydroxyl band.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.24; H, 10.04.

Similar oxidation of X gave 20-isosmilagenone (XXII), crystallizing as rods from methanol, m.p. 162–163°, $[\alpha]^{25}D$ −54.9°. The infrared spectrum was similar to that of XXI except that the bands between 850 and 1350 Kaysers resembled those in Fig. 5.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.14; H, 10.18.

Refluxing 20-isosarsasapogenone and 20-isosmilagenone in ethanol containing 10% concentrated hydrochloric acid gave the known sarsasapogenone (XIX),[43] m.p. 222–223°, and smilagenone (XX),[44] m.p. 187°.

Similar oxidation of VII and VIII also yielded XIX and XX, respectively.

Oxidation with CrO_3–Acetic Acid.—The general procedure used in all experiments was as follows: 5.0 g. of steroid was dissolved in a minimal quantity of glacial acetic acid containing 1.25 g. of sodium acetate. The solution was cooled to 15°. To it was added slowly with stirring 5.0 g. of CrO_3 in a solution of 80% acetic acid–20% water. The temperature was allowed to rise to 22°, and the solution was kept at this temperature for 1 hour. After addition of water, the usual ethereal extraction was applied, with one *exception*. Removal of acetic acid with bicarbonate was omitted. Instead it was removed by heating on the steam-bath *in vacuo*. The residue thus obtained was treated with *t*-butyl alcohol containing KOH for 3 hours at room temperature with constant stirring.[20] After addition of water, the product was given the usual ethereal extraction. This is designated the *neutral fraction*. The aqueous alkaline layer from this operation was acidified and extracted with ether. All bicarbonate washes were omitted. This is the *acid fraction*.

Oxidation of VII and VIII.—The neutral fractions yielded XIX and XX; the acid fractions were negligible.

Oxidation of IX and X.—In both cases, the crude product after oxidation had acidic properties. After the crude acids were treated with *t*-butyl alcohol–KOH, the neutral fraction in both cases gave a good yield (50%) of the known 16-pregnen-3,20-dione (XXIII).[11] The analytical sample after repeated crystallization from acetone melted at 200–202°, $[\alpha]^{25}D$ +88°, $\lambda_{max.}$ 239 mμ, log ε 3.95. The infrared spectrum was identical with that of an authentic specimen showing (a) no hydroxyl bands, (b) a 3-ketone band at 1716 Kaysers and a conjugated 20-ketone band at 1670 Kaysers, (c) C=CH bands at 818, 827 and 3050 Kaysers.

Oxidation of XIII and XIV.—These yielded only an acid fraction, which was not further investigated.

Oxidation of XV and XVI.—These after oxidation and alkaline hydrolysis both yielded 16-pregnen-3,20-dione in the neutral fraction in 50–55% over-all yield.

(+)- and (−)-α-Methylglutaric Acid from 20-Isosarsasapogenin (IX) and 20-Isosmilagenin (X).—The alkaline aqueous *t*-butyl alcohol solution remaining from the oxidation and hydrolysis of IX above was passed through a column of Dowex 50 to liberate free acids. The acidic solution thus obtained was concentrated on the steam-bath to a small volume, and was essentially free from any volatile acid or alcohol. The aqueous concentrate was extracted 6 times with ether, the ether dried with anhydrous sodium sulfate, and evaporated. The residue was sublimed *in vacuo* at 80–100° and the sublimate crystallized from pentane containing a small amount of ether. In this manner IX yielded (+)-α-methylglutaric acid, m.p. 78–80°, $[\alpha]^{25}D$ +17° (ethanol) and X gave (−)-α-methylglutaric acid, m.p. 78–80°, $[\alpha]^{25}D$ −18° (lit.[6] gives m.p. 78.5–81°, $[\alpha]^{20}D$ +18° for (+)- and $[\alpha]^{20}D$ −20° for (−)-α-methylglutaric acids).

Conversion of VII and VIII to 16,22-Epoxycoprostan-3β-ol-3-(3,5-dinitrobenzoate).—Smilagenin acetate, 5 g., was catalytically hydrogenated as described previously. The resultant XIV-3 monoacetate was not isolated. The presence of a free 26 hydroxyl was shown by the typical pair of bands at approximately 3500 and 3650 Kaysers. The crude product was dissolved in 20 ml. of pyridine, and 5.0 g. of *p*-toluenesulfonyl chloride was added with gentle heating. After standing for 17 hours at room temperature, water was added, followed by the usual ethereal extraction. *Note.* It is essential that all the excess *p*-toluenesulfonyl chloride be decomposed. This is best accomplished by adding 2 ml. of water and then allowing the mixture to stand overnight. Then more water is added, followed by extraction.

The resultant crude XIV-3 acetate-26-tosylate was not isolated. Its infrared spectrum showed absence of hydroxyl, presence of acetate at 1735 Kaysers, and presence of tosylate, as shown by bands at 665, 815, 1100, 1182 and 1192 Kaysers.

The crude tosylate was dissolved in 100 ml. of acetone and 10 g. of sodium iodide was added. The mixture was heated overnight in a bomb at 100°. After cooling, the solution showed no free iodine. The acetone was removed by evaporation, and the residue triturated with petroleum ether. This dissolved the crude 26-iodo-XIV-3-acetate. After removal of solvent, the residue was refluxed for 1 hour in glacial acetic acid in the presence of 30 g. of zinc dust. Addition of water followed by the usual ethereal extraction and then ethanolic KOH hydrolysis gave 16,22-epoxycoprostan-3β-ol. The compound was amorphous. Scheer, *et al.*,[6] have prepared it in pure, crystalline form. The infrared spectrum showed a single non-bonded hydroxyl band at 3670 Kaysers, indicating that the C_{26}-hydroxyl had been removed.

The amorphous 16,22-epoxycoprostan-3β-ol was dissolved in 40 ml. of pyridine, and 5.0 g. of 3,5-dinitrobenzoyl chloride was added. The mixture was heated for 1 hour on the steam-bath. After it had cooled, water was added, followed by the usual ethereal extraction. The residue was dissolved in benzene and chromatographed on acid-washed alumina (50 g.). Elution with benzene gave 2.6 g. of cream-white product. Further elution with chloroform and alcohol gave negligible quantities. Recrystallization from acetone of the product from the benzene eluates gave the crystalline 16,22-epoxycoprostan-3β-ol-3-(3,5-dinitrobenzoate) as needles, m.p. 236–237°, $[\alpha]^{25}D$ +6.3°.

In a similar manner sarsasapogenin acetate yielded a compound with identical properties. The derivatives from VII and VIII had identical infrared spectra and identical X-ray powder diffraction patterns.

Anal. Calcd. for $C_{34}H_{48}N_2O_7$: C, 68.51; H, 8.12. Found: C, 68.38; H, 8.20.

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PHILADELPHIA 18, PENNA.

(43) R. E. Marker, *et al.*, THIS JOURNAL, **61**, 943 (1939), give m.p. 226°.

(44) R. E. Marker, *et al.*, *ibid.*, **62**, 2525 (1940), give m.p. 187–188°.