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

Steroidal Sapogenins. XXVII. Preparation and Properties of 20-Isosapogenins[2]

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This paper describes the preparation and properties of a number of 20β,25D- and 20β,25L-sapogenins. The latter are characterized by being much more dextrorotatory than their 25D-analogs. Both series have infrared spectra which differ from each other and from those of the analogous 20α-compounds. Sapogenins of the 20β-series are characterized by a spiroketal side chain which is much more labile than the 20α-series. Thus the latter group is not attacked by mild CrO_3 oxidation. Sapogenins of the 20β,25L-series are cleaved to acidic intermediates which on alkaline treatment yield Δ^{16}-20-ketopregnenes. Compounds of the 20β,25D-group behave similarly, but in addition also yield on oxidation a new group of sapogenins which contain an additional hydroxyl tentatively placed at C_{20}. 20-Isohecogenin is an exception and forms only the hydroxylated derivative. The cleavage fragments of 20β,25L- and 20β,25D-sapogenins gave (+)- and (−)-α-methylglutaric acids, thus establishing the C_{25}-configuration of both groups.

In several recent publications[3a,b,c] we have described the preparation of two members of a new class of steroidal sapogenins which we called 20-isosapogenins. Shortly after our initial publications on this subject, several other laboratories independently published preliminary communications on this subject.[4a,b,c]

The present paper reports data on the physical properties and reactions of a large number of 20-isosapogenins prepared from natural 25D- and 25L-sapogenins.[5a,b] The various 20-isosapogenins were prepared from their pseudosapogenin analogs by acetic acid isomerization as described previously.[3a,c]

Table I gives the code used for the various 20-isosapogenins and their naturally-occurring analogs. Table II presents melting point and optical rotation data as well as the analytical data. Referring to Table II it will be noted that the melting points of the various 20-isosapogenins (20β-series) were, with a few exceptions, lower and less sharp than their naturally occurring (20α-series) analogs. This has been noted previously by Callow and James[4a] and Dickson, *et al.*[4b] On heating 20-isohecogenin (VIII) and 20-isodiosgenin (IV) just to their melting points and then obtaining the infrared spectra of the melted compounds, we observed that there had occurred a partial isomerization to the pseudosapogenins. Samples of 20-isosarsasapogenin (XIV) and 20-isosmilagenin (XVI), heated under these conditions, did not show formation of pseudosapogenin and it will be noted that XIV and XVI as well as their acetates melt sharply. On the whole, the melting points of the various 20-isosapogenins cannot be regarded as a reliable or reproducible physical constant (*cf.* also references 4a, b).

The optical rotations of the 20-isosapogenins show some characteristic differences. The 20β,25D-sapogenins on the whole do not differ considerably from their naturally-occurring 20α,25D-analogs. However, the 20β,25L-sapogenins are much more dextrorotatory than their 20α,25L-, 20β,25D- and 20α,25D-isomers (*cf.* Table II, compare XI and XII, XIII and XIV, XIX and XX).

The infrared spectra of the various 20-isosapogenin acetates are also of considerable interest. Both the 20β,25D- and 20β,25L-series have in common absorption bands in the region 1160–1170, 1150–1160, 1070–1080, 1040–1045, 1010–1020 and 860–870 cm.$^{-1}$. Characteristic differences also are present. The sapogenins of the 25L-series have peaks near 1180–1185 and 982–984 cm.$^{-1}$ lacking in the 25D-series. The latter group have characteristic absorption bands near 970–975 and 785 cm.$^{-1}$, absent in the 25L-series. Both groups of 20-isosapogenins have strong bands near 920 and 900 cm.$^{-1}$ which also show a characteristic change in frequency. The 25L-series bands occur at 903–905 and 918–919 cm.$^{-1}$ whereas the bands of the 25D-group are found between 921–922 and at 895–897 cm.$^{-1}$. Table III presents some of the characteristic infrared absorption bands of a number of 20-isosapogenins between 1200–1785 cm.$^{-1}$.

The complete infrared absorption curves of these steroids will be presented elsewhere.[6] The spectra of most of the naturally occurring (20α) sapogenin acetates have been published previously.[7] Comparison with the infrared spectra of the 20β-series indicated marked differences. Particularly noteworthy was the absence in the 20β-series of the reversal of intensities of the peaks in the region of 900 and 920 cm.$^{-1}$ which were so characteristic of the 20α,25D- and 20α,25L-sapogenins.[7,8a,b]

We have confirmed and extended some of the reactions of the sapogenins of the 20β-series which were previously reported only for XIV and XVI.[3a,c] These reactions are summarized in Fig. 1. Refluxing the various 20β-sapogenins listed in Table I with ethanol containing 1% hydrochloric acid rapidly converted them to the corresponding 20α-series. Refluxing the 20β-group with acetic anhydride caused formation of the corresponding pseudosapogenin di- or triacetates. As mentioned previously,

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

(2) Paper XXVI, M. E. Wall, C. S. Fenske, J. J. Willaman, D. S. Correll, B. G. Schubert and H. S. Gentry, U. S. Dept. Agr., Agr. Research Service Circ. ARS-73-4 (1955).

(3) (a) M. E. Wall, C. R. Eddy and S. Serota, THIS JOURNAL, **76**, 2849 (1954); (b) M. E. Wall and S. Serota, *ibid.*, 2850 (1954); (c) M. E. Wall, S. Serota and C. R. Eddy, *ibid.*, **77**, 1230 (1955).

(4) (a) R. K. Callow and V. H. T. James, *Chemistry and Industry*, 691 (1954); (b) D. H. W. Dickson, *et al.*, *ibid.*, 692 (1954); (c) J. B. Ziegler, W. E. Rosen and A. C. Shabica, THIS JOURNAL, **76**, 3865 (1954).

(5) The C_{25}-isomerism of naturally occurring sapogenins was established by the researches of: (a) I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **75**, 4871 (1953), and further developed by (b) V. H. T. James, *Chemistry and Industry*, 1388 (1953).

(6) C. R. Eddy, *et al.*, *Anal. Chem.*, in press.

(7) C. R. Eddy, M. E. Wall and M. K. Scott, *ibid.*, **25**, 266 (1953).

(8) (a) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *ibid.*, **24**, 1337 (1952); (b) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953).

TABLE I

CODE AND IUPAC NOMENCLATURE FOR SAPOGENINS DISCUSSED IN THIS PAPER

Modified IUPAC nomenclature[a]	20α		20β	
5α,22ε,25D-Spirostane-3β,6α-diol	I	Chlorogenin	II	20-Isochlorogenin
5-22ε-Spirosten-3β-ol	III	Diosgenin	IV	20-Isodiosgenin
5α,22ε,25D-Spirostane-2α,3β-diol	V	Gitogenin	VI	20-Isogitogenin
5α,22ε,25D-Spirostan-12-one-3β-ol	VII	Hecogenin	VIII	20-Isohecogenin
5α,22ε,25D-Spirostane-12-one-2α,3β-diol	IX	Manogenin	X	20-Isomanogenin
22ε,25L-Spirostane-2β,3β-diol	XI	Markogenin	XII	20-Isomarkogenin
22ε,25L-Spirostan-3β-ol	XIII	Sarsasapogenin	XIV	20-Isosarsasapogenin
22ε,25D-Spirostan-3β-ol	XV	Smilagenin	XVI	20-Isosmilagenin
5α,22ε.25D-Spirostan-3β-ol	XVII	Tigogenin	XVIII	20-Isotigogenin
5-22ε,25L-Spirosten-3β-ol	XIX	Yamogenin	XX	20-Isoyamogenin

[a] The nomenclature used is essentially that of the report on "Steroid Nomenclature" issued by the NRC Subcommittee on Steroid Nomenclature. The use of the terms 20α or 20β to denote configuration at C_{20} and of 25D or 25L for configuration at C_{25} was originated by G. Mueller and B. Riegel whom we wish to thank for sending us a prepublication copy of their manuscript.

TABLE II

PHYSICAL CONSTANTS OF 20α- AND 20β-SAPOGENINS AND ANALYSES OF 20β-SAPOGENINS

Compound 20α	Compound 20β	M.p.° 20α	M.p.° 20β	$[\alpha]^{25}D$ 20α[a]	$[\alpha]^{25}D$ 20β[b]	MD 20β − 20α	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Descript.	Cryst. from
I	II	276	205–209	− 64°	−49°	+ 65	$C_{27}H_{44}O_4$	74.95	74.78	10.25	10.26	Needles	Acetone
I diac.[c]	II diac.	158	167–172	− 34	−40	− 31	$C_{31}H_{48}O_6$	72.06	72.16	9.36	9.46	Rods	Methanol
III	IV	208	192–195	−123	−98	+103	$C_{27}H_{42}O_3$	78.21	77.50	10.21	10.23	Plates	Methanol
III ac.	IV ac.	199–202	190–195	−115	−97	+ 82	$C_{29}H_{44}O_4$	76.27	76.47	9.71	9.75	Needles	Methanol
V	VI	272	222–228	− 56	−56	0	$C_{27}H_{44}O_4$	74.95	75.05	10.25	10.78	Needles	Methanol
V diac.	VI diac.	244–245	199–205	− 85	−82	+ 15	$C_{31}H_{48}O_6$	72.06	71.96	9.36	9.35	Plates	Methanol
VII	VIII	268	218–222	+ 18	+14	− 17	$C_{27}H_{42}O_4$	75.31	74.91	9.83	9.96	Rods	Methanol
VII ac.	VIII ac.	245	210–217	+ 4	+ 8	+ 18	$C_{29}H_{44}O_5$	73.69	73.58	9.38	9.40	Needles	Methanol
IX	X	246	210–220	0	+ 2	+ 9	$C_{27}H_{42}O_5$	72.61	71.94	9.48	9.77	Plates	Methanol
IX diac.	X diac.	264	200–220	− 42	−31	+ 58	$C_{31}H_{46}O_7$	70.16	70.16	8.74	8.57	Needles	Methanol
XI	XII	256–257	189–192	− 64	+18	+354	$C_{27}H_{44}O_4$	74.95	74.73	10.25	10.44	Plates	Acetone
XI diac.	XII diac.	185–186	166–169	− 78	− 5	+372	$C_{31}H_{48}O_6$	72.06	72.03	9.36	9.39	Rods	Methanol
XIII	XIV	200	176–177	− 79	+36	+480	$C_{27}H_{44}O_3$	77.83	77.70	10.65	10.62	Plates	Acetone
XIII ac.	XIV ac.	144–145	167–168	− 65	+35	+458	$C_{29}H_{46}O_4$	75.95	75.94	10.11	9.94	Plates	Methanol
XV	XVI	188–189	185	− 68	−54	+ 58	$C_{27}H_{44}O_3$	77.83	77.92	10.65	10.74	Prisms	Methanol
XV ac.	XVI ac.	150–151	160	− 58	−43	+ 69	$C_{29}H_{46}O_4$	75.95	75.77	10.11	10.05	Plates	Methanol
XVII	XVIII	208	190–195	− 61	−62	− 4	$C_{27}H_{44}O_3$	77.83	78.18	10.65	10.52	Rods	Acetone
XVII ac.	XVIII ac.	206–208	189–195	− 66	−65	+ 5	$C_{29}H_{46}O_4$	75.94	76.01	10.11	10.28	Needles	Methanol
XIX	XX	201	165–175	−123	−10	+515	.....	...	...	...	...	.....	
XIX ac.	XX ac.	182	185–186	−113	−11	+465	$C_{29}H_{44}O_4$	76.27	76.52	9.71	9.79	Plates	Methanol

[a] Rotations in chloroform converted to dioxane by adding +6°. [b] Rotations in dioxane. [c] ac = acetate.

TABLE III

CHARACTERISTIC INFRARED ABSORPTION BANDS OF SOME TYPICAL 20-ISOSAPOGENINS

Code	Wave numbers, $cm.^{-1}$														
II	..	..	1159	1074	..	1036	..	..	974	965	921	897	..	860	787
IV	..	..	1154	1073	..	1043	1015	..	972	959	919	896	..	860	780
VI	..	1165	1155	1075	..	1041	1015	..	969	..	921	897	..	861	788
VIII	..	1164	1155	1076	1066	1053	1014	..	971	956	922	897	..	862	785
X	..	1165	1154	1076	1065	1044	1014	..	972	955	922	897	..	860	785
XII	1185	1171	..	1084	..	1041	..	984	..	..	918	905	871	..	..
XIV	1182	1162	1152	1080	1062	1046	1020	982	..	..	918	903	865	..	..
XVI	..	1162	1156	1073	..	1043	1015	..	969	..	921	895	..	860	787
XVIII	..	1164	1155	1075	..	1042	1017	..	973	959	922	896	..	860	787
XX	1179	..	1155	1082	1068	1052	1027	992, 979	..	962	917	904	870	..	..

partial conversion of the 20β-sapogenins to pseudosapogenins could be produced by heating them to their melting point temperatures. The side chains of the 20β-sapogenins were not affected by acetylation in the presence of pyridine either at room or elevated temperatures.

Oxidation of members of the 20β,25L-series, XII diacetate and XIV with CrO_3 in acetic acid at 15°, resulted in the formation of amorphous carboxylic acids. On alkaline cleavage these gave Δ^{16}-20 ketone pregnene derivatives. Acidification of the alkaline liquors followed by ethereal extraction and subsequent purification gave in both cases (+)-α-methylglutaric acid. Similar oxidation of members of the 20β,25D-series gave a more complex picture. Thus VI, XVI and XVIII all formed amorphous acids which on alkaline cleavage gave the corresponding Δ^{16}-pregnene and (−)-α-methylglutaric acid. However, in each case a non-acidic product was obtained from the oxidation which, prior to the alkaline cleavage, could be easily separated from the acid fraction by chromatography. In the case of 20-isohecogenin (VIII) the non-acidic product was the only product of the oxidation isolated and no Δ^{16}-pregnene could be obtained.

The structures of the non-acidic oxidation products of VI, VIII, XVI and XVIII have been elucidated only partially although infrared evidence in-

dicated that all were similar. The data obtained from the oxidation of 20-isogitogenin diacetate (VI diacetate) was typical of the group. The infrared spectrum of the crystalline non-acidic component XXI, obtained during the oxidation of VI diacetate, showed that a new hydroxyl function was present, no additional carbonyl other than acetate, and a complex spectrum in the 850–1200 cm.$^{-1}$ region with many strong bands associated with the spiroketal side chain.[8a,b] These differed from those obtained from analogous 20α- and 20β-sapogenins, being characterized in particular by strong bands near 990–995 and 925 cm.$^{-1}$ and a rather broad but much weaker band between 900–905 cm.$^{-1}$. The optical rotation of XXI was of an order similar to those found for V and VI diacetates and hence was consistent with a closed spiroketal side chain.[9] Elementary analysis (*cf.* Experimental section) gave results in good agreement for a sapogenin diacetate having an extra oxygen function. That this extra oxygen was due to a tertiary hydroxyl function was deduced from the infrared spectra and from the fact that the newly formed hydroxyl was resistant to conditions which would oxidize primary and secondary hydroxyl groups. In addition the new hydroxyl could not be acetylated by heating in pyridine–acetic anhydride. Tentatively we have placed the new hydroxyl at C_{20}. Further work is in progress to elucidate more definitely the location of this hydroxyl as well as the various stereochemical problems inherent in such a molecule as shown in Fig. 1. Catalytic hydrogenation of 20-isohecogenin (VIII) and 20-isotigogenin (XVIII) gave dihydro-20-isorockogenin (XXII) and dihydro-20-isotigogenin (XXIII). Compound XXIII also was obtained by catalytic hydrogenation of 20-isodiosgenin (IV). As found previously with the hydrogenation products of XIV and XVI,[3a,c] XXII and XXIII were identical with the compounds obtained by hydrogenation of the analogous pseudosapogenin diacetates followed by alkaline hydrolysis. Oxidation of XXIII gave only an acidic reaction product. The infrared spectrum of this product showed that no tertiary hydroxyl was present. Alkaline hydrolysis gave 16-allopregnene-3,20-dione. Marker[10] previously had oxidized dihydropseudotigogenin (which we now know is identical to XXIII) to the intermediate oxidation product, tigone, and thence obtained the Δ^{16}-20-keto-allopregnene. Similar oxidation and hydrolysis of the hecogenin derivative XXII yielded no side chain cleavage products. Some time previously we had noted that similar treatment of dihydropseudohecogenin diacetate also gave no reaction and the compound was recovered unchanged.[11,12]

The data presented in this paper confirm the conclusions previously presented[3a,c] in regard to the stereochemistry at C_{20} and C_{25}. It seems certain that 20-isosapogenins have the 20β-configuration shown in Fig. 1.

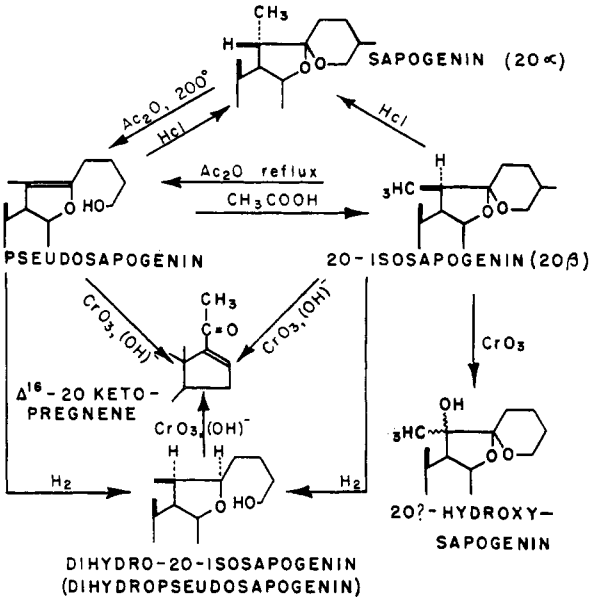

Fig. 1.

The lability of the spiroketal side chain in sapogenins of the 20β-series can be ascribed to the instability introduced by the strong interaction of the methyl groups attached to C_{18} and C_{20}. From the researches of Callow and James[4a] and Dickson and co-workers[4b] it would seem that pseudosapogenins are the probable intermediates in the acid-catalyzed reactions of the 20β-series. Whether pseudosapogenins are intermediates in the oxidative cleavage of the 20β-side chain is less clear since dihydro-20-isosapogenins also can be oxidized and in this case one cannot postulate a pseudosapogenin intermediate.[13]

The configuration of 20β-sapogenins at C_{25} is established as identical with that of the corresponding 20α-series[5a,b] as a result of the isolation of (+)- and (−)-α-methylglutaric acids from the oxidation of 20β,25 L- and 20β,25 D-sapogenins, respectively. The question of the conformation at C_{25} is intimately linked with the problem of whether 25D- and 25L-sapogenins are identical or different at C_{22}. Although we and other research groups have expressed some opinion on this subject in preliminary communications[3a,4c,5a,14] it is our present opinion that the information at hand does not permit of a clear-cut solution to this question.

Experimental

Physical Measurements.—Melting points were obtained with a Kofler micro-hot-stage. Optical rotations of 20-isosapogenins were conducted in dioxane in order to avoid any changes which might occur from the presence of free

(9) Opening the spiroketal side chain invariably results in a large positive rotational shift. This has been observed for pseudosapogenins and dihydrosapogenins (references 3a, c, and 5a and Djerassi, *et al.*, *J. Org. Chem.*, **16**, 303 (1951), and for "Tigone" (unpublished work from this Laboratory).

(10) R. E. Marker, *et al.*, THIS JOURNAL, **63**, 774, 779 (1941).

(11) This experiment was carried out by Dr. E. S. Rothman of this Laboratory.

(12) The anomalous behavior of the hecogenin derivatives may be due to interactions between the 12 carbonyl and C_{20}. Molecular models indicate the two groups can approach each other.

(13) The hydrogen attached to the tertiary C_{20} is in a favorable stereochemical position for oxidative attack in both 20-isosapogenins and their dihydro-20-iso analogs. The vulnerability of hydrogen atoms attached to tertiary carbons toward oxidative attack is well known. It is not unlikely that the cleavage of the C_{20}–C_{22} bond in the 20β-series is initiated by an attack on the C_{20}-hydrogen atom as also may be the case for the formation of the new 20?-hydroxysapogenins.

(14) D. A. H. Taylor, *Chemistry and Industry*, 1066 (1954).

acids in the chloroform. All other rotations were conducted in chloroform. Infrared spectra were determined in carbon disulfide solution, concentration 10.0 g. per liter, using a Perkin–Elmer model 21 spectrophotometer.

Preparation of 20-Isosapogenins.—The procedure used for the preparation of 20-isodiosgenin was typical and will be given in detail. Ten grams of diosgenin acetate was refluxed 5 hours in 50 ml. of acetic anhydride containing 4.25 g. of pyridine hydrochloride.[15] Several volumes of water were added, and the pseudodiosgenin diacetate extracted with ether. The ethereal solution was washed with sodium carbonate, and then water until neutral and then dried over sodium sulfate. The crude product was not isolated.[16] Conversion to the pseudodiacetate was virtually complete as indicated by absence of the typical sapogenin bands and presence of the characteristic pseudosapogenin peak at 1685 cm.$^{-1}$.[17]

The ethereal solution was evaporated to dryness, and the residue saponified by refluxing one hour in a solution of 250 ml. of methanol containing 12.5 g. of potassium hydroxide in a nitrogen atmosphere. The alkaline solution was neutralized with acetic acid with cooling, and then 250 ml. of glacial acetic acid was added. Crystallization commenced in a few minutes[18] and the solution was allowed to stand overnight. After filtering 3.0 g. of crystalline product, m.p. 188–195°, was obtained. Dilution of the filtrate with water and extraction with ether followed by evaporation and crystallization from methanol gave 3.2 g. of similar crystals, making a total of 6.2 g., yield 68%. The resinous residual product was refluxed with ethanolic hydrochloric acid yielding 1.2 g. of diosgenin, m.p. 200–203°, infrared spectrum identical to reference curve.

All the 20-isosapogenins listed in Table II were prepared in a similar manner. The corresponding acetates were prepared by dissolving the 20-isosapogenin in pyridine, adding an equal volume of acetic anhydride and allowing the solution to stand overnight at room temperature followed by the usual work-up. In all of the work with 20-isosapogenins or their acetates it was essential to avoid use of mineral acids, nor is chromatography to be recommended. All pertinent analytical data for the 20-iso series are given in Table II. The characteristic infrared bands for the acetates are shown in Table III.

Conversion of 20-Isosapogenins to Sapogenins.—The data for 20-isotigogenin (XVIII) were typical. To a solution of 100 ml. of ethanol containing 200 mg. of XVIII was added 1 ml. of concentrated hydrochloric acid. The solution was refluxed one hour, cooled, and after adding water was given the usual ethereal extraction. The washed and dried ether solution was concentrated to dryness. Tigogenin XVII was obtained in quantitative yield, m.p. 200°, infrared spectrum identical to reference material.

Conversion of 20-Isosapogenins to Pseudosapogenins. (a) Heating.—Small samples, 6.7 mg., of 20-isodiosgenin (IV), 20-isohecogenin (VIII), 20-isosarsasapogenin (XIV) and 20-isosmilagenin (XVI) were placed in 2-ml. volumetric flasks. The samples were placed in an oil-bath at room temperature and slowly heated to their respective melting points. The temperatures approximated those obtained on the Kofler hot-stage. The samples were cooled rapidly and dissolved in 2 ml. of carbon disulfide and the infrared spectra obtained. Pseudosapogenins always have a double hydroxyl peak at approximately 3630–3640 cm.$^{-1}$ (unbonded C_3-hydroxyl) and near 3530 cm.$^{-1}$ (C_{26}-bonded hydroxyl),[3c] a characteristic peak near 1685 cm.$^{-1}$ [17] and a relatively simple spectrum with few strong peaks in the region 1400–700 cm.$^{-1}$. Sapogenins and 20-isosapogenins have only a single hydroxyl peak near 3620–3640 cm.$^{-1}$, no peak at 1685, and a very complex system of strong bands in the 1400–700 cm.$^{-1}$ region. Based on these considerations the infrared spectra of IV and VIII showed greater than 50% conversion to pseudosapogenin, that of XIV no conversion, and the spectrum of XVI showed less than 10% conversion.

(b) **Refluxing with Acetic Anhydride.**—The following experiment was typical: 9.0 g. of 20-isodiosgenin was refluxed one hour in 200 ml. of acetic anhydride. After cooling, the acetic anhydride solution was extracted six times with hexane (200-ml. portions) and the hexane solution washed with sodium carbonate and water. After drying, the hexane extracts were concentrated to approximately 100 ml., whereupon crystals of pseudodiosgenin diacetate appeared. After several recrystallizations from methanol, 2.25 g. of pseudodiosgenin diacetate, m.p. 99–101° (lit.[19] 100–101°) was obtained with infrared spectra agreeing with that of an authentic sample. In a similar manner VI, VIII, XIV, XVI and XVIII were converted to crystalline pseudoacetates.

Hydrogenation of 20-Isosapogenins and Pseudosapogenins.—The following experiment was typical of the reaction conditions. 20-Isohecogenin (VIII), 1.3 g., was dissolved in 50 ml. of warm acetic acid. After cooling, the solution was hydrogenated in the presence of 0.65 g. of PtO_2 at 50 pounds pressure for 16 hours at room temperature. After removing the catalyst, the acetic acid was evaporated and the residue refluxed one hour in methanol–10% KOH. The alkaline solution was diluted with water, filtered, and washed. The insoluble precipitate was dried; weight 0.8 g. Of this, 0.4 g. was crystallized from acetone; yield 0.3 g., plates, m.p. 185–186°, $[\alpha]^{25}D$ +17° (pyridine). The product was dihydro-20-isorockogenin (XXII) (16,22-epoxy-20β,22ξ,25D-cholestane-3β,12β?,26-triol).

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.77; H, 10.81.

The same product was obtained by hydrogenation of **pseudohecogenin diacetate**[20] followed by saponification.

The triacetate of XXII was prepared by room temperature acetylation in pyridine solution in the usual manner. The product crystallized from 90% methanol–10% water as cubes, m.p. 109–112°, $[\alpha]^{25}D$ −4.8°.

In a similar manner catalytic hydrogenation of both IV and XVIII gave dihydro-20-isotigogenin (XXIII), as plates from methanol; m.p. 195–196°, $[\alpha]^{25}D$ +20° (pyridine) (lit.[19] gives 203–205° but the method of preparation was not valid, *cf.* footnote 20). Hydrogenation of pseudotigogenin diacetate followed by saponification gave the same compound, XXIII.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.34; H, 11.07.

The diacetate of XXIII was crystallized from methanol; m.p. 122–123°, $[\alpha]^{25}D$ −15°.

Oxidation of 20-Isosapogenins and Dihydro-20-isosapogenins.—The conditions for CrO_3–acetic acid oxidation and alkaline cleavage of the spiroketal side chain previously were presented in detail.[3c] The oxidation of VI diacetate is typical of the 20β,25D-series. Two and two-tenths grams was treated with CrO_3–acetic acid in the usual manner.[3c] The oxidation product was chromatographed on Florisil. Elution with benzene gave 0.74 g. as plates from methanol; m.p. 253–254°, $[\alpha]^{25}D$ −98°. The infrared spectrum showed presence of a strong hydroxyl band near 3510 cm.$^{-1}$,[21] a strong diacetate peak near 1735 cm.$^{-1}$ and a number of very strong peaks in the fingerprint region, 1069, 1060, 1021 1001, 999 and 927 cm.$^{-1}$. The product could not be acetylated in pyridine–acetic anhydride and was recovered unchanged after heating in this mixture.

Anal. Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.09. Found: C, 69.53; H, 9.20.

On the above basis the compound has been designated tentatively as a 20-hydroxyl derivative of gitogenin acetate with configuration at C_{20} and C_{22} not stipulated.

(15) W. G. Dauben and G. J. Fonken, THIS JOURNAL, **76**, 4618 (1954).

(16) In other experiments intermediates were isolated at each step but experience indicated that maximal yields were obtained in non-isolation runs.

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

(18) This was not typical of every 20-isosapogenin and in fact only 20-isodiosgenin and 20-isotigogenin crystallized out of the reaction mixture.

(19) R. E. Marker, T. Tsukamoto and D. L. Turner, THIS JOURNAL, **62**, 2525 (1940).

(20) R. E. Marker and co-workers, *ibid.*, **69**, 2171 (1947), hydrogenated pseudohecogenin in acetic acid and obtained a product, m.p. 224–226°, which they designated as dihydropseudorockogenin (XXII). The method of preparation was not valid. Unless the 26-hydroxyl group of pseudosapogenins is protected, immediate rearrangement takes place on treatment with acid. Moreover, glacial acetic acid produces compounds other than 20-isosapogenins (unpublished information from this Laboratory) unless diluted with alcohol.

(21) We have found that the 17α-hydroxyl also is found in this region whereas secondary hydroxyl groups give bands near 3620–3640 cm.$^{-1}$.

Further elution of the Florisil column with chloroform gave 1.4 g. of amorphous material which was acidic. Alkaline cleavage with KOH in *t*-butyl alcohol in the usual manner[3c] gave a neutral fraction. This was identified as 16-allopregnene-2α,3β-diol-20-one as needles from methanol; m.p. 228–229° (reference 20, p. 2184, gives m.p. 228–230°), $[\alpha]^{25}D$ +30.6°, λ_{max} 239 mμ, log ϵ 4.0, infrared spectrum identical to authentic specimen prepared by oxidation of pseudogitogenin diacetate followed by hydrolysis.

Oxidation products of other members of the 20β,25D-series have not been studied in as great detail. However, oxidation of 20-isohecogenin (VIII) resulted in a poor yield of a new compound, m.p. 233–235°, tentatively identified as a 3-keto-20?-hydroxysapogenin. The infrared spectrum showed a strong hydroxyl peak at 3500 cm.$^{-1}$ and strong peaks near 1069, 1053, 1024, 990 and 924 cm.$^{-1}$ similar to the 20?-hydroxygitogenin derivative.

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 73.19; H, 9.34.

No Δ^{16}-20-ketone derivative could be isolated, the major portion of the oxidation and saponification products being resinous. Similar oxidation of XVI gave a 20? hydroxy derivative, crystallized as rods from hexane; m.p. 188–189°, $[\alpha]^{25}D$ −72°. The infrared spectrum shows hydroxyl at 3480 cm.$^{-1}$, 3-ketone at 1715 and strong "fingerprint" bands at 1070, 1020, 993 and 925 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.98; H, 10.05.

In addition, an amorphous acidic fraction was obtained which on saponification gave 16-pregnene-3,20-dione, m.p. 200–202°, $[\alpha]^{25}D$ +89°, λ_{max} 239 mμ, log ϵ, 3.95 (lit.[22] gives m.p. 199–201°).

Oxidation of XVIII gave a 20?-hydroxy derivative with infrared spectrum similar to those previously described. It crystallized from methanol; m.p. 175–185°. Sufficient quantities for further purification were not available. The amorphous acid fraction on alkaline cleavage gave 16-allopregnene-3,20-dione as plates from methanol; m.p. 213.5–214.5°, $[\alpha]^{25}D$ +73°, λ_{max} 239 mμ, log ϵ 3.95 (lit.[19] gives m.p. 208–211°).

After oxidation and saponification, (−)-α-methylglutaric was recovered from the cleavage products of VI diacetate, XVI and XVIII, by methods described in detail previously.[3c] The product was obtained as crystals from ether–pentane; m.p. 78–80°, $[\alpha]^{25}D$ −18° (lit.[5a] gives m.p. 78.5–81°, $[\alpha]^{25}D$ −20°).

Similar oxidation was applied to the 20β,25L-compounds XII and XIV. No 20?-hydroxy derivatives could be found. The only products obtained were amorphous acids which on alkaline cleavage yielded in the neutral fraction 16-pregnen-3,20-dione, m.p. 201–202°, identical in all respects to an authentic specimen, and 16-pregnen-2β,3β-diol-20-one, crystallized as plates from acetone–methanol; m.p. 196–198°, $[\alpha]^{25}D$ +28°, λ_{max} 239 mμ, log ϵ 3.91.

In both cases we obtained (+)-α-methylglutaric acid, m.p. 78–80°, $[\alpha]^{25}D$ +16° (lit.[5a] gives m.p. 78.5–81° and $[\alpha]^{25}D$ +18°).

CrO_3 oxidation of the dihydro 20-isosapogenins from XIV, XVI and XVIII led to isolation of 16-pregnene-3,20-dione in the first two cases and 16-allopregnene-3,20-dione in the latter case. Similar oxidation of dihydro-20-isohecogenin followed by alkaline treatment gave no Δ^{16}-20-keto derivative.

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

Steroidal Sapogenins. XXVIII.[2] Conversion of Steroidal Sapogenins to Δ^{16}-20-Keto-pregnenes[3]

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The three-step conversion of steroidal sapogenins (I) to Δ^{16}-20-ketopregnenes (VII) has been systematically studied. Treatment of I with acetic anhydride gave pseudosapogenin (II) in high yield. Oxidation of II with CrO_3 or H_2O_2 gave the oxidation intermediates III and smaller quantities of VII, 16,17-α-epoxides (X), and some unidentified products. Alkaline hydrolysis of pure III with *t*-butyl alcohol–potassium hydroxide proceeded quantitatively. This reagent had no effect on pure compounds of type VII with or without a C-12 carbonyl. This paper presents complete physical properties of a number of type VII compounds prepared from various sapogenins and of their C_{16-17}-saturated analogs.

Pregnene and allopregnene derivatives with the Δ^{16}-20-keto moiety and saturated analogs are excellent sources for the preparation of cortisone or cortisone analogs.[4–6] This paper presents the results of studies leading to improved procedures for the preparation of these compounds from steroidal sapogenins. In addition a complete description of the physical properties of these compounds is presented. Many of the compounds described were first prepared by Marker and co-workers,[7a–c] although in most cases only melting points were given. The methods subsequently presented by other workers[8a–d] as well as the procedures described herein are fundamentally only variants of Marker's procedures. These involve essentially a three-step process as shown in Fig. 1. In the standard sequence a sapogenin (I) is treated with acetic anhydride to give the pseudosapogenin acetate (IIa) which is oxidized with CrO_3 to the intermediate V. V can be treated in a variety of ways to yield the desired Δ^{16}-20-ketopregnene (VII). In addition VII can be obtained by oxidation of dihydropseudosapogenin (III)[7b] or the equivalent dihy-

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

(2) Paper XXVII, M. E. Wall and H. A. Walens, THIS JOURNAL, **77**, 5661 (1955).

(3) A preliminary announcement of these researches has been presented previously; M. E. Wall, H. E. Kenney, H. W. Jones and E. S. Rothman, Fifth Meeting-in-Miniature, Philadelphia Section A.C.S. Jan. 29, 1953, Abstracts of Papers, p. 10.

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