Acetylation of Vb with pyridine–acetic anhydride afforded the diacetate (Vc) (Reichstein's Compound D diacetate), m.p. 217–220°; recrystallized from chloroform–ether, m.p. 219–222°, $[\alpha]_D$ +72° (dioxane) (lit.[6] m.p. 223–224°, $[\alpha]$ 72.3 ± 2° (dioxane)); infrared spectrum, acetate, 5.72 μ, carbonyl, 5.80–5.82 μ, and hydroxyl, 2.9 μ.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 67.17; H, 8.88.

Monosemicarbazone of 5α-Pregnane-3β,17α,21-triol-11,-20-dione.—5α-Pregnane-3β,17α,21-triol-11,20-dione (2.2 g.) reacted at room temperature with 1.48 g. of semicarbazide hydrochloride and 0.84 g. of anhydrous sodium acetate in 66 cc. of purified methanol. The initial reaction mixture is a suspension which after some time dissolves and then reprecipitates. After 48 hours, 0.79 g. of anhydrous sodium acetate and 40 cc. of water were added to the cooled reaction mixture. The product was filtered and washed with water until free of chloride ion; yield 2.2 g., m.p. 302–303°; infrared spectrum, hydroxyl, amide 2.9–3.2 μ and carbonyl 5.84, 6.02 μ.

Anal. Calcd. for $C_{22}H_{35}O_5N_3$: N, 9.95. Found: N, 9.57.

Monosemicarbazone of 5α-Pregnane-3β,11β,17α,21-tetrol-20-one.—The monosemicarbazone of Va (2.2 g.) was dissolved in 500 cc. of refluxing, purified methanol. Reduction was carried out at the reflux point under an atmosphere of N_2 with 50 cc. of sodium borohydride solution (3 g. of $NaBH_4$, 0.69 g. of NaOH, 54 cc. of H_2O) added dropwise to the refluxing solution. Refluxing was continued 0.5 hour after addition of the borohydride solution was complete at which time a second 50-cc. portion of sodium borohydride solution was added dropwise and refluxing was maintained 1.5 hours after the sodium borohydride solution was complete. The cooled solution was diluted with 164 cc. of water containing 8.3 cc. of glacial acetic acid. The reaction mixture was concentrated *in vacuo* in absence of air to approximately 200 cc. The product was filtered and washed with water; yield 1.87 g., m.p. 299–301°; infrared spectrum, 2.9 μ, 3.05 μ hydroxyl and amide; 6.00 μ, 6.30 NH; no ketonic carbonyl.

5α-Pregnane-3β,11β,17α,20-tetrol-20-one (VI).—The reduced semicarbazone III (1.87 g.) was stirred as a suspension in 13.4 cc. of glacial acetic acid, 7 cc. of water and 2 cc. of pyruvic acid. After approximately 6 hours, solution was complete. Reprecipitation took place shortly thereafter. After a total reaction time of 23 hours the reaction mixture was transferred to a separatory funnel with 250 cc. of ethyl acetate. Sodium bicarbonate (21 g.) and 25 cc. of water was added and the mixture shaken; an additional 25 cc. of water was added and, after shaking, the aqueous layer was removed. The ethyl acetate was then washed with 50 cc. of saturated sodium bicarbonate solution followed by 75 cc. of saturated sodium chloride solution. The combined washes were extracted three times with 50 cc. of ethyl acetate. The combined ethyl acetate solutions were dried by shaking with granular drierite and the solvent removed in the absence of air on the oil-pump. The residue was taken up in 80% methanol and concentrated again to the point of crystallization. After standing overnight in the ice-box the product was filtered; yield 0.88 g., m.p. 218–223°, $[\alpha]^{24}_D$ +52° (dioxane).

A sample recrystallized from methanol–water, m.p. 221–224°; from dioxane–water, m.p. 233–235°, $[\alpha]^{24}_D$ +55° (dioxane), lit., m.p. 220–225°, $[\alpha]_D$ +52° (dioxane); infrared spectrum, 2.94–3.0 μ hydroxyl, 5.85 μ carbonyl; sulfuric acid chromogen spectrum λ_{max} 333, 417, 510 mμ; lit.[11] λ_{max} 285, 330, 415 mμ; 510 mμ.

Anal. The compound crystallizes from dioxane–water with 1 mole of dioxane of crystallization which is lost only on prolonged drying (24 hours) at a temperature of 103°.

Anal. Calcd. for $C_{21}H_{34}O_5 \cdot C_4H_8O_2$: C, 66.04; H, 9.31. Found: C, 66.50; H, 8.82. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 69.14; H, 9.05.

(11) A. Zaffaroni and R. B. Burton, *J. Biol. Chem.*, **193**, 749 (1951).

RAHWAY, N. J.

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC.]

The Stereochemistry of Steroidal Sapogenins. II[1]

BY J. B. ZIEGLER, W. E. ROSEN AND A. C. SHABICA

RECEIVED JULY 30, 1954

The complexity of the stereochemistry of the E and F rings of the steroidal sapogenins is considerably greater than was originally realized. Assuming identical configurations at C-16 and C-17, the asymmetric centers at C-20, C-22 and C-25 permit the existence of eight diastereoisomers for each nuclear type; four of these are now known. Studies of the reactions of these isomers, together with assumptions of mechanisms for their formation and interconversion, have permitted assignment of absolute configurations which appear to be consistent with all established experimental facts.

The stereochemistry of the nucleus in the various steroidal sapogenins[2] has for the most part been well delineated.[3] However, the stereochemistry of the E and F rings is much less well known. The asymmetric centers at C-20, C-22 and C-25 permit the existence of a maximum of eight side-chain diastereoisomers, even assuming that all sapogenins have the same configurations at C-16 and C-17.

It has been recognized that certain naturally occurring pairs of sapogenins exist, one member ("normal" series) of each pair being convertible to the other member ("iso" series) by vigorous treatment with mineral acid.[4–7] Evidence derived from degradative studies indicated that the seat of this isomerism lay in the side chain, and the assumption was made that the actual locus was the C-22 or spiro position; reports[5a,8] of the identity of the pseudo and dihydro derivatives of, for example, sarsasapogenin ("normal" series) and smilagenin ("iso" series) were consistent with this hypothesis. It would appear that for some time the other asym-

(1) Paper I is a Communication to the Editor of THIS JOURNAL, **76**, 3865 (1954).

(2) The term "sapogenin" will hereafter be used to mean "steroidal sapogenin."

(3) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publ. Corp., New York, N. Y., 1949, Chapt. VIII.

(4) Early evidence for these transformations was not convincing. Only recently have they been established by infrared spectroscopy[7,18,21] and by degradation.[14]

(5) (a) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846 (1939); (b) G. A. R. Kon, H. R. Soper and A. M. Woolman, *J. Chem. Soc.*, 1201 (1939).

(6) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith, and C. H. Ruof, THIS JOURNAL, **69**, 2185 (1947).

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

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

metric centers in the side chain were not seriously considered as possibly playing a role (see, however, a very early paper by Farmer and Kon[9]). Very recently there has been a remarkable resurgence of interest in this topic, which has at long last drawn attention to the importance of the asymmetric centers at C-20 and C-25, in addition to that at C-22. The first publication in this recent series was that of Wall, *et al.*,[7] dealing with the newly-isolated markogenin ("normal" series) and its side-chain isomer samogenin ("iso" series). They showed that the respective pseudo compounds were not identical; although these authors did not mention a configurational difference at C-25, one may be deduced from this fact. This paper was followed closely by that of Scheer, *et al.*,[10] who demonstrated the non-identity of the pseudo compounds of sarsasapogenin and smilagenin through careful determination of physical constants and through an elegant oxidative degradation of these substances to give (+)-α-methylglutaric acid and the levorotatory isomer, respectively, as remnants of the side chain containing carbon atoms 22 through 27 inclusive. This clear demonstration of the C-25 isomerism of sarsasapogenin and smilagenin obviated the necessity for assumption of C-22 isomerism to explain the non-identity of these substances, but did not eliminate the possibility that such isomerism might also exist. Shortly thereafter, James[11] extended this type of degradative attack to diosgenin and hecogenin and showed that these sapogenins had the same configuration at C-25 as smilagenin. Furthermore, he deduced that these three sapogenins had the D-configuration at C-25 relative to D-glyceraldehyde; it followed, therefore, that sarsasapogenin had the L-configuration.

The next development was the appearance in rapid succession of communications[12-16] from several laboratories all reporting the preparation of sapogenins isomeric with the usual naturally-occurring types by *very mild* acid treatment of the corresponding pseudosapogenins. Recent authors have variously designated this "abnormal" class as 20-isosapogenins,[12,13] *ana*sapogenins[15] and neosapogenins[14,16,17] and have shown that representatives may be synthesized from sapogenins of both "normal" and "iso" series; it seems reasonably certain that every naturally-occurring sapogenin will be shown to have its "neo" analog. Marker and Lopez[19] were probably the first to obtain examples of this class, although they failed to realize their full significance.

As a class, the neosapogenins are distinguished by an unusual degree of chemical reactivity. Thus, (1) they are converted to the sapogenins by relatively mild treatment with alcoholic hydrochloric acid[12,14-16,31]; (2) they are readily oxidized by chromic acid–acetic acid to 21-carbon Δ^{16}-20-one compounds whereas the sapogenins are unaffected by this treatment (the dihydrosapogenins behave in the same way as their corresponding sapogenins)[12]; (3) they are converted readily to pseudosapogenin diacetates by refluxing with acetic anhydride, in contrast to the more vigorous conditions required for conversion of the sapogenins.[12,14,15,31] Since the neosapogenin–sapogenin interconversion has been established for compounds of both "normal" (25-L) and "iso" (25-D) series, it is clear that configurational changes at C-20 and/or C-22 must be involved in this interconversion. Various interpretations of the data have been made, in connection with configurations at C-20 and C-22. With regard to C-20, two British groups[14,15] take the view, based on examination of models, that since excessive hindrance would exist between C-18 and C-21 if the C-21 methyl group were in the β-orientation, only α-orientation is possible; therefore, they suggest that the sapogenins and neosapogenins are alike at C-20 and differ only at C-22 (no definite configurations were assigned). This concept suffers from the disadvantage that it affords no ready explanation for the great difference in reactivity between the two classes. On the other hand, Wall, *et al.*,[12] as well as ourselves,[16] have concluded that the sapogenins have the α-C-21-methyl and the neosapogenins have the β-C-21-methyl; the difference between the degree of C-18-methyl–C-21-methyl interaction thus parallels the difference in chemical reactivity between the two series.

With regard to C-22, Wall, *et al.*,[13] hold that the sapogenins and the corresponding neosapogenins have the same configuration. This accommodates their conviction that configuration at C-22 changes during conversion from the "normal" to the "iso" series (*e.g.*, sarsasapogenin → smilagenin). Adherence to this classical concept leads to considerable difficulty when the attempt is made to formulate a rational and consistent mechanism for the formation and interconversion of the sapogenins and the corresponding neosapogenins, and for the conversion from the "normal" to the "iso" series. Furthermore, despite claims[13] to the contrary, there is no unequivocal evidence pointing to a configurational difference at C-22 between sarsasapogenin and smilagenin. Evidence cited[13] for such a difference includes (1) infrared data, (2) conversion of "normal" series to "iso" series sapogenins, and (3) reported differences in behavior between "normal" and "iso" series sapogenins on bromination. Neither of the first two points is decisive, since other at least equally acceptable interpretations of the experimental data are possible. With regard to the third point it has been stated[13,20] that sarsasapogenin acetate forms a 23,23-dibromo de-

(9) S. N. Farmer and G. A. R. Kon, *J. Chem. Soc.*, 414 (1937).

(10) I. Scheer, R. Kostic and E. Mosettig, THIS JOURNAL, **75**, 4871 (1953).

(11) V. H. T. James, *Chemistry and Industry*, 1388 (1953).

(12) M. E. Wall, C. R. Eddy and S. Serota, THIS JOURNAL, **76**, 2849 (1954).

(13) M. E. Wall and S. Serota, *ibid.*, **76**, 2850 (1954).

(14) R. K. Callow and V. H. T. James, *Chemistry and Industry*, 691 (1954).

(15) D. H. W. Dickson, J. Elks, R. M. Evans, A. G. Long, J. F. Oughton and J. E. Page, *ibid.*, 692 (1954).

(16) J. B. Ziegler, W. E. Rosen and A. C. Shabica, THIS JOURNAL, **76**, 3865 (1954).

(17) The naturally-occurring neotigogenin of Noller,[18] which is a typical "normal" series sapogenin analogous to sarsasapogenin, should not be confused with these highly acid-sensitive compounds.

(18) L. H. Goodson and C. R. Noller, THIS JOURNAL, **61**, 2420 (1939).

(19) R. E. Marker and J. Lopez, *ibid.*, **69**, 2373 (1947).

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

rivative under proper conditions, whereas the acetates of various sapogenins of the "iso" series[13,20] and, in particular smilagenin,[13] form only 23-monobromo derivatives. This evidence formed the major basis for the assignment by Wall, *et al.*,[13] of specific configurations at C-22 to sarsasapogenin (ring F oxygen α) and smilagenin (ring F oxygen β). However, the structure of the dibromosarsasapogenin has never been proven and therefore the formation of this substance does not necessarily bear on C-22 configuration. Furthermore, in contrast to the earlier report,[13] we have now found that *smilagenin acetate also forms a dibromo derivative* under conditions similar to those used in preparing dibromosarsasapogenin acetate. Treatment with zinc dust in hot acetic acid regenerates the original sapogenin acetate, as is the case with the dibromosarsasapogenin acetate. In consequence, the question of C-22 isomerism between these two sapogenins is completely open, insofar as direct experimental evidence is concerned.

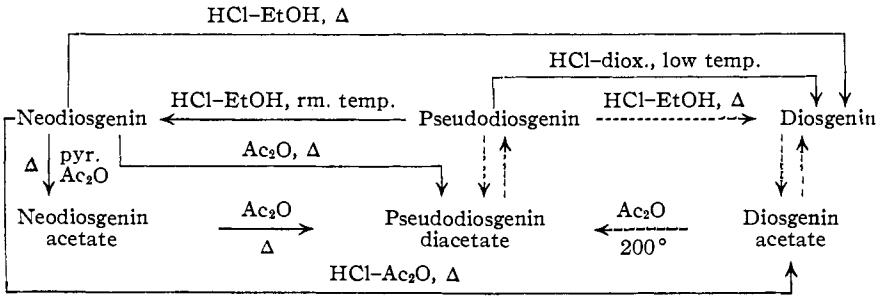


Fig. 1.—Dashed arrows represent previously reported conversions; solid arrows, new conversions.

The authors' conclusions[16] with regard to configuration at C-20 and C-22 were based also on studies of neosapogenin-type compounds, but whereas Wall, *et al.*,[13] asserted that neosapogenins differed from sapogenins only at C-20, and Callow and James[14] assigned the difference solely to C-22, we felt that mechanistic requirements as well as consistency required a difference at *both* C-20 and C-22.

It was observed[16,31] that neodiosgenin separated immediately from an ethanolic or methanolic solution of pseudodiosgenin on addition of a few drops of hydrochloric acid. When the mild acid treatment of pseudodiosgenin was carried out in dioxane, wherein the first-formed neodiosgenin remained in solution, diosgenin was the resultant product. Neodiosgenin was converted also into diosgenin by short reflux of an ethanolic acid solution. The acetylation reactions of neodiosgenin were particularly interesting. Acetic anhydride in excess pyridine afforded neodiosgenin acetate; acetic anhydride reflux in the absence of pyridine gave pseudodiosgenin diacetate; acetic anhydride with a trace of mineral acid converted neodiosgenin to diosgenin acetate. A flow sheet of the conversions in the diosgenin series is given in Fig. 1.

The formation of neodiosgenin and its conversion to diosgenin can be interpreted as an example of the initial formation of a kinetically favored but thermodynamically unfavored product (neodiosgenin) which, when permitted to reach equilibrium, goes over to the thermodynamically favored product, diosgenin. Isolation of pseudodiosgenin diacetate from acetic anhydride reflux of neodiosgenin suggests that acetic acid is sufficiently strong to establish the equilibrium between neodiosgenin and pseudodiosgenin, but not to effect cyclization to diosgenin; mineral acid apparently catalyzes not only the opening of the neodiosgenin ring F, but also the rapid cyclization of pseudodiosgenin to the stable diosgenin system.

There is growing evidence that ionic addition reactions in general involve atoms or groups (here H and OR) situated in the *trans* relationship to each other.[21] The acid-catalyzed cyclization reactions under consideration are conceived to be polar in nature, with *trans* addition occurring across the C-20, C-22 double bond[22] of the pseudosapogenin. In the initial cyclization of pseudodiosgenin to neodiosgenin, it would appear that attack of a solvated proton at C-20 from the less hindered rear face of the molecule is kinetically favored and leads to structure II. However, on prolonged treatment with acid, a process of equilibration leads to the thermodynamically more stable III; the driving force for the conversion of neodisogenin (II) to diosgenin (III) is furnished mainly by hindrance between the C-18 and C-21 methyl groups and secondarily by the less stable axial conformation[23–25] of the C-27 methyl group in structure II. All of these considerations apply also to the interconversion of isomers[12,31] in the smilagenin series (VII, VIII, IX) and also, no doubt, to other "iso" series sapogenins.

The conversions[12,31] of pseudosarsasapogenin(IV) to neosarsasapogenin (V) by mild acid treatment, and either IV or V to sarsasapogenin (VI) by more vigorous ethanolic acid treatment are unquestionably similar to the corresponding reactions in the diosgenin and smilagenin series. Neosarsasapogenin, however, is rendered less stable *only* by the hindrance between C-18 and C-21, since the methyl group at C-25 is equatorial. Conversion to sarsasapogenin under equilibrating conditions relieves the great strain of C-18, C-21 interaction, but requires that the methyl group at C-25 assume the less stable axial conformation. On the basis of conformational concepts, therefore, the ultimate conversion of sarsasapogenin (axial methyl group) to smilagenin (equatorial methyl group) by *long* acid treat-

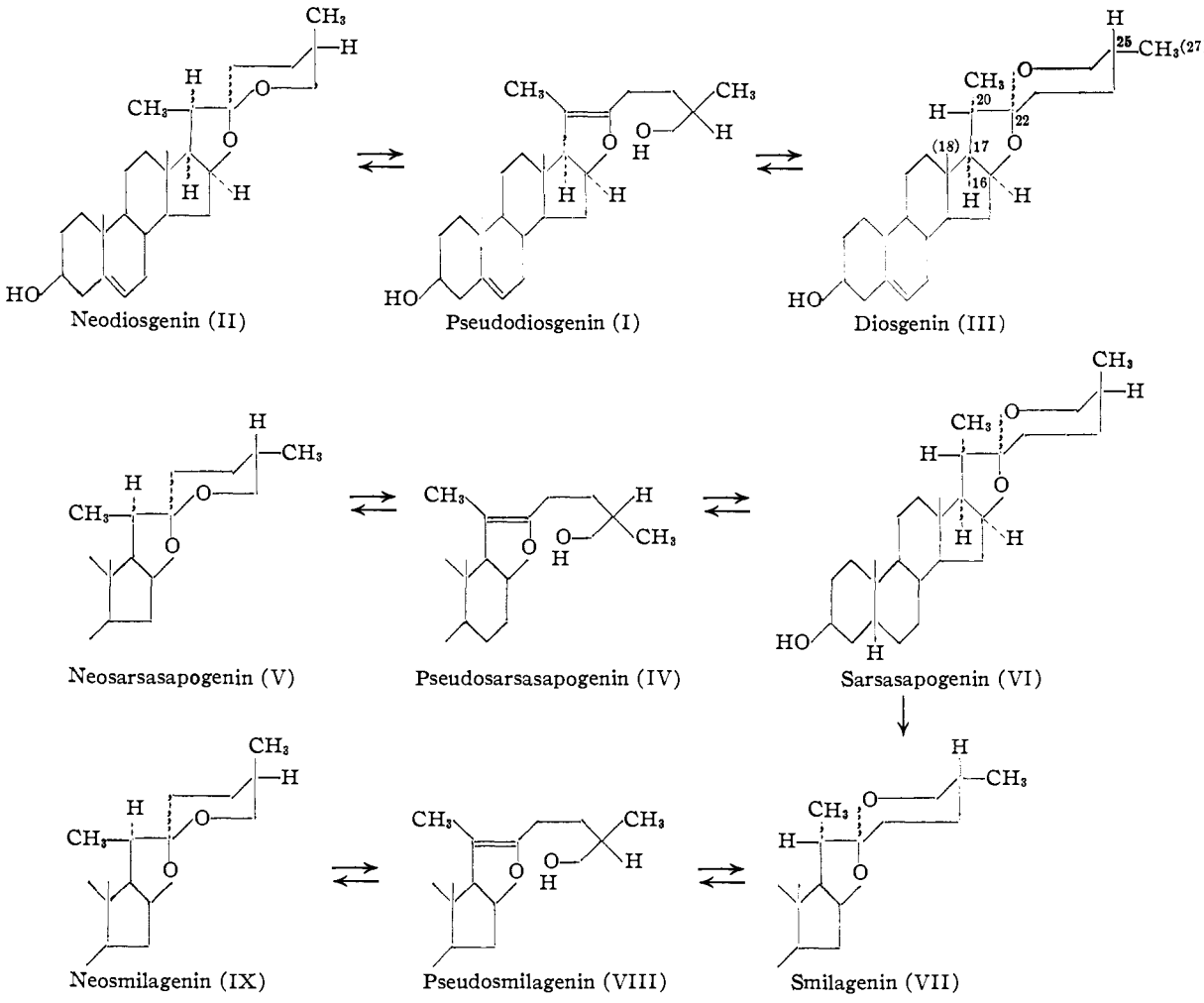
(21) See for example P. D. Bartlett in Gilman, "Organic Chemistry," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 51.

(22) The C-20, C-22 double bond requires the *cis* fusion of rings D and E on steric grounds, presumably in the β-configuration[27] (see also ref. 3, p. 666).

(23) The axial conformation of the methyl group at C-25 coincides with hindrance between the C-18 and C-21 methyl groups only if the absolute configuration of the steroid nucleus is as drawn; evidence that the steroid nucleus actually is drawn correctly in an absolute sense is presented later in this paper.

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

(25) Conformational analysis of six-membered rings containing oxygen seems to be on firm ground: (a) O. Hassel and B. Hottar, *Acta Chem. Scand.*, 1, 929 (1947); (b) J. A. Mills, *Chemistry and Industry*, 633 (1954).



ment is understandable. This transformation was first reported by Marker[5] and recently claimed by Wall[13] to have been confirmed. We also have confirmed this reaction and feel that although the driving force for the reaction is apparent from the greater energy of the axial methyl group at C-25, the mechanism is most subtle.[26]

Absolute Configuration.—This work, together with previous studies, makes possible the assignment of absolute configuration to the steroid nucleus. Two groups[27a,b] have determined the absolute configuration of sterols at C-20 by correlation through citronellal with D-glyceraldehyde. The configurations at C-20 of cholesterol and diosgenin have been shown to be the same.[28] The present work has clearly established the configuration of the steroid nucleus in the sapogenins relative to C-20, and therefore the absolute configuration of the nucleus (formulas I through IX). Formulas III, VI and VII depict the absolute configuration at C-20 as determined by the two groups cited[27a,b] above, while II, V and IX have the opposite configuration at this point.

Infrared Absorption Spectra.—Examination of infrared spectra of the sapogenins and neosapogenins thus far available reveals few general correlations between particular spectral features and specific configurations and/or conformations in the side chain. On the basis of the structural assignments made in this paper, previous correlations[29,30] of the relative intensities of the bands near 920 and 900 cm.$^{-1}$ with configurations at C-22 in the sapogenins cannot hold. All of the relatively acid-stable, naturally-occurring sapogenins (*e.g.*, III, VI, VII) are formulated as having the same configuration at C-22, but sarsasapogenin ("normal" series, 25-L) has the band near 900 cm.$^{-1}$ much weaker than that near 920 cm.$^{-1}$, while the relative order of magnitude of the corresponding bands in smilagenin and diosgenin (both "iso" series, 25-D) is reversed. Furthermore, in passing from neodiosgenin (II) to diosgenin[29,30] (III) or from neosmilagenin (IX, Fig. 2)

(26) J. W. Cornforth, *Ann. Rept. on Progr. Chem. (Chem. Soc. London)* **50**, 219 (1954), in noting the work of Scheer, Kostic and Mosettig[10] on differences at C-25 between sarsasapogenin and smilagenin stated that if VI is convertible to VII by acid, and if VI and VII differ *only* at C-25, and if the C-27 methyl group were axial a proton attached to the oxygen atom of ring F "would be rather well placed to initiate a displacement reaction with inversion of configuration at C-25."

(27) (a) B. Riniker, D. Arigoni and O. Jeger, *Helv. Chim. Acta*, **37**, 546 (1954); (b) J. W. Cornforth, I. Youhotsky and G. Popjàk, *Nature*, **173**, 536 (1954).

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

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

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

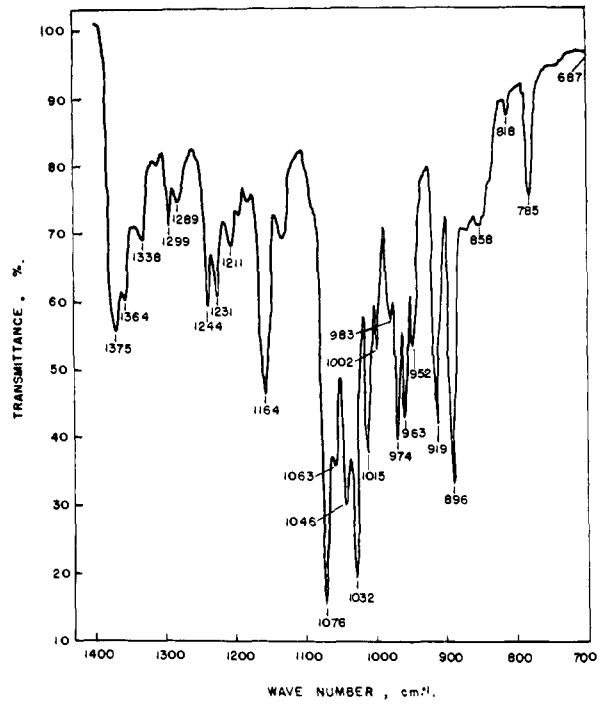


Fig. 2.—Infrared absorption spectrum of neosmilagenin: 1% in CS_2, 1-mm. cell.

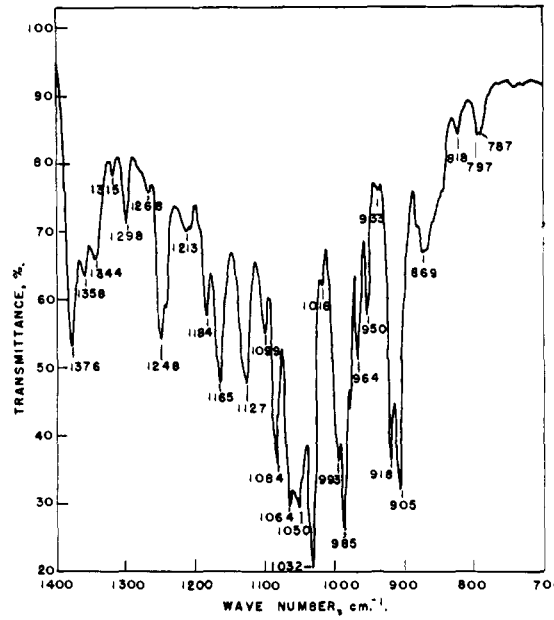


Fig. 3.—Infrared absorption spectrum of neosarsasapogenin: 1% in CS_2, 1-mm. cell.

to smilagenin[29,30] (VII), configuration at C-22 is inverted but the relative order of magnitude of the bands near 920 cm.$^{-1}$ and 900 cm.$^{-1}$ remains the same; in these neosapogenins, however, the relative intensities of these bands are more nearly equal. On the other hand, in passing from neosarsasapogenin (V, Fig. 3) to sarsasapogenin[29,30] (VI), the relative order of magnitude of these bands *is* reversed as configuration at C-22 is inverted. Actually, it would appear that within the class of ordinary, relatively acid-stable sapogenins, the relative intensity of these bands may instead be correlated with the conformation of the C-27 methyl group (axial in "normal" sapogenins; equatorial in "iso" sapogenins). This cannot be extended to include the neosapogenins, however.

There are two points of uncertain significance in connection with the fingerprint region of the available neosapogenin spectra which are worthy of mention. Firstly, it seems that there is a characteristic difference between the spectra of the ordinary sapogenins of the "iso" series and their neosapogenin analogs in the vicinity of 970–985 cm.$^{-1}$. All ordinary sapogenins have strong bands in the vicinity of 980–985 cm.$^{-1}$.[29,30] However, the spectra of neodiosgenin[31] (II), neosmilagenin[12,31] (IX, Fig. 2), neotigogenin,[15,17] 11-ketoneotigogenin[15] and neohecogenin[14,15] are characterized by the strong reduction or absence of this band and the appearance of a band of medium intensity near 974 cm.$^{-1}$. It may be noted that the E and F rings of these neosapogenins are under maximum strain owing to configuration at C-20 and conformation at C-25. On the other hand, sarsasapogenin[29,30] (VI) ("normal" series) and neosarsasapogenin[12,31] (V, Fig. 3) both have the usual strong band near 985 cm.$^{-1}$; neosarsasapogenin has only the single element of ring strain involving configuration at C-20. Secondly, neosarsasapogenin displays a pronounced shift of the 900 cm.$^{-1}$ band from 895 to 905 cm.$^{-1}$.

Of especial interest are comparisons between the spectra of diosgenin[29,30] (III), smilagenin[29,30] (VII) and sarsasapogenin[29,30] (VI) and their neosapogenin analogs (respectively: II, IX, Fig. 2, and V, Fig. 3) in the "methyl" region near 1360–1380 cm.$^{-1}$. Bands in this region in steroids have been assigned[30,32] to C–H bending vibrations of methyl groups. All of these sapogenins show a strong band near 1380 cm.$^{-1}$; Jones, *et al.*,[30] have assigned this band in the steroidal sapogenins to the "unperturbed" C-18, C-19 and C-21 methyl groups. In addition to this band, the neosapogenins show a strong band, quite well resolved, near 1358–1364 cm.$^{-1}$. This same band is observed in the spectra of all sapogenin *3-acetates* studied; in this instance, it has been assigned[30,32] to the acetate group. However, its appearance in spectra of sapogenin *3-alcohols* appears to be confined to the neosapogenins, and the suggestion is made that it may be due to interaction between the C-18 and C-21 methyl groups in these substances.

Use of 0.5% solutions in carbon tetrachloride is especially suitable for a detailed study of this region of the spectrum.

Acknowledgment.—The authors are grateful to Dr. Monroe E. Wall, of the Agricultural Research Service, United States Department of Agriculture, Philadelphia, Penna., for the generous gift of samples of sarsasapogenin and pseudosarsasapogenin. We are indebted to Mr. Louis Dorfman, head of our Analytical Group, and his staff for various determinations and for discussion of technical matters; microanalyses were by Mr. George Robertson and Mrs. Barbara Pita, infrared absorption spectra by Mrs. Violet Loire and optical rotations by Mr. Ir-

(31) See Experimental section.

(32) R. N. Jones and A. R. H. Cole, THIS JOURNAL, **74**, 5648 (1952).

ving Barrack. Thanks are also due to Mr. Robert Wolf, Staff Photographer, and to Miss Ruth Cole for preparation of the illustrations. We thank Mr. William Schweikart for technical assistance.

Experimental[33]

Pseudodiosgenin Diacetate from Pseudodiosgenin.—A sample of pseudodiosgenin[34,35] prepared in this Laboratory had m.p. 165–168° and $[\alpha]^{25}D$ −39°.

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.61): C, 78.21; H, 10.21. Found: C, 78.26; H, 9.91.

The infrared absorption spectrum (1% in chf.) showed a medium band[36] at 1694 cm.$^{-1}$.

A suspension of 1.0 g. of pseudodiosgenin in 9 ml. of acetic anhydride containing 3 drops of pyridine was heated on the steam-bath for one hour. The clear solution was concentrated to dryness *in vacuo* and the residue was crystallized from methanol, yielding 1.03 g. of white platelets, m.p. 98–100°. Recrystallization from isopropyl alcohol gave 0.79 g. of white platelets, m.p. 98–100°, $[\alpha]D$ −39°.

Anal. Calcd. for $C_{31}H_{46}O_5$ (498.68): C, 74.66; H, 9.30. Found: C, 74.94; H, 9.33.

The infrared spectrum showed a medium band[36] at 1692 cm.$^{-1}$ on the shoulder of the strong acetate carbonyl band at 1732 cm.$^{-1}$.

Neodiosgenin (II) from Pseudodiosgenin (I).—Three drops (approximately 0.1 ml.) of concentrated hydrochloric acid was added to a room temperature solution of 10.00 g. of pseudodiosgenin in 300 ml. of absolute ethanol, causing a white precipitate to form in about 30 seconds. After the mixture had stood overnight, the white powder was collected, washed once with ethanol and dried, giving 8.35 g. (83.5%) of product, m.p. 188–193°. Crystallization from ethanol raised the melting point to 199–202°, $[\alpha]^{26}D$ −122°.

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.61): C, 78.21; H, 10.21. Found: C, 77.82; H, 10.15.

The infrared absorption spectrum differed significantly from that of diosgenin; the 982 cm.$^{-1}$ band was reduced to a shoulder, a medium band appeared at 973 cm.$^{-1}$, and the 960 cm.$^{-1}$ band was intensified.

Similar treatment of pseudodiosgenin dipropionate, m.p. 84.5–85.5°, gave only a 55% recovery of starting material.

Diosgenin (III) from Neodiosgenin (II).—A solution of 0.60 g. of neodiosgenin in 60 ml. of absolute ethanol containing 3 ml. of concentrated hydrochloric acid was refluxed for two hours, diluted with 120 ml. of water, and allowed to stand in the refrigerator overnight. The white solid was collected and dried giving a quantitative yield of material, m.p. 196–206°. The product depressed the melting point of neodiosgenin 10°, gave no depression of melting point with authentic diosgenin, and had an infrared spectrum superimposable with that of authentic diosgenin. One crystallization from ethyl acetate raised the melting point to 206–211°.

Diosgenin (III) from Pseudodiosgenin (I).—A solution of 0.60 g. of pseudodiosgenin in 60 ml. of absolute ethanol was treated with 3 ml. of concentrated hydrochloric acid. A heavy precipitate formed which disappeared on heating to the boiling point. The solution was refluxed for 2.5 hours and excess water was added. After chilling, the white solid was collected, washed with water and dried. The product weighed 0.57 g., m.p. 199–203°. Recrystallization from ethyl acetate yielded 0.42 g. of crystalline material, m.p. 200–208°. A second recrystallization from the same solvent gave 0.30 g., m.p. 208–211°. This material did not depress the melting point of authentic diosgenin, and the infrared spectra were identical.

Diosgenin (III) from Pseudodiosgenin (I) by Mild Acid Treatment.—To a solution of 0.20 g. of pseudodiosgenin in 10 ml. of dioxane was added three drops of concentrated hydrochloric acid. The white precipitate which formed went back into solution almost completely after standing at room temperature for 30 minutes and in the refrigerator for 30 minutes. Dilution with water precipitated 0.18 g. of white solid, m.p. 184–204°; one crystallization from ethanol improved the melting point to 190–199°. A mixture with diosgenin (m.p. 206–211°) melted at 197–206°, whereas with neodiosgenin (m.p. 199–201°) there was a depression to 184–190°. An infrared spectrum established the product unambiguously as diosgenin.

Neodiosgenin Acetate from Neodiosgenin (II).—A clear colorless solution of 0.50 g. of neodiosgenin in 20 ml. of dry pyridine and 15 ml. of acetic anhydride was heated on the steam-bath under a calcium chloride drying tube for one hour; the solution acquired a light yellow color. Solvent was removed at reduced pressure and the yellow residue was charcoaled and crystallized from ethanol, affording 0.33 g. (60%) of white crystals shrinking at 188° and melting at 192–195°. One recrystallization from methanol gave 0.28 g. of long prisms, shrinking at 191° and melting at 194–196.5° [a mixture melting point with diosgenin acetate (m.p. 198°) depressed to 185–190°], $[\alpha]^{23}D$ −119°.

Anal. Calcd. for $C_{29}H_{44}O_4$ (456.64): C, 76.27; H, 9.71. Found: C, 76.52; H, 9.47.

The infrared spectrum was clearly different from diosgenin acetate and similar to neodiosgenin (*e.g.*, a shoulder at 980 cm.$^{-1}$, a medium band at 973 cm.$^{-1}$, and a strong band at 960 cm.$^{-1}$).

A second crop of 0.10 g. (18%) of neodiosgenin acetate, m.p. 185–188°, was obtained from the ethanol mother liquors, raising the total crude yield to 78%.

Pseudodiosgenin Diacetate from Neodiosgenin (II).—The suspended white solid slowly dissolved when 0.50 g. of neodiosgenin was refluxed in 50 ml. of acetic anhydride. After the solution had been refluxed for 1.5 hours and allowed to stand overnight at room temperature, it was evaporated to dryness at reduced pressure. The white solid residue was charcoaled and crystallized from methanol, giving 0.32 g. (53%) of white plates, m.p. 98–99°; there was no depression in melting point on admixture with authentic pseudodiosgenin diacetate. One recrystallization from methanol afforded 0.27 g. of white plates, m.p. 99–100°, $[\alpha]^{23}D$ −41°; the infrared spectrum was identical with the spectrum of authentic pseudodiosgenin diacetate.

Pseudodiosgenin Diacetate from Neodiosgenin Acetate.—A solution of 93 mg. of neodiosgenin acetate in 10 ml. of acetic anhydride was refluxed for 1.5 hr. The acetic anhydride was removed at reduced pressure and the residue was charcoaled and crystallized from methanol. The 57 mg. (56%) of white plates which separated melted at 97–99°, and the melting point was not depressed on admixture with authentic pseudodiosgenin diacetate.

Diosgenin Acetate from Neodiosgenin (II).—Five drops of concentrated hydrochloric acid was added to a suspension of 0.40 g. of neodiosgenin in 20 ml. of acetic anhydride, and the suspension was heated on the steam-bath for one hour; the white crystalline solid dissolved within ten minutes. The dark red solution was evaporated to dryness at reduced pressure, and the tan residue was taken up in ether–methanol, charcoaled, filtered, and concentrated to 10 ml. When the solution cooled, 0.17 g. (39%) of white crystals separated, m.p. 189–192°. A mixture melting point with diosgenin acetate showed no depression, whereas a mixture melting point with neodiosgenin acetate (m.p. 194°) depressed to 178–186°. The infrared spectrum was identical with that of diosgenin acetate, confirming the melting point data. Chromatography of the evaporated mother liquors gave only oils or semi-solids.

Neosarsasapogenin (V) from Pseudosarsasapogenin (IV).—A solution of 0.50 g. of pseudosarsasapogenin in 50 ml. of methanol was treated dropwise with 7 ml. of water with agitation. To the faintly hazy solution was added 5 drops

(33) The diosgenin was commercial material obtained from the Treemond Company, New York, N. Y. Smilagenin (as acetate) was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, and was stated by them to have been isolated from species of *Smilax*. Sarsasapogenin and pseudosarsasapogenin were obtained from Dr. M. E. Wall of the Agricultural Research Service of the United States Department of Agriculture, Eastern Regional Research Laboratory, Philadelphia, Penna. Infrared absorption spectra were determined on a double-beam Perkin–Elmer model 21 infrared spectrophotometer using a sodium chloride prism. 1% solutions in carbon disulfide were used. Melting points were taken on an electrically-heated aluminum block by the capillary tube method and are uncorrected. All rotations were taken in 1% chloroform solution.

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

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

(36) A. L. Hayden, P. B. Smelzer and I. Scheer, *Anal. Chem.*, **26**, 550 (1954).

of concentrated hydrochloric acid with agitation. Scratching induced crystallization after about one minute. After addition of 10 more drops of water, copious crystallization ensued. After chilling in the refrigerator overnight, the precipitate was collected, washed with 50% aqueous methanol and dried. There was obtained 0.42 g. of product which melted at about 100°, resolidified at 110° and remelted at 174–175°. Recrystallization from methanol afforded 0.34 g. of white plates which melted at 175–179° after slight shrinking at 100°. The melting point was depressed on admixture with sarsasapogenin. The analytical sample was dried for two hours at 65° *in vacuo* over P_2O_5; $[\alpha]^{23}D$ +37°.

Anal. Calcd. for $C_{27}H_{44}O_3$ (458.66): C, 77.83; H, 10.65. Found: C, 78.07; H, 10.35.

The infrared absorption spectrum of this material was distinct from that of sarsasapogenin, especially in the vicinity of 900–920 cm.$^{-1}$ (see Discussion).

When this preparation was repeated without addition of water prior to addition of acid, no precipitation occurred. The product obtained by precipitation with water after one hour at room temperature apparently consisted of sarsasapogenin contaminated with neosarsasapogenin and a trace of pseudosarsasapogenin, as judged by examination of the infrared absorption spectrum.

Sarsasapogenin (VI) from Neosarsasapogenin (V).—A solution of 0.10 g. of neosarsasapogenin in 10 ml. of absolute ethanol was treated with 0.5 ml. of concentrated hydrochloric acid and refluxed for 1.5 hours. Water was added until the hot solution became cloudy, and cooling induced crystallization. The mixture was chilled, and the crystals were collected, washed with water and dried. The product weighed 0.7 g., m.p. 195–198°. A mixture with authentic sarsasapogenin (m.p. 194–198°) melted at 195–200°. The infrared absorption spectrum of the reaction product was identical with that of authentic sarsasapogenin.

Neosmilagenin (IX) from Pseudosmilagenin (VIII).—A sample of pseudosmilagenin was prepared by a method similar to the usual procedure of Marker.[34] The crude product so obtained had m.p. 137–140°, and the infrared absorption spectrum (1% in chf.) showed the usual band[36] at 1694 cm.$^{-1}$.

To a solution of 2.00 g. of pseudosmilagenin in 200 ml. of methanol was added 45 ml. of water, gradually and with agitation. To the faintly cloudy solution at room temperature was added 20 drops of concentrated hydrochloric acid with shaking; precipitation of an amorphous solid soon began and, after about 15 minutes, the original solution had turned to a thick slurry. After standing at room temperature for a total of 2.5 hours, the precipitate was collected and washed with water. Crystallization of the damp product from methanol yielded 1.08 g. of elongated prisms, m.p. 168–169°. Recrystallization from methanol raised the melting point to 170.5–172°. A mixture of this substance with smilagenin (m.p. 175–180°) melted at 154–156°. The analytical sample was dried for four hours at 80° *in vacuo* over P_2O_5; $[\alpha]^{21}D$ −72°.

Anal. Calcd. for $C_{27}H_{44}O_3$ (458.66): C, 77.83; H, 10.65. Found: C, 77.49; H, 10.61.

The infrared absorption spectrum of this substance was distinct from that of smilagenin, especially near 980 cm.$^{-1}$ (see Discussion).

Smilagenin (VII) from Neosmilagenin (IX).—A sample of 0.10 g. of neosmilagenin was treated with hydrochloric acid in ethanol as described above for the analogous reaction of neosarsasapogenin. The product weighed 0.09 g., m.p. 183–186°. A mixture with authentic smilagenin (m.p. 177–181°) melted at 180–184°. The infrared absorption spectrum was identical with that of an authentic specimen of smilagenin.

Isosarsasapogenin (Smilagenin) (VII) from Sarsasapogenin (VI).—The procedure used was essentially that of Marker and Rohrmann,[5a] except that the crude product was isolated by filtration after dilution with water. From 1.5 g. of sarsasapogenin there was obtained 0.7 g. of crude isosarsasapogenin in the form of long, hair-like white needles, m.p. 145–160°, after recrystallization from methanol. After successive recrystallizations from acetone and methanol, there remained 0.25 g. of needles, m.p. 174–180°. A final recrystallization from methanol gave 0.21 g., m.p. 176–180°. A mixture of this substance with sarsasapogenin (m.p. 192–195°) melted at 178.5–182° (*cf.* Marker and Rohrmann[5a]). However, a mixture of the acetate (prepared in the customary manner in pyridine–acetic anhydride), m.p. 149–151°, with sarsasapogenin acetate (m.p. 144–145°), melted at 114–116°. Also, a mixture of this isosarsasapogenin acetate (m.p. 149–151°) with authentic smilagenin acetate (m.p. 152–153°) melted at 150–151°. The infrared absorption spectra of isosarsasapogenin and authentic smilagenin were identical, as were the spectra of the respective acetates.

Dibromosarsasapogenin Acetate.—This substance was prepared essentially according to the procedure of Djerassi, *et al.*,[20] except that the reaction mixture was allowed to stand overnight at room temperature. From 5.00 g. of sarsasapogenin acetate there was obtained 4.5 g. of crude product, m.p. 231° dec. Crystallization from chloroform–methanol gave 3.67 g., m.p. 238° dec. After a second recrystallization from the same solvent, the analytical sample was dried for 4 hours at 80° *in vacuo* over P_2O_5.

Anal. Calcd. for $C_{29}H_{44}O_4Br_2$ (616.50): Br, 25.93. Found: Br, 25.33.

The infrared absorption spectrum indicated the presence of the spiroketal ring system.

This dibromo acetate, on refluxing for 19 hours in 5% methanolic potassium hydroxide, gave no ketonic material, but did yield the previously unreported dibromosarsasapogenin, m.p. 213–215° dec., $[\alpha]^{24}D$ −65°.

Anal. Calcd. for $C_{27}H_{42}O_3Br_2$ (574.47): C, 56.45; H, 7.37; Br, 27.82. Found: C, 56.34; H, 7.57; Br, 26.71.

Acetylation with acetic anhydride–pyridine (room temperature) gave dibromosarsasapogenin acetate, m.p. 237–238° dec.

Sarsasapogenin Acetate from Dibromosarsasapogenin Acetate.—The debromination of 0.5 g. of the dibromo compound was carried out with zinc dust in glacial acetic acid according to the procedure of Djerassi, *et al.*[20] After recrystallization from methanol the product had m.p. 145–146.5°; a mixture with authentic sarsasapogenin acetate (m.p. 144–146°) melted at 144–146.5°. The infrared absorption spectrum of the product was identical with that of an authentic specimen of sarsasapogenin acetate.

Dibromosmilagenin Acetate from Smilagenin Acetate.—To a solution of 1.00 g. (2.18 millimoles) of smilagenin acetate in 15 ml. of glacial acetic acid was added dropwise 1.03 g. (0.35 ml., 6.4 millimoles) of bromine; the first molar equivalent of bromine (0.12 ml., 2.2 millimoles) was taken up immediately. The red-brown reaction mixture stood at room temperature for 18 hours, slowly darkening and evolving hydrogen bromide, and depositing long white prisms. The prisms were collected and washed with acetic acid and methanol to give 0.59 g. (44%) of product, m.p. 217.5–219.5° (slight dec.). Dilution of the combined mother liquors and washings with water precipitated 0.76 g. of white powder which afforded an additional 0.47 g. (35%) of white prisms, m.p. 214–216° (slight dec.) on one crystallization from ether–methanol. The same product was obtained in slightly lower yield when chloroform–acetic acid was used as reaction solvent.

A sample crystallized from ether–methanol and melting at 217–218.5° (sl. dec.) was dried under vacuum at 65° over P_2O_5; $[\alpha]^{21}D$ −64°.

Anal. Calcd. for $C_{29}H_{44}O_4Br_2$ (616.50): C, 56.49; H, 7.19; Br, 25.93. Found: C, 56.47; H, 7.21; Br, 26.20.

The infrared spectrum was clearly different from that of monobromosmilagenin acetate.[5a]

Smilagenin Acetate from Dibromosmilagenin Acetate.—A solution of 0.50 g. of dibromosmilagenin acetate in 15 ml. of glacial acetic acid was refluxed for one hour in the presence of 3.0 g. of zinc dust. The zinc dust was removed by filtration and washed with 10 ml. of warm acetic acid. The combined colorless acetic acid solution was diluted with 100 ml. of water and allowed to stand for one-half hour. The white crystalline product was collected, washed with water, and crystallized from methanol, giving 0.32 g. (87%) of white needles melting 149–150°; a mixture with authentic smilagenin acetate (m.p. 151–152°) melted 150.5–151.5°. Infrared spectroscopy established its identity with smilagenin acetate.

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