chromatographed on 15 g. of ethyl acetate-washed alumina; yield 0.21 g., m.p. 165–170°. The analytical sample was recrystallized from methanol; m.p. 170–171°, $[\alpha]D$ +100°, λ_{max}^{EtOH} 240 mμ, log ε 4.18; $\lambda_{max}^{CS_2}$ 5.72, 5.98 and 8.2 μ.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.67; H, 8.95.

17α-Hydroxyprogesterone (XII).—A sample (150 mg.) of the monoenol acetate XI in 5 cc. of chloroform was treated at 0° with 30 cc. of 0.1 *N* perbenzoic acid (in chloroform) and left for 3 hours at room temperature. After washing with dilute sodium hydroxide and water, the solvent was removed and the residue was warmed on the steam-bath for 3 minutes with 2 cc. of 4% methanolic potassium hydroxide. Dilution with water and extraction with ether furnished 125 mg. of crude product which was chromatographed on 5 g. of ethyl acetate-washed alumina and recrystallized from methanol; yield 55 mg., m.p. 218–220°, $[\alpha]D$ +100°. The identity of this substance with 17α-hydroxyprogesterone synthesized by an alternate procedure[22] was established by infrared comparison and mixture melting point determination.

(22) G. Rosenkranz, J. Pataki, St. Kaufmann, J. Berlin and C Djerassi, THIS JOURNAL, **72**, 4081 (1950).

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Digitogenin

BY DONALD L. KLASS, MARY FIESER AND LOUIS F. FIESER

RECEIVED JANUARY 24, 1955

Digitogenin has been related directly to gitogenin by elimination of the most hindered hydroxyl group. Gitogenin has been shown to be 22a,5α-spirostane-2α,3β-diol by a partial synthesis through the 2β,3β-diol. Evidence for the location of the third hydroxyl in digitogenin at C_{15} is presented; this group probably has the β-configuration. A new sapogenin, the normal isomer of digitogenin, has been isolated from a commercial preparation of digitonin.

When this work was initiated, more than two years ago, digitogenin (Ia) was tentatively regarded as a 5α,22a-spirostane-2,3,15-triol,[1] but only the position (but not configuration) of the 2,3-glycol group was known with reasonable certainty from an indirect correlation of digitogenin with gitogenin, 5α,22a-spirostane-2,3-diol.[2] The third hydroxyl group was shown to be adjacent to an asymmetric center bearing a hydrogen atom and was originally placed at C_6,[3] and then at C_{15},[4] mainly on the basis of exclusion evidence.[5]

We have achieved a direct conversion of digitogenin into gitogenin in the following way. On treatment with ethyl chloroformate in dioxane–pyridine the 2,3-dicathylate (Ib) is obtained in good yield.[6] Oxidation with sodium dichromate gives a keto dicathylate II, which forms a 2,4-dinitrophenylhydrazone without difficulty, but which is reduced by the Huang-Minlon modification of the Wolff–Kishner reaction in very low yield (4%). The carbonyl group is eliminated, however, without difficulty by desulfuration of the ethylenethioketal, and the product is the dicathylate of gitogenin (IIIb), from which the free diol is obtained on hydrolysis.

In the meantime the probable structure of gitogenin has been shown to be 22a,5α-spirostane-2α,-3β-diol (IIIa) by a partial synthesis[7] involving a sequence of reactions for which the stereochemistry has been established in the cholesterol series[8] and by non-identity with the 2α,3α-diol and the 2β,3α-diol, prepared a few years ago by partial synthesis.[9] We have prepared the fourth possible isomer, the 2β,3β-diol, by *cis*-hydroxylation of Δ²-22a,5α-spirostene (IV)[9] with silver acetate, iodine and moist acetic acid.[10] Although two products are possible, only one diol could be isolated from the reaction (57% yield), and since it differs from the 2α,3α-diol, it is evidently the 2β,3β-diol. As expected, it forms an acetonide, which also differs from the acetonide of the 2α,2α-diol, and it is readily oxidized to gitogenic acid.[11] When the 2β,3β-diol is heated with sodium ethoxide in a sealed tube at 180°, the 2β-hydroxyl group (axial) rearranges to the more stable 2α-configuration (equatorial) with formation of gitogenin. The identity was established by mixed melting point and infrared comparisons. However, gitogenin prepared by this partial synthesis or from digitogenin (above) does not give a purple color with concentrated sulfuric acid, a test which is said to be characteristic for this sapogenin.[7,9] The color reaction probably is due to an impurity,[12] since gitogenin isolated from *Digitalis purpurea*[13] gives only light yellow to colorless solutions in the reagent.

Further evidence that the 2- and 3-hydroxyl groups of digitogenin are *trans* to each other is that the sapogenin is not dehydrated on sublimation

(1) Nomenclature: *Chem. Ind.*, June 23, 1951, SN 1.

(2) R. Tschesche, *Ber.*, **68**, 1090 (1935).

(3) R. Tschesche and A. Hagedorn, *ibid.*, **69**, 797 (1936).

(4) R. E. Marker, D. L. Turner and P. R. Ulshafer, THIS JOURNAL, **64**, 1843 (1942).

(5) For reviews of early literature see Elsevier's "Encyclopaedia of Organic Chemistry," Vol. 14, Series III, 1940, p. 286; L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Chapt. VIII, Reinhold Publ. Corp., New York, N. Y., 1949.

(6) Acetylation with acetic anhydride and sodium acetate (reflux temperature) gives a mixture of di- and triacetates, from which only the latter has been obtained pure [A. Windaus and K. Weil, *Z. physiol. Chem.*, **121**, 62 (1922)]. The diacetate is formed on treatment with warm acetic anhydride and pyridine [H. B. MacPhillamy, THIS JOURNAL, **62**, 3518 (1940)].

(7) J. Herran, G. Rosenkranz and F. Sondheimer, *ibid.*, **76**, 5531 (1954).

(8) L. F. Fieser and M. A. Romero, *ibid.*, **75**, 4716 (1953).

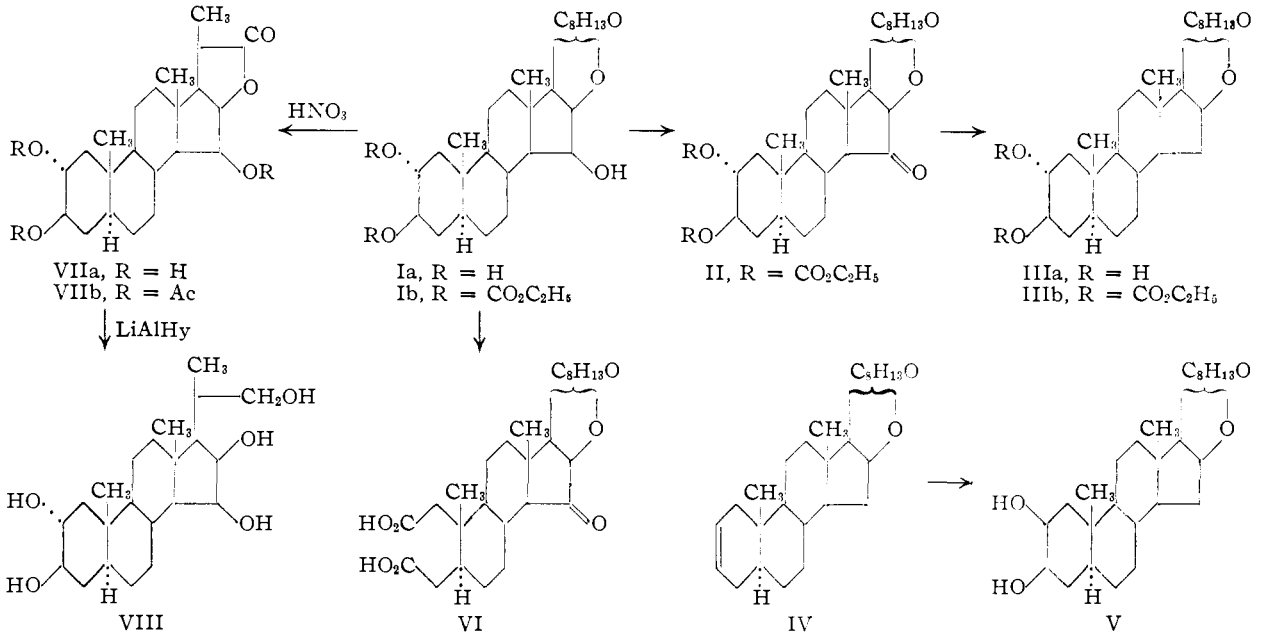
(9) J. Pataki, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 5375 (1951).

(10) For examples of this method see D. Ginsburg, *ibid.*, **75**, 5746 (1953), and L. B. Barkley, *et al.*, *ibid.*, **76**, 5014 (1954).

(11) Our diol differs from a diol assigned the 2β,3β-configuration by J. Herran, *et al.*[7] However, the method used in this other partial synthesis could well lead to mixtures and the only evidence presented for the presumed structure is oxidation to gitogenic acid and non-identity with the other possible 2,3-diols. The rate of oxidation with lead tetraacetate has been reported [C. Djerassi and R. Ehrlich, *J. Org. Chem.*, **19**, 1351 (1954)].

(12) Possibly Δ⁴-yuccagenin (personal communication of Dr. F. Sondheimer).

(13) We are indebted to Dr. W. A. Jacobs, Rockefeller Institute, for this material.



from potassium bisulfate[14] and does not form an acetonide under usual conditions. Location of the third hydroxyl group at C_{15} is confirmed by infrared examination of digitogenic acid (VI)[15] and the corresponding oxime. The former substance shows strong bands at 5.74 and at 5.84 μ (as well as the bands in the 10–12 μ region characteristic of the side chain[16]). The oxime lacks the band at 5.74 μ, which must correspond therefore to the carbonyl band, but has the band at 5.84 μ, corresponding to the carboxyl group. Since a band near 5.7 μ is characteristic of carbonyl groups attached to 5-membered rings,[17] the third hydroxyl group is evidently in ring D and at position 15, adjacent to the asymmetric center at C_{14}. There is one seeming inconsistency with this formulation, for Barton and co-workers[18] have found that 15-ketones with the natural (*trans*) C/D ring fusion do not epimerize in the presence of strong base, a behavior which contrasts with the ready isomerization[2] of digitogenic acid (VI) to digitoic acid, presumably 14-isodigitogenic acid. Likewise our keto dicathylate (II) is isomerized readily and the change in molecular rotation corresponds with that noted in the isomerization of VI. One possible but unlikely explanation is that the C/D ring juncture is *cis* in digitogenin even though it is known to be *trans* in tigogenin and in gitogenin, and that an inversion occurs during formation or desulfurization of the thioketal of II. Another factor which may be responsible for the difference in stability of 15-ketones in the sapogenins and sterols is the presence in the former of an additional ring fused to the D ring. Models indicate more steric hindrance between the angular methyl group at C_{13} and ring E in the C/D *trans*-sapogenins than in the C/D *cis*-sapogenins.

Two other laboratories also have presented evidence for location of the third hydroxyl group at C_{15}. Warren and Canham[19] have noted that the trihydroxy lactone VII, a known oxidation product of digitogenin,[20] on titration in an alkaline medium with sodium periodate consumes 3.84 equivalents of oxygen and therefore corresponds to a tetrahydroxy acid with two glycol groupings (C_2, C_3; C_{15}, C_{16}). Djerassi and Grossnickle[21] recently have degraded digitogenin through the pseudo derivative[22] into a Δ^{16}-pregnene-20-one derivative, whose absorption maximum (λ_{max} 231 mμ, log E 3.97) is displaced from that of Δ^{16}-20-ketones (λ_{max} $\sim$ 239 mμ), presumably owing to substitution in the γ-position (C_{15}) of the chromophore grouping.

The fact that the C_{15}-hydroxyl group does not react with ethyl chloroformate suggests that it has the β-configuration (axial with respect to ring C), since polar hydroxyl groups generally are resistant to cathylation.[23] This hydroxyl group also resists tosylation; however, it is converted readily into the trifluoroacetate. We have attempted to relate the group to the 16β-oxygen function of the lactone VII.[24] Reduction of either VIIa or VIIb with lithium aluminum hydride (tetrahydrofuran solu-

(14) The present observation confirms previous reports of Marker [R. E. Marker, *et al.*, THIS JOURNAL, **64**, 1843 (1942); **69**, 2183 (1947)].

(15) Prepared according to H. Kiliani and B. Merk, *Ber.*, **34**, 3562 (1901).

(16) M. E. Wall, *et al.*, *Anal. Chem.*, **24**, 1337 (1952); R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953).

(17) R. N. Jones, P. Humphries and K. Dobriner, *ibid.*, **72**, 956 (1950).

(18) C. S. Barnes, D. H. R. Barton and G. F. Laws, *Chemistry & Industry*, 616 (1953); D. H. R. Barton and G. F. Laws, *J. Chem. Soc.*, 52 (1954).

(19) F. L. Warren and P. A. S. Canham, *Chemistry & Industry*, 727 (1954).

(20) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 2724 (1939).

(21) C. Djerassi and T. T. Grossnickle, *Chemistry & Industry*, 728 (1954).

(22) Marker[4] had reported previously that digitogenin is not converted to a pseudo derivative. We also have been able to prepare the pseudo derivative as an amorphous powder by the conventional method.

(23) L. F. Fieser, *et al.*, THIS JOURNAL, **74**, 3309 (1952).

(24) Prepared by oxidation of digitogenin triacetate with fuming nitric acid, a method used for the degradation of tomatidine to tigogenin lactone [R. Kuhn, I. Löw and H. Trischmann, *Ber.*, **85**, 416 (1952)]. Oxidation with chromic acid according to Marker[4] proceeds in much lower yield.

tion) gives the pentaol VIII, which readily forms an acetonide, obtained in two polymorphic forms (see Experimental). This reaction cannot involve the glycol group at C_2 and C_3, but models indicate that acetonide formation is possible between the C_{16}- and C_{22}-hydroxyl groups as well as the C_{15}- and C_{16}-hydroxyl groups. In the hope of eliminating the former possibility, we carried out the same sequence of reactions on tigogenin lactone,[25] and were surprised to find that the triol (corresponding to VIII, but lacking the C_2- and C_{15}-hydroxyl groups) readily forms an acetonide. As far as we are aware this example is the first reported case of a seven-membered acetonide.[26] We tried to distinguish between the two possible formulas for the acetonide of VIII by establishing the presence of a primary hydroxyl group by oxidation to the corresponding acid. Oxidation of the acetonide with nitrogen dioxide in chloroform, a reagent which is fairly selective for primary hydroxyl groups,[27] gave a mixture from which no single product could be isolated, but which appeared from infrared analysis to contain a carboxylic acid derivative of the acetonide and also the lactone VIIa.

A new sapogenin, isomeric with digitogenin, has been isolated during this investigation from a commercially available digitonin preparation.[28] Hydrolysis followed by cathylation and subsequent chromatography furnished pure digitogenin dicathylate together with minor amounts of gitogenin dicathylate and the dicathylate of a new sapogenin, $C_{27}H_{44}O_5$, m.p. 277.5–279.5°, α_D −82° Py. This substance is isomerized to digitogenin on prolonged treatment with ethanolic hydrochloric acid, a reaction characteristic of a sapogenin with the normal or neo configuration of the side chain.[29] Moreover it shows the intense band at 10.9 μ in the infrared, also characteristic of neosapogenins. Consequently we propose the name neodigitogenin for this new sapogenin. This compound was not obtained from Merck's digitonin;[30] only digitogenin and a trace of gitogenin were isolated after hydrolysis of this material. Therefore it is not an artifact that results from acid treatment of digitogenin.

Acknowledgment.—This work was part of a program supported by grants from the National Institutes of Health and the National Science Foundation.

Experimental[31]

Digitogenin (Ia) and Neodigitogenin.—Hydrolysis of digitonin (14.1 g.)[28] was carried out in methanol (400 ml.) containing concentrated hydrochloric acid (35 ml.); after a 17-hour reflux period, the solution was concentrated until crystals separated and then cooled and diluted with water. The precipitated solid (5.1 g.) melted over the range 268–283° with decomposition and after five recrystallizations from methanol was still obviously impure digitogenin (m.p. 277–285°). Somewhat purer digitogenin (m.p. 280–287° dec.) could be obtained by application of the same procedure to Digitonin Merck.

The precipitated solid (3.84 g.) was dissolved in anhydrous dioxane (100 ml.) containing anhydrous pyridine (16 ml.) and treated with an excess of ethyl chloroformate at 0°. The mixture was allowed to stand overnight at room temperature and then diluted with water (200 ml.) containing concd. hydrochloric acid (8 ml.). The sapogenin derivatives were then extracted with ether in the usual manner. The oily residue was then chromatographed on alumina (170 g.). Early fractions (petroleum ether–benzene, 70:30) yielded oils; the fractions extracted by petroleum ether–benzene (60:40) were solids and on crystallization from methanol afforded **gitogenin dicathylate** (71 mg.) as plates, m.p. 203–205° (open tube), α_D −86° Chf (*c* 1.13), λ^{Chf} 5.77, 7.8, 10.22, 10.95, 11.19, 11.55 μ. The same product also was prepared by cathylation of gitogenin by the usual procedure.

Anal. Calcd. for $C_{33}H_{52}O_8$ (576.75): C, 68.72; H, 9.09. Found: C, 68.63; H, 8.96.

Fractions eluted by petroleum ether–benzene (60:40) contained mixtures of this dicathylate and the dicathylate of digitogenin.

Fractions extracted by petroleum ether–benzene (50:50) and by benzene after recrystallization from methanol yielded 3.11 g. of **digitogenin 2,3-dicathylate (Ib)** as short, prismatic needles, m.p. 174–176° (open tube), α_D 91° Chf (*c* 1.72), α_D −77° Py (*c* 1.72), λ^{Chf} 2.86, 5.76, 7.86, 8.79 (sharp), 10.24, 10.95, 11.22, 11.56 μ.

Anal. Calcd. for $C_{33}H_{52}O_9$ (592.75): C, 66.86; H, 8.84. Found: C, 67.16; H, 8.82.

Hydrolysis with methanolic hydroxide in the usual manner followed by one crystallization from methanol furnished digitogenin (Ia) as fibrous needles, m.p. 288–291° dec. (open tube),[32] 293–296° (evacuated tube), α_D −80° Chf (*c* 1.29), α_D −61° Py (*c* 1.18), λ^{Chf} 2.86, 8.78 (sharp), 10.23, 10.94, 11.19, 11.55 μ. Recrystallization did not affect the constants.

Fractions eluted by benzene–ether (90:10) and by ether after two crystallizations from methanol afforded 290 mg. of **neodigitogenin 2,3-dicathylate** as needles, m.p. 225–228°, α_D −102° Chf (*c* 1.50), α_D −91° Py, λ^{Chf} 2.84, 5.76, 7.84, 8.78 (sharp), 10.19, 10.96, 11.21, 11.7 μ.

Anal. Calcd. for $C_{33}H_{52}O_9$ (592.75): C, 66.86; H, 8.84. Found: C, 67.10; H, 8.57.

Hydrolysis with methanolic potassium hydroxide followed by two crystallizations from methanol gave **neodigitogenin** as needles, m.p. 277.5–279.5° (open tube), m.m.p. with digitogenin 275–283° dec., α_D −82° Py (*c* 1.25), λ^{Chf} 2.8, 8.78 (sharp), 10.19, 10.94, 11.21, 11.7 μ. No color with concd. sulfuric acid.

Anal. Calcd. for $C_{27}H_{44}O_5$ (448.62): C, 72.28; H, 9.89. Found: C, 72.01; H, 10.10.

Isomerization of Neodigitogenin to Digitogenin.—Neodigitogenin (173 mg.) was dissolved in 4 ml. of ethanol and treated with a mixture of 4 ml. each of concd. hydrochloric acid and ethanol. The solution was refluxed for 72 hr. with addition of 2 ml. of concd. acid at the 24- and the 48-hr. interval. The solution then was cooled and diluted with water. The dried precipitate was treated with ethyl chloroformate in the usual manner, and the cathylate derivative purified by chromatography followed by crystallization from petroleum ether. Digitogenin 2,3-dicathylate was isolated in this way in 58% yield (133 mg.) and identified by mixed melting point and infrared comparisons.

Conversion of Digitogenin to Gitogenin.—A solution of digitogenin 2,3-dicathylate (1.66 g.) was shaken with 1.41 g. of sodium dichromate dihydrate with cooling until the oxidant had dissolved and was then allowed to stand 9 hr. at room temperature. Lower temperatures decreased the yield. Addition of water precipitated the crude product,

(25) R. Tschesche and A. Hagedorn, *Ber.*, **68**, 1412 (1935).

(26) *Cf.* S. A. Barker and E. J. Bourne, *J. Chem. Soc.*, 905 (1952).

(27) See J. M. Sugihara, *Advances in Carbohydrate Chemistry*, **8**, 41 (1953).

(28) Analar grade, British Drug Houses, Ltd.

(29) It is uncertain at the present time whether the isomerization involves inversion at C_{22} or C_{25}: J. W. Cornforth, *Ann. Repts. Chem. Soc.*, **50**, 218 (1953); D. A. H. Taylor, *Chemistry & Industry*, 1066 (1954); M. E. Wall and S. Serota, THIS JOURNAL, **76**, 2850 (1954); Z. B. Ziegler, W. E. Rosen and A. C. Shabica, *ibid.*, **76**, 3865 (1954).

(30) We thank Merck and Co., Inc., for generous gifts of digitonin.

(31) Unless stated otherwise melting points were taken in evacuated Pyrex capillary tubes in a Hershberg apparatus and are reported without stem corrections. Microanalyses were performed in the laboratory of Mr. S. M. Nagy at the Massachusetts Institute of Technology.

(32) C. R. Noller and S. Lieberman, THIS JOURNAL, **63**, 2131 (1941), report m.p. 289–293°, with shrinking at 285°.

which was crystallized from ether–methanol to give **22a,5α-spirostane-2α,3β-15-one dicathylate** (II, 77% yield, 1.25 g.), glistening plates, m.p. 224.5–226°, α_D −79° Chf (*c* 1.87), λ^{Chf} 5.75 (shoulder), 5.77, 7.83, 10.21, 10.9 (weak), 11.17, 11.5 μ.

Anal. Calcd. for $C_{33}H_{50}O_9$ (590.73): C, 67.09; H, 8.53. Found: C, 67.00; H, 8.45.

The **2,4-nitrophenylhydrazone** of II, prepared by the method of Djerassi,[33] formed yellow crystals, m.p. 268–270° dec. (open tube), α_D −288° Chf (*c* 0.60), λ^{Chf} 10.22, 10.9, 11.22, 11.49 μ.

Anal. Calcd. for $C_{39}H_{54}O_{12}N_4$ (770.85): C, 60.76; H, 7.06. Found: C, 60.68; H, 7.01.

The ethylenethioketal of II was made by treatment of the lactone (1.0 g.) with ethanedithiol (3 ml.) with catalysis by one drop of 70–72% perchloric acid. Methanol was added after 20 minutes until the yellow color was discharged, followed by water, which precipitated an oil. This was taken up in ether, washed with cold 5% potassium hydroxide and then water. A semisolid (1.02 g.) was recovered from the dried ether solution. Direct crystallizations from ether–methanol gave the ethylenethioketal as short, flat spars (66% yield). In another experiment the derivative was separated by chromatography and the yield was less (43%) but the product somewhat more pure; m.p. 244.5–246° (open tube), α_D −110° Chf (*c* 1.51), λ^{Chf} 5.75, 7.74, 10.22, 10.90, 11.19, 11.5 μ.

Anal. Calcd. for $C_{35}H_{54}O_3S_2$ (666.90): C, 63.03; H, 8.16. Found: C, 62.94; H, 8.14.

The ethylenethioketal of the dicathylate (300 mg.) was refluxed in 100 ml. of absolute ethanol with Raney nickel (6 g.) for 12 hr. The solution then was refluxed with ethanolic potassium hydroxide to effect hydrolysis of the cathyl groups. The product was precipitated by water, dried, and then acetylated by treatment with acetic anhydride–pyridine at room temperature overnight. The crude acetate was chromatographed and from the fractions eluted with petroleum ether–benzene (50:50 and 25:75) pure **gitogenin diacetate** was obtained by crystallization from ether–methanol (69% yield); m.p. 251–254°, α_D −96° Chf (*c* 1.92).

Anal. Calcd. for $C_{31}H_{48}O_6$ (516.69): C, 72.06; H, 9.36. Found: C, 71.92; H, 9.31.

This material was identical with gitogenin diacetate prepared from a sample of gitogenin isolated by Dr. W. A. Jacobs from *Digitalis purpurea* leaves and which had the following constants; m.p. 251–254°, α_D −97° Chf (*c* 1.22).

Gitogenin, obtained by hydrolysis in the usual manner, crystallized from ethanol as fibrous needles, m.p. 271.5–275° dec. (open tube), α_D −67° Chf, λ^{Chf} 2.8, 10.22, 10.95, 11.22, 11.64 μ.

Anal. Calcd. for $C_{27}H_{44}O_4$ (432.62): C, 74.95; H, 10.25. Found: C, 74.65; H, 10.37.

This material was identical with authentic gitogenin (Dr. W. A. Jacobs). Since it did not give a purple color with concd. sulfuric acid, we investigated the homogeneity of a sample of gitogenin (m.p. 263–269°)[34] which gives a strong color in the reagent. After purification through the dicathylate, hydrolysis to the free sapogenin and chromatography the diol was obtained as needles, m.p. 271.5–275° dec. (open tube), α_D −70° Chf (*c* 1.02). This purified material still gave a positive color test, but the intensity was about one-fortieth of that of the original sample. The impurity must be present in small amounts since it is not detectable in the infrared spectrum.

Gitogenin also was obtained in low yield (4%) by reduction of the keto dicathylate II with hydrazine hydrate in triethylene glycol by the usual Huang-Minlon conditions.

22a,5α-Spirostane-2β,3β-diol (V).—Δ^2-22a,5α-Spirostene (IV) was prepared according to the literature[9] from tigogenin (68.4% yield, m.p. 181–184°, (open tube), α_D −31° Chf (*c* 3.62)). A modification of the *cis*-hydroxylation method of Woodward and Brutcher[35] was employed. A solution of the spirostene (2.47 g., 0.00619 mole) in warm glacial acetic acid (250 ml.) was treated with reagent grade silver acetate (2.32 g., 0.0139 mole). The temperature was adjusted to 35°, and while the mixture was stirred vigorously finely powdered iodine (1.65 g., 0.0065 mole) was added all at once. After 15 min. a mixture of 12 ml. of glacial acetic acid and 0.5 ml. of water was added. The reaction was heated on the bath with stirring for 3 hr., and then cooled and saturated with sodium chloride; the precipitated silver salts were filtered and washed with acetic acid. The filtrates were evaporated to dryness under reduced pressure. The semisolid residue was acetylated (acetic anhydride–pyridine) and then chromatographed on 80 g. of alumina. Early fractions (petroleum ether–benzene, 90:10) contained starting material (49 mg.). Fractions eluted by petroleum ether–benzene (25:75) and by benzene after two crystallizations from methanol afforded **22a,5α-spirostane-2β,3β-diol diacetate**, 0.94 g. (23% yield), short needles, m.p. 216–217.5°, α_D −36° Chf (*c* 1.89), λ^{Chf} 5.78, 7.95, 10.22, 10.94, 11.19, 11.60 μ, negative tetranitromethane test.

Anal. Calcd. for $C_{31}H_{48}O_6$ (516.69): C, 72.06; H, 9.36. Found: C, 71.88; H, 9.24.

Hydrolysis of the mother liquors and of late fractions of the chromatograph followed by chromatography yielded an additional quantity of the free diol (0.92 g., 34%).

Hydrolysis of the purified diacetate with methanolic potassium hydroxide followed by crystallization from methanol furnished **22a,5α-spirostane-2β,3β-diol**, as needles, m.p. 243.5–245.5°, α_D −48.5° Chf (*c* 1.55), λ^{Chf} 2.80, 10.22, 10.92, 11.19, 11.60 μ, light yellow color in concd. sulfuric acid.

Anal. Calcd. for $C_{27}H_{44}O_4$ (432.62): C, 74.95; H, 10.25. Found: C, 74.89; H, 10.01.

Oxidation of the diol (203 mg.) in glacial acetic acid (3.5 ml.) and chloroform (1 ml.) with Kiliani solution (1.61 g.)[15] gave an acid fraction which on crystallization from aqueous acetic acid gave gitogenic acid (119 mg., 55% yield), short needles, m.p. 239–242°, α_D −56° Chf (*c* 1.45), identical with an authentic sample prepared from gitogenin.

The acetonide was prepared by refluxing the diol (225 mg.) and *p*-toluenesulfonic acid (100 mg.) in reagent grade acetone in a Soxhlet apparatus (1:1 mixture of anhydrous magnesium sulfate and Drierite in thimble) for 18 hr. After neutralization with 10% potassium carbonate solution, the mixture was concentrated to a volume of 50 ml. and extracted with ether in the usual manner. The solid residue so obtained (239 mg.) was crystallized from ether as small plates, 185 mg. (75% yield), m.p. 249–251.5°, α_D −37° Chf (*c* 1.51), λ^{Chf} 10.22, 10.94, 11.20, 11.48, 11.60, 12.08 μ.

Anal. Calcd. for $C_{30}H_{48}O_4$ (472.68): C, 76.22; H, 10.24. Found: C, 76.19; H, 10.24.

Isomerization of 2β,3β-Diol V to Gitogenin (III).—Sodium (0.7 g.) was added to a solution of the diol V (168 mg.) in 17 ml. of absolute ethanol; the mixture was heated in a sealed tube at 184° for 24 hr., cooled to room temperature, and the product precipitated by water, washed with water, and dried. A chloroform solution was placed on alumina; elution with chloroform–methanol (75:25) gave impure gitogenin (128 mg.), m.p. 260–266°. This was acetylated (acetic anhydride–pyridine, room temperature overnight), and then purified by chromatography. Material eluted with petroleum ether–benzene (50:50) after crystallization from ether–methanol afforded pure gitogenin diacetate (109 mg., 54% yield) as fibrous needles, m.p. 252–255°, α_D −95° Chf (*c* 1.51), λ^{CS_2} 5.78, 8.1, 8.2, 10.22, 10.9, 11.19, 11.64 μ. This material is identical with authentic gitogenin (see above). On hydrolysis the free sapogenin was obtained, m.p. and m.m.p. 270–275° dec., α_D −67° Chf (*c* 1.35).

Digitogenic Acid and the Oxime.—**The acid** was prepared by the procedure of Kiliani[15] and on crystallization from acetic acid furnished needles, m.p. 211–219° dec. (open tube), m.p. 221–224° (evacuated tube), α_D −43° Chf (*c* 2.16), λ^{Chf} 3.2 (broad), 5.74, 5.84, 10.21, 10.9 (weak), 11.17, 11.5 μ. **The oxime**[36] was obtained from ethanol–water (50:50) as micro-needles, m.p. 172–175° dec. (open tube), λ^{Chf} 3.2 (broad), 5.84, 10.21, 10.9 (weak), 11.17, 11.5 μ.

Isomerization of 22a,5α-Spirostane-2α,3β-diol-15-one Dicathylate (II).—The keto dicathylate II (40 mg.) was refluxed 20 min. in a solution of 0.25 ml. of concd. hydrochloric acid in 10 ml. of absolute ethanol, and then concentrated. **22a,5α-14-Isospirostane-2α,3β-diol-15-one dica-**

(33) C. Djerassi, THIS JOURNAL, **71**, 1007 (1949).

(34) Source unknown to us; kindly supplied by Syntex S. A.

(35) R. B. Woodward and F. V. Brutcher, unpublished work.

(36) H. Kiliani, *Arch. Pharm.*, **232**, 334 (1894).

thylate, 33 mg., separated as short needles, m.p. 217–220°, and was crystallized from ethanol; m.p. 221–223°, m.m.p. with starting material 187–215°, α_D −115° Chf (*c* 1.44), λ^{Chf} 5.78, 7.87, 10.24, 10.96, 11.25, 11.58 μ.

Anal. Calcd. for $C_{33}H_{50}O_9$ (590.73): C, 67.09; H, 8.53. Found: C, 66.91; H, 8.38; C, 66.81; H, 8.50.

The 2,4-dinitrophenylhydrazone of the isoketo dicathylate, prepared by treatment with Brady solution and purified by chromatography, separated from chloroform–methanol as bright yellow needles, m.p. 240–242° dec., m.m.p. with the 2,4-dinitrophenylhydrazone of II 223–235° dec., α_D −420° Chf (*c* 0.99), λ^{Chf} 10.24, 10.96, 11.24, 11.55 μ.

Anal. Calcd. for $C_{39}H_{54}O_{12}N_4$ (770.85): C, 60.76; H, 7.06. Found: C, 60.71; H, 7.00.

22a,5α-14-Isospirostane-2α,3β-diol-15-one was obtained by hydrolysis of the 2,3-dicathylate with methanolic potassium hydroxide in the usual manner. It separated as microneedles, from dilute acetone, m.p. 200–205°, α_D −106° Chf (*c* 1.07), λ^{Chf} 2.8, 5.76, 10.24, 10.96, 11.24, 11.6 μ. Recathylation yielded the isoketone dicathylate.

Anal. Calcd. for $C_{27}H_{42}O_5$ (446.61): C, 72.61; H, 9.48. Found: C, 72.77; H, 9.53.

The substance was recovered unchanged on attempted conversion to the ethylenethioketal (perchloric acid catalysis). Under drastic conditions unidentified oils were obtained which lacked the sapogenin side chain bands in the infrared.

Digitogenin 2,3-Dicathylate-15-trifluoroacetate.—A solution of 260 mg. of digitogenin 2,3-dicathylate (Ib) in trifluoroacetic anhydride (2.5 ml.) was allowed to stand for 2 hr. at room temperature, after which excess anhydride was removed under reduced pressure. Dry benzene (5 ml.) was added and solvent again removed. The oily residue was crystallized from ether–methanol; m.p. 186–189° (86% yield). Repeated recrystallization furnished the pure derivative, m.p. 193–196° (open tube), α_D −110° Chf (*c* 1.18), λ^{Chf} 5.63, 5.77, 7.85, 10.24, 10.92, 11.17, 11.56 μ.

Anal. Calcd. for $C_{35}H_{51}O_{10}F_3$ (688.76): C, 61.03; H, 7.46. Found: C, 60.87; H, 7.52.

The dicathylate Ib was recovered unchanged on treatment with *p*-toluenesulfonyl chloride (pyridine or triethylamine catalysis) at room temperature or at 90–95°.

Digitogenin Lactone Triacetate (VIIb).—Crude digitogenin (4.65 g., m.p. 271–282°), obtained by acidic hydrolysis of Digitonin Merck was refluxed in acetic anhydride (140 ml.) with anhydrous sodium acetate (4 g.) for 8 hr. After addition of water, the precipitated oil was extracted by ether in the usual way and then chromatographed. Fractions eluted with petroleum ether–benzene (50:50) yielded 112 mg. of gitogenin diacetate, m.p. and m.m.p. 251–254°, α_D −97° Chf (*c* 1.61), satisfactory analysis. Elution with benzene and crystallization from petroleum ether yielded digitogenin triacetate (3.4 g.), needles, m.p. 193.5–196° (evacuated tube), α_D −117° Chf (*c* 2.71), λ^{Chf} 5.78, 8.0, 10.21, 10.91, 11.17, 11.53 μ.

A solution of the triacetate (1.79 g.) in reagent grade chloroform (40 ml.) was treated with fuming nitric acid (28 ml.) and then refluxed on the steam-bath for 45 min. The cooled mixture was washed with water and then the chloroform layer was diluted with ether and washed with bicarbonate solution and saturated salt solution until neutral to litmus. The dried solution was evaporated to dryness, and the residue crystallized twice from acetone–petroleum ether. The lactone triacetate (VIIb, 30% yield) formed fine, white needles, m.p. 287–291°, α_D −122° Chf (*c* 1.36), λ^{Chf} 5.65, 5.78, 8.0 μ.

Anal. Calcd. for $C_{28}H_{40}O_8$ (504.60): C, 66.64; H, 7.99. Found: C, 66.79; H, 7.96.

A sample of the triacetate was saponified with ethanolic potassium hydroxide; acidification and dilution precipitated the lactone triol (**VIIa**), which crystallized from acetone as short needles, m.p. 285–289° dec., α_D −114° Py (*c* 1.35), λ^{Di} 2.88, 5.65, λ^{Nujol} 3.0 (broad), 5.69, 8.80 (sharp) μ. Apparently the compound is hygroscopic when crystallized from acetone and retains solvent when crystallized from ethanol.

Bisnorallocholane-2α,3β,15β,16β,22-pentaol (VIII).—The lactone triacetate (VIIb, 1.70 g.), dissolved in anhydrous tetrahydrofuran, was added to a suspension of lithium aluminum hydride (5.0 g.) in the same solvent (50 ml.). The mixture was refluxed for 24 hr., and then the excess hydride decomposed by dropwise addition of water. A 1:1 mixture of chloroform–tetrahydrofuran was added until two phases separated, followed by dilution with 20% sulfuric acid until both phases were clear. The aqueous layer was extracted with chloroform and the extract added to the organic layer. The combined extracts were washed with water, 10% bicarbonate solution, and water, dried by azeotropic distillation with chloroform, and evaporated to dryness. The pentaol VII was obtained after two crystallizations from ethanol as glistening plates, 68% yield, m.p. 255.6–258.5°, α_D −2° Py (*c* 1.45), λ^{Nujol} 2.96, 8.82 (sharp) μ. The pentaol was obtained in this way from the lactone triol, but in lower yield (51%).

Anal. Calcd. for $C_{22}H_{38}O_5$ (382.52): C, 69.07; H, 10.01. Found: C, 69.13; H, 10.16.

Only an intractable oil was obtained on attempted acetylation.

The pentaol (575 mg.) forms an **acetonide** (64% yield) when shaken in acetone solution with finely powdered, anhydrous copper sulfate for 3 days at room temperature. The filtered solution was concentrated and crystalline product crystallized from chloroform as short needles, m.p. 240–243°, α_D −5° Py (*c* 1.88), λ^{Nujol} 2.98, 13.18, 13.30, 13.40 μ.

Anal. Calcd. for $C_{25}H_{42}O_5$ (422.59): C, 71.05; H, 10.02. Found: C, 71.18; H, 9.95.

The acetonide on hydrolysis with ethanol–concd. hydrochloric acid gives the pentaol (71% yield).

When the acetonide was chromatographed in the standard way it was obtained in another form; short needles, m.p. 260–263°, α_D −7° Py (*c* 1.78), λ^{Nujol} 2.98 μ, fine structure in 8–12 μ region identical with that of the lower-melting form, no triple peak in 13 μ region.[37] On acidic hydrolysis it is converted into the pentaol. The two forms when mixed gave intermediate melting points.

Anal. Calcd. for $C_{25}H_{42}O_5$ (422.59): C, 71.05; H, 10.02. Found: C, 70.81; H, 10.04.

Digitogenin is recovered in high yield when subjected to the same conditions, or after being refluxed in acetone in the presence of *p*-toluenesulfonic acid for 96 hr., as in the preparation of the acetonide of 22a,5α-spirostane-2β,3β-diol.

We were unable to effect oxidation of the acetonide with the Heyn platinum black catalyst, which is usually selective for oxidation of primary hydroxyl groups.[38] Intractable mixtures were obtained on sodium dichromate oxidation. Oxidation in chloroform solution with liquid nitrogen dioxide gave an oil whose infrared spectrum suggested the presence of a carboxyl group and a five-membered lactone.[39]

Bisnorallocholane-3β,16β-22-triol.—Tigogenin acetate was oxidized by the procedure used above for oxidation of digitogenin triacetate. Tigogenin lactone acetate was obtained as needles, m.p. 219–222° (open tube), α_D −45° Chf (*c* 1.51), λ^{Chf} 5.65, 5.77, 8.0, 21% yield. The lactone was reduced with lithium aluminum hydride by the procedure described above; the triol was obtained in 75% yield as hexagonal plates, m.p. 247–250°, α_D +15° Py (*c* 1.90), λ^{Nujol} 3.08 μ.

Anal. Calcd. for $C_{22}H_{38}O_3$ (350.52): C, 75.38; H, 10.93. Found: C, 75.56; H, 10.95.

The triacetate was prepared by acetylation with acetic anhydride–pyridine (overnight, room temperature). It separated from ether–methanol as large crystals, m.p. 117–119°, α_D +53° Chf (*c* 2.55), λ^{Chf} 5.78, 8.0 μ.

Anal. Calcd. for $C_{28}H_{44}O_6$ (476.63): C, 70.55; H, 9.31. Found: C, 70.46; H, 9.10.

When the triol was treated with acetone and anhydrous copper sulfate under the conditions used to form the acetonide of the pentaol, an acetonide was obtained in 52% yield; flat needles from acetone, m.p. 189.5–191.5° (open tube), α_D −33° Py (*c* 1.63), λ^{Nujol} no OH or CO band.

Anal. Calcd. for $C_{25}H_{42}O_3$ (390.59): C, 76.87; H, 10.84. Found: C, 76.88; H, 10.92.

The triol was obtained on acidic hydrolysis. The acetonide is not changed by chromatography on alumina.

CAMBRIDGE, MASS.

(37) Differences in the infrared spectra of polymorphs, particularly in the fingerprint region, have already been observed; *cf.* A. A. Ebert, Jr., and H. B. Gottlieb, THIS JOURNAL, **74**, 2806 (1952).

(38) *Cf.* K. C. Tsou and A. M. Seligman, *ibid.*, **74**, 3066 (1952); **75**, 1042 (1953).

(39) Similar oxidation of cholanyl alcohol gave cholanic acid in 81% yield.