of attempts to remove the methoxyl group by heating with strong aqueous mineral acids either caused general decomposition or failed to change the dienone absorption in the ultraviolet.

Anal. Calcd. for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27; CH_3O–, 10.61. Found: C, 69.65; H, 8.18; CH_3O–, 10.51.

Treatment of Xb with anhydrous copper sulfate in acetone by standard procedures[1] reconverted the glycol back to its acetonide IXb in essentially quantitative yield.

C-Methyl Determinations.—Compounds of Xa and Xb along with model compounds of established structure (Ia), and the norketone corresponding to Ia but without the methyl group in the 4-position[20], were submitted without structural identification to an independent analyst[21] for Kuhn–Roth C-methyl determination. The observed number of C-methyl groups for Xa, Xb, Ia and the norketone were 1.19, 1.18, 1.31 and 0.58 indicating that both Xa and Xb correspond to Ia in the number of C-methyl groups present.

(20) Prepared as outlined in footnote 25 of the Woodward paper cited in reference 1.

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

C-22 Isomeric Dihydrosapogenins from Kryptogenin

BY IRVING SCHEER, MALCOLM J. THOMPSON AND ERICH MOSETTIG

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In the reduction of kryptogenin in acidic media three compounds have been obtained: dihydrotigogenin and two new sapogenin derivatives. It is shown that the new compounds are isomeric at C-22 and are 22,26-epoxycholestane-3β,16ξ-diol and 22,26-epoxy-22-isocholestane-3β,16ξ-diol. The preparation of cholestane-3β,22,26-triol and cholestane-3β,26-diol-22-one is described. The latter was converted to 22,26-epoxycholestan-3β-ol and 22,26-epoxy-22-isocholestan-3β-ol.

The reduction of kryptogenin (I) yields a variety of products, depending on the experimental conditions employed. Thus, Marker, *et al.*,[1] obtained diosgenin when reducing I with sodium in ethanol, tigogenin (II) and dihydrotigogenin (III) when using platinum oxide in acid media, and dihydrokryptogenin with the same catalyst in a neutral medium. On the other hand, Kaufmann and Rosenkranz[2] obtained "16-dihydrokryptogenin" (XVII) by hydrogenating I in neutral solution with Raney nickel catalyst. In our hands the hydrogenation of kryptogenin (I) with platinum oxide in glacial acetic acid at 25° gave a mixture of at least three compounds which were separated by chromatography on alumina. The main product was dihydrotigogenin (III) and was obtained in a yield of about 80%. The two other products each being formed in an approximate yield of 10% were unknown derivatives.[3] We could show that these new compounds differ only in their configuration at C-22 and have the structures of a 22,26-epoxycholestane-3β,16ξ-diol (IV) and a 22,26-epoxy-22-isocholestane-3β,16ξ-diol (V).[4] The reduction of kryptogenin diacetate under similar conditions yielded only dihydrotigogenin diacetate as previously reported.[1] The infrared spectra of IV and V exhibited strong hydroxyl absorption at 3571 cm.$^{-1}$ (unassociated) and at 3401 cm.$^{-1}$ (associated), no carbonyl absorption, and differed decidedly in the fingerprint region. The bands characteristic for sapogenins were absent, and there was no resemblance to the infrared spectra of dihydrosapogenins. Elemental analysis established for IV and V the empirical formula $C_{27}H_{46}O_3$, *i.e.*, they are isomeric with dihydrotigogenin. Esterification readily gave diesters, indicating that each, IV and V, contained two hydroxyl groups and a third oxygen in an inert ether-like linkage. Oxidation of IV and V with chromic acid led to the diketo-compounds VI and VII, respectively. The strong bands at 1712 and at 1736 cm.$^{-1}$ suggested a 3-ketone and a 16-ketone. Reduction of VI and VII, either catalytic (with PtO_2 in acetic acid) or with sodium borohydride in methanol, gave nearly quantitatively the dialcohols IV and V, respectively, *i.e.*, in either instance only one isomer.

The deoxylation of 22,26-epoxycholestane-3β,16ξ-diol (IV) and 22,26-epoxy-22-isocholestane-3β,16ξ-diol (V) yielded 22,26-epoxycholestane (VIII) and 22,26-epoxy-22-isocholestane (IX), respectively. Compounds VIII and IX contain only two asymmetric centers (C_5 and C_{22}) not found in the original kryptogenin. The remote possibility that these isomers differ at C_5 was eliminated by showing that 5,6-dihydrokryptogenin (XII) was also reduced to III, IV and V.

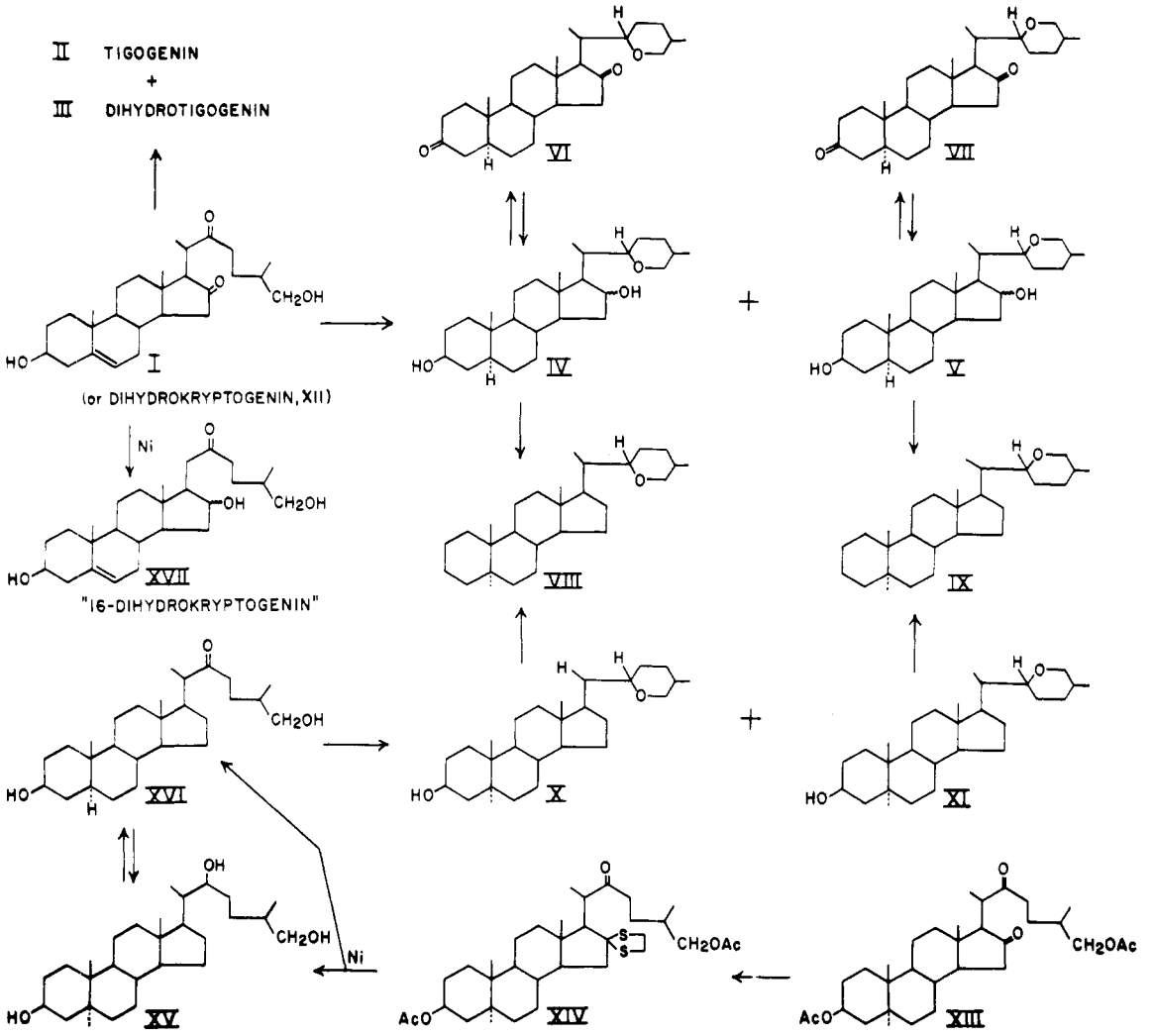
In order to confirm the structures of VIII and IX, these compounds were also prepared by a different route. Treatment of 5,6-dihydrokryptogenin diacetate (XIII) with ethane dithiol gave a monoethylenethioketal derivative. Infrared analysis showed that it was not the C-22 carbonyl group that had reacted and that XIV was a C_{16}-ethylenethioketal. Desulfurization with freshly prepared Raney nickel in dioxane yielded a mixture from which after hydrolysis and chromatography on alumina cholestane-3β,22,26-triol (XV) and cholestane-3β,26-diol-22-one (XVI) were obtained in an approximate ratio of 6:1. For the routine preparation of XVI the crude mixture from the desulfurization of XIV was oxidized gently with chromic acid to give XVI as the sole product after saponification. Catalytic reduction (PtO_2, acetic acid) of XVI led to a mixture of the triol XV, 22,26-epoxycholestan-3β-ol (X) and 22,26-epoxy-22-isocholestan-3β-ol (XI). By chromatography and fractional crystallization compounds

(1) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. J. P. Goldsmith and C. H. Ruof, THIS JOURNAL, **69**, 2167 (1947).

(2) S. Kaufmann and G. Rosenkranz, *ibid.*, **70**, 3502 (1948).

(3) In one instance when for some reason the reduction did not go to completion a small amount of tigogenin (II) could be isolated.

(4) The configuration at C-22 as shown in the structural formulas V and V is arbitrary. The naming of V as an iso compound is provisional.



X, XI and XV were obtained in the respective yields of 23, 43 and 15%. Deoxylation of X and XI gave VIII and IX showing that X and XI were the 16-deoxy analogs of IV and V. Reduction of XVI with sodium borohydride in methanol gave the triol XV in excellent yield. Deoxylation of XV gave cholestane.

It should be noted that no definite assignment of configuration at C-16 of IV and V can be made at this time. Hirschmann, *et al.*,[5] had postulated that reduction of a 16-ketosteroid leads to a 16β-hydroxy compound. Hence we were inclined to assign to IV and V the 16β-hydroxy configuration. The $\Delta M_D^{16\text{-OH}-H}$ values for IV and V, however, are -72 and $-70°$, respectively (Table I), agreeing fairly well with the average value $\Delta M_D^{16\alpha\text{-OH}-H}$ $-60°$ in ethanol which Fukushima, *et al.*,[6] attributed to a 16α-hydroxyl group. Furthermore, these authors have shown that the contribution of a 16α-acetoxy group is about $-300°$ and of a 16β-acetoxy approximately $+100°$. The contribution of the 16-acetoxy group in our 22,26-epoxycholestane derivatives (see acetates of X and IV and of XI and V, respectively) is approximately $+200°$. Even though no configuration can be assigned to the 16-oxygen functions of IV and V on the basis of the anomalous rotational data, the indications are very strong that the configuration is the same in both series.

(5) H. Hirschmann, F. B. Hirschmann and M. A. Daub, This Journal, **74**, 539 (1952); see also leading references in this paper.

(6) D. K. Fukushima and T. F. Gallagher, *ibid.*, **73**, 196 (1951).

Experimental[7]

Reduction of Kryptogenin.—A mixture of 10.0 g. of rigorously purified kryptogenin (I, m.p. 192–193°, $[\alpha]^{20}D$ -198 to $-200°$), 1.0 g. of Adams catalyst and 350 ml. of glacial acetic acid was shaken with hydrogen at room temperature and atmospheric pressure for 24 hr. Three molecular equivalents of hydrogen was absorbed. The crystalline residue obtained from the mixture was chromatographed on benzene-washed alumina. The first few fractions eluted with benzene–chloroform 9:1 yielded 5.0 g. of a mixture melting above 175°. This material was fractionally crystallized from ethyl acetate to give 1.0 g. of **22,26-epoxycholestane-3β,16ξ-diol (IV)** as thin, white needles, melting at 265–268°, $[\alpha]^{20}D$ $-22°$.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.59; H, 11.22.

IV was obtained in several polymorphic forms melting at 240–240.5°, 265–268°, 276–277°. Their identity was established by infrared analysis, optical rotation and interconversion of one form to another by seeding and crystalli-

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

TABLE I

MOLECULAR ROTATION ANALYSIS

Compound	$[\alpha]^{CHCl_3}D$	M_D	ΔM_D M_D 16-OH − H	M_D 22-"normal" − 22-"iso"
22,26-Epoxycholestane (VIII)	− 1.5°	− 6°		−141°
22,26-Epoxycholestan-3β-ol (X)	− 5.5	− 22		−115
22,26-Epoxycholestane-3β,16ξ-diol (IV)	−22	− 94	− 72°	−117
22,26-Epoxy-22-isocholestane (IX)	+35	+135		
22,26-Epoxy-22-isocholestan-3β-ol (XI)	+23	+ 93		
22,26-Epoxy-22-isocholestane-3β,16ξ-diol (V)	+ 5.5	+ 23	− 70	
22,26-Epoxycholestan-3β-ol acetate	−14	− 62	M_{D16}-OAc − H	−133
22,26-Epoxycholestane-3β,16ξ-diol diacetate	+31	+156	+218	
22,26-Epoxy-22-isocholestan-3β-ol acetate	+16	+ 71		−116
22,26-Epoxy-22-isocholestane-3β,16ξ-diol diacetate	+54	+272	+201	

zation. **The diacetate of IV** (acetic anhydride–pyridine, reflux, 30 min.) was obtained as white plates from methanol, m.p. 152–153°, $[\alpha]^{20}D$ +31°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 74.32; H, 10.09.

The di-3,5-dinitrobenzoate of IV (3,5-dinitrobenzoyl chloride–pyridine, 4 hr., steam-bath) was obtained crystalline from acetone–methanol, m.p. 268–269°, $[\alpha]^{20}D$ +19°. *Anal.* Calcd. for $C_{41}H_{50}O_{13}N_4$: C, 61.02; H, 6.25; N, 6.94. Found: C, 61.24; H, 6.13; N, 7.03.

Further elution of the column with benzene–chloroform 9:1 and 1:1 afforded material melting below 175° which was recrystallized from ethyl acetate to give 4.4 g. of **dihydrotigogenin (III)**, m.p. 169–171° (lit.[1] m.p. 170°).

The identity of III was established by comparison (melting point, optical rotation, infrared spectrum, diacetate) with an authentic sample prepared from tigogenin.

The material remaining after the separation of IV from the 5.0 g. of solids melting above 175° was rechromatographed on benzene-washed alumina. Elution with benzene–ether 3:1 afforded material melting above 230° which upon recrystallization from ethyl acetate gave an additional 0.2 g. of **IV**. Further elution of the column with benzene–ether 3:1 and 1:1 gave material melting above 175°. Crystallization of this material from ethyl acetate yielded 1.0 g. of **22,26-epoxy-22-isocholestane-3β,16ξ-diol (V)**, m.p. 212–215°, $[\alpha]^{20}D$ +5.5°. *Anal.* Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.18; H, 11.37.

The diacetate of V was obtained crystalline from methanol, m.p. 179–182°, $[\alpha]^{20}D$ +54°. *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 74.07; H, 9.75.

The di-3,5-dinitrobenzoate of V was crystallized from acetone–methanol, m.p. 235–237, $[\alpha]^{20}D$ +36°. *Anal.* Calcd. for $C_{41}H_{50}O_{13}N_4$: C, 61.02; H, 6.25; N, 6.94. Found: C, 60.87; H, 6.37; N, 6.93.

Elution of the column with chloroform yielded an additional 2.2 g. of **III**.

Thus from 10.0 g. of kryptogenin (I) was obtained 1.2 g. (12.4%) of 22,26-epoxycholestane-3β,16ξ-diol (IV), 1.0 g. (10.3%) of 22,26-epoxy-22-isocholestane-3β,16ξ-diol (V) and 6.6 g. (68%) of dihydrotigogenin III.

The infrared spectra of IV and V in chloroform solution (3%) were distinctly different. IV showed a weak band at 1129 cm.$^{-1}$ and twin bands at 907 and 871 cm.$^{-1}$, while V showed a strong absorption maximum at 1129 cm.$^{-1}$ and twin bands at 905 and 895 cm.$^{-1}$, other differences useful for distinguishing the two isomers were medium bands at 978, 967 and 957 cm.$^{-1}$ in IV which appeared at 983, 969 and 954 cm.$^{-1}$ in V.

In another reduction experiment under these same conditions, 0.4 g. of **tigogenin (II)** was isolated from 12.0 g. of kryptogenin in addition to III, IV and V which were obtained in their usual proportions. The tigogenin was identified by direct comparison with an authentic sample.

Reduction of 5,6-Dihydrokryptogenin.—A mixture of 1.0 g. of 5,6-dihydrokryptogenin (XII), 0.1 g. of Adams catalyst and 50 ml. of glacial acetic acid was shaken with hydrogen at room temperature and atm. pressure for 24 hr. Two molecular equivalents of hydrogen were absorbed. Separation of the reduction mixture as described above yielded 0.1 g. of **IV**, 0.09 g. of **V** and 0.6 g. of **III**.

22,26-Epoxycholestane-3,16-dione (VI).—To a stirred solution of 160 mg. of 22,26-epoxycholestane-3β,16ξ-diol (IV) in 200 ml. of acetone at 20°, was added, dropwise, an 8 *N* solution of chromic acid in dilute sulfuric acid (*ca.* 40%) until a persistent orange-brown coloration indicated oxidation was complete.[8] This addition required 45 minutes. The mixture was diluted with 2 l. of water and the white crystalline precipitate was collected, washed with water and dried to yield 140 mg. of **22,26-epoxycholestane-3,16-dione VI**, m.p. 167–169°. Recrystallization from ethanol raised the melting point to 172–173°, $[\alpha]^{20}D$ − 107°, ν^{CHCl_3} 1736 cm.$^{-1}$ strong (16-ketone) and 1716 cm.$^{-1}$ strong (3-ketone).

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.28; H, 10.26.

The dioxime (hydroxylamine hydrochloride, ethanol–pyridine, steam-bath, 2 hr.) was obtained as white needles from ether–petroleum ether (30–60°), m.p. 246–248° dec.

Anal. Calcd. for $C_{27}H_{44}O_3N_2$: C, 72.93; H, 9.97. Found: C, 72.60; H, 9.75.

The disemicarbazone (semicarbazide hydrochloride, methanol–pyridine–water, steam-bath, 16 hr.) was obtained crystalline from dilute methanol, begins to decompose at 278° but does not melt even at 320°. *Anal.* Calcd. for $C_{29}H_{48}O_3N_6$: C, 65.87; H, 9.15. Found: C, 65.65; H, 9.01.

Reduction of VI in the presence of Adams catalyst and acetic acid or with sodium borohydride in methanol yielded the original diol IV in quantitative yield.

22,26-Epoxy-22-isocholestane-3,16-dione (VII).—Oxidation of 140 mg. of 22,26-epoxy-22-isocholestane-3β,16ξ-diol (V) as described above for the preparation of VI gave 130 mg. of **22,26-epoxy-22-isocholestane-3,16-dione (VII)** as white needles, m.p. 138–175°. Three recrystallizations from methanol afforded 65 mg. of constantly melting material, m.p. 171–175°, $[\alpha]^{20}D$ −104°, ν^{CHCl_3} 1736 cm.$^{-1}$ strong (16-ketone) and 1716 cm.$^{-1}$ strong (3-ketone). A polymorphic form was also obtained which melts at 133–135°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 77.97; H, 10.19.

Treatment of VII under the conditions which yielded a disemicarbazone from VI afforded a monosemicarbazone, **22,26-epoxy-22-isocholestane-3,16-dione 3-semicarbazone**, m.p. 236–243° dec. The infrared spectrum of this derivative exhibits the typical bands of a semicarbazone[9] and in addition retains the strong absorption band at 1736 cm.$^{-1}$ attributed to the 16-ketone.

Anal. Calcd. for $C_{28}H_{45}O_3N_3$: C, 71.30; H, 9.61. Found: C, 71.60; H, 9.80.

Reduction of VII in the presence of Adams catalyst and acetic acid yielded the original diol V.

22,26-Epoxycholestane (VIII).—To a solution of 0.3 g. of 22,26-epoxycholestane-3β,16ξ-diol (IV) in 12 ml. of dry pyridine at 0° was added an ice-cold solution of 0.6 ml. of methanesulfonyl chloride in 3 ml. of pyridine, and the mixture was kept at 3° overnight. The orange solution was poured into ice and water, and the oily precipitate was extracted with ether. The ethereal solution was washed with cold 5% hydrochloric acid, water, 2% sodium bicarbonate solution, water and dried over sodium sulfate. The solution was evaporated *in vacuo* to an oil which was dissolved in 30 ml. of benzene, distilled to a volume of 18 ml., and 18 ml. of dry ether and 4 ml. of a 1.6 *M* solution of lithium aluminum hydride in ether were added and the mixture was

(8) A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.*, 2548 (1953).

(9) W. H. T. Davison and P. E. Christie, *ibid.*, 3389 (1955).

refluxed overnight. The reaction mixture was cooled and after the addition of a few drops of ethyl acetate, treated with 10 ml. of 6 *N* hydrochloric acid. The aqueous layer was separated, extracted with ether and the extracts combined with the benzene–ether layer. The combined extracts were washed with 10% sodium bicarbonate solution and with water, dried over sodium sulfate and evaporated to dryness *in vacuo*. The oily residue was crystallized from methanol to yield 0.2 g. of **22,26-epoxycholestane (VIII)**, m.p. 149–152°. One recrystallization gave 0.18 g. of analytically pure material, m.p. 151.5–152.5°, $[\alpha]^{20}D$ −1.5°.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.71; H, 11.97.

The reduction of 22,26-epoxycholestan-3β-ol (X) in a similar manner also yielded **VIII**.

22,26-Epoxy-22-isocholestane (IX).—As in the preparation of VIII above, 150 mg. of 22,26-epoxy-22-isocholestane-3β,16ξ-diol (V) in 5 ml. of dry pyridine was treated with 0.3 ml. of methanesulfonyl chloride in 1.5 ml. of dry pyridine at 3°. The oil thus obtained was dissolved in 15 ml. of benzene and distilled to a volume of 9 ml. To the solution was added 9 ml. of dry ether and 2 ml. of a 1.6 *M* solution of lithium aluminum hydride in ether, and the mixture was refluxed overnight. The oil obtained from the reduction mixture gave a slight positive test with tetranitromethane and was dissolved in 10 ml. of dioxane and shaken under hydrogen in a stainless steel bomb for 24 hr. at 150° and 1500 p.s.i. in the presence of 2 ml. of Raney nickel in ethanol. The mixture was filtered and concentrated *in vacuo* to a colorless oil which gave a negative test with tetranitromethane. Crystallization from methanol yielded 70 mg. of **22,26-epoxy-22-isocholestane (IX)**, m.p. 159–163°, $[\alpha]^{20}D$ + 35°. Recrystallization did not alter the melting point.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.70; H, 11.87.

The reduction of 22,26-epoxy-22-isocholestan-3β-ol (XI) in a similar manner, also yielded **IX**.

Cholestane-3β,26-diol-16,22-dione 3,26-Diacetate 16-Ethylenethioketal (XIV).—To a solution of 3.0 g. of 5,6-dihydrokryptogenin diacetate (XIII) in 2 ml. of ethanedithiol was added 1.5 ml. of a solution of boron fluoride in ether (Eastman Kodak Co. practical 45% BF_3 in ether). The mixture was hand stirred and set to a stiff paste in about one minute. The paste was thinned with methanol and the white solid collected and washed with methanol. Recrystallization from ethanol gave 3.1 g. of **cholestane-3β,26-diol-16,22-dione 3,26-diacetate 16-ethylenethioketal (XIV)** as white needles, m.p. 193–194°, $[\alpha]^{20}D$ −37°, ν^{CS_2} 1739 cm.$^{-1}$ strong (acetate) and 1706 cm.$^{-1}$ strong (22-ketone).

Anal. Calcd. for $C_{33}H_{52}O_5S_2$: C, 66.85; H, 8.84; S, 10.82. Found: C, 67.00; H, 8.91; S, 11.10.

Saponification of XIV with 2% potassium hydroxide in methanol for 2 hr. at reflux temperature afforded **cholestane-3β,26-diol-16,22-dione-16-ethylenethioketal (kryptogenin 16-ethylenethioketal)** in quantitative yield as white needles from methanol, m.p. 194–195°, $[\alpha]^{20}D$ −28°. For analysis a sample was recrystallized from ethyl acetate, m.p. 195–197°.

Anal. Calcd. for $C_{29}H_{48}O_3S_2$: C, 68.45; H, 9.51; S, 12.60. Found: C, 68.30; H, 9.39; S, 12.30.

Cholestane-3β,26-diol-22-one (XVI).—A mixture of 1.0 g. of cholestane-3β,26-diol-16,22-dione 3,26-diacetate 16-ethylenethioketal (XIV), 4 teaspoons of Raney nickel and 150 ml. of dioxane was refluxed for 16 hr. The Raney nickel was filtered and washed several times with hot dioxane. The combined filtrate and washings were concentrated to dryness *in vacuo* to yield a solid residue. The residue was dissolved in 300 ml. of acetone at 20° and oxidized by dropwise addition of an 8 *N* solution of chromic acid in sulfuric acid and water until a persistent orange color remained. The solution was poured into 3 l. of water and extracted with ether. The ethereal solution was washed with water, 2% aqueous sodium bicarbonate, water, dried over sodium sulfate and concentrated to dryness *in vacuo*. The resultant residue was dissolved in 120 ml. of ethanol and saponified by refluxing for 5 hr. with 6.0 g. of potassium bicarbonate in 100 ml. of water. The solution was concentrated *in vacuo* to one-half its volume and extracted with ether. The ethereal solution was washed with water, dried over sodium sulfate and concentrated to dryness *in vacuo* to yield a crystalline residue which was chromatographed on benzene-washed alumina. The fractions eluted with benzene–chloroform 9:1 and 6:1 were combined and crystallized from benzene to yield 0.6 g. of **cholestane-3β,26-diol-22-one (XVI)** as white needles, m.p. 173–175°, $[\alpha]^{20}D$ +6°, ν^{CHCl_3} 1706 cm.$^{-1}$ strong (22-ketone).

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.54; H, 11.05.

The diacetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as white needles from methanol, m.p. 102.5–103.5°, $[\alpha]^{20}D$ −8.5°. *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 74.35; H, 10.08.

The oxime (hydroxylamine hydrochloride, pyridine–ethanol, steam-bath, 4 hr.) was obtained as thin plates from dilute methanol, m.p. 192–194°. *Anal.* Calcd. for $C_{27}H_{47}O_3N$: C, 74.78; H, 10.92. Found: C, 74.62; H, 10.89.

Cholestane-3β,22,26-triol (XV). a. From XIV.—Three grams of cholestane-3β,26-diol-16,22-dione 3,26-diacetate 16-ethylenethioketal (XIV) was reduced with Raney nickel as described above. The dioxane solution was concentrated to dryness *in vacuo*. The residue was dissolved in 200 ml. of ethanol and refluxed for 5 hr. with 12.0 g. of potassium bicarbonate in 140 ml. of water. The solution was cooled, diluted with water and filtered. The crystalline precipitate was triturated with benzene and recrystallized from methanol–benzene to yield 1.2 g. (47.5%) of **cholestane-3β,22,26-triol (XV)** as white needles, m.p. 229–232°, $[\alpha]^{20}D$ +15° (EtOH).

Anal. Calcd. for $C_{27}H_{48}O_3$: C, 77.09; H, 11.50. Found: C, 76.91; H, 11.53.

The triacetate of XV (acetic anhydride–pyridine, 18 hr., 25°) was obtained as needles from methanol, m.p. 98–100°. *Anal.* Calcd. for $C_{33}H_{54}O_6$: C, 72.49; H, 9.96. Found: C, 72.68; H, 9.72.

The benzene solution which was recovered after the trituration of the crystalline precipitate from the Raney nickel was chromatographed on benzene-washed alumina. Elution with benzene yielded 0.20 g. (8%) of **XVI**, m.p. 172–175°. One hundred milligrams of another crystalline material was obtained from a fraction eluted with benzene–chloroform 9:1. This compound melted at 163–171°, exhibited strong absorption in chloroform at 1678 cm.$^{-1}$ (—C=C—?) and gave a positive tetranitromethane test. Reduction of this material (PtO_2, acetic acid) yielded **22,26-epoxy-22-isocholestan-3β-ol (XI)** from methanol, m.p. 150–153°. Concentration of the mother liquor gave an unsharply melting compound which is very likely the impure C-22-isomer of XI. The unsaturated parent compound is probably a 22,26-epoxide with a double bond at C-20,22 or at C-22,23, but no further work has been done with this compound.

b. From XVI.—A mixture of 0.050 g. of XVI, 0.150 g. of sodium borohydride, 5 ml. of methanol and 0.5 ml. of water was allowed to stand overnight at room temperature. Three drops of acetic acid were added, and the mixture was diluted with water and filtered to yield 0.045 g. (90%) of **cholestane-3β,22,26-triol (XV)**, m.p. 210–220°. Crystallization from methanol–benzene afforded 0.040 g. of **XV** as white needles, m.p. 227–230°, identical with the material obtained from procedure a above.

Cholestane from Cholestane-3β,22,26-triol.—Reduction of 0.150 g. of cholestane-3β,22,26-triol as previously described[10] for the reduction of tetrahydrotigogenin afforded 0.080 g. (60%) of cholestane, identical with an authentic sample.

22,26-Epoxy-22-isocholestan-3β-ol (XI).—A mixture of 1.0 g. of cholestane-3β,26-diol-22-one (XVI), 0.2 g. of Adams catalyst and 100 ml. of glacial acetic acid was shaken with hydrogen at room temperature and atm. pressure for 3 hr. until hydrogenation was complete. The crystalline residue obtained from the reaction was triturated with 50 ml. of benzene. The benzene-insoluble material yielded 0.10 g. of **cholestane-3β,22,26-triol (XV)**. The benzene-soluble material was chromatographed on benzene-washed alumina and a mixture was obtained from the fraction eluted with benzene–chloroform 98:2. From the fraction eluted with ethanol an additional 0.05 g. of XV was obtained. Repeated recrystallization of the mixture from the benzene–chloroform eluate yielded 0.42 g. of **22,26-epoxy-22-isocho-**

(10) I. Scheer and E. Mosettig, This Journal, **77**, 1820 (1955).

lestan-3β-ol (XI), m.p. 152–158°. Another recrystallization from methanol yielded constant melting material, 0.33 g., m.p. 155–158°, but still not pure. From the combined recrystallization mother liquors was obtained 0.10 g. of pure **XI**, m.p. 161–163°, $[\alpha]^{20}D$ +23°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.42; H, 11.64.

The acetate was obtained as white crystals from dilute methanol, m.p. 131–133.5°, $[\alpha]^{20}D$ +16°. *Anal.* Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.02; H, 10.92.

22,26-Epoxycholestan-3β-ol (X).—The mother liquors from the above reduction and subsequent purification procedures were combined and concentrated to dryness *in vacuo*. The residue was crystallized from ethyl acetate to yield thick plates of **X**, m.p. 215–219°. Recrystallization from methanol yielded 0.23 g. of **22,26-epoxycholestan-3β-ol (X)** as needles, m.p. 211–214°, $[\alpha]^{20}D$ −5.5°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.51; H, 11.78.

The acetate was obtained as needles from methanol, m.p. 188.5–190°, $[\alpha]^{20}D$ −14°. *Anal.* Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.46; H, 10.73.

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

Steroidal Sapogenins. XXXVI. Side Chain Bromination Isomers of Diosgenin and Tigogenin[2]

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Bromination of diosgenin acetate with 3 moles of bromine in acetic acid solution for 2 to 3 hr. gave a mixture of 5α,6β,23a-tribromodiosgenin acetate (I) and the corresponding 23b isomer II which could be separated by fractional crystallization. Treatment of I with sodium iodide in ethanol gave 23a-bromodiosgenin acetate (III). Similar treatment of II yielded 23b-bromodiosgenin acetate (IV). Catalytic hydrogenation of III and IV gave, respectively, 23a-bromotigogenin acetate (V) and 23b-bromotigogenin acetate (VI). V and VI were also obtained by monobromination of tigogenin acetate. Prolonged treatment of diosgenin with $3^1/_3$ to $3^1/_2$ moles of bromine gave two isomeric tetrabromide isomers, arbitrarily designated tetrabromodiosgenin acetates A (VII) and B (VIII). Treatment of VII and VIII with sodium iodide gave the same dibromodiosgenin acetate (IX) which on catalytic hydrogenation gave dibromotigogenin acetate (X). Bromination of tigogenin acetate gave two dibromotigogenin acetate isomers, A (X) and B (XI). Dibromotigogenin acetate A was identical with the product obtained by hydrogenation of dibromodiosgenin acetate IX. Treatment of X and XI with sodium iodide in ethanol resulted in conversion of XI to X.

Bromination of the steroidal sapogenin side chain has received sporadic attention in the past 15 years. Marker and Rohrmann,[3] who first investigated the problem, stated that only one bromine atom could be introduced in the side chain of 25D ("iso") or 25L ("normal") sapogenins. Later Djerassi[4] and co-workers found that two atoms of bromine could be introduced in the side chain of sarsasapogenin (25L series). More recently Ziegler, Rosen and Shabica[5] have found that smilagenin (25D) series) can also be dibrominated in the side chain. In addition there have been several reports of the existence of bromine side chain isomers.[6] Recently Barton, Page and Shoppee[7] have on the basis of infrared spectra in the 700–500 cm.$^{-1}$ region assigned the equatorial position to the 23a compounds of the type described by Mueller and Norton[6a] and Dickson and Page.[6b] The 23b isomers were accordingly designated axial. The location of the bromine in the various side-chain brominated sapogenins has been placed at C_{23}[8] and accepted by subsequent workers. Although this assignment is logical, since the bromine atoms are located on a carbon atom adjacent to a potential carbonyl, it must be emphasized that the C_{23} assignment is not based on a rigid structure proof.

The experiments to be reported in this paper were the outgrowth of our interest in the stereochemistry of the spiroketal side chain.[9] On bromination of diosgenin acetate with 3 moles of bromine in acetic acid at 15–20°, a precipitate was noted. After filtration, the products in both the precipitate and filtrate fractions were isolated. It was apparent from the properties of the two fractions that they were isomeric. Since the infrared spectra of the soluble and insoluble fractions resembled, respectively, those of the 23a and 23b series described by Dickson and Page,[6b] we designated the acetic acid soluble fraction as 5α,6β,23a-tribromodiosgenin acetate (I) and the insoluble, precipitate fraction as 5α,6β,23b-tribromodiosgenin acetate (II). The structural assignments of I and II were on the following evidence. Bromine analysis showed that three bromine atoms were present. The infrared absorption bands between 800–840 cm.$^{-1}$ characteristic of the Δ^5-ethylenic moiety in diosgenin[10] and

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(2) Presented in part at the Delaware Valley Regional Meeting, American Chemical Society, February 16, 1956. Paper XXXV in this series, to *J. Org. Chem.*, in press.

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