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Reaction of the Steroidal Sapogenin Spiroketal System with Ethanedithiol[1]

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Ethanedithiol in the presence of boron trifluoride etherate reacts with the spiroketal system of steroidal sapogenins to form the corresponding furostane 26-ethylenethioketal. Desulfurization leads to the furostane which in the 5α series was transformed into cholestan-16β-ol. Structural formulas are proposed to explain these reactions.

The spiroketal ring system, characteristic of the steroidal sapogenins, was first recognized by Marker and Rohrmann[4] who observed its stability to most reagents which were not acidic. While the spiroketal grouping is opened[4,5] under the strongly acid conditions prevailing in the Clemmensen reduction, the ketone function of carbonyl-containing sapogenins can be removed readily[6] by the Wolff-Kishner reduction, including[7] Huang-Minlon's modification.[8] Subsequently, the formation of mercaptals and desulfurization with Raney nickel, introduced by Hauptmann in the steroid series,[9] was also employed with sapogenins and the required sapogenin mercaptals were prepared using zinc chloride,[10] hydrogen bromide[11] hydrogen chloride,[12] or perchloric acid[13] as the condensing agent without affecting the spiroketal moiety. In an attempt to utilize the boron trifluoride procedure[14] for the synthesis of certain steroidal sapogenin ethylenethioketals,[15] it was observed that reaction with the side chain had occurred since the characteristic infrared bands[16] associated with the spiroketal system had disappeared. In view of the importance of steroidal sapogenins in connection with the synthesis of steroid hormones[17] and the relative paucity of reactions which the spiroketal system undergoes,[18,19] we have undertaken a more detailed

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(4) R. E. Marker and E. Rohrmann, *J. Am. Chem. Soc.*, **61**, 846 (1939).

(5) See also R. E. Marker and E. Rohrmann, *J. Am. Chem. Soc.*, **62**, 896 (1940).

(6) See for instance R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith, and C. H. Ruof, *J. Am. Chem. Soc.*, **69**, 2167 (1947).

(7) See C. Djerassi, H. Martinez, and G. Rosenkranz, *J. Org. Chem.*, **16**, 1278 (1951).

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(14) L. F. Fieser, *J. Am. Chem. Soc.*, **76**, 1945 (1954).

(15) See C. Djerassi, T. T., Grossnickle, and L. B. High, *J. Am. Chem. Soc.*, **78**, 3166 (1956), footnote 34.

(16) R. N. Jones, E. Katzenellenbogen, and K. Dobriner, *J. Am. Chem. Soc.*, **75**, 158 (1953); C. R. Eddy, M. E. Wall, and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).

(17) For recent review see C. Djerassi, Proc. Fourth Internat. Congress Biochem., Vienna, September 1958, Symposium IV.

(18) See L. F. Fieser and M. Fieser, *Natural Products Related to Phenanthrene*, Reinhold Publishing Corp., New York, 1949, chapter VIII.

(19) For recent references see E. S. Wallis and R. S. Miner, *J. Org. Chem.*, **21**, 715 (1956); R. K. Callow and P. N. Massy-Beresford, *J. Chem. Soc.*, 4482 (1957).

examination of its behavior toward boron trifluoride etherate and certain mercaptans.

Tigogenin acetate (Ia) upon treatment with ethanedithiol or propane-1,3-dithiol in the presence of boron trifluoride etherate[14] furnished the sulfur-containing products IVa and IV, whose structure will be discussed below. Desulfurization of either substance with Raney nickel catalyst led to the sulfur-free product subsequently shown to be 5α-furostan-3β-ol acetate (VIIIa) which represented the key intermediate for all further work. The identical compound VIIIa could also be obtained from either diosgenin acetate (II) or hecogenin acetate (III) by the following routes, thus establishing the generality of this reaction and the fact that it involved only the spiroketal side chain. Condensation of diosgenin acetate (II) with ethanedithiol–boron trifluoride etherate yielded Va, which was desulfurized with Raney nickel catalyst to 5-furosten-3β-ol acetate (VII) and then hydrogenated in acetic acid solution with platinum oxide to 5α-furostan-3β-ol acetate (VIIIa). In the case of hecogenin acetate (III), it was interesting to determine whether the 12-keto function would complicate matters but the reaction with either ethanedithiol or propane-1,3-dithiol proceeded smoothly *via* the 12-mercaptals (VIa and VIb) and after desulfurization provided VIIIa. Sarsasapogenin acetate (5β, Ia) was converted to the compound eventually shown to be 5β-furostan-3β-ol acetate (5β, VIIIa) by an analogous series of reactions.

The analytical composition of the sulfur containing intermediates (IV, V, VI) as well as of the desulfurization products VII and VIIIa indicated the loss of one oxygen atom. The ether nature of the single oxygen atom derived from the spiroketal grouping was demonstrated as follows. The acetate VIIIa exhibited no hydroxyl absorption in the infrared and saponification gave the corresponding alcohol VIIIb which could be reacetylated to the starting acetate VIIIa. Oxidation of the alcohol VIIIb led to the ketone VIIIc which was reduced by the Huang-Minlon modification[8] of the Wolff-Kishner procedure to 5α-furostane (VIIId), which did not exhibit any hydroxyl or carbonyl absorption in the infrared and did not possess any active hydrogen atom (Zerewitinoff determination). The identical ether VIIId could also be obtained by treatment of deoxytigogenin (Ic)[20] with ethanedithiol–boron trifluoride etherate, followed by desulfurization or most directly by transforming tigogenone (Ib)[21] into the 3-ethylenethioketal IVc and then desulfurizing.

In order to determine the structure of the ether ring and to establish that no skeletal rearrangement had occurred, it was necessary to accomplish interconversion with a known steroid and ether cleavage experiments appeared to offer the most direct avenue. For this purpose the ether subsequently shown to be VIIId (5α-furostane) was selected and a variety of reagents were examined, of which the following proved unsatisfactory in initial experiments: boron trifluoride etherate–acetic acid,[22] zinc chloride–acetic anhydride,[23] hydrogen iodide,[24] or hydrogen bromide–acetic anhydride.[25] On the other hand, treatment of the ether VIIId with *p*-toluenesulfonic acid in acetic anhydride[26] led to an oily mixture of isomeric monounsaturated acetates ($C_{29}H_{48}O_2$). Careful chromatography yielded a small amount of a crystalline isomer which was shown to be Δ^{23}-cholesten-16β-ol acetate (IX) since ozonolysis furnished isovaleraldehyde and (after treatment with alkaline hydrogen peroxide and acidification) 16β-hydroxybisnorallocholanic 22 → 16 lactone (X).[27] Catalytic hydrogenation led to the acetate XIa, which was hydrolyzed by means of lithium aluminum hydride to cholestan-16β-ol (XIb)[28] and further oxidized to cholestan-16-one (XII). The identical sequence of reactions could be performed with the oily ether cleavage product which also gave some of the crystalline alcohol XIb and thence the ketone XII.

The conversion of the ethanedithiol–boron trifluoride etherate condensation products *via* the ether VIIId to cholestan-16β-ol (XIb) demonstrates that no skeletal rearrangement was involved in ether formation and furthermore, that one terminus of the ether ring must be at C-16. The isolation of a 16β-ol was of some mechanistic importance since if ether formation had proceeded by a displacement reaction at C-16, a 16α-hydroxy derivative would have resulted almost certainly.

There now remained the question regarding the other point of attachment of the ether ring. Mechanistically, initial attack by sulfur could be assumed to proceed at C-22(A) to give an intermediate such as B. Displacement by the second sulfur atom on C-22 of B and eventual ether ring closure would lead to a 16,26-oxide[29] (C) and such a formulation was originally favored by us. While the olefin IX is apparently only a minor constituent of the iso-

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(21) W. A. Jacobs and E. E. Fleck, *J. Biol. Chem.*, **88**, 545 (1930).

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(25) R. B. Turner, V. R. Mattox, L. L. Engel, B. McKenzie, and E. C. Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

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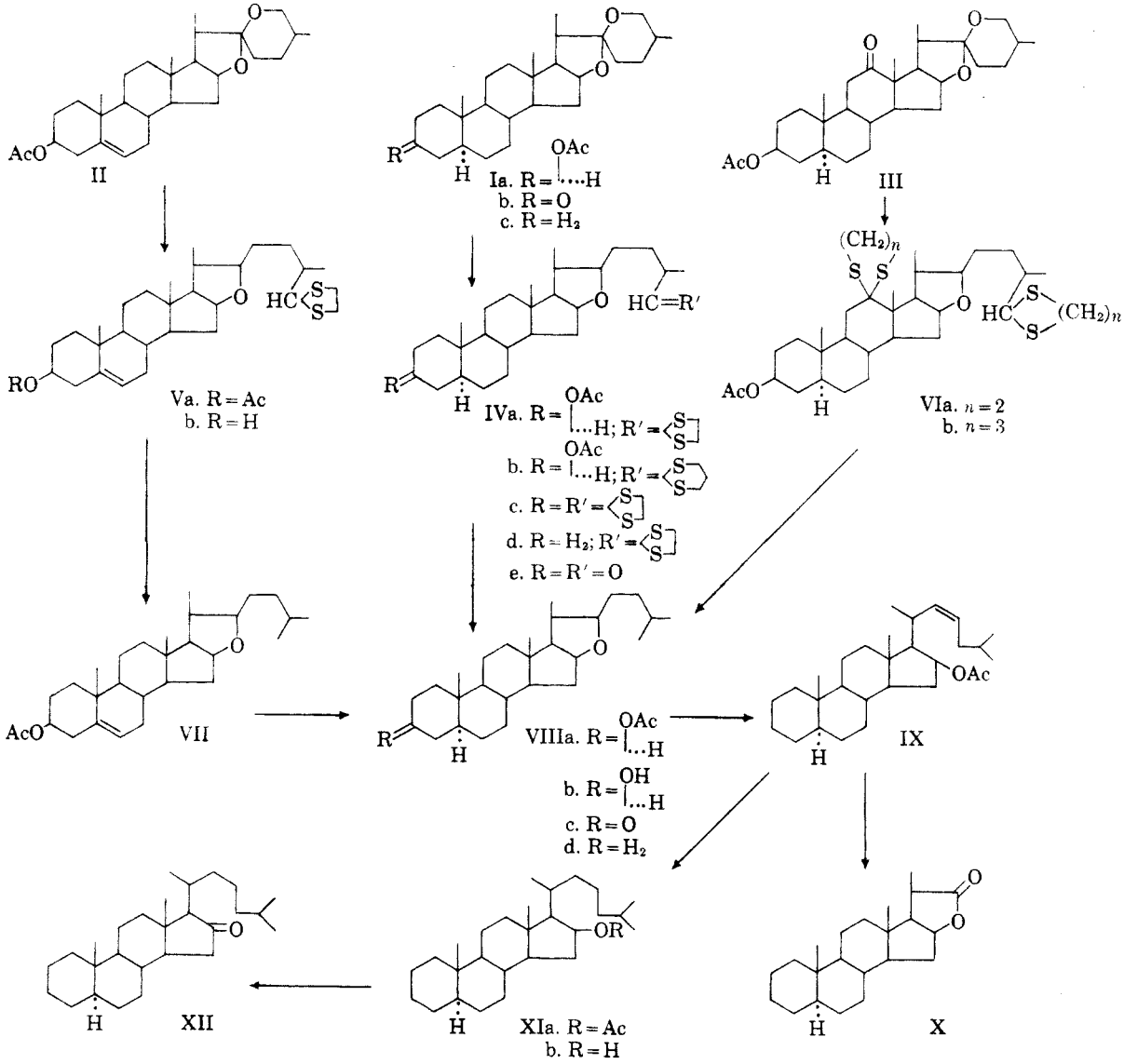
(27) R. Tschesche and A. Hagedorn, *Ber.*, **68**, 1412 (1935).

(28) I. Scheer and E. Mosettig, *J. Am. Chem. Soc.*, **77**, 1820 (1955).

(29) The oxide could also have been eight-membered by terminating at C-25 since the intimate steps (*e.g.*, initial formation of an intermediate Δ^{25}-olefin) involved in this ether ring closure are not known.



meric mixture of unsaturated acetates produced in the ether cleavage, its formation from C could be rationalized readily by assuming a transannular hydrogen transfer (D) which is known to occur frequently in medium-sized rings.

At this stage of the investigation, our attention was directed[30] to the fact that structures such as E had not been excluded as possible representations for the products of the boron trifluoride–catalyzed reaction of ethanedithiol and steroidal sapogenins. A substance of this type could be formed by attack of the second sulfur atom of B at C-26[31] in which case the desulfurization products (VII, VIII) would be furostane derivatives. Such 16,22-oxides were prepared[32a] originally in the steroidal 5β-series, but subsequently[32b] the corresponding 5α-isomer (VIIIb) has also been synthesized by an unambiguous route. Through the kind cooperation of Dr. E. Mosettig of the National Institutes of Health, samples of authentic 5α- and 5β-furostan-3β-ol were secured and were found to be identical with the corresponding specimens derived from the desulfurization of the ethanedithiol condensation products.

The identification of the desulfurization products (VIII) as 16,22-oxides demonstrates that the reaction of ethanedithiol (or propane-1,3-dithiol) with the steroidal spiroketal side chain in the presence of boron trifluoride etherate proceeds by formation of a new sulfur-containing ring (*e.g.*, IVa).

Very recently,[33] there has been suggested and

(30) We acknowledge with pleasure a profitable discussion on the subject of this paper with Prof. D. H. R. Barton, Imperial College of Science and Technology, London.

(31) Ring closure at C-25 rather than at C-26 (*e.g.*, E) was not excluded since the reaction might conceivably proceed by addition of the mercaptan to a Δ^{25}-olefin intermediate. Dimeric structures were eliminated by Rast molecular weight determinations.

(32) (a) I. Scheer, R. B. Kostic, and E. Mosettig, *J. Am. Chem. Soc.*, **77**, 641 (1955); (b) G. Sato, H. G. Latham, and I. Scheer, *J. Org. Chem.*, **21**, 689 (1956).

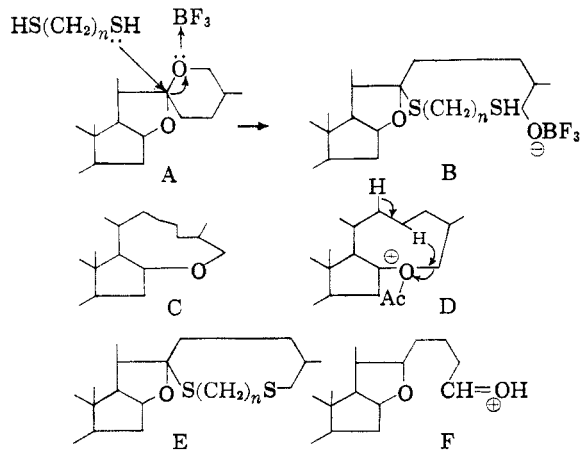
(33) R. B. Woodward, F. Sondheimer and Y. Mazur, *J. Am. Chem. Soc.*, in press. We are greatly indebted to Professor Woodward for calling this work to our attention and to Dr. F. Sondheimer for providing experimental details prior to publication.

TABLE I

Product	Yield, %[a]	M.P., °C.	$[\alpha]_D$	Empirical Formula	Analysis: Calcd. C	Calcd. H	Calcd. S	Found C	Found H	Found S
5α-furostan-3β-ol acetate 26-ethylenethioketal (IVa)[b]	70	123–124.5	−7°	$C_{31}H_{50}O_3S_2$	69.63	9.43	11.98	69.33	9.24	12.19
5β-furostan-3β-ol acetate 26-ethylenethioketal (5β, IVa)	69	121–123	+1.8°	$C_{31}H_{50}O_3S_2$	69.63	9.43	11.98	69.52	9.30	12.01
5α-furostan-3β-ol acetate 26-trimethylenethioketal (IVb)	38	130.5–132	−10°	$C_{32}H_{52}O_3S_2$	70.04	9.55	11.68	70.09	9.69	11.94
5α-furostane 3,26-bisethylene-thioketal (IVc)	60	159–160.5	+11°	$C_{31}H_{50}OS_4$	65.68	8.89	22.62	66.01	8.90	22.73
5α-furostane 26-ethylenethioketal (IVd)	54	96.5–98	+8°	$C_{29}H_{48}OS_2$	73.06	10.15	13.45	73.12	10.25	13.59
5-furosten-3β-ol acetate 26-ethylenethioketal (Va)	55	140–142	−31°	$C_{31}H_{48}O_3S_2$	69.89	9.08	12.04	69.51	9.19	12.81
5-furosten-3β-ol 26-ethylenethioketal (Vb)[c]	80	148–149	−22°	$C_{29}H_{46}O_2S_2$	70.98	9.45	13.07	70.62	9.84	12.60
5α-furostan-3β-ol acetate 12,26-bisethylenethioketal (VIa)	75	165–166.5	+39°	$C_{33}H_{52}O_3S_4$	63.46	8.33	20.05	63.97	8.57	19.94
5α-furostan-3β-ol acetate 12,26-bistrimethylenethioketal (VIb)	60	226–227.5	+24°	$C_{35}H_{56}O_3S_4$	64.64	8.68	19.72	63.93	8.27	19.29

[a] After recrystallization. [b] The substance exhibited λ_{max}^{EtOH} 239 mμ (log ε 3.11) and λ_{max}^{EtOH} 227 mμ (log ε 3.08). [c] Obtained from Va by saponification (18 hr., 20°) with 5% methanolic potassium hydroxide or by heating for 25 hr. under reflux with ethanol-water–concd. hydrochloric acid (6:1:1).

experimental evidence presented for a new mechanism for the acid-catalyzed isomerization of the steroidal sapogenin side chain which is postulated to proceed *via* F–the conjugate acid of the aldehyde of a dihydrosapogenin. Consequently, the possibility existed that our boron trifluoride-ethanedithiol reaction simply involved capture of this aldehyde intermediate in the form of its thioketal (*e. g.* IVa, V, VI). This was actually shown to be the case by oxidizing dihydrotigogenin by the reported procedure[33] to the 3-keto-26-aldehyde IVe and treating the crude product directly with ethanedithiol in the presence of boron triflouride or a trace of perchloric acid (known[13] not to affect the sapogenin side chain). In each case, there was obtained the identical 3,26-bis-thioketal IVc, which had already been isolated above in one step from tigogenone (Ib) and ethanedithiol-boron trifluoride. There remains no question, that the structures of our condensation products are represented by IVa, V and VI and that the formation of these substances constitutes additional support for the Woodward-Sondheimer-Mazur mechanism[33] of steroidal sapogenin side chain isomerization.



EXPERIMENTAL[34]

Reaction of steroidal sapogenins with ethanedithiol and propane-1,3-dithiol in the presence of boron trifluoride. Two typical procedures will be given below while the pertinent physical constants and analytical results are summarized in Table I.

A mixture of 4.2 g. of tigogenin acetate (Ia), 8.65 g. of ethanedithiol, and 7.6 cc. of boron trifluoride etherate was left at room temperature for 2 hr.,[35] diluted with benzene, washed with *N* sodium hydroxide and water, dried, and evaporated *in vacuo*. Crystallization from acetone furnished 3.5 g. of the adduct IVa (see Table I).

The dark colored solution which formed upon mixing 1.0 g. of hecogenin acetate (III) with 2.7 g. of propane-1,3-dithiol[36] and 2.5 cc. of boron trifluoride etherate was diluted with much benzene after 1 hr. Extraction with alkali and water, followed by drying and evaporation *in vacuo* left an oily residue which was chromatographed in 1:1 hexane-benzene solution on 30 g. of alumina. Elution with benzene provided 1.18 g. of crystalline product (VIb), m.p. 225–227° (see Table I).

Substitution of β-mercaptoethanol for ethanedithiol yielded only unchanged sapogenin and this also applied to use of β-mercaptoethanol in conjunction with aluminum chloride or zinc chloride.

(34) Melting points were determined on the Kofler block. Unless noted otherwise, rotations were measured in chloroform solution. We are indebted to Mrs. Dolores Phillips for the infrared measurements and to Dr. A. Bernhardt, Mülheim, Germany, for the microanalyses.

(35) All of the tigogenin acetate dissolved only after 30 min.

(36) J. R. Meadow and E. E. Reid, *J. Am. Chem. Soc.*, **56**, 2177 (1934); S. D. Simpson, *Can. J. Research*, **25B**, 20 (1947).

5α-Furostane 3,26-bisethylenethioketal (IVc) via dihydrotigogenin. A solution of dihydrotigogenin[37] (1.59 g.) in 100 ml. of benzene containing 10 ml. of glacial acetic acid was cooled in ice and treated over a 15 min. period with a solution composed of sodium dichromate (10 g.), concentrated sulfuric acid (2.5 ml.) and 60% acetic acid (140 ml.). Stirring was continued with cooling for an additional 1.75 hr. Excess oxidizing agent was then reduced with aqueous ferrous sulfate. After separating the benzene solution, the aqueous mixture was extracted with ether and the combined solvents were washed repeatedly with water. The straw colored oily residue, obtained by removing the dry (sodium sulfate) solvents *in vacuo*, weighed 1.5 g.

The crude 3-keto-26-aldehyde (0.70 g.) was treated with 3 ml. of ethanedithiol and one drop of perchloric acid (70–72%). After 2 hr., the mixture was diluted with ether and washed successively with 2*N* sodium hydroxide and water. Removal of solvent *in vacuo* afforded a straw colored oil. Chromatography on Merck activated alumina and elution with petroleum ether-benzene (1:4) gave 105 mg. of crystalline 5α-furostane 3,26-bisethylenethioketal melting at 158–160°. Recrystallization from ethyl acetate afforded 50 mg. of colorless needles, m.p. 159–160.5°. The product (IVc) was identical (mixture melting point and infrared comparison) with a specimen prepared by reacting tigogenone with ethanedithiol in the presence of boron trifluoride etherate (Table I).

5α-Furostan-3β-ol acetate (VIIIa). (*a*) *By desulfurization of 5α-furostan-3β-ol acetate 12,26-bisethylenethioketal* (VIa). A solution of 1.0 g. of the thioketal VIa in 100 cc. of ethanol was heated under reflux for 4 hr. with 14 g. of W-4 Raney nickel catalyst.[38] Filtration of the catalyst, concentration of the filtrate and chilling afforded 0.53 g. (74%) of colorless needles, m.p. 82–84.5°. Further recrystallization from ethanol led to the analytical sample, m.p. 83.5–84.5°, $[\alpha]_D$ −14°, $\lambda_{max}^{CHCl_3}$ 5.75 and 7.90 μ.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.32; H, 10.82.

The corresponding 12-trimethylenethioketal VIb was desulfurized under the same conditions in 67% yield to 5α-furostan-3β-ol acetate (VIIIa).

In one experiment, where W-2 Raney nickel[38] was employed and the ratio between VIa and catalyst was only 1:5, there was isolated in about 60% yield a monothioketal which proved to be different from 5α-furostan-3β-ol acetate 26-ethylenethioketal (IVa) and which is, therefore, assigned the structure 5α-furostan-3β-ol acetate 12-ethylenethioketal. The substance crystallized from aqueous acetone as colorless plates, m.p. 155–157°, $[\alpha]_D$ +30°, $\lambda_{max}^{CHCl_3}$ 5.78 and 7.95 μ.

Anal. Calcd. for $C_{31}H_{50}O_3S_2$: C, 69.63; H, 9.43; S, 11.98. Found: C, 69.91; H, 9.53; S, 11.62.

(*b*) *By desulfurization of 5α-furostan-3β-ol acetate 26-trimethylenedithioketal* (IVb). A solution of 0.2 g. of the acetate IVb in 30 cc. of ethanol was heated under reflux for 2 hr. with 2 g. of W-4 Raney nickel catalyst and processed in the usual manner to provide 67% of 5α-furostan-3β-ol acetate (VIIIa), m.p. 82–83.5°.

(*c*) *By desulfurization of 5α-furostan-3β-ol acetate 26-ethylenethioketal* (IVa). Desulfurization was accomplished by refluxing the acetate (1.5 g.) for 18 hr. in 150 ml. of ethanol with 12 g. of W-4 Raney nickel. Isolation and crystallization from ethanol afforded 0.78 g. (75%) of colorless crystalline product, m.p. 80–83°.

The analogous *5β-furostan-3β-ol acetate* (5β, VIIIa) was prepared by an identical procedure employing 5β-furostan-3β-ol acetate 26-ethylenethioketal (5β, IVa) (0.8 g.), ethanol (75 ml.), and W-4 Raney nickel (6 g.). The product crystallized as colorless needles from ethanol; weight 0.5 g., m.p. 80–82°. Recrystallization from ethanol gave a pure sample, m.p. 82–83°, $[\alpha]_D$ −4.5°.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 77.93; H, 11.76.

(*d*) *By hydrogenation of 5-furosten-3β-ol acetate* (VII). The desulfurization of 5-furosten-3β-ol acetate 26-ethylenethioketal (Va) proceeded in 60% yield when conducted in boiling ethanol (2.5 hr.) with eight times the weight of W-2 Raney nickel.[38,39] Recrystallization from ethanol gave 5-furosten-3β-ol acetate (VII), m.p. 130–132°.

Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.74; H, 10.38.

The above unsaturated ether VII (1.0 g.) was hydrogenated over a 2-hr. period in 100 cc. of glacial acetic acid with 0.2 g. of platinum oxide catalyst using 3 atm. of hydrogen. Filtration of the catalyst, evaporation of the solvent, and recrystallization from ethanol yielded 0.8 g. of 5α-furostan-3β-ol acetate (VIIIa), m.p. 78–81°.

In procedures *b–d* compound VIIIa was identified by mixture melting point and infrared comparison with the specimen produced *via* route *a*.

5α-Furostan-3β-ol (VIIIb). Saponification of the acetate VIIIa was accomplished by heating a solution of 1.0 g. of VIIIa in 60 cc. of 5% methanolic potassium hydroxide under reflux for 1 hr., concentrating the solution to one-half its volume and cooling. Filtration provided 0.8 g. of the alcohol VIIIa (m.p. 147–149°), which was recrystallized from hexane whereupon the melting point was raised to 148.5–150°, $[\alpha]_D$ −10°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.45; H, 11.34.

Conversion of 5β-furostan-3β-ol acetate (5β, VIIIa) (0.3 g.) to *5β-furostan-3β-ol* (5β, VIIIb) (0.2 g.) was carried out in similar fashion. An analytical sample recrystallized from methanol exhibited a melting point of 136–138°, $[\alpha]_D$ −4.1°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.36; H, 11.41.

The product was identical (mixture melting point and infrared comparison) with an authentic sample of 5β-furostan-3β-ol (5β, VIIIb).[32a]

A mixture melting point determination with VIIIb and an authentic sample of 5α-furostan-3β-ol (VIIIb)[32b] was undepressed.

Acetylation of a sample of the alcohol VIIIb with boiling acetic anhydride–pyridine led to the original acetate VIIIa, m.p. 82–84.5°.

5α-Furostan-3-one (VIIIc). The oxidation of 0.5 g. of the alcohol VIIIb was carried out in 10 cc. of glacial acetic acid with 50 mg. of chromium trioxide dissolved in 5 cc. of 80% acetic acid. Dilution with water, extraction with ether, and purification by filtration through alumina and recrystallization from aqueous ethanol led to colorless plates of the ketone VIIIc, m.p. 134–135°, $[\alpha]_D$ +15°, $\lambda_{max}^{CHCl_3}$ 5.84 μ.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.24; H, 11.64.

5α-Furostane (VIIId). (*a*) *By desulfurization of 5α-furostane 26-ethylenthioketal* (IVd). The desulfurization of 0.3 g. of IVd (see Table I) in 100 cc. of ethanol was performed in the usual manner (2 hr.) with 3 g. of W-4 Raney nickel and led in 87% yield to 5α-furostane, which crystallized from ethanol as colorless leaflets, m.p. 95–96°, $[\alpha]_D$ −9°, no infrared hydroxyl or carbonyl absorption.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.91; H, 11.91; active hydrogen, 0.00.

(*b*) *By desulfurization of 5α-furostane 3,26-bisethylenethioketal* (IVc). The desulfurization of the thioketal IVc (see Table I) was conducted in the usual manner except that an extended reflux time (30 hr.) was necessary for optimum yields (69–76%).

(*c*) *By Wolff-Kishner reduction of 5α-furostan-3-one*

(37) R. E. Marker and E. Rohrmann, *J. Am. Chem. Soc.*, **61**, 1516 (1939).

(38) A. A. Pavlic and H. Adkins, *J. Am. Chem. Soc.*, **68**, 1471 (1946).

(39) With W-4 Raney nickel, some reduction of the 5,6-double bond was also observed.

(VIIIc). A mixture of 70 mg. of the ketone VIIIc, 70 mg. of potassium hydroxide, 1 cc. of triethylene glycol, and 0.1 g. of 85% hydrazine hydrate was heated for 3 hr. at 120–130°, the condenser was removed, the temperature raised to 195° whereupon refluxing was continued for 3.5 hr. Dilution with water, extraction with ether, and purification of the ether extract by chromatography on acid-washed alumina (hexane elution) followed by recrystallization from ethanol afforded 40 mg. of the ether VIIId, m.p. 92.5–94°, undepressed upon admixture with the specimens prepared according to *a* or *b*. The infrared spectra of all three samples were identical.

Ether cleavage of 5α-furostane (VIIId). A solution of 950 mg. of the ether VIIId and 500 mg. of *p*-toluenesulfonic acid monohydrate in 50 cc. of acetic anhydride was heated under reflux for 30 min. and then poured unto ice. After standing for 5 hr., the product was extracted with ether, washed until neutral, dried, and evaporated to yield 1.05 g. of a pale orange colored gum. Chromatography on 100 g. of Merck acid-washed alumina and elution with hexane gave 570 mg. of unreacted ether VIIId, followed by 490 mg. of a heavy, colorless oil which was eluted partly by hexane and partly by hexane-benzene mixtures (9:1). For analysis, a sample of the oil was rechromatographed and distilled at 180° and 0.005 mm.; yellow color with tetranitromethane, $\lambda_{max}^{CHCl_3}$ 5.78 and 7.95 μ.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29; O, 7.46. Found: C, 80.74; H, 11.05; O, 7.99.

Further elution with hexane-benzene (4:1) provided 75 mg. of crystals, which were recrystallized from ethanol to give an analytical sample of Δ^{23}-cholesten-16β-ol acetate (IX), m.p. 87–90°, $[\alpha]_D$ +18°, $\lambda_{max}^{CHCl_3}$ 5.77 and 7.90 μ.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.08; H, 10.94.

When the ether cleavage was conducted with anhydrous *p*-toluenesulfonic acid (first heated at 100° and 40 mm. and subsequently distilled at a bath temperature of 200° and 0.005 mm.) and the time prolonged to 45 min., the results were substantially the same except that the amount of unreacted starting material was reduced and nearly 70% of oily, unsaturated acetate could be isolated.

Ozonolysis of Δ^{23}-cholesten-16β-ol acetate (IX). Ozone was passed for 15 min. at −80° through a solution of 119 mg. of the olefin IX in 2 cc. of methylene chloride and the solvent was then removed cautiously *in vacuo*. Water (20 cc.) was added and the mixture was steam distilled into a suspension of 2,4-dinitrophenylhydrazine in aqueous sulfuric acid. The distillate was extracted with benzene and the benzene solution passed through a short column of alumina. The combined benzene eluates were evaporated to give 35 mg. of crude, yellow 2,4-dinitrophenylhydrazone which was rechromatographed on alumina, eluted with hexane-benzene (2:1) and recrystallized from aqueous acetone, m.p. 122–124°. The melting point was depressed by over 30° upon admixture with acetone 2,4-dinitrophenylhydrazone but was undepressed when mixed with authentic isovaleralde hyde 2,4-dinitrophenylhydrazone (lit.,[40] m.p. 123°). The infrared spectra of the two samples were identical.

The residue from the stream distillation was heated for 30 min. with 10 cc. of 10% sodium hydroxide and 3 cc. of 30% hydrogen peroxide. After an additional hour at room temperature, the mixture was acidified, extracted with chloroform, and the chloroform extracts washed with sodium bicarbonate solution and water, dried, and evaporated. Chromatography on 3 g. of Merck acid-washed alumina, elution with hexane-benzene (1:1) and recrystallization from aqueous ethanol provided 13 mg. of 16β-hydroxybisnorallocholanic 22 → 16 lactone (X),[27] m.p. 198–199°, with sublimation and crystal change from needles to very fine hair-like crystals at 160°, $\lambda_{max}^{CHCl_3}$ 5.66 u. Identity with an authentic specimen[41] was established by mixture melting point determination and infrared comparison.

Hydrogenation of Δ^{23}-cholesten-16β-ol acetate (IX). Reduction of 54 mg. of the olefin IX in 5 cc. of acetic acid at 29.5° and 1 atm. of hydrogen with 8 mg. of platinum oxide resulted in the uptake of 3.19 cc. (1.01 equivalents) of hydrogen within 4 min. After 10 min., the catalyst was filtered, the solvent was evaporated, and the residue crystallized twice from ethanol to provide 30 mg. of cholestan-16β-ol acetate (XIa), m.p. 116–117°, $[\alpha]_D$ +63°. The analytical sample was lost in transit but characterization was completed by conversion to XIb and XII.

The above acetate was converted in 90% yield to *cholestan-16β-ol* (XIb) by heating for 45 min. with an ethereal solution of lithium aluminum hydride. Recrystallization from aqueous methanol gave the analytical sample, m.p. 110–113°, then resolidifying and melting at 120–120.5°, $[\alpha]_D$ +30°. The mixture melting point with an authentic sample[28,42] was undepressed and the infrared spectra were identical.

For further identification, 9.7 mg. of cholestan-16β-ol (XIb) was oxidized in 0.5 cc of acetone with a few drops of 8*N* chromic acid–sulfuric acid–water solution[43] and after 2 min. the resulting cholestan-16-one (XII) was isolated by ether extraction and recrystallized from aqueous methanol, m.p. 94–95°, $\lambda_{max}^{CHCl_3}$ 5.75 μ, strong negative single Cotton effect typical[44] of 16-keto steroids (*c*, 0.09 in methanol, trough at $[\alpha]_{320}$ −2900°, peak at $[\alpha]_{277.5}$ +3100°). The infrared spectrum was identical with that of an authentic sample prepared by oxidation of cholestan-16β-ol.[28,42]

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.91; H, 12.06.

When the hydrogenation was performed with the oily unsaturated acetate obtained in the ether cleavage and the crude product saponified (using lithium aluminum hydride) followed by chromatography, there was isolated cholestan-16β-ol (XIb) as well as two isomers, m.p. 114–115° (depressed to 85–110° upon admixture with cholestan-16β-ol) and m.p. 143–145°. Unfortunately insufficient material was available for further work but one of these substances may represent the expected "reverse" ether cleavage product with the oxygen atom in the side chain.

Anal. Calcd. for $C_{27}H_{48}O$: C, 83.43; H, 12.45; O, 4.12. Found: sample, m.p. 114–115°: C, 82.95; H, 12.15; O, 4.59; sample, m.p. 143–145°: C, 83.22; H, 12.31; O, 4.18.

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(40) S. M. McElvain *The Characterizati of Organic Compounds*, MacMillan Co., New York, 1953, p. 207.

(41) We are greatly indebted to Dr. Hans Hirschman, Western Reserve School of Medicine for this sample.

(42) We are grateful to Dr. Irving Scheer, National Institutes of Health, for this specimen.

(43) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, **39** (1946).

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