A FACILE ETHERIFICATION PROCEDURE FOR 3β-HYDROXY-Δ^5-STEROIDS

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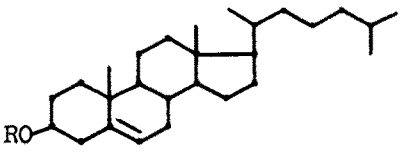
A simplified procedure for the etherification of 3β-hydroxy-Δ^5-steroids has been elaborated and a mechanism for the reaction has been proposed. The reaction conditions employed (trialkyl-orthoformates-perchloric acid) have been shown to also formylate secondary hydroxyl functions, promote elimination of tertiary hydroxy groups and with prolonged reaction time to esterify carboxylic acids and initiate ester-ether exchange. A study of cholestan-3β-ol under these reaction conditions has been made.

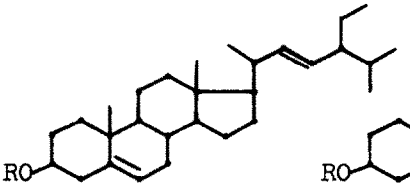
In the course of a detailed study[1] of the reactions of steroids with <u>in situ</u> generated dialkoxycarbonium ions, a unique process has been observed for the etherification of 3β-hydroxy-Δ^5-steroids. Since the known methods for the preparation of 3β-alkoxy-Δ^5-steroids are somewhat cumbersome,[2,3,4,5,6] an investigation of the application and scope of this simplified etherification procedure was undertaken. The advent of a new method[7] for cleavage of such ethers in high yield may reemphasize their potential utility as a protective group for the 3β-hydroxy-Δ^5-steroid system.

When a solution of cholest-5-en-3β-ol (I) in trimethylorthoformate was treated with 72% perchloric acid (2.1 equivalents),

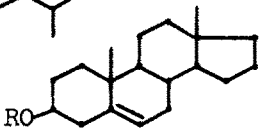
a highly crystalline compound immediately precipitated from the reaction mixture and was identified as 3β-methoxycholest-5-ene (II).[5] Analysis of the reaction mixture by thin layer chromatography indicated the absence of I or any significant amount of by-products. When the reaction was investigated with a variety of other trialkylorthoformates, the appropriate ethers were isolated. Table I summarizes the various ethers prepared from cholest-5-en-3β-ol (I), stigmasta-5,22-dien-3β-ol (III) and androst-5-en-3β-ol (IV). This procedure offers the advantages of brevity of reaction times (3-15 min.), simplified product isolation and reasonable yields (75-85%).



I, R = H
II, R = CH_3-
V, R = C_2H_5-
VI, R = CH_2=$CHCH_2$-
VII, R = $(CH_3)_2CHCH_2$-
VIII, R = $CH_3(CH_2)_{17}$-



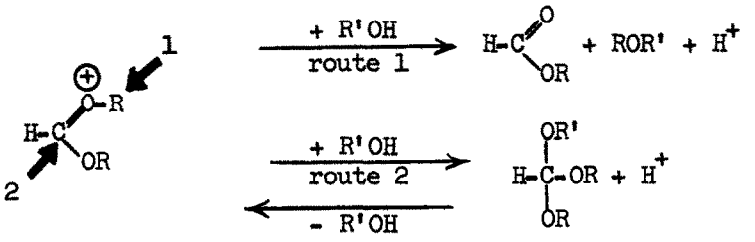
III, R = H
IX, R = CH_3-
X, R = $CH_3(CH_2)_3$-



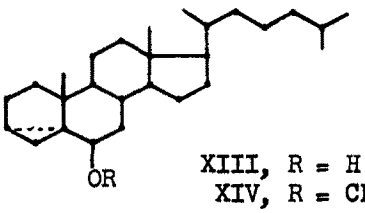
IV, R = H
XI, R = CH_3CH_2-
XII, R = $CH_3(CH_2)_4$-

The mechanism for the formation of 3β-ethers warrants discussion since the ambident nature of dialkoxycarbonium cations offers mechanistic alternatives. The general subject of the attack of reagents on ambident cations has been reviewed recently by Hünig.[8] From the examples discussed the reaction of alcohols with dialkoxycarbonium cations may proceed by either of two routes illustrated as follows; route 1, attack of alcohol on the alkyl

group giving rise to an ether; and, route 2, attack of alcohol at the carbon having the alkoxyl groups.



It can be seen that route 1 is a irreversible process while route 2 is reversible. On first consideration, the attack of 3β-hydroxy-Δ^5-steroids on dialkoxycarbonium cations might be thought to proceed through route 1, direct etherification. However, an added consideration implicit in route 2 is the generation of the steroidal homoallylic cation by acid attack on the mixed orthoester. Subsequent addition of alcohol at C3 would give rise to the 3β-alkoxy-Δ^5-ethers. It is well known[3,9] that alcohols attack these steroidal homoallylic cations at C6 to give 6β-alkoxy-3α,5α-cyclo-5α-steroids; however, these products are encountered in strictly buffered solution, reactions in strong acid solution yield 3β-alkoxy-Δ^5-steroids.[3]

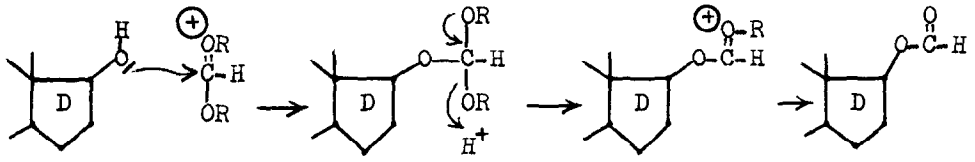


XIII, R = H
XIV, R = CH_3

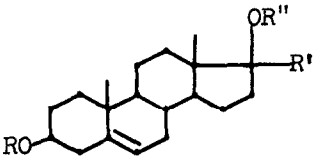
The reaction of either 3α,5α-cyclo-5α-cholestan-6β-ol (XIII) or its 6β-methyl ether (XIV) with trimethylorthoformate-perchloric acid rapidly gave 3β-methoxycholest-5-ene (II) indicating the

greater stability of the 3β-methoxy-Δ^5-ethers under these strong acidic conditions. It has been emphasized in the preparation of II that no by-products were observed. Since no 3β-formate, 3,5-diene or 3α-methoxy derivative were isolated, the generation of the homoallylic cation from the mixed orthoester must proceed very rapidly. It is this mechanism for the formation of the 3β-alkoxy-Δ^5-ethers which we prefer.

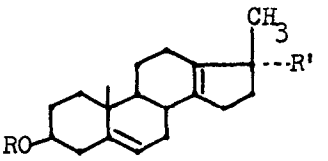
The fate of the 17β-hydroxyl function in several 3β-hydroxy-Δ^5-steroids undergoing the etherification reaction was next studied. With androst-5-ene-3β,17β-diol (XV), a mixture of two products was isolated, the previously described[10] 3β-methoxyandrost-5-en-17β-ol (XVI) and its 17β-formate (XVII). The formylation of a secondary C17-hydroxyl group undoubtedly proceeds through the mixed orthoformate in a manner illustrated as follows:



Change of the C17-hydroxyl group to tertiary character resulted in its elimination and concomitant methyl migration, such that 17α-methylandrost-5-ene-3β,17β-diol (XVIII) was converted into 3β-methoxy-17,17-dimethyl-18-norandrosta-5,13-diene (XIX). A similar migration was observed with 17α-ethynylandrost-5-ene-3β,17β-diol (XX) leading to the isolation of 17α-ethynyl-3β-methoxy-17β-methyl-18-norandrosta-5,13-diene (XXI) and 3β-ethoxy-17α-ethynyl-17β-methyl-18-norandrosta-5,13-diene (XXII), the ether isolated being dependent on the specific alkylorthoformate employed in the reaction.



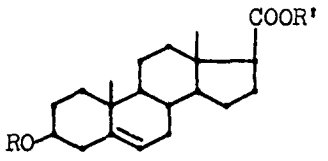
XV, R = R' = R'' = H
XVI, R = CH_3; R' = R'' = H
XVII, R = CH_3; R' = H; R'' = $\overset{O}{\overset{\|}{C}}H$
XVIII, R = H; R' = CH_3; R'' = H
XX, R = H; R' = C≡CH; R'' = H



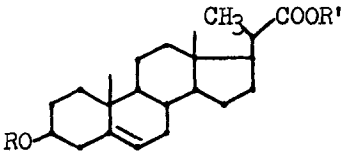
XIX, R = R' = CH_3
XXI, R = CH_3; R' = C≡CH
XXII, R = C_2H_5; R' = C≡CH

The migration of the 13β-methyl group under these acidic conditions, with and without subsequent aromatization of ring D has been studied by numerous investigators, and was in confusion until the recent papers of Dvolaitzky et al.[11] The lack of aromatic ultraviolet absorption maxima and the observed chemical shift for the migrated methyl group (NMR) definitely established the structure of ethers XIX, XXI and XXII. The acetylenic proton in XXI and XXII could also be easily discerned in the NMR spectrum.

In further application methyl 3β-hydroxyeti-5-enate (XXIII) was subjected to the orthoformate etherifying conditions and its 3β-methyl ether (XXIV)[12] was easily isolated from the reaction mixture. The identical derivative could be isolated under extended reaction conditions (6-7 hr.) from 3β-acetoxyeti-5-enic acid (XXV),

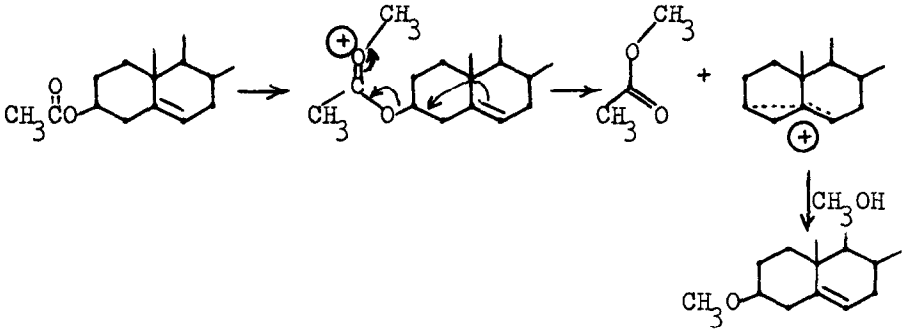


XXIII, R = H; R' = CH_3
XXIV, R = CH_3; R' = CH_3
XXV, R = $CH_3\overset{O}{\overset{\|}{C}}$; R' = H



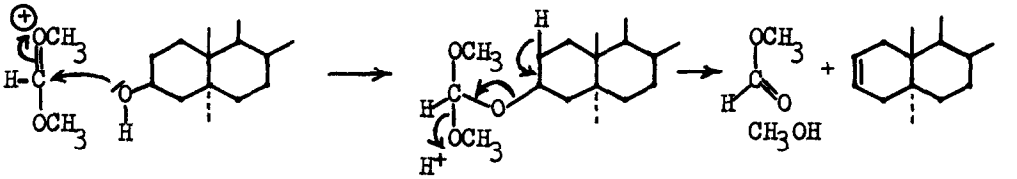
XXVI, R = $CH_3\overset{O}{\overset{\|}{C}}$; R' = H
XXVII, R = CH_3; R' = CH_3

thus demonstrating that the reactions conditions both esterified the carboxylic acid function and effectively replaced an acetoxyl with a methyl ether grouping. In another example in the bile acid series, the reaction of 3β-acetoxybisnorchol-5-enic acid (XXVI) with trimethylorthoformate-perchloric acid gave the known methyl 3β-methoxybisnorchol-5-enate (XXVII).[13] Since the acetate-ether interchange in the bile acid series proceeds with retention of configuration at C3, the most appealing mechanism for the process must invoke the homoallylic cation. This can be generated by alkylation of the acetate function[14] with subsequent removal of the elements of methyl acetate. The cation could react with the alcohol present to give the observed ether.

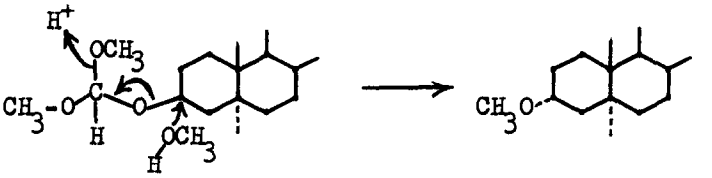


Since the presence of the 5,6-double bond in the compounds studied dictated the exclusive formation of the 3β-alkoxy-Δ^5-ethers, cholestan-3β-ol (XXVIII) was studied under these reaction conditions. When the reaction was carried out and the complex reaction mixture submitted to preparative thin layer chromatography, five components were isolated and identified in the designated proportions; cholest-2-ene (XXIX, 38%),[15] 3α-methoxycholestane (XXX, 9%),[16] 3β-formyloxycholestane (XXXI, 37%),[17] 3β-methoxycholestane (XXXII, 10%),[18] and cholestan-3β-ol (XXXIII, 6%). It appears from our observations

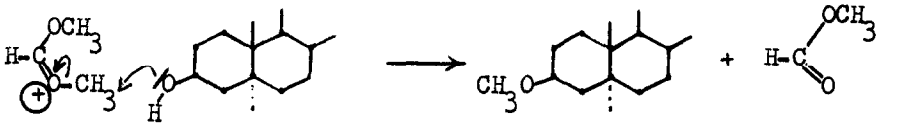
that the formation of cholestan-3β-ol arose from the partial hydrolysis of the 3β-formate on the preparative plates. The presence of cholest-2-ene could be explained by the formation of the mixed orthoformate and its subsequent reaction with acid.



The 3α-methoxy derivative undoubtedly arose from a displacement reaction as illustrated below, while the formate also arose from



the mixed orthoformate as discussed in the 17β-hydroxyl formylation described previously. Finally, the presence of the 3β-methyl ether indicated the alternate attack of the steroid hydroxyl group on dimethoxycarbonium ion (originally cited as route 1).



In addition to limitations on the etherification reaction discussed thus far, it must be emphasized that compounds containing carbonyl groupings are sensitive to dialkoxycarbonium ion attack.[1] Thus, etherification of a 3β-hydroxy-Δ^5-steroid may be accompanied by more complex reactions involving carbonyl and adjacent methylene groups if both structural features are present in the compound under study. Results of these investigations will be reported shortly.

Table 1

Ethers of 3β-Hydroxy-Δ^5-Steroids

Compound	Physical Constants				Literature Reference of Analytical Data
	Found		Literature		
	m.p.°	α_D	m.p.°	α_D	
3β-Methoxycholest-5-ene (II)	83-84	-43°	84	-46°	5
3β-Ethoxycholest-5-ene (V)	88	-40°	88.5	-39°	4,5
3β-Allyloxycholest-5-ene (VI)	76-77	-39°	78	-33°	4
3β-Isobutoxycholest-5-ene (VII)	111-112	-32°	113	-29°	4
3β-Octadecyloxycholest-5-ene (VIII)	59-61	-18°	-	-	Calcd. for $C_{45}H_{82}O$(639.11): C, 84.56; H, 12.93. Found: C, 84.15; H, 13.38.
3β-Methoxystigmasta-5,22-diene (IX)	119-120	-49°	122	-	3
3β-n-Butoxystigmasta-5,22-diene (X)	105-106	-44°	-	-	Calcd. for $C_{33}H_{56}O$(468.78) C, 84.54; H, 12.04. Found: C, 84.83; H, 12.21.
3β-Ethoxyandrost-5-ene (XI)	72-73	-71°	-	-	Calcd. for $C_{21}H_{34}O$(302.48): C, 83.38; H, 11.33. Found: C, 83.06; H, 11.49.
3β-n-Pentyloxyandrost-5-ene (XII)	57-58	-64°	-	-	Calcd. for $C_{24}H_{40}O \cdot \frac{1}{2}H_2O$(353.57): C, 81.53; H, 11.70. Found: C, 81.47; H, 11.86.

Experimental[19]

General Preparation for Compounds Listed in Table 1. - A solution (or suspension) of the steroid (1.0 g.) in trialkylorthoformate (10 ml.) was stirred at room temperature and 72% perchloric acid (2.1 equivalents) was added. The reaction was terminated after 3-15 minutes by pouring the reaction mixture into a saturated aqueous sodium bicarbonate solution. The precipitated material was collected, dried and recrystallized from an appropriate solvent (usually methanol). When two liquid phases were encountered after terminating the reaction, methylene chloride was added and the organic layer separated, dried (anhydrous sodium sulfate) and evaporated yielding a solid which was recrystallized as above. The preparation of the stearyl ether required the reaction mixture to be kept at 60°. The purity of the ethers was established by TLC using the system cyclohexane-ethyl acetate (95:5). The elimination of colored impurities could be accomplished by passage of a methylene chloride solution of the ether through Magnesol.

3β-Methoxycholest-5-ene (II)

a) From 3α,5α-Cyclo-5α-cholestan-6β-ol (XIII). - A solution of 3α,5α-cyclo-5α-cholestan-6β-ol (0.5 g.) in trimethyl orthoformate (5 ml.) was stirred at room temperature. The addition of 72% perchloric acid (0.3 ml.) caused an immediate precipitation. After stirring the suspension for 3 min., the reaction mixture was poured into a saturated aqueous sodium bicarbonate solution and filtered, 0.50 g., m.p. 68-70°. The infrared spectrum of this compound was isentical to that an authentic sample of II and TLC indicated but one spot identical to the standard.

b) From 6β-Methoxy-5α,6α-cyclo-5α-cholestane (XIV). - A suspension of 6β-methoxy-3α,5α-cyclo-5α-cholestane (XIV, 1.0 g.) in trimethylorthoformate (10 ml.) was stirred at room temperature. When 72% perchloric acid (0.6 ml.) was added, the crystalline nature of the suspension appeared to change and stirring was continued for 5 min. After the addition of a saturated sodium bicarbonate solution, the reaction mixture was poured into water and filtered to give 3β-methoxycholest-5-ene (II, 0.88 g.). Recrystallization from methanol afforded (0.64 g.), m.p. 86-87°.

3β-Methoxyandrost-5-en-17β-ol (XVI) and 17β-Formyloxy-3β-methoxyandrost-5-ene (XVII). - 72% Perchloric acid (0.5 ml.) was added to a stirred solution of androst-5-ene-3β,17β-diol (XV, 1.0 g.) in trimethylorthoformate (20 ml.) and after 10 min., pyridine (1.0 ml.) was added. After the addition of water, the precipitated solid was filtered to yield 0.93 g. of a mixture of two components as demonstrated by TLC. The mixture was dissolved in a minimum amount of methylene chloride and administered to two 200 x 200 x 1 mm. plates and developed by a solvent system of ethyl acetate-cyclohexane 30:70. The least polar component (0.53 g.), 17β-formyloxy-3β-methoxyandrost-5-ene (XVII), was not stable to the conditions of the plate and itself was partially hydrolyzed to XVI. Therefore the crude formate isolated from the plate was chromatographed on Florisil and the material eluted with 2% acetone-hexane was crystallized from methanol to give the formate XVII (0.32 g.), m.p. 118-120.5°; $[\alpha]_D$ -83°; NMR: 50 (S)[3]; 62 (S)[3]; 202 (S)[3]: 278 (M)[1]; 323 (M)[1]; 485 (D) J~2)[1] cps.

Anal. Calcd. for $C_{21}H_{32}O_3$ (332.47): C, 75.86; H, 9.70. Found: C, 76.13; H, 10.07.

The more polar zone was removed from the plate and the compound recovered by trituration with acetone. Evaporation of the solvent and crystallization from methanol-water gave the 17β-hydroxy compound XVI (0.15 g.), m.p. 143-144°; $[\alpha]_D$ -62°; NMR: 46 (S)[3]; 62 (S)[3]; 202 (S)[3]; 217 (M)[1]; 320 (M)[1] cps. (literature[10] m.p. 142.5-143°; $[\alpha]_D$ -51°).

3β-Methoxy-17,17-dimethyl-18-norandrosta-5,13-diene (XIX). - 17α-Methylandrost-5-ene-3β,17β-diol (XVIII, 1.0 g.) in trimethylorthoformate (10 ml.) was stirred vigorous and 72% perchloric acid (0.5 ml.) was added. After 10 min. the reaction was terminated by pouring into a saturated aqueous sodium bicarbonate solution. Filtration afforded XIX (0.93 g.) which as witnessed by TLC was one component. Crystallization from methanol-water and then methanol gave 3β-methoxy-17,17-dimethyl-18-norandrosta-5,13-diene (0.54 g.), m.p. 93-94°; (a sublimed sample exhibited the same melting point), $[\alpha]_D$ -203°; NMR: 58 (S)[9]; 203 (S)[3]; and 327 (M)[1] cps.

Anal. Calcd. for $C_{21}H_{32}O$ (300.47): C, 83.94; H, 10.73. Found: C, 83.35; H, 10.81.

17α-Ethynyl-3β-methoxy-17β-methyl-18-norandrosta-5,13-diene (XXI). - An ice cooled suspension of 17α-ethynylandrost-5-ene-3β,17β-diol (XX, 5.0 g.) in trimethylorthoformate (50 ml.) was treated with 72% perchloric acid (3.8 ml.). After stirring for 30 min., pyridine (4.0 ml.) was added and the reaction mixture poured into water. The oil that separated was extracted with hexane and passed through Magnesol. Evaporation of the solvent gave oily crystals which were redissolved in hexane and chromatographed on Florisil. The fraction eluted with 2% acetone-hexane was recrystallized from methanol to

yield 17α-ethynyl-3β-methoxy-17β-methyl-18-norandrosta-5,13-diene (1.32 g.), m.p. 91-92.5° (the melting point after sublimation was 90-90.5°); $[\alpha]_D$ -170°; NMR: 58 (S)[3]; 75 (S)[3]; 127 (S)[1]; 202 (S)[3] and 303 (M)[1] cps.

Anal. Calcd. for $C_{22}H_{30}O$ (310.46): C, 85.11; H, 9.74. Found: C, 85.05; H, 9.63.

3β-Ethoxy-17α-ethynyl-17β-methyl-18-norandrosta-5,13-diene (XXII). - In a procedure identical to that for the preparation of XXI with the exception that triethylorthoformate (50 ml.) was used in the reaction, 3β-ethoxy-17α-ethynyl-17β-methyl-18-norandrosta-5,13-diene (XXII) was obtained on recrystallization (methanol-water) of the hexane eluate of a Florisil column yielding 0.53 g., m.p. 80-81°; $[\alpha]_D$ -155°; NMR: 58 (S)[3]; 72 (T, J~8)[3]; 75 (S)[3]; 127 (S)[1]; 211 (Q)[2] and 323 (M)[1] cps.

Anal. Calcd. for $C_{23}H_{32}O$ (324.49): C, 85.13; H, 9.94. Found: C, 84.61; H, 9.95.

Methyl 3β-methoxyeti-5-enate (XXIV). (a). From XXIII. - A suspension of methyl 3β-hydroxyeti-5-enate (XXIII, 1.0 g.) in trimethylorthoformate (20 ml.) was treated with 72% perchloric acid (0.6 ml.). After 10 min. pyridine (1.0 ml.) was added and the reaction mixture diluted with water. Filtration afforded a solid which was dissolved in methylene chloride and passed through Magnesol. Evaporation of the solvent gave a glass which crystallized to yield XXIV (0.79 g.), m.p. 134-135° (lit.[12] m.p. 133-134°, $[\alpha]_D$ -23°).

(b). From XXV. - 3β-Acetoxyeti-5-enic acid (XXV, 1.0 g.) in trimethylorthoformate (10 ml.) was treated with 72% perchloric acid (0.52 ml.). Stirring was continued for 7 hr., the volume of

trimethylorthoformate was kept near 10 ml. by addition of the ortho-ester during the reaction. The dark brown reaction mixture was neutralized with pyridine (2.0 ml.) and then poured into water and filtered. The crude yellow solid was dissolved in methylene chloride and passed through a Magnesol pad. Evaporation and recrystallization from methanol afforded XXIV (0.50 g.), m.p. 129-130°.

Methyl 3β-methoxybisnorchol-5-enate (XXVII). - A suspension of 3β-acetoxybisnorchol-5-enic acid (XXVI, 1.0 g.) in trimethylortho-formate (20 ml.) was stirred for 6 hr. after the addition of 72% perchloric acid (0.5 ml.). Additional trimethylorthoformate was used to keep the volume near 20 ml. Pyridine (0.5 ml.) was added to terminate the reaction and the reaction mixture was then poured into water. The product was filtered and crystallized from methanol to yield methyl 3β-methoxybisnorchol-5-enate (0.12 g.), m.p. 118-120.5°; $[\alpha]_D$ -63°; NMR: 43 (S)[3]; 61 (S)[3]; 71 (D, J~7)[3]; 202 (S)[3]; 218 (S)[3] and 322 (M)[1] cps. (lit.[13] m.p. 117-118°, $[\alpha]_D$ -63.3°).

The Reaction of Cholestan-3β-ol (XXVIII) with Trimethylorthoformate Perchloric Acid. - To a suspension of 5α-cholestan-3β-ol (0.5 g.) in trimethylorthoformate (10 ml.) was added 72% perchloric acid (0.3 ml.). Solution was effected immediately and after 5 min. the reaction was terminated by pouring the reaction mixture into a saturated sodium bicarbonate solution. The resulting gum was extracted with methylene chloride. After washing the extract with water and drying over magnesium sulfate, evaporation in vacuo gave 500 mg. of a low melting solid.

Two 20 x 20 cm. glass plates were coated with a 0.5 mm. layer of Silica Gel G adsorbent and 100 mg. of the crude reaction mixture was streaked horizontally on each of the plates. The development was carried out with cyclohexane-ethyl acetate (97.5:2.5). Detection along both sides and a narrow strip through the center of each plate with a 10% phosphomolybdic acid-methanol spray disclosed the areas covered by five constituents. Each zone was removed by scraping the glass plates free of adsorbent in each zone and removing the compounds by acetone filtration of the solid. In the order of increasing polarity the following compounds were isolated: Cholest-2-ene (XXIX), 72 mg.; m.p. 71-72°, NMR C_2C_3 protons 333 cps, (lit.[15] m.p. 75-76°); 3α-methoxycholestane (XXX), 16 mg., m.p. 59-60°, NMR 3α-methoxy group 197 cps, (lit.[16] m.p. 62.5-63.5°); 3β-formyloxycholestane (XXXI), 70 mg., m.p. 79-80°, NMR formyl proton 477 cps J≈1 cps, (lit.[17] m.p. 83-84°); 3β-methoxycholestane (XXXII), 18 mg., m.p. 88-89°, NMR 3β-methoxyl group 198 cps, (lit.[18] m.p. 82.5-83°); and cholestan-3β-ol (XXXIII), 11 mg., m.p. 115-117° for a recovery of 187 mg.

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19. All melting points are uncorrected. The analyses were carried out by L. M. Brancone and associates. The spectral, NMR (Varian A-60-TMS standard), and optical rotational data(chloroform, 25°C) were supplied by William Fulmor and associates. The thin layer chromatograms were carried out at room temperature on glass plates coated with Silica Gel G (Merck, Darmstadt) prepared according to Stahl, E., Chem. Ztg., 82, 323 (1958). The solvent system employed was cyclohexane-ethyl acetate (95:5). Detection was accomplished with 10% phosphomolybdic acid spray. Florisil® (Floridin Corporation) is a synthetic magnesium silicate adsorbent. Magnesol® (Food Machinery Chemical Corp.) is a hydrous magnesium silicate. The orthoformates used in this study are commercially available or were supplied through the generosity of Kay-Fries Chemicals, Inc., New York, N.Y.