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Molecular Rearrangements in the Steroids. XIII. The Non-reductive Scission of Rings E and F of the Steroidal Sapogenins

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A new reaction of diosgenin acetate has been discovered in which both rings, E and F, are opened under the influence of hydrogen chloride in acetic anhydride. Presumptive generality of this reaction has been demonstrated. Several new compounds have been prepared and characterized. Mechanisms have been proposed for this new reaction, for the isomerization of steroidal sapogenins to pseudo genins and for the complex metallic hydride reduction of ring F of the spiro ketal system. The stereochemistry at C-16 in the steroid molecule has been briefly explored.

In the course of studies in this laboratory directed toward an elucidation of the mechanism of the isomerization of the steroidal sapogenins to their corresponding pseudo genin counterparts, a new reaction of the spiro ketal side chain system has been discovered. This discovery has resulted in the preparation of several new compounds and has cast light on some of the details of mechanism in the reactions of compounds of this type.

Gould, Staeudle, and Hershberg[2] postulated an ionic mechanism in which electrophilic attack by acetylonium ion at the F ring oxygen accounted for the observed opening of this ring which was previously accomplished[3] in acetic anhydride at 200° by Marker and his students. On this basis several Lewis acids were investigated as additives to the acetic anhydride and sapogenin reaction mixture. It was found that the temperature necessary to achieve isomerization was lowered from the previous 200° to the reflux temperature of acetic anhydride, 139°. Large excesses of the Lewis acids resulted in greatly diminished yields. The authors proposed that this latter effect was attributable to attack at the furanoid E ring.

Dauben and his associates[4,5] published details of their researches in which pyridinium chloride was used as the sole additive to the acetic anhydride and sapogenin reaction mixture. They found that use of this compound gave excellent yields and a product of good purity. Reaction time was relatively short in refluxing acetic anhydride. In the belief that these results indicated the formation of an intermediate containing chlorine, possibly at C-22, capable of undergoing subsequent dehydrohalogenation to form the observed 20(22) double bond, we set out to isolate this postulated compound.

The principal product of several reactions between diosgenin acetate (I) and an acetic anhydride solution saturated with anhydrous hydrogen chloride was indeed a chlorine-containing compound (II). Microanalysis results corresponded to an empirical formula of $C_{31}H_{47}ClO_5$. However, the stability of this compound and its resistance to all but the most vigorous measures to dehalogenate or dehydrohalogenate it made it clear that this was no tertiary halide or anything resembling such. Overnight reflux of II in acetic acid containing silver acetate resulted in the formation of III, a halogen-

(1) Present address: CIBA Pharmaceutical Products Inc., Summit, N. J. This article is based upon a dissertation submitted by Mr. Robert S. Miner, Jr., in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Princeton University.

(2) Gould, Staeudle and Hershberg, *J. Am. Chem. Soc.*, **74**, 3685 (1952).

(3) Marker and Rohrmann, *J. Am. Chem. Soc.*, **62**, 518, 898 (1940).

(4) Dauben, Eastham, Micheli, Takemura, Mandell and Chemerda, *J. Am. Chem. Soc.*, **75**, 3255 (1953).

(5) Dauben and Fonken, *J. Am. Chem. Soc.*, **76**, 4618 (1954).

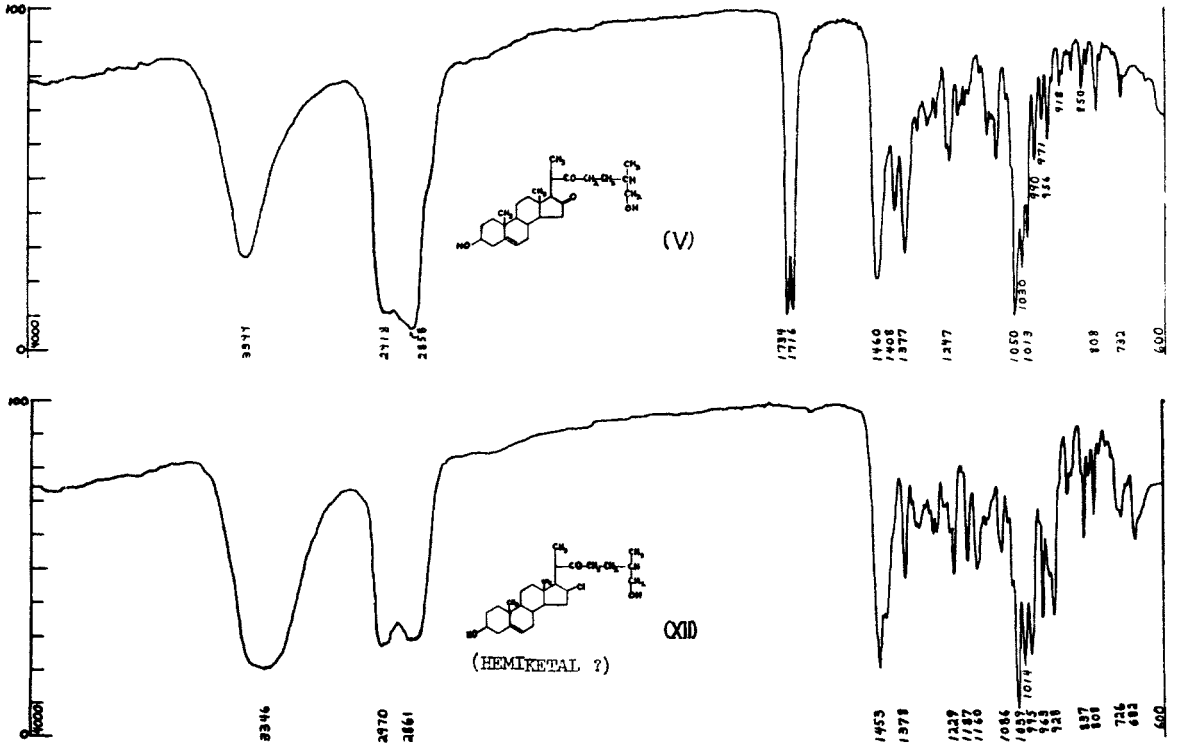
free compound corresponding to the empirical formula $C_{33}H_{50}O_7$. Mild saponification of III with carbonate produced IV, $C_{27}H_{44}O_4$. Treatment of IV with very dilute acetic acid produced diosgenin (VI).

Kaufmann and Rosenkranz[6] prepared a compound of the same empirical formula as IV by catalytic hydrogenation of kryptogenin (V). Furthermore their compound also underwent recyclization with dilute acetic acid to form diosgenin. Comparison of melting points and specific optical rotations made undeniable the identity of IV and the 16-dihydrokryptogenin of Kaufmann and Rosenkranz. Thereby III is identified as the corresponding triacetate, 3,16,27-trihydroxy-5-cholesten-22-one triacetate. II is thus a chloro-diacetate, the location of the chlorine being undetermined between the alternatives of C-16 and C-27. Decision in favor of location at C-16 is made largely on the basis of, the aforementioned replacement by silver acetate, since a primary halide would probably offer even greater resistance to replacement as well as undergoing rearrangement.

Since a halogen atom located at C-16 is not flanked on either side by a "configuration retaining group"[7] it is very likely that the conversion of II to III is accompanied by Walden inversion at this center. The fusion of rings D and E of the sapogenins being established as *cis-beta* it may be stated with certainty that the acetate group at C-16 in III and the hydroxyl group similarly located in IV are both in *beta* configuration. Similarly it may be stated that the chlorine at C-16 in II is most probably *alpha* oriented. Thus II may be identified as 16α-chloro-3,27-dihydroxy-5-cholesten-22-one diacetate.

The infrared spectrum of II, Figure 4, shows two distinctly resolved carbonyl absorptions, one at 1740 cm.$^{-1}$ corresponding to the carbonyl absorption of the acetates, and the other at 1720 cm.$^{-1}$ which is due to the absorption of an aliphatic ketone,[8] in this case located at C-22. This information in addition to the absence of the four strong bands associated with the spiro ketal structure, plus the striking similarity of the spectrum of II to that of kryptogenin diacetate serves to confirm our postulated open chain structure for II. The same resolution of carbonyl bands is also evident in the infrared spectrum of III.

In the spectrum of IV however, Figure 3, there is no evidence for any carbonyl absorption whatever. Marker[9] had postulated the existence of an intermediate in the formation of bethogenin (16α-methoxydiosgenin) from kryptogenin as a hemiketal in which the F ring had been reconstituted. Kaufmann and Rosenkranz[6] had postulated that IV could exist in any of three forms, open chain dihydroxy-ketone, E ring hemiketal, or F ring hemi-

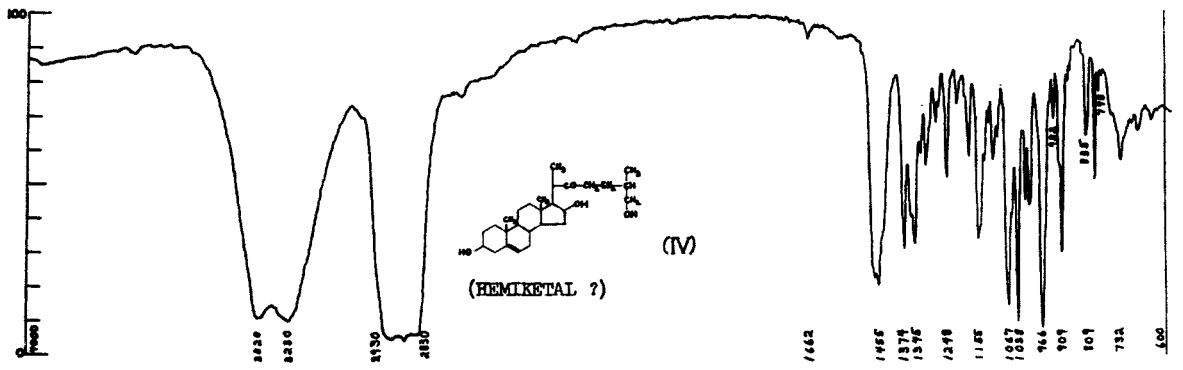


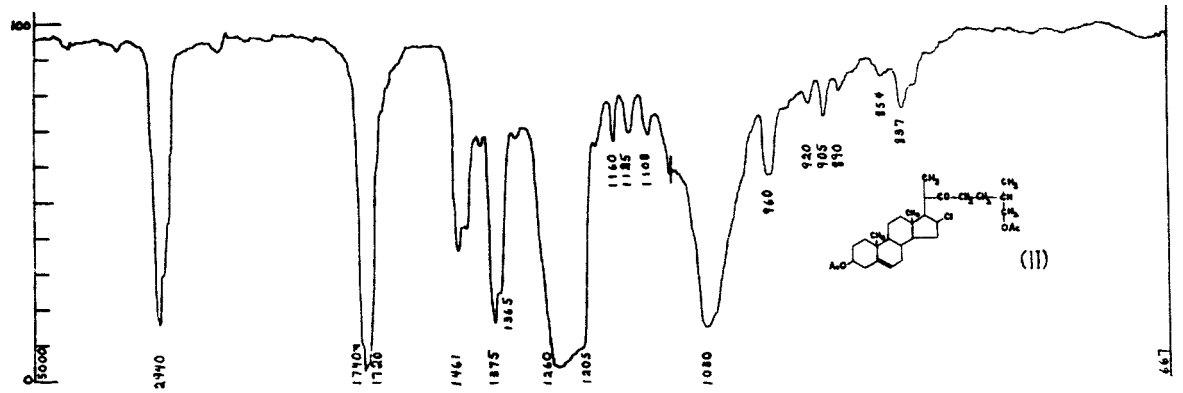
(6) Kaufmann and Rosenkranz, *J. Am. Chem. Soc.*, **70**, 3502 (1948).

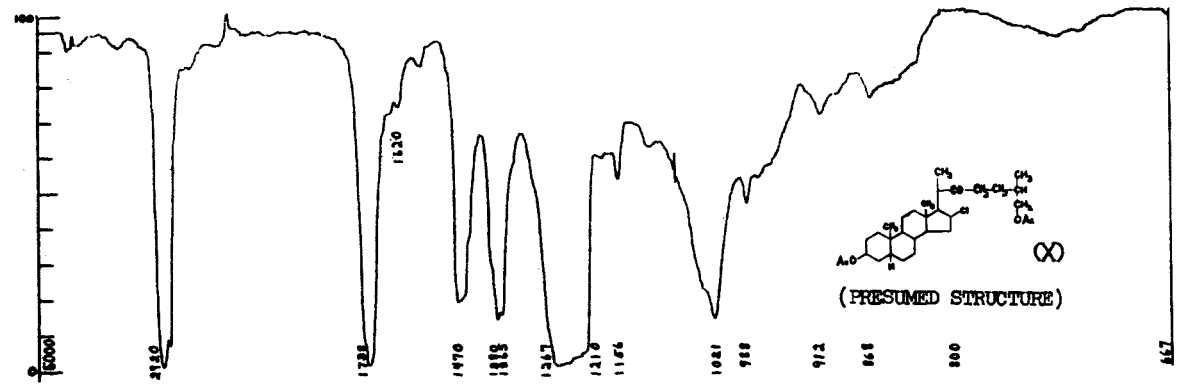
(7) Ingold, *Structure and Mechanism in Organic Chemistry*, Cornell University Press, Ithaca, 1953, p. 387-391.

(8) Jones, Katzenellenbogen and Dobriner, *J. Am. Chem. Soc.*, **75**, 158 (1953).

(9) Marker, Wagner, Ulshafer, Wittbecker, Goldsmith and Ruof, *J. Am. Chem. Soc.*, 69, 2210 (1947).







INFRARED SPECTRAL FIGURES

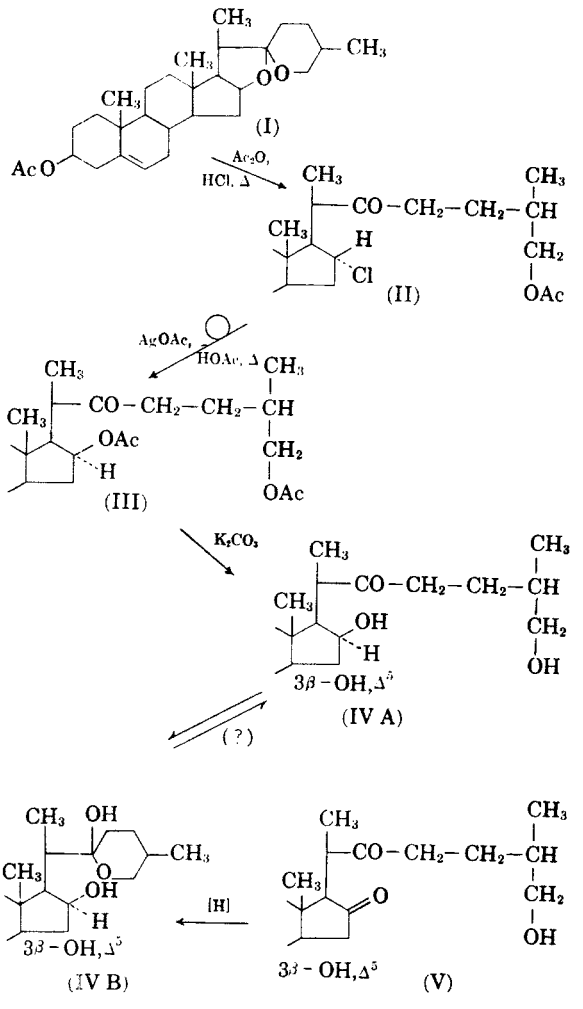
The spectra submitted herewith were prepared on two different instruments, both Perkin-Elmer Model 21 infrared spectrophotometers. One was linear with respect to wavelength and the other linear with respect to frequency, hence the differences in resolution noted from one figure to another. The following pertinent data are therefore presented by way of explanation. The mulls were prepared by the usual technique with Nujol.

Number	Solvent	Linearity	Identification
1.	Mull	Frequency	Kryptogenin, (V)
2.	Mull	Frequency	Compound XII
3.	Mull	Frequency	Compound IV
4.	$CHCl_3$	Wavelength	Compound II
5.	$CHCl_3$	Wavelength	Presumable saturated analog of II, (X)

ketal. Here for the first time was some definite physical evidence in support of the hemiketal structure, ring indeterminate however.

In an effort to determine whether the hydroxyl group at C-16 or the one at C-27 had participated in this ring closure, II was subjected to mild carbonate saponification producing XII, $C_{27}H_{43}ClO_3$, the 16α-chloro-3,27-diol. Infrared spectral analysis, Figure 2, disclosed that this compound also exists as a hemiketal. Here the only possibility was participation by the hydroxyl group at C-27. Room temperature acetylation of XII with acetic anhydride and pyridine produced the 3-acetate of XII, $C_{29}H_{45}ClO_4$, designated XIII. This compound also showed no carbonyl absorption which could be as-

CHART ONE



sociated with C-22. These cases present very strong evidence for the F ring hemiketal structure in XII and XIII. By analogy of structure and spectra it is highly probable that IV, Figure 3, also exists as the *F ring* hemiketal. Overnight reflux in acetic anhydride converts XIII to II.

In these infrared spectral studies it is surprising to note the failure of kryptogenin, Figure 1, to form a hemiketal. Here there is clear resolution of the bands attributable to the two carbonyl groups at C-16 and C-22, the former appearing at about the same frequency as the carbonyl absorption of the acetate group, *i.e.*, 1734 cm.$^{-1}$ Failure to form the F ring hemiketal in this case is not readily explicable on any basis other than possibly that of steric hindrance.

A well known analogy to the formation of F ring hemiketals in all of these compounds is found in carbohydrate chemistry where the pyranoid ring is the structure of choice in those situations where both alternatives, pyranoid and furanoid are possible. Further precedent for this type of behavior is also found in the recently studied oxo-cyclo desmotropism of aldosterone where both forms have actually been isolated in the crystalline state[10] as shown by dual melting points and mull type infrared spectra. All of the chloroform solution spectra of aldosterone are reported to have a strong carbonyl absorption band.

In Marker's early work[11] he showed that hydrogenation of the spiro ketal side chain over platinum in the presence of a trace of acid resulted in the reductive scission of the F ring to form the dihydrogenin. This type of compound was resistant to further reduction, even under Clemmensen conditions. However if the spiro ketal compound was subjected to Clemmensen reduction both the E and the F rings were opened with formation of the tetrahydrogenin. Wilson's study[12] shows the cleavage of tetrahydrofuran rings to proceed at a rate of the order of one hundred times that of the cleavage of tetrahydropyran rings. Thus it seems logical to conclude that strongly acid conditions favor cleavage of ring E before ring F. It has also been shown[13] that the cleavage of anisole by anhydrous hydrogen chloride in solvents of low dielectric constant does not proceed unless some other compound is added which reacts to permit ionization of the HCl.

Especially interesting in this connection are two recent publications by Doukas and Fontaine[14,15] concerning the reduction of the F ring of steroidal sapogenins by complex metal hydrides in the presence of hydrogen chloride and their failure to react in the absence of hydrogen chloride. In view of the results reported here, it seems that a possible mechanism for this reaction might be postulated. Electrophilic attack by protons at both oxygen atoms, opens ring E first to produce a hemiketal and a carbonium ion at C-16. This ion reacts with chloride ions *largely via* unhindered approach from the rear of the molecule to form the 16α-chloro compound analogous to XII. Further electrophilic attack by a second proton at the F ring oxygen then cleaves this ring in a concerted mechanism which also involves liberation of a proton from the hemiketal hydroxyl and an electron pair shift to produce a carbonyl group at C-22. The carbonyl then is reduced by the complex hydride with formation of an alcoholate. This group eliminates one mole of metallic chloride in a typical Williamson reaction with Walden inversion at C-16 to re-establish ring E.

A similar mechanism is postulated for the formation of II (see Chart Two) from diosgenin acetate

(10) Harman, Ham, DeYoung, Brink, and Sarett, *J. Am. Chem. Soc.*, **76**, 5035 (1954).

(11) Marker and Rohrmann, *J. Am. Chem. Soc.*, **61**, 846 (1939).

(12) Wilson, *J. Chem. Soc.* (*London*), **1945**, 48, 63.

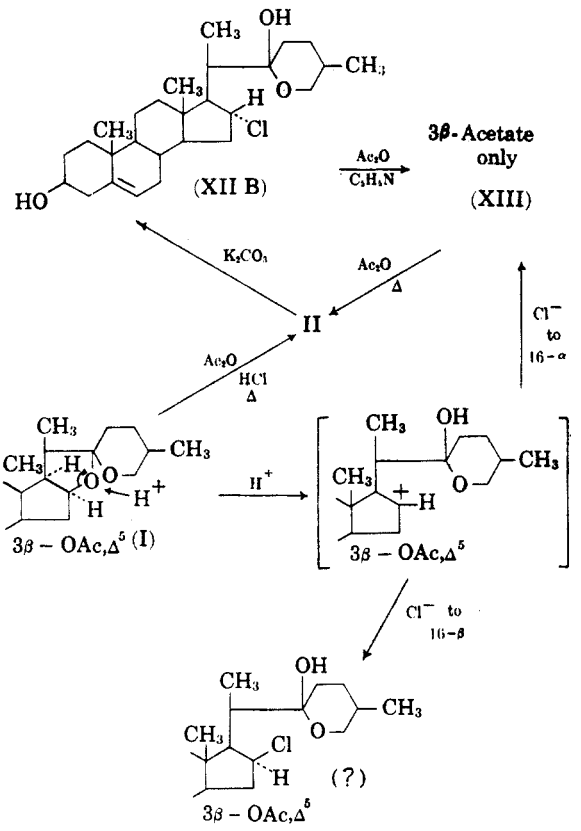
(13) Walvekar, Phalnikar, and Bhide, *J. Indian Chem. Soc.*, **20**, 131 (1943).

(14) Doukas and Fontaine, *J. Am. Chem. Soc.*, **73**, 5917 (1951).

(15) Doukas and Fontaine, *J. Am. Chem. Soc.*, **75**, 5355 (1953).

(I) with a few changes in details. Cleavage of ring F may be brought about by a proton attack or by acetylonium ion attack since both are necessarily present. If the former is the case, then esterification of the hydroxyl group which is formed takes place subsequent to and distinctly separate from the reaction of cleavage. The carbonyl and the 16α-chloride remain intact.

CHART TWO



Finally it seems in order to postulate an alternative mechanism for the conversion of genins to pseudo genins in acetic anhydride, either with or without the addition of pyridinium chloride. Nucleophilic attack at the spiro ketal carbon atom by chloride ion or by acetate ion alone in the absence of pyridinium chloride cleaves F ring with the formation of a 22-chloride or 22-acetate, the negatively charged oxygen attached to C-27 soon adding an acetylonium ion to form the ester. This compound then undergoes thermal elimination of the elements of one mole of acid, hydrochloric or acetic as the case may be, to form the 20(22) unsaturation. Thus the role of the pyridinium chloride is dual, the suppression of hydrogen ion concentration thereby protecting the E ring from cleavage, and also the furnishing of an adequate concentration of nucleophilic ions at a temperature much lower than that required to produce adequate ionization of acetic anhydride.

An account of this research would not be complete without mentioning two other reactions which were carried out but not completely clarified as to the identity of their products. The saponification of II with potassium hydroxide in methanol with overnight reflux produced an unidentified mixture of compounds which were arbitrarily designated VII and VIII. Treatment of this mixture with a trace of dilute acid converted it to diosgenin (VI). Secondly the treatment of smilagenin acetate with acetic anhydride and hydrogen chloride apparently produced a scission of both rings, E and F, analogous to that experienced with diosgenin acetate, but no pure crystalline product was isolated. Evidence for this statement is found in a comparison of the infrared spectra found in Figures 4 and 5. All attempts to purify the products of these reactions both by crystallization and by chromatography over acid-washed alumina have failed thus far. It is hoped that these details can yet be clarified.

EXPERIMENTAL

Melting points are uncorrected. They were determined in a flame-heated brass block at a heating rate not exceeding three degrees Centigrade per minute for at least the final twenty degrees before melting occurred. The microanalyses were done by the staff of the Microanalytical Laboratory of CIBA Pharmaceutical Products Inc., Summit, New Jersey.[16] Optical rotations and a number of the infrared spectra were determined and recorded also by appropriate CIBA personnel. The raw materials for this work in the form of various sapogenins and their esters were obtained from several generous donors to whom we are deeply grateful.[17]

16-Chloro-3,27-dihydroxy-5-cholesten-22-one diacetate (II). A suspension of 150 g. of diosgenin acetate in 1.5 liters of acetic anhydride was subjected to addition of hydrogen chloride gas for a period of four hours. After the addition of hydrogen chloride had started, the heat of reaction was supplemented sufficiently to produce reflux (70–80°) and continued thus. After four hours the HCl addition was discontinued and the reaction mixture was refluxed for an additional three hours during which time the reflux temperature rose to 100° or slightly above. The run was then quenched by pouring into at least 10 liters of water and permitted to stand overnight. Next day the aqueous phase was discarded and the gummy residue was dissolved in 2 liters of ether, dried, decolorized with carbon, and filtered. The filtrate was concentrated to a total volume of 0.5 liters or a bit less and the separated crystals were filtered

(16) Grateful acknowledgment is hereby made of the leave of absence and the generous financial assistance provided by CIBA Pharmaceutical Products Inc., Summit, N. J., to the junior author of this paper during his graduate studies. We also wish to thank Dr. Emil Schlittler and Mr. Louis Dorfman for making available to the authors the services of the CIBA Microanalytical and Infra Red Laboratories.

(17) Thanks for these materials are especially extended to Dr. W. H. Fischer, Dr. A. C. Shabica, Dr. J. A. Nelson, Dr. J. B. Ziegler, Mr. F. B. Arentz and Dr. Lincoln Werner of CIBA; to Dr. E. F. Elslager of Parke-Davis and Company; to Dr. Irving Scheer and Dr. William Wildman of the National Institutes of Health; and to Dr. Monroe Wall of the Eastern Regional Research Laboratory, U. S. D. A.

off, washed with the minimal amount of ether, and dried. Usually a second fraction of crystalline product could be isolated by further concentration. It was washed, dried, and combined with the first fraction. The crude yield varied from 25 to 30 g. Recrystallization twice from absolute ethanol gave 15–20 g. of pure II, m.p. 211–212°, $[\alpha]_D^{26}$ +14° in chloroform.

Anal. Calc'd for $C_{31}H_{47}ClO_5$: C, 69.57; H, 8.85; Cl, 6.63. Found: C, 69.70; H, 9.08; Cl, 6.35.

3,16,27-Trihydroxy-5-cholesten-22-one triacetate (III). A charge of 0.5 g. of II, 0.2 g. of silver acetate, and 250 ml. of glacial acetic acid was refluxed in darkness for 24 hours, care being taken to exclude moisture from the system. The mixture then was concentrated *in vacuo* to a volume of about 15 ml., the insoluble silver chloride was removed by filtration, and the filtrate was concentrated again *in vacuo* to dryness. The residue was recrystallized in 2 ml. of methanol to give 0.35 g. of material of insufficient purity for microanalysis. Repeated recrystallization from methanol gave pure III, m.p. 167.0–167.5°, $[\alpha]_D^{24}$ +13° in chloroform, Beilstein test negative.

Anal. Calc'd for $C_{33}H_{50}O_7$: C, 70.93; H, 9.02. Found: C, 70.79; H, 9.14.

3,16,27-Trihydroxy-5-cholesten-22-one (IV). A charge of 0.25 g. of III, 10 ml. of methanol, 0.4 g. of anhydrous potassium carbonate, and 2 ml. of water was refluxed for three hours and then was poured into excess water. The solids were filtered, washed with water, partially dried, and recrystallized from the minimal amount of methanol. The first fraction yield was 0.10 g. of pure IV, m.p. 175–176°, $[\alpha]_D^{25}$ −43° in ethanol. (*Cf.* 16-dihydrokryptogenin of K. & R.: m.p. 173–175°, $[\alpha]_D^{20}$ −47° in $CHCl_3$).

Anal. Calc'd for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.74; H, 10.52.

If this procedure is modified to the extent that 1.2 ml. of 20% acetic acid is added to the cooled reaction mixture before quenching into water, the product is diosgenin (VI), identified by melting point, admixture melting point with an authentic sample, and by infrared spectrum comparison.

16-Chloro-3,27-dihydroxy-5-cholesten-22-one (XII). A charge of 1.0 g. of II, 45 ml. of methanol, 1.2 g. of anhydrous potassium carbonate, and 6 ml. of water was refluxed for two hours and then poured over excess ice. Solids were filtered, washed, and dried to yield 0.75 g. of crude product melting over a long range. Recrystallization from the minimal amount of absolute ethanol gave a first fraction yield of 0.21 g. of pure XII, m.p. 133–134°, $[\alpha]_D^{26}$ −62° in chloroform.

Anal. Calc'd for $C_{27}H_{43}O_3Cl$: C, 71.89; H, 9.61; Cl, 7.86. Found: C, 72.14; H, 9.99; Cl, 7.62.

16-Chloro-3,27-dihydroxy-5-cholesten-22-one 3-acetate (XIII). To a solution of 1.35 g. of crude XII in 7 ml. of pyridine was added a charge of 10 ml. of acetic anhydride. After thorough mixing the reactants were permitted to stand overnight at room temperature. The following day the batch was poured over excess ice and allowed to stand several hours. Solids were filtered, washed, dried, and recrystallized in the minimal amount of absolute ethanol. First fraction yield was 0.76 g. of pure XIII, m.p. 102–103°, $[\alpha]_D^{25}$ −57° in chloroform.

Anal. Calc'd for $C_{29}H_{45}ClO_4$: C, 70.63; H, 9.20; Cl, 7.19. Found: C, 70.94; H, 9.48; Cl, 6.74.

Overnight reflux of XIII in acetic anhydride reconverted it to II, identified by melting point and admixture melting point with an authentic sample of II.

KOH saponification of II. A charge of 1.50 g. of II, 65 ml. of methanol, 3.0 g. of potassium hydroxide pellets, and 6 ml. of water was refluxed overnight and then poured onto excess ice. The solids were filtered, washed, and dried to give a yield of crude product of 1.20 g. Beilstein test was negative on this material arbitrarily designated VII and VIII, m.p. 150–152° (unsharp). The melting point was not improved by recrystallization.

Anal. Calc'd for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 76.84; H, 10.62; Calc'd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21.

Acid-catalyzed recyclization of VII and VIII. To a solution of 0.1 g. of VII and VIII in 10 ml. of methanol was added 4 ml. of 25% acetic acid in water. The solution was rewarmed on the steam-bath to boiling and then was poured onto excess ice. The solids were filtered, washed, and dried. The crude residue, m.p. 203–204°, showed no depression on determination of admixture melting point with an authentic sample of diosgenin (VI), m.p. 201–205°.

Dehydrohalogenation of II with silver oxide. A charge of 5.0 g. of II intimately mixed and ground with 5.0 g. of silver oxide was carefully heated *in vacuo* to 139° at which temperature a violent exothermic reaction took place. The charred mass was further heated at 145–150° for 42 hours *in vacuo*. After cooling, the vacuum was broken and the residue was extracted exhaustively with acetone and the extracts were evaporated to dryness. Recrystallization from the minimal amount of methanol gave three fractions of crystalline material of closely comparable purity, totalling 2.62 g. XIV, m.p. 203–205°, halogen free.

Anal. Calc'd for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.83; H, 9.24.

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